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## Morphological and anatomical characterisation of black alder *Alnus glutinosa* (L.) Gaertn. ectomycorrhizas

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**Abstract** Ectomycorrhizal types of black alder [*Alnus glutinosa* (L.) Gaertn.] collected over a 3-year period within an alder forest were characterised by morphological and anatomical features. Of the total of 16 types, 14 are described for the first time in this paper. Eight identified types belong to the genera *Russula*, *Lactarius*, *Naucoria*, and *Cortinarius*, while eight further types remained unidentified. In some cases, similarities of mantle features indicate relationships to identified mycorrhizas. Mycorrhizas of *Naucoria escharoides* and *N. subconspersa* were not distinguished. Two unidentified mycorrhizal types exhibited hyphal mantle structures very similar to these *Naucoria* species. Within the genus *Cortinarius*, mycorrhizas of *C. cf. helvelloides* were easily distinguished from all other *Cortinarius*-like mycorrhizas described on *Alnus*, which in general showed little anatomical variation. Two further unidentified mycorrhizas, “*Alnirhiza lilacina*” and “*A. violacea*”, probably also belong to *Cortinarius*. The ectomycorrhiza of *Russula pumila* was the only identified type within the genus *Russula*, but the unidentified type “*Alnirhiza cremicolor*” also likely belongs to this genus. Three *Lactarius* species were present in the experimental plot. Two species (*L. obscuratus* and *L. omphaliformis*) had indistinguishable mycorrhizal types, but were easily differentiated from the mycorrhizas of *L. lilacinus*, which caused intracellular penetration of Hartig net hyphae into epidermal and cortical cells. All other mycorrhizal types of black alder exhibited a paraepidermal Hartig net without penetration of root cells. Two unidentified mycorrhizal types “*Alnirhiza atroverrucosa*” and “*A.*

*cystidiobrunnea*”, already described from North American *Alnus rubra* as unnamed morphotypes, showed no similarity to identified mycorrhizas. All 16 mycorrhizal types appeared to be specific or at least typical for alders, since they have not yet been reported from other tree species.

**Key words** Alder mycorrhizas · Characterisation of ectomycorrhizas · Morphology · Anatomy · *Alnus glutinosa*

### Introduction

Mycorrhizas of black alder [*Alnus glutinosa* (L.) Gaertn.] were studied during investigations into the ecology of black alder stands in Northern Germany within the interdisciplinary project Ecosystem Research in the Bornhoeved Lakes District. In addition to their actinorhizal symbiosis with *Frankia* spp., alders are known to form both arbuscular (AM) and ectomycorrhizas (EM) (Harley and Smith 1983). AM within the genus *Alnus* have been described from *Alnus glutinosa* (Truszkowska 1953; Mejskrik 1971; Hall et al. 1979; Rose 1980; Beddiar 1984) and several other alder species (Arveby 1988; Chatarpaul et al. 1989; Russo 1989; Lumini et al. 1994), whereby they seemed to be restricted to seedlings (Beddiar 1984; Arveby 1988) or very wet locations (Truszkowska 1953) within natural habitats or were found on mine spoils (Mejskrik 1971; Lumini et al. 1994). Within the present experimental plot, AM were not observed on either seedlings or roots of older trees from continuously flooded sublocations (Pritsch 1996); therefore further investigations focused on EM.

Alder forests bear a rich fungal flora with many apparently alder-specific species (Bujakiewicz 1989). Of the approximately 1000 fungal species described from ecologically diverse alder stands in western and central Europe, an estimated 100 species are potential mycorrhizal fungi (Boyle 1996).

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Culture syntheses with alders and a variety of ectomycorrhizal fungi showed that, in contrast to many other tree species, alders are rather specific concerning their ectomycorrhizal partners (Molina 1979, 1981). So far approximately 50 ectomycorrhizal types have been described from alders world-wide and about 20 have been identified. However, some of these types have only been synthesised under laboratory conditions or in the greenhouse (Molina 1979, 1981; Godbout and Fortin 1983; Brunner et al. 1990; Miller et al. 1991, 1992) and have not yet been described from natural alder stands.

Naturally occurring alder ectomycorrhizas have been described mainly from North America (Neal et al. 1968b; Froidevaux 1973; Massicotte et al. 1986; Miller et al. 1988, 1991, 1992) but also from Japan (Masui 1926) and New Zealand (Mejstrik and Benecke 1969). Very few ectomycorrhizas of European alder species have yet been characterised (Treu 1990; Agerer et al. 1993), and no systematic descriptions of black alder mycorrhizas existed until now.

Thorough characterisation of mycorrhizal species by morphological and anatomical description of mycorrhizas (Miller et al. 1991) and identification of their fungal partners (Agerer 1991) is a prerequisite for recognising mycorrhizal diversity in ecosystems and for comparison of data obtained by other researchers. Therefore, in this study, the EM of black alder were first characterised. Two complementary methods were used, namely a morphological anatomical study and PCR/RFLP analyses of both EM and fruitbodies. The morphological and anatomical results are presented in this paper, and molecular biological data can be found in Pritsch et al. (1997).

## Materials and methods

### Sampling sites

The investigated 60-year-old black alder stand at Lake Belau (30 km south of Kiel, Schleswig-Holstein, Germany) is an experimental plot of the ecosystem research programme carried out by the Ecosystem Research Centre of the University of Kiel. A description of the site is given by Pritsch et al. (1997). Roots were collected monthly over 3 years (1992–1994) from the litter layer, in which 80–90% of all alder fine roots were growing. After cleaning of the roots in the laboratory, alder roots were easily differentiated from others by the presence of actinorhizal nodules and the typical broom-like branched root system.

### Morphology of ectomycorrhizas

Cleaned mycorrhizas were examined with a stereomicroscope and photographed with an MC100 microscope camera (Zeiss, Oberkochen) with EPY64T (Kodak) film to describe and document morphological features. Fresh mycorrhizas were either prepared for direct microscopy of the hyphal mantle (hand sections or mantle mounts) according to Agerer (1991) or fixed in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer. For embedding, mycorrhizas were postfixed with OsO<sub>4</sub>, and dehydrated with the series 10, 20, 50, 70, 95, 100% acetone in water. Embedding in ERL

followed the “low viscosity, longer pot life” variant described by Spurr (1969). Semi-thin tangential and cross sections were cut with glass knives using an RMC MT7 ultramicrotome and stained with New Fuchsin/Crystal Violet (NF/CV).

Total mantle views, handsections and sections from resin-embedded mycorrhizas were examined and photographed with a Zeiss Axioplan light microscope equipped with an MC100 camera (Zeiss) at × 400–1000 magnification. Sulpho-vanilline was used for the test of colour reactions within members of the Russulaceae. According to Miller et al. (1991), other reagents recommended for differentiating chemical reactions in fungal tissues also stain unmycorrhized alder roots, making them unsuitable as stains for alder mycorrhizas.

### Nomenclature

For identified ectomycorrhizas, the nomenclature of fungal partners follows Moser (1983). Unidentified mycorrhizas were termed according to Agerer (1991) using the genus of the tree species completed by “rhiza” and a describing epithet. Mantle structures were described following the nomenclature recommended by Agerer (1991), using “plectenchyma” for loose hyphal mantles or mantles with cylindrical hyphal cells and “pseudoparenchyma” for mantle structures built up by densely layered hyphae with a polygonal or epidermoid shape.

### Reference specimens and voucher material

Reference specimens of mycorrhizas and the sporocarps of identified mycorrhizas are preserved in the herbarium of K. Pritsch. Voucher specimens of ectomycorrhizas are deposited in the collection of the State Botanical Collection, Munich, Germany.

## Results

According to morphological/anatomical features of hyphal mantle structures, 14 EM types could be differentiated. Complementary molecular biological PCR/RFLP-analyses (Pritsch et al. 1997) revealed that these 14 EM types represent 16 EM species, eight of which could be identified within the four mycorrhizal genera *Naucoria*, *Cortinarius*, *Russula*, and *Lactarius*. Several of the 16 EM morphotypes showed similar morphology and anatomy, namely *Naucoria escharoides*/N. *subconspersa*, *Lactarius obscuratus*/L. *omphaliformis*, *Cortinarius alneus*/"*Alnirhiza lilacina*". In these cases, a morphotype description is given only once, with differentiating features, if any present, marked separately. The following key is provided for a quick determination of the described EM types.

### Determination key of black alder mycorrhizas occurring at the experimental plot

- 1 white or whitish with nuances of purple, lilac, blue, green, yellow, grey, or light brown → 6
- 1\* yellow, orange or brown → 2
- 2 yellow, yellowish-brown, orange → 4
- 2\* brown → 3
- 3 mantle overall dark brown, surface warty, apex blunt covered by mantle, outer mantle layer pseudopar-

enchymatous, inner layer plectenchymatous → “*Alnirhiza atroverrucosa*”

3\* mantle brown in mature parts, surface with cystidia, apex acute without or with unpigmented hyphae, outer and inner mantle plectenchymatous → “*Alnirhiza cystidiobrunnea*”

4 laticifers present, surface smooth, orange or yellowish brown → 5

4\* laticifers absent, surface warty, light yellow → *Russula pumila*

5 orange-brown, intracellular penetration of Hartig net hyphae into cortical cells → *Lactarius lilacinus*

5\* yellow, yellow-brownish, Hartig net paraepidermal → *Lactarius obscuratus* or *L. omphaliformis*

6 surface rather smooth to warty, emanating hyphae sparse, rhizomorphs lacking, outer mantle layer pseudoparenchymatous, inner mantle layer pseudoparenchymatous → “*Alnirhiza cremicolor*”

6\* surface woolly or cottony, emanating hyphae abundant or rhizomorphs present, outer and inner mantle layers plectenchymatous → 7

7 mantle whitish with grey or brown shades, hyphae of outer mantle layer not inflated, parallel to the root axis → 10

7\* mantle with purple, lilac, or bluish shades, air bubbles often enclosed, hyphae of outer mantle layer inflated, without parallel arrangement → 8

8 young EM with purple, older EM with rust-like shades, emanating hyphae and hyphal bundles numerous and with pigmented septa in older parts → “*Alnirhiza violacea*”

8\* young EM with faint lilac shade, older EM without rust-like shades, emanating hyphae and hyphal bundles numerous, hyphal bundles without pigmented septa → 9

9 EM conspicuously thick, mantle thickness exceeding 50 µm, mantle with bluish, green, yellow or lilac shades → *Cortinarius cf. helvelloides*

9\* EM not conspicuously thick, mantle thickness not exceeding 50 µm, lilac shade of mantle hyphae distinct → “*Alnirhiza lilacina*” or barely visible → *Cortinarius cf. alneus*

10 rhizomorphs regularly branched, surface felt-like, outer mantle layer net-like, middle layer of mantle with densely and strictly parallel arranged hyphae, inner layer plectenchymatous, colour cream-white → “*Alnirhiza texta*”

10\* rhizomorphs when present irregular, surface woolly, outer mantle of loosely net-like to parallel-arranged hyphae, inner layer of irregularly shaped, densely arranged hyphae, colour whitish → *Naucoria escharoides* or *N. subconspersa*, greyish → “*Alnirhiza cana*”, light brownish → “*Alnirhiza suffusa*”

## Description of the morphotypes

*Cortinarius cf. helvelloides* (Fr.) Fr. + *Alnus glutinosa*

### Morphology:

Tips unbranched, bent to hook-shaped, occasionally straight; apex rounded; up to 2 mm in diameter, length up to 15 mm. Colour: basically beige, with blue, green, yellow shades; silvery patches with enclosed air; injured parts rusty; apex lilac; old parts with metallic and rusty appearance. Surface: numerous woolly emanating hyphae agglutinated with soil particles; after their removal spongy to velvety. Hyphal bundles: irregular, flattened; white, milky exudates after scratching the mantle of young mycorrhizas.

### Section of mantle:

Not stratified, dense plectenchyma; thickness at the apex 20 µm, in differentiated parts 50–150 µm.

### Tangential view of mantle:

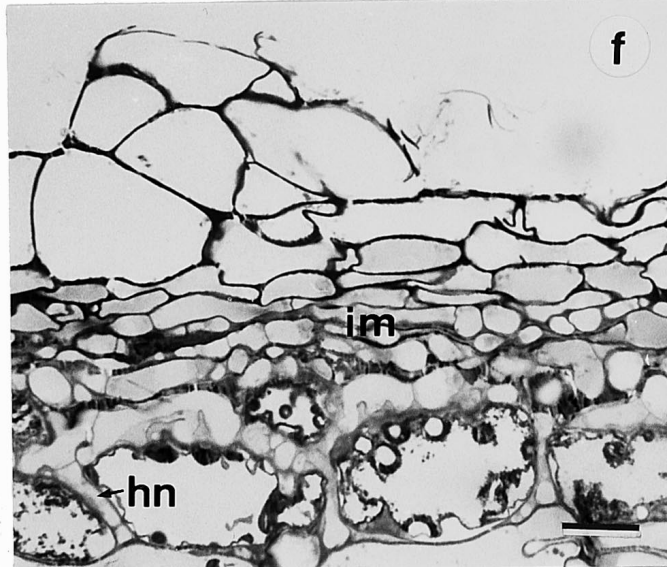
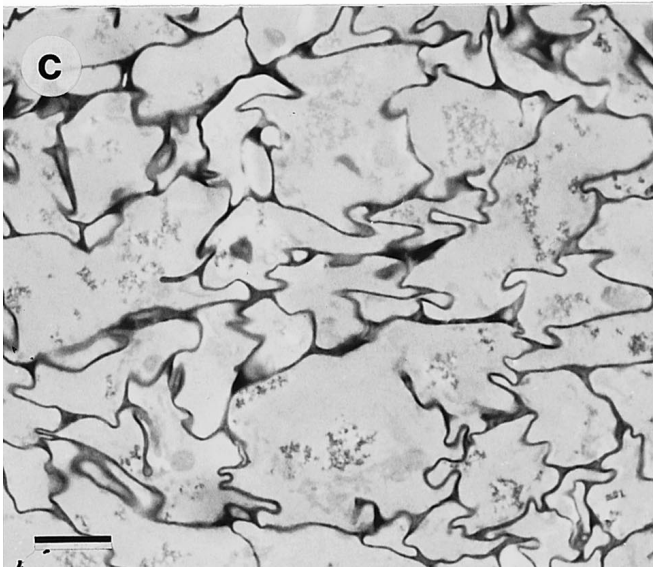
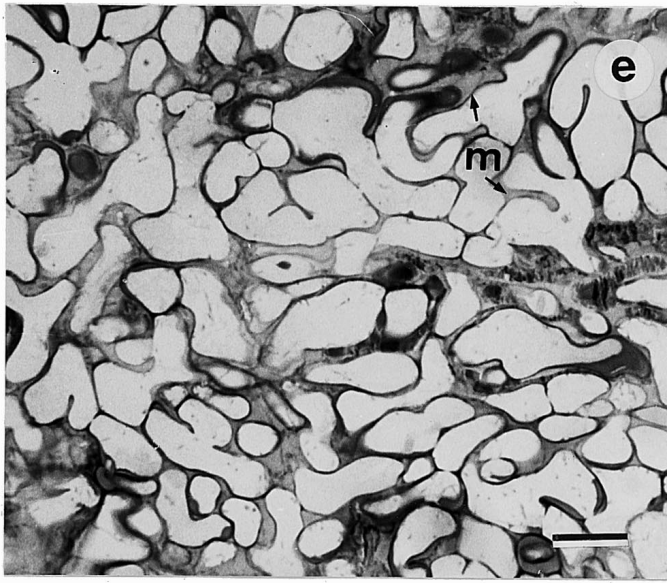
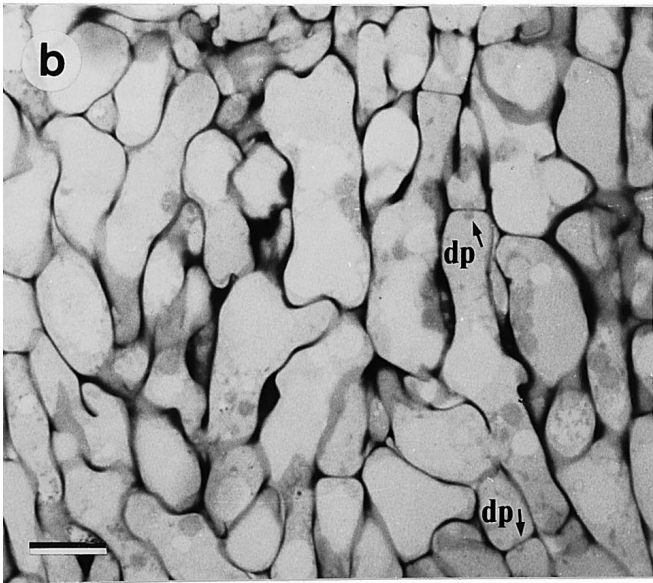
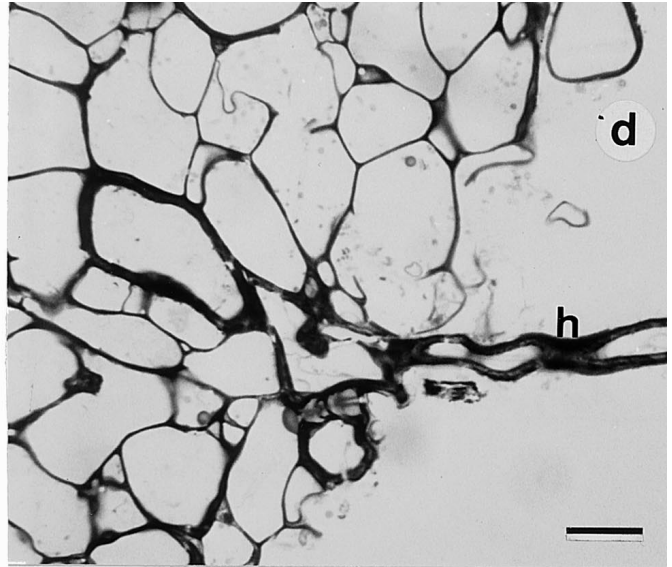
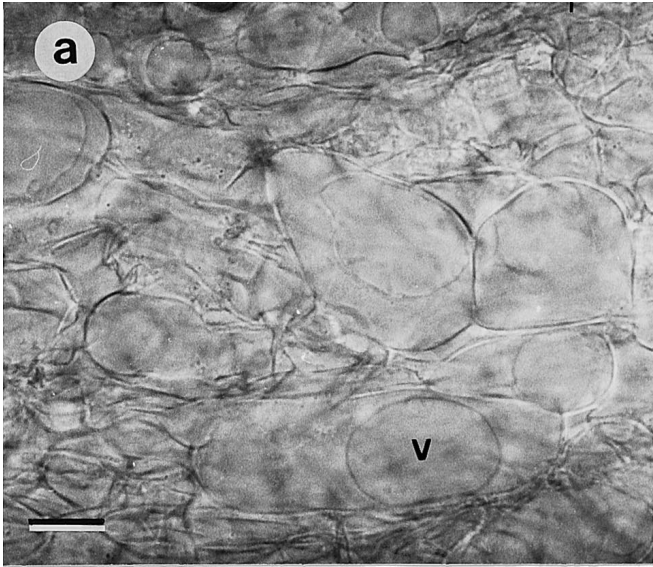
Young parts built up by cylindrical hyphae (4–10 × 15–39 µm) (Fig. 1b) with up to 1-mm-thick walls; clamps present only in outer parts, but dolipores visible in outer and inner parts; fully differentiated parts with highly inflated, densely packed hyphae, often containing huge, refractive vacuoles (15–75 µm in diameter) (Fig. 1a). Older parts with partially lysed or folded cell walls (Fig. 1c) resulting in an irregular pattern of large cells with diameters up to 75 µm and of completely collapsed hyphae with diameters smaller than 1 µm. Hartig net paraepidermal, with one row of hyphae, 1–4 µm thick.

### Emanating hyphae:

Clamps present, dolipores well visible; walls smooth, not or slightly thickened (up to 1 µm); simple anastomoses without septum abundant; diameter 4–8 µm, at the mantle surface up to 15 µm; distance between septa 25–200 µm; rhizomorph-like hyphal bundles consisting of undifferentiated, parallel-arranged, partially agglutinated, anastomosing, emanating hyphae.

*Cortinarius cf. alneus* (Mos.) Mos. + *Alnus glutinosa* “*Alnirhiza lilacina*”

With the exception of the more vivid lilac coloration of “*Alnirhiza lilacina*” and the dark brown spots after injuries of mycorrhizas of *C. cf. alneus*, both mycorrhizas exhibit identical anatomy and will therefore be described together.



◀ **Fig. 1** Light micrographs of EM of *Alnus glutinosa* with *Cortinarius helvelloides* (a–c) and unidentified ectomycorrhiza “*Alnirhiza atroverrucosa*” (d–f); **a** mantle mount of fresh mycorrhiza showing inflated cells with high roundish vacuole (v); **b** semi-thin tangential section through young part of mantle, hyphal septa with dolipores (dp); **c** semi-thin section through older part of mantle with folded cell walls; **d** semi-thin tangential section through outer pseudoparenchymatous mantle layer and mantle surface with emanating, thick-walled hypha (h); **e** semi-thin tangential section through inner plectenchymatous mantle layer with homogenous matrix material (m) between hyphae; **f** semi-thin median longitudinal section through “wart-like” structures at the surface, plectenchymatous inner mantle (im) and paraepidermal Hartig net zone (hn); bar 10  $\mu\text{m}$

#### Morphology:

Tips unbranched, often bent; apex rounded; diameter 0.3–0.8 mm, length 3–10 mm, rarely more than 15 mm. Colour: whitish, silvery when air enclosed, very young tips with a faint lilac shade, (only *C. cf. alneus* with dark brown patches when injured); older EM with brown root surface shining through mantle. Surface: woolly, cottony with many trapped air bubbles, felt-like, covered with irregular hyphal bundles; mantle at the basis and at the tip often thin or lacking. Emanating hyphae: numerous, often emerging in flat angles as hyphal bundles of fan-like or irregular shape; strongly agglutinated with soil particles.

#### Section of mantle:

Not stratified, plectenchyma loose in the outer part becoming gradually denser in the inner part; thickness 20–50  $\mu\text{m}$ .

#### Tangential view of mantle:

Surface with net-like arranged hyphae, (diameter 3–6  $\mu\text{m}$ ); hyphae branched, often inflated (diameter 10–15  $\mu\text{m}$ ) (Fig. 2a); simple anastomoses without septa; walls smooth, not thickened, neighbouring walls partially agglutinated; clamps present, dolipores recognisable. Inner mantle parts with gradually more densely arranged hyphae; hyphae cylindrical with straight or slightly curved walls in young parts (Fig. 2b), irregularly shaped and often collapsed in older parts (Fig. 2c). Hyphal walls smooth, not or slightly thickened; clamps sometimes still recognisable; diameter of hyphae 1–3.5  $\mu\text{m}$  in collapsed parts, 11–17  $\mu\text{m}$  in turgescient parts, distance between septa 3–90  $\mu\text{m}$ ; variable proportion of collapsed hyphae with a patchy distribution over differentiated parts of the mantle. Innermost part with lobed, loosely arranged hyphae surrounded by homogeneous matrix material; Hartig net paraepidermal, one-layered, 0.5  $\mu\text{m}$  thick. Symbiosis in basal parts often not fully established, with hyphae growing loosely at the surface without forming Hartig net.

#### Emanating hyphae:

Often combined to hyphal bundles by agglutination of cell walls and anastomoses; clamps present, dolipores visible; vacuoles often filled with rounded droplets, appearing yellow-greenish in fresh, and dark violet in embedded sections stained with NF/CV; diameter 3–6  $\mu\text{m}$ , inflated hyphae with diameter up to 15  $\mu\text{m}$ , distance between septa 15–150  $\mu\text{m}$ .

#### “*Alnirhiza violacea*”

#### Morphology:

Tips unbranched, bent to tortuous; apex rounded; 0.35–0.8 mm in diameter, length up to 13 mm. Colour: young mycorrhizas whitish with violet to purple shades and silvery patches when air entrapped, older mycorrhizas rust-like, purplish, brownish. Surface: spongy when air bubbles entrapped, after their removal woolly, cottony, felt-like, covered with emanating hyphae and hyphal bundles, strongly agglutinated with soil particles. Emanating hyphae abundantly combined to irregular hyphal bundles, with flattened or roundish cross section.

#### Section of mantle:

Not stratified, loose to dense plectenchyma; mantle thickness apically up to 50  $\mu\text{m}$ , in fully differentiated parts 40–110  $\mu\text{m}$ .

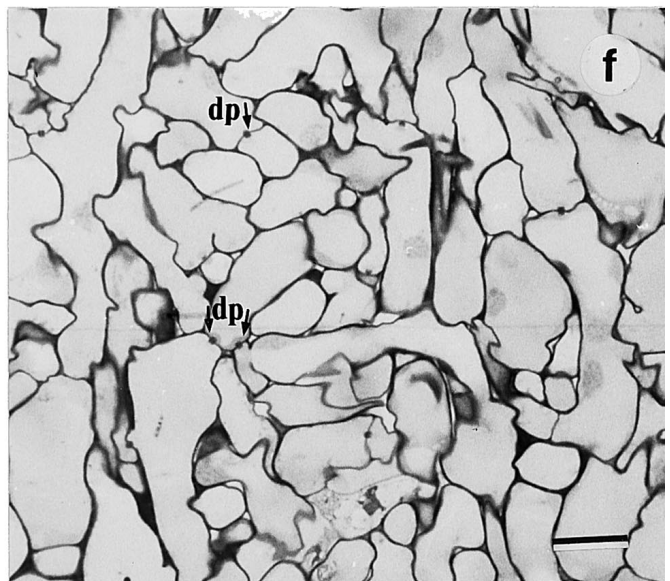
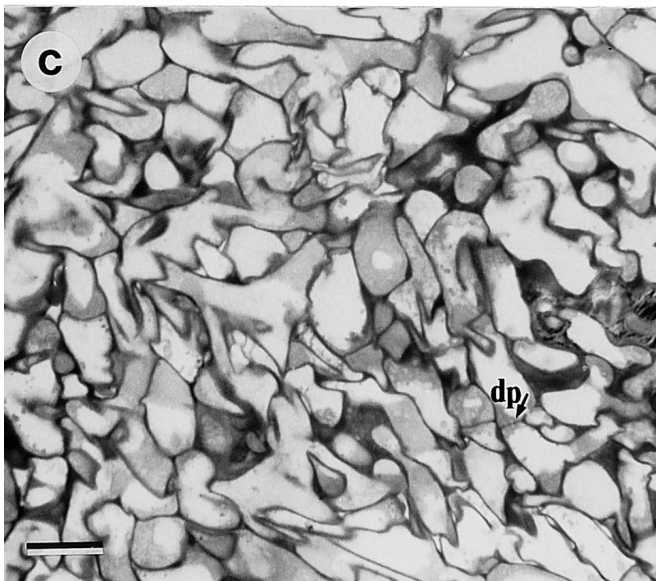
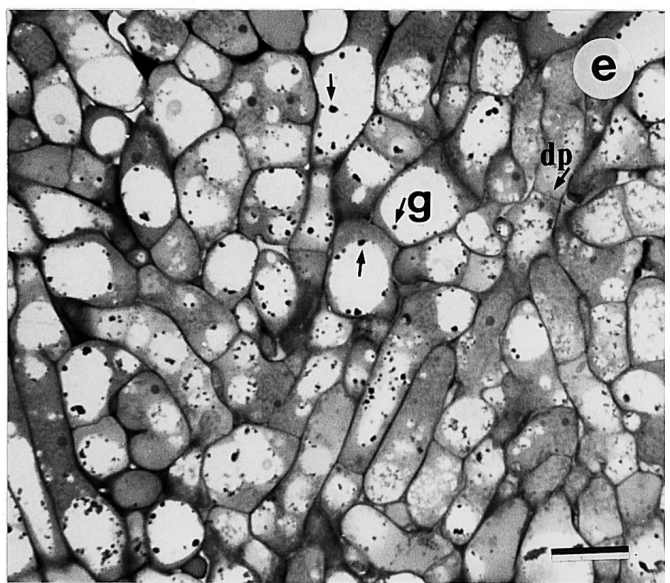
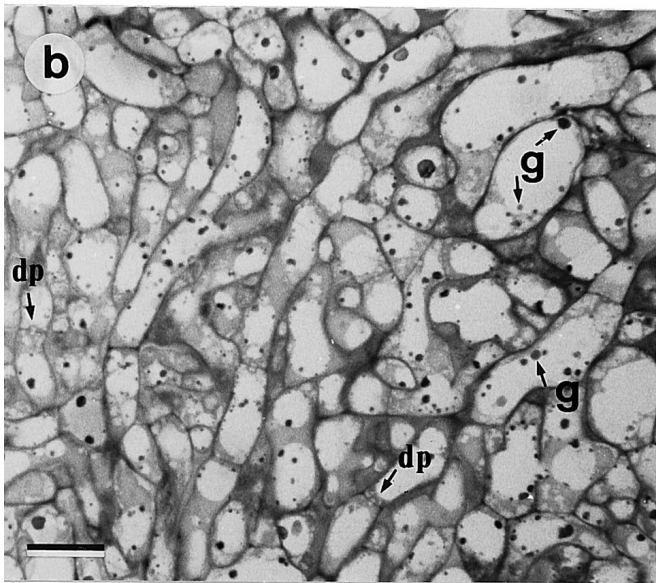
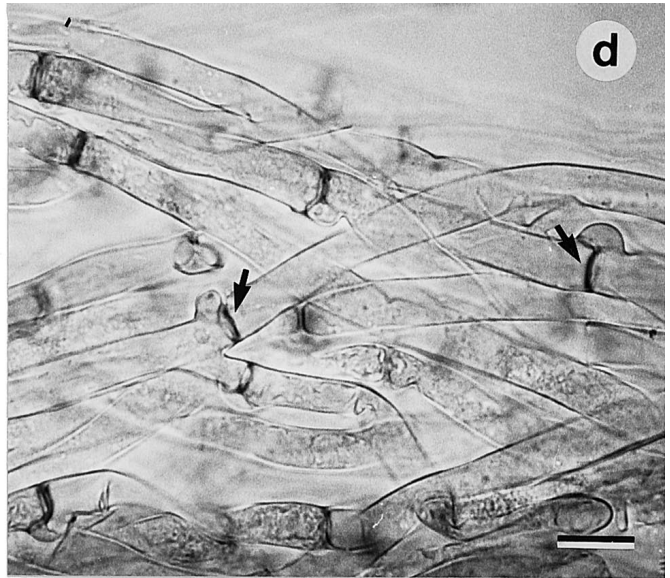
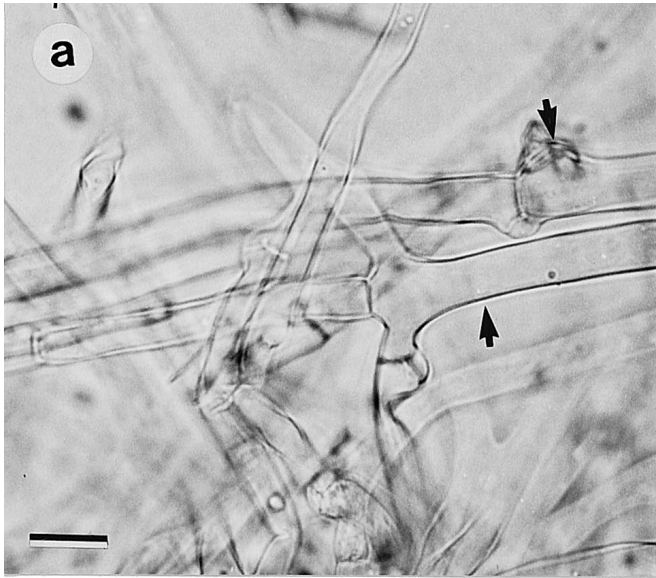
#### Tangential view of mantle:

Surface with loosely, net-like arranged hyphae; hyphae branched, septate, clamps present, dolipores visible; anastomoses simple without septum; walls smooth, partly thickened (up to 1  $\mu\text{m}$ ), often agglutinated to walls of neighbouring hyphae; hyphae often inflated, 4–14  $\mu\text{m}$  in diameter. Towards deeper mantle layers hyphae densely arranged, cylindrical or inflated; young parts with straight walls (Fig. 2e), fully differentiated parts with increasingly folded walls, resulting in an irregular shape of hyphal cells (Fig. 2f). Innermost part of the mantle with loosely arranged often branched or lobed hyphae, surrounded by homogeneous matrix material. Hartig net paraepidermal, hyphae broadly lobed, 1–4  $\mu\text{m}$  thick.

#### Emanating hyphae:

Forming irregular, rope-like interwoven, slightly differentiated rhizomorphs; up to 300  $\mu\text{m}$  in diameter; single inflated hyphae with diameters up to 14  $\mu\text{m}$ ; distance between septa 20–250  $\mu\text{m}$ ; especially conspicuous





◀ **Fig. 2** Light micrographs of unidentified ectomycorrhizas of “*Alnirhiza lilacina*” (a–c) and “*A. violacea*” (d–f); **a** inflated hyphae (arrows) emanating from mantle surface; **b** semi-thin tangential section through young part of mantle, vacuoles filled with dark-stained granules (g, arrows), septa with dolipores (dp, arrows); **c** semi-thin tangential section through older part with irregularly shaped cells, septa with dolipores (dp, arrow); **d** hyphae of undifferentiated rhizomorphs, showing dark pigmentation of septa (arrows); **e** semi-thin tangential section through young part of mantle, dark stained granules within vacuoles (g, arrows), septa with dolipores (dp, arrow); **f** semi-thin tangential section through older part of hyphal mantle with folded cell walls, septa with dolipores (dp); bar 10 µm

brown pigmentation of septa in older rhizomorphs, resulting in a stripe-like pattern (Fig. 2d). Hyphae with clamps, septae with recognisable dolipores; vacuoles of fresh hyphae with yellow-greenish, round droplets, which appear darkly stained on semi-thin sections of embedded EM; hyphal walls thin or thickened (up to 1.5 µm), often agglutinated to neighbouring walls, unpigmented or with brown pigmentation (fresh material); hyphae 3–7 µm in diameter.

*Naucoria escharoides* (Fr.: Fr.) Kummer + *Alnus glutinosa*

*Naucoria subconspersa* Kühn. + *Alnus glutinosa*

“*Alnirhiza suffusa*”

“*Alnirhiza cana*”

Morphology and anatomy of all four mycorrhizas is very similar. In contrast to *N. escharoides*/*N. subconspersa*, young mycorrhizas of both “*A. suffusa*” and “*A. cana*” possess brownish respectively greyish pigmented hyphae, thus appearing slightly coloured, while it is difficult or impossible to distinguish older mycorrhizas. Anatomically, the general architecture of the hyphal mantle is as described for *N. escharoides*/*N. subconspersa*. Both “*A. suffusa*” and “*A. cana*” can be distinguished from the former by their stripe-like thickened, pigmented hyphal walls. Because of the morphological and anatomical similarities, molecular biological features are more reliable for unambiguous determination of these types (Pritsch et al. 1997).

#### Morphology:

Unbranched tips or systems monopodial to irregularly pinnately branched (length up to 4 cm); with flexuous mean axes and side branches; length of mean axes up to 25 mm, diameter up to 1.2 mm; length of side branches up to 15 mm, diameter 0.4–0.8 mm; apex acute or rounded, with or without mantle. Colour: depending upon age, whitish with red apex when young, hyaline, light brown with root surface shining through when older, occasionally reddish all over. Surface: woolly, felt-like, or covered with hyphal bundles. Emanating

hyphae: hyaline, agglutinated with soil particles; combined to loose, irregularly shaped hyphal bundles; some hyphae short, cystidia-like with thin walls.

#### Section of mantle:

Two-layered (Fig. 3c); outer layer loose, plectenchymatous; inner layer dense, plectenchymatous to almost pseudoparenchymatous; mantle thickness 10–50 µm, inner layer representing 30–50% of the thickness.

#### Tangential view of mantle:

Surface with net-like arranged hyphae; in outer mantle layer, hyphae arranged in parallel to the root axis (Fig. 3a), straight, cylindrical, thin walled, clamps present, 2.5–6 µm in diameter, distance between septa 30–100 µm and more. In the transition zone between outer and inner layer, hyphae no longer parallel arranged, more often septate and branched, clamps rare; diameter 2.5 × 3–30 µm. Hartig net: paraepidermal, with delicate “palmetti” structure, diameter of hyphae between epidermal cells 0.5–2 µm.

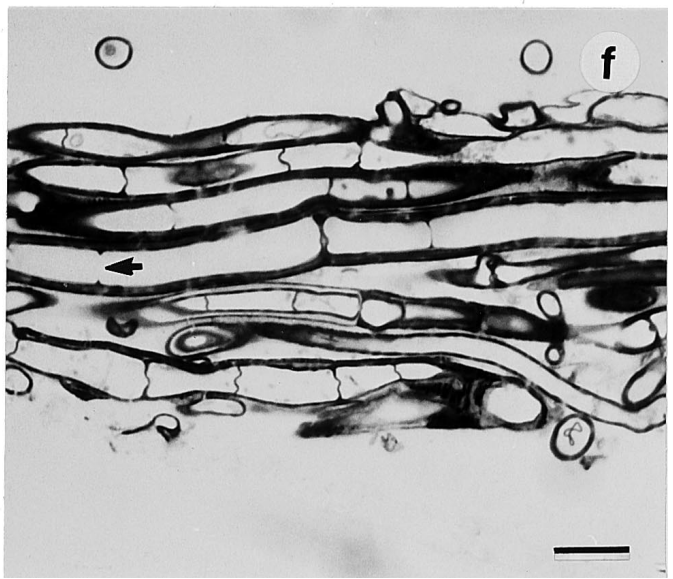
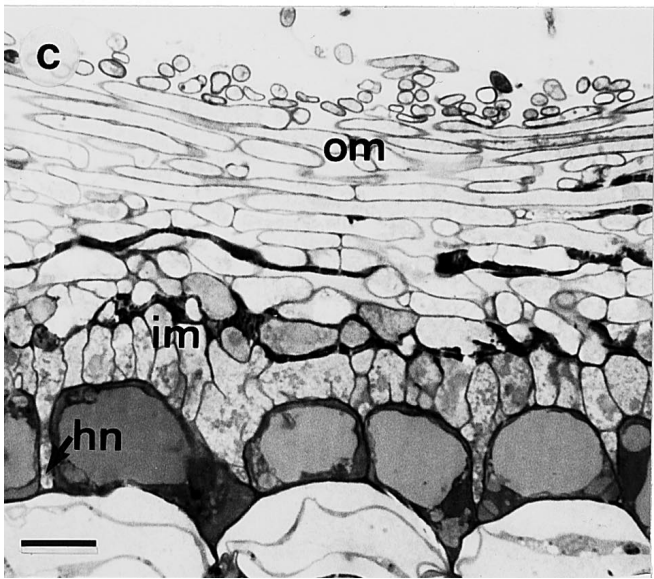
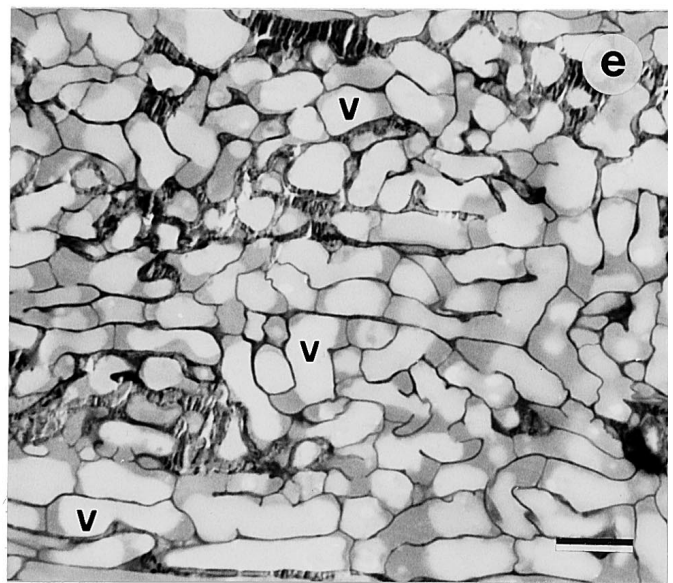
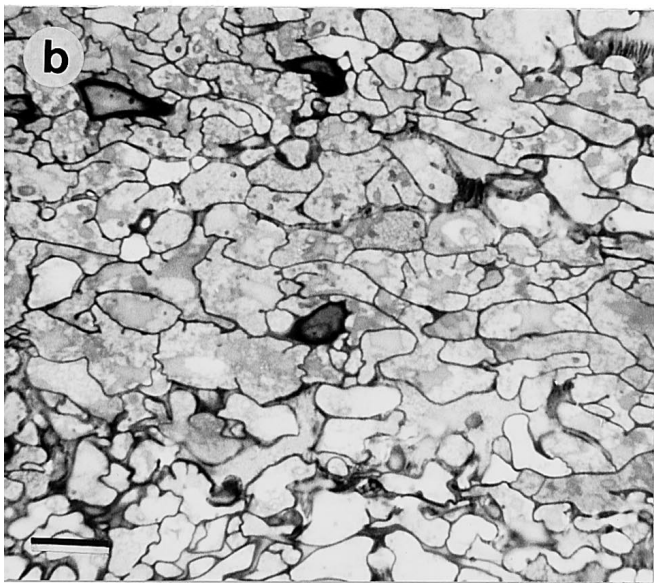
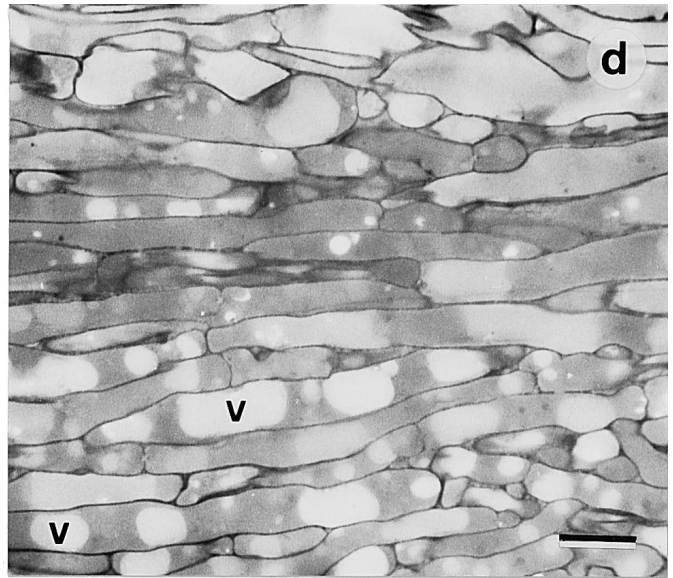
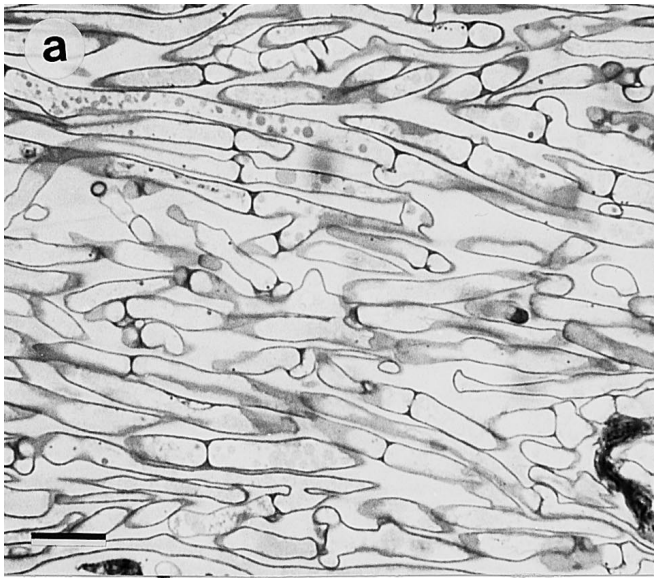
#### Emanating hyphae:

Clamps present, smooth and thin-walled (or thickened in the cases of “*A. cana*” and “*A. suffusa*”), branched; anastomoses abundant, without septa; vacuoles filled with round droplets or granular contents; diameter of hyphae 2.5–4 µm, distance between septa 20–210 µm; short hyphae with rounded tip up to 200 µm long; hyphal bundles irregular consisting of parallel-arranged, partly agglutinated and anastomosing, undifferentiated hyphae.

#### “*Alnirhiza texta*”

#### Morphology:

Unbranched tips or systems monopodial to irregularly pinnately branched; mean axes and side branches straight to flexuous; length of side branches up to 25 mm, diameter 0.5–1.2 mm; diameter of mean axes up to 1.5 mm; apex subacute with red tip, most often covered by mantle. Colour: whitish, when young, patches with fine air bubbles enclosed silvery shining, sometimes root reddish pigmented, giving the whole mycorrhiza a reddish-coloured appearance, older tips dull brown. Surface: felt-like with interwoven hyphae, covered with numerous short and sparse longer emanating hyphae; only rarely agglutinated with soil particles. Rhizomorphs abundant, regular, branched, round in cross-section with defined emanation point.





◀ **Fig. 3** Light micrographs of ectomycorrhizas of *A. glutinosa* with *Naucoria escharoides* (a–c) and unidentified ectomycorrhiza “*Alnirhiza texta*” (d–f); **a** semi-thin tangential section through plectenchymatous outer mantle layer; **b** semi-thin section through irregularly pseudoparenchymatous inner mantle layer; **c** median longitudinal section showing two-layered mantle [outer (*om*)/inner mantle (*im*)] and paraepidermal Hartig net (*hn*); **d** semi-thin tangential section through middle layer of the mantle with parallel-arranged hyphae with unstained vacuoles (*v*); **e** semi-thin tangential section through inner mantle layer, irregular plectenchyma, hyphae with unstained vacuoles (*v*); **f** semi-thin median longitudinal section through rhizomorph, thick-walled hyphae, septa present or partially lysed (*arrow*); *bar* 10  $\mu\text{m}$

#### Section of mantle:

Three-layered, plectenchymatous; outer mantle net-like, middle layer with dense, parallel-arranged hyphae, inner layer irregularly lobed; mantle thickness 35–50  $\mu\text{m}$ .

#### Tangential view of mantle:

Surface and outer mantle layer with net-like arranged, abundantly branched, often inflated hyphae; clamps and dolipores present; simple anastomoses without septum; hyphal walls thin, smooth, often agglutinated to walls of neighbouring hyphae; 3.5–6  $\mu\text{m}$ , inflated hyphae 10–15  $\mu\text{m}$  in diameter. Middle mantle layer with densely arranged, cylindrical hyphae, oriented in parallel to the root surface and length axis (Fig. 3d); vacuoles roundish with homogeneous, rarely with granular content; walls smooth and thin; clamps sometimes still recognisable; diameter 4–8  $\mu\text{m}$ , distance between septa 20–90  $\mu\text{m}$ . In the inner mantle layer hyphae loosely and irregularly arranged, branched (Fig. 3e), with Hartig net-like wall ingrowth at the root surface; diameter 4–10  $\mu\text{m}$ , distance between septa 6–40  $\mu\text{m}$ . Hartig net paraepidermal, delicate “palmetti” structure.

#### Emanating hyphae:

With clamps, smooth and thin walled; longer hyphae branched and with simple anastomoses; shorter hyphae with rounded to club-shaped tip. Rhizomorphs slightly differentiated (Fig. 3f); hyphae loosely rope-like arranged, outer hyphae regularly interwoven, inner hyphae more or less parallel arranged, clamps sometimes missing, often with thick walls (up to 1.5  $\mu\text{m}$ ); single hyphae with greater diameter (8–14  $\mu\text{m}$ ), septa most often present; rhizomorphs 100–300  $\mu\text{m}$  in diameter.

#### “*Alnirhiza cystidiobrunnea*”

This mycorrhiza was previously described by Miller et al. (1991) as “type 1” from *A. rubra*. As an additional feature, one undifferentiated rhizomorph was detected

on a single tip in the present studies. All other features were equivalent to the ones described by Miller and co-workers.

*Lactarius obscuratus* (Lasch) Fr. + *Alnus glutinosa*  
*Lactarius omphaliformis* Romagn. + *Alnus glutinosa*

Both mycorrhizas exhibited identical features. Mycorrhizas of *L. obscuratus* on *A. rubra* were described by Froidevaux (1973) and Miller et al. (1991), which differ in some features from the two *Lactarius* species collected on *A. glutinosa* in our experimental plot. For this reason a brief description is given, valid for both *L. obscuratus* and *L. omphaliformis*.

#### Morphology:

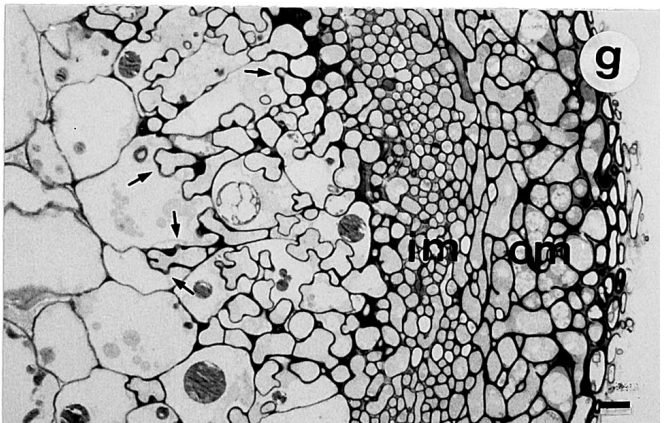
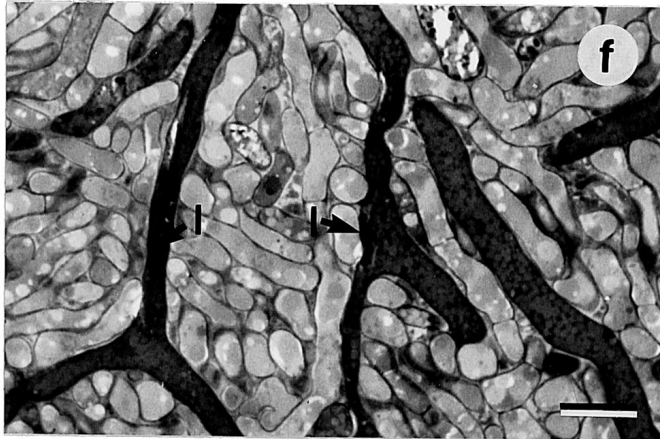
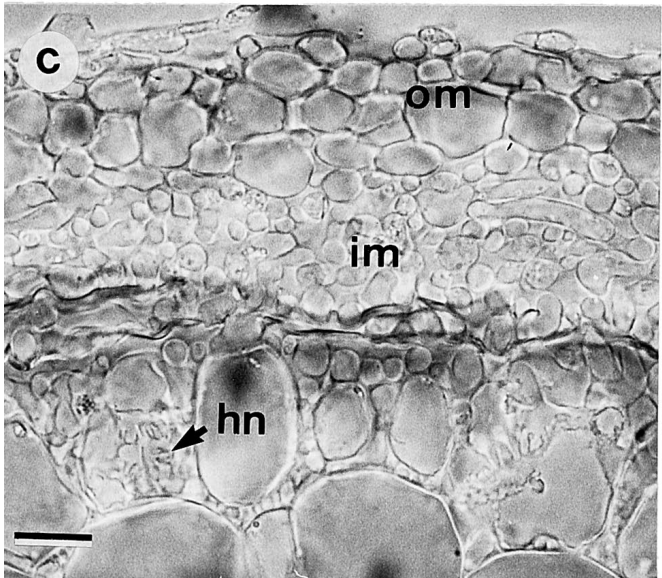
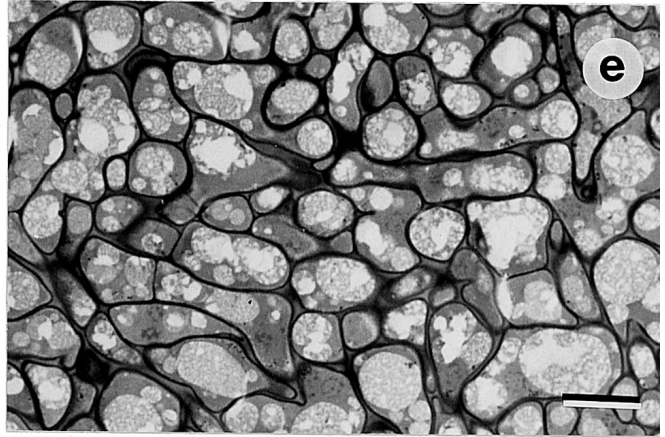
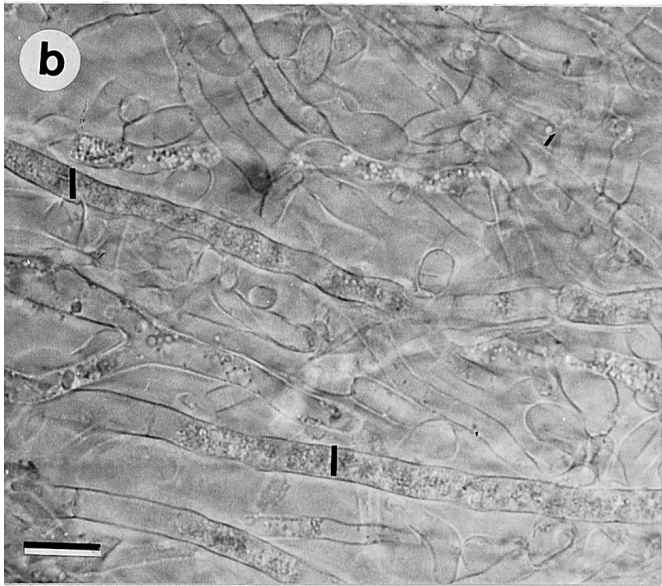
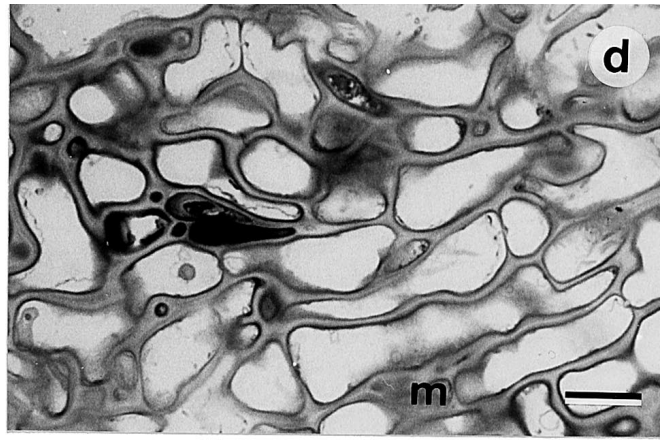
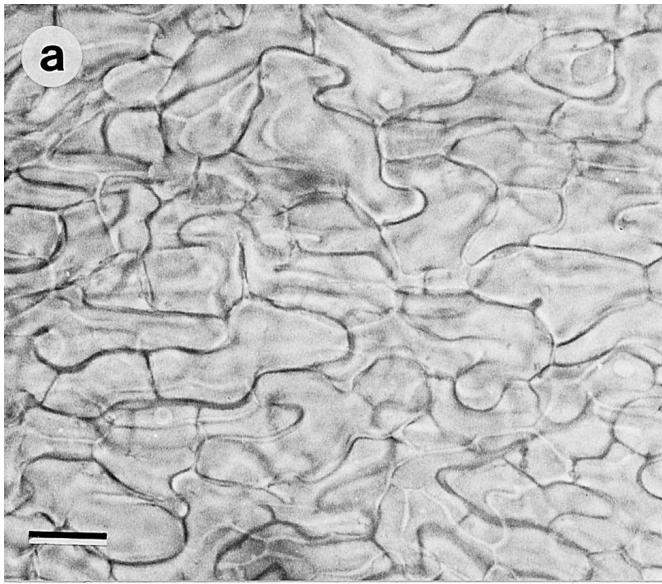
Single, unbranched tips or systems monopodial to pinate; axes straight or flexuous; apex rounded and covered with hyphal mantle; diameter of main axes up to 1.3 mm; diameter of side branches 0.4–0.8 mm, length up to 20 mm. Colour: yellow-brownish when young, dull brown to bronze when ageing, injured parts with rusty patches. Surface: smooth, shining, free of soil particles. Emanating hyphae: sparse, hyaline, fragile, sometimes emerging in fine bundles, agglutinated with soil particles.

#### Section of mantle:

Two-layered (Fig. 4c), outer layer intermediate between compact plectenchyma and irregular pseudoparenchyma, inner layer plectenchymatous; mantle thickness 15–25  $\mu\text{m}$ .

#### Tangential view of mantle:

Outer mantle layer with densely arranged cylindrical to epidermoid hyphae of irregular shape (Fig. 4a); walls smooth, with homogeneous, yellow wall appositions, thickness 1–2  $\mu\text{m}$ ; diameter of hyphae 5–32  $\mu\text{m}$ . Inner layer with cylindrical hyphae, partly parallel arranged to the root length axis (Fig. 4b); space between hyphae filled with homogeneous matrix; no clamps observed; walls smooth, thin; diameter of hyphae 3–6  $\mu\text{m}$ , distance between septa 15–25  $\mu\text{m}$ ; laticifers distributed net-like over inner layer, branched, filled with globular droplets; staining of globules with sulphovanilline dark purple; diameter of laticifers 3–8  $\mu\text{m}$ . Hartig net paraepidermal, one layered, “palmetti” structure, diameter of Hartig net hyphae 1.5–5  $\mu\text{m}$ .



◀ **Fig. 4** Light micrographs of ectomycorrhizas of *A. glutinosa* with *Lactarius omphaliformis* (a–c) and *Lactarius lilacinus* (d–g); **a** mantle preparation, plan view of outer mantle, with irregular pseudoparenchyma to plectenchyma; **b** mantle mount, plan view of inner mantle with plectenchymatous structure, laticifers (*l*) present; **c** cross section, two-layered mantle [outer (*om*)/inner mantle (*im*)] and Hartig net (*hn*); **d** semi-thin tangential section through mantle surface with irregularly arranged, mostly dead hyphae surrounded by homogeneous matrix material (*m*); **e** semi-thin tangential section through outer mantle layer; **f** semi-thin tangential section through inner mantle layer with dark-stained laticifers (*l*); **g** semi-thin cross section showing two-layered mantle [outer (*om*)/inner mantle (*im*)] and intracellular penetration (*ar*) of Hartig net hyphae into cortical cells; *bar* 10  $\mu\text{m}$

#### *Emanating hyphae:*

Usually thin-walled, rarely with yellow wall apposition; clamps present; diameter 3–5  $\mu\text{m}$ .

*Lactarius lilacinus* (Lasch) Fr. + *Alnus glutinosa*

#### *Morphology:*

Usually large systems or clusters of up to several hundred tips, rarely single tips; systems monopodially pyramidal or irregularly monopodially branched; axes straight or flexuous; apex rounded, sometimes with subapical thickening and club shaped, covered with hyphal mantle; main axes up to 1.8 mm in diameter; side branches 0.5–0.8 mm in diameter, length up to 14 mm. Colour: vivid yellow-orange when young, dark orange to orange-brown when ageing. Surface: smooth, shining, without adherent soil particles. White, milky exudates after scratching the hyphal mantle. Emanating hyphae: sparse, hyaline, fragile, mainly emerging from older parts as loose hyphal bundles, intensively agglutinated with soil particles.

#### *Section of mantle:*

Plectenchymatous two-layered mantle; hyphae of outer layer with greater diameter than in the inner layer (Fig. 4g); mantle thickness 50–90  $\mu\text{m}$ , outer layer 30–55  $\mu\text{m}$ , inner layer 20–35  $\mu\text{m}$ .

#### *Tangential view of mantle:*

Surface covered with lobed, branched, inflated mostly dead hyphae, imbedded in homogeneous matrix material (Fig. 4d); diameter at septa 4–7  $\mu\text{m}$ , diameter of inflated hyphae up to 12  $\mu\text{m}$ , distance between septa 15–45  $\mu\text{m}$ . In outer mantle layer, hyphae irregularly arranged (Fig. 4e); space between hyphae decreasing with depth; hyphae inflated, lobed, branched, septate, without clamps, dolipores visible; on semi-thin sections stained with NF/CV cytoplasm dark violet, vacuoles ap-

pearing light; diameter of hyphae 3–14  $\mu\text{m}$ , decreasing to 2–7  $\mu\text{m}$  with depth, distance between septa 3–45  $\mu\text{m}$ . Inner layer with densely arranged hyphae; net-like structure within the transition zone from outer to inner parts (Fig. 4f), changing to more-or-less parallel arrangement in the middle part; innermost part with loosely arranged, broadly lobed or branched hyphae; hyphae septate, without clamps, with abundant anastomoses, partially with lobed hyphae resembling a coarse Hartig net; 2–7  $\mu\text{m}$  in diameter, distance between septa 6 to >50  $\mu\text{m}$ .

#### *Laticifers:*

Sparse in outer layer, abundant in inner layer; sometimes branched, septa rare, often partially lysed; staining of embedded EM with NF/CV resulting in vivid blue coloration of cytoplasm and fainter staining of vacuoles filled with numerous droplets or homogeneous content (Fig. 4f); dark purple staining reaction of vacuole contents in fresh EM with sulphovanilline; 4–6  $\mu\text{m}$  in diameter. Hartig net: hyphae broadly lobed, 3–5  $\mu\text{m}$  in diameter; extending 2–3 layers deep into root cortex (Fig. 4g); intracellular penetration of cortical cells in all developmental stages except apex; no obvious disintegration of penetrated cortical cells observed.

*Russula pumila* Rouzeau & Massart + *Alnus glutinosa*

#### *Morphology:*

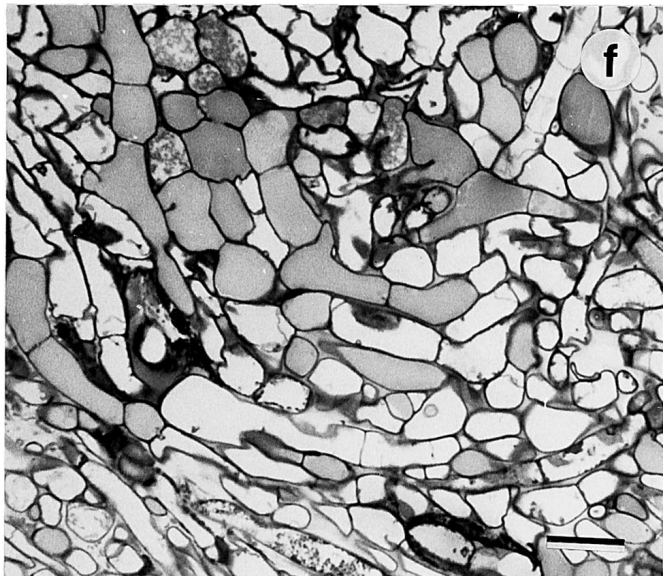
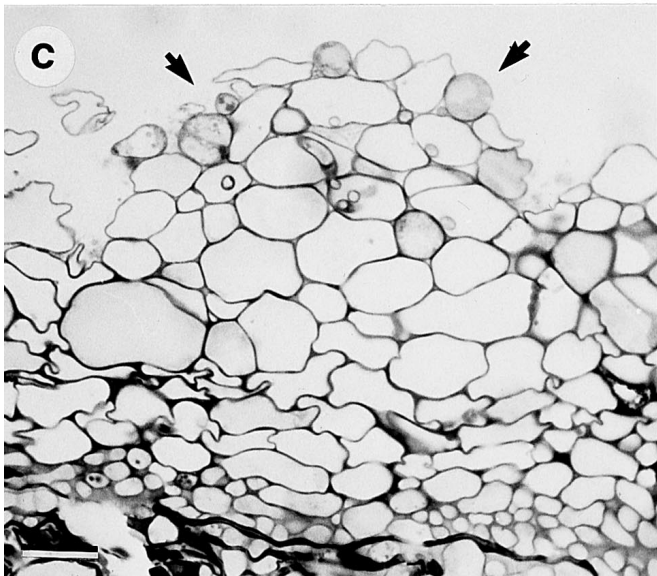
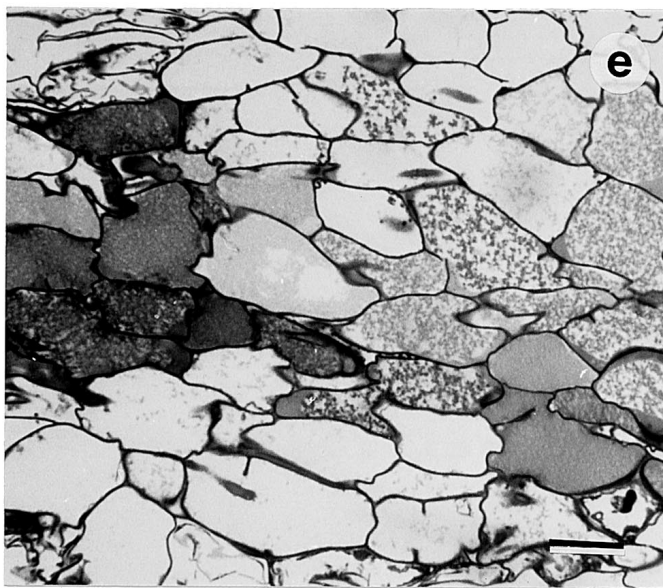
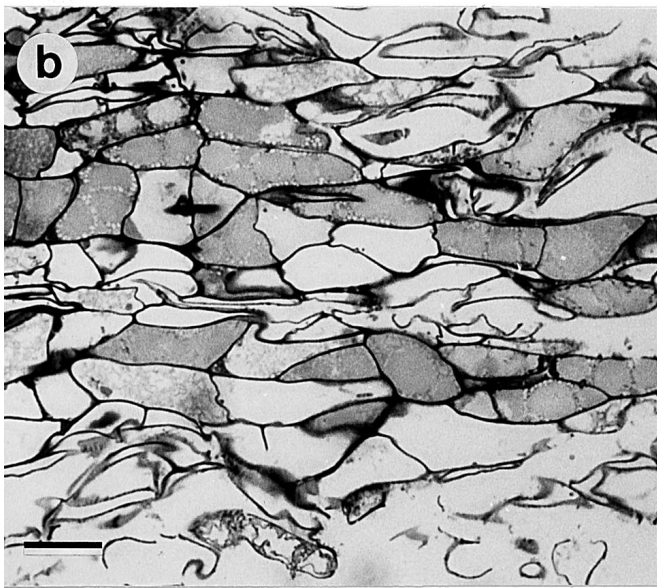
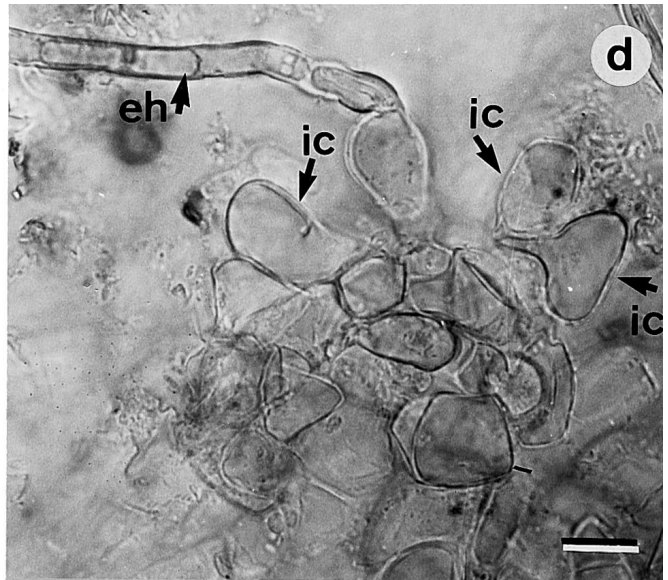
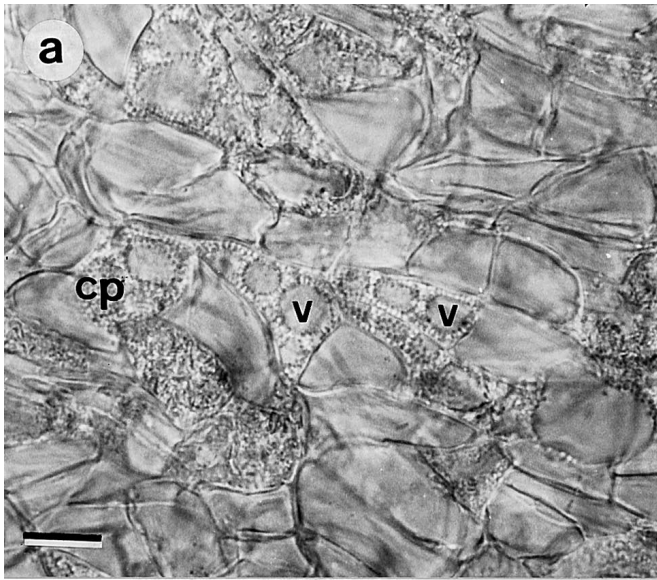
Mostly single unbranched tips, rarely irregularly pinnate systems branched at the basis; axes thickset, straight or flexuous; apex blunt, covered with hyphal mantle, occasionally root tip breaking through mantle; 0.5–1.5 mm in diameter, length up to 10 mm. Colour: young tips light yellow, yellow-brown when ageing. Surface smooth to fine warty. Emanating hyphae sparse, fragile, hyaline, often very short.

#### *Section of mantle:*

Two-layered (Fig. 5c), outer layer an angular pseudoparenchyma, inner layer plectenchyma; mantle thickness 25–70  $\mu\text{m}$ .

#### *Tangential view of mantle:*

Surface papillous, covered with thin-walled, hyaline often grouped dead cells of irregular, variable shape. Outer layer with two cell types (Fig. 5a,b): (1) angular, polygonal or somewhat elongated cells, often dead without cytoplasm; diameter 5–10  $\times$  6–20  $\mu\text{m}$ ; (2) single or grouped to linear-arranged cells with fine droplets in the cytoplasm, but not in the vacuoles; 2–8  $\mu\text{m}$  in diam-





◀ **Fig. 5** Light micrographs of ectomycorrhizas of *A. glutinosa* with *Russula pumila* (a–c) and unidentified ectomycorrhiza “*Alnirhiza cremicolor*” (d–f); **a** mantle mount of fresh mycorrhiza, plan view of outer mantle layer with cells containing round vacuoles (*v*) surrounded by granular cytoplasm (*cp*); **b** semi-thin tangential section through outer mantle layer of irregular polygonal pseudoparenchyma; **c** semi-thin cross section through “wart-like” arranged hyphal cells on mantle surface (*arrows*); **d** mantle preparate of fresh mycorrhiza showing mantle surface with papilla formed by a group of inflated cells (*ic*) and emanating hypha (*eh*); **e** semi-thin tangential section through outer mantle layer, irregular pseudoparenchyma; **f** semi-thin tangential section through plectenchymatous inner mantle part; *bar* 10  $\mu\text{m}$

eter; older parts often with collapsed hyphae. Inner layer with cylindrical densely, at the root surface loosely, net-like arranged, branched or lobed hyphae; walls smooth, thin; without clamps; diameter 2–5  $\mu\text{m}$ . Hartig net paraepidermal, one-layered, “palmetti” structure, diameter of lobes 1–2  $\mu\text{m}$ .

#### *Emanating hyphae:*

Unbranched or branched, without clamps, dolipores often visible; walls thin and smooth; 2–4  $\mu\text{m}$  in diameter, distance between septa 12–40  $\mu\text{m}$ ; some emanating hyphae with defined lengths between 15–50  $\mu\text{m}$ .

#### “*Alnirhiza cremicolor*”

#### *Morphology:*

Unbranched tips or irregularly pinnate systems, axes flexuous; apex rounded, covered with hyphal mantle; diameter of main axes up to 1.2 mm; diameter of side branches 0.4–1 mm, length up to 18 mm. Colour: cream-white when young, dull light brown when ageing. Surface: smooth to sparsely woolly, with wart like protrusions, which appear as light patches on older EM; with slightly agglutinated soil particles. Emanating hyphae: sparse but regularly present, short, hyaline, occasionally combined to small bundles, agglutinated with soil particles.

#### *Section of mantle:*

Usually two-layered; outer layer pseudoparenchymatous, inner layer plectenchymatous; at the apex only one-layered, plectenchymatous; mantle thickness apically 20–40  $\mu\text{m}$ , in differentiated parts 40–70  $\mu\text{m}$ .

#### *Tangential view of mantle:*

Surface papillous, covered with variably shaped, roundish hyphae, without cytoplasmic contents; often grouped to wart-like assemblages (Fig. 5d); short hy-

phae and cystidia also present. Outer mantle layer with angular or rounded hyphal cells, elongated in direction of the root axis (Fig. 5e), diameter 3–17  $\times$  8–48  $\mu\text{m}$ ; walls smooth, mostly thin, single cells or small groups of cells with thick walls up to 2  $\mu\text{m}$ ; hyphae often collapsed towards deeper layers. Inner mantle with branched, cylindrical hyphae (Fig. 5f); without clamps, dolipores often visible; walls smooth, with yellow appositions (fresh material); diameter 2–9  $\mu\text{m}$ , distance between septa 5–20  $\mu\text{m}$ ; innermost layer with lobed hyphae and wall ingrowths. Hartig net one-layered, paraepidermal, “palmetti” structure; diameter 1–3  $\mu\text{m}$ .

#### *Emanating hyphae:*

Branched, tortuous, emerging from thick-walled cells and separated by a basal septum; walls thin or with thick, smooth, yellow appositions; with simple septa or with clamps; diameter 2–7  $\mu\text{m}$ . Also cystidia-like hyphae present, with or without wall appositions, without clamps, length 15–50  $\mu\text{m}$ . Papillae consisting of rounded or acute ending, unseptate inflated cells on mantle surface.

#### “*Alnirhiza atroverrucosa*”

This mycorrhiza is very likely identical with a mycorrhiza formed by a Basidiomycete (Neal et al. 1968a), which was only briefly characterised by Neal et al. (1968b) from *A. rubra* and designated “clavate with a dark-brown, distinctively roughened mantle”. Therefore a complete description is given here.

#### *Morphology:*

Unbranched tips or irregular pinnate to monopodial systems; axes flexuous; apex rounded, covered with hyphal mantle, sometimes root apex breaking through mantle; diameter of main axes and side branches 0.8–1.2 mm, length up to 25 mm. Colour: light brown when young, dark brown to blackish-brown when fully differentiated; wart-like elements appearing darker. Surface: rough with warts. Emanating hyphae: single, mostly emerging from older mantle parts; darkly pigmented, thick, branched.

#### *Section of mantle:*

Two-layered (Fig. 1f), outer layer pseudoparenchymatous consisting of 1–4 layers of cells, inner layer plectenchymatous; thickness 30–70  $\mu\text{m}$ .



*Tangential view of mantle:*

Surface with groups of inflated cell (“warts”); majority of hyphal cells thick-walled (up to 1.5  $\mu\text{m}$ ), often without cytoplasm; surface with irregularly distributed, single angularly shaped cells with very thick walls (2  $\mu\text{m}$ ), from which emanating hyphae emerge (Fig. 1d). Outer layer with polygonal cells (Fig. 1d); walls up to 1.5  $\mu\text{m}$  thick, straight or curved; diameter 5–40  $\mu\text{m}$ . Inner mantle layer with cylindrical, branched or lobed hyphae (Fig. 1e); septate, without clamps, anastomosing, dolipores often recognisable; walls smooth; space between hyphae filled with homogeneous matrix; diameter 3–7  $\mu\text{m}$ . Hartig net: one-layered, paraepidermal, 1–4  $\mu\text{m}$  thick.

*Emanating hyphae:*

With clamps, between clamp-bearing septa simple septa present; 6–7  $\mu\text{m}$  in diameter. Hyphae emerging from single thick-walled cells; often branched; hyphae straight, rarely tortuous; hyphal tip with thin unpigmented wall, in basal direction gradually thicker, brown, walls smooth; wall thickness up to 2  $\mu\text{m}$ .

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**Discussion**

## General remarks

All black alder mycorrhizas described within the present study exhibited a paraepidermal Hartig net, restricted to the depth of one cell, with the exception of *L. lilacinus*, which is discussed below. Sometimes, radial and cross sections give the false impression that the Hartig net hyphae completely surround the epidermal cells forming a periepidermal Hartig, because epidermal cells were sectioned tangentially as is illustrated in a cross section of *L. omphaliformis* (Fig. 4). In most cases, mycorrhizas of alders seem to exhibit a paraepidermal or periepidermal Hartig net of one cell layer (Godbout and Fortin 1983). Nevertheless, deeper Hartig net penetration has been observed occasionally on *A. rubra* by Miller et al. (1991) and *A. sinuata* by Helm et al. (1996). More specific comparisons to other anatomical studies of alder mycorrhizas are given below.

Mycorrhizas of *Naucoria* and anatomically similar unidentified mycorrhizas

Several members within the genus *Naucoria* (syn. *Alnicola*) have been suspected to form mycorrhizas with alders (Reid 1984) but no corresponding mycorrhizal form has been described until now. *Naucoria escharoides* and *N. subconspersa*, which can be distinguished by their sporocarp morphology, produced anatomically identical mycorrhizas, which were collected beneath fruitbodies of the respective species. Neither the sporocarps nor the mycorrhizas could be differentiated by

the molecular biological studies (Pritsch et al. 1997). Until further indications on the taxonomic position of the two species are available, the EM of *N. escharoides* and *N. subconspersa* are treated here as one morphotype corresponding to two putatively different species.

The EM of *N. escharoides* and *N. subconspersa* showed features similar to “type B” mycorrhizas of *A. japonica* (Masui 1926) except for the absence of rhizomorphs. The combination of an outer plectenchymatous and an inner pseudoparenchymatous mantle exhibited by these mycorrhizas has also been reported from subtype K EM of *A. viridis* (Mejstrik and Benecke 1969).

A mantle anatomy very similar to EM of *N. escharoides* and *N. subconspersa* was also exhibited by “*Alnirhiza cana*” and “*A. suffusa*”, suggesting that these unidentified types belong to the genus *Naucoria*. “*Alnirhiza texta*” is morphologically similar to *N. escharoides* and *N. subconspersa*, but has differentiated rhizomorphs and a more compact hyphal mantle with hyphae strictly orientated in parallel in the middle layer of the mantle. However, without rhizomorphs “*A. texta*” is easily confused with mycorrhizas of the *Naucoria* spp. In contrast to “*A. cana*” and “*A. suffusa*”, which were shown to be closely related to *N. escharoides*/*N. subconspersa* by molecular biological studies, the relationship of “*A. texta*” with the genus *Naucoria* is unclear (Pritsch et al. 1997).

Mycorrhizas of *Cortinarius* on *Alnus* and anatomically similar unidentified mycorrhizas

All *Cortinarius*-mycorrhizas and anatomically similar unidentified mycorrhizas described here exhibited folded cell walls and collapsed hyphae in mature and older parts of the mantle on both fixed and fresh material. Other *Cortinarius* mycorrhizas described on alder do not show such features (Godbout and Fortin 1983; Brunner et al. 1990; Miller et al. 1991). Whether these collapsed hyphae reflect normal ageing processes of the respective mycorrhizae or are a consequence of adverse soil conditions in our experimental plot is not clear, since no reference specimens of other black alder stands were investigated. Within the genus *Cortinarius*, the EM of *C. cf. helvelloides* showed a unique macroscopic appearance with a thick, conspicuously blue-shaded mantle, making these mycorrhizas unambiguously identifiable. None of the bluish mycorrhizas described by others, i.e. *Byssocorticium atrovirens*, *Cortinarius hercynicus*, *Hydnellum peckii* (Agerer 1987–97) and *Alpova diplophloeus* with a blue bruising reaction after squeezing the mantle (Miller et al. 1988), exhibits similar anatomical features. *Cortinarius cf. alneus* and “*A. lilacina*” would probably not have been separated without additional molecular biological studies (Pritsch et al. 1997). Apart from small colour differences of young mantles, both types had identical anatomical features. “*Alnirhiza violacea*” was also similar in colour and mantle architecture. Nevertheless this type was dis-

tinguished by a thicker mantle and pigmented cell walls. The similarities of mantle anatomy and molecular biological features (Pritsch et al. 1997) suggest a close relationship of “*A. lilacina*” and “*A. violacea*” to the genus *Cortinarius*. Alder mycorrhizas with similar anatomy were described from *C. bibulus* on *A. rubra* by Miller et al. (1991). Although fruitbodies of *C. bibulus* were present in our experimental plot, its mycorrhizas were not found.

#### Mycorrhizas of *Lactarius* on *Alnus*

EM of three species within the genus *Lactarius* were found. The morphologically and anatomically indistinguishable EM of *L. obscuratus* and *L. omphaliformis* were only differentiated by DNA polymorphism (Pritsch et al. 1997). Kraigher et al. (1995) obtained similar results with mycorrhizas of *L. lignyotus* and *L. picinus*, which were also difficult or impossible to differentiate anatomically but could be separated by PCR/RFLP. The EM of *L. obscuratus* described by Froidevaux (1973) and Miller et al. (1991) differ from the description given here in some details. Miller et al. (1991) found numerous clamps within the hyphal mantle which were absent in the Northern German *L. obscuratus* EM. Furthermore, they mentioned a prosenchymatous mantle, while an irregularly pseudoparenchymatous mantle was observed in the present study. Whether the North American and European *L. obscuratus* are the same species should be carefully investigated at the molecular level, since some taxonomic limits between species are in conflict within the genus *Lactarius* section *Obscurati* (Krieglsteiner 1981). The mycorrhiza of *L. alpinus* described by Treu (1990) on alpine *A. viridis* shows a mantle anatomy very similar to that of *L. obscuratus* and *L. omphaliformis*, but is of lighter colour and has very pronounced, macroscopically visible laticifers.

Mycorrhizas of *L. lilacinus* were easily distinguished from the former two species by intracellular penetration of Hartig net hyphae into epidermal and cortical cells. Similar, intracellular infection of cortical cells in naturally occurring *Lactarius* mycorrhizas have also been reported by Brand (1991) from mycorrhizas of *L. acris* on *Fagus sylvatica*. He suspected that these infections were caused by a secondary fungus such as in the case of *L. subdulcis* (Brand and Agerer 1988), but found no anatomical proof of this. In the case of *L. lilacinus*, neither anatomical nor molecular biological studies support the theory of a secondary mycobiont, suggesting that this mycobiont penetrates host cells. The overall appearance of *L. lilacinus* EM, healthy looking and of normal lifespan, did not reveal pathogenicity.

Mycorrhizas of *Russula* and anatomically similar unidentified mycorrhizas on *Alnus*

*Russula pumila*, a suspected alder-associated species (Jahn et al. 1976), was the only identified member of

this genus. Mycorrhizas of *R. pumila* are stout in appearance similar to other EM described within this genus (Agerer 1987–97). According to their mantle anatomy, *R. pumila* EM belong to the group of *Russula* mycorrhizas with an angular pseudoparenchymatous mantle structure such as *R. fellea* on beech (described as “*Fagirhiza granulosa*”, Brand and Agerer 1988), *R. mairei* on beech (Brand 1991) and *R. ochroleuca* on spruce (Agerer 1986). Similarly to mycorrhizas of *R. mairei*, single cells of *R. pumila* in the outer hyphal mantle have a granular content but, in contrast, these latex-containing cells form patches or rows of several cells and are not stainable with sulphovanilline.

The morphological appearance of “*Alnirhiza cremicolor*” with groups of dead cells at the mantle surface forming papilla and the pseudoparenchymatous hyphal mantle suggests a possible relationship with the genus *Russula*. This unidentified mycorrhiza showed no similarities to alder mycorrhizas described by other authors.

Unidentified mycorrhizas on *Alnus* with unique features not attributable to identified genera

“*Alnirhiza atroverrucosa*”, which is possibly identical with a “clavate, dark brown” mycorrhiza described by Neal et al. (1968b), also has similarities with the unidentified spruce mycorrhiza “*Piceirhiza nigra*” (Gronbach 1988; Haug and Pritsch 1992). In contrast to “*Piceirhiza nigra*”, “*Alnirhiza atroverrucosa*” never exhibits cystidia and has emanating hyphae of conspicuously greater diameter (6–7 µm in contrast to 2.5–5.5 µm in “*P. nigra*”). Whether these anatomical differences justify classification into two different species needs to be examined at the molecular level.

According to their identical morphological and anatomical features “*Alnirhiza cystidiobrunnea*” is the same morphotype as “type 1” of *A. rubra* described by Miller et al. (1991). Interestingly, “*Alnirhiza cystidiobrunnea*” (Miller et al. 1991, type 1) and “*Alnirhiza atroverrucosa*” (Neal et al. 1968b, “clavate, dark brown mycorrhiza”) both seem to be common on North American *A. rubra* and on European *A. glutinosa*. Whether these morphotypes belong to the same species must also be investigated by direct comparison of anatomical and molecular features.

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