Ectomycorrhizal diversity of Chinese pine (*Pinus tabulaeformis*) in North China

Dissertation

zur Erlangung des Doktorsgrades der Naturwissenschaften

(Dr. rer. nat.)

Der Fakultät für Biologie der Ludwig-Maximilians-Universität München

Vorgelegt von Jie Wei

München, März 2010

Datum der Mündlichen Prüfung: 28.05.2010
Erstguachter: Prof. Dr. Reinhard Agerer
Zweitgutachter: Dr. Peter Döbbeler

Acknowledgments

I would like to express my gratitude to my supervisor Prof. Dr. Reinhard Agerer, who gave me the chance to begin my career in systematic mycology, guided me gradually on the way of science, encouraged me to be open and confident to science and life, whose expertise in fungi, respects for culture, and patience, added considerably to my experience. I appreciate his assistance in writing reports (i.e., grant proposals, scholarship applications and this dissertation). I doubt that I will ever be able to convey my appreciation fully, but I owe him my eternal gratitude.

A very special thanks goes out to Rita Verma for her continuous help from the beginning when I first came to our laboratory, till now as the study has been finished. I learned much extra knowledge (e.g. German and European cultures, history, and language) from her and her friend, and experienced many wonderful moments besides research with her and her family, e.g. Christmas, October festival, Ostern, I would benefit from these invaluable experiences which should be lodged in the beautiful memory in my life.

I am particularly grateful to Dr. Peter Döbbeler, who was so kind to write reference letters for me each time without hesitation.

I feel so lucky that I work in a group with friendly and supportive atmosphere, I must thereby acknowledge all the contributors, Dr. Derek Peršoh, Dr. Philomena Bodensteiner, Dr. Nourou Soulemane Yorou, Sebastian Gardt, Dr. Stefan Raidl for their suggestions for my research, Claudia Bubenzer-Hange, Anina Neumann for their technical assistance during this study. Without these exchanges of knowledge and skills, this study would not have been successful.

I would also like to convey thanks to Prof. Yan Wei for providing the financial means and laboratory facilities during the field surveys, and to Dr. Fan Yongjun for his assistance during sampling trips.

Apart from the colleagues, I would also like to thank my parents for the support they provided me through my entire life, my sister and her family to take care of my parents through the duration of my research in Germany, and a German friend, he showed me many beautiful

places in Munich and other cities. This big contrast let me make up my mind to do more work to improve my home city’s environment.

In conclusion, I recognize that this research would not have been possible without the financial assistance of DAAD.

Summary

- This present study characterized a much higher ECM diversity of Chinese Pine compared with the previous similar investigations, such as fungal speices of *Inocybe* (paper **I**), *Tomentella* (papers **II**, **V**), Sebacinales (paper **VII**), Pyronemataceae (paper **IV**), and *Tuber* (paper **VII**).
- Four fungal species belonging to genera *Humaria*, *Geopora* and *Trichophae* of Pyronemataceae that form ECM with Chinese Pine have been firstly reported in this study (paper **IV**). This study also provided detailed descriptions of ECM of *Geopora* and *Trichophaea*.
- Two species of Sebacinales (paper **VI**) represent also the first reports about fungi in this group forming ECM with Chinese Pine, additional anatomical diversity of sebacinoid ECM has been presented compared to other related studies.
- "*Pinirhiza tomentelloides*" (paper **II**) is the first detailed description of *Tomentella* ECM on Chinese Pine, three other *Tomentella* ECM on Chinese Pine (paper **V**) indicate that *Tomentella* species could be very common in north China. Furthermore, a key to thelephoroid ECM in paper **V** therefore demonstrates the structural diversity of ECM in Thelephoraceae and facilitates the identification of thelephoroid ECM.
- Most *Tuber* species have been reported from southwest China, few are known in north China. *Tuber* ECM occurring on Chinese Pine (paper **VII**) have provided additional information about the *Tuber* diversity and ecological distribution in China. In addition, paper **VII** contributes also to the knowledge about the phylogenetic value of ECM mantle-type in taxonomy. Among 16 valid *Tuber* species in China, only ECM of two species have been well studied, further detailed description of *Tuber* ECM could facilitate the identification of *Tuber* species in China since the already described *Tuber* ECM in China could be easily distinguished from each other (a key given in paper **VII**).
- The combination of surveys of detailed morpho-anatomical features and molecular approaches is a powerful tool in ECM fungal community studies (papers **II**, **IV**, **V**, **VI**, **VII**).

1. Introduction

1.1 Ectomycorrhizal association

Ectomycorrhizal associations (abbreviated as ECM) are mutualistic associations between roughly 7750 fungal species (Rinaldi et al. 2008) and more than 6000 plant species of Gymnosperms or Angiosperms (5600 Angiosperm species and 285 Gymnosperm species in 145 genera and 26 families, Brundrett 2009). ECM are characterised by the presence of a mantle from which emanating hyphae, rhizomorphs or cystidia could develop, and by a Hartig net (Agerer 1995, Fig. 1). The extramatrical mycelium grows either as scattered simple hyphae (emanating hyphae) from the mantle into the soil or it can be united to undifferentiated rhizomorphs with a small reach or to highly organized, root-like organs with vessel-like hyphae for efficient water and nutrient transport from distances of decimetres (Brownlee et al. 1983, Agerer 1999, Agerer 2006). Cystidia, sterile and variously shaped hyphal ends, are possibly at least in some species appropriate for preventing animal attacks (Agerer 2006). Hartig net is caused by hyphal penetration between host cells and branching. It is therefore a labyrinthine network of specialised fungus hyphae, with frequent ramifications (or wall ingrowths) that forms a layer between the walls of adjacent root epidermal or cortex cells. Hartig net is considered to be the major site of nutrient exchange between the fungus and host plant (Smith and Read 1997).

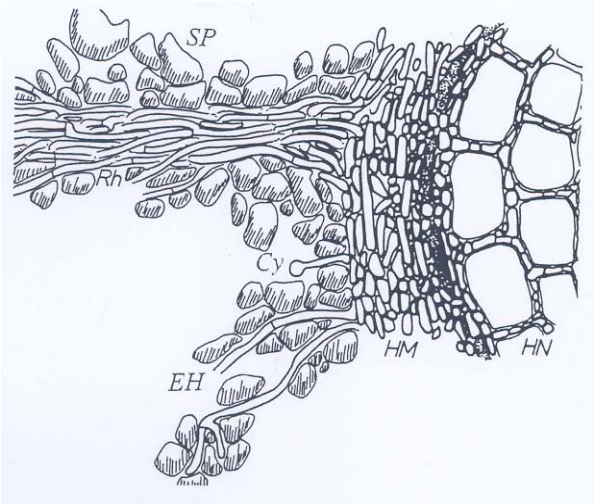


Fig. 1 ECM structure in cross section. *EH* emanating hyphae; *Rh* rhizomorph; *Cy* cystidia; *HM* haphal mantle; *HN* Hartig-net; *SP* soil particle. Modified from Agerer 2009 (with permission).

1.2 Ectomycorrhiza identification - from traditional classic methods to modern molecular approaches

1.2.1 Morpho-anatomical identification

Anatomy of ECM has been studied over 120 years since Gibelli (1883) and Frank (1885). But it lasted almost a century until the structure of ECM was recognized as being essential for studies on fungal relationships (Giraud 1979, Godbout and Fortin 1985). The first detailed ECM descriptions at species level using plan views of mantle and rhizomorph organization originate from the late 1960s. Schramm (1966) characterized ECM of *Astraeus hygrometricus* (Pers.) Morgan and *Thelephora terrestris* Ehrh., while Chilvers (1968) those of *Cenococcum geophilum* Fr. and *Octaviania densa* (Rodway) G. Cunn.. Since then, many short and several detailed descriptions of ECM have been published (de Roman et al. 2005, Rinaldi et al. 2008).

A first attempt to summarize ectomycorrhizal features for definition and delimitation of fungal relationships originates from Agerer (1995). In the last decades, some more compilations have been published that focus on ECM anatomy of selected fungal groups and conclude that rhizomorph and mantle features are an aid for delimitation and recognition of fungal relationships at different systematic levels (e.g. Agerer 1999, 2006, Agerer and Iosifidou 2004, Beenken 2004a, b, Eberhardt et al. 2000, Hahn et al. 2000). ECM fungal identification of most of these studies are based on the fruitbodies which are directly connected through rhizomorphs with aimed ECM, or by synthesis of ECM in artificial culture using already identified fungal cultures and seedlings of supposed partner tree species. Four anatomic complexes are informative for recognition of fungal relationships: (a) structure of outer mantle layers as seen in plan view; (b) structure of rhizomorphs; (c) shape of cystidia; and (d) features of emanating hyphae. Some additional non-anatomical useful characters such as chemical reactions and colour of ECM were also addressed (Agerer 2006).

For detailed studies, ECM are firstly sorted into morphotypes according to mainly their colour, the habit of mantle surface, type of ramification, exploration type, presence of mantle hydrophobicity, presence of sclerotia, and shape of unramified ends (Agerer 1987–2008). Then the morphotypes are assigned to anatomotypes in terms of mainly of different combinations of individual characteristics of the four anatomic feature complexes mentioned above (a-d). An unidentified new anatomotype which has been described morpho-

anatomically in detail will be given a binomial name referring to the host genus by that the ECM has most likely been formed (Gronbach and Agerer 1986). General features of already described ECM in different genera have been summarized in appendix 1 according to Agerer (2006), which provides basic information for identification or determination of ECM at different taxonomical levels.

Despite over 100 years of investigation on the subject, the number of species described using morpho-anatomical features is relatively small (around 343 species, de Roman et al. 2005). Identification of ECM fungi using this way faces many challenges: (1) The diversity of fungal relationships contributing to the thousands of ectomycorrhizal species, however, the informative ectomycorrhizal features are limited. For example, fungal groups with limited features for distinction of their fruitbodies (e.g. *Cortinarius*) anatomically can not be expected to offer discriminating differences on their ECM with of course considerably less informative structures. Identification of ECM fungi according to morpho-anatomical features of their ECM has been proven unsuccessfully in some fungal groups (Erős-Honti et al. 2008, Kovács & Jakucs 2006). (2) ECM which are morpho-anatomically well studied are presently restricted only to some fungal groups (see appendix 1). An affiliation of non-identified, but comprehensively described ECM to higher hierarchical levels is sometimes impossible due to the lack of distinctly useful features. (3) Morpho-anatomical data are very patchy under different descriptive systems, such as brief description lacking important features, therefore complete comparisons are difficult; (4) Morphotyping and anatomotyping are financially inexpensive, but they are very time-consuming and need well trained personnel.

1.2.2 Molecular identification

In a hierarchical sequence of less to higher exactness for ECM identification, Restriction Fragment Length Polymorphism (RLFP) takes after morphotyping and anatomotyping the third position (e.g. Agerer et al. 1996, Beenken 2001a, b, c, Fischer et al. 2004, Gardes et al. 1991, Gardes and Bruns 1993, Kennedy et al. 2003, Kraigher et al. 1995, Mleczko 2004, Raidl and Müller 1996). RFLP is applied to compare ECM with fruitbodies that are supposed as being the fungal agents for ECM formation. Identical restriction patterns which are generated by cleavage of DNA with restriction enzymes (e.g. Alu I, EcoR I, Hap II, Hinf I), are followed by size separation of the resulting fragments via gel electrophoresis. However this method provides low taxonomic resolution (Kennedy et al. 2003). Nowadays

the fastest, most convenient and most precise way to identify root associated fungi is without doubt sequencetyping, i.e. selective fungal DNA-sequence analysis (each anatomotype of ECM could obtain at least one responsible sequence-type). The use of DNA sequences provides 50-200 times more characters compared to RFLP-based methods when utilizing the ITS region (Tedersoo 2007). Sequence analysis allowing a large-scale sequence have become dominant due to falling prices and improved sequence quality.

Two suitable DNA regions, nuclear Large Subunit (nLSU) and Internal Transcribed Spacer (ITS) region, have been widely used to compare sequences for primer design (Egger 1995, Gardes and Bruns 1993, Glen et al. 2001, Martin and Rygiewicz 2005, Tedersoo 2007, White et al. 1990), for molecular-phylogenetic analyses for identification of ECM fungi (Erős-Honti et al. 2008, Kovács et al. 2006, Kõljalg et al. 2001, Jakucs et al. 2005, Tedersoo et al. 2006, Urban et al. 2003), for reconstruction of the evolution of fungi (James et al. 2006), for inferring the transformation of fungal lifestyles (Binder et al. 2006, Hosaka et al. 2006, Larsson et al. 2006, Matheny et al. 2006, Moncalvo et al. 2006, Perry et al. 2007, Weiß et al. 2004), and for estimation of relative ages of ECM fungi (Hibbett and Matheny 2009). Because usually (1) both regions are easily amplifiable; (2) nLSU region allows alignment of sequences from all fungal phyla; (3) ITS region provides sufficient resolution to discriminate between sister species; (4) many fungal sequences of ITS region and nLSU regions are available in public databases (Tedersoo 2007).

Public databases which are often cited in ECM studies, such as European Molecular Biology Laboratory (EMBL, http://www.embl.de/index.php), National Centre of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp/), as well as UNITE database (http://unite.ut.ee/, Kõljalg et al. 2005), allow to compare the obtained sequences to publicly available sequences data deposited in these databases. However results of sequence-comparison (blast search) in these databases can be difficult to interpret, because (1) different levels of variation may occur in the same DNA region of one and the same taxon (Nilsson et al. 2006), resulting in similar matches to different taxa; (2) many sequences in these databases have been identified only to higher systematic levels rather than to species; (3) many fungi are known only from environmental collections named as environmental samples in these databases; (4) sequences of fungi from many taxa are not available in these databases; (5) many sequences are misidentified (Nilsson et al. 2006).

Molecular phylogeny has great success in inferring fungal relationship and in revealing many new putatively ECM-forming fungal lineages in Ascomycota and Basidiomycota (e.g. Binder and Hibbett 2006, Hansen et al. 2005, Larsson et al. 2004, Matheny et al. 2006, Perry et al. 2007, Weiß et al. 2004). However ECM status of many of these lineages have to be proven yet. But more importantly, these molecular phylogenetic studies provide little information about the morpho-anatomical, and possibly functionally important features of ECM.

An increasing number of studies has proven that the combination of morpho-anatomical features of ECM and molecular identification as well as molecular phylogeny is essential for taxonomy of ECM fungi at different systematic levels (e.g. Erős-Honti et al. 2008, Kovács et al. 2006, Kõljalg et al. 2001, Jakucs et al. 2005, Tedersoo et al. 2006, Urban et al. 2003).

1.3 Chinese Pine and its ECM diversity

Chinese Pine (*Pinus tabulaeformis* Carr., Pinaceae) is a major and widespread component of coniferous forests in northern China, which extend from northeast China (Liaoning province) to northwest China (Qinghai province) between 102° and 122°E longitude and 32° and 43°N latitude (Wu 1995; Fig. 2).

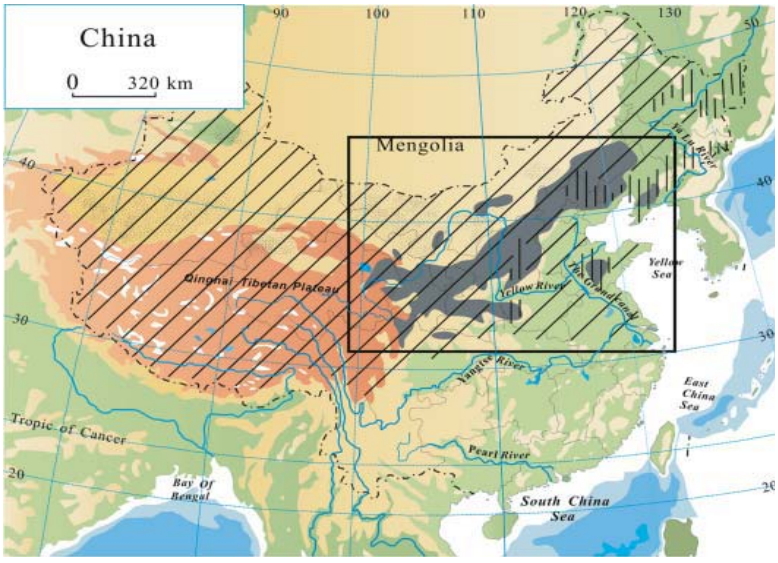


Fig. 2 The present-day distribution of *Pinus tabulaeformis* in China (shaded dark grey), cited from Chen et al. 2008.

Although Chinese Pine is the main tree species that is used for reforestation in north China, the ECM fungal diversity of this species keeps still little known. Huang (1990) has found ECM of *Suillus luteus*, and possibly erroneously *Suillus grevillei* on Chinese pine. Bai (2006a) reported 11 species of fruitbodies belonging to genera of *Boletus*, *Gyroporus*, *Russula*, *Suillus*, *Tricholoma*, *Tylopilus*, and *Xerocomus* that could form ECM with Chinese Pine. Some other investigations (e.g. Bai et al. 2001, Bai et al. 2006b) have similar results. However these studies showed only patchily the ECM fungal diversity to be expected of *Pinus tabulaeformis*, because they are all dependant on the presence of fruitbodies, and only few of them applied anatomical examination of ECM mantle preparations. No study combining morpho-anatomical comparison and molecular methods have been applied on Chinese Pine yet.

1.4 Aim of the thesis

The goal of the present dissertation is to show ECM fungi diversity of Chinese Pine in natural ecosystems combining detailed descriptions of ECM morpho-anatomically and molecular approaches, to provide basic information for addressing ECM fungal communities and for selecting the suitable ECM fungal inocula for potential reforestation of Chinese Pine in north China in the future.

- To obtain basic information of the diversity of ECM fungi with *Pinus tabulaeformis* in natural habitats (**I**, **II**, **III**, **IV**, **V**, **VI**, **VII**);
- To discover new lineages of ECM fungi which have not been reported before as occurring on Chinese Pine and to provide detailed descriptions of ECM, giving general features of ECM to facilitate the ECM identification and determination at different systematic levels (**IV**, **VI**);
- To confirm the ECM status of some fungal lineages which form resupinate or hypogeous fruitbodies. These are very common ECM fungal lineages, but have not yet been reported on Chinese Pine (**II**, **V**, **VII**).

2. Results

2.1 Sample data

Over 200 soil samples and 140 specimens of fruitbodies were collected during the trips during 2007–2008. Over 150 soil samples were treated to obtain ECM systems, 90 of them were then used to classify ECM into anatomotypes, 80 specimens of fruitbodies which are possible agents of forming ECM were microscopically examined, 15 anatomotypes (see appendix 4) were described in detail. DNA was successfully extracted from 32 anatomotypes, thirteen ITS and nine LSU sequences were obtained and deposited at GenBank with accession numbers (see appendix 4).

2.2 ECM fungi with Chinese Pine

Fungi which form ECM with *Pinus tabulaeformis* as revealed in this study belong to the following fungal orders (table 2) according to Hibbett et al. (1997) and Blackwell et al. (2006).

2.3 Potential ECM fungi with Chinese Pine according to anatomotypes (primary results)

Appendix 3 shows the ECM fungi identified morpho-anatomically by ECM features at genus or species level with *Pinus tabulaeformis* in different sampling sites, but these are only preliminary results about the occurrence of ECM anatomotypes. The richness, species composition, ECM communities and other related ecological studies have not been fulfilled yet, because the present study has a different focus, and time of this study was limited. However, the results of this study provide basic information about the ECM diversity of Chinese Pine, and this is the first step before other studies will be envisaged in the future, such as selection of best inocula for synthesis with Chinese Pine seedlings for reforestation, and ecological investigation.

Table 2. ECM of *Pinus tabulaeformis* in different groups of Basidiomycota and Ascomycota

BASIDIOMYCOTA (Agaricomycotina)	Samples in this study
Agaricales	"*Pinirhiza inocyboides*" and "*Pinirhiza tricholomoides*" (papers **I** and **III**); *Cortinarius* sp., and *Chroogomphus* sp. (see appendices 2 and 3) (not studied in detail yet)
Boletales	*Suillus* spp. (see appendices 2 and 3) (not studied in detail yet)
Cantharellales	*Cantharellus subalbidus* (likely, see appendix 2) (not studied in detail yet)
Russulales	*Russula* spp. (see appendices 2 and 3) (not studied in detail yet) *Lactarius deliciosus* (see appendices 2 and 3) (not studied in detail yet)
Sebacinales	"*Pinirhiza multifurcata*", "*Pinirhiza nondextrinoidea*" (paper **VI**) *Tomentella* spp. (papers **II** and **V**) *Thelephora* spp. (see appendices 2 and 3)
Thelephorales	(not studied in detail yet)
ASCOMYCOTA	Samples in this study
Dothideomycetes	*Cenococcom geophilum* (not studied in detail yet)
Pezizales Pyronemataceae	"*Pinirhiza humarioides*", "*Pinirhiza daqingensis*", "*Pinirhiza geoporoides*", "*Pinirhiza trichophaeoides*" (paper **IV**)
Tuberaceae	"*Pinirhiza pubulata*", "*Pinirhiza puborchii*", "*Pinirhiza ongensis*" (paper **VII**)

2.4 Pubilications

This dissertation is based on the following publications in a chronological order, which are referred to in the text by their Roman numeral (**I-VII**):

I Wei J, Agerer R (2008) "*Pinirhiza inocyboides*" + *Pinus tabulaeformis* Carr. Descriptions of Ectomycorrizae 11/12: 89–96

II Wei J, Agerer R (2008) "*Pinirhiza tomentelloides*" + *Pinus tabulaeformis* Carr. Descriptions of Ectomycorrizae 11/12: 97–102

III Wei J, Agerer R (2008) "*Pinirhiza tricholomoides*" + *Pinus tabulaeformis* Carr. Descriptions of Ectomycorrizae 11/12: 103–112

IV Wei J, Peršoh D, Agerer R (2009) Four Ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*) – morpho-anatomical and molecular-phylogenetic analyses. Mycological Progress. In press.

V Wei J, Agerer R (2009) Three Ectomycorrhizae of Thelephoraceae on Chinese Pine (*Pinus tabulaeformis*) and a key to thelephoroid Ectomycorrhizae. Nova Hedwigia. In press.

VI Wei J, Agerer R (2010) Two sebacinoid Ectomycorrhizae on Chinese Pine. Mycorrhiza (submitted)

VII Wei J, Agerer R (2010) Three *Tuber* ectomycorrhizae on Chinese Pine. Mycoscience (submitted)

2.4.1 *"Pinirhiza inocyboides" + Pinus tabulaeformis* Carr.

I

Agerer R, Danielson RM, Egli S, Ingleby K, Luoma D, Treu R (eds)
DESCRIPTIONS OF ECTOMYCORRHIZAE *11/12:* 89–96 (2008)

"Pinirhiza inocyboides"
+ *Pinus tabulaeformis* Carr.

Jie Wei, Reinhard Agerer, Department Biology I and GeoBioCenter^{LMU}, Department Biology I and GeoBio-Center^{LMU}, Organismic Biology: Mycology, University of München, Menzinger Str. 67, D-80638 München, Germany.

Short description

The ectomycorrhizae are yellowish brown and semitransparent with plectenchymatous outer mantle layers composed of squrrosely branched and partially inflated hyphae with a gelatinous matrix; mantle hyphae lack clamps. Emanating hyphae with frequent clamps; these clamps are typically large in lateral view and with a hole; anastomoses of emanating hyphae variable, open or septate with clamps, with a rather short bridge or with a relatively long bridge; cell walls of emanating hyphae thin and colourless.

Morphological characters (Fig. 3a): *Mycorrhizal systems* dichotomous, with 1-4(5) orders of ramification, main axis 0.4-0.5 mm diam., mycorrhizal systems up to 3.8 mm in length, of short distance exploration type, hydrophilic. – *Unramified ends* straight, cylindric, not inflated, up to 3.2 mm long, (0.3)0.4 mm diam., loosely to densely cottony, reddish brown or yellowish brown, very tips pale yellow or white, old parts brownish, mantle semi-transparent, dots lacking, often with substrate particles attached to the surface. – *Emanating hyphae* abundant, not straight, not specificly distributed. – *Rhizomorphs* lacking. – *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figs. 1, 2a): *Mantle* plectenchymatous throughout; hyphal walls thin, colourless, smooth, all hyphae of mantle without clamps. - *Outer mantle layers* (Fig. 1a) plectenchymatous, with a gelatinous matrix and few soil particles, hyphae loosely arranged forming a hyphal net connecting emanating hyphae and outer mantle layers (mantle type B to E, according to Agerer 1987-2006, 1991), hyphae not cylindric, mostly squarrosely branched, hyphal cells (2)3-4(5) µm diam., cell wall 0.3 µm, simple septate (in contrast to the clamp-bearing emanating hyphae). – *Middle mantle layers* (Fig. 1b), densely plectenchymatous without special patterns, hyphae 3-5(7) µm diam., frequently branched. – *Inner mantle layers* (Fig. 2a) with more or less ring-like structures, hyphal shape and dimensions almost identical to those of middle mantle layers, 3.5-5(7) µm diam., hyphal cells infrequently with lipid droplets.-*Very tip* with the same structural characters as other parts of the mantle.

Anatomical characters of emanating elements (Figs. 2b-g, 3b,c): *Rhizomorphs* lacking. – *Emanating hyphae* (Figs. 2b-g, 3b,c) thin-walled, cell walls 0.3 µm wide, hyphae 2-3.5 µm diam., colourless, not straight; septa frequently with clamps and also with simple septa, clamps in dorsal view oval, as broad as the hypha, in lateral view a semicircle to a large semicircle, as broad as its hypha or broader, clamps with a hole; hyphal ramification abundant, Y shaped or ca. 90°, one or two diam. below the septum or from the clamp, side branch hyphae often with clamps close to the point of ramification; hyphae infrequent, with short protrusions; hyphae of emanating system frequently connected with each other by anastomoses, anastomoses variable, often open or very infrequent with a contact clamp, with a short bridge or with a relatively long bridge; distal ends not differentiated; infrequently finely warty hyphae present, these hyphae are

otherwise similar to remaining emanating hyphae in dimension and form of clamps (relatively large and with a hole), one hyphal system with both, warty and smooth surface observed. – *Cystidia* lacking.

Anatomical characters, longitudinal section: *Mantle* (15)18-23(26) µm wide, remnants of calyptra cells present in 1-2 rows, close to root cells, different mantle layers not discernible; pseudoparenchymatously organized, hyphae tangentially 4-10 µm, radially 2-7 µm. – *Tannin cells* present, 1-2 rows, extremly irregularly shaped. – *Cortical cells* rectangular to radially-oval to elliptic, oriented obliquely, tangentially (20)26-40(60) µm, radially 15-26(40) µm, mean tangential length CCt 35 µm, shape ratio CCq 1.8. – *Hartig net* present, protruding towards endodermis, hyphal cells around tannin cells roundish to varaibly shaped, 3-4 µm thick, 1-2 rows; hyphal cells around cortical cells roundish to slightly beaded, in one row, Hartig net in plan view of palmetti type.

DNA extraction, amplification, and sequencing: DNA of the ectomycorrhizae on *Pinus tabulaeformis* roots was extracted using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany). The rDNA ITS region was amplified using the PCR primers ITS1F and ITS4 (White et al.1990, Gardes & Bruns 1993). The obtained PCR product was purified using the QIAquick protocol (Qiagen, Hilden, Germany). The fragment was sequenced using the same primers as mentioned above. The sequencing was performed by the sequencing service of the Institute for Genetics, Department Biology I (Ludwig-Maximilians-University, Munich) using BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1. ITS regions showed total base-pair length of 696 bp. The sequence of this species is as follows.

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAG
GATCATTACCGAATYGTTGACATGAGTTGTTGCTGGCCTTCAAACGGGGG
CATGTGCACGCTCTGTTTACACATCCACCCACCCCTGTGCACCTTTTGTA
GTTCTGTGGTCTGGAGGCTCTGCTTCCCTTCCGTGGCTCTACGTCTTTAC
ACACACACATTAAAGAAGTCTTGTGGAATGTATACCGCGTTTAACGCAAT
ACAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAATCTTTGAACGCACCTTGCGCCCTTTGGCTATTCCGAAGGGCATGCCT
GTTTGAGTATCATGAACACCTCAACTCTCACAGTTTCTTGTGACAAGTTG
GACTTGGGGGTTTTGTTGGCCTGTGGTCAGCTCTCCTCAAATGAATTAGC
TTGCCGGTGTCTGGCGGCATCATGGGTGTGATAACCATCTACGCTTGTGA
TCGTCTGCGAGGTAAACCTCTGGTCTGCGGAGGTTCGCTGGAGCTCATAG
ATGTCTCTCCTCGGTGAAGACAGCTGTCTGAAGTTCGATCTCAAATCAGG
TAGGACTACCCGCTGAACTTAAGCATATCA

The newly created rDNA ITS sequence as queried by galaxieBlast under NJ, Screen and Unique in UNITE showed that this fungus is located between some species of *Tomentella,* such as *Tomentella sublilacina, T. terrestris, T. subtestacea, T. fuscocinerea,* and two species of *Thelephora,* like *Thelephora terrestris* and *T. caryophyllea.*

The newly created rDNA ITS sequence as queried by BlastN search in GenBank retrieved best matches with two samples of uncultured ectomycorrhizae *Tomentella,* the accession numbers are EF218823.1(query coverage 100%; sequence identity 100%) and EF218830.1 (query coverage 99%; sequence identity 100%). Other most similar ITS sequences were uncultured Thelephoraceous ectomycorrhiza or identified and unidentified species blonging to genera of *Thelephora* and *Tomentella.*

Colour reactions with different reagents: *Preparations of mantle*: Melzer's reagent: n. r. (= no reaction); lactic acid: n. r.; sulfo-vanillin: n. r.; KOH: n. r.; guaiac: n. r.; $FeSO_4$: n. r.

Reference specimen for Pinus ectomycorrhiza: China, Inner Mongolia, Heilihe National Natural Reserve, (N 41° 24', E 118° 27'), pure *Pinus tabulaeformis* stand, myc. exc. and isol. by Jie Wei, 12.09.2007, JW 19 (in M). On its possible genus affiliation (comp. epitheton 'inocyboides') was concluded by morphological and anatomical comparisons using DEEMY (AGERER & RAMBOLD 2004-2008, and AGERER 2006).

Discussion: The ectomycorrhiza presented in this study shows typical features of the genus *Inocybe* (AGERER 2006, AGERER & RAMBOLD 2004-2008): As far as presently known, the ectomycorrhizae of this genus are characterized by plectenchymatous often slightly gelatinous mantles without clamps, emanating hyphae with large clamps with holes and simple septa in addition, often granules or droplets within hyphae of inner mantle layers, and lacking rhizomorphs.

This supposed generic affiliation is in contrast to results obtained by DNA-sequencing (s. above). Although a 100% identity to 'uncultured (!) *Tomentella* mycorrhizae' or close to this figure could be found, the features of "*P. inocyboides*" do not show any similarities to ectomycorrhizal characters known to date for any species of thelephoralean genera. As "*P. inocyboides*" was found being associated with rhizomorphs and hyphae that showed similarities to thelephoroid ectomycorrhizae, a mixture of both hyphae in the ectomycorrhizae used for DNA extraction, and resulting in a prevalence of thelephoroid DNA is very likely.

Ectomycorrhizae of *Inocybe.* subg. *Mallocybe* Kuyper, *I. terrigena* (Fr.) Kuyper, *I. fuscomarginata* Kühn. (BEENKEN et al.1996a, b), and *I. heimii* Bon (LASZLO1999) also have a gelatinous matrix in which the hyphal cells of the outer mantle layers are embedded. But *I. obscurobadia* (J. Favre) Grund & D.E. Stuntz (BEENKEN et al.1996c), *I. appendiculata* Kühn (BEENKEN et al.1996d,), *I. lacera* (Fr.: Fr) Kumm. (CRIPPS 1997), and *I. avellana* Horak (INGLEBY 1999) are without such a gelatinous matrix.

In *Inocybe appendiculata, I. terrigena, I. obscurobadia, I. fuscomarginata* and *I. avellana*, hyphae with short protrusions, anastomoses of emanating hyphae with an open bridge could also be found in "*P. inocyboides*", but anastomoses with contact clamps were only observed in this ectomycorrhiza. Emanating hyphae that are finely rough in places have not been mentioned to date for any ectomycorrhiza of the genus *Inocybe* and appear as a specific feature of this suggested *Inocybe* ectomycorhiza. Hyphae of the inner mantle of this ectomycorrhiza possess infrequent lipid droplets which were also described in *I. avellana, I. terrigena, I. fuscomarginata, I. obscurobadia, I. appendiculata* and *I. lacera* (INGLEBY 1999, BEENKEN et al.1996a, b, c, d, CRIPPS 1997).

"*Pinirhiza inocyboides*" is yellowish brown, and looks like a light coloured *Tomentella* or a *Thelephora* mycorrhiza, but a frequent feature of *Tomentella* ectomycorrhizae are brown, light brown or black. The mantle of "*Pinirhiza inocyboides*" is, however, light coloured and even semitransparent, which is not yet reported for any comprehensively described *Tomentella ecto*mycorrhiza: All hyphae of "*Pinirhiza inocyboides*" are colourless, whereas most of the comprehensively described *Tomentella* mycorrhizae form light yellow to brown hyphae (AGERER1996, AGERER et al. 2001, RAIDL & MÜLLER 1996, JAKUCS & AGERER 1999, 2001).

Up to now there are only a few comprehensive descriptions of ectomycorrhizae of the genus *Thelephora*. All of them focus on *Thelephora terrestris* and *Thelephora terrestris* like ectomycorrhizae (DE ROMANet al. 2005). According to AGERER & WEISS (1989), the most important features of *T. terrestris* are at first off-white, later light brown ectomycorrhizae, the netted hyphae below the mantle surface, hyphae with clamps, slightly differentiated rhizomorphs with emanating hyphae similar to emanating hyphae of the mantle (some septa of them are amyloid), and proximally abruptly thick-walled cystidia. The features of "*Pinirhiza inocyboides*" do not fit to those of *T. terrestris*. In addition, mantle hyphae with clamps and clamps without a distinctive hole are also important characteristics to distinguish *Thelephora terrestris* ectomycorrhizae from those of *Inocybe*.

In general, we conclude due to morphological and anatomical examinations that "*Pinirhiza inocyboides*" is an ectomycorrhiza of the genus *Inocybe.* But an exact identification was not possible. Therefore this ectomycorrhiza received a binomial name, "*Pinirhiza inocyboides*",

as practiced for unidentified ectomycorrhizae (GRONBACH & AGERER 1986, AGERER 1987-2006), referring to the host *Pinus* and to the genus by that the ectomycorrhiza has most likely been formed.

We are keen to get the fruitbodies connected with this kind of ectomycorrhiza to further prove our conclusion.

Acknowledgements: We are very grateful to the German Academic Exchange Service (DAAD) for the financial support of this study. The field work and equipment were supported by Prof. Yan Wei (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China). We also thank Dr. Philomena Bodensteiner (Ludwig-Maximilians-Universität, München) for her useful advices during writing this paper. Fan Yongjun (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) assisted the collection of the material.

References: **AGERER** R (1987-2006) Colour Atlas of Ectomycorrhizae. 1st-13th delivery. Einhorn, Schwäbisch Gmünd. – **AGERER R** (1991) Characterization of Ectomycorrhiza. In Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods in Microbiology 23: 25-73. – **AGERER R** (1996) Ectomycorrhizae of *Tomentella albomarginata* (*Thelephoraceae*) on Scots pine. Mycorrhiza 6: 1-7. – **AGERER R** (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5: 67-107. – **AGERER R,** BOUGHER NL (2001) *Tomentella brunneorufa* M. J. Larsen+*Eucalyptus* spec. Descriptions of Ectomycorrhizae 5: 205-212. – **AGERER R**, RAMBOLD G (2004-2008, First posted on 2004-06-01; most recent update: 2008-01-04) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de – München, Germany. – **AGERER** R, WEISS M (1989) Studies on ectomycorrhizae. XX. Mycorrhizae formed by *Thelephora terrestris* on Norway spruce. Mycologia 81: 444-453. – **BEENKEN L**, AGERER R, BAHNWEG G (1996a) *Inocybe fuscomarginata* Kühn. + *Salix* spec./*Populus nigra* L. Descriptions of Ectomycorrhizae 1: 41-46. – **BEENKEN L**, AGERER R, BAHNWEG G (1996b) *Inocybe terrigena.* (Fr.) Kuyper + *Pinus sylvestris* L.. Descriptions of Ectomycorrhizae 1: 53-58. – **BEENKEN L**, AGERER R, BAHNWEG G (1996c) *Inocybe obscurobadia* (J.Favre) Grund & D.E.Stuntz + *Picea abies* (L.) Karst.. Descriptions of Ectomycorrhizae 1: 47-52. – **BEENKEN L**, AGERER R, BAHNWEG G (1996d) *Inocybe appendiculata* Kühn. + *Picea abies* (L.). Karst. Descriptions of Ectomycorrhizae 1: 35-40. – **CRIPPS CL** (1997) *Inocybe lacera* (Fr.:Fr) Kumm. + *Populus tremuloides* Michx. Descriptions of Ectomycorrhizae 2: 19-23. – **DE ROMAN M,** CLAVERIA V, DE MIGUEL AM (2005) A revision of the descriptions of ectomycorrhizas published since 1961. Mycol Res 109(10): 1063-1104. – **GARDES M,** BRUNS T (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118. – **GRONBACH E**, AGERER R (1986) Charakterisierung und Inventur der Fichten-Mykorrhizen im Höglwald und deren Reaktion auf saure Beregnung. Forstwiss Cbl 105: 329-335. – **INGLEBY K** (1999) *Inocybe avellana* Horak + *Shorea leprosula* Miq. Descriptions of Ectomycorrhizae 4: 55-60. – **JAKUCS E**, AGERER R (1999) *Tomentella pilosa* (Burt) Bourdot & Galzin + *Populus alba* L.. Descriptions of Ectomycorrhizae 4: 135-140. – **JAKUCS E**, AGERER R (2001) *Tomentella subtestacea* Bourdot & Galzin + *Populus alba* L. Descriptions of Ectomycorrhizae 5: 213-219. – **LASZLO M** (1999) *Inocybe heimii* Bon + *Fumana procumbens* (Dun.) Gr.Godr. Descriptions of Ectomycorrhizae 4: 61-65. – **RAIDL S**, MÜLLER WR (1996) *Tomentella ferruginea* (Pers.) Pat. + *Fagus sylvatica* L. Descriptions of Ectomycorrhizae 1: 161-166. – **WHITE TJ**, BRUNS TD, LEE SB, TAYLOR JW (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In *PCR Protocols*: a guide to methods and applications (M.A. Innis, D. H. Gelfand, J.N. Sninsky & T.J. White, eds): 315-322. Academic Press, San Diego.

Captions – Fig. 1 – a. Plan view of outer mantle layer; plectenchymatous with a gelatinous matrix, hyphae squarrosely branched and partially inflated. – ***b***. Plan view of middle mantle layer; hyphae more densely packed. – ***Fig. 2 – a.*** Plan view of inner mantle layer; hyphal shape and dimensions identical to middle mantle layers. – ***b.*** Hypha with short protrusions. – **c** Anastomosis open with a short bridge H shaped and with a short protrusion (arrow). – ***d.*** Anastomosis closed with a contact clamp. – ***e.*** Ramification of emanating hyphae. – ***f.*** Distal part of emanating hyphae with anastomosis close to hyphal tip. – ***g.*** One hyphal system with finely warty and smooth surfaces; anastomoses with a reversely oriented clamp. – ***Fig. 3 – a.*** Habit of ectomycorrhiza with many emanating hyphae and covered with soil particles. – ***b.*** Frequently ramified emanating hyphae (one or two diam. below the septum), once connected with an anastomosis, some simple septa pesent. – ***c.*** Ramification of a hypha from a clamp. *(All Figs. from JW 19, in M)*

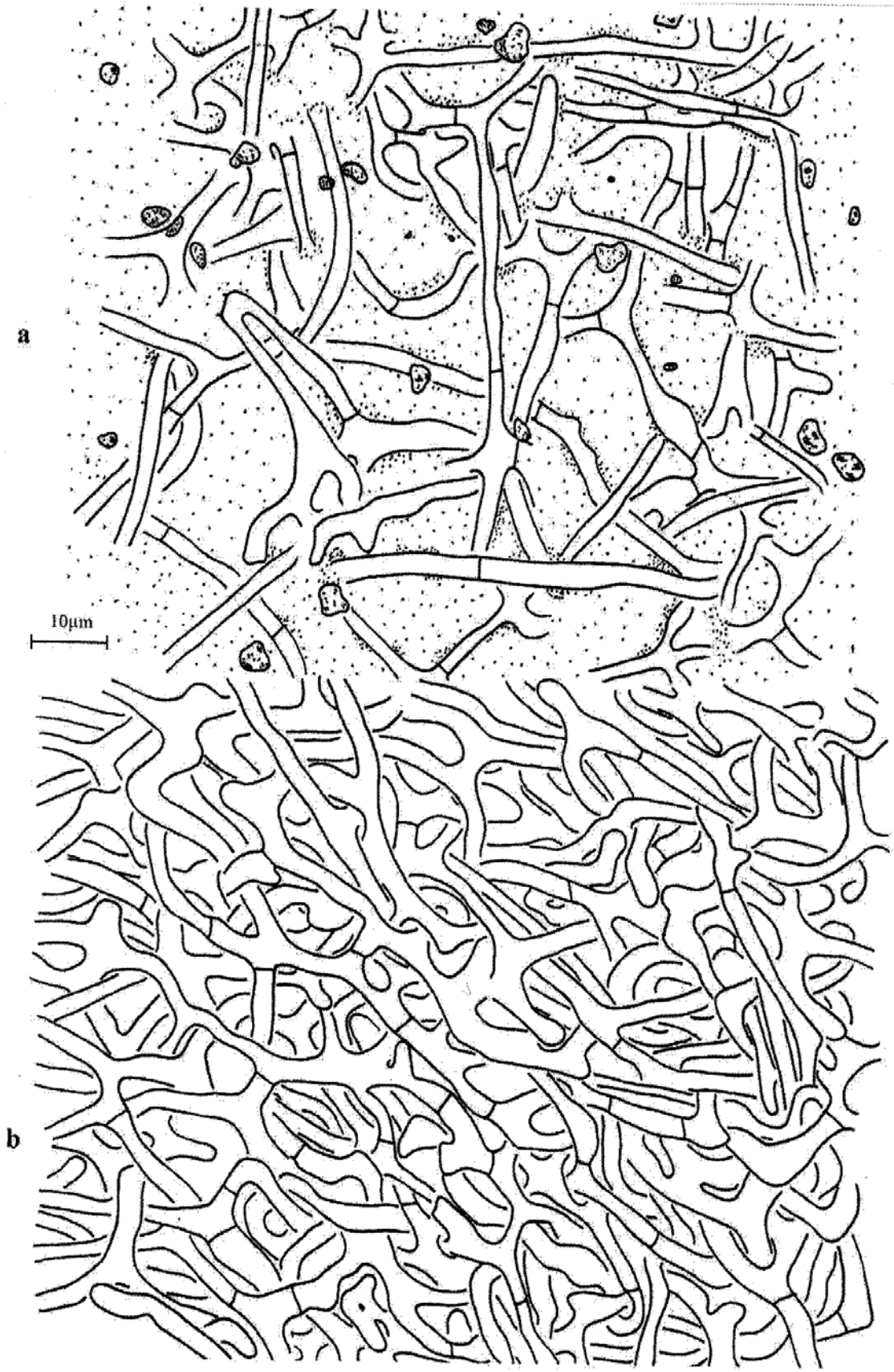


Fig. 1 – "Pinirhiza inocyboides" + Pinus tabulaeformis

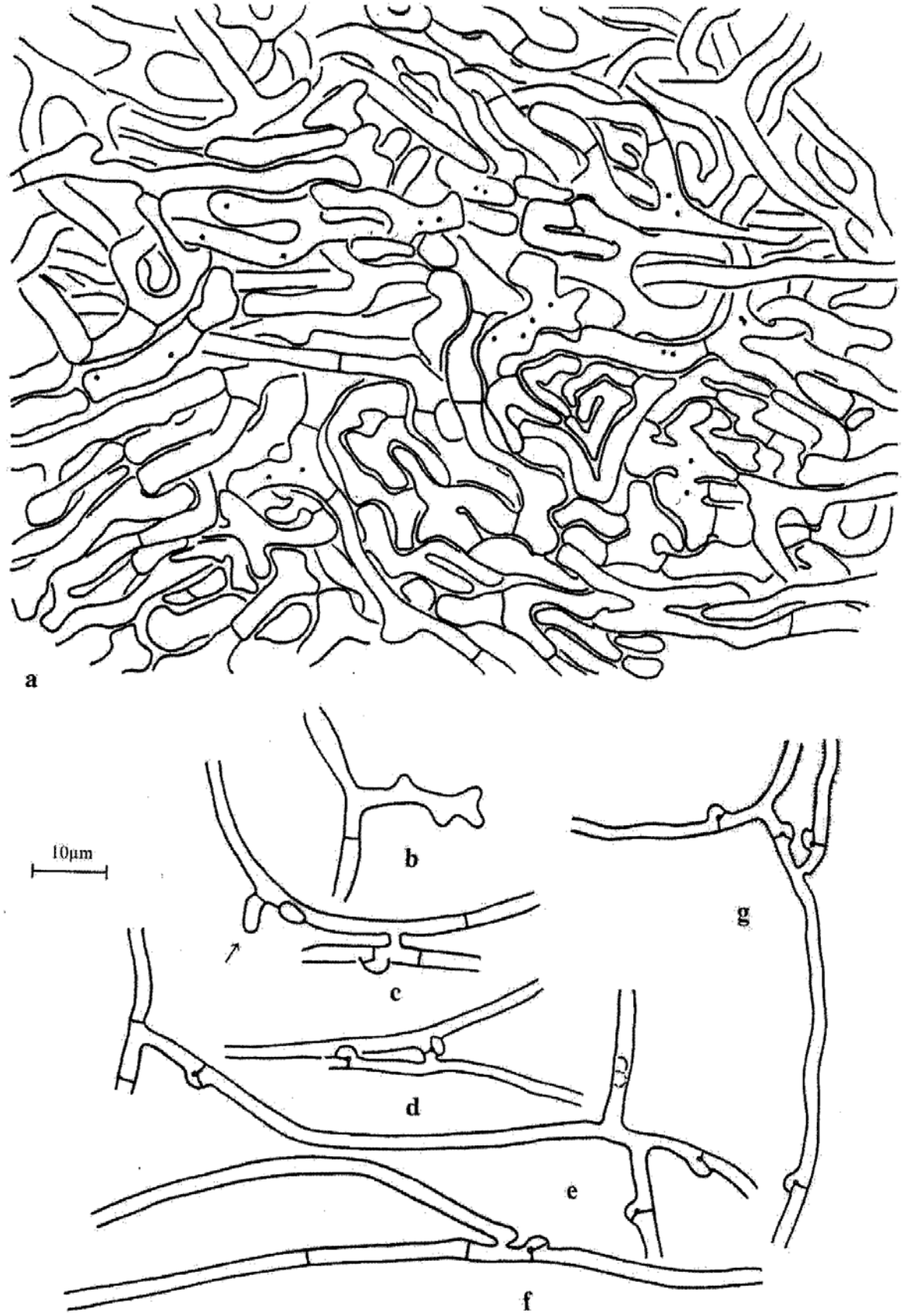


Fig. 2 – "Pinirhiza inocyboides" + Pinus tabulaeformis

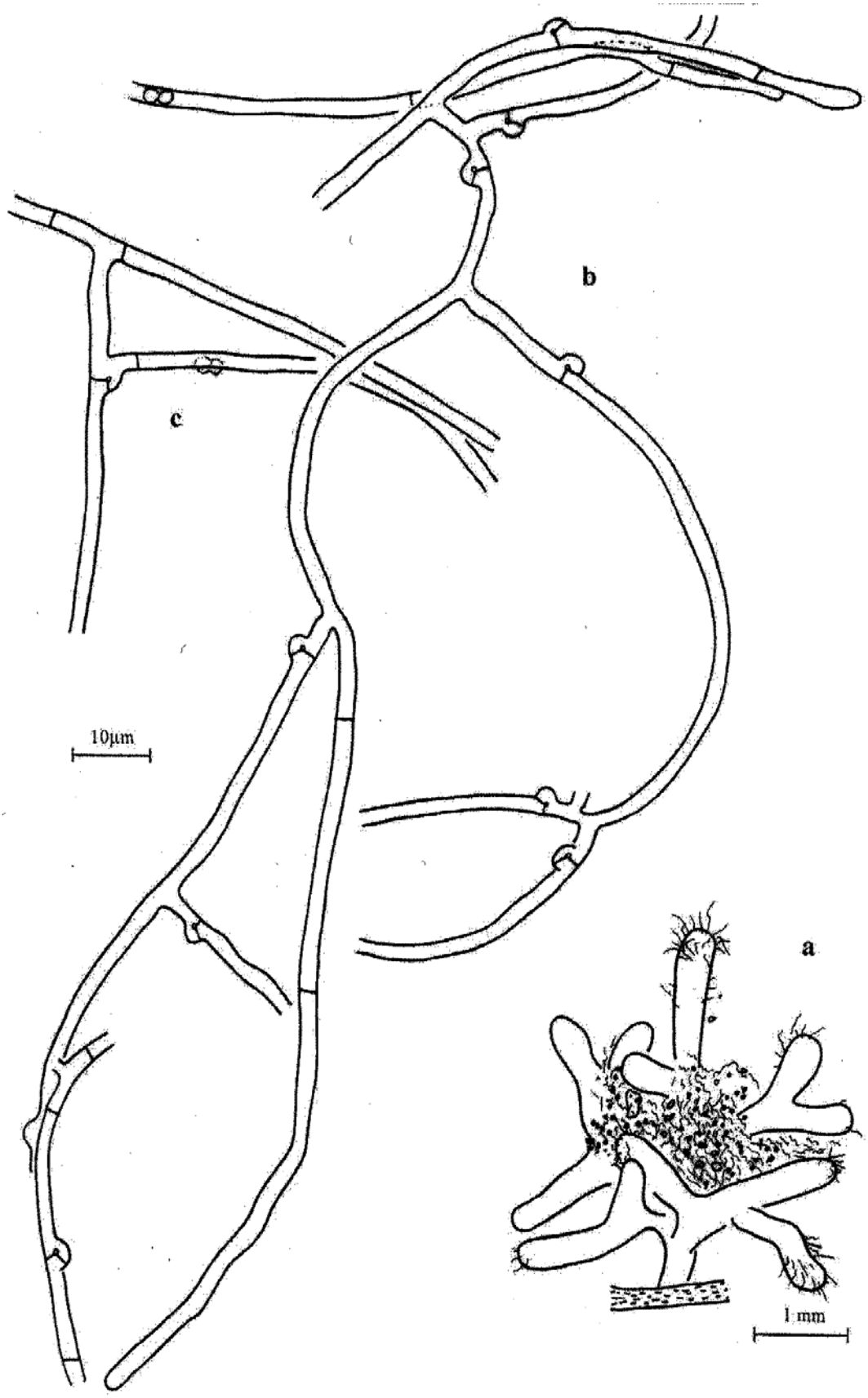


Fig. 3 – "Pinirhiza inocyboides" + Pinus tabulaeformis

2.4.2 “*Pinirhiza tomentelloides* ” + *Pinus tabulaeformis* Carr.

II

Agerer R, Danielson RM, Egli S, Ingleby K, Luoma D, Treu R (eds)
DESCRIPTIONS OF ECTOMYCORRHIZAE *11/12:* 97–102 (2008)

"Pinirhiza tomentelloides"
+ *Pinus tabulaeformis* Carr.

Jie Wei, Reinhard Agerer, Department Biology I and GeoBio-Center^{LMU}, Organismic Biology: Mycology, University of München, Menzinger Str. 67, D-80638 München, Germany.

Short description

Ectomycorrhiza black, dichotomous, with club-shaped unramified ends. Surface of mantle loosely to densely woolly in places. Two mantle layers discernible, hyphae in both layers plasmatically brown and with brown cell walls; outer mantle layers pseudoparenchymatous with angular cells, hyphal cells rosette-like arranged and with mounds of flattened cells on the mantle surface; hypha-like cystidia growing out from these flattened cells, thick-walled, without septa and with ramified ends; inner mantle layers plectenchymatous without pattern, hyphae without clamps. Emanating hyphae thin, clampless, brown, and smooth. Rhizomorphs lacking.

Morphological characters (Fig. 1a): *Mycorrhizal systems* up to 3 mm long, dichotomous, with 0-4 orders of ramification, of short distance exploration type, hydrophilic. – *Main axes* 0.4-0.5 mm diam. – *Unramified ends* straight, slightly inflated between young tips and older parts, cylindrical to club-shaped at youngest stage of ramification; 0.7-2 mm long, 0.35-0.4 mm diam. – *Surface of unramified ends* with very small black granules (under 50× magnification of stereoscope) caused by groups of flattened cells, and covered with many soil particles, mantle not transparent, loosely to even densely woolly, brownish or black emanating hyphae not specificly distributed, complete mycorrhiza system, whatever young or old, black. – *Rhizomorphs* lacking. – *Cystidia* loosely distributed, hypha-like.(it is difficult to decide under stereoscope whether they are emanating hyphae or cystidia). – *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Figs. 2, Fig. 3): Two mantle layers discernible, both with membranaceously and plasmatically brown hyphae; bluish black granules lacking. – *Outer mantle layers* (Figs. 2a, 3) pseudoparenchymatous with angular cells arranged rosette-like and with mounds of flattened cells on the mantle surface (mantle type O, according to Agerer 1987-2006, 1991), these flattened cells mostly roundish, diameter of those cells 9-25 µm, walls ca. 0.5 µm, bearing cystidia-like hyphae (Fig. 3); outer mantle layer cells variable in size, 3-12x3-18 µm, cell walls 0.5 µm thick, smooth. – *Inner mantle layers* plectenchymatous, (Fig. 2b), hyphae irregularly arranged, no special pattern discernible; cells 2.3-3.5 µm diam.; clamps lacking. – *Very tip* like other parts of mantle.

Anatomical characters of emanating elements (Figs.1b, 3): *Rhizomorphs* lacking. – *Emanating hyphae* (Fig. 1b) infrequent, membranaceously brown and plasmatically brownish, clamps lacking; hyphae 2 µm diam., cell wall 0.2 µm; hyphae bent; ramification infrequent, angle of ramification approximately 90°; anastomoses open with a very short bridge or bridge almost lacking. – *Cystidia* (Fig. 3) frequent, straight to bent, hypha-like due to irregular ramification at distal ends, but septa lacking, growing out from the mounds of flattened cells, 2.7-4 µm diam., 80-170 µm long, cell walls 0.5-1 µm, smooth, cells membranaceously and plasmatically brown.

Anatomical characters, longitudinal section: *Mantle* 15-25 µm thick, remnants of calyptra cells present in 1-2 rows, close to root cells, different mantle layers not discernible; pseudoparenchymatous to plectenchymatous, hyphae tangentially (2)6-17(25) µm, radially 2.5-8(10) µm. – *Tannin cells* present, in 1-2 rows, extremly irregularly shaped. – *Cortical cells* tangentially-oval, elliptic or cylindrical and oriented in parallel to root axis to radially-oval to elliptic and oriented obliquely, tangentially (10)20-30 µm, radially (7)12-23 µm, mean tangential length CCt 21.5 µm, shape ratio CCq 1.4; *Hartig net* present, protruding towards endodermis, hyphal cells around tannin cells roundish to slightly elongate, 3-4 µm thick, in 1-2 rows; hyphal cells around cortical cells roundish to slightly beaded, in one row, Hartig net in plan view of palmetti type.

DNA extraction, amplification, and sequencing failed.

Colour reactions with different reagents: *Preparations of mantle*: Melzer's reagent: n. r. (= no reaction); lactic acid: n. r.; sulfo-vanillin: n. r.; KOH: n. r.; guaiac: n. r.; $FeSO_4$: n. r.

Autofluorescence: not studied

Reference specimen for Pinus ectomycorrhiza: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand (N 38° 46', E 105° 54') He Lanshan National Natural Reserve, located in Yin Chuan, China.; myc. exc. and isol. by Jie Wei, 16.08.2007, JW 38a (in M). Its tree genus affiliation was determined by morphological and anatomical comparisons using DEEMY (Agerer & Rambold 2004-2008).

Discussion: The distinctive characteristics of this black ectomycorrhiza are the outermost pseudoparenchymatous mantle layers with rosette-like arranged angular cells and mounds of flattened cells from which brown and thick-walled, non-septate, hypha-like cystidia with irregularly ramified ends grow. Emanating hyphae are thin, brown, simple-septate, smooth and with thin walls, rhizomorphs are lacking.

This ectomycorrhiza resembles "*Quercirhiza tomentellocystidiata*" (Azul & Agerer 2006), *Tomentella stuposa* (Link) Stalpers (Jakucs et al. 2005), and "*Fagirhiza setifera*" (Brand 1991) due to its rosette-like or star-like arrangement of hyphal cells in the pseudoparenchymatous outer mantle layers.

Tomentella stuposa, "*Fagirhiza setifera*" and "*Pinirhiza tomentelloides*" posses mounds of globose to roundish cells on the mantle surface, "*Quercirhiza tomentellocystidiata*" does not form such cells at all. "*Quercirhiza tomentellocystidiata*", *Tomentella stuposa* and "*Fagirhiza setifera*" have clamped emanating hyphae, which are thicker (5-8 µm, 4-5.5 µm and 5-9 µm, respectively) and possess thicker walls (1-1.5 µm, 0.5-1-1.5 µm and 0.5-1.5 µm, respectively) than those of "*Pinirhiza tomentelloides*" (2 µm in diam., walls 0.2 µm thick). In addition, emanating hyphae of "*Pinirhiza tomentelloides*" are clampless. Cystidia are lacking in *T. stuposa* ectomycorrhizae but present in case of "*Quercirhiza tomentellocystidiata*", "*Pinirhiza tomentelloides*"and "*Fagirhiza setifera*". Cystidia of "*Quercirhiza tomentellocystidiata*" are bottle-shaped with a straight neck, posses thin cell walls (0.3-0.5 µm), and are obviously shorter (18.5-31 µm), than those of "*Pinirhiza tomentelloides*" and "*Fagirhiza setifera*", which are long (80-170 µm and 40-80 µm, respectively) and have thick cell walls (0.5-1 µm and 0.5-1.5 µm, respectively).The cystidia of "*Fagirhiza setifera*" are awl-shaped, those of "*Pinirhiza tomentelloides*" are hypha-like, but resemble cystidia due to the lack of septa and their relatively limited length; in contrast to typical cystidia their distal ends are irregularly ramified with distally thin walls. *Tomentella stuposa* forms undifferentiated rhizomorphs, whereas rhizomorphs were found neither in "*Quercirhiza tomentellocystidiata*" nor in "*Pinirhiza tomentelloides*" nor in "*Fagirhiza setifera*" . Similarly, hyphal cells of outer mantle with dense aggregations of bluish granules were found only in case of *Tomentella stuposa*.

This ectomycorrhiza is probably a tomentelloid species according to its morphological and anatomical characteristics.

Acknowledgements: We are very grateful to the German Academic Exchange Service (DAAD) for the financial support of this study. The field work and equipment were supported by Prof. Yan Wei (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China). We also thank Dr. Philomena Bodensteiner (Ludwig-Maximilians-Universität, München) for her useful advices during writing this paper. Fan Yongjun (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) assisted the collection of the material.

References: **Agerer R** (1987-2006) Colour Atlas of Ectomycorrhizae. 1st – 13th delivery. Einhorn, Schwäbisch Gmünd. – **Agerer R** (1991) Characterization of Ectomycorrhiza. In: Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods Microbiol 23: 25-73. – **Agerer R**, Rambold G (2004-2008, First posted on 2004-06-01; most recent update: 2008-01-04) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de – München, Germany. – **Azul AM**, Agerer R (2006) "*Quercirhiza tomentellocystidiata*" + *Quercus suber* L. Descr Ectomyc 9/10: 115-119. – **Brand F** (1991) Ektomykorrhizen an *Fagus sylvatica*. Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. Libri Botanici, vol. IHW, Eching. – **Jakucs E**, Kovács GM, Agerer R, Romsics C, Erös-Honti Z (2005) Morphological-anatomical characterization and molecular identification of *Tomentella stuposa* ectomycorrhizae and related anatomotypes. Mycorrhiza 15: 247-258.

Captions – Fig. 1 – a. Habit of ectomycorrhiza. – ***b.*** Thin emanating hyphae with ramifications. – ***Fig. 2 – a.*** Plan view of outer, pseudoparenchymatous mantle layer, hyphal cells arranged rosette-like. – ***b***. Plan view of inner, plectenchymatous mantle layer, without pattern. – ***Fig. 3.*** Mantle surface with mounds of flattened cells from which hypha-like cystidia with ramified ends grow. *All Figs. from JW 38a (in M)*

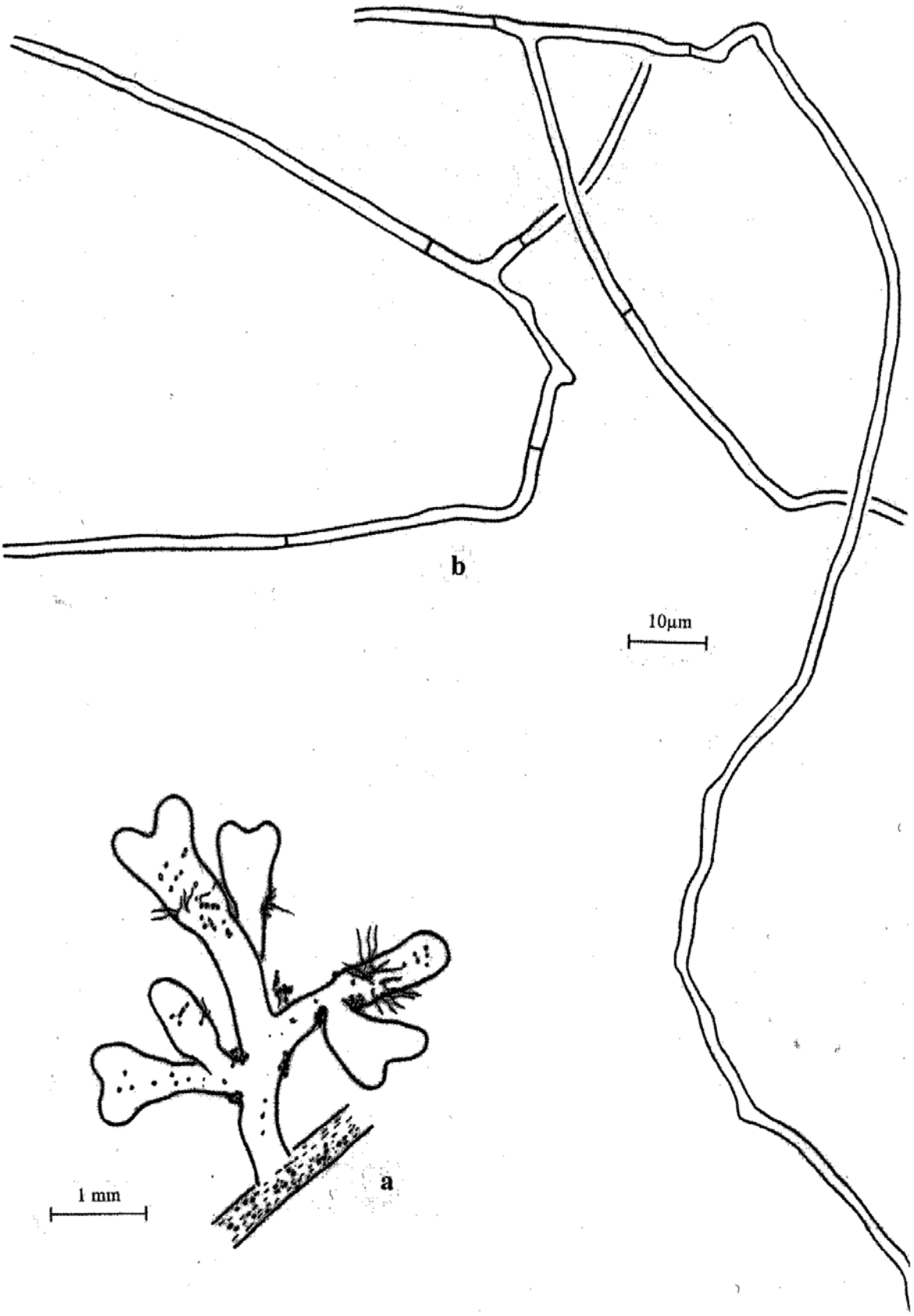


Fig. 1 – "Pinirhiza tomentelloides" + Pinus tabulaeformis

100

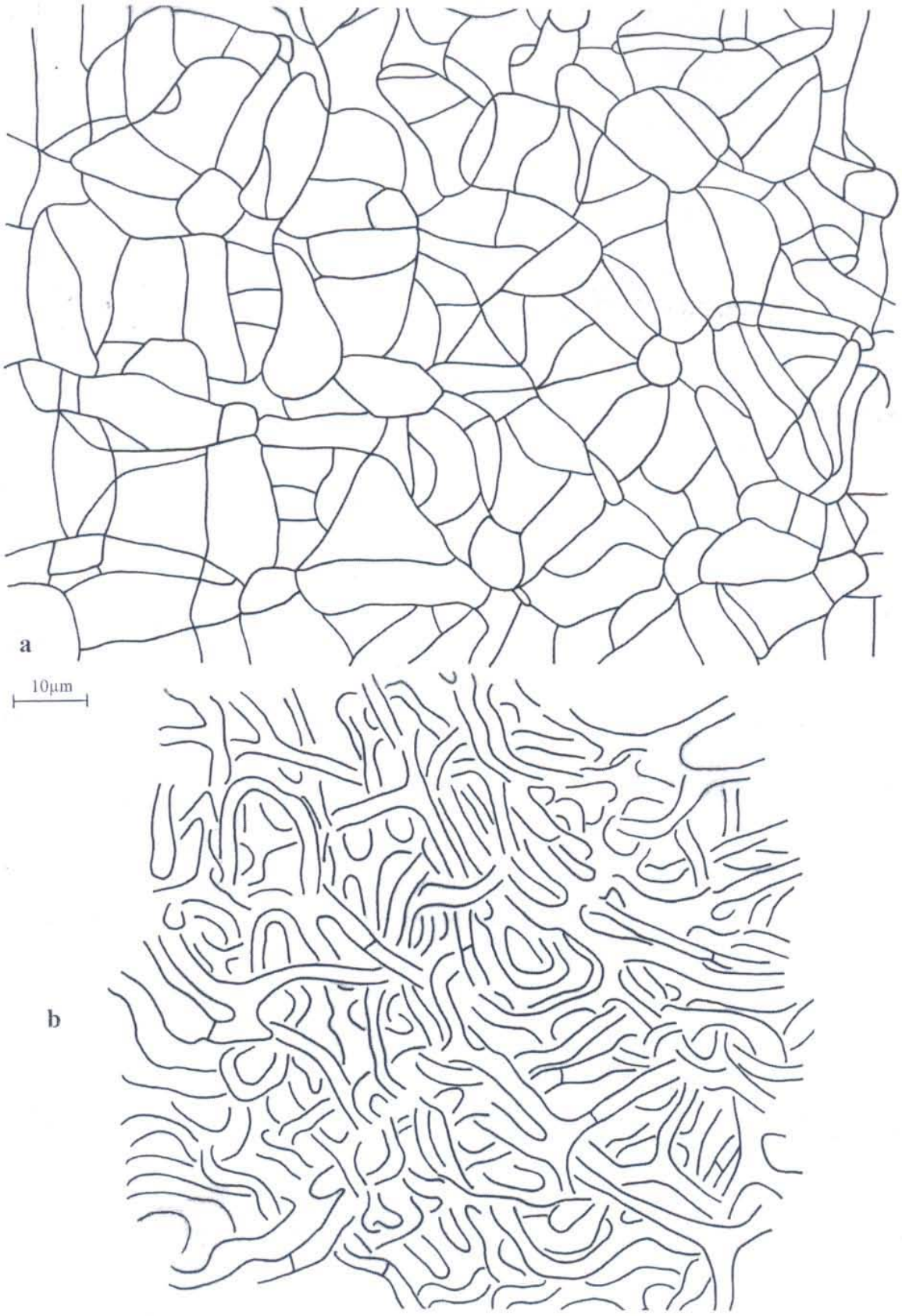


Fig. 2 – "Pinirhiza tomentelloides" + Pinus tabulaeformis

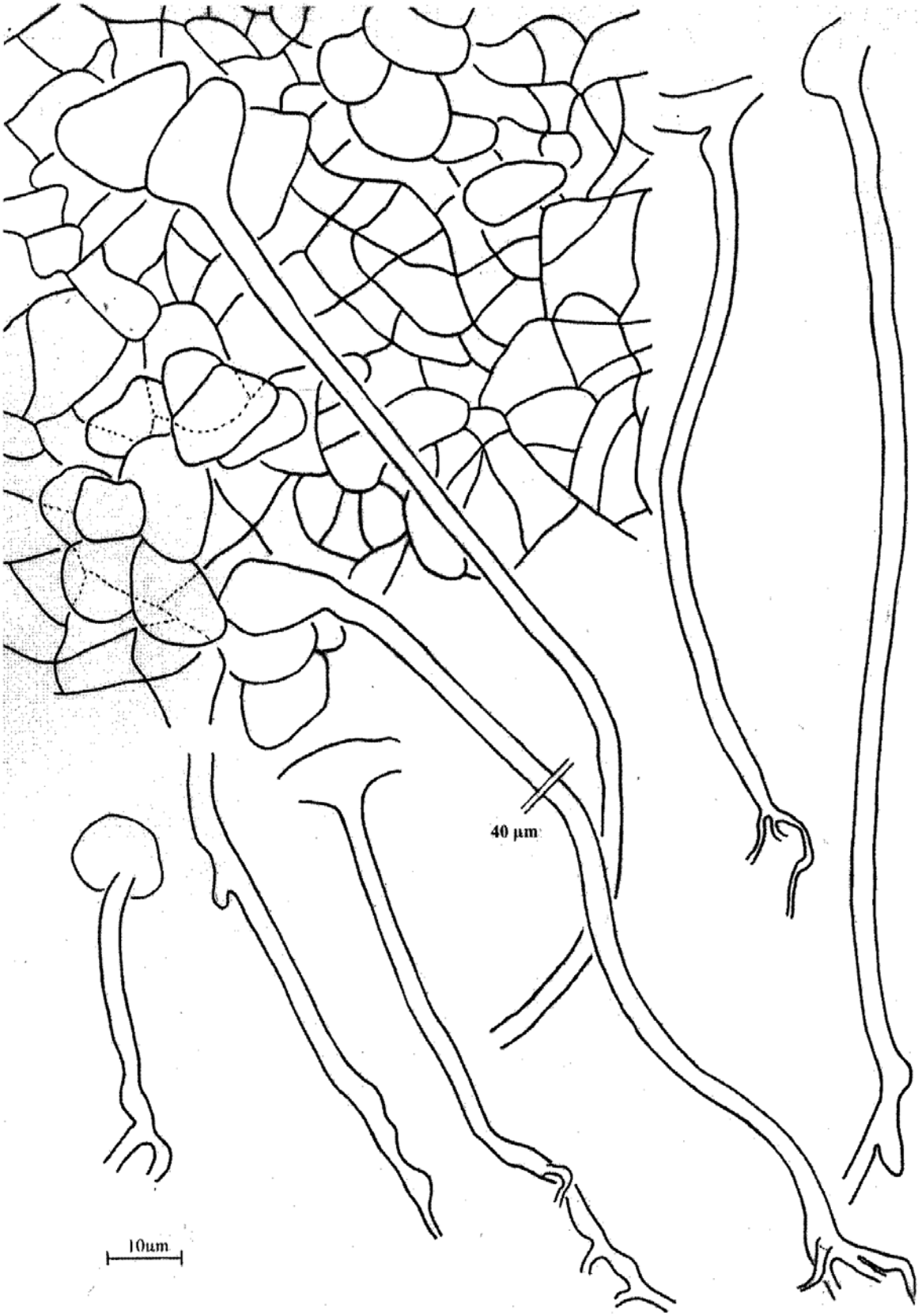


Fig. 3 – "Pinirhiza tomentelloides" + Pinus tabulaeformis

2.4.3 “*Pinirhiza tricholomoides* ” + *Pinus tabulaeformis* Carr.

III

Agerer R, Danielson RM, Egli S, Ingleby K, Luoma D, Treu R (eds)
DESCRIPTIONS OF ECTOMYCORRHIZAE *11/12:* 103–112 (2008)

"Pinirhiza tricholomoides"
+ Pinus tabulaeformis Carr.

Jie Wei, Reinhard Agerer, Stefan Raidl, Department Biology I and GeoBio-Center[LMU], Organismic Biology: Mycology, University of München, Menzinger Str. 67, D-80638 München, Germany.

Short description

Mycorrhizae silvery, dichotomous, attached with abundant white and stringy rhizomorphs, which are slightly differentiated with enlarged central hyphae and nodia, covered with adhering soil. Rhizomorph-forming hyphae with slightly yellow walls, with open anastomoses, without clamp connections. Peripheral hyphae of rhizomorphs smooth or with crystalline ornamentation, often ramified, then becoming thinner and thinner to 1.8 µm diam. The outer mantle plectenchymatous with gelatinous matrix, composed of slightly thick-walled hyphae with open anastomoses. Inner mantle rather dense, more compact than outer mantle. Hyphae of all mantle layers clampless.

Morphological characters (Fig. 1a): *Mycorrhizal systems* dichotomous, with 0-3(4) orders of ramification, solitary or in small numbers, main axis 0.5-0.6 mm diam., smooth subtype of medium distance exploration type. – *Unramified ends* straight or sometimes bent, cylindric, not inflated, up to 4,5 mm long, 0.4-0.6 mm diam., silvery white because of enclosed air, hydrophobic, later yellowish brown or ochre, very tips pale yellow, mantle not transparent, dots lacking, not carbonizing, often with substrate particles attached to the surface, densely or loosely woolly. – *Emanating hyphae* infrequent, not specifically distributed. – *Rhizomorphs* abundant, silvery white, up to 0.3 mm diam., connection to mantle distinct, ramifications frequent at restricted points, no specific origin, occasionally growing along roots, surface smooth, round in cross-section. – *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Fig. 2): Mantle plectenchymatous, two layers discernible, in both layers all hyphae slightly thick-walled and colouress, blue granules absent, anastomoses open, all hyphae clampless. - *Outer mantle layers* (Fig. 2a) plectenchymatous, at places with ring-like arranged hyphal bundles (mantle type A to B, according to Agerer 1987-2006, 1991, Agerer & Rambold 2004-2008) or without pattern, with gelatinous matrix, hyphae sinuous, sticky matrix on hyphal surface present, angles of hyphal ramification ca. 90°, septa thinner than cell walls, hyphae not constricted at septa, cylindrical, 3-4 µm diam., cells 10-50 µm long, cell walls 0.5(0.9) µm thick, surface with many soil particles otherwise smooth. – *Middle mantle layer* not discernible. – *Inner mantle layers* (Fig. 2b) plectenchymatous, without pattern, cells 3-5 µm diam. – *Very tip* with the same structural characters as in other parts of mantle.

Anatomical characters of emanating elements (Figs. 1b, 3-5): Clamps lacking, intrahyphal hyphae present, septal pores mostly indistinct, sometimes discernible, backwards oriented ramifications not found. – *Rhizomorphs* (Fig. 1b, 3, 4) of type C (according to Agerer 1987-2006, 1991, 1999), slightly differentiated, with distinct nodia at branching points, surface rather compact with very few emanating hyphae, with conical side-branches originating from central

hyphae, hyphae of rhizomorphs with slightly yellowish walls, with open anastomoses; central hyphae somewhat enlarged in some parts of rhizomorphs, up to 6 µm diam., cell walls 0.5(0.9) µm thick; peripheral hyphae 3-4 µm diam., cells 50-60 µm long, surface sometimes with few soil particles, emanating hyphae of rhizomorphs 3-4 µm diam., cell walls 0.5 µm, thinning to 1.8 µm diam. after ramification and there thin-walled, few short hyphae showing swollen tips. – *Emanating hyphae* (Fig. 5) with open anastomoses, with a short bridge or bridge almost lacking, cell wall of anastomoses as thick as remaining cell walls, ramifications infrequent, angle of ramifications ca. 90°, one side-branch at septum, 1-2 diam. below the septum; hyphae 2.5-3.5 µm diam., cell wall 0.5 µm, hyphal ends simple, cell walls as thick as in other parts of hyphae, gelatinous, surface with soil particles. – *Cystidia* lacking.

Anatomical characters, longitudinal section: *Mantle* (20)25-30(35) µm thick, remnants of calyptra cells present in 1-2 rows, close to root cells, different mantle layers not discernible; plectenchymatously organized, hyphae tangentially 3-15(30) µm, radially 3-6 µm, septal pores visible in mantle and emanating hyphae. – *Tannin cells* present, 1-2 rows, extremly irregularly shaped. - *Cortical cells* round to tangentially-oval to -elliptic, oriented parallel to root axis, tangentially 40-90 µm, radially (20)30-40(55) µm, mean tangential length CCt 65 µm, shape ratio CCq 2.1. – *Hartig net* present, mostly not protruding towards endodermis, one or half a row of cortical cells adjoining endodermis free of Hartig net, Hartig net cells roundish to slightly elongate, hyphal cells around tannin cells 3-4 µm thick, 1-2 rows; hyphal cells around cortical cells roundish to slightly cylindrical, 2-3 µm thick, in one row, Hartig net in plan view infrequently lobed, lobes 2.5-3(4) µm wide.

DNA extraction, amplification, and sequencing: DNA of the mycorrhizae on *Pinus tabulaeformis* roots was extracted using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany). The rDNA ITS region was amplified using the PCR primers ITS1F and ITS4 (White et al.1990, Gardes & Bruns 1993). The obtained PCR product was purified using the QIAquick protocol (Qiagen, Hilden, Germany). The fragment was sequenced using the same primers as mentioned above. The sequencing was performed by the sequencing service of the Institute for Genetics, Department Biology I (Ludwig-Maximilians-University, Munich) using BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1. ITS regions showed total base-pair lengths of 711 bp. Sequence deposited in GenBank with the designation EU781654.

The newly created rDNA ITS sequence as queried by BlastN search in GenBank retrieved best matches with samples from an undetermined uncultured fungus, the accesion number of this fungus in GenBank is EU292410.1. The most similar ITS sequences of identified *Tricholoma* species were those of *T. moseri* Singer (query coverage 100%; sequence identity 95%), *T. terreum* (Schaeff.:Fr.) Kummer (query coverage 98%; sequence identity 94%), and *T. myomyces* (query coverage 98%; sequence identity 96%). Other sequences of genera *Lepista*, *Collybia*, *Lyophyllum* and *Hypsizyqus*, generally accepted as saprotrophs, are with lower similarities (maximal query coverage 95%, maximal identity 89%).

Colour reactions with different reagents: *Preparations of mantle*: Melzer's reagent: n. r. (= no reaction); lactic acid: n. r.; sulfo-vanillin: n. r.; KOH: n. r.; guaiac: n. r.; $FeSO_4$: n. r.

Reference specimen for Pinus ectomycorrhiza: The mycorrhiza was collected in a pure, artificial, 20-30 years-old *Pinus tabulaeformis* stand at Daqing Mountain (40°34' -40° 57' N, 110° 25'-112° 30' W), located in Inner Mongolia, China, myc. exc. and isol. by Jie Wei, 24. July, 2007, JW 71 (in M). Its genus affiliation was determined by morphological and anatomical comparisons using DEEMY – an Information System for Characterization and Determination of Ectomycorrhizae (Agerer & Rambold 2004-2008), and by the comparison of the newly generated ITS sequence with sequences published previously via BlastN search in GenBank using the newly generated sequence as query.

Discussion: Common features of many of the *Trichloma* mycorrhizae that have been cha-

racterized to date AGERER (2006) are a stringy, with silvery mantle surrounded by conspicuous extramatrical hyphae, rhizomorphs and a plectenchmatous outer mantle layer. These characters were also observed in this ectomycorrhiza on *Pinus tabulaeformis*. The diversity of rhizomorph organization is however large in the genus *Tricholoma*.

The results of the examination of the morphological and antomical features and the comparison of DNA data suggest that this species belongs to the genus *Tricholoma*, but an exact identification was not possible. Therefore this ectomycorrhiza received a binomial name, "*Pinirhiza tricholomoides*", as practiced for unidentified ectomycorrhizae (GRONBACH & AGERER 1986, AGERER 1987-2006), referring to the host *Pinus* and to the genus by that the ectomycorrhiza has most likely been formed.

Detailed descriptions of *Tricholoma* ectomycorrhizae are pulished for the following species:

Tricholoma acerbum with *Fagus sylvatica* (WALLER & AGERER 1993), *T. aurantium* with *Picea abies* (UHL 1988), *T. auratum* with *Pinus silvestris* (UHL 1988), *T. imbricatum* with *Larix decidua* (TREU 1990), *T. flavobrunneum* with *Betula pendula* (UHL 1988), *T. magnivelare* with *Pinus contorta* (LEFEVRE & MÜLLER 1998), *T. saponaceum* with *Picea abies* (UHL 1988), *T. scalpturatum* with *Populus tremuloides* (CRIPPS 1997), *T. sciodes* with *Fagus sylvatica* (BRAND 1991), *T. sejunctum* with *Pinus strobus* (UHL 1988), *T. sulphureum* with *Picea abies* (AGERER 1987) and *T. vaccinum* with *Picea abies* (AGERER 1987, UHL 1988).

The to date described *Tricholoma* ectomycorrhizae are compared using the following key:

1 Rhizomorphs undifferentiated
  2 Anastomoses closed by a contact-septum
    T. sulphureum (Bull.:Fr.) Kummer
  2* Anastomoses open
    3 Emanating hyphae of mantle with clamps
      T. sejunctum (Sow.:Fr.) Quél.
    3* Emanating hyphae of mantle without clamps
      T. sciodes (Secr.) Mart.
1* Rhizomorphs differentiated
  4 Rhizomorphs without clamps
    5 Emanating hyphae of mantle with clamps
      T. saponaceum (Fr.) Kummer
    5* Emanating hyphae of mantle without clamps
      6 Anastomoses of emanating hyphae closed by a simple septum
        7 Rhizomorphs with crystalline ornamentation
          T. magnivelare (Peck) Redhead
        7* Rhizomorphs without crystalline ornamentation
          T. aurantium (Schff.:Fr.) Ricken
      6* Anastomoses of emanating hyphae open
        8 Rhizomorphs lacking cell wall pigment
          T. scalpturatum (Fr.) Quél.
        8* Rhizomorphs composed of hyphae with yellowish cell walls or with brownish pigment
          9 Inner mantle layer pseudoparenchymatous
            T. auratum (Paul.:Fr.) Quél.
          9* Inner mantle layer plectenchymatous
            Pinirhiza tricholomoides
  4* Rhizomorphs with clamps
    10 Thick hyphae up to 10 µm diam., septa not dissolved
      11 Hyphae of outer mantle with yellowish colour
        T. flavobrunneum (Fr.) Kummer
      11* Hyphae of outer mantle colourless
        T. vaccinum (Pers.:Fr.) Kummer
    10* Thick hyphae more than 10 µm, up to 25 µm wide, septa dissolved
      12 Hyphae of outer mantle with clamps
        T. imbricatum (Fr.:Fr.) Kummer
      12* Hyphae of outer mantle without clamps
        T. acerbum (Bull.:Fr.) Quél.

Acknowledgements: We are very grateful to the German Academic Exchange Service (DAAD) for the financial support of this study. The field work and equipment were supported by Prof. Yan Wei (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China). We also thank Dr. Philomena Bodensteiner (Ludwig-Maximilians-Universität, München) for her useful advices during writing this paper. Fan Yongjun (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) assisted the collection of the material.

References: **Agerer R** (1987) Studies on ectomycorrhizae IX. Mycorrhizae formed by *Tricholoma sulphureum* and *T. vaccinum* on spruce. Mycotaxon 28: 327-360. – **Agerer** R (1987-2006) Colour Atlas of Ectomycorrhizae. 1st-13th delivery. Einhorn, Schwäbisch Gmünd. – **Agerer R** (1991) Characterization of Ectomycorrhizae. In Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods in Microbiology 23:25-73. – **Agerer R** (1995) Anatomical characteristics of ectomycorrhizae: an attempt towards a natural classification. In Mycorrhiza: structure, function, molecular biology and biotechnology

(A. K. Varma & B. Hock, eds): 685-734. Springer Verlag, Berlin. – **Agerer R** (1999) Never change a functionally successful principle: the evolution of Boletales s. l. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendtnera 6: 5-91. – **Agerer R** (2001) Exploration types of ectomycorrhizae – a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11: 107-114. – **Agerer R**, Rambold G (2004-2008, First posted on 2004-06-01; most recent update: 2008-01-04) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de - München, Germany. – **Brand F** (1991) Ektomykorrhizen an *Fagus sylvatica* Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. Libri Botanici 2: 64-69. – **Cripps C** (1997) *Tricholoma scalpturatum* (Fr.) Quél. + *Populus tremuloides* Michx. Descriptions of Ectomycorrhizae 2: 73-78. – **Gardes M,** Bruns T (1993) ITS primers with enhanced specificity for basidiomycetes: application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118. – **Gronbach E**, Agerer R (1986) Charakterisierung und Inventur der Fichten-Mykorrhizen im Höglwald und deren Reaktion auf saure Beregnung. Forstwiss Cbl 105: 329-335. – **Lefevre CK**, Müller WR (1998) *Tricholoma magnivelare* (Peck) Redhead + *Pinus contorta* Dougl. var. *latifolia* Engelm. In Goodman DM, Durall DM, Trofymow JA, Berch S (eds) A Manual of Concise Descriptions of North American Ectomycorrhizae: including microscopic and molecular characterization. CDE 18. Mycologue Publications, Sidney, BC. – **Treu R** (1990) Charakterisierung und Identifizierung von Ektomykorrhizen aus dem Nationalpark Berchtesgaden. Bibliotheca Mycologica 134: 1-196. – **Uhl M** (1988) Studies on ectomycorrhizae XVI. Ectomycorrhizae formed by *Tricholoma flavobrunneum* and *Betula pendula* and *Tricholoma auratum* and *Pinus sylvestris*. Mycotaxon 33: 1-21. – **Waller K**, Agerer R (1993) Ektomykorrhizen von *Dermocybe cinnamomeolutea* (Cortinariaceae) und *Tricholoma acerbum* (Tricholomataceae). Sendtnera 1: 23-38. – **White TJ**, Bruns TD, Lee SB, Taylor JW (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In *PCR Protocols*: a guide to methods and applications (M.A. Innis, D. H. Gelfand, J.N. Sninsky & T.J. White, eds): 315-322. Academic Press, San Diego.

Captions: Fig. 1 – a. Habit of ectomycorrhiza with rhizomorphs, covered with adhering soil particles. – ***b.*** Young rhizomorph in surface view with an outgrowing hypha. – ***Fig. 2 – a.*** Plan view of outer mantle layer; plectenchymatous with gelatinous matrix. – ***b.*** Plan view of inner mantle layer, hyphae more densely packed. – ***Fig. 3 – a.*** Rhizomorph with a short, conical side-branch with an open anastomosis near a hyphal tip. – ***b.*** Rhizomorph with a short hypha showing a strongly swollen tip. – ***c.*** Hyphal ramification of a rhizomorph, showing a backward oriented ramification of an emanating hypha, branching again and running along the thin rhizomorph. – ***d.*** Central hyphae of the slightly differentiated rhizomorph. – ***Fig. 4 – a.*** Ramifications of emanating hyphae of the rhizomorph, hyphae becoming thinner and thinner . – ***b.*** Emanating hypha, a young side-branch of the rhizomorph. – ***Fig. 5 – a.*** Emanating hyphae with open anastomoses. – ***b.*** Emanating hyphae of the mantle with slightly gelatinous surface, hyphal ends simple. *All Figs from JW 71 (in M)*

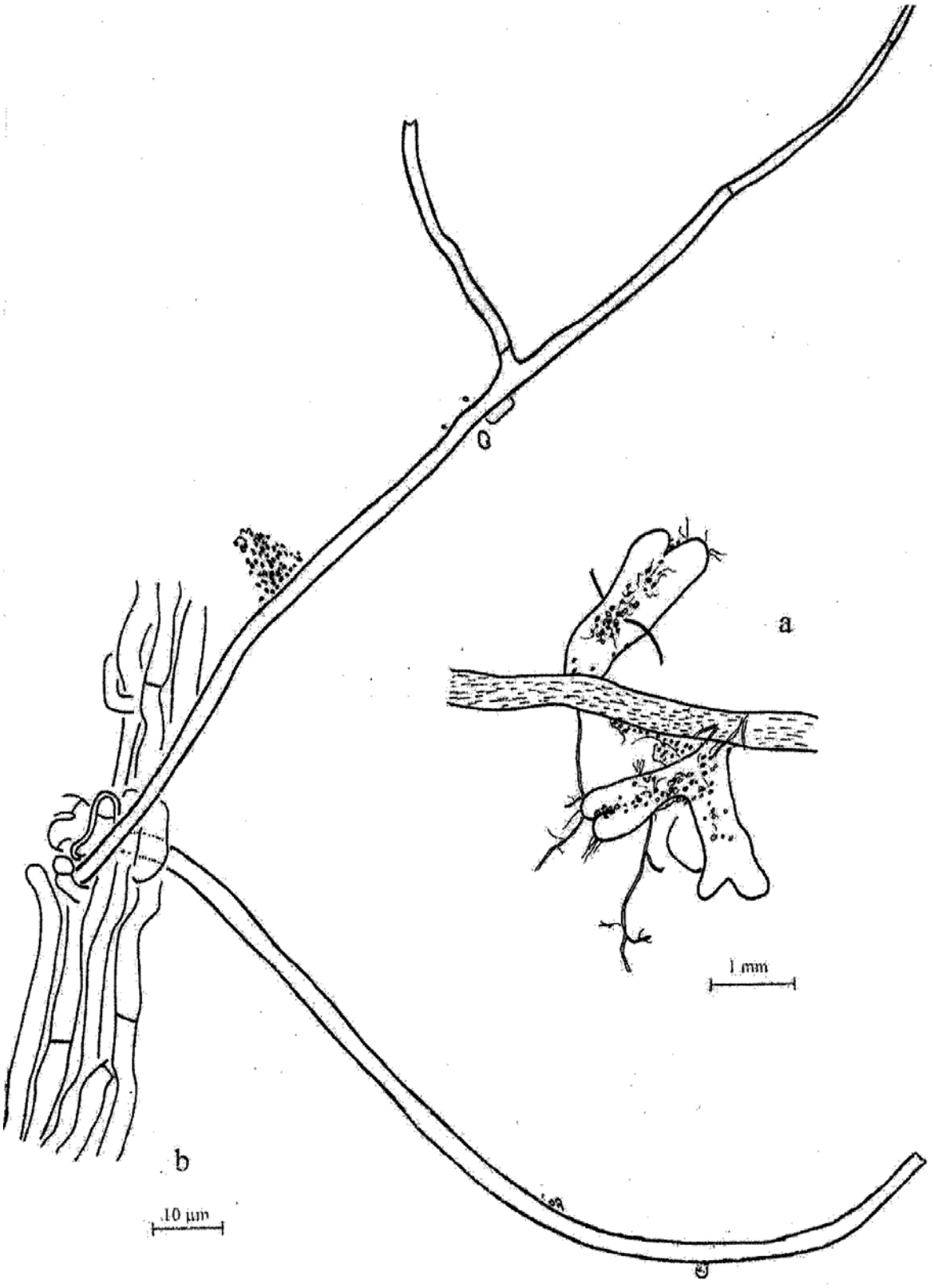


Fig. 1 – "Pinirhiza tricholomoides" + Pinus tabulaeformis

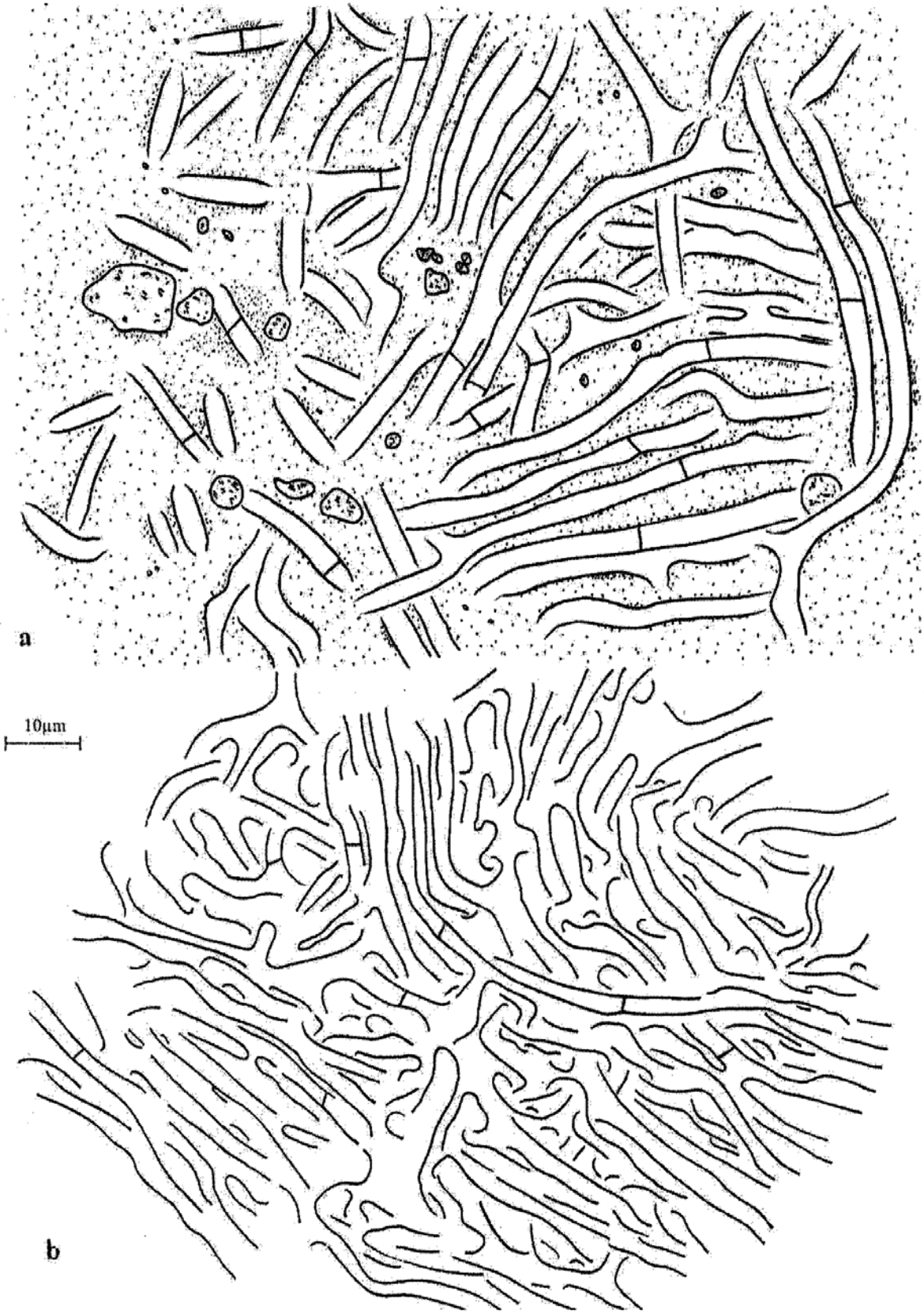


Fig. 2 – "Pinirhiza tricholomoides" + Pinus tabulaeformis

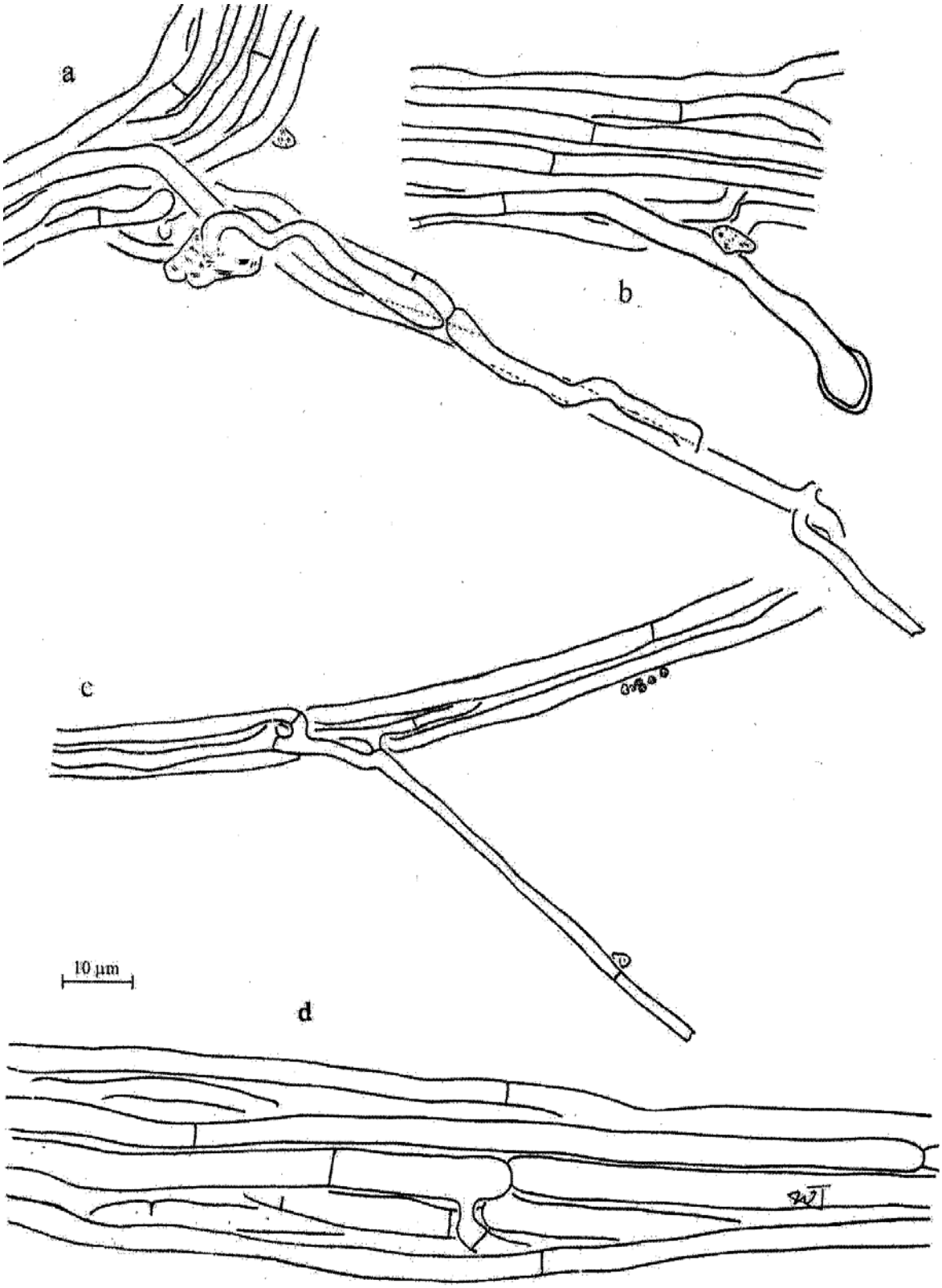


Fig. 3 – "Pinirhiza tricholomoides" + Pinus tabulaeformis

110

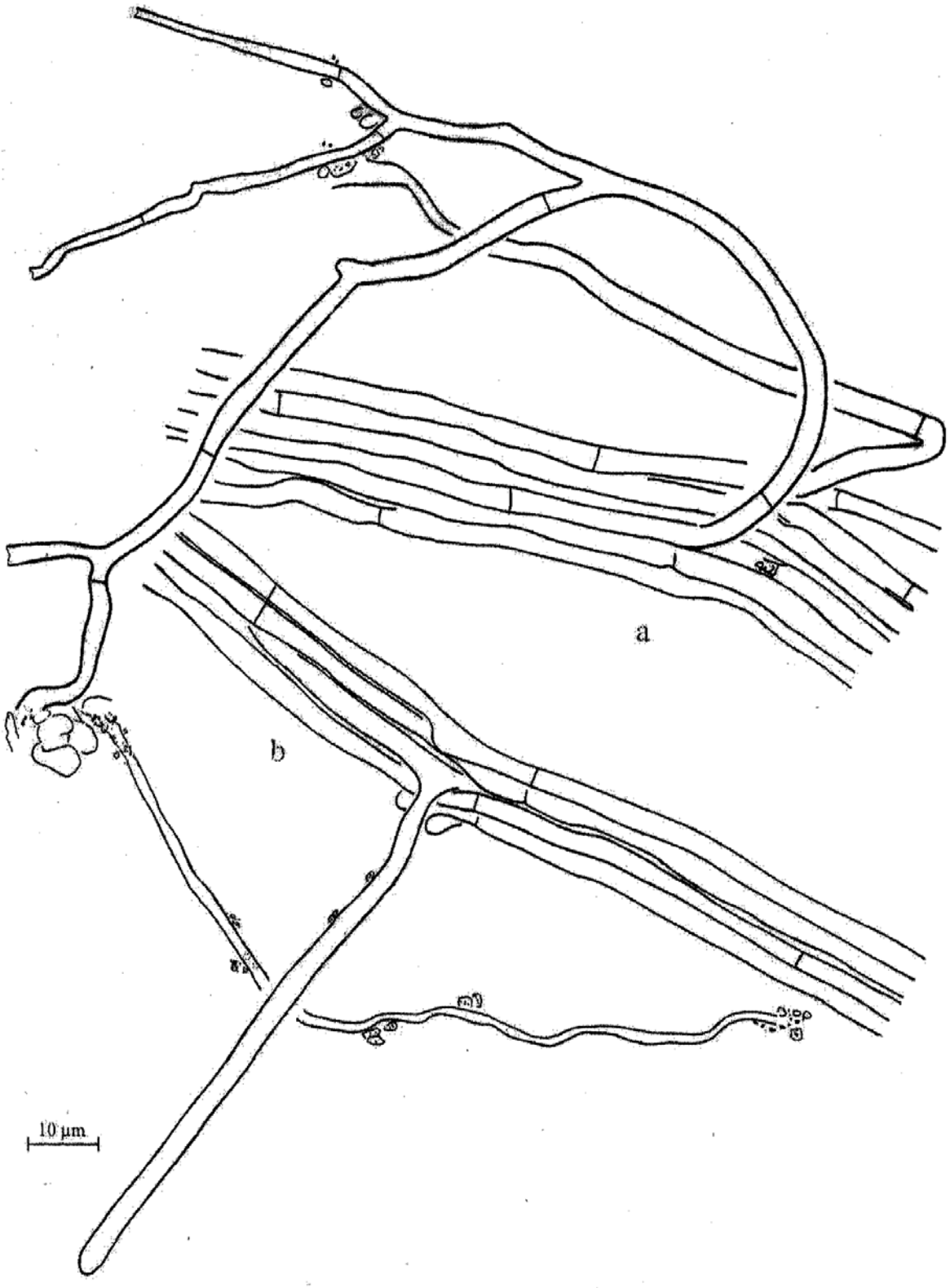


Fig. 4 – "Pinirhiza tricholomoides" + Pinus tabulaeformis

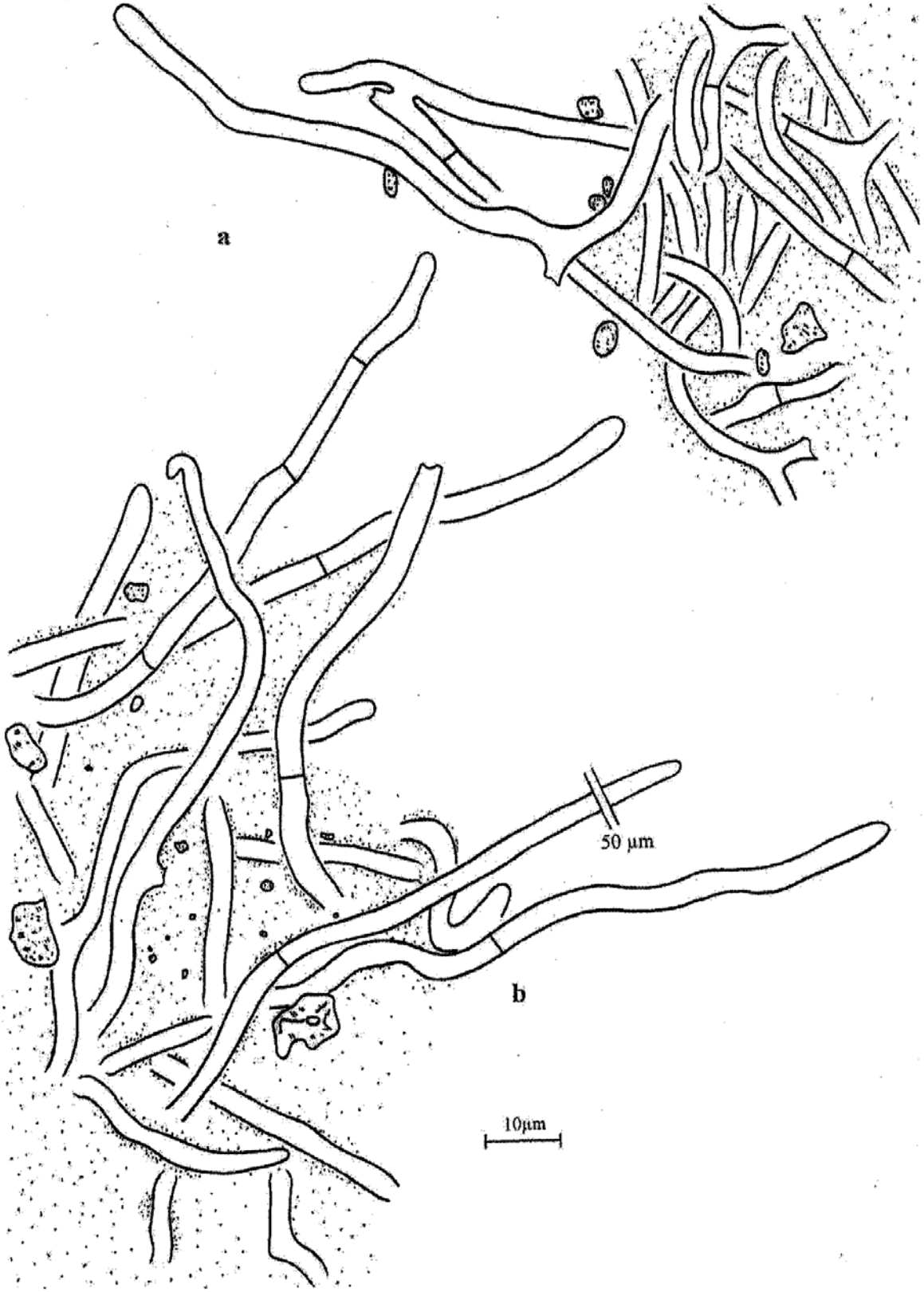


Fig. 5 – "Pinirhiza tricholomoides" + Pinus tabulaeformis

2.4.4 Four Ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*), morpho-anatomical and molecular phylogenetic analyses

IV

Mycol Progress
DOI 10.1007/s11557-009-0637-x

ORIGINAL ARTICLE

Four ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*): morpho-anatomical and molecular-phylogenetic analyses

Jie Wei · Derek Peršoh · Reinhard Agerer

Received: 29 July 2009 / Revised: 21 October 2009 / Accepted: 5 November 2009

Abstract Morphological and anatomical characters of four ectomycorrhizae with affinities to the genera *Humaria*, *Geopora*, and *Trichophaea* of Pyronemataceae (Pezizomycetes, Ascomycota) on Chinese Pine (*Pinus tabulaeformis*) are described. The ectomycorrhizae are yellowish brown to brown, and have pseudoparenchymatous outer mantle layers and partially warty emanating hyphae with thick walls and without clamps. Intrahyphal hyphae are present, and no rhizomorphs are formed. The four ectomycorrhizae are distinguishable by differences in cell shape of outer mantle layers and the presence of cystidia. Ectomycorrhizae of a possible *Humaria* species (*Pinirhiza humarioides*) lack cystidia and have irregularly inflated cells on the outer mantle layer that are connected with thin septa. The two ectomycorrhizae showing probable affinities to *Geopora* species ("*P. daqingensis*" and "*P. geoporoides*") possess row-like arranged cells in the outer mantle layer and cell heaps, and differ by the presence or absence of cystidia as well as by the structure of the inner mantle layers. Ectomycorrhizae likely having been formed by a *Trichophaea* species ("*P. trichophaeoides*") have oval to polygonal cells and no cystidia. The possible taxa affiliations were assessed by molecular-phylogenetic analyses of the internal transcribed spacer (ITS) and partial large subunit (LSU) nrDNA. Morphological and anatomical characters are discussed against the background of the LSU phylogeny.

Keywords Anatomy · Ectomycorrhiza · Morphology · Molecular-phylogenetic analyses · Pyronemataceae

Introduction

The majority of taxa in Pyronemataceae have traditionally been considered as being saprotrophic (Perry et al. 2007), but a few ectomycorrhizal fungi were also reported in this family: *Genabea, Genea*, *Geopora*, *Humaria*, *Pulvinula*, *Sphaerosporella*, and *Trichophaea* (Amicucci et al. 2001; Danielson 1984; Erős-Honti et al. 2008; Fujimura et al. 2005; Jakucs et al., 1998; Smith et al. 2006; Tedersoo et al. 2006). While *Geopora* and *Wilcoxina* form E-strain mycorrhizae (ectendomycorrhiza) (Fujimura et al. 2005; Yu et al. 2001), *Tarzetta catinus* (Holmsk.) Korf & J.K. Rogers and *Geopyxis carbonaria* (Alb. & Schw.: Fr.) Sacc. have been hypothesized as ectomycorrhizal associates of *Fagus sylvatica* L. (Tedersoo et al. 2006) and *Picea abies* L. (Vrålstad et al. 1998).

Ectomycorrhizal anatomy is little studied in Pyronemataceae, with detailed morpho-anatomical descriptions being only available for species of *Genea*, *Humaria,* and *Tricharina*. While most of the species form pseudoparenchymatous outer mantle layers, as in *Genea*, *Humaria*, *Trichophaea*, and *Geopora* (Erős-Honti et al. 2008; Tedersoo et al. 2006), ectomycorrhizae (ECM) of *Pulvinula* and *Tricharina gilva* (Boud. ex Cooke) Eckblad (later identified as *Wilcoxina mikolae* Chin S. Yang & H.E. Wilcox by Egger 1996) form plectenchymatous outer mantle layers (Amicucci et al. 2001; Ingleby et al. 1990). Adistinction by ECM features is difficult, especially between *Genea* and *Humaria*, because the ECM of both genera share common morpho-anatomical features, like angular cells in the outer mantle, and emanating hyphae

Electronic supplementary material The online version of this article (doi:10.1007/s11557-009-0637-x) contains supplementary material, which is available to authorized users.

J. Wei (✉) · D. Peršoh · R. Agerer
Department Biology I, Division of Organismic Biology: Mycology, University of Munich,
Menzinger Str. 67,
80638 Munich, Germany
e-mail: weijie211@yahoo.com

Published online: 15 December 2009

being colorless and smooth when young and yellowish brown and warty when old (Erős-Honti et al. 2008; Tedersoo et al. 2006). In addition, identifications of ECM of *Genea* or *Humaria,* which were merely based on morpho-anatomical features, remain questionable, hence studies combining morphological and molecular approaches were demanded for a state-of-the-art identification of these ECM (Erős-Honti et al. 2008).

In the course of an investigation of the ECM communities on Chinese Pine (*Pinus tabulaeformis* Carr.), we found four anatomotypes, all of which have pseudoparenchymatous outer mantle layers, thick-walled, yellowish to brownish, warty emanating hyphae without clamps, and lack rhizomorphs. They are similar to some ECM previously reported in Pyronemataceae. In this study, their morpho-anatomical features are described in detail and molecular-phylogenetic analyses are applied to unravel their phylogenetic position. This is the first report of ECM in Pyronemataceae on Chinese Pine.

Materials and methods

Specimen sampling, ECM morphology and anatomy

Soil samples were collected in pure Chinese Pine forests at Helan Mountain (Yinchuan City, Ningxia Hui Nationality Autonomous Region, China) and at Daqing Mountain (Huhhot City, Inner Mongolia Autonomous Region, China) throughout 2 years. ECM systems were assigned to anatomotypes and described according to Agerer (1987–2008, 1991). Anatomical studies are based on at least 5 ECM for each anatomotype, and drawings were performed with the aid of a Normarski interference contrast microscope (Standard 14; Zeiss West Germany) connected with a drawing tube. All drawings were made at a magnification of ×1,000. Reference specimens of the mycorrhizae are deposited in M (see Holmgren et al. 1990).

DNA sequencing

One unramified end, previously fixed in CTAB, from each of the four morphotypes was used for DNA extraction following careful microscopical examination to ensure that the isolated DNA originates from the respective anatomotype. DNA of ECM was extracted using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. The nuclear rDNA (nrDNA) ITS and LSU regions were amplified using the PCR primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) and LROR and LR5 (Moncalvo et al. 2000), respectively. The obtained PCR product was purified using the QIAquick protocol (Qiagen), and fragments were sequenced applying the same primers as for the PCR. Sequencing was performed by the sequencing service of the Department Biology I (Ludwig-Maximilians-Universität, München) using BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1.

Sequence analyses

The most similar sequences were searched for in UNITE (Kõljalg et al. 2005, http://unite.ut.ee/) and GenBank (http://www.ncbi.nlm.nih.gov/) using megablast (Zhang et al. 2000). The 100 sequences most similar to each obtained LSU sequence were downloaded from GenBank. Duplicates, i.e. identical sequences found as closest relatives of different query sequences, were omitted. Using the software BioEdit v7.0.5 (Hall 2005), the sequences were automatically aligned. The alignment was revised manually and columns not alignable with certainty were excluded from the following analyses. A total of 209 unique LSU sequences were retained for further molecular phylogenetic analyses. RAxML (Stamatakis 2006) was used for searching the most likely tree and for mapping of the bootstrap support values (500 replicates) upon this tree. The GTRCAT model of substitution was applied for both analyses having Maximum Likelihood as optimality criterion. The most parsimonious trees were searched for by executing batch files generated by PAUPRat (Sikes and Lewis 2001) in PAUP* v4.0 (Swofford 2003), with weighting mode set to multiplicative. Twenty replicates and 500 iterations were conducted. A consensus tree was calculated of all trees with equal (minimal) length and the posterior probabilities were noted for each branch. The following positions, according to DQ220352 (*Humaria hemisphaerica* (F.H. Wigg.) Fuckel), were alignable with certainty throughout all 209 taxa included in the LSU alignment: 21–61, 75–95, 112–170, 185–407, 411–533, and 544–579. The ITS nrDNA sequence from "*P. humarioides*" was additionally aligned with 62 of the best matching sequences found by "megablast". The alignment subjected to the molecular-phylogenetic analysis included the reliably alignable positions: 110–144, 277–286, 290–300, 302–401, 403–480, 505–513, and 515–527 (according to EU819538, *H. hemisphaerica*).

Results

Morpho-anatomical descriptions

"Pinirhiza humarioides"

Morphological characters (Fig. 1a) Mycorrhizal systems up to 5 mm long, main axes 0.4–0.5 mm diam.,

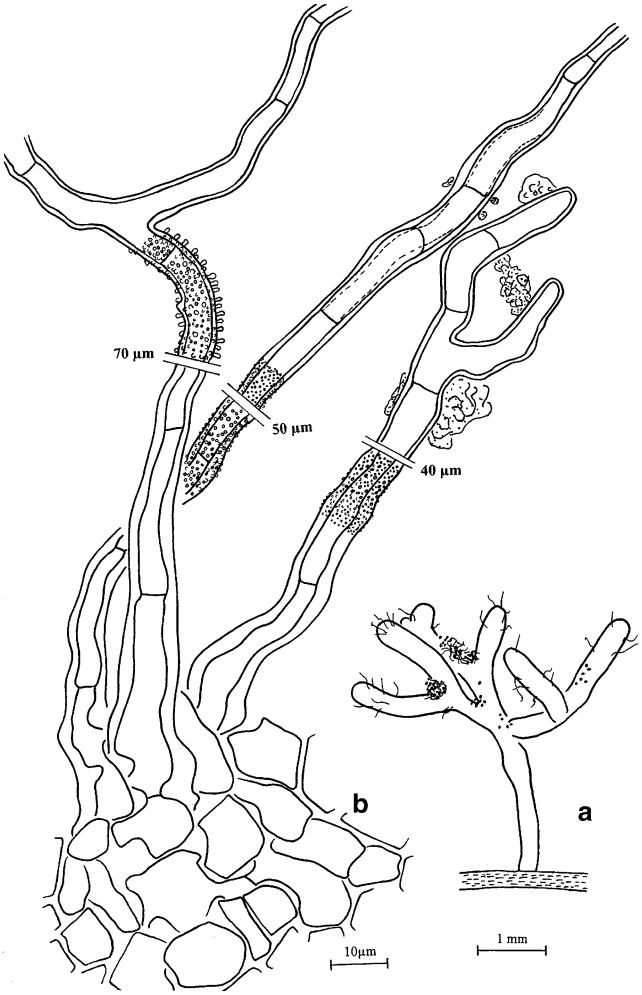


Fig. 1 "*Pinirhiza humarioides*": **a** habit of ectomycorrhiza, with few emanating hyphae and soil particles, **b** emanating hyphae with partially distinctly warty and partially smooth surface, intrahyphal hyphae present; note the cylindric, sometimes capitate warts

dichotomous, ramification orders 0–2 (3), hydrophilic, short distance exploration type. *Unramified ends* mostly straight, cylindric, sometimes slightly inflated at very tips, 0.4–1.3 mm long, 0.4 mm diam., brown when young, dark brown to black when older; surface of unramified ends loosely woolly, covered with few soil particles, mantle not transparent. *Emanating hyphae* infrequent, brownish under dissecting microscope. *Cystidia* lacking. *Rhizomorphs* lacking. *Sclerotia* absent.

Anatomical characters of mantle in plan views (Figs. 2 and 3) Mantle surface (Fig. 2a, b) formed by a very thin, at places incomplete, pseudoparenchymatous layer composed of inflated, often irregularly shaped cells arranged in rows, 3–10 µm diam., with very thick cell walls, (1) 3–5 (11) µm wide, septa small and thin, surface with many soil particles. *Outer mantle layer* (Fig. 3a) pseudoparenchymatous with angular cells (mantle type L/M, according to Agerer 1987−2008, 1991; Agerer and Rambold 2004–2009), neighboring cells sometimes connected by small and thin septa like in mantle surface, cells membranaceously yellowish to brownish, with infrequent solitary cells filled with granular contents, surface smooth, variable in dimension, 8–23 µm long, 5–8.5 (12) µm wide, cell walls 0.5–1.8 (2.5) µm. *Inner mantle layer* (Fig. 3b) plectenchymatous, hyphal cells 3.5–5 µm diam., membranaceously yellowish. *Very tip* (apex of the tip) organized like remaining parts.

Anatomical characters of emanating elements (Figs. 1b and 4) Rhizomorphs lacking. *Emanating hyphae* (Figs. 1b and 4) infrequent, originating from a hyphal cell of outer mantle, distal of ramification point always sinuous; septa simple and frequent, thinner than walls; walls thick, uneven; ramifications frequent, angle of ramification acute to rectangular, side-branches thinner in diameter than the main hypha; main hyphae 7–11 µm diam., up to 14 µm at the base, cell walls 2–2.5 µm; side-branches 3–5.5 µm diam., cell walls 0.5–1.5 µm; cells (15) 23–35 (45) µm long; surface of hyphae with alternating smooth and warty

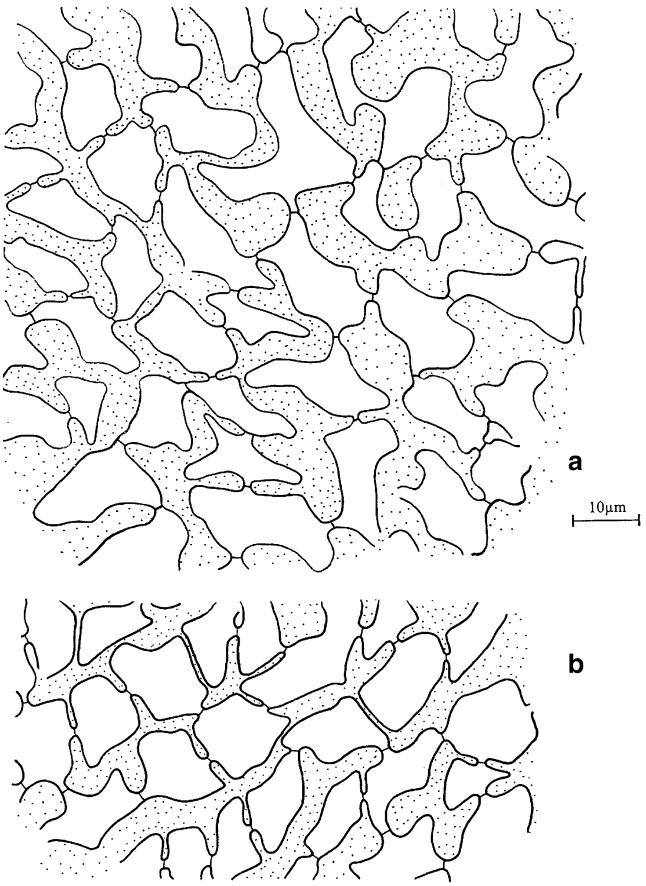


Fig. 2 Surface layer of outer mantle of "*Pinirhiza humarioides*": **a** hyphal cells variable in shape, cells connected by thin septa and arranged in irregular rows, **b** with more regularly-shaped hyphal cells like in outer mantle layer

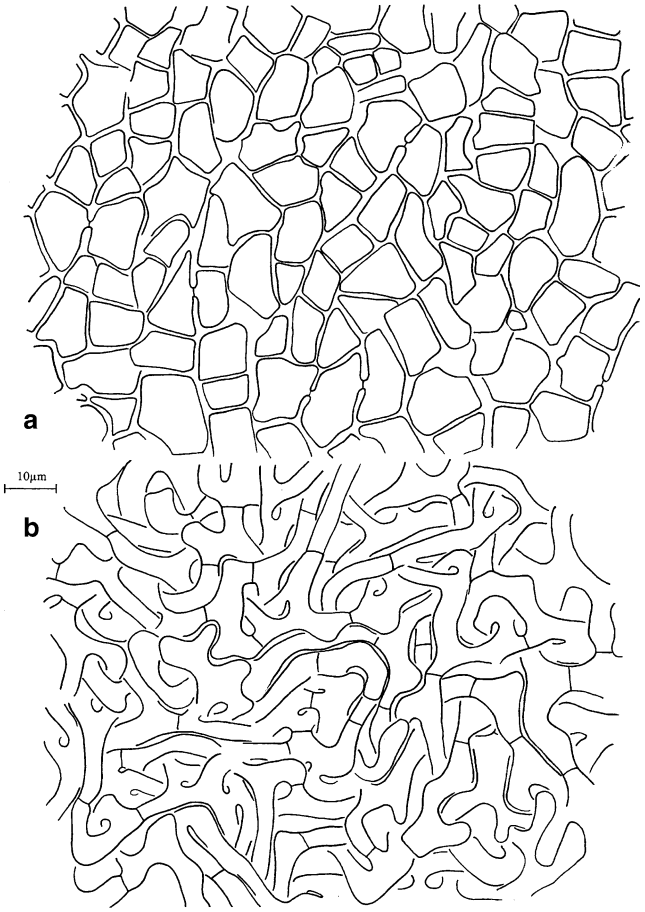


Fig. 3 Mantle layers of "*Pinirhiza humarioides*": **a** outer mantle layer with angular cells, some of them connected by thin septa, cells arranged in rows, **b** plectenchymatous inner mantle layer

portions, rough areas with cylindrical or capitate warts up to 1 µm long and up to 1.1 µm diam. at apex; tip of hyphae simple or ramified, often with some adhering soil particles; hyphae brownish when old, yellowish to colourless when young. *Cystidia* lacking. *Clamydospores* lacking.

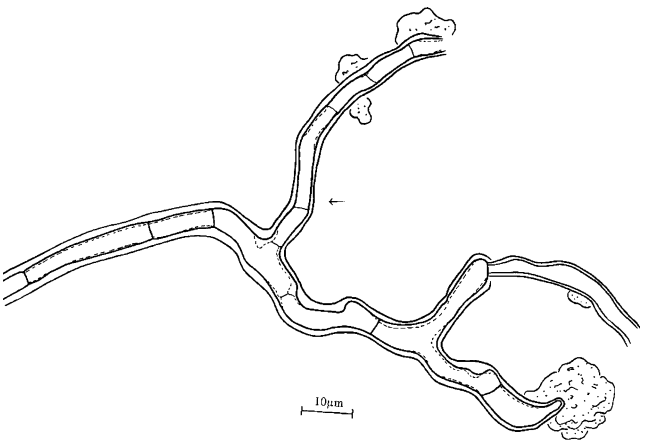


Fig. 4 Emanating hyphae of "*Pinirhiza humarioides*": angle of ramification ca.90°, one hyphal branch is thinner than the main hypha (*arrow*)

Colour reactions with different reagents Preparations of mantle: Melzer's reagent: n.r. (= no reaction); lactic acid: n.r.; KOH: n.r.; $FeSO_4$: n.r.

Reference specimen The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Helan Mountain, Suyukou National Reserve located in Yinchuan City, Ningxia Hui Nationality Autonomous Region, China, myc. exc. and isol. by Jie Wei, 09.09.2008, JW 189d (in M). Sequences obtained: ITS (GQ281479) and LSU (GQ281475).

"Pinirhiza daqingensis"

Morphological characters (Fig. 5a) Mycorrhizal systems 3–5 (8) mm long, main axes 0.4–0.5 mm diam., dichotomous or irregularly ramified, ramification order 0–6, contact to short distance exploration type, hydrophilic. *Unramified ends* straight, irregularly inflated, constricted between old and young parts, 1.0–3.5 mm long, 0.3–

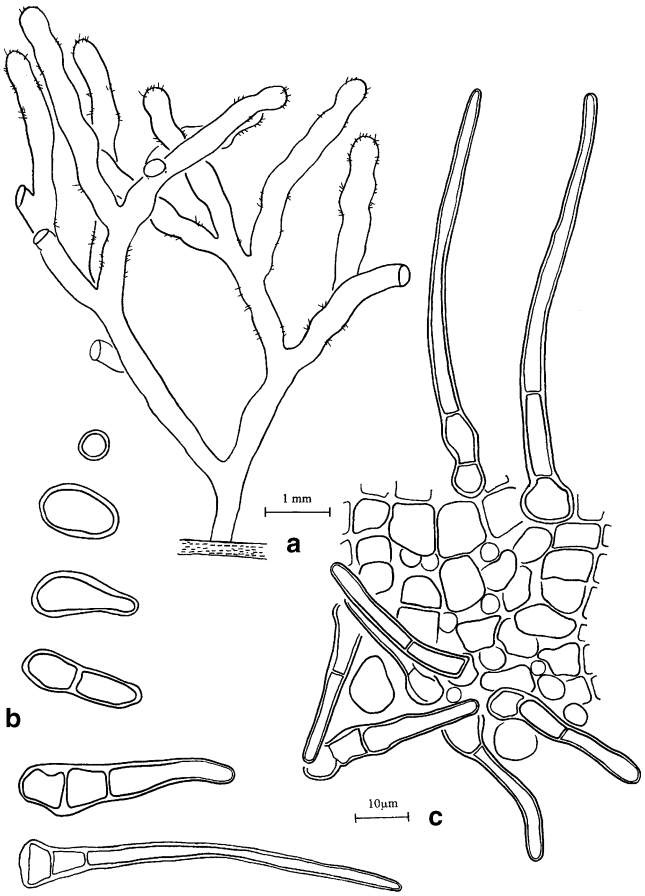


Fig. 5 "*Pinirhiza daqingensis*": **a** habit of ectomycorrhiza, surface of mantle partially densely short spiny, **b** different developmental stages from roundish cells to cystidia, **c** awl-shaped cystidia on outer mantle layer together with some roundish cells arranged in patches

0.5 mm diam., younger parts yellowish brown, older parts reddish brownish, mantle not transparent, cortical cells not visible. *Surface of unramified ends* loosely short-spiny, cystidia not specifically distributed, concolorous to mantle. *Emanating hyphae* infrequent. *Rhizomorphs* lacking. *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figs. 6 and 7) Outer mantle layers (Fig. 6a, b) pseudoparenchymatous with angular cells and many roundish cells on the mantle surface, solitary or arranged in groups that can bear prominent cystidia (mantle type K, according to Agerer 1987–2008, 1991; Agerer and Rambold 2004–2009), some small areas of mantle slightly depressed; hyphal cells partially arranged in rows, sometimes also star-like structures present; roundish cells 4–11 µm in diam., cell walls 0.5 µm thick, cells of the outer mantle layer 6–15 µm wide and 13–24 µm long; cells membranaceously yellowish to brownish, surface smooth. *Inner mantle layers* (Fig. 7) plectenchymatous with few angular cells, cylindric hyphae 6–8 µm diam., angular cells 12.5–25 µm long, 5.5–15 µm wide, colourless and smooth. *Very tip* similar to remaining parts of the mantle.

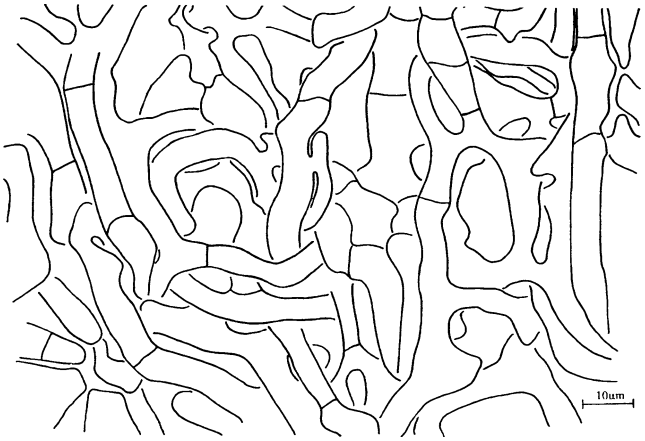


Fig. 7 "*Pinirhiza daqingensis*": plan view of inner plectenchymatous mantle layer

Anatomical characters of emanating elements (Figs. 5b, c and 8) Rhizomorphs lacking. *Emanating hyphae* (Fig. 8)

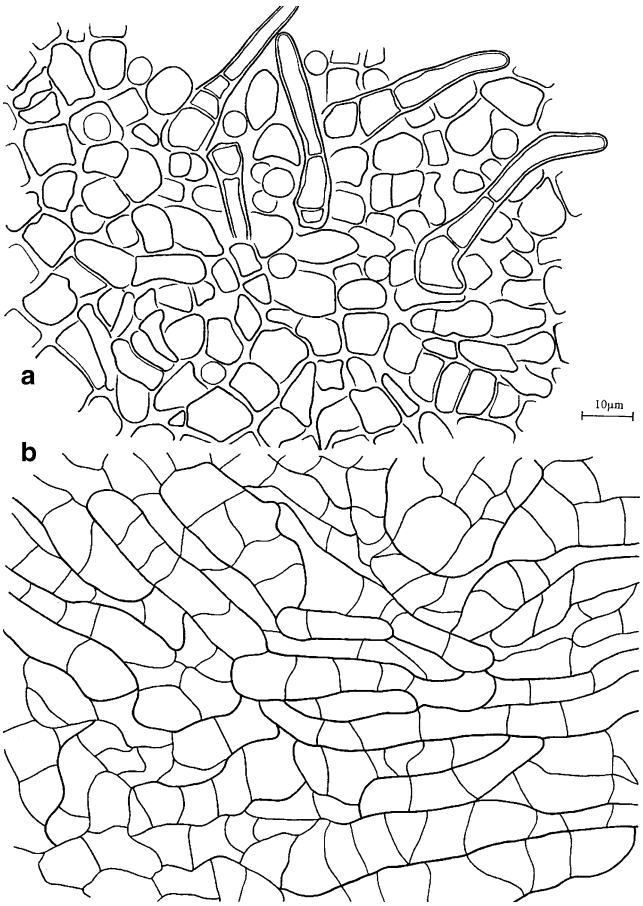


Fig. 6 Outer mantle layer of "*Pinirhiza daqingensis*": **a** some cells forming heaps and cystidia, **b** plan view of outer mantle layer, hyphal cells arranged in rows (thickness of cell walls not shown)

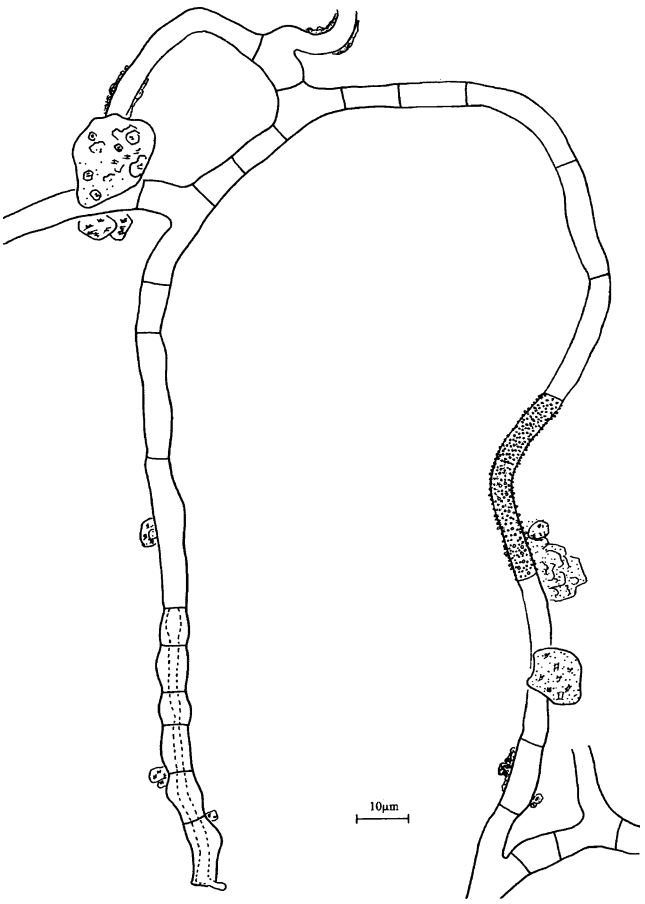


Fig. 8 Emanating hyphae of "*Pinirhiza daqingensis*" with frequent simple septa and some adhering soil particles, warts and intrahyphal hyphae

infrequent, (3) 6.5–7.5 µm diam., cell walls 0.5 µm, hyphal cells short, distance of septa (9) 13–17 (20) µm, septa simple; hyphae frequently ramified, angle of ramification acute or sometimes ca. 90°; surface mucilaginous with many soil particles and warty, warts cylindrical, up to 1.5 µm long and 0.5–1 µm wide; hyphae membranaceously yellowish; intrahyphal hyphae present. *Cystidia* (Fig. 5b, c) very frequent (on very tip infrequent), bottle- to awl-shaped, with a strongly inflated base and a long torn-out neck, neck separated from inflated body by a septum, inflated body 10–11.5 µm diam. and with 0.5–1 µm thick walls, necks 2.5–3.5 µm wide and with 0.5 µm thick walls, cell wall at very tip thinner than at remaining parts; cystidia 21–54 (90) µm long, neck with 1–3 septa, membranaceously yellowish to brownish, some crystals present on surface. *Chlamydospores* lacking.

Colour reactions with different reagents Preparations of mantle: Melzer's reagent: n.r.; lactic acid: n.r.; KOH: n.r.; $FeSO_4$: n.r.

Reference specimen The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Daqing Mountain, Guluban located in Huhhot city, Inner Mongolia Autonomous Region, China, myc. exc. and isol. by Jie Wei, 07.26. 2007, JW 76a (in M). Sequences obtained: ITS (GQ281480) and LSU (GQ281476).

"Pinirhiza geoporoides"

Morphological characters (Fig. 9a) Mycorrhizal systems dichotomous, with 0–3 orders of ramification, solitary or in few numbers, main axis 0.4–0.5 mm diam., contact to short distance exploration type, hydrophilic. *Unramified ends* straight or bent, cylindric, not inflated, 0.5–1.8 mm long, 0.35 mm diam., yellowish brown, older parts dark brown; mantle not transparent, loosely woolly. *Emanating hyphae* infrequent. *Rhizomorphs* absent. *Cystidia* lacking. *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Figs. 10 and 11) Outer mantle layers (Fig. 10a, b) pseudoparenchymatous with angular cells, in some parts cells arranged in rows and in some parts forming heaps, and with few solitary, roundish, thick-walled (0.5 µm) cells (mantle type K, according to Agerer 1987−2008, 1991; Agerer and Rambold 2004–2009), surface of mantle with a gelatinous matrix gluing many soil particles, roundish cells 5–7 µm in diam., other cells 12–23.5 µm long, and 6.5–13 µm wide, walls 0.3–0.5 µm thick, surface smooth, membranaceously brownish, plasmatically brownish when old, but colorless when young. *Middle mantle layer* (Fig. 11a) transitional

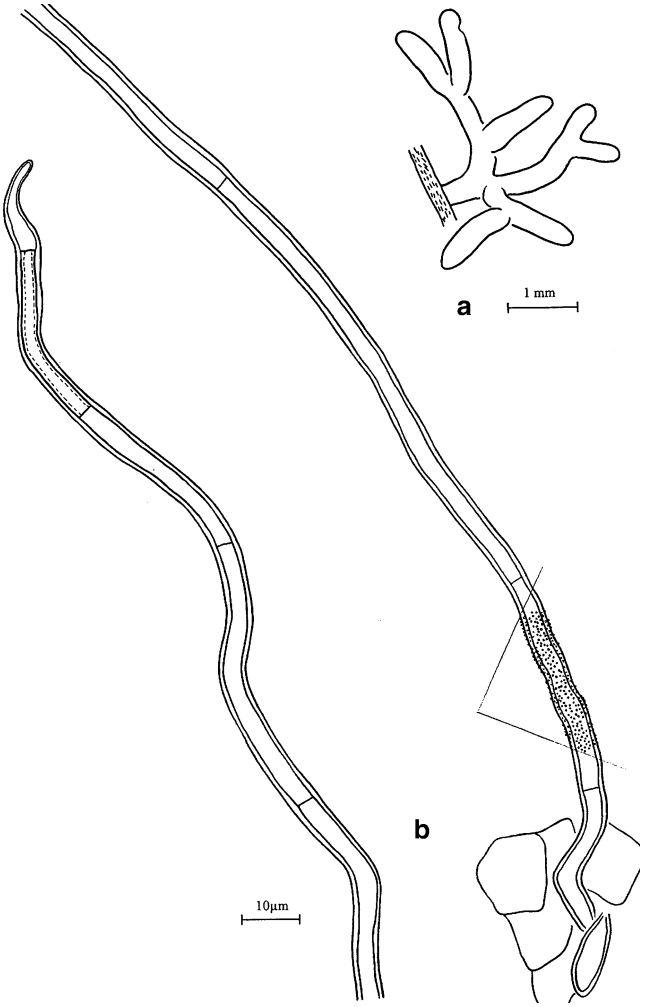


Fig. 9 "*Pinirhiza geoporoides*": **a** habit of ectomycorrhiza, **b** emanating hyphae originating from cells of outer mantle, surface partially warty, intrahyphal hyphae present

between pseudoparenchymatous with mostly angular cells and few irregularly shaped cells and short hyphal cells intermixed, some parts forming ring-like structures, cells membranaceously yellowish to brownish, 7–15 µm long and 6.5–11 µm wide. *Inner mantle layers* (Fig. 11b) plectenchymatous, hyphae ring-like arranged, cells 3–3.5 µm wide, with some thicker hyphae up to 5.5 µm diam., cell walls thin, hyphae connected by anastomoses, membranaceously yellowish. *Very tip* like remaining parts of mantle.

Anatomical characters of emanating elements (Fig. 9b) Rhizomorphs lacking. *Emanating hyphae* (Fig. 9b) very infrequent, originating directly from an outer mantle layer cell, at the base not thicker than at other parts, 5–5.5 µm diam., cells (23) 27–45 (60) µm long, thick-walled, walls 1–1.5 µm, brownish, finely warty, simple septa, few soil particles adhering, intrahyphal hyphae present. *Cystidia* lacking. *Chlamydospores* lacking.

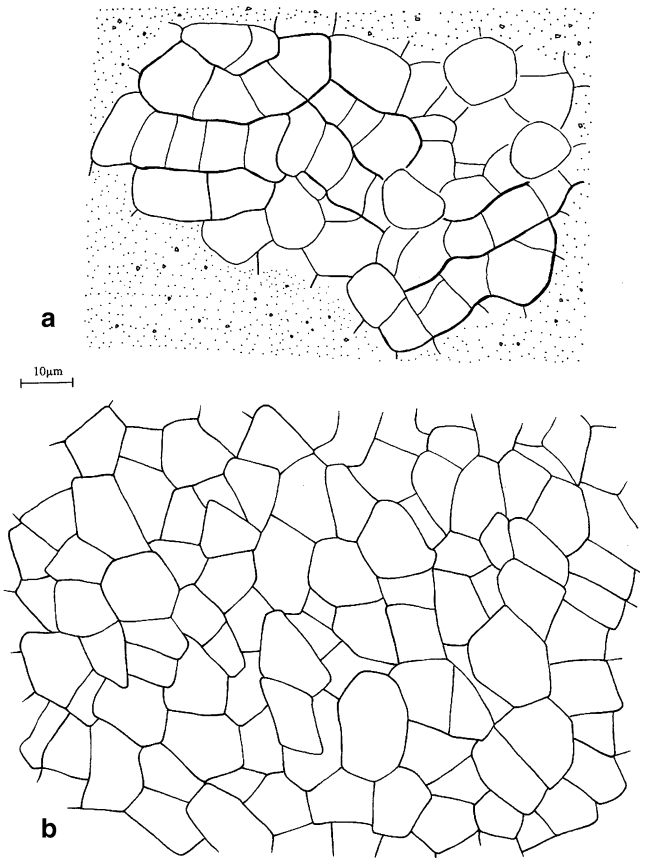


Fig. 10 Outer mantle layer of "*Pinirhiza geoporoides*",: **a** some hyphal cells arranged in rows, surface of outer mantle with matrix and few adhering particles (shown only at the margins of the mantle piece), **b** plan view of outer mantle layer

Colour reactions with different reagents Preparations of mantle: Melzer's reagent: n.r.; lactic acid: n.r.; KOH: n.r.; $FeSO_4$: n.r.

Reference specimen The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Daqing Mountain, Guluban located in Huhhot city, Inner Mongolia Autonomous Region, China, myc. exc. and isol. by Jie Wei, 06.08.2008, JW 96a (in M). Sequences obtained: ITS (GQ281481) and LSU (GQ281477).

"Pinirhiza trichophaeoides"

Morphological characters (Fig. 12a) Mycorrhizal systems 1.2–2.5 mm long, dichotomous, with 0–3 orders of ramification, solitary or in small numbers, main axes 0.3–0.45 mm diam., short distance exploration type, hydrophilic. *Unramified ends* straight, cylindric, not inflated, 0.2–1.8 mm long, 0.3–0.4 mm diam., reddish brown, very tips lighter, greyish, older parts dark brown or black; mantle not transparent, loosely to densely long-woolly. *Emanating hyphae* frequent. *Rhizomorphs* absent. *Cystidia* lacking. *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Figs. 13, 14) Outer mantle layers (Fig. 13a) pseudoparenchymatous with angular cells and with oval to polygonal cells forming heaps, and also with solitary, small, roundish cells, 6–8 µm in diam., with 0.5–1 µm thick walls (mantle type K, according to Agerer 1987–2008, 1991; Agerer and Rambold 2004–2009), cells of outer mantle layer 9–23 µm long and 4–15 µm wide, walls 0.5–1 µm; surface smooth, membranaceously brownish, plasmatically brownish when old, but colourless when young, mantle surface with many soil particles. *Middle mantle layers* (Fig. 13b) pseudoparenchymatous with angular cells, without special arrangement, cells membranaceously yellowish to brownish, 10–22 µm long and 5–12 µm wide. *Inner mantle layers* (Fig. 14) transitional between pseudoparenchymatous with epidermoid cells and plectenchymatous, without pattern, cells membranaceously yellowish to brownish, plasmatically brownish, cells 3.5–7 µm diam. *Very tip* like remaining parts of mantle.

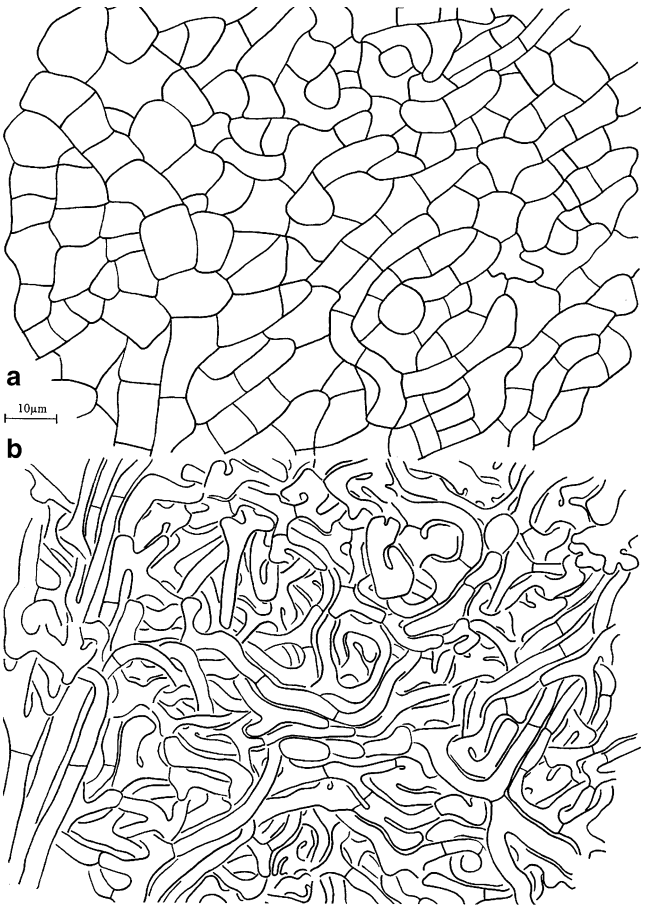


Fig. 11 "*Pinirhiza geoporoides*",: **a** plan view of middle mantle layer, cells of some parts arranged in rows, **b** plan view of inner mantle layer, plectenchymatous with ring-like structures

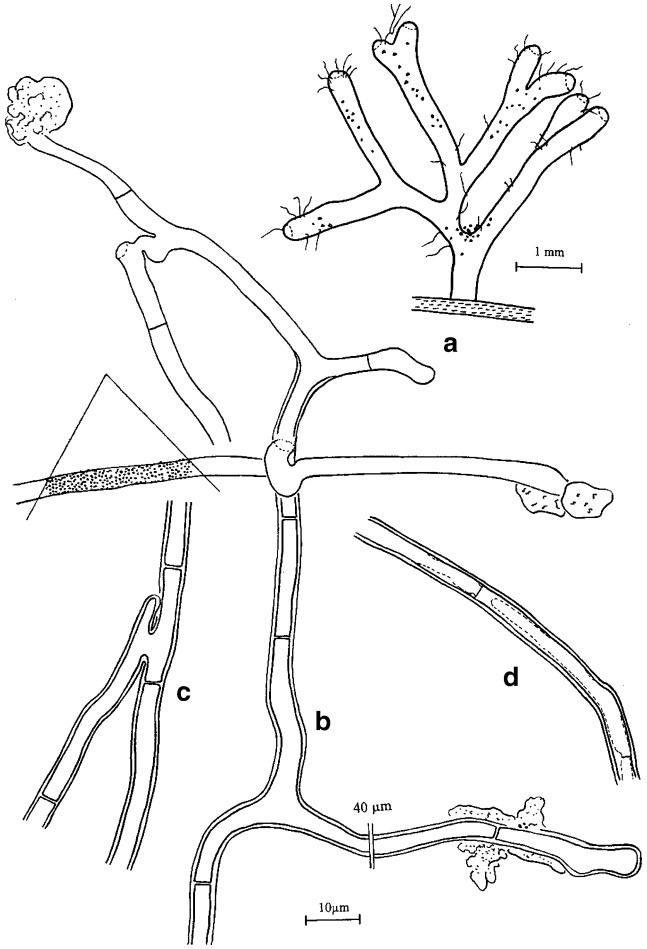


Fig. 12 "*Pinirhiza trichophaeoides*",: **a** habit of ectomycorrhiza, surface of mantle partially densely woolly, **b** emanating hyphae with partially warty and partially smooth surface and with some adhering soil particles, anastomoses open and with a long bridge, **c** emanating hyphae, anastomosis open and with a short bridge, **d** intrahyphal hyphae

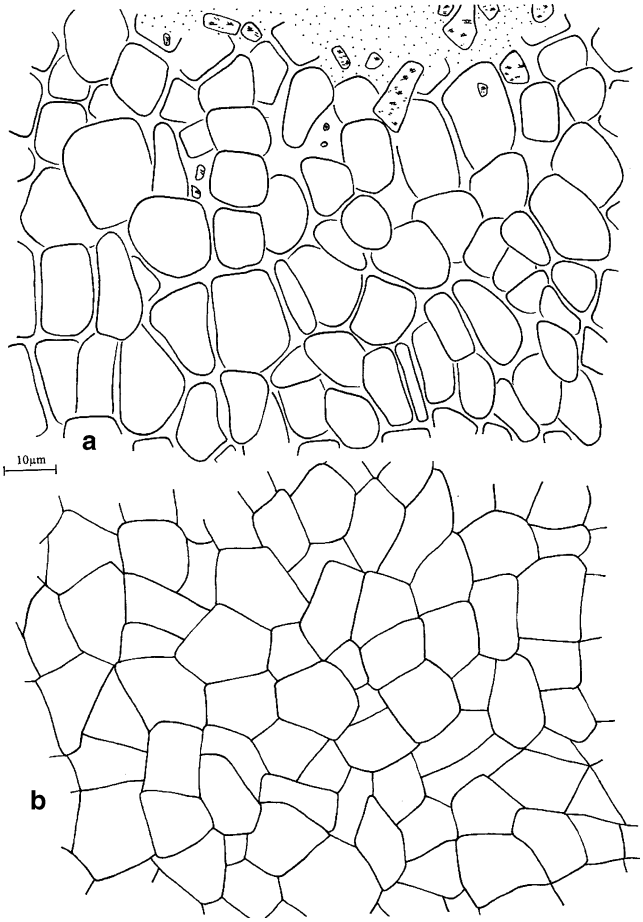


Fig. 13 "*Pinirhiza trichophaeoides*",: **a** plan view of outer mantle layer, roundish and polygonal cells forming heaps, **b** plan view of middle mantle layer

Anatomical characters of emanating elements (Fig. 12b–d) *Rhizomorphs* lacking. *Emanating hyphae* (Fig. 12b–d) frequent, originating directly from an outer mantle layer cell, 3.5–5 (8) µm diam., cells (18) 30–55 (65) µm long, cell walls 0.5–1 (1.5) µm; frequently ramified, angle of ramification ca. 90°; anastomoses open with a short or long bridge, anastomoses smooth; septa simple, thinner than walls; hyphae partially smooth and partially warty, often smooth near the origin; hyphae plasmatically brownish when old, membranaceously yellowish to colorless when young; very tip of hyphae simple, sometimes slightly swollen, often with adhering soil particles; intrahyphal hyphae present. *Cystidia* lacking. *Clamydospores* lacking.

Colour reactions with different reagents Preparations of mantle: Melzer's reagent: n.r.; lactic acid: n.r.; KOH: n.r.; $FeSO_4$: n.r.

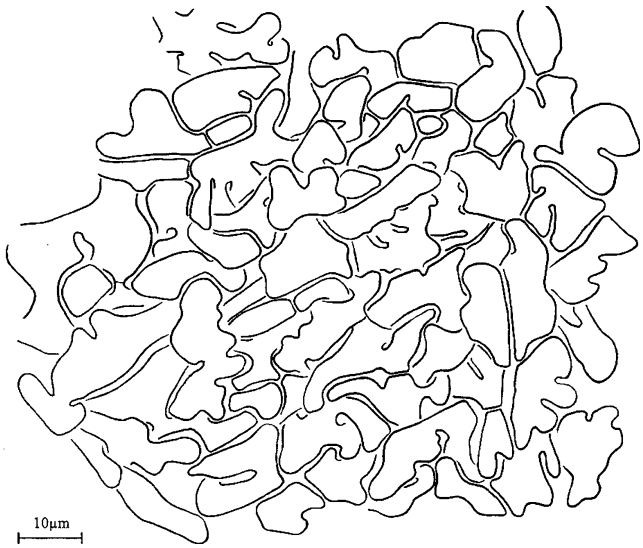


Fig. 14 Plan view of inner mantle layer of "*Pinirhiza trichophaeoides*"

Reference specimen The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Helan Mountain, Suyukou National Reserve located in Yinchuan City, Ningxia Hui Nationality Autonomous Region, China, myc. exc. and isol. by Jie Wei, 16.08.2007, JW 44a (in M). Sequences obtained: ITS (GQ281482) and LSU (GQ281478).

Sequence analyses

The results of the Blast searches among the sequences deposited in GenBank and in UNITE were ambiguous for both loci, i.e. a reliable assignment of any sequence to a certain taxon was not possible. Therefore, these results are not discussed in detail here, but the results of the molecular-phylogenetic analyses of all best matching LSU nrDNA sequences are presented below. The results of the analyses of the ITS regions were in accordance with those of the LSU analyses, but revealed additional information in the following cases. Eleven sequences showing more than 80% similarity and at least 75% coverage to GQ281482 ("*Pinirhiza trichophaeoides*") were found by the Blast Search in GenBank, the best matching sequence of which was an uncultured orchid mycorrhiza described as a member of Pyronemataceae (AY634164), with 90% similarity of a fragment accounting for 99% of the total sequence. Eight of the best matches were obtained from uncultured mycorrhizae and one from *Trichophaea woolhopeia* (Cooke & W. Phillips) Arnould (typus generis). Morpho-anatomical description in detail is available for 1 of the 11 sequences: "*Quercirhiza quadratum*" (EU822505). Detailed morpho-anatomical description with reference to the ITS sequence (EU024883) of BP98701 (*Genea* ECM) is also available, the sequence of which is identical to AJ969624, an ITS sequence of specimen TL6764 (*Genea verrucosa*), an LSU sequence of which is included in our analyses.

Of the 503 reliably alignable positions of the LSU nrDNA Alignment, 268 were variable and 226 were parsimony informative. The 50% Majority Rule Consensus Tree (2,057 steps, CI=0.245, RI=0.773) was calculated of the 3,341 most parsimonious trees (2,050 steps). The likelihood of the most likely tree (length: 0.682483) found was −1,295.151293 and the substitution rates estimated by RAxML were: A↔C: 0.964615, A↔G: 2.335736, A↔T: 0.825886, C↔G: 0.464748, C↔T: 2.895288, and G↔T: 1.

The topology of the molecular-phylogenetic trees calculated from the LSU sequence data is similar to the results of an extensive study on the relationships within the Pyronemataceae (Perry et al. 2007), and therefore not discussed in detail here. Nevertheless, the trees calculated using Parsimony Ratchet and RAxML are both available as electronic supplementary material (ESM) files (Figs. 17 and 18). The topologies of both trees are largely in accordance with respect to the obtained sequences. Therefore, the results are presented for the RAxML analysis, only, if not explicitly stated otherwise. Two sections from the RAxML tree, concerning the ECM being subject of this study, are shown in Figs. 15 and 16.

"*Pinirhiza trichophaeoides*" clusters with *Trichophaea woolhopeia* with 100% bootstrap support (BS) (Fig. 15). The sister group to this grouping includes further species of *Trichophaea*, *Sphaerosporella* spp., and *Anthracobia subatra* (Rehm) M.M. Moser rendering *Trichophaea* and *Sphaerosporella* paraphyletic. Sister group to these two clades, the monophyly of which is supported with 90% BS, form additional species of *Anthracobia*. Accordingly, neither genus included in the *Trichophaea-Sphaerosporella-Anthracobia* clade appears to be of monophyletic origin. The majority of *Geopora* spp. included form a clade (50% BS) together with "*Pinirhiza geoporoides*" (Fig. 15) and three sequences deposited as *Pezizales* spp. described by Tedersoo et al. (2006) as species of *Geopora*. "*Pinirhiza daqingensis*" is sister group to this clade, to which three *Tricharina* spp. cluster as sister group, in turn. At the root of the *Geopora-Tricharina* clade (92% BS), a second *Geopora* lineage, *G. pellita* (Cooke & Peck) T. Schumacher branches off. Because sequences of the type species of *Tricharina*, *T. gilva*, form one of two sister groups to the *Geopora-Tricharina* clade, neither *Geopora* nor *Tricharina* represent monophyla according to their current circumscriptions. According to the Parsimony Ratchet analysis, "*Pinirhiza daqingensis*" and *G. pellita* form a monophyletic group nested within a clade comprising two *Tricharina* sequences at the basis (ESM, Fig. 18). "*Pinirhiza humarioides*" is nested within the *Genea-Humaria* clade with 100% BS (Fig. 16). It clusters with an uncultured ECM from Japan as sister group to *Humaria hemisphaerica* and *Humaria* ECM from Europe with moderate support (75%). Molecular-phylogenetic analysis of the ITS region from "*P. humarioides*" resulted in a similar tree topology (results not shown), according to which it also clustered as sister group to a clade of *Humaria* spp., but with 83% BS.

Discussion

The four yellowish brown to brown ECM have pseudoparenchymatous outer mantles, dark coloured, warty, thick-walled and clampless emanating hyphae, and lack rhizomorphs. Nevertheless, they can be separated according to their anatomical features.

1 Surface of mantle with frequent bottle-shaped to awl-shaped cystidia; emanating hyphae with frequent simple septa, cells (9) 13–17 (20) µm long
"*P. daqingensis*"

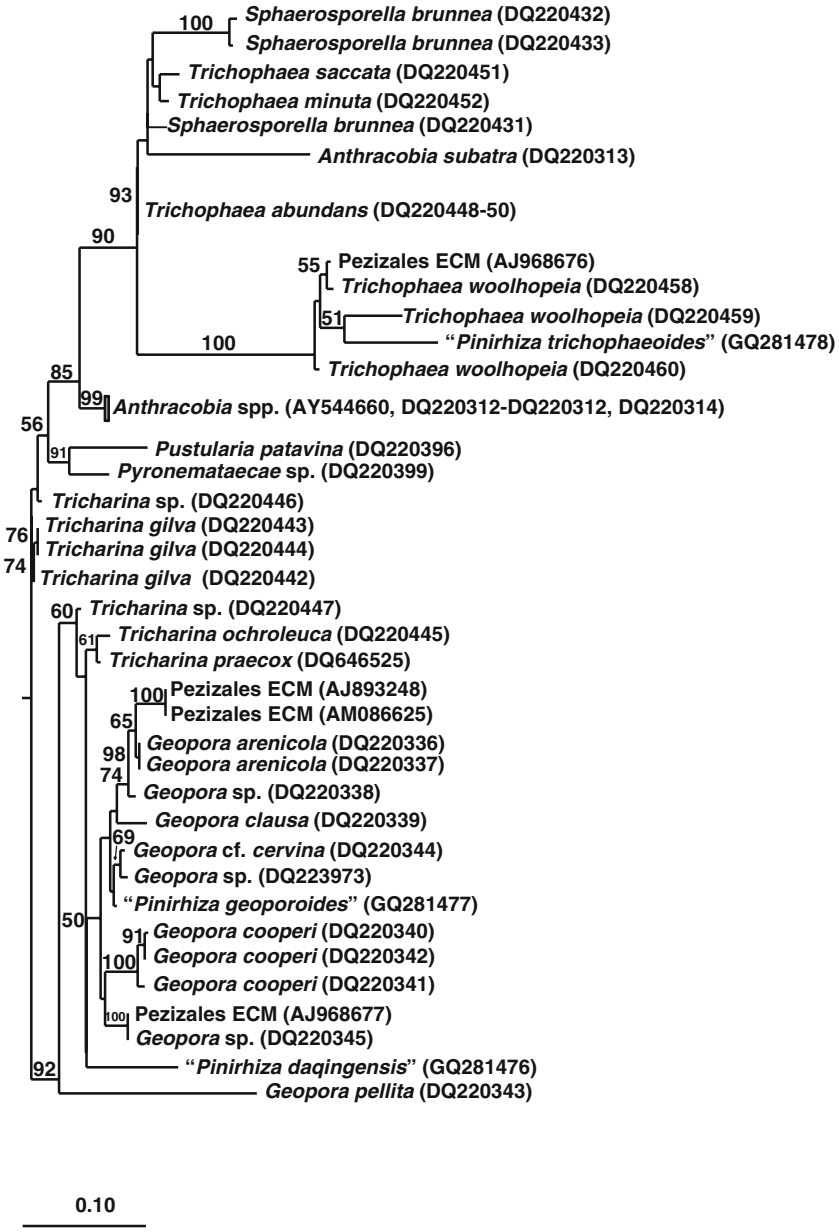


Fig. 15 Molecular-phylogenetic placement of "*P. trichophaeoides*", "*Pinirhiza geoporoides*" and "*P. daqingensis*" among selected Pyronemataceae. Section of the best scoring tree found by the RAxML analysis of LSU nrDNA sequences. Bootstrap support values above 50% are noted *above* or to the *left* of the respective branches. GenBank accession numbers are given in parentheses following the species names

1* Surface of mantle without cystidia; emanating hyphae with less frequent simple septa (cells mostly >20 µm long)

2 Mantle with a very thin, at places incomplete, pseudoparenchymatous surface layer composed of inflated, often irregularly shaped cells arranged in rows

"*P. humarioides*"

2* Mantle such a surface layer lacking, but instead with heaps of oval to polygonal cells

3 Hyphal cells in outer mantle layer or in middle mantle layer arranged in distinct rows; inner mantle layer plectenchymatous with ring-like arranged hyphae

"*P. geoporoides*"

3* Hyphal cells in outer mantle layer or in middle mantle layer not arranged in distinct rows; inner mantle layer pseudoparenchymatous with epidermoid cells

"*P. trichophaeoides*"

"*Pinirhiza daqingensis*" is furnished with many solitary, thick-walled, roundish cells laying on the outer mantle layer and bearing abundant bottle- to awl-shaped cystidia. The hyphal cells of the outer mantle are arranged in distinct rows, and the cells of its emanating hyphae are unusually short and are. at 6–8 µm ,very wide. All other ECM described here lack cystidia and, if roundish cells lie on the mantle surface, they mostly form heaps and possess thinner walls than those of "*P. daqingensis*". Very evident features of "*P. humarioides*" are the irregularly shaped cells of the

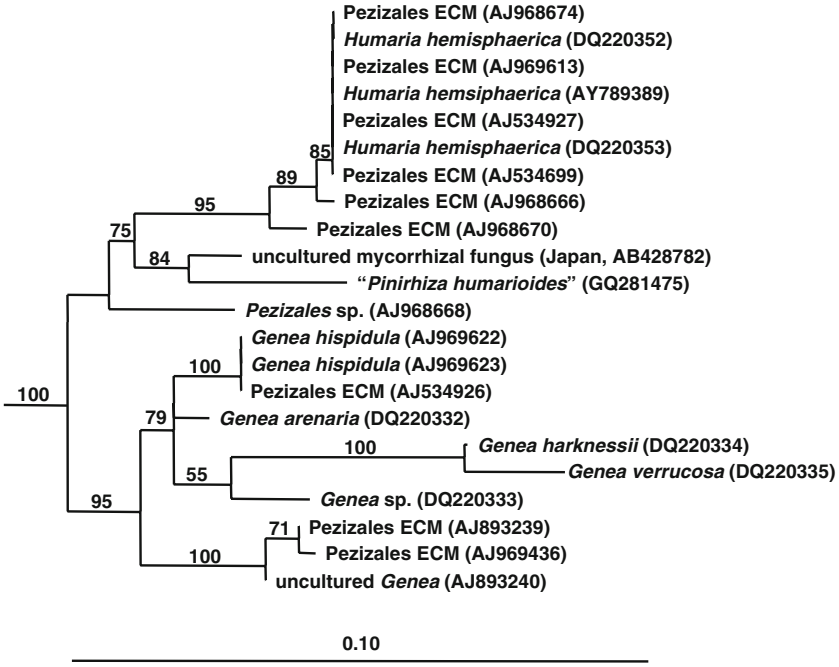


Fig. 16 Molecular-phylogenetic placement of "*P. humarioides*" among selected Pyronemataceae. Section of the best scoring tree found by the RAxML analysis of LSU nrDNA sequences. Bootstrap support values above 50% are noted *above* or to the *left* of the respective branches. GenBank accession numbers are given in parentheses following the species names

outermost mantle layer with very thick cell walls. Although this layer may appear discontinuous, it is very distinctive due to thin septa between cells that indicate row-like arrangements of apparently formerly distinct hyphae. Also very specific areo the emanating hyphae, with a thicker main hypha in comparison to the side-branch and with warts that sometimes even form a head-like inflation at their tip. Although "*P. geoporoides*" and "*P. trichophaeoides*" are both characterized by heaps of cells on a pseudoparenchymatous mantle with roundish to angular cells, they differ in the arrangement of the mantle cells, forming distinctive rows in "*P. geoporoides*", which are lacking in "*P. trichophaeoides*". The structure of the inner mantle layer also tells both ECM apart. The ring-like arrangement of a plectenchymatous layer is characteristic for "*P. geoporoides*", whereas that of "*P. trichophaeoides*" is pseudoparenchymatous with irregularly shaped, even epidermoid cells. Many frequently perpendicularly branched side-branches of emanating hyphae are typical of "*P. trichophaeoides*" in contrast to those of "*P. geoporoides*", which are only scarcely ramified. In addition, the emanating hyphae of "*P. geoporoides*" are 5–5.5 µm wide, whereas those of "*P. trichophaeoides*" measure only 3.5–5 µm, but can exceptionally reach 8 µm in diameter.

The data background was insufficient to obtain conclusive results based on the Blast Search results alone. From the results of the phylogenetic analyses, however, first congruencies of morpho-anatomical and molecular data arise, even though the taxon selection as represented by the sequence data available from GenBank is still rather fragmentary (Brock et al. 2009).

"*Pinirhiza humarioides*" clusters within a clade consisting of *Humaria* and *Genea* spp. in the molecular-phylogenetic analyses of the nrDNA LSU (Fig. 16) and ITS (100% bootstrap support, data not shown). ECM in this *Humaria* and *Genea* clade are all brown in color, with a pseudoparenchymatous mantle with angular cells, some of which are connected by thin septa and show partially smooth and partially warty emanating hyphae, which corresponds very well to the results of the molecular-phylogenetic analyses. Except for *Genea hispidula*, all ECM of *Humaria* and *Genea* described in detail show the typical mantle of "*P. humarioides*", the surface of which consists of inflated, often irregularly shaped, extremely thick-walled cells, serially connected by very thin septa (Brand 1991; Erős-Honti et al. 2008; Jakucs et al. 1998; Tedersoo et al. 2006). However, "*Pinirhiza humarioides*" has a clearly plectenchymatous inner mantle layer, whereas all those ECM have a pseudoparenchymatous inner mantle layer with epidermoid cells, or a transitional type between plectenchymatous and pseudoparenchymatous. This is consistent with the phylogenetic position of "*Pinirhiza humarioides*" clustering with an undescribed ECM fungus from Japan (AB428782) as a sister group to the *Humaria* clade and even more distant to *Genea* (Fig. 16). Therefore, neither morpho-anatomical nor molecular data support or reject an inclusion of "*P. humarioides*" in the genus *Humaria*. Nevertheless, an assignment of "*P. humarioides*" to the currently monophyletic genus *Genea* would render *Genea* paraphyletic according to the molecular-phylogenetic analyses.

"*Pinirhiza geoporoides*" and "*Pinirhiza daqingensis*" cluster within the well-supported *Geopora-Tricharina* clade. Their exact position, however, remains unclear due to the generally low supported internal branches of the RAxML tree and a diverging topology in the parsimony tree. Like the two "*Pinirhiza* spp.", the two morpho-anatomically described ECM of this clade, *Pezizales* spp. (AJ893248 and AM086625), have a pseudoparenchymatous outer mantle, the cells of which are row-like arranged (Tedersoo et al. 2006). However, it could not be ascertained, based on the available data and descriptions, whether the species of the *Geopora-Tricharina* clade show a similar anatomy with regard to the outer mantle.

"*Pinirhiza trichophaeoides*" forms a 100% supported monophylectic clade (Fig. 15) with *Trichophaea woolhopeia* (typus generis) and a *Pezizales* sp. described as *Trichophaea* sp. (Tedersoo et al. 2006). "*P. trichophaeoides*" is similar to this *Pezizales sp*. with regard to the pseudoparenchymatous outer mantle layer with heaps of oval to polygonal cells (Tedersoo et al. 2006), while comparative data for *T. woolhopeia* are missing. *Sphaerosporella brunnea* (Alb. & Schwein.) Svrček & Kubička, the only morpho-anatomically well-described species of *Sphaerosporella*, representatives of which cluster in the sister group, was also reported to have a pseudoparenchymatous outer mantle layer (Danielson 1984). However, the corresponding photographs may also be interpreted to show a plectenchymatous structure. Nevertheless, the *Dichobotrys* anamorph and smooth ascospores are common features of both genera (Perry et al. 2007). *Anthracobia* is the only genus in the *Geopora-Tricharina-Trichophaea-Sphaerosporella* clade not reported to form mycorrhiza. While the *Trichophaea-Sphaerosporella* clade is positioned distant to the *Geopora-Tricharina* clade according to the Parsimony analysis, one *Anthracobia* sequence (DQ220313, *Anthracobia subatra*) also clusters there within *Trichophaea*. Therefore, it seems likely that future studies will reveal at least *A. subatra* and possibly further *Anthracobia* spp. to form ECM like their closest relatives.

An assignment of "*Pinirhiza trichophaeoides*" to *T. woolhopeia* could not be made because ITS rDNA sequences (GQ281482, DQ200835) of both taxa showed 81% identity at most (92% query coverage) and the branch lengths in both LSU based trees indicate that the corresponding clade may actually include more than one species. In accordance with the similarities among the corresponding ITS sequences, "*P. trichophaeoides*" morpho-anatomically resembles an ECM described in detail as "*Quercirhiza quadratum*" on *Quercus ilex* L. subsp. *ballota* (Desf.) Samp (Águeda et al. 2008) in having heaps of oval to polygonal cells on a pseudoparenchymatous outer mantle layer composed of angular cells. Emanating hyphae of both ECM are frequent, partially warty, and show abundant rectangular ramifications. However, "*P. trichophaeoides*" differs from "*Q. quadratum*" with regard to the cell shape of the inner mantle layer. In "*P. trichophaeoides*", it has epidermoid cells whereas those of "*Q. quadratum*" are roundish to polygonal. Unfortunately, we could not include this ECM in the molecular-phylogenetic analyses because nrDNA LSU data of "*Q. quadratum*" are not available. Molecular-phylogenetic analyses based on ITS nrDNA would have been fruitless because the few closely related sequences included only three identified ones and a reliably alignable outgroup sequence could not be found. Phylogenetic relationships inferred from such a 'four taxon tree' would predominantly depend on which taxon is chosen as outgroup: a decision not feasible based on the available data. The close relationship of the ITS sequences (100% maximum identity and 84% query coverage with *T. woolhopeia* (DQ200835) of "*Q. quadratum*", however, indicates that a third taxon with similar anatomical ECM characteristics may probably be closely related to *T. woolhopeia*. Common features of the ECM discussed here (heaps of oval to polygonal cells on a pseudoparenchymatous outer mantle layer composed of angular cells, abundantly rectangular ramified emanating hyphae) may be distinctive characters for taxa of the *T. woolhopeia* clade.

The four analyzed ECM clearly belong to the family Pyronemataceae according to the molecular-phylogenetic studies. ECM of this family are hydrophilic and possibly belong to the contact or short-distance exploration type with very few rough and clampless emanating hyphae (Agerer 2001, 2006). The genera *Humaria*, *Geopora*, and *Trichophaea* show a pseudoparenchymatous outer mantle layer (Águeda et al. 2008; Erős-Honti et al. 2008; Tedersoo et al. 2006), which is reminiscent of the shape and arrangement of the outer mantle layer cells to the ECM described here. The three genera could be separated by morpho-anatomical characters, because each of the corresponding molecular-phylogenetic clades unifies similar taxa, while taxa of different clades differ morpho-anatomically. ECM in the *Humaria* group are distinguished by the presence of angular to epidermoid cells connected by short and thin septa in outer mantle layer. ECM of the *Geopora* group may be characterized by row-like arranged angular cells. ECM in *Trichophaea* have oval to polygonal cells forming heaps on the mantle and frequently almost rectangularly ramified emanating hyphae.

Further brown ECM with pseudoparenchymatous outer mantle occur outside the Pyronemataceae, e.g., in the genus *Tomentella* or in unidentified *Tomentella*-like ECM on *Picea*, *Pinus*, and *Quercus* (Agerer and Rambold 2004–2009), in *Coltriciella*, and in *Coltricia* (Tedersoo et al. 2007). Only three of these have clampless hyphae and an outer mantle layer composed of angular cells and lacking

rhizomorphs: "*Pinirhiza cyaneoviridis*" (Golldack et al. 1998), "*P. tomentelloides*" (Wei and Agerer 2008), and an unknown species of *Coltricia*. *Coltricia* sp. ECM (Tedersoo et al. 2007) differ from "*P. humarioides*" in lacking irregularly shaped cells with very thick cell walls of the outermost mantle layer, from "*P. daqingensis*" by the lack of bottle- to awl-shaped cystidia, and from "*P. geoporioides*" and "*P. trichophaeoides*" in lacking heaps of cells on the outer mantle and in having thicker emanating hyphae (5–10 μm) than "*P. geoporioides*" (5–5.5 μm) and "*P. trichophaeoides*" (3.5–5 (8) μm). "*P. tomentelloides*" differs from all Pyronemataceae ECM described here in having rosette-like arranged outer mantle cells and hypha-like cystidia with irregular ramification at the distal ends. "*P. cyaneoviridis*" forms blue granules within the outer mantle cells, which is not known from the presented nor from any hitherto characterized Pyronemataceae ECM (Agerer 2006), and by having heap cells with very thick walls (0.5–3 μm) in comparison to those of "*P. geoporoides*" (0.5 μm) and "*P. trichophaeoides*" (0.5–1 μm). The ECM identified as *Coltricia* aff. *oblectans* by Tedersoo et al. (2007) show abundant cystidia like "*P. daqingensis*", but these differ in their shape, being cylindrical in *Coltricia* aff. *oblectans* and bottle- to awl-shaped as in "*P. daqingensis*". Furthermore, the mantle type of *Coltricia* aff. *oblectans* is transitional between plectenchymatous and pseudoparenchymatous. Some *Tuber* spp. show pseudoparenchymatous mantles with angular cells and lack clamps and rhizomorphs, i.e. *Tuber aestivum* Vitt. (Müller et al. 1996a; Zambonelli et al. 1995), *T. brumale* Vitt. (Fischer et al. 2004; Zambonelli et al. 1995), *T. indicum* Cooke & Massee (Comandini and Pacioni 1997; Zambonelli et al. 1997), *T. mesentericum* Vitt. (Rauscher et al. 1995; Zambonelli et al. 1995), and *T. uncinatum* Chat. (Müller et al. 1996b), but these species are characterized by typically awl-shaped cystidia.

Acknowledgements This study was financially supported by German Academic Exchange Service (DAAD). We thank Prof. Yan Wei (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) for his support of field work and for providing equipment, Fan Yongjun (Inner Mongolia Agricultural University) for assistance in the field, and the Managment Authority of Helan Mountain and Heilihe Natural Reserve for sampling permission. We thank two anonymous reviewers, whose suggestions significantly improved the manuscript.

References

Agerer R (1987–2008) Colour Atlas of Ectomycorrhizae. 1st – 14th delivery. Einhorn, Schwäbisch Gmünd, Germany

Agerer R (1991) Characterization of Ectomycorrhizae. In: Norris JR, Read DA, Varma AK (eds) Techniques for the study of mycorrhiza. Methods in microbiology, vol 23. Academic, London, pp 25–73

Agerer R (2001) Exploration types of ectomycorrhizae: a proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. Mycorrhiza 11(2):107–114

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progr 5:67–107

Agerer R, Rambold G (2004–2009, First posted on 2004-06-01; most recent update: 2009-01-26) DEEMY - An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de - München, Germany

Águeda B, Agerer R, De Miguel AM, Parladé J (2008) "*Quercirhiza quadratum*" + *Quercus ilex* L. subsp.*ballota* (Scop.). Desf Samp Descr Ectomyc 11(12):113–123

Amicucci A, Zambonelli A, Guidi C, Stocchi V (2001) Morphological and molecular characterisation of *Pulvinula constellatio* ectomycorrhizae. FEMS Microbiol Lett 194:121–125

Brand F (1991) *Genea hispidula*. In: Agerer R (ed) Colour Altas of Ectomycorrhizae, plate 57. Einhorn, Schwäbisch Gmünd

Brock PM, Döring H, Bidartondo MI (2009) How to know unknown fungi: the role of a herbarium. New Phytol 181:719–724

Comandini O, Pacioni G (1997) Mycorrhizae of Asian black truffles, *Tuber himalayense* and *T. indicum*. Mycotaxon 63:77–86

Danielson RM (1984) Ectomycorrhiza formation by the operculate discomycete *Sphaerosporella brunnea* (Pezizales). Mycologia 76:454–461

Egger KN (1996) Molecular systematics of E-strain mycorrhizal fungi: *Wilcoxina* and its relationship to *Tricharina* (Pezizales). Can J Bot 74:773–779

Erős-Honti Z, Kovacs GM, Szedlay G, Jakucs E (2008) Morphological and molecular characterization of *Humaria* and *Genea* ectomycorrhizae from Hungarian deciduous forests. Mycorrhiza 18:133–143

Fischer C, Suz LM, Martin MP (2004) *Tuber brumale* Vitt. + *Quercus ilex* L. Descr Ectomyc 7(8):135–141

Fujimura KE, Smith JE, Horton TR, Weber NS, Spatafora JW (2005) Pezizalean mycorrhizas and sporocarps in ponderosa pine (*Pinus ponderosa*) after prescribed fires in eastern Oregon, USA. Mycorrhiza 15:79–86

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes -applications to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Golldack J, Münzenberger B, Hüttl RF (1998) "*Pinirhiza cyaneoviridis*" + *Pinus sylvestris* L. Descr Ectomyc 3:49–54

Hall T (2005) BioEdit, biological sequence alignment editor for Win95/98/NT/2K/XP. Ibis therapeutic, Carlsbad

Holmgren PK, Holmgren NH, Barnett LC (1990) Index Herbariorum. Part I. Herbaria of the World. 8th edn. Regnum Vegetabile 120. New York Botanical Garden, New York (http://www.nybg.org/bsci/ih/ih.html)

Ingleby K, Mason PA, Last FT, Fleming LV (1990) Identification of ectomycorrhizas. ITE research publication 5. HMSO, London

Jakucs E, Agerer R, Bratek Z (1998) *Genea verrucosa* Vitt. + *Quercus spec*. Descr Ectomyc 3:19–23

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166:1063–1068

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst Biol 49:278–305

Müller WR, Rauscher T, Agerer R, Chevalier G (1996a) *Tuber aestivum* Vitt. + *Corylus avellana* L. Descr Ectomyc 1:167–172

Müller WR, Rauscher T, Agerer R, Chevalier G (1996b) *Tuber uncinatum* Chat. + *Corylus avellana* L. Descr Ectomyc 1:179–183

Perry BA, Hansen K, Pfister DH (2007) A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycol Res 111:549–571

Rauscher T, Agerer R, Chevalier G (1995) Ektomykorrhizen von *Tuber melanosporum*, *Tuber mesentericum* und *Tuber rufum* (Tuberales) an *Corylus avellana*. Nova Hedwigia 61(3-4):282–322

Sikes DS, Lewis PO (2001) PAUPRat: A tool to implement Parsimony Ratchet searches using PAUP*. (http://viceroy.eeb.uconn.edu/paupratweb/pauprat.htm)

Smith ME, Trappe JM, Rizzo DM (2006) *Genea*, *Genabea* and *Gilkeya* gen.nov.: ascomata and ectomycorrhiza formation in a *Quercus* woodland. Mycologia 98(5):699–716

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer, Sunderland

Tedersoo L, Hansen K, Perry BA, Kjøller R (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytol 170:581–596

Tedersoo L, Suvi T, Beaver K, Saar I (2007) Ectomycorrhizas of *Coltricia* and *Coltriciella* (Hymenochaetales, Basidiomycota) on Caesalpiniaceae, Dipterocarpaceae and Myrtaceae in Seychelles. Mycol Progr 6:101–107

Vrålstad T, Holst-jensen A, Schumacher T (1998) The postfire discomycete *Geopyxis carbonaria* (Ascomycota) is a biotrophic root associate with Norway spruce (*Picea abies*) in nature. Mol Ecol 7:609–616

Wei J, Agerer R (2008) "*Pinirhiza tomentelloides*" + *Pinus tabulaeformis* Carr. Descr Ectomyc 11(12):97–102

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MA, Gelfand DH, Sninsky JN, White TJ (eds) *PCR Protocols*: a guide to methods and applications. Academic, San Diego, pp 315–322

Yu TE, Egger KN, Peterson RL (2001) Ectendomycorrhizal associations-Characteristics and functions. Mycorrhiza 11:167–177

Zambonelli A, Salomoni S, Pisi A (1995) Caratterizzazione anatomo-morphologica delle microrrize di *Tuber borchii*, *Tuber aestivum*, *Tuber mesentericum*, *Tuber brumale*, *Tuber melanosporum* su *Pinus pinea*. Micol Ital 1995(2):119–137

Zambonelli A, Tibiletti E, Pisi A (1997) Caratterizzazione anatomo-morfologica delle micorrize die *Tuber indicum* Cooke & Massee su *Pinus pinea* L. e *Quercus cerris* L. Micol Ital 26(1):29–36

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214

Supplementary file

Fig. 17 Molecular-phylogenetic relationships among selected Pyronemataceae. Best scoring tree found by the RAxML analysis of LSU nrDNA sequences. Bootstrap support values above 50% are noted above or to the left of the respective branches. GenBank accession numbers are given in parentheses following the species names. Sequence names with * are from this study.


100 Sphaerosporella sp. (DQ220432)
Sphaerosporella sp. (DQ220433)
Trichophaea saccata (DQ220451)
Trichophaea minuta (DQ220452)
Sphaerosporella hinnulea (DQ220431)
Anthracobia subatra (DQ220313)
Trichophaea abundans (DQ220449)
93 Trichophaea abundans (DQ220450)
Trichophaea abundans (DQ220448)
90
55 Pezizales ECM (AJ968676)
Trichophaea woolhopeia (DQ220458)
51 Trichophaea woolhopeia (DQ220459)
100 "Pinirhiza trichophaeoides" (GQ281478) *
Trichophaea woolhopeia (DQ220460)
85
Anthracobia macrocystis (DQ220310)
Anthracobia macroscytis (DQ220311)
Anthracobia macrocystis (AY544660)
99 Anthracobia tristis (DQ220314)
Anthracobia sp. (DQ220312)
56
91 Pustularia patavina (DQ220396)
Pyronemataecae sp. (DQ220399)
Tricharina sp. (DQ220446)
Tricharina gilva (DQ220443)
74 Tricharina gilva (DQ220444)
Tricharina gilva (DQ220442)
60 Tricharina sp. (DQ220447)
61 Tricharina ochroleuca (DQ220445)
Tricharina praecox (DQ646525)
100 Pezizales ECM (AJ893248)
Pezizales ECM (AM086625)
65 Geopora arenicola (DQ220336)
Geopora arenicola (DQ220337)
74 Geopora sp. (DQ220338)
Geopora clausa (DQ220339)
69 Geopora cf. cervina (DQ220344)
Geopora sp. (DQ223973)
50 "Pinirhiza geoporoides" (GQ281477) *
91 Geopora cooperi (DQ220340)
100 Geopora cooperi (DQ220342)
Geopora cooperi (DQ220341)
100 Pezizales ECM (AJ968677)
Geopora sp. (DQ220345)
92 "Pinirhiza daqingensis" (GQ281476) *
72 Geopora pellita (DQ220343)
94 Scutellinia barlae (DQ220415)
67 Scutellinia trechispora (DQ220424)
92 Scutellinia subhirtella (DQ220423)
98 Scutellinia scutellata (DQ220421)
Scutellinia scutellata (DQ247806)
88 Scutellinia sp. (DQ220422)
58 Scutellinia blumenaviensis (DQ220416)
66 Cheilymenia fimicola (DQ220322)
52 Kotlabaea deformis (DQ220356)
Scutellinia erinaceus (DQ220417)
81
87 Ramsbottomia asperior (DQ220406)
Ramsbottomia asperior (DQ220408)
94 Ramsbottomia sp. (DQ220409)
65
99 Miladina lecithina (DQ220371)
Miladina lecithina (DQ220372)
100 Pyronema omphalodes (DQ220397)
Pyronema domesticum (DQ247805)
100 Rhodoscypha ovilla (DQ220411)
Rhodoscypha ovilla (DQ220412)
88 Leucoscypha leucotricha (DQ220365)
63 Rhodotarzetta rosea (DQ220413)
90 Neotiella rutilans (DQ220377)
Octospora axillaris (DQ220378)
93
uncultured soil fungus (EU861601)
54 uncultured soil fungus (EU861652)
70 uncultured soil fungus (EU861714)
99 uncultured soil fungus (EU861611)
96 uncultured soil fungus (EU861704)
Octospora lilacina (DQ220381)
50
Aleuria rhenana (DQ220309)
100 Sowerbyella rhenana (EU669267)
Sowerbyella rhenana (EU669266)
Sowerbyella radiculata (DQ220429)
100 Sowerbyella radiculata (DQ220430)
76 Sowerbyella radiculata (DQ220428)
100 Sowerbyella imperialis (DQ220426)
Sowerbyella imperialis (DQ220427)
Pyronemataceae sp. (DQ220466)
100 Melastiza flavorubens (DQ220368)
Melastiza flavorubens (DQ220369)

Fig. 17a

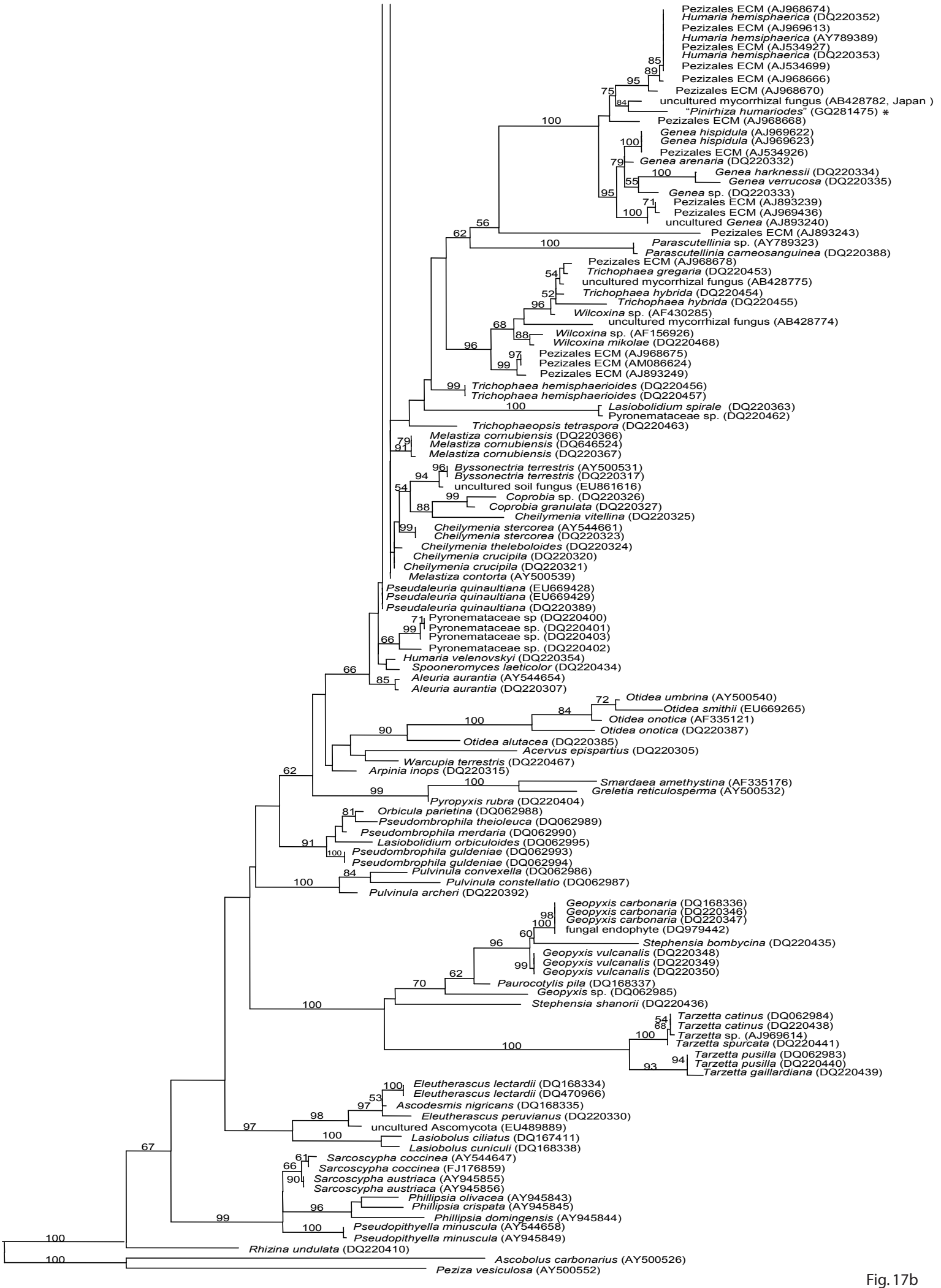

Pezizales ECM (AJ968674)
Humaria hemisphaerica (DQ220352)
Pezizales ECM (AJ969613)
Humaria hemsiphaerica (AY789389)
Pezizales ECM (AJ534927)
Humaria hemisphaerica (DQ220353)
Pezizales ECM (AJ534699)
Pezizales ECM (AJ968666)
Pezizales ECM (AJ968670)
uncultured mycorrhizal fungus (AB428782, Japan)
"Pinirhiza humariodes" (GQ281475) *
Pezizales ECM (AJ968668)
Genea hispidula (AJ969622)
Genea hispidula (AJ969623)
Pezizales ECM (AJ534926)
Genea arenaria (DQ220332)
Genea harknessii (DQ220334)
Genea verrucosa (DQ220335)
Genea sp. (DQ220333)
Pezizales ECM (AJ893239)
Pezizales ECM (AJ969436)
uncultured Genea (AJ893240)
Pezizales ECM (AJ893243)
Parascutellinia sp. (AY789323)
Parascutellinia carneosanguinea (DQ220388)
Pezizales ECM (AJ968678)
Trichophaea gregaria (DQ220453)
uncultured mycorrhizal fungus (AB428775)
Trichophaea hybrida (DQ220454)
Trichophaea hybrida (DQ220455)
Wilcoxina sp. (AF430285)
uncultured mycorrhizal fungus (AB428774)
Wilcoxina sp. (AF156926)
Wilcoxina mikolae (DQ220468)
Pezizales ECM (AJ968675)
Pezizales ECM (AM086624)
Pezizales ECM (AJ893249)
Trichophaea hemisphaerioides (DQ220456)
Trichophaea hemisphaerioides (DQ220457)
Lasiobolidium spirale (DQ220363)
Pyronemataceae sp. (DQ220462)
Trichophaeopsis tetraspora (DQ220463)
Melastiza cornubiensis (DQ220366)
Melastiza cornubiensis (DQ646524)
Melastiza cornubiensis (DQ220367)
Byssonectria terrestris (AY500531)
Byssonectria terrestris (DQ220317)
uncultured soil fungus (EU861616)
Coprobia sp. (DQ220326)
Coprobia granulata (DQ220327)
Cheilymenia vitellina (DQ220325)
Cheilymenia stercorea (AY544661)
Cheilymenia stercorea (DQ220323)
Cheilymenia theleboloides (DQ220324)
Cheilymenia crucipila (DQ220320)
Cheilymenia crucipila (DQ220321)
Melastiza contorta (AY500539)
Pseudaleuria quinaultiana (EU669428)
Pseudaleuria quinaultiana (EU669429)
Pseudaleuria quinaultiana (DQ220389)
Pyronemataceae sp (DQ220400)
Pyronemataceae sp. (DQ220401)
Pyronemataceae sp. (DQ220403)
Pyronemataceae sp. (DQ220402)
Humaria velenovskyi (DQ220354)
Spooneromyces laeticolor (DQ220434)
Aleuria aurantia (AY544654)
Aleuria aurantia (DQ220307)
Otidea umbrina (AY500540)
Otidea smithii (EU669265)
Otidea onotica (AF335121)
Otidea onotica (DQ220387)
Otidea alutacea (DQ220385)
Acervus epispartius (DQ220305)
Warcupia terrestris (DQ220467)
Arpinia inops (DQ220315)
Smardaea amethystina (AF335176)
Greletia reticulosperma (AY500532)
Pyropyxis rubra (DQ220404)
Orbicula parietina (DQ062988)
Pseudombrophila theioleuca (DQ062989)
Pseudombrophila merdaria (DQ062990)
Lasiobolidium orbiculoides (DQ062995)
Pseudombrophila guldeniae (DQ062993)
Pseudombrophila guldeniae (DQ062994)
Pulvinula convexella (DQ062986)
Pulvinula constellatio (DQ062987)
Pulvinula archeri (DQ220392)
Geopyxis carbonaria (DQ168336)
Geopyxis carbonaria (DQ220346)
Geopyxis carbonaria (DQ220347)
fungal endophyte (DQ979442)
Stephensia bombycina (DQ220435)
Geopyxis vulcanalis (DQ220348)
Geopyxis vulcanalis (DQ220349)
Geopyxis vulcanalis (DQ220350)
Paurocotylis pila (DQ168337)
Geopyxis sp. (DQ062985)
Stephensia shanorii (DQ220436)
Tarzetta catinus (DQ062984)
Tarzetta catinus (DQ220438)
Tarzetta sp. (AJ969614)
Tarzetta spurcata (DQ220441)
Tarzetta pusilla (DQ062983)
Tarzetta pusilla (DQ220440)
Tarzetta gaillardiana (DQ220439)
Eleutherascus lectardii (DQ168334)
Eleutherascus lectardii (DQ470966)
Ascodesmis nigricans (DQ168335)
Eleutherascus peruvianus (DQ220330)
uncultured Ascomycota (EU489889)
Lasiobolus ciliatus (DQ167411)
Lasiobolus cuniculi (DQ168338)
Sarcoscypha coccinea (AY544647)
Sarcoscypha coccinea (FJ176859)
Sarcoscypha austriaca (AY945855)
Sarcoscypha austriaca (AY945856)
Phillipsia olivacea (AY945843)
Phillipsia crispata (AY945845)
Phillipsia domingensis (AY945844)
Pseudopithyella minuscula (AY544658)
Pseudopithyella minuscula (AY945849)
Rhizina undulata (DQ220410)
Ascobolus carbonarius (AY500526)
Peziza vesiculosa (AY500552)
0.10

Fig. 17b

Supplementary file

Fig. 18 Molecular-phylogenetic relationships among selected Pyronemataceae. 50% Majority Rule Consensus Tree (2057 steps, CI=0.245, RI=0.773) of the 3341 most parsimonious trees (2050 steps) found by Parsimony Ratchet. Posterior Probabilities were 100% except where indicated otherwise. GenBank accession numbers are given in parentheses following the species names. Sequence names with * are from this study.

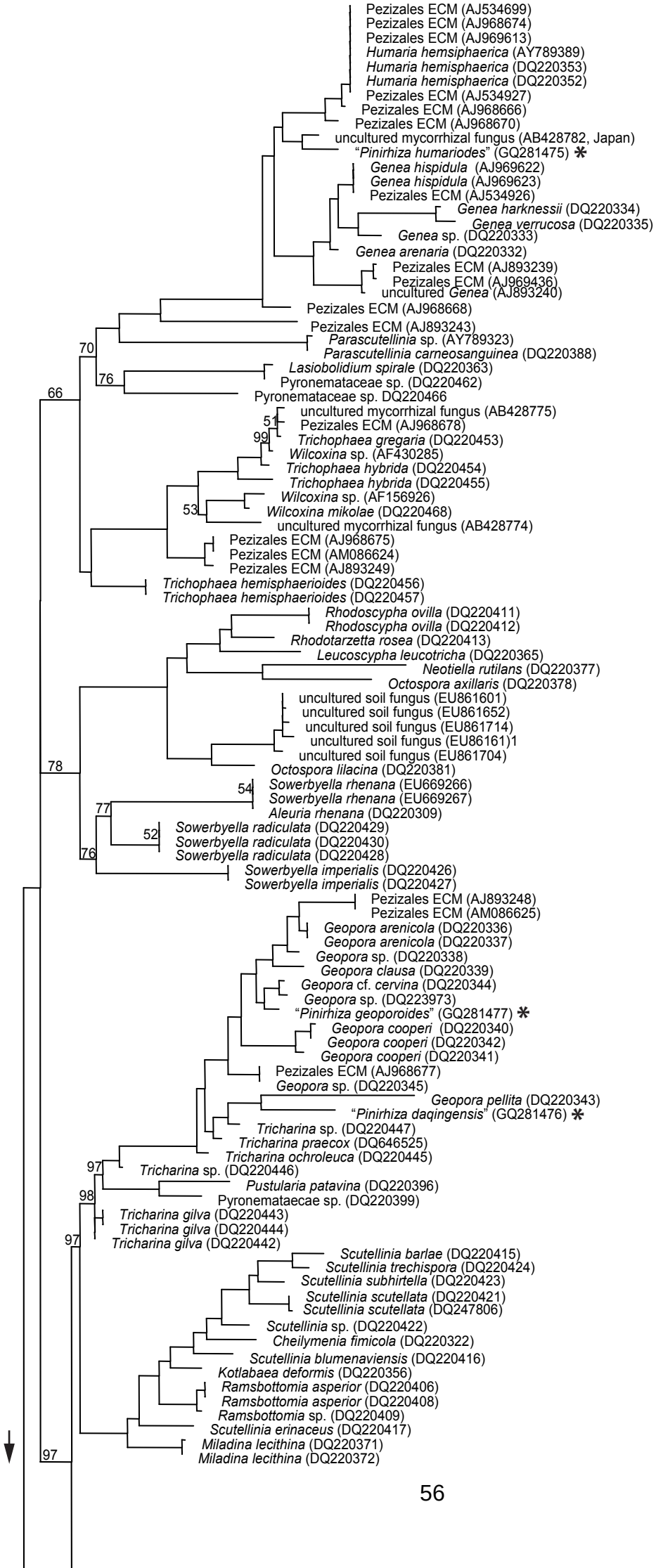

Pezizales ECM (AJ534699)
Pezizales ECM (AJ968674)
Pezizales ECM (AJ969613)
Humaria hemsiphaerica (AY789389)
Humaria hemisphaerica (DQ220353)
Humaria hemisphaerica (DQ220352)
Pezizales ECM (AJ534927)
Pezizales ECM (AJ968666)
Pezizales ECM (AJ968670)
uncultured mycorrhizal fungus (AB428782, Japan)
"Pinirhiza humariodes" (GQ281475) *
Genea hispidula (AJ969622)
Genea hispidula (AJ969623)
Pezizales ECM (AJ534926)
Genea harknessii (DQ220334)
Genea verrucosa (DQ220335)
Genea sp. (DQ220333)
Genea arenaria (DQ220332)
Pezizales ECM (AJ893239)
Pezizales ECM (AJ969436)
uncultured Genea (AJ893240)
Pezizales ECM (AJ968668)
Pezizales ECM (AJ893243)
Parascutellinia sp. (AY789323)
Parascutellinia carneosanguinea (DQ220388)
Lasiobolidium spirale (DQ220363)
Pyronemataceae sp. (DQ220462)
Pyronemataceae sp. DQ220466
uncultured mycorrhizal fungus (AB428775)
Pezizales ECM (AJ968678)
Trichophaea gregaria (DQ220453)
Wilcoxina sp. (AF430285)
Trichophaea hybrida (DQ220454)
Trichophaea hybrida (DQ220455)
Wilcoxina sp. (AF156926)
Wilcoxina mikolae (DQ220468)
uncultured mycorrhizal fungus (AB428774)
Pezizales ECM (AJ968675)
Pezizales ECM (AM086624)
Pezizales ECM (AJ893249)
Trichophaea hemisphaerioides (DQ220456)
Trichophaea hemisphaerioides (DQ220457)
Rhodoscypha ovilla (DQ220411)
Rhodoscypha ovilla (DQ220412)
Rhodotarzetta rosea (DQ220413)
Leucoscypha leucotricha (DQ220365)
Neotiella rutilans (DQ220377)
Octospora axillaris (DQ220378)
uncultured soil fungus (EU861601)
uncultured soil fungus (EU861652)
uncultured soil fungus (EU861714)
uncultured soil fungus (EU86161)1
uncultured soil fungus (EU861704)
Octospora lilacina (DQ220381)
Sowerbyella rhenana (EU669266)
Sowerbyella rhenana (EU669267)
Aleuria rhenana (DQ220309)
Sowerbyella radiculata (DQ220429)
Sowerbyella radiculata (DQ220430)
Sowerbyella radiculata (DQ220428)
Sowerbyella imperialis (DQ220426)
Sowerbyella imperialis (DQ220427)
Pezizales ECM (AJ893248)
Pezizales ECM (AM086625)
Geopora arenicola (DQ220336)
Geopora arenicola (DQ220337)
Geopora sp. (DQ220338)
Geopora clausa (DQ220339)
Geopora cf. cervina (DQ220344)
Geopora sp. (DQ223973)
"Pinirhiza geoporoides" (GQ281477) *
Geopora cooperi (DQ220340)
Geopora cooperi (DQ220342)
Geopora cooperi (DQ220341)
Pezizales ECM (AJ968677)
Geopora sp. (DQ220345)
Geopora pellita (DQ220343)
"Pinirhiza daqingensis" (GQ281476) *
Tricharina sp. (DQ220447)
Tricharina praecox (DQ646525)
Tricharina ochroleuca (DQ220445)
Tricharina sp. (DQ220446)
Pustularia patavina (DQ220396)
Pyronemataecae sp. (DQ220399)
Tricharina gilva (DQ220443)
Tricharina gilva (DQ220444)
Tricharina gilva (DQ220442)
Scutellinia barlae (DQ220415)
Scutellinia trechispora (DQ220424)
Scutellinia subhirtella (DQ220423)
Scutellinia scutellata (DQ220421)
Scutellinia scutellata (DQ247806)
Scutellinia sp. (DQ220422)
Cheilymenia fimicola (DQ220322)
Scutellinia blumenaviensis (DQ220416)
Kotlabaea deformis (DQ220356)
Ramsbottomia asperior (DQ220406)
Ramsbottomia asperior (DQ220408)
Ramsbottomia sp. (DQ220409)
Scutellinia erinaceus (DQ220417)
Miladina lecithina (DQ220371)
Miladina lecithina (DQ220372)
70
76
66
51
99
53
78
54
77
52
76
97
98
97
97

Fig. 18a

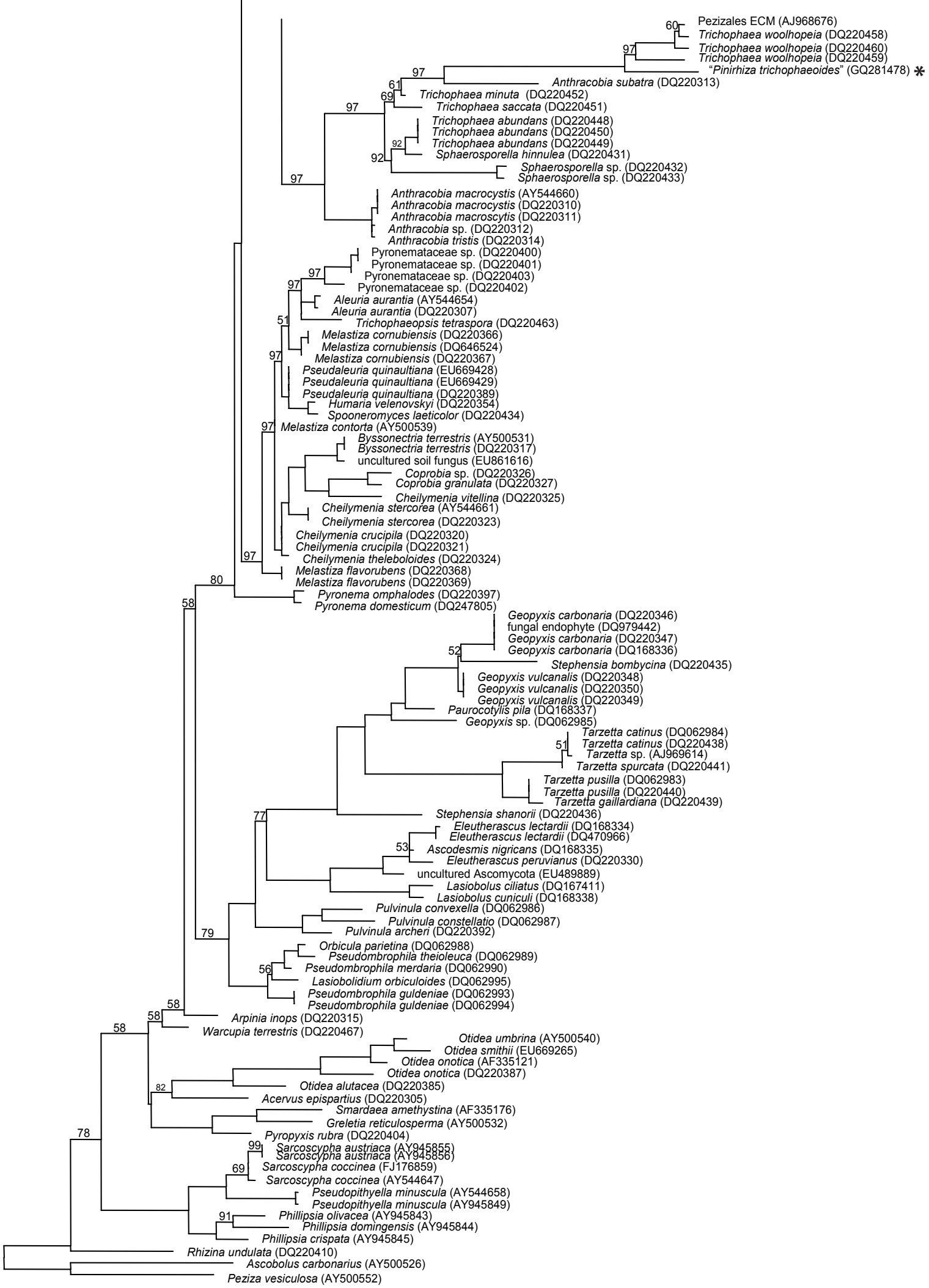

Pezizales ECM (AJ968676)
Trichophaea woolhopeia (DQ220458)
Trichophaea woolhopeia (DQ220460)
Trichophaea woolhopeia (DQ220459)
"Pinirhiza trichophaeoides" (GQ281478) *
Anthracobia subatra (DQ220313)
Trichophaea minuta (DQ220452)
Trichophaea saccata (DQ220451)
Trichophaea abundans (DQ220448)
Trichophaea abundans (DQ220450)
Trichophaea abundans (DQ220449)
Sphaerosporella hinnulea (DQ220431)
Sphaerosporella sp. (DQ220432)
Sphaerosporella sp. (DQ220433)
Anthracobia macrocystis (AY544660)
Anthracobia macrocystis (DQ220310)
Anthracobia macroscytis (DQ220311)
Anthracobia sp. (DQ220312)
Anthracobia tristis (DQ220314)
Pyronemataceae sp. (DQ220400)
Pyronemataceae sp. (DQ220401)
Pyronemataceae sp. (DQ220403)
Pyronemataceae sp. (DQ220402)
Aleuria aurantia (AY544654)
Aleuria aurantia (DQ220307)
Trichophaeopsis tetraspora (DQ220463)
Melastiza cornubiensis (DQ220366)
Melastiza cornubiensis (DQ646524)
Melastiza cornubiensis (DQ220367)
Pseudaleuria quinaultiana (EU669428)
Pseudaleuria quinaultiana (EU669429)
Pseudaleuria quinaultiana (DQ220389)
Humaria velenovskyi (DQ220354)
Spooneromyces laeticolor (DQ220434)
Melastiza contorta (AY500539)
Byssonectria terrestris (AY500531)
Byssonectria terrestris (DQ220317)
uncultured soil fungus (EU861616)
Coprobia sp. (DQ220326)
Coprobia granulata (DQ220327)
Cheilymenia vitellina (DQ220325)
Cheilymenia stercorea (AY544661)
Cheilymenia stercorea (DQ220323)
Cheilymenia crucipila (DQ220320)
Cheilymenia crucipila (DQ220321)
Cheilymenia theleboloides (DQ220324)
Melastiza flavorubens (DQ220368)
Melastiza flavorubens (DQ220369)
Pyronema omphalodes (DQ220397)
Pyronema domesticum (DQ247805)
Geopyxis carbonaria (DQ220346)
fungal endophyte (DQ979442)
Geopyxis carbonaria (DQ220347)
Geopyxis carbonaria (DQ168336)
Stephensia bombycina (DQ220435)
Geopyxis vulcanalis (DQ220348)
Geopyxis vulcanalis (DQ220350)
Geopyxis vulcanalis (DQ220349)
Paurocotylis pila (DQ168337)
Geopyxis sp. (DQ062985)
Tarzetta catinus (DQ062984)
Tarzetta catinus (DQ220438)
Tarzetta sp. (AJ969614)
Tarzetta spurcata (DQ220441)
Tarzetta pusilla (DQ062983)
Tarzetta pusilla (DQ220440)
Tarzetta gaillardiana (DQ220439)
Stephensia shanorii (DQ220436)
Eleutherascus lectardii (DQ168334)
Eleutherascus lectardii (DQ470966)
Ascodesmis nigricans (DQ168335)
Eleutherascus peruvianus (DQ220330)
uncultured Ascomycota (EU489889)
Lasiobolus ciliatus (DQ167411)
Lasiobolus cuniculi (DQ168338)
Pulvinula convexella (DQ062986)
Pulvinula constellatio (DQ062987)
Pulvinula archeri (DQ220392)
Orbicula parietina (DQ062988)
Pseudombrophila theioleuca (DQ062989)
Pseudombrophila merdaria (DQ062990)
Lasiobolidium orbiculoides (DQ062995)
Pseudombrophila guldeniae (DQ062993)
Pseudombrophila guldeniae (DQ062994)
Arpinia inops (DQ220315)
Warcupia terrestris (DQ220467)
Otidea umbrina (AY500540)
Otidea smithii (EU669265)
Otidea onotica (AF335121)
Otidea onotica (DQ220387)
Otidea alutacea (DQ220385)
Acervus epispartius (DQ220305)
Smardaea amethystina (AF335176)
Greletia reticulosperma (AY500532)
Pyropyxis rubra (DQ220404)
Sarcoscypha austriaca (AY945855)
Sarcoscypha austriaca (AY945856)
Sarcoscypha coccinea (FJ176859)
Sarcoscypha coccinea (AY544647)
Pseudopithyella minuscula (AY544658)
Pseudopithyella minuscula (AY945849)
Phillipsia olivacea (AY945843)
Phillipsia domingensis (AY945844)
Phillipsia crispata (AY945845)
Rhizina undulata (DQ220410)
Ascobolus carbonarius (AY500526)
Peziza vesiculosa (AY500552)
0.10

Fig. 18b

2.4.5 Three Ectomycorrhizae of Thelephoraceae on Chinese Pine (*Pinus tabulaeformis*) and a key to thelephoroid Ectomycorrhizae

V

Three Ectomycorrhizae of Thelephoraceae on Chinese Pine (*Pinus tabulaeformis*) and a key to thelephoroid Ectomycorrhizae

Jie Wei*, Reinhard Agerer

Department Biology I, Division of Organismic Biology: Mycology, University of München, Menzinger Str. 67, D-80638 München, Germany

*Corresponding author: E-mail: weijie211@yahoo.com, phone: 00498917861196

Abstract: Morphological and anatomical characters of three thelephoroid ectomycorrhizae of the family Thelephoraceae on Chinese Pine (*Pinus tabulaeformis*) are described in detail. They are all brown and without rhizomorphs, and show high diversity regarding the structure of outer mantle layers and shape of cystidia. "*Pinirhiza acuminata*" is plectenchymatous throughout all mantle layers and has a hyphal net and acuminate hyphae on the mantle surface; the other two ectomycorrhizae "*Pinirhiza heilihensis*" and "*Pinirhiza fibulocystidiata*" have pseudoparenchymatous outer mantles with angular cells, but they can be differentiated by cell dimensions. The most important character to distinguish both are cystidia, "*Pinirhiza fibulocystidiata*" possesses typical fibulocystidia, whereas those of "*Pinirhiza heilihensis*" are awl-shaped and without septa. A key to thelephoroid ECM of the family Thelephoraceae is presented.

Key words: Anatomy, morphology, thelephoroid ectomycorrhiza, Thelephoraceae

Introduction

Species of *Pseudotomentella*, *Thelephora*, *Tomentella* and *Tomentellopsis* of the family Thelephoraceae (Thelephorales, Agaricomycetes) have been reported to form ectomycorrhizae (ECM). Some ECM not identified to species-level but considered as being caused by Thelephoraceae, are described as *Afzeliaerhiza*, *Fagirhiza*, *Piceirhiza*, *Pinirhiza*, *Populirhiza*, *Quercirhiza,* and *Uapacaerhiza* (Table 1). Only a few ECM studies considered comprehensively anatomical details of *Pseudotomentella*, *Thelephora* and *Tomentellopsis*.

ECM of these genera show plectenchymatous outer mantle layers with or without ring-like arrangement. Pseudoparenchymatous mantles are unknown yet.

Tomentella species belong to the most frequently studied ECM fungi because they are common and often dominant on the root system of coniferous and deciduous forests (Jakucs & Erős-Honti 2008, Kõljalg et al. 2000, Tedersoo et al. 2003, Tedersoo et al. 2007a). Some studies have been published to provide features that could generally characterize *Tomentella* ECM. Agerer (2006) referred to (a) dark brown ECM with pseudoparenchymatous mantles and clamps or with cystidia, (b) dark brown ECM with pseudoparenchymatous mantles and blue granules that turn green in KOH, irrespective of the presence of clamps or cystidia. Jakucs & Erős-Honti (2008) reviewed brown ECM that have been characterized in detail and either fit the features compiled by Agerer (2006) or have been proven by nuclear rDNA (nrDNA) analysis as representing a *Tomentella* species. They compiled further distinctive characteristics, like angular or star-like arranged outer mantle cells, groups of globular cells on the mantle surface, clamped cystidia, and nodally ramified rhizomorphs densely entwined with very thin peripheral hyphae.

More than 30 *Tomentella* or *Tomentella*-like ECM (Tab. 1) have been studied in detail microscopically since 1988. Most of them share common features summarized by Agerer (2006) and by Jakucs & Erős-Honti (2008). Some, however, that have been identified as *Tomentella* species using nrDNA sequence analyses, present plectenchymatous outer mantle layers as known in other ectomycorrhizal genera of Thelephoraceae. How to identify these thelephoroid ECM morpho-anatomically is still an open question. It appears therefore helpful for future diversity studies to provide a traditional key of determination for thelephoroid ECM, in addition to the already existing and continuously updated synoptic key for all ECM described to date that includes thelephoroid ECM as well (Agerer & Rambold 2004–2009).

After the first detailed description of *Tomentella*-like ECM on Chinese Pine (Wei & Agerer 2008), some additional *Tomentella*-like ECM have been found. We present three of them in this study, compare anatomically all ECM of Thelephoraceae (Tab. 1) and present a key to facilitate their identification.

Table 1 *Tomentella* and *Tomentella*-like ECM described morpho-anatomically in detail since 1988

ECM or fungal partner	Host	Collecting sites	References
"*Afzeliaerhiza beninensis*"	*Afzelia africana* Smith	Benin, Borgou	Yorou & Agerer 2008
"*Fagirhiza asteromustrata*"	*Fagus sylvatica* L.	Hungary, Bükk-Őserdő	Jakucs et al. 2008
"*Fagirhiza setifera*"	*Fagus sylvatica* L.	Germany, Baden-Württemberg	Brand 1991
"*Fagirhiza spinulosa*"	*Fagus sylvatica* L.	Germany, Baden-Württemberg	Brand 1991
"*Fagirhiza stellata*"	*Fagus sylvatica* L.	Italy, Trient	Di Marino et al. 2008
"*Piceirhiza obscura*"	*Picea abies* (L.) H. Karst.	Germany	Gronbach 1988
"*Piceirhiza nigra*"	*Picea abies* (L.) H. Karst.	Germany	Berg 1989, Gronbach 1988, Haug & Pritsch 1992
"*Pinirhiza ligulata*"	*Pinus sylvestris* L.	Poland, Zmiaca	Mleczko 2004b
"*Pinirhiza amyloidea*"	*Pinus sylvestris* L.	Poland, Zmiaca	Mleczko 2004a
"*Pinirhiza cyaneoviridis*"	*Pinus sylvestris* L.	Germany, Thuringia	Golldack et al. 1998
"*Pinirhiza dimorpha*"	*Pinus sylvestris* L.	Germany, Brandenburg	Golldack et al. 1999
"*Pinirhiza tomentelloides*"	*Pinus tabulaeformis* Carr.	China, Ningxia	Wei & Agerer 2008
"*Populirhiza asperula*"	*Populus alba* L.	Hungary, Tompa	Jakucs et al. 2005
Pseudotomentella humicola M.J. Larsen	*Picea abies* (L.) H. Karst.	Norway, Akershus	Di Marino et al. 2007
Pseudotomentella tristis (P. Karst.) M.J. Larsen	*Salix herbacea* L.	Italy, Südtirol	Agerer 1994
"*Quercirhiza ateracusrugosa*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2006a
"*Quercirhiza aurateroocystidiata*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2006b
"*Quercirhiza cumulosa*"	*Quercus ilex* L. subsp.*ballota* (Desf.) Samp	Spain, Navarra	De Roman et al. 2002a
"*Quercihiza flavocystidiata*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2006c
"*Quercirhiza nodulosomorpha*"	*Quercus suber* L.	Portugal, Grândola	Azul et al. 1999
"*Quercirhiza squamosa*"	*Quercus robur*	Slovenia	Palfner & Agerer 1996
"*Quercirhiza summatriangularis*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2006d
"*Quercirhiza stellata*"	*Quercus ilex* L. Subsp *ballota* (Desf.) Samp	Spain, Navarra	De Roman et al. 2002b
"*Quercirhiza tomentellocumulata*"	*Quercus suber* L.	Portual, Concelho de Santiago do Cacém	Azul et al. 2008a
"*Quercirhiza tomentellocystidiata*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2006e
"*Quercirhiza tomentelloepidermoidea*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2008b
"*Quercirhiza tomentelloflexuosa*"	*Quercus suber* L.	Portugal, Distrito de Setúbal	Azul et al. 2006f
"*Quercirhiza tomentellofuniculosa*"	*Quercus suber* L.	Portugal, Distrito de Évpra	Azul et al. 2006g
"*Quercirhiza tomentelloreticulata*"	*Quercus suber* L.	Portugal, Distrito de Évpra	Azul et al. 2008c
"*Quercihiza tomentellostellata*"	*Quercus suber* L.	Portugal, Distrito de Évpra	Azul et al. 2008d
Thelephora terrestris Ehrh.	*Picea abies* L. *Picea sitchensis* (Bong.) Carr.	Germany, Bavaria Britain	Agerer & Weiss 1989, Ingleby et al. 1990
Tomentella brunneorufa M.J.Larsen	*Eucalyptus* sp.	Australia	Agerer & Bougher 2001
T. ferruginea (Pers.)Pat.	*Fagus sylvatica* L.	Germany, Bayern	Raidl & Müller 1996
T. galzinii Bourdot (sub nom "*Quercirhiza fibulocystidiata*")	*Quercus cerris* L.	Hungary	Jakucs et al.1997, Kõljalg et al. 2001
T. pilosa (Burt) Bourdot & Galzin	*Populus alba* L.	Hungary, Tompa	Jakucs & Agerer 1999
T. stuposa (Link) Stalpers	*Quercus cerris* L., *Picea abies* (L.) H. Karst.	Hungary, Püspökladány Germany, Bayern	Jakucs et al. 2005
T. sublilacina (Ellis & Holw.) Wakef. (Sub nom *T. albomarginata*)	*Pinus sylvestris* L.	Germany, Hessen	Agerer 1996a
T. subtestacea Bourdot & Galzin	*Populus alba* L.	Hungary, Tompa	Jakucs & Agerer 2001
Tomentellopsis submollis (Svrcek) Hjortstam (sub nom "*Fagirhiza rosea*")	*Fagus sylvatica* L.	Germany, Bavaria	Brand 1991
"*Uapacaerhiza wariensis*"	*Uapaca guineensis* Müll.Arg.	Benin, Borgou	Yorou et al. 2008

Material and methods

Soil samples were collected in pure Chinese Pine forests at Helan Mountain (Ningxia, China) and in Heilihe National Reserve (Inner Mongolia, China) during two years. ECM systems were assigned to anatomotypes and described according to Agerer (1987–2008, 1991a). Anatomical studies and drawings were performed with the aid of a Normarski interference contrast microscope (Standard 14, ZEISS West Germany) connected with a drawing tube. All drawings were made at a magnification of 1000 ×. Reference specimens of the ECM are deposited in M (see Thiers 2009).

One unramified end previously fixed in CTAB from each morphotype was used for DNA extraction and before extraction carefully examined microscopically to ensure that the DNA products are corresponding to the aimed anatomotypes. DNA of ECM was extracted with DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany). The nrDNA ITS region was amplified using the PCR primers ITS1F and ITS4 (Gardes & Bruns 1993, White et al. 1990). The obtained PCR product was purified using the QIAquick protocol (Qiagen, Hilden, Germany) and fragments were sequenced applying the same primers for PCR. Sequencing was performed by the sequencing service of the Department Biology I (University of München) using BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1. Fungal identification was carried out by searching highly similar sequences (BlastN) in the GenBank (http://www.ncbi.nlm.nih.gov/) and UNITE (http://unite.ut.ee/) databases (Kõljalg et al. 2005).

Results

Morpho-anatomical descriptions

"*Pinirhiza acuminata*"

Morphological characters (Fig. 1a): *Mycorrhizal systems* 4.2–5.4 mm long, dichotomous, with 0–4 orders of ramification, main axis 0.35–0.4 mm diam., hydrophilic, short distance exploration type. *Unramified ends* straight or bent, 0.4–1.1 mm long, 0.35–0.4 mm diam., young mycorrhiza brown, old parts dark brown. *Surface of unramified ends* smooth or loosely woolly, mantle not transparent. *Emanating hyphae* infrequent. *Rhizomorphs* lacking. *Cystidia* lacking. *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Figs. 2–4): Plectenchymatous throughout. *Outer mantle layers* (Figs.2–3) plectenchymatous, without pattern, with a gelatinous matrix with few gluing soil particles (mantle type B/C, according to Agerer 1987–2008, 1991a, Agerer & Rambold 2004–2009), surface with a hyphal net, hyphae of net cylindrical to irregularly inflated at places, uneven in diam., 2–5.5 µm wide, cell walls 0.3 µm, some containing colourless granules; simple septa frequent, clamps infrequent; frequently ramified; tips of hyphae simple or ramified; some hyphae of mantle surface with horn-shaped and acuminate outgrowths; hyphae in outer mantle 3.5–6.5 µm wide with irregular inflations at places up to 13 µm, cell walls thin, 0.3 µm; surface smooth. *Middle mantle layers* (Fig. 4a) plectenchymatous, without pattern, hyphae 3.5–5 µm diam., cell walls 0.3 µm, with simple septa, clamps not observed. *Inner mantle layers* (Fig. 4b) plectenchymatous, compact, hyphae arranged ring-like at places, cells 2.5–4 µm diam., cell walls 0.3 µm, with simple septa, clamps not observed. *Very tip* like remaining parts of the mantle.

Anatomical characters of emanating elements (Figs. 1b–f): *Rhizomorphs* lacking. *Emanating hyphae* (Figs. 1b–f) infrequent, (1.5) 2.5–4.5 µm diam., cell walls 0.3 µm; with frequent clamps and infrequent simple septa, clamps in lateral view in the shape of a semicircle and more than half in diam. than their hypha, reversal clamps present; occasionally with elbow-like structures; membranaceously yellowish, smooth; ramification approximately rectangular, very tip simple, and intrahyphal hyphae common. *Cystidia* lacking.

Chemical reactions: *Mantle preparations*: Melzer's reagent: no reaction.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Helan Mountain, Suyukou National Reserve located in Yinchuan City, Ningxia Hui Nationality Autonomous Region, China, myc. exc. and isol. by Jie Wei, 09.09.2008, JW191a (in M). Accession number in GenBank: GQ979995.

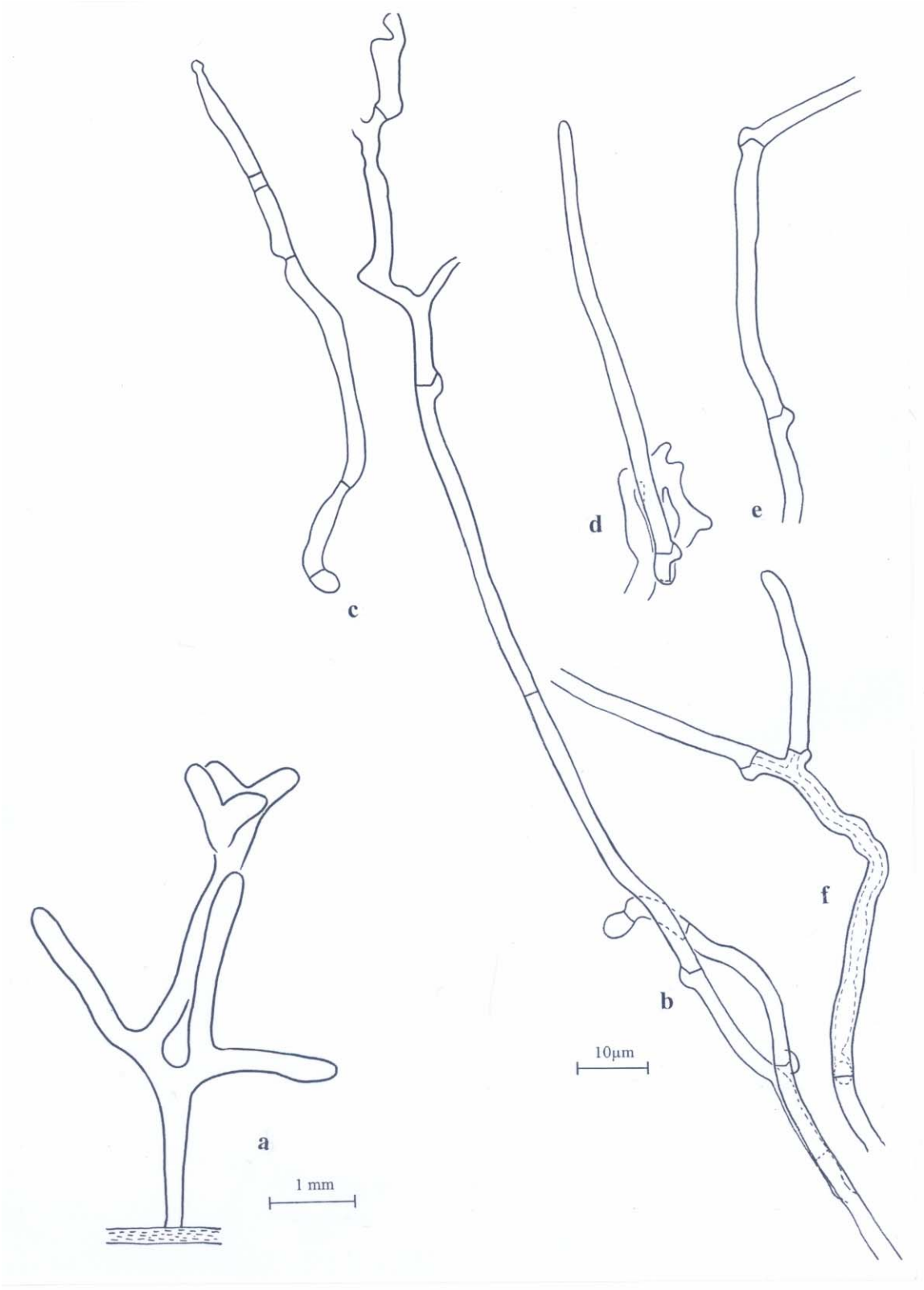


Fig. 1 "*Pinirhiza acuminata*", habit of ECM and emanating hyphae. a habit of ECM, surface smooth; b emanating hyphae with both clamps and simple septa, and with ramified ends; c hypha of mantle surface, very end occasionally with constriction forming a globular structure; d a hypha from the mantle; e reversed clamps on emanating hyphae; f intrahyphal hypha.

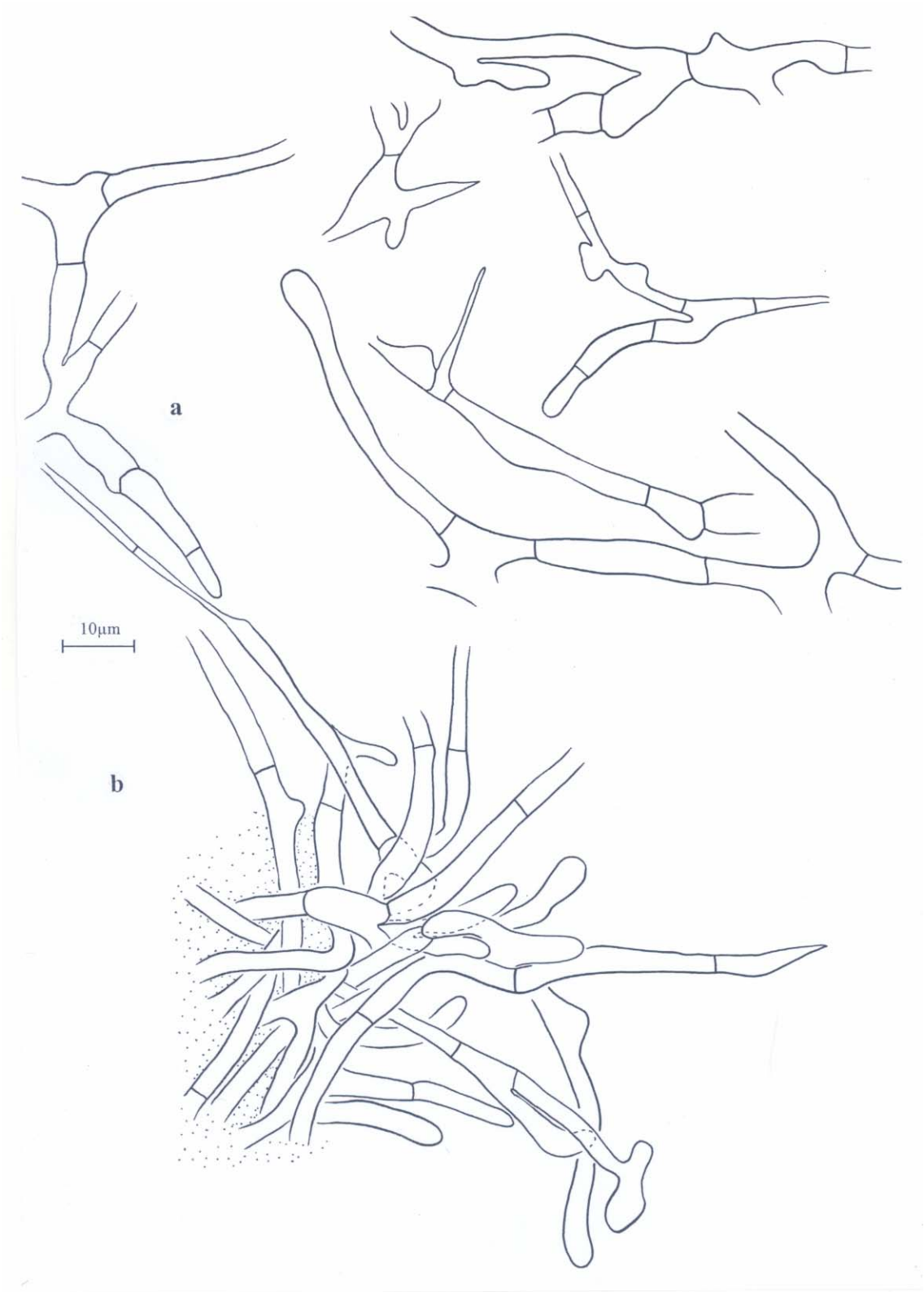


Fig. 2 "*Pinirhiza acuminata*", the hyphal net as well as the hyphae of mantle surface.
a hyphae of the hyphal net on mantle surface, some of them with horn-shaped, acuminate outgrowths, some of them irregularly inflated and ramified; b acuminate hyphae with simple or ramified ends originating from the mantle surface (only a small portion of the mantle shown).

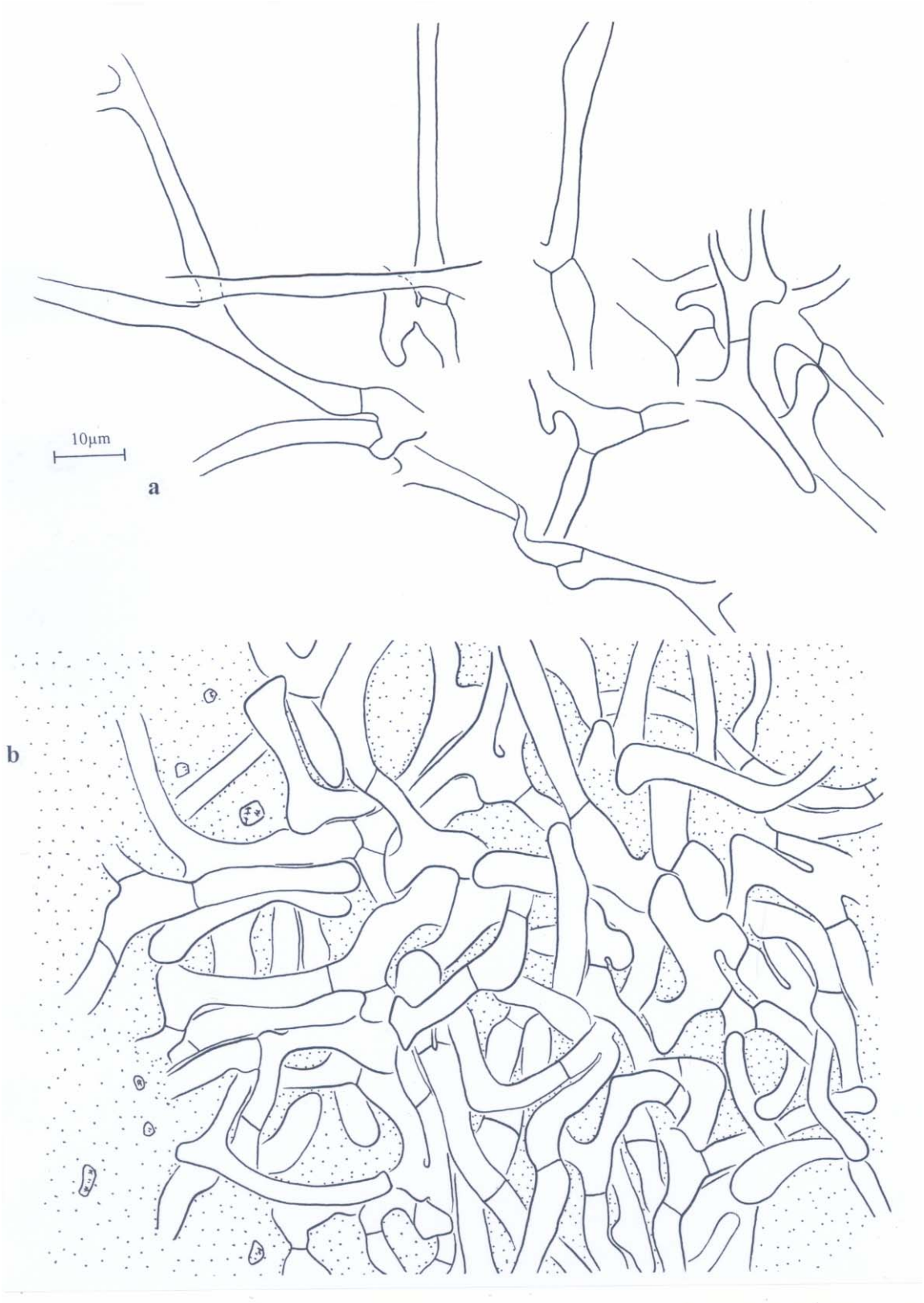


Fig. 3 "*Pinirhiza acuminata*", hyphae of the hyphal net and the plan view of outer mantle layer. a hyphal net consisting of ramified and irregularly shaped hyphae, some of them with clamps; b outer mantle layer with hyphae laying in a gelatinous matrix, some soil particles glued to the matrix.

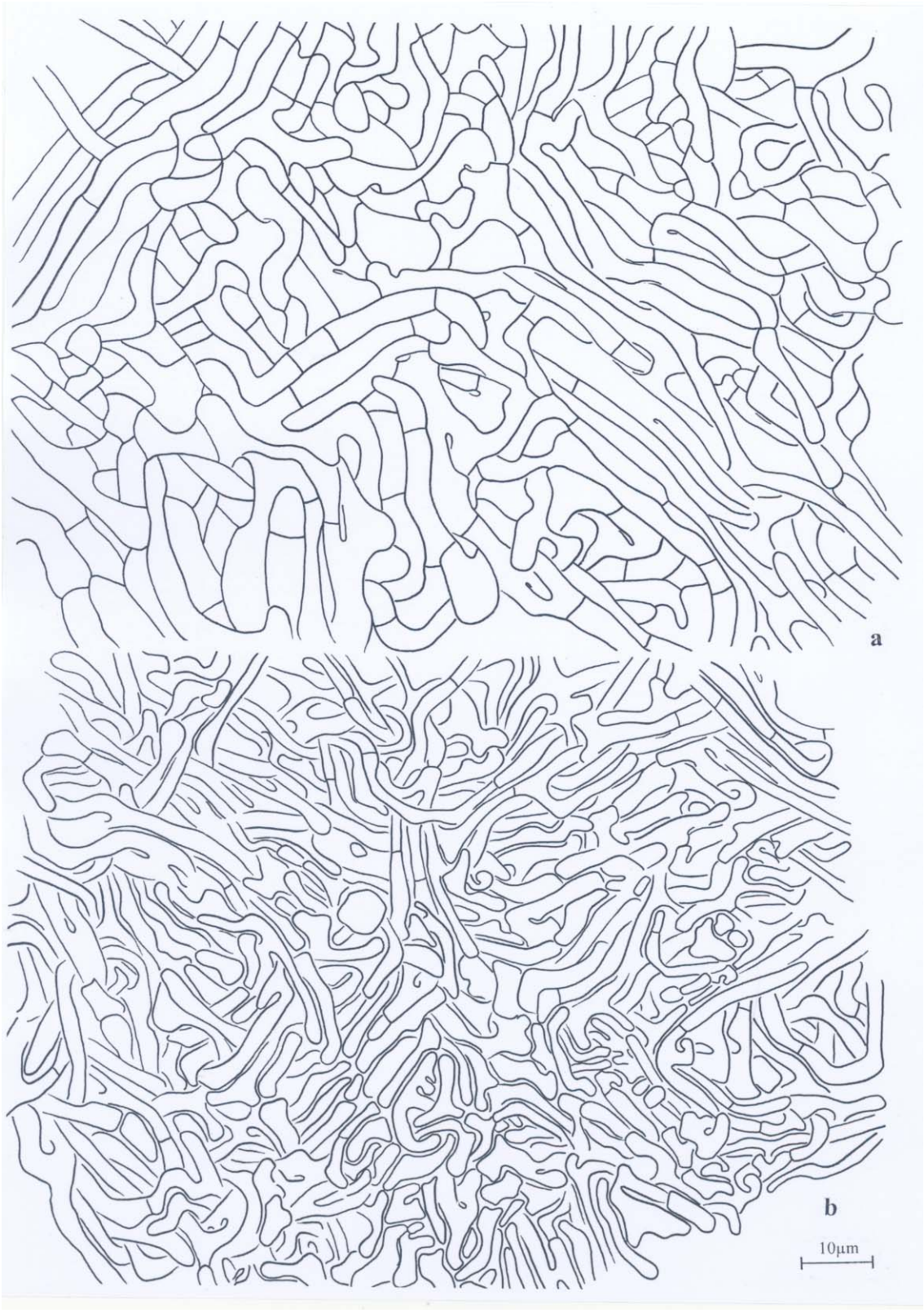


Fig. 4 "*Pinirhiza acuminata*", plan view of middle and inner mantle layers.
a middle mantle layer; b inner mantle layer.

"Pinirhiza heilihensis"

Morphological characters (Fig. 5a): *Mycorrhizal systems* 4–5.5 mm long, irregularly dichotomous, with 0–4(5) orders of ramification, main axis 0.3–0.4 mm diam., hydrophilic, short distance exploration type. *Unramified ends* straight, 0.35–1.3 mm long, 0.3–0.35 mm diam., young parts brown, old parts dark brown. *Surface of unramified ends* smooth or loosely woolly at places, mantle not transparent. *Emanating hyphae* infrequent. *Rhizomorphs* lacking. *Cystidia* infrequent. *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Fig. 6): *Outer mantle layer* (Fig. 6a) pseudoparenchymatous with distinctly elongate angular cells, star-like, with few solitary, roundish, thick-walled (0.5–0.8 µm) cells of 5–8 µm diam. on the surface (mantle type K, according to Agerer 1987–2008, 1991a, Agerer & Rambold 2004–2009), cystidia originating from these cells, surface with a matrix with few soil particles gluing; angular cells of the outer mantle layer 13.5–30(40) µm long, 6–11 µm wide, membranaceously brownish, cell walls 0.8 µm. *Inner mantle layer* (Fig. 6b) plectenchymatous, some parts with ring-like structures, cells 3–4.5 µm diam., cell walls 0.3 µm, membranaceously yellowish, with simple septa, clamps not found. *Very tip* like remaining parts of the mantle.

Anatomical characters of emanating elements (Figs. 5b, 7): *Rhizomorphs* lacking. *Emanating hyphae* (Fig. 7) frequent, brownish, straight to sometimes sinuous, 5–8 µm diam., cell walls 0.5–1 µm, with clamps and simple septa; short branches or outgrowths with pale and thickened walls (1.5–2 µm) occasionally present; intrahyphal hyphae common; ramification frequent, ramification Y-shaped or ca.90˚; surface with very fine warts; anastomoses as long bridges with a clamp close to one of the anastomosing hyphae; backwardly oriented clamps present. *Cystidia* (Fig. 5b) awl-shaped with a wide base, infrequent, membranaceously yellowish, at the base 7–8 µm wide, at the very tip 2–2.5 µm, 35–100 µm long, cell walls 0.3 µm, surface smooth, without septa, without contents.

Chemical reactions: *Mantle preparations*: Melzer's reagent: no reaction.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Heilihe National Reserve located in Chi Feng city, Inner Mongolia, China, myc. exc. and isol. by Jie Wei, 28.08.2008, JW179b (in M). Accession number in GenBank: GQ979997.

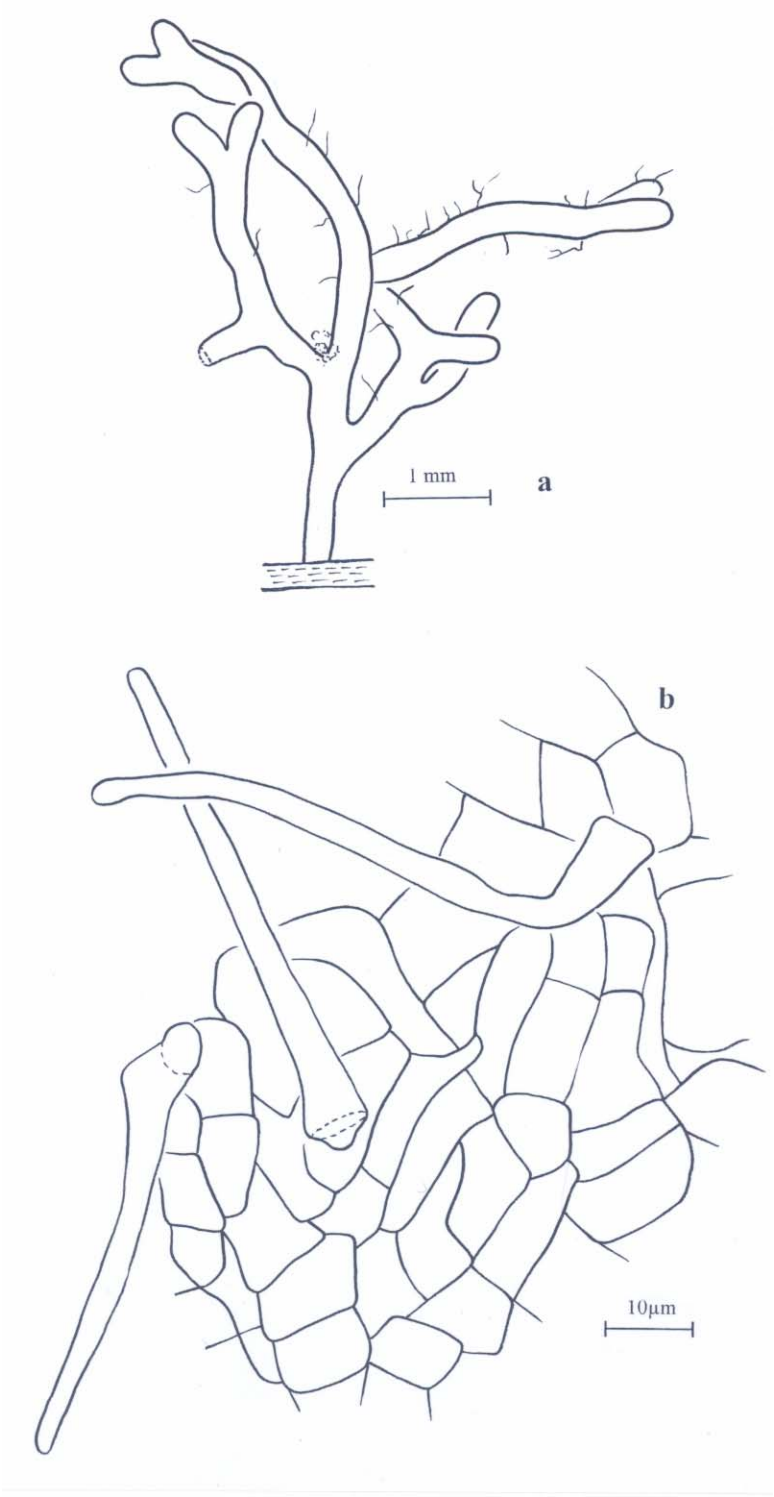


Fig. 5 "*Pinirhiza heilihensis*", habit of ECM and cystidia. a habit of ECM, surface of mantle loosely woolly; b awl-shaped cystidia without any septa.

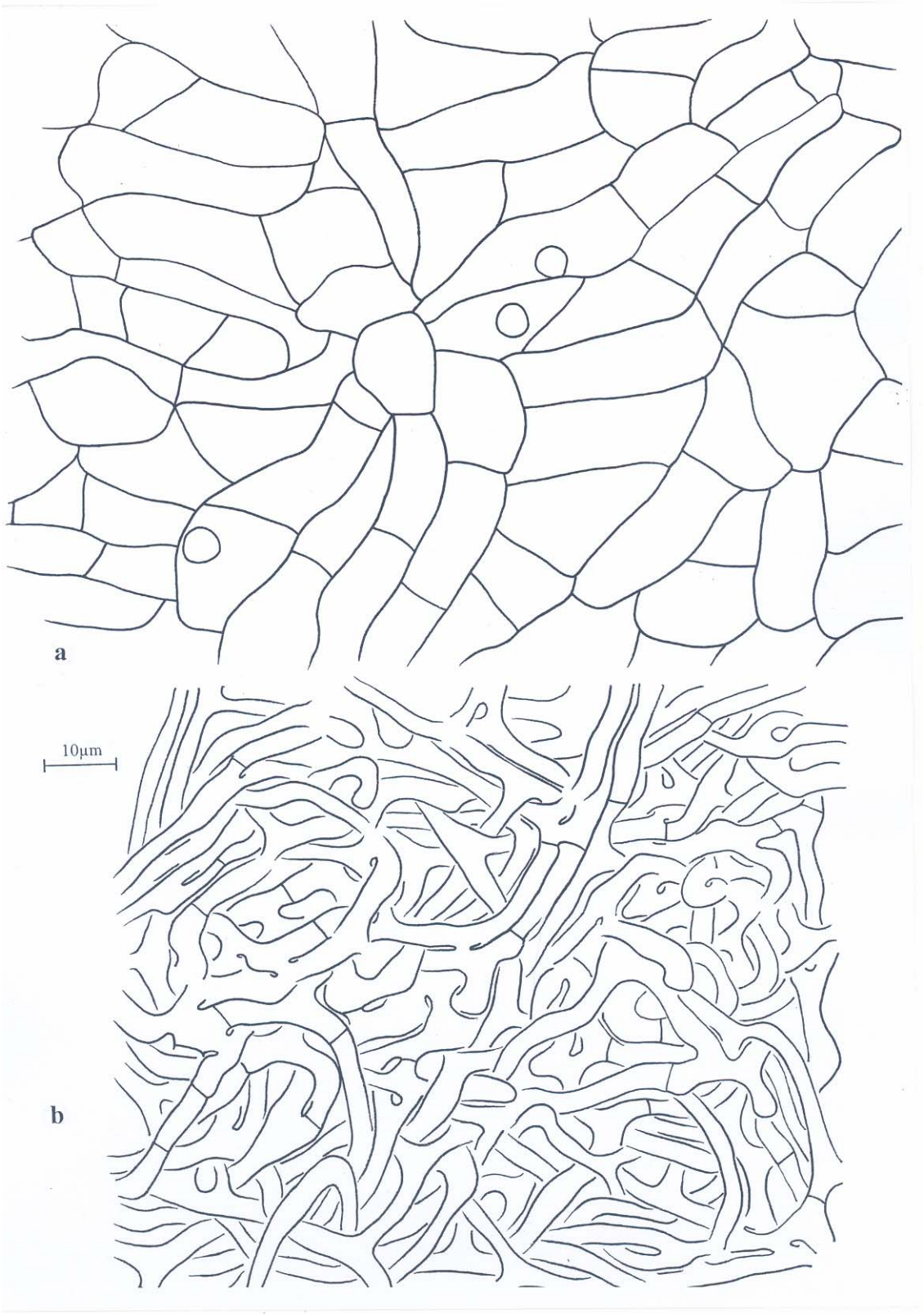


Fig. 6 "*Pinirhiza heilihensis*", plan view of outer and inner mantle layers. a outer mantle layer with angular cells, cells arranged rosette-like, few round cells on the mantle surface; b plectenchymatous inner mantle layer with partially ring-like arranged hyphae.

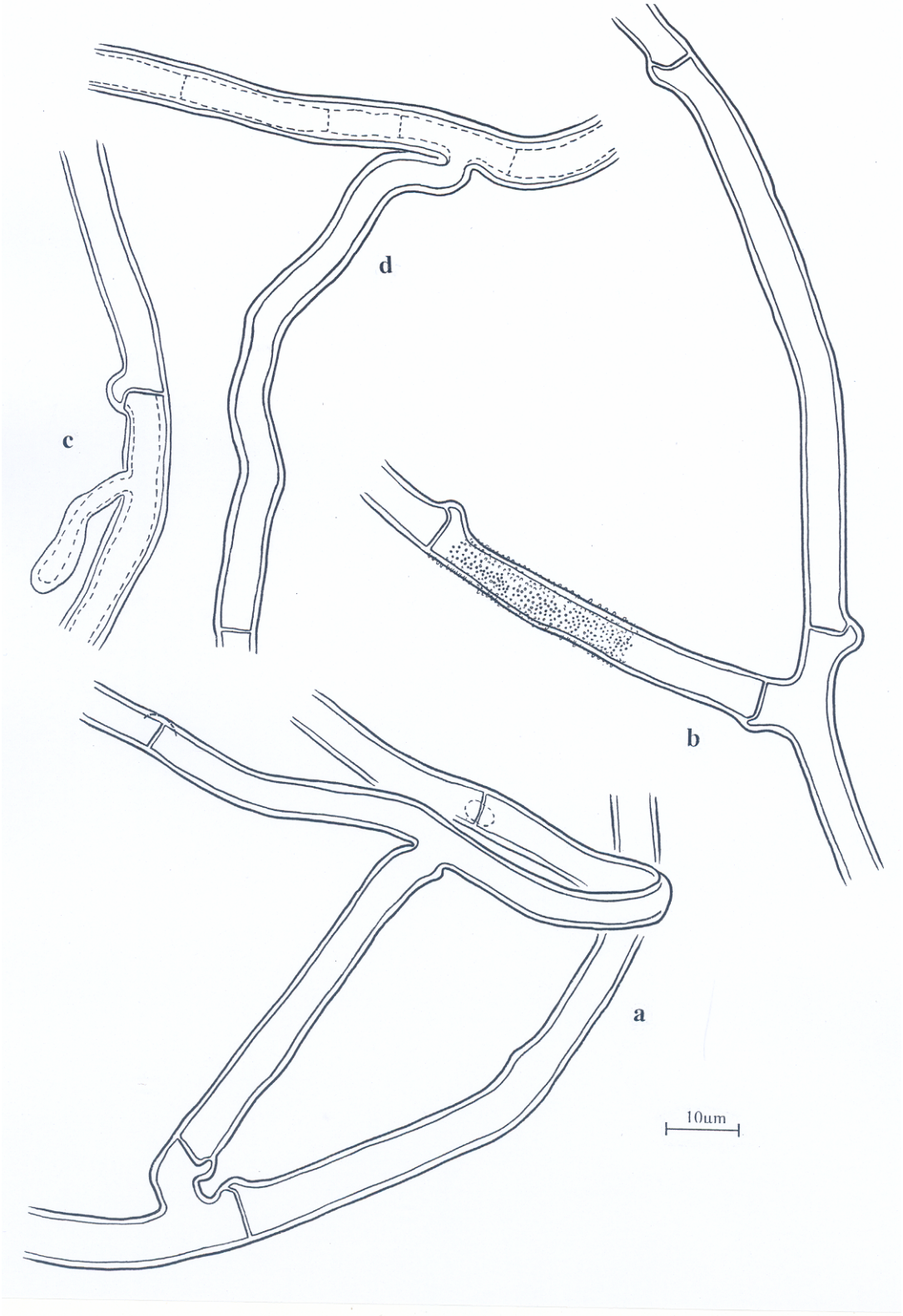


Fig. 7 "*Pinirhiza heilihensis*", emanating hyphae. a anastomosis closed by a clamp near one side of the emanating hypha, with a long bridge; b Y-shaped ramification, surface with fine warts; c emanating hypha with an outgrowth, thick-walled at tip; d intrahyphal hypha.

"Pinirhiza fibulocystidiata"

Morphological characters (Fig. 8a): *Mycorrhizal systems* 0.6–1.0 mm long, dichotomous, with 0–2 orders of ramification, main axis 0.3–0.4 mm diam., hydrophilic, contact exploration type. *Unramified ends* straight, strongly inflated at young parts, young portions 0.5–0.7 mm long, 0.3–0.4 mm diam., brown with yellow tint, old parts dark brown to black, very tip lighter than other parts, yellowish. *Surface of unramified ends* smooth and somewhat shiny, mantle not transparent. *Emanating hyphae* not found. *Rhizomorphs* lacking. *Cystidia* not easily discernible under a stereoscope. *Sclerotia* lacking.

Anatomical characters of mantle in plan views (Fig. 9): *Outer mantle layers* (Fig. 9a) pseudoparenchymatous with angular cells bearing cystidia (mantle type L**,** Agerer 1987–2008, 1991a, Agerer & Rambold 2004–2009), mantle surface with a matrix with few soil particles adhering; hyphal cells 4–14 µm long, and 3–9 µm wide, cell walls thin, 0.3 µm. *Inner mantle layers* (Fig. 9b) plectenchymatous, at places hyphae arranged ring-like, cells 2.5–4.5 µm diam., cell walls 0.3 µm, with simple septa, clamps not observed. *Very tip* like remaining parts of the mantle.

Anatomical characters of emanating elements (Fig. 8b): *Rhizomorphs* lacking. *Emanating hyphae* not found. *Cystidia* (Fig. 8b) frequent, originating from cells of mantle; fibulocystidia-type with an intercalar clamp, capitate with an abrupt inflation; basal parts wider, 4.5–6 µm, distal 2.5–4.5 µm, straight and short, 16–22 µm long; cell walls thin, 0.3 µm; with brown, heterogeneously distributed contents, normally more dense in distal parts.

Chemical reactions: *Mantle preparations*: Melzer's reagent: no reaction.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Helan Mountain, Suyukou National Reserve located in Yinchuan City, Ningxia Hui Nationality Autonomous Region, China, myc. exc. and isol. by Jie Wei, 20.08.2007, JW49a (in M). Accession number in GenBank: GQ979996.

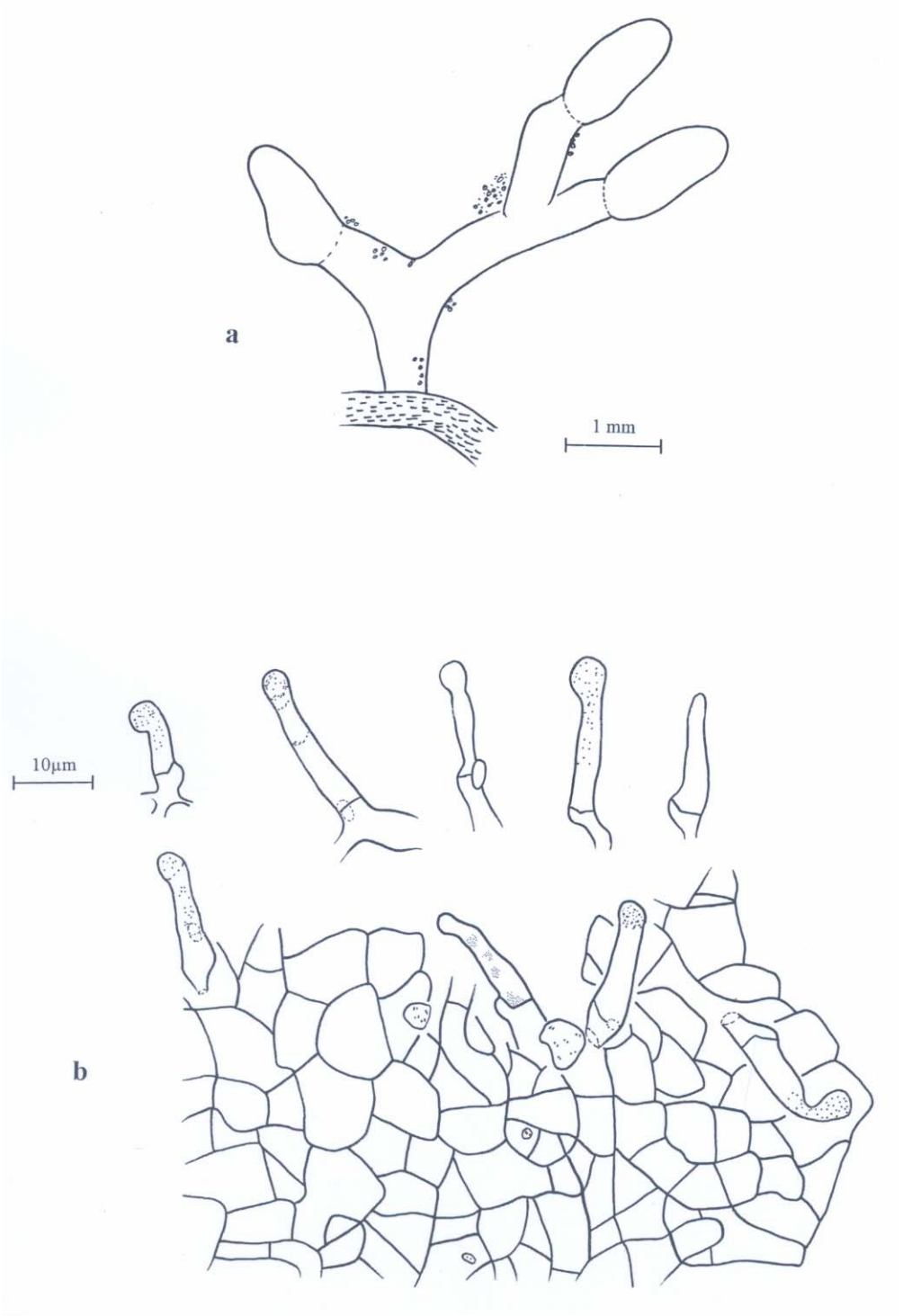


Fig. 8 "*Pinirhiza fibulocystidiata*", habit of ECM and cystidia. a habit of ECM, note young inflated parts; b cystidia, growing on the mantle, as well as separately drawn, with brownish contents, very tip of them capitate with an abrupt inflation; note unevenly distributed, mainly in the apical inflations concentrated brownish contents.

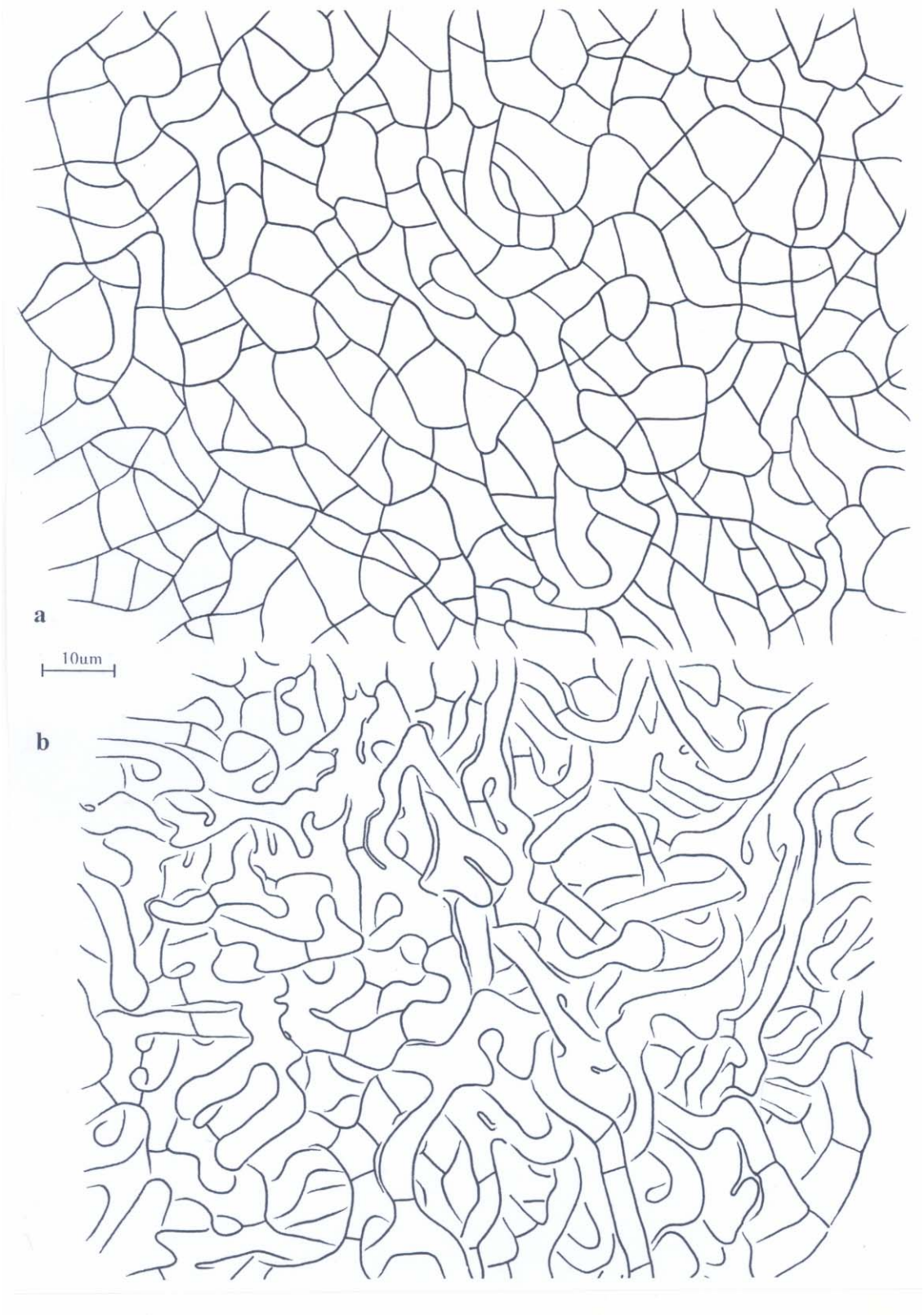


Fig. 9 "*Pinirhiza fibulocystidiata*", plan view of outer and inner mantle layers. a pseudoparenchymatous outer mantle layer with angular cells; b plectenchymatous inner mantle layer.

nrDNA sequence analyses by Blast Search in GenBank and UNITE

The first 100 matched sequences in GenBank show at least 92% maximum identity and at least 92% query coverage with "*Pinirhiza acuminata*" and are all in Thelephoraceae including uncultured Thelephoraceae ECM (or Thelephoraceae clones) and uncultured *Tomentella* ECM (or *Tomentella* isolates). The sequence of "*Pinirhiza acuminata*" matches best with FJ554019 and EU668276 with 98% identity and 100% query coverage designated each as an uncultured *Tomentella*. The only identified species of the first 100 most similar entries is *Tomentella atramentaria* (DQ974772, EF644114, AF272904 and EF644115) that show 93%–100% query coverage and 93%–95% maximum identity with our sequence of "*P. acuminata*". Also two uncultured *Thelephora* spp. are mentioned in these first 100 matched sequences, sequence DQ482000 matches that of "*P. acuminata*" with 95% identity and 100% coverage, that of isolate FJ816756 shows 100% query coverage and 94% maximum identity. The 37 retrieved sequences from UNITE as compared to the sequence of "*Pinirhiza acuminata*", are all *Tomentella* species. The first 15 sequences, are from *T. atramentaria*, *T. badia*, *T. bryophila*, *T. cinerascens*, *T. stuposa*, *T.* spp. and *T. subclavigera*, and show at least 91% identity (excluding sequences locked by the authors). The sequence of "*Pinirhiza acuminata*" matches best with *Tomentella atramentaria* (UDB000235, Russia) and *T. badia* (UDB000961, Norway) both with 95% identity.

The first 100 matched sequences with "*P. heilihensis*" from GenBank reveal at least 91% query coverage and at least 90% maximum identity and are all in Thelephoraceae including uncultured Thelephoraceae ECM (or Thelephoraceae clones) and uncultured *Tomentella* ECM (or *Tomentella* isolates). The sequence of "*P. heilihensis*" matches best with an uncultured ECM (FJ196988) with 98% identity and 100% query coverage mentioned as a member of Thelephoraceae. The only identified species of the 100 matched sequences is *Tomentella ramosissima* (U83480) that shows 97% maximum identity and 100% query coverage.

The 37 retrieved sequences from UNITE as compared to the sequence of "*P. heilihensis*" are all *Tomentella* species. The first 15 sequences, *T. fuscocinerea*, *T. lapida*, *T. lateritia*, *T. lilacinogrisea*, *T. stuposa*, *T. subclavigera*, and *T. subtestacea*, show at least 90% identity (excluding sequences locked by the authors). The sequence of "*P. heilihensis*" matches best with *Tomentella lapida* (UDB000270, Norway) with 98% identity.

The first 100 matched sequences of "*P. fibulocystidiata*" in GenBank show at least 89% query coverage and at least 92% maximum identity and are all in Thelephoraceae including uncultured Thelephoraceae ECM (or Thelephoraceae clones) and uncultured *Tomentella* ECM (or *Tomentella* isolates).The sequence of "*P. fibulocystidiata"* matches best with DQ150117 supposed as being a member of Thelephoraceae with 99% identity and100% query coverage. Only two identified sequences provided in GenBank, are *Tomentella ferruginea* (EU819497) and *T. cinerascens* (U83483), both match the sequence of "*P. fibulocystidiata*" with 100% coverage and 92% maximum identity.

The 37 retrieved sequences shown in UNITE as compared to the sequence of "*P. fibulocystidiata*" are all *Tomentella* species. The first 15 sequences from *T. coerulea*, *T. galzinii*,*T. subtestacea*, *T.* spp., and *T. viridula* show at least 94% identitiy (excluding sequences locked by the authors). The sequence of "*P. fibulocystidiata*" matches best with *Tomentella viridula* (UDB000261, Sweden) with 96% identity, with *Tomentella subtestacea* (UDB000034, Denmark) and *T. galzinii* (UDB000264, Finland; UDB000263, Russia) with 95 % identity each.

Discussion

"*Pinirhiza acuminata*" differs from "*P. heilihensis*" and "*P. fibulocystidiata*" - apart from lacking cystidia - in having a mantle that is plectenchymatous throughout with a hyphal net laying on the mantle, and in the presence of acuminate hyphae in this net. It resembles some brown *Tomentella* ECM, "*Afzeliaerhiza beninensis*" (Yorou & Agerer 2008), "*Quercirhiza tomentellofuniculosa*" (Azul et al. 2006g), "*Quercirhiza tomentelloreticulata*" (Azul et al. 2008c), *Tomentella brunneorufa* (Agerer & Bougher 2001), *Tomentella ferruginea* (Raidl & Müller 1996), and "*Uapacaerhiza wariensis*" (Yorou et al. 2008), in having a plectenchymatous outer mantle and in the lack of awl-shaped cystidia. "*Quercirhiza tomentelloreticulata*" differs distinctly from "*Pinirhiza acuminata*" by a hyphal net composed of strongly ramified, very thin, frequently bent hyphae and in the lack of clamps. "*Q. tomentellofuniculosa*" and *T. ferruginea* can easily be distinguished from "*P. acuminata*" by their frequent rhizomorphs peripherally covered by repeatedly branched very thin hyphae and by thick central hyphae. "*Afzeliaerhiza beninensis*", *Tomentella brunneorufa,* and "*U. wariensis*" lack acuminate hyphae on the mantle surface and possess slightly differentiated or

undifferentiated rhizomorphs. "*Pinirhiza acuminata*" is also similar to *Pseudotomentella humicola* ECM (Di Marino et al. 2007) due to its plectenchymatous mantle, and the presence of acuminate outer mantle hyphae, but differs by the lack of star-like arranged heaps of hyphae. Blast search of ITS nrDNA in GenBank and UNITE indicate that "*Pinirhiza acuminata*" could be a member of *Tomentella*, but an identification to species level was not possible.

"*Pinirhiza heilihensis*" and "*P. fibulocystidiata*" form both pseudoparenchymatous outer mantle layers with angular cells lacking a hyphal net. Both have cystidia, but they differ in their shape. Cystidia are awl-shaped with a wide base, lack a basal clamp and contents in "*P. heilihensis*". Fibulocystidia are specific for "*P. heilihensis*". They reveal an intercalar clamp, are inflated at their tips and show brown contents. The dimension of outer mantle cells as well as their arrangement keeps both ECM apart, too: star-like arranged (13.5–30 (40) µm long, 6–11 µm wide) cells with thick walls (0.8 µm) are characteristic for "*P. heilihensis*", whereas those of "*P. fibulocystidiata*" are smaller (4–14 µm long, 3–9 µm wide) with thin walls (0.3 µm) and are arranged without a special pattern.

"*Pinirhiza heilihensis*" resembles closely some *Tomentella*-like ECM, as "*Piceirhiza obscura*", "*Quercirhiza ateracusrugosa*" and "*Fagirhiza setifera*" in having a pseudoparenchymatous outer mantle with awl-shaped cystidia and in having clamped emanating hyphae, but differs from "*Piceirhiza obscura*" in having angular cells in outer mantle instead of epidermoid cells. It is different from "*Quercirhiza ateracusrugosa*" and "*Fagirhiza setifera*" by thin-walled (0.3 µm) cystidia in comparison to those of both latter ECM (0.5–1.5 µm). "*Pinirhiza fibulocystidiata*" is similar to some *Tomentella* ECM, as *T. galzinii*, *T. pilosa* and *T. subtestacea,* in having a pseudoparenchymatous outer mantle with angular cells and in possessing cystidia with an intercalary clamp, but differs in cystidia of deviating dimensions (Jakucs et al. 1997, Jakucs & Agerer 1999, 2001). The cystidia of "*P. fibulocystidiata*" are 16–22 µm shorter than those of *T. galzinii* (40–55 µm), *T. pilosa* (55–60 µm), and *T. subtestacea* (25–30 µm). *Tomenella subtestacea* and "*P. fibulocystidiata*" have in common brownish contents in the cystidia, but *T. subtestacea* can be kept apart from "*P. fibulocystidiata*" due to a hyphal net on the mantle surface. ITS nrDNA sequence comparisons by Blast Search in GenBank show that "*P. heilihensis*" and "*P. fibulocystidiata*" are likely ECM of Thelephoraceae, and could be members of *Tomentella* as indicated by Blast Search in UNITE.

Key for supposed thelephoroid ECM according to anatomical features (this study, including those compiled in Tab. 1)

1 Outer mantle layer plectenchymatous
2 Cystidia present
3 Cystidia awl-shaped; middle mantle plectenchymatous; mycorrhizae brownish
Thelephora terrestris
3* Cystidia hypha-like, slightly fusiform; middle mantle pseudoparenchymatous with star-like arranged angular cells; mycorrhizae yellow
"***Quercirhiza flavocystidiata***"
2* Cystidia lacking
4 Emanating hyphae without clamps
5 Mantle surface covered with star-like arranged heaps of hyphae
Pseudotomentella humicola
5* Mantle surface not covered by star-like arranged heaps of hyphae, ring- or star-like structures in outer mantle layers may be present
6 Outer mantle layer with distinct ring-like pattern, mantle surface without strongly ramified, very thin, frequently bent hyphae; ECM reddish pink
Tomentellopsis submollis
6* Outer mantle layer without a distinct ring-like pattern, if an indistinct ring-like pattern discernible, then mantle surface with strongly ramified, very thin, frequently bent hyphae; ECM not reddish pink
7 Outer mantle with an indistinct ring-like pattern, covered by a net composed of strongly ramified, very thin, frequently bent hyphae
"***Quercirhiza tomentelloreticulata***"
7* Outer mantle layer without a ring-like pattern, and without a surface net
Pseudotomentella tristis
4* Emanating hyphae with clamps
8 Rhizomorphs lacking; mantle surface with acuminate hyphal cells
"***Pinirhiza acuminata***"
8* Rhizomorphs present; acuminate hyphal cells on mantle surface lacking
9 Emanating hyphae only with a basal clamp ***Tomentella brunneorufa***
9* Emanating hyphae with frequent clamps
10 Rhizomorphs not differentiated "***Uapacaerhiza wariensis***"

10* Rhizomorphs with thicker central hyphae

11 Rhizomorphs enveloped by multiply branched, thin, irregularly shaped hyphae

12 Mantle with a delicate and star-like arranged hyphal net "***Quercirhiza tomentellofuniculosa***"

12* Mantle without a delicate and star-like arranged hyphal net ***Tomentella ferruginea***

11* Rhizomorphs not enveloped by multiply branched, thin, irregularly shaped hyphae "***Afzeliaerhiza beninensis***"

1* Outer mantle layer pseudoparenchymatous

13 Mantle with cystidia

14 Cystidia with an intercalary clamp

15 Cystidia up to 30 µm long, with brownish contents

16 Cystidia originate from a hyphal net forming also aculeate horn-shaped cells ***Tomentella subtestacea***

16* Cystidia originate from the cells of mantle surface "***Pinirhiza fibulocystidiata***"

15* Cystidia more than 40 µm long, with greenish blue granules or without granules

17 Mantle surface with a hyphal net ***Tomentella pilosa***

17* Mantle surface without a hyphal net ***Tomentella galzinii***

14* Cystidia of different shape

18 Cystidia bottle-shaped

19 Outer mantle without heaps of thick-walled cells "***Quercirhiza tomentellocystidiata***"

19* Outer mantle with heaps of thick-walled cells

20 Cells of heaps more regularly assembled on mantle surface, awl-shaped cystidia present in addition "***Piceirhiza nigra***

20* Cells of heaps patchy assembled, a second type of cystidia lacking

21 Cells of heaps big (9–21 µm) "***Fagirhiza spinulosa***"

21* Cells of heaps small (5–13 (17) µm) "***Pinirhiza dimorpha***"

18* Cystidia not bottle-shaped

22 Cystidia awl-shaped

23 Cystidia with a clamp at the base "***Quercirhiza aurateroocystidiata***"

23* Cystidia without clamps

24 Emanating hyphae without clamps, outer mantle with epidermoid cells, a special arrangement lacking "***Piceirhiza obscura***"

24* Emanating hyphae with clamps, outer mantle layer with angular cells, star- to rosette-like arranged

25 Cystidia thin walled (0.3 μm) "***Pinirhiza heilihensis***"

25* Cystidia thick walled (0.5–1.5 μm)

26 Middle mantle layer plectenchymatous "***Quercirhiza ateracusrugosa***"

26* Middle mantle layer pseudoparenchymatous "***Fagirhiza setifera***"

22* Cystidia club-shaped or hypha-like

27 Cystidia club-shaped, very end simple "***Quercirhiza nodulosomorpha***"

27* Cystidia hypha-like, very end ramified "***Pinirhiza tomentelloides***"

13* Mantle without cystidia

28 Emanating hyphae without clamps "***Pinirhiza cyaneoviridis***"

28* Emanating hyphae with clamps

29 Cells of outer mantle layer irregular with some epidermoid cells "***Quercirhiza tomentelloepidermoidea***"

29* Cells of outer mantle layer roundish or angular

30 Cells of outer mantle roundish "***Quercirhiza tomentellocumulata***"

30* Cells of outer mantle angular

31 Rhizomorphs present

32 Rhizomorphs not differentiated, thicker central hyphae lacking

33 Cells of outer mantle rosette-like arranged ***Tomentella stuposa***

33* Cells of outer mantle without discernible pattern

34 Mantle surface with heaps of global cells "***Pinirhiza amyloidea***"

34* Mantle surface without heaps of global cells ***Tomentella sublilacina***

32*Rhizomorphs slightly differentiated, thicker central hyphae present

35 Cells of outer mantle layer star- to rosette-like arranged

36 Mantle surface with groups of globose cells "***Populirhiza asperula***"

36* Mantle surface without groups of globose cells

37 Mantle surface with a hyphal net

"***Fagirhiza asteromustrata***"

37* Mantle surface without a hyphal net

"***Quercirhiza tomentelloflexuosa***"

35* Cells of outer mantle layer without pattern

38 Mantle surface with mounds of flattened cells

"***Quercirhiza cumulosa***"

38* Mantle surface without mounds of flattened cells

"***Fagirhiza stellata***"

31* Rhizomorphs lacking

39 Cells of outer mantle layer star-like arranged, with short cystidia-like hyphae "***Pinirhiza ligulata***"

39* Cells of outer mantle layer not star-like arranged, without short cystidia-like hyphae

40 Mantle surface with mounds of flattened cells

"***Quercirhiza squamosa***"

40* Mantle surface without mounds of flattened cells

41 Mantle surface with a hyphal net composed of angular cells

"***Quercirhiza stellata***"

41* Mantle surface with a hyphal net composed of cylindrical hyphae

"***Quercirhiza summatriangularis***"

This key shows that ECM of Thelephoraceae are characterized by heterogeneous mantle types. Plectenchymatous mantles occur in *Pseudotomentella*, *Thelephora*, and *Tomentellopsis* and in a few identified species of the genus *Tomentella* as well as in some ECM designated with an ECM specific binomen (i.e., with the suffix -rhiza) that have been proven by nrDNA analyses as belonging to the genus *Tomentella*. The greater portion of *Tomentella* species form pseudoparenchymatous mantles. This agrees with already published data (Agerer 2006).

Some of the Thelephoraceae ECM resemble those of other relationships, but they can be rather easily distinguished. ECM of *Thelephora terrestris* are characterized by typical awl-shaped and thick-walled cystidia with a basal clamp. Plectenchymatous mantles with awl-shaped cystidia also occur in ECM of the genera *Albatrellus*, *Gomphidius,* and *Chroogomphus* (Agerer & Rambold 2004–2009). ECM of *Albatrellus ovinus* (Agerer 1996b),

however, are hydrophobic, form distally forked cystidia without a basal clamp, clampless emanating hyphae, and phlegmacioid rhizomorphs, whereas ECM of *Thelephora terrestris* (Agerer & Weiss 1989), *Gomphidius* (Agerer 1991b) and *Chroogomphus* (Agerer 1990) are hydrophilic, have clamped emanating hyphae, simple-ended cystidia with a basal clamp and possess thelephoroid (Agerer 1999) rhizomorphs when rhizomorphs are formed at all. But ECM of *Thelephora terrestris* differ from Gomphidiaceae ECM in lacking globular cells on the mantle surface.

There are a few thelephoroid ECM known to date that could be confounded with *Tuber* ECM. Epidermoid outer mantle cells with awl-shaped cystidia occurring in "*Piceirhiza obscura*", have also been found in *Tuber borchii*, *T. brumale*, *T. magnatum*, and *T. melanosporum* (Agerer 2006, Agerer & Rambold 2004–2009, Giraud 1990). "*Piceirhiza obscura*" has clampless emanating hyphae as in *Tuber* ECM. However, cystidia of "*Piceirhiza obscura*" are browinish and smooth, whereas those of these *Tuber* ECM are colourless and covered by infrequent tiny, acicular wall-structures close to their apex. Cystidia of *Tuber* species quite frequently originate from short hyphal bridges laying on the pseudoparenchymatous mantle, what is not the case in "*P. obscura*".

Angular outer mantle cells with awl-shaped cystidia without clamps occurring in "*Fagirhiza setifera*", "*Pinirhiza heilihensis*", "*Piceirhiza nigra*", and "*Quercirhiza ateracusrugosa*", have also been observed in three *Tuber* ECM, *T. aestivum*, *T. mesentericum* and *T. uncinatum* (Agerer 2006, Agerer & Rambold 2004–2009, Giraud 1990). But clamped emanating hyphae as well as some distinctive characteristics, such as roundish cells on the mantle surface as in all four *Tomentella* ECM mentioned above, star- to rosette-like arrangement of outer mantle cells as in "*Fagirhiza setifera*" and "*Pinirhiza helihensis*" keep these four *Tomentella* ECM distinctly apart from *Tuber* ECM.

Some brown thelephoroid ECM with pseudoparenchymatous outer mantle layers consisting of angular cells and lacking cystidia are similar to ECM of *Genea* and *Humaria* (Brand 1991, Erős-Honti et al. 2008, Jakucs et al. 1998, Tedersoo et al. 2006, Wei et al. 2009), *Trichophaea* (Águeda et al. 2008, Wei et al. 2009), and *Coltricia* sp. (Tedersoo 2007b). But all *Tomentella* or *Tomentella*-like ECM with above mentioned features have, except for "*Pinirhiza cyaneoviridis*", clamped emanating hyphae, in contrast to those of *Humaria,*

Genea, Trichophaea, and *Coltricia*. "*Pinirhiza cyaneoviridis*" can be distinguished by its heaps of thick-walled cells (0.5–3 µm) and the presence of amyloidity and blue granules. Furthermore, all *Tomentella* or *Tomentella*-like ECM in question differ from ECM of *Genea* and *Humaria* in the lack of small septa connecting outer mantle cells, and in the lack of a discontinuous surface layer consisting of irregularly shaped, thick-walled cells, except for *Genea hispidula*, (Brand 1991, Erős-Honti et al. 2008, Tedersoo et al. 2006, Wei et al. 2009), from ECM of *Trichophaea* (Águeda et al. 2008, Wei et al. 2009) in the lack of heaps of polygonal cells on mantle surface and of frequently ramified clampless emanating hyphae. Most of the *Tomentella* or *Tomentella*-like brown thelephoroid ECM with pseudoparenchymatous outer mantle layers and lacking cystidia posses rhizomorphs, except for "*Pinirhiza ligulata*", "*Quercirhiza squamosa*", "*Q. stellata*", and "*Q. summatriangularis*", whereas ECM of *Coltricia* sp., *Genea*, *Humaria* and *Trichophaea* do not form rhizomorphs. "*Pinirhiza ligulata*" differs from ECM of *Coltricia* sp., *Genea*, *Humaria* and *Trichophaea* by short, thick-walled cystidia-like hyphae and star-like arranged cells in outer mantle, "*Quercirhiza squamosa*" by mounds of flattened and dark stained cells on outer mantle, "*Q. stellata*" by a hyphal net of angular cells on the mantle surface, and "*Q. summatriangularis*" by a distinct hyphal net forming triangular rings on the mantle surface.

Acknowledgements

This study was financially supported by German Academic Exchange Service (DAAD). We thank also Prof. Yan Wei (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) for his support of field work and equipment, Fan Yongjun (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) for his field assistance, the Managment Authority of Helan Mountain National Reserve and Heilihe National Reserve for the allowance of field sampling.

References

AGERER, R. (1987–2008) Colour Atlas of Ectomycorrhizae. 1st – 14th delivery. Einhorn, Schwäbisch Gmünd.

AGERER, R. (1990) Studies on ectomycorrhizae XXIV. Ectomycorrhizae of *Chroogomphus helveticus* and *C. rutilus* (Gomphidiaceae, Basidiomycetes) and their relationship to those of *Suillus* and *Rhizopogon*. Nova Hedwigia **50**: 1–63.

AGERER, R. (1991a) Characterization of Ectomycorrhizae. In Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods in Microbiology vol 23, pp 25–73. Acad Press, London et al.

AGERER, R. (1991b) Studies on ectomycorrhizae. XXXIV. Mycorrhizae of *Gomphidius glutinosus* and of *G. roseus* with some remarks on Gomphidiacea (Basidiomycetes). Nova Hedwigia **53**: 127–170.

AGERER, R. (1994) *Pseudotomentella tristis* (Thelephoraceae). Eine Analyse von Fruchtkörper und Ektomykorrhizen. Z Mykol **60**: 143–158.

AGERER, R. (1996a) Ectomycorrhizae of *Tomentella albomarginata* (Thelephoracea) on Scots pine. Mycorrhiza **6**: 1–7.

AGERER, R. (1996b) *Albatrellus ovinus* (Schaeff.: Fr.) Kotl. & Pouz. + *Picea abies* (L.) Karst. Descr Ectomyc **1**: 23–28.

AGERER, R. (1999) Never change a functionally successful principle: the evolution of Boletales s.1. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendtnera **6**: 5-91.

AGERER, R. (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress **5**: 67–107.

AGERER, R. & N.L. BOUGHER (2001) *Tomentella brunneorufa* + *Eucalyptus* sp. Descr Ectomyc **5**: 205–212.

AGERER, R. & G. RAMBOLD (2004–2009, First posted on 2004-06-01; most recent update: 2009-01-26) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de – München, Germany.

AGERER, R. & M.WEISS (1989) Studies on ectomycorrhizae. XX. Mycorrhizae formed by *Thelephora terrestris* on Norway spruce. Mycologia **81**: 444–453.

ÁGUEDA, B., R. AGERER, A.M. DE MIGUEL & J. PARLADÉ (2008) "*Quercirhiza quadratum*" + *Quercus ilex* L. subsp. *ballota* (Scop.) Desf. Samp.Descr Ectomyc **11/12**: 113–123.

AZUL, A.M., R. AGERER & H. FREITAS (1999) "*Quercirhiza nodulosomorpha*" + *Quercus suber* L. Descr Ectomyc **4**: 103–108.

AZUL, A.M., R. AGERER & H. FREITAS (2006a) "*Quercirhiza ateracusrugosa*" + *Quercus suber* L. Descr Ectomyc **9/10**: 75–79.

AZUL, A.M., R. AGERER & H. FREITAS (2006b) "*Quercirhiza aurateroc ystidiata*" + *Quercus suber* L. Descr Ectomyc **9/10**: 81–86.

AZUL, A.M., R. AGERER , M.P. MARTIN & H. FREITAS (2006c) "*Quercirhiza flavocystidiata*" + *Quercus suber* L. Descr Ectomyc **9/10**: 93–97.

AZUL, A.M., R. AGERER & H. FREITAS (2006d) "*Quercirhiza summatriangularis*" + *Quercus suber* L. Descr Ectomyc **9/10**: 111–114.

AZUL, A.M., R. AGERER & H. FREITAS (2006e) "*Quercirhiza tomentellocystidiata*" + *Quercus suber* L. Descr Ectomyc **9/10**: 115–119.

AZUL, A.M., R. AGERER & H. FREITAS (2006f) "*Quercirhiza tomentelloflexuosa*" + *Quercus suber* L. Descr Ectomyc **9/10**: 121–126.

AZUL, A.M., R. AGERER , M.P. MARTIN & H. FREITAS (2006g) "*Quercirhiza tomentellofuniculosa*"+ *Quercus suber* L. Descr Ectomyc **9/10**: 127–134.

AZUL, A.M., R. AGERER , M.P. MARTIN & H. FREITAS (2008a) "*Quercirhiza tomentellocumulata*" + *Quercus suber* L. Descr Ectomyc **11/12**: 125–130.

AZUL, A.M., R. AGERER , M.P. MARTIN & H. FREITAS (2008b) "*Quercirhiza tomentelloepidermoidea*" + *Quercus suber* L. Descr Ectomyc **11/12**: 131–134.

AZUL, A.M., R. AGERER, M.P. MARTIN & H. FREITAS (2008c) "*Quercirhiza tomentelloreticulata*" + *Quercus suber* L. Descr Ectomyc **11/12**: 135–139.

AZUL, A.M., R. AGERER , M.P. MARTIN & H. FREITAS (2008d) "*Quercirhiza tomentellostellata*" + *Quercus suber* L. Descr Ectomyc **11/12**: 141–146.

BERG, B. (1989) Charakterisierung und Vergleich von Ektomykorrhizen gekalkter Fichtenbestände. Dissertation, University of München.

BRAND, F. (1991) Ektomykorrhizaen an *Fagus sylvatica*– Charakterisierung und Identifizierung, ökologische Kennzeichnung und unsterile Kultivierung. Libri Botanici Vol 2. IHW. Eching.

DE ROMAN, M., R. AGERER & A.M. DE MIGUEL (2002a) "*Quercirhiza cumulosa*" + *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. Descr Ectomyc **6**: 13–18.

DE ROMAN, M., R. AGERER & A.M. DE MIGUEL (2002b) "*Quercirhiza stellata*" + *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. Descr Ectomyc **6**: 19–24.

DI MARINO, E., U. KÕLJALG & R. AGERER (2007) The ectomycorrhizae of *Pseudotomentella humicola* on *Picea abies*. Nova Hedwigia **84** (3): 429–440.

DI MARINO, E., L. MONTECCHIO & R. AGERER (2008) "*Fagirhiza stellata*" + *Fagus sylvatica* L. Descr Ectomyc **11/12**: 59–69.

ERŐS-HONTI, Z., G.M. KOVACS, G. SZEDLAY & E. JACUCS (2008). Morphological and molecular characterization of *Humaria* and *Genea* ectomycorrhizae from Hungarian deciduous forests. Mycorrhiza **18**: 133–143.

GARDES, M. & T.D. BRUNS (1993) ITS primers with enhanced specificity for basidiomycetes – applications to the identification of mycorrhizae and rusts. Mol Ecol **2**: 113–118.

GIRAUD, M. (1990) Mycorhizes: prelevement et analyse. In : Verlhac A, Giraud M, Leteinturnier J (eds) La truffe guide pratique. Ctfl FPGV, Reims, pp 77–88.

GOLLDACK, J., B. MÜNZENBERGER & R.F. HÜTTL (1998) "*Pinirhiza cyaneoviridis*" + *Pinus sylvestris* L. Descr Ectomyc **3**: 49–54.

GOLLDACK, J., B. MÜNZENBERGER & R.F. HÜTTL (1999) "*Pinirhiza dimorpha*" + *Pinus sylvestris* L. Descr Ectomyc **4**: 73–78.

GRONBACH, E. (1988) Charakterisierung und Identifizierung von EktomyKorrhizen in einem Fichtenbestand mit Untersuchungen zur Merkmalsvariabilität in sauer beregneten Flächen. Bibl Mycol **125**: 35–45.

HAUG, I. & K. PRITSCH (1992) Ectomycorrhizal tpyes of spruce (*Picea abies* (L.) Karst.) in the Black Forest. A microscopical atlas. Kernforschungszentrum Karlsruhe.

INGLEBY, K., P.A. MASON, F.T. LAST & L.V. FLEMING (1990) Identification of ectomycorrhizas. ITE research publication 5. HMSO, London.

JAKUCS, E., R. AGERER & Z. BRATEK (1998) *Genea verrucosa* Vitt. + *Quercus* spec. Descr Ectomyc **3**: 19–23.

JAKUCS, E. & R. AGERER (1999) *Tomentella pilosa* (Burt) Bourdot & Galzin + *Pinus sylvestris* L. Descr Ectomyc **4**: 135–140.

JAKUCS, E. & R. AGERER (2001) *Tomentella subtestacea* Bourdot & Galzin + *Populus alba* L. Descr Ectomyc **5**: 213–219.

JAKUCS, E., R. AGERER & Z. BRATEK (1997) "*Quercirhiza fibulocystidia*" + *Quercus* spp. Descr Ectomyc **2**: 67–72.

JAKUCS, E., G.M. KOVÁCS, R. AGERER, ROMSICS C & Z. ERŐS-HONTI (2005) Morphological– anatomical characterization and molecular identification of *Tomentella stuposa* ectomycorrhizae and related anatomotypes. Mycorrhiza **15**: 247–258.

JAKUCS, E. & Z. ERŐS-HONTI (2008) Morphological-antomical characterization and identification of *Tomentella* ectomycorrhizas. Mycorrhiza **18**: 277–285.

JAKUCS, E., S. GANYEC & Z. ERŐS-HONTI (2008) "*Fagirhiza asteromustrata*" + *Fagus sylvatica* L. Descr Ectomyc **9/10**: 31–35.

KÕLJALG, U., A. DAHLBERG, A.F.S. TAYLOR, E. LARSSON, N. HALLENBERG & J. STENLID et al (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Mol Ecol **9**: 1985–1996.

KÕLJALG, U., E. JAKUCS, K. BOKA & R. AGERER (2001) Three ectomycorrhiza with cystidia formed by different *Tomentella* species as revealved by rDNA ITS sequences and amatomical characteristics. Folia Cryptog. Estonia Facs. **38**: 27–39.

KÕLJALG, U., K.H. LARSSON, K. ABARENKOV, R.H. NILSSON, I.J. ALEXANDER, U. EBERHARDT, S. ERLAND, K. HOILAND, R. KJOLLER, E. LARSSON, T. PENNANEN, R.SEN, A.F.S. TAYLOR, L. TEDERSOO, T. VRALSTAD & B.M.URSING (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol **166**: 1063–1068.

MLECZKO, P. (2004a) "*Pinirhiza amyloidea*" + *Pinus sylvestris* L. Descr Ectomyc **7/8**: 59–68.

MLECZKO, P. (2004b) "*Pinirhiza ligulata*" + *Pinus sylvestris* L. Descr Ectomyc **7/8**: 79–86.

RAIDL S & W.R. MÜLLER (1996) *Tomentella ferruginea* (Pers.) Pat. + *Fagus silvatica* L. Descr Ectomyc **1**: 61–66.

PALFNER G & R. AGERER (1996) "*Quercirhiza squamosa*" eine nichtidentifizierte Ektomycorrhiza an *Quercus robur*. Sendtnera **3**: 137–145.

TEDERSOO, L., U. KÕLJALG, N. HALLENBERG & K-H LARSSON (2003) Fine scale distribution of ectomycorrhizal fungi and roots across substrate layers including coarse woody debris in a mixed forest. New Phytol **159**: 153–165.

TEDERSOO, L., T. SUVI, K. BEAVER & U. KÕLJALG (2007a) Ectomycorrhizal fungi of the Seychelles: diversity patterns and host shifts from the native *Vateriopsis seychellarum* (Dipterocarpaceae) and *Intsia bijuga* (Caesalpiniaceae) to the introduced *Eucalyptus robusta* (Myrtaceae), but not *Pinus caribea* (Pinaceae). New Phytol: **175**: 321–333.

TEDERSOO, L., T. SUVI, K. BEAVER & I. SAAR (2007b) Ectomycorrhizas of *Coltricia* and *Coltriciella* (Hymenochaetales, Basidiomycota) on Caesalpiniaceae, Dipterocarpaceae and Myrtaceae in Seychelles. Mycol Progress **6**:101–107.

TEDERSOO, L., K. HANSEN, B.A. PERRY & R. KJØLLER (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytol **170**: 581–596.

THIERS, B. (2009, continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. sciweb.nybg.org/science2/IndexHerbariorum.asp.

WEI, J. & R. AGERER (2008) "*Pinirhiza tomentelloides*" + *Pinus tabulaeformis* Carr. Descr Ectomyc **11/12**: 97–102.

WEI, J., D. PERŠOH & R. AGERER (2009) Four Ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*) – morpho-anatomical and molecular–phylogenetic analyses. Subm.

WHITE, T.J., T.D. BRUNS, S.B. LEE & J.W. TAYLOR (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In *PCR Protocols*: a guide to methods and applications (M.A. Innis, D. H. Gelfand, J.N. Sninsky & T.J. White, eds):315–322. Academic Press, San Diego.

YOROU, N.S. & R. AGERER (2008) "*Afzeliaerhiza beninensis*" + *Afzelia africana* Smith Descr Ectomyc **11/12**: 1–8.

YOROU NS, R. AGERER & S. RAIDL (2008) "*Uapacaerhiza wariensis*"+ *Uapaca guineensis* Müll.Arg. Descr Ectomyc **11/12**: 147–153.

2.4.6 Two sebacinoid Ectomycorrhizae on Chinese Pine

VI

Two sebacinoid Ectomycorrhizae on Chinese Pine

Jie Wei, Reinhard Agerer

Department Biology I, Division of Organismic Biology: Mycology, University of Munich, Menzinger Str. 67, 80638 Munich, Germany

Abstract

Sebacinoid fungi show a broad mycorrhizal capacity, therefore they play a very important role in natural systems. Worldwide, fungi of Sebacinales are present under different environmental conditions and associate with diverse plant hosts, however are hitherto poorly studied in China. Two sebacinoid ectomycorrhiza (ECM), "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*", are described in detail morphologically and anatomically in present study. They share a plectenchymatous outer mantle with multiply ramified hyphae in a gelatinous matrix, clampless, thin, thick-walled emanating hyphae with mostly Y-shaped ramifications and triangular inflations at the point of ramification. "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" can be distinguished by thick cells in mantle layers, the ramification of emanating hyphae, presence or absence of rhizomorphs, as well as the differing colour reaction in Melzer's reagent. The putative molecular phylogenetic relationships of "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" were inferred by analyses of the partial large subunit nuclear rDNA (nLSU), however an affiliation to fungal species was not possible. This is the first report of sebacinoid ECM on Chinese pine.

Key words: anatomy, sebacinoid ectomycorrhiza, morphology, molecular phylogenetic analyses

Introduction

The order Sebacinales, assigned to the Agaricomycetes (Blackwell et al. 2006), ecologically is characterized by the capacity of its members to form a diversity of mycorrhizae (table 1). Fungi of Sebacinales are present under different environmental conditions and associate with diverse plant hosts, however, are hitherto poorly studied in China.

Most of our knowledge on Sebacinales and their diverse host species comes from molecular ecology studies from direct amplification of fungal ribosomal DNA of environmental samples (Selosse et al. 2007). Sebacinoid fungi form similar structure of hyphal coils (pelotons) in cortical cell as in orchid mycorrhiza (OM), ericoid mycorrhiza (ERM), and ectendomycorrhiza (EEM) (e.g. Selosse et al. 2004, 2007, Setaro et al. 2006, Talor et al 2003). Hyphal coils show at present limited information to make further taxonomical analyses, although additional evidence of features like clampless hyphae with imperforate parenthesomes have been applied (e.g. Selosse et al. 2007, Setaro et al. 2006). However morpho-anatomical features of ECM which are recognized as being important for function and can also be used to hypothesize fungal relationships at different taxonomic levels (Agerer 2006) provide us a hard-won chance to study in detail the sebacinoid group whose fruitbody are limited studied till now (Weiß and Oberwinkler 2001, Weiß et al. 2004).

That an ectomycorrhizal status might be a common feature in the Sebacinaceae has already been assumed by Weiß and Oberwinkler (2001), and has been repeatedly proven (table 1). Nevertheless, morpho-anatomical features of ECM are still little known in Sebacinales. Sebacinoid ECM have been described in detail only by Urban et al. (2003) providing characterizations of *Sebacina incrustans* on *Picea abies* and a sebacinoid sp. ECM on *Tilia* as well as by Azul et al. (2006) who published a description of a sebacinoid ECM on *Quercus suber* under the name "*Quercirhiza dendrohyphidiomorpha*" (* in table 1).

During the study of the ectomycorrhizal diversity on Chinese pine (*Pinus tabulaeformis* Carr.), two sebacinoid ECM have been found. The aim of our work is to characterize these two anatomotypes morpho-anatomically, to identify them by molecular analyses of nLSU and ITS sequence, and to compare them in detail with previously published descriptions. This is the first report of sebacinoid ECM on Chinese pine.

Table 1. Sebacinoid mycorrhiza

MC	Host	Type of Ecosystem	Geographic Localization	Edaphic Condition	Reference
	Betula sp.	wooded meadow	Estonia	mollisihumi- rendzic leptosol	Tedersoo et al. 2006
	Corylus avellana L., *Carpinus betulus* L.	deciduous forest	Lorraine France	rendzine	Selosse et al. 2002a
	Eucalyptus marginata Donn ex Sm.	*Eucalyptus* forest	Western Australia	---	Avis et al. 2003
	Picea abies L. *	deciduous forest	Vienna, Austria	calcareous soil	Urban et al. 2003
E	*Pinus sylvestris* L.	mixed forest	Eastern Austria	serpentine soil	Urban et al. 2008
C	*Pinus thunbergii* Parl.	coastal pine forests	Korea	maritime sand	Obase et al. 2009
M	*Quercus douglasii* Hook & Arn.	oak woodlands	Yuba, USA	metavolcanic rocks, pH 5.7–6.2	Smith et al. 2007
	Quercus ilex L.	mediterranean forest	Corsica, France	alocrisols, pH 5.7–6.4	Richard et al. 2005
	Quercus macrocarpa Michaux	oak savanna	Cedar Creek, USA	metalliferous soils, pH 6.55	Glen et al. 2002
	Quercus suber L. *	oak forest	Portugal	---	Azul et al. 2006
	Tilia sp. *	deciduous forest	Vienna, Austria	calcareous soil	Urban et al. 2003
	Cephalanthera damasonium (Mill.) Druce	deciduous forest	France	calcareous soil	Julou et al. 2005
	Epipactis microphylla (Ehrh.) Schinz &Thell.	deciduous forest	France	calcareous soil	Selosse et al. 2004
O	*Hexalectris spicata* (Walter) Barnhart	dessert	USA	---	Taylor et al. 2003
M	*Neottia nidus-avis* (L.) Rich.	mixed forest	France	---	Selosse et al. 2002b
	Neottia nidus-avis	pine forest	Bavaria, Germany	Leptosols, pH 7	Bidartondo et al. 2004
	Agauria sp., *Andromeda* spp.				
E	*Calluna vulgaris* (L.) Hull				
R	*Chiogenes hispidula* (Linn.) Torr.		Austria, Estonia,		
M	*Empetrum nigrum* L.*, Erica* spp.	---	France, Spain,	---	Selosse et al. 2007
	Gaultheria spp.		La Réunion Island		
	Kalmia sp.		Canada, Argentina		
	Rhododendron spp.				
	Vaccinium spp.				
	Calypogeia muelleriana (Schiffn.) K. Müll.	---	Germany, France	soil covered by needle litter	
J	*Lophozia incisa* (Schrad.) Dum.	---	Swedish Lapland	sandy soil	
M	*Lophozia sudetica* (Nees) Grolle	---	Pyrenean	wet soil	Kottke et al. 2003
	Arbutus unedo L.	forest	France	---	Selosse et al. 2007
E	*Cavendishia nobilis* var*. capitata*	mountain rain forest	Andes in southern	schists and sandstones,	Setaro et al. 2006
E	(Bentham) Luteyn		Ecuador	pH 3–5.5	
M	*Orthilia secunda* (L.) House	forest, lake	France, Canada	---	Selosse et al. 2007
	Pyrola chlorantha Sw.	forest	France	---	Selosse et al. 2007

Note: MC-mycorrhiza class; ECM- ectomycorrhiza; OM-orchid mycorrhiza; ERM-ericoid mycorrhiza; JM-jungermannioid mycorrhiza; EEM-ectendomycorrhiza; --- means information has been not provided in the original papers. ECM (host species with *) have been described morphologically and anatomically, other ECM are only identified by molecular identification.

Materials and methods

Sampling and morpho-anatomical characterization

Soil samples were collected in pure Chinese pine forests at Helan Mountain (Yinchuan City, Ningxia Hui Nationality Autonomous Region, China) and at Heilihe National Reserve (Chi Feng City, Inner Mongolia Autonomous Region, China) throughout two years. ECM systems were assigned to anatomotypes and described according to Agerer (1987–2008, 1991). Anatomical studies are based on at least five ECM systems for each anatomotype. Drawings were performed with the aid of a Normarski interference contrast microscope (Standard 14, ZEISS West Germany) connected with a drawing tube. All drawings were made at a magnification of 1000 ×. Reference specimens of the mycorrhizae are deposited in M (for herbarium abbreviation see Thiers 2009).

DNA extraction, PCR and sequencing

One unramified end, previously fixed in CTAB, from each of the two morphotypes was used for DNA extraction following careful microscopical examination in order to ensure that the isolated DNA originated from the respective anatomotype. DNA of ECM was extracted using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer´s instructions. The nLSU and internal transcribed spacer (ITS) region were amplified using the PCR primer pairs LROR and LR5 (Moncalvo et al. 2000) as well as ITS1F and ITS4 (Gardes and Bruns 1993, White et al.1990), respectively. The obtained PCR product was purified using the QIAquick protocol (Qiagen, Hilden, Germany) and fragments were sequenced applying the PCR primers. Sequencing was performed by the sequencing service of the Department Biology I (Ludwig-Maximilians-Universität, München) using BigDye Terminator Ready Reaction Cycles Sequencing Kit v3.1.

Sequence alignments and molecular analyses

Using the obtained nLSU sequence of the two ECM as query megablast searches (Zhang et al. 2000) were performed in GenBank (http://www.ncbi.nlm.nih.gov/). The 100 most similar sequences each were downloaded. Duplicates, i.e. identical sequences found as closest matches of different query sequences, were omitted. Using the software BioEdit v7.0.5 (Hall 2005) the sequences were automatically aligned. The alignment was revised manually and columns not alignable with certainty were excluded from the following analyses. 47 unique nLSU sequences were retained for further molecular phylogenetic analyses. RAxML Web-

Servers (the CIPRES Portal v1.14 at the San Diego Supercomputing Center http://8ball.sdsc.edu:8889/cipres-web/Home.do, Stamatakis 2006, Stamatakis et al. 2008) was used for calculation of the most likely trees and the bootstrap support values (500 replicates). The GTRGAMMAI model of substitution with default was applied for nLSU analyses with Maximum Likelihood as optimality criterion. The most parsimonious trees were searched for by executing batch files generated by PAUPRat (Sikes and Lewis 2001) in PAUP* v4.0 (Swofford 2003), with weighting mode set to multiplicative. ITS sequence comparisons were performed in GenBank using megablast and in UNITE (Kõljalg et al. 2005, http://unite.ut.ee/) using BlastN.

Results

Morpho-anatomical descriptions

"*Pinirhiza multifurcata*"

Morphological characters (Fig. 1a): *Mycorrhizal systems* unramified to dichotomous, with 0–2(3) orders of ramification, solitary or in small numbers, main axis 0.35–0.4 mm diam., hydrophilic, of short distance exploration type. *Unramified ends* straight or sometimes bent, cylindric, not inflated, (0.9)2.5(3.8) mm long, 0.35–0.4 mm diam., greyish orange-brown, very tips whitish, older parts dark brown to black, mantle opaque to semitransparent, not carbonizing, surface not smooth, bumpy, loosely hairy. *Emanating hyphae* moderately frequent to frequent, not specifically distributed. *Cystidia* not distinct under stereoscope magnification. *Rhizomorphs* not found. *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figs. 2a–c): plectenchymatous throughout, hyphae in all layers colourless and clampless. *Outer mantle layers* (Fig. 2a) plectenchymatous with mutiply branched and irregularly inflated hyphae in a matrix (mantle type E/C, according to Agerer 1987–2008, 1991, Agerer and Rambold 2004–2009), hyphae 2.5–5 µm diam. with up to 8 µm thick cells, cell walls 0.3–0.5 µm, surface smooth; in few hyphae septa with a large pore discernible; surface of mantle with many soil particles. *Middle mantle layer* (Fig. 2b) plectenchymatous, areas with longer hyphae intermixed with few lobed, multiply branched ones, 2–3.5(7) µm diam., cell walls 0.3µm, in few hyphae septa with a large pore discernible. *Inner mantle layers* (Fig. 2c) densely plectenchymatous, hyphae frequently

ramified and irregular in diam., some inflated, bent or curved; hyphal cells 2–4.5 µm wide, walls 0.3µm. *Very tip* like remaining parts of the mantle.

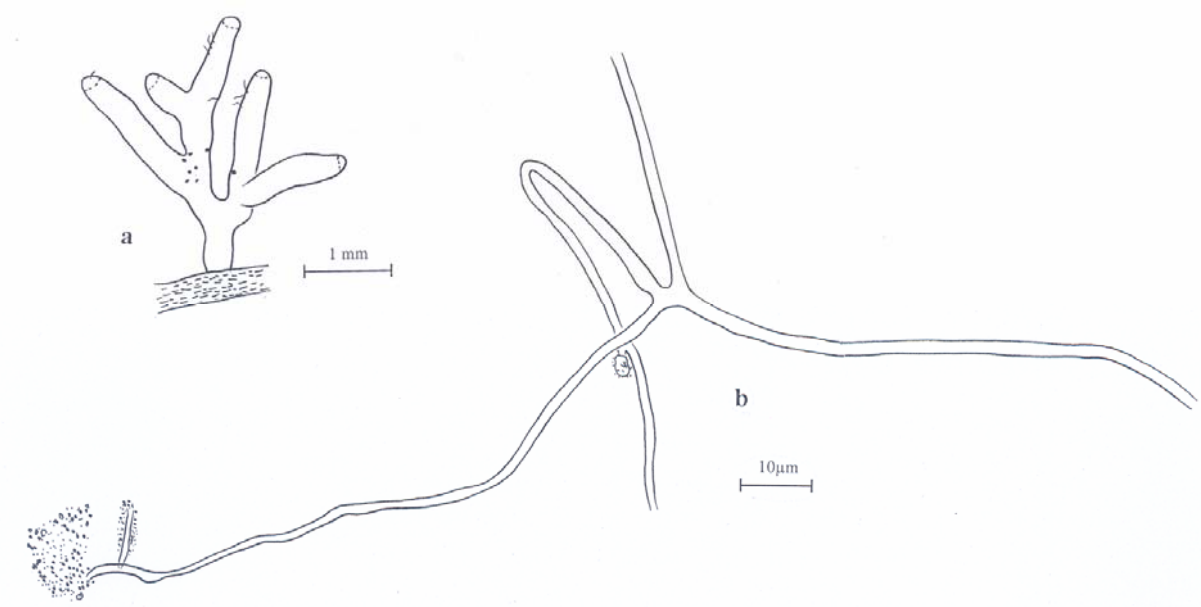


Fig. 1 "*Pinirhiza multifurcata*", habit of ECM and emanting hyphae. a habit of ECM, surface with infrequent emanating elements; b emanating hyphae with trifurcate ramification.

Anatomical characters of emanating elements (Figs. 1b, 3): *Rhizomorphs* lacking. *Emanating hyphae* (Figs. 1b, 3) similar to cystidia (s. below), infrequent to frequent, colourless, surface smooth, mostly straight, cylindrical or occasionally with elbow-like structures or irregular inflations, not constricted at septa, septa simple, very infrequent, clamps lacking, septa slightly thinner than hyphal walls; most hyphae without septa; ramifications frequent, with 1–2 side branches, occasionally polytomies with three branches (Fig. 1b); ramification rectangular to Y-shaped with triangular inflations at points of ramification; hyphal dimensions very variable, (1)1.5–2.5(3.5) µm diam., slightly thick-walled, cell walls 0.3–0.8 µm; no simple emanating hyphae found. *Cystidia* lacking, but emanating hyphae might be considered as cystidia due to their extraordinary type of ramification, their rather thick walls in comparison to their diam., and the almost lacking septa.

Colour reactions with different reagents (preparations of mantle): Melzer's reagent: emanating hyphae (cystidia) dextrinoid; Lactic acid: n. r.; KOH: n. r.; $FeSO_4$: n. r.

Anatomical characters of longitudinal section: Mantle plectenchymatous, 5–10 µm wide. Mantle of very tip plectenchymatous, 5–10 µm wide. Tannin cells in 1 row, irregularly

tangentially cylindrical. Cortical cells in 2–3 rows, radially oval to roundish, and 2–3 rows with Hartig net. Hartig net around tannin cells and cortical cells in 1–2 rows, palmetti-like in plan view, lobes 1–2 (3.5) µm broad.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* forest (altitude 1700–2300 m, precipitation 200–400 mm per year, calcareous soil) at Helan Mountain, Suyukou National Reserve located in Yinchuan City, Ningxia Hui National Autonomous Region, China, myc. exc. and isol. by Jie Wei, 20.08.2007, JW 54a (in M). GenBank sequence accession numbers: GU269908 (nLSU), GU269910 (ITS).

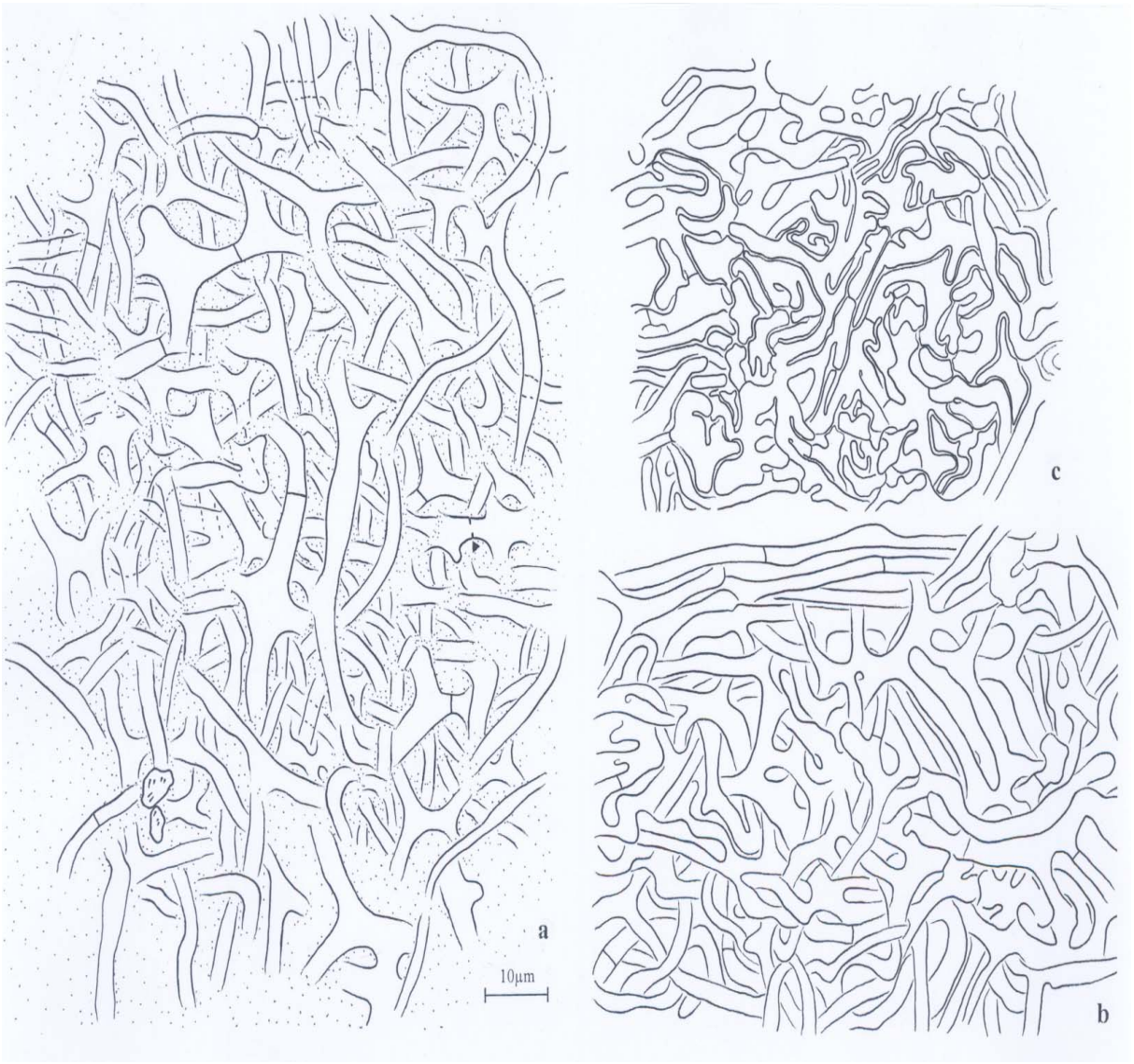


Fig. 2 "*Pinirhiza multifurcata*", plan view of outer, middle and inner mantle. a outer mantle layer with some multiply branched and irregularly inflated hyphal cells in a gelatinous matrix, septa with big pore in few hyphae discernible (arrowhead); b middle mantle layer with multi-ramified hyphae; c inner mantle layer.

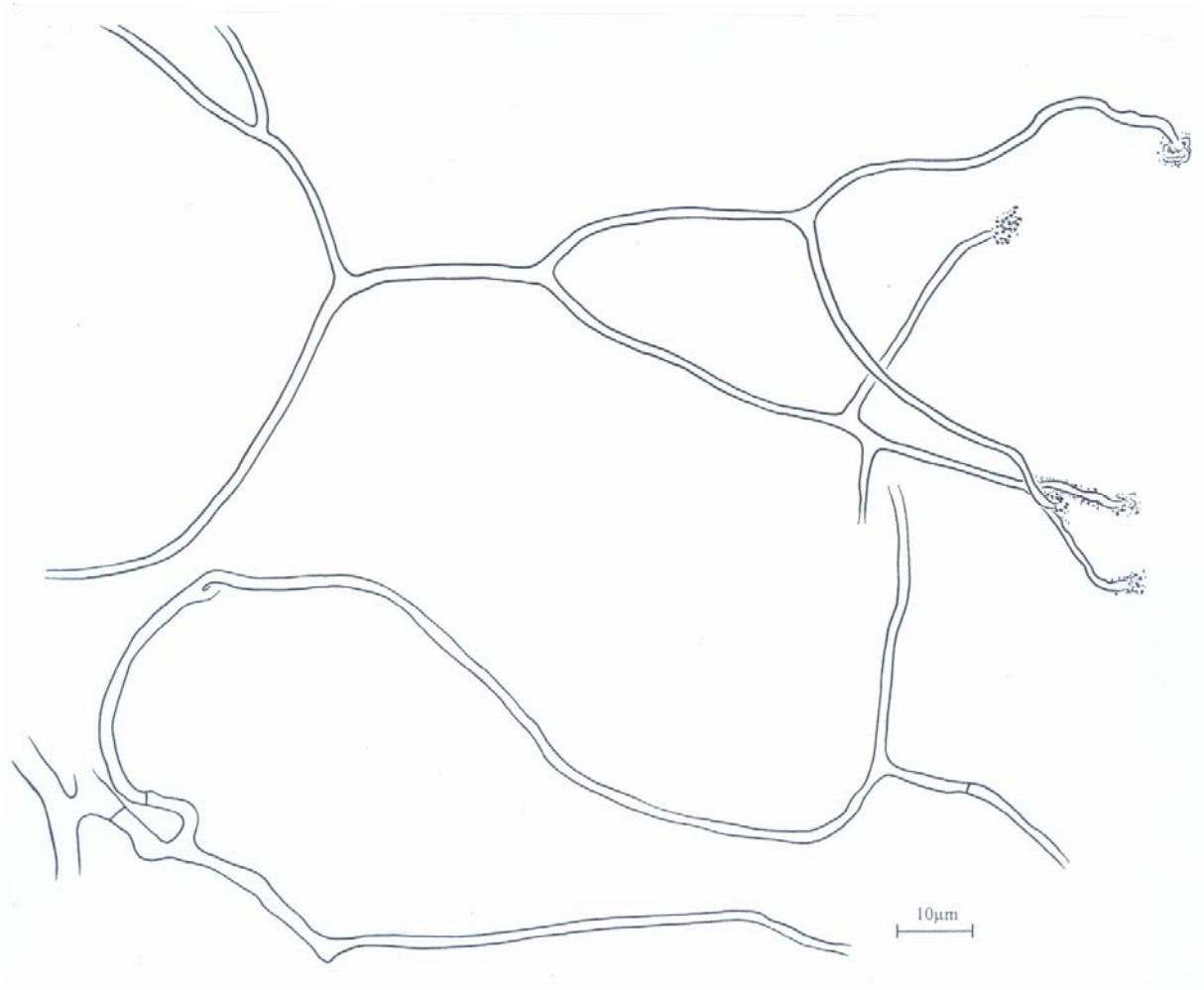


Fig. 3 "*Pinirhiza multifurcata*", emanating hyphae originated from the multiply ramified hyphae of outer mantle, with simple septa, and frequently ramified, Y-shaped emanating hyphae with triangular inflation at ramification points without septa.

"*Pinirhiza nondextrinoidea*"

Morphological characters (Fig . 4a): *Mycorrhizal systems* dichotomous, with 0–3 orders of ramification, in small numbers, main axis 0.4–0.45 mm diam., hydrophilic, of short distance exploration type or of the smooth subtype of medium distance exploration type. *Unramified ends* straight, cylindric, not inflated, 0.4–0.6 mm long, 0.35–0.4 mm diam., young parts cinnamon-brownish, older parts black, mantle surface with many soil particles, densely woolly. *Emanating hyphae* abundant, not specifically distributed. *Cystidia* not distinct under stereoscope magnification. *Rhizomorphs* infrequent, yellowish, 0.04–0.07 mm diam., no side branches observed, margin densely woolly. *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figs. 5a, b): plectenchymatous throughout, hyphae in all layers colourless and clampless. *Outer mantle layers* (Fig. 5a) plectenchymatous with squarrosely branched hyphae and with a matrix (mantle type E/C, according to Agerer 1987–2008, 1991, Agerer and Rambold 2004–2009), emanating hyphae originating from these squarrosely branched hyphae; hyphae 1.5–3 µm diam., cell walls 0.3 µm; all hyphae with simple septa, in few hyphae septa with a large pore discernible (Fig. 5a); hyphae smooth; mantle surface covered by many soil particles. *Inner mantle layers* (Fig. 5b) densely plectenchymatous, hyphae frequently ramified, 2–3 µm diam., cell walls 0.3 µm, with simple septa, surface smooth. *Very tip* similar to remaining parts of the mantle.

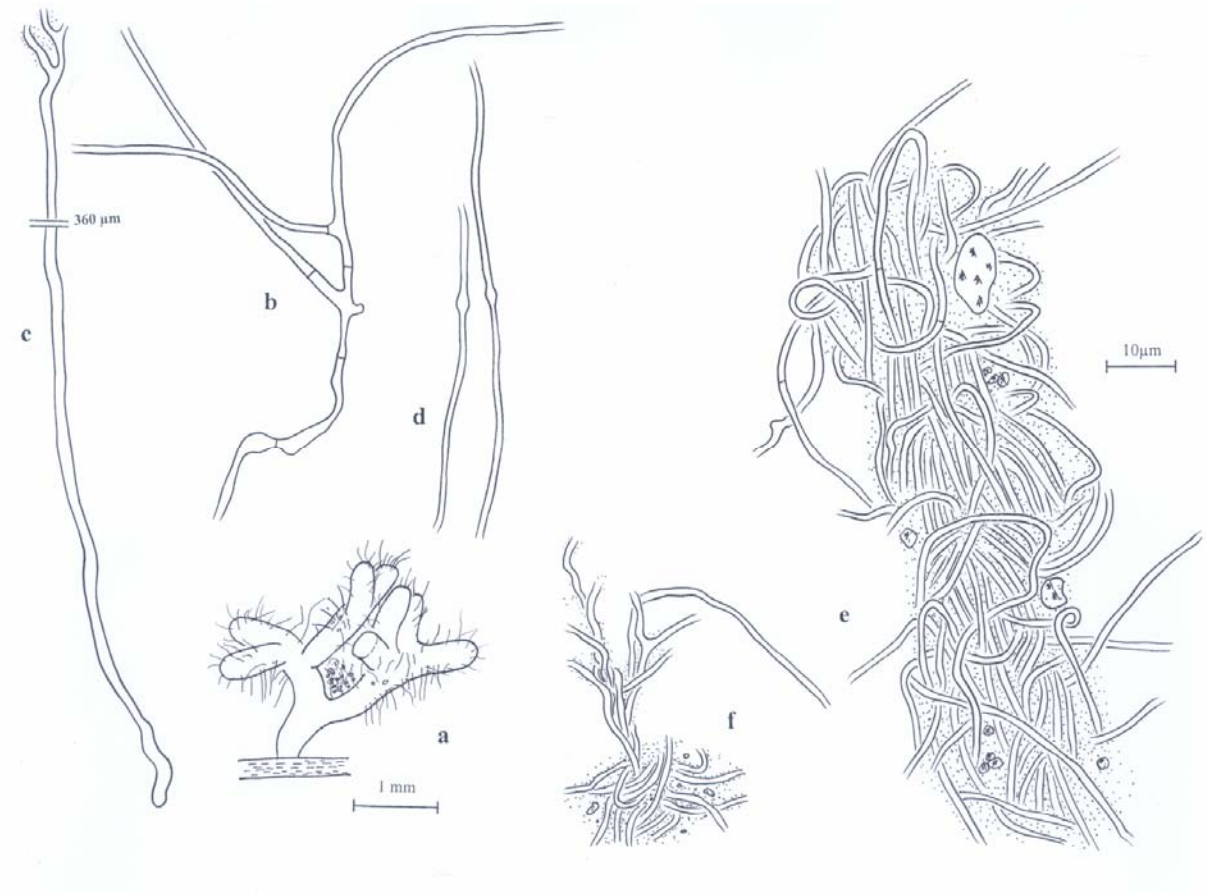


Fig. 4 "*Pinirhiza nondextrinoidea*", habit of ECM, emanating hyphae and rhizomorph. a habit of ECM, surface of mantle densely woolly; b emanating hyphae with Y-shaped ramification and inflated at ramified points, septa simple; c emanating hyphae with a ramified end; d emanating hyphae with inflations; e rhizomorph composed of uniform hyphae with gelatinous matrix covered with many soil particles; f some inner hyphae of rhizomorph having the same diameter as remaining hyphae.

Anatomical characters of emanating elements (Figs. 4b–f): *Rhizomorphs* (Figs. 4e, f) infrequent, with gelatinous matrix, not differentiated (type A/B according to Agerer 1987–2008, 1991, Agerer and Rambold 2004–2009); surface of rhizomorphs with many soil particles; in diameter and features all hyphae similar to emanating hyphae, very thin, 1–2 µm diam., thick-walled, cell wall 0.3–0.5 µm, colourless and clampless; most hyphae with a smooth surface. *Emanating hyphae* (Fig. 4b–d) abundant, colourless, thick-walled, thin, of variable diam., 1–2 µm, cell walls 0.3–0.5 µm; hyphae mostly straight, cylindrical, not constricted at septa, with infrequent, simple septa, septa as thick as hyphal walls; occasionally irregular inflations and elbow-like structures present; ramifications infrequent, Y-shaped or nearly rectangular, with triangular inflations at the points of ramification; surface of most hyphae smooth and gelatinous, with some soil particles, small crystals present on few hyphae; only few hyphae with ramified ends observed. *Cystidia* lacking.

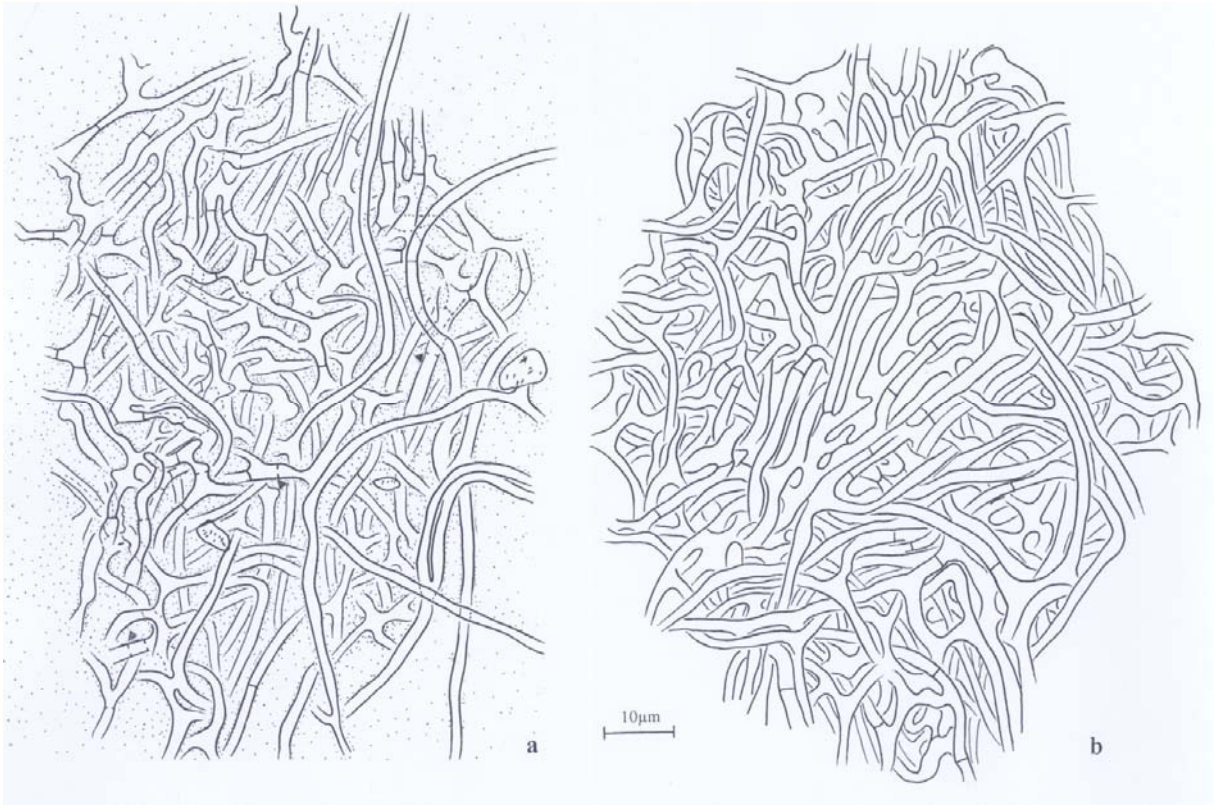


Fig. 5 "*Pinirhiza nondextrinoidea*", plan view of mantle layers. a outer mantle layer with squarrosely ramified hyphae in a gelatinous matrix gluing with some soil particles, some emanating hyphae originated from these squarrosely ramified hyphae, some septa with a large pore discernible (arrow heads); b inner mantle layer.

Colour reactions with different reagents (preparations of mantle): Melzer's reagent: n.r.; Lactic acid: n. r.; KOH: n. r.; $FeSO_4$: n. r.

Anatomical characters of longitudinal section: Mantle plectenchymatous, 9–22 µm wide. Mantle of very tip plectenchymatous, 15–20 µm wide. Tannin cells in 1–2 rows, oval to irregularly tangentially cylindrical. Cortical cells in 2–3 rows, oval to roundish to sometimes tangentially elongated, and 2–3 rows with Hartig net. Hartig net around tannin cells and cortical cells in 1–2 rows, at places reaching the endodermis, globular protrusions occasionally observed in cortical cells, palmetti-like in plan view, lobes 1–2.5 µm broad.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand (altitude 700–800 m, precipitation ca. 500–600 mm per year, calcareous soil) in Heilihe National Reserve, Chi Feng City, Inner Mongolia, China, myc. exc. and isol. by Jie Wei, 08.09.2008, JW 185b (in M). GenBank sequence accession numbers: GU269909 (nLSU), GU269911 (ITS).

Sequence analyses

Topology of nLSU RAxML and PAUPRat trees

The likelihood of the most likely tree found was -2976.528891 and the substitution rates estimated by RAxML were: A↔C: 1.467049, A↔G: 5.056997, A↔T: 0.706806, C↔G: 0.641633, C↔T: 14.877343, and G↔T: 1. Of the 569 reliably alignable positions of the nLSU Alignment, 215 were variable and 166 were parsimony informative. The 50% Majority Rule Consensus Tree was calculated of the 8944 most parsimonious trees.

The topologies of both the RAxML (Fig. 6) and PAUPRat trees (Fig. 7 as supplementary file) as generated by analyses of nLSU are largely concordant. The RAxML tree indicates that the genus *Sebacina* is polyphyletic. It could be basically splitted into two clades I and II, with 97% and 100% BS support, respectively. Clade I comprises the *Sebacina*/*Tremellodendron* complex which includes mainly sebacinoid OM such as with *Neottia nidus-avis*, *Hexalectris spicata*, *Epipactis helleborine*, few sebacinoid ECM, the *Sebacina incrustans* (type species) - an ECM fungus (Urban et al. 2003, Tedersoo et al. 2006), as well as two *Tremellodendron* species and one ERM with 95% BS, in addition two *Craterocolla cerasi* isolates and three

Chaetospermum camelliae isolates. Two *Craterocolla cerasi* isolates form a sister clade to the *Sebacina*/*Tremellodendron* complex with moderate support (54% BS), three *Chaetospermum camelliae*-isolates are again a sister clade to the *Sebacina*/*Tremellodendron* complex and two *Craterocolla cerasi* isolates receiving moderate support (66% BS). Clade II comprises *Sebacina vermifera* as well as some ERM, *Pyriformospora indica* and a *Rhizoctonia* sp.. Two *Tremella*-species represent the outgroup.

Molecular-phylogenetic position of "*Pinirhiza multifurcata*" *and* "*Pinirhiza nondextrinoidea*"

The RAxML tree (Fig. 6) shows that "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" are nested in the *Sebacina-Tremellodendron* complex in clade I. They are located in different subclades, both of which received no significant bootstrap support, however. "*Pinirhiza multifurcata*" is very close to an ECM of *Dryas octopetala* (AY452681) with 99% BS support. "*Pinirhiza nondextrinoidea*" clusters with an ECM of Sebacinaceae (AM161532) with 55% BS.

ITS-sequence comparisons of "*Pinirhiza multifurcata*" *and* "*Pinirhiza nondextrinoidea*" *with sequences obtained from GenBank and UNITE*

Molecular phylogenetic analyses of ITS-sequences are not meaningful because only two fully identified sequences occurred among the first 100 most similar sequences each obtained in megablast searches in GenBank with our ECM sequences as query. Those identified sequences were not the most similar entities compared to our ECM sequences, however. The first 100 matches in GenBank have at least 89% query coverage and at least 90% maximum identity with the sequences of "*Pinirhiza multifurcata*" and "*P. nondextrinoidea*" and belong nearly exclusively to the order Sebacinales (except for a *Tomentella* ECM (EU668944) and few unclassified, uncultured ECM) including mostly uncultured sebacinoid ECM, sebacinoid OM, and few Sebacinales isolates.

The best matches regarding the sequence of "*Pinirhiza multifurcata*" are two uncultured sebacinoid ECM (EF218817 and EU645627) and two sebacinoid OM (AF440651 and AF440656) with 97% identity and 100% query coverage each. The only identified species among the 100 most similar sequences is *Sebacina* aff. *epigaea* (AF490393) that shows 95% maximum identity and 95% query coverage. The sequence of "*Pinirhiza nondextrinoidea*" is

most similar to three uncultured sebacinoid ECM (FJ210755, FJ196964, and AM161532) with 96% identity and at least 98% query coverage. The two to species level identified isolates among its 100 best matches represent *Sebacina epigaea* (AJ966754) and *S*. aff. *epigaea* (AF490393) that show 95% maximum identity and 90% query coverage, and 91% maximum identity and 100% query coverage, respectively.

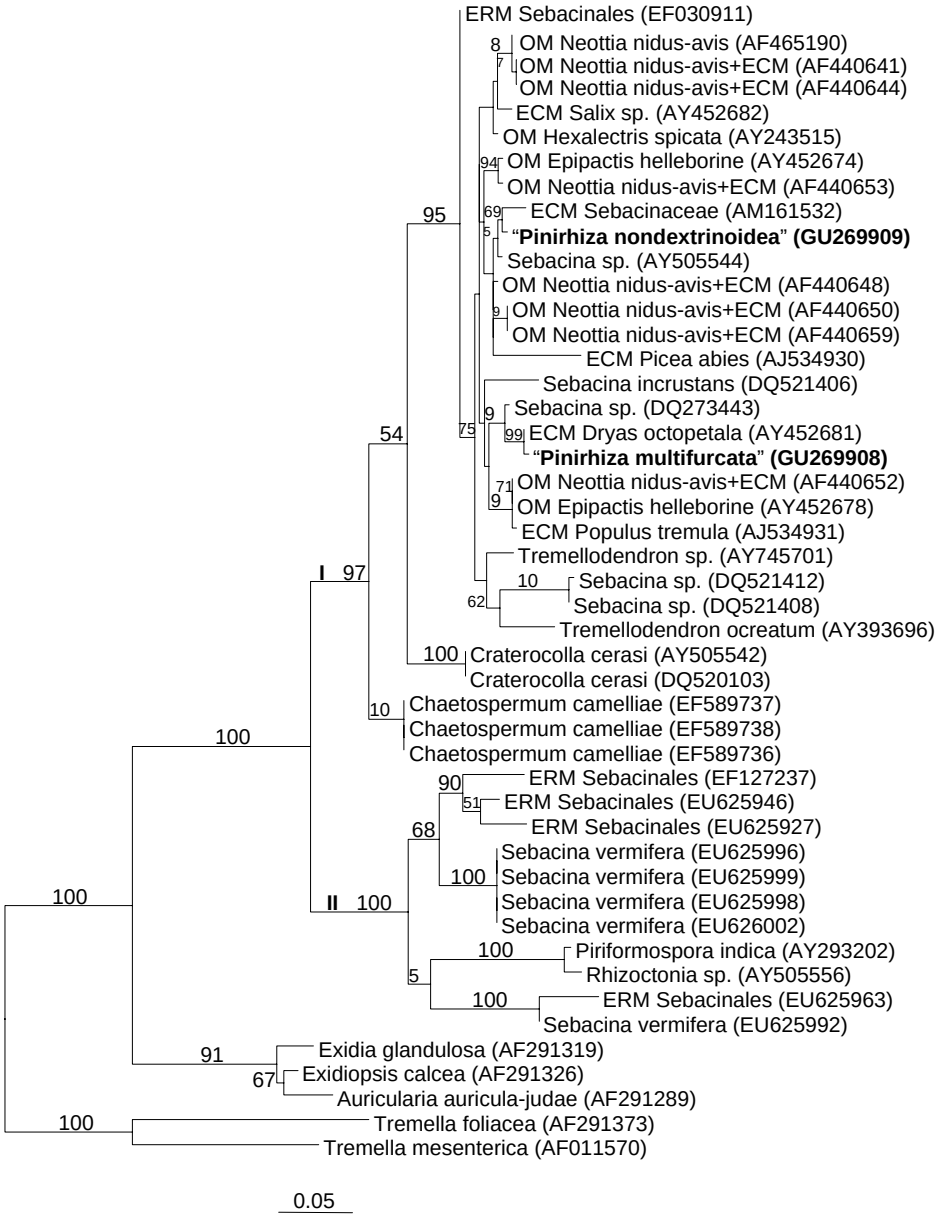


Fig. 6 Molecular-phylogenetic placement of "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" among selected Sebacinales inferred by the RAxML analysis of nLSU sequences. Bootstrap support values above 50% are noted above or left of the respective branches. GenBank accession numbers are given in parentheses following the species names.

Sequences obtained from UNITE using BlastN belong to different genera such as *Sebacina*, *Tomentella*, *Mycena*, *Amanita*, *Cortinarius*, and *Russula*. The sequence of "*Pinirhiza*

multifurcata" is most similar to two *Sebacina* spp. from Denmark (UDB00073 and UDB00074) with 89% (685 score, 0 E-value) and 88% (626 score, e-180 E-value) similarity, respectively. The similarity scores of sequences from other genera are even worse (at most 270 score) compared with the sequence of "*Pinirhiza multifurcata*". The sequence of "*Pinirhiza nondextrinoidea*" is most similar to that of *Sebacina epigaea* from Estonia (UDB000975) with 95% (832 score, 0 E-value) similarity, while sequences of members of other genera have clearly lower (at most 268 score) similarity values.

Discussion

Morpho-anatomical features

"*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" share a number of features. They are hydrophilic, all hyphae have simple septa and are colourless, multiply ramified hyphae are present at least in the outer mantle. The outer mantle layer and its surface show a gelatinous matrix. The emanating hyphae are thick-walled, variable in diameter (1–3.5 µm in "*Pinirhiza multifurcata*" and 1–2 µm in "*Pinirhiza nondextrinoidea*"), infrequently simple septate, and have triangular inflations at their points of ramification. Nevertheless, "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" can easily be distinguished from each other. "*Pinirhiza multifurcata*" is greyish orange-brown with a bumpy surface that is loosely covered by emanating hyphae, whereas "*Pinirhiza nondextrinoidea*" is cinnamon-like ochre brown, has an even surface, and a densely woolly coverage of emanating hyphae. Anatomically, "*Pinirhiza multifurcata*" differs from "*Pinirhiza nondextrinoidea*" in having thick cells in outer and middle mantle layers and by the lack of rhizomorphs. Emanating hyphae of "*Pinirhiza multifurcata*" ramify frequently bifurcately and range from 1 to 3.5 µm in diameter, whereas those of "*Pinirhiza nondextrinoidea*" have infrequent ramifications and are between 1–2 µm wide. In addition, exclusively the emanating hyphae of "*Pinirhiza multifurcata*" are dextrinoid.

"*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" are similar to three sebacinoid ECM that have been described in detail: *Sebacina incrustans* (Pers.) Tul. and C. Tul. on *Picea abies*, *Sebacinoid* sp. on *Tilia* sp. (Urban et al. 2003), and "*Quercirhiza dendrohyphidiomorpha"* on *Quercus suber* (Azul et al. 2006). They can be separated according to their anatomical features, however. "*Pinirhiza multifurcata*" differs from the

ECM of *Sebacinoid* sp. in the lack of a pseudoparenchymatous outer mantle, in mostly bifurcate ramifications in emanating hyphae rather than polytomies as in the latter, and thinner cell walls of emanating hyphae ("*Pinirhiza multifurcata*": 0.3–0.5 µm, exceptionally up to 0.8 µm; *Sebacinoid* sp.: up to 1.3 µm). From the *Sebacina incrustans* ECM "*Pinirhiza multifurcata*" differs by the lack of a superficial hyphal net of thick-walled, lobed and frequently branched hyphae, from "*Quercirhiza dendrohyphidiomorpha*" in having a plectenchymatous rather than a pseudoparenchymatous middle mantle layer.

"*Pinirhiza nondextrinoidea*" can be distinguished from all these other sebacinoid ECM by the presence of rhizomorphs. Furthermore, it differs from the ECM of *S. incrustans* by thinner-walled (0.3–0.5 µm versus 0.8 µm) emanating hyphae and the lack of a superficial net consisting of frequently branched thick cells (2.5–6 µm) that are present in *S. incrustans* ECM. It differs from ECM of the *Sebacinoid* sp. by lacking the pseudoparenchymatous outer mantle and rather thin-walled emanating hyphae (0.3–0.5 µm versus up to 1.3 µm). In addition, the emanating hyphae of "*Pinirhiza nondextrinoidea*" are not dextrinoid unlike the slightly dextrinoid ones in "*Quercirhiza dendrohyphidiomorpha*". This chemical reaction was not checked in *Sebacina incrustans* ECM and *Sebacinoid* sp. ECM (Urban et al. 2003).

Concerning the emanating hyphae which are frequently ramified and clampless, "*Pinirhiza multifurcata*", *S. incrustans* ECM, ECM of *Sebacinoid* sp., and "*Quercirhiza dendrohyphidiomorpha*" are similar to two *Trichophaea*-ECM, "*Pinirhiza trichophaeoides*" (Wei et al. 2009) and "*Quercirhiza quadratum*" (Águeda et al. 2008). With the exception of the ECM of *Sebacinoid* sp. that forms a pseudoparenchymatous outer mantle with epidermoid cell, the sebacinoid ECM have plectenchymatous outer mantles, *Trichophaea* ECM have angular cells in the outer mantle. Emanating hyphae of these sebacinoid ECM are colourless, generally smooth, and mostly with Y-shaped or trifurcate ramifications and at least those of "*Pinirhiza multifurcata*" and "*Quercirhiza dendrohyphidiomorpha*" are dextrinoid. The emanating hyphae of the two *Trichophaea*-ECM are partly warty, brownish, have mostly rectangular ramifications, and are not dextrinoid.

Molecular-phylogenetic analyses

The two well supported clades of Sebacinales in the RAxML tree generated by the analysis of nLSU (Fig. 6) concur with the results of Weiß and Oberwinkler (2001), Urban et al. (2003),

Weiß et al. (2004), and Selosse et al. (2009). "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" are members of the Sebacinales, because both cluster in the *Sebacina*/*Tremellodendron* complex in clade I with good support (95% BS), and they appear as two different species according to the RaxML tree.

Our two "*Pinirhiza*"-collections do not belong to the ERM fungi complex (at most 88% identity and 100% query coverage compared with the sequences of "*Pinirhiza*" spp.) in the nLSU tree (clade II). This corresponds well with the results of ITS-sequence comparisons in which no sequences of *Sebacina vermifera*/ ERM fungi were among the most similar sequences obtained by megablast search using our sequences as queries.
Due to the lack of species names of included sequences and the insufficient BS-support of the clades "*Pinirhiza multifurcata*" and "*Pinirhiza nondextrinoidea*" are positioned in, the assignment of the two "*Pinirhiza*"-isolates described here to fungal genera or species is impossible to date, however.

Although the sequence of "*Pinirhiza multifurcata*" is very similar to a sequence of a Sebacinaceae ECM of *Dryas octopetala* (99% BS), it keeps unknown according to our present knowledge whether both fungal partners of "*Pinirhiza multifurcata*" and of *D. octopetala* ECM are the same species. "*Pinirhiza nondextrinoidea*" is placed in a very weakly supported clade (55% BS) with a sebacinoid ECM on *Fagus sylvatica*. As an identification to species-level is impossible, the two ECM for the present are named with artificial binomina as has frequently been done for unidentified, comprehensively described ECM since Gronbach and Agerer (1986).

The presence of two ECM of Sebacinales in China supports the suggestion that the Sebacinales have a wide geographical distribution and host spectrum (Weiß et al. 2004).

Acknowledgements This study was financially supported by the German Academic Exchange Service (DAAD). We thank also Prof. Yan Wei (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) for his support regarding field work and equipment, Fan Yongjun (Inner Mongolia Agricultural University, Huhhot, Inner Mongolia, China) for his field assistance, Derek Peršoh (University of Munich) for his useful suggestions on phylogenetic analyses, Philomena Bodensteiner for her help on improving writing, and the Managment Authority of Helan Mountain National Reserve and Heilihe

National Reserve for the allowance of field sampling. We thank two anonymous reviewers, whose suggestions significantly improved the manuscript.

References

Agerer R (1987–2008) Colour Atlas of Ectomycorrhizae. 1st – 14th delivery. Einhorn, Schwäbisch Gmünd

Agerer R (1991) Characterization of Ectomycorrhizae. In Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods in Microbiology, vol 23, pp 25–73. Acad Press, London et al.

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5:67–107

Agerer R, Rambold G (2004–2009, First posted on 2004-06-01; most recent update: 2009-01-26) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de - München, Germany

Águeda B, Agerer R, De Miguel AM, Parladé J (2008) "*Quercirhiza quadratum*" + *Quercus ilex* L. subsp.*ballota* (Scop.) Desf. Samp. Descr Ectomyc 11/12:113–123

Avis PG, McLaughlin DJ, Dentinger BC, Reich PB (2003) Long-term increase in nitrogen supply alters above and below-ground ectomycorrhizal communities and increases the dominance of *Russula* spp. in a temperate oak savanna. New Phytol 160:239–253

Azul AM, Agerer R, Freitas H (2006) "*Quercirhiza dendrohyphidiomorpha*" + *Quercus suber* L. Descr Ectomyc 9/10:87–91

Bidartondo M, Burghardt B, Gebauer G, Bruns TD, Read DJ (2004) Changing partners in the dark:isotopic and molecualr evidence of ectomycorrhizal liaisons between forest orchids and trees. Proc R Soc Lond B 271:1799–1806

Blackwell M, Hibbett D, Taylor JW, Spatafora JW (2006) Research Coordination Networks: a phylogeny for kingdom Fungi (Deep Hypha). Mycologia 98 (6):829–837

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - applications to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118

Glen M., Tommerup I.C., Bougher N.L. O'Brien P.A. (2002) Are Sebacinaceae common and widespread ectomycorrhizal associates of *Eucalyptus* species in Australian forests? Mycorrhiza 12:243–247

Gronbach E, Agerer R (1986) Charakterisierung und Inventur der Fichten-Mykorrhizen im Höglwald und deren Reaktion auf saure Beregnung. Forstwiss Cbl 105:329–335

Hall T (2005) BioEdit, biological sequence alignment editor for Win95/98/NT/2K/XP. Carlsbad, California: Ibis therapeutic

Julou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse MA (2005) Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. New Phytol 166:639–653

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 166:1063–1068

Kottke I, Beiter A, Weiß M, Haug I, Oberwinkler F, Nebel M (2003) Heterobasidiomycetes form symbiotic associations with hepatics: *Jungermanniales* have sebacinoid mycobionts while *Aneura pinguis* (Metzgeriales) is associated with a *Tulasnella* species. Mycol Res 107:957–968

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst Biol 49:278–305

Obase K, Cha JY, Lee JK, Lee SY, Lee JH, Chun KW (2009) Ectomycorrhizal fungal communities associated with *Pinus thunbergii* in the eastern coastal pine forests of Korea. Mycorrhiza 20:39–49

Richard F, Millot S, Gardes M, Selosse MA (2005) Diversity and specificity of ectomycorrhizal fungi retrieved from an old-growth Mediterranean forest dominated by *Quercus ilex*. New Phytol 166:1011–1023

Selosse MA, Bauer R, Moyersoen B (2002a) Basal hymenomycetes belonging to the Sebacinaceae are ectomycorrhizal on temperate deciduous trees. New Phytol 155:183–195

Selosse MA, Dubois MP, Alvarez N (2009) Do Sebacinales commonly associate with plant roots as endophytes? Mycol Res 113:1062–1069

Selosse MA, Setaro S, Glatard F, Rhichard F, Urcelay C, Weiß M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. New Phytol 174 (4):864–878

Selosse MA, Weiß M, Jany JL, Tillier A (2002b) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. Mol Ecol 11:1831–1844

Selosse MA, Faccio A, Scappaticci G, Bonfante P (2004) Chlorophyllous and Achlorophyllous Speciemens of *Epipactis microphylla* (Neottieae, Orchidaceae) Are

Associated with Ectomycorrhizal Septomycetes, including Truffles. Micro Ecol 47:416–426

Setaro S, Weiß M, Oberwinkler F, Kottke I (2006) Sebacinales form ectendomycorrhizas with Cavendishia nobilis, a member of the Andean clade of Ericaceae, in the mountain rain forest of southern Ecuador. New Phytol 169:355–365

Sikes DS, Lewis PO (2001) PAUPRat: A tool to implement Parsimony Ratchet searches using PAUP*. (http://viceroy.eeb.uconn.edu/paupratweb/pauprat.htm)

Smith ME, Douhan GW, Rizzo DM (2007) Ectomycorrhizal community structure in a xeric Quercus woodland based on rDNA sequence analysis of sporocarps and pooled roots. New Phytol 174:847–863

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Stamatakis A, Hoover P, Rougement J (2008) A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 75:758–771

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

Taylor DL, Bruns TD, Szaro TM, Hodges SA (2003) Divergence in mycorrhizal specialization within Hexalectris spicata (Orchidaceae), a nonphotosynthetic desert orchid. Am J Bot 90:1168–1179

Tedersoo L, Suvi T, Larsson E, Kõljalg U (2006) Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycol Res 110:734–748

Thiers B (2009, continuously updated) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. sciweb.nybg.org/science2/IndexHerbariorum.asp.

Urban A, Weiss M, Bauer R (2003) Ectomycorrhizas involving sebacinoid mycobionts. Mycol Res 107(1):3–14

Urban A, Puschenreiter M, Strauss J, Gorfer M (2008) Diversity and structure of ectomycorrhizal and co-associated fungal communities in a serpentine soil. Mycorrhiza 18:339–354.

Wei J, Peršoh D, Agerer R (2009) Four Ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*) – morpho-anatomical and molecular–phylogenetic analyses. Mycological Progress (in Press)

Weiß M, Oberwinkler F (2001) Phylogenetic relationships in Auriculariales and related groups- hypotheses derived from nuclear ribosomal DNA sequences. Mycol Res 105:403–415

Weiß M, Selosse MA, Rexer KH, Urban A, Oberwinkler F (2004) *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol Res 108 (9):1003–1010

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MA, Gelfand DH, Sninsky JN, White TJ (eds) PCR Protocols: a guide to method and applications. Academic Press, San Diego, pp315–322

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7:203–214

Supplementary file

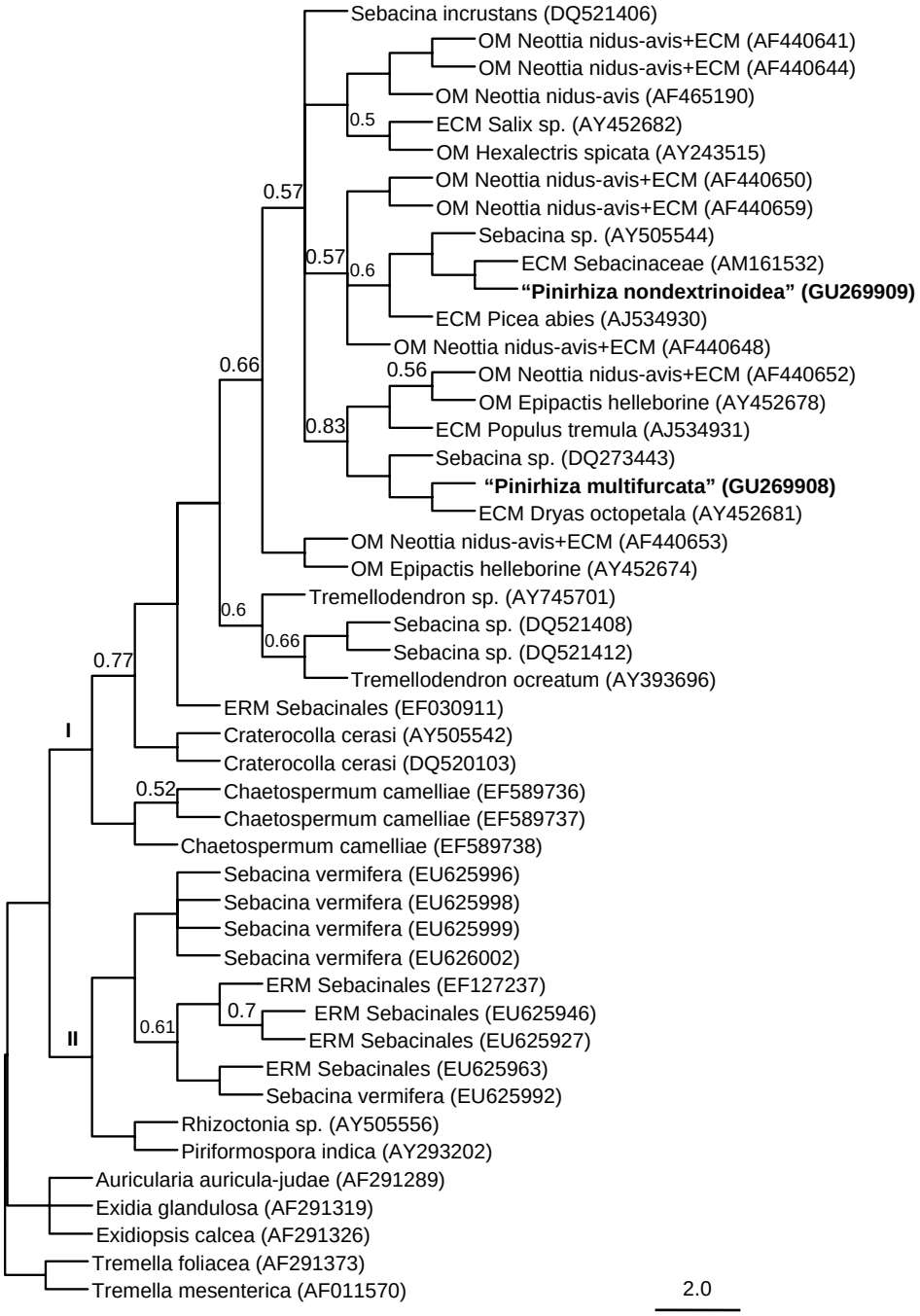


Fig. 7 Molecular-phylogenetic placement of “*Pinirhiza multifurcata*” and “*Pinirhiza nondextrinoidea*” among selected Sebacinales inferred by the PAUPRat analysis of nLSU sequences. Posterior Probability of nodes are 1.0 except where indicated otherwise. GenBank accession numbers are given in parentheses following the species names.

2.4.7 Three *Tuber* Ectomycorrhizae on Chinese Pine

VII

Three *Tuber* Ectomycorrhizae on Chinese Pine

Jie Wei[1]*, Wei Yan[2], Reinhard Agerer[1]

[1] Department Biology I, Division of Organismic Biology, Mycology, University of Müchen, Menzinger Str. 67, D-80638 Müchen, Germany

[2] Mycorrhizal research team, Forestry Colleage, Inner Mongolia Agricultural University, Xinjian East Road 275, 010019 Huhhot, Inner Mongolia, China

*Corresponding author: E-mail: weijie211@yahoo.com, phone: 00498917861196

Abstract

Three anatomotypes of *Tuber* ectomycorrhizae on Chinese pine are presented in this study. They all have pseudoparenchymatous outer mantle layers, but differ from each other in the shape of mantle cells and the presence of cystidia. "*Pinirhiza pubulata*" has angular cells in outer mantle and abundant cystidia, "*Pinirhiza puborchii*" forms in the outer mantle layer irregular epidermoid cells with few angular cells connected by septa and abundant cystidia, whereas "*Pinirhiza ongensis*" has epidermoid cells in outer mantle and lacks cystidia. The fungal partners of these three ectomycorrhizae are members of the genus *Tuber* inferred by molecular phylogenetic analyses of LSU nrDNA and by structural similarity to already studied ectomycorrhizae of this genus. "*Pinirhiza ongensis*" could be caused by a species of the *Tuber liaotongense* complex, fungal partners of "*Pinirhiza pubulata*" and "*Pinirhiza puborchii*" could only be assigned to species groups according to the phylogenetic analyses of ITS region. Molecular phylogenetic analyses of ITS region indicate that *Tuber* ECM with angular cells in outer mantle are not monophyletic. It can be assumed that detailed morpho-anatomical ECM studies combined with molecular methods will quickly increase our knowledge about distribution and ecology of *Tuber* species in China.

Keywords *Tuber* ectomycorrhizae, morphology, anatomy, molecular phylogeny

Introduction

Many *Tuber* species have been reported to form ectomycorrhizae (ECM) in terms of recent reviews of ECM fungal diversity (Agerer 2006, Rinaldi et al. 2008, de Roman et al. 2005). *Tuber* ECM have been comprehensively studied morpho-anatomically in Europe since at least two decades (e.g. Blaschke 1987, 1988, Fischer et al. 2004, Giraud 1990, Granetti 1995, Kovács and Jakucs 2006, Müller et al. 1996a, b, Rauscher and Agerer 1995, Rauscher et al. 1996, Zambonelli et al. 1993, 1995, 1999). In comparison, only two *Tuber* ECM from Asia have been described in detail till now, i.e. ECM of *T. indicum* Cooke & Massee and *T. himalayense* B.C. Zhang & Minter (*T. himalayense* is synonymous to *T. indicum* according to Wang et al. 2006) with *Quercus pubescens* (Comandini and Pacioni 1997).

Tuber ECM are generally easily distinguished from ECM of other genera in presenting frequently typical awl-shaped cystidia connected to a pseudoparenchymatous hyphal mantle, lack of rhizomorphs and lack of clamps. The genus *Tuber* can be divided into two entities and a transitional type with respect to ectomycorrhizal structures (Agerer 2006). One group is characterized by pseudoparenchymatous mantles with angular cells, including *T. aestivum* Vitt. (Müller et al. 1996a), *T. excavatum* Vitt. (Giraud 1990), *T. mesentericum* Vitt. (Granetti 1995), and *T. uncinatum* Chat. (Müller et al. 1996b). The other group forms pseudoparenchymatous mantles composed of epidermoid cells, including *T. borchii* Vitt. (sub nomine *T. albidum* Pico., Rauscher et al. (1996)), *T. brumale* Vitt. (Fischer et al. 2004), *T. macrosporum* Vitt. (Granetti 1995), *T. maculatum* Vitt. (Zambonelli et al.1999), *T. puberulum* Berk. & Broome (Blaschke 1987, 1988, Kovács and Jakucs 2006). Mantle cells of *Tuber indicum* ECM are irregularly polygonal and represent a transitional type between typical angular and epidermoid (Comandini and Pacioni 1997). Cystidia are generally present. An exception are *T. rufum* ECM which lack cystidia (Palenzona et al. 1972, Rauscher & Agerer 1995). These morpho-anatomical descriptions of *Tuber* ECM provide useful taxonomical information and can distinctly facilitate ECM identification at least at *Tuber*-level, but whether mantle and cystidia types fit to molecular-phylogenetic results obtained by DNA sequencing is still an open question.

Tuber studies in China are mainly based on collections of fruitbodies in southwest China. *Tuber* diversity and distribution are little known in north China mainly because of the hypogeous habitat of *Tuber* fruitbodies, which also hinders comprehensive study of *Tuber* diversity and distribution in whole China (Garcia-Montero et al. 2010). ECM investigation

combining morpho-anatomical features and a molecular approach can play a very important role to reveal diversity and distribution of *Tuber* species. In the course of ECM investigation on Chinese Pine (*Pinus tabulaeformis* Carr.), three *Tuber* ECM have been found. We provide detailed descriptions of these ECM and apply phylogenetic analyses of LSU rDNA and ITS region to unravel their relationship, and try to increase our knowledge about the taxonomical value of mantle types and presence of cystidia in the genus *Tuber*.

Material and methods

Soil samples were collected in pure Chinese Pine forests at Heilihe National Reserve (Chi Feng City, Inner Mongolia Autonomous Region, China) and in Daqing Mountain (Huhhot City, Inner Mongolia Autonomous Region, China) throughout two years. ECM systems were assigned to anatomotypes and described according to Agerer (1987–2008, 1991). Anatomical studies and drawings were performed with the aid of a Normarski interference contrast microscope (Standard 14, ZEISS West Germany) connected with a drawing tube. All drawings were made at a magnification of 1000 ×. Reference specimens of the mycorrhizae are deposited in M (see Thiers 2009).

DNA isolation, amplification and sequencing of the LSU and ITS regions were carried out as described previously (Wei et al. 2009)

Sequence alignments and molecular-phylogenetic analyses

The most similar sequences were searched for in GenBank (http://www.ncbi.nlm.nih.gov/) using megablast (Zhang et al. 2000). The 100 sequences most similar to each obtained LSU and ITS sequence were downloaded from GenBank. Duplicates, i.e. identical sequences found as closest relatives of different query sequences, were omitted, additional sequences of some already described *Tuber* species from GenBank were added for ITS analyses. Using the software BioEdit v7.0.5 (Hall 2005), the sequences were automatically aligned. The alignment was revised manually and columns not alignable with certainty were excluded from the following analyses. RAxML Web-Servers (the CIPRES Portal v1.14 at the San Diego Supercomputing Center, http://8ball.sdsc.edu:8889/cipres-web/Home.do; Stamatakis 2006, Stamatakis et al. 2008) was used for calculation of the most likely trees and the bootstrap support values (500 replicates). The GTR model of substitution was applied for LSU and ITS

region analyses having Maximum Likelihood as optimality criterion. The most parsimonious trees were searched for by executing batch files generated by PAUPRat (Sikes and Lewis 2001) in PAUP* v4.0 (Swofford 2003), with weighting mode set to multiplicative.

Results

Morpho-anatomical descriptions

"*Pinirhiza pubulata*"

Morphological characters (Figure 1a), *Mycorrhizal systems* with 0.3–0.37 mm wide main axis, dichotomous to irregularly dichotomous, 0–5 ramification orders, in small numbers, hydrophilic, of contact exploration type. *Unramified ends* 1.5–4 mm long, 0.3–0.37 mm diam., straight, not inflated, cylindric, younger mycorrhizae yellowish brown, older parts brownish, mantle surface densely short spiny. *Cystidia* abundant. *Emanating hyphae* not distinct under stereoscope. *Rhizomorphs* lacking. *Sclerotia* absent.

Anatomical characters of mantle in plan views (Figures 1c, 2, 3). *Outer mantle layers* (Figure 2) pseudoparenchymatous, most of cells angular, a few irregular (mantle type L, according to Agerer 1987–2008, 1991, Agerer & Rambold 2004–2009), with a matrix gluing some soil particles on its surface; hyphal cells (6) 8×10 (16)–5×8 (12.5) µm, cell wall 0.5–1 µm, 4–8 cells in a square of 20×20 µm; cystidia bearing hyphal bridges between mantle cells occasionally occurring on the mantle surface (Figure 1c); colourless and smooth. *Middle mantle layers* (Figure 3a) pseudoparenchymatous, cell shape similar to cells of the outer mantle, (5) 8–12 (18) µm long, 5–14 µm wide, cell wall 0.5–1 µm, 4–8 cells in a square of 20×20 µm, smooth and colourless. *Inner mantle layers* (Figure 3b) pseudoparenchymatous with epidermoid cells, hyphal cells 7–18 µm long, 2.5–5 µm wide, 4–5 cells in a square of 20×20 µm, cell wall 0.3–0.5 µm, smooth and colourless. *Very tip* organized like remaining parts.

Anatomical characters of emanating elements (Figures 1b, c): *Rhizomorphs* lacking. *Emanating hyphae* (Figure 1b), infrequent to frequent, 3–4 µm wide, cell wall 0.5 µm, ends sometimes inflated (up to 5.5 µm); septa simple, as thick as cell walls; surface gluing soil particles, very end simple or sometimes ramified. *Cystidia* (Fig. 1c) originating from hyphal cells on outer mantle layer or from hyphae on mantle surface, one type, awl-like, abundant, straight with slightly broadened bases and tapering tips, (40) 75–110 µm long, with one to two simple septa, septa as thick as cell walls; diameter at the base 2.5–3.5 µm (not including

the base cell) and at the apex 1–1.5 μm, slightly thick-walled, cell wall 0.5 μm; very tip simple, not ramified; surface smooth.

Colour reactions with different reagents, Preparations of mantle: Melzer's reagent: n. r. (=no reaction); lactic acid: n.r.; KOH: n. r.; $FeSO_4$: n. r.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Daqing Mountain, Huhhot city, Inner Mongolia, China, myc. exc. and isol. by Jie Wei, 08.06.2008, JW 97b (in M). Sequence accession number in GenBank: GU722190 for LSU region, GU722193 for ITS region.

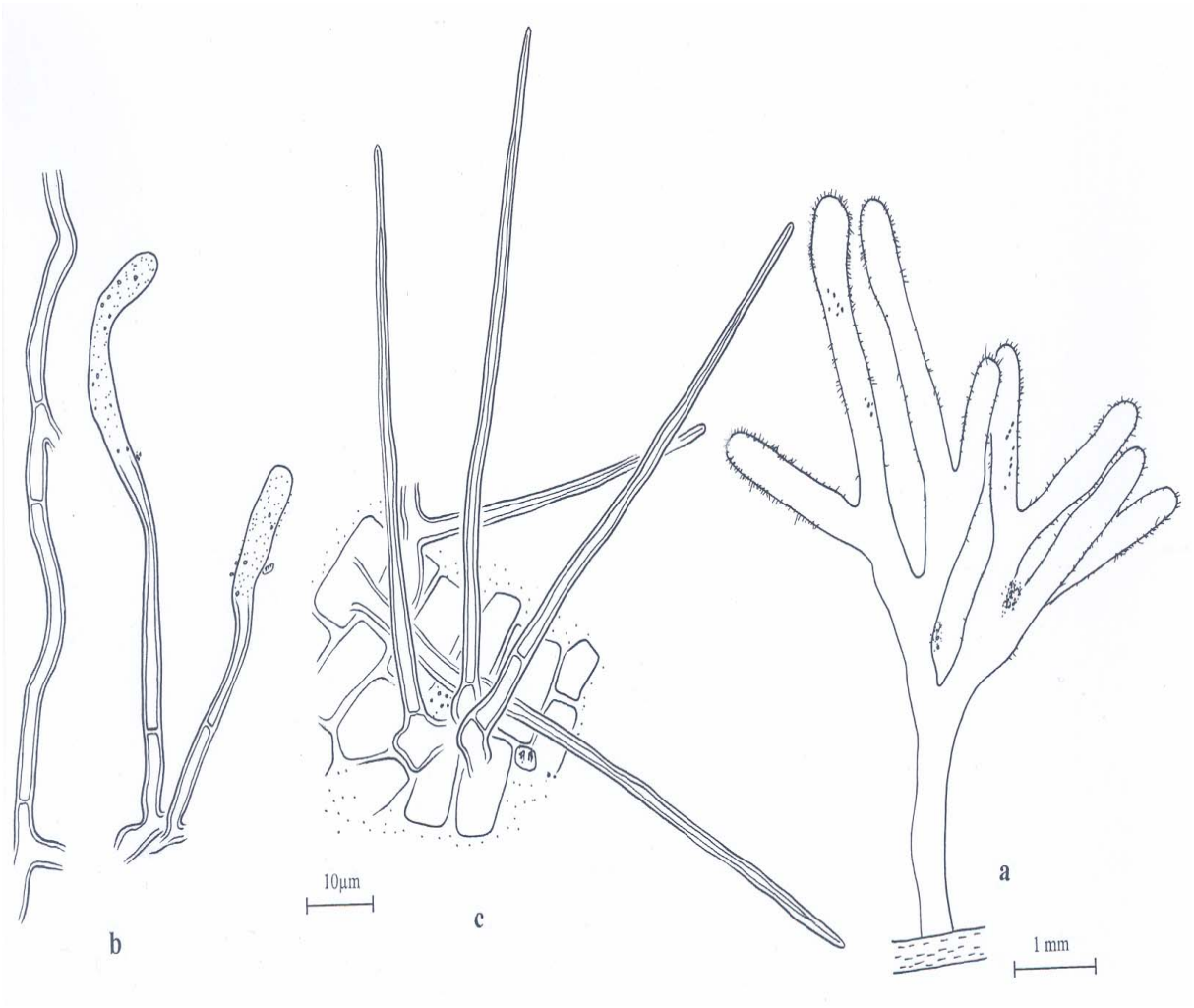
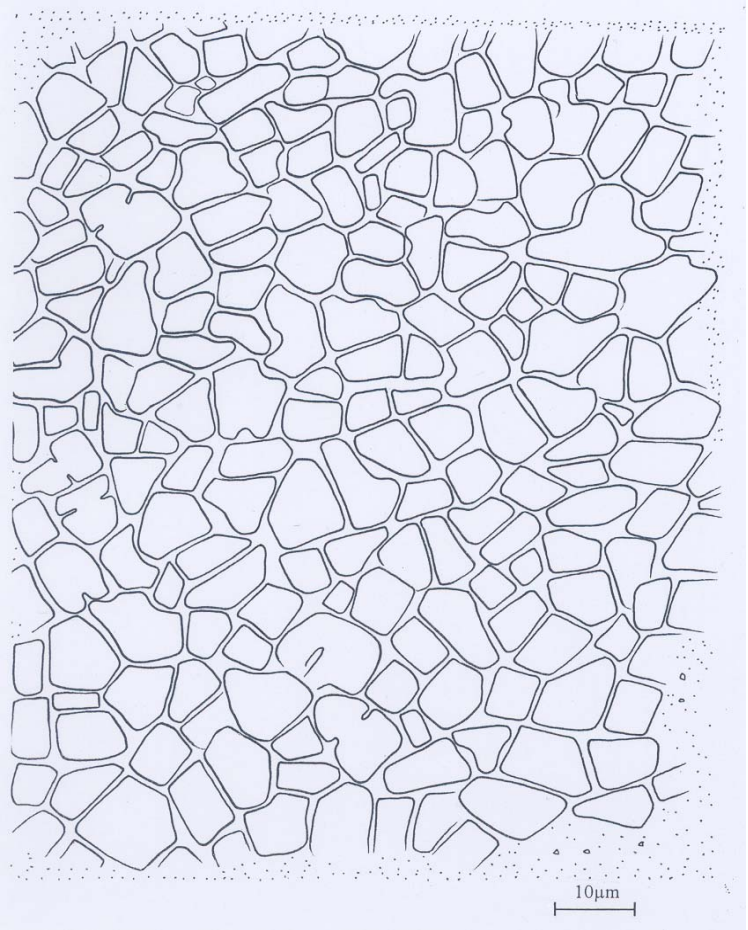
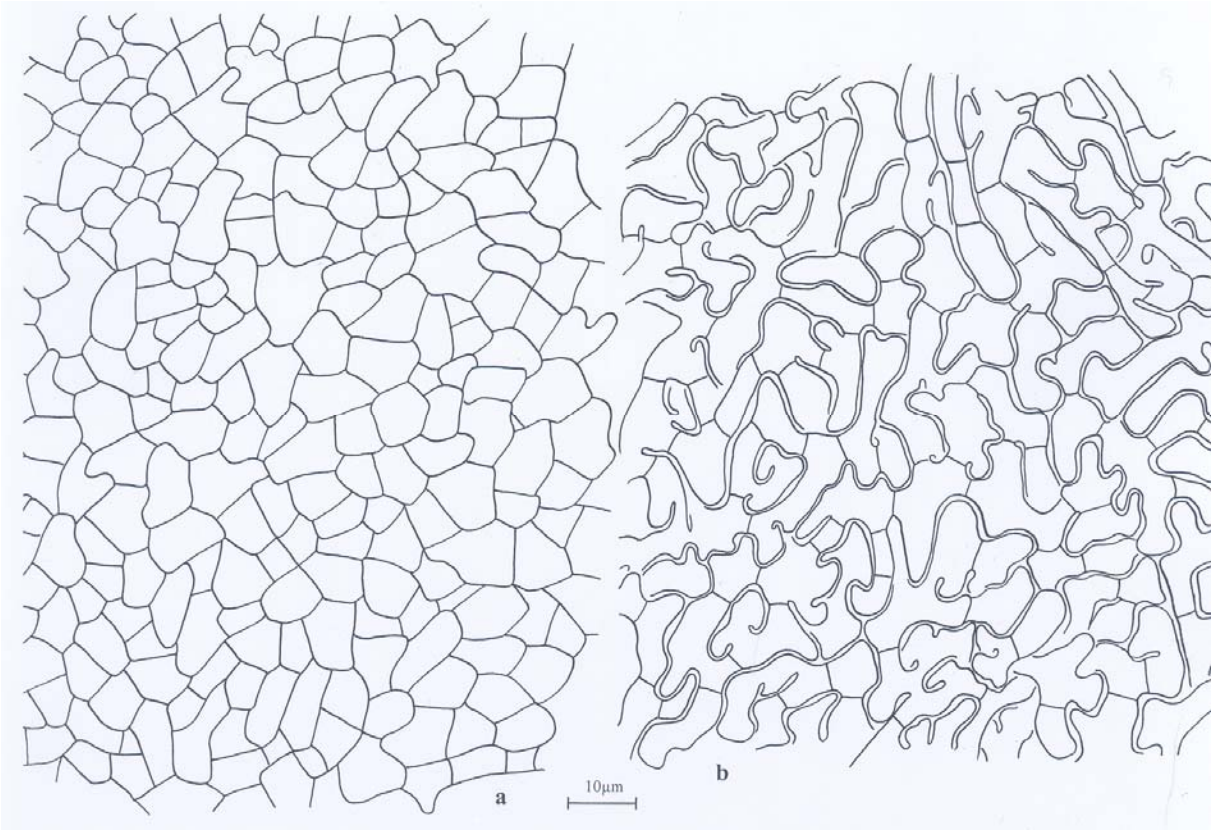


Figure 1 "*Pinirhiza pubulata*", habit of ECM and cystidia. a habit of ECM, surface with abundant cystidia; b emanating hyphae, with two short ones with inflated ends covered by soil particles; c awl-shaped cystidia with simple septa originating directly from outer mantle cells or from hyphae on mantle surface



Figures 2–3 "*Pinirhiza pubulata*" Figure 2 (left) plan view of outer mantle layer with mostly angular hyphal cells and few irregular-shaped cells with gelatinous walls. Figure 3 (below) a plan view of middle mantle layer (cell wall thickness not shown); b plan view of inner mantle layer with epidermoid cells.



"Pinirhiza puborchii"

Morphological characters (Figure 4a): *Mycorrhizal systems* with 0.4–0.5 mm wide main axis, dichotomous, ramification orders 0–5, in small numbers, hydrophilic, of contact exploration type. *Unramified ends* 0.6–1.6 mm long, 0.3–0.5 mm wide, straight, not inflated, cylindric, younger mycorrhizae yellowish, very tips lighter, older reddish brown, mantle surface densely short spiny. *Cystidia* abundant, not specificly distributed. *Emanating hyphae* not distinct under stereoscope. *Rhizomorphs* lacking. *Sclerotia* absent.

Anatomical characters of mantle in plan views (Figures 5, 6): *Mantle surface* with a gelatinous matrix gluing many soil particles. *Outer mantle layers* (Figure 5) pseudoparenchymatous with irregularly shaped epidermoid cells (mantle type Q, according to Agerer 1987–2008, 1991, Agerer & Rambold 2004–2009), embedded in a dense matrix; cells 3–6.5 (10) µm diam., cell wall variable 1.5–3 µm, 5–7 cells in a square of 20×20 µm; cystidia bearing hyphal bridges between mantle cells occuring occasionally on the mantle surface; colourless and smooth. *Middle mantle layers* (Figure 6a) pseudoparenchymatous with angular to epidermoid cells, cells 7–18 µm long, 4–10 µm wide, cell wall 1 µm, 4–6 cells in a square of 20×20 µm. *Inner mantle layers* (Figure 6b) pseudoparenchymatous with epidermoid cells, hyphal cells 4–7.5 µm diam., cell wall 0.5–1 µm, 4–6 cells in a square of 20×20 µm. *Very tip* like remaining parts.

Anatomical characters of emanating elements (Figures 4b, c): *Rhizomorphs* lacking. *Emanating hyphae* (Figure 4c) infrequent, 3–4 µm wide, sometimes inflated near the end (up to 5 µm), such ends covered with soil particles; with simple septa, septa as thick as cell wall, cell wall 0.5–0.8 µm; very end simple; hyphal surface smooth. *Cystidia* (Figure 4b) of one type, awl-like, abundant, originating directly from the cells of outer mantle or occassionally from hyphae on the mantle surface, straight with broad base and tapering to rounded tip, (30) 70–90 (150) µm long, with one to three simple septa, septa as thick as cell wall, located near the base, in the middle or near the apex, diameter at the base 4–5.5 µm (not including the basal cell) and at the apex 1.5–2.5 µm, slightly thick-walled, cell wall 0.5–0.8 µm, at apex thinner, 0.5 µm; very ends simple; surface smooth, colourless.

Colour reactions with different reagents: *Preparations of mantle*: Melzer's reagent: n. r.; lactic acid: n.r.; KOH: n. r.; $FeSO_4$: n. r..

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Heilihe National Reserve, Chi Feng city, Inner Mongolia, China, myc. exc. and isol. by Jie Wei, 09.12.2007, JW 29a (in M). Sequence accession number in GenBank: GU722192 for LSU region, GU722194 for ITS region.

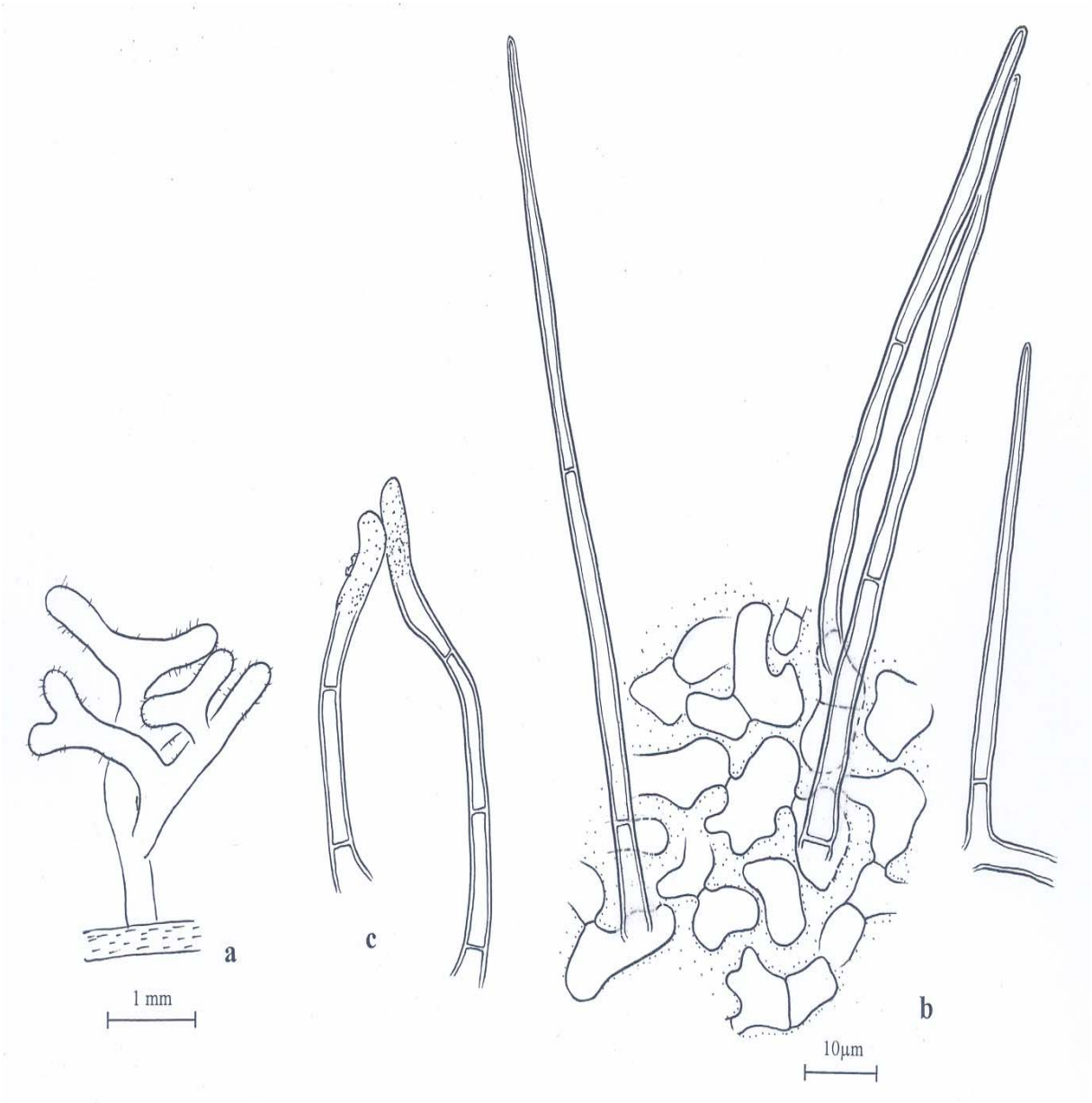
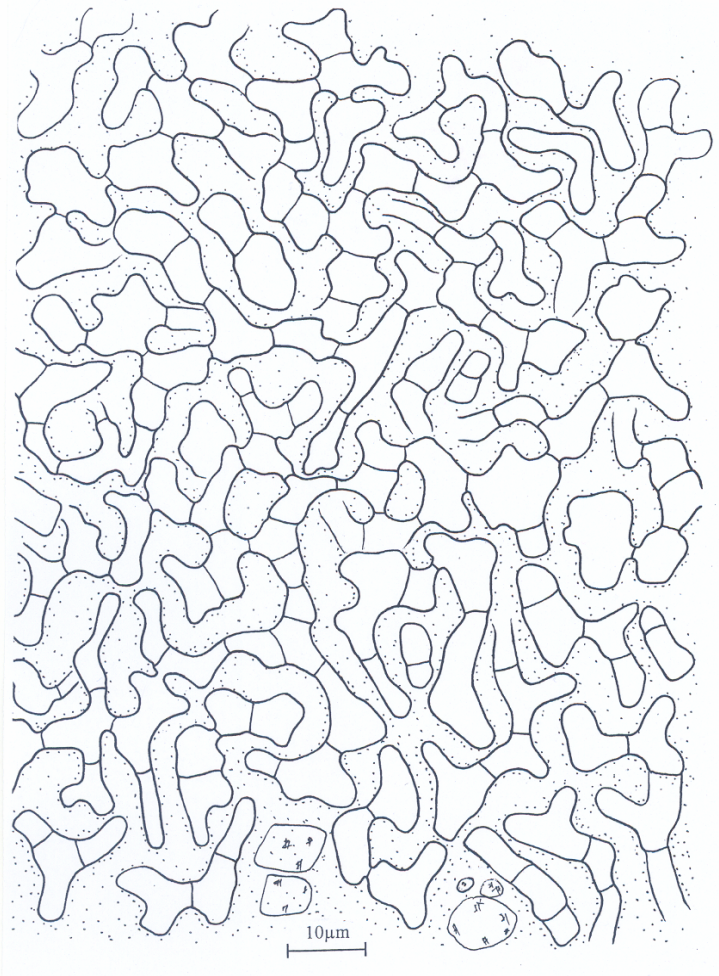
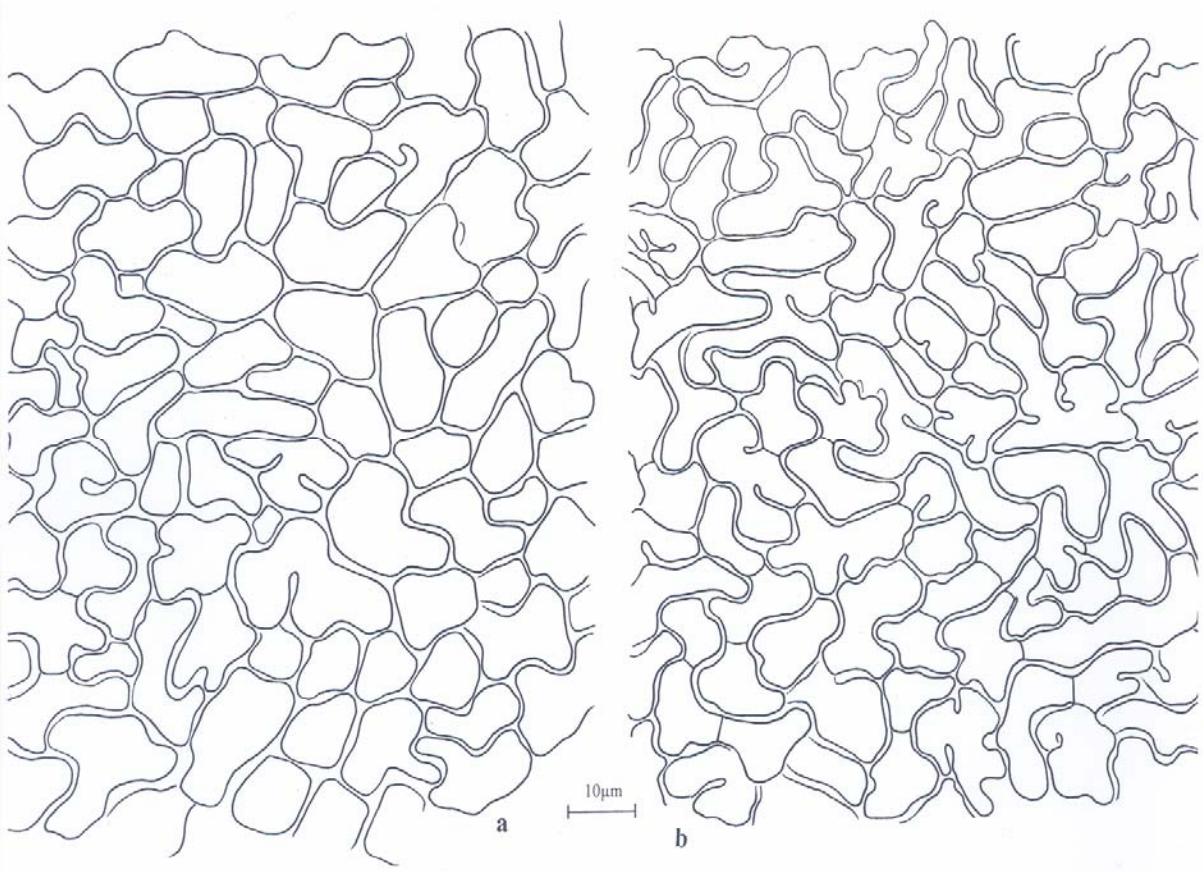


Figure 4 "*Pinirhiza puborchii*", habit of ECM and emanating elements. a habit of ECM, surface of mantle densely spiny; b awl-shaped cystidia with simple septa originating from outer mantle cells or from hyphae on mantle surface; c emanating hyphae with inflated ends covered by soil particles.



Figures 5–6 "*Pinirhiza puborchii*" Figure 5 (left) outer mantle layer with epidermoid cells and few angular cells connected by septa in a gelatinous matrix gluing with some soil particles. Figure 6 (below) a plan view of middle mantle layer; b plan view of inner mantle layer.



"Pinirhiza ongensis"

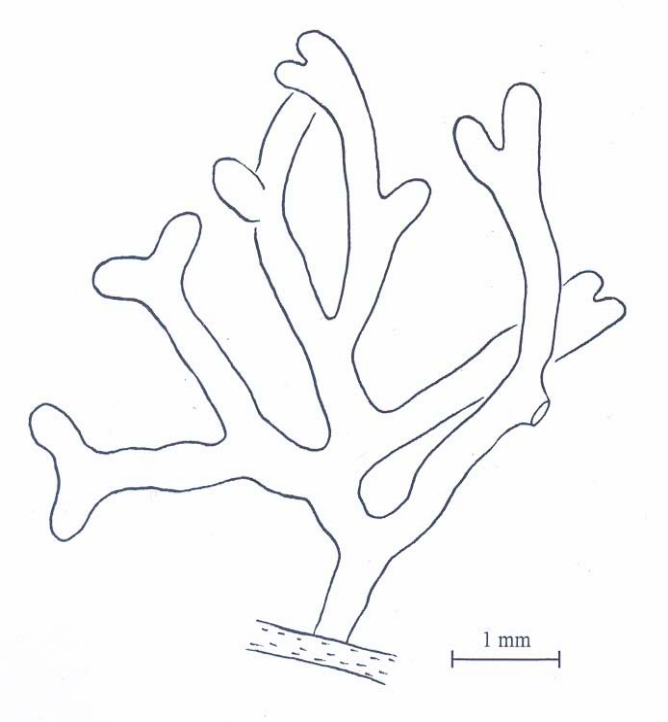
Morphological characters (Figure 7): *Mycorrhizal systems* unramified to dichotomous, with 0–5 orders of ramification, in small numbers, main axis 0.5–0.6 mm diam., hydrophilic, of contact exploration type. *Unramified ends* straight, cylindric, not inflated, 0.4–3 mm long, 0.4–0.5 mm diam., yellowish brown when young, older parts dark brown, mantle not transparent, surface smooth. *Emanating hyphae* not observed. *Cystidia* not observed. *Rhizomorphs* lacking. *Sclerotia* not observed.

Anatomical characters of mantle in plan views (Figure 8, 9): *Mantle surface* with a robust hyphal net (Figure 8a), distributed at places, hyphae 2.5–5 µm diam, cell wall 0.5 µm, septa simple, frequent, cells 4–12 µm long, hyphae of this net frequently ramified. *Outer mantle layers* (Figure 8b) pseudoparenchymatous with epidermoid cells (mantle type Q, according to Agerer 1987–2008, 1991, Agerer & Rambold 2004–2009), hyphal cells connected and separated by septa, cells elongate, 4–12 µm long, 2.5–5 µm wide, cell walls 1–2 µm, 5–9 cells in a square of 20×20 µm, covered by a gelatinous matrix gluing some soil particles. *Middle mantle layer* (Figure 9a) pseudoparenchymatous, hyphal cells similar to cells in outer mantle in shape and dimension, irregualarly shaped to epidermoid, 5–12 µm long, 2.5–5 µm wide, cell wall 0.5 µm, 6–9 cells in a square of 20×20 µm. *Inner mantle layers* (Figure 9b) pseudoparenchymatous, sometimes occurring also elongate cells, hyphal cells 2–4 µm wide, cell walls 0.3 µm, without matrix. *Very tip* with the same structural characters as in remaining parts.

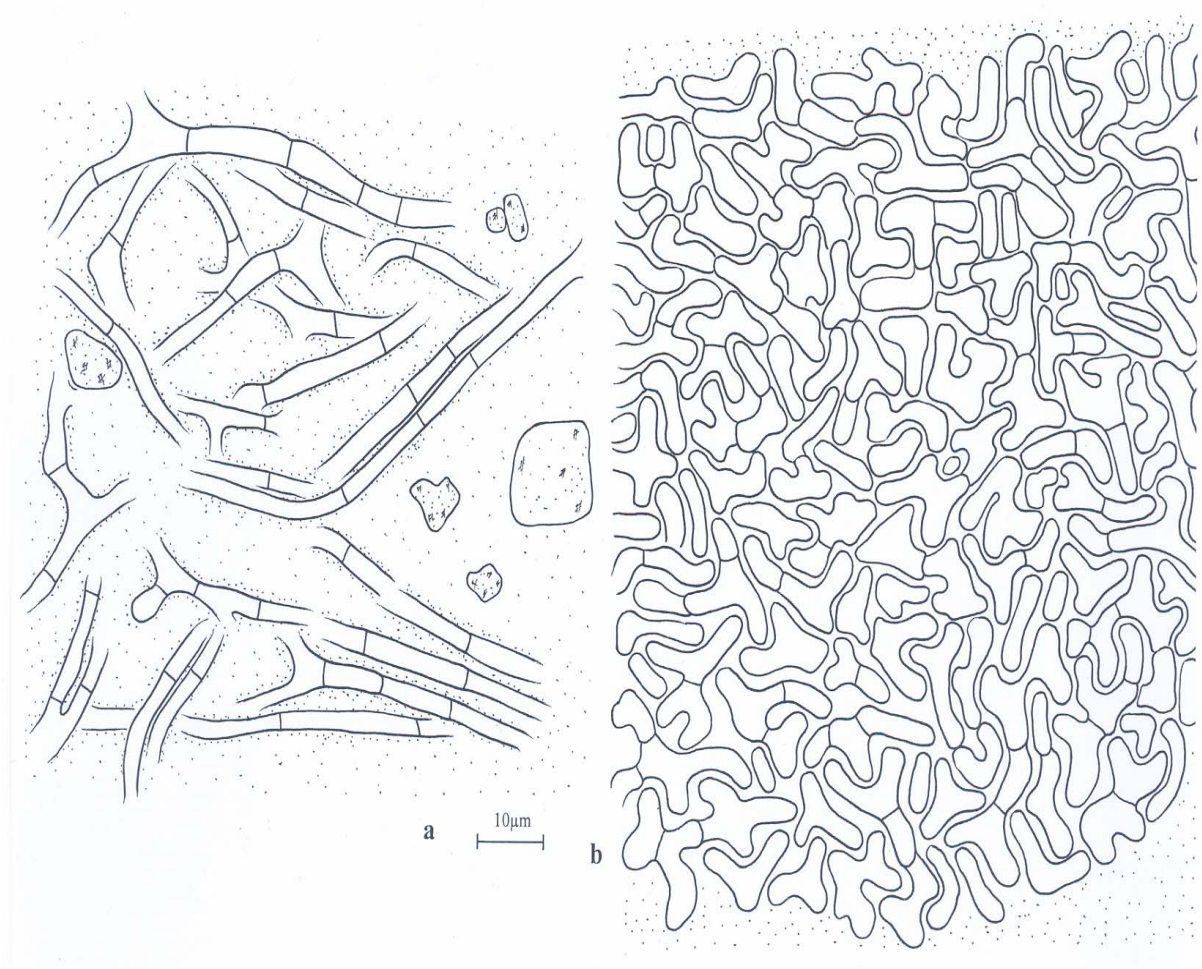
Anatomical characters of emanating elements: *Rhizomorphs* lacking. *Emanating hyphae* lacking. *Cystidia* lacking.

Colour reactions with different reagents: *Preparations of mantle*: Melzer's reagent: no reaction (n.r.); Lactic acid: n. r.; KOH: n. r.; $FeSO_4$: n. r.

Reference specimen: The mycorrhiza was collected in a pure *Pinus tabulaeformis* stand at Heilihe National Reserve, Chi Feng city, Inner Mongolia, China, myc. exc. and isol. by Jie Wei, 08.26.2008, JW 156a (in M). Sequence accession number in GenBank: GU722191 for LSU region, GU722195 for ITS region.



Figures 7–8 "*Pinirhiza ongensis*"
Figure 7 (left) habit of ectomycorrhiza, surface of mantle smooth.
Figure 8 (below) a robust hyphal net on the mantle surface; b outer mantle layer with epidermoid cells in a gelatinous matrix.



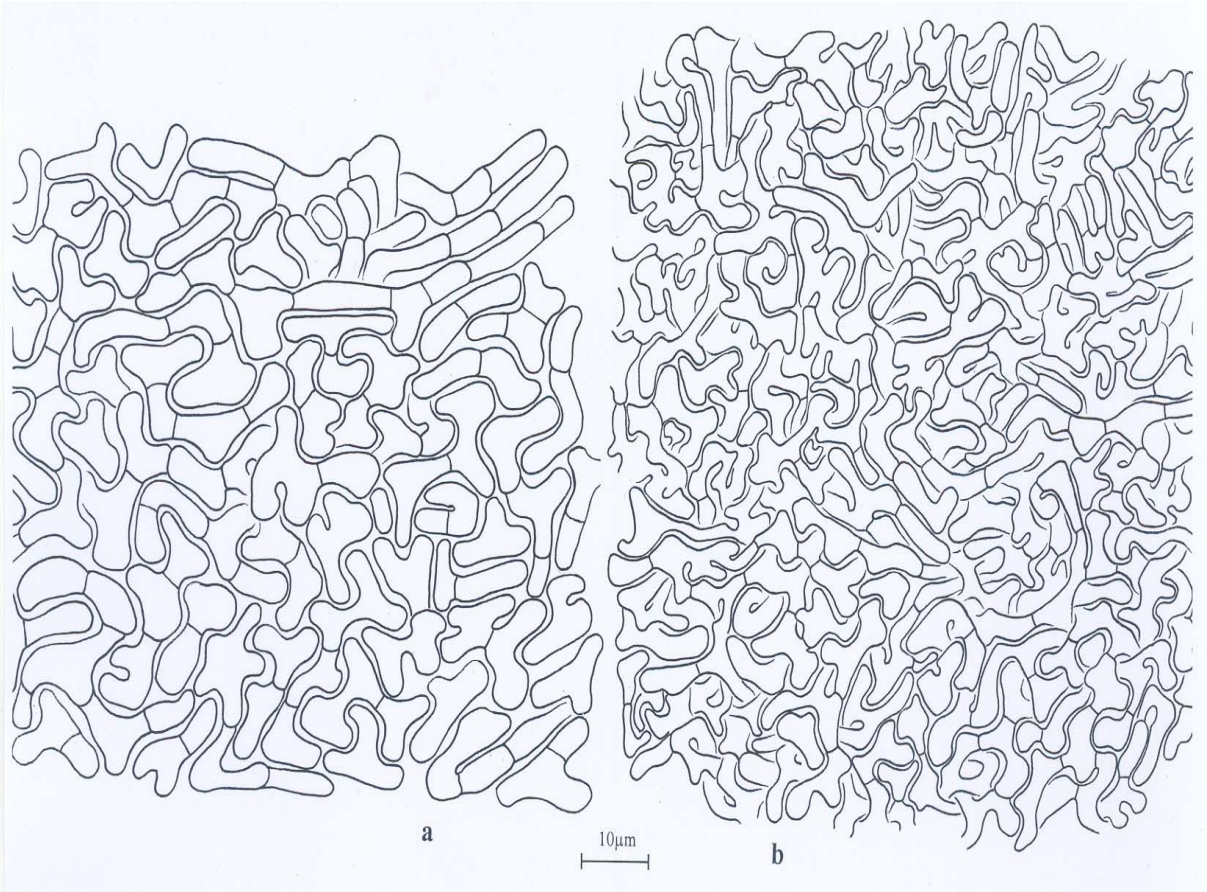


Figure 9 "*Pinirhiza ongensis*", plan view of middle and inner mantle layers. a middle mantle layer; b inner mantle layer.

Phylogenetic analyses

LSU nrDNA topology of RAxML and PAUPRat tree and placement of "*Pinirhiza pubulata*", "*Pinirhiza puborchii*", and "*Pinirhiza ongensis*"

The topologies of both RAxML tree and PAUPRat tree for LSU nrDNA are largely in accordance with respect to the obtained sequences. One section from the RAxML (Figure 10) and PAUPRat (Figure 11, supplementary file) trees, concerning the ECM being subject of this study, is shown. Due to the better support, only Figure 10 showing RAxML analysis is considered in more detail. "*Pinirhiza pubulata*", "*P. puborchii*", and "*P. ongensis*" cluster together with sequences from *Tuber* fruitbodies and *Tuber* ECM forming a clade with 80% bootstrap (BS), with *Choiromyces venosus* and *Choiromyces alveolatus* as a sister clade, the whole clade is supported with 99% BS.

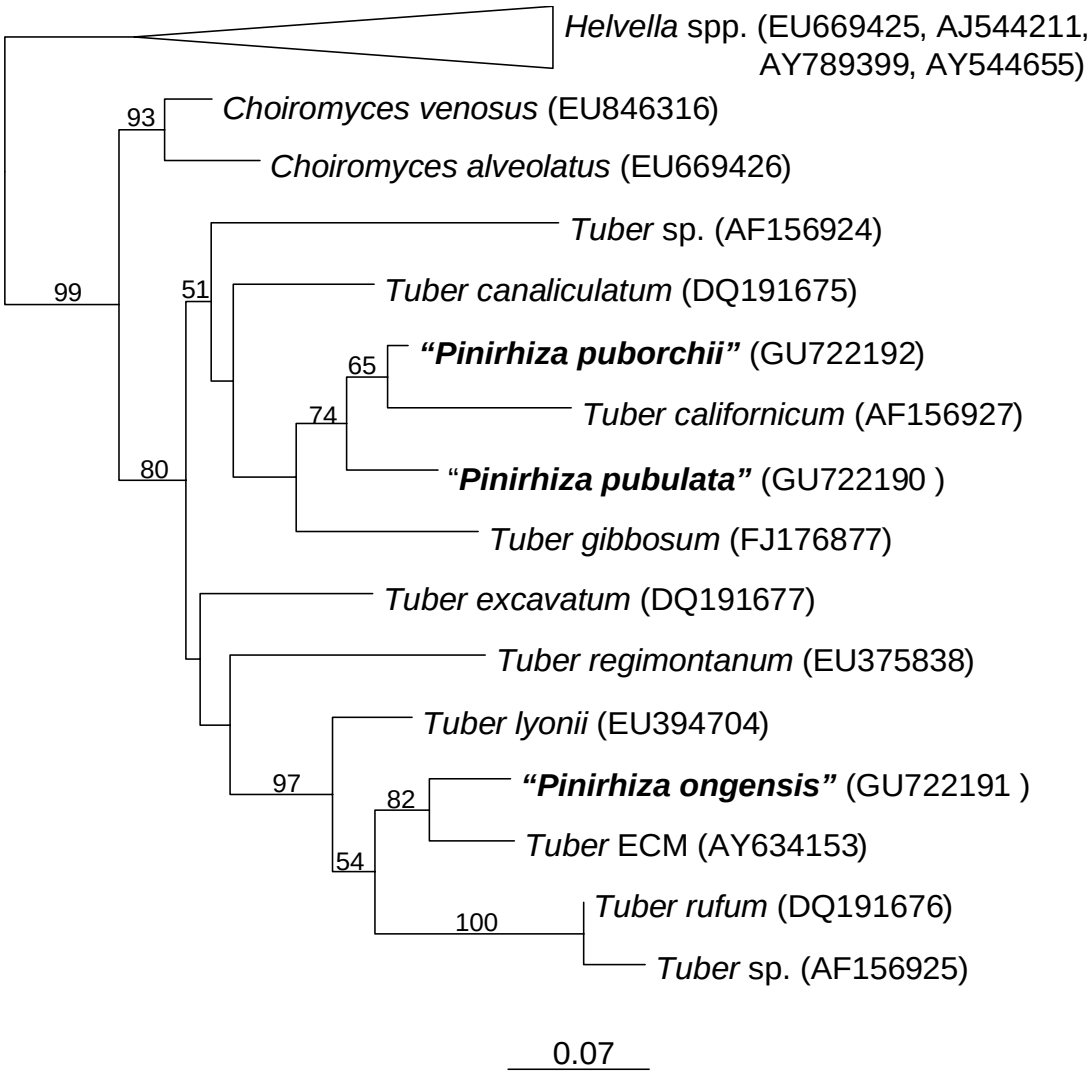


Figure 10 Molecular-phylogenetic placement of "*Pinirhiza pubulata*", "*Pinirhiza puborchii*", and "*Pinirhiza ongensis*" among Tuberaceae by the RAxML analysis of LSU nrDNA. Bootstrap support values above 50% are noted above or left of the respective branches. GenBank accession numbers are given in parentheses following the species names.

ITS sequences and phylogenetic placement of the *Tuber* ECM

Specific insertion-deletion patterns in the ITS-1 region of the multiple-aligned sequences have been observed. Only partial ITS-1 region was included in the analyses. For inferring molecular phylogenies, a 400-character long alignment was used. RAxML tree is shown here (Figure 12), however PAUPRat tree is presented as a supplementary file (Figure 13).

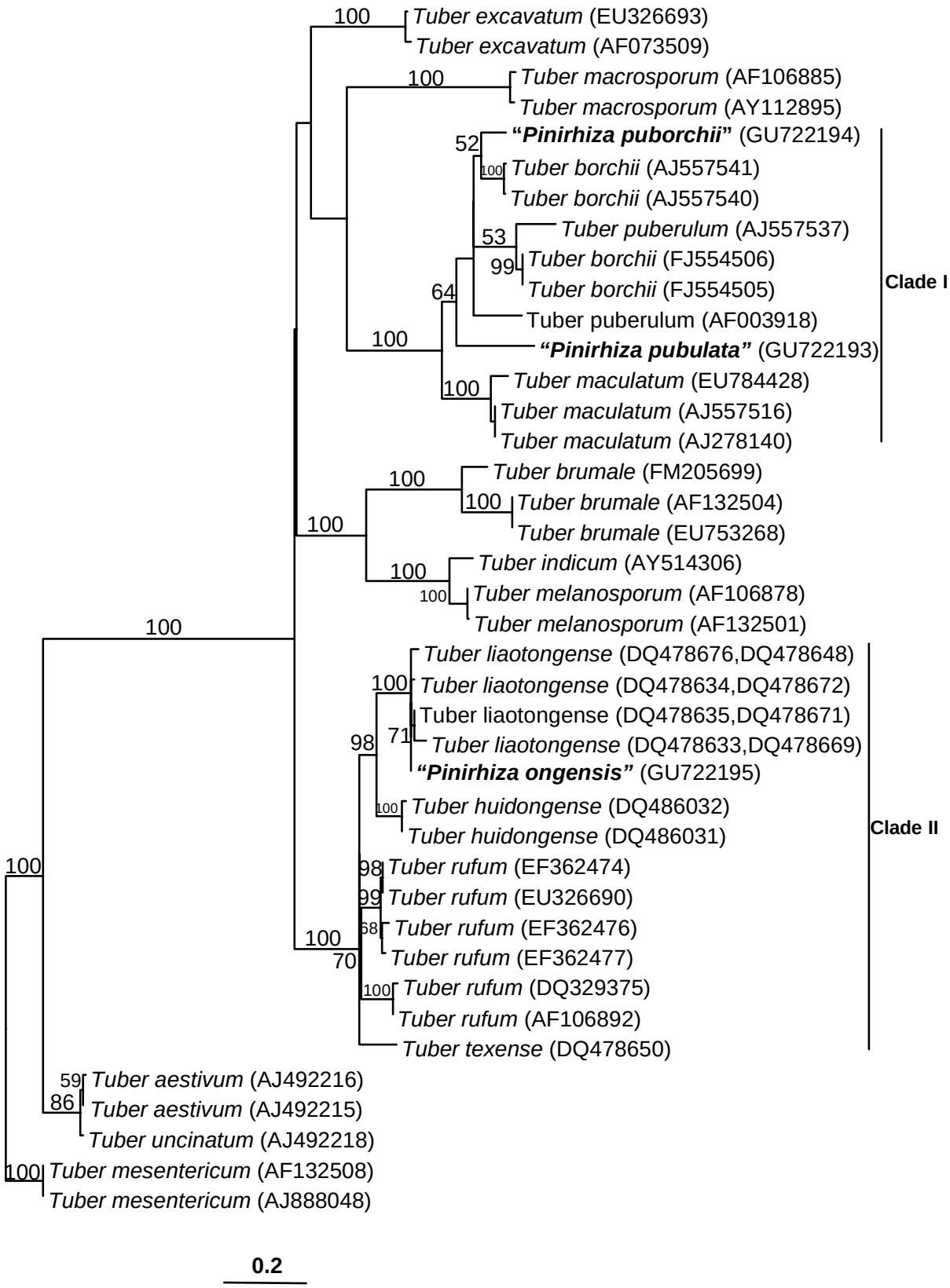


Figure 12 Molecular-phylogenetic placement of "*Pinirhiza pubulata*", "*Pinirhiza puborchii*", and "*Pinirhiza ongensis*" among different clades in genus *Tuber* by the RAxML analysis of partial ITS region. Bootstrap support values above 50% are noted above or left of the respective branches. GenBank accession numbers are given in parentheses following the species names.

Sequences of our ECM fall into two well supported clades in RAxML tree of ITS sequences (Figure 12). Clade I with 100% BS is divided into a *Tuber puberulum-borchii* and a *T. maculatum* subclade. The sequences of "*Pinirhiza pubulata*", placed separately between *T. puberulum-borchii* and *T. maculatum* subclades, is sistering to *T. puberulum-borchii* subclade with 64% BS. "*Pinirhiza puborchii*" clusters within *T. puberulum-borchii* subclade, and is close to two *Tuber borchii* sequences with 52% BS. Clade II with 100% BS consists of a *T. liaotongense* subclade, a *T. huidongense* subclade, a *T. rufum* subclade, and a *T. texense* subclade. The *T. huidongense* subclade is a sister clade to the *T. liaotongense* subclade with 98% BS, and the *T. rufum* subclade (including *T. texense*) is with very low support a sister clade to the combined subclades of *T. liaotongense* and *T. huidongense*. The sequence of "*Pinirhiza ongensis*" clusters in *T. liaotongense* subclade which is including four sequences resigned as *T. liaotongense* by Wang et al. (2007) with 100% BS, but "*Pinirhiza ongensis*" is basal to two of the *Tuber liaotongense* sequences that show 71% BS.

Discussion

Although "*Pinirhiza pubulata*", "*P. puborchii*", and "*P. ongensis*" showed pseudoparenchymatous outer mantle layers with a gelatinous matrix, they can be easily distinguished from each other. "*P. pubulata*" differs distinctively from "*P. puborchii*" and "*P. ongensis*" in having mostly angular cells in outer and middle mantle layers (althouth irregular-shaped cells are present), whereas "*P. puborchii*" and "*P. ongensis*" have mostly epidermoid cells in outer and middle mantle layers. "*P. pubulata*" and "*P. puborchii*" could be distinguished from "*P. ongensis*" in having typical awl-shaped cystidia which are thicker (4–5.5 μm) at the base in "*P. puborchii*" but are thin (2.5–3.5 μm) at the base in "*P. pubulata*", whereas cystidia are lacking in "*P. ongensis*". "*Pinirhiza ongensis*" differs from "*P. pubulata*" and "*P. puborchii*" in having a robust hyphal net which is absent in the latter two ECM.

"*Pinirhiza pubulata*", "*P. puborchii*", and "*P. ongensis*" are morpho-anatomically similar to *Tuber* ECM already studied in detail (Blaschke 1987, 1988,
Fischer et al. 2004, Giraud 1990, Müller et al. 1996a, b, Rauscher and Agerer 1995, Rauscher et al. 1996, Zambonelli et al. 1993, 1995, 1999), and they are all members of *Tuber* according to the molecular-phylogenetic analysis of LSU nrDNA (Figure 10).

"*Pinirhiza pubulata*" resembles *Tuber aestivum* (Müller et al. 1996a), *T. excavatum* (Giraud 1990), *T. mensentericum* (Rauscher and Agerer 1995), and *T. uncinatum* (Müller et al. 1996b) in having angular cells in outer mantle and in having awl-shaped cystidia. But "*Pinirhiza pubulata*" differs from *T. aestivum* and *T. uncinatum* in having shorter ((40) 75–110 µm) and straight cystidia than those of *T. aestivum* and *T. uncinatum* which are longer ((300) 450–800 (900) µm) and according to Müller et al. (1996a, b) curled. It differs from *T. mensentericum* in lacking basally branched cystidia and they are in "*P. pubulata*" in addition shorter in comparison to those of *T. mesentericum* with 450–1250 (1510) µm. Comparisons between "*P. pubulata*" and *T. excavatum* are impossible because detailed descriptions of the latter species are not available. The sequence of "*P. pubulata*" clusters in clade I between the *T. puberulum-borchii* and *T. maculatum* subclades. "*Pinirhiza pubulata*" can neither be assigned to the *T. puberulum-borchii* subclade nor to the *T. maculatum* subclade. An identification to species level is therefore impossible. The epitheton "pubulata" refers to the position between the two subclades.

"*Pinirhiza pubulata*" does not cluster within *T. excavatum*, *T. mensentericum,* and *T. uncinatum* in our phylogenetic analysis of ITS sequences (see Figure 12). This indicates that *Tuber* ECM with angular cells in the outer mantle layers are not monophyletic (see Figure 12).

"*Pinirhiza puborchii*" is similar to ECM of *Tuber borchii* (Rauscher et al 1996), *T. brumale* (Fischer et al. 2004), *T. melanosporum* (Rauscher & Agerer 1995), *T. macrosporum* (Zambonelli et al. 1993, 1995), *T. maculatum* (Zambonelli et al. 1999), *T. magnatum* (Zambonelli et al. 1993, 1995), *T. puberulum* (Blaschke 1987, 1988, Kovács and Jakucs 2006) in having epidermoid cells in outer mantle and awl-shaped cystidia. "*P. puborchii*" differs from ECM of *T. melanosporum* and *T. macrosporum* in having simple and short cystidia ((30) 70–90 (150)µm) rather than those in *T. melanosporum* and *T. macrosporum* that are ramified at the base and long (290–420 µm). "*P. puborchii*" is highly similar to *Tuber borchii* ECM, but differs from *Tuber borchii* ECM slightly in lacking a clear superficial hyphal net on the mantle surface. "*P. puborchii*" and *Tuber borchii* ECM differ from ECM of *T. brumale, T. maculatum, T. magnatum,* and *T. puberulum* in irregularly epidermoid cells of outer mantle which are connected by septa in "*P. puborchii*" and *T. borchii,* whereas the cells of outer mantle in *T. brumale, T. maculatum, T. magnatum,* and *T. puberulum* ECM are typically epidermoid. The sequence of "*Pinirhiza puborchii*" clusters in clade I within the *T. puberulum-borchii* subclade, but not with *T. brumale* and *T. maculatum,* and is related to

Tuber borchii with weak support according to the phylogenetic analysis of ITS region (see Figure 12). An identification to species level is not possible yet as *T. borchii* and *T. puberulum* sequences are intermixed. The epitheton "puborchii" refers to a possible affiliation of this ECM to either of the species.

"*Pinirhiza ongensis*" is similar to ECM of *Tuber rufum* (Rauscher and Agerer 1995) concerning lacking cystidia and epidermoid cells in outer mantle layers. However differences concern the hyphal net in both ECM, hyphae of hyphal net in "*P. ongensis*" are shorter (4–12 µm) and thinner (2.5–5 µm) than those ((10) 18–32 (40) µm long, (2) 4–8 (10) µm wide) in *T. rufum* ECM. Phylogenetic analysis of ITS region (Figure 12) indicates that "*Pinirhiza ongensis*" could be formed by *Tuber liaotongense* complex rather than of *Tuber rufum*, however *Tuber liaotongense* and *T. rufum* subclades cluster together with 100% BS in our study. This corresponds very well with the results that both, "*P. ongensis*" and *Tuber rufum* ECM (Rauscher and Agerer 1995), lack cystidia. In addition, *T. huidongense* and *T. texense* cluster also together, which is consistent with the conclusions of Wang et al. (2007) that *Tuber huidongense*, *T. liaotongense*, and *T. texense* belong to the *T. rufum* group. Therefore it could be expected that ECM of *Tuber huidongense* and *Tuber texense* also lack cystidia. However a possible *Tuber rufum* ECM in a recent description by Kovács and Jakucs (2006) showed infrequent cystidia. Many more species have to be investigated to test the value of cystidia characteristics of mycorrhizae of this group. As "*P. ongensis*" clusters closely with *T. liaotongense*, the ECM could probably be caused by this species, but the low sequence identity of 90-96% (megablast in GenBank), makes this conclusion improbable. Therefore the ECM received the special name "*P. ongensis*", considering the possibility that the ECM could be formed by a different species of the *T. liaotongense-T. huidongense* subclade.

Among 16 *Tuber* species proven to occur in China (Garcia-Montero et al. 2009), only ECM of *Tuber indicum* (Comandini and Pacioni 1997, Garcia-Montero et al. 2008), *Tuber himalayense* (Comandini and Pacioni 1997), synonymous to *T. indicum* according to Wang et al. (2006), and *T. pseudoexcavatum* (Garcia-Montero et al. 2008) have been studied morpho-anatomically. However, all features of these three *Tuber* spp. ECM have been obtained exclusively by synthesis experiments in artificial culture. Here we present a key to *Tuber* ECM known to occur at least as fruitbodies in China including the ECM of *Tuber* of this study.

1 Cystidia lacking

"*Pinirhiza ongensis*"

1* Cystidia present

2 Cystidia with infrequent to frequent right angle-like ramification

3 Outer mantle layer with polygonal to epidermoid cells *Tuber indicum*

3*Outer mantle with typical epidermoid cells *Tuber pseudoexcavatum*

2* Cystidia without right angle-like ramification

4 Outer mantle layer mainly with angular cells "*Pinirhiza pubulata*"

4*Outer mantle layer with mainly epidermoid cells "*Pinirhiza puborchii*"

Further detailed descriptions of *Tuber* ECM will contribute to solving taxonomic and phylogenetic problems within the genus *Tuber* in China. *Tuber* ECM found in north China and reported in our study provide useful information about distribution and ecology of *Tuber* species in China. It can be assumed that detailed morpho-anatomical ECM studies combined with molecular methods will quickly increase our knowledge about distribution and ecology of *Tuber* species in China.

Acknowledgements This study was financially supported by German Academic Exchange Service (DAAD). We thank also Fan Yongjun (Baotou Normal College, Baotou, Inner Mongolia, China) for his field assistance, Derek Peršoh (University of Munich) for his useful suggestions on phylogenetic analyses, the Managment Authority of Daqing Mountain National Reserve and Heilihe National Reserve for the allowance of field sampling.

References

Agerer R (1987–2008) *Colour Atlas of Ectomycorrhizae*. 1st – 14th delivery. Einhorn, Schwäbisch Gmünd

Agerer R (1991) Characterization of Ectomycorrhizae. In Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods in Microbiology, vol 23, pp. 25–73. Acad Press, London et al.

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycol Progress 5:67–107

Agerer R, Rambold G (2004–2009, First posted on 2004-06-01; most recent update, 2009-01-26) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de München, Germany

Blaschke H (1987) Vorkommen und Charakterisierung der Ektomykorrhizaassoziation *Tuber puberulum* mit *Picea abies*. Z Mykol 53:283–288

Blaschke H (1988) *Tuber puberulum*. In, Agerer R (ed) *Colour atlas of Ectomcorrhizae*, plate 22. Einhorn. Schwäbisch Gmünd

Comandini O, Pacioni G (1997) Mycorrhizae of asian black truffles, *Tuber himalayense* and *T. indicum*. Mycotaxon 63:77–86

De Roman M, Claveria V, de Miguel AM (2005) A revision of the descriptions of ectomycorrhizas published since 1961. Mycol Res 109:1063–1104

Fischer CR, Suz LM, Martin MP, Colinas C (2004) *Tuber brumale* Vitt. + *Quercus ilex* L. Descr Ectomyc 7/8:135–141

García-Montero LG, Díaz P, Di Massimo G, García-Abril A (2010) A review of research on Chinese *Tuber* species. Mycol Progress DOI 10.1007/s11557-009-0647-8

García-Montero LG, Di Massimo G, Manjón JL, García-Abril A (2008) New data on ectomycorrhizae and soils of the Chinese truffles Tuber pseudoexcavatum and Tuber indicum, and their impact on truffle cultivation. Mycorrhiza 19:7–14

Giraud M (1990) Mycorrhizes, prelevement et analyse. In , Verlhac A, Giraud M, Leteinturnier J (eds) La truffe guide pratique. Ctfl FPGV, Reims, pp 77–88

Granetti B (1995) Caratteristiche morfologiche biometriche e strutturali delle micorrize di *Tuber* di interesse economico. Micol Ital 24 (2):101–117

Hall T (2005) BioEdit, biological sequence alignment editor for Win95/98/NT/2K/XP. Carlsbad, California, Ibis therapeutic

Kovács GM, Jakucs E (2006) Morphological and molecular comparison of white truffle ectomycorrhizae. Mycorrhiza 16:567–574

Müller WR, Rauscher T, Agerer R, Chevalier G (1996a) *Tuber aestivum* Vitt. + *Corylus avellana* L. Descr Ectomyc 1:167–172

Müller WR, Rauscher T, Agerer R, Chevalier G (1996b) *Tuber uncinatum* Chat. + *Corylus avellana* L. Descr Ectomyc 1:179–183

Palenzona M, Chevalier G, Fontana A (1972) Sintesi micorrizica tra i miceli in coltura di *Tuber brumale, T. melanosporum, T. rufum* e semenzali di conifere e latifoglie. Allionia 18:41–52

Rauscher T, Agerer R (1995) Ektomykorrhizen von *Tuber melanosporum, Tuber mesentericum* und *Tuber rufum* (Tuberales) an *Corylus avellana*. Nova Hedwigia 61: 281–322

Rauscher T, Müller WR, Agerer R, Chevalier G (1996) *Tuber borchii* Vitt. + *Corylus avellana* L. Descr Ectomyc 1:173–178

Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity, separating the wheat from the chaff. Fungal Divers 33:1–45

Sikes DS, Lewis PO (2001) PAUPRat, A tool to implement Parsimony Ratchet searches using PAUP*. (http,//viceroy.eeb.uconn.edu/paupratweb/pauprat.htm)

Stamatakis A (2006) RAxML-VI-HPC, maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690

Stamatakis A, Hoover P, Rougement J (2008) A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 75:758–771

Swofford DL (2003) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts

Thiers B (2009, continuously updated) Index Herbariorum, A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. sciweb.nybg.org/science2/IndexHerbariorum.asp.

Wang YJ, Tan ZM, Zhang DC, Murat C, Jeandroz S, Tacon FL (2006) Phylogenetic and populational study of the *Tuber indicum* complex. Mycol Res 110:1034–1045

Wang YJ, Tan ZM, Murat C, Jeandroz S, Le Tacon F (2007) Molecular taxonomy of Chinese truffles belonging to the *Tuber rufum* and *Tuber puberulum* groups. Fungal Divers 24:301–328

Wei J, Peršoh D, Agerer R (2009) Four ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*), morpho-anatomical and molecular-phylogenetic analyses. *Mycol Progress* DOI10.1007/s11557-009-0637-x

Zambonelli A, Salomoni S, Pisi A (1993) Caratterizazione anatomo-morfologica delle micorrhize di *Tuber* spp. su *Quercus pubescens* Willd. Micol Ital 3:73–90

Zambonelli A, Salomoni S, Pisi A (1995) Caratterizazione anatomo-morfologica delle micorrhize de *Tuber borchii*, *Tuber aestivum*, *Tuber mesentericum*, *Tuber brumale*, *Tuber melanosporum* su *Pinus pinea*. Micol Ital 2:119–137

Zambonelli A, Iotti M, Amicucci A, Pisi A (1999) Caratterizzazione anatomo-morfologica delle micorrize di *Tuber maculatum* Vittad. Su *Ostrya carpinifolia* Scop. Micol Ital 28:29–35

Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214

Supplementary files

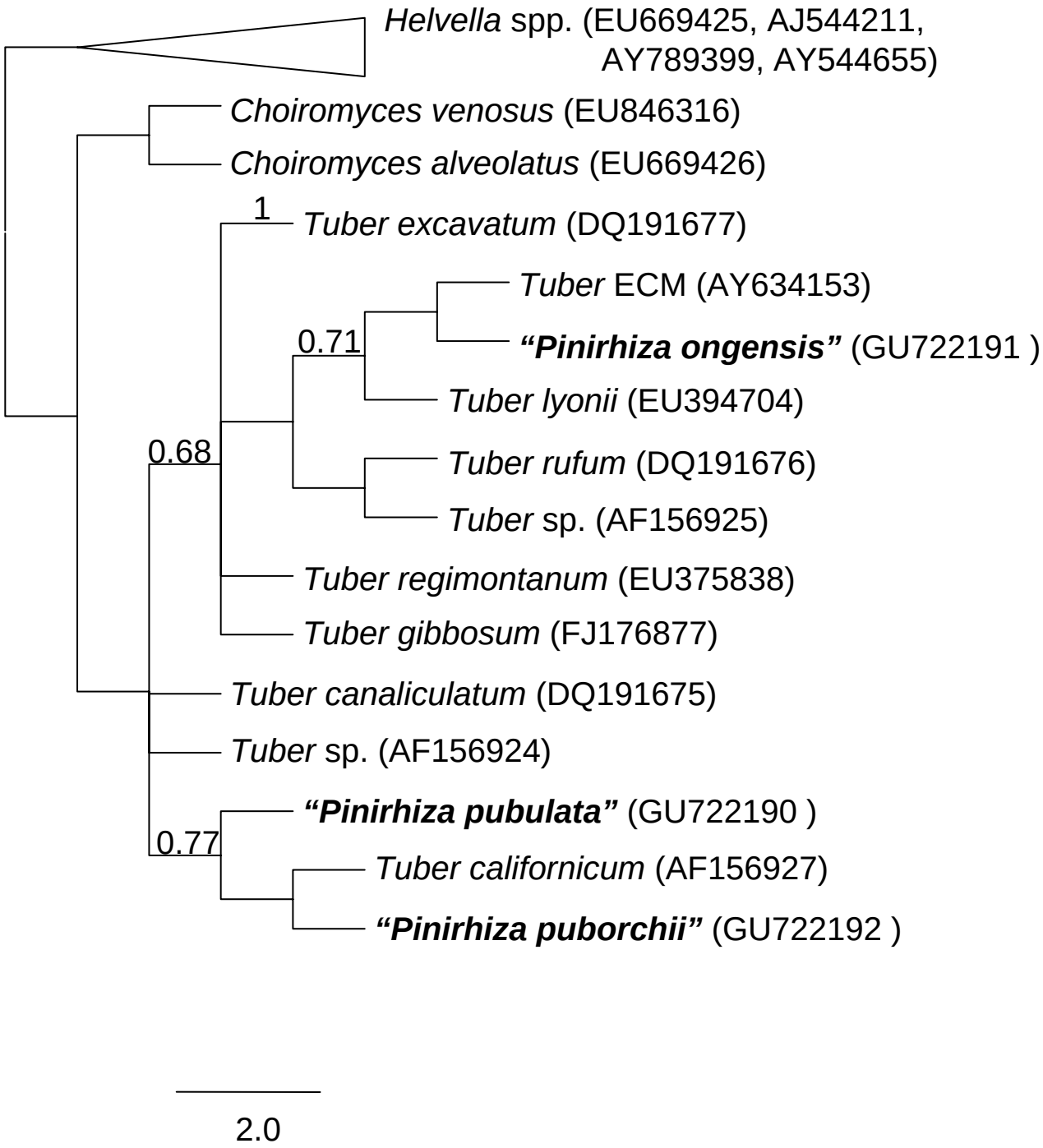


Figure 11 Molecular-phylogenetic placement of "*Pinirhiza pubulata*", "*Pinirhiza puborchii* ", and "*Pinirhiza ongensis*" among different clades in the genus *Tuber* by PAUPrat analysis of partial LSU region. Posterior Probability of nodes were 1.0 except where indicated otherwise. GenBank accession numbers are given in parentheses following the species names.

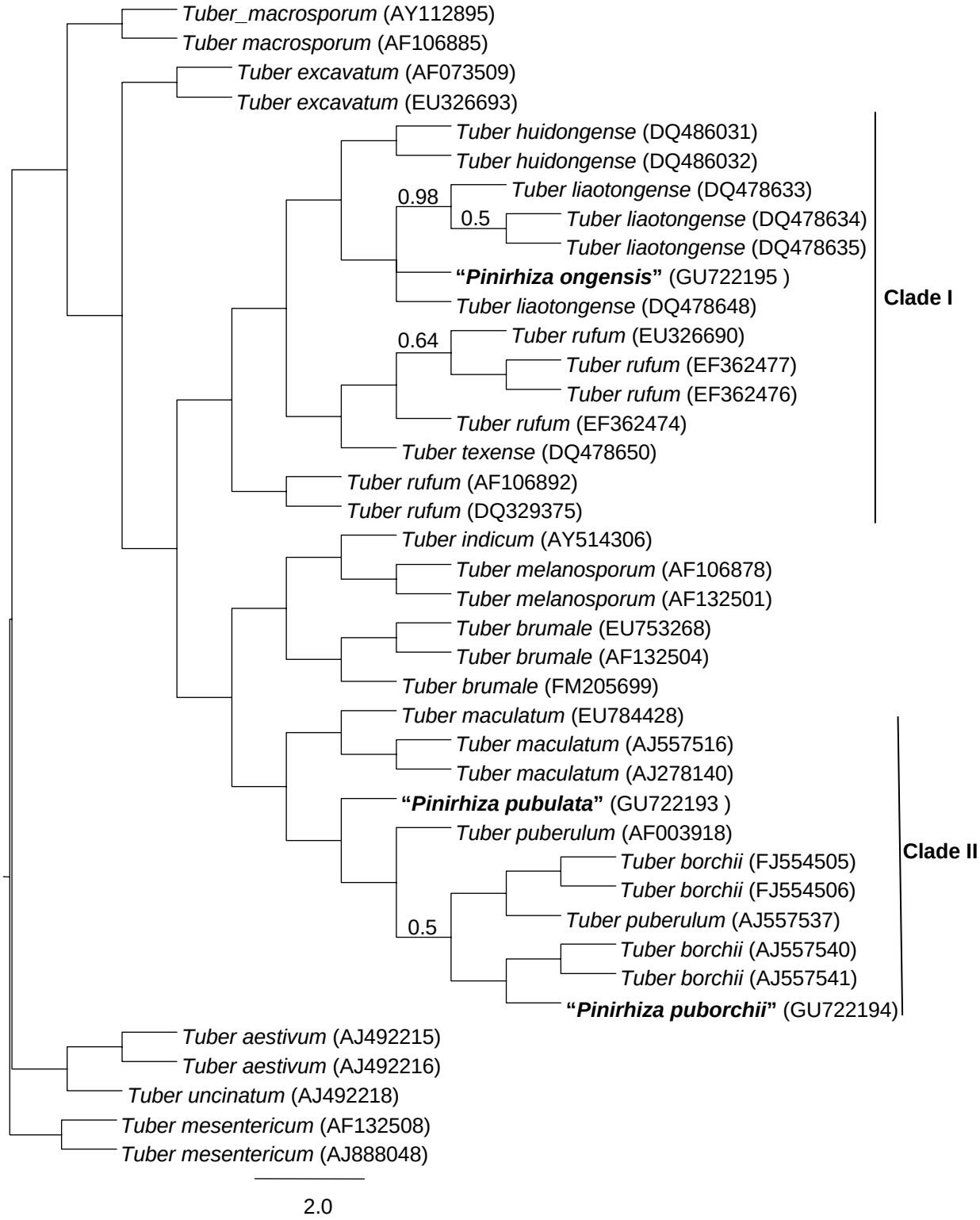


Figure 13 Molecular-phylogenetic placement of "*Pinirhiza pubulata*", "*Pinirhiza puborchii*", and "*Pinirhiza ongensis*" among different clades in the genus *Tuber* by PAUPRat analysis of partial ITS region. Posterior Probability of nodes were 1.0 except where indicated otherwise. GenBank accession numbers are given in parentheses following the species names.

3. General discussion

The seven publications in this thesis cover two main topics: Publications **I-VII** are about morphological and anatomical identification of ECM fungi on Chinese Pine, and publications **II**, **VI-VII** consider in addition molecular identification of the fungal partners of ECM formed on Chinese Pine.

3.1 Morpho-anatomical identification of ECM and molecular evidence

Although shared ECM features to distinguish fungal relationships are often limited, some special features or combinations of features are very helpful to affiliate ECM to relationships at different hierarchical levels (Agerer 2006). The ECM characterized in this doctoral thesis can be divided into two groups concerning the mantle type. Group I reveals plectenchymatous outer mantle layers, i.e. "*Pinirhiza acuminata*", "*P. inocyboides*", "*P. multifurcata*", "*P. nondextrinoidea*", and "*P. tricholomoides*". Group II possesses pseudoparenchymatous outer mantle layers, i.e. "*Pinirhiza daqingensis*", "*P. fibulocystidiata*", "*P. geoporoides*", "*P. heilihensis*", "*P. humarioides*", "*P. ongensis*", "*P. puborchii*", "*P. pubulata*", "*P. tomentelloides*" and "*P. trichophaeoides*".

The ECM forming fungus of "*P. inocyboides*" (paper **I**) belongs according to the combination of the following features to *Inocybe*. According to Agerer (2006) the hitherto described ECM of the genus *Inocybe* lack rhizomorphs, their emanating hyphae are furnished by many secondary septa and prominent clamps with a hole, but clamps do not occur in outer mantle. A molecular phylogenetic support was not possible due to the failure of amplifying its DNA product (reasons are discussed below).

"*Pinirhiza multifurcata*" and "*P. nondextrinoidea*" (paper **VI**) are similar to three sebacinoid ECM that have been described in detail: *Sebacina incrustans* (Pers.) Tul. and C. Tul. on *Picea abies*, *Sebacinoid* sp. on *Tilia* sp. (Urban et al. 2003), and "*Quercirhiza dendrohyphidiomorpha"* on *Quercus suber* (Azul et al. 2006a), in having clampless emanating hyphae or cystidia which are ramified bi- or trifurcately and somewhat inflated at the points of ramification, and in showing in addition mutiply branched hyphae at least in outer mantle, however they can be separated according to the differences of mantle layers as

well as the presence of rhizomorphs (comparative discussion see paper **VI**). "*P. multifurcata*" and "*P. nondextrinoidea*" are members of Sebacinales according to the phylogenetic analyses of nLSU, but an identification to species level was not possible, because the only two identified ITS sequences obtained from GenBank have low identity with the ITS sequences of the two ECM.

"*Pinirhiza acuminata*" resembles some brown *Tomentella* ECM, "*Afzeliaerhiza beninensis*" (Yorou and Agerer 2008), *Pseudotomentella humicola* (Di Marino et al. 2007), "*Quercirhiza tomentellofuniculosa*" (Azul et al. 2006b), "*Quercirhiza tomentelloreticulata*" (Azul et al. 2008), *Tomentella brunneorufa* (Agerer and Bougher 2001), *Tomentella ferruginea* (Raidl and Müller 1996), and "*Uapacaerhiza wariensis*" (Yorou et al. 2008), in having a plectenchymatous outer mantle and in the lack of awl-shaped cystidia, however they could be distinguished from each other according to the key in paper **V**. Blast searches using ITS sequence in GenBank and UNITE indicate that "*P. acuminata*" could be a member of *Tomentella*, but an identification to species level was not possible.

"*Pinirhiza tricholomoides*" (paper **III**) is hydrophobic, has plectenchymatous mantle layers throughout, clampless emanating hyphae and slightly differentiated and no ramarioid or boletoid rhizomorphs, but lack cystidia and chlamydospores. The results of the examination of the morphological and antomical features and the comparison of DNA data suggest that "*P. tricholomoides*" belongs to the genus *Tricholoma*, but an identification to species was not possible. "*P. tricholomoides*" differs from all ECM of *Tricholoma* species which have been studied morpho-anatomically in detail (see the key in paper **III**).

"*Pinirhiza tomentelloides*" (paper **II**), "*P. humarioides*", "*P. geoporoides*", "*P. daqingensis*", "*P. trichophaeoides*" (paper **IV**), and "*P. pubulata*" (paper **VII**) could be members of genera *Tomentella* in Basidiomycota or in Ascomycota like *Humaria*, *Genea*, *Geopora*, *Trichophaea*, and *Tuber* in being brownish to brown and hydrophilic, and in having pseudoparenchymatous outer mantles with angular cells and clampless emanating hyphae.

"*Pinirhiza tomentelloides*" is a *Tomentella* ECM according to the combination of the following features: brown color, outer mantle layer with angular cells, cells of mantle in star-like pattern, groups of globular cells on mantle surface. This feature complex fits the criteria to indicate *Tomentella* ECM (Jakucs and Erős-Honti 2008). "*P. tomentelloides*" differs from

all described *Tomentella* ECM in having hypha-like cystidia which ramified near the end and are clampless (for comparison see the key in paper **V)**, molecular identification is not achieved, as the responsible sequence has not been obtained.

"*Pinirhiza humarioides*" resembles *Humaria* and *Genea* ECM (Brand 1991, Erős-Honti et al. 2008, Jakucs et al. 1998, Tedersoo et al. 2006), because its special features of irregularly shaped cells with very thick cell walls in the outermost mantle layer occur only in ECM of these two genera till now. However, "*P. humarioides*" has a clearly plectenchymatous inner mantle layer, whereas all other mentioned ECM have a pseudoparenchymatous inner mantle layer with epidermoid cells, or a transitional type between plectenchymatous and pseudoparenchymatous. But an unambiguous identification to either genus is impossible because *Genea* and *Humaria* ECM are very similar in morpho-anatomical features (Erős-Honti et al. 2008). Phylogenetic analyses of nLSU indicate that the fungal partner of "*P. humarioides*" is a member of *Humaria*.

"*Pinirhiza trichophaeoides*" is similar to "*Quercirhiza quadratum*" on *Quercus ilex* L. subsp. *ballota* (Desf.) Samp (Águeda et al. 2008) in having heaps of oval to polygonal cells on a pseudoparenchymatous outer mantle layer composed of angular cells. Emanating hyphae of both ECM are frequent, partially warty, and show abundant rectangular ramifications. However, "*P. trichophaeoides*" differs from "*Q. quadratum*" with regard to the cell shape of the inner mantle layer. In "*P. trichophaeoides*", it has epidermoid cells whereas those of "*Q. quadratum*" are roundish to polygonal. Phylogenetic analyses indicate that "*P. trichophaeoides*" is a member of *Trichophaea*. However identification to species level was not possible because of rare identified sequences obtained from GenBank.

"*Pinirhiza geoporoides*" and "*P. daqingensis*" cluster within the well supported *Geopora-Tricharina* clade in phylogenetic analyses (paper **IV**). Their exact position, however, remains unclear due to the generally low supported internal branches in likehood tree and a diverging topology in the parsimony tree. Like the two "*Pinirhiza* spp.", the two morpho-anatomically described ECM of this clade, *Pezizales* spp. (AJ893248 and AM086625), have a pseudoparenchymatous outer mantle, the cells of which are row-like arranged (Tedersoo et al. 2006). However, it could not be ascertained based on the available data and descriptions, whether the species of the *Geopora-Tricharina* clade show a similar anatomy with regard to the outer mantle.

"*Pinirhiza pubulata*" has angular cells bearing awl-shaped cystidia which are typical features for *Tuber* ECM. It resembles *Tuber aestivum* (Müller et al. 1996a), *T. excavatum* (Giraud 1990), *T. mensentericum* (Rauscher and Agerer 1995), and *T. uncinatum* (Müller et al. 1996b), whereas "*P. pubulata*" differs from them in deviating length of cystidia, and in the absence of basal branches of cystidia (for detail see paper **VII**). The sequence of "*P. pubulata*" clusters in *Tuber* clade in phylogenetic analyses of nLSU, and clusters in between the *T. puberulum-borchii* and *T. maculatum* subclades in phylogenetic analyses of ITS sequence. "*P. pubulata*" can neither be assigned to the *T. puberulum-borchii* subclade nor to the *T. maculatum* subclade. An identification to species level is therefore impossible.

Except brown colour and angular cells in outer mantle, "*P. fibulocystidiata*" and "*P. heilihensis*" have clamped emanating elements (cystidia or emanating hyphae), which places "*P. fibulocystidiata*" and "*P. heilihensis*" in *Tomentella* according to Jakucs and Erős-Honti (2008). They are two new anatomotypes according to the comprehensive comparison in paper **V**. ITS sequence analyses indicate they could be two *Tomentella* species.

Two ECM, "*P. puborchii*" and "*P. ongensis*", show pseudoparenchymatous outer mantles with epidermoid cells and clampless emanating hyphae. "*P. puborchii*" is similar to ECM of *Tuber borchii* (Rauscher et al 1996), *T. brumale* (Fischer et al. 2004), *T. melanosporum* (Rauscher & Agerer 1995), *T. macrosporum* (Zambonelli et al. 1993, 1995), *T. maculatum* (Zambonelli et al. 1999), *T. magnatum* (Zambonelli et al. 1993, 1995), *T. puberulum* (Blaschke 1987, 1988, Kovács and Jakucs 2006) in having epidermoid cells in outer mantle and awl-shaped cystidia. "*P. puborchii*" differs from them in deviating length of cystidia, the absence of basal ramification of cystidia, the presence of a hyphal net on the mantle surface, as well as in the shape of mantle cells (see the comparative discussion in paper **VII**). Phylogenetic analyses of nLSU sequence reveal that "*P. puborchii*" is a *Tuber* ECM. The sequence of "*P. puborchii*" clusters within the *T. puberulum-borchii* subclade in phylogenetic analyses of ITS region, and is related to *Tuber borchii* with weak support (paper **VII)**. An identification to species level is not possible yet as *T. borchii* and *T. puberulum* sequences are intermixed.

"*Pinirhiza ongensis*" is similar to ECM of *Tuber rufum* (Rauscher and Agerer 1995) concerning lacking cystidia and having epidermoid cells in outer mantle layers. However

differences concern the hyphal net in both ECM. The phylogenetic analysis of the ITS region indicates that "*P. ongensis*" could be formed by species of the *Tuber liaotongense* complex rather than of *Tuber rufum*, however *Tuber liaotongense* and *T. rufum* subclades cluster together with 100% BS in our study. As "*P. ongensis*" clusters closely with *T. liaotongense*, the ECM could probably be caused by this species, but the low sequence identity of 90-96% (megablast in GenBank), makes this conclusion improbable.

Key to all ECM described in this thesis

1 Outer mantle in plan view plectenchymatous
2 Emanating hyphae with clamps
3 Some hyphae of mantle surface with horn-shaped and acuminate outgrowths "*Pinirhiza acuminata*"
3*Hyphae of mantle surface without horn-shaped and acuminate outgrowths "*Pinirhiza inocyboides*"
2* Emanating hyphae clampless
4 Multiply branched hyphae lacking in all mantle layers, emanating hyphae not inflated at the points of ramification "*Pinirhiza tricholomoides*"
4*Multiply branched hyphae at least in outer mantle layers, emanating hyphae somewhat inflated at the points of ramification
5 Rhizomorph present, emanting hyphae not dextrinoid "*Pinirhiza nondextrinoidea*"
5* Rhizomorph lacking, emanating hyphae dextrinoid "*Pinirhiza multifurcata*"

1* Outer mantle in plan view pseudoparenchymatous
6 Outer mantle layer with angular cells
7 Cystidia present
8 Cystidia with an intercalar clamp, capitate with an abrupt inflation "*Pinirhiza fibulocystidiata*"
8* Cystidia without clamps, different shape
9 Cystidia hypha-like with ramified ends "*Pinirhiza tomentelloides*"
9* Cystidia not hypha-like, with simple ends
10 Cystidia bottle shaped "*Pinirhiza daqingensis*"

10*Cystidia awl-shaped

11 Emanating hyphae with clamps, cells of outer mantle star-like arranged "*Pinirhiza heilihensis*"

11*Emanating hyphae without clamps, cells of outer mantle with no discernible special arrangement "*Pinirhiza pubulata*"

7* Cystidia lacking

12 Mantle with a very thin, at places incomplete, pseudoparenchymatous surface layer composed of inflated, often irregularly shaped cells arranged in rows "*Pinirhiza humarioides*"

12*Mantle such a surface layer lacking, but instead with heaps of oval to polygonal cells

13 Hyphal cells in outer mantle layer or in middle mantle layer arranged in distinct rows; inner mantle layer plectenchymatous with ring-like arranged hyphae "*Pinirhiza geoporoides*"

13* Hyphal cells in outer mantle layer or in middle mantle layer not arranged in distinct rows; inner mantle layer pseudoparenchymatous with epidermoid cells "*Pinirhiza trichophaeoides*"

6* Outer mantle layer with epidermoid cells

14 Cystidia present, mantle surface with an indistinct hyphal net "*Pinirhiza puborchii*"

14* Cystidia lacking, mantle surface with a distinct hyphal net "*Pinirhiza ongensis*"

Because morpho-anatomical descriptions of ECM on Chinese Pine are limited, and no other anatomotypes on Chinese Pine have been described up to now in detail, all 15 ECM in this study are designated as new ones. They are named binomially the first time, as none of these *Pinus tabulaeformis* ECM are known on any other pine species (Agerer & Rambold 2004–2009). The key to these 15 anatomotypes could provide basic knowledge for determination of ECM anatomotypes on Chinese Pine and be useful for other kind of studies on Chinese Pine, e.g. for investigating ECM fungal diversity and richness, species composition, for selecting suitable inoculums for reforestation, for estimating the dynamics of ECM fungi composition in a long term or under climate changes.

3.2 Molecular identification of ECM fungi

The main problem during these studies was the question of identification to species-level of the ECM forming fungus, because of the insufficient identified sequences deposited in official sequence data bases, many sequences especially for ITS sequences are not identified to species or have been named as uncultured environment samples (see results of papers **II**, **V**, **VI**). A comparison to fruitbody DNA from the sample sites was impossible due to a very limited availability of fruitbody diversity of ECM fungi during the sample period for the ECM. This problem could be caused by insufficient comparable sequences from responsible fruitbodies at the collection areas, because three of the sampling sites (Helan Mountain, Wula Mountain, and Daqing Mountain) are arid regions with limited rainfall that hinders the occurrence of fruitbodies. But Heilihe National Reserve is in semi-humid area. Here, the most frequent fruitbodies collected were *Suillus bovinus*, others have been infrequently found but comparing to similar vegetation types, most of the species to expected were absent (Mou 2000). Some fungi produce hypogeous fruitbodies like *Genea*, *Geopora*, *Trichophaea* (paper **IV**) and *Tuber* (paper **VII**) and have to be searched for applying sample forks and scratching intensely the upper soil layer (Læssøe and Hansen, 2007). This was avoided to leave the study area undisturbed. Fruitbodies of some other fungi form only a rather thin and inconspicuous layers on the underside of dead twigs or stems like *Tomentella* (papers **III**, **V**) are easily overlooked (Kõljalg 1996). And more importantly, the fungal diversity in Inner Mongolia has been only scarcely studied, making fruitbody identification difficult, and this resulted in a nearly complete lack of such sequences in public databases.

ECM fungi have been successfully identified to genus level using ITS sequence comparisons for some very common genera like *Tricholoma* (paper **II**), *Tomentella* (paper **V**), and *Tuber* (paper **VII**), because the most obtained sequences from GenBank through magablast search fall with high sequence coverage and maximal identity with sequences of our ECM into these genera. However, for some fungal genera, an unambiguous identification even to genus level using ITS sequences was not possible, because sequences similar to those of the compared ECM sequences fall into different genera (papers **IV**, **VI**), blast search using nLSU sequences faces the similar problem (papers **IV**, **VI**, **VII**), in this case a phylogenetic analysis is necessary.

Partial nLSU regions are conserved, also easier to align unambiguously, and provide higher-level taxonomic information (Tedersoo 2007) (comp. papers **V**, **VI**, and **VII**). However, ITS sequence has high variability between different species in the same genus and is difficult to align unambiguously because of the unequal rate of evolution in the ITS (Nilsson et al. 2008) , so only 5.8s and ITS2 regions have been used to for phylogenetic analyses (paper **VII**).

14 of 15 of our anatomotypes have been successfully sequenced, and anatomotypes fit well with sequencetype (see appendix 4) in our study except one (paper **I**). The sequence of "*Pinirhiza inocyboides*" is not congruent with its anatomotype inasmuch it falls according to blast search in GenBank into a clade with *Thelephora* spp.. But the structure of "*P. inocyboides*" represents all features that are well known for ECM of the genus *Inocybe* (Agerer 2006). It seems that the studied ECM was covered by or showed an intermixed growth with hyphae of another fungus which was more easily amplified than the ECM forming fungus. As more and more ECM investigations apply exclusively molecular methods and no microscopical studies, much caution should be observed and considered that some of the results of the databases or of molecular identification of not thoroughly studied ECM could be misleading. Only the combination of detailed morpho-anatomical studies of ECM with molecular identification by comparison of sequence data is sufficient for further conclusions.

Future studies will have to pay more attention to the following points: (1) more fruitbodies of ECM-forming fungi under Chinese pine have to be collected in order to make an unambiguous identification of ECM possible through sequence comparison; (2) more researches on species richness, diversity, and composition of ECM-communities should be made, which are important for selecting desirable inocula to promote survival and growth of Chinese pine in the field.

4. References

Agerer R (1987–2008) Colour Atlas of Ectomycorrhizae. 1st – 14th delivery. Einhorn, Schwäbisch Gmünd

Agerer R (1991) Characterization of Ectomycorrhizae. In Norris JR, Read DJ, Varma AK (eds.) Techniques for the study of mycorrhiza. Methods in Microbiology, vol 23, pp 25–73. Acad Press, London et al.

Agerer R (1995) Anatomical characteristics of identified ectomycorrhizas: an attempt towards a natural classification. In: Varma K, Hock B (des) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin Heidelberg New York, pp 685–734

Agerer R (1999) Never change a functionally successful principle: the evolution of Boletales s.1. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendtnera 6: 5–91.

Agerer R (2006) Fungal relationships and structural identity of their ectomycorrhizae. Mycological Progress 5: 67–107.

Agerer R (2009) Bedeutung der Ektomykorrhiza für Waldökosysteme. Rundgespräche der Kommission für Ökologie, Bd. 37, Ökologische Rolle von Pilzen, S. 111–121.

Agerer R, Bougher NL (2001) *Tomentella brunneorufa* + *Eucalyptus* sp. Descriptions of Ectomycorrhizae 5: 205–212.

Agerer R, Iosifidou P (2004) Rhizomorph structure of Hymenomycetes: a possibility to test DNA-based phylogenetic hypotheses? In: Agerer R, Piepenbring M, Blanz P (eds) Frontiers in basidiomycota mycology. IHW-Verlag, Eching, pp 249–302.

Agerer R, Kraigher H, Javornik B (1996) Identification of ectomycorrhizae of *Hydnum rufescens* on Norway spruce and the variability of the ITS region of *H. rufescens* and *H. repandum* (Basidiomycetes). Nova Hedwigia 63: 183–194.

Agerer R, Rambold G (2004–2009, First posted on 2004-06-01; most recent update: 2009-01-26) DEEMY – An Information System for Characterization and Determination of Ectomycorrhizae. www.deemy.de - München, Germany

Águeda B, Agerer R, De Miguel AM, Parladé J (2008) "*Quercirhiza quadratum*" + *Quercus ilex* L. subsp.*ballota* (Scop.) Desf. Samp. Descriptions of Ectomycorrhizae11/12: 113–123.

Azul AM, Agerer R, Freitas H (2006a) "*Quercirhiza dendrohyphidiomorpha*" + *Quercus suber* L. Descriptions of Ectomycorrhizae 9/10: 87–91.

Azul AM, Agerer R, Martin MP, Freitas H (2006b) "*Quercirhiza tomentellofuniculosa*"+ *Quercus suber* L. Descriptions of Ectomycorrhizae9/10: 127–134.

Azul AM, Agerer R, Martin MP, Freitas H (2008) "*Quercirhiza tomentelloreticulata*" + *Quercus suber* L. Descriptions of Ectomycorrhizae11/12: 135–139.

Bai SL (2006a) Study on Distribution and Selection of Ectomycorrhizal Fungi in Daqingshan Mountain, Inner Mongolia. Dissertation.

Bai SL, Liu Y, Zhou J, Dong Z, Fan R (2006b) Resources investigation and ecological study on ectomycorrhizal fungi in Daqingshan Mountains,Inner Mongolia. Acta Ecologica Sinica (3): 838–841.

Bai SL, Yan W, Ma RH, Wang TN (2001) Investigation of ECM Fungi Resources in Mt. Daqing and Mt. Manhan. Journal of Mountain Research 19 (1): 44–47.

Beenken L (2001a) *Russula vesca* Fr. + *Quercus robur* L. Descriptions of Ectomycorrhizae 5: 187–192.

Beenken L (2001b) *Russula vinosa* Fr. + *Picea abies* (L.) H. Karst. Descriptions of Ectomycorrhizae 5: 193–198.

Beenken L (2001c) *Russula virescens* (Schaeff.) Fr. + *Quercus robur* L. Descriptions of Ectomycorrhizae 5: 199–203.

Beenken L (2004a) Die Gattung Russula. Untersuchungen zu ihrer Systematik anhand von Ektomykorrhizen. Dissertation, University of München.

Beenken L (2004b) Les ectomycorhizes du genre *Russula*. Bulletin de la Société mycologique de France 120: 293–333.

Binder M, Hibbett DS (2006) Molecular systematics and biological diversification of Boletales. Mycologia 98: 971–981.

Blackwell M, Hibbett D, Taylor JW, Spatafora JW (2006) Research Coordination Networks: a phylogeny for kingdom Fungi (Deep Hypha). Mycologia 98 (6): 829–837.

Blaschke H (1987) Vorkommen und Charakterisierung der Ektomykorrhizaassoziation *Tuber puberulum* mit *Picea abies*. Zeitschrift für Mykologie 53: 283–288.

Blaschke H (1988) *Tuber puberulum*. In, Agerer R (ed) Colour atlas of Ectomcorrhizae, plate 22. Einhorn. Schwäbisch Gmünd.

Brand F (1991) *Genea hispidula*. In: Agerer R (ed.) Colour Altas of Ectomycorrhizae, plate 57. Einhorn, Schwäbisch Gmünd

Brownlee C, Duddridge JA, Malbari A, Read DJ (1983) The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming

interplant connections and providing pathways for assimilate and water transport. Plant Soil 71: 433–443.

Brundrett MC (2009) Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. Plant Soil 320: 37–77.

Chen KM, Richard JA, Richard IM, Tian XM, Liu JQ (2008) Phylogeography of *Pinus tabulaeformis* Carr. (Pinaceae), a dominant species of coniferous forest in northern China. Molecular Ecology 17: 4276–4288.

Chilvers GA (1968) Some distinctive types of eucalypt mycorrhiza. Australian Journal of Botany 16: 49–70.

De Roman M, Claveria V, De Miguel AM (2005). A revision of the descriptions of ectomycorrhizas published since 1961. Mycological Research 109 (10): 1063–1104.

Di Marino E, Kõljalg U, Agerer R (2007) The ectomycorrhizae of *Pseudotomentella humicola* on *Picea abies*. Nova Hedwigia 84 (3): 429–440.

Eberhardt U, Oberwinkler F, Verbeken A, Rinaldi AC, Pacioni G, Comandini O (2000) *Lactarius* ectomycorrhizae on *Abies alba*: morphological description, molecular characterization, and taxonomic remarks. Micologia 92: 860–873.

Egger KN (1995) Molecular analysis of ectomycorrhizal fungal communities. Canadian Journal of Botany 73:1415–1422.

Erős-Honti Z, Kovacs GM, Szedlay G, Jakucs E (2008) Morphological and molecular characterization of *Humaria* and *Genea* ectomycorrhizae from Hungarian deciduous forests. Mycorrhiza 18: 133–143.

Fischer C, Suz LM, Martin MP (2004) *Tuber brumale* Vitt. + *Quercus ilex* L. Descriptions of Ectomycorrhizae 7/8: 135–141.

Frank AB (1885) Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Berichte der Deutschen botanischen Gesellschaft 3: 128–145.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - applications to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Gardes M, White TJ, Fortin J, Bruns TD, Taylor JW (1991) Identification of indigenous and introduced symbilotic fungi in ectomycorrhizae by amplification of nuclear and mitochondrial ribosomal DNA. Canadian Journal of Botany, 69: 180–190.

Gibelli G (1883) Nuovi studii sulla malattia del Castagno detta dell' inchiostro. Mem R Accad Sci Ist Bologna 4: 287–314.

Giraud M (1979) Etude comparative des mycorhizes d'arbres producteurs de truffe ou non en zone truffiere. Mem de fin d'etudes, Diplôme d' Agaronomie Approfondie, E.N.S.A. Rennes, INRA, Clermont-Fd., 56 pp

Godbout C, Fortin JA (1985) Synthesized ectomycorrhizae of aspen : fungal genus level of structural characterization. Canadian Journal of Botany 63: 252–262.

Giraud M (1990) Mycorrhizes, prelevement et analyse. In , Verlhac A, Giraud M, Leteinturnier J (eds) La truffe guide pratique. Ctfl FPGV, Reims, pp 77–88.

Glen M, Tommerup IC, Bougher NL, O'Brien P (2001) Specificity, sensitivity and discrimination of primers for PCR-RFLP of larger basidiomycetes and their applicability to identification of ectomycorrhizal fungi in *Eucalyptus* forests and plantations. Mycological research 105: 138–149

Gronbach E, Agerer R (1986) Charakterisierung und Inventur der Fichten-Mykorrhizen im Höglwald und deren Reaktion auf saure Beregnung. Forstwissenschaftliches Centralblatt 105: 329–335.

Hahn C, Agerer R, Wanner G (2000) Anatomische und ultrastrukturelle Analyse von *Ramaricium alboochraceum*, einer seltenen Art der Gomphales and seine verwandtschaftliche Beziehung zu *Geastrum* und *Gautieria*. Hoppea 61:115–125

Hansen K, Lobuglio KF, Pfister DH (2005) Evolutionary relationships of the cup-fungus genus *Peziza* and *Pezizaceae* inferred from multiple nuclear genes: RPB2, ß-tubulin and LSU rDNA. Molecular Phylogenetics and Evolution 36: 1–23.

Hansen K (2006) Systematics of the Pezizomycetes-the operculate discomycetes. Mycologia 98: 1029–1040

Hibbett DS, Matheny PB (2009) The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. BMC Biology 7: 13

Hibbett DS, Pine EM, Langer E, Langer G, Donoghue MJ (1997). Evolution of gilled mushrooms and puffballs inferred from ribosomal DANN sequences. Proceedings of the National Academy of Sciences of the USA, 94: 12002–12006.

Hosaka K, Bates ST, Beever RE, Castellano MA, Colgan III W, Dominguez LS, Nouhra ER, Geml J, Giachini AJ, Kenney SR, Simpson NB, Spatafora JW, Trappe JM (2006) Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders. Mycologia 98 (6): 949–959.

Huang NL (1998) Colored illustrations of macrofungi (mushrooms) of China. Chinese Agricultural Press.

Huang YC (1990) Studies on classification and physiology of the ectomycorrhizae of some *Pinus* species in China. Dissertation.

Jakucs E, Agerer R, Bratek Z (1998) *Genea verrucosa* Vitt. + *Quercus spec*. Descriptions of Ectomycorrhizae 3: 19–23.

Jakucs E, Erős-honti Z (2008) Morphological-antomical characterization and identification of *Tomentella* ectomycorrhizas. Mycorrhiza 18: 277–285.

Jakucs E, Kovács GM, Agerer R, Romsics C, Erős-honti Z (2005) Morphological– anatomical characterization and molecular identification of *Tomentella stuposa* ectomycorrhizae and related anatomotypes. Mycorrhiza 15: 247–258.

James TY, Kauff F, Schoch CL et al. (2006) Reconstructing the early evolution of fungi using a six-gene phylogeny. Nature 443/19: 818–822.

Kennedy PG, Izzo AD, Bruns TD (2003) There is high potential for the formation of common mycorrhizal networks between understroey and canopy trees in a mixed evergreen forest. Journal of Ecology 91: 1071–1080.

Kõljalg U (1996) Tomentella (Basidiomycota) and related genera in Temperate Eurasia. Synopsis Fungorum. Oslo, Fungiflora.

Kõljalg U, Jakucs E, Boka K, Agerer R (2001) Three ectomycorrhiza with cystidia formed by different *Tomentella* species as revealved by rDNA ITS sequences and amatomical characteristics. Folia Cryptogamic Estonia Facs. 38: 27–39.

Kõljalg U, Larsson KH, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Hoiland K, Kjoller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vralstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166: 1063–1068.

Kovács GM, Jakucs E (2006) Morphological and molecular comparison of white truffle ectomycorrhizae. Mycorrhiza 16: 567–574.

Kraigher H, Agerer R, Javornik B (1995) Ectomycorrhizae of *Lactarius lignyotus* on Norway spruce, characterized by anatomical and molecular tools. Mycorrhiza 5: 175–180.

Læssøe T, Hansen K (2007) Truffle trouble: what happened to the *Tuberales*? Mycological Research 111: 1075–1099.

Larsson KH, Larsson E, Kõljalg U (2004) High phylogenetic diversity among corticioid homobasi-diomycetes. Mycological Research 108: 983–1002.

Larsson KH, Parmasto E, Fischer M, Langer E, Nakasone KK, Redhead SA (2006) Hymenochaetales: a molecular phylogeny for the hymenochaetoid clade. Mycologia 98 (6): 926–936.

Martin KJ, Rygiewicz PT (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. BMC Microbiology 5: 28

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, Denitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS (2006) Major clades of Agaricales: a multi-locus phylogenetic overview. Mycologia 98: 984–997.

Mleczko P (2004) *Rhodocollybia butyracea* (Bull.:Fr) Lennox + *Pinus sylvestris* L. Descriptions of Ectomycorrhizae 7/8: 101–108.

Moncalvo JM, Nilsson RH, Koster B, Dunham SM, Bernauer T, Matheny PB, Porter TM, Margaritescu S, Weiß M, Garnica S, Danell E, Langer G, Langer E, Larsson E, Larsson KH (2006) The cantharelloid clade: dealing with incongruent gene trees and phylogenetic reconstruction methods. Mycologia 98 (6):937–948.

Mou XL (2000) The Macrofungi in China. Science and Technology Press, Henan et al.

Müller WR, Rauscher T, Agerer R, Chevalier G (1996a) *Tuber aestivum* Vitt. + *Corylus avellana* L. Descriptions of Ectomycorrhizae 1: 167–172.

Müller WR, Rauscher T, Agerer R, Chevalier G (1996b) *Tuber uncinatum* Chat. + *Corylus avellana* L. Descriptions of Ectomycorrhizae 1: 179–183.

Nilsson RH, Ryberg M, Kristiansson E, Abarenkov K, Larsson K-H, Köljalg U (2006) Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLoS One 1(1): e59.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson K-H (2008) Intraspecific ITS variability in the Kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evolutionary Bioinfomratics 4: 193–201.

Perry BA, Hansen K, Pfister DH (2007) A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycological Research 111: 549–571.

Raidl S, Müller WR (1996) *Tomentella ferruginea* (Pers.) Pat. + *Fagus silvatica* L. Descriptions of Ectomycorrhizae 1: 61–66.

Rauscher T, Agerer R (1995) Ektomykorrhizen von *Tuber melanosporum*, *Tuber mesentericum* und *Tuber rufum* (Tuberales) an *Corylus avellana*. Nova Hedwigia 61: 281–322.

Rauscher T, Müller WR, Agerer R (1996) *Tuber borchii* Vitt. + *Corylus avellana* L.. Descriptions of Ectomycorrhizae 1: 173–178.

Rinaldi AC, Comandini O, Kuyper TW (2008) Ectomycorrhizal fungal diversity, separating the wheat from the chaff. Fungal Diversity 33:1–45.

Schramm JR (1966) Plant colonization studies on black wastes from anthracite mining in Pennsylvania. Trans Am Philos Soc 56: 5–189.

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. 2nd ed. Academic Press, San Diego, London et al.

Tedersoo L, Hansen K, Perry BA, Kjøller R (2006) Molecular and morphological diversity of pezizalean ectomycorrhiza. New Phytologist 170: 581–596.

Tedersoo L (2007) Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Dissertation.

Urban A, Weiss M, Bauer R (2003) Ectomycorrhizas involving sebacinoid mycobionts. Mycological Research 107(1): 3–14.

Weiß M, Selosse MA, Rexer KH, Urban A, Oberwinkler F (2004) *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycological Research 108 (9): 1003–1010.

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. In: Innis MA, Gelfand DH, Sninsky JN, White TJ (eds) PCR Protocols: a guide to method and applications. Academic Press, San Diego, pp 315–322.

Wu CY (1995) *Vegetation of China*, 2nd edn. Science Press, Beijing.

Yorou NS, Agerer R (2008) "*Afzeliaerhiza beninensis*" + *Afzelia africana* Smith. Descriptions of Ectomycorrhizae11/12: 1–8.

Yorou NS, Agerer R, Raidl S (2008) "*Uapacaerhiza wariensis*"+ *Uapaca guineensis* Müll.Arg.. Descriptions of Ectomycorrhizae11/12: 147–153.

Zambonelli A, Salomoni S, Pisi A (1993) Caratterizazione anatomo-morfologica delle micorrhize di *Tuber* spp. su *Quercus pubescens* Willd. Micol Ital 3:73–90

Zambonelli A, Salomoni S, Pisi A (1995) Caratterizazione anatomo-morfologica delle micorrhize de *Tuber borchii*, *Tuber aestivum*, *Tuber mesentericum*, *Tuber brumale*, *Tuber melanosporum* su *Pinus pinea*. Micol Ital 2:119–137

Zambonelli A, Iotti M, Amicucci A, Pisi A (1999) Caratterizzazione anatomo-morfologica delle micorrize di *Tuber maculatum* Vittad. Su *Ostrya carpinifolia* Scop. Micol Ital 28:29–35

Appendix 1: ECM-forming fungi and general features of already described ECM

Basidiomycota	
Agaricales	
Agaricaceae	(*Gymnogaster*, *Setschelliogaster*)
Amanitaceae *Amanita*	(*Amarrendia*, *Torrendia*) HO, ET long distance, MTY A, B, P, RH boletoid, EH with or without clamps
Cortinariaceae *Cortinarius* *Dermocybe* *Descolea* *Descomyces* *Rozites* *Stephanopus*	(*Anamika*, *Cribbea*, *Mackintoshia*) HO, ET short distance or medium-distance fringe subtype, MTY A,B,C, RH lacking or uniform-loose or phlegmacioid, EH with clamps HO, ET or medium-distance fringe subtype, MTY A,B RH uniform-loose, EH with clamps HI, MTY D, RH lacking, EH with clamps, CY capitate HI, MTY D, RH lacking, EH with clamps, CY capitate HI, MTY C, with amyloid gelatinous matrix, RH lacking, EH with clamps strongly bent, HO, MTY B, RH phlegmacioid, EH with clamps
Entolomataceae *Entoloma*	HI, ET medium-distance smooth subtype, MTY B, hyphae growing in parallel bundles, RH uniform-compact, EH with clamps
Hygrophoraceae *Hygrophorus*	HI, MTY B, M, RH lacking, EH with clamps
Hydangiaceae *Laccaria*	HI, MTY B, RH uniform-compact, EH with clamps, anastomoses pear-shaped
Inocybaceae ***Inocybe***	(*Auritella*) HI, ET short distance, MTY A, B, C, RH lacking, EH with clamps, clamps large, half or more than half semi-circle, with a hole
Lyophyllaceae *Lyophyllum*	HO, ET medium-distance fringe subtype, MTY B, RH agaricoid, EH with clod-like crystalline and clamps
Trichomomataceae *Tricholoma*	(*Leucopaxillus*) HO, ET medium-distance fringe subtype, MTY A, B, RH uniform-loose, uniform-compact, phlegmacioid or thelephoroid, EH clamps lacking
Hymenogastraceae *Alnicola* *Hebeloma* *Naucoria*	HI, ET medium-distance smooth subtype, MTB A/B, RH uniform-loose, EH with clamps HO, ET short or medium-distance fringe subtype, MTY B, RH lacking or uniform-loose, EH with clamps MTY B, RH uniform-loose, EH with clamps
Boletales	
Boletaceae	(*Aureoboletus*, *Austroboletus*, *Boletellus*, *Boletochaete*, *Bothia*, *Chalciporus*, *Fistulinella*, *Gastroboletus*, *Gastroleccinum*, *Gastrotylopilus*, *Heimioporus*, *Leccinellum*, *Mycoamaranthus*,

	Octaviania, *Paxillogaster*, *Phylloporus*, *Retiboletus*, *Rhodactina*, *Royoungia*, *Rubinoboletus*, *Setogyroporus*, *Sinoboletus*, *Tubosaeta*) **HO, ET long distance, MTY A, B, C, RH boletoid with nodes, clamps lacking**
Boletus *Leccinum* *Tylopilus* *Chamonixia*	MTY A,B, C, EH smooth or covered with crystals MTY A, RH with short inflated cells MTY A, EH smooth MTY A, blue in FEA
Suillaceae *Boletinus* *Suillus* *Truncocolumella*	(*Psiloboletinus*) **HO, ET long distance, MTY A, B, C, RH boletoid with nodes, clamps lacking** MTY F, with brownish drops, large crystals, EH clamp lacking MTY F, RH boletoid, EH clampless, drops of exuded pigment ET medium distance smooth, MTY A, RH uniform compact, EH clamps present
Calostomataceae	(*Calostoma*)
Gomphidiaceae *Chroogomphus* *Gomphidius*	(*Cystogomphus*, *Gomphogaster*) **HI, MTY D/F, CY awl-shaped with a basal clamp, globular cells on mantle surface, septa of inner mantle layers and of EH partially amyloid, a three-way relation to ECM of the genera *Rhizopogon* and *Suillus*, produce peloton-like haustoria within foreign ECM** RH lacking RH thelephoroid
Paxillaceae *Alpova* *Gyrodon* *Melanogaster* *Paxillus*	(*Austrogaster*, *Paragyrodon*) **HO, ET long distance, RH boletoid with nodes, EH clamps present** MTY A, RH with globular inflations, EH with crystals MTY A, F, with sclerotia MTY A, RH with globular inflations, CY clavate, capitate, EH with crystals MTY B, CY clavate, with sclerotia
Diplocystidiaceae	(*Diplocystis*)
Astraeaceae *Astraeus*	 MTY A, RH boletoid with nodes, EH clamps present
Sclerodermataceae *Pisolithus* and *Scleroderma*	(*Chlorogaster*, *Horakiella*) HO, ET long distance, MTY A/B, RH boletoid with nodes, thicker RH with short and inflated cells, EH clamps present
Phallomycetidae (Gomphales, Hysterangiales)	
Hysterangiales	
Hysterangiaceae *Hysterangium*	(*Aroramyces*) HO, ET medium-distance mat subtype, CY oleoacanthocystidia, RH ramarioid with CY, and yellowish globular cells, EH with or without clamps
Gallaceaceae	(*Austrogautieria*, *Gallacea*, *Hallingea*)
Mesophelliaceae	(*Annebbia*, *Castoreum*, *Chondrogaster*, *Gummiglobus*, *Gummivena*, *Malajczukia*, *Mesophellia*, *Nothocastoreum*)
Gomphales	**HO, ET medium distance mat subtype, RH ramarioid, CY oleoacanthocystidia, irregular globular yellowish cells**
Gomphaceae	

Gauteria *Gomphus* *Ramaria*	MTY B, CY and yellowish globular cells on RH, EH clamps lacking CY on mantle, EH with clamps MTY A, B, C, CY and yellowish globular cells on RH, EH clamps frequent lacking
Clavariadelphaceae *Clavariadelphus*	 MTY B, CY and yellowish globular cells on RH, EH with clamps
Thelephorales	
Bankeraceae *Bankera* *Boletopsis* *Hydnellum* *Phellodon* *Sarcodon*	**HO, carbonizing, ET medium-distance mat subtype, MTY A (ring to star-like), formation of chlamydospores** MTY star-like, chlamydospores Oidia-like, EH lacking MTY ring-like, EH with clamps, clamps with inflation chlamydospores thick radially spitting walls or thick walled MTY star-like, chlamydospores with concentrically, and asymmetrically splitting walls, EH lacking MTY ring-like, chlamydospores star-like with hollow warts, with clamps
Thelephoraceae *Pseudotomentella* *Thelephora* ***Tomentella*** *Tomentellopsis*	 HI, MTY B/C, RH lacking, EH clampless, partially amyloid HI, ET medium distance, MTY D, CY awl-shape with a basal clamp, EH with clamps, partially amyloid Brownish to dark brown, HI, ET contact, short or medium distance smooth subtype, RH lacking, uniform-loose or thelephoroid, CY lacking, awl-shaped or capitate, with or without cystidia; CR lacking or amyloid HO, MTY A, RH uniform-compact
Polyporales	
Atheliaceae *Amphinema* *Byssoporia* *Byssocorticium* *Piloderma* *Tylospora*	 HO, ET medium-distance fringe subtype, MTY B, RH uniform-loose EH with clamps HI/HO, ET medium-distance smooth subtype, MTY B, C, RH thelephoroid HO, ET short distance, MTY B, RH lacking, EH clampless HO, ET short distance, MTY A/B, RH uniform-loose, EH clampless HI, ET short distance, MTY C, RH lacking, EH clamps present
Rusullales	
Russulaceae *Arcangeliella* *Lactarius* *Russula*	(*Cystangium*, *Gymnomyces*, *Hydnangium*, *Macowanites*, *Multifurca*) HI, MTY P, with laticifers, RH lacking, CY present, EH clampless HI, MTY B, C, H, I, P, Q, with laticifers, CY lacking, RH lacking, uniform-compact, russuloid, EH clampless MTY A, D, H, K/O, P and Q, CY russuloid (with apical knob) or russuloid and awl-shaped, or lacking, EH clampless
Albatrellaceae *Ablatrellus* *Polyporoletus*	(*Corditubera*, *Leucogaster*, *Leucophleps*, *Mycolevis*) **MTY D, RH phlegmacioid, CY awl-shaped, thick walled cells amyloid** CY distally forked CY distally acuminate
Cantharellales	

Cantharellaceae *Cantharellus* *Craterellus*	**HI, MTY B, hyphae with oily droplets** RH compact, EH with clamps RH lacking, EH without clamps
Clavulinaceae	(*Clavulina*, *Membranomyces*)
Hydnaceae *Hydnum* *Sistotrema*	**HO, ET medium distance, MTY A, with oily droplet, RH ramarioid, EH with clamps** Often with short obtuse outgrowths of clams in outer mantle Hyphae in a gelatinous matrix
Sebacinales	
Sebacinaceae ***Sebacina***	(*Craterocolla*, *Efibulobasidium*, *Tremellodendron*, *Tremelloscypha*) HI, MTY D/E, EH or CY dichotomously, tritomously or quadritomously ramified, dextrinoid or not, inflated at the points of ramification
Hymenochaetales	
Hymenochaetaceae *Coltricia* *Coltriciella*	**brown to dark brown, HI, EH without clamps, RH lacking** MTY P or transform between PL and PS, with or without cystidia MTY transform between PL and PS, with cystidia
Tremellomycetidae	
Tulasnellaceae	(*Tulasnella*)
Ascomycota	
Eurotiomycetes **Eurotiales** Elaphomycetaceae *Elaphomyces* *Pseudotulostoma*	 HI, MTY A, C, E, RH lacking, EH without clamps HI, MTY B?, RH lacking, EH clampless
Pyrenomycetes Chaetosphaeriaceae	 (Chloridium)
Dothideomycetes *Cenococcum*	 black, HI, MTY G, RH lacking, EH lacking
Leotiomycetes	(*Meliniomyces*)
Helotiales	(*Leptodontidium*, *Phaeangium*)
Pezizales	
Discinaceae *Hydnotrya*	(*Gyromitra*) HI, ET contact, MTY L/M, RH lacking
Pezizaceae *Pachyphloeus* *Peziza* *Terfezia*	(*Amylascus*, *Cazia*, *Delastria*, *Eremiomyces*, *Glischroderma*, *Hydnobolites*, *Hydnotryopsis*, *Kalaharituber*, *Muciturbo*, *Mycoclelandia*, *Plicaria*, *Ruhlandiella*, *Tirmania*, *Underwoodia*) **HI, MTY L, RH lacking, EH clampless** HI, MTY L HI, MTY L, inner mantle with angular cells HI, MTY M

Sarcosphaera	HO, MTY B, inner mantle with angular cells
Helvellaceae	(*Barssia*, *Wynnella*) **HI, RH lacking, EH clampless**
Balsamia	MTY B
Helvella	MTY M
Leucangium	**MTY L**
Tuberaceae	(*Choiromyces*, *Dingleya*, *Labyrinthomyces*, *Loculotuber*, *Paradoxa*, *Reddellomyces*)
Tuber	HI, MTY L, M, P, and Q, CY infrequent to abundant, awl shaped, EH clampless
Morchellaceae	(*Fisherrula*)
Pyronemataceae	(*Geopyxis*, *Gilkeya*, *Nothojafnea*, *Otidea*, *Paurocotylis*, *Pseudaleuria*, *Sowerbyella*, *Sphaerosoma*, *Stephensia*, *Tarzetta*) **HI, EH clampless**
Genea-Humaria	brown, MTY L with a surface layer composed of irregularly shaped cells conneted by thin septa, EH partially warty and smooth
Geopora	brown, MTY L with cell heaps
Pulvinula	brown, MTY B
Sphaerosporella	MTY L
Sphaerozone	MTY B
Trichophaea	brown, MTY L
Wilcoxina	brown, MTY B, transition between PL and PS
Zygomycota	
Endogone *Diversispora*	

Note: The nomenclature used in this table is adopted from Binder et al. (2006), Blackwell et al. (2006), Hansen (2006), Hosaka et al. (2006), Larsson et al. (2006), Matheny et al. (2006), Moncalvo et al. (2006). The content of this table integrates Agerer (2006) and Rinaldi (2008). Taxa are listed regarding order and family. Genera (in parentheses) after the familiy name have been reported as ECM-forming fungi by molecular-phylogenetic analyses, molecular identification of ECM sequences, synthesis experiments, or have been recorded in different references without any further information, however no further morph-anatomical feature have been provided (detail see Rinaldi et al. 2008). Morpho-anatomical features in bold indicate the general features for the family. Genera in bold indicate the anatomotypes described in this study are belonging to these genera. These features have been summarized from already described species in this family to present, but it does not mean that species in the whole family have these features.

The abbreviations used for features of ECM and brief description of the main features are according to Agerer (1987–2008, 2006):
PL: plectenchymatous
PS: pseudoparenchymatous
EH: emanating hyphae
HI: hydrophilic
HO: hydrophobic
CY: cystidia

MTY: mantle type, assigned to A–Q (except J) according to the structures of outer mantle layers as seen in plan view, A–I: plectenchymatous series. - A: with ring-like structures; B: without distinctive patterns; C: with gelatinous matrix; D: with cystidia; E: with squarrosely branched hyphae; F: with globular cells; G: with star-like tightly glued hyphae; H: with inflated cells; I: with rather short and slightly tortuous cells. - K–Q: pseudoparenchymatous series; K, L, O with angular cells; K: with roundish mounded cells or with rosette-like structures; N: with solitary cells stainable in sulpho-vanillin; O: with heaps of flattened or bowl-shaped cells; P: with a hyphal net; Q: with a hyphal net.

RH: rhizomorph, types A-F. A: uniform loose; B: uniform compact; C: with central, slightly thick hyphae (thelephoroid) or additional with ampullate, inflated hyphae (ramarioid); D: with a few randomly distributed hyphae (phlegmacioid); E: with thick hyphae [and ladder-like hyphae (russuloid)]; F: with vessel-like hyphae (boletoid).

ET: exploration types based on amount of EH and RH structure
Contact: mantle smooth with only a few EH; *Short distance*: voluminous envelope of EH, RH lacking; *Medium-distance*: (1) fringe subtype: rhizomorphs with fringy (hairy) margins, RH of type A or exceptionally type C, D; (2) mat subtype: rhizomorphs with fringy (hairy) margin, ECM forming dense mats, RH of type A, C, exceptionally type D; (3) smooth subtype: rhizomorphs smooth, of RH type B, C, D, exceptionally type E. *Long distance*: with RH of type F

Appendix 2: Fruitbodies of potential ECM fungi of Basidiomycota under *Pinus tabulaeformis* in different sampling sites (Huang 1998, Mou 2000)

ECM Fungi	Daqing Mountain	Wula Mountain	Helan Mountain	Heilihe National Reserve
Cantharellus				*C. subalbidus* A.H. Sm. & Morse
Chroogomphus				*C. rutilus* (Schaeff.) O.K. Mill.
Cortinarius			*C.* sp.	
Hebeloma		*H. sinuosum* (Fr.) Quél.		
Inocybe			*I.* sp1, *I.* sp2, *I.* sp3	*I.* cf *geophylla*
Lactarius				*L. deliciosus* (L.) Gray
Russula	*R.* sp.			*R. rubra* (Fr.) Fr. *R. alutacea* (Fr.) Fr.
Suillus	*S. granulatus* (L.) Roussel	*S. luteus* (L.) Roussel	*S. granulatus*	*S. bovinus* (Pers.) Roussel *S.* cf. *placidus*
Thelephora	*T. caryophyllea* (Schaeff.) Pers.			
Tricholoma	*T.* cf. *virgatum*	*T.* cf. *virgatum*	*T.* cf. *virgatum*	*T.* cf. *virgatum*

Appendix 3: ECM, not yet studied in detail, with *Pinus tabulaeformis* and their potential genus affiliation due to morpho-anatomical comparisons with already published decriptions in different sampling sites

ECM Fungi	Daqing Mountain	Wula Mountain	Helan Mountain	Heilihe National Reserve
Basidiomycota				
Cortinarius			*C*. sp.	
Chroogomphus				*C*. sp.
Inocybe	"*P. inocyboides*"	"*P. inocyboides*"	"*P. inocyboides*" , *I*. sp.	"*P. inocyboides*"
Lactarius				*L*. sp.
Russula	*R*. sp2			*R*. sp1, *R*. sp2
Sebacinales	*S*. sp1		"*P.multifurcata*"	"*P. nondextrinoidea*"
Suillus	*S*. sp1	*S*. sp2	*S*. sp1	*S*. sp3
Thelephora	*T*. sp1		*T*. sp2	
Tomentella	*T*. sp.	"*P.tomentelloides*" *T*. sp.	"*P. tomentelloides*", "*P. fibulocystidiata*", *T*. sp.	"*P. heilihensis*" *T*. sp.
Tricholoma	"*P.tricholomoides*"	"*P. tricholomoides*"	"*P. tricholomoides*"	"*P.trichophaeoides*"
Ascomycota				
Cenococcom geophilum	C. g.	C. g.	C. g.	C. g.
Geopora	"*P. daqingensis*" "*P. geoporoides*"			
Humaria			"*P. humarioides*"	
Trichophaea			"*P. trichophaeoides*"	
Tuber	"*P. pubulata*"	"*P. pubulata*"	*T*. sp.	"*P. puborchii*", "*P. ongensis*"

Appendix 4: Already described anatomotypes of ECM with *Pinus tabulaeformis* as well as the accession number in GenBank

Sampling Number	Resigned Name	Accession Number in GenBank	Publication
JW19	"*Pinirhiza inocyboides*"	-	**I**
JW38a	"*Pinirhiza tomentelloides*"	-	**II**
JW71a	"*Pinirhiza tricholomoides*"	EU292410 (ITS)	**III**
JW189d	"*Pinirhiza humarioides*"	GQ281479 (ITS), GQ281475 (LSU)	**IV**
JW76a	"*Pinirhiza daqingensis*"	GQ281480 (ITS), GQ281476 (LSU)	**IV**
JW96a	"*Pinirhiza geoporoides*"	GQ281481 (ITS), GQ281477 (LSU)	**IV**
JW44a	"*Pinirhiza trichophaeoides*"	GQ281482 (ITS), GQ281478 (LSU)	**IV**
JW191a	"*Pinirhiza acuminata*"	GQ979995 (ITS)	**V**
JW49a	"*Pinirhiza fibulocystidiata*"	GQ979996 (ITS)	**V**
JW179b	"*Pinirhiza heilihensis*"	GQ979997 (ITS)	**V**
JW54a	"*Pinirhiza multifurcata*"	GU269910 (ITS), GU269908 (LSU)	**VI**
JW185b	"*Pinirhiza nondextrinoidea*"	GU269911 (ITS), GU269909 (LSU)	**VI**
JW29a	"*Pinirhiza puborchii*"	GU722194 (ITS), GU722192 (LSU)	**VII**
JW97b	"*Pinirhiza pubulata*"	GU722193 (ITS), GU722190 (LSU)	**VII**
JW156a	"*Pinirhiza ongensis*"	GU722195 (ITS), GU722191 (LSU)	**VII**

Note: A "-" means that the sequence has not been submitted in GenBank, or it has not been successfully obtained.

Curriculum vitae

Jie Wei March 2010

Date and place of birth: 31.03.1980, Huhhot (Inner Mongolia, China)

Current contact: Department Biologie I und GeoBio-Center^{LMU}; Organismische Biologie: Mykologie, Menzinger Str. 67; D-80638 München, Tel. 089 / 17861-196,

Email weijie211@yahoo.com

Education:

03 /2007- 05/2010	Department of Biology I, Ludwig-Maximilians-Universität München, PhD
09/2003-07/2006	Forest science, Inner Mongolia Agricultural University, Master degree, Title of study: Ectomycorrhizal diversity of *Pinus tabulaefomis* in Nursery in Huhhot
09/1999-07/2003	Gardening, Inner Mongolia Agricultural University, Bachelor degree
09/1994-07/1999	Gymnasium

Selective publications

Wei J, Agerer R (2008) "*Pinirhiza inocyboides*" + *Pinus tabulaeformis* Carr. Descriptions of Ectomycorrhizae 11/12 : 89-96.

Wei J, Agerer R (2008) "*Pinirhiza tomentelloides*" + *Pinus tabulaeformis* Carr. Descriptions of Ectomycorrhizae: 97-102.

Wei J, Agerer R, Raidl S (2008) "*Pinirhiza tricholomoides*" +*Pinus tabulaeformis* Carr. Descriptions of Ectomycorrhizae:103-112.

Wei J, Peršoh D, Agerer R (2009) Four Ectomycorrhizae of Pyronemataceae (Pezizomycetes) on Chinese Pine (*Pinus tabulaeformis*) – morpho-anatomical and molecular-phylogenetic analyses. Mycological Progress, in press.

Wei J, Agerer R (2009) Three Ectomycorrhizae of Thelephoraceae on Chinese Pine (*Pinus tabulaeformis*) and a key to thelephoroid Ectomycorrhizae. Nova Hedwigia, in press.

Wei J, Agerer R (2009) Two sebacinoid Ectomycorrhizae on Chinese Pine. Mycorrhiza, submitted.

Wei J, Agerer R (2010) Three Tuber Ectomycorrhizae on Chinese Pine. Mycoscience, submitted.

Scholarships

2007-2010	Scholar of the German Academic Exchange Service (DAAD)

Practices:

10/2007	one-week mycological excursion in Italy (Region Emilia Romana)
10/2008	one-week mycological excursion in Italy (Region Lazio)