

Ectomycorrhizal fungal communities of native and non-native *Pinus* and *Quercus* species in a common garden of 35-year-old trees

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Abstract Non-native tree species have been widely planted or have become naturalized in most forested landscapes. It is not clear if native trees species collectively differ in ectomycorrhizal fungal (EMF) diversity and communities from that of non-native tree species. Alternatively, EMF species community similarity may be more determined by host plant phylogeny than by whether the plant is native or non-native. We examined these unknowns by comparing two genera, native and non-native *Quercus robur* and *Quercus rubra* and native and non-native *Pinus sylvestris* and *Pinus nigra* in a 35-year-old common garden in Poland. Using molecular and morphological approaches, we identified EMF species from ectomycorrhizal root tips and sporocarps collected in the monoculture tree plots. A total of 69 EMF

species were found, with 38 species collected only as sporocarps, 18 only as ectomycorrhizas, and 13 both as ectomycorrhizas and sporocarps. The EMF species observed were all native and commonly associated with a Holarctic range in distribution. We found that native *Q. robur* had ca. 120% higher total EMF species richness than the non-native *Q. rubra*, while native *P. sylvestris* had ca. 25% lower total EMF species richness than non-native *P. nigra*. Thus, across genera, there was no evidence that native species have higher EMF species diversity than exotic species. In addition, we found a higher similarity in EMF communities between the two *Pinus* species than between the two *Quercus* species. These results support the naturalization of non-native trees by means of mutualistic associations with cosmopolitan and novel fungi.

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Introduction

Ectomycorrhizas are an important symbiosis by which many plant species in north temperate and boreal conditions cope with infertile soils. Many ectomycorrhizal fungal (EMF) species can form ectomycorrhizas with a variety of tree species (Smith and Read 2008), and closely related tree species tend to form similar EMF species communities (Horton and Bruns 1998; Ishida et al. 2007). However, different tree species of the same genus may still possess specific EMF species (i.e., Rusca et al. 2006). One of the factors making EMF species community structures similar among closely related trees is the co-evolution of both partners: fungi and host trees (Kretzer et al. 1996).

Moreover, the speciation process during the co-evolution of tree and of fungi (Cairney 2000) and their movement into geographically and climatically distant regions (Grubisha et al. 2007) might also influence the divergence in EMF assemblages for closely related tree species. How native and non-native species of the same genus are affected in this regard remains unknown. The colonization process of non-native tree species partially consists of the niche partitioning of locally existing EMF species toward the non-native tree roots. The recognition and mycorrhiza formation of native EMF species with a new tree species is controlled by many unknown factors as demonstrated by Parladé et al. (1996) who also found incompatibility among some fungal strains of the same EMF species and *Pseudotsuga menziesii* seedlings.

Frequently trees grow outside of their natural ranges as a result of intentional or unintentional human activity. For example, red oak (*Quercus rubra* L.) and Austrian black pine (*Pinus nigra* Arn.) are widespread exotic species. Both tree species survive well outside their native areas (Białobok and Chylarecki 1965; Bellon et al. 1977; Zhou et al. 1997; Demchik and Sharpe 2000; Gebhardt et al. 2007) and are widely used in forestry. Furthermore, *Q. rubra* is considered an invasive tree species in Europe (Daubree and Kremer 1993; Petit et al. 2004; Křivánek and Pyšek 2006), although reasons for its invasiveness remain uncertain. One of the potential reasons making *Q. rubra* successful is its broad ecological range for climatic, soil moisture and N availability conditions and, in turn, competitive abilities against native tree species (Zerbe and Wirth 2006). Exotic plant species may be abundant in novel environments as they do not experience the same negative feedback with soil communities (Reinhart and Callaway 2006). This is a key factor in alien plant invasions and considered as “enemy escape.” It is unclear how non-native tree species may utilize mycorrhizal fungi to cope with novel environments, and it is also unknown to what extent they form similar EMF assemblages as their native counterparts.

Non-native trees may form symbioses with EMF species in exotic locations through acceptance of native EMF species, increased reliance on cosmopolitan EMF species, or co-introduction non-native trees with non-native EMF species. The invasive *Picea engelmannii* found in North America (Cullings et al. 2000) and *Q. rubra* growing in Germany accepted a broad range of native EMF species (Gebhardt et al. 2007) as did non-native *Eucalyptus* sp. growing in the Seychelles Islands (Tedersoo et al. 2007). Kohout et al. (2010) also demonstrated that non-native *Pinus strobus* effectively adopted EMF assemblages with native fungi in a mesocosm experiment in Czech Republic. Similarly, Parladé et al. (1996) demonstrated that 18 EMF species out of 27 collected in northern Spain were able to colonize roots of non-native *Pseudotsuga menziesii* in a

pure culture test. On the other hand, the intended introduction of *Pinus* spp. into Australia failed as a result of lack of pine-specific EMF species (Nuñez et al. 2009). However, Dickie et al. (2010a) showed that non-native *Pinus contorta* was successfully introduced into New Zealand mostly by means of the co-invasion of EMF species and forming ectomycorrhizal associations with cosmopolitan EMF species rather than the acceptance of novel fungi. This was also demonstrated by Tedersoo et al. (2007) for *Pinus caribea* which maintained EMF species co-introduced with seedlings in the Seychelles Islands.

Ectomycorrhizal fungal species are more host specific than arbuscular mycorrhizal fungi (Smith and Read 2008), and the absence of ectomycorrhizas originally hindered the success of many *Pinus* species to new regions of the world (Brisco 1959; Poynton 1979 in Reinhart and Callaway 2006). However, it has been shown that EMF species can migrate or be moved by human activities to remote novel places (see Vellinga et al. 2009). The global introduction and distribution of EMF species may facilitate the introduction and finally lead to the invasion of certain trees. This potential was shown by Richardson et al. (1994) for *Pinus* introduced into the Southern Hemisphere and also by Chou-Chou and Grace (1983) who observed a co-occurrence of *Rhizopogon vinicolor* (an EMF species originally from North America) with *P. menziesii* grown in New Zealand.

This suggests a key role for EMF in the establishment of non-native tree species in novel places. It has been shown how the non-native tree species use mutualistic symbioses to cope with growing in novel places (see above citations). However, to what extent the EMF assemblages of non-native tree species are shared with their native counterparts remains unknown.

To gain insights into the ability of two native and non-native trees in the genera *Pinus* and *Quercus* to share common EMF species when grown in a common garden, we addressed the following questions: (a) Is the EMF species richness higher for native tree species than for non-native tree species? (b) Is the EMF community more similar within a tree genus than between tree genera, and is it more similar for *Pinus* spp. as the two are less separated geographically than the *Quercus* spp? (c) Do native species possess a higher number of unique EMF species? (d) Do the cosmopolitan EMF species dominate on the non-native tree species?

Materials and methods

Study site

The study was carried out at the Siemianice Experimental Forest in central Poland (52°14.87' N, 18°06.35' E;

150 m a.s.l.). Fourteen tree species were planted as monocultures (20×20 m plots; trees were planted at 1×1 m spacing) in 1970–1971 after 80-year-old Scots pine stands were clear-cut (including removal of the root systems), on two adjacent sites differing in soil properties (a coniferous forest site and a mixed forest site). Each site has three replicates of nine tree species, with four species shared between the two sites. In the present study, we used only the coniferous forest site to avoid putative effects of edaphic factors on EMF community structure. The coniferous forest site comprises plots of *Quercus robur*, *Q. rubra*, *Pinus sylvestris* and *P. nigra* (with three replicates each) as well as plots of five species not considered in this study (*Betula pendula*, *Carpinus betulus*, *Larix decidua*, *Picea abies*, and *P. menziesii*). The mean annual temperature is 8.2°C and the mean annual precipitation is 591 mm. The growing season (calculated as the number of days with an average temperature above 5°C) lasts on average 213 days and snow cover lasts 50–60 days. Details of soil characteristics in tree plots are presented in Table 1. Details of the experimental area and description of various aspects of tree effects on seedling mycorrhizas (Dickie et al. 2006), fungal phenology (Dickie et al. 2010b), plant invasions (Knight et al. 2008), calcium and earthworms (Reich et al. 2005; Dauer et al. 2007), leaf and fine root litter decomposition (Hobbie et al. 2006, 2010; Goebel et al. 2011), soil nutrients and biogeochemistry (Hobbie et al. 2007), root distribution and lifespan (Withington et al. 2003, 2006; Dauer et al. 2009), and soil organic matter (Hobbie et al. 2007) have been addressed in previous studies of these forest stands.

Sporocarp survey and identification

Sporocarps of all ectomycorrhizal fungi were collected for identification approximately once a month during three growing seasons from May to November in 2004,

2005, and 2006. Identification was carried out using macroscopic and microscopic characters according to standard procedures. The nomenclature follows Knudsen and Vesterholt (2008), Legon et al. (2005), and Index Fungorum (www.indexfungorum.org). The sporocarp collections were deposited in the Herbaria of the University of Łódź and the University of Szczecin, Poland.

All the species found as sporocarps were included in the analysis. Although some sporocarps may have been linked to trees growing in neighboring plots (ex. individuals of *R. vinicolor*, a species specific to *P. menziesii*, found in a *Q. robur* plot), these occurrences were likely very low and did not impact the results of the analysis.

Ectomycorrhizal survey and morphotype assessment

Soil samples (ca. 30 cm³ each) for EMF species evaluation were collected during late spring (May/June), late summer (August/September), and fall (beginning of November) in 2004 and in 2005. For each sampling, we collected nine soil samples from each monoculture plot of the four tree species studied (12 plots total, 108 samples/collection date). The samples were collected in a 5×5-m subplot, with about 1.5 m grid spacing between soil cores. The subplot was in the central part of the plot to avoid any possible contamination by tree roots from neighboring plots. Samples were placed in tagged plastic bags and stored at –10°C until analysis. In total, we collected 162 soil samples for each tree species studied. Separation and evaluation of ectomycorrhizal root tips was conducted under a dissecting microscope. All fine roots from each soil sample were collected and rinsed under tap water using a 1-mm sieve just before ectomycorrhizal morphotypes assessment. Ectomycorrhizal morphotypes were described based on macroscopic observations according to Agerer (1987–2003) and Ingleby et al. (1990) and also were compared to a database used in the Laboratory of

Table 1 Selected soil characteristics of organic horizon (O horizon) and upper mineral horizons (means, $n=3$) of four tree species growing in monoculture plots at Siemianice, Poland

Species	Soil pH		SOM (%)		N _{total} (%)		C _{org} (%)		C/N		P (mg/100 g)		K (mg/100 g)	
	O horizon	0–20 cm	O horizon	0–20 cm	O horizon	0–20 cm	O horizon	0–20 cm	O horizon	0–20 cm	O horizon	0–20 cm	O horizon	0–20 cm
<i>Pinus nigra</i>	4.08	4.11	57.55	1.39	1.25	0.05	33.37	0.8	26.98	17.05	10.95	0.02	26.14	0.48
<i>Pinus sylvestris</i>	3.95	4.01	61.5	1.64	1.28	0.05	35.67	0.95	28.13	19.11	11.81	0.21	23.73	0.47
<i>Quercus rubra</i>	4.86	4.18	55.19	1.32	1.45	0.05	32	0.77	22.29	15.03	20.65	0.27	64.38	0.81
<i>Quercus robur</i>	4.77	4.15	50.74	1.48	1.64	0.06	29.47	0.86	17.96	14.42	18.5	0.11	47.98	1.0

Mycorrhizal Research at the Institute of Dendrology (Iwański et al. 2006; Rudawska et al. 2006; Trocha et al. 2006). Each ectomycorrhizal morphotype was described in detail (color and structure of the surface of the mantle, shape of the ectomycorrhizal root tip, extramatrical hyphae color and structure, and occurrence of other features, e.g., cistidia), photographed, and stored in a plastic tube in the -10°C until molecular analysis.

Ectomycorrhizal morphotype molecular identification

Total genomic DNA was extracted from each ectomycorrhizal morphotype or pieces of selected sporocarps (ca. 20 mg of dried tissue) using the PLANT&FUNGI DNA Purification Kit (EURx, Poland) according to manufacturer's protocol. For the morphotype analyses, we used from two to four root tips per morphotype (depending on the sequence quality obtained further) of each tree species. In total, each morphotype had at least two replicates or, in the case of its presence on four tree species, it had eight replicates. This allowed for an analysis of intramorphotype variation of the sequenced regions. Ectomycorrhizas formed by *Cenococcum geophilum* were excluded from molecular identification and were designated based on their unique morphological features. Internal transcribed spacer regions (ITS1-5.8S-ITS2) were amplified via the polymerase chain reaction (PCR) using primers: fungal specific ITS1-F (Gardes and Bruns 1993) and universal ITS4 (White et al. 1990). The PCR reactions were performed in a 10- μl volume mixture consisting of 1 \times PCR buffer (Novazym, Poland), 1.5 mM MgCl_2 (Novazym, Poland), 0.2 mM of each dNTP (Novazym, Poland), 0.5 μM of each primer, 0.02 mg/ml BSA (Promega, Madison, WI, USA), 0.25 U of *Taq* Polymerase (Novazym, Poland), and 5 μl of DNA aliquot (undiluted or 2 \times or 4 \times diluted). Reactions were performed in a T3 Thermocycler (Biometra, Germany) using the following temperature profile: 1 min 93°C (initial denaturation), 1 min 95°C (denaturation), 1 min 60°C (annealing), 2 min 72°C (elongation), 10 min 72°C (final elongation), and 7°C (pause). Steps 2 to 4 were repeated 35 times. PCR products were purified by the MinElute PCR Purification Kit (Qiagen, Canada) according to the manufacturer's instructions and directly sequenced using primers ITS 1 and/or ITS 4 (White et al. 1990). DNA sequencing was undertaken using the ABI Prism Big Dye Terminator Sequencing Kit version 3.1 (Applied Biosystems, Foster City, CA, USA). Cycle sequencing was performed using a 2720 Thermal Cycler followed by capillary electrophoresis in an ABI Prism 3130XL Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

In cases of sequence heterogeneity, ITS amplicons were cloned using the pGEM-T easy vector system (Promega, Madison, WI, USA) and DH5 α *Escherichia coli* competent cells (Invitrogen, Ltd., Paisley, UK) according to the manufactures' protocols. Transformed cells were incubated on Petri dishes containing LB Broth EZmix TM Powder (Sigma-Aldrich) with 1.5% agar (AppliChem GmbH, Darmstadt, Germany) at 37°C overnight. About ten white clones were randomly selected for each transformation effort and subjected to colony PCR using the M13 forward (5'-TGTA AACGACGGCCAGT-3') and reversed (5'-CAGGAAACAGCTATGACC-3') primer pair and *Taq* DNA polymerase (Novazym, Poland). Reactions were performed in the following temperature profile: 3 min 96°C (initial denaturation), 20 s 95°C (denaturation), 15 s 53°C (annealing), 1 min 10 s 72°C (elongation), 5 min 72°C (final elongation), and 7°C (pause). Steps from 2 to 4 were repeated 36 times. PCR products with amplicons of different sizes, confirmed by agarose gel electrophoresis, were subjected to another round of sequencing PCR using T7 primer (5'-TAATAC GACTCACTATAGGG-3') and to DNA sequencing (as described above). All sequencing results were compared to those deposited in GenBank and UNITE using the *blastn* algorithm.

All of the sequences obtained from studied morphotypes were verified by analysis of chromatograms using CodonCode Aligner (CodonCode Corporation). This included replicates within morphotypes, unknown species of *Cortinarius* sporocarps, and from selected sporocarps of known species: *Elaphomyces muricatus* (JF834198), *Lactarius rufus* (JF834199), *Russula betularum* (JF834200), and *Russula fragilis* (JF834201) collected in the study plots. Then all of those sequences with reference sequences were separated at a 97% similarity using assembly process under default criteria in CodonCode Aligner. Contigs obtained this way were aligned with ClustalW (CodonCode Aligner) and exported into neighbor joining using a number of differences model ("Appendix 2"). Selected sequences of studied ectomycorrhizal morphotypes (accession numbers HM015465–HM015482) and of selected sporocarps (accession numbers HQ115586–HQ115590; JF834198–JF834201) were published in GenBank.

Statistics

The relative abundance of each ectomycorrhizal morphotype was assessed by counting ectomycorrhizal root tips in each sample and expressing each ectomycorrhizal morphotype as a percentage of all fresh-looking ectomycorrhizal root tips collected from all samples of each tree

species. After molecular verification, if it was found that different morphotypes were formed by the same EMF species, the EMF root tips of those morphotypes were summed and recalculated for the appropriate relative abundance. To assess the efficiency of ectomycorrhizal sampling, we constructed a species accumulation curve (Mao Tau), Chao 2, and first- and second-order Jackknife (Jackknife-1 and Jackknife-2, respectively) estimators of true species richness using the EstimateS program version 8.2.0 (Colwell 2009).

To examine the similarity of EMF communities among the tree species investigated, the Jaccard similarity indices were calculated: $P = (2c/a+b) \times 100\%$, where a is the number of EMF species for tree A, b is the number of EMF species for tree B, and c is the number of EMF species shared between tree species A and tree species B. Jaccard indices were calculated including both belowground (ectomycorrhizas) and aboveground (sporocarps) EMF species.

Different EMF species (only for ectomycorrhizas) diversity indices (Shannon, Simpson, dominance, and evenness) among tree species studied were calculated using PAST 1.8 (Hammer et al. 2001). Comparison of belowground EMF species compositions was calculated using detrended correspondence analysis (DCA) by CANOCO software (Ter Braak and Šmilauer 2002).

Results

Ectomycorrhizal identification

In total, we described 31 ectomycorrhizal morphotypes on the four tree species studied (“Appendix 1”). Each morphotype found on each tree species was subjected to molecular analysis to identify the EMF species. The same ectomycorrhizal morphotypes collected from different host species were used as well to study any intra-morphotype variation among tree species (“Appendix 1”). After searching in GenBank and UNITE, the ITS sequences obtained from ectomycorrhizas and selected sporocarps were aligned with their best *blastn* matches.

All studied sequences and their best matches were separated at a 97% similarity using assembly process under other default criteria in CodonCode Aligner. We also incorporated sequences of selected sporocarps *E. muricatus*, *L. rufus*, *R. betularum*, and *R. fragilis* (JF834198–JF834201) collected in the study plots. The following sequences required assembling at either a 94% or 92% similarity threshold: HM015476 (morphotype 16 from *Q. robur*) and best match *Humaria hemisphaerica* (UDB000988) at 94%

and HM015475 (morphotype 15 from *Q. rubra*), HQ115589 and HQ115590 (sporocarps of *Cortinarius* sp. 1/51 and of *Cortinarius* sp. 1/52, respectively) and their best match *Cortinarius vibratilis* (UDB002397) at 92%. Finally, we obtained 31 groups (contigs), each of which contained a specific reference, and thus were identified as different 31 species. EMF morphotypes and sporocarps that formed contigs at the similarity <97% with their reference sequences were not identified to the species level (“Appendix 1”).

An additional phylogenetic analysis for *Cortinariaceae* and *Russulaceae* was conducted using MEGA4.1 software (Trocha et al. 2006; Fig. 1). Using a molecular approach, among 31 ectomycorrhizal morphotypes described on four tree species (“Appendix 1”), we identified 31 EMF species (Table 2; Fig. 2). In some cases, different morphotypes were formed by the same EMF species, e.g., morphotype 8 and 53 (*Paxillus involutus*), whereas, in other cases, the same morphotype was formed by different EMF species depending on the host species (“Appendix 1”).

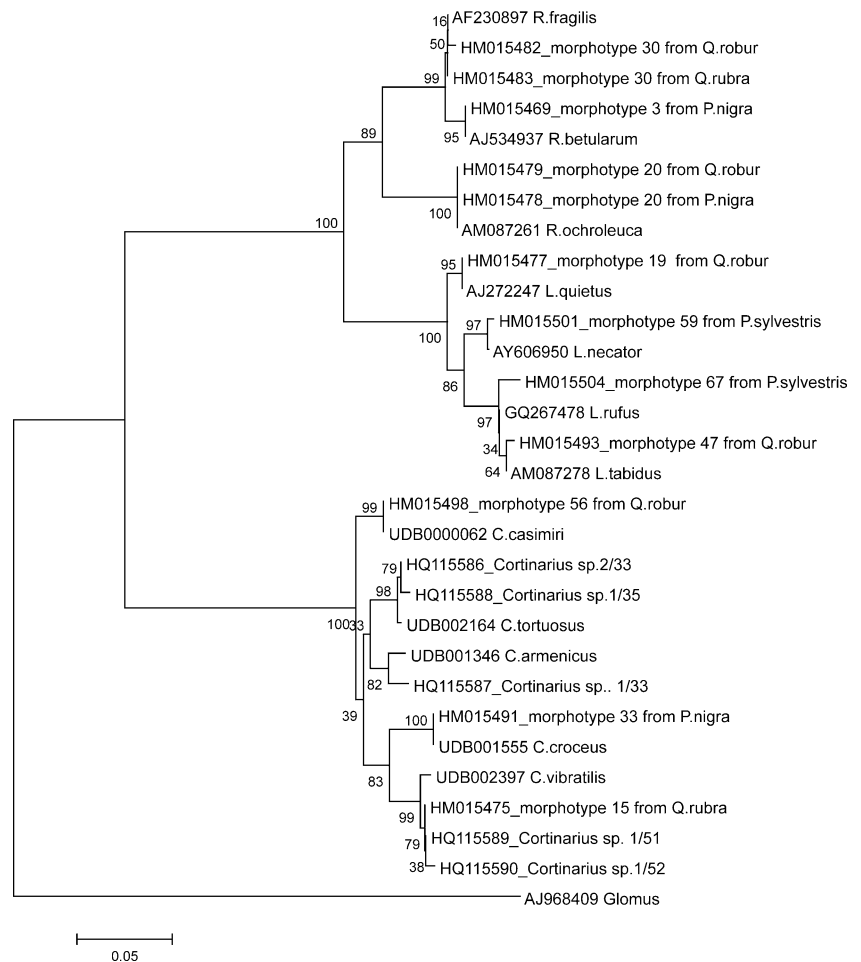
Efficiency of ectomycorrhizal sampling

Species area curves (Mao-Tau) for the four tree species studied revealed an asymptotic pattern in total numbers of EMF species (data not shown). The total number estimator Chao-2 for *Q. robur* was 16.5, Jackknife-1 was 19.99, and Jackknife-2 was 18.98. Hence, the observed number of EMF species belowground ranged from around 80% to 97% of the estimated total number. The total number estimator Chao-2 for *Q. rubra* was 11.00, Jackknife-1 was 11.99, and Jackknife-2 was 12.00. The observed EMF species total number comprised between 83% and 91% of the estimated richness. For *P. sylvestris*, Chao-2 was 15.98, Jackknife-1 was 15.98, and Jackknife-2 was 18.94. Thus, the observed EMF species total number comprised from about 69% to 81% of the estimated richness. *P. nigra* richness estimators were Chao-2 10.99, Jackknife-1 11.99, and Jackknife-2 13.96. The observed EMF species richness varied between 72% and 91% of estimated richness. Thus, we assumed that the ectomycorrhizal sampling efforts were sufficient for further analyses and discussion.

Sporocarps identification

Undesignated sporocarps of *Cortinarius* spp. were subjected to molecular identification the same way as ectomycorrhizas. The ITS of specimens representing five collections were sequenced (HQ155586–HQ115590), compared to NCBI and UNITE databases, and using

Fig. 1 Phylogenetic analysis of *Cortinariaceae* and *Russulaceae* constructed using MEGA4.1 applying Kimura's two-parameter model. GenBank accession numbers from ectomycorrhizas or sporocarps studied and their best *blastn* matches are shown and all bootstrap values are indicated



blastn search and phylogenetic analysis (Fig. 1), identified as four different species (“Appendix 1”). Molecular identification of the sporocarps of *Cortinarius* sp. 1/41 and *Cortinarius* sp. 1/49 (Fig. 2) was not possible as they were collected in a very poor condition. Remaining sporocarps were identified using standard procedures (see “Materials and methods”), and the data are presented in Fig. 2.

Comparison between sporocarp and ectomycorrhizal surveys

Combining DNA barcoding and morphotyping, 69 taxa of EMF species were recovered from root tips and found as sporocarps on the tree plots of the four host species studied (Fig. 2). Out of 69 EMF species, 38 EMF species were found only as sporocarps and 18 EMF only as ectomycorrhizas, whereas 13 were found both belowground and aboveground (Fig. 2). *Basidiomycota* were the most abundant EMF taxa both belowground and aboveground (Fig. 2). Among EMF species, we identified 25

species of *Basidiomycota*, five of *Ascomycota*, and one unidentified EMF (Fig. 2) on the four tree species. Aboveground, we found 49 species of *Basidiomycota* and two of *Ascomycota*. The most common fungal families, both belowground and aboveground, were *Cortinariaceae* (13 species) and *Russulaceae* (12 species; “Appendix 1”). The number of unique EMF species belowground was eight for *Q. robur* and three for *Q. rubra*, whereas for sporocarps the numbers were 19 and one, respectively (Fig. 2). *P. sylvestris* had six EMF species belowground and two aboveground, whereas *P. nigra* had four EMF species found as ectomycorrhizas and five found as sporocarps (Fig. 2).

The most frequently occurring EMF species (hereafter called multi-host) that occurred belowground on four tree species studied were *C. geophilum* Fr., *P. involutus* (Batsch) Fr., and *Thelephoraceae* sp. 1 (Table 2; Fig. 2). The EMF species with the highest frequency aboveground were *Amanita gemmata* (Fr.) Bertill. and *P. involutus*; both species were recorded under all tree species studied

Table 2 Relative abundance (\pm SE in parentheses) of below-ground EMF species on native (*Q. robur* and *P. sylvestris*) and non-native tree species (*Q. rubra* and *P. nigra*) growing in a common garden experiment in Poland; $n=162$

Morphotype identity	Relative abundance (%) of EMF species on host tree			
	<i>Quercus robur</i>	<i>Quercus rubra</i>	<i>Pinus sylvestris</i>	<i>Pinus nigra</i>
<i>Cenococcum geophilum</i>	61.97 (\pm 4.5)	97 (\pm 0.6)	36.3 (\pm 5.3)	44.5 (\pm 9.7)
<i>Paxillus involutus</i>	1 (\pm 0.9)	1 (\pm 0.03)	19.5 (\pm 1.6)	9.3 (\pm 4.4)
<i>Thelephoraceae</i> sp. 1	0.21 (\pm 0.09)	0.6 (\pm 0.1)	13.2 (\pm 1.1)	19.7 (\pm 3.1)
<i>Russula ochroleuca</i>	0.37 (\pm 0.37)			9.3 (\pm 6.4)
<i>Scleroderma citrinum</i>	0.25 (\pm 0.15)	0.07 (\pm 0.06)		
<i>Lactarius rufus</i>			0.7 (\pm 0.6)	
<i>Tomentellopsis submollis</i>	0.17 (\pm 0.08)	0.2 (\pm 0.05)		
<i>Lactarius tabidus</i>	0.07 (\pm 0.06)			
<i>Tylospora asterophora</i>			5.2 (\pm 3.1)	1.9 (\pm 1.9)
<i>Boletus edulis</i>	0.9 (\pm 0.8)	0.03 (\pm 0.03)		
<i>Lactarius quietus</i>	33 (\pm 4.1)			
<i>Russula fragilis</i>	0.37 (\pm 0.33)	0.37 (\pm 0.3)		
<i>Tomentella sublilacina</i>			1.1 (\pm 1.1)	
<i>Tuber puberulum</i>		0.4 (\pm 0.35)		
<i>Xerocomus badius</i>			7.3 (\pm 3.8)	4.8 (\pm 2.7)
<i>Atheliaceae</i> sp. 1			3.5 (\pm 1.7)	3.8 (\pm 2)
<i>Elaphomyces muricatus</i>	0.04 (\pm 0.03)			
<i>Lactarius necator</i>			0.3 (\pm 0.3)	
<i>Thelephora terrestris</i>			2.1 (\pm 0.9)	3.4 (\pm 0.8)
<i>Tomentella botryoides</i>		0.03 (\pm 0.03)		
<i>Tomentellopsis</i> sp. 1			9.6 (\pm 1.8)	
<i>Clavulina</i> sp. 1	0.36 (\pm 0.17)			
<i>Cortinarius</i> cf. <i>vibratilis</i>		0.13 (\pm 0.03)		
<i>Cortinarius casimiri</i>	0.21 (\pm 0.14)			
<i>Cortinarius croceus</i>				1.8 (\pm 1.7)
<i>Humaria</i> cf. <i>hemispaerica</i>	1.1 (\pm 0.9)			
<i>Pezizales</i> sp. 1	0.15 (\pm 0.14)			
<i>Russula betularum</i>				1.6 (\pm 1.5)
<i>Scleroderma</i> sp. 1	0.21 (\pm 0.1)			
<i>Pseudotomentella griseopergamacea</i>			1.1 (\pm 1.1)	
Unidentified EMF			0.17 (\pm 0.1)	

(Fig. 2). The remaining EMF species were less frequent or rare, either as ectomycorrhizas or sporocarps. The most abundant EMF species was *C. geophilum* for all tree species studied (36.3–97%), followed by *Lactarius quietus* on *Q. robur*, (33%), *Thelephoraceae* sp. 1 on *P. nigra* (19.7%) and *P. involutus* on *P. sylvestris* (19.5%; Table 2). Less abundant were *Tomentellopsis* sp. 1 on *P. sylvestris* (9.6%), and *P. involutus* and *Russula ochroleuca* on *P. nigra* (9.3% for each; Table 2). The other EMF species had low or very low (<1%) relative abundance (Table 2).

Q. robur was displayed the highest EMF species richness, both belowground and aboveground (Table 3) among the four tree species. *P. nigra* and *Q. rubra* hosted

the lowest number of EMF species on their roots (Table 3). The lowest richness of EMF species as sporocarps was found under *P. sylvestris* (Table 3). *Q. robur* had ca. 100% higher total richness than non-native *Q. rubra* (Fig. 2; Table 3). *P. sylvestris* had ca. 30% more of the belowground and ca. 50% less of the aboveground EMF species richness as that of non-native *P. nigra* (Table 3).

EMF species community similarity and host preferences

Based on belowground EMF species occurrence and their relative abundances, we also calculated EMF

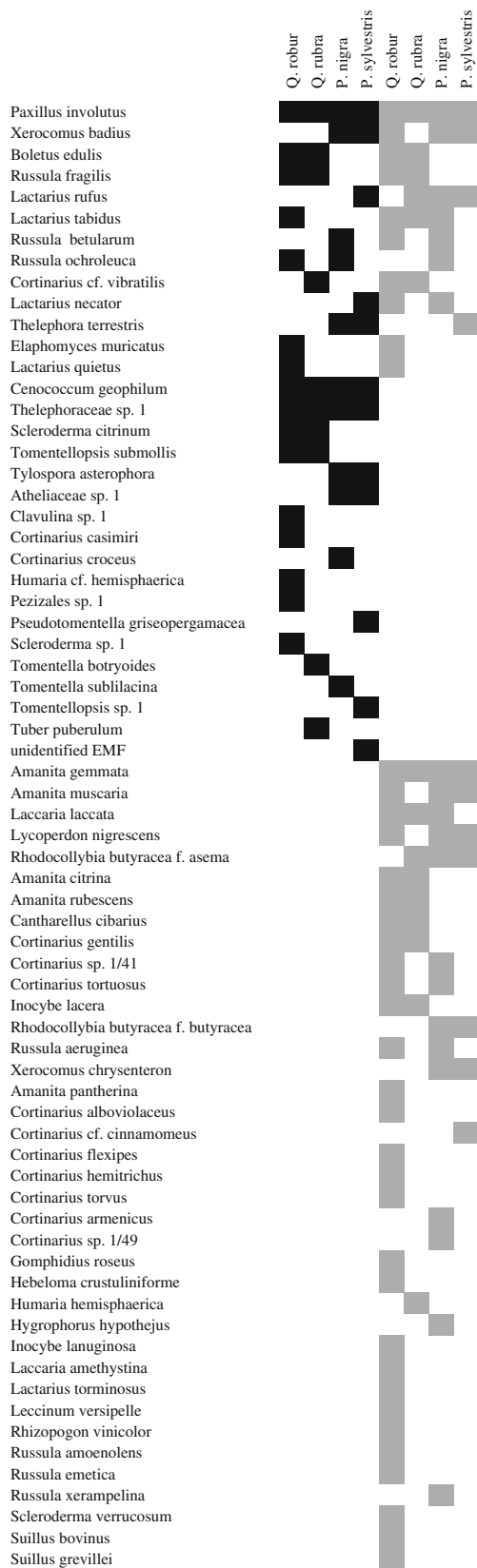


Fig. 2 Visualization of ectomycorrhizal species occurrence on tree species studied. Each row of the matrix represents an ectomycorrhizal species while each column represents a tree species. Black squares were used for belowground EMF species and gray squares for the aboveground sporocarps

species dominance, Shannon and Simpson diversity indices, and evenness (Table 3). *P. sylvestris* and *P. nigra* displayed the highest EMF species diversity (both Shannon and Simpson), whereas *Q. rubra* displayed the lowest diversity (Table 3). The dominance index was the highest for *Q. rubra* and the lowest for *P. sylvestris* (Table 3). The evenness index was the highest for *P. nigra* followed by *P. sylvestris*, *Q. robur*, and the lowest on *Q. rubra* (Table 3).

Jaccard similarity indices showed that *Pinus* species shared the highest number of EMF species among all four species (61%), followed by the two *Quercus* species (46%). The lowest number of EMF species was shared between *P. sylvestris* and *Q. robur* (21%). Additionally, detrended correspondence analysis showed a higher resemblance between *P. sylvestris* and *P. nigra* EMF communities than between *Q. robur* and *Q. rubra* EMF communities (data not shown). The least divergent EMF species was *C. geophilum* for all tree species. The most common EMF species for both oaks were *Tomentellopsis submollis* and *R. fragilis*, whereas for the pines they were *Thelephora terrestris* and *Xerocomus badius*. Several EMF species exclusively associated with particular tree species: *Lactarius tabidus*, *L. quietus*, *E. muricatus*, *Clavulina* sp. 1, *Cortinarius casimiri*, *H. cf. hemisphaerica*, *Pezizales* sp. 1, and *Scleroderma* sp. 1 to *Q. robur*; *Tuber puberulum*, *Tomentella botryoides*, and *C. cf. vibratilis* to *Q. rubra*; *Cortinarius croceus* and *R. betularum* to *P. nigra*; and *L. rufus*, *Tomentella sublilacina*, *Lactarius necator*, *Tomentellopsis* sp. 1, and *Pseudotomentella griseopergamacea* to *P. sylvestris*.

Discussion

Plant host identity is a key factor influencing ectomycorrhizal fungal community structure with greater phylogenetic distance of the hosts leading to greater dissimilarity of fungal communities between host species (Ishida et al. 2007; Tedersoo et al. 2008). Additionally, native host species that have had a relatively extended period of co-evolution with the fungal mycorrhizal community may have more diverse fungal communities than those of introduced host species. To test this assumption, we

Table 3 EMF species richness (above-, belowground, and total) and EMF species diversity (only for belowground) indices including Shannon and Simpson, evenness and dominance for four tree species studied

Host tree	Richness (S)			Diversity			
	Belowground	Aboveground	Total	Dominance (<i>D</i>)	Shannon (<i>H</i>)	Simpson (1- <i>D</i>)	Evenness ($e^{H/S}$)
<i>P. sylvestris</i>	13	12	21	0.22	1.92	0.78	0.52
<i>P. nigra</i>	10	22	28	0.29	1.77	0.71	0.58
<i>Q. robur</i>	16	37	49	0.53	0.94	0.47	0.16
<i>Q. rubra</i>	10	14	21	0.98	0.17	0.02	0.12

compared EMF species communities between a native and exotic oak (*Q. robur* and *Q. rubra*) and a native and exotic pine (*P. sylvestris* and *P. nigra*) growing in a common garden experimental forest stand in Poland.

We found that native and non-native tree species within the genera *Pinus* and *Quercus* shared many EMF species and exhibited no systematic difference in diversity across genera. In *Quercus*, there was clearly higher richness and diversity in the native *Q. robur* than in the exotic, *Q. rubra*, while in *Pinus*, the total richness was higher for non-native *P. nigra* whereas the belowground richness and diversity was lower for that tree species (Table 3). In contrast to our study, Gebhardt et al. (2007) found a higher total number of distinctive EMF species (32 vs. 22 in this study) in a 46-year-old *Q. rubra* stand in Germany, indicating that the number of EMF species in *Q. rubra* is not uniformly low outside its natural range. Non-native tree species were able to form ectomycorrhizal assemblages with fungi that naturally occurred on the study site. Similar EMF species communities within the genera *Quercus* and *Pinus* probably results from sharing the same habitat conditions, stand history and successional status, as well as the same type of adjacent forest (Cline et al. 2005; Ishida et al. 2007). The similarity also results from a high proportion of EMF species with a broad host range (found in both angiosperms and gymnosperms). All this, in turn, may explain successful adaptation of the trees to local conditions and their competitive growth and survival (Reich et al. 2005).

Ectomycorrhizal fungal species like *C. geophilum* and *P. involutus* were present on all hosts, while fungi with stronger host preferences (unique EMF species) were present on one or two tree species (Table 2; Fig. 2). A similar pattern was found by Newton (1991) who found that *B. pendula* and *Q. robur* shared only three EMF species (*C. geophilum*, *P. involutus*, and *Scleroderma citrinum*) out of 41 described in that study. Horton and Bruns (1998) also observed that Douglas fir (*P. menziesii*) and bishop pine

(*Pinus muricata*) growing in a mixed stand shared some EMF species, while some of them were also unique to each tree species. The fact that different tree species growing in the same habitat conditions share EMF species may indicate that these fungi exhibit a broad ecological profile and do not display narrow host preferences. However, studies on EMF species communities of co-occurring species of *Quercus* and *Pinus sabiniana* showed that the multi-host species might be much less dominant (Smith et al. 2009).

The dominance of *C. geophilum* on the four tree species in this study may be affected by the high competitive abilities and wide ecological amplitude of this fungus (Pigott 1982; Jonsson et al. 1999; Izzo et al. 2005). Moreover, that *C. geophilum* reaches its highest relative abundance on *Q. rubra* (Table 2) rather than the other tree species studied may be of interest. The dominance index was the highest for *Q. rubra*, while the evenness index was the lowest for that tree species indicating that the belowground EMF community was strongly affected by *C. geophilum* (Table 3). Walker et al. (2005, 2008) showed that *C. geophilum* was the most frequent and abundant EMF species on *Q. rubra* seedlings growing in North America. However, the abundance of *C. geophilum* was much lower (<37%) in the studies of Walker et al. (2008) than on *Q. rubra* in our study. Hence, the dominance of *C. geophilum* on *Q. rubra* in a novel habitat (see also Gebhardt et al. 2007) may be of ecological interest. Moreover, it has been also discovered that *C. geophilum* is a complex species (see Douhan et al. 2007). Thus, quantifying root tips typical of this EMF species based on its unique morphology may underestimate the actual number of EMF species.

At least half of the EMF species recorded in this study are known to occur naturally both in Europe and North America. Most of them were recorded in association with oaks in the present study (e.g., *C. geophilum*, *Boletus edulis*, *P. involutus*, *R. fragilis*, *S. citrinum*; “Appendix 1”). Some of the taxa found in this study also formed

ectomycorrhizas with *Q. rubra* within its natural distribution range, e.g., *A. gemmata*, *C. geophilum*, *Laccaria laccata* (Walker et al. 2005). Holarctic distribution of these symbionts may enhance successful introduction of *Q. rubra* on the European continent. However, the finding that *T. puberulum*, a species of European distribution (Jeandroz et al. 2008), forms ectomycorrhizas with *Q. rubra* shows that the tree is able to accept new symbionts as well.

It is well documented that the lack of EMF species hinders both introductions and invasions of Pinaceae in the southern hemisphere (see Nuñez et al.). In our study, we found similar EMF community composition of non-native *P. nigra* and native *P. sylvestris* (see “Results”) as well as a considerable number of EMF species found uniquely in association with *P. nigra*. This could result from the mainly European distribution of both tree species, partial overlap of their ranges, and pan-European distribution of many of their ectomycorrhizal symbionts. In the mountainous region in Spain, where both pines co-occur naturally, sporocarps of at least 115 EMF species were found in stands of *P. nigra* (Martínez de Aragón et al. 2007), and two thirds of them were also common for *P. sylvestris*. Eight of these species were also present in our experimental plots, although none of them was found in association with *P. nigra*. Most of them are known to occur in East Europe; thus, potentially *P. nigra* occurrence here should not be limited by the lack of ectomycorrhizal fungal inoculum. However, as shown by Bonfante et al. (1998), strains originating from a specific site show better symbiotic capabilities in association with host plants growing in the same type of environment. The robust occurrence of native EMF symbionts on *Q. rubra* and *P. nigra* growing in our experiment may be due to the fact that initial introduction was conducted via acorns and seeds, since most contemporary stands were established using acorns and seeds from old domestic plantations. Tedersoo et al. (2007) found that introduced *P. caribea* in the Seychelles maintained EMF species co-introduced with seedlings. Considerable similarity in EMF species community structure between *Q. robur* and *Q. rubra* and between *P. sylvestris* and *P. nigra* (Jaccard similarity index and DCA—see “Results”) confirms the observations that tree species of the same genus or family tend to form similar EMF assemblages (Newton and Haigh 1998; Ishida et al. 2007). The similarity of EMF species communities within the same tree genus may be an effect of host plant preferences toward to genus-specific or even species-specific ectomycorrhizal fungi (Allen 1991; Tedersoo et al. 2008; Smith et al. 2009). Kennedy et al. (2003) showed that dominant canopy (*Pseudotsuga menziesii*) and understorey (*Lithocarpus densiflora*) shared around 30% of EMF species suggesting a high potential for

common mycorrhizal networks to form between trees. Ishida et al. (2007) found the most similar EMF structures for closely related tree species or due to their successional status. However, the EMF species communities of closely related tree species may differ from each other significantly as well, as shown by Morris et al. (2008) for *Quercus* spp. and Korkama et al. (2006) for clones of *Picea abies*.

Data presented in this study indicate that lack of sufficient geographic barriers for numerous EMF fungi, which exhibit broad distribution and low host specificity, a long history of introduction and afforestation of studied tree species, as well as trade of potted seedlings, allow for effective growth of exotic tree species outside their natural ranges. Cullings et al. (2000) found no specific EMF species colonizing roots of invasive *Picea engelmannii* trees growing together with native *Pinus contorta* in the Yellowstone National Park. Parladé et al. (1996) found that many EMF species native to Spain formed ectomycorrhizas with introduced *Pseudotsuga menziesii*. Tedersoo et al. (2007) also observed that native EMF species colonized introduced eucalypts in the Seychelles. However, Dickie et al. (2010a) found that invasive *P. contorta* had no mutualistic associations with native EMF in New Zealand. On the other hand, exotic EMF species present on roots of introduced trees may also invade novel territories if they find new hosts (Díez 2005).

In summary, the results presented in this study show that tree species, both non-native and native belonging to the same genus, share EMF species and form similar EMF species communities. This supports naturalization of non-native trees by means of mutualistic associations with cosmopolitan and novel fungi. We found no systematic difference in fungal richness of native hosts compared to non-native hosts across genera. Native *Pinus sylvestris* and non-native *Pinus nigra* have more similar EMF communities than native *Quercus robur* and non-native *Quercus rubra*. Our research also demonstrates that dominant EMF species is the same for different host genera or for different host species within the same genus.

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Appendix 1

Table 4 Identification of ectomycorrhizal morphotypes associated with *Q. robur*, *Q. rubra*, *P. sylvestris*, and *P. nigra*

Ectomycorrhizas					
Morphotype no.	Specimen NCBI acc. no.	Host tree	Best match acc. no. (NCBI and/or UNITE)	Identity (%)	Morphotype identity
2	HM015465	<i>P. nigra</i>	Uncultured <i>Thelephoraceae</i> (AF430259)	564/573 (98)	<i>Thelephoraceae</i> sp. 1
2	HM015466	<i>P. sylvestris</i>	Uncultured <i>Thelephoraceae</i> (AF430259)	602/636 (95)	<i>Thelephoraceae</i> sp. 1
2	HM015467	<i>Q. robur</i>	Uncultured <i>Thelephoraceae</i> (AF430259)	554/580 (96)	<i>Thelephoraceae</i> sp. 1
2	HM015468	<i>Q. rubra</i>	Uncultured <i>Thelephoraceae</i> (AF430259)	540/565 (96)	<i>Thelephoraceae</i> sp. 1
3	HM015469	<i>P. nigra</i>	<i>Russula betularum</i> (AJ534937)	503/503 (100)	<i>Russula betularum</i>
8	HM015470	<i>P. nigra</i>	<i>Paxillus involutus</i> (EU819126)	540/565 (96)	<i>Paxillus involutus</i>
8	HM015471	<i>Q. robur</i>	<i>Paxillus involutus</i> (EU8191416)	775/793 (98)	<i>Paxillus involutus</i>
53	HM015494	<i>P. sylvestris</i>	<i>Paxillus involutus</i> (EU8191416)	621/629 (99)	<i>Paxillus involutus</i>
53	HM015495	<i>Q. rubra</i>	<i>Paxillus involutus</i> (FR750011)	591/606 (98)	<i>Paxillus involutus</i>
11	HM015472	<i>P. nigra</i>	<i>Tylospora asterophora</i> (AF052557)	445/463 (96)	<i>Tylospora asterophora</i>
12	HM015473	<i>P. sylvestris</i>	<i>Pseudotomentella griseopergamacea</i> (UDB001617)	593/594 (99)	<i>Pseudotomentella griseopergamacea</i>
13	HM015474	<i>Q. robur</i>	<i>Scleroderma areolatum</i> (FM213352) and <i>S. verrucosum</i> (UDB000044)	661/671 (99) and 609/613 (99)	<i>Scleroderma</i> sp. 1
15	HM015475	<i>Q. rubra</i>	<i>Cortinarius vibratilis</i> (UDB002397)	509/544 (93)	<i>Cortinarius</i> cf. <i>vibratilis</i>
16	HM015476	<i>Q. robur</i>	<i>Humaria hemisphaerica</i> (UDB000988)	373/401 (93)	<i>Humaria</i> cf. <i>hemisphaerica</i>
19	HM015477	<i>Q. robur</i>	<i>Lactarius quietus</i> (AJ272247)	650/664 (98)	<i>Lactarius quietus</i>
20	HM015478	<i>P. nigra</i>	<i>Russula ochroleuca</i> (AM087261)	560/587 (95)	<i>Russula ochroleuca</i>
20	HM015479	<i>Q. robur</i>	<i>Russula ochroleuca</i> (AM087261)	606/609 (99)	<i>Russula ochroleuca</i>
28	HM015480	<i>Q. rubra</i>	<i>Tuber puberulum</i> (AJ969626)	481/482 (99)	<i>Tuber puberulum</i>
29	HM015481	<i>Q. robur</i>	<i>Tomentellopsis submollis</i> (AJ410773)	562/565 (99)	<i>Tomentellopsis submollis</i>
30	HM015482	<i>Q. robur</i>	<i>Russula fragilis</i> (AF230897)	548/564 (97)	<i>Russula fragilis</i>
30	HM015483	<i>Q. rubra</i>	<i>Russula fragilis</i> (AF230897)	564/572 (99)	<i>Russula fragilis</i>
31	HM015484	<i>P. nigra</i>	Uncultured <i>Atheliaceae</i> (EU557324)	409/432 (95)	<i>Atheliaceae</i> sp. 1
31	HM015485	<i>P. sylvestris</i>	Uncultured <i>Atheliaceae</i> (EU557324)	576/578 (99)	<i>Atheliaceae</i> sp. 1
31a	HM015486	<i>P. sylvestris</i>	<i>Tomentella subhilarina</i> (UDB000970)	575/578 (99)	<i>Tomentella subhilarina</i>
32	HM015487	<i>Q. robur</i>	<i>Boletus edulis</i> (DQ131622)	557/558 (99)	<i>Boletus edulis</i>
32	HM015488	<i>Q. rubra</i>	<i>Boletus edulis</i> (HM57930)	548/596 (92)	<i>Boletus edulis</i>
32	HM015489	<i>P. nigra</i>	<i>Xerocomus badius</i> (AJ889926)	521/528 (99)	<i>Xerocomus badius</i>
32	HM015490	<i>P. sylvestris</i>	<i>Xerocomus badius</i> (AJ889926)	521/526 (99)	<i>Xerocomus badius</i>
33	HM015491	<i>P. nigra</i>	<i>Dermocybe crocea</i> (U56038) and <i>Cortinarius croceus</i> (UDB001555)	532/552 (96) and 520/524 (99)	<i>Cortinarius croceus</i>
45	HM015492	<i>Q. rubra</i>	<i>Tomentella botryoides</i> (UDB000256)	568/568 (100)	<i>Tomentella botryoides</i>
47	HM015493	<i>Q. rubra</i>	<i>Lactarius tabidus</i> (AM087278)	581/584 (99)	<i>Lactarius tabidus</i>
54	HM015496	<i>Q. robur</i>	<i>Scleroderma citrinum</i> (GQ166907)	503/513 (98)	<i>Scleroderma citrinum</i>
55	HM015497	<i>Q. robur</i>	<i>Clavulina</i> sp. (AJ534709)	530/550 (96)	<i>Clavulina</i> sp. 1
56	HM015498	<i>Q. robur</i>	<i>Cortinarius casimiri</i> (UDB000062)	484/487 (99)	<i>Cortinarius casimiri</i>
57	HM015499	<i>Q. robur</i>	<i>Elaphomyces muricatus</i> JF834198	508/520 (98)	<i>Elaphomyces muricatus</i>
58	HM015500	<i>Q. robur</i>	Uncultured <i>Pezizales</i> (AJ969619)	530/538 (99)	<i>Pezizales</i> sp. 1
59	HM015501	<i>P. sylvestris</i>	<i>Lactarius necator</i> (AY606950)	455/461 (99)	<i>Lactarius necator</i>
60	HM015502	<i>P. nigra</i>	<i>Thelephora terrestris</i> (UDB000971)	558/583 (96)	<i>Thelephora terrestris</i>
60	HM015503	<i>P. sylvestris</i>	<i>Thelephora terrestris</i> (UDB000971)	575/590 (97)	<i>Thelephora terrestris</i>
67	HM015504	<i>P. sylvestris</i>	<i>Lactarius rufus</i> (GQ267478)	509/618 (95)	<i>Lactarius rufus</i>
24	HM015505	<i>P. sylvestris</i>	<i>Tomentellopsis echinospora</i> (UDB000183)	349/355 (98)	<i>Tomentellopsis</i> sp. 1
Sporocarps					
Specimen name	Specimen acc. no.	Host tree	Best match acc. no. (NCBI and/or UNITE)	Identity (%)	Sporocarp identity
<i>Cortinarius</i> 2/33	HQ115586	<i>P. nigra</i>	<i>Cortinarius tortuosus</i> (UDB002164)	578/581 (98)	<i>Cortinarius tortuosus</i>
<i>Cortinarius</i> 1/33	HQ115587	<i>P. nigra</i>	<i>Cortinarius armenicus</i> (UDB001346)	558/572(97)	<i>Cortinarius armenicus</i>
<i>Cortinarius</i> 1/35	HQ115588	<i>Q. robur</i>	<i>Cortinarius tortuosus</i> (UDB002164)	581/591 (98)	<i>Cortinarius tortuosus</i>
<i>Cortinarius</i> 1/51	HQ115589	<i>Q. rubra</i>	<i>Cortinarius vibratilis</i> (UDB002397)	530/561 (94)	<i>Cortinarius</i> cf. <i>vibratilis</i>
<i>Cortinarius</i> 1/52	HQ115590	<i>Q. robur</i>	<i>Cortinarius vibratilis</i> (UDB002397)	578/626 (92)	<i>Cortinarius</i> cf. <i>vibratilis</i>

Morphotype and sporocarps identities are based on CodonCode Aligner of the sequences and their best matches at the 97% threshold. Morphotypes and specimen marked as cf. species were aligned at the <97% threshold

Appendix 2

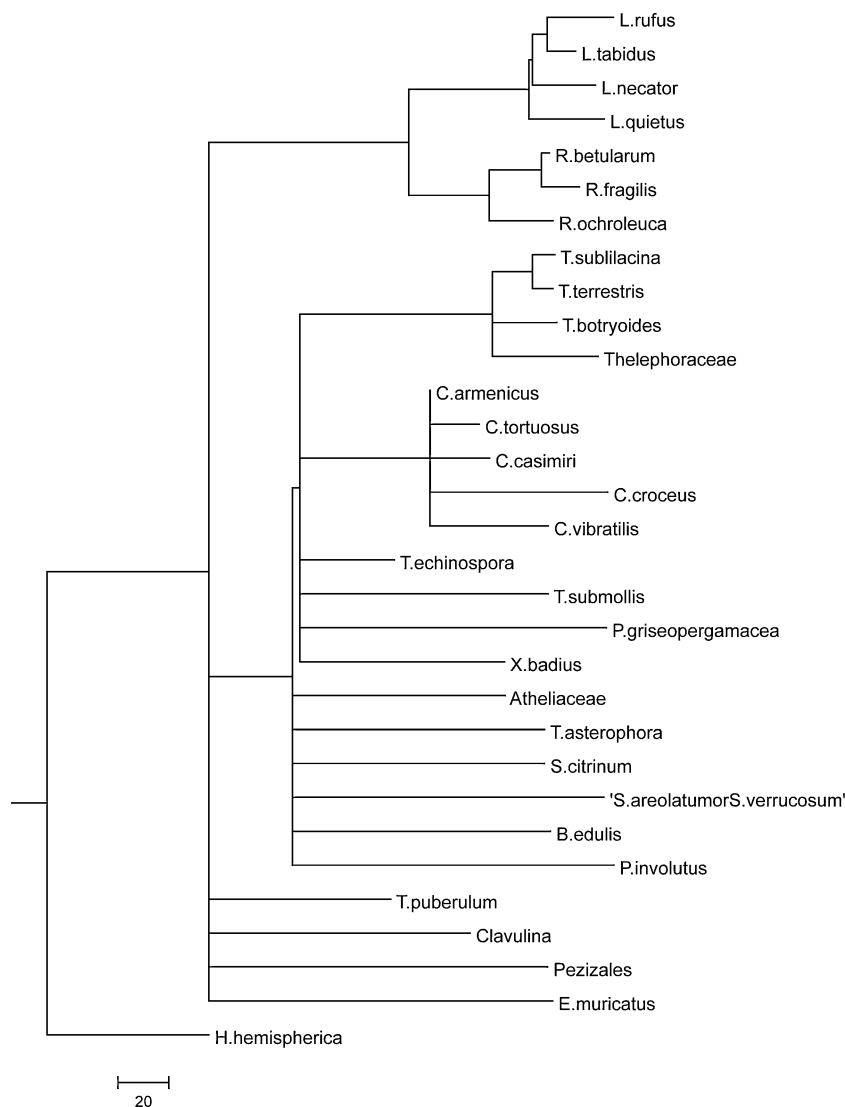


Fig. 3 Dendrogram of contigs derived from CodonCode Aligner using ITS sequences obtained from EMF morphotypes, selected sporocarps, and their best matches from GenBank, UNITE matches, and sporocarps collected in the study plots

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