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Morphological and molecular diversity and abundance of tomentelloid ectomycorrhizae in broad-leaved forests of the Hungarian Plain

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Abstract The most common representatives of tomentelloid ectomycorrhizae (EM) collected in broad-leaved forests (*Populus* and *Quercus*) of the Hungarian Plain during a 4-year project are demonstrated. Eighteen specimens of nine tomentelloid EM morphotypes were investigated. Five of these, introduced here for the first time, were characterized by microscopical–morphological and anatomical methods. Molecular identification was carried out using sequence analysis of the nrDNA ITS region. Altogether, 54 ITS sequences (6 previously published and 12 new sequences from our mycorrhizae and 36 from GenBank derived from fruitbodies) were compared by phylogenetic analyses using neighbor-joining, maximum parsimony and maximum likelihood methods. Identification to species level was successful in the case of six EM morphotypes (*Tomentella galzinii*, *T. subtestacea*, *T. sublilacina*, *T. pilosa*, *T. ferruginea* and *T. stuposa*), and the possible taxonomic position of the remaining three was approached. These results are supported by morphology, as compared with literature data. Relative abundance of the EM morphotypes within the soil samples was estimated. Our results confirm that tomentelloid EM are constant, diverse and abundant members of the EM communities in temperate-continental broad-leaved forests.

Keywords Tomentella · Ectomycorrhiza · DNA sequences · ITS · Morphology

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Introduction

The term ‘tomentelloid fungi’ is used to describe the species of the order Thelephorales (Basidiomycetes) with resupinate sporocarps belonging to the genus *Tomentella* and some related genera (Köljalg et al. 2000). Though tomentelloids have a worldwide distribution, the majority of the species has been reported from the boreal and temperate coniferous and broad-leaved forests of Eurasia (Corner 1968; Stalpers 1993; Köljalg 1996).

The basidiocarps of these fungi are easily overlooked since they form mainly a thin, sometimes spiderweb-like, inconspicuous layer on the surface of the soil or often on the underside of dead plant debris (Köljalg 1996). Previously, therefore, the tomentelloids were declared as rare, saprotrophic, wood decaying fungi (Larsen 1974). However, in the 1980s, it was proved that tomentelloid fungi can form ectomycorrhizae (EM) (Danielson and Pruden 1989). All species investigated up to now turned out to be mycorrhizal, forming symbioses with different gymnosperms and woody angiosperms and even with orchids (Danielson and Pruden 1989; Brand 1991; Visser 1995; Gardes and Bruns 1996; Bradbury et al. 1998; Agerer 1996a; Raidl and Müller 1996; Taylor and Bruns 1997, 1999; Kranabetter and Wylie 1998; Horton and Bruns 1998; Erland and Taylor 1999; Köljalg et al. 2000; Jakucs 2002). Tomentelloid EM are generally characterized by brownish black, brown or yellow pseudoparenchymatous mantles, emanating hyphae with or without clamps and often a blue colour reaction in KOH (Agerer 1995; Agerer et al. 1995). According to Köljalg et al. (2000), those EM are defined as ‘tomentelloid’ which have melanized hyphae and suit one or more of the following three criteria: presence of clamp connections, cystidia and a greenish-blue colour reaction in KOH.

Despite the rarity of sporocarps, analyses of EM root samples carried out in the last decade show that tomentelloid fungi belong to the most frequent and widespread EM partners of gymnosperm and angiosperm trees in the forests of Europe and North America (Mehmann et al. 1995; Gardes and Bruns 1996; Dahlberg et al. 1997; Jonsson et al.

1999). Brand et al. (1994) reported 28% abundance of an unidentified tomentelloid EM *Piceirhiza nigra* in limed spruce stands. Gardes and Bruns (1996) found *Tomentella sublilacina* to colonize ca. 15% of the EM root tips in a *Pinus muricata* stand in the United States. Köljalg et al. (2000) demonstrated six tomentelloid taxa in Swedish boreal forests, which represented about 1–8% of the EM community in more than a half of the sites examined. According to Kaldorf et al. (2004), *Tomentella ellisii* represented more than 30% of the ectomycorrhizal community in different hybrid aspen stands in Germany. In drought-adapted *Populus alba* forests of the Hungarian Plain, brownish black tomentelloids were minority (<10%) or minority codominant (10–50%) components of the EM community, although no sporocarps could have been detected during a 4-year-long project (Jakucs 2002).

A common observation of mycorrhizal studies has been that no correlation exists between the abundance of fruitbodies and mycorrhiza (Agerer 1990; Dahlberg et al. 1997). Investigations based upon collecting only sporocarps may lead to a rough underestimation of the diversity, abundance and ecological role of tomentelloid fungi. Therefore, when investigating fungal community structure, direct analysis of EM in soil samples is needed. As few *Tomentella* EM have been described and characterized morphologically in detail (listed in Jakucs et al. 2004), a morphological approach (Agerer 1991) within this group has its limits. However, by combining morphological and molecular techniques (e.g. nrDNS ITS sequence analysis), identification of the species and examination of their role in forest ecosystems may be successful also for unknown morphotypes (Gardes and Bruns 1996; Dahlberg et al. 1997; Köljalg et al. 2000; Jakucs et al. 2004).

In this study, morphological and molecular taxonomical characteristics and relative abundance of nine tomentelloid EM morphotypes, represented by 18 specimens collected during a 4-year-long project from poplar and oak forests of the Hungarian Plain, are investigated using microscopic methods and nrDNA ITS sequence analysis. Our aim was to obtain knowledge on the diversity and abundance of the most common tomentelloid EM mycobionts of broad-leaved forests in order to approach their ecological significance in temperate forest communities.

Materials and methods

Sampling

Ectomycorrhizae have been isolated from soil samples collected in three sampling sites in Hungary. Two of these (Tompa and Kelebia) are about 10 km apart in the southern part of the Hungarian Plain near the Serbian border, at an altitude of 120–130 m. The average rainfall in the region is 550 mm/year. The sampling plot in Tompa is a *P. alba* stand with trees of 35–40 m height mixed with *Acer negundo* L., *Celtis occidentalis* L., *Fraxinus excelsior* L., *Robinia pseudoacacia* L., *Sambucus nigra* L. and *Ulmus laevis* Pall. The forest has been established replacing an autochthon zonal Convallario–Quercetum roboris stand. The soil is sandy, mixed with loess and with a quite thick (about 10 cm) moder-type humus layer containing 0.4% lime, pH 7.1–7.6. The forest near Kelebia is a younger plantation with *P. alba* trees no higher than 30 m mixed with *C. occidentalis*, *Crataegus monogyna* Jacq., *R. pseudoacacia* and *Prunus* sp. The soil is sandy with a thinner (about 4 cm) moder-type humus layer than in the Tompa stand, containing 2.4–2.6% lime, pH 7.9.

The third sampling plot (Püspökladány) is in the central part of the Hungarian Plain within the territory of the National Park Hortobágy at an altitude of 85–90 m. The average rainfall in the region is 525 mm/year. The forest established on solonetz soil (pH 6.5) consists of a 47- and a 27-year-old *Quercus robur* L. stand with *Ligustrum vulgare* L. and *Fraxinus* spp. and a 67-year-old *Quercus cerris* L. stand with *Prunus spinosa* L. and *Rosa* sp. in the understorey.

At all sites, mycorrhizal soil samples were taken five times during the period of 1997–2000 (see Table 1). Each time, two or three soil samples were taken (as repetitions) randomly from a 25×25-m square at each sampling plot. Soil cubes (20×20×20 cm) were cut with a sharp knife from the upper layer of the forest soil. During our 4-year investigation, 15 soil samples were taken from 'Tompa', 13 from 'Kelebia' and 17 from Püspökladány. Within the soil samples altogether, more than 15 different tomentelloid EM morphotypes could be distinguished. The nine most frequent morphotypes represented by 18 specimens are included in this study.

After transport to the laboratory, soil samples were stored at 4°C for not more than 1 week. Roots were washed out from the soil cubes with tap water over a sieve, and EM were separated and selected in water under a dissecting microscope. Relative abundance of the morphotypes was calculated as described below. EM tips were fixed in FEA for further microscopy (Agerer 1991). For DNA analysis, three EM tips from each morphotype were fixed in CTAB buffer (2% CTAB, 20 mM EDTA pH 8, 100 mM Tris–HCl pH 9 and 1.4 mM NaCl). Type specimens fixed in FEA had been preserved in the collection of E. Jakucs, and voucher specimens were deposited in the Hungarian Natural History Museum, Budapest.

Herbarium numbers, collection data and GenBank accession numbers of the EM investigated are listed in Table 1.

Estimation of relative abundance

The relative abundance of the investigated tomentelloid EM morphotypes to all mycorrhizal tips within each soil sample was estimated visually using the semiquantitative method of Gardes and Bruns (1996) with modifications as described elsewhere (Jakucs 2002). Relative abundance of the morphotypes is expressed as average percentage values of the three repetitive soil cubes taken from one sampling site. Abundance values were classified as follows: A—

Table 1 List of the representative samples of the studied tomentelloid EM

Herbarial number	Collection site	Time of collection	Host	Reference	GenBank number
BP 92148	Tompa	02. 06. 1998	*Populus alba*	Köljalg et al. 2001	AJ421252
BP 92153	Tompa	08. 10. 1998	*Populus alba*	Köljalg et al. 2001	AJ421250
BP 92154	Püspökladány	09. 04. 1998	*Quercus cerris*	Köljalg et al. 2001	AJ421251
BP 96971	Püspökladány	08. 04. 1998	*Quercus cerris*	Jakucs et al. 2004	AY635168
BP 96972	Tompa	02. 06. 1998	*Populus alba*	Jakucs et al. 2004	AY635169
BP 96975	Tompa	02. 06. 1998	*Populus alba*	this study	AY874389
BP 97486	Kelebia	08. 10. 1998	*Populus alba*	this study	AY874390
BP 96976	Kelebia	08. 10. 1998	*Populus alba*	this study	AY874388
BP 96977	Kelebia	08. 10. 1998	*Populus alba*	this study	AY874386
BP 96981	Tompa	08. 10. 1998	*Populus alba*	Jakucs et al. 2004	AY635175
BP 96982	Püspökladány	07. 06. 1999	*Quecus robur*	this study	AY874384
BP 96983	Püspökladány	07. 06. 1999	*Quercus cerris*	this study	AY874383
BP 96984	Kelebia	07. 06. 1999	*Populus alba*	this study	AY874387
BP 97487	Tompa	07. 06. 1999	*Populus alba*	this study	AY874385
BP 96986	Tompa	07. 06. 1999	*Populus alba*	this study	AY874379
BP 96987	Püspökladány	24. 04. 2000	*Quecus robur*	this study	AY874380
BP 96988	Püspökladány	24. 04. 2000	*Quecus robur*	this study	AY874382
BP 96989	Püspökladány	10. 06. 2001	*Quecus robur*	this study	AY874381

minor component (<10% of the total EM tips in the soil cube); B—minority codominant (10–50%); C—majority codominant (50–90%); D—dominant (>90%).

Morphological characterization

Tomentelloid EM morphotypes were characterized and described using the microscopical morphological methods of Agerer (1991). The mycorrhizal system was examined by stereomicroscopy. The mantle structure and the hyphal and rhizomorphal characteristics were studied by DIC (Nomarski) microscopy, including microscopic drawings and digital photodocumentation.

DNA analysis

DNA extraction

DNA isolation and amplification were carried out as described previously (Jakucs et al. 2004). The mycorrhizal tips were thoroughly ground with micropestles and sand in Eppendorf tubes in lysis buffer (2% CTAB, 20 mM EDTA pH 8, 100 mM Tris–HCl pH 9, and 1.4 mM NaCl). After incubation at 65°C for 45 min, the samples were extracted twice with an equal volume of chloroform and centrifuged for 15 min at 12,000 *g* after both extractions. The DNA was precipitated with two volumes of ethanol, and after incubation for at least 8 h at −20°C it was pelleted by centrifugation at 12,000 *g* for 30 min. The pellet was washed with 70% ethanol, dried and redissolved in 30 µl sterile Milli-Q water (Millipore).

PCR and sequencing of the ITS region

The amplification of the ITS region was carried out with the fungus-specific primer pair ITS1F–ITS4 (Gardes and Bruns 1993). Reaction mixture contained 0.1 volume of 10× PCR buffer (MBI Fermentas), 200 µM each of dATP, dCTP, dGTP and dTTP (Sigma), 0.5 µM of each primer, 1 U Taq DNA polymerase (MBI Fermentas) and the 10× diluted target DNA. The amplifications were performed with DNA-Engine thermocycler (MJ Research), programmed for a denaturation step at 93°C for 3 min, followed by 35 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 52°C and extension for 1 min at 72°C; the thermal cycling was ended by a final extension for 10 min at 72°C.

For direct sequencing, PCR products were purified with a PCR Clean up-M Kit (Viogene). For cycle sequencing, an ABI PRISM 3.1 BigDye Terminator Kit (Perkin Elmer) was used according to the instructions of the manufacturer using both primers. Electrophoresis was carried out on an ABI PRISM 3100 Genetic Analyser.

Sequence analyses and phylogenetic inference

Sequences were compiled from electropherograms using Pregap4 and Gap4 (Staden et al. 2000).

Related sequences were selected from the GenBank database using a BLAST homology search (Altschul et al. 1990). Multiple alignments were made with the ClustalX program (Thompson et al. 1997) and manually edited subsequently if necessary. Phylogenies were inferred by maximum likelihood (ML), neighbor-joining (NJ) and maximum parsimony (MP) methods using PAUP* 4.0 (Swofford

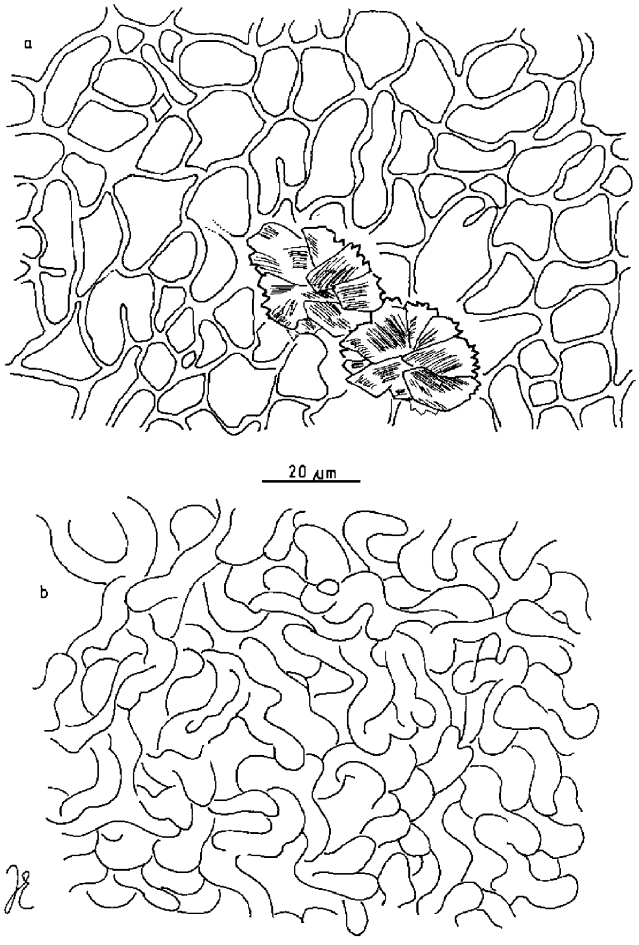

Fig. 1 Anatomy of the Mt. 3 mycorrhiza. **a** Outer layer of mantle organized from large, angular to globase, thick-walled cells and scales of remnant cell debris formed on surface. **b** Plectenchymatous-epidermoid inner layer

2003). For the ML analysis, optimal parameters (substitution model, base frequencies, the shape parameter of the gamma distribution and the proportion of invariant sites) were estimated by hierarchical likelihood ratio test with the Modeltest 3.06 (Posada and Crandall 1998). In NJ analyses (Saitou and Nei 1987), distances were calculated with the optimal evolution model estimated with the ML analysis. When looking for the most parsimonious trees, nucleotides were treated as unordered, equally weighted characters; gaps were treated as 'fifth base'. The starting tree for branch swapping was obtained via random stepwise addition procedure. The topology was optimized during the heuristic search by the TBR algorithm, with STEEPEST function not in effect, MULTREES function in effect and topological constraints not enforced. Branches were collapsed if the maximum branch length was zero.

The phylogenies inferred in MP and NJ analyses were statistically tested by bootstrapping (Felsenstein 1985) using 100 and 1,000 replicates, respectively. All trees gained were visualized and edited by the TreeView program (Page 1996).

Results

Morphological and anatomical description of the ectomycorrhizae

In the studied samples, nine different tomentelloid EM morphotypes (Mt.) could be distinguished. Four of these (Mt. 1, 2, 6, 8) have been morphologically described previously (see Discussion). The brief morphological–anatomical description and image documentation of the remaining five morphotypes (Mt. 3, 4, 5, 7, 9), presented for the first time in this study, are given below.

Characterization of Mt. 3 (Figs. 1, 2) (specimen examined: BP 96986): EM brown to black, ramifying system monopodial pyramidal. Surface densely woolly with large, straight emanating hyphae (Fig. 2). Outer mantle layer pseudoparenchymatous, organized from large, angular to globase, thick-walled (2–3 µm) cells (Fig. 1a). Inner layers plectenchymatous-epidermoid, with thin cell walls (Fig. 1b). At some places, scales of remnant cell debris are formed on mantle surface.

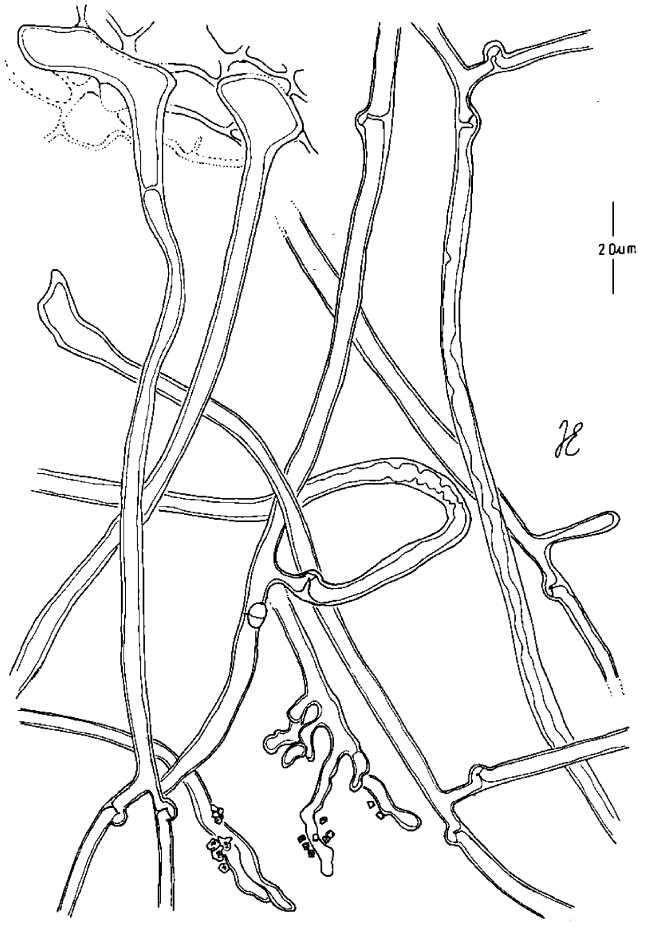

Fig. 2 Emanating hyphae of the Mt. 3 mycorrhiza. Extremely robust, thick-walled emanating hyphae with clamps, large, extending foot cells and with irregularly ramifying ends, sticking soil particles

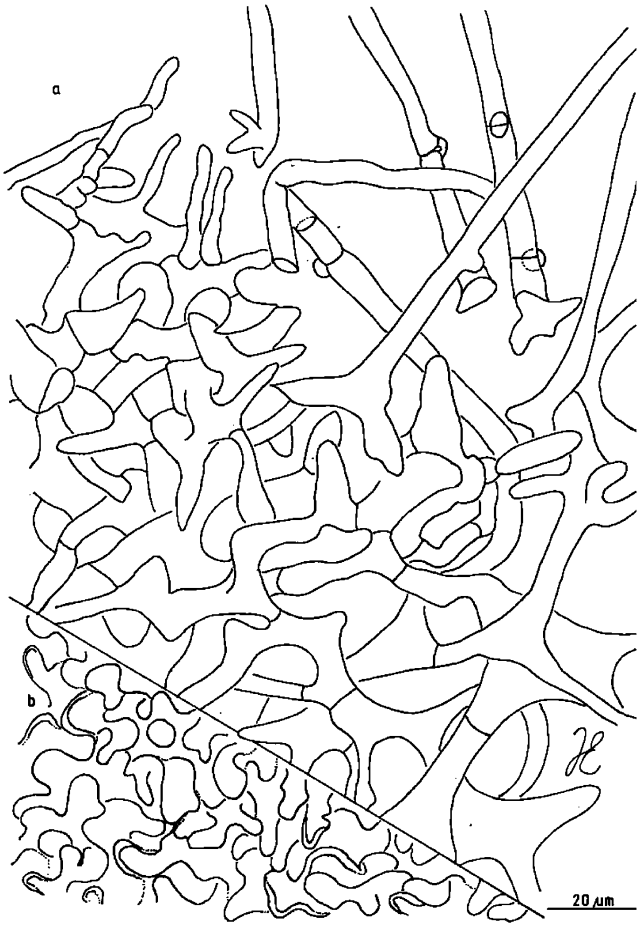

Fig. 3 Anatomy of the Mt. 4 mycorrhiza. **a** Outer layer of mantle with hyphal network formed of extending triangular, horn-shaped cells and origin of emanating hyphae. **b** Transient of outer layer to epidermoid medial layer

Emanating hyphae straight, slightly bent, ramifying, extremely robust (up to 10 µm in diameter), thick-walled (1–2 µm), with clamps and large, extending foot cells (25–40 µm in diameter) rooting in the angular layer of the mantle and with irregularly ramifying ends sticking soil particles (Fig. 2).

Characterization of Mt. 4 (Figs. 3, 4, 5) (specimen examined: BP 96987): EM brown, ramifying system monopodial pyramidal. Surface woolly with straight emanating hyphae. Mantle at older parts covered by a gelatinous matrix or at distinct places by a network of hyphae formed of extending triangular, horn-shaped cells emanating hyphae originate from (Fig. 3a). Outer layer of mantle pseudoparenchymatous, cells irregularly angular, elongated or transient to epidermoid (Fig. 3a, b). Medial layer (Fig. 4a) with small (1–3 µm), unevenly thick-walled epidermoid cells. Inner layers plectenchymatous without pattern (Fig. 4b). Young emanating hyphae (Fig. 5) long, extremely straight and rigid, thin-walled, with clamps and large, extending foot cells rooting in the surface hyphal network, 8–10 µm in diameter at basal part and 4–5 µm near the tip. Some short, cystidium-like emanating hyphae also appear on surface (Fig. 3a). Older hyphae thick-walled (up to 1 µm), often with elbow-like protrusions, thickened walls forming cap-like structures at the tip. Rhizomorphs not observed.

Characterization of Mt. 5 (Figs. 6, 7) (specimen examined: BP 96983): EM dark brown, ramifying system abundant and dense, monopodial pyramidal. Surface of EM smooth or rarely woolly. Mantle pseudoparenchymatous, with groups of globase cells on the surface (Fig. 6a). Outer layer of mantle angular, with thick-walled cells (1–2 µm), cell walls unevenly pigmented (Fig. 6b). Inner layers plectenchymatous (Fig. 7a). Emanating hyphae (Fig. 7b) with clamps, somewhat twisted in shape, slightly constricted at septa, cell walls of hyphae 1–1.5 µm thick, hyphal ends club-shaped with thicker walls (up to 3 µm) than other parts of the hyphae.

Characterization of Mt. 7 (Figs. 8, 9, 10) (specimen examined: BP 96984): EM dark brown to black, ramifying system monopodial pyramidal. Surface of EM rarely wooly. Mantle pseudoparenchymatous, with a

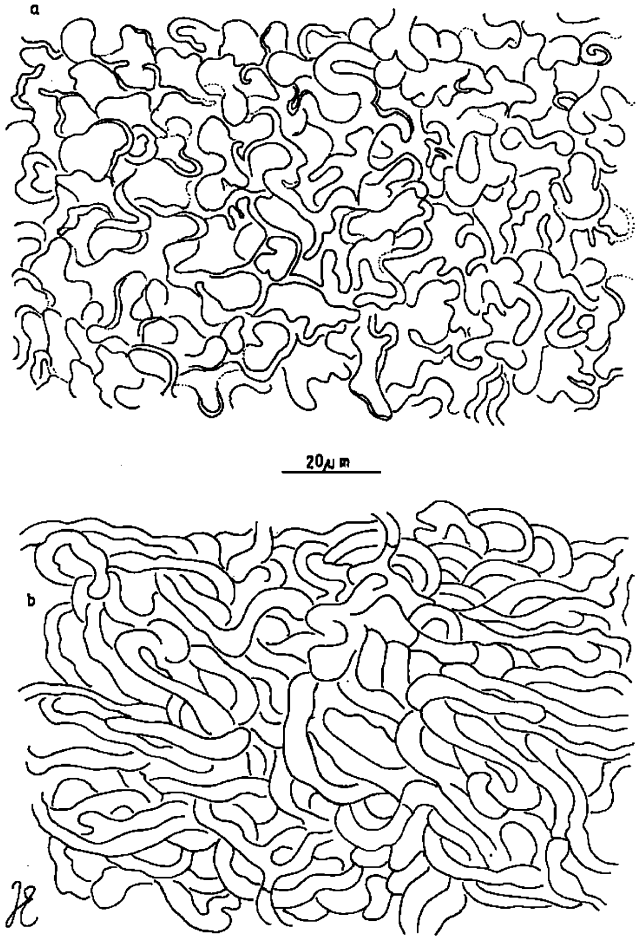

Fig. 4 Anatomy of the Mt. 4 mycorrhiza. **a** Medial layer with small, unevenly thick-walled epidermoid cells. **b** Inner plectenchymatous layer

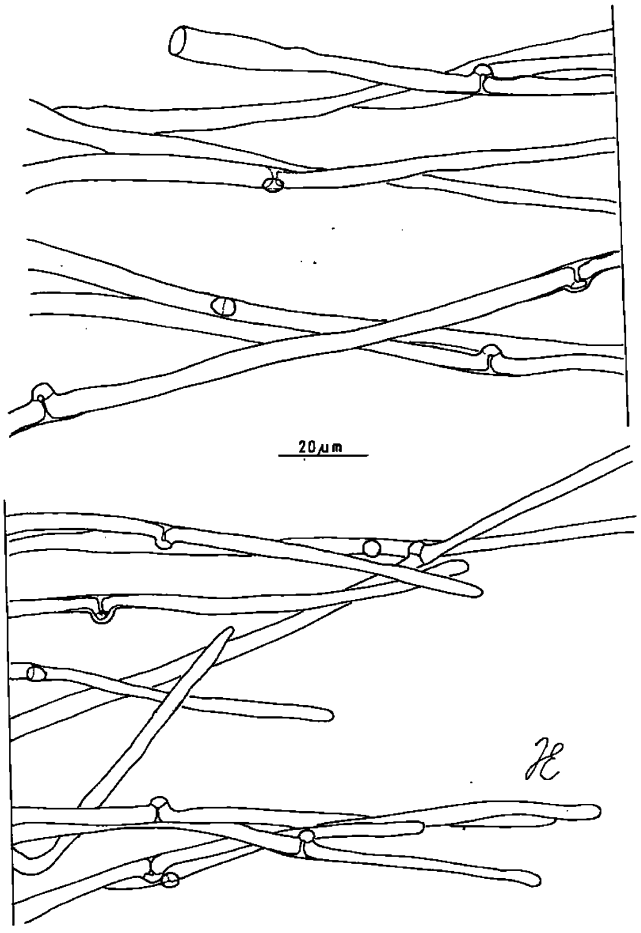

Fig. 5 Emanating hyphae of the Mt. 4 mycorrhiza. Straight, thin-walled young emanating hyphae with clamps and large, extending foot cells rooting in the surface hyphal network

surface hyphal network (Fig. 8a) formed by hyphae of uneven diameter (0.6–1.2 µm) and cystidium-like hyphae, without clamps, and at some places on mantle surface with scales formed by groups of remnant cells (Fig. 8c). Outer mantle layer angular (Fig. 8b). Middle layer formed by smaller cells than the outer one, epidermoid to plectenchymatous (Fig. 9a). Inner layer plectenchymatous, with hyphae larger than in middle layer (Fig. 9b). Emanating hyphae (Fig. 9c) with rare clamps, narrower than those of surface network and even in diameter (2–5 µm), simple septa frequent. Rhizomorphs, originating from middle mantle layer (Fig. 9a), turning from yellow to dark brown with age. They are not differentiated in inner structure (Fig. 10b) but form nodia (Fig. 10d) at ramifications and are covered by a layer of thin, curling marginal hyphae (1–2 µm in diameter), much thinner than central ones (5–6 µm in diameter) (Fig. 10c). Hyphae in rhizomorph thin walled, clamped (Fig. 10a).

Characterization of Mt. 9 (Figs. 11, 12) (specimen examined: BP 97486): EM dark brown to black, ramifying system monopodial pinnate or pyramidal. Surface of EM densely wooly. Mantle pseudoparenchymatous, showing a star-like pattern of cells under stereomicroscope. Cells of outer mantle layer (Fig. 11a) angular or triangular, thick-walled (2–3 µm thick), organized in groups forming star-shaped scales, centre of stars emerging from the surface as peaks (Figs. 11a, 12a). Some cells of outer mantle layer densely filled with dark-blue droplets or granula, probably consisting of thelephoric acid or its derivatives. Inner layers of mantle (Fig. 11b) epidermoid, with thin-walled cells, lacking blue granula. Emanating hyphae (Fig. 12a, b) without clamps, cell walls 0.5–1 µm thick, some hyphae originating with foot cells from mantle. Rhizomorphs and cystidia lacking.

Molecular identification

A 415-character-long alignment of the nrDNA ITS was used in the phylogenetic analysis. Optimal likelihood parameters selected for this dataset according to the hierarchical likelihood ratio test are as follows: HKY+G substitution model; transitions/transversion ratio 4.9135 (κ=9.6543);

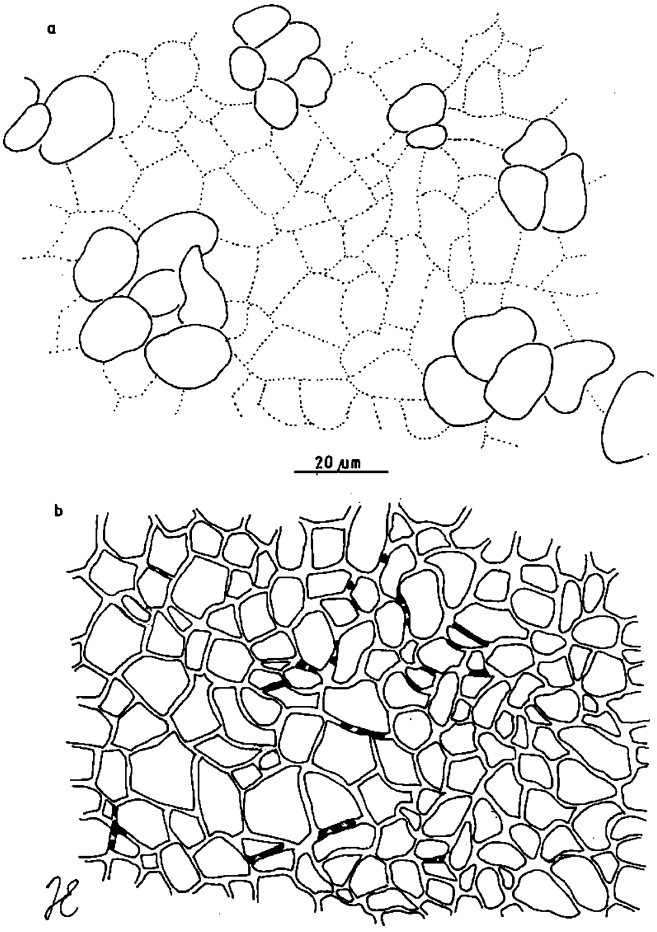

Fig. 6 Anatomy of the Mt. 5 mycorrhiza. **a** Surface of mantle with groups of globose cells. **b** Outer layer of mantle with thick-walled angular cells, cell walls unevenly pigmented

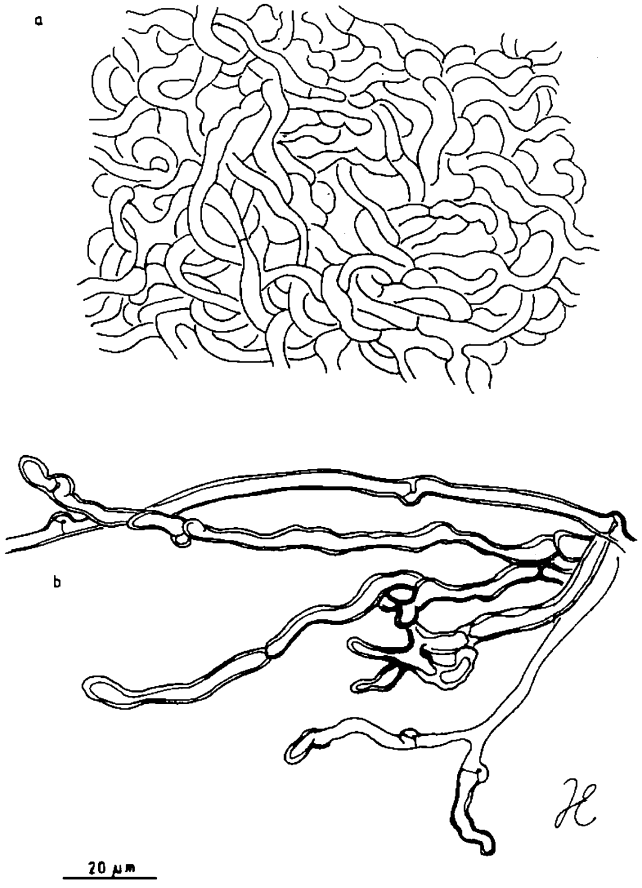

Fig. 7 Anatomy of the Mt. 5 mycorrhiza. **a** Plectenchymatous inner layer of mantle. **b** Emanating hyphae with clamps and club-shaped hyphal ends with thicker walls than other parts of the hyphae

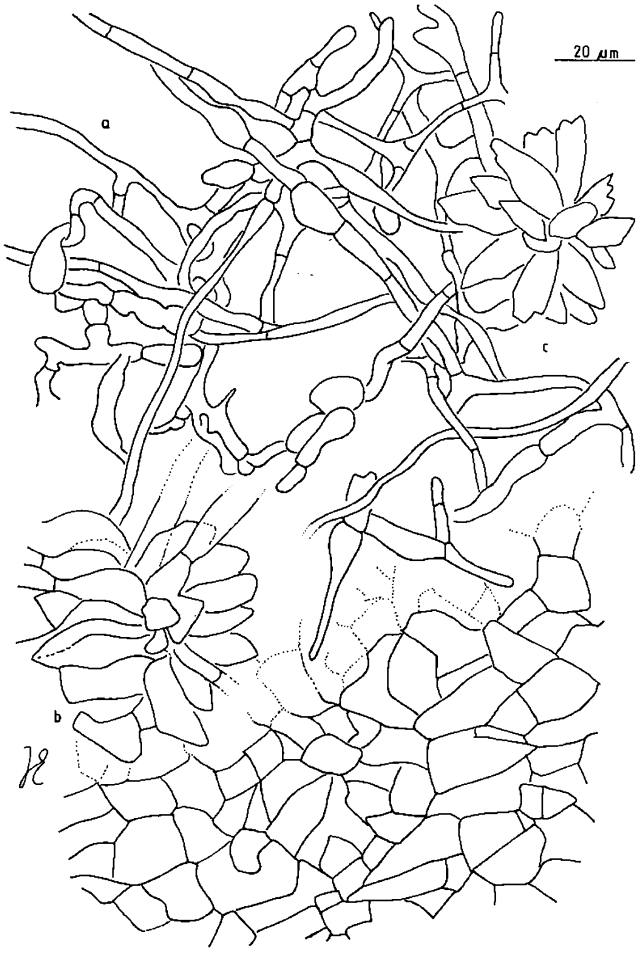

Fig. 8 Anatomy of the Mt. 7 mycorrhiza. **a** Hyphal network on the surface of the mantle. **b** Angular outer mantle layer. **c** Scales formed of groups of remnant cells on mantle surface

frequencies of nucleotide A, C, T and G—0.2161, 0.2624, 0.2871 and 0.2344, respectively. Rates of substitution types at variable sites are assumed to follow the gamma distribution with the shape parameter (α) of 0.47278. The score (ln L) of the resulting phylogeny found by the heuristic maximum likelihood search was −4,283.90581.

In the maximum parsimony search, 196 of the 415 characters turned out to be parsimony-informative (154 constant, 65 parsimony-uninformative). With the heuristic search, 94 equally parsimonious trees were found in a single island with the tree length of 956. For the ML tree, with the indication of the bootstrap values of the NJ and the MP searches, see Fig. 13.

The topologies of the phylogenetic trees constructed by the three different methods are remarkably similar. The only difference between the results of the parsimony-based and the neighbor-joining methods is the different bootstrap values supporting certain clades. The sequences of our samples cluster together according to the different morphological–anatomical characters: different morphotypes are members of cladistically distinct groups, but sequences of the same types are very close together in all of the three trees, and their groups are always supported by bootstrap values higher than 90%.

Diversity and abundance of the mycorrhizae

The relative abundance of the different specimens within all mycorrhizal root tips in the soil samples is summarized in Table 2.

Discussion

Within the tomentelloid EM investigated here, nine different morphotypes (Mt.) could be distinguished, which segregated into distinct clades on the phylogenetic tree. Some of these were morphologically characterized and identified by molecular methods previously. Mt. 8, forming two neighbouring clades of the tree representing two anatomotypes identified as *Tomentella stuposa* and a close taxon, is described elsewhere in detail (Jakucs et al. 2004). Three yellow-ochre morphotypes with a pseudoparenchymatous-angular mantle, clamped cystidia and rhizomorphs (Mt. 1, 2 and 6) are identical with *Tomentella galzinii*, *T. subtestacea* and *T. pilosa*, respectively. The morphological and molecular characterization of these was also published earlier

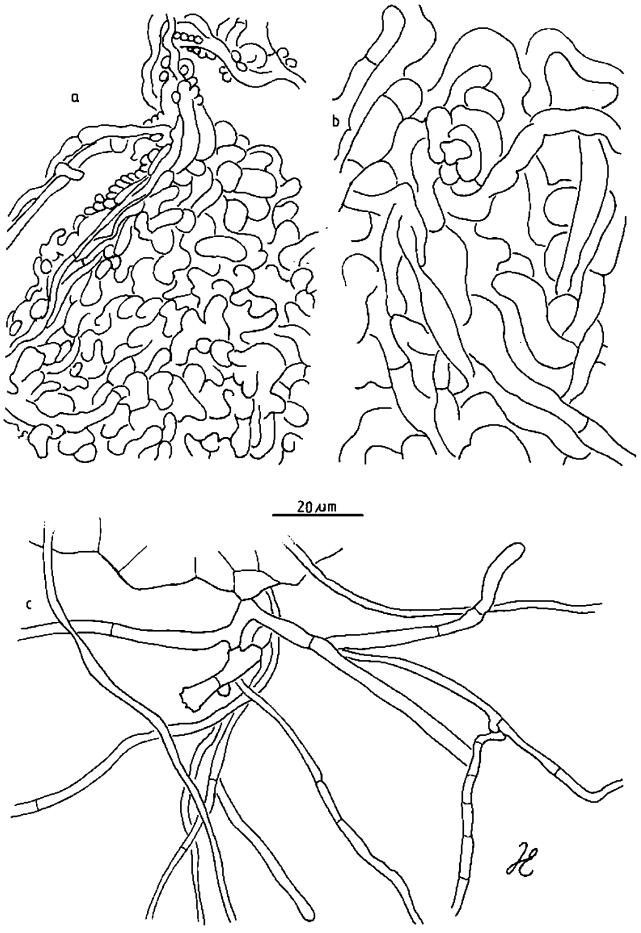

Fig. 9 Anatomy of the Mt. 7 mycorrhiza. **a** Epidermoid to plectenchymatous middle layer of mantle layer and origin of a rhizomorph. **b** Inner layer with larger hyphae than in middle layer. **c** Narrow emanating hyphae with rare clamps and frequent simple septa

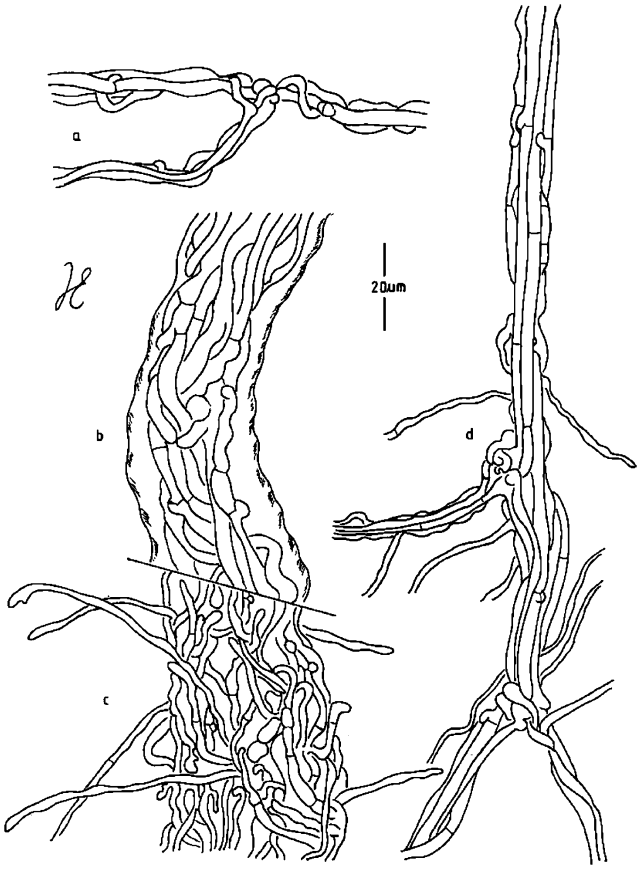

Fig. 10 Rhizomorph of Mt. 7 mycorrhiza. **a** Young, branching rhizomorph organized from thin walled, clamped hyphae. **b** Inner layer of rhizomorph not differentiated in structure. **c** Surface of rhizomorph covered by thin, curling marginal hyphae. **d** Rhizomorphs forming nodia at ramifications

(Jakucs et al. 1997, 1998; Jakucs and Agerer 1999, 2001; Jakucs 2002a, b; Köljalg et al. 2001).

Mt. 7 (isolated from *P. alba*) clustered together in the phylogenetic tree with the GenBank sequence of *Tomentella ferruginea* and shows high morphological similarity to the *T. ferruginea* EM formed on *Fagus sylvatica* described by Raidl and Müller (1996) and Raidl (1997, 1998). Plan views of the mantle, rhizomorphs and hyphal structure are identical. However, some differences from the original description can be observed. The outer mantle layer of *T. ferruginea* is described as plectenchymatous (Raidl and Müller 1996), while our specimen has a pseudoparenchymatous-angular mantle, with a hyphal network on the surface. However, Raidl revised his original characterization and agreed with our interpretation. Comparing his voucher specimen with our images, he also observed the angular layer below the hyphal layer in his original preparation, though only at the mature parts of the mycorrhiza (Raidl, personal communication). So the contradiction could be solved, and, by this correction, it can be stated that all currently described *Tomentella* EM have pseudoparenchymatous mantles (at least in older parts of the mantle), and the molecular identification of Mt. 7 as *T. ferruginea* is also confirmed morphologically.

The unknown morphotypes could partly be identified comparing their ITS sequences with known fruitbody sequences from the database.

The three sequences of Mt. 4 clustered together with *T. sublilacina* in a well supported clade. *T. sublilacina* has often been detected in EM communities using molecular methods and found to be rather abundant in pine and spruce stands (Gardes and Bruns 1996; Kranabetter and Wylie 1998; Jonsson et al. 1999; Köljalg et al. 2000). A detailed morphological description of the EM of this species formed with *Pinus sylvestris* was published by Agerer (1996a,b) as *Tomentella albomarginata*, which is a synonym of *T. sublilacina* (Köljalg 1996). The identification of the *T. albomarginata* EM 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 (Agerer 1996a). Although the description of Agerer (1996a) does not contradict the morphology observed in our samples, some characteristics are interpreted differently. The outer mantle

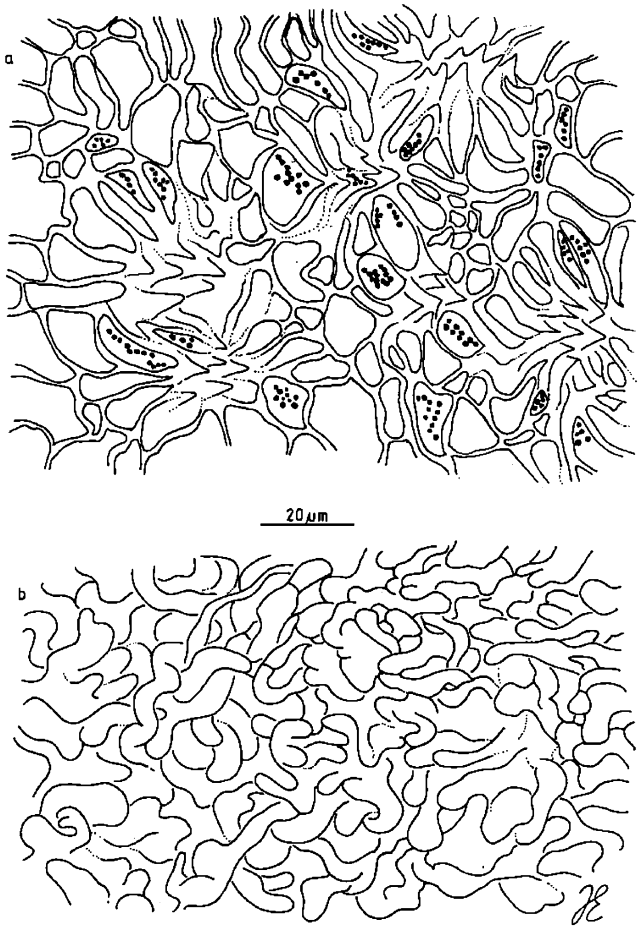

Fig. 11 Anatomy of the Mt. 9 mycorrhiza. **a** Outer layer of mantle with angular or triangular, thick-walled cells organized in groups forming star-shaped scales, emerging from the surface as peaks. Some cells densely filled with dark-blue pigment droplets. **b** Epidermoid inner layers of mantle

Fig. 12 Anatomy of the Mt. 9 mycorrhiza. **a** Emanating hyphae without clamps originating with foot cells from mantle. **b** Anastomosis of hyphae

layer of *T. albomarginata* described by Agerer (1996a) is 'pseudoparenchymatous, cells often irregularly angular or roundish, in part elongated, no pattern recognizable'. The surface network of hyphae formed of extending triangular, horn-shaped cells has not been detected during the previous description. Neither was the epidermoid medial mantle layer formed of thick-walled cells emphasized, although these could be found in the drawings (Agerer 1996a). In our samples, this epidermoid layer seemed to be the most conspicuous characteristic of the mantle. In contrast to the earlier findings, no rhizomorphs were observed in our samples. (According to Köljalg (1996), hyphal cords are also absent from the fruitbodies of *T. sublilacina*.) The identification of Mt. 4 as *T. sublilacina* can most likely be accepted as there is strong molecular evidence supported by three own mycorrhizal and four database fruitbody sequences. However, based on the level of the difference within the clade, we could conclude that this group consists of at least two different species, but in this case, these must be closely related ones. By morphological comparison, it cannot be decided exactly whether Mt. 4 is conspecific with *T. albomarginata* described by Agerer (1996a) or not.

Mt. 3 clustered together with *Tomentella bryophila* (and *T. lapida*), supported well by bootstrap analysis. So it is probable that Mt. 3 belongs to the *T. bryophila* group. However, as not every *Tomentella* species is represented in DNA databases, the identity cannot be confirmed. In any case, the fruitbody characteristics do not contradict this identification because *T. bryophila* (and also *T. lapida*) lacks rhizomorphs and cystidia and may occur under *Populus* (Köljalg 1996), like the Mt. 3 mycorrhiza. Comparing the morphology of Mt. 3 with known *Tomentella* EM, no identity with determined EM can be proven. However, the thick-walled pseudoparenchymatous-angular brown mantle, the robust, thick-walled, clamped emanating hyphae with enlarged foot cells and the scales of remnant cell debris formed on the mantle surface ('squama') are very similar to *Quercirhiza squamosa* (Palfner and Agerer 1996).

Mt. 5 formed a distinct clade in the phylogenetic tree which is separated from other species included in this study.

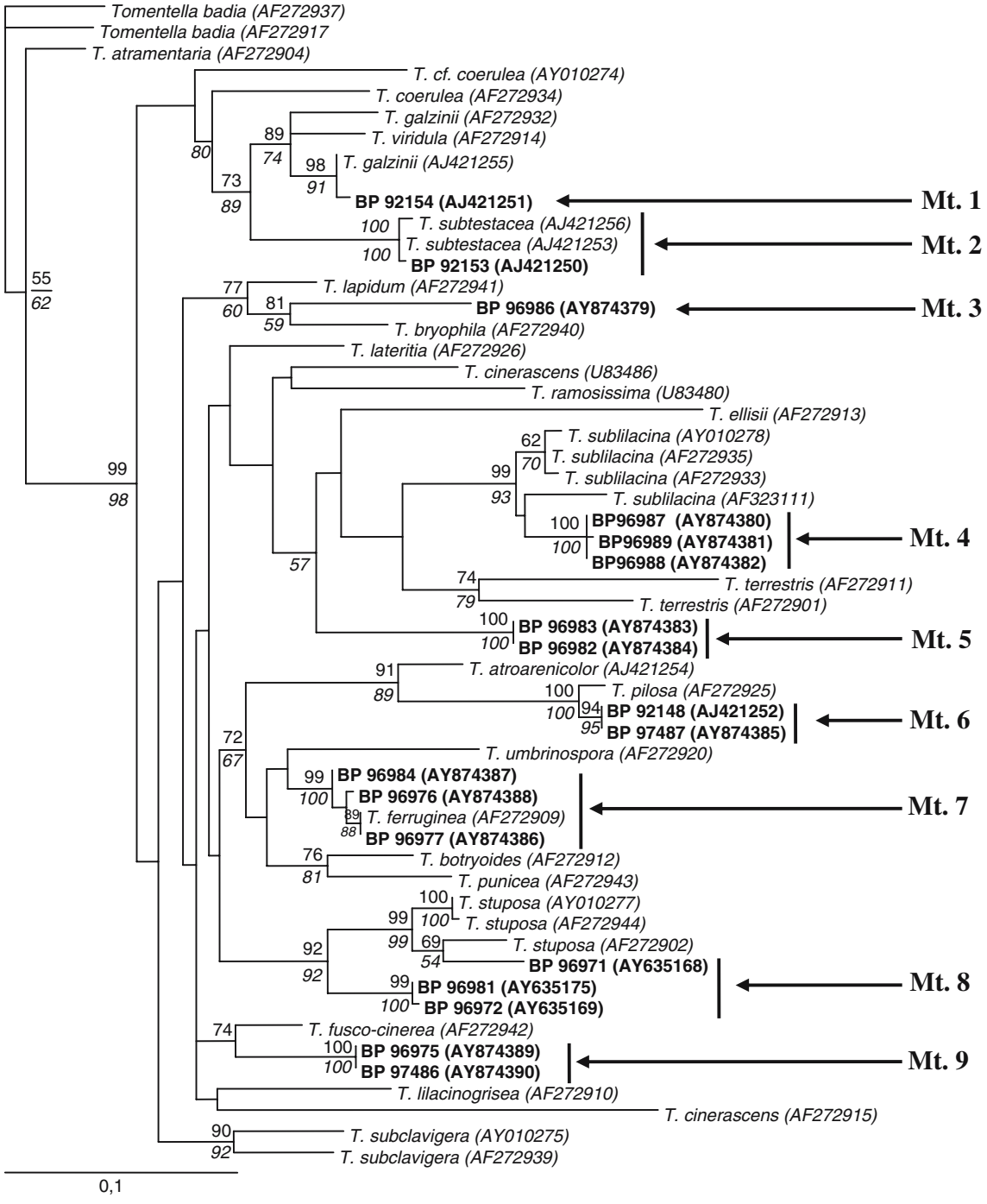

Fig. 13 Maximum likelihood tree of 54 rDNA-ITS sequences from *Tomentella* species including 36 fruitbody and 18 (12 new) own mycorrhizal sequences of tomentelloid EM. Bootstrap values higher than 50% originating from neighbor-joining (*above*) and maximum parsimony (*below*) trees are indicated

Although this EM shows some morphological similarities (e.g. groups of globose cells on the surface of the angular mantle) to *T. stuposa* and its relatives (Jakucs et al. 2004), its position in the tree is far from that of the former ones. This EM cannot be identified presently as ITS sequence data of *Tomentella* species are insufficient.

Although Mt. 9 clustered into a joint clade with *Tomentella fuscocinerea*, insufficient molecular data make it impossible to determine this EM now. However, it is remarkable that the hyphae of this EM have simple septa (without clamps) and the mantle cells contain dark-blue pigment droplets. Only a few *Tomentella* species (*T. fuscocinerea*, *T. badia* and *T. cinereoumbrina*) have simple septate hyphae in the fruitbody, and, among these three, only *T. badia* contains blue pigments (Köljalg 1996). Nevertheless, *T. badia* stands very far from Mt. 9 in the

Table 2 Morphotypes and relative abundance of the mycorrhizae

Herbarial number	Morphotype	Relative abundance	Herbarial number	Morphotype	Relative abundance
BP 92153	Mt. 2	B	BP 96984	Mt. 7	A
BP 96986	Mt. 3	A	BP 97486	Mt. 9	A
BP 97487	Mt. 6	B	BP 92154	Mt. 1	A
BP 92148	Mt. 6	B	BP 96987	Mt. 4	A
BP 96972	Mt. 8	B	BP 96988	Mt. 4	A
BP 96981	Mt. 8	B	BP 96989	Mt. 4	A
BP 96975	Mt. 9	B	BP 96982	Mt. 5	C
BP 96976	Mt. 7	B	BP 96983	Mt. 5	D
BP 96977	Mt. 7	A	BP 96971	Mt. 8	C

A Minor component (<10%), *B* minority codominant (10–50%), *C* majority codominant (50–90%), *D* dominant (>90%)

phylogenetic tree, but *T. fuscocinerea* cannot be excluded unambiguously as the mycobiont of this EM.

According to the abundance data, seven morphotypes (Mt. 1, 2, 3, 4, 6, 7 and 9) were minor components (A) or minority codominants (B) within the examined soil samples. Mt. 5 (not identified) and Mt. 8 (identified as *T. stuposa*) were majority codominant or dominant (C and D) components on the examined roots of oak trees in Püspökladány. No exact conclusions can be drawn from these sporadic data about the abundance of these mycorrhiza in the whole forest community, as it is well known that EM are distributed heterogeneously in the soil, showing random patterns (Ozinga et al. 1997; Agerer et al. 2002; Kaldorf et al. 2004). Spatial d8stribution of fungal communities can only be examined using extended, large-scale sampling methods (Dahlberg et al. 1997). However, it is remarkable that in a 4-year-long investigation of the EM community structure of the *Populus* forests of Tompa and Kelebia (Jakucs 2002), tomentelloid EM were present in all soil samples taken at any time during the 4 years, at least as minor or minority codominant components. Abundance data varied from 1 to 90%, but, on average, from 5 to 20%. This exceeds the results of Köljalg et al. (2000), who detected 2–5% abundance of tomentelloid EM in Swedish boreal forests. We often found more (two or four) different tomentelloids within one soil sample. Repetitive sampling shows that at the same plots, the same morphotypes can be detected for years.

Compared to literature data, tomentelloid EM are as important or show even higher diversity and abundance in the drought-adapted poplar and oak stands of the Hungarian Plain (Jakucs 2002) than in the coniferous and deciduous forests of northern, Pacific and Atlantic territories (Gardes and Bruns 1996; Dahlberg et al. 1997; Jonsson et al. 1999; Brand et al. 1994; Köljalg et al. 2000; Kaldorf et al. 2004). This suggests that tomentelloid EM may have advantages in semiarid environments, probably because of their melanized cell walls, which are regarded as an adaptation to soil drying (Pigott 1982). The frequent root colonization by other melanized fungi, like the DSE ones detected in the same area (Kovács and Bagi 2001; Kovács and Szigetvári 2002), could also support such a relation, although the drought tolerance of tomentelloid mycobionts must be directly proven. However, we can unambiguously state that *Tomentella* species are important and frequent EM fungi in the abiotically stressed environments of the Hungarian Plain. Our results also confirm that this fungal group is a general and abundant member of the EM communities in temperate-continental broad-leaved forests.

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