

Host specificity and biodiversity of ectomycorrhizal fungi in pure and mixed stands of Scots pine (*Pinus sylvestris* L.) and beech (*Fagus sylvatica* L.)

Von der Fakultät für Umweltwissenschaften und Verfahrenstechnik
der Brandenburgischen Technischen Universität Cottbus zur Erlangung des akademischen Grades
eines Doktors der Naturwissenschaften
genehmigte Dissertation

vorgelegt von

Diplom-Biologe
Ben Bubner
aus Hoyerswerda

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Gutachter: apl. Prof. Dr. Ingrid Kottke

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Thesis Part I

Introduction

I.1. Structure of thesis

This work analyses the extent of host specialisation in ectomycorrhizas, a mutualistic symbiosis between fungi and roots of forest trees. It consists of five parts:

- Part I: Introduction
- Part II: Fungal species identification
- Part III: Manuscript 1: The concept of specificity guilds reveals dominance of host specific fungi in pure and mixed stands of *Pinus sylvestris* L. and *Fagus sylvatica* L.
- Part IV: Manuscript 2: Individual tree genotypes do not contribute to ectomycorrhizal biodiversity in a pure stand of beech (*Fagus sylvatica* L.)
- Part V: Synopsis

Section I.2 of the introductory Part I reviews the general concepts of specificity phenomena and how host specificity might contribute to the explanation of biodiversity patterns in ectomycorrhizal communities. This theoretical background is followed by the presentation of the research site, the Kahlenberg site in the North-eastern lowlands of Germany (section I.3). Knowledge of the general research questions and the layout of the research site are the necessary prerequisites to understand the specific research questions that can be analyzed under the conditions of the Kahlenberg site. Therefore, the formulation of the specific research questions of this work and the outline of the data Parts II, III and IV are presented in section 1.4 at the end of the introductory Part I.

Parts II, III and IV present original data. Part II contains anatomical/morphological descriptions of 34 ectomycorrhizas found at Kahlenberg site and three case studies of species identification including molecular methods. Part III consists of Manuscript 1 that is supposed to be submitted to the Journal *Mycorrhiza*. Part IV consists of Manuscript 2 that has been prepared for submission to the Journal *Trees*.

Part V concludes this thesis by summarizing the distinctive methodological advances and the results of this work. The last section presents an outlook with two proposals for future research directions.

Each part is provided with a separate numbering of figures and tables.

I.2. Theoretical background

Importance of ectomycorrhizae

The mycorrhizal symbiosis is one of the most important mutualisms in terrestrial environments. The term mutualism denotes an interaction between two organisms in which both partners benefit from each other (Begon et al. 2006). Mycorrhizal mutualism involves plant roots as phytobiont and a fungus as mycobiont, whereby the plant provides the fungus with carbohydrate and the fungus provides the plant with nutrients foraged in the soil (Smith and Read 2008). Colonization of the earth's surface was enabled by the association of fungi and early land plants even before roots have evolved (Pirozynski and Malloch 1975; Fitter and Moyersoen 1996; Wang et al. 2010). After evolution of mycorrhizas, vascular plants were able to spread over the earth's surface, forming the many vegetation types known today. The mycorrhizal status is the normal status of a terrestrial plant. The few plant groups without mycorrhizal fungi (e.g. *Brassicaceae*, *Cyperaceae*) are derived from predecessor plants with mycorrhizal status (Brundrett 2002; Wang and Qiu 2006).

Among several types of mycorrhizas the most important are endomycorrhizas (= arbuscular mycorrhizas = AM) and ectomycorrhizas. In endomycorrhizas, fungi of the phylum *Glomeromycota* enter the root cells while in ectomycorrhizas, fungi of the phyla *Ascomycota* and *Basidiomycota* colonize the root apoplast without entering the root cells (Brundrett 2004). Another major difference refers to the global diversity patterns of the fungal and plant partners. Endomycorrhizas are formed by 74 % of vascular plant species so that most mycorrhizal plants are endomycorrhizal (Brundrett 2009). On the fungal side less than two hundred fungal species are involved mostly in the genus *Glomus* and related genera (Brundrett 1991). In ectomycorrhizas the opposite pattern is found. Only two percent of vascular plant species are ectomycorrhizal (Brundrett 2009). These are mainly woody plants (e.g. *Pinaceae*, *Fagaceae*, and *Dipterocarpaceae*) that can potentially associate with thousands of fungal species (Tedersoo et al. 2010). In a typical local ectomycorrhizal community the number of host plant species is at least an order of magnitude lower than the number of fungal partner species (Bruns 1995).

Despite the lower number of plant species as compared to endomycorrhizas, ectomycorrhizas play an important role in the world's ecosystems since temperate and boreal forests are dominated by ectomycorrhizal tree species in the families *Pinaceae*, *Fagaceae*, *Betulaceae*, and *Myrtaceae* (genus *Eucalyptus*). Mixed and pure forests of these trees cover large areas of the higher latitudes in the northern and southern hemispheres (Malloch et al. 1980; Brundrett 1991; Tedersoo et al. 2010). In the northern hemisphere these forests cover soils that contain the largest global stock of organic carbon (Post et al. 1982; Read and Perez-Moreno 2003). Thus, in connection with their hosts ectomycorrhizal fungi contribute to the functioning of global biogeochemical cycles on earth. Apart from their global importance ectomycorrhizal fungi have an indirect economical impact through their mutualism with forest trees. Especially ectomycorrhizal conifer forests of the northern hemisphere provide timber wood

and pulpwood (FAO 2011). Understanding the biodiversity pattern of ectomycorrhizal fungi will help to understand how different patterns contribute to forest health and productivity.

Diversity patterns and fungus-host associations

Biodiversity patterns in ectomycorrhizal communities await explanations both on the global and the local scale. One biodiversity parameter is the species richness that equals the species number in a community. The relatively high number of species on the fungal side has already been mentioned. A second parameter to describe diversity is the distribution of relative species abundances (Volkov et al. 2007). Typical for local ectomycorrhizal communities is the dominance of a few fungal species that are supplemented by a long list of many rare species (Taylor 2002). It is important for understanding ectomycorrhizal community patterns that this distribution is not a general pattern for all groups of sessile organisms. For instance relative abundances of (mostly AM) tree species in tropical rain forests are more evenly distributed. Despite high numbers of trees species in a local rainforest community, each species is represented by similar numbers of individuals with a low proportion of rare species (Volkov et al. 2007).

Interestingly, at the global scale and at least at the level of genera, ectomycorrhizal distributions show a pattern that is different from the local scale. Most ectomycorrhizal genera are cosmopolitan (Dickie and Moyersoen 2008; Tedersoo et al. 2008), while local communities show the mentioned dominance pattern with many rare species. From this follows that in a global metacommunity ectomycorrhizal genera are more evenly distributed than species at the local community. Opposite species abundance patterns in local and metacommunities have been observed for trees in tropical rain forests and for the sessile organisms in tropical coral reefs (Volkov et al. 2007). In tropical tree communities the proportion of rare tree species is lower in the local community than in the metacommunity, while in the single coral reef the proportion of rare species is larger than in the metacommunity. In that respect the abundance pattern in coral reefs is similar to the abundance pattern in ectomycorrhizal communities: cosmopolitan species at the global scale and dominant species at the local scale.

Dickie and Moyersoen (2008) speculate that host preferences of fungi might control species composition of a local ectomycorrhizal community. Specialisation of fungi for certain tree species might be a driving force both for species richness and species abundance patterns in ectomycorrhizal communities (Newton and Haigh 1998; Dickie 2007). Not surprisingly, the phenomenon of preferred fungus host associations has been a topic from the very beginning of ectomycorrhizal research (Melin 1923, 1948). These observations were based on the regular occurrences of fruiting bodies of ectomycorrhizal fungi beneath certain tree species, as they are listed in species descriptions in field guides (Moser 1983; Breitenbach and Kränzlin 1991; Dähncke 1993).

The question of specificity

Despite the general recognition of specificity phenomena in fruiting body occurrences, not at last by the collectors of edible mushrooms, many authors assume that generalist fungi, i.e. fungi that are able to colonize a broad range of host species, prevail in ectomycorrhizal communities. Assumptions on the prevalence of generalist fungi are based on several theoretical frameworks and observations. One prediction deals with the recognition of ectomycorrhizas as diffuse mutualism. Diffuse mutualism defines a beneficial interaction in which more than one partner species are involved on both sides. The opposite is a pair-wise mutualism that is restricted to two species, one on the host side, and one on the symbiont side. While the pair-wise mutualism involves a high degree of specialisation, diffuse mutualisms are supposed to require generalist species on the symbiont side (Hoeksema and Bruna 2000; Hoeksema and Kummel 2003; Stanton 2003). If ectomycorrhizas are considered to be a diffuse mutualism then ectomycorrhizal fungi have to be generalists.

The second reason for the assumption of generalist prevalence is the comparison with endomycorrhizal communities involving AM fungi as symbionts. The few recognized species of AM fungi colonize a broad range of host species (Smith and Read 2008). As generalist AM fungi are dominant in many ecosystems and associate with much more host species than ectomycorrhizal fungi, it is often asserted that unspecificity is the general status for all mycorrhizal associations including ectomycorrhizal associations (Brundrett 1991 and references therein).

Another concept that requires prevalence of generalists and is also derived from observations in AM communities is the concept of Common Mycorrhizal Networks (CMN). It has been observed that hyphae of AM fungi are able to connect different plants and even different plant species in grasslands (Heap and Newman 1980; Francis and Read 1984; Haystead et al. 1988). It was postulated that plant roots and AM mycelia are integrated into Common Mycorrhizal Networks (CMN) that can transport nutrients between plants (Newman 1988; He et al. 2003; Whitfield 2007). Since AM fungi are generalists they can form both intra and interspecific CMNs. The concept of CMNs has been expanded to ectomycorrhizal fungi after it has been shown that nutrients can be transported between trees of different species via ectomycorrhizal mycelium, both in pot experiments (Finlay and Read 1986; Arnebrant et al. 1993) and under field conditions (Simard et al. 1997a; Simard et al. 1997b). The existence of a wood-wide web has been postulated that connects all trees in a mixed forest via an interspecific CMN (Sen 2000; Wiemken and Boller 2002). Prerequisite for interspecific CMNs in mixed forests is that dominant ectomycorrhizal fungi are generalists. Because of the attractiveness of the concept of interspecific CMNs several authors claimed, that lack of specificity is a general trait of ectomycorrhizal communities (Read 1997; Bruns et al. 2002; Kennedy et al. 2003; Selosse et al. 2006).

Determining host specificities in below-ground communities

Not only theoretical considerations, but also practical problems contribute to the difficulty in determining the extent of host specificities in ectomycorrhizal communities. Early

compilations of fungus-host association were based almost exclusively on the observations of fruiting bodies beneath putative host trees (Trappe 1962; Molina et al. 1992; Newton and Haigh 1998). Because large study areas can be easily covered with this method it is still used in recent studies (Roy et al. 2008; Buée et al. 2011). However, this approach can be problematic when fungi that form ectomycorrhizas do not occur as fruiting bodies above-ground (Gardes and Bruns 1996; Dahlberg et al. 1997; Peter et al. 2001). Either they form below ground fruiting bodies as true and false truffles, e.g. *Tuber*, *Hydnotrya*, *Rhizopogon* (Kretzer et al. 2003; Murat et al. 2005; Tedersoo et al. 2006) or they have inconspicuous resupinate fruiting bodies, such as *Thelephora*, *Tomentella*, *Sistotrema* (Kõljalg et al. 2000; Nilsson et al. 2006; Jakucs and Erős-Honti 2008).

Some ectomycorrhizal fungi have no fruiting bodies at all. The most important member of this group is *Cenococcum geophilum*. It is not only the most widespread ectomycorrhizal fungus but also dominates many ectomycorrhizal communities (Jany et al. 2002; Richard et al. 2005). Another cause for the disparity below-ground and above-ground views is the sporadic appearance of fruiting bodies even in groups that have epigeous fruiting. Depending on the weather and soil conditions many species do not form sporocarps every year, though they are present as ectomycorrhiza (Jonsson et al. 2000; Horton and Bruns 2001).

Besides the disparity between below- and above-ground views, an additional problem occurs in fruiting bodies surveys of mixed stands. Ectomycorrhizal fungi fruiting in forests with several tree species are often considered to be non-specific. However, it is possible that they colonize the roots of only a single host (Bruns et al. 2002). Considering all these reasons together, it is clear that for any analysis of ectomycorrhizal biodiversity patterns including fungus-host associations, ectomycorrhizal communities have to be observed directly at the roots.

Confusion of specificity and preferences

Although specificity phenomena are recognized, it is difficult to find an explicit definition for the term specificity. Even in a widely cited review on specificity phenomena (Molina et al. 1992) the term specificity is not defined. One practical reason for this situation is that no single fungal species associates exclusively with a single host. Hence, all ectomycorrhizal fungi associate with a range of possible hosts. In the review of Molina et al. (1992) fungi of narrow host range are distinguished from fungi with a broad or intermediate host range. Since the compilation of host ranges was mainly based on fruiting body reports the designation of host affinities relies on the principle of presence/absence in a certain type of forest. The more hosts are reported for a fungus the wider is its host range. In such compilations frequency does not play a role (see Trappe 1962). For instance, when a fungus is reported ten times from an angiosperm host and one time from a conifer, it is counted as generalist, because it does not exclusively occur on angiosperm hosts.

In ectomycorrhizal community studies that observe specificity phenomena below-ground the term specificity is often replaced by the term preference (Tedersoo et al. 2008; Tedersoo et al. 2009; Lang et al. 2011). Since ectomycorrhizal community studies involve distinguishing and

counting thousands of root tips they provide a better basis for quantitative description of specificity phenomena in mixed stands. One outcome in these studies is that some fungal species are more often found on the roots of one tree species than on the other. This behaviour is called preference. It implies that a host-preferring fungus is found to a low percentage on roots of a non-preferred host (Lang et al. 2011). One problem in the usage of the term preference is that some authors use it completely interchangeably with the term specificity (e.g. Kernaghan et al. 2003; Ishida et al. 2007; Smith et al. 2009)

The distinction of specificity and preference would be academic, if there wouldn't be an underlying principle, which would make the distinction useful. One question is whether the observation of host preferences in mixed stands is a special case for a species that is otherwise host specific. Host specificity would denote the more general case that a fungal species is only present when also the putative host species is present but is otherwise absent in stands without the host. Note, that in this treatment of specificity and preference the same fungal species is considered. Specificity can then be viewed as an expression of the physiological adaptation of the fungal species to the host species which is loose enough to allow occasional host switches in mixed stands (= host preference).

Deterministic and stochastic concepts for the explanation of ectomycorrhizal biodiversity patterns

Many authors assume that there is a positive relationship between host number and the number of ectomycorrhizal fungal species in a defined area and that this correlation is caused by specificity phenomena (Nantel and Neumann 1992; Kernaghan et al. 2003; Dickie 2007; Ishida et al. 2007). This would explain why on a global scale much more ectomycorrhizal fungal species exist than AM fungal species, the latter being generalists. The fundamental underlying principle for this kind explanation is niche theory: the more niches, in this case host species, are present, the more species, in this case fungi of different host affinities, can be observed (Dickie 2007). Niche theory is per se deterministic, because it assumes that the presence of a species can be explained by a cause, i.e. the adaptation to a niche (Chesson 1991, 2000). In that sense most explanations for ectomycorrhizal biodiversity are deterministic and host specificity is one of them (Dickie 2007).

However, not all authors in the field of theoretical ecology agree that deterministic explanations are the only approach towards biodiversity and species distributions and claim that also stochastic processes play a role (Hubbell 2001; Tilman 2004; Rosindell et al. 2011). Limits to deterministic explanations of biodiversity occur also in ectomycorrhizal research and can be related to the discussion of host specificity. One example is the already mentioned assumption of generalists dominance in ectomycorrhizal communities (Read 1997; Bruns et al. 2002; Kennedy et al. 2003; Selosse et al. 2006). If most ectomycorrhizal species are generalists, a major deterministic niche, namely host specialisation, has to be excluded. A similar situation occurs in pure stands where high ectomycorrhizal species richness cannot be explained by different host species. Niches that are not related to host specialisation are mentioned in two reviews on ectomycorrhizal species richness in pure stands (Bruns 1995; Kennedy 2010). They include soil horizons, distribution of mineral nutrients and organic

matter. However, both reviews discuss also processes that are independent of niches and involve stochastic components.

These processes include competition and dispersal related effects. Competition alone would lead to the exclusion of one of the competitors and thus species richness would be reduced (Hardin 1960; Chesson 2000). Therefore competition theory has to be complemented by dispersal related processes that always involve a stochastic component. Theories that explain species distributions by dispersal processes are the theory of island biogeography (MacArthur and Wilson 1967), the neutral theory of biodiversity (Hubbell 2001) and lottery models (Sale 1977; Chesson and Warner 1981). While the first two theories are intended to explore species distributions at larger geographic scales, lottery models might be useful to explain ectomycorrhizal biodiversity patterns at the level of a forest plot (Kennedy 2010).

Extreme cases of unexplained species distributions are the frequent occurrences of several ectomycorrhizal species within a single soil core (a few hundred cm³). Within this small volume many environmental factors potentially contributing to niches are the same, especially in a pure stand where only a single species of host roots is present. One solution would be to look for ever finer niches such as vertical compartmentalization or individual genotype differences of the host. Failure to explain the presence of species by these niches would point to the need to transfer the ideas of competition and dispersal to ectomycorrhizal community studies.

Species distinction by morphotyping and molecular methods

The analysis of below-ground ectomycorrhizal biodiversity requires determination of the fungal species directly on the host root. One approach is recording and comparing morphological and anatomical features of the fungal structures on mycorrhized roots (mantle, rhizomorphs, emanating hyphae). Several catalogues of characteristics that are crucial for the distinction of the fungal partners have been developed (Ingleby et al. 1990; Goodman et al. 1996-1999). The most widespread used system was proposed by Agerer (1991) that led to the publication of a colour atlas and a series of descriptions of ectomycorrhizae (Agerer 1987-2006; Agerer et al. 1998-2006).

A direct determination of the fungal species is only possible when a soil sample is excavated together with a fruiting body and the mycelium of the fruiting body can be visually linked to the mycelium of the described ectomycorrhiza. Because of the above mentioned disparity of fruiting body and ectomycorrhiza occurrence, a direct linkage of ectomycorrhizal and fruiting body mycelium is rarely observed so that many ectomycorrhizal descriptions are distinguished by provisional morphotype names. The method of characterizing an ectomycorrhizal community by morphotypes has been referred to as "morphotyping" (Conn and Dighton 2000; Agerer and Göttlein 2003; Baier et al. 2006). Determining abundance of each morphotype requires counting of ten thousands of root tips (e.g. Taylor and Alexander 1990; Lazaruk et al. 2008; Kranabetter et al. 2009; Azul et al. 2010).

The approach of morphotyping is limited when species within a genus have uniform mycorrhizal morphology and anatomy or when the species name is explicitly required in an

investigation. Therefore, morphotyping has been complemented or replaced by the molecular method of ITS sequencing. ITS (Internal Transcribed Spacer) is a region on the nuclear DNA that is located between the ribosomal RNA genes (Horton and Bruns 2001). Once the fungal ITS-sequence is obtained from an ectomycorrhizal sample it can be compared with database entries for fruiting bodies. The reference database for determining ectomycorrhizal fungi is UNITE (Kõljalg et al. 2005; Abarenkov et al. 2010). ITS sequencing of ectomycorrhizal fungi does not always lead to a species name, for instance if the fruiting body sequence is not yet in the database or the taxonomic status of the fruiting body sequence is unclear. Even in this case different sequence types of closely related species can be much more reliably distinguished than morphotypes.

While ITS sequencing is standard for ectomycorrhizal identification, it is often used in combination with morphotyping (e.g. Jakucs et al. 2005; Tedersoo et al. 2006; Nieto and Carbone 2009). Mycorrhized root tips are distinguished as morphotypes and abundances are calculated as in classical morphotyping studies. Subsequently, selected root tips per morphotype are sequenced to give each morphotype a species name (e.g. Toljander et al. 2006; Courty et al. 2008; Blom et al. 2009; Diedhiou et al. 2009; Ryberg et al. 2011). Because this method relies on an exact delineation of morphotypes, which requires long-time experience, more and more studies are published, that are based on datasets in which each sampled root tip is sequenced. For budget constraints the number of sequenced root tip is usually much smaller (a few hundreds to a few thousands root tips) than in morphotyping (tens of thousands root tips). Most studies of this kind use randomized samplings scheme and do not distinguish morphotypes at all (Kjøller and Clemmensen 2009; Peay et al. 2010; Peay et al. 2011).

The disadvantage of the combined morphotyping/sequencing approach is obvious. Even if there is a good match of morphotype and sequence among the 3-5 sequenced root tips, it is not clear if this would be the case for all root tips of this morphotype. And in the case of pure sequencing studies, the agglomerated knowledge on morphology and anatomy is completely neglected. A mix of both approaches would be to sequence all collected root tips while preselecting the sequenced tips on the basis of morphotypes. It would be a methodological improvement to compare the match between morphotype and sequences in a study that sequences all collected root tips and uses morphotypes as selection criterion.

I.3. Study site

The study site for this thesis was established in the course of a joint research project of the German Federal Ministry of Research and Education (Münzenberger et al. 2005). The joint project run between 1999 and 2004 and explored the conditions for sustainable forestry in the North-eastern Lowlands of Germany. Forests in these areas are dominated by pine plantations (Müller et al. 2005). One aim of sustainable forestry is to reduce the dependency on pine plantation by promoting broad leaved trees of the natural vegetation. The northern parts of the state Brandenburg in Germany belong to the Baltic distribution area of European beech

(*Fagus sylvatica*, L. Fig. 1). Thus, beech is the natural forest tree dominating in this area. Therefore, beech is the preferred tree species for transforming pine plantations into more natural forests (Münzenberger et al. 2005).

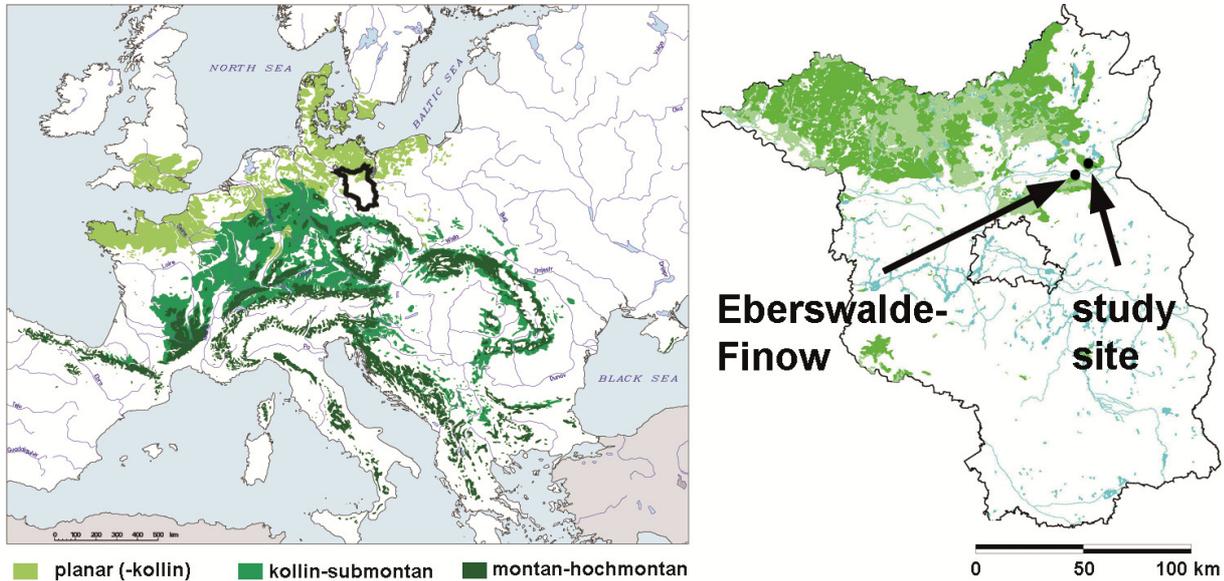


Fig. 1: The natural distribution of beech and the location of the Kahlenberg site

The site is shown in relation to the natural distribution of beech in Europe and in the state Brandenburg of Germany (maps adapted from Bohn and Neuhäusl (2003) and Jenssen et al. (2007)).

The process of transforming a pine plantation into a beech forest takes decades and is difficult to observe directly. Therefore, a "false time series" was established at the "Kahlenberg" site near Eberswalde-Finow, 100 km northeast of Berlin (Fig. 1). It comprised four stands representing different stages in the transformation process (Fig. 2): a pure pine stand (91 years), a younger mixed stand (pine 83 years, beech 40 years), an old mixed stand (pine 121 years, beech 80 years) and a pure beech stand (108 years). Ages of trees refer to the 01.01.2007 (H. K. Sakowski, personal communication). The pure pine stand represents pine monocultures as the starting condition. With the two mixed stands intermediate stages of the transformation process were studied. The pure beech stand was established as understorey of a Scots pine monoculture that has been finally converted into a beech stand. Therefore, it represents the endpoint of this forest transformation approach.

In the joint project various studies have been carried out on these four stands considering for example the impact of forest transformation on physical soil properties (Buczko et al. 2002; Buczko et al. 2005) and on the distribution of humus forms (Bens et al. 2006). One study analyzed the change of the ectomycorrhizal community along the "false time series" on the basis of morphotyping (Rumberger et al. 2004; Rumberger 2005). This approach involved counting ten thousands of root tips allowing statistical analysis of the changing community pattern. However, species resolution was poor, since many morphotypes could not be

determined to species level. Therefore, a subsequent project aimed at determining the ectomycorrhizal fungal species by a combination of morphotyping and molecular methods at the same four stands. The data of this project (DFG, Mu1035/9-2) was generated and analysed as basis for the work presented here.

The four stands of the Kahlenberg site were selected to represent a "false time series". They also provide an excellent study design for evaluation of host specificities of ectomycorrhizal species in pure and mixed stands. The four stands are unified by the same history of forestry: One century ago, they constituted Scots pine plantations that were established on the ground of a former beech forest. Furthermore, the close vicinity of the stands (the largest distance among the sites is approximately one kilometre) allows considering them as a single site with the same climatic conditions and the same underground. The main difference between the stands is the different stocking with the two host species Scots pine and beech. Thus, the two mixed stands correspond to the traditional study design for the analysis of host preferences.

By determining the percentage of an ectomycorrhizal fungal species on either Scots pine or beech roots the host preference of this fungus can be described. What is new for the study of host preferences is that the two neighbouring stands of pure Scots pine and beech allow a direct comparison of the fungal host associations in pure and mixed stands. For example, it can be studied whether the same fungi that prefer Scots pine in a mixed pine/beech stand are completely absent from the pure beech stand or not.

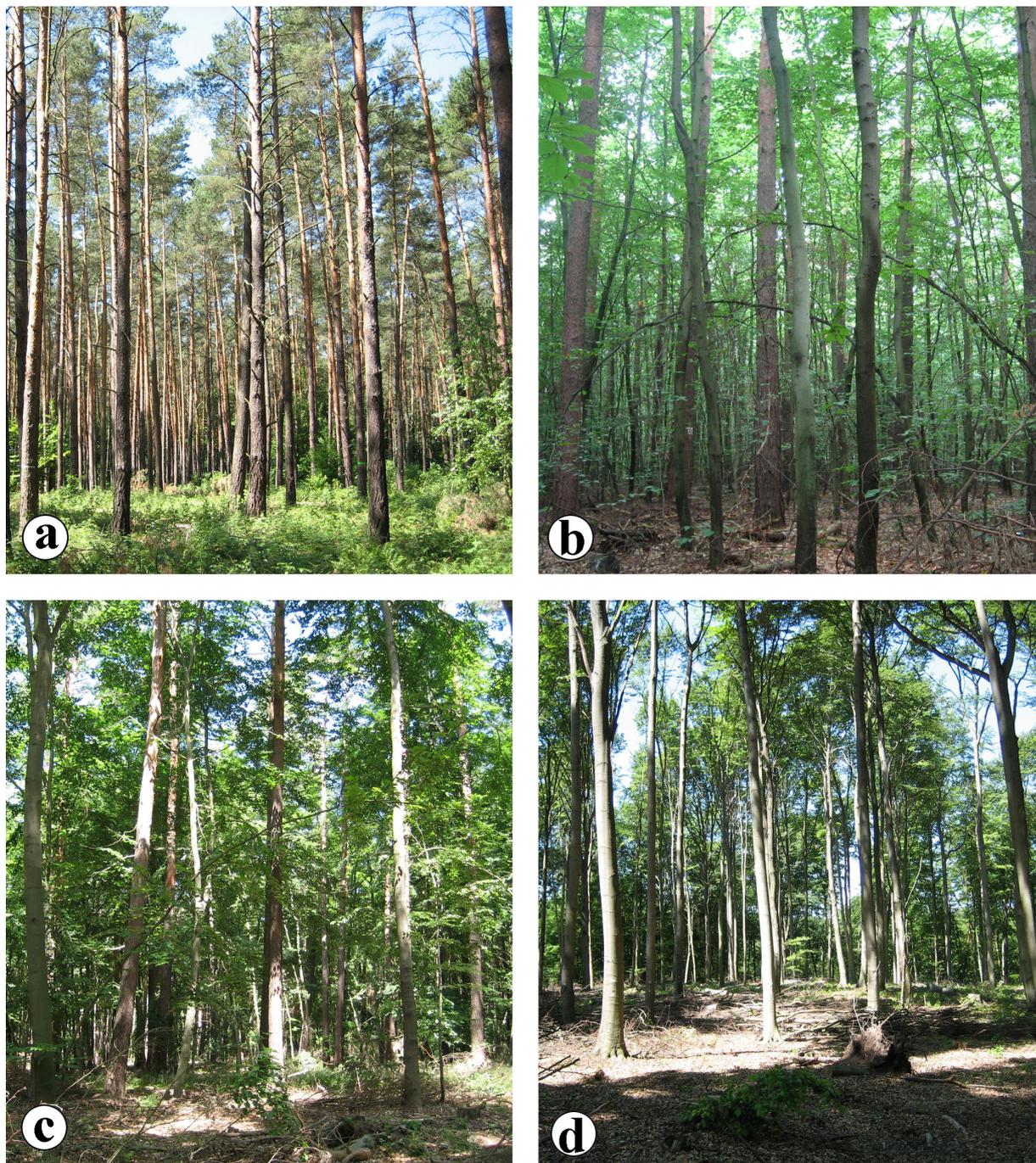


Fig. 2: Analyzed stands

a) pure pine stand, b) young mixed stand, c) old mixed stand, d) pure beech stand

I.4. Research questions and relation of the Parts II, III and IV to each other

In the following, the three data chapters Part II, III and IV are shortly outlined.

Part II - Species identification

Determining the fungal species is the necessary basis for any analysis of ectomycorrhizal biodiversity. In Part II of this thesis ectomycorrhizas are described morphologically and it will be explored how the distinction of ectomycorrhizal fungal species can be improved by using molecular methods. One basis is the morphological and anatomical description of the ectomycorrhizas found on the Kahlenberg site.

The second basis for Part II is the contribution of the thesis author to three published manuscripts. These three papers are case studies for the distinction of ectomycorrhizal fungi. Case study 1 describes the new ectomycorrhizal species *Acephala macrosclerotiorum* which is a relative of endophytic root fungi (Münzenberger et al. 2009). Case study 2 analyses the relationship of the ectomycorrhizal species *Hydnotrya tulasnei* to its sister species *H. bailii* (Stielow et al. 2010). Both *A. macrosclerotiorum* and *H. tulasnei* are prominent members of the ectomycorrhizal communities at the Kahlenberg site. In the third case study an ectomycorrhiza of *Sistotrema spec.* is characterized by morphology and molecular data (Münzenberger et al. 2012). Although this species is not a member of the ectomycorrhizal communities at the Kahlenberg site it is a representative of Scots pine associated fungi of the North-eastern Lowlands of Germany. The contributions of the author of this thesis to the three manuscripts will be listed.

The species identifications presented in Part II are an important prerequisite for the analyses in Part III and IV. Methodologically, the two manuscripts rely on sequenced root tips that were preselected on the basis of morphological/anatomical characters. Therefore, Part II also contains an analysis of the match of determinations by morphotypes and sequence types for all root tips sequenced in Part III and Part IV.

Part III - Manuscript 1: The concept of specificity guilds reveals dominance of host specific fungi in pure and mixed stands of *Pinus sylvestris* L. and *Fagus sylvatica* L.

Part III is an unpublished manuscript to be submitted to the Journal *Mycorrhiza*. It introduces the concept of specificity guilds for assessing host specificities. The term host specificity is used inconsistently in the literature and is often confused with the term host preference. In part III the term host preference refers to fungi that are predominantly found on a single host species (either Scots pine or beech) in the mixed stands of the Kahlenberg site but these fungi might occasionally also be found on co-occurring non-target hosts. Host preferring fungi, together with specialists that are found exclusively on the same tree species, form a guild of host specific fungi. Corresponding to the two tree species, two host specific guilds are distinguished at Kahlenberg site: pine specific and beech specific. These host specific guilds

are contrasted by the generalist guild, which consists of fungi that are found at similar proportions at beech and pine roots. The designation of the host specific guilds in the two mixed stands at Kahlenberg is confirmed by the data of the two pure stands. A fungus with preferences in the mixed stand is only found in the pure stand of the preferred host, but does not occur in the pure stand of the non-target host.

With this new definition of specificity guilds as background, the question can be answered whether host specific or generalist fungi dominate the Kahlenberg site. Beyond the conceptual innovation of specificity guilds the technical strength of part III is the reliance on sequenced root tips. Only sequenced root tips were included in the datasets for the assignment of fungi to specificity guilds. Furthermore, reliance on sequences improved distinction of fungal species via phylogram supported species determination.

Phylograms helped to explain contradictions of host specificities in comparison to reports in the literature. On the Kahlenberg site, one member of the beech specific guild, *Laccaria* cf. *laccata*, behaved like a beech specialist although it is considered to be a generalist in the literature. The phylogram of the genus *Laccaria* suggests that *Laccaria laccata* is in fact a species complex that consists of cryptic species which belong to different specificity guilds. Altogether 15 phylograms are presented as online supplemental material. These 15 phylograms were the basis for determining all 40 ectomycorrhizal species at the Kahlenberg site.

The results based on the assignment of specificity guilds are used to discuss several aspects of specificity phenomena. One aspect is the contrasting guild memberships in closely related species. Two other aspects are the relationship of specificity guilds to the concept of host ranges and interspecific CMNs (Common Mycorrhizal Networks).

Part IV- Manuscript 2: Individual tree genotypes do not contribute to ectomycorrhizal biodiversity in a pure stand of beech (*Fagus sylvatica* L.)

Part IV is an unpublished manuscript to be submitted to the Journal *Trees*. It explores the limits of niche theory for explaining the paradox of fungal species biodiversity in a seemingly uniform environment. In Part III the contribution of different host species to ectomycorrhizal biodiversity is reflected by the formation of generalist and host specific guilds. Both generalists and host specific fungi enrich the ectomycorrhizal community in mixed stands. In pure stands fungal specialisation to different host species cannot influence biodiversity because only a single host species is present. Besides fungal specialisation to different host species, it could be possible that fungal species are adapted to different genotypes within the same host species. The manuscript in part IV tests the hypothesis that specialisation of fungal species to individual genotypes of a single host species might explain biodiversity in a pure stand and an individual soil core.

For this purpose, ectomycorrhizal data of the 2007 season in the pure beech stand of the Kahlenberg site has been combined with soil parameters measured at the same transect points. In addition to the 10 transect point sampling scheme used for manuscript 1 (Part III), three soil cores of the pure beech stand have been examined in detail. All root segments in a soil

core have been collected (30-40 per core) and the ectomycorrhizal species on each root segment have been determined by sequencing. The root genotypes were distinguished by microsatellite PCR and connected to the genotypes of surrounding beech trees. This allowed concluding which individual beech sent its roots to the respective soil core.

Since the genotype hypothesis assumes a specialisation of fungal species to host genotypes it belongs to the category of niche theories. The results of the hypothesis test will be used to discuss explanations for ectomycorrhizal biodiversity that go beyond deterministic niche theories.

Thesis Part II

Fungal species identification

II.1. Outline of Part II - Fungal species identification

Part II is one of three parts of this thesis that contain original data. However, in contrast to Part III and Part IV its structure does not follow the classical paper form of Methods/Results/Discussion. The structure of Part II presents a logic concept of identifying the fungal species on a mycorrhized root tip.

When a soil is inspected under a dissecting microscope, the first information on a mycorrhiza is its physical appearance. Therefore, the ability of a researcher to differentiate different types of ectomycorrhizas is the crucial step to select the ectomycorrhizas of interest for further identification by molecular methods. Section II.2 contains not only anatomical and morphological descriptions of the ectomycorrhizas found at Kahlenberg site, but also microphotographs of morphotypes. It serves to illustrate the variety of morphotypes but also the difficulty to differentiate them from morphological appearance alone.

An overview over the method of ITS sequencing is given in section II.3. This section does not present a detailed description of the laboratory method (this can be found in the method sections of Part III and IV) but rather serves to assess the advantages and difficulties of this method. It will give the background why the usual criterion of database match is not always sufficient for species differentiation and how species differentiation can be improved by phylogenetic analysis.

In cases where no database matches exist, because the corresponding fungus has not yet been described, an even more detailed analysis is necessary. It involves cultivation techniques and inoculation tests and a new species description if the species is yet unknown. Three case studies are presented in sections II.4 to II.6. Each case study is based on a published manuscript to which the author of this thesis contributed as co-author. The first study (section II.4) presents the description of the new species *Acephala macrosclerotiorum* based on morphological, anatomical and genetic evidence as well as evidence from axenic co-cultivation with potential host plants.

Section II.5 contains a taxonomic study in the ectomycorrhizal genus *Hydnotrya* based on fruiting body morphology and molecular data both from fruiting bodies and ectomycorrhizas. The molecular data of ectomycorrhizas originate from Kahlenberg site and helped to confirm species status of *Hydnotrya tulasnei*. Both *H. tulasnei* and *A. macrosclerotiorum* are prominent members of the ectomycorrhizal communities at Kahlenberg site. Observations that relate to host specificity are highlighted in both case studies of section II.4 and II.5.

In the third case study (section II.6) a combination of morphological/anatomical and genetic methods has been used to describe and identify the ectomycorrhizal fungus EW63 of the genus *Sistotrema*. The proof of ectomycorrhizal status applied axenic cultivation with potential host trees. This proof of ectomycorrhizal status is important because the genus *Sistotrema* contains also saprotrophic fungi. Although the fungus EW63 has not been found at Kahlenberg site, it is an example of host specificity because it shows an interesting behaviour towards its host *Pinus sylvestris*. As supplement to the published manuscript, proof of the

ectomycorrhizal status of type species of the genus *Sistotrema*, *S. confluens*, is presented together with a description of its ectomycorrhiza collected in the field.

When species are determined either by database match, phylogenetic analysis or by a new description it remains to assess how good morphotype/anatomotype data really fit to the molecular species identification. The match of species descriptions from Kahlenberg site and identification by ITS-sequencing will be compared in section II.7. However, in section II.7 only the match of morphological and molecular data will be analysed. A complete analysis of the molecular data of the ectomycorrhizal fungi is the major component of Part III and Part IV.

In the final section II.8, the discussion focuses on how the results of this comparison serve a justification that only molecularly identified root tips can be used for the data analysis in Part III and Part IV.

II.2. Descriptions of 34 ectomycorrhizas at Kahlenberg site

The physical appearance of a mycorrhized root can be described as morphotypes and anatomotypes. The term morphotype refers to the physical appearance as it can be seen through a dissecting microscope at a maximum magnification of 75 x. Characters used for the distinction of morphotypes are the type and order of ramification, colour and transparency of the hyphal mantle, presence/absence of rhizomorphs or emanating hyphae and the diameters of main axis and branches. These characters helped to sort ectomycorrhizal types prior sequencing in the spring 2006 and fall 2006 field campaign at the Kahlenberg site. The term anatomotype refers to the physical appearance of the hyphal cells as it can be seen through a light transmission microscope at a maximum magnification of 1000x. Preparations of the hyphal mantle and/or rhizomorphs and emanating hyphae are used for the descriptions of cellular structures. Anatomical characters of ectomycorrhizas have been recorded at the winter 2007 and spring 2007 field campaign at the Kahlenberg site.

Based on anatomical characters 34 short descriptions were prepared in reference to the system proposed by Prof. Agerer (Agerer 1987-2006; Agerer 1991; Agerer et al. 1998-2006). Morphological and anatomical characters were recorded according to the check lists published on the website of the online key DEEMY (<http://www.deemy.de>). The text of the short descriptions follows the examples published in the Descriptions of Ectomycorrhiza (Agerer et al. 1998-2006) and contains three paragraphs:

- a) general morphology
- b) anatomy of the mantle
- c) anatomy of emanating elements (hyphae, cystids, and rhizomorphs)

Morphological characters were observed with an Olympus SHZ10 at varying magnifications between 7x and 75x and anatomical characters were observed with a Zeiss Axioskop at 1000x magnification and oil immersion. Ectomycorrhizal types are named with a laboratory code. If available, the biological species name is given as it emerged from molecular identification of the specimens described here (results of Part III, for molecular methods see section II.3 and III.3). The host name refers to the host on which the individual mycorrhiza used for the description was found. It is not a statement on host specificity.

BB05 *Cenococcum geophilum* Fr.; Scots pine; Fig. 1a, Fig 5a, b

- a) Ectomycorrhizas are black and woolly with many emanating hyphae, sclerotia are rare. They appear as single tips or are dichotomously ramified.
- b) The hyphal mantle is completely plectenchymatic. The outer and middle mantle layer contains membranously dark brown hyphae with thick walls (up to 4 μm) that are star-like arranged and are tightly glued together (mantle type G). The hyphae of inner mantle layer show no distinct pattern.
- c) Emanating hyphae are straight. They are membranously brown and have thick walls (up to 2 μm) with a smooth to warty surface.

BB06 *Xerocomus badius* (Fr.) Kühn. ex Gilb; Scots pine; Fig. 1b, c, Fig. 5c, d

- a) The ectomycorrhiza has a silvery appearance and is dichotomously ramified. Its colour turns to ochre when air is removed from the hyphal mantle. Many silvery rhizomorphs emanate from the smooth surface. Rhizomorphs are hairy and ramify frequently at distinct points. They are connected to the mantle at distinct points, often at the older part of the mycorrhiza. Emanating hyphae are rare.
- b) The hyphal mantle is plectenchymatic in all mantle layers. Hyphal bundles of the outer mantle layer are arranged in ring-like structures (mantle type A) and have smooth surface covered with few soil particles. Ring-like structures are not as prominent as is in morphotype BB28, but more elongated and rectangular. Short hyphal ends are prominent at the outer mantle surface. Hyphae of the outer mantle layer ramify at angles of 90°. They have different shapes: 1) ampullate at both ends of the cell; 2) ampullate at one end of the cell; 3) cylindrical, constricted at septa; 4) slightly inflated at middle part of the cell. Their diameter ranges from (2) 2.5-4.5 μm with a cell wall thickness of 0.5 μm . Hyphae of the middle mantle layer are arranged in rings and have a smooth surface (\varnothing 3-6 (6.5) μm , wall thickness 0.5-1 μm). Hyphae of the inner mantle are arranged both in rings and as broad streaks of parallel hyphae (\varnothing 3-6 μm , wall thickness 0.5-1 μm). Cell walls of the mantle hyphae are colourless to slightly membranously yellowish.
- c) Rhizomorphs are highly differentiated; thick hyphae (\varnothing 5-6 (6.5) μm , wall thickness 0.5 μm) form central vessel-like structures with partially dissolved septa (rhizomorph type F). Nodia are present. The surface of the peripheral hyphae (\varnothing 3 μm , wall thickness 0.5 μm) is

smooth or slightly covered with soil particles. Cell walls of the rhizomorph hyphae are membranously yellowish.

BB11 *Acephala macrosclerotiorum* Münzenberger et Bubner; Scots pine; Fig. 1d, Fig. 5e

a) The surface of the brown (root colour) to dark brown mycorrhizas is shiny but not smooth. The mycorrhizas occur as single, not ramified, straight tips with numerous black emanating hyphae. Older mycorrhizas are often covered with black lens-shaped or flat sclerotia that are oval in diameter. Although the mantle is complete it is so thin that cortical cells of the root are visible at younger mycorrhizas.

b) Because of the low thickness of the mantle (2-3 cells), only an inner and an outer mantle layer are differentiated. The outer layer is characterized by an undifferentiated hyphal system (plectenchymatic), with some ring-like arranged hyphae (mantle type A) and a gelatinous matrix (mantle type C). Hyphae ramify at angles of 90° and occur in three shapes: 1) broad cells (not cylindrical), 2) broad cells with tapering ends, 3) cylindrical cells with constrictions at septa (\varnothing 2-5 (7.5) μm , wall thickness 0.5-1 μm). The inner mantle layer is also plectenchymatic with ring-like arranged hyphae and a gelatinous matrix (\varnothing 3.5-5 μm , wall thickness 0.5 μm). Cells both of the outer and the inner layer are membranously brown with a smooth surface.

c) Emanating hyphae are frequent and without clamps. They are membranously dark brown and septate. Hyphae are 29-60 μm long with a diameter of 3.5-4 μm and varying wall thickness (0.5-2 μm). Hyphal surface is smooth or rough from round warts ($\varnothing \leq 0.5$ μm) or irregular structures of unknown nature (0.5 x 1.0 μm). Some hyphae appear to have two-layered cell walls but this could also be a gelatinous envelope. Anastomoses with short bridges were observed. The bridge was closed with a simple septum.

This ectomycorrhizal type was described as morphotype Pinirhiza sclerotia by Wöllecke (2001). It is the only ectomycorrhizal type with frequent sclerotia at the Kahlenberg site. The outer layer of the sclerotium is pseudoparenchymatic with membranously brown cell walls. A detailed description of the sclerotia is given in Münzenberger et al. (2009). This publication contains also a species description of the fungal partner *Acephala macrosclerotiorum*, which will be discussed in section II.4.

BB14 *Russula ochroleuca* (H.C.Hall) Pers.; beech; Fig. 1e, Fig. 5f

a) At beech roots, the morphotype BB14 occurs as single mycorrhized root tips without ramification and with straight ends. The surface is densely warty at the basis of the mycorrhiza (toward the unmycorrhized root) and appears to be of greenish yellow colour. At younger root tips warts are less prominent, so that the surface appears to be smooth and of light yellow colour. Emanating hyphae are rare.

b) The outer hyphal mantle is pseudoparenchymatic and consists of angular cells and heaps of flattened cells (mantle type O). Cells are frequently triangular and arranged in rosettes. Both mantle and heap cells have a smooth surface (0.5-1.5 μm wall thickness). Like the outer layer,

the middle mantle is pseudoparenchymatic but consists of polygonal, occasionally rounded cells with a smooth surface (0.5-1 μm wall thickness). The inner mantle layer is plectenchymatic and the hyphae are arranged without a special pattern.

c) The rare emanating hyphae have a smooth surface, or occasionally they are covered with soil particles. Hyphae have frequently elbow-like protrusions or are irregularly inflated (\varnothing (2)2.5-3 μm , wall thickness 0.5 μm). Although simple septa can occur, clamps have not been observed. Both emanating hyphae and the mantle cells are membraneously yellowish.

BB22 (BB17, BB69) *Lactarius tabidus* Fr.; Scots pine; Fig. 1f, g, Fig. 6a, b

a) Mycorrhizae are dichotomously ramified (second order ramifications) and have straight ends. Their colour ranges from orange to yellowish brown with a bright upper end. Together with the smooth surface and the lack of emanating elements, the presence of laticifers identifies this morphotype as *Lactarius sp.*

b) The outer mantle layer is pseudoparenchymatic. Angular to irregular cells are overlaid with hyphal net (mantle type P in transition to H, cell wall thickness 0.5-1 μm). Hyphae of the middle mantle layer are of irregular or cylindrical shape (cell wall thickness <0.5 μm). The inner mantle layer is plectenchymatic and the hyphae are arranged without a special pattern. In all mantle layers, hyphae are colourless to slightly membraneously yellowish and have a smooth surface.

c) Emanating elements are not observed.

BB 23 *Thelephora terrestris* Ehrh. ex Willd.: Fr.; Scots pine; Fig. 1h, i, j, Fig. 6c, d

a) This mycorrhiza features second order dichotomous ramifications and has both cystids and rhizomorphs as emanating elements. The colour is light brown with hydrophobic patches of silvery appearance. Cortex cells of the root are not visible through the tick mantle. At the maximum magnification of the dissecting microscope of 75x the mycorrhizal surface appears prickly, caused by the presence of cystids. The single ends are straight (1.3-1.8 mm, \varnothing 0.5 mm).

b) The outer mantle is plectenchymatic, formed by a net of coarse and irregularly shaped hyphae (mantle type H). Hyphae are often inflated (\varnothing 4-7 μm , wall thickness 0.5 μm) and relatively short for a plectenchymatic mantle (20-30 μm). The middle and inner mantle layers are also plectenchymatic without large differences to the outer layer. Hyphae of the inner layer are of smaller diameter (usually 4 μm). In all layers hyphae have a smooth surface and are colourless to slightly membraneously yellowish.

c) Rhizomorphs have a silvery appearance. They are 50 μm in diameter and have hairy edge. Ramifications are observed. Hyphae of the rhizomorphs are colourless and have a smooth surface (\varnothing 4 μm , 40-70 μm long, cell wall thickness 0.5-1 μm). Septa have clamps. Central hyphae are not differentiated (rhizomorph type A). Anastomoses are occasionally observed between hyphae of the rhizomorphs. The other prominent emanating elements are the two

types of cystids. The first cystid type has no septa but carries an apical knob. Cell walls of the apical knob are as thick as the cell walls of the remaining cystid (\varnothing 2 to 2.5 μm , more than 100 μm long, cell wall thickness 0.5-1 μm , wall thickness not changing over the length). Cystids of the first type are colourless and have a smooth surface. The second type of cystids has no apical knobs. They have septa with clamps. Because of their length of more than 100 μm they could also be classified as emanating hyphae.

BB24 *Cortinarius* (subgenus *Dermocybe*) sp. 1; Scots pine; Fig. 1k, l, m, Fig. 6e, f

a) Mycorrhizae occur as single unramified tips that have a silvery greenish to yellowish colour. When air is removed from the hyphal mantle the tips appear ochre to brownish. The large numbers of emanating hyphae cause a woolly to hairy appearance of the mantle surface. The numerous rhizomorphs are silvery greenish to yellowish and protrude at small angles from the mantle surface. They are fan-shaped and ramify into more delicate filaments. Mantle colour and shape of the rhizomorphs identify the morphotype as *Cortinarius* sp.

b) Hyphal mantle is plectenchymatic in all mantle layers. Hyphae of the outer mantle are ring-like arranged (mantle type A, \varnothing 2.5-4 μm , wall thickness \leq 0.5 μm). They have a smooth surface and ramify at angles from 45°-90°. Clamps are present but not frequent. While hyphae of the middle mantle are ring-like arranged (\varnothing 2.5-4 μm , wall thickness 0.5-1 μm) hyphae of the inner mantle layer do not show a distinct pattern (\varnothing 3-8 μm , wall thickness 0.5-1 μm).

c) Rhizomorphs are undifferentiated and of uniform diameter, nodia are not present (rhizomorph-type A). Surfaces of both the central (\varnothing 3.5-4 μm , wall thickness $<$ 0.5 μm) and the peripheral hyphae (\varnothing 3 μm , wall thickness $<$ 0.5 μm) are smooth. The inconspicuously formed emanating hyphae (\varnothing 2.5-4 μm , wall thickness 0.5 μm) ramify frequently and carry clamps at the septa. Ramifications are Y-shaped and occur adjacent to a septum or one hyphal diameter below the septum. Clamps are oval and as broad as the hyphae in dorsal view. In lateral view they are smaller than a semicircle and have occasionally holes. Secondary septa (without clamps) are not observed. Emanating hyphae are of equal diameter or slightly constricted at septa. Wall thickness of the apical cell of emanating hyphae is usually uniform. Only one cell with a slightly thicker cell wall at the apex was observed. Anastomoses are frequent. They are either closed (septum with clamp) or open with short bridges.

BB28=BB08 *Xerocomus* spp.; beech; Fig. 1n, o, Fig. 7a, b

a) Besides single tips, these mycorrhizae form large systems with monopodial-pyramidal ramifications (first to second order ramifications). Mycorrhizae have silvery yellowish colour and a smooth hydrophobic surface (no cystids). Rhizomorphs are of the same colour as the root tips and ramify frequently. Emanating hyphae are rare. This morphotype has been identified as *Xerocomus* sp. The morphological appearance of BB06 and BB28 is very similar. When morphotypes were differentiated for preselecting prior sequencing in part III and part IV, morphotypes with pure silvery appearance were collected as BB06 and morphotypes with a yellowish silvery appearance were collected as BB28 or BB08 (BB08 even more yellowish than BB28).

b) Hyphal mantle is plectenchymatic in all layers. In the outer mantle layer, hyphae are arranged in bundles forming rings (mantle type A). The surface is smooth or covered with soil particles. Many hyphae are ampullate at septa but also inflation in the middle of the cells and septa are observed (\varnothing 3-4 μm , wall thickness 0.5-1 μm). Both in the middle and the inner mantle layer hyphae are arranged in ring-like structures. In the middle layer the cells are broader than in the inner layer (\varnothing 5-9 μm middle layer, (2)3-4(8) μm inner layer, cell wall thickness 0.5-1 μm for both layers). Cell walls in all layers are colourless to slightly membranously yellowish.

c) Rhizomorphs are highly differentiated: broad hyphae with partially dissolved septa form a core (rhizomorph type F). Nodia, internodia and conical side branches are observed. Central hyphae are 3-5 μm wide and have 0.5-1 μm walls. Peripheral hyphae have no special shape and a smooth surface that can be covered by soil particles (\varnothing 2-3 μm). Cell walls of the rhizomorphs are colourless to slightly membranously yellowish.

BB38; *Lactarius sp.*; beech; Fig. 2a, b, Fig. 7c

a) Mycorrhizae form monopodial systems with first order, occasionally second order ramifications. Their appearance is pale yellowish with a smooth surface, emanating elements are lacking. The whitish net of laticifers indicates that this morphotype belongs to the fungal genus *Lactarius*.

b) The outer mantle layer is pseudoparenchymatic. Its angular cells are arranged without special pattern (wall thickness 0.5-1(1.5) μm) but irregular and epidermoid cells occur. The outer layer is overlaid by a plectenchymatic hyphal net (mantle type P). Cells of the hyphal net (\varnothing (2)3-5 μm) form ring- and star-like structures. The surface of the outer mantle cells are occasionally covered by soil particles. Cells of the pseudoparenchymatic middle mantle layer are epidermoid and have a smooth surface (wall thickness 0.5-1 μm). Hyphae of the plectenchymatic inner mantle are arranged without special pattern (wall thickness 0.5-1 μm). Laticifers were observed in the middle and inner mantle. They ramify frequently. They are mostly straight but some are worm-like (\varnothing (2.5)3-5(6) μm , wall thickness 0.5 μm). Hyphen throughout the mantle are colourless to slightly membranously yellowish.

c) Emanating elements are not observed.

BB45 *Lactarius sp.*; beech; Fig. 2c, d, Fig. 7d

a) The analyzed mycorrhizal system is relatively large: 8 mm long and 0.7 mm diameter of the main axis. The mycorrhiza is brown to dark brown with a smooth semitransparent surface. After scratching a whitish to greyish latex is exuded. The system is monopodial-pyramidally ramified with first order ramifications only. The ends are straight or slightly bent (1.0-2.1 mm long, 0.5 mm wide). Emanating elements are not observed. Due to its conspicuous laticifers this morphotype is identified as *Lactarius sp.*

b) The outer mantle layer is pseudoparenchymatic. It contains angular colourless cells (14-19 μm long and 7-17 μm wide) that have smooth surface (mantle type L). Cells of the middle

mantle layer are similar, but they are more roundish and slightly membranously yellowish. The inner mantle is plectenchymatic without special patterns. Hyphae have a smooth surface and are slightly membranously brownish (\varnothing 3.5-5 μm , longer than 30 μm). Laticifers are found in the inner mantle layer directly adjacent to cortical cells of the root. They have a distinctly larger diameter than the mantle cells (up to 10 μm) and are 55-65 μm long. Laticifers are straight, have septa and ramify occasionally.

c) Emanating elements are not observed.

BB53 *Russula puellaris* Fr.; Scots pine, Fig. 2 e; Fig. 7e, f

a) Mycorrhizae are dichotomously ramified, with first order ramifications. They appear orange to brown, have a smooth surface and the mantle is not transparent. Ends are 0.8-1 mm long and 0.5 mm thick. Rhizomorphs are not observed. This morphotype was classified as *Russula sp.*

b) The outer mantle is pseudoparenchymatic (mantle type P or L). Angular cells (7-12 μm long and 6-10 μm wide, wall thickness 0.5-1 μm) have a smooth surface and are colourless to slightly membranously yellowish. Although hyphae occur at the surface of the outer mantle it is not clear, whether they form a true hyphal net or are simply emanating hyphae. The middle mantle layer is between pseudoparenchymatic and plectenchymatic. The main difference to the outer mantle is that the length/width ratio is larger. The inner mantle is plectenchymatic with streaks of parallel hyphae. Cell colour in the middle and inner mantle is the same as in the outer mantle.

c) Emanating hyphae are present but rare (\varnothing 2.5 μm , wall thickness 0.5 μm). Hyphae are mostly straight and simple, only one ramification was found at the observed specimen. Cells have a smooth surface, are of membranously yellowish colour and do not carry clamps.

BB57 *Tuber puberulum* Berk. & Broom; beech; Fig. 2f, g, Fig. 8a, b

a) This mycorrhizal type is a larger system (3.6 mm long, diameter of main branch 0.2 mm). It is monopodial-pinnately ramified with second order ramification. The ends are relatively thin and straight or slightly sinuously bent. Besides the ramified system also solitary tips are present (\varnothing 0.2 mm, length 0.5-0.8 mm). The mycorrhizae are of brown colour with dark brown spots. The surface is shiny and not transparent. A prominent surface character is the presence of numerous, relatively long cystidia that give the mycorrhizae a densely long-spiny appearance at larger magnification under the dissecting microscope.

b) The outer mantle layer is pseudoparenchymatic and consists of epidermoid cells (mantle type M). Cells are sometimes elongated so that a transition to mantle type H can be observed (plectenchymatic with inflated hyphae). The irregularly shaped hyphae (10-30 μm long, 6-15 μm wide, cell wall thickness 1.5 μm) are colourless to slightly membranously yellowish. Their cell walls are gelatinized but smooth. Cells of the middle and inner mantle display the same characters. The only difference is found in the inner layer whose cells are truly plectenchymatic. At the transition to root cortex cells finger-like protrusions are observed.

c) Cystidia are very long (105 μm , \varnothing 4-7 μm , cell wall thickness 1 μm), but differ from emanating hyphae by lack of ramification. They are colourless and have occasionally a colourless finely granular content. At the basis of each cystidium a foot cell is found, that is followed by a second septum (17-35 μm above the foot cell) so that each cystidium possesses two basal cells.

BB70; beech; Fig. 2h, Fig. 8c, d

a) Mycorrhizae are yellowish light brown to orange brown. They occur as solitary straight tips; but occasional ramifications are also observed. Their surface is smooth with very few emanating hyphae. The hyphal mantle is opaque to semitransparent so that cortex (tannin) cells are visible as dark spots.

b) The outer mantle layer is pseudoparenchymatic and consists of angular to roundish cells (wall thickness 0.5-1(1.5) μm). Heaps of flat cells with slightly thicker cell walls ((0.5)1.5-2 μm) are located on the mantle surface (mantle type O). The mantle type and colour indicate a resemblance to the mycorrhizal type BB14 *Russula ochroleuca*. The only prominent differences are that the surface cell heaps consist of triangular cells arranged in rosettes and that the mantle cells are more roundish. This is even more true for the pseudoparenchymatic middle mantle that consists of roundish and polygonal cells (wall thickness 0.5-1(1.5) μm). The inner mantle is plectenchymatic without special arrangement of the hyphae. With the exception of the cell heaps, all mantle cells are colourless to slightly membranously yellowish with a smooth surface.

c) Because of the unclear nature of the few emanating hyphae (e.g. parasitizing hyphae of another species) they are not separately described.

BB71; Scots pine; Fig. 2i, j, Fig. 8e

a) Mycorrhizae occur as solitary tips with a low number of emanating hyphae. Hyphal mantle is very thin and transparent and has light brown to brown colour.

c) The mantle is consistently plectenchymatic. Because it is very thin only an outer and an inner layer are differentiated. Hyphae of the outer mantle form a net of irregularly shaped hyphae (mantle type H, cell wall thickness 0.5 μm). They have a smooth surface and are frequently constricted at septa. The inner mantle consists of elongated cells that are arranged without special pattern, but also broad streaks of parallel hyphae are observed. Cells in both mantle layers are colourless to slightly membranously brownish.

c) Emanating hyphae are colourless to slightly membranously brownish. They are straight and sometimes tortuously bent. Their tips are flat and have thickenings at the very end so that they appear to be broken at septa. Septa in the middle of the hyphae carry clamps with a hole. At dorsal view the clamps are cylindrical and as broad as the hyphae. At lateral view clamps are lower than the hyphal diameter and have the shape of a semicircle. Hyphae are constricted at the contact point with the clamp. Secondary septa (without clamps) are frequent and sometimes thicker than the adjacent cell walls.

BB72 *Russula ionochlora* Romagn., *R. vesca* Fr.; beech; Fig. 2k, l, m, Fig. 8f, Fig. 9a

a) Mycorrhizae are yellowish brown with a silvery shine. The systems are monopodial-pinnately ramified with first order, occasionally second order ramifications. Besides ramified systems also solitary tips are observed. The surface is densely short-spiny due to the presence of numerous cystidia. Further emanating elements like rhizomorphs or emanating hyphae are not observed.

b) Hyphal mantle is plectenchymatic throughout all mantle layers. The outer mantle carries a net of unspecialized hyphae (cell wall thickness 1 µm) from which the cystidia emerge (mantle type D). The other cells of the outer mantle have thinner walls (< 0.5 µm), form an undifferentiated hyphal system and are covered with soil particles. Hyphae of the middle and the inner mantle have a smooth surface (cell wall thickness < 0.5 µm) and are arranged without special pattern. Cell walls of all mantle cells are colourless to slightly membranously yellowish.

c) Two types of cystidia are observed: 1) bristle-like cystidia (cystidia type A) and 2) flask-shaped cystidia with an apical knob (cystidia type D). The diameter of type A cystidia (length 40-80 µm) changes from 2-2.5 µm at the basis to 1 µm at the tapering end (wall thickness 0.5 µm). Type A cystidia emerge from a foot cell. In some cases two cystidia emerge from the same foot cell. This arrangement corresponds to cystidia type E. At the tip of flask shaped cystidia (cystidia type D) a knob can be found. It is located symmetrically in the middle of apex and its cell wall is as thick as the cell wall of the main body of the cystidium (0.5 µm). The surfaces of both cystidia types are smooth, although bristle-like cystidia are sometimes covered by soil particles. Cell walls of both cystidia types are colourless to slightly membranously yellowish.

According to Beenken (2004), *Russula vesca*, has very similar anatomical features, especially the two types of cystidia. Root tips from the mycorrhizal system used for the ectomycorrhizal description could not be sequenced. Fig. 8f and Fig. 9a show two different root tips that were collected as morphotype BB72. Both could be successfully sequenced. One was determined to be *R. ionochlora* (Fig. 8f) the other was determined to be *R. vesca* (Fig. 9a).

BB75=BB37; *Laccaria cf. laccata*; beech; Fig. 2n, o, Fig. 9b

a) Mycorrhizae occur as monopodial-pinnately ramified systems with first order ramifications but solitary tips are also observed. The ends are straight or bent and slightly beaded. The colour is ochre to pale yellowish with a silvery shine. Emanating hyphae give the surface a very loosely woolly appearance.

b) The outer hyphal mantle is plectenchymatic and consists of a network of coarse, irregularly shaped hyphae. This mantle is overlaid by a net of elongated hyphae (mantle type H). Hyphae of the net are 3-4 µm wide, 6 µm at ramifications. The remaining hyphae of the outer mantle (ø 3-5 (5.5) µm, wall thickness 0.5-1 µm) ramify at angles of 120° or 45° and are constricted at septa. Surface of the cells is smooth but sometimes covered with soil particles. Apart from the hyphal net, the outer mantle is two cell layers thick. The middle mantle layer is very

similar and consists only of a single cell layer. Hyphae of the plectenchymatic inner mantle are arranged without special pattern.

c) Emanating hyphae (\varnothing 2-3 μm , wall thickness 0.5-1 μm) possess clamps and ramify Y-shaped or at an angle of 90° . Ramifications start in a distance of 1-3 hyphal diameters from a septum. Clamps have a smooth surface. At the dorsal view they are cylindrical and as broad as the hyphae. At lateral view clamps are smaller than a semi-circle and lower than the corresponding hyphae. Some clamps show holes. Many emanating hyphae are straight without a conspicuous shape, but some hyphae are tortuous or have elbow-like protrusions. Secondary septa (without clamps) are rare and do not show a specific distribution. Surface of the secondary hyphae is smooth but sometimes covered with soil particles. They are colourless to slightly membranously yellowish.

BB79 *Lactarius rufus* (Scop.: Fr.) Fr. Scots pine; Fig. 3a, b, Fig. 9c, d

a) Mycorrhizae are dichotomously ramified with first order ramifications. Solitary tips are also observed. The mycorrhizae are dark orange or orange-brown with a smooth surface. The hyphal mantle is semitransparent, i.e. the colour of the root surface (reddish brown) is recognizable at older tips. Unramified ends are straight and often constricted between older and younger parts. Presence of laticifers identifies this morphotype as *Lactarius sp.*

b) The outer and middle mantle layers are pseudoparenchymatic and consist of epidermoid cells (mantle type M). Transition between outer mantle cells (2.5-8 μm x 13-22 μm , wall thickness (0.5)1-1.5(2) μm) and middle layer cells (2.5-11 μm x 11-19 μm , wall thickness 0.5-1 μm) is continuous with more compact cells in the middle layer. The inner mantle layer is plectenchymatic (diameter of hyphae 2.5-4 μm , wall thickness 0.5-1 μm). All mantle layers contain a gelatinous matrix and cell walls are colourless to membranously yellowish. Most laticifers are found in the inner mantle layer, but they are also present in the outer and middle mantle and even on the mantle surface (two observations). Laticifers are straight (\varnothing 2.5-4 μm , cell wall thickness 0.5 μm), sometimes tortuously bent, and ramify frequently. Parts of laticifers have colourless to slightly yellowish content. Septa are present.

c) Emanating elements are not observed.

BB80 *Cortinarius* (subgenus *Telamonia*) *sp.* 2; Scots pine; Fig. 3c, d, e, Fig. 9e, f

a) Mycorrhizae occur as coralloid systems. In some cases they are dichotomously ramified with first to second order ramifications. Apical tips are cylindrical, club-shaped or inflated. Surface is loosely woolly and appears silvery, whitish or yellowish with darker areas. Emanating hyphae and hairy rhizomorphs are frequent. Rhizomorphs protrude from the mantle at distinct points. They ramify frequently and they are colourless to yellowish.

b) Hyphal mantle is plectenchymatic in all mantle layers. Hyphae of the outer mantle do not present a special pattern. Because it is difficult to decide whether the matrix is gelatinous or not both mantle types B or C are possible. Hyphal cells (\varnothing 3-6(9) μm , wall thickness 0.5 μm) are constricted at septa and frequently more or less inflated. Strictly cylindrical cells are rare.

Crystals (3-6 μm x 2-5 μm) are found on the mantle surface, either directly on the hyphae or in the matrix. Surface of the hyphae is rough, either from very small crystals, soil particle or granulated pigments. Hyphae ramify Y-shaped, but also angle of 45° and 90° were observed. Hyphae of the middle mantle layer (\varnothing 3-5(12) μm , wall thickness 0.5 μm) and hyphae of the inner mantle layer (\varnothing (2)3-4(5) μm , wall thickness 0.5-1 μm) are arranged without a special pattern. In some parts of the middle mantle layer ring-like structures can be observed. Mantle hyphae are membranously yellowish. They became brownish with sulpho-vanillin. Matrix stained pinkish.

c) Rhizomorphs are highly differentiated: broad central hyphae with partially dissolved septa form a vessel-like core (rhizomorph type F). Central hyphae (\varnothing 4 μm , wall thickness 0.5 μm) ramify occasionally. Surface of peripheral hyphae (\varnothing 3 μm , wall thickness 0.5 μm) is smooth or rough, either by very small crystals, soil particles or granulated pigments. Besides being rough, peripheral hyphae carry angular or irregularly shaped crystals (3-4 μm x 2-2.5 μm). Inside hyphae pigment droplets are observed. Emanating hyphae (\varnothing <2-4(5.5) μm) have no special shape with some hyphae being irregularly inflated. Their ends are simple or slightly inflated, with the same cell wall thickness as the remaining hyphae. They ramify Y-shaped or at angles of 90°. Lateral branches are thinner than or as broad as main branches. Ramifications occur at larger distances from septa, adjacent to septa or one to two hyphal diameters below the septum. Septa of emanating hyphae carry clamps. At dorsal view, clamps are cylindrical and of small diameter than the hyphae. At lateral view, clamps are smaller than a semicircle and thinner than the corresponding hyphae. Holes were observed. Surface of emanating hyphae is smooth or rough, either from very small crystals, soil particles or granulated pigments. Larger, irregularly shaped or rhomboid crystals (\varnothing 2-5(8) μm x 1.5-4(7) μm) were attached to the surface. Open anastomoses with short bridges are observed in rhizomorphs and emanating hyphae. The bridge has the same diameter as the other hyphae and a smooth surface. Cell wall thickness of anastomoses is the same as in the remaining rhizomorph. Emanating hyphae and hyphae of the rhizomorphs are membranously yellowish and stain brownish with sulpho-vanillin

BB81 *Hydnotriza tulasnei* (Berk.) Berk. & Broome; beech; Fig. 3f, Fig. 10a

a) Mycorrhizae are monopodial-pinnately ramified systems with first order ramifications. The unramified ends are tortuously bent or straight. They have a smooth surface with shiny yellowish brown colour and spots of darker colour. Because of the transparent hyphal mantle the spots are probably underlying tannin cells of the root cortex. Emanating elements are not observed.

b) The hyphal mantle is very thin, so that only inner and outer mantles are differentiated. The mantle consists of epidermoid cells in both layers (mantle type M) and contains a gelatinous matrix. Cell walls in the outer layer are somewhat larger than in the inner layer (9-18(26) μm x 12-29(39) μm and 3.5-10(22) μm x 17-20(35) μm). Cell wall thickness is 0.5 to 1.5 μm in both layers. Cell wall colour ranges from membranously yellowish to brownish.

The main differences to similar smooth mycorrhizas of *Lactarius* are the lack of laticifers and the epidermoid shape of the mantle cells.

BB82 *Genea hispidula* Berk. ex Tul.; beech; Fig. 3g, h, Fig. 10b, c

a) Mycorrhizae are monopodial-pinnately ramified with first order ramifications. Solitary tips are also observed. Mycorrhizae are dark brown and have a shiny granulated surface. Coarse emanating hyphae give the surface a very loosely woolly appearance.

b) The outer mantle is pseudoparenchymatic (mantle type O) and consists of relatively large angular cells (10-15(17) μm x 16-21(30) μm , wall thickness 1-2 μm) that are overlaid by heaps of flattened cells (\varnothing 7-12(15) μm). Although the middle mantle layer is also pseudoparenchymatic it contains smaller, roundish or polygonal cells (7-13 μm x 11-19 μm , wall thick 0.5-1 μm). The inner mantle is plectenchymatic. Besides elongated cells (\varnothing 4-6(12) μm , length 10-25 μm , wall thickness 0.5-1.5 μm) it contains nests of roundish cells (3.5-5 μm x 5-6.5(9) μm). Cell walls of mantle hyphae are membraneously brown to reddish brown.

c) Emanating hyphae (\varnothing 5-7(10) μm , wall thickness 1-1.5 μm) have a smooth surface and are membraneously brown to reddish brown. Both intrahyphal hyphae and two layered cell walls have been observed. Ramifications are rare, only one has been observed.

BB83 *Pezizaceae*; beech; Fig. 3i, j, Fig. 10d, e

a) Mycorrhizae are monopodial-pinnately ramified with first to second order ramifications. Both the main axis and the unramified ends are tortuously bent. The surface appears silvery with underlying pale ochre colour. At larger magnifications the surface is loosely woolly due to many emanating hyphae. The habit of the morphotype BB83 superficially resembles *Xerocomus spp.* but is clearly different by having a pseudoparenchymatic mantle.

b) As already mentioned the hyphal mantle is plectenchymatic. Because it is very thin only an outer and an inner layer are differentiated. It is not clear whether the angular cells of the outer layer (9-14(19) μm x 14-36 μm , wall thickness 0.5-1 μm) are overlaid by a hyphal net or whether the elongated hyphae on the mantle surface present collapsed emanating hyphae (mantle type L or O). The cells of the inner mantle are of similar shape but smaller (8-14 μm x 14-20 μm , wall thickness 0.5-1 μm) wall than in the outer mantle. Mantle hyphae are membraneously yellowish.

c) Emanating hyphae have a smooth surface or are occasionally covered with soil particles (\varnothing 3.5-5(8) μm , length 15-32 μm , wall thickness $<0.5\mu\text{m}$). Y-shaped ramifications (or rarely at 90°) occur at larger distance to a septum or 1-2 hyphal diameter below a septum. The lateral branch is wider than main branch. Hyphal cells are cylindrical or inflated (\varnothing 3.5-5(8) μm , length 15-32 μm , wall thickness $<0.5\mu\text{m}$), sometimes with elbow-like protrusions. Directly adjacent to thick septa, the longitudinal cell walls are thicker. The cell walls of hyphal ends are occasionally thickened. Anastomoses were observed. They are either open or closed by septum and have short bridges. Both diameter and wall thickness of the bridges correspond to the remaining hyphae. Emanating hyphae are colourless or slightly membraneously yellowish.

BB84; Scots pine; Fig. 3k; Fig. 10f

a) Mycorrhizae occur as solitary tips. Due to the smooth, transparent mantle they are reddish brown (colour of the root). The very thin mantle and the lack of further characters could be an indicator that this not an own morphotype but the beginning of root colonization by a morphotype with a thicker "aged" mantle.

b) Only two plectenchymatic layers are differentiated. Hyphae of the outer mantle are ring-like arranged (mantle type A) and ramify Y-shaped, or at angles of 90° or 120°. Cells are slightly ampullate (\varnothing 3.5-4 μm , 5-6 μm at inflations, wall thickness 0.5 μm), sometimes constricted at septa, and have a smooth surface. Hyphae of the inner mantle are also ring-like arranged (\varnothing 4-5 μm , wall thickness 0.5 μm). Mantle hyphae are colourless to slightly membranously yellowish.

c) Emanating elements are not observed.

BB85 *Hydnотrya tulasnei*; beech; Fig. 3l, Fig. 11a

a) Mycorrhizae occur as solitary tips. Beginning first order ramification has been observed. The colour is pale ochre and the surface is shiny and smooth. Due to the assumed presence of laticifers this morphotype was morphologically identified as *Lactarius sp.*

b) The outer mantle layer is pseudoparenchymatic and consists of epidermoid cells (2-6 (18) μm x 7-35 μm , wall thickness 0.5-1 μm) that carry a hyphal net of laticifers (mantle type Q). The shape of the hyphal net is unspecific. Cells of the middle mantle layer are also pseudoparenchymatic but smaller (3-6 μm x 8-29 μm , wall thickness < 0.5 μm). The inner mantle is plectenchymatic (\varnothing 2-5 μm , length 6-31 μm , wall thickness < 0.5 μm) but also contains nests of pseudoparenchymatic cells. Besides from the hyphal net, laticifers (\varnothing 2-5 μm cell wall thickness < 0.5 μm) are observed in all mantle layers. Mantle cells are membranously yellowish.

c) Emanating elements are not observed.

This mycorrhiza is similar to BB81 (*Hydnотrya tulasnei*) but has been described as separate morphotype because a hyphal net and laticifers have been observed. Because of the assumed presence of laticifers, BB85 was originally identified as *Lactarius sp.* Together with the ectomycorrhizal types BB81 and BB94 it is the only type with a smooth surface and epidermoid mantle cells. A root tip of the analyzed specimen of BB85 has been sequenced and is determined to be *Hydnотrya tulasnei*. Thus, BB81 and B85 belong to the same species. The epidermoid mantle and the morphological appearance correspond to the short description of *Hydnотrya tulasnei* ectomycorrhiza in (Tedersoo et al. 2006). It has to be concluded that elongated cells of the hyphal net (not observed for BB81) have been misinterpreted as laticifers.

BB86; beech; Fig. 3m, n, o, Fig. 11b

a) Mycorrhizae occur as solitary tips or with a single ramification. They are light yellow and have a densely woolly surface due to many emanating hyphae. Two colourless rhizomorphs were observed. They were not directly connected to the mantle surface.

b) Hyphal mantle is plectenchymatic in all layers. The outer mantle consists of a net of coarse, irregularly shaped hyphae (mantle type H). Hyphae do not have clamps and ramify at angles of 90°. The cell shape is cylindrical with a smooth surface (\varnothing 4-5(10) μm , wall thickness 0.5-1 μm). Hyphae both of the middle mantle (\varnothing 3-5.5 μm , wall thickness 0.5-1 μm) and the inner mantle (\varnothing 2-3 μm , wall thickness 0.5 μm) are arranged without a special pattern. Occasionally ring-like structures are observed in the inner mantle. Mantle hyphae are colourless to slightly membraneously yellowish.

c) Emanating hyphae are smooth (\varnothing 3-3.5 μm , wall thickness <0.5-1 μm). Although they do not have a special shape, elbow-like protrusions have been observed. Anastomoses and ramifications have not been observed. Emanating hyphae are colourless to slightly membraneously yellowish. Rhizomorphs are undifferentiated. Margins are smooth and the hyphae compactly arranged (rhizomorph type B). Central, not vessel-like hyphae ramify at angles of 90° or below 45° (\varnothing 4.5-5 μm , wall thickness 1 μm). Two septa with an enlarged porus have been observed. Septa are not as thick as the longitudinal cell walls. The smooth peripheral hyphae have no conspicuous shape (\varnothing 4.5-5 μm , wall thickness 1-1.5 μm).

BB87; Scots pine; Fig. 4a, b, Fig. 11c

a) Mycorrhizae occur as solitary root tips and are yellowish brown. Due to numerous emanating hyphae the surface appears densely woolly. The hyphal mantle is transparent (root cortex cells are visible).

b) Hyphal mantle is complete, but very thin (2-3 cell layers) so that only two plectenchymatic mantle layers are differentiated. Hyphae of the outer mantle are arranged without a special pattern (mantle type B). They have a smooth surface and ramify at angles of 90°. Cells are cylindrical or slightly inflated in the middle (\varnothing 3-4 (7) μm , wall thickness <0.5 μm). As in the outer mantle, hyphae of the inner mantle are arranged without a special pattern. Besides the cylindrical cells, also inflated and irregularly shaped cells occur (\varnothing (2)3-5(7) μm). Clamps have been observed and mantle hyphae are colourless to slightly membraneously yellowish.

c) The most obvious character of the emanating hyphae (\varnothing 2.5-3 μm , wall thickness 0.5 μm) is their warty surface. Larger warts are spherical (\varnothing <0.5- <1 μm), smaller warts have a smaller curvature. Cells have no special shape. They are cylindrical and are occasionally constricted at septa (\varnothing 2.5-3 μm , wall thickness 0.5 μm). The cell wall consists of single layer of constant thickness and they are colourless to slightly membraneously yellowish. Emanating hyphae ramify occasionally at angles of 90°. Ramification points are 1-3 hyphal diameters below a septum. Main and lateral branch have the same diameter. Clamps of the emanating hyphae have a smooth surface. At dorsal view, they are oval and have the same diameter as the hyphae. At lateral view, clamps have the shape of semicircle that is as high as the hyphae.

Some clamps have a hole. Secondary septa are observed and are partially crowded (not evenly distributed). They can be thicker than the longitudinal cell walls. Besides clamps, anastomoses were observed. Their short bridge has a smooth surface and is closed by a septum with a clamp.

The described emanating hyphae, especially the warty surface are typical for *Amphinema byssoides*. At two of three dissected root tips a second, untypical, type of emanating hyphae was observed. The main difference is their smooth surface and that they ramify rarely (90° or below). Furthermore, the cell wall thickness (0.5-1 µm) is not constant and two-layered cell walls are observed. Cell walls are inflated between two septa (ø 3.5 - 5 µm). Secondary septa are thinner than the longitudinal cell walls but have sometimes circular thickenings.

BB88; *Tomentella sublilacina* (Ell. & Holw.) Wakef., Scots pine, Fig. 4c, Fig. 11d, e, f

a) Mycorrhizae are dichotomously or monopodial-pyramidally ramified and have first order ramifications. Unramified ends are straight or slightly tortuously bent. Hyphal mantle is transparent and thin so that the reddish brown colour is caused by the underlying root surface. Mantle is partially hydrophobic, that gives the mantle a slightly silvery appearance. Emanating hyphae are present.

b) The mantle is plectenchymatic in all layers. Hyphae of the outer mantle are irregularly arranged without a special pattern (mantle type B). Cells are occasionally slightly inflated (ø 2.5-5 µm, wall thickness 0.5 µm). Cells of the middle and inner mantle are similar to the outer mantle. In all layers hyphae are colourless to slightly membranously yellowish.

c) Emanating hyphae (ø 1-3 µm, wall thickness 0.5 - 1 µm) are slightly membranously yellowish and have a smooth surface. They are straight but occasionally ampullately thickened at septa. Ramifications occur distant from septa. Clamps have been observed. At dorsal view, they are cylindrical and have the same diameter as the corresponding hyphae. At lateral view, clamps appear as semicircles. Rhizomorphs have not been observed.

BB89 *Cortinarius* (subgenus *Telamonia*) sp.2; Scots pine, Fig. 4d, e, Fig. 12a

a) The most distinct feature of this mycorrhiza is the dense maze of rhizomorphs and emanating hyphae which makes it difficult to determine the type of ramification but also allows to classify the morphotype as *Cortinarius*.. As far it is recognizable, mycorrhizae are irregularly pinnately (first order ramification) or dichotomously (second to third order ramifications) ramified. Unramified ends are straight to slightly tortuously bent and 0.4-0.6 mm in diameter. They are of light yellow to ochre colour. The dense network of emanating hyphae results in a hydrophobic, silvery shining surface. Rhizomorphs ramify frequently and have smooth margins (not fan-like).

b) All mantle layers are plectenchymatic. Hyphae of the outer mantle are irregularly arranged with no conspicuous pattern (mantle type B). Some hyphae form ring-like structures or are ramified (angles between 90° and 120°). Hyphae are colourless to slightly membranously

yellowish (\varnothing 4-7 μm , length 15 to 50 μm , wall thickness 1 μm). Middle and inner mantle have the same characteristics as the outer mantle.

c) Emanating hyphae (\varnothing 3.5-4 μm , length 60-110 μm , wall thickness 0.5 μm) ramify Y-shaped or at angles lower than 90°. They have clamps and anastomoses, a smooth surface and are slightly constricted at septa. Rhizomorphs have smooth margins and lack vessel-like central hyphae (rhizomorph type B). Both central and peripheral hyphae (\varnothing 4.5-5.5 μm , length 100 μm , wall thickness 0.5-1 μm) have a smooth surface. Both emanating hyphae and hyphae of the rhizomorphs are colourless to slightly membranously yellowish.

BB91 *Russula puellaris* Fr.; beech; Fig. 4f, g, Fig. 12b

a) Mycorrhizae occur as solitary tips of light brown colour. Tips are straight, 0.7-1.4 mm long and 0.3 mm thick. The surface is smooth but due to emanating hyphae very loosely woolly. Among the Scots pine morphotypes it is similar to BB53, among the beech morphotypes it resembles BB72, but lacks the cystidia.

b) The outer mantle is pseudoparenchymatic and contains angular to epidermoid cells that are slightly elongated (mantle type L to M). Cells have a smooth surface and are membranously yellowish (7-9 μm x 9-17 μm , wall thickness 0.5 μm). The middle mantle is also pseudoparenchymatic but contains more elongated cells (transition to mantle type H). The inner mantle layer is plectenchymatic and consists of elongated cells (\varnothing 3 μm , length 18-30 μm).

c) Emanating hyphae (\varnothing 2.5 μm , cell walls 0.5 μm) are straight and do not ramify. They have septa but no clamps and are colourless to slightly membranously yellowish. Hyphal ends are occasionally slightly inflated.

Mycorrhizal type BB53 and BB91 are very similar. Both have a smooth pseudoparenchymatic mantle with a few emanating hyphae. The main difference is that BB91 has more irregular cells in the outer mantle, which appears to be epidermoid. Furthermore, the analyzed specimen of BB53 was collected from Scots pine, whereas the analyzed specimen of BB91 was collected from beech roots. The analyzed specimens of BB53 and BB91 have been identified by sequencing as *Russula puellaris*.

BB92 *Laccaria cf. laccata*; beech; Fig. 12c

a) Mycorrhizae are monopodial-pyramidally ramified with first order ramifications or they occur as solitary tips. The studied system is 3 mm long. Its unramified tips are straight or slightly tortuously bent and 0.5-0.8 mm long and 0.3 mm thick. Surface of mycorrhizae is smooth and the mantle not transparent (no cortex cells) visible. Mantle colour is bright grey.

b) Mantle is plectenchymatic in all layers.

c) Because of their rareness, emanating hyphae are not separately described.

This morphotype resembles the morphotype BB75 that was sequenced to be *Laccaria cf. laccata*. The main differences are the brighter colour and the almost complete lack of

emanating hyphae. These differences are apparently due to the variability of *Laccaria* cf. *laccata* because BB92 was also sequenced to be *Laccaria* cf. *laccata*.

BB94; Scots pine; Fig. 4h, Fig. 12d

a) Mycorrhiza occurs as solitary, once dichotomously ramified tips. The mantle is light ochre and smooth but not transparent. Unramified ends are straight and 1 mm long and 0.5 mm thick. Emanating elements have not been observed.

b) The outer mantle is pseudoparenchymatic and consists of epidermoid cells (mantle type M). Cells are slightly elongated (4-8 μm x 4-5 μm , wall thickness 0.5 μm), have a smooth surface and are colourless to slightly membranously yellowish. Cells of the middle mantle have same characteristics. The inner mantle is plectenchymatic without a special pattern. Cells have diameter of 3 μm and are 11-22 μm long and colourless.

BB95, Scots pine; Fig. 4i, j, k, Fig. 12e, f

a) Mycorrhizae occur as solitary tips or are dichotomously ramified with first order ramifications. Unramified ends are straight (\emptyset 0.4 mm, length 1.3 mm). They are of light brown colour and have a reddish tip. The mantle is semi-transparent and emanating hyphae cause a very loosely woolly appearance at the base of the mycorrhiza.

b) The hyphal mantle is plectenchymatic in all layers. Hyphae of the outer mantle are net-like arranged (mantle type E). It is not clear whether the outermost hyphae form a true hyphal net or represent collapsed emanating hyphae. Cells have light brownish colour and a smooth surface (\emptyset 4-5 μm , length 25 μm , wall thickness 0.5 μm). They are ramified at angles of 120° or 90° (or below 90°). Some hyphae are slightly inflated in the middle between septa. Rarely, clamps have been observed. Middle and inner mantle layer are very similar to the outer mantle but cells are colourless. Additionally ring-like structures have been observed in the inner mantle layer.

c) Emanating hyphae are light brown and slightly bent (\emptyset 2.5 μm , length 100 μm , wall thickness 0.5 μm). They do not ramify. Clamps are present. At dorsal view they are cylindrical and of slightly smaller diameter than hyphae. At lateral view claps form semicircles that are slightly larger than the hyphal diameter

BB96 *Lactarius subdulcis* (Bull.: Fr.) Gray; beech; Fig. 4l, Fig. 13a, b

a) Mycorrhizae occur as monopodial-pyramidally ramified systems and have first order ramifications. This mycorrhizal type was frequent in the analyzed soil core. Unramified ends are straight (\emptyset 0.3-0.5 mm, length 0.5-1.3 mm). The mantle is light brown, smooth, semitransparent (root cortex visible at young, apical tips) and shiny in certain spots. Emanating elements are not observed.

b) The outer mantle is pseudoparenchymatic and consists of angular cells that are overlaid by a hyphal net (mantle type P). Cells of the hyphal net have a diameter of 3-5 μm and distinct

cell walls. Mantle cells have equal width and length but are of very different size (6-25 μm , the larger sizes are more frequent, cell wall thickness 0.5-1 μm). They have a smooth surface and are of membranously yellowish colour. Cells of the middle mantle are similar to the outer mantle layer. The inner mantle is plectenchymatic. Its hyphae are smooth and have a diameter of 3.5-5 μm .

c) Emanating elements are not present.

BB98; beech; Fig. 4m, o, Fig. 13c, d

a) Mycorrhizae occur as solitary tips or are monopodial-pinnately ramified with first order ramifications. Unramified ends are straight to slightly tortuously bent (\varnothing 0.3 mm, length 0.6-1.7 mm). Mantle surface is of light brown to light greyish colour. The mantle is not transparent and has a smooth surface. Emanating hyphae and white rhizomorphs are present.

b) The outer mantle layer is plectenchymatic. Hyphae are irregularly arranged with a net of coarse hyphae as outermost cell layer (between mantle type B and H). Net-like structures are probably emanating hyphae. Hyphae of the outer mantle have no special shape (\varnothing 4-5 μm , length at least 30 μm , wall thickness 0.5 μm). They are straight, but sometimes inflated in the middle. Hyphae are colourless to slightly membranously yellowish and have a smooth surface. They ramify at angles of 90°, sometimes 120°. The middle and the inner mantle layer are plectenchymatic with the same characteristics as the outer mantle.

c) Emanating hyphae (\varnothing 3 μm , length more than 100 μm , wall thickness 0.5 μm) have clamps and ramify Y-shaped or at angles of 90°. Ramifications are located one or two hyphal diameters below a septum. At dorsal view clamps are cylindrical and have the same diameter as hyphae. In lateral view they have the shape of a semicircle that is as large as the hyphal diameter. Anastomoses are observed between hyphae, in most cases they are open (no septum at the bridge). Emanating hyphae are colourless to slightly membranously yellowish and have a smooth surface. Rhizomorphs are round in cross section (\varnothing 30-65 μm). They ramify into smaller filaments and are indistinctly connected to the mantle, that is emanating hyphae unify at some distance from the mantle to form a rhizomorph. Central hyphae of the rhizomorphs are not vessel-like enlarged (rhizomorph type A or B). Both central and peripheral hyphae are ampullately enlarged at hyphal ends. Diameter at the inflations is 6 μm , at the remaining cell 2.5-3.5 μm (length 70-80 μm). Septa have clamps and sometimes globular thickenings.

BB100 *Laccaria laccata* cf.; beech; Fig. 4 o, Fig. 13e

a) Mycorrhizae occur as solitary tips or are monopodial-pinnately ramified. When they ramify they have first order ramifications. Unramified ends are straight (\varnothing 0.3 mm, length 0.7-2 mm). They are grey to ochre and are slightly violet coloured at the very apical end. Although cortex cells are not recognizable, the mantle appears to be semi-transparent. Surface is partially densely woolly from emanating hyphae.

b) The outer mantle is plectenchymatic and consists of irregularly arranged hyphae (mantle type H). Net-like structures on the mantle surface originate from collapsed emanating hyphae. Hyphae are slightly inflated (\varnothing 3.5-4 μm , length 10-20 μm , wall thickness 0.5 μm) and have a smooth surface. They are colourless or slightly membranously brownish. Middle mantle layer and inner mantle layer have the same characteristics as the outer mantle. In the inner mantle layer also ring-like structures are observed.

c) Emanating hyphae are numerous (\varnothing 3 μm , length 30-40 μm , wall thickness 1 μm) and evenly distributed along the root. They are straight or occasionally slightly tortuously bent and have straight surface. They can be constricted at septa or ampullate. Ramifications occur at angles below 90°. Septa have always clamps (i. e. no secondary septa). At dorsal view clamps are oval with a maximum width of the hyphal diameter. At lateral view clamps form a semicircle.

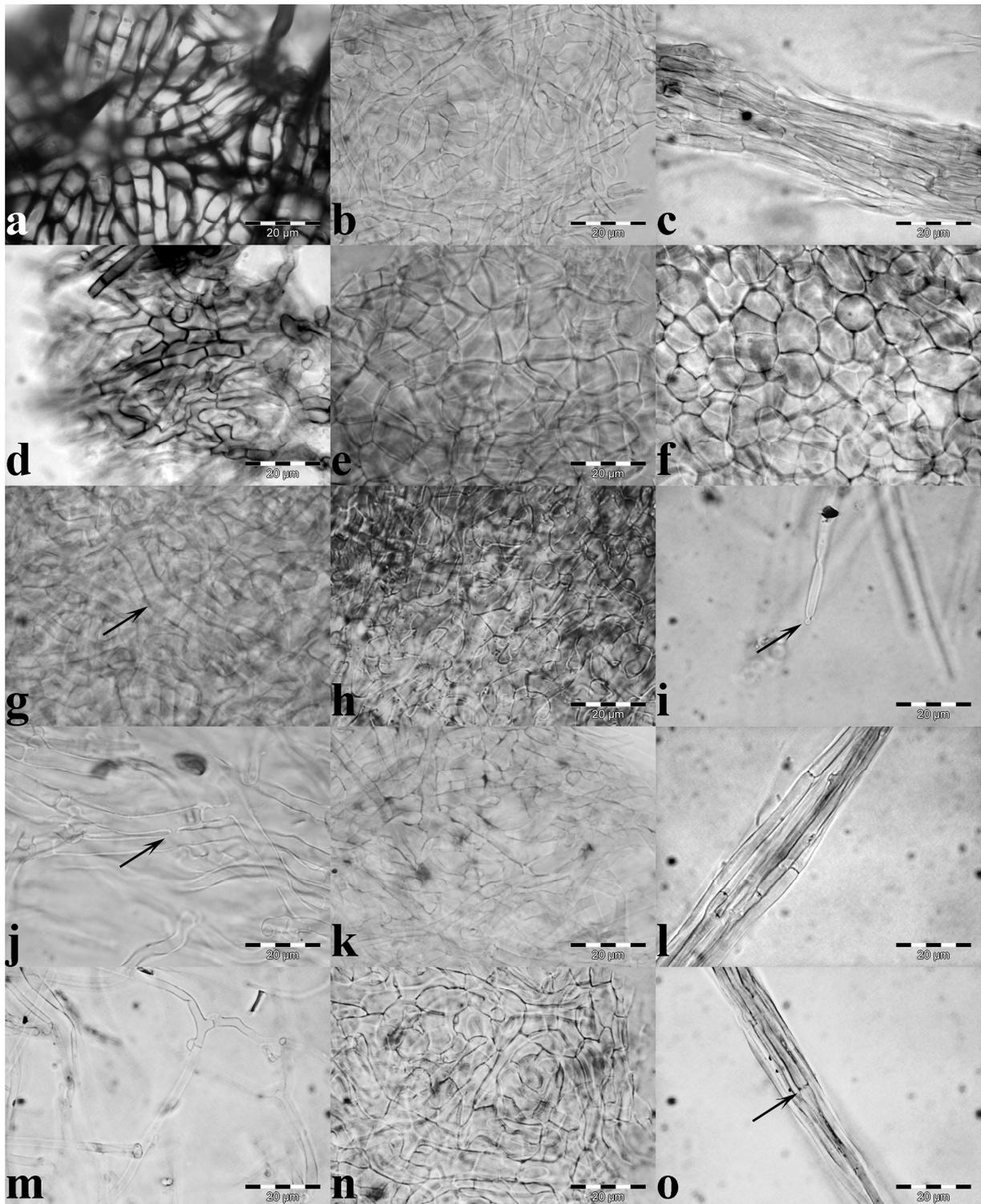


Fig. 1: Anatomotypes I, mantle preparations at 1000x magnification

- | | | |
|---------------------------------|----------------------------|----------------------------|
| a) BB05 outer mantle layer | b) BB06 outer mantle layer | c) BB06 rhizomorph |
| d) BB11 outer mantle layer | e) BB14 outer mantle layer | f) BB22 outer mantle layer |
| g) BB22 inner mantle, laticifer | h) BB23 outer mantle layer | i) BB23 beaded cystid |
| j) BB23 rhizomorph, anastomosis | k) BB24 outer mantle layer | l) BB24 rhizomorph |
| m) BB24 emanating hyphae | n) BB28=BB08 outer mantle | o) BB28=BB08 rhizomorph |

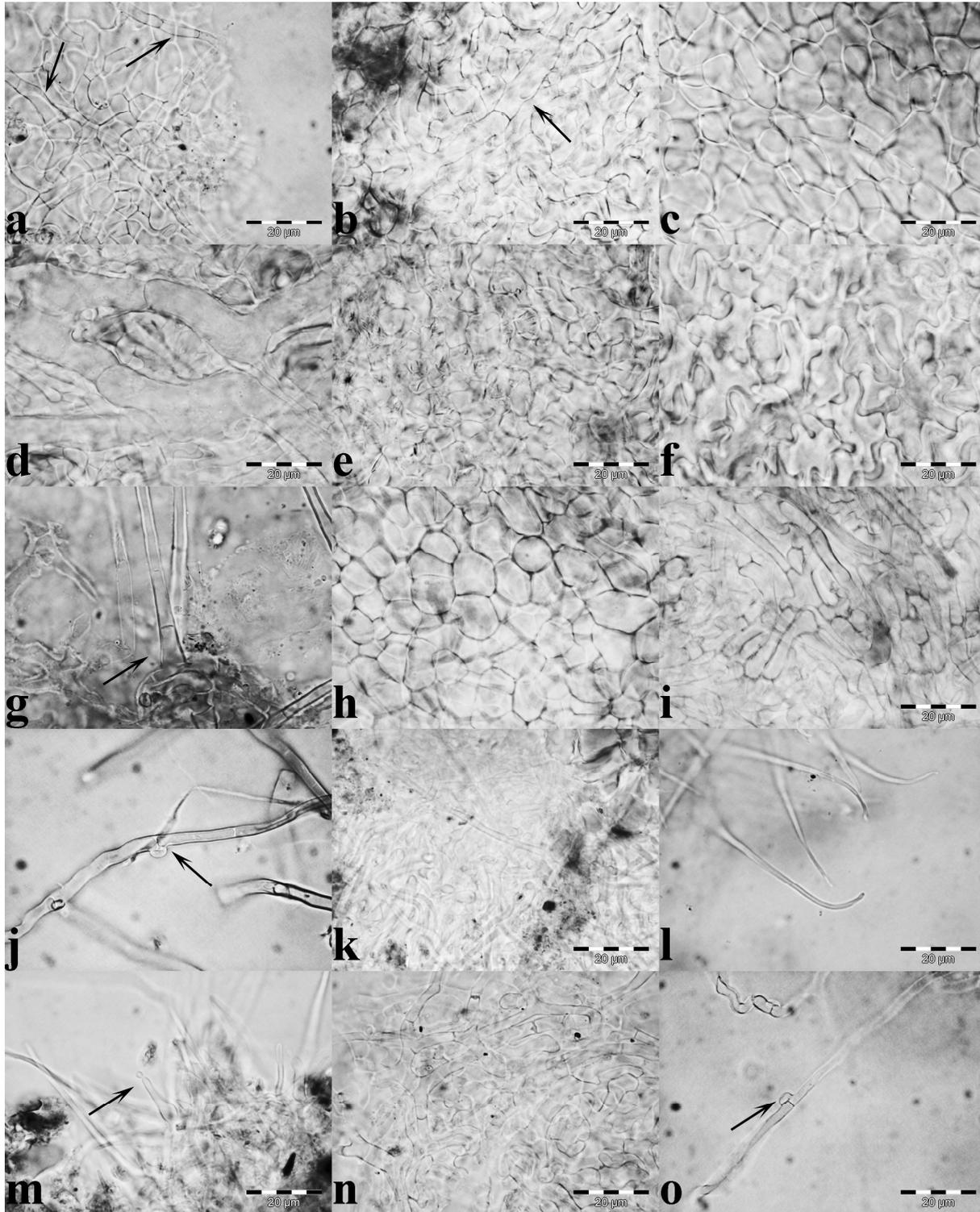


Fig. 2: Anatomotypes II, mantle preparations at 1000x magnification

- | | | |
|--|----------------------------------|----------------------------|
| a) BB38 outer mantle layer, net | b) BB38 middle mantle, laticifer | c) BB45 outer mantle layer |
| d) BB45 inner mantle layer, laticifer | e) BB53 outer mantle layer | f) BB57 outer mantle layer |
| g) BB57 cystidia, foot cell | h) BB70 outer mantle layer | i) BB71 outer mantle layer |
| j) BB71 emanating hypha, clamp w. hole | k) BB72 outer mantle, net | l) BB72 cystidium type A |
| m) BB72cystid type D | n) BB75=37 outer mantle, net | o) BB75=37 emanating hypha |

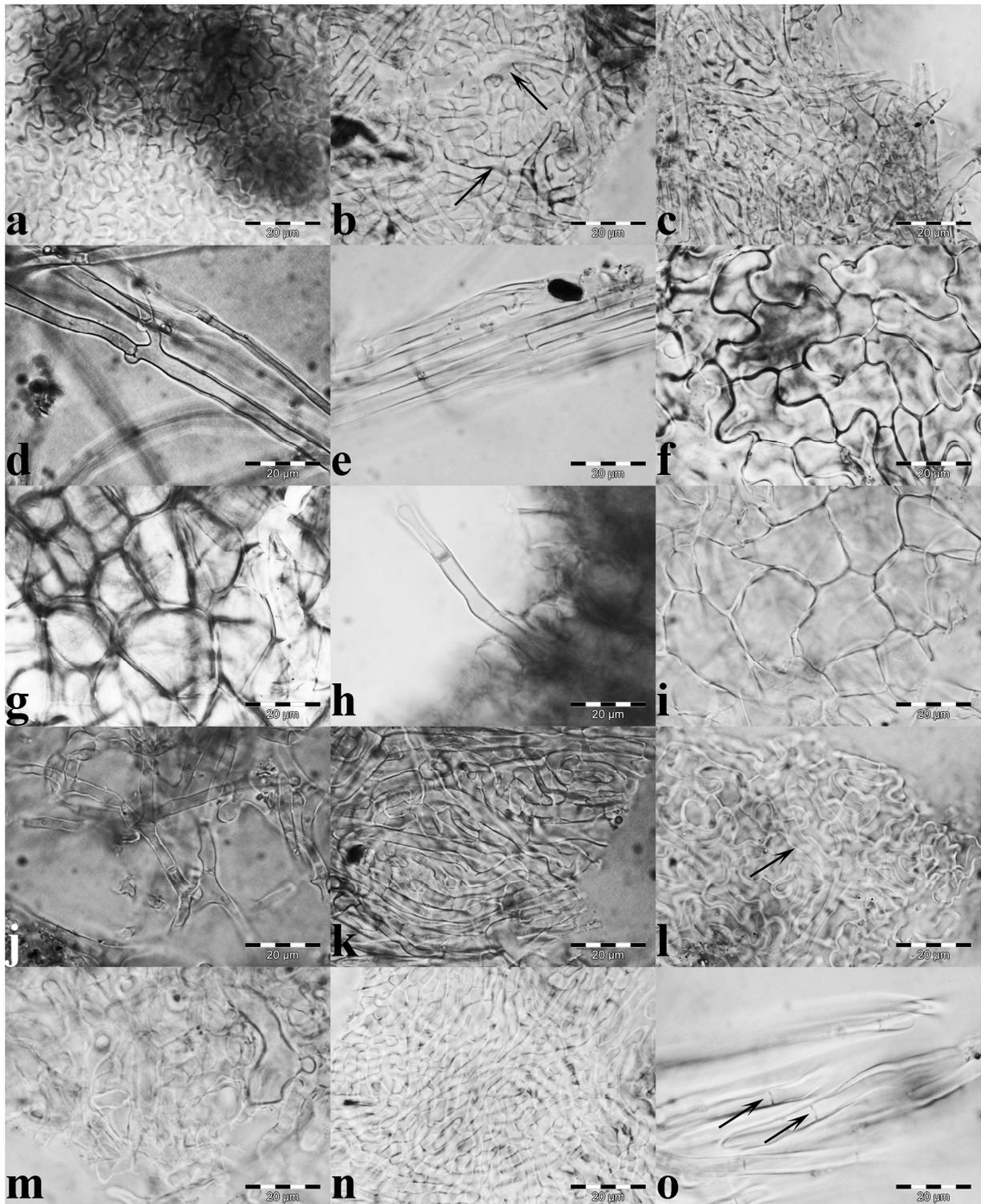


Fig. 3: Anatomotypes III, mantle preparations at 1000x magnification

- | | | |
|--|---------------------------------|---------------------------------|
| a) BB79 outer mantle layer | b) BB79 inner mantle, laticifer | c) BB80 outer mantle layer |
| d) BB80 emanating hyphae, ramification | e) BB80 rhizomorph | f) BB81 outer mantle layer |
| g) BB82 outer mantle layer | h) BB82 outer mantle layer | i) BB83 outer mantle layer |
| j) BB83 emanating hyphae | k) BB84 outer mantle layer | l) BB85 outer mantle, laticifer |
| m) BB86 outer mantle layer | n) BB86 inner mantle layer | o) BB86, rhizomorph, porus |

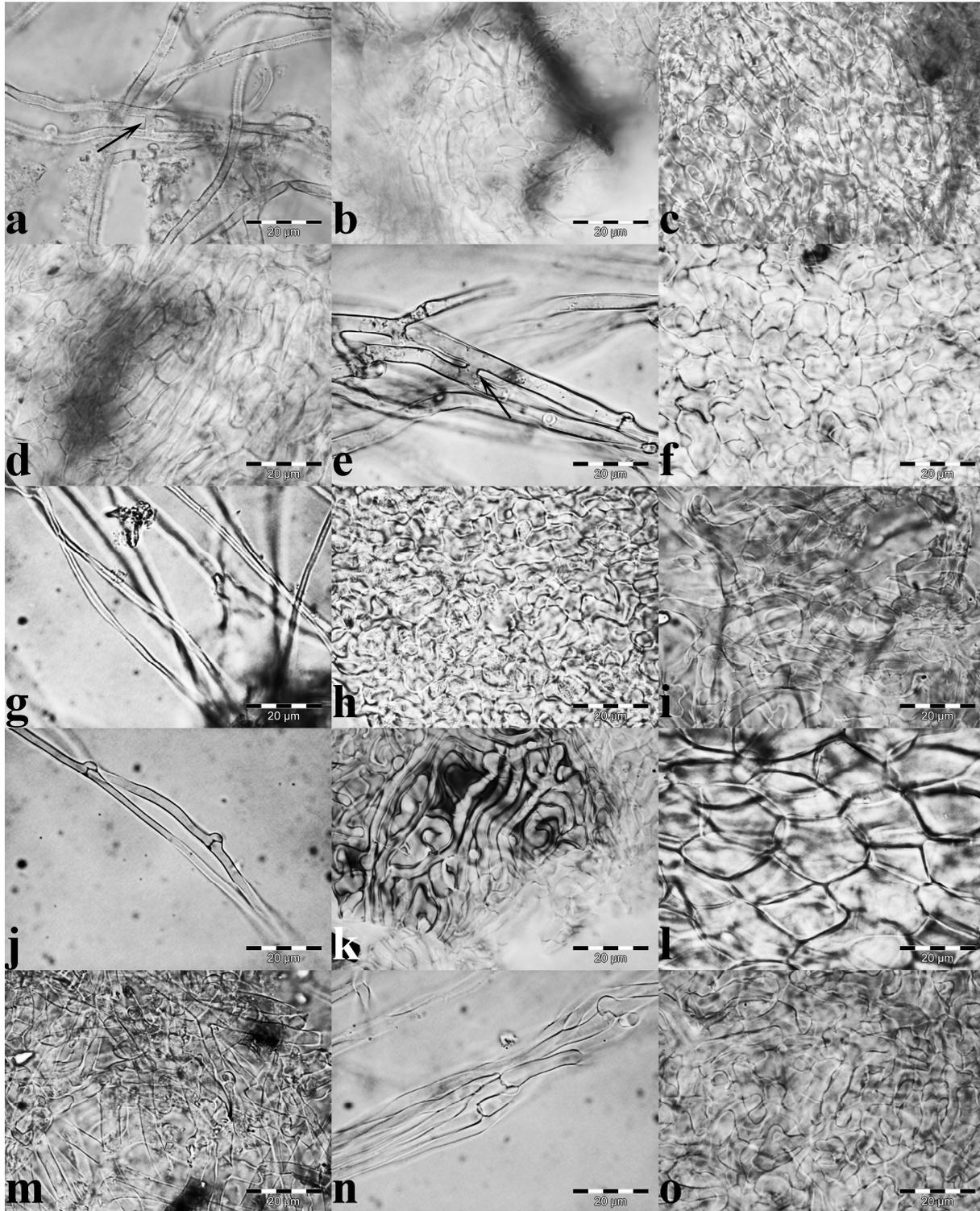


Fig. 4: Anatomotypes IV, mantle preparations at 1000x magnification

- | | | |
|---------------------------------------|---------------------------------|-----------------------------|
| a) BB87 emanating hyphae, anastomosis | b) BB87 outer mantle layer | c) BB88 outer mantle layer |
| d) BB89 outer mantle layer | e) BB89 rhizomorph, anastomosis | f) BB91 outer mantle layer |
| g) BB91 emanating hyphae | h) BB94 outer mantle layer | i) BB95 outer mantle layer |
| j) BB95 emanating hyphae with clamps | k) BB95 inner mantle layer | l) BB96 outer mantle layer |
| m) BB98 outer mantle layer | n) BB98 rhizomorph | o) BB100 outer mantle layer |

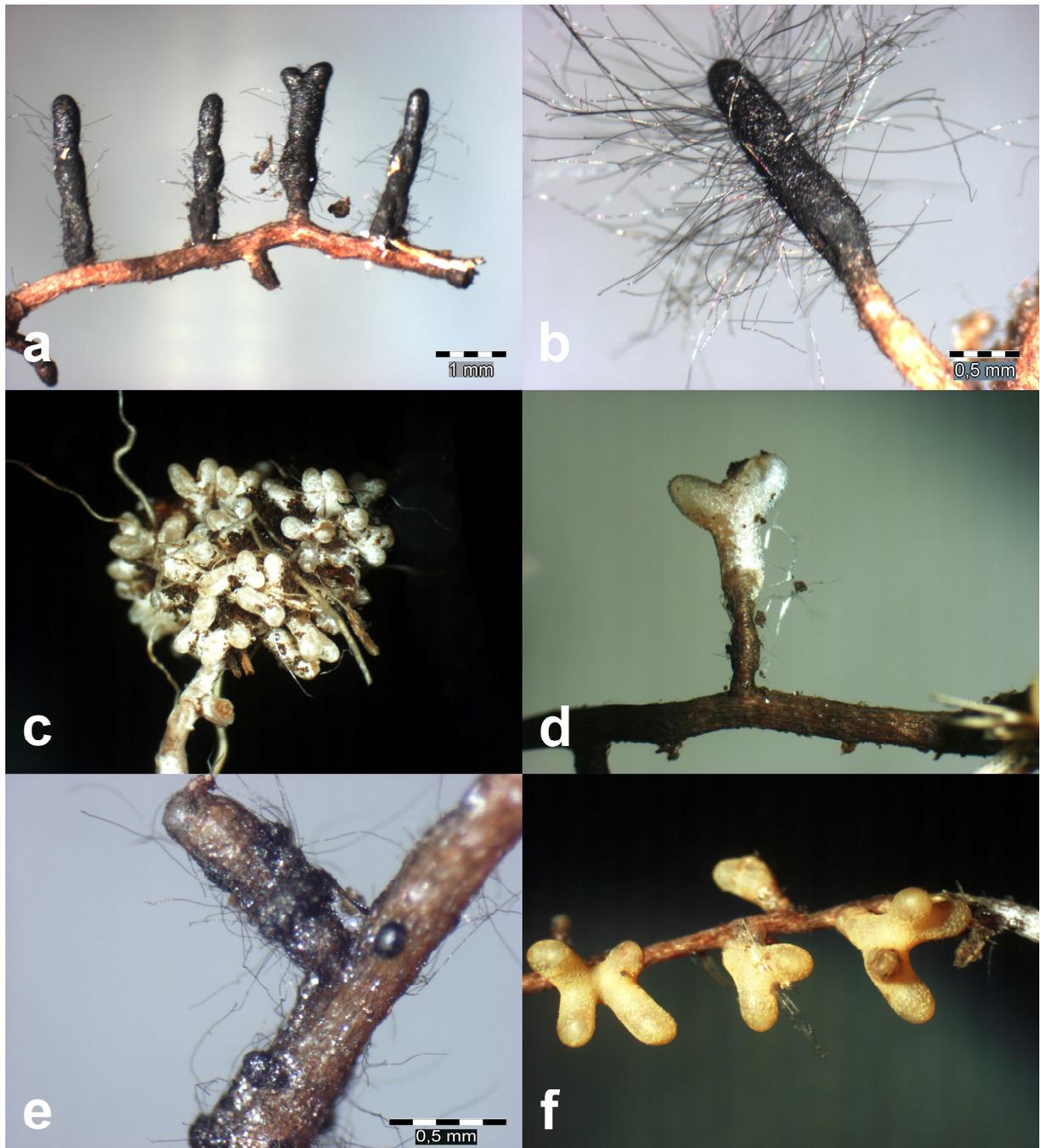


Fig. 5: Morphotypes I

Microphotographs of this and the following plates are taken with a dissecting microscope Olympus SHZ10 and a digital camera. Samples were mounted in a water bath.

- a) BB05 *Cenococcum geophilum* 15 x; Scots pine
- b) BB05 *Cenococcum geophilum** 30x; Scots pine; microphotograph by Melanie Dartsch
- c) BB06 *Xerocomus badius* magnification n. a.; Scots pine; mycorrhizal system
- d) BB06 *Xerocomus badius* magnification n. a.; Scots pine; solitary, dichotomously ramified tip
- e) BB11 *Acephala macrosclerotiorum** 50x; Scots pine; microphotograph by Guido Vogt
- f) BB14 *Russula ochroleuca* magnification n. a., Scots pine

* molecular identification from the depicted root

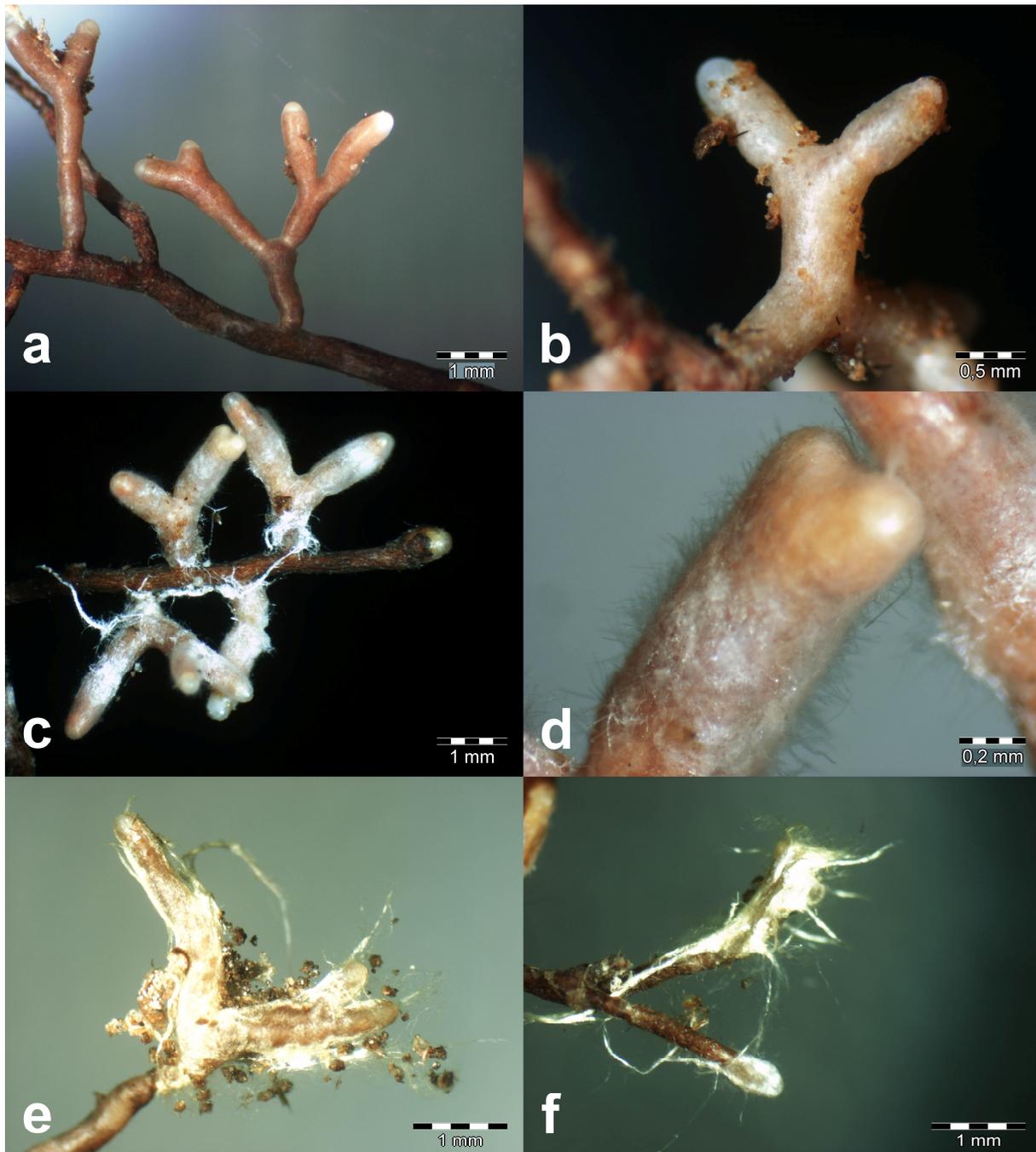


Fig. 6: Morphotypes II

- a) BB 22 *Lactarius tabidus** 15x; Scots pine
- b) BB 22 *Lactarius tabidus** 30x; Scots Pine
- c) BB23 *Thelephora terrestris** 15x; Scots Pine
- d) BB23 *Thelephora terrestris** 70x; Scots Pine; detail of Fig. 6c
- e) BB24 *Cortinarius (Dermocybe) sp. 1** 20x; Scots pine
- f) BB24 *Cortinarius (Dermocybe) sp. 1** 20x; Scots pine

* molecular identification from the depicted root

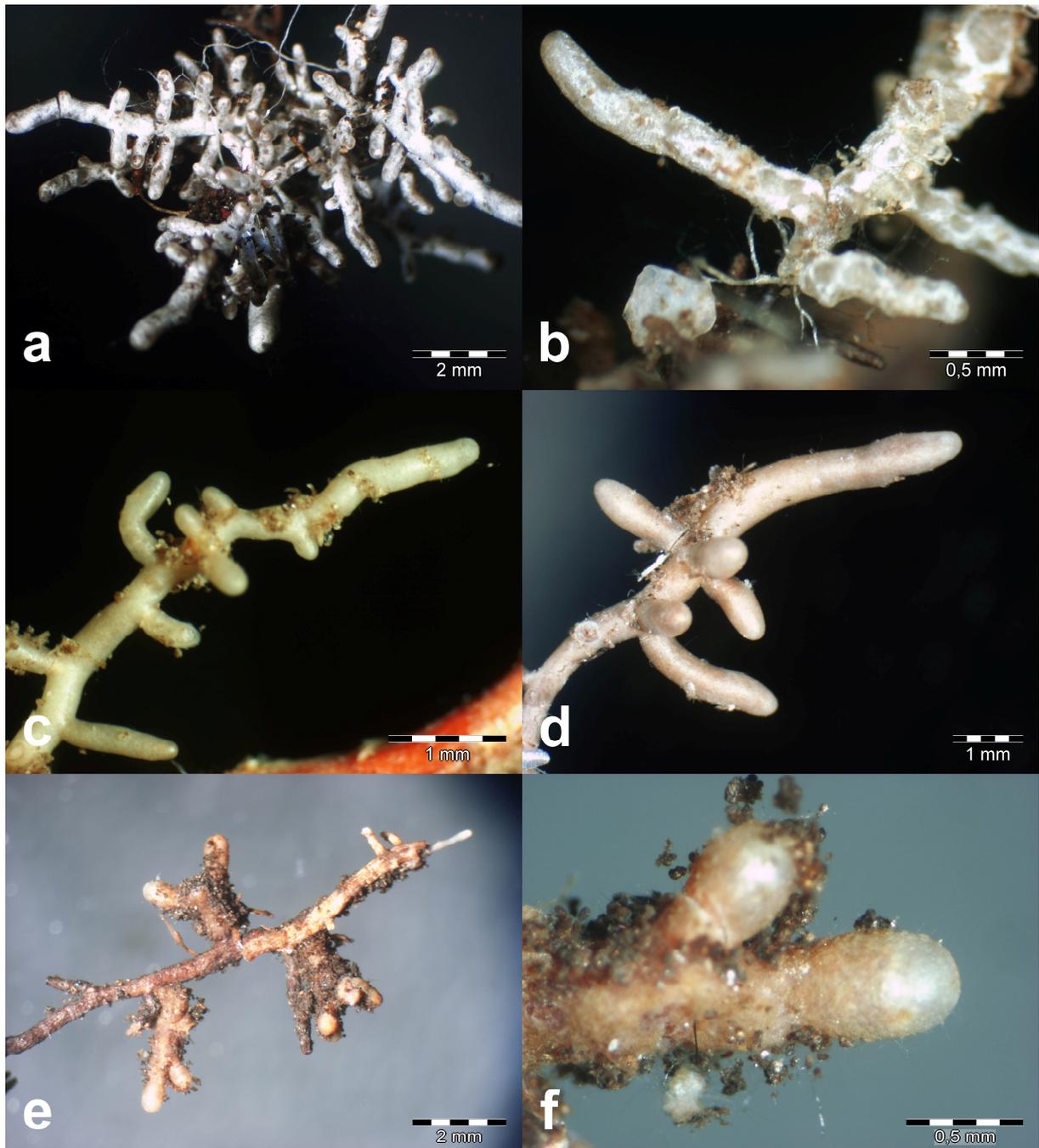


Fig. 7: Morphotypes III

- a) BB28 *Xerocomus pruinatus** 10x; beech
- b) BB28 *Xerocomus cisalpinus** 40x; beech
- c) BB38 *Lactarius sp.* 25x; beech
- d) BB45 *Lactarius sp.* 15x beech
- e) BB53 *Russula puellaris** 10x; Scots pine
- f) BB53 *Russula puellaris** 50x; Scots pine, detail of Fig. 7e

* molecular identification from the depicted root

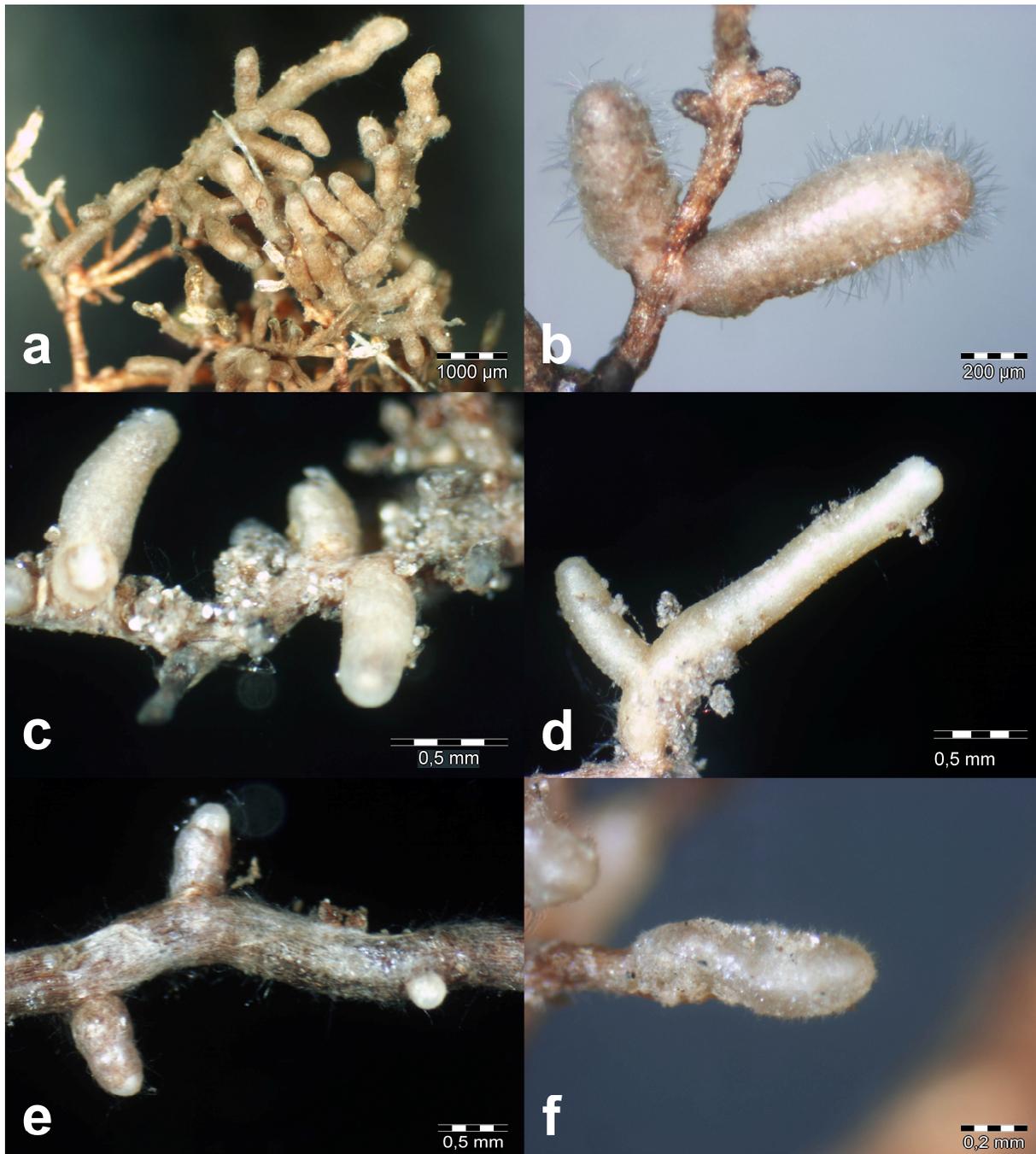


Fig. 8: Morphotypes IV

- a) BB57 *Tuber puberulum** 10x; beech
- b) BB57 *Tuber puberulum** 40x; beech; detail of Fig. 8a
- c) BB70 *Russula ochroleuca** 50x, beech
- d) BB70 *Russula ochroleuca** 40x, beech
- e) BB71 *Tomentella sublilacina** 30x, Scots pine
- f) BB72 *Russula ionochlora* 70x, beech; BB72 has also been identified as *R. vesca* (Fig. 9a)

* molecular identification from the depicted root



Fig. 9: Morphotypes V

- a) BB72 *Russula vesca** 20x; beech
- b) BB75 *Laccaria laccata* cf.* 40x; beech
- c) BB79 *Lactarius rufus** 30x, beech
- d) BB79 *Lactarius rufus** 60x, beech, detail of Fig. 9d
- e) BB80 *Cortinarius (Dermocybe) sp. 2* 25x;
- f) BB80 *Cortinarius (Dermocybe) sp. 2** 2 30x

* molecular identification from the depicted root



Fig. 10: Morphotypes VI

- a) BB81 *Hydnotrya tulasnei** 25x; beech
- b) BB82 *Genea hispidula** 20 x; beech
- c) BB82 *Genea hispidula** 50 x; beech
- d) BB83 *Pezizaceae** 15x; beech
- e) BB83 *Pezizaceae** 40x; beech; detail of Fig. 10d
- f) BB84 50x; Scots pine

* molecular identification from the depicted root

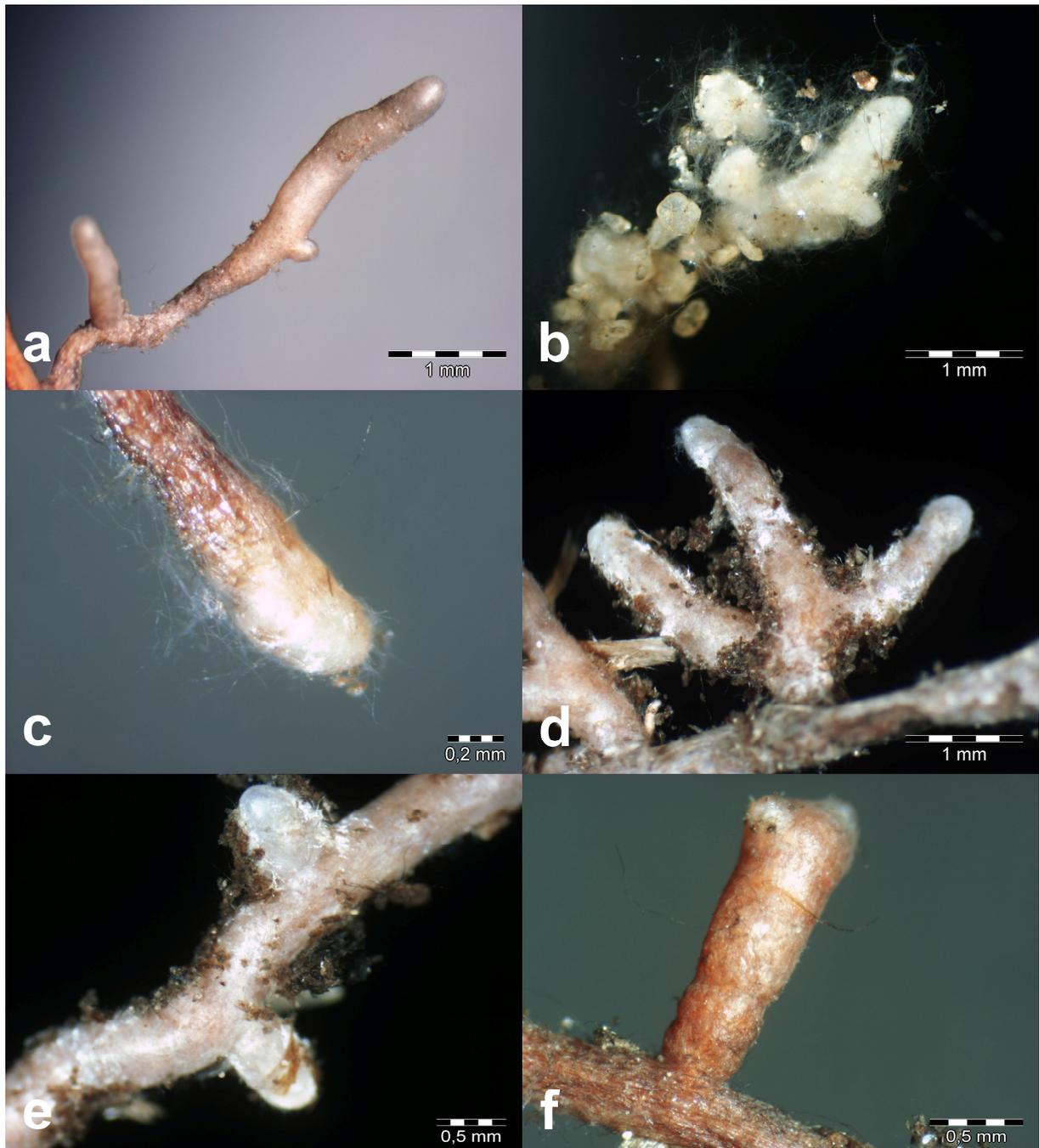


Fig. 11: Morphotypes VII

- a) BB85 *Hydnotrya tulasnei** 25x; beech; compare with Fig. 10a
- b) BB86 25x; beech
- c) BB87 60x; beech
- d) BB88 *Tomentella sublilacina** 25x; Scots pine
- e) BB88 *Tomentella sublilacina** 30x; Scots pine; compare with Fig. 8e (BB71)
- f) BB88 *Tomentella sublilacina** 40x; Scots pine; very young mycorrhiza, transparent mantle

* molecular identification from the depicted root

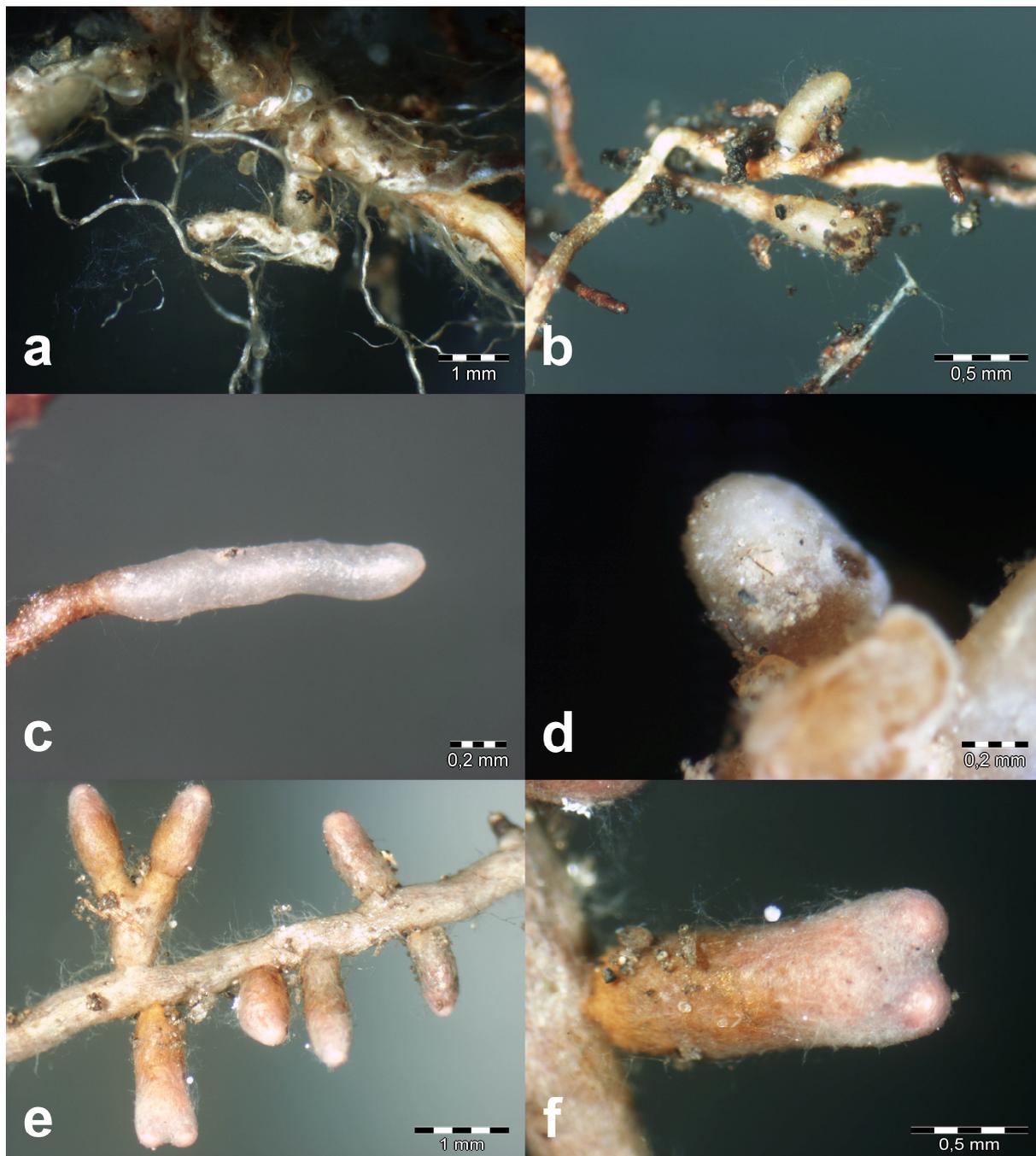


Fig. 12: Morphotypes VIII

- a) BB89 *Cortinarius (Telamonia) sp.2** 15x; Scots pine
- b) BB91 *Russula puellaris** 40x; beech; compare with Fig. 7e and f
- c) BB92 *Laccaria laccata** 60x beech, compare with Fig. 9b (BB75)
- d) BB94 70x; Scots pine
- e) BB95 20x; Scots pine
- f) BB95 50x; Scots pine; detail of Fig. 12e

* molecular identification from the depicted root



Fig. 13: Morphotypes IX

a) BB96 *Lactarius subdulcis** 20x; beech

b) BB96 *Lactarius subdulcis** 30x; beech; detail of Fig. 13a; compare with Fig. 7c and 7d

c) BB98 20x; beech

d) BB98 40x; beech; detail of Fig. 13 c

e) BB100 *Laccaria laccata* cf.* 20x; beech; compare with Fig. 9b and 12 c

f) Unmycorrhized root tip of Scots pine 25x; Note the numerous root hairs. This rootlet originates from a 12 weeks old seedling in sterile culture. Under field conditions of Kahlenberg site, unmycorrhized rootlets with root hairs were very rare.

* molecular identification from the depicted root

II.3. ITS sequencing of ectomycorrhizas

Limits of morphological/anatomical species identification

The above listed descriptions lead in some cases directly to the determination of the genus or in some cases even to determination of the fungal species. At the Kahlenberg site this was possible for *Russula ochroleuca* (BB14) on Scots pine roots (not on beech roots!) and for *Cenococcum geophilum* (BB05). At the genus level *Xerocomus* species could be easily distinguished from other genera, because of their silvery appearance with many rhizomorphs and the plectenchymatic mantle with ring-like structures. However, species distinction within *Xerocomus* was not possible even with a combination of morphological and anatomical characteristics. But even at the genus level it was difficult to differentiate genera. At Kahlenberg site many ectomycorrhizal types with a smooth appearance and pseudoparenchymatic outer mantle were observed on beech roots that were suspected to belong to the group of *Lactarius/Russula* species. *Lactarius* and *Russula* species are usually differentiated by the presence or absence of laticiferous hyphae (=laticifers). If they are inconspicuous or only present in the plectenchymatic inner mantle layer, laticifers can be overlooked so that *Lactarius sp.* is misidentified as *Russula sp.* The other way round, elongated cells of a hyphal net or the plectenchymatic inner mantle can be mistaken for laticifers so that *Russula sp.* is misidentified as *Lactarius sp.* The described problems in identifying the fungal mycorrhiza partner are well known for a long time. They can only be resolved by using molecular techniques. The standard procedure for identifying fungi is the analysis of the ITS region of the nuclear ribosomal DNA (Horton and Bruns 2001; Begerow et al. 2010; Schoch et al. 2012).

Organization of ITS region

Each cell in each organism harbours numerous ribosomes, the place of cellular protein synthesis. Each ribosome contains a large and small subunit that both comprise proteins and structural RNA, the ribosomal RNA (rRNA). The small ribosomal subunit of fungi contains 18S and 5.8S rRNA, whereas the large ribosomal subunit contains 28S rRNA. The nucleotide sequence of the ribosomal rRNA is encoded on nuclear DNA in cassettes called ribosomal DNA (rDNA). Each cassette codes for 18S rDNA (small subunit) and 28S rDNA (large subunit). The 18S and 28S rDNA sequences are separated by a non-coding spacer called ITS (internal transcribed spacer, Fig. 14). Because ITS also contains a region coding for the 5.8S rRNA, it is actually separated into two spacers: ITS1 and ITS2. When they are used for species identification usually all three parts, ITS1, 5.8S rDNA and ITS2 are analysed together and are referred to as ITS region. Ribosomal DNA is organized in tandem repeats. This means that several rDNA cassettes, each comprising 18S rDNA, ITS region and 28S rDNA, are lined up next to each other.

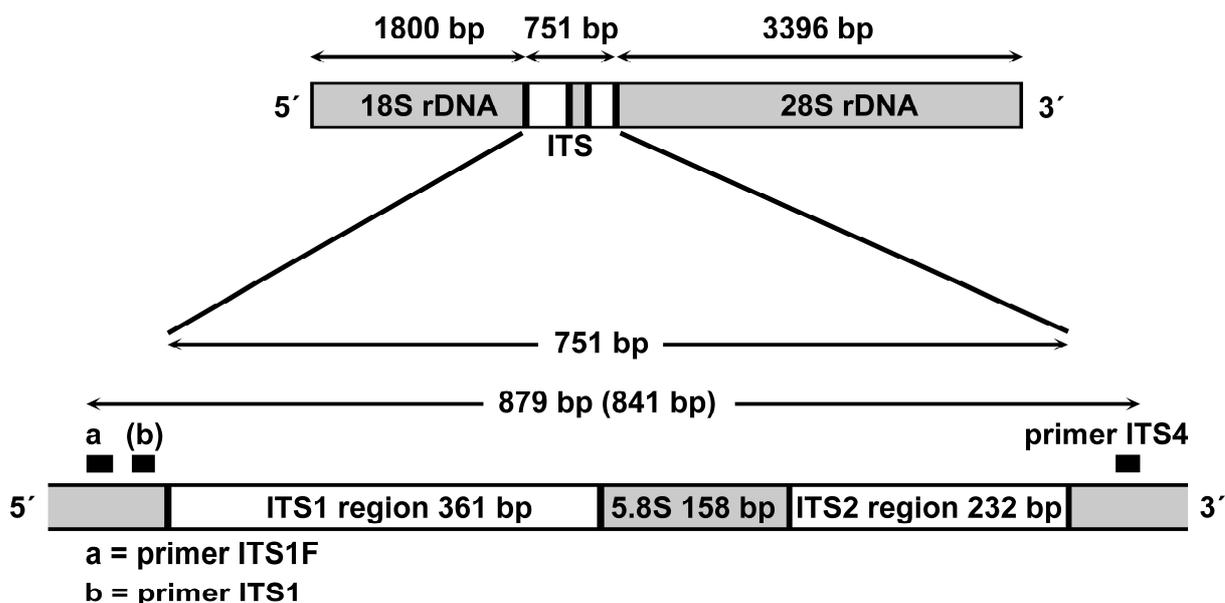


Fig. 14: Organisation of a single repeat of ribosomal DNA and the ITS region.

Sequence length in base pairs (bp) refer to the yeast (*Saccharomyces cerevisiae*) reference genome.

The organisation of the rDNA and the ITS region is described in detail because it entails crucial characteristics for species identification. 18S rDNA and 28S rDNA sequences are highly conserved, that is they contain nucleotide sequences that are the same for all fungal species. This is necessary for the PCR (**P**olymerase **C**hain **R**eaction) which is used to amplify the DNA of the ITS region. PCR needs primers to start amplification. Primers are oligonucleotides of 15-30 base pair length that bind to DNA stretches with the same nucleotide sequence. The ITS region lays between two highly conserved priming sequences so that a set of two standard primers, usually ITS1F and ITS4 (Gardes and Bruns 1993; O'Donnell 1993) can be used for most fungal species without having a priori information on their identity. The amplified PCR-product is always longer than the ITS-region. It contains also the conserved 3'-end of the 18S rDNA and the conserved 5'-end of 28S rDNA. The PCR product is 879 base pairs long in the yeast *Saccharomyces cerevisiae*, while the ITS region with the species specific information is 751 bp long (Fig. 14).

ITS RFLP

There are two possibilities to analyze amplified PCR-products of the ITS-region. One is the analysis of RFLPs, the other is sequencing. RFLP stands for **R**estriction **F**ragment **L**ength **P**olymorphism which is basically a fingerprint pattern generated from the PCR-product. This fingerprint is produced by digesting the PCR-products with restriction enzymes. At different species the restriction sites are located at different positions within the ITS region resulting in a pattern of restriction fragments of different length and number when the digest is applied to an agarose gel. These RFLP patterns are identical within specimens of the same fungal species. ITS-PCR-RFLP method has been successfully applied to determine and compare the

structure (species richness, diversity indices) of ectomycorrhizal communities (Gardes and Bruns 1996; Timonen et al. 1997; Taylor and Bruns 1999; Peter et al. 2001).

Identification of the species is possible when the RFLP pattern of a mycorrhized root matches the RFLP pattern of a fruiting body. Due to a lack of standardization, identification was only successful if the fruiting body has been analyzed by the same laboratory so that in most cases identification was only possible if the fruiting body was found on the site of the ectomycorrhizal study (e.g. Gehring et al. 1998; Horton et al. 1999; Jonsson et al. 1999).

Although RFLP analysis and sequencing of ITS were developed at the same time, RFLP analysis had initially the advantage of being cheaper and some molecular studies of ectomycorrhizal communities used a combination of RFLP analysis and sequencing. The majority of root tips were analyzed by ITS RFLP and only selected root tips were sequenced (e.g. Lilleskov et al. 2002; Nara et al. 2003; Douglas et al. 2005). Technological advances made sequencing available at lower costs, so that today sequencing of the ITS PCR product is the standard procedure for ectomycorrhizal species identification. Nevertheless, the reader should be aware of the distinction of ITS RFLP analysis and ITS sequencing because also papers based on ITS RFLP will be cited as molecular ITS studies at appropriate places of this thesis.

ITS sequences and BLAST search

Sequencing of DNA means to determine the order of the four bases Adenine (A), Thymine (T), Cytosine (C) and Guanine (G) within the DNA. Due to the distinct nature of the bases a sequence can be written as words with a length of several hundred letters. In contrast to human language, the words contain only the letters A, T, C, and G. Sequences can be compared to each other and they can be stored in and retrieved from online databases.

The basic principle of species identification by ITS sequences is that the sequence is the same between individuals of the same species, but differs between species (Begerow et al. 2010). Identification of a fungal ITS sequence produced from a mycorrhized root is achieved, when it is identical to the sequence of a fruiting body of a known species collected from the same site. As already outlined in the introduction (section I.1), fruiting body production and mycorrhiza formations do not correspond to each other, so that in many ectomycorrhizal studies fruiting bodies are not available from the study site. Therefore, the query sequence is compared with sequences in publicly available online databases. The easy access to large online data bases with reference sequences is the main advantage of ITS sequencing over ITS RFLP analysis. A database can be searched for matches between query and reference sequences by using BLAST (Basic Local Alignment Search Tool) and related algorithms (Altschul et al. 1990; Zhang et al. 2000). Result of a BLAST search is a list with matching database entries. A 100% match means that query and reference are equal at all positions of the ITS sequence.

The standard database for sequences from all kind of organisms and all kind of sources is GenBank (<http://www.ncbi.nlm.nih.gov>). It also contains fungal ITS sequences. Since scientific journals require that all sequences used for a manuscript are made publicly available

in online databases, GenBank contains large amounts of environmental data without proper species designation. Therefore it can happen that a 100% BLAST match does not lead to species identification. It is possible that the matching database entry is yet another unidentified mycorrhized root tip. In order to circumvent this problem, databases can be used that contain sequences of identified fruiting bodies (e.g. Bruns et al. 1998, Cullings and Vogler 1998). The most widely used database for ITS based identification of ectomycorrhizas is UNITE (<http://unite.ut.ee>). Explicitly founded as a reference database for ectomycorrhizal fungi (Kõljalg et al. 2005), today it also collects reference sequences of other fruiting body forming fungi (Abarenkov et al. 2010).

Phylogenetic analysis

Most ectomycorrhizal community studies rely on ITS BLAST matches for species identification. However, even when a 100% BLAST match provides a species name, some ambiguities remain. There are two possibilities. Either identical sequences have different species names (error type I) or different sequences have the same species name (error type II). While the error type I is discovered by screening the list of BLAST search hits for contradicting names, the second case remains often undiscovered. Possible reasons for both type I and type II errors are taxonomic uncertainties in a group of fungal species.

Taxonomic ambiguities can only be evaluated with a phylogenetic analysis. In a thorough phylogenetic analysis, it is not sufficient to align all hits of a BLAST search. In order to test the position of the assumed species in relation to related species, it is necessary to base the phylogenetic analysis on existing taxonomic knowledge about the corresponding group of fungi. This would involve using sequences of published phylogenetic analyses. Because this method requires an active literature review, also type II errors, i.e. two different sequences have the same name, can be detected. As a result of a phylogenetic analysis, one of the two different sequences has to be given a new name. Two examples of name correction in database entries are explained in section II. 4 and section II.5. A sequence called *Phialocephala sp. 6* has been renamed *Acephala sp. 6* (section II.4) and a sequence called *Hydnotrya tulasnei* has been renamed *H. bailii* (section II.5).

At Kahlenberg site 40 ectomycorrhizal species are found that have been analyzed in 15 phylograms. These 15 phylograms can be found in Part III. The results of Part III have been anticipated in the morphology plates of Fig. 5 to Fig. 13 which list for each morphotype the sequenced based identification.

Beyond phylogenetic analyses

Phylogenetic analyses are also useful when no BLAST match is found at all. In these cases a phylogenetic analysis helps to establish the closest relatives for which sequence information is available. They also help to evaluate whether the sequence is missing in the database because a known species has not yet an ITS database entry or whether the sequence belongs to a completely unknown species. When the sequence belongs to a new species and more material than a mycorrhized root is present (i. e. fruiting body, culture) it can be considered to write a

new species description. A necessary prerequisite to describe a new ectomycorrhizal species is to prove that it really forms ectomycorrhizas and the sequence is not a by-product of parasitic or saprotrophic fungi. This proof involves three necessary steps:

- 1) isolation of the fungal strain
- 2) in-vitro cultivation of the fungal strain
- 3) re-infection of host roots in order to confirm that the "artificial mycorrhiza" has the same morphology and anatomy than the natural mycorrhiza.

This method has been the traditional method of proofing the ectomycorrhizal status of a fungus (Melin 1923; Molina 1979; Malajczuk et al. 1982; Tedersoo et al. 2010). ITS-sequencing can confirm whether the strain of interest is present in all three stages.

In the following three sections (II.4 to II.6) three case studies are presented that are based on published manuscripts. They involve cultivation techniques but also show the limits of cultivation. These three case studies also illustrate which efforts can be necessary to determine the species names for an ectomycorrhizal fungus found in a community study. The included summaries of results are not identical to the abstracts of the published manuscripts but highlight those results that are relevant to the topic of species identification. The subsequent comments of the thesis author stress points that were not, or only marginally, treated in the published manuscripts.

II.4. Case study 1: Species description of *Acephala macrosclerotiorum*

Münzenberger B, **Bubner B**, Wöllecke J, Sieber TN, Bauer R, Fladung M, Hüttl RF (2009) The ectomycorrhizal morphotype Pinirhiza sclerotia is formed by *Acephala macrosclerotiorum* sp. nov., a close relative of *Phialocephala fortinii*. Mycorrhiza 19:481-492

Summary of results

The ectomycorrhizal fungus EW76 was isolated from Scots pine (*Pinus sylvestris*). It showed both in field collections (named Pinirhiza sclerotia) and in resynthesized ectomycorrhizas the unusual characteristic of sclerotia directly attached to the surface of the mycorrhized root. Ultrastructural microphotographs of the mantle and the sclerotia from the synthesized mycorrhizas confirmed that it was an ectomycorrhizal fungus by showing a Hartig net that reached to the root endodermis. Genetic analysis of the ITS region revealed that it was related but not equal to the root endophyte *Phialocephala fortinii*. Axenic resynthesis of EW76 was compared with a strain of *Phialocephala fortinii* (strain75). EW76 formed ectomycorrhizas on Scots pine seedlings but not on clonally propagated plants of the angiosperm *Populus tremula* x *tremuloides* Esch5. *Phialocephala* strain 75 did not form ectomycorrhizas on either hosts.

Although fruiting bodies were not observed EW76 was described as new species based on the following four observations: 1) ability to form host specific ectomycorrhizas, 2) characteristics of hyphal mantle of mycorrhized roots, 3) characteristics of culture on plates, and 4) phylogenetic analysis of ITS region.

Contributions of the author of this thesis

The thesis author sequenced the ITS region and 28S rDNA of cultures of EW76 (*Acephala macrosclerotiorum*) and strain 75 (*Phialocephala fortinii sensu lato*). He proposed and organized the experiments on axenic mycorrhization of *Populus* Esch5. This experiment served to compare potential mycorrhiza formation with a published ectomycorrhiza of *Phialocephala fortinii sensu lato* on *Populus* Esch5. He also proposed to describe EW76 as new species and to involve Thomas N. Sieber as specialist of the *Phialocephala/Acephala* species complex (PAC) to help with phylogenetic analysis and species description. Fig. 1a was taken during the work on mycorrhiza descriptions of the Kahlenberg site as presented in section II.2 of this thesis. Fig. 5e in section II.2 of this thesis shows exactly the same root at larger magnification. This ectomycorrhiza was collected from Scots pine roots at the old mixed stand at Kahlenberg site. The depicted root tip was sequenced and the ITS sequence was identical with the type sequence of the cultured EW76=*Acephala macrosclerotiorum* (accession EU882732).

The following sections present commentaries by the thesis author.

Discussion of species delimitation

The discovery and description of *Acephala macrosclerotiorum* is an example of how important it is to rely not only on databases. It is also a case study for the usefulness of the classical method of isolation, cultivation and re-infection for the prove of ectomycorrhizal status. While the presentation in Münzenberger et al. (2009) was result oriented, the species delimitation of *Acephala macrosclerotiorum* is presented here as the puzzle of discovery that it was.

Correction for an intron sequence

An ectomycorrhiza from Scots pine was described as Pinirhiza sclerotia by Jens Wöllecke in 2001 (Wöllecke 2001). A tissue culture could be established from this ectomycorrhizal type and has been cultivated as EW76 in the strain collection of Babette Münzenberger. The sample was collected in the Scots pine forest "Hubertusstock" in Northern Brandenburg at a distance of ca. 20 km from the Kahlenberg site in the year 2004.

The tissue culture of EW76 was ITS sequenced. Because of an intron between the positions of the forward primers ITS1F and ITS1 (see Fig. 14), the PCR product of the standard primers ITS1F and ITS4 was with 1600 base pairs ca. 1000 base pairs longer than expected. The type

sequence published by the thesis author in GenBank (accession EU882732) contains a large part of this intron so that the actual ITS sequence starts at position 890. Because EW76 is an ascomycete and the insertion region is part of the 18S, the presence of an intron is not unusual: introns in the 18S rDNA of ascomycetes have been previously observed and it has been speculated that their presence in ascomycete might be rather the rule than the exception (Gargas and DePriest 1996; Gargas et al. 1996).

Although the intron did not interrupt the actual ITS sequence, a large PCR product is difficult to sequence because the sequencing reaction is aborted after 1000 bp read length. The existence of an intron was a critical discovery for the sequencing efforts at Kahlenberg site, since the ectomycorrhizal type Pinihriza sclerotia from Hubertusstock was also present at Kahlenberg site (described as BB11, see section II.2). In order to avoid problems with large PCR products, all BB11 morphotypes were amplified with the primers ITS1 (not ITS1F) and ITS4. The resulting PCR product (568 bp) contained the complete ITS sequence but no intron and could be sequenced with the standard protocols.

Conflicting database results

After discovery of the intron and solving sequencing problems by using the primer ITS1 for PCR, sequence analysis could be performed according to the usual algorithms. A BLAST search of the EW76 sequence in GenBank resulted in a 99.2% match with a fungal sequence from Scots pine roots in Lithuania, that was listed under the species name *Phialocephala fortinii* (AY606280). A literature review revealed that *Phialocephala fortinii* was mostly considered to be a root endophyte (Grünig et al. 2004; Grünig et al. 2006; Grünig et al. 2007; Grünig et al. 2008). However, one publication described *Phialocephala fortinii* as ectomycorrhizal on the hybrid aspen clone *Populus tremula x tremuloides* Esch5 (Kaldorf et al. 2004). Fig. 2c and 3c in this publication show a morphotype and mantle anatomy of an ectomycorrhiza that was called EM5. The morphotype EM5 is similar to EW76/Pinihriza sclerotia/BB11 with one major difference: It lacked the sclerotia attached to the root surface.

The thesis author speculated, if EW76/Pinihriza sclerotia/BB11 and EM5 (Kaldorf et al. 2004) were the same species, they still could show different morphologies on *Pinus* and *Populus*. For instance, the type of ramification of the same fungus is different on the conifer *Pinus sylvestris* and the angiosperm *Fagus sylvatica*, namely dichotomous versus monopodial (Pillukat and Agerer 1992). Therefore, it could be possible that EW76 formed sclerotia on *Pinus* but not on *Populus*.

Mycorrhiza synthesis and mycorrhizal status of *Phialocephala fortinii*

At the time of the speculation on species identity with *Phialocephala fortinii*, the proof that EW76/Pinihriza sclerotia/BB11 was indeed ectomycorrhizal had been completed according to the principles of isolation, cultivation and re-infection. The isolated fungus EW76 had been cultivated and was used for inoculation of roots of Scots pine seedlings. The resulting ectomycorrhizas were analyzed by morphology and anatomy and they showed the same

characteristics, including Hartig net, as the natural ectomycorrhizas described as *Pinirhiza sclerotia* and BB11.

In order to test the hypothesis that EW76 lacks sclerotia on angiosperms and can form a morphotype similar to the natural ectomycorrhiza EM5 (supposedly formed by *Phialocephala fortinii*), an inoculation experiment was performed with EW76 and *Populus* Esch5, the host species of EM5. The arrangement of this resynthesis was unusual in the respect to the genetic identity of the natural and the in-vitro host. The host from the field experiment of Kaldorf et al. (2004), *Populus tremula x tremuloides* Esch5 is a clone that can be propagated by sterile in-vitro methods (Kaldorf et al. 2002). Therefore, clonal plantlets of *Populus* Esch5 used for the synthetic ectomycorrhizas have the same individual genotype as the roots of the field ectomycorrhizas. This excludes any variation in mycorrhization that could be potentially contributed by different individual host genotypes. In contrast, individual host genotypes differ when seedlings are used for comparing natural and synthetic ectomycorrhizas. Clonally propagated plantlets of *Populus* Esch5 were provided by Matthias Fladung, Großhansdorf.

Inoculation of *Populus* Esch5 with EW76 did not result in ectomycorrhizas or in the production of sclerotia. Because any variation of the host genotype could be excluded, it had to be concluded that EW76 was not identical with EM5 and therefore also not with *Phialocephala fortinii*. However, it left the question whether *Phialocephala fortinii* is ectomycorrhizal at all. Since *Phialocephala* strains are frequent root endophytes, it might be possible that the sequence of EM5 does not originate from the ectomycorrhizal fungus but from a co-habiting *Phialocephala* strain. In this situation, experiments of isolation, cultivation and re-infection help to clarify hypotheses raised by database matches. A culture of "strain 75" was provided by Francois Buscot. This strain had been isolated from *Populus* Esch5 roots colonized by the morphotype EM5 and had been cultivated at the Helmholtz Centre for Environmental Research (UFZ), Halle. Inoculation of *Populus* Esch5 with "strain75" did not result in ectomycorrhiza formation. Therefore, the principle of isolation, cultivation and re-infection failed to prove the ectomycorrhizal status of *Phialocephala fortinii* while it succeeded for EW76.

EW76 in the genus *Acephala*

The results of the inoculation experiments delivered the motivation to describe ectomycorrhizal EW76 as a new species which is discriminated against endophytic *Phialocephala fortinii*. A valid description necessitates not only a latin diagnosis but also a phylogenetic comparison with related species which was performed by Thomas N. Sieber. In Fig. 7 of Münzenberger et al. (2009) EW76 did not group together with a cluster of the previously established *Phialocephala fortinii/Acephala applanata* species complex (Grünig et al. 2008). This complex contained not only sequences of endophytic *Phialocephala* species and the newly described endophytic genus *Acephala* (Grünig and Sieber 2005) but also the sequence of the morphotype EM5 (AJ510268) from Kaldorf et al. (2004). This suggests, together with the failure to form ectomycorrhizas experimentally, that the sequence AJ510268 does not belong to the fungus that caused the ectomycorrhizal morphotype EM5.

Despite exclusion of EW76 from the *Phialocephala fortinii/Acephala applanata* species complex, the authors of Münzenberger et al. (2009), including the thesis author, decided to describe the new species as belonging to genus *Acephala*. This decision is related to the history of the genus *Acephala*; which was erected in a differential diagnosis in comparison to *Phialocephala fortinii* based on the morphology of colonies of cultivated root endophytes (Grünig and Sieber 2005). The major argument is that *Phialocephala* sporulates with conidiophores which are organized as heads of phialids (cephalos=head) while *Acephala* does not sporulate and conidiophores are completely lacking (acephala = without head). Because of the lack of conidiophores on agar plates, EW76 was placed into the genus *Acephala*.

From the point of view of the thesis author it is doubtful whether absence or presence of sporulation on agar plates is sufficient for erecting a separate genus, especially since *Acephala* and *Phialocephala* are very closely related based on molecular data, as the name PAC = *Phialocephala fortinii* *Acephala applanata* Complex suggests (Grünig et al. 2007). It also remains to note that the type strain of *Phialocephala fortinii* did only sporulate (i. e. showing the name giving conidiophores) after incubation at 5°C for 6 to 12 months (Wang and Wilcox 1985). However, phylogenetic analysis also showed that EW76 is a new species and that a species description is justified. Since a species name needs a genus affiliation and the evidence did not justify to erect another new genus, the species name *Acephala macrosclerotiorum* was accepted as compromise.

Ectomycorrhizal status and phylogenetic relationships

In Fig. 7 of Münzenberger et al. (2009), the EW76 type sequence EU882732 clusters with 100% bootstrap support with two other sequences, AY606280 (Menkis et al. 2004) and EU434833 (Grünig et al. 2009). These sequences match with EW76 99,2% and 99,4%, respectively. The GenBank entry AY606280 was originally named *Phialocephala fortinii* (see above) and has been renamed *Acephala* sp. 6 by Tomas Sieber and colleagues (see GenBank accession AY606280). The high sequence similarity and the 100% boot strap support makes it probable that *Acephala* sp. 6 and *Acephala macrosclerotium* are the same species or at least very closely related species. Since it is improbable that the mycorrhizal status changes within a species it can be assumed that the two specimens of *Acephala* sp. 6 are ectomycorrhizal. This view is also shared by Tedersoo et al. (2010, Table 1). Because the two specimens of *Acephala* sp.6 are cultures that were established by placing root tips on agar plates (Menkis et al. 2004), it is possible that their mycorrhizal status has been overlooked.

It has been observed several times before that species thought to be closely related belong to different genera based on molecular data and that this split coincided with the mycorrhizal status of this species, i.e mycorrhizal vs. nonmycorrhizal (Moncalvo et al. 2002). An example is the separation of lignicole *Tapinella* from ectomycorrhizal *Paxillus* (Bresinsky et al. 1999; Hahn and Agerer 1999). This allows the assumption that the mycorrhizal status does not change within a genus. However, the type species of the genus *Acephala applanata* is a root endophyte (Grünig and Sieber 2005). The thesis author raises the hypothesis that among the species related to *Phialocephala fortinii* a new genus has to be erected that contains only

ectomycorrhizal species including *Acephala macrosclerotiorum* and that it is separated from endophytic species of the *Phialocephala/Acephala* species complex.

Clarification of the generic status will require further collections of *Acephala macrosclerotiorum* and related species with an exact proof of their mycorrhizal status through re-synthesis experiments and analysis of the Hartig net. The discovery of further occurrences will be improved by the description published in Münzenberger et al. (2009), because now the fungus can be morphologically separated from roots infected by unidentified fungi. In this respect it was also necessary to give EW76 a species name because it improves exchange between scientists when a morphotype can be related to a species name instead of referring to an "unknown ectomycorrhizal fungus".

Host specificity

Originally, the synthesis experiment with *Acephala macrosclerotiorum* and *Populus* Esch5 was a control experiment for presence and absence of sclerotia. The failure to form ectomycorrhizas with the angiosperm *Populus* Esch5 and the readiness of ectomycorrhiza formation on Scots pine indicates a host specificity for the genus *Pinus*. Field observations also point to an affinity to pine trees. All specimens of the morphotype EW76/Pinirhiza sclerotia/BB11 originate from Scots pine roots that have been collected at three different sites in the state of Brandenburg (Kahlenberg, Hubertusstock, Bayerswald). When the two specimens of *Acephala* sp. 6 (AY606280, EU434833) are included into the species *A. macrosclerotiorum* the picture gets even broader. Both specimens are from Lithuania, the first isolated from roots of *Pinus sylvestris* the latter from roots of *Picea abies*. Thus, *A. macrosclerotiorum* is not only a local strain but widely spread and always found on roots of Pinaceae. This strongly suggests an affinity to Pinaceae but not to angiosperms.

As already mentioned the fungus *Acephala macrosclerotiorum* is also present at Kahlenberg site. Since Kahlenberg site comprised four stands with various combinations of the Pinaceae *Pinus sylvestris* and the angiosperm *Fagus sylvatica*, collections on this site can help to clarify the question of host specificity under field conditions. Especially on the two mixed stands it can be tested whether the affinity to Pinaceae is a site effect or a true host effect. If it is a true host effect then *A. macrosclerotiorum* will be found only on Scots pine roots. The result of this field survey is presented in part III.

II. 5. Case study 2: Phylogenetic analysis in the genus *Hydnotrya*

Stielow B, **Bubner B**, Hensel G, Münzenberger B, Hoffmann P, Klenk HP, Göker M (2010) The neglected hypogeous fungus *Hydnotrya bailii* Soehner (1959) is a widespread sister taxon of *Hydnotrya tulasnei* (Berk.) Berk. & Broome (1846). *Mycological Progress* 9:195-203

Summary of results

Hypogeous sporocarps of a *Hydnotrya* species were collected by G. Hensel in the Harz mountains, Germany. They were clearly distinct from *Hydnotrya tulasnei* fruiting bodies and matched best to a neglected description of the species *H. bailii* (Soehner 1959). Although sequencing of the type material collected by Soehner and Bail in the 1950s was not successful, fresh fruiting bodies and a herbarium specimen from 2003 with the same morphology as the type material of *H. bailii* yielded identical ITS sequences. Phylogenetic analysis including root tip sequences from the Kahlenberg site confirmed the separation of *H. bailii* from *H. tulasnei* and confirmed the species identification of the Kahlenberg sequences as *H. tulasnei*. It also allowed to correct species names of published sequences. Comparison of sampling sites indicate a different ecology and distribution of both species. *H. tulasnei* was found in temperate lowlands under angiosperm hosts, while *H. bailii* is found in mountainous or boreal areas under *Picea abies*.

Contributions of the author of this thesis

The author sequenced ectomycorrhizal root tips from Kahlenberg site (*H. tulasnei*) and the type material from *H. bailii* fruiting bodies collected by Soehner and Bail in the 1950ies (historical herbarium material) and by Gunnar Hensel (fresh material). A preliminary phylogenetic analysis by the thesis author indicated that *H. bailii* is a distinct species that is closely related but well separated from *H. tulasnei*. The phylogenetic trees in Stielow et al. (2010) were constructed by Markus Göker.

The following sections present commentaries by the thesis author.

Duplicate sequencing of herbarium material

The results of Stielow et al. (2010) are based on a methodological approach that is rarely used: sequencing of the same sample in two different laboratories. The critical sequences of the neglected species *H. bailii* were generated by B. Stielow at DSMZ, Braunschweig and B. Bubner at ZALF, Müncheberg (Table 1). They used different protocols for DNA extraction and PCR. Sequencing of the fresh fruiting bodies collected by G. Hensel in the Harz mountains resulted in 100% identical sequences in both laboratories (GQ149464, GQ140237). This proofed that the difference to sequences of *H. tulasnei* was not due to sequencing errors but marked a species boundary. Also sequencing of a 5 year old dried herbarium specimen from the Black Forest resulted in sequences 100% identical to each other (GQ149465, GQ140238) and to the Harz sequences. These results underline the reliability and comparability of ITS sequencing both from fresh and dried material.

However, working in two laboratories demonstrated also the limits of sequencing of dried herbarium specimens. With special permission of the Botanische Staatssammlungen München type material of *H. bailii* and other *Hydnotrya* species from the Herbarium Soehner could be used for DNA extraction. These five specimens were between 60 and 100 years old. DNA

extraction did not yield amplifiable DNA and PCR failed in both laboratories. This failure together with the success on the five year old fruiting body from the black forest confirms earlier results that sequencing of herbarium material of fruiting bodies is possible in principle but that success rates decline rapidly with the age of the material (Brock et al. 2009).

Cultivation

Ectomycorrhizal morphology and anatomy of *Hydnotrya* ectomycorrhizas were shortly sketched by Tedersoo et al. (2006) but a formal description is not found in the literature (but see short descriptions of BB81 and BB85 in section II.2). Furthermore, the proof of ectomycorrhizal status on the basis of isolation, cultivation and re-infection is still missing. These two facts provided the motivation for trying isolation of the fungus *H. bailii* from fruiting bodies collected by Gunnar Hensel in the Harz mountains. Although the same methods have been used that were successful for the isolation of *Acephala macrocephala* (Münzenberger et al. 2009), no culture of *H. bailii* could be established. Difficulties in isolating *Hydnotrya bailii* are also reported by Vohnik et al. (2007) who isolated not only *H. bailii* (AM261522) from fruiting bodies but also a strain related to the genus *Meliniomyces*. A resynthesis experiment of the *H. bailii* strain and *Picea abies* and *Vaccinium corymbosum* did not result in mycorrhiza formation in both hosts. The example of *H. bailii* shows that it is not always possible to proof the ectomycorrhizal status by the principles of isolation, cultivation and re-infection. Critical stage for this proof is the possibility to cultivate a fungus.

Importance of *H. bailii* description for identification efforts at Kahlenberg site

H. bailii is not present at Kahlenberg site. Nevertheless, its rediscovery was a valuable contribution for the confirmation of *H. tulasnei* at Kahlenberg site. At Kahlenberg site several sequences from mycorrhized beech roots matched with 99.8% (1 bp difference) to a sequence of *Hydnotrya tulasnei* fruiting body from Denmark (AJ969620). This BLAST match alone is accepted in most ectomycorrhizal studies as proof of species identity. However, database researches for phylogenetic tree construction discovered a *Hydnotrya tulasnei* fruiting body sequence from Bohemia, Czech Republik (AM261522) that was only to 95.2 % identical to the ectomycorrhizal sequence from Kahlenberg. This contradiction indicated that one of two fruiting bodies was not correctly identified and named and that identification of the ectomycorrhizal sequences was ambiguous.

The phylogenetic analysis presented in Fig. 6 of Stielow et al. (2010) unambiguously proofs that AM261522 from Bohemia is *H. tulasnei* but belongs to the resurrected species of *H. bailii*. It also confirmed that the ectomycorrhizal sequences from Kahlenberg are correctly identified as *H. tulasnei*. In the already mentioned cultivation report (Vohnik et al. 2007), AM261522 was identified on the grounds of fruiting body morphology. The misinterpretation of this specimen as *H. tulasnei* is not surprising because at the time of publication of (Vohnik et al. 2007) an easily accessible differential diagnosis of *H. bailii* was not available. The GenBank entry AM261522 has been renamed from *H.tulasnei* to *H. bailii*.

Host specificity in the genus *Hydnotrya*

Availability of *Hydnotrya* sequences in GenBank allows broadening the picture on host specificity as compared to data from a single study (Table 1). In using the results of the phylogenetic analysis in Stielow et al. (2010) also sequences can be incorporated that are listed without species names in their GenBank entries (e.g. AJ534700). It has to be noted that it makes a difference whether the host is determined by affiliation of the fruiting body to a host tree or by directly sequencing the mycorrhized root tip. In the case of mycorrhized root tips the host status is directly proven when the species name of the plant root is known which is given in pure stands. Statement on host affiliation of fruiting bodies is only an indirect proof because for many GenBank entries a description of the plant community is missing, so that it is not known whether other potential hosts would be available.

Despite these restrictions, a pattern of host specificity emerges from Table 1. *H. bailii* is associated with *Picea abies*, while *Hydnotrya tulasnei* is associated with *Fagus sylvatica*. Co-occurrence of *H. tulasnei* with *Coryllus avellana* indicates that it is not strictly specific to *Fagus sylvatica* but colonizes a wider host spectrum within the angiosperms. Thus, the two closely related *Hydnotrya* species associate with a different host spectrum. However, a conclusive statement on host specificity can be only given if data from more locations is available. This data should record the host affiliation by directly sequencing the fungus and the host from the mycorrhized roots. It has to be noted that one of the two *H. tulasnei* sequences of the Kahlenberg site originates from *Pinus sylvestris* roots (GQ215698). Since many more root tips of *H. tulasnei* have been sequenced at Kahlenberg site, linking this data with the host names can solve the question whether the occurrence on *Pinus sylvestris* is just an exception and the specificity to angiosperm can be confirmed. Results of this analysis will be presented in part III of this thesis.

Table 1: Hosts of *Hydnотrya bailii* and *H. tulasnei* sequences

The data combines sequences from Stielow et al. (2010) and other publications. In the case of two accession numbers per line, pieces of the same specimen were DNA extracted and sequenced by different methods in two different laboratories (B = B. Bubner at ZALF Mönchberg, S=B. Stielow at DSMZ Braunschweig). In the columns source, f.b. denotes a fruiting body as source for the sequence and m. r. a mycorrhized root tip.

accession no.	host	source	site	reference
<i>Hydnотrya bailii</i>				
GQ149465 B , GQ140238 S	<i>Picea abies</i>	f. b.	Hinterzarten, Black Forest, Germany	(Stielow et al. 2010)
GQ149464 B , GQ140237 S	<i>Picea abies</i>	f. b.	Schierke, Harz, Germany	(Stielow et al. 2010)
AM261522	<i>Picea abies</i>	f. b.	Táborsko region, Bohemia, Czech Republic	(Vohnik et al. 2007)
AJ534700 ^a	Mixed forest dominated by <i>Picea abies</i>	m. r.	Järvselja, Estonia	(Tedersoo et al. 2003)
<i>Hydnотrya tulasnei</i>				
GQ149454 B	<i>Fagus sylvatica</i>	m. r.	Kahlenberg, Germany	(Stielow et al. 2010)
GQ215698 B	<i>Pinus sylvestris</i>	m. r.	Kahlenberg, Germany	(Stielow et al. 2010)
GQ140240 S	<i>Coryllus avellana</i>	f. b.	Freyburg, Saxony-Anhalt, Germany	(Stielow et al. 2010)
AJ969616	<i>Fagus sylvatica</i>	m. r.	Lille Bogeskov, Denmark	(Tedersoo et al. 2006)
AJ969620	<i>Fagus sylvatica</i>	f. b.	Hareskoven, Denmark	(Tedersoo et al. 2006)
AJ969621	<i>Fagus sylvatica</i>	f. b.	Hareskoven, Denmark	(Tedersoo et al. 2006)
EU784276	not specified	f. b.	Surrey, Southern England	(Brock et al. 2009)

^a GenBank entry as *Pezizales sp. B48*

II.6. Case study 3: Ectomycorrhizas in the genus *Sistotrema*

Münzenberger B, Schneider B, Nilsson HR, **Bubner B**, Larsson KH, Hüttl RF (2012) Morphology, anatomy, and molecular studies of the ectomycorrhiza formed axenically by the fungus *Sistotrema* sp. (Basidiomycota). *Mycological Progress* 11:817-826

Summary of results

The publication of Münzenberger et al. (2012) describes studies on a single species within the genus *Sistotrema*. A fungus was isolated from mycorrhized roots of *Pinus sylvestris* in Meuro (Southern Brandenburg, Germany) as early as 1998 and was since then cultivated as EW63 in the culture collection of the ZALF, Müncheberg. It was used for ectomycorrhizal synthesis on axenically grown *Pinus sylvestris* seedlings. Morphology and anatomy was described from the synthesized ectomycorrhizas. ITS sequencing revealed that EW63 was not related to *Amphinema byssoides*, as was first suspected by ectomycorrhizal morphology, but rather to the genus *Sistotrema*. Beate Schneider collected a resupinate *Sistotrema* sp. fruiting body at a different Scots pine stand near Schlabendorf, Southern Brandenburg, in 2008. It proved to be sequence identical with EW63. Morphological analysis of the fruiting body by Karl-Henrik Larsson placed it close to the species *Sistotrema albopallescens*. However, in the phylogenetic analysis of ITS and 28S rDNA sequences performed by R. Henrik Nilsson, *S. albopallescens* and EW63 did not group together. Therefore, EW63 could not be designated a species name. As long as the status of *S. albopallescens* is not clarified by further fruiting body collections it is also not possible to describe the solitary *Sistotrema* sp. fruiting body from Schlabendorf as a new species.

Contributions by the author of this thesis

Anatomical and morphological analysis of the synthesized mycorrhiza EW63 was performed and the result section "Description of ectomycorrhiza" was written by the author of this thesis. It follows the layout of the short descriptions prepared for the ectomycorrhizal types of the Kahlenberg site. The contribution includes the Figs 5 b, c, d. Furthermore, ITS of the cultured fungus was sequenced to confirm sequencing results by Beate Schneider.

The following sections present commentaries and additional results of the thesis author.

Discussion in relation to species delimitation and mycorrhizal status

The paper of Münzenberger et al. (2012) demonstrates the difficulties of species identification but also how much useful information can be generated without species identification. Although BLAST searches resulted in 100% matches of EW63 to ectomycorrhizal sequences they did not match a described fruiting body. Even the discovery

of the fruiting body did not lead to species determination because it only demonstrated that the taxonomy and systematics of the genus *Sistotrema* are in a state of flux that did not justify to designate the name of an existing species or to describe a new species.

Despite the failure to determine a species name, the paper of Münzenberger et al. (2012) constitutes a valuable contribution to the discussion of mycorrhizal status within the genus *Sistotrema*. A first indication that members of genus *Sistotrema* form ectomycorrhizas was published for the species *S. alboluteum* and *S. musicola* (Nilsson et al. 2006) followed by a publication about an unknown *Sistotrema* species which is not identical with *S. alboluteum* or *S. musicola* (DiMarino et al. 2009). In all three species, the ectomycorrhizal status was confirmed by establishing the identity of ITS/28S rDNA sequence in fruiting bodies and mycorrhized root tips. The publication of Münzenberger et al. (2012) goes beyond the results of Nilsson et al. (2006) and DiMarino et al. (2009) by using the principles isolation, cultivation and re-infection to proof that *Sistotrema* sp. EW 63 is a true mycorrhiza-forming fungus.

Nilsson et al. (2006) note that the genus *Sistotrema* is polyphyletic because it contains both saprotrophic and ectomycorrhizal species. (Münzenberger et al. 2012) showed that *Sistotrema* sp. EW 63 belongs to a clade that contains the type species of the genus, *S. confluens* and the ectomycorrhizal species *S. alboluteum* and *S. musicola*. *S. confluens* differs in fruiting body morphology from all other members of the genus *Sistotrema* (resupinate fruiting bodies) by forming both resupinate and stipitate fruiting bodies (Fig. 15a). However, from its placement in the same clade as the ectomycorrhizal species, Nilsson et al. (2006) speculated that *S. confluens* might be ectomycorrhizal. In the following section evidence is presented that *Sistotrema confluens* is indeed ectomycorrhizal.

Short description of a natural *Sistotrema confluens* ectomycorrhiza

Fruiting bodies of *Sistotrema confluens* were collected near Streuberg, Vogtland, Germany on 15.09.2007. The species was identified on the basis of the remarkable fruiting body morphology (Fig. 15a). Fruiting bodies grew next to a road under birch (*Betula pendula*) and aspen (*Populus tremula*). The fruiting bodies were collected together with the first 5 cm of top soil. Morphology and anatomy of ectomycorrhizas was analyzed two days later in the laboratory. Mycorrhized root tips and fruiting bodies were ITS sequenced according to the methods described in Part III. One hundred percent match of root tip and fruiting body sequence indicates that the analyzed ectomycorrhiza is formed by *Sistotrema confluens*. Fruiting body tissue was placed on MMN 1/10 agar as described for EW63 (Münzenberger et al. 2012). Unfortunately cultivation of *Sistotrema confluens* failed.

Morphology: Ectomycorrhizas of *Sistotrema confluens* were monopodial-pyramidally ramified or occurred as solitary tips (Fig. 15b, c). The observed system was 2.7 mm long; the unramified ends were 0.4 to 0.6 mm long and 0.3 mm in diameter. The colour was light yellowish brown and the hydrophobic surface had a silvery shine. Abundant emanating hyphae gave the surface a cottony to woolly appearance.

Mantle anatomy: The mantle is plectenchymatic in all mantle layers. Hyphae of the outer mantle layer are arranged without a special pattern (mantle type B, Fig. 15d) but streaks of parallel hyphae are present. A hyphal net is observed at the mantle surface. It cannot be unambiguously clarified whether the surface hyphae (\varnothing 3.5 μm) are a mantle characteristic or just collapsed emanating hyphae. Hyphae of the outer mantle are mostly cylindrical (\varnothing 3-6.5 μm , length 15-60 μm , wall thickness 0.5 μm). Some cells are irregularly shaped or have slightly inflated ends or constrictions at septa. Hyphae ramify at angles of 120° . Surface of hyphae is smooth and they are colourless to slightly membranously brownish. The arrangement of the middle and the inner mantle hyphae is similar to the outer mantle. Hyphae of the middle mantle layer are shorter (\varnothing 4-6 μm , length 12-25 μm , wall thickness 0.5 μm) and hyphae of the inner mantle layer (\varnothing 4-6 μm , length 10-30 μm wall thickness 0.5 μm) are accompanied by nests of pseudoparenchymatic cells.

Emanating elements: Emanating hyphae (\varnothing 3 μm , more than 100 μm long, wall thickness 0.5 μm) have clamps and ramify at angles of 90° . Ramifications occur one or two hyphal diameters below septum. At dorsal view clamps are cylindrical and have the same diameter as hyphae. In lateral view they have the shape of a semicircle that is as high as the hyphal diameter (Fig. 15f). At secondary septa (without clamps) cells are ampullately inflated (Fig. 15e), but ampullate inflations are also observed at clamps. Cell surface is smooth and the cells are colourless to slightly membranously brownish. Parallel streaks of emanating hyphae were observed, but true rhizomorphs are absent.



Fig. 15: Morphology and anatomy of *Sistotrema confluens* ectomycorrhizas

a) fruiting body, identified by morphology and ITS sequencing

b) ectomycorrhizas beneath fruiting body, 10x, **x** *Sistotrema confluens*, **xx** *Cenococcum geophilum*

c) morphology of ectomycorrhizal root tip, 40x, depicted root was sequenced, sequence identical to fruiting body; the black hyphae (arrow) could belong to *C. geophilum*

d) anatomy: outer mantle layer, 1000x

e) anatomy: emanating hyphae with ampullate inflation (arrow), 1000x

f) anatomy: emanating hypha with clamp, 1000x

Comparison of *Sistotrema confluens* with EW63 and importance of *S. confluence* description

Because of the failure to cultivate *Sistotrema confluens*, the strength of evidence that it forms ectomycorrhiza is comparable to the publication of Nilsson et al. (2006) and DiMarino et al. (2009). This evidence is supported by the mantle characteristics that are very similar between *S. confluence* and EW63. One difference is that mantle cells were shorter and more densely packed in *S. confluens* as compared to EW63. Another difference, mantle thickness (semitransparent for EW63, no cortical cells visible at *S. confluens*), is probably due to ontogenetic age. EW63 was analyzed 15 weeks after inoculation of Scots pine seedlings, the natural ectomycorrhiza of *S. confluens* was of unknown age. However, the occasional occurrence of dark septate hyphae (Fig. 15c) suggests an aging mycorrhiza.

The most conspicuous characteristic which both species have in common is the presence of ampullately inflated ends of emanating hyphae. It is interesting to note that, while (Nilsson et al. 2006) did not include a formal description of *S. alboluteum* and *S. musicola* ectomycorrhizas, they pointed to the presence of ampullate thickenings in mantle hyphae. Ampullate hyphae are also described for the rhizomorphs of an unknown *Sistotrema* species (DiMarino et al. 2009). DiMarino et al. (2009) also speculate that ampullate hyphae are a common feature for all members of the cantharelloid clade to which ectomycorrhizal *Sistotrema* species belong. The observations on *S. confluens* underline the assumption that ampullate hyphae are common for all ectomycorrhizal *Sistotrema* species.

Host specificity within ectomycorrhizal *Sistotrema* species

A remarkable outcome in relation to the thesis topic of host specificity is that the sequence of *Sistotrema* sp. EW63, though lacking a species name, appeared in several other sequence based ectomycorrhizal studies (Table 2). All ITS sequences originate from mycorrhized root tips and were 100% identical to the fruiting body sequence of *Sistotrema* sp. EW63 from Schlabendorf (accession no. FR838002). The sites are located in Europe, Southern Asia and Southern North-America. Thus, the fungus is almost globally distributed. Despite the wide geographic range all sites have in common that a *Pinus* species is the dominating forest tree and the only ectomycorrhizal host tree. The two stands of *Pinus sylvestris* (Schlabendorf and Culbin Forest) are even monoculture stands. This indicates that *Sistotrema* sp. EW63 has an affinity to the host genus *Pinus*.

The status of pine affinity deserves further attention, especially in comparison to other ectomycorrhizal *Sistotrema* species. The described *S. confluens* was found under deciduous trees (*Betula pendula* and *Populus tremula*) while *S. alboluteum* and *S. musicola* grew in mixed forest composed of Pinaceae and deciduous trees in Estland and Finland (Nilsson et al. 2006). Furthermore, the unknown *Sistotrema* species of DiMarino et al. (2009) was described from the deciduous tree *Castanea sativa*. This indicates that altogether four ectomycorrhizal *Sistotrema* species have an affinity to angiosperms or are at least not specialized to the genus *Pinus*. Whether *Sistotrema* sp. EW63 has a host specificity that deviates from the remaining ectomycorrhizal *Sistotrema* species can only be clarified by further fruiting body collections with information on host identity. In mixed forests whose hosts cannot be distinguished by

fine root morphology (as is the case for *Betula* and *Populus*), the host has to be determined by sequencing plant DNA directly from the mycorrhized root.

Table 2: Hosts of identical ITS sequences in GenBank

All sequences originate from ectomycorrhizal root tips and are identical with the fruiting body sequence of *Sistotrema* sp. EW63 (FR838002). All locations are stands where *Pinus* spp. is the only ectomycorrhizal host.

Accession No.	Host	Site	Reference
FR865901	<i>Pinus sylvestris</i>	Schlabendorf, Germany	(Münzenberger et al. 2012)
GU289428	<i>Pinus sylvestris</i>	Culbin Forest, Scotland	(Pickles et al. 2010)
DQ822795	<i>Pinus muricata</i>	Point Reyes, California	(Peay et al. 2007)
HM021160	<i>Pinus muricata</i>	Point Reyes, California	(Peay et al. 2011)
AB587739	<i>Pinus thunbergerii</i>	Kangwon-do, South Korea	(Obase et al. 2011)

II.7. Match of sequence types to described ectomycorrhizas

Application of the match of sequences and ectomycorrhizal descriptions (morphotypes)

The preceding three sections (II.4 to II.6) illustrated how via sequencing and phylogenetic analysis a species name can be assigned to an unknown mycorrhiza. However, in most ectomycorrhizal studies more root tips are collected than can be sequenced. Therefore, many ectomycorrhizal studies rely on a combination of morphotyping and sequencing (e.g. Richard et al. 2005; Twieg et al. 2009; Pickles et al. 2010; Lang et al. 2011). By that method most collected root tips (usually several thousand) are differentiated as morphotypes and only a subset of the collected root tips of each morphotype (3-10) is then sequenced. If all sequenced tips of a morphotype yield the same sequence, the remaining root tips of this morphotype are considered to have the same sequence.

In the two studies presented in part III and IV of this thesis, the number of collected root tips was reduced with the aim to sequence all collected root tips. However, either the PCR or the sequencing reaction failed so that not all collected root tips yielded a valid sequence. In part III, 426 of 661 collected root tips could be sequenced and identified as ectomycorrhizal fungi (64.4 %). In part IV, only 39 of 87 collected root tips could be sequenced and identified as ectomycorrhizal fungi (44.8 %). Since all collected root tips have been preselected on the basis of morphological data, the mycorrhized tips without sequences could be identified if their morphotypes could unambiguously assigned to a species as identified by sequencing. This is only possible, if there is a good match of identifications by morphotype and sequencing data in the datasets of 426, respectively 39, sequenced ectomycorrhizal root tips.

Match of sequences and morphotypes at all four stands at Kahlenberg site

Altogether 426 root tips could be sequenced at all four stands of Kahlenberg site. The complete analysis can be found in part III. Here the goodness of match between morphotype and sequence data will be shown for eighteen ectomycorrhizal species. These eighteen species are the species with more than four identified root tips. In most cases more than one morphotype has been distinguished for a single species. Table 3 lists the collected morphotypes for each sequenced species. The goodness of match between morphotype and sequence data for a selected species is presented by the percentage of the most frequent morphotype among the sequenced root tips (Table 3, column 3).

In only one species, *Cenococcum geophilum*, all sequenced root tips belong to a single morphotype (in this case BB05) so that match between sequences and morphotypes is 100 %. *Acephala macrosclerotiorum* is a species with one mismatch between sequence identification and morphotype. One of the 25 root tips was collected as BB05. The remaining root tips were collected as morphotype BB11, that is, 96% of all sequenced tips belong to the most frequent morphotype BB11. The single misidentification as BB05 can be explained by the similarity of BB05 and BB11. Both morphotypes feature pitch black emanating hyphae so that confusion is not unlikely.

Table 3: Match of sequencing data and morphotypes at all four stands

Data originate from all four stands at Kahlenberg site. Only species with more than four collected root tips are listed. Number behind species or morphotype names indicate the number of collected root tips. The column morphotypes lists the morphotype names under which the sequenced species was collected. In each line the most frequent morphotype is listed first, the following morphotypes appear in descending order according to the number of root tips. Root tip numbers behind morphotype names (middle column) sum up to the sequenced root tip number of the corresponding species (left column). In the right column the most frequent morphotype is listed separately together with its percentage among the sequenced root tips.

* These morphotypes are not covered by the section on ectomycorrhizal type descriptions (section II.2). They were only differentiated on the basis of morphological appearance (i.e. type of ramification, colour, surface structure, presence of rhizomorphs or emanating hyphae), in order to preselect root tips for sequencing.

species as identified by sequencing	morphotypes among the root tips of a sequenced species	percentage of the most frequent morphotype
<i>Cenococcum geophilum</i> : 9	BB05: 9	BB05: 100%
<i>Acephala macrosclerotiorum</i> : 25	BB11: 24, BB05: 1	BB11: 96%
<i>Cortinarius (Dermocybe) sp1</i> : 11	BB24: 10, BB28: 1	BB24: 91%
<i>Xerocomus cisalpinus</i> : 35	BB28=BB08: 29, BB06: 5, BB37: 1	BB28: 83%
<i>Laccaria amethystina</i> : 5	BB75=BB37: 4, BB100: 1	BB75: 80%
<i>Genea hispidula</i> : 7	BB82: 5, BB05: 1, BB52*: 1	BB82: 71%
<i>Xerocomus badius</i> : 33	BB06: 22, BB28=BB08: 6, BB22: 1, BB23:1, BB24:1, BB80:1 B84:1	BB06: 67%
<i>Russula ionochlora</i> : 35	BB72: 21, BB28=BB08: 10, BB38: 2, BB06:1 BB22:1	BB72: 60%
<i>Russula ochroleuca</i> : 56	BB14: 31, BB70: 5, BB39*: 3, BB45: 3, BB54*: 2, BB06: 2, BB22: 1, BB26*: 1, BB27*: 1, BB28=BB08: 1, BB34*: 1, BB38: 1, BB52*: 1, BB53: 1, BB60*: 1, BB61*:1	BB14: 55%
<i>Lactarius subdulcis</i> : 11	BB45: 6, BB22:1, BB26*: 1, BB39*: 1, BB70: 1, BB96: 1	BB45: 55%
<i>Xerocomus pruinatus</i> : 23	BB28: 12 BB06: 7, BB14: 1, BB22: 1, BB23: 1, BB 61*: 1	BB28: 52%
<i>Russula vesca</i> : 5	BB72: 2, BB06: 1 BB38: 1, BB28=BB08: 1	BB72: 40%
<i>Thelephora terrestris</i> : 10	BB23: 4, BB06: 1, BB14: 1 BB19*: 1, BB20*: 1, BB64*: 1, BB88: 1	BB23: 40%
<i>Lactarius tabidus</i> : 27	BB22: 10, BB38: 10, BB45: 3, BB23: 1, BB26*: 1, BB39*: 1, BB61*: 1	BB22: 37% BB38: 37%
<i>Laccaria cf. laccata</i> : 16	BB75=BB37: 5, BB34*: 4, BB38: 3, BB28=BB08: 2, BB43* :1, BB92: 1	BB75: 31%
<i>Russula puellaris</i> : 13	BB53: 4, BB61*: 2, BB14: 1, BB22: 1, BB38: 1, BB45: 1, BB57: 1, BB63*: 1, BB91:1	BB53: 31%
<i>Tomentella sublilacina</i> : 39	BB22: 8, BB38: 6, BB88: 5, BB39*: 4, BB53: 4, BB75=37: 3, BB06: 2, BB61*: 2, BB71: 2, BB34*: 1, BB42*: 1, BB23: 1	BB22: 21%
<i>Hydnotrya tulasnei</i> : 23	BB34*: 4, BB27*: 3, BB38: 3, BB26*: 2, BB39*: 2, BB81*: 2, BB85: 2, BB14: 1, BB22: 1, BB51*: 1, BB52*: 1, BB70: 1	BB34: 17%

Apart from *C. geophilum* and *A. macrosclerotiorum* only three further species have a high correspondence of sequence and morphotype (Fig. 15). In these five species at least 80% of the sequenced root tips belong to the most frequent morphotype, so that this morphotype can be considered to represent a single species. Next to the species group of high correspondence, a species group of low correspondence and a group of no correspondence of sequence and morphotype can be defined (Fig. 15). The group of low correspondence comprises six species in which 50-80 % of the sequenced root tips belong to the most frequent morphotype. Despite high number of mismatches, the most frequent morphotype can still be considered as typical for the species. The group of no correspondence comprises seven species in which less than 50% of the sequenced root tips belong to the most frequent morphotype. Because of the lack of dominance of a single morphotype, it is not possible to determine a morphotype that represents the sequenced species.

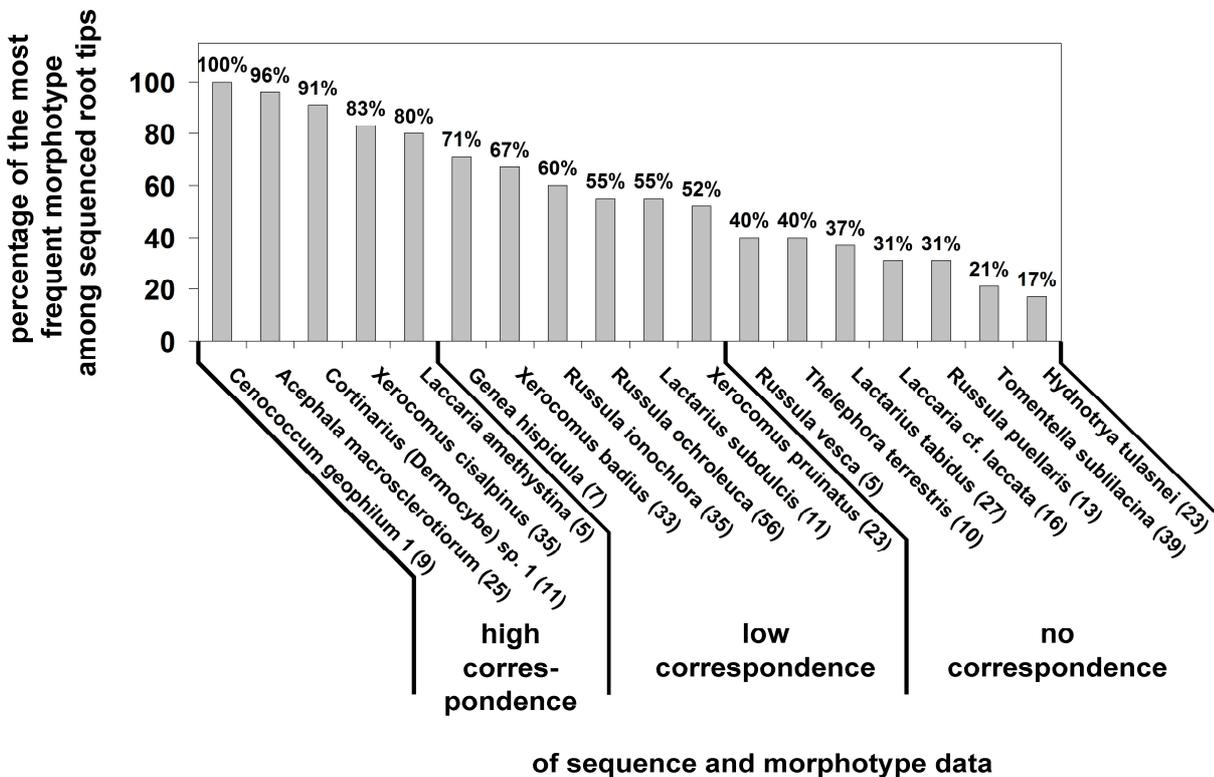


Fig. 15: Three groups of species according to the percentage of the most frequent morphotype within a species

18 species are sorted in groups based on the data presented in Table 3. The data originates from all four stands at Kahlenberg site (Part III). The number in parentheses represents the number of sequenced tips per species.

Species with interesting mismatches of sequences and morphotypes

Two *Xerocomus* species (*X. badius* and *X. pruinosus*) belong to the species group of low correspondence of sequences and morphotypes (Fig. 15). The main reason for the mismatch of sequence and morphotype data is that the *Xerocomus*-like mycorrhizas have been collected

as two ectomycorrhizal types, BB06 and BB28, which were only separated on the basis of slight colour differences. If only a single *Xerocomus* morphotype would have been distinguished, the match between the sequenced root tips of a *Xerocomus* species and the corresponding morphotype would have been 100%. However, because there are three frequent *Xerocomus* species present at Kahlenberg site (see Part III) this single morphotype would have been comprised three true species. It has to be concluded that the *Xerocomus* species cannot be distinguished by morphotypes.

Hydnotrya tulasnei and *Tomentella sublilacina* are found in the species group of no correspondence of sequences and morphotypes. They have not been recognized as morphotypes at all, neither during the morphotyping campaigns of this study nor in the morphotype based predecessor study (Rumberger et al. 2004; Rumberger 2005). Their recognition was also complicated by absence of descriptions in the online key DEEMY. Their presence and abundance on Kahlenberg site, *T. sublilacina* is the second most frequent species (see Part III), has been only recognized after sequencing. *H. tulasnei*, although identified as well defined species by phylogenetic analysis (see section II.5), could only be described after comparison of ectomycorrhizal type descriptions and the sequencing results. It is a smooth ectomycorrhiza of ochre colour that changes from light grey to dark grey (ectomycorrhizal types BB81 and 85, Fig. 10a, 11a). The main difference in comparison to many other smooth ectomycorrhizas of the *Russula/Lactarius* group is the epidermoid mantle. This is an explanation why the list of *Hydnotrya* sequences contains so many different morphotypes (Table 3). These morphotypes are all smooth ectomycorrhizas of different colours, but the distinctive mantle characteristics remained unnoticed.

A similar case is observed in *Tomentella sublilacina*. Despite its widespread occurrence in ectomycorrhizal ITS RFLP studies (Kõljalg et al. 2000), no formal description including anatomical characters exists for this species (but see description of *Tomentella cf. sublilacina* on *Alnus acuminata* (Pritsch et al. 2010)). Although slightly hydrophobic (Fig. 11d, e), in the absence of a silvery shine (Fig. 8e, 11f), the morphology is similar to many other smooth ectomycorrhizal types. An additional complication is the regular occurrence on both hosts. As illustrated for *R. ochroleuca* below, different hosts contribute to the variability of ectomycorrhizal morphology. Therefore, *T. sublilacina* sequences have been collected as many different morphotypes.

Match of sequences and morphotypes in three selected soil cores

The data in Table 3 includes not only morphotypes differentiated according to the ectomycorrhizal descriptions in section II.2 (including anatomical characteristics) but also root tips that were distinguished only on the basis of morphological appearance (ramification, colour, mantle surface). Therefore, it could be speculated that the match of sequences and morphotypes is low, because the morphotypes were not thoroughly enough distinguished. Table 4 presents a different dataset of sequences and morphotypes. These root tips were collected for the analysis presented in Part IV. All root tips stem from three soil cores of the pure beech stand and were collected during the field campaign of 2007. The methodological

difference to the data in Table 3 is that all collected root tips were classified according the 34 ectomycorrhizal type descriptions of section II.2, including anatomical characteristics.

Due to the low number of soil cores, only seven different species could be identified by sequencing. The equal number of seven ectomycorrhizal types promises a good match of sequences and morphotypes (Table 4). However, this was only partially true. While two species could be reliably distinguished by morphotypes (100% match), the next best species, *R. ochroleuca* matches with four out of five tips to the ectomycorrhizal type BB70. This is remarkable because the morphotype BB14 and not BB70 was considered to represent the morphology of *R. ochroleuca* ectomycorrhizas (see below). If the morphotype BB28 and BB06 would have been collected as a single *Xerocomus*-like morphotype the match with the *Xerocomus cisalpinus* would have been 100% and not 57% as is Table 3. However, a single *Xerocomus*-like morphotype would have also comprised *Xerocomus pruinatus*, which was collected as BB28. Thus, even with a more detailed morphotype analysis, the distinction of *Xerocomus* species remains problematic.

Another problem that cannot be solved with anatomical analysis is the distinction of smooth ectomycorrhizas of the *Lactarius/Russula* type. *Lactarius subdulcis* has been collected as three different ectomycorrhizal types. Two of those, BB22 and BB45, were considered to belong to two different types of *Lactarius*, according to the ectomycorrhizal type descriptions in section II.2. The third ectomycorrhizal type, BB70, was considered to be a *Russula* with similarities to *R. ochroleuca*. This means that the laticifers that are typical for a *Lactarius* species have not been recognized at the two ectomycorrhizal root tips collected as BB70. It is possible that in some *Lactarius* species the laticifers in the ectomycorrhizal mantle are less prominent than in others.

Following conclusion can be drawn from the data in Table 4: although the number of mismatches is lower when all collected root tips are distinguished on the basis of detailed descriptions (as compared to the data in Table 3), the match between morphotypes and sequence type is not sufficient to identify all species by their morphotypes.

Table 4: Correspondence of sequencing data and morphotypes in three soil cores

The data originate from a detail study of three selected soil cores collected in the beech pure stand (part IV). The morphotypes have been separated on the base of morphological and anatomical characters. Numbers behind species or morphotype names indicates the number of analyzed root tips.

species as identified by sequencing	morphotypes among the root tips of a sequenced species	percentage of the most frequent morphotype
<i>Laccaria cf. laccata</i> : 10	BB75: 10	BB75: 100%
<i>Russula ionochlora</i> : 11	BB72: 8	BB72: 100%
<i>Russula ochroleuca</i> : 5	BB70: 4, BB06: 1	BB70: 80%
<i>Russula sp. aff. ionochlora</i> : 3	BB28: 2, BB06: 1	BB28: 66%
<i>Xerocomus cisalpinus</i> : 7	BB06: 4, B28: 3	BB06: 57%
<i>Lactarius subdulcis</i> : 5	BB45: 2, BB70: 2, BB22: 1	BB45: 40%
<i>Xerocomus pruinatus</i> : 1	BB28: 1	-

Mismatch of sequences and morphotypes for *Russula ochroleuca*

Possible reasons for mismatch between sequence and morphotypes are illustrated in the case of *R. ochroleuca*. It is supposed to form one of the most typical ectomycorrhizal morphotypes with yellow flocks on a yellow surface (Pillukat and Agerer 1992). However, its yellow colour is more apparent on Scots pine roots than on beech roots (Fig. 16a, b). Both ectomycorrhizal morphotypes in Fig. 16a and Fig. 16b have been collected as the morphotype BB14, which, as exception from the rule, could also be identified as species from morphological appearance. Despite the clear morphological characteristics of BB14, only 55% of the root tips sequenced as *R. ochroleuca* have been morphologically identified as *R. ochroleuca*. Many *R. ochroleuca* root tips had a morphotype diagnosis other than BB14. Fig. 16c shows an *R. ochroleuca* mycorrhiza that has been collected as morphotype BB39, which encompasses dark mycorrhizas with a smooth surface. The mycorrhized root tip probably became darker and lost its typical flocks in the aging process.

Besides complete misinterpretation because of different colour, some morphotypes distinctions were questionable from the point of description. Ectomycorrhizal type BB70 has been both described as morphotype and anatomotype from beech roots (Fig. 1e). It was noted in the description, that apart from the lacking flocks on the surface the anatomy was very similar to BB14 on beech roots. Figs. 16d and Fig. 16e shows two mycorrhizas collected as BB70 from different soil cores in the pure beech stand. Although their surfaces were smooth they were sequenced as *R. ochroleuca*. It seems that presence or absence of flocks is not an unambiguous characteristic to distinguish *R. ochroleuca* from similar smooth ectomycorrhizal types. This corresponds to an earlier report of the occasional absence of yellow flocks on *R. ochroleuca* ectomycorrhizas (Pillukat and Agerer 1992).

Another reason for misinterpretation of morphotypes is again related to ontogenetic stage. The morphotype BB60 (Fig. 16f) had no special characteristics apart from having a very thin mantle. It has been speculated at its collection that it represents the beginning of the mycorrhization process of a new root tip that not yet had developed specific characteristics. After sequence based identification as *R. ochroleuca* and not another unknown species, the microphotograph in Fig. 16f has been re-examined. It can be observed that the base of the swollen part of the root turns already yellow. Thus BB60 is indeed an early stage of the species *R. ochroleuca* which lacks the complete mantle and the characteristic yellow flocks.

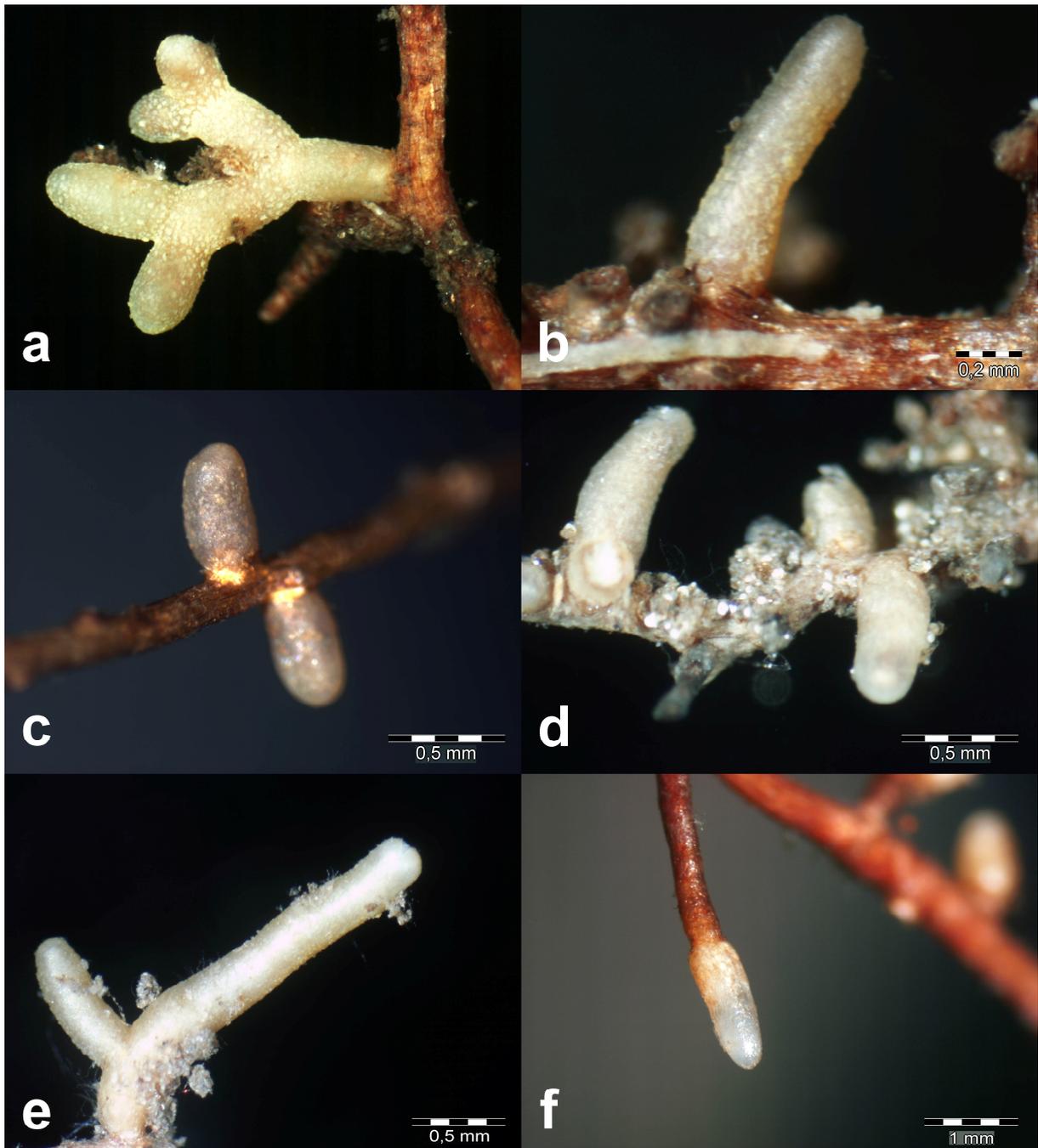


Fig. 16: Different ectomycorrhizal types are identified as the same species: *Russula ochroleuca*

- a) BB14 magnification n. a.; Scots pine; this ectomycorrhiza has been identified by morphology as *R. ochroleuca*
 b) **BB14*** 60x; beech; root tips from this root system have been used for the ectomycorrhizal type description in section II.2
 c) **BB39*** 50x; beech
 d) **BB70*** 50x; beech; root tips from this system have been used for ectomycorrhizal type description
 e) **BB70*** 40x; beech
 f) **BB60*** 20x; Scots pine; beginning of the mycorrhization

* molecular identification from the depicted root

II.8. Discussion

Reliance on sequences

A basic problem for all ectomycorrhizal studies is not all collected mycorrhized root tips can be analyzed by molecular methods. This would be unpractical and is often unnecessary. However, the approach of analyzing only a subset of all collected mycorrhized tips is only acceptable if there is a good match between morphotype and molecular data. In the best case, there should be a 1:1 match, i. e. all mycorrhizas with the same sequence (or same RFLP pattern in older reports) should belong to the same morphotype and vice versa. In published reports this agreement is very different. It reaches from very good (e.g. Baar et al. 1999; Grogan et al. 2000; Sakakibara et al. 2002; Twieg et al. 2007) over sufficient for ecological analysis (e.g. Rosling et al. 2003; Wang and Guo 2010) to very bad (e.g. Jonsson et al. 2000; Mah et al. 2001). For example, Mah et al. (2001) reports 22 ITS RFLP genotypes from 8 morphotypes so that each morphotype comprised three putative species. The extent of this mismatch is comparable to the results in section II.7 (Table 3 and Table 4).

Note that all above mentioned studies used a combination of morphotyping and molecular methods. This means that the ecological analysis is based on counted root tips that were distinguished by morphotypes. These morphotypes were subsequently identified by ITS sequences or RFLP patterns. In these studies a natural bias exists toward a good match of morphotypes and molecular data because a bad match means that the ectomycorrhizal species could not be identified by morphotyping which in turn makes it difficult to draw ecological conclusion from the morphotype data. Thus, it is possible that results with bad match of morphotypes and molecular data do not reach the stage of publication.

The match of sequences and morphotypes as presented in section II.2 (Table 3 and 4) is so low, that a morphotype cannot be represented by a single sequence type. Therefore, it is not possible to assign a species name to those root tips that could not be sequenced (235 unidentified root tips in Part III, 48 unidentified root tips in part IV). Since correct species assignments are important for the arguments in Part III and IV, only sequenced root tips can be used in the ecological analyses of Part III and IV.

A plea for morphology and anatomy

The decision to rely for analyses in Part III and Part IV only on sequenced tips is not an argument for neglecting knowledge on ectomycorrhizal morphology and anatomy. Quite the opposite is true. Only a minority of studies will be supported by enough funding to sequence the ten thousands of root tips that were usually counted in morphotype based community studies. Therefore, studies will rely on sampling schemes that allow reducing the number of collected root tips to an amount that can be readily sequenced.

Within the last years several studies have been published that base their analysis only on sequenced root tips. In order to circumvent the problem with the match of sequences and morphotypes these studies do not distinguish morphotypes at all (e.g. Kjølner and

Clemmensen 2009; Kennedy and Hill 2010; Peay et al. 2010; Peay et al. 2011). Instead, they use random sampling schemes. For instance, the first 8-10 root tips of a soil core encountered under a dissection microscope are collected and sequenced (Kennedy and Hill 2010; Peay et al. 2011). This means, although these studies collect hundreds of sequences, they cannot compare the match of sequences and morphotypes. Thus, to the knowledge of the thesis author, Table 3 presents the first comparison of sequences and morphotypes for a completely sequenced dataset.

While the thesis author assumes that the number of ectomycorrhizal community studies sequencing all collected root tips will increase, he doubts that all ecological questions can be solved with random sampling schemes, because randomization means that more and more root tips have to be collected for useful statements on biodiversity. A solution to reconcile the requirements of complete sequencing and the constraints of limited resources is the pre-selection of sequenced root tips on the basis of morphological and anatomical characteristics.

One selection scheme that includes distinction of morphotypes is to collect one root tip per morphotype in a soil core. This is the method that is applied in Part III and Part IV of this study. When using this sampling scheme the capability to distinguish morphotypes is essential. If fewer morphotypes are recognized than are actually present in a soil core, sequencing will not represent the true biodiversity. However, in cases of hardly distinguishable morphotypes, e. g. morphotypes within the genera *Russula*, *Lactarius*, *Xerocomus*, species distinction is often impossible. It can be helpful to observe small differences in colour or general habit to collect more morphotypes that can be distinguished with certainty. This oversampling has been used of instance by Lilleskov et al. (2002) and was also partially applied by the thesis author. If the small colour differences do not represent species differences, more morphotypes will be collected than species are present, as has been illustrated for *R. ochroleuca* (Fig. 16). Thus, the improved coverage of biodiversity will be traded against an increased mismatch of sequences and morphotypes.

In order to improve the distinction of morphotypes, it is necessary to increase the knowledge on their morphology and anatomy. It is conspicuous that one of the best matches of sequences and morphotypes was obtained for *Acephala macrosclerotiorum* (Table 3). Because of its description as new species (section II.4) it drew attention to the thesis author, so that it was safely distinguished from the other ectomycorrhizal fungus with dark septate hyphae (*Cenococcum geophilum*). In this respect, it is not only important to record outstanding characters (in the case of *A. macrosclerotiorum* the sclerotia) but also the morphological/anatomical variability (in the case of *A. macrosclerotiorum* the occasional lack of sclerotia). Knowledge on the variability would have also helped to reduce the number of morphotypes that have been collected for the species *R. ochroleuca* or to recognize the morphotypes of *Hydnotrya tulasnei* and *Tomentella sublilacina*. The improvement of the match of sequences and morphotypes from Table 3 to Table 4 demonstrates that the knowledge of anatomical characteristics help to increase the accuracy of morphotype selection.

The best way to increase our knowledge on the variability of anatomy/morphology is to publish comparisons of sequence data and morphotypes. At the introduction of molecular

techniques to ectomycorrhizal community studies it was usual practice to publish the match between ITS-RFLP pattern and the collected morphotype (e.g. Sakakibara et al. 2002). Unfortunately, in many recent ectomycorrhizal community studies only the sequencing data are presented even if a combination of morphotyping and sequencing is used (e.g. Ishida et al. 2007; Tedersoo et al. 2008). With neglect of morphology a chance is lost to improve our knowledge on anatomical and morphological variability. Only knowledge on ectomycorrhizal variability will improve the match of morphotypes and sequence types in the pre-selection process and in the end the precision of sequence based conclusions.

Thesis Part III

Manuscript 1

The concept of specificity guilds reveals dominance of host specific fungi in pure and mixed stands of *Pinus sylvestris* L. and *Fagus sylvatica* L.

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III.1. Abstract

There have been long lasting debates whether ectomycorrhizal communities are dominated by generalist or host specific fungi. In this study we apply the concept of specificity guilds to determine the ratio of generalist and host specific fungi in two mixed stands and one pure stand each of Scots pine (*Pinus sylvestris*, L.) and beech (*Fagus sylvatica* L.) in north-eastern Germany. Ectomycorrhizal partners were determined to species level by ITS sequencing, database comparison and phylograms. We assigned fungal species to three specificity guilds: generalist, pine specific and beech specific. These assignments were based on the relative association with the two hosts in the mixed stands. The majority of species, i.e. 67%, belonged to host specific guilds (five pine specific species, seven beech specific species), 33% were generalists (six species). Most fungi of the host specific guilds expressed preferences, i.e. in the mixed stands they also colonized non-target roots while these host preferring fungi were absent from the pure stand of the non-target host. This interesting behaviour may indicate improved competitiveness of fungi when associated with their preferential hosts. Only four out of 18 species associated exclusively with either pine or beech roots, i.e. they are specialists. The most notable beech specialist is *Laccaria cf. laccata* although *L. laccata s.l.* is traditionally considered a generalist. Phylogenetic analysis suggests that the *L. laccata* complex consists of cryptic species that belong to different host specific guilds. This demonstrates the importance of in depth delineation of fungal species by phylograms for assigning specificity guilds.

keywords:

cryptic species, ITS sequencing, *Laccaria laccata*, phylogram, specificity guild, *Xerocomus*

III.2. Introduction

Ectomycorrhizal fungi form associations with roots of selected conifer (Pinaceae) and broad leaved trees (mainly Fagales) in the temperate and boreal zones (Smith and Read 2008). There have been long lasting debates on the question of specificity among the fungus-plant associations especially whether ectomycorrhizal communities are dominated by generalist (Horton and Bruns 1998; Selosse et al. 2006; Read 1997) or host specific fungi (Dickie 2007; Smith et al. 2007; Ishida et al. 2007). Information on tree species in the neighbourhood of fruiting bodies as provided by local floras served as basis for compilations of host specificities (Molina et al. 1992; Trappe 1962; Newton and Haigh 1998; Halling 2001). With the use of data from pure stands of different host species, generalist fungi were distinguished from host specific fungi associating with a limited number of hosts (Lee and Kim 1987; Buée et al. 2011).

The disadvantage of fruiting body surveys is that ambiguous host assignment and presence/absence data can lead to overestimation of generalists. Molecular identification in combination with morphotyping allows distinguishing ectomycorrhizal fungi directly on the roots. In studies of mixed stands it was discovered that many fungal species express specificity phenomena as preferences for certain host trees (Tedersoo et al. 2008; Ishida et al. 2007; Lang et al. 2011; Smith et al. 2009). Preference means that a fungus is found mainly on roots of one host with occasional occurrences on accompanying tree species.

It is not clear whether host preferences in molecular root focused studies correspond to the concept of host specificity developed in fruiting body surveys. A direct comparison between both types of studies is complicated by the fact that in many molecular studies, only a fraction of sequences was determined to species level (see Ishida et al. 2007; Dickie and Moyersoen 2008; Tedersoo et al. 2008; Peay et al. 2010). By improving molecular fungal identification by use of phylograms our key question about specificity focused on whether a host preferring fungus from mixed stands can be found in pure stands of the non-preferred host. This question can be answered by exploring neighbouring ectomycorrhizal communities both in mixed and pure stands.

In this study we supplement a survey of ectomycorrhizal communities in two mixed stands of Scots pine (*Pinus sylvestris*) and beech (*Fagus sylvatica*) with surveys in neighbouring pure stands of pine and beech. Comparison with existing data on specificities is improved by explicit species identification of the molecular data. We rely not only on database matches for species identification but construct phylograms for each fungal species. We suggest and use the concept of “specificity guilds” uniting the concepts of host specificity and host preferences for exploration of specificity phenomena among beech and pine.

III.3. Material and methods

Site description

The study area is situated in the North-eastern Lowlands of Germany (52°52'N, 13°53'E) approx. 100 km northeast of Berlin. This area would be naturally covered by beech forest (Bohn and Neuhäusl 2003; Jenssen et al. 2007) but forestry has promoted pine plantations for the last two hundred years leading to a coexistence of pine, beech and mixed stands (Fig. 1). Four stands at max. one km distance were chosen: young mixed stand (pine 83 y./beech 40 y.), old mixed stand (pine 121 y./beech 80 y.), pure pine (91 y.), pure beech (108 y., ages valid as of 01.01.2007). The mean annual precipitation of the area varies from 562 to 577 mm and the mean annual temperature is 8.3°C (minimum in January -17.1 °C). The soil type in all four stands was classified as weakly podsollic cambisol (Rumberger et al. 2004).

Sampling procedure

At each of the four stands samples were collected along an 81m transect with 10 transect points at a distance of 9 m. Soil samples were collected with a soil corer (6 cm wide, 40 cm long) during four field campaigns: spring 2006 (May), fall 2006 (October), winter 2007 (January), and spring 2007 (April/May). Each sampling took place at the same transect points with 50 cm distance between the cores of the four collecting dates. Soil cores were divided into four vertical compartments (Of, Oh, A, B) and stored in separate plastic bags at 4°C. All ten soil cores of a stand were collected on the same day and processed in the laboratory within a week. Deviating from this general procedure, in winter 2007 only three transect points per stand were sampled.

In order to prepare selection for sequencing, root tips were sorted under a stereo-microscope and divided into morphotypes according to branching, colour, texture and presence of emanating hyphae or rhizomorphs. Morphotypes were assigned to exploration types according to Agerer (2001). Roots from the mixed stands were sorted according to host species. Distinction of pine and beech roots was based on root morphology. All morphotypes found in winter and spring 2007 were also characterized as anatomotypes, i.e. by mantle preparation and microscopy with an Axioskop microscope (Zeiss, Oberkochen, Germany), according to the principles demonstrated in Agerer (1987-2002) and the online key DEEMY (<http://www.deemy.de>). Representative sequencing of root tips in a soil core followed two differing sampling schemes. In spring and fall 2006 one tip per morphotype was sequenced. In winter and spring 2007 one tip per morphotype and vertical compartment (Of, Oh, A, B) was sequenced.

Amplification and sequencing of the ITS-region

Single mycorrhizae were homogenized using glass micro mortar and pestle. DNA was extracted using DNeasy Plant Mini Kit (Quiagen, Hilden, Germany), PCR was performed

with Accuprime[®] Taq Polymerase System (Invitrogen, Karlsruhe, Germany) using the supplied buffer II with following final concentrations: 1.5 mM MgCl₂ and 0.2 mM of each dNTP and 500 nM of each of the forward primer ITS1F (Gardes and Bruns 1993) or ITS1 (White et al. 1990) and the reverse primer ITS4 (O'Donnell 1993). The total volume of the reaction was 25 µl, comprising 5µL of template DNA. A second PCR assay used Recombinant Taq Polymerase (Invitrogen) with the same primers but 4 mM MgCl₂ final concentration and 50 µl total volume. The thermocycler, a GeneAmp[®] PCR system 9700 (ABI, Darmstadt, Germany), was programmed as follows: 3 min denaturation at 94°C, ten cycles with decreasing annealing temperature (94°C for 30 s, 60–50°C for 45 s, and 68°C or 72°C for 60 s), 35 cycles with constant annealing temperature (94°C for 30 s, 50°C for 45 s, and 68°C or 72°C for 60 s), and 7 min strand completion at 68°C or 72°C. PCR-products were sent to GATC Biotech AG (Konstanz, Germany) for sequencing with primers ITS1F/ITS1 and ITS4. Sequences were assembled with the Lasergene[®] Software Package (DNASTAR, Madison, USA).

Species determination by phylograms

Sequences were grouped into sequence types (= molecular operational taxonomical units) with at least 99 % identity. The UNITE database (Kõljalg et al. 2005, <http://unite.ut.ee>) was searched using the BLAST algorithm. Fungal groups not represented in UNITE, were searched in GenBank (Zhang et al. 2000, <http://www.ncbi.nlm.nih.gov>). Sequence types matching a database entry with at least 99% were given a preliminary species name, for sequences with less than 99% match the name of the next related genus or family was given. To confirm species names, phylograms were constructed using reference sequences from UNITE or sequences published in the taxonomic literature for the corresponding group. Only when both sources were not available GenBank results from the BLAST search were used. Sequences were aligned with the ClustalW algorithm implemented in the program BioEdit (Hall 1999) version 7.0.9.0. Alignments were edited and abridged to contain only the ITS region. Using the program PAUP* 4.0b10 (Sinauer, Sunderland, USA) a Neighbour-Joining analysis with 1000 bootstrap replications was performed. For comparison reasons selected alignments were analyzed under Maximum-Likelihood criterion using the RAxML web server, version 7.0.4 (Stamatakis et al. 2008). One hundred bootstrap replicates were computed via the web server. Both kinds of analysis resulted in unrooted trees that were visualized using the program TreeGraph 2 (Stöver and Müller 2010). Based on phylogram supported species names literature was screened for fruiting body reports on host specificity of the corresponding species.

Creation of specificity guilds and designation of fungal species therein

In this study a fungus is defined as host preferring when at least 75% of root tips are found on a single host in the mixed stands. It often remains unclear whether a group of host preferring includes also specialists, i.e. fungi that grow exclusively on one host (Tedersoo et al. 2008; Ishida et al. 2007; Lang et al. 2011; Smith et al. 2009). In this study we explicitly distinguish

host preferring fungi that are found occasionally on non-target hosts from host specialist. In observing pure and mixed stands specialists can be further differentiated:

1. *specialist/mixed stands*: The fungus is found exclusively on one host both in the corresponding pure stand and in the mixed stands

2. *specialist/pure stands*. The fungus is found exclusively on one host, and occurs only in the corresponding pure stand.

In order to avoid confusion with the broader usage of the term preference we introduce the term specificity guild. A host specific guild comprises three groups of fungi: host preferring fungi as defined above, specialists/mixed stands and specialist/pure stands. In following the tradition of assessing specificity phenomena on a continuum from specific to unspecific (Molina et al. 1992) we include a generalist guild into the concept of specificity guilds. The generalist guild is reserved for species with a frequency of more than 25% or less than 75% for both hosts in the mixed stands. According to the tree species on our sites, three specificity guilds are distinguished: pine specific guild, beech specific guild and generalist guild (pine + beech).

III.4. Results

Species determination

We analyzed 661 mycorrhizae. PCR products could be obtained from 539 tips. Up to three PCR assays with slightly different conditions (primers, polymerase) were run until a sample was considered to yield no PCR signal. Readable sequences could be generated for 463 PCR products (64.4 %) of which 426 were considered to belong to ectomycorrhizal fungi. Forty sequence types were distinguished by 15 phylograms (Supplementary Fig. S1-15). 31 types could be designated to a species name or at least to close affiliation. Seven sequence types could be assigned to genus level. One sequence type was identified on the family level (Pezizaceae) and one sequence type could be grouped to corticioid fungi (Table 1).

A 100% database match with UNITE references did not lead to unambiguous species identification of *Laccaria cf. laccata*. Although three UNITE-sequences of *L. laccata* clustered with our ectomycorrhizal sequences, further *L. laccata* specimens clustered with *L. bicolor* and *L. proxima* specimens, indicating lacking species delimitation within those three taxa (Fig. 2). This is further confirmed by a fruiting body collected as *L. proxima* (DNA991) at a distance of 50 km from the study site that clustered with a *L. laccata* sequence from Norway. For *Laccaria amethystina* species designation by database search could be confirmed by the phylogram since all ectomycorrhizal and reference sequences formed a single cluster.

Xerocomus species comprised a second difficult group. Their ectomycorrhizae cannot be distinguished morphologically and until recently fruiting body based taxonomy was confusing (Peintner et al. 2003). The most recent revisions of the *X. chrysenteron* and *X. subtomentosus* species complexes (Peintner et al. 2003; Taylor et al. 2006; Taylor et al. 2007) are reflected in Fig. 3 by separate clusters for the UDB sequences of each newly recognized species. At our sites, two frequent members of *X. chrysenteron* species complex, *X. pruinatus* and *X. cisalpinus* (Fig. 3), can be distinguished, that occur together with *X. badius* in the mixed stands. A third member of the *X. chrysenteron* complex, *X. porosporus* was detected as a single root in the pure beech stand.

Distribution of species in specificity guilds

Only species with more than four sequenced mycorrhizae (18 on total) were assigned to specificity guilds (Fig. 4). Five species were assigned to the pine specific guild, seven species to the beech specific guild and six species to the generalist guild. Thus, the majority of species (12 out of 18 or 67 %) belonged to the host specific guilds of either pine or beech (Fig. 5). Among the 12 species in the host specific guilds, only four species are specialists found exclusively on either Scots pine or beech (Fig.4). Two of the four specialists, *Genea hispidula* and *Lactarius subdulcis*, are found only in the beech stand (category beech specialists, pure stands). They are the only two species, among 18, that were found exclusively in one stand. All other species occurred in the mixed stands and at least in one of the pure stands. The other two specialists, *Acephala macrosclerotiorum* and *Laccaria cf. laccata* occurred in pure stands and in the mixed stands but exclusively on Scots pine or beech roots, respectively.

The majority of species in the host specific guilds, four in the pine specific guild and four in the beech specific guild, are host preferring fungi, i.e. they are found occasionally on roots of the other host (Fig. 4). It is remarkable that the occurrences on the non-preferred host are only observed in the mixed stands and were absent from the pure stand of the non-preferred host. The only exception is *Hydnotrya tulasnei* in the beech specific guild, for which a two mycorrhizas were found in the pure pine stand. However, designation to the beech specific guild is justified because altogether 20 of 23 mycorrhizae occurred on beech roots (Fig.4). Among the six species in the generalist guild, only two species, *Russula ochroleuca* and *Tomentella sublilacina* were found on both the pure pine and the pure beech stand (category generalist, two pure stands). Since these species were able to grow on both hosts without the presence of the other, these are the only two species (13%) that behaved on our sites as true generalists. The other four species in the generalist guild (*Laccaria amethystina*, *Russula puellaris*, *Lactarius tabidus* and *Xerocomus pruinatus*) were absent from one of the pure stands (category generalist, one pure stand, Fig. 4). It is remarkable that species in the genera *Laccaria*, *Russula*, *Lactarius* and *Xerocomus* belong to different specificity guilds (Table 2).

Exploration types in the mixed stands

The six species of the generalist guilds are found on 51% of the sequenced root tips in the mixed stands (Fig. 6). Four of these species, namely *Russula ochroleuca*, *Russula puellaris*,

Lactarius tabidus and *Tomentella sublilacina* belong to the contact exploration type (Table 3). The other two generalists, *Laccaria amethystina* and *Xerocomus pruvinatus* belong to medium and long distance exploration type, respectively. These two species represent 10% of all sequenced root tips in the mixed stands (Fig. 6).

Specificity in fruiting body based compilations

Specificity data based on published reports from fruiting body collections could be assembled for 16 of the 18 analyzed species (Table 3). Among the twelve fungi considered to be generalist, six species (including *Laccaria cf. laccata*) belong to host specific guilds as determined by this study. On the other hand, the four species presumed to preferably associate with angiosperms (*Xerocomus cisalpinus*, *Lactarius subdulcis*, *Russula ionochlora*, *Genea hispidula*) were assigned to the beech specific guild by this study (Table 3).

III.5. Discussion

Can the concept of specificity guilds reconcile the preference concept in molecular studies and the concept of host specificity in fruiting body surveys?

Most fungi in the host specific guilds are able to grow on the roots of the non-preferred host, but only in the mixed stands (Fig. 4). This corresponds to the phenomenon of host preference as it was described from molecular studies in mixed stands (Tedersoo et al. 2008; Lang et al. 2011). However, if only pure stands are considered, members of the host specific guilds grow exclusively in one of the pure stands. This behaviour in the pure stands corresponds to the presence/absence definition of specificity as used by Newton and Haigh (1998).

Because the same fungus shows host preferences in the mixed stands and host specificities in the pure stands, specificity and preference can be viewed as expressions of the same host affinity. This same host affinity finds its expression in our designation of specificity guilds: a guild is defined "as a group of species that exploit the same class of environmental resources in a similar way" (Root 1967; Simberloff and Dayan 1991; de Kroon and Olf 1995). In the sense of this definition Scots pine would not be an environmental resource for fungi of the beech specific guild and vice versa. This would explain why beech specific fungi are found on pine roots in the mixed stands but not in the pure pine stand where the suitable environmental resource (i.e. beech roots) is absent.

Is guild membership characteristic for a fungal genus?

Xerocomus: *X. badius* belonged to the pine specific guild although literature references suggest generalist status (Molina et al. 1992). Even with this ambiguity it can be excluded that *X. badius* belongs to a beech specific guild. In contrast to *X. badius*, we assigned *X. cisalpinus* (*X. chrysenteron* complex) to the beech specific guild and, thus, confirm the suggestion of angiosperm affinities (Peintner et al. 2003; Lehr and Schreiner 2006). Despite membership in

the beech specific guild we report for the first time that *X. cisalpinus* is able to colonize also roots of *Pinus* in mixed *Pinus/Fagus* stands (Fig. 4). *X. pruinus* was equally frequent on beech and pine in the mixed stands and, therefore, assigned to the generalist guild which is in correspondence to literature reports (Meltzer and Rothe 2003; Peintner et al. 2003). Guild membership reflects the genetic distance of the three *Xerocomus* species. The two closely related species of the *X. chrysenteron* complex, *X. cisalpinus* and *X. chrysenteron*, occur at a much higher percentage on beech roots than the more distantly related *X. badius* (Fig. 3) which belongs to the pine specific guild.

In the other two genera with more than two species, *Lactarius* and *Russula*, species are either members of the generalist or the beech specific guilds (Table 2). Together with observations in previous studies (Marjanovic et al. 2010; den Bakker et al. 2004; Sato et al. 2007), it seems the rule rather the exception that guild membership (generalist or host specific) is not a genus synapomorphy. This means that within a genus different specificities can be observed. The most important prerequisite for such a statement is exact delineation of species within a species. This cannot be achieved by pure database comparisons but requires species delineation by phylograms as presented in this study.

Can cryptic species explain contradicting specificity guilds?

Laccaria. In our study *Laccaria cf. laccata* is not only member of the beech specific guild but is the only species that is found exclusively on beech roots in the mixed stands (Table 2, Fig 4). This specialist behaviour is in sharp contrast to the traditional view of *Laccaria laccata* as typical generalist (Trappe 1962; Román de et al. 2005; Molina et al. 1992; Lee and Kim 1987). An explanation might be provided by the complicated taxonomy of the genus *Laccaria* generally and of the *L. laccata* complex specifically which is caused by relative simple sporocarp morphology together with phenotypic flexibility over the wide geographical range of the species (Mueller 1991). Estimation of species numbers differs depending on author from 18 to 43 worldwide (Singer 1986; Mc Nabb 1972) and from 7 to 11 in Europe (Moser 1983; Breitenbach and Kränzlin 1991). Despite the widespread use of *L. bicolor* as model organism (Martin et al. 2008; Martin and Selosse 2008) and in contrast to North American species (Osmundson et al. 2005) a modern phylogenetic analysis for European *Laccaria* species is lacking. Our *Laccaria* phylogram (Fig. 2) suggests that *L. laccata* consists of several cryptic species. This is in correspondence with earlier reports on intrataxonomic variation within *L. laccata* species complex (Singer 1977; Gardes et al. 1990; Gardes et al. 1991). It has been shown in *Strobilomyces* that cryptic species differ in their host specificities (Sato et al. 2007). Therefore, we hypothesize that *Laccaria cf. laccata* is one of several cryptic species within the *Laccaria laccata* species complex that belong to different specificity guilds. The hypothesis of host specific cryptic species in a presumably generalist species has been tested for *Laccaria amethystina*. Roy et al. 2008 could not find cryptic species under different host trees and confirmed the status of *L. amethystina* as a single generalist species. This is in correspondence with the single *L. amethystina* cluster in our phylogram (Fig.2.) and designation of *L. amethystina* to the generalist guild (Fig. 4). Thus, if we accept that the *L.*

laccata complex contains host specific cryptic species, the genus *Laccaria* comprises members of host specific and generalist guilds.

Cenococcum: *C. geophilum 1* was not a dominating species as in many other temperate forests (e.g. Blom et al. 2009; Gebhardt et al. 2009; Moser et al. 2009). Its designation to the pine specific guild is in contrast to its generalist status (Trappe 1964) and frequent reports of *Cenococcum geophilum* in beech forests (Grebenc and Kraigher 2007; Kreisel 1957). A second genotype (*C. geophilum 2*, Table 1, Suppl. Fig. S3) met the genetic distance criterion for cryptic species separation in *Cenococcum* (Douhan and Rizzo 2005). Although generalization is not possible, it is remarkable that the single record of *C. geophilum 2* occurred on a beech root. In reference to the hypothesis for *Laccaria cf. laccata*, we speculate that the pine specific guild membership of *C. geophilum 1* can be explained by host specific cryptic species within the *C. geophilum* species complex.

Are specificity guilds indicative of host ranges?

Host range is a semi-quantitative criterion to describe specificity phenomena on a global scale and comprises the three categories broad, intermediate and narrow host range (Molina et al. 1992). Ectomycorrhizal species of broad host range associate with many tree species both within angiosperms and Pinaceae, species of intermediate host range accompany either angiosperms or Pinaceae and species of narrow host range are restricted to a single host genus (Molina et al. 1992).

Assignment of fungi to pine or beech specific guilds in this study does not imply that they are narrow host range species restricted to the genera *Pinus* or *Fagus* on a global scale. Species in the local pine specific guild could be species of intermediate host range, i.e. they could grow on other genera within the Pinaceae and would be members of a global Pinaceae specific guild. Similarly, most species in the beech specific guild could be able to grow on other angiosperm hosts, i.e. they would be members of global angiosperm associated guild. One species with a narrow host range on a global scale could be *Lactarius subdulcis*. It was a beech specialist in our study and is exclusively associated with beech in other studies (Trappe 1962; Rineau et al. 2010; Buée et al. 2011; Agerer 2006), so that *Lactarius subdulcis* could belong to a narrow host range guild restricted to the genus *Fagus*.

The term specialist is used in this study to designate fungi that are found exclusively on one of two host species. In the context of host ranges, a specialist in the sense that it is restricted to a single host species on a global scale has not been observed so far (Smith and Read 2008; Molina et al. 1992). On the other end of the specificity scale, members of our generalist guild are species with a broad host range. *Russula ochroleuca* and *Tomentella sublilacina*, the generalists found in all four stands, including the pure pine and the pure beech stand, are reported from many angiosperm and Pinaceae hosts (Trappe 1962, Molina et al. 1992, foot note for Table 3).

Why are there so many members in the host specific guilds?

The majority of ectomycorrhizal species belonged to the host specific guild (Fig 5). This contradicts the claim of generalist dominance in ectomycorrhizal communities (Read 1997; Selosse et al. 2006; Kennedy et al. 2003). One reason for this conflict might be that general conclusions on specificity phenomena have been based on fruiting body based compilations of fungus/host associations. Field guides and fungal floras (Moser 1983; Breitenbach and Kränzlin 1991; Dähncke 1993) list occurrences in mixed stands, and consider the corresponding fungi as generalists, although looking at the roots preferences for one host can be recognized (Table 3). Therefore, fruiting body reports tend to overestimate the importance of generalists.

The second reason for the high proportion of host specific guilds can be found in the large genetic distance of the two host species in our study. Molecularly guided studies suggesting dominance of generalists analyzed communities of closely related hosts within Pinaceae (Horton and Bruns 1998; Cullings et al. 2000; Hubert and Gehring 2008) or within Caesalpinaceae (Diedhiou et al. 2010). In contrast, studies that observed dominance of host specific fungi in mixed stands included hosts of larger phylogenetic distance: Rosales/Fagales/Myrtales (Tedersoo et al. 2008), Fagales/Malvales (Lang et al. 2011) Fagales/Pinaceae (Ishida et al. 2007; Smith et al. 2009). Thus, the tendency of an ectomycorrhizal community to split in host specific guilds is higher for hosts with larger phylogenetic distance.

Is there support for the concept of interspecific Common Mycorrhizal Networks (CMN)?

The concept of interspecific Common Mycorrhizal Networks (CMN, (Simard et al. 1997; Sen 2000; Wiemken and Boller 2002) assumes that individuals of different tree species can exchange nutrients via ectomycorrhizal hyphae. This assumption has been used to support the claim of generalist dominance in ectomycorrhizal communities (Read 1997; Selosse et al. 2006; Kennedy et al. 2003). When ectomycorrhizal fungi should play a major role for transferring nutrients between different tree species (He et al. 2003; Whitfield 2007) it is necessary that they belong to the generalist guild. Furthermore, they should have interconnecting structures (extraradical mycelium and rhizomorphs) as they are typical for mycorrhizae of medium and long distance exploration type sensu Agerer (2001). In a review on 18 vitro experiments with CMNs all fungi belonged to the medium or long distance exploration type (He et al. 2003). In our study, fungi that are both in the general guild and of medium or long distance exploration type comprise the minority of sequenced root tips in the mixed stands (Fig. 6). Therefore, an interspecific CMN may not play a large ecological role in the mixed stands of Scots pine and beech. Discrepancy between our observation and the CMN concept shows that evaluation of specificity phenomena should not only be guided by a theoretical concept, but accompanied by careful observations in field studies.

III.6. Acknowledgements

We thank Monika Roth and Renate Krüger for excellent technical assistance. Judith Golldack gave support in the morphological/anatomical distinction of ectomycorrhizae and Peter Lentzsch helped with sequence analysis. Martin Schmidt shared his knowledge on the fungal flora of Brandenburg and provided fruiting bodies for sequence comparisons. We thank Ingrid Kottke for improving an earlier version of this manuscript. Finally, we thank M. T. Lavin-Zimmer for assisting with the English language.

III.7. Tables and figures

Table 1: List of the 40 ectomycorrhizal sequence types

The column “match” gives the most frequent value for the match between query and reference sequence. Notes on host (last column) are only presence/absence (p = Scots pine, b = beech).

species name/sequence type	match in %	accession no. of reference	quantity of tips	accession no. of GenBank submission	host
<i>Acephala macrosclerotiorum</i>	100	EU882732	25	HM189696-720	p
<i>Amanita sp.</i>	97.5	FJ596814	2	HM189721-2	p
<i>Cenococcum geophilum 1</i>	99.6	EU285479	9	HM189723-31	p, b
<i>Cenococcum geophilum 2</i>	98.2	FJ152539	1	HM189732	b
<i>Corticoid fungus</i>	100	FM992888	2	HM189733-4	p
<i>Cortinarius (Dermocybe) sp. 1</i>	98.2	AY669585	11	HM189735-45	p, b
<i>Cortinarius (Telamonia) sp. 2</i>	96.7	AY669664	2	HM189746-47	p
<i>Genea hispidula</i>	100.0	UDB001408	7	HM189748-54	p
<i>Hydnotrya tulasnei</i>	99.5	UDB000095	23	GQ149454-62, GQ215698-700, HM189755-65	b, p
<i>Hydnum elliposporum</i>	100	AY817138	3	HM189766-68	b
<i>Inocybe napipes</i>	99.7	UDB000607	1	HM189769	b
<i>Inocybe sp. aff. praetervisa</i>	99.1	AM882720	3	HM189770-72	b
<i>Laccaria amethystina</i>	100	UDB002418	5	HM189773-77	b, p
<i>Laccaria cf. laccata</i>	100	UDB000104	16	HM189778-93	b

<i>Lactarius necator</i>	100	UDB000361	1	HM189794	b
<i>Lactarius rufus</i>	100	UDB001601	2	HM189795-96	p
<i>Lactarius subdulcis</i>	100	UDB001601	11	HM189797-807	b
<i>Lactarius tabidus</i>	99.9	UDB000385	27	HM189808-34	b, p
<i>Lactarius vellereus</i>	99.9	UDB002494	1	HM189835	b
<i>Pezizaceae</i>	100.0	AJ969437	3	HM189836-38	b
<i>Russula integra</i>	100	UDB000357	2	HM189839-40	b, p
<i>Russula ionochlora</i>	100	GQ924690	35	HM189841-75	b, p
<i>Russula ochroleuca</i>	100	UDB000046	56	HM189876-931	b, p
<i>Russula puellaris</i>	99.4	UDB000031	13	HM189932-44	b, p
<i>Russula sp. aff. ionochlora</i>	96.6	GQ924690	4	HM189945-48	b
<i>Russula velenovskyi</i>	100	UDB001640	3	HM189949-51	p
<i>Russula vesca</i>	100	UDB000340	5	HM189952-56	b, p
<i>Scleroderma citrinum</i>	100	EU784414	1	HM189957	b
<i>Thelephora terrestris</i>	100	UDB000971	10	HM189958-67	b, p
<i>Tomentella sp. 1</i>	96.1	AF430259	1	HM189968	b
<i>Tomentella sp. 2</i>	97.9	AJ889982	1	HM189969	b
<i>Tomentella sublilacina</i>	99.8	UDB000970	39	HM189970-190008	p, b
<i>Tomentellopsis sp.</i>	100	DQ377434	3	HM190009-11	p
<i>Tuber puberulum</i>	100	UDB001385	2	HM190012-13	b
<i>Tylopilus felleus</i>	99.9	UDB000680	3	HM190014-16	p
<i>Tylospora asterophora</i>	99.8	UDB000841	1	HM190017	p
<i>Xerocomus badius</i>	99.8	UDB000050	33	HM190018-50	p, b
<i>Xerocomus cisalpinus</i>	99.6	UDB002180	35	HM190051-85	p, b
<i>Xerocomus porosporus</i>	99.7	UDB000475	1	HM190086	b
<i>Xerocomus pruinatus</i>	100	UDB000049	23	HM190087-109	p, b

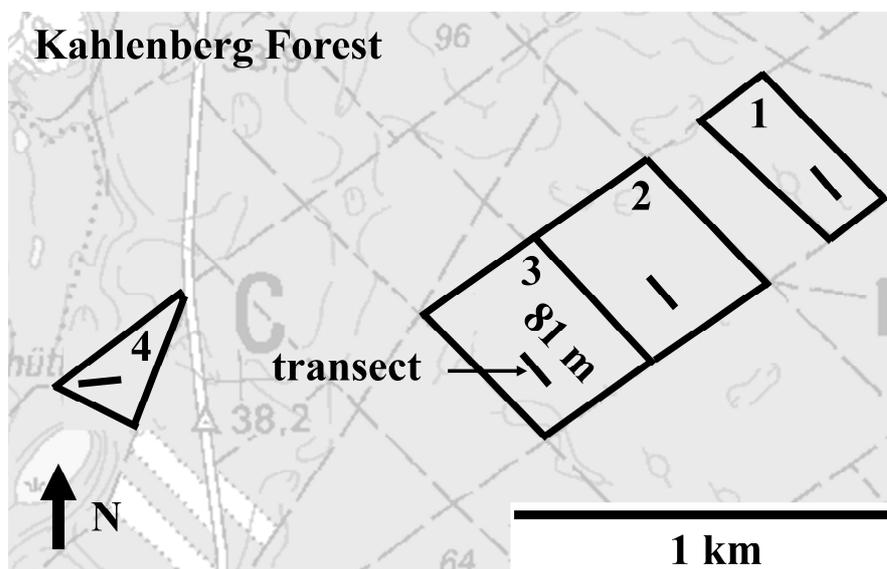
Table 2: Specificity guilds within fungal genera

genus	specificity guild		
	generalist	pine specific	beech specific
<i>Laccaria</i>	<i>L. amethystina</i>		<i>L. cf. laccata</i>
<i>Lactarius</i>	<i>L. tabidus</i>		<i>L. subdulcis</i>
<i>Russula</i>	<i>R. ochroleuca</i>		<i>R. ionochlora</i>
	<i>R. puellaris</i>		<i>R. vesca</i>
<i>Xerocomus</i>	<i>X. pruinatus</i>	<i>X. badius</i>	<i>X. cisalpinus</i>

Table 3: Comparison of specificity guilds with specificity data from literature

species	specificity guild (this study)	specificity based on fruiting body associations (literature)	reference	exploration type according to Agerer (2001)
<i>Acephala macrosclerotiorum</i>	pine	no fruit bodies	(Münzenberger et al. 2009)	medium distance
<i>Xerocomus badius</i>	pine	generalist	(Molina et al. 1992)	long distance
<i>Cortinarius (Dermocybe) sp. 1</i>	pine	-	-	medium distance
<i>Thelephora terrestris</i>	pine	generalist	(Molina et al. 1992)	medium distance
<i>Cenococcum geophilum 1</i>	pine	generalist	(Molina et al. 1992; Trappe 1964)	short distance
<i>Russula puellaris</i>	generalist	generalist	(Moser 1983; Kreisel 1957)	contact
<i>Tomentella sublilacina</i>	generalist	generalist	No metadata ^{a)}	short distance
<i>Russula ochroleuca</i>	generalist	generalist	(Molina et al. 1992)	contact
<i>Lactarius tabidus</i>	generalist	generalist (as <i>L. theiogalus</i>)	(Trappe 1962)	contact
<i>Xerocomus pruinaus</i>	generalist	generalist	(Peintner et al. 2003; Meltzer and Rothe 2003)	long distance
<i>Laccaria amethystina</i>	generalist	generalist	(Molina et al. 1992)	medium distance
<i>Hydnotrya tulasnei</i>	beech	generalist	(Molina et al. 1992)	contact
<i>Russula vesca</i>	beech	generalist	(Molina et al. 1992)	contact
<i>Xerocomus cisalpinus</i>	beech	angiosperms	(Peintner et al. 2003; Lehr and Schreiner 2006)	long distance
<i>Russula ionochlora</i>	beech	angiosperms, especially beech	(Moser 1983)	short distance
<i>Laccaria cf. laccata</i>	beech	generalist	(Molina et al. 1992; Roy et al. 2008)	medium distance
<i>Genea hispidula</i>	beech	angiosperm	(Molina et al. 1992)	short distance
<i>Lactarius subdulcis</i>	beech	angiosperm	(Dähncke 1993; Trappe 1962)	contact

^a Two lines of evidence speak for generalist behaviour of *Tomentella sublilacina*. First, UNITE data: among 14 fruiting body collections seven were found under broad leaved trees (including beech), four under conifers, and three in mixed forests. Second, ectomycorrhizal reports based on molecular identification indicate a variety of hosts: *Pinus pinaster* (Nieto and Carbone 2009), *Fagus sylvatica* (Shi et al. 2002), and *Tsuga heterophylla* (Wright et al. 2009).

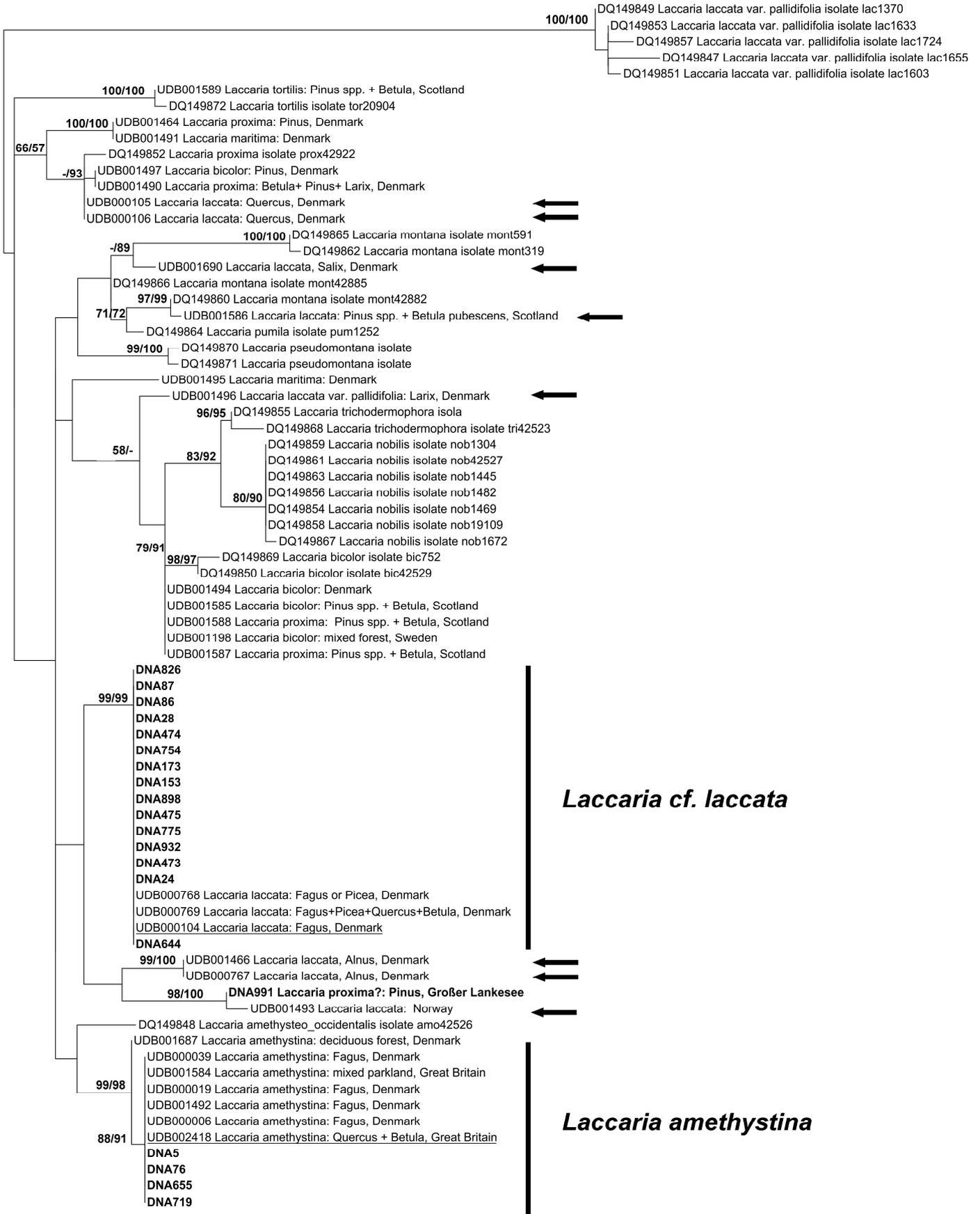


- 1 young mixed stand (pine 83 y, beech 40 y)**
2 pure pine stand (91 y)
3 old mixed stand (pine 121 y, beech 80 y)
4 pure beech stand (108 y)

Fig. 1: Location of the stands and the transects within the stands

Fig. 2: Ambiguous species delimitation in the genus *Laccaria* (next page)

Phylogram is based on a 645 bp alignment of ITS region. For reference sequences from UNITE-database (UDB...) host tree species and country of origin are listed. Reference sequences from GenBank are selected from Osmundson et al. (2005) and are of North American origin. Sequences in bold face are from roots except for DNA991 that stems from a fruiting body collected at a distance of 50 km from the study site. The best BLAST-search matches are underlined. *L. laccata* sequences (black arrows) appear at different positions in the phylogram. Therefore, the species designation remains ambiguous. The Maximum Likelihood tree was generated with RAxML algorithm and 100 bootstrap replicates. Bootstrap values above 50 are indicated. A second tree was generated with the Neighbour-Joining algorithm and 1000 bootstrap replicates. For those nodes equal in both trees the NJ-bootstrapping values are given as a second number on the node.



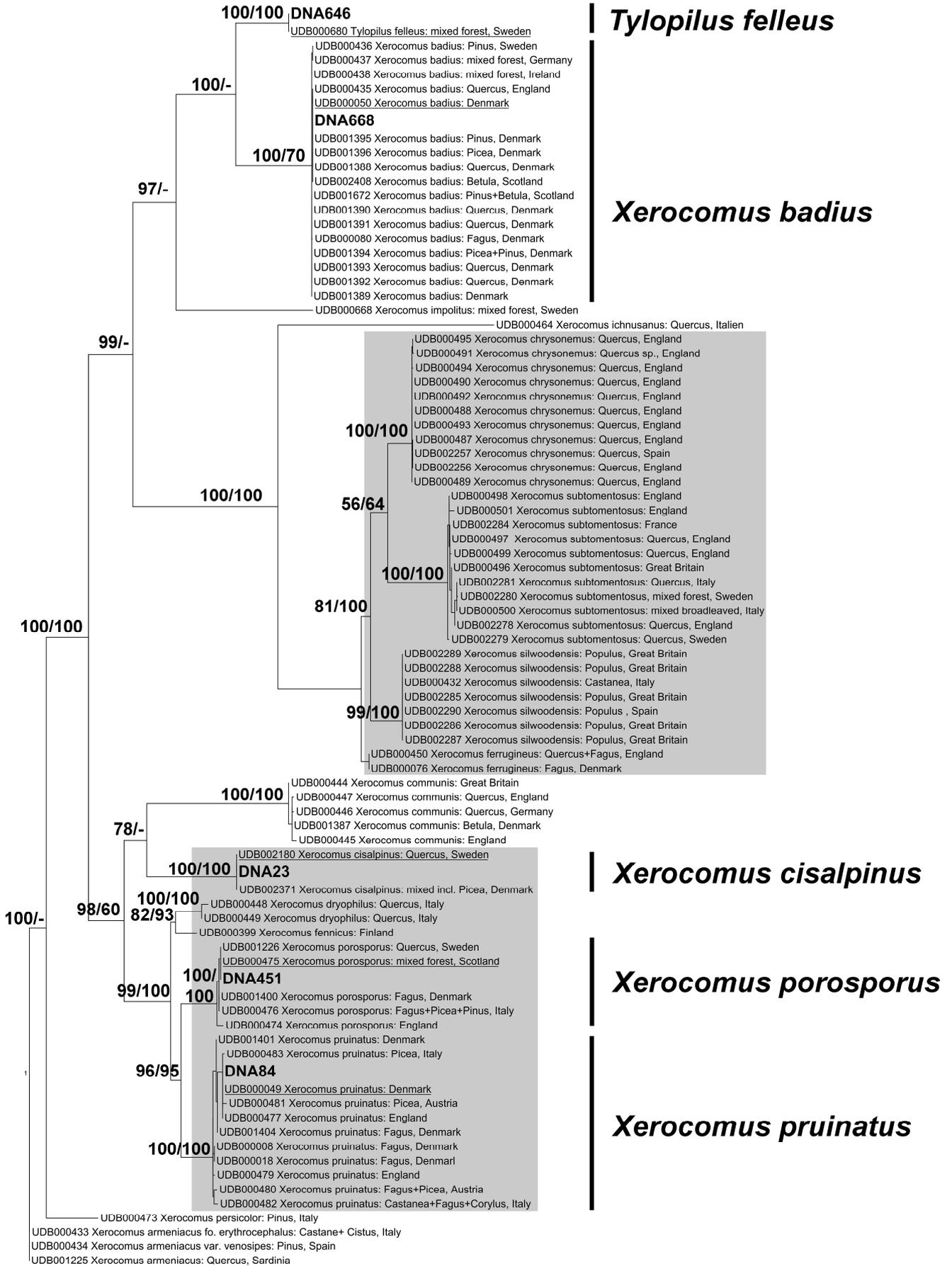


Fig. 3: Unambiguous species delimitation in the genus *Xerocomus* (previous page)

The phylogram is based on an 834 bp alignment of ITS region. Reference sequences are from UNITE-database (UDB). For *Xerocomus ferrugineus* only two UDB sequences are included, for all other species all UDB entries are used. The best BLAST-search matches are underlined. The Maximum Likelihood tree was generated with RAxML algorithm and 100 bootstrap replicates. Bootstrap values above 50 are indicated. A second tree was generated with the Neighbour-Joining algorithm and 1000 bootstrap replicates. For those nodes equal in both trees the NJ-bootstrapping values are given as a second number on the node. Upper shaded area: *X. subtomentosus* complex, lower shaded area: *X. chrysenteron* complex.

Fig. 4: Specificity guilds by stands (next page)

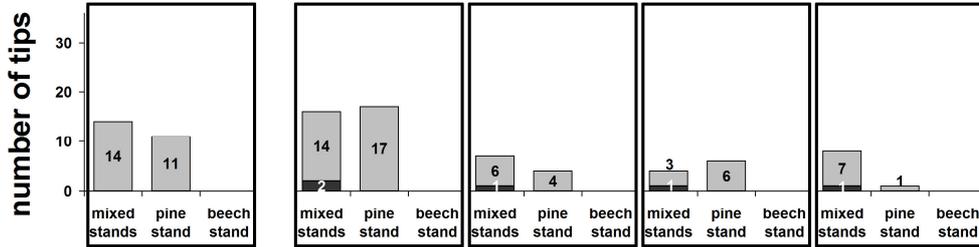
Data for 18 species with more than four sequenced root tips is presented. Main criterion for the distinction of specificity guild was the host frequency in the mixed stands. When at least 75% of tips were found on one host in the mixed stands (left column in each block) the fungus is considered to prefer this host and is assigned to a host specific guild. Note that all five members of the pine specific guild are absent from the pure beech stand and six of seven members of the beech specific guild are absent from the pure pine stand. The number on the columns refers to the absolute number of tips.

pine specific guild

■ pine ■ beech

pine specialist
(mixed stands)

pine preferring



Acephala macro-sclerotiorum

Xerocomus badius

Cortinarius (Dermocybe) terrestris

Thelephora terrestris

Cenococcum geophilum 1

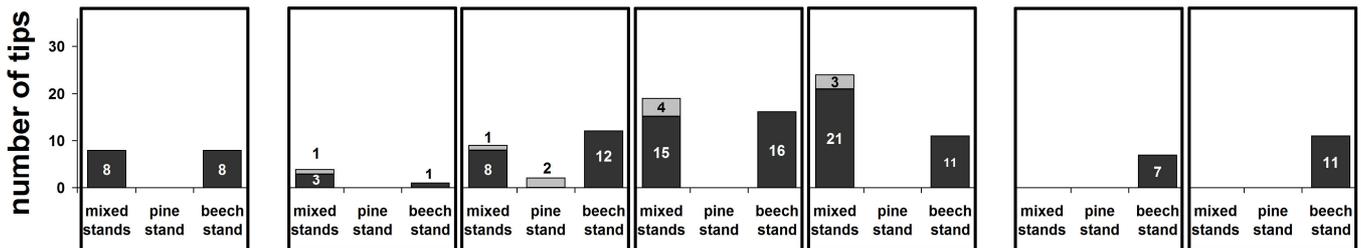
beech specific guild

■ pine ■ beech

beech specialist
(mixed stands)

beech preferring

beech specialist
(pure stand)



Laccaria cf. laccata

Russula vesca

Hydnotrya tulasnei

Xerocomus cisalpinus

Russula ionochlora

Genea hispida

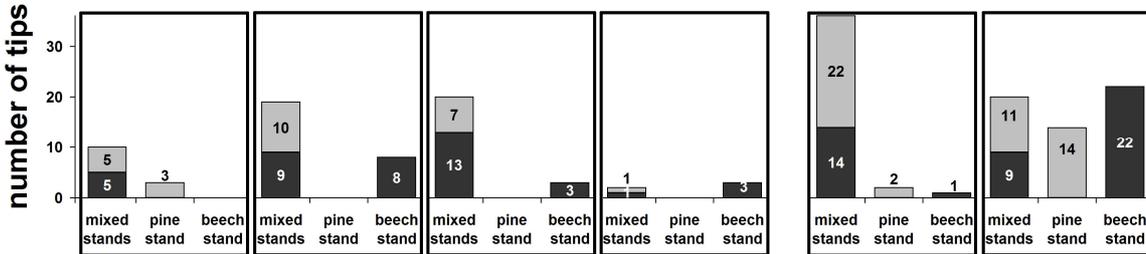
Lactarius subdulcis

generalist guild

■ pine ■ beech

generalist, one pure stand

generalist, two pure stands



Russula puellaris

Lactarius tabidus

Xerocomus pruinosus

Laccaria amethystina

Tomentella sublilacina

Russula ochroleuca

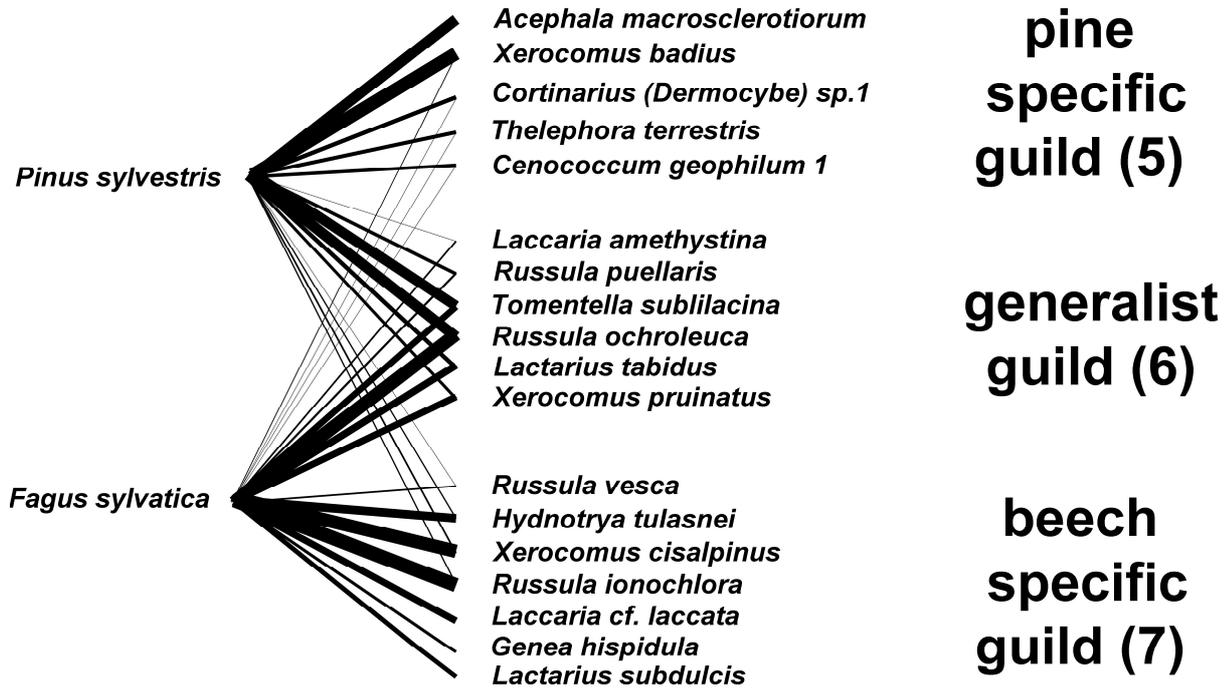


Fig. 5: Specificity guilds by host

Strength of linking lines is proportional to the number of root tips shown in Fig.4. Numbers in parentheses refer to the number of species in the guild.

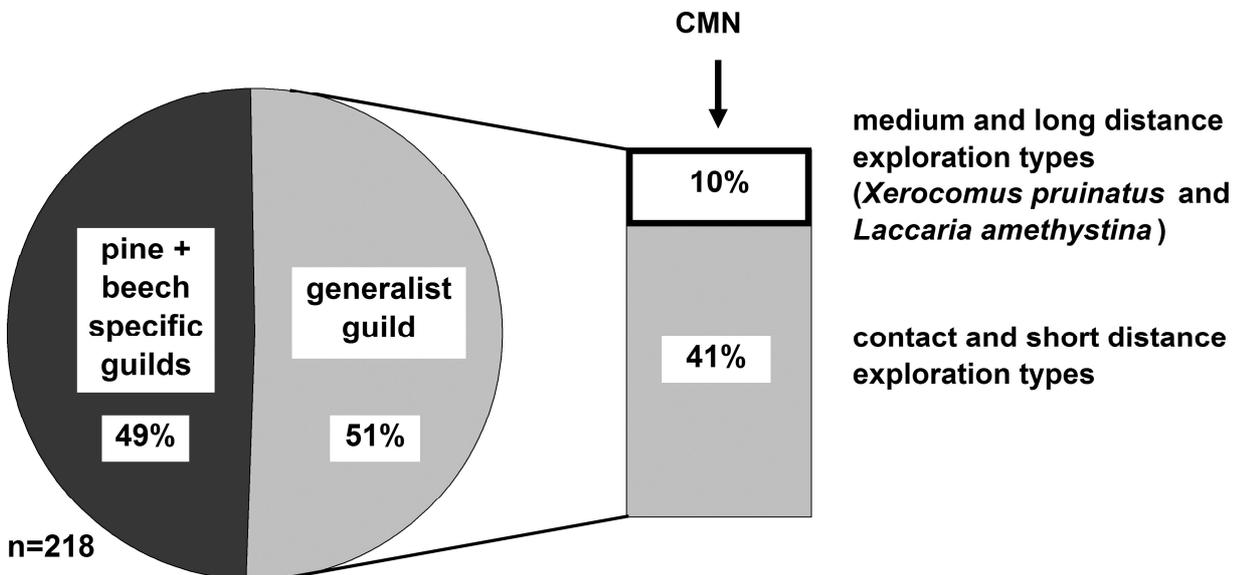


Fig. 6: Fungal specificity guilds in the mixed stands and the potential contribution to an interspecific Common Mycorrhizal Network (CMN)

The percentages refer to the sum of all root tips for the 16 species in the mixed stands. Basis for calculation are the designations to exploration types according to Agerer (2001) as listed in Table 3.

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III.9. Online supplemental material

The online supplemental material of manuscript 1 consists of 15 Supplementary Figures S1-S15 (pp. 117-133) and one Supplementary Table S1 (pp. 134-144). The 15 phylograms are headed by a list that contains all ectomycorrhizal fungi in alphabetical order together with the number of the corresponding phylogram. The online supplemental material is concluded by a separate list of all references used in the figure captions.

Supplementary Figures S1-S15: Identification of all 40 ectomycorrhizal species by phylograms

Ectomycorrhizal fungi

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23	<i>Russula ochroleuca</i>	Fig. S8	121
24	<i>Russula puellaris</i>	Fig. S8	121
25	<i>Russula sp. aff. ionochlora</i>	Fig. S8	121
26	<i>Russula velenovskyi</i>	Fig. S8	121
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33	<i>Tomentellopsis sp.</i>	Fig. S10	125
34	<i>Tuber puberulum</i>	Fig. S2	113
35	<i>Tylopilus felleus</i>	Fig. S15	132
36	<i>Tylospora asterophora</i>	Fig. S12	128
37	<i>Xerocomus badius</i>	Fig. S15	132
38	<i>Xerocomus cisalpinus</i>	Fig. S15	132
39	<i>Xerocomus porosporus</i>	Fig. S15	132
40	<i>Xerocomus pruinatus</i>	Fig. S15	132

Root associated fungi

		page	
1	<i>Basidiomycete (Mycena p. p.) sp. 1</i>	Fig. S12, Fig. S13	128, 129
2	<i>Basidiomycete sp. 2</i>	Fig. S12, Fig. S13	128, 129
3	<i>Helotiales (Mollisia) sp. 1</i>	Fig. S2	113
4	<i>Helotiales sp. 3</i>	Fig. S2	113
5	<i>Helotiales sp. 2</i>	Fig. S2	113
6	<i>Meliniomyces bicolor</i>	Fig. S9	123
7	<i>Meliniomyces variabilis</i>	Fig. S9	123
8	<i>Mitosporic ascomycota</i>	Fig. S2	113
9	<i>Phialocephala fortinii</i>	Fig. S2	113

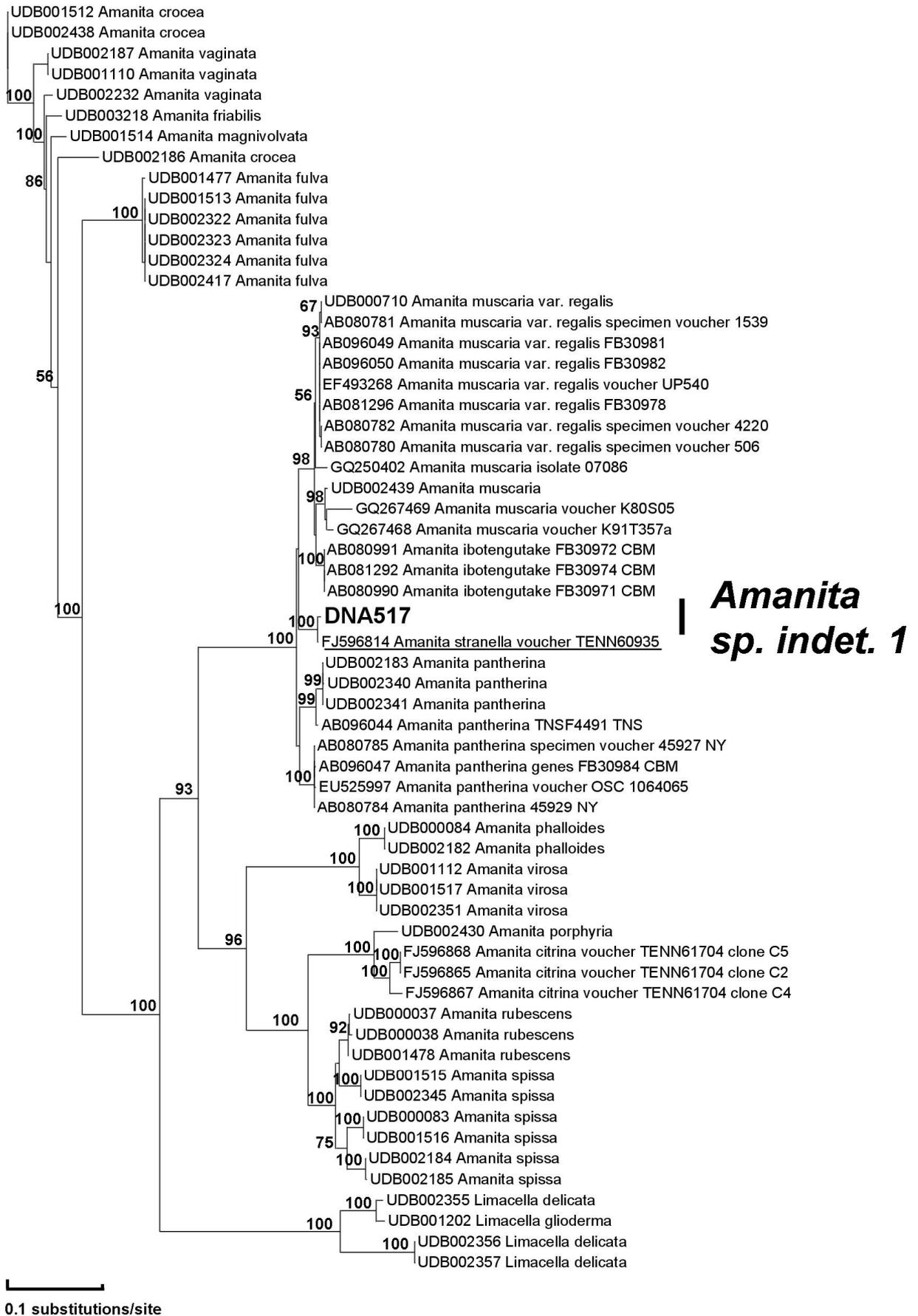
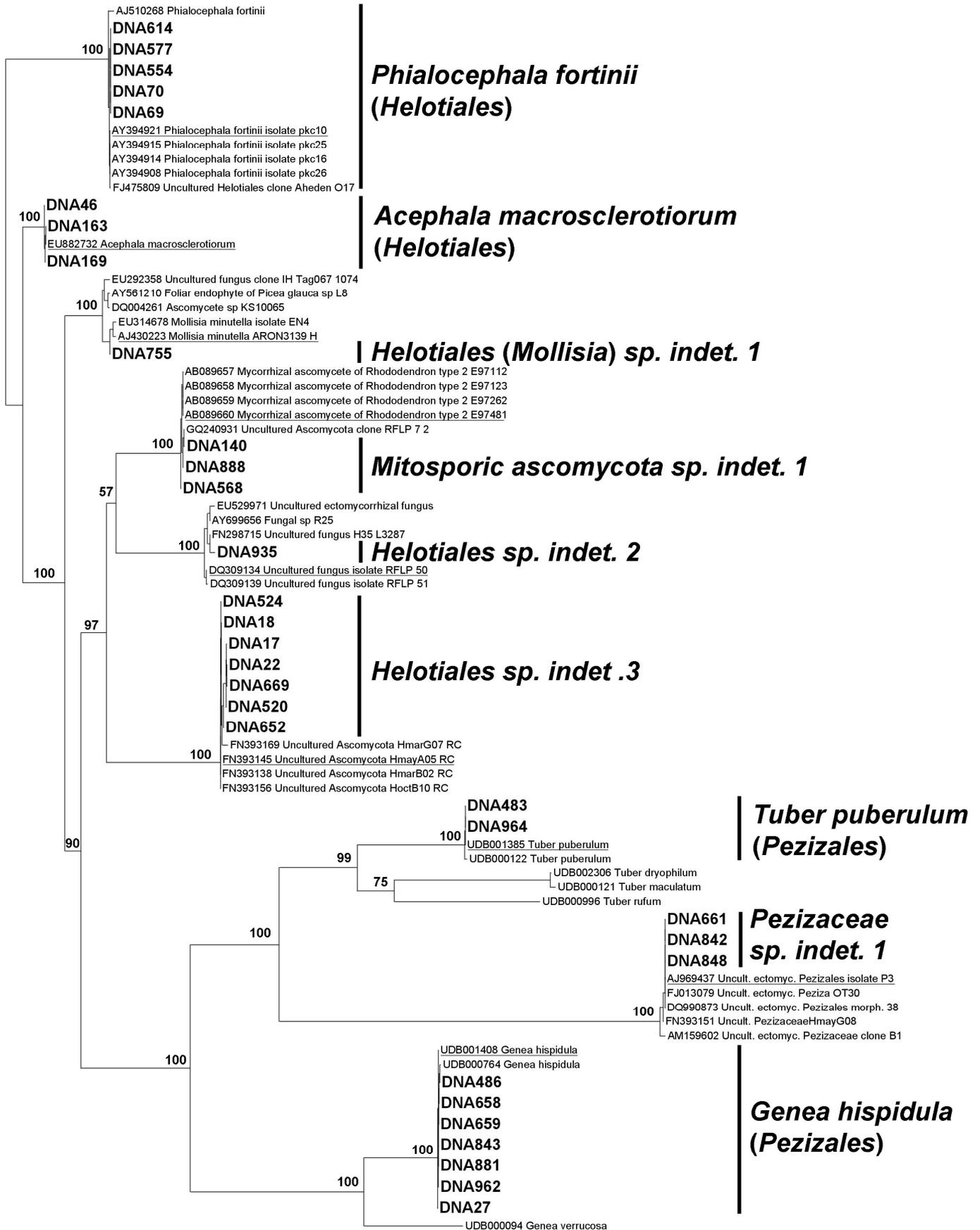


Fig. S1: *Amanita* sequence type (previous page)

Reference sequences are from UNITE and GenBank and were selected according to Moncalvo et al. (2001, Fig. 2). The best BLAST-search match is underlined. The unrooted Neighbour-Joining tree is based on a 705 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated. Since the best GenBank match is only 97.5% identical to the query sequence, a species name cannot be designated.

Fig. S2: *Ascomycota* sequence types (next page)

Reference sequences for *Genea* and *Tuber* are the UNITE entries for these genera. For the sequence types without close UNITE matches four to six closest GenBank matches were used as reference. The best BLAST-search matches are underlined. *Acephala macrosclerotiorum* has been described as new species (Münzenberger et al. 2009). The unrooted Neighbour-Joining tree is based on a 594 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.



0.1 substitutions/site

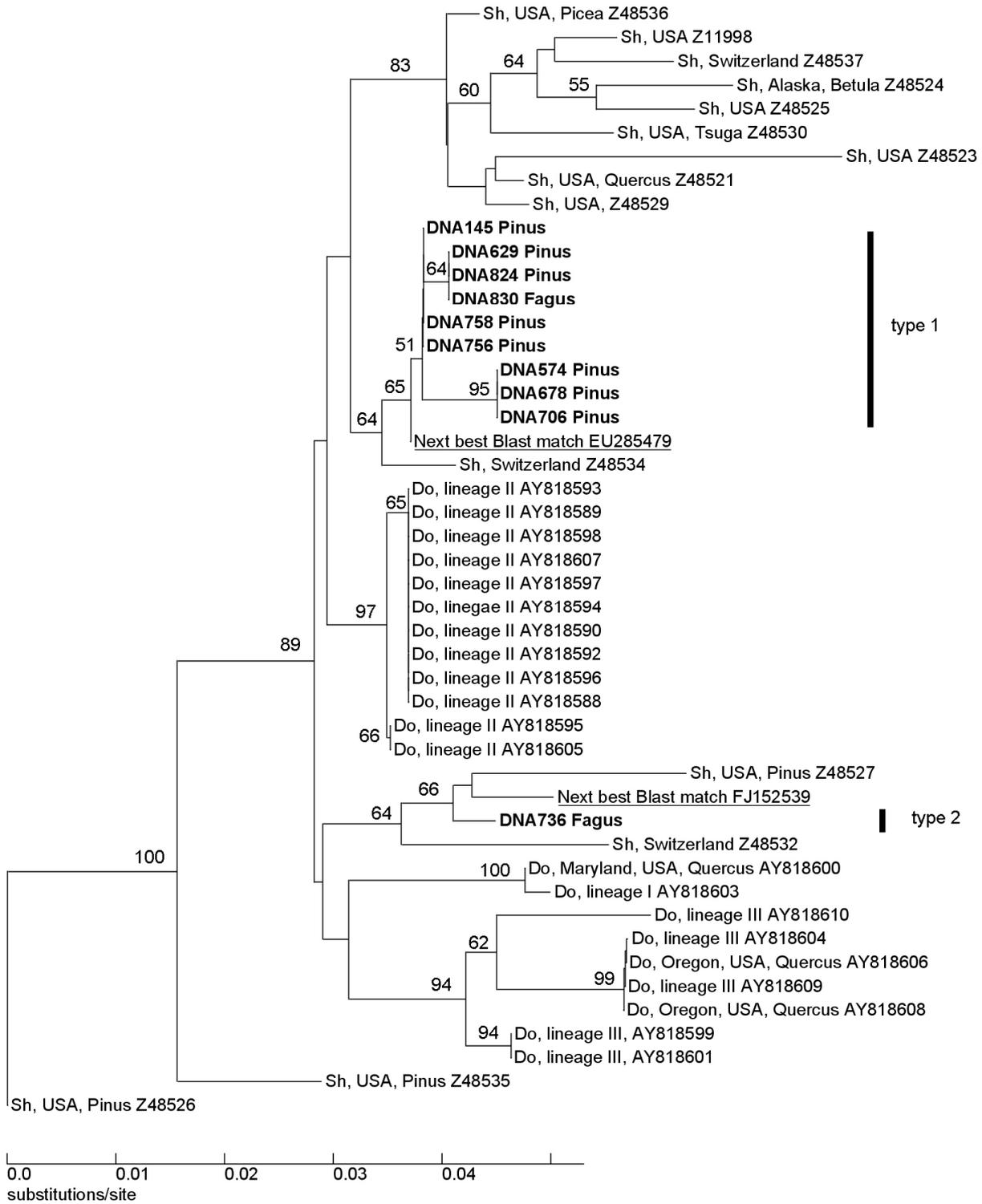


Fig. S3: *Cenococcum geophilum* sequence types (previous page)

Reference sequences are from Douhan and Rizzo (2005, prefix Do) and Shinohara et al. (1999, prefix Sh). The best matches of BLAST-searches in GenBank are underlined. Sh-sequences originate in different regions of Europe and United States; the host tree species is indicated. Do-sequences originate from one Californian site under *Quercus spec.* (for three exceptions the origin is indicated). Note that the genetic distance between Douhan and Rizzo's lineages is about the same as between the query sequences type 1 and type 2. Following Douhan and Rizzo's interpretation of their lineages representing cryptic species, the sampled *Cenococcum* sequences are separated into two sequence types. Further note, that with two exceptions the query sequences originate from *Pinus*, while *Cenococcum* is typically considered to be a generalist. The unrooted Neighbour-Joining tree is based on a 443 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.

Fig. S4: *Cortinarius* sequence types (next page)

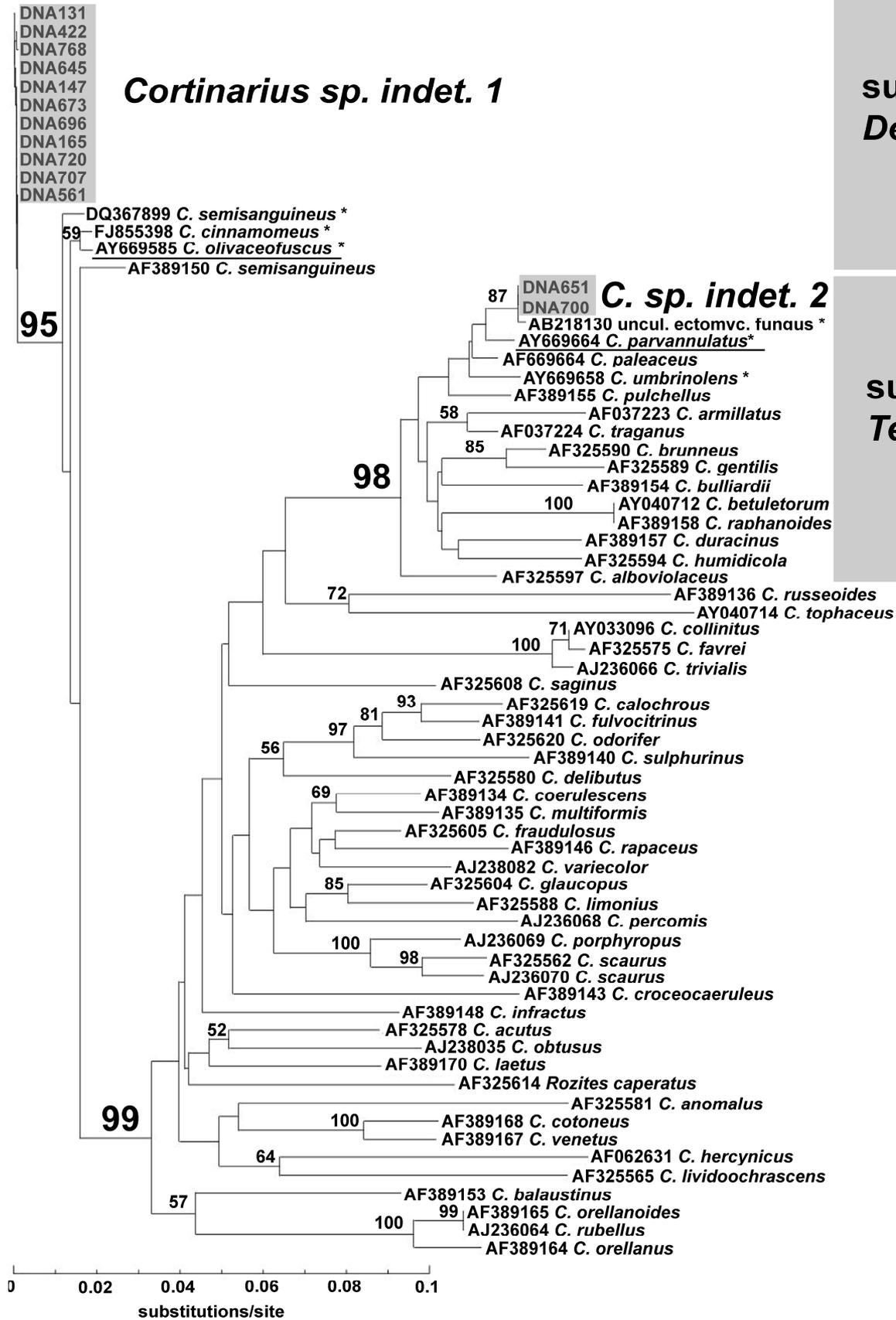
Reference sequences are from Peintner et al. (2004). The best BLAST-search matches with species names are underlined. *C. sp. indet. 1* shows only 98% similarity with *C. olivaceofuscus*. The nodes for the species closest to *C. sp. indet. 2* are not supported by bootstrap values. Therefore, for both *Cortinarius* sequence types no species names can be designated. Their grouping into two different subgenera can be confirmed. Further subgenera as recognized by Peintner et al. (2004) are not depicted. The unrooted Neighbour-Joining tree is based on a 575 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.

subgenus
Dermocybe

subgenus
Telamonia

Cortinarius sp. indet. 1

C. sp. indet. 2



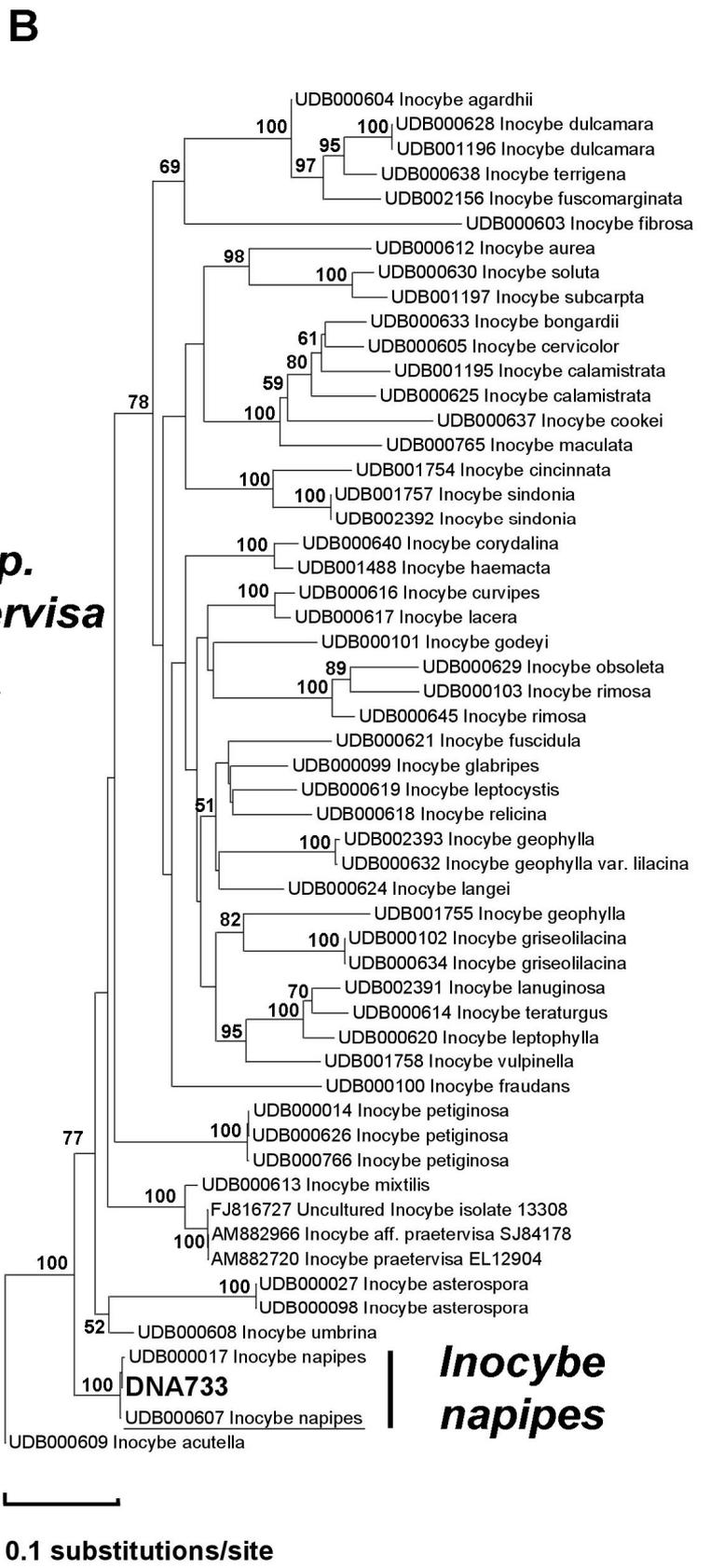
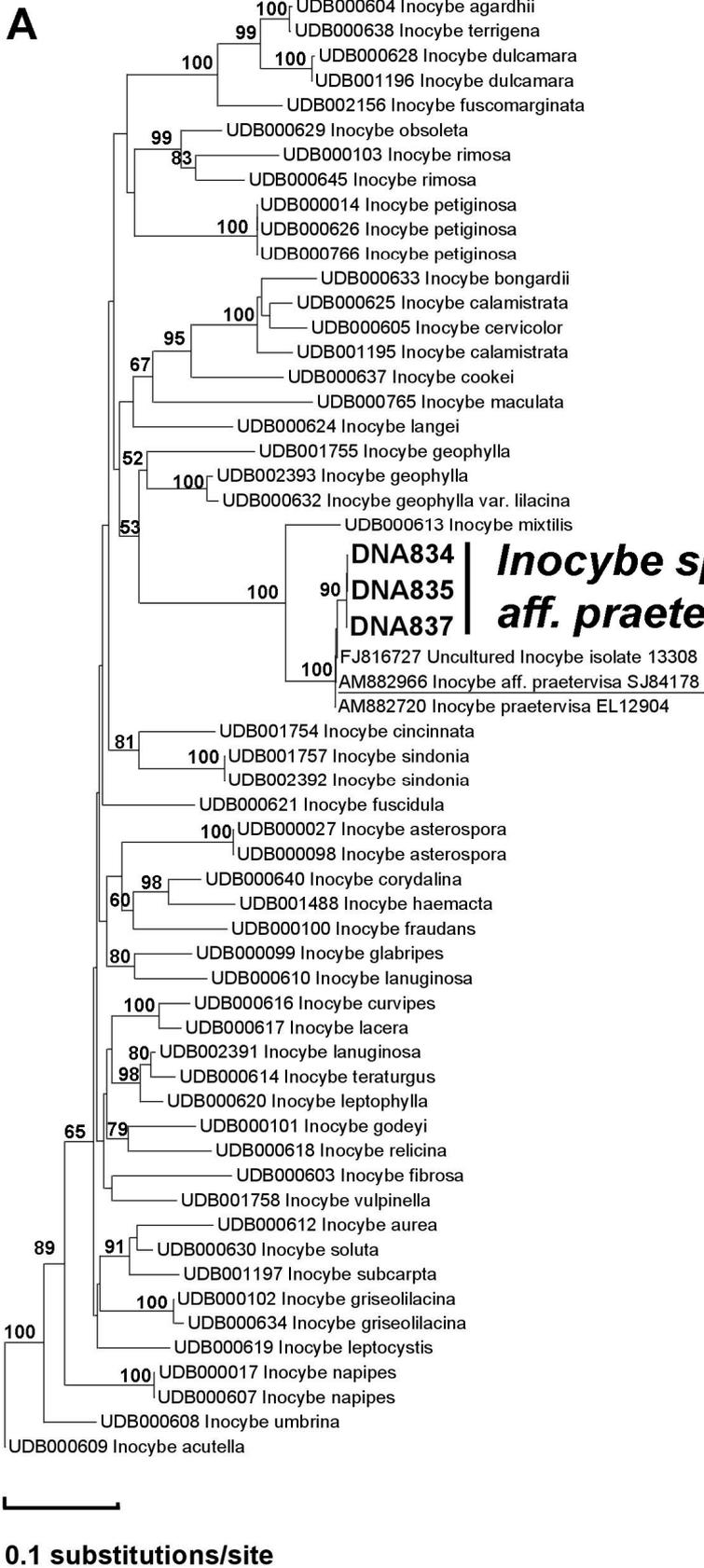
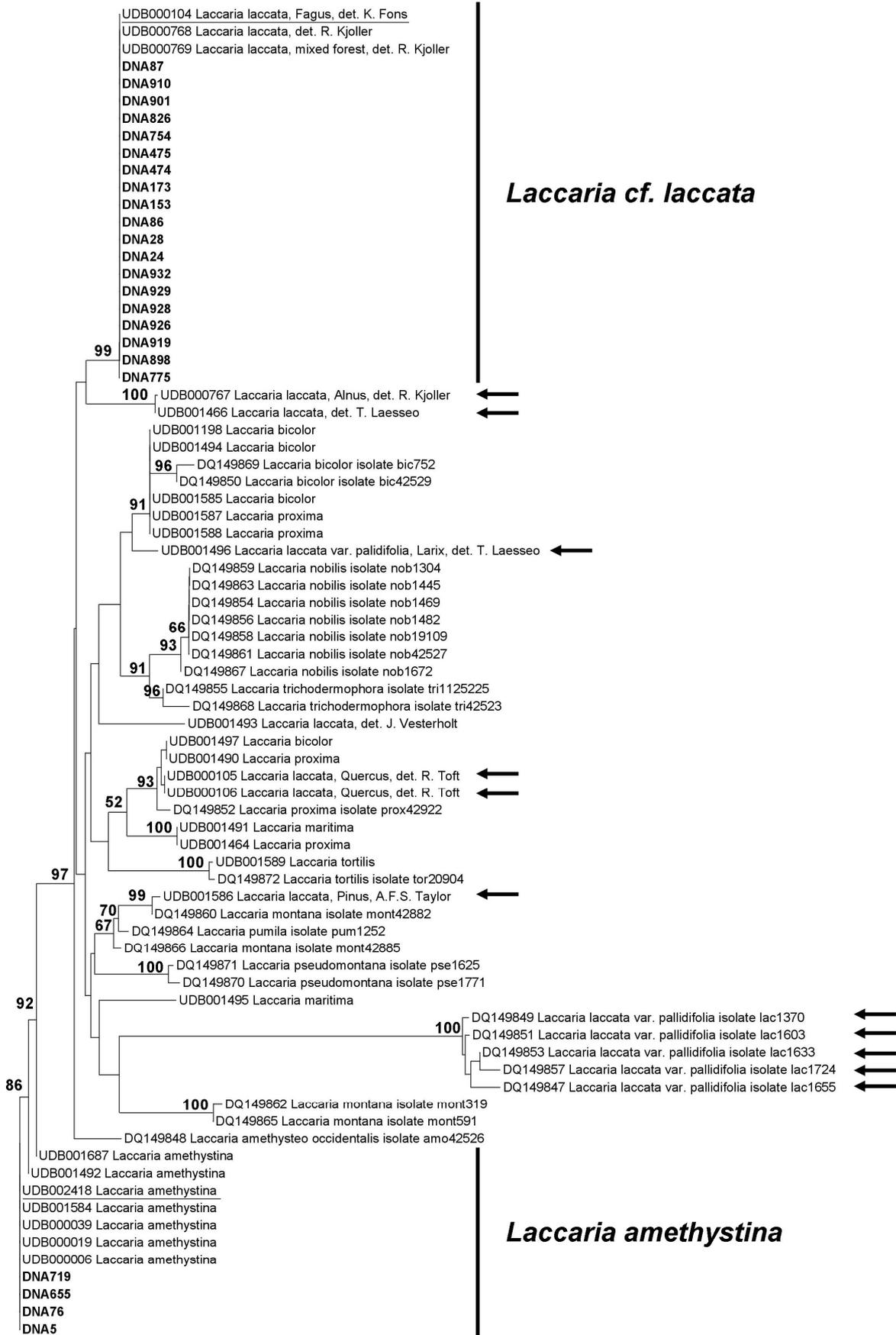


Fig. S6: *Inocybe* sequence types (previous page)

Reference sequences are from UNITE database (except AM882966, AM882720 and FJ816727 from GenBank). The best BLAST-search matches are underlined. For the query sequences only parts of the ITS region could be sequenced. A) For DNA834/835/837 the alignment comprises 441bp of the 5.8S and ITS2 regions. B) For DNA733 the alignment comprises 476 bp of ITS1 and 5.8S regions. For each alignment a separate unrooted Neighbour-Joining with 1000 bootstrap replications was constructed. Bootstrap values above 50 are indicated. The separation of DNA834/835/837 from AM882966 and AM882720 is well supported by high bootstrap values. That is the sequence type shows close affinities to *I. praetervisa* but the exact species designation remains questionable.

Fig. S7: *Laccaria* sequence types (next page)

Reference sequences from UNITE-database (UDB) indicate the host tree species and the name of person who determined the species name. Reference sequences from GenBank are selected according to Osmundson et al. (2005). The best BLAST-search matches are underlined. Sequences of morphologically determined fruit bodies of *L. laccata* (black arrows) appear at different positions in the phylogram. Therefore the species designation of *L. laccata* remains ambiguous. The unrooted Neighbour-Joining tree is based on a 625 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.



0.01 substitutions/site

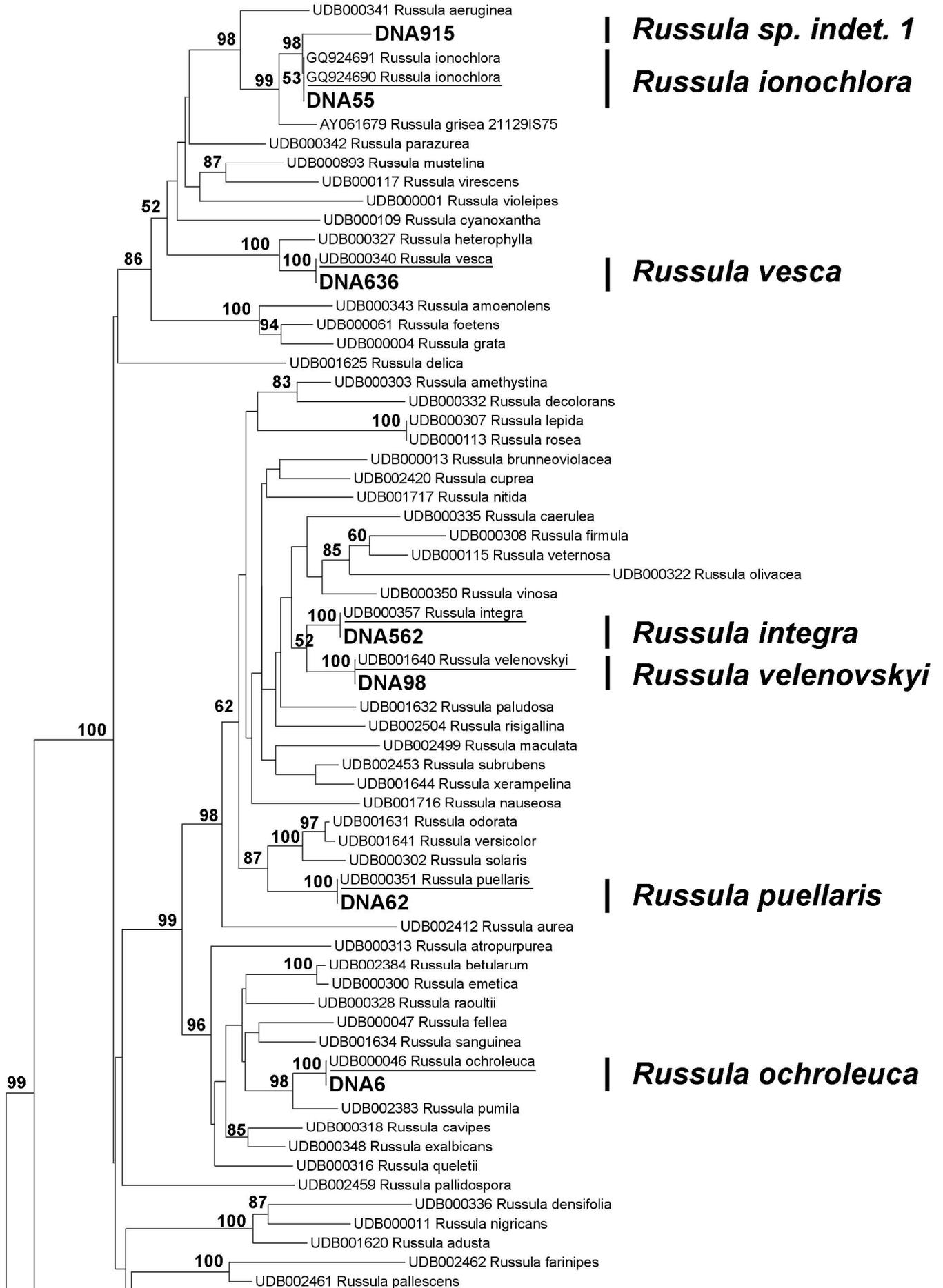


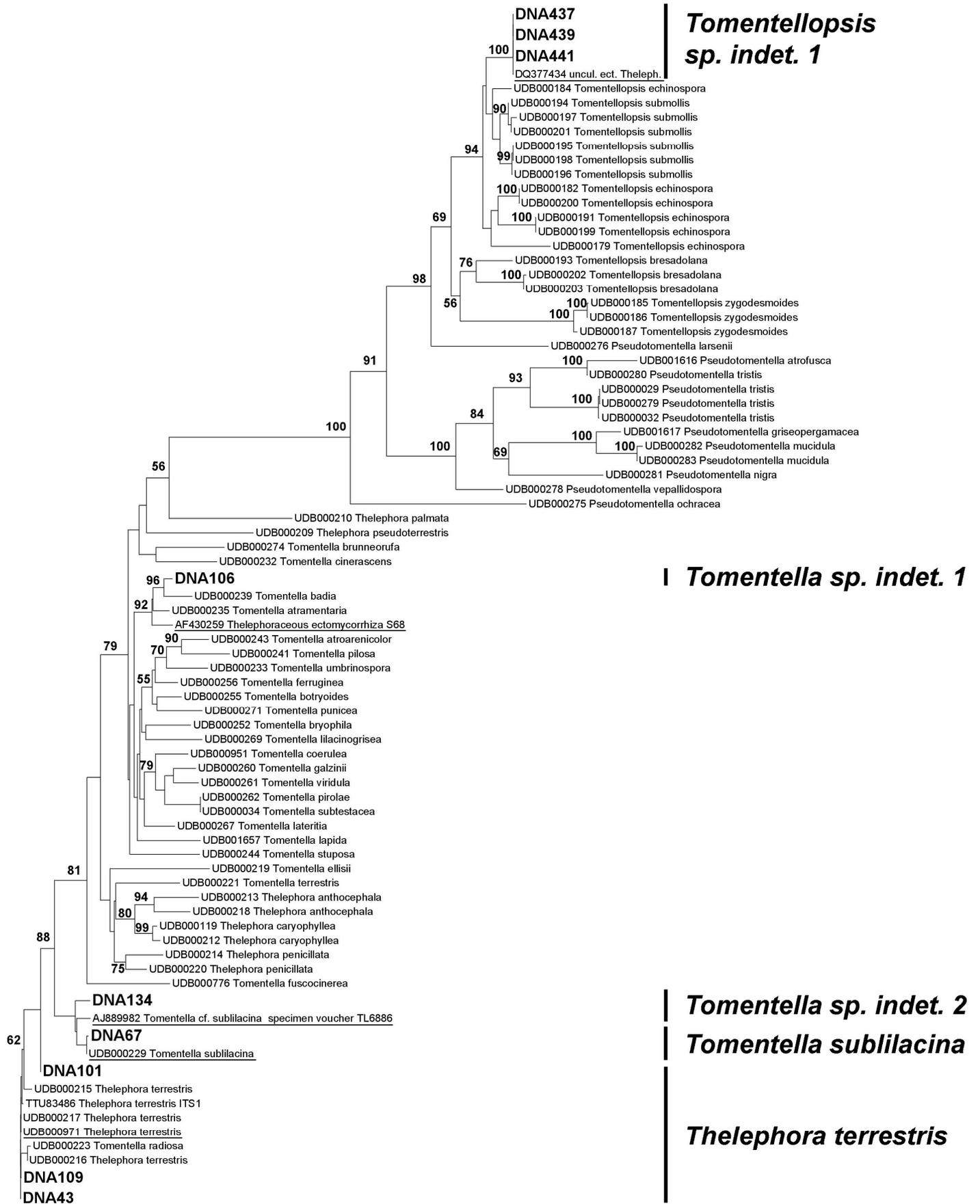


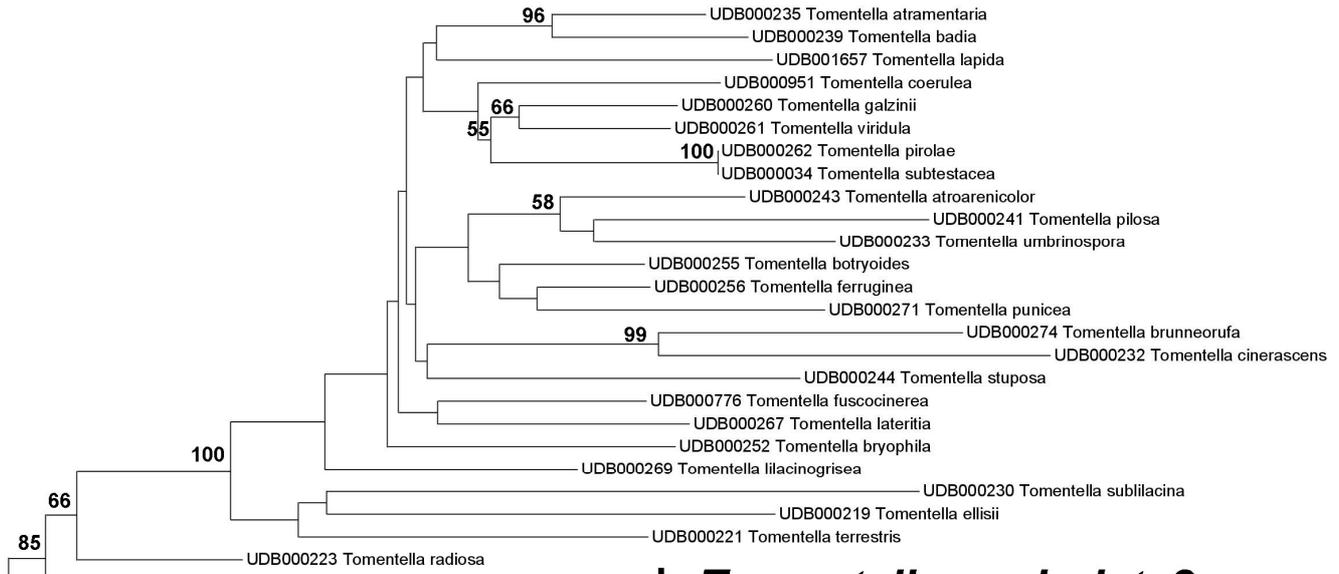
Fig. S9: *Meliniomyces* sequence types (previous page)

Reference sequences are from a BLAST-search in GenBank. The best BLAST-search matches are underlined. The unrooted Neighbour-Joining tree is based on a 512 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated for groups of interest only.

Fig. S10: Sequence types related to *Thelephora* (next page)

Reference sequences are from UNITE-database. For *Thelephora* and *Tomentellopsis* all UNITE entries are used. For *Tomentella* only one entry per species is given. The best BLAST-search matches are underlined. In the case of no close match in UNITE the best match in GenBank is given. For *Tomentellopsis* all query sequences are depicted, for *Thelephora* one sequence for each of two variations. DNA101 is an 186bp fragment that matches to *Thelephora terrestris*. One of 39 *Tomentella sublilacina* query sequences is used. A detailed phylogram with all *T. sublilacina* query sequences and 11 *T. sublilacina* references is depicted in Fig. S11. The unrooted Neighbour-Joining tree is based on a 600 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.





- DNA134**
 AJ889982 *Tomentella cf. sublilacina* specimen voucher TL6886
- DNA89**
DNA178
 UDB000228 *Tomentella sublilacina*
 UDB000227 *Tomentella sublilacina*
- DNA490**
DNA527
DNA555
DNA777
DNA838
DNA839
 UDB000229 *Tomentella sublilacina*
 UDB000970 *Tomentella sublilacina*
- DNA556**
 UDB000777 *Tomentella sublilacina*
DNA729
DNA576
DNA498
DNA496
DNA732
DNA730
 UDB000968 *Tomentella sublilacina*
DNA132
DNA632
DNA762
DNA633
DNA124
DNA149
DNA518
DNA146
DNA174
DNA549
DNA550
 UDB001662 *Tomentella sublilacina*
- DNA449**
DNA497
 UDB000033 *Tomentella sublilacina*
DNA499
DNA772
 UDB000969 *Tomentella sublilacina*
- DNA545**
DNA543
DNA540
DNA68
DNA122
DNA170
 UDB000030 *Tomentella sublilacina*
 UDB002378 *Tomentella sublilacina*
- DNA761**
DNA541
DNA67

Tomentella sp. indet. 2

Tomentella sublilacina

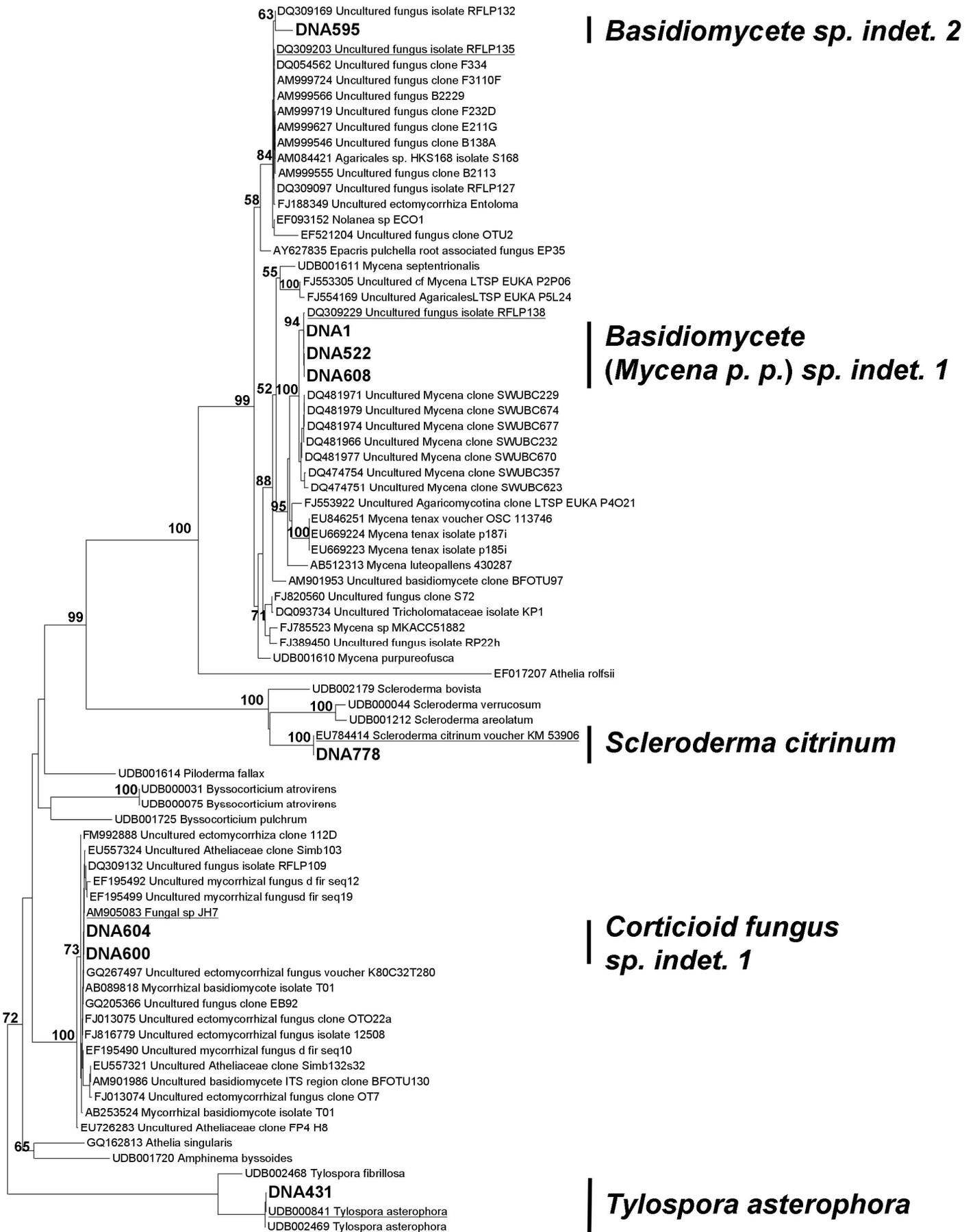
0.01 substitutions/site

Fig. S11: Detailed phylogram for the *Tomentella sublilacina* sequence type (previous page)

Not all 39 *T. sublilacina*-query sequences are exactly identical. Almost all have one or two deviating bases (at different positions). These 39 sequences are compared with 11 *T. sublilacina* references together with all *Tomentella* species (one sequence per species) stored in UNITE. DNA134 und DNA67 have been used in Fig S10. There is intraspecific variation in *T. sublilacina* but all 11 reference sequences of this species make up a group (together with the 39 query sequences) that is separated from the other *Tomentella* species. DNA 134 remains separated from *T. sublilacina* (as in Fig. S10) but also from other *Tomentella* species stored in UNITE. The unrooted Neighbour-Joining tree is based on a 567 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.

Fig. S12: *Tylospora*- and *Scleroderma* sequence types and unknown basidiomycetes (next page)

Most reference sequences are from GenBank. *Tylospora*- and *Mycena*-sequences and sequences of corticioid fungi are from UNITE-database (UDB...). The best BLAST-search matches are underlined. Detailed phylograms for DNA1/522/595/622 and DNA600/604 are depicted in Fig. S13 and S14. The unrooted Neighbour-Joining tree is based on a 577 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.



0.1 substitutions/site

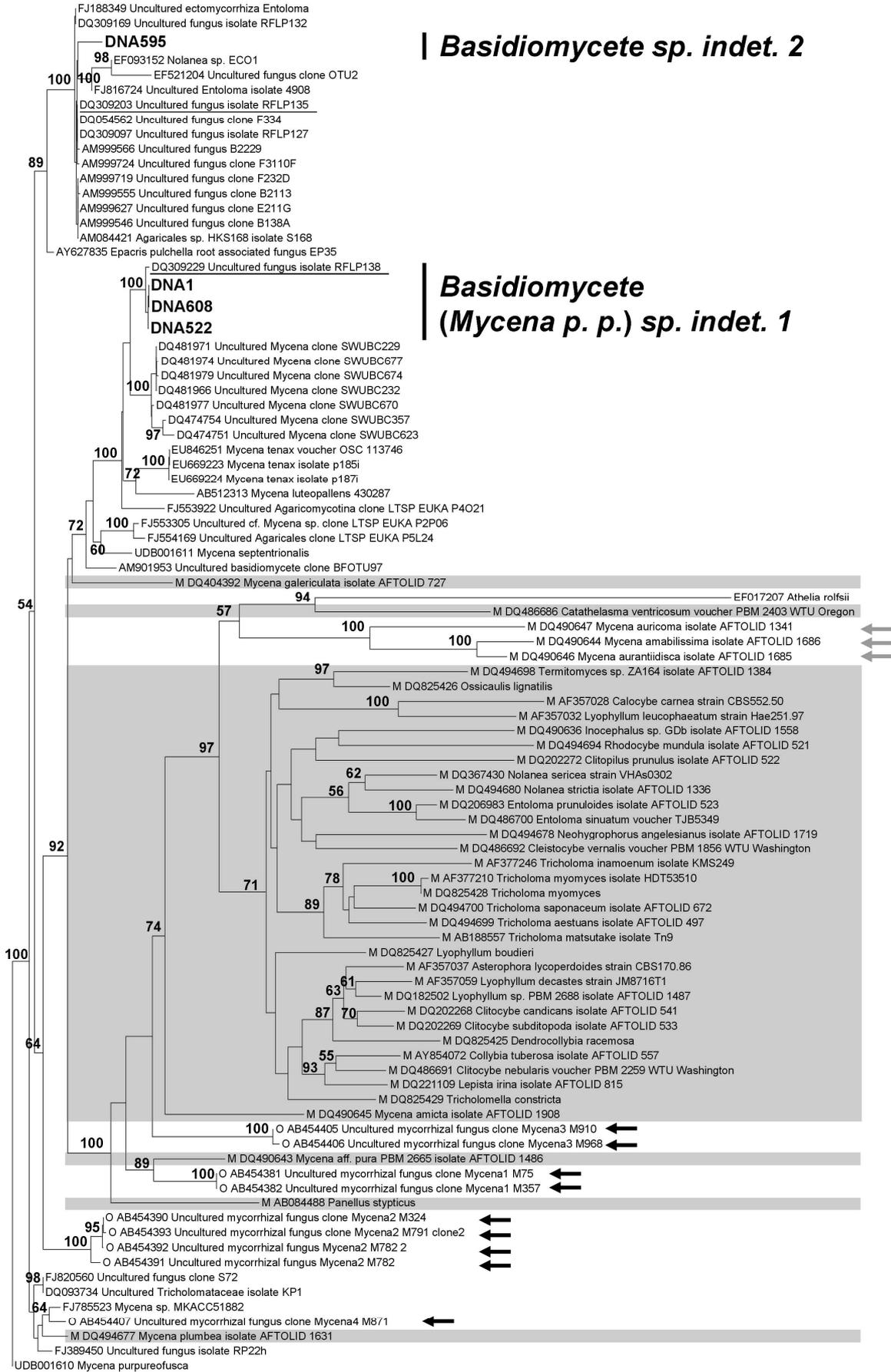
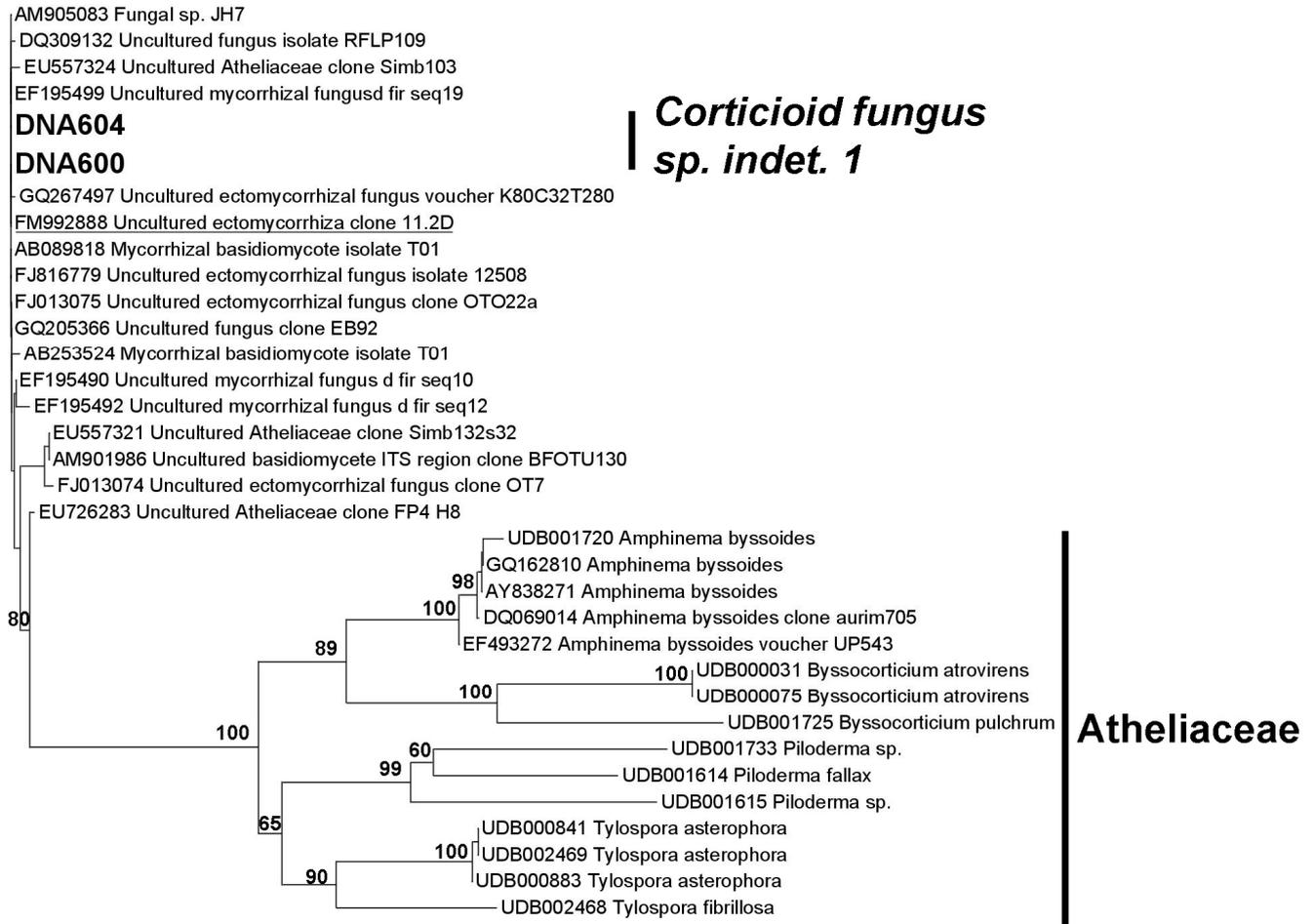


Fig. S13: Unknown basidiomycetes, extension of Fig. S12 (previous page)

Reference sequences are from BLAST-search in GenBank. Matches 1 to 16 for DNA522 and 1 to 11 for DNA595 were used (as in Fig. S12). UDB001610 und UDB001611 are from UNITE database. The best matches of BLAST-searches are underlined. For further comparison sequences of Matheny et al. (2006), Table 1, were incorporated (prefix M). The sequences in the grey boxes form the tricholomatoid clade in Matheny et al. According to Matheny et al. some *Mycena* sequences do not belong to the tricholomatoid clade (grey arrows), which is a sign for the paraphylie of this taxon. Ogura-Tsujita et al. (2009) report, that mycorrhizae on heterotrophic orchid are formed by four formerly unknown *Mycena*-species. Nine of their sequences are used for this phylogram (black arrows, prefix O). Since the genus designation is based solely on sequence analysis and Matheny et al suggest the paraphylie of *Mycena*, these fungi do not belong to *Mycena sensu strictu*. However, the observation of *Ogura-Tsujita et al.* shows that fungi related to *Mycena* can form close associations with roots. Although DNA1, DNA608 and DNA522 do not cluster with the tricholomatoid clade of Matheny et al. (2006) they cluster with one sequence of a morphologically determined *Mycena* species (UDB001611). Therefore these sequences are designated *Mycena pro parte* (p. p.). Since the morphotype is not clear (the roots were collected as the DS-morphotype BB05) and the *Mycena*-related sequences of Ogura-Tsujita et al. (2009) do not belong to true ectomycorrhizas these three sequences are considered to belong to root associated fungi but not to true ectomycorrhizal fungi. DNA595 does not cluster with any morphologically determined specimens. Therefore no genus designation is given. It was collected as DS-morphotype BB05 and is therefore considered to be a root associated fungus. The unrooted Neighbour-Joining tree is based on a 643 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.



L

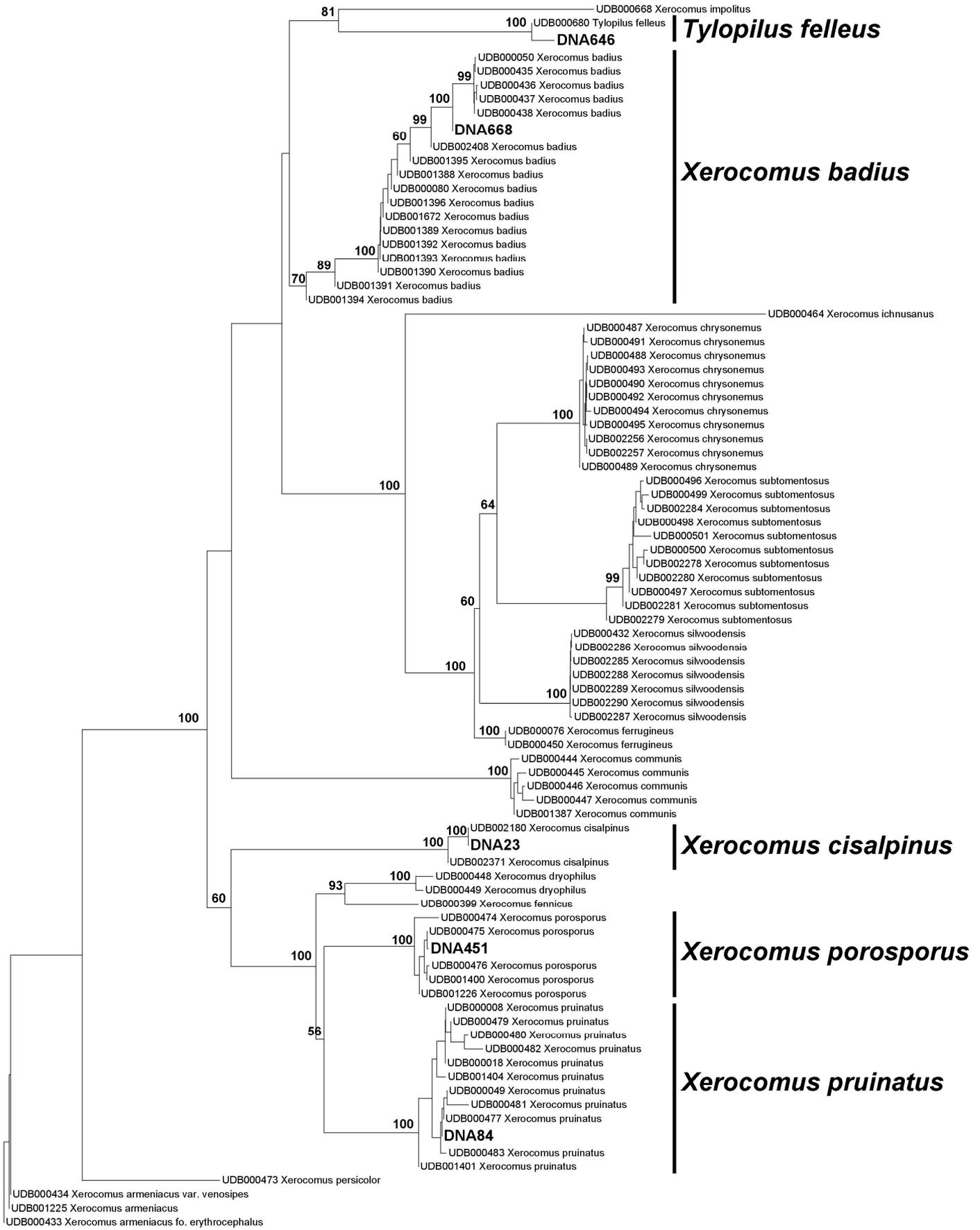
0.01 substitutions/site

Fig. S14: Corticoid fungus, extension of Fig. S12

Reference sequences are the matches 1 to 17 of a BLAST-search in GenBank for DNA601 and all UNITE sequences of the Atheliaceae sensu Larsson (2007). For better coverage of the Atheliaceae three *Amphinema byssoides* sequences from GenBank are included. DNA600 and DNA604 do not cluster with Atheliaceae. Since the genetic distance of the query sequence to the corticoid Atheliaceae is low they are considered to represent a corticoid fungus. The unrooted Neighbour-Joining tree is based on a 612 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.

Fig. S15: Xerocomus and Tylopilus sequence types, Neighbour-Joining (next page)

Reference sequences are from UNITE-database. For *Xerocomus ferrugineus* only two sequences are included, for all other species all entries are used. The unrooted Neighbour-Joining tree is based on an 834 bp alignment of the ITS region with 1000 bootstrap replications. Bootstrap values above 50 are indicated.



0.1 substitutions/site

Supplementary Table S1**Table S1: List of collected and sequenced specimens**

Sequences in bold face appear in the phylograms of Supplementary Figs. S1-S15.

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
ectomycorrhizal fungi						
BB11_301_Ah_Pi_150506	46	<i>Acephala macrosclerotiorum</i>	568	98.8	EU882732	HM189696
BB11_306_Oh_Pi_150506	71	<i>Acephala macrosclerotiorum</i>	526	98.8	EU882732	HM189697
BB05_205_Aeh_020506	111	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189698
BB11_107_Of_Pi_080506	163	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189699
BB11_108_Of_Pi_080506	169	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189700
BB11_201_Of_091006	415	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189701
BB11_208_Of_091006	435	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189702
BB11_107_Of_Pi_231006	533	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189703
BB11_110_Aeh_Pi_231006	557	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189704
BB11_307_Oh_Pi_011106	596	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189705
BB11_307_Oh_Pi_011106	598	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189706
BB11_203_Of_150107	648	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189707
BB11_203_Of_150107	649	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189708
BB11_203_Of_150107	650	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189709
BB11_206_Oh_150107	664	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189710
BB11_201_Of_160407	690	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189711
BB11_204_Of_160407	698	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189712
BB11_210_Of_160407	715	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189713
BB11_210_Oh_160407	716	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189714
BB11_108_Of_Pi_230407	760	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189715
BB11_109_Oh_Pi_230407	770	<i>Acephala macrosclerotiorum</i>	523	100	EU882732	HM189716
BB11_110_Of_Pi_230407	776	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189717
BB11_107_Of_Pi_230407	779	<i>Acephala macrosclerotiorum</i>	568	99.8	EU882732	HM189718
BB11_304_Of_Pi_070507	792	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189719

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB11_307_Of_Pi_070507	811	<i>Acephala macrosclerotiorum</i>	568	100	EU882732	HM189720
BB22_105_Oh_Pi_231006	517	<i>Amanita sp.</i>	703	97.5	FJ596814	HM189721
BB06_202_Of_160407	693	<i>Amanita sp.</i>	259	96.9	FJ596814	HM189722
BB05_105_Of_Pi_080506	145	<i>Cenococcum geophilum 1</i>	534	99.6	EU285479	HM189723
BB05_304_Of_Pi_011106	574	<i>Cenococcum geophilum 1</i>	533	98.9	EU285479	HM189724
BB05_103_Of_Pi_150107	629	<i>Cenococcum geophilum 1</i>	534	99.6	EU285479	HM189725
BB05_306_Of_Pi_150107	678	<i>Cenococcum geophilum 1</i>	533	98.9	EU285479	HM189726
BB05_207_Of_160407	706	<i>Cenococcum geophilum 1</i>	533	98.9	EU285479	HM189727
BB05_106_Of_Pi_230407	756	<i>Cenococcum geophilum 1</i>	518	99.6	EU285479	HM189728
BB05_106_Oh_Pi_230407	758	<i>Cenococcum geophilum 1</i>	534	99.6	EU285479	HM189729
BB05_308_Oh_Pi_070507	824	<i>Cenococcum geophilum 1</i>	488	99.6	EU285479	HM189730
BB05_309_Of_Fa_070507	830	<i>Cenococcum geophilum 1</i>	534	99.4	EU285479	HM189731
BB05_103_Of_Fa_230407	736	<i>Cenococcum geophilum 2</i>	508	98.2	FJ152539	HM189732
BB68_308_Of_Pi_011106	600	<i>Corticoid fungus</i>	637	100	FM992888	HM189733
BB68_308_Of_Pi_011106	604	<i>Corticoid fungus</i>	603	100	FM992888	HM189734
BB24_102_Of_Pi_080506	131	<i>Cortinarius (Dermocybe) sp. 1</i>	687	98.2	AY669585	HM189735
BB24_105_Of_Pi_080506	147	<i>Cortinarius (Dermocybe) sp. 1</i>	725	98.2	AY669585	HM189736
BB24_107_Oh_Pi_080506	165	<i>Cortinarius (Dermocybe) sp. 1</i>	725	98.5	AY669585	HM189737
BB24_203_Of_091006	422	<i>Cortinarius (Dermocybe) sp. 1</i>	540	98.0	AY669585	HM189738
BB28_301_Oh_Fa_011106	561	<i>Cortinarius (Dermocybe) sp. 1</i>	451	97.8	AY669585	HM189739
BB24_203_Of_150107	645	<i>Cortinarius (Dermocybe) sp. 1</i>	725	98.2	AY669585	HM189740
BB24_106_Oh_Pi_150107	673	<i>Cortinarius (Dermocybe) sp. 1</i>	652	98.2	AY669585	HM189741
BB24_203_Of_160407	696	<i>Cortinarius (Dermocybe) sp. 1</i>	688	98.4	AY669585	HM189742
BB24_207_Oh_160407	707	<i>Cortinarius (Dermocybe) sp. 1</i>	451	98.4	AY669585	HM189743
BB24_101_Of_Pi_230407	720	<i>Cortinarius (Dermocybe) sp. 1</i>	451	98.4	AY669585	HM189744
BB24_109_Oh_Pi_230407	768	<i>Cortinarius (Dermocybe) sp. 1</i>	725	98.2	AY669585	HM189745
BB80_203_Aeh_150107	651	<i>Cortinarius (Telamonia) sp. 2</i>	633	96.7	AY669664	HM189746
BB89_204_Aeh_160407	700	<i>Cortinarius (Telamonia) sp. 2</i>	633	96.8	AY669664	HM189747
BB05_407_Ah_220506	27	<i>Genea hispidula</i>	675	99.8	UDB001408	HM189748
BB52_410_Oh_161006	486	<i>Genea hispidula</i>	685	100.0	UDB001408	HM189749

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB82_406_Ah_150107	658	<i>Genea hispidula</i>	722	100.0	UDB001408	HM189750
BB82_406_Ah_150107	659	<i>Genea hispidula</i>	685	100.0	UDB001408	HM189751
BB82_401_Oh_210507	843	<i>Genea hispidula</i>	685	100.0	UDB001408	HM189752
BB82_404_Oh_210507	881	<i>Genea hispidula</i>	685	100.0	UDB001408	HM189753
BB82_410_Oh_210507	962	<i>Genea hispidula</i>	684	100.0	UDB001408	HM189754
BB51_402_Ah_220506	8	<i>Hydnotrya tulasnei</i>	777	99.5	UDB000095	GQ149454
BB34_402_Bv_220506	10	<i>Hydnotrya tulasnei</i>	777	99.5	UDB000095	GQ149455
BB38_301_Ah_Fa_150506	47	<i>Hydnotrya tulasnei</i>	777	99.5	UDB000095	HM189755
BB38_305_Ah_Fa_150506	65	<i>Hydnotrya tulasnei</i>	777	99.5	UDB000095	HM189756
BB39_101_Aeh_Fa_080506	130	<i>Hydnotrya tulasnei</i>	777	99.1	UDB000095	HM189757
BB27_105_Aeh_Fa_080506	151	<i>Hydnotrya tulasnei</i>	777	99.2	UDB000095	HM189758
BB22_202_Aeh_091006	419	<i>Hydnotrya tulasnei</i>	739	99.3	UDB000095	GQ215699
BB34_403_Ah_161006	450	<i>Hydnotrya tulasnei</i>	740	99.5	UDB000095	GQ149456
BB27_404_Ah_161006	460	<i>Hydnotrya tulasnei</i>	739	90.0	UDB000095	GQ149457
BB34_405_Ah_161006	462	<i>Hydnotrya tulasnei</i>	740	99.5	UDB000095	GQ149458
BB26_405_Ah_161006	463	<i>Hydnotrya tulasnei</i>	739	99.5	UDB000095	HM189759
BB34_410_Ah_161006	487	<i>Hydnotrya tulasnei</i>	308	98.7	UDB000095	HM189765
BB26_107_Bsh_Fa_231006	538	<i>Hydnotrya tulasnei</i>	777	99.3	UDB000095	HM189760
BB39_107_Bsh_Fa_231006	539	<i>Hydnotrya tulasnei</i>	777	99.3	UDB000095	HM189761
BB27_108_Aeh_Fa_231006	547	<i>Hydnotrya tulasnei</i>	777	99.3	UDB000095	HM189762
BB52_401_Oh_161006	620	<i>Hydnotrya tulasnei</i>	734	99.6	UDB000095	GQ149459
BB81_406_Ah_150107	656	<i>Hydnotrya tulasnei</i>	777	99.3	UDB000095	GQ149460
BB81_406_Ah_150107	657	<i>Hydnotrya tulasnei</i>	740	99.3	UDB000095	GQ149461
BB14_208_Aeh_160407	712	<i>Hydnotrya tulasnei</i>	740	99.5	UDB000095	GQ215700
BB70_108_Aeh_Pi_230407	766	<i>Hydnotrya tulasnei</i>	777	99.3	UDB000095	GQ215698
BB38_305_Ah_Fa_070507	803	<i>Hydnotrya tulasnei</i>	732	99.4	UDB000095	HM189763
BB85_404_Ah_210507	886	<i>Hydnotrya tulasnei</i>	740	99.2	UDB000095	HM189764
BB85_406_Ah_210507_R25	895	<i>Hydnotrya tulasnei</i>	740	99.5	UDB000095	GQ149462
BB43_404_Ah_220506	16	<i>Hydnum ellipsosporum</i>	685	100.0	AY817138	HM189766
BB53_401_Bv_161006	624	<i>Hydnum ellipsosporum</i>	492	100.0	AY817138	HM189767

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB86_404_Bv_210507	887	<i>Hydnum elliposporum</i>	648	100.0	AY817138	HM189768
BB75_102_Aeh_Fa_230407	733	<i>Inocybe napipes</i>	429	99.7	UDB000607	HM189769
BB38_309_Ah_Fa_070507	834	<i>Inocybe sp. aff. praetervisa</i>	400	99.1	AM882720	HM189770
BB38_309_Ah_Fa_070507	835	<i>Inocybe sp. aff. praetervisa</i>	400	99.1	AM882720	HM189771
BB38_309_Bv_Fa_070507	837	<i>Inocybe sp. aff. praetervisa</i>	402	99.4	AM882720	HM189772
BB37_402_Oh_220506	5	<i>Laccaria amethystina</i>	730	100	UDB002418	HM189773
BB37_307_Of_Pi_150506	76	<i>Laccaria amethystina</i>	730	100	UDB002418	HM189774
BB75_406_Of_150107	655	<i>Laccaria amethystina</i>	692	99.7	UDB002418	HM189775
BB75_101_Of_Fa_230407	719	<i>Laccaria amethystina</i>	692	100	UDB002418	HM189776
BB100_410_Ah_210507	963	<i>Laccaria amethystina</i>	145	100.0	UDB002418	HM189777
BB34_406_Bv_220506	24	<i>Laccaria cf. laccata</i>	730	100	UDB000104	HM189778
BB34_407_Ah_220506	28	<i>Laccaria cf. laccata</i>	692	100	UDB000104	HM189779
BB38_308_Ah_Fa_150506	86	<i>Laccaria cf. laccata</i>	692	100	UDB000104	HM189780
BB34_308_Bv_Fa_150506	87	<i>Laccaria cf. laccata</i>	692	99.9	UDB000104	HM189781
BB34_310_Ah_Fa_150506	96	<i>Laccaria cf. laccata</i>	507	100	UDB000104	HM189782
BB38_105_Bsh_Fa_080506	153	<i>Laccaria cf. laccata</i>	730	100	UDB000104	HM189783
BB38_108_Aeh_Fa_080506	173	<i>Laccaria cf. laccata</i>	730	100	UDB000104	HM189784
BB43_407_Ah_161006	473	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM189792
BB28_407_Ah_161006	474	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM189785
BB08_407_Ah_161006	475	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM189786
BB75_403_Bv_150107	644	<i>Laccaria cf. laccata</i>	692	100	UDB000104	HM189787
BB75_105_Bsh_Fa_230407	754	<i>Laccaria cf. laccata</i>	730	100	UDB000104	HM189788
BB92_109_Bsh_Fa_230407	775	<i>Laccaria cf. laccata</i>	730	99.9	UDB000104	HM189789
BB75_308_Ah_Fa_070507	826	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM189790
BB75_407_Ah_210507_R28	898	<i>Laccaria cf. laccata</i>	693	99.9	UDB000104	HM189791
BB75_408_Ah_210507_R48	932	<i>Laccaria cf. laccata</i>	730	99.9	UDB000104	HM189793
BB85_106_Aeh_Fa_150107	675	<i>Lactarius necator</i>	761	100	UDB000361	HM189794
BB79_203_Of_150107	647	<i>Lactarius rufus</i>	763	100	UDB001601	HM189795
BB79_308_Ah_Pi_070507	827	<i>Lactarius rufus</i>	726	100	UDB001601	HM189796
BB45_407_Ah_220506	29	<i>Lactarius subdulcis</i>	771	100	UDB000048	HM189797

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB39_407_Of_161006	469	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189798
BB45_407_Of_161006	470	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189799
BB45_408_Ah_161006	476	<i>Lactarius subdulcis</i>	771	100	UDB000048	HM189800
BB45_401_Oh_161006	617	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189801
BB26_401_Ah_161006	623	<i>Lactarius subdulcis</i>	437	100	UDB000048	HM189805
BB96_401_Oh_210507	846	<i>Lactarius subdulcis</i>	771	99.9	UDB000048	HM189802
BB45_407_Ah_210507_R36	913	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189807
BB70_407_Ah_210507_R42	923	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189803
BB45_408_Ah_210507_R50	934	<i>Lactarius subdulcis</i>	728	100	UDB000048	HM189806
BB22_408_Ah_210507_R55	940	<i>Lactarius subdulcis</i>	734	99.9	UDB000048	HM189804
BB38_404_Oh_220506	14	<i>Lactarius tabidus</i>	418	99.8	UDB000385	HM189829
BB38_406_Ah_220506	21	<i>Lactarius tabidus</i>	489	100	UDB000385	HM189830
BB45_409_Oh_220506	35	<i>Lactarius tabidus</i>	497	100	UDB000385	HM189834
BB22_103_Of_Pi_080506	135	<i>Lactarius tabidus</i>	722	99.9	UDB000108	HM189808
BB38_103_Oh_Fa_080506	139	<i>Lactarius tabidus</i>	766	99.9	UDB000385	HM189809
BB23_104_Oh_Pi_080506	144	<i>Lactarius tabidus</i>	766	99.9	UDB000385	HM189810
BB38_106_Of_Fa_080506	155	<i>Lactarius tabidus</i>	664	99.7	UDB000385	HM189811
BB45_106_Of_Fa_080506	157	<i>Lactarius tabidus</i>	721	99.9	UDB000385	HM189831
BB26_402_Oh_161006	445	<i>Lactarius tabidus</i>	435	99.7	UDB000385	HM189832
BB39_404_Oh_161006	455	<i>Lactarius tabidus</i>	728	99.9	UDB000385	HM189812
BB22_103_Oh_Pi_231006	503	<i>Lactarius tabidus</i>	206	99.5	UDB000385	HM189813
BB38_104_Of_Fa_231006	511	<i>Lactarius tabidus</i>	689	99.9	UDB000385	HM189814
BB61_104_Of_Pi_231006	512	<i>Lactarius tabidus</i>	689	99.9	UDB000385	HM189815
BB22_104_Of_Pi_231006	513	<i>Lactarius tabidus</i>	689	99.9	UDB000385	HM189816
BB22_104_Oh_Pi_231006	514	<i>Lactarius tabidus</i>	767	99.7	UDB000385	HM189817
BB45_106_Oh_Fa_231006	528	<i>Lactarius tabidus</i>	723	99.9	UDB000385	HM189818
BB38_106_Oh_Fa_231006	529	<i>Lactarius tabidus</i>	766	99.9	UDB000385	HM189819
BB22_103_Of_Pi_150107	628	<i>Lactarius tabidus</i>	686	99.9	UDB000385	HM189820
BB38_403_Of_150107	639	<i>Lactarius tabidus</i>	197	100	UDB000385	HM189821
BB38_406_Ah_150107	663	<i>Lactarius tabidus</i>	686	99.9	UDB000385	HM189822

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB38_106_Oh_Fa_150107	671	<i>Lactarius tabidus</i>	686	99.9	UDB000385	HM189823
BB22_106_Oh_Pi_150107	672	<i>Lactarius tabidus</i>	686	99.9	UDB000385	HM189824
BB22_103_Of_Pi_230407	734	<i>Lactarius tabidus</i>	766	99.9	UDB000385	HM189825
BB22_103_Aeh_Fa_230407	741	<i>Lactarius tabidus</i>	495	100	UDB000385	HM189826
BB22_104_Oh_Fa_230407	744	<i>Lactarius tabidus</i>	770	99.3	UDB000385	HM189827
BB22_104_Aeh_Pi_230407	745	<i>Lactarius tabidus</i>	729	99.9	UDB000385	HM189833
BB38_404_Oh_210507	882	<i>Lactarius tabidus</i>	722	100	UDB000385	HM189828
BB86_306_Bv_Fa_150107	686	<i>Lactarius vellereus</i>	723	99.9	UDB002494	HM189835
BB83_406_Ah_150107	661	<i>Pezizaceae</i>	732	100.0	AJ969437	HM189836
BB83_401_Oh_210507	842	<i>Pezizaceae</i>	695	100.0	AJ969437	HM189837
BB83_401_Ah_210507	848	<i>Pezizaceae</i>	695	100.0	AJ969437	HM189838
BB45_301_Ah_Fa_011106	562	<i>Russula integra</i>	743	100	UDB000357	HM189839
BB14_301_Ah_Pi_011106	563	<i>Russula integra</i>	743	100	UDB000357	HM189840
BB28_410_Ah_220506	41	<i>Russula ionochlora</i>	390	100	GQ924690	HM189870
BB22_303_Of_Pi_150506	55	<i>Russula ionochlora</i>	683	100	GQ924690	HM189841
BB08_309_Ah_Fa_150506	93	<i>Russula ionochlora</i>	683	100	GQ924690	HM189842
BB38_310_Bv_Fa_150506	97	<i>Russula ionochlora</i>	633	99.8	GQ924690	HM189843
BB28_406_Ah_161006	465	<i>Russula ionochlora</i>	646	100	GQ924690	HM189871
BB08_406_Ah_161006	468	<i>Russula ionochlora</i>	646	100	GQ924690	HM189872
BB38_303_Ah_Fa_011106	570	<i>Russula ionochlora</i>	683	100	GQ924690	HM189844
BB72_303_Ah_Fa_011106	571	<i>Russula ionochlora</i>	683	100	GQ924690	HM189845
BB72_307_Ah_Fa_011106	599	<i>Russula ionochlora</i>	683	100	GQ924690	HM189846
BB08_310_Ah_Fa_011106	613	<i>Russula ionochlora</i>	646	100	GQ924690	HM189847
BB28_310_Ah_Fa_011106	615	<i>Russula ionochlora</i>	646	100	GQ924690	HM189848
BB28_310_Bv_Fa_011106	616	<i>Russula ionochlora</i>	646	100	GQ924690	HM189849
BB72_403_Bv_150107	643	<i>Russula ionochlora</i>	639	100	GQ924690	HM189850
BB72_303_Bv_Fa_150107	654	<i>Russula ionochlora</i>	683	100	GQ924690	HM189851
BB72_406_Ah_150107	662	<i>Russula ionochlora</i>	683	100	GQ924690	HM189852
BB72_306_Oh_Pi_150107	679	<i>Russula ionochlora</i>	683	100	GQ924690	HM189853
BB72_306_Oh_Fa_150107	680	<i>Russula ionochlora</i>	683	100	GQ924690	HM189854

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB72_306_Bv_Fa_150107	683	<i>Russula ionochlora</i>	683	100	GQ924690	HM189855
BB28_104_Oh_Fa_230407	743	<i>Russula ionochlora</i>	683	100	GQ924690	HM189856
BB72_104_Aeh_Fa_230407	747	<i>Russula ionochlora</i>	646	99.7	GQ924690	HM189857
BB72_104_Bsh_Fa_230407	748	<i>Russula ionochlora</i>	683	100	GQ924690	HM189858
BB72_106_Bsh_Fa_230407	759	<i>Russula ionochlora</i>	683	100	GQ924690	HM189859
BB28_302_Bv_Fa_070507	787	<i>Russula ionochlora</i>	641	100	GQ924690	HM189873
BB28_302_Bv_Fa_070507	788	<i>Russula ionochlora</i>	640	100	GQ924690	HM189860
BB72_307_Ah_Pi_070507	815	<i>Russula ionochlora</i>	636	100	GQ924690	HM189861
BB72_307_Bv_Fa_070507	819	<i>Russula ionochlora</i>	646	100	GQ924690	HM189862
BB72_309_Ah_Fa_070507	832	<i>Russula ionochlora</i>	646	100	GQ924690	HM189863
BB72_309_Bv_Fa_070507	836	<i>Russula ionochlora</i>	646	100	GQ924690	HM189864
BB72_310_Bv_Fa_070507	841	<i>Russula ionochlora</i>	646	100	GQ924690	HM189865
BB06_403_Ah_210507_R02	855	<i>Russula ionochlora</i>	636	100	GQ924690	HM189866
BB72_403_Bv_210507_R15	871	<i>Russula ionochlora</i>	646	99.4	GQ924690	HM189874
BB72_404_Ah_210507	885	<i>Russula ionochlora</i>	646	99.8	GQ924690	HM189867
BB72_407_Ah_210507_R34	909	<i>Russula ionochlora</i>	646	100	GQ924690	HM189875
BB72_408_Ah_210507_R58	944	<i>Russula ionochlora</i>	640	100	GQ924690	HM189868
BB72_408_Bv_210507_R66	953	<i>Russula ionochlora</i>	646	100	GQ924690	HM189869
BB14_402_Oh_220506	6	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189876
BB45_402_Ah_220506	7	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189877
BB34_403_Ah_220506	12	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189878
BB14_202_Of_020506	100	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189879
BB60_204_Of_020506	104	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189880
BB06_204_Of_020506	105	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189881
BB53_208_Oh_020506	120	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189882
BB54_209_Aeh_020506	123	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189883
BB54_210_Aeh_020506	126	<i>Russula ochroleuca</i>	693	100	UDB000046	HM189884
BB14_101_Of_Pi_080506	128	<i>Russula ochroleuca</i>	687	100	UDB000046	HM189931
BB08_205_Of_091006	428	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189885
BB14_205_Oh_091006	429	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189886

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB14_209_Of_091006	438	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189887
BB14_402_Of_161006	443	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189888
BB14_402_Oh_161006	444	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189889
BB14_403_Oh_161006	447	<i>Russula ochroleuca</i>	690	100	UDB000046	HM189890
BB26_403_Ah_161006	452	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189891
BB45_403_Ah_161006	453	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189892
BB14_404_Oh_161006	457	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189893
BB52_404_Oh_161006	458	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189930
BB39_405_Ah_161006	461	<i>Russula ochroleuca</i>	698	100	UDB000046	HM189894
BB45_409_Oh_161006	480	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189895
BB39_409_Oh_161006	481	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189896
BB14_101_Oh_Pi_231006	491	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189897
BB14_101_Aeh_Fa_231006	494	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189898
BB14_101_Aeh_Pi_231006	495	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189899
BB39_304_Of_Fa_011106	578	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189900
BB14_304_Oh_Pi_011106	580	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189901
BB14_308_Oh_Pi_011106	602	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189902
BB38_308_Oh_Pi_011106	605	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189903
BB61_308_Oh_Pi_011106	607	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189904
BB14_401_Oh_161006	619	<i>Russula ochroleuca</i>	692	100	UDB000046	HM189905
BB27_401_Ah_161006	621	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189906
BB70_103_Of_Fa_150107	631	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189907
BB14_103_Of_Fa_150107	634	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189908
BB14_403_Of_150107	640	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189909
BB14_209_Oh_150107	667	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189910
BB14_109_Of_Fa_150107	685	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189911
BB14_205_Oh_160407	701	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189912
BB14_205_Aeh_160407	702	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189913
BB14_206_Aeh_160407	703	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189914
BB14_209_Of_160407	714	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189915

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB14_101_Oh_Pi_230407	721	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189916
BB14_101_Oh_Fa_230407	723	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189917
BB14_101_Aeh_Fa_230407	725	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189918
BB22_103_Oh_Pi_230407	737	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189919
BB14_103_Oh_Pi_230407	738	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189920
BB14_106_Oh_Fa_230407	757	<i>Russula ochroleuca</i>	736	100	UDB000046	HM189921
BB14_304_Oh_Pi_070507	793	<i>Russula ochroleuca</i>	693	100	UDB000046	HM189922
BB14_304_Ah_Pi_070507	795	<i>Russula ochroleuca</i>	693	100	UDB000046	HM189923
BB70_401_Oh_210507	845	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189924
BB70_401_Ah_210507	849	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189925
BB14_402_Bv_210507	852	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189926
BB06_403_Ah_210507_R07	862	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189927
BB70_407_Ah_210507_R31	903	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189928
BB70_408_Ah_210507_R54	939	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189929
BB61_305_Oh_Pi_150506	62	<i>Russula puellaris</i>	735	99.7	UDB000351	HM189932
BB14_208_Of_020506	118	<i>Russula puellaris</i>	735	99.4	UDB000351	HM189933
BB63_109_Oh_Fa_080506	176	<i>Russula puellaris</i>	669	99.2	UDB000351	HM189934
BB57_207_Oh_091006	433	<i>Russula puellaris</i>	697	99.5	UDB000351	HM189935
BB53_109_Bsh_Fa_231006	553	<i>Russula puellaris</i>	735	99.5	UDB000351	HM189936
BB45_302_Ah_Fa_011106	564	<i>Russula puellaris</i>	735	99.5	UDB000351	HM189937
BB38_305_Ah_Fa_011106	588	<i>Russula puellaris</i>	735	99.5	UDB000351	HM189938
BB53_307_Of_Pi_011106	592	<i>Russula puellaris</i>	735	99.4	UDB000351	HM189939
BB61_307_Oh_Pi_011106	597	<i>Russula puellaris</i>	692	99.5	UDB000351	HM189940
BB22_208_Aeh_160407	711	<i>Russula puellaris</i>	698	99.4	UDB000351	HM189941
BB53_109_Aeh_Pi_230407	773	<i>Russula puellaris</i>	735	99.4	UDB000351	HM189942
BB91_109_Bsh_Fa_230407	774	<i>Russula puellaris</i>	735	99.4	UDB000351	HM189943
BB53_307_Oh_Pi_070507	813	<i>Russula puellaris</i>	698	99.4	UDB000351	HM189944
BB72_309_Ah_Fa_150107	687	<i>Russula sp. aff. ionochlora</i>	646	96.6	GQ924690	HM189948
BB28_309_Ah_Fa_150107	688	<i>Russula sp. aff. ionochlora</i>	646	96.4	GQ924690	HM189947
BB06_403_Bv_210507_R14	869	<i>Russula sp. aff. ionochlora</i>	646	96.6	GQ924690	HM189945

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB28_407_Ah_210507_R36	915	<i>Russula sp. aff. ionochlora</i>	683	96.6	GQ924690	HM189946
BB14_201_Oh_020506	98	<i>Russula velenovskyi</i>	737	100	UDB001640	HM189949
BB22_201_Aeh_091006	417	<i>Russula velenovskyi</i>	699	100	UDB001640	HM189950
BB22_201_Oh_160407	692	<i>Russula velenovskyi</i>	700	100	UDB001640	HM189951
BB28_103_Aeh_Fa_231006	508	<i>Russula vesca</i>	681	100	UDB000340	HM189952
BB72_103_Ah_Fa_150107	636	<i>Russula vesca</i>	722	100	UDB000340	HM189953
BB72_103_Bsh_Fa_150107	637	<i>Russula vesca</i>	685	100	UDB000340	HM189954
BB38_403_Of_150107	638	<i>Russula vesca</i>	677	100	UDB000340	HM189956
BB06_103_Oh_Pi_230407	740	<i>Russula vesca</i>	722	100	UDB000340	HM189955
BB28_110_Aeh_Fa_230407	778	<i>Scleroderma citrinum</i>	714	100	EU784414	HM189957
BB64_301_Oh_Pi_150506	43	<i>Thelephora terrestris</i>	701	100	UDB000971	HM189958
BB23_305_Ah_Pi_150506	64	<i>Thelephora terrestris</i>	701	100	UDB000971	HM189959
BB19_203_Of_020506	101	<i>Thelephora terrestris</i>	186	97.2	TTU83486	HM189967
BB14_205_Of_020506	109	<i>Thelephora terrestris</i>	661	99.3	UDB000971	HM189960
BB20_205_Oh_020506	110	<i>Thelephora terrestris</i>	701	100	UDB000971	HM189961
BB23_204_Of_091006	424	<i>Thelephora terrestris</i>	663	100	UDB000971	HM189962
BB06_204_Of_091006	425	<i>Thelephora terrestris</i>	663	100	UDB000971	HM189963
BB88_204_Oh_160407	699	<i>Thelephora terrestris</i>	701	100	UDB000971	HM189964
BB23_301_Of_Pi_070507	781	<i>Thelephora terrestris</i>	663	100	UDB000971	HM189965
BB23_301_Of_Fa_070507	782	<i>Thelephora terrestris</i>	663	100	UDB000971	HM189966
BB05_204_Oh_020506	106	<i>Tomentella sp. 1</i>	671	96.1	AF430259	HM189968
BB38_102_Aeh_Fa_080506	134	<i>Tomentella sp. 2</i>	703	97.9	AJ889982	HM189969
BB53_305_Of_Pi_150506	67	<i>Tomentella sublilacina</i>	704	98.8	UDB000970	HM190007
BB22_305_Of_Pi_150506	68	<i>Tomentella sublilacina</i>	704	98.8	UDB000970	HM190008
BB61_309_Of_Pi_150506	89	<i>Tomentella sublilacina</i>	654	99.8	UDB000970	HM189970
BB06_209_Of_020506	122	<i>Tomentella sublilacina</i>	666	99.3	UDB000970	HM189971
BB06_210_Of_020506	124	<i>Tomentella sublilacina</i>	704	99.1	UDB000970	HM189972
BB22_102_Of_Pi_080506	132	<i>Tomentella sublilacina</i>	704	99.7	UDB000970	HM189973
BB22_105_Of_Pi_080506	146	<i>Tomentella sublilacina</i>	704	99.3	UDB000970	HM189974
BB23_105_Oh_Pi_080506	149	<i>Tomentella sublilacina</i>	664	99.6	UDB000970	HM189975

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB22_108_Oh_Pi_080506	170	<i>Tomentella sublilacina</i>	704	99.1	UDB000970	HM189976
BB42_109_Of_Fa_080506	174	<i>Tomentella sublilacina</i>	658	99.5	UDB000970	HM189977
BB22_110_Of_Pi_080506	178	<i>Tomentella sublilacina</i>	704	100	UDB000970	HM189978
BB34_403_Oh_161006	449	<i>Tomentella sublilacina</i>	666	99.3	UDB000970	HM189979
BB39_101_Of_Fa_231006	490	<i>Tomentella sublilacina</i>	667	100	UDB000970	HM189980
BB38_102_Of_Fa_231006	496	<i>Tomentella sublilacina</i>	704	100	UDB000970	HM189981
BB39_102_Of_Fa_231006	497	<i>Tomentella sublilacina</i>	667	99.7	UDB000970	HM189982
BB37_102_Of_Fa_231006	498	<i>Tomentella sublilacina</i>	628	99.8	UDB000970	HM189983
BB53_102_Oh_Pi_231006	499	<i>Tomentella sublilacina</i>	704	99.7	UDB000970	HM189984
BB38_105_Oh_Fa_231006	518	<i>Tomentella sublilacina</i>	704	99.8	UDB000970	HM189985
BB38_106_Oh_Fa_231006	527	<i>Tomentella sublilacina</i>	704	100	UDB000970	HM189986
BB22_108_Of_Pi_231006	540	<i>Tomentella sublilacina</i>	704	99.1	UDB000970	HM189987
BB38_108_Of_Fa_231006	541	<i>Tomentella sublilacina</i>	662	99.3	UDB000970	HM189988
BB22_108_Oh_Pi_231006	543	<i>Tomentella sublilacina</i>	704	99.1	UDB000970	HM189989
BB38_108_Oh_Fa_231006	545	<i>Tomentella sublilacina</i>	704	99.1	UDB000970	HM189990
BB39_109_Of_Fa_231006	549	<i>Tomentella sublilacina</i>	704	99.5	UDB000970	HM189991
BB38_109_Of_Fa_231006	550	<i>Tomentella sublilacina</i>	704	99.5	UDB000970	HM189992
BB39_110_Of_Fa_231006	555	<i>Tomentella sublilacina</i>	704	100	UDB000970	HM189993
BB22_110_Oh_Pi_231006	556	<i>Tomentella sublilacina</i>	704	99.8	UDB000970	HM189994
BB61_304_Of_Pi_011106	576	<i>Tomentella sublilacina</i>	704	99.8	UDB000970	HM189995
BB71_103_Of_Pi_150107	632	<i>Tomentella sublilacina</i>	704	99.3	UDB000970	HM189996
BB71_103_Of_Pi_150107	633	<i>Tomentella sublilacina</i>	667	99.1	UDB000970	HM189997
BB37_102_Of_Fa_230407	729	<i>Tomentella sublilacina</i>	704	99.8	UDB000970	HM189998
BB88_102_Oh_Pi_230407	730	<i>Tomentella sublilacina</i>	704	99.7	UDB000970	HM189999
BB88_102_Oh_Pi_230407	732	<i>Tomentella sublilacina</i>	704	99.7	UDB000970	HM190000
BB88_108_Of_Pi_230407	761	<i>Tomentella sublilacina</i>	704	99.1	UDB000970	HM190001
BB88_108_Of_Pi_230407	762	<i>Tomentella sublilacina</i>	667	99.1	UDB000970	HM190002
BB37_109_Oh_Fa_230407	772	<i>Tomentella sublilacina</i>	704	99.7	UDB000970	HM190003
BB88_110_Oh_Pi_230407	777	<i>Tomentella sublilacina</i>	704	100	UDB000970	HM190004
BB53_310_Of_Pi_070507	838	<i>Tomentella sublilacina</i>	667	100	UDB000970	HM190005

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB53_310_Of_Pi_070507	839	<i>Tomentella sublilacina</i>	667	100	UDB000970	HM190006
BB06_209_Of_091006	437	<i>Tomentellopsis sp.</i>	646	100	DQ377434	HM190009
BB06+BB14_209_Of_091006	439	<i>Tomentellopsis sp.</i>	646	100	DQ377434	HM190010
BB06_210_Of_091006	441	<i>Tomentellopsis sp.</i>	610	99.8	DQ377434	HM190011
BB57_410_Oh_161006	483	<i>Tuber puberulum</i>	557	100.0	UDB001385	HM190012
BB57_410_Ah_210507	964	<i>Tuber puberulum</i>	556	100.0	UDB001385	HM190013
BB06_203_Of_091006	420	<i>Tylophilus felleus</i>	673	99.8	UDB000680	HM190014
BB06_203_Of_150107	646	<i>Tylophilus felleus</i>	711	99.9	UDB000680	HM190015
BB06_203_Of_160407	695	<i>Tylophilus felleus</i>	711	99.9	UDB000680	HM190016
BB22_206_Aeh_091006	431	<i>Tylospora asterophora</i>	627	99.8	UDB000841	HM190017
BB06_304_Of_Pi_150506	58	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190018
BB08_306_Oh_Pi_150506	72	<i>Xerocomus badius</i>	615	99.8	UDB000050	HM190048
BB06_309_Of_Pi_150506	88	<i>Xerocomus badius</i>	528	99.8	UDB000050	HM190049
BB06_202_Of_020506	99	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190019
BB24_204_Aeh_020506	107	<i>Xerocomus badius</i>	530	99.6	UDB000050	HM190020
BB06_208_Oh_020506	119	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190021
BB23_101_Of_Pi_080506	127	<i>Xerocomus badius</i>	653	100	UDB000050	HM190022
BB22_101_Of_Pi_080506	129	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190023
BB06_107_Oh_Pi_080506	161	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190024
BB06_108_Oh_Pi_080506	168	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190025
BB06_202_Of_091006	418	<i>Xerocomus badius</i>	615	99.8	UDB000050	HM190026
BB08_204_Of_091006	426	<i>Xerocomus badius</i>	615	100.0	UDB000050	HM190027
BB06_206_Oh_091006	430	<i>Xerocomus badius</i>	615	100.0	UDB000050	HM190028
BB08_207_Of_091006	432	<i>Xerocomus badius</i>	615	99.8	UDB000050	HM190029
BB06_207_Oh_091006	434	<i>Xerocomus badius</i>	615	99.8	UDB000050	HM190030
BB06_208_Oh_091006	436	<i>Xerocomus badius</i>	615	100.0	UDB000050	HM190031
BB06_101_Oh_Pi_231006	492	<i>Xerocomus badius</i>	616	99.8	UDB000050	HM190032
BB28_102_Aeh_Fa_231006	502	<i>Xerocomus badius</i>	616	100.0	UDB000050	HM190033
BB08_107_Oh_Pi_231006	534	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190034
BB06_107_Oh_Pi_231006	536	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190035

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB06_310_Oh_Pi_011106	612	<i>Xerocomus badius</i>	611	99.8	UDB000050	HM190050
BB06_209_Oh_150107	668	<i>Xerocomus badius</i>	653	100.0	UDB000050	HM190036
BB06_204_Of_160407	697	<i>Xerocomus badius</i>	616	100.0	UDB000050	HM190037
BB80_206_Bv_160407	704	<i>Xerocomus badius</i>	616	100.0	UDB000050	HM190038
BB84_207_Oh_160407	708	<i>Xerocomus badius</i>	616	100.0	UDB000050	HM190039
BB06_207_Oh_160407	709	<i>Xerocomus badius</i>	616	100.0	UDB000050	HM190040
BB06_208_Of_160407	710	<i>Xerocomus badius</i>	616	99.8	UDB000050	HM190041
BB06_209_Of_160407	713	<i>Xerocomus badius</i>	616	99.8	UDB000050	HM190042
BB06_210_Oh_160407	717	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190043
BB06_108_Oh_Pi_230407	763	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190044
BB28_108_Oh_Fa_230407	765	<i>Xerocomus badius</i>	653	99.8	UDB000050	HM190045
BB06_304_Oh_Pi_070507	794	<i>Xerocomus badius</i>	615	99.8	UDB000050	HM190046
BB06_307_Oh_Pi_070507	812	<i>Xerocomus badius</i>	616	99.8	UDB000050	HM190047
BB28_401_Ah_220506	3	<i>Xerocomus cisalpinus</i>	781	99.6	UDB002180	HM190051
BB28_402_Bv_220506	9	<i>Xerocomus cisalpinus</i>	780	99.6	UDB002180	HM190052
BB28_403_Bv_220506	13	<i>Xerocomus cisalpinus</i>	735	99.3	UDB002180	HM190053
BB28_406_Bv_220506	23	<i>Xerocomus cisalpinus</i>	780	100.0	UDB002180	HM190054
BB28_407_Ah_220506	30	<i>Xerocomus cisalpinus</i>	742	99.7	UDB002180	HM190055
BB28_408_Ah_220506	34	<i>Xerocomus cisalpinus</i>	780	99.7	UDB002180	HM190056
BB28_302_Ah_Fa_150506	53	<i>Xerocomus cisalpinus</i>	781	99.7	UDB002180	HM190057
BB28_305_Bv_Fa_150506	66	<i>Xerocomus cisalpinus</i>	471	99.8	UDB002180	HM190083
BB08_308_Ah_Fa_150506	85	<i>Xerocomus cisalpinus</i>	734	99.6	UDB002180	HM190058
BB06_105_Oh_Fa_080506	150	<i>Xerocomus cisalpinus</i>	780	99.7	UDB002180	HM190059
BB28_106_Aeh_Fa_080506	160	<i>Xerocomus cisalpinus</i>	780	99.9	UDB002180	HM190060
BB08_404_Ah_161006	459	<i>Xerocomus cisalpinus</i>	738	99.9	UDB002180	HM190061
BB28_405_Ah_161006	464	<i>Xerocomus cisalpinus</i>	743	99.9	UDB002180	HM190062
BB28_409_Oh_161006	479	<i>Xerocomus cisalpinus</i>	743	99.9	UDB002180	HM190084
BB28_301_Of_Fa_011106	558	<i>Xerocomus cisalpinus</i>	780	99.6	UDB002180	HM190063
BB08_301_Oh_Pi_011106	559	<i>Xerocomus cisalpinus</i>	780	99.6	UDB002180	HM190064
BB28_302_Ah_Fa_011106	565	<i>Xerocomus cisalpinus</i>	781	99.9	UDB002180	HM190065

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB08_304_Of_Pi_011106	572	<i>Xerocomus cisalpinus</i>	780	99.6	UDB002180	HM190066
BB06_304_Of_Pi_011106	573	<i>Xerocomus cisalpinus</i>	780	99.6	UDB002180	HM190067
BB08_304_Of_Fa_011106	579	<i>Xerocomus cisalpinus</i>	780	99.6	UDB002180	HM190068
BB28_403_Ah_150107	641	<i>Xerocomus cisalpinus</i>	780	99.9	UDB002180	HM190069
BB28_403_Bv_150107	642	<i>Xerocomus cisalpinus</i>	735	100	UDB002180	HM190070
BB28_406_Ah_150107	660	<i>Xerocomus cisalpinus</i>	742	99.9	UDB002180	HM190085
BB28_108_Bsh_Fa_230407	767	<i>Xerocomus cisalpinus</i>	779	99.6	UDB002180	HM190071
BB28_301_Ah_Fa_070507	783	<i>Xerocomus cisalpinus</i>	742	100	UDB002180	HM190072
BB28_301_Ah_Fa_070507	784	<i>Xerocomus cisalpinus</i>	742	99.7	UDB002180	HM190073
BB06_304_Bv_Pi_070507	797	<i>Xerocomus cisalpinus</i>	742	99.7	UDB002180	HM190074
BB28_306_Bv_Fa_070507	809	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM190075
BB28_307_Ah_Fa_070507	817	<i>Xerocomus cisalpinus</i>	743	99.9	UDB002180	HM190076
BB37_308_Ah_Fa_070507	828	<i>Xerocomus cisalpinus</i>	743	99.9	UDB002180	HM190077
BB28_308_Bv_Fa_070507	829	<i>Xerocomus cisalpinus</i>	735	100	UDB002180	HM190078
BB06_402_Ah_210507	851	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM190079
BB06_403_Bv_210507_R11	866	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM190080
BB28_405_Bv_210507	893	<i>Xerocomus cisalpinus</i>	743	99.9	UDB002180	HM190081
BB28_407_Ah_210507_R47	930	<i>Xerocomus cisalpinus</i>	745	99.6	UDB002180	HM190082
BB28_403_Ah_161006	451	<i>Xerocomus porosporus</i>	782	99.7	UDB000475	HM190086
BB28_404_Oh_220506	15	<i>Xerocomus pruinatus</i>	807	99.9	UDB000049	HM190087
BB06_308_Ah_Pi_150506	82	<i>Xerocomus pruinatus</i>	747	100.0	UDB000049	HM190088
BB61_308_Ah_Pi_150506	84	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190089
BB23_103_Of_Pi_080506	138	<i>Xerocomus pruinatus</i>	675	100.0	UDB000049	HM190090
BB06_104_Oh_Pi_080506	142	<i>Xerocomus pruinatus</i>	579	99.0	UDB000049	HM190091
BB28_107_Ah_Fa_080506	166	<i>Xerocomus pruinatus</i>	750	100.0	UDB000049	HM190092
BB28_404_Oh_161006	454	<i>Xerocomus pruinatus</i>	770	100.0	UDB000049	HM190093
BB28_109_Of_Fa_231006	551	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190094
BB06_305_Of_Pi_011106	584	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190095
BB06_306_Of_Pi_150107	676	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190096
BB28_306_Oh_Fa_150107	681	<i>Xerocomus pruinatus</i>	807	99.7	UDB000049	HM190097

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB28_109_Of_Fa_150107	684	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190098
BB28_101_Of_Fa_230407	718	<i>Xerocomus pruinatus</i>	769	100.0	UDB000049	HM190099
BB06_101_Oh_Pi_230407	722	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190100
BB28_103_Of_Fa_230407	735	<i>Xerocomus pruinatus</i>	679	100.0	UDB000049	HM190101
BB14_103_Oh_Fa_230407	739	<i>Xerocomus pruinatus</i>	679	100.0	UDB000049	HM190102
BB22_104_Of_Pi_230407	742	<i>Xerocomus pruinatus</i>	578	99.3	UDB000049	HM190103
BB06_105_Of_Pi_230407	750	<i>Xerocomus pruinatus</i>	770	99.9	UDB000049	HM190107
BB28_105_Aeh_Fa_230407	753	<i>Xerocomus pruinatus</i>	770	99.9	UDB000049	HM190108
BB06_109_Oh_Pi_230407	769	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190104
BB28_109_Oh_Fa_230407	771	<i>Xerocomus pruinatus</i>	807	100.0	UDB000049	HM190105
BB28_305_Of_Fa_070507	799	<i>Xerocomus pruinatus</i>	770	100.0	UDB000049	HM190106
BB28_408_Ah_210507_R57	942	<i>Xerocomus pruinatus</i>	770	100.0	UDB000049	HM190109

root associated fungi

BB27_401_Of_220506	1	<i>Basidiomycete (Mycena pp.) sp. 1</i>	679	99.5	DQ309229	HM190110
BB64_105_Oh_Fa_231006	522	<i>Basidiomycete (Mycena pp.) sp. 1</i>	749	99.4	DQ309229	HM190111
BB11_308_Ah_Pi_011106	608	<i>Basidiomycete (Mycena pp.) sp. 1</i>	712	99.6	DQ309229	HM190112
BB05_307_Of_Fa_011106	595	<i>Basidiomycete sp. 2</i>	659	95.5	DQ309203	HM190113
BB05_106_Of_Fa_230407	755	<i>Helotiales (Mollisia) sp. 1</i>	585	100.0	AJ430223	HM190114
BB28_408_Ah_210507_R51	935	<i>Helotiales sp. 2</i>	566	98.5	DQ309134	HM190115
BB05_404_Ah_220506	17	<i>Helotiales sp. 3</i>	703	100	FN393145	HM190116
BB34_405_Ah_220506	18	<i>Helotiales sp. 3</i>	740	100	FN393145	HM190117
BB65_406_Ah_220506	22	<i>Helotiales sp. 3</i>	702	99.9	FN393145	HM190118
BB05_105_Oh_Fa_231006	520	<i>Helotiales sp. 3</i>	705	100	FN393145	HM190119
BB39_105_Aeh_Fa_231006	524	<i>Helotiales sp. 3</i>	740	100	FN393145	HM190120
BB05_103_Of_Fa_150107	630	<i>Helotiales sp. 3</i>	704	99.9	FN393145	HM190121
BB11_303_Oh_Fa_150107	652	<i>Helotiales sp. 3</i>	706	99.9	FN393145	HM190122
BB11_106_Of_Fa_150107	669	<i>Helotiales sp. 3</i>	703	99.6	FN393145	HM190123
BB60_108_Aeh_Pi_080506	171	<i>Meliniomyces bicolor</i>	594	99.8	FN179335	HM190124
BB23_106_Of_Pi_080506	162	<i>Meliniomyces variabilis</i>	571	99.6	EF093178	HM190125

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB05_105_Oh_Pi_231006	516	<i>Meliniomyces variabilis</i>	606	99.6	EF093178	HM190126
BB60_107_Oh_Pi_231006	537	<i>Meliniomyces variabilis</i>	497	98.2	EF093178	HM190127
BB05_305_Oh_Pi_011106	586	<i>Meliniomyces variabilis</i>	606	99.6	EF093178	HM190128
BB06_308_Oh_Pi_011106	606	<i>Meliniomyces variabilis</i>	571	99.9	EF093178	HM190129
BB62_103_Bsh_Fa_080506	140	<i>Mitosporic ascomycota</i>	560	99.6	AB089660	HM190130
BB62_303_Oh_Fa_011106	568	<i>Mitosporic ascomycota</i>	598	99.6	AB089660	HM190131
BB98_404_Bv_210507	888	<i>Mitosporic ascomycota</i>	549	99.4	AB089660	HM190132
BB06_306_Of_Pi_150506	69	<i>Phialocephala fortinii</i>	602	99.8	AY394921	HM190133
BB05_306_Of_Fa_150506	70	<i>Phialocephala fortinii</i>	602	99.3	AY394921	HM190134
BB64_110_Of_Pi_231006	554	<i>Phialocephala fortinii</i>	602	99.8	AY394921	HM190135
BB05_304_Of_Fa_011106	577	<i>Phialocephala fortinii</i>	602	99.8	AY394921	HM190136
BB64_310_Of_Fa_011106	614	<i>Phialocephala fortinii</i>	565	99.8	AY394921	HM190137
Contamination						
BB28_408_Ah_210507_R56	941	<i>Cryptococcus wieringae</i>	616	99.8	AF444383	
BB45_407_Ah_210507_R33	907	<i>Davidiella macrospora</i>	545	1000	EU167591	
BB22_103_Aeh_Fa_231006	509	<i>Exophiala sp. CPC 12173</i>	652	99.7	EU035422	
BB05_304_Of_Fa_150506	60	<i>Fagus sylvatica</i>				
BB05_108_Aeh_Fa_231006	546	<i>Fagus sylvatica</i>				
BB38_403_Oh_220506	11	<i>Gyoeerffiyella sp. PB1-R3-D Fr</i>	551	97.2	EF601602	
BB05_109_Of_Fa_231006	548	<i>Malassezia restricta</i>	769	99.7	AJ437695	
BB72_302_Ah_Fa_070507	786	<i>Malassezia restricta</i>	732	99.9	AJ437695	
BB05_106_Aeh_Fa_231006	532	<i>Phialophora sessilis</i>	733	97.9	AY857541	
no PCR product						
BB33_401_Ah_220506	2	no PCR product				
BB43_401_Of_220506	4	no PCR product				
BB05_405_Ah_220506	19	no PCR product				
BB42_406_Ah_220506	20	no PCR product				
BB42_407_Of_220506	25	no PCR product				
BB52_407_Ah_220506	26	no PCR product				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB05_408_Of_220506	31	no PCR product				
BB31_408_Oh_220506	32	no PCR product				
BB52_408_Ah_220506	33	no PCR product				
BB05_409_Of_220506	36	no PCR product				
BB28_409_Ah_220506	37	no PCR product				
BB39_409_Bv_220506	38	no PCR product				
BB34_409_Bv_220506	39	no PCR product				
BB37_410_Of_220506	40	no PCR product				
BB22_301_Oh_Pi_150506	42	no PCR product				
BB05_301_Oh_Pi_150506	44	no PCR product				
BB61_301_Ah_Pi_150506	45	no PCR product				
BB28_301_Ah_Fa_150506	48	no PCR product				
BB64_302_Oh_Pi_150506	50	no PCR product				
BB05_302_Oh_Fa_150506	51	no PCR product				
BB38_302_Oh_Fa_150506	52	no PCR product				
BB05_303_Of_Fa_150506	54	no PCR product				
BB23_303_Ah_Pi_150506	56	no PCR product				
BB38_303_Ah_Pi_150506	57	no PCR product				
BB05_304_Of_Pi_150506	59	no PCR product				
BB28_306_Oh_Fa_150506	73	no PCR product				
BB38_306_Bv_Fa_150506	74	no PCR product				
BB08_307_Of_Pi_150506	75	no PCR product				
BB05_307_Of_Fa_150506	77	no PCR product				
BB61_307_Oh_Pi_150506	78	no PCR product				
BB28_307_Oh_Fa_150506	80	no PCR product				
BB28_308_Of_Fa_150506	81	no PCR product				
BB14_308_Ah_Pi_150506	83	no PCR product				
BB05_309_Of_Fa_150506	90	no PCR product				
BB28_309_Oh_Fa_150506	91	no PCR product				
BB05_309_Of_Fa_150506	92	no PCR product				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB05_310_Of_Fa_150506	94	no PCR product				
BB23_310_Oh_Pi_150506	95	no PCR product				
BB23_203_Of_020506	102	no PCR product				
BB06_203_Aeh_020506	103	no PCR product				
BB06_205_Of_020506	108	no PCR product				
BB05_206_Of_020506	113	no PCR product				
BB14_206_Oh_020506	114	no PCR product				
BB06_207_Oh_020506	115	no PCR product				
BB22_207_Aeh_020506	116	no PCR product				
BB54_207_Bv_020506	117	no PCR product				
BB14_209_Of_020506	121	no PCR product				
BB14_210_Oh_020506	125	no PCR product				
BB22_104_Oh_Pi_080506	143	no PCR product				
BB05_105_Of_Fa_080506	148	no PCR product				
BB39_105_Aeh_Fa_080506	152	no PCR product				
BB05_106_Oh_Pi_080506	158	no PCR product				
BB24_106_Aeh_Pi_080506	159	no PCR product				
BB32_107_Aeh_Fa_080506	167	no PCR product				
BB32_108_Aeh_Fa_080506	172	no PCR product				
BB22_109_Oh_Pi_080506	175	no PCR product				
BB28_109_Aeh_Fa_080506	177	no PCR product				
BB24_110_Of_Pi_080506	179	no PCR product				
BB28_110_Aeh_Fa_080506	181	no PCR product				
BB11_203_Of_091006	421	no PCR product				
BB24_204_Oh_091006	427	no PCR product				
BB14_209_Oh_091006	440	no PCR product				
BB28_402_Ah_161006	446	no PCR product				
BB38_403_Oh_161006	448	no PCR product				
BB38_406_Ah_161006	466	no PCR product				
BB52_406_Ah_161006	467	no PCR product				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB39_407_Of_161006	471	no PCR product				
BB34_407_Ah_161006	472	no PCR product				
BB34_408_Ah_161006	477	no PCR product				
BB52_409_Oh_161006	482	no PCR product				
BB05_410_Oh_161006	484	no PCR product				
BB28_410_Bv_161006	488	no PCR product				
BB06_102_Oh_Pi_231006	501	no PCR product				
BB39_103_Oh_Fa_231006	507	no PCR product				
BB28_104_Oh_Pi_231006	515	no PCR product				
BB08_105_Oh_Pi_231006	519	no PCR product				
BB66_105_Oh_Fa_231006	521	no PCR product				
BB39_106_Of_Fa_231006	530	no PCR product				
BB62_106_Aeh_Fa_231006	531	no PCR product				
BB24_108_Oh_Pi_231006	542	no PCR product				
BB52_109_Aeh_Fa_231006	552	no PCR product				
BB59_303_Oh_Pi_011106	567	no PCR product				
BB38_304_Ah_Fa_011106	582	no PCR product				
BB28_304_Ah_Fa_011106	583	no PCR product				
BB72_305_Ah_Fa_011106	587	no PCR product				
BB64_306_Oh_Pi_011106	590	no PCR product				
BB28_306_Ah_Fa_011106	591	no PCR product				
BB28_307_Of_Fa_011106	593	no PCR product				
BB05_103_Oh_Fa_150107	635	no PCR product				
BB05_306_Of_Fa_150107	677	no PCR product				
BB06_306_Oh_Pi_150107	682	no PCR product				
BB84_202_Of_160407	694	no PCR product				
BB84_207_Of_160407	705	no PCR product				
BB72_101_Aeh_Fa_230407	726	no PCR product				
BB28_101_Aeh_Fa_230407	727	no PCR product				
BB11_102_Oh_Pi_230407	731	no PCR product				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB06_104_Oh_Pi_230407	746	no PCR product				
BB11_105_Of_Pi_230407	749	no PCR product				
BB06_305_Oh_Pi_070507	800	no PCR product				
BB53_305_Oh_Pi_070507	801	no PCR product				
BB28_305_Ah_Fa_070507	802	no PCR product				
BB37_306_Oh_Fa_070507	804	no PCR product				
BB11_306_Oh_Pi_070507	806	no PCR product				
BB11_307_Oh_Pi_070507	814	no PCR product				
BB53_307_Ah_Pi_070507	816	no PCR product				
BB72_307_Ah_Fa_070507	818	no PCR product				
BB79_308_Oh_Pi_070507	823	no PCR product				
BB05_308_Oh_Fa_070507	825	no PCR product				
BB05_309_Ah_Fa_070507	833	no PCR product				
BB72_310_Ah_Fa_070507	840	no PCR product				
BB70_401_Oh_210507	844	no PCR product				
BB70_403_Oh_210507	853	no PCR product				
BB72_404_Bv_210507	889	no PCR product				
BB28_405_Ah_210507	890	no PCR product				
BB28_405_Bv_210507	891	no PCR product				
BB28_405_Bv_210507	892	no PCR product				
BB28_409_Ah_210507	958	no PCR product				
BB05_409_Ah_210507	959	no PCR product				
BB70_409_Ah_210507	960	no PCR product				
BB57_410_Oh_210507	961	no PCR product				
BB28_410_Ah_210507	965	no PCR product				
BB28_410_Ah_210507	966	no PCR product				
sequence not readable						
BB05_302_Of_Fa_150506	49	sequence not readable				
BB38_304_Ah_Fa_150506	61	sequence not readable				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB22_305_Oh_Pi_150506	63	sequence not readable				
BB38_307_Oh_Fa_150506	79	sequence not readable				
BB06_206_Of_020506	112	sequence not readable				
BB05_102_Of_Fa_080506	133	sequence not readable				
BB61_103_Of_Pi_080506	136	sequence not readable				
BB05_103_Of_Fa_080506	137	sequence not readable				
BB38_104_Of_Fa_080506	141	sequence not readable				
BB22_106_Of_Pi_080506	154	sequence not readable				
BB05_106_Of_Fa_080506	156	sequence not readable				
BB22_107_Of_Pi_080506	164	sequence not readable				
BB06_201_Of_091006	414	sequence not readable				
BB11_201_Oh_091006	416	sequence not readable				
BB26_402_Of_161006	442	sequence not readable				
BB28_404_Oh_161006	456	sequence not readable				
BB28_408_Bv_161006	478	sequence not readable				
BB38_410_Oh_161006	485	sequence not readable				
BB28_101_Of_Fa_231006	489	sequence not readable				
BB08_101_Oh_Pi_231006	493	sequence not readable				
BB22_102_Oh_Pi_231006	500	sequence not readable				
BB45_103_Oh_Pi_231006	504	sequence not readable				
BB45_103_Oh_Fa_231006	505	sequence not readable				
BB38_103_Oh_Fa_231006	506	sequence not readable				
BB66_104_Of_Fa_231006	510	sequence not readable				
BB60_105_Aeh_Pi_231006	523	sequence not readable				
BB28_105_Bsh_Fa_231006	525	sequence not readable				
BB28_106_Oh_Fa_231006	526	sequence not readable				
BB11_107_Oh_Pi_231006	535	sequence not readable				
BB62_108_Oh_Fa_231006	544	sequence not readable				
BB11_301_Oh_Pi_011106	560	sequence not readable				
BB38_302_Ah_Fa_011106	566	sequence not readable				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB64_303_Ah_Pi_011106	569	sequence not readable				
BB60_304_Of_Pi_011106	575	sequence not readable				
BB45_304_Oh_Fa_011106	581	sequence not readable				
BB28_305_Oh_Fa_011106	585	sequence not readable				
BB06_306_Oh_Pi_011106	589	sequence not readable				
BB05_307_Of_Fa_011106	594	sequence not readable				
BB72_308_Oh_Pi_011106	601	sequence not readable				
BB05_308_Oh_Pi_011106	603	sequence not readable				
BB72_309_Of_Fa_011106	609	sequence not readable				
BB28_309_Of_Fa_011106	610	sequence not readable				
BB38_309_Of_Fa_011106	611	sequence not readable				
BB28_401_Oh_161006	618	sequence not readable				
BB45_401_Ah_161006	622	sequence not readable				
BB28_303_Ah_Fa_150107	653	sequence not readable				
BB84_209_Of_150107	665	sequence not readable				
BB84_209_Of_150107	666	sequence not readable				
BB11_106_Oh_Fa_150107	670	sequence not readable				
BB05_106_Aeh_Fa_150107	674	sequence not readable				
BB87_309_Ah_Pi_150107	689	sequence not readable				
BB71_201_Of_160407	691	sequence not readable				
BB05_101_Aeh_Fa_230407	724	sequence not readable				
BB24_102_Of_Pi_230407	728	sequence not readable				
BB11_105_Oh_Pi_230407	751	sequence not readable				
BB05_105_Oh_Fa_230407	752	sequence not readable				
BB88_108_Oh_Pi_230407	764	sequence not readable				
BB28_107_Oh_Fa_230407	780	sequence not readable				
BB28_302_Ah_Fa_070507	785	sequence not readable				
BB72_303_Ah_Fa_070507	789	sequence not readable				
BB72_303_Ah_Fa_070507	790	sequence not readable				
BB72_303_Bv_Fa_070507	791	sequence not readable				

field assignment	DNA	species designation	bp	%	database reference	GenBank-submission
BB14_304_Ah_Fa_070507	796	sequence not readable				
BB94_304_Bv_Pi_070507	798	sequence not readable				
BB06_306_Oh_Pi_070507	805	sequence not readable				
BB72_306_Ah_Fa_070507	807	sequence not readable				
BB06_306_Bv_Pi_070507	808	sequence not readable				
BB05_308_Of_Fa_070507	820	sequence not readable				
BB05_308_Of_Pi_070507	821	sequence not readable				
BB95_308_Of_Pi_070507	822	sequence not readable				
BB28_309_Oh_Fa_070507	831	sequence not readable				
BB96_401_Ah_210507	847	sequence not readable				
BB45_402_Oh_210507	850	sequence not readable				
BB72_404_Ah_210507	883	sequence not readable				
BB28_404_Ah_210507	884	sequence not readable				
BB22_103_Ah_230407	967	sequence not readable				

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Thesis Part IV

Manuscript 2

Individual tree genotypes do not explain ectomycorrhizal biodiversity in a pure stand of beech (*Fagus sylvatica* L.)

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IV.1. Abstract

Niche differentiation is a common explanation for high ectomycorrhizal diversity. In monocultures and on small spatial scales the number of variable factors that may provide niches decreases. Still, even in the restricted volume of a soil core, typically more than one ectomycorrhizal species is found. We tested the hypothesis, that roots of different individual beech genotypes provide niches on a small spatial scale in a pure beech (*Fagus sylvatica* L.) stand in the North-eastern Lowlands of Germany. Fourteen ectomycorrhizal species, as determined by ITS sequencing and phylograms were patchily distributed along an 81 m long transect with ten transect points. All root segments in the three species richest soil cores and the surrounding beeches were genotyped by microsatellite PCR. In each of the three soil cores roots of two host genotypes were present that corresponded to the two closest mature trees. We found that the different root genotypes did not carry different sets of ectomycorrhizal species even at the high species resolution provided through our study. Therefore, the hypothesis of tree genotypes contributing to biodiversity in a soil sample has to be rejected. In the absence of other niche based explanations (soil parameters were homogenously distributed among transect points, no vertical compartmentalization), we propose that stochastic processes, such as spore dispersal might have contributed to the biodiversity in the analyzed soil cores. To the best of our knowledge, this is the first report that links ectomycorrhizal biodiversity in a soil core to the individual genotype of an angiosperm host.

keywords

ectomycorrhiza, *Fagus sylvatica*, microsatellite PCR, niches, ribosomal DNA, stochastic explanations, tree genotype

IV.2. Introduction

In forest ecosystems ectomycorrhizal species richness can be very high. In stands with several tree species parts of the biodiversity can be explained by fungal host specificities (Molina et al. 1992; Dickie 2007; Ishida et al. 2007). However, also in monoculture stands with an area of less than one hectare, 30-45 fungal species can be observed (e.g. Kjølner 2006; Diedhiou et al. 2009; Pena et al. 2010). This situation is similar to the paradox of the plankton (Hutchinson 1961). Many species of phytoplankton compete for light, CO₂ and nutrients in a relatively unstructured environment. According to the competitive exclusion principle (Hardin 1960), competition should lead to the prevalence of a single species that outcompetes all the others. In pure forest stands the situation is comparable, since all ectomycorrhizal fungi rely essentially on the same carbohydrate resource, the fine roots of a single tree species (Bruns 1995).

In ecological theory, coexistence of species is approached by two types of explanations: stochastic and deterministic (Agren and Fagerstrom 1984; Chave 2004). Stochastic explanations rely on dispersal as the basic mechanism. Biodiversity in an area is explained as equilibrium between the rate of immigration into the area and the rate of local extinctions, independently of species adaptations to this area. Examples for such theories are the theory of island biogeography (MacArthur and Wilson 1967), lottery models (Sale 1977; Chesson and Warner 1981) and the neutral theory of biodiversity (Hubbell 2001). While recently the latter received much attention in theoretical ecology (Leigh 2007; Rosindell et al. 2011), only a few reports and reviews consider stochastic explanations for ectomycorrhizal biodiversity (Bruns 1995; Peay et al. 2007; Kennedy 2010).

In contrast to stochastic approaches, deterministic explanations rely on niche adaptations, so that competitive exclusion is prevented by specialization (Chesson 2000; Palmer et al. 2003). Niche theory is much more accepted for explanations of ectomycorrhizal biodiversity. Fungi have been shown to be specialized for the quantity and distribution of soil organic matter (Conn and Dighton 2000; Dighton et al. 2000), N availability (Lilleskov et al. 2002a; Lilleskov et al. 2002b; Avis et al. 2003), micro-sites such as rotten logs (Goodman and Trofymow 1998; Tedersoo et al. 2003; Tedersoo et al. 2008), access to mineral nutrients (Agerer and Göttlein 2003; Toljander et al. 2006) and avoidance of predation by micro fauna (Böllmann et al. 2010). However, in the restricted soil volume harvested by a soil corer (usually a few 100 cm³) many of the mentioned factors can be assumed to be homogenous. Still, 2 to 12 species can be found in a soil core (Douglas et al. 2005; Toljander et al. 2006; Courty et al. 2008; Blom et al. 2009). Even if fungal preferences for certain soil horizons are taken into account (Dickie et al. 2002; Rosling et al. 2003; Baier et al. 2006; Scattolin et al. 2008) some portions of biodiversity in pure forest stands remain unexplained.

In pursuing the deterministic approach, one could look for further, subtle niches in a given soil volume. One of such rarely looked at niches is the intraspecific genetic variation of tree roots. Several studies suggest an influence of host genetic differences below the species level on the composition of ectomycorrhizal communities (Tagu et al. 2001; Van der Heijden and

Kuyper 2001; Tagu et al. 2005; Gehring et al. 2006; Korkama et al. 2006; Sthultz et al. 2009; Leski et al. 2010). Therefore, we hypothesise that fungal species richness in a pure forest stand can be explained by fungal specialization for individual tree genotypes. A soil core with two or more ectomycorrhizal species would be expected to contain roots of at least two individual trees with a preference of the fungi for one of the tree genotypes. We tested this genotype niche hypothesis in a pure beech (*Fagus sylvatica*, L.) stand in North-eastern Lowlands of Germany and discuss the results in the light of an alternative explanation.

IV.3. Material and methods

Site description

The study site is located in the North-eastern Lowlands of Germany (52°52'N, 13°53'E) approx. 50 km northeast of Berlin in a forest protection area called Schorfheide Chorin. This area is naturally covered by beech (*Fagus sylvatica* L.) forests (Bohn and Neuhäusl 2003; Jenssen et al. 2007), but reforestation in the 18th and 19th century promoted expansion of Scots Pine (*Pinus sylvestris* L.) monocultures. The investigated pure beech stand was founded on the site of a pure pine stand at the turn of 19th to 20th century, forming today a 113 year old pure beech stand. As the edaphic and climatic conditions were described in detail by Rumberger et al. (2004), only a short characterization is given here: The mean annual precipitation of the area ranges from 562 to 577 mm and the mean annual temperature was 8.3°C (minimum was in January with -17.1 °C). The soil type was classified as weakly podsolc cambisol.

Sampling and morphotyping

Morphotyping along the transect: Ten soil cores (diameter 6 cm, length 40 cm) were collected along an 81 m transect at every 9 m distance in May 2007. Each soil core was divided in four vertical compartments: the organic horizons Of and Oh, the mineral layer Ah and the subsoil Bv. One root tip per morphotype was sampled for each horizon and soil core and air dried for later sequencing. Morphotypes were distinguished under a stereo-microscope according to branching, colour, surface texture and presence of emanating hyphae or rhizomorphs. Distinction of morphotypes was supplemented by mantle preparation and microscopy with an Axioskop microscope (Zeiss, Oberkochen, Germany), according to the principles demonstrated in (Agerer 1987-2002) and the online key DEEMY (<http://www.deemy.de>).

Intensified morphotyping: The three transect points with the highest morphotype number (T3, T7, T8), were selected for intensified morphotyping. All root fragments in the soil core were collected and numbered. Root fragments varied from small clusters of fine roots to coarse roots with attached fine roots. For each root fragment one root tip per morphotype (up to three morphotypes were found on one root fragment) was collected for fungal sequencing.

Root sampling in T3, T7, and T8: After morphotyping, root fragments were air dried and stored for later host genotyping by microsatellite PCR. Since we were interested in assigning ectomycorrhizal species to tree individuals, only those root fragments were genotyped for which successful fungal partner identification by sequencing was possible.

Tree sampling: In order to identify the source trees of the root segments, pieces of cambium of adult beech trees were collected for microsatellite PCR in April 2008. Around each transect point all adult trees within a 15 m radius were sampled. While the area around T7 and T8 was free of understory, T3 was surrounded by beech seedlings and saplings. In order to explore whether offspring contributed to the roots in core T3, buds of all young trees (5-15 years) within 10 m distance (beech 5-10, 15) and of all seedlings (2 years) within 40 cm distance (beech 11-14) were collected.

Soil parameters

Soil cores were collected at the same transect points 50 cm apart from the soil cores for the ectomycorrhizal analysis in spring 2006. After removal of roots, soil samples were dried and ground. HNO₃ digestion under pressure preceded the measurement of element contents. Ca, Mg, P, Mn, Fe, and Al were measured by ICP-OES (inductively coupled plasma optical emission spectroscopy) on Unicam 701. K was measured by AAS (atomic absorption spectroscopy) on Unicam 932. N and C were measured directly from dried samples on CNS elemental analyzer Vario EL (Elementar, Hanau, Germany). Before pH-measurements soil samples were diluted in ddH₂O at v/v ratio from 1:2.5 to 1:20 depending on sample.

DNA extraction

Three different DNA extraction methods were used.

Mycorrhizae: single mycorrhized root tips were homogenized using glass micro mortar and pestle. DNA was extracted with DNeasy Plant Mini Kit (Quiagen, Hilden, Germany) following the suppliers instruction (Quiagen). DNA extractions from mycorrhized root tips contain both fungal and beech DNA.

Root segments. Dried root segments were homogenized with beads in a Retsch mill. The DNA extraction protocol is based on lysis with alkyl trimethyl ammonium bromide (ATMAB), phase separation with dichlormethan and alcohol precipitation following the protocol of Dumolin et al. (1995).

Aboveground beech: buds (54-196 mg) and cambium (4-31 mg) were homogenized in 200 mg quart sand (VEB Laborchemie, Apolda, Germany) with mortar and pestle. DNA extraction followed the CTAB-based protocol of Ahmad et al. (2004).

Fungal species determination

PCR was performed with Accuprime[®] Taq Polymerase System (Invitrogen, Karlsruhe, Germany) using the supplied buffer II with following final concentrations: 1.5 mM MgCl₂ and

0.2 mM of each dNTP and 500 nM of each of the forward primer ITS1F (Gardes and Bruns 1993) and the reverse primer ITS4 (O'Donnell 1993). Total reaction volume was 25 μ l, comprising 5 μ l of template DNA. The thermocycler, a GeneAmp[®] PCR system 9700 (ABI, Darmstadt, Germany), was programmed as following: 3 min denaturation at 94°C, ten cycles with decreasing annealing temperature (94°C for 30 s, 60–50°C for 45 s, and 68°C for 60 s), 35 cycles with constant annealing temperature (94°C for 30 s, 50°C for 45 s, and 68°C for 60 s), and 7 min strand completion at 68°C. PCR-products were sent to GATC Biotech AG (Konstanz, Germany) for sequencing with primers ITS1F and ITS4. Sequences were assembled with the Lasergene[®] Software Package (DNASTAR, Madison, USA). Sequences were grouped into sequence types (= molecular operational taxonomical units) with at least 99 % identity. The UNITE (Abarenkov et al. 2010) and GenBank (Zhang et al. 2000) databases were searched using the BLAST algorithm. Sequence types matching a database entry with at least 99% were given a species name.

Fingerprints of plant material

Microsatellite PCR was carried out using Taq DNA polymerase (Invitrogen, Darmstadt, Germany) with following final concentrations: primers 0.2 μ M, dNTPs 100 μ M, and MgCl₂ 1.8 mM. Total reaction volume was 25 μ l with 1 μ l DNA as template. Six microsatellite loci were amplified (Table 2) by PCR on the Thermocycler UNO II (Biometra, Göttingen, Germany) with the following cycle parameters: three min denaturation at 94°C, 35 cycles (94°C for 30s, 55/60°C for 45s, 72°C for 60s) and ten minutes strand completion at 72°C. Obtained PCR products together with standards were applied to a polyacrylamide gel electrophoresis (PAGE) and made visible by silver staining as described in Ziegenhagen et al. (2003). Locus-specific allelic standards were run in every 5 to 10 lanes of the gels.

Statistical analysis

SPSS 19 was used for testing normal distribution of soil parameters and correlation analysis. PCOrd 6 was used for Canonical Correspondence Analysis (CCA), Mantel-tests and cluster analysis of ectomycorrhizal diversity and soil parameters.

CCA: The main matrix was ectomycorrhizal diversity based on presence/absence. The secondary matrix (soil parameters) was divided into four separate matrices (one for each horizon), for reasons of matrix algebra. The number of columns in each secondary matrix was further reduced by combining C and N content as C/N ratio and omission of Mn. Four CCAs with the same main matrix (fungal species) and four different secondary matrices were run.

Mantel tests: Four tests were run to determine the significance of the correlation between the main matrix and the four secondary matrices. p-values were calculated using Mantel's asymptotic approximation method.

Cluster analyses: Separate analyses were run for the species dataset and the soil parameters dataset (Euclidean distance, Ward's method).

IV.4. Results

Identification of fungal species

Along the transect of 10 soil cores, 14 sequence types could be detected (Fig.1). Species names could be determined for 11 sequence types based on direct database matches and phylograms (Suppl. Table S1). Among the remaining three, one sequence type could be only determined to family level (*Pezizaceae*). The other two were closely related *Russula* species. *Russula ionochlora* was determined by sequence comparison with morphologically determined fruiting bodies collected at a distance of 50 km from the research site. The status of *Russula sp. aff. ionochlora*, is unclear (Suppl. Fig. S1). The 23 bp difference to *R. ionochlora* would be high enough for designating another species, but the close clustering suggests intraspecific variation of the genome as observed by Simon and Weiss (2008). Here we treat it as a closely related but yet unknown species.

Distribution of fungal species on the transect

The three transect points with the highest numbers of morphotypes, T3, T7 and T8 were also the transect points with the highest numbers of genetically identified species (four, five and six, respectively). They are not found in a single cluster, but in the same sub-cluster 1 (Fig.1). T3 is the only soil core that shares three ectomycorrhizal species both with T7 and T8. Clustering did not directly relate to the position on the transect, but in some cases, clusters of transect points are also spatially close to each other as is the case for T7 and T8. Due to dominance of dead roots no mycorrhizal species could be determined for T9. The most unique soil core is T10 with the only occurrences of *Laccaria amethystina* and *Tuber puberulum* on the transect. In the intensively studied soil cores T3, T7, T8 all identified fungi are found in the mineral horizons A and B. There is no vertical compartmentalization. Most fungi are found in the A horizon, only *Russula ionochlora* and *R. sp. aff. ionochlora* are found both in the A and B horizon.

Contribution of soil parameters to explanation of ectomycorrhizal diversity

Eleven soil parameters were analyzed for four horizons resulting in 44 tests for normal distribution and spatial correlation. Only three tests out of 44 show deviation from normal distribution. Another three out of 44 tests indicated a spatial correlation of soil parameters with transect position (Table 1). The prevalence of normal distributions and the near complete lack of spatial correlations indicate a homogeneous distribution of soil parameters along the transect.

In a Canonical Correspondence Analysis (CCA) there were only weak correlations between the ordination scores of the fungal diversity matrix and separate soil parameters in horizon Oh, A and B (Fig. 2, above). The fermentation horizon Of is omitted because no ectomycorrhizal species could be identified for this horizon. Mantel test detected no significant correlations between fungal diversity matrix and the three soil parameter matrices

Oh, A and B. Results of cluster analyses for fungal species and soil parameters are not congruent. While according to species similarity, T3, T7 and T8 are in the same sub-cluster they are in different sub-clusters according to soil parameter similarities (Fig. 2 below). Note that cluster analysis and CCA show the same species similarities (e.g. T2/T5 or T7/T8). The homogenous distribution of soil parameters and the weak correlations of soil parameters and fungal distribution speak against an explanatory value of soil parameters for ectomycorrhizal diversity.

Fingerprints of trees around T3, T7, T8

We looked for individual tree genotypes as an explanation for ectomycorrhizal diversity in the soil cores T3, T7 and T8. Therefore, we tested whether we can distinguish the trees in a 15 m radius around the three transect points by microsatellite PCR. Two of the six tested microsatellite markers showed polymorphism (Table 2). The loci FS4-46 and FS1-15 showed enough variation to distinguish individual trees. Among 26 beech individuals (old growth trees, saplings and seedlings) 21 genotypes were found (Table 3). Eleven of the 14 old growth trees have unique genotypes, only tree 18 and 20 are genetically identical. No fingerprint could be generated from beech 19. The high number of genotypes demonstrates that individual trees can be genetically separated with the two loci FS4-46 and FS1-15.

Fingerprints of root segments in soil cores T3, T7, T8

Sixty nine root segments were collected. Fingerprints were only generated for root segments with a successfully identified fungal partner. All genotyped roots segments originate from the mineral horizons Ah and Bv. Among 39 genotyped root segments six genotypes were found, two genotypes in each of the three soil cores. One genotype dominated in core T3 and T7, while two genotypes are evenly distributed in core T8 (Fig. 3). In core T3, nine root segments belonged to beech 3 at 7 m distance, while a single root segment was identical with beech 1 at 9 m distance. Despite the proximity of beech offspring, no roots of seedlings or saplings (beech 5 to 15) were detected in core T3. In core T7, a single root segment belonged to beech 17 at 11 m distance. The majority of 16 root segments belonged to one genotype that was identical to beech 18 and 20, the only two adult beeches with an identical genotype. The distance of 6 m and 12 m, respectively, allows assuming that most root segments of the dominating genotype are from beech 18. In core T8, three root segments belong to tree 22 at 8 m distance. The second root genotype was identified for seven root segments but did not match to any tree genotype. Since no fingerprint could be generated for beech 19 for technical reasons, and beech 19 is the only tree without fingerprint, we consider the root genotype to belong to tree 19 (Table 3 and Fig. 2).

Distribution of ectomycorrhizal species on root genotypes

In core T8, all three ectomycorrhizal species growing on roots of beech 22 are also found on roots of beech19 (Fig. 4). Interestingly the dominating species in soil core T8 is *Laccaria cf. laccata*, which is not present on the remaining transect. There is only one occurrence in the neighbouring soil core T7. The single root segment of beech 17 in core T7 is colonized by *R. ochroleuca* which also occurs on roots of the beech 18/20 genotype. In core T3, the ectomycorrhizal sequence type *Russula sp. aff. ionochlora* is found on the single root segment of beech 1. The closely related *R. ionochlora* colonizes three root segments of beech 3. In summary, there is no indication that the root genotypes can be separated by the colonization of different ectomycorrhizal species.

IV.5. Discussion

In our beech stand, there is no large variation in the measured soil parameters among transect points, so that measured soil parameters cannot explain the distribution of fungi along the transect. Therefore, we looked for other factors explaining biodiversity. One of these factors is vertical compartmentalisation which was looked at in the intensively sampled soil cores T3, T7 and T8. Because thickness of organic soil coverage was thin in comparison to the mineral soil, few roots were found in organic horizons so that ectomycorrhizal fungi could be determined only in the mineral soil. In contrast, most studies on vertical compartments analyzed coniferous stands with a thick organic coverage which allowed a comparison of biodiversity between organic and mineral horizons (Dickie et al. 2002; Rosling et al. 2003; Tedersoo et al. 2003; Baier et al. 2006; Genney et al. 2006; Scattolin et al. 2008). In these studies the largest differences are found between organic and mineral horizons but not within mineral horizons. Thus, it is reasonable to assume that within the mineral soil of our beech stand the number of four to six species per soil core cannot be explained by vertical compartmentalization.

Based on microsatellite analysis we were able to analyze whether different tree genotypes are responsible for the diversity within the soil cores T3, T7, T8. Since only two of six tested microsatellite markers are variable, we were not able to perform an analysis of similarity of tree genotypes. However, two markers provided sufficient information to distinguish individual trees above and below ground. Based on this capability we tested the hypothesis that individual tree genotypes host different ectomycorrhizal species within a soil core. For the six tree genotypes found in three soil cores we have to reject this hypothesis: the tree genotypes do not harbour different ectomycorrhizal species. A similar test of the hypothesis that tree genotypes are responsible for ectomycorrhizal biodiversity in defined soil volumes has only been reported by Saari et al. (2005). They also had to reject the theory that host genotype is responsible for the ectomycorrhizal species composition in a monoculture stand. It is noteworthy that Saari et al. (2005) analyzed a mature stand of a Pinaceae host (*Pinus sylvestris*) while we analyzed a mature stand of an angiosperm host (*Fagus sylvatica*). This is remarkable as ectomycorrhizas of Pinaceae and angiosperm hosts differ in many aspects, e.g.

host specific fungal communities (Ishida et al. 2007; Smith et al. 2009; Yamada et al. 2010), location of the Hartig net (Smith and Read 1997) and expression patterns of symbiosis related genes (Heller et al. 2008). Despite these differences, the host genotype hypothesis has to be rejected both for a Pinaceae and an angiosperm host. This allows the generalization that individual host genotype is not a niche that explains ectomycorrhizal biodiversity in a restricted soil volume of a mature pure stand.

In this study we excluded several niches as the source of ectomycorrhizal biodiversity in a pure stand. The question remains what causes biodiversity in a homogenous habitat. Ectomycorrhizal distribution is very patchy with patches varying in size from 3-10 meters (Lilleskov et al. 2004; Lian et al. 2006; Pickles et al. 2010). One explanation might be the changing availability of the primary resource, the fine roots, that is caused by root turnover rate within months (Pregitzer 2002). When new fine roots appear in spring these can be considered as microhabitat that can be newly colonized (Hoeksema and Kummel 2003). Ectomycorrhizal fungi arrive at their new habitats either by extension of neighbouring mycelia or as spores (Dahlberg and Stenlid 1995; Redecker et al. 2001). Small patches are thought to be typical for species that propagate by spores while large patches stem from vegetative growth (Hirose et al. 2004). Although our experimental layout with 10 transect points allows only limited generalization, both modes of propagation could be active at our study site. The distance between the transect points T7 and T8 is, with 9 m distance, in the range of typical patch sizes, so that their similarity can be explained by mycelial extension.

However, the question remains how the fungi arrived at T7 and T8. The only fungus that is evenly distributed along the transect is *R. ochroleuca*, all the others are more patchily distributed. Spores can be transported over large distances either by animals or by air (Smith and Read 2008; Bruns et al. 2009). It could be a stochastic process on which habitat, i. e. patch of emerging fine root, which fungus arrives. Thus, chance in long distance spore propagation could have contributed to the observation that *Laccaria cf. laccata* became the dominating fungus in core T7, while it was absent from the remaining transect. One theoretical model that deals with stochastic arrivals in a local community from an outside meta community is the lottery model, that was first proposed for the sessile communities of coral reefs (Sale 1977), but could be also applied to ectomycorrhizal communities (Hoeksema and Kummel 2003; Kennedy and Hill 2010).

It is not in the scope of this paper to develop a model for local ectomycorrhizal diversity. But one scheme is obvious in publications on small scale distributions of ectomycorrhizal fungi. Parts of the observed distributions could be accounted for with deterministic explanations, such as competition, while other patterns remained unexplained (Koide et al. 2005; Gebhardt et al. 2009; Pickles et al. 2010). The discussions in these papers do not consider individual host genotypes as possible cause of nondeterministic patterns. With our result we can confirm that host genotypes indeed do not play a role at the local scale of a soil core. We do not want to propose that stochastic processes are the only explanation for ectomycorrhizal diversity. As was recently proposed in theoretical ecology, niche theory and models involving stochastic processes are not exclusive but complementary to each other (Chisholm and Pacala 2010;

Vellend 2010; Rosindell et al. 2011). For ectomycorrhizal research this means that future studies have to be designed to test models that involve stochastic processes.

IV.6. Acknowledgements

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IV.7. Tables and figures

	Ca	Mg	P	Mn	Fe	Al	K	C	N	C/N	pH
Of	2.80±1.2 (0.188)	0.62±0.1 (0.210)	0.40±0.13 (0.235)	0.65±0.39 (0.140)	10.07±3.3 9	4.16±0.57 (0.703)	1.07±0.16 (0.055)	10.9±5.8 (0.223)	0.57±0.28 (0.251)	18.7±1.7 (0.743)	4.0±0.3 (0.677)
Oh	1.43±0.48 (0.421)	0.52±0.05 (0.092)	0.36±0.05 (0.918)	0.31±0.23 (0.339)	6.46±1.25 (0.786)	4.45±0.53 (0.449)	0.89±0.08 (0.569)	6.8±3.0 (0.073)	0.39±0.16 (0.158)	17.5±2.0 (0.980)	3.6±0.2 (0.938)
A	0.64±0.13 (0.053)	0.52±0.08 (0.055)	0.24±0.02 (0.556)	0.18±0.07 (0.433)	4.67±0.32 (0.767)	4.84±0.55 (0.987)	0.77±0.08 (0.012)	1.4±0.3 (0.637)	0.07±0.01 (0.118)	20.0±1.4 (0.025)	3.7±0.1 (0.432)
B	0.66±0.16 (0.929)	0.58±0.09 (0.779)	0.21±0.05 (0.132)	0.14±0.05 (0.174)	4.74±0.41 (0.812)	5.25±0.63 (0.168)	0.81±0.11 (0.773)	0.4±0.2 (0.373)	0.03±0.01 (0.769)	16.3±4.1 (0.84)	4.2±0.1 (0.824)

Table 1: Distribution of soil parameters

Values in brackets are the significance values of normality tests (Shapiro-Wilk), values above 0.05 indicate normal distribution. Highlighted averages (bold) do not originate from normal distributions. Shaded fields indicate a significant spatial correlation with transect position. Units are mg/g dry mass for Ca, Mg, P, Mn, Fe, Al and K. C and N are given as percentage in dry mass.

Table 2: Primer pairs for microsatellite PCR

microsatellite locus	reference	annealing temperature	polymorphism
FS1-15	(Tanaka et al. 1999)	60°C	Yes
FS4-46	(Tanaka et al. 1999)	60°C	Yes
FS1-03	(Tanaka et al. 1999)	60°C	No
FS1-25	(Tanaka et al. 1999)	60°C	No
FS3-04	(Tanaka et al. 1999)	60°C	No
MFC7	(Pastorelli et al. 2003)	55°C	No

Table 3: Fingerprints of trees

All trees and saplings within a 15 m radius around each transect point were probed. At T3 also seedlings were probed. Seedling 8, 9, 10, 15 were larger seedlings suspected to reach with their root to the sampling point. Seedlings 11-14 were found within 40 cm of the transect point. At T7 and T8 no seedlings were present within a 1 m radius. Fingerprints of grey shaded trees are found among the root segments in soil cores T3, T7, T8.

	individual beech	FS4-46 length in bp	FS1-15 length in bp	
T3	old trees	1	238, 269	121
		2	-	111, 119
		3	236, 272	119
		4	229	111
	young trees	5	-	-
		6	-	111, 113
		7	238	111, 115
		8	229, 238	111, 119
		9	238	111, 119
		10	238	119
		15	-	111, 119
	seedlings	11	229	111
		12	-	111, 121
		13	123, 272	117
14		229, 238	111	
T7	old trees	16	238	119
		17	272	113, 119
		18	238	111
		20	238	111
		21	272	111, 115
T8	old trees	19	-	-
		22	238, 272	111
		23	229	121
		24	-	111, 113
		25	229	107, 119
	young tree	26	-	113, 121

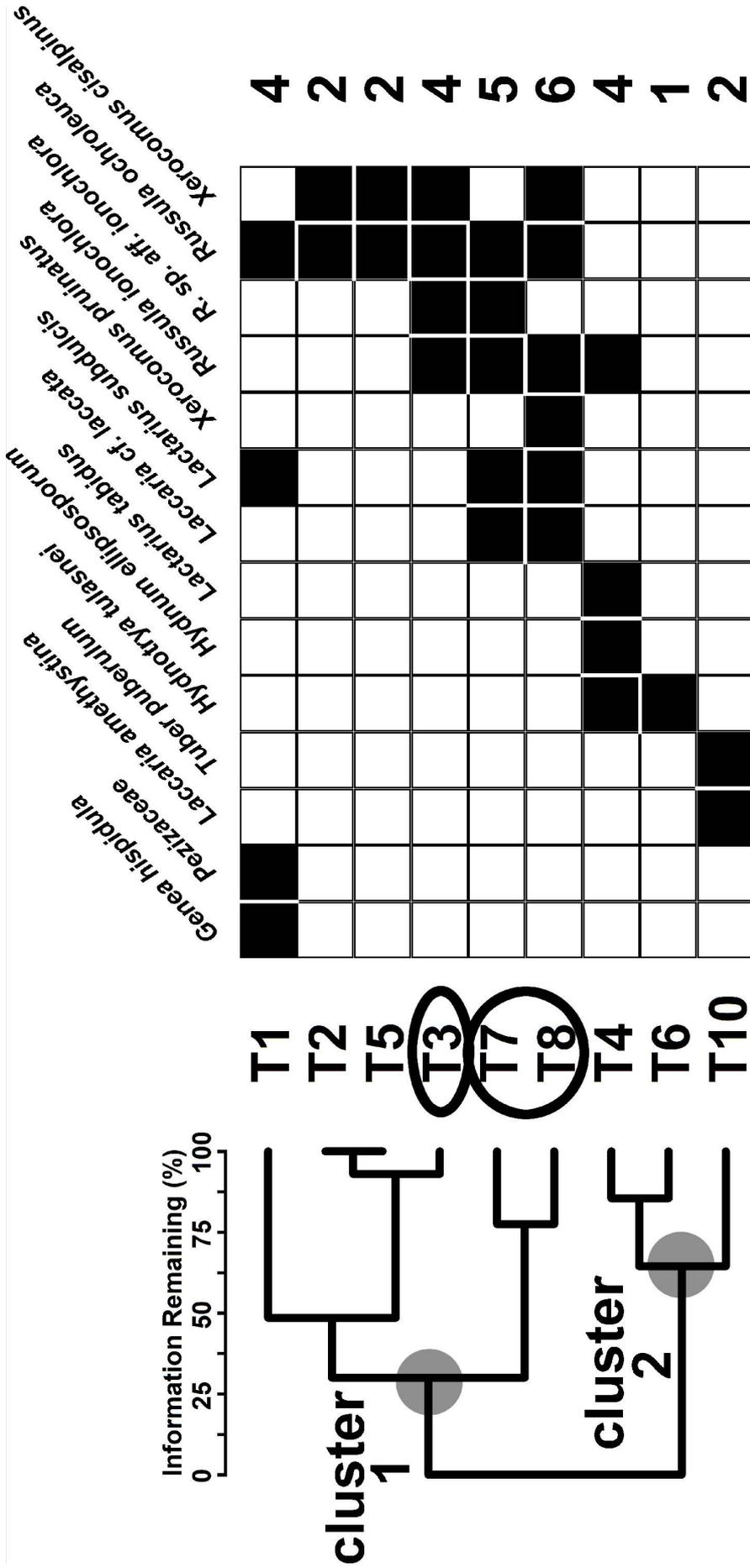


Fig. 1: Distribution of ectomycorrhizal fungi on the transect by two-way cluster analysis

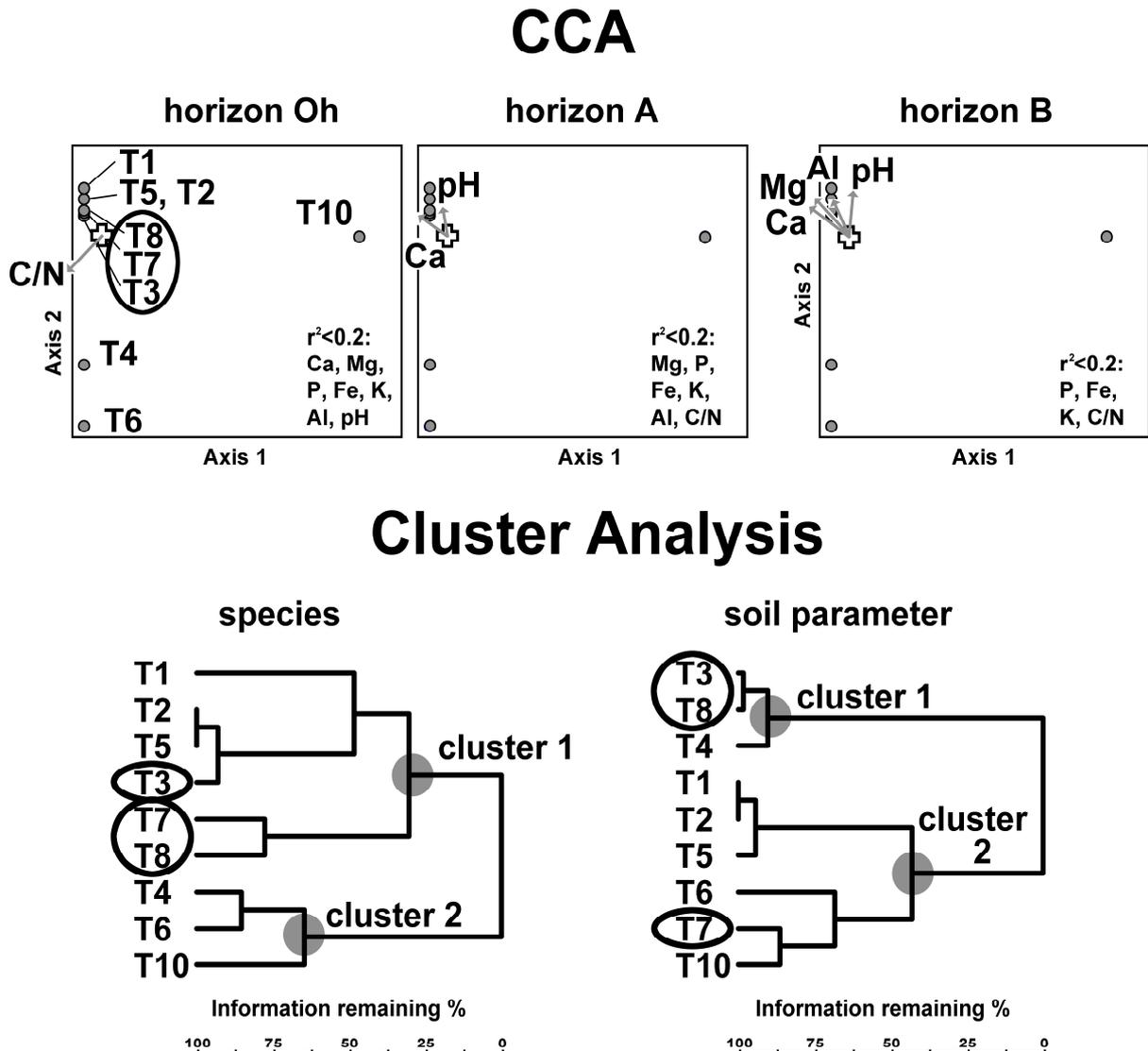


Fig. 2: Relation of soil parameters and ectomycorrhizal diversity

Results of CCA are shown as joint plots of the same main matrix (diversity) and three secondary matrices (soil parameters for Oh, A, B). Only parameters with combined $r^2 > 0.2$ (correlation with axis 1 and 2) are drawn into the plot. In the cluster analysis, soil parameters of all horizons were analysed in a single run.

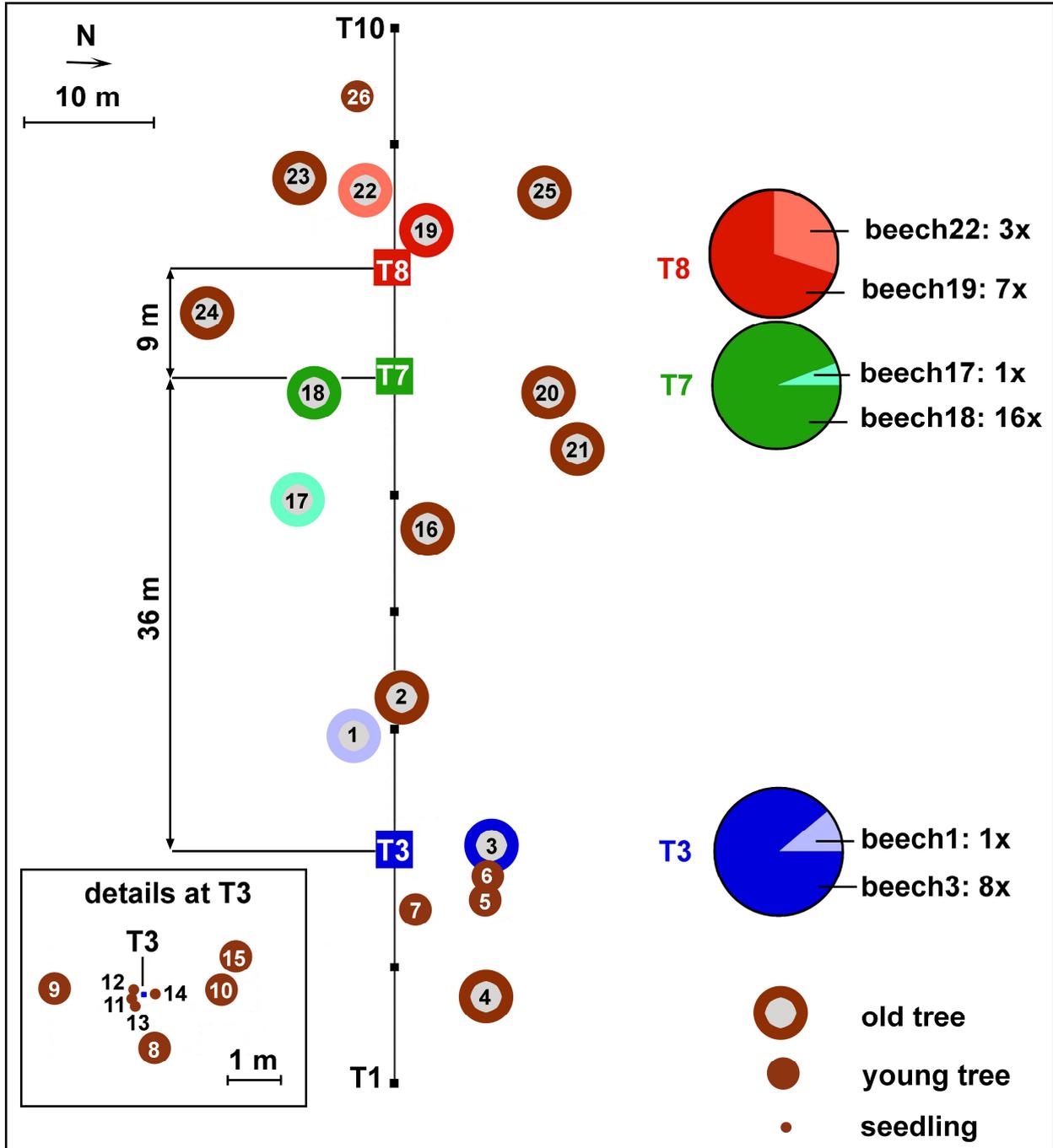


Fig. 3: Allocation of root segments to trees

All trees and saplings within a radius of 15 m around transect points T3, T7 and T8 were genotyped. In addition, all seedlings within a radius of 1 m around T3 were genotyped. The root segment genotypes in the soil cores T3, T7 and T8 are depicted in the pie charts on the right hand side.

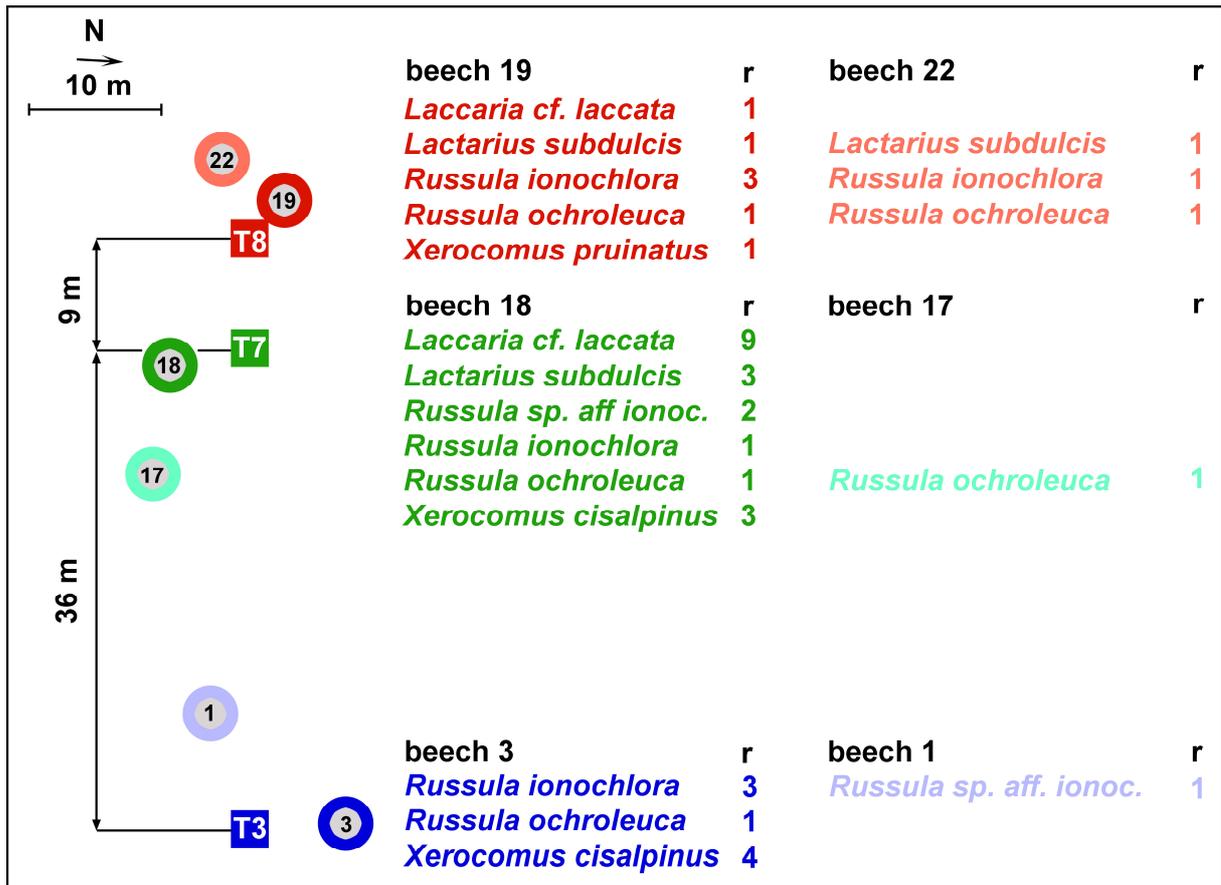


Fig. 4: Ectomycorrhizal species on root segments

Ectomycorrhizal species are sorted according to the genotype of the root segments and the soil core. The column denoted with "r" lists the quantity of root segments.

IV.8. References

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IV.9. Online supplemental material

Supplementary Table S1: Species designation

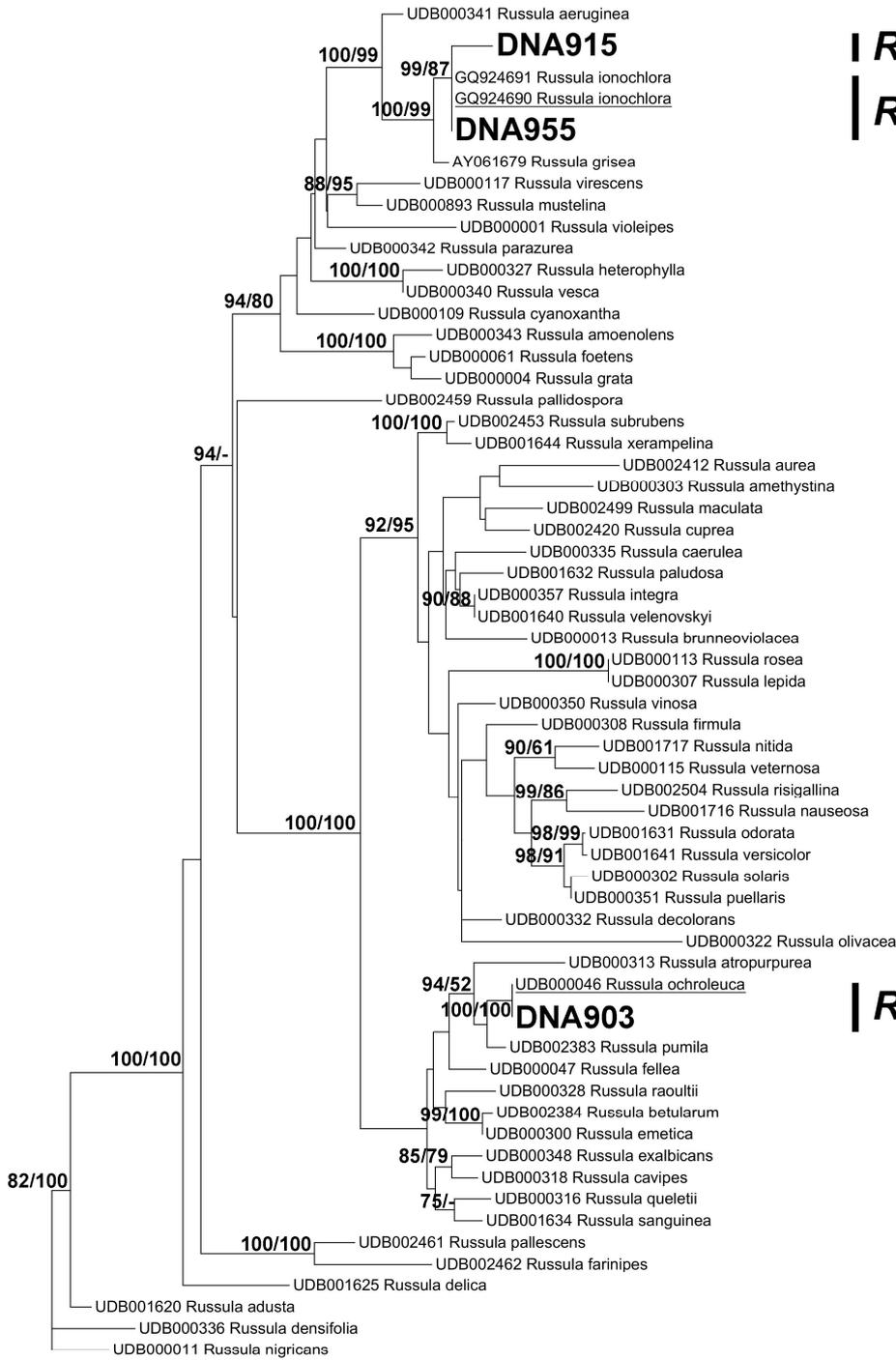
Colour coding corresponds to tree genotypes in Fig. 2.

field assignment	DNA	species name	bp	%	Database reference	GenBank accession
sequenced tips in T3, T7, T8						
BB75_407_Ah_210507_R28	898	<i>Laccaria cf. laccata</i>	693	99.9	UDB000104	HM189791
BB75_407_Ah_210507_R29	901	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM355997
BB75_407_Ah_210507_R34	910	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM355998
BB75_407_Ah_210507_R35	912	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM356599
BB75_407_Ah_210507_R36	914	<i>Laccaria cf. laccata</i>	693	100	UDB000104	HM356000
BB75_407_Ah_210507_R40	919	<i>Laccaria cf. laccata</i>	730	99.9	UDB000104	HM356001
BB75_407_Ah_210507_R45	926	<i>Laccaria cf. laccata</i>	730	99.9	UDB000104	HM356002
BB75_407_Ah_210507_R46	928	<i>Laccaria cf. laccata</i>	693	99.9	UDB000104	HM356003
BB75_407_Ah_210507_R47	929	<i>Laccaria cf. laccata</i>	730	99.9	UDB000104	HM356004
BB75_408_Ah_210507_R48	932	<i>Laccaria cf. laccata</i>	730	99.9	UDB000104	HM189793
BB70_407_Ah_210507_R42	923	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189803
BB70_407_Ah_210507_R47	931	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM356005
BB22_408_Ah_210507_R55	940	<i>Lactarius subdulcis</i>	734	99.9	UDB000048	HM189804
BB45_408_Ah_210507_R50	934	<i>Lactarius subdulcis</i>	728	100	UDB000048	HM189806
BB45_407_Ah_210507_R36	913	<i>Lactarius subdulcis</i>	734	100	UDB000048	HM189807
BB72_408_Ah_210507_R58	944	<i>Russula ionochlora</i>	640	100	GQ924690	HM189868
BB72_408_Ah_210507_R62	947	<i>Russula ionochlora</i>	174	100	GQ924690	HM356006
BB72_408_Bv_210507_R66	953	<i>Russula ionochlora</i>	646	100	GQ924690	HM189869
BB72_408_Bv_210507_R67	954	<i>Russula ionochlora</i>	646	100	GQ924690	HM356007
BB72_408_Bv_210507_R68	955	<i>Russula ionochlora</i>	683	100	GQ924690	HM356008
BB72_403_Bv_210507_R15	871	<i>Russula ionochlora</i>	646	99.4	GQ924690	HM189874
BB72_403_Bv_210507_R23	880	<i>Russula ionochlora</i>	646	99.4	GQ924690	HM356017
BB72_407_Ah_210507_R34	909	<i>Russula ionochlora</i>	646	100	GQ924690	HM189875
BB06_403_Ah_210507_R07	862	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189927
BB70_407_Ah_210507_R31	903	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189928

BB70_407_Ah_210507_R37	916	<i>Russula ochroleuca</i>	700	99.8	UDB000046	HM356009
BB70_407_Ah_210507_R41	921	<i>Russula ochroleuca</i>	307	100	UDB000046	HM356010
BB70_408_Ah_210507_R54	939	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189929
BB06_403_Bv_210507_R14	869	<i>Russula sp. aff. ionochlora.</i>	646	96.6	GQ924690	HM189945
BB28_407_Ah_210507_R36	915	<i>Russula sp. aff. ionochlora</i>	683	96.6	GQ924690	HM189946
BB28_407_Ah_210507_R46	927	<i>Russula sp. aff. ionochlora</i>	646	96.6	GQ924690	HM356011
BB06_403_Bv_210507_R11	866	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM190080
BB06_403_Bv_210507_R16	872	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM356012
BB06_403_Bv_210507_R21	878	<i>Xerocomus cisalpinus</i>	743	99.7	UDB002180	HM356013
BB28_407_Ah_210507_R47	930	<i>Xerocomus cisalpinus</i>	745	99.6	UDB002180	HM190082
BB06_403_Bv_210507_R19	875	<i>Xerocomus cisalpinus</i>	743	100	UDB002180	HM356014
BB28_407_Ah_210507_R35	911	<i>Xerocomus cisalpinus</i>	743	99.9	UDB002180	HM356015
BB28_407_Ah_210507_R44	925	<i>Xerocomus cisalpinus</i>	667	99.8	UDB002180	HM356016
BB28_408_Ah_210507_R57	942	<i>Xerocomus pruinaeus</i>	770	100	UDB000049	HM190109

sequenced tips in the remaining transect

BB82_401_Oh_210507	843	<i>Genea hispidula</i>	685	100	UDB001408	HM189752
BB85_404_Ah_210507	886	<i>Hydnotrya tulasnei</i>	740	99.2	UDB000095	HM189764
BB85_406_Ah_210507_R25	895	<i>Hydnotrya tulasnei</i>	740	99.5	UDB000095	GQ149462
BB86_404_Bv_210507	887	<i>Hydnum ellipsosporum</i>	648	100	AY817138	HM189768
BB100_410_Ah_210507	963	<i>Laccaria amethystina</i>	145	100	UDB002418	HM189777
BB96_401_Oh_210507	846	<i>Lactarius subdulcis</i>	771	99.9	UDB000048	HM189802
BB38_404_Oh_210507	882	<i>Lactarius tabidus</i>	722	100	UDB000385	HM189828
BB83_401_Oh_210507	842	<i>Pezizaceae</i>	695	100	AJ969437	HM189837
BB83_401_Ah_210507	848	<i>Pezizaceae</i>	695	100	AJ969437	HM189838
BB72_404_Ah_210507	885	<i>Russula ionochlora</i>	885	99.8	GQ924690	HM189867
BB70_401_Oh_210507	845	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189924
BB70_401_Ah_210507	849	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189925
BB14_402_Bv_210507	852	<i>Russula ochroleuca</i>	699	100	UDB000046	HM189926
BB57_410_Ah_210507	964	<i>Tuber puberulum</i>	556	100	UDB001385	HM190013
BB06_402_Ah_210507	851	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM190079
BB28_405_Bv_210507	893	<i>Xerocomus cisalpinus</i>	743	99.6	UDB002180	HM190081



| *Russula sp. aff. inonochlora*
| *Russula ionochlora*

| *Russula ochroleuca*

0.1 substitutions/site

Supplementary Fig. S1: Species designation for *Russula ectomycorrhizas* (previous page)

Phylogram is based on a 709 bp alignment of ITS region. Reference sequences are from the UNITE database except for *Russula ionochlora* that stem from fruiting bodies collected for this study in the State of Brandenburg; Germany. One fruiting body (GQ924690) was collected by D. Wernigk in a mixed forest near lake Lubowsee at 20.07.2008. The second (GQ924691) was collected by M. Schmidt in an alder stand with oaks near lake Große Lankesee at 30.08.2008. Both fruiting bodies were determined by H. Streese, Berlin as *Russula ionochlora*. Despite 23 differing positions, DNA915 and DNA955 are closely related and it is possible that the differences represent sequence heterogeneity between ITS amplicons of the same species. The Maximum Likelihood tree was generated with RAxML algorithm and 100 bootstrap replicates. Bootstrap values above 50 are indicated. A second tree was generated with the Neighbour-Joining algorithm and 1000 bootstrap replicates. For those nodes equal in both trees the NJ-bootstrapping values are given as a second number on the node.

Thesis Part V

Synopsis

V. 1. Distinctive presuppositions for analysis of ectomycorrhizal host specificity

This thesis focused on host specificity of ectomycorrhizal fungal species of four forest stands at site Kahlenberg in the North-eastern Lowlands of Germany. Ectomycorrhizal fungi live in a mutualistic symbiosis with roots of different species of forest trees. Host specificity is one factor that can explain biodiversity in an ectomycorrhizal community, i. e. number and distribution of ectomycorrhizal fungal species on tree roots. This section summarizes the theoretical and methodological presuppositions which have been applied for this thesis.

Species level at the fungal side

Although biodiversity can be considered at different levels (individual genotypes, species or higher taxonomic levels), this thesis is restricted to the biodiversity of species at the fungal side.

Two levels of biodiversity at the host side (species and genotypes)

At the side of the host trees, two levels of biodiversity were considered. Part III analysed how the diversity of fungal species is influenced by the composition of the host species Scots pine (*Pinus sylvestris* L.) and beech (*Fagus sylvatica* L.). Part IV left the species level at the host side and analyzed whether the diversity of fungal species is influenced by differing individual genotypes within the same species, in this case the individual beech genotypes in a pure beech stand.

Sampling in mixed and pure stands

Previous studies on host specificities analyzed either mixed stands or pure stands of differing host tree species. To the best knowledge of the author this is the first study that explores mixed and pure stands simultaneously and in close vicinity. The special condition of the Kahlenberg site is that the two pure stands (one Scots pine stand and one beech stand) harbor separately the same fungal species that grow together in the two mixed stand (Scots pine and beech). The close vicinity allows considering all four stands as subplots of a single site.

Specificity guilds

The study design of mixed and pure stands requires the concept of specificity guilds to reconcile different observations in mixed and pure stands. The guild of host specific fungi contains both specialists (occurring only on the roots of one host species) and host preferring fungi (majority of occurrences on host species with occasional switches to a non-target host in the mixed stands). Three specificity guilds were distinguished: pine specific guild, beech specific guild, generalist guild.

Sequencing of all mycorrhized root tips

All root tips used for the analysis in Part III and IV were ITS sequenced. In contrast to other ectomycorrhizal studies that sequence all collected root tips and use random sampling schemes, collected root tips were chosen on the basis of morphotypes. This procedure allowed comparing the match of the sequence based and morphotype based identifications for a complete study (Part II).

Species identification by in-depth phylogenetic analysis

The usual practice for fungal ectomycorrhizal studies is to compare the fungal ITS sequence with a database entries. A BLAST hit with 99% match (in some cases 97%) is considered sufficient to assume species identity of query and reference sequence. This thesis goes beyond the reliance on database comparisons by performing a phylogenetic analysis for each assumed ectomycorrhizal sequence type.

V.2. Summary of results

High percentage of identified sequence types

Forty ectomycorrhizal sequence types could be distinguished at the site Kahlenberg, of which 31 (78%) could be identified to the species level or at least as closely affiliated to a species. All sequences generated in the course of this work were submitted as new entries to GenBank (477 submissions).

High percentage of host specific fungi

The majority of species (66%) belonged either to the pine or to the beech specific guild. Even in the mixed stands, 49% of sequenced root tips were colonized by fungi of the host specific guilds. Thus, host specific fungi are a major component of the ectomycorrhizal community at the Kahlenberg site.

Observed specificity phenomena

With the improvements in fungal species identification by using phylograms following specificity phenomena have been observed:

1. A supposedly generalist species can include several cryptic species with different host specificities.
2. The switch from host specific to generalist species occurs frequently within a fungal genus.
3. In a mixed stand, a member of a host specific guild is able to switch occasionally to roots of the non-target host.

From host species to individual host genotypes

The high percentage of host specific fungi indicates a strong influence of the host species on the fungal species composition at Kahlenberg site. In extending the idea of host adaptation to genotype differences, the individual host genotypes of beech roots were identified by microsatellite analysis in three soil core of the pure beech stand (Part IV). Each soil core contained the roots of two neighbouring beech trees.

No influence of individual host genotypes or soil parameters on the fungal species diversity in a pure beech stand

Up to six different species were identified in a single soil core. However, no specialisation to one of the two individual beech root genotypes could be detected. Furthermore, no relationship between fungal species diversity and soil parameter could be established by using cluster analysis and canonical correspondence analysis.

V.3. Outlook

The combination of deterministic and stochastic explanations

The results on fungal specialisation at different levels of host diversity provide an input to the discussion of deterministic and stochastic explanations of ectomycorrhizal diversity. As it is explained in section IV.5., several niches could be excluded to explain the distribution of the ectomycorrhizal fungi in the pure beech stand of the Kahlenberg site. It has been proposed that dispersal related processes may lead to a stochastic distribution along the observed transect. It is not the intent of this work to question the importance of niches and deterministic theories on species distributions. In fact, the results of Part III clearly demonstrate that host specificity is a major component that determines the fungal community at the Kahlenberg site. If the membership in a host specific guild is viewed as a physiological adaptation to the host, then presence of host specific fungi is a striking example that niches influence the distribution of fungal species.

The phenomenon of occasional host switches in the mixed stands of pine and beech might serve as an illustration how niche theory and dispersal related stochasticity can be set in relation to each other. Let it be assumed that a fungus of the pine specific guild at Kahlenberg site is well adapted to Scots pine but can potentially colonize beech roots. However, the pine specific fungus will be a weaker competitor for beech roots than a beech specific fungus. In the pure beech stand the pine specific fungus is completely outcompeted by beech specific fungi, so that it is not able to grow on beech roots. In the two mixed stands, the pine specific fungus competes successfully and colonizes intensively the pine roots. If young unmycorrhized beech roots extend by chance to a nest of mycorrhized pine roots, the beech roots will encounter only the mycelium of the pine specific fungus, so that competition with beech specific fungi is locally excluded. As a result, the pine specific fungus will be able to

colonize the beech roots. The competition and exclusion of the pine specific fungus in the beech stand is related to adaptation and to deterministic niche theory while the assumed undirected growth of the beech root is an illustration of a dispersal process that involves stochasticity. Thus, the described explanation is a model for explanations of ectomycorrhizal diversity patterns that include both deterministic and stochastic elements. The proof of this model would require the observation of root growth and mycorrhiza-formation in situ. The presented model of occasional host switches in mixed stands has been inspired by a proposal of Melin (1923, pp. 512-513) to explain the occurrence of pine specific fungi in mixed stands of Scots pine and birch.

Meta-analysis of specificity phenomena

It has been discussed in section III.5 that the high number of host specific fungi at the Kahlenberg site might be due to the large phylogenetic distance of the two hosts Scots pine and beech. In differing host communities and in differing parts of the world the distribution of fungi in host specific and generalist guilds can deviate from the results in this thesis. In order to assess the global importance of adaptations of ectomycorrhizal fungi to host species, further field studies on host specificities are necessary. While broadening the empirical basis is one way, the global importance of host specific fungi can also be assessed by re-evaluating the existing knowledge of fungus-host associations. One possible approach is to use Ecological Network theory (not to confuse with the physical connections of Common Mycorrhizal Networks). By meta-analysis of existing studies, networks of interactions between different species can be recognized. Ecological Networks have been used to assess specificity phenomena in non-symbiotic mutualisms such as seed disperser or pollinator networks on regional and global scales (Bascompte et al. 2003; Olesen et al. 2007; Thebault and Fontaine 2010). Extending the methods of Ecological Network theory to the interactions of ectomycorrhizal fungi and their hosts might help to evaluate whether host specificities influence not only local communities, as demonstrated in this work, but also shape global distributions of ectomycorrhizal fungi.

Abbreviations and glossary

ampullate	One end of a fungal hypha is formed like a flask.
anatomotype	Anatomotype refers to features of ectomycorrhizas as they can be seen at 1000 x magnification through a microscope. These are details of cell shape, content and surface. Observation of anatomical characteristics requires dissecting the fungal mantle or preparation of thin sections.
anastomosis	Pl. anastomoses. It describes a fusion of two hyphae.
BTU	B randenburgische T echnische U niversität, Cottbus
CCA	C anonical C orrespondence A nalysis
clamp	Short form for clamp connection. It is a blister-like structure at the septum between two hyphal cells of some basidiomycete fungi. Presence of clamps is indicative for some, but not all, basidiomycete fungi. Absence, however, does not exclude basidiomycetes.
CMN	C ommon M ycorrhizal N etwork
corticoid	Basidiomycetes with simple crust-like fruiting bodies (resupinate). Originally thought to be represented by the family Corticiaceae, corticoid fungi are members of many different lineages.
cystidium	Pl. cystidia. Cells that differ from remaining mantle hyphae and protrude from the mantle surface.
deterministic	An observation can explained by "cause and effect". In relation to ectomycorrhizal community studies it means that a fungal species distribution can be explained by adaptations to the environment.
DFG	D eutsche F orschungs G emeinschaft
DSMZ	D eutsche S ammlung von M ikroorganismen und Z ellkulturen, Braunschweig
generalist	An ectomycorrhizal fungus that colonizes the roots of a wide range of host trees
GFZ	G eo F orschungs Z entrum, Potsdam
ITS	I nternal T ranscribed S pacers (of rDNA)
laticifer	Hypha containing latex in the fungal genus <i>Lactarius</i>
mantle	Sheath of fungal hyphae around a mycorrhized root tip
monopodial-pinnate	Branching pattern of an ectomycorrhizal system with a main axis and side branches. Side branches protrude in one plane, giving the system a flat shape.

monopodial-pyramidal	Branching pattern of an ectomycorrhizal system with a main axis and - side branches. Side branches protrude in different planes, giving the system a three-dimensional shape.
morphotype	Morphotype refers to features of an ectomycorrhiza as they can be seen at lower magnification through a dissecting microscope. In the strict usage of the term, a morphotype is distinct from the anatomotype of a mycorrhiza. In many publications, including this thesis, the term is used in the broader sense for the physical appearance of a mycorrhiza including both morphological and anatomical characteristics.
mutualism	This term denotes an interaction of at least two organisms in which all partners gain a net benefit. Mutualism does not require a close "living together", i.e. not all mutualisms are symbioses. An example for a non-symbiotic mutualism is the seed dispersal by birds. Birds are provided with food, while the plants gain a means for dispersing their seeds.
phialid	special form of a conidiophore in the endophytic fungal genus <i>Phialocephala</i>
plectenchymatic	Fungal mantle hyphae form a network. Cells are elongated.
OTU	O perational T axonomic U nit, see also sequence type
PCR	P olymerase C hain R eaction
pseudoparenchymatic	Fungal mantle hyphae form a "false tissue". Cells are roundish.
rDNA	r ibosomal D eoxyribo N ucleic A cid
resupinate	Describes as fungus whose fruiting body lies directly on the underground with the hymenium (the spore bearing cell layer) facing upward.
rhizomorph	A bundle of fungal hyphae that explore the soil. They bear a superficial resemblance to roots.
RFLP	R estriction F ragment L ength P olymorphism
rRNA	r ibosomal R ibo N ucleic A cid
secondary septum	A structure of emanating hyphae. It describes a septum without clamps between two septa with clamps. By definition, they can be only found at basidiomycete hyphae that have regularly clamps. Ascomycetes do not have clamps and therefore no secondary septa.
sequence type	All ITS sequences that share at least 99% sequence similarity belong to a sequence type. A sequence type is thought to represent a species and is given a provisional name. Another term describing the same fact is OTU (Operational Taxonomical Unit).
symbiosis	Pl. symbioses. This term means "living together" of two or more organisms. In contrast to colloquial usage in German language not all

symbioses provide a benefit to both partners. An example is an organism that uses another organism as habitat without harming it or giving it an advantage (= commensalism). Mycorrhiza is considered to be a mutualistic symbiosis because both fungus and plant benefit.

stipitate

Describes a fungus with a fruiting body composed of head and stalk.

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Erklärungen:

Hiermit erkläre ich an Eides Statt, dass ich die Dissertation selbstständig verfasst habe und dass ich alle in Anspruch genommenen Hilfsmittel angegeben habe.

Hiermit erkläre ich, dass die Veröffentlichung der Dissertation keine bestehenden Schutzrechte verletzt.

Ben Bubner, Müncheberg, den 05.12.2012

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