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Six new species of *Sporothrix* from hardwood trees in Poland

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Abstract

Sporothrix (*Sordariales*, *Ascomycota*) is a well-supported monophyletic lineage within the *Ophiostomatales*, species of which occur in a diverse range of habitats including on forest trees, in the soil, associated with bark beetles and mites as well as on the fruiting bodies of some *Basidiomycota*. Several species have also been reported as important human and animal pathogens. During surveys of insect- and wound-associated *Ophiostomatales* from hardwood trees in Poland, many isolates with affinity to *Sporothrix* were recovered. In the present study, six undescribed *Sporothrix* spp. collected during these surveys are characterized based on their morphological characteristics and multi-locus phylogenenetic inference. They are described as *Sporothrix cavum*, *Sporothrix cracoviensis*, *S. cryptarchum*, *S. fraxini*, *S. resoviensis*, and *S. undulata*. Two of the *Sporothrix* spp. reside in the *S. gossypina*-complex, while one forms part of the *S. stenoceras*-complex. One *Sporothrix* sp. is a member of lineage F, and two other species grouped outside any of the currently defined species complexes. All the newly described species were recovered from hardwood habitats in association with sub-cortical insects, wounds or woodpecker cavities. These species were morphologically similar, with predominantly asexual states having hyaline or lightly pigmented conidia, which produce holoblastically on denticulate conidiogenous cells. Five of the new taxa produce ascomata with necks terminating in long ostiolar hyphae and allantoid ascospores without sheaths. The results suggest that *Sporothrix* species are common members of the *Ophiostomatales* in hardwood ecosystems of Poland.

Keywords

6 new species, bark beetle-associated fungi, *Ophiostomatales*, phylogeny, tree wounds

Introduction

Sporothrix was established by Hektoen and Perkins (1900) based on the morphological description of the human pathogen, *Sporothrix schenckii*. Species of *Sporothrix* (*Ascomycota*, *Ophiostomatales*, *Ophiostomataceae*) were first accommodated in *Sporotrichum* (De Beurmann and Gougerot 1911). Until the latter half of the 20th century, these fungi were also treated in various other genera, including *Cephalosporium*, *Cladosporium* (Hedgcock 1906; Münch 1907; Lagerberg et al. 1927; Melin and Nannfeldt 1934; Siemaszko 1939; Davidson 1942; Bakshi 1950; Mathiesen-Käärik 1953; Hunt 1956), *Cylindrocephalum*, *Hormodendron* (Robak 1932), *Hyalodendron* (Goidànich 1935; Georgescu et al. 1948), and *Rhinotrichum* (Georgescu et al. 1948; Sczerbin-Parfenenko 1953), in order to accommodate the asexual morphs of *Ophiostoma*. de Hoog (1974) published a monograph of the *Sporothrix* species and proposed the placement of *S. schenckii* as the asexual morph of *O. stenoceras*. That monograph expanded the concept of *Sporothrix* and included new *Sporothrix* species causing human infections as well as those associated with wood and bark beetles.

de Hoog et al. (1985) recognized that *Sporothrix* is not a homogenous group. As DNA sequencing technology was applied to resolve taxonomic relationships for fungi, evidence emerged that *S. schenckii* is phylogenetically related to species of *Ophiostoma* (Berbee and Taylor 1992; Hausner et al. 1993, 2000). In these studies, species producing only sporothrix-like asexual states were treated as members of the *S. schenckii*–*O. stenoceras* complex in *Ophiostoma sensu lato* (De Beer et al. 2003; Villarreal et al. 2005; Roets et al. 2006; Zipfel et al. 2006; De Meyer et al. 2008; Linnakoski et al. 2010; Kamgan Nkuekam et al. 2012). The genus *Sporothrix* was recently redefined and emended based on the analysis of partial 18S and 28S rDNA sequences for species in the *Ophiostomatales* (De Beer et al. 2016). *Sporothrix* was consequently separated from species of *Ophiostoma* and various complexes were defined within *Sporothrix*. *Sporothrix* is now defined as one of nine relatively clearly defined genera in the *Ophiostomataceae* (De Beer and Wingfield 2013; De Beer et al. 2013a, 2013b, 2016).

As currently recognized, *Sporothrix* includes 56 species (De Beer et al. 2016; Ngubane et al. 2018; Wang et al. 2019; Musvuugwa et al. 2020), which are characterized by their dark brown to black, globose ascomata with elongated necks up to 1600 µm, occasionally terminating in an ostiole, often surrounded by ostiolar hyphae. Ascospores are usually curved and lunate to reniform, without a sheath (De Beer and Wingfield 2013). The asexual states have conidiophores that proliferate sympodially and produce hyaline or occasionally pigmented conidia on denticulate conidiogenous cells (De Beer and Wingfield 2013).

Sporothrix includes a large assemblage of species that are widely distributed across various climatic zones of the world (De Beer and Wingfield 2013; De Beer et al. 2016). Species also occupy a wide range of habitats. The greatest numbers of species are found on bark, in the infructescences of *Protea* spp. and on the wood of different forest trees (e.g., Roets et al. 2008, 2009, 2013; De Errasti et al. 2016). Other species have been described from soil, bark beetles, ambrosia beetles, mites, and from the fruiting bodies

of basidiomycetes (e.g., Constantinescu and Ryman 1989; Marmolejo and Butin 1990; De Meyer et al. 2008; Roets et al. 2008; De Errasti et al. 2016). Several species are also well-known as human and animal pathogens (Travassos and Lloyd 1980; Summerbell et al. 1993; Barros et al. 2004; Lòpez-Romero et al. 2011; Zhang et al. 2015).

Jankowiak et al. (2019a) conducted the first extensive survey of fungal associates of hardwood-infesting bark and ambrosia beetles in Poland. In the same year, *Ophiostomatales* associated with wounds on hardwood trees were also studied in Poland (Jankowiak et al. 2019b). These studies reported several *Sporothrix* species, which were apparently new to science, but names were not provided for them. In addition, one unknown *Sporothrix* species was isolated from cavities of woodpeckers in Poland (Jankowiak et al. 2019c). In this study, morphological characters and DNA sequence data for the ITS region (ITS1–5.8S–ITS2) and three protein coding genes (β-tubulin, calmodulin, translation elongation factor 1-α) were analyzed to characterize six new species of *Sporothrix*. These were compared with closely related known species and formal descriptions have been provided for them.

Materials and methods

Fungal isolates

The collection details for the isolates included in the present study (Table 1) are provided in previous studies (Jankowiak et al. 2019a, 2019b, 2019c). The cultures are maintained in the culture collection of the Department of Forest Ecosystems Protection, University of Agriculture in Krakow, Poland, and in the culture collection of the Natural Resources Institute Finland (Luke), Helsinki, Finland. The ex-type isolates and representative isolates of the new species described were deposited in the culture collection (**CBS**) of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands. Dried cultures were deposited as holotype specimens in the Mycological Herbarium (**O**), Natural History Museum, University of Oslo, Norway.

Microscopy and growth studies

Morphological characters were examined for selected isolates as well as for the herbarium specimens selected as types. Cultures were grown on 2% Malt Extrat Agar (**MEA**) made up of 20 g Bacto malt extract, 20 g agar Bacto agar powder (Becton Dickinson and Company, Franklin Lakes, USA) in 1 l deionized water. In attempts to induce the formation of ascomata, autoclaved twigs of host trees including the bark were placed at the centres of agar plates containing MEA. Fungal cultures were derived from single spores. To promote the production of ascomata, single conidial isolates were crossed in all possible combinations, following the technique described by Grobbelaar et al. (2009). These cultures were incubated at 25 °C and monitored regularly for the appearance of fruiting structures.

Table 1. Isolates used in the present study.

Fungal species	Previous identification[A]	Isolate no.			Source	Site	GenBank accessions[E]			
		CBS[B]	O-F[C]	KFL=NRFI[D]			ITS1-5.8S-ITS2	βT	TEF 1-α	CAL
Sporothrix cracoviensis sp. nov	*Sporothrix* sp. 7	CBS 147940		KFL17FRJTD	Adult of *Trypodendron domesticum* on *Fagus sylvatica*	Krzeszowice	MH283148	MH283365	MH283500	MH283526
		CBS 147939		KFL2114bRJTD	Adult of *Trypodendron domesticum* on *Fagus sylvatica*	Krzeszowice	MH283149	MH283366	MH283501	MH283527
		CBS 147941	O-F-258629	KFL2514aRJTD[F]	Adult of *Trypodendron domesticum* on *Fagus sylvatica*	Krzeszowice	**MW768963**	MH283367	MH283502	MH283528
		CBS 147942[ET]	O-F-258628	KFL2514bRJTD	Adult of *Trypodendron domesticum* on *Fagus sylvatica*	Krzeszowice	**MW768964**	MH283368	MH283503	MH283529
Sporothrix fraxini sp. nov	*Sporothrix* sp. 8	CBS 147936[ET]	O-F-258630	KFL21BS16bRJHV	Gallery of *Hylesinus varius* on *Fraxinus excelsior*	Zbylitowska Góra	MH283150	MH283370	MH283504	MH283530
		CBS 147938[F]	O-F-258631	KFL21BS16dRJHV	Gallery of *Hylesinus varius* on *Fraxinus excelsior*	Zbylitowska Góra	**MW768968**	MH283371	**MW768973**	MH283531
		CBS 147937		KFL21BS16cRJHV	Gallery of *Hylesinus varius* on *Fraxinus excelsior*	Zbylitowska Góra	MH283151	MH283372	MH283505	MH283532
Sporothrix resoviensis sp. nov	*Sporothrix* sp. 10	CBS 147927[ET]	O-F-258632	KFL204ABRZN16AO	Wound on *Betula pendula*	Borownica	MH740962	MH741100	MH741189	MH741228
Sporothrix cryptarchum sp. nov.	*Sporothrix* sp. 11			KFL1097NOL16RJ	Wound on *Alnus incana*	Wierzchosławice	MH740963	MH741101	MH741190	MH741229
				KFL1146NDB16RJ	Wound on *Quercus robur*	Ispina	MH740964	MH741102	MH741191	MH741230
		CBS 147935		KFL48716NDBRJ	Wound on *Quercus robur*	Wierzchosławice	**MW768967**	MH741103	MH741192	**MW768977**
		CBS 147934[ET]	O-F-258633	KFL410DB16bRJCU	Adult of *Cryptarcha undata*	Wierzchosławice	**MW768966**	MH741104	MH741193	MH741231
		CBS 147933[E]	O-F-258634	KFL404DB16aRJCU	Adult of *Cryptarcha undata*	Wierzchosławice	**MW768965**	MH741105	MH741194	MH741232
Sporothrix undulata sp. nov.	*Sporothrix* sp. 12	CBS 147931[E]	O-F-258636	KFL13NDB15bRJ	Wound on *Quercus robur*	Wierzchosławice	MH740965	MH741106	**MW768974**	**MW768978**
		CBS 147930		KFL12NDBCZ15RJ	Wound on *Quercus rubra*	Wierzchosławice	MH740967	MH741108	MH741196	**MW768979**
		CBS 147928		KFL221NBK16RJ	Wound on *Fagus sylvatica*	Czajowice	MH740970	MH741112	MH741199	MH741235
		CBS 147932		KFL430NDB16RJ	Wound on *Quercus robur*	Ispina	MH740971	MH741113	MH741200	MH741236
				KFL1099NOLCZ16RJ	Wound on *Alnus incana*	Wierzchosławice	MH740973	MH741115	MH741202	MH741237
				KFL1140NDB16bRJ	Wound on *Quercus robur*	Ispina	MH740975	MH741117	MH741203	MH741238
				KFL6117NWB17RJ	Wound on *Salix fragilis*	Babimost	**MW768970**	MH741119	MH741204	**MW768980**
		CBS 147929[ET]	O-F-258635	KFL398DB16RJEG	Adult of *Epuraea guttata*	Wierzchosławice	MH740976	MH741121	MH741205	MH741239
				KFL404DB16bRJCU	Adult of *Cryptarcha undata*	Wierzchosławice	**MW768969**	MH741124	MH741208	MH741242
Sporothrix cavum sp. nov	*Sporothrix* sp. 18	CBS 147943[ET]	O-F-258637	KFL42215aDRJ	Cavity of *Dendrocopos major* on *Salix fragilis*	Kraków	MF782813	MF782850	**MW768972**	**MW768976**
			O-F-258638	KFL35614DRJ[F]	Cavity of *Dendrocopos medius* on *Malus domestica*	Książ Wielki	MF782814	MF782851	**MW768971**	**MW768975**

[A] Isolates collected and identified during previous surveys in Poland (Jankowiak et al. 2019a, 2019b, 2019c). *Sporothrix* sp. 18 in the study of Jankowiak et al. (2019c) was labelled as *Sporothrix* sp.
[B] CBS Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands.
[C] Herbarium of the Natural History Museum, University of Oslo, Norway,
[D] KFL Culture collection of the Department of Forest Ecosystems Protection, University of Agriculture in Krakow, Poland; NRIF The Natural Resources Institute Finland (Luke), Helsinki, Finland.
[E] ITS1-5.8S-ITS2-ITS2 = the internal transcribed spacer 1 and 2 regions of the nuclear ribosomal DNA gene, 5.8S rRNA gene; βT= Beta-tubulin; TEF1-α = Translation elongation factor 1-alpha; CAL = Calmodulin.
[F] Isolates used in growth and morphological studies; [T]type strain
Sequences obtained during the survey in this study are indicated in bold.

Morphological features were examined by mounting fungal tissue in 80% lactic acid on glass slides, and fruiting structures were observed using a Nikon Eclipse 50*i* microscope (Nikon Corporation, Tokyo, Japan) with an Invenio 5S digital camera (DeltaPix, Maalov, Denmark) to capture photographic images. Microscopy followed the technique described by Kamgan Nkuekam et al. (2011). Colour designations were based on the colour charts of Kornerup and Wanscher (1978).

For each taxonomically relevant structure, fifty measurements were made, when possible, using the Coolview 1.6.0 software (Precoptic, Warsaw, Poland). Averages, ranges and standard deviations were calculated for the measurements, and these are presented in the format '(min–)(mean–SD)–(mean+SD)(–max)'.

Growth characteristics for the novel species were determined by analysing the radial growth for 12 isolates (two for each species) (Table 1). Agar disks (5 mm diam.) were cut from the actively growing margins of fungal colonies and these disks were placed at the centres of plates containing 2% MEA. Four replicate plates for each of the six putative new species were incubated at temperatures between 5, and 35 °C at 5 °C intervals. The radial growth (two measurements perpendicular to each other per plate) was determined 14 d after inoculation, and growth rates were calculated as mm/d.

PCR, sequencing and phylogenetic analyses

DNA extractions were performed as described by Jankowiak et al. (2019d). For sequencing and phylogenetic analyses, four loci were amplified: the internal transcribed spacer region (ITS, consisting of ITS1, 5.8S, and ITS2), beta tubulin (βT), calmodulin (CAL), and the translation elongation factor 1-alpha (TEF1-α). The primers used for PCR and sequencing of the various gene regions were as follows: ITS1-F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for ITS; T10 (O'Donnell and Cigelnik 1997) or Bt2a together with Bt2b (Glass and Donaldson 1995) for βT; F-728F (Carbone and Kohn 1999) and EF2 (O'Donnell et al. 1998) were used for TEF1-α; CL1 and CL2a (O'Donnell et al. 2000) or CL3F and CL3R (De Beer et al. 2016) were used for CAL. PCR and sequencing protocols were as described by Jankowiak et al. (2019d), other than the annealing temperature being optimised for some individual reactions. All analyses were run independently for each gene partition (Figs 1–4). Resulting trees were visually compared for topological incongruence. Gene partitions showing no topological incongruence (βT, CAL) were combined and presented as a concatenated construct (Fig. 5).

For phylogenetic analyses, sequence alignments were performed using the online version of MAFFT v7 (Katoh and Standley 2013). The ITS, βT, CAL, and TEF1-α datasets were aligned using the E-INS-i strategy with a 200PAM/κ=2 scoring matrix, a gap opening penalty of 1.53 and an offset value of 0.00. The alignments were checked manually with BioEdit v.2.7.5 (Hall 1999). The resulting alignments and trees were deposited into TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S27966).

Phylogenetic trees were inferred for each of the datasets using three different methods: Maximum likelihood (ML), Maximum Parsimony (MP) and Bayesian inference (BI). For ML and BI analyses, the best-fit substitution models for each aligned dataset

were established using the corrected Akaike Information Criterion (AICc) in jModelTest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). ML analyses were carried out with PhyML 3.0 (Guindon et al. 2010), utilizing the Montpelier online server (http://www.atgc-montpellier.fr/phyml/). The ML analysis included bootstrap analysis (1000 bootstrap pseudoreplicates) in order to assess node support values and the overall reliability of the tree topology. The best evolutionary substitution model was GTR+I+G for ITS (-lnL = 4497.47), GTR+G for CAL (-lnL = 4112.25) and TEF1-α (-lnL = 4218.36), HKY+G for βT (-lnL = 2641.05) and HKY+I+G for combined βT-CAL (-lnL 6798.48).

MP analyses were performed using PAUP* 4.0b10 (Swofford 2003). Gaps were treated as fifth state. Bootstrap analysis (1000 bootstrap replicates) was conducted to determine the levels of confidence for the nodes within the inferred tree topologies. Tree bisection and reconnection (TBR) was selected as the branch swapping option. The tree length (TL), Consistency Index (CI), Retention Index (RI), Homoplasy Index (HI) and Rescaled Consistency Index (RC) were recorded for each analysed dataset after the trees were generated.

BI analyses using Markov Chain Monte Carlo (MCMC) methods were carried out with MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003). Four MCMC chains were run for 10 million generations applying the best-fit model for each dataset. Trees were sampled every 100 generations, resulting in 100,000 trees. Tracer v1.4.1 (Rambaut and Drummond 2007) was utilized to determine the burn-in value for each dataset. The remaining trees were utilised to generate a 50% majority rule consensus tree, which allowed for calculating posterior probability values for the nodes.

Results

Phylogenetic Analyses

Alignments for the ITS dataset contained 575 characters; for the βT 303 characters; for CAL 543 characters; and for TEF1-α 812 characters; for the concatenated combined dataset 826 (including gaps), of which respectively 202, 123, 271, 439, 390 were parsimony-informative. The exon/intron arrangement of the βT data included exons 5 and 6, interrupted by intron 5. The exon/intron arrangement of the CAL data included exons 4 and 5, interrupted by intron 4. The aligned TEF1-α gene region consisted of intron 3 and exons 4 and 5, but lacked intron 4.

DNA sequence data were generated for 24 isolates considered in this study (Table 1). Blast analyses of the ribosomal DNA sequences placed all the isolates in *Sporothrix*. Based on phylogenetic analyses of the ITS (Fig. 1), the isolates emerged as six undescribed taxa. Phylogenetic analysis of the ITS indicated that the unknown species resided in two previously defined *Sporothrix* species complexes, including the *S. gossypina*- and *S. stenoceras*- species complexes, and lineage "F". Additionally, isolates representing two new species grouped outside any of the currently defined species

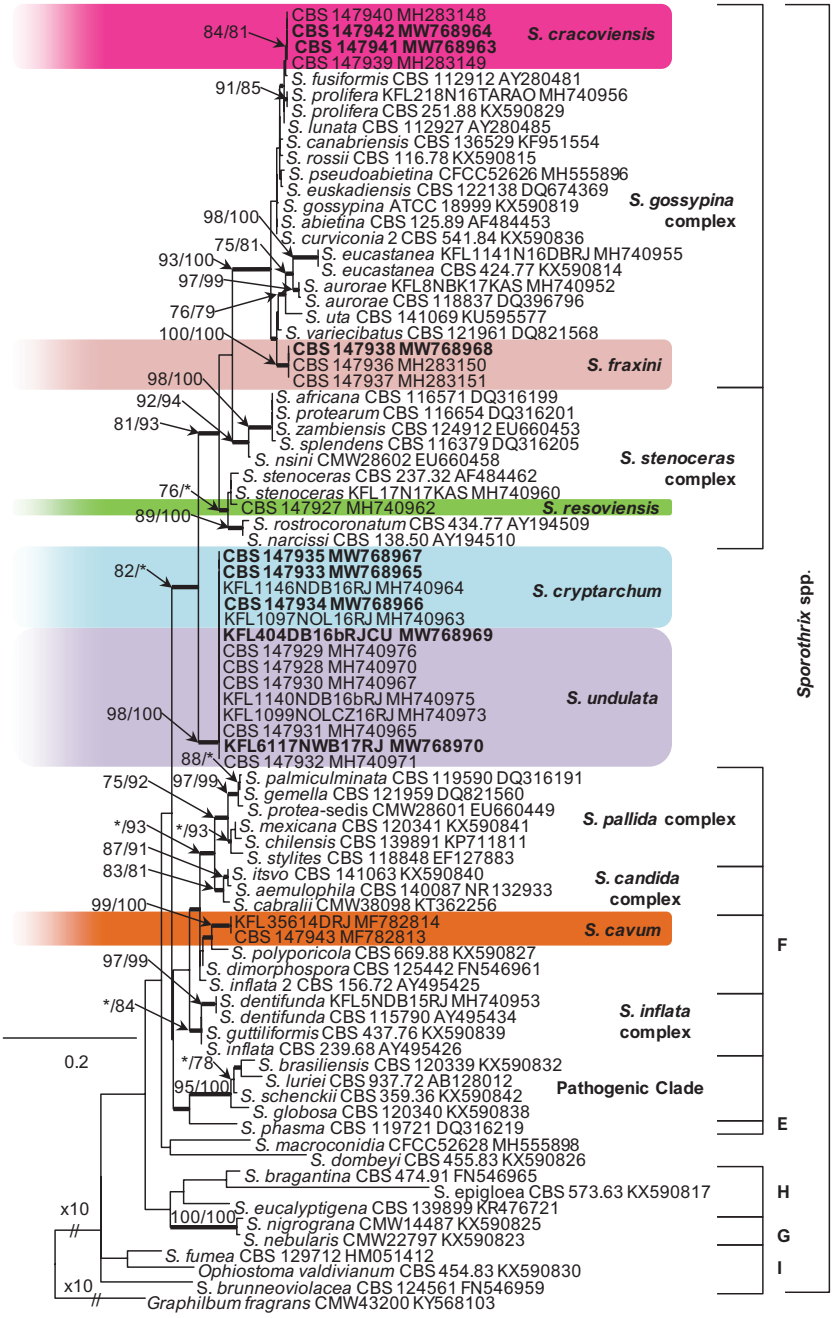

Figure 1. Phylogram obtained from Maximum Likelihood (ML) analyses of the ITS1-5.8S-ITS2 data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

complexes (Fig. 1). Based on the availability of sequence data for these complexes, different datasets were assembled and analysed separately for each species complex.

Seven isolates from hardwood-infesting bark beetles identified as *Sporothrix* 7 and *Sporothrix* 8 by Jankowiak et al. (2019a) resided in the *S. gossypina*-complex (Fig. 1). All three gene regions (ITS, βt, CAL) separated *Sporothrix* sp. 8 from the other known species with strong statistical support (Figs 2–4). The ITS and βt gene regions grouped isolates of this species together with the ex- type isolate of *S. variecibatus*, while CAL gene region placed it with *S. aurorae* (Figs 1–3). Isolates representing *Sporothrix* sp. 7 had ITS sequences that were almost identical to the ITS sequences for *S. fusiformis*, *S. lunata* and *S. prolifera* (Fig. 1). In the βt and CAL trees (Figs 2, 3), *Sporothrix* sp. 7 formed lineages that clearly separated this species from the known species in the *S. gossypina* complex, and although there were differences in the βt sequence compared to other species, the node lacked statistical support (Fig. 2). The combined analyses of the βt and CAL datasets clearly distinguish *Sporothrix* sp. 7 and *Sporothrix* sp. 8 into separate lineages within the *S. gossypina*-complex (Fig. 5).

The single isolate from a wound on *Betula pendula* identified as *Sporothrix* sp. 10 by Jankowiak et al. (2019b), resided in *S. stenoceras*-complex and grouped closely with *S. stenoceras* sensu stricto based on analysis of ITS, βt, CAL, and TEF1-α gene regions (Figs 1–4). All three gene regions separated *Sporothrix* sp. 10 from *S. stenoceras*, although this separation was not statistically supported by the ITS gene region (Figs 1–4). The combined analyses of the βt and CAL datasets clearly distinguish *Sporothrix* sp. 10 into separate lineages within the *S. stenoceras*-complex (Fig. 5).

Two isolates from woodpecker cavities identified as *Sporothrix* sp. 18 by Jankowiak et al. (2019c), belonged to the lineage F defined by De Beer et al. (2016) based on the ITS tree. All the three gene regions (ITS, βt, CAL) separated *Sporothrix* sp. 18 from the other known species in lineage F with strong statistical support (Figs 1–4). The combined analyses of the βt and CAL datasets clearly distinguish *Sporothrix* sp. 18 into separate lineages within the *Sporothrix* spp. (Fig. 5).

Fourteen isolates from wounds on different species of hardwood trees and nitidulid beetles identified as *Sporothrix* sp. 11 and *Sporothrix* sp. 12 by Jankowiak et al. (2019b) did not group in any of the defined *Sprothrix* species complexes based on analysis of ITS gene region and formed a monophyletic lineage within *Sporothrix* (Fig. 1). Isolates of *Sporothrix* sp. 11 had ITS sequences that were identical with ITS sequences noted in *Sporothrix* sp. 12. In the βt, CAL, and TEF1-α trees (Figs 2–4), *Sporothrix* sp. 11 and *Sporothrix* sp. 12 formed well-supported lineages that clearly separated these two putative new species from each other. The combined analyses of the βt and CAL datasets also separated *Sporothrix* sp. 11 and Sporothrix sp. 12 from the other known species in *Sporothrix* spp. and also from each other (Fig. 5).

Morphological characteristics

The six new taxa in *Sporothrix* emerging from the phylogenetic studies showed differences in colony colour. The cultures of *Sporothrix* spp. 7, 8, 10 and 11 were white.

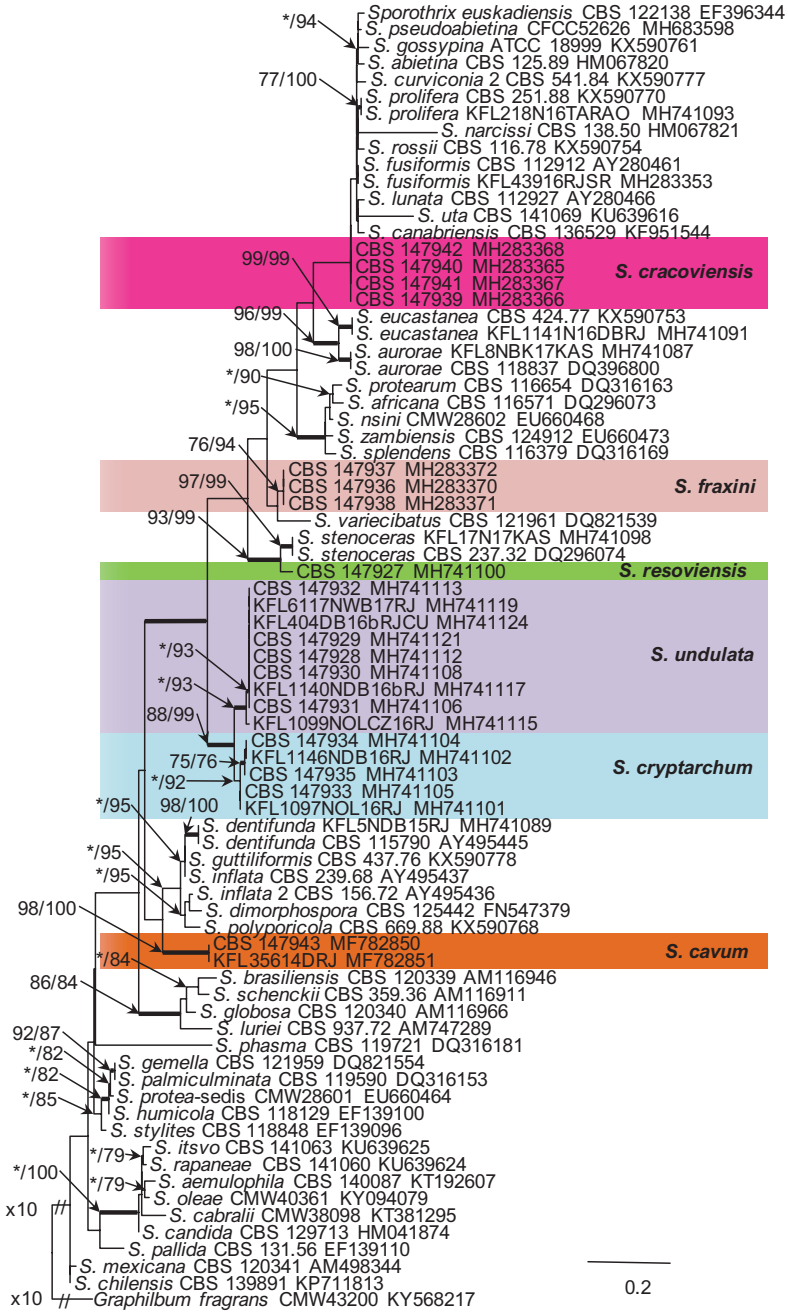

Figure 2. Phylogram obtained from Maximum Likelihood (ML) analyses of βT data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

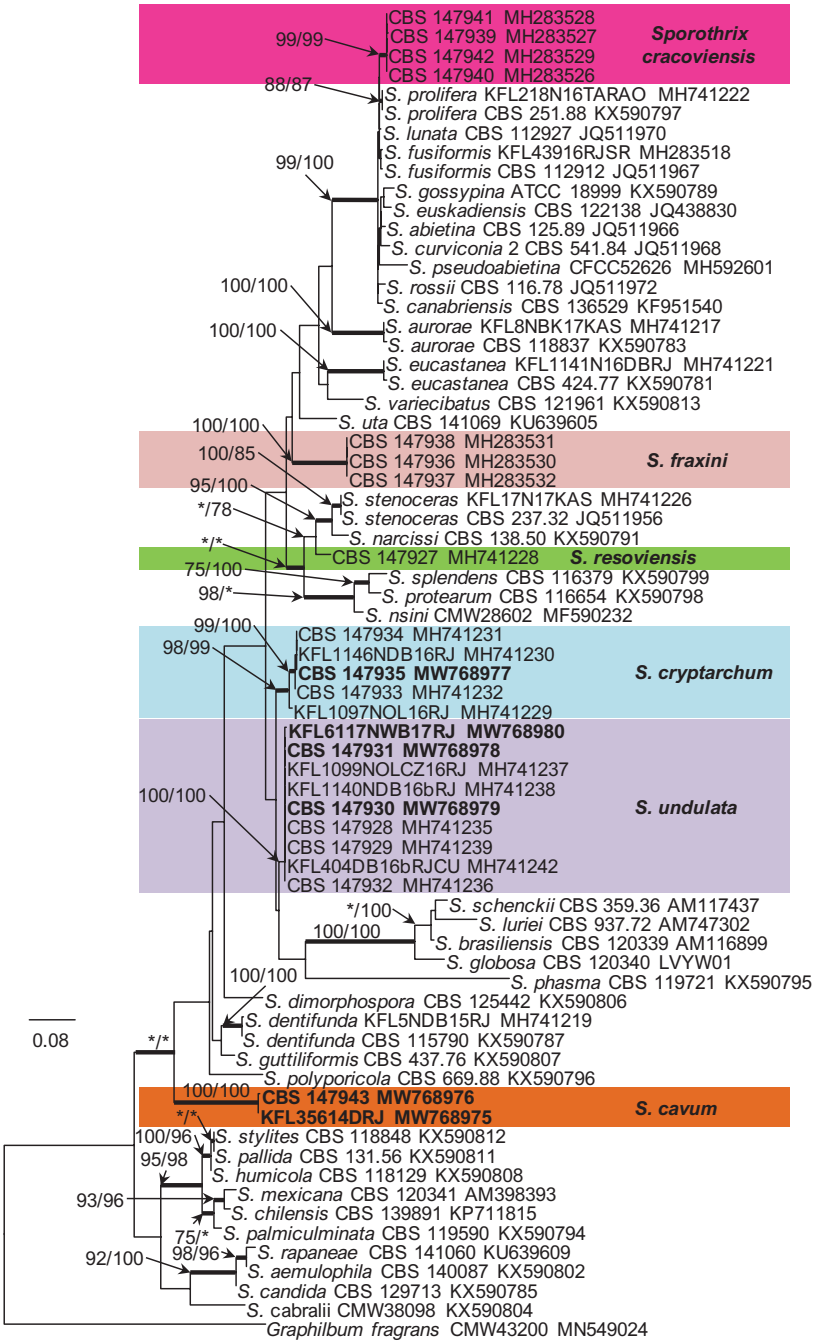

Figure 3. Phylogram obtained from Maximum Likelihood (ML) analyses of CAL data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

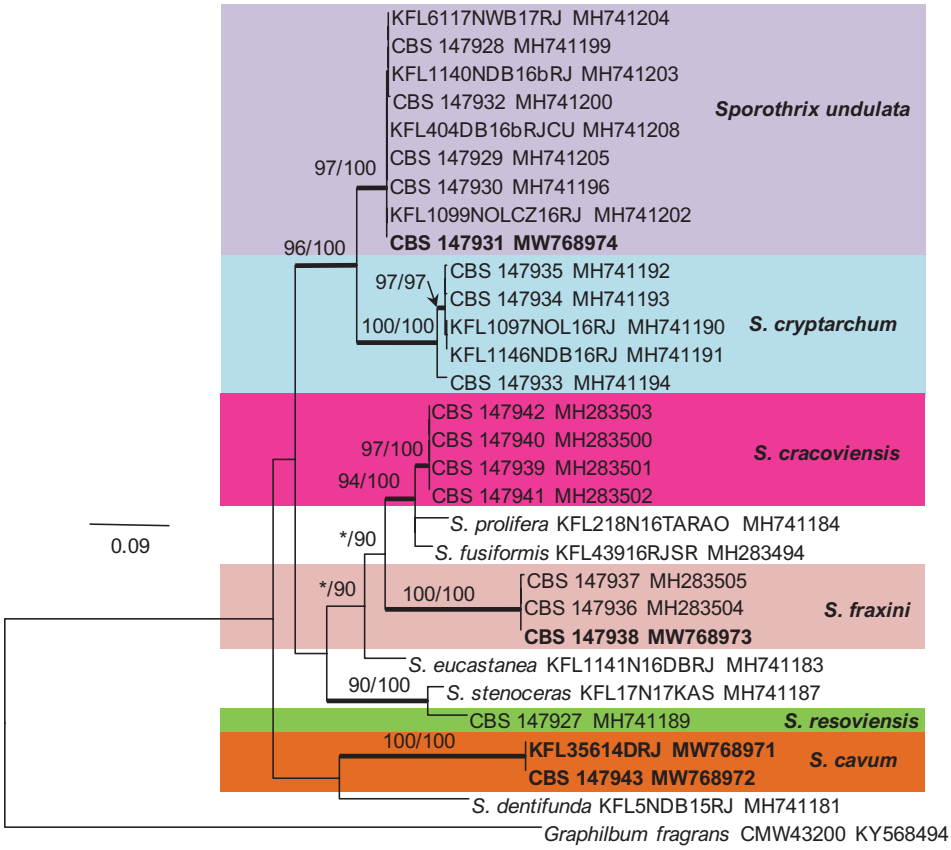

Figure 4. Phylogram obtained from Maximum Likelihood (ML) analyses of TEF1-α data for the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

The cultures of *Sporothrix* sp. 12 were white or pigmented (white grey) whereas cultures of *Sporothrix* sp. 18 were greyish green. With the exception of *Sporothrix* sp. 7 cultures that had an optimum growth at 25 °C followed by 20 °C, all of the undescribed taxa displayed optimum growth at 25 °C followed by 30 °C.

All the new taxa emerging from this study produced micronematous conidiophores and hyaline or pigmented conidia formed holoblastically on denticulate conidiogenous cells. *Sporothrix* sp. 11 and *Sporothrix* sp. 12 were characterized by the formation of hyaline and pigmented conidia. Other than *Sporothrix* sp. 18, which remained asexual, a sexual morph was induced in all five of the other emerging taxa. Ascomata were black and globose with straight necks and up to 700 µm long. Ostiolar hyphae were well-developed and up to 74 µm long. Ascospores were allantoid (*Sporothrix* sp. 7, 8) or kidney-shaped (*Sporothrix* spp. 10–12), and they lacked sheaths.

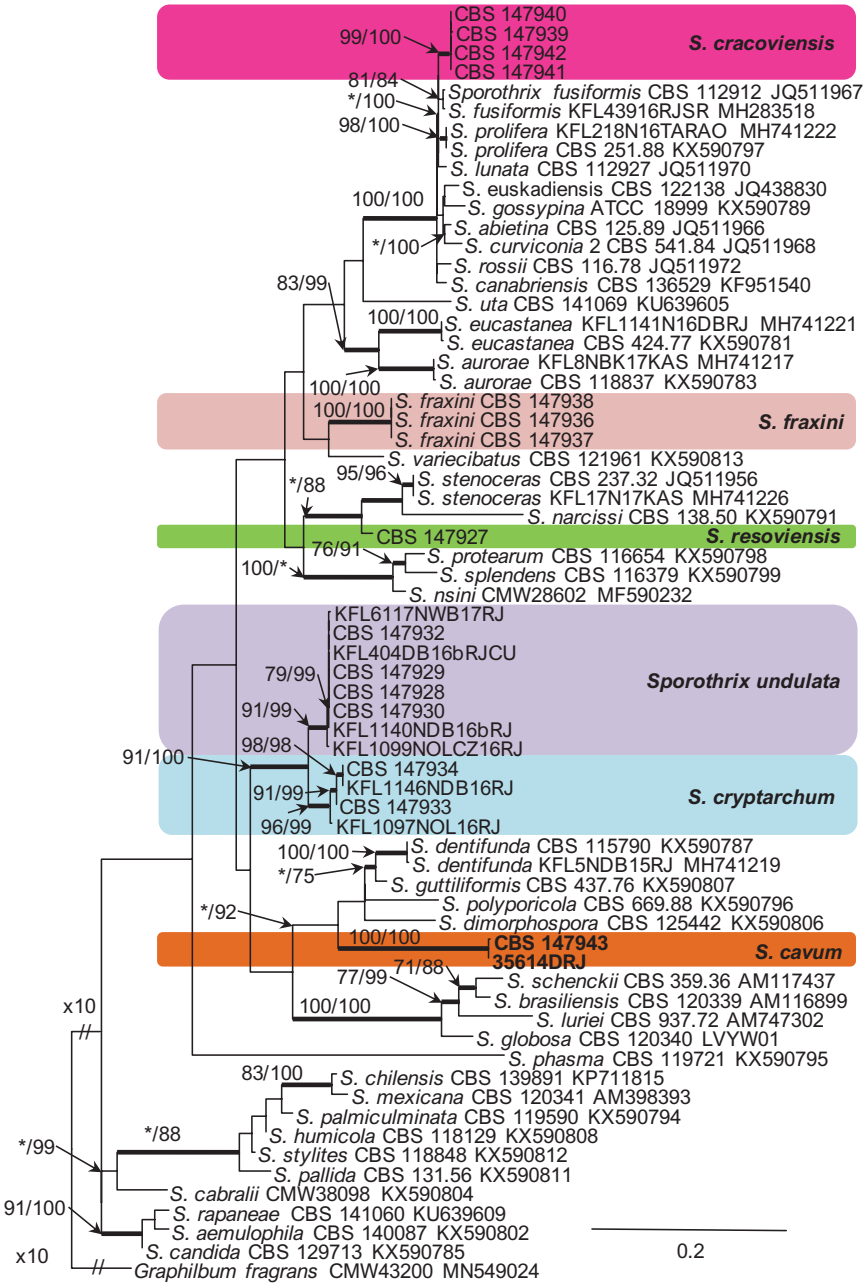

Figure 5. Phylogram obtained from Maximum Likelihood (ML) analyses of the combined βT and CAL sequences of the *Sporothrix* spp. Sequences obtained during this study are presented in bold type. The Bootstrap values ≥ 75% for ML and Maximum Parsimony (MP) analyses are presented at nodes as follows: ML/MP. Bold branches indicate posterior probabilities values ≥ 0.95 obtained from Bayesian Inference (BI) analyses. * Bootstrap values <75%. The tree is drawn to scale (see bar) with branch length measured in the number of substitutions per site. *Graphilbum fragrans* represent the outgroup.

Taxonomy

Sporothrix cracoviensis **R. Jankowiak, sp. nov.**
MycoBank No: 840460
Fig. 6

Etymology. From Latin, referring to the capital of Małopolskie Voivodeship and the former capital of Poland (Cracovia in Latin, Kraków in Polish); the region where this fungus was collected.

Type. Poland, Małopolskie Province, Krzeszowice, from adult *Tryopodendron domesticum* beetle on *Fagus sylvaticum*, January 2014, *R. Jankowiak* (O-F-258628 ***holotype***, culture ex-type CBS 147942).

Description. Sexual and asexual structures produced on sterilised beech twigs on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal bases* black, globose, (66–)89–153(–245) µm diam., with brown hyphal hairs, 12 to 165 µm long and 1 to 1.8 µm wide at the base; *ascomatal necks* black, straight or curved, (187–)272–462(–611) µm long, diameter (9–)10.4–16.7(22.5) µm at the apex and (26.8–)29.9–50.5(–63.9) µm at the base. *Ostiolar hyphae* present, pale brown, septate, straight or slightly waved, tapering towards the apex or sporadically dichotomous branching at the tip, (7–)8–16(–22) in number (17.8–)29.6–48.4(–64.5) µm long, (0.3–)0.5–1(–1.5) µm at the apex and (1.2–)1.6–2.3–(3) µm at the base. *Asci* evanescent. *Ascospores* one-celled, allantoid in side view (2.8–)3.1–3.8(–5.1) × (1–)1.1–1.4(–1.6) µm, elliptical in front view (2.8–)3.1–4.2(–4.8) × (1–)1.2–1.5(–1.8) µm, sometimes with residual sheath up to 1 µm thick, accumulated in creamy-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous, simple or branched, straight, simple or branched, bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastics, cylindrical, terminal, lateral or intercalary, straight or curved, tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on visible denticles, (4.2–)17.5–43.1(–72.2) µm long, (0.8–)1.1–1.7(–2.1) µm wide at the base. Apical part with denticles (0.8–)1.3–3.7(–7.3) µm long and (1.2–)1.7–3.7(–7.3) µm wide. Conidia hyaline, unicellular, smooth, obovoid to clavate, sometimes slightly curved, with slightly pointed bases, (2.8–)3.2–6.4(–8.7) × (1.1–)1.4–2.1(–2.7) µm, formed directly on denticles. *Culture characteristics*: Cultures showing optimal growth at 25 °C (1 mm/d) with somewhat slower growth by at 20 °C (0.8 mm/d), white, flat, floccose, growing in a circular pattern with smooth margins.

Host tree. *Fagus sylvatica*.

Insect vector. *Trypodendron domesticum*, *T. signatum*.

Distribution. Poland

Additional specimen examined. Poland, Małopolskie Province, Krzeszowice, from adult *Tryopodendron domesticum* beetle on *Fagus sylvaticum*, January 2014, *R. Jankowiak* (O-F-258629, cultures CBS 147941).

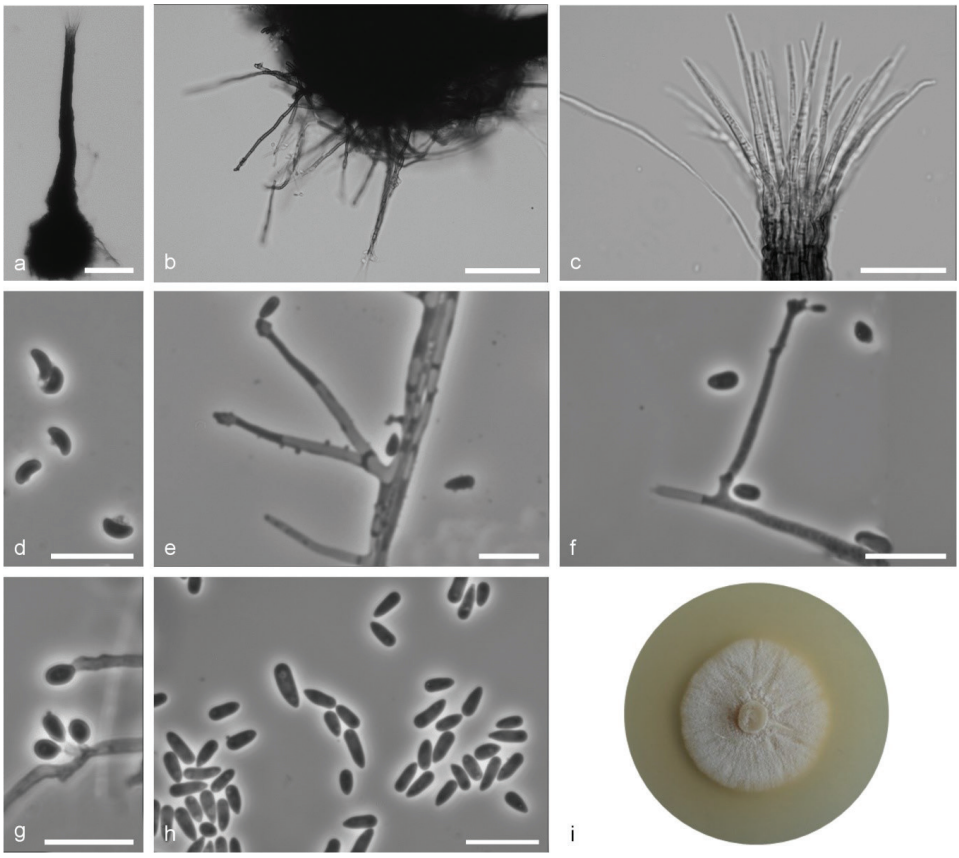

Figure 6. *Sporothrix cracoviensis* sp. nov. (CBS 147942) **a** ascoma **b** ascomatal base **c** ostiolar hyphae **d** ascospores **e, f** conidiogenous cell with an inflated cluster of denticles at the apex **g** conidiogenous cells arising directly from hyphae **h** conidia **i** fourteen-day-old culture on MEA. Scale bars: 50 μm (**a, b**), 25 μm (**c**), 10 μm (**d–h**).

Notes. *Sporothrix cracoviensis* is phylogenetically distinct from the other *Sporothrix* species based on the βT, CAL and TEF1-α sequences. This species is closely related to *S. fusiformis*, *S. lunata* and *S. prolifera*. *Sporothrix cracoviensis* has smaller ascomatal necks (187–611 μm) compared to *S. fusiformis* (301–1168) μm (Aghayeva et al. 2004). Their conidial dimensions and shapes showed also differences. *Sporothrix fusiforme* has fusiforme conidia (Aghayeva et al. 2004), whereas *S. cracoviensis* has obovoid to clavate conidia. *Sporothix lunata* has also different shape of conidia (crescent) (Aghayeva et al. 2004) compared to *S. cracoviensis* (obovoid to clavate). In addition, *S. lunata* has smaller conidia (2.3–6.2 × 0.8–1.6 μm) (Aghayeva et al. 2004) compared to *S. cracoviensis* (2.8–8.7 μm × 1.1–2.7 μm). *Sporothrix prolifera* could be distinguished from *S. cracoviensis* by its smaller ascomatal base (*S. prolifera*: 65–90 μm (Kowalski and Butin 1989); *S. cracoviensis*: 66–245 μm) and smaller ascomatal necks (*S. prolifera*: 75–160 μm (Kowalski and Butin 1989); *S. cracoviensis*: 187–611 μm). In addition,

S. prolifera has shorter ostiolar hyphae (*S. prolifera*: 15–30 µm (Kowalski and Butin 1989); *S. cracoviensis*: 26.8–63.9 µm) and shorter and wider ascospores (*S. prolifera*: 3.2–3.8 × 1.8–2 µm (Kowalski and Butin 1989); *S. cracoviensis*: 2.8–5.1 × 1–1.6 µm). The conidia of *S. prolifera* are also smaller (*S. prolifera*: 4–5.8 × 1.6–2.2 µm (Kowalski and Butin 1989) compared to *S. cracoviensis* (2.8–8.7 × 1.1–2.7 µm).

Sporothrix cracoviensis was represented by four isolates collected from adult *Trypodendron domesticum* beetles on *Fagus sylvatica*. It corresponds to *Sporothrix* sp. 7 in the study of Jankowiak et al. (2019a).

***Sporothrix fraxini* R. Jankowiak, sp. nov.**
MycoBank No: 840463
Fig. 7

Etymology. From Latin, referring to the genus name of the host (*Fraxinus excelsior*).

Type. Poland, Małopolskie Province, Zbylitowska Góra, from the gallery of *Hylesinus varius* on *Fraxinus excelsior*, May 2016, *R. Jankowiak* (O-F-258630 ***holotype***, culture ex-type CBS 147936).

Description. Sexual and asexual structures produced on sterilized ash twigs and on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal base* black, globose, (89–)110–161(–216) µm diam., with brown hyphal hairs, 14 to 65 µm long and 1.1 to 2.1 µm wide at the base; *ascomatal necks* black, straight or curved, (222–)332–461(–526) µm long, diameter (10.1–)11.3–16(–20.4) µm at the apex and (26.2–)29.1–41.4(–53) µm at the base. *Ostiolar hyphae* present, pale brown, septate, straight or rather tortuous, tapering towards the apex or sporadically dichotomous branching at the tip, (8–)10–20(–24) in number (21.4–)31.1–52.1(–73.6) µm long, (0.4–)0.7–1.1(–1.4) µm at the apex and (1.4–)1.8–2.4–(3.1) µm at the base. *Asci* evanescent. *Ascospores* one-celled, allantoid in side view (2.7–)2.9–3.5(–4.4) × (0.9–)1–1.4(–1.8) µm, elliptical in front view (2.2–)2.9–3.8(–4.7) × (0.8–)1.2–1.6(–1.8) µm, sometimes with residual sheath up to 1 µm thick, accumulated in white-color mass at the tip of the neck. *Conidiophores* hyaline, micronematous, simple or branched, straight, simple or branched, bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical terminal or intercalary, straight or curved, tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on hardly visible denticles, (13.6–)14.6–47.7(–99.6) µm long, (0.9–)1.2–1.6(–1.9) µm wide at the base. Apical part (0.8–)1.7–5.1(–10.6) µm long and (0.8–)1.1–2(–3) µm wide. *Conidia* hyaline, unicellular, smooth, obovoid to ellipsoidal, ends slightly rounded or truncate, (2.6–)3.4–5(–6.8) × (0.8–)1.1–1.6(–2) µm, formed directly on denticles. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1 mm/d) followed by at 30 °C (0.9 mm/d), white, flat, growing in a circular pattern with smooth margins, with sparse aerial mycelium, often fading around the edges.

Host tree. *Fraxinus excelsior*.

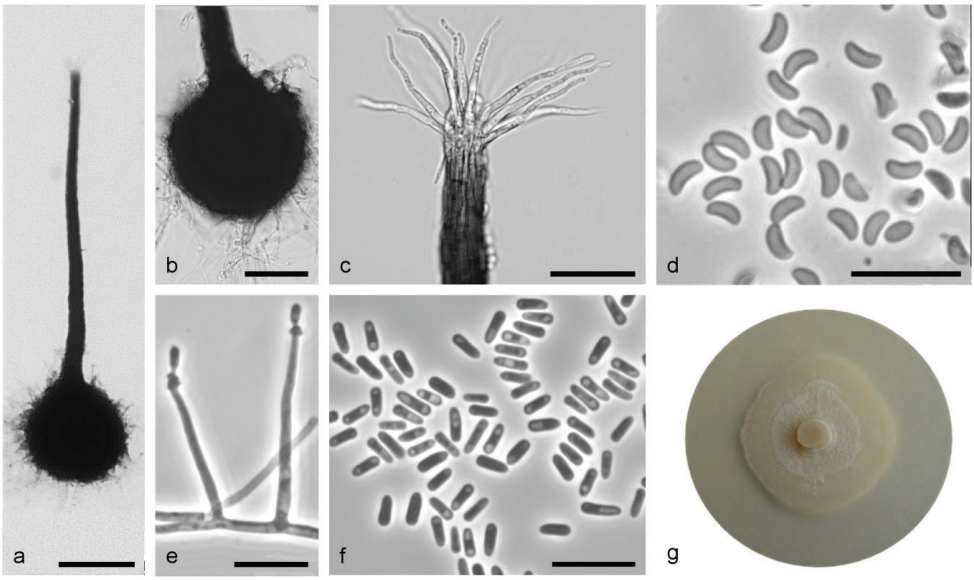

Figure 7. *Sporothrix fraxini* sp. nov. (CBS 147936) **a** ascoma **b** ascomatal base **c** ostiolar hyphae **d** ascospores **e** conidiogenous cell with an inflated cluster of denticles at the apex **f** conidia **g** fourteen-day-old culture on MEA. Scale bars: 100 µm (**a**), 50 µm (**b**), 25 µm (**c**), 10 µm (**d–f**).

Insect vector. *Hylesinus crenatus*, *H. varius*.

Distribution. Poland

Additional specimen examined. Poland, Małopolskie Province, Zbylitowska Góra, from the gallery of *Hylesinus varius* on *Fraxinus excelsior*, May 2016, *R. Jankowiak* (O-F-258631, cultures CBS 147938).

Notes. This species is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. *Sporothrix fraxini* is closely related to *S. variecibatus*. However, *S. variecibatus* does not produce a sexual morph, and has narrower conidia (2–3 µm) (Roets et al. 2008) compared to *S. fraxini* (0.8–2 µm). In addition, the conidia of *S. variecibatus* are clavate while *S. fraxini* has obovoid to ellipsoidal conidia.

Sporothrix fraxini was represented by three isolates collected from the galleries of *Hylesinus varius* on *Fraxinus excelsior*. It corresponds to *Sporothrix* sp. 8 in the previous study of Jankowiak et al. (2019a).

Sporothrix resoviensis **R. Jankowiak & A. Ostafińska, sp. nov.**
MycoBank No: 840475
Fig. 8

Etymology. From Latin, referring to the capital of Podkarpackie Voivodeship (Resovia in Latin, Rzeszów in Polish), the region from which this fungus was collected.

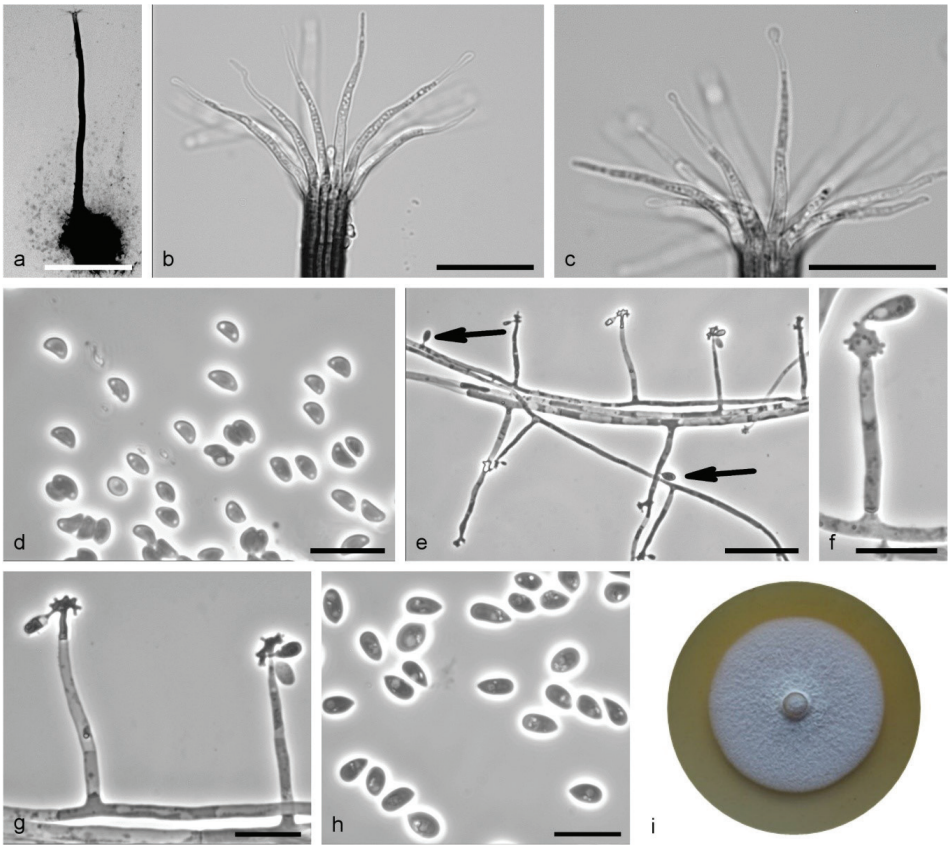

Figure 8. *Sporothrix resoviensis* sp. nov. (CBS 147927) **a** ascoma **b, c** ostiolar hyphae **d** ascospores **e–g** conidiogenous cell with an inflated cluster of denticles at the apex **h** conidia **i** fourteen-day-old culture on MEA. Scale bars: 250 µm (**a**), 25 µm (**b, c**), 10 µm (**d**), 25 µm (**e**), 10 µm (**f–h**).

Type. Poland, Podkarpackie Province, Borownica, from the wound on *Betula pendula*, June 2016, *A. Ostafińska*, (O-F-258632 ***holotype***, culture ex-type CBS 147927).

Description. Sexual and asexual structures produced on sterilised birch twigs and on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal bases* black, globose, (87–)113–184(–232) µm diam., with brown hyphal hairs, 14 to 44 µm long and 0.9 to 2.2 µm wide at the base; *ascomatal necks* black, straight or curved, often extended at the base, (228–)378–624(–700) µm long, diameter (10–)11.2–17(–20.2) µm at the apex and (26.2–)34–47.7(–56) µm at the base. *Ostiolar hyphae* present, pale brown, septate, straight or curved, tapering towards the apex and often swollen at the tip, (7–)9–15(–18) in number, (15.7–)26.1–47.7(–67.6) µm long, (0.3–)0.7–1.5(–2.5) µm at the apex and (1.3–)2–3–(3.4) µm at the base. *Asci* evanescent. *Ascospores* one-celled, kidney-shaped to almost triangular in side view (2.7–)3.2–3.9(–4.4) × (1.4–)1.7–2.1(–2.3) µm, oblong-elliptical in front view (2.6–)3–3.8(–4.9) × (1.4–)1.7–2.2(–2.6) µm, without residual

sheath accumulated in white-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous, straight, simple and bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, swollen apical part forming conidia by sympodial proliferation on easily visible denticles, (3.1–)9.3–57(–120.1) µm long, (1–)1.1–1.6(–2.2) µm wide at the base. Apical part (1.3–)1.9–3.5(–4.4) µm long and (1.4–)2.4–3.9(–4.5) µm wide. *Conidia* hyaline, unicellular, smooth, obovate to ellipsoidal, pointed at the base, (3.9–)4.3–6.7(–8.5) × (2.1–)2.4–3.4(–4) µm, formed singly on denticles or on the side of vegetative hyphae. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1.8 mm/d) followed by at 30 °C (1.7 mm/d), white, growing in a circular pattern with smooth margins, funiculose and woolly.

Host trees. *Betula pendula*.

Insect vector. unknown.

Distribution. Poland.

Note. *Sporothrix resoviensis* is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. This species grouped most closely with *S. stenoceras* but can be distinguished by its larger ascospores (*S. resoviensis*: 2.7–4.4 × 1.4–3.3 µm; *S. stenoceras*: 2.0–2.9 × 1.3–1.4 µm (Robak 1932). Perithecia developing on the agar medium and twigs have significantly shorter necks (*S. resoviensis*: 228–700 µm; *S. stenoceras*: 450–1500 µm (Robak 1932). *Sporothrix resoviensis* has larger conidia (3.9–8.5 × 2.1–4 µm) compared to *S. stenoceras* (3.4–6.9 × 2–3.4 µm). This new species also differs from *S. stenoceras* based on culture morphology, where *S. resoviensis* produces wooly cultures, different to the sparse and flat mycelium of *S. stenoceras* (Robak 1932).

Sporothrix resoviensis was represented by one isolate collected from a wound on *Betula pendula*. It corresponds to *Sporothrix* sp. 10 in the study of Jankowiak et al. (2019b).

***Sporothrix cryptarchum* R. Jankowiak & A. Ostafińska, sp. nov.**

MycoBank No: 840477

Fig. 9

Etymology. Referring to the genus name of the beetle, *Cryptarcha* sp. (*Coleoptera*: *Nitidulidae*), with which this fungus is associated.

Type. Poland, Małopolskie Province, Wierzchosławice, from *Cryptarcha undata* on *Quercus robur*, June 2016, *R. Jankowiak*, (O-F-258633 ***holotype***, culture ex-type CBS 147934).

Description. Sexual and asexual structures produced on the sterilised oak twigs and on the surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal bases* black, globose, (55–)115–172(–210) µm diam., with brown hyphal hairs, 15 to 141 µm long and 0.9 to 3.8 µm wide at the base; *ascomatal necks* black, straight or curved, (126–)198–

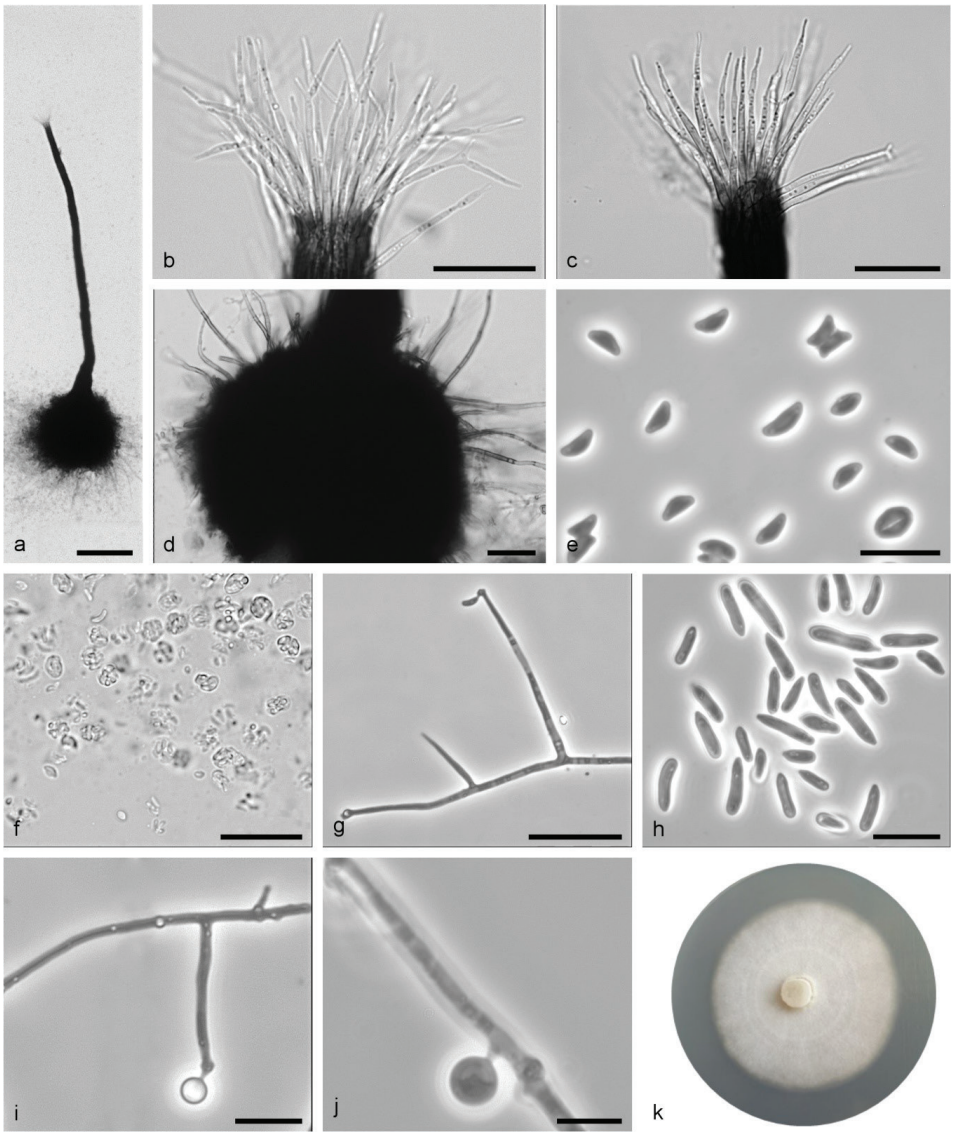

Figure 9. *Sporothrix cryptarchum* sp. nov. (CBS 147934) **a** ascoma **b** ascomatal base **c, d** ostiolar hyphae **e** ascospores **f** asci **g** conidiogenous cell with an inflated cluster of denticles at the apex **h** conidia **i** globose conidia arising on long conidiophore **j** globose conidia arising directly from hyphae **k** fourteen-day-old culture on MEA. Scale bars: 100 µm (**a**), 25 µm (**b–d**), 10 µm (**e**), 25 µm (**f, g**), 10 µm (**h, i**), 5 µm (**j**).

412(–544) µm long, diameter (10.9–)13–19(–23.8) µm at the apex and (17.6–)29.3–47.6(–59.6) µm at the base. *Ostiolar hyphae* present, pale brown, with small granules, septate, straight or curved, simple or dichotomous branching, tips tapering or sometimes thickened, (9–)13–24(–31) in number, (15.8–)30.5–51.8(–60.9) µm long, (0.2–)0.3–0.5(–0.7) µm at the apex and (0.9–)1.6–2.4–(3) µm at the base. *Asci* subglobose to

ovoid, (5.5–)6.7–9(–11) × (4–)4.9–6.2(–7.2) µm. *Ascospores* one-celled, kidney-shaped to almost triangular in side view in side view (3.2–)3.8–4.7(–5.8) × (0.8–)1–1.3(–1.5) µm, elliptical in front view (3.1–)3.6–4.4(–5) × (1–)1.2–1.6(–1.8) µm, sometimes with residual sheath up to 0.6 µm thick, accumulated in white-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous, simple or occasionally branched and bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on narrow denticles, (2.2–)13.9–51.2(–102.8) µm long, (0.7–)1.2–1.8(–2.2) µm wide at the base. Apical part (0.6–)1.4–3.1(–5.3) µm long and (1–)1.7–3(–3.8) µm wide, single denticles often below. Conidia of two types: 1) abundant in cultures, often produced, hyaline, unicellular, smooth, obovate to ellipsoid, pointed at the base, (3.3–)4.6–8.1(–10.3) × (1–)1.3–1.9(–2.2) µm, formed directly on denticles; 2) sparse in cultures, subhyaline to lightly pigmented, unicellular, smooth, subglobose to globose, (2.3–)3.1–4.1(–4.5) µm diam, formed singly, either directly on the side of vegetative hyphae or on short lateral branches. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1.3 mm/d) followed by at 30 °C (1.1 mm/d), mostly pigmented or white or pig, flat, growing in a circular pattern with smooth margins.

Host tree. *Alnus glutinosa*, *Quercus robur*.

Insect vector. *Cryptarcha undata*, *C. strigata*.

Distribution. Poland.

Additional specimen examined. Poland, Małopolskie Province, Wierzchosławice, from *Cryptarcha undata* on *Quercus robur*, June 2016, *R. Jankowiak*, (O-F-258634, cultures CBS 147933).

Notes. This species is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. *Sporothrix cryptarchum* is phylogenetically closely related to *S. undulata* (*Sporothrix* sp. 12) described in the present study. This species also shares morphological similarities such as kidney-shaped ascospores and two morphological forms of conidia with *S. undulata*. However, *S. cryptarchum* has narrow ascospores (0.8–1.5 µm) compared to *S. undulata* (1.1–2 µm). It also has distinct ostiolar hyphae, with those in *S. cryptarchum* often dichotomously branching while in *S. undulata* these hyphae occur only sporadically and do not have dichotomous branching. Both species produce hyaline and pigmented conidia. However, *S. cryptarchum* cultures are predominantly hyaline whereas those in pure cultures of *S. undulata* are mostly pigmented. Their conidial shapes in these two species are similar but their dimensions are distinct. *Sporothrix cryptarchum* has conidia that are smaller than those of *S. undulata*. In addition, cultures of *S. cryptarchum* are white and grow in a circular pattern with smooth margins while those of *S. undulata* grow in a circular pattern with undulate margins and some have grey pigmentation.

Sporothrix cryptarchum was represented by four isolates collected from Poland. It corresponds to *Sporothrix* sp. 11 in the study of Jankowiak et al. (2019b). *Sporothrix cryptarchum* was isolated from wounds on hardwood trees and nitidulid beetles (*Coleoptera*: *Nitidulidae*), which visited fresh wounds on *Quercus robur*.

***Sporothrix undulata* R. Jankowiak & A. Ostafińska, sp. nov.**
MycoBank No: 840478
Fig. 10

Etymology. Referring to the aerial mycelium growing in undulating concentric zones on MEA.

Type. Poland, Małopolskie Province, Wierzchosławice, from *Epuraea guttata* on *Quercus robur*, June 2016, *R. Jankowiak*, (O-F-258635 ***holotype***, culture ex-type CBS 147929).

Description. Sexual and asexual structures produced on sterilised oak twigs and on surface of malt agar in Petri dishes. *Ascomata* abundant, superficially or partly embedded in the agar, single or in groups; *ascomatal base* black, globose, (65–)95–186(–223) µm diam., with brown hyphal hairs, 8 to 134 µm long and 1.2 to 3.1 µm wide at the base; *ascomatal necks* black, straight or curved, (114–)174–482(–697) µm long, diameter (9.1–)12.3–18.7(–24.2) µm at the apex and (14.7–)22–40.3(–58.7) µm at the base. *Ostiolar hyphae* present, pale brown, with small granules, septate, straight or slightly waved, tapering towards the apex or sporadically dichotomously branched at the tip, (9–)16–28(–31) in number, (29.4–)39.9–59.5(–72) µm long, (0.4–)0.6–1(–1.1) µm at the apex and (1.5–)1.8–2.7–(3.3) µm at the base. *Asci* subglobose to ovoid, (5.7–)6.7–8.5(–9.4) × (3.4–)4.4–5.8(–6.4) µm. *Ascospores* one-celled, kidney-shaped to almost triangular in side view (3.4–)3.8–4.6(–4.9) × (1.1–)1.4–1.7(–2) µm, elliptical in front view (3.2–)3.5–4.5(–5.6) × (0.9–)1.5–2.1(–2.8) µm, sometimes with residual sheath up to 0.6 µm thick, accumulated in white-colored mass at the tip of the neck. *Conidiophores* hyaline, micronematous or semimacronematous, simple or occasionally branched and bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, slightly tapering towards the apex, swollen apical part forming conidia by sympodial proliferation on small or hardly visible denticles, (5.2–)11.3–50.4(–112.2) µm long, (0.9–)1.3–1.8(–2.1) µm wide at the base. Apical part (1.1–)1.6–3.4(–5.9) µm long and (1.1–)1.7–3.5(–5.4) µm wide. *Conidia* of two types: 1) sparsely in cultures, hyaline, unicellular, smooth, ellipsoid, pointed at the base, (3.2–)4.2–7.8(–11.7) × (1.4–)1.7–2.4(–3.5) µm, formed directly on denticles; 2) abundant in cultures, subhyaline to lightly pigmented, unicellular, smooth, subglobose to globose, sometimes pointed at the base, (2.1–)2.9–4.2(–5.5) µm diam, formed singly or in chains, either directly on the side of vegetative hyphae, on short lateral branches or denticles. *Culture characteristics*: Cultures showing optimum growth at 25 °C (1.2 mm/d) with growth somewhat slower at 20 °C and 30 °C (0.9 mm/d), white or white grey, flat, growing in a circular pattern with undulate margins.

Host tree. *Alnus glutinosa*, *Carpinus betulus*, *Fagus sylvatica*, *Quercus robur*, *Quercus rubra*, *Salix fragilis*.

Insect vector. *Cryptarcha undata*, *Epuraea guttata*.

Distribution. Poland.

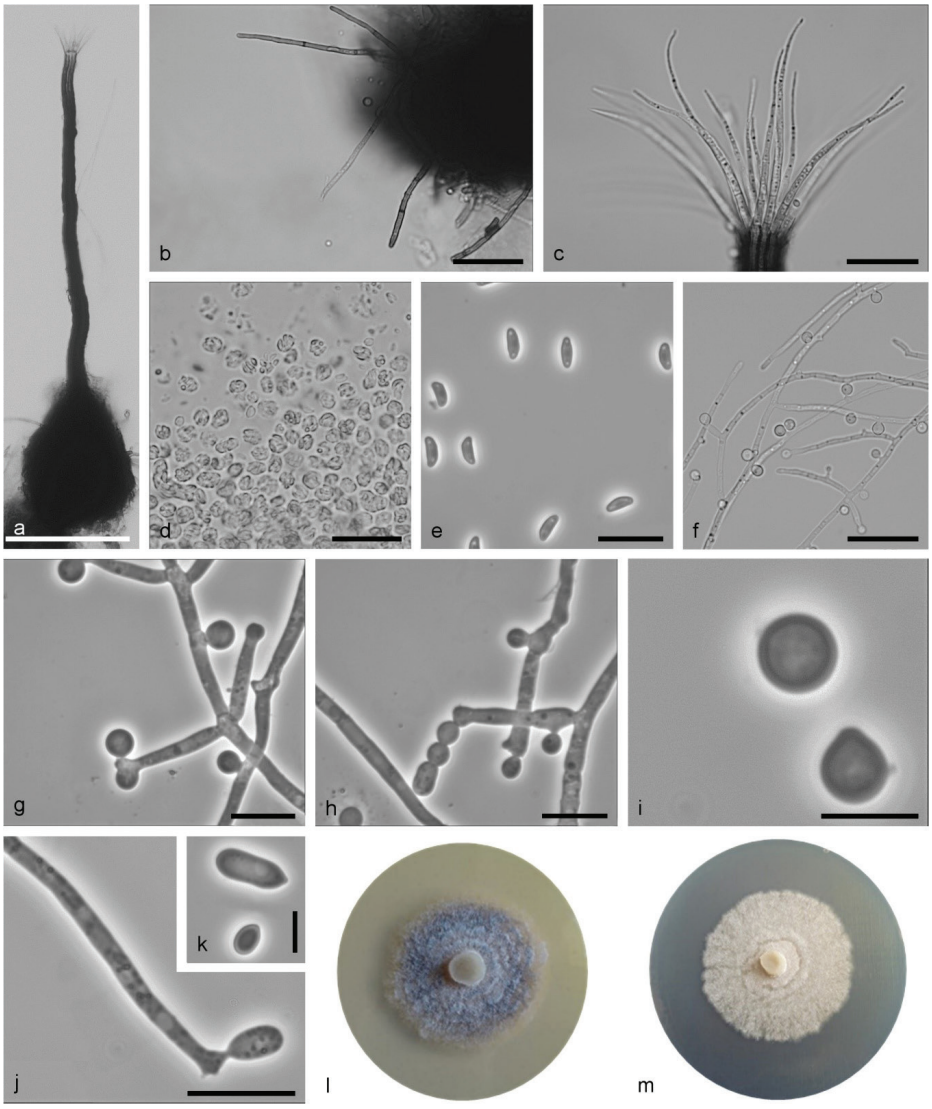

Figure 10. *Sporothrix undulata* sp. nov. (CBS 147929) **a** ascoma **b** ascomatal base **c** ostiolar hyphae **d** asci **e** ascospores **f–h** globose conidia arising on long conidiophore or directly from hyphae **i** globose conidia **j** conidiogenous cell with an inflated cluster of denticles at the apex **k** conidia **l–m** fourteen-day-old culture on MEA (left- pigmented CBS 147929, right – white KFL404DB16bRJCU). Scale bars: 100 µm (**a**), 25 µm (**b–d**), 10 µm (**e**), 25 µm (**f**), 10 µm (**g, h**), 5 µm (**i**), 10 µm (**j**), 5 µm (**k**).

Additional specimen examined. Poland, Małopolskie Province, Wierzchosławice, from wound on *Quercus robur*, October 2015, *R. Jankowiak* (O-F-258636, cultures CBS 147931).

Notes. This species is phylogenetically distinct from the other *Sporothrix* species based on the ITS, βT, CAL and TEF1-α sequences. *Sporothrix undulata* is

phylogenetically closely related to *S. cryptarchum* described in this study. The morphological differences between *S. undulata* and *S. cryptarchum* are described in the section above treating *S. cryptarchum*.

Sporothrix undulata was represented by nine isolates collected from Poland. It corresponds to *Sporothrix* sp. 12 in the study of Jankowiak et al. (2019b). In this study *S. undulata* was isolated from wounds on hardwood trees and from adults of nitidulid beetles (*Coleoptera*: *Nitidulidae*), which visited wounds on *Quercus robur*.

***Sporothrix cavum* R. Jankowiak sp. nov.**
Mycobank: 840479
Fig. 11

Etymology. From Latin, referring to the hollow cavities produced by woodpeckers and from which this fungus was collected.

Type. Poland, Małopolskie Province, Kraków, from the cavity of *Dendrocopos major* on *Salix fragilis*, December 2015, *R. Jankowiak*, (O-F-258637 ***holotype***, culture ex-type CBS 147943).

Description. Sexual morph not observed. Asexual structures produced on sterilized beech twigs placed on the surface of malt agar in Petri dishes. *Conidiophores* hyaline, micronematous, simple, straight, simple or branched, bearing several conidiogenous cells, either borne on vegetative hyphae or on upright hyphae. *Conidiogenous cells* blastic, cylindrical, terminal, lateral or intercalary, straight or curved, slightly tapering toward the apex, swollen apical part forming conidia by sympodial proliferation on well-developed denticles, (2.8–)11.5–32.8(–54.4) µm long, (0.7–)1.1–1.7(–2.4) µm wide at the base. Apical part with denticles (1.2–)1.5–2.8(–4.4) µm long and (1.4–)1.8–2.6(–3.1) µm wide, individual denticles often formed below aplical part. *Conidia* hyaline, unicellular, smooth, obovoid, with pointed bases, (3.1–)3.6–5.5(–7.8) × (1.7–)2–2.7(–3.2) µm, formed on terminal or lateral denticles, either directly on the side of vegetative hyphae. *Culture characteristics*: Cultures having optimum growth at 25 °C (1.7 mm/d) followed by at 30 °C (1.5 mm/d), growing well at 35 °C (0.6 mm/d), greyish green, with a darker centre, flat, growing in a circular pattern with smooth margins and abundant aerial mycelium.

Host tree. *Malus domestica*, *Salix fragilis*

Insect vector. unknown

Distribution. Poland

Additional specimen examined. Poland, Małopolskie Province, Książ Wielki, from the cavity of *Dendrocopos medius* on *Malus domestica*, (O-F-258638, cultures exparatype KFL=NRFI 35614DR).

Notes. This species is phylogenetically distinct from the other *Sporothrix* species based sequences for the ITS, βT, CAL and TEF1-α regions. *Sporothrix cavum* is related to *S. polyporicola* based on analyses of the ITS sequences. However, *S. cavum* in contrast to *S. polyporicola*, does not produce a sexual morph (Constantinescu and

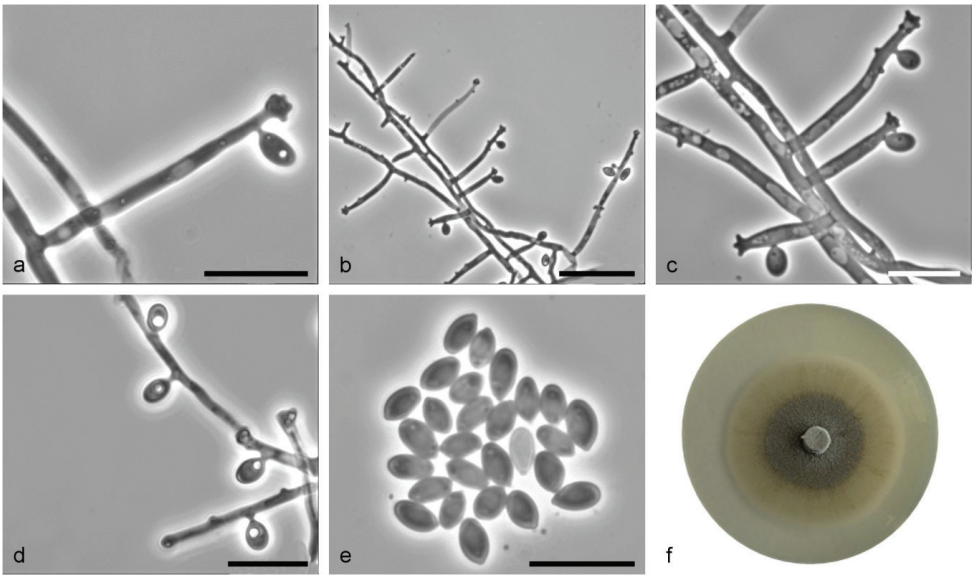

Figure 11. *Sporothrix cavum* sp. nov. (CBS 147943) **a–c** conidiogenous cell with an inflated cluster of denticles at the apex and below apex **d** conidiogenous cells arising directly from hyphae **e** conidia **f** fourteen-day-old culture on MEA. Scale bars: 10 μm (**a**), 25 μm (**b**), 10 μm (**c–e**).

Ryman 1989). In addition, *S. cavum* has obovoid and short conidia (3.1–7.8 μm), whereas *S. polyporicola* has clavate and longer conidia (6–14 μm) (Constantinescu and Ryman 1989).

Sporothrix cavum was represented by two isolates collected from the cavities produced by the woodpeckers *Dendrocopos major* on *Salix fragilis* and *Dendrocopos medius* on *Malus domestica*. It corresponds to *Sporothrix* sp. 18 in the study of Jankowiak et al. (2019c).

Discussion

Our work (Jankowiak et al. 2019a, 2019b, 2019c; this study) has led to the discovery of six novel *Sporothrix* species associated with hardwood trees in Poland. Description of these new species brings the total number of species in this genus to 62, of which 16 occur in Poland. These include the six species described here as well as *S. aurorae* (Jankowiak et al. 2019b), *S. cantabriensis* (Jankowiak et al. 2017), *S. dentifunda* (Aghayeva et al. 2005, Jankowiak et al. 2019b), *S. eucastaneae* (Jankowiak et al. 2019a, 2019b, 2021), *S. fusiformis* (Jankowiak et al. 2019a, 2019b), *S. inflata* (Jankowiak et al. 2012; Jankowiak and Bilański 2013a, 2013b), *S. inflata* '2' (Jankowiak et al. 2019a, 2019b), *S. prolifera* (Kowalski and Butin 1989; Jankowiak et al. 2019a, 2019b), *S. stenoceras*, (Kowalski and Butin 1989; Jankowiak and Bilański 2013b, Jankowiak et al. 2019b) and *S. variecibatus* (Jankowiak and Bilański 2013b).

All of the species described in this study are morphologically similar, having asexual states with hyaline or lightly pigmented conidia produced holoblastically on denticulate conidiogenous cells or directly from the hyphae. Where ascomata were present, these tended to have globose bases with elongated necks terminating in long ostiolar hyphae and allantoid or kidney-shaped ascospores not surrounded by hyaline sheaths. All of the newly described species grew optimally at 25 °C and all also grew well at 30 °C on MEA. *Sporothrix undulata* and *S. cavum* differed from the other four species in having pigmented as opposed to white cultures on MEA. All of the newly described species were recovered from hardwood ecosystems in Poland in association with bark and ambrosia beetles, nitidulid beetles, naturally occurring tree wounds or woodpecker cavities.

The six species described in this study can easily be distinguished from each other and from the other species of *Sporothrix* based on the DNA sequence comparisons. Analyses of the ITS sequence data were insufficient to distinguish between *S. cryptarchum* and *S. undulata* or between *S. cracoviensis* and *S. fusiformis*. However, analyses of sequence data for the protein-coding genes, including the βT, CAL and TEF1-α showed that *S. cracoviensis*, *S. cryptarchum*, and *S. undulata* represent distinct taxa. Furthermore, the two closely related species, *S. cryptarchum* and *S. undulata* formed a new and well-supported lineage in *Sporothrix* including species infecting wounds on a variety of hardwood trees. The species in this lineage are characterised by having both hyaline as well as pigmented conidia and kidney-shaped ascospores.

The asexual morphs of the *Sporothrix* species described in this study had variable morphology. All species had hyaline conidia produced holoblastically on denticulate conidiogenous cells that proliferate sympodially or arise directly from hyphae. *Sporothrix cryptarchum* and *S. undulata* also had pigmented globose conidia formed singly or in chains, either directly on the sides of the vegetative hyphae or on short lateral branches. The presence of two different conidial types has previously been found in other *Sporothrix* species, including *Sporothrix dimorphospora* and *S. brunneoviolacea* (Madrid et al. 2010) as well as *S. brasiliensis*, *S. globose*, and *S. mexicana* (Marimon et al. 2007).

Recently, Jankowiak et al. (2019b) provided evidence that fresh wounds on hardwood trees in Europe are preferred habitats for some *Sporothrix* species. These authors isolated 15 *Sporothrix* species from trees belonging to 12 species of angiosperms. Likewise, nine *Sporothrix* species have been described from fresh wounds on non-native *Eucalyptus* spp. and various genera of native trees in South Africa (Kamgan Nkuekam et al. 2012; Musvuugwa et al. 2016, 2020; Osorio et al. 2016).

Three species of wound-associated *Sporothrix* spp. collected during a survey reported in the study of Jankowiak et al. (2019b) were included in the present study. The greatest number of isolates (194) obtained during that survey were those of *S. undulata*. This species was found as a common associate of bleeding wounds on *Quercus robur* and *Salix fragilis*, suggesting that they might have some level of pathogenicity. The other species inhabiting wounds on hardwood trees that was collected during the survey of Jankowiak et al. (2019a) was *S. cryptarchum* (34 isolates). Transfer of this species to the sampled tree wounds was most likely by nitidulid (*Coleoptera*, *Nitidulidae*) beetles as previously noted by Jankowiak et al. (2019b) who suggested

that these insects commonly transmit *Ophiostomatales*, including *Sporothrix* species to tree wounds in Poland. Likewise, Kamgan Nkuekam et al. (2012) have demonstrated that the nitidulid beetles *Brachypeplus depressus* and *Carpophilus* spp. vector *S. candida* and *S. fumea* in the *Eucalyptus* plantations of South Africa. This association is also consistent with other studies providing compelling evidence that nitidulid beetles act as vectors of the well-known pathogens, such as *Bretziella fagacearum* (De Beer et al. 2017; Jagemann et al. 2018) and *Ceratocystis albifundus* (Heath et al. 2009).

The second largest number of isolates (81 in total) included in this study represented two species in the *S. gossypina*-complex, bringing the total number of species in that complex to 15 (De Beer et al. 2016; Wang et al. 2019). *Sporothrix cracoviensis* was represented by 45 isolates from the ambrosia beetles *Trypodendron domesticum* and *T. signatum* collected on *Fagus sylvatica* (Jankowiak et al. 2019a). This is not unusual given that an association between ambrosia beetles has recently been recorded by De Errasti et al.(2016) in a study on *Nothofagus pumelo* in Patagonia. The other species residing in this complex collected during the survey of Jankowiak et al. (2019a) is *S. fraxini* (36 isolates). This fungus was found on *Fraxinus excelsior* in association with the bark beetles *Hylesinus crenatus* and *H. varius* (Jankowiak et. al. 2019a).

The Polish study by Jankowiak et al. (2019a) revealed that, apart from *S. cracoviensis* and *S. fraxini*, five other *Sporothrix* species (*S. fusiformis*, *S. prolifera*, *S. eucastanea*, *Sporothrix* sp. 4, *Sporothrix* sp. 9) were associated with bark beetles. These findings confirm that most species in the *S. gossypina* complex are associated with galleries of conifer-infesting bark beetles worldwide (De Beer et al. 2016). The other species in the *S. gossypina*-complex were isolated from the stained oak wood (Kowalski and Butin 1989; Aghayeva et al. 2004), cankers caused by *Cryphonectria parasitica* on chestnut (Davidson 1978), a hardwood tree native to South Africa (Musvuugwa et al. 2016), and from mites infesting the infructescences (flower heads) of *Protea* in South Africa (Roets et al. 2008).

Sporothrix cavum, the remaining taxon collected from hardwood trees during the surveys that formed the basis of the present study, resided in lineage F defined by De Beer et al. (2016). This lineage includes three species, namely *S. polyporicola*, *S. dimorphospora*, and *S. inflata* '2'. Two of these species (*S. dimorphospora*, and *S. inflata* '2') are known from soil and *S. polyporicola* was isolated from basidiocarps of the polypores *Fomitopsis pinicola* and *Amaropostia stiptica* (Constantinescu and Ryman 1989; Madrid et al. 2010). The results of the present study show that species in this complex also accommodate wood-inhabiting *Sporothrix* species. Other than the fact that *S. cavum* was isolated from cavities on *Salix fragilis* and *Malus domestica* made by woodpeckers (Jankowiak et al. 2019c), nothing is known regarding the ecology or distribution of the fungus. It could, for example, be introduced into these cavities by arthropods or have some relationship with the woodpeckers themselves.

The results of this study have substantially expanded our knowledge of *Sporothrix* and the ecology of species in this genus. Broadly, the results suggest that *Sporothrix* species are common members of the *Ophiostomatales* in hardwood ecosystems in Poland. Furthermore, interesting questions have arisen that should shape future investigations regarding these fungi.

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Four new species in the *Tremella fibulifera* complex (Tremellales, Basidiomycota)

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Abstract

Samples of species close to *Tremella fibulifera* from China and Brazil are studied, and *T. fibulifera* is confirmed as a species complex including nine species. Five known species (*T. cheejenii*, *T. fibulifera* s.s., *T. "neofibulifera"*, *T. lloydiae-candidae* and *T. olens*) and four new species (*T. australe*, *T. guangxiensis*, *T. latispora* and *T. subfibulifera*) in the complex are recognized based on morphological characteristics, molecular evidence, and geographic distribution. Sequences of eight species of the complex were included in the phylogenetic analyses because *T. olens* lacks molecular data. The phylogenetic analyses were performed by a combined sequence dataset of the internal transcribed spacer (ITS) and the partial nuclear large subunit rDNA (nLSU), and a combined sequence dataset of the ITS, partial nLSU, the small subunit mitochondrial rRNA gene (mtSSU), the translation elongation factor 1-α (TEF1), the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2). The eight species formed eight independent lineages with robust support in phylogenies based on both datasets. Illustrated description of the six species including *Tremella fibulifera* s.s., *T. "neofibulifera"* and four new species, and discussions with their related species, are provided. A table of the comparison of the important characteristics of nine species in the *T. fibulifera* complex and a key to the whitish species in *Tremella* s.s. are provided.

Keywords

Multi-gene, phylogeny, taxonomy, Tremellaceae

Introduction

Tremella Pers. is characterized by being parasitic on or associated with other fungi or lichens (Bandoni and Oberwinkler 1983; Chen 1998; Pippola and Kotiranta 2008; Malysheva et al. 2015; Zhao et al. 2019) and by having a haploid unicellular yeast stage and diploid stage in its life cycle (Boekhout et al. 2011; Weiss et al. 2014; Liu et al. 2015a; Zhao et al. 2019). *Tremella* s.l. includes approximately 90 species and about 50 are recognized as lichenicolous species (Kobayasi 1939; Bandoni 1958, 1987; Lowy 1971; Bandoni and Oberwinkler 1983; Diederich 1996; Chen 1998; Kirk et al. 2008; Pippola and Kotiranta 2008; Millanes et al. 2011, 2012, 2014, 2015, 2016; Malysheva et al. 2015; Zamora et al. 2016; Zhao et al. 2019). *Tremella* s.l. is polyphyletic and was divided into five groups by Chen (1998) including Mesenterica group, Fuciformis group, Indecorata group, Foliacea group and Aurantia group based on morphological characteristics and molecular data of ITS rDNA and nLSU rDNA sequencing. Then species in Mesenterica group and Fuciformis group were allocated to *Tremella* s.s., and species in Indecorata group, Foliacea group and Aurantia group were emended as *Pseudotremella* Xin Zhan Liu et al., *Phaeotremella* Rea and *Naematelia* Fr., respectively (Liu et al. 2015b; Wedin et al. 2016; Spirin et al. 2018). Besides, *Tremella* s.l. contained lichenicolous species that defined as *Tremella* clade I, clade II, clade III, and several single *Tremella* species lineages based on rDNA sequences (Millanes et al. 2011; Liu et al. 2015a, b).

Although *Tremella* s.l. was separated into several genera due to its polyphyletism, it is still somewhat confusing because taxonomic positions of some *Tremella* species are uncertain in Tremellales, especially some species recently described from lichens (Ariyawansa et al. 2015; Malysheva et al. 2015; Millanes et al. 2015; Zamora et al. 2016, 2018). These lichenicolous species were described as *Tremella*, but they were not clustered into *Tremella* s.s. in the phylogeny (Ariyawansa et al. 2015; Malysheva et al. 2015; Millanes et al. 2015; Zamora et al. 2016, 2018). Recently, Zhao et al. (2019) described four new *Tremella* species based on the phylogenetic relationship of 19 species in *Tremella* s.s., and Li et al. (2020) published a new yeast species of *Tremella* s.s. based on multi-gene analysis.

In this study, samples of species morphologically similar to *Tremella fibulifera* characterized by cerebriform whitish basidioma and abundant clamp complexes from China and Brazil are studied. Based on morphology, geographic distribution and phylogenetic analyses *T. fibulifera* is confirmed as a species complex, which was previously mentioned by Bandoni and Oberwinkler (1983) and Malysheva et al. (2015), and nine species are involved in the complex including five known species (*T. cheejenii*, *T. fibulifera* s.s., *T. "neofibulifera"*, *T. lloydiae-candidae* and *T. olens*) and four new species (*T. australe*, *T. guangxiensis*, *T. latispora* and *T. subfibulifera*) in the present study. The aim of this paper is to outline the *T. fibulifera* complex and describe two known species (*T. fibulifera* s.s., *T. "neofibulifera"*) and the four new species based on our collections.

Materials and methods

Sampling and morphological analysis

The studied specimens were collected from Rondônia and Pernambuco states in Brazil, Yunnan, Taiwan, Guangxi, Jilin Provinces in China. They are deposited at the herbaria of Beijing Forestry University (**BJFC**), Institute of Botânica in São Paulo (**SP**) and Universidade Federal de Pernambuco, Departamento de Micologia (**URM**). Macro-morphological illustrations refer to Chen (1998) and Zamora et al. (2017) and microscopic structures refer to Pippola and Kotiranta (2008) and Malysheva et al. (2015). Special color terms followed Petersen (1996). Handmade sections of dried basidioma were examined by a Nikon Eclipse 80i (Japan) microscope (magnification × 1000) after being mounted in 5% KOH for five minutes and treated with 1% Phloxine B ($C_{20}H_4Br_4Cl_2K_2O_5$). Microscopic structures were photographed using a Nikon Digital Sight DS-L3 (Japan) or Leica ICC50 HD (Japan) camera. Microscopic structures were examined and measured in the mix solution of 5% KOH and 1% Phloxine B. To represent variation in the size of spores, 5% of measurements were excluded from each end of the range, and are given in parentheses. Stalks were excluded for basidia measurements and apices were excluded for basidiospores measurements. Length and width of at least 30 basidia and basidiospores from each specimen were measured to micrometers.

Abbreviations as follows: **L** = mean length (arithmetic average of all basidia or spores length), **W** = mean width (arithmetic average of all basidia or spores width), **Q** = L/W ratio for each specimen studied, **n (a/b)** = number of spores (a) measured from given number of specimens (b).

Molecular phylogeny

Dry specimens were used to extract DNA after pretreatment using TissuePrep (Jie Ling, China) by CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) or directly using the DNA easy Plant Mini Kit (Qiagen, China), according to the manufacturer's instructions with some modifications. The internal transcribed spacer regions (**ITS**), partial nuclear large subunit rDNA (nLSU), the translation elongation factor 1-α (TEF1), the largest and second largest subunits of RNA polymerase II (RPB1 and RPB2), the small subunit mitochondrial rRNA gene (mtSSU) sequences were amplified with primer pairs listed in the Table 1. All newly generated sequences were submitted to GenBank (Table 2).

Polymerase chain reaction (**PCR**) cycling schedule for ITS, mtSSU and TEF1 included an initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 40 s, 54–56 °C (ITS) and 56–58 °C (mtSSU, TEF1) for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min, for RPB1 and RPB2 included an initial denaturation at 95 °C for 3 min, followed by 9 cycles at 94 °C for 45 s or 1 min , 58 °C for

Table 1. Sequencing primers used in this study.

Pairs of primer	Nucleotide sequence (5'–3')	Reference
ITS		
ITS5	5'–GGAAGTAAAAGTCGTAACAAGG–3'	White et al. 1990
ITS4	3'–TCCTCCGCTTATTGATATGC–5'	
ITS1	5'–TCCGTAGGTGAACCTGCGG–3'	
Partial nLSU		
LR0R	5'–ACCCGCTGAACTTAAGC–3'	Hopple and Vilgalys 1994
LR7	5'–TACTACCACCAAGATCT–3'	
TEF1		
983F	5'–GCYCCYGGHCAYCGTGAYTTYAT–3'	Rehner 2001
1567R	3'–ACHGTRCCRATACCACCRATCTT–5'	Rehner 2001
2218R	3'–ATGACACCRACRGCRACRGTYTG–5'	Rehner and Buckley 2005
RPB1		
Af	5'–GARTGYCCDGGDCAYTTYGG–3'	Stiller and Hall 1997
Cr	3'–CCNGCDATNTCRTTRTCCATRTA–5'	Matheny et al. 2002
RPB2		
5F	5'–GAYGAYMGWGATCAYTTYGG–3'	Matheny 2005
6F	5'–TGGGGKWTGGTYTGYCCTGC–3'	Matheny 2005
7R	3'–CCCATWGCYTGCTTMCCCAT–5'	Matheny 2005
7CR	3'–CCCATRGCTTGYTTRCCCAT–5'	Matheny 2005
Fcrypto	5'–TGGGGYATGGTTTGTCCKGC–3'	Ye et al. 2012
Rcrypto	3'–CCCATGGCTTGTTTRCCCATYGC–5'	Ye et al. 2012
mtSSU		
MS1	5'–CAGCAGTCAAGAATATTAGTCAATG–3'	White et al. 1990
MS2	3'–GCGGATTATCGAATTAAATAAC–5'	White et al. 1990

45 s or 60 °C for 1 min and 72 °C for 1.5 min, then followed by 35 cycles at 95 °C for 1 min, 53 °C or 55 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min, for partial nLSU included an initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50–52 °C for 1 min, 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. PCR products were purified at the Beijing Genomics Institute (BGI), China or at the Plataforma Tecnológica de Genômica e Expressão Gênica do Centro de Biociências (UFPE) with the same primers.

Newly generated sequences in this study were aligned with additional related sequences downloaded from GenBank (Table 2) using MAFFT 7.0 online service with the Q-INS-i strategy (Katoh et al. 2019, http://mafft.cbrc.jp/alignment/server/). Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps were manually adjusted to optimize the alignment using the default parameters in BioEdit (Hall 1999). Those positions deemed ambiguous to align were excluded manually. Multi-genes were concatenated as a combined file by Mesquite version 3.2. (Maddison and Maddison 2017). Phylogenetic analyses were applied to the ITS + partial nLSU dataset and the combined ITS+partial nLSU+mtSSU+TEF1+RPB1+RPB2 dataset. Sequences of *Cryptococcus depauperatus* (Petch) Boekhoutet et al. were used as outgroup, which referred to Malysheva et al. (2015). The final concatenated sequence alignments were deposited in TreeBase (https://treebase.org/treebase-web/home.html; submission ID 28280 for ITS + partial nLSU

Table 2. Information on sequences used in this study.

Species	Sample no.	GenBank accessions						References
		ITS	Partial nLSU	mtSSU	TEF1	RPB1	RPB2	
***Tremella austral* sp. nov.**	**Dai 11539**	**MT445847**	–	–	**MT445759**	**–**	–	**Present study**
***T. austral* sp. nov.**	**Wu 154**	**MT445848**	**MT425188**	**MT483749**	**MT445760**	**–**	**MT445753**	**Present study**
T. basidiomaticola	CBS 8225	MH712822	MH712786	–	–	–	–	Zhao et al. 2019
T. basidiomaticola	CGMCC 2.5724^{T}	MH712820	MH712784	–	–	–	–	Zhao et al. 2019
T. basidiomaticola	CGMCC 2.5725	MH712821	MH712785	–	–	–	–	Zhao et al. 2019
T. brasiliensis	CBS 6966^{R}	AF444429	AF189864	KF036694	KF037200	–	KF036938	Liu et al. 2015a
T. brasiliensis	CBS 8212	KY105674	KY109886	–	–	–	–	Vu et al. 2016
T. cerebriformis	ZRL 20170101	MH712823	MH712787	–	–	–	–	Zhao et al. 2019
T. cerebriformis	ZRL 20170269	MH712824	MH712788	–	–	–	–	Zhao et al. 2019
T. cheejenii	GX 20172598	MH712825	MH712789	–	–	–	–	Zhao et al. 2019
T. cheejenii	GX 20172640	MH712826	MH712790	–	–	–	–	Zhao et al. 2019
T. dysenterica	LE 303447	KP986509	KP986542	–	–	–	–	Malysheva et al. 2015
T. dysenterica	VLA M 18599	KP986531	–	–	–	–	–	Malysheva et al. 2015
T. erythrina	HMAS 255317	MH712827	MH712791	–	–	–	–	Zhao et al. 2019
T. erythrina	HMAS 279591	MH712828	MH712792	–	–	–	–	Zhao et al. 2019
***T. fibulifera* s.s.**	**SP 211759**	**MT445850**	**MT425190**	**MT483750**	–	–	–	**Present study**
***T. fibulifera* s.s.**	**Alvarenga 471**	**MT445851**	**MT425191**	–	–	–	–	**Present study**
T. flava	CBS 8471^{R}	KY105681	KY105681	KF036699	KF037205	KF036527	KF036943	Liu et al. 2015a
T. flava	CCJ 907	AF042403	AF042221	–	–	–	–	Zhao et al. 2019
T. fuciformis	CBS 6970^{R}	KY105683	AF075476	KF036701	KF037207	KF036529	–	Liu et al. 2015a
T. fuciformis	CCJ1080	AF042410	AF042228	–	–	–	–	Malysheva et al. 2015
T. globispora	CBS 6972^{R}	AF444432	AF189869	KF036703	KF037208	KF036531	KF036947	Liu et al. 2015a
T. globispora	UBC 586	AF042425	AF042243	–	–	–	–	Zhao et al. 2019
***T. guangxiensis* sp. nov.**	**Wu 3**	**MT445843**	**MT425186**	**MT483748**	**MT445756**	**MT445746**	**MT445752**	**Present study**
***T. guangxiensis* sp. nov.**	GX 20172028	MH712829	MH712793	–	–	–	–	Zhao et al. 2019
***T. latispora* sp. nov.**	**Dai 17574**	**MT445852**	**MT425192**	**MT483751**	**MT445761**	**MT445750**	**MT445754**	**Present study**
***T. latispora* sp. nov.**	**Dai 17568**	**MT445853**	**MT425193**	**MT483752**	**MT445762**	**MT445751**	**MT445755**	**Present study**
T. laurisilvae	S-F 102408(AM4)	JN053467	JN043572	–	–	–	–	Zhao et al. 2019
T. lloydiae-candidae	VLA M 11702	KP986536	KP986559	–	–	–	–	Malysheva et al. 2015
T. lloydiae-candidae	VLA M 11703	KP986559	KP986560	–	–	–	–	Malysheva et al. 2015
T. mesenterica	CBS 6973^{R}	AF444433	AF444433	KF036705	KF037210	KF036533	KF036949	Liu et al. 2015a
T. mesenterica	FO 24610	AF042447	AF042265	–	–	–	–	Zhao et al. 2019
T. "neofibulifera"	**Wu 248**	**MT445844**	**MT425187**	–	**MT445757**	**MT445747**	–	**Present study**
T. "neofibulifera"	**Wu 243**	**MT445845**	–	–	–	**MT445748**	–	**Present study**
T. "neofibulifera"	**Wu 244**	**MT445846**	–	–	**MT445758**	**MT445749**	–	**Present study**
T. "neofibulifera"	LE 303445	KP986518	KP986547	–	–	–	–	Malysheva et al. 2015
T. resupinata	CBS 8488^{T}	AF042421	AF042239	KF036708	KF037212	KF036535	KF036951	Liu et al. 2015a
T. saccharicola	DMKU-SP23^{T}	AB915385	AB909021	–	–	–	–	Khunnamwong et al. 2019
T. saccharicola	DMKU-SP40	AB915386	AB909022	–	–	–	–	Khunnamwong et al. 2019
T. salmonea	GX 20172637	MH712851	MH712815	–	–	–	–	Zhao et al. 2019
T. samoensis	LE 303465	KP986508	KP986541	–	–	–	–	Malysheva et al. 2015
T. samoensis	VLA M 18603	KP986532	KP986555	–	–	–	–	Malysheva et al. 2015
T. shuangheensis	CBS 15561	MK050285	MK050285	MK050285	MK849087	MK849223	MK849362	Li et al. 2020

Species	Sample no.	GenBank accessions						References
		ITS	Partial nLSU	mtSSU	TEF1	RPB1	RPB2	
***T. subfibulifera* sp. nov.**	**Alvarenga 334**	**MT445849**	**MT425189**	–	–	–	–	**Present study**
T. taiwanensis	CBS 8479[R]	AF042412	AF042230	KF036709	KF037213	KF036536	KF036952	Liu et al. 2015a
T. taiwanensis	GX 20170625	MH712854	MH712818	–	–	–	–	Zhao et al. 2019
T. tropica	CBS 8483[R]	KY105697	KY109908	KF036710	KF037214	KF036537	KF036953	Liu et al. 2015a
T. tropica	CBS 8486	KY105696	KY109909	–	–	–	–	Liu et al. 2015a
T. yokohamensis	JCM 16989	HM222926	HM222927	–	–	–	–	Zhao et al. 2019
T. yokohamensis	CBS 11776	KY105698	KY109910	–	–	–	–	Malysheva et al. 2015
Cryptococcus depauperatus	CBS 7841[T]	FJ534881	FJ534911	AJ568017	KF037150	KF036471	–	Zhao et al. 2019

The samples used in this study are in bold.

dataset; submission ID 27553 for ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2 dataset) and the taxonomic novelties in MycoBank (http://www.MycoBank.org).

Phylogenetic constructions of Maximum likelihood (ML), Maximum parsimony (MP), and Bayesian analyses were performed in the CIPRES Science Gateway portal Version 3.3 (Miller et al. 2012) using tool of RAxML-HPC BlackBox 8.2.6, PAUP on XSEDE (4.a165) and Mrbayes on XSEDE 3.2.6 respectively. All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using heuristic search option with TBR branch swapping and 1000 random sequence additions. MrModeltest 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for both datasets for Bayesian analyses using MrBayes3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for two runs from random starting trees for 3 million generations (ITS + nLSU) and for 5 million generations (ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2) until the split deviation frequency value < 0.01, and trees were sampled every 100 generation. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

Phylogenetic trees were viewed by FigTree v. 1.4.2 (Rambaut 2012) and edited by Adobe Illustrator CS6 (Guide 2012). Branches that received bootstrap support for Maximum parsimony (BP), Maximum likelihood (BS) and Bayesian posterior probabilities (BPP) greater than or equal to 50% (BP/BS) and 0.95 (BPP) were considered as significantly supported, respectively.

Results

Phylogeny

The ITS + partial nLSU dataset included 50 fungal specimens representing 27 species. The dataset has an aligned length of 2282 total characters including gaps, of which 1777 characters are constant, 128 variable characters are parsimony-uninformative, and 377 are parsimony-informative. MP analysis yielded four equally parsimonious trees (TL = 1394, CI = 0.529, RI = 0.792, RC = 0.419, HI = 0.471). The best model

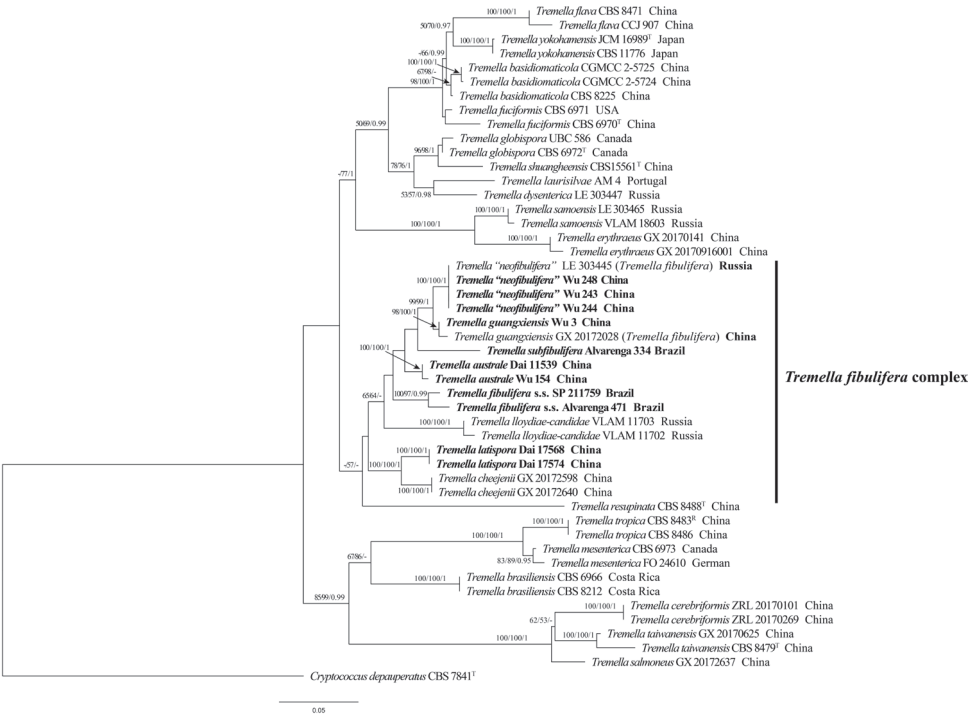

Figure 1. The Maximum likelihood tree showing phylogenetic relationship of species in *Tremella* s.s. based on the ITS + partial nLSU dataset. Bootstrap support values for MP and ML greater than 50% and BI greater than 0.95 are given at each node respectively. The samples used in this study are in bold.

for the ITS + partial nLSU dataset estimated and applied in the BI analysis was GTR. BI and ML analyses generated similar topologies as MP analysis, with an average standard deviation of split frequencies = 0.002648 (BI). The best tree obtained from the ML analysis with bootstrap values for BP, BS and BPP is shown in Fig. 1. The phylogeny shows that eight species are clustered into the *T. fibulifera* complex and four new species form four independent lineages with robust support.

The combined dataset of ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2 has an aligned length of 5113 total characters including gaps, of which 3332 characters are constant, 383 variable characters are parsimony-uninformative, and 1398 are parsimony-informative. MP analysis yielded two equally parsimonious trees (TL = 4519, CI = 0.607, RI = 0.730, RC = 0.443, HI = 0.393). The best model for the combined dataset estimated and applied in the BI analysis was GTR+I+G. BI analysis generated similar topology to MP and ML analysis, with an average standard deviation of split frequencies = 0.008566 (BI). The best tree obtained from the ML analysis with bootstrap values for BP, BS and BPP is shown in Fig. 2. The phylogeny results in similar topology to the phylogeny based on the ITS + partial nLSU sequences, which supports four new species separated from *T. fibulifera* s.s. and *T. "neofibulifera"*.

Taxonomy

Tremella fibulifera Möller, Botanische Mittheilungen aus den Tropen 8: 170 (1895)

Figs 3A, 4

Basidioma. Sessile, when fresh gelatinous, pale whitish, lobed to irregularly cerebriform, becoming pale yellowish when dry, 0.5–2.5 cm in diameter, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to thick-walled, 2.0–5.0 μm in diameter, branched, interwoven, with abundant clamp connections and medallion clamp connections (clamp complexes), thick-walled hyphae usually present near to base of basidioma; hyphidia hyaline, smooth, thin-walled, branched; swollen cells, vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 13.0–18.0(–22.0) × 9.0–16.0 μm, L = 15.7 μm, W = 14.8 μm, Q = 1.06 (n = 30/1), sometimes their width greater than length, usually longitudinally septate, rarely obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 100 μm long, 1.5–2.0 μm in diameter, slightly protuberant at apex; probasidia thin-walled, subglobose to ellipsoid, mostly proliferating directly from basidial clamps; basidiospores hyaline, thin-walled, mostly ellipsoid to slightly ovoid, apiculate, with oil drops, 7.0–10.0 × 6.0–7.0 μm, L = 8.4 μm, W = 6.5 μm, Q = 1.29–1.40 (n = 60/2), germinating by germ tubes or secondary spores; conidia occasionally present in cluster, originating from conidiophores, hyaline, thin-walled, ellipsoid to subglobose, 2.0–3.0 × 1.0–2.5 μm.

Specimens examined. Brazil Rondônia, Municipality of Jaru, in mixed forest near the airport, 9°40'S, 61°50'W, on wood, associated with old pyrenomycete stromata and litter, 10 October 1986, M. Capelari & R. Maziero 944 (SP211759, duplicate BJFC028110); Pernambuco, Recife, Jardim Botânico do Recife, on angiosperm wood, 16 May 2017, R. L. M. Alvarenga 471 (URM).

Notes. *Tremella fibulifera* was probably a species complex including *T. olens* originally from Australia and *T. neofibulifera* originally from Japan because they shared cerebriform whitish basidioma and abundant clamp complexes (Möller 1895; Bandoni and Oberwinkler 1983; Malysheva et al. 2015). Two specimens (SP211759, Alvarenga

Table 3. A morphological comparison of taxa in the *Tremella fibulifera* complex.

Taxa	Basidia (μm)	Basidiospores (μm)	Conidia (μm)	Hyphidia	Distribution	Reference
T. fibulifera	12.0–16.0	7.0–10.0	3.5	Unknown	Brazil	Möller 1895
T. fibulifera	15.0–18.0 × 9.0–13.0	8.0–9.0 × 5.0–8.0	Not observed	Unknown	Brazil	Bandoni and Oberwinkler 1983
T. fibulifera s.s.	13.0–18.0 × 9.0–16.0	7.0–10.0 × 6.0–7.0	2.0–3.0 × 1.0–2.5	Branched	Brazil	Present study
T. australe	14.0–19.0 × 13.0–17.0	8.0–10.0 × 6–8.0	Absent	Present	China	Present study
T. cheejenii	12.0–17.0 × 13.0–18.0	5.0–10.0 × 4.5–8.0	2.2–4.0 × 1.8–3.0	Branched	China	Zhao et al. 2019
T. guangxiensis	14.0–17.0 × 14.0–16.0	8.0–9.5 × 6.0–7.5	2.0–3.2 × 1.8–3.0	Branched	China	Present study
T. latispora	17.2–24.0 × 17.0–23.0	10.1–11.8 × 9.9–11.4	2.8–3.6 × 1.8–3.0	Present	China	Present study
T. lloydiae-candidae	14.0–20.0 × 13.0–16.0	7.5–10	Absent	Unknown	Japan, Russia	Malysheva et al. 2015
T. olens	Unknown	12.7–14.5	Absent	Unknown	Australia	Hooker 1860
T. neofibulifera	13.2–15.5 × 9–10	5.5–8.5 × 4.5–5.5	Absent	Unknown	Japan	Kobayasi 1939
T. "*neofibulifera*"	14.0–16.0 × 13.0–17.0	8.0–10.0 × 6.0–8.0	Absent	Parallel	China, Russia	Present study
T. subfibulifera	14.4–20.3 × 12.8–16.3	5.4–9.8 × 4.2–6.0	2.0–3.0 × 0.5–1.0	Absent	Brazil	Present study

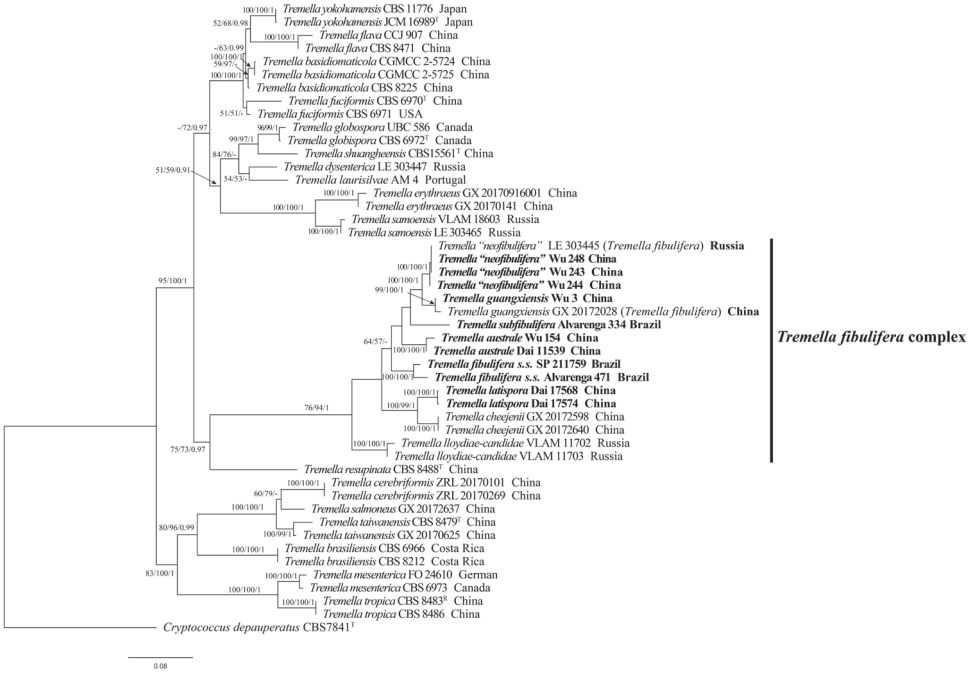

Figure 2. The Maximum likelihood tree showing phylogenetic relationship of species in *Tremella* s.s. based on the combined ITS + partial nLSU + mtSSU + TEF1 + RPB1 + RPB2 dataset. Bootstrap support values for MP and ML greater than 50% and BI greater than 0.95 are given at each node respectively. The samples used in this study are in bold.

471) from Brazil bearing the common feature of the complex formed a distinct lineage in our phylogenies (Figs 1, 2). Morphologically, the two specimens agree well with *T. fibulifera* except for the presence of conidia (Table 3). However, conidia are unstable in *T. fibulifera*. Möller (1895) described the anamorph of *T. fibulifera*, but the conidia were not observed when Bandoni and Oberwinkler (1983) re-described *T. fibulifera* based on the type designated by Möller (1895). Furthermore, *T. fibulifera* was originally described from Blumenau, Brazil, which is very close to the location of SP211759, Rondônia, Brazil. Therefore, we treat Alvarenga 471 and SP211759 as the representatives of *T. fibulifera* s.s. In addition, *T. fibulifera* s.s. are different from *T. subfibulifera* and *T. australe* by 8.51%, 9.87% sequence differences in the ITS sequences and 2.10%, 1.57% in the partial nLSU sequences respectively.

Tremella australe **F. Wu, L.F. Fan & Y.C. Dai, sp. nov.**
MycoBank No: 839825
Figs 3B, 5

Holotype. China Yunnan, Ruili, on fallen angiosperm branch, 23 April 2018, F. Wu 154 (BJFC028064).

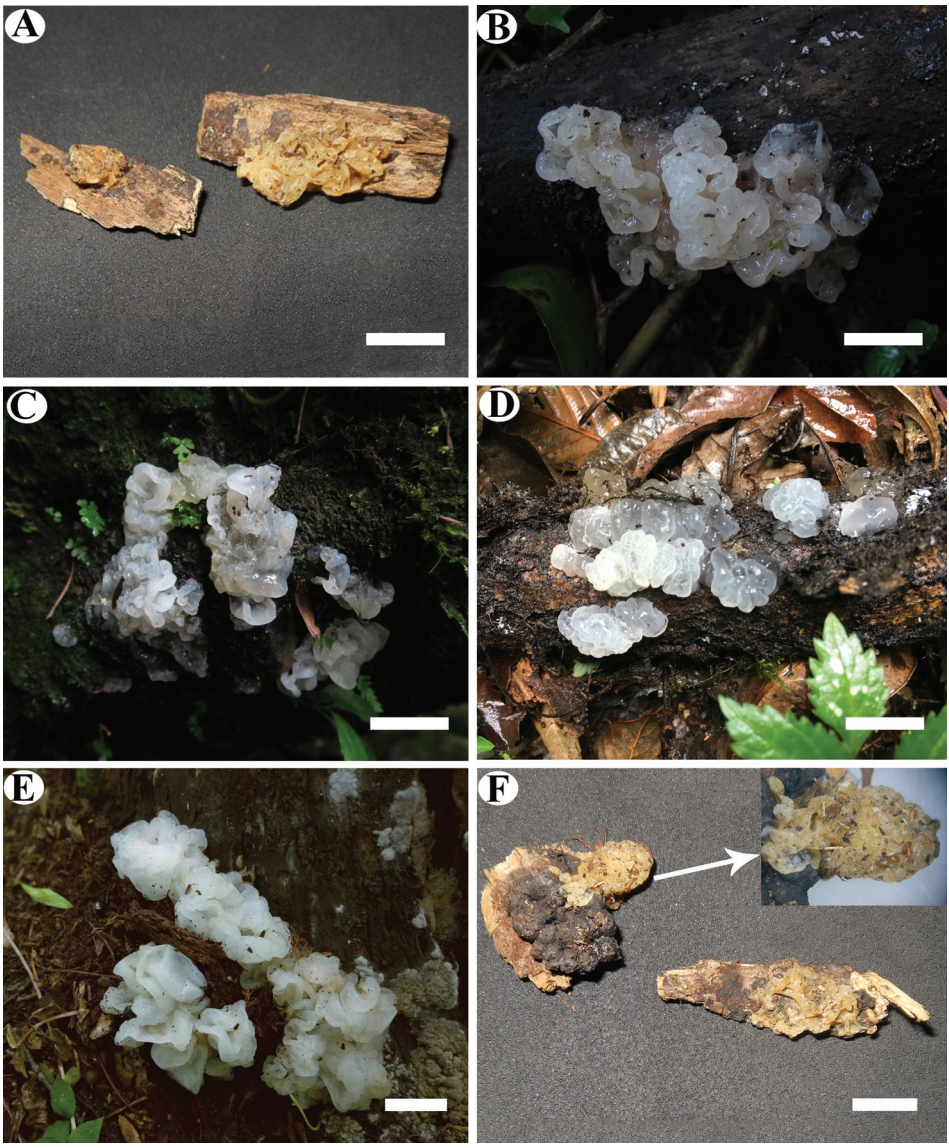

Figure 3. Basidioma **A** *Tremella fibulifera* (Alvarenga 471) **B** *T. australe* (Wu 154) **C** *T. guangxiensis* (Wu 3) **D** *T. latispora* (Dai 17568) **E** *T. "neofibulifera"* (Wu 244) **F** *T. subfibulifera* (Alvarenga 334). Scale bars: 1 cm (**A–F**).

Etymology. Refers to the distribution of this species in South Asia.

Basidioma. Sessile, when fresh soft gelatinous, creamy-white to beige, translucent, cerebriform, with thick and undulate lobes, up to 4.0 cm long, 2.0 cm broad and 2.0 cm high from base, distinctly shrinking into a film and becoming pale yellow when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to slightly thick-walled, 1.5–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp

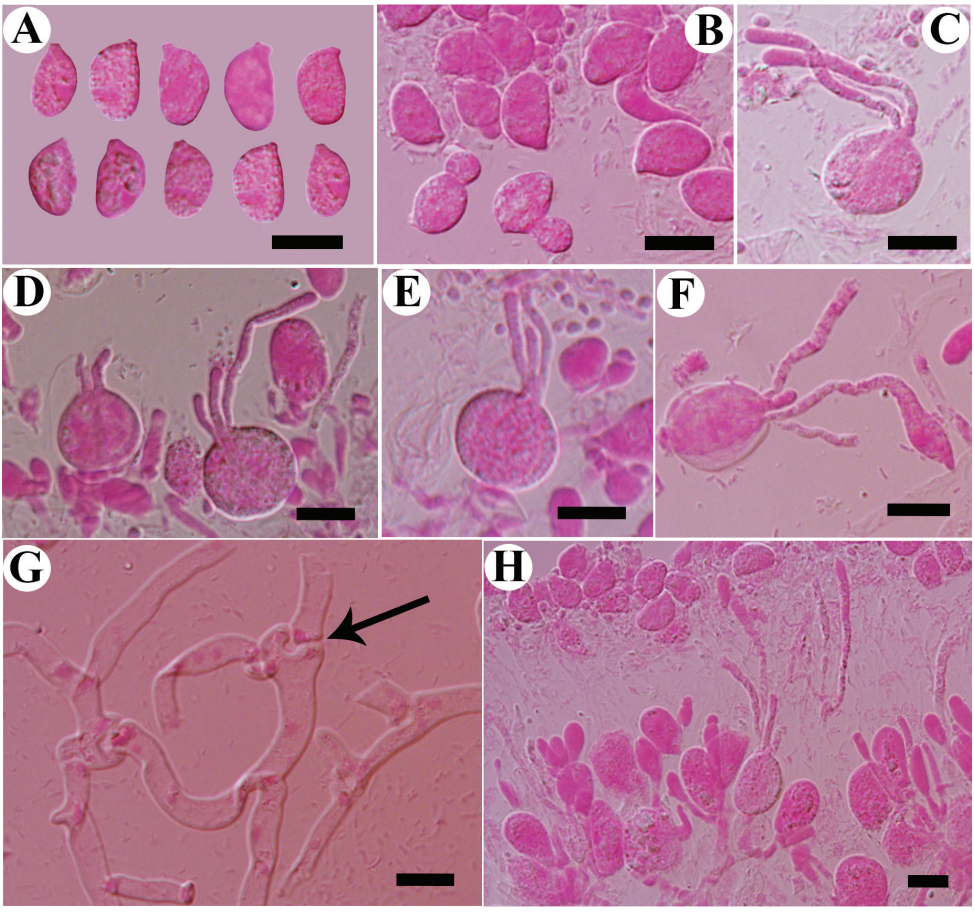

Figure 4. Microscopic structures of *Tremella fibulifera* s.s. (SP 211759) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C–F** basidia at different stages **G** hyphae with clamp connections and clamp complexes; **H** a section of hymenium. Scale bars: 10 μm (**A–H**).

complexes and anastomoses, slightly thick-walled hyphae usually present near to base of basidioma and sometimes swollen up to 8.5 μm; hyphidia hyaline, smooth, thin-walled, usually derived from the same hyphae with basidia; swollen cells, vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 14.0–19.0 × 13.0–17.0(–18.0) μm, L = 16.3 μm, W = 15.8 μm, Q = 1.03 (n = 30/1), sometimes their width greater than length, usually longitudinally septate, 2–4-celled, with obvious oil drops; sterigmata up to 20 μm long, 1.0–2.5 in diameter, slightly protuberant at apex; probasidia thin-walled, globose to subglobose, mostly proliferating directly from basidial clamps; basidiospores hyaline, thin-walled, broadly ellipsoid to ellipsoid, apiculate, with oil drops, 8.0–10.0 × 6.0–8.0 μm, L = 8.6 μm, W = 7.3 μm, Q = 1.18–1.28 (n = 60/2), germinating by germ tubes or secondary spores; conidia absent.

Additional specimen examined. (***paratype***) China Taiwan, Yilan, Linmei Road, on fallen angiosperm branch, 20 June 2009, Y.C. Dai 11539 (BJFC007408).

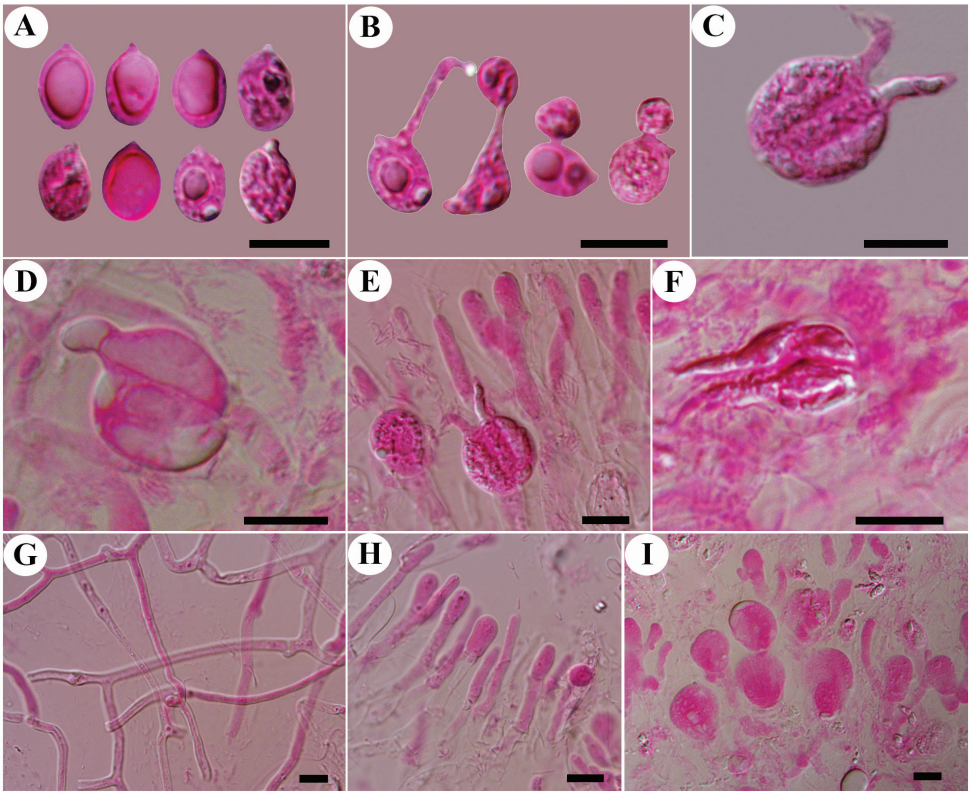

Figure 5. Microscopic structures of *Tremella australe* (Wu 154) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C–F** basidia at different stages **G** hyphae with clamp connections and clamp complexes **H** hyphidia **I** a section of hymenium. Scale bars: 10 μm (**A–I**).

Notes. *Tremella australe* formed an independent lineage with high support in our phylogenies (Figs 1, 2). The species is easily confused with *T. guangxiensis* by sharing whitish, translucent cerebriform basidioma and similar basidia and basidiospores, but *T. guangxiensis* has branched hyphidia and umbelliform conidiophores. Besides, *T. australe* are different from *T. subfibulifera*, *T. guangxiensis* and *T. "neofibulifera"* by 7.82%, 5.94% and 6.82% sequence differences in the ITS sequences and 2.13%, 3.43% and 1.25% in the partial nLSU sequences respectively.

Tremella guangxiensis **F. Wu, L.F. Fan & Y. C. Dai, sp. nov.**
MycoBank No: 839827
Figs. 3C, 6

Holotype. China. Guangxi, Jinxiu, Dayao Mountain, on angiosperm tree, 15 July 2017, F. Wu 3 (BJFC026009).

Etymology. Refers to the distribution of the species in Guangxi, China.

Basidioma. Sessile, when fresh soft gelatinous, milky to creamy-white, translucent, pustulate to irregularly cerebriform, with thick and undulate lobes, up to 4.0 cm long, 4.0 cm broad and 1.5 cm high from base, distinctly shrinking into a film and becoming lightly yellowish when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to slightly thick-walled, 2.0–6.0 µm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses, slightly thick-walled hyphae usually present near to base of basidioma and sometimes swollen up to 9.0 µm; hyphidia hyaline, smooth, thin-walled, branched; swollen cells present, hyaline, smooth and various in the shape, sometimes slightly concave; vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 14.0–17.0 × (13.6–)14.0–16.0(–17.0) µm, L = 15.9 µm, W = 14.8 µm, Q = 1.07 (n = 30/1), sometimes their

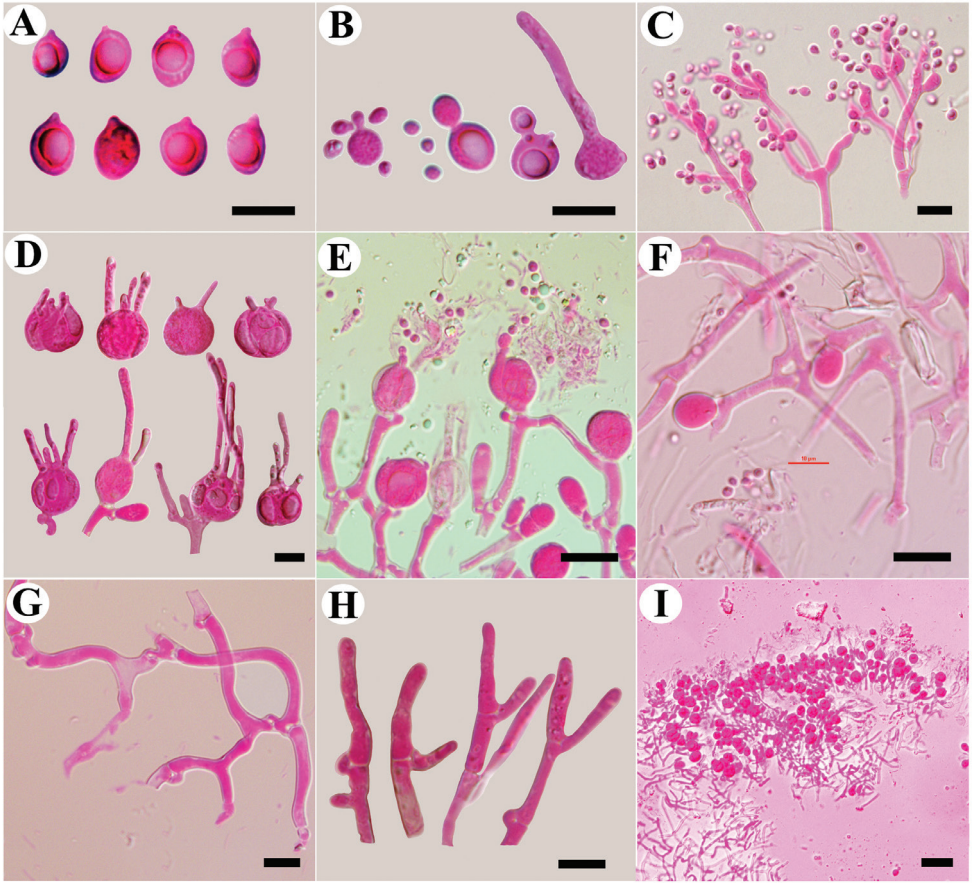

Figure 6. Microscopic structures of *Tremella guangxiensis* (Wu 3) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C** conidia and conidiophores **D** basidia at different stages **E, F** probasidia **G** hyphae with clamp connections and clamp complexes **H** hyphidia **I** a section of hymenium. Scale bars: 10 µm (**A–H**); 20 µm (**I**).

width greater than length, usually longitudinally septate, rarely obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 60 μm long, 1.5–2.0 in diameter, slightly protuberant at apex; probasidia thin-walled, clavate to ellipsoid, proliferating from terminal hyphae; basidiospores hyaline, thin-walled, broadly ellipsoid to slightly ovoid, apiculate, with oil drops, (7.5–)8.0–9.5 × 6.0–7.5(–8.0) μm, L = 8.7 μm, W = 6.8 μm, Q = 1.28 (n = 30/1), germinating by germ tubes or secondary spores; conidia massively present, originating from umbelliform conidiophores, hyaline, thin-walled, ovoid to broadly ellipsoid or fusiform to cylindrical, 2.0–3.2 × 1.8–3.0 μm.

Notes. *Tremella guangxiensis* is closely related *T. "neofibulifera"* in our phylogenies (Figs 1, 2). The most distinctive characteristic of the species is branched hyphidia and umbelliform conidiophores, but *T. "neofibulifera"* has parallel hyphidia and lacks of conidia. In addition, *T. guangxiensis* are different from *T. australe* and *T. "neofibulifera"* by 6.35% and 5.09% sequence differences in the ITS sequences and 3.39% and 1.97% in the partial nLSU sequences respectively.

Tremella latispora **F. Wu, L.F. Fan & Y. C. Dai, sp. nov.**
MycoBank No: 839828
Figs 3E, 7

Holotype. China. Yunnan, Xinping, Shimenxia Park, on stump of *Lithocarpus*, 16 June 2017, Y.C. Dai 17574 (BJFC025106).

Etymology. Refers to the species having wide basidiospores.

Basidioma. Sessile, when fresh soft gelatinous, creamy-white to lvory, translucent, pustulate to irregularly cerebriform, with thick and undulate lobes, up to 4.0 cm long, 2.0 cm broad and 1.0 cm high from base, distinctly shrinking into a film and becoming whitish to pale yellow when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, thin- to thick-walled, 1.5–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses, thick-walled hyphae usually present near to base of basidioma and sometimes swollen up to 7.5 μm; hyphidia hyaline, smooth, thin-walled, usually derived from the same hyphae with basidia; swollen cells, vesicles and haustoria absent; mature basidia thin-walled, globose to subglobose, with a basal clamp connection, 17.2–24.0(–27.0) × 17.0–23.0(–24.3) μm, L = 19.5 μm, W = 20.8 μm, Q = 0.94 (n = 30/1), commonly their width greater than length, usually longitudinally septate, occasionally obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 60 μm long, 1.5–2.0 in diameter, slightly protuberant at apex; probasidia thin-walled, ellipsoid to subglobose, proliferating from terminal hyphae; basidiospores hyaline, thin-walled, globose to subglobose, apiculate, with oil drops, (9.0–)10.1–11.8(–12.0) × (9.6–)9.9–11.4(–11.7) μm, L = 11.0 μm, W = 10.7 μm, Q = 1.03 (n = 30/1), germination by germ tubes or secondary spores; conidia massively present, originating from umbelliform conidiophores, hyaline, thin-walled, ovoid to oblong or globose to subglobose, 2.8–3.6 × 1.8–3.0 μm.

Additional specimen examined. (***paratype***) China Yunnan, Xinping, Shimenxia Park, on stump of *Lithocarpus*, 16 June 2017, Y.C. Dai 17568 (BJFC025100).

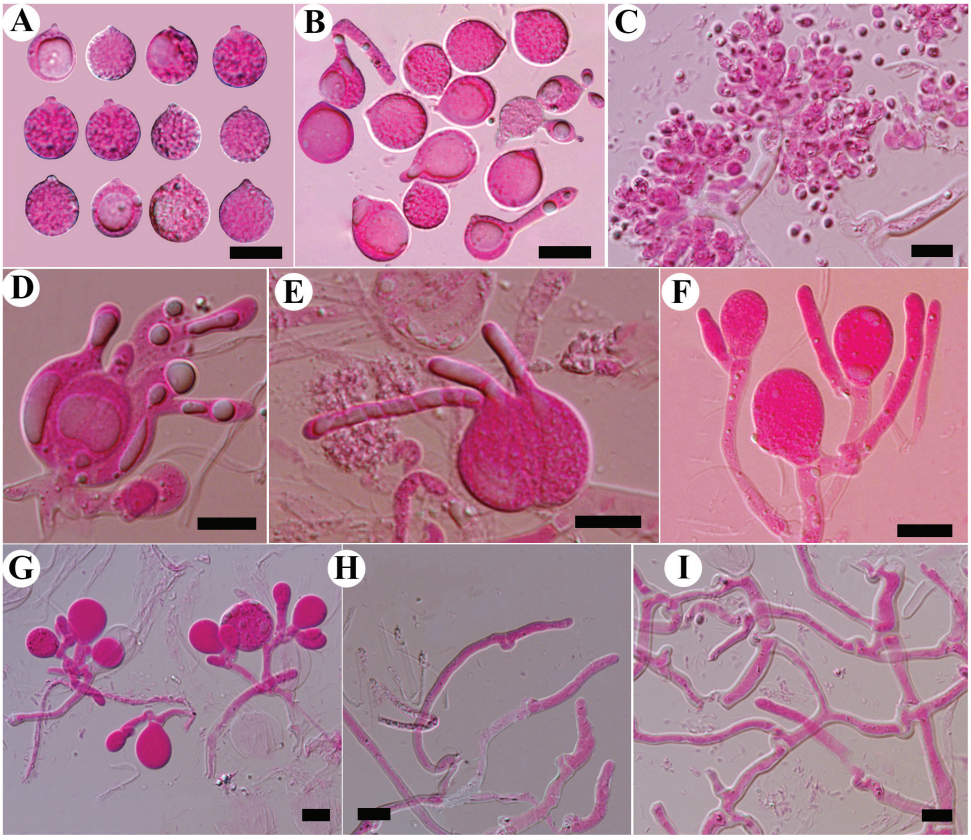

Figure 7. Microscopic structures of *Tremella latispora* (Dai 17568) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C** conidia and conidiophores **D, E** basidia **F, G** probasidia **H, I** hyphae with clamp connections and clamp complexes. Scale bars: 10 μm (**A–I**).

Notes. Phylogenetically, *Tremella latispora* formed a distinct lineage closely related to *T. cheejenii* (Figs 1, 2). Morphologically, the species has significantly larger basidia and basidiospores than *T. cheejenii* or other similar species (Table 3), and it has globose to subglobose basidiospores rather than more or less ellipsoid basidiospores in other species. And *T. latispora* are different from *T. cheejenii* and *T. fibulifera* s.s. by 4.63% and 5.09% sequence differences in the ITS sequences and 3.39% and 2.95% in the partial nLSU sequences respectively.

***Tremella "neofibulifera"* Kobayasi, Scientific Report, Tokyo Bunrika Daigaku, Section 4: 15 (1939)**
Figs 3D, 8

Basidioma. Sessile, when fresh soft gelatinous, creamy-white to pale yellowish, irregularly cerebriform or slightly foliose, with undulate lobes, up to 4.5 cm long, 2.0 cm

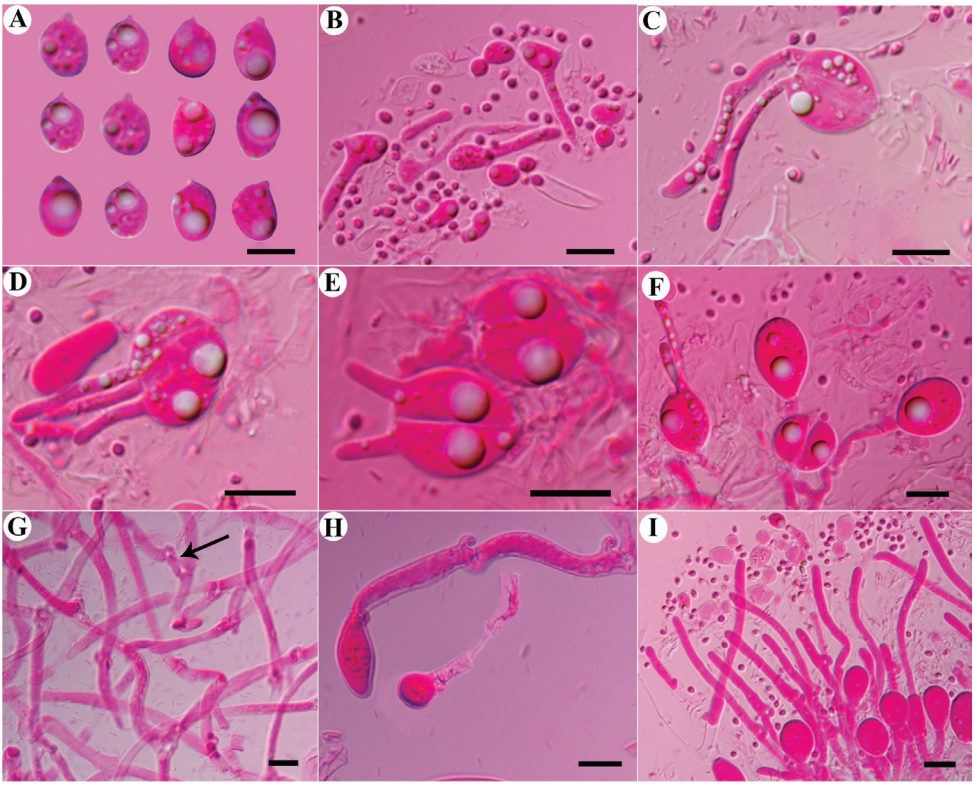

Figure 8. Microscopic structures of *Tremella "neofibulifera"* (Wu 248) **A** basidiospores **B** germination tubes of basidiospores and secondary spores **C–E** basidia at different stages **F** probasidia **G** hyphae with clamp connections and clamp complexes **H** vesicles **I** parallel hyphidia in hymenium. Scale bars: 10 μm (**A–I**).

broad and 2.5 cm high from base, becoming firmly gelatinous and invisible yellowish when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth thin- to slightly thick-walled, 2.0–6.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses, slightly thick-walled hyphae usually present near to base of basidioma, sometimes swollen up to 8.5 μm; hyphidia hyaline, smooth, thin-walled, arranged in cluster, usually parallel; vesicles infrequent, thick-walled; swollen cells and haustoria absent; mature basidia thin-walled, ovoid to subglobose, with a basal clamp connection, 14.0–16.0 × 13.0–17.0 μm, L = 14.9 μm, W = 14.8 μm, Q = 1.01 (n = 30/1), sometimes their width greater than length, usually longitudinally septate, rarely obliquely septate, 2–4-celled, with obvious oil drops; sterigmata up to 70 μm long, 1.5–2.0 in diameter, slightly protuberant at apex; probasidia thin-walled, ellipsoid to subglobose, usually proliferating from terminal hyphae; basidiospores hyaline, thin-walled, ellipsoid to broadly ellipsoid, apiculate, with oil drops, 8.0–10.0 × 6.0–8.0 μm, L = 8.9 μm, W = 6.5 μm, Q = 1.37 (n = 30/1), germination by germ tubes or secondary spores; conidia absent.

Specimens examined. China Jilin, Helong, Quanshuidong Forest Farm, on stump of *Quercus*, 15 July 2017, F. Wu 243 (BJFC031046); F. Wu 244 (BJFC031047); F. Wu 248 (BJFC031051).

Notes. Three specimens listed above from Northeast China together with LE303445 from Far East of Russia formed a distinct lineage closely related to *T. guangxiensis* in our phylogenies (Figs 1, 2). *T. neofibulifera* was originally described from Japan (Kobayasi 1939), and our studied East Asian samples have similar morphology to *T. neofibulifera* except bigger basidiospores (Table 3). We fail to loan the type of *T. neofibulifera*, and for the time being we treat our studied East Asia samples as *T. "neofibulifera"*. The current *T. "neofibulifera"* differs from other similar species of the *Tremella fibulifera* complex by the parallel hyphidia and the presence of vesicles. In addition, *T. "neofibulifera"* are different from *T. guangxiensis*, *T. australe*, *T. subfibulifera* and *T. fibulifera* s.s. by 3.15%, 5.25%, 7.14%, and 8.19% sequence differences in the ITS sequences and 2.04%, 1.32%, 3.18%, and 2.41% in the partial nLSU sequences respectively.

Tremella subfibulifera **Alvarenga, F. Wu, L.F. Fan & Y.C. Dai, sp. nov.**
MycoBank No: 839829
Figs 3F, 9

Holotype. Brazil. Pernambuco, Recife, Jardim Botânico do Recife, on angiosperm wood, 17 June 2016, R. L. M. Alvarenga 334 (URM).

Etymology. Refers to the species being similar to *Tremella fibulifera*.

Basidioma. Sessile, when fresh gelatinous, pale white, foliose to irregularly cerebriform, with undulate lobes, up to 3.0 cm long, 2.0 cm broad and 1.0 cm high from base, becoming firmly gelatinous and pale yellowish when dry, broadly attached to substratum.

Internal features. Hyphae hyaline, smooth, slightly thick-walled, 2.0–4.0 μm in diameter, branched, interwoven, with abundant clamp connections, clamp complexes and anastomoses; hyphidia, swollen cells, vesicles and haustoria absent; mature basidia thin-walled, subglobose to broadly ellipsoid, with a basal clamp connection, (14.0–)14.4–20.3(–21.0) × (9.0–)12.8–16.3(–17.8) μm, L = 17.63 μm, W = 15.05 μm, Q = 1.17 (n = 30/1), sometimes their width greater than length, usually longitudinally or obliquely septate, 2–4-celled, with obvious oil drops; mature sterigmata often collapsed, juvenile sterigmata up to 15.0 μm long, 2.0–4.0 μm in diameter, slightly protuberant at apex; probasidia thin-walled, clavate to ellipsoid, guttulate, proliferating from terminal hyphae; basidiospores hyaline, thin-walled, ellipsoid apiculate, with oil drops, (5.0–)5.4–9.8(–10.0) × (4.0–)4.2–6.0(–6.4) μm, L = 8.0 μm, W = 5.3 μm, Q = 1.50 (n = 30/1); conidia massively present, originating from umbelliform conidiophores, hyaline, thin-walled, variously shaped, ellipsoid, fusiform to cylindrical, 2.0–3.0 × 0.5–1.0 μm.

Notes. *Tremella subfibulifera* nested in the clade of the *T. fibulifera* complex, and formed an independent lineage. It resembles *T. fibulifera* s.s., but *T. fibulifera* s.s. has larger basidiospores (7.0–10.0 × 6.0–7.0 μm vs. 5.4–9.8 × 4.2–6.0 μm) and the pres-

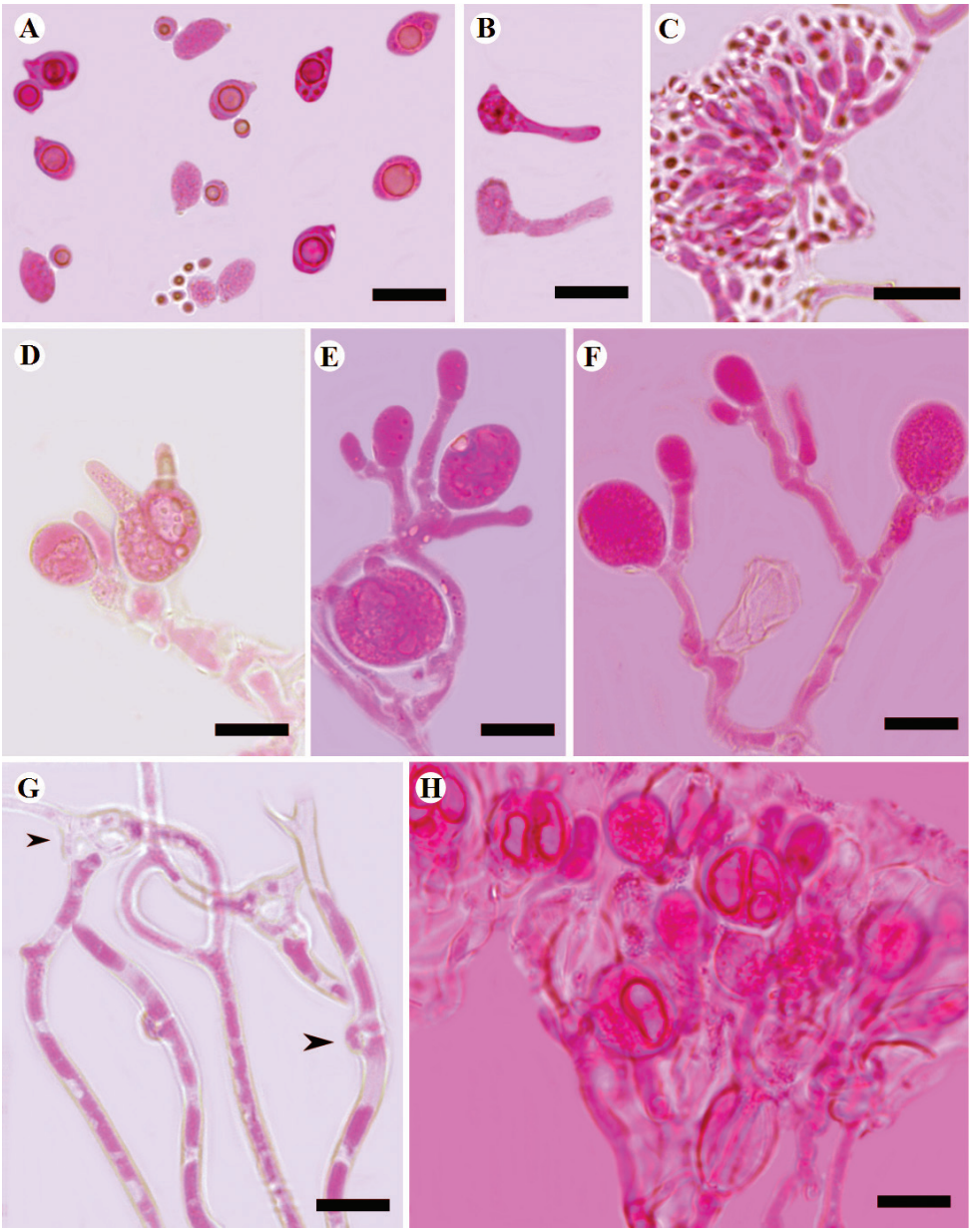

Figure 9. Microscopic structures of *Tremella subfibulifera* (Alvarenga 334) **A** basidiospores and secondary spores **B** germination tubes of basidiospores **C** conidia and conidiophores **D–F** basidia and probasidia **G** hyphae with clamp connections and clamp complexes **H** a section of hymenium. Scale bars: 10 μm (**A–H**).

ence of branched hyphidia (Table 3). In addition, *T. subfibulifera* are different from *T. australe* and *T. fibulifera* s.s. by 6.19% and 7.85% sequence differences in the ITS sequences and 2.23% and 2.10% in the partial nLSU sequences respectively.

Discussion

Tremella fibulifera was originally described from Blumenau of Brazil (Möller 1895); later two similar species, *T. olens* and *T. neofibulifera*, were respectively described from Tasmania of Australia and Simotuke of Japan (Hooker 1860; Kobayasi 1939). The Russian Far East specimen LE303445 was identified as *T. fibulifera* by Malysheva et al. (2015). Our results demonstrated the Northeastern Chinese specimens and Russian Far East specimen formed an independent lineage, and this lineage is distantly related to the lineage formed by two Brazilian specimens, SP 211759 and Alvarenga 471 (Figs 1, 2). The location of SP 211759 is near to the type locality of *T. fibulifera*. So, we treat SP 211759 and Alvarenga 471 as representatives of *T. fibulifera* s.s. Molecular data are not available from type or type locality specimens of *T. neofibulifera*. Neither is its type re-examined, but the Northeastern Chinese specimens have more or less similar morphology as the description of *T. neofibulifera*, so we temporarily treat Northeast Chinese specimens and Russian Far East specimen as *T. "neofibulifera"*.

The Southern Chinese specimen GX20172028 was also identified as *Tremella fibulifera* by Zhao et al. (2019), but it clustered with another Southern Chinese specimen Wu 3 into a distinct lineage which is closely related to *T. "neofibulifera"* (Figs 1, 2). *T. guangxiensis* is different from *T. "neofibulifera"* by 5.09% sequence differences in the ITS sequences and 1.97% in the partial nLSU sequences respectively. In addition, the Southern Chinese specimens have translucent basidioma, branched hyphidia and umbelliform conidiophores, and they are readily distinguished from *T. "neofibulifera"*. So, these two specimens are identified as a new species *T. guangxiensis*.

Seven species, *Tremella fibulifera*, *T. olens*, *T. "neofibulifera"*, *T. guangxiensis*, *T. australe*, *T. latispora* and *T. subfibulifera* have cerebriform whitish basidioma and abundant clamp complexes, and they nested in the same clade. So, we treat these seven species as members of the *T. fibulifera* complex.

Tremella lloydiae-candidae Wojewoda and *T. cheejenii* Xin Zhan Liu & F.Y. Bai also have whitish basidioma and similar micro-morphology with *T. fibulifera*, but clamp complexes were not observed (Malysheva et al. 2015; Zhao et a. 2019), and we did not examine their types. Because these two species are nested in the same clade as other species of the *T. fibulifera* complex with robust support in our phylogenies (Figs 1, 2), we treat them as members of the *T. fibulifera* complex, too.

Currently, more than 30 morphological characteristics are applied for identification species of *Tremella* s.s. (Chen 1998; Zhao et al. 2019), and some features including basidioma color and basidia shape are variable at different stages. The shape and size of basidiospores are relatively stable characteristics for each species, but they are very similar among some species in the *T. fibulifera* complex; that is why several taxa were previously treated as *T. fibulifera* s.l. (Malysheva et al. 2015; Zhao et al. 2019). Consequently, combined morphology and molecular evidence are essential to distinguish species within the complex, and ITS + partial nLSU dataset are selected for species delimitation.

Key to the whitish species in *Tremella* s. s.

1	Basidiospores > 10 μm long	**2**
–	Basidiospores < 10 μm long	**5**
2	Basidioma resupinate	***T. resupinata***
–	Basidioma pustulate to irregularly cerebriform or foliose	**3**
3	Basidiospores > 17 μm long	***T. cerebriformis***
–	Basidiospores < 17 μm long	**4**
4	Basidiospores > 12 μm long	***T. olens***
–	Basidiospores < 12 μm long	***T. latispora***
5	Basidia with stalks	**6**
–	Basidia without stalks	**8**
6	Basidia < 13 μm wide	***T. yakohamensis***
–	Basidia > 13 μm wide	7
7	Basidiospores mostly broader than long	***T. globispora***
–	Basidiospores mostly longer than broad	***T. cheejenii***
8	Basidia with sterigmata shorter than 35 μm	**9**
–	Basidia with sterigmata longer than 35 μm	**11**
9	Basidiospores < 6 μm wide	***T. subfibulifera***
–	Basidiospores > 6 μm wide	**10**
10	Hyphae with clamp complexes and anastomoses	***T. australe***
–	Hyphae without clamp complexes and anastomoses	***T. lloydiae-candidae***
11	Basidioma filamentous lobes, conjunctive as a ball	***T. hainanensis***
–	Basidioma pustulate to irregularly cerebriform or foliose	**12**
12	Basidiospores < 6 μm wide	***T. fuciformis***
–	Basidiospores > 6 μm wide	13
13	Hyphidia parallel; conidia absent	***T. "neofibulifera"***
–	Hyphidia branched; conidia present	**14**
14	Basidioma pustulate to irregularly cerebriform; basidia with sterigmata up to 60 μm; conidia originating from umbelliform conidiophores	***T. guangxiensis***
–	Basidioma lobed to irregularly cerebriform; basidia with sterigmata up to 100 μm; conidia not originating from umbelliform conidiophores	***T. fibulifera* s.s.**

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Novel saprobic *Hermatomyces* species (Hermatomycetaceae, Pleosporales) from China (Yunnan Province) and Thailand

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Abstract

During our survey of the diversity of woody litter fungi in China and Thailand, three *Hermatomyces* species were collected from dead woody twigs of *Dipterocarpus* sp. (Dipterocarpaceae) and *Ehretia acuminata* (Boraginaceae). Both morphology and multigene analyses revealed two taxa as new species (*Hermatomyces turbinatus* and *H. jinghaensis*) and the remaining collections as new records of *H. sphaericus*. *Hermatomyces turbinatus* is characterized by 1) dimorphic conidia, having circular to oval lenticular conidia and 2) turbinate conidia consisting of two columns with two septa composed of 2–3 cells in each column. *Hermatomyces jinghaensis* is characterized by dimorphic conidia, having circular to oval lenticular conidia and clavate or subcylindrical to cylindrical conidia and consisting of one or two columns with 6–8 cells in each column. Phylogenetic analyses of combined LSU, ITS, *tub*2, *tef*1-α and *rpb*2 sequence data supports the placement of these new taxa within Hermatomycetaceae with high statistical support.

Keywords

2 new species, hyphomycetes, phylogeny, taxonomy, woody litter fungi

Introduction

Over the past few decades, the number of studies using a molecular-based approach to study microfungal diversity in the greater Mekong subregion (GMS) has increased rapidly, especially on freshwater and woody litter fungi from China (Yunnan Province) and Thailand (Hapuarachchi et al. 2019; Dong et al. 2020; Li et al. 2020; Monkai et al. 2020; Wanasinghe et al. 2020, 2021; Mortimer et al. 2021). Hyde et al. (2018) reported that about 96% of fungi from Thailand are new to science. Feng and Yang (2018) estimated 104,000 fungal species currently exist in Yunnan Province, China; however, only about 6,000 are extant. Therefore, further studies need to be conducted to fill gaps in knowledge regarding the diversity, taxonomy and phylogeny of microfungi in the GMS. Supporting this obligation, we have begun to study plant-based ascomycetes in GMS. The current study accounts for hermatomyces-like ascomycetes recovered from the woody litter in China (Yunnan Province) and Thailand.

Hermatomyces was introduced by Spegazzini (1911) with *H. tucumanensis* as the type species. Doilom et al. (2017) accommodated *Hermatomyces* in Lophiotremataceae based on combined LSU, SSU, *tef*1-α and *rpb*2 sequence data. Later, Hashimoto et al. (2017) validated Hermatomycetaceae (Hermatomycetaceae Locq. 1984 was not validly published, Art. 39.1) to accommodate the genus *Hermatomyces*. This genus is known only by its asexual morph that is characterized by sporodochial conidiomata and dimorphic (lenticular or cylindrical) conidia of one or two types. The lenticular conidia are globose to subglobose, hyaline to pale brown peripheral cells with dark brown central cells, and the cylindrical conidia is hyaline, cylindrical to subcylindrical or turbinate and consisting of 1–4 columns of 2–12 cells (Spegazzini 1911; Tibpromma et al. 2016; Hashimoto et al. 2017; Hyde et al. 2019; Pem et al. 2019; Phukhamsakda et al. 2020).

Based on morphological comparisons and phylogenetic affinities, Koukol et al. (2018) revised *Hermatomyces* species and described five new species (viz. *H. bifurcatus*, *H. constrictus*, *H. megasporus*, *H. sphaericoides* and *H. verrucosus*) and one new combination, *H. reticulatus*, from Panama. Accordingly, *H. chromolaenae*, *H. saikhuensis*, *H. tectonae* were treated as *H. sphaericus* and *H. subiculosus*, *H. chiangmaiensis*, *H. thailandicus* were synonymized with *H. reticulatus*, *H. krabiensis* and *H. indicus*, respectively (Koukol et al. 2018). These are probably species complexes that need more detailed study. Subsequent studies introduced *H. bauhiniae*, *H. biconisporus*, *H. clematidis*, *H. trangensis* and *H. truncates* into *Hermatomyces* (Tibpromma et al. 2018; Hyde et al. 2019; Koukol et al. 2019; Nuankaew et al. 2019; Phukhamsakda et al. 2020). Currently, 24 species are recognized in *Hermatomyces* (Koukol et al. 2018, 2019; Nuankaew et al. 2019; Delgado et al. 2020; Phukhamsakda et al. 2020; Table 2).

Our investigation led to the discovery of three *Hermatomyces* species, including two novel species, on dead woody-based substrates. Morphological illustrations and multi-gene phylogenetic analyses with ML, MP and BI of combined LSU, ITS, *tub*2, *tef*1-α and *rpb*2 sequence data are used to confirm the phylogenetic placement of the novel species within *Hermatomyces*.

Materials and methods

Sample collection, examination and isolation

Woody litter samples were collected from China (Yunnan Province) during the dry season (December 2019) and Thailand (Tak Province) during the wet season (August 2019). Samples were brought to the laboratory using plastic Ziploc bags. Fungal specimens were then examined using a stereomicroscope (Olympus SZ61, China). Pure cultures were obtained via single spore isolation on potato dextrose agar (PDA) following the methods described in Senanayake et al. (2020). Cultures were incubated at 25 °C for three weeks. Micro-morphological structures were photographed using a Nikon compound microscope (Nikon ECLIPSE Ni) fitted with a Canon (EOS 600D) digital camera. Measurements were taken using the Tarosoft (R) Image Frame Work program. Figures were processed using Adobe Photoshop CS6. Type specimens were deposited in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (**KUN-HKAS**). Ex-type living cultures were deposited at the Culture Collection of Mae Fah Luang University (**MFLUCC**) and Kunming Institute of Botany Culture Collection (**KUMCC**).

DNA extraction, amplification and sequencing

DNA extraction, amplification, sequencing, sequence alignment and phylogenetic analyses follow the methods of Dissanayake et al. (2020) with the following details. Two partial rDNA genes and three protein coding genes were used in our study, including internal transcribed spacer region (ITS) using primer pair ITS5/ITS4 (White et al. 1990), 28S large subunit nuclear ribosomal (LSU) using primer pair LR0R/LR5 (Vilgalys and Hester 1990), translation elongation factor 1-alpha gene (*tef*1-α) using primer pair EF1-983F/EF1-2218R (Rehner and Buckley 2005), RNA polymerase II second largest subunit (*rpb*2) using primer pair fRPB2-5F/fRPB2-7cR (Liu et al. 1999) and β-tubulin (*tub*2) using primer pair T1/T22 (O'Donnell and Cigelnik 1997). Amplification reactions were performed in a total volume of 25 µL of PCR mixtures containing 8.5 µL ddH_2O, 12.5 µL 2× PCR MasterMix (TIANGEN Co., China), 2 µL DNA template and 1 µL of each primer. The PCR thermal cycle program for LSU, ITS, *tef*1-α and *rpb*2 were set as described in Tibpromma et al. (2018). The PCR amplification condition of *tub*2 was set as denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 45 seconds, annealing at 56 °C for 50 seconds and extension at 72 °C for 1 minute, with a final extension step at 72 °C for 10 minutes. PCR products were sent to the Qingke Company, Kunming City, Yunnan Province, China, for sequencing. Sequences were deposited in GenBank (Table 1).

Sequence alignment and phylogenetic analyses

Representative species used in the phylogenetic analyses were selected based on previous publications (Koukol et al. 2018; Nuankaew et al. 2019; Delgado et al. 2020;

Table 1. GenBank accession numbers of sequences used for the phylogenetic analyses.

Organism	Strain number	GenBank accession numbers					Reference
		LSU	ITS	*tub2*	*tef1-α*	*rpb2*	
Anteaglonium globosum	ANM 925.2[T]	GQ221879	NA	NA	GQ221925	NA	Mugambi and Huhndorf (2009)
A. parvulum	MFLUCC 14-0821	KU922915	NA	NA	KU922921	NA	Jayasiri et al. (2016)
Hermatomyces amphisporus	CBS 146610	LR812664	LR812664	NA	NA	NA	Delgado et al. (2020)
H. amphisporus	CBS 146611	NA	LR812663	LR812674	LR812658	LR812669	Delgado et al. (2020)
H. amphisporus	CBS 146612	NA	LR812665	LR812675	LR812659	LR812670	Delgado et al. (2020)
H. amphisporus	CBS 146613	LR812662	LR812662	LR812673	LR812657	LR812668	Delgado et al. (2020)
H. amphisporus	CBS 146614	LR812666	LR812666	LR812676	LR812660	LR812671	Delgado et al. (2020)
H. amphisporus	CBS 146615	LR812667	LR812667	LR812677	LR812661	LR812672	Delgado et al. (2020)
H. bauhiniae	MFLUCC 16-0395[T]	MK443378	MK443382	NA	MK443384	MK443386	Hyde et al. (2019)
H. biconisporus	KUMCC 17-0183[T]	MH260296	MH275063	NA	MH412771	MH412755	Tibpromma et al. (2018)
H. bifurcatus	CCF 5899	LS398262	LS398262	LS398441	LS398416	LS398343	Koukol et al. (2018)
H. bifurcatus	CCF 5900[T]	LS398263	LS398263	LS398442	LS398417	LS398344	Koukol et al. (2018)
H. clematidis	MFLUCC 17-2085[T]	MT214556	MT310603	NA	MT394735	MT394684	Phukhamsakda et al. (2020)
H. constrictus	CCF 5904[T]	LS398264	LS398264	LS398443	LS398418	LS398345	Koukol et al. (2018)
H. indicus	MFLUCC 14-1143[T1]	KU764692	KU144920	NA	KU872754	KU712488	Doilom et al. (2017)
H. indicus	MFLUCC 14-1144	KU764693	KU144921	NA	KU872755	KU712489	Doilom et al. (2017)
H. indicus	MFLUCC 14-1145	KU764694	KU144922	NA	KU872756	KU712490	Doilom et al. (2017)
H. iriomotensis	KH 361[T]	LC194367	LC194483	NA	LC194394	LC194449	Hashimoto et al. (2017)
H. jinghaensis	**HKAS 112167[T]**	**MW989519**	**MW989495**	**NA**	**MZ042642**	**NA**	**This study**
H. krabiensis	MFLUCC 16-0249[T]	KX525742	KX525750	NA	KX525758	KX525754	Tibpromma et al. (2016)
H. krabiensis (*H. chiangmaiensis*)	MFLUCC 16-2817[T2]	KY559394	NA	NA	NA	NA	Tibpromma et al. (2017)
H. megasporus	CCF 5897	NA	LS398265	LS398444	LS398419	LS398346	Koukol et al. (2018)
H. megasporus	CCF 5898[T]	LS398266	LS398266	LS398445	LS398420	NA	Koukol et al. (2018)
H. nabanheensis	KUMCC 16-0149[T]	KY766059	KY766058	NA	KY766061	NA	Hyde et al. (2017)
H. pandanicola	MFLUCC 16-0251[T]	KX525743	KX525751	NA	KX525759	KX525755	Tibpromma et al. (2016)
H. reticulatus	CCF 5893	LS398267	LS398267	LS398446	LS398421	LS398347	Koukol et al. (2018)
H. reticulatus (*H. subiculosus*)	MFLUCC 15-0843[T3]	KX259523	KX259521	NA	KX259527	KX259529	Hyde et al. (2016)
H. sphaericoides	CCF 5896	NA	LS398271	LS398448	LS398425	LS398351	Koukol et al. (2018)
H. sphaericoides	CCF 5908[T]	LS398273	LS398273	LS398450	LS398427	LS398352	Koukol et al. (2018)
H. sphaericoides	CCF 5907	NA	LS398272	LS398449	LS398426	NA	Koukol et al. (2018)
H. sphaericoides	CCF 5895	LS398270	LS398270	LS398447	LS398424	LS398350	Koukol et al. (2018)
H. sphaericus	PMA 116080	LS398281	LS398281	LS398454	LS398431	LS398356	Koukol et al. (2018)
H. sphaericus	PMA 116081	NA	LS398283	LS398455	LS398432	LS398357	Koukol et al. (2018)
H. sphaericus	PRM 946201	NA	LS398284	LS398456	LS398433	LS398358	Koukol et al. (2018)
H. sphaericus	PRC 4116	NA	LS398275	NA	NA	NA	Koukol et al. (2018)
H. sphaericus	PRC 4105	NA	LS398286	NA	NA	NA	Koukol et al. (2018)
H. sphaericus	PRC 4104	NA	LS398278	LS398453	LS398430	LS398355	Koukol et al. (2018)
H. sphaericus	PRC 4100	NA	LS398277	LS398452	LS398429	LS398354	Koukol et al. (2018)
H. sphaericus	PRC 4106	NA	LS398279	NA	NA	NA	Koukol et al. (2018)
H. sphaericus	PMA 116085	NA	LS398280	NA	NA	NA	Koukol et al. (2018)
H. sphaericus	PMA 116082	NA	LS398285	NA	NA	NA	Koukol et al. (2018)
H. sphaericus	KZP 462	NA	LS398287	LS398457	LS398434	LS398359	Koukol et al. (2018)
H. sphaericus	PRC 4117	NA	LS398276	NA	NA	NA	Koukol et al. (2018)
H. sphaericus (*H. chromolaenae*)	MFLUCC 16-2818[T4]	KY559393	NA	NA	NA	NA	Tibpromma et al. (2017)
H. sphaericus (*H. saikhuensis*)	MFLUCC 16-0266[T5]	KX525740	KX525748	NA	KX525756	KX525752	Tibpromma et al. (2016)
H. sphaericus (*H. saikhuensis*)	MFLUCC 16-0267	KX525741	KX525749	NA	KX525757	KX525753	Tibpromma et al. (2016)
H. sphaericus (*H. tectonae*)	MFLUCC 14-1140[T6]	KU764695	KU144917	NA	KU872757	KU712486	Doilom et al. (2017)
H. sphaericus (*H. tectonae*)	MFLUCC 14-1141	KU764696	KU144918	NA	KU872758	NA	Doilom et al. (2017)
H. sphaericus (*H. tectonae*)	MFLUCC 14-1142	KU764697	KU144919	NA	NA	KU712487	Doilom et al. (2017)

Organism	Strain number	GenBank accession numbers					Reference
		LSU	ITS	*tub*2	*tef*1-α	*rpb*2	
H. sphaericus	**MFLUCC 21-0036**	**MW989516**	**MW989492**	**MZ042643**	**MZ042639**	**MZ042636**	**This study**
H. sphaericus	**KUMCC 20-0231**	**MW989517**	**MW989493**	**MZ042644**	**MZ042640**	**MZ042637**	**This study**
H. trangensis	BCC 80741[T]	KY790600	KY790598	NA	KY790606	KY790604	Nuankaew et al. (2019)
H. trangensis	BCC 80742	KY790601	KY790599	NA	KY790607	KY790605	Nuankaew et al. (2019)
H. tucumanensis	CCF 5912	LS398288	LS398288	LS398458	LS398435	LS398360	Koukol et al. (2018)
H. tucumanensis	CCF 5913	LS398289	LS398289	LS398459	LS398436	LS398361	Koukol et al. (2018)
H. tucumanensis	CCF 5915	LS398290	LS398290	LS398460	LS398437	LS398362	Koukol et al. (2018)
H. turbinatus	**MFLUCC 21-0038[T]**	**MW989518**	**MW989494**	**MZ042645**	**MZ042641**	**MZ042638**	**This study**
H. verrucosus	CCF 5903[T]	LS398292	LS398292	LS398462	LS398439	LS398364	Koukol et al. (2018)
H. verrucosus	CCF 5892	LS398291	LS398291	LS398461	LS398438	LS398363	Koukol et al. (2018)

Phukhamsakda et al. 2020). Sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/), and their accession numbers are listed in Table 1. The newly generated sequences in this study were assembled by BioEdit 7.0.9.0 (Hall 1999). Individual gene regions were separately aligned in MAFFT v.7 web server (http://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019). The alignments of each gene were improved by manually deleting the ambiguous regions plus gaps and combined using BioEdit 7.2.3. Final alignments containing LSU, ITS, *tub*2, *tef*1-α and *rpb*2 were converted to NEXUS format (.nxs) using CLUSTAL X (2.0) (Thompson et al. 1997) and processed for Bayesian and maximum parsimony analysis. The FASTA format was changed into PHYLIP format via the Alignment Transformation Environment (ALTER) online program (http://www.sing-group.org/ALTER/) and used for maximum likelihood analysis (ML).

ML was carried out in CIPRES Science Gateway v.3.3 (http://www.phylo.org/portal2/; Miller et al. 2010) using RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis 2014) with the GTRGAMMA substitution model and 1,000 bootstrap iterations. Maximum parsimony analysis (MP) was performed in PAUP v. 4.0b10 (Swofford 2002) with the heuristic search option and Tree-Bisection-Reconnection (TBR) of branch-swapping algorithm for 1,000 random replicates. Branches with a minimum branch length of zero were collapsed, and gaps were treated as missing data (Hillis and Bull 1993).

Bayesian analysis was executed in MrBayes v.3.2.2 (Ronquist et al. 2012). The model of evolution was estimated using MrModeltest v. 2.3 (Nylander et al. 2008) via PAUP v. 4.0b10 (Ronquist and Huelsenbeck 2003). The SYM+I+G for LSU and ITS; HKY+I for *tub*2; GTR+I+G for *tef*1-α and *rpb*2 were used in the final command. Markov chain Monte Carlo sampling (MCMC) in MrBayes v.3.2.2 (Ronquist et al. 2012) was used to determine posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002). Bayesian analyses of six simultaneous Markov chains were run for 2,000,000 generations and trees were sampled and printed to output at every 200 generations (resulting in 10,001 total trees). The first 25% of sampled trees were discarded as part of the burn-in procedure, the remaining 7,501 trees were used to create the consensus tree and the average standard deviation of split frequencies was set as 0.01.

Phylogenetic trees were visualized in FigTree v1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/; Rambaut 2012), the tree was edited using Microsoft PowerPoint before

Table 2. Synopsis of the morphological characteristics of *Hermatomyces* species.

Species	Lenticular conidia size (µm)	Cylindrical / turbinate conidia feature			Host	Country	Reference
		Shape	Length × width (µm)	Number of columns (cells)			
Hermatomyces amphisporus	27–36(–38) × 18–29(–31)	Cylindrical, pyriform or turbinate	30–38 × 20–26	2(–4) (6–12 cells)	*Cyathea* sp., *Sabal minor*	Mexico, USA	Castañeda-Ruiz and Heredia (2000); Delgado et al. (2020)
H. bauhiniae	25–36 × 15–20	Cylindrical	20–28 × 8–11	1 (2–3-septate)	*Bauhinia variegata*	Thailand	Hyde et al. (2019)
H. biconisporus	28–34 × 15–25	Cylindrical	32–39 × 14.5–26	1–2 (3–4 cells)	*Pandanus* sp.	China	Tibpromma et al. (2018)
H. bifurcatus	(24–)30–36.5(–41) × (18–)21.5–26(–28)	Cylindrical	Apex: 7–16 × 7–12 Basal: 9–14 × 13–18.5	2 (2–3 cells)	Unknown	Panama	Koukol et al. (2018)
H. chromolaenae	9.2–10.4 × 10.2–11.5	NA	NA	NA	*Chromolaena odorata*	Thailand	Tibpromma et al. (2017)
H. clematidis	30–45 × 24–31	Cylindrical	29–35 × 12–14	1–2 (5–6 cells)	*Clematis sikkimensis*	Thailand	Phukhamsakda et al. (2020)
H. constrictus	(22–)25.5–29.5(–32) × 19–23.5(–27.5)	Cylindrical	Lower cells: (20–)24–30.5(–37) × 12–17 Upper cells: (16–)20–26(–30) × 8–14	1 (2 cells)	*Bauhinia cumanensis*	Panama	Koukol et al. (2018)
H. dimorphus	35–55 × 15–20	Cylindrical	15–40 × 10–15	4 (7 cells)	Unknown	India	Rao and de Hoog (1986)
H. indicus	18–30 × 11.5–19	Turbinate	22.4–35.4 × 11.4–21.6	2 (6–7 cells)	*Phoenix rupicola*	India	Prasher and Prasher (2014)
H. iriomotensis	30–36 × 20–27	Cylindrical	20.5–33 × 7–12.5	1–2 (3–7 cells)	Unknown	Japan	Hashimoto et al. (2017)
H. jinghaensis	**30–40 × 25–30**	**Clavate, subcylindrical**	**33–43 × 11–13**	**1–2 (6–8 cells)**	**Unknown**	**China**	**This study**
H. krabiensis	24.3–32.5 × 12.1–21.3	Cylindrical	20.4–26.4 × 8.6–19.7	1–2 (2–3 cells)	*Pandanus odorifer*	Thailand	Tibpromma et al. (2016)
H. megasporus	(45–)49–56(–59) × (31–)37–46	Cylindrical	(37–)49.5–60.5(67–) × 18–28(–32)	2 ((5–)6–7(–10) cells)	Unknown	Panama	Koukol et al. (2018)
H. nabanheensis	20.2–25.1 × 16.6–20.7	Cylindrical	15.3–26.8 × 12.1–18.2	1–2 (2–3 cells)	*Pandanus* sp.	China	Hyde et al. (2017)
H. pandanicola	12–15.7 × 20–30.1	Cylindrical	13.2–20.6 × 8.9–11.9	2 (2 cells)	*Pandanus odorifer*	Thailand	Tibpromma et al. (2016)
H. reticulatus	3–40(–45) × 25–34(–41)	NA	NA	NA	Unknown	Thailand, Panama	Hyde et al. (2016); Koukol et al. (2018)
H. saikhuensis	14.2–21.4 × 11.2–19.3	NA	NA	NA	*Pandanus odorifer*	Thailand	Tibpromma et al. (2016)
H. sphaericoides	(20.5–)24.5–28(–31) × (20–)23–26(–29)	NA	NA	NA	Unknown	Panama	Koukol et al. (2018)
H. sphaericus (PMA 116080)	(21–)24–29(–32.5) × (18–)21–27(–31.5)	NA	NA	NA	Various host plants	Tropical or subtropical	Koukol et al. (2018)
H. sphaericus	**27–29 × 26–28**	NA	NA	NA	***Dipterocarpus* sp., *Ehretia acuminata***	**China, Thailand**	**This study**
H. tectonae	(23–)26–29(–33) × (19–)23–25(–28)	Cylindrical	(27–)31–32(–35) ×(21–)23	2 (6 cells)	*Tectona grandis*	Thailand	Doilom et al. (2017)
H. trangensis	27.5–35 × 25–32.5	NA	NA	NA	*Arenga pinnata*	Thailand	Nuankaew et al. (2019)
H. truncates	(26–)31.5–36.5(–37) × 22–27(–30)	Cylindrical	Lower cells: 14–22.5(–28) × 8.5–14.5 Upper cells: 12–18(–30) × (6–)8–12.5	1 (2–3 cells)	*Averrhoa carambola*	Ghana, Panama	Koukol et al. (2019)
H. tucumanensis	(22–)27–35 × 18–25	Obclavate or subcylindrical	(21–)23–26(–28.5) × 7–14	2 (3–6 cells)	Unknown	Panama	Koukol et al. (2018)

Species	Lenticular conidia size (µm)	Cylindrical / turbinate conidia feature			Host	Country	Reference
		Shape	Length × width (µm)	Number of columns (cells)			
H. turbinatus	**24–30 × 17–21**	**Turbinate**	**27–36 × 19–28**	**2 (2–3 cells)**	***Dipterocarpus* sp.**	**Thailand**	**This study**
H. uniseriatus	27–36 × 15.5–24	Cylindrical	19–34 × 10–12.5	1 (3–4 cells)	*Smilax campestris*	Argentina	Leão-Ferreira et al. (2013)
H. verrucosus	23–30(–39) × 21–29.5	NA	NA	NA	Unknown	Panama	Koukol et al. (2018)

NA: absent

being saved in PDF format and finally converted to JPG format using Adobe Illustrator CS6 (Adobe Systems, USA). The finalized alignments and trees were deposited in TreeBASE, submission ID: TB2:S28514 (http://purl.org/phylo/treebase/phylows/study/TB2:S28514).

Ex-type strains are indicated with superscript "T", and newly generated sequence is shown in bold. NA represents sequences that are unavailable in GenBank. Abbreviations:

ANM A.N. Miller;
BCC BIOTEC Culture Collection, Bangkok, Thailand;
CBS Centraal Bureau voor Schimmel cultures, Utrecht, The Netherlands;
CCF Culture Collection of Fungi, Charles University, Prague, Czech Republic;
HKAS The herbarium of Cryptogams Kunming Institute of Botany Academia Sinica;
KH K. Hirayama;
KUMCC Culture Collection of Kunming Institute of Botany, Kunming, China;
KZP O. Koukol;
MFLUCC Mae Fah Luang University Culture Collection, Chiang Rai, Thailand;
PMA Herbarium of the University of Panama, Panama City, Panama;
PRC Herbarium of the Charles University, Prague, Czech Republic;
PRM Herbarium of the National Museum, Prague, Czech Republic.

T1 Type of *Hermatomyces thailandicus*;
T2 Type of *H. chiangmaiensis*;
T3 Type of *H. subiculosus*;
T4 Type of *H. chromolaenae*;
T5 Type of *H. saikhuensis*;
T6 Type of *H. tectonae*.

Results

Phylogenetic analysis

The phylogenetic analysis was conducted using 57 strains in Hermatomycetaceae, and two outgroup taxa *Anteaglonium globosum* (ANM 925.2) and *A. parvulum* (MFLUCC 14-0821) in Pleosporales (Table 1). The aligned sequence matrix comprised five gene regions (LSU: 887 bp, ITS: 530 bp, *tub*2: 606 bp, *tef*1-α: 952 bp and *rpb*2: 1,028 bp) and a total of 4,003 characters (including gaps), of which 3,207 characters were

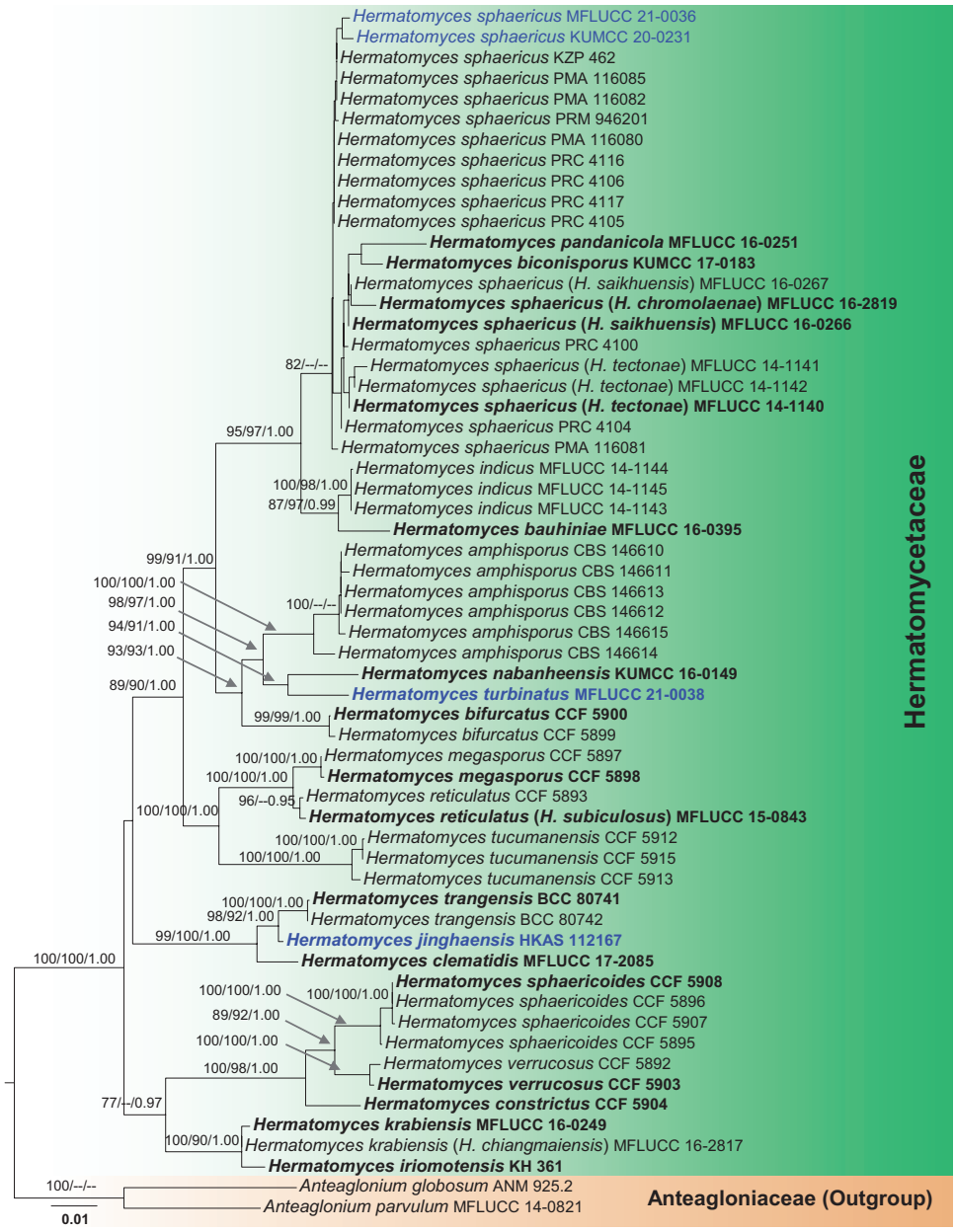

Figure 1. Phylogenetic RAxML tree based on analysis of a combined LSU, ITS, *tub*2, *tef*1-α and *rpb*2 and dataset. Bootstrap support values for ML and MP equal to or higher than 75% and Bayesian PP equal to or greater than 0.95 are shown at nodes. Hyphens (--) represent support values less than 75% / 0.95 BYPP. The ex-type strains are in bold and the new isolate in this study is in blue bold. The tree is rooted with *Anteaglonium globosum* (ANM 925.2) and *A. parvulum* (MFLUCC 14-0821). The scale bar represents the expected number of nucleotide substitutions per site.

constant, 174 variable characters were parsimony-uninformative and 622 characters were parsimony-informative. The Kishino-Hasegawa test shows length = 1,388 steps with CI = 0.671, RI = 0.884, RC = 0.593 and HI = 0.329. The RAxML analysis of the combined dataset yielded a best scoring tree with a final ML optimization likelihood value of -13406.555506. Estimated base frequencies were as follows: A = 0.241874, C = 0.266701, G = 0.257552, T = 0.233873; substitution rates AC = 1.188604, AG = 4.826453, AT = 1.273226, CG = 0.855218, CT = 11.409386, GT = 1.00; gamma distribution shape parameter α = 0.16102.

In the phylogenetic tree obtained from ML, MP and BI analysis (Fig. 1) the maximum likelihood analysis resulted in trees largely with similar topology and clades as in the maximum parsimony and Bayesian analyses. The new species, *Hermatomyces turbinatus*, is sister to *H. nabanheensis* (KUMCC 16-0149) with high support (94% ML, 91% MP and 1.00 BYPP, Fig. 1). *Hermatomyces jinghaensis* is nested between *H. trangensis* and *H. clematidis* with a strongly supported monophyletic group (98% ML, 92% MP, 1.00 PP; Fig. 1). New isolates of *H. sphaericus* (KUMCC 20-0231; MFLUCC 21-0036) clustered with remaining *H. sphaericus* strains as a monophyletic group (Fig. 1). The topology of the phylogenetic tree is in accordance with recent phylogenetic studies discussing species in Hermatomycetaceae (Nuankaew et al. 2019; Phukhamsakda et al. 2020).

Taxonomy

Hermatomyces turbinatus **G.C. Ren & K.D. Hyde, sp. nov.**
MycoBank No: 558166
Facesoffungi Number No: FoF09735
Figure 2

Etymology. Referring to the turbinate shape of the conidia.

Holotype. HKAS 112724.

Description. *Saprobic* on woody litter of *Dipterocarpus* sp. (Dipterocarpaceae) **Sexual morph** Undetermined. **Asexual morph** *Colonies* on natural substrate forming sporodochial conidiomata, superficial, scattered, small groups, circular or oval, sterile mycelial outer zone enclosing a black-brown velvety margin, sparse, black sporulating center, shiny, glistening, circular or oval, conidia readily liberated when agitated. *Mycelium* superficial, branched, septate, hyaline to pale brown, 2–3 µm wide. *Conidiophores* 6–8 × 2–3 µm, micronematous, straight or flexuous, smooth, short, pale brown. *Conidiogenous cells* 3–5 × 2–3 µm, monoblastic, integrated, terminal, determinate, often arising directly on the superficial mycelium, subsphaerical, ovoid or ampulliform, hyaline to pale brown, smooth finely verruculose. *Conidia* dimorphic, solitary, smooth-walled. *Lenticular conidia* 24–30 × 17–21 µm (x = 27 × 20 µm, n = 20), 12–15 µm thick, thick-walled, circular to oval in front view, smooth, solitary, muriform, central cells dark brown to black, peripheral cells hyaline to pale brown,

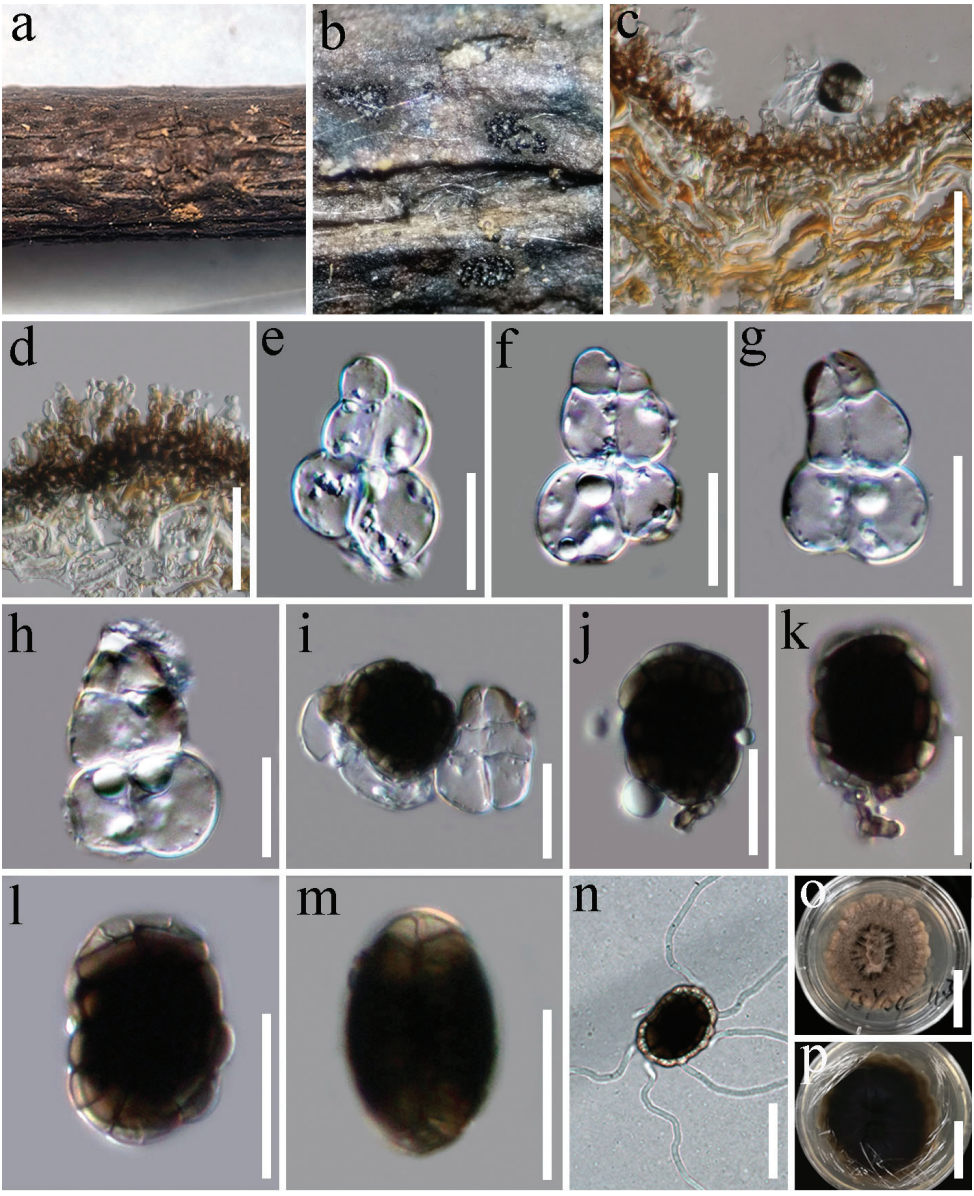

Figure 2. *Hermatomyces turbinatus* (HKAS 112724, holotype) **a, b** sporodochia on natural substrate **c** vertical section of sporodochium **d** conidiophores and conidiogenous cells **e–h** turbinate conidia **i** turbinate and mature lenticular conidia **j–m** mature lenticular conidia **n** germinated conidium **o, p** culture characters on PDA. Scale bars: 30 μm (**c**); 20 μm (**d–n**); 30 mm (**o, p**).

forming a weakly ring, sometimes slightly constricted at septa, obovoid or oblong in lateral view, arranged in 2 rows, a row of composed of 4–6 cells, end cells pale brown to hyaline, middle cells dark brown. *Turbinate conidia* turbinate, pyriform, 27–36 μm

in length, 19–28 µm wide in broadest part of lower cells, (x = 32 × 23 µm, n = 20), asymmetrical with the upper cells smaller than lower cells, thick-walled, smooth, septate, constricted distinct at septa, consisting of two columns with two septa composed of 2–3 rectangular to globose cells in each column, usually upper part of terminal cells dark brown, becoming hyaline towards the lower side, two cells hyaline in the lower cells swollen with oil globules.

Known host and distribution. *Dipterocarpus* sp. (Thailand).

Culture characteristics. *Colonies on PDA*, reaching 30–40 mm diam., after 3 weeks at 25–30 °C, circular, convex with papillate and radially furrowed at the center, rough, labate, crenate edge, fluffy, dense, gray black, in reverse darkens at the center, pale brown to gray at edge.

Material examined. Thailand, Tak Province. Ban Na Sam Ngao District, on woody litter of *Dipterocarpus* sp. (Dipterocarpaceae), 22 August 2019, G. C. Ren, TSY04 (HKAS 112724, ***holotype***), ex-type living culture, MFLUCC 21-0038.

Notes. *Hermatomyces turbinatus* is introduced as a new species based on its distinct morphology, which is supported by phylogenetic analyses. In the phylogenetic analyses, *H. turbinatus* is distinct from extant species in this genus and formed a sister clade to *H. nabanheensis* with strong support (94% ML, 91% MP, 1.00 PP; Fig. 1). *Hermatomyces turbinatus* differs from *H. nabanheensis* in having turbinate conidia with two columns, while *H. nabanheensis* has cylindrical conidia with one or two columns. *Hermatomyces turbinatus* has two conidial types, and its lenticular conidia are similar to *H. tectonae* in shape and size. However, the turbinate conidia of *H. turbinatus* have 2 columns of 2–3 cells in each column, while the turbinate conidia of *H. tectonae* have 2 columns of 3 cells in each column. We also compared the morphological characters of *H. turbinatus* to other species of *Hermatomyces* (Table 2). Despite no molecular data being available for the three species viz. *H. dimorphus*, *H. uniseriatus* and *H. truncates*, *H. turbinatus* nonetheless differs from these species in conidial characteristics (Table 2).

***Hermatomyces jinghaensis* G.C. Ren & K.D. Hyde, sp. nov.**
MycoBank No: 558165
Facesoffungi Number No: FoF09736
Figure 3

Etymology. The species epithet "*jinghaensis*" refers to the location where the species was collected.

Holotype. HKAS 112167.

Description. *Saprobic* on unidentified woody litter. **Sexual morph** Undetermined. **Asexual morph** *Colonies* on natural substrate forming sporodochial conidiomata, superficial, scattered, small groups, circular, sterile mycelial outer zone enclosing a black velvety margin, dense, thick, black sporulating center, shiny, glistening, circular or oval, conidia readily liberated when agitated. *Mycelium* superficial, branched, septate,

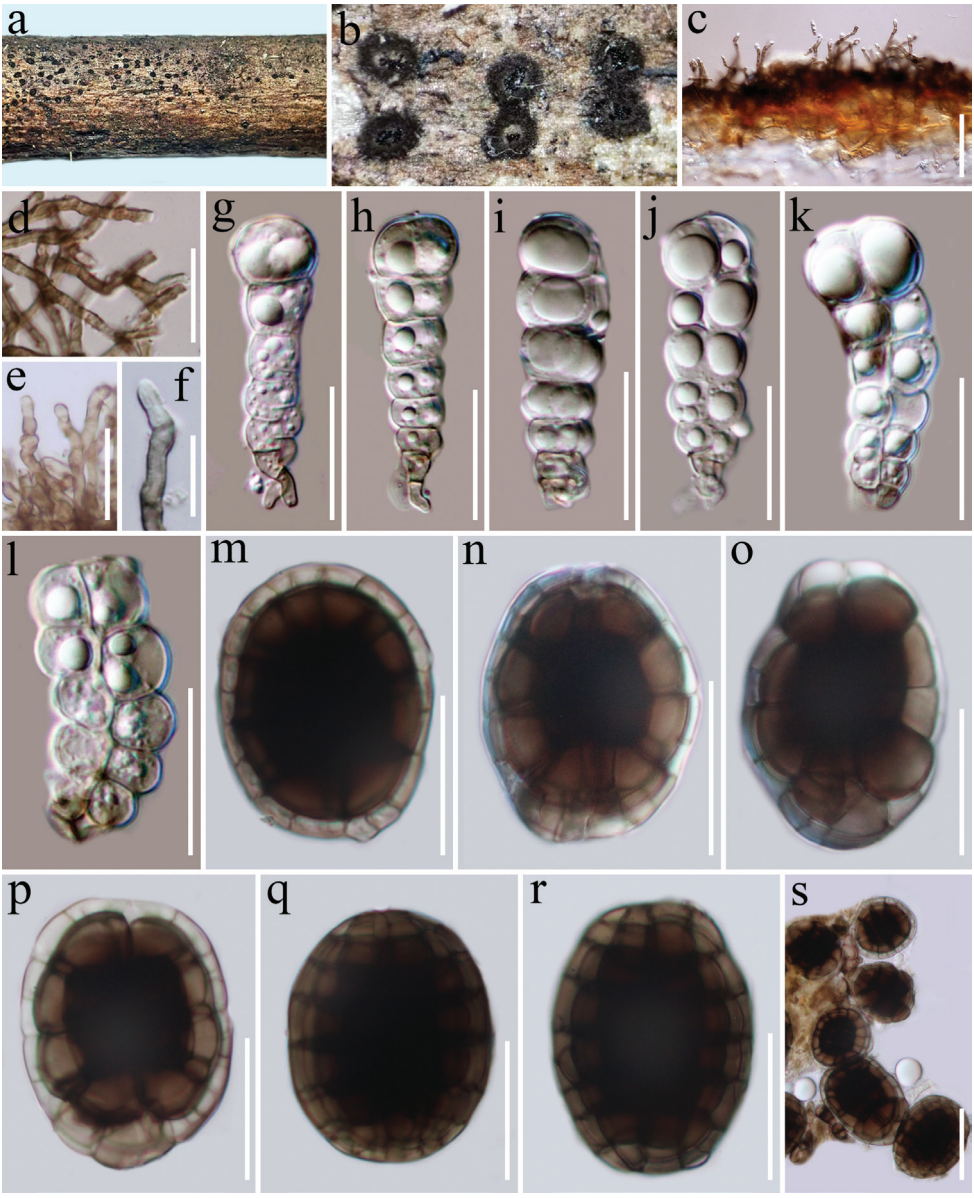

Figure 3. *Hermatomyces jinghaensis* (HKAS 112167, holotype) **a, b** sporodochia on natural substrate **c** vertical section of sporodochium **d** conidiophores **e, f** conidiogenous cells **g–l** cylindrical conidia **m–s** mature lenticular conidia. Scale bars: 50 μm (**c**); 30 μm (**d**); 20 μm (**e–r**); 30 μm (**s**).

hyaline to pale brown, 2–3 μm wide. *Conidiophores* 30–45 × 2–3 μm, mononematous, cylindrical, straight or flexuous, smooth, pale brown. *Conidiogenous cells* 4–6 × 2–3 μm, monoblastic, integrated, terminal, determinate, often arising directly on the superficial mycelium, cylindrical, ampulliform, hyaline to pale brown, smooth finely verruculose. *Conidia* dimorphic solitary, smooth-walled. *Lenticular conidia* 30–40 × 25–30 μm

(x = 37 × 28 µm, n = 20), 21–25 µm thick, thick-walled, circular to oval in front view, smooth, solitary, muriform, central cells brown to dark brown, peripheral cells hyaline to subhyaline, forming a wide and distinct ring, sometimes slightly constricted at septa, obovoid or oblong in lateral view, central cells brown to dark brown, peripheral cells pale brown to brown. *Cylindrical conidia* 33–43 µm in length, 11–13 µm wide in broadest part of lower cells (x = 39 × 12 µm, n = 20), clavate or subcylindrical, straight or flexuous, septate, constricted distinct at the septa, with large guttules, consisting of one or two columns, each column with 6–8 cells, apical cell rectangular to globose, smooth, hyaline, smooth, basal cells acute, rectangular to cylindrical, pale brown.

Known host and distribution. Unidentified woody litter (China)

Material examined. China, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong, Jingha (21°78.06'N, 101°05.61'E), on unidentified woody litter, 19 December 2019, D.N. Wanasinghe, DW57 (HKAS 112167, ***holotype***), no living culture.

Notes. *Hermatomyces jinghaensis* is introduced as a new species based on its distinct morphology and the phylogenetic results of a combined LSU, ITS, *tub*2, *tef*1-α and *rpb*2 dataset. *Hermatomyces jinghaensis* nested with *H. clematidis* and *H. trangensis* in a strongly supported monophyletic group (99% ML, 100% MP, 1.00 PP; Fig. 1). *Hermatomyces jinghaensis* is characterized by both lenticular and cylindrical conidia. *Hermatomyces jinghaensis* differs from *H. clematidis* in having cylindrical conidia with one or two columns, each of which has 6–8 cells with large guttules, while the latter has 5–6 cells for each column conidia. *Hermatomyces trangensis* differs from *H. jinghaensis* in having only lenticular conidia.

***Hermatomyces sphaericus* (Sacc.) S. Hughes 1953.**
MycoBank No: 298410
Facesoffungi Number No: FoF05259
Figure 4

Description. *Saprobic* on woody litter of *Dipterocarpus* sp. (Dipterocarpaceae) and *Ehretia acuminata* (Boraginaceae). **Sexual morph** Undetermined. **Asexual morph** *Colonies* on natural substrate forming sporodochial conidiomata, superficial, circular or irregular, scattered or crowded, consisting of a velvety, dense, annular, gray brown, sterile mycelial outer zone and a black, glistening, abundantly sporulating granulose center, with conidia readily liberated when agitated. *Mycelium* 2–2.5 µm wide, superficial, composed of a tightly network of branched, septate, smooth or finely verruculose, hyaline or pale brown hyphae. *Conidiophores* 10–13 × 2–4 µm (x = 12 × 3 µm, n = 10) micronematous, cylindrical or forked, smooth, hyaline or pale brown, often corresponding to conidiogenous cells. *Conidiogenous cells* 5–8 × 3–5 µm (x = 7 × 4 µm, n = 20), monoblastic, integrated, terminal, cylindrical, hyaline to pale brown, smooth or finely verruculose. *Conidia* of one type, 27–29 × 26–28 µm (x = 28 × 27 µm, n = 30) µm, 19–24 µm thick, solitary, lenticular, globose, subglobose in front view, muriform, smooth, central cells brown, dark brown, outer ring of peripheral cells narrow, pale brown to brown, often constricted at septa, disk-shaped in lateral view,

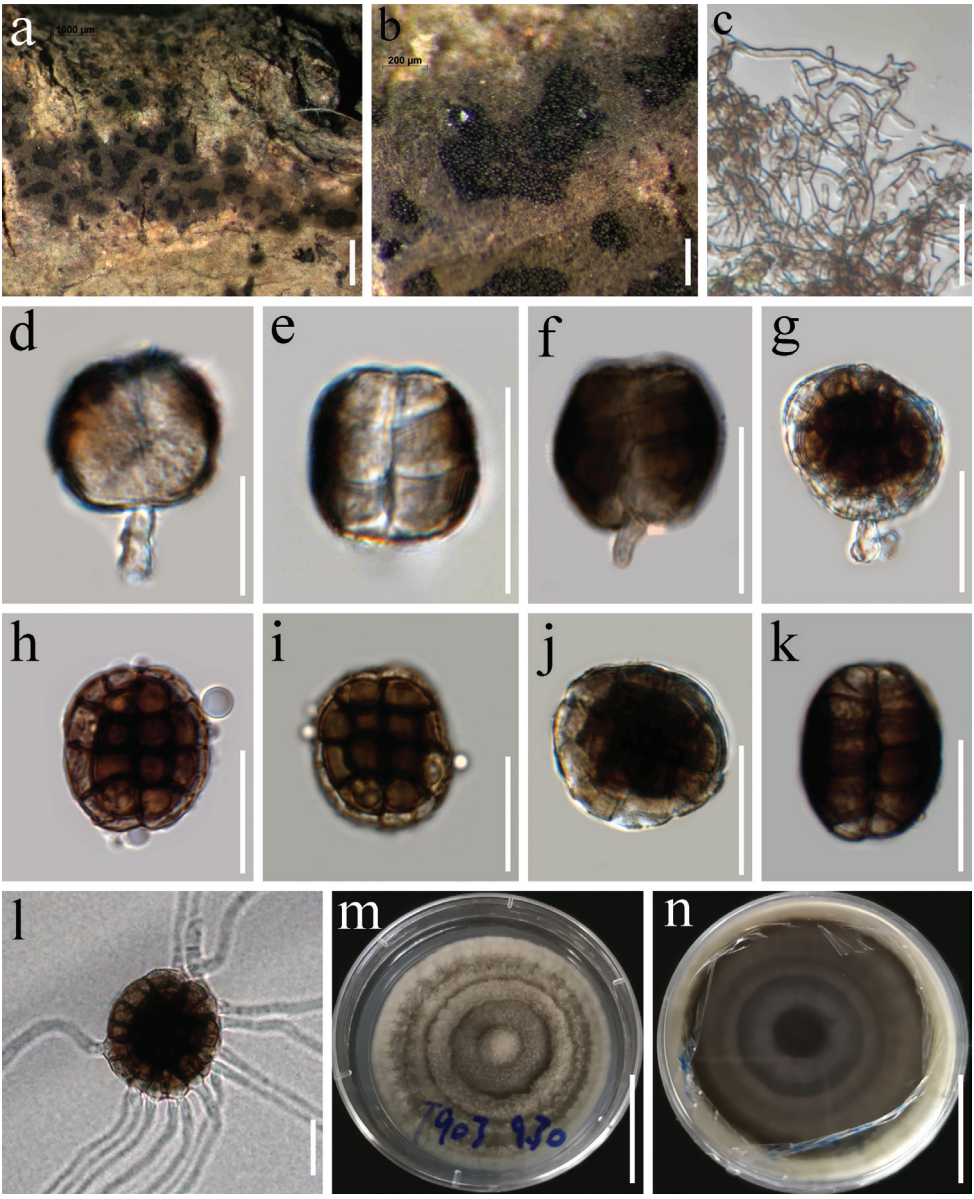

Figure 4. *Hermatomyces sphaericus* (HKAS 112725) **a, b** colonies on the natural substrate **c** mycelia **d–g** young conidia **h–k** mature conidia (**h–j** surface view **k** thickness view) **l** germinated conidium **m, n** culture characters on PDA. Scale bars: 1000 µm (**a**); 200 µm (**b**); 20 µm (**c–i, l**); 30 µm (**j, k**); 3 cm (**m, n**).

consisting of two rows, each row with 4–6 cells, hyaline to light brown at lower and upper cells, middle cells brown to black brown.

Known host and distribution. Tropical and subtropical regions of Central and South America, Africa, Asia, Oceania and North America. The species were found

as saprobes on Acanthaceae, Apocynaceae, Arecaceae, Asteraceae, Dipterocarpaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Leguminosae, Mimosaceae, Nyctaginaceae, Oxalidaceae, Pandanaceae, Pinaceae, Rhamnaceae, and Sterculiaceae (Zhang et al 2009; Koukol et al. 2018, 2019).

Culture characteristics. Colonies on PDA, reaching 35–40 mm diam., after 3 weeks at 25–30 °C, with circular, umbonate, fluffy, velvety, entire edge, a circular raised band, gray white, in reverse dark gray, black toward the center.

Material examined. Thailand, Tak Province, Tha Song Yang District, on woody litter of *Dipterocarpus* sp. (Dipterocarpaceae), 22 August 2019, G. C. Ren, T903 (HKAS 112725), living culture, MFLUCC 21-0036; China, Yunnan Province, Xishuangbanna (21°55.19'N, 101°15.24'E), on woody litter of *Ehretia acuminata* (Boraginaceae), 4 August 2020, G. C. Ren, JH39 (HKAS 112166), living culture, KUMCC 20-0231.

Notes. The characters of our new strain of *Hermatomyces sphaericus* (KUMCC 20-0231, MFLUCC 21-0036) are similar to the type collection (K(M)–IMI 37763) in having gray black to black sporodochia, mononematous, pale brown, smooth, monoblastic, integrated, terminal, cylindrical, hyaline to pale brown conidiogenous cells and globose to subglobose conidia (Hughes 1953). A multigene phylogeny indicates that novel strains clustered within the *H. sphaericus* clade (Fig. 1). We name our strain (KUMCC 20-0231, MFLUCC 21-0036) as *H. sphaericus*, which has been reported from different plant families and genera (Koukol et al. 2018). However, we consider this might be a species complex that need further detailed studies. Our study provides the new host records of *H. sphaericus* on *Dipterocarpus* sp. (Dipterocarpaceae) and *Ehretia acuminata* (Boraginaceae), and updates sequence data for the new collections of *H. sphaericus*.

Discussion

This study introduces two new species of woody-based litter fungi; *Hermatomyces jinghaensis* from Yunnan, China and *Hermatomyces turbinatus* on *Dipterocarpus* sp. from Thailand. We also report for the first time two new records of *H. sphaericus* on *Dipterocarpus* sp. and *Ehretia acuminata* in China and Thailand.

Hermatomyces (Hermatomycetaceae) is different from other similar genera in its sporodochial conidiomata and in having one to two (lenticular and cylindrical conidia) unusual conidial types (Spegazzini 1911). All species of *Hermatomyces* have lenticular conidia with similar characteristics, whereas some species have cylindrical and turbinate conidia, which have greater variance in shape, size, number of columns and cells. Koukol et al. (2018, 2019) have reported that multiple species may occur together on a single sample, a phenomenon we observed, which may complicate morphological identification and separation for culturing. Therefore, molecular sequence data are more reliable for the identification of *Hermatomyces* species (Tibpromma et al. 2016, 2017, 2018; Nuankaew et al. 2019; Phukhamsakda et al. 2020).

Hermatomyces sphaericus was introduced by Hughes (1953), which may be the most widespread of species in *Hermatomyces* distributed across many subtropical and tropical regions worldwide (Wijayawardene et al. 2014; Doilom et al. 2017; Koukol et al. 2018, 2019; Hyde et al. 2019; Jayasiri et al. 2019; Nuankaew et al. 2019; Phukhamsakda et al. 2020). This species has been reported as saprobic on dead plant tissues of several host families (Tibpromma et al. 2016, 2017; Doilom et al. 2017; Jayasiri et al. 2019). In addition, Koukol et al. (2018) reported that *H. sphaericus* (ARIZ: PS0053) was isolated from seeds of *Apeiba membranacea* (Malvaceae), suggesting this species could be an endophyte. Previous studies have indicated that *H. sphaericus* is not restricted to any single host (Koukol et al. 2018, 2019; Jayasiri et al. 2019), whereas other species of *Hermatomyces* are saprobic on a limited number of hosts and are limited to specific regions (Rao and de Hoog 1986; Leão-Ferreira et al. 2013; Prasher and Prasher 2014; Hyde et al. 2016, 2017, 2019; Tibpromma et al. 2016, 2017, 2018; Doilom et al. 2017; Hashimoto et al. 2017; Koukol et al. 2018, 2019; Nuankaew et al. 2019; Delgado et al. 2020; Phukhamsakda et al. 2020; Table 2). In this study, our new strains of *H. sphaericus* had slight morphological differences in lenticular conidia size compared to the type strains and other strains of *H. sphaericus* (Hughes 1953, Table 2). As reported by Koukol et al. (2018), *H. sphaericus* is a plurivorous species, and accordingly the phenotypic variation among strains could be influenced by environmental factors and culture conditions or it could have speciated in isolated polulations (Hyde et al. 2020).

Species delineation in *Hermatomyces*, especially in the *H. sphaericus* clade, is subject to much controversy due to species inconsistency in morphological and phylogenetic status. Koukol et al. (2018) synonymized *H. chromolaenae*, *H. saikhuensis* and *H. tectonae* under *H. sphaericus* based on morphological and molecular comparisons and suspected that *H. pandanicola* could either be a hybrid species or incorrect sequences were used in the analysis. Koukol et al. (2019) considered that during isolation of *H. biconisporus*, a conidium of *H. sphaericus* might have been taken instead, leading to contamination when extracting DNA and the misinterpretation of its taxonomic placement. Phukhamsakda et al. (2020) further confirmed that *H. biconisporus*, *H. pandanicola* and *H. sphaericus* should be treated as the same species based on Genealogical Concordance Phylogenetic Species Recognition (GCPSR) analysis.

Hermatomyces had long been treated as "*incertae sedis*" within Ascomycota (Wijayawardene et al. 2012). Doilom et al. (2017) placed *Hermatomyces* in Lophiotrematacеae baed on phylogenetic analyses, and consequently, Hashimoto et al. (2017) revised the family Lophiotrematacеae based on morphological observations and phylogenetic analyses, and *Hermatomyces* was accepted in the family Hermatomycetaceae, as monophyletic. Recent studies and our study indicate *Hermatomyces* to be highly polyphyletic, and *Hermatomyces* morphology has evolved, which is mainly characterized by lenticular and cylindrical conidia (Fig. 1; Koukol et al. 2018, 2019; Hyde

et al. 2019; Phukhamsakda et al. 2020). Support for a single *H. sphaericus* species (Fig. 1) lacks internal statistical support and includes *H. biconisporus*, *H. chromolaenae*, *H. pandanicola*, *H. saikhuensis* and *H. tectonae* and we suspect that this is a species complex. Tibpromma et al. (2018) also noted that *H. sphaericus* could be a species complex including several species and did not accept the synonymy of *H. saikhuensis* and *H. tectonae* in *H. sphaericus* owing to their significant base-pair differences.

In this study, we combined two non-translated loci (LSU, ITS) and three protein-coding regions (*tub*2, *tef*1-α and *rpb*2) to carry out phylogenetic analysis for *Hermatomyces* species in order to validate phylogenetic placement of the taxa within *Hermatomyces*. In our phylogenetic analyses, *H. tectonae*, *H. chromolaenae*, *H. biconisporus*, *H. pandanicola* and *H. saikhuensis* grouped together with strains of *H. sphaericus* (PRC 4100, PRC 4104, PMA 116081). *Hermatomyces saikhuensis* and *H. chromolaenae* are characterized by one conidium type (lenticular) similar to *H. sphaericus*, however, they differ in the shape, color and size of conidia (Tibpromma et al. 2016, 2017; Table 2). *Hermatomyces tectonae*, *H. biconisporus* and *H. pandanicola* are characterized by dimorphic conidia which differ from *H. sphaericus* (Tibpromma et al. 2016, 2018; Doilom et al. 2017; Koukol et al. 2018; Table 2). *Hermatomyces sphaericus* (PRC 4100, PRC 4104, PMA 116081) did not have a morphological description for inter-species comparison (Koukol et al. 2018). Further taxon sampling and more sequence data are needed to elucidate this clade.

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Two new species of *Hirsutella* (Ophiocordycipitaceae, Sordariomycetes) that are parasitic on lepidopteran insects from China

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Abstract

Hirsutella are globally distributed entomopathogenic fungi that offer important economic applications in biological control and biomedicine. *Hirsutella* was suppressed in favour of *Ophiocordyceps* affected by the ending of dual nomenclature for pleomorphic fungi in 2011. Currently, *Hirsutella* has been resurrected as a genus under Ophiocordycipitaceae. In this study, we introduce two new species of *Hirsutella*, based on morphological and phylogenetic analyses. *Hirsutella flava* and *H. kuankuoshuiensis* are pathogenic on different species of larval Lepidoptera in China. *Hirsutella flava* primarily differs from related species by its awl-shaped base; long and narrow neck, 24–40.8 × 2.2–2.5 µm; long and narrow cymbiform or fusoid conidia, 6.5–10 × 2.1–4.3 µm. *Hirsutella kuankuoshuiensis* has two types of phialides and distinctive 9.9–12.6 × 2.7–4.5 µm, clavate or botuliform conidia. The distinctions amongst the new species and phylogenetic relationships with other *Hirsutella* species are discussed.

Keywords

entomopathogenic fungi, *Hirsutella*, *Ophiocordyceps*, two new taxa

Introduction

The entomopathogenic fungal genus *Hirsutella* Pat. was erected by Patouillard (1892) based on the type species *H. entomophila.* The genus was introduced to the family Ophiocordycipitaceae and its sexual morph was linked to *Ophiocordyceps* (Sung et al.

2007a; Simmons et al. 2015). In *Hirsutella* sensu stricto, conidiation is synnematous and phialides typically have a swollen base that tapers abruptly into a long neck producing either a single conidium or 2–3 conidia coated with mucus. The colour of the synnemata ranges from ash-grey or brown to dark brown. The size and shape of the hyaline conidia vary from citriform to oblong, subcylindric, globose, rhombic, or reniform (Luangsa-ard et al. 2017; Quandt et al. 2014). These taxa are important pathogens of agricultural pests and are used as popular traditional medicine and a nutritious food in many Asian countries (Evans 1974; Quandt et al. 2014; Hyde et al. 2019). Several common species of *Hirsutella*, such as *H. thompsonii* and *H. rhossiliensis*, are potentially important biological control agents for nematodes and mites (Jaffee 1992; Van der Geest 2010; Hyde et al. 2019). Further uses involve the development and application of several effective bioactive secondary metabolites (Mazet and Vey 1995; Lang et al. 2005; Qu et al. 2017).

Research on *Hirsutella* originated in the 1920s. Through the 1950s, Speare (1920), Petch (1924) and subsequent researchers reported 25 new species of the genus. However, many of these species were not described in detail and lacked adequate drawings, as well as holotypes. In addition, many specimens were damaged or lost during wartime (Zou et al. 2016a). In the 1970s and 1980s, Miner, Samson and Evans re-examined the status of *Hirsutella* and established the modern scientific definition for the genus (Minter and Brady 1980; Evans and Samson 1982, 1984). Since the beginning of the 21st century, the taxonomy, molecular evolution and phylogeny of *Hirsutella* have been addressed by a small number of Chinese and international studies, with sporadic reports of new species (Seifert 2004; Xiang et al. 2006; Zou et al. 2010). However, it is likely that further new species remain to be discovered, and specific information on insect hosts, pathogenicity and habitats are lacking (Sung et al. 2007a; Hoyos-Carvajal et al. 2009).

Quandt et al. (2014) proposed that *Hirsutella* should be suppressed in favour of *Ophiocordyceps* affected by the ending of dual nomenclature for pleomorphic fungi in 2011 (McNeill et al. 2012). *Ophiocordyceps* is the type genus in the family Ophiocordycipitaceae (Hypocreales, Sordariomycetes) (Sung et al. 2007a). The main characteristics of the sexual morphs of *Ophiocordyceps* are fibrous, hard, pliant-to-wiry, dark stromata with superficial to immersed perithecia (Sung et al. 2007a; Xiao et al. 2019). Most of the sexual species of *Ophiocordyceps* were transferred from the genus *Cordyceps* (Cordycipitaceae) by Sung et al. (2007a). Since many species of *Hirsutella* are closely related to *Cordyceps*, the asexual morphs in most of the species in *Ophiocordyceps* have hirsutella-like features (Kepler et al. 2013; Quandt et al. 2014; Maharachchikumbura et al. 2015, 2016). Therefore, *Hirsutella* was treated as a separate genus from *Ophiocordyceps* before the taxonomic revision (Sung et al. 2007a; McNeill et al. 2012; Quandt et al. 2014). For example, some new species only known from a *Hirsutella* morph have been accepted into *Ophiocordyceps* (Simmons et al. 2015a; Qu et al. 2018b).

In recent years, the taxonomic transitions of Ophiocordycipitaceae changed rapidly under the new rules. Quandt et al. (2014) included *Ophiocordyceps*, *Tolypocladium*, *Polycephalomyces*, *Purpureocillium*, *Drechmeria* and *Harposporium* in Ophiocordycipi-

taceae based on morphological and phylogenetic analyses. In the paper "Outline of Ascomycota: 2017", the genus *Hymenostilbe* was added into the Ophiocordycipitaceae families (Wijayawardene et al. 2018). According to the latest taxonomic report, the number of genera included in Ophiocordycipitaceae has increased to ten, and among them, *Hirsutella*, *Paraisaria* and *Perennicordyceps* are new additions (Hyde et al. 2020). The taxonomic revision of Ascomycota is continuing. Further research into the phylogeny of these organisms is needed. Examples include investigating the new resources to supplement the available taxonomic information and perform phylogenetic research.

During an investigation of the genetic resources of entomopathogenic fungi in southwest China, we collected two specimens of Lepidoptera insects that were infected by fungi. Two hirsutella-like species were isolated and their gene sequences and morphological traits were shown to be related to *Hirsutella* sensu stricto. In this study, two new species of *Hirsutella* are introduced.

Materials and methods

Specimens

The specimens HKAS112884 and HKAS112885 were deposited at the Kunming Institute of Botany, Chinese Academy of Sciences (KIB), Kunming, China. The isolated strains of their asexual stage were deposited at the Institute of Fungal Resources of Guizhou University (**GZAC**), Guiyang, China. More information about these specimens is shown in Suppl. material 1: Table S1.

Fungal isolation and culture

The fungi were isolated as described by Qu et al. (2018). The surface of specimens was rinsed with sterile water, followed by surface sterilisation with 75% ethanol for 3-5 s. Parts of the insect body were cut off and a piece of tissue was inoculated in haemocoel on a PDA plate for 20 days at 16 °C.

LM and SEM observation

For light microscopy (LM) observations and imaging, the morphological characteristics of mycelia were observed using an optical microscope (OM, BK5000, OPTEC, Chicago, IL, USA) after staining with a lactic acid/phenol cotton blue solution. The captured images of new species were edited and digitally contrasted using Paint Shop Pro v. 5.0.1 (Corel, Ottawa, Canada).

Electron microscopy was performed as described by Qu et al. (2018). Briefly, 1 cubic cm of hyphae with conidia were cut from the fungus on PDA cultures, fixed with 4% glutaraldehyde at 4 °C overnight, and then washed three times with phosphate buffer saline (PBS) (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM

KH_2PO_4, pH 7.4) for 10 min each time. Fixed hyphae and conidia were dehydrated using 50%, 70%, 90% and 100% ethanol, 10 min for each concentration, and were finally dehydrated with super-critical carbon dioxide. After being sprayed with gold, the conidia and mucilage were examined by scanning electron microscopy (SEM) (S-3400N, Hitachi, Tokyo, Japan) and photographed.

DNA extraction, PCR amplification and sequencing

Axenic and fresh mycelia (0.05–0.1 g) of the new species were transferred to 1.5 ml Eppendorf tubes for genomic DNA extraction using a Fungal DNA MiniKit (Omega Bio-Tek, Norcross, GA, USA). The universal known primers were used for PCR amplification: (1) NS1/NS4 for the partial small subunit ribosomal RNA gene region (SSU) (White et al. 1990), (2) LROR/LR5 for the partial large subunit rDNA gene region (LSU) (Vilgalys and Hester 1990; Rehner and Samuels 1994), (3) ITS4/ITS5 for the internal transcribed spacer gene region (ITS) (White et al. 1990), (4) 983F/2218R for the partial translation elongation factor 1-alpha gene region (TEF1α) (Sung et al. 2007b) and (5) CRPB1A/RPB1Cr for the partial RNA polymerase II largest subunit gene region (RPB1) (Castlebury et al. 2004).

Molecular phylogeny

To construct a phylogeny of major lineages, 71 representative species were chosen to represent the ecological diversity of *Hirsutella* and *Ophiocordyceps* based on previous phylogenetic studies (Simmons et al. 2015b; Xiao et al. 2017; Qu et al. 2018; Xiao et al. 2019). *Tolypocladium inflatum* and *T. ophioglossoides* were selected as the outgroup taxa and are classified within Ophiocordycipitaceae (Xiao et al. 2019). The sequences used in this study were combined with published data on hirsutella-like species and Ophiocordycipitaceae. All the other sequences were collected from GenBank and the accession numbers are shown in Table 1.

All the sequences were edited for multi-alignment using the BioEdit Sequence Alignment Editor v.7.0.5.3 (Hall 1999) with the Clustal X v.1.83 software package (Thompson et al. 1999). Gaps were excluded from the phylogenetic analysis based on previous research (Qu et al. 2018). The ITS, SSU, LSU, TEF1α and RPB1 regions were aligned in combined datasets using MAFFT v.7 (Katoh and Standley 2013, http://mafft.cbrc.jp/alignment/server/). The Akaike Information Criterion (AIC) in jModeltest 0.1.1 (Guindon and Gascuel 2003; Posada 2008) was used to select the nucleotide substitution model for each region. The combined data included a 4778 bp character set of the five regions and were analysed. Maximum likelihood phylogenetic analyses were conducted in RAxML (Stamatakis et al. 2008) with the recommended partition parameters to determine the best tree topology. The bootstrap support values were achieved after 500 search replicates and summarised in TreeGraph. Bayesian Posterior Probabilities (BPP) were estimated in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with the same partition parameters. In this analysis, two runs of

Table 1. GenBank accession numbers for sequences used in the phylogenetic analysis.

Species	Insecta	Voucher	GenBank accession no.				
			ITS	LSU	SSU	RPB1	TEF1α
Hirsutella cf. *haptospora*	Diptera: Itonididae	ARSEF 2228	KM652166	KM652118	KM652075	KM652041	KM652001
H. changbeisanensis	Homoptera: leafhopper	GZUIFR-hir160527	KY415578	KY415586			KY415592
H. citriformis	Hemiptera: Delphacidae	ARSEF 490	KM652151	KM652103			KM651987
H. citriformis (*Cixiidae*)	Hemiptera: Cixiidae	ARSEF 1035	KM652153	KM652105	KM652064	KM652030	KM651989
H. citriformis (*Psyliidae*)	Hemiptera: Psyllidae	ARSEF 2598	KM652155	KM652107			KM651991
H. cryptosclerotium	Hemiptera: Pseudococcidae	ARSEF 4517	KM652157	KM652109	KM652066	KM652032	KM651992
H. flava	Lepidoptera:	**GZUIFR-hir100627-1**	KY415598	KY415599		KY945366	KY415601
	Lepidoptera:	**GZUIFR-hir100627-2**	MF623036	MF623042			MF623046
	Lepidoptera:	**GZUIFR-hir100627-3**	MF623037	MF623043			MF623047
H. fusiformis	Coleoptera: Curculionidae	ARSEF 5474		KM652110	KM652067	KM652033	KM651993
H. guyana	Hemiptera: Cicadellidae	ARSEF 878	KM652158	KM652111	KM652068	KM652035	KM651994
H. haptospora	Acari: Uropodina	ARSEF 2226	KM652159			KM652036	KM651995
H. illustris	Hemiptera: Aphididae	ARSEF 5539	KM652160	KM652112	KM652069	KM652037	KM651996
H. kirchneri	Acari: Eriophyidae	ARSEF 5551	KM652161	KM652113	KM652070		KM651997
H. kuankuoshuiensis	Lepidoptera:	**GZUIFR 2012KKS3-1**	KY415575	KY415582		KY945360	KY415590
	Lepidoptera:	**GZUIFR 2012KKS3-2**	MF623038	MF623044			MF623048
	Lepidoptera:	**GZUIFR 2012KKS3-3**	MF623039	MF623045			MF623049
H. leizhouensis	Lepidoptera: Pyralidae	GZUIFR-hir140506	KY415573	KY415580			KY415587
H. lecaniicola	Hemiptera: Coccidae	ARSEF 8888	KM652162	KM652114	KM652071	KM652038	KM651998
H. liboensis	Lepidoptera: Cossidae	ARSEF 9603	KM652163	KM652115	KM652072		
H. necatrix	Acari	ARSEF 5549	KM652164	KM652116	KM652073	KM652039	KM651999
H. nodulosa	Lepidoptera: Pyralidae	ARSEF 5473	KM652165	KM652117	KM652074	KM652040	KM652000
H. radiata	Diptera	ARSEF 1369		KM652119	KM652076	KM652042	KM652002
H. repens nom. inval.	Hemiptera: Delphacidae	ARSEF 2348	KM652167	KM652120	KM652077		KM652003
H. rhossiliensis (*Heteroderide*)	Tylenchida: Heteroderidae	ARSEF 2931	KM652168	KM652121	KM652078	KM652043	KM652004
H. rhossiliensis	Tylenchida: Criconematidae	ARSEF 3747	KM652170	KM652123	KM652080	KM652045	KM652006
H. satumaensis	Lepidoptera: Pyralidae	ARSEF 996	KM652172	KM652125	KM652082	KM652047	KM652008
H. sinensis	Lepidoptera: Hepialidae	ARSEF 6282	KM652173	KM652126	KM652083	KM652048	KM652009
H. strigosa(*Cicadellidae*)	Hemiptera: Cicadellidae	ARSEF 2197	KM652175	KM652129	KM652085	KM652050	KM652012
H. strigosa(*Delphacidae*)	Hemiptera: Delphacidae	ARSEF 2044	KM652174	KM652128			KM652011
H. subulata	Lepidoptera: Microlepidoptera	ARSEF 2227	KM652176	KM652130	KM652086	KM652051	KM652013
H. thompsonii (*Eriophyidae*)	Acari: Eriophyidae	ARSEF 253	KM652179	KM652133	KM652088		KM652016
H. thompsonii (*Tetranychidae*)	Acari: Tenuipalpidae	ARSEF 3323	KM652188	KM652143	KM652096	KM652059	KM652024
H. thompsonii var. *synnematosa*	Acari: Tetranychidae	ARSEF 5412	KM652193	KM652148	KM652100		
H. thompsonii var. *thompsonii*	Acari: Eriophyidae	ARSEF 137	KM652177	KM652131	KM652087	KM652052	KM652014
H. versicolor	Hemiptera: Membracidae	ARSEF 1037		KM652150	KM652102	KM652063	KM652029
Ophiocordyceps acicularis	Coleoptera	OSC 110988		EF468804	EF468951	EF468853	EF468745
O. agriotidis	Coleoptera	ARSEF 5692	JN049819	DQ518754	DQ522540	DQ522368	DQ522322
O. aphodii	Coleoptera	ARSEF 5498		DQ518755	DQ522541		DQ522323
O. appendiculata	Coleoptera	NBRC 106960	JN943326	JN941413	JN941728	JN992462	AB968577
O. brunneipunctata	Coleoptera (Elateridae)	OSC 128576		DQ518756	DQ522542	DQ522369	DQ522324
O. clavata	Coleoptera	NBRC 106962	JN943328	JN941415	JN941726	JN992460	AB968587
O. cochlidiicola	Insect	HMAS199612	AB027377	KJ878884	KJ878917	KJ878998	KJ878965
O. communis	Coleoptera	NHJ 12581		EF468831	EF468973		EF468775
O. dipterigena	Diptera (adult fly)	OSC 151912		KJ878887	KJ878920	KJ879001	KJ878967
O. elongata	Lepidoptera (larva)	OSC 110989		EF468808		EF468856	EF468748

Species	Insecta	Voucher	GenBank accession no.				
			ITS	LSU	SSU	RPB1	TEF1α
O. entomorrhiza	Lepidoptera	KEW 53484	JN049850	EF468809	EF468954	EF468857	EF468749
O. evansii	Hymenoptera (Pachycondylaharpax)	Ophsp 858		KC610770	KC610796	KP212916	KC610736
O. forquignonii	Diptera (adult fly)	OSC 151908		KJ878889	KJ878922	KJ879003	
O. geometridicola	Lepidoptera (Geometridae)	TBRC 8095		MF614648		MF614663	MF614632
O. gracilis	Lepidoptera (larva)	EFCC 8572	JN049851	EF468811	EF468956	EF468859	EF468751
O. heteropoda	Hemiptera (cicada nymph)	OSC 106404		AY489722	AY489690	AY489651	AY489617
O. irangiensis	Hymenoptera (adult ant)	OSC 128579		EF469076	EF469123	EF469089	EF469060
O. konnoana	Coleoptera (larva)	EFCC 7315			EF468959	EF468861	EF468753
O. lanpingensis	Hepialus (larva)	YHOS0707		KC417461	KC417459	KC417465	KC417463
O. lloydii	Hymenoptera (Camponotus)	OSC 151913		KJ878891	KJ878924	KJ879004	KJ878970
O. macroacicularis	lepidopterans (larvae)	NBRC 105888	AB968401	AB968417	AB968389		AB968575
O. melolonthae	Coleoptera (Scarabeidae larva)	OSC 110993		DQ518762	DQ522548	DQ522376	DQ522331
O. multiperitheciata	Lepidoptera (larva)	BCC 69008		MF614657			MF614641
O. myrmicarum	Formicidae (adult ant)	ARSEF 11864			KJ680150	KJ680151	JX566973
O. nigrella	Lepidoptera (larva)	EFCC 9247	JN049853	EF468818	EF468963	EF468866	EF468758
O. pauciovoperitheciata	Lepidoptera (larva)	TBRC 8106		MF614652			MF614633
O. pseudoacicularis	Lepidoptera (larva)	TBRC 8102		MF614646		MF614661	MF614630
O. ramosissimum	Lepidoptera (larva)	GZUHHN8	KJ028007		KJ028012	KJ028017	KJ028014
O. robertsii	Lepidoptera (Hepialidae larva)	KEW 27083		EF468826			EF468766
O. sinensis	Lepidopteran pupa	EFCC7287	JN049854		EF468971	EF468874	EF468767
O. sporangifera	Lepidoptera (Cossidae)	MFLUCC 18-0492	MH725818	MH725832	MH725814	MH727392	MH727390
O. stylophora	Coleoptera (Elateridae larva)	OSC 111000	JN049828	DQ518766	DQ522552	DQ522382	DQ522337
O. xuefengensis	Lepidoptera (Hepialidae larva)	GZUH2012HN11	KC631803		KC631788	KC631799	KC631794
Tolypocladium inflatum	Coleoptera (larva)	OSC 71235	JN049844	EF469077	EF469124	EF469090	EF469061
T. ophioglossoides	Fungi (Elaphomyces sp.)	NBRC 106332	JN943322	JN941409	JN941732	JN992466	

four chains each were executed simultaneously for 5,000,000 generations, with sampling every 500 generations. TreeGraph was used to compute the BPP from a summary of 7,501 trees retained after a burn-in of the first 2,500 trees collected.

Results

Phylogenetic analyses

The tree was constructed with maximum likelihood and Bayesian posterior probabilities with *Tolypocladium inflatum* and *T. ophioglossoides* as the outgroup taxa based on RPB1, *tef1*, ITS, 18S rDNA and 28S rDNA gene datasets (SSU: 1391 bp, LSU: 903 bp, ITS: 721 bp, TEF1α: 946 bp and RPB2: 817 bp) (Fig. 1). In this phylogenetic tree, *Hirsutella flava* and *H. kuankuoshuiensis* formed a separate clade from the other species with credible bootstrap values (85% ML and 0.90 PP), suggesting that these two species are truly related. Within a separate branch, *H. flava* and *H. kuanku-*

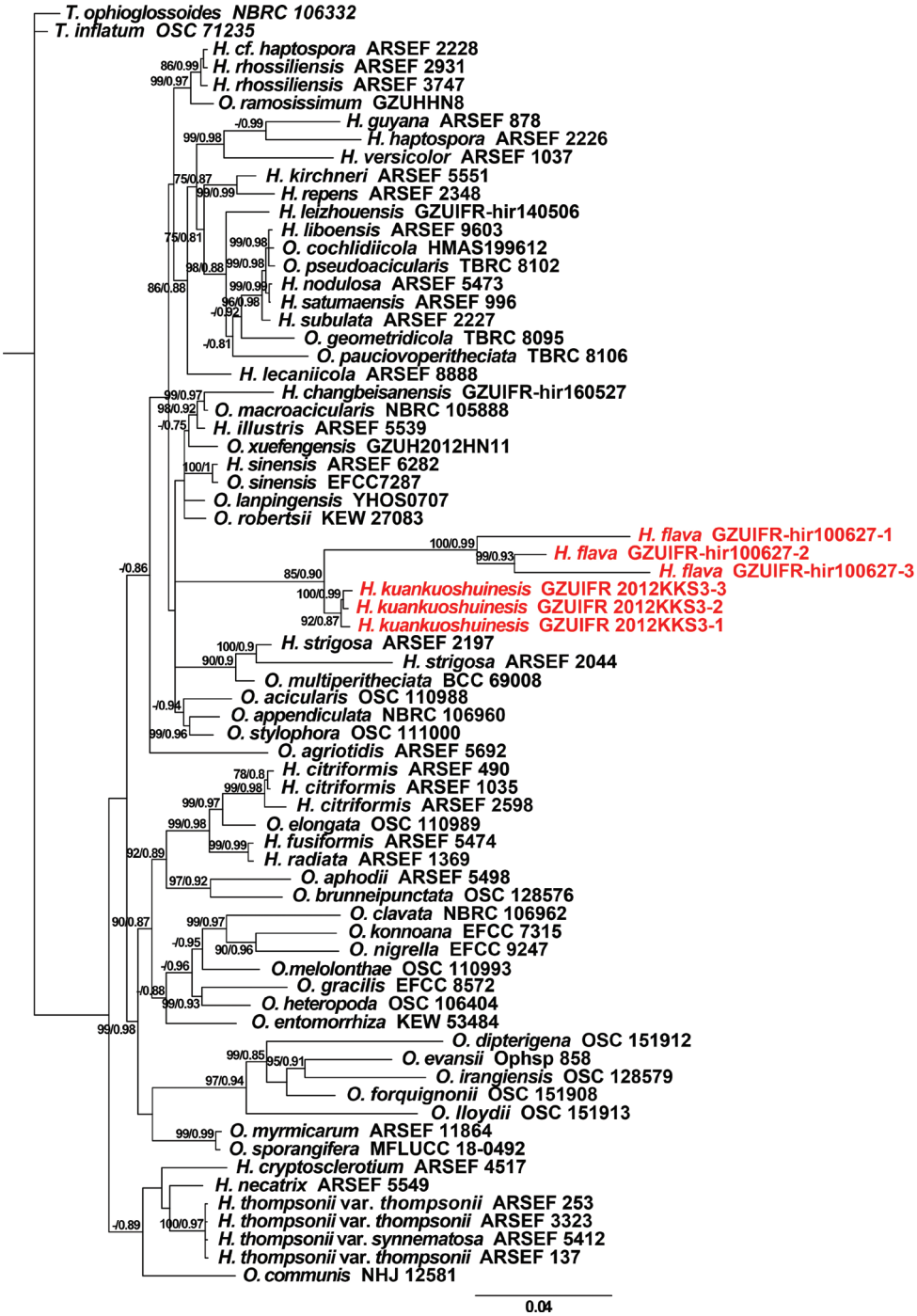

Figure 1. Phylogenetic tree of *Hirsutella* species combined with RPB1, *tef1*, ITS, 18S rDNA and 28S rDNA datasets, using the maximum likelihood method. Numbers below the branches are bootstrap percentage values, based on 10,000 replicates, ML/BPP.

oshuiensis were allied with the *H. sinensis* and *H. strigosa* clade, distant from the other hirsutella-like species, particularly the *H. thompsonii* clade. A molecular phylogenetic analysis further confirmed the differences among the two new species and other related species. Based on the morphological characteristics and molecular phylogenetic analysis, these two new species are introduced as new members of *Hirsutella* species in the Ophiocordycipitaceae family.

Taxonomy

Hirsutella flava **X. Zou, J.J. Qu, Z.A. Chen & Z.Q. Liang, sp. nov.**
MycoBank No: 819552
Fig. 2

Diagnosis. Characterised by phialides slender awl-shaped and tapered; a width of base 24–40.8 × 2.2–2.5 μm; tapering to narrow neck, 7.2–9 μm long × 0.5 μm wide. Conidia narrow cymbiform, long fusoid or limoniform, 6.5–10 × 2.1–4.3 μm.

Type. China, Zhejiang Province, Tianmu Mountain National Nature Reserve (30°18'N, 119°28'E, approximately 600–1200 m a.s.l.), 27 June 2010, presented by Prof. Zhuan Chen. The holotype has been deposited at KIB (HKAS112884). Sequences from isolated strains (GZUIFR-hir100627-1, GZUIFR-hir100627-2 and GZUIFR-hir100627-3) have been deposited in GenBank.

Description. *Synnemata* extending from the head of insect, 3–10 cm × 0.5–1 mm, simple or irregularly branched, dark brown and changing to faint yellow toward the apex; no conidiation was observed (Fig. 2A). The fungus grows slowly at 22 ± 1 °C on Czapek-Dox agar medium to a diam. of 8–12 mm; the colony surface was flat and flocculent with white aerial hyphae. On PDA agar, fungal colonies grew quickly to a diam. of 15–23 mm after 20 d at 22 ± 1 °C, when the colonies were blanket-like with rough mycelia, radiating beam-like from the centre; centre lunate concave, pale yellow; colony surface with yellowish liquid exudation (Fig. 2B, C). *Mycelium* hyaline, smooth, septate, 3.6–4.5 μm wide. *Conidiogenous cells* form directly from the mycelial end, monophialidic or polyphialidic, and borne perpendicular or at acute angles (80°–85°) to the subtending hyphae. *Phialides* slender awl-shaped and tapered, width of the base 24–40.8 × 2.2–2.5 μm, tapering to a narrow neck, 7.2–9 μm long × 0.5 μm wide. *Conidia* narrow cymbiform, long fusoid or limoniform, 6.5–10 × 2.1–4.3 μm; single- or double-enveloped in a hyaline mucus, thickness 2.0–3.0 μm (Fig. 2D–K).

Host. Larva of a species of Lepidoptera.

Habitat and distribution. On decaying leaves in broadleaved forests, Zhejiang Province, China.

Etymology. Refers to the yellow colour (Lat. 'flava') of the holotype and colony.

Teleomorph. Unknown.

Remarks. This species is allied with the *H. sinensis* and *H. strigosa* clade. The phialides of *H. flava* are subulate, and the necks are slenderer. In particular, the colony

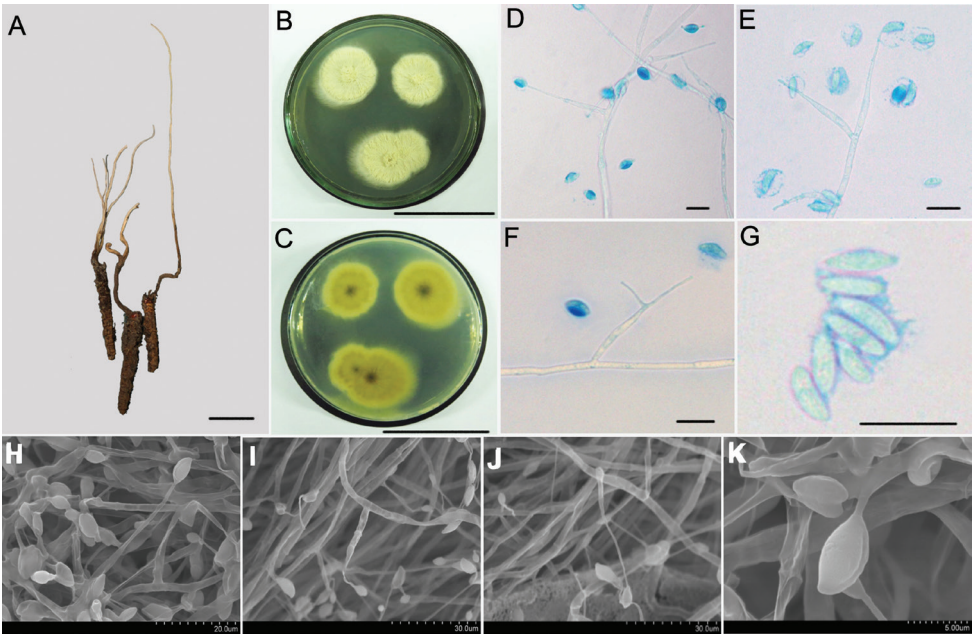

Figure 2. Morphological characteristics of *Hirsutella flava* **A** the infected insect specimens with a long and single synnemata (HKAS112884) **B, C** colonial morphology on PDA agar media for 20 d **B** shows the front of the colony and **C** shows the back of the colony **D–G** LM images of the general morphology of conidiogenous cells and conidia **H–K** SEM images showing conidiogenous cells and conidial structure; Scale bars: 1 cm (**A**); 5 cm (**B, C**), 10 μm (**D–G**); the rest of the bars are shown in the figure. LM, light microscopy; PDA, potato dextrose agar; SEM, scanning electron microscopy.

morphology of this fungus is unique among the *Hirsutella* species. The colony surface appears very rough, and the hyphae are gathered into outwardly radiating filamentous bundles of varying sizes.

***Hirsutella kuankuoshuiensis* X. Zou, J.J. Qu & Z.Q. Liang, sp. nov.**
MycoBank No: 819591
Fig. 3

Diagnosis. *Hirsutella kuankuoshuiensis* differs from other species in this genus primarily by its clavate, narrow fusiform or botuliform conidia and subulate or slender columnar phialide.

Type. China, Guizhou Province, Suiyang County, Kuankuoshui Nature Reserve (28°08'N, 107°02'E, approximately 1400 m a.s.l.), July 2012, collected by X. Zou. The holotype has been deposited at KIB (HKAS112885). Sequences from isolated strains (GZUIFR-2012KKS3-1, GZUIFR-2012KKS3-2 and GZUIFR-2012KKS3-3) have been deposited in GenBank.

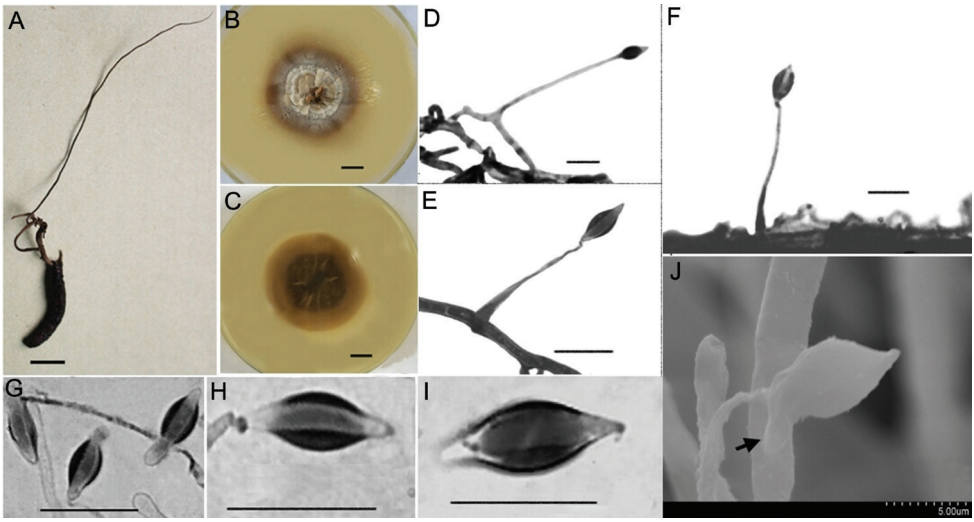

Figure 3. Morphological characteristics of *Hirsutella kuankuoshuiensis* **A** the insect specimens with single and thin synnemata (HKAS112885) **B, C** colonial morphology on PDA agar media for 20 d **B** shows the front of the colony and **C** shows the back of the colony **D–G** LM images showing conidiogenous cells and conidia **D, E** the structure of conidiogenous cells on mycelia **F** the images of conidiogenous cells on synnemata (optical microscope) **H–J** conidial morphology (LM) **G** conidia with mucilage (SEM). Scale bars: 10 mm (**A–C**); bar of **G** was shown in the figure; the rest of the bars were 10 μm. LM, light microscopy; PDA, potato dextrose agar; SEM, scanning electron microscopy.

Description. *Synnemata* are single, extending from the head of insect; 8.6 cm long, dark brown and changing to brown towards the apex; no conidiation was observed (Fig. 3A). The fungus spreads slowly on PDA agar at 20–22 °C and grows to a diam. of 22–30 mm after 14 d; the colony is round, centre of surface with brown dense bulges and grey-white sparse flocculent aerial hyphae. Colony margin is flat with radial groove; a large amount of brown pigment secreted into the medium causes the back of colony to appear dark brown; thickness 10–12 mm (Fig. 3B, C). *Mycelium* hyaline, smooth, septate, 1.5–3.0 μm wide. *Conidiogenous cells* monophialidic, hyaline, borne perpendicular or at an acute angle to the subtending hyphae. *Phialides* subulate or slender columnar, tapering gradually to a long and narrow neck, 30–45 × 1–3 μm long. *Conidia* clavate, narrow fusiform or botuliform without a diaphragm, 9.9–12.6 × 2.7–4.5 μm, single- or double-enveloped in a hyaline mucus, thickness 2.0–3.0 μm (Fig. 3D–J).

Etymology. Referring to the locality of the specimen, kuankuoshui (Lat. 'kuankuoshuiensis').

Host. Lepidoptera larva.

Habitat and distribution. On the decaying leaves of broadleaved forests, Guizhou Province, China.

Teleomorph. Unknown.

Remarks. This species possesses two types of conidiogenous cells and long fusiform or clavate without diaphragm conidia (9.9–12.6 × 2.7–4.5 μm), which is extremely rare in *Hirsutella* species. In addition, *H. kuankuoshuiensis* could produce long thin synnemata on the culture media that contain few or no conidia.

Discussion

Previous taxonomic studies have shown that the *Hirsutella* species are reconstructed in five main groups, and clustering taxa shared the same phialide structures (Simmons et al. 2015b; Qu et al. 2017; Qu et al. 2018). In general, the *H. nodulosa* lineage possesses phialides with apical helical twists. The *H. citriformis* clade is primarily represented by a squat ovoid base and a single slender neck. The *H. thompsonii* clade, the most widely studied hirsutella-like species and a potential biocontrol agent for mite pests, has a small cylindrical or round phialide, usually less than 25 μm, while the *H. sinensis* clade includes isolates that originate from a variety of taxa, including nematodes, mites and both hemi (Hemiptera) and holometabolous (Coleoptera, Lepidoptera) insect hosts (Simmons et al. 2015b). The majority of these species share a cylindrical base and an average phialide length greater than 40 μm. In our phylogenetic tree, these five typical branches of *Hirsutella* were more dispersed owing to the addition of more *Ophiocordyceps* species. *Hirsutella flava* and *H. kuankuoshuiensis* formed a separate clade that is represented by the subulate phialides and narrow fusiform conidia and have a close relationship with the *H. sinensis* and *H. strigosa* clades. In addition, this separate clade is distant from the *H. thompsonii* and *H. citriformis* clades. Species in these clades primarily share similarly large phialides and long fusiform conidia (Qu et al. 2018).

The phylogenetic tree confirmed the distinction between two new species and extant species. Among the species with an awl-shaped base and a long narrow neck, *H. flava* differs in its subulate phialides (e.g. *H. danubiensis* Balazy et al., 2008; *H. tunicate* Ciancio et al., 2013), cylindrical phialides (e.g. *H. changbeisanensis* Liang, 1991; *H. strigosa* Petch, 1939) and two types of conidiogenous cells (e.g. *H. stilbelliformis* Evans & Samson, 1982; *H. shennongjiaensis* Zou et al., 2016b) (Suppl. material 1: Table S2). In addition, *H. flava* is unique in the colony morphology of isolated strains. The fungus spreads more quickly than other hirsutella-like species on PDA media, and the colony surface appears very rough, owing to the hyphae being gathered into outwardly radiating filamentous bundles of varying sizes. *H. flava* could be distinguished from similar species by the shape and size of the conidiogenous cells. Morphological comparisons of relevant taxa are shown in Suppl. material 1: Table S2.

Hirsutella kuankuoshuiensis possesses two types of conidiogenous cells and long fusiform or clavate conidia, which are unique to *Hirsutella*. Furthermore, this species can readily produce long thin synnemata on culture media, but it produces few or no conidia. There are five other species similar to this species: *H. shennongjiaensis* (Zou et al. 2016), *H. stilbelliformis* var. *stilbelliformis* (Evans and Samson 1982), *H. sporodochialis*

(Evans and Samson 1984), *H. subramanianii* (Samson and Evans 1985), and *H. zhangjiajiensis* (Liang et al. 2005). Among them, the conidia of *H. shennongjiaensis* are primarily rod-like and slender; *H. stilbelliformis* var. *stilbelliformis* has a larger base with thorny phialides, greater than 50 μm long; *H. sporodochialis* has longer conidia; *H. subramanianii* has hymenopteran hosts and thinner stick-shaped conidia, 10–13.5 × 1.8–2.5 μm; and *H. zhangjiajiensis* conidia are lanceolate or resemble an orange segment (Suppl. material 1: Table S3). Within the framework of the available data for the genus, the phylogenetic tree and the morphological analysis confirmed the status of *Hirsutella flava* and *H. kuankuoshuiensis* as new species.

Acknowledgements

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Supplementary material 1

Tables S1–S3. A total of 71 taxa were selected to represent the morphological and ecological diversity of *Hirsutella* asexual morphs and *Ophiocordyceps*

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Data type: phylogenetic data

Explanation note: The GenBank accession numbers of all species are shown in Table 1.

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RESEARCH ARTICLE

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Refining the picture: new records to the lichen biota of Italy

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Abstract

Based on the analysis of both historical and recent collections, this paper reports an annotated list of taxa which are new to the lichen biota of Italy or of its administrative regions. Specimens were identified using a dissecting and a compound microscope; routine chemical spot tests and standardized thin-layer chromatography (TLC or HPTLC). The list includes 225 records of 153 taxa. Twenty taxa are new to Italy, the others are new to one or more administrative regions, with 15 second records and 5 third records for Italy. Some of the species belong to recently-described taxa, others are poorly known, sterile or ephemeral lichens which were largely overlooked in Italy. Several species are actually rare, either because of the rarity of their habitats (e.g. old-growth forests), or because in Italy they are at the margins of their bioclimatic distribution. The picture of the lichen biota of Italy has now new pixels, but its grain is still coarse. Further analysis of historical collections, increased efforts in the exploration of some areas, and the taxonomic revision of critical groups are still necessary to provide more complete distributional data for new biogeographic hypotheses, taxonomic and ecological research, and biodiversity conservation.

Keywords

Alps, biodiversity, floristics, herbarium specimens, rarity

Introduction

The lichen biota of Italy is among the best known worldwide thanks to a long-lasting tradition of lichenological studies (Nimis 2018) that has experienced a strong boost after the publication of the first modern checklists (Nimis 1993, 2016), and of the first computer-aided keys (Nimis and Martellos 2020). This is reflected in the steep increase of the number of species known to occur in Italy, more than 550 species having been added between 1993 and 2016 (Nimis 2016). Since 2016, new records (both for the country and for its administrative regions) are constantly being published every year (e.g. Ravera et al. 2020a, b, 2021), which indicates that the exploration of the lichen biota of Italy is still incomplete, and that distributional data of many species are still lacunose (e.g. Martellos et al. 2020). More information is available for the regions of the North, Tuscany and Sardinia (Nimis 2016), all of which are known to host more than 1.000 species each, while most regions of Southern and Central Italy are still insufficiently explored, which may hamper accurate estimations of species rarity (Nimis et al. 2018a), analyses of species richness and composition patterns (Marini et al. 2011), as well as species distribution models (Guttová et al. 2019).

In this work we report the results of the analysis of recent collections and the re-evaluation of herbarium specimens, mainly collected during the last twenty years, in the light of recent taxonomical progress. Twenty taxa are new to Italy, 133 are new to different administrative regions, thus providing a substantial contribution to the knowledge of the lichen biota of Italy.

Materials and methods

Based on the analysis of herbarium material (BOLO, GZU, M, MOD, TSB, RO, UPS, Herb. Nascimbene and other private herbaria), an annotated list of taxa which are new to the lichen biota of Italy or of its administrative regions, has been prepared. The specimens were identified in the laboratory using a dissecting and a compound microscope. Routine chemical spot tests were performed for most specimens. In some cases (i.e. for sterile crustose lichens) standardized thin-layer chromatography (TLC) was used, following the protocols of Orange (2010), or HPTLC, following Arup et al. (1993).

Nomenclature, as well as synonimization of old records, mainly follow ITALIC 6.0 – The information system on Italian Lichens (Nimis and Martellos 2021), which is continuously updated online. This source was used also for retrieving ecological and distributional information for each taxon.

Taxa are subdivided in two alphabetically ordered lists: 1) new to Italy, 2) new to administrative regions of Italy (see Fig. 1). For each taxon, the following information is included:

1. administrative region;
2. collection locality, elevation, and substrate. When available, longitude and latitude are reported in DMS. Coordinates available on the original label of the specimens in other formats were converted to this system;

Figure 1. Administrative regions of Italy

3. collection date, collector(s) name (Leg.), and Herbarium code. Institutional herbaria are abbreviated according to Index Herbariorum (Thiers 2016). The private herbarium of J. Nascimbene is abbreviated as Herb. Na;
4. short note on ecology, distribution and/or taxonomy.

Results

Taxa new to Italy

***Agonimia vouauxii* (B. de Lesd.) M. Brand & P. Diederich**

Abruzzo • Chieti Prov., Majella National Park, main ridge of the Majella Massif; 42°06'56"N, 14°06'54"E; 2450 m; 12 Jul. 2018; Nascimbene leg.; on soil; Na 5842.

A taxon described from maritime Northern France, where it colonises organic waste like paper, leather, etc.; elsewhere it was reported from soil rich in calcium in open vegetation type.

***Bacidina adastra* (Sparrius & Aptroot) M. Hauck & V. Wirth**

Emilia-Romagna • Parma Prov., Baganzola; 44°51'07"N, 10°18'22"E; 60 m; 03 Mar. 2015; Nascimbene leg.; on *Tilia* sp.; Na 4486, GZU.

This species, similar to *B. arnoldiana*, was described from the Netherlands (Sparrius and Aptroot 2003) and is mainly bound to anthropised environments where it usually grows on acid or neutral, eutrophicated bark, as in the case of our specimen, collected in an urban area.

***Cetrelia chicitae* (W.L. Culb.) W.L. Culb. & C.F. Culb.**

Veneto • Belluno Prov., Cansiglio Forest, Pian Canaje; 46°06'11"N, 12°24'35"E; 1050 m; 12 Oct. 2020; Nascimbene leg.; on *Fagus sylvatica*; Na 6978. – **Friuli Venezia Giulia** • Udine Prov., Lake of Sauris; 46°26'32"N, 12°40'37"E; 1000 m; 03 Sep. 2003; G. Caniglia leg.; on *Fagus sylvatica*; Na 5141.

According to Obermayer and Mayrhofer (2007) this is the rarest species of *Cetrelia* in the Eastern Alps.

***Circinaria serenensis* (Cl. Roux & M. Bertrand) A. Nordin**

Abruzzo • L'Aquila Prov., Campotosto lake, near Campotosto; 1340 m; 12 Aug. 1996; on limestone; TSB 24336 • L'Aquila Prov., Gran Sasso Massif, Campo Imperatore, 3 km from bifurcation Fonte Velica-C. d. Monte, road to Rifugio Abruzzi; 1500 m; 08 Sep. 1996; Nimis and Tretiach leg.; on limestone; TSB 24460. – **Basilicata** • Potenza Prov., M. Volturino above Marsico Vetere, road to the lift; 1300 m; 11 Apr. 1996; Nimis & Tretiach leg.; on limestone; TSB 22128. – **Campania** • Caserta Prov., road between Sella di Perrone and Campitello Matese, pass between M. Porco and M. La Gallinola; 16 Apr. 2000; Nimis & Tretiach leg.; on limestone; TSB 32108. – **Marche** • Pesaro Prov., Montefeltro, Eremo di Madonna del Faggio above Calvillano di Carpegna; 1300 m; 20 Aug. 1998; Nimis leg.; on limestone; TSB 23373. – **Piemonte** • Cuneo Prov., Cottian Alps, Vallone dell'Arma, on the ridge SE above colle Valcavera; 2470 m; 23 Jul. 2000; Nimis & Tretiach leg.; on limestone; TSB 33966.

A recently-described calcicolous species similar to *C. calcarea*, with optimum in the montane-subalpine belts. The species, which is apparently widespread in the Western Alps (France), might have been filed under *C. calcarea* in the past, and should be looked for throughout the Alps, including the Italian Alps.

***Collema glebulentum* (Nyl. ex Cromb.) Degel.**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Bona; 46°17'35"N, 11°45'36"E; 1740 m; 28 Aug. 2020; Nascimbene leg.; on periodically submerged porphyric rock; Na 6895.

A species found in very humid situations near or above treeline, as in the case of this record, collected on a boulder along a mountain stream.

***Enchylium coccophorum* (Tuck.) Otálora, P.M. Jørg. & Wedin**

Emilia-Romagna • Bologna Prov., Gessi Bolognesi Natural Park, San Lazzaro di Savena, Dolina della Spipola; 44°26'49"N, 11°22'45"E; 195 m; 07 May 2021; Nascimbene leg.; on terricolous bryophytes above gypsum; Na 7230.

An almost cosmopolitan species of dry areas, found on calciferous soil in dry grasslands, which can be easily mistaken for *E. tenax.* It might be more widespread in Italy, especially in dry Mediterranean areas of the South.

***Fuscopannaria confusa* (P.M. Jørg.) P.M. Jørg.**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Ceremana; 46°17'19"N, 11°43'35"E; 1760 m; 10 Sep. 2012; Nascimbene leg.; on *Salix caprea*; Na 2819.

A rare European species with a boreal to temperate-high montane distribution, found on branches of various trees and shrubs near the ground, but also on rocks in very humid places, like in the spray zone of waterfalls. Our specimen was found on bark of *Salix caprea* in a very humid old-growth spruce forest, together with other suboceanic cyanolichens (e.g. *Pannaria conoplea*, *Peltigera collina*, *Nephroma parile*).

***Gyalideopsis helvetica* van den Boom & Vězda**

Trentino-Alto Adige • Trento Prov., Stelvio National Park, Val de la Mare along the path from the parking place at the hydro-electric power station just below Malga Mare; 46°24'58"N, 10°40'53"E; 1990 m; 27 Jul. 2006; Thor leg.; on a *Larix* log; UPS L-166763.

When sterile, as in the case of our collection, the species is recognised by the thallus which consists of a thin film interrupted by patches of concave goniocystangia (van den Boom and Vězda 2000). Described from Switzerland, but widely distributed in Europe, North America and Asia.

***Lecanora leptacinella* Nyl.**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Passo Rolle, northern slopes of Mt. Tognazza; 46°17'20"N, 11°47'05"E; 2100 m; 01 Sep. 2002; Hafellner leg.; on bryophytes (moribund *Polytrichum sexangulare*); GZU – Ha 63986.

This inconspicuous species settles in a narrow ecological niche, growing on the upper parts of moribund *Polytrichum*-gametophytes, in the Alps only at high altitudes. For a detailed treatment see Obermayer and Poelt (1994).

***Myriospora scabrida* (Hedl. ex H. Magn.) K. Knudsen & Arcadia**

Toscana • Livorno Prov., Arcipelago Toscano National Park, Elba Island, M. Capanne; 42°46'16"N, 10°10'02"E; 1000 m; 30 Aug. 1982; Mayrhofer leg.; on granite; GZU – Ma 3923.

A species with an epilithic thallus consisting of pale grey to pale brown areolate to subsquamulose areoles, and 1–2 mm wide apothecia with brown, somewhat raised discs; usually on acidic schistose rocks .

***Protoblastenia szaferi* J. Nowak**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, Monte Tinisa, Le Forcelle; 46°25'10"N, 12°42'10"E; 1900 m; 19 Aug. 1994; Hafellner leg.; on calcareous rock; GZU – Ha 78786.

The species is recognised by its flat, hardly protruding, orange apothecia dispersed on a whitish to cream-coloured semi-endolithic thallus. It grows on steep, shaded faces of limestone. For a detailed treatment see Hafellner (2006).

***Rhizocarpon intermediellum* Räsänen**

Lombardia • Brescia Prov., Adamello Natural Park, Passo Gallinera; 46°10'49"N, 10°24'49"E; 2320 m; 16 Jun. 2004; Nascimbene leg.; on siliceous rock; Na 5104.

An arctic to nemoral-alpine species morphologically resembling *R. norvegicum*, but ascospores submuriform, larger (to 20 µm long), with 1–4 transverse septa and 1 incomplete longitudinal septum. It colonises basic siliceous rocks and schists with low content of calcium in exposed situations, and starts the life-cycle on various crustose lichens. Most records from the Alps are in the central-eastern part (Nimis et al. 2018a), but the species is likely to have been overlooked in some regions.

***Rhizocarpon postumum* (Nyl.) Arnold**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, road from Timau to Passo di M. Croce Carnico, 1100 m; 28 Oct. 1982; P.L. Nimis leg.; M. Tretiach det.; on Werfen sandstone; TSB 2857.

A species recalling *Rh. distinctum* in the small-sized apothecia, but medulla not amyloid and with stictic acid, apothecia flat, smooth, less than 0.5 mm across, ascospores small (mostly less than 25 µm long), submuriform; on siliceous rocks, often close to streams and waterfalls; widespread in Europe, but rather rare or not always distinguished, with scattered records from the Alps.

***Rinodina interpolata* (Stirt.) Sheard**

Calabria • Reggio Calabria Prov., Aspromonte, Pietra Impiccata; 1750 m; 12 Jul. 1988; Josef Poelt leg.; M. Giralt and H. Mayrhofer rev.; on north–facing, deep overhangs of siliceous rock; GZU.

This species is characterized by a pale brown thallus, discrete, sessile apothecia and narrowly ellipsoid *Physcia*-type ascospores grading into the *Physconia*-type at maturity, with a poorly developed torus. It grows on hard siliceous rocks, usually on sheltered and shaded, vertical or overhanging cliffs and boulders. Described from Scotland where it is uncommon (Mayrhofer and Poelt 1979; Giavarini et al. 2009) and also known from scattered localities in middle and southern Scandinavia and Iceland (Mayrhofer and Moberg 2002). Very rare in Western and Central Europe (Giralt et al. 1997; Wirth et al. 2013), being known from mid- to rather high altitudes in Portugal (Giralt 2001).

***Solorina bispora* Nyl. var. *subspongiosa* (Zschacke) Frey**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'25"N, 11°50'34"E; 2030 m; 10 Apr. 2021; Nascimbene leg.; on soil; Na 7160.

This is probably a morph with a dominant cyanobacterial photobiont in the *S. bispora*-group, growing on basic to subneutral soil at high elevations. A detailed DNA analysis of this group is being carried out by colleagues from Graz.

***Staurothele sapaudica* Asta, Clauzade & Cl. Roux**

Veneto • Belluno Prov., Dolomiti d'Ampezzo Natural Park, Tofana di Rozes; 46°32'29"N, 12°03'01"E; 2870 m; 05 Aug. 2020; Nascimbene leg.; on dolomite (dolomia principale) with water seepage; Na 6886, TSB42610.

This species, described from the Western Alps (France), is found on periodically moist calcareous rocks, e.g. along streams at high elevations, or in shaded-humid situations. Our specimen was collected on dolomite washed by melting water in the nival belt.

***Thelidium schibleri* Zschacke**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, above Rolle pass, near Baita Cervino; 46°17'51"N, 11°55'15"E; 2090 m; 14 Sep. 2020; Nascimbene leg.; on sedimentary, calcareous-arenaceous rocks; Na 7131.

A critical taxon, hitherto known only from the type locality in Switzerland where it was collected on calcareous rocks in upland areas. Similar to other *Thelidium*-species, this taxon would require further research to clarify its taxonomic position. Our specimen was collected in a very humid wall of the early Triassic Werfen-formation composed of carbonatic, terrigenous and mixed, varicolored deposits that are sometimes dolomitized. The ecological conditions of the site are similar to those of the type locality, where the species was found together with *Polyblastia cupularis*, as in our case.

***Thelidium subabsconditum* Eitner**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Castellazzo; 46°18'29"N, 11°47'51"E and 46°18'26"N, 11°47'43"E; 2198 m; 14 Sep. 2020; Nascimbene leg.; on calcareous rocks; Na 7198.

This species is widespread in the Alps (Nimis et al. 2018a) on inclined surfaces of compact calciferous rocks in rather shaded, non-eutrophicated situations near or above treeline. Since it was not always distinguished from similar species, it has an apparently incomplete distribution in the Alps.

***Toensbergia leucococca* (R. Sant.) Bendiksby & Timdal**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Ceremana; 46°17'16"N, 11°43'15"E; 1550 m; 10 Sep. 2012; Nascimbene leg.; on *Picea abies* in an old-growth forest; Na 3141. – **Friuli Venezia Giulia** • Udine

Prov., Carnic Alps, Sauris Lake, Bosco della Stua; 46°26'35"N, 12°42'50"E; 1100 m; 16 Aug. 1994; Hafellner leg.; on *Alnus incana*; GZU – Ha 84342.

This is an obligately sterile species with a thallus consisting of scattered, whitish, adnate areoles and usually marginal soralia, containing alectorialic acid and therefore turning reddish with age. It is widespread in the Holarctic region from the boreal to the nemoral-montane zone, including the Alps (most records being from the eastern part), but was still largely overlooked in Italy, where it may be more widespread, at least in the Alps.

***Usnea flavocardia* Räsänen**

Sardegna • Oristano Prov., Seneghe, Montiferru, Nuraghe Ruju; 40°06'49"N, 08°34'55"E; 780 m; 10 Mar. 2014; Nascimbene leg.; on *Quercus ilex*; Na 4403.

A Mediterranean-Atlantic species growing on deciduous trees (e.g. *Fagus*, *Quercus*, *Salix* and *Sorbus*). In Europe it is known from France, Great Britain, Greece, Ireland, Netherlands, Portugal, Spain (Randlane et al. 2009), and Germany (Otte 2011). Our specimen was collected in a montane, old-growth, unmanaged forest hosting several suboceanic and oceanic lichens such as *Lobaria pulmonaria*, *Ricasolia amplissima*, and *R. virens*.

Taxa new to administrative regions of Italy

***Acarospora freyi* H. Magn.**

Basilicata • Potenza Prov., Mt. Volturino above Marsico Vetere; 1500 m; 11 Apr. 1996; Nimis and Tretiach leg.; K. Knudsen rev.; on siliceous rocks, parasitic of *Aspicilia* sp.; TSB 22197. – **Calabria** • Cosenza Prov., Sila Greca, loc. Finaita; 1000 m; 15 Jul. 1988; Nimis, Tretiach and M. Castello leg.; K. Knudsen rev.; on siliceous rock, parasitic on *Aspicilia* sp.; TSB 10995.

Probably overlooked and more widespread, both in the Alps and in the Apennines, with optimum near and above treeline, this lichen starts the life-cycle on species of *Aspicilia*, especially *A. candida* and *A. polychroma*, on calciferous rocks which are at least partly decalcified on the surface.

***Acarospora gallica* H. Magn.**

Trentino-Alto Adige • Bolzano/Bozen Prov., Venosta valley, near Silandro/Schlanders; 46°37'50"N, 10°46'46"E; 750–800 m; 12 Sep. 1970; Josef Poelt leg.; on south-facing slopes, on rock; GZU.

A probably holarctic species of base-rich, weakly calciferous siliceous substrata, such as calcareous sandstone, brick, and roofing tiles, usually at relatively low elevations; much overlooked or confused with other species and certainly more widespread in Italy.

***Acarospora irregularis* H. Magn.**

Basilicata • Potenza Prov., Melfi, below the castle; 530 m; 14 Apr. 1997; Nimis and Tretiach leg.; K. Knudsen rev.; on basaltic rocks; TSB 29919 (as *Acarospora oligospora*).

A species known from Central Europe (Austria, Czech Republic, Hungary and Slovakia), as well as Greece and Sardinia, which was often confused with *A. nitrophila* and related species.

***Acarospora laqueata* Stizenb.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Colle Cesta; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2020; Nascimbene leg.; on selciferous calcareous rocks (Formazione di Fonzaso); Na 7245. – **Sicilia** • Palermo Prov., Madonie, Piano Battaglia; 37°52'47"N, 14°01'31"E; 1600 m; 31 May 1988; Josef Poelt leg.; on calcareous rocks; GZU.

On hard calcareous rocks, both on vertical faces and at the top of birds' perching sites in dry-continental areas, below the subalpine belt.

***Acarospora similis* H. Magn.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Erera-Brendol; 46°09'44"N, 11°58'57"E; 1710 m, 18 Oct. 2020; Nascimbene leg.; on a wooden fence near a hut; Na 7011. **Second record for Italy.**

The only previous record of this lignicolous species from Italy is that of a specimen collected on a horizontal wood fence in a vineyard near Merano, at c. 500 m (Nimis 2016). Our sample was collected on a similar substrate but at higher elevation, indicating that in Italy this species could span a wide altitudinal range.

***Acolium marcianum* (B. de Lesd.) M. Prieto & Wedin**

Emilia-Romagna • Bologna Prov., Camugnano, Alto Reno Terme; 44°08'00"N, 11°05'44"E and 44°08'07"N, 10°53'56"E; 870–890 m; 2018; S. Gambini leg.; on old *Castanea sativa* in traditional orchards; Na 6817.

A rare lichen usually growing on pertusarioid silicicolous species, mainly Tyrrhenian in Italy. Our specimen was collected in old-growth *Castanea*-stand near Bologna (Pezzi et al. 2020).

***Agonimia gelatinosa* (Ach.) M. Brand & Diederich**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'25"N, 11°50'34"E; 2030 m; 10 Apr. 2021; Nascimbene leg.; on terricolous bryophytes; Na 7158. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Cordin delle Vette; 46°05'20"N, 11°51'09"E; 1950 m; 13 Jun. 2021; Nascimbene leg.; on terricolous bryophytes; Na 7224.

A species growing on plant debris and mosses in calcareous dry grasslands, with optimum above treeline.

***Agonimia opuntiella* (Buschardt & Poelt) Vězda**

Veneto • Belluno Prov., Feltre, Vincheto di Celarda Natural Reserve; 46°36'39"N, 11°04'29"E; 310 m, Aug. 2011; Nascimbene leg.; among mosses on isolated *Ulmus* sp.;

Na 2558. • Belluno Prov., Feltre, Vincheto di Celarda Natural Reserve; 46°00'50"N, 11°58'41"E; 310 m, Aug. 2011; Nascimbene leg.; among mosses on isolated *Populus* sp.; Na 2560.• Belluno Prov., Feltre, Rocchetta di San Vittore; 46°00'11"N, 11°56'43"E; 400 m; 16 May 2021; Nascimbene leg.; on plant debris and mosses; Na 7203.

A mild-temperate species that in Italy occurs along a wide altitudinal gradient, from the Mediterranean to the montane belt (Nimis 2016) both on terricolous mosses and plant debris over calcareous substrata and amongst mosses on basal parts of old trees.

***Alyxoria ochrocincta* (Werner) Ertz**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'36"N, 14°21'23"E; 1000 m; Jul. 2009; Nascimbene leg.; on wood; Na 2416.

A Mediterranean species occurring in shaded and humid situations, as in the case of our sample.

***Anaptychia bryorum* Poelt**

Piemonte • Cuneo Prov., Cottian Alps, W ridge of Mt. Nebin, ca. 1 km E of Colle di Sempeyre; 2380 m; Summer 2000; Nimis and Tretiach leg.; TSB 32957.

An arctic-alpine to boreal-montane, probably circumpolar species found amongst mosses and moribund plants on base-rich siliceous substrata in the alpine and subalpine belts.

***Anema tumidulum* Henssen ex P.M. Jørg., M. Schultz & Guttová**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Erera-Brendol, climbing area near San Vito-Primolano; 45°57'06"N, 11°43'28"E; 370 m; 26/10/2019; Nascimbene leg.; compact calcareous rock with water seepage; Na 6816. • Belluno Prov., Dolomiti Bellunesi National Park, Erera-Brendol, climbing area near San Vito-Primolano; 46°09'39"N, 11°58'35"E; 1700 m; 18/10/2020; Nascimbene leg.; compact calcareous rock with water seepage; Na 7031.

Apparently widespread in Central Europe, but poorly collected elsewhere, growing on steeply inclined, sunny surfaces of calcareous or basic siliceous rocks with periodical water seepage after rain.

***Arthonia calcicola* Nyl.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, between Piadoch and Cima Dodici, near Busa delle Vette; 46°06'28"N, 11°50'23"E; 2150 m; 19 Jun. 2021; Nascimbene leg.; on marly limestone (Rosso Ammonitico Superiore); Na 7242.

An early coloniser on exposed calcareous rocks below the subalpine belt; overlooked and probably more common, especially in the EU-Mediterranean belt. It also occurs in warm-dry Alpine valleys.

***Arthonia vinosa* Leight.**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'55"N, 08°21'24"E; 950–1000 m; Jul. 2009; Nascimbene leg.; on *Abies alba*; Na 2538. • Ibidem; 23 Oct. 2015; Nascimbene leg.; Na 4717, 4720.

A mild-temperate lichen found near the base of old trees, mostly on rough bark, especially of *Quercus* sp., more rarely on wood, in very humid and closed-canopied forests. Included in the Italian red list of epiphytic lichens as "Near-threatened" (Nascimbene et al. 2013).

***Aspicilia candida* (Anzi) Hue**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Passo delle Vette Grandi; 46°05'24"N, 11°50'37"E; 2010 m; 27 Dec. 2003; Nascimbene leg.; on a selciferous carbonatic rock; Na 84. • Ibidem; 26 Jul. 2004; Nascimbene leg.; Na 85. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Cima Dodici; 46°06'30"N, 11°50'30"E; 2200 m; 13 Jun. 2020; Nascimbene leg.; on a selciferous carbonatic rock; Na 6834.

On weakly calciferous rocks, mostly near or above treeline. Our samples were collected on the selciferous carbonatic rock of the late Jurassic formation called "Formazione di Fonzaso".

***Aspicilia grisea* Arnold**

Lombardia • Brescia Prov., Adamello Natural Park, Edolo, Passo Gallinera; 46°10'49"N, 10°24'49"E; 2320 m; 16 Jun. 2004; Nascimbene leg.; on siliceous rocks; Na 5393. • Brescia Prov., Adamello Natural Park, Presanella-group, Passo del Tonale, S above the pass towards Passo del Paradiso; 46°15'10"N, 10°34'45"E; 1950 m; 24 Jul. 2006; Hafellner and Muggia leg.; gentle N-exposed slope, on inclined faces of large granitic boulders; GZU – Ha 85838. **Second record for Italy.**

A chemically variable species found on siliceous rocks, sometimes also on pebbles, widespread in the Alps (Nimis et al. 2018a), but very much overlooked in Italy.

***Aspicilia mashiginensis* (Zahlbr.) Oxner**

Lombardia • Brescia Prov., Adamello Natural Park, Edolo, Passo Gallinera; 46°10'49"N, 10°24'49"E; 2320 m; 16 Jun. 2004; Nascimbene leg.; on siliceous rocks; Na 5394. • Ibidem; 27 Jul. 2006; Hafellner leg.; GZU – Ha 85843, Ha 85844.

A species with grey thalli showing somewhat effigurate margins, the central parts covered in short, thick, hollow papillate structures gradually breaking down into flattened propagules (Poelt 1994). It is restricted to subvertical to overhanging faces of siliceous cliffs from treeline to the nival belt. At the investigated site several thalli have been found, colonised by parasitic *Lecidea tessellata*.

***Bacidia arceutina* (Ach.) Rehm & Arnold**

Veneto • Belluno Prov., Feltre, Vincheto di Celarda Natural Reserve; 46°00'49"N, 11°58'37"E; 310 m; Aug. 2011; on *Populus* sp. in a riparian forest; Nascimbene leg.; on *Populus* sp. in a riparian forest; Na 2555. – **Sardegna** • Nuoro Prov., Barbagia Seulo, State Forest of Monte Arbo, S. Girolamo Valley; 900–1100 m; 16 Jul. 1987; Josef Poelt leg.; GZU. • Oristano Prov., Catena del Marghine, Nuraghe Ortachis, Badde Salighe; 40°21'01"N, 08°54'24"E; 1020 m; 11 Mar. 2014; Nascimbene leg.; on *Quercus ilex* in a forest site rich in *Lobaria pulmonaria*; Na 4830.

A temperate to humid subtropical species found on subneutral bark of broad-leaved trees in open deciduous woodlands near rivers, very rarely calcicolous or muscicolous.

***Bacidina delicata* (Leight.) V. Wirth & Vězda**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Ceremana; 46°17'21"N, 11°43'21"E; 1640 m; Sep. 2012; Nascimbene leg.; on *Picea abies* in old growth forest; Na 3143. • Ibidem; 46°17'26"N, 11°43'14"E; 1780 m; Sep. 2012; Nascimbene leg.; on *Picea abies* in old growth forest; Na 3144.

A mainly Mediterranean-Atlantic to humid subtropical species that was found in a very humid situation together with several other suboceanic lichens.

***Bacidina egenula* (Nyl.) Vězda**

Trentino-Alto Adige • Trento Prov., Val di Fassa, Mazzin; 46°27'34"N, 11°42'00"E; 1400–1450 m; 07 Apr. 1979; Hafellner leg.; on dolomitic rocks in open forest habitat; GZU – Ha 4617.

A mild-temperate to humid subtropical species, most common on pebbles over moist ground in areas with siliceous substrata; certainly overlooked and probably more widespread in Tyrrhenian Italy, with outposts in the Alps.

***Bellemerea subsorediza* (Lynge) R. Sant.**

Lombardia • Brescia Prov., Adamello Natural Park, Passo del Tonale, on the north side of Monticello di Mezzo, below the funicular; 1950 m; 24 Jul. 2006; Hafellner and Muggia leg.; on granite; TSB 38540. **Third record for Italy.**

On siliceous rocks in open lichen communities near or above treeline (i.e. near glaciers). Known from Trentino-Alto Adige (Nimis 2016) and probably more widespread in the Alps, but overlooked, being mostly sterile.

***Blastenia gennargentuae* Vondrák**

Calabria • Reggio Calabria Prov., Aspromonte Massif, Pietra Impiccata; 1750 m; Summer 1988; Nimis and Tretiach leg.; on siliceous rocks; TSB 12205. **Second record for Italy.**

A recently-described species of siliceous rocks in the Mediterranean mountains. The record from Calabria (Aspromonte) was cited by Nimis (1993: 167) under *B. festivella*.

***Blastenia monticola* Arup & Vondrák**

Piemonte • Cuneo Prov., Ligurian Alps above Colle Bertrand, W above Upega; 1960 m; Summer 2000; Nimis & Tretiach leg.; on conifers; TSB 33234.

A recently described species growing on acid bark, mostly of conifers, or on the branches of shrubs in the subalpine belt. Most previous records of *B. herbidella* from the Alps (see Nimis 1993, 2016) could refer to this species, which is probably widespread throughout the Alps.

***Blastenia psychrophila* Halıcı & Vondrák**

Veneto • Belluno Prov., Casera Razzo; 1750 m; Summer 1981; Nimis leg.; on siliceous rocks; TSB 1678. – **Friuli Venezia Giulia** • Udine Prov., near the summit of Mt. Paularo; about 2000 m; Summer 2000; P. Fragiacomo and Nimis leg.; on siliceous rocks; TSB 1707.

A recently-described species growing on base-rich siliceous rocks in the southern and Central European mountains, mostly above or near treeline. Several earlier records of *B. crenularia* (see Nimis 1993, 2016) from alpine-subalpine situations probably refer to this species.

***Buellia atrocinerella* (Nyl.) Scheid.**

Toscana • Livorno Prov., Arcipelago Toscano National Park, Elba island, Madonna di Monserrato; 42°47'02"N, 10°23'31"E; 120 m; 26 Nov. 1989; Mayrhofer and J. Sattler leg.; C. Scheidegger det.; on siliceous rock, radiolarite; GZU – Ma 8696. **Second record for Italy.**

A Mediterranean species of hard siliceous rocks in warm-dry habitats, sometimes growing on other crustose lichens.

***Calicium adspersum* Pers.**

Emilia-Romagna • Bologna Prov., Vergato, Lizzano in Belvedere, Camugnano, Montese, Alto Reno Terme; 44°08'00"N, 10°53'01"E; 890 m; 2018; S. Gambini leg.; on old *Castanea sativa* trees in traditional orchards; Na 6012. • Ibidem; 44°13'28"N, 10°55'41"E; 900 m; S. Gambini leg.; Na 6013. • Ibidem; 44°13'17"N, 10°55'48"E; 950 m; S. Gambini leg.; Na 6018. • Ibidem; 44°07'58"N, 11°05'40"E; 860 m; S. Gambini leg.; Na 6022. • Ibidem; 44°08'00"N, 11°05'44"E; 870 m; S. Gambini leg.; Na 6014. • Ibidem; 44°12'54"N, 11°05'56"E; 870 m; S. Gambini leg.; Na 6015. • Ibidem; 44°08'07"N, 10°53'56"E; 670 m; S. Gambini leg.; Na 6016. • Ibidem; 44°13'45"N, 10°55'36"E; 890 m; S. Gambini leg.; Na 6017. • Ibidem; 44°19'45"N, 11°02'41"E; 840 m; S. Gambini leg.; Na 6018. • Ibidem; 44°07'55"N, 10°54'09"E; 600 m; S. Gambini leg.; Na 6019.

A holarctic, temperate species found on bark, rarely on wood of deciduous trees, especially *Quercus* sp., often in fissures of the bark, more rarely on conifers.

Calogaya arnoldii* (Wedd.) Arup, Frödén & Søchting subsp. *arnoldii

Trentino-Alto Adige • Trento Prov., Stenico, trail near the offices of the Adamello-Brenta Natural Park; 46°03'22"N, 10°50'25"E; 720 m; 08 Apr. 2021; Nascimbene leg.; on limestone; Na 7243.

A well-distinct taxon of the critical *C. saxicola*-complex, found on steeply inclined surfaces of calciferous rocks (limestone, dolomite, calcareous schists) in open habitats; certainly more widespread in Italy. For further details see Gaya et al. (2001).

Calogaya rouxii **(Gaya, Nav.-Ros. & Llimona) provisionally placed here, ICN art. 36.1b**

Piemonte • Cuneo Prov., Cottian Alps, W ridge of Mt. Nebin, c. 1 km E of Colle di Sempeyre; 2380 m; Summer 2000; Nimis and Tretiach leg.; on calciferous siliceous rocks; TSB 32977.

A species with an Alpine distribution, growing mainly on the top of calcareous boulders in sunny and nutrient-enriched sites, often with *C. biatorina* and *Rusavskia elegans*. The Italian distribution is poorly known, as the species was frequently confused with *C. arnoldii*, but probably it is widespread throughout the calcareous Alps, and also occurs in the Central Apennines. All of the Italian records prior to 2008 were under *C. arnoldii* (see Nimis 2016).

Caloplaca coccinea **(Müll. Arg.) Poelt**

Valle d'Aosta • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N, 06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on steep, N-exposed cliffs and boulders of Jurassic limestone; GZU – Ha 85852. – **Piemonte •** Cuneo Prov., Western Alps, Cottian Alps, crest SW above Colle dell' Agnello; 44°40'54"N, 06°58'35"E; 2830 m; 25 Jul. 2000; Hafellner leg.; on outcrops of calcareous schists on steep slope exposed to the SE; GZU – Ha 59417.

The thallus of *Caloplaca coccinea* is somewhat variable, ranging from entirely immersed and uncoloured to semi-immersed with orange particles. Diagnostic are the semi-immersed to sessile, vivid red apothecia, a colour unique among the alpine caloplacoid lichens. The species, which does not belong to *Caloplaca s.str.*, mostly settles on steeply inclined to vertical rock faces of limestone cliffs and outcrops from the lower alpine to the nival belt.

Caloplaca marmorata **(Bagl.) Jatta**

Toscana • Livorno Prov., Island of Pianosa, east coast; 10 m; 22 Mar. 2005; Muggia leg.; on limestone near the sea; TSB 36720.

This lichen has a complex nomenclatural history: Nimis (2016) states that the type of *Callopisma marmoratum* Bagl. (MOD-TSB), the basionym of *Xanthocarpia marmorata* (Bagl.) Frödén, Arup & Søchting, has nothing to do with a *Xanthocarpia*, clearly corresponding with the lichen called *Caloplaca subochracea* (M. Choisy & Werner) Clauzade & Cl. Roux, which, according to Roux & Coll. (2014), is not identical with *Blastenia subochracea* (Wedd.) Arup, Søchting & Frödén. The species, which does not belong to *Caloplaca s.str*. nor to *Blastenia*, and whose DNA analysis is pending, has a Mediterranean distribution on compact limestone, being locally very abundant in coastal situations, and extremely rare far from the coast. The colour of the thallus, based on which several infraspecific taxa were distinguished (see e.g. Roux et al. 2014), is variable depending on exposure to sunlight, and intermediate forms are frequent. A new name for the lichen called *Xanthocarpia marmorata* auct. will be also proposed in a forthcoming paper.

***Caloplaca turkuensis* (Vain.) Zahlbr.**

Veneto • Belluno Prov., Dolomiti d'Ampezzo Natural Park, near Ra Stua; 46°36'53"N, 12°05'54"E; 1600 m; 27 Aug. 2005; Thor leg.; U. Arup conf.; on *Acer pseudoplatanus*, on a steep gravelly slope; GT19355, UPS.

A mild-temperate lichen found on old deciduous trees, often near the base of the trunks, often overlooked, or confused with *C. cerina* (Šoun et al. 2011). It differs from *C. monacensis* in the well-developed, areolate, rarely fertile thallus with a thick layer of small granules, although molecular data suggest that it could be a sorediate-blastidiate morph of *C. monacensis* (Vondrák *in litt.*).

***Candelariella kuusamoënsis* Räsänen**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Erera-Brendol; 46°09'44"N, 11°58'57"E; 1710 m; 18 Oct. 2020; Nascimbene leg.; on a wooden fence near the hut; Na 7199.

A boreal-montane, poorly understood lichen growing on the top of poles and wooden fences, on plant debris and soil, more rarely on rocks in upland areas; certainly more widespread in the Alps. The delimitation of this species is problematic: most of the material distributed in exsiccata belongs to other species, and it is doubtful whether the material called "*C. kuusamoensis*" by Central and Southern European authors really corresponds to the type material, which in itself resembles a luxuriant *C. vitellina* growing on mosses (Westberg, *in litt.*).

***Cetrelia monachorum* (Zahlbr.) W.L. Culb. & C.F. Culb.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Mt. Avena, Col Melon; 46°02'39"N, 11°50'37"E; 1300 m; 27 Feb. 1997; Nascimbene leg.; on *Fagus sylvatica*; Na 356. • Belluno Prov., Cansiglio Forest, Due Ponti; 46°06'39"N, 12°25'04"E; 1100 m; Jan. 2002; Nascimbene leg.; on *Salix* sp.; Na 357. • Ibidem; 1100 m; 18 Dec. 2004; G. Caniglia leg.; on *Fagus sylvatica*; Na 5143. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Soladen; 46°03'45"N, 11°50'38"E; 870 m; 16 Feb. 2020; Nascimbene leg.; on *Fagus sylvatica*; Na 6959, 6961, 6962. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Col dei Cavai; 46°04'23"N, 11°50'02"E; 1360 m; 21 Jan. 1994; Nascimbene leg.; on *Fagus sylvatica*; Na 354. • Belluno Prov., Dolomiti Bellunesi National Park, Longarone, Cajada Forest; 46°14'02"N, 12°14'37"E; 1300 m; Jul. 2010; Nascimbene leg.; on *Abies alba*; Na 2359. • Belluno Prov., Lamon, Senaiga valley near Chioè; 46°02'34"N, 11°42'39"E; 460 m; 17 Mar. 2013; Nascimbene leg.; on *Picea abies*; Na 2979. – **Sardegna** • Nuoro Prov., Gennargentu National Park, Punta Salinas, Baunei (Ogliastra); 40°06'03"N, 09°41'39"E; 450 m; Jul. 2010; Nascimbene leg.; on *Juniperus*; Na 2712.

A species with the imbricaric acid syndrome (major) and perlatolic acid (minor), mainly found on the bark of broad-leaved trees, more rarely on conifers and silicicolous mosses in humid, old, mostly montane forests; probably the most common species of *Cetrelia* in Italy.

***Chaenotheca brachypoda* (Ach.) Tibell**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'36"N, 14°21'23"E; 1000 m; Jul. 2009; Nascimbene leg.; on wood; Na 2487.

On decorticated stumps of deciduous and coniferous trees, more rarely on bark and siliceous rocks in old humid forests, on faces slightly protected from rain. Included in the Italian red list of epiphytic lichens as "Endangered" (Nascimbene et al. 2013).

***Chaenotheca brunneola* (Ach.) Müll. Arg.**

Emilia-Romagna • Bologna Prov., Camugnano; 44°07'58"N, 11°05'40"E; 870 m; 2018; S. Gambini leg.; on old *Castanea sativa* trees in traditional orchards; Na 6519. • Ibidem; 44°08'00"N, 11°05'44"E; 860 m; 2018; S. Gambini leg.; Na 6520.

This species is included in the Italian red list of epiphytic lichens as "Near-threatened" (Nascimbene et al. 2013).

***Chaenotheca phaeocephala* (Turner) Th. Fr.**

Emilia-Romagna • Bologna Prov., Montese; 44°13'45"N, 10°55'36"E; 840 m; 2018; S. Gambini leg.; on old *Castanea sativa* trees in traditional orchards; Na 6020.

A cool-temperate, holarctic lichen mainly found on old *Quercus* sp. in open woodlands, in bark fissures seldom wetted by rain. Our specimen was collected on a very old *Castanea* tree near Bologna (Pezzi et al. 2020).

***Chaenotheca stemonea* (Ach.) Müll. Arg.**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'54"N, 14°21'27"E; 940–1000 m; Jul. 2009; Nascimbene leg.; on *Abies alba*; Na 2404. • Ibidem; 22–23 Oct. 2015; Nascimbene leg.; Na 4670, 4691, 4715.

A cool-temperate to boreal-montane, circumpolar lichen found in rain-protected hollows of conifer trunks inside forests, especially near the ground, both on bark and wood.

***Chaenotheca trichialis* (Ach.) Th. Fr.**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'54"N, 14°21'27"E; 1000 m; Jul. 2009; Nascimbene leg.; on *Abies alba*; Na 2403.

A holarctic species found on acid-barked deciduous trees, conifers and wood in forests and woodlands; widespread in upland areas throughout the country, but most common in the Alps.

***Chaenothecopsis debilis* (Sm.) Tibell**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Canali, Villa Welsperg; 46°11'57"N, 11°52'06"E; 1000 m; 31 May 2012; Nascimbene leg.; on old *Tilia* sp.; Na 2757.

This species was collected in the park surrounding an historical building, on the trunk of an old lime-tree that presented some wood areas protected from rain (i.e. dry conditions). The species was probably overlooked in Italy, as indicated by its current scattered distribution pattern across the country.

***Cladonia arbuscula* (Wallr.) Flot.**

Marche • Fermo Prov., Montefortino; 1846; D. Marzialetti leg.; Gheza det.; BOLO – Herb. Bertoloni (as *C. rangiferina*).

A circumpolar, boreal-subarctic-subalpine lichen, one of the most abundant elements of lichen-rich tundra-like vegetation on mineral soil in exposed habitats. This specimen was likely collected in the summit area of the mountains near Montefortino where altitude exceeds 2000 m.

***Cladonia mitis* Sandst.**

Marche • Fermo Prov., Montefortino; no date [probably 1846]; D. Marzialetti leg.; Gheza det.; BOLO – Herb. Bertoloni (as *C. rangiferina*).

A typical member of subalpine-alpine tundras, perhaps more common at higher altitudes than *C. arbuscula*. This specimen was probably collected in the same stand of *C. mitis* (see above). This is the southernmost record for Italy.

***Diplotomma chlorophaeum* (Leight.) Kr.P. Singh & S.R. Singh**

Calabria • Cosenza Prov., between Frascineto and Villapiana Scalo, along the Raganello river; 39°48'46"N, 16°19'54"E; 200 m; 04 Jun. 1979; Mayrhofer leg.; on rock; GZU – Ma 1142.

A temperate, perhaps holarctic early coloniser of basic siliceous rocks and roofing tiles; overlooked, and certainly more widespread in Italy.

***Diplotomma lutosum* A. Massal.**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Castellazzo; 46°18'28"N, 11°47'56"E; 2270 m; 14 Sep. 2020; Nascimbene leg.; on carbonatic rocks; Na 7134. • Ibidem; 46°18'26"N, 11°47'43"E; 2330 m; 14 Sep. 2020; Nascimbene leg.; Na 7135. – **Veneto** • Belluno Prov., Dolomiti Bellunesi National Park, near Passo Falzarego; 46°30'49"N, 12°00'26"E; 2170 m; 26 Sep. 1985; Mayrhofer leg.; on carbonatic rock; GZU – Ma 8313. – **Basilicata** • Potenza Prov., Piana del Pollino, NW Serra delIe Ciavole; 39°55'09"N, 16°12'57"E; 1900 m; 02 Jun. 1979; Josef Poelt leg.; on rock; GZU – Po 1180.

An apparently widespread but rare, or at least rarely distinguished species, characterised by four-celled spores and a J+ blue medulla .

***Diplotomma murorum* (A. Massal.) Coppins**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Passo Rolle, near Baita Segantini; 46°17'56"N, 11°48'13"E; 2200 m; 14 Sep. 2020; Nascimbene leg.; on arenaceous calciferous rocks (Werfen-formation), parasitic on *Caloplaca erythrocarpa*; Na 7119.

A mild-temperate lichen starting the life-cycle on species of the *Caloplaca teicholyta*-complex, the peculiar biology of which deserves further study. It was certainly overlooked in Italy, as indicated by its current scattered distribution pattern across the country. It is similar to *D. scheideggerianum* that has shorter spores and usually grows on *Leproplaca*-species.

***Diromma dirinellum* (Nyl.) Ertz & Tehler**

Lazio • Latina Prov., Borgo Montello; 41°30'28"N, 12°46'22"E; 30 m; Dec. 2011; Nascimbene leg.; on *Quercus cerris*; Na 2967. • Ibidem; 41°27'46"N, 12°47'18"E; 30 m; Dec. 2011; Nascimbene leg.; Na 2968. • Ibidem; 41°30'21"N, 12°45'48"E; 30 m; Dec. 2011; Nascimbene leg.; Na 2969.

A rare species living as a parasite on *Dirina ceratoniae*, strictly confined to the Mediterranean belt. Our specimen was collected on isolated trees in an agricultural landscape near the Tyrrhenian coast.

***Eiglera flavida* (Hepp) Hafellner**

Liguria • Imperia Prov., Western Alps, Alpi Liguri, mountain ridge S above the village Monesi, on the ridge W above Col Garezzo; 44°02'50"N, 07°46'25"E; 1850 m; 21 Jul. 2000; Hafellner leg.; on small outcrops of calcareous schist in subalpine pasture, on almost horizontal faces of low outcrops; GZU – Ha 84338.

A very inconspicuous species which, on account of its mostly ochraceous to grey thallus and minute black aspicilioid apothecia, is often detected only in screenings of larger collections on limestone pebbles or rock pieces of low outcrops from higher altitudes. Diagnostic are the blue-green epihymenium and asci with a distinct tholus reacting intensely blue with Lugol's reagent, which is the distinguishing character from otherwise similar *Hymenelia*-species.

***Eiglera homalomorpha* (Nyl.) Hafellner & Türk**

Trentino-Alto Adige • Trento Prov., Dolomites, Sass Becè, Pordoi; 46°28'50"N, 11°48'36"E; 2300 m; 25 Oct. 1984; Hafellner leg.; on a dolomitic rock; GZU. **Second record for Italy.**

This species is locally common in the Alps on limestone near the ground, such as on basal parts of steep cliffs in upland areas.

***Elixia flexella* (Ach.) Lumbsch**

Veneto • Belluno Prov., Dolomiti d'Ampezzo Natural Park, Passo Tre Croci; 46°33'24"N, 12°12'55"E; 1750 m; 25 Sep. 1985; Mayrhofer leg.; GZU – Ma 8304. – **Friuli Venezia Giulia** • Udine Prov., Carnic Alps, Monte Tinisa; 46°25'00"N, 12°42'00"E; 1750 m; 19 Aug. 1994; Hafellner leg.; on wood; GZU – Ha 76953.

This species may easily be mistaken for a non-lichenised ascomycete with minute black, angular apothecia, as the thallus is regularly present as an endoxylic discontinuous crust only. Vertical faces of coniferous stumps in upper montane forests are the preferred ecological niche, the ecology being similar to that of *Calicium trabinellum*, which is present as accompanying species on the cited specimen from Friuli.

***Fellhanera subtilis* (Vězda) Diederich & Sérus.**

Veneto • Belluno Prov., Feltre, Vincheto di Celarda Natural Reserve; 46°01'17"N, 11°58'37"E; 310 m; Aug. 2011; Nascimbene leg.; on *Populus* sp. in a riparian forest; Na 5397. **Second record for Italy.**

Our specimen was collected at the bottom of the Piave Valley along the river, in a particular microclimatic condition (very humid and cold in winter, and dry-warm in summer). The species is currently included in the Italian red list of epiphytic lichens as "Endangered" (Nascimbene et al. 2013).

***Flavoplaca limonia* (Nimis & Poelt) Arup, Frödén & Søchting**

Toscana • Livorno Prov., Island of Pianosa, road from the prison to the east coast; 10 m; 22 Mar. 2005; Muggia and Tretiach leg.; on calcareous rocks; TSB 36728.

This species, described from the calcareous cliffs along the coast of the Island of Marettimo, is also known from inland localities, and is certainly more widespread in Italy; earlier records might be under *Caloplaca citrina* s.lat.

***Frutidella caesioatra* (Schaer.) Kalb**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, Mt. Crostis; 46°34'20"N, 12°53'20"E; 2240 m; 17 Aug. 1994; Hafellner leg.; on saxicolous mosses; GZU – Ha 84341.

This species, characterized by mostly whitish-grey minute almost granular areoles and lead-grey to blackish convex immarginate apothecia, is found on cushions of bryophytes on siliceous boulders and outcrops, mostly on N-facing slopes, from treeline to the alpine belt.

***Frutidella furfuracea* (Anzi) M. Westb. & M. Svensson**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, Monte Tinisa; 46°25'00"N, 12°42'00"E; 1750 m; 19 Aug. 1994; Hafellner leg.; on *Larix decidua*; GZU – Ha 76938.

Diagnostic for this species are the mostly brownish thallus with greenish-brownish, punctiform, flat, farinose soralia in combination with the lead-grey to blackish, convex, immarginate apothecia (recalling those of the type species of the genus, *F. caesioatra*, but larger and less convex). It grows on long-time moist sites from the montane belt to treeline, mostly on bark, more rarely on wood.

***Fuscopannaria ignobilis* (Anzi) P.M. Jørg.**

Molise • Isernia Prov., Staffoli, near Vastogirardi; 41°45'36"N, 14°18'27"E; 1006 m; 23 Oct. 2015; Nascimbene leg.; on *Salix alba*; Na 4775.

This Mediterranean-Atlantic species was found in cracks of the bark of large trees (near the base of the boles) abundantly colonised by *Lobaria pulmonaria*, along a tree row near a small river.

***Fuscopannaria praetermissa* (Nyl.) P.M. Jørg.**

Valle d'Aosta • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N, 06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on soil among cliffs and boulders of Jurassic limestone on a N-exposed slope; GZU – Ha 75410. – **Toscana** • Pistoia

Prov., Northern Appenines, Abetone, Val di Luce, Alpe Tre Potenze; 44°07'30"N, 10°37'60"E; 1500–1820 m; 27 Oct. 1978; Hafellner leg.; on saxicolous bryophytes; GZU – Ha 4344.

An arctic-alpine to boreal-montane, circumpolar lichen found on calciferous soil, mosses and plant debris, with optimum near and above treeline. It is usually sterile, with dark-grey to brownish thalli mainly consisting of small, suberect squamules which likely also act as vegetative diaspores. In old herbarium specimens, the triterpenoids often crystallize into long, translucent needles resembling glassy hairs, a diagnostic character to distinguish this species from sterile thalli of *Massalongia carnosa*.

***Gyalecta erythrozona* Lettau**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Passo delle Vette Grandi; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2020; Nascimbene leg.; on a selciferous carbonatic rock (Formazione di Fonzaso); Na 7200. **Second record for Italy.**

A species of the *G. leucaspis*-group characterised by the entire (rather than radially incised) apothecial margins, and the elongate-fusiform (rather than acicular) ascospores. It is widespread in the Holarctic region, and in the Central European orobiomes it mostly occurs near or above treeline. In the Alps it seems to be most frequent in the eastern sector (Nimis et al. 2018a). Our specimen was collected on a steeply inclined, N-exposed rock in very moist, shaded, conditions, under overhangs.

***Gyalecta foveolaris* (Ach.) Schaer.**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, Monte Bivera; 46°26'40"N, 12°37'45"E; 2250 m; 30 Jul. 1993; Hafellner leg.; terricolous in crevices; GZU – Ha 32694a. – **Veneto** • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'25"N, 11°50'34"E; 2030 m; 10 Apr. 2021; Nascimbene leg.; on terricolous bryophytes; Na 7159. – **Valle d'Aosta** • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N, 06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on soil; GZU – Ha 75411.

A circumpolar, arctic-alpine lichen occasionally found in the lower alpine belt of calcareous mountains, where it mostly settles on long-time humid, subvertical soil stripes. The ecology is similar to that of *Ramonia melathelia* which may grow next on plant remnants.

***Gyalidea fritzei* (Stein) Vězda**

Trentino-Alto Adige • Bolzano/Bozen Prov., Sciliar Natural Park, Bad Ratzes; 46°31'41"N, 11°35'03"E; 1300 m; 25 Aug. 2006; Nascimbene leg.; on siliceous rock along a creek; Na 4836. **Third record for Italy.**

This is an overlooked, but certainly not common silicicolous species typical of periodically inundated sites.

***Halecania lecanorina* (Anzi) M. Mayrhofer & Poelt**

Trentino-Alto Adige • Bolzano/Bozen Prov., Sciliar Natural Park, Castelrotto; 46°30'42"N, 11°35'17"E; 2200 m; 18 Jul. 2007; Nascimbene leg.; on plant debris among dolomitic rocks; Na 4167.

An often overlooked, but certainly uncommon species, perhaps more widespread in the Alps over calcareous substrata, with optimum near treeline.

***Heppia adglutinata* (Kremp.) A. Massal.**

Veneto • Belluno Prov., Dolomiti d'Ampezzo Natural Park, Croda del Becco; 46°40'02"N, 12°04'56"E; 2400 m; 02 Aug. 1997; Nascimbene leg.; on calcareous soil; Na 641. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Busa di Cavaren; 46°05'44"N, 17°49'40"E; 1950 m; 20 Nov. 1994; Nascimbene leg.; on calcareous soil; Na 642.

A cool-temperate to boreal-montane, circumpolar, ephemeral lichen of disturbed calciferous soil in dry, open grasslands.

***Hymenelia heteromorpha* (Kremp.) Lutzoni**

Lombardia • Brescia Prov., Adamello Natural Park, Val Paghera di Vezza along the path from Rifugio alla Cascata to Rifugio Aviolo; 46°12'38"N, 10°24'49"E; 1800 m; 25 Jul. 2006; Thor leg.; , on mortar; UPS-L-166762.

A probably holarctic species found on dolomite and hard limestone in rather sheltered situations, with optimum near treeline, as in the case of our record.

***Hypotrachyna afrorevoluta* (Krog & Swinscow) Krog & Swinscow**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Canali; 46°13'06"N, 11°54'32"E; 1200 m; 08 Aug. 2013; Nascimbene leg.; on *Abies alba*; Na 3807. **First record from the Italian Alps.**

This species, widely distributed on both Hemispheres, is very similar to *H. revoluta* (for the main differences see Masson 2005), and some Italian records of the latter could refer to it. It seems to be widespread along the northern side of the Alps (Clerc 2006; Nimis et al. 2018a).

***Lecania cyrtellina* (Nyl.) Sandst.**

Veneto • Belluno Prov., Feltre, Villabruna; 46°03'05"N, 11°55'40"E; 350 m; 25 Jan. 2020; Nascimbene leg.; on *Malus* sp.; Na 6809. – **Abruzzo** • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'54"N, 14°21'27"E; 1000 m; Jul. 2009; Nascimbene leg.; on an isolated tree with *Athallia alnetorum* and *Lecanora horiza*; Na 2476.

A species found on the base-rich bark of more or less isolated deciduous trees, not always distinguished from *L. cyrtella* by Italian authors.

***Lecanora cavicola* Creveld**

Trentino-Alto Adige • Bolzano/Bozen Prov., Val di Roja, east ridge of the Outer Nockenkopf, just below the summit; 46°49'25"N, 10°27'50"E; 2700 m; 22 Apr. 1984;

Hafellner leg.; on overhanging siliceous rock; GZU – Ha 12476. • Bolzano/Bozen Prov., Upper Venosta Valley/Vintschgau, NE slopes of the Elferspitze; 46°46'55"N, 10°29'35"E; 2650 m; 25 Jul. 2006; Hafellner leg.; on overhanging siliceous rock; GZU – Ha 12329. – **Lombardia** • Brescia Prov., Adamello Natural Park, Edolo, Passo Gallinera; 46°10'55"N, 10°24'45"E; 2340 m; 25 Jul. 2006; Hafellner leg.; on overhanging rock faces of cliffs of siliceous schist on the crest; GZU – Ha 85845.

This sorediate and only rarely fertile species has a peculiar chemistry (atranorin and alectorialic acid, Poelt and Leuckert 1984), the latter causing a reddish miscoloration of the freshly yellowish-ochre areoles after some time of storage in the herbarium. It is a characteristic colonizer of overhangs of siliceous rocks, from treeline high up in the alpine belt, where *Aspicilia mashiginensis*, *Lecanora orbicularis*, *L. swartzii*, *Psorinia conglomerata*, and *Sporastatia polyspora* are among the accompanying species. It is fairly common in the siliceous mountains near the eastern border areas of the Alps, but apparently much rarer in the central and western parts.

***Lecanora epibryon* (Ach.) Ach. var. *bryopsora* Doppelb. & Poelt**

Lombardia • Brescia Prov., Adamello Natural Park, Passo del Tonale, Cima di Cadì, on the top and below on the south-east ridge; 46°16'33"N, 10°34'15"E; 2590 m; 28 Jul. 2006; Hafellner and Muggia leg.; on soil and plant debris; TSB 38476, GZU. – **Trentino-Alto Adige** • Bolzano/Bozen Prov., Dolomites, Puez-Odle Natural Park, Ortisei/St.Ulrich, M. Seceda; 46°36'05"N, 11°44'17"E; 2470 m; 02 Sep. 2002; Hafellner leg.; on crevices and plant debris; GZU.

This is a sorediate lichen known from Piedmont (Nimis et al. 2018a), but certainly more widespread in the Alps. Difficult to recognise, being often sterile, it is perhaps just a sorediate morph of *L. epibryon* (Nimis 2016).

***Lecanora horiza* (Ach.) Linds.**

Trentino-Alto Adige • Trento Prov., Val del Merlo, Monte Bondone Natural Reserve; 46°00'25"N, 11°02'04"E; 1640 m; Oct. 2003; Nascimbene leg.; on *Fagus sylvatica* in a thermophilous open stand; Na 4095.

A mainly Mediterranean species found on smooth bark of broad-leaved trees. It is rarer in the Alps than in the Mediterranean mountains, as indicated by the few records from northern Italy. Our specimen was collected on isolated *Fagus sylvatica* trees.

***Lecanora lojkaeana* Szatala**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Passo delle Vette Grandi; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2020; Nascimbene leg.; on a selciferous carbonatic rock under overhangs; Na 7201. **Second record for Italy.**

A rarely collected species known from the Alps, the Central European mountains and Scandinavia, found beneath underhanging surfaces of siliceous rocks in upland areas; perhaps overlooked and more widespread in the Alps, being almost always sterile. Our specimen was collected on flint nodules in overhanging rocks of the late Jurassic formation “Formazione di Fonzaso”.

***Lecanora silvae-nigrae* V. Wirth**

Lombardia • Brescia Prov., Adamello Natural Park, Edolo, Passo Gallinera; 46°10'55"N, 10°24'45"E; 2340 m; 25 Jul. 2006; Hafellner leg.; on inclined siliceous schist faces; GZU – Ha 85842. – **Toscana** • Pistoia Prov., Abetone, Val di Luce, Alpe Tre Potenze; 44°07'60"N, 10°37'60"E; 1500–1820 m; 27 Oct. 1978; Josef Poelt leg.; on siliceous rock; TLC: usnic, protocetraric, rangiformic, norrangiformic (analysed by H. Vänskä); GZU.

This species recalls *L. alpigena*, a large-fruiting species of the *L. polytropa*-group, but apothecial discs and margins are more discolourous, the discs being mostly reddish-brown, and the margins react P+ orange due to protocetraric acid;.the apothecia are often arranged in scattered groups on a poorly developed thallus. It grows on siliceous boulders including metal-rich rocks, mostly in the upper montane to lower alpine belts.

***Lempholemma intricatum* (Arnold) Zahlbr.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'25"N, 11°50'34"E; 2030 m; 10 Apr. 2021; Nascimbene leg.; on marly limestone (Rosso Ammonitico Superiore); Na 7167.

On steeply inclined surfaces of calcareous or basic siliceous rocks in seepage tracks, mostly in humid areas, as in our site.

***Lepra schaereri* (Hafellner) Hafellner**

Lombardia • Brescia Prov., Adamello Natural Park, Edolo, Passo Gallinera; 46°10'49"N, 10°24'49"E; 2320 m; 16 Jun. 2004; Nascimbene leg.; on base-rich siliceous rock; Na 5390, 5392.

This species seems to be restricted to high-elevation sites of the Alps and the Apennines, reaching the nival belt.

***Lepra teneriffensis* (Vain.) Hafellner**

Sardegna • Olbia-Tempio Prov., Archipelago della Maddalena, Island of Caprera; 41°12'22"N, 09°27'48"E; near sea level; 14 Apr. 2003; Tretiach leg.; T. Craighero and M. Tretiach rev.; on siliceous rocks; TSB 43336, 43337. • Olbia-Tempio Prov., Arcipelago della Maddalena, Island of Spargi; 41°12'33"N, 09°27'48"E; 14 Apr. 2003; Tretiach leg.; T. Craighero and M. Tretiach rev.; on siliceous rock; TSB 43335. **Second records from Italy.**

A Mediterranean-Macaronesian silicicolous species, for Italy previously known only from the island of Linosa.

***Leptogium byssinum* (Hoffm.) Nyl.**

Lombardia • Brescia Prov., Adamello Natural Park, Breno, Passo di Crocedomini, Val Fredda, Passo di Val Fredda, above the saddle on the south-east side of Mt. Frerone; 2340 m; 26 Jul. 2006; Hafellner and Muggia leg.; on soil over siliceous substrata; TSB 38503. **Second record from Italy.**

An inconspicuous, perhaps overlooked, ephemeral lichen, previously known in Italy only from Trentino-Alto Adige/Südtirol (Nimis 2016; Nimis et al. 2018a).

***Lobothallia alphoplaca* (Wahlenb.) Hafellner**

Emilia-Romagna • Parma Prov., Gravene, Prato Grande, Borgotaro; 44°28'53"N, 09°46'18"E; 500 m; 26 Jun. 1980; G. Caniglia leg.; on siliceous rocks; Na 5385.

A widespread species with an apparently disjunct distribution in mountain areas of the Northern Hemisphere, found on compact siliceous rocks wetted by rain in upland areas. It is locally abundant in the Alps, rarer in the Apennines.

***Massalongia carnosa* (Dicks.) Körb.**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Cima Bocche; 46°20'03"N, 11°44'45"E; 2100 m; 04 Sep. 2020; Nascimbene leg.; on bryophytes on porphyric rock, and directly on rock; Na 6910. • Trento Prov., Stelvio National Park, Val de la Mare, Bosco di Celvestré near Malga Prabon; 46°24'22"N, 10°41'40"E; 1780 m; 27 Jul. 2006; Thor leg.; on mosses over siliceous boulders; UPS-L-166815. – **Toscana** • Pistoia Prov., Abetone, Val di Luce, Alpe Tre Potenze; 44°07'30"N, 10°37'60"E; 1500–1820 m; 27 Oct. 1978; Josef Poelt leg.; on saxicolus bryophytes; GZU.

A circumpolar, arctic-alpine to boreal-montane lichen that at first sight is similar to *Fuscopannaria praetermissa*, but the thalli are mostly paler brown, often forming small rosettes, and the isidia-like lobules are less dense and mostly marginal. As the thalli are devoid of lichen substances, no extruding needle-like crystals develop with age, a diagnostic character to distinguish sterile herbarium specimens of both species under the dissecting microscope. Fertile thalli with red-brown, sessile apothecia are not rare, as in the specimen from Tuscany.

***Micarea globulosella* (Nyl.) Coppins**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'36"N, 14°21'23"E; 940 m; 22 Oct. 2015; Nascimbene leg.; on *Abies alba*; Na 5086, 5087.

A temperate to probably circumboreal-montane species found on bark of conifers and *Quercus* spp. in humid forests, more rarely on wood. Included in the Italian red list of epiphytic lichens as "Data Deficient" (Nascimbene et al. 2013).

***Miriquidica instrata* (Nyl.) Hertel & Rambold**

Lombardia • Brescia Prov., Adamello Natural Park, Passo del Tonale towards Passo del Paradiso; 46°15'10"N, 10°34'45"E; 1950 m; 24 Jul. 2006; Hafellner & Muggia leg.; on inclined faces of big granitic boulders, parasitic on *Lecanora alpigena*, *Lecidea confluens*, *Rhizocarpon geographicum*; GZU – Ha 85837. – **Sardegna** • Sassari Prov., Mt. Limbara; 1250–1300 m; 07 May 1986; Josef Poelt leg.; parasitic on *Rhizocarpon geographicum*; GZU. • Ibidem; Mayrhofer leg.; GZU – Ma 6379.

This species is morphologically characterised by brown, slightly concave to flat, often compound areoles with paler greyish margins, recalling those of *M. intrudens*, but has aspicilioid apothecia, while the latter is sorediate. In early stages of development, it

grows parasitically on a wide range of silicicolous crusts, including species of *Aspicilia*, *Lecanora*, *Lecidea* and *Rhizocarpon*. Later on, the lichenicolous behaviour may not be obvious. Preferred microhabitats are horizontal to slightly inclined rock faces of siliceous boulders, from the montane to the lower alpine belt.

***Mycobilimbia epixanthoides* (Nyl.) Hafellner & Türk**

Lombardia • Como Prov., Lanzo d'Intelvi, Foresta Monte Generoso; 45°57'23"N, 09°01'33"E; 1097 m; 26 Oct. 2018; Gheza leg.; on *Acer pseudoplatanus*; Herb. Gheza. • Brescia Prov., Paisco Loveno, Foresta Legnoli; 46°03'29"N, 10°14'52"E; 1286 m; 04 Aug. 2019; Gheza leg.; on *Acer pseudoplatanus*; Herb. Gheza. • Brescia Prov., Cisano, Foresta Gardesana Occidentale, surroundings of Malga Lorina; 45°48'30"N, 10°39'39"E; 1333 m; 02 Aug. 2019; Gheza leg.; on *Fagus sylvatica*; Herb. Gheza.

On mossy trunks of deciduous trees, more rarely on siliceous rocks, with a few records from the Italian Alps, probably locally overlooked. The three sites are located in the Prealps of Lombardy suggesting that the whole prealpine area of this region could potentially host this species. It was found in moist broadleaved forest stands.

***Mycocalicium subtile* (Pers.) Szatala**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'36"N, 14°21'23"E; 1000 m; Jul. 2009; Nascimbene leg.; on *Abies alba*; Na 2410.

A saprophyte on dry, hard wood, especially of conifers, in open situations, mostly in the montane and subalpine belts.

***Naetrocymbe fraxini* (A. Massal.) R.C. Harris**

Lombardia • Brescia Prov., Adamello Natural Park, Val Paghera di Vezza, along the path from Rifugio alla Cascata to Rifugio Aviolo; 46°12'06"N, 10°24'52"E; 1730 m; 25 Jul. 2006; Thor leg.; on *Sorbus aucuparia*; UPS-L-166798.

A mild-temperate species found on smooth bark of (mostly) deciduous trees; most probably non-lichenised.

***Opegrapha vermicellifera* (Kunze) J.R. Laundon**

Veneto • Belluno Prov., Feltre, Villabruna; 46°03'00"N, 11°55'46"E; 385 m; 02 Dec. 2017 and 26 Nov. 2017; Nascimbene leg.; on *Juglans regia* and *Carpinus betulus*; Na 5293, 5301. – **Abruzzo** • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'54"N, 14°21'26"E; 950 m; 23 Oct. 2015; Nascimbene leg.; on *Abies alba*; Na 4716, 4721, 4722, 4763.

A mild-temperate lichen found on old trees in humid areas, especially near rivers, on faces seldom wetted by rain. In the Italian Alps it seems to be rare. In both regions our specimens were collected along a north exposed, deep valley, very close to a river.

***Pertusaria flavicans* Lamy**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2020; Nascimbene leg.; on selciferous carbonatic rocks; Na 6972. • Ibidem; 12 Jul. 2020; Nascim-

bene leg.; Na 6975. • Belluno Prov., Dolomites, Arabba, Porta Vescovo; 46°27'43"N, 11°53'12"E; 2400 m; 16 Apr. 1979; Hafellner leg.; on basic siliceous rocks; GZU – Ha 4587 (as host of *Sclerococcum saxatile* under the name of the lichenicolous fungus).

This chemically variable species grows on lime-free but mineral-rich siliceous rocks, mostly on sheltered, steeply inclined surfaces. The specimens from Passo delle Vette Grandi belong to the chemotype with stictic acid in addition to the common thiophaninic acid, and were collected on flint nodules or decalcified strata in overhanging rocks of the late Jurassic formation "Formazione di Fonzaso".

***Pertusaria glomerata* (Ach.) Schaer.**

Valle d'Aosta • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N, 06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on plant remains; GZU – Ha 75436.

A rather conspicuous species characterised by perithecioid apothecia sunken into yellowish-white, subglobose thalline warts reacting K+ yellow turning red, due to norstictic acid (Hanko 1983). It is regularly found encrusting bryophytes and plant debris over calciferous soil, mostly in the lower alpine belt.

***Phaeophyscia pusilloides* (Zahlbr.) Essl.**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Val Canali, Villa Welsperg; 46°11'57"N, 11°52'06"E; 1000 m; 31 May 2012; Nascimbene leg.; on *Tilia* sp.; Na 2752.

A temperate species found on isolated deciduous trees with nutrient-rich bark, in montane valleys as in the case of our specimen.

***Placidiopsis pseudocinerea* Breuss**

Lombardia • Brescia Prov., Adamello Natural Park, Edolo, Passo Gallinera; 46°10'49"N, 10°24'49"E; 2320 m; 16 Jun. 2004; Nascimbene leg.; on soil; Na 5388.

An arctic-alpine, circumpolar lichen growing on soil and on moribund bryophytes on siliceous, base-rich or slightly calciferous soil (e.g. on calcareous schist), with optimum near and above treeline. It is certainly widespread through the Italian Alps, and also occurs in the central Apennines.

***Placynthium dolichoterum* (Nyl.) Trevis.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'25"N, 11°50'34"E; 2030 m; 10 Apr. 2021; Nascimbene leg.; on marly limestone (Rosso Ammonitico Superiore); Na 7166.

A poorly known species of the *P. nigrum* complex growing in humid-sheltered situations near or above treeline on basic siliceous or slightly calciferous rocks, as in our collection site.

***Placynthium filiforme* (Garov.) M. Choisy**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Col dei Cavai; 46°04'23"N, 11°50'02"E; 1450 m; 15 Oct. 2001; Nascimbene leg.; on calcare-

ous rock; Na 1259. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Erera-Brendol; 46°09'39"N, 11°58'35"E; 1700 m; 18 Oct. 2020; Nascimbene leg.; on calcareous rock; Na 7029.

A Mediterranean (-montane) to mild-temperate lichen growing on steeply inclined seepage tracks of calcareous rocks, with a rather wide altitudinal range. Our specimens were collected on the Jurassic "Calcari Grigi" and "Rosso Ammonitico Superiore" formations.

***Polysporina urceolata* (Anzi) Brodo**

Veneto • Belluno Prov., Carnic Alps, M. Tiarfin; 46°28'10"N, 12°35'50"E; 2200 m; 27 Jul. 1993; Hafellner leg.; on calcareous rocks; GZU – Ha 32725.

A rarely recorded species. The taxonomic value of the genus *Polysporina* is so far unresolved (Westberg et al. 2015) and the generic placement of the species treated here appears to be provisional.

***Porina byssophila* (Körb. ex Hepp) Zahlbr.**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Colle Cesta; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2020; Nascimbene leg.; on selciferous calcareous rocks (Formazione di Fonzaso); Na 6829.

A mild-temperate to humid subtropical species found on calcareous rocks in damp and shaded habitats. Our specimen was collected on an N-exposed selciferous carbonatic rock in very moist, shaded conditions, together with other species with a trentepohlioid photobiont (e.g. *Dirina massiliensis*, very abundant, *Gyalecta erythrozona*, and *G. hypoleuca*).

***Protoblastenia aurata* Poelt & Vězda**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park; 46°18'28"N, 11°47'48"E; 2270 m; 14 Sep. 2020; Nascimbene leg.; on compact calcareous rocks; Na 7078. **Third record for Italy.**

A rarely collected species of calciferous rocks in upland areas. Its distribution in Italy, as well as in the Alps, is currently poorly known (Nimis et al. 2018a).

***Protoblastenia terricola* (Anzi) Lynge**

Valle d'Aosta • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N, 06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on soil in open alpine vegetation; GZU – Ha 84337.

This is the terricolous counterpart of the closely related saxicolous *P. siebenhaariana*, indicated by a sister position of both taxa in a phylogenetic reconstruction (Ekman and Blaalid 2011). It occasionally transgrades from mineral-rich soil layers covering strongly weathered calciferous rocks to solid rock, and then distinguishing both taxa may be difficult.

***Protoparmeliopsis muralis* var. *dubyi* (Müll. Arg.) Hafellner & Türk**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Erera-Brendol; 46°09'44"N, 11°58'57"E; 1710 m; 18 Oct. 2020; Nascimbene leg.; on a

wooden fence near a hut; Na 7010. • Belluno Prov., Dolomites, Arabba, Porta Vescovo; 46°27'43"N, 11°53'12"E; 2400 m; 16 Apr. 1979; Hafellner leg.; on basic siliceous rock; GZU – Ha 4600.

This lichen usually grows on weakly calciferous or base-rich siliceous rocks in upland areas, with optimum near and above treeline, but it may also occur on wood, as in the case of our collection. In the Italian Alps it is fairly common.

***Pseudothelomma ocellatum* (Körb.) M. Prieto & Wedin**

Veneto • Belluno Prov., Dolomites, Croda da Lago; 46°29'32"N, 12°06'11"E; 2100 m; 08 Oct. 2006; D. Cester leg.; on a decaying stump of *Larix*; Na 1786.

A circumboreal-montane species growing on hard rotting wood, e.g. on poles and fences, more rarely on *Larix* and *Pinus cembra* in the subalpine belt. This floristic note rectifies the erroneous attribution to Trentino-Alto Adige by Nascimbene et al. (2008).

***Psoroma tenue* Henssen var. *boreale* Henssen**

Veneto • Belluno Prov., Dolomiti d'Ampezzo Natural Park, Foses; 46°38'23"N, 12°05'54"E; 2100 m; Aug. 2002; Nascimbene leg.; on soil; Na 1339, 1340.

An arctic-alpine, circumpolar lichen weak in competition, found on wet, naked soil, near glaciers or late snow-beds over acidic substrata.

***Psorotichia lugubris* (A. Massal.) Arnold**

Friuli Venezia Giulia • Udine Prov., Julian Pre-Alps, high Torre-Valley above Tanataviele, gorge of Rio Zaturran; about 750 m; 28 Nov. 1992; V. Calatayud and M. Tretiach leg.; on limestone in shade; TSB 16661. **Second record for Italy.**

Perhaps related to *P. murorum*, but with a verruculose to squamulose thallus and inconspicuous apothecia which are at first immersed, later prominent, and somewhat wider ascospores; overall distribution poorly known, with a few scattered records from the Alps.

***Punctelia jeckeri* (Roum.) Kalb**

Sardegna • Nuoro Prov., Gennargentu National Park, Punta Salinas, Baunei, Ogliastra; 40°06'03"N, 09°41'39"E; 450 m; Jul. 2010; Nascimbene leg.; on *Juniperus*; Na 2652.

This recently-resurrected species was often not distinguished from *P. subrudecta* in earlier studies. The predominantly marginal soralia and pruinose lobe tips are diagnostic. It grows on bark of isolated deciduous trees and is certainly widespread throughout Italy, including Mediterranean regions.

***Pyrenodesmia erodens* (Tretiach, Pinna & Grube) Søchting, Arup & Frödén**

Sicilia • Palermo Prov., Madonie Mnts., top of Pizzo Carbonara; 1979 m; 17 Aug. 2011; L. Muggia and Ha. Weingartmann leg.; on limestone; TSB 42254.

This specis grows on exposed, subvertical faces of limestone and dolomite, including old monuments, in dry sites of the montane and subalpine belts. The total distribution extends to the Irano-Turanian Region.

***Ramalina subgeniculata* Nyl.**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'57"N, 08°21'16"E; 990 m; 22 Oct. 2015; Nascimbene leg.; on *Quercus cerris*; Na 4489, 4493.

A Mediterranean-Macaronesian species mainly found on twigs of shrubs and young trees in warm-humid Mediterranean areas. Our specimen was collected in a humid *Quercus cerris* forest.

***Ramonia melathelia* (Nyl.) Ertz**

Valle d'Aosta • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N, 06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on plant remains; GZU – Ha 75426.

This species is regularly found on plant remnants in *Caricion firmae*–communities, mostly in humid, N-exposed sites. The ecology is similar to that of *Gyalecta foveolaris*, which has also been observed in the collection site.

***Rhizocarpon atroflavescens* Lynge**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Colle Cesta; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2020; Nascimbene leg.; on selciferous calcareous rocks (Formazione di Fonzaso); Na 6831, 6833.

A cool-temperate to arctic-alpine species growing on steeply inclined surfaces of base-rich, or weakly calciferous siliceous rocks. In Italy it is common in the subalpine and alpine belts, especially in the Alps. In our collection site it was abundant.

***Rhizocarpon furax* Poelt & V. Wirth**

Piemonte • Torino Prov., Mountains W of Pinerolo, northeastern slopes and ridges of the Punta Cialáncia; 44°52'60"N, 07°07'20"E; 2350 m; 26 Jul. 2001; Hafellner leg.; on siliceous boulders, parasitic on *Lecidea lapicida* agg.; GZU – Ha 69377, 69378.

An invader of the thalli of *Lecidea lapicida* s lat. It may be distinguished from the macroscopically similar *R. geographicum* by the frequently angular apothecia with at least partly rough, umbonate to subgyrose discs, and the soon pigmented, mostly 3-septate ascospores. It is widespread in the siliceous Alps, mainly in the lower alpine belt, but apparently it was often overlooked.

***Rhizocarpon norvegicum* Räsänen**

Trentino-Alto Adige • Trento Prov., Paneveggio-Pale di San Martino Natural Park, Cima Bocche; 46°20'48"N, 11°45'30"E; 2560 m; 13 Sep. 2020; Nascimbene leg.; porphyritic blocks of a military trench of WW1; Na 7202. • Bolzano/Bozen Prov.,

Melago, Vallelunga; 46°49'53"N, 10°39'57"E; 1920 m; Hafellner leg.; on iron-containing rocks; GZU – Ha 12395 (2 specimens). • Bolzano/Bozen Prov., Rojental, east ridge of the Outer Nockenkopf, just below the summit; 46°49'25"N, 10°27'50"E; 2700 m; 22 Apr. 1984; Hafellner leg.; on iron-containing rocks; GZU – Ha 12473. **Second records for Italy.**

A pioneer species of schistose, slightly calciferous or basic eruptive rocks in upland areas, which occasionally starts the life-cycle on members of Acarosporaceae, e.g. *Acarospora sinopica* when on metal-rich schists.

***Rinodina albana* (A. Massal.) A. Massal.**

Sicilia • Messina Prov., Nebrodi National Park, Cesarò, along the road to Monte Soro, 0.8 km WSW of the top; 37°55'43"N, 14°41'08"E; 1740 m; 05 May 2012; J. Malíček, I. Frolov and J. Vondrák leg.; *Fagus sylvatica* forest, on *Fagus sylvatica*; Herb. Malíček 7589.

A temperate species found on isolated deciduous trees with more or less smooth bark.

***Rinodina aspersa* (Borrer) J.R. Laundon**

Piemonte • Vercelli Prov., Borgosesia, basal part of M. Fenera; 22 May 1876; A. Carestia leg.; on porphyric rocks; RO (as *Rinodina trachytica*). **Third record for Italy.**

On hard siliceous rocks near the ground in cold-humid habitats, sometimes on walls, mostly below the montane belt.

***Rinodina capensis* Hampe**

Veneto • Belluno Prov., Dolomites, Passo Tre Croci; 46°33'24"N, 12°11'46"E; 1720–1780 m; 25 Sep. 1985; Mayrhofer leg.; on *Picea abies*; GZU – Ma 8298.

A cool-temperate to boreal-montane pioneer species, mostly found on smooth bark, but also on wood, with optimum in the subalpine and montane belts.

***Rinodina castanomela* (Nyl.) Arnold**

Veneto • Belluno Prov., Dolomites, saddle of M. Sief, Col di Lana; 46°30'23"N, 11°57'19"E; 2200 m; 30 Aug. 1981; T. Feuerer leg.; on sand-lime rocks (Wengen formation, middle Triassic); Herb. Feuerer 12181, HBG. **Second record for Italy.**

An arctic-alpine to boreal-montane, perhaps circumpolar lichen found under overhanging cliffs of weakly calcareous or basic siliceous rocks, marl and calciferous schist near or above treeline.

***Rinodina castanomelodes* H. Mayrhofer & Poelt**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, near Casera Novarzutta, north of Lateis, Sauris; 1600 m; 10 Sep. 1987; Josef Poelt leg.; on calcareous rocks; GZU.

An arctic-alpine to boreal-montane, perhaps circumpolar lichen found on limestone, marl and calcareous schists at and above treeline; widespread but not common in the Alps, where it can reach the nival belt, and also reported from the mountains of southern Italy.

***Rinodina cretica* H. Mayrhofer**

Sicilia • Palermo Prov., Madonie, slopes above Geraci Siculo, SW Castelbuono; 1200 m; Jun. 1988; M. Pietschmann leg.; on calcareous rocks; M. **Second record for Italy.**

A Mediterranean calcicolous species, probably somehow more widespread in southern Italy.

***Rinodina dubyana* (Hepp) J. Steiner**

Liguria • Imperia Prov., Ligurian Alps, Monte de la Guardia; 1600 m; 14 Sep. 1970; H. Wunder leg.; on rock; M 1345.

A mainly temperate species found on steeply inclined to underhanging, sunny surfaces of limestone and dolomite wetted by rain, sometimes also on pebbles on the ground, with optimum below the subalpine belt.

***Rinodina luridescens* (Anzi) Arnold**

Liguria • Genova Prov., "in montibus di Reppia"; Caldesi leg.; MOD.

A Mediterranean-Atlantic lichen described from Tuscany, found on hard siliceous rocks subject to frequent humid winds, often near the coast; not uncommon in some parts of Mediterranean Italy.

***Rinodina obnascens* (Nyl.) H. Olivier**

Trentino-Alto Adige • Bolzano/Bozen Prov., Venosta valley/Vintschgau, near Lasa; 900 m; 05 Sep. 1992; Hafellner leg.; on siliceous rocks; GZU – Ha 30594.

A Mediterranean-Atlantic lichen found on weakly inclined to horizontal surfaces of siliceous rocks wetted by rain, starting the life-cycle especially on *Aspiciliella intermutans*, but sometimes on other lichens, e.g. *Rhizocarpon*-species.

***Rinodina oleae* Bagl.**

Emilia-Romagna • Parma Prov., Parma, Strada Ugozzolo, Case Nuove; 44°49'51"N, 10°20'21"E; 45 m; 03 Mar. 2015; Nascimbene leg.; on *Tilia* sp.; Na 4898, 4899.

A submediterranean-Mediterranean epiphytic lichen which was overlooked or confused with similar species in the past. Our specimen was collected in an urban environment of the Po-Plain.

***Rinodina olivaceobrunnea* C.W. Dodge & G.E. Baker**

Calabria • Reggio Calabria Prov., Aspromonte, Pietra Impiccata; 1700–1750 m; 12 Jul. 1988; Josef Poelt leg.; GZU.

An arctic-alpine, circumpolar species found on soil, bryophytes and plant debris in tundra-like environments over siliceous substrata; certainly widespread throughout the Alps, and also reported from the high Mediterranean mountains.

***Rinodina pityrea* Ropin & H. Mayrhofer**

Calabria • Catanzaro Prov., Serre di Catanzaro, Serra S. Bruno; 38°34'36"N, 16°19'45"E; 780 m; 14 Jul. 1988; R. Türk leg.; on *Populus* sp.; Herb. Türk 10040.

A temperate species found on asbestos-cement and mortar, often on walls, more rarely on dust-impregnated bark; easy to overlook, being often sterile.

***Rinodina roscida* (Sommerf.) Arnold**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, M. Tinisa, near the summit; 46°24'48"N, 12°43'07"E; 2100 m; 29 Jul. 1993; Hafellner leg.; plant debris in crevices; GZU – Ha.

An arctic-alpine, circumpolar species found on soil, bryophytes and plant debris over calcareous substrata in tundra-like habitats; widespread throughout the Alps.

***Rinodina teichophila* (Nyl.) Arnold**

Emilia-Romagna • Parma Prov., M. Testanello; Aug. 1899; C. Zanfrognini leg.; on rock; MOD. • Parma Prov., M. Santa Donna; Aug. 1899; C. Zanfrognini leg.; on rock; MOD. • Bologna Prov., Lizzano, Fiammineda; Aug. 1896; C. Zanfrognini leg.; on rock; MOD.

A widespread species growing on base-rich siliceous rocks, mostly on more or less calciferous sandstone, especially in nutrient-enriched situations such as on walls, tiles, brick or gravestones, mostly below the montane belt, also found in large conurbations.

***Rinodina tephraspis* (Tuck.) Herre**

Lombardia • Sondrio Prov., near Liro river; 1100 m; M. Anzi leg.; on mica schist (*Ad saxa micaceo-schistosa juxta flumen Liri*); UPS, Anzi: Lich. rar. Lang. exs. 561 (as *Rinodina confragosa*). **Second record for Italy.**

This exsiccatum was erroneously cited by Mayrhofer and Poelt (1979) and Mayrhofer (1984) under *Rinodina arnoldii*. The species grows on siliceous rocks in upland areas, in moist and often shaded situations such as near waterfalls, rapids, gorges and shores of lakes, often associated with Cyanobacteria (*Stigonema*).

***Rinodina turfacea* (Wahlenb.) Körb.**

Veneto • Belluno Prov., Carnic Alps, near the saddle between Col Marende and M. Tiarfin; 2000 m; 27 Jul. 1993; Hafellner leg.; on mosses and plant debris; GZU – Ha 32752.

An arctic-alpine, circumpolar lichen found on soil rich in humus and plant remains in tundra-like habitats.

***Rinodinella dubyanoides* (Hepp) H. Mayrhofer & Poelt**

Puglia • Foggia Prov., Gargano, Monte Saraceno; 41°41'48"N, 16°03'04"E; 100 m; 25 May 1972; H. Wunder leg.; on calcareous rock; M.

A mild-temperate to Mediterranean species found on hard, compact calcareous rocks, mostly on steeply inclined faces wetted by rain.

***Rostania ceranisca* (Nyl.) Otálora, P.M. Jørg. & Wedin**

Trentino-Alto Adige • Bolzano/Bozen Prov., Dolomiti di Sesto Natural Park, Tre Cime di Lavaredo; 46°37'14"N, 12°17'13"E; 2354 m; 19 Sep. 2020; Nascimbene leg.; on calcareous soil; Na 7007. **Third record for Italy.**

An arctic-alpine lichen that typically grows over frost-disturbed, weakly calcareous soil above treeline, as in the case of our collection site.

***Sarcogyne fallax* H. Magn.**

Trentino-Alto Adige • Bolzano/Bozen Prov., Passo della Mendola; 30 Apr. 1965; Josef Poelt leg.; on rock; GZU.

A mainly mild-temperate lichen found on steeply inclined to underhanging surfaces of base-rich siliceous rocks, more rarely on calcareous rocks.

***Scutula circumspecta* (Vain.) Kistenich, Timdal, Bendiksby & S. Ekman**

Veneto • Belluno Prov., Feltre, Vincheto di Celarda Natural Reserve; 46°00'49"N, 11°58'37"E; 310 m; 2005; Nascimbene leg.; on *Sambucus nigra*; Na 2326.

A mild-temperate lichen growing on old trees in open, humid woodlands below the subalpine belt, more rarely on primarily acid, but nutrient-enriched bark. Our material was collected in a humid riparian forest.

***Scytinium aragonii* (Otálora) Otálora, P.M. Jørg. & Wedin**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Cordin delle Vette, Busa delle Vette; 46°05'17"N, 11°51'06"E; 1950 m; 31 Oct. 2020; Nascimbene leg.; on pleurocarpous mosses in rock crevices at the base of a N-exposed calcareous wall; Na 7057. • Ibidem; 46°05'20"N, 11°51'09"E; 1950 m; 13 Jun. 2021; Nascimbene leg.; Na 7231.

A recently-described species, widespread throughout Europe growing on pleurocarpous mosses close to the base of trunks, over mossy walls or calcareous rocks within forests, or on mosses in rock fissures within dry subalpine grasslands.

***Scytinium imbricatum* (P.M. Jørg.) Otálora, P.M. Jørg. & Wedin**

Trentino-Alto Adige • Bolzano/Bozen Prov., Sciliar Natural Park, Tuffal, Fiè; 46°30'18"N, 11°32'51"E; 1700 m; Jul. 2006; Nascimbene leg.; on terricolous mosses and soil in alpine grasslands; Na 4285. • Bolzano/Bozen Prov., Sciliar Natural Park, Rifugio Bolzano; 46°30'33"N, 11°34'00"E; Jul. 2007; Nascimbene leg.; on terricolous mosses and soil in alpine grasslands; Na 3033. – **Veneto** • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Colle Cesta, near Rifugio Dal Piaz; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2021; Nascimbene leg.; on terricolous mosses; Na 7234.

This species seems to be bound to high elevations and is likely to be widespread in the Alps, as well as in the higher mountains of the Apennines.

***Scytinium massiliense* (Nyl.) Otálora, P.M. Jørg. & Wedin**

Veneto • Belluno Prov., Feltre, Rocchetta di San Vittore; 46°00'11"N, 11°56'43"E; 400 m; 16 May 2021; Nascimbene leg.; on limestone; Na 7216.

A mild-temperate to Mediterranean species found on steeply inclined surfaces of calcareous rocks with periodical seepage of water.

***Sphinctrina leucopoda* Nyl.**

Abruzzo • Chieti Prov., Abetina di Rosello Natural Reserve; 41°52'57"N, 08°21'16"E; 1000 m; Jul. 2009; Nascimbene leg.; parasitic on *Lepra albescens* on *Quercus cerris*; Na 2405, 2406.

A non-lichenized parasite on the thalli of epiphytic crustose lichens, certainly declining.

***Sticta limbata* (Sm.) Ach.**

Trentino-Alto Adige • Trento Prov., Val Noana; 46°07'52"N, 11°50'31"E; 1200 m; 23 Jul. 2014; Nascimbene leg.; on *Fagus sylvatica* in a mixed, humid *Fagus sylvatica-Abies alba* forest; Na 4430.

A humid subtropical to Mediterranean-Atlantic species growing on bark, often associated with bryophytes, on mossy rocks and soil in very humid situations, certainly worthy of protection in Italy, being included in the Italian red list of epiphytic lichens as "Vulnerable" (Nascimbene et al. 2013). In Northern Italy, it seems to be restricted to the eastern Alps where it is extremely rare.

***Thelidium dionantense* (Hue) Zschacke**

Veneto • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, near Passo delle Vette Grandi; 46°05'25"N, 11°50'34"E; 2010 m; 09 May 2021; Nascimbene leg.; on marly limestone (Rosso Ammonitico Superiore); Na 7227. • Belluno Prov., Dolomiti Bellunesi National Park, Vette Feltrine, Colle Cesta, near Rifugio Dal Piaz; 46°05'24"N, 11°50'37"E; 2010 m; 13 Jun. 2021; Nascimbene leg.; on marly limestone (Rosso Ammonitico Superiore); Na 7228.

On steeply inclined surfaces of calciferous rocks in upland areas, with several scattered records throughout the Alps (outside the Italian territory); known from the Central Apennines, probably more widespread in the Italian Alps.

***Thelidium zwackhii* (Hepp) A. Massal.**

Trentino-Alto Adige • Trento Prov., Dolomites, Boè Group, Antersass; 46°31'12"N, 11°48'48"E; 2710 m; 24 Jul. 2019; Nascimbene leg.; on humid mineral soil; Na 6896.

A mainly temperate, ecologically broad-ranging pioneer species found on both calcareous and siliceous rocks and on thin layers of soil, occasionally also in periodically submerged sites. Our specimen was collected on mineral, poorly developed sandy soil abundantly colonised by *Carex bicolor*, indicative of periodically inundated conditions.

***Thelocarpon lichenicola* (Fuckel) Poelt & Hafellner**

Veneto • Belluno Prov., Dolomites, Croda da Lago; 46°29'32"N, 12°06'11"E; 2100 m; 08 Oct. 2006; D. Cester leg.; on *Placynthiella uliginosa* on wood in *Larix*-forest; Na 1939.

On clay soil in disturbed sites, often in *Calluna*-heaths, doubtfully lichenised.

***Varicellaria rhodocarpa* (Körb.) Th. Fr.**

Valle d'Aosta • Aosta Prov., Western Alps, Monte Bianco (Mont Blanc) group, Val Veny W of Courmayeur, ridge W above the Rifugio Elisabetta Soldini; 45°45'45"N,

06°50'15"E; 2250 m; 30 Jul. 2001; Hafellner leg.; on plant remains; GZU – Ha 75427 (fertile material).

Alpine thalli are usually thick, whitish pustulate, virtually sterile crusts reacting C+ red. The pustules soon become rough (sorediate state) and later rather frequently contain inconspicuous (often slightly pink), immersed ascomata, indicating that in some genera (e.g., *Varicellaria*, *Lepra*) soralia may be derived from ascomata. In the Alps, the species is mostly a coloniser of plant remains in alpine mats over acidic soils, with a preference for wind-exposed ridges. Over superficially decalcified substrata it may also occur on limestone or calcareous schists, as in the case of our site.

***Variospora australis* (Arnold) Arup, Søchting & Frödén**

Basilicata • Potenza Prov., Piana del Pollino, NW Serra delle Ciavole; 39°55'09"N, 16°12'57"E; 1850 m; 02 Jun. 1979; Mayrhofer leg.; on calcareous rocks; GZU – Ma 21936. • Potenza Prov., Mt. Pollino, near Bosco di Chiaromonte; 1700 m; 02 Jun. 1979; Hafellner leg.; on calcareous rocks; GZU – Ha 4696.

On exposed calciferous rocks near or above treeline, e.g. on the top of large, isolated boulders and on steeply inclined to vertical surfaces.

***Verrucaria praetermissa* (Trevis.) Anzi**

Veneto • Belluno Prov., Feltre, Torbiera di Lipoi; 46°02'15"N, 11°57'23"E; 320 m; 20 Apr. 2021; Nascimbene leg.; on periodically submerged calcareous stones along a small creek near the peat bog; Na 7171.

A probably circumboreal freshwater species, submerged only for very short periods, mostly found along creeks, on mineral-rich siliceous rocks, more rarely on calcareous substrata.

***Violella fucata* (Stirt.) T. Sprib.**

Friuli Venezia Giulia • Udine Prov., Carnic Alps, Sauris Lake, Bosco della Stua; 46°26'35"N, 12°42'50"E; 1100 m; 16 Aug. 1994; Hafellner leg.; on *Alnus incana*; GZU – Ha 84339.

This species forms sterile thalli with whitish convex areoles reacting K+ yellow, P+ orange-red and UV- (due to the presence of atranorin and fumarprotoetraric acid), and becoming apically sorediate with coarse soredia, the outermost ones often being slightly bluish. Frequently the thalli are parasitized by *Tremella lichenicola* and the presence of its galls is a good hint as to the identity of the host. *V. fucata* colonises both the bark of a wide range of trees and wood (e.g. rotting snags), in Central Europe from the colline to the montane belt.

***Xylographa pallens* (Nyl.) Harm.**

Veneto • Belluno Prov., Croda da Lago; 46°29'32"N, 12°06'11"E; 2100 m; 08 Oct. 2006; D. Cester leg.; on a stump; Na 1933, 3052.

Widespread in the Northern Hemisphere on wood, especially in exposed habitats becoming dry in summer, mainly in montane to subalpine coniferous forests, with a

few scattered records from the Alps (Nimis et al. 2018a). The samples collected on a slightly decomposed stump contain stictic acid.

***Xylographa vitiligo* (Ach.) J.R. Laundon**

Basilicata • Potenza Prov., Piana del Pollino, NW Serra delle Ciavole; 39°55'09"N, 16°12'57"E; 1900 m; 02 Jun. 1979; Mayrhofer leg.; on *Pinus leucodermis*; GZU – Ma 1180.

A mainly boreal-montane species found on decaying, decorticated but still hard wood, mostly of conifers, especially near the base, or on fallen trunks, with optimum near treeline.

Discussion

The list includes 225 records of 153 taxa. Twenty taxa are new to Italy, the others are new to one or more administrative regions; the latter include 15 second records and 5 third records for Italy.

The administrative regions with most new records are Veneto (61 records, 50 taxa), Trentino Alto Adige (38, 32 taxa), Emilia-Romagna (22, 10 taxa), Lombardia (19, 15 taxa), and Abruzzo (14, 14 taxa). From each of the other 14 regions, 12 to 1 new records are reported.

Most records come from bark (72 records, 43 taxa), calcareous rocks (47, 34 taxa) and siliceous rocks (39, 29 taxa). Fewer come from soil, plant debris, dead wood and bryophytes.

Most records are from the alpine belt (61 records, 55 taxa), followed by the subalpine (53, 44 taxa) and montane (53, 36 taxa) belts. This is due to the fact that the research activity of most of the authors is mainly centred on the Alps (e.g. Nascimbene et al. 2017; Nimis et al. 2018a; Saiz et al. 2021).

The species listed in this paper can be subdivided into the following main groups:

1. Recently-described or -resurrected species, such as *Bacidina adastra*, *Blastenia gennargentuae*, *B. monticola*, *B. psychrophila*, *Calogaya rouxii*, *Circinaria serenensis*, *Flavoplaca limonia*, *Fuscopannaria praetermissa*, *Gyalideopsis helvetica*, *Hypotrachyna afrorevoluta*, *Lecanora silvae-nigrae*, *Placidiopsis pseudocinerea*, *Protoblastenia aurata*, *Psoroma tenue* var. *boreale*, *Punctelia jeckeri*, *Pyrenodesmia erodens*, *Ramonia interjecta*, *Rhizocarpon furax*, *Scytinium aragonii*, *S. imbricatum*.
2. Sterile or ephemeral species, such as *Aspicilia grisea*, *A. mashiginensis*, *Bellemerea subsorediza*, *Gyalideopsis helvetica*, *Lecanora cavicola*, *L. epibryon* var. *bryopsora*, *L. lojkaeana*, *Lepra schaereri*, *L. teneriffensis*, *Leptogium byssinum*, *Opegrapha vermicellifera*, *Pertusaria flavicans*, *Pseudothelomma ocellatum*, *Rinodina pityrea*, *Toensbergia leucococca*, *Violella fucata*, *Varicellaria rhodocarpa*, *Xylographa vitiligo*. Several of these species are not generally rare, but were simply overlooked, undercollected or not identified at species level in previous studies mostly because a traditional recognition approach based on macro- and microscopic characteristics is not sufficient, these taxa requiring a DNA-based identification or the definition of secondary metabolites.

3. Species belonging to taxonomically critical groups, such as *Anema tumidulum*, *Candelariella kuusamoënsis*, *Diplotomma*-species, *Lecania cyrtellina*, *Lempholemma intricatum*, *Polysporina urceolata*, *Psorotichia lugubris*, *Staurothele sapaudica*, *Thelidium*-species, which were probably not recognized or misidentified in previous studies.

4. Species of biogeographic interest, which in Italy (or in some regions) are near the limits of their climatic optima. Most of the nationally or regionally rare lichens belong to an oceanic-suboceanic element with tropical affinities, or to a small set of continental species with their optima in the dry steppe biome, which suggests that many rare species can persist in microrefugia, i.e. sites with microclimates that support small populations of species beyond the boundaries of the climatic limits of their main distributions. This is the case of *Bacidina delicata*, *Fuscopannaria praetermissa*, *Lecanora horiza* and *Sticta limbata*, which are at the limit of their bioclimatic ranges (suboceanic and/or Mediterranean) in a continental-alpine region such as Trentino-Alto Adige, of *Fuscopannaria ignobilis*, mainly Tyrrhenian, a suboceanic species which is obviously restricted to a few humid sites in Molise, of *Rinodina oleae*, a mainly Mediterranean species, which is at the limit of its bioclimatic range in Emilia, and of *Usnea flavocardia*, a subatlantic species restricted to a few sites in Tyrrhenian Italy. Another example is that of *C. arbuscula* and *C. mitis*, two arctic-alpine to boreal-montane species which are widespread and common in the Alps, but are near their southern distributional limit in the Marche region (Northern Apennines). Similar is the case of *R. olivaceobrunnea*, an arctic-alpine species which finds the southernmost limit of its Italian distribution in the mountains of Calabria. As already observed by Aptroot and van Herk (2007) in the Netherlands, these species are those for which climate change is most likely to modify their relative patterns of commonness/rarity.

5. Species bound to rare habitats, such as old-growth forests. This is the case of e.g. *Arthonia vinosa*, *Calicium adspersum*, *Cetrelia chicitae*, *Chaenotheca brachypoda*, *C. brunneola*, *C. phaeocephala*, *Chaenothecopsis debilis*, *Mycobilimbia epixanthoides*, *Scutula circumspecta*, *Sphinctrina leucopoda*, plus some of the species listed under the previous point. Also in this case, these species are generally rare, their rarity being mainly due to the strong contraction of their habitat.

Our results indicate that, even in historically well-explored areas it is still possible to discover several new species. Even small, previously well-studied sites may provide interesting surprises. This is the case of the small plot in the Vette Feltrine (Pre-Alps, Dolomiti Bellunesi National Park), which consists of a few square meters of rock outcrops, within a peculiar site with abundant precipitations due to humid air masses from the Adriatic Sea that originate frequent fog. The collections were carried out on a NE exposed slope where the rock outcrops contain both a calciferous (dominant) and a siliceous (flint nodules and strata) component, with an alternation of exposed and protected overhanging parts that correspond to diverse microhabitats for lichens, also providing refugia for microthermic species. Another emblematic case is represented by the Paneveggio-Pale di San Martino Natural Park, an area with a very humid climate and heterogeneous geological features, that was intensively explored by Ferdinand Arnold at the end of the 19th century (Dalla Torre and Sarnthein 1902) and also in recent

times in the framework of studies mainly focused on lichen ecology in forest ecosystems (e.g. Nascimbene et al. 2008; Nascimbene et al. 2009). On one hand, these examples indicate that repeated, intensive, surveys are needed to reach exhaustive knowledge even of small sites. On the other hand, they indicate that further exploration should prioritise areas with rare climatic conditions and heterogeneous rock composition, corroborating the view that high geo-diversity, even at a small spatial scale, corresponds to high lichen diversity (Spitale and Nascimbene 2013).

Conclusion

The picture of the lichen biota of Italy now has new pixels, but its grain is still coarse. On one hand, herbaria, especially when digitized, are an irreplaceable tool for further data mining allowing the re-evaluation of old records in the light of the progress of phylogenetic hypotheses and taxonomy, and should be therefore sustained and implemented with new records (see e.g. Crisci et al. 2020). On the other hand, professional floristics should gain more consideration in the scientific community, acknowledging its fundamental role in providing and updating occurrence and distributional data, which are the basis for new biogeographic hypotheses, taxonomic and ecological research, and biodiversity conservation.

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Morphological and phylogenetic evidence for two new species of *Russula* subg. *Heterophyllidia* from Guangdong Province of China

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Abstract

Two new species of *Russula* subg. *Heterophyllidia* from Guangdong Province of China were described and illustrated based on morphological characters, and their identity supported by molecular phylogeny. *R. luofuensis* is morphologically characterized by a grayish yellow to brownish orange pileus center with a purplish gray to grayish magenta margin, a surface that is cracked and broken into small golden-brown patches, subglobose to broadly ellipsoid basidiospores with warts fused in short or long chains and a suprapellis composed of hyphal extremities with inflated, ellipsoid or globose cells and attenuated terminal cell. *R. subbubalina* is distinguished by the blanched almond to dark salmon pileus that is cracked with age, subglobose to broadly ellipsoid basidiospores with wart fused in short or long chains and frequently connected by line connections, a suprapellis with hyphal ends composed of inflated or ellipsoid cells and attenuated terminal cell, and pileocystidia that are mainly clavate and sometimes with round or ellipsoid appendage. The phylogenetic analyses based on ITS-nrLSU-mtSSU-*TEF1* dataset were performed using maximum likelihood and Bayesian analysis. In terms of morphological features and molecular data, the former species belongs to subsect. *Virescentinae*, whereas the latter comes under subsect. *Heterophyllinae*.

Keywords

Luofu Mountain, new species, phylogeny, Russulaceae, taxonomy

Introduction

Russula Pers. is the largest genus of Russulaceae, estimated at least to contain 2000 species, which has resulted in many complex and multilevel classifications (Buyck et al. 2018; Adamčík et al. 2019; Wijayawardene et al. 2020). Recent molecular phylogenetic studies have indicated eight subgenera within the genus *R.* subg. *Glutinosae* Buyck & X.H. Wang, *R.* subg. *Archaeae* Buyck & V. Hofst., *R.* subg. *Compactae* (Fr.) Bon, *R.* subg. *Crassotunicatae* Buyck & V. Hofst., *R.* subg. *Heterophyllidiae* Romagnesi, *R.* subg. *Malodorae* Buyck & V. Hofst., *R.* subg. *Brevipedum* Buyck & V. Hofst., and *R.* subg. *Russula* (Buyck et al. 2018, 2020). The infrageneric classification system of *Russula* based on a multi-locus phylogenetic analysis was followed in this study. This genus is globally distributed and occurs across a wide range of habitats from Arctic tundra to tropical forests and forms ectomycorrhizal relationships with diverse plants (Knudsen and Borgen 1982; Buyck et al. 1996; Looney et al. 2018). Some species of *Russula* are famous edible fungi and are also commercially traded worldwide (Looney et al. 2018; Wang 2020). According to recent statistics on the resource diversity of Chinese macrofungi, there are 78 edible *Russula* species in China (Wu et al. 2019).

Guangdong Province is located in the southern coastal area of China, which is one of the Chinese provinces with tropical and subtropical climates. The climate can be divided into the middle subtropical, the southern subtropical, and the tropical climate zones, from north to south. The annual average temperature in Guangdong Province is 19–24 °C and the annual average rainfall is 1500–2000 mm. Abundant moisture, moderate to high temperatures, and variegated physiography support luxuriant and highly diversified plant growth. Broad-leaved evergreen forests, intermixed with coniferous and deciduous trees, cover much of the land. During the rainy season, the forest ecosystem can facilitate the fruiting of most ectomycorrhizal fungi, among which the members of *Russula* are very common. Recently, 16 new species and one epitype of *Russula* from Guangdong Province have been reported (Das et al. 2017; Zhang et al. 2017; Song et al. 2018a, b; Li et al. 2019; Yuan et al. 2019; Zhou et al. 2020). Obviously, Guangdong Province has become a hotspot in research on biodiversity of Chinese *Russula*, which makes it more vital for us to continue to explore it.

Northern hemisphere species within subg. *Heterophyllidia* are mainly characterized by the mostly medium to large basidiomata, equal lamellae, mild to strongly acrid taste, white or cream and rarely ochre spore print, basidiospores with inamyloid or partly amyloid suprahilar spot, mostly abundant gloeocystidia that are typically mucronate to obtuse-rounded, and absence of primordial hyphae. During a survey of the habitat diversity and geographic distribution of *Russula* in Guangdong Province, some interesting specimens of subg. *Heterophyllidia* were found that were different from known species. In this study, two new species from Guangdong Province are presented based on the morphological characters and molecular data.

Materials and methods

Morphological study

Fresh specimens were collected and photographed in Luofu Mountain Provincial Nature Reserve, Guangdong Province, South China. Collections were dried at 45–55 °C and deposited in the herbarium of the Research Institute of Tropical Forestry, Chinese Academy of Forestry (RITF). The macromorphological characters were described based on detailed notes and photographs. The color codes mostly refer to Kornerup and Wanscher (1981). The description templates and terminology of the micromorphological characters were taken from Adamčík et al. (2019). Estimates of spore ornamentation density from scanning electron microscopy pictures follow Adamčík and Marhold (2000). The hymenial cystidia density estimates refer to Buyck (1991). Experiments were performed on dried specimens using a ZEISS Imager M2 (Carl Zeiss AG; Germany). The basidiospores were observed and measured in Melzer's reagent from a lateral view excluding ornamentation. After pretreatment of dried specimens in 5% potassium hydroxide (KOH), other micromorphological characters were identified and measured in Congo red. The coloring of the cystidia contents was observed in a sulfovanillin (SV) solution (Caboň et al. 2017). The pileipellis were examined in cresyl blue to verify the presence of ortho- or metachromatic reactions (Buyck 1989). The structure and ornamentation of the basidiospores were illustrated using a scanning electron microscopy (SEM-JEOL JSM-6510). Basidiospore measurements are presented as (Min–)AV-SD–AV–AV+SD(–Max), where Min is the minimum value, Max is the maximum value, AV is the average value, SD is the standard deviation, and Q represents the length/width ratio of the basidiospores.

Molecular study

The total genomic DNA was extracted from dry specimens following an improved CTAB protocol (Zhou and Liang 2011). We amplified and sequenced the following four loci with standard primer sets: 600 base pairs of the ITS region of rDNA using the primers ITS1 and ITS4 (White et al. 1990); 900 base pairs of the nuclear ribosome large subunit (nrLSU) using the primers LROR and LR5 (Vilgalys and Hester 1990); 600 base pairs of the ribosomal mitochondrial small subunit (mtSSU) with primers MS1 and MS2 (White et al. 1990); 900 base pairs of the translation elongation factor 1-alpha (TEF1) using primers EF1-F and EF1-R (Morehouse et al. 2003). Successful PCR products were subjected to automated DNA sequencing on an ABI 3730 DNA analyser using an ABI BigDye 3.1 terminator cycle sequencing kit (Shanghai Sangon Biological Engineering Technology and Services CO., Ltd, Shanghai, China). The newly generated sequences were submitted to GenBank database (Table 1).

Phylogenetic analysis

Species in the subg. *Heterophyllidia* with high similarity to our new species and partially representative species that are closely related to subsect. *Heterophyllinae* (Fr.) Jul. Schäff. and subsect. *Virescentinae* Singer were selected for phylogenetic analyses. *Russula maguanensis* J. Wang, X.H. Wang, Buyck & T. Bau and *R. substriata* J. Wang, X.H. Wang, Buyck & T. Bau were used as outgroup. NCBI accession numbers and references of sequences used in the phylogenetic tree are listed in Table 1. Initial sequence alignment was performed using the online version MAFFT 7.0 (http://mafft.cbrc.jp/alignment/server/) with manual evaluations and adjustments in BioEdit when necessary to obtain reliable and reasonable results (Hall 1999). The final aligned result was submitted to TreeBASE (S27792). Maximum likelihood (ML) and Bayesian analysis (BA) were implemented for the phylogenetic analyses. The maximum likelihood was carried out by using RAxML-HPC2 on XSEDE (8.2.12) through the CIPRES Science Gateway (www.phylo.org). The ML analysis was executed by applying the rapid bootstrap algorithm with 1000 replicates to affirm the consistency of the results under the GAMMA model. Bootstrap support (BS) ≥70% on the final tree was regarded as significant. The BA was performed on XSEDE (MrBayes 3.2.7a) through the CIPRES Science Gateway (www.phylo.org) under the GTR model. Four independent Markov chains were run for a total of 50000000 generations, trees were sampled every 100 generations, and the first 25% of the trees were discarded as the burn-in phase of each analysis. The Bayesian posterior probability (PP) values were obtained from the 50% majority-rule consensus trees, and nodes with PP ≥0.95 were considered to be significantly supported.

Results

Phylogeny

Both the ML analysis and BA of combined ITS-nrLSU-mtSSU-*TEF1* sequences dataset resulted in similar tree topologies, and only the ML tree is shown in Fig. 1. The posterior probabilities for the BA are also shown along the branches. The phylogenetic analyses confirmed that both subsect. *Virescentinae* and subsect. *Heterophyllinae* were a monophyletic group; each strongly supported by BS (100%) and PP (1). Additionally, the monophyly of the remaining 4 subsections of subg. *Heterophyllidia* was also significantly supported.

The samples of the two new species, *R. luofuensis* and *R. subbubalina*, formed each a strongly supported clade (BS 100%, PP 1.00) and were clearly distinct from known and sequenced species of the subg. *Heterophyllidia*. *R. luofuensis* clustered together with Chinese species *R. albidogrisea* J. W. Li & L. H. Qiu, which is sister to a clade comprising *R. viridirubrolimbata* J. Z. Ying, *R. parvovirescens* Buyck, D. Mitch. & Parrent and

Table 1. GenBank accession numbers for sequences used in phylogenetic tree. The newly generated sequences are in bold.

Taxon	Voucher	Location	ITS	nrLSU	mtSSU	*TEF1*	Reference
R. aeruginea	AT2003017	Sweden	DQ421999	DQ421999	–	–	Buyck et al. 2008
R. albidogrisea	K15091234	China	KY767807	–	–	MN617847	Das et al. 2017
R. albidogrisea	RITF1871	China	MW397095	MW397128	MW403841	–	Unpublished
R. amoena	SAV F–3147	Slovakia	MT017544	–	MT417190	MT417211	Wisitrassameewong et al. 2020
R. aureoviridis	H16082612	China	KY767809	–	–	MN617846	Das et al. 2017
R. aureoviridis	RITF4709	China	**MW646980**	**MW646992**	**MW647003**	**MW650849**	This work
R. bella	SFC20170819-05	South Korea	MT017552	–	MT196931	MT199655	Wisitrassameewong et al. 2020
R. bubalina	K15052614	China	MG018742	–	–	–	Li et al. 2019
R. bubalina	RITF1863	China	MW397097	–	MW403843	–	Unpublished
R. crustosa	BPL265	USA	KT933966	KT933826	–	–	Looney et al. 2016
R. cyanoxantha	FH 12-201	Germany	KR364093	KR364225	–	–	De Crop et al. 2017
R. cyanoxantha	RITF4682	China	**MW646981**	**MW646993**	**MW647004**	–	This work
R. dinghuensis	GDGM45244	China	KU863579	–	–	MN617848	Zhang et al. 2017
R. dinghuensis	RITF5142	China	**MW646982**	**MW646994**	**MW647005**	–	This work
R. grisea	UE2005.08.16-01	Sweden	DQ422030	DQ422030	–	–	Buyck et al. 2008
R. grisea	FH12234	Germany	KT934006	KT933867	–	–	Looney et al. 2016
R. heterophylla	UE20.08.2004-2	Sweden	DQ422006	DQ422006	–	–	Buyck et al. 2008
R. ilicis	563IC52	Europe	AY061682	–	–	–	Miller and Buyck 2002
R. lakhanpalii	AG 17-1584	India	MN262088	–	–	–	Ghosh et al. 2020
R. lakhanpalii	RITF2600	China	**MW646983**	**MW646992**	**MW647006**	**MW650850**	This work
R. lotus	RITF499	China	MK860699	MW397129	MK860706	–	Song et al. 2019
R. luofuensis	**RITF4706**	**China**	**MW646973**	**MW646985**	**MW646996**	**MW650842**	**This work**
R. luofuensis	**RITF4707**	**China**	**MW646974**	**MW646986**	**MW646997**	**MW650843**	**This work**
R. luofuensis	**RITF4708**	**China**	**MW646975**	**MW646987**	**MW646998**	**MW650844**	**This work**
R. luofuensis	**RITF4712**	**China**	**MW646976**	**MW646988**	**MW646999**	**MW650845**	**This work**
R. luofuensis	**RITF4714**	**China**	**MW646977**	**MW646989**	**MW647000**	**MW650846**	**This work**
R. maguanensis	XHW4765	China	MH724918	MH714537	–	MH939983	Wang et al. 2019
R. mustelina	FH12226	Germany	KT934005	KT933866	–	–	Looney et al. 2016
R. orientipurpurea	SFC20170819-08	South Korea	MT017550	–	MT196926	MT199651	Wisitrassameewong et al. 2020
R. orientipurpurea	SFC20170725-37	South Korea	MT017548	–	MT196927	MT199652	Wisitrassameewong et al. 2020
R. pallidula	RITF2613	China	MH027958	MH027960	MW403845	**MW650852**	Chen et al. 2019, This work
R. pallidula	RITF3331	China	MH027959	MH027961	MW403846	**MW650853**	Chen et al. 2020, This work
R. parvovirescens	SDRM 6280	USA	MK532789	–	–	–	Unpublished
R. phloginea	CNX530524068	China	MK860701	MK860704	MK860708	MK894877	Song et al. 2019
R. phloginea	CNX530524304	China	MK860700	MK860703	MK860707	MK894876	Song et al. 2019
R. prasina	HMAS 281232	China	MH454351	–	–	–	Hyde et al. 2019
R. pseudobubalina	GDGM70632	China	MF433036	–	–	–	Li et al. 2019
R. subbubalina	**RITF4710**	**China**	**MW646978**	**MW646990**	**MW647001**	**MW650847**	**This work**
R. subbubalina	**RITF4715**	**China**	**MW646979**	**MW646991**	**MW647002**	**MW650848**	**This work**
R. subpallidirosea	RITF4083	China	MK860697	MK860702	MK860705	MK894875	Song et al. 2019
R. substriata	XHW4766	China	MH724921	MH714540	–	MH939986	Wang et al. 2019
R. vesca	RITF5038	China	**MW646984**	–	**MW647007**	**MW650851**	**This work**
R. vesca	BPL284	USA	KT933978	KT933839	–	–	Looney et al. 2016
R. virescens	HJB9989	Belgium	DQ422014	DQ422014	–	–	Buyck et al. 2008
R. viridicinnamomea	K15091418	China	MK049972	–	–	MN617850	Yuan et al. 2019
R. viridicinnamomea	RITF3324	China	MW397098	MW397130	MW403847	–	Unpublished
R. viridirubrolimbata	HBAU 15011	China	MT337526	–	–	–	Deng et al. 2020
R. werneri	IB1997/0786	Europe	DQ422021	DQ422021	–	–	Unpublished
R. xanthovirens	GDGM 71145	China	MG786056	–	–	–	Song et al. 2018b

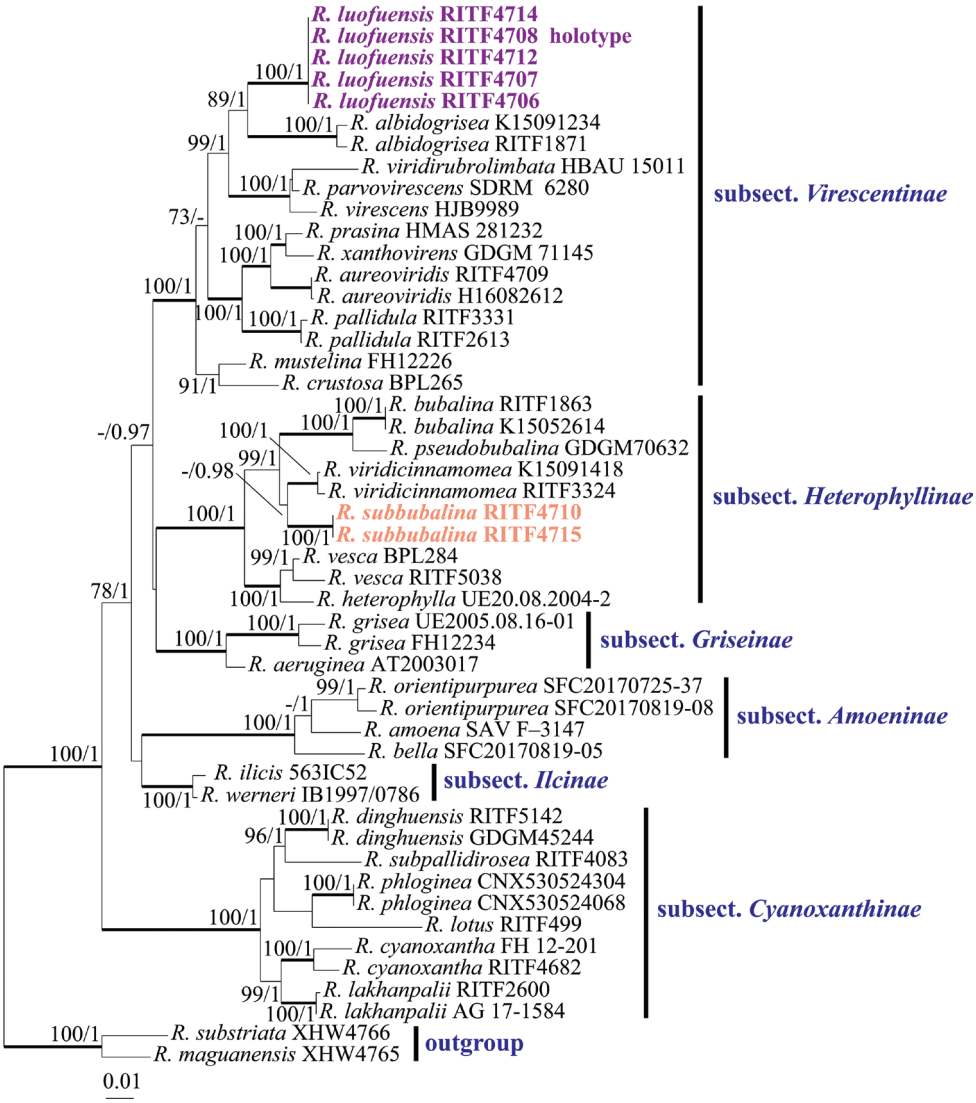

Figure 1. Phylogenetic tree of based on the ITS-nrLS-mtSSU-*TEF1* dataset. Species in the subg. *Heterophyllidia* with high similarity to our new species and partially representative species that are closely related to subsect. *Heterophyllinae* and subsect. *Virescentinae* were selected. *Russula maguanensis* and *R. substriata* were used as outgroup. Bootstrap support (BS) ≥70% are shown. Bayesian Posterior Probabilities (PP) ≥0.95 are given. Infrageneric classification follows Buyck et al. (2018).

R. virescens (Schaeff.) Fr. with 99% bootstrap support and 1.00 posterior probabilities. Our second species, *R. subbubalina* clustered with Chinese species *R. viridicinnamomea* F. Yuan & Y. Song and formed a sister clade to two Chinese species (*R. bubalina* J.W. Li & L.H. Qiu and *R. pseudobubalina* J.W. Li & L.H. Qiu) with 99% bootstrap support and 1.00 posterior probabilities.

Taxonomy

***Russula luofuensis* B. Chen & J. F. Liang, sp. nov.**
MycoBank No: MB838836
Figs 2A–D, 3 and 4

Diagnosis. Basidiomata medium-sized to large; grayish yellow to brownish orange pileus center, purplish gray to grayish magenta towards the margin, surface cracking and broken into small golden-brown patches, peeling to 1/2 of the radius; subglobose to broadly ellipsoid basidiospores with warts fused in short or long chains; hymenial gloeocystidia mainly clavate; suprapellis composed of hyphal extremities with inflated, ellipsoid or globose cells and attenuated terminal cell; pileocystidia always one-celled, apically typically obtuse.

Holotype. China. Guangdong Province, Huizhou City, Boluo County, Luofu Mountain Provincial Nature Reserve, 23°15'47.13"N, 114°3'45.42"E, 90 m asl., in mixed Fagaceae forests of *Cyclobalanopsis* and *Castanopsis*, 22 August 2020, leg. CB446 (RITF4708).

Etymology. The species name refers to the type locality, Luofu Mountain Provincial Nature Reserve.

Description. Basidiomata medium-sized to large; pileus 35–80 mm in diameter; initially hemispheric when young, applanate to convex, convex with a depressed center after mature; margin incurved, not cracked, striation short and inconspicuous; surface dry, glabrous, peeling to 1/2 of the radius, cracking and broken into small golden-brown patches, patches crowded towards the center, with smaller patches towards the margin; grayish yellow (4B5) to brownish orange (5C5) in the center, purplish gray (13B2) to grayish magenta (13B3) towards the margin. **Lamellae** adnate to subfree, 2–4 mm deep, 8–10 at 1 cm near the pileus margin, white (1A1) to cream; lamellulae absent; furcations occasional near the stipe; edge entire and concolor. **Stipe** 30–50 × 10–25 mm, cylindrical, slightly inflated towards the base, white (1A1), with yellowish (2A2) tinge at the base, and medulla initially stuffed becoming hollow. **Context** 2–3 mm thick in half of the pileus radius, white (1A1), unchanging when bruised, taste mild, odor inconspicuous. **Spore print** pale yellowish (2A2).

Basidiospores (5.0–)5.8–6.6–7.5(–8.6) × (4.5–)5.4–6.2–7.0(–8.0) µm, Q = (1.0–)1.02–1.08–1.14(–1.26), subglobose to broadly ellipsoid; ornamentation of medium-sized, moderately distant to dense [6–8(–9) in a 3 µm diameter circle] amyloid warts or spines, 0.3–0.6 µm high, locally reticulate, frequently fused in short or long chains [2–3(–4) in the circle], occasionally to frequently connected by line connections [1–2(–3) in the circle]; suprahilar spot medium-sized, amyloid. **Basidia** (35.0–)36.7–39.8–42.8(–45.5) × (9.0–)9.5–10.0–10.5(–11.2) µm, mostly 4-spored, some 2- and 3-spored, clavate; basidiola clavate or subcylindrical, ca. 6.5–11.5 µm wide. **Hymenial gloeocystidia on lamellae sides** dispersed to moderately numerous, ca. 600–900/mm^2, (59.0)63.2–71.3–79.3(83.6) × (7.0)7.7–8.8–9.9(10.5) µm, clavate or narrowly clavate, apically mainly obtuse, occasionally acute, often with 3–10 µm long appendage, thin-walled; contents heteromorphous or granulose, mainly in the middle and upper part,

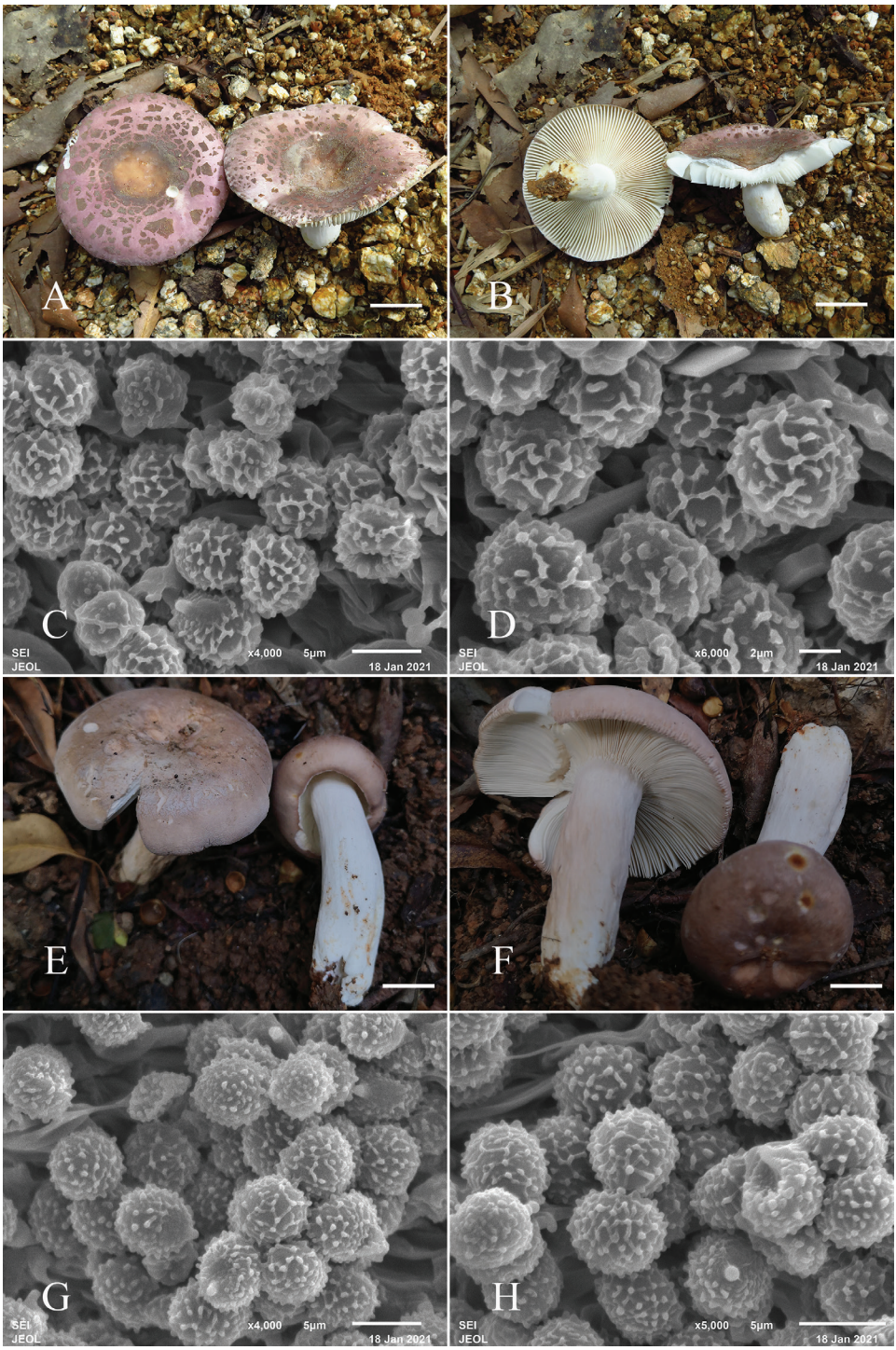

Figure 2. Fruiting bodies (**A, B**) and basidiospores (**C, D**) of *Russula luofuensis* (RITF4708). Fruiting bodies (**E, F**) and basidiospores (**G, H**) of *R. subbubalina* (RITF 3715). Scale bars: 20 mm (**A, B, E, F**).

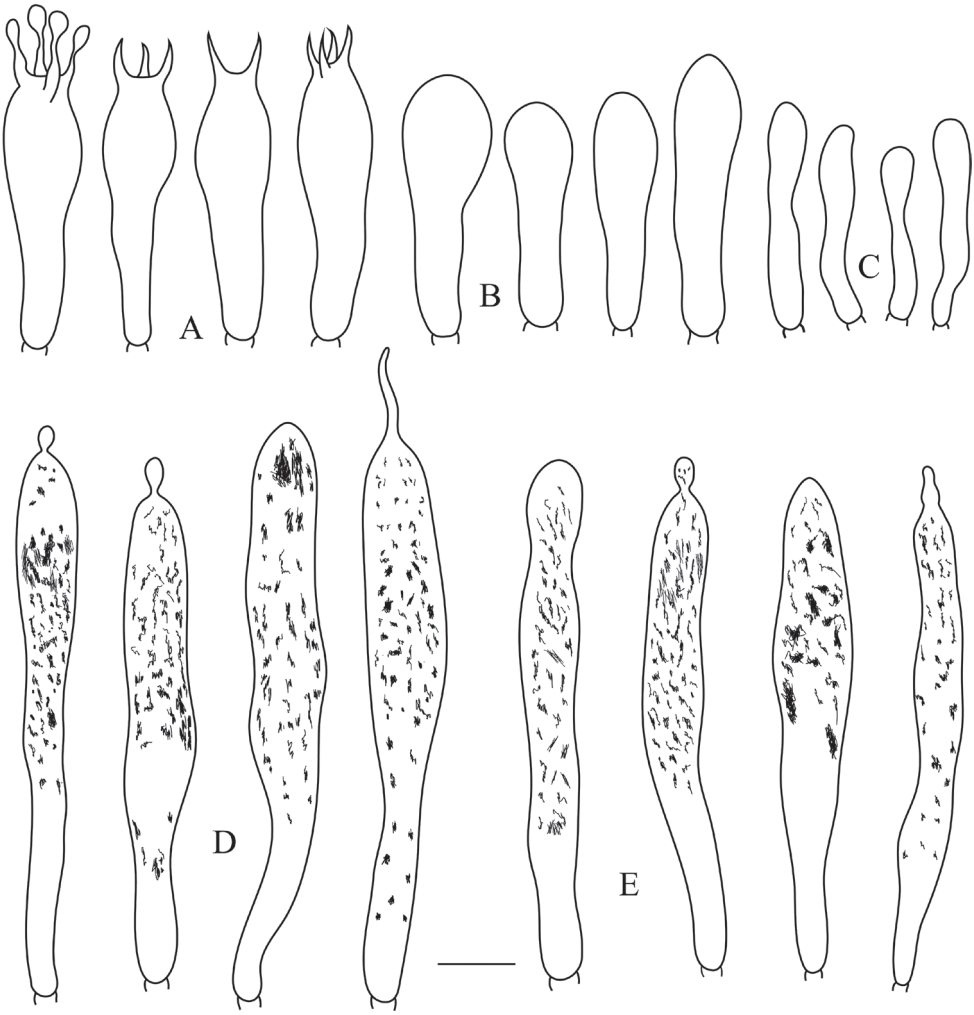

Figure 3. *Russula luofuensis* (RITF 4708) **A** basidia **B** basidiola **C** marginal cells **D** hymenial gloeocystidia on lamellae sides **E** hymenial gloeocystidia on lamellae edges. Scale bar: 10 μm.

turning reddish black in SV. **Hymenial gloeocystidia on lamellae edges** often smaller, (49.5–)56.2–64.3–72.4(–80.2) × (6.2–)7.3–8.3–9.4(–10.0) μm, clavate, or subcylindrical, sometimes fusiform, apically mainly obtuse, occasionally mucronate, sometimes with 3–6 μm long appendage thin-walled; contents heteromorphous, turning reddish black in SV. **Marginal cells** (15.2–)19.8–23.5–27.2(–30.6) × (3.5–)4.0–4.8–5.6(–7.0) μm, subcylindrical or clavate, often flexuous. **Pileipellis** orthochromatic in cresyl blue, not sharply delimited from the underlying context, 260–300 μm deep, two-layered; suprapellis 120–150 μm deep, hyphal endings composed of inflated, ellipsoid or globose cells with attenuated terminal cells; subpellis 120–160 μm deep, composed of repent, intricate, 2–6 μm wide hyphae. Hyphal terminations near the pileus margin typically

unbranched, occasionally flexuous, thin-walled; terminal cells (9.2–)18.6–28.2–37.8(–50.8) × (3.2–)3.9–5.0–6.1(–8.2) μm, mainly narrowly lageniform, occasionally clavate or cylindrical, apically attenuated or constricted, occasionally obtuse; subterminal cells frequently shorter and wider, ca. 4–9 μm wide, typically unbranched. Hyphal terminations near the pileus center similar to those near the pileus margin; terminal cells (10.2–)18.4–27.4–36.4(–44.8) × (3.2–)3.6–4.7–5.8(–6.8) μm, mainly lageniform, occasionally fusiform or subcylindrical, apically attenuated or constricted; subterminal cells often shorter and wider, rarely branched, ca. 4–7 μm wide. **Pileocystidia** near the pileus margin always one-celled, (23.3–)27.9–35.0–42.2(–47.5) ×3.5–4.8–6.0(–8.3) μm, mainly clavate, occasionally subcylindrical or fusiform, apically typically obtuse, occasionally acute, often with round or ellipsoid, 3–6 μm long appendage, thin-walled; contents heteromorphous or granulose, turning reddish black in SV. Pileocystidia near the pileus center similar in size, always one-celled, (24.6–)27.2–34.8–42.5(–48.2) × 3.0–4.2–5.4(–6.8) μm, thin-walled, mainly clavate, occasionally fusiform, apically often obtuse or occasionally acute, occasionally with 2–4 μm long appendage, contents heteromorphous or granulose, turning reddish black in SV. **Cystidioid hyphae** In subpellis and context with heteromorphous contents, oleiferous hyphae in subpellis with refringent contents.

Additional specimens examined. China. Guangdong Province, Huizhou City, Boluo County, Luofu Mountain Provincial Nature Reserve, 23°15'44.11"N, 114°3'16.77"E, 120 m asl., in mixed Fagaceae forests of *Cyclobalanopsis* and *Castanopsis*, 22 August 2020, leg. CB444 (RITF4706); ibid., 22 August 2020, leg. CB445 (RITF4707); ibid., 22 August 2020, leg. CB450 (RITF4712); ibid., 22 August 2020, leg. CB452 (RITF4714).

Notes. The combination of morphological features and phylogenetic analysis place *R. luofuensis* in subsect. *Virescentinae*. Phylogenetically, our new species *R. luofuensis* is clustered with *R. albidogrisea* with 89% bootstrap support and 1.00 posterior probabilities, which is also from Guangdong Province of China. However, *R. albidogrisea* differs from *R. luofuensis* in having a white to grayish pileus with acute, even to slightly undulate margin, often smaller basidiospores [(5.1–)5.3–5.6–6.0(–6.4) × (4.6–)4.8–5.1–5.3(–5.6) μm], longer hymenial gloeocystidia on lamellae sides (35–50 × 5–11 μm) and hymenial gloeocystidia on lamellae edges (37–46 × 9–12 μm, Das et al. 2017).

Given cracking surface, *R. viridirubrolimbata*, *R. parvovirescens*, *R. virescens* and *R. crustosa* Peck of subsect. *Virescentinae* resemble *R. luofuensis*. However, *R. viridirubrolimbata*, originally described from China, can be distinguished by a light yellowish olive to yellowish olive pileus center with a pinkish red to light jasper red margin and absence of hymenial gloeocystidia on lamellae edges (Ying 1983; Deng et al. 2020). The American species *R. parvovirescens* possesses a greenish brown to metallic bluish green pileus with green patches (Buyck et al. 2006). *Russula virescens* (originally reported from Europe) is distinct in its green to yellowish green pileus (Sarnari 1998). *Russula albidogrisea*, originally reported from North America, has a brownish-yellow, greenish or subolivaceous pileus with small spot-like areolae or pseudo-verrucae, shorter basidia [(29–)30–32–33.5(–35) × (7.5–)8–9.5–10.5(–11) μm] and absence of hymenial gloeocystidia on the lamellar edges (Adamčík et al. 2018).

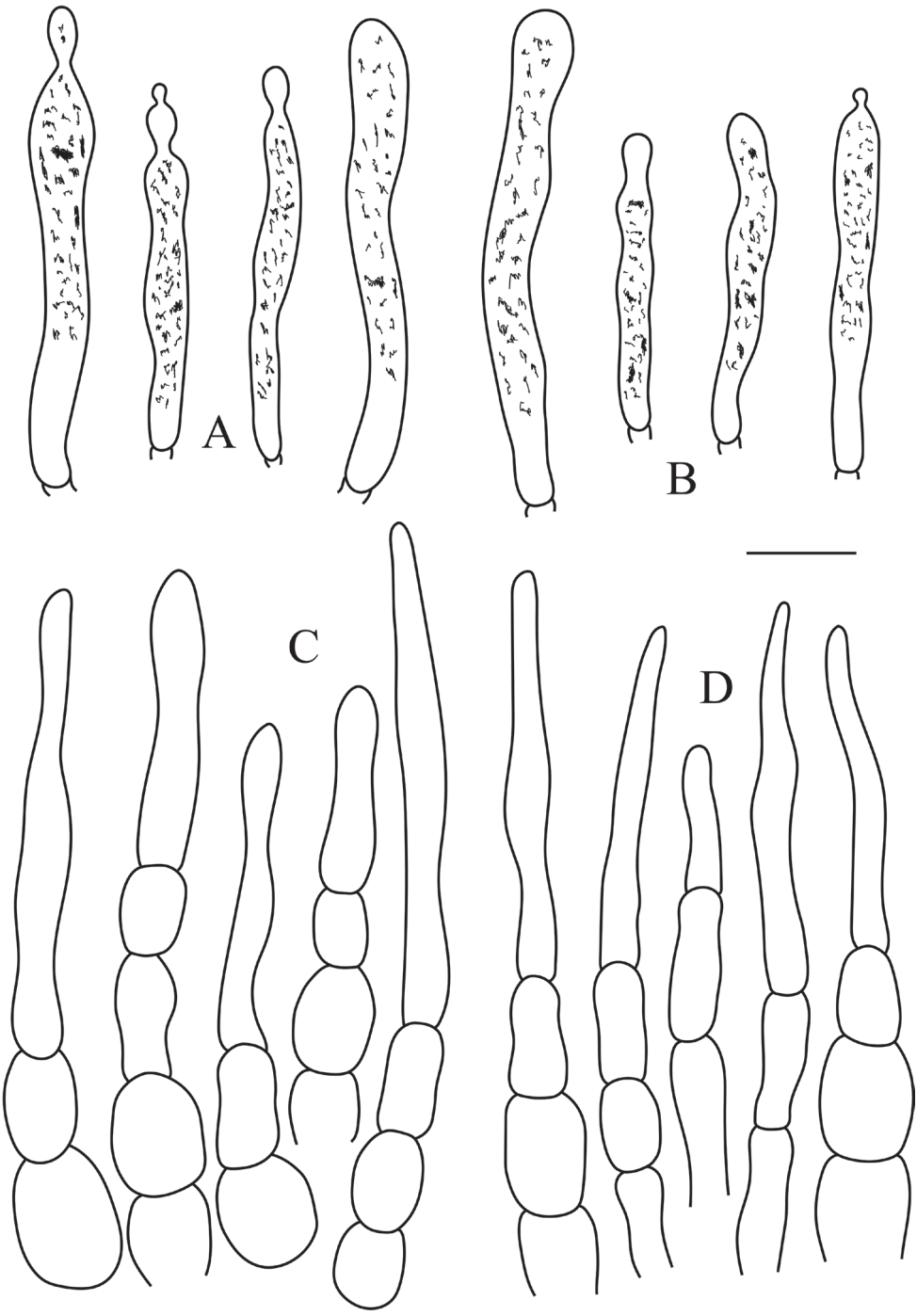

Figure 4. *Russula luofuensis* (RITF 4708) **A** pileocystidia near the pileus margin **B** pileocystidia near the pileus center **C** hyphal terminations near the pileus margin **D** hyphal terminations near the pileus center. Scale bar: 10 μm.

Russula subbubalina **B. Chen & J. F. Liang, sp. nov.**
MycoBank No: MB838837
Figs 2E–H, 5 and 6

Diagnosis. Basidiomata medium-sized to large; dark salmon pileus with rusty spots when young, blanched almond with a cracked margin after maturation, surface pruinose in some parts; adnate to slightly adnexed lamellae; subglobose to broadly ellipsoid basidiospores with warts fused in short or long chains and frequently connected by line connections; clavate or ellipsoid basidiola; hymenial gloeocystidia clavate or fusiform, apically mainly obtuse; suprapellis with hyphal ends composed of inflated or ellipsoid cells and attenuated terminal cell; pileocystidia mainly clavate, apically typically obtuse, sometimes with round or ellipsoid appendage.

Holotype. China. Guangdong Province, Huizhou City, Boluo County, Luofu Mountain Provincial Nature Reserve, 23°15'43.80"N, 114°3'5.40"E, 220 m asl., in mixed Fagaceae forests of *Cyclobalanopsis* and *Castanopsis*, 22 August 2020, leg. CB448 (RITF4710).

Etymology. Referred to its morphological resemblance to *R. bubalina*.

Description. Basidiomata medium-sized to large; pileus 50–100 mm in diameter; initially hemispheric when young, applanate to convex, convex with a slightly depressed center after mature; margin incurved, cracked with age, striation short and inconspicuous; surface dry, glabrous, peeling to 1/4 of the radius, pruinose in some part; dark salmon with rusty spots when young, blanched almond after maturation, shallower at the margin. **Lamellae** adnate to slightly adnexed, 3–5 mm deep, 11–13 at 1 cm near the pileus margin, white (1A1) to cream; lamellulae sometimes present and irregular in length; furcations present especially near the stipe; edge entire and concolor. **Stipe** 30–55 × 5–15 mm, cylindrical, slightly inflated towards the base, white (1A1) to blanched almond, with rusty tinge towards the base, and medulla initially stuffed becoming hollow. **Context** 3–4 mm thick in half of the pileus radius, white (1A1), unchanging when bruised, taste mild, odor inconspicuous. **Spore print** white (1A1) to cream.

Basidiospores (5.2–)5.6–6.2–6.8(–7.2) × (4.5–)4.9–5.3–5.7(–6.2) µm, Q = (1.0–)1.08–1.17–1.25(–1.38), subglobose to broadly ellipsoid; ornamentation of relatively small, moderately distant to dense [6–8(–9) in a 3 µm diameter circle] amyloid warts or spines, 0.3–0.5 µm high, locally reticulate, fused in short or long chains [2–3(–4) in the circle], frequently connected by line connections [3–4(–5) in the circle]; suprahilar spot medium-sized, amyloid. **Basidia** (30.5–)31.7–34.8–37.8(–43.0) × (6.3–)7.5–8.1–8.8(–9.4) µm, mostly 4-spored, some 2- and 3-spored, clavate; basidiola clavate or ellipsoid, ca. 5.5–10 µm wide. **Hymenial gloeocystidia on lamellae sides** Moderately numerous, ca. 800–1000/mm^2, (41.0)49.1–56.7–64.3(68.5) × (6.5)7.2–8.1–9.0(10.0) µm, clavate or fusiform, apically mainly obtuse, occasionally acute, sometimes with 4–10 µm long appendage, thin-walled; contents heteromorphous or granulose, turning reddish black in SV. **Hymenial gloeocystidia on lamellae edges** Often longer, (40.5–)52.6–

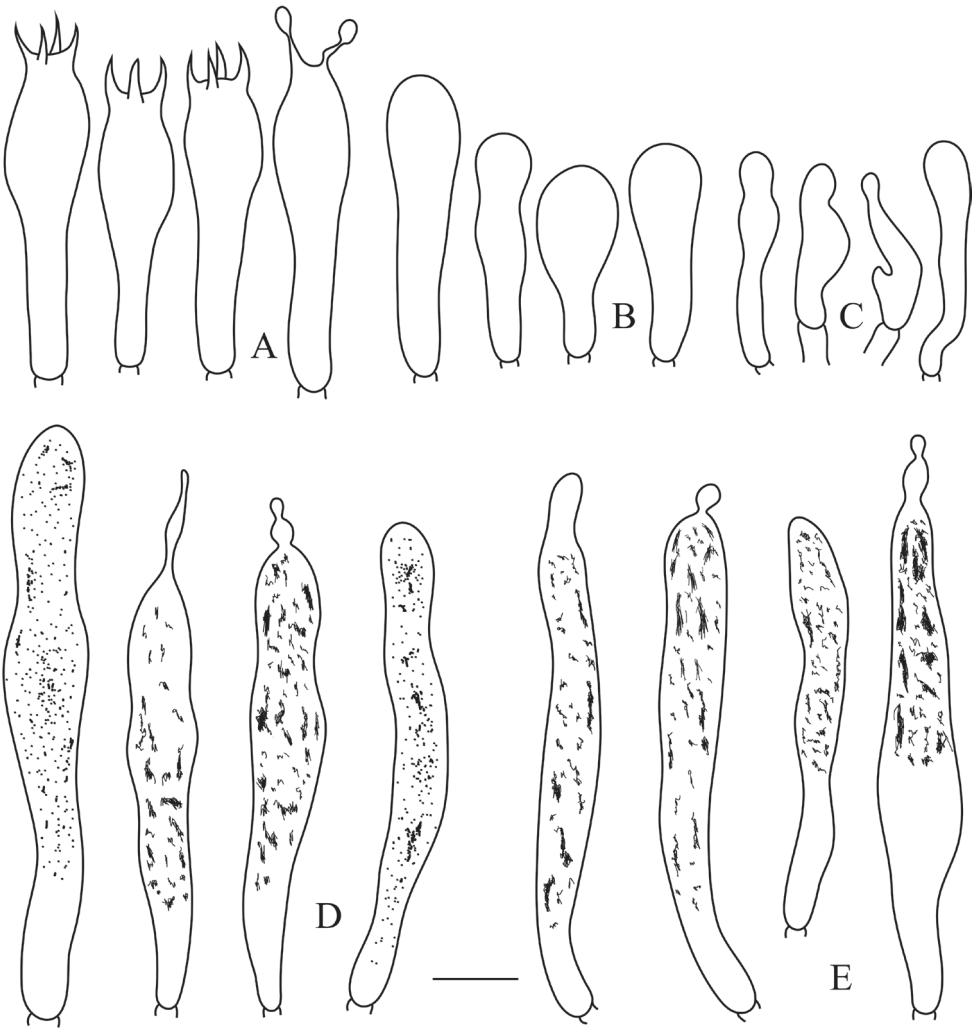

Figure 5. *Russula subbubalina* (RITF 4710) **A** basidia **B** basidiola **C** marginal cells **D** hymenial gloeocystidia on lamellae sides **E** hymenial gloeocystidia on lamellae edges. Scale bar: 10 µm.

63.0–73.5(–83.6) × (4.6–)6.7–8.1–9.6(–10.8) µm, mainly clavate, occasionally fusiform, apically typically obtuse, sometimes with 3–8 µm long appendage, thin-walled; contents heteromorphous-crystalline, turning reddish black in SV. **Marginal cells** (14.0–)19.0–23.4–27.7(–34.2) × (3.4–)3.7–4.5–5.3(–5.8) µm, clavate, lageniform or fusiform, often flexuous. **Pileipellis** Orthochromatic in cresyl blue, sharply delimited from the underlying context, 400–450 µm deep, two-layered; suprapellis180–200 µm deep, hyphal endings composed of inflated or ellipsoid cells with attenuated terminal cells; subpellis 240–260 µm deep, composed of horizontally oriented, relatively dense, intricate, 3–6 µm wide hyphae. Hyphal terminations near the pileus margin sometimes branched, occasionally flexuous,

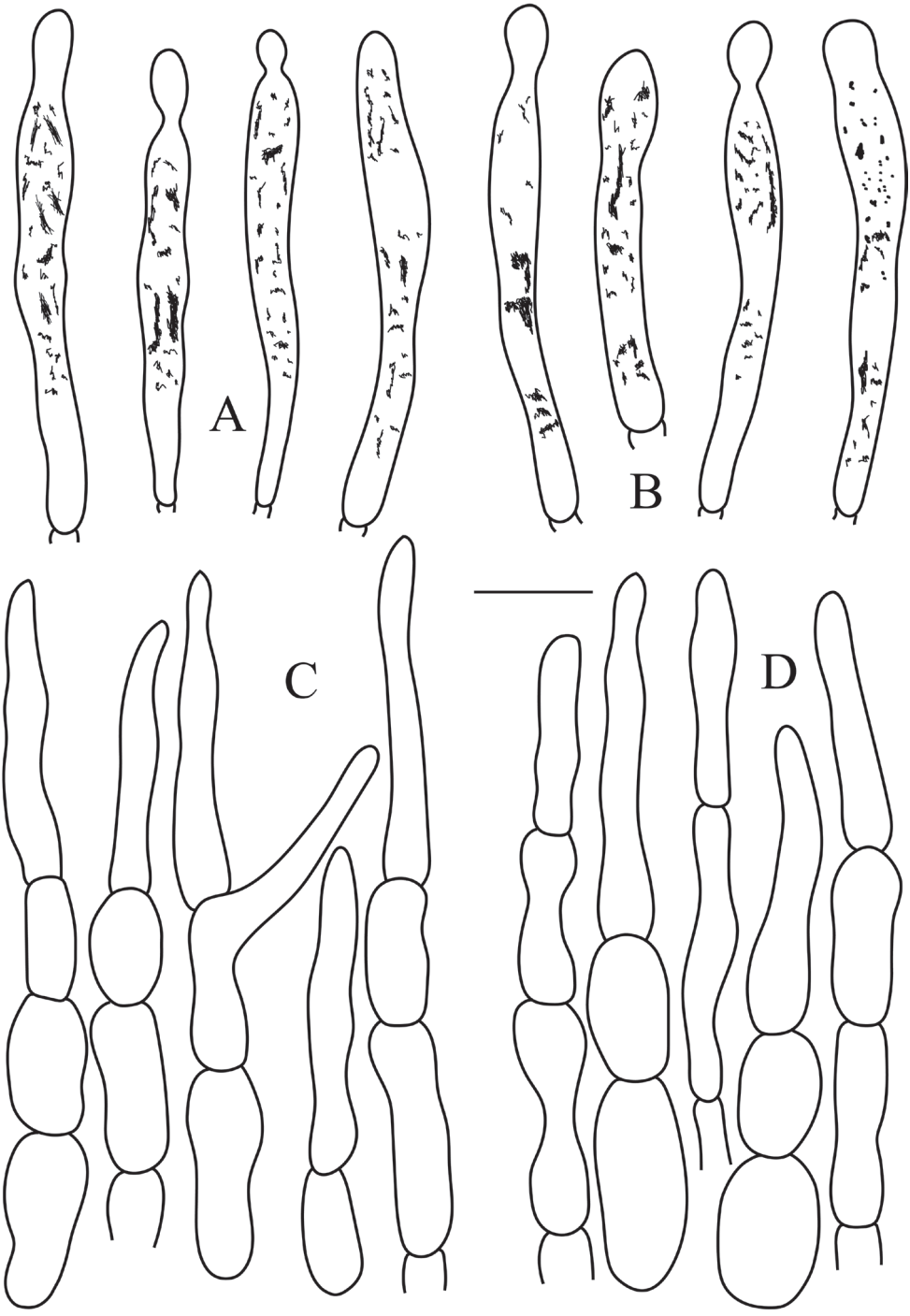

Figure 6. *Russula subbubalina* (RITF 4710) **A** pileocystidia near the pileus margin **B** pileocystidia near the pileus center **C** hyphal terminations near the pileus margin **D** hyphal terminations near the pileus center. Scale bar: 10 μm.

thin-walled; terminal cells (14.8)20.9–26.6–32.3(38.0) × 3.5–4.0–4.6(5.5) μm, mainly narrowly lageniform, occasionally cylindrical, apically attenuated or constricted; subterminal cells frequently shorter and wider ca. 3–8 μm wide, occasionally branched. Hyphal terminations near the pileus center similar to those near the pileus margin; terminal cells (14.3–)17.5–22.7–27.8(–33.7) × (3.4–)3.7–4.1–4.6(–5.0) μm, lageniform, clavate or cylindrical, apically attenuated or constricted, sometimes obtuse; subterminal cells often wider, rarely branched, ca. 4–8 μm wide. **Pileocystidia** near the pileus margin always one-celled, (27.9–)35.1–40.5–45.9(–48.9) × (3.8–)4.2–4.7–5.3(–5.7) μm, mainly clavate, occasionally fusiform, apically typically obtuse, sometimes with round or ellipsoid 2–6 μm long appendage, thin-walled; contents heteromorphous, turning reddish black in SV. Pileocystidia near the pileus center similar in shape, always one-celled, (23.7–)25.6–31.8–38.0(–46.0) × (3.3–)4.2–4.8–5.4(–6.0) μm, thin-walled, mainly clavate, occasionally fusiform or subcylindrical, apically typically obtuse, sometimes with 4–6 μm long appendage, contents granulose, turning reddish in SV. **Cystidioid hyphae** In subpellis and context with granulose contents, oleiferous hyphae frequent in subpellis with yellowish contents.

Additional specimens examined. China. Guangdong Province, Huizhou City, Boluo County, Luofu Mountain Provincial Nature Reserve, 23°15'41.70"N, 114°3'5.21"E, 240 m asl., in mixed Fagaceae forests of *Cyclobalanopsis* and *Castanopsis*, 22 August 2020, leg. CB453 (RITF4710).

Notes. Both morphology and phylogeny place *R. subbubalina* clearly in subsect. *Heterophyllinae*. In our phylogenetic tree, *R. viridicinnamomea* is the sister taxon to *R. subbubalina* but differs from it by the typically smaller basidiomata (30–50 μm), an emerald green-tinged buff pileus with undulate and tearing margin and longer hymenial gloeocystidia on the lamellae edges (36.5–63 × 4–12 μm, Yuan et al. 2019).

Morphologically, *R. subbubalina* may be confused in the field with two recently reported new species: *R. bubalina* and *R. pseudobubalina* also from Guangdong Province of China. However, *R. bubalina* has the typically smaller basidiomata (35–54 μm), a striate pileus margin and basidiospores with warty ornamentations not forming reticulum (Li et al. 2019), whereas *R. pseudobubalina* possesses the typically smaller basidiomata (31–46 μm), never forked lamellae, basidiospores with isolated warts, and often shorter hymenial gloeocystidia on the lamellae edges (23.4–37.8–65.5 × 6.2–8.3–10.0 μm, Li et al. 2019).

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Greetings from belowground: two new species of truffles in the genus *Pachyphlodes* (Pezizaceae, Pezizales) from México

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Abstract

Pachyphlodes is a lineage of ectomycorrhizal, hypogeous, sequestrate ascomycete fungi native to temperate and subtropical forests in the Northern Hemisphere. *Pachyphlodes* species form ectomycorrhizae mainly with Fagales hosts. Here we describe two new species of *Pachyphlodes*, *P. brunnea*, and *P. coalescens*, based on morphological and phylogenetic analysis. *Pachyphlodes brunnea* is distributed in the states of Tamaulipas and Nuevo León in northern México, occurring with *Quercus* and *Juglans* species. It is characterized by its dark brown peridium, white gleba, and spores with capitate columns. *Pachyphlodes coalescens* is distributed in the states of Michoacán and Tlaxcala in central and southwestern México co-occurring with *Quercus* and is distinguished by its reddish-brown peridium, light yellow gleba, and spore ornamentation. Both species, along with *P. marronina*, constitute the Marronina clade. This clade contains North American species characterized by a brown peridium and spores ornamented with capitate spines to coalesced spine tips that form a partial perispore.

Keywords

Ascomycota, hypogeous, new taxa, sequestrate fungi, systematics, truffles

Introduction

Pachyphlodes Zobel, 1854 (Pezizaceae, Pezizales) is characterized by truffle-like ascomata with a thick peridium of large isodiametric cells and globose spores ornamented with spines or columns. The spores are either naked or covered with a perispore (Tulasne and Tulasne 1844; Healy et al. 2018). There are currently 16 recognized *Pachyphlodes* species and two varieties in the genus (Kirk 2016; Ting et al. 2019). *Pachyphlodes* was known as *Pachyphloeus*, but this name was declared illegitimate (Healy et al. 2018), so its species were transferred to the oldest legitimate name *Pachyphlodes* (Doweld 2013a, b). *Pachyphlodes* species are distributed across the Northern Hemisphere; in North America and Europe; they form ectomycorrhizae with hosts in the Betulaceae, Fagaceae, and Juglandaceae in temperate and subtropical regions (Smith et al. 2007; Lindner and Banik 2009; Stefani et al. 2009; Tedersoo et al. 2009, 2010; Bonito et al. 2011; García-Guzmán et al. 2017). With the use of molecular techniques, the number of *Pachyphlodes* species has nearly doubled from eight species and two varieties in 2000 to 16 species and two varieties in 2020. Four species have been described from México; Cázares et al. (1992) reported *P. citrina* (Berk. and Broome) Doweld (unverified by molecular methods) from Nuevo León and *P. virescens* (Gilkey) Doweld (unverified by molecular methods) from Nuevo León and Tamaulipas; Healy et al. (2009) reported *Pachyphlodes* cf. *carnea* from Nuevo León, and described a new species *P. marronina* Healy, Bonito & Guevara from Nuevo León, Tamaulipas and Tlaxcala. Healy et al. (2009) remarked on morphological differences between the *P. marronina* collections from the upper Midwestern USA and the *P. marronina* collections from México and proposed they may be part of a species complex in need of further analysis. With the aim to solve this species complex, here we report new collections along with results from further analyses that support the description of the Mexican collections as two new species of *Pachyphlodes* in the Marronina clade.

Materials and methods

Morphological observations

Ascomata of *P. brunnea* were collected from the state of Tamaulipas, while *P. coalescens* collections were found across the states of Michoacán and Tlaxcala. All the specimens are deposited in the following herbaria: Oregon State University (**OSC**), Instituto Tecnológico de Ciudad Victoria (**ITCV**) and Herbario Nacional de México (**MEXU**). Macroscopic characters were described from fresh specimens under natural light, and colors of fresh ascomata are described in general terms by the authors. Microscopic characters were described from razor-blade sections of fresh specimens mounted in 5% KOH and Melzer's reagent. Fifty measurements were taken per structure; measurements of structures are length by width (this is the order of appearance in the descriptions). For scanning electron microscopy (SEM), ascospores were scraped from

the dried gleba onto double-sided tape, which was mounted directly on an SEM stub, coated with platinum-palladium, and examined and photographed with a HITACHI TM 3000 scanning electron microscope, or they were prepared and imaged as outlined in Healy et al. (2018).

DNA sequencing and phylogenetic analyses

A tissue sample from collection MEXU 26842 was sent to the Canadian Center of Barcoding (CCDB) for extraction, amplification, and sequencing of the Internal Transcribed Spacer (ITS). DNA was extracted from JT32454, JT32623, and ITCV-GGG-896 at the University of Minnesota with a modified CTAB method (Healy et al. 2009). The ITS1-5.8s-ITS2 (ITS) region was amplified with ITS1 and ITS4 (White et al. 1990) and ITS1f (Gardes and Bruns 1993). DNA sequences were deposited in GenBank (Table 1). Sequences were edited in Geneious 7.1 (Kearse et al. 2012) or Sequencher 4.0 (Gene Codes, Ann Arbor, MI). As done in Piña Páez et al. (2018), the distribution of species was complemented with soil DNA data from central and south México through a BLASTn search against the Mexican Soil Fungi Database in Geneious 10.1. This database includes ITS2 sequences of soil fungi from México and has been partially published in Argüelles-Moyao and Garibay-Orijel (2018).

Phylogenetic analyses of ITS rDNA have been implemented to describe and resolve species delimitation in *Pachyphlodes* (Healy et al. 2015; Li et al. 2019; Liu et al. 2020). Phylogenetic analyses utilizing the 28S rDNA, ß-Tubulin, and RPB2 markers showed that *Pachyphlodes* is a member of the *Pezizaceae*, that *Plicariella* (Sacc.) Rehm (as *Scabropezia* Dissing and Pfister) is sister to *Pachyphlodes*, and that the sister lineage to *Pachyphlodes* and *Plicariella* is *Amylascus* Trappe (Hansen et al. 2005). Healy et al. (2018) showed that *Plicariella* is within or sister to the Melanoxanthus clade of *Pachyphlodes.* Our phylogenetic analysis consisted of 42 sequences from 16 described species, including nine sequences from type specimens of *Pachyphlodes* and from *Amylascus* Trappe. *Amylascus* was selected as an outgroup based on previous phylogenetic analyses. DNA sequences were aligned with MAFFT v 6.822 (Katoh and Toh 2010) and manually improved in SE-AL v2.0a11 (Rambaut 2007) for a final alignment with 754 positions. Phylogenetic inferences were estimated with maximum likelihood in RAxML 7.2.8 (Stamatakis 2006) with a GTR + G model of nucleotide substitution. For Bayesian posterior probability, priors were selected with jModeltest 2.1.4 (Darriba et al. 2012), under the Aikake information criterion, and posterior probability was estimated in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) with 20,000,000 generations with trees sampled every 1000 generations. The first 25% of samples were discarded as burn-in, and stationarity was checked in Tracer (Rambaut and Drummond 2007). RAxML and MrBayes were both runs on the Cipres Portal (Miller et al. 2010). Trees were visualized and optimized in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/), and font and color were added in Adobe Illustrator vCS4 (Adobe Systems, Inc., San Jose, CA). Alignment is available in OSF (Open Space Framework, to be uploaded prior to journal submission).

Table 1. Accession and voucher numbers of sequences included in the phylogenetic analysis. Herbarium collection with * indicates holotypes and ** indicates paratypes.

Species	Herbarium	Country	GenBank
Amylascus	OSC:H5626	Australia	JX414224, KJ720812
Amylascus	MEL2364119A	Australia	KT318375
Pachyphlodes annagardnerae	ISC:RH46*	USA: IA	JN102472
	ISC:RHAM14	USA: IA	JN102375
Pachyphlodes austro-oregonensis	SOC775*	USA: OR	JX414191
Pachyphlodes brunnea	ITCV896*	Mexico	HQ324990
	JG3757	Mexico	EU427551
	OSC:JT32623	Mexico	MT461399
	DUKE	Mexico	JN102443
Pachyphlodes carnea	OSC43593	USA: CA	JX414189
	FLAS-F-63788	USA: CA	MT461396
Pachyphlodes cinnabarina	HMAS-96735*	China	MK192830
	BJTC-FAN946	China	MK192831
	BJTC-FAN1157	China	MK192829
Pachyphlodes citrina	FLAS:JBP-2011-09-10	France	KJ720747
	FLAS-F-59182	England	JN102468
	OSC:JRWL 2197	Italy	EU543196
Pachyphlodes coalescens	MEXU-26842*	Mexico	KJ595000
	TXLM:JT32454	Mexico	EU543209
Pachyphlodes conglomerata	FLAS-F-66164	Spain	KJ720788
	MA-29354	Spain	JN102487
Pachyphlodes depressa	BJTC:FAN302*	China	KP027405
	BJTC:FAN324	China	KP027406
Pachyphlodes ligerica	FLAS-F-62613	France	MT461402
Pachyphlodes marronina	MIN-925598	USA: IA	KJ720786
	MIN-925612	USA: IA	JN102364
	HUH-258432*	USA: IA	EU427549
Pachyphlodes melanoxantha	FLAS-F-61135	England	JX414217
	FLAS-F-66172	France	KJ720792
	FLAS-F-66167	Spain	KJ720793
Pachyphlodes nemoralis	FLAS-F-61964	France	MT461400
	FLAS-F-66166	Spain	MF462328
	FLAS-F-59181*	England	JN102469
	S-F-133989	Sweden	JX414218
Pachyphlodes oleifera	FLAS-F-64137	Spain	KJ720787
	MA-82461*	Spain	JQ996421
Pachyphlodes pfisteri	FLAS-F-59179*	USA: ME	JN102474
Pachyphlodes thysellii	OSC 80959**	USA: WA	EU543197
	FLAS-F-66243	USA: MN	JN102479
Pachyphlodes virescens	FLAS-F-60565	USA: CA	MT461401
	OSC JT13043	USA: CA	JX414219
Pachyphlodes wulushanensis	BJTC-FAN923*	China	MK192827

Results

The nucleotide substitution model selected by jModeltest was TPM1uf+I+G. The final optimization likelihood was –lnL 4774.259669, and the most likely tree is shown in Fig. 1. Both the Maximum Likelihood and Bayesian analyses (Fig. 1) show that *P. brunnea* forms a new strongly supported clade (100/1), which includes sequences from

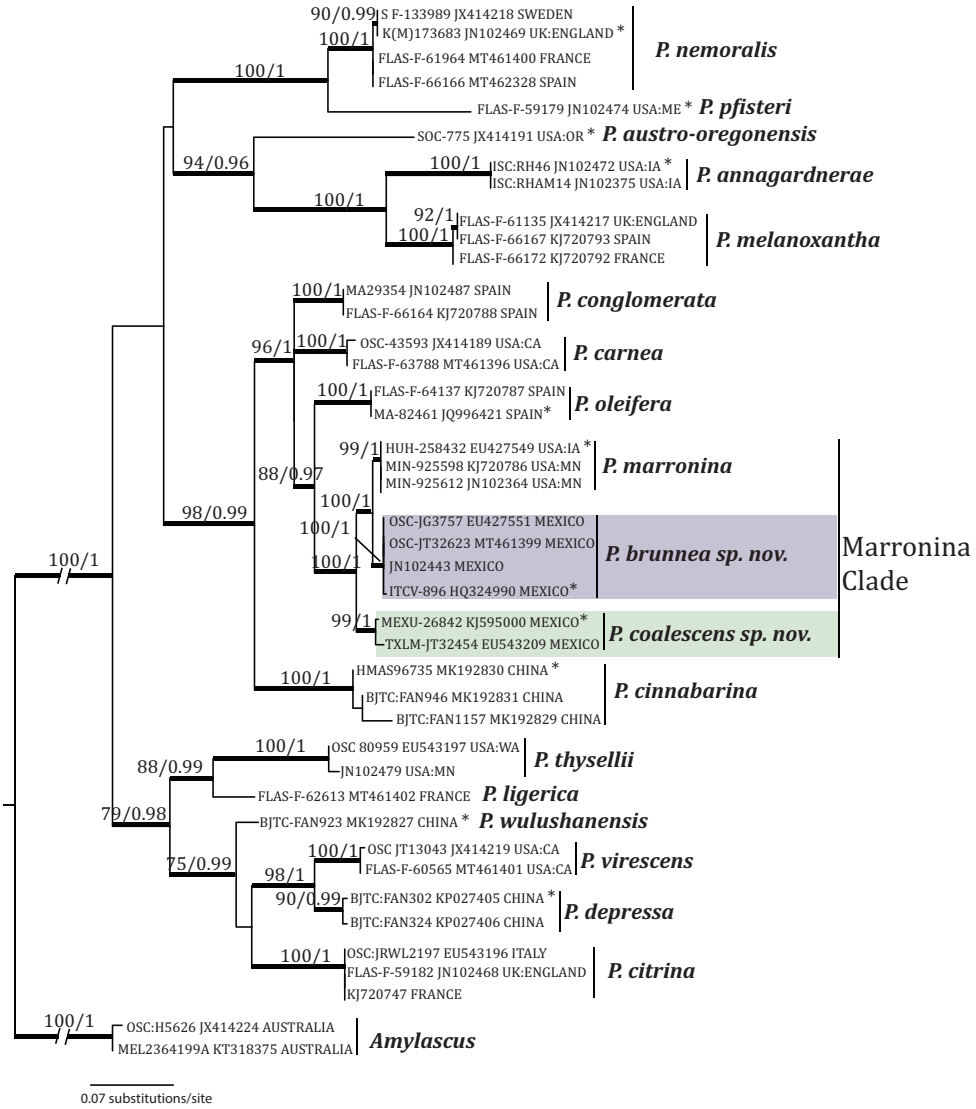

Figure 1. The most likely tree generated from RAxML analysis of the ITS sequences of 18 *Pachyphlodes* species, rooted with *Amylascus*. Thickened branches denote >70% bootstrap support (left of slash) and >0.95 posterior probability (right of slash) from Bayesian analysis. New species are in shaded boxes, and Marronina clade demarcated. Terminals contain GenBank accession number, herbarium number, and country/state of collection. Asterisks denote sequences from holotypes.

voucher collections and ectomycorrhizae. This clade is placed as a sister taxon of *P. marronina*, which also forms a strongly supported clade (99/1). The sister taxon (100/1) of these two species is *P. coalescens*, which is also a new strongly supported clade (99/1).

Taxonomy

Pachyphlodes brunnea **Guevara, Piña Páez & Healy, sp. nov.**
MycoBank No: 835665
ITS barcode GenBank: HQ324990 (Holotype), EU427551, MT461399, JN102443
Fig. 2a–d

Type. México, Tamaulipas, Ciudad Victoria, Torre de Microondas "Las Mulas", 23°37'00"N, 99°14'31"W, alt. 1549 m, under *Quercus polymorpha* Schlecht. & Cham., *Quercus* sp. and *Juglans* sp., hypogeous, solitary or in groups of 2, 11 November 2006, col. G. Guevara (holotype: ITCV 896).

Diagnosis. *Pachyphlodes brunnea* is be recognized by the dark brown ascomata and two-layered. Thick (474–570 µm) peridium, white gleba when immature, spores ornamented with capitate columns growing under *Quercus* and with an odor similar to raw potatoes.

Etymology. Latin, brunnea in reference to the brown peridium.

Description. ***Ascomata*** subglobose to ovoid, 15–17 × 10–15 mm, surface dry, with an irregular basal depression, surface dark brown when fresh (Fig. 2a), with geometric, angular, or pyramidal warts 1 mm wide, with flattened, elevated, or rounded top. Gleba solid (Fig. 2b), marbled with white sterile veins separating brownish, fertile tissue, overall brownish when dried. Odor of corn starch-like or of raw potatoes.

Peridium of two layers. Outer peridium 125–570 µm thick, of textura angularis, with warts up to 300–500 (–800) µm high, outermost cells up to 42 µm broad, some ventricose or irregular, radial arrangement in some areas, walls 2–3 (–5) µm thick, reddish-brown to orange-brown in 5% KOH, innermost cells up to 10 µm broad, walls 1–2 µm thick, hyaline in 3% KOH. Inner peridium 120–500 (–700) µm thick, composed of hyaline, septate, interwoven hyphae (textura intricata), 5–12 µm broad, thin-walled 1–2 µm thick. ***Asci*** 8-spored, clavate, subclavate, subfusoid or irregular, 120–238 × 30–45 µm including pedicel, hyaline in 5% KOH, walls 1 µm thick, asci are scattered. ***Paraphyses*** not detected. ***Ascospores*** irregularly biseriate to uniseriate, hyaline in 5% KOH, globose, including ornamentation 18–22 µm broad, mean = 20 µm; excluding ornamentation 12–18 (–20) µm broad, mean = 15 µm. Ornamentation averaging 1.5 (–2.0) µm high, capitate columns, consisting of columns with a boarder, rounded tip.

Distribution and ecology. Known only from northeastern México (Tamaulipas, Nuevo Leon). Ascomata hypogeous always associated with *Quercus polymorpha*, and DNA (JN102443) of this species were recovered from sampled roots of oak (JN102443) from Chipinque National Park in Nuevo León. No DNA sequences of this species were found in soil in central or southern México.

Specimens examined. México, Tamaulipas, Ciudad Victoria, Torre de Microondas "Las Mulas", 23°37'00"N, 99°14'31"W, alt. 1549 m, under *Quercus polymorpha*, *Quercus* sp. and *Juglans* sp., hypogeous, solitary or in pairs, November 11, 2006, col. G. Guevara (ITCV 891; No ITS); Carretera Victoria, El Madrono, 23°36'3"N,

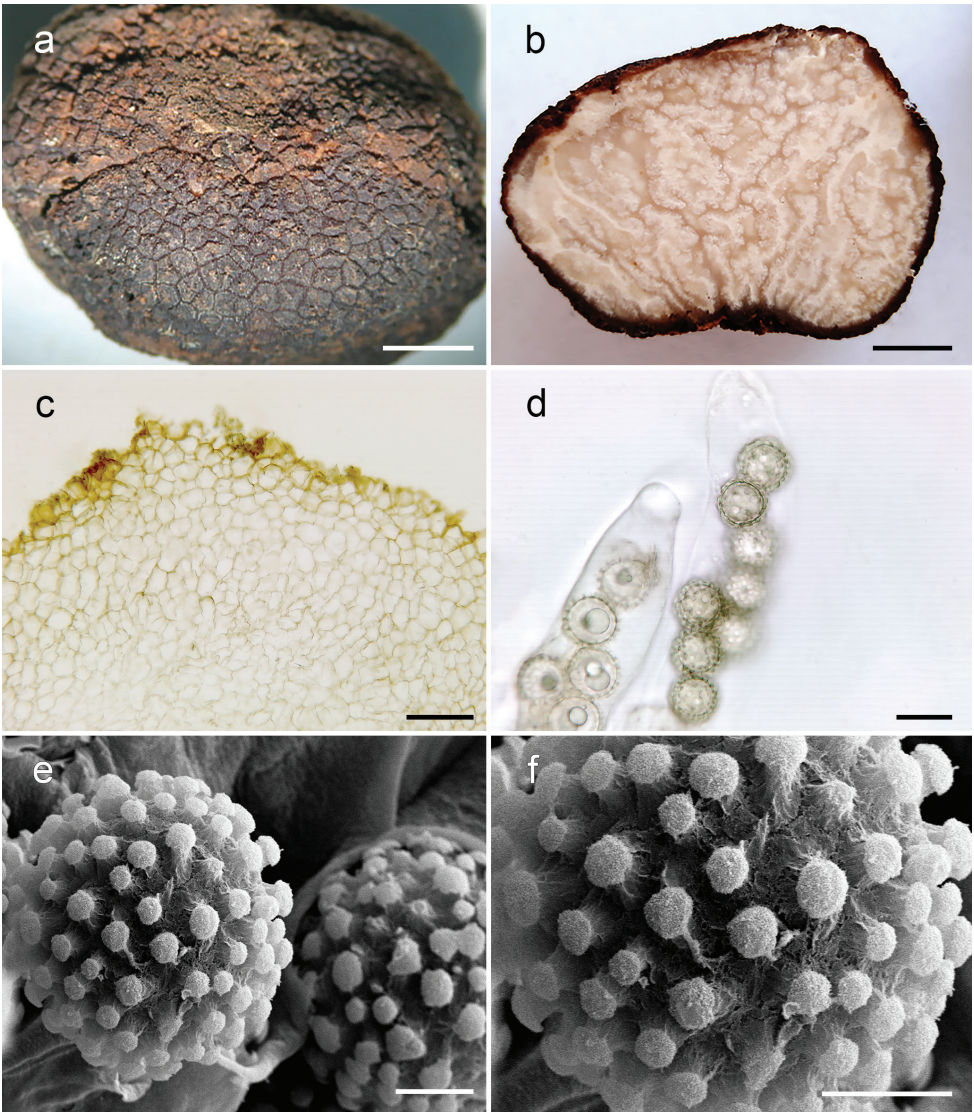

Figure 2. *Pachyphlodes brunnea* (Holotype: ITCV 896) **a** ascoma dried **b** gleba in cross-section **c** peridium in cross-section, showing a wart composed of isodiametric cells **d** light microscopy of asci and spores **e, f** SEM microscopy of spores in surface view. Scale bars: 3 mm (**a, b**), 20 µm (**c, d**), 5 µm (**e, f**).

99°13'8"W, alt. 1460 m, under *Quercus canbyi* Trel., *Q. polymorpha*, and *Q. laeta* Liebm., hypogeous, August 1, 2008, col. G. Bonito (JT32623; GenBank MT461399). Nuevo León, Municipio de Santiago, El Cercado September 14, 1983, col J. García (UNL 3757; GenBank EU427551).

Taxonomic comments. The ITS sequences of *Pachyphlodes brunnea* are similar to those of *P. marronina* (97.79% of identity and 12 nucleotide differences in ITS region), which is why it was originally described as *P. marronina.* However, the peridium color

and geographic location of these two species differ considerably. Spore ornamentation also separates them. The fresh peridium of *P. marronina* is red with indistinct warts, while that of *P. brunnea* is dark brown with distinct angular warts. The angular to pyramidal warts in the peridium of *P. brunnea* aretaller (300–800 μm) than the lower, indistinct warts on *P. marronina* (160–270 μm). The spines in *P. marronina* are taller (1.5–3.0 μm) than *P. brunnea* (1.5–2.0 μm), conferring a different aspect to the spores overall (Fig. 2e, f). *Pachyphlodes brunnea* superficially resembles *P. melanoxantha* (Tul. & C. Tul. ex Berk.) Doweld and *P. annagardnerae* R.A. Healy & M.E. Sm., but the latter two are black to the unaided eye, purple under transmitted light, have acute tipped spiny spores, and *P. melanoxantha* is said to have a nauseous odor (Berkeley 1844). In contrast, *P. brunnea* is dark brown to the unaided eye, yellowish-brown under transmitted light, and has a pure white gleba with capitate spore spines and a pleasant odor. *Pachyphlodes annagardnerae* has no perceptible odor.

Pachyphlodes coalescens **Piña Páez, R.A. Healy & Cázares, sp. nov.**
MycoBank No: 835666
GenBank KJ720784, KJ595000 (Holotype).
Fig. 3 a–e

Type. México, Michoacán, road Morelia-Atécuaro, Morelia, 19°36'0"N, 101°10'58.8"W, alt. 2280 m, under *Quercus deserticola* Trel., hypogeous, solitary, 30 September 2012, col. R. Garibay-Orijel (holotype: MEXU 26842).

Diagnosis. *Pachyphlodes coalescens* can be recognized by the brown ascomata and two-layered, thick (600–700 μm) peridium, and a gleba marbled with light yellow, meandering, sterile veins alternating with dark brown fertile veins, spores ornamented with truncated spines, that have material deposited at the tips, which accumulates and coalesces with neighboring tip material to form a broad, meandering, roughened, reticulum that hides the underlying spines, growing under *Quercus*.

Etymology. Named for the process that produces the spore ornamentation: material deposited on the spine tips coalesces to form a meandering reticulum, from Latin coalecere, to grow together.

Description. ***Ascomata*** irregularly subglobose, slightly compressed, 12 × 14 mm, surface with flat, polygonal warts with 4–6 sides, each wart about 2.5–3.0 mm broad, orange-brown when fresh (Fig. 3a), dark reddish-brown when dried, areole 6 × 4 mm where internal sterile veins emerge. Gleba light yellow with translucent yellowish sterile veins when fresh becoming cream with light brown veins when dried (Fig. 3b).

Peridium of two layers. Outer peridium 440–500 μm thick, composed of textura angularis, with warts up to 220 μm high, outermost cells up to 30 μm broad, walls 1 μm broad, orange-brown in 5% KOH, interior cells up to 22 μm broad with notably thinner cell walls <0.5 μm, hyaline (Fig. 3c). Inner peridium about 175–190 μm thick, composed of hyaline, septate, interwoven hyphae 4.5–6.5 μm broad, thin-walled <0.5 μm. ***Paraphyses*** filiform, septate, with swollen tips, 200–210 × 8.75 μm,

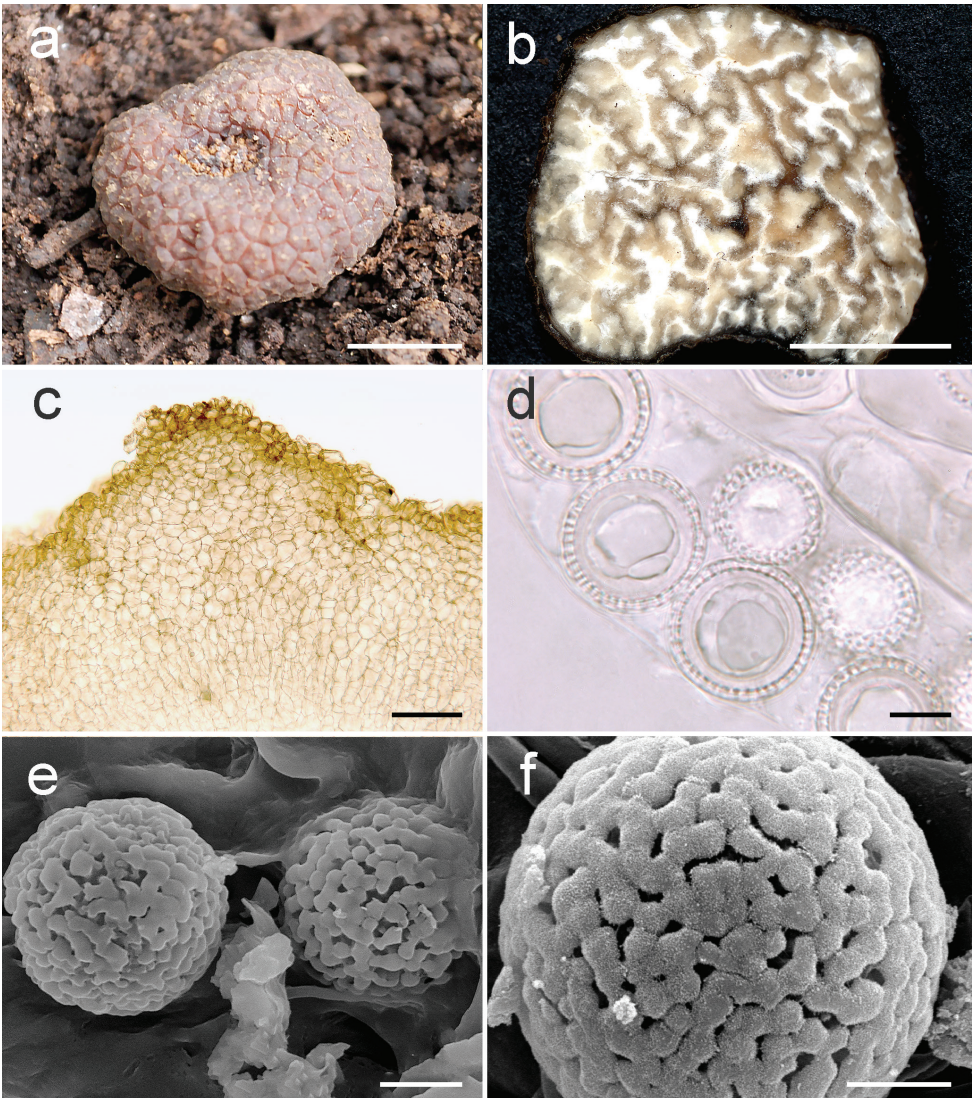

Figure 3. *Pachyphlodes coalescens* (Holotype: MEXU 26842) **a** ascoma fresh **b** gleba in cross-section **c** peridium in cross-section, showing a wart composed of isodiametric cells **d** light microscopy of asci and spores **e, f** SEM microscopy of spores in surface view. Scale bars: 5 mm (**a, b**), 100 µm (**c**), 10 µm (**d, e**), 5 µm (**f**).

10–14 µm broad at the apex, pale green with granular contents, thin-walled <0.5 µm. ***Asci*** 8-spored, irregularly distributed in fertile brown veins among interwoven hyphae, pyriform to cylindrical with a short pedicel, 180–195 µm long including pedicel, 40–50 µm wide, pedicel 22–26 × 10–12 µm, widening at the base, hyaline in 5% KOH, walls <0.5 µm (Fig. 3d). Spores irregularly biseriate to uniseriate. No reaction of asci in Melzer's reagent. ***Ascospores*** (Fig. 3e, f) globose, hyaline to light yellow, size range

including ornaments 20–23 µm, averaging 21.20 µm, spores excluding ornaments 16–18 µm, averaging 17.70 µm. Ornamentation averaging 1.80 µm high, of short capitate spines that accumulate material at the tips that coalesces to produce a nearly solid covering over the spore by maturity.

Distribution and ecology. Ascomata hypogeous, known from Michoacán and Tlaxcala co-occurring with *Quercus deserticola* Trel, *Quercus rugosa* Née, and *Q. crassifolia* Humb. & Bonpl. DNA sequences have also been found in *Quercus* dry forests or xerophilous pine-oak forests in Libres in Puebla, Tequila volcano in Jalisco, and Cerro del Águila in Michoacán, all in central-southwestern México.

Specimens examined. México, Tlaxcala, 1 km east of San Francisco Temezontla, Municipio Panotla, alt. 2600 m, under *Quercus rugosa* Née, and *Q. crassifolia* Humb. & Bonpl., September 20, 2007, col. E. Cázares (JT32454; GenBank EU543209).

Taxonomic comments. *Pachyphlodes coalescens* has a texture and peridial structure of the peridium similar to the other two species of the Marronina clade (*P. brunnea* and *P. marronina*) clade, but they vary in other macroscopic or microscopic characteristics. Ascomata of *Pachyphlodes brunnea* are dark brown to brownish black, whereas *P. coalescens* ascomata are orange-brown. In addition, they differ in spore size (*P. brunnea* 18–22 µm vs. *P. coalescens* 20–23 µm), and the spore ornamentation of *P. brunnea* is of discreet, capitate columns, whereas in *P. coalescens*, it is of spines with additional material that is so thickly deposited at the apices as to form a broad, meandering perispore that nearly covers the spore surface. *Pachyphlodes coalescens* are similar to *P. marronina*, but the latter has smaller spores (19–22 µm) ornamented with coarse, mostly discreet, truncate to capitate spines, whereas *P. coalescens* has short spines fully connected at the tips via the material deposited at the apex of each spine (see above). The spore ornamentation of *P. coalescens* is similar to that of *P. nemoralis* Hobart, Bóna & A. Paz and *P. pfisteri* Tocci, M.E. Sm. & Healy, which otherwise differ strongly in color, peridium structure, and phylogenetic placement.

Discussion

The *Pachyphlodes marronina* original description included collections from Iowa, U.S.A., Nuevo León and Tlaxcala, México. Cryptic diversity within this species was addressed by Healy et al. (2009) concerning molecular differences between the *P. marronina* collection from the U.S. and the Mexican collections. We now have additional molecular, geographical, and morphological evidence that the *P. marronina* complex includes three distinct species across North America: *P. brunnea*, *P. coalescens*, and *P. marronina*. Our evidence indicates that *P. brunnea* is associated with *Quercus* on the basis of molecular information from an ectomycorrhizal sequence (JN102443). However, no direct evidence exists that *Quercus* is the ectomycorrhizal host for the other two species. Their habitat descriptions suggest these two species associate with *Quercus*, but we need more environmental data to corroborate the association.

The three members of the Marronina clade (Fig. 1) have an ornamented peridium with flat warts, which are more conspicuous in *P. brunnea* and *P. coalescens.* The struc-

ture and composition of the peridium are also similar; all three have a two-layered peridium composed of an outermost layer of textura angularis and an inner layer of texture intricata. The biseriate to uniseriate arrangement of the spores in the asci is similar across the three species. *Pachyphlodes brunnea* resembles *P. marronina* in spore ornamentation; both species have spores with spines columns that are joined by the accumulation of material at the apex of the spines. However, the shorter spines in *P. brunnea* confer a clumpier appearance overall. The spore ornamentation in *P. coalescens* is different from the other two Marronina clade members but is simply the result of the coalescence of spine tip material, which occurs only occasionally in *P. marronina*. The late-stage spore ornamentation of the coalescence of spore tip material is also seen in *P. nemoralis* and *P. pfisteri* (Healy et al. 2015), but these species otherwise differ in color, peridium structure, and phylogenetic placement (Fig. 1).

The sister species of the Marronina clade is *P. oleifera* (Fig. 1), a European taxon with very distinct morphological characters, peridium with coarse warts, an unusual gray blueish gleba, and finely verrucose spores (Cabero and Pérez-Pérez 2012). Another characteristic of *P. oleifera* that separates it from the rest of the known species of *Pachyphlodes* is the oily content in all the microscopic structures, particularly hymenial cells.

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RESEARCH ARTICLE

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Two new brown rot polypores from tropical China

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Abstract

Brown-rot fungi are types of fungi that selectively degrade cellulose and hemicellulose from wood and are perhaps the most important agents involved in the degradation of wood products and dead wood in forest ecosystem. Two new brown-rot species, collected from southern China, are nested within the clades of *Fomitopsis* sensu stricto and *Oligoporus* sensu stricto, respectively. Their positions are strongly supported in the Maximum Likelihood phylogenetic tree of the concatenated the internal transcribed spacer (ITS) regions, the large subunit of nuclear ribosomal RNA gene (nLSU), the small subunit of nuclear ribosomal RNA gene (nuSSU), the small subunit of mitochondrial rRNA gene (mtSSU), the largest subunit of RNA polymerase II (RPB1), the second largest subunit of RNA polymerase II (RPB2) and the translation elongation factor 1-α gene (TEF1) sequences. *Fomitopsis bambusae*, only found on bamboo, is characterised by its resupinate to effused-reflexed or pileate basidiocarps, small pores (6–9 per mm), the absence of cystidia, short cylindrical to oblong-ellipsoid basidiospores measuring 4.2–6.1 × 2–2.3 µm. *Oligoporus podocarpi* is characterised by white to pale cream pore surface, round or sometimes angular pores (5–6 per mm), broadly ellipsoid to reniform basidiospores measuring 3.8–4.2 × 2–2.3 µm and growing on *Podocarpus*. Illustrated descriptions of these two novel species, *Fomitopsis bambusae* and *Oligoporus podocarpi*, are provided.

Keywords

Brown-rot fungi, multi-gene phylogeny, phylogeny, taxonomy

Introduction

Wood-inhabiting basidiomycota can be grouped into two categories, white-rot and brown-rot fungi, according to their ability for decaying or decomposing wood. Brown-rot fungi selectively degrade cellulose and hemicellulose from wood and decayed mate-

rial becomes reddish-brown or tan, crisp, causing massive cracks in the middle of a longitudinal crisscross. However, white-rot fungi can degrade all the components of wood and decayed material, become white or pale-yellow or light reddish-brown and expose the fibrous structure. The number of brown rot fungi is remarkably smaller compared to white rot fungi (Zhang 2003; Wu et al. 2020). Gilbertson (1981) has estimated that approximately 6% of the wood-rotting basidiomycetes in North America give a brown rot. On the other hand, Dai (2012) demonstrated that 14% of Chinese polypores in northern China can cause a brown rot (Cui et al. 2019). Brown-rot fungi are perhaps the most important agents involved in the degradation of wood products and in the degradation of dead wood in forest ecosystems. It is worth emphasising that the diversity of brown rot fungi is higher in high-latitude areas than in low-latitude areas and the number of brown rot fungi decreases from north to south in China (Zhou and Dai 2012; Dai et al. 2015), so that brown-rot fungi are infrequent in tropical areas.

As a cosmopolitan brown-rot genus of polypores, *Fomitopsis* P. Karst., was established by Karsten, based on *F. pinicola* (Sw.) P. Karst. (Karsten 1881). The genus was classified in the Fomitopsidaceae morphologically (Jülich 1981) and belonged to the *Antrodia* clade phylogenetically (Binder et al. 2005; Ortiz-Santana et al. 2013; Han et al. 2016). Han et al. (2016) confirmed that species, previously belonging to *Fomitopsis* sensu lato, were embedded in seven lineages and eleven species form the core group of *Fomitopsis*. In addition, four species *Fomitopsis caribensis* B.K. Cui & Shun Liu, *F. eucalypticola* B.K. Cui & Shun Liu, *F. ginkgonis* B.K. Cui & Shun Liu and *F. roseoalba* A.M.S. Soares, Ryvarden & Gibertoni were introduced as new species and *F. bondartsevae* (Spirin) A.M.S. Soares & Gibertoni was proposed as a new combination (Soares et al. 2017; Tibpromma et al. 2017; Liu et al. 2019). In the latest study, ten species have been recognised in the *Fomitopsis pinicola* complex (Haight et al. 2019; Liu et al. 2021). So far, 25 species have been accepted in *Fomitopsis* sensu stricto (s. str.).

Oligoporus Bref. (Polyporales, Basidiomycetes) was typified with *O. farinosus* Bref., 1888 (Syn. *O. rennyi* (Berk. & Broome) Kotl.) (Brefeld 1888). Recent phylogenetic analyses have demonstrated that *Oligoporus* and *Tyromyces* belong to different clades and that they were grouped within families Dacryobolaceae Jülich and Incrustoporiaceae Jülich (Binder et al. 2013; Floudas and Hibbett 2015; Justo et al. 2017). Shen et al. (2019) have proved *Oligoporus* s. str. is different from *Postia* s. str. in morphology and molecular phylogenetic analysis. Meanwhile, species in *Postia* s. str. have a broad host range growing both on angiosperm and gymnosperm wood, but *Oligoporus* s. str. grows only on gymnosperm wood (Donk 1971; Ryvarden and Melo 2014; Shen et al. 2019). So far, only two species have been accepted in *Oligoporus* s. str. (Shen et al. 2019).

During our investigations of brown-rot fungi in China, eight specimens were collected from Hainan Province in tropical China. Morphological examination shows these collections to represent two brown-rot polypores, corresponding to *Fomitopsis s.s.* and *Oligoporus s.s*. After phylogenetic analyses of the internal transcribed spacer (ITS) regions, the large subunit of nuclear ribosomal RNA gene (nLSU), the small subunit of nuclear ribosomal RNA gene (nuSSU), the small subunit of mitochondrial rRNA gene (mtSSU), the largest subunit of RNA polymerase II (RPB1), the second largest

subunit of RNA polymerase II (RPB2) and the translation elongation factor 1-α gene (TEF1) sequences, two new species were confirmed as belonging to *Fomitopsis* s.s. and *Oligoporus* s.s.. In this paper, we describe and illustrate these two new species.

Materials and methods

Morphological studies

The examined specimens were deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (**BJFC**) in Beijing, China. Macro-morphological descriptions were based on the field notes and measurements of herbarium specimens. Colour terms followed Petersen (1996). Micro-morphological data were obtained from the dried specimens and observed under a light microscope following Chen et al. (2017) and Shen et al. (2019). Sections were studied at a magnification up to 1000× using a Nikon Eclipse 80i microscope with phase contrast illumination (Nikon, Tokyo, Japan). Drawings were made with the aid of a drawing tube. Microscopic features, measurements and drawings were made from slide preparations stained with Cotton Blue and Melzer's Reagent. Spores were measured from sections cut from the tubes. To present the variation of spore size, 5% of measurements were excluded from each end of the range and are given in parentheses. The following abbreviations are used: IKI = Melzer's Reagent, IKI– = neither amyloid nor dextrinoid, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of basidiospores (a) measured from given number (b) of specimens.

DNA extraction and sequencing

A cetyltrimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd, Beijing, China) was used to extract the total genomic DNA from dried specimens according to the manufacturer's instructions with some modifications (Song and Cui 2017; Xing et al. 2018). The ITS regions were amplified with the primer pairs ITS5 (GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (TCC TCC GCT TAT TGA TAT GC) (White et al. 1990). The nLSU regions were amplified with the primer pairs LR0R (ACC CGC TGA ACT TAA GC) and LR7 (TAC TAC CAC CAA GAT CT) (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The nuSSU regions were amplified with the primer pairs NS1(CCG GAG AGG GAG CCT GAG AAA C) and NS4 (CCC GTG TTG AGT CAA ATT A) (White et al. 1990). The mtSSU regions were amplified with the primer pairs MS1 (CAG CAG TCA AGA ATA TTA GTC AAT G) and MS2 (GCG GAT TAT CGA ATT AAA TAA C) (White et al. 1990). RPB1 was amplified with the primer pairs RPB1-Af (GAR TGY CCD GGD CAY TTY GG) and RPB1-Cr (CCN GCD ATN TCR TTR TCC

ATR TA) (Matheny et al. 2002). RPB2 was amplified with the primer pairs fRPB2-5F (GAY GAY MGW GAT CAY TTY GG) and fRPB2-7CR (CCC ATR GCT TGY TTR CCC AT) (Matheny 2005). TEF1 was amplified with the primer pairs EF1-983F (GCY CCY GGH CAY CGT GAY TTY AT) and EF1-1567R (ACH GTR CCR ATA CCA CCR ATC TT) (Rehner and Buckley 2005). The PCR procedure followed that of Liu et al. (2019). The PCR products were purified with a Gel Extraction and PCR Purification Combo Kit (Spin-column) in Beijing Genomics Institute, Beijing, P.R. China. The purified products were then sequenced on an ABI-3730-XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the same primers as in the original PCR amplifications. The sequence quality was checked following Nilsson et al. (2012). All newly-generated sequences were submitted to GenBank and were listed in Tables 1 and 2.

Phylogenetic analyses

New sequences, deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Table 1), were aligned with additional sequences retrieved from GenBank (Table 1) using BioEdit 7.0.5.3 (Hall 1999) and ClustalX 1.83 (Thompson et al. 1997), followed by manual adjustment. Sequence alignment was deposited at TreeBase (http://purl.org/phylo/treebase/; submission ID 28131). In phylogenetic reconstruction, sequences of *Laetiporus zonatus* B.K. Cui & J. Song, obtained from GenBank, were used as outgroups in the phylogeny of *Fomitopsis* (Fig. 1) while sequences of *Antrodia serpens* (Fr.) P. Karst. were used as outgroups in the phylogeny of *Oligoporus* (Fig. 2).

Maximum Parsimony (MP) analysis was applied to those two phylogenies and trees construction procedure were performed in PAUP* version 4.0b10 (Swofford 2002). Settings for phylogenetic analyses in this study followed the approach of Zhu et al. (2019) and Song and Cui (2017). All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated.

Maximum Likelihood (ML) analysis was conducted with RAxML-HPC252 on Abe through the CIPRES Science Gateway (www.phylo.org) and involved 100 ML searches. All model parameters were estimated by the programme. Only the best Maximum Likelihood tree from all searches was kept. The Maximum Likelihood bootstrap values (ML-BS) were performed using a rapid bootstrapping with 1000 replicates. The phylogenetic tree was visualised using Treeview (Page 1996).

MrModeltest 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for two combined matrices to reconstruct phylogenetic analyses as a 6-gene dataset (ITS+nLSU+nuSSU+mtSSU+RPB2+TEF1) and a 7-gene dataset

Table 1. A list of species, specimens and GenBank accession numbers of sequences used in the phylogeny of *Fomitopsis*.

Species	Sample no.	GenBank accessions						References
		ITS	nLSU	nuSSU	mtSSU	tef1	rpb2	
Antrodia heteromorpha	Dai 12755	KP715306	KP715322	KR605908	KR606009	KP715336	KR610828	Chen and Cui (2015)
Antrodia serpens	Dai 14850	MG787582	MG787624	MG787731	MG787674	MG787849	MG787798	Chen et al. (2018)
Buglossoporus quercinus	JV 1406/1	KR605801	KR605740	KR605899	KR606002	KR610730	KR610820	Han et al. (2016)
Buglossoporus quercinus	LY BR 2030	KR605799	KR605738	KR605897	KR606000	KR610728	KR610818	Han et al. (2016)
Daedalea quercina	Dai 2260	KR605792	KR605731	KR605885	KR605988	KR610718	KR610808	Han et al. (2016)
Daedalea quercina	Dai 12659	KP171208	KP171230	KR605887	KR605990	KR610719	KR610810	Han et al. (2015)
Fomitopsis bambusae	**Dai 22110**	**MW937874**	**MW937881**	**MW937867**	**MW937888**	**MZ082980**	**MZ082974**	**Present study**
Fomitopsis bambusae	**Dai 22114**	**MW937875**	**MW937882**	**MW937868**	**MW937889**	**MZ082981**	**MZ082975**	**Present study**
Fomitopsis bambusae	**Dai 22116**	**MW937876**	**MW937883**	**MW937869**	**MW937890**	**—**	**—**	**Present study**
Fomitopsis bambusae	**Dai 21942**	**MW937873**	**MW937880**	**MW937866**	**MW937887**	**MZ082979**	**—**	**Present study**
Fomitopsis betulina	Cui 10756	KR605797	KR605736	KR605894	KR605997	KR610725	KR610815	Han et al. (2016)
Fomitopsis betulina	Dai 11449	KR605798	KR605737	KR605895	KR605998	KR610726	KR610816	Han et al. (2016)
Fomitopsis bondartsevae	X 1207	JQ700277	JQ700277	—	—	—	—	Soares et al. (2017)
Fomitopsis bondartsevae	X 1059	JQ700275	JQ700275	—	—	—	—	Soares et al. (2017)
Fomitopsis cana	Cui 6239	JX435777	JX435775	KR605826	KR605934	KR610661	KR610761	Li et al. (2013)
Fomitopsis cana	Dai 9611	JX435776	JX435774	KR605825	KR605933	KR610660	KR610762	Li et al. (2013)
Fomitopsis caribensis	Cui 16871	MK852559	MK860108	MK860124	MK860116	MK900482	MK900474	Liu et al. (2019)
Fomitopsis durescens	Overholts 4215	KF937293	KF937295	KR605835	KR605941	—	—	Han et al. (2014)
Fomitopsis durescens	O 10796	KF937292	KF937294	KR605834	KR605940	KR610669	KR610766	Han et al. (2014)
Fomitopsis eucalypticola	Cui 16594	MK852560	MK860110	MK860126	MK860118	MK900483	MK900476	Liu et al. (2019)
Fomitopsis eucalypticola	Cui 16598	MK852562	MK860113	MK860129	MK860121	MK900484	MK900479	Liu et al. (2019)
Fomitopsis ginkgonis	Cui 17170	MK852563	MK860114	MK860130	MK860122	MK900485	MK900480	Liu et al. (2019)
Fomitopsis ginkgonis	Cui 17171	MK852564	MK860115	MK860131	MK860123	MK900486	MK900481	Liu et al. (2019)
Fomitopsis hemitephra	O 10808	KR605770	KR605709	KR605841	KR605947	KR610675	—	Han et al. (2016)
Fomitopsis iberica	O 10810	KR605771	KR605710	KR605842	KR605948	KR610676	KR610771	Han et al. (2016)
Fomitopsis iberica	O 10811	KR605772	KR605711	KR605843	—	KR610677	KR610772	Han et al. (2016)
Fomitopsis meliae	Dai 10035	KR605774	KR605713	KR605847	KR605952	KR610683	—	Han et al. (2016)
Fomitopsis meliae	Ryvarden 16893	KR605776	KR605715	KR605849	KR605954	KR610681	KR610775	Han et al. (2016)
Fomitopsis mounceae	DR-366	KF169624	—	—	—	KF178349	KF169693	Haight et al. (2019)
Fomitopsis mounceae	JAG-08-19	KF169626	—	—	—	KF178351	KF169695	Haight et al. (2019)
Fomitopsis nivosa	JV 0509/52 X	KR605779	KR605718	KR605853	KR605957	KR610686	KR610777	Han et al. (2016)
Fomitopsis nivosa	Man 09	MF589766	MF590166	—	—	—	—	Liu et al. (2019)
Fomitopsis ochracea	ss5	KF169609	—	—	—	KF178334	KF169678	Haight et al. (2016)
Fomitopsis ochracea	ss7	KF169610	—	—	—	KF178335	KF169679	Haight et al. (2016)
Fomitopsis ostreiformis	IRET 22	KY449363	—	—	—	—	—	Thangamalai et al. (2018)
Fomitopsis ostreiformis	LDCMY 21	KY111252	—	—	—	—	—	Thangamalai et al. (2018)

Species	Sample no.	GenBank accessions						References
		ITS	nLSU	nuSSU	mtSSU	tef1	rpb2	
Fomitopsis palustris	Cui 7597	KP171213	KP171236	KR605854	KR605958	KR610687	KR610778	Han et al. (2015)
Fomitopsis palustris	Cui 7615	KR605780	KR605719	KR605855	KR605959	KR610688	KR610779	Han et al. (2015)
Fomitopsis pinicola	Cui 10532	KP171214	KP171237	KR605858	KR605962	KR610691	KR610782	Han et al. (2015)
Fomitopsis pinicola	Cui 10312	KR605781	KR605720	KR605856	KR605960	KR610689	KR610780	Han et al. (2016)
Fomitopsis roseoalba	AS 1496	KT189139	KT189141	—	—	—	—	Tibpromma et al. (2017)
Fomitopsis roseoalba	AS 1566	KT189140	KT189142	—	—	—	—	Tibpromma et al. (2017)
Fomitopsis schrenkii	JEH-144	KF169621	—	—	—	MK236355	MK208857	Haight et al. (2019)
Fomitopsis schrenkii	JEH-150	KF169622	—	—	—	MK236356	MK208858	Haight et al. (2019)
Fomitopsis subtropica	Cui 10154	JQ067652	JX435773	—	—	—	—	Li et al. (2013)
Fomitopsis subtropica	Cui 10578	KR605787	KR605726	KR605867	KR605971	KR610698	KR610791	Han et al. (2016)
Laetiporus zonatus	Dai 13633	KX354481	KX354508	KX354547	KX354589	KX354635	KX354676	Jie and Cui (2017)
Laetiporus zonatus	Cui 10404	KF951283	KF951308	KX354551	KX354593	KX354639	KT894797	Jie and Cui (2017)
Niveoporofomes spraguei	JV 0509/62	KR605786	KR605725	KR605864	KR605968	KR610697	KR610788	Han et al. (2016)
Niveoporofomes spraguei	4638	KR605784	KR605723	KR605862	KR605966	KR610696	KR610786	Han et al. (2016)
Rhodofomes rosea	Cui 10633	KR605782	KR605721	KR605860	KR605964	KR610693	KR610784	Han et al. (2016)
Rhodofomes rosea	JV 1110/9	KR605783	KR605722	KR605861	KR605965	KR610694	KR610785	Han et al. (2016)
Rhodofomitopsis feei	Ryvarden 37603	KC844850	KC844855	KR605838	KR605944	KR610670	KR610768	Han and Cui (2015)
Rhodofomitopsis feei	Oinonen 6011906	KC844851	KC844856	KR605837	KR605943	KR610671	KR610767	Han and Cui (2015)
Rubellofomes cystidiatus	Cui 5481	KF937288	KF937291	KR605832	KR605938	KR610667	KR610765	Han et al. (2014)
Rubellofomes cystidiatus	Yuan 6304	KR605769	KR605708	KR605833	KR605939	KR610668	—	Han et al. (2016)

(ITS+nLSU+nuSSU+mtSSU+RPB1+RPB2+TEF1) for Bayesian Inference (BI). Bayesian Inference was calculated with MrBayes 3.2.6 (Ronquist et al. 2012), with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites. Four Markov chains were run for two runs from random starting trees for one million generations and trees were sampled every 100 generations. The burn-in was set to discard 25% of the trees. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Posterior Probabilities (BPP) greater than or equal to 75% (MP and ML) and 0.95 (BPP) were considered as significantly supported.

Results

Molecular phylogeny

The phylogeny of *Fomitopsis*, based on a combined 6-gene (ITS, nLSU, nuSSU, mtSSU, RPB2, TEF1) dataset, included sequences from 64 fungal samples repre-

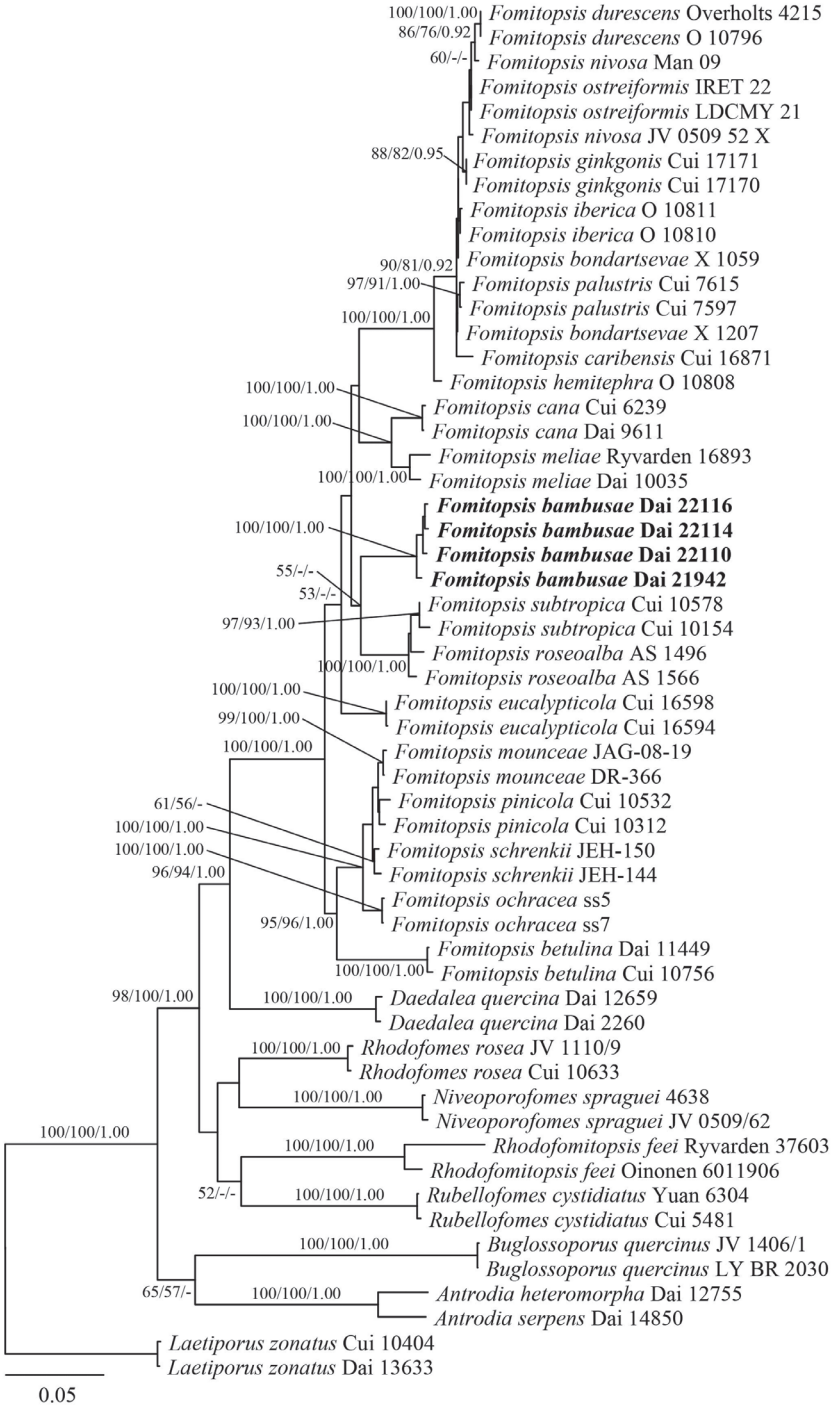

Figure 1. Maximum Likelihood phylogenetic tree of the new *Fomitopsis* species, based on multi-genes sequences data. Branches are labelled with bootstrap values (MP/ML) higher than 50% and posterior probabilities (BI) more than 0.90, respectively. Bold names: New species.

Table 2. A list of species, specimens and GenBank accession numbers of sequences used in the phylogeny of *Oligoporus*.

Species	Sample no.	GenBank accessions							References
		ITS	nLSU	nuSSU	mtSSU	TEF1	RPB2	RPB1	
Amaropostia stiptica	Cui 10043	KX900906	KX900976	KX901119	KX901046		KX901219	KX901167	Shen et al. (2019)
Amaropostia stiptica	Cui 10981	KX900907	KX900977	KX901120	KX901047		KX901220	KX901168	Shen et al. (2019)
Amylocystis lapponica	HHB-13400	KC585237	KC585059						Ortiz-Santana et al. (2013)
Amylocystis lapponica	OKM-4118	KC585238	KC585060						Ortiz-Santana et al. (2013)
Antrodia serpens	Dai 7465	KR605813	KR605752	KR605913	KR606013	KR610742	KR610832		Han et al. (2016)
Antrodia serpens	Dai 14850	MG787582	MG787624	MG787731	MG787674	MG787849	MG787798		Chen et al. (2018)
Calcipostia guttulata	Cui 10028	KF727433	KJ684979	KX901139	KX901066	KX901277	KX901237	KX901182	Shen et al. (2019)
Calcipostia guttulata	KHL 11739(GB)	EU118650	EU118650						Larsson direct submission
Cyanosporus caesius	Dai 12605	KX900883	KX900953	KX901096	KX901021		KX901206	KX901159	Shen et al. (2019)
Cyanosporus caesius	Dai 12974	KX900884	KX900954	KX901097	KX901022	KX901258	KX901207	KX901160	Shen et al. (2019)
Cyanosporus subcaesius	KA12-1375	KR673585							Kim et al. (2015)
Cyanosporus subcaesius	K(M)32713	AY599576							Yao et al. (2005)
Cystidiopostia hibernica	Cui 2658	KX900905	KX900975	KX901118	KX901045		KX901218		Shen et al. (2019)
Cystidiopostia hibernica	K(M)17352	AJ006665							Yao et al. (2005)
Cystidiopostia pileata	Cui 5721	KF699127	KX900960	KX901121	KX901049	KX901268	KX901221	KX901169	Shen et al. (2019)
Cystidiopostia pileata	Cui 10034	KX900908	KX900956	KX901122	KX901050	KX901269	KX901222	KX901170	Shen et al. (2019)
Fuscopostia duplicata	Cui 10366	KF699124	KJ684975	KR605927	KR606026	KR610755	KR610844	KX901173	Han et al. (2016)
Fuscopostia duplicata	Dai 13411	KF699125	KJ684976	KR605928	KR606027	KR610756	KR610845	KX901174	Han et al. (2016)
Fuscopostia fragilis	Cui 10020	KX900912	KX900982	KX901126	KX901054	KX901270	KX901226		Shen et al. (2019)
Fuscopostia fragilis	Cui 10088	KF699120	KJ684977	KX901127	KT893749		KT893745		Han et al. (2016)
Oligoporus podocarpi	**Dai22042**	**MW937877**	**MW937884**	**MW937870**	**MW937891**	**MZ082982**	**MZ082976**	**MZ005579**	**Present study**
Oligoporus podocarpi	**Dai22043**	**MW937878**	**MW937885**	**MW937871**	**MW937892**	**MZ082983**	**MZ082977**	**MZ005580**	**Present study**
Oligoporus podocarpi	**Dai22044**	**MW937879**	**MW937886**	**MW937872**	**MW937893**	**MZ082984**	**MZ082978**	**MZ005581**	**Present study**
Oligoporus rennyi	KEW 57	AY218416	AF287876						Ortiz-Santana et al. (2013)
Oligoporus rennyi	MR 10497	JX090117							Ortiz-Santana et al. (2013)
Oligoporus sericeomollis	Cui 9560	KX900919	KX900989	KX901140	KX901067			KX901183	Shen et al.(2019)
Oligoporus sericeomollis	Cui 9870	KX900920	KX900990	KX901141	KX901068			KX901184	Shen et al. (2019)

Species	Sample no.	GenBank accessions							References
		ITS	nLSU	nuSSU	mtSSU	TEF1	RPB2	RPB1	
Osteina obducta	Cui 9959	KX900923	KX900993	KX901143	KX901070		KX901239		Shen et al. (2019)
Osteina obducta	Cui 10074	KX900924	KX900994	KX901144	KX901071		KX901240		Shen et al. (2019)
Osteina undosa	Dai 7105	KX900921	KX900991	KX901142	KX901069		KX901238		Shen et al. (2019)
Osteina undosa	L-10830	KC585396	KC585229						Ortiz-Santana et al. (2013)
Postia hirsuta	Cui 11180	KJ684971	KJ684985		KX901039				Shen and Cui (2014)
Postia hirsuta	Cui 11237	kj684970	KJ684984	KX901113	KX901038	KX901266			Shen and Cui (2014)
Postia lactea	Cui 9319	KX900894	KX900964	KX901106	KX901031	KX901262	KX901213	KX901165	Shen et al. (2019)
Postia lactea	Cui 11511	KX900893	KX900963	KX901105	KX901030	KX901261	KX901212	KX901164	Shen et al. (2019)
Postia lowei	Cui 9585	KX900898	KX900968	KX901110	KX901035				Shen et al. (2019)
Postia lowei	X1373	KC595941							Ortiz-Santana et al. (2013)
Postia ochraceoalba	Cui 10802	KM107903	KM107908	KX901115	KX901041		KX901216		Shen et al. (2015)
Postia ochraceoalba	Cui 10825	KM107902	KM107907	KX901114	KX901040		KX901215		Shen et al. (2015)
Spongious gloeoporus	Cui 9507	KM107901	KM107906	KX901132	KX901059		KX901231		Shen et al. (2015)
Spongious gloeoporus	Cui 10401	KX900915	KX900985	KX901133	KX901060		KX901232		Shen et al. (2015)
Spongiporus floriformis	Cui 10292	KM107899	KM107904	KX901131	KX901058	KX901274	KX901230	KX901178	Shen et al. (2015)
Spongiporus floriformis	Dai 13887	KX900914	KX900984	KX901130	KX901057	KX901273	KX901229	KX901177	Shen et al. (2019)

senting 29 taxa. They were downloaded from GenBank and generated in the present study (Table 1). The dataset had an aligned length of 4718 characters, including gaps (680 characters for ITS, 1343 characters for nLSU, 1013 characters for nuSSU, 547 characters for mtSSU, 648 characters for RPB2, 487 characters for TEF1), of which 3346 characters were constant, 1860 were variable and parsimony-uninformative, and 1212 were parsimony-informative. Maximum parsimony analysis yielded one equally-parsimonious tree (TL = 3802, CI = 0.544, RI = 0.787, RC = 0.428, HI = 0.456) and the MP tree is shown in Fig. 1. The best model for the combined ITS+nLSU+nuSSU+mtSSU+RPB2+TEF1 sequence dataset was estimated and applied in the Bayesian analysis was GTR+I+G with equal frequency of nucleotides, lset nst = 6 rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis resulted in a concordant topology with an average standard deviation of split frequencies = 0.008975.

The phylogeny of *Oligoporus*, combined 7-gene (ITS, nLSU, nuSSU, mtSSU, RPB1, RPB2, TEF1) dataset, included sequences from 43 fungal samples representing 21 taxa. They were downloaded from GenBank and generated in the present study (Table 2). The dataset had an aligned length of 5772 characters,

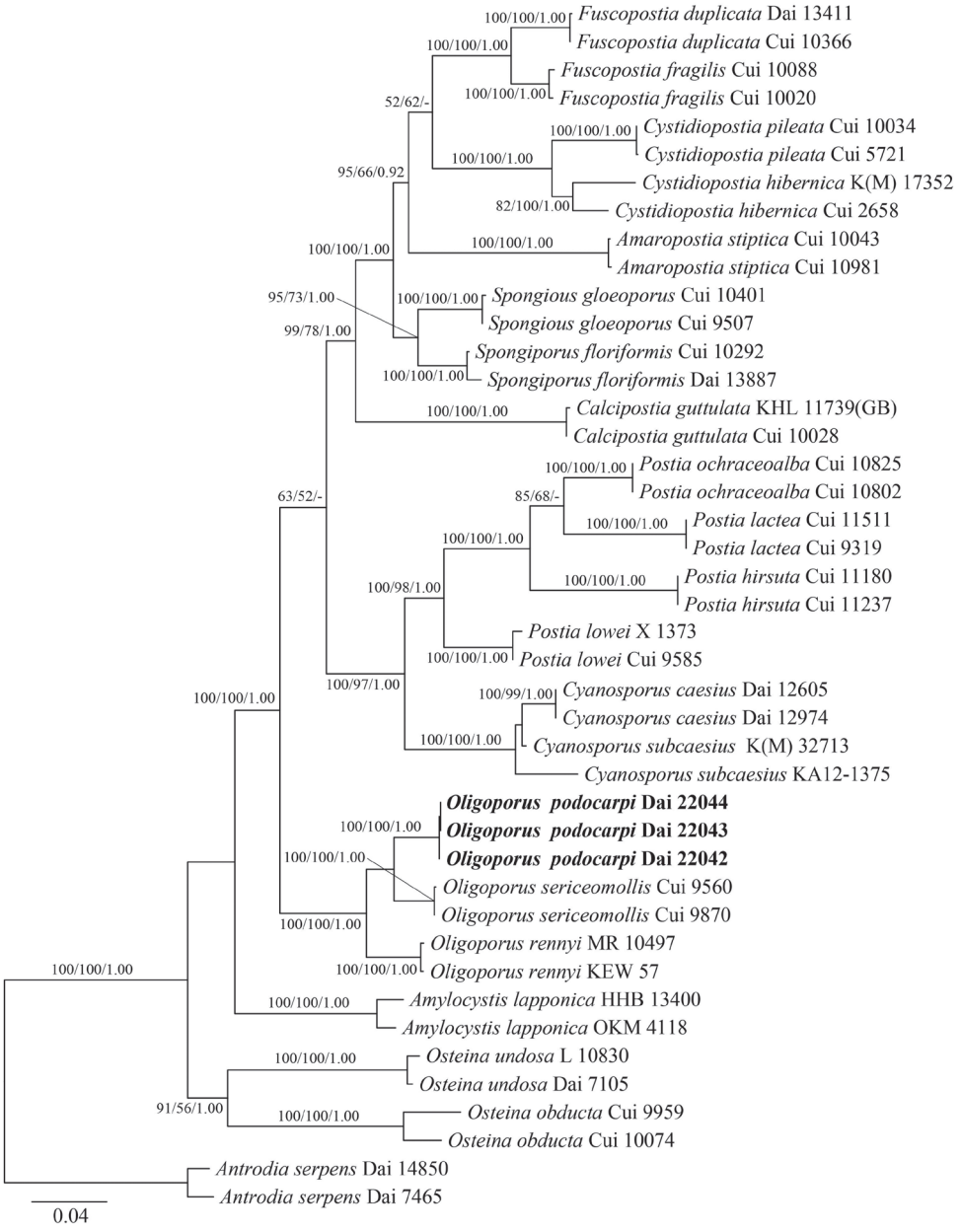

Figure 2. Maximum Likelihood phylogenetic tree of the new *Oligoporus* species, based on multi-genes sequences data. Branches are labelled with bootstrap values (MP/ML) higher than 50% and posterior probabilities (BI) more than 0.90, respectively. Bold names: New species.

including gaps (612 characters for ITS, 1302 characters for nLSU, 1009 characters for nuSSU, 491 characters for mtSSU, 1231 characters for RPB1, 648 characters for RPB2, 479 characters for TEF1), of which 4127 characters were constant, 129

Table 3. A comparison of species in the *Fomitopsis*.

Species	Holotype	Basidiocarps	Pileal surface	Pore surface	Pore (per mm.)	Hyphal system	Cystidia/ cystidioles	Basidiospores	References
F. abieticola	China	Annual to perennial; pileate	Cream to pinkish buff	Cream to pinkish buff when fresh, becoming buff to curry-yellow when dry	Round to angular, 2–4	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 17.5–50.2 × 4.3–9.5 µm	Oblong-ellipsoid to ellipsoid, 7–9 × 4–5 µm.	Liu et al. (2021)
F. bambusae	**China**	**Annual, resupinate to effused-reflexed or pileate**	**Pluish grey when fresh, pale mouse-grey to greyish-sepia when dry**	**Bluish-grey to pale mouse-grey when fresh, becoming mouse-grey to dark grey when dry**	**Round to angular, 6–9**	**Dimitic**	**Cystidia absent; fusoid cystidioles present, 11–18 × 2.5–4 µm**	**Cylindrical to oblong ellipsoid, 4.2–6.1 × 2–2.3 µm**	**Present study**
F. betulina	Norway	Annual; pileate	Whitish to mouse-coloured or brownish	White to pale brownish	Round to angular, 3–5	Di-trimitic	Absent	Cylindrical, slightly allantoid, 5–6 × 1.5–1.7 µm.	Ryvarden and Melo (2014)
F. bondartsevae	Russia	Annual; effused-reflexed to pileate			Round to angular, 2–3	Trimitic	Cystidia absent; fusoid cystidioles present, 18–26 × 4.5–6 µm	Cylindrical, 6–7.2 × 2.2–2.5 µm.	Spirin (2002)
F. cana	China	Annual; resupinate to effused-reflexed or pileate	Pale mouse-grey to dark grey, azonate	Cream to straw coloured turning mouse-grey to dark grey	Angular, 5–8	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 9–16 × 3–5 µm	Cylindrical to oblong ellipsoid,5–6.2× 2.1–3 µm.	Li et al. (2013)
F. caribensis	Puerto Rico.	Annual; pileate, sessile	White to cream buff when fresh, cream buff to curry-yellow at base	White to cream when fresh, becoming cream to pinkish-buff when dry	Round to angular, 6–9	Dimitic	Cystidia absent; fusoid cystidioles occasional, hyaline, thin-walled, 12.5–23.5 × 2.5–4 µm	Cylindrical to oblong-ellipsoid, 6–7.5 × 2.3–3.1 µm.	Liu et al. (2019)
F. durescens	USA	Annual; sessile	Cream coloured to pale buff, drying tan	White to cream coloured, ochraceous on drying	Round to angular, 4–5	Trimitic	Cystidia absent; fusoid cystidioles present, 14–16 × 5–6 µm	Narrowly cylindrical, 6–8 × 1.5–2.5 µm	Gilbertson and Ryvarden (1986)
F. eucalypticola	Australia	Annual to biennial; effused-reflexed to pileate	Cream to salmon-coloured when young, straw yellow to clay-pink	Cream to yellow when fresh, buff to clay-buff when dry	Round to angular, 3–5	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 15–36 × 2–5.3 µm	Cylindrical to oblong-ellipsoid, 5.8–9.1 × 2.7–5 µm.	Liu et al. (2019)
F. ginkgonis	China	Annual; pileate, imbricate	Dirty greyish-brown to mouse-grey	Pinkish-buff to cinnamon-buff	Round to angular, 3–6	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 12.5–27.6 × 2.8–4.1 µm	Cylindrical, 7.2–9 × 2.2–3 µm.	Liu et al. (2019)
F. hemitephra	New Zealand	Perennial; solitary, attached by a broad lateral base	Tobacco brown or fuscous.	White or straw to isabelline	Round or slightly angular, 6–7	Trimitic	Cystidia absent; cystidioles, 6–8 × 3.5–4 µm	Elliptic-oblong, 4–6 × 2–2.5 µm.	Cunningham (1965)

Species	Holotype	Basidiocarps	Pileal surface	Pore surface	Pore (per mm.)	Hyphal system	Cystidia/ cystidioles	Basidiospores	References
F. hengduanensis	China	Annual to perennial; pileate	Pale dark grey to reddish-brown at base and cream to flesh-pink towards the margin	white to cream when fresh, becoming buff to straw-yellow	Round to angular, 6–8	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 13.2–36.5 × 2.5–5.4 µm	Oblong-ellipsoid to ellipsoid, 5.2–6 × 3.2–3.6 µm.	Liu et al. (2021)
F. iberica	Portugal	Annual; sessile, dimidiate, single or imbricate	White to cream when young. drying honey-coloured to brown	Pale, white, cream to straw-coloured	Round to ellipsoid, 3–4 per mm	Trimitic	Cystidia absent; pointed cystidioles present, 20–27 × 4–5–5 µm	Cylindrical to distinctly fusoid, 6–8 × 2.8–3.7 µm.	Melo and Ryvarden (1989)
F. kesiyae	Vietnam	Annual; pileate	Buff yellow to orange-yellow buff	White to cream when fresh, olivaceous buff to cinnamon-buff when dry	Round to angular, 6–8	Dimitic	Cystidia absent; fusoid cystidioles occasionally present, 11.5–30.4 × 2.6–6 µm	Oblong-ellipsoid to ellipsoid, 4.8–5.3 × 3–3.5 µm.	Liu et al. (2021)
F. massoniana	China	Annual; effused-reflexed to pileate	Buff-yellow to apricot-orange	White to cream when fresh, cream to buff	Round, 5–7	Dimitic	Cystidia absent; fusoid cystidioles occasionally present, 14.8–36 × 3.8–6 µm	Oblong-ellipsoid, 6.2–7.3 × 3.3–4 µm.	Liu et al. (2021)
F. meliae	USA	Annual or biennial; sessile, pilei single to imbricate, dimidiate	Ivory to tan or cinereous	Ochraceous	Round to angular, 5–7	Trimitic	Cystidia absent; fusoid cystidioles present, 15–23 × 4–5 µm	Cylindrical, slightly fusiform, tapering to the apex, 6–8 × 2.5–3 µm.	Gilbertson (1981)
F. mounceae	Canada	Perennial; pileate	Brownish-orange to black at base and pale orange to greyish-orange towards the margin	Yellowish-white, greyish-yellow, pale orange to light ochraceous buff, bright reddish-brown when dry	Round, 3–5	Dimitic	Cystidia obclavate to subfusiform with subacute or rounded apices, 16–35 × 3–6.5 µm	Ellipsoid to cylindrical, 5.8–6.6 × 3.4–4 µm.	Haight et al. (2019)
F. nivosa	Brazil	Annual to biennial; sessile, dimidiate, single to imbricate	Cream to pale sordid brown or tan	Cream to pale sordid brown or tan	Round to angular, 6–8	Trimitic	Cystidia absent; cystidioles broadly rounded, subapically contracted, 12–15 × 4–5 µm	Cylindrical, 6–9 – 2–3 µm	Gilbertson and Ryvarden (1986)
F. ochracea	Canada	Perennial; pileate	Brownish-grey to greyish-brown at base and orange white to pale orange towards the margin	Pale yellow, pale orange, light ochraceous buff, reddish-brown when dry	Round, 4–5	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 20–40 × 4–6.5 µm	Broadly ellipsoid, 5.1–5.9 × 3.6–4 µm.	Stokland and Ryvarden (2008); Haight et al. (2019)
F. ostreiformis	Singapore	Annual; sessile or effuse-reflexed	Greyish pileal surface	White or greyish-white	Round to angular, 3–4	Trimitic	Cystidia absent; cystidioles present, 10–17 × 2.8–4 µm	Cylindrical, 4.2–5.6 × 1.4–2.6 pm	De (1981); Hattori (2003)

Species	Holotype	Basidiocarps	Pileal surface	Pore surface	Pore (per mm.)	Hyphal system	Cystidia/ cystidioles	Basidiospores	References
F. palustris	USA	Perennial; sessile, horizontal, applanate	Dingy ochraceous to ochraceous buff, suffused dingy brownish-vinaceous	Vinaceous drab to brownish-vinaceous but pallid ochraceous near the margin	Angular, 7–9	Dimitic	absent	Cylindrical, 3.7–4.7 × 2–2.5 µm.	Corner (1989); Hattori (2003)
F. pinicola	Europe	Perennial; pileate	Brownish-orange to black at base and buff-yellow to cinnamon towards the margin	Cream coloured becoming citric yellow when bruised	Round, 4–6	Trimitic	Cystidia present, 18–90 × 3–9 µm	Cylindrical-ellipsoid, 6–9 × 3–4.5 µm.	Ryvarden and Melo (2014); Haight et al. (2019)
F. roseoalba	Brazil	Annual; pileate, resupinate to effused-reflexed	White to pink when fresh, cream to greyish when dry	White to cream when fresh and ochraceous when dried	Round to angular, 4–6	Trimitic	absent	Ellipsoid to sub-cylindrical, 3–4.9 × 1.8–2 µm.	Tibpromma et al. (2017)
F. schrenkii	USA	Perennial; effused-reflexed to pileate	Greyish-orange to olive brown at base and greyish-orange to greyish-yellow towards the margin	Pale yellow, pale orange, cream buff, reddish-brown when dry	Round, 3–4	Dimitic	Cystidia cylindrical, subulate, or subfusiform with subacute, 16–30 × 3–8 µm	Ellipsoid to broadly cylindrical, 5.7–6.7 × 3.7–4.2 µm.	Haight et al. (2019)
F. subpinicola	China	Annual; pileate	Apricot-orange, scarlet to fuscous	White to cream when fresh, turning buff yellow to buff when dry	Round, 6–8	Dimitic	Cystidia absent; fusoid cystidioles occasionally present, 14.5–34.6 × 3.2–7.2 µm	Oblong-ellipsoid to ellipsoid, 4.3–5.5 × 2.7–3.3 µm.	Liu et al. (2021)
F. subtropica	China	Annual; resupinate to effused-reflexed or pileate	Straw-yellow when young, becoming pale mouse-grey to flesh-pink with age.	Cream to straw coloured or pale pinkish	Angular, 6–9	Trimitic	Cystidia absent; fusoid cystidioles occasionally present, 9–15 × 3–4 µm	Cylindrical to oblong-ellipsoid, 3.2–4 × 1.8–2.1 µm.	Li and Cui (2013)
F. tianshanensis	China	Annual to perennial; effused-reflexed to pileate	Dark bluish-grey to yellowish-brown	Cream to pinkish-buff when fresh, becoming faint yellow to light pink when dry	Round to angular, 1–3	Dimitic	Cystidia absent; fusoid cystidioles occasionally present, 15.5–44 × 3.3–6.5 µm	Oblong-ellipsoid, 6.3–7 × 3.2–3.8 µm.	Liu et al. (2021)

were variable and parsimony-uninformative and 1516 were parsimony informative. Maximum parsimony analysis yielded four equally-parsimonious trees (TL = 3925, CI = 0.600, RI = 0.784, RC = 0.471, HI = 0.400) and a strict consensus tree of these trees is shown in Fig. 2. The best model for the combined ITS+nLSU +nuSSU+mtSSU+RPB1+RPB2+TEF1 sequence dataset was estimated and applied in the Bayesian analysis was GTR+I+G with equal frequency of nucleotides, lset nst = 6 rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). Bayesian analysis resulted in a concordant topology with an average standard deviation of split frequencies = 0.008567.

In our phylogenies (Figs 1 and 2), five samples on bamboo formed an independent lineage in the *Fomitopsis* s.s. clade with strong support (100% ML, 100% MP, 1.00 BPPs) and are distant from other taxa in the genus. Both morphology and rDNA sequence data confirmed that the five samples represent a new species in *Fomitopsis*. Meanwhile, three samples on *Podocarpus* were nested in the *Oligoporus* s.s. clade and formed an independent lineage with a robust support (100% ML, 100% MP, 1.00 BPPs). Both morphology and rDNA sequence data confirmed that the three samples represent a new species in *Oligoporus*.

Taxonomy

Fomitopsis bambusae **Y.C. Dai, Meng Zhou & Yuan Yuan, sp. nov.**
MycoBank No: 839359
Figs 3, 4

Diagnosis. *Fomitopsis bambusae* is characterised by resupinate to effused-reflexed or pileate, soft corky basidiocarps with bluish-grey pores, small pores measuring 6–9 per mm, cylindrical to oblong ellipsoid basidiospores measuring 4.2–6.1 × 2–2.3 μm and growing on dead bamboo.

Type. China. Hainan, Haikou, Jinniuling Park, on dead bamboo, 18.XI.2020, Yu-Cheng Dai leg., *Dai 22116* (holotype BJFC036008).

Etymology. *Bambusae* (Lat.): refers to the species growing on bamboo.

Fruiting body. Basidiocarps annual, resupinate to effused-reflexed or pileate, separable from the substrate, without odour or taste and soft corky when fresh, corky and light in weight when dry. Pilei semicircular, projecting up to 1 cm, 1.5 cm wide and 5 mm thick at base; resupinate part up to 14 cm long, 6 cm wide and 2 mm thick at centre. Pileal surface bluish-grey when fresh, pale mouse-grey to greyish-sepia when dry, glabrous to slightly velutinate, rough, azonate; margin acute, incurved when dry. Pore surface bluish-grey to pale mouse-grey when fresh, becoming mouse-grey to dark grey when dry; sterile margin up to 1 mm wide; pores round to angular, 6–9 per mm; dissepiments thin, entire. Context white to cream, corky, up to 3.5 mm thick. Tubes paler than pore surface, corky, up to 1.5 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae IKI–, CB–; tissue unchanged in KOH.

Context. Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, 1.5–3 μm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a narrow lumen to subsolid, occasionally branched, interwoven, 2–4.5 μm in diam.

Tubes. Generative hyphae hyaline, thin- to slightly thick-walled, rarely branched, 1.5–2.5 μm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a narrow lumen to subsolid, occasionally branched, flexuous, interwoven, 2–3 μm in diam. Cystidia absent; fusoid cystidioles present, hyaline, thin-walled, 11–18 × 2.5–4 μm. Basidia short clavate to barrel-shaped, bearing four sterigmata and a basal clamp connection, 13–19 × 4.5–5.5 μm; basidioles dominant, in shape similar to basidia, but smaller.

Figure 3. Basidiocarps of *Fomitopsis bambusae* (holotype Dai 22116). Scale bar: 1.0 cm.

Spores. Basidiospores cylindrical to oblong ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (4–)4.2–6.1(–6.5) × (1.9–)2–2.3(–2.6) µm, L = 4.917 µm, W = 2.109 µm, Q = 2.26–2.41 (n = 90/3).

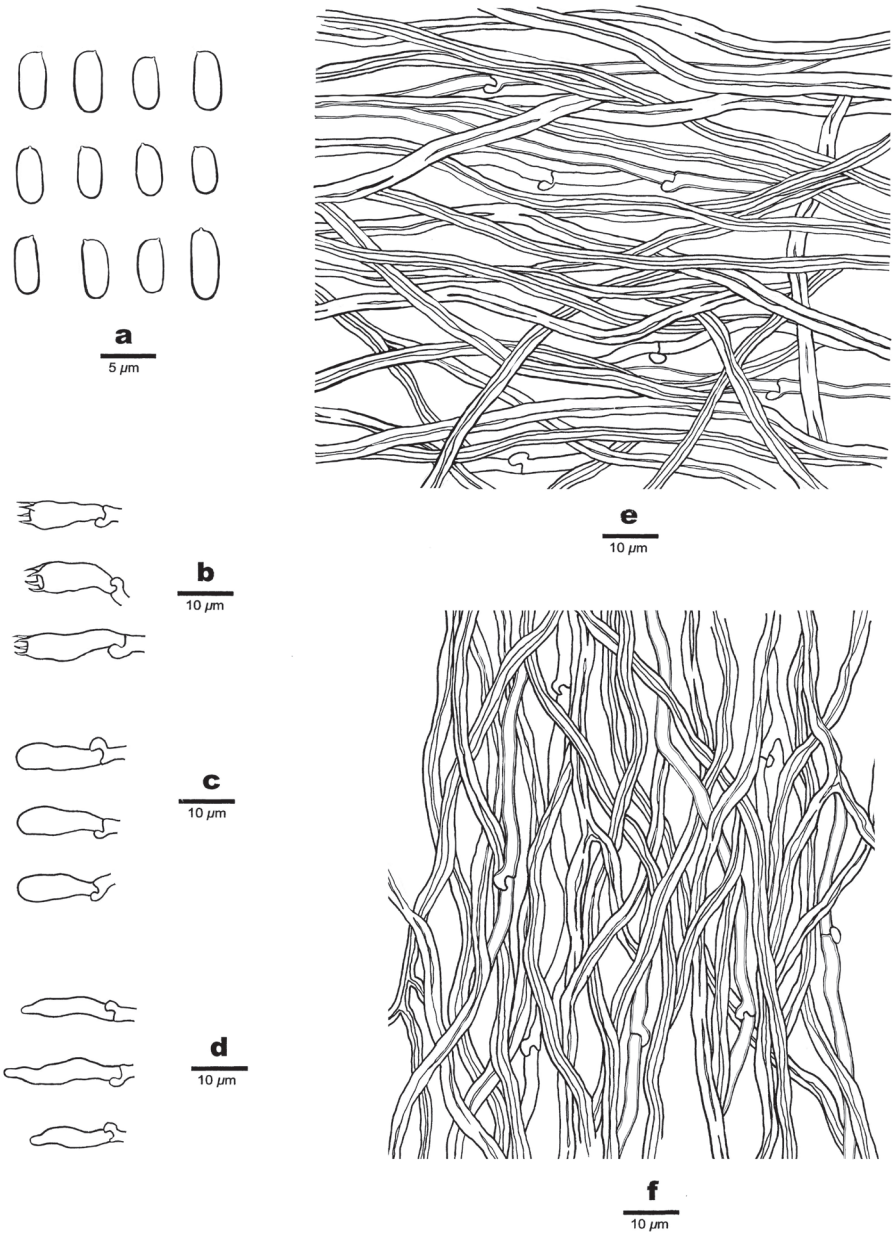

Figure 4. Microscopic structures of *Fomitopsis bambusae* (drawn from the holotype) **a** basidiospores **b** basidia **c** basidioles **d** cystidioles **e** hyphae from context **f** hyphae from trama.

Type of rot. Brown rot.

Additional specimens (paratypes) examined. China. Hainan, Haikou, Jinniuling Park, on dead bamboo, 7.XI.2020, Yu-Cheng Dai leg., *Dai 21942* (BJFC035841), 18.XI.2020, *Dai 22104* (BJFC035996), *Dai 22110* (BJFC036002) and *Dai 22114* (BJFC036006).

Table 4. A comparison of species in the *Oligoporus*.

Species	Basidiocarps	Pore (per mm)	Pore surface	Cystidia	Cystidioles	Basidiospores size (μm)	Basidiospores shape	Reference
Oligoporus podocarpi	**Resupinate**	**Round to angular, 5–6**	**White to pale cream**	**Thick-walled with apically encrusted**	**Absent**	**3.8–4.2 × 2–2.5**	**Allantoid to oblong ellipsoid**	**Present study**
O. rennyi	Resupinate	Angular, 2–4	White or cream, then pale brown	Absent	Absent	4.8–6 × 2.5–3.5	Oblong ellipsoid	Ryvarden and Melo (2014); Shen et al. (2019)
O. sericeomollis	Resupinate	Round and angular, 4–6	White or discoloured yellowish or tan	Thick-walled with apically encrusted	Present, thin-walled	4–5 × 2–2.5	Oblong cylindrical to ellipsoid	Ryvarden and Melo (2014); Shen et al. (2019)

Oligoporus podocarpi **Y.C. Dai, Chao G. Wang & Yuan Yuan, sp. nov.**
MycoBank No: 839360
Figs 5, 6

Diagnosis. *Oligoporus podocarpi* is characterised by soft fresh basidiocarps, becoming rigid upon drying, a monomitic hyphal system with hyaline clamped generative hyphae, the presence of apically encrusted cystidia, broadly ellipsoid to reniform, dextrinoid, cyanophilous basidiospores measuring 3.8–4.2 × 2–2.3 μm, and growing on rotten wood of *Podocarpus*.

Type. China. Hainan, Changjiang, Hainan Tropical Rainforest National Park, Bawangling, rotten wood of *Podocarpus imbricatus*, 10.XI.2020, Yu-Cheng Dai leg., *Dai 22042* (holotype BJFC035938).

Etymology. *Podocarpi* (Lat.): referring to the species growing on wood of *Podocarpus imbricatus*.

Fruiting body. Basidiocarps annual, resupinate, adnate, soft corky, with mushroom odour when fresh, becoming rigid when dry, mild taste, up to 3 cm long, 2 cm wide and 2.3 mm thick at the centre. Pore surface snow white when fresh, becoming cream to buff upon drying, somewhat glancing; sterile margin indistinct, thinning out, up to 0.3 mm wide; pores round to angular, 5–6 per mm; dissepiments thin, entire. Subiculum white, fibrous to soft corky when dry, up to 0.3 mm thick. Tubes concolorous with the pore surface, hard corky to brittle when dry, up to 2 mm long.

Hyphal structure. Hyphal system monomitic; generative hyphae with clamp connections, smooth, hyaline, IKI–, CB–; tissues unchanged in KOH.

Subiculum. Generative hyphae thick-walled with a wide lumen, occasionally branched, flexuous, interwoven, 2.5–3.8 μm in diam.

Tubes. Generative hyphae thin- to thick-walled, occasionally branched, subparallel along the tubes to loosely interwoven, 2–3.1 μm in diam. Cystidia present, ventricose, very thick-walled, some apically encrusted. Basidia short clavate, sometimes with an intermediate constriction, with four sterigmata and a basal clamp connection, 12.5–16 × 4–5 μm; basidioles in shape similar to basidia, but smaller.

Spores. Basidiospores broadly ellipsoid to reniform, hyaline, thin- to slightly thick-walled, smooth, often with one guttule, dextrinoid, CB+, (3.5–)3.8–4.2(–4.5) × 2–2.3(–2.5) μm, L = 3.98 μm, W = 2.14 μm, Q = 1.82–1.90 (n = 90/3).

Type of rot. Brown rot.

Additional specimens (paratypes) examined. China. Hainan, Changjiang, Hainan Tropical Rainforest National Park, Bawangling; rotten wood of *Podocarpus imbricatus*, 10.XI.2020, Yu-Cheng Dai leg., *Dai 22043* (BJFC035939) and *Dai 22044* (BJFC035940).

Discussion

In this study, two new species, *Fomitopsis bambusae* and *Oligoporus podocarpi*, are described, based on morphological features and molecular data. The phylogenetic analysis of *Fomitopsis* (Fig. 1), inferred from ITS+nLSU+nuSSU+mtSSU+PRB2+TEF1 sequences, provides strong support (100% ML, 100% MP, 1.00 BPPs) for the placement of *F. bambusae* in *Fomitopsis* s.s. Besides, *Fomitopsis bambusae* formed a distinct and independent lineage, which is clearly distinguishable phylogenetically from all other known species of the genus. *Fomitopsis roseoalba* A.M.S. Soares and *F. subtropica* B.K. Cui & Hai J. Li are potentially the most closely related. Meanwhile, *F. roseoalba* is distinguished from *F. bambusae* by its larger pores (4–6 per mm vs. 6–9 per mm) and smaller basidiospores (3–4.9 × 1.8–2 μm vs. 4.2–6.1 × 2–2.3 μm, Tibpromma et al. 2017); *F. subtropica* is different from *F. bambusae* by smaller basidiospores (3.2–4 × 1.8–2.1 μm vs. 4.2–6.1 × 2–2.3 μm, Li et al. 2013).

Morphologically, *Fomitopsis bambusae*, *F. cana* (Blume & T. Nees) Imazeki, *F. caribensis*, *F. hemitephra* (Berk.) G. Cunn. and *F. nivosa* (Berk.) Gilb. & Ryvarden share approximately the same-sized pores (6–9 per mm). However, *Fomitopsis cana* differs from *F. bambusae* by its trimitic hyphal system, slightly larger basidiospores (5–6.2 × 2.1–3 μm, L = 5.81 μm, W = 2.6 μm vs. 4.2–6.1 × 2–2.3 μm, L = 4.917 μm, W = 2.109 μm) and grows on angiosperm wood rather than bamboo (Li et al. 2013). *Fomitopsis caribensis* differs from *F. bambusae* by larger basidiospores (6–7.5 × 2.3–3.1 μm vs. 4.2–6.1 × 2–2.3 μm, Liu et al. 2019). *Fomitopsis hemitephra* is distinguished from *F. bambusae* by its perennial habitat, woody hard basidiocarps (Cunningham 1965). *Fomitopsis nivosa* differs from *F. bambusae* by having longer basidiospores (6–9 × 2–3 μm vs. 4.2–6.1 ×2–2.3 μm, Gilbertson and Ryvarden 1986). In addition, *Fomitopsis bambusae* may be confused with *F. ostreiformis* (Berk.) T. Hatt. in having similar-sized basidiospores and also growing on bamboo, but *F. ostreiformis* differs from *F. bambusae* by the larger pores (3–4 per mm vs. 6–9 per mm) and trimitic hyphal system (De 1981).

Our phylogeny of *Oligoporus* (Fig. 2), based on ITS+nLSU+nuSSU+mtSSU+PRB1+PRB2+TEF1 sequence, demonstrated *Oligoporus* s.s. formed a monophyletic lineage with a robust rating (100% ML, 100% MP, 1.00 BPPs), which is distant from *Postia* s.s. Though *Oligoporus* and *Postia* are similar to each other in morphological characteristics, some significant differences remain. For instance, *Postia* s.s. has effuse-

Figure 5. Basidiocarps of *Oligoporus podocarpi* (holotype Dai 22042). Scale bar: 1.0 cm.

reflexed to pileate basidiocarps, thin-walled and acyanophilous basidiospores (Donk 1971; Ryvarden and Melo 2014; Shen et al. 2019), while *Oligoporus* s.s. has resupinate basidiocarps, slightly thick-walled and cyanophilous basidiospores (Shen et al.

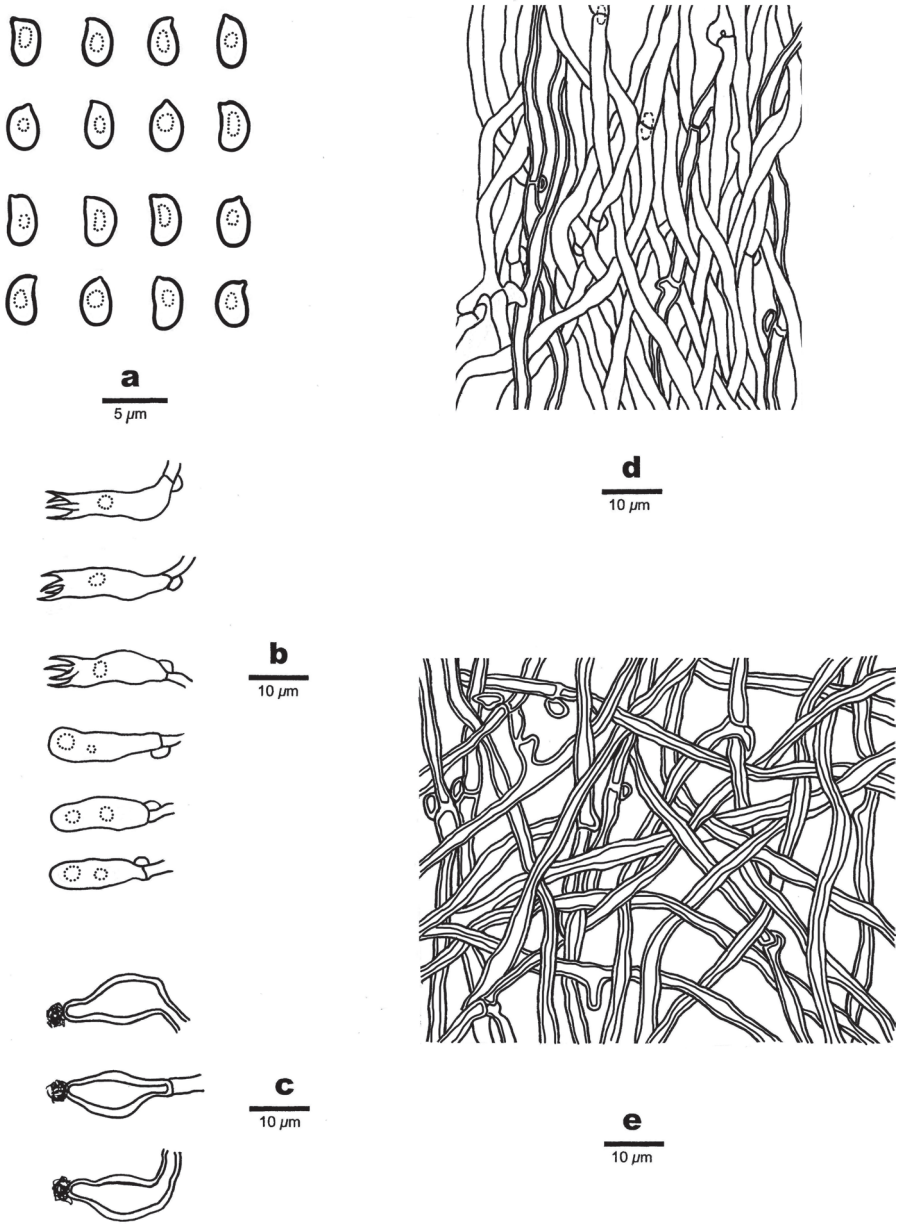

Figure 6. Microscopic structures of *Oligoporus podocarpi* (drawn from the holotype) **a** basidiospores **b** Basidia and basidioles **c** cystidia **d** hyphae from subiculum **e** hyphae from trama.

2019). Phylogenetically, *Oligoporus podocarpi* is nested in the *Oligoporus* s.s. clade with a strong support (100% ML, 100% MP, 1.00 BPPs) and related to *O. rennyi* (Berk. & Broome) Donk and *O. sericeomollis* (Romell) Bondartseva (Fig. 2). These three species, representing *Oligoporus* s.s., have resupinate basidiocarps, white to cream pore

surface and thick-walled, dextrinoid, cyanophilous basidiospores. However, *Oligoporus rennyi* differs from *O. podocarpi* in the very fragile dry basidiocarps, the lack of cystidia and the presence of chlamydospores (Donk 1971; Ryvarden and Melo 2014). *Oligoporus sericeomollis* is different from *O. podocarpi* by fragile dry basidiocarps, longer basidiospores (4–5 × 2–2.5 μm vs. 3.8–4.2 × 2–2.3 μm) and the extremely bitter taste (Núñez and Ryvarden 2001; Ryvarden and Melo 2014). Mophologically, *Oligoporus podocarpi* is similar to *Postia simanii* (Pilát) Jülich, *Cystidiopostia hibernica* (Berk. & Broome) B.K. Cui, L.L. Shen & Y.C. Dai and *Rhodonia rancida* (Bres.) B.K. Cui, L.L. Shen & Y.C. Dai by resupinate basidiocarps, white to cream pore surface (Jülich 1982; Núñez and Ryvarden 2001; Ryvarden and Melo 2014; Shen et al. 2019). However, *Postia simanii* has smaller pores (6–8 per mm) and allantoid, thin-walled basidiospores measuring 4–5.3 × 0.8–1.2 μm (Jülich 1982; Ryvarden and Melo 2014). *Cystidiopostia hibernica* and *Rhodonia rancida* are different from *Oligoporus podocarpi* by larger pores (2–3 per mm in *C. hibernica*, 2–4 per mm in *R. rancida*) and allantoid, thin-walled basidiospores (4.3–6 × 1.4–1.9 μm in *C. hibernica*, 5–7 × 2–2.5 μm in *R. rancida*) (Ryvarden and Melo 2014; Shen et al. 2019).

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