MycoKeys 50: 1–77 (2019)
doi: 10.3897/mycokeys.50.32432
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Solving the taxonomic identity of *Pseudotomentella tristis* s.l. (Thelephorales, Basidiomycota) – a multi-gene phylogeny and taxonomic review, integrating ecological and geographical data

Sten Svantesson[1,2,3], Karl-Henrik Larsson[4], Urmas Kõljalg[5,6], Tom W. May[3], Patrik Cangren[1,2], R. Henrik Nilsson[1,2], Ellen Larsson[1,2]

1 *Department of Biological and Environmental Sciences, University of Gothenburg, Box 463, 405 30 Göteborg, Sweden* **2** *Gothenburg Global Biodiversity Centre, Box 461, 405 30 Göteborg, Sweden* **3** *Royal Botanic Gardens Victoria, Birdwood Ave, Melbourne, Victoria 3004, Australia* **4** *The Mycological Herbarium, Natural History Museum, University of Oslo, P.O. Box 1172, Blindern, 0318 Oslo, Norway* **5** *Natural History Museum, University of Tartu, 14a Ravila, 50411 Tartu, Estonia* **6** *Institute of Ecology and Earth Sciences, University of Tartu, 14a Ravila, 50411 Tartu, Estonia*

Corresponding author: *Sten Svantesson* (sten.svantesson@bioenv.gu.se)

Academic editor: *O. Raspé* | Received 15 December 2018 | Accepted 8 March 2019 | Published 4 April 2019

Citation: Svantesson S, Larsson K-H, Kõljalg U, May TW, Cangren P, Nilsson RH, Larsson E (2019) Solving the taxonomic identity of *Pseudotomentella tristis* s.l. (Thelephorales, Basidiomycota) – a multi-gene phylogeny and taxonomic review, integrating ecological and geographical data. MycoKeys 50: 1–77. https://doi.org/10.3897/mycokeys.50.32432

Abstract

P. tristis is an ectomycorrhizal, corticioid fungus whose name is frequently assigned to collections of basidiomata as well as root tip and soil samples from a wide range of habitats and hosts across the northern hemisphere. Despite this, its identity is unclear; eight heterotypic taxa have in major reviews of the species been considered synonymous with or morphologically similar to *P. tristis*, but no sequence data from type specimens have been available.

With the aim to clarify the taxonomy, systematics, morphology, ecology and geographical distribution of *P. tristis* and its morphologically similar species, we studied their type specimens as well as 147 basidiomata collections of mostly North European material.

We used gene trees generated in BEAST 2 and PhyML and species trees estimated in STACEY and ASTRAL to delimit species based on the ITS, LSU, Tef1α and mtSSU regions. We enriched our sampling with environmental ITS sequences from the UNITE database.

We found the *P. tristis* group to contain 13 molecularly and morphologically distinct species. Three of these, *P. tristis*, *P. umbrina* and *P. atrofusca*, are already known to science, while ten species are here described as new: *P. sciastra* **sp. nov.**, *P. tristoides* **sp. nov.**, *P. umbrinascens* **sp. nov.**, *P. pinophila* **sp. nov.**, *P. alnophila* **sp. nov.**, *P. alobata* **sp. nov.**, *P. pluriloba* **sp. nov.**, *P. abundiloba* **sp. nov.**, *P. rotundispora* **sp. nov.** and *P. media* **sp. nov**.

We discovered *P. rhizopunctata* and *P. atrofusca* to form a sister clade to all other species in *P. tristis* s.l. These two species, unlike all other species in the *P. tristis* complex, are dimitic.

In this study, we designate epitypes for *P. tristis*, *P. umbrina* and *Hypochnopsis fuscata* and lectotypes for *Auricularia phylacteris* and *Thelephora biennis*. We show that the holotype of *Hypochnus sitnensis* and the lectotype of *Hypochnopsis fuscata* are conspecific with *P. tristis*, but in the absence of molecular information we regard *Pseudotomentella longisterigmata* and *Hypochnus rhacodium* as doubtful taxa due to their aberrant morphology. We confirm *A. phylacteris*, *Tomentella biennis* and *Septobasidium arachnoideum* as excluded taxa, since their morphology clearly show that they belong to other genera. A key to the species of the *P. tristis* group is provided.

We found *P. umbrina* to be a common species with a wide, Holarctic distribution, forming ectomycorrhiza with a large number of host species in habitats ranging from tropical forests to the Arctic tundra. The other species in the *P. tristis* group were found to be less common and have narrower ecological niches.

Keywords

Corticioid fungi, ectomycorrhiza, taxonomy, species complex, molecular systematics, species tree, STACEY, UNITE database

Introduction

Species of the genus *Pseudotomentella* Svrček are recognised by their smooth, corticioid, membranaceous basidiomata, bi- or trifurcately echinulate basidiospores and their lack of cystidia (Larsen 1971a, Stalpers 1993, Kõljalg 1996). All species, for which a life strategy has been confirmed, are ectomycorrhizal (Agerer 1994, Kõljalg et al. 2000, Cline et al. 2005, Di Marino et al. 2007, Trocha et al. 2012, Branco et al. 2013, Malysheva et al. 2016) and the genus is widely distributed throughout the northern hemisphere (Larsen 1971a, Kõljalg 1996). Basidiomata are formed on the underside of dead wood, turf and stones, where their spores are probably dispersed by insects, as found by a study on a species in the closely related genus *Tomentella* Pers. ex Pat. (Lilleskov and Bruns 2005).

Pseudotomentella tristis (P.Karst.) M.J.Larsen is characterised by its brown to bluish-grey, sometimes green-tinged basidiomata, simple septate, monomitic hyphal system, wide subicular hyphae and large, yellow to brown basidiospores (Larsen 1971a, Stalpers 1993, Kõljalg 1996). In this current, morphological delimitation of the species, it is probably the most commonly collected *Pseudotomentella* species in the world: out of 1038 herbarium specimens registered in GBIF (13–08–2018), 497 are attributed to *P. tristis* – the second most common species being *Pseudotomentella mucidula* (P.Karst.) Svrček with a total of 230 specimens. Even though there is no taxonomic study currently linking the type of *P. tristis* to molecular information, it is also a name frequently assigned to sequences recovered in molecular ecology studies of ectomycorrhizal com-

munities in soil and on root tip samples (e.g. Kõljalg et al. 2000, Cline et al. 2005, Hrynkiewicz et al. 2009, Walbert et al. 2010, Izumi and Finlay 2011, Argüelles-Moyao et al. 2017), some of which even report it to constitute one of the most common species found (Kõljalg et al. 2000, Izumi and Finlay 2011). Entries in international sequence and specimen databases from 15 countries in the northern hemisphere indicate that it is a very widespread species (Kõljalg et al. 2005, Clark et al. 2015, GBIF 13–08–2018, Nilsson et al. 2019). Concordantly, it also has a very large ecological amplitude: sequences attributed to *P. tristis* have been encountered in habitats and with hosts ranging from the Swedish tundra with *Salix polaris* Wahlenb. (Hrynkiewicz et al. 2009) to the neotropics of Mexico with *Abies religiosa* (Kunth) Schltdl. & Cham. (Argüelles-Moyao et al. 2017).

Taxonomically and nomenclaturally, *P. tristis* is a species with a long history. Based on French material, Bulliard (1790) described *Auricularia phylacteris* Bull. – a fungus with a large corticioid basidiome, a plicated base and an initially pale, but with maturity darkening hymenium. Fries (1821) described *Thelephora biennis* Fr. with reference to *A. phylacteris*.

In 1828, Fries introduced the name *Thelephora umbrina* Fr. to describe a soft, brown, effused basidiome, which he stated that he had seen alive (Fries 1828).

Karsten (1889) raised a subspecies, *Hypochnus subfuscus* ssp. *tristis* P.Karst., that he had previously described, based on Finnish material (Karsten 1883), to the level of species, giving it the name *Hypochnus tristis* (P.Karst.) P.Karst. In his protologue, he wrote of it as having a wool or felt-like basidiome and a thick, blackish hymenium, with hues of olive brown or green, coloured brown by the detaching spores. He noted the spores to be roundedly angular, aculeate, yellow or brown and 8–12 µm in diameter. In 1889, Karsten also described *Hypochnopsis fuscata* P.Karst. – a second species from Finland, whose description is similar to that of *H. tristis*, except that, according to the author, the colour of the hymenium is bluish-black and the spores are smooth, bluish with a dark wall and 3–4 µm in diameter (Karsten 1889).

Bresadola (1897) described the species *Hypochnus sitnensis* Bres. from a Hungarian basidiome with a soft, chestnut brown colour, a smoke-coloured hymenium and spores similar in size to those of *H. tristis*.

Berkeley and Broome (1873) created the new name *Thelephora arachnoidea* Berk. & Broome for their sparse description of a basidiome with a powdery, grey hymenium and a soft, black subiculum, based on a collection made in Sri Lanka.

Burt (1926) described *Hypochnus rhacodium* Berk. & M.A. Curtis ex Burt from a US specimen. He wrote of it as having a crust-like and brittle texture, fuscous to dusky drab appearance and spores measuring 6–7 µm in diameter.

Following the original descriptions of *A. phylacteris*, *T. biennis*, *T. umbrina*, *H. tristis*, *H. fuscata*, *H. sitnensis*, *T. arachnoidea* and *H. rhacodium*, a large number of publications were made, proposing new combinations and synonymisations (Gmelin 1792, de Candolle 1815, Fries 1821, 1849, 1874, Quélet 1888, Saccardo 1888, Bresadola 1903, 1916, von Höhnel and Litschauer 1906, 1908, Burt 1916, Donk 1933, Litschauer 1933, Rogers 1948, Parker-Rhodes 1956, Svrček 1958).

No new, morphologically similar species were published until 1967, when Larsen, based on a basidiome he collected in USA, described *Pseudotomentella longisterigmata* M.J.Larsen – a fungus with a greyish-green hymenium and unusually long sterigmata. In his description of *P. longisterigmata*, Larsen also combined *Thelephora umbrina* to *Pseudotomentella*. He then proceeded to synonymise all other species similar to *P. umbrina* described thus far (Larsen 1968). Hjortstam (1969), however, argued that *Thelephora umbrina* and *Hypochnus tristis* represented different species that could be separated mainly based on the colour and texture of basidiomata: *T. umbrina* could be recognised by its pale to dark chocolate brown hymenium and softer texture and *H. tristis* could be distinguished by its dark greenish-blue, sometimes brownish-tinted hymenium and firmer texture. Larsen (1971a) disagreed: writing that he had observed a continuum of variation between the distinct character states defined by Hjortstam as characteristic of the two taxa, he considered them as one. Larsen (1971a) further proposed that the taxon in question should have the species epithet *tristis* instead of *umbrina*, with reference to his interpretation of Fries's (1828) original description and to Rogers and Jackson (1943), who claimed *Thelephora tristis* to be a synonym of *Coniophora olivacea* (Fr.) P.Karst. He consequently made the combination *Pseudotomentella tristis* M.J.Larsen (1971a) and urged that the use of *Thelephora umbrina* and all its homotypic synonyms be discontinued.

Larsen (1971b) described one additional species, *Pseudotomentella atrofusca* M.J.Larsen, based on a blackish-brown, soft basidiome with spores 5.5–6.6 µm in diameter, from the US. Kõljalg (1996) considered *P. longisterigmata* to be a synonym of *P. atrofusca*. Beside *P. tristis*, *P. atrofusca* is the only name left in common use today (GBIF 13–08–2018). It is employed for small-spored specimens in both North America and Europe, but is considerably less frequently collected (GBIF 13–08–2018).

Thus, in conclusion, ten names have so far been associated with the *P. tristis* group, as here defined. Of these, only two – *P. tristis* and *P. atrofusca* – remain in use today. *Pseudotomentella tristis* is under the currently employed, morphological delimitation regarded as a common species with a very wide geographic distribution and ecological amplitude. The purpose of the present study is to molecularly delimit species within the *P. tristis* group, describe their morphology and present knowledge on their ecology and geographical distribution – describing new species and designating types as needed.

Methods

Taxon sampling and information

We collected specimens of basidiomata extensively throughout Sweden, Norway and Estonia in the period 2010–2017. For the majority of the Swedish and Norwegian specimens, we recorded the vegetation type of each locality, following Fremstad (1997) and Pålsson (1998). We then sorted this information into the habitats "tundra", "co-

niferous forest", "deciduous forest" and "mixed forest" and the soil pH types "low", "intermediate" and "high", following Pålsson (1998) and Halvorsen (2015). We also recorded potential hosts of each specimen, as indicated by nearby ectomycorrhiza-forming plants. The Swedish specimens were photographed, weather permitting. We complemented the fresh material by examining all collections identified as *P. tristis*, *P. atrofusca* and *Pseudotomentella* sp. in GB and TU, along with collections identified as *P. tristis* in TUR and H, and relevant type specimens in S, H, BPI and ARIZ. Permission to extract DNA was granted. In addition, we studied Fries' collection of *Thelephora umbrina* in situ at UPS. Taxonomic author abbreviations follow IPNI (26–11–2018) and herbarium codes follow Index Herbariorum (Thiers 2018). Abbreviations of journal titles follow BPH Online (26–11–2018) and abbreviations of book titles follow Stafleu and Cowan (1976–1988).

Morphological data

We studied all specimens macroscopically and at 20× magnification under a dissecting microscope. Photos of micromorphological characters and measurements were made using an Axioskop 2 microscope (Zeiss, Oberkochen, Germany), equipped with an AxioCam MRc camera (Zeiss) at 400× and 1000× magnifications and in the ZEN Blue software (http://www.zeiss.com/microscopy/int/home.html). Measurements were made on dried material, mounted in 3% (potassium hydroxide) KOH and in Melzer's reagent. We examined a minimum of three specimens per species, whenever the total number of specimens allowed it, and we measured 20–30 micromorphological structures of each type. Measurements were made to the nearest 0.1 µm, except basidial length, which was measured to the nearest µm. As a necessary aid in identification, we present the values recorded both as spans of the lowest to the highest value and as mean values. For the spans, the 5% smallest and largest measurements are denoted in brackets, in the cases where they differed from the remaining 90%. We calculated the mean values for each specimen omitting the 5% tails and values presented for each species are hence a span of such data. Spore measurements include lobes but exclude echinuli and the hilar appendage. We did not measure abnormally large spores, originating from two-sterigmate basidia. Measurements of basidial width were made at the widest part of the tip of the basidia; basidial length excludes sterigmata. We obtained the width of hyphae from unbroken, internodal sections.

The spore measurements follow Kõljalg (1996) in the recognition of the dorsal side of basidiospores observed in face view as the frontal face (Fig. 1) and the sides of the spores seen in side-view as the lateral faces. Great care has been taken to correctly identify these faces while conducting measurements and not tilted versions of the same, as inclusion of such would undoubtedly lead to an increased margin of error.

For optimal usage of the species descriptions in this article, we recommend readers to utilise the online version, where high resolution pictures are available.

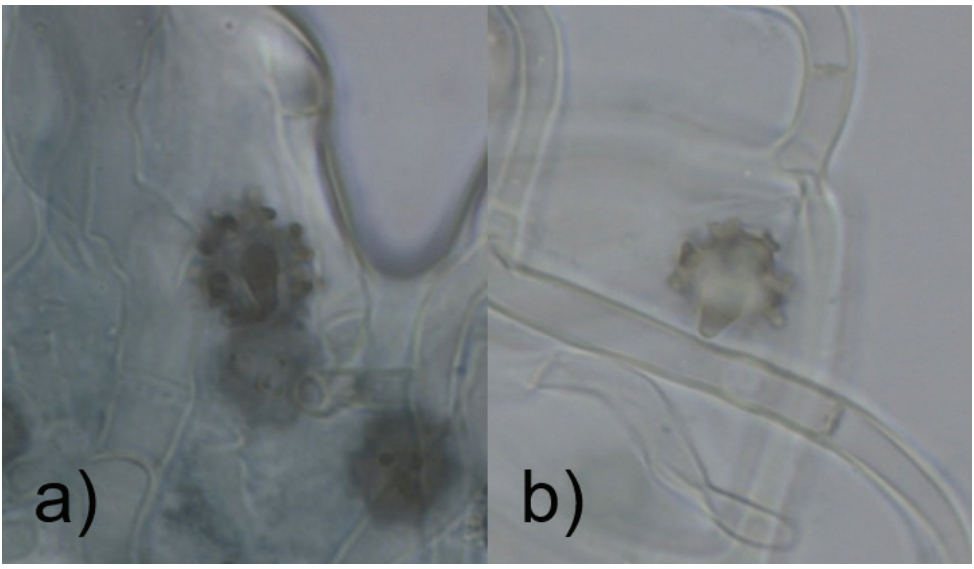


Figure 1. Angle of spore faces. Dorsal side of spores, seen in **a** frontal face and **b** tilted frontal face.

Molecular data

We generated sequences from four regions for the study: the complete ITS region, including the 5.8S gene, and about 1200 bases of the 5′end of the LSU nuclear ribosomal DNA; about 600 bases of translation elongation factor subunit 1 alpha (Tef1α); and approximately 500 bases of the mtSSU. DNA extractions, PCR reactions and sequencing were performed as described in Larsson et al. (2018). The primers used to amplify the complete ITS region and the 5′end of the LSU region were ITS1F (Gardes and Bruns 1993), LR21, LR0R and LR7 (Hopple and Vilgalys 1999); for Tef1α we used EF983F and EF1567R (Rehner and Buckley 2005); and for mtSSU we used MS1 and MS2 (White et al. 1990). Primers used for sequencing were ITS1, ITS4, MS1, MS2 (White et al. 1990), Ctb6 (https://nature.berkeley.edu/brunslab/tour/primers.html), Lr5 (Hopple and Vilgalys 1999), EF983F and EF1567R.

To assemble the DNA sequences, we used Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA). We aligned them in AliView 1.18 (Larsson 2014), utilising the L-INS-i strategy as implemented in MAFFT v. 7.017 (Katoh and Standley 2013) and manually adjusted the resulting multiple sequence alignments. Only a few sequences in the Tef1α dataset contained introns. They proved unalignable between species and were removed. In addition, we complemented the nrDNA dataset with representatives of all ITS genotypes belonging to the same 3% Species Hypothesis in the UNITE database (Kõljalg et al. 2005, Parrent and Vilgalys 2007, Peintner et al. 2007, Bidartondo and Read 2008, Krpata et al. 2008, Obase et al. 2009, Tedersoo et al. 2009, Bacher et al. 2010, Cox et al. 2010, Benucci et al. 2011, Obase et al. 2011, Huang et al. 2012, Kranabetter et al. 2012, Sun et al. 2012, Těšitelová et al. 2012, LeDuc et al. 2013,

Leonardi et al. 2013, Põlme et al. 2013, Tedersoo et al. 2013, Malysheva et al. 2014, Taylor et al. 2014, Guichon 2015, Malysheva et al. 2016, Argüelles-Moyao et al. 2017, Rosenthal et al. 2017, Nilsson et al. 2019). The sequences generated for this article were deposited in GenBank, with accession numbers MK290647–MK290732 and MK312643–MK312663 (Table 1). The specimens they originate from are indicated with an asterisk (*) in the lists of examined specimens.

The species described in this article have been provided with links to the UNITE Species Hypotheses they are part of, in the cases where such exist. Upon publication of the article, the Species Hypotheses will be updated with their new names and the DNA sequences generated for the article will be made available in GenBank. At the next update of UNITE, the ITS sequences will then be copied from GenBank and clustered into the appropriate Species Hypotheses.

Molecular analyses

We used SplitsTree 4.14.4 (Huson and Bryant 2006) to explore the amount of intragenic conflict and possible presence of intragenic recombination, and RDP4 (Martin et al. 2015) to test for recombination. In RDP, all DNA regions were initially submitted to testing with the methods RDP, GENECONV, Chimaera and MaxChi, with Bonferroni correction and 0.01 as the significance level. We submitted sequences with significant signs of recombination to a second round of testing that made use of all recombination methods. Sequences with a positive result for more than two methods with p-values ≤ 10^{-5} in the second round were regarded as probable recombinants.

To generate Bayesian phylogenetic trees from the alignments, we used BEAST 2.4.7 (Bouckaert et al. 2014), employing the standard version of the programme for gene tree estimation, and STACEY 1.2.4 (Jones 2017) for species tree inference under the multispecies coalescent model. We prepared the xml-files for the BEAST 2 runs in BEAUti 2.4.7 (Bouckaert et al. 2014). The following minimal partitions were assumed per unlinked genetic region (Table 2): ITS1, 5.8S, ITS2, LSU (nrDNA); Tef1α first positions, Tef1α second positions, Tef1α third positions (Tef1α); mtSSU (mtSSU). We used the automated best-fit tests implemented in PAUP 4.0a (Swofford 2002) to select optimal substitution models and substitution model partitions for each minimal partition. Using three substitution schemes, the following partitions and models had the highest ranking, according to BIC scores: ITS1+ITS2 (HKY+I+G), 5.8S+LSU (GTR+I+G), Tef1α first positions (JC+I), Tef1α second positions (K80+I), Tef1α third positions (HKY) and mtSSU (GTR+G). The BEAST 2 and STACEY analyses did not converge under the GTR model and invariant site fraction parameter (I), however, such that we used HKY+G for the two nrDNA substitution model partitions and the mtSSU region. Omitting the I parameter in a rerun of the partition test for Tef1α yielded the result JC+G for first+second positions and HKY for the third positions and these were hence the partitions and models we adopted in the BEAST 2 analyses.

Table 1. DNA regions included per analysis and collection. Accession numbers of DNA sequences generated for this study start with "MK" and for sequences obtained from UNITE with "UDB"; all other sequences were acquired from GenBank. Type collections are shown in boldface. The respective gene trees included all available sequences, whereas collections and accession numbers whose sequences were included in the STACEY analysis are marked with * and those included in both the STACEY and ASTRAL analyses with **.

Species	Collection no.	Country of origin	Acc. no. ITS	Acc. no. LSU	Acc. no. mtSSU	Acc. no. Tef1α
*P. abundiloba***	**O F110312**	Norway	MK290731	MK290731	MK290669	MK312646
*P. abundiloba**	TU 110852	Estonia	UDB014123			
*P. alnophila***	**O F110313**	Norway	MK290715	MK290715	MK290661	
*P. alnophila**	–	China	UDB012458			
P. alnophila	–	China	UDB012511			
*P. alobata***	**O F110315**	Norway	MK290695	MK290695	MK290665	MK312657
*P. alobata***	SS425	Sweden	MK290696	MK290696	MK290664	MK312658
P. alobata	KHL11873	Sweden	MK290693			
P. alobata	O F110316	Norway	MK290694			
P. alobata	TU 115626	Slovenia	UDB020318			
*P. atrofusca***	**ML7553**	USA	MK290732		MK290651	
*P. atrofusca**	–	China	HQ850125			
P. atrofusca	–	China	HQ850126			
P. atrofusca	–	China	HQ850127			
*P. media***	**TU115609**	Estonia	MK290714	MK290714	MK290653	
*P. media**	–	Canada	KC840631			
P. media	TU 115608	Estonia	UDB016437			
P. media	–	Italy	HM044465			
P. media	–	Italy	HM044464			
P. media	–	Russia	UDB007475			
*P. pinophila***	**SS358**	Sweden	MK290708	MK290708	MK290654	
*P. pinophila***	SS419	Sweden	MK290710		MK290655	MK312655
P. pinophila	O F110328	Norway	MK290709	MK290709		
P. pinophila	SS440	Sweden	MK290711			
P. pinophila	SS418	Sweden	MK290712			
P. pinophila	O F110330	Norway	MK290713			
P. pinophila	–	R. o. Korea	AB506089			
P. pinophila	–	China	AB636446			
P. pinophila	–	R. o. Korea	AB587761			
*P. pluriloba***	**US 4263**	Finland	MK290698	MK290698	MK290672	MK312650
*P. pluriloba***	SS439	Sweden	MK290699	MK290699	MK290671	MK312649
P. pluriloba	–	USA	KF617867			
P. pluriloba	–	Canada	JN652992			
*P. rotundispora***	**SS413**	Sweden	MK290674	MK290674	MK290657	MK312651
*P. rotundispora***	SS394	Sweden	MK290728	MK290728	MK290656	
P. rotundispora	SS393	Sweden	MK290729			
P. rotundispora	KHL17682	Norway	MK290730			
P. rotundispora	TU100138	Estonia	MK290727			
P. rotundispora	–	UK	EU668195			
P. rotundispora	–	Italy	DQ990858			
P. rotundispora	–	Italy	JX625330			

Species	Collection no.	Country of origin	Acc. no. ITS	Acc. no. LSU	Acc. no. mtSSU	Acc. no. Tef1α
P. rotundispora	–	Austria	EF644141			
*P. sciastra***	**SS359**	Sweden	MK290686		MK290666	MK312662
*P. sciastra***	SS420	Sweden	MK290689		MK290667	MK312661
*P. sciastra***	SS312	Sweden	MK290687			MK312663
P. sciastra	O F110317	Norway	MK290684	MK290684		
P. sciastra	O F110318	Norway	MK290688	MK290688		
P. sciastra	TU 124213	Estonia	UDB028204	UDB028204		
P. sciastra	TU 124211	Estonia	UDB028202	UDB028202		
P. sciastra	TU 110153	Turkey	UDB004970	UDB004970		
P. sciastra	O F110322	Norway	MK290685			
P. sciastra	SS423	Sweden	MK290690			
P. sciastra	KHL17308b	Sweden	MK290691			
P. sciastra	TAA 187322	UK	UDB001616			
P. sciastra	TU 110113	Turkey	UDB004951			
P. sciastra	TU 100644	Estonia	UDB016813			
P. sciastra	–	USA	KP814390			
P. sciastra	–	USA	EF619790			
*P. tristis***	**SS193**	Sweden	MK290679	MK290679	MK290662	
*P. tristis***	LK 54/13	Finland	MK290683		MK290663	MK312659
P. tristis	KHL15084	Norway	MK290682	MK290682		
P. tristis	O F110300	Norway	MK290676	MK290676		
P. tristis	TU108134	Estonia	MK290677			
P. tristis	O F110297	Norway	MK290678			
P. tristis	O F110298	Norway	MK290680			
P. tristis	KHL16367	Norway	MK290681			
P. tristis	TAAM 159485	Estonia	AF274771			
P. tristis	TU 115642	Slovenia	UDB020327			
P. tristis	TU 115439	Estonia	UDB016304			
P. tristoides	**O F110306**	Norway	MK290692	MK290692		
P. tristoides	–	Estonia	UDB008832			
P. tristoides	–	Czechia	GU327494			
*P. umbrina***	**SS351**	Sweden	MK290700	MK290700	MK290659	MK312654
*P. umbrina***	SS239	Sweden	MK290704		MK290660	
*P. umbrina***	SS221	Norway	MK290703			MK312653
P. umbrina	O F110268	Norway	MK290702	MK290702		
P. umbrina	O F110296	Norway	MK290701			
P. umbrina	SS280	Sweden	MK290705			
P. umbrina	SS174	Sweden	MK290706			
P. umbrina	TU 115344	Finland	UDB011636			
P. umbrina	TU 115209	Norway	AF274772			
P. umbrina	TU 108084	Canada	UDB015056			
P. umbrina	–	Denmark	AJ889979			
P. umbrina	–	USA	FJ803973			
*P. umbrinascens***	**SS335**	Sweden	MK290697	MK290697	MK290670	MK312647
*P. umbrinascens**	–	Italy	HM370480			
P. umbrinascens	–	Italy	HM370468			
P. sp. 1**	SS285	Sweden	MK290716	MK290716	MK290658	MK312652
P. sp. 1*	–	Mexico	KF041350			

Species	Collection no.	Country of origin	Acc. no. ITS	Acc. no. LSU	Acc. no. mtSSU	Acc. no. Tef1α
P. sp. 1	–	Russia	KJ769286			
P. sp. 1	–	Russia	KP783455			
P. sp. 2**	SS169	Sweden	MK290707	MK290707	MK290668	MK312648
P. sp. 3	–	Estonia	UDB002898			
P. sp. 3	–	Estonia	UDB002899			
*P. flavovirens***	KHL17461	Finland	MK290722		MK290648	MK312644
P. flavovirens	KHL16310	Sweden	MK290723	MK290723		
*P. griseopergamacea***	LLSS883	Norway	MK290721	MK290721	MK290649	
P. griseopergamacea	SS401	Sweden	MK290720			
*P. humicola***	SS345	Sweden	MK290724	MK290724	MK290650	MK312643
P. humicola	SS212	Sweden	MK290675	MK290675		
*P. mucidula***	LLSS1155	Norway	MK290725		MK290673	MK312656
P. mucidula	LLSS1123	Norway	MK290726	MK290726		
*P. nigra***	KHL16273	Finland	MK290718	MK290718	MK290647	MK312645
P. nigra	LLSS838	Norway	MK290719	MK290719		
*P. rhizopunctata***	SS129	Sweden	MK290717	MK290717	MK290652	
P. rhizopunctata	–	Canada	KP889924			
*P. vepallidospora***	TU 115205	Norway	UDB000278	UDB000278		
P. vepallidospora	–	Germany	HM146848			

Table 2. Partitions and models used in the STACEY analysis.

DNA region	Minimal partitions	Substitution model partitions	Substitution model	Clock model partitions	Clock model	Tree-estimation partitions
nrDNA	ITS1	ITS1+ITS2	HKY+G	ITS1	Lognormal, relaxed	ITS1+5.8S+ITS2+LSU
	5.8S	5.8S+LSU	HKY+G	5.8S	Lognormal, relaxed	ITS1+5.8S+ITS2+LSU
	ITS2	ITS1+ITS2	HKY+G	ITS2	Lognormal, relaxed	ITS1+5.8S+ITS2+LSU
	LSU	5.8S+LSU	HKY+G	LSU	Lognormal, relaxed	ITS1+5.8S+ITS2+LSU
Tef1α	Tef1α 2nd pos.	Tef1α 1st pos.+ Tef1α 2nd pos.	JC+G	Tef1α 2nd pos.	Lognormal, relaxed	Tef1α 1st pos.+ Tef1α 2nd pos.+ Tef1α 3rd pos.
	Tef1α 2nd pos.	Tef1α 1st pos.+ Tef1α 2nd pos.	JC+G	Tef1α 2nd pos.	Lognormal, relaxed	Tef1α 1st pos.+ Tef1α 2nd pos.+ Tef1α 3rd pos.
	Tef1α 3rd pos.	Tef1α 3rd pos.	HKY	Tef1α 3rd pos.	Lognormal, relaxed	Tef1α 1st pos.+ Tef1α 2nd pos.+ Tef1α 3rd pos.
mtSSU	mtSSU	mtSSU	HKY+G	mtSSU	Lognormal, relaxed	mtSSU

The substitution rate of each partition was estimated independently of the others in each BEAST 2 run. We set all individuals as separate species in the STACEY analysis. We set the trees of the minimal nrDNA partitions as linked, as did we for the Tef1α minimal partitions. We set the clock models of all minimal partitions as unlinked and

a lognormal, relaxed clock model was assumed for each, as test runs had shown that all partitions had a coefficient of variation well above 0.1 (i.e. implying a relatively high rate variation amongst branches). The clock rate of each partition was estimated in the runs, using a lognormal prior, with a mean set to one in real space. We set the growth rate prior to lognormal, with a mean of 5 and a standard deviation of 2. The Collapse Height prior of the STACEY analysis was set to 10^{-5} and a lognormal prior with a mean of -7 and a standard deviation of 2 was set to the PopPriorScale parameter.

We ran the Markov Chain Monte Carlo (MCMC) chains of the mtSSU and Tef1α regions for 10 million generations with tree and parameter files sampled every 1000 generations. For the nrDNA and STACEY analysis, we ran the MCMC chains for 100 million generations and sampled it every 5000 generations, and for 1 billion generations and sampled it every 25000 generations, respectively. All analyses converged well in advance of the 10% burn-in threshold and had effective sampling size values well above 200 for all parameters. Chain mixing was found to be satisfactory as assessed in Tracer 1.6.0 (Rambaut et al. 2014). After discarding the burn-in trees, maximum clade credibility trees were identified by TreeAnnotator 2.4.7 (Bouckaert et al. 2014). Posterior probabilities of the clusterings of the species trees output by STACEY were analysed in the associated software SpeciesDelimitationAnalyser version 1.8.0, with burn-in set to 10%, simcutoff to 1 and collapseheight to 10^{-5}. The estimated similarity matrix was visualised by the R-script plot.simmatrix.R (Jones et al. 2015).

To generate Maximum Likelihood (ML) gene trees, we used PhyML 3.1 (Guindon et al. 2010). We set the substitution model to GTR+I+G for the Tef1α, nrDNA and mtSSU regions, since it was the best-fit model output by the automated model test in PAUP, using AICc and three substitution schemes. The tree topology search was conducted using NNI+SPR, with ten random starting trees. Non-parametric bootstrap analyses with 1000 replicates were performed on the resulting trees. We also inferred a species tree from the ML gene trees, using ASTRAL III (Zhang et al. 2018), with node support calculated as local posterior probabilities (Sayyari and Mirarab 2016).

The Bayesian and ML gene trees comprised the entire nrDNA, mtSSU and Tef1α datasets of this study, while the STACEY and ASTRAL species trees, including the ML trees underlying the latter, contained subsets thereof (Table 1) to avoid destabilising the analyses with large amounts of missing data.

We visually prepared the resulting trees from the Bayesian and ML analyses in FigTree 1.4.3 (Rambaut 2012) and Inkscape (Albert et al. 2018).

Results

Molecular species delimitation

The STACEY analysis retrieved 13 well-supported clades, based on DNA from specimens morphologically belonging to the *P. tristis* group. We interpret these as species (Fig. 2). The corresponding clades in the ASTRAL analysis were also supported, when present

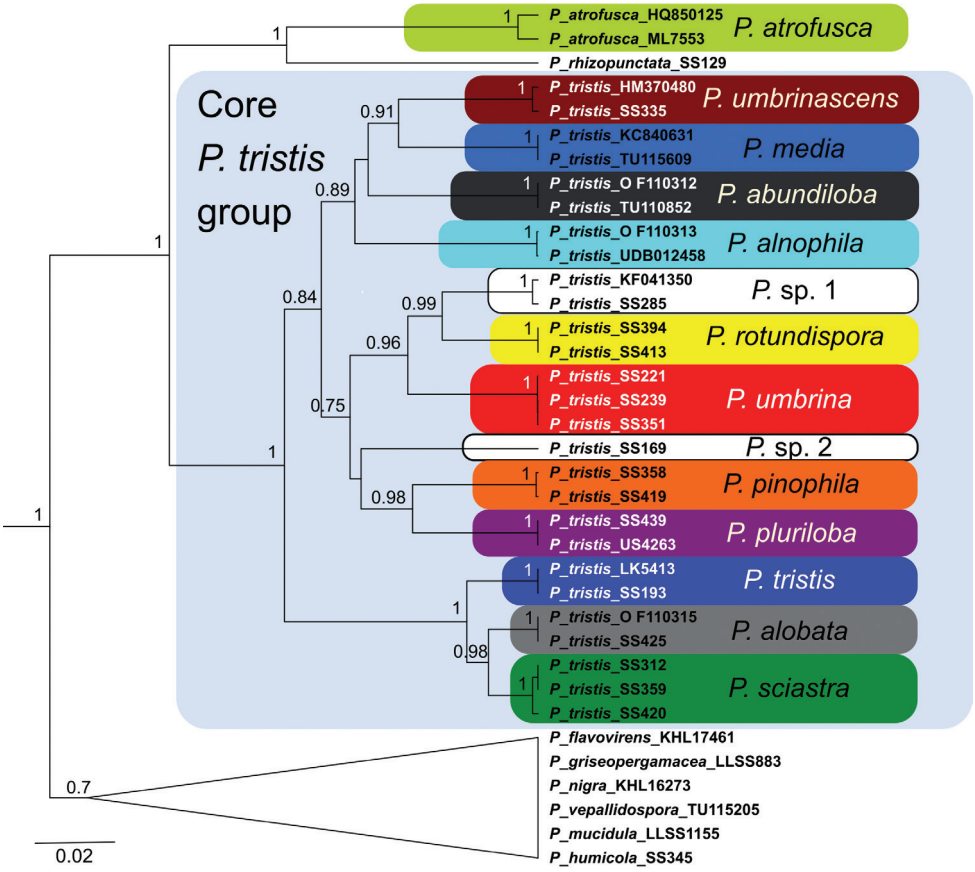


Figure 2. STACEY species tree of the *P. tristis* group. Numbers at nodes denote posterior probability values (only values > 0.70 are shown). The branch lengths are scaled in estimated number of substitutions/site.

as more than one leaf node (Fig. 4). We found three of the delimited species, *P. tristis*, *P. umbrina* and *P. atrofusca*, to constitute previously described taxa, while nine species are described as new to science: *P. sciastra*, *P. umbrinascens*, *P. pinophila*, *P. alnophila*, *P. alobata*, *P. pluriloba*, *P. abundiloba*, *P. rotundispora* and *P. media*. We chose not to describe *P.* sp. 1, since the only collection available is too small to be suitable as a type. The same applies to *P.* sp. 2, which in addition was retrieved as a singleton by both analyses.

The delimitation of the species recognised in the species tree equals the clusters output by SpeciesDelimitationAnalyser (Fig. 3). As displayed by the similarity matrix, each cluster has internal support and zero posterior probability of the included individuals belonging to any other cluster. In the case of specimens SS419 and SS420 and sequences HQ850125, HM370480, UDB012458 and KF041350, however, the similarity matrix shows weak support for them to belong to their respective clusters; but given the strong support for the corresponding species in the species tree, we interpret these as instances of intraspecific genetic structure.

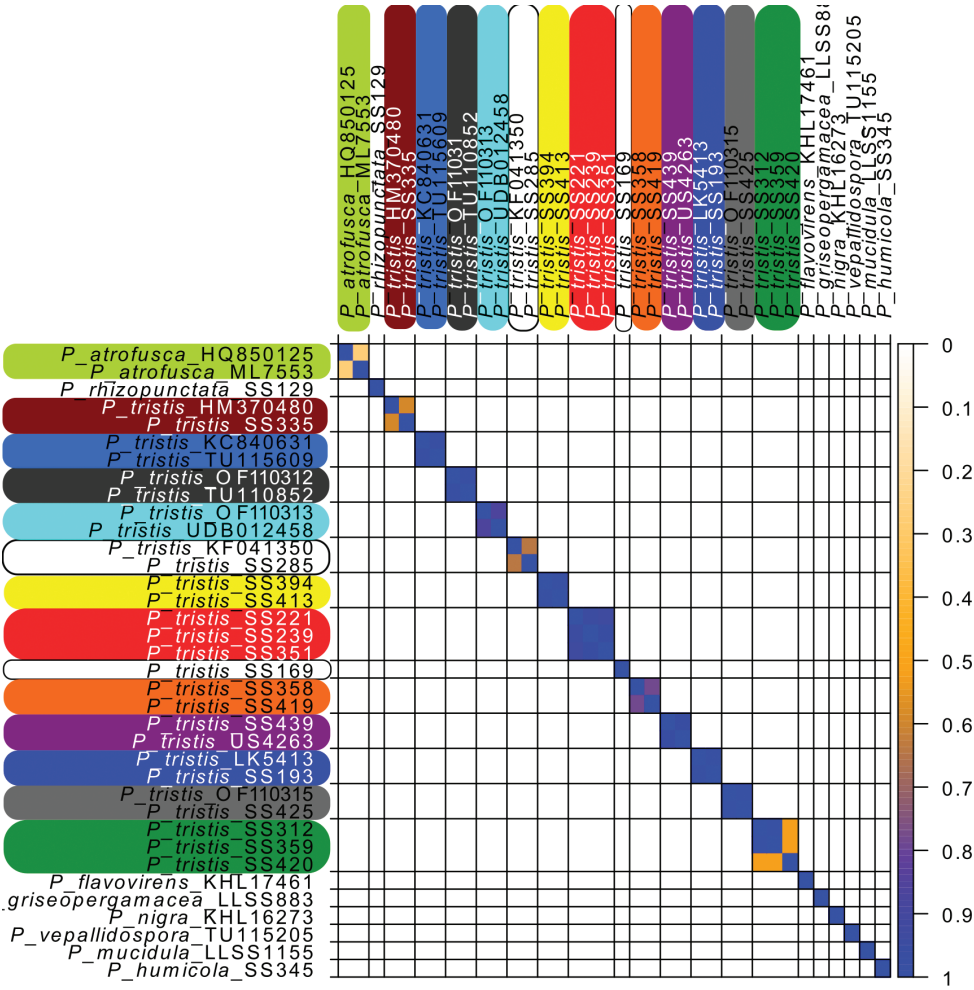


Figure 3. Pairwise similarity matrix between the clusters of the STACEY species tree. The species are colour-coded the same as in Fig. 2. Values between 0 and 1 denote posterior probability.

In addition to the species delimited based on clades in the species tree, we recognised two species, *P. tristoides* and *P.* sp. 3, based on their presence as highly supported nodes in the nrDNA gene tree (Fig. 5). Their sequences could not be included in the species tree analyses, due to lack of data for the other genetic regions. We did not describe *P.* sp. 3, since it has no physical material tied to it.

Phylogenetic relationships

The species tree analyses were congruent. The trees retrieved show that the species in the *P. tristis* group, with the addition of *Pseudotomentella rhizopunctata* E.C.Martini & Hen-

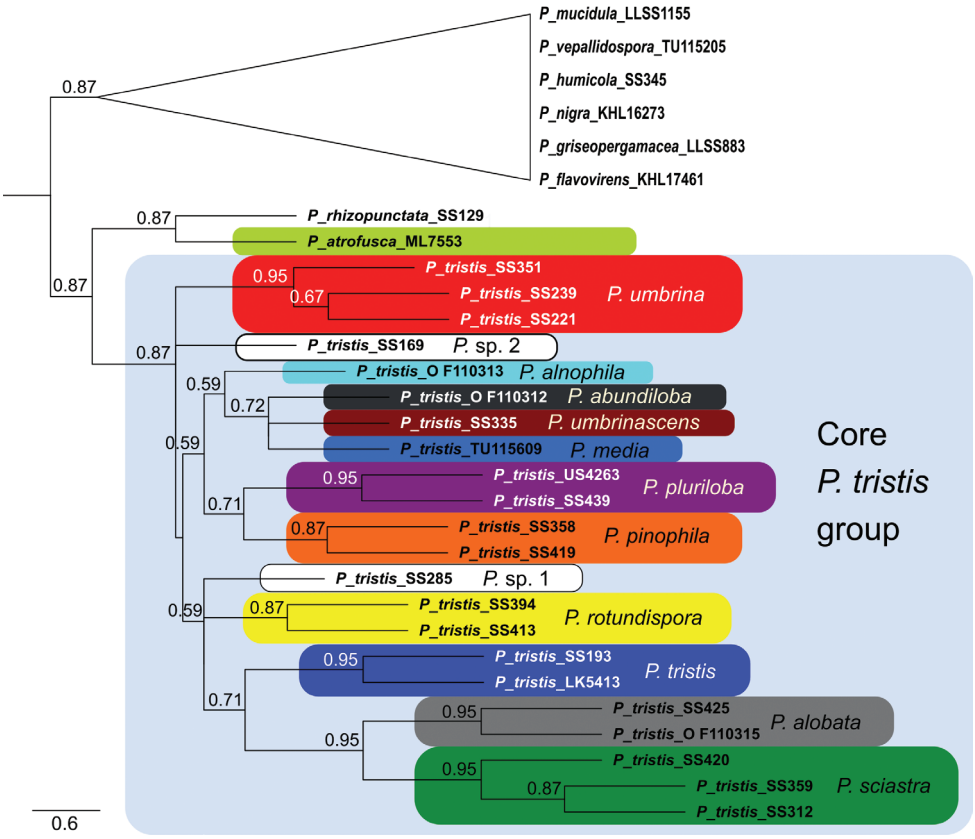


Figure 4. ASTRAL species tree of the *P. tristis* group. Numbers between 0.5 and 1 denote local posterior probability values (only values > 0.5 are shown). The internal branch lengths are scaled in coalescent units, while the length of the terminal branches is a standard value set by the programme.

tic, form a monophyletic clade with high support (Figs 2, 4). Its two daughter clades, one containing *P. rhizopunctata* and *P. atrofusca* and the other containing the remaining species of the *P. tristis* group (the "core *P. tristis* group"), were also well supported. The phylogenetic relationships within the core *P. tristis* group, however, were not; only the clades (*P. sciastra*, *P. alobata*), ((*P. sciastra*, *P. alobata*), *P. tristis*) and (*P. pinophila*, *P. pluriloba*) were supported by both analyses. In addition, the clades ((*P. rotundispora*, *P.* sp. 1), *P. umbrina*) and (*P. rotundispora*, *P.* sp. 1) were supported by the STACEY analysis.

No signal of intragenic recombination was detected in RDP4, but as indicated by the low phylogenetic resolution present also in the gene trees (Fig. 5, Suppl material 1: Figs S1– S4) and the network-like structure between splits observed in SplitsTree (Suppl material 1: Figs S5–S7) for the included genetic regions, there is considerable intragenic conflict between species. The exception to this pattern is the highly sup-

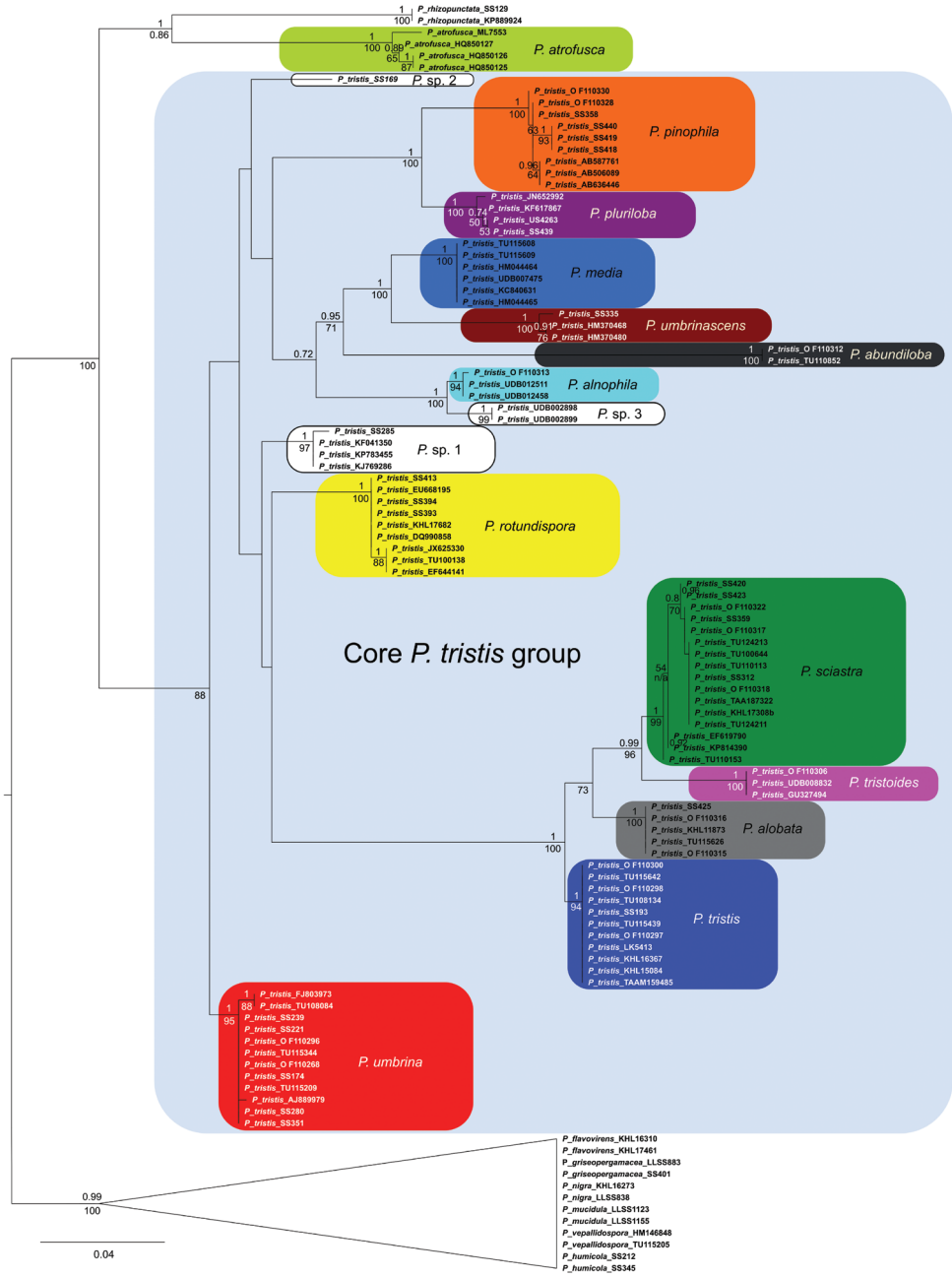


Figure 5. Nuclear ribosomal DNA phylogeny of the *P. tristis group*. ML phylogram with bootstrap support values (only values > 50 are shown), and posterior probability values added from congruent Bayesian tree (only values > 0.7 are shown). Branch lengths are scaled in substitutions/site.

ported, long branch of the clades (((*P. sciastra*, *P. tristoides*), *P. alobata*), *P. tristis*) and ((*P. sciastra*, *P. alobata*), *P. tristis*) in the nrDNA and Tef1α trees, respectively. In the neighbour nets of the nrDNA and Tef1α regions, this clade is reminiscent of the "dog-bone" shape displayed by paralogy, a hypothesis that is reinforced by its placement at the very root of the Tef1α tree. The branch in question thus constitutes the only – but in itself a major – incongruence between the gene trees.

A methodological observation to future users of STACEY with limited amounts of data is that support for species-level nodes decreases dramatically unless at least one included leaf taxon has a complete coverage of all the genetic regions used.

Type studies

All of the 13 newly described and previously described species can be distinguished morphologically (Table 3), although the differences exhibited by some species pairs are small. We found the previously designated lectotype of *P. tristis* and neotype of *P. umbrina* to fall within the morphological variation of sequenced material, with which they could hence be epitypified. The lectotypes of *H. fuscata* and *H. sitnensis*, however, display the morphological characteristics of *P. tristis*, with which we thus considered them conspecific. Of the seven type specimens studied, we were only able to generate ITS sequence data for *P. atrofusca*. The European collections, studied of *P. atrofusca*, all belong to *P. sciastra*.

The type collections of *P. longisterigmata* and *H. rhacodium* differ morphologically from all other specimens studied. Their aberrant morphology, with extremely long sterigmata and a very hard and thick basidiome, respectively (see further under "Taxonomy"), however, suggest that they may be misshapen forms of other species.

Septobasidium arachnoideum (Berk. & Broome) Bres. was accepted in *Septobasidium* by the thorough study of Couch (1938) and hence, we consider it excluded from Thelephorales Corner ex Oberw. We designated a plate by Bulliard (1790) as lectotype of *A. phylacteris* and *T. biennis*. The morphology displayed by this plate and stated in the original descriptions of these species does not match any *Pseudotomentella* species known to date (Larsen 1971a, Stalpers 1993, Kõljalg 1996).

Morphology

We were able to discern a few morphological patterns amongst the clades of the nrDNA trees. The most pronounced is perhaps the lack of hyphal cords and skeletal hyphae in the species of the core *P. tristis* group. These are characters that are present in *P. atrofusca* and *P. rhizopunctata* (Larsen 1971a, Martini and Hentic 2003) and indeed in all other simple-septate *Pseudotomentella* species (Larsen 1971a, Kõljalg 1996). Generally, spore shape and dimensions, the width of subicular hyphae and the length of echinuli proved to be the most taxonomically informative characters. Subhymenial hyphal width, basidial dimensions and sterigmal length were moderately useful for

Table 3. The most taxonomically informative micromorphological characters. Summary statistics for each species is marked in bold. "Frontal" and "lateral" refer to the corresponding spore faces and "L" and "W" denote mean length and width, respectively. All measurements are in µm, with the 5% smallest and largest values denoted in brackets, when differing from the remaining 90%.

Species/ collection	Frontal length	Frontal L	Frontal width	Frontal W	Lateral length	Lateral L	Lateral width	Lateral W	Length echinuli	L echinuli	Width subic. hyphae	W subic. hyphae
***P. abundiloba* sp. nov.**	**(8.8–) 9.2–10.5**	**9.8**	**(8.0–) 8.6–10.7 (–10.8)**	**9.5–9.6**	**(8.9–) 9.3–10.1 (–10.5)**	**9.7–9.8**	**(6.7–) 7.0–8.1 (–8.2)**	**7.3–7.7**	**(0.9–) 1.1–1.8 (–1.9)**	**1.2–1.5**	**(4.3–) 4.8–6.9 (–7.2)**	**5.5–6.1**
holotype	(8.8–) 9.2–10.5	9.8	(8.0–) 8.6–10.7 (–10.8)	9.6	(9.2–) 9.3–10.1 (–10.2)	9.7	(6.7–) 7.0–7.7 (–7.8)	7.3	(0.9–) 1.1–1.8 (–1.9)	1.5	(5.0–) 5.5–6.9 (–7.2)	6.1
TU 110852	(9.0–) 9.3–10.2	9.8	(8.9–) 9.0–10.2 (–10.3)	9.5	(8.9–) 9.3–10.1 (–10.5)	9.8	(7.0–) 7.4–8.1 (–8.2)	7.7	1.0–1.7	1.2	(4.3–) 4.8–6.2	5.5
***P. alnophila* sp. nov. holotype**	**(8.8–) 9.0–10.1 (–10.4)**	**9.5**	**9.2–10.2 (–10.6)**	**9.8**	**9.0–10.6**	**9.6**	**(6.6–) 6.9–8.1 (–8.2)**	**7.7**	**(0.8–) 0.9–1.7**	**1.2**	**4.0–5.0 (–5.1)**	**4.5**
***P. alobata* sp. nov.**	**(9.0–) 9.1–10.7**	**9.7–10.1**	**(8.4–) 8.9–10.5 (–10.7)**	**9.5–9.8**	**(8.9–) 9.1–10.3**	**9.7–9.9**	**(6.5–) 6.7–8.2**	**7.1–7.4**	**1.2–1.8 (–1.9)**	**1.4–1.7**	**(4.3–) 4.6–7.4 (–7.6)**	**5.6–5.9**
holotype	9.5–10.7	10.1	(9.1–) 9.2–10.4 (–10.7)	9.8	(9.6–) 9.7–10.1	9.9	6.8–8.2	7.4	1.2–1.8	1.4	(4.7–) 5.0–6.9	5.9
TU 115626	(9.0–) 9.3–10.5 (–10.6)	9.8	(8.8–) 9.0–10.5	9.5	(8.9–) 9.1–10.3	9.7	(6.7–) 6.8–7.7	7.2	(1.2–) 1.3–1.8 (–1.9)	1.6	(4.3–) 4.6–7.4 (–7.6)	5.8
O F110316	(9.0–) 9.1–10.4	9.7	(8.4–) 8.9–10.3 (–10.5)	9.5	9.5–10.1 (–10.3)	9.8	(6.5–) 6.7–7.5 (–7.6)	7.1	(1.4–) 1.6–1.8 (–1.9)	1.7	(4.7–) 4.8–6.3 (–6.8)	5.6
***P. atrofusca* holotype**	**(6.1–) 6.2–7.0 (–7.1)**	**6.6**	**(5.8–) 6.3–7.2 (–7.3)**	**6.8**	**6.3–6.9 (–7.3)**	**6.5**	**(4.0–) 4.1–4.8 (–5.0)**	**4.4**	**0.6–0.9 (–1.1)**	**0.8**	**(1.7–) 1.8–2.8**	**2.3**
***P. longisterigmata* holotype**	**(9.7–) 10.0–11.7**	**11.0**	**(9.4–) 9.8–11.7**	**10.7**	**10.3–11.5 (–11.7)**	**10.9**	**(6.7–) 7.5–9.1**	**8.5**	**1.2–1.8 (–2.1)**	**1.5**	**4.9–7.2**	**6.2**
***P. media* sp. nov.**	**(7.8–) 8.0–9.5**	**8.9–9.3**	**(8.3–) 8.5–9.9 (–10.1)**	**9.2–9.8**	**(8.8–) 8.9–9.6**	**9.1–9.4**	**(6.6–) 7.0–7.9**	**7.3–7.6**	**(0.8–) 0.9–1.6**	**1.1–1.2**	**(3.6–) 3.7–5.0 (–5.4)**	**4.1–4.6**
holotype	(8.5–) 8.7–9.5	9.3	(9.0–) 9.1–9.9	9.8	(8.8–) 8.9–9.6	9.4	7.0–7.7 (–7.8)	7.3	(0.8–) 0.9–1.3 (–1.4)	1.1	(3.6–) 3.7–4.6	4.1
TU 115608	(7.8–) 8.0–9.4 (–9.5)	8.9	(8.3–) 8.5–9.9 (–10.1)	9.2	8.9–9.4 (–9.6)	9.1	(6.6–) 7.2–7.9	7.6	(0.8–) 0.9–1.6	1.2	(3.8–) 4.4–5.0 (–5.4)	4.6
***P. pinophila* sp. nov.**	**(7.7–) 7.9–10.2 (–10.3)**	**8.6–9.1**	**(7.7–) 8.3–10.1 (–10.2)**	**8.8–9.4**	**(8.2–) 8.3–9.7 (–9.8)**	**8.7–9.0**	**(5.7–) 5.8–6.8 (–7.0)**	**6.3–6.6**	**(0.6–) 0.8–1.4 (–1.5)**	**0.9–1.1**	**3.0–4.9**	**3.6–4.1**
holotype	(7.7–) 7.9–10.2 (–10.3)	9.1	8.3–10.1 (–10.2)	9.4	8.4–9.5 (–9.8)	9.0	(6.0–) 6.1–6.8 (–6.9)	6.3	(0.8–) 0.9–1.4 (–1.5)	1.1	3.2–3.9 (–4.3)	3.6
O F110330	8.0–9.4 (–9.7)	8.7	(8.6–) 8.9–9.9	9.3	(8.5–) 8.6–9.4	8.8	6.1–7.0	6.5	(0.6–) 0.8–1.1	0.9	(3.4–) 3.5–4.9	4.1
O F110305	(8.1–) 8.3–8.9 (–9.4)	8.6	(7.7–) 8.4–9.4 (–9.6)	8.8	(8.2–) 8.3–9.7 (–9.8)	8.7	(5.7–) 5.8–6.8 (–6.9)	6.3	0.8–1.4	1.1	3.0–4.4 (–4.8)	3.7

Species/ collection	Frontal length	Frontal L	Frontal width	Frontal W	Lateral length	Lateral L	Lateral width	Lateral W	Length echinuli	L echinuli	Width subic. hyphae	W subic. hyphae
***P. pluriloba* sp. nov.**	**(9.0–) 9.1–10.8 (–10.9)**	**9.8**	**(9.2–) 9.3–10.9 (–11.1)**	**10.2**	**9.0–10.4 (–10.8)**	**9.6–9.8**	**(6.7–) 6.8–8.5 (8.6)**	**7.5–7.6**	**(0.9–) 1.0–1.9**	**1.4**	**(3.9–) 4.0–5.9 (–6.8)**	**4.8–5.1**
holotype	(9.0–) 9.1–10.4 (–10.8)	9.8	(9.2–) 9.3–10.9 (–11.1)	10.2	9.0–10.4 (–10.8)	9.8	(6.7–) 6.8–8.5 (–8.6)	7.6	(1.0–) 1.1–1.9	1.4	(4.1–) 4.7–5.9 (–6.8)	5.1
SS439	(9.2–) 9.3–10.8 (–10.9)	9.8	9.5–10.9 (–11.0)	10.2	(9.3–) 9.4–9.9 (–10.4)	9.6	6.9–7.9 (–8.1)	7.5	(0.9–) 1.0–1.8 (–1.9)	1.4	(3.9–) 4.0–5.4 (–5.8)	4.8
***P. rhacodia* comb. nov. syntype**	**(7.8–) 8.0–9.1 (–9.3)**	**8.3**	**(7.7–) 7.8–8.9 (–9.0)**	**8.3**	**(7.9–) 8.2–8.9**	**8.5**	**(5.4–) 5.9–6.8 (–7.0)**	**6.3**	**(0.9–) 1.0–1.6 (–1.7)**	**1.3**	**(5.6–) 5.7–7.3 (–8.0)**	**6.5**
***P. rotundispora* sp. nov.**	**(6.7–) 7.0–8.2 (–8.4)**	**7.5–7.6**	**7.0–8.6**	**7.7–7.9**	**7.0–8.2 (–8.3)**	**7.6–7.9**	**(5.2–) 5.3–6.0 (–6.1)**	**5.6–5.7**	**0.5–1.1 (–1.3)**	**0.8**	**3.0–4.4 (–4.6)**	**3.4–3.8**
holotype	(6.7–) 7.0–8.1 (–8.4)	7.6	7.1–8.5 (–8.6)	7.9	(7.1–) 7.2–8.2	7.9	5.5–6.0	5.7	0.7–0.9 (–1.1)	0.8	3.0–4.1 (–4.3)	3.4
SS394	(6.9–) 7.1–8.2 (–8.3)	7.6	(7.0–) 7.3–8.6	7.8	7.0–8.1 (–8.3)	7.7	(5.2–) 5.3–6.0 (–6.1)	5.7	0.5–1.0	0.8	(3.1–) 3.4–4.4 (–4.6)	3.8
SS393	7.0–7.9 (–8.0)	7.5	7.0–8.2 (–8.4)	7.7	7.3–8.2	7.6	5.3–5.8	5.6	0.5–1.1 (–1.3)	0.8	3.1–3.8 (–4.1)	3.5
***P. sciastra* sp. nov.**	**(6.0–) 6.1–7.9 (–8.1)**	**6.6–7.3**	**6.3–8.2**	**6.7–7.7**	**(6.2–) 6.5–7.7 (–8.0)**	**6.8–7.3**	**(4.3–) 4.4–6.0 (–6.2)**	**4.6–5.4**	**(0.5–) 0.6–1.2 (–1.4)**	**0.8–0.9**	**(3.9–) 4.4–6.6 (–6.8)**	**5.0–5.8**
holotype	6.5–7.9 (–8.1)	7.3	(6.8–) 7.0–8.1 (–8.2)	7.7	(6.5–) 6.7–7.7 (–8.0)	7.3	4.7–6.0 (–6.2)	5.4	0.6–1.2 (–1.3)	0.8	(4.5–) 4.8–6.4 (–6.8)	5.7
O F110317	(6.5–) 6.6–7.9 (–8.0)	7.2	(6.9–) 7.0–8.2	7.6	(6.7–) 7.0–7.6 (–7.8)	7.3	4.8–5.9 (–6.1)	5.4	(0.5–) 0.6–1.2 (–1.4)	0.9	(4.7–) 4.9–6.6 (–6.7)	5.8
TAA 187322	(6.0–) 6.1–7.0 (–7.1)	6.6	6.3–7.3 (–7.6)	6.7	(6.2–) 6.5–7.1 (–7.3)	6.8	(4.3–) 4.4–5.6 (–5.7)	4.6	0.6–1.1 (–1.4)	0.8	(3.9–) 4.4–5.8 (–6.0)	5.0
P. tristis	**7.7–9.1 (–9.2)**	**8.3–8.5**	**8.0–9.3 (–9.6)**	**8.4–8.6**	**7.7–9.0 (–9.1)**	**8.3–8.5**	**5.6–) 6.0–6.8 (–7.0)**	**6.3–6.5**	**(0.8–) 0.9–1.9**	**1.4**	**(4.5–) 4.6–7.4**	**5.4–6.2**
epitype	(7.7–) 8.1–8.8 (–9.0)	8.5	(8.0–) 8.1–9.0	8.6	(7.7–) 8.0–9.0	8.5	(5.6–) 6.1–6.8	6.5	(0.8–) 1.0–1.9	1.4	4.6–6.4 (–6.9)	5.7
lectotype	7.7–8.8	8.3	8.2–9.1	8.6	(7.9–) 8.0–8.8	8.3	6.0–6.7 (–7.0)	6.5	1.1–1.8	1.4	(4.5–) 4.7–7.4	5.9
TAA 159485	(7.8–) 7.9–9.1 (–9.2)	8.4	8.0–8.9 (–9.2)	8.4	8.0–8.7	8.4	6.1–6.7 (–7.0)	6.4	0.9–1.8	1.4	(5.0–) 5.4–6.1 (–6.4)	5.7
L. Kosonen 54/13	8.0–9.1 (–9.2)	8.5	8.1–9.2 (–9.6)	8.6	8.1–8.9	8.4	6.1–6.6	6.3	(0.9–) 1.1–1.7	1.4	(4.6–) 4.7–6.2 (–6.3)	5.4
KHL15084	7.9–9.1	8.3	(8.1–) 8.3–9.3	8.6	7.7–8.9 (–9.1)	8.3	(5.9–) 6.0–6.8	6.4	(0.8–) 1.0–1.8	1.4	(5.2–) 5.3–7.0 (–7.4)	6.2
***H. fuscata* lectotype**	**(7.8–) 8.0–9.0**	**8.5**	**(8.1–) 8.4–9.1**	**8.7**	**(7.9–) 8.1–9.2 (–9.4)**	**8.5**	**6.0–6.5**	**6.3**	**(1.1–) 1.2–1.6 (–1.7)**	**1.4**	**5.4–6.4**	**5.9**
***H. sitnensis* holotype**	**7.9–9.2 (–9.3)**	**8.5**	**(7.7–) 8.1–9.2**	**8.6**	**(7.7–) 8.1–8.9 (–9.2)**	**8.5**	**(5.9–) 6.3–6.9**	**6.5**	**1.0–1.8**	**1.5**	**5.5–6.9 (–7.1)**	**6.1**

Species/ collection	Frontal length	Frontal L	Frontal width	Frontal W	Lateral length	Lateral L	Lateral width	Lateral W	Length echinuli	L echinuli	Width subic. hyphae	W subic. hyphae
***P. tristoides* sp. nov. holotype**	**7.7–8.6 (–8.8)**	**8.2**	**(7.4–) 7.7–9.3 (–9.5)**	**8.5**	**(7.9–) 8.0–8.6**	**8.2**	**6.0–6.5 (–6.7)**	**6.3**	**(0.5–) 0.7–0.9 (–1.1)**	**0.8**	**(4.7–) 4.9–7.1 (–7.6)**	**6.0**
P. umbrina	**7.7–9.3 (–9.4)**	**8.3–8.7**	**(7.6–) 7.9–9.1 (–9.4)**	**8.4–8.7**	**8.0–9.3 (–9.6)**	**8.4–8.7**	**(5.1–) 5.6–6.7 (–6.9)**	**6.0–6.1**	**(0.7–) 0.8–1.5**	**1.1–1.2**	**3.3–4.8 (–5.3)**	**4.0–4.3**
epitype	(8.0–) 8.4–9.1	8.7	(7.7–) 8.1–9.0 (–9.1)	8.5	(8.0–) 8.2–9.1 (–9.6)	8.6	(5.1–) 5.8–6.7 (–6.8)	6.1	0.9–1.5	1.2	(3.7–) 3.8–4.7 (–4.9)	4.3
neotype	(7.9–) 8.0–9.3 (–9.4)	8.7	8.2–9.1	8.7	8.1–9.3	8.7	(5.4–) 5.8–6.5 (–6.7)	6.0	(0.7–) 0.9–1.4	1.2	3.3–4.7 (–5.3)	4.0
O F110268	7.7–8.9 (–9.0)	8.3	(7.6–) 7.9–9.1 (–9.4)	8.4	8.0–9.1 (–9.5)	8.4	(5.3–) 5.6–6.7 (–6.9)	6.1	0.8–1.3	1.1	3.5–4.8 (–4.9)	4.1
***P. umbrinascens* sp. nov. holotype**	**(8.5–) 8.7–9.4 (–9.6)**	**8.9**	**(8.4–) 8.7–9.2 (–9.3)**	**8.9**	**8.5–9.2 (–9.4)**	**8.9**	**(5.7–) 6.0–6.5**	**6.2**	**(0.9–) 1.0–1.9 (–2.0)**	**1.6**	**3.1–) 3.2–4.3 (–4.8)**	**3.7**

Table 4. Ecological data based on Scandinavian collection information and worldwide UNITE metadata.

Host	pH	Habitat	Basidiomata collections	Soil and root tip sequences
Abies alba, *Alnus rubra*, *Betula nana*, *B. pubescens* ssp. *czerepanovii*, *B. pubescens* ssp. *pubescens*, *Dryas octopetala*, *Fagus sylvatica*, *Picea abies*, *P. glauca*, *Picea mariana*, *Pinus banksiana*, *P. pinaster*, *P. sylvestris*, *Pseudotsuga menziesii*, *Pyrola media*, *Quercus petraea*, *Salix polaris*, *Tsuga canadensis*	Low to high	Tundra Deciduous forest Coniferous forest Mixed forest	74	62
Castanea sativa, *Cedrus libani*, *Neottia ovata*, *Picea abies*, *Quercus* sp.	Intermediate to high	Deciduous forest Coniferous forest Mixed forest	24	5
Betula pendula, *Fagus sylvatica*	Intermediate to high	Deciduous forest Mixed forest	19	2
Pinus densiflora, *P. massoniana*, *P. sylvestris*, *P. thunbergii*	High	Coniferous forest Mixed forest	9	3
Castanea sp., *P. tremula*	High	Deciduous forest Coniferous forest Mixed forest	5	4
Betula pendula, *Larix decidua*, *Picea glauca*	–	–	2	4
–	High	Coniferous forest Mixed forest	5	0
Alnus incana, *A. mandschurica*	Intermediate	Deciduous forest	2	2
Pseudotsuga menziesii	Intermediate	Mixed forest	2	2
Rhododendron decorum	–	–	1	3
Cephalanthera damasonium, *Populus alba*	Intermediate	Mixed forest	1	2
Corylus avellana	High	Deciduous forest	1	2
–	High	Mixed forest	2	0

distinguishing between species, whereas the Q value (spore length/width) was considerably less so. The presence of wide subicular hyphae, a blue green reaction in the hymenium and subhymenium and amyloid material present in and on the same, in the species of the clade containing *P. tristis*, *P. alobata*, *P. sciastra* and *P. tristoides*, is also worthy of notice. These are, however, characters also present in species of other clades, e.g. (*P. pinophila*, *P. pluriloba*) and (*P. umbrina*, *P. rotundispora*), where they are not shared amongst all species included and may thus represent plesiomorphic characters.

The specimens examined of *P. sciastra* display considerable morphological variation; the spore and subicular hyphal measurements of TAA187322 deviate markedly from those of SS359 and O F110317. Interestingly, *P. sciastra* is also more genetically variable than the other species studied.

For many species, we recorded a blue-green reaction of subhymenial hyphae, basidia, encrusting material and sometimes also of spores, to occur in KOH. We only observed the reaction occurring close to air bubbles or adjacent to the edges of cover glasses and we did not record it close to the centre of preparations free from air bubbles, unless they had been made slowly and hence had allowed air to come into contact with the entire samples before the application of a cover glass. The same structures often, but not always, also had an amyloid reaction in Melzer's reagent. Both the blue green and the amyloid reaction could be used as species-separating characters (see further under "Taxonomy"). When present, the encrustation was most prevalent on the bases of basidia, but common also on the upper part of subhymenial hyphae. Occasionally, it was also appearing on subicular hyphae.

Ecology and geographical distribution

We found the majority of the collections and sequences included in this study to belong to *P. umbrina* (Table 4). As shown by the origin of these (Fig. 6), *P. umbrina* is distributed across at least 12 countries in Europe and North America, where it has been found growing with 18 different hosts. It is present in Arctic/alpine vegetation above the treeline as well as in coniferous, deciduous and mixed forests and has been encountered on soils with pH ranging from low to high.

The other species in the *P. tristis* group have been encountered markedly fewer times, with a smaller number of hosts, in less diverse habitats and mostly within a smaller geographical range. They have all been collected on soil with intermediate to high pH or both. Similarly to *P. umbrina*, however, many species seem to form ectomycorrhiza with a range of hosts; they have been collected on the root tips of both broadleaved and coniferous trees, as well as orchid species. Three species seem to have a limited host range: *P. pinophila* has only been found inhabiting the roots of *Pinus* L. species, while *P. alnophila* and *P.* sp. 2 have been found exclusively on the roots of *Alnus* Mill. Two species, meanwhile, now have a different confirmed geographical distribution than previously documented: the only verified sequences and basidiomata of *P. tristis* here studied originate in Europe, while *P. atrofusca* now have no confirmed findings there – the only validated findings are currently the Arizona holotype and three Chinese root tip sequences.

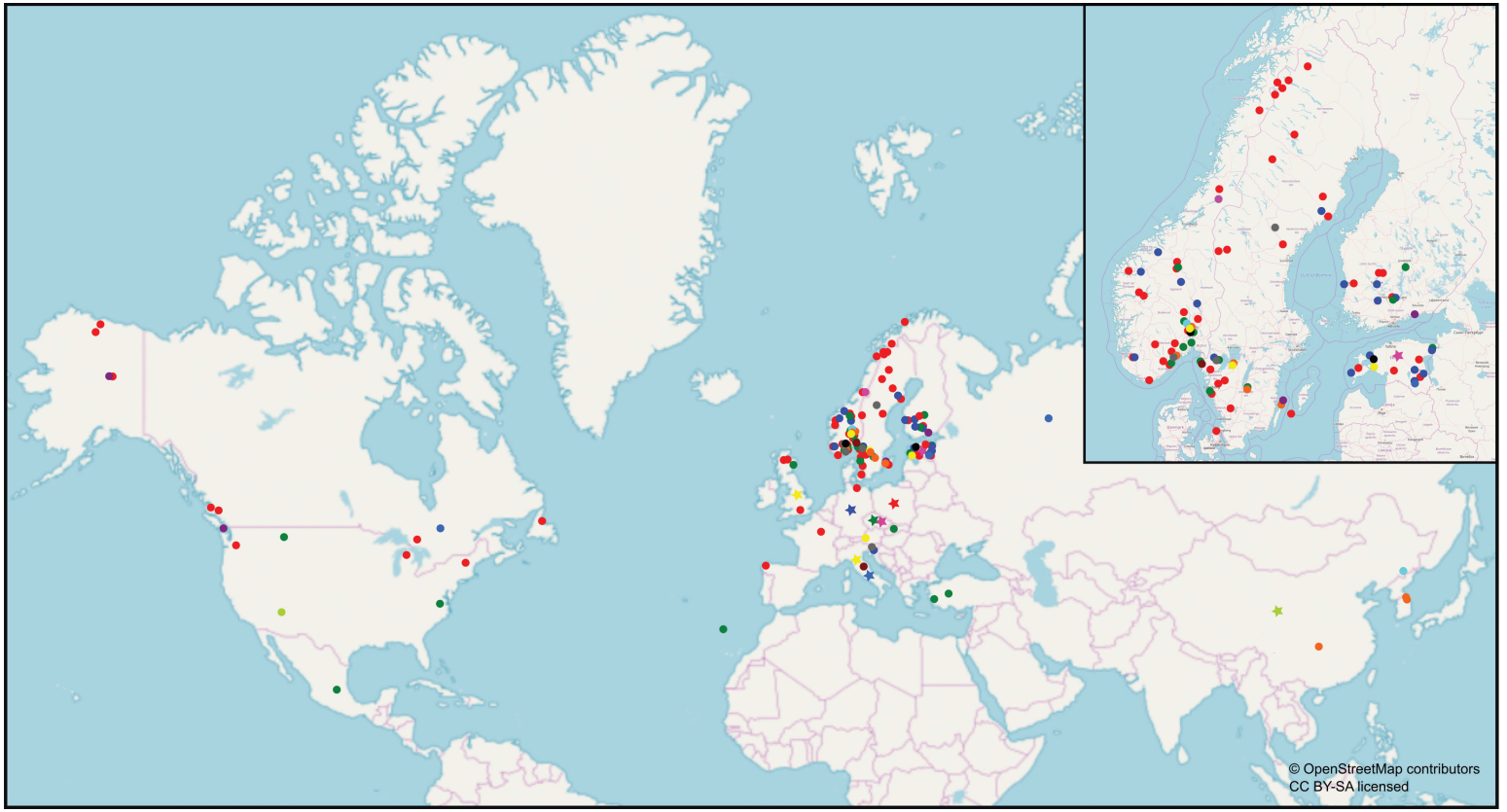


Figure 6. World distribution of new and previously described species in the *P. tristis* group, excluding doubtful taxa. Red – *P. umbrina*; pink – *P. tristoides*; dark green – *P. sciastra*; pale green – *P. atrofusca*; dark blue – *P. tristis*; pale blue – *P. media*; orange – *P. pinophila*; yellow – *P. rotundispora*; grey – *P. alobata*; black – *P. abundiloba*; brown – *P. umbrinascens*; turquoise – *P. alnophila*; purple – *P. pluriloba*.

Taxonomy

We provide descriptions of ten species new to science and of previously described accepted, dubious and excluded species in the *P. tristis* group. A worldwide key to all recognised and dubious species is also presented.

Key to the species in the *P. tristis* group

Pseudotomentella species with brownish spores and subicular hyphae, lacking clamps and chlamydospores.

1 Basidiome with hyphal cords containing skeletal hyphae, mean width of subicular hyphae 2.3 µm ..... ***P. atrofusca***
– Basidiome lacking hyphal cords and skeletal hyphae, mean width of subicular hyphae 3.4–6.5 µm **2**
2 Basidiome when dried hard and brittle ..... ***H. rhacodium***
– Basidiome when dried soft cottony or soft, yet rather firm and compact and ± elastic **3**
3 Basidiome when dried brown in all parts; blue or green colours are completely lacking in immature parts and in the subhymenium of mature parts. No blue green reaction in KOH (though basidia might be very pale green) **4**
– Basidiome when dried with blue or green colours in immature parts and in the subhymenium of mature parts. Subhymenial hyphae and basidia with blue green (often strong) reaction in KOH, in the presence of air **5**
4 Mean length of echinuli 1.1–1.2 µm, mean length of sterigmata 9.6–10.5 µm, spores with three-six lobes or corners (rarely unlobed), basidia very pale greenish in KOH, sometimes with a slightly brown or blue hue, subiculum orange brown, immature hymenium and subhymenium initially pale brown ..... ***P. umbrina***
– Mean length of echinuli 1.6 µm, mean length of sterigmata 8.6 µm, spores with three-four lobes or corners (rarely five-six lobes), basidia pale brown to brown in KOH, sometimes with a greyish hue, subiculum pale yellowish-brown to pale orange brown, immature hymenium and subhymenium initially yellowish-white to pale brown ..... ***P. umbrinascens***
5 Subicular hyphae narrow: mean width < 5 µm **6**
– Subicular hyphae wide: mean width > 5 µm **10**
6 Spores short: mean length ≤ 7.8 µm ..... ***P. rotundispora***
– Spores long: mean length ≥ 8.7 µm **7**

7 Basidiome when dried soft cottony in texture, hymenium bluish-grey (sometimes with a slightly brown hue) also when mature, close to *Alnus* ..... ***P. alnophila***

– Basidiome when dried soft, yet rather firm and compact and ± elastic, mature hymenium various shades of brown, with various hosts **8**

8 Mean width of subicular hyphae 3.6–4.1 µm, mean width of subhymenial hyphae 3.9–4.0 µm, mean lateral spore width 6.3–6.6 µm, spores commonly roundedly star-shaped, often close to *Pinus*..... ***P. pinophila***

– Mean width of subicular hyphae > 4.1 µm, mean width of subhymenial hyphae > 4.0 µm, mean lateral spore width ≥ 7.3 µm, spores generally angular-nodulose, with various hosts **9**

9 Mean width of subicular hyphae 4.8–5.1 µm, noticeably wider than subhymenial hyphae, frontal face of spores with mean dimensions approximately 9.8 × 10.2 µm..... ***P. pluriloba***

– Mean width of subicular hyphae 4.1–4.6 µm, with ± the same width as subhymenial hyphae, frontal face of spores with mean dimensions approximately 8.9–9.3 × 9.2–9.8 µm..... ***P. media***

10 Spores short: mean length < 8.5 µm..... **11**

– Spores long: mean length > 9.7 µm..... **13**

11 Mean spore length 6.7–7.3 µm, spores star-shaped ..... ***P. sciastra***

– Mean spore length 8.2–8.6 µm, spores angular to nodulose..... **12**

12 Mean length of echinuli 0.8 µm (maximal length 1.1 µm), mean sterigmal length approximately 8.6 µm ..... ***P. tristoides***

– Mean length of echinuli 1.4 µm (maximal length 1.7–1.9 µm), mean sterigmal length 9.4–10.2 µm ..... ***P. tristis***

13 Mean lateral spore dimensions 10.9 × 8.5 µm, sterigmata very long – mean length 14.7 µm..... ***P. longisterigmata***

– Mean lateral spore dimensions 9.6–9.9 × 7.1–7.7 µm, sterigmata normal – mean length 10.0–12.3 µm..... **14**

14 Spores with three-five lobes or corners, mean width of subicular hyphae 4.8–5.1 µm, mean sterigmal length 11.5–12.3 µm, mean frontal spore width 10.2 µm..... ***P. pluriloba***

– Spores either unlobed or with four-seven lobes or corners, mean width of subicular hyphae 5.5–6.1 µm, mean sterigmal length 10.0–11.5 µm, mean frontal spore width 9.5–9.8 µm **15**

15 Spores unlobed, amyloid reaction observed in encrustation on basidia and subhymenial hyphae ..... ***P. alobata***

– Spores with four-seven lobes or corners, amyloid reaction not seen in encrustation on basidia and subhymenial hyphae..... ***P. abundiloba***

Accepted taxa

***Pseudotomentella abundiloba* Svantesson, sp. nov.**
MycoBank No.: MB828974
Fig. 7

Type. NORWAY. Oslo (county): Oslo (municipality), Bygdøy, Hengsåsen, boreonemoral mixed forest on soil with high pH, 22 September 2010, S. Svantesson (holotype: O F110312!, GenBank Acc. No. ITS: MK290731).

UNITE SH. SH032598.07FU

Etymology. The name refers to the spores, which are abundantly lobed.

Description. Basidiomata annual, resupinate, membranaceous, effused to several tens of centimetres in diameter. Mature parts continuous, with a rather firm, fibrous and compact, yet quite soft and elastic texture. Hymenium smooth, but sometimes strongly undulating; brown with a pinkish hue. Immature parts discontinuous, byssoid, with a cottony texture. Subhymenium and hymenium of immature parts blue grey to brown grey. Subiculum well developed, loose, fibrous, orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium. All characters recorded in dried state.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic; clamp connections and reaction in Melzer's reagent absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (4.3–) 4.8–6.9 (–7.2) µm wide, with a mean width of 5.5–6.1 µm; orange brown to dark brown in both KOH and water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (4.0–) 4.1–6.8 (–7.2) µm wide, with a mean width of 5.5–5.7 µm; in the upper parts, hyaline to orange brown or orange green in KOH, with a blue green reaction in the presence of air; in the lower parts, pale orange brown to orange brown in KOH, unchanged in air; in water with strongly granular contents, orange green.

Encrustation granular, inamyloid; hyaline to orange brown or orange green in KOH, blue green in the presence of air; orange green in water; common to rare, usually scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (63–) 64–92 (–93) × (8.7–) 10.0–14.4 (14.9) µm; mean dimensions: 70–81 × 11.0–12.0 µm. Sterigmata (8.4–) 9.0–12.9 (–13) µm long, with a mean length of 10.0–11.5 µm. Colours and reactions the same as for the upper parts of the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

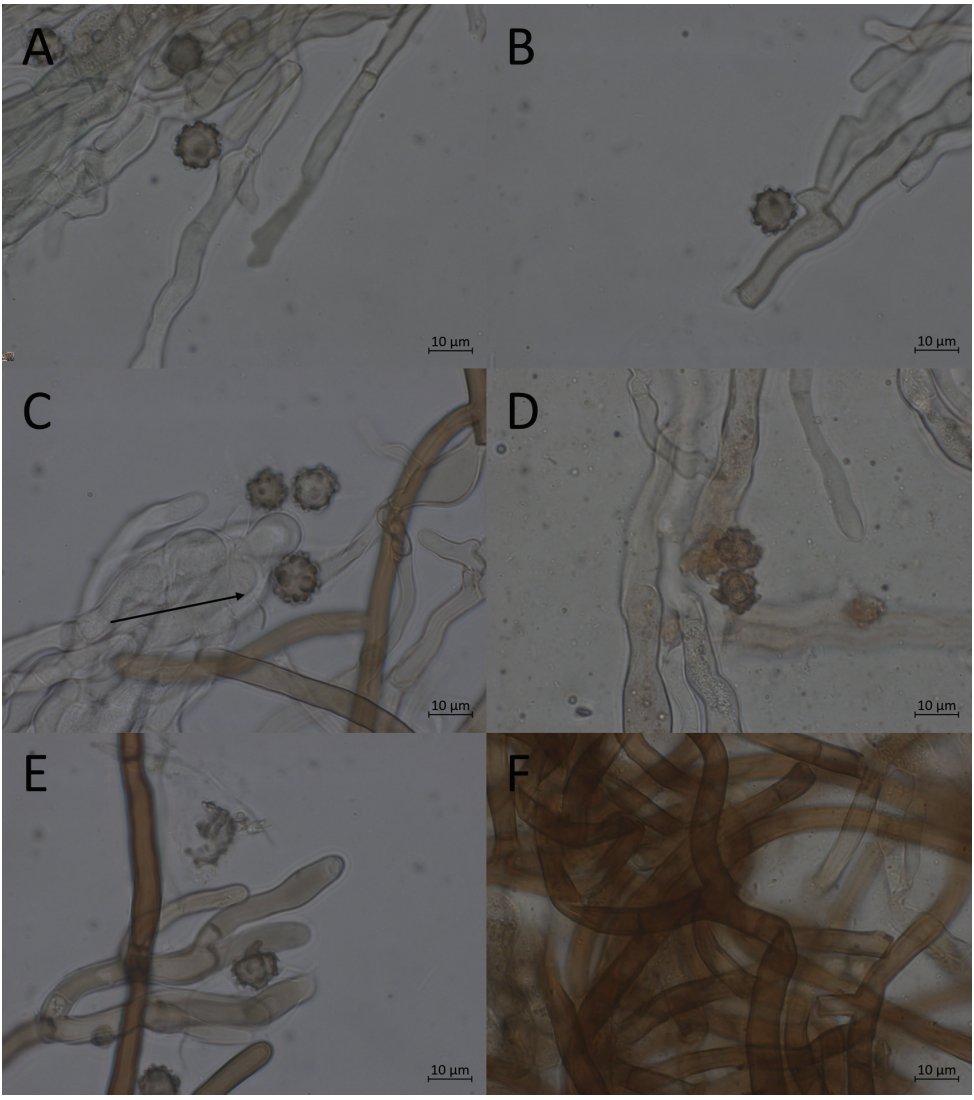


Figure 7. Micromorphological features of *P. abundiloba* in KOH. **A, B** basidiospores in frontal face (TU 110852) **C** in tilted frontal face (TU 110852) **D, E** in lateral face (TU 110852) **F** subicular hyphae (holotype).

Basidiospores in frontal face generally with a subcircular basic shape and a star-shaped, angular, nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with four-seven, low but distinct, rounded to square lobes or corners; unlobed, broadly ovoid spores and rounded, heart-shaped spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (8.8–) 9.2–10.5 ×

(8.0–) 8.6–10.7 (–10.8) µm; mean dimensions: 9.8 × 9.5–9.6 µm; Q-value: 0.9–1.2; mean Q-value: 1.0. Echinuli (0.9–) 1.1–1.8 (–1.9) µm long, with a mean length of 1.2–1.5 µm. Lateral face ellipsoid to semicircular, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: (8.9–) 9.3–10.1 (–10.5) × (6.7–) 7.0–8.1 (–8.2) µm; mean dimensions: 9.7–9.8 × 7.3–7.7 µm; Q-value: 1.2–1.4 (–1.5); mean Q-value: 1.3. Colour in KOH pale orange green to orange brown, in the presence of air sometimes with a blue green reaction; in water pale orange green; inamyloid.

Chlamydospores lacking.

Habitat. The type collection was obtained in an old, mixed forest on soil with high pH. No additional sequences are available in UNITE.

Distribution. Basidiomata encountered in: Estonia and Norway.

Remarks. Within the *P. tristis* group, the basidiomata of *P. abundiloba* are recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae, long, abundantly lobed spores and inamyloid encrustation on subhymenial hyphae and basidia. *Pseudotomentella abundiloba*, *P. pluriloba* and *P. media* can appear similar, but none of them has abundantly lobed spores. *Pseudotomentella media* further differs by having smaller spores and narrower subicular hyphae, while *P. pluriloba* has narrower subicular hyphae, longer sterigmata and frontally wider spores and *P. alobata* has amyloid encrustation on its subhymenial hyphae and basidia.

Additional specimens studied. ESTONIA. Lääne: Ridala, between Uneste and Võnnu, Ehmja-Turvalepa Special Conservation Area, nutrient-rich, boreonemoral forest, 25 September 2012, L. Tedersoo (TU 110852*).

***Pseudotomentella alnophila* Svantesson, sp. nov.**
MycoBank No.: MB828998
Fig. 8

Type. NORWAY. Buskerud: Ringerike, Juveren N, boreonemoral *Alnus incana* forest on soil with intermediate pH, 25 September 2010, S. Svantesson and N. Svensson (holotype: O F110313!, GenBank Acc. No. ITS: MK290715).

UNITE SH. SH218588.07FU

Etymology. The name refers to the ectomycorrhizal association of the species, which always seems to be with *Alnus*.

Description. Basidiomata annual, resupinate, membranaceous, effused. Mature parts continuous, with a soft cottony texture. Hymenium smooth; blue grey, sometimes with a slightly brown hue. Immature parts discontinuous, byssoid, with a soft cottony texture. Subhymenium and hymenium of immature parts pale blue grey to blue grey. Subiculum thin to well developed, loose, fibrous, orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium. All characters recorded in dried state.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

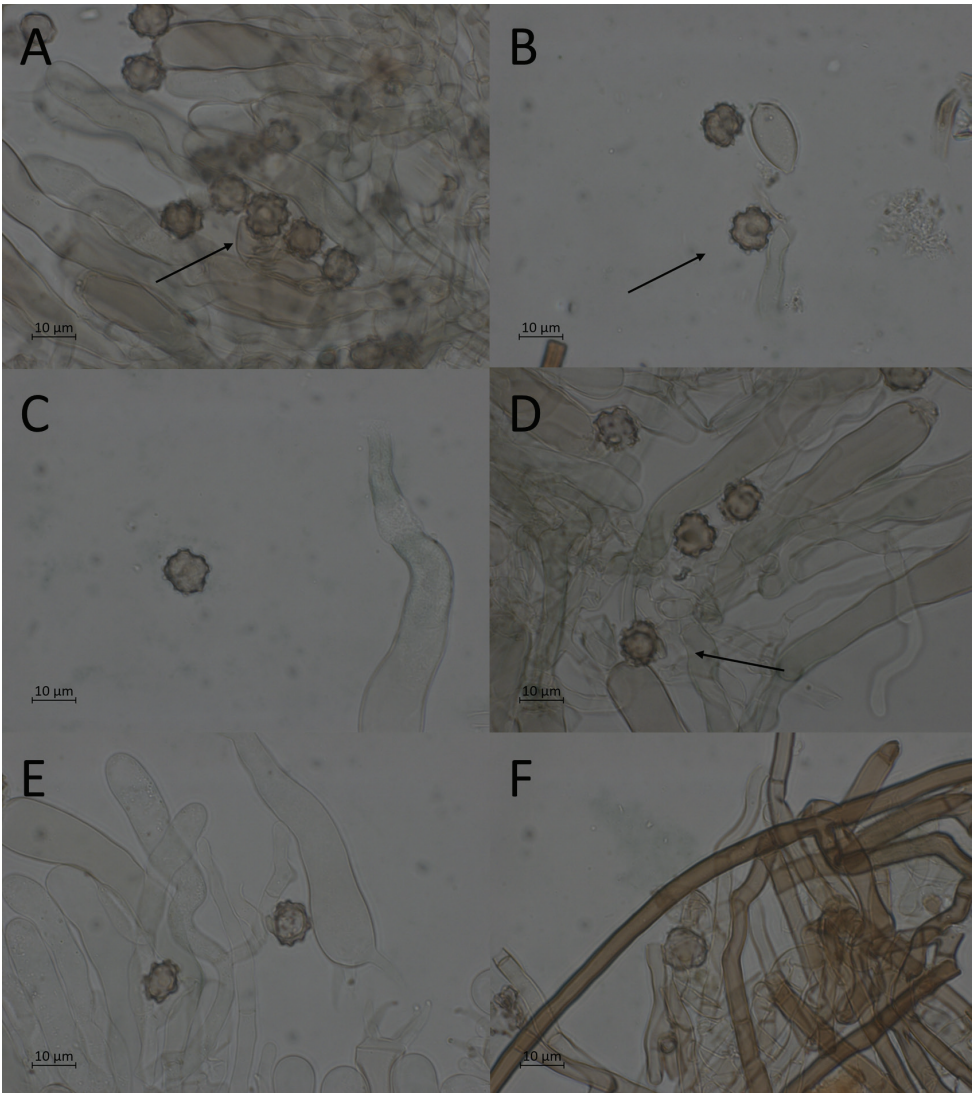


Figure 8. Micromorphological features of *P. alnophila* in KOH. Holotype: **A, B, C** basidiospores in frontal face **D, E** in lateral face **E** subicular hyphae.

Hyphal system monomitic, clamp connections and reaction in Melzer's reagent absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae 4.0–5.0 (–5.1) µm wide, with a mean width of 4.5 µm; orange brown to dark brown in KOH and orange to orange brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.2–) 3.4–5 (–5.6) µm wide, with a mean

width of 4.1 µm; hyaline to pale orange brown in KOH, blue green in the presence of air; pale green in water, with strongly granular contents.

Encrustation not seen.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (66–) 67–93 (–100) × (11.2–) 11.3–14.2 (–15.0) µm; mean dimensions: 83 × 12.8 µm. Sterigmata (9.5–) 11–14.5 (–14.8) µm long, with a mean length of 8.6 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with three-five distinct corners or rounded to square lobes; broadly ovoid spores and rounded, heart-shaped spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (8.8–) 9.0–10.1 (–10.4) × 9.2–10.2 (–10.6) µm; mean dimensions: 9.5 × 9.8 µm; Q-value: 0.9–1.0; mean Q-value: 1.0. Echinuli (0.8–) 0.9–1.7 µm long, with a mean length of 1.2 µm. Lateral face ellipsoid to ovoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: 9.0–10.6 × (6.6–) 6.9–8.1 (–8.2) µm; mean dimensions: 9.6 × 7.7 µm; Q-value: 1.2–1.3 (–1.4); mean Q-value: 1.3. Colour in KOH pale orange brown to pale orange green, in the presence of air occasionally with a blue green reaction; in water pale green to pale orange green; inamyloid.

Chlamydospores lacking.

Habitat. The only specimens recorded to date of *P. alnophila* is the type collection and one other collection from the same locality, which is a mature and, at the collection site pure, stand of *Alnus incana* on clay soil with intermediate pH. In addition, UNITE sequence metadata show that the species forms ectomycorrhiza with at least *Alnus mandschurica* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Norway. Soil or root tip samples confirm presence also in: China.

Remarks. Within the *P. tristis* group, the basidiomata of *P. alnophila* can be recognised by their lack of hyphal cords and skeletal hyphae and their soft cottony texture after drying, bluish to greenish colour of immature parts, narrow hyphae, long spores, bluish-grey mature hymenium (sometimes with a slightly brown hue) and their association with *Alnus*. *Pseudotomentella pluriloba*, *P. media* and *P. pinophila* are similar, but they all have basidiomata that are compact and rather firm after drying and whose mature parts are some shade of brown, without any bluish hue. *Pseudotomentella pluriloba* also has slightly longer spores and echinuli and wider subicular hyphae, while *P. media* and *P. pinophila* have generally slightly smaller microcharacters. *Pseudotomentella pinophila* also has a different spore shape. In addition, neither of these species has been recorded as being associated with *Alnus*.

Additional specimens studied. NORWAY. Buskerud: Ringerike, Juveren N, boreonemoral, *Alnus incana* forest on soil with intermediate pH, 25 September 2010, S. Svantesson and N. Svensson (O F110314).

***Pseudotomentella alobata* Svantesson, sp. nov.**
MycoBank No.: MB828999
Fig. 9

Type. SWEDEN. Dalsland, Mellerud, Skållerud, Norgekullen SW, coniferous forest on soil with high pH, 20 September 2017, S. Svantesson 425 (holotype: GB!, GenBank Acc. No. ITS: MK290696).

UNITE SH. SH030577.07FU

Etymology. The name refers to the spores, which commonly lack lobation.

Description. Basidiomata annual, resupinate, membranaceous, effused – often to several tens of centimetres in diameter. Mature parts continuous, with a cottony texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; brown, purplish-brown or blue-greyish-brown when fresh, brown with a pinkish hue when dried. Immature parts discontinuous, byssoid, with a cottony texture both when fresh and when dried. Subhymenium and hymenium of immature parts blue to blue grey when fresh and blue grey to brown grey when dried. Subiculum well developed, loose, fibrous, orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (4.3–) 4.6–7.4 (–7.6) µm wide, with a mean width of 5.6–5.9 µm; orange in both KOH and water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.1–) 3.4–6.9 µm wide, with a mean width of 4.0–4.5 µm; hyaline to pale green in KOH, blue green in the presence of air; yellow to pale orange yellow in water, with strongly granular contents.

Encrustation granular, amyloid; purple in KOH, dark blue green in the presence of air; dark brown in water; usually common and scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (63–) 64–91 (–98) × (10.2–) 10.5–14.2 (–14.3) µm; mean dimensions: 74–77 × 11.3–12.1 µm. Sterigmata 8.5–12.1 (–12.4) µm long, with a mean length of 10.0–10.3 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

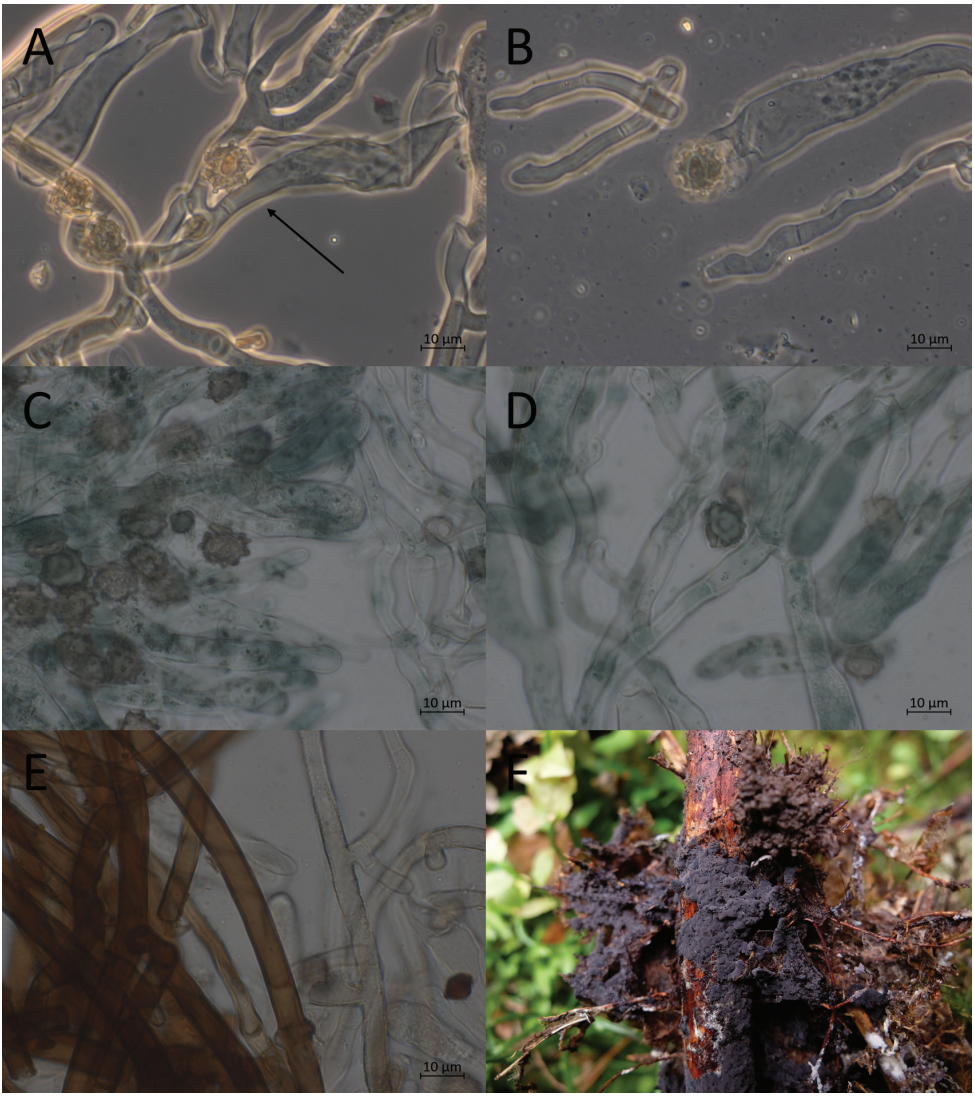


Figure 9. Morphological features of *P. alobata*, mounted in KOH and macroscopically. **A, B** basidiospores in frontal face (O F110315) **C, D** in lateral face (O F110316) **E** subicular hyphae (TU 115626) **F** mature basidiome (holotype).

Basidiospores in frontal face generally with a subcircular basic shape and an unlobed or occasionally weakly pronounced, rounded, heart-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Subcircular, three-five-lobed spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (9.0–) 9.1–10.7 × (8.4–) 8.9–10.5 (–10.7) µm; mean dimensions: 9.7–10.1 × 9.5–9.8 µm; Q-value: (0.9–) 1.0–1.1; mean Q-value: 1.0. Echinuli 1.2–1.8 (–1.9) µm long, with a mean length of 1.4–1.7 µm. Lat-

eral face ellipsoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: (8.9–) 9.1–10.3 × (6.5–) 6.7–8.2 µm; mean dimensions: 9.7–9.9 × 7.1–7.4 µm; Q-value: (1.2–) 1.3–1.5; mean Q-value: 1.3–1.4. Colour in KOH pale brownish-yellow, in the presence of air often with a blue green reaction; in water pale greenish-yellow to pale orange yellow; occasionally amyloid.

Chlamydospores lacking.

Habitat. Data on habitat are scarce to date, but recent Scandinavian collections have been made in old growth coniferous or mixed forests on soil with high pH.

Distribution. Basidiomata encountered in: Norway, Slovenia and Sweden. No sequences originating from soil or root tip samples in UNITE.

Remarks. Within the *P. tristis* group, the basidiomata of *P. alobata* are recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae, long, unlobed spores and amyloid encrustation on subhymenial hyphae and basidia. *Pseudotomentella abundiloba*, *P. pluriloba* and *P. media* can appear similar, but none of them has spores which generally are unlobed. *P. media* further differs by having smaller spores and narrower subicular hyphae, while *P. pluriloba* has narrower subicular hyphae, longer sterigmata and frontally wider spores than *P. alobata*. *Pseudotomentella abundiloba* sometimes has encrusted subhymenial hyphae and basidia, but without amyloid reaction.

Additional specimens studied. NORWAY. Telemark: Bamble, Rognsflaugane, boreonemoral, mixed forest on soil with high pH, 2 September 2010, K.-H. Larsson and S. Svantesson (O F110316*); Telemark: Tokke, Dalen, Huvestad, boreonemoral, mixed forest on soil with high pH, 28 September 2010, S. Svantesson and N. Svensson (O F110315*);

SLOVENIA. Radovljica: Triglav National Park, Pokljuka plateau, transition zone between secondary spruce forest (in parts with remnants of primary *Fagus sylvatica/Acer pseudoplatanus* forest) and natural *Larix decidua* stand with individual trees of *Pinus mugo*, *Sorbus aucuparia* and *Salix* sp., 1530 m a.s.l., 20 September 2012, U. Kõljalg (TU 115626*);

SWEDEN. Ångermanland: Edsele, Djupdalsmyran, Stordjupdalen, on *Picea abies*, 29 August 2002, K.-H. Larsson 11873* (GB 0087566).

Pseudotomentella atrofusca **M.J.Larsen, Bull. Torrey Bot. Club. 98: 39 (1971)**
Fig. 10

Type. UNITED STATES. Arizona: Fort Valley, Coconino, on *Pinus ponderosa* Laws., 21 September 1967, R. L. Gilbertson 7553 (holotype: ARIZ!, GenBank Acc. No. ITS: MK290732; isotype: SSMF 685–4578).

UNITE SH. SH005338.07FU

Description. Basidiome annual, resupinate, membranaceous, effused. Mature parts continuous, with a cottony texture. Hymenium smooth, brown. Immature parts discon-

tinuous, byssoid, with a cottony texture. Subhymenium and hymenium of immature parts initially whitish-grey to whitish-grey brown, when more mature blue grey to brown grey. Subiculum thin, loose, fibrous, pale brown; often forms the outer edge of the basidiome, extending noticeably beyond the hymenium. All characters recorded in dried state.

Hyphal cords connecting to the edge of the basidiome and thinning out underneath; whitish to pale brown. Individual cords dimitic; formed by a sheathing layer of skeletal hyphae and two layers of generative hyphae; the outer generative hyphae thinner and the inner ones wider, the latter often swollen interseptally. Skeletal hyphae 1.1–1.4 (–1.5) µm wide, with a mean width of 1.3 µm. Outer generative hyphae (2.2) 2.3–2.9 µm wide, with a mean width of 2.6 µm. Inner generative hyphae (3.8) 3.9–5.3 (5.5) µm wide, with a mean width of 4.5 µm. All hyphae pale yellowish-brown in both KOH and water.

Hyphal system dimitic, clamp connections and reaction in Melzer's reagent absent from all hyphae.

Subicular hyphae of two kinds: (1) generative hyphae noticeably long and straight, thick-walled; forming a loose tissue, in which (2) skeletal hyphae occur sparsely (most common in areas to where hyphal cords attach). Generative hyphae (1.7–) 1.8–2.8 µm wide, with a mean width of 2.3 µm; pale yellowish-brown to yellowish-brown in both KOH and water. Skeletal hyphae with the same width and colour as in the hyphal cords.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (1.8–) 2.1–3.0 µm wide, with a mean width of 2.5 µm; hyaline to pale green in KOH, blue green in the presence of air; hyaline to pale blue green in water, with strongly granular contents.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (33–) 34–56 (–59) × (5.8–) 6.2–7.8 (7.9) µm; mean dimensions: 44 × 7.2 µm. Sterigmata 4.4–5.6 (–6.8) µm long, with a mean length of 5.1 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes star or cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with five distinct, square lobes, but depending on the precise angle sometimes perceived as three; four-lobed spores occasionally occurring; abnormally large spores originating from two-sterigmate basidia infrequently seen. Frontal dimensions: (6.1–) 6.2–7.0 (–7.1) × (5.8–) 6.3–7.2 (–7.3) µm; mean dimensions: 6.6 × 6.8 µm; Q-value: 0.9–1.0; mean Q-value: 1.0. Echinuli 0.6–0.9 (–1.1) µm long, with a mean length of 0.8 µm. Lateral face ellipsoid to ovoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: 6.3–6.9 (–7.3) × (4.0–) 4.1–4.8 (–5.0) µm; mean dimensions: 6.5 × 4.4 µm; Q-value: (1.3–) 1.4–1.6; mean Q-value: 1.5. Colour in both KOH and water pale brownish-yellow to pale blue green; inamyloid.

Chlamydospores lacking.

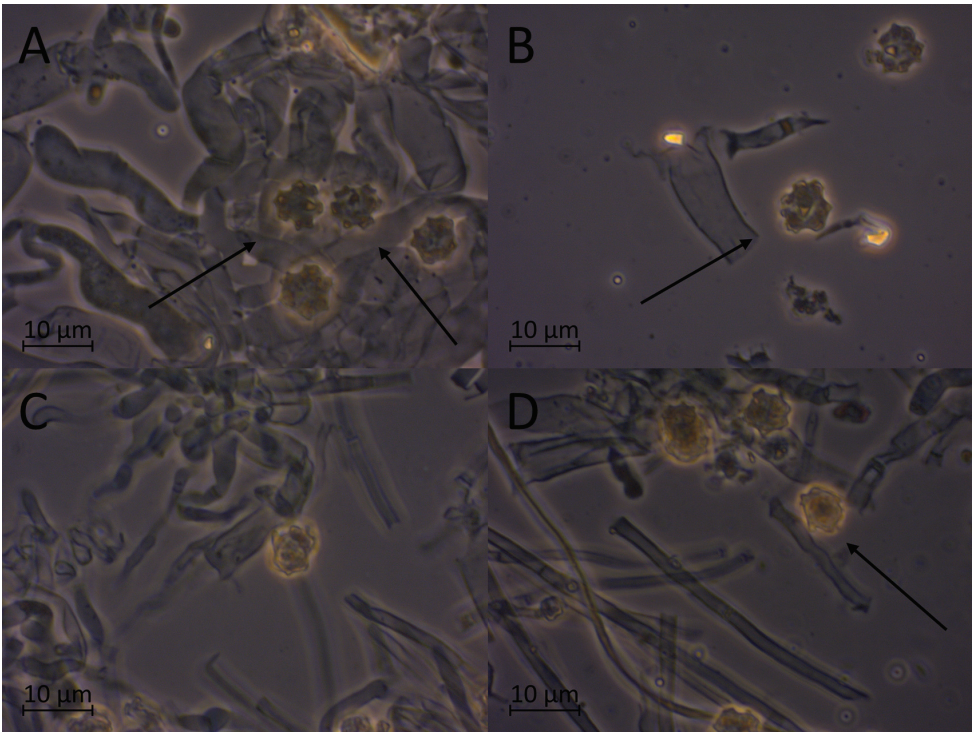


Figure 10. Micromorphological features of *P. atrofusca* in KOH. Holotype: **A, B** basidiospores in frontal face **C, D** in lateral face.

Habitat. The only specimen recorded to date of *P. atrofusca* is the type collection, which was collected on wood of *Pinus ponderosa*. Available UNITE sequences originate from root tips of *Rhododendron decorum* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiome encountered in: United States. Soil or root tip samples confirm presence also in: China.

Remarks. Within the *P. tristis* group, the basidiome of *P. atrofusca* can be recognised by its hyphal cords and skeletal hyphae. These features make it unique within the group and the risk for confusion with any other described species should be small. Outside the *P. tristis* group, *P. rhizopunctata* is somewhat similar, but differs from *P. atrofusca* by the presence of chlamydospores on its hyphal cords.

Pseudotomentella media **Svantesson & Kõljalg, sp. nov.**
MycoBank No.: MB829000
Fig. 11

Type. ESTONIA. Valga: Otepää, Trommi, 12 September 2012, U. Kõljalg (holotype: TU 115609!, GenBank Acc. No. ITS: MK290714).

UNITE SH. SH005336.07FU

Etymology. The name refers to the middling morphological characters of the species, relative to other species in the *P. tristis* group.

Description. Basidiomata annual, resupinate, membranaceous, effused to approximately ten centimetres in diameter. Mature parts continuous, with a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; brown with a reddish hue, both when fresh and when dried. Immature parts discontinuous, byssoid, with a cottony texture when dried. Subhymenium and hymenium of immature parts blue to greenish-blue when fresh and pale blue grey or blue grey to grey brown when dried. Subiculum well developed, loose, fibrous, pale brown to pale orange brown; forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (3.6–) 3.7–5.0 (–5.4) µm wide, with a mean width of 4.1–4.6 µm; orange brown to brown in both KOH and water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.2–) 3.4–5.6 (–5.7) µm wide, with a mean width of 4.3–4.4 µm; pale brown, pale orange brown, pale greenish-brown or hyaline in KOH, blue green in the presence of air; orange green to brown in water, with strongly granular contents. Some subhymenial hyphae with a pink colour in water and an amyloid reaction.

Encrustation granular, probably amyloid (hard to observe due to the colour); blackish in KOH, dark blue green in the presence of air; blackish in water; scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate or sometimes narrowly clavate or clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (57–) 58–84 (–87) × (8.8–) 9.6–11.8 (–12.5) µm; mean dimensions: 74–77 × 10.2–10.7 µm. Sterigmata (9.4–) 9.5–11.4 (–11.7) µm long, with a mean length of 10.0–10.8 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. A majority of the spores with three-six indistinct corners to distinct, square lobes; unlobed subellipsoid spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (7.8–) 8.0–9.5 × (8.3–) 8.5–9.9 (–10.1) µm; mean dimensions: 8.9–9.3 × 9.2–9.8 µm; Q-value: 0.9–1.0; mean Q-value: 1.0. Echinuli (0.8–) 0.9–1.6 µm long, with a mean length of 1.1–1.2 µm. Lateral face ellipsoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: (8.8–) 8.9–9.6 × (6.6–) 7.0–7.9 µm; mean dimensions: 9.1–9.4 × 7.3–7.6 µm; Q-value: 1.2–1.3;

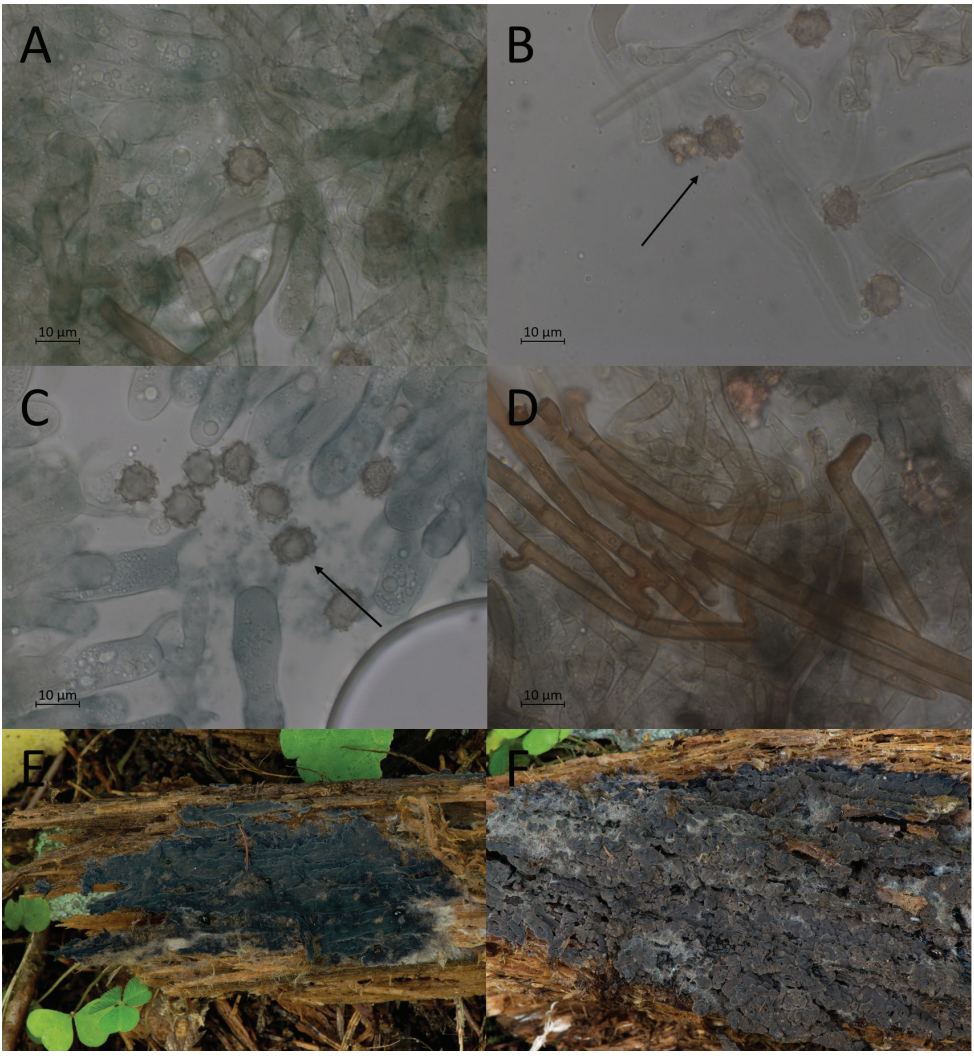


Figure 11. Morphological features of *P. media*, mounted in KOH and macroscopically. **A, B** basidiospores in frontal face (TU 115608) **C** in lateral face (TU 115608) **D** subicular hyphae (TU 115608) **E** younger basidiome (TU 115608) **F** mature basidiome (holotype).

mean Q-value: 1.2–1.3. Colour in KOH pale brown to brown or pale orange brown to orange brown, in the presence of air sometimes blue green; in water orange brown to brown; inamyloid.

Chlamydospores lacking.

Habitat. *P. media* has been found to form ectomycorrhiza with at least *Betula pendula*, *Larix decidua* and *Picea glauca* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Estonia. Soil or root tip samples confirm presence also in: Canada, Italy and Russia.

Remarks. Within the *P. tristis* group, the basidiomata of *P. media* can be recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, narrow subicular hyphae, brown mature hymenium, long, laterally wide, angular-nodulose spores and subhymenial hyphae that are of ± the same width as the subicular hyphae. *Pseudotomentella pinophila* and *P. pluriloba* can appear similar, but the spores of *P. pinophila* are laterally narrower and generally star-shaped, while *P. pluriloba* has wider subicular hyphae, larger spores and subhymenial hyphae that are noticeably narrower than the subicular hyphae.

Additional specimens studied. ESTONIA. Valga: Otepää, Trommi, 12 September 2012, U. Kõljalg (TU 115608*).

***Pseudotomentella pinophila* Svantesson, sp. nov.**
MycoBank No.: MB829001
Fig. 12

Type. SWEDEN. Småland: Jönköping, Svarttorp, Ramlaklint, boreonemoral, mixed, old-growth forest, on soil with intermediate pH, 12 September 2016, S. Svantesson 358 (holotype: GB!, GenBank Acc. No. ITS: MK290708).

UNITE SH. SH005337.07FU

Etymology. The name refers to the ectomycorrhizal association of the species, which often seems to be with *Pinus*.

Description. Basidiomata annual, resupinate, membranaceous, effused – often to several tens of centimetres in diameter. Mature parts continuous, with a cottony texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; pale brown to pale greenish-brown when fresh, pale reddish-brown when dried. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of immature parts blue to grey when fresh, pale blue or blue grey to dark blue grey or brown grey, sometimes with a green hue, when dried. Subiculum well-developed, loose, fibrous, pale orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae 3.0–4.9 µm wide, with a mean width of 3.6–4.1 µm; pale orange brown to pale pinkish-brown in both KOH and water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.1–) 3.2–4.7 (–5.2) µm wide, with a mean width of 3.9–4.0 µm; hyaline to pale green or occasionally pale brown in KOH, blue green in the presence of air; pale green to pale orange in water, with strongly granular contents; sometimes with an amyloid reaction in the cell walls.

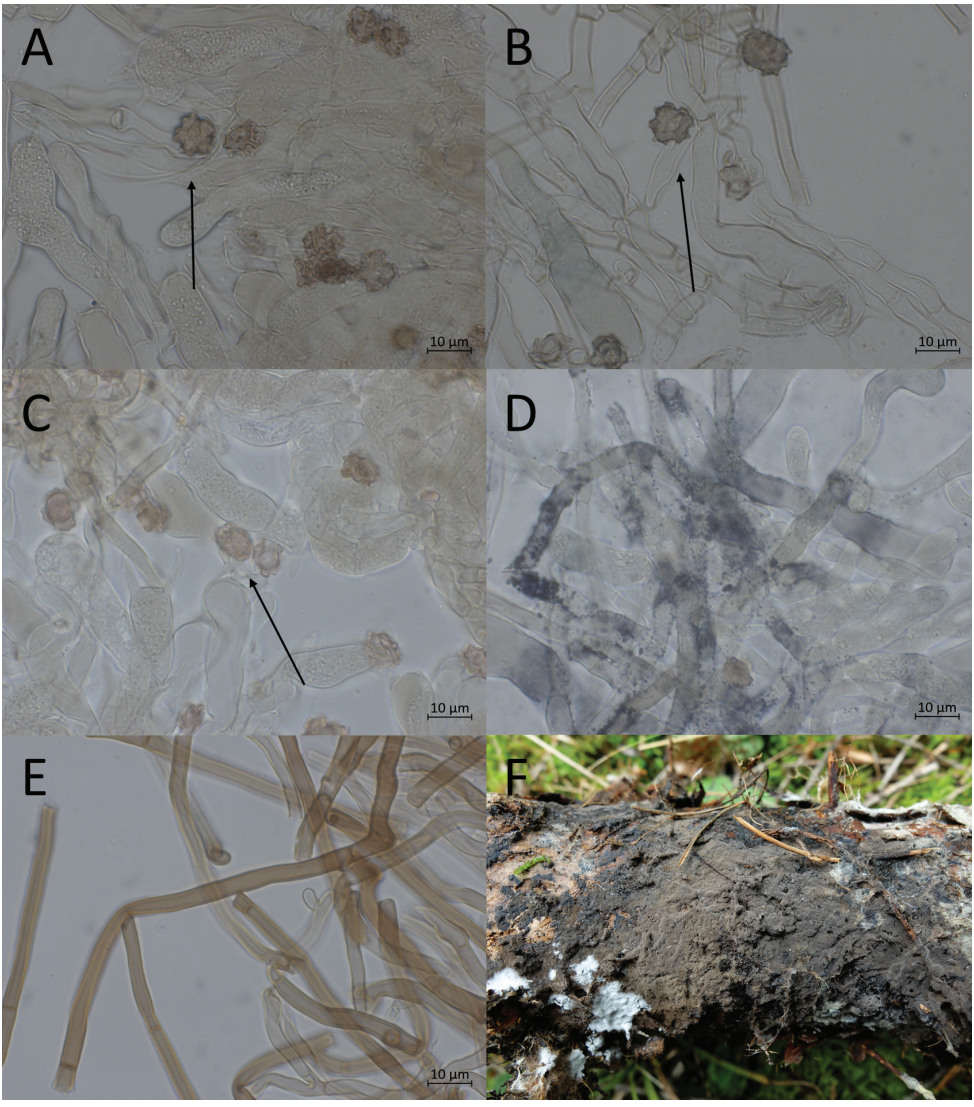


Figure 12. Morphological features of *P. pinophila*, mounted in KOH and macroscopically. **A, B** basidiospores in frontal face (holotype) **C** in lateral face (O F110330) **D** encrusted subhymenial hyphae (O F110305) **E** subicular hyphae (O F110330) **F** mature basidiome (SS418).

Encrustation granular, amyloid; bluish-black in both KOH and water; common to rare, usually scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (53–) 58–74 (–83) × (9.0–) 9.1–11.7 (–12.1) µm; mean dimensions: 65–67 × 9.9–10.3 µm. Sterigmata (8.2–) 8.8–10.5 (–11.9) µm long, with

a mean length of 9.6–10.0 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face commonly with a subcircular basic shape and a roundedly star-shaped, sometimes roundedly cross-shaped or angular to nodulose outline, covered in bi- or trifurcate, occasionally singularly attached, echinuli. Lobes distinct, rounded to square; predominantly five, but commonly also three or four; six-lobed or subellipsoid, unlobed spores and spores with corners instead of lobes infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (7.7–) 7.9–10.2 (–10.3) × (7.7–) 8.3–10.1 (–10.2) µm; mean dimensions: 8.6–9.1 × 8.8–9.4 µm; Q-value: 0.9–1.1; mean Q-value: 1.0. Echinuli (0.6–) 0.8–1.4 (–1.5) µm long, with a mean length of 0.9–1.1 µm. Lateral face ellipsoid to ovoid, usually with evenly rounded edges, sometimes with angular edges or one-three lobes. Lateral dimensions: (8.2–) 8.3–9.7 (–9.8) × (5.7–) 5.8–6.8 (–7.0) µm; mean dimensions: 8.7–9.0 × 6.3–6.6 µm; Q-value: 1.3–1.6; mean Q-value: 1.3–1.4. Colour in KOH pale green to orange brown, in the presence of air sometimes with a blue green reaction; in water pale green to orange brown or brown; occasionally amyloid.

Chlamydospores lacking.

Habitat. Data on habitat are scarce to date, but recent Scandinavian collections have been made in old coniferous or mixed forests on soil with high pH. *Pseudotomentella pinophila* has been found to form ectomycorrhiza with at least *Pinus densiflora*, *Pinus massoniana*, *Pinus sylvestris* and *Pinus thunbergii* (Kõljalg et al. 2005, Nilsson et al. 2019). It should be noted however that, although the only hitherto documented hosts of *P. pinophila* belong to the genus *Pinus* and *P. sylvestris* has indeed been present at nearly all Nordic localities of collection, a few of these collections were made at localities where *Pinus* was not recorded as a possible host.

Distribution. Basidiomata encountered in: Norway and Sweden. Soil or root tip samples confirm presence also in: China and Republic of Korea.

Remarks. Within the *P. tristis* group, the basidiomata of *P. pinophila* can be recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, narrow subicular hyphae, brown mature hymenium, long, laterally narrow and commonly star-shaped spores. *Pseudotomentella sciastra*, *P. pluriloba* and *P. media* can appear similar. Even though *P. sciastra* has star-shaped spores, it also has wider subicular hyphae than *P. pinophila* and, while *P. pluriloba* and *P. media* both share the characters of narrow hyphae, long spores and hymenia that are brown when mature with *P. pinophila*, they differ by having angular-nodulose spores, which are also laterally wider than the spores of *P. pinophila*.

Additional specimens studied. NORWAY. Akershus: Asker, Skaugumåsen, boreonemoral, mixed forest on on soil with high pH, 23 September 2010, S. Svantesson (O F110327); Oslo (county): Oslo (municipality), Bygdøy, Hengsåsen, boreonemoral, mixed forest on soil with high pH, 22 September 2010, S. Svantesson (O F110328*); Oslo (county): Oslo (municipality), Gressholmen, boreonemoral, mixed forest on soil

with high pH, 20 September 2010, S. Svantesson (O F110329); Telemark: Bamble, Rognsflaugane, boreonemoral, mixed forest on soil with high pH, 2 September 2010, K.-H. Larsson and S. Svantesson (O F110305); Akershus: Asker, Esvika, Løkenes, boreonemoral, mixed forest on soil with high pH, 15 August 2010, K.-H. Larsson and N. Svensson (O F110330*);

SWEDEN. Västergötland: Götene, Österplana, Hönsäter, coniferous forest on soil with high pH, 14 September 2017 S. Svantesson 418*, 419* (GB); Öland: Borgholm, Böda, Hagudden, mixed forest on soil with high pH, 5 October 2017 S. Svantesson 440* (GB).

***Pseudotomentella pluriloba* Svantesson, sp. nov.**
MycoBank No.: MB829018
Fig. 13

Type. FINLAND. Uusimaa: Loviisa, Rutosinpyhtää, Marinkylä, rotten trunk on the ground (*Picea*), 30 September 2010, U. Söderholm 4263 (holotype: H 6018127!, GenBank Acc. No. ITS: MK290698).

UNITE SH. SH030565.07FU

Etymology. The name refers to the several lobes of the spores.

Description. Basidiomata annual, resupinate, membranaceous, effused to approximately ten centimetres in diameter. Mature parts continuous, with a cottony texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; brown to purplish-brown when fresh, reddish-brown when dried. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of immature parts blue when fresh, blue grey to brown grey after drying. Subiculum well developed, loose, fibrous, brown with an orange hue; forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (3.9–) 4.0–5.9 (–6.8) µm wide, with a mean width of 6.8–5.1 µm; orange brown in both KOH and water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (2.7–) 2.9–5.3 (–5.4) µm wide, with a mean width of 4.0–4.2 µm; pale orange green to hyaline in KOH, blue green in the presence of air; pale orange green to hyaline in water, with strongly granular contents.

Encrustation granular, amyloid, concolourous with the hyphae in both KOH and water; usually common and scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight

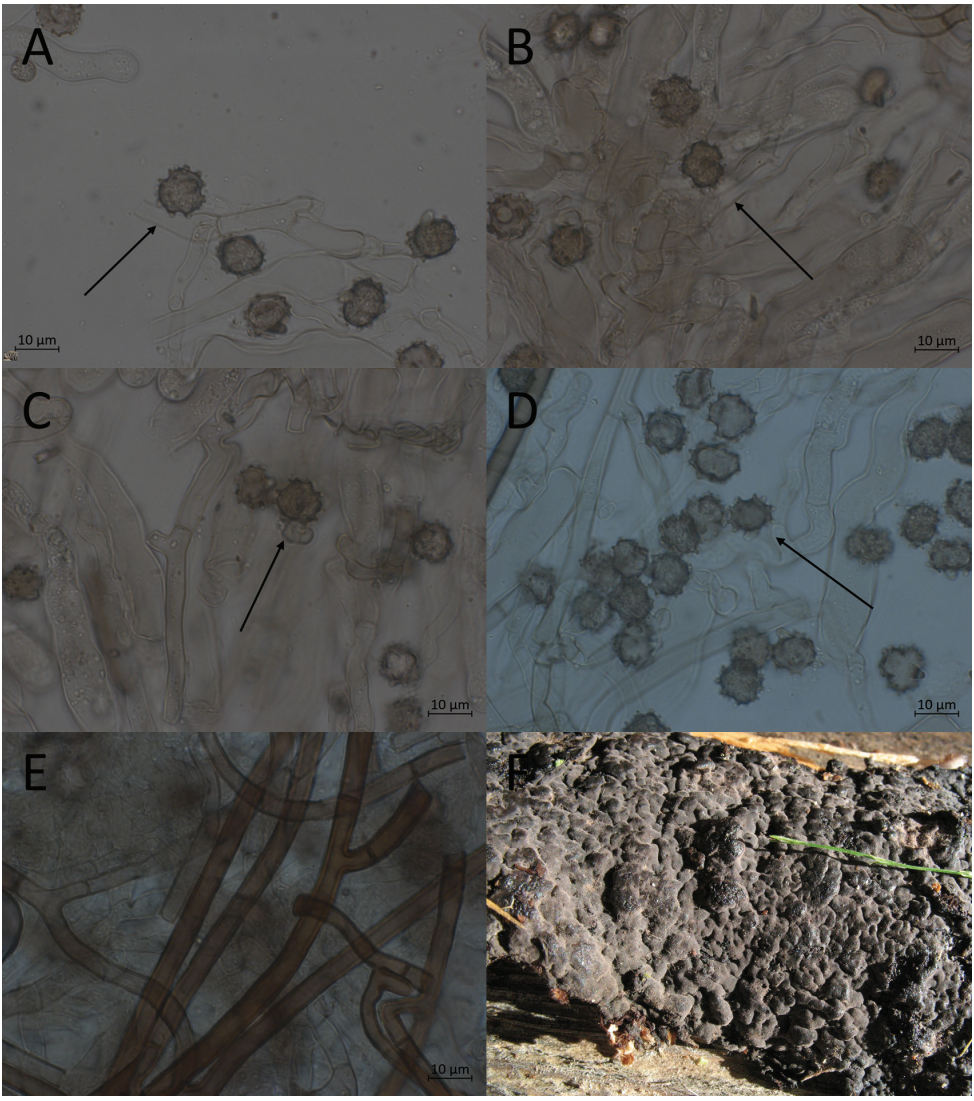


Figure 13. Morphological features of *P. pluriloba*, mounted in KOH and macroscopically. Holotype: **A, B, C** basidiospores in frontal face **D** in lateral face **E** subicular hyphae **F** mature basidiome.

constrictions. Dimensions: (55–) 58–87 (–94) × (10.3–) 10.7–13.3 (–13.4) µm; mean dimensions: 68–73 × 11.8–12.1 µm. Sterigmata (9.8–) 10.1–13.7 (–14.5) µm long, with a mean length of 11.5–12.3 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with three-five distinct corners

or rounded to square lobes; unlobed subcircular, unlobed subellipsoid or rounded, heart-shaped spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (9.0–) 9.1–10.8 (–10.9) × (9.2–) 9.3–10.9 (–11.1) µm; mean dimensions: 9.8 × 10.2 µm; Q-value: 0.9–1.0 (–1.1); mean Q-value: 1.0. Echinuli (0.9–) 1.0–1.9 µm long, with a mean length of 1.4 µm. Lateral face ellipsoid, usually with evenly rounded edges, rarely with one-three lobes. Lateral dimensions: 9.0–10.4 (–10.8) × (6.7–) 6.8–8.5(8.6) µm; mean dimensions: 9.6–9.8 × 7.5–7.6 µm; Q-value: 1.2–1.4; mean Q-value: 1.3. Colour in KOH pale orange green, in the presence of air often with a pale blue green reaction; in water pale orange; occasionally amyloid.

Chlamydospores lacking.

Habitat. Data on habitat are scarce to date, but recent Scandinavian collections have been made in mature to old coniferous or mixed forests on soil with intermediate pH. *Pseudotomentella pluriloba* has been found to form ectomycorrhiza with at least *Pseudotsuga menziesii* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Finland and Sweden. Soil or root tip samples confirm presence also in: Canada and the United States.

Remarks. Within the *P. tristis* group, the basidiomata of *P. pluriloba* are recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae and noticeably narrower subhymenial hyphae, long, moderately lobed spores and amyloid encrustation on subhymenial hyphae and basidia. *Pseudotomentella abundiloba*, *P. alobata* and *P. media* can appear similar, but *P. media* differs by having smaller spores and narrower subicular hyphae which are ± the same width as its subicular hyphae, while *P. abundiloba* and *P. alobata* have frontally narrower spores with different lobation than *P. pluriloba*, as well as wider subicular hyphae and shorter sterigmata.

Additional specimens studied. SWEDEN. Öland: Borgholm, Böda, Trollskogen, mixed forest on soil with intermediate pH, 5 October 2017, S. Svantesson 439* (GB).

***Pseudotomentella rotundispora* Svantesson, sp. nov.**
MycoBank No.: MB829020
Fig. 14

Type. SWEDEN. Västergötland: Götene, Medelplana, Eriksberg, boreonemoral, mixed forest on soil with high pH, 17 October 2016, S. Svantesson 413 (holotype: GB!, GenBank Acc. No. ITS: MK290674).

UNITE SH. SH030562.07FU

Etymology. The name refers to the appearance of the spores, which often have rounded or weakly pronounced lobes and comparably short echinuli.

Description. Basidiomata annual, resupinate, membranaceous, effused – often to several tens of centimetres in diameter. Mature parts continuous, with a cottony

texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; brown to greenish-brown when fresh, concolourous when dried, but then sometimes with a red hue. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of immature parts blue to blue green or grey when fresh, pale blue grey to grey blue when dried. Subiculum well developed, loose, fibrous, pale brown to pale orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae 3.0–4.4 (–4.6) µm wide, with a mean width of 3.4–3.8 µm; brown to orange brown in both KOH and water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (2.7–) 3.0–4.6 (–6.3) µm wide, with a mean width of 3.6–3.7 µm; pale brown to brown in KOH, often with orange or green hues, blue green in the presence of air; brown to orange brown in water, with strongly granular contents; some subhymenial hyphae with a pink colour in water and an amyloid reaction in Melzer's reagent.

Encrustation granular, probably amyloid (hard to observe due to the colour); blackish in KOH, dark blue green in the presence of air; blackish in water; scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate or sometimes narrowly clavate or clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: 40–66 (–69) × 8.2–10.6 (–11.1) µm; mean dimensions: 54–60 × 8.8–9.7 µm. Sterigmata (6.6–) 7.4–11.0 (–11.5) µm long, with a mean length of 8.5–10.2 µm.

Colours and reactions the same as for the upper parts of subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face very variable. Generally with a subcircular basic shape and an angular, nodulose, star-shaped or occasionally cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with three-seven, commonly three or five, indistinct corners to distinct, usually rounded lobes; spores with angular or square lobes infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: (6.7–) 7.0–8.2 (–8.4) × 7.0–8.6 µm; mean dimensions: 7.5–7.6 × 7.7–7.9 µm; Q-value: 0.9–1.1; mean Q-value: 1.0. Echinuli 0.5–1.1 (–1.3) µm long, with a mean length of 0.8 µm. Lateral face ellipsoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: 7.0–8.2 (–8.3) × (5.2–) 5.3–6.0 (–6.1) µm; mean dimensions: 7.6–7.9 × 5.6–5.7 µm; Q-value: 1.3–1.5; mean Q-value: 1.4. Colour in KOH pale brown to brown or pale orange brown to orange brown, in the presence of

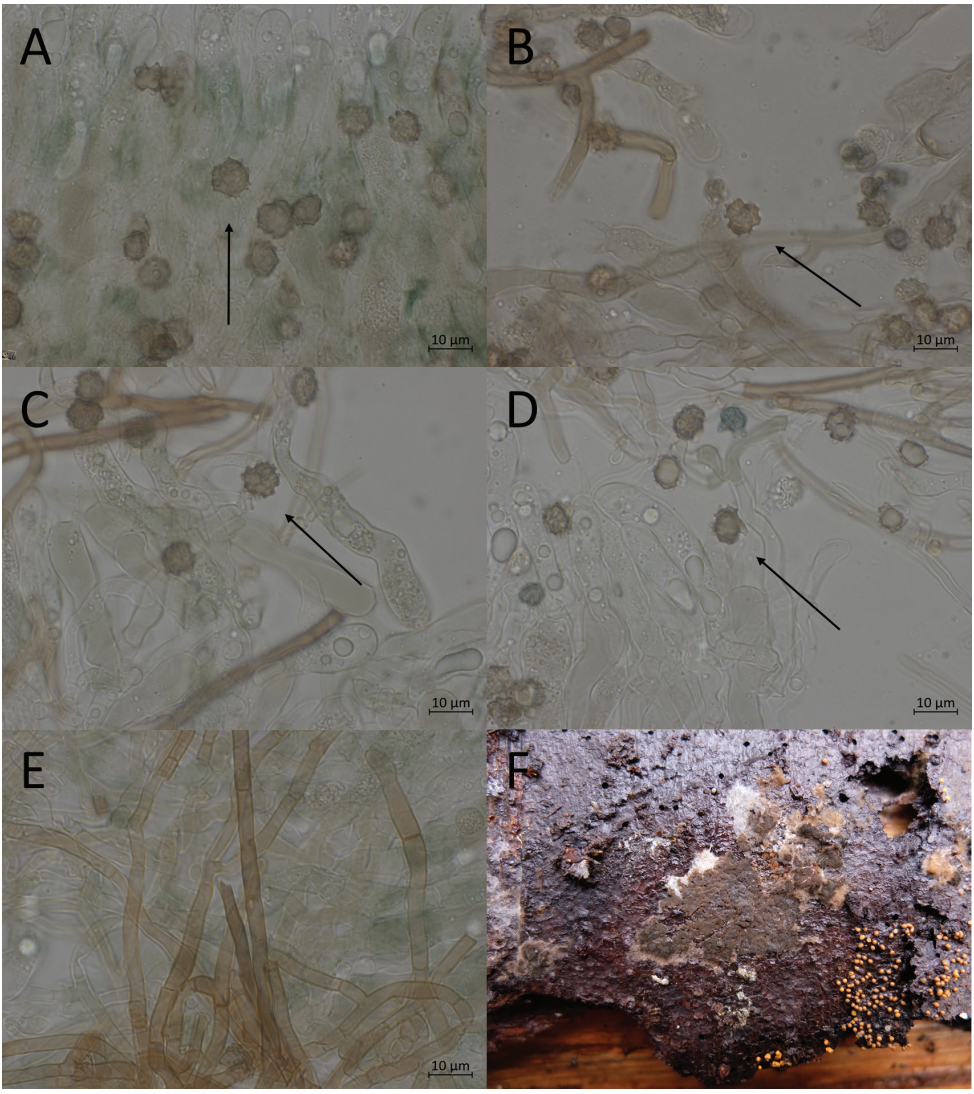


Figure 14. Morphological features of *P. rotundispora*, mounted in KOH and macroscopically. **A** (holotype) **B** (SS393) **C** (holotype) basidiospores in frontal face **D** in lateral face (holotype) **E** subicular hyphae (holotype) **F** mature basidiome (holotype).

air sometimes with a blue green reaction; in water brown to orange brown; occasionally with an amyloid reaction.

Chlamydospores lacking.

Habitat. Data on habitat are scarce to date, but recent Scandinavian collections have been made in old, coniferous, deciduous or mixed forests on soil with high pH. *Pseudotomentella rotundispora* has been found to form ectomycorrhiza with at least *Castanea* sp. and *Populus tremula* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Estonia, Norway and Sweden. Soil or root tip samples confirm presence also in: Austria, Italy and the United Kingdom.

Remarks. Within the *P. tristis* group, the basidiomata of *P. rotundispora* can be recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, narrow subicular hyphae and short spores. The other species within the group can appear similar, but have either wider hyphae, longer spores or both.

Additional specimens studied. ESTONIA. Lääne: Hanila, Puhtu-Laelatu Nature Reserve, Puhtu peninsula, deciduous forest with *Populus*, *Tilia*, *Quercus* and *Picea*, 11 August 2005, U. Kõljalg (TU 100138*);

NORWAY. Oslo (county): Oslo (municipality), Bygdøy, Dronningberget, mixed forest on soil with high pH, 30 September 2017, K.-H. Larsson 17682* (O);

SWEDEN. Västergötland: Götene, Medelplana, Eriksberg, boreonemoral, mixed forest on soil with high pH, 17 October 2016, S. Svantesson 393*, 394* (GB).

***Pseudotomentella sciastra* Svantesson & Kõljalg, sp. nov.**
MycoBank No.: MB829025
Fig. 15

Type. SWEDEN. Småland: Jönköping, Svarttorp, Ramlaklint, boreonemoral, mixed, old-growth forest, on soil with intermediate pH, 12 September 2016, S. Svantesson 359 (holotype: GB!, GenBank Acc. No. ITS: MK290686).

UNITE SH. SH030554.07FU

Etymology. The name refers to the dark, star-like appearance of the spores.

Description. Basidiomata annual, resupinate, membranaceous, effused – often to several tens of centimetres in diameter. Mature parts continuous, with a cottony texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; blue grey when fresh and brown with a pinkish hue when dried. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of immature parts blue grey when fresh or occasionally green or even yellow; blue grey to brown grey when dried. Subiculum well developed, loose, fibrous, pale orange brown to brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (3.9–) 4.4–6.6 (–6.8) µm wide, with a mean width of 5.0–5.8 µm; brown to orange brown in KOH, orange brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae 2.9–5.0 (–6.0) µm wide, with a mean width

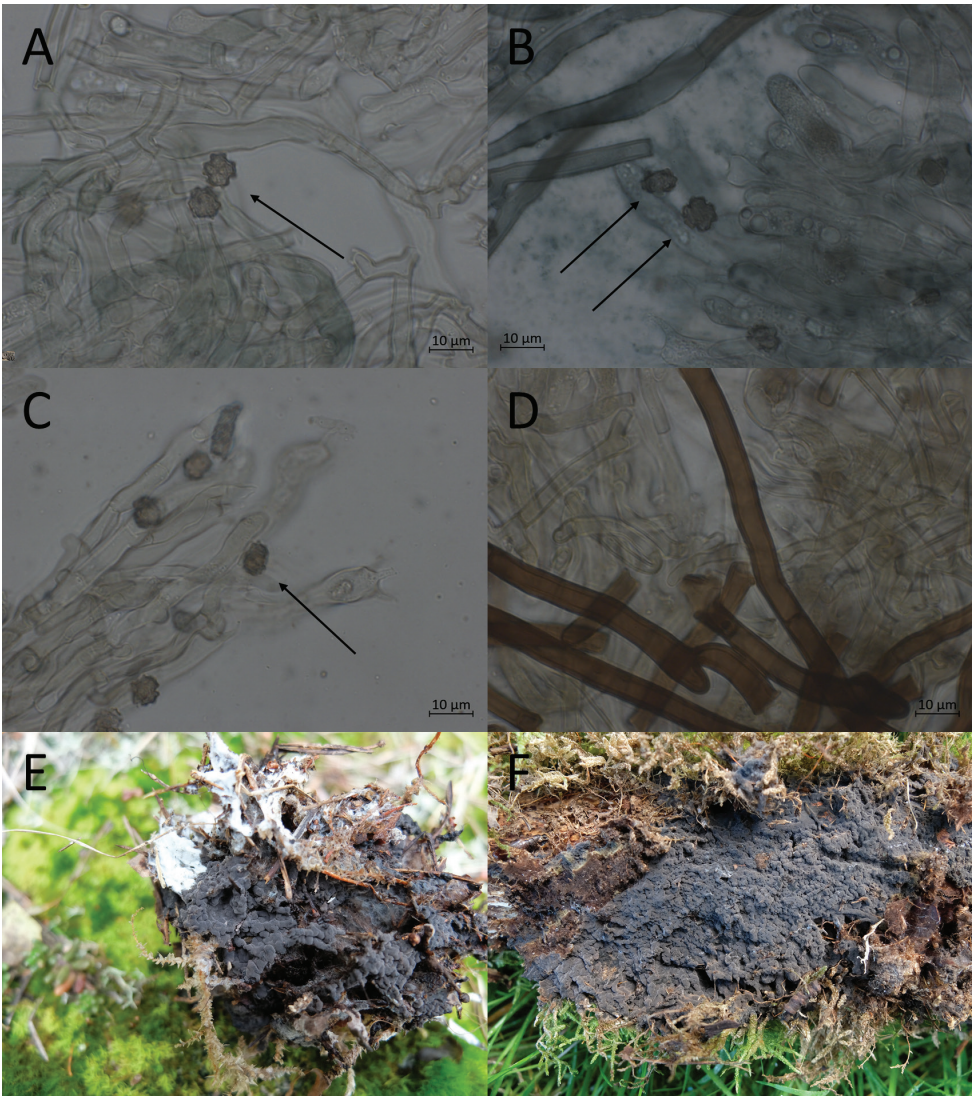


Figure 15. Morphological features of *P. sciastra*, mounted in KOH and macroscopically. **A** basidiospore in frontal face (O F110317) **B** in frontal and lateral faces (O F110317) **C** in lateral face (O F110317) **D** subicular hyphae (O F110317) **E** (SS420) **F** (SS312) mature basidiomata.

of 3.8–4.0 µm; hyaline to pale green in KOH, blue green in the presence of air; pale orange green to pale yellowish-green in water, with strongly granular contents.

Encrustation granular, probably amyloid (hard to observe due to the colour); blackish-brown in KOH, dark blue green in the presence of air; blackish-brown in water; scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: 42–67 (–68) × 7.3–9.0 (–9.3) µm; mean dimensions: 54–55 × 7.8–8.1 µm. Sterigmata (6.0–) 6.3–8.9 (–9.1) µm long, with a mean length of 7.4–7.9 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face with a subcircular basic shape and a star- or cross-shaped, sometimes angular to nodulose outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with four-six distinct, rounded to more often square lobes or rarely corners; abnormally large spores originating from two-sterigmate basidia infrequently occurring. Frontal dimensions: (6.0–) 6.1–7.9 (–8.1) × 6.3–8.2 µm; mean dimensions: 6.6–7.3 × 6.7–7.7 µm; Q-value: 0.9–1.1; mean Q-value: 1.0. Echinuli (0.5–) 0.6–1.2 (–1.4) µm long, with a mean length of 0.8–0.9 µm. Lateral face ellipsoid to ovoid, with evenly rounded edges or one-three lobes. Lateral dimensions: (6.2–) 6.5–7.7 (–8.0) × (4.3–) 4.4–6.0 (–6.2) µm; mean dimensions: 6.8–7.3 × 4.6–5.4 µm; Q-value: 1.2–1.6 (–1.7); mean Q-value: 1.3–1.5. Colour in KOH brown to yellow brown, in the presence of air often with a green to blue green reaction; in water pale greenish to pale greenish-orange; occasionally amyloid.

Chlamydospores lacking.

Habitat. Recent Scandinavian collections have been made in mature to old coniferous, deciduous or mixed forests on soil with intermediate to high pH. *Pseudotomentella sciastra* has been found to form ectomycorrhiza with at least *Castanea sativa*, *Cedrus libani*, *Neottia ovata*, *Picea abies* and *Quercus* sp. (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Estonia, Finland, Norway, Sweden, Turkey and the United Kingdom. Soil or root tip samples confirm presence also in: the Czech Republic, Mexico, Portugal (Madeira) and the United States.

Remarks. All studied European specimens previously identified as *P. atrofusca* belong to *P. sciastra*. The two species display considerable morphological differences (see key).

Within the *P. tristis* group, the basidiomata of *P. sciastra* are recognised by their lack of hyphal cords and skeletal hyphae and their soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae and small, star-shaped spores. *Pseudotomentella pinophila* is similar, but has narrower subicular hyphae and larger spores.

Additional specimens studied. ESTONIA. Ida-Virumaa: Illuka, Puhatu Nature Reserve, Poruni primeval forest, wetlands, 1 October 2006, U. Kõljalg (TU 100644*); [Saare,] Saarema, Kihelkonna, Hülgera, Sampling area G4422, 25 September 2015, A. Saitta (TU 124211*, TU 124213*);

FINLAND. Etelä-Häme: Jyväskylä, Korpilahti, Oittila, on dead trunk of *Ulmus glabra*, 3 September 2014, U. Söderholm 4755 (H 6052710); Kanta-Häme: Lammi, Lammi Biological Station, Leib-rich forest, 12 September 2001, K.-H. Larsson (TU 108754*);

NORWAY. Oslo (county): Oslo (municipality), Bygdøy, Hengsåsen, boreonemoral, mixed forest on soil with high pH, 16 August 2010, K.-H. Larsson and N. Svensson (O F110317*); Østfold: Moss, Jeløya, boreonemoral, mixed forest on soil with high pH, 26 September 2010, S. Svantesson and N. Svensson (O F110318*); Oppland: Dovre, Grimsdalen, Austre Stakkstosætra, *Pinus sylvestris* forest, 26 August 2010, K.-H. Larsson and S. Svantesson (O F110301); Vestfold: Larvik, Kvelde, Jordstøyp, boreonemoral, mixed forest on soil with intermediate pH, 1 October 2010, K.-H. Larsson (O F110302); Ibidem, on soil with high pH, 1 September 2010, K.-H. Larsson and S. Svantesson (O F110303, F110304); Aust-Agder, Risør, Glupedalen, boreonemoral, mixed forest on soil with high pH, 10 September 2010, S. Svantesson and N. Svensson (O F110319, F110320, F110321); Aust-Agder: Tvedestrand, Eidbo, boreonemoral, mixed forest on soil with intermediate pH, 10 September 2010, S. Svantesson and N. Svensson (O F110322*); Oslo (county): Oslo (municipality), Gressholmen, boreonemoral, mixed forest on soil with high pH, 20 September 2010, S. Svantesson (O F110323, F110324); Oslo (county): Oslo (municipality), Killingen, boreonemoral, mixed forest on soil with high pH, 22 September 2010, S. Svantesson (O F110325); Buskerud: Ringerike, Ulltveit Nature Reserve, boreonemoral, coniferous forest on soil with high pH, 25 September 2010, S. Svantesson and N. Svensson (O F110326);

SWEDEN. Småland: Jönköping, Svarttorp, Ramlaklint, boreonemoral, mixed, old-growth forest, on soil with intermediate pH, 12 September 2016, S. Svantesson 360 (GB); Bohuslän: Tanum (municipality), Tanum (parish), Lammön, boreonemoral, deciduous forest on soil with high pH, 6 September 2016, S. Svantesson 312* (GB); Västergötland: Göteborg, Askim, Årekärrslunden, 24 October 2015, K.-H. Larsson 17308b* (GB); Dalsland, Mellerud, Skållerud, Österbo, mixed forest on soil with high pH, 20 September 2017, S. Svantesson 420* (GB); Ibidem, Norgekullen SW, coniferous forest on soil with high pH, 20 September 2017, S. Svantesson 423* (GB);

TURKEY. [Antalya: Elmalı,] Ciglikara, 2009, L. Tedersoo (TU 110153*); [Isparta:] Yukan-Gökdere [=Yukarı Gökdere], 2009, L. Tedersoo (TU 110113*);

UNITED KINGDOM. Scotland, Aberdeenshire: Inverurie, Burnhervie, in a small group of planted *Populus* trees, 16 September 2005, I. J. Alexander (TAA 187322*).

***Pseudotomentella tristis* (P. Karst.) M.J.Larsen, Nova Hedwigia 22(1–2): 613 (1971) [1972]**
Fig. 16

Homotypic names. *Hypochnus subfuscus* ssp. *tristis* P.Karst., Meddeland. Soc. Fauna Fl. Fenn. 9: 71 (1883). *Hypochnus tristis* (P.Karst.) P.Karst. Bidrag Kännedom Finlands Natur Folk. 48: 440 (1889). *Tomentella tristis* (P.Karst.) Höhn. & Litsch., Sitzungsber. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Cl., Abt. 1 115: 1572 (1906). Type. FINLAND. Tavastia australis [= Etelä-Häme]: Tammela, Mustiala, ad Betulam, 19 August 1865, P. A. Karsten (lectotype: Herbarium P. A. Karsten 3036 [H 6018703]!, designated by M.J. Larsen in Nova Hedwigia 22(1–2): 613 (1971) [1972]); SWE-

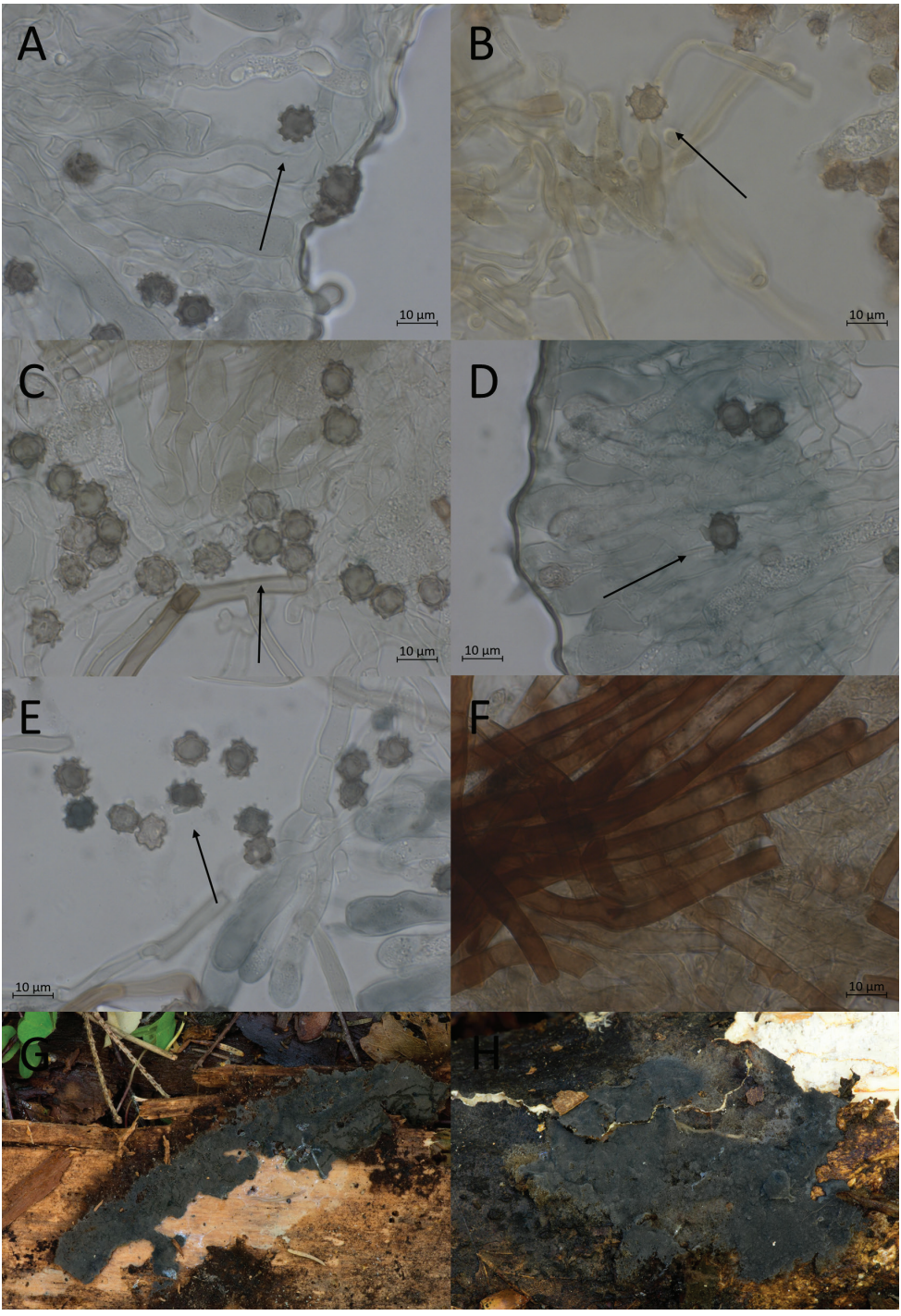


Figure 16. Morphological features of *P. tristis*, mounted in KOH and macroscopically. A (LK54/13) **B** (lectotype) **C** (epitype), basidiospores in frontal face **D** (epitype) **E** (TAA 159485) in lateral face **F** subicular hyphae (epitype) **G** young basidiome (TU 115439) **H** mature basidiome (TU 115642).

DEN. Västerbotten: Vännäs, Orrböle, boreal, mixed forest on soil with high pH, 28 August 2015, S. Svantesson 193 (EPITYPE: GB!, here designated, MycoBank Typification No. MBT384911, GenBank Acc. No. ITS: MK290679).

Heterotypic names. ***Hypochnopsis fuscata*** P.Karst., Bidrag Kännedom Finlands Natur Folk 48: 443 (1889). *Hypochnus fuscatus* (P.Karst.) Sacc., Syll. fung. 9: 244 (1891). Type. FINLAND. Tavastia australis: Messuby [Tavastia australis = Etelä-Häme; Messuby is part of the city of Tampere], September 1860, P.A. Karsten (lectotype: Herbarium P.A. Karsten 770 [H 6059014]!, designated by M.J. Larsen in Nova Hedwigia 22(1–2): 616 (1971) [1972]); SWEDEN. Västerbotten: Vännäs, Orrböle, boreal, mixed forest on ground with high pH, 28 August 2015, S. Svantesson 193 (EPITYPE: GB!, here designated, MycoBank Typification No. MBT384955, GenBank Acc. No. ITS: MK290679).

Hypochnus sitnensis Bres., Atti Imp. Regia Accad. Roveretana. 3(1): 115 (1897)

Type. SLOVAKIA [Hungary at the time of collection]. Prenčow, Sitno, infra filagorum, in trunco putr. Fagi, 11 September 1895, Andr. Kmet (holotype: S F15178!).

UNITE SH. SH030560.07FU

Description. Basidiomata annual, resupinate, membranaceous, effused – often to several tens of centimetres in diameter. Mature parts continuous, with a cottony texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; blue grey to purplish-brown when fresh, blue grey or blue-greenish grey to brown, with a reddish hue, when dried. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of immature parts blue green, blue or blue grey when fresh and pale grey blue or pale blue grey to grey blue or blue grey when dried, sometimes with a green hue. Subiculum well developed, loose, fibrous, orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (4.5) 4.6–7.4 µm wide, with a mean width of 5.4–6.2 µm; orange brown to dark brown in KOH, orange brown to brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae 3.2–6.2 (7.2) µm wide, with a mean width of 3.9–4.5 µm; pale orange brown to pale green in KOH, blue green in the presence of air; pale green to pale greenish-orange in water, with strongly granular contents.

Encrustation granular, probably amyloid (hard to observe due to the colour); blackish in KOH, dark blue green in the presence of air; blackish in water; scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight

constrictions. Dimensions: 51–76 (–84) × (8.1) 8.3–13.7 (–14.6) µm; mean dimensions: 56–62 × 9.6–11.6 µm. Sterigmata (8.0) 8.3–11.3 (13.3) µm long, with a mean length of 9.4–10.2 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an unlobed, angular, weakly nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, occasionally singularly attached echinuli. A majority of the spores normally unlobed or with three-five indistinct corners to rounded lobes; subcircular spores with more pronounced, sometimes square lobes or ovoid to subellipsoid spores also common in some specimens; subcircular, six-lobed spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: 7.7–9.1 (9.2) × 8.0–9.2 (9.6) µm; mean dimensions: 8.3–8.6 × 8.4–8.8 µm; Q-value: 0.9–1.1; mean Q-value: 1.0–1.1. Echinuli (0.8) 0.9–1.9 µm long, with a mean length of 1.4 µm. Lateral face ellipsoid to narrowly ovoid or sometimes semicircular in shape, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: (7.7) 8.0–9.0 × (6.0) 6.1–6.8 (7.0) µm; mean dimensions: 8.3–8.5 × 6.3–6.5 µm; Q-value: 1.2–1.4 (–1.5); mean Q-value: 1.3. Colour in KOH brown to orange brown, in the presence of air often with a blue green reaction; in water greenish-orange to orange brown; occasionally amyloid.

Chlamydospores lacking.

Habitat. Data on habitat are scarce to date, but recent Scandinavian collections have been made in mature to old deciduous or mixed forests on soil with intermediate to high pH. *Pseudotomentella tristis* has been found to form ectomycorrhiza with at least *Betula pendula* and *Fagus sylvatica* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Estonia, Finland, Norway, Slovakia, Slovenia and Sweden. Soil or root tip samples confirm presence also in: Germany.

Remarks. We here select a Swedish specimen to serve as an epitype for both *P. tristis* and *H. fuscata*. This decision is based on four reasons: first, the present study has found *P. tristis* and *H. fuscata* to be conspecific; secondly, the lectotypes of these species were both collected in Finland (within the same county); thirdly we have found *P. tristis* to occur at several localities in both Finland and Sweden; and fourthly, the Swedish material chosen is both ampler and displays more variation with respect to maturity of the basidiome than the single available recent Finnish collection.

The type specimen of *H. sitnensis* was collected in Slovakia, i.e. far from the type locality of *P. tristis*. It displays the morphological characters of *P. tristis*, apart from the absence of an amyloid reaction in the encrusting material found on basidia and subhymenial hyphae. This might be an artefact of, for example, its drying conditions, time or intraspecific variation, but since the specimen is in too poor a condition to allow DNA sequencing with currently available methods, this cannot be ascertained. We therefore consider it a synonym of *P. tristis* and suggest it be epitypified in due course with locally sampled material that matches the type.

In the case of *H. sitnensis*, there is only one collection matching the locality and habitat description of the protologue as well as the collector stated. It predates the publication of the species. This collection must hence be regarded as a holotype.

P. A. Karsten 770 is the lectotype of *H. fuscata*, as designated by Larsen in Nova Hedwigia (1971), but his note has been placed in P. A. Karsten 769, which has created confusion amongst mycologists studying these specimens. Mature spores that fall within the morphological span of *P. tristis* can easily be found in all collections that match Karsten's (1889) description of the species, with respect to locality and date. It would hence seem that the smooth, small, bluish spores he writes of in the protologue (see Introduction) probably were immature ones, studied in the presence of air.

Within the *P. tristis* group, basidiomata of *P. tristis* itself can be recognised by their lack of hyphal cords and skeletal hyphae and their soft, rather elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae, medium sized, commonly angular to nodulose spores and relatively long echinuli and sterigmata. *Pseudotomentella tristoides* is similar but has shorter echinuli and sterigmata, *P. sciastra* has smaller, star-shaped spores and *H. rhacodium* (only known from the type) has hard, brittle basidiomata after drying.

Additional specimens studied. ESTONIA. Valga: Otepää, Kääriku, Välkjärve, 10 September 2012, U. Kõljalg (TU 115439*); Tartumaa: Võnnu, Terikeste, on fallen branch of *Picea abies* in mixed forest, 20 August 1996, U. Kõljalg (TAAM 159485*); Lääne: Vormsi, road from Diby to Norrby, deciduous forest with *Betula* and *Corylus*, 27 September 2008, U. Kõljalg (TU 108134*);

FINLAND. Kanta-Häme: Lammi, Lammi Biological Station, Leib-rich forest, 12 September 2001, U. Kõljalg (TU 108757*); Satakunta: Luvia, Säppi, on fallen decayed *Betula*, 11 September 2013, L. Kosonen 54/13* (TUR);

NORWAY. Møre og Romsdal: Nesset, Eikesdal, Ljåstranda, rich, deciduous forest, 18 September 2011, K.-H. Larsson 15084* (O); Oppland: Vinstra, Liadalen, rich, deciduous forest, 24 September 2013, K.-H. Larsson 16367* (O); Hedmark: Ringsaker, Liberget, 24 August 1984, K.-H. Larsson 5901 (GB 87563); Sogn og Fjordane: Stryn, Flostranda Nature Reserve, boreonemoral, deciduous forest on ground with high pH, 25 September 2013, K.-H. Larsson (O F110297*); Rogaland: Forsand, Rössdalen, boreonemoral, deciduous forest on ground with high pH, 29 September 2012, K.-H. Larsson and S. Svantesson (O F110298*); Oppland: Nord-Fron, Liadalane Nature Reserve, boreonemoral, deciduous forest on ground with intermediate pH, 24 September 2013, K.-H. Larsson (O F110299, F110300*);

SLOVENIA. Upravna enota Kočevje: Rahjenavski Rog virgin forest reserve, S and E edge of the reserve, beech-silver fir old growth forest, 21 September 2012, S. Kõljalg; U. Kõljalg (TU 115642*);

SWEDEN. Västerbotten: Vännäs (municipality), Vännäs (parish), Orrböle, boreal, mixed, secondary, mature forest, on ground with high pH, 28 August 2015, S. Svantesson 188 (GB); Dalsland: Ödskölt, S of lake Ivägsjön, on deciduous wood, 22 September 1990, K. Hjortstam 17197 (K.-H. Larsson private collection).

***Pseudotomentella tristoides* Svantesson & K.H.Larss., sp. nov.**
MycoBank No.: MB829030
Fig. 17

Type. NORWAY. Nord-Tröndelag: Snåsa, Bergsåsen, boreal, deciduous forest on soil with intermediate pH, 28 August 2012, K.-H. Larsson (holotype: O F110306!, GenBank Acc. No. ITS: MK290692).

UNITE SH. SH030566.07FU

Etymology.

The name refers to the overall similarity between this species and *P. tristis*.

Description. Basidiome annual, resupinate, membranaceous, effused to approximately ten centimetres in diameter. Mature parts continuous, with a rather firm, fibrous and compact, yet quite soft and elastic texture. Hymenium smooth; brown with a reddish hue. Immature parts discontinuous, byssoid with a cottony texture. Subhymenium and hymenium of immature parts initially pale greyish-blue, when more mature dark blue grey. Subiculum well-developed, loose, fibrous, brown with an orange hue; forms the outer edge of the basidiome, extending noticeably beyond the hymenium. All characters recorded in dried state.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (4.7–) 4.9–7.1 (–7.6) µm wide, with a mean width of 6.0 µm; orange brown to dark brown in KOH, orange brown to brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.1–) 3.2–5.3 (–5.4) µm wide, with a mean width of 4.6 µm; pale yellowish-brown in KOH, pale green to blue green in the presence of air; pale green to pale orange green in water, with strongly granular contents.

Encrustation granular, amyloid, concolourous with the hyphae in both KOH and water; scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (49–) 54–72 (–75) × (7.3–) 7.9–10.0 (–10.5) µm; mean dimensions: 63 × 9.1 µm. Sterigmata (7.6–) 7.8–9.9 (–10.5) µm long, with a mean length of 8.6 µm. Colours and reactions the same as for the subhymenial hyphae.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. A majority of the spores with three-five indistinct corners to distinct, square lobes; subellipsoid, ovoid and subcircular spores with a rather evenly rounded outline occasionally occurring, as well as subcircular, six-lobed spores; abnormally large spores originating from two-sterigmate basidia infrequently seen. Frontal dimensions: 7.7–8.6 (–8.8) × (7.4–) 7.7–9.3 (–9.5) µm; mean dimensions: 8.2 × 8.5 µm; Q-value: 0.9–1.1; mean Q-value: 1.0. Echinuli (0.5–) 0.7–0.9

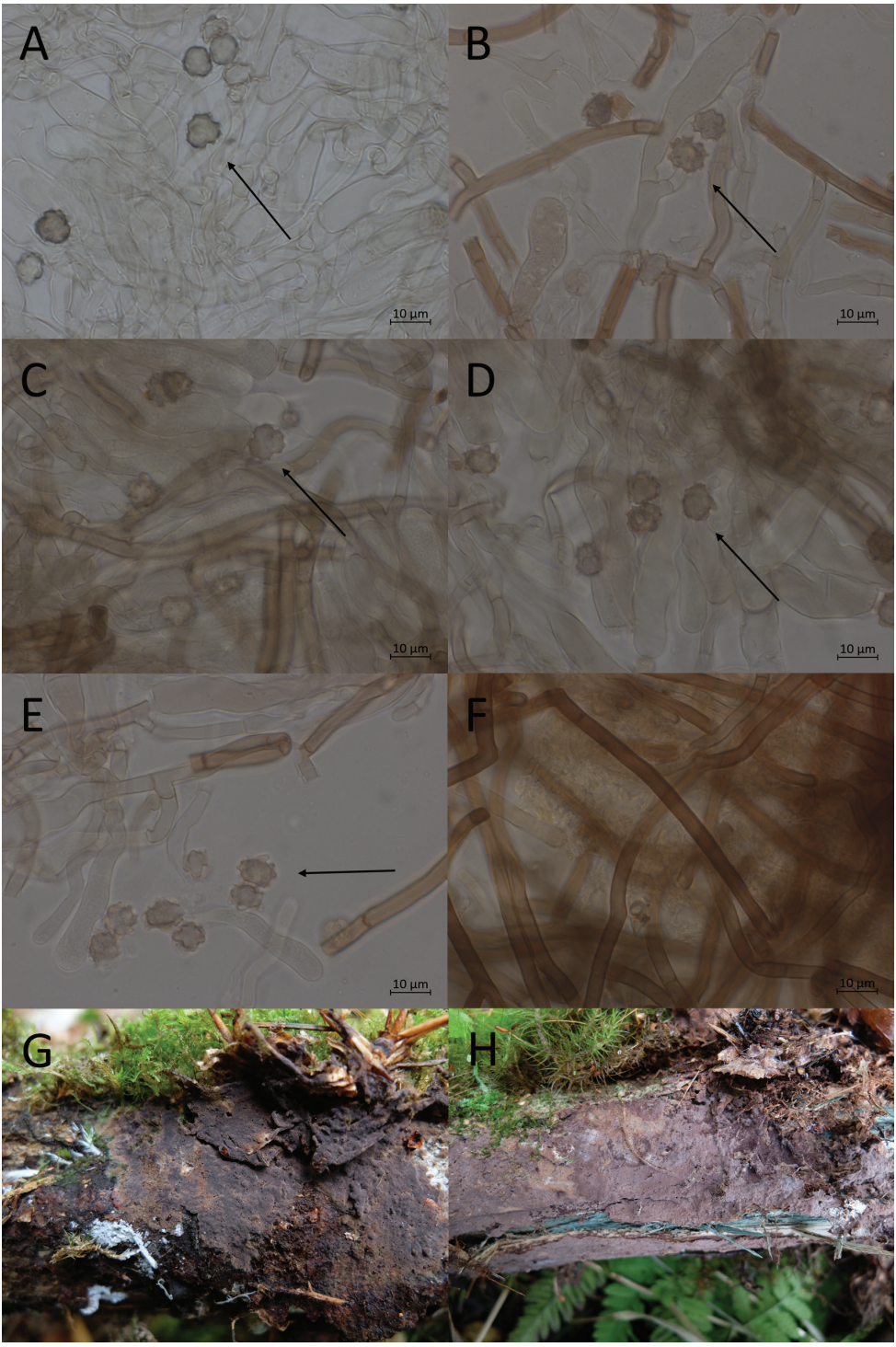


Figure 17. Micromorphological features of *P. tristoides* in KOH. Holotype: **A, B, C** basidiospores in frontal face **D, E** in lateral face **E** subicular hyphae.

(–1.1) µm long, with a mean length of 0.8 µm. Lateral face ellipsoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: (7.9–) 8.0–8.6 × 6.0–6.5 (–6.7) µm; mean dimensions: 8.2 × 6.3 µm; Q-value: 1.2–1.4; mean Q-value: 1.3. Colour in KOH brown to yellow brown, in the presence of air often with a green to blue green reaction; in water pale greenish to pale greenish-orange; occasionally amyloid.

Chlamydospores lacking.

Habitat. The only specimen recorded to date of *P. tristoides* is the type collection, which was obtained in an old, mixed forest on soil with intermediate pH. UNITE sequence metadata show that the species forms ectomycorrhiza with at least *Populus alba* and *Cephalanthera damasonium* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Norway. Soil or root tip samples confirm presence also in: Estonia and the Czech Republic.

Remarks. Within the *P. tristis* group, the basidiome of *P. tristoides* can be recognised by its lack of hyphal cords and skeletal hyphae and its soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae, medium sized, angular-nodulose spores and relatively short echinuli and sterigmata. *Pseudotomentella tristis* is similar but has longer echinuli and sterigmata, *P. sciastra* has smaller, star-shaped spores and *H. rhacodium* (only known from the type) has hard, brittle basidiomata after drying.

Pseudotomentella umbrina (Fr.) M.J.Larsen, Canad. J. Bot. 45: 1298 (1967)

Fig. 18

Homotypic names. *Thelephora umbrina* Fr. Elench. fung. 1: 199 (1828), non Pers. (1801), sanctioned name [Fries explicitly excluded *T. umbrina* Pers. from his concept]. *Hypochnus umbrinus* (Fr.) Fr. [basionym not cited], Summa veg. Scand.: 337 (1849), non Wallr. (1833), illegitimate name [combination also made by Quélet (1888) and Burt (1916)]. *Corticium umbrinum* (Fr.) Fr., Hymenomyc. eur.: 658 (1874). *Coniophora umbrina* (Fr.) Sacc., Syll. fung. 6: 652 (1888) [as (Alb. & Schwein.) Fr.]. *Tomentella umbrina* (Fr.) Litsch., Bull. Soc. Mycol. France. 49(1): 52 (June 20, 1933) [combination also made by Donk, Meded. Bot. Mus. Herb. Rijks Univ. Utrecht. 9: 29 (before July 7 1933)]. *Prillieuxia umbrina* (Fr.) Park.-Rhodes 1956 Ann. Bot. (Oxford). 20(78): 258. 1956, invalid name, basionym not cited. *Tomentellastrum umbrinum* (Fr.) Svrček, Ceská Mykol. 12(2): 70 (1958).

Type. SWEDEN. Småland: Femsjö, E. Fries (neotype: Herb. Fries [UPS F003106]!, designated by E.A. Burt in Ann. Missouri Bot. Gard 3: 213 (1916)); Småland: Hylte, Femsjö, Femsjö Church Nature Reserve, boreonemoral, mixed forest on soil with intermediate pH, 7 September 2016, S. Svantesson 351 (EPITYPE: GB!, here designated, MycoBank Typification No. MBT384818, GenBank Acc. No. ITS: MK290700).

UNITE SH. SH030549.07FU

Description. Basidiomata annual, resupinate, membranaceous, effused – often to several tens of centimetres in diameter. Mature parts continuous, with a cottony

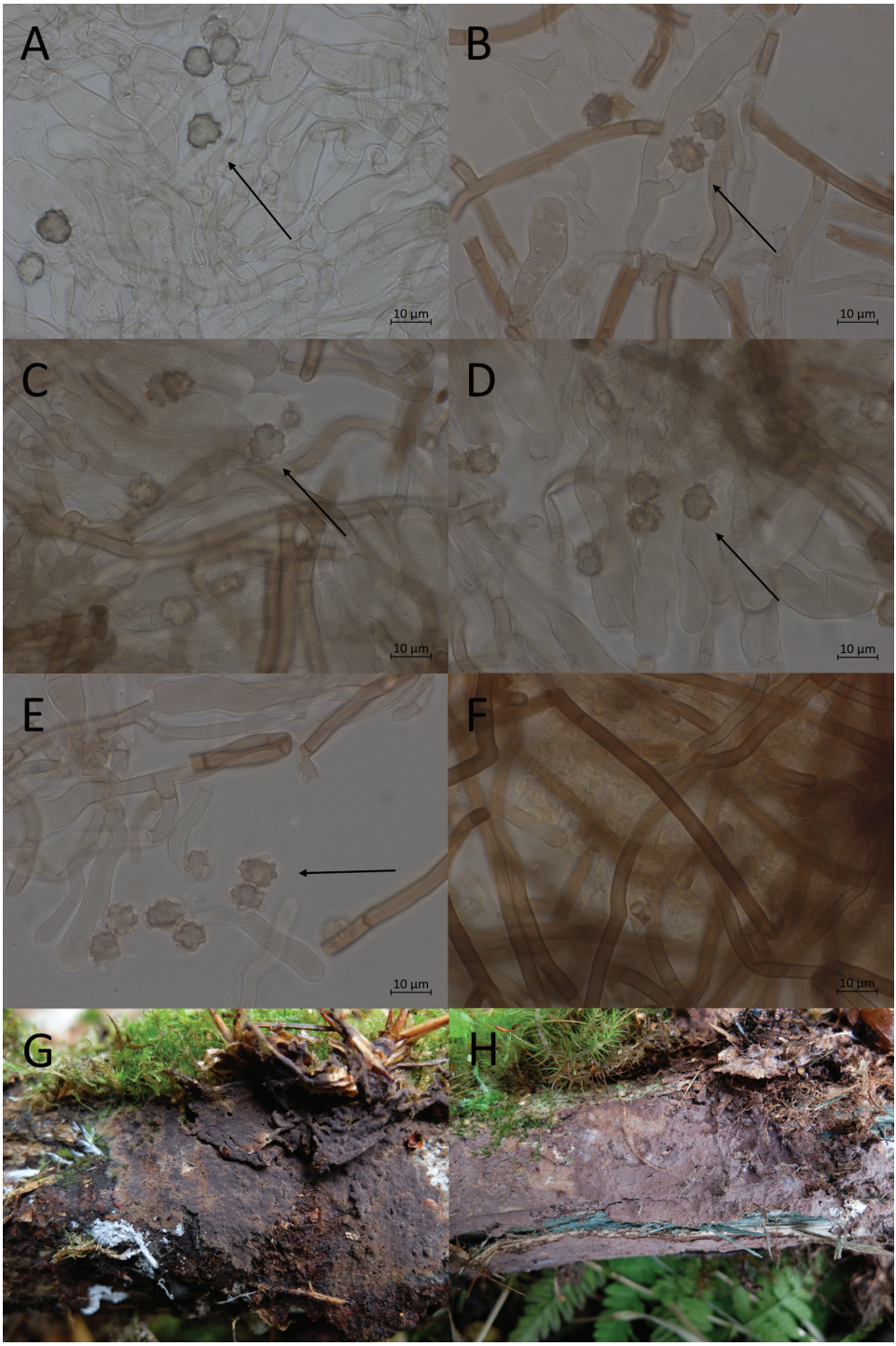


Figure 18. Morphological features of *P. umbrina*, mounted in KOH and macroscopically. **A** (O F110268) **B** (epitype) **C** (epitype) basidiospores in frontal face **D, E** in lateral face (epitype) **F** subicular hyphae (epitype) **G** (epitype) **H** (SS174) mature basidiomata.

texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth, but sometimes strongly undulating; blue grey or purplish-grey to pale brown or brown when fresh, pale brown to brown when dried, sometimes with a reddish or greyish hue. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of immature parts pale blue grey or pale purplish-grey to pale brown when fresh, pale brown when dried. Subiculum well developed, loose, fibrous, orange brown; often forms the outer edge of basidiomata, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections and reaction in Melzer's reagent absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae 3.3–4.8 (–5.3) µm wide, with a mean width of 4.0–4.3 µm; orange brown to brown in KOH, orange brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (2.8–) 2.9–5.0 (–5.9) µm wide, with a mean width of 3.7–4.2 µm; in the upper parts, pale green in KOH, sometimes with a faintly blue or brown hue; in the lower parts, orange brown to brown; in water, orange brown, with strongly granular contents.

Encrustation lacking.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (54–) 57–71 (–77) × (8.3–) 8.5–10.9 (–12.4) µm; mean dimensions: 60–64 × 9.5–10.3 µm. Sterigmata (8.7–) 8.8–11.1 (–11.7) µm long, with a mean length of 9.6–10.5 µm. Colour for the great majority very pale green in KOH, sometimes with a faintly blue or brown hue (but not the blue green reaction present in other species), for a small number formed directly from subicular hyphae brown; sometimes with granular contents; in water orange brown and with strongly granular contents.

Cystidial organs lacking.

Basidiospores in frontal face generally with a broadly subellipsoid, triangular or subcircular basic shape and an unlobed, angular, nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, occasionally singularly attached, echinuli. A majority of the spores normally with three-six indistinct corners to distinct, square lobes; broadly ellipsoid, unlobed spores infrequently occurring (but dominate in some collections), as well as abnormally large spores originating from two-sterigmate basidia. Frontal dimensions: 7.7–9.3 (–9.4) × (7.6–) 7.9–9.1 (–9.4) µm; mean dimensions: 8.3–8.7 × 8.4–8.7 µm; Q-value: (0.9–) 1.0–1.1; mean Q-value: 1.0. Echinuli (0.7–) 0.8–1.5 µm long, with a mean length of 1.1–1.2 µm. Lateral face ellipsoid to narrowly ovoid or sometimes semicircular in shape, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: 8.0–9.3 (–9.6) × (5.1–) 5.6–6.7 (–6.9) µm; mean dimensions: 8.4–8.7 × 6.0–6.1 µm; Q-value: (1.2–) 1.3–1.6 (–1.7); mean Q-value: 1.4–1.5. Colour in KOH pale green to pale brown; in water orange brown to brown; inamyloid.

Chlamydospores lacking.

Habitat. *P. umbrina* has a wide ecological amplitude. Recent Scandinavian collections have been made in young to old deciduous, mixed and coniferous forests on soil with low to high pH, as well as on the tundra. The species has been found to form ectomycorrhiza with at least *Abies alba*, *Alnus rubra*, *Betula nana*, *Betula pubescens* ssp. *czerepanovii*, *Betula pubescens* ssp. *pubescens*, *Dryas octopetala*, *Fagus sylvatica*, *Picea abies*, *Picea glauca*, *Picea mariana*, *Pinus banksiana*, *Pinus pinaster*, *Pinus sylvestris*, *Pseudotsuga menziesii*, *Pyrola media*, *Quercus petraea*, *Salix polaris* and *Tsuga canadensis* (collection data; Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Canada, Estonia, Finland, Norway, Sweden and the United Kingdom. Soil or root tip samples confirm presence also in: France, Poland, Spain and the United States.

Remarks. The nomenclatural situation surrounding *P. umbrina* is complex. Fries described *Thelephora umbrina*, explicitly excluding *Thelephora umbrina* Pers., but synonymising it with *Thelephora umbrina* var. *lignatilis* Alb & Schwein. These names might represent different species or not, but in either case do not threaten *Thelephora umbrina* Fr., due to the sanctioning.

A large number of names synonymous with *P. umbrina* have been illegitimately or superfluously published. Fries himself (1847), as well as Quélet (1888) and Burt (1916), seem to have overlooked Wallroth's (1833) combination of *Hypochnus umbrinus* (Alb. & Schwein.) Wallr. from *Himantia umbrina* Alb. & Schwein, (1805) and hence created illegitimate name combinations. The status of Donk's (1933) combination of *Tomentella umbrina* (Fr.) Donk versus Litschauer's (1933) remains hard to evaluate due to the fact that, although 20 June is known to be the date of Litschauer's publication, 7 Julyis when Donk defended the thesis wherein he published his combination; the publication date of Donk's thesis was probably at an unknown point in time prior to that of his dissertation. Combinations based on *Thelephora umbrina* where the combining authors cite Alb. & Schwein as authors of the basionym (e.g. Saccardo 1888) have to be considered miscitations, since there is no *Thelephora umbrina* Alb. & Schwein.

Our interpretation of *Thelephora umbrina* Fr. as the basionym follows Burt (1916), Litschauer (1933), Svrcek (1958) and Larsen (1967), although Rogers and Jackson (1943) considered it to be a synonym of *Coniophora olivacea*. The name is not used here in the sense of what we today interpret as *C. olivacea*, but in the sense of Burt's (1916) type selection.

The material in the Fries Herbarium cited by Burt (1916), as the type of *Thelephora umbrina* Fr., constitutes a collection made by Fries at locus classicus, Femsjö, but in Fries's own handwriting, it is identified as *Corticium umbrinum* (Fr.) Fr., a name he combined *T. umbrina* to in 1874 (Fries 1874). Therefore, Burt's typification cannot be considered a lectotype, but must be regarded as a neotype. We here designate an epitype from Femsjö, which matches the neotype morphologically.

Within the *P. tristis* group, basidiomata of *P. umbrina* can be recognised by their brown colour – blue or green colours are completely absent from immature parts and

from the subhymenium of mature parts – their soft, rather elastic texture after drying and their microcharacters. *Pseudotomentella umbrinascens* is very similar but has slightly different microcharacters (see key). *Hypochnus rhacodium* (only known from the type) is also similar but has hard, brittle basidiomata after drying.

Additional specimens studied. CANADA. Newfoundland: Crooked Knife, mixed forest with *Betula*, *Alnus* and *Picea*, 99 m a.s.l., 10 September 2008, U. Kõljalg (TU 108084*);

ESTONIA. Põlva: Vastse-Kuuste, older *Pinus-Picea* mixed forest between Kiidjärve and Taevaskoja, near Maarja village, 22 September 2005, U. Kõljalg (TU 100329, 100339, 100340); Saare: Muhu, Kesselaid, Karjalasma forest, *Picea abies* forest, 28 August 1998, Erast Parmasto (TAAM 174051); Põlva: Vastse-Kuuste, coniferous forest with *Pinus* and *Picea* along road between Kiidjärve and Taevaskoja, east of Ahja river, 18 August 2005, U. Kõljalg (TU 100194); Viljandi: Pääsmä laas, Sooma National Park, on a fallen *Betula* trunk over Halliste river, 7 September 2000, U. Kõljalg (TU 108538);

FINLAND. Kanta-Häme: Lammi, Kotinen Virgin Forest, 10 September 2001, U. Kõljalg (TU 108742, 108743, 108744); Etelä-Häme, Ruovesi, Siikaneva swamp islands, 14 September 1999, U. Kõljalg (TAAM 159809, 159810); Satakunta: Ilkaalinen, under *Picea* log and mosses, 29 August 2010, U. Kõljalg (TU 115017); Varsinais-Suomi: Parainen, Kuggö, 24 October 2009, P. Kunttu (TU115344*);

NORWAY. Oppland, Dovre, Hjerkinn, low alpine vegetation under *Salix phyllicifolia*, *Salix lapponica* and *Betula nana*, on soil with low pH, 14 September 2014, S. Svantesson 216, 221* (GB); Akershus: Asker, Skaugumåsen, boreonemoral, mixed forest on moderately alkaline, moderately nutrient-rich ground under, 23 September 2010, S. Svantesson (O F110268*); Troms: Kvænangen, Kvænangselva, boreal mixed forest on soil with low pH, 31 August 2013, B. Larsson and K.-H. Larsson (O F110269); Ibidem, boreal, deciduous forest on soil with intermediate pH, 31 August 2013, B. Larsson and K.-H. Larsson (O F110270, F110271); Oppland: Dombås, Hjerkinnholen, boreal, mixed forest on soil with low pH, 30 September 2013, K.-H. Larsson (O F110272, F110273, F110274, F110275, F110276, F110277); Sogn og Fjordane: Leikanger, Flætene-Vesterheim, boreonemoral, mixed forest on soil with low pH, 2 October 2012, K.-H. Larsson and S. Svantesson (O F110278, F110279, F110280); Sogn og Fjordane: Eid, Eitrefjellet, deciduous forest on soil with high pH, 25 September 2013, K.-H. Larsson (O F110281); Oppland, Dovre, Grimsdalen, Storberget, subalpine *Betula pubescens* ssp. *czerepanovii* forest on soil with low pH, 26 August 2010, K.-H. Larsson and S. Svantesson (O F110282, F110283, F110284, F110285, F110286); Aust-Agder: Tvedestrand, Eidbo, boreonemoral, mixed forest on soil with intermediate pH, 10 September 2010, S. Svantesson and N. Svensson (O F110307); Aust-Agder: Åmli, Gangsei W, boreonemoral, mixed forest on soil with low pH, 09 September 2010, S. Svantesson and N. Svensson (O F110308); Telemark: Drangedal, Asgjerdstigfjellet, boreonemoral, deciduous forest on on soil with intermediate pH, 28 September 2010, S. Svantesson and N. Svensson (O F110309,

F110310); Vest-Agder: Mandal, Uføra, nemoral, deciduous forest on soil with high pH, 26 September 2012, K.-H. Larsson and S. Svantesson (O F110287); Sogn og Fjordane: Leikanger, Kvinnafossen, boreonemoral, mixed forest on soil with high pH, 2 October 2012, K.-H. Larsson and S. Svantesson (O F110288); Nord-Tröndelag: Grong, Gartlandselva, boreal, coniferous forest on soil with low pH, 27 August 2012, K.-H. Larsson (O F110289, F110290, F110291, F110292, F110293, F110294); Nordland: Saltdal, Nystadneslia, boreal, mixed forest on soil with intermediate pH, 24 August 2012, K.-H. Larsson (O F110295, F110296*); Telemark: Tokke, Dalen, Huvestad, boreonemoral, mixed forest on soil with high pH, 28 September 2010, S. Svantesson and N. Svensson (O F110311); Akershus: Nannestad, Tomte farm, 3 September 2004, U. Kõljalg (TU 100005, 100007); Telemark: Sauherad, E of Nordagutu, W slope of Bjørndalsfjell along path to Svanastøl, 24 September 2003, K.-H. Larsson 12094 (TU); Buskerud: Nes, Alungruken, 25 September 1997, J. Stokland (TU 115209*), Rogaland: Forsand, Rössdalen, on *Salix* sp., 14 October 1998, K. Hjortstam 17918 (K.-H. Larsson private collection);

SWEDEN. Lycksele Lappmark: Storuman, Blaiken N, boreal, mixed, old-growth forest on fertile, moderately alkaline ground, 26 August 2015, S. Svantesson 137 (GB); Västerbotten: Umeå, Stora Tuvan, older, boreal, mixed forest on soil with low pH, 28 August 2015, S. Svantesson 174*, 175 (GB); Lule Lappmark: Gällivare, Ritsem, subalpine *Betula pubescens* ssp. *czerepanovii* forest on soil with low pH, 11 August 2016, S. Svantesson 234, 239*, 240 (GB); Lule Lappmark: Jokkmokk, Slappejaure NO, middle alpine vegetation on soil with high pH, 14 August 2016, S. Svantesson 255, 256 (GB); Lule Lappmark: Jokkmokk, Unna Duvgge, low alpine vegetation on soil with intermediate pH, 15 August 2016, S. Svantesson 277 (GB); Lule Lappmark: Jokkmokk, Ajajaure N, low alpine vegetation on soil with high pH, 16 August 2016, S. Svantesson 279, 280* (GB); Halland: Kungsbacka, Släp, Särö Västerskog, old growth *Pinus* and *Quercus* forest, under a *Pinus* log, 1 October 1999 U. Kõljalg (TAAM 159818); Ångermanland; Sollefteå, Sörgraninge mångfaldspark, Språngsjöberget, 9 September 2014, K.-H. Larsson 16608 (GB); Västergötland: Alingsås, Simmenäshalvön, Gräskärr, on *Picea*, 5 October 2008, B. and K. Hjortstam 20311, 20332 (K.-H. Larsson private collection); Ibidem, on wood of *Quercus* on the ground, 13 September 2004, K. Hjortstam 18795 (K.-H. Larsson private collection); Ibidem, on *Picea* bark, 17 October 2001, K. Hjortstam 18531 (K.-H. Larsson private collection); Västergötland: Vårgårda, Nårunga, Sandviksås, on branch of *Quercus robur*, 8 November 2000, Björn Nordén (TU 115240);); Öland: Böda, Fagerör, under log of *Pinus sylvestris*, 15 October 2016, E. Larsson 387-16 (GB); Öland: Böda, Bryum Sandvik, under log of *Pinus sylvestris*, 15 October 2016, E. Larsson 384B-16 (GB);

UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND. Scotland, Invernesshire: Glen Strathfarrar National Nature Reserve, ancient *Pinus sylvestris* forest with a few oak trees, 14 September 2005, U. Kõljalg (TU 100304); Scotland, Morayshire: Culbin Forest, planted *Pinus sylvestris* forest on sand dunes, 13 September 2005, U. Kõljalg (TU 100292).

***Pseudotomentella umbrinascens* Svantesson, sp. nov.**
MycoBank No.: MB829031
Fig. 19

Type. SWEDEN. Bohuslän: Tanum (municipality), Tanum (parish), Greby Kleva, boreonemoral, deciduous forest on soil with high pH, RT90: E1236840, N6518916, 6 September 2016, S. Svantesson 335 (holotype: GB!, GenBank Acc. No. ITS: MK290697)

UNITE SH. SH030563.07FU

Etymology. The name refers to the overall morphological similarity to *P. umbrina*.

Description. Basidiome annual, resupinate, membranaceous, effused to approximately ten centimetres in diameter. Mature parts continuous, with a cottony texture when fresh and a rather firm, fibrous and compact, yet quite soft and elastic texture when dried. Hymenium smooth; greenish-brown when fresh, pale brown when dried. Immature parts discontinuous, byssoid with a cottony texture, both when fresh and when dried. Subhymenium and hymenium of the immature parts initially yellowish-white to pale brown, in the dried basidiome, when more mature pale brown. Subiculum well developed, loose, fibrous, pale yellowish-brown to pale orange brown; forms the outer edge of the basidiome, extending noticeably beyond the hymenium.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections and reaction in Melzer's reagent absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae 3.1–) 3.2–4.3 (–4.8) µm wide, with a mean width of 3.7 µm; pale orange brown to brown in KOH, orange brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae 3.6–5.7 (–7.1) µm wide, with a mean width of 4.5 µm; pale grey brown to grey brown or brown in KOH; orange brown to pale green in water (but not with the blue green reaction present in other species), with strongly granular contents.

Encrustation not seen.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; clavate to narrowly clavate, sometimes clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: (57–) 58–71 (–75) × (8.8–) 9.5–11.5 (–12.5) µm; mean dimensions: 64 × 10.6 µm. Sterigmata 8.1–9.5 (–10.1) µm long, with a mean length of 8.6 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition sometimes with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a triangular or subcircular basic shape and an angular to cross-shaped or sometimes nodulose outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with three-four distinct, often rounded lobes; subcircular, five-lobed spores infrequently occurring, as well as abnormally large spores originating from two-sterigmate basidia. Frontal

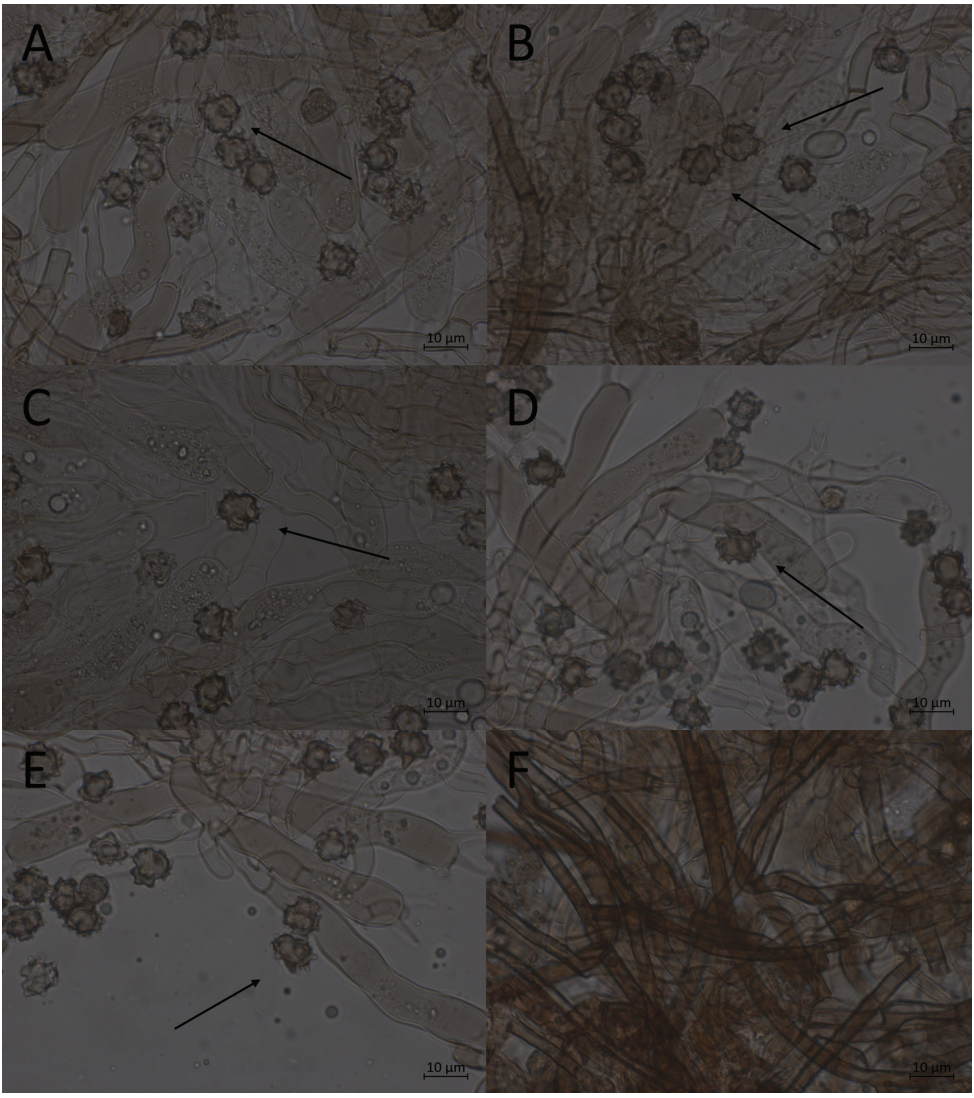


Figure 19. Micromorphological features of *P. umbrinascens* in KOH. Holotype: **A, B, C** basidiospores in frontal face **D, E** in lateral face **E** subicular hyphae.

dimensions: (8.5–) 8.7–9.4 (–9.6) × (8.4–) 8.7–9.2 (–9.3) µm; mean dimensions: 8.9 × 8.9 µm; Q-value: 1.0 (–1.1); mean Q-value: 1.0. Echinuli (0.9–) 1.0–1.9 (–2.0) µm long, with a mean length of 1.6 µm. Lateral face ellipsoid to narrowly ovoid or sometimes semicircular in shape, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: 8.5–9.2 (–9.4) × (5.7–) 6.0–6.5 µm; mean dimensions: 8.9 × 6.2 µm; Q-value: 1.3–1.5 (–1.6); mean Q-value: 1.4. Colour in KOH pale brown to pale greenish-brown colour; in water pale brownish-orange to pale greenish-orange; inamyloid.

Chlamydospores lacking.

Habitat. The only specimen recorded to date of *P. umbrinascens* is the type collection, which was obtained in an old, coastal, deciduous forest on soil with high pH. UNITE sequence metadata shows that the species forms ectomycorrhiza with at least *Corylus avellana* (Kõljalg et al. 2005, Nilsson et al. 2019).

Distribution. Basidiomata encountered in: Sweden. Soil or root tip samples confirm presence also in: Italy.

Remarks. Within the *P. tristis* group, basidiomata of *P. umbrinascens* can be recognised by their brown colour, their soft, rather elastic texture after drying and their microcharacters. Blue or green colours are completely absent from immature parts and from the subhymenium of mature parts. *Pseudotomentella umbrina* is very similar but has slightly different microcharacters (see key). *Hypochnus rhacodium* (only known from the type) is also similar but has basidiomata that are hard and brittle after drying.

Dubious taxa

Pseudotomentella longisterigmata **M.J.Larsen, Canad. J. Bot. 45: 1298 (1967)**
Fig. 20

Type. UNITED STATES. Washington: Olympic Peninsula, Sol Duc River, on coniferous wood, 25 August 1957, J. L. Lowe 8061 (holotype: BPI US0291345!; isotype: SYRF).

Description. Basidiome annual, resupinate, membranaceous, effused to approximately ten centimetres in diameter. Mature parts continuous, with a cottony to rather firm, fibrous and compact, yet quite soft and elastic texture. Hymenium smooth; bluish-grey to brownish-grey. Immature parts discontinuous, byssoid with a cottony texture. Subhymenium and hymenium of immature parts bluish-grey. Subiculum well-developed, loose, fibrous, orange brown; forms the outer edge of the basidiome, extending noticeably beyond the hymenium. All characters recorded in dried state.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae 4.9–7.2 µm wide, with a mean width of 6.2 µm; orange brown to brown in KOH, orange to orange brown in water.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.0–) 3.2–4.9 (–6.1) µm wide, with a mean width of 3.9 µm; pale brownish-green in KOH, blue green in the presence of air; brownish-green in water, with strongly granular contents.

Encrustation granular, probably amyloid (hard to observe due to the colour); dark brownish-green in KOH, dark blue green in the presence of air; blackish in water; scattered in occurrence on the upper parts of subhymenial hyphae and on the lower parts of basidia.

Basidia with four very long, slightly curved to hypha-like sterigmata, occasionally two-sterigmate; clavate or sometimes clavopedunculate, thin-walled, with one-

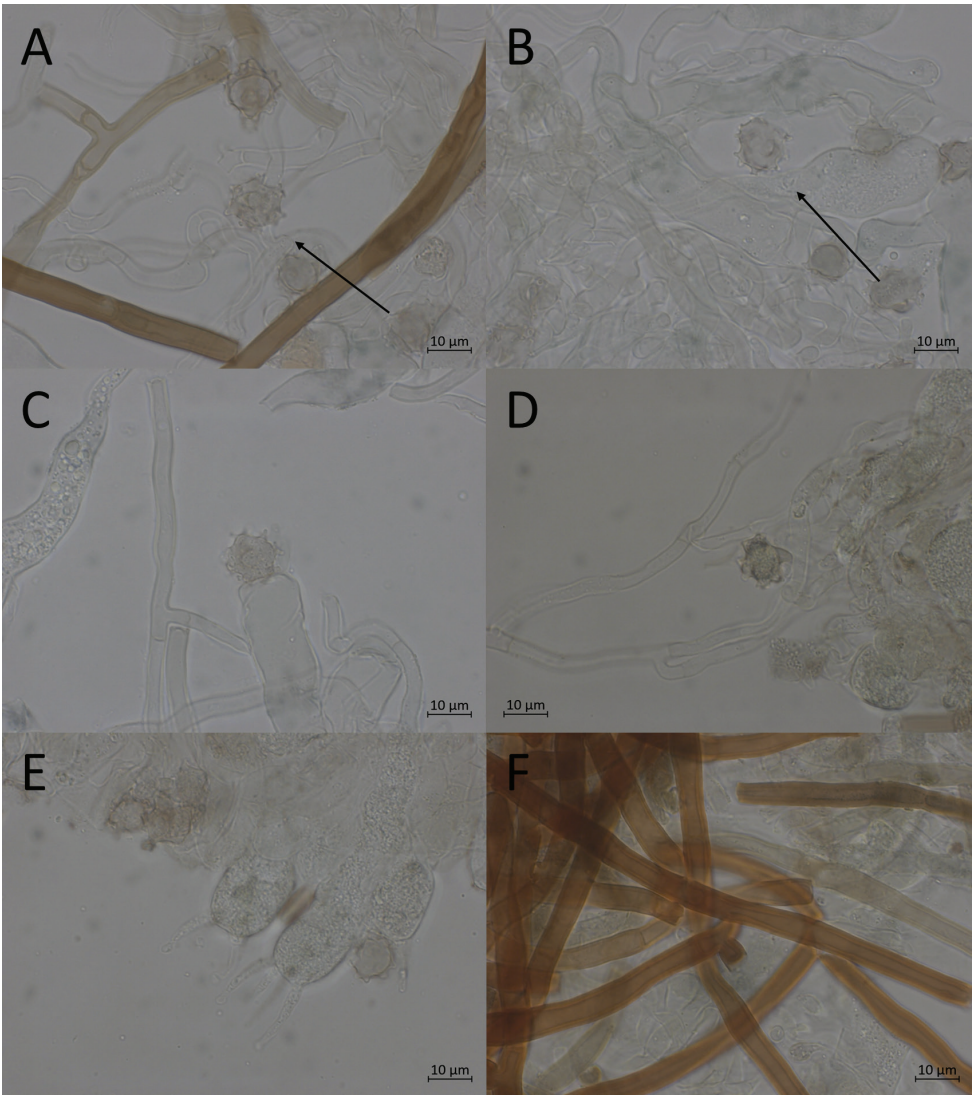


Figure 20. Micromorphological features of *P. longisterigmata* in KOH. Holotype: **A, B, C** basidiospores in frontal face **D** in lateral face **E** basidia **F** subicular hyphae.

three slight constrictions. Dimensions: (73–) 77–110 (–121) × (12.3–) 13.0–15.1 (–16.3) µm; mean dimensions: 91 × 13.9 µm. Sterigmata (11.2–) 11.7–17.9 (–19.3) µm long, with a mean length of 14.7 µm. Colours and reactions the same as for the subhymenial hyphae, but in addition often with granular contents in KOH.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. A majority of the spores with three-five indistinct lobes; unlobed subellipsoid or broadly ovoid spores present to a lesser extent, as

well as subcircular, six or seven-lobed spores; abnormally large spores originating from two-sterigmate basidia infrequently occurring. Frontal dimensions: (9.7–) 10.0–11.7 × (9.4–) 9.8–11.7 µm; mean dimensions: 11.0 × 10.7 µm; Q-value: (0.9–) 1.0 (–1.1); mean Q-value: 1.0. Echinuli 1.2–1.8 (–2.1) µm long, with a mean length of 1.5 µm. Lateral face ellipsoid to ovoid, usually with evenly rounded edges, sometimes with one-three lobes. Lateral dimensions: 10.3–11.5 (–11.7) × (6.7–) 7.5–9.1 µm; mean dimensions: 10.9 × 8.5 µm; Q-value: 1.2–1.6; mean Q-value: 1.3. Colour in KOH pale brown to pale greenish-brown, in the presence of air often with a green to blue green reaction; in water brown; occasionally amyloid.

Chlamydospores lacking.

Habitat. The only specimen of *P. longisterigmata* recorded to date is the type collection, which was collected on coniferous wood in a coastal forest in the state of Washington, United States.

Distribution. Basidiomata encountered in: the United States.

Remarks. The type collection is large and in seemingly good condition but repeated attempts at obtaining a useful DNA sequence from it proved unfruitful. The specimen exhibits a peculiar morphology, where the basidia carry sterigmata that are unusually long for the *P. tristis* group. They are often cylindrical rather than tapering and hence resemble generative hyphae – a growth form that basidia are sometimes seen reverting into in corticioid basidiomata formed under unfavourable conditions. It is therefore doubtful whether *P. longisterigmata* is a true species, but since this presently cannot be ascertained and, in order to stimulate its recollection, the name is here retained as a separate species.

There are relatively few spores in the hymenium of the holotype and many of them are immature. A more mature fruiting body of the species would hence probably have a browner colour.

Within the *P. tristis* group, the basidiome of *P. longisterigmata* can be recognised by its lack of hyphal cords and skeletal hyphae, its soft, yet rather firm and compact and ± elastic texture after drying, bluish to greenish colour of immature parts, wide subicular hyphae, large spores and long sterigmata. *Pseudotomentella alobata*, *P. pluriloba* and *P. abundiloba* are similar but all have smaller spores and shorter sterigmata.

Hypochnus rhacodium **Berk. & M.A.Curtis ex Burt, Ann. Missouri Bot. Gard. 13(3): 322 (1926).**

Fig. 21

Type. UNITED STATES OF AMERICA. Pennsylvania: on underside of decaying logs of apparently a frondose species, E. Michener 1435 (syntypes: Mo. Bot. Gard. Herb. 5095 [BPI US0291002]!; FH Curtis Herb. 4061; K Curtis Herb. 4061, designated by E.A. Burt in Ann. Missouri Bot. Gard. 13: 322 (1926)).

Description. Basidiome annual, resupinate, membranaceous, effused. Mature parts continuous, with a hard and rather brittle texture. Hymenium smooth; pale

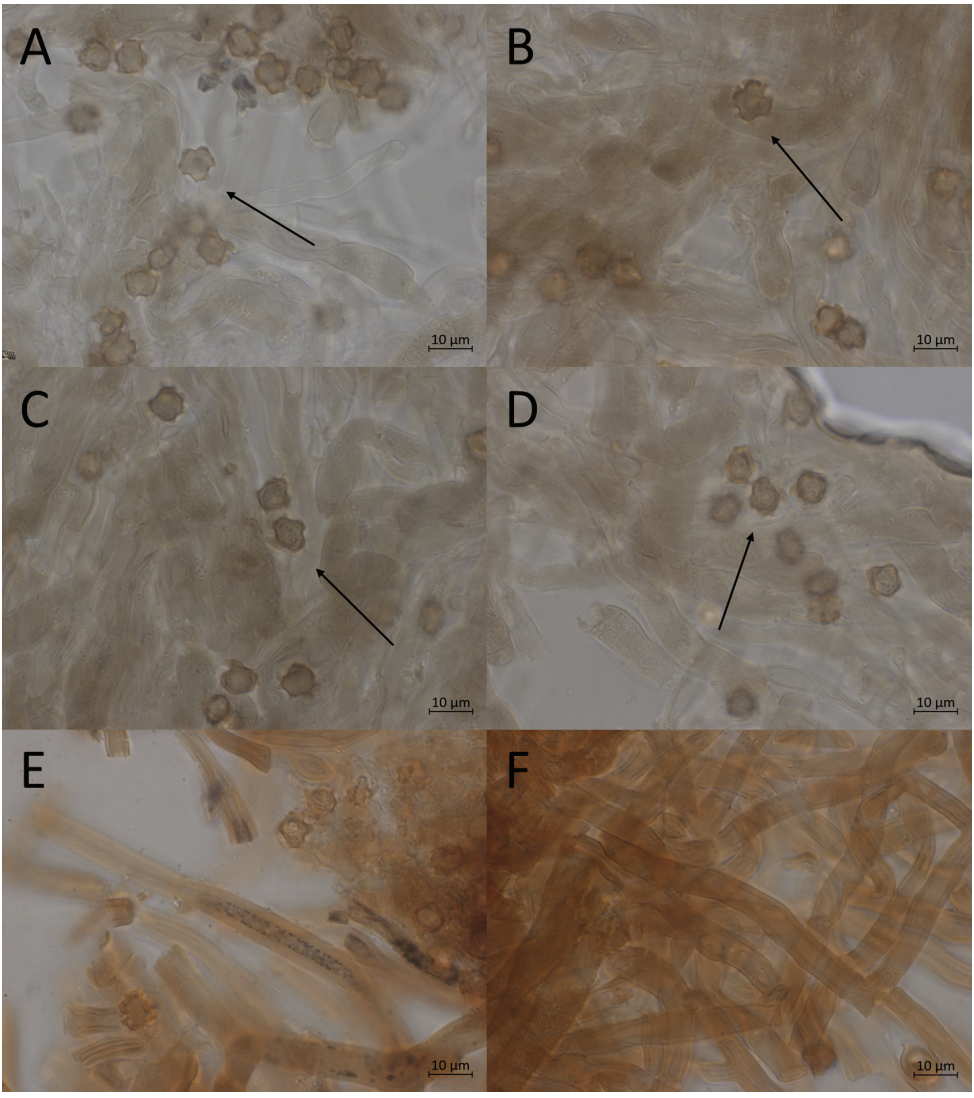


Figure 21. Micromorphological features of *H. rhacodium* in KOH. Syntype: **A, B** basidiospores in frontal face **C, D** in lateral face **E** subicular hyphae with granulation and **F** without.

brown to brown with a pink hue. Subiculum loose, fibrous and dark brown with an orange hue. All characters recorded in dried state.

Hyphal cords lacking, but loose bundles of subicular hyphae sometimes present.

Hyphal system monomitic, clamp connections and reaction in Melzer's reagent absent from all hyphae.

Subicular hyphae noticeably long and straight, thick-walled; forming a loose tissue. Individual hyphae (5.6–) 5.7–7.3 (–8.0) µm wide, with a mean width of 6.5 µm;

pale to dark orange brown in both KOH and water, sometimes with granular contents which turn blue green in the presence of air.

Subhymenial hyphae often somewhat sinuous, thin to thick-walled; forming a rather dense tissue. Individual hyphae (3.7–) 3.8–5.3 (–6.1) µm wide, with a mean width of 4.7 µm; brown to pale orange brown or pale green in KOH (but not with the blue green reaction present in other species); orange brown to brown in water, with strongly granular contents.

Encrustation granular, probably amyloid (hard to observe due to the colour); brownish-black in KOH, dark blue green in the presence of air; brownish-black in water; scattered in occurrence on the subicular hyphae.

Basidia with four slightly curved sterigmata, occasionally two-sterigmate; narrowly clavate or sometimes narrowly clavopedunculate, thin-walled, with one-three slight constrictions. Dimensions: 73–105 (–109) × (8.8–) 8.9–10.1 (–11.2) µm; mean dimensions: 94 × 9.6 µm. Sterigmata (8.5–) 9.5–12.1 (–12.5) µm long, with a mean length of 10.9 µm. Colours and reactions the same as for the subhymenial hyphae.

Cystidial organs lacking.

Basidiospores in frontal face generally with a subcircular basic shape and an angular to nodulose or sometimes cross-shaped outline, covered in bi- or trifurcate, sometimes singularly attached, echinuli. Nearly all spores with three-five distinct, rounded to square lobes; abnormally large spores originating from two-sterigmate basidia infrequently occurring. Frontal dimensions: (7.8–) 8.0–9.1 (–9.3) × (7.7–) 7.8–8.9 (–9.0) µm; mean dimensions: 8.3 × 8.3 µm; Q-value: 0.9–1.1; mean Q-value: 1.0. Echinuli (0.9–) 1.0–1.6 (–1.7) µm long, with a mean length of 1.3 µm. Lateral face ellipsoid to ovoid or sometimes subcylindrical, usually with angular edges, sometimes with one-three lobes. Lateral dimensions: (7.9–) 8.2–8.9 × (5.4–) 5.9–6.8 (–7.0) µm; mean dimensions: 8.5 × 6.3 µm; Q-value: 1.2–1.4 (–1.6); mean Q-value: 1.3. Colour in KOH brown to orange brown, in the presence of air with a blue green reaction; in water greenish-orange to orange brown; inamyloid.

Chlamydospores lacking.

Habitat. The only specimen of *H. rhacodium* recorded to date is the type collection, which was collected in Pennsylvania, United States. No further information on habitat or any further locality description is available.

Distribution. Basidiome encountered in: the United States.

Remarks. The hymenium of *H. rhacodium* is very thick and dense in comparison to all other *Pseudotomentella* species. It consists of tightly packed basidia, which are overlapping in length and have a total thickness equalling four-six basidial lengths. All other morphological characters fit well within the *P. tristis* group, thus suggesting an abnormal basidiome.

Within the *P. tristis* group, the basidiome of *H. rhacodium* can be recognised by its lack of hyphal cords and skeletal hyphae and its hard and brittle texture after drying. This feature makes it unique within the group and the risk for confusion with any other described species should hence be small.

Excluded taxa

Septobasidium arachnoideum (Berk. & Broome) Bres., Ann. Mycol. 14 (3-4): 241 (1916)

Homotypic names. *Thelephora arachnoidea* Berk. & Broome, J. Linn. Soc., Bot. 14: 64 (1873) [1875]. *Hypochnus arachnoideus* (Berk. & Broome) Bres., Ann. Mycol. 1(2): 108 (1903). *Tomentella arachnoidea* (Berk. & Broome) Höhn. & Litsch., Wiesner Festschrift: 77 (1908).

Type. CEYLON [Nowadays Sri Lanka]. Habgalla, Feb. 1868, [M. J. Berkeley and C. E. Broome] No. 539 (K).

Remarks. Bresadola (1916) combined *Thelephora arachnoidea* Berk. & Broome to *Septobasidium*, and the species was accepted by Couch (1938) in his detailed review of the genus. We thus have no reason to believe that this species belongs in *Pseudotomentella*.

Tomentella biennis (Fr.) A.M.Rogers Mycologia 40(5): 634 (1948)

Homotypic names. *Auricularia phylacteris* Bull., Herb. France 10: plate 436, fig. 2 (1790). *Thelephora phylacteris* (Bull.) J.F.Gmel., Syst. nat. 2 (2): 1441 (1792) [combination also made by de Candolle (1815)]. *Thelephora biennis*, Fr., Syst. mycol. 1: 449 (1821), sanctioned name. *Phylacteria biennis* (Fr.) Bigeard, Fl. champ. sup. France 2: 452 (1913).

Type. Bulliard JBF (1790) Herbier de la France, ou Collection complette des plantes indigenes de ce royaume; Avec leurs Détails Anatomiques, leurs propriétés, et leurs usages en Medecine. Tome 10, plate 436, figure 2, LECTOTYPE of *Auricularia phylacteris*, here designated (Mycobank Typification No. MBT384912), LECTOTYPE of *Thelephora biennis*, here designated (MBT384913).

Remarks. Bulliard (1790) described *Auricularia phylacteris* and Gmelin (1792) combined it to *Thelephora*. Seemingly, both Fries and de Candolle (1815) overlooked Gmelin's combination. Fries (1821) created the name *Thelephora biennis*, citing under it *A. phylacteris* and *T. phylacteris* DC, but seems to attribute it to de Candolle in the index of the Systema Mycologicum 3 (Fries 1832). This is probably an error and since Fries is the original author of the name, we agree with Petersen (1975) that the authorship is *Thelephora biennis* Fr.

Fries (1821) indicated "v.ic.", which would be a reference to the plate in Bulliard (1790). There are no specimens under any of the aforementioned names known to have been examined by Bulliard, de Candolle or Fries. Consequently Bulliard's plate of *A. phylacteris* (1790), mentioned by Fries (1821), is here designated as the lectotype of *A. phylacteris* and *T. biennis*.

The protologue of *T. biennis* describes and the plate of *A. phylacteris* depicts a species which is plicated at the lower part of the basidiome, yellowish-white when young,

brown when older and which eventually turns black. It is further described as biennial and growing up from the ground and on to stones and branches, if they are present in its vicinity. It is hence doubtful whether the species in question belongs to the Thelephorales at all and, even though it has been synonymised with *P. umbrina* by Burt (1916), Litschauer (1933) and Svrček (1958), it does not match any *Pseudotomentella* described to date.

Discussion

In a world where unseen and undescribed new phyla hide in a grain of soil (Nilsson et al. 2016) and where visible, morphologically delimited taxa increasingly turn out to constitute cryptic species (e.g. Kroken and Taylor 2001, Shivas and Cai 2012), it is reassuring to note that some visible, molecularly delimited species can still be separated morphologically. Similarly to many other fungal species, we, nevertheless, found *P. tristis* s.l. to constitute a complex of closely related and morphologically very similar species (e.g. Lücking et al. 2014, Jeppson et al. 2017 and Larsson et al. 2018). Thus Hjortstam's (1969) interpretation of *P. tristis* and *P. umbrina* as two different species was indeed correct, but so was Larsen's (1971a) argument that the variation he observed could not be separated into two species; under the name *P. tristis* are hiding no less than 13 species exhibiting morphological characteristics so close in range that they would indeed seem like a continuum to all mycologists without the aid of molecular analysis methods. In the light of our resurrection of *P. umbrina* as a separate taxon and the reviewed delimitation of *P. tristis* and *P. atrofusca*, this study not only proves the importance of combining molecular analysis methods with careful morphological studies, but also shows the power of these in conjunction with type studies. In the case of *H. rhacodium* and *P. longisterigmata*, the problems that can arise from species descriptions based solely on morphology are also clearly demonstrated.

From the perspective of functionality and usability of the international DNA sequence databases, it is satisfying to acknowledge that, while metadata from ecological studies have been very useful for understanding the molecularly delimited taxa presented here, future ecological studies querying such databases now have more reliable names to use. One species in the *P. tristis* group – *P. umbrina* – was indeed found to be widespread, have a wide ecological amplitude and, at least in northern Europe, to be commonly occurring. This is not to say that all the other species in the group are less widely distributed or have narrower ecological niches; some species, for example, *P. pinophila*, *P. tristis* itself and *P. sciastra*, have been collected in widely separated countries and habitats, but in comparison to *P. umbrina* they have rarely been encountered so far. More material is needed to establish the frequency of occurrence and ecological niches of all species in the species complex – information that might prove a helpful complement to morphology in the process of species identification, given the high degree of similarity between some species. With the current knowledge, it is quite paradoxical that the combination *P. tristis* was made by an American mycologist (Larsen

1971a), while the species in question now has no confirmed findings in North America. In contrast, *P. atrofusca*, a species believed to be widely distributed in Europe (GBIF 13–08–2018) and documented from the Russian far east (Kõljalg 1996), is now only known with certainty from the North American type collection and three sequences from China. South East Asia and Russia generally constitute large white spaces on the mycological map, even though findings so far indicate that species in the *P. tristis* group do occur in these areas. Even after taking the ecological knowledge gap into account, it is interesting to note that, unlike species in ectomycorrhizal genera such as *Leccinum* Gray and *Hygrophorus* Fr. that show strong host preferences and have more limited distribution ranges (den Bakker et al. 2004, Moreau et al. 2018), most species in the *P. tristis* group are able to form ectomycorrhiza with a broad range of hosts and are widely distributed. The fact that all species, except for *P. umbrina*, seem to be restricted to areas where soil pH is intermediate or high is possibly a factor that could help explain their difference in occurrence frequency.

The present study clarifies the application of the name *P. tristis*. In doing so, however, it renders hundreds of previous molecular ecology studies obscure with respect to this particular name. The name of *P. tristis* has served as something of a wastebasket for any and all *Pseudotomentella* species, owing both to the obscure nature of the underlying taxonomy and to the noisy state of taxonomic annotations in the public sequence databases. Thus, while the present study clarifies the use of the name *P. tristis*, it also raises doubts about previous molecular ecology results in the context of this name. To the extent that previous studies have relied on UNITE Species Hypotheses identifiers rather than Latin names when reporting molecular ecology results, this problem will be solved automatically. However, any study that tied species occurrences only to Scientific names may, from now on, convey incorrect information in the context of *Pseudotomentella*.

To the extent that it can be assessed given the moderate phylogenetic resolution, it is intriguing that the morphological characters that differ between species (e.g. spore size and shape, subicular hyphal width) do not seem to have a strong phylogenetic link. Whether these absences of patterns have the same cause, for example, an old rapid radiation, with extensive gene flow or are just artefacts of time and chance – causing both intragenic mutational conflict and genetic drift towards evolutionarily neutral shifts in morphology – is unclear, but could possibly be resolved by analysis of additional genetic regions. This may also shed some light on the presence of paralogous relationships between some of the taxa in the group and would possibly resolve some species into additional new species. The considerable genetic and morphological variation exhibited by *Pseudotomentella sciastra*, for example, may well indicate a species complex. Both ASTRAL and STACEY should be robust with the relatively small datasets used in the present study, in the sense that the employed datasets should not include less species than the analyses support. Additional specimen sampling may, however, aid in the distinction between populations and any possible, additional species and would thus, besides widened gene sampling, also be preferable in an extended study of the group.

Concerning morphology, the presence of amyloid material in and on basidia and subhymenial hyphae of Thelephorales species does not seem to have been reported.

This is surprising, given its possible usefulness as a discriminatory character between species. Whether the cause of this is rarity or obscurity remains to be revealed by further studies in the field. Similarly worthy of notice is the blue green reaction observed in the same micromorphological structures of some species. Such a reaction has been mentioned by others studying *Tomentella* and *Pseudotomentella* (Larsen 1971a, Kõljalg 1996), but we would like to draw attention to the observation that the reaction in question here only seems to occur in the presence of air and also to its probable usefulness as a species-separating character. Finally, this study demonstrates clearly the necessity of applying a well-developed and consistent methodology when assessing the morphological characters of closely related species. It cannot be emphasised enough how important it is for those who endeavour to correctly identify Thelephorales species to carefully measure spores using the methodology originally described by Kõljalg (1996) and further explained in the Methods sections of this paper.

Acknowledgements

Funding for this study was received from The Swedish Taxonomy Initiative (2014-152 4.3), Göran Gustafssons Stiftelse för natur och miljö i Lappland, Stiftelsen Lars Hiertas Minne, Helge Ax:son Johnsons Stiftelse, Wilhelm & Martina Lundgrens Vetenskapsfond and Kapten Carl Stenholms donationsfond.. The authors gratefully acknowledge the curators of herbaria ARIZ, BPI, H, S, TU and TUR for granting and arranging loans. Stefan Ekman and Svengunnar Ryman are cordially thanked for their assistance during the visit to UPS. We are very grateful to Björn Larsson and Natalia Svensson for help with collecting specimens, Unto Söderholm for the information and photograph of *P. pluriloba* and Seppo Huhtinen and Viacheslav Spirin for assistance with locating and distributing collections. Finally, SS wishes to express his sincere gratitude to Bengt Oxelman for discussions on species concepts and the use of STACEY, to Solveig Bua Løken for the same, as well as inspiration for the map and to Bengt Oxelman and Alanna Main for comments on the manuscript.

References

Agerer R (1994) Characterization of ectomycorrhiza. In: Norris JR, Read DJ, Varma AK (Eds) Techniques for mycorrhizal research. Academic Press, 25–74.

Albert M et al. (2018) Inkscape 0.92.3. https://inkscape.org/

Argüelles-Moyao A, Garibay-Orijel R, Márquez-Valdelamar LM, Arellano-Torres E (2017) *Clavulina-Membranomyces* is the most important lineage within the highly diverse ectomycorrhizal fungal community of *Abies religiosa*. Mycorrhiza 27(1): 53–65. https://doi.org/10.1007/s00572-016-0724-1

Bacher M, Zoell M, Peintner U (2010) Ectomycorrhizal status of *Larix decidua*-, *Picea abies*- and *Pinus cembra*-nursery plants in South Tyrol. Forest Observer 5: 3–30.

Benucci GMN, Raggi L, Albertini E, Grebenc T, Bencivenga M, Falcinelli M, Di Massimo G (2011) Ectomycorrhizal communities in a productive *Tuber aestivum* Vittad. orchard: composition, host influence and species replacement. FEMS Microbiology Ecology 76(1): 170–184. https://doi.org/10.1111/j.1574-6941.2010.01039.x

Berkeley MJ, Broome CE (1873) [1875] Enumeration of the Fungi of Ceylon. Part II., containing the remainder of the Hymenomycetes, with the remaining established tribes of Fungi. Botanical Journal of the Linnean Society 14(73): 29–64. https://doi.org/10.1111/j.1095-8339.1873.tb00301.x

Bidartondo MI, Read DJ (2008) Fungal specificity bottlenecks during orchid germination and development. Molecular Ecology 17(16): 3707–3716. https://doi.org/10.1111/j.1365-294X.2008.03848.x

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ (2014) BEAST 2: A software platform for Bayesian evolutionary analysis. PLOS Computational Biology 10(4): e1003537. https://doi.org/10.1371/journal.pcbi.1003537

BPH Online (2018) http://www.huntbotanical.org/databases/show.php?1 [Accessed 26 November 2018]

Branco S, Bruns TD, Singleton I (2013) Fungi at a small scale: spatial zonation of fungal assemblages around single trees. PLOS ONE 8(10): e78295. https://doi.org/10.1371/journal.pone.0078295

Bresadola G (1897) Hymenomycetes Hungaricia Kmetiani. Atti dell'Imperial Regia Accademia Roveretana 3(1): 66–120.

Bresadola G (1903) Fungi Polonici a cl. viro B. Eichler lecti. Annales Mycologici 1(2): 97–131.

Bresadola G (1916) Synonymia et adnotanda mycologica. Annales Mycologici 14(3–4): 221–242.

Bulliard JBF (1790) Herbier de la France, ou Collection complette des plantes indigenes de ce royaume; Avec leurs Détails Anatomiques, leurs propriétés, et leurs usages en Medecine. Tome 10. Printed by the author, plates 433–480.

Burt EA (1916) The Thelephoraceae of North America VI. Annals of the Missouri Botanical Garden 3(2): 203–241. https://doi.org/10.2307/2989976

Burt EA (1926) The Thelephoraceae of North America XV. Annals of the Missouri Botanical Garden 13 (3): 173–354. https://doi.org/10.2307/2394065

de Candolle AP (1815) Flore française, ou, Descriptions succinctes de toutes les plantes qui croissent naturellement en France: disposées selon une nouvelle méthode d'analyse, et précédées par un exposé des principes élémentaires de la botanique. Tome 2. Desray, 1–600.

Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2015) GenBank. Nucleic Acids Research 44(1): 67–72. https://doi.org/10.1093/nar/gkv1276

Cline ET, Ammirati JF, Edmonds RL (2005) Does presence of mature trees influence ectomycorrhizal fungus communities of Douglas-fir seedlings? New Phytologist 166(3): 993–1009. https://doi.org/10.1111/j.1469-8137.2005.01387.x

Couch JN (1938) The genus *Septobasidium*. The University of North Carolina Press, 1–480.

Cox F, Barsoum N, Lilleskov EA, Bidartondo MI (2010) Nitrogen availability is a primary determinant of conifer mycorrhizas across complex environmental gradients. Ecology Letters 13(9): 1103–1113. https://doi.org/10.1111/j.1461-0248.2010.01494.x

Den Bakker HC, Zuccarello GC, Kuyper TW, Noordeloos ME (2004) Evolution and host specificity in the ectomycorrhizal genus *Leccinum*. New Phytologist 163(1): 201–215. https://doi.org/10.1111/j.1469-8137.2004.01090.x

Di Marino E, Kõljalg U, Reinhard A (2007) The ectomycorrhizae of *Pseudotomentella humicola* on Picea abies. Nova Hedwigia 84(3–4): 429–440. https://doi.org/10.1127/0029-5035/2007/0084-0429

Donk MA (1933) Revision der Niederländischen Homobasidiomycetae-Aphyllophoraceae II. Mededeelingen uit het Botanisch Museum en Herbarium der Rijksniversiteit te Utrecht 9: 1–298.

Fremstad E (1997) Vegetasjonstyper i Norge. NINA Temahefte 12. Norsk institutt for naturforskning, 1–279.

Fries EM (1821) Systema Mycologicum 1. Berling, 1–520.

Fries EM (1828) Elenchus Fungorum 1. Moritz, 1–238.

Fries EM (1832) Systema Mycologicum 3 (Index). Moritz, 1–202.

Fries EM (1849) Summa Vegetabilium Scandinaviae. v.2. A. Bonnier, 261–572.

Fries EM (1874) Hymenomycetes Europaei. Berling, 1–756.

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizas and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

GBIF (2018) https://www.gbif.org [Visited on 13 August 2018]

Gmelin JF (1792) Systema Naturae. v.2(2). GE Beer, 885–1661.

Guichon SHA (2015) Mycorrhizal fungi: unlocking their ecology and role in the establishment and growth performance of different conifer species in nutrient-poor coastal forests. PhD Thesis, the University of British Columbia, Canada, 1–219. https://doi.org/10.14288/1.0221252

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Systematic Biology 59(3): 307–21. https://doi.org/10.1093/sysbio/syq010

Halvorsen R (2015) NiN natursystemnivået – oversettelse fra 'Håndbok i registrering av livsmiljøer i skog' (MiS) til NiN versjon 2.0 og arealstatistikk for naturtyper i skogsmark. – Natur i Norge, Artikkel 5 (versjon 2.0.4, 3. utg.). Artsdatabanken, 1–104. http://www.artsdatabanken.no

Hjortstam K (1969) Studies in the Swedish species of the genus *Tomentella* (Thelephoraceae). Svensk Botanisk Tidskrift 63(4): 491–495.

von Höhnel FXR, Litschauer V (1906) Beiträge zur Kenntnis der Corticieen. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Wien. Mathematisch-naturwissenschaftliche Classe. Abteilung 1 115(8): 1549–1620.

von Höhnel FXR, Litschauer V (1908) Österreichische Corticieen. In: Linsbauer K (Ed.) Wiesner-Festschrift. Verlagsbuchhandlung Carl Konegen (Ernst Stülpnagel), 56–81.

Hopple Jr JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: divergent domains, outgroups, and monophyly. Molecular Phylogenetics and Evolution 13(1): 1–19. https://doi.org/10.1006/mpev.1999.0634

Hrynkiewicz K, Baum C, Leinweber P (2009) Mycorrhizal community structure, microbial biomass P and phosphatase activities under *Salix polaris* as influenced by nutrient availability. European Journal of Soil Biology 45(2): 168–175. https://doi.org/10.1016/j.ejsobi.2008.09.008

Huang J, Nara K, Lian C, Zong K, Peng K, Xue S, Shen Z (2012) Ectomycorrhizal fungal communities associated with Masson pine (*Pinus massoniana* Lamb.) in Pb–Zn mine sites of central south China. Mycorrhiza 22(8): 589–602. https://doi.org/10.1007/s00572-012-0436-0

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biololgy and Evolution 23(2): 254–267. https://doi.org/10.1093/molbev/msj030

Izumi H, Finlay RD (2011) Ectomycorrhizal roots select distinctive bacterial and ascomycete communities in Swedish subarctic forests. Environmental Microbiology 13(3): 819–830. https://doi.org/10.1111/j.1462-2920.2010.02393.x

Jeppson M, Altés A, Moreno G, Nilsson RH, Loarce Y, de Bustos A, Larsson E (2017) Unexpected high species diversity among European stalked puffballs – a contribution to the phylogeny and taxonomy of the genus *Tulostoma* (Agaricales). MycoKeys 21: 33–88. https://doi.org/10.3897/mycokeys.21.12176

Jones G (2017) Algorithmic improvements to species delimitation and phylogeny estimation under the multispecies coalescent. Journal of Mathematical Biology 74(1–2): 447–467. https://doi.org/10.1007/s00285-016-1034-0

Jones G, Aydin Z, Oxelman B (2015) DISSECT: an assignment-free Bayesian discovery method for species delimitation under the multispecies coalescent. Bioinformatics 31(7): 991–998. https://doi.org/10.1093/bioinformatics/btu770

Karsten PA (1883) Symbolae ad Mycologiam fennicam, XI. Meddelanden af Societas pro Fauna et Flora Fennica 9: 68–71.

Karsten PA (1889) Kritisk öfversigt af Finlands Basidsvampar (Basidiomycetes; Gastero- & Hymenomycetes). Bidrag till Kännedom af Finlands Natur och Folk 48: 1–470.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7, improvements in performance and usability. Molecular Biology and Evolution 30(4):772–80. https://doi.org/10.1093/molbev/mst010

Kõljalg U (1996) *Tomentella* (Basidiomycota) and related genera in Temperate Eurasia. Synopsis Fungorum 9: 1–213.

Kõljalg U, Dahlberg A, Taylor AF, Larsson E, Hallenberg N, Stenlid J, Larsson K-H, Fransson PM, Kårén O, Jonsson L (2000) Diversity and abundance of resupinate thelephoroid fungi as ectomycorrhizal symbionts in Swedish boreal forests. Molecular Ecology 9(12): 1985–1996. https://doi.org/10.1046/j.1365-294X.2000.01105.x

Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing BM (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytologist 166 (3): 1063–1068. https://doi.org/10.1111/j.1469-8137.2005.01376.x

Kranabetter JM, Stoehr MU, O'Neill GA (2012) Divergence in ectomycorrhizal communities with foreign Douglas-fir populations and implications for assisted migration. Ecological Applications 22(2): 550–60. https://doi.org/10.1890/11-1514.1

Kroken S, Taylor JW (2001) A gene genealogical approach to recognize phylogenetic species boundaries in the lichenized fungus *Letharia*. Mycologia 93(1): 38–53. https://doi.org/10.2307/3761604

Krpata D, Peintner U, Langer I, Fitz WJ, Schweiger P (2008) Ectomycorrhizal communities associated with *Populus tremula* growing on a heavy metal contaminated site. Mycological Research 112(Pt 9) 1069–1079. https://doi.org/10.1016/j.mycres.2008.02.004

Larsen MJ (1967) *Tomentella* and related genera in North America: III. New species of *Tomentella* and *Pseudotomentella*. Canadian Journal of Botany 45(8): 1297–1307. https://doi.org/10.1139/b67-137

Larsen MJ (1968) Tomentelloid fungi of North America. State University College of Forestry at Syracuse University, Technical Publication 93:1–157

Larsen MJ (1971a) [1972] The genus *Pseudotomentella* (Basidiomycetes, Thelephoraceae s. str.) Nova Hedwigia 22(1–2): 599–619.

Larsen MJ (1971b) Notes on tomentelloid fungi III. New species of *Pseudotomentella*. Bulletin of the Torrey Botanical Club 98(1): 38–41. https://doi.org/10.2307/2483496

Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30(22): 3276–3278. https://doi.org/10.1093/bioinformatics/btu531

Larsson E, Vauras J, Cripps CL (2018) *Inocybe praetervisa* group – a clade of four closely related species with partly different geographical distribution ranges in Europe. Mycoscience 59(4): 277–287. https://doi.org/10.1016/j.myc.2017.11.002

LeDuc SD, Lilleskov EA, Horton TR, Rothstein DE (2013) Ectomycorrhizal fungal succession coincides with shifts in organic nitrogen availability and canopy closure in post-wildfire jack pine forests. Oecologia 172(1): 257–269. https://doi.org/10.1007/s00442-012-2471-0

Leonardi M, Iotti M, Oddis M, Lalli G, Pacioni G, Leonardi P, Maccherini S, Perini C, Salerni E, Zambonelli A (2013) Assessment of ectomycorrhizal fungal communities in the natural habitats of *Tuber magnatum* (Ascomycota, Pezizales). Mycorrhiza 23(5): 349–358. https://doi.org/10.1007/s00572-012-0474-7

Lilleskov EA, Bruns TD (2005) Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. Mycologia 97(4): 762–769. https://doi.org/10.1080/15572536.2006.11832767

Litschauer V (1933) Additamenta ad floram Asiae Minoris Hymenomycetum. Phylacteriaceae Bulletin Trimestriel de la Société Mycologique de France 49(1): 52–75.

Lücking R, Dal-Forno M, Sikaroodi M, Gillevet PM, Bungartz F, Moncada B, Yánez-Ayabaca A, Chaves JL, Coca LF, Lawrey JD (2014) A single macrolichen constitutes hundreds of unrecognized species. Proceedings of the National Academy of Sciences 111(30): 11091–11096. https://doi.org/10.1073/pnas.1403517111

Malysheva VF, Malysheva EF, Kovalenko AE, Pimenova EA, Gromyko MN, Bondarchuk SN (2014) Ectomycorrhizal fungal diversity of *Pinus koraiensis* in the forests of the Central Sikhote-Alin based on rDNA sequence analysis of mycorrhizal tips. Mikologiya i Fitopatologiya 48(6): 372–385.

Malysheva EF, Malysheva VF, Kovalenko AE, Pimenova EA, Gromyko MN, Bondarchuk SN, Voronina EY (2016) Below-ground ectomycorrhizal community structure in the postfire

successional *Pinus koraiensis* forests in the central Sikhote-Alin (the Russian Far East). Botanica Pacifica 5(1): 19–31. https://doi.org/10.17581/bp.2016.05102

Martin DP, Murrell B, Golden M, Khoosal A, Muhire B (2015) RDP4: Detection and analysis of recombination patterns in virus genomes. Virus Evolution 1(1): vev003. https://doi.org/10.1093/ve/vev003

Martini E, Hentic R (2003) *Pseudotomentella rhizopunctata* sp. nov., une nouvelle espèce de champignon tomentelloïde chlamydosporée. Bulletin de la Société Mycologique de France 119(1-2): 19–29.

Moreau P-A, Bellanger J-M, Lebeuf R, Athanassiou Z, Athanasiades A, Lambert H, Schwarz C, Larsson E, Loizides M (2018) Hidden diversity uncovered in *Hygrophorus* sect. *aurei* (Hygrophoraceae), including the Mediterranean *H. meridionalis* and the North American *H. boyeri*, spp. nov. Fungal Biology 122(8): 817–836. https://doi.org/10.1016/j.funbio.2018.04.009

Nilsson RH, Wurzbacher C, Bahram M, Coimbra VRM, Larsson E, Tedersoo L, Eriksson J, Duarte Ritter C, Svantesson S, Sánchez-García M, Ryberg M, Kristiansson E, Abarenkov K (2016) Top 50 most wanted fungi. MycoKeys 12: 29–40. https://doi.org/10.3897/mycokeys.12.7553

Nilsson RH, Larsson K-H, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47(D1): D259–D264. https://doi.org/10.1093/nar/gky1022

Obase K, Lee JK, Lee SY, Chun KW (2011) Diversity and community structure of ectomycorrhizal fungi in *Pinus thunbergii* coastal forests in the eastern region of Korea. Mycoscience 52(6): 383–391. https://doi.org/10.1007/s10267-011-0123-6

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(1): 39–49. https://doi.org/10.1007/s00572-009-0262-1

Pålsson L (Ed.) (1998) Vegetationstyper i Norden, 3. uppl. Nordisk Ministerråd, 1–706.

Parker-Rhodes AF (1956) Distribution of Fungi in a Small Wood. Annals of Botany (Oxford) 20(78): 251–264. https://doi.org/10.1093/oxfordjournals.aob.a083521

Parrent JL, Vilgalys R (2007) Biomass and compositional responses of ectomycorrhizal fungal hyphae to elevated CO2 and nitrogen fertilization. New Phytologist 176(1): 164–174. https://doi.org/10.1111/j.1469-8137.2007.02155.x

Peintner U, Iotti M, Klotz P, Bonuso E, Zambonelli A (2007) Soil fungal communities in a *Castanea sativa* (chestnut) forest producing large quantities of *Boletus edulis* sensu lato (porcini): where is the mycelium of porcini? Environmental Microbiology 9(4): 880–889. https://doi.org/10.1111/j.1462-2920.2006.01208.x

Petersen RH (1975) Specific and infraspecific names for fungi used in 1821. Part I. Introduction, A & B. Mycotaxon 1(3): 149–188.

Põlme S, Bahram M, Yamanaka T, Nara K, Dai YC, Grebenc T, Kraigher H, Toivonen M, Wang PH, Matsuda Y, Naadel T, Kennedy PG, Kõljalg U, Tedersoo L (2013) Biogeography of ectomycorrhizal fungi associated with alders (Alnus spp.) in relation to biotic and abiotic variables at the global scale. New Phytologist 198(4): 1239–1249. https://doi.org/10.1111/nph.12170

Quélet L (1888) Flore mycologique de la France et des pays limitrophes. Octave Doin, 1–492.
Rambaut A (2012) FigTree v. 1.4.3. http://tree.bio.ed.ac.uk/software/figtree/
Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. http://tree.bio.ed.ac.uk/software/tracer/
Rehner SA, Buckley E (2005) A Beauviera phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97(1): 84–98. https://doi.org/10.1080/15572536.2006.11832842
Rogers DP (1948) On *Zygodesmus* (under "Brief Notes"). Mycologia 40(5): 633–635. https://doi.org/10.1080/00275514.1948.12017735
Rogers DP, Jackson HS (1943) Notes on the synonymy of some North American Thelephoraceae and other resupinates. Farlowia 1(2): 263–336.
Rosenthal LM, Larsson K-H, Branco S, Chung JA, Glassman SI, Liao HL, Peay KG, Smith DP, Talbot JM, Taylor JW, Vellinga EC, Vilgalys R, Bruns TD (2017) Survey of corticioid fungi in North American pinaceous forests reveals hyperdiversity, underpopulated sequence databases, and species that are potentially ectomycorrhizal. Mycologia 109(1): 115–127. https://doi.org/10.1080/00275514.2017.1281677
Saccardo PA (1888) Sylloge fungorum omnium hucusque cognitorum. v.6. Printed by the author, 1–928.
Sayyari E, Mirarab S (2016) Fast coalescent-cased computation of local branch support from quartet frequencies. Molecular Biology and Evolution 33(7): 1654–1668. https://doi.org/10.1093/molbev/msw079
Shivas R, Cai L (2012) Cryptic fungal species unmasked. Microbiology Australia 33(1): 35–36.
Stafleu FA, Cowan RS (1976–1988) Taxonomic Literature. A selective guide to botanical publications and collections with dates, commentaries and types. 2.ed (TL2). Bohn, Scheltema & Holkema.
Stalpers JA (1993) The aphyllophoraceous fungi I. Keys to the species of the Thelephorales. Studies in Mycology 35:1–168.
Sun L, Pei K, Wang F, Ding Q, Bing Y, Gao B, Yu Zheng, Liang Y, Ma K (2012) Different distribution patterns between putative ercoid mycorrhizal and other fungal assemblages in roots of *Rhododendron decorum* in the southwest of china. PLOS ONE 7(11): e49867. https://doi.org/10.1371/journal.pone.0049867
Svrček M (1958) Contribution to the taxonomy of the resupinate Thelephoraceous Fungi. Ceská Mykologie 12(2): 66–77.
Swofford DL (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0a, build 159. Sinauer Associates, Sunderland, MA.
Taylor DL, Hollingsworth TN, McFarland JW, Lennon NJ, Nusbaum C, Ruess RW (2014) A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. Ecological Monographs 84(1): 3–20. https://doi.org/10.1890/12-1693.1
Tedersoo L, Mett M, Ishida TA, Bahram M (2013) Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. New Phytologist 199(3): 822–831. https://doi.org/10.1111/nph.12328

Tedersoo L, Suvi T, Jairus T, Ostonen I, Põlme S (2009) Revisiting ectomycorrhizal fungi of *Alnus*: differential host specificity, diversity and determinants of the fungal community. New Phytologist 182(3): 727–735. https://doi.org/10.1111/j.1469-8137.2009.02792.x

Těšitelová T, Těšitel J, Jersáková J, RÍhová G, Selosse MA (2012) Symbiotic germination capability of four *Epipactis* species (Orchidaceae) is broader than expected from adult ecology. American Journal of Botany 99(6): 1020–1032. https://doi.org/10.3732/ajb.1100503

The International Plant Names Index (IPNI). http://www.ipni.org [Accessed 26 November 2018]

Thiers B (2018) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/ [Accessed 26 November 2018]

Trocha LK, Kałucka I, Stasińska M, Nowak W, Dabert M, Leski T, Rudawska M, Oleksyn J (2012) Ectomycorrhizal fungal communities of native and non-native *Pinus* and *Quercus* species in a common garden of 35-year-old trees. Mycorrhiza 22(2): 121–34. https://doi.org/10.1007/s00572-011-0387-x

Walbert K, Ramsfield TD, Ridgway HJ, Jones EE (2010) Ectomycorrhizal species associated with *Pinus radiata* in New Zealand including novel associations determined by molecular analysis. Mycorrhiza 20(3): 209–215. https://doi.org/10.1007/s00572-009-0277-7

Wallroth CFW (1833) Flora Cryptogamica Germaniae. v.2. JL Schrag, 1–923.

White TJ, Bruns T, Lee L, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sininski JJ, White J (Eds) PCR Protocols, A guide to Methods and Applications. Academic Press, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang C, Rabiee M, Sayyari E, Mirarab S (2018) ASTRAL-III: Polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinformatics 19(Supplement 6): 1–153. https://doi.org/10.1186/s12859-018-2129-y

Supplementary material 1

Solving the taxonomic identity of *Pseudotomentella tristis* s.l. (Thelephorales, Basidiomycota)

Authors: Sten Svantesson, Karl-Henrik Larsson, Urmas Kõljalg, Tom W. May, Patrik Cangren, R. Henrik Nilsson, Ellen Larsson

Data type: (phylogenetic trees/neighbour nets)

Explanation note: Phylogenetic trees (BEAST and PHYML) of the Tef1α and mtSSU regions and neighbour nets of the nrDNA, Tef1α and mtSSU alignments.

Link: https://doi.org/10.3897/mycokeys.50.32432.suppl1

MycoKeys 50: 79–91 (2019)
doi: 10.3897/mycokeys.50.32517
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Re-collection of *Dermea prunus* in China, with a description of *D. chinensis* sp. nov.

Ning Jiang[1], Cheng-Ming Tian[1]

1 *The Key Laboratory for Silviculture and Conservation of the Ministry of Education, Beijing Forestry University, Beijing 100083, China*

Corresponding author: *Cheng-Ming Tian* (chengmt@bjfu.edu.cn)

Academic editor: *Kevin Hyde* | Received 18 December 2018 | Accepted 24 March 2019 | Published 4 April 2019

Citation: Jiang N, Tian C-M (2019) Re-collection of *Dermea prunus* in China, with a description of *D. chinensis* sp. nov. MycoKeys 50: 79–91. https://doi.org/10.3897/mycokeys.50.32517

Abstract

Dermea was protected against its synonym, *Foveostroma*, due to its well-circumscribed generic concept and more frequent use. We describe and illustrate *Dermea chinensis* **sp. nov.** based on its morphological characteristics and a molecular analysis of the internal transcribed spacer (ITS) and large subunit (LSU) sequence data. *Dermea chinensis* is isolated from *Betula albosinensis* with sexual and asexual morphs and can be distinguished from *D. molliuscula* on *Betula* trees by its aseptate and wider ascospores. The connection between the two morphs is proved based on sequence data. Here, we describe the asexual morph of *D. pruni* for the first time based on morphological and molecular data from the same host and country of origin, and compare it with other species of *Prunus*.

Keywords

Betula, Dermateaceae, new species, *Prunus*

Introduction

Dermea Fr. (Dermateaceae, Helotiales) was first proposed based on *D. cerasi* (Fries, 1825), which is the sexual morph of the type species of *Micropera* Lév. (Léveillé, 1846) and *Foveostroma* DiCosmo (DiCosmo 1978), namely *M. drupacearum* and *F. drupacearum*, respectively. Due to the well-circumscribed concept and its more frequent use, *Dermea* was protected as the legitimate generic name (Johnston et al. 2014).

Groves (1946) accepted 16 species in *Dermea* and proposed a key for this genus based mainly on the characteristics of apothecia, asci, ascospores, and conidia, along with host associations. Subsequently, *Dermea tumifaciens* (Ramakrishnan & Ramakrishnan, 1948), *D. pruni* (Groves, 1951), *D. grovesii* (Reid & Pirozynski, 1966), *D. rhytidiformans* (Funk &

Kuijt, 1970), *D. tetrasperma* (Funk, 1976), *D. abietinum* (Johnston et al., 2014), *D. boycei* (Johnston et al., 2014), *D. stellata* (Johnston et al., 2014), and *D. persica* (Mehrabi et al., 2018) were added to this genus. However, *D. balsamea* and *D. peckiana*, which were accepted by Groves (1946), were later synonymised with *D. abietinum* and *D. stellata*, respectively (Johnston et al. 2014). Thus, 23 species were included in this genus before this study.

Dermea is a well-characterized genus with hard, leathery, dark brown to black apothecia; cylindrical to clavate-cylindrical, usually eight-spored asci; and ellipsoid-fusiform to ellipsoidal, hyaline to yellowish-brown, aseptate to 3-septate ascospores (Groves 1946; Mehrabi et al. 2018). The asexual morph of *Dermea* contains rather diverse conidiomatal structures, which usually accompany the apothecia (Groves 1946; Mehrabi et al. 2018). Additionally, two kinds of conidia are characterized: elongate-fusiform to sickle-shaped macroconidia and bacillary to filiform microconidia (Groves 1946; Mehrabi et al. 2018).

Dermea species are generally considered highly host-specific (Groves 1946, 1951). The plant genus *Prunus* is the major host for *Dermea*, with *D. cerasi*, *D. padi*, *D. prunastri*, and *D. pruni* described from them (Groves 1946, 1951). However, ascospores in *D. pruni* are larger than those from the other three species (Groves 1951). *Dermea cerasi*, *D. padi*, and *D. prunastri* can be easily distinguished by the macroconidial and microconidial dimensions (Groves 1946). Among these four species, *D. cerasi*, *D. padi*, and *D. prunastri* were recognized based on both sexual and asexual fruiting bodies (Groves 1946), but *D. pruni* was proposed only with a sexual morph based on a specimen (Teng #3352, preserved in the herbarium of the University of Michigan) collected from China (Groves 1951). Hence, the re-collection of *D. pruni* specimens aiming for an asexual morph from the original host and country seems meaningful. Additionally, few sequence data are available for most *Dermea* species, and considering that the host associations may be incorrect and that many geographical areas are still insufficiently studied, the synonymies and actual numbers of *Dermea* species are still unclear.

Dermea species were considered pathogenic to their hosts (Groves 1951; Abeln et al. 2000). For example, *D. abietinum* (syn. *D. balsamea*) caused hemlock dieback (Dodge 1932) and *D. prunastri* was considered the cause of greengage plums die-back (Dowson 1913). However, members of *Dermea* have not been recently reported to cause serious plant diseases.

During our fungal collection surveys conducted in China, we collected several *Dermea* specimens from two species of tree, *Betula albosinensis* and *Prunus cerasifera* f. *atropurpurea*. We identified fungi species using both morphological and molecular approaches; as a result, a novel species and the asexual morph of *D. pruni* are described herein for the first time.

Materials and methods

Sample collections and fungal isolates

Fresh specimens of *Dermea* were collected from tree barks during our fungal collection trip in China. We obtained single ascospore and conidia isolates by removing a mucoid spore mass from apothecia or conidiomata and spreading the suspension on the surface

of 2% malt extract agar (MEA; 20 g malt extract, 20 g agar, 1 L water). After inoculation, agar plates were incubated at 25 °C to induce germination of spores. Single germinating spores were then transferred to clean plates under a dissecting microscope with a sterile needle. Specimens and isolates were deposited in the Museum of Beijing Forestry University (BJFC). Axenic cultures are maintained in the China Forestry Culture Collection Center (CFCC).

Morphological analysis

Species identification was based on the morphological characters of apothecia and conidiomata produced on natural substrates. Cross-sections were prepared manually using a double-edged blade under a Leica stereomicroscope (M205 FA). Photomicrographs were captured with a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Ri2 high-definition colour camera, using differential interference contrast (DIC) illumination and the Nikon software, NIS-Elements D Package 3.00. Measurements of ascospores and conidia are reported as the maximum and minimum in parentheses and the range representing the mean ± standard deviation of the number of measurements is given in parentheses. Cultural characteristics of isolates incubated on MEA in the dark at 25 °C were recorded.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from axenic living cultures on MEA with cellophane using a modified CTAB method (Doyle and Doyle 1990). The internal transcribed spacer (ITS) region was amplified with primers ITS1 and ITS4 (White et al. 1990), and the large subunit (LSU) region with the primers LR0R and LR5 (Vilgalys and Hester 1990). Amplification of ITS and LSU were accomplished by an initial step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 51 °C, and 40 s at 72 °C, with a final extension of 10 min at 72 °C. DNA sequencing was performed on an ABI PRISM 3730XL DNA Analyzer using BigDye Terminater Kit 3.1 (Invitrogen) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analyses

Sequences from this study and reference sequences obtained from GenBank (Table 1) were aligned and edited manually using MEGA6 (Tamura et al. 2013). The alignments were concatenated for phylogenetic analyses. Maximum parsimony (MP) analyses were conducted with PAUP 4.0b10 (Swofford 2003), using 1000 heuristic search replicates with random-additions of sequences along with the tree bisection and reconnection (TBR) branch swapping algorithm (MULTREES option in effect, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command

Table 1. Strains and NCBI GenBank accession numbers used in this study. Strains from this study are in bold.

Species	Strain	Genbank	
		ITS	LSU
Davidhawksworthia ilicicola	CBS 734.94	KU728517	KU728556
Davidhawksworthia ilicicola	CBS 261.95	KU728516	KU728555
Dermea acerina	CBS 161.38	AF141164	DQ247801
Dermea ariae	CBS 134.46	AF141158	NA
Dermea cerasi	CBS 136.46	AF141159	NA
Dermea chinensis	**CFCC 53008**	**MK330013**	**MK626645**
Dermea chinensis	**CFCC 53009**	**MK330014**	**MK626646**
Dermea chinensis	**CFCC 53010**	**MK330015**	**MK626647**
Dermea hamamelidis	CBS 137.46	AF141157	NA
Dermea padi	CBS 140.46	AF141160	NA
Dermea persica	MFLU 16-0259	MH104719	MH104720
Dermea prunastri	CBS 143.46	AF141162	NA
Dermea pruni	**CFCC 53006**	**MK330016**	**MK626648**
Dermea pruni	**CFCC 53007**	**MK330017**	**MK626649**
Dermea viburni	CBS 145.46	AF141163	NA
Mollisia dextrinospora	ICMP 18083	HM116746	HM116757
Neofabraea inaequalis	CBS 326.75	KR859081	KR858872
Neofabraea kienholzii	CBS 126461	KR859082	KR858873
Neofabraea malicorticis	CBS 122030	KR859086	KR858877
Neofabraea perennans	CBS 102869	KR859087	KR858878
Pezicula aurantiaca	CBS 201.46	KR859102	KR858893
Pezicula cornina	CBS 285.39	KR859163	KR858915
Pezicula cinnamomea	CBS 239.96	KR859124	KR858955
Pezicula eucrita	CBS 259.97	KR859179	KR858971
Pezicula neosporulosa	CBS 101.96	KR859223	KR859015
Pezicula pseudocinnamomea	CBS 101000	KR859235	KR859027
Pezicula sporulosa	CBS 224.96	KR859261	KR859053
Phlyctema vincetoxici	CBS 123727	KF251207	KF251710
Phlyctema vincetoxici	CBS 123743	KF251208	KF251711
Pseudofabraea citricarpa	CBS 130533	KR859281	KR859075
Pseudofabraea citricarpa	CBS 130297	KR859279	KR859073

was set to minbrlen, maxtrees were set to 5000. All equally parsimonious trees found were saved in the MP analyses. Other calculated parsimony scores were tree length (TL), consistency index (CI), retention index (RI), and rescaled consistency (RC). MP bootstrap analyses with 1000 replicates were performed in the same manner, with 10 rounds of heuristic search replicates with random addition of sequences and subsequent TBR branch swapping during each bootstrap replicate. ML analyses were conducted using RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012), using the ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates. Taxonomic novelties were deposited in MycoBank.

Results

Phylogenetic analyses

The alignment based on the combined sequence dataset (ITS and LSU) contained 1431 characters. Of these, 1136 characters were constant, 103 variable characters were parsi-

mony-uninformative, and 192 parsimony informative. The MP analyses resulted in five equally most parsimonious trees, with the first tree (TL = 601, CI = 0.647, RI = 0.807, RC = 0.522), which is shown in Figure 1. Tree topologies of the best tree revealed by the ML analyses was identical to those of the MP tree (not shown). The two species from this study appeared in two distinct clades, and three strains of *Dermea chinensis* from the *Betula albosinensis* cluster in a well-supported clade (MP/ML = 100/100) (Fig. 1).

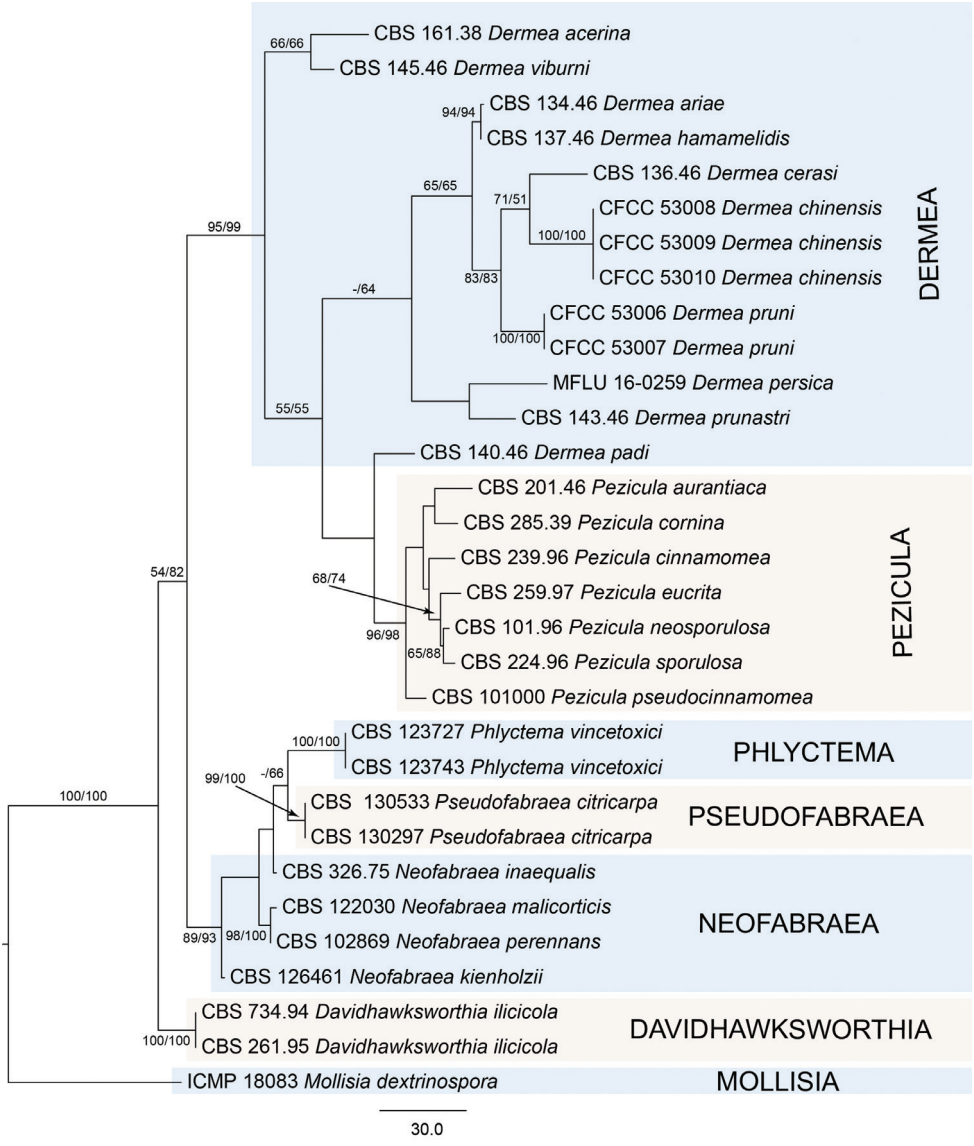


Figure 1. Phylogram of *Dermea* and related genera based on combined ITS and LSU sequence data. Values above or below the branches indicate maximum parsimony and maximum likelihood bootstrap support. Scale bar: 30 nucleotide substitutions.

Taxonomy

Dermea chinensis **C.M. Tian & N. Jiang, sp. nov.**
MycoBank: MB828880
Figures 2, 3

Diagnosis. *Dermea chinensis* differs from *D. molliuscula* by its wider ascospores

Holotype. CHINA. SHAANXI PROVINCE, Ankang City, Huoditang forest park, 33°26'12"N, 108°26'42"E, 1650 m a.s.l., on branches of *Betula albosinensis*, N. Jiang & C.M. Tian leg., 18 Jul 2018 (holotype BJFC-S1729). Ex-type culture from sexual fruiting body: CFCC 53008; living culture from asexual fruiting body: CFCC 53009.

Etymology. Named after the country where it was first discovered, China.

Description. *Sexual morph*: *apothecia* erumpent, scattered or sometimes gregarious, circular, sinuate, sessile to substipitate, 2.1–3.5 mm wide, 0.8–1.2 mm high (av. = 2.7 × 0.9 mm, *n* = 10), dark brown to black, hard, leathery to horny in consistency, hymenium at the first concave, becoming plane or convex, roughened, sometimes cracked, occasionally slightly umbilicate; tissue of the basal stroma pseudoparenchymatous, composed of closely interwoven hyphae with elongated cells about 8 µm in diameter, hyaline to brownish, thick walled, curving towards the outside, forming a darker, pseudoparenchymatous excipulum of thick-walled cells about 8 µm in diameter; subhymenium a narrow zone of closely interwoven hyphae about 3 µm in diameter. *Asci* 85–118 × 14–19 µm (av. $\bar{x}$= 96.5 × 16.4 µm, *n* = 10), cylindric-clavate, tapering below into a short stalk, 8-spored. Paraphyses hyaline, filiform, septate, simple or branched, 1.5–2.5 in diameter, the tips slightly swollen up to 4 µm and glued together forming a yellowish epithecium. *Ascospores* (14.2–)16.3–17.1(–18.6) × (7.3–)7.5–8.5(–8.9) µm, l/w = (1.8–)1.9–2.2(–2.3) (*n* = 50), ellipsoid-fusiform, hyaline to yellowish-brown, straight or slightly curved, aseptate, irregular biseriate. *Asexual morph*: *conidial fruiting bodies* erumpent, gregarious, columnar to subconical, 0.5–2.5 mm wide, 0.4–0.7 mm high (av. = 1.6 × 0.6 mm, *n* = 10), yellowish, furfuraceous to glabrous, tearing open irregularly and widely at the top, waxy in consistency, more fresh when moist, usually containing 3–8 more or less lobed cavity. *Conidiophores* 7–18 × 2–3.5 µm, hyaline, aseptate, unbranched, tapering to a slender tip. *Conidiogenous cells* 5–15 × 1.5–3 µm, determinate, phialidic, cylindrical, hyaline. *Conidia* (54–)60–72(–78) × (3.2–)3.5–4(–4.2) µm, hyaline, fifiform, straight or curved, one-celled. *Microconidia* absent.

Culture characters. On MEA at 25 °C colonies grow slowly, reaching 50 mm diameter within 60 d, pale yellow at first, gradually turning dark brown with scanty aerial mycelium.

Habitat and host range. On dead corticated branches of *Betula albosinensis*.

Additional specimen examined. CHINA. SHAANXI PROVINCE, Ankang City, Qinling Mountain, 33°26'12"N, 108°26'42"E, 1570 m a.s.l., on branches of *Betula albosinensis*, N. Jiang & C.M. Tian leg., 15 Jul 2018 (BJFC-S1730, living culture CFCC 53010).

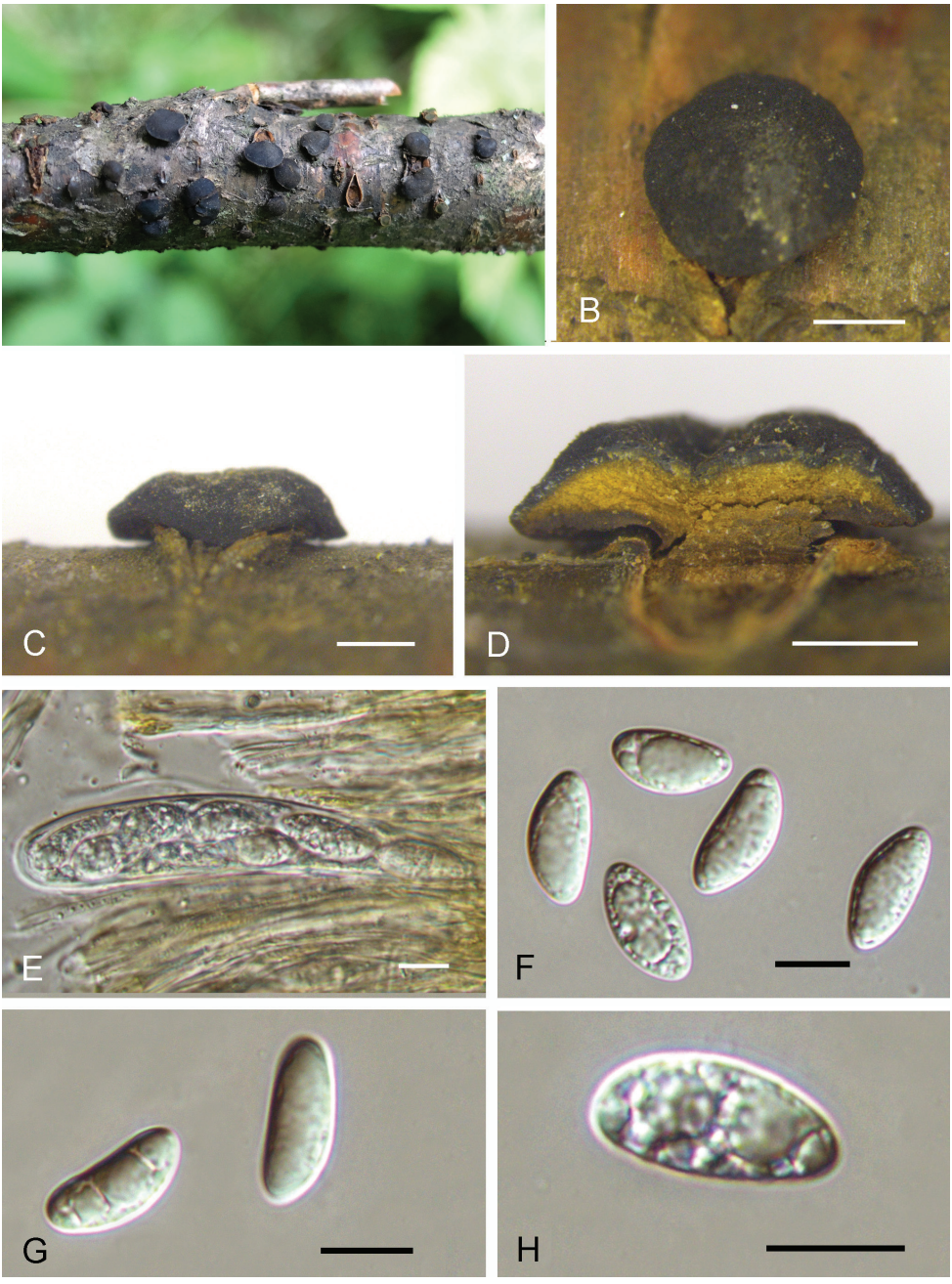


Figure 2. Sexual morph of *Dermea chinensis* from *Betula albosinensis* (BJFC-S1729, holotype) **A–C** apothecia on the natural substrate in surface view **D** longitudinal section through apothecium **E** ascus and paraphyses **F–H** ascospores. Scale bars: 1 mm (**B–D**); 10 μm (**E–H**).

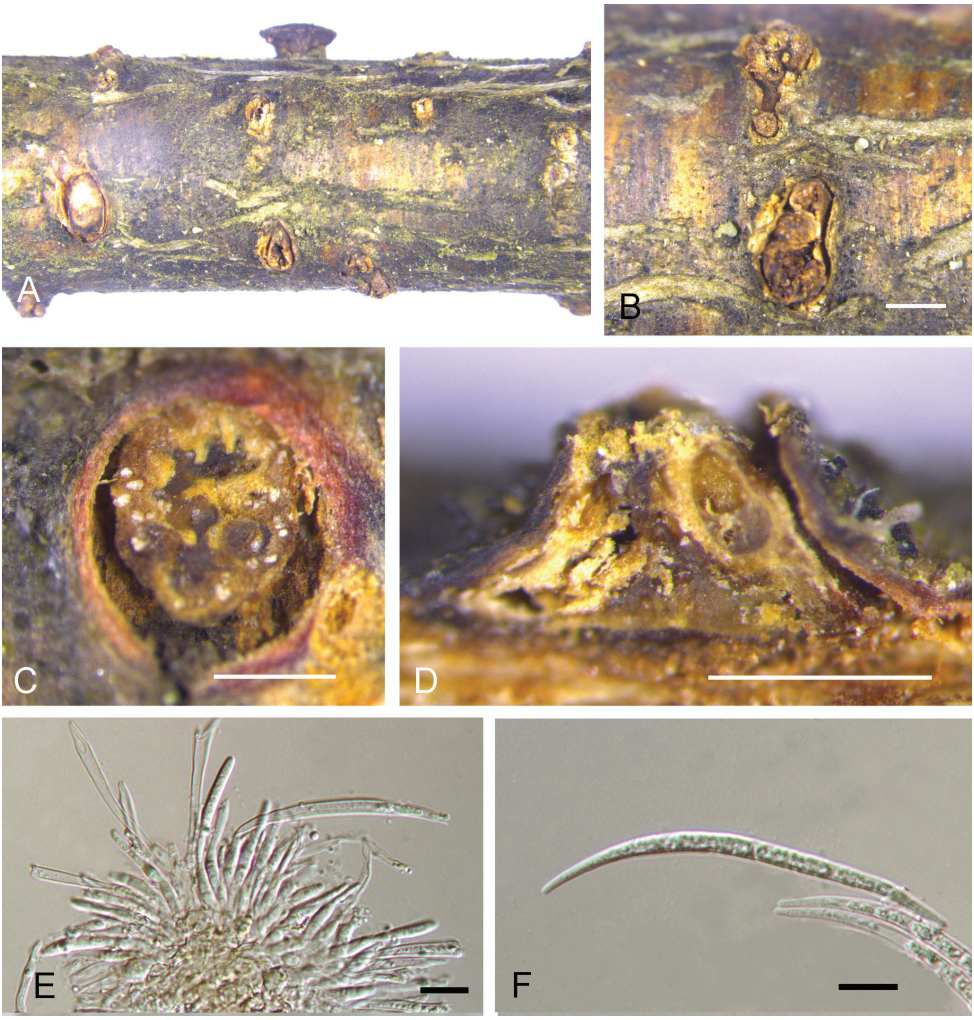


Figure 3. Asexual morph of *Dermea chinensis* from *Betula albosinensis* (BJFC-S1729, holotype) **A, B** conidiomata on the natural substrate in surface view **C** transverse section through conidioma **D** longitudinal section through conidioma **E, G** conidiophores **F, H** conidia. Scale bars: 1 mm (**B**); 0.5 mm (**C, D**); 10 μm (**E–H**).

Notes. Three isolates of *D. chinensis* were obtained from *Betula albosinensis* cluster in a well-supported clade (MP/ML = 100/100) and appeared closely related to *D. cerasi* from *Prunus* branches. *Dermea chinensis* and *D. cerasi* are similar in macroconidia dimensions (54–78 × 3.2–4.2 μm in *D. chinensis* vs 40–60 × 2.5–4.5 μm in *D. cerasi*) but different in ascospore dimensions (14.2–18.6 × 7.3–8.9 μm in *D. chinensis* vs 15–20 × 5–7.5 μm in *D. cerasi*) and host associations (Groves 1946). Furthermore, the two species are separated by 51 bp differences in their ITS. *Dermea molliuscula*, which occurs in the USA and Canada, is the other species inhabiting

Betula trees. However, *D. chinensis* is distinguished from *D. molliuscula* by aseptate ascospores and in width (7.3–8.9 μm in *D. chinensis* vs 4–7 μm in *D. molliuscula*) (Groves 1946).

***Dermea pruni* (Teng) J.W. Groves, Mycologia 43(6): 721. 1952.**
Figure 4

Description. *Sexual morph*: see Groves (1952). *Asexual morph*: *conidial fruiting bodies* erumpent, gregarious, pulvinate, 0.6–2.3 mm wide, 0.2–0.35 mm high (av. = 1.8 × 0.28 mm, *n* = 10), yellowish, furfuraceous to glabrous, tearing open irregularly and widely at the top, waxy in consistency, more fresh when moist, usually containing up to 30 more or less lobed cavities. *Conidiophores* 4–15 × 1.5–2.5 μm, hyaline, aseptate, unbranched, tapering to a slender tip. *Conidiogenous cells* 3.5–15 × 1.5–2.5 μm, determinate, phialidic, cylindrical, hyaline. *Conidia* (62–)75–88(–95) × (2–)2.5–3.3(–3.5) μm, hyaline, fifiform, straight or curved, two-celled. *Microconidia* absent.

Culture characters. On MEA at 25 °C colonies grow slowly, reaching 50 mm diameter within 50 d, at first pale yellow, gradually becoming dark brown with scanty aerial mycelium.

Habitat and host range. On dying stems and branches of *Prunus cerasifera* f. *atropurpurea*.

Specimens examined. CHINA. SHAANXI PROVINCE, Ankang City, Qinling Mountain, 33°26'7"N, 108°26'48"E, 1570 m asl, on branches of *Prunus cerasifera* f. *atropurpurea*, N. Jiang & C.M. Tian leg., 23 Jul 2018 (BJFC-S1727, living culture CFCC 53006). CHINA. SHAANXI PROVINCE, Ankang City, Qinling Mountain, 33°26'7"N, 108°26'48"E, 1570 m asl, on branches of *Prunus cerasifera* f. *atropurpurea*, N. Jiang & C.M. Tian leg., 23 Jul 2018 (BJFC-S1728, living culture CFCC 53007).

Notes. *Dermea pruni* was proposed based on a specimen collected from *Prunus* branches in Sichuan province, China. However, no living culture or DNA data were available (Groves 1951). In addition, the asexual morph was not included in the original description (Groves 1951). During our fungal collection trip in China, two *Dermea* specimens were accidentally discovered on a common road tree, *Prunus cerasifera* f. *atropurpurea* in Shaanxi province, which borders Sichuan province, the original collection province of the holotype. Asexual fruiting bodies were observed on the whole trees, from stems to branches. However, no sexual morph was found, even though we investigated all *Prunus* trees along the road. Conidial size was compared among our collections, *D. cerasi*, *D. padi*, and *D. prunastri*, which can distinguish them (Table 2). Considering that our collections and the type specimen (Teng #3352, preserved in the herbarium of the University of Michigan) of *D. pruni* were collected from the same hosts and from nearby regions (Groves 1951), our specimens were identified and treated here as *D. pruni*. However, more detailed taxonomic studies are needed, including DNA extraction from the holotype of *D. pruni* to compare ITS sequences of our collections and the holotype.

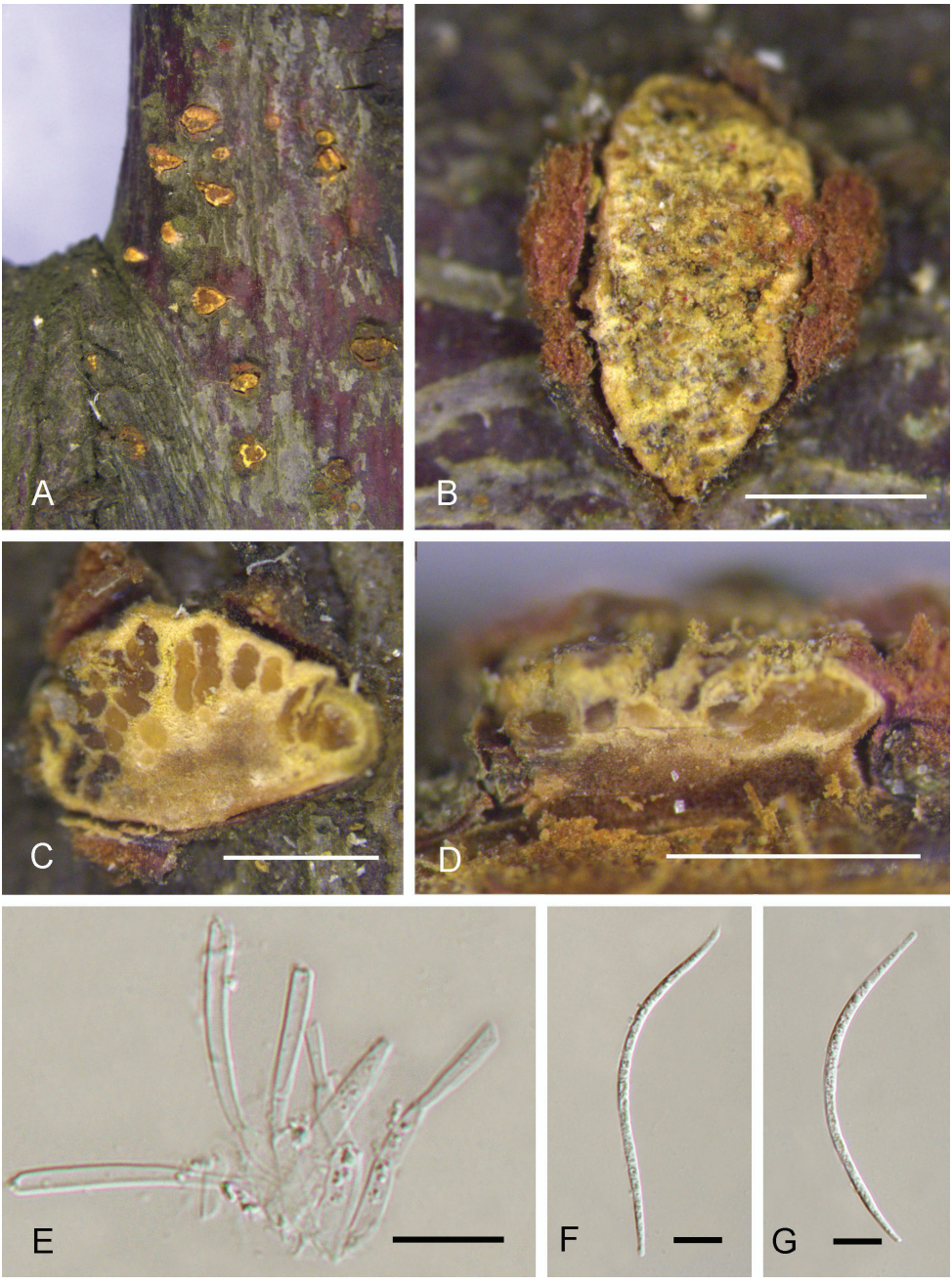


Figure 4. Asexual morph of *Dermea pruni* from *Prunus cerasifera* f. *atropurpurea* (BJFC-S1727) **A, B** conidiomata on the natural substrate in surface view **C** transverse section through conidioma **D** longitudinal section through conidioma **E** conidiophores **F** conidia. Scale bars: 1 mm (**B, C**); 0.5 mm (**D**); 10 μm (**E, F**).

Table 2. Comparison of phenotypic characters of currently accepted *Dermea* species.

Species	Host genera	Ascospores dimension (μm); septation	Macroconidia dimension (μm); septation	Microconidia dimension (μm)	Reference
D. abietinum	*Abies*; *Tsuga*	20–30 × 6–8; 1–4-celled	60–75 × 4–5; 1–4-celled	11–22 × 1.0–1.5	Groves 1946; Johnston 2014
D. acerina	*Acer*	13–20 × 5–8; 1–4-celled	15–25 × 5–8; 1-celled	6–10 × 1.0–2.0	Groves 1946
D. ariae	*Sorbus*	12–18 × 3–5; 1–4-celled	15–20 × 2.0–4.0; 1–2-celled	NA	Groves 1946
D. bicolor	*Amelanchier*	12–15 × 3–4; 1–2-celled	15–20 × 2.5–4.0; 1–2-celled	NA	Groves 1946
D. boycei	*Pseudotsuga*	16–28 × 4–7; 1–4-celled	42–56 × 3–4; 1–4-celled	8–14 × 1–2	Funk 1967; Johnston 2014
D. cerasi	*Prunus*	15–20 × 5–7.5; 1–4-celled	40–60 × 2.5–4.5; 1–2-celled	12–23 × 1.0–1.5	Groves 1946
D. chinensis	*Betula*	14–19 × 7–9; 1-celled	54–78 × 3.2–4.2; 1-celled	NA	This study
D. chionanthi	*Chionanthus*	18–25 × 7–9; 1–2(–4)-celled	25–35 × 5–7; 1–2-celled	NA	Groves 1946
D. grovesii	*Picea*	16.5–21.5 × 6–5; 1–3-celled	60–95 × 6.5–8; 7–11-celled	NA	Reid and Pirozynski 1966
D. hamamelidis	*Hamamelis*	15–20 × 5.0–7.5; 1–4-celled	18–25 × 4.5–6.0;1–2-celled	NA	Groves 1946
D. libocedri	*Libocedrus*	15–20 × 6–8; 1–4-celled	42–65 × 4–6; 1–4-celled	10–18 × 1.0–1.5	Groves 1946
D. molliuscula	*Betula*	15–20 × 4–7; 1–4-celled	50–75 × 2.5–3.5; 1–4-celled	7–12 × 1.0–1.5	Groves 1946
D. padi	*Prunus*	15–20 × 5–7; 1–4-celled	20–28 × 2.5–4.0; 1–2-celled	4–6 × 1.5	Groves 1946
D. persica	NA	NA	20–25 × 2.5–3.5; 1-celled	NA	Mehrabi et al. 2018
D. piceina	*Picea*	12–14 × 6–8; 1–2(–4)-celled	22–40 × 3–5; 1–4-celled	9–15 × 1.0–1.5	Groves 1946
D. pinicola	*Pinus*	13–18 × 5.0–7.5; 1–2-celled	30–40 × 4–6; 1–4-celled	NA	Groves 1946
D. prunastri	*Prunus*	15–20 × 5.0–7.5; 1–4-celled	20–30 × 5–7; 1-celled	7–10 × 1.5	Groves 1946
D. pruni	*Prunus*	15–20 × 8–10; 1(–4)-celled	62–95 × 2–3.5; 2-celled	NA	Groves 1951; This study
D. rhytidiformans	*Abies*	18–28 × 8–11; 1-celled	25–65 × 3.5–5.5; 1–4-celled	10–22 × 1.5	Funk and Kuijt 1970
D. stellata	*Nemopanthus*	12–18 × 4–6; 1–2(–4)-celled	40–55 × 2.5–4.5; 1–2-celled	8–13 × 1.5–2.0	Groves 1946; Johnston 2014
D. tetrasperma	*Pseudotsuga*	14–17 × 4–6; 1-celled	15–22 × 5–6; 1-celled	NA	Funk 1976
D. tulasnei	*Fraxinus*	15–20 × 6–8; 1–4-celled	25–40 × 6–8; 1-celled	NA	Groves 1946
D. tumifaciens	*Capparis*	13 × 5.4 / 10–19 × 4.8–9.6; 2-celled	18 × 7 / 15–22 × 4–9; 2- celled	NA	Ramakrishnan and Ramakrishnan 1948
D. viburni	*Viburnum*	14–18 × 3.5–5.5; 1–2-celled	30–45 × 2.5–4.0; 1–4-celled	NA	Groves 1946

Discussion

In this study, we collected several *Dermea* specimens from China and morphologically and molecularly examined them. *Dermea chinensis* from *Betula* trees is introduced, which can be distinguished from *D. molliuscula* by aseptate and wider ascospores, and from other species by host association (Table 2). Four *Dermea* species, *D. cerasi*, *D. padi*, *D. prunastri*, and *D. pruni* have been reported from *Prunus* trees

(Groves 1946, 1951). These four species can be obviously distinguished by both morphological and molecular approaches. We update the asexual morph and molecular data of *D. pruni*.

The genus *Pezicula* is a phylogenetically close to *Dermea* species and has recently been confirmed based on an ITS-28S-16S rDNA analysis (Mehrabi et al. 2018). However, *Pezicula* is characterized by typically bright-coloured, yellowish to ochraceous, more fleshy-waxy apothecia, broader and more clavate asci, and more broadly ellipsoid to oblong-ellipsoid or ovoid ascospores (Grove 1946). Our phylogenenetic analysis of *Dermea* and related genera based on the combined ITS and LSU sequence data (Fig. 1) showed that *Pezicula* is well-supported as a separate clade with high values (MP/ML = 96/98). *Dermea* was thought to be a monophyletic group (Abeln et al. 2000), but *Dermea* was not well-supported, as *D. persica* was included in the analysis (Mehrabi et al. 2018). We added additional DNA sequence data in our study (Fig. 1), which indicates that *Dermea* is not monophyletic.

Species of *Dermea* are well-circumscribed by morphological characteristics. However, only 10 species (Table 1) are currently characterized by molecular data, and most species remain unconfirmed by phylogenetic examination. Hence, DNA data from type or ex-strains and newly obtained collections are essential in subsequent taxonomic work.

Acknowledgements

This study was financed by the National Natural Science Foundation of China (Project No.: 31670647). We are grateful to Chungen Piao and Minwei Guo (China Forestry Culture Collection Center (CFCC), Chinese Academy of Forestry, Beijing) for support with strain preservation during this study.

References

Abeln ECA, de Pagter MA, Verkley GJM (2000) Phylogeny of *Pezicula*, *Dermea* and *Neofabraea* inferred from partial sequences of the nuclear ribosomal RNA gene cluster. Mycologia 92: 685–693. https://doi.org/10.2307/3761426

DiCosmo F (1978) A revision of *Corniculariella*. Canadian Journal of Botany 56: 1665–1690. https://doi.org/10.1139/b78-197

Dodge BO (1932) Notes on three hemlock fungi. Mycologia 24: 421–430. https://doi.org/10.2307/3753985

Dowson WJ (1913) On a disease of greengage trees caused by *Dermatella prunastri* Pers. New Phytologist 12: 207–216. https://doi.org/10.1111/j.1469-8137.1913.tb05695.x

Groves JW (1946) North American species of *Dermea*. Mycologia 38: 351–431.https://doi.org/10.1080/00275514.1946.12024065

Groves JW (1951) A *Dermea* on *Prunus* from China. Mycologia 43: 719–722.https://doi.org/10.1080/00275514.1951.12024165

Fries EM (1825) Systema orbis vegetabilis. Typographia academica 1: 1–374.

Funk A (1976) The genus *Dermea* and related conidial states on Douglas fir. Canadian Journal of Botany 54: 2852–2856. https://doi.org/10.1139/b76-306

Funk A, Kuijt J (1970) *Dermea rhytidiformans* n. sp., the fungus associated with the cork-bark of alpine fir. Canadian Journal of Botany 48: 1481–1483. https://doi.org/10.1139/b70-223

Johnston PR, Seifert KA, Stone JK, Rossman AY, Marvanová L (2014) Recommendations on generic names competing for use in Leotiomycetes (Ascomycota). IMA Fungus 5: 91–120. https://doi.org/10.5598/imafungus.2014.05.01.11

Léveillé JH (1846) Descriptions des champignons de l'herbier du Muséum de Paris. Annales des Sciences Naturelles Botanique 5: 249–305.

Mehrabi M, Asgari B, Wijayawardene NN, Hyde KD (2018) Description of *Dermea persica* (Dermateaceae, Helotiales), a new asexual Ascomycete from Iran, and an updated key to *Dermea* species. Phytotaxa 367 (1): 25–37. https://doi.org/10.11646/phytotaxa.367.1.3

Ramakrishnan TS, Ramakrishnan K (1948) Additions to fungi of Madras IV. Proceedings of the Indian Academy of Sciences (Section B) 27: 33–46.

Reid J, Pirozynski KA (1966) Notes on some interesting North American fungi. Canadian Journal of Botany 44: 645–653. https://doi.org/10.1139/b66-0777

Silvestro D, Michalak I (2012) raxmlGUI: A graphical front-end for RAxML. Organisms Diversity & Evolution 12: 335–337. https://doi.org/10.1007/s13127-011-0056-0

Stamatakis E (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl4466

Swofford DL (2003) PAUP*: Phylogenetic Analyses Using Parsimony and Other Methods, Version 4.0b10. Sinauer Associates, Sunderland.

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC (2000) Phylogenetic species recognition and species concepts in fungi. Fungal Genetics and Biology 31: 21–32. https://doi.org/10.1006/fgbi.2000.1228

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee S, Taylor JM (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: A Guide to the Methods and Applications. New York: Academic Press, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

MycoKeys 50: 93–133 (2019)
doi: 10.3897/mycokeys.50.32653
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Differential patterns of ophiostomatoid fungal communities associated with three sympatric *Tomicus* species infesting pines in south-western China, with a description of four new species

Hui Min Wang[1], Zheng Wang[1], Fu Liu[1], Cheng Xu Wu[1], Su Fang Zhang[1], Xiang Bo Kong[1], Cony Decock[2], Quan Lu[1], Zhen Zhang[1]

1 *Key Laboratory of Forest Protection, National Forestry and Grassland Administration; Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China* **2** *Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL), Earth and Life Institute, Microbiology, B-1348 Louvain-la-Neuve, Belgium*

Corresponding author: *Zhen Zhang* (zhangzhen@caf.ac.cn); *Quan Lu* (luquan@caf.ac.cn)

Academic editor: *Kevin Hyde* | Received 27 December 2018 | Accepted 9 March 2019 | Published 9 April 2019

Citation: Wang HM, Wang Z, Liu F, Wu CX, Zhang SF, Kong XB, Decock C, Lu Q, Zhang Z (2019) Differential patterns of ophiostomatoid fungal communities associated with three sympatric *Tomicus* species infesting pines in south-western China, with a description of four new species MycoKeys 50: 93–133. https://doi.org/10.3897/mycokeys.50.32653

Abstract

Bark beetles and their associated fungi, which cause forest decline and sometimes high mortality in large areas around the world, are of increasing concern in terms of forest health. Three *Tomicus* spp. (*T. brevipilosus*, *T. minor* and *T. yunnanensis*) infect branches and trunks of *Pinus yunnanensis* and *P. kesiya* in Yunnan Province, in south-western China. *Tomicus* spp. are well known as vectors of ophiostomatoid fungi and their co-occurrence could result in serious ecological and economic impact on local forest ecosystems. Nonetheless, knowledge about their diversity, ecology, including pathogenicity and potential economic importance is still quite rudimentary. Therefore, an extensive survey of ophiostomatoid fungi associated with these *Tomicus* species infesting *P. yunnanensis* and *P. kesiya* was carried out in Yunnan. Seven hundred and seventy-two strains of ophiostomatoid fungi were isolated from the adult beetles and their galleries. The strains were identified based on comparisons of multiple DNA sequences, including the nuclear ribosomal large subunit (LSU) region, the internal transcribed spacer regions 1 and 2, together with the intervening 5.8S gene (ITS) and the partial genes of β-tubulin (*TUB2*), elongation

factor 1α (*TEF1-a*) and calmodulin (*CAL*). Phylogenetic analyses were performed using maximum parsimony (MP) as well as maximum likelihood (ML). Combinations of culture features, morphological characters and temperature-dependent growth rates were also employed for species identification. Eleven species belonging to five genera were identified. These included six known species, *Esteya vermicola*, *Leptographium yunnanense*, *Ophiostoma brevipilosi*, *O. canum*, *O. minus* and *O. tingens* and four novel taxa, described as *Graphilbum anningense*, *O. aggregatum*, *Sporothrix pseudoabietina* and *S. macroconidia*. A residual strain was left unidentified as *Ophiostoma* sp. 1. The overall ophiostomatoid community was by far dominated by three species, representing 87.3% of the total isolates; in decreasing order, these were *O. canum*, *O. brevipilosi* and *O. minus*. Furthermore, the ophiostomatoid community of each beetle, although harbouring a diversity of ophiostomatoid species, was differentially dominated by a single fungal species; *Ophiostoma canum* was preferentially associated with and dominated the ophiostomatoid community of *T. minor*, whereas *O. brevipilosi* and *O. minus* were exclusively associated with and dominated the ophiostomatoid communities of *T. brevipilosus* and *T. yunnanensis*, respectively. Eight additional species, representing the remaining 12.7% of the total isolates, were marginal or sporadic. These results suggested that sympatric *Tomicus* populations are dominated by distinct species showing some level of specificity or even exclusivity.

Keywords

Esteya vermicola, *Graphilbum*, *Leptographium*, *Ophiostoma*, species-specific association, *Sporothrix*, taxonomy

Introduction

Associations between insects and microorganisms are increasingly recognised as one of the major issues in forest ecology and forest health around the world (Wingfield et al. 2016). Many bark beetles are well known as tree pests causing various levels of tree mortality and forest decline in large areas of the world, mostly in temperate areas (Jankowiak 2006, Wingfield et al. 2017). These bark beetles are well known vectors of variably pathogenic fungi, forming symbiosis-like relationships (Six 2003, Lu et al. 2009).

The pine shoot beetles, *Tomicus* Latreille (syn. *Blastophagus* Eichhoff, *Myelophilus* Eichhoff, Scolytidae, Coleoptera), are destructive insects with a range spanning the Eurasian pine forests, seriously affecting tree growth and causing a great threat to the forest ecosystems (Kirkendall et al. 2008, Lieutier et al. 2015). Currently, eight species are recorded worldwide, i.e. *T. armandii* Li and Zhang (Li et al. 2010), *T. brevipilosus* Eggers, *T. destruens* Wollaston, *T. minor* Hartig, *T. pilifer* Spessivtsev, *T. piniperda* L., *T. puellus* Reitter, and *T. yunnanensis* Kirkendall and Faccoli (Kirkendall et al. 2008). They all occur in China except *T. destruens* and five of them, viz. *T. armandii*, *T. brevipilosus*, *T. minor*, *T. pilifer* and *T. yunnanensis*, are sympatric in forests of the Yunnan Province (Li et al. 1997, 2010, Kirkendall et al. 2008; Ye 2011). *Tomicus brevipilosus*, *T. minor* and *T. yunnanensis* have overlapping geographical distribution, host range and infection periods. They aggregately infect branches and trunks of two indigenous pines, *Pinus yunnanensis* and *P. kesiya* (Li et al. 1997, 2006, Chen et al. 2009, 2010, Lu et al. 2012, 2014), causing locally extensive tree decline or mortality (Ye and Dang 1986, Ye 1991, 2011). Since the 1980s, damage caused by these bark beetles has resulted in losses of more than 93,000 m^3 of pinewood (Ji et al. 2007).

Generally, two or three pine shoot beetles co-occur underneath the bark or in shoots of a single host tree, either simultaneously but with spatially isolated galleries or successively, during differential infesting peaks. Spatial and chorological differentiation would reduce competition between beetles, but their co-occurrence also could enhance cooperation (Lu et al. 2012, Chen et al. 2015). *Tomicus yunnanensis* is considered to be the most aggressive species in Yunnan, causing primary infestations of healthy *P. yunnanensis* trees and eventually tree death (Ye and Lieutier 1997, Kirkendall et al. 2008, Chen et al. 2010, 2015, Lu et al. 2014). Although *T. brevipilosus* is able to infect healthy trees, it preferably colonises trunks already infested by *T. yunnanensis* or both *T. yunnanensis* and *T. minor* (Chen et al. 2010, 2015). *Tomicus minor* is often regarded as a secondary, opportunist species infesting trees already weakened by *T. yunnanensis* or/and *T. brevipilosus* (Ye and Ding 1999, Lieutier et al. 2003, Chen et al. 2009).

Pine shoot beetles such as *T. piniperda*, *T. minor* and *T. destruens* are commonly associated with ophiostomatoid fungi (Masuya et al. 1999, Kim et al. 2005, Jankowiak 2006, 2008). Fifteen ophiostomatoid fungi were reported associated with *T. piniperda* in Europe (Mathiesen 1950, Lieutier et al. 1989, Gibbs and Inman 1991, Solheim and Långström 1991, Jankowiak 2006, Jankowiak and Bilański 2007) and 11 were documented in eastern Asia (Japan and Korea) (Masuya et al. 1999, Kim et al. 2005). *Ophiostoma minus* was shown to be the dominant species associated with *T. piniperda* in Europe and Japan (Mathiesen 1950, Lieutier et al. 1989, Gibbs and Inman 1991, Masuya et al. 1999, Jankowiak 2006). *Leptographium wingfieldii* was shown to be the strongest pathogenic one (Gibbs and Inman 1991) in Europe. *Tomicus minor* also infests various pines in Europe and Asia. Fifteen (Mathiesen-Käärik 1953, Masuya et al. 1999, Jankowiak 2008) and 11 (Masuya et al. 1999) ophiostomatoid species have been reported to be associated with this beetle species in Europe and Japan, respectively. *Ophiostoma canum* was recorded as a frequent/dominant species in association with *T. minor*, both in Europe and Japan (Mathiesen 1950, 1951, Rennerfelt 1950, Francke-Grosmann 1952, Masuya et al. 1999) but seems to represent a weak pathogen to *P. sylvestris* (Solheim et al. 2001). Additionally, six ophiostomatoid fungi were documented associated with *T. destruens* in Europe (Lieutier 2002, Sabbatini Peverieri et al. 2006, Ben Jamaa et al. 2007).

Despite the fact that *Tomicus* spp. have caused serious losses to forest ecosystems in south-western China, there are no systematic studies of their ophiostomatoid associates but only a few sporadic reports. So far, nine ophiostomatoid species have been reported as being associated with *Tomicus* spp. in Yunnan. Six species (*Leptographium yunnanense*, *Ophiostoma ips*, *O. minus*, *O. quercus*, *S. abietina* and *S. nebularis*) were recorded to be associated with *T. yunnanensis* (Ye et al. 2000, Zhou et al. 2000, 2013, Chang et al. 2017). Two species (*Graphilbum fragrans* and *O. tingens*) were recorded as being associated with *T. minor* (Zhou et al. 2013, Pan et al. 2017), whereas only a single species (*O. brevipilosi*) was recorded as being associated with *T. brevipilosus* (Chang et al. 2017). Amongst them, *L. yunnanense* was the first species newly described from the area (Zhou et al. 2000) and is likely the most virulent one (Liao and Ye 2004, Gao et al. 2017). Until now, the relative abundance with which these fungi occur, their host (pine and beetle) relationships, and their pathogenicity remain unknown.

The symbiosis between bark beetles and ophiostomatoid fungi enhances their pathogenicity. The fitness of bark beetle populations may depend in part on the degree of the fungal partners' pathogenicity and the resulting weakening of the tree (Christiansen et al. 1987, Kirisits 2004, Linnakoski et al. 2012), although this has been questioned by some (Six and Wingfield 2011). Therefore, the question remains whether there is any link between the differential aggression of the pine shoot beetles and the differential virulence of their fungal associates, especially in circumstances where various beetle species co-exist.

The aim of this study was to describe the diversity of ophiostomatoid fungal communities associated with three pine shoot beetles and their galleries infesting *P. yunnanensis* and *P. kesiya* in forest ecosystems of Yunnan Province. We also analysed the degree of beetle/ophiostomatoid fungi specificity. Such studies will enable us to understand the aggressive nature of the beetles and the pathogenicity of the associated fungi and the interactions, ultimately helping to address the current situation of ceaseless outbreaks and rapid expansion of the pests.

Materials and methods

Sample collection and fungus isolation

Samples of galleries in bark and shoots and adults of *Tomicus* spp. were collected from *P. yunnanensis* and *P. kesiya* at five sites in Yunnan Province (Fig. 1, Table 1) from December 2016 to March 2017. Beetles were placed individually in sterilised Eppendorf tubes and their galleries were placed in sterile envelopes and stored at 4°C until processed within one week.

Isolations from beetles and their galleries were carried out on 2% malt extract agar (MEA: 20 g Biolab malt extract, 20 g Biolab agar and 1 000 ml deionised water) with 0.05% NaClO added, in 9-cm Petri dishes as described by Seifert et al. (1993). Hyphal tips of emerging colonies were cut and transferred to MEA plates in order to obtain pure strains. The strains were grown routinely on 2% MEA at 25 °C. Representative

Table 1. Basic information on the sample collection plots in China.

Location	Host	Insect vector	longitude\latitude	altitude(m)	No. of examained samples
Xiangyun,Yunnan	*Pinus yunnanensis*	*Tomicus yunnanensis*, *T. minor*	25°21'25.8"N, 100°51'49"E	2255.4	447
Puer,Yunnan	*P. kesiya*	*T. brevipilosus*, *T. minor*	22°56'36.1"N, 101°14'36.7"E	1400.7	346
Qujing,Yunnan	*P. yunnanensis*	*T. yunnanensis*, *T. minor*, *T. brevipilosus*	25°28'51"N, 103°46'32"E	2068.2	102
Anning,Yunnan	*P. yunnanensis*	*T. yunnanensis*, *T. minor*, *T. brevipilosus*	24°53'32"N, 102°24'23"E	1939.9	138
Yuxi, Yunan	*P. yunnanensis*	*T. yunnanensis*, *T. minor*	24°18'23"N, 102°34'37"E	1908.1	85

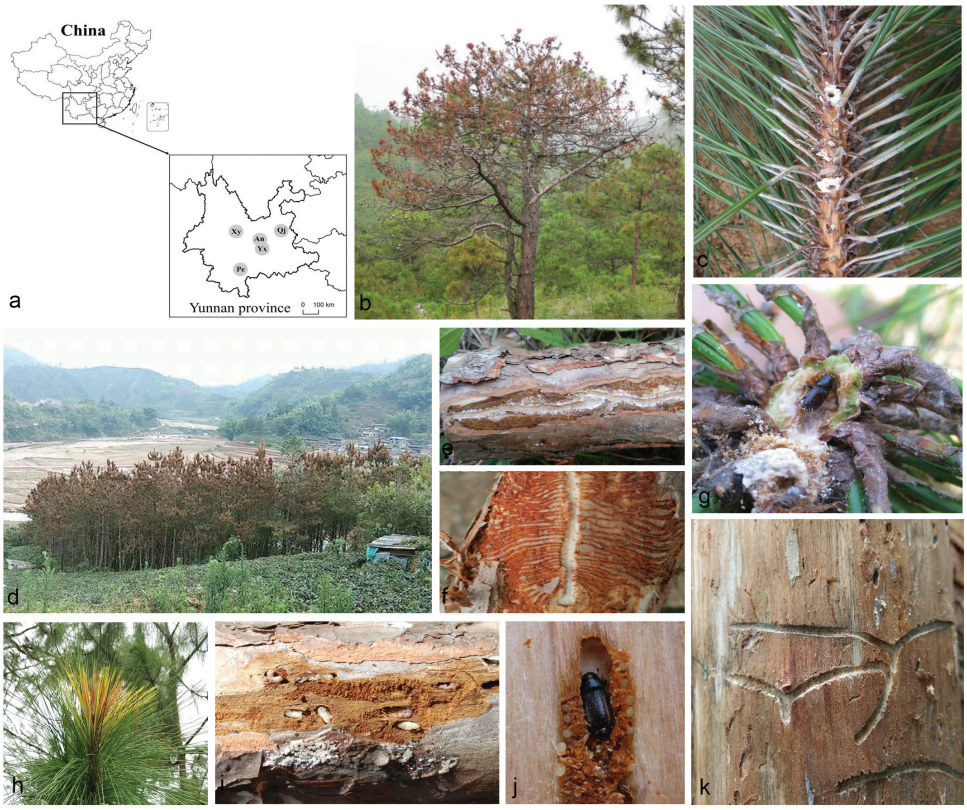


Figure 1. A Map showing the 11 species of ophiostomatoid fungi detected from Yunnan Province, China **B, D** disease symptoms on *Pinus yunnanensis* and *P. kesiya* trees infested by *Tomicus* spp. (*T. yunnanensis*, *T. minor* and *T. brevipilosus*) and ophiostomatoid fungi **C, G, H** exposed branches of *Tomicus* spp. on *P. yunnanensis* and *P. kesiya* **E, F, I–K** galleries of *Tomicus* spp. on *P. yunnanensis* and *P. kesiya*.

cultures of each morphotype were deposited in the China Forestry Culture Collection Center (CFCC, part of the National Infrastructure of Microbial Resources) and the culture collection of the Chinese Academy of Forestry (CXY) (Table 2).

Morphology and growth studies

Morphological characterisation of both the sexual and asexual reproduction forms was performed on 2% MEA media incubated 3–6 weeks at 25 °C in the dark. Slide cultures were made to observe all microscopic characters (sexual/asexual structures) using a BX51 OLYMPUS microscope with differential interference contrast. Fifty measurements were made of each relevant structure and the ranges were calculated. Standard

Table 2. Representative strains of the ophiostomatoid fungi associated with three *Tomicus* spp. in Yunnan Province, China, and three *E. vermicola* strains used in this study.

Group	Taxon	Strain no.	Host	Location	Beetle	GenBank no.				
						LSU	ITS\ ITS2–LSU5	BT	EF	CAL
A	*Esteya vermicola*	CFCC52625 (CXY1893)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325143	–	MH697597	MH605999	–
		ATCC74485	Japanese black pine	Taiwan, China	*Bursaphelenchus xylophilus*	–	–	–	GQ995674	–
		CNU120806	soil	Korea	saprophytic nematodes	EU627684	–	FJ490553	GQ995671	–
		CBS 115803	oak	Czech Republic	*Scolytus intricatus*	–	–	FJ490552	GQ995672	–
B	***Graphilbum anningense***	CFCC52631 (CXY1939)	*P. yunnanensis*	Anning	*T. yunnanensis*	MH325162	MH555903	MH683595	–	–
		CFCC52632 (CXY1940)	*P. yunnanensis*	Anning	*T. yunnanensis*	MH325164	MH555901	MH683596	–	–
		CFCC52633 (CXY1944)	*P. yunnanensis*	Anning	*T. minor*	MH325163	MH555902	MH683597	–	–
C	*Leptographium yunnanense*	CFCC52619 (CXY1897)	*P. kesiya*	Ninger	*T. brevipilosus*	MH325138	MH487721	MH603933	MH606000	–
		CFCC52620 (CXY1900)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325139	MH487724	MH603934	MH606001	–
		CFCC52621 (CXY1904)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325140	MH487726	MH603935	MH606003	–
		CFCC52622 (CXY1908)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325142	MH487725	MH603938	MH606002	–
		CFCC52623 (CXY1917)	*P. kesiya*	Puer	*T. brevipilosus*	MH325137	MH487723	MH603936	MH606004	–
		CFCC52624 (CXY1925)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325141	MH487722	MH603937	MH606005	–
D	*Ophiostoma brevipilosi*	CFCC52596 (CXY1828)	*Pinus kesiya*	Puer	*T. brevipilosus*	MH325134	MH555904	MH619527	–	–
		(CXY1806) CFCC52597	*P. kesiya*	Puer	*T. brevipilosus*	MH325135	MH555905	MH619528	–	–
		CFCC52598 (CXY1808)	*P. kesiya*	Puer	*T. brevipilosus*	MH325136	MH555906	MH619529	–	–

E	*O. canum*	CFCC52601 (CXY1858)	*P. yunnanensis*	Xiangyun	*T. minor*	MH325151	MH555889	MH619521	–	–
		CFCC52602 (CXY1848)	*P. yunnanensis*	Xiangyun	*T. minor*	MH325152	MH555890	MH619522	–	–
		CFCC52603 (CXY1857)	*P. yunnanensis*	Xiangyun	*T. minor*	MH325153	MH555891	MH619523	–	–
F	***O. aggregatum***	CFCC52615 (CXY1876)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325146	MH555894	MH603927	–	–
		CFCC52616 (CXY1875)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325145	MH555893	MH603929	–	–
		CFCC52617 (CXY1874)	*P. kesiya*	Puer	*T. minor*	MH325147	MH555895	MH603928	–	–
G	*O. minus*	CFCC52606 (CXY1885)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325154	MH578163	MH619524	–	–
		CFCC52607 (CXY1877)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325155	MH578164	MH619525	–	–
		CFCC52608 (CXY1881)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325156	MH578165	MH619526	–	–
H	*O. tingens*	CFCC52611 (CXY1866)	*P. yunnanensis*	Xiangyun	*T. minor*	MH325148	MH578166	MH603931	–	–
		CFCC52612 (CXY1865)	*P. yunnanensis*	Xiangyun	*T. minor*	MH325149	MH578167	MH603932	–	–
		CFCC52613 (CXY1868)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325150	MH578168	MH603930	–	–
I	*Ophiostoma* sp. 1	CFCC52618 (CXY1936)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325144	MH555892	MH683600	–	–
J	***Sporothrix macroconidia***	CFCC52628 (CXY1894)	*P. yunnanensis*	Xiangyun	*T. yunnanensis*	MH325157	MH555898	MH697594	–	MH592598
		CFCC52629 (CXY1895)	*P. kesiya*	Ninger	*T. brevipilosus*	MH325158	MH555899	MH697595	–	MH592599
		CFCC52630 (CXY1896)	*P. kesiya*	Ninger	*T. brevipilosus*	MH325159	MH555900	MH697596	–	MH592600
K	***S. pseudoabietina***	CFCC52626 (CXY1937)	*P. yunnanensis*	Qujing	*T. minor*	MH325160	MH555896	MH683598	–	MH592601
		CFCC52627 (CXY1938)	*P. yunnanensis*	Qujing	*T. minor*	MH325161	MH555897	MH683599	–	MH592602

Species names in bold are species newly described in this study.
CFCC: China Forestry Culture Collection Center, Beijing, China;
CXY (Culture Xingyao): Culture collection of the Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry.
Sequences missing data are indicated by [–]

deviation (*SD*), minimum (min) and maximum (max) measurements are presented as (min–) (mean–*SD*) – (mean+*SD*) (–max).

The optimal growth temperature of the various strains was determined by placing a 5-mm (diam.) plug from an actively growing fungal colony upside down at the centre of an MEA plate. For each strain, three replicates were incubated at temperatures ranging from 5 to 35 °C at five-degree intervals, for 8d. The diameter of each colony was measured daily. Culture characters were recorded on MEA incubated at 25 °C for 8 d and 20 d. Colour descriptions were made by reference to Rayner (1970).

DNA extraction and sequencing

DNA was extracted from actively growing mycelium scraped from seven-day-old cultures using sterile scalpels and transferred to 2 ml Eppendorf tubes. DNA extraction and purification were performed using the Invisorb Spin Plant Mini Kit (Invitek, Berlin, Germany), following the manufacturer's protocols.

DNA sequences were determined for six gene regions: the nuclear ribosomal large subunit region (LSU), the internal transcribed spacer regions 1 and 2, including the intervening 5.8S gene (ITS), as well as segments of the β-tubulin (*TUB2*), elongation factor 1α (*TEF1-a*) and calmodulin (*CAL*) genes. DNA fragments were amplified using the primer pairs LROR/LR5 (Vilgalys and Hester 1990), ITS1/ITS4 (White et al. 1990), ITS3/LR3 (Vilgalys and Hester 1990, White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), EF1/EF2 (Jacobs et al. 2004) and CL1/CL2a (Zhang et al. 2015), respectively. PCR reactions were conducted in 25 µl volumes (2.5 mM $MgCl_2$, 1× PCR buffer, 0.2 mM dNTP, 0.2 mM of each primer and 2.5 U Taq-polymerase enzyme). PCR amplifications were carried out in a thermocycler (Applied Biosystems, Foster City, California, USA). The reaction conditions for these six gene regions were similar to those described in the references used for primer design. PCR products were cleaned with an MSB Spin PCR apace Kit (250), following the manufacturer's instructions.

Phylogenetic analyses

BLAST searches for the obtained sequences were performed in NCBI GenBank and published sequences of closely related species were downloaded. Alignments of the genes were made using MAFFT 7.0 (Katoh and Standley 2013) and the E-INS-i strategy and edited manually in MEGA 5.2 (Tamura et al. 2011). Phylogenetic analyses were performed using maximum parsimony (MP) as well as maximum likelihood (ML).

ML analyses were implemented using RAxML v. 7.0.3 (Stamatakis 2006), under the GTR-GAMMA model. Support for the nodes was estimated from 1 000 bootstrap replicates. The results were subsequently exported to Figtree v.1.4.2 to visualise the trees.

MP analyses were implemented in PAUP* 4.0b10 (Swofford 2003). The most parsimonious trees were identified by a heuristic search of 1 000 random addition sequence replicates, using the tree-bisection-recognition (TBR) algorithm for branch

swapping. Branch support was assessed by 1 000 bootstrap replicates. Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were used to evaluate the trees.

Results

Fungal isolation and sequence comparisons

Three *Tomicus* species occurred on *P. yunnanensis* and *P. kesiya* in the areas studied, either independently or concomitantly in individuals of the host trees (Fig. 1). In total, 772 strains of ophiostomatoid fungi (*Hyalorhinocladiella*-like, *Ophiostoma*, *Pesotum*-like, *Leptographium*-like and *Sporothrix*-like) were isolated from 223 adult beetles (20% of the strains) and 890 galleries (80% of the strains). Galleries or adults of *T. yunnanensis* yielded 297 strains whereas 247 strains were retrieved from galleries or adults of *T. minor* and 228 strains from galleries or adults of *T. brevipilosus* (Table 3).

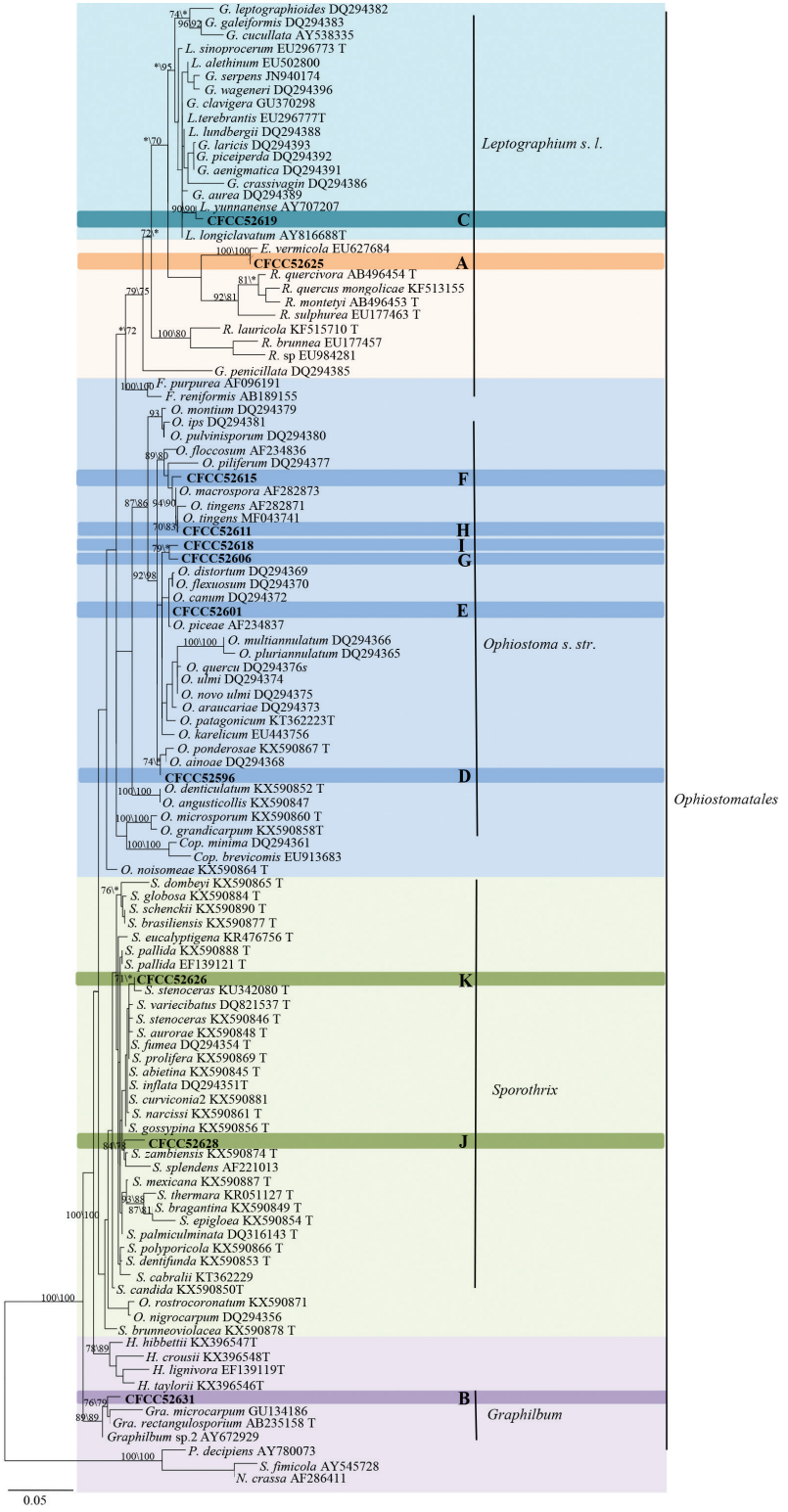
The LSU sequence was used to search for preliminary affinities using the BLASTn search option in GenBank. As a result, these strains were found to be distributed over 5 genera and 11 tentative species/groups (A–K) (Table 2).

Phylogenetic analyses

The degrees of polymorphism of LSU, ITS, *TUB2*, *TEF1-a* and *CAL* make them variably suitable for genus or species discrimination amongst ophiostomatoid fungi. The LSU sequence is a suitable marker to infer the generic affinities (de Beer and Wingfield 2013,

Table 3. Strain numbers of various ophiostomatoid fungi obtained from three *Tomicus* spp. and their galleries collected in Yunnan Province.

Group	Fungi species	*Tomicus yunnanensis*	*T. minor*	*T. brevipilosus*	Total no. strains\samples
A	*Ophiostoma brevipilosi*	0	0	224	224
B	*O. canum*	52	201	0	253
C	*O. minus*	197	0	0	197
D	*O. tingens*	4	26	0	30
E	*O. aggregatum*	3	2	0	5
F	*Ophiostoma* sp. 1	1	0	0	1
G	*Leptographium yunnanense*	30	0	2	32
H	*Esteya vermicola*	1	0	0	1
I	*Sporothrix pseudoabietina*	4	15	0	19
J	*S. macroconidia*	1	0	2	3
K	*Graphilbum anningense*	4	3	0	7
	Total no. strains	297	247	228	772
	Total no. samples	455	324	339	1118


G. leptographioides DQ294382
G. galeiformis DQ294383
G. cucullata AY538335
L. sinoprocerum EU296773 T
L. alethinum EU502800
G. serpens JN940174
G. wageneri DQ294396
G. clavigera GU370298
L.terebrantis EU296777T
L. lundbergii DQ294388
G. laricis DQ294393
G. piceiperda DQ294392
G. aenigmatica DQ294391
G. crassivagin DQ294386
G. aurea DQ294389
L. yunnanense AY707207
CFCC52619
C
L. longiclavatum AY816688T
E. vermicola EU627684
CFCC52625
A
R. quercivora AB496454 T
R. quercus mongolicae KF513155
R. montetyi AB496453 T
R. sulphurea EU177463 T
R. lauricola KF515710 T
R. brunnea EU177457
R. sp EU984281
G. penicillata DQ294385
Leptographium s. l.
F. purpurea AF096191
F. reniformis AB189155
O. montium DQ294379
O. ips DQ294381
O. pulvinisporum DQ294380
O. floccosum AF234836
O. piliferum DQ294377
CFCC52615
F
O. macrospora AF282873
O. tingens AF282871
O. tingens MF043741
CFCC52611
H
CFCC52618
I
CFCC52606
G
O. distortum DQ294369
O. flexuosum DQ294370
O. canum DQ294372
CFCC52601
E
O. piceae AF234837
O. multiannulatum DQ294366
O. plurianmulatum DQ294365
O. quercu DQ294376s
O. ulmi DQ294374
O. novo ulmi DQ294375
O. araucariae DQ294373
O. patagonicum KT362223T
O. karelicum EU443756
O. ponderosae KX590867 T
O. ainoae DQ294368
CFCC52596
D
O. denticulatum KX590852 T
O. angusticollis KX590847
O. microsporum KX590860 T
O. grandicarpum KX590858T
Cop. minima DQ294361
Cop. brevicomis EU913683
O. noisomeae KX590864 T
Ophiostoma s. str.
Ophiostomatales
S. dombeyi KX590865 T
S. globosa KX590884 T
S. schenckii KX590890 T
S. brasiliensis KX590877 T
S. eucalyptigena KR476756 T
S. pallida KX590888 T
S. pallida EF139121 T
CFCC52626
K
S. stenoceras KU342080 T
S. variecibatus DQ821537 T
S. stenoceras KX590846 T
S. aurorae KX590848 T
S. fumea DQ294354 T
S. prolifera KX590869 T
S. abietina KX590845 T
S. inflata DQ294351T
S. curviconia2 KX590881
S. narcissi KX590861 T
S. gossypina KX590856 T
CFCC52628
J
S. zambiensis KX590874 T
S. splendens AF221013
S. mexicana KX590887 T
S. thermara KR051127 T
S. bragantina KX590849 T
S. epigloea KX590854 T
S. palmiculminata DQ316143 T
S. polyporicola KX590866 T
S. dentifunda KX590853 T
S. cabralii KT362229
S. candida KX590850T
O. rostrocoronatum KX590871
O. nigrocarpum DQ294356
S. brunneoviolacea KX590878 T
Sporothrix
H. hibbettii KX396547T
H. crousii KX396548T
H. lignivora EF139119T
H. taylorii KX396546T
CFCC52631
B
Gra. microcarpum GU134186
Gra. rectangulosporium AB235158 T
Graphilbum sp.2 AY672929
Graphilbum
P. decipiens AY780073
S. fimicola AY545728
N. crassa AF286411
0.05

de Beer et al. 2013a, 2016); it allowed confirming the preliminary placement of our strains based on morphological characters (Fig. 2). The ITS region would be useful to place strains within the *Ophiostoma s. l.* complex, but the degree of polymorphism does not allow distinguishing species. Usually, *TUB2*, *TEF1-a* and *CAL* regions are better markers to identify and, where appropriate, to show the genetic diversity within ophiostomatoid fungi (Zipfel et al. 2006, de Beer and Wingfield 2013, de Beer et al. 2016).

On the basis of the LSU blast searches, one to six strains of each tentative species (A–K) were selected for sequencing of five additional DNA markers (ITS, ITS2-LSU, *TUB2*, *TEF1-a* and *CAL*) to infer more accurate identification and phylogenetic affinities. Six sequence datasets (LSU, ITS, ITS2-LSU, *TUB2*, *TEF1-a* and *CAL*) were generated for a total of 31 representative strains (Table 2) and the sequences were deposited in GenBank. Resulting alignments were deposited in TreeBASE (submission no: 24032). The topologies generated by the ML and MP analyses were highly concordant and the ML phylograms are presented for all the individual genes, incorporating nodal supports of both the ML and MP analyses.

The LSU dataset consisted of 109 sequences, 11 sequences obtained in this study and 98 downloaded from GenBank. The phylogenetic analyses confirmed the morphology-based placement of our strains into *Esteya*, *Graphilbum*, *Leptographium*, *Ophiostoma* and *Sporothrix* (Fig. 2).

Group A consisted of a single strain. LSU-based phylogenetic analysis showed this strain to be close to *E. vermicola* (Fig. 2). *TUB2* and *TEF1-a* data analysis confirmed the strain's close affinities to *E. vermicola* (Fig. 3a, b), that could justify conspecificity.

Group B strains nested within the *Graphilbum* lineage in the LSU-based phylogenetic analysis (Fig. 2). Phylogenetic analysis based on LSU, ITS and *TUB2* concordantly showed that the group B strains formed a single, well-supported clade related to but distinct from *Gra. rectangulosporium* and *Gra. microcarpum* (Fig. 4a, b); this would warrant its recognition as a distinct, undescribed species.

Group C strains were shown to belong to the *Leptographium* lineage in the LSU-based phylogenetic analysis (Fig. 2). The ITS2-LSU dataset consisted of six of our own sequences and 49 reference sequences downloaded from GenBank. Within the *Leptographium* lineage, group C strains nested in the *L. lundbergii*-complex; they were related to *L. yunnanense*, *L. lundbergii* and *L. conjunctum* (Fig. 5a). *TUB2*- and *TEF1-a* based analysis confirmed their close affinities with *L. yunnanense*, although forming a slightly divergent clade (Fig. 5b, c). *TUB2* and *TEF1-a* sequences of group C strains showed some polymorphisms, which could be considered as falling within the natural diversity of *L. yunnanense*.

Figure 2. Phylograms obtained from ML analysis of LSU sequences, showing fungal associates with pines infected by *Tomicus yunnanensis*, *T. minor* and *T. brevipilosus* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 743 positions, including gaps.

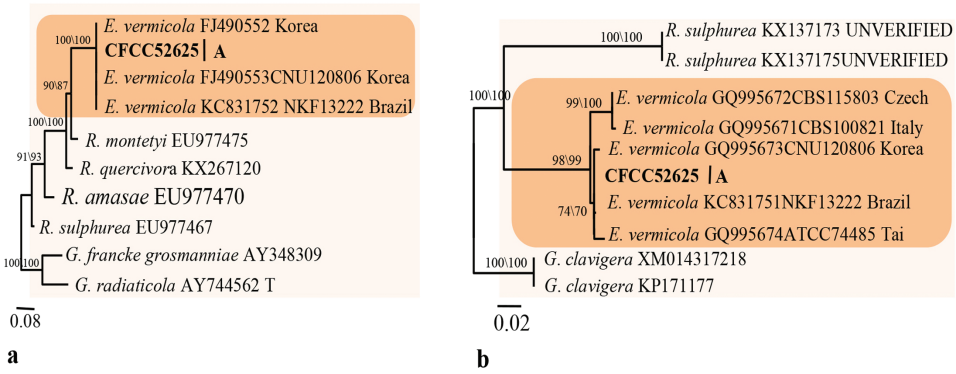


Figure 3. Phylograms obtained from ML analysis of β-tubulin **A** and elongation factor **B** sequences of *Esteya*, showing fungal associates with pines infected by *Tomicus yunnanensis* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 320 (**A**), 856 (**B**) positions, including gaps.

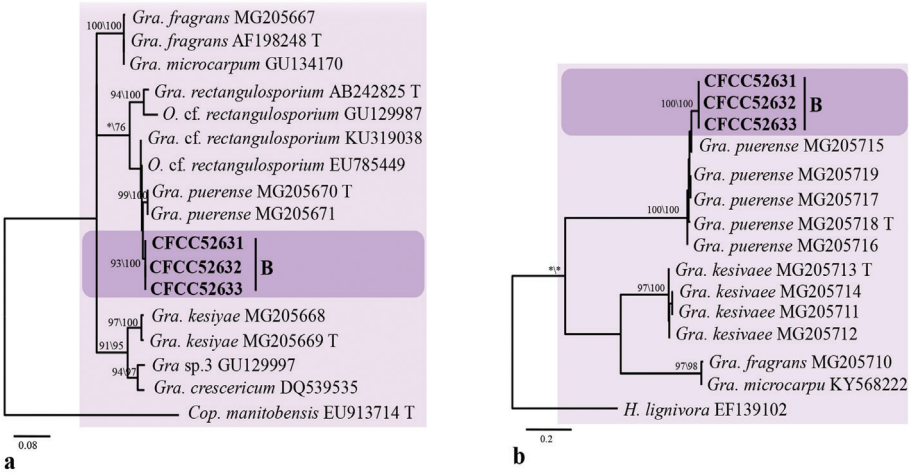


Figure 4. Phylograms obtained from ML analysis of ITS sequences **A** and β-tubulin sequences **B** of *Graphilbum* showing fungal associates with pines infected by *Tomicus yunnanensis* and *T. minor* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 515 (**A**), 481 (**B**) positions, including gaps.

The six strains from groups D to I nested within the *Ophiostoma* lineage based on the LSU phylogenetic tree (Fig. 2). The ITS dataset comprised species from all lineages discovered in this study. Analysis of this dataset yielded the phylograms shown in Fig. 6. Sixteen ITS sequences generated in this study were compared with 61 sequences

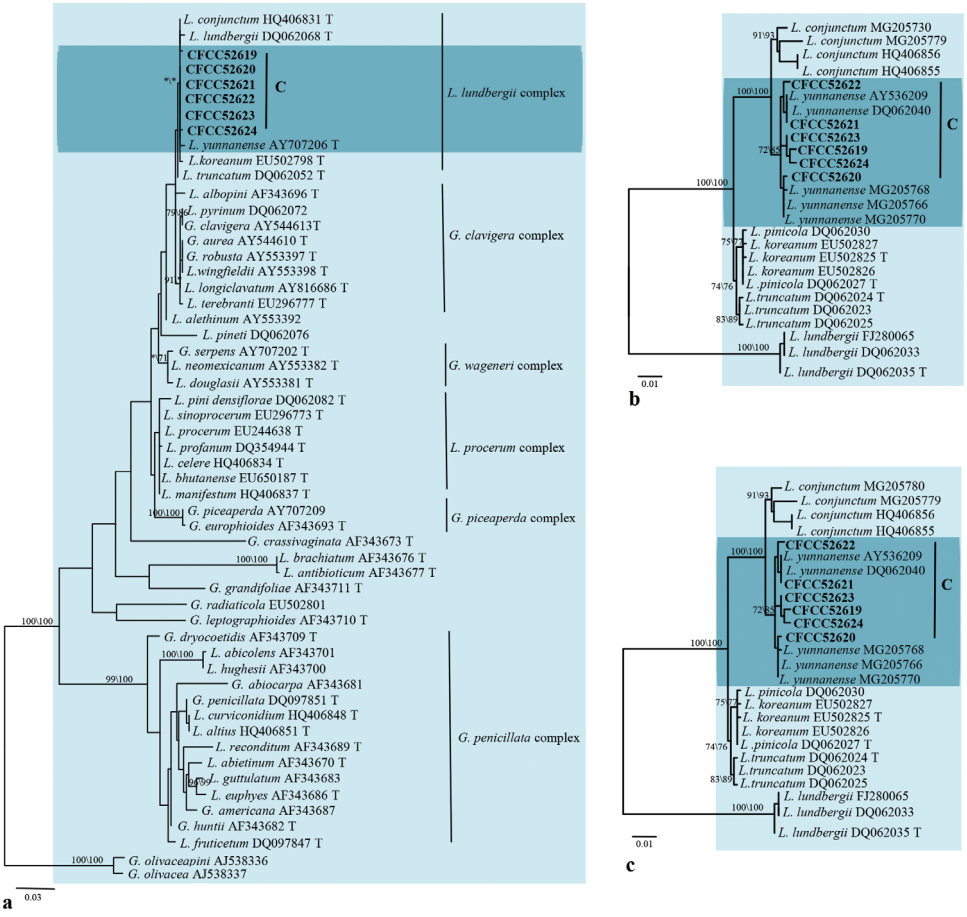


Figure 5. Phylograms obtained from ML analysis of ITS2-28S **A** β-tubulin **B** and elongation factor **C** sequences of *Leptographium*, showing fungal associates with pines infected by *Tomicus yunnanensis* and *T. brevipilosus* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 641 (**A**), 358 (**B**), 639 (**C**) positions, including gaps.

retrieved from GenBank, representing the major groups of *Ophiostoma* (de Beer and Wingfield 2013, Linnakoski et al. 2016).

The ITS- and *TUB2*-based phylogenetic inferences (Figs 6, 7a, b) showed that the strains of groups D and E nested within the *O. clavatum*- and *O. piceae*-complex (de Beer and Wingfield 2013, Yin et al. 2016, Linnakoski et al. 2016), in which they were positioned in the near vicinity of the *O. brevipilosi* and *O. canum* clades, respectively. From these results, and considering their morphological features, we concluded that the strains of groups D and E are conspecific with *O. brevipilosi* and *O. canum*, respectively.

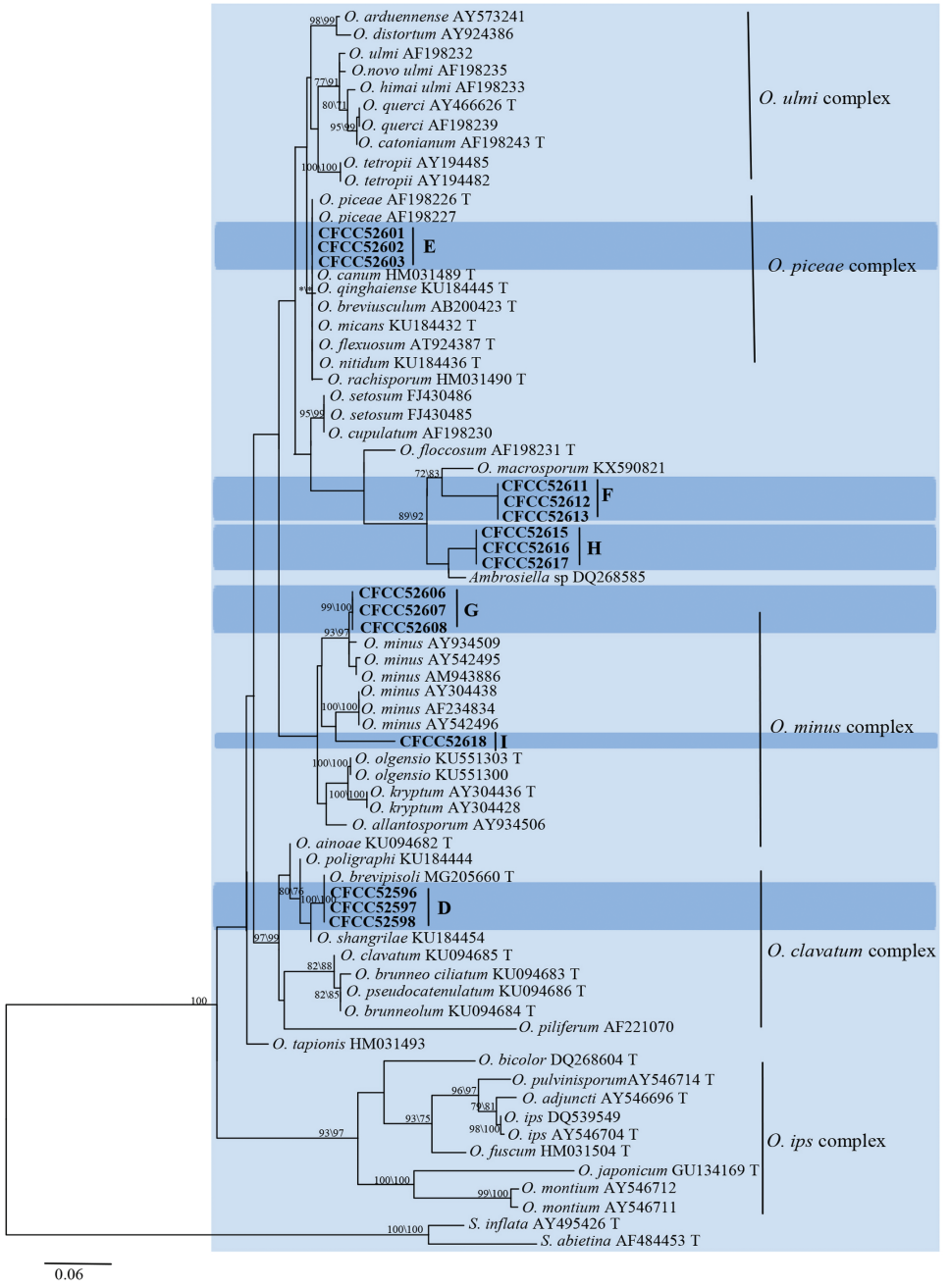


Figure 6. Phylograms obtained from ML analysis of ITS sequences of *Ophiostoma*, showing fungal associates with pines infected by *Tomicus yunnanensis*, *T. minor* and *T. brevipilosus* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 633 positions, including gaps.

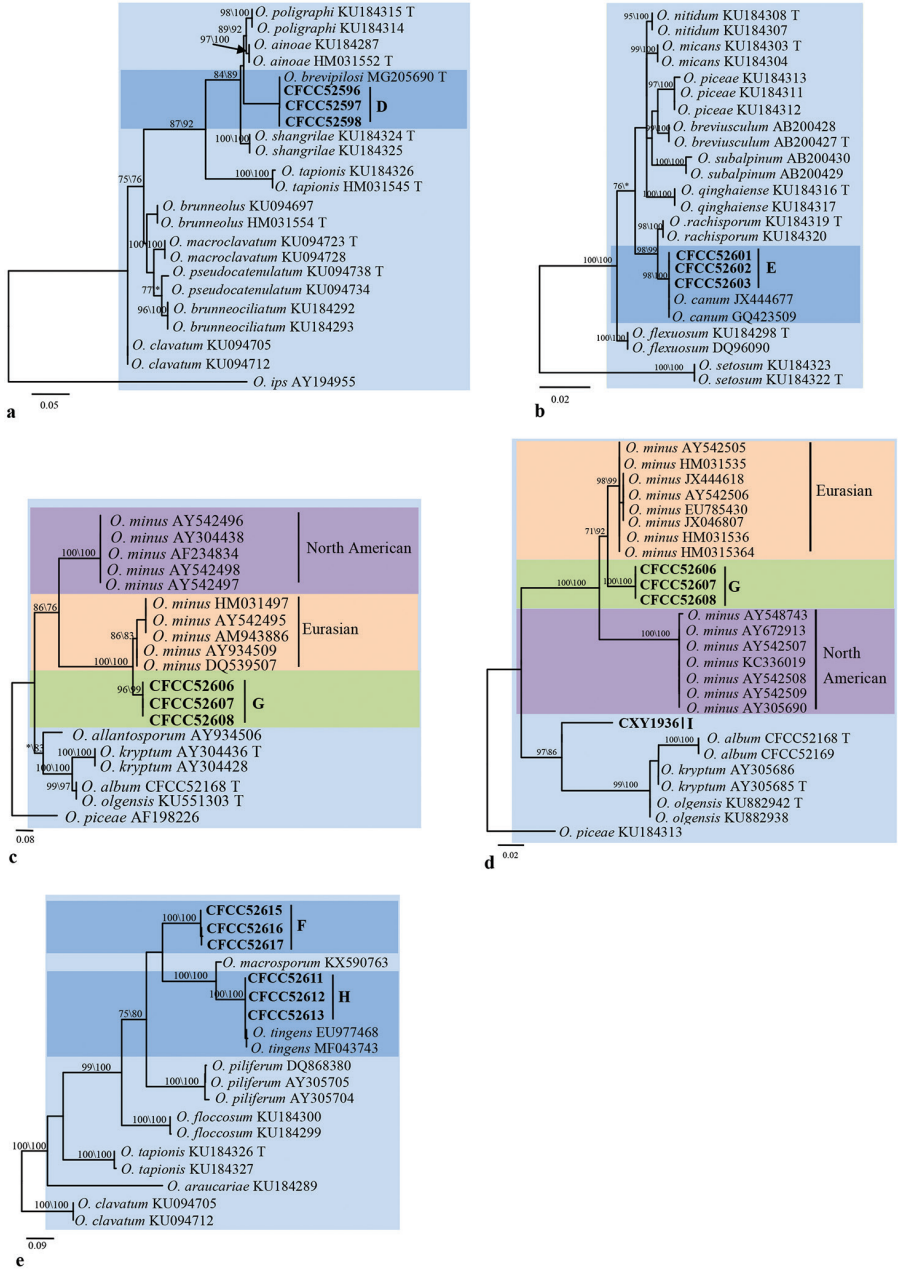


Figure 7. Phylograms obtained from ML analysis of β-tubulin sequences of *Ophiostoma* **A, B, D, E** and ITS sequences of *O. minus*-complex **C** showing fungal associates with pines infected by *Tomicus yunnanensis*, *T. minor* and *T. brevipilosus* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 455 (**A**), 430 (**B**), 541 (**C**), 378 (**D**), 423 (**E**) positions, including gaps.

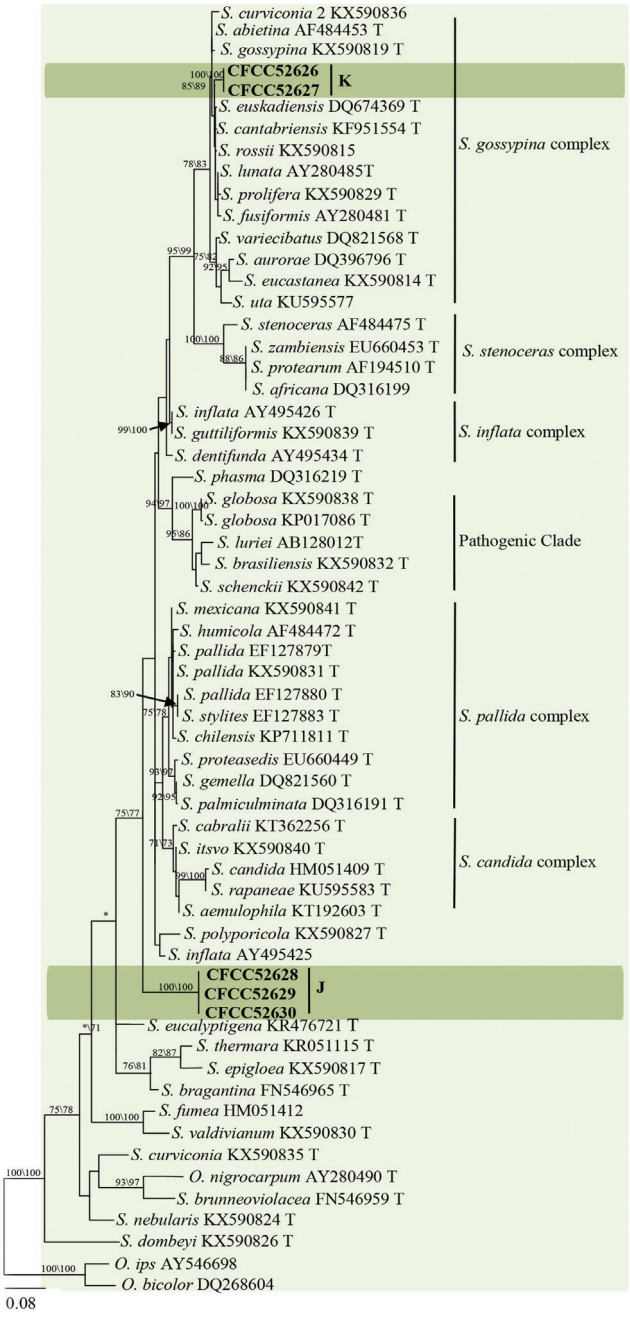


Figure 8. Phylograms obtained from ML analysis of ITS sequences of *Sporothrix*, showing fungal associates with pines infected by *Tomicus yunnanensis*, *T. minor* and *T. brevipilosus* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 546 positions, including gaps.

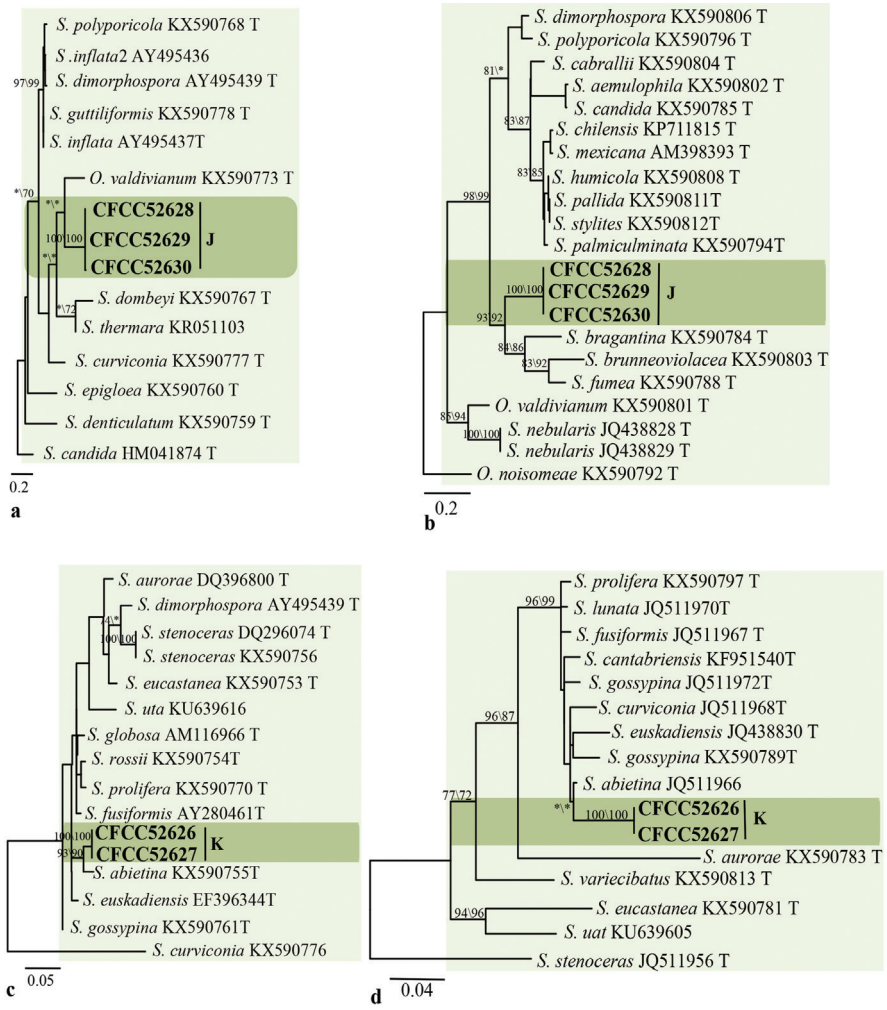


Figure 9. Phylograms obtained from ML analysis of β-tubulin **A, C** and calmodulin **B, D** sequences of *Sporothrix*, showing fungal associates with pines infected by *Tomicus yunnanensis*, *T. minor* and *T. brevipilosus* in Yunnan Province, China. Novel sequences obtained in this study are printed in bold type. Bootstrap values ≥ 70% for ML and MP are indicated above branches. Bootstrap values < 70% are indicated by the symbol *. Strains representing ex-type sequences are marked with 'T'; ML, maximum likelihood; MP, maximum parsimony and the final alignment of 284(**A**), 622(**B**), 260(**C**), 675(**D**) positions, including gaps.

In the ITS-based phylogenetic analysis, strains of groups G and I were grouped with the *O. minus* complex (Fig. 6). ITS- and *TUB2*-based phylogenetic analyses consistently showed that group G strains formed a well-supported subclade between the North American and European subclades within the *O. minus* lineage (Fig. 7c, d). The strains of group G are therefore identified as *O. minus*. The ITS- and *TUB2*-based phylogenetic analyses consistently showed that the single strain of group I formed a branch that is related to, but distinct from the *O. minus*, *O. kryptum* and *O. olgensis* clades (Figs 6, 7d). Hence, this strain is interpreted as belonging to a distinct, undescribed *Ophiostoma*.

The remaining two groups (F and H) were not placed in any defined complex. Phylogenetic analyses, based on ITS and *TUB2* sequences, consistently showed that the group H strains clustered in the near vicinity of the *O. tingens* clade whereas group F strains formed a clade related to, but distinct from the *O. macrosporum* and *O. tingens* clades (Figs 6, 7e). Thus, the strains in group H should be identified as *O. tingens* whereas the strains of group F represent an undescribed *Ophiostoma*.

Strains of groups J and K nested within the *Sporothrix* lineage in LSU-based phylogenetic analysis (Fig. 2). The phylograms resulting from the analyses of individuals are shown in Fig. 8 (ITS), Fig. 9a, c (*TUB2*) and Fig. 9b, d (CAL).

The ITS-based analyses showed that group K strains belonged to the *S. gossypina*-complex whereas the group J strains were not placed in any species complex as defined by de Beer et al. (2016) (Fig. 8). Both groups formed independent, well-supported clades in ITS-, *TUB2*- and *CAL*-based phylogenetic analyses (Figs 8, 9). It could be deduced from results of multiple phylogenies that both groups represent novel species.

Morphology and taxonomy

From a morphological perspective, strains of groups D, E and G appeared, overall, concordant with the descriptions or our own observations of reference strains, namely of *O. brevipilosi*, *O. canum* and *O. minus*, respectively. However, although strains of groups A, C, and H are phylogenetically close to *E. vermicola*, *L. yunnanense* and *O. tingens*, respectively, justifying, for the time being, conspecificity, their phenotype deviated slightly from published descriptions and/or our own observation of type material. The description of these species is extended. Strains of groups B, F, J and K revealed unique combinations of phenotypes, allowing morphological distinction from their closest phylogenetic relatives; consequently, they are described below as new species. The strain of the stand-alone group I also may represent an undescribed species; however, we refrain from describing it for the time being, waiting for more material to become available.

Taxonomy

Esteya vermicola **J.Y. Liou, J.Y. Shih & Tzean, Mycol. Res. 103(2): 243. 1999.**
MycoBank MB450702
Fig. 10

Description. Sexual form: unknown.

Asexual form: *Hyalorhinocladiella*-like. *Conidiophores* mononematous, micronematous; *conidiophorous cells* solitary, integrated, flask-shaped, with an inflated base (3.6–) 4.6–6.1 (–7.1) µm in diam., the fertile hyphoid part (9.1–) 12.2–19.0 (–22.5) × (1.4–) 1.9–3.1 (–4.7) µm, often crooked due to successive conidial development; *conidia*

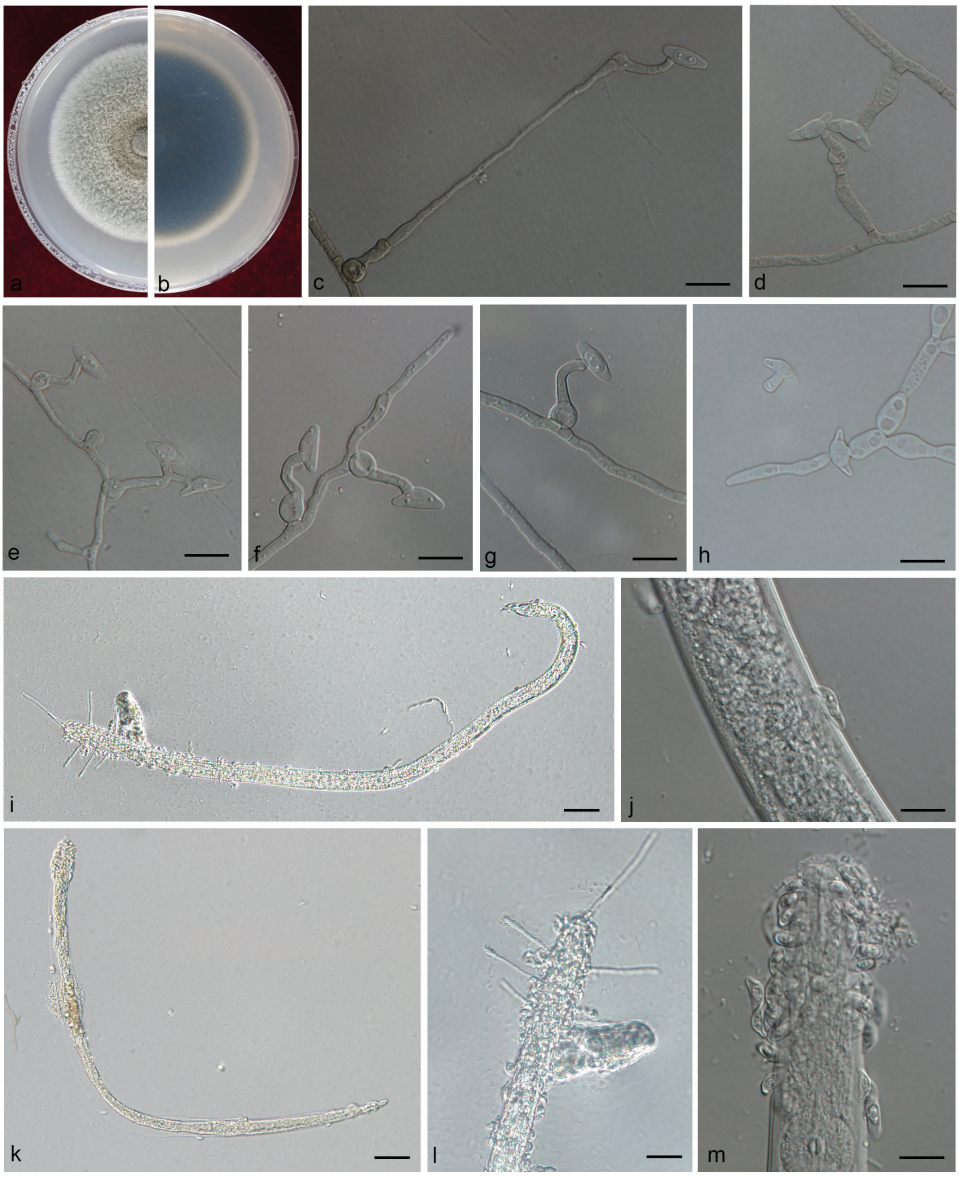


Figure 10.A–H Morphological characters of *Esteya vermicola* **A, B** upper and reverse of cultures on 2% MEA 20 d after inoculation **C–H** conidiogenous cells with lunate conidia **I–M** the cuticle of a nematode attached by many lunate conidia. Scale bars: 20 µm (**I, K, L**); 10 µm (**C–H, J, M**).

1-celled, asymmetrically ellipsoidal in face view, concave, lunate in side view, with a layer of adhesive mucus on the concave surface, ending slightly apiculate, hyaline, smooth, (8.0–) 10–12 (–13.1) × (3.3–) 3.4–4.5 (–5.1) µm, containing an ovoid endospore-like structure.

Culture characteristics. Colonies on 2% MEA in the dark reaching 31 mm in diam. in 8 days at 25 °C, growth rate up to 5 mm/day at the fastest; colony margin smooth. Mycelium compact, somewhat floccose in the margin, white at first, gradually discolouring to greyish-green, eventually dark green. Optimal growth temperature 25 °C, growth at 5 °C and 35 °C.

Known substrate and host. Galleries of *Tomicus yunnanensis* in *Pinus yunnanensis*.

Known insect vector. *Tomicus yunnanensis*.

Known distribution. Yunnan Province, China.

Specimen examined. CHINA, Yunnan, *Tomicus yunnanensis* galleries in *Pinus yunnanensis*, Dec. 2016, HM Wang, CFCC 52625 = CXY 1893.

Note. *Esteya vermicola* is known only from an asexual, *Hyalorhinocladiella*-like state producing lunate and bacilliform conidia (Liou et al. 1999, Kubátová et al. 2000, Wang et al. 2009, 2014) that we also observed in various strains of *E. vermicola* with a different origin (Taiwan, Korea, Czech Republic). Our strain was identified as *E. vermicola* based on phylogenetic inferences and morphological characters. However, our strain differed from previous descriptions (Liou et al. 1999) in having only lunate conidia *in vitro*. The size of the lunate conidia of our strains (mostly 10 - 12 × 3.4 - 4.5 µm) was similar to that reported for *E. vermicola*, *viz.* 9.9–11.9 × 3.4–4.5 µm vs 8.2-11.1 × 3.5–3.7 µm (Taiwan, Liou et al. 1999), 9.3–12.4 × 3.0–3.2 µm (Czech Republic, Kubátová et al. 2000), 7.7–12.1 × 3.0–3.8 µm (Korea, Wang et al. 2009) or 8.7–11.9 × 3.0–3.6 µm (Brazil, Wang et al. 2014).

This is the first report of *E. vermicola* from continental China. The species was originally isolated from Japanese black pine infected by the pinewood nematode *Bursaphelenchus xylophilus*, in Taiwan (Liou et al. 1999). Since then, its distribution range has been extended to Japan and Korea, Europe (Czech Republic, Italy) and both North (USA) and South America (Brazil) (Liou et al. 1999, Kubátová et al. 2000, Wang et al. 2009, 2014, Li et al. 2018). This species is associated with various vectors, including the pinewood nematode, *Oxoplatypus quadridentatus* and the bark beetle *Scolytus intricatus*. It was isolated also from wooden packaging material infested by *Bursaphelenchus rainulfi*.

Graphilbum anningense **H. Wang, Q. Lu & Z. Zhang, sp. n.**
MycoBank MB828884
Fig. 11

Etymology. '*anningense*' (Latin), referring to the type locality.

Type. CHINA, Yunnan, *Tomicus yunnanensis* galleries in *Pinus yunnanensis*, Apr. 2017, HM Wang, holotype CXY 1939, culture ex-holotype CFCC 52631 = CXY 1939.

Description. Sexual form: unknown.

Asexual forms: *Pesotum*-like and *Hyalorhinocladiella*-like. *Pesotum*-like *conidiophores* abundant on 2% MEA, macronematous, synnematous, (150–) 210–293 (–336) µm long including conidiogenous apparatus, the base dark brown, slightly widened, (6.7–)

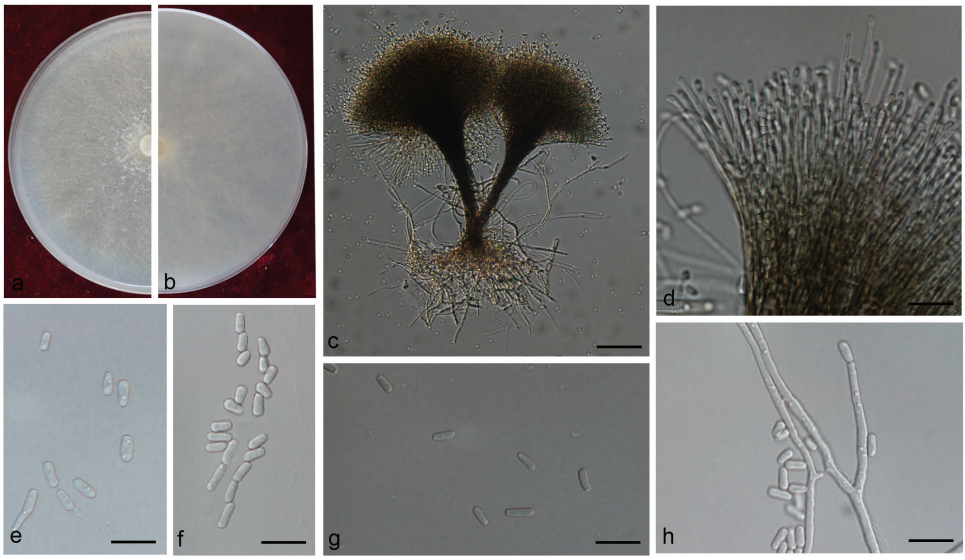


Figure 11. Morphological characters of *Graphilbum anningense* sp. n. **A, B** Upper and reverse of cultures on 2% MEA 8 d after inoculation **C, D, G** conidiogenous cells of Pesotum-like macronematal asexual state and conidia **E, F, H** conidiogenous cells of *Hyalorhinocladiella*-like asexual state and conidia. Scale bars: 50 μm (**C**); 20 μm (**D**); 10 μm (**E–H**).

7.9–18.8 (–29.0) μm wide anchored in the media by brown rhizoid-like hyphae, the apex slightly enlarging, fan-shaped; *conidiogenous cells* hyaline, thin-walled, aseptate, (15.3–) 21.0–35.5 (–42) × (0.7–) 1.1–1.9 (–2.3) μm; *conidia* 1-celled, clavate, ellipsoid to ovoid with truncate base and rounded apex, hyaline, smooth, (3.1–) 3.6–6.3 (–9.7) × (1.4–) 1.6–2.2 (–2.5) μm.

Hyalorhinocladiella-like: *conidiogenous cells* macronematous or semi-macronematous, mononematous, hyaline, simple or loosely branched, thin-walled, aseptate, (4.5–) 10.8–29.0 (–47) × (1.5–) 1.7–2.3 (–2.6) μm; *conidia* hyaline, clavate to ellipsoid, with obtuse ends, 1-celled, aseptate, smooth, (3.7–) 4.5–6.4 (–9.0) × (1.4–) 1.7–2.3 (–2.9) μm.

Culture characteristics. Colonies on 2% MEA in the dark reaching 90 mm in diam. in 6 days at 25 °C, growth rate up to 19.5 mm/day at the fastest; colony margin smooth. Mycelium superficial to flocculose or floccose, hyaline; reverse hyaline to pale yellowish. Optimal growth temperature 30 °C, slow growth at 40 °C, no growth at 5 °C.

Known substrate and hosts. Galleries of *Tomicus yunnanensis* and *T. minor* in *Pinus yunnanensis*.

Known insect vectors. *Tomicus yunnanensis*, *T. minor*.

Known distribution. Yunnan Province, China.

Additional specimens examined. CHINA, Yunnan, *Tomicus yunnanensis*, *T. minor* galleries in *Pinus yunnanensis*, Apr. 2017, HM Wang, CFCC 52632 = CXY 1940, CFCC 52633 = CXY 1944.

Note. *Graphilbum anningense* is characterised by a *Pesotum*-like and a *Hyalorhinocladiella*-like asexual state. It is phylogenetically closely related to *Gra. rectangulosporium*. However, *Gra. rectangulosporium* produced a sexual state *in vitro* (Ohtaka et al. 2006) which has not been observed in *Gra. anningense*. Other morphologically similar species include *Gra. fragrans*, *Gra. crescericum*, *Gra. kesiyae* and *Gra. puerense*. *Graphilbum kesiyae* and *Gra. puerense* also produce a *Pesotum*-like and a *Hyalorhinocladiella*-like asexual state. *Graphilbum anningense* and *Gra. kesiyae* differ by the size of their synnemata, whose length ranges do not overlap, *viz*. 210–293 µm and 112.5–173 µm long (Harrington et al. 2001), respectively. They also differ by their optimal growth temperature, respectively 30°C and 25°C. The synnemata of *Gra. puerense*, 206–357 µm long (Chang et al. 2017), are marginally longer than those of *Gra. anningense*. *Graphilbum fragrans* and *Gra. crescericum* produce only a *Leptographium*-like and/or a *Hyalorhinocladiella*-like asexual state *in vitro* (Harrington et al. 2001, Chang et al. 2017).

Graphilbum anningense was isolated from galleries of *T. yunnanensis* and *T. minor* infesting *P. yunnanensis*. Previously, *Gra. fragrans* had been reported from *T. yunnanensis* infesting *P. yunnanensis* and from *Pissodes* spp. infesting *Tsuga dumosa* and *P. armandii* in China (Paciura et al. 2010, Zhou et al. 2013). *Graphilbum kesiyae* and *Gra. puerense* were isolated from galleries of *Polygraphus aterrimus*, *Po. szemaoensis* and *Ips acuminatus* infesting *P. kesiya* (Chang et al. 2017). Although the geographic distribution of these four Graphilbum species overlaps, their hosts and vectors are nevertheless, as far as it is known, different (Chang et al. 2017).

Leptographium yunnanense **X.D. Zhou, K. Jacobs, M.J. Wingf. & M. Morelet, Mycoscience 41(6): 576. 2000.**
MycoBank MB 466542
Fig. 12

Description. Sexual form: unknown.

Asexual form: *Leptographium*-like. *Conidiophores* occurring singly or in groups of up to three, arising from the superficial mycelium, erect, macronematous, mononematous, (93.5–) 159–412 (–544) µm long, without rhizoid-like structures; *stipes* simple, cylindrical, not constricted at septa, 1-6-septate, pale olivaceous at the base, (12–) 19.0–128 (–245) × (3.3–) 4.1–6.1 (–7.3) µm; *conidiogenous apparatus* (33.0–) 65.5–119.5 (–168.0) µm long (high), with 2 to 3 series of cylindrical branches; *primary branches* hyaline to pale olivaceous, smooth, cylindrical, 2–3 septate, (11.5–) 18.2–37.7 (–56.0) µm long and (3.0–) 3.7–5.9 (–7.7) µm wide; *secondary branches* hyaline, 0–2 septate, (10.3–) 14.5–30.0 (–50.1) µm long, (2.8–) 3.4–5.5 (–7.3) µm wide; *conidiogenous cells* discrete, 2–3 per branch, cylindrical, (10.2–) 13.2–29.6 (–57.4) × (2.2–) 2.9–3.9 (–4.4) µm; *conidia* 1-celled, oblong to obovoid with truncate bases, hyaline, (5.8–) 7.0–10.4 (–13.0) × (2.9–) 3.6–5.3 (–6.4) µm.

Figure 12. Morphological characters of *Leptographium yunnanense* **A, B** upper and reverse of cultures on 2% MEA 8 d after inoculation **D, I** conidiophore on 2% MEA **C, E–H** conidiogenous cells of *Leptographium*-like asexual state and conidia. Scale bars: 50 μm (**D, I**); 10 μm (**C, E–H**).

Culture characteristics. Colonies on 2% MEA medium fast growing in the dark, reaching 76 mm in diam. in 8 days at 25 °C, growth rate up to 20 mm/day at the fastest; colony margin smooth. Hyphae submerged in agar with aerial mycelium, greenish-olivaceous to olivaceous, smooth, straight; reverse hyphae umber-brown to dark olivaceous. Optimal growth temperature 25 °C, slow growth at 5 °C and 30 °C.

Known substrate and hosts. *Tomicus yunnanensis* and its galleries in *Pinus yunnanensis*, galleries of *T. brevipilosus* in *P. kesiya*.

Known insect vectors. *Tomicus brevipilosus*, *T. yunnanensis*.

Known distribution. Yunnan Province, China.

Specimens examined. CHINA, Yunnan, adults of *Tomicus yunnanensis* and their galleries in *Pinus yunnanensis*, *Tomicus brevipilosus* galleries in *P. kesiya*. Apr. 2017, HM Wang, CFCC 52619 = CXY 1897, CFCC 52620 = CXY 1900, CFCC 52621 = CXY 1904, CFCC 52622 = CXY 1908, CFCC 52623 = CXY 1917, CFCC 52624 = CXY 1925.

Note. The sole reproductive structure formed on MEA in *L. yunnanense* is a *Leptographium*-like state. Our strains were identified as *L. yunnanense*, based on phylogenetic evidence and secondarily, on morphological features. However, our strains slightly deviated from *L. yunnanense* in having longer conidiophores, mainly 159–412 μm *vs* mostly 74–227 (–233) μm (Zhou et al. 2000) or 80–240 μm (Yamaoka et al. 2008). Furthermore, our strains grew faster than reported for the species, 76 mm *vs* 44 mm in 8 days at 25 °C (Zhou et al. 2000).

Although our strains were slightly genetically and morphologically divergent, we are of the opinion that they enter into the current *L. yunnanense* species concept (e.g. *sensu* Zhou et al. 2000). Yamaoka et al. (2008) showed the genetic diversity of *L. yunnanense* in Yunnan to be higher than in other places, that which is confirmed by the present study.

Leptographium yunnanense was originally described from Yunnan Province with only an asexual state (Zhou et al. 2000). Subsequently, mating of strains from different origins (Thailand, China and Japan) yielded the sexual state, which is formed by neckless ascocarps and cucullate ascospores (Yamaoka et al. 2008).

Leptographium yunnanense was the third most abundant species associated with *T. yunnanensis* in our study. A few strains also were isolated from *T. brevipilosus* infesting *P. kesiya* and none from *T. minor*.

Ophiostoma aggregatum **H. Wang, Q. Lu & Z. Zhang, sp. n.**
MycoBank MB828885
Fig. 13

Etymology. '*aggregatum*' (Latin), reflects to the conidiophores aggregated in clusters.

Type. CHINA, Yunnan, from *Tomicus minor* galleries in *Pinus yunnanensis*, Dec. 2016, HM Wang, holotype CXY 1876, culture ex-holotype CFCC 52615 = CXY 1876.

Description. Sexual form: unknown.

Asexual form: *Leptographium*-like. *Conidiophores* macronematous, mononematous, gathered in groups up to 5, (28.5–) 34–45.5 (–52) μm long; *stipes* cylindrical, 1–2 septate, not constricted at septa, umber-brown to dark olivaceous, (6.3–) 7.3–14.5 (–18) μm long × (2.2–) 3.1–4.6 (–5.8) μm wide. *Conidiogenous apparatus* (22–) 26.5–31 (–34) μm long, with 2–3 series of cylindrical branches; primary branches olivaceous, smooth, cylindrical all over, (5.9–) 7.2–13.5 (–20.5) × (3–) 3.3–4.2 (–4.6) μm; *conidiogenous cells* discrete, 2–3 per branch, aseptate, cylindrical, hyaline to pale umber, (5.8) 7.2–12.1 (–18.5) × (2.1–) 2.8–4.0 (–4.7) μm; *conidia* 1-celled, globose, elliptical with truncate bases, hyaline to pale umber, (4.0–) 4.8–5.9 (–6.3) × (3.1–) 4.0–5.0 (–5.6) μm.

Culture characteristics. Colonies on 2% MEA fast growing in the dark, reaching 90 mm in diam. in 8 days at 25 °C, growth rate up to 13 mm/day at the fastest; colony margin smooth. Hyphae submerged and aerial, umber-brown to dark olivaceous, flocculose or floccose; reverse hyphae umber-brown to dark olivaceous. Optimal growth temperature 25 °C, able to grow at 5 °C and 30 °C. No growth at 35 °C.

Known substrate and hosts. Galleries of *Tomicus yunnanensis* and *T. minor* in *Pinus yunnanensis*.

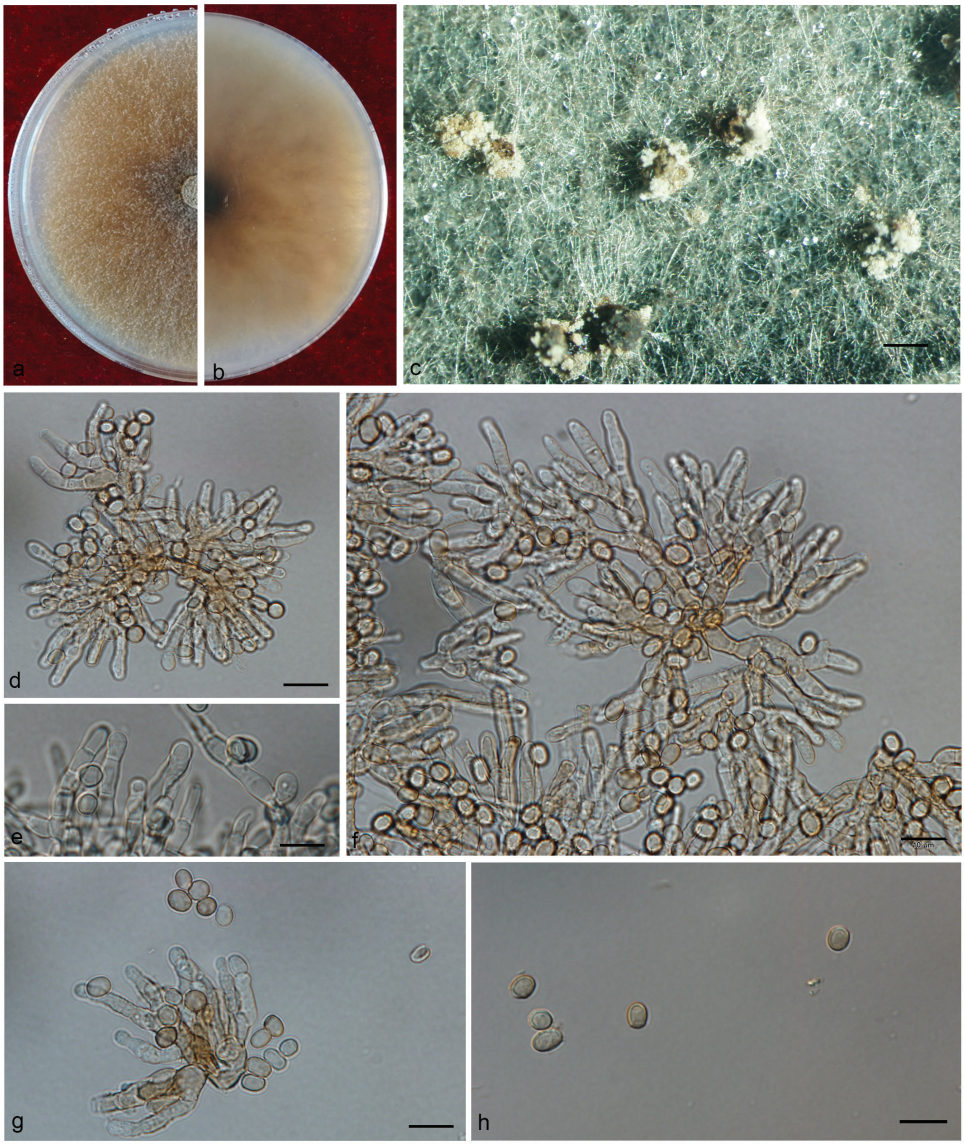


Figure 13. Morphological characters of *Ophiostoma aggregatum* sp. n. **A, B** upper and reverse of cultures on 2% MEA 8 d after inoculation **C** conidiomata on 2% MEA (bar = 50 μm) **D–H** conidiogenous cells of *Leptographium*-like asexual state and conidia. Scale bars: 20 μm (**C**); 10 μm (**D–H**).

Known insect vectors. *Tomicus minor*, *T. yunnanensis*.

Known distribution. Yunnan Province, China.

Additional specimens examined. CHINA, Yunnan, from *Tomicus yunnanensis* and *T. minor* galleries in *Pinus yunnanensis*, Dec. 2016, Apr. 2017, HM Wang, CFCC 52616 = CXY 1875, CFCC 52617 = CXY 1874.

Note. *Ophiostoma aggregatum* produced a single asexual, *Leptographium*-like state *in vitro*. This species is phylogenetically closely related to *O. macrosporum*, *O. tingens*,

O. floccosum, *O. tapionis* and *O. piliferum* in LSU-, ITS- and *TUB2*-based phylogenetic inferences. *Ophiostoma aggregatum* and *O. tingens* are shown to be sympatric in Yunnan pine forest; both taxa were isolated from galleries and adults of *T. minor* and *T. yunnanensis* infesting *P. yunnanensis* (Table 2). *Ophiostoma tingens* was also reported from *T. minor* infesting *P. yunnanensis* in Yunnan (Pan et al. 2017).

Ophiostoma aggregatum and *O. tingens* differ in their asexual state. *Ophiostoma aggregatum* only produces a *Leptographium*-like state. Inversely, the asexual states of *O. tingens* are variable. Our strains produced a *Pesotum*-like and a *Sporothrix*-like state whereas previously, Francke-Grosmann (1952) and de Beer et al. (2013b) reported a *Hyalorhinocladiella*- to *Raffaelea*-like state in European strains. The origin of this variability and its importance for taxonomy is uncertain.

Ophiostoma macrosporum, *O. floccosum*, *O. tapionis* and *O. piliferum* also differ from *O. aggregatum* by their asexual state. *Ophiostoma macrosporum* and *O. floccosum* produce a *Pesotum*-like asexual state, *O. tapionis* a *Hyalorhinocladiella*-like state and *O. piliferum* produces a *Sporothrix*-like state (Francke-Grosmann 1952, Upadhyay 1981, Yamaoka et al. 2004, Linnakoski et al. 2008).

Ophiostoma macrosporum and *O. tingens* were both originally described in *Trichosporium* as *T. tingens* var. *macrosporum* and *T. tingens* (Lagerberg 1927, Francke-Grosmann 1952). Batra (1967) transferred these two species into *Ambrosiella*. It is only recently that the morphological characteristics were found to agree with those of *Ophiostoma* (de Beer et al. 2013b). *Ophiostoma macrosporum* has been reported from various *Pinus* spp. (including *P. sylvestris*) infected by *Ips acuminatus* in Europe (Francke-Grosmann 1952, Batra 1967).

***Ophiostoma tingens* (Lagerb. & Melin) Z.W. de Beer & M.J. Wingf., Svensk Skogsvårdsförening Tidskr. 25:233. 1927.**

MycoBank: MB801091

Fig. 14

Description. Sexual form: unknown.

Asexual forms: *Pesotum*-like and *Sporothrix*-like. *Pesotum*-like: *conidiophores* macronematous, synnematous; synnemata simple, anchored into the substrate by brown rhizoid-like hyphae, (333–) 344–584 (–684) µm long including *conidiogenous apparatus*, the base dark brown, slightly widened, (16.7–) 17–50.5 (–65.5) µm wide, the apex cream-coloured or pale brown, slightly widening; *conidia* hyaline, globose to elliptical, 1-celled, smooth, (2.7–) 3.6–7.2 (–8.0) × (2.8–) 4.3–6.1 (–7.0) µm.

Sporothrix-like: *conidiophores* semi-macronematous, mononematous, hyaline, simple or loosely branched, smooth, bearing terminal denticulate *conidiogenous cells* (8.3–) 15.6–30.0 (–42.5) × (1.1–) 1.7–3.1 (–4.7) µm; *conidia* hyaline, globose to elliptical, obovoid with pointed bases and rounded apices, 1-celled, smooth, (2.6–) 4.0–6.8 (–8.7) × (2.2–) 3.5–5.5 (–7.4) µm.

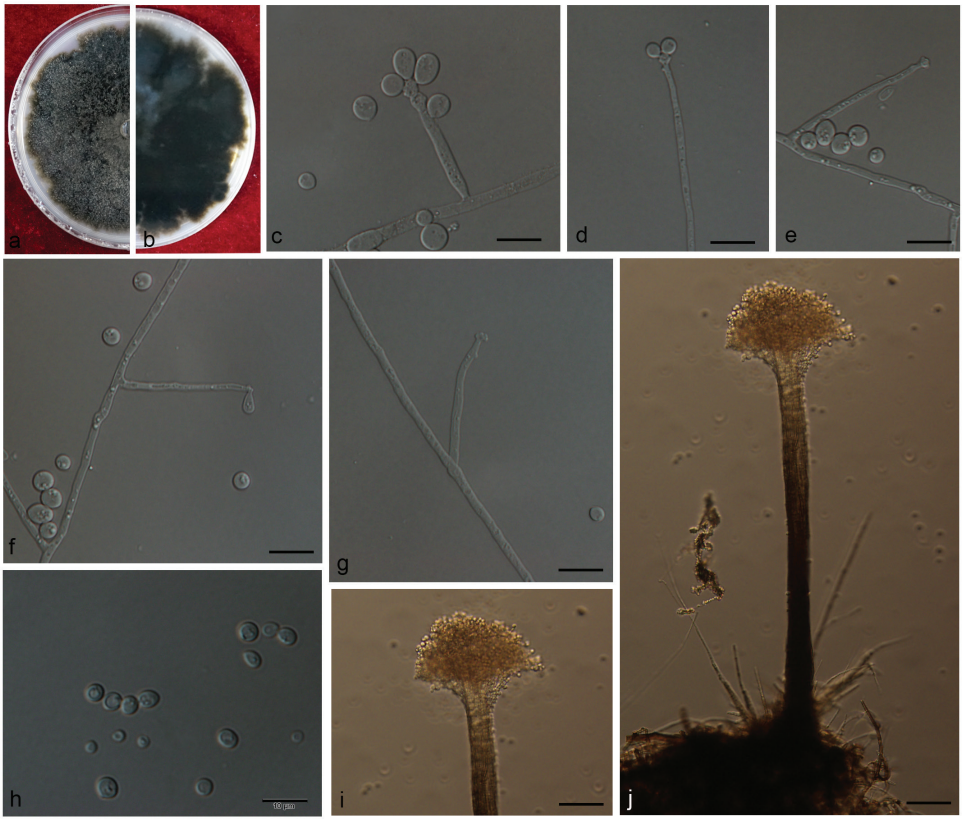


Figure 14. Morphological characters of *Ophiostoma tingens* **A, B** upper and reverse of cultures on 2% MEA 20 d after inoculation **C–G** conidiogenous cells of *Sporothrix*-like asexual state and conidia **H–J** conidiogenous cells of *Pesotum*-like macronematal asexual state and conidia. Scale bars: 10 μm (**C–H**); 50 μm (**I, J**).

Culture characteristics. Colonies on 2% MEA medium slow growing in the dark, reaching 39 mm in diam. in 8 days at 25 °C, growth rate up to 5 mm/day at the fastest; colony margin anomalous. Hyphae appressed to flocculose, black; reverse hyphae also black. Optimal growth temperature 25 °C, no growth at 5 °C and 30 °C.

Known substrate and hosts. Galleries of *Tomicus yunnanensis* and *T. minor* in *Pinus yunnanensis*.

Known insect vectors. *Tomicus yunnanensis*, *T. minor*.

Known distribution. Yunnan Province, China; Europe.

Specimens examined. CHINA, Yunnan, from *Tomicus minor* and *T. yunnanensis* galleries in *Pinus yunnanensis*, Feb. 2017, Nov. 2016, HM Wang, CFCC 52611 = CXY 1866, CFCC 52612 = CXY 1865, CFCC 52613 = CXY 1868.

Note. Our strains of *O. tingens* were identified based on phylogenetic affinities and morphological features. (cf. above under note for *O. aggregatum*.)

Ophiostoma tingens has been reported from sapwood of various *Pinus* spp. (including *P. sylvestris*) infested by *T. minor*, *T. piniperda* and *Ips sexdentatus* in Europe (Francke-Grosmann 1952, Batra 1967, Jankowiak 2008). The species was recorded in Yunnan Province in China in 2017, associated with *T. minor* infesting *P. yunnanensis* (Pan et al. 2017).

Sporothrix macroconidia **H. Wang, Q. Lu & Z. Zhang, sp. n.**
MycoBank: MB828886
Fig. 15

Etymology. '*macroconidia*' (Latin), referring to the large conidia of this fungus.

Type. CHINA, Yunnan, from *Tomicus yunnanensis* galleries in *Pinus yunnanensis*, Dec. 2016, collected by HM Wang, holotype CXY 1894, culture ex-holotype CFCC 52628 = CXY 1894.

Description. Sexual form: unknown.

Asexual form: *Sporothrix*-like. *Conidiophores* semi-macronematous, mononematous; *conidiogenous cells* hyaline, simple or loosely branched, thin-walled, aseptate, bearing denticles forming a rachis (4.1–) 11.0–24.5 (–36.5) × (1.4–) 2.1–3.4 (–4.9) μm; *conidia* hyaline, cylindrical, ellipsoid to ovoid, 1-celled, smooth, (3.6–) 4.8–7.4 (–9.9) × (2.5–) 3.2–4.9 (–9.9) μm, solitarily or aggregating in slimy masses.

Culture characteristics. Colonies on 2% MEA medium slow growing in the dark, reaching 34 mm in diam. in 8 days at 25 °C, growth rate up to 5 mm/day at the fastest; colony margin smooth. Hyphae appressed to flocculose, white; reverse hyaline to pale yellowish. Optimal growth temperature 25 °C, little growth at 5 °C and 35 °C.

Known substrates and hosts. Galleries of *Tomicus yunnanensis* and *T. brevipilosus* in *Pinus yunnanensis* and *P. kesiya*.

Known insect vectors. *Tomicus yunnanensis*, *T. brevipilosus*.

Known distribution. Yunnan Province, China.

Additional specimens examined. CHINA, Yunnan, from *Tomicus brevipilosus* galleries in *Pinus kesiya*, Dec. 2016, Jan. 2017, HM Wang, CFCC 52629 = CXY 1895, CFCC 52630 = CXY 1896.

Note. *Sporothrix macroconidia* is closely related to *O. valdivianum*, *S. bragantina*, *S. brunneoviolacea* and *S. fumea* in phylogenetic analyses inferred from LSU, ITS, *TUB2* and *CAL* DNA sequence data. It differs from these species by its conidia, which are larger than those of the other four species, mostly 4.8–7.4 × 3.2–4.9 μm and 4–6 × 2 μm in *O. valdivianum* (Butin and Aquilar 1984), 4–6 × 2–2.5 μm in *S. bragantina* (Pfenning and Oberwinkler 1993), 3–7 × 1.5–3 μm in *S. brunneoviolacea* (Madrid et al. 2010) and 1.5–2.0 × 0.5–1.0 μm in *S. fumea* (Nkuekam et al. 2012). In addition, a sexual state was observed *in vitro* for *O. valdivianum*, *S. bragantina* and *S. fumea*, which was not observed in *S. macroconidia* and *S. brunneoviolacea*.

Sporothrix macroconidia was found associated with *T. yunnanensis* infesting *P. yunnanensis* and with *T. brevipilosus* infesting *P. kesiya*. The other four similar species have

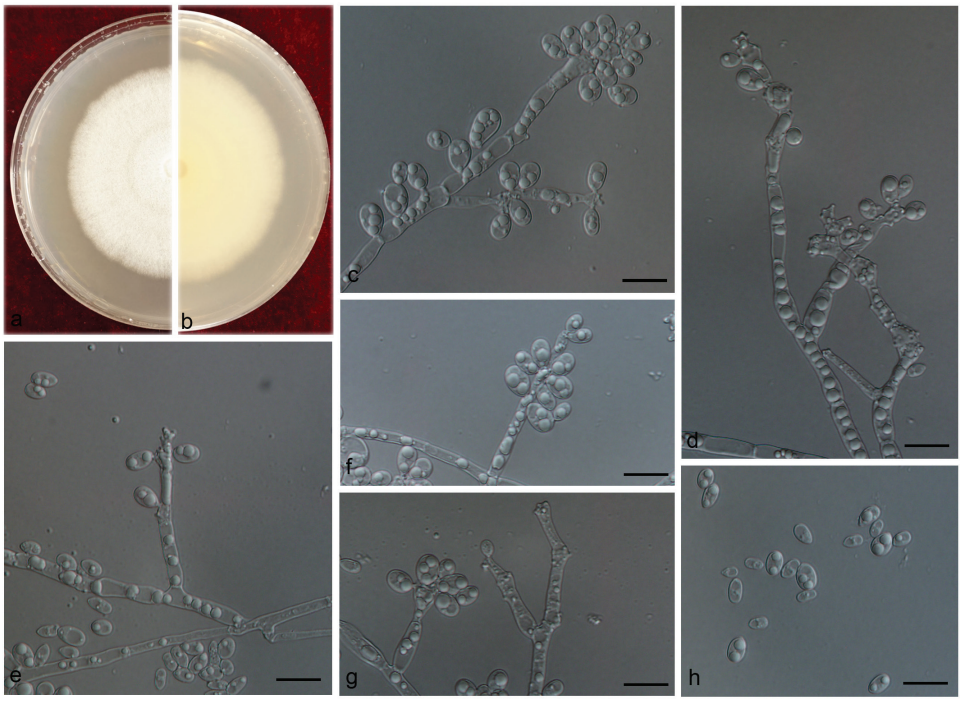


Figure 15. Morphological characters of *Sporothrix macroconidia* sp. n. **A, B** Upper and reverse of cultures on 2% MEA 20 d after inoculation **C–H** conidiogenous cells of *Sporothrix*-like asexual state and conidia. Scale bars: 10 μm (**C–H**).

very different ecology and known geographic distributions. *Sporothrix fumea* was isolated from *Eucalyptus cloeziana* infested by *Phoracantha* beetles in South Africa (Nkuekam et al. 2012), whereas *O. valdivianum*, *S. bragantina* and *S. brunneoviolacea* were obtained from soil or *Nothofagus* in Europe and South America (Butin and Aquilar 1984, Pfenning 1993, Madrid et al. 2010).

Sporothrix pseudoabietina **H. Wang, Q. Lu & Z. Zhang, sp. n.**
MycoBank: MB828887
Fig. 16

Etymology. '*pseudoabietina*' (Latin), referring to the phylogenetic affinities to *S. abietina*.

Type. CHINA, Yunnan, from *T. minor* galleries in *P. yunnanensis*, Apr. 2017, HM Wang, holotype CXY 1937, culture ex-holotype CFCC 52626 = CXY 1937.

Description. Sexual form perithecial: on 2% MEA, *perithecia* superficial or partially immersed, with a globose base extending into a cylindrical neck, often terminated by ostiolar hyphae; bases (85–) 110–152 (–168) μm diam., black, the outer layer with dark brown hyphal ornamentation; apical neck mild to dark brown at the

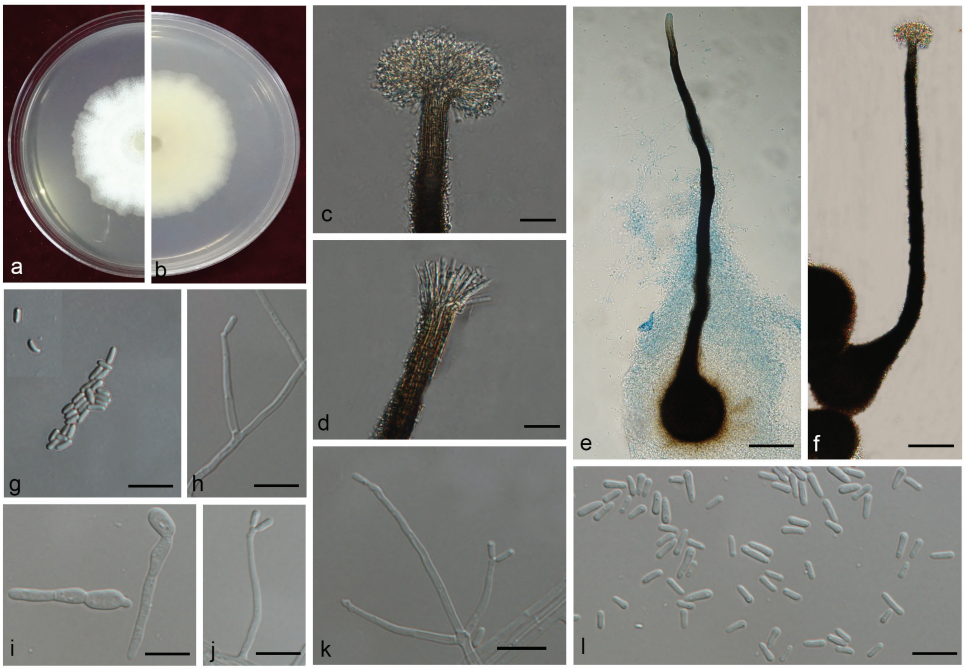


Figure 16. Morphological characters of *Sporothrix pseudoabietina* sp. n. **A, B** upper and reverse of cultures on 2% MEA 20 d after inoculation **C, D** ostiolar hyphae present **E, F** perithecium **G** ascospores of sexual state **H–I** conidiogenous cells of *Sporothrix*-like asexual state and conidia. Scale bars: 20 μm (**C, D**); 50 μm (**E, F**); 10 μm (**G–I**).

base, pale brown to pale yellow or hyaline toward the apex, straight or slightly curved, (172–) 560–985 (–1039) μm long, (37–) 41–62 (–78) μm wide at the base, (9.3–) 12.5–17.5 (–20) μm wide at the apex; *ostiolar* hyphae numerous, hyaline, divergent, (19.5–) 21.5–38.0 (–43) μm long; *asci* not seen; *ascospores* hyaline, 1-celled, orange-shaped in lateral view, ellipsoid in face view, circular in polar view, (2.9 –) 3.4–4.4 (–5.3) × (0.8–) 1.0–1.5 (–1.9) μm, without mucilaginous sheath.

Asexual form: *Sporothrix*-like. *Conidiophores* semi-macronematous to mononematous; *conidiogenous cells* hyaline, simple or loosely branched, smooth, bearing denticles disposed in a dense rachis (16.0–) 20.5–30.5 (–34.5) × (1.2–) 1.6–2.0 (–2.3) μm; *conidia* 1-celled, clavate, ellipsoid to ovoid, hyaline, (3.0–) 4.0–7.0 (–9.0) × (1.0–) 1.1–3.1 (–4.8) μm.

Culture characteristics. Colonies on 2% MEA slow growing in the dark, reaching 23 mm in diam. in 8 days at 25 °C, growth rate up to 2.5 mm/day at the fastest; colony margin smooth. Hyphae appressed to flocculose or floccose, white; reverse hyaline to pale yellowish. Optimal growth temperature 25 °C; very slow growth at 35 °C; no growth at 5 °C.

Known substrate and hosts. Galleries of *Tomicus yunnanensis* and *T. minor* in *Pinus yunnanensis*.

Known insect vectors. *Tomicus yunnanensis*, *T. minor*.

Known distribution. Yunnan Province, China.

Additional specimen examined. CHINA, Yunnan, *Tomicus minor* galleries in *Pinus yunnanensis*, Apr. 2017, HM Wang, CFCC 52627 = CXY 1938.

Note. *Sporothrix pseudoabietina* is characterised by a perithecial sexual form and a *Sporothrix*-like asexual state. Multiple phylogenetic inferences (LSU, ITS, *TUB2* and *CAL*) showed that *S. pseudoabietina* belonged to the *S. gossypina* complex, in which it is closely related to *S. abietina*. However, it can be distinguished from this species, based on both morphological and physiological features. The conidia of *S. pseudoabietina* (4.0–7.0 × 1.1–3.1 µm) are wider than those of *S. abietina* (4–7.5 × 1–2 µm) (Marmolejo and Butin 1990). Perithecia are known from *S. abietina* but only on natural substrates and not *in vitro* on artificial media, contrary to those from *S. pseudoabietina*. The perithecial neck in *S. pseudoabietina* is much longer than that of *S. abietina*, *viz*. mostly 560–985 µm and 450–650 µm, respectively. Ostiolar hyphae of *S. abietina* and *S. pseudoabietina* also differ in number, numerous *vs* 7–10 and size, mostly 13–19 µm and in *S. pseudoabietina* 21.5–38.0 µm (Fig. 11c, d). In addition, no growth of *S. abietina* was observed at 35 °C, but *S. pseudoabietina* can grow at 35 °C.

The hosts and geographic distributions of *S. pseudoabietina* and *S. abietina* are also very different. *Sporothrix pseudoabietina* was found associated with *T. minor* and *T. yunnanensis* infecting *P. yunnanensis*, whereas *S. abietina* was reported from *Abies vejari* attacked by *Pseudohylesinus* sp. in Mexico (Marmolejo and Butin 1990).

Discussion

In this study, 772 strains of ophiostomatoid fungi were isolated from galleries and adults of three pine shoot beetles, *T. brevipilosus*, *T. minor* and *T. yunnanensis*, inhabiting *P. yunnanensis* and *P. kesiya* in forests in Yunnan Province, south-western China. Multiple phylogenetic analyses and morphological features allowed the identification of 11 species from 5 genera. Six species corresponded to known taxa (*E. vermicola*, *L. yunnanense*, *O. brevipilosi*, *O. canum*, *O. minus* and *O. tingens*), whereas four species are proposed as new, *Gra. anningense*, *O. aggregatum*, *S. pseudoabietina* and *S. macroconidia*. A single strain remained unnamed.

The global ophiostomatoid fungal communities, associated with these three *Tomicus* species in pine forest, were dominated by far by three species, which are, in decreasing order of isolates, *O. canum*, *O. brevipilosi* and *O. minus*. Furthermore, these three ophiostomatoid species are not equally associated with the three *Tomicus* species but show variable degrees of preference or specificity.

Overall, *O. canum* was the most frequently isolated species in our study (253 out of the 772 strains). It was preferably (79.4% of the *O. canum* strains) isolated from galleries and adults of *T. minor*, infesting both *P. yunnanensis* and *P. kesiya* (Table 3) and dominated the ophiostomatoid community associated with this beetle (81.4%, 201 strains of *O. canum* out of 247 strains in the community, Table 3).

This is the first report of this species in China. It was previously reported in eastern Asia but only in Japan (Masuya et al. 1999). *Ophiostoma canum* was also shown to be

the dominant species associated with *T. minor*, both in Europe and Japan (Masuya et al. 1999, Jankowiak 2008). In addition, this species was found in association with other bark beetles in Finland and Russia, e.g. *Hylastes brunneus*, *Hylurgops palliatus*, *Ips typographus*, *Pityogenes chalcographus* and *Trypodendron lineatum* (Linnakoski et al. 2010). The close association between *O. canum* and *T. minor* appears stable over an extensive geographical distribution and tree host range, indicating likely intimate relationships.

Ophiostoma brevipilosi represented the second most frequently isolated species in our survey (224 out of 772 strains), occurring exclusively in galleries and adults of *T. brevipilosus*, dominating this beetle's ophiostomatoid community (98.2%, 224 strains of *O. brevipilosi* out of 228 strains in the community, Table 3). The occurrence or fitness of *O. brevipilosi* is therefore strongly linked to the presence of *T. brevipilosus*.

Ophiostoma brevipilosi was described originally from Yunnan, based on six strains, all isolated from *T. brevipilosus* (Chang et al. 2017). It belongs to the recently defined O. clavatum complex (Linnakoski et al. 2016). It is only known from this area of south-western China.

Ophiostoma minus was the third most frequently isolated species overall (197 strains out of 772), occurring exclusively in galleries and adults of *T. yunnanensis* infesting *P. yunnanensis*, dominating this beetle ophiostomatoid community (66.3%, 197 strains of *O. minus* out of 297 strains in the community, Table 3).

Ophiostoma minus, first reported as a blue-stain agent in Europe (Munch 1907), is a widely distributed species, also recorded from North America and East Asia (Japan and China) (Hedgcock 1906, Gorton and Webber 2000, Gorton et al. 2004, Lu et al. 2009, Linnakoski et al. 2010). It infests various pines and is transported by various bark beetles. This species was predominantly associated with *T. piniperda* in Europe (Jankowiak 2006) and Japan (Masuya et al. 1999) and with the southern pine beetle, *Dendroctonus frontalis*, in the southern states of the USA (Klepzig 1998, Gorton and Webber 2000, Gorton et al. 2004).

Ophiostoma minus was deemed to have two allopatric populations, *viz*. a North American and a Eurasian population (Gorton et al. 2004). In ITS/*TUB2* phylogenetic inferences, the North American and Eurasian populations of *O. minus* were resolved as two closely related clades (Gorton et al. 2004, Lu et al. 2009). ITS and *TUB2*-based phylogenetic inferences (Fig. 7c, d) also resolved our strains as a third distinct clade, which could thus be interpreted as a third allopatric population. The question of translating these populations into a Linnaean taxonomic rank, however, remains open.

Tomicus yunnanensis galleries and adult beetles harboured the highest diversity of ophiostomatoid fungi; ten of the 11 species identified were isolated from galleries and adults of this beetle. Three species were exclusively found with this beetle (*O. minus*, *E. vermicola*, *Ophiostoma* sp. 1). By comparison, galleries and adults of *T. minor* and of *T. brevipilosus* yielded less species; five species were isolated from *T. minor*, none of which was associated exclusively with this beetle and three species from *T. brevipilosus*, of which one was exclusive, *O. brevipilosi*. Five species are shared by both *T. yunnanensis* and *T. minor* and two species by both *T. yunnanensis* and *T. brevipilosus*, but none by *T. minor* and *T. brevipilosus* and also none by all three pine shoot beetles (Table 3, Fig. 17).

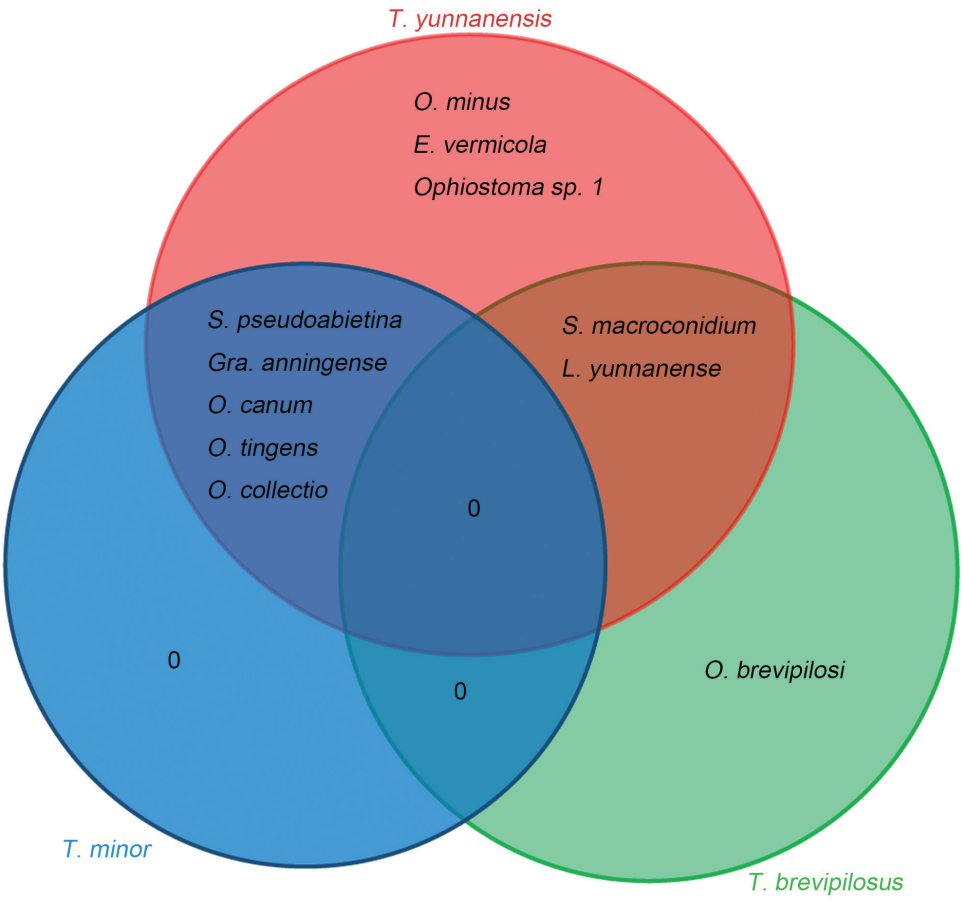


Figure 17. Venn diagram showing overlaps of the ophiostomatoid fungal communities associated with three pine shoot beetles.

The ectosymbiosis between bark beetles and fungi is widespread and diverse. Some fungi are highly specific and associated with a single beetle species, forming a 'species-specific association' (Six and Paine 1999, Six 2012), while others can be associated with many vectors (Kostovcik et al. 2014). The species-specific associations include, for instance, *Ips typographus* and *Endoconidiophora polonica*, *I. cembrae* and *End. laricicola* (Harrington et al. 2002) or *I. subelongatus* and *End. fujiensis* (Marin et al. 2005, Meng et al. 2015). The present study showed that species-specific associations might occur with various sympatric beetles that share the same niche. The association of *T. brevipilosus* and *O. brevipilosi* seems to be species-specific in the pine forest of Yunnan, where both taxa are, so far, endemic. In the pine forest of Yunnan, the Chinese 'population' of *O. minus* is also specifically associated with *T. yunnanensis*, whereas the two other *O. minus* 'populations' are associated, at least preferably, with *Dendroctonus frontalis* and *T. piniperda* (Gorton et al. 2004, Jankowiak 2006). The genetically distinct 'populations' might originate from both

the allopatric distribution and vector specificity and both factors could support recognition of three distinct taxa. In the pine forest of Yunnan, the association of *O. canum* with *T. minor* is preferential but not exclusive.

Up to now, no data have been provided proving the pathogenicity of these ophiostomatoid species to both indigenous pines, except for *L. yunnanense* (Liao and Ye 2004, Gao et al. 2017). Pathogenicity tests have been done by artificial inoculation of the dominant species into seedlings of the two pines. The results preliminarily showed that the virulence of *O. minus* and *O. brevipilosi* was significantly stronger than that of *O. canum*. This is similar to the relative aggressive nature of the three *Tomicus* species. Thus, we suspect there might be some link between beetle aggression and fungus virulence (Christiansen et al. 1987, Kirisits 2004).

Conclusions

This study provides evidence for the diversity of ophiostomatoid fungi associated with *T. yunnanensis*, *T. minor* and *T. brevipilosus* in Yunnan pine forest in south-western China. Eleven species were identified, of which four were new to science. The diversity is the highest in the galleries and adults of *T. yunnanensis* and the poorest in the galleries and adults of *T. brevipilosus*.

Three species, namely *O. brevipilosi*, *O. canum* and *O. minus*, dominate the ophiostomatoid communities; each is associated predominantly with one species of *Tomicus*, namely *T. brevipilosus*, *T. minor* and *T. yunnanensis*, respectively. In this regard, this study has revealed differential associations between beetles living sympatrically, concomitantly or sequentially, in the same ecological niche, which indicates a certain level of specificity of the relationships between the fungi and the beetles. However, the parameters behind these (partial) species-specific relationships remain unknown.

Increased study of the biodiversity, biogeography and ecology of ophiostomatoid fungi in China, in particular of those associated with *Tomicus* spp., would facilitate comparison with well-known species associated with other *Tomicus* spp. in other neighbouring or distant geographical areas, e.g. in European countries, Japan and Korea and allow a better understanding of the occurrence and mechanisms behind the outbreak of infections, enabling the development of effective management methods to alleviate the subsequent plant losses.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Project No.: 31770693 and 31770682). We are very grateful to Shuangcheng Li and Hongxun Wang for their help in field survey and collection. Cony Decock gratefully acknowledges the financial support received from the Belgian State–Belgian Federal Science Policy through the BCCM programme.

References

Batra LR (1967) Ambrosia fungi: a taxonomic revision, and nutritional studies of some species. Mycologia 59: 976–1017. https://doi.org/10.2307/3757271

Ben Jamaa ML, Lieutier F, Yart A, et al. (2007) The virulence of phytopathogenic fungi associated with the bark beetles *Tomicus piniperda* and *Orthotomicus erosus* in Tunisia. For. Pathol. 37: 51–63. https://doi.org/10.1111/j.1439-0329.2007.00478.x

Butin H, Aquilar M (1984) Blue-Stain Fungi on Nothofagus from Chile-Including Two New Species of *Ceratocystis* Ellis and Halst. Journal of Phytopathology 109(1): 80–89. https://doi.org/10.1111/j.1439-0434.1984.tb04233.x

Chang R, Duong TA, Taerum SJ, et al. (2017) Ophiostomatoid fungi associated with conifer-infesting beetles and their phoretic mites in Yunnan, China. MycoKeys 28: 19. https://doi.org/10.3897/mycokeys.28.21758

Chen P, Li L, Liu H (2009) Interspecific competition between *Tomicus yunnanensis* and *T. minor* (Col，Scolytidae) during shoot-feeding period in Yunnan of China. Journal of West China Forestry Science 38(3): 52–60.

Chen P, Li L, Liu H (2010) Host preference and competition in bark beetles, *Tomicus yunnanensis* and *T. minor* in breeding period. Journal of West China Forestry Science 39(1) 15–20.

Chen P, Lu J, Haack RA, Ye H (2015) Attack pattern and reproductive ecology of *Tomicus brevipilosus* (Coleoptera: Curculionidae) on *Pinus yunnanensis* in south-western china. Journal of Insect Science 15(1). https://doi.org/10.1093/jisesa/iev014

Christiansen E, Waring RH, Berryman AA (1987) Resistance of conifers to bark beetle attack: searching for general relationships. Forest Ecology and Management 22: 89–106. https://doi.org/10.1016/0378-1127(87)90098-3

de Beer ZW, Duong TA, Wingfield MJ (2016) The divorce of *Sporothrix* and *Ophiostoma*: solution to a problematic relationship. Studies in mycology 83: 165–191. https://doi.org/10.1016/j.simyco.2016.07.001

de Beer ZW, Wingfield MJ (2013) Emerging lineages in the Ophiostomatales. In: KA Seifert, ZW de Beer, MJ Wingfield. The Ophiostomatoid Fungi: Expanding Frontiers. Utrecht, The Netherlands CBS, 21–46.

de Beer ZW, Seifert KA, Wingfield MJ (2013a) The ophiostomatoid fungi: Their dual position in the sordariomycetes. In: Seifert KA, de Beer ZW, Wingfield MJ (Eds) The ophiostomatoid fungi: Expanding frontiers. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, 1–19.

de Beer ZW, Seifert KA, Wingfield MJ (2013b) A nomenclator for ophiostomatoid genera and species in the Ophiostomatales and Microascales. In: Seifert KA, de Beer ZW, Wingfield MJ (Eds) The ophiostomatoid fungi: Expanding frontiers. CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands, 245–322.

Francke-Grosmann H (1952) Uber die Ambrosiazucht der beiden Kiefernborkenka¨fer Myelophilus minor Htg. und *Ips acuminatus* Gyll. Medd. Statens Skogforskningsinst 41: 1–52

Gao X, Zhou X, Wang H, Kong, et al. (2017) Influence of severe drought on the resistance of *Pinus yunnanensis*, to a bark beetle-associated fungus. Forest Pathology 47(4): e12345. https://doi.org/10.1111/efp.12345

Gibbs JN, Inman A (1991) The pine shoot beetle *Tomicus piniperda* as a vector of blue stain fungi to windblown pine. Forestry 64: 239–249. https://doi.org/10.1093/forestry/64.3.239

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied Environmental Microbiology 61: 1323–1330. DOI: 10.0000/PMID7747954

Gorton C, Webber JF (2000) Reevaluation of the status of the blue-stain fungus and bark beetle associate *Ophiostoma minus*. Mycologia 92: 1071–1079. https://doi.org/10.2307/3761474

Gorton C, Kim SH, Henricot B, et al. (2004) Phylogenetic analysis of the blue-stain fungus *Ophiostoma minus* based on partial ITS rDNA and β-tubulin gene sequences. Mycological Research 108: 759–765. https://doi.org/10.1017/S0953756204000012

Harrington TC, McNew D, Steimel J, et al. (2001) Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 111–136. https://doi.org/10.2307/3761610

Harrington TC, Pashenova NV, McNew DL, et al. (2002) Species delimitation and host specialization of *Ceratocystis laricicola* and *C. polonica* to Larch and Spruce. Plant Disease 86: 418–422. https://doi.org/10.1094/PDIS.2002.86.4.418

Hedgcock GG (1906) Studies upon some chromogenic fungi which discolor wood. Missouri Botanical Garden Annual Report 17: 59–114. https://doi.org/10.2307/2400089

Jacobs K, Bergdahl DR, Wingfield MJ, et al. (2004) *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycol Res 108(4): 411–418. https://doi.org/10.1017/S0953756204009748

Jankowiak R (2006) Fungi associated with *Tomicus piniperda* in Poland and assessment of their virulence using Scots pine seedlings. Annals of forest science, 63(7): 801–808. https://doi.org/10.1051/forest:2006063

Jankowiak R (2008) Fungi associated with *Tomicus minor* on *Pinus sylvestris* in Poland and their succession into the sapwood of beetle-infested windblown trees. Canadian journal of forest research 38(10): 2579–2588. https://doi.org/10.1139/X08-101

Jankowiak R, Bilański P (2007) Fungal flora associated with *Tomicus piniperda* L. in an area close to a timber yard in southern Poland. Journal of applied entomology 131(8): 579–584. https://doi.org/10.1111/j.1439-0418.2007.01194.x

Ji M, Dong XQ, Liu HP, et al. (2007) Preliminary Study on Remote Sensing Detection of Yunnan Pine Forest Damaged by *Tomicus piniperda*. Journal of West China Forestry Science 36(1): 87–90.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular biology and evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kim JJ, Lim YW, Breuil C, et al. (2005) A new *Leptographium* species associated with *Tomicus piniperda* infesting pine logs in Korea. Mycological research 109(3): 275–284. https://doi.org/10.1017/S0953756204002060

Kirkendall LR, Faccoli M, Ye H (2008) Description of the Yunnan shoot borer, *Tomicus yunnanensis* Kirkendall and Faccoli sp. n.(Curculionidae, Scolytinae), an unusually aggressive pine shoot beetle from southern China, with a key to the species of *Tomicus*. Zootaxa 1819: 25–39.

Kirisits T (2004) Fungal associates of European bark beetles with special emphasis on the ophiostomatoid fungi. In: Lieutier F, Day KR, Battisti A, Grégoire JC, Evans HF (Eds) Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis. Kluwer Academic Publishers, The Netherlands, 181–235. https://doi.org/10.1007/978-1-4020-2241-8_10

Klepzig KD (1998) Competition between a biological control fungus, *Ophiostoma piliferum*, and symbionts of the southern pine beetle. Mycologia 90: 69–75. https://doi.org/10.1080/00275514.1998.12026880

Kubátová A, Novotný D, Prášil K, et al. (2000) The nematophagous hyphomycete *Esteya vermicola* found in the Czech Republic. Czech Mycology 52(3): 227–235.

Kostovcik M, Bateman CC, Kolarik M, et al. (2014) The ambrosia symbiosis is specific in some species and promiscuous in others: evidence from community pyrosequencing. Isme Journal 9(1): 126. https://doi.org/10.1038/ismej.2014.115

Lagerberg, T (1927) Biological and practical researches into blueing in pine and spruce. Sven Skogsvardsforen Tidskr 25, 145–272.

Li LS, Wang HL, Chai X, Shu N (1997) Study on the Biological Characteristics of *Tomicus piniperda* and Its Damage. Yunnan Forestry Science and Technology 2: 1–7.

Li LS, Liu HP, Chen P, et al. (2006) Comparison study on ecological characteristics of *Tomicus piniperda* and *T. minor*. Journal of West China Forestry Science 35(1): 1–5.

Li X, Zhang Z, Wang HB, et al. (2010) *Tomicus armandii* Li & Zhang (Curculionidae, Scolytinae), a new species from China. Zootaxa 2572:57–64.

Li Y, Huang YT, Kasson MT, et al. (2018) Specific and promiscuous ophiostomatalean fungi associated with Platypodinae ambrosia beetles in the southeastern United States. Fungal Ecology 35: 42–50. https://doi.org/10.1016/j.funeco.2018.06.006

Liao ZY, Ye H (2004) Action mechanisms of phytotoxin produced by *Leptographium yunnanense* associated with *Tomicus*. Forest Pest and Disease 23(1): 20–22.

Lieutier F (2002) Mechanisms of resistance in conifers and bark beetle attack strategies. In: Wagner MR, Clancy KM, Lieutier F, Paine TD (Eds) Mechanisms and Deployment of Resistance in Trees to Insects. Kluwer, Dordrecht, 31–75. https://doi.org/10.1007/0-306-47596-0_2

Lieutier F, Ye H, Yart A (2003) Shoot damage by *Tomicus* sp. (Coleoptera: Scolytidae) and effect on *Pinus yunnanensis* resistance to subsequent reproductive attacks in the stem. Agric. Forest Entomol 5: 227–233. https://doi.org/10.1046/j.1461-9563.2003.00182.x

Lieutier F, Yart A, Garcia J, et al. (1989) Champignons phytopathogènes associés à deux coléoptères scolytidae du pin sylvestre (*Pinus sylvestris* L.) et étude préliminaire de leur agressivité envers l'hôte, Ann. Sci. For. 46: 201–216 https://doi.org/10.1051/forest:19890301 [in French with English summary].

Lieutier F, Langstrom B, Faccoli M (2015) The genus *Tomicus*. In F. E. Vega and R. W. Hofstetter (eds.), Bark beetles: biology and ecology of native and invasive species. Elsevier, Amsterdam, The Netherlands, 371–426. https://doi.org/10.1016/B978-0-12-417156-5.00010-1

Linnakoski R, Jankowiak R, Villari C, Kirisits T, Solheim H, et al. (2016). The *Ophiostoma clavatum* species complex: a newly defined group in the Ophiostomatales including three novel taxa. Antonie van Leeuwenhoek 109: 987–1018. https://doi.org/10.1007/s10482-016-0700-y

Linnakoski R, Beer ZWD, Niemelä P, et al. (2012) Associations of conifer-infesting bark beetles and fungi in Fennoscandia. Insects 3(1): 200–227. https://doi.org/10.3390/insects3010200

Linnakoski R, de Beer ZW, Ahtiainen J, et al. (2010) *Ophiostoma* spp. associated with pine-and spruce-infesting bark beetles in Finland and Russia. Persoonia: Molecular Phylogeny and Evolution of Fungi 25: 72. https://doi.org/10.3390/insects3010200

Linnakoski R, de Beer ZW, Rousi M, et al. (2008) Fungi, including *Ophiostoma karelicum* sp. n. associated with *scolytus ratzeburgi* infesting birch in finland and russia. Mycological Research 112(12): 1475–1488. https://doi.org/10.1016/j.mycres.2008.06.007

Liou JY, Shih JY, Tzean SS (1999) *Esteya*, a new nematophagous genus from Taiwan, attacking the pinewood nematode (*Bursaphelenchus xylophilus*). Mycological Research 103(2): 242–248. https://doi.org/10.1017/S0953756298006984

Lu M, Zhou XD, De Beer ZW, et al. (2009) *Ophiostomatoid* fungi associated with the invasive pine-infesting bark beetle, *Dendroctonus valens*, in China. Fungal Diversity 38: 133-145.

Lu J, Zhao T, Ye H, et al. (2014) The shoot-feeding ecology of three *Tomicus* species in Yunnan Province, southwestern China. Journal of Insect Science 14: 37. https://doi.org/10.1093/jis/14.1.37

Lu RC, Wang HB, Zhang Z, et al. (2012) Coexistence and competition between *Tomicus yunnanensis* and *T. minor* (coleoptera: scolytinae) in yunnan pine. Psyche A Journal of Entomology 2012(3): S17. https://doi.org/10.1155/2012/185312

Madrid H, Gené J, Cano J, et al. (2010) *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 102(5): 1193–1203. https://doi.org/10.3852/09-320

Marmolejo JG, Butin H (1990) New conifer-inhabiting species of *Ophiostoma* and *Ceratocystiopsis* (Ascomycetes, Microascales) from Mexico. Sydowia 42: 193–199.

Marin M, Preisig O, Wingfield BD, et al. (2005) Phenotypic and DNA sequence data comparisons reveal three discrete species in the *Ceratocystis polonica* species complex. Mycological Research 109(10): 1137–1148. https://doi.org/10.1017/S095375620500362X

Masuya H, Kaneko S, Yamaoka Y, et al. (1999) Comparisons of ophiostomatoid fungi associated with *Tomicus piniperda* and *T. minor* in Japanese red pine. Journal of Forest Research 4(2): 131–135. https://doi.org/10.1007/BF02762237

Mathiesen A (1950) Über einige mit Borkenkäfern assoziierten Bläuepilze in Schweden. Oikos 2: 275–308. https://doi.org/10.2307/3564798

Mathiesen A (1951) Einige neue Ophiostoma-Arten in Schweden. Sven. Bot. Tidskr 45: 203–232.

Mathiesen-Käärik A (1953) Eine Übersicht über die gewöhnlichsten mit Borkenkäfern assoziierten Bläuepilze in Schweden und einige für Schweden neue Bläuepilze, Meddn. St. Skogforsk Inst, 431–74.

Meng XJ, Lu Q, Liu XW, et al. (2015) The species specific associations between *Ips subelongatus* and ophiostomatoid fungi. Acta Ecologica Sinica 35(2): 313–323.

Munch E (1907) Die Blaufaule des Nadelholzes. Naturwiss. Ztschr. F. Forst .U.Landw 5: 531–573.

Nkuekam G K, de Beer Z W, Wingfield M J, et al. (2012) A diverse assemblage of *Ophiostoma* species, including two new taxa on eucalypt trees in South Africa. Mycological progress 11(2): 515–533. https://doi.org/10.1007/s11557-011-0767-9

Ohtaka N, Masuya H, Yamaoka Y, et al. (2006) Two new *Ophiostoma* species lacking conidial states isolated from bark beetles and bark beetle-infested Abies species in Japan. Botany 84(2): 282–293. https://doi.org/10.1139/b05-164

Paciura D, Zhou XD, de Beer ZW, et al. (2010) Characterisation of synnematous bark beetle-associated fungi from China, including *Graphium carbonarium* sp. n. Fungal Diversity 40(1): 75–88. https://doi.org/10.1007/s13225-009-0004-x

Pan Y, Chen P, Lu J, et al. (2017) First report of blue-stain in *Pinus yunnanensis* caused by *Ophiostoma tingens* associated with *Tomicus minor* in china. Journal of Plant Pathology 99(3): 805.

Pfenning L, Oberwinkler F (1993) *Ophiostoma bragantinum* n. sp., a possible teleomorph of *Sporothrix inflata*, found in Brazil. Mycotaxon 46: 381–385.

Rayner RW (1970) A mycological colour chart. CMI and British Mycological Society, Kew, Surrey, UK.

Rennerfelt E (1950) Uber den Zusammenhang zwischen dem Verblauendes Holzes und den Insekten. Oikos 2: 120–137. https://doi.org/10.2307/3564666

Seifert KA, Webber JF, Wingfield MJ (1993) Methods for studying species of *Ophiostoma* and *Ceratocystis*. *Ceratocystis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. Minnesota, U.S.A., The American Phytopathological Society Press, 255–259.

Sabbatini Peverieri G, Capretti P, Tiberi R (2006) Associations between *Tomicus destruens* and *Leptographium* spp. in *Pinus pinea* and *P. pinaster* stands in Tuscany, central Italy. For. Pathol 36, 14–20. https://doi.org/10.1111/j.1439-0329.2006.00427.x

Six DL (2003) Bark beetle-fungus symbiosis. In: Bourtzis K, Miller TA (Eds) Insect Symbiosis. CRC Press, Washington D.C., 97–114. https://doi.org/10.1201/9780203009918.ch7

Six DL (2012) Ecological and Evolutionary Determinants of Bark Beetle–Fungus Symbioses. Insects 3: 339–366. https://doi.org/10.3390/insects3010339

Six DL, Paine TDA (1999) Phylogenetic comparison of ascomycete mycangial fungi and *Dendroctonus* bark beetles (Coleoptera: Scolytidae). Ann Entomol Soc Am 92: 159–166. https://doi.org/10.1093/aesa/92.2.159

Six DL, Wingfield MJ (2011) The role of phytopathogenicity in bark beetle-fungus symbioses: a challenge to the classic paradigm. Annu Rev Entomol 56: 255–272. https://doi.org/10.1146/annurev-ento-120709-144839

Solheim H, Långström B (1991) Blue-stain fungi associated with *Tomicus piniperda* in Sweden and preliminary observations on their pathogenicity, Ann. Sci. For. 48: 149–156. https://doi.org/10.1051/forest:19910203

Solheim H, Krokene P, Långström B (2001) Effects of growth and virulence of associated blue-stain fungi on host colonization behavior of the pine shoot beetles *Tomicus minor* and *T. piniperda*. Plant Pathol 50: 111–16. https://doi.org/10.1046/j.1365-3059.2001.00541.x

Stamatakis A (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Swofford DL (2003) PAUP*: phylogenetic analysis using parsimony, Version 4. Sinauer Associates, Sunderland, MA. https://doi.org/10.1007/BF02198856

Tamura K, Peterson D, Peterson N, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739. https://doi.org/10.1093/molbev/msr121

Upadhyay HP (1981) A monograph of *Ceratocystis* and *Ceratocystiopsis*. Univ. Georgia Press. https://doi.org/10.1007/BF02858713

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Wang CY, Fang ZM, Wang Z, et al. (2009) High infection activities of two *Esteya vermicola* isolates against pinewood nematode. African Journal of Microbiology Research 3(10): 581–584. https://doi.org/10.1007/s12275-007-0122-7

Wang X, Wang T, Wang J, et al. (2014) Morphological, molecular and biological characterization of *Esteya vermicola*, a nematophagous fungus isolated from intercepted wood packing materials exported from Brazil. mycoscience 55(5): 367–377. https://doi.org/10.1016/j.myc.2014.01.002

Wingfield M J, Garnas J R, Hajek A, et al. (2016) Novel and co-evolved associations between insects and microorganisms as drivers of forest pestilence. Biological invasions 18(4): 1045–1056. https://doi.org/10.1007/s10530-016-1084-7

Wingfield MJ, Barnes I, De Beer ZW, et al. (2017) Novel associations between ophiostomatoid fungi, insects and tree hosts: current status–future prospects. Biological Invasions: 1–14. https://doi.org/10.1007/s10530-017-1468-3

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and application. Academic Press, San Diego, USA, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yamaoka Y, Masuya H, Ohtaka N, et al. (2004) Three new *Ophiostoma* species with *Pesotum* anamorphs associated with bark beetles infesting Abies species in Nikko, Japan. Mycoscience 45(4): 277–286. https://doi.org/10.1007/S10267-004-0179-7

Yamaoka Y, Chung WH, Masuya H, et al. (2008) The teleomorph of *Leptographium yunnanense*, discovered in crosses among isolates from Thailand, China, and Japan. Mycoscience 49(4): 233–240. https://doi.org/10.1007/S10267-008-0412-X

Yin M, Wingfield MJ, Zhou X, et al. (2016) Multigene phylogenies and morphological characterization of five new *Ophiostoma* spp. associated with spruce-infesting bark beetles in China. Fungal biology 120(4): 454–470. https://doi.org/10.1016/j.funbio.2015.12.004

Ye H (1991) On the bionomy of *Tomicus piniperda* (L.) (Coleoptera：Scolytidae)in the Kunming region of China. Journal of Applied Entomology 112: 366–369. https://doi.org/10.1111/j.1439-0418.1991.tb01069.x

Ye H, Zhou X, Lu J, Yang L, Ding H (2000) A preliminary study on the occurrence and pathogenicity of the fungi associated with *Tomicus*. Forest Research, Beijing 13: 451–454.

Ye H (2011) *Tomicus yunnanensis*. Kunming: Yunnan Science and Technology Publishing Press.

Ye H, Dang CL (1986) The study on the feature of the bark beetle (*Blastophagus piniperda* L.) injuring the pine tree (*Pinus yunnanensis*). Journal of Yunnan University 8(2): 218–221.

Ye H, F Lieutier (1997) Shoot aggregation by *Tomicus piniperda* L (Col: Scolytidae) in Yunnan, southwestern China. Ann. Forest Sci 54: 635–641. https://doi.org/10.1051/forest:19970704

Ye H, Ding XS (1999) Impacts of *Tomicus minor* on distribution and reproduction of *Tomicus piniperda* (Col., Scolytidae) on the trunk of the living *Pinus yunnanensis* trees. J. Appl. Entomol 123: 329–333. https://doi.org/10.1046/j.1439-0418.1999.00353.x

Zhang Y, Hagen F, Stielow B, et al. (2015) Phylogeography and evolutionary patterns in *Sporothrix* spanning more than 14 000 human and animal case reports. Persoonia 35: 1–20. https://doi.org/10.3767/003158515X687416

Zhou XD, de Beer ZW, Wingfield MJ (2013) Ophiostomatoid fungi associated with conifer infecting bark beetles in China. In: Seifert KA, de Beer ZW, Wingfield MJ (Eds) Ophiostomatoid fungi: Expanding frontiers. CBS, Utrecht, The Netherlands, 91–98.

Zhou XD, Jacobs K, Wingfiled MJ, et al. (2000) A new *Leptographium* species associated with *Tomicus piniperda* in South Western China. Mycoscience 41: 573–578. https://doi.org/10.1007/BF02460923

Zipfel RD, Beer ZW de, Jacobs K, et al. (2006) Multigene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Studies in Mycology 55: 75–97. https://doi.org/10.3114/sim.55.1.75