

Research Article

Recommendations on approving the name "Entomosporium", with a new species, E. dichotomanthes from China (Leotiomycetes, Drepanopezizaceae)

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Abstract

The phytopathogenic genus, *Entomosporium* can cause serious leaf diseases worldwide. *Entomosporium* has long been regarded as a synonym of *Diplocarpon*. However, different morphologies between *Entomosporium* and *Diplocarpon* make this doubtful. Based on morpho-phylogenetic analyses, the placement of the genus was re-evaluated in this study. The combined the internal transcribed spacer gene region (ITS) and the 28S large subunit ribosomal RNA gene region (LSU) phylogenetic analysis shows that *Entomosporium* is an independent clade within Drepanopezizaceae and formed a sister clade to the generic type *Diplocarpon*. Moreover, *Hymenula* and *Pseudopeziza* do not cluster in Drepanopezizaceae. We propose to resurrect the name *Entomosporium*, and exclude *Hymenula cerealis* and *Pseudopeziza medicaginis* from Drepanopezizaceae and propose to treat them under Ploettnerulaceae. A new species, *E. dichotomanthes* is also introduced from China based on morpho-molecular analyses which is associated with *Dichotomanthes tristaniicarpa*.

Key words: *Diplocarpon, Hymenula cerealis, plant pathogen, phylogeny, Pseudopeziza medicaginis*

Introduction

Entomosporium Lév, a synonym of *Diplocarpon* F.A. Wolf, is a member of the strongly plant-pathogenic family Drepanopezizaceae (Holtslag et al. 2003; Nunes et al. 2016; Johnston et al. 2019; Wöhner and Emeriewen 2019). The *Entomosporium* species causes entomosporium leaf disease worldwide and frequently occurs as an epidemic (Bogo et al. 2018). As many species are described without molecular data, the relationship with *Diplocarpon* species remains unclear. Although *Diplocarpon* species are common and widespread, studies on *Diplocarpon* have predominantly focused on their phytopathology, with the taxonomy utilizing molecular markers being largely overlooked (Wijay-awardene et al. 2017; Ekanayaka et al. 2019).



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Historically, Diplocarpon has undergone several revisions. Diplocarpon was erected by Wolf (1912), with the type species D. rosae (syn. Asteroma rosae) which caused black spot disease on Rose. The sexual stage of Diplocarpon forms cup-like apothecia, in which the asci develop. The ascospores are hyaline, 2-celled, marssonina-like and oblong-elliptical (Wolf 1912; Stowell and Backus 1967). The asexual stage comprises acervuli that develop on leaf surfaces accompanied by typical black dot disease (Frick 1943). Conidia of D. rosae are hyaline, oblong-elliptical, 2-celled with one constricted septum. By the interpretations of the morphology of these taxa, a broad concept of species circumscription was employed. Some members of Bostrichonema, Entomosporium, Gloeosporium and Marssonina were treated as synonyms of Diplocarpon. For example, Ascochyta coronariae (= D. coronariae), Bostrichonema alpestre (= D. alpestre), Dothidea impressa (= D. impressa), Leptothyrium fragariae (= D. fragariae) and Phacidium saponariae (= D. saponariae) were transferred into Diplocarpon (Johnston et al. 2014; Braun 2018; Crous et al. 2020). In addition, all members in Entomopeziza, Entomosporium and Morthiera were regarded as congruent with D. mespili (Stowell and Backus 1966; Gamundí et al. 2004; Johnston et al. 2014).

Although genera, such as Entomopeziza, Entomosporium and Morthiera have morphological similarities to Diplocarpon, it is perplexing that they are considered as synonyms. For example, 15 epithets of Entomosporium were regarded as D. mespili, as the sexual stage of Entomosporium morphologically resembles Diplocarpon (Naoui 2013; Johnston et al. 2014). However, Entomosporium produces cruciform, insect-like, 2-6-celled conidia, which is distinct from the conidia of Diplocarpon (Stowell and Backus 1966). Moreover, Entomosporium species are widely distributed in Argentina, Australia, Brazil, Canada, China, India, Israel, Italy, Japan, New Zealand, North America, Pakistan, and South Africa, on a wide host range of Rosaceae (Stowell and Backus 1966; Cariddi et al. 2009; Batool et al. 2014). Diplocarpon on the other hand, is mostly or specifically parasitic on herbaceous Rosaceae or low shrubs. The proposal to adopt Diplocarpon over Entomosporium is doubtful (Horie and Kobayashi 1980; Wijayawardene et al. 2021). The hypothesis that Diplocarpon mespili did not speciate with its worldwide spread should be re-evaluated (Chethana et al. 2021). An example of evidence is that Entomosporium sp. from Japan has more lateral cells (2-4) (Horie and Kobayashi 1979). Chen et al. (2022) introduced a new species with insect-like conidia but under the name "Diplocarpon". In Index Fungorum (https://www.indexfungorum.org, 23 Nov 2023), 12 Diplocarpon species are recorded, namely D. alpestre, D. coronariae, D. earlianum, D. fragariae, D. hymenaeae, D. impressum, D. mali, D. mespili, D. mespilicola, D. polygoni, D. rosae and D. saponariae.

We are studying the pathogens of urban and forest tree species in Yunnan Province (Thiyagaraja et al. 2024) and in this study *Entomosporium* leaf disease was found in *Dichotomanthes tristaniicarpa* and has not been reported before. *Dichotomanthes* is endemic to Yunnan and Sichuan provinces in China (Zhou et al. 2000). It belongs to Rosaceae, with only one species *D. tristaniicarpa* which is a rare evergreen shrub tree, and is used as ornamental and medicinal plants (Tang et al. 2010; Yang et al. 2018). The ITS sequence blastn search of the newly generated sequences showed the close hits to *Diplocarpon*, and

identified it as a new species based on the evidence from both morphology and phylogeny. Since the increasing number of members and updating molecular data of *Diplocarpon*, this study has provided an opportunity for a better understanding of the taxonomy of the genus. In this study, we interpret the relationship between *Entomosporium* and *Diplocarpon*, and further re-evaluate the taxonomy of Drepanopezizaceae.

Materials and methods

Sampling, isolation and morphological observations

Leaves with lesions of Dichotomanthes tristaniicarpa were collected from Yunnan Province. For single-spore isolation, the fruit bodies were transferred to sterilized water in a centrifuge tube using a syringe needle, then crushed into pieces using pipette tips. Subsequently, 200 µL of the spore suspension was transferred to potato dextrose agar (PDA) using a micropipette (Zhang et al. 2013). For tissue isolation, the leaves were washed with distilled water for 1 minute and then air-dried. The margins of the disease lesions were cut into fragments $(0.5 \times 0.5 \text{ cm})$ under aseptic conditions. These fragments were surface-sterilized with 75% ethanol for 30 seconds, followed by dipping in 1% sodium hypochlorite for 40 seconds. They were then rinsed three times in sterile demineralized distilled water before being transferred onto a PDA plate, with four fragments per plate (Senanayake et al. 2020). The Petri dishes were incubated in the dark at 25 °C. Specimens were deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS). Morphological observations were performed using Nikon SMZ745T dissecting microscope (DM) and Nikon Eclipse 80i compound microscope, equipped with IMG Camera SC2000C. Index Fungorum and Facesoffungi numbers were obtained as in Index Fungorum (https://www.indexfungorum.org/) and Jayasiri et al. (2015) and the details of the fungus were deposited in the Greater Mekong Subregion database (Chaiwan et al. 2021).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted by using Lysis Buffer for Microorganism to Direct PCR (Takara), following the user manual. PCR amplifications were performed in T100 Thermal Cycler (T100TM, Bio-Rad, USA) with ingredients of 21 µL Golden-Star T6 Super PCR Mix (Tsingke), 1 µL (10 µM) of each primer and 2 µL DNA template. Amplification conditions include 3 min initial denaturation at 95 °C, followed by 35 cycles of 95 °C denaturation for 15 s, 53 °C ~ 56 °C annealing for 15 min, 72 °C extension for 20 s, followed by a final extension at 72 °C for 5 min. The primer set ITS5/ITS4 (White et al. 1990) was used to amplify the internal transcribed spacer gene region (ITS); and LROR/LR5 for the 28S large subunit ribosomal RNA gene region (LSU) (Vilgalys and Hester 1990; White et al. 1990) and 983F/2218R for translation elongation factor 1-alpha gene region (*tef*-α) (Rehner and Buckley 2005). PCR products were purified and sequenced by Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China.

Phylogenetic analyses

Reverse and forward sequences were assembled using Chromas Pro (2.1.8) and initial identification was subjected to the NCBI (https://www.ncbi.nlm.nih. gov/) using BLAST search. Sequences of similar taxa were retrieved from the NCBI, and additional reference sequence selections based on Johnston et al. (2019) were downloaded from the DataStore (https://datastore.landcareresearch.co.nz/). The alignment was constructed with the online tool MAFFT v.7 (http://mafft.cbrc.jp/alignment/server) (Katoh and Standley 2013), and refined using BioEdit v. 7.7.1 (Hall 1999). The final combined data matrix was converted by the online tool ALTER (https://www.sing-group.org/ALTER/) (Glez-Peña et al. 2010). A quick Phylogenetic analysis was conducted using OFPT (Zeng et al. 2023) following its default protocol. The final Phylogenetic analyses were conducted on the CIPRES Science Gateway platform (https://www.phylo.org), using tools of RAxML-HPC v.8 on XSEDE (8.2.12) for maximum likelihood (ML) and MrBayes on XSEDE (3.2.7a) for Bayesian inference (BI). In the Bayesian inference, the best optimal substitution model was determined by using ModelFinder (Kalyaanamoorthy et al. 2017) under the Bayesian information criterion (BIC). The final phylogenetic tree was visualized with FigTree v. 1.4.4 and edited using Adobe Photoshop CS6 version 10.0. Sequences of the new strain generated in this study are deposited in GenBank (Table 1).

Results

A total of 50 ingroup taxa from Drepanopezizaceae, Hyaloscyphaceae, Ploettnerulaceae and Vibrisseaceae were used in the phylogenetic tree analysis, of which 20 species were from the type (Fig. 1). In total, 31 isolates contained all extant species that have available molecular data within Drepanopezizaceae. The combined LSU and ITS yield a 1409 bp alignment, with the best substitution models for each summarised as TIM2e+I+G4 and TIM2+F+R3, respectively.

Phylogenetic analysis demonstrated that *Diplocarpon* divided into two phylogenetically close relative clades, *Diplocarpon* and *Entomosporium*. *Diplocarpon* clade is composed of *D. coronariae* (from China, Japan, Korea and the USA), *D. earlianum* (unknown country) and *D. rose* (from China, Germany and an unknown country). Those three species have common characteristics of two-celled conidia. The new species *Entomosporium dichotomanthes* (from China), along with *E. mespili* (from England, Korea and an unknown country) and *E. mespilicola* (from China) consisted of clade *Entomosporium*, which showed insect-like conidia. Moreover, *Hymenula cerealis* and *Pseudopeziza medicaginis* were within Ploettnerulaceae.

Sequence comparison reveals the intergeneric and interspecific variation (Fig. 2). ITS sequence shows a high nucleotide variation within *Diplocarpon*, with an average of 58.1, compared to *Diplocarpon*, the *Entomosporium*, *Blumeriella*, *Drepanopeziza* and *Thedgonia* have an average of 62.6, 68, 71.3 and 76, respectively. The sequence comparison results align with the phylogenetic analysis, indicating that the closely related species exhibit less nucleotide variation. The LSU sequences have a lower variation. The *Diplocarpon* has an average of 28, and the *Drepanopeziza*, *Blumeriella*, *Entomosporium* and *Thedgonia* have an average of 35.5, 37, 40, and 44, respectively. The interspecific variation of *Drepanopeziza* and *Entomosporium* is 26.8 and 41.3.

Table 1. GenBank accession numbers used in the phylogenetic analyses.

		_	GenBank accession number			
Species	Strain	Country	ITS	LSU		
Acephala applanata	CBS 109321T	Switzerland	NR_119482	KF951051		
Blumeriella hiemalis	CBS 146.35	USA	MH855609	MH867119		
B. kerriae	JS20160615	United Kingdom	KY929501	-		
Cadophora fascicularis	CBS 146382	Germany	NR_170729	MN339414		
Cheirospora botryospora	MFLUCC 17-1399	China	MN535816	MN535856		
Collembolispora aristata	CPC 21145T	Czech Republic	NR_111830	NG_042760		
Cylindrosporium concentricum	CBS:157.35	Australia	MH855615	MH867125		
Diplocarpon coronariae	Satoko Kanematsu Dip-ap6-3	Japan	AB609188	-		
D. coronariae	CS 01	Korea	AB494960	AB494964		
D. coronariae	5C11	USA	MW364818	-		
D. coronariae	NL1	China	KY672995	-		
D. earlianum	CBS 162.32	Unknown	MH855259	MH866712		
D. rosae	CBS 163.31	Unknown	MH855164	MH866612		
D. rosae	CBS 829.72	Netherlands	-	MH872311		
D. rosae	CFCC6814	Unknown	KP099199	-		
D. rosae	DortE4 [⊤]	Germany	Genome	Genome		
Drepanopeziza balsamiferae	14-19	USA	MN315242	-		
D. brunnea	Marbr1	Unknown	genome	genome		
D. ribis	CBS:200.36	Netherlands	MH855774	MH867284		
D. salicis	CBS:405.64	Switzerland	MH858467	MH870102		
D. tremulae	CBS 408.64	Switzerland	MH858468	MH870103		
D. triandrae	CBS 409.64	Switzerland	MH858469	MH870104		
Entomosporium dichotomanthes	HKAS 131154	China	PP333041	PP333042		
E. mespili	CBS 166.28	England	_	MH877689		
E. mespili	CBS 402.65	Unknown	_	MH870277		
E. mespili	KACC 42361	Korea	EF600984	_		
E. mespili	KACC 42436	Korea	EF600985	_		
E mespilicola	CF 2	China	OM237437	MW809414		
E mespilicola	CE 3	China	0M237438	MW809415		
E mespilicola		China	OM237436	MW809413		
Hvaloscypha ericae	LIAMH 6735 ^T	Canada	AF284122	MH018947		
	CBS 145341 ^T	Czech Republic	M7520780	NG 081311		
	CBS 145337	Czech Republic	MZ520785	M7520774		
H minuta	G M 2015-04-06 2T		KV760526	-		
		Einland	E 1477050	E1/77059		
	CBS 132 341	lanan	NP 171200	NG 070830		
	CRS 540.63	United Kingdom	MH858350	MH860071		
	CBS:176.44	Netherlands	MH856125	MH867637		
	AFTOL ID 1207	Unknown	MF830123	F 1176094		
L. puchernina Meliniamyosa veriabilia		Olikilowii	ND 101010	FJ170004		
		Callaua	NR_121313	NG_073010		
Mycochaelophora gentianae	MAFF 239231	Japan	NR_121201	AB490937		
Neospermospora avenae	CBS 227.38	USA	MW298276	NG_07/377		
Oculimacula yallundae	CBS 110665	South Africa	MW810278	MW/15035		
Pseudaegerita corticalis	ICMP 15324'	New Zealand	EF029224	-		
Pseudopeziza medicaginis	CBS 283.55	USA	MH85/484	MH869025		
Rhexocercosporidium carotae	CBS 418.65 ^T	Norway	NR_111086	MH870289		
Rhynchosporium agropyri	CBS:146762	Switzerland	MW298346	MW298448		
I hedgonia ligustrina	CBS 132025	Korea	GU269839	GU253856		
T. ligustrina	CBS:148.59 ^T	Netherlands	NR_175086	NG_078647		
T. ligustrina	CPC 10530	Netherlands	FJ839628	FJ839665		
Vibrissea truncorum	AFTOL-ID 1322 ^T	Canada	EU434854	FJ176874		
Ypsilina buttingtonensis	CPC 39109'	United Kingdom	NR_170831	M1373355		

Type strains are marked with "T", and strains from the present study are in black bold.



0.04

Figure 1. Maximum likelihood phylogenetic tree inferred from combined LSU and ITS sequence data of Drepanopezizaceae and its closely related families. The tree is artificially rooted with *Leuconeurospora capsici* (CBS:176.44) and *Leuconeurospora pulcherrima* (AFTOL-ID 1397). Maximum likelihood bootstrap values \geq 65% and Bayesian Posterior Probabilities (BYPP) \geq 0.90 are given at the nodes. Novel taxon is in bold. Type sequences are labeled asterisk (*).

Taxonomy

Drepanopezizaceae Baral MycoBank No: 828889

Facesoffungi Number: FoF05864 Fig. 3

Type. Drepanopeziza (Kleb.) Jaap 1914.

Description. *Sexual morph*: Ascomata small-sized, up to 2 mm in diameter, apothecial, cupulate, margin often protruding, with or without lobes, sessile and mostly immersed. Excipulum is composed of cells of textura angularis. Paraphyses hyaline, thin-walled, aseptate or septate, apically swollen. Asci 4–8-spored, clavate or cylindrical, apex obtuse to conical, with or without apical



Figure 2. Intergeneric and interspecific variation analysis **A** mean of ITS sequence variation within genera **B** means of LSU sequence variation within genera **C** ITS sequence variation of the query sequence and the subject, "S" is the subject, "x" is the mean value of nucleotide variation within species.

ring. Ascospores ellipsoid to fusoid, aseptate or 1–2-septate. **Asexual morph:** Conidiomata solitary to gregarious or confluent, mostly epiphyllous, acervulus. Conidiogenesis holoblastic. Conidia hyaline, thin-walled.

Notes. Drepanopezizaceae was described with sexual and asexual morphs. Both life morphs were found as parasitic on leaves of various dicotyledons, and rarely on herbaceous (Johnston et al. 2019). The sexual morph is recognized by the cupulate, apothecial ascomata, and the paraphyses with swollen apical (Harada et al. 1974; Williamson and Bernard 1988; Spiers and Hopcroft 1998). The asexual morph is acervular but varies in conidial shape among genera (Crous et al. 2009; Khodadadi et al. 2022). The family name was first time used by Batista and Maia (1960), but was invalid because of unavailable diagnosis or description (Johnston et al. 2019). It was difficult to trace back the history of members accommodated in the family until Johnston et al. (2019), validated the family name based on the phylogenetic analysis (Table 2).

Entomosporium Lév. 1857 MycoBank No: 8180 Facesoffungi Number: FoF15505

Type. Entomosporium maculatum Lév. 1856.

Description. Sexual morph: Ascomata small-sized, apothecial, cupulate, epiphyllous. Excipulum composed of cells of textura angularis. Paraphyses numer-



Figure 3. Morphology of genera in Drepanopezizaceae. *Diplocarpon*: a ascomata, b asci, paraphyses and ascospore (**a**, **b** *D. rosae*, redraw from Wolf 1912) **c** acervulus and conidia (*D. rosae*, redraw from Lee and Shin 2000), *Entomosporium* **d** ascomata **e** asci and paraphyses (**d**, **e** *E. maculatum*, redraw from Stowell and Backus 1967) **f** acervulus **g** conidia (**f**, **g** *E. mespilicola*, redraw from Chen et al. 2022), *Drepanopeziza* **h** ascomata (*Dr. populorum*, redraw from Spiers and Hopcroft 1998) **i** asci, paraphyses and ascospore **j** conidiogenous cells and conidia (**i**, **j** *Dr. ribis*, redraw from https://www.centrodeestudiosmicologicosasturianos.org), *Blumeriella* **k** ascomata **l** asci, paraphyses and ascospore (**k**, **l** *B. haddenii*, redraw from Williamson and Bernard 1988) **m** acervulus **n** conidia (**m**, **n** *B. jaapii*, redraw from https://www.forestryimages.org), *Thedgonia* **o** acervulus and conidia (*T. ligustrina*, redraw from Crous et al. 2009) **p** *Hymenula*: conidiogenous cells and conidia (*H. gramineum* redraw from Wiese and Ravenscroft 1978), *Pseudopeziza* **q** ascomata (*P. trifolii*, redraw from Kirchner and Boltshauser 1987) **r** asci, paraphyses and ascospore (*P. ribis*, redraw from https://www.pesticidy.ru/pathogens_genus/Pseudopeziza).

Batista and Maia (1960)	d Maia Wijayawardene et al. 0) (2017) Johnston et al. (2019) Ekan		Ekanayaka et al. (2019)	Wijayawardene et al. (2022)	Zhu et al. (2023)	This study	
The family name was used	Blumeriella	Erected the	Blumeriella	Blumeriella	Blumeriella	Blumeriella	Blumeriella
	Diplocarpon	family	Diplocarpon	Diplocarpon	Diplocarpon	Diplocarpon	Diplocarpon
	Drepanopeziza		Drepanopeziza	Drepanopeziza	Drepanopeziza	Drepanopeziza	Drepanopeziza
	Felisbertia	- - -	Felisbertia	Felisbertia	Felisbertia	Felisbertia	Entomosporium
	Leptotrochila		Leptotrochila	Leptotrochila	Leptotrochila	Hymenula	Felisbertia
	Pseudopezicula		Pseudopeziza	Marssonina	Pseudopeziza	Leptotrochila	Leptotrochila
	Pseudopeziza		Spilopodia	Pseudopezicula	Spilopodia	Pseudopeziza	Spilopodia
	Spilopodia		Spilopodiella	Spilopodiella	Spilopodiella	Spilopodia	Spilopodiella
	Spilopodiella		Thedgonia	Spilopodia		Spilopodiella	Thedgonia
		1		Thedgonia		Thedgonia	

Table 2. Main versions of classification of Drepanopezizaceae and its accepted genera.

The taxa used in the phylogenetic analysis are labeled in bold.

ous, hyaline, thin-walled, septate, apically swollen, simple or branched, longer than aci. Asci 8-spored, bitunicate to uniseriate, thick-walled, clavate, short pedicel, apex obtuse, amyloid, with apical ring. Ascospores 2-celled, ellipsoidal, smooth, hyaline, thick-walled, unequal, the upper cell slightly lager. **Asexual morph:** Conidiomata solitary to gregarious or confluent, mostly epiphyllous, acervulus. Conidiogenesis hyaline, cylindrical, holoblastic. Conidia 2–6-celled, hyaline, thin-walled, cruciform or insect-like, basal cell developed from the conidiogenous cell, cylindrical, globose to obovate, and other cells attached basal cell in both upper sides and apex, apical cell larger, globose to subglobose, lateral cells globose to ellipsoidal, smaller than the apical and basal cells, the apical and basal cells with a tubular appendage.

Notes. Entomosporium was erected by Leveille in 1856, based on E. maculatum from leaves of Pyrus communis (Rosaceae), and was characterized by 4-celled, cross-like conidia (Stowell and Backus 1966; Horie and Kobayashi 1980). Historically, Entomosporium is composed of multiple morphologically indistinguishable species. Sivanesan and Gibson combined all species to E. mespili, but they did not mention their taxonomic basis (Horie and Kobayashi 1980). Atkinson recorded the process by which ascospores from a cupulate fungi formed the conidia of E. maculatum, and named the species as Fabraea maculata, while he later proposed that F. maculata may be identical to E. mespili (Atkinson 1897, 1909). Taxonomic status changed for the morphologically similar taxa, viz. Diplocarpon, Entomopeziza, Fabraea and Marssonina (Stowell and Backus 1967; Johnston et al. 2014). After versions, Jørstad (1945) combined Diplocarpon and Entomosporium, and recognized Atkinson's collection as the type. This opinion was also discussed by Stowell and Backus (1967), but they failed the verification through experiments. The mystery of Entomosporium associated with sexual morph is still not confirmed by molecular data, since no new collection was found in sexual stage in recent decades.

Entomosporium dichotomanthes H.D. Yang, Jayaward & K.D. Hyde, sp. nov. Index Fungorum: IF901675 Facesoffungi Number: FoF15506

Fig. 4

Etymology. The species epithet '*dichotomanthes*' refers to the host *Dichotomanthes tristaniicarpa* in which the holotype was collected. **Holotype.** HKAS 131154.



Figure 4. Entomosporium dichotomanthes (HKAS 131154, holotype) **a, b** disease symptoms on the leaves, **c**-**e** conidiomata **f, g** conidiogenous cells and conidia,**h**-**k** conidia. Scale bars: 50 µm (**e**); 10 µm (**f**-**k**).

Description. Parasitic on leaf of *Dichotomanthes tristaniicarpa* in terrestrial habitat. *Leaf spots:* appear as tiny black spots or irregular black stripes on the upper side of the mature leaf when young, without injured disease symptoms. Later the spot enlarged to circular lesions or large dead areas with black edege. The area around the black spots remains green. **Sexual** *morph*: Not determined. **Asexual morph: Conidiomata** dark brown to black, stromatic, acervular, epiphyllous, solitary to gregarious or confluent, subcuticular to rounded or irregular in outline, rugose, erumpent through the cuticle. **Conidiomatal wall** mixed with host plant tissue, of several layers loose textura angularis cell. **Conidiophores** hyaline to pale brown, cylindrical, branched. **Conidiogenous cells** $5.0-8.4 \times 2.8-4.4 \mu m$, hyaline, cylindrical, holoblastic. **Conidia** hyaline, 3-4-celled, cruciform, the basal cell developed from the conidiogenous cell, and other cells attached to basal cell in both upper sides and apex. Basal cells $6.7-12.1 \times 4.5-8.6$ (avg. = 9.9×7.1 , n = 30) μm , cylindrical, globose to subglobose, the end with a tubular appendage. Lateral cells $3.5-7.5 \times 2.5-4.1$ (avg. = 5.7×4.1 , n = 20) μm , subglobose to ellipsoidal, the end with a tubular appendage.

Material examined. CHINA, Yunnan Province, Kunming City, Longchuanqiao park, 25°8'15.65"N, 102°47'13.70"E, on living leaf of *Dichotomanthes tristanii-carpa*, 14 December 2021, YHD 239-5 (HKAS 131154); YHD 202.

Notes. Entomosporium dichotomanthes is characterized by having three to four cells of conidia. Its morphology resembles *D. mespili* and *D. mespilicola*, but has different host plants association and distribution. *E. dichotomanthes* is easily detectable on the host substrate in the mountains around the lake of Longchuanqiao Park. However, we couldn't find this fungus on nearby plants of the host, or on other plants in the mountains. We also failed to isolate the culture by using both single spore isolation and tissue isolation methods which indicates *E. dichotomanthes* strictly rely on *D. tristaniicarpa*.

Discussion

The taxonomic status and the phylogenetic relationship of Diplocarpon and Entomosporium in Drepanopezizaceae were assessed in this study. We included all extant species with molecular data in Drepanopezizaceae, as well as most genera of its sister family Ploettnerulaceae for the first time. Upon molecular phylogenetic analysis, Diplocarpon divided into two distinct clades representing Entomosporium and Diplocarpon. Sequence comparison reveals the average nucleotide variation of Blumeriella, Drepanopeziza, Entomosporiummm, and Thedgonia is higher than Diplocarpon which means intergeneric variation is greater than interspecific variation. Moreover, Diplocarpon and Entomosporium have a high nucleotide variation compared to the more speciose genus Drepanopeziza. Consequently, Entomosporium recovered separately from Diplocarpon and should not assign all species to E. mespili. On the plant host (Table 3), D. rose is commonly reported on Roses, D. earlianum on strawberries and D. coronaria on apple trees. However, Entomosporium has a wide host range of woody plants like shrubs and trees, such as apple, hawthorn, saskatoon and pear (Holtslag et al. 2004; Nunes et al. 2016; Thurn et al. 2019). Likewise, the morphology features of conidia sustained the difference, Entomosporium displays insect-like conidia while Diplocarpon produces 2-celled conidia. Johnston et al. (2014) stated that Entomosporium and Diplocarpon are conspecific due to the linked asexual and sexual morphs, but this was not confirmed by molecular data. Conclusively, our study based on morphology coupled with molecular data supported the division

Current name	Original name	Host		Disease	Symptom	Location	Reference
D. coronaria	Diplocarpon mali	Malus	Rosaceae	Apple blotch disease	premature leaf fall of Apple	India	Goyal et al. (2018)
D. coronaria	Diplocarpon mali	Malus	Rosaceae	Apple blotch disease	dark brown and irregularly shaped blotches or lesions	China	Lian et al. (2021)
D. coronaria	Marssonina coronaria	Malus	Rosaceae	Apple blotch disease	tiny yellow spots at first, become grayish brown circular lesions	Korea	Lee et al. (2011)
D. coronaria	Marssonina coronaria	Malus baccata	Rosaceae	Leaf spot disease	initially light brown to brown lesions without a distinctive margin, later reddish black to purple, finally appearing as a yellow blotch with green islands	Korea	Lee and Shin (2000)
D. coronaria	Diplocarpon coronaria	Malus	Rosaceae	Apple blotch disease	dark brown and irregularly shaped blotches or lesions	Germany	Wöhner et al. (2021)
D. coronaria	Diplocarpon mali	Malus	Rosaceae	Apple blotch disease	-	Japan	Tanaka et al. (2000)
D. coronariae	Diplocarpon coronariae	Malus spp.	Rosaceae	Apple blotch disease	brown to black spots, with frond- like edges or surrounded by a yellow halo	America	Khodadadi et al. (2022)
D. earlianum	Diplocarpon earlianum	Strawberry	Rosaceae	Leaf scorch	reddish-purple lesions	Canada	Dhanvantari (1967)
D. earlianum	Diplocarpon earlianum	Strawberry	Rosaceae	Leaf scorch disease	_	México	Garay-Serrano et al. (2021)
D. earlianum	Diplocarpon earlianum	Fragaria x ananassa	Rosaceae	Leaf scorch disease	_	America	Xue et al. (1996)
D. fragariae	Diplocarpon fragariae	Strawberry	Rosaceae	Leaf scorch disease	_	México	Garay-Serrano et al. (2021)
D. fragariae	Marssonina fragariae	Duchesnea chrysantha, Fragaria × ananassa, Potentilla fragarioides, Potentilla feyniana	Rosaceae	Leaf spot disease	initially reddish to brown, later dark brown, central area surrounded by yellowish halo	Korea	Lee and Shin (2000)
D. mespili	Diplocarpon mespili	Pyrus communis	Rosaceae	Entomosporium leaf disease	reddish, purple, to dark brown spots	Southern Brazil	Nunes et al. (2016)
D. mespili	Diplocarpon mespili	Eriobotrya japonica	Rosaceae	Entomosporium leaf disease	circular, bright red spots on young leaves, turned to purple blotches with ash brown grey centers, and coalesced to form large dead areas on leaf surfaces	Pakistan	Batool et al. (2014)
D. mespili	Entomosporium maculatum	Cydonia oblonga	Rosaceae	Entomosporium leaf disease	-	Southwestern Romania	Borcean and Imbrea (2021)
D. mespili	Entomosporium mespili	Photinia x fraseri	Rosaceae	Entomosporium leaf disease	reddish-colored lesions	Georgia	Mims et al. (2000)
D. mespili	Entomosporium mespili	Pyrus communis	Rosaceae	Entomosporium leaf disease	-	Southern Brazil	Bogo et al. (2018)
D. mespili	Entomosporium mespili	Amelanchier alnifolia	Rosaceae	Entomosporium leaf and berry spot disease	-	Canada	Holtslag et al. (2003)
D. mespili	Entomosporium mespili	Photinia glabra	Rosaceae	Entomosporium leaf disease	Initially appeared as minute circular spots, later several small spots coalesced to make large necrotic blotches	Korea	Seo et al. (2010)
D. mespili	Entomosporium mespili	Cydonia oblonga	Rosaceae	Entomosporium leaf disease	circular reddish-brown spots at first, coalesced producing large necrotic areas, the leaves turned yellow or reddish and fell prematurely	Southern Italy	Cariddi et al. (2009)
D. mespili	Entomosporium sp.	Amelanchier asiatica	Rosaceae	Entomosporium leaf disease	black shiny pustule spots	Japan	Horie and Kobayashi (1979)
D. mespili	Entomosporium sp.	Eriobotrya japonica	Rosaceae	Entomosporium leaf disease	yellowish or reddish spots with a greenish halo around	Japan	Horie and Kobayashi (1979)
D. mespili	Entomosporium sp.	Pyrus communis	Rosaceae	Entomosporium leaf and fruit spot disease	reddish to purple at the beginning, later irregular dark brown to black necrotic patches on the leaf, sunken irregular black spot on the fruit	India	Altaf et al. (2019)
D. mespili	Entomosporium mespili	Crataegus	Rosaceae	small, irregularly shaped spots or larger lesions	_	America	Thurn et al. (2019)

Table 3. Diplocarpon species documented from different countries and plant host.

Current name	Original name	Host		Disease	Symptom	Location	Reference	
D. mespili	Diplocarpon mespili	Pyrus pyraster	Rosaceae		_	Bulgaria	Velinova and Tashev (2017)	
D. mespilicola	Diplocarpon mespilicola	Crataegus pinnatifida	Rosaceae	Entomosporium leaf disease	brown spots	China	Chen et al. (2022)	
D. rosae	Diplocarpon rosae	Rose	Rosaceae	Black spot disease	black spots	United Kingdom	Knight and Wheeler (1977)	
D. rosae	Diplocarpon rosae	Rosa multiflora	Rosaceae	Black spot disease	black spots	Germany	Von Malek and Debener (1998)	
D. rosae	Diplocarpon rosae	Rose	Rosaceae	Black spot disease	black spots	North America	Whitaker et al. (2007)	
D. rosae	Diplocarpon rosae	Rose	Rosaceae	Black spot disease	-	Belgium	Leus et al. (2002)	
D. rosae	Diplocarpon rosae	Rosa rugosa	Rosaceae	Black spot disease	black spot lesions	Canada	Bolton and Svejda (1979)	
D. saponariae	Diplocarpon saponariae	Silene latifolia	Caryophyllaceae	Leaf spot disease	pale yellow to pale brown spots, sometimes purple-bordered, regular or irregularly rounded, sometimes elongated	Turkey	Erdoĝdu and Hüseyin (2009)	

of these genera. Entomosporium leaf disease is mainly associated with Entomosporium species (Holtslag et al. 2003; Seo et al. 2010; Nunes et al. 2016). Hence, the classification system of Diplocarpon was revised. We propose to recover the validity of the genus name "Entomosporium", to accommodate species that have insect-like conidia species in Drepanopezizaceae. Furthermore, we introduced a new species E. dichotomanthes from China. Its taxonomical placement is basal in the "Entomosporium" clade supported by high bootstrap. The disease symptom appeared as black spots or irregular black stripes on the upper side of the mature leaf of Dichotomanthes tristaniicarpa, which was easily recognizable. We also generated the first sequence of the $tef1-\alpha$ gene for Entomosporium, from E. dichotomanthes. However, we used only LSU and ITS sequences data in our study, since scant $tef1-\alpha$ sequences data are available for reference taxa that cannot be used in this phylogenetic analysis. The blast against NCBI shows the tef1- α sequences have highest similarity with D. coronariae (MT674914) and Hyaloscypha fuckelii (MT254572), gained the value of 884/948(93%) and 860/948(91%), respectively.

Correspondingly, Hymenula cerealis and Pseudopeziza medicaginis were not clustered in Drepanopezizaceae in our phylogenetic tree. However, Hymenula was recovered within Drepanopezizaceae in Zhu et al. (2023). Further, the type material of H. cerealis (= Cephalosporium gramineum, CBS 132.34) was used in their study and obtained a good statistical support (MLBP/BIPP = 96%/100%), in the phylogenetic analyses conducted based on the combined five-gene data set. However, only H. cerealis as well as a small group of taxa from both Drepanopezizaceae and Ploettnerulaceae were applied in their phylogenetic analysis. The disease caused by Hymenula is cephalosporium stripe on herbaceous plants that is different from Drepanopezizaceae (Wiese and Ravenscroft 1978; Zhu et al. 2023). Similar situations with Hymenula, Pseudopeziza cause black spot disease mostly found on Alfalfa and Red clover (Fabaceae), not on Rosaceae plants (Jones 1919; Meyer and Luttrell 1986; Yuan et al. 2007). The taxonomy of Pseudopeziza is confusing (Meyer and Luttrell 1986). There are 135 species epithets that have been linked to Pseudopeziza in Index Fungorum (https://www.indexfungorum.org), of which many names have been transferred

to other families, such as Diaporthaceae, Ploettnerulaceae and Rhytismataceae. Only three sequences labeled as *Pseudopeziza* were accessible in the Gen-Bank, and *P. medicaginis* (CBS 283.55) was used in this study.

Morphologically, *Hymenula* was only found in the asexual stage. Meanwhile, the asexual morph of Drepanopezizaceae does not share highly persuasive common morphological characteristics for delimiting its generic members. The morphology of *P. medicaginis* fits Drepanopezizaceae (Jones 1919), but differs in having indistinctive swollen apical paraphyses (Meyer and Luttrell 1986). Thus, we propose to exclude *H. cerealis* and *P. medicaginis* from Drepanopezizaceae and to treat them under Ploettnerulaceae.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Four new species of *Russula* subsect. *Cyanoxanthinae* from China (Russulales, Russulaceae)

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Abstract

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Four new species of Russula subsect. Cyanoxanthinae, viz. Russula atrochermesina Y.L. Chen & J.F. Liang, R. lavandula Y.L. Chen, B. Chen & J.F. Liang, R. lilaceofusca Y.L. Chen & J.F. Liang and R. perviridis Y.L. Chen, B. Chen & J.F. Liang, from China are proposed, based on morphological and molecular evidence. Russula atrochermesina can be distinguished by its violet pileus with tuberculate-striate margin, distant lamellae that stain greyish-yellow when bruised, basidiospores ornamented by isolated warts, wide hymenial cystidia on lamellae edges, cystidia content negative reaction in sulphovanillin and branched subterminal cells in pileipellis. Russula lavandula has a purplish-white to violet red pileus with a yellow centre, frequently present lamellulae and furcations, stipe often with pale yellow near the base, isolated basidiospores ornamentation and unbranched cuticular hyphal terminations, while R. lilaceofusca is characterised by its lilac brown to dark brown pileus, crowded lamellae with lamellulae and furcations, stipe often turning reddish-yellow when bruised, subreticulate basidiospores ornamentation and clavate hymenial cystidia often with capitate appendage whose contents that change to reddish-black in sulphovanillin. Russula perviridis is characterised by its large basidiomata, smooth pileus surface, frequently present lamellulae and furcations, stipe with yellow-brown tinge, globose to broadly ellipsoid basidiospores with subreticulate ornamentation, long hymenial cystidia that turn greyish-black in sulphovanillin and symbiotic with Quercus semecarpifolia. Phylogenetic analyses, based on multi-gene ITS+LSU+mtSSU+rpb2, indicate that R. atrochermesina, R. lavandula, R. lilaceofusca and R. perviridis are closely related to R. pallidirosea and R. purpureorosea, R. banwatchanensis, R. lakhanpalii and R. nigrovirens, respectively.

Key words: Ectomycorrhiza, edible fungi, morphology, new species, phylogeny

Introduction

Russula Per. is amongst the most ecologically and economically important groups of macrofungi (Looney et al. 2018; Caboň et al. 2019). More than 800 valid species in the genus have been described, but this figure is far below the 2,000 estimated species number (Adamčík et al. 2019). *Russula* is currently

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divided into nine subgenera, viz. Russula subg. Archaeae Buyck and V. Hofst., R. subg. Brevipedum Buyck & V. Hofst., R. subg. Compactae (Fr.) Bon, R. subg. Crassotunicatae Buyck & V. Hofst., R. subg. Cremeoochraceae Buyck & X.H. Wang, R. subg. Glutinosae Buyck & X.H. Wang, R. subg. Heterophyllidiae Romagn., R. subg. Malodorae Buyck & V. Hofst. and R. subg. Russula Pers. (Buyck et al. 2018; Buyck et al. 2020; Buyck et al. 2024). The subsect. Cyanoxanthinae Singer belongs to R. subg. Heterophyllidiae and is characterised by the following set of characters: medium to large basidiomata, lamellulae and furcations mostly present, mild to acrid context taste, white to cream spore print, basidiospores with inamyloid suprahilar spots, metachromatic pileipellis in cresyl blue and mainly one-celled pileocystidia. In the wild mushroom markets in south-western China, mushrooms known locally as "Mitangjun" have always been named as R. cyanoxantha (Schaeff.) Fr. However, they comprise several species that differ from R. cyanoxantha. The species diversity in this subsection has remained unresolved for a long time on the Asian continent, including in China. In the past decade, the number of new species described within this subsection from East and Southeast Asia has increased due to the use of DNA sequence information (Zhao et al. 2015; Zhang et al. 2017; Song et al. 2019; Ghosh et al. 2020; Khatua et al. 2021; Crous et al. 2022; Song 2022).

We aim to clarify the species diversity and geographical distribution of the genus *Russula* in China. From 2012 to 2023, several large-scale fungal surveys were conducted in different regions of China and some interesting specimens were collected. We conducted ITS analyses and found that they belong to the subsect. *Cyanoxanthinae* and are distinct from described species. Subsequently, through morphological comparisons and further multi-gene tree analyses, it was confirmed that they are new species. Here, we propose four new species and provide their morphological descriptions, related illustrations and molecular phylogenetic positions.

Materials and methods

Sampling and morphological studies

The specimens studied in this paper were collected and photographed from five subtropical provinces in China during 2012–2023. Samples were dried at 50 °C and deposited in the Herbarium of the Research Institute of Tropical Forestry, Chinese Academy of Forestry (RITF).

Macro-morphological features were described using detailed field notes and photographs of fresh basidiomata. Colour designations followed those in the Methuen Handbook of Color (Kornerup and Wanscher 1981). The description of microscopic morphological features followed the template of Adamčík et al. (2019). All micromorphological features were observed using a ZEISS Imager M2 microscope (Carl Zeiss AG, Oberkochen, Germany) with oil immersion lenses at a magnification of 1000×. Basidiospores were observed and measured in Melzer's Reagent in side view excluding ornamentation. Other microscopic structures were pretreated in 5% potassium hydroxide (KOH) and then stained with 1% Congo red solution. The pileipellis was examined in cresyl blue for the presence of ortho- or metachromatic reactions (Buyck 1989). Sulphovanillin (SV) was used to determine variation in the cystidia content (Caboň et al. 2017). Measurements of basidiospores are expressed as (Min–)AV-SD–AV– AV+SD(–Max), where Min = the minimum value, Max = the maximum value, AV = average, SD = standard deviation and Q stands for the length/width ratio of the basidiospores. A scanning electron microscope (SEM. JEOL JSM-SU8020) was used to illustrate the structure and ornamentation of the basidiospores.

DNA extraction, amplified and sequenced

A rapid extraction kit for fungal DNA (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to extract total DNA from dried specimens. A total of four nuclear loci including ITS (internal transcribed spacer region of ribosomal DNA), LSU (ribosomal nuclear large subunit), mtSSU (ribosomal mitochondrial small subunit) and *rpb2* (second largest subunit of RNA polymerase II) were amplified and sequenced. The ITS regions were amplified with the ITS1/ITS4 primer pairs (White et al. 1990). The LSU regions were amplified with the LR0R/LR5 primer pairs (Vilgalys and Hester 1990). The *rpb2* regions were amplified with the fRPB2-6F/fRPB2-7cR primer pairs (Matheny 2005). The mtSSU regions were amplified with the MS1/MS2 primer pairs (White et al. 1990). Amplifications were performed in 25 μ I reactions containing 1.5 μ I of Taq DNA polymerase (5U/ μ I, Thermostable DNA polymerase, BBI), 5 μ I of 5× PCR Buffer, 2.5 μ I of dNTP (200 μ mol/I), 3.5 μ I MgCl₂, 1 μ I of each primer (5 μ mol/I), 2 μ I of DNA template and 8.5 μ I of ddH₂O.

The thermal cycling conditions for ITS, LSU, *rpb2* and mtSSU included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s (denaturation); 53 °C for 30 s (ITS), 55 °C for 45 s (LSU), 54 °C for 45 s (mtSSU), 56 °C for 1 min (*rpb2*) (annealing), 72 °C for 1 min (elongation) and a final extension at 72 °C for 10 min. PCR products were purified using Bioteke's Purification Kit (Bioteke Corporation, Beijing, China) and sequenced with 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 have been submitted to the GenBank database. All sequences used in this study are shown in Table 1.

DNA sequence alignments and molecular phylogenetic analyses

Representative sequences of species belonging to the subsect. *Cyanoxanthinae* were obtained from the GenBank database, based on recent published studies (Crous et al. 2022; Song 2022). Two species of subsect. *Substriatinae*, *R. maguanensis* J. Wang, X.H. Wang, Buyck & T. Bau and *R. substriata* J. Wang, X.H. Wang, Buyck & T. Bau, were used as outgroups. Sequences of four genes were aligned separately using MAFFT 7.0 (http://mafft.cbrc.jp/alignment/server/) and the alignment was manually refined using BioEdit v.7.0.9 (Hall 1999).

Phylogenetic analyses, based on a multi-gene matrix, were conducted using both Randomised Accelerated Maximum Likelihood (RAxML) and Bayesian Inference (BI). A partition homogeneity test (PHT) was performed using heuristic searches with PAUP* 4.0a (Swofford 2003) to evaluate incongruence amongst individual genes. The sequences were then concatenated using Phyutility v.2.2 (Smith and Dunn 2008) for multi-gene analyses as no supported conflicts (P < 0.5). The final aligned dataset was submitted to TreeBASE (31001).

 Table 1. GenBank accession numbers for sequences used in this study. The newly-generated sequences are labelled in bold; holotypes are labelled by letter T in parentheses.

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Taxon	Voucher	Location	ITS	LSU	rpb2	mtSSU	References
R. atrochermesina	RITF6878 (T)	Yunnan, south-western China	OR907106	OR907057	OR914538	OR934536	This study
R. atrochermesina	RITF6460	Yunnan, south-western China	OR907107	OR907056		OR934535	This study
R. banwatchanensis	BBH 49228 (T)	Thailand	MT940813	MT940823	MT965687		Crous et al. (2022)
R. cyanoxantha	UE29.09.2002-2	France	DQ422033	DQ422033	DQ421970		Buyck et al. (2008)
R. cyanoxantha	FH 12-201	Germany	KR364093	KR364225	KR364341		De Crop et al. (2017)
R. dinghuensis	K15052704 (T)	Guangdong, southern China	KU863581	MK881922		MK882050	Zhang et al. (2017)
R. dinghuensis	RITF6860	Guangdong, southern China	OR907119	OR907061	OR914549	OR934507	This study
R. dinghuensis	RITF5885	Guangdong, southern China	OR907120		OR914550	OR934503	This study
R. dinghuensis	RITF5142	Jiangxi, central China	OR907123	OR907058	OR914551	OR934502	This study
R. dinghuensis	RITF6855	Guangxi, southern China	OR907121	OR907060	OR914552	OR934504	This study
R. dinghuensis	RITF6740	Guangxi, southern China	OR907122	OR907059	OR914553	OR934506	This study
R. dinghuensis	RITF6859	Guangdong, southern China	OR907124	OR907062	OR914554	OR934505	This study
R. flavobrunnea var. violaceotincta	71/BB 06.050	Madagascar		KU237468	KU237754	KU237312	Buyck et al. (2018)
R. fusiformata	K15052703 (T)	Guangdong, southern China	MK049978	MK881942		MK882070	Song (2022)
R. fusiformata	RITF6671	Zhejiang, eastern China	OR907108	OR907049	OR914567	OR934501	This study
R. fusiformata	RITF6487	Jiangxi, central China	OR907109		OR914563	OR934500	This study
R. lakhanpalii	CAL 1795 (T)	India	NR_173867				
R. lakhanpalii	RITF2600	Hubei, central China	OR907090	OR907076		OR934525	This study
R. lakhanpalii	RITF6973	Yunnan, south-western China	OR907089		OR914542	OR934526	This study
R. lakhanpalii	RITF6868	Yunnan, south-western China	OR907091	OR907078	OR914543	OR934528	This study
R. lakhanpalii	RITF6474	Yunnan, south-western China	OR907092	OR907077	OR914544	OR934527	This study
R. lakhanpalii	RITF6940	Yunnan, south-western China	OR907088	OR907079		OR934529	This study
R. langei	450/BB 07.792	France		KU237510	KU237796	KU237356	Buyck et al. (2018)
R. lavandula	RITF6329	Yunnan, south-western China	OR907083	OR907065	OR914534	OR934513	This study
R. lavandula	RITF6340	Yunnan, south-western China	OR907085	OR907066	OR914535	OR934515	This study
R. lavandula	RITF3196	Yunnan, south-western China	OR907086	OR907067		OR934512	This study
R. lavandula	RITF3282 (T)	Yunnan, southwestern China	OR907087	OR907068	OR914537	OR934511	This study
R. lavandula	RITF6349	Yunnan, south-western China	OR907084	OR907069	OR914536	OR934514	This study
R. lilaceofusca	RITF6330 (T)	Yunnan, south-western China	OR907102	OR907075	OR914539	OR934530	This study
R. lilaceofusca	RITF2645	Hubei, central China	OR907093	OR907074	OR914540	OR934531	This study
R. lilaceofusca	RITF2631	Hubei, central China			OR914541		This study
R. lilaceofusca	RITF3761	Guizhou, south-western China	OR907094				This study
R. lilacina	MMCR00191	Thailand	MT940809	MT940819	MT965685		Crous et al. (2022)
R. lotus	HKAS79209 (T)	Guangdong, southern China	MG214688	MG214695			Li and Deng (2018)
R. lotus	RITF3330	Yunnan, south-western China		OR907050			This study
R. maguanensis	HKAS 102277 (T)	Yunnan, south-western China	MH724918	MH714537	MH939989		Wang et al. (2019)
R. nigrovirens	HKAS 55222 (T)	Yunnan, south-western China	KP171173				Zhao et al. (2015)
R. nigrovirens	RITF6408	Yunnan, south-western China	OR907095	OR907073	OR914568	OR934521	This study
R. pallidirosea	UTC 00274382 (T)	USA	NR_153259				Kropp (2016)
R. perviridis	RITF3131 (T)	Yunnan, south-western China	OR907098	OR907072	OR914548	OR934523	This study
R. perviridis	RITF2912	Xizang, western China	OR907100	OR907070	OR914547	OR934522	This study
R. perviridis	RITF6983	Yunnan, south-western China	OR907099				This study
R. perviridis	RITF6982	Yunnan, south-western China	OR907101	OR907071	OR914545	OR934524	This study
R. perviridis	RITF6734	Sichuan, south-western China			OR914546		This study
R. perviridis	RITF3143	Yunnan, south-western China	OR907097				This study
R. perviridis	RITF6790	Xizang, western China	OR907096				This study
R. phloginea	CNX530524068 (T)	Yunnan, south-western China	MK860701	MK860704		MK860708	Song et al. (2019)

Taxon	Vouchor	Location		Poforonaca			
Taxon	voucher	Location	ITS	LSU	rpb2	mtSSU	References
R. phloginea	RITF6904	Yunnan, south-western China	OR907113				This study
R. phloginea	RITF6905	Yunnan, south-western China	OR907116	OR907051	OR914555	OR934516	This study
R. phloginea	RITF6906	Yunnan, south-western China	OR907117	OR907055	OR914558	OR934520	This study
R. phloginea	RITF6907	Yunnan, south-western China	OR907114	OR907052	OR914556	OR934517	This study
R. phloginea	RITF6908	Yunnan, south-western China	OR907115	OR907054	OR914557	OR934518	This study
R. phloginea	RITF6914	Yunnan, south-western China	OR907118	OR907053	OR914559	OR934519	This study
R. pseudocyanoxantha	CUH AM177 (T)	India	NR_173166				Khatua et al. (2021)
R. purpureorosea	H17050506 (T)	Guangdong, southern China	MK049976	MK881941		MK882069	Song (2022)
R. purpureorosea	RITF6835	Guangdong, southern China	OR907104	OR907082	OR914565	OR934533	This study
R. purpureorosea	RITF6834	Guangdong, southern China	OR907103	OR907081	OR914566	OR934534	This study
R. purpureorosea	RITF5886	Guangdong, southern China	OR907105	OR907080	OR914564	OR934532	This study
R. purpureoviridis	BBH 49226 (T)	Thailand		MT940817	MT965684		Crous et al. (2022)
R. subpallidirosea	K15052818 (T)	Guangdong, southern China	KU863582	MK881923		MK882051	Zhang et al. (2017)
R. subpallidirosea	RITF3219	Yunnan, south-western China	OR907111	OR907063	OR914560	OR934508	This study
R. subpallidirosea	RITF3343	Yunnan, south-western China	OR907112		OR914562	OR934509	This study
R. subpallidirosea	RITF6264	Yunnan, south-western China	OR907110	OR907064	OR914561	OR934510	This study
R. substriata	HKAS 102278 (T)	Yunnan, south-western China	MH724921	MH714540	MH939992		Wang et al. (2019)
R. variata	BPL241	USA	KT933959	KT933818	KT933889		Looney et al. (2016)

A mixed-model (partitioned) scheme was used for ML and BI analyses, ITS, LSU, mtSSU and rpb2. The best-fit model of sequence evolution for each gene was estimated using MrModelTest 2.3 (Nylander 2004), based on the Akaike Information Criterion. The best model for the BI analysis of the ITS and LSU datasets was the GTR+I+G model; the SYM+G model was optimal for rpb2; and the GTR+I model was optimal for mtSSU. RaxML 7.0.3 (Stamatakis 2006) and MrBayes v.3.2 (Ronquist et al. 2012) were used to perform the RAxML and BI analyses, respectively. Maximum Likelihood analysis was conducted using non-parametric bootstrapping with 1,000 replicates. Bootstrap support (BS) above 70% (Nuhn et al. 2013) was considered significant. BI analysis was performed using the Metropolis-coupled Markov Chain Monte Carlo method. Four chains were run for 1,500,000 generations and trees were sampled every 100 generations. Other parameters were kept at their default settings. The analysis was terminated when the average standard deviation of the splitting frequency was below 0.01 and the PSRF values for all parameters were near 1. Bayesian posterior probabilities (BPP) were calculated after discarding the first 25% of the samples as burn-in and branches with BPP over 0.95 were considered significantly supported.

Results

Phylogeny

The four-gene dataset (ITS+LSU+mtSSU+*rpb2*) included 65 samples representing 22 species. The matrix contains a total of 2,786 nucleotide sites, including 652 ITS sites, 896 LSU sites, 516 mtSSU sites and 722 *rpb2* sites. The tree topologies of both the RAxML and BI analyses were similar and only the tree inferred by the ML analysis is displayed, but with both BS and BPP values (Fig. 1).



Figure 1. The RAxML likelihood tree based on the four-gene dataset (ITS+LSU+mtSSU+*rpb2*). Support values (BS > 70%, BPP > 0.95) are displayed above or below the branches, with the maximum values of BS (= 100%) and BPP (= 1.0) represented by asterisks (*). The four new species are marked with different colours.

Our molecular phylogenetic analyses show that *Cyanoxanthinae* can be divided into two major supported lineages and one species from Madagascar. All samples from China fell into major clades and represent 12 species, of which four are new species. The four new species proposed each formed a well-supported branch and were distinct from other related taxa. *Russula atrochermesina* Y.L. Chen & J.F. Liang, sp. nov. (BS = 99%, BPP = 1.00) was closely related to *R. pallidirosea* Kropp and *R. purpureorosea* Y. Song. *Russula lavandula* Y.L. Chen, B. Chen & J.F. Liang, sp. nov. (100% BS and 1.00 BPP) was sister to *R. banwatchanensis* Sommai, Pinruan, Somrith. & Luangsa-ard (80% BS and 0.97 BPP). *Russula lilaceofusca* Y.L. Chen & J.F. Liang, sp. nov. (BS = 96%, BPP = 1.00) was allied to *R. lakhanpalii* (90% BS and 1.00 BPP). *Russula perviridis* Y.L. Chen, B. Chen & J.F. Liang, sp. nov. (BS = 97%, BPP < 0.95) was close to *R. nigrovirens* Q. Zhao, Y.K. Li & J.F. Liang (BS = 83%, BPP < 0.95).

Taxonomy

Russula atrochermesina Y.L. Chen & J.F. Liang, sp. nov. MycoBank No: 851267 Figs 2A–C, 3A–D, 4, 5

Diagnosis. *Russula atrochermesina* can be distinguished by its violet pileus with tuberculate-striate margin, distant lamellae that stain greyish-yellow when bruised, wide hymenial cystidia on lamellae edges, cystidia content negative reaction in sulphovanillin, branched subterminal cells in pileipellis.

Holotype. CHINA, Yunnan Province, Chuxiong Yi Autonomous Prefecture, Lufeng City, Tuoan Township, 25°14'35"N, 101°46'23"E, alt. 2250 m, 7 Sep 2023, Y.L. Chen (RITF6878).

Etymology. 'atrochermesina' refers to the dark purple colour of pileus.

Description. *Basidiomata* medium to large-sized; pileus 75–115 mm in diameter, initially hemispherical when young, convex to applanate when mature, margin tuberculate-striate, not cracked; surface dry, glabrous, peeling readily, violet white (16A2) to pale violet (16A3), dark violet (16F6) in the centre. *Lamellae* adnate, 5–8 per cm near pileus margin, cream, staining greyish-yellow (2B5) when bruised; lamellulae present and irregular in length; furcations frequently present near the stipe; edge entire and concolorous. *Stipe* 80–170 × 25–40 mm, cylindrical to subcylindrical, white (1A1), often with yellowish-white (1A2) tinge, solid. *Context* white (1A1), unchanging when bruised, compact, 5 mm thick in half of the pileus radius; taste mild; odour inconspicuous. *Spore print* not observed.

Basidiospores $(6.3-)6.7-7.2-7.6(-8.7) \times (5.3-)5.8-6.2-6.5(-7.4) µm, Q = (1.05-)1.11-1.15-1.20(-1.25), subglobose to broadly ellipsoid, hyaline in 5% KOH; ornamentation of small to medium, dense (5-9 in a 3 µm diam. circle) amyloid warts, less than 0.6 µm high, mostly isolated or fused in pairs, occasionally fused by short lines; suprahilar plage inamyloid.$ **Basidia**(33.0-)37.0-43.0-48.5(-52.0) × (9.0-)10.0-11.5-12.5(-14.0) µm, clavate, 2- to 4-spored, thin-walled; basidiola clavate, ca. 8.0-12.5 µm wide.**Hymenial cystidia on lamellae sides**moderately numerous, (52.5-)55.0-60.5-65.5(-69.5) × (7.0-)8.0-8.5-9.5(-10.0) µm, fusiform, apically acute, usually with an appendage, thin-walled; contents heteromorphous, yellow in sulphovanillin.**Hymenial cystidia on lamellae edges**longer and wider than those on lamellae sides, (54.5-)58.0-65.0-71.5(-75.5) × (8.0-)9.0-10.5-11.5(-13.0) µm, fusiform, apically acute, usually with an appendage, thin-walled; contents crystalline, yellow in sulphovanillin.**Marginal cells**undifferentiated.**Pileipellis**hyphae of



Figure 2. SEM photos of basidiospores of the four new species **A–C** *Russula atrochermesina* (RITF6878, holotype) **D–F** *Russula lavandula* (RITF6329) **G–I** *Russula lilaceofusca* (RITF6330, holotype) **J–L** *Russula perviridis* (RITF3131, holotype). Scale bars: 20 μm (**G**); 10 μm (**A**, **B**, **D**, **E**, **H**, **J**, **K**); 5 μm (**C**, **F**, **I**, **L**).

all tissues metachromatic in cresyl blue, sharply delimited from the underlying context, two-layered, gelatinised; suprapellis 260–400 µm deep, composed of densely arranged and erect hyphal terminations; subpellis 150–200 µm deep, composed of horizontally orientated and 2–9 µm wide hyphae. Hyphal terminations near the pileus margin branched, thin-walled; terminal cells (9.0–)13.5–18.0–22.5(–27.5) × (2.5–)3.5–4.0–5.0 µm, mainly clavate, occasionally cylindrical, apically mostly obtuse; subterminal cells usually shorter, ca. 3.0–5.5 µm wide, branched. Hyphal terminations near the pileus centre slightly narrower than those near the pileus margin; terminal cells (10.0–)13.5–17.5–21.5(–26.5) × (2.5–)3.0–3.5–4.0(–5.0) µm, cylindrical, slightly attenuated. *Pileocystidia* near the pileus margin always 1-celled, (22.5–)28.5–36.0–44.0(–50.0) × (4.0–)4.5–5.0–6.0(–6.5) µm, dispersed, clavate, occasionally fusiform,



Figure 3. Fruiting bodies photos of four new species **A–D** *Russula atrochermesina* (A-C-RITF6878, holotype, D-RITF6460) **E–I** *Russula lavandula* (E-F-RITF6329, G-H-RITF6340, I-RITF3282, holotype) **J–L** *Russula lilaceofusca* (J-K-RITF6330, holotype, L-RITF2631) **M–R** *Russula perviridis* (M-N-RITF3131, holotype, O-P-RITF6982, Q-R-RITF6983). Scale bars: 40 mm (**A**, **B**, **D–G**, **I**, **J**, **M–O**); 5 mm (**C**, **L**); 20 mm (**H**, **K**, **P**, **Q**, **R**).



Figure 4. *Russula atrochermesina* (RITF6878, holotype) **A** basidia **B** basidiola **C** hymenial cystidia on lamellae sides **D** hymenial cystidia on lamellae edges. Scale bar: 10 μm.



Figure 5. *Russula atrochermesina* (RITF6878, holotype) **A** hyphal terminations near the pileus margin **B** hyphal terminations near the pileus centre **C** pileocystidia near the pileus margin **D** pileocystidia near the pileus centre. Scale bar: 10 µm.

apically usually obtuse, sometimes with a round or elliptical appendage, thinwalled; contents crystalline, no reaction in sulphovanillin. Pileocystidia near the pileus centre slightly narrower than those near the pileus margin, (30.0-)32.0- $38.0-44.0(-51.5) \times (3.5-)4.0-4.5-5.0(-5.5) \mu m$.

Habitat. On the ground under mixed forests dominated by *Castanopsis* sclerophylla and *Pinus yunnanensis*.

Known distribution. South-western China (Yunnan Province).

Additional specimens examined. CHINA, Yunnan Province, Chuxiong Yi Autonomous Prefecture, Lufeng City, Tuoan Township, 25°14'34"N, 101°45'39"E, alt. 2300 m, 28 Jul 2022, X.L. Gao (RITF6460).

Notes. Phylogenetic analyses showed that R. atrochermesina is related to R. pallidirosea, R. purpureorosea and R. purpureoviridis Khamsuntorn, Lueangjaroenkit, Sommai & Pinruan. However, R. pallidirosea from American Samoa has a pallid to pinkish pileus and smaller hymenial cystidia on the lamellae edges $(40.0-55.0 \times 5.0-7.0 \ \mu\text{m})$ and is associated with tropical trees (Kropp 2016). Russula purpureorosea differs in the following set of characters: pale pinkish-purple pileus, lamellae furcations absence, narrower hymenial cystidia on lamellae edges (54.0-60.0-95.0 × 7.0-8.5-10.0 μ m) and unbranched hyphal terminations in the pileipellis (Song 2022). Russula purpureoviridis, originally described from Thailand, differs in a greyish-green pileus, narrower hymenial cystidia on lamellae edges (45.0-77.5 × 7.5-10.0 µm), constricted terminal cells in the pileipellis and larger pileocystidia (52.5-87.5 × 5.0-8.5 µm) (Crous et al. 2022). The morphology of *R. atrochermesina* is similar to *R. fusiformata*. However, R. fusiformata lacks lamellulae and furcations and has narrower hymenial cystidia on lamellae edges (35.5-52.0-78.0 × 5.0-9.0-11.0 µm) and shorter and wider terminal cells near the pileal centre $(5.5-14.0 \times 3.0-8.0 \mu m)$ (Song 2022).

Russula lavandula Y.L. Chen, B. Chen & J.F. Liang, sp. nov. MycoBank No: 851264 Figs 2D–F, 3E–I, 6, 7

Diagnosis. *Russula lavandula* is characterised by its purplish-white to violet red pileus with a yellow centre, frequently present lamellulae and furcations, stipe often with pale yellow near the base, isolated basidiospores ornamentation and unbranched cuticular hyphal terminations. It is mainly distinguished from *R. lotus* Fang Li by its frequently present lamellulae and furcations, subglobose to broadly ellipsoid basidiospores, moderately numerous and narrower hymenial cystidia on lamellae sides and shorter cuticular terminal cells.

Etymology. 'lavandula' refers to the colour of its pileus similar to lavender.

Holotype. CHINA, Yunnan Province, Kunming City, Wild Duck Lake, 25°07'34"N, 102°51'42"E, alt. 2100 m, 27 Jul 2014, H.J. Li (RITF3282).

Description. *Basidiomata* medium-sized; pileus 40–80 mm in diameter, initially hemispherical when young, convex to applanate with a depressed centre after maturity; margin incurved, striation short or inconspicuous, cracked after maturity; surface dry, glabrous, peeling readily, locally cracking into pale yellow (2A3), purplish-white (14A2) to greyish-magenta (13D5 or 14E6) patches, rose (13B3), purplish-white (14A2) to violet red (14B6), sometimes white (1A1) at the margin, yellowish-white (1A2) to golden yellow (4C7) in the centre. *Lamellae* adnate to slightly adnexed, 10–13 per cm near pileus margin, white (1A1), unchanging when bruised, 3–4 mm wide; lamellulae usually present and irregular in length; furcations frequently present throughout the lamellae; edge entire and concolorous. *Stipe* 40–60 × 16–25 mm, cylindrical, flexuous and tapering towards the base, white (1A1), often with pale yellow tinge near the base, solid.



Figure 6. *Russula lavandula* (RITF3282, holotype) **A** basidia **B** hymenial cystidia on lamellae sides **C** hymenial cystidia on lamellae edges **D** basidiola. Scale bar: 10 µm.

Context white (1A1), unchanging when bruised, 3–4 mm thick in half of the pileus radius; taste mild; odour inconspicuous. **Spore print** not observed.

Basidiospores (6.5–)7.1–7.7–8.3(–9.2) × (5.9–)6.4–6.9–7.5(–8.0) μ m, Q = (1.01–)1.05–1.11–1.17(–1.24), subglobose to broadly ellipsoid, hyaline in 5% KOH; ornamentation of small, moderately distant to dense (6–8 in a 3 μ m diam. circle) amyloid warts, less than 0.5 μ m high, mostly isolated, occasionally connected by short line connections or ridges, not forming a reticulum;



Figure 7. *Russula lavandula* (RITF3282, holotype) **A** pileocystidia near the pileus margin **B** pileocystidia near the pileus centre **C** hyphal terminations near the pileus margin **D** hyphal terminations near the pileus centre. Scale bar: 10 µm.

suprahilar plage indistinct, inamyloid. **Basidia** (24.0–)28.5–33.5–39.0(–43.5) × (6.0–)7.0–8.5–9.5(–10.5) μ m, clavate, 2- to 4-spored, thin-walled; basidiola clavate or subcylindrical, ca. 5–10 μ m wide. **Hymenial cystidia on lamellae** sides moderately numerous, (42.5-)47.0-54.5-62.5(-64.5) × (5.0-)6.5-7.5-8.5 µm, clavate or fusiform, apically mostly obtuse, partially acute, sometimes with a 4-6 µm and papillate appendage, thin-walled; contents granulose or heteromorphous, reddish-black in sulphovanillin. Hymenial cystidia on lamellae edges shorter, but wider than those on lamellae sides, (35.0-)39.0-46.0-52.5(-56.0) × (6.0-)7.0-8.1-9.2(-10.0) µm, mostly clavate or subcylindrical, apically mostly obtuse, occasionally with 2-5 µm long, papillate or moniliform appendage. Marginal cells undifferentiated. Pileipellis only hyphae of suprapellis metachromatic in cresyl blue, sharply delimited from the underlying context, 300-450 µm deep, two-layered, gelatinised; suprapellis 180-200 µm deep, composed of densely arranged and prostrate to erect hyphal terminations; subpellis 140-260 µm deep, composed of horizontally orientated, intricate and 3-5 um wide hyphae. Hyphal terminations near the pileus margin unbranched, thinwalled, occasionally flexuous; terminal cells (12.5-)14.5-19.5-24.5(-26.0) \times 4.5-5.0-5.5(-6.5) µm, mainly cylindrical, occasionally lageniform, apically mostly obtuse; subterminal cells usually shorter and slightly wider, ca. 4-7 um wide, unbranched. Hyphal terminations near the pileus centre shorter and narrower than those near the pileus margin; terminal cells (11.0-)13.5-17.0- $20.5(-21.5) \times (3.5-)4.0-4.2-4.5 \ \mu m$, clavate or cylindrical, apically obtuse; subterminal cells usually wider, ca. 4-6 µm, unbranched. Pileocystidia near the pileus margin always 1-celled, (20.0-)27.0-36.0-45.0(-48.0) × 4.5-5.5-6.0(-6.5) µm, clavate, occasionally fusiform, apically usually obtuse, sometimes with 2-6 µm long, round or elliptical appendage, thin-walled; contents crystalline, reddish-black in sulphovanillin. Pileocystidia near the pileus centre similar to those near the pileus margin, $(20.0-)26.0-36.0-45.5(-52.5) \times 4.0-$ 5.0-5.5(-6.0) µm.

Habitat. On the ground under mixed forests of *Pinus yunnanensis*, *Lithocarpus dealbatus* and *Quercus* spp.

Known distribution. South-western China (Yunnan Province).

Additional specimens examined. CHINA, Yunnan Province, Chuxiong Yi Autonomous Prefecture, Lufeng City, Guangtong Town, 25°14'43"N, 101°45'53"E, 18 Sep 2022, X.L. Gao (RITF6329); ibid, 25°14'48"N, 101°45'28"E, alt. 2400 m, 24 Sep 2022, X.L. Gao (RITF6340); ibid, 25 Sep 2022, X.L. Gao (RITF6349); Kunming City, Wuhua District, Qiongzhu Temple, 25°3'58"N, 102°37'29"E, alt. 2150 m, 28 Jul 2012, Y.J. Hao (RITF3196).

Notes. *Russula lavandula* is phylogenetically related to Thai *R. banwatchanensis* and Indian *R. pseudocyanoxantha* Paloi, K. Acharya & S. Khatua (Fig. 1). However, *R. lavandula* can be easily distinguished from them by its cracked pileus. Moreover, *R. banwatchanensis* differs in its darker coloured pileus, lack of lamellulae, thick-walled basidia and often longer pileocystidia of 42.5–127.0 × 2.5–5.0 µm (Crous et al. 2022) and *R. pseudocyanoxantha* differs in its darker coloured pileus, lack of lamellae furcations and association with *Shorea robusta* (Khatua et al. 2021). *Russula lavandula* can be easily confused with four Chinese species, via. *R. lotus*, *R. phloginea* J. Song & J.F. Liang, *R. purpureorosea* and *R. subpallidirosea* J.B. Zhang and L.H. Qiu in the field. However, *R. lotus*, originally described from southern China, can be easily distinguished by its absence of lamellae furcations, broadly ellipsoid to ellipsoid basidiospores, dispersed and wider hymenial cystidia on lamellae sides (52.0–70.0 × 10.0–16.0 µm) and longer cuticular terminal cells (10.0–40.0 × 4.0–8.0 µm) (Li and

Deng 2018). *Russula phloginea*, which occurs in subalpine areas, differs in having lamellae furcations only present near the stipe, smaller basidiospores of $(6.0-)6.5-8.0 \times 5.0-6.5 \mu m$, longer hymenial cystidia of $(48.0-)60.0-78.5(-79.5) \times 7.5-9.5(-10.0) \mu m$ and pileocystidia with a moniliform apex (Song et al. 2019). *Russula purpureorosea* and *R. subpallidirosea*, can be distinguished from *R. lavandula* by their rosy brown, pale pinkish-purple or pale greyish-pink pileus centre and occurrence at low altitudes (Zhang et al. 2017; Song 2022). Besides, *R. purpureorosea* lacks lamellae furcations and has shorter terminal cells of the pileipellis ($6.5-15.5 \times 2-5.5 \mu m$) and wider pileocystidia ($17.0-53.0 \times 4.5-9.0 \mu m$) (Song 2022). In addition, *R. cyanoxantha*, can be confused with *R. lavandula*. However, *R. cyanoxantha* can be distinguished by its uncracked pileus cuticle, longer hymenial cystidia up to 100 µm and slender cuticular hyphal end cells of $2-3 \mu m$ (Bon 1988; Sarnari 1998).

Russula lilaceofusca Y.L. Chen & J.F. Liang, sp. nov.

MycoBank No: 851265 Figs 2G-I, 3J-L, 8, 9

Diagnosis. *Russula lilaceofusca* is mainly characterised by its lilac brown to dark brown pileus, crowded lamellae with the presence of lamellulae and furcations, stipe often turning reddish-yellow when bruised, subreticulate basid-iospores ornamentation and clavate hymenial cystidia often with capitate appendage whose contents change to reddish black in sulphovanillin. It can differ from *R. cyanoxantha* in shorter hymenial cystidia and wider cuticular terminal cells and differ from *R. fusiformata* in frequently present lamellulae and furcations, clavate hymenial cystidia on lamellae edges that have no reaction in sulphovanillin and occasionally branched cuticular hyphal terminations.

Holotype. CHINA, Yunnan Province, Chuxiong Yi Autonomous Prefecture, Lufeng City, G30).

Etymology. 'lilaceofusca' refers to a lilac brown pileus.

Description. *Basidiomata* medium-sized; pileus 40–60 mm in diameter, initially hemispherical when young, convex to applanate after mature; margin incurved, no striation, not cracked; surface dry, glabrous, reddish-white (8A2), lilac (15B4), brown (7E5) to dark brown (7F5). *Lamellae* adnate, very crowded, 20–24 per cm near pileus margin, cream, unchanging when bruised; lamellulae present and irregular in length; furcations frequently present; edge entire and concolorous. *Stipe* 35–50 × 8–17 mm, cylindrical to subcylindrical, slightly expanded towards the base, white (1A1), staining reddish-yellow (4A6) when touched, solid. *Context* white (1A1), unchanging when bruised, soft; taste mild; odour inconspicuous. *Spore print* not observed.

Basidiospores (6.5–)7.0–7.8–8.7(–10.5) × (5.5–)6.1–6.9–7.7(–9.4) µm, Q = (1.05–)1.09–1.13–1.17(–1.26), subglobose to broadly ellipsoid; ornamentation of small to medium, dense (5–9 in a 3 µm diam. circle) amyloid warts, less than 0.5 µm high, subreticulate, connected by short line connections or ridges; suprahilar plage large, inamyloid. **Basidia** (26.5–)30.5–38.5–47.0(– 56.0) × (9.5–)10.5–12.5–14.0(–15.5) µm, clavate or ellipsoid, 1- to 4-spored, thin-walled; basidiola clavate or ellipsoid, ca. 8.0–13.0 µm wide. **Hymenial cystidia on lamellae sides** dispersed to moderately numerous, (39.5–)43.5–48.5–


Figure 8. *Russula lilaceofusca* (RITF6330, holotype) **A** hymenial cystidia on lamellae sides **B** basidiola **C** marginal cells **D** basidia **E** hymenial cystidia on lamellae edges. Scale bar: 10 µm.

53.5(-60.0) × (8.0-)9.5-10.5-11.5(-12.0) µm, mostly clavate, apically mostly obtuse, often with a 2.0-4.5 µm round appendage, thin-walled; contents granulose or heteromorphous, reddish-black in sulphovanillin. *Hymenial cystidia on lamellae edges* shorter and narrower than those on lamellae sides, (31.0-)36.0-41.5-47.0(-54.0) × (7.0-)7.5-8.5-10.0(-10.5) µm, mostly clavate, apically mostly obtuse, occasionally with a 2-3 µm long round or elliptical appendage, thin-walled; contents heteromorphous, reddish-black in sulphovanillin. *Marginal cells* (13.5-)15.0-18.5-22.0(-26.0) × 4.0-5.0-5.5(-6.5) µm, lageniform, clavate or subcylindrical. *Pileipellis* only hyphae of suprapellis metachromatic in cresyl blue, sharply delimited from the underlying context, two-layered, gelatinised; suprapellis 130-200 µm deep, composed of loosely arranged and erect hyphal terminations; subpellis 90-200 µm deep, composed of horizontally orientated and intricate hyphae. Hyphal terminations near the pileus margin occasionally branched, thin-walled, often flexuous; terminal cells (11.0-)12.5-18.5-24.0(-32.0) × (3.0-)3.5-4.0-5.0(-5.5) µm, mainly lageniform or cylin-



Figure 9. *Russula lilaceofusca* (RITF6330, holotype) **A** hyphal terminations near the pileus margin **B** hyphal terminations near the pileus centre **C** pileocystidia near the pileus margin **D** pileocystidia near the pileus centre. Scale bar: 10 µm.

drical, apically obtuse, sometimes attenuated or constricted; subterminal cells usually longer and slightly wider, ca. $3.5-7.0 \mu m$ wide, occasionally branched. Hyphal terminations near the pileus centre slightly shorter than those near the pileus margin; terminal cells $(9.5-)11.5-16.0-20.0(-25.5) \times (3.0-)3.5-4.0-5.0(-5.5) \mu m$, lageniform or cylindrical, apically obtuse, sometimes attenuated or constricted; subterminal cells usually equal or slightly wider, ca. $2.8-6.6 \mu m$,

occasionally branched. *Pileocystidia* near the pileus margin always 1-celled, $(23.5-)26.5-35.0-43.5(-51.0) \times (3.5-)4.0-4.5-5.5 \ \mu\text{m}$, subfusiform or cylindrical, apically usually obtuse, always with 2–3 μ m long, round or elliptical appendage, thin-walled; contents heteromorphous, reddish-black in sulphovan-illin. Pileocystidia near the pileus centre similar to those near the pileus margin, $(30.5-)34.5-39.5-44.5(-53.0) \times 3.5-4.0-4.5(-5.5) \ \mu\text{m}$.

Habitat. On the ground of broad-leaved forests dominated by *Quercus* spp. or mixed forests with *Pinus* spp.

Known distribution. Central (Hubei Province) and south-western China (Guizhou and Yunnan Provinces).

Additional specimens examined. CHINA, Guizhou Province, Zunyi City, Kuankuoshui National Nature Reserve, 28°14'29"N, 107°11'59"E, alt. 1750 m, 27 Sep 2014, H.J. Li (RITF3761); Hubei Province, Shennongjia Forest District, Jianglongping, 31°25'46"N, 110°20'18"E, alt. 1500 m, 9 Aug 2015, Y.K. Li (RITF2645); ibid, 11 Aug 2015, Y.K. Li (RITF2631).

Note. Phylogenetic analyses showed that *R. lilaceofusca* was closely related to the Indian species *R. lakhanpalii* and *R. variata* from the United States. However, *R. lakhanpalii* differs in having a yellowish-white to pale yellow areolate pileus with orange-brown centre, cystidia that show no change in sulphovanillin and longer and wider pileocystidia of $(27.0-)40.0-63.0-86.5(-123.0) \times (4.0-)4.5-5.5-6.5(-7.0) \mu m$ near the pileus margin (Ghosh et al. 2020). *Russula variata* differs in having a green pileus and basidiospores with higher warts of 0.4–1.0 µm (Hessler 1960; Burge 1979). In morphology, Chinese *R. fusiformata* Y. Song can be easily confused with this species. However, *R. fusiformata* can be distinguished by its striate pileus margin, absence of lamellulae and furcations, fusiform hymenial cystidia on the lamellae edges, cystidia that show a negative reaction in sulphovanillin and unbranched terminal cells in the pileipellis (Song 2022). *Russula lilaceofusca* is similar to *R. cyanoxantha*, but *R. cyanoxantha* has a greenish-violet pileus, longer hymenial cystidia up to 100 µm and slender cuticular hyphal end cells of 2–3 µm (Romagnesi 1967; Bon 1988; Sarnari 1998).

Russula perviridis Y.L. Chen, B. Chen & J.F. Liang, sp. nov.

MycoBank No: 851266 Figs 2J–L, 3M–R, 10, 11

Diagnosis. *Russula perviridis* is characterised by its large basidiomata, smooth pileus surface, frequently present lamellulae and furcations, a coarser stipe with yellow-brown tinge, globose to broadly ellipsoid basidiospores with locally reticulate ornamentation, long hymenial cystidia that turn greyish-black in sulphovanillin and is symbiotic with *Quercus semecarpifolia*. It differs from *R. dinghuensis* in longer and wider hymenial cystidia on lamellae edges, subreticulate basidiospores ornamentation and is associated with *Quercus semecarpifolia*. It differs from *R. nigrovirens* in frequently present furcations, subreticulate basidiospores ornamentation, longer and wider hymenial cystidia and related host plants.

Holotype. CHINA, Yunnan Province, Diqing Tibetan Autonomous Prefecture, Shangri La County, Bitahai Nature Reserve, 27°49'42"N, 99°59'30"E, alt. 3600 m, 20 Aug 2014, Q. Zhao (RITF3131).



Figure 10. *Russula perviridis* (RITF3131, holotype) **A** basidia **B** basidiola **C** marginal cells **D** hymenial cystidia on lamellae sides **E** hymenial cystidia on lamellae edges. Scale bar:10 µm.

Etymology. 'perviridis' refers to a dark green pileus.

Description. *Basidiomata* large-sized; pileus 90–150 mm in diameter, initially hemispherical to when young, applanate with a depressed centre when mature; margin usually incurved, striation short or inconspicuous, cracked when mature; surface dry, smooth and glabrous, peeling readily, greyish-green (27E5–30C5) to dark green (30F5), sometimes yellow-brownish (2C5) near the centre, greyish-yellow (2C3) or greenish-white (26A2) at the margin. *Lamellae* adnate to subfree, 9–12 per cm near pileus margin, white to cream, staining yellow-ish-brown (5E6) when bruised, 6–8 mm wide; lamellulae present and irregular in length; furcations frequently present near the stipe to half of the lamellae;



Figure 11. *Russula perviridis* (RITF3131, holotype) **A** pileocystidia near the pileus margin **B** pileocystidia near the pileus centre **C** hyphal terminations near the pileus margin **D** hyphal terminations near the pileus centre. Scale bar: 10 µm.

edge entire and concolorous. *Stipe* $70-90 \times 20-40$ mm, cylindrical, white (1A1) with yellow-brown (2C5) tinge, solid. *Context* white (1A1), unchanging when bruised, 4-9 mm thick in half of the pileus radius, relatively soft; taste mild; odour inconspicuous. *Spore print* not observed.

Basidiospores (5.9-)7.1-7.7-8.3(-9.7) × (5.2-)6.1-6.8-7.4(-8.4) µm, Q = (1.00-)1.07-1.14-1.21(-1.31), globose to broadly ellipsoid, hyaline in 5% KOH; ornamentation of small, dense (5-10 in a 3 µm diam. circle) amyloid warts, less than 0.5 µm high, reticulate, connected by short line connections or ridges; suprahilar plage large, inamyloid. Basidia (26.0-)34.5-42.0-59.0(-55.0) × (8.0-)9.5-11.0-12.0(-13.0) µm, clavate, 2- to 4-spored, thin-walled; basidiola clavate, ca. 6-11 µm wide. Hymenial cystidia on lamellae sides moderately numerous, (56.5-)62.5-69.5-77.0(-81.5) × (6.5-)7.0-7.5-8.0 µm, fusiform or clavate, apically mostly obtuse, sometimes acute, thin-walled; contents heteromorphous, grevish-black in sulphovanillin. Hymenial cystidia on lamellae edges similar to those on lamellae sides, (45.0)54.0-64.5-74.5(-79.5) × 6.0-7.5-8.5(-10.0) µm, mostly clavate, occasionally fusiform, apically mostly obtuse, sometimes with 3-5 µm long appendage, thin-walled; contents granulose or heteromorphous, greyish-black in sulphovanillin. Marginal cells (23.0-)24.5- $27.5-33.5 \times (4.5-)5.5-6.0-7.0 \mu m$, clavate or subcylindrical, occasionally flexuous. Pileipellis only hyphae of suprapellis weakly metachromatic in cresyl blue, sharply delimited from the underlying context, 300-360 µm deep, two-layered, not gelatinised; suprapellis 180-200 µm deep, composed of erect and densely arranged hyphal terminations; subpellis 140-180 µm deep, composed of horizontally orientated, intricate and 3-5 µm wide hyphae. Hyphal terminations near the pileus margin occasionally branched, thin-walled, sometimes flexuous; terminal cells (17.0-)20.0-25.5-31.5(-38.0) × 3.0-4.0-4.5(-6.0) µm, usually clavate or subcylindrical, apically tapering; subterminal cells usually shorter, ca. 4-6 µm wide, occasionally branched. Hyphal terminations near the pileus centre shorter than those near the pileus margin; terminal cells (13.0-)14.5-20.5-26.5(-32.0) × 3.5-4.0-4.5 µm. Pileocystidia near the pileus margin always 1-celled, (36.5-)40.5-47.5-55.0(-60.0) × (3.0-)3.5-4.0-4.5 µm, fusiform or subcylindrical, apically usually obtuse, sometimes with 2-6 µm long, round or elliptical appendage, thin-walled; contents heteromorphous or granulose, greyish-black in sulphovanillin. Pileocystidia near the pileus centre similar to those near the pileus margin, $(26.5-)28.0-36.0-44.0(-50.5) \times 3.0-3.5-4.5 \,\mu m$.

Habitat. On the ground under broad-leaved forests dominated by *Quercus* semecarpifolia.

Known distribution. South-western (Sichuan and Yunnan Provinces) and western China (Xizang Autonomous Region).

Additional specimens examined. CHINA, Sichuan Province, Ganzi Tibetan Autonomous Prefecture, Daocheng County, Yading Village, 4 Aug 2022, X.L. He (RITF6734); Yunnan Province, Diqing Tibetan Autonomous Prefecture, Deqin County, Wudi Lake, 27°48'47"N, 99°42'50"E, alt. 3300 m, 11 Sep 2023, Y.L. Chen & J.Y. Liang (RITF6982, 6983); Shangri La City, Bita Sea Scenic Area, 27°49'39"N, 99°59'28"E, alt. 3600 m, 15 Jul 2014, Q. Zhao (RITF3143); Xizang Autonomous Region, Linzhi City, Motuo County, 29°22'13"N, 95°26'59"E, alt. 1800 m, 15 Jul 2014, Q. Zhao (RITF2912); Lhasa City, Mozhugongka County, Zhaxigang Township, 29°42'10"N, 92°4'44"E, alt. 4500 m, 7 Aug 2023, Y. Zhang (RITF6790).

Notes. Phylogenetic analyses showed that R. perviridis is related to two European species, R. langei Bon and R. cyanoxantha and the Chinese species R. nigrovirens. However, R. langei has a violaceous pileus, a white stipe with a lilac tinge and narrower cuticular hyphal terminations (2.0-3.0 µm) (Bon 1988), whereas R. cyanoxantha has non-reticulate basidiospore ornamentation and longer hymenial cystidia up to 100 µm (Bon 1988; Sarnari 1998). Russula nigrovirens differs in having patches on the pileus surface, furcations rarely present near the stipe, basidiospore ornamentation that does not form a reticulum and smaller hymenial cystidia ($46.0-55.0 \times 6.5-8.5 \mu m$) on the lamellae edges (Zhao et al. 2015). Morphologically, R. perviridis has a green pileus as in Chinese R. dinghuensis J.B. Zhang & L.H. Qiu. However, R. dinghuensis differs in its shorter and narrower hymenial cystidia on lamellae edges (45.0-52.0 × 4.0-6.0 µm) and isolated basidiospore warts (Zhang et al. 2017). Furthermore, R. perviridis can be clearly distinguished from similar Cyanoxanthinae species in its habitat. Russula perviridis is distributed in subalpine areas (over 2,000 m) and is associated with Quercus semecarpifolia. However, R. nigrovirens is associated with Picea spp., Rhododendron spp., Sorbus spp. and Abies spp. and *R. dinghuensis* is distributed in low-altitude areas (below 1,000 m).

Key to known species in R. subsect. Cyanoxanthinae in Asia

1	Lamellulae absent or rare2
-	Lamellulae irregularly inserted, but frequent (more than usual in subg. Heterophyllidiae)
2	Association with Pinus merkusii
_	Association with FagaceaeR. fusiformata
3	Lamellae and context changing pale purplish-pink when bruised, hyme-
	nial cystidia on lamellae edges numerous and short (35.0-57.5 × 10.0-
	10.5 μm) R. lilacina
-	Lamellae and context unchanging when bruised, hymenial cystidia on
	lamellae edges dispersed to moderately numerous and long (45.0–77.5 \times
	7.5–10.0 μm)
4	Basidiospores ornamentation mainly subreticulate to reticulate5
-	Basidiospores ornamentation mainly isolated7
5	Lamellae instant (9–12/cm at pileus margin), average length of hymenial
	cystidia on lamellae edges over 50 μm R. perviridis
-	Lamellae crowed to very crowed (14-27/cm at pileus margin), average
	length of hymenial cystidia on lamellae edges less than 50 μm 6
6	Hymenial cystidia on lamellae sites short and wide of (39.5–)43.5–48.5–
	$53.5(-60.0) \times (8.0-)9.5-10.5-11.5(-12.0) \ \mu\text{m}$, clavate, pileocystidia near
	the pileus margin short and narrow [(23.5-)26.5-35.0-43.5(-51.0) \times
	(3.5–)4.0–4.5–5.5] μm <i>R. lilaceofusca</i>
-	Hymenial cystidia on lamellae sites short and wide of (45-)47.2-548.5-
	61.8(-75) × (6-)6.6-7.3-7.9(-8) μ m, cylindrical to subclavate to fu-
	siform, pileocystidia near the pileus margin long and wide [(27-)39.8-
	63.1-86.5(-123) × (4-)4.4-5.4-6.4(-7)] μm <i>R. lakhanpalii</i>
7	Lamellae furcations absent or rare8
-	Lamellae furcations irregularly inserted, but frequent (more than usual in
	subg. Heterophyllidiae)12

-	Appearing in subalpine forest dominated by <i>Picea</i> spp., <i>Rhododendron</i> spp., <i>Sorbus</i> spp. and <i>Abies</i> spp R. nigrovirens
-	Appearing in evergreen broad-leaved forest dominated by <i>Shorea robusta</i> , <i>Lithocarpus corneus</i> . <i>Quercus</i> spp
9	Hymenial cystidia on lamellae edges narrow (not exceeding 7 μ m wide)
-	Hymenial cystidia on lamellae edges wide (most cells exceeding / μm wide)9
10	Basidiospores broadly ellipsoid to ellipsoid, occasionally subglobose
-	Basidiospores subglobose to broadly ellipsoid11
11	Lamellae adnate, context unchanging when bruising or with $FeSO_4$, hyme-
	nial cystidia on lamellae edges short (36–64.5 × 7.2–10.8 μm), pileocys-
	tidia short and narrow (18.2–32 \times 3.5–5.4 $\mu m)$ $\textbf{R.}$ pseudocyanoxantha
-	Lamellae adnexed to subdecurrent, context changing light yellow when
	bruising or with FeSO_4 , hymenial cystidia on lamellae edges long (54–60–
	$95 \times 7-8.5-10 \ \mu$ m), pileocystidia short and narrow (17-53 × 4.5-9 μ m)
	R purpureorosea
	n pupuler occu
12	Basidiospores globose to subglobose (Q < 1.15) R. banwatchanensis
12 -	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13
12 - 13	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45)
12 - 13	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45) <i>R. subpallidirosea</i>
12 - 13 -	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45) <i>R. subpallidirosea</i> Basidiospores subglobose to broadly ellipsoid (Q = 1.15–1.30) 14
12 - 13 - 14	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45)13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45)
12 - 13 - 14 -	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45)
12 - 13 - 14 - 15	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45) <i>R. subpallidirosea</i> Basidiospores subglobose to broadly ellipsoid (Q = 1.15–1.30)
12 - 13 - 14 - 15	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45)13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45)
12 - 13 - 14 - 15	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = $1.15-1.45$) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = $1.30-1.45$) <i>R. subpallidirosea</i> Basidiospores subglobose to broadly ellipsoid (Q = $1.15-1.30$) 14 Pileocystidia often with moniliform appendage
12 - 13 - 14 - 15	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = $1.15-1.45$) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = $1.30-1.45$)
12 - 13 - 14 - 15	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45)
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12 - 13 - 14 - 15	Basidiospores globose to subglobose (Q < 1.15) <i>R. banwatchanensis</i> Basidiospores subglobose to ellipsoid (Q = 1.15–1.45) 13 Basidiospores broadly ellipsoid to ellipsoid (Q = 1.30–1.45)

Discussion

Our multi-gene phylogenetic analyses (Fig. 1) indicate that species with white spore print, basidiospores ornamented by warts with inamyloid suprahilar spot, metachromatic pileipellis and one-celled pileocystidia constitute subsect. *Cyanoxanthinae*. The analyses also suggest that *Cyanoxanthinae* can be divided into two major lineages, which may be distinguishable by the connections of basidiospores ornamentation. Clade 1 represented by *R. lilaceofusca* and *R. perviridis* has more connections between the warts. Clade 2 represented by *R. lavandula* and *R. atrochermesina* has more isolated warts. Additional studies are need to determine the critical point for the number or density of connections. There are no other morphological differences that can clearly correspond to these two lineages.

We recommend several main characteristics that can be used to distinguish species within *Cyanoxanthinae*. The first, lamellulae and furcations are crucial characters in our view. Except for *R. fusiformata* having completely regular lamellae, the remaining species in *Cyanoxanthinae* have inserted lamellulae or furcations. The density of lamellulae and furcations may require our attention in future research. The second, the species of *Cyanoxanthinae* have different shape of basidiospores (Q values). Four Chinese species, *R. dinghuensis*, *R. nigrovirens*, *R. lotus* and *R. subpallidirosea* have ellipsoid basidiospores (Q > 1.3), while other species have broadly ellipsoid basidiospores (Q < 1.3).

The subsect. *Cyanoxanthinae* is a widely distributed group, ranging from tropical forests to temperate regions and have formed ectomycorrhizal relationships with various host plants. All known species come from the Northern Hemisphere, except for one species from Madagascar. Australia is an unexplored continent for studying this group. For the level of species, there seems to be restricted life zones and specific hosts. For example, we found that *R. perviridis* is only distributed in the subalpine zone and only forms a symbiotic relationship with *Quercus semecarpifolia*. The European *R. cyanoxantha* is well-known to many people in China. Despite our team conducting surveys in the southern provinces of China (subtropical regions) for over a decade, we have not found it there. This means it is a strictly temperate species.

Currently, it is known that there are 16 species in the subsect. *Cyanoxanthinae* distributed in Asia, including the four new species described in this article. These species are all found from tropical or subtropical regions and, so far, there have been no new species found from temperate Asia. In view of the wide distribution of members of the subsection in Europe, more attention needs to be paid to temperate Asia.

Amongst the 12 known species in China, we can only confirm that *R*. *phloginea* is edible and more support is needed for the edibility of the remaining species.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Writing the original draft: Yanliu Chen; improving the manuscript: Junfeng Liang; providing fund projects: Junfeng Liang and Bin Chen; collecting studied specimens: Yanliu Chen, Bin Chen, Ruoxi Liang, Shengkun Wang, Mengya An, Jinhua Zhang, Jingying Liang, Yaxin Wang and Xuelian Gao. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

ITS

Authors: Yanliu Chen, Bin Chen, Ruoxi Liang, Shengkun Wang, Mengya An, Jinhua Zhang, Jingying Liang, Yaxin Wang, Xuelian Gao, Junfeng Liang

Data type: fas

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Link: https://doi.org/10.3897/mycokeys.107.123304.suppl1

Supplementary material 2

LSU

Authors: Yanliu Chen, Bin Chen, Ruoxi Liang, Shengkun Wang, Mengya An, Jinhua Zhang, Jingying Liang, Yaxin Wang, Xuelian Gao, Junfeng Liang

Data type: fas

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Supplementary material 3

RPB2

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Data type: fas

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Supplementary material 4

SSU

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Data type: fas

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Research Article

Three new species of *Pestalotiopsis* (Amphisphaeriales, Sporocadaceae) were identified by morphology and multigene phylogeny from Hainan and Yunnan, China

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Abstract

Pestalotiopsis fungi are widely distributed all over the world, mainly as plant pathogens, endophytes or saprobes from multiple hosts. In this study, the sequence data analysis based on internal transcribed spacer (ITS), partial beta-tubulin (*tub2*) and partial regions of translation elongation factor 1 alpha (*tef1a*) combined with morphological characteristics was used to identify strains isolated from the diseased leaves of *Aporosa dioica* and *Rhaphiolepis indica*, as well as some rotted leaves from Yunnan and Hainan Provinces in China as three new species, viz., *Pestalotiopsis aporosae-dioicae* **sp. nov.**, *P. nannuoensis* **sp. nov.** and *P. rhaphiolepidis* **sp. nov.**

Key words: New species, Pestalotiopsis, taxonomy

Introduction

Pestalotiopsis was separated from Pestalotia by Steyaert in 1942 and belongs to the Sporocadaceae, Amphisphaeriales, Ascomycota (Stevaert 1949). At present, a total of 420 records of Pestalotiopsis have been recorded in the Index Fungorum (http://www.indexfungorum.org/, accessed on 26 Jun 2024). Pestalotioid fungi are a cosmopolitan group of fungi, which have important relationships with different plants as plant pathogens, saprobes or endophytes, and are widely distributed in temperate and tropical regions (Maharachchikumbura et al. 2011, 2012, 2014). As important plant pathogens, pestalotioid species can cause many plant diseases and great economic losses to people (Zhang et al. 2012a; Maharachchikumbura et al. 2013; Jayawardena et al. 2016; Liu et al. 2017; Yang et al. 2017; Diogo et al. 2021; Prasannath et al. 2021). In the past, gray blight disease of tea trees caused by pestalotioid species had caused huge yield losses in southern India (Joshi et al. 2009). In addition, pestalotioid species can also cause the leaf spot of Taxus chinensis in China, leaf blight of Elettaria cardamomum in India, and dieback and stem girdling in young eucalyptus plants in Portugal (Biju et al. 2018; Li et al. 2021;



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Wang et al. 2021). Therefore, the study of pathogenic pestalotioid species can provide research basis for the treatment and inhibition of diseases and avoid significant economic losses.

At first, Pestalotiopsis resembling those taxa having a relationship with Pestalotia were also referred to as pestalotioid fungi. Pestalotioid fungi are characterized by multiseptate and fusiform conidia with appendages at one end or both, frequently with some melanized cells. (Bonthond et al. 2018; Liu et al. 2019). Traditionally, pestalotioid species have been classified mainly according to color intensity of the median conidial cell and the hosts (Maharachchikumbura et al. 2014). But the development of DNA based phylogenetic analysis has brought the traditional classification system into dispute. Maharachchikumbura et al. (2014) applied molecular data to the classification of Pestalotiopsis. By the difference of multilocus phylogenetic analyses, conidial pigment color, and conidiophores, this group was divided into three genera, Pestalotiopsis, Pseudopestalotiopsis, and Neopestalotiopsis. Neopestalotiopsis differs from Pestalotiopsis and Pseudopestalotiopsis in that two upper median cells are darker than the lowest median cell of the conidia, and its indistinct conidiophores. Pseudopestalotiopsis can be easily distinguished from Pestalotiopsis due to its three darker median cells. In recent years, many novel species have been introduced into this group by the use of phylogeny approaches together with morphology (Akinsanmi et al. 2017; Liu et al. 2017; Nozawa et al. 2017; Ariyawansa and Hyde 2018; Jiang et al. 2018; Tibpromma et al. 2018; Tsai et al. 2018).

We conducted extensive sampling in southern China to investigate fungal diversity and explore fungal resources. This study aimed to identify *Pestalotiopsis* which was isolated from diseased leaves of *Aporosa dioica* and *Rhaphiolepis indica*, as well as some rotted leaves collected from Hainan and Yunnan Provinces by morphological characters and molecular phylogeny, and three new species of *Pestalotiopsis* were described and illustrated.

Materials and methods

Sample collection and isolation

The isolates used in this study were obtained from diseased or rotted leaves collected in Yunnan and Hainan Provinces from March to May 2023. Cut 5 × 5 mm small square leaves from the fungal infection part of each sample of diseased or rotted leaves and put them into sterile containers respectively. First, immerse all the small square leaves of each sample in 75% ethanol for disinfection for 1 min, and rinse with sterilized water one time after pouring out the ethanol. Then immerse all the small square leaves of each sample in 5% so-dium hypochlorite solution for disinfection for 30s, and pour out the sodium hypochlorite solution, rinse them repeatedly with sterilized water three times. After pouring out the sterilized water, pick them up with sterilized tweezers and put them on sterilized filter paper to dry. The sterilized leaves were plated on PDA plates (PDA: 20 g agar, 20 g dextrose, 200 g potato, 1000 ml distilled water, pH 7.0) with sterilized tweezers, then 4 small leaves were placed symmetrically on the surface of each medium, with the disease spot facing down,

close to the medium, and the serial number and date were marked on the medium after sealing with a sealing film. The PDA plate was cultured in a constant temperature incubator at 25 °C and the growth of fungi was observed and recorded every day. After 2 to 3 days of culture, the agar with mycelium on the edges of the colony was purified onto a new PDA plate and cultured for 1 to 2 weeks.

Morphological and cultural characterization

The PDA plates were photographed on days 7 and 14 with a digital camera (Canon Powershot G7X). The morphological characteristics of fungi were observed with Olympus SZX10 stereomicroscope and Olympus BX53 microscope, then the fungal structures such as conidiomata, conidiophores, conidiogenous cells, conidia, and appendages, were photographed with an Olympus DP80 high-definition color digital camera. The microstructures are measured with the Digimizer software (https://www.digimizer.com/), and the number of samples measured is generally 20–30. All strains were stored in sterilized 10% glycerol at 4 °C. Voucher specimens have been preserved in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). Ex-holotype living cultures have been preserved in the Shandong Agricultural University Culture Collection (SAUCC). A taxonomy and description of the new species has been uploaded to MycoBank (http://www.mycobank.org/).

DNA extraction, PCR amplification, and sequencing

The genomic DNA was extracted from the colonies cultured on PDA by CTAB (cetyl trimethyl ammonium bromide) method and BeaverBeads Plant DNA Kit (Cat. No.: 70409-20; BEAVER Biomedical Engineering Co., Ltd.) (Guo et al. 2000; Wang et al. 2023). Fungal DNA was amplified using polymerase chain reaction (PCR) using three pairs of primers including internal transcribed spacer (ITS), partial beta-tubulin (tub2) and partial regions of translation elongation factor 1 alpha (tef1a) (White et al. 1990; Glass and Donaldson 1995; O'Donnell et al. 1998; Carbone and Kohn 1999). The reaction was amplified at 25 µL reaction volume, including 12.5 µL 2× Hieff Canace® Plus PCR Master Mix (Shanghai, China) (with dye) (Yeasen Biotechnology, Shanghai, China, Cat No. 10154ES03), 1 µL forward primer, 1 µL reverse primer, and 1 µL genomic DNA template, add distilled deionized water to a total volume 25 µL. The PCR amplification products were detected by electrophoresis in 2% agarosegels and the amplification effect was determined by observing the fragments with UV light (Zhang et al. 2022). Then use a Gel Extraction Kit (Cat: AE0101-C) (Shandong Sparkjade Biotechnology Co., Ltd.) for gel recovery. DNA sequencing and primer synthesis were completed by Tsingke Biotechnology Co., Ltd. (Qingdao, China). The bidirectional sequencing results of the three primers were examined and spliced using MEGA v. 7.0 (Kumar et al. 2016). The sequences of all three new species have been uploaded to GenBank, and the Genebank numbers of all strain sequences used in this study are shown in Table 1.

	Isolate	Origin	Substrate	Ge	nBank access	D (
Species				ITS	tub2	tef1a	Keterences
Neopestalotiopsis magna	MFLUCC 12-0652*	France	Pteridium sp.	KF582795	KF582793	KF582791	(Maharachchikumbura et al. 2014)
Pestalotiopsis abietis	CFCC 53013	China	Abies fargesii	MK397015	MK622282	MK622279	(Gu et al. 2021)
	CFCC 53011*	China	Abies fargesii	MK397013	MK622280	MK622277	
	CFCC 53012	China	Abies fargesii	MK397014	MK622281	MK622278	
P. adusta	MFLUCC 10-146	Thailand	Syzygium sp.	JX399007	JX399038	JX399071	(Maharachchikumbura
	ICMP 6088*	Fiji	Refrigerator door	JX399006	JX399037	JX399070	et al. 2012)
P. aggestorum	LC8186	China	Camellia sinensis	KY464140	KY464160	KY464150	(Liu et al. 2017)
	LC6301*	China	Camellia sinensis	KX895015	KX895348	KX895234	
P. anhuiensis	CFCC 54791*	China	Cyclobalanopsis glauca	ON007028	ON005056	ON005045	(Jiang et al. 2022b)
P. anacardiacearum	IFRDCC 2397*	China	Mangifera indica	KC247154	KC247155	KC247156	(Sajeewa et al. 2013)
P. arengae	CBS 331.92*	Singapore	Arenga undulatifolia	KM199340	KM199426	KM199515	(Maharachchikumbura et al. 2014)
P. arceuthobii	CBS 434.65*	USA	Arceuthobium campylopodum	KM199341	KM199427	KM199516	(Maharachchikumbura et al. 2014)
P. aporosae-dioicae	SAUCC224004*	China	Aporosa dioica	OR733506	OR912985	OR912988	This study
	SAUCC224005	China	Aporosa dioica	OR733505	OR912986	OR912989	
P. appendiculata	CGMCC 3.23550*	China	Rhododendron decorum	OP082431	OP185516	OP185509	(Gu et al. 2022)
P. australis	CBS 114193*	New South Wales	Grevillea sp.	KM199332	KM199383	KM199475	(Maharachchikumbura et al. 2014)
	CBS 111503	South Africa	Protea neriifolia	KM199331	KM199382	KM199557	
P. australasiae	CBS 114141	New South Wales	Protea sp.	KM199298	KM199410	KM199501	(Maharachchikumbura et al. 2014)
	CBS 114126*	New Zealand	Knightia sp.	KM199297	KM199409	KM199499	
P. biciliata	CBS 236.38	Italy	Paeonia sp.	KM199309	KM199401	KM199506	(Maharachchikumbura et al. 2014)
	CBS 124463*	Slovakia	Platanus hispanica	KM199308	KM199399	KM199505	
P. brachiata	LC2988*	China	Camellia sp.	KX894933	KX895265	KX895150	(Liu et al. 2017)
	LC8188	China	Camellia sp.	KY464142	KY464162	KY464152	
P. brassicae	CBS 170.26*	New Zealand	Brassica napus	KM199379	NA	KM199558	(Maharachchikumbura et al. 2014)
P. camelliae	MFLUCC 12-0277*	China	Camellia japonica	JX399010	JX399041	JX399074	(Zhang et al. 2012b)
P. camelliae-oleiferae	CSUFTCC08*	China	Camelliae oleiferae	OK493593	OK562368	OK507963	(Li et al. 2021)
	CSUFTCC09	China	Camelliae oleiferae	OK493594	OK562369	OK507964	
P. cangshanensis	CGMCC 3.23544*	China	Rhododendron delavayi	OP082426	OP185517	OP185510	(Gu et al. 2022)
P. castanopsidis	CFCC 54430*	China	Castanopsis lamontii	OK339732	OK358508	OK358493	(Jiang et al. 2022b)
P. chamaeropis	CBS 186.71*	Italy	Chamaerops humilis	KM199326	KM199391	KM199473	(Maharachchikumbura et al. 2012)
P. changjiangensis	CFCC 54314*	China	Castanopsis tonkinensis	OK339739	OK358515	OK358500	(Jiang et al. 2022b)
	CFCC 52803	China	Cyclobalanopsis sp.	OK339741	OK358517	OK358502	
	CFCC 54433	China	Castanopsis hainanensis	OK339740	OK358516	OK358501	
P. chiangmaiensis	MFLU 22-0164*	Thailand	Phyllostachys edulis	OP497990	OP752137	OP753374	(Sun et al. 2023)
P. chiaroscuro	BRIP 72970*	Australia	Sporobolus natalensis	OK422510	OK423752	OK423753	(Crous et al. 2022)
P. chinensis	MFLUCC 12-0273	China	Taxus sp.	JX398995	NA	NA	(Maharachchikumbura et al. 2012)
P. clavata	MFLUCC 12-0268*	China	Buxus sp.	JX398990	JX399025	JX399056	(Maharachchikumbura et al. 2012)
P. colombiensis	CBS 118553*	Colombia	Eucalyptus urograndis	KM199307	KM199421	KM199488	(Maharachchikumbura et al. 2014)

Table 1. GenBank numbers used in the phylogenetic analysis of Pestalotiopsis.

0	Isolate	Origin	Substrate	GenBank accession			Deferences
Species				ITS	tub2	tef1a	References
P. cyclobalanopsidis	CFCC 54328*	China	Cyclobalanopsis glauca	OK339735	OK358511	OK358496	(Jiang et al. 2022b)
	CFCC 55891	China	Cyclobalanopsis glauca	OK339736	OK358512	OK358497	
P. daliensis	CGMCC 3.23548*	China	Rhododendron decorum	OP082429	OP185518	OP185511	(Gu et al. 2022)
P. dianellae	CPC 32261	Australia	Dianella sp.	MG386051	MG386164	NA	(Crous et al. 2017)
P. digitalis	MFLU 14-0208*	New Zealand	Digitalis purpurea	KP781879	KP781883	NA	(Liu et al. 2015)
P. dilucida	LC3232*	China	Camellia sinensis	KX894961	KX895293	KX895178	(Liu et al. 2017)
	LC8184	China	Camellia sinensis	KY464138	KY464158	KY464148	
P. diploclisiae	CBS 115449	China	Psychotria tutcheri	KM199314	KM199416	KM199485	(Maharachchikumbura
	CBS 115587*	China	Diploclisia glaucescens	KM199320	KM199419	KM199486	et al. 2014)
P. disseminata	CBS 143904	New Zealand	Persea americana	MH554152	MH554825	MH554587	(Liu et al. 2017)
P. diversiseta	MFLUCC 12-0287*	China	Rhododendron sp.	JX399009	JX399040	JX399073	(Maharachchikumbura et al. 2012)
P. doitungensis	MFLUCC 14-0090*	Thailand	Dendrobium sp.	MK993574	MK975837	MK975832	(Ma et al. 2019)
P. dracontomelonis	MFLU 14-0207*	Thailand	Dracontomelon sp.	KP781877	NA	KP781880	(Liu et al. 2015)
P. dracaenae	HGUP 4037*	China	Dracaena fragrans	MT596515	MT598645	MT598644	(Ariyawansa et al. 2015)
P. dracaenicola	MFLUCC 18-0913*	Thailand	Dracaena sp.	MN962731	MN962733	MN962732	(Chaiwan et al. 2020)
P. eleutherococci	HMJAU 60189*	China	Eleutherococcus brachypus	NR182556	NA	NA	(Tian et al. 2022)
P. endophytica	MFLU 20-0607*	Thailand	Magnolia garrettii	MW263946	NA	MW417119	(De Silva et al. 2021)
P. ericacearum	IFRDCC 2439*	China	Rhododendron delavayi	KC537807	KC537821	KC537814	(Zhang et al. 2013)
P. etonensis	BRIP 66615*	Australia	Sporobolus jacquemontii	MK966339	MK977634	MK977635	(Crous et al. 2020)
P. ficicola	SAUCC230046*	China	Ficus microcarpa	OQ691974	OQ718749	OQ718691	(Zhang et al. 2023)
P. foliicola	CFCC 57359	China	Castanopsis faberi	ON007030	ON005058	ON005047	(Jiang et al. 2022b)
	CFCC 57360	China	Castanopsis faberi	ON007031	ON005059	ON005048	
	CFCC 54440*	China	Castanopsis faberi	ON007029	ON005057	ON005046	
P. furcata	MFLUCC 12-0054*	Thailand	Camellia sinensis	JQ683724	JQ683708	JQ683740	(Watanabe et al. 2018)
P. fusoidea	CGMCC 3.23545*	China	Rhododendron delavayi	OP082427	OP185519	OP185512	(Gu et al. 2022)
P. formosana	NTUCC 17-009*	China	Poaceae sp.	MH809381	MH809385	MH809389	(Akinsanmi et al. 2017)
P. gaultheriae	IFRD 411-014*	China	Gaultheria forrestii	KC537805	KC537819	KC537812	(Maharachchikumbura et al. 2014)
P. gibbosa	NOF 3175*	Canada	Gaultheria shallon	LC311589	LC311590	LC311591	(Watanabe et al. 2018)
P. grandis-urophylla	E-72-02	Brazil	Eucalyptus sp.	KU926708	KU926716	KU926712	(Carvalho et al. 2019)
	E-72-03	Brazil	Eucalyptus sp.	KU926709	KU926717	KU926713	
	E-72-04	Brazil	Eucalyptus sp.	KU926710	KU926718	KU926714	
	E-72-06	Brazil	Eucalyptus sp.	KU926711	KU926719	KU926715	
P. guangdongensis	ZHKUCC 22-0016*	China	Arenga pinnata	ON180762	ON221548	ON221520	(Xiong et al. 2022)
P. guangxiensis	CFCC 54308*	China	Quercus griffithii	OK339737	OK358513	OK358498	(Jiang et al. 2022b)
	CFCC 54300	China	Quercus griffithii	OK339738	OK358514	OK358499	
P. grevilleae	CBS 11412/*	Australia	<i>Grevillea</i> sp.	КМ199300	KM199407	KM199504	(Maharachchikumbura et al. 2014)
P. guizhouensis	CFCC 54803	China	Cyclobalanopsis glauca	ON007035	ON005063	ON005052	(Jiang et al. 2022b)
	CFCC 57364	China	Cyclobalanopsis glauca	ON007036	ON005064	ON005053	
P. hawaiiensis	CBS 114491*	USA	Leucospermum sp.	KM199339	KM199428	KM199514	(Maharachchikumbura et al. 2014)
P. hispanica	CBS 115391	Portugal	Eucalyptus globulus	MH553981	MH554640	MH554399	(Maharachchikumbura et al. 2014)

	Isolate	ate Origin		GenBank accession			
Species			Substrate	ITS	tub2	tef1a	References
P. hollandica	CBS 265.33*	The Nethelands	Sciadopitys verticillata	KM199328	KM199388	KM199481	(Maharachchikumbura et al. 2014)
P. humicola	CBS 336.97*	Papua New Guinea	Soil	KM199317	KM199420	KM199484	(Maharachchikumbura et al. 2014)
P. hunanensis	CSUFTCC18	China	Camellia oleifera	OK493600	OK562375	OK507970	(Li et al. 2021)
	CSUFTCC15*	China	Camellia oleifera	OK493599	OK562374	OK507969	
P. hydei	MFLUCC 20-0135	Thailand	Litsea elliptica	MW266063	MW251112	MW251113	(Huanaluek et al. 2021)
P. iberica	CAA 1005	Spain	Pinus sylvestris	MW732250	MW759034	MW759037	(Monteiro et al. 2021)
	CAA 1006	Spain	Pinus radiata	MW732249	MW759036	MW759039	
	CAA 1004*	Spain	Pinus radiata	MW732248	MW759035	MW759038	
P. intermedia	MFLUCC 12-0259*	China	Unidentified tree	JX398993	JX399028	JX399059	(Maharachchikumbura et al. 2012)
P. inflexa	MFLUCC 12-0270*	China	Unidentified tree	JX399008	JX399039	JX399072	(Maharachchikumbura et al. 2012)
P. italiana	MFLU 14-0214*	Italy	Cupressus glabra	KP781878	KP781882	KP781881	(Liu et al. 2015)
P. jesteri	CBS 109350*	Papua New Guinea	Fragraea bodenii	KM199380	NA	KM199554	(Maharachchikumbura et al. 2014)
P. jiangxiensis	LC4399*	China	Camellia sp.	KX895009	KX895341	KX895227	(Liu et al. 2017)
P. jiangsuensis	CFCC 59538	China	Pinus massoniana	OR533577	OR539191	OR539186	(Li et al. 2024)
P. jinchanghensis	LC8190	China	Camellia sinensis	KY464144	KY464164	KY464154	(Liu et al. 2017)
	LC6636*	China	Camellia sinensis	KX895028	KX895361	KX895247	
P. kandelicola	NCYUCC 19-0354	China	Kandelia candel	MT560723	MT563100	MT563102	(Hyde et al. 2020)
	NCYUCC 19-0355*	China	Kandelia candel	MT560722	MT563099	MT563101	
P. kaki	KNU-PT-1804*	Korea	Diospyros kaki	LC552953	LC552954	LC553555	(Das et al. 2020)
P. kenyana	LC6633	China	Camellia sinensis	KX895027	KX895360	KX895246	(Maharachchikumbura
	CBS 442.67*	Kenya	Coffea sp.	KM199302	KM199395	KM199502	et al. 2014)
P. knightiae	CBS 114138*	New Zealand	Knightia sp.	KM199310	KM199408	KM199497	(Maharachchikumbura et al. 2014)
	CBS 111963	New Zealand	Knightia sp.	KM199311	KM199406	KM199495	
P. krabiensis	MFLUCC 16-0260*	Thailand	Pandanus sp.	MH388360	MH412722	MH388395	(Tibpromma et al. 2018)
P. leucadendri	CBS 121417*	South Africa	Leucadendron sp.	MH553987	MH554654	MH554412	(Liu et al. 2019)
P. licualicola	HGUP 4057*	China	Licuala grandis	KC492509	KC481683	KC481684	(Geng et al. 2013)
P. lijiangensis	CFCC 50738*	China	Castanopsis carlesii	KU860520	NA	NA	(Zhou et al. 2018)
P. linearis	MFLUCC 12-0271*	China	Trachelospermum sp.	JX398992	JX399027	JX399058	(Maharachchikumbura et al. 2012)
P. linguae	ZHKUCC 22-0159	China	Pyrrosia lingua	OP094104	OP186108	OP186110	(Li et al. 2023)
P. lithocarpi	CFCC 55893	China	Lithocarpus chiungchungensis	OK339743	OK358519	OK358504	(Jiang et al. 2022b)
	CFCC 55100*	China	Lithocarpus chiungchungensis	OK339742	OK358518	OK358503	
P. longiappendiculata	LC3013*	China	Camellia sinensis	KX894939	KX895271	KX895156	(Liu et al. 2017)
P. loeiana	MFLU 22-0167*	Thailand	Unidentified tree	OP497988	OP713769	OP737881	(Sun et al. 2023)
P. lushanensis	LC8182	China	Camellia sp.	KY464136	KY464156	KY464146	(Liu et al. 2017)
	LC8183	China	Camellia sp.	KY464137	KY464157	KY464147	
	LC4344*	China	Camellia sp.	KX895005	KX895337	KX895223	
P. macadamiae	BRIP 63739b	Australia	Macadamia integrifolia	KX186587	KX186679	KX186620	(Akinsanmi et al. 2017)
	BRIP 63741a	Australia	Macadamia integrifolia	KX186586	KX186678	KX186619	
	BRIP 63738b*	Australia	Macadamia integrifolia	KX186588	KX186680	KX186621	
P. malayana	CBS 102220*	Malaysia	Macaranga triloba	KM199306	KM199411	KM199482	(Maharachchikumbura et al. 2014)
P. manyueyuanensis	NTUPPMCC 18- 165*	Taiwan	Ophocordyceps sp.	OR125060	OR126306	OR126313	(Hsu et al. 2024)

	Isolate	Origin	Substrate	GenBank accession			D (
Species				ITS	tub2	tef1a	Keterences
P. menhaiensis	CGMCC 3.18250*	China	Ophocordyceps sp.	KU252272	KU252488	KU252401	(Li et al. 2024)
P. microspora	SS1-033I	Canada	Cornus canadensis	MT644300	NA	NA	(Zhao and Li 1995)
P. montellica	MFLUCC 12-0279	China	dead plant material	JX399012	JX399043	JX399076	(Maharachchikumbura et al. 2012)
P. monochaeta	CBS 144.97*	The Nethelands	Quercus robur	KM199327	KM199386	KM199479	(Maharachchikumbura et al. 2014)
	CBS 440.83	The Nethelands	Taxus baccata	KM199329	KM199387	KM199480	
P. multicolor	CFCC59981	China	Taxus chinensis	OQ626676	0Q714336	0Q714341	(Wang et al. 2024)
P. nanjingensis	CSUFTCC16*	China	Camellia oleifera	OK493602	OK562377	OK507972	(Li et al. 2021)
P. nanningensis	CSUFTCC10*	China	Camellia oleifera	OK493596	OK562371	OK507966	(Li et al. 2021)
P. nannuoensis	SAUCC232203*	China	Unknown host	OR733504	OR863909	OR912991	This study
	SAUCC232204	China	Unknown host	OR733503	OR863910	OR912992	
P. novae-hollandiae	CBS 130973*	Australia	Banksia grandis	KM199337	KM199425	KM199511	(Maharachchikumbura et al. 2014)
P. neolitseae	NTUCC 17-011*	China	Neolitsea villosa	MH809383	MH809387	MH809391	(Akinsanmi et al. 2017)
P. oryzae	CBS 171.26	Italy	Unknown host	KM199304	KM199397	KM199494	(Maharachchikumbura
	CBS 353.69*	Denmark	Oryza sativa	KM199299	KM199398	KM199496	et al. 2014)
	CBS 111522	USA	Telopea sp.	KM199294	KM199394	KM199493	
P. pallidotheae	MAFF 240993*	Japan	Pieris japonica	AB482220	NA	NA	(Watanabe et al. 2010)
P. pandanicola	MFLUCC 16-0255*	Thailand	Pandanus sp.	MH388361	MH412723	MH388396	(Tibpromma et al. 2018)
P. papuana	CBS 331.96*	Papua New Guinea	Coastal soil	KM199321	KM199413	KM199491	(Maharachchikumbura et al. 2014)
	CBS 887.96	Papua New Guinea	Cocos nucifera	KM199318	KM199415	KM199492	
P. parva	CBS 278.35	Thailand	Delonix regia	KM199313	KM199405	KM199509	(Maharachchikumbura
	CBS 265.37*	Thailand	Delonix regia	KM199312	KM199404	KM199508	et al. 2014)
P. phoebes	SAUCC230093*	China	Phoebe zhennan	OQ692028	OQ718803	0Q718745	(Zhang et al. 2023)
P. pini	MEAN 1092	Portugal	Pinus pinea	MT374680	MT374705	MT374693	(Silva et al. 2020)
P. photiniicola	GZCC 16-0028*	China	Photinia serrulata	KY092404	KY047663	KY047662	(Chen et al. 2017)
P. pinicola	KUMCC 19-0183*	China	Pinus armandii	MN412636	MN417507	MN417509	(Tibpromma et al. 2019)
P. portugallica	CBS 393.48*	Portugal	Unknown host	KM199335	KM199422	KM199510	(Maharachchikumbura et al. 2014)
P. rhaphiolepis	SAUCC367701*	China	Rhaphiolepis indica	OR733502	OR863906	OR912994	This study
	SAUCC367702	China	Rhaphiolepis indica	OR733501	OR863907	OR912995	
P. rhizophorae	MFLUCC 17-0416*	Thailand	Rhizophora mucronata	MK764283	MK764349	MK764327	(Norphanphoun et al. 2019)
P. rhodomyrti	HGUP4230*	China	Rhodomyrtus tomentosa	KF412648	KF412642	KF412645	(Song et al. 2013)
P. rhododendri	IFRDCC 2399*	China	Rhododendron sinogrande	KC537804	KC537818	KC537811	(Zhang et al. 2013)
P. rosea	MFLUCC 12-0258*	China	Pinus sp.	JX399005	JX399036	JX399069	(Maharachchikumbura et al. 2012)
P. rosarioides	CGMCC 3.23549*	China	Rhododendron decorum	OP082430	OP185520	OP185513	(Gu et al. 2022)
P. sabal	ZHKUCC 22-0035*	China	Sabal mexicana	ON180775	ON221561	ON221533	(Xiong et al. 2022)
P. sequoiae	MFLUCC 13-0399*	Italy	Sequoia sempervirens	KX572339	NA	NA	(Li et al. 2016)
P. scoparia	CBS 176.25*	China	Chamaecyparis sp.	KM199330	KM199393	KM199478	(Maharachchikumbura et al. 2014)
P. shaanxiensis	CFCC 57356	China	Quercus variabilis	ON007027	ON005055	ON005044	(Jiang et al. 2022b)
	CFCC 54958*	China	Quercus variabilis	ON007026	ON005054	ON005043	
P. shoreae	MFLUCC 12-0314*	Thailand	Shorea obtusa	KJ503811	KJ503814	KJ503817	(Song et al. 2014)
P. sichuanensis	CGMCC 3.18244*	China	Camellia sinensis	KX146689	KX146807	KX146748	(Wang et al. 2019)
P. silvicola	CFCC 57363	China	Cyclobalanopsis kerrii	ON007034	ON005062	ON005051	(Jiang et al. 2022b)

	Isolate	Origin	Substrate	GenBank accession			.
Species				ITS	tub2	tef1a	References
P. silvicola	CFCC 55296*	China	Cyclobalanopsis kerrii	ON007032	ON005060	ON005049	(Jiang et al. 2022b)
	CFCC 54915	China	Cyclobalanopsis kerrii	ON007033	ON005061	ON005050	
P. smilacicola	MFLU 22-0165*	Thailand	Smilax sp.	OP497991	OP762673	OP753376	(Sun et al. 2023)
P. sonneratiae	CFCC 57394*	China	Sonneratia apetala	ON114184	ON086816	ON086812	(Jiang et al. 2022a)
P. spatholobi	SAUCC231201*	China	Spatholobus suberectus	OQ692023	OQ718798	OQ718740	(Zhang et al. 2023)
P. spathuliappendiculata	CBS 144035*	Australia	Phoenix canariensis	MH554172	MH554845	MH554607	(Liu et al. 2019)
P. spathulata	CBS 356.86*	Chile	Gevuina avellana	KM199338	KM199423	KM199513	(Maharachchikumbura et al. 2014)
P. suae	CGMCC 3.23546*	China	Rhododendron delavayi	OP082428	OP185521	OP185514	(Gu et al. 2022)
P. taxicola	CFCC59976	China	Taxus chinensis	OQ626673	0Q714333	0Q714338	(Wang et al. 2024)
P. telopeae	CBS 113606	Australia	Telopea sp.	KM199295	KM199402	KM199498	(Maharachchikumbura et al. 2014)
	CBS 114161*	Australia	Telopea sp.	KM199296	KM199403	KM199500	
	CBS 114137	Australia	Protea sp.	KM199301	KM199469	KM199559	
P. thailandica	MFLUCC 17-1616*	Thailand	Rhizophora mucronata	MK764285	MK764351	MK764329	(Norphanphoun et al. 2019)
P. terricola	CBS 141.69*	Pacific islands	Soil	MH554004	MH554680	MH554438	(Liu et al. 2019)
P. trachicarpicola	OP068*	China	Trachycarpus fortunei	JQ845947	JQ845945	JQ845946	(Zhang et al. 2012a)
P. trachycarpicola	BJFUCC42	China	Taxus chinensis	0Q626674	0Q714334	0Q714339	(Zhang et al. 2012a)
P. tumida	CFCC 55158*	China	Rosa chinensis	OK560610	OM158174	OL814524	(Peng et al. 2022)
P. unicolor	MFLUCC 12-0275	China	Unidentified tree	JX398998	JX399029	JX399063	(Maharachchikumbura
	MFLUCC 12-0276*	China	Rhododendron sp.	JX398999	JX399030	NA	et al. 2012)
P. verruculosa	MFLUCC 12-0274*	China	Rhododendron sp.	JX398996	NA	JX399061	(Maharachchikumbura et al. 2012)
P. yunnanensis	HMAS 96359*	China	Podocarpus macrophyllus	AY373375	NA	NA	(Wei et al. 2013)
P.yanglingensis	LC3412	China	Camellia sinensis	KX894980	KX895312	KX895197	(Liu et al. 2017)
	LC4553*	China	Camellia sinensis	KX895012	KX895345	KX895231	

Notes: New species established in this study are shown in bold. Those marked "*" in the table are represented as ex-type or ex-epitype strains. NA: Not available.

Phylogenetic analyses

According to the latest publication of this genus, the reference sequences used in this study (Table 1) were obtained from the National Center for Biotechnology Information (NCBI) (Li et al. 2024). Reference sequences and sequences obtained from the sequenced strains were aligned and manually corrected by MAFFT 7 online service with the Auto strategy (http://mafft.cbrc.jp/alignment/ server/) (Katoh et al. 2019). Based on maximum likelihood (ML) and Bayesian inference (BI) algorithms, the phylogenetic analysis of multilabel data was carried out. Run ML and BI on the CIPRES Science Gateway portal (https://www. phylo.org/) (Miller et al. 2012). ML was performed on RaxML-HPC2 of XSEDE (8.2.12) (Stamatakis 2014), 1000 fast bootstrap repeats were performed using GTRGAMMA model of nucleotide evolution. MrModeltest v.2.3 (Nylander 2001) is used to screen the optimal evolutionary model, and BI was performed on XSEDE (3.2.7a) (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2012; Ronquist et al. 2012). When the mean standard deviation of separation frequency is less than 0.01, output the topology. View and adjust phylogenetic trees in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree) and beautify the phylogenetic trees with Adobe Illustrator CC 2019. The names of the isolates in this study are marked in red in the phylogenetic tree.

Result

Phylogenetic analyses

By analyzing the sequence data sets of ITS, tub2 and tef1a, the interspecific relationships of Pestalotiopsis were inferred. The phylogenetic analysis of Pestalotiopsis strains contained 183 sequences, using Neopestalotiopsis magna (MFLUCC 12-0652) as the outgroup. A total of 1579 characters including gaps (523 of ITS, 530 of tub2 and 526 of tef1a) were included in the phylogenetic analysis. There were 915 constant, 185 variable but parsimony non-informative, and 479 parsimony informative characters. In Bayesian inference, GTR + I + G is used as the optimal evolutionary model of ITS and tub2, and HKY + I + G is used as the optimal evolutionary model of tef1a. The final ML optimization likelihood was -15175.820563. The trees obtained by the ML and BI methods are similar, and the ML tree with the best score was shown in Fig. 1, the Maximum Likelihood Bootstrap Values and Bayesian Inference Posterior Probabilities (MLBS/BIPP) are marked at the node position of the phylogenetic tree. On the basis of previous studies, six strains of Pestalotiopsis were imported into the phylogenetic analysis in this study. The six new strains introduced in this study were divided into three monophyletic branches in the phylogenetic tree, representing three new species of Pestalotiopsis, P. aporosae-dioicae sp. nov., P. nannuoensis sp. nov. and P. rhaphiolepidis sp. nov. Finally, the 183 strains were divided into 135 species clades in the phylogenetic map.

Taxonomy

Pestalotiopsis aporosae-dioicae C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov. MycoBank No: 851279 Fig. 2

Type. CHINA, Yunnan Province, Jinghong City, Sancha River (22°10'10"N, 100°51'49"E), from diseased leaves of *Aporosa dioica*, 19 Mar 2023, C.Z. Yin, Z.X. Zhang and X.G. Zhang, holotype HMAS 352667, ex-type living culture SAUCC224004.

Etymology. Referring to the name of the host plant Aporosa dioica.

Description. Conidiomata in culture on PDA, 600–1000 μ m diam, globular, solitary, black conidial masses permeated above the mycelium. Conidiophores mostly degenerated into conidiogenous cells, hyaline. Conidiogenous cells smooth, clavate, hyaline, aggregative, 16.1–22.2 × 3.9–5.5 μ m. Conidia fusiform, 4-septate, slightly curved or straight, 25.6–35.2 × 5.0–7.1 μ m; basal cell conical, hyaline, rough, thin-walled, 3.9–9.7 μ m; three median cells subcylindrical, light brown or brown, rough, thick-walled, the first median cell from base 4.9–7.0 μ m, the second median cell 4.8–7.0 μ m, the third median cell 4.6–6.9 μ m, together 14.9–20.2 μ m; apical cell subcylindrical, hyaline, smooth, thin–walled, 4.7–8.3 μ m; basal appendage tubular, single, centric, straight or slightly bent, unbranched, 4.0–13.2 μ m; apical appendages tubular, 2–4, straight or bent, unbranched, 8.8–31.7 μ m. Sexual morph not observed.

Culture characteristics. After 14 days of dark cultivation at 25 °C on PDA, the colony diameter reached 90 mm, and the growth rate is 6.2–6.6 mm/day. Colonies filamentous to circular, aerial mycelium on surface raised, white, dense, forms multiple rings from the middle to the edge, fruiting bodies black; reverse yellow, brown in parts.



Figure 1. A Maximum Likelihood phylogram of *Pestalotiopsis* based on ITS, *tub2* and *tef1a* gene sequences, and MFLUCC 12-0652 of *Neopestalotiopsis magna* as the tree root of *Pestalotiopsis*. The Maximum Likelihood Bootstrap Value (left, MLBV≥70%) and Bayesian Inference Posterior Probability (right, BIPP≥0.90), separated by a slash line, are marked at the node. The scale bar at the top left represents 0.1 nucleotide changes at each site. Some shortened branches are represented by double slashes and the number of fold times. The strains in this study are shown in red.



Figure 2. *Pestalotiopsis aporosae-dioicae* (holotype: HMAS 352667) **a** leaves of host *Aporosa dioica* **b**, **c** the front and back of the colony after 14 days of culture on PDA **d** conidiomata on PDA **e**, **f** conidiophores and conidiogenous cells **g-m** conidia. Scale bars: 10 μm (**e-m**).

Additional specimen examined. CHINA, Yunnan Province, Jinghong City, Sancha River, from diseased leaves of *Aporosa dioica*, 19 Mar. 2023, C.Z. Yin, Z.X. Zhang and X.G. Zhang, living culture SAUCC224005.

Notes. According to phylogenetic trees based on ITS, *tub2* and *tef1a*, *Pestalotiopsis aporosae-dioicae* sp. nov. was closely related to *P. arengae* in a well support branch (ML/BI = 100/1). *P. aporosae-dioicae* was different from *P. arengae* by 14/508 bp in ITS, 51/529 bp in *tub2*, and 10/465 bp in *tef1a*. Morphologically, *P. aporosae-dioicae* was different from *P. arengae* by having thinner conidia (*P. aporosae-dioicae*: $25.6-35.2 \times 5.0-7.1$ vs. *P. arengae*: $25.0-32.0 \times 7.0-9.5 \mu$ m) and longer basal appendages (*P. aporosae-dioicae*: 4.0-13.2 vs. *P. arengae*: $1.5-3.0 \mu$ m) (Maharachchikumbura et al. 2014). Therefore, *Pestalotiopsis aporosae-dioicae* was identified as a new species of *Pestalotiopsis* by morphological and phylogenetic comparison.

Pestalotiopsis nannuoensis C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov. MycoBank No: 851280

Fig. 3

Type. CHINA, Yunnan Province, Menghai County, Nannuo Mountain (21°55'25"N, 100°35'41"E), from rotted leaves, 18 Mar 2023, C.Z. Yin, Z.X. Zhang and X.G. Zhang, holotype HMAS 352668, ex-type living culture SAUCC232203.

Etymology. Referring to the collection site of the holotype, Nannuo Mountain. **Description.** Conidiomata in culture on PDA, 750–900 μm diam, subsphaeroidal, solitary, black conidial masses permeated above the mycelium. Conidiophores mostly degenerated into conidiogenous cells, hyaline, simple. Conidiogenous cells oval, hyaline, rough, aggregative, 10.6–19.4 × 2.2–3.4 μm. Conidia fusiform or subcylindrical, straight or slightly curved, 4-septate, 21.7–27.2 × 3.6–5.0 μm; basal cell conical, hyaline, rough, thinwalled, 3.9–5.4 μm; three median cells subcylindrical, brown, rough, thickwalled, the first median cell from base 4.4–6.2 μm, the second median cell 4.1–5.3 μm, the median third cell 4.5–5.7 μm, together 13.0–17.2 μm; apical cell conical or subcylindrical, hyaline, smooth, thin-walled, 2.9–4.6 μm; basal appendage tubular, single, centric, straight or slightly bent, unbranched, 6.8–9.2 μm; apical appendages tubular, 1–2, straight or bent, unbranched, 15.6–26.2 μm. Sexual morph not observed.

Culture characteristics. After 7 days of dark cultivation at 25 °C on PDA, the colony diameter reached 75 mm, and the growth rate is 9.5–11.5 mm/day. Colonies filamentous to circular, with filiform margin, aerial mycelium on surface rugged, white, dense, fruiting bodies black; reverse white.

Additional specimen examined. CHINA, Yunnan Province, Menghai County, Nannuo Mountain, from rotted leaves, 18 Mar 2023, C.Z. Yin, Z.X. Zhang and X.G. Zhang, living culture SAUCC232204.

Notes. *Pestalotiopsis nannuoensis* sp. nov. formed an independent clade (ML/BI = 100/1) in the phylogenetic tree based on ITS, *tub2* and *tef1a*, and was closely related to *P. diversiseta*. *P. nannuoensis* was different from *P. diversiseta* by 46/508 bp in ITS, 83/529 bp in *tub2*, and 59/465 bp in *tef1a*. Morphologically, *P. nannuoensis* was different from *P. diversiseta* by having shorter and thinner conidia (*P. nannuoensis*: 21.7–27.2 × 3.6–5.0 vs. *P. diversiseta*: 27.0–34.0 × 5.5–8.0 µm), and the number of apical appendages (*P. nannuoensis*: 1–2 vs. *P. diversiseta*: 3–5). (Maharachchikumbura et al. 2012). Therefore, *Pestalotiopsis nannuoensis* was identified as a new species of *Pestalotiopsis* by morphological and phylogenetic comparison.

Pestalotiopsis rhaphiolepidis C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov. MycoBank No: 851281 Fig. 4

Type. CHINA, Hainan Province, Jianfeng Town (18°42'35"N, 108°52'35"E), from diseased leaves of *Rhaphiolepis indica*, 11 Apr 2023, C.Z. Yin, Z.X. Zhang and X.G. Zhang, holotype HMAS 352669, ex-type living culture SAUCC367701. **Etymology.** Referring to the name of the host plant *Rhaphiolepis indica*.



Figure 3. *Pestalotiopsis nannuoensis* (holotype: HMAS 352668) **a**, **b** the front and back of the colony after 14 days of culture on PDA **c** conidiomata on PDA **d**–**f** conidiophores and conidiogenous cells **g**–**k** conidia. Scale bars: 10 µm (**d**–**k**).

Description. Conidiomata in culture on PDA, 600–1000 µm diam, globular, solitary, black conidial masses permeated above the mycelium. Conidiophores mostly degenerated into conidiogenous cells, simple, hyaline. Conidiogenous cells fusiform, rough, discrete, $9.8-17.1 \times 2.4-3.3$ µm. Conidia fusiform, straight or slightly curved, 4-septate, $18.0-23.1 \times 3.8-5.1$ µm; basal cell conical, hyaline, rough, thin-walled, 3.3-5.1 µm; three median cells subcylindrical, light brown or brown, rough, thick-walled, the first median cell 3.7-5.6 µm, together 10.1-15.6 µm; apical cell subcylindrical or conical, hyaline, smooth, thin-walled, 2.8-4.7 µm; basal appendage tubular, single, centric, straight or slightly bent, unbranched, 4.7-9.8 µm; apical appendages tubular, 2-3, straight or bent, unbranched, 5.2-18.5 µm. Sexual morph not observed.



Figure 4. *Pestalotiopsis rhaphiolepidis* (holotype: HMAS 352669) **a** leaves of host *Rhaphiolepis indica* **b**, **c** the front and back of the colony after 14 days of culture on PDA **d** conidiomata on PDA **e**–**g** conidiogenous cells and conidia **h**–**n** conidia. Scale bars: 10 µm (**e**–**n**).

Culture characteristics. After 7 days of dark cultivation at 25 °C on PDA, the colony diameter reached 90 mm, and the growth rate is 11.8–13.5 mm/day. Colonies filamentous to circular, flat, center raised, aerial mycelium on surface, with irregular edges, white, medium dense, fruiting bodies black; reverse white, multilayer rings from the middle to the edge.

Additional specimen examined. CHINA, Hainan Province, Jianfeng Town, from diseased leaves of *Rhaphiolepis indica*, 11 Apr 2023, C.Z. Yin, Z.X. Zhang and X.G. Zhang, living culture SAUCC367702.

Notes. According to phylogenetic trees based on ITS, *tub2* and *tef1a*, *Pestalotiopsis rhaphiolepidis* sp. nov. was closely related to *P. inflexa* in a well support branch (ML/BI = 98/1). *P. rhaphiolepidis* was different from *P. inflexa*

by 9/508 bp in ITS, 30/529 bp in *tub2*, and 16/465 bp in *tef1a*. Morphologically, *P. rhaphiolepidis* was different from *P. inflexa* by having shorter and thinner conidia (*P. rhaphiolepidis*: $18.0-23.1 \times 3.8-5.1$ vs. *P. inflexa*: $24.0-31.0 \times 6.0-9.0 \mu$ m) and shorter apical appendages (*P. rhaphiolepidis*: 5.2-18.5 vs. *P. inflexa*: $20.0-30.0 \mu$ m) (Maharachchikumbura et al. 2011). Therefore, *Pestalotiopsis rhaphiolepidis* was identified as a new species of *Pestalotiopsis* by morphological and phylogenetic comparison.

Discussion

Pestalotiopsis fungi are widely distributed and have been found all over the world, with 12,072 samples and 59,207 sequences were included in the Global-Fungi database (https://globalfungi.com/, accessed on 26 Jun 2024; Asia, 58.81%, North America, 20.84%, Europe, 5.86%, Africa, 5.38%, South America, 4.49%, Australia, 3.59%, Pacific Ocean, 0.78%, Atlantic Ocean, 0.21%, Antarctica, 0.05%). In this study, we obtained six strains of Pestalotiopsis from diseased and rotted leaves collected from Yunnan and Hainan Provinces in China. Based on phylogenetic analysis and morphological characteristics, we identified six strains as three new species of Pestalotiopsis, P. aporosae-dioicae, P. nannuoensis and P. rhaphiolepidis. It is worth noting that the plant hosts of Pestalotiopsis fungi are abundant, such as Theaceae, Arecaceae, and Fagaceae (Maharachchikumbura et al. 2014; Jiang et al. 2022b). We first reported the new hosts of Aporosa dioica (Phyllanthaceae) and Rhaphiolepis indica (Rosaceae) by identifying the Pestalotiopsis fungi. This suggests that there were more potential new species of Pestalotiopsis to be discovered in these two host plants. Pestalotiopsis nannuoensis was found on rotted leaves and its host is unknown. Pestalotiopsis fungi are mostly plant pathogens, and the relationship between the three newly discovered species and their hosts and their effects on cash crops needs further study (Zhang et al. 2012a; Maharachchikumbura et al. 2013; Jayawardena et al. 2016; Liu et al. 2017; Yang et al. 2017; Diogo et al. 2021; Prasannath et al. 2021).

Since Steyaert introduced Pestalotiopsis into Sporocadaceae (Amphisphaeriales, Ascomycota) in 1949, more and more species of Pestalotiopsis have been discovered (Stevaert 1949; Akinsanmi et al. 2017; Liu et al. 2017; Nozawa et al. 2017; Ariyawansa and Hyde 2018; Jiang et al. 2018; Tibpromma et al. 2018; Tsai et al. 2018). However, due to the similarity of the spore structure, the classification of Pestalotiopsis is unclear, and the traditional identification method was very complicated work. With the development of molecular technology, the identification method combining morphology and phylogeny has been accepted by more and more taxonomists. Maharachchikumbura et al. (2014) applied phylogenetic analysis in the classification of Pestalotiopsis to make the classification more clearly. Therefore, phylogenetic analyses based on ITS, tub2 and tef1a, including maximum likelihood (ML) and Bayesian inference (BI), have been widely used. Meanwhile, the size of spores, length, position, origin and number of branches of apical appendages and basal appendages are important for the classification of Pestalotiopsis fungi. Taking several subjects in this study as examples, Pestalotiopsis aporosae-dioicae and P. rhaphiolepidis can be identified as new species by morphological comparison with other species in a well-supported clade (Maharachchikumbura et al. 2011, 2014). Although Pestalotiopsis nannuoensis was

an independent clade, it had the basic characteristics of *Pestalotiopsis*, and was significantly different from several closely related species in spore size and number of apical appendages, so it was also identified as a new species (Maharachchikumbura et al. 2012). Based on the results of this study, we believe that we will isolate more potential *Pestalotiopsis* fungi in the future. With the development of biotechnology and the deepening of the research on *Pestalotiopsis* fungi, it has become an important research focus and direction to sequence the genome of *Pestalotiopsis* fungi, annotate its structure and function, and explore the types and applications of secondary metabolites.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: CY. Data curation: CY. Formal analysis: ZZ. Funding acquisition: XZ. Investigation: CY. Methodology: CY. Project administration: XZ. Resources: CY. Software: CY. Supervision: LM, SW. Validation: ZZ. Visualization: CY. Writing - original draft: CY. Writing - review and editing: CY.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Original and spliced sequences of three genes of all strains of the genus *Pestalotiopsis* for phylogenetic analysis

Authors: Changzhun Yin, Zhaoxue Zhang, Shi Wang, Liguo Ma, Xiuguo Zhang Data type: zip

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Research Article

Molecular and morphological data reveal two new polypores (Polyporales, Basidiomycota) with reddish brown to orange basidiomata from China

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Abstract

Two taxonomically controversial polypore genera with reddish brown to orange basidiomata that stain reddish with KOH solution, *Aurantiporus* and *Hapalopilus*, are revised based on additional sampling, morphological examination, and phylogenetic analysis of a combined dataset of ITS1-5.8S-ITS2-nLSU sequences. *Hapalopilus* is a monophyletic genus belonging to Phanerochaetaceae, whereas *Aurantiporus* is a polyphyletic genus belonging to Meruliaceae. *Hapalopilus* and *Aurantiporus* s. str. are circumscribed, and two new species – *Aurantiporus orientalis* and *Hapalopilus tabuliformis* – are described and illustrated from temperate China. In addition, four new combinations, viz. *Aurantiporus alboaurantius*, *A. mutans*, *A. tropicus* and *Luteoporia albocitrina*, are proposed based on morphology and phylogenetic analysis. The relationships between *Aurantiporus* and *Hapalopilus* are discussed.

Key words: Phlebioid clade, phylogeny, taxonomy, wood-rotting fungi

Introduction

Polypores are important wood-decaying fungi and have distribution in all the forest ecosystems; around 2670 polypores have been reported worldwide and some have economic values (Papp and Dai 2022; Wu et al. 2019, 2022; Yuan et al. 2023; Zhao et al. 2024). *Aurantiporus* Murrill and *Hapalopilus* P. Karst. are polypore genera with limited species producing reddish brown to orange basid-iomata and belonging to the phlebioid clade of Polyporales (Chen et al. 2021). *Aurantiporus* was treated as a synonym of *Hapalopilus* by Ryvarden (1991), and its type species was accepted as *Hapalopilus croceus* (Pers.) Donk (Donk 1933). Until now, the name of *Hapalopilus croceus* is still accepted by some mycologists (Langer et al. 2015; Ryvarden and Melo 2017; Redr et al. 2020).

Aurantiporus was typified as *Polyporus pilotae* Schwein. (Murrill 1905) and is characterized by annual, bright-colored basidiomata staining more or less red with KOH solution, a monomitic hyphal system with agglutinated clamped hy-



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The genus *Hapalopilus* was established by Karsten (1881) and typified by *Polyporus nidulans* Fr. (= *Hapalopilus rutilans* (Pers.) Murrill 1904). Morphologically, the genus is known by its distinctive annual, colorful, soft basidiomata with a reddish to violet coloration upon contact with KOH solution, a monomitic hyphal system, generative hyphae bearing clamp connections, and hyaline, thin-walled, smooth basidiospores which are negative in Melzer's reagent and Cotton Blue (Miettinen et al. 2016; Ryvarden and Melo 2017; Chen et al. 2021). The color change in KOH solution has been attributed to the presence of polyporic acid in *H. rutilans*, which renders the fungus poisonous (Kraft et al. 1998; Villa et al. 2013).

Previously, most polypore species with reddish KOH reaction were placed in Hapalopilus on a morphological basis (Gilbertson and Ryvarden 1986; Zmitrovich et al. 2006; Ryvarden and Melo 2017) and therefore Hapalopilus was considered polyphyletic in early molecular studies (Ko et al. 2001). Niemelä et al. (2005) established Erastia Niemelä & Kinnunen to accommodate H. salmonicolor (Berk. & M.A. Curtis) Pouzar, and recently two additional species, H. aurantiacus (Rostk.) Bondartsev & Singer and H. ochraceolateritius (Bondartsev) Bondartsev & Singer, were combined into this genus (Zmitrovich 2018; Zíbarová et al. 2021). Furthermore, the phylogenetic studies demonstrated that H. ochraceolateritius belonged to Irpicaceae (Justo et al. 2017; Chen et al. 2021; Li et al. 2022). However, the phylogenetic position of *Erastia* and its type species was uncertain. Based on molecular and morphological analyses, Miettinen et al. (2016) redefined Hapalopilus as monophyletic with a narrow concept that included its type species and three additional species within Phanerochaetaceae. Although this revised classification enhanced the clarity of phylogenetic relationships within Hapalopilus in Phanerochaetaceae, other species initially described in Hapalopilus were found in different clades of Polyporales, highlighting unresolved taxonomic issues (Ko et al. 2001; Justo et al. 2017; Chen et al. 2021).

To better understand the morphological variation and phylogeny of the above bright-colored polypores with basidiomata staining red in KOH solution, and especially the uncertain phylogenetic position of some species in *Aurantiporus* and *Hapalopilus*, we examined specimens from Asia and North America. Based on morphology and new molecular data, we provide an updated phylogeny of *Aurantiporus* and *Hapalopilus*. As a result, two new species are described and four new combinations are proposed in this study.

Materials and methods

Morphological studies

The specimens used in this study are deposited at the Fungarium of the State Key Laboratory of Efficient Production of Forest Resources, Beijing Forestry University, China (BJFC), the private herbarium of Josef Vlasák (JV), and the National Museum Prague of Czech Republic (PRM). Macro-morphological descriptions are based on field notes and voucher specimens. Our morphological studies follow Miettinen et al. (2016) and Westphalen et al. (2022). The following abbreviations are used: IKI = Melzer's reagent, IKI-= neither amyloid nor dextrinoid, CB = Cotton Blue, CB-= acyanophilous in Cotton Blue, L = arithmetic average length of all measured spores, W = arithmetic average of all measured spore width, Q = L/W ratios among the studied specimens, and n (a/b) = number of spores (a) measured from a given number of specimens (b). Color terms follow Anonymous (1969) and Petersen (1996).

Molecular studies and phylogenetic analysis

Total genomic DNA was extracted from dried specimens using the CTAB plant genomic DNA extraction kit DN14 (Aidlab Biotechnologies Co., Ltd, Beijing, China), following the manufacturer's guidelines with some modifications (Shen et al. 2019; Sun et al. 2020). The ITS1-5.8S-ITS2 region was amplified using the primer pairs ITS4 and ITS5, and the nLSU region was amplified using the primer pairs LROR and LR7 (White et al. 1990). The PCR procedures for the ITS1-5.8S-ITS2 and nLSU regions followed Wang et al. (2023) and Zhang et al. (2023). The PCR products were purified and sequenced at the Beijing Genomics Institute, China (BGI) with the same primers. All newly generated sequences were submitted to GenBank and are listed in Table 1.

Sequences generated for this study and additional sequences downloaded from GenBank were partitioned to ITS1, 5.8S, ITS2 and nrLSU, and then aligned separately using MAFFT v.74 (http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2017) with the G-INS-I iterative refinement algorithm. Following manual optimization in BioEdit 7.0.5.3 (Hall 1999), the separate alignments were concatenated using PhyloSuite v. 1.2.3 (Zhang et al. 2020; Xiang et al. 2023). The combined ITS1-5.8S-ITS2-nLSU dataset was analyzed to confirm the phylogenetic position of target species within the phlebioid clade of Polyporales (Fig. 1). Sequences of Heterobasidion annosum (Fr.) Bref. and Stereum hirsutum (Willd.) Pers. were used as outgroups following Chen et al. (2021) and Liu et al. (2023). The resulting alignment was deposited at TreeBase (submission ID 31520; Reviewer access URL: http://purl.org/ phylo/treebase/phylows/study/TB2:S31520?x-access-code=49bae894df9bffc4280d3da656868775&format=html). Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used for the phylogenetic analysis. ModelFinder v. 2.2.0 with Corrected Akaike information criterion (AICc) was applied to estimate the best-fit partition scheme and evolutionary model for BI (Kalyaanamoorthy et al. 2017).

Maximum Likelihood (ML) analysis was performed in RAxML v.8.2.10 (Stamatakis 2014). All parameters in the ML analysis used default settings, and statistical support values were obtained using rapid bootstrapping with 1000 replicates.

Bayesian Inference (BI) analysis was run with four chains for two runs and performed for two million generations sampling every 1000 generations in MrBayes v3.2.7 (Ronquist et al. 2012), until the split deviation frequency value was less than 0.01. A burn-in of 25% was used before computing the consensus tree.

Species nome	Complee (Veueber	Country	GenBank Accession no.		
Species name	Samples/voucher	Country	ITS no.	nLSU no.	
Alboefibula bambusicola	Chen 2304 (holotype)	China	MZ636926	MZ637091	
Aurantiopileus mayaensis	MCW 373/12	Brazil	OL630487	OL630487	
A. mayaensis	TJB10228 (holotype)	Belize	HM772140	HM772139	
A. mayaensis	JV1504/128	Costa Rica	KT156706	/	
Aurantiporus albidus	CIEFAP-117	Argentina	KY948739	KY948848	
A. albidus	F32	Argentina	MT076170	/	
A. 'albidus'	Cui 16664	Australia	ON682353	ON680805	
A. 'albidus'	Cui 16665	Australia	ON682354	ON680806	
A. alboaurantius	Cui 2877	China	KF845954	KF845947	
A. alboaurantius	Cui 4136 (holotype)	China	KF845955	KF845948	
A. croceus	H6-27	Lithuania	MH571407	/	
A. croceus	VPapp 300518-1	Hungary	MT876120	/	
A. croceus	BRNM737561	Czech	JQ821320	JQ821317	
A. 'croceus'	TVR 7	USA	MW020539	/	
A. 'croceus'	PUL00031376	USA	OM747650	/	
A. 'croceus'	57362583	USA	OM473901	/	
A. mutans	JV0509/123	USA	MN318460	/	
A. mutans	JV0309/83a	USA	MN318458	/	
A. mutans	JV0309/83b	USA	MN318459	/	
A. orientalis	Dai 23714 (holotype)	China	PP702380	PP623071	
A. pseudoplacentus	Miettinen 18997	USA	KY948744	KY948902	
A. pseudoplacentus	PRM 899297 (holotype)	USA	JN592496	JN592504	
A. pulcherrimus	MR80	Argentina	OL630488	OL630488	
A. roseus	Dai 13573 (holotype)	China	KJ698635	KJ698639	
A. roseus	CLZhao 4762	China	PP392925	/	
A. sp. (A. 'croceus')	Miettinen 16483	Malaysia	KY948745	KY948901	
A. sp. (A. 'priscus')	Dai 4686	China	PP916606	/	
A. sp. (A. 'priscus')	Dai 22793	China	ON413717	ON413719	
A. sp. (A. 'priscus')	VS6295	Russia	MN318461	/	
A. tropicus	JV1707/5T	Costa Rica	MN318455	/	
A. venustus	MCW 391/12	Brazil	OL630489	OL635577	
Bjerkandera adusta	HHB-12826-Sp	USA	KP134983	KP135198	
Byssomerulius corium	FP-102382	USA	KP135007	KP135230	
Ceriporia gossypinum	Dai 23392 (holotype)	China	OQ476824	OQ476770	
C. viridans	Dai 17003	China	OQ476847	OQ476790	
Ceriporiopsis gilvescens	BRNM 710166	Czech	FJ496684	FJ496720	
C. semisupina	Cui 10222 (holotype)	China	KF845956	KF845949	
C. semisupina	Cui 7971	China	KF845957	KF845950	
Crustodontia chrysocreas	HHB-6333-Sp	USA	KP135358	KP135263	
Crystallicutis serpens	HHB-15692-Sp	USA	KP135031	KP135200	
Efibula tropica	He 6008	China	MW580947	MW580937	
Erastia aurantiaca	BR4112	France	MN318464	1	
E. aurantiaca	Dai 18399	Vietnam	PP715440	/	

Gustafson176

Dai 23109

JV1609/12TDK

Miettinen 16992

VS4749

FLAS-F-61674

Unknown

China

Czech

USA

Russia

USA

AY986499

PP715441

MN318463

KY948741

MN318462

MH212041

Table 1. Taxa information and GenBank accession numbers of sequences used in this study.

E. aurantiaca

E. ochraceolateritia

E. ochraceolateritia

E. ochraceolateritia

E. ochraceolateritia

E. salmonicolor

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/

/ KY948891

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		0	GenBank Accession no.		
Species name	Samples/voucher	Country	ITS no.	nLSU no.	
E. salmonicolor	JV0904/46	USA	JN592500	JN592507	
E. salmonicolor	MC13	USA	MW619631	/	
Gloeoporus hainanensis	Dai 15268 (holotype)	China	KU360401	KU360411	
G. thelephoroides	JV 1808 26	French Guiana	OQ476858	OQ476799	
Hapalopilus eupatorii	F. Dammrich 10744	Germany	KX752620	KX752620	
H. eupatorii	K 132752	UK	KX008364	KX081076	
H. percoctus	H 7008581 (holotype)	Botswana	KX752597	KX752597	
H. ribicola	H 6045691	Finland	KX752616	/	
H. ribicola	H 6045697	Finland	KX752617	/	
H. rutilans	Dai 23591	China	OL469801	OL469800	
H. rutilans	H 6012735	Finland	KX752614	/	
H. rutilans	H 6013411	Finland	KX752615	/	
H. tabuliformis	Dai 24535	China	PP715438	PP623072	
H. tabuliformis	Dai 24540 (holotype)	China	PP715439	PP623073	
Heterobasidion annosum	Dai 20962	Belarus	ON417163	ON417213	
Irpex lacteus	Dai 11230	China	OQ476863	OQ476805	
Leptoporus mollis	Dai 21062	Belarus	MW377302	MW377381	
Luteoporia albocitrina	JV1704/103	Costa Rica	MN318457	/	
L. albocitrina	Dai 19507 (holotype of L. citriniporia)	Sri Lanka	MT872218	MT872216	
L. albocitrina	Dai 19622	Sri Lanka	MT872219	MT872217	
L. albomarginata	Dai 15229 (holotype)	China	KU598873	KU598878	
L. albomarginata	Dai 15240	China	KU598874	KU598879	
L. albomarginata	GC 1702-1	China	LC379003	LC379155	
L. lutea	CHWC 1506-68	China	MZ636997	MZ637157	
L. lutea	GC 1409-1	China	MZ636998	MZ637158	
L. straminea	CLZhao 5794	China	OM897115	OM897114	
L. straminea	CLZhao 18947 (holotype)	China	MW732407	MW724799	
L. tenuissima	Dai 20429	China	PP356578	PP356576	
L. tenuissima	Dai 25825 (holotype)	China	PP356579	PP356577	
Meruliopsis taxicola	Dai 22625	China	OL457966	OL457436	
Mycoacia fuscoatra	HHB-10782-Sp	USA	KP135365	KP135265	
M. nothofagi	HHB-4273-Sp	USA	KP135369	KP135266	
Odoria alborubescens	BP106943	Hungary	MG097864	MG097867	
O. alborubescens	BRNU 627479	Czech	JQ821319	JQ821318	
O. alborubescens	PC 0706595	France	MG097863	/	
Pappia fissilis	BRNM 699803	Czech	HQ728292	HQ729002	
P. fissilis	MUcc 814	Czech	HQ728291	HQ729001	
P. fissilis	HHB-9530-Sp	USA	KY948774	/	
Phaeophlebiopsis caribbeana	HHB-6990	USA	KP135415	KP135243	
Phanerochaete chrysosporium	HHB-6251-Sp (holotype)	USA	KP135094	KP135246	
P. inflata	Cui 7712	China	JX623930	JX644063	
Phanerochaetella angustocystidiata	Wu 9606-39	China	MZ637020	GQ470638	
Phlebia austroasiana	Dai 17556 (holotype)	China	ON135439	ON135443	
P. poroides	CLZhao 16121 (holotype)	China	MW732405	MW724797	
P. radiata	AFTOL-484	Unknown	AY854087	AF287885	
P. rufa	FBCC297	Sweden	LN611092	LN611092	
P. setulosa	HHB-6891-Sp	USA	KP135382	KP135267	
P. tomentopileata	CLZhao 9563 (holotype)	China	MT020765	MT020743	
P. tremellosa	FBCC82	Finland	LN611124	LN611124	
Phlebicolorata brevispora	FBCC1463 (holotype)	USA	LN611135	LN611135	
Phlebiopsis gigantea	FCUG 1417	Norway	MZ637051	AF141634	

Species name	Semales (Veueber	Country	GenBank Accession no.		
Species name	Samples/voucher	Solution Country Generation 1209-46 China KY6 121 USA AY2 1en 11038 Finland FN9 nen 9472 Finland FN9 133888 Unknown AY8 93-216T USA JN7 inen 7487 Finland H00	ITS no.	nLSU no.	
Resiniporus pseudogilvescens	Wu 1209-46	China	KY688203	MZ637268	
Rhizochaete fouquieriae	KKN-121	USA	AY219390	GU187608	
Skeletocutis amorpha	Miettinen 11038	Finland	FN907913	FN907913	
S. chrysella	Miettinen 9472	Finland	FN907916	FN907916	
Stereum hirsutum	FP-133888	Unknown	AY854063	/	
Trametopsis cervina	TJV-93-216T	USA	JN165020	JN164796	
Tyromyces chioneus	Miettinen 7487	Finland	HQ659244	HQ659244	
Newly generated sequences for this stud	lv are in bold.				

Trees were viewed in FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Branches that received bootstrap support for ML and Bayesian Posterior Probabilities (BPP) greater than or equal to 75% (ML) and 0.95 (BPP) were considered to be significantly supported.

Results

Molecular phylogeny

The combined ITS1-5.8S-ITS2-nLSU dataset of the phlebioid clade included sequences from 107 specimens (Phanerochaetaceae, Irpicaceae, and Meruliaceae) representing 68 taxa and the outgroup (Table 1). ModelFinder suggested SYM+I as the best-fit model for 5.8S, and GTR+F+I+G4 as the best-fit models for ITS1, ITS2, and nrLSU for the Bayesian analysis. BI analysis yielded an almost identical topology to the ML analysis, with an average standard deviation of split frequencies of 0.006514, and thus only the ML tree (Fig. 1) is presented with branch support values for ML and BI when these were greater than or equal to 50% and 0.90, respectively.

The phylogeny of the phlebioid clade (Fig. 1) revealed that Hapalopilus is fully supported and clustered within Phanerochaetaceae as a monophyletic genus that includes five species (100% ML, 1.00 BPP; Fig. 1). Similarly, Erastia includes three species and forms a monophyletic clade within Irpicaceae (100% ML, 1.00 BPP; Fig. 1). It is also obvious from the genetic analysis that Aurantiporus is highly polyphyletic in its current concept, representing three separate clades within Meruliaceae. Notably, the sampled sequences of the so-called A. croceus (Pers.) Murrill appear to represent three different species from different continents (USA, Europe, and Malaysia), but all well nested within Aurantiporus s. str. (Fig. 1). In addition, two specimens from Northern China, annotated as Hapalopilus tabuliformis, form a distinct lineage with robust support and are stably nested within Hapalopilus (100% ML, 1.00 BPP; Fig. 1). Another specimen from Northeast China, annotated as Aurantiporus orientalis, grouped together with so-called A. croceus from North America and Europe (the type species of Aurantiporus) with robust support (100% ML, 1.00 BPP; Fig. 1). Hapalopilus albocitrinus (Petch) Ryvarden is nested in Luteoporia F. Wu et al. and is transferred to the latter genus in the present study. Ceriporiopsis alboaurantia C.L. Zhao et al., Hapalopilus mutans (Peck) Gilb. & Ryvarden and H. tropicus I. Lindblad & Ryvarden nested in the Aurantiporus s. str. clade, are transferred to Aurantiporus in this study.



Figure 1. Maximum likelihood tree (ML) illustrating the phylogeny of the phlebioid clade within the Polyporales based on a combined ITS1-5.8S-ITS2-nLSU dataset. Branches are labelled with ML bootstrap values higher than 50% and Bayesian Posterior Probabilities (BPP) more than 0.90. New species and new combinations are in bold. Black triangles represent the generic types.

Taxonomy

Aurantiporus orientalis Y.C. Dai, Xin Zhang, Ghobad-Nejhad & Yuan Yuan, sp. nov. MycoBank No: 853592

Figs 2, 3

Holotype. CHINA, Jilin Province, Antu County, Changbaishan Nature Reserve, on living tree of *Quercus mongolica*, 4 July 2022, Dai 23714 (BJFC 038959).

Etymology. Orientalis (Lat.): refers to the species occurring in East Asia.

Diagnosis. Aurantiporus orientalis is characterized by pileate, imbricate, triquetrous basidiomata with apricot-orange pores when fresh, that become honey yellow upon drying and reddish in KOH solution, large pores 1-2 per mm, the presence of cystidioles, broadly ellipsoid basidiospores measuring $3.4-4 \times 2.5-3 \mu m$, and growing on *Quercus* in Northeast China.

Fruitbody. Basidiomata annual, pileate, imbricate, inseparable from the substrate, watery to soft corky and without odor or taste when fresh, shrinking and becoming brittle to hard corky upon drying. Pilei triquetrous, projecting up to 10 cm, 15 cm wide and 3 cm thick at base. Pileal surface orange-yellow when fresh, becoming honey-yellow upon drying, matted. Pore surface apricot-orange when fresh, becoming fuscous to date brown upon drying; sterile margin distinct, concolorous with pileal surface, up to 3 mm wide; pores angular to irregular, 1–2 per mm; dissepiments thin, lacerate. Context clay-buff and hard corky when dry, up to 2.5 cm thick, becoming reddish in KOH solution. Tube layer concolorous with pore surface, brittle to rigid, up to 5 mm deep.

Hyphal structure. Hyphal system monomitic; generative hyphae bearing clamp connections, richly encrusted with fine yellowish crystals, IKI–, CB–; tissue becoming reddish in KOH solution.

Context. Generative hyphae hyaline, slightly thick- to thick-walled, occasionally branched, flexuous, interwoven, $2.5-5 \mu m$ in diam.

Tubes. Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, flexuous to straight, interwoven, 2–4 μ m in diam. Cystidia absent; cystidioles present, clavate to fusoid, thin-walled, smooth, 16–24 × 4–5.5 μ m; basidia clavate, bearing four sterigmata and a basal clamp connection, 21– 26 × 5–7 μ m; basidioles similar in shape to basidia, but smaller.



Figure 2. Basidiomata of Aurantiporus orientalis (Dai 23714).



Figure 3. Microscopic structures of *Aurantiporus orientalis* (Dai 23714, holotype) **a** basidiospores **b** basidia and basidioles **c** cystidioles **d** hyphae from context **e** hyphae from tube trama.

Spores. Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, some with one or two guttules, IKI–, CB–, $(3.3–)3.4-4(-4.1) \times 2.5-3 \mu$ m, L = 3.69 μ m, W = 2.76 μ m, Q=1.34 (n = 30/1).

Ecology and distribution. Growing on living tree of *Quercus mongolica*. Known from the type location only.

Type of rot. White rot.

Specimens examined/studied. The holotype.

Hapalopilus tabuliformis Y.C. Dai, Xin Zhang, Ghobad-Nejhad & Yuan Yuan, sp. nov. MycoBank No: 853593 Figs 4, 5

Holotype. CHINA. Inner Mongolia Autonomous Region, Alxa County, Beisi Forest Park, on fallen branch of *Pinus tabuliformis*, 18 September 2022, Dai 24540 (BJFC 039782). **Etymology.** *Tabuliformis* (Lat.): refers to the species growing on *Pinus tabuliformis*.

Diagnosis. Hapalopilus tabuliformis is characterized by resupinate to effused-reflexed basidiomata having a pale pink to buff-yellow pileal surface and purple coloration in KOH solution, small pores 3–5 per mm, the absence of cystidioles, long and narrow basidia measuring $18-31 \times 3.2-5.8 \mu m$, broadly ellipsoid basidiospores measuring $3.2-4 \times 2.6-3.2 \mu m$, and growing on *Pinus tabuliformis* in western China.

Fruitbody. Basidiomata annual, resupinate to effused-reflexed, adnate, soft corky and without odor or taste when fresh, becoming brittle to hard corky upon drying. Pilei projecting up to 0.9 cm, 1.2 cm wide and 3 mm thick at base. Pileal surface pale pink to buff-yellow when fresh, becoming honey-yellow when dry. Pore surface light vinaceous gray to grayish violet when fresh, becoming buff to grayish brown when dry; margin cream to pale ochraceous, fimbriate and thinning out when resupinate, up to 1 mm wide; pores angular to irregular, 3–5 per mm; dissepiments thin, entire to lacerate. Context honey and corky when dry, up to 2 mm thick, becoming purple in KOH solution. Tube layer concolorous with pore surface, corky, up to 1 mm deep.

Hyphal structure. Hyphal system monomitic; generative hyphae bearing clamp connections, richly encrusted with fine yellowish crystals (dissolved in KOH solution), IKI–, CB–; tissue becoming purple in KOH solution.

Context. Generative hyphae hyaline, slightly thick- to thick-walled, occasionally branched, interwoven, $2-4.3 \mu m$ in diam.

Tubes. Generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, interwoven, flexuous, $3-5.9 \mu m$ in diam. Cystidia and cystidioles absent. Basidia clavate to pyriform, bearing four sterigmata and a basal clamp connection, $18-31 \times 3.2-5.8 \mu m$; basidioles similar in shape to basidia, but smaller.

Spores. Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, usually with a guttule, IKI-, CB-, $(3-)3.2-4(-4.2) \times (2.5-)2.6-3.2(-3.4) \mu$ m, L= 3.68 µm, W = 2.76 µm, Q=1.25 (n=60/2).

Ecology and distribution. Growing on fallen branches of *Pinus tabuliformis*. Known from the type location only.

Type of rot. White rot.

Additional specimen examined. CHINA. Inner Mongolia Autonomous Region, Alxa County, Beisi Forest Park, on fallen branch of *Pinus tabuliformis*, 18 September 2022, Dai 24535 (BJFC 039777).



Figure 4. Basidiomata of Hapalopilus tabuliformis (Dai 24540).



Figure 5. Microscopic structures of Hapalopilus tabuliformis (Dai 24540, holotype) a basidiospores b basidia c basidioles, d hyphae from context e hyphae from tube trama.

Aurantiporus alboaurantius (C.L. Zhao, B.K. Cui & Y.C. Dai) Y.C. Dai, Xin Zhang, Ghobad-Nejhad & Yuan Yuan, comb. nov.

MycoBank No: 853599

- Ceriporiopsis alboaurantia C.L. Zhao, B.K. Cui & Y.C. Dai, Phytotaxa 164: 22 (2014) (Basionym)
- = Phlebicolorata alboaurantia (C.L. Zhao, B.K. Cui & Y.C. Dai) C.L. Zhao, J. Fungi 9 (3, no. 320): 32 (2023)

Description. See Cui and Zhao (2014).

Ecology and distribution. Growing on fallen trunk of Cunninghamia. Known from subtropical forests in southeast China.

Type of rot. White rot.

Notes. *Ceriporiopsis pseudoplacenta* Vlasák & Ryvarden and *C. alboaurantia* were recently described from USA (Vlasák et al. 2012) and China (Cui and Zhao 2014), respectively. However, Vampola and Vlasák (2021) recombined *C. pseudoplacenta* into *Aurantiporus* following morphological analyses, and they considered *Aurantiporus priscus* Niemelä et al. described from Europe two months later (Niemelä et al. 2012) as a taxonomic synonym of *A. pseudoplacentus* (Vlasák & Ryvarden) J. Vlasák & P. Vampola. Our updated phylogeny with enhanced taxon sampling also indicates that both species are nested within the *Aurantiporus* s. str. clade (Fig. 1), but it is not sure that they are conspecific. All these species resemble the type species of *Aurantiporus* by sharing dense agglutinated tubes, shrinking and darkening upon drying, and a monomitic hyphal system with ellipsoid, smooth basidiospores. Hence, the above combination is proposed.

Phlebicolorata C.L. Zhao, typified with *P. brevispora* (Nakasone) C.L. Zhao, was established to include the generic type and *A. pseudoplacentus*, *C. alboaurantia*, and *A. roseus* (C.L. Zhao & Y.C. Dai) Zmitr. (Zhao et al. 2023). However, the latter three species are nested in the *Aurantiporus* s. str. clade in our phylogeny (Fig. 1). Similar results were obtained by Liu et al. (2022).

Aurantiporus mutans (Peck) Y.C. Dai, Xin Zhang, Vlasák, Ghobad-Nejhad & Yuan Yuan, comb. nov. MycoBank No: 854371

Polyporus mutans Peck, Rep. (Annual) Trustees State Mus. Nat. Hist., New York 41: 77 (1888) (Basionym)

- = Poria mutans (Peck) Peck, Ann. Rep. Reg. N.Y. St. Mus. 43: 85 (1890)
- = Hapalopilus mutans (Peck) Gilb. & Ryvarden, N. Amer. Polyp., Vol. 1 Abortiporus - Lindtneri (Oslo): 337 (1986)

Description. See Gilbertson and Ryvarden (1986).

Ecology and distribution. Growing on dead hardwoods, usually on *Castanea*. Known from eastern North America from Canada to Florida and Australia.

Aurantiporus tropicus (I. Lindblad & Ryvarden) Y.C. Dai, Xin Zhang, Vlasák, Ghobad-Nejhad & Yuan Yuan, comb. nov. MycoBank No: 854372

Hapalopilus tropicus I. Lindblad & Ryvarden, Mycotaxon 71: 342 (1999) (Basionym)

Description. See Lindblad and Ryvarden (1999).

Ecology and distribution. Growing on dead deciduous wood. Known from tropical wet forests in Costa Rica.

Notes. Hapalopilus mutans was first described as Polyporus mutans from New York, USA, and was recognized by resupinate, colorful basidiomata with a reddish coloration in KOH solution (Lowe 1966; Gilbertson and Ryvarden 1986). Hapalopilus tropicus was originally described from the tropical forests of Costa Rica, and unlike the other *Hapalopilus* species, it is not reactive in KOH solution (Lindblad and Ryvarden 1999). However, *Hapalopilus tropicus* mostly resembles *H. mutans* by having resupinate, colorful basidiomata that turn red upon bruising, dense agglutinated tubes, shrinking and darkening upon drying, and a monomitic hyphal system with ellipsoid, smooth, thin-walled basidiospores (Lowe 1966; Gilbertson and Ryvarden 1986; Lindblad and Ryvarden 1999). However, according to the present study (see discussion), the above morphological characteristics fit the definition of *Aurantiporus*. Moreover, our phylogeny (Fig. 1) confirms that *Hapalopilus mutans* and *H. tropicus* grouped together with *Aurantiporus roseus* within the *Aurantiporus* s. str. clade, which was distant from *H. rutilans* (the type of *Hapalopilus*). Thus, the above combinations are proposed.

Luteoporia albocitrina (Petch) Y.C. Dai, Xin Zhang, Vlasák, Ghobad-Nejhad & Yuan Yuan, comb. nov.

MycoBank No: 854431

- Poria albocitrina Petch, Ann. R. bot. Gdns Peradeniya 7(4): 286 (1922) (Basionym)
- ≡ Hapalopilus albocitrinus (Petch) Ryvarden, in Ryvarden & Johansen, Prelim. Polyp. Fl. E. Afr. (Oslo): 359 (1980)
- = Luteoporia citriniporia Z.B. Liu & Yuan Yuan, Phytotaxa 46(1): 36 (2020)

Description. See Ryvarden and Johansen (1980) and Liu and Yuan (2020).

Ecology and distribution. Growing on dead deciduous wood. Known from Costa Rica, Rwanda, Kenya and Sri Lanka.

Notes. *Hapalopilus albocitrinus* is a tropical species originally described as *Poria albocitrina* from Sri Lanka (Petch 1922), and it is characterized by resupinate, bright-colored basidiomata with a reddish coloration in KOH solution, swollen hyphae covered with crystals at the tips and cylindrical to oblong ellipsoid basidiospores (Petch 1922; Ryvarden and Johansen 1980). *Luteoporia citriniporia* is not only morphologically similar to *H. albocitrinus*, but also have an overlapping distribution. So, we consider that *Luteoporia citriniporia* and *H. albocitrinus* represent a single species, hence the above recombination is proposed.

Specimens examined. Aurantiporus alboaurantius: CHINA. Fujian Province, Wuyishan County, Longfenggu Forest Park, alt. 500 m, on fallen trunk of *Cunninghamia*, 27 August 2006, Cui 4136 (BJFC 000412, holotype); Longchuan Valley, alt. 500 m, on fallen trunk of *Cunninghamia*, 16 October 2005, Cui 2877 (BJFC 000416, paratype). *A. mutans*: USA, Pennsylvania, Wilkes-Barre, Ricketts Glen State Park, on black cherry, 11 September 2003, JV 0309/83a,b (JV, PRM); Pike County, Promised Land State Park, on *Quercus* sp., 13 September 2005, JV 0509/123 (JV, PRM). *A. pseudoplacentus*: USA, Washington, Forks, Bogachiel State Park, on trunk of *Picea sitchensis*, 6 August 2003, JV0308/68 (PRM 899297, holotype; BJFC 020510, isotype). *A. tropicus*: COSTA RICA, Puntarenas Province, Santa Elena, JV1707/5-T (JV, PRM). *Luteoporia albocitrina*: SRI LANKA. Colombo, Dombagaskanola Forest Reserve, on rotten angiosperm wood, 27 February 2019, Dai 19507 (BJFC 031186); Avissawella, Salgala Forest, on rotten angiosperm wood, 3 March 2019, Dai 19622 (BJFC0 31299); COSTA RICA, Puntarenas Province, Tarcoles, on rotten angiosperm wood, 22 April 2017, JV 1704/103 (JV).

Discussion

Despite the controversial history of *Aurantiporus* and *Hapalopilus*, there is certainty in the placement of the new species *Aurantiporus orientalis* and *Hapalopilus tabuliformis*. This placement is consistent with the type species of their corresponding genera (Fig. 1). Both species are found in the temperate forests of China and show bright-colored basidiomata with a reddish coloration in KOH solution.

Our phylogenetic analysis (Fig. 1) corroborates that *Erastia* and *Hapalopilus* are monophyletic and nested in different clades of Polyporales. *Erastia* was established to accommodate *Hapalopilus* species growing on coniferous wood (Niemelä et al. 2005), and it is nested in Irpicaceae including its type and two additional species in our phylogeny (Fig 1.). Conversely, *Hapalopilus* is nested in Phanerochaetaceae as monophyletic clade which is in accordance with Miettinen et al. (2016), and includes five species here. In conclusion, *Erastia* and *Hapalopilus* are independent genera within Irpicaceae and Phanerochaetaceae, respectively.

Hapalopilus tabuliformis is an independent lineage within Hapalopilus, as indicated by the phylogenetic analysis of the combined ITS1-5.8S-ITS2-nLSU dataset (Fig. 1). Morphologically, H. eupatorii (P. Karst.) Spirin & Miettinen resembles H. tabuliformis by having resupinate to effused-reflexed basidiomata, fimbriate margin and similar sized ellipsoid basidiospores, but differs from H. tabuliformis by shorter basidia (14-18 µm vs. 18-31 µm) and longer basidiospores (3.4–4.5 µm vs. 3.2–4 µm; Miettinen et al. 2016; Zíbarová et al. 2021). Hapalopilus rutilans and H. ribicola (P. Karst.) Spirin & Miettinen differ from H. tabuliform is by basidiospore sizes $(4-5 \times 2.3-3 \mu m in H. ribicola and 3.2-5.1 \times 1.2 \mu m in H. ribicola and 3.2-5.1 \mu m in H. ribicola and$ 2-2.7 µm in H. rutilans vs. 3.2-4 × 2.6-3.2 µm in H. tabuliformis) and shorter basidia (16.5–20.5 × 4.5–6 µm in *H. ribicola* and 18–22 × 5–6.5 µm in *H. ruti*lans vs. 18-31 × 3.2-5.8 µm in H. tabuliformis; Miettinen et al. 2016; Ryvarden and Melo 2017). Moreover, the former two species have a wide distribution in Europe and commonly grow on deciduous trees (Miettinen et al. 2016), while H. tabuliformis is known from China and grow on Pinus. Hapalopilus percoctus differs from H. tabuliformis by having pileate basidiomata, longer basidiospores (3.8-4.6 µm vs. 3.2-4 µm), and is known to grow on dicots in the Southern Hemisphere (Miettinen et al. 2016).

Aurantiporus is found to be highly polyphyletic in the family Meruliaceae, as shown in our phylogenetic analysis as well as in previous studies (Floudas and Hibbett 2015; Chen et al. 2021; Liu et al. 2022). The type species of Aurantiporus was erected by Murrill (1905) as Polyporus pilotae Schwein. (Schweinitz 1832) described from North America, and was later considered by himself as a synonym of Polyporus croceus (Pers.) Fr. (=Boletus croceus Pers.; Persoon 1796; Fries 1815). Nevertheless, it is noteworthy that the sequences sampled from North America and Europe, and named as Hapalopilus croceus in GenBank, represent a species complex (Fig. 1). We do not doubt that Aurantiporus croceus or A. pilotae (Schwein.) Murrill belong to Aurantiporus rather than Hapalopilus. In addition, similar uncertainty exists within Aurantiporus priscus, which was synonymized as A. pseudoplacentus by Vampola and Vlasák (2021). Our phylogenetic analysis illustrated that they make form two closely related separate subclades (Fig. 1). However, Aurantiporus priscus in our phylogeny is represented by specimens from China and Russia Far East, with no sequences available from the type locality (Poland). So, further study

is needed, especially the sequences from type locality are very important to confirm the species complex.

The new species Aurantiporus orientalis nested in the Aurantiporus s. str. clade (Fig. 1) and grouped together with the generic type (A. pilotae from North America, Fig. 1). Morphologically, these species share pileate, bright orange-red colored basidiomata and grow on Quercus. However, the generic type (A. pilotae from North America) differs from A. orientalis by the absence of cystidioles (Murrill 1905; Koszka and Papp 2020). Aurantiporus pseudoplacentus, A. mutans, and A. tropicus are similar to A. orientalis in having orangish basidiomata and similar shape and size of basidiospores, but A. pseudoplacentus, A. mutans, and A. tropicus differ from A. orientalis by the absence of cystidioles (Lowe 1966; Gilbertson and Ryvarden 1986; Lindblad and Ryvarden 1999; Vlasák et al. 2012). In addition, A. alboaurantius, A. roseus, and A. orientalis share a monomitic hyphal system with ellipsoid, thin-walled basidiospores and the presence of cystidioles (Cui and Zhao 2014; Zhao et al. 2015). However, A. alboaurantius and A. roseus differ from A. orientalis in having resupinate basidiomata and bigger basidiospores (4-5 × 3-3.3 µm in A. alboaurantius and 4-5.2 × 3.3-3.8 µm in A. roseus vs. 3.4-4 × 2.5-3 µm in A. orientalis; Cui and Zhao 2014; Zhao et al. 2015).

From a morphological perspective, Hapalopilus is characterized by pileate to resupinate, colorful, and soft to cottony corky basidiomata when fresh, brittle when dry, a monomitic hyphal system with generative hyphae bearing clamp connections, and covered with granular, golden yellow pigment that dissolves in KOH solution (Miettinen et al. 2016). Aurantiporus s. str. differs from Hapalopilus by having larger, watery and fleshy basidiomata when fresh, slower drying process, often shrinking significantly in size, denser agglutinated tubes, darker and hard when dried, generative hyphae somewhat colored, and usually covered with oily matter not dissolving in KOH solution (Jahn 1974; Zmitrovich et al. 2006; Wu et al. 2010; Niemelä et al. 2012; Vlasák et al. 2012). Moreover, the traditional concept of Aurantiporus has caused difficulty in defining certain species, such as A. pulcherrimus (Rodway) P.K. Buchanan & Hood, A. fissilis (Berk. & M.A. Curtis) H. Jahn, and A. alborubescens (Romell) H. Jahn, which were also combined into the genus Tyromyces P. Karst. due to morphological similarities (Reid 1967; Yao et al. 1999; Buchanan and Ryvarden 2000; Ryvarden and Melo 2017). These species seem to be phylogenetically distant from the type species of Aurantiporus, Hapalopilus, and Tyromyces (Binder et al. 2013; Floudas and Hibbett 2015; Liu et al. 2023). Recently, the genus Odoria V. Papp & Dima was established to accommodate A. alborubescens (Papp and Dima 2017), and Pappia Zmitr. was established for A. fissilis (Zmitrovich 2018), while the phylogenetic affiliations of other Aurantiporus species remained unclear. Our current phylogeny strongly supports the treatment of A. fissilis in the monophyletic genus Pappia. Additionally, the distinctive characteristics of whitish pileate basidiomata, shrinking significantly in size when dry, a pleasant and sweet smell, and the presence of chlamydospores, differentiate Pappia fissilis as a unique entity in both Aurantiporus and Tyromyces (Ryvarden and Melo 2017; Chen et al. 2021).

The genus Aurantiopileus D.L. Lindner & T.J. Baroni, typified as A. mayaensis Ginns, was erected by Ginns et al. (2010), and unlike Aurantiporus, it has cystidia. Confusingly, Aurantiopileus mayaensis was somehow combined into Aurantiporus by Zmitrovich (2018). In our phylogenetic analysis (Fig. 1), the two genera *sensu typi* stand apart from each other and are clustered in different clades. Nevertheless, *A. mayaensis* is nested with *Aurantiporus albidus* Rajchenb. & Cwielong described from Argentina, and both species are characterized by large, watery, and fleshy basidiomata when fresh, shrink and hard when dried which fit well with the concept of *Aurantiporus* in morphology (Rajchenberg 1995; Ginns et al. 2010). Regarding the available GenBank sequences attributed to *Aurantiporus albidus*, two Australian specimens (Cui 16664 and Cui 16665) and two Argentinean specimens (CIEFAP-117 and F32) formed two lineages in our phylogeny (Fig. 1). It seems that the Australian samples represent another taxon rather than *Aurantiporus albidus*. Notably, *Aurantiporus albidus* stands out microscopically from other *Aurantiporus* species in its strongly agglutinated generative hyphae covered with hyaline, resinous material and thickwalled, amyloid basidiospores, and may be a distinct species as suggested by Rajchenberg in (1995). However, it is certain that taxa of *Aurantiporus* and *Aurantiporus* s. str. nested in two clades in Meruliaceae.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Two new species of *Collybiopsis* (Agaricales, Omphalotaceae) from Eastern North America

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Abstract

Two small gymnopoid fungi from the southern Appalachian Mountains and Massachusetts, *Collybiopsis complicata* **sp. nov.** and *C. prolapsis* **sp. nov.**, are identified and described. A new generic nrITS-LSU phylogeny of *Collybiopsis* places *C. complicata* and *C. prolapsis* in a small clade together with *C. minor*, and an unknown taxon from Arkansas. This clade adds to the growing circumscription of *Collybiopsis* (= *Marasmiellus*).

Key words: Gymnopus, Marasmiaceae, new species, Omphalotaceae, phylogeny, taxonomy

Introduction

Although the southern Appalachian Mountains of the Eastern United States have been explored by numerous mycologists for the last century, not all agarics have been recorded or described. This is especially true of the Great Smoky Mountains National Park (GSMNP) and adjacent regions where L. R. Hesler, together with visiting workers, collected for much of the 20th century, and where the senior author and additional visitors have collected and described fungi for the past 50 years or more (Hesler 1959; Petersen 1977; Desjardin 1989; Mata et al. 2007). Nonetheless, smaller agarics from this species-rich region often remain overlooked. Especially problematic are small litter- and wood-decomposing agarics that dominate the moist understory of the varied conifer-hardwood forests of the southern Appalachian Mountains. Herein, we name and describe two such fungi within *Collybiopsis, C. complicata* and *C. prolapsis*, place them within a larger *Collybiopsis* phylogeny, and note that while originally identified from the southern Appalachian area, *C. complicata*, at least, has a wider distribution.

Species within *Collybiopsis* and *Gymnopus* were previously included within *Collybia* s.l., a large polyphyletic genus within the Omphalotaceae consisting of transient basidiomes, convex and often non-striate pilei, variably attached lamellae and robust, non-filiform stipes (compared to *Marasmius*) (Halling 1983; Wilson and Desjardin 2005; Mata et al. 2007). With the advent of molecular methods of assessing fungal relationships, it became clear that morphology alone was insufficient to delineate modern taxonomic relationships and that traditional genera of small saprobic "collybiod" fungi (*Marasmius*, *Marasmiellus* and *Collybia*) were polyphyletic. *Collybia* s.s. was segregated from *Collybia* and remaining taxa



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Ronald H. Petersen & Karen W. Hughes. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). were transferred to Gymnopus ("gymnopoid fungi": (Antonín and Noordeloos 1993, 1997). Wilson and Desjardin (2005) examined nrLSU-based phylogenetic relationships among the gymnopoid and marasmioid fungi and designated two unresolved clades, /gymnopus and /marasmiellus. /Marasmiellus contained the type species of Marasmiellus and was dominated by members of Gymnopus section Vestipedes (stipe surface usually with a vesture, hyphae sometimes diverticulate or coralloid). Mata et al. (2007) used molecular data to examine structure within Gymnopus, arriving at clades A-N of "gymnopoid fungi", confirming placement of the generic type species of Marasmiellus, Marasmiellus juniperinus, within Gymnopus clade D (Mata et al. 2004). Species within Clade D (/Marasmiellus of Wilson and Desjardin 2005) were transferred to Marasmiellus (Oliveira et al. 2019), then Collybiopsis (Petersen and Hughes 2021). Other gymnopoid taxa were subsequently segregated from Gymnopus including Paragymnopus, Mycetinis (Petersen and Hughes 2017), Paramycetinis (Petersen and Hughes 2020), and Pseudomarasmius (Petersen and Hughes 2020). The most recent treatment of the Omphalotaceae overall which included 191 sequences within the Omphalotaceae showed Mycetinis and Paramycetinis as basal to Marasmiellus and sister to a diverse group of taxa including Lentinula and Rhodocollybia (Petersen and Hughes 2020). The terms "gymnopoid" and marasmielloid" thus refer to taxa that historically belonged to Gymnopus or Marasmiellus (spore print white, stipe central, without volva or annulus; lamellae variously attached) but marasmielloid fungi tend to have pale pilei, prostrate and diverticulate hyphae and cheilocystidia arising from horizontal hyphae in the hymenium. Ultimately, both gymnopoid and marasmielloid fungi are defined molecularly as belonging to Gymnopus or Marasmiellus.

Materials and methods

Macromorphology

Below, colors of basidiomatal structures within quotation marks ("") are from Ridgway (1912) and matching colors from Kornerup and Wanscher (1967) are cited alphanumerically by plate, column, and row (i.e. "28E5").

Micromorphology

Observations of microscopic structures were made with an Olympus BX60 research microscope fitted with phase contrast microscopy (PhC). Photos were produced using an Olympus Q-color 5 camera/computer attachment. All micromorphology was accomplished with squash mounts of minute amounts of basidioma tissue in 3% aqueous KOH; in some cases, enough material existed to make a second mount in Melzer's reagent (cited as IKI) to test for amyloidity.

Molecular procedures

DNA was extracted from either dried herbarium specimens or from cultures grown in PD Broth (24g/I Difco Potato Dextrose: Thermo Fisher Scientific, Waltham, Massachusetts) using an E.N.Z.A HP Fungal DNA kit (Omega Bio-Tek Inc., Norcross, GA). The nrITS (Schoch et al. 2012) and nrLSU regions were PCR-amplified using procedures outlined in Hughes et al. (2020b). Primers ITS1F, ITS4, ITS2, and ITS3 were used in various combinations to amplify the whole nrITS region or fragments of the region (White et al. 1989; Gardes and Bruns 1996). Primers LROR and LR5 were used to amplify the 5' end of the nrLSU region (Cubeta et al. 1991). PCR products were confirmed by gel electrophoresis. Five μ L of the PCR product were treated with 2ul ExoSAP-IT (Thermo-Fisher Scientific) using the manufacturer's directions. Sanger dideoxy sequencing reactions were performed using BigDye Terminator 3.1 (Thermo-Fisher Scientific) following manufacturers' directions but with cycles increased to 35. Sanger sequencing was performed by the University of Tennessee UT Genomics Core, College of Arts and Sciences.

NrITS and nrLSU sequences (Table 1) were concatenated in Geneious R11.1.5 (Geneious 2017) and aligned manually in Aliview (Larsson 2014). The Alignment for *Collybiopsis* had 177 sequences with 1875 columns, 905 distinct patterns, 605 parsimony-informative sites, and 1172 constant sites. The model of evolution was estimated using Model Finder (Kalyaanamoorthy et al. 2017) in W-IQ Tree (Trifinopoulos et al. 2016) as GTR+F+I+G4. This model was implemented in the generation of a Fast ML tree with 1000 bootstrap replicates, using the web version of IQTree tree (http://iqtree.cibiv.univie.ac.at/) (Fig. 1).

In addition, Bayesian analysis was performed on the *Collybiopsis* alignment in Geneious 11.1.5 using the MrBayes plugin (Huelsenbeck and Ronquist 2001) with a GTR model of evolution (4 Gamma Categories, nst=6, and basefreq=estimated). The MCMC search was carried out with 4 chains for 1,100,000 generations with sampling every 1000 generations. The first 100,000 trees were discarded when likelihood values had reached convergence. Convergence was assessed by ensuring that the average standard deviation of split frequencies was below 0.01. Posterior probabilities were estimated by sampling trees generated after likelihood values had reached equilibrium.

The alignment for the *C. complicata* subgroup contained 34 sequences with 366 distinct patterns, 182 parsimony-informative sites and 1510 invariant sites. The best-fit model of evolution was estimated using Model Finder in W-IQ-Tree as TIM2+F+I+G4. This model was implemented in the generation of a Fast ML tree with 1000 bootstrap replicates, using the web version of IQTree tree (http://iqtree. cibiv.univie.ac.at/) (Fig. 2). Bayesian analysis was performed as described above.

Taxa used in both analyses are given in Table 1. Collections retained at TENN have both a field number (TFB = Tennessee Field Book) and a TENN accession number (TENN-F-XXXXXX).

Results

The phylogenetic position of *C. prolapsis* and *C. complicata* within *Collybiopsis* varies between Maximum Likelihood and Bayesian analyses. In Maximum Likelihood analysis, the *C. prolapsis/C. complicatus* clade is sister to a large clade containing elements of *Collybia* sects. *Subfumosae/Vestipedes* (Fig. 1.). In Bayesian analysis, the *C. prolapsis/C. complicatus* clade is sister to a clade containing *C. ramealis*. This is the only major difference between the topologies of the two phylogenetic analyses. Other differences include minor differences in the position of Gymnopus sp. 17, an unnamed *Collybiopsis*, and *C. quercophila*. The nrITS sequences for collections within *C. complicata* (Table 1) are genetically identical with the exception of a 1bp C/T transition in TENN-F-065811 (0.17% difference). In contrast, GenBank accession OR500517 has 6 differing base pairs (1.05%).

The pileipellis structure of *C. prolapsis* and *C. complicata* is similar to that of the infrageneric *Collybia* sects. *Subfumosae* and *Vestipedes* (Halling 1983) on the one hand, and to the cheilocystidial structure of these two species to the *Collybiopsis ramealis* group (Petersen and Hughes 2021) on the other. Cheilocystidial morphology also comes close to that of *C. minor*, somewhat morphologically disjunct from the *C. ramealis* group. In all cases, however, molecular sequences clearly separate the *C. ramealis* clade, the *C. complicata/prolapsis* clade and the *Collybiopsis subfumosae/vestipedes* clade.

Name	Location1	Isolate	Voucher	ITS GenBank Identifier	LSU GenBank Identifier	Figure
Collybiopsis aff. villosipes	Australia: Perth	N.L.Bougher NLB470	PERTH:8872252	MT537088	MT537088	Fig. 1
Collybiopsis biforma	USA: TN	TFB13814	TENN-F-065189	KJ416249	KJ189569	Fig. 1
Collybiopsis biforma	USA: NC	TFB13890	TENN-F-065586	KJ416248	KJ189570	Fig. 1
Collybiopsis biforma	USA: TN GSMNP	TFB14250	TENN-F-068108	KJ416246	KJ189568	Fig. 1
Collybiopsis biformis	USA: TN, GSMNP	TFB14251	TENN-F-068109	KJ416245	KJ189567	Fig. 1
Collybiopsis brunneigracilis	Indonesia: Java	AWW01	AWW01-SFSU	AY639412	no	Figs 1, 2
Collybiopsis californica	USA: CA	DED8372	SFSU-F-024526	MN413337	no	Fig. 1
Collybiopsis californica	USA: OR	iNAT-143113059	no	OQ781003	no	Fig. 1
Collybiopsis californica	USA: WA	iNat-29416590	no	OK346494	no	Fig. 1
Collybiopsis californica	USA: CA	none	SFSU Wright2941	MN413335	no	Fig. 1
Collybiopsis californica	USA: CA	none	SFSU Wright 866	MN413336	no	Fig. 1
Collybiopsis californica	Canada: BC	TFB05787	TENN-F-052617	MN413338	no	Fig. 1
Collybiopsis complicata	USA: Tennessee, GSMNP	TFB09168	TENN-F-055766	DQ450029	no	Figs 1, 2
Collybiopsis complicata	USA: North Carolina, Macon Co.	TFB13916	TENN-F-065811	OR500517	OR500517	Figs 1, 2
Collybiopsis complicata as Marasmiellus sp.	USA: MA, World's End, Boston Harbor Islands	HUH-F-00964493	FH:BHI-F447	MF161269	no	Figs 1, 2
Collybiopsis complicata as Marasmiellus sp.	USA: MA, World's End, Boston Harbor Islands	HUH-F-00964494	FH:BHI-F401	MF161247	no	Figs 1, 2
Collybiopsis complicata as Marasmiellus sp.	USA: MA, World's End, Boston Harbor Islands	HUH-F-00964495	FH:BHI-F034	MF161165	no	Figs 1, 2
Collybiopsis confluens	USA: NC	TFB14075	TENN-F-067822	KP710281	KP710281	Fig. 1
Collybiopsis confluens	Germany: Thuringia	TFB14114	TENN-F-067864	KP710296	KJ189573	Fig. 1
Collybiopsis confluens	Germany: Thuringia	TFB14115	TENN-F-067865	KP710292	KJ189578	Fig. 1
Collybiopsis confluens	Canada: NB	TFB14389	TENN-F-069053	KP710279	KJ189584	Fig. 1
Collybiopsis confluens	Canada: NB	TFB14409	TENN-F-09073	KP710278	KJ189585	Fig. 1
Collybiopsis dichroa	USA: NC	TFB01860	TENN-F-048680	MW396869	MW396869	Fig. 1
Collybiopsis dichroa	USA: SC	TFB05459	TENN-F-051775	MW396868	MW396868	Fig. 1
Collybiopsis dichroa	USA: NC, GSMNP	TFB09623h1,h2	TENN-F-056584	MW396865- MW396866	MW396865- MW396866	Fig. 1
Collybiopsis dichroa	USA:NC	TFB10009h1	TENN-F-056721	KY026654	KY026654	Fig. 1
Collybiopsis dichroa	USA: NC	TFB13873	TENN-F-065569	MW396867	MW396867	Fig. 1
Collybiopsis dichroa	USA: TN, GSMNP	TFB14111ss1	TENN-F-067859	KY026696	KY026696	Fig. 1
Collybiopsis dichroa	USA: TN, GSMNP	TFB14111ss2	TENN-F-067859	KY026697	KY026697	Fig. 1

Table 1. Collections used in phylogenetic analyses.

Name	Location1	Isolate	Voucher	ITS GenBank Identifier	LSU GenBank Identifier	Figure
Collybiopsis disjuncta	USA: MS	TFB14281	TENN-F-068136	KY019643	KY019643	Fig. 1
Collybiopsis disjuncta Type	USA: CT	TFB14339	TENN-F-069172	KJ416252	PP430330	Fig. 1
Collybiopsis eneficola	Canada: Newfoundland	10-09-21AV04	TENN-F-069122	KJ128265	no	Fig. 1
Collybiopsis eneficola	USA:AK	NA	MICH:PK6975	KP710270	no	Fig. 1
Collybiopsis eneficola	USA:AK	NA	MICH:PK6976	KP710271	no	Fig. 1
Collybiopsis enificola Type	Canada: Newfoundland	09-09-26AV13	TENN-F-069123	NR_137613	NG_059502	Fig. 1
Collybiopsis filamentipes Type	USA: TN	TFB13962	TENN-F-065861	MN897832	MN897832	Fig. 1
Collybiopsis filamentipes Env. Samp.	Canada: Alberta	KTRF390	NA	MG433317	no	Fig. 1
Collybiopsis furtiva	USA: North Carolina, Highlands	DED3973	SFSU-F-024523	MN413339	no	Fig. 1
Collybiopsis furtiva Type	USA:	DED4425	SFSU DED4425	DQ450031	AF042650	Fig. 1
Collybiopsis furtiva	USA: North Carolina, Macon Co.	DED4584	SFSU-F-024508	MN413340	no	Fig. 1
Collybiopsis furtiva	USA: North Carolina, Coweeta	DED5796h1	SFSU-F-024524h1	MN413341	no	Fig. 1
Collybiopsis furtiva	USA: North Carolina, Coweeta	DED5796h2	SFSU-F-024524h2	MN413342	no	Fig. 1
Collybiopsis furtiva	USA: GA	TFB04796	TENN-F-051097	MN413343	MW396879	Fig. 1
Collybiopsis gibbosa	Australia: NT	NA	MEL:2382838	KP012713	KP012713	Fig. 1
Collybiopsis gibbosa	Brazil: Amapa	NA	URM 90012	KY061202	KY061202	Fig. 1
Collybiopsis gibbosa	Brazil: Amapa	NA	URM 90006	KY061203	KY061203	Figs 1, 2
Collybiopsis hasanskyensis Type	Russia: Far Eastern	TFB11846	TENN-F-060730	MN897829	no	Fig. 1
Collybiopsis hasanskyensis	Russia: Far Eastern	TFB11847	TENN-F-060731	MN897830	no	Fig. 1
Collybiopsis indocta	Argentina	TFB08605	TENN-F-054944	MW396870	MW396870	Fig. 1
Collybiopsis juniperina	Argentina	TFB10782	TENN-F-058988	KY026661	KY026661	
something wrong Collybiopsis vaillantii	USA: TN, GSMNP	TFB13739	TENN-F-065155	KY026676	KY026676	Fig. 1
Collybiopsis juniperina	USA: LA	TFB9889	TENN-F-59540	AY256708	AY256708	Fig. 1
Collybiopsis luxurians	Switzerland	TBF04283ss10	TENN-F:050619	KJ416240	PP430331	Figs 1, 2
Collybiopsis luxurians	USA: LA	TFB09121	TENN-F-055748	KY026649	KY026649	Figs 1, 2
Collybiopsis luxurians	USA: NC	TFB10350	TENN-F:057910	AF505765	not done	Figs 1, 2
Collybiopsis luxurians	USA: NC	TFB14060	TENN-F:067806	MW396871	MW396871	Figs 1, 2
Collybiopsis melanopus Type	Indonesia: Java	AWW54	SFSU:AWWilson 54	AY263425, NR_137539	AY639422., NG_060624	Fig. 1
Collybiopsis menehune		AWW15	SFSU:AWWilson 15	AY263443	AY639424	Fig. 1
Collybiopsis menehune	USA: HI	TFB11587	DEH2320	DQ450043	no	Fig. 1
Collybiopsis menehune	India		CUH:AM074	KJ778753	no	Fig. 1
Collybiopsis menehune Type	Indonesia: Java	DED5866	SFSU: DED5866	AY263426	no	Fig. 1
Collybiopsis mesoamericana	Costa Rica	REH7379	NYBG REH7379	AF505768	no	Fig. 1
Collybiopsis mesoamericana	Costa Rica	TFB10411	TENN-F-058106	DQ450036	no	Fig. 1
Collybiopsis minor	USA: South Carolina	TFB05434	TENN-F-051792	MW396872	MW396872	Fig. 1
Collybiopsis minor Type	USA: TN, GSMNP	TFB11930	TENN-F-067806	MN413334, NG_228867	MW396880	Figs 1, 2
Collybiopsis neotropica	Costa Rica	TFB10416	TENN-F-058113	AF505769	no	Fig. 1

Name	Location1	Isolate	Voucher	ITS GenBank Identifier	LSU GenBank Identifier	Figure
Collybiopsis nonnulla	USA: MS	TFB14278	TENN-F-068133	KY026701	no	Fig. 1
Collybiopsis nonnulla	USA: MS	TFB14492	TENN-F-069193	MW396873	MW396873	Fig. 1
Collybiopsis nonnulla v. attenuatus	Cameroon	NA	RAK369.2	MN930621	no	Fig. 1
Collybiopsis nonnulla v. attenuatus	Cameroon	NA	RAK372.2	MN930622	no	Fig. 1
Collybiopsis nonnullus v. attenuatus	Indonesia: Java	AWW05	SFSU: AWWilson05	AY263445	AY639445	Fig. 1
Collybiopsis nonnullus v. attenuatus	Indonesia: Java	AWW55	SFSU: AWWilson55	AY263446	no	Fig. 1
Mycetinis opacus	USA: TN	BM888	TENN-F-070567	MW396878	no	Fig. 1
Mycetinis opacus	USA: MS	TFB09071	TENN-F-054871	MW396877	MW396877	Fig. 1
Collybiopsis parvula	Costa Rica	TFB10422	TENN-F-058116	AF505774	no	Fig. 1
Collybiopsis parvula	Mexico	NA	SR83-10MX	KT697977	no	Fig. 1
Collybiopsis parvula Type	Costa Rica	TFB10419	TENN-F-058113	NR_119584, DQ450060	no	Fig. 1
Collybiopsis parvula	Costa Rica	TFB10421	TENN-F-058115	DQ450061	no	Fig. 1
Collybiopsis parvula	Costa Rica	TFB10425	TENN-F-058119	DQ450062	no	Fig. 1
Collybiopsis peronata	Belgium, Dinante	TFB13743	TENN-F-065121	KY026677	KY026677	Fig. 1
Collybiopsis peronata	USA: GA	TFB14617	TENN-F-069322	KY026738	KY026738	Fig. 1
Collybiopsis peronata	Unknown	NA	CBS223.37	MH855896	no	Fig. 1
Collybiopsis polygramma	Puerto Rico	TFB09628	TENN-F-056589	DQ450028	no	Figs 1, 2
Collybiopsis polygramma	Korea	TFB12806	SFC20120821-64	KJ609162	no	Figs 1, 2
Collybiopsis polygramma	Puerto Rico	NA	PR2542TN	AY842954	no	Figs 1, 2
Collybiopsis polygramma	India	NA	CUH:AM082	KJ778752	no	Figs 1, 2
Collybiopsis polygramma	Brazil: Amapa	NA	URM90015	KY074640	no	Figs 1, 2
Collybiopsis polygramma	Brazil: Para	NA	URM90016	KY074641	no	Figs 1, 2
Collybiopsis polygramma	Brazil: Para	NA	URM90017	KY074642	no	Figs 1, 2
Collybiopsis polygramma	China, Hunan	NA	MHHNU 30912	MK214392	no	Figs 1, 2
Collybiopsis polygramma	China, Jiangxi	NA	HFJAU0425	MN258643	no	Figs 1, 2
Collybiopsis pseudoluxurians holotype	USA: Mississippi	TFB14290	TENN-F-068144	KY026702, NR_137863	KJ416242	Figs 1, 2
Collybiopsis pseudoomphalodes	Costa Rica	REH7348	NYBG REH7348	AF505762	no	Figs 1, 2
Collybiopsis pseudoomphalodes	Puerto Rico	NA	PR24TN	AY842957	no	Figs 1, 2
Collybiopsis peronata	Russia:	LE-BIN1364	no voucher specimen	KY026755	KY026755	Fig. 1
Collybiopsis quercophilia	Slovakia	TFB14570	TENN-F-069267	KY026729	KY026729	Fig. 1
Collybiopsis quercophilia	USA: CA	TFB14615	TENN-F-069320	KY026736	KY026736	Fig. 1
Collybiopsis quercophilia	USA: CA	TFB14616	TENN-F-069321	KY026737	KY026737	Fig. 1
Collybiopsis quercophilia	USA: CA	NA	SFSU:25220	KY026761	KY026761	Fig. 1
Collybiopsis ramealis	Belgium	BR72_41	BR <bel>:72-41</bel>	MW396875	MW396875	Fig. 1
Collybiopsis ramealis	UK: Scotland	TFB03772	TENN-F-050509	MN413350	MW396885	Fig. 1
Collybiopsis ramealis	UK: Scotland	TFB06989	TENN-F-055908	MN413372	MW396883	Fig. 1
Collybiopsis ramealis	Sweden	TFB13520	TENN-F-062867	JF313670	OR500520	Fig. 1
Collybiopsis ramealis	Belgium	TFB13759	TENN-F-065136	MN413344	MN413344	Fig. 1
Collybiopsis ramealis	Belgium	TFB13769	TENN-F-065145	MN413345	MN413345	Fig. 1
Collybiopsis ramealis	Germany	TFB14140c1	TENN-F-067890	MN413355	OR500518	Fig. 1
Collybiopsis ramealis	Germany	TFB14150c1	TENN-F-067900c1	MN413363	OR500519	Fig. 1
Collybiopsis ramealis	Germany	TFB14163h1	TENN-F-067912	MN413351	MN413351	Fig. 1
Collybiopsis ramealis	Germany	TFB14163h2	TENN-F-067913	MN413352	MN413352	Fig. 1
Collybiopsis ramealis	Slovakia	TFB14555	TENN-F-069251	MW405779	MW396884	Fig. 1

Name	Location1	Isolate	Voucher	ITS GenBank Identifier	LSU GenBank Identifier	Figure
Collybiopsis ramealis	Slovakia	TFB14556	TENN-F-069252	MN413369	MN413369	Fig. 1
Collybiopsis ramealis	Slovakia	TFB14559h1	TENN-F-069255h1	MN413370	PP430332	Fig. 1
Collybiopsis ramealis	Slovakia	TFB14559h2	TENN-F-069255h2	MN413371	PP430332	Fig. 1
Collybiopsis readiae	New Zealand	TFB07571	TENN-F-053687	DQ450034	no	Fig. 1
Collybiopsis readiae	New Zealand: Buller District	TFB13056	TENN-F-061061	KJ416244	no	Fig. 1
Collybiopsis readiae	New Zealand	NA	PDD: 95844	HQ533036	no	Fig. 1
Collybiopsis sp.	Australia: Christmas Island	N.L.Bougher NLB 1292	PERTH:08827494	ON715771	ON715771	Fig. 1
Collybiopsis sp. (Gymnopus sp. 17)	USA: CT	TFB14334h1	TENN-F-068185	KY026707	KY026707	Fig. 1
<i>Collybiopsis</i> sp. (Gymnopus sp. 17)	USA: CT	TFB14334h2	TENN-F-068185	KY026708	KY026708	Fig. 1
Collybiopsis sp. "prolapsis"	USA: Georgia	TFB04800	TENN-F-051101	MW396874	MW396874	Figs 1, 2
Collybiopsis sp. (Gymnopus sp.)	USA: WV	NA	WRW05-1170	KY026764	KY026764	Fig. 1
Collybiopsis sp. (VC-2017f)	Brazil: Paraiba	NA	URM 90043	KY321573	KY321573	Figs 1, 2
Collybiopsis sp. (VC-2017f)	Brazil: Paraiba	NA	URM 90042	KY321574	KY321574	Figs 1, 2
Collybiopsis sp. (VC-2017f)	Brazil: Paraiba	NA	URM 90045	KY321575	KY321575	Figs 1, 2
Collybiopsis sp. (VC-2017f)	Brazil: Para	NA	URM 90051	KY321568	KY321568	Fig. 1
Collybiopsis sp. (VC-2017f)	Brazil: Para	NA	URM 90053	KY321570	KY321570	Fig. 1
Collybiopsis sp. Env. Samp.	USA: California	Environmental Sample	none	DQ273359	no	Fig. 1
Collybiopsis stenophylla	USA: North Carolina, Macon Co.	TFB11558	TENN-F-059443	DQ450032	no	Fig. 1
Collybiopsis stenophylla	USA: North Carolina, Macon Co.	TFB11559	TENN-F-059444	DQ450033	no	Fig. 1
Collybiopsis stenophyllus	USA: Georgia	TFB04798	TENN-F-051099	MN413330	MW396879	Fig. 1
Collybiopsis stenophyllus	Belgium	TFB13770	TENN-F-065146	MN413346	MW396882	Fig. 1
Collybiopsis stenophyllus	USA: Tennessee, GSMNP	TFB13998	TENN-F-065943	MN413331	MW396886	Fig. 1
Collybiopsis subcyathiformis	Brazil: Para	NA	URM90023	KY404982	KY404982	Fig. 1
Collybiopsis subcyathiformis	Brazil: Para	NA	URM 90022RNA	KY404983	KY404983	Fig. 1
Collybiopsis subnuda	USA: TN, GSMNP	TFB12577	TENN-F-061138	KY026667	FJ750262	Fig. 1
Collybiopsis subnuda	USA: NC, Macon Co.	TFB14043	TENN-F-065984	MW396876	MW396876	Fig. 1
Collybiopsis subnuda	USA: WV	NA	WRW08-462	KY026765	KY026765	Fig. 1
Collybiopsis trogioides Type	Indonesia: Java	AWW51	AWW51-SFSU	NR_152884	NG_228715	Fig. 1
Collybiopsis vaillantii	USA: TN, GSMNP	TFB13739	TENN-F-065115	KY026676	KY026676	Fig. 1
Collybiopsis velosipes	USA: CA	TFB09539	TENN-F-056252	DQ450058	no	Fig. 1
Collybiopsis villosipes	New Zealand: Fiordland	TFB12836	TENN-F-060951	KJ416255	FJ750264	Fig. 1
Collybiopsis villosipes	USA: CA	inaturalist.org/ observations/2708886	NA	MF163171	no	Fig. 1
Colybiopsis minor	USA: South Carolina	TFB06284	TENN-F-052933	MW405778	MW396881	Fig. 1
Environmental Sample	USA: Oregon	clone FON_f09	none	HM488468	no	Fig. 1
Environmental Sample-soil	USA: Oregon	clone FON_h10	none	HM488469	no	Fig. 1
Gymnopanella nothofagi	Chile: Aisen	PSL 411	SG0163624	KT906426	KT906426	Fig. 1
Gymnopanella nothofagi	Chile: Aisen	PSL 414	SG0163625	KT906425	KT906425	Fig. 1
Collybiopsis obscuroides	Sweden: Jamtland	NA	GB-0053811	KX958398	KX958398	Fig. 1

Name	Location1	Isolate	Voucher	ITS GenBank Identifier	LSU GenBank Identifier	Figure
Collybiopsis obscuroides	Norway: Svalbard	NA	GB-0150514	KX958399	KX958399	Fig. 1
Gymnopus peronata	Canada: BC	NA	UBC F28402	KP454027	no	Fig. 1
Gymnopus sp. (VC-2017k)	Brazil: Paraiba	NA	URM 90054	KY404984	KY404978	Fig. 1
Gymnopus sp.	Japan: Okinawa	Ns8-1	none	LC504922	no	Fig. 1
Gymnopus sp. (root sample)	Sweden	olrim406	none	KY352520	no	Fig. 1
Gymnopus sp.	USA: MS, Boston Harbor Islands	BHI-F523a	FH:BHI-F523a	MF161290	no	Fig. 1
<i>Gymnopus</i> sp. (Not in Collybiopsis paper)	Costa Rica	TFB10494	TENN-F-058602	KY026660	no	Fig. 1
Marasmiaceae sp.	USA: FL	NA	FLAS-F-69007	OP163218	no	Fig. 1
Marasmiellis sp.	USA: Arkansas	RA7L5-13a (leaf litter)	none	MK234195	no	Figs 1, 2
Marasmiellus foliiphila	India	none	CUH AM090	KP317637	no	Fig. 1
Marasmiellus foliiphila	India	none	CUH AM101	KP317638	no	Fig. 1
Marasmiellus sp.	Mexico: Oaxaca	P196 (soil)	NA	KR135355	no	Fig. 1
Marasmiellus sp.	USA:	14147	NA	MW023100	no	Fig. 1
Mycetinis copelandii	USA: CA	TFB08084h1	TENN-F-55408 haplotype h1	KY696750	KY696750	Fig. 1
Mycetinis copelandii	USA: CA	TFB08084h2	TENN-F-55408 haplotype h2	KY696751	KY696751	Fig. 1
Mycetinis kallioneus	Norway: Svalvard	NA	GB-0150513	KX958397	KX958397	Fig. 1
Mycetinis opacus	USA: MS	TFB14490h1	TENN-F-069200 h1	KY696768	KY696768	Fig. 1
Mycetinis opacus	USA: MS	TFB14490h2	TENN-F-069200 h2	KY696769	KY696769	Fig. 1
Mycetinis salalis	Canada: BC, Vancouver Island	NA	DAOM:175251	KX752265	KX752265	Fig. 1
Mycetinis scorodonius	Sweden	TFB03785	TENN-F-050522	KY696731	KY696731	Fig. 1
Mycetinis scorodonius	USA: NC	TFB03071	TENN-F-050689	KY696733	KY696733	Fig. 1
Mycetinis scorodonius	USA: TN, GSMNP	TFB03708	TENN-F-050696	KY696734	KY696734	Fig. 1
Mycetinis scorodonius	Canada: Nova Scotia	TFB05031	TENN-F-051442	KY696739	KY696739	Fig. 1
Mycetinis scorodonius	USA: NY	TFB04969	TENN-F-053466	KY696741	KY696741	Fig. 1
Mycetinis scorodonius	Canada: Nova Scotia	TFB05025	TENN-F-053467	KY696742	KY696742	Fig. 1
Mycetinis scorodonius	USA: NT	TFB04939	TENN-F-053471	KY696746	KY696746	Fig. 1
Mycetinis scorodonius	USA: ME	TFB05005	TENN-F-053474	KY696748	KY696748	Fig. 1
Paramycetinis austrobrevipes	Australia: Tasmania	TFB03585	TENN-F-053181	KY026638	KY026638	Fig. 1
Paramycetinis austrobrevipes	Australia: Tasmania	TFB03591	TENN-F-053146	KY026637	KY026637	Fig. 1
Paramycetinis austrobrevipes Type	Australia: Tasmania	TFB04033	TENN-F-050135	KY026622	KY026622	Fig. 1
Paramycetinis caulocystidiatus Type	New Zealand	TFB07148	TENN-F-054050	KY026645	KY026645	Fig. 1
Paramycetinis caulocystidiatus	New Zealand	TFB07572	TENN-F-053683	KY026642	KY026642	Fig. 1
Paramycetinis caulocystidiatus	New Zealand	TFB07588	TENN-F-053721	KY026643	KY026643	Fig. 1
Paramycetinis caulocystidiatus	New Zealand	TFB07589	TENN-F-053725	KY026644	KY026644	Fig. 1
Collybiopsis melanopus	Not Indicated	NA	CUH AM093	KP100305	KP100305	Fig. 1
Collybiopsis mesoamericanus Type	Costa Rica	TFB11005	TENN-F-058613	NR_119583	KY019632	Fig. 1
Collybiopsis folliphilia Type	India	NA	CUH:AM090	NR_154176	NG_060320	Fig. 1



Figure 1. nrITS-nrLSU based Maximum Likelihood consensus tree with 1000 bootstrap replicates. The tree was generated using the web version of IQTree tree (http://iqtree.cibiv.univie.ac.at/) using the best-fit model of evolution (GTR+F+I+G4, AIC criteria). Colors represent branch bootstrap support. Red = 95-100% bootstrap support, Purple = 90-94.9% bootstrap support, Blue = 80-89.9% bootstrap support, Aqua = 70-79.9% bootstrap support and Green = 60-69.9 bootstrap support.



Figure 2. NrITS-nrLSU based Maximum Likelihood consensus tree of the *C. complicata/C. prolapsis* clade with 1000 bootstrap replicates. The tree was generated using the web version of IQTree tree (http://iqtree.cibiv.univie.ac.at/) using the best-fit model of evolution (TIM2+F+I+G4, AIC criteria). Included taxa were selected based on the Bayesian analysis which showed the *C. complicata* group and the *C. ramealis* group as sister taxa. Bootstrap support is indicated below the branches and Bayesian Posterior probabilities above the branches.

Taxonomy

Collybiopsis complicata R.H. Petersen, sp. nov. Index Fungorum: IF901185 Figs 3–11

Holotype. Tennessee, Blount Co., Great Smoky Mountains National Park, Metcalf's Bottoms Picnic Area, 9.VI.1997, coll. Ronald H. Petersen, TFB 9168 (TENN-F-055766).

Diagnosis. 1) Basidiomata marasmielloid/gymnopoid, gracile, small, with slender stipe; 2) pileus pigmented, especially over disc; 3) pileipellis composed of stalked-coralloid structures and lobed repent hyphae; 4) stipe fully vestured; 5) clamp connections present; 6) cheilocystidia prominent, similar to pileipellis elements; 7) basidiospores $5-8 \times 3-4 \mu m$.

Etymology. Cheilocystidia with complex, branched structure; also complex distribution, from southern Appalachians to New England.

Description. Basidiomata (Fig. 3) scattered-gregarious >50 basidiomata in <1² m, marasmielloid or miniature collybioid. Pileus 6–14 mm broad, shallowly convex to plane by maturity, matt, delicately rivulose-striate; disc "snuff brown" (5E8), outward "sayal brown" (6C5) to fleshy tan. Lamellae free to adnexed but seceding in drying to become removed from stipe apex, total lamellae 47-64; through lamellae 24–26, in two major ranks with no anastomosis, 1.5–2 mm broad, subventricose to ventricose by maturity, knife-edged, dull pale tan-gray (near "tilleul buff" 7B2), bleaching to off-white, often (but not exclusively) becoming pale cream-colored with necropigment; lamellulae in one rank, less than $\frac{1}{2}$ the length through lamellae. Stipe $7-30(-45) \times 0.7-1.2$ mm, terete and remaining so upon drying, minutely vestured overall, somewhat darker than lamellae apically, downward fleshy tan to fleshy brown, drying matt; vesture on upper stipe of scattered delicate squamules composed of individual caulocystidia, on lower stipe a solid turf (but never felty), straw-colored to "light ochraceous buff" (5A4); insertion insititious on fine twigs and leaves; superficial litter-binding mycelium not observed. Odor and taste negligible.

Pileipellis a thatch of occasional repent encrusted hyphae (Fig. 4) and dominant interlocking, stalked-lobose pileocystidia and lobed-diverticulate, repent hyphae (Fig. 5B, C); *Pileocystidia* (Figs 5A, D) 27-37 × 5-14 µm, stalked, branching in lobose-coralloid configurations, apparently hyaline, thin- to firm-walled, arising from clamp connections. Subsurface pileipellis hyphae 2.5-10 µm diam, firm-walled, conspicuously clamped, ornamented with small flakes (in profile) but no profile calluses or stripes. *Pleurocystidia* (Fig. 6) common, arising from clamp connections, $32-40 \times 7-9 \mu m$, stalked-fusiform with apex often broadly submammillate; contents homogeneous. Basidioles clavate. Basidia (Fig. 7) $35-60 \times 7-9 \mu m$, clavate to weakly urniform or with somewhat expanded subcapitate apex, (2-)4-sterigmate, arising from clamp connections; sterigmata robust, slightly curved; contents heterogeneous, with scattered, refringent granules. **Basidiospores** (Fig. 8B) (5.5-)6.5-8.5(-10) × 3-4(-5) µm (Q = 1.56-2.50; $Q^m = 2.03$; $L^m = 8.02 \mu m$), ellipsoid, flattened adaxially, thin-walled, inamyloid; contents (dried) homogeneous. Lamellar edge sterile. Cheilocystidia (Figs 9, 10) plentiful, 25-40 × 4-15 µm, stalked, lobose-coralloid branched, often with diverticulate-lobed termini, hyaline, thin-walled. Stipe medullary hyphae



Figure 3. Collybiopsis complicata. Basidiomata. TFB 13916 (TENN-F-065811). Scale bar: 10 mm.



Figure 4. Collybiopsis complicata. Pileipellis elements; repent, encrusted hyphae. Sx TFB 9168 (TENN-F-055766). Scale bars: 10 mm.

 $4-14~\mu m$ diam, strictly parallel, free (not involved in slime matrix), firm-walled, inconspicuously clamped; contents heterogeneous (multigranular). Stipe cortical hyphae $3-5~\mu m$ diam, apparently adherent, obscurely clamped, moderately dextrinoid (more or less hyaline without IKI, reddish brown with IKI), firm- to thick-walled (wall $-0.8~\mu m$ thick), clamped, producing side branches elongating



Figure 5. *Collybiopsis complicata*. Pileipellis elements; pileocystidia **A** cluster of pileocystidia **B**, **C** "Diverticulate" repent hyphae **D** two lobose hyphal termini. Note clamp connections. TFB 9168 (TENN-F-055766). Scale bars: 10 µm.

into caulocystidia. **Caulocystidia** from upper stipe (Figs 8 A, 11) in delicate, interrupted patches $-80 \times 4-10 \mu m$, cylindrical to vermiform, usually somewhat gnarled distally with broadly rounded apex, thick-walled (wall often occluding cell lumen), arising as non-septate side branches from slender (1.5–3.5 μm diam, wall $-1.0 \mu m$ thick), superficial stipe surface hyphae, strongly dextrinoid (dark brownish red with IKI). Caulocystidia from lower stipe similar, a dense turf of free individuals (not involved in slime matrix), weakly to strongly dextrinoid, $-200 \times 3-8 \mu m$, thick-walled (wall/lumen distinction often impossible in IKI;



Figure 6. Collybiopsis complicata. Pleurocystidia Note clamp connection in C. TFB 9168 (TENN-F-055766). Scale bars: 10 µm.

wall $-1.0 \ \mu m$ thick, less dextrinoid than cell contents), tapering gradually to an acutely rounded apex, often gnarled and sometimes forked. Ample evidence of clamp connections in pileipellis, subpellis and pileus trama (i.e. hook cells of disarticulated clamps common and obvious) occasional complete clamps observed at basidial bases.

Habitat. Tsuga debris and adjacent hardwood leaves.


Figure 7. Collybiopsis complicata. Basidia. TFB 9168 (TENN-F-055766). Scale bars: 10 µm.

Specimens examined. Massachusetts, Plymouth County, Boston Harbor, World's End Peninsula, coll LA Kappler, 23.VIII.2015, (HUH) BHI 447 (HUH-F-00964493), Boston Harbor, World's End Peninsula, Rocky Neck, coll D. Healewaters & LA Kappler, 12.VIII.2015, (HUH) BHI 401 (HUH-F-00964494); World's End Peninsula, coll D. Healewaters et al., 14.IX.2013, (HUH) BHI 034 (HUH-F-00964495. North Carolina, Macon Co., vic. Highlands, Bull Pen Rd., Ellicott Rock Trailhead, 35°01.010'N, 83°08.190'W, 20.VII. 2011, coll RHP, TFB 13916 (TENN-F-065811). Tennessee, Blount Co., GSMNP, Metcalf's Bottoms Picnic Area, 9.VI.1997, coll. RHP, TFB 9168 (TENN 55766).

Commentary. The strongly modified "*Rameales*-structure" of the pileipellis structures of *C. complicata* resembles that of species of traditional *Marasmiellus* [viz. *C. ramealis* complex, (Petersen and Hughes 2021)]. This construction comprises a thatch of stalked, ventricose-rostrate structures with lobose, molar-shaped



Figure 8. *Collybiopsis complicata* **A** Caulocystidia **B** Basidiospores. TFB 9168 (TENN-F-055766). Scale bars: 10 μm (**A**); 5 μm (**B**).

outgrowths. These pileipellis structures conform closely to those of *Marasmiellus* sect. *Dealbati* subsect. *Dealbatini* sensu Singer (Singer 1973) [Type = *Marasmius dealbatus* Berk. & Curt.], of which *Marasmius stenophyllus* (*C. stenophylla*, Fig. 2) is a member (Desjardin 1997). *M. stenophyllus* (section *Dealbati*) was transferred to *Gymnopus* sensu lato (Mata et al. 2004), then to *Collybiopsis* (Petersen and Hughes 2021). Both Desjardin (Desjardin 1989; 1997) and Hesler (1959) examined type material of *M. subsynodicus* Murrill, considered by Singer (Singer 1973) and Desjardin (Desjardin 1997) to be synonymous with *M. stenophyllus* (Mont.) Singer). *Marasmius dealbatus* remains unplaced in modern classifications.

Parenthetically, in *C. complicata* collections TENN-F-055766 and HUH-F-00964493, the lobose individual pileipellis elements dominated the pileipellis, while in TENN-F-065811 (also *C. complicata*), these structures were only occasional in a pileipellis dominated by encrusted filamentous hyphae.

The pileipellis structure of *Marasmius* Sect. *Androsacei* (= *Gymnopus* sect. *Androsacei*, see Noordeloos and Antonín (2008), is also described as a combination of diverticulate, repent hyphae and *siccus*-type broom cells (a "Rameales-structure"). Our experience with this, however, indicates that the diverticulae (= setulae) of pileipellis cells are much finer, usually vermiform, refringent (PhC) and <1 µm broad. Cheilocystidia of *C. complicata* are very similar to pileipellis structures, but as a rule are somewhat less complexly branched. Morphologically, the pileipellis of *C. complicata* might be forced into section *Androsacei*, but phylogenetically, it is distant from that group in Gymnopus S.S.



Figure 9. Collybiopsis complicata. Individual cheilocystidia. TFB 9168 (TENN-F-055766). Scale bars: 10 µm.

Mata et al. (2007) initially designated TENN-F-055766 (GenBank DQ450029, *C. complicata*) as *Marasmiellus* sp. aff. *pluvius*, but while basidiomata of *Marasmiellus* pluvius Redhead (Redhead 1982) have similar stature, they are smaller and are gregarious on *Pseudotsuga* and *Thuja* needle beds in southern British Columbia. Further, the pileipellis of *Ma. pluvius* does not include stalked-lobose structures, but is a "compactly interwoven layer of densely diverticulate



Figure 10. Collybiopsis complicata. Clusters of cheilocystidia. HUH. HUH-F-00964494. Scale bars: 10 µm.

hyphae" (Redhead 1982); "Rameales-structure"). Cheilocystidia of Ma. pluvius are stalked-vesciculose to clavate with narrow, vermiform setulae quite similar to those of the Ma. ramealis complex. Basidiospores of Ma. pluvius are longer and significantly narrower than those of C. complicata and caulocystidia exhibit a significantly thicker wall than those of C. complicata. While perhaps morphologically congeneric with C. complicata in Collybiopsis, the two species are quite different microscopically and a second GenBank accession under the name Marasmiellus aff. pluvius (MK277736; NL-5034: nrLSU sequence only) is 0.22% different from other C. complicata nrLSU sequences. Marasmiellus pluvius has not yet been transferred to Collybiopsis, but DNA sequences will probably support such transfer when they become available, although specific placement in a phylogeny remains unknown.

The nrITS sequences for collections within *C. complicata* (Table 1) are genetically identical with the exception of a 1bp C/T transition in TENN-F-065811 from Macon County, GA (0.17% difference). This lack of nrITS variation includes three nrITS sequences of specimens collected in a study of Boston (MA) Harbor Islands (Haelewaters et al. 2018), and one from the Great Smoky Mountains National Park TENN-F-055766. *Collybiopsis complicata*, therefore, seems distributed extensively in temperate Eastern North America. The nearest taxon



Figure 11. *Collybiopsis complicata.* Caulocystidia. TFB 9168 (TENN-F-055766) **A** caulocystidia from upper stipe **B**, **C**, **D** individual caulocystidia. Scale bars: 10 μm.

to *C. complicata*, GenBankaccession MK234195, differs by 6 base pairs (1.05%) and while this falls within the commonly accepted criteria of 2% divergence for conspecificity (Hughes et al. 2009), the lack of variation within *C. complicata* across a wide geographic range (Boston Harbor Islands vs. Southern Appalachians) argues that *C. complicata* does not include MK234195. Possibly, *C. complicata* is a recently-diverging taxon that has not accumulated geographical differences.

Collybiopsis prolapsis

Index Fungorum: IF902313 Figs 12–19

Holotype. UNITED STATES, Georgia, Rabun Co., vic. Clayton, Warwoman Dell Picnic Area, 34°52'57.81"N, 83°20'57.99"W, 15.VI.1992, coll. Scott A. Gordon, TFB 4800 (TENN-F-051101).

Diagnosis. 1) Basidiomata diminutive, collybioid or marasmielloid, saprophytic on hardwood litter; 2) clamp connections ubiquitous; 3) cheilocystidia "prolapsed," similar to "ramealis" type, with an abrupt bouquet of branched diverticula; 4) stipe without vesture (i.e. not similar to *Gymnopus* sect. *Vestipedes*; 5) resupinate patch significant, with diminutive, white hyphal ropes; 6) necropigment weak over hymenophore; 7) nrITS sequence unique, but quite similar to that of *C. complicata* and *C. minor*.

Etymology. Pileo- and cheilocystidia structures with swollen, subspherical excrescences, reminiscent of a prolapse.

Description. Basidiomata diminutive (Fig. 12). *Pileus* 8–12 mm broad, when fresh rich deep brown (6E6-7, "Brussels brown," "Sudan brown"), shallowly convex to plane, very vaguely finely sulcate, minutely radially fibrillose; drying grayish brown, more or less unicolorous. *Lamellae* subdistant, adnate (with very slight non-lamellate hymenium decurrent on stipe apex for less than a millimeter), not ventricose (straight from stipe to margin), total lamellae 30–40, through lamellae 4–10, "off-white" to "cream" to "brown orange", 6C4 in age; lamellulae rudimentary. *Stipe* when fresh reported as concolorous with pileus, when dried more or less concolorous with lamellae, terete, hardly



Figure 12. Collybiopsis prolapsis A basidioma B basidiospores, Scale bars: 8 mm (A); $5 \mu M$ (B).



Figure 13. Collybiopsis prolapsis. Dermatocystidia. Scale bars: 10 μ M.

twisted, glabrous-shining to somewhat wispy very near base; stipe base with extensive (-1.5 sq cm) resupinate patch, now creamy off-white and appearing varnished and with a few small, off-white synnematoid mycelial ropes. **Odor** and **taste** not recorded.



Figure 14. Collybiopsis prolapsis. Repent pileipellis hyphae. Scale bars: 5 µM.

Pileipellis a repent layer of free (with no evidence of slime matrix or individual slime sheath), filamentous hyphae of the following types: 1) dermatocystidia (Fig. 13) clavate to fusiform to inflated and tapering distally, 7–11.5 μ m diam, smooth (Fig. 13A, B) to ornamented in annular pattern (Fig. 13C, E), apparently arising at a clamp connection; contents homogeneous to heterogeneous with scattered inclusion (Fig. 13D); 2) repent hyphae 3.5–8 μ m diam, thin- to firm-walled, varying as follows: a) minutely roughened (Fig. 14A); b) ornamented with individual scabs, flattened in flake-like scales, individual lumps –2 μ m high (Fig. 14B), spiculate structures (Fig. 14C) and/or annular ornamentation with profile calluses (Fig. 14D); 3) smooth, repent hyphae



Figure 15. Collybiopsis prolapsis. Smooth, repent hyphae with 'Subfumosae' side branches. Scale bars: 5 µM.

3.5–7.5 µm diam, with occasional filamentous side branches (Fig. 15A–C); branches simple, lobate to branched, not arising from clamp connections; and 4) thick-walled, usually somewhat inflated hyphae 5.5–10.5 µm diam, with thick-walled cog-like warts, $1.5-2.5 \times 1-2.5$ µm (Fig. 16A, B); intermediate forms (Fig. 16C) occasional. Pileal and lamellar tramae interwoven; hyphae 3–8.5 µm diam, firm-walled, frequently and conspicuously clamped, in lamellar trama with slender hyphae 2–3.5 µm diam. *Pleurocystidia* (Fig. 17)



Figure 16. Collybiopsis prolapsis. Dermatocystidia, thick-walled, inflated, often ornamented. Scale bars: 7.5 µM.

24–34 × 7–11 µm, abundant, stalked-fusiform, near lamellar edge ampulliform with rounded apex, conspicuously clamped; contents homogeneous to heterogeneous with crystal-like inclusions. **Basidia** (Fig. 18) more or less stalked-columnar (not urniform, not clavate), 27–35 × 8–12 µm, 4-sterigmate (sterigmata slender, slightly curved), conspicuously clamped; contents homogeneous to minutely heterogeneous. **Basidiospores** (Fig. 12B) (9–)9.5–10.5 × 4–4.5(–5) µm (Q = 2.10–2.50; Q^m = 2.05; L^m = 9.75 µm), elongate-ellipsoid, somewhat tapered proximally, thin-walled, hyaline; contents homogeneous to heterogeneous-subrefringent. **Cheilocystidia** (Fig. 19 A–G) ventricose-rostrate to stalked-globose, hyaline, firm- to thick-walled (wall –1 µm thick, especially laterally, smooth), conspicuously clamped, apically producing a cluster of diverticula; diverticula –16 × 1–2.5(–4) µm, repeatedly dichotomously



Figure 17. Collybiopsis prolapsis. Pleurocystidia. Scale bars: 30 uM.

branched, often inflated somewhat apically (Fig. 19 A, B). *Stipe medullary hyphae* thick-walled, occasionally conspicuously clamped, of two types: 1) $4.5-8.5 \,\mu$ m diam, seldom branched; contents heterogeneous (multigranular); and, 2) 2–3.5 μ m diam, occasionally branched; contents homogeneous. Stipe cortical hyphae similar to slender medullary hyphae. *Resupinate* patch composed of tightly interwoven hyphae in a slime matrix; hyphae of two types, both inconspicuously clamped: 1) 3–5.5 μ m diam, thick-walled (wall –1 μ m thick, refringent; PhC); and 2) 2.5–4 μ m diam, thick-walled (wall –0.7 μ m thick, non-refringent), frequently branched.

Commentary. Specimen notes on undried specimens for the holotype specimen, TENN-F-051101, report lamellae as brownish-orange (6C4) in age. Similar pigmentation is currently evident on dried material, presumably a necropigment (approximately "Light ochraceous salmon," "Light salmon orange," more or less characteristic of the *Collybiopsis ramealis* complex.)

The pileipellis is a poorly developed *Gymnopus* structure (Hughes and Petersen 2015), with only a few "diverticulate" hyphal termini as described by Halling (1983); typical of section *Subfumosae*. Conversely, the typical *Ramealis* pileipellis structure is quite different. There, the typical diverticula are cog-like



Figure 18. Collybiopsis prolapsis. Basidia. Scale bars: 30 uM.

(not wart-like) and the surrounding hyphal walls thickened (vaguely similar to those shown in Fig. 16A, B). Clamp connections are common and conspicuous throughout. The thick walls of stipe medullary hyphae appears to be laminate, often peeling into narrow shards (as in peeling a banana).

Cheilocystidia, while highly distinctive, are not totally unique. *Collybiopsis straminipes* cheilocystidia are similar, but the specimens examined (Desjardin and Petersen 1989, including the type) were clampless and from spruce-fir zone. A clamped variety (*Marasmius straminipes* var. *fibulatus* Desjardin & R.H. Petersen) is from lower elevation and hardwood (*Quercus* litter) substrate.

Discussion

The fragmentation and rearrangement of the agaricoid Omphalotaceae, *Marasmius* and *Marasmiellus* is ongoing as molecular data identifies new taxa and associations (Owings and Desjardin 1997; Wilson and Desjardin 2005; Matheny et al. 2006; Mata et al. 2007; Hughes and Petersen 2015; Petersen and Hughes 2016; Petersen and Hughes 2017; Petersen and Hughes 2021). This understudied group of small gymnopoid mushrooms will continue to enlarge as environmental studies identify new members and define their niches.



Figure 19. Collybiopsis prolapsis. Cheilocystidia. Clusters with diverticula (A, B) Individual Cheilocystidia (C–G).Scale bars: 16 uM (A, B).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Karen W. Hughes: Molecular characterizations, phylogenetic analysis, wrote parts of paper. Ronald H. Petersen: Morphological analyses, wrote parts of paper.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Morphological and phylogenetic analyses reveal two new *Alternaria* species (Pleosporales, Pleosporaceae) in *Alternaria* section from Cucurbitaceae plants in China

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Abstract

Alternaria species are commonly found as saprophytes, endophytes and plant pathogens. During a survey of small-spored Alternaria in China, two new species were discovered from Cucurbitaceae plants collected in Hubei and Sichuan provinces. This study identified two new species of Alternaria using seven genes (ITS, GAPDH, TEF1, RPB2, Alt a 1, EndoPG, and OPA10-2) for phylogenetic analyses and morphological characteristics. The two new species A. jingzhouensis and A. momordicae were described and illustrated. Alternaria jingzhouensis sp. nov., associated with Citrullus lanatus, is characterized by producing muriform, ellipsoidal, flask-shaped, rostrate, and beaked conidia. It differs from A. koreana, A. ovoidea, and A. baoshanensis by bearing conidia in a simple conidiogenous locus with occasionally longer beaks in a chain, and from A. momordicae sp. nov. by having shorter beaks. Alternaria momordicae sp. nov. from Momordica charantia was distinct from A. koreana, A. ovoidea, and A. baoshanensis by producing muriform, long ellipsoid or ovoid to obclavate, sometimes inverted club-shaped conidia on a single conidiogenous locus with a wider body and longer beak in a chain, and distinct from A. jingzhouensis sp. nov. by a longer beak conidia. These two species were clearly distinguished from other species in the section Alternaria based on DNA based phylogeny and morphological characteristics. The morphological features were discussed and compared to relevant species in the present paper.

Key words: Morphology, novel species, phylogeny, small-spored Alternaria, taxonomy

Introduction

The Cucurbitaceae, also called cucurbits or the gourd family, consists of approximately 975 species belonging to 98 genera (Xu and Chang 2017). There are 35 genera with 151 species in China (Raven and Wu 2022). This family includes highly nutritious vegetables with significant economic value, such as cucumber, pumpkin, and so on. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a popular fruit worldwide, and its seeds contain high levels of proteins,

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lipids and medicinal properties (Wani et al. 2011, Maoto et al. 2019). China is the world's leading producer of watermelons (Qiang et al. 2024). Bitter gourd (Momordica charantia L.) is normally cultivated in China for its fruit as a popular vegetable and traditional medicine (Sun et al. 2023). Alternaria-like leaf blight can severely affect the crop production of Cucurbitaceae (Maheswari and Sankaralingam 2010; Ma et al. 2021). Many Alternaria species have been reported to be associated with cucurbit plants, including A. alternata (Fr.) Keissl. (Chen et al. 1993; Zhao et al. 2016a, 2016b; Ma et al. 2021), A. baoshanensis J.F. Li, Phookamsak & Jeewon (Li et al. 2023), A. brassicae (Berk.) Sacc. (Simmons 2007), A. brassicae var. nigrescens (Peglion) Sacc. & Traverso (Simmons 2007), A. caudata Cooke & Ellis (Simmons 2007), A. cucumericola E.G. Simmons & C.F. Hill (Simmons 2007), A. cucumerina (Ellis & Everh.) J.A. Elliott (Chen et al. 1993; Zhang 2003; Simmons 2007; Ma et al. 2021), A. cylindrorostra T.Y. Zhang (Zhang 2003; Simmons 2007), A. gaisen Nagano ex Bokura (Ma et al. 2021), A. granulosa (Bubák) E.G. Simmons (Simmons 2007), A. hydrangea D. F. Pei & J. X. Deng (Liu et al. 2022), A. infecotria E.G. Simmons (Ma et al. 2021), A. loofahae E.G. Simmons & Aragaki (Simmons 2007), A. nigrescens (Peglion) Neerg. (Simmons 2007), A. peponicola (Rabenh.) E.G. Simmons (Zhang 2003; Simmons 2007), A. peponis Yatel (Simmons 2007), and A. tenuissima (Kunze) Wiltshire (Chen et al. 1993; Zhao et al. 2016a, 2016b; Ma et al. 2021).

The genus *Alternaria* Nees von Esenbeck (1816) is categorized according to its morphological characteristics, typified by *A. alternata* with muriform and catenulate conidia (Simmons 2007). Simmons (1992) applied standard criteria to achieve solid taxonomic outcomes for *Alternaria* species, primarily relying on the sporulation patterns and developmental morphology of conidia. In 2007, Simmons illustrated approximately 276 species (148 large-spored species and 128 small-spored species) and provided a final summary of morphological taxonomy on *Alternaria*. The small-spored species fall into 10 subsections containing the type species of *A. alternata* (Simmons 2007). In 2003, Zhang identified approximately 80 small-spored species associated with specific host plant families in China.

To date, the utilization of multigene phylogenetic analyses has played a crucial role in understanding the *Alternaria* genus (Pryor and Gilbertson 2000; Pryor and Bigelow 2003; Hong et al. 2005; Runa et al. 2009; Woudenberg et al. 2013, 2014; Lawrence et al. 2013, 2014, 2016; Poursafar et al. 2018). The genus contains 24 internal clades (sections) and six monotypic lineages (Woudenberg et al. 2013) using type or referenced strains collected by Simmons (2007), which has recently been updated to 29 sections (Li et al. 2023). Small-spored *Alternaria* species are also frequently isolated from Cucurbitaceae in China (Ma et al. 2021). Woudenberg et al. (2015) provided a clear and stable species classification of section *Alternaria* based on the genomic and multi-loci analyses, from which the species commonly produce concatenated conidia (Norphanphoun et al. 2021; Li et al. 2022; Gou et al. 2022). Consequently, the combination of morphology and molecular techniques provides a better understanding of species in section *Alternaria* (Aung et al. 2020).

During the investigation of small-spored Alternaria species in China, two new taxa were isolated from gourd plants of Citrullus lanatus and Momordica charantia. The aim of this study was to characterize and differentiate both taxa using morphology and multigene sequence analyses. This research sought to enhance understanding of Alternaria species diversity within the Cucurbitaceae family, offering crucial taxonomic information for species conservation efforts.

Materials and methods

Isolation

Leaves of *Citrullus lanatus* and *Momordica charantia* with necrotic spots were collected from Jingzhou, Hubei in 2022 and Deyang City, Sichuan Province in 2016 China, respectively. To facilitate isolation, the specimens were carefully enclosed in sterile plastic bags and transported to the laboratory. Subsequently, the tissues were accurately divided into small segments, arranged on moist filter papers within Petri dishes, and incubated at 25 °C to promote spore production. After sporulation, spores of *Alternaria* were individually collected using sterilized glass needles under a stereo microscope (Shunyu SZM series) and transferred onto potato dextrose agar (PDA) plates. Each distinct culture was purified and preserved in test-tube slants maintained at 4 °C. Additionally, dried cultures derived from individual spores and reference strains were stored in the Fungi Herbarium of Yangtze University (YZU), located in Jingzhou, Hubei, China.

Morphology

To study the features of colonies, the strains were grown on PDA at 25 °C for 7 days without light. To examine the characteristics of the conidia (size, shape, sporulation, etc.), fresh mycelia were transferred to potato carrot agar (PCA) and V8 juice agar (V8A) plates and then placed in an incubator at 22 °C with an 8-hour light cycle for 7 days (Simmons 2007). A total of 50 conidia were randomly selected and photographed for the morphological determination after mounting the conidia into lactophenol picric acid under an ECLIPSE Ni-U microscope system (Nikon, Japan). The sporulation patterns and morphological characteristics were also recorded.

DNA extraction, PCR amplification and sequencing

Fresh mycelia growing on PDA were used to extract genomic DNA with the CTAB method, as described by Watanabe et al. (2010). To amplify multigene fragments, including the internal transcribed spacer rDNA region (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), translation elongation factor 1 alpha (TEF1), RNA polymerase second largest subunit (RPB2), Alternaria major allergen gene (Alt a 1), endopolygalacturonase gene (EndoPG), and an anonymous gene region (OPA10-2), primer pairs were employed including ITS5/ITS4 (White et al. 1990), gpd1/gpd2 (Berbee et al. 1999), EF1-728F/ EF1-986R (Carbone and Kohn 1999), RPB2-5F/RPB2-7cR (Liu et al. 1999), Altfor/Alt-rev (Hong et al. 2005), PG3/PG2b (Andrew et al. 2009) and OPA10-2L/OPA10-2R (Andrew et al. 2009), respectively. The PCR reaction mixture was 25 µL, including 21 µL of 1.1×Tag PCR Star Mix from TSINGKE, 2 µL of template DNA, and 1 µL of each primer. The amplification process was carried out in an Eppendorf Mastercycler, following the protocols outlined by Woudenberg et al. (2015). After a successful amplification, the PCR products were purified and sequenced by TSINGKE company (Beijing, China). The obtained sequences were assembled using BioEdit v. 7.2.3 (Hall 1999) and primarily aligned with PHYDIT v.3.2 (Chun 1995) then deposited into GenBank (https://www.ncbi.nlm.nih.gov/) (Table 1).

Species	Strain	Host/Substrate	Courter	GenBank accession numbers							
Species			Country	ITS	GAPDH	TEF1	RPB2	Alt a 1	EndoPG	OPA10- 2	
A. alternantherae	CBS 124392	Solanum melongena	China	KC584179	KC584096	KC584633	KC584374	KP123846	np	np	
A. alternata	CBS 916.96T	Arachis hypogaea	India	AF347031	AY278808	KC584634	KC584375	AY563301	JQ811978	KP124632	
	CBS 106.34T	Linum usitatissimum	Unknown	Y17071	JQ646308	KP125078	KP124771	KP123853	KP124000	KP124608	
	CBS 102596T	Citrus jambhiri	USA	KP124328	KP124183	KP125104	KP124796	KP123877	KP124030	KP124637	
	CBS 121336T	Allium sp.	USA	KJ862254	KJ862255	KP125141	KP124833	KJ862259	KP124067	KP124676	
	CBS 121547T	Pyrus bretschneideri	China	KP124372	KP124224	KP125150	KP124842	KP123920	KP124076	KP124685	
	CBS 119543T	Citrus paradisi	USA	KP124363	KP124215	KP125139	KP124831	KP123911	KP124065	KP124674	
	CBS 918.96R	Dianthus chinensis	UK	AF347032	AY278809	KC584693	KC584435	AY563302	KP124026	KP124633	
	CBS 127671T	Stanleya pinnata	USA	KP124381	KP124233	KP125159	KP124851	KP123929	KP124085	KP124694	
	CBS 121455T	Broussonetia papyrifera	China	KP124368	KP124220	KP125146	KP124838	KP123916	KP124072	KP124681	
	CBS 117.44T	Godetia sp.	Denmark	KP124303	KP124160	KP125079	KP124772	KP123854	KP124001	KP124609	
	CBS 127672T	Astragalus bisulcatus	USA	KP124382	KP124234	KP125160	KP124852	KP123930	KP124086	KP124695	
	CBS 102.47R	Citrus sinensis	USA	KP124304	KP124161	KP125080	KP124773	KP123855	KP124002	KP124610	
	CBS 102599T	Minneola tangelo	Turkey	KP124330	KP124185	KP125106	KP124798	KP123879	KP124032	KP124639	
	CBS 102595T	Citrus jambhiri	USA	FJ266476	AY562411	KC584666	KC584408	AY563306	KP124029	KP124636	
	CBS 103.33T	Soil	Egypt	KP124302	KP124159	KP125077	KP124770	KP123852	KP123999	KP124607	
A. arborescens	CBS 126.60	Wook	UK	KP124397	KP124249	KP125175	KP124867	JQ646390	KP124101	KP124710	
	CBS 119545T	Senecio skirrhodon	New Zealand	KP124409	KP124260	KP125187	KP124879	KP123956	KP124113	KP124723	
	CBS 101.13T	Peat soil	Switzerland	KP124392	KP124244	KP125170	KP124862	KP123940	KP124096	KP124705	
	CBS 105.24	Solanum tuberosum	Unknown	KP124393	KP124245	KP125171	KP124863	KP123941	KP124097	KP124706	
	CBS 119544T	Avena sativa	New Zealand	KP124408	JQ646321	KP125186	KP124878	KP123955	KP124112	KP124722	
	CBS 105.49	Contaminant blood culture	Italy	KP124396	KP124248	KP125174	KP124866	KP123944	KP124100	KP124709	
	CBS 112749	Malus domestica	South Africa	KP124401	KP124253	KP125179	KP124871	KP123948	KP124105	KP124715	
A. baoshanensis	MFLU 21- 0124T	Curcubita moschata	China	MZ622003	OK236706	OK236613	OK236659	OK236760	np	np	
	MFLU 21- 0296	C. moschata	China	MZ622004	OK236707	OK236612	OK236660	OK236759	np	np	
A. breviconidiophora	MFLUCC 21-0786T	Digitalis sp.	Italy	MZ621997	OK236698	OK236604	OK236651	OK236751	np	np	
A. burnsii	CBS 118817T	Tinospora cordifolia	India	KP124424	KP124274	KP125202	KP124893	KP123971	KP124128	KP124738	
	CBS 118816T	Rhizophora mucronata	India	KP124423	KP124273	KP125201	KP124892	KP123970	KP124127	KP124737	
A. ellipsoidialis	MFLUCC 21-0132T	<i>Brassica</i> sp.	Italy	MZ621989	OK236690	OK236596	OK236643	OK236743	np	np	
A. eupatoriicola	MFLUCC 21-0122T	Eupatorium cannabinum	Italy	MZ621982	OK236683	OK236589	OK236636	OK236736	np	np	
A. falcata	MFLUCC 21-0123T	Atriplex sp.	Italy	MZ621992	OK236693	OK236599	OK236649	OK236746	np	np	
A. gaisen	CBS 632.93R	Pyrus pyrifolia	Japan	KC584197	KC584116	KC584658	KC584399	KP123974	AY295033	KP124742	
	CBS 118488R	P. pyrifolia	Japan	KP124427	KP124278	KP125206	KP124897	KP123975	KP124132	KP124743	

Table 1. Alternaria strains used in this study and their GenBank accession numbers.

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Species	Strain	Host/Substrate	Country	GenBank accession numbers							
				ITS	GAPDH	TEF1	RPB2	Alt a 1	EndoPG	OPA10- 2	
A. gossypina	CBS 102601T	Minneola tangelo	Colombia	KP124433	KP124282	KP125212	KP124903	KP123979	KP124138	KP124749	
	CBS 104.32T	Gossypium sp.	Zimbabwe	KP124430	JQ646312	KP125209	KP124900	JQ646395	KP124135	KP124746	
A. jacinthicola	CBS 878.95	Arachis hypogaea	Mauritius	KP124437	KP124286	KP125216	KP124907	KP123983	KP124142	KP124753	
	CBS 133751T	Eichhornia crassipes	Mali	KP124438	KP124287	KP125217	KP124908	KP123984	KP124143	KP124754	
A. jingzhouensis sp. nov.	YZU 221144T	Citrullus lanatus	China	OR883772	OR887690	OR887686	OR887688	OR887694	OR887692	OR887684	
	YZU 221145	C. lanatus	China	OR901948	OR914170	OR914166	OR914168	OR914174	OR914172	OR914176	
A. koreana	SPL2-1T	Atractylodes ovata	Korea	LC621613	LC621647	LC621715	LC621681	LC631831	LC631844	LC631857	
	SPL2-4	A. ovata	Korea	LC621615	LC621649	LC621717	LC621683	LC631832	LC631845	LC631858	
A. longipes	CBS 121333R	Nicotiana tabacum	USA	KP124444	KP124293	KP125223	KP124914	KP123990	KP124150	KP124761	
	CBS 540.94R	N. tabacum	USA	AY278835	AY278811	KC584667	KC584409	AY563304	KP124147	KP124758	
A. minimispora	MFLUCC 21-0127T	Citrullus lanatus	Thailand	MZ621980	OK236705	OK236587	OK236634	OK236734	np	np	
A. momordicae sp. nov.	YZU 161378T	Momordica charantia	China	OR883774	OR887691	OR887687	OR887689	OR887695	OR887693	OR887685	
	YZU 161379	M. charantia	China	OR901949	OR914171	OR914167	OR914169	OR914175	OR914173	OR914177	
A. muriformispora	MFLUCC 21-0784T	Plantago sp.	Italy	MZ621976	OK236677	OK236583	OK236630	OK236730	np	np	
A. obpyriconidia	MFLUCC 21-0121T	Vicia faba	Italy	MZ621978	OK236680	OK236585	OK236633	OK236732	np	np	
A. ovoidea	MFLUCC 0782T	Dactylis glomerata	Italy	MZ622005	OK236708	OK236614	OK236661	OK236761	np	np	
	MFLU 21- 0298	D. glomerata	Italy	MZ622006	OK236709	OK236615	OK236662	OK236762	np	np	
A. orobanches	MFLUCC 21-0137T	Orobanche sp.	Italy	MZ622007	OK236710	np	np	OK236763	np	np	
	MFLU 21- 0303	Orobanche sp.	Italy	MZ622008	OK236711	np	np	OK236764	np	np	
A. phragmiticola	MFLUCC 21-0125T	Phragmites sp.	Italy	MZ621994	OK236696	OK236602	OK236649	OK236749	np	np	
A. rostroconidia	MFLUCC 21-0136T	Arabis sp.	Italy	MZ621969	OK236670	OK236576	OK236623	OK236723	np	np	
A. salicicola	MFLUCC 22-0072T	Salix alba	Russia	MZ621999	OK236700	OK236606	OK236653	OK236753	np	np	
A. tomato	CBS 103.30	Solanum lycopersicum	Unknown	KP124445	KP124294	KP125224	KP124915	KP123991	KP124151	KP124762	
	CBS 114.35	S. lycopersicum	Unknown	KP124446	KP124295	KP125225	KP124916	KP123992	KP124152	KP124763	
A. torilis	MFLUCC 14-0433T	Torilis arvensis	Italy	MZ621988	OK236688	OK236594	OK236641	OK236741	np	np	

Notes: Novel species proposed in this study are marked in bold. Ex-type strains are marked 'T'. Representative strains are marked 'R'. No products are 'np'.

Phylogenetic analyses

Preliminary BLAST searches on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi) indicated that the current species are highly similar to species within the *Alternaria* genus. Subsequently, sequence data of 57 *Alternaria* strains and *A. alternantherae* Holcomb & Antonop. CBS 124392 (outgroup) were retrieved from the GenBank database and referenced from relevant publications (Woudenberg et al. 2015; Li et al. 2022; Romain et al. 2022) (Table 1). The gene sequences were concatenated and edited manually with equal weight in MEGA v.11.0.13 (Tamura et al. 2021), and gaps were treated as

missing data. Bayesian inference (BI) analysis was carried out using MrBayes v. 3.2.6 (Ronquist et al. 2012). This analysis employed a Markov Chain Monte Carlo (MCMC) algorithm to estimate Bayesian posterior probabilities. The best-fit evolutionary model (GTR+I+G) was determined using MrModeltest v. 2.3 (Nylander 2004, Posada and Crandall 1998) with the Akaike Information Criterion (AIC). In MrModeltest, the file "MrModelblock" was executed in the PAUP path (Swofford 2002) and the MrMt path (Nylander 2004). Bayesian analyses included two parallel runs for 10,000,000 generations (ngen) with the stop rule option and a sampling frequency set to every 100 generations (samplefreq=100). The run was stopped when the standard deviation of split frequencies reached a value below 0.01. The first 25% of sampled trees were discarded as burn-in. Additionally, a maximum likelihood (ML) analysis was performed using RAxML v.7.0.3 (Stamatakis et al. 2008). The GTRGAMMAI model was implemented using ML+ rapid bootstrap setting with 1000 replications to assess branch support. The tree was visualized with FigTree v1.4.3 (Rambaut 2016). Nodes in the phylogram displayed branch support values equal to or above 0.60/60% for posterior probability (PP)/bootstrap (BS) values.

Results

Phylogenetic analyses

The dataset includes a total of 58 *Alternaria* strains with 3627 characters in total after alignment. The dataset consists of 533 characters for ITS, 574 for *GAPDH*, 216 for *TEF1*, 757 for *RPB2*, 444 for *EndoPG*, 469 for *Alt a* 1, and 634 for OPA10-2. Both Bayesian inference (BI) and maximum likelihood (ML) analyses yielded similar topologies. The ML tree was selected for discussing the placement of our new species (Fig. 1). The results indicated that all *Alternaria* strains in the present study fell into *Alternaria* section with PP values of 1.0. The present four strains separated into two individual clades sister to *A. koreana* 0. Hassan, B.B.N.D. Romain, J.S. Kim & T. Chang, *A. ovoidea* J.F. Li, Camporesi, Bhat & Phookamsak, *A. baoshanensis*, and *A. orobanches* J.F. Li, Camporesi, Phookamsak & Jeewon (Bayesian posterior probability (BI-BPP)/Maximum-Likelihood bootstrap proportions (ML-BS) = 0.64/74%).

The clade containing YZU 161378 and YZU 161379 was closely related to *A. baoshanensis*, *A. koreana*, *A. ovoidea*, and forming a distinct branch. While another clade, YZU 221144 and YZU 221145 was found to be independent with a posterior probability (PP) of 1.00 and bootstrap (BS) values of 68%, and it was closely related to *A. orobanches*. These results suggest that the present strains represent two new taxa.

Taxonomy

Alternaria jingzhouensis S.L.L. Aung & J.X. Deng, sp. nov. MycoBank No: 851272 Fig. 2

Type. CHINA, Hubei Province, Jingzhou city, Yangtze University (west campus) on infected leaves of *Citrullus lanatus* 2022, F.Y Liu, (YZU-H-2022030, holotype), ex-type culture YZU 221144.

A. alternata CBS 102599 ^T Minneola tangelo ^{0,9874} A. alternata CBS 102595 ^T Citrus jambhiri		
-A. alternata CBS916.96 ^T Arachis hypogaea		
0.99/ A. alternata CBS 102.47 ^R Citrus sinensis		
0.80/ A. alternata CBS 103.33 ^T Soil		
A. alternata CBS 127672 ^T Astragalus bisulcatus		
0.68/ A. alternata CBS102596 ^T Citrus jambhiri		
-A. alternata CBS 117.44 ^T Godetia sp.	A. alternata	
A. alternata CBS918.96 ^T Dianthus chinensis		
0.93/-		
0.9485 937 JA. alternata CBS121547 ^T Pyrus bretschneideri		
0.99/61 A. alternata CBS 127671 ^T Stanleya pinnata		
0.921-A. alternata CBS106.34 ^T Linum usitatissimum		
1.0/100 r.A. alternata CBS 121336 ^T Allium sp.		
A. alternata CBS 119543 ^T Citrus paradisi		1
A. arborescens CBS119545 ^T Senecio skirrhodon		
0.51/95 A. arborescens CBS 119544 ^T Avena sativa		
0.99 1.0/- A. arborescens CBS 112749 Malus domestica		
1.4m A. arborescens CBS 105.24 Solanum tuberosum	A. arborescens species complex (AASC)	
A arborescens CBS101.13 ^T Peat soil		
1.094 0.66/- A arborescens CBS126 60 Wood		
A whorescens CBS 105 49 Blood culture contaminant		
Alternaria sp. YZU 161379		
Alternaria sp. YZU 161378 ^T	Alternaria momordicae	
1.0/100 A ovoidea MFLU 21-0298 Dactulis glomerata		
1.079 A ovoidea MELUCC 0782 ^T Dactulis glomerata	A. ovoidea	
-162 A baoshanansis MELLICC 21-0124 ^T Curcubita moschata		
1.0/100 A baoshamensis MET LU 21-0/206 Curcubita moschata	A. baoshanensis	
A. buosnanensis MI LO 21-0250 Carcubia mosenara		
0.84/4 0.82/- 0.82/- 0.82/- 0.82/- 0.82/- 0.82/-	A. koreana	4
Atternation NTU 201145		0
Alternaria sp. 120 221145	Alternaria jingzhouensis	
1.0/68 A crobanches MELLICC 21-0137 ^T Orobanche sp		2
1.0/100 A. orobanches MELU 21-0303 Orobanche sp.	A. orobanches	2
A gaison CBS 118458R Davis matifalia		
4 gaisen CBS 632 03 ^R Paras prifolia	A. gaisen	2
0.96/- A. guisen CBS 052.55 Tyrus pyrijonu		2
1.0001A. gossipina CBS 102001 Minimedia langelo	A. gossypina	
1.079 A. gossyphia CBS 104.52 Gossyphian sp.		
A longings CDS 140.54 "Micoliana tabagum	A. longipes	
A torilis MELLICC 14-0433T Torilis amonsis	A torilis	
A minimizerore MELLICC 21-0127 Citrallus lengtus	4 minimizzora	
0.65/	A rostrocovidia	
0.99/84 A. roshoconidia MELUCC 21-0150° Arabis sp.	A. obmriconidia	
A allingoidialig MELLICC 21-0121 Pressing sp	4 ollinsoidialis	
A felore AFT LICC 21 0122 drasher on	A faloata	
A. Jaccata MFLUCC 21-0125 ⁻ Alliplet sp.	A. Jucana A. phragmiticola	
A amatorianal MELUCC 21-0125 Fundamines sp.	A. ematoriicola	
A sugar and a sugar and a sugar	1. braviconidianhora	
1.0/100 A. Divicional ophical MELLICC 21-0/84 Digitals sp.	A muriformisnora	
4. millijormispora MFLOCC 21-0764 Planago sp.	2. maryornispora	-
1.0/100 - A. Jacinthicola CBS 155/51- Electronormia crassipes	A. jacinthicola	1
1.099 A humani CDS 118816T Phizawhana muana ata		
10/100 A. Durinsu CDS 110610 ⁻ Kni20pnora mucronala	A. burnsii	
10700 4 towato CDS 114 25 Colorem International		-
A. Iomato CBS 114.55 Solaniim lycopersicum	A. tomato	1
0.01	1 anlinian la	
A. sancicola MFLUCC 22-00/24 Saitx alba	A. SAUCICOIA	0
UBS 1/4397 Solanim melongena	A alternantherae	

Figure 1. Phylogenetic tree of the *Alternaria* species most related to the new taxa based on maximum likelihood analysis using the combined gene sequences of ITS, *GAPDH*, *TEF1*, *RPB2*, *Alt a 1*, *EndoPG* and OPA10-2 which rooted with *Alternaria alternantherae* (CBS 124392) from sect. *Alternantherae*. The Bayesian posterior probabilities >0.60 (PP) and bootstrap support values >60 (BS) are given at the nodes (PP/BS). The novel species are highlighted in bold. Ex-type isolates are marked with a superscript T and Representative isolates are marked with a superscript R.

Etymology. Named after the collecting locality, Jingzhou (Hubei, China) **Description.** *Colonies* on PDA (7 d at 25 °C) pale luteous to amber in the center, white at the edges, light to moderate rosy buff or pale saffron in reverse, cottony surface and 49–52 mm in diam., at 25 °C for 7 days (Fig. 2A, B). On PCA (7 d at 22 °C), *conidiophores* arising from substrate, simple, straight or flexuous, light to olivaceous buff, 41–99 (–151) × 3.5–5 μ m ($\bar{x} = 73 \times 4.4$



Figure 2. *Alternaria jingzhouensis* sp. nov. (ex-type YZU 221144) **A**, **B** seven-day-old culture on PDA **C**, **D** conidiophores and conidia on PCA and V8A, respectively. Scale bars: 25 μm (**E**, **F**); 50 μm (**C**, **D**).

 μ m, n = 20), conidiogenous cells 5–11 × 3–6 μ m (\overline{x} = 8 × 4 μ m, n = 20), monoto polytretic, terminal, determinate, cylindrical, olivaceous buff, smooth, thinwalled, apically doliiform, with 1 conidiogenous locus cicatrized on conidial secession, sometimes swollen near conidiogenous loci; conidia 3-5 units per chain, arising from the apex or near the apex of the conidiophores or terminal hyphae, muriform, ellipsoidal, flask-shaped, rostrate, beaked, 28-51 × 11-21 μ m ($\bar{x} = 38 \times 16.4$, n = 50), with 1–4 transverse septa with 0–2 branching (Fig. 2C, E); On V8A (7 d at 22 °C), conidiophores 40-94 × 4-7 μm (x = 58 × 5, n = 20), simple, straight or flexuous, light to olivaceous buff; conidiogenous cells $5-13 \times 3-6 \mu m$ ($\overline{x} = 8 \times 4 \mu m$, n = 20), mono- to polytretic, terminal, determinate, cylindrical, olivaceous buff, smooth, thin-walled, apically doliiform, with 1 conidiogenous locus, sometimes swollen near conidiogenous loci cicatrized on conidial secession; conidia 3-5 units per chain, arising from the apex or near the apex of the conidiophores or terminal hyphae, muriform, ellipsoidal, flask-shaped, rostrate, beaked, $22-51 \times 3-16 \mu m$ ($\bar{x} = 33.9 \times 13.2$, n = 50), 1–6 transverse septa with 0-2 branching (Fig. 2D, F).

Additional isolate examined. CHINA, Hubei Province, Jingzhou city, Yangtze University (west campus) on infected leaves of *Citrullus lanatus* 2022, F.Y Liu, living culture YZU 221145.

Notes. Phylogenetically, *A. jingzhouensis* sp. nov. is different from its sister species *A. baoshanensis*, *A. koreana*, *A. momordicae* sp. nov., *A. orobanches* and *A. ovoidea* based on sequences derived from seven genes (Fig. 1). After conducting a nucleotide pairwise comparison as recommended by Jeewon and Hyde (2016), the present species can be readily distinguished from the closet species *A. koreana*, *A. momordicae* sp. nov. and *A. orobanches* constructed on any of the ITS, *GAPDH*, *TEF1*, *RPB2*, *Alt a 1*, *EndoPG*, and OPA10-2 genes, which has 1 bp difference in the ITS region, 1 bp in *GAPDH*, 1 bp in *TEF1*, 7 pb in *RPB2*, 9 bp in *Alt a1*, 10 bp in *EndoPG*, and 4 bp in OPA10-2 when compared with *A. koreana*, 1 bp in *GAPDH*, 4 bp in *RPB2*, and 11 bp in OPA10-2 when compared with *A. momordicae* sp. nov. and 49 bp differences in the ITS region when compared with sister species *A. orobanches*. Morphologically, the species is distinct from *A. baoshanensis*, *A. koreana*, and *A. ovoidea* as it produces conidia on a simple conidiogenous locus with occasionally longer beaks in a chain of 3–5 units, and from *A. momordicae* sp. nov. by having shorter beaks (Table 2).

Table 2. Conidial features of the novel Alternaria species proposed here and their closest relatives in section Alternaria.

Species		Conidia	Madium	Poforonoo				
Species	Shape	Body (µm)	Beak (µm)	Septa	per chain	weatum	Reference	
A. baoshanensis	Subglobose to ellipsoidal, or subcylindrical to obpyriform	25-60 × 12-22	Short beak	3-6	1-3	PCA	Li et al. (2023)	
A. jingzhouensis sp. nov.	Ellipsoidal, flask-shaped,	28−51 × 11−21	2-7(-15)	1-4	3-5	PCA	Present study	
	rostrate, beaked	22−51 × 3−16	3-7	1-6	3-5	V8A	Present study	
A. koreana	Obovate to long ellipsoid	12.9-61.2×8.6-20.7	4.5-9.1	2-8	1-2	SNA	Romain et al. (2022)	
A. momordicae sp. nov.	Obclavate, inverted club-shaped	6-42 × 4-34	2-19.5	1-5	3-4	PCA	Present study	
		24−61 × 10−17	3-25 .5	1-5	3-4	V8A	Present study	
A. orobanches	Obclavate to ovoid	20-50 × 10-20	-	3-6	1-2	PCA	Li et al. (2023)	
A. ovoidea	Ovoid	48-65 × 15.5-30	-	1-3	1	PDA	Li et al. (2022)	

Alternaria momordicae S.L.L. Aung & J.X. Deng, sp. nov.

MycoBank No: 851270 Fig. 3

Type. CHINA, Sichuan Province, Deyang city infected leaves of *Momordica charantia*. 2016, J.X Deng, (YZU-H-2016001, holotype), ex-type culture YZU 161378. **Etymology.** Refers to the host genus, *Momordica*.

Description. *Colonies* on PDA (7 d at 25 °C) greyish yellow-green, light white at the edge, buff to salmon in reverse, surface compact, 50-55 mm in diam. (Fig. 3A, B). On PCA (7 d at 22 °C), *conidiophores* arising from substrate, simple, straight or flexuous, septate, olivaceous buff to olivaceous, $26.5-93 \times 3-4 \mu m$ ($\overline{x} = 59.5 \times 3.8 \mu m$, n = 20); *conidiogenous cells* $5-10 \times 3-5 \mu m$ ($\overline{x} = 7 \times 4 \mu m$, n = 20), mono- to polytretic, terminal, determinate, cylindrical, olivaceous buff to olivaceous, smooth, thin-walled, apically doliiform, with 1 conidiogenous locus cicatrized on conidial secession, sometimes swollen near conidiogenous loci; *conidia* 3-4 units per chain, arising from the apex or near the apex of the conidiophores or terminal hyphae, muriform, long ellipsoid or ovoid to obclavate, sometime inverted club-shaped, $6-42 \times 4-34 \mu m$ ($\overline{x} = 32.8 \times 13.5 \mu m$, n = 50), 1-5 transverse septa, apical beak $2-19.5 \mu m$ long and 1-2 septa (Fig. 3C, E);



Figure 3. Alternaria momordicae sp. nov. (ex-type YZU 161378) **A**, **B** seven-day-old culture on PDA **C**, **D** conidiophores and conidia on PCA and V8A, respectively **E**, **F** conidia on PCA and V8A, respectively. Scale bars: 25 μm (**E**, **F**); 50 μm (**C**, **D**).

On V8A(7 d at 22 °C), **conidiophores** straight or curved, smooth-walled, olivaceous buff $23-63(-208) \times 3-5 \ \mu m$ ($\overline{x} = 64.9 \times 4.2 \ \mu m$, n = 20); **conidiogenous cells** $5-13 \times 3-4 \ \mu m$ ($\overline{x} = 7 \times 4 \ \mu m$, n = 20), mono- to polytretic, terminal, determinate, cylindrical, olivaceous buff, smooth, thin-walled, apically doliiform, with 1 conidiogenous locus cicatrized on conidial secession, sometimes swollen near conidiogenous loci; **conidia** $3-4 \ units$ per chain, muriform, long ellipsoid or ovoid to obclavate, inverted club-shaped, $24-61\times10-17 \ \mu m$ ($\overline{x} = 39 \times 14.3 \ \mu m$, n = 50), 1–5 transverse septa with apical beak 3–25.5 $\ \mu m$ long and 1–2 septa (Fig. 3D, F).

Additional isolate examined. CHINA, Sichuan Province, Deyang city infected leaves of *Momordica charantia*. 2016, J.X Deng, living culture YZU 161379.

Notes. After the combined dataset of ITS, *GAPDH*, *TEF1*, *RPB2*, *Alt a 1*, *EndoPG* and OPA10-2 gene fragments, *A. momordicae* sp. nov. is readily distinguished from its sister species *A. baoshanensis*, *A. jingzhouensis* sp. nov., *A. koreana*, and *A. ovoidea*, (Fig. 1). After a nucleotide pairwise comparison as suggested by Jeewon and Hyde (2016), the present species can be readily distinguished from the closet species *A. koreana* and others related a novel species based on any of the ITS, *GAPDH*, *TEF1*, *RPB2*, *Alt a 1*, *EndoPG*, and OPA10-2 genes, which has 1 bp difference in the ITS region, 1 bp in *GAPDH*, 1 bp in *TEF1*, 4 bp in *RPB2*, 8 bp in *Alt a1* and 10 bp in *EndoPG* when compared with *A. koreana* and 1 bp in *GAPDH*, 4 bp in *RPB2*, and 11 bp in OPA10-2 when compared with *A. jingzhouensis* sp. nov.. Morphologically, *A. momordicae* sp. nov. produces conidia on PCA that are

significantly shorter than those on V8A. It can be distinguished from *A. baoshanensis*, *A. koreana*, and *A. ovoidea* by producing conidia on a single conidiogenous locus with a wider body and longer beak in a chain of 3–4 units. Additionally, it differs from *A. jingzhouensis* sp. nov. by having a longer beak (Table 2).

Discussion

Most of the Alternaria species published before the year 2000s relied on morphology to characterize the species status (Simmons 2007). In this study, two new Alternaria species, A. jingzhouensis and A. momordicae, have been identified and illustrated using the morphological method of Simmons (2007) and phylogenetic analysis of seven gene loci. Both resemble the type small-spored species of A. alternata in morphology but are easily distinguished by short chains, which also differentiate them from each other and their phylogenetically closely related species of A. baoshanensis, A. koreana, A. ovoidea and A. orobanches by the chain formation of sporulation patterns (Table 2). In recent publications, the Alternaria species descriptions have not followed the morphological standard created by Simmons (2007) (Romain et al. 2022; Li et al. 2022). Simmons (2007) classified the genus Alternaria into small-spored and largespored taxa based on morphology. Andrew et al. (2009) noted that phylogenetic studies have confirmed a distinct separation between large- and small-spored Alternaria species. Woudenberg et al. (2015) identified 35 morphospecies as synonyms of A. alternata, but their relationships remain unclear due to inconsistencies and lack of detailed morphological information. Accurate identification and classification of species within these small-spored Alternaria species require strong identification through multigene sequence analysis (Kgatle et al. 2018). Li et al. (2023) described that recent studies using combined multi-locus phylogeny suggest that certain A. alternata species classified under section Alternaria may not constitute a monophyletic group in DNA sequence-based phylogenies. To reduce potential misidentification of morphological characteristics within this section, this study utilized PCA and V8A media for 7 days at 22 °C to identify Alternaria species, following Simmons' (2007) recommendations. These media effectively promote typical morphological characteristics. Hence, it is strongly recommended to use the standard of morphological identification for further describing small-spored and large-spored Alternaria in order to reduce taxonomic ambiguity caused by different temperatures and substrates.

With the development of molecular studies, the species-group was re-defined and the section *Alternaria* was introduced and updated (Pryor and Gilbertson 2000; Lawrence et al. 2013; Woudenberg et al. 2013; Li et al. 2023). The section *Alternaria* is one of the small-spored *Alternaria* species groups and comprises 11 phylogenetic species and one species complex (Woudenberg et al. 2015). The two new *Alternaria* species are identified as members of section *Alternaria* according to the multigene sequence analysis of ITS, *GADPH*, *RPB2*, *TEF1*, *Alt a 1, EndoPG* and OPA10-2 gene sequences, which are close to *A. baoshanensis* (Li et al. 2023) from *Curcubita moschata* (Cucurbitaceae), *A. koreana* (Romain et al. 2022) from *Atractylodes ovata* (Compositae), *A. orobanches* (Li et al. 2023) from *Orobanche* sp. (Orobanchaceae), and *A. ovoidea* (Li et al. 2022) from *Dactylis glomerata* (Poaceae). Three genes, *GAPDH*, *RPB2*, and OPA10-2, provide more informative data for the classification of the current species. Small-spored Alternaria species have been frequently reported on Cucurbitaceae plants worldwide, including A. alternata (Chen et al. 1993; Zhao et al. 2016a, 2016b; Ma et al. 2021), A. baoshanensis (Li et al. 2023), A. caudata (Simmons 2007), A. gaisen (Ma et al. 2021), A. infecotria (Ma et al. 2021), A. peponicola (Zhang 2003; Simmons 2007), and A. tenuissima (Chen et al. 1993; Zhao et al. 2016a, 2016b; Ma et al. 2021). The present two small-spored species, A. jingzhouensis sp. nov. and A. momordicae sp. nov., were first found on C. lanatus and M. charantia, respectively, in China. Pathogenicity tests were performed on detached and living leaves for the two new species, which showed weak pathogenicity (data not shown). However, they did exhibit a certain level of aggressiveness on cucurbit plants. The two species, A. jingzhouensis sp. nov. and A. momordicae sp. nov., were found to be non-pathogenic to their host plants, possibly due to their saprophytic or weakly pathogenic nature when encountering resistance from C. lanatus and M. charantia. These findings provide valuable insights into Alternaria leaf diseases in Cucurbitaceae.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Sein LLA conceived and designed the study; Sein LLA, Liu FY, Gou YN, Zin MN, Yu ZH, conducted the experiments; Sein LLA, Deng JX wrote the manuscript and revised.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Three new *Melanogaster* species (Boletales, Paxillaceae) from southwestern China based on morphological and molecular evidence

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Abstract

Three newly discovered Melanogaster species, namely M. cyaneus, M. digingensis, and M. truncatisporus, are introduced and illustrated based on both morphological and molecular data from Sichuan and Yunnan provinces in China. A multigene phylogenetic analysis (nrITS, nrLSU, and rpb2) was performed mainly to verify the placement of the new species in Melanogaster. A second, nrITS-only phylogenetic analysis comprising more Melanogaster species for which only ITS sequences were available, was used to infer the relationship between the new species and as many known Melanogaster species as possible. Specimens of M. cyaneus, M. digingensis, and M. truncatisporus formed three independent clades in a phylogenetic tree inferred from the ITS data set. The robust support from ITS for these clades and genetic similarity with other species being lower than 93.2% suggest that these three species are indeed distinct from the other Melanogaster species in the phylogeny. Morphologically, M. cyaneus is characterized by its blue or bluish gleba, light brown to yellowish brown peridium, and subglobose to globose basidiospores, 6.2-15 × 4.6-9.0 µm. Melanogaster digingensis is distinguished from other Melanogaster species by its pale yellow to brown-yellow peridium and obovate to subglobose basidiospores, 3.0-5.1 × 2.0-4.0 µm. Melanogaster truncatisporus is diagnosed by its subglobose to globose or irregularly elongate-pyriform basidiomata, pale yellow to deeply orange-yellow peridium, and subglobose to globose or pyriform, truncate basidiospores. Additionally, infrageneric classification based on the number of peridium layers, the average thickness of the peridium, and the average length and width of basidiospores was tested with M. cyaneus, M. digingensis, and M. truncatisporus. Orthogonal partial least squares discriminant (OPLS-DA) analysis placed the three new species within the Melanogaster, Rivulares, and Variegati sections, respectively. However, the morphologically circumscribed sections were not monophyletic in the phylogenetic tree. Therefore, the current infrageneric classification should be abandoned.

Key words: False truffles, gasteroid Boletales, phylogeny, taxonomy, three new species

Introduction

Melanogaster Corda, belonging to Paxillaceae within the Boletales (Basidiomycota), stands out as one of the ecologically significant groups of hypogeous fungi. According to He et al. (2019), Melanogaster encompasses approximately 26 species. However, recent discoveries by taxonomists worldwide have identified new species, expanding the count to 34 as listed in Index Fungorum (https:// www.indexfungorum.org/, accessed on November 10, 2023). While Melanogaster species are predominantly distributed in the Northern Hemisphere, an exception is noted with Melanogaster guercus L., reported by Trappe et al. (2009) in the Southern Hemisphere. Melanogaster species typically establish ectomycorrhizal associations (EcM) with various plant families, including Betulaceae, Cistaceae, Fagaceae, Pinaceae, and Salicaceae (Comandini et al. 2006; Krisztián 2008; Perič and Moreau 2010; Türkoğlu and Castellano 2013; Lacheva 2015). These fungi are recognized by their peridium, varying in color from brownish to yellowish, occasionally featuring mycelial strands at the base or on the surface. The sequestrate hymenophore is composed of rounded to irregular locules of varying sizes, filled with black or dark brown basidiospores embedded in a gel and separated by whitish to yellowish veins or walls. Melanogaster basidiospores exhibit a range of shapes, including globose, ellipsoid, pyriform, or cirriform. Most species in the genus emit distinctive odors, ranging from sweet and pleasant to garlic-like or nauseating (Castellano et al. 1986; Montecchi and Sarasini 2000; Cázares et al. 2008; Trappe et al. 2009; de la Fuente et al. 2021; Alvarado et al. 2021; Xu et al. 2022). Melanogaster can be differentiated from related sequestrate Paxillaceae such as Alpova C.W. Dodge, Neoalpova Vizzini, and Paralpova Cabero & P. Alvarado based on differences in peridium structure. In Melanogaster, the peridium exhibits prostrated or interwoven hyphae, contrasting with the pseudoparenchymatous structure with inflated hyphae found in Alpova, Neoalpova, and Paralpova. Another false-truffle genus, Rhizopogon Fr. (Rhizopogonaceae), differs from Melanogaster by the absence of gel in the locules and the hyaline basidiospores (yellow to dark brown in Melanogaster). The relationships among these genera have been extensively discussed and Melanogaster has been placed in the Paxillinae, along with Gyrodon Opat., Paxillus Fr., and Alpova by Trappe (1975), Grubisha et al. (2001), Binder and Hibbett (2006), Vizzini et al. (2010), Moreau et al. (2011, 2013), and Alvarado et al. (2021).

Knapp (1954) proposed a subdivision of the genus *Melanogaster* into three groups based on the spore length (L), namely the *ambiguous* group (L > 10 μ m), the *variegatus* group (7 < L < 10 μ m), and the *microsporus* group (L < 7 μ m). Then, Svrček (1958) proposed the subdivision into three sections, namely sect. *Melanogaster* (L> 10 μ m), sect. *Variegati* (6 < L < 10 μ m), and sect. *Rivulares* (L < 6 μ m). Those sections were later validated by Moreau et al. (2011).

In China, the first specimen of *Melanogaster* was gathered from the Jinshajiang Valley in Yunnan Province, southwestern China, in 1915. Initially identified as *M. variegatus* (Vittad.) Tul. & C. Tul. by Keissler and Lohwag in 1937, it was later erected as a distinct species named *M. ovoidisporus* Y. Wang (Wang et al. 1995). Up to now, twelve species have been reported from China: *M. fusisporus* Y. Wang, *M. natsii* Y. Wang, K. Tao & B. Liu, *M. obovatisporus* B. Liu, K. Tao & Ming C. Chang, *M. ovoidisporus* Y. Wang, *M. shanxiensis* B. Liu, K. Tao & Ming C. Chang, *M. spinisporus* Y. Wang, *M. subglobisporus* K. Tao, Ming C. Chang & B. Liu, and *M. utriculatus* Y. Wang, Castellano & Trappe, *M. minobovatus*, *M. panzhihuaensis*, *M. quercicola*, *M. tomentellus* L. Fan, X. Y. Yan & Y. Y. Xu (Liu et al. 1989; Wang et al. 1995; Wang et al. 2005; Xu et al. 2022). China seems to have a rich variety of *Melanogaster* species; however, most of the previous records relied primarily on morphological evidence (Xu et al. 2022). During our survey of hypogeous fungi in Yunnan and Sichuan provinces, located in southwest China, from 2019 to 2021, we uncovered three novel species of *Melanogaster* under *Castanea mollissima* BI. and *Quercus aquifolioides* Rehd. et Wils. Through a comprehensive analysis encompassing both morphology and phylogenetic considerations (including *Alpova*, *Neoalpova*, *Paralpova*, and *Melanogaster*), we introduce three new species: *Melanogaster cyaneus*, *M. diqingensis*, and *M. truncatisporus*.

Materials and methods

Fungal materials

The *Melanogaster* specimens were collected from Yunnan and Sichuan Provinces in China. They were photographed in the field, placed in sterilized plastic tubes and boxes, returned to the laboratory, and stored at 4 °C. Macroscopic and microscopic descriptions were based on fresh basidiomes following the methods of Wan et al. (2016). Hand-cut sections were mounted in 5% (w/v) aqueous KOH solution, Cotton blue, or Congo red solutions and examined with an OLYMPUS BH-2 compound microscope. At least 50 basidiospores were measured of selected specimens, and the measurements are presented in the following format: L, W, Q, representing the extreme values of length, width, length to width ratio, $L_m = L \pm S.D.$, $W_m = W \pm S.D.$ and $Q_m = Q \pm S.D.$ For scanning electron microscopy (SEM), spores were scraped from the gleba of dried specimens onto double-sided tape, which was mounted directly on a SEM stub, coated with gold-palladium, examined and photographed with an SEM JSM-5600LV (JEOL, Tokyo, Japan).

The dried specimens were deposited in the Herbarium of Biotechnology and Germplasm Resources Institute of the Yunnan Academy of Agricultural Sciences (YAAS), and the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS), Yunnan, China.

DNA extraction, PCR, and sequencing

About 10–20 mg of dried gleba were placed in a 1.5 mL tube together with one 3 mm in diameter tungsten carbide bead, and crushed by shaking two to four times for 50 s at 30Hz with a Mixer Mill MM301 (Haan, Germany). Total DNA was extracted using the CTAB method described by Hofstetter et al. (2002). The following primer pairs were used for PCR amplification: the primer pair ITS4/ITS5 was used to amplify the nuclear ribosomal internal transcribed spacer region (White et al. 1990), the primers bRPB2-5F/bRPB2-7.1R for the second largest subunit of RNA polymerase II gene (RPB2) (Matheny 2005; Matheny et al. 2007), and the LR0R/LR5 primers (Vilgalys and Hester 1990; Cubeta et al. 1991) for the 28S nrDNA region (nrLSU). Amplifications were carried out in 25 μ L reaction containing 12.5 μ L 2×Taq Plus Master Mix II (Vazyme Biotech Co. Ltd, China), 9.5 μ L ddH₂0, 1 μ L 10 μ M of forward and reverse primers, and 1 μ L template DNA. Standard cycles of denaturation at 94 °C for 45 seconds, annealing for 45

seconds at different temperatures depending on the primer set (50 °C for RPB2, 54 °C for nrLSU, and 55 °C for ITS), and elongation at 72 °C for 1.5 min, followed by a final elongation step at 72 °C for 10 min. Post-cycling, samples were held at 4 °C. PCR products were sent to Shanghai Sangon Biotechnology (Shanghai, China) for purification and sequencing.

Phylogenetic analyses

The newly generated sequences were edited and assembled using SegMan II (SeqMan Pro, DNAStar) with generic-level identifications for sequences corroborated via BLAST queries of GenBank. A total of 89 sequences (including ITS1-2, 5.8S, nrLSU, and RPB2) were used in the molecular phylogenetic analyses (Table 1), including 20 sequences newly generated in this study and 82 downloaded from GenBank. Paragyrodon sphaerosporus and Paxillus involutus were selected as outgroup for the multilocus phylogenetic analyses while Alpova alpestris, Alpova concolor, and Alpova cinnamomeus were selected as outgroup for the ITS analysis. A sequence (AJ555527) of M. tuberiformis served as the reference for delineating 5.8S, ITS1, and ITS2. The ITS, 5.8S, nrLSU and RPB2 sequences were separately aligned using MAFFT ver. 7 (Katoh and Standley 2013) on the online server accessed at https:// mafft.cbrc.jp/alignment/server/, with the G-INS-I algorithm. The obtained alignment was manually refined in BioEdit, and ambiguously aligned sites were pinpointed using Gblocks 0.91b (Castresana 2000), using default options, except "Allowed Gap Positions" = half. After Gblocks, 90%, 99%, 99.5%, and 99% of the positions were kept for ITS1-2, 5.8S, nrLSU, and RPB2, respectively. The ITS1-2, 5.8S, nrLSU, and RPB2 alignments were 537, 157, 886, 715 bp long, respectively (including gaps). The alignments were deposited in Figshare (doi: 10.6084/m9.figshare.25440544). Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI) to validate the affiliation of our specimens with Melanogaster, relying on multigene sequences, which included Alpova, Paralpova, and Neoalpova (Fig. 1). Additionally, an ITS dataset (including 159 bp 5.8S, and 527 bp ITS1+ITS2) was compiled to infer the phylogenetic relationships between Melanogaster species. The ML analyses were performed with RAxML 8.0.14 (Stamatakis et al. 2005; Stamatakis 2014) with all parameters at default settings, except a mixed-model partitioning (with the same character sets as in the BI analyses), GTRGAMMA+I for all character sets, and 1,000 bootstrap pseudoreplicates. Partitioned BI analyses were performed with MrBayes v.3.1.2 (Ronguist and Huelsenbeck 2003) based on the following best-fit substitution models estimated by jModelTest2 on Cipres XSEDE (2.1.6, https://www.phylo.org; Miller et al. 2010). For the multi-gene dataset, 5.8S: K80+G; ITS1-2: HKY+I+G; nrLSU and RPB2: GTR+I+G). For the ITS-only dataset, 5.8S: HKY+I+G; and ITS1-2: GTR+I+G. Two independent runs of four chains were conducted for 2.5 106 generations (ITS dataset) and 5 10⁶ generations (multiple-gene dataset), with trees sampled every 100 generations. The average standard deviation of split frequency (ASDSF) values at the conclusion of the runs were 0.009248 for the multi-gene tree and 0.001889 for the ITS tree. After discarding the samples from the burn-in phase (first 25% of trees), a 70% majority-rule consensus tree was constructed and posterior probabilities computed. No outgroup was
Spanias	Isolate/ Strain/ Clone/Voucher	Country	Genbank accession numbers			Poforonoo
Species			ITS	nrLSU	RPB2	Reference
Alpova alpestris	S123	France	HQ714711	/	HQ714846	Moreau et al. 2013
Alpova alpestris	S159	France	HQ714721	/	HQ714853	Moreau et al. 2013
Alpova cf. cinnamomeus	PAM09082702	France	HQ714779	/	HQ714901	Moreau et al. 2013
Alpova cinnamomeus	BROWN FP73	USA	KF835996	/	/	Hayward et al. 2014
Alpova cinnamomeus	HRL1384	Canada	MN594282	MN594298	MN594770	Unpublished
Alpova concolor	UBC F14673	USA	KF835997	/	/	Hayward et al. 2014
Alpova concolor	OSC 65696	USA	NR_154686	/	/	Unpublished
Alpova corsicus	S287	France	HQ714769	/	HQ714893	Hayward et al. 2014
Alpova corsicus	S288	France	HQ714770	/	HQ714894	Moreau et al. 2011
Alpova komovianus	PAM10081201	Montenegro	JQ436850	/	JQ436862	Moreau et al. 2013
Neoalpova arenicola	JC150513NR	Spain	MN594292	MN594304	MN594775	Unpublished
Neoalpova cf. rubescens	JC140920BT	Spain	MN594294	MN594305	MN594776	Unpublished
Neoalpova montecchii	JC181021NR	Spain	MN594296	MN594306	MN594777	Alvarado et al. 2021
Paralpova artikutzensis	AH 49154	Spain	NR_173892	MN594307	MN594778	Alvarado et al. 2021
Melanogaster ambiguus	B-2220	Hungary	AJ555510	/	/	Unpublished
Melanogaster ambiguus	51745	Hungary	AJ555511	/	/	Unpublished
Melanogaster ambiguus	B-1599	Hungary	AJ555512	/	/	Unpublished
Melanogaster ambiguus	B-1613	Hungary	AJ555513	/	/	Unpublished
Melanogaster ambiguus	B-2409	Hungary	AJ555514	/	/	Unpublished
Melanogaster ambiguus	Ch12	Poland	KX438335	/	/	Unpublished
Melanogaster ambiguus	JC180719NR	Spain	MN594286	MN594299	MN594771	Unpublished
Melanogaster ambiguus	OSC158337	Poland	MN984308	/	/	Unpublished
	MES304					-
Melanogaster ambiguus	MTH1	Germany	MN994353	/	/	Unpublished
Melanogaster broomeanus	JC091213NR	Spain	MN594287	MN594300	MN594772	Unpublished
Melanogaster broomeanus	OTU_718s	United Kingdom	MT095837	/	/	Arraiano-Castilho et al. 2021
Melanogaster broomeanus	OTU_719s	United Kingdom	MT095838	/	/	Arraiano-Castilho et al. 2021
Melanogaster cyaneus	TJ75_1 (TYPE)	China	ON427476	ON427489	ON533869	This study
Melanogaster cyaneus	TJ75_2	China	ON427477	ON427490	ON533870	This study
Melanogaster diqingensis	WXH_9068 (TYPE)	China	ON427482	ON427495	ON533874	This study
Melanogaster euryspermus	OSC158352 DS1257	USA	MN984309	/	/	Unpublished
Melanogaster euryspermus	OSC158364 DS1555	USA	MN984310	/	/	Unpublished
Melanogaster euryspermus	OSC158339 JLF1044	USA	MN984311	/	/	Unpublished
Melanogaster euryspermus	OSC158325 JLF1129	USA	MN984312	/	/	Unpublished
Melanogaster euryspermus	OSC158351 JLF1456	USA	MN984313	/	/	Unpublished
Melanogaster euryspermus	OSC158317 JMT22778	USA	MN984314	/	/	Unpublished
Melanogaster euryspermus	OSC158333 MES110	USA	MN984315	/	/	Unpublished
Melanogaster intermedius	B-1770	Hungary	AJ555515	/	/	Unpublished
Melanogaster intermedius	RBG Kew K(M)130202	England	EU784372	/	/	Brock et al. 2009
Melanogaster intermedius	MT48	Germany	KX168661	/	/	Unpublished
Melanogaster luteus	S328/PAM09082801	France	HQ714780	/	HQ714902	Moreau et al. 2011
Melanogaster luteus	S407/Mon06	Montenegro	HQ714794	/	/	Moreau et al. 2011
Melanogaster macrosporus	cl-94	USA	AJ555526	/	/	Unpublished
Melanogaster macrosporus	B-2254	USA	AJ555528	/	/	Unpublished

Table 1. Specimen information and GenBank accession numbers for sequences used in this study.

Cracico	Isolate/ Strain/	Country	Genbank accession numbers			Deference
Species	Clone/Voucher		ITS	nrLSU	RPB2	Reference
Melanogaster minobovatus	BJTC FAN911	China	NR_186967	/	/	Xu et al. 2022
Melanogaster natsii	OSC82168 JMT7491	USA	MN984331	/	/	Unpublished
Melanogaster natsii	OSC158336 MES297	USA	MN984332	/	/	Unpublished
Melanogaster panzhihuaensis	HMAS 81915	China	NR_186968	/	/	Xu et al. 2022
Melanogaster rivularis	S190/PAM08090514	France	HQ714731	/	HQ714862	Moreau et al. 2011
Melanogaster rivularis	S285/PAM08090514	France	HQ714767	/	HQ714891	Moreau et al. 2011
Melanogaster rivularis	LIP PAM08090514 (TYPE)	France	NR_132848	/	/	Moreau et al. 2011
Melanogaster sp.	Melanog002FRA	France	KU924526	/	/	Unpublished
Melanogaster sp.	Melanog006FRA	France	KU924529	/	/	Unpublished
Melanogaster sp.	Melanog007FRA	France	KU924530	/	/	Unpublished
Melanogaster sp.	Melanog008FRA	France	KU924531	/	/	Unpublished
Melanogaster sp.	Melanog011FRA	France	KU924533	/	/	Unpublished
Melanogaster sp.	Melanog012FRA	France	KU924534	/	/	Unpublished
Melanogaster sp.	Melanog018FRA	France	KU924535	/	/	Unpublished
Melanogaster sp.	Melanog019FRA	France	KU924536	/	/	Unpublished
Melanogaster sp.	MT15	Germany	KX168646	/	/	Unpublished
Melanogaster sp.	MFS-1003	Chile	KY462394	,	/	Brock et al. 2009
Melanogaster sp.	LMKR1187	United Kingdom	MF352733	/	/	Suz et al. 2017
Melanogaster sp.	JC110118BT	Spain	MN594288	/	/	Unpublished
Melanogaster sp.	OSC AHF420	France	MN984333	/	/	Unpublished
Melanogaster sp.	OSC158378 DS1755	USA	MN984334	/	/	Unpublished
Melanogaster sp	0SCIG1042	USA	MN984335	,	/	Unpublished
Melanogaster sp.	MVC 753_ FLAS-F-65878	Chile	MT366708	/	/	Unpublished
Melanogaster sp. OK-2022a	oka331	Turkey	OP548647	/	/	Unpublished
Melanogaster sp. OK-2022a	oka332	Turkey	OP548648	/	/	Unpublished
Melanogaster spinisporus	BJTC FAN1092	China	MW598537	/	/	Xu et al. 2022
Melanogaster spinisporus	BJTC FAN938	China	MW598546	/	/	Xu et al. 2022
Melanogaster spinisporus	BJTC FAN941-A	China	MW598548	/	/	Xu et al. 2022
Melanogaster spinisporus	BJTC FAN941-B	China	MW598549	/		Xu et al. 2022
Melanogaster subglobisporus	HMAS83329	China	MW598534	,	/	Xu et al. 2022
Melanogaster truncatisporus	TJ83	China	ON427478	, 0N427491	, 0N533871	This study
Melanogaster truncatisporus	T.187 (TYPF)	China	ON427479	ON427492	ON533872	This study
Melanogaster truncatisporus	T.I109	China	ON427480	ON427493	ON533873	This study
Melanogaster truncatisporus	15346	China	ON427481	ON427494	/	This study
Melanogaster tuberiformis	B-1295	Romania	Δ 1555527	/	/	Unpublished
Melanogaster tuberiformis	IC110120PT	Spain	MNI504290	/ MNI504202	/ MNI50/1772	Unpublished
Melanogaster variogatus	22640	Gungany	A 1555522	/	/	Unpublished
Melanogaster variegatus	23040 D 1420	llungany	AJJJJJJZZ	/	/	Unpublished
	D-1430	Hungary	AJ555523	/	/	Unpublished
	D-1000	Hungary	AJ555524	/	/	Unpublished
Melanogaster variegatus	B-1225	Hungary	AJ555533	/	/	Unpublished
Melanogaster variegatus	B-1348	Hungary	AJ555534	/	/	Unpublished
Melanogaster variegatus	B-2312	Hungary	AJ555535	/	/	Unpublished
Melanogaster variegatus	JC180617BT	Spain	MN594290	MN594303	MN594774	Unpublished
Uncultured Melanogaster	MFT57	Germany	FJ403505	/	/	Pena et al. 2010
Paragyrodon sphaerosporus	MB06-066	USA	GU187540	GU187593	GU187803	Binder et al. 2010
Paxillus involutus	Bel10.4	France	KF261366	/	JQ436854	Jargeat et al. 2014
Note: The symbol "/" means th	at the sequence was n	ot available, a	and sequence	es newly gene	erated for this	study are in bold.

specified when running the BI analyses, but the obtained tree was rerooted with the outgroups used in the ML analysis.

The trees were visualized with TreeView32 (Page 2001), exported in PDF format, and edited in Adobe Illustrator CS6. Clades with bootstrap support (BS) \geq 70% and Bayesian posterior probabilities (PP) \geq 0.90 were considered significantly supported (Alfaro et al. 2003).

Subdivision of the genus *Melanogaster* based on morphological characteristics

In order to explore the correspondence between morphological features on the subdivision of *Melanogaster* from China (including the three new species described herein) and other parts of the world, the main morphological features (including the number of peridium layers, average thickness of peridium, average length, and width of basidiospores) were selected for statistical analysis (Table 2). Using the three sections (*Melanogaster, Rivulares,* and *Variegati*) as the dependent variable (*Y*), and the morphological features of the species as independent variables (*X*), we conducted a visual analysis of subdivision of different species using Orthogonal partial least squares discriminant analysis (OPLS-DA) using SIMCA 14.0.

Results

Molecular data analyses

The multi-gene dataset (ITS, 5.8S, nrLSU and RPB2) contained 27 specimens (7 novel specimens from our collections) and had an aligned length of 2,295 characters. ML and BI yielded identical tree topologies and only the tree inferred from the ML analysis is shown (Fig. 1). The ITS dataset contained 79 specimens, of which 7 were newly sequenced, and had an aligned length of 683 characters. ML and BI analyses produced identical tree topologies and only the tree derived from the ML analysis is shown (Fig. 2). The four-gene dataset and ITS (ITS1-2 and 5.8S) resulted in ML trees with a log-likelihood of -9919.6 and -5052.3, respectively. The general topology of the trees (Figs 1, 2) is congruent with those already published by Moreau et al. (2011, 2013). The phylogenetic tree derived from the four-gene dataset (Fig. 1), validates the classification of our specimens in Melanogaster. Our 7 specimens were resolved as 3 strongly supported species-level clades or branches, different from all known species included in the analysis (Fig. 2). Furthermore, two sequences (Melanogaster sp. MVC 753 FLASF 65878 and Melanogaster sp. MES 1003) from Chile, a sequence (Melanogaster sp. JC110118BT) from Spain, two sequences (Melanogaster sp. OSC AHF420 from France, Melanogaster sp. OSC LG1042 from the USA), and two sequences (Melanogaster sp. OK 2022a oka331 and Melanogaster sp. OK 2022a oka332) from Turkey formed four distinct, species-level clades/lineages labelled M. sp1 to M. sp4 (Fig. 2). These clades exhibited less than 98.4% similarity (M. sp1 = 98.3%, M. sp2 = 93.47%, M. sp3 = 94.06%, and M. sp4 = 96.63%) in their ITS sequences compared to other species of Melanogaster. This observation suggests the likely presence of four additional undescribed species.

0	Peridium			Basidi	ospores	•
Species	layers	Min. (µm)	Max. (µm)	L _m (µm)	W _m (µm)	Country
M. quercicola	2	450	600	13.1	6.5	China
M. utriculatus	2	300	400	13	9	Japan
M. fusisporus	2	250	420	12.3	5.2	China
M. shanxiensis	2	180	420	12.2	6.1	China
M. obovatus	2	350	600	12.2	6	China
M. macrosporus	1	85	315	11.5	5.7	Hungary
M. panzhihuaensis	1	150	220	10.4	6.1	China
M. natsii	2	200	250	10	6	China
M. coccolobae	2	170	230	9.9	7.5	Mexico
M. subglobisporus	2	130	300	9.8	7	China
M. spinisporus	1	360	750	9.6	7.3	China
M. cyaneus	2	100	400	9.5	7	China
M. tomentellus	1	250	350	9.4	4.5	China
M. ovoidisporus	2	250	450	7.1	4.3	China
M. truncatisporus	2	200	450	7	4.5	China
M. minobovatus	2	350	500	6.1	4.7	China
M. broomeanus	1	150	400	6	3.9	China
M. luteus	2	100	250	6	2.75	France, Montenegro
M. minysporus	1	160	240	5.5	4	Mexico
M. rivularis	2	100	135	5.5	2.9	France
M. diqingensis	2	100	320	3.8	3.2	China

Table 2. The main morphological characterization of Melanogaster species.

Notes: The new species introduced in this study are presented in bold.



Figure 1. The phylogram of *Melanogaster* and closely related genera obtained with RAxML (including ITS1, ITS2, 5.8S, nrLSU and RPB2). *Paragyrodon sphaerosporus* MB06 066 and *Paxillus involutus* Bel10 10 were selected as the outgroup. Nodes were annotated with ML BS > 70%, Bayesian PP > 0.90, or "-" in case of non-significant support value. New species are in bold font.

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Figure 2. The ITS phylogram of *Melanogaster* obtained with RAxML (including ITS1, ITS2, and 5.8S). The six *Alpova* cessions were chosen as the outgroup. Nodes were annotated with ML BS > 70%, Bayesian PP > 0.90, or "-" in case of non-significant support value. New species are in bold font. The sections are as defined by Moreau et al. 2011 on the basis of basidiospore length.

Taxonomy

Melanogaster cyaneus **T. J. Yuan, Shu H. Li, & Raspé, sp. nov.** MycoBank No: 901868 Fig. 3a-d

Diagnosis. *Melanogaster cyaneus* is diagnosed by its blue or bluish gleba, rhizomorphs on the base, thinner peridium (100–400 μ m), and longer and wider basidiospores (6.2–15 × 4.6–9.0 μ m).

Etymology. The epithet cyaneus refers to the blue or bluish gleba.

Holotype. CHINA. Sichuan Province: Panzhihua City, Yanbian County, Shuanglong village, 26°49'12"N, 101°33'7.1028"E, elevation 1,970 m, in mainly reddish brown soils under *Castanea mollissima* Bl., 16 Aug. 2020, collected by M. Yang (KUN- HKAS129200, holotype; YAAS-TJ75-1, isotype). **Description.** *Basidiomata* 2.5–4.0 × 1.5–2.5 cm, hypogeous or semi-hypogeous, subglobose to ellipsoidal, occasionally elongate; light brown to yellowish brown, with mycelial strands attached in the base (Fig. 3a). *Peridium* two-layered, outer layer 20–75 µm thick, composed of interwoven hyphae, orange-yellow to reddish, 2–4 µm broad, thick-walled, clamp connections present; inner layer 145– 335 µm thick, composed of interwoven, strongly gelatinized hyphae, 2–5 µm broad, pale-yellow, intermixed with massive inflated cells, 6–12 µm broad, with clamp connections present. *Gleba* solid, gelatinous, milk-white when immature, blue or bluish at mature; trama plates pale-yellow, of gelatinized hyphae (Fig. 3b); locules small, filled with black spores (Fig. 3c). *Basidia* exhibit limited revival, appearing clavate, hyaline, 4-spores, randomly distributed, gelatinized at maturity. *Basidiospores* subglobose to globose, 6.2–15 × 4.6–9.0 µm (L_m × W_m = 9.5 ± 3.0 × 7.0 ± 2.0, *Q*= 1.0–2.0, *Q_m* =1.4 ± 0.6, n = 75), smooth, hyaline when immature, light yellow to reddish at maturity, surfaces display distinctive spots, and optical microscopy reveals a notable hilar appendage, 0.5–1.0 µm in diam (Fig. 3d).

Other material examined. CHINA, Sichuan province, Panzhihua City, Yanbian County, Shuanglong village, 26°49'12"N, 101°33'7.1029"E, elevation 2,000 m, under *Castanea mollissima* Bl. in evergreen hill forest, in mainly reddish-brown soils, 16 Aug. 2020, collected by M. Yang (YAAS TJ75-2).

Notes. *M. broomeanus* Berk (Türkoğlu and Castellano 2013; Uzun et al. 2014), *M. shanxiensis* and *M. obovatisporus* (Liu et al. 1989), are similar to *M. cyaneus* in morphology. *M. cyaneus* (basidiomata light brown to yellowish brown), *M. broomeanus* (basidiomata yellow-brown to deep brown), *M. shanxiensis* (basidiomata brown to rust-brown) and *M. obovatisporus* (basidiomata brown to brownish-black), but the specimens of *M. cyaneus* clustered in an independent clade with strong support (BS = 100%, PP = 1.0; Fig. 2), supporting it as a distinct species. Additionally, DNA analysis revealed *M. cyaneus* shared less than 92% ITS similarity with other *Melanogaster* species.

Melanogaster diqingensis T. J. Yuan, Shu H. Li, & Raspé, sp. nov.

MycoBank No: 901869 Fig. 3e-h

Diagnosis. Melanogaster diqingensis is diagnosed by the combination of medium-sized, pale-yellow to orange or brown-yellow basidiomata with scaled, lobed or concave surface, pale yellow gleba, and obovate to subglobose, smooth basidiospores $(3.0-5.1 \times 2.0-4.0 \ \mu m, Q = 1.0-1.8)$.

Etymology. The epithet *diqingensis* refers to the prefecture of the type locality. **Holotype.** CHINA. Yunnan Province: Diqing Autonomous Prefecture, Shangri-La County, Baishuitai village, 27°30'14.2236"N, 100°2'50.5716"E, elevation 2,380 m, in brown soil under *Quercus aquifolioides* Rehd. et Wils., 25 Sep. 2020, collected by X. H. Wang (KUN- HKAS121212, holotype; YAAS-WXH_9068, isotype).

Description. Basidiomata $0.5-2.5 \times 0.2-2.2$ cm, hypogeous, globose, subglobose or ellipsoidal, pale-yellow to orange or brown-yellow, scaled, lobed or concave in the surface, without visible rhizomorphs (Fig. 3e). Odor faint. Peridium two-layered, outer layer 40–80 µm thick, composed of interwoven hyphae, 3.5-7.5 µm broad, with fusoid to cylindrical terminal cells, deeply orange-yellow walls toward surface, with clamp connections; inner layer 150–260 µm thick,



Figure 3. Photographs of *Melanogaster* species. *Melanogaster cyaneus* (YAAS-TJ75-1, holotype) **a** basidiomata **b** LM of Peridium **c** LM of basidiospores **d** SEM of basidiospore. *Melanogaster diqingensis* (YAAS-WXH_9068, holotype) **e** basidiomata **f** LM of Peridium **g** LM of basidiospores **h** SEM of basidiospore. *Melanogaster truncatisporus* (YAAS-TJ87, holotype) **i** basidiomata **j** LM of peridium **k** LM of basidiospores **l** SEM of ascospore. Scale bars: 2 cm (**a**, **e**, **i**); 20 µm (**b**, **f**, **g**); 10 µm (**c**, **g**, **k**); 1 µm (**d**, **h**, **l**).

composed of interwoven hyphae, 5–10 µm broad, with inflated cells, 7.5–15 µm broad, bright pale yellow, with abundant clamp connections. **Gleba** solid, milk-white when immature, pale yellow at maturity, hard when dried; trama plates of hyaline or yellowish, gelatinized hyphae (Fig. 3f); locules 2–3 mm in diam (Fig. 3g). **Basidia** exhibit limited reviving, appearing clavate, hyaline, 4–spored. **Basidiospores** obovate to subglobose, smooth, $3.0-5.1 \times 2.0-4.0 \text{ µm}$ (L_m × W_m = $3.8 \pm 1.0 \times 3.2 \pm 0.8$, Q = 1.0-1.8, $Q_m = 1.2 \pm 0.6$, n = 75), hyaline (immature) to light yellow, dark brown (mature) in KOH 5%, with truncate-cupped base and very short hilar appendage in optical microscopy, 0.5-1.5 µm in diam (Fig. 3h).

Notes. Six *Melanogaster* species, namely *M. subglobisporus*, *M. natsii*, *M. spinisporus* (Wang et al. 1995), *M. rivularis*, *M. luteus=M. microsporus* (Moreau et al. 2011), are similar to *M. diqingensis* in morphology and related by phylogeny. The colors of their basidiomata are similar to *M. diqingensis*, i.e. rust brown to deep brown in *M. subglobisporus*, yellow-brown in *M. natsii*, grayish brown to light brown in *M. spinisporus*, and bright golden yellow in *M. minysporus*, but the basidiomata of all of latter species are without scales, lobes or concave area, by which they were easily differentiated from *M. diqingensis*. *M. rivularis* (anthracite-black gleba), and *M. luteus* (club-shaped or cylindro-elliptical to cylindrical basidiospores) also are easily differentiated from *M. diqingensis*. The thickness of the peridium (\leq 300 µm) is similar among *M. subglobisporus*, *M. ovoidisporus*, and *M. coccolobae*, However, the size of basidiospores provide a clear distinction between these

species (*M. subglobisporus* $8-11 \times 7-9 \mu m$, *M. ovoidisporus* $5-7 \times 3.5-5.8 \mu m$ and *M. coccolobae* $6.2-12 \times 5.2-10 \mu m$). Phylogenetically, *M. diqingensis* and *M. subglobisporus* formed an independent and strongly supported clade (BS = 78%, PP = 1.0; Fig. 2), but *M. diqingensis* shared less than 93.2% ITS similarity with *M. subglobisporus*, supporting *M. diqingensis* as a distinct species.

Melanogaster truncatisporus T. J. Yuan, Shu H. Li, & Raspé, sp. nov.

MycoBank No: 901870 Fig. 3i-l

Diagnosis. *Melanogaster truncatisporus* is diagnosed by the combination of medium-sized basidiomata with orange-yellow peridium that becomes reddish brown to dark brown in age, and truncate basidiospores.

Etymology. The epithet *truncatisporus* refers to the truncate basidiospores.

Holotype. CHINA. Yunnan Province: Nujiang Autonomous Prefecture, Lanping County, Zhongpai township, Xinchangping village, 26°54'15"N, 99°10'32"E, elevation 1990 m, in mainly brown soils under *Castanea mollissima* and *Pinus yunnanensis* Franch., 26 Oct. 2020, collected by T. J. Yuan (KUN-HKAS129199, holotype; YAAS-TJ87, isotype).

Description. Basidiomata 1.5–3.0 × 0.4–2.3 cm, hypogeous or semi-hypogeous, subglobose to oval, occasionally irregular-elongated, yellowish when young, reddish brown to dark brown at maturity, smooth or slightly velvety surface, lobed or indented at the base, attached mycelial strands, occasionally extending to the surface, dark brown, rhizomorphs not distinct (Fig. 3i). Peridium two-layered, outer layer 50-100 µm thick, composed of interwoven hyphae, orange-yellow, with clamp connections, and fusoid to cylindrical terminal cells, 4-5 µm broad; inner layer 150-350 µm thick, composed of interwoven hyphae, 3-5 µm broad, pale yellow, intermixed with massive inflated cells, ellipsoidal or irregular, 3-20 µm broad. Gleba solid, pale brown when young, blackish brown to black at maturity, separated by white or pale yellow trama when young, which becomes deep brown at maturity, hard when dried; trama plates of hyaline or yellowish gelatinized hyphae (Fig. 3j); locules 2-4 mm in diameter, polygonal to irregular (Fig. 3k). Basidia poorly recovered, hyaline, 4-spored, occurring randomly in the locules (Fig. 3k). Basidiospores subglobose to globose or irregularly elongate-pyriform, $3.5-9.5 \times 3.0-7.0 \mu m (L_m \times W_m = 7.0 \pm 2.5 \times 4.5 \pm 1.0 \pm$ 2.0, Q = 1.0-2.5, $Q_m = 1.5 \pm 1.0$, n = 65), hyaline when immature, becoming dark brown at maturity, smooth, with truncate-cupped base and short hilar appendage, $1-2 \mu m$ in diam in optical microscopy (Fig. 3I).

Habitat, phenology, and distribution. hypogeous to semi-hypogeous under *Castanea mollissima* and *Pinus yunnanensis*, in mixed forest, in late autumn. So far found in Lanping and Gongshan counties, Yunnan Province, China.

Other material examined. CHINA. Yunnan Province: Nujiang autonomous Prefecture, Lanping County, 26°54'17"N, 99°10'31"E, elevation 2,030 m, in mainly brown soils under *Castanea mollissima* and *Pinus yunnanensis*, 26 Oct. 2020, collected by T. J. Yuan (YAAS TJ83 and YAAS TJ109); CHINA. Yunnan Province: Nujiang Autonomous Prefecture, Gongshan County, 28°1'19"N, 98°37'2"E, elevation 1,800 m, in mainly brown soils under *Castanea mollissima* and *Pinus yunnanensis*, 25 Sep. 2020, collected by Li, S. H. (YAAS L5346).

Notes. Four Melanogaster species, namely M. minysporus (Cázares et al. 2008), M. broomeanus Berk (Türkoğlu and Castellano 2013, Uzun et al. 2014), M. obovatisporus (Liu et al. 1989), and M. variegatus (Krisztián 2008), are similar in morphology and related to M. truncatisporus by phylogeny. M. truncatisporus can be easily differentiated by its peridium thickness (M. truncatisporus, 200-450 µm vs M. minysporus, 160-240 µm) and the size of basidiospores (*M. truncatisporus*, $3.5-9.5 \times 3.0-7.0 \mu m$ vs *M. minysporus*, $5-6.5 \times 3-5 \mu m$). M. truncatisporus has a two-layered peridium and M. broomeanus has a single-layered peridium. The difference in basidiospore size is evident, with 3.5-9.5 \times 3.0–7.0 µm for *M. truncatisporus* and 7–9 \times 4 µm for *M. broomeanus*. Also, *M.* truncatisporus basidia typically contain 4 spores, whereas those of M. obovatisporus consistently contain 8 spores. Basidiospore size (especially minimum size) is also a diagnostic character to separate M. truncatisporus from M. variegatus $(3.5-9.5 \times 3.0-7.0 \mu m$ for the former, and $7.5-10 \times 5.5-8 \mu m$ for the latter). Phylogenetically, the specimens of *M. truncatisporus* clustered in an independent clade with strong support (BS = 100%, PP = 1.0; Fig. 2), supporting it as a new species. Additionally, *M. truncatisporus* (holotype ITS sequence ON427479) shared less than 93.2% similarity with ITS sequences of other Melanogaster species.

Subdivision of the genus Melanogaster in sections based on morphology

The main characters to identify *Melanogaster* species are the number of layers of the peridium, thickness of the peridium, and average length and width of basidiospores (Table 2). A discriminant analysis based on five variables, namely the number of layers of the peridium, the minimum and maximum thickness of the peridium, and the length and width of basidiospores, was performed by OPLS-DA (Fig. 4). In this analysis, the three sections, *Melanogaster, Variegati*, and *Rivulares*, were separated in the 3D principal component space by the average length and average width of basidiospores, and the minimum thickness of the peridium. The cumulative contribution of the first three principal components exceeded 80.5%. The primary factors determining the distribution of species in the 3-dimensional space are the average length and width of spores. The three new species (*M. cyaneus, M. diqingensis*, and *M. truncatisporus*) were placed into the section *Melanogaster, Rivulares*, and *Variegati* by OPLS-DA, respectively.

Discussion

In this paper, we introduce three novel species of hypogeous fungi belonging to *Melanogaster* (Paxillaceae, Boletales): *M. cyaneus*, *M. diqingensis*, and *M. truncatisporus*. The first two species were discovered in Yunnan Province, while the latter was collected in Sichuan Province, in southwest China. Morphologically, *M. cyaneus* stands out with its distinctive blue or bluish gleba and light brown to yellowish brown basidiomata. *M. diqingensis* can be identified by its pale yellow to brown-yellow basidiomata, featuring a scaled, lobed, or concave surface. *M. truncatisporus* is characterized by its distinct pale yellow to deeply orange-yellow peridium and subglobose to globose or pyriform basidiospores. These characteristics differentiate them from other *Melanogaster* species found in China. Phylogenetically, our analysis based on an ITS rDNA dataset, including ITS1-2 and 5.8S, revealed that specimens of *M. cyaneus* and *M. truncatisporus* clustered into two independent clades with strong support (BS = 100%, PP = 1.0). *M. diqingensis* and *M. subglobisporus* formed a separate and well-supported clade (BS = 78%, PP = 1.0; Fig. 2). Moreover, these three species shared less than 93.2% ITS similarity with other *Melanogaster* species. Both the phylogenetic tree and morphological features concur to support these three species as new additions to *Melanogaster*.

Knapp (1954) and Moreau et al. (2011) proposed a division of European Melanogaster species into three sections based on the average length of basidiospores. These sections were defined as follows: section Melanogaster, with basidiospores longer than 10 µm; section Variegati, with basidiospores measuring 6-10 µm; and section Rivulares, with basidiospores ranging from 5 to 6 µm. Our multivariate statistical analysis based on wider taxon sampling and three morphological characters instead of only spore length allowed to separate species in line with Moreau et al. (2011) sections (Fig. 4). However, in the phylogenetic tree (Fig. 2), the three morphologically delineated sections were not monophyletic. For example, M. panzhihuaensis described from China was placed within the Melanogaster section based on morphology (Fig. 4), but shared a sister relationship with M. minobovatus, also from China, which was assigned to section Rivulares by the multivariate morphological analysis (Fig. 4). This placement is distinct from other species classified within the Melanogaster section, such as M. ambiguus (Spain and China), M. intermedius (UK), M. euryspermus (USA), M. tuberiformis (Spain), M. natsii (China), M. subglobisporus (China), and M. macrosporus (Hungary) (Wang et al. 1995; Frank et al. 2006; Brock et al. 2009; Alvarado et al. 2021; Xu et al. 2022).

A set of 19 *Melanogaster* sequences from Germany, France, and the USA (as detailed in Table 1) were included in our phylogenetic analyses. The phylogenetic position of certain specimens based on these sequences was problematic, particularly concerning *M. intermedius* B-1770, and *M. variegatus* 23640 and B-1688 from Hungary, as well as *M. natsii* OSC82168 JMT7491 and OSC158336 MES297 from the USA. All those accessions belonged in species-level clades including other accessions identified as different species. The ITS rDNA sequences of *Melanogaster* sp. (Melanog002FRA, Melanog006FRA, Melanog007FRA, Melanog008FRA, Melanog011FRA, and Melanog012FRA), along with *Melanogaster* sp. (Melanog018FRA and Melanog019FRA) from France, clustered with *M. rivularis* and *M. luteus* clades, respectively. This observation is substantiated by robust support from high bootstrap values (BS = 97 and BS = 93) and posterior probability from Bayesian inference (PP = 1.0) in Fig. 2. Consequently, we recommend classifying the first six unidentified specimens as *M. rivularis* species and the last two specimens as *M. luteus*.

Melanogaster sp. MVC 753 FLASF 65878 (Chile) and Melanogaster sp. MES 1003 (Chile) exhibited less than 98.3% similarity in the ITS region when compared to *M. macrosporus*. Furthermore, an ITS sequence (*Melanogaster* sp. JC110118BT) from Spain, two ITS sequences (*Melanogaster* sp. OSC AHF420 from France and *Melanogaster* sp. OSC LG1042 from the USA), and two ITS sequences (*Melanogaster* sp. OK 2022a oka331 and *Melanogaster* sp. OK 2022a oka332) from Turkey exhibited less than 96.7% similarity to ITS sequences of other *Melanogaster* species. Consequently, those accessions represent four distinct species-level lineages, designated as *M.* sp1 to *M.* sp4, respectively (Fig. 2). This



Figure 4. OPLS-DA discriminant analysis of three sections including Chinese species, based on their main morphological characteristics. Lm = mean spore length; Wm = mean spore width; Peridium Tmin = minimum thickness of peridium.

observation suggests the presence of four undescribed species. More research is therefore needed to validate and properly describe those putative novel species.

Key to the Melanogaster species from China

1a	Basidiospores verrucose
1b	Basidiospores smooth
2a	Basidiomata brown to deep brown, peridium $250-350 \ \mu m$ thick, surface minutely green tomentose; basidiospores elongate fusiform <i>M. tomentellus</i>
2b	Basidiomata light brown with some red-brown or brick-red when fresh, peridium 360-750 µm thick, basidiospores ellipsoid to obovoid, subglo-
	bose
3a	Basidiospores light yellow or yellow4
3b	Basidiospores brown to dark brown or reddish brown5
4a	Basidiomata brick-red with dark green to black-green gleba at maturity
4b	Basidiomata red-brown to brown with yellow-green gleba at maturity
5a	Basidiospores mostly longer than 10 µm6
5b	Basidiospores mostly shorter than 10 μm 7

6а	Basidiomata yellow-brown to brown, gleba brown, basidiospores fusi-
	form M. fusisporus
6b	Basidiomata brown to rust-brown, gleba brownish, basidiospores long
	obovoid
6c	Basidiomata light brown to yellowish brown, gleba bluish, basidiospores
	subglobose to globose
6d	Basidiomata yellow-brown, gleba brown to dark brown, basidiospores li-
	moniform or ellipsoid M. natsii
7a	Basidiospores oblong to cylindrical
7b	Basidiospores obovate or obovate to subglobose
7c	Basidiospores subglobose to globose9
8a	Basidiospores red-brown, 8.7–12.4 × 5.2–7.6 µm M. panzhihuaensis
8b	Basidiospores dark brown to brownish, 6.5–8 × 3.8–4.7 μm
	M. obovatisporus
8c	Basidiospores yellowish brown to brown, 8.7–10.6 × 6.6–8.0 μ m
8d	Basidiospores dark brown, 5.1–6.9 × 4.2–5.1 µm
9a	Basidiomata reddish brown to dark brown, gleba brownish-black or black,
	basidiospore 3.5–9.5 × 3.0–7.0 μm <i>M. truncatisporus</i>
9b	Basidiomata pale yellow to orange, gleba pale yellow, basidiospore 3.0-
	5.1 × 2.0−4.0 µm <i>M. diqingensis</i>

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Tian-Jun Yuan performed the research and wrote the manuscript, Olivier Raspé supervised the work and revised the manuscript, Hong-Mei Luo and Kai-Mei Su collected specimens, Shu-Hong Li provided the fundings.

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Data availability

All of the data that support the findings of this study are available in the main text, or in publicly accessible repositories as indicated in the text.

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Research Article

Morphology and multigene phylogeny revealed four new species of *Xylodon* (Schizoporaceae, Basidiomycota) from southern China

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Abstract

Fungi are one of the most diverse groups of organisms on Earth, amongst which wood-inhabiting fungi play a crucial role in ecosystem processes and functions. Four new wood-inhabiting fungi, Xylodon cremeoparinaceus, X. luteodontioides, X. poroides and X. wumengshanensis are proposed, based on morphological features and molecular evidence. Xylodon cremeoparinaceus is distinguished by a cream hymenial surface with a pruinose hymenophore, a monomitic hyphal system with clamped generative hyphae and ellipsoid basidiospores. Xylodon luteodontioides is characterised by flavescens hymenophore surface with odontioid hymenophore, monomitic hyphal system with clamped generative hyphae and ellipsoid basidiospores. Xylodon poroides bears coriaceous basidiomata with a poroid hymenophore surface, monomitic hyphal system with clamped generative hyphae and ellipsoid basidiospores. Xylodon wumengshanensis is a distinct taxon by its grandinoid hymenophore surface, monomitic hyphal system with clamped generative hyphae and ellipsoid basidiospores. Sequences of ITS and nLSU rRNA markers of the studied samples were generated and phylogenetic analyses were performed using the Maximum Likelihood, Maximum Parsimony, and Bayesian Inference methods. The phylogram, based on the ITS+nLSU rDNA gene regions, included three genera within the Schizoporaceae as Fasciodontia, Lyomyces and Xylodon. The four new species were grouped into the genus Xylodon. The topology, based on the ITS sequences, revealed that Xylodon cremeoparinaceus was grouped closely with X. pruinosus, X. detriticus and X. ussuriensis. The taxon X. luteodontioides was sister to X. nesporii. The species X. poroides separated from X. pseudotropicus, while X. wumengshanensis was grouped with four taxa: X. patagonicus, X. radula, X. subtropicus and X. taiwanianus.

Key words: Biodiversity, China, phylogenetic analyses, wood-inhabiting fungi, Yunnan Province

Introduction

Fungi are well-known as a diverse group of microorganisms that play important roles in forest ecosystems (Phookamsak et al. 2019). Wood-inhabiting fungi are essential to natural ecosystems for nutrient cycling and maintaining plant

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diversity (Drinkwater et al. 2017; Horwath 2017; Hyde et al. 2018; Wu et al. 2022a, b; Guan et al. 2023; Yuan et al. 2023; Deng et al. 2024a, b; Zhang et al. 2024). Schizoporaceae Jülich includes many variations of the fruiting body types amongst Hymenochaetales Oberw. (Larsson et al. 2006; Wu et al. 2022a; Guan et al. 2023; Zhang et al. 2024), in which it comprises many representative wood-inhabiting fungal taxa, including hydnoid, corticioid and polyporoid basidiomes with diverse hymenophoral and cystidial morphology (Yurchenko and Wu 2016; Riebesehl and Langer 2017; Yurchenko et al. 2017; Cui et al. 2019; Riebesehl et al. 2019; Jiang et al. 2021; Wu et al. 2022a, b; Guan et al. 2023; Deng et al. 2024a, b; Zhang et al. 2024). In addition, members of Schizoporaceae are widely distributed, causing white rot (Langer 1994; Luo et al. 2022; Guan et al. 2023; Zhang et al. 2024).

Xylodon (Pers.) Gray is a large genus of corticioid fungi, having a cosmopolitan distribution (Bernicchia and Gorjón 2010; Guan et al. 2023; Yurchenko et al. 2024; Zhang et al. 2024). Species of Xylodon inhabit dead wood of various sizes, from twigs several millimetres in diameter to large fallen trunks and cause white rot (Girometta et al. 2020; Greslebin and Rajchenberg 2000; Kotiranta and Saarenoksa 2000; Guan et al. 2023). Sometimes basidiomata of Xylodon species appear on living parts of trees (Yurchenko 2008) and non-woody plant remains, for example, fern rachises (Kotiranta and Saarenoksa 2000), herb stems and fallen leaves (Viner et al. 2018) and dead polypore basidiomata (Viner et al. 2023). The genus is known from almost all types of world biomes where wooden plant debris occurs, from humid to semi-arid and from seashore to the upper limit of wooden vegetation in altitudinal gradients (Yurchenko et al. 2024). This genus is typified by X. quercinus (Pers.) Gray (Bernicchia and Gorjón 2010) and characterised by the resupinate or effuse basidiomata with a smooth, tuberculate, grandinioid, odontioid, coralloid, irpicoid or poroid hymenophore; a monomitic or dimitic hyphal system with clamped generative hyphae; the presence of different types of cystidia; utriform or suburniform basidia; and cylindrical to ellipsoid to globose basidiospores (Gray 1821; Bernicchia and Gorjón 2010; Zhang et al. 2024). Based on the MycoBank database (http://www.mycobank.org, accessed on 19 May 2024) and the Index Fungorum (http://www. indexfungorum.org, accessed on 19 May 2024), Xylodon has been registered with 234 specific and infraspecific names and the actual number of the species has reached 109 species (Chevallier 1826; Kuntze 1898; Wu 1990, 2000, 2001, 2006; Hjortstam and Ryvarden 2007, 2009; Xiong et al. 2009, 2010; Bernicchia and Gorjón 2010; Tura et al. 2011; Dai 2012; Lee and Langer 2012; Yurchenko et al. 2013; Yurchenko and Wu 2014a, b; Zhao et al. 2014; Chen et al. 2016; Kan et al. 2017a, b; Riebesehl and Langer 2017; Wang and Chen 2017; Viner et al. 2018; Riebesehl et al. 2019; Shi et al. 2019; Dai et al. 2021; Luo et al. 2021a, 2022; Qu and Zhao 2022; Qu et al. 2022; Viner and Miettinen 2022; Guan et al. 2023; Wang and Zhou 2024; Yurchenko et al. 2024; Zhang et al. 2024).

Classification of taxa in the kingdom *Fungi* has been updated continuously, based on the frequent inclusion of data from DNA sequences in many phylogenetic studies (Yurchenko et al. 2020). For the past few years, the genus *Xylodon* was generally studied by molecular systematics and it was included in the *Hyphodontia* s.l. (Hjortstam and Ryvarden 2009; Yurchenko and Wu 2016; Riebesehl and Langer 2017; Wang and Chen 2017; Riebesehl et al. 2019; Qu et al. 2022; Guan et al. 2023). *Hyphodontia* s.l. was shown to be a polyphyletic

genus and a broad concept employed by some mycologists due to a lack of rDNA sequences for many taxa, in which Xylodon and Kneiffiella P. Karst included rich species (Hjortstam and Ryvarden 2009; Riebesehl and Langer 2017; Riebesehl et al. 2019; Luo et al. 2022; Zhang et al. 2024). Based on the molecular systematics research, two clades, the Xylodon-Lyomyces-Rogersella and the Xylodon-Schizopora-Palifer clades were described and the related species of Lyomyces P. Karst., Palifer Stalpers & P.K. Buchanan, Rogersella Liberta & A.J. Navas Schizopora Velen. and Xylodon, within both clades were suggested to be mixed (Yurchenko et al. 2013). The research comprised the representative sequences and taxa of Hyphodontia s.l., such as Lyomyces, Palifer, Rogersella, Schizopora and Xylodon, in which the result demonstrated that it was hard to distinguish the two genera Xylodon and Schizopora on the basis of the morphological and phylogenetic information; therefore, the authors proposed that the related species of Schizopora should be united into the genus Xylodon (Riebesehl and Langer 2017). For the phylogenetic relationship of Xylodon species, it was confirmed that the two genera Lagarobasidium Jülich and Xylodon should be synonymous, based on the molecular data from the ITS and nLSU regions, in which the three species X. pumilius (Gresl. & Rajchenb.) K.H. Larss., X. magnificus (Gresl. & Rajchenb.) K.H. Larss. and X. rickii (Gresl. & Rajchenb.) K.H. Larss. were combined into Xylodon (Viner et al. 2018). All the taxa of the genera Odontipsis Hjortstam & Ryvarden and Palifer were placed in the genus Xylodon, based on the molecular analyses of 28S and ITS data, in which they proposed four new species of Xylodon as X. exilis Yurchenko, Riebesehl & Langer, X. filicinus Yurchenko & Riebesehl, X. follis Riebesehl, Yurchenko & Langer and X. pseudolanatus Nakasone, Yurchenko & Riebesehl (Riebesehl et al. 2019). Based on the multiple loci in Hyphodontia s.l., Fasciodontia Yurchenko& Riebesehl, Hastodontia (Parmasto) Hjortstam & Ryvarden, Hyphodontia J. Erikss., Lyomyces, Kneiffiella and Xylodon in Hymenochaetales, they were divided into four clades and three new taxa were found from China, in which X. gossypinus C.L. Zhao & K.Y. Luo and X. brevisetus (P. Karst.) Hjortstam & Ryvarden grouped together (Luo et al. 2021a). Based on the morphological descriptions and molecular analyses, three new species, namely Xylodon angustisporus Viner & Ryvarden, X. dissiliens Viner & Ryvarden and X. laxiusculus Viner & Ryvarden, were described in Africa and placed in the genus Xylodon (Viner et al. 2021). A phylogenetic and taxonomic study focusing on the genus Xylodon (Hymenochaetales) newly described one species of this genus from southern China and this research enriched the fungal diversity worldwide (Zhang et al. 2024). Since the 1810s, a total of 234 species have been proposed for the genus Xylodon (http://www.indexfungorum.org/Names/Names. asp?pg=1, accessed on 19 May 2024). Inspiringly, new species have been described in the genus at an accelerated pace after the inflection point around the year 1890 and 2007 on the trend curve of species number (Fig. 1), which is due to advances in morphological taxonomy and molecular phylogeny (Luo et al. 2022; Qu et al. 2022; Guan et al. 2023; Yurchenko et al. 2024; Zhao et al. 2024; Zhang et al. 2024).

During investigations on the wood-inhabiting fungi in the Yunnan Province of China, samples representing four additional species belonging to *Xylodon* were collected. To clarify the placement and relationships of these species, we carried out a phylogenetic and taxonomic study on *Xylodon*, based on the ITS and



Figure 1. Trends in the accumulative number of species of Xylodon recorded in the world and China.

nLSU sequences. These specimens are identified as four undescribed species of *Xylodon* and the detailed description, illustrations and phylogenetic analysis results of the new species are provided here.

Materials and methods

Morphology

Fresh basidiomata of the fungi growing on the angiosperm branches were collected from the Honghe, Lincang, Puer, Wenshan and Zhaotong of Yunnan Province, P.R. China. Specimens were dried in an electric food dehydrator at 40 °C (Hu et al. 2022), then sealed and stored in an envelope and deposited in the Herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China. Macromorphological descriptions were based on field notes and photos captured in the field and lab. Colour terminology followed Petersen (1996). Micromorphological data were obtained from the dried specimens when observed under a light microscope following the previous study (Guan et al. 2023). The following abbreviations are used: KOH = 5% potassium hydroxide water solution, CB = Cotton Blue, CB- = acyanophilous, IKI = Melzer's Reagent, IKI- = both inamyloid and indextrinoid, L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied and n = a/b (number of spores (a) measured from given number (b) of specimens).

Molecular phylogeny

The EZNA HP Fungal DNA Kit (Omega Biotechnologies Co., Ltd., Kunming, China) was used to extract DNA with some modifications from the dried specimens. The nuclear ribosomal ITS region was amplified with primers ITS5 and ITS4 (White et al. 1990). The PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s and 72 °C for 1 min, with a final extension of 72 °C for 10 min. The nuclear nLSU region was amplified with primer pair LR0R and LR7 (Rehner and Samuels 1994). The PCR procedure for nLSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C for 1 min and 72 °C for 1.5 min with a final extension of 72 °C for 10 min. The PCR procedure for ITS and nLSU followed a previous study (Zhao and Wu 2017). All newly-generated sequences were deposited in NCBI GenBank (https://www.ncbi.nlm.nih.gov/ genbank/) (Table 1).

The sequences were aligned in MAFFT version 7 (Katoh et al. 2019) using the G-INS-i strategy. The alignment was adjusted manually using AliView version 1.27 (Larsson 2014). Sequences of *Hymenochaete ochromarginata* P.H.B. Talbot and *H. rubiginosa* (Dicks.) Lév. retrieved from GenBank were used as the outgroups in the ITS+nLSU analysis (Fig. 2); Sequences of *Lyomyces sambuci* (Pers.) P. Karst. retrieved from GenBank were used as the outgroups in the ITS analysis (Fig. 3) (Guan et al. 2023; Zhang et al. 2024).

Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were applied to the combined three datasets following a previous study (Zhao and Wu 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 1,000 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 bootstrap (BT) analysis with 1000 pseudo replicates (Felsenstein 1985). Descriptive tree statistics - tree length (TL), composite consistency index (Cl), composite retention index (RI), composite rescaled consistency index (RC) and composite homoplasy index (HI) - were calculated for each maximum parsimonious tree generated. The combined dataset was also analysed using Maximum Likelihood (ML) in RAxML-HPC2 through the CIPRES Science Gateway (Miller et al. 2012). Branch support (BS) for the ML analysis was determined by 1000 bootstrap pseudo replicates.

MrModelTest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each dataset for the purposes of Bayesian Inference (BI) which was performed using MrBayes 3.2.7a with a GTR+I+G model of DNA substitution and a gamma distribution rate variation across sites (Ronquist et al. 2012). A total of four Markov chains were run for two runs from random starting trees for 1.3 million generations for ITS+nLSU (Fig. 2) and 16 million generations for ITS (Fig. 3) with trees and parameters sampled every 1,000 generations. The first quarter of all of the generations were discarded as burn-in. A majority rule consensus tree was computed from the remaining trees. Branches were considered as significantly supported if they received a Maximum Likelihood bootstrap support value (BS) of \geq 70%, a maximum parsimony bootstrap support value (BT) of \geq 70% or a Bayesian posterior probability (BPP) of \geq 0.95.

 Table 1. Names, specimen numbers, references and corresponding GenBank accession numbers of the taxa used in this study.

Species name	Specimen No.	GenBank ac	cession No.	References
		ITS	nLSU	_
Fasciodontia brasiliensis	MSK-F 7245a	MK575201	MK598734	Yurchenko et al. (2020)
F. bugellensis	KAS-FD 10705a	MK575203	MK598735	Yurchenko et al. (2020)
F. bugellensis	MSK-F 7353	MK575205	MK598736	Yurchenko et al. (2020)
F. yunnanensis	CLZhao 6280	MK811275	MZ146327	Luo and Zhao (2021)
F. yunnanensis	CLZhao 6385	MK811277	_	Luo and Zhao (2021)
Hymenochaete ochromarginata	He 47	KU978861	JQ279666	Unpublished
H. rubiginosa	He 458	JQ279580	_	He and Li (2013)
Lyomyces albopulverulentus	CLZhao 21478	OP730712	OP730724	Guan et al. (2023)
L. niveus	CLZhao 6431	MZ262541	MZ262526	Luo et al. (2021b)
L. niveus	CLZhao 6442	MZ262542	MZ262527	Luo et al. (2021b)
L. ochraceoalbus	CLZhao 4385	MZ262535	MZ262521	Luo et al. (2021b)
L. ochraceoalbus	CLZhao 4725	MZ262536	MZ262522	Luo et al. (2021b)
L. sambuci	KAS-JR7	KY800402	KY795966	Yurchenko et al. (2017)
L. sambuci	83SAMHYP	JX857721		Yurchenko et al. (2017)
L. yunnanensis	CLZhao 9375	OP730710		Guan et al. (2023)
L. yunnanensis	CLZhao 10041	OP730709	_	Guan et al. (2023)
X. asiaticus	CLZhao 10368	OM959479	_	Zhang et al. (2024)
X. attenuatus	Spirin 8775	MH324476		Wang et al. (2021)
X. asperus	Spirin 11923	OK273838		Viner et al. (2021)
X. apacheriensis	Canfield 180	KY081800		Wang et al. (2021)
X. acuminatus	Larsson 16029	ON197552		Viner et al. (2023)
X. acvstidiatus	LWZ 20180514-9	MT319474		Wang et al. (2021)
X. afromontanus	H 7006811	00645463		Yurchenko et al. (2024)
X. angustisporus	Rvvarden 50691b	OK273831		Viner et al. (2021)
X. astrocvstidiatus	TNM F24764	NR154054		Yurchenko and Wu (2014b)
X. australis	LWZ 20180509-8	MT319503		Wang et al. (2021)
X. bambusicola	CLZhao 11310	MW394660		Ma and Zhao (2021)
X. borealis	JS 26064	AY463429		Larsson et al. (2004)
X. brevisetus	JS 17863	AY463428		Larsson et al. (2004)
X. cremeoparinaceus	CLZhao 23388	PP537951		Present study
X. crystalliger	KUN 2312	NR166242		Viner et al. (2018)
X. cymosus	Miettinen 19606	ON197554		Viner et al. (2023)
X. cystidiatus	FR-0249200	MH880195		Wang et al. (2021)
X. damansaraensis	I W7 20180417-23	MT319499		Wang et al. (2021)
X daweishanensis	Cl 7hao 18357	OP730715		Guan et al. (2023)
X detriticus	Zíbarová 30 10 17	MH320793		Wang et al. (2021)
X dissiliens	Ryvarden 44817	OK273856		Viner et al. (2021)
X echinatus	OM 18237	00645464		Yurchenko et al. (2024)
X filicinus	MSK-F 12869	MH880100		Wang et al. (2021)
X fissuratus	Cl Zhao 9407	0P730714		Guan et al. (2023)
X flavinorus	FR-02/0707	MH880201		Wang et al. (2023)
X flocoulosus	Cl 7hao 18212	MM/020776		$\frac{1}{10000000000000000000000000000000000$
X. noccurosus	FR-02/081/	MH880204		Wang at al. (2022)
X. doeocystidiifer	BIS M-5232	00645467		Yurchenko et al. (2024)
X aossyninus	CI Zhao 8375	M7663804		Luc et al. (2021a)
X. grandineus	CLZhao 6425	OM338090		Luo et al. (20270)

Species name	Specimen No.	GenBank acc	ession No.	References
		ITS	nLSU	-
X. hastifer	K(M) 172400	NR166558	_	Riebesehl and Langer (2017)
X. heterocystidiatus	Wei 17-314	MT731753	_	Unpublished
X. hjortstamii	Gorjon 3187	ON188816	_	Unpublished
X. hyphodontinus	KAS-GEL9222	MH880205	_	Riebesehl et al. (2019)
X. jacobaeus	MA-Fungi 91340	MH430073	_	Wang et al. (2021)
X. kunmingensis	TUB-FO 42565	MH880198	_	Wang et al. (2021)
X. laceratus	CLZhao 9892	OL619258	—	Qu et al. (2022)
X. lagenicystidiatus	LWZ 20180515-14	MT319633	—	Wang et al. (2021)
X. lagenicystidiatus	LWZ 20180513-16	MT319634	_	Wang et al. (2021)
X. lanatus	CFMR FP-101864-A	OQ645474	_	Yurchenko et al. (2024)
X. laxiusculus	Ryvarden 44877	OK273827	_	Viner et al. (2021)
X. lenis	Wu 890714-3	KY081802	_	Yurchenko et al. (2024)
X. luteodontioides	CLZhao 3207	MH114740	_	Present study
X. luteodontioides	CLZhao 18494	PP505422	_	Present study
X. macrosporus	CLZhao 10226	MZ663809	—	Luo et al. (2021a)
X. magallanesii	MA: Fungi:90397	MT158729	—	Fernandez-Lopez et al. (2020)
X. mantiqueirensis	MV 529	OQ645478	_	Yurchenko et al. (2024)
X. mollissimus	LWZ 20160318-3	KY007517	_	Kan et al. (2017)
X. montanus	CLZhao 8179	OL619260	_	Qu et al. (2022)
X. neotropicus	MV 580	OQ645479	_	Yurchenko et al. (2024)
X. nesporii	LWZ 20180921-35	MT319655	_	Wang et al. (2021)
X. nesporii	LWZ 20190814-17a	ON063679	_	Wang et al. (2023)
X. niemelaei	LWZ 20150707-13	MT319630	_	Wang et al. (2021)
X. nongravis	GC 1412-22	KX857801	—	Wang et al. (2021)
X. nothofagi	ICMP 13842	AF145583	_	Wang et al. (2021)
X. ovisporus	LWZ 20170815-31	MT319666	—	Wang et al. (2021)
X. papillosus	CBS 114.71	MH860026	—	Vu et al. (2019)
X. paradoxus	Dai 14983	MT319519	—	Wang et al. (2021)
X. patagonicus	ICMP 13832	AF145581	_	Wang et al. (2021)
X. poroides	CLZhao 17845	PP505420	PP657608	Present study
X. pruinosus	Spirin 2877	MH332700	—	Wang et al. (2021)
X. pruniaceus	Ryvarden 11251	OK273828	_	Viner et al. (2021)
X. pseudolanatus	FP-150922	MH880220	—	Wang et al. (2021)
X. pseudotropicus	Dai 10768	KF917543	—	Wang et al. (2021)
X. pseudotropicus	Dai 16167	MT326536	_	Wang et al. (2021)
X. puerensis	CLZhao 8142	OP730720	—	Guan et al. (2023)
X. punctus	CLZhao 17691	OM338092	_	Luo et al. (2022)
X. punctus	CLZhao 17908	OM338093	_	Luo et al. (2022)
X. punctus	CLZhao 17916	OM338094	—	Luo et al. (2022)
X. quercinus	Spirin 12030	OK273841	_	Viner et al. (2021)
X. raduloides	FCUG 2433	AF145570	_	Wang et al. (2021)
X. ramicida	Spirin 7664	NR138013	_	Unpublished
X. reticulatus	Wu 1109-178	KX857805	_	Wang et al. (2021)
X. reticulatus	GC 1512-1	KX857808	_	Wang et al. (2021)
X. rimosissimus	Ryberg 021031	DQ873627	_	Wang et al. (2021)
X. rhizomorphus	Dai 12367	NR154067	_	Zhao et al. (2014)
X. rhododendricola	LWZ 20180513-9	MT319621	_	Wang et al. (2021)
X. serpentiformis	LWZ 20170816-15	MT319673	_	Wang et al. (2021)
X. sinensis	CLZhao 9197	MZ663810	—	Luo et al. (2021a)

Qi Yuan & Changlin Zhao: Four new species of Xylodon

Species name	Specimen No.	GenBank ac	References	
		ITS	nLSU	-
X. sinensis	CLZhao 11120	MZ663811	_	Luo et al. (2021a)
X. spathulatus	LWZ 20180804-10	MT319646	_	Wang et al. (2021)
X. subclavatus	FO 42167	MH880232	-	Wang et al. (2021)
X. subflaviporus	TNM F29958	NR184880	-	Chen et al. (2017)
X. submucronatus	Renvall 1602	OK273830	_	Viner et al. (2021)
X. subserpentiformis	LWZ 20180512-16	MT319486	_	Wang et al. (2021)
X. subtilissimus	Spirin 12228	ON188818	_	Unpublished
X. subtropicus	LWZ 20180510-24	MT319541	_	Wang et al. (2021)
X. taiwanianus	CBS 125875	MH864080	_	Vu et al. (2019)
X. tropicus	CLZhao 3351	OL619261	_	Qu et al. (2022)
X. ussuriensis	KUN 1989	NR166241	_	Unpublished
X. verecundus	KHL 12261	DQ873642	-	Wang et al. (2021)
X. victoriensis	LWZ 20180510-29	MT319487	-	Wang et al. (2021)
X. wenshanensis	CLZhao 15729	OM338097	_	Luo et al. (2022)
X. wumengshanensis	CLZhao 32517	PP645439	PP826351	Present study
X. xinpingensis	CLZhao 9174	MW394657	-	Ma and Zhao (2021)
X. yarraensis	LWZ 20180510-5	MT319639	_	Wang et al. (2021)
X. yunnanensis	LWZ 20180922-47	MT319660	_	Wang et al. (2021)



Figure 2. Maximum Parsimony strict consensus tree illustrating the phylogeny of four new species and related species in *Xylodon* within Schizoporaceae, based on ITS+nLSU sequences. Branches are labelled with Maximum Likelihood boot-strap values \geq 70%, parsimony bootstrap values \geq 50% and Bayesian posterior probabilities \geq 0.95, respectively.



Figure 3. Maximum parsimony strict consensus tree illustrating the phylogeny of the four new species and related species in *Xylodon*, based on ITS sequences. Branches are labelled with Maximum Likelihood bootstrap values \ge 70%, parsimony bootstrap values \ge 50% and Bayesian posterior probabilities \ge 0.95, respectively.

Results

Molecular phylogeny

The ITS+nLSU dataset (Fig. 2) comprised sequences from 36 fungal specimens representing 27 taxa. The dataset had an aligned length of 2130 characters, of which 1312 characters were constant, 301 were variable and parsimony-uninformative and 517 were parsimony-informative. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 2445, CI = 0.5051, HI = 0.4949, RI = 0.6113 and RC = 0.3088). The best model of nucleotide evolution for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was found to be GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis. The Bayesian analysis had an average standard deviation of split frequencies = 0.005704 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 337. The phylogram, based on the ITS+nLSU rDNA gene regions (Fig. 2), included three genera viz. *Fasciodontia, Lyomyces* and *Xylodon*, within the family Schizoporaceae (Hymenochaetales), in which four new species were grouped into the genus *Xylodon*.

The ITS+nLSU dataset (Fig. 3) comprised sequences from 98 fungal specimens representing 88 taxa. The dataset had an aligned length of 748 characters, of which 219 characters were constant, 168 were variable and parsimony-uninformative and 361 were parsimony-informative. Maximum parsimony analysis yielded 100 equally parsimonious trees (TL = 3719, CI = 0.2533, HI = 0.7467, RI = 0.4268 and RC = 0.1081). The best model of nucleotide evolution for the ITS dataset estimated and applied in the Bayesian analysis was found to be GTR+I+G. Bayesian analysis and ML analysis resulted in a similar topology as in the MP analysis. The Bayesian analysis had an average standard deviation of split frequencies = 0.015679 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 3443. The phylogenetic tree (Fig. 3), inferred from the ITS+nLSU sequences, highlighted that X. cremeoparinaceus was grouped closely with X. pruinosus (Bres.) Spirin & Viner, X. detriticus (Bourdot) K.H. Larss., Viner & Spirin and X. ussuriensis Viner. The taxon X. luteodontioides was sister to X. nesporii (Bres.) Hjortstam & Ryvarden. The species X. poroides was sister to X. pseudotropicus (C.L. Zhao, B.K. Cui & Y.C. Dai) Riebesehl, Yurch. & Langer. The species X. wumengshanensis was grouped with four taxa: X. patagonicus J. Fernández-López, Telleria, M. Dueñas & M.P. Martín, X. radula (Fr.) Tura, Zmitr., Wasser & Spirin, X. subtropicus (Che C. Chen & Sheng H. Wu) Che C. Chen & Sheng H. Wu and X. taiwanianus (Sheng H. Wu) Hjortstam & Ryvarden.

Taxonomy

Xylodon cremeoparinaceus Q. Yuan & C.L. Zhao, sp. nov. MycoBank No: 854061

Figs 4, 5

Holotype. CHINA. Yunnan Province, Zhaotong, Zhaoyang District, Fenghuang Mountain Forest Park, GPS coordinates 27°29'N, 103°68'E, altitude 2872 m, on the fallen branch of angiosperm, leg. C.L. Zhao, 24 August 2022, CLZhao 23388 (SWFC).

Etymology. *cremeoparinaceus* (Lat.): referring to the cream hymenial surface with pruinose hymenophore.



Figure 4. Basidiomata of Xylodon cremeoparinaceus (holotype). Scale bars: 1 cm (A); 2 mm (B).

Description. Basidiomata annual, resupinate, adnate, farinaceous, without odour or taste when fresh, up to 2.5 cm long, 1.5 cm wide, 50–80 um thick. Hymenial surface reticulate, white to cream when fresh, turning to cream upon drying. Sterile margin white, up to 1 mm wide.





Hyphal system monomitic, generative hyphae with clamp connections, colourless, thin-walled, frequently branched, interwoven, 1.5–2 μ m in diameter; IKI–, CB–, tissues unchanged in KOH. Numerous crystals present amongst generative hyphae.

Cystidia capitate, colourless, thin-walled, smooth, slightly constricted at the neck, with a globose tip, $18.5-25 \times 3.5-6.5 \mu m$; basidia subclavate, with 4 sterigmata and a basal clamp connection, $13.5-17.5 \times 3-3.5 \mu m$.

Basidiospores ellipsoid, colourless, thin-walled, smooth, with one drop, cyanophilous, IKI–, $3.5-4.5 \times 2.5-3.5 \mu$ m, L = 3.71μ m, W = 2.82μ m, Q = 1.31 (n = 30/1).

Xylodon luteodontioides **Q. Yuan & C.L. Zhao, sp. nov.** MycoBank No: 854060 Figs 6, 7

Holotype. CHINA. Yunnan Province, Puer, Laiyanghe National Forestry Park, GPS coordinates 22°60'N, 101°00'E, altitude 1500 m, on the fallen branch of angiosperm, leg. C.L. Zhao, 30 September 2017, CLZhao 3207 (SWFC).



Figure 6. Basidiomata of Xylodon luteodontioides (holotype). Scale bars: 1 cm (A); 2 mm (B).

Etymology. *luteodontioides* (Lat.): referring to the flavescent hymenophore surface with odontioid hymenophore.

Description. Basidiomata annual, resupinate, adnate, coriaceous, without odour and taste when fresh and up to 7 cm long, 4 cm wide, 100 μ m thick. Hymenial surface odontioid, buff when fresh, to buff to olivaceous-buff upon drying. Sterile margin slightly buff and up to 1 mm wide.



Figure 7. Microscopic structures of *Xylodon luteodontioides* (holotype): basidiospores (**A**), basidia (**B**), basidioles (**C**), schizopapillate cystidia (**D**), capitate cystidia (**E**), a section of hymenium (**F**). Scale bars: 20 μ m (**A**–**F**).

Hyphal system monomitic, generative hyphae with clamp connections, colourless, thin-walled, branched, 2.5–3.5 μ m in diameter; IKI–, CB–, tissues unchanged in KOH. Cystidia of two types: (1) schizopapillate cystidia, colourless, thin-walled, smooth, $29.5-37 \times 2.5-3.5 \mu m$; (2) capitate cystidia, colourless, thin-walled, smooth, $38.5-44.5 \times 3.5-4 \mu m$; basidia subclavate, with 4 sterigmata and a basal clamp connection, $19.5-26 \times 3.5-4 \mu m$.

Basidiospores ellipsoid, colourless, thin-walled, smooth, CB–, IKI–, $3.5-4.5 \times 2.5-3.5 \mu$ m, L = 4.07 μ m, W = 2.92 μ m, Q = 1.39–1.45 (n = 60/2).

Additional specimens examined (*paratypes*). CHINA. Yunnan Province, Honghe, Pingbian Country, Daweishan National Nature Reserve, GPS coordinates 22°93'N, 103°69'E, altitude 1800 m, on the fallen branch of angiosperm, leg. C.L. Zhao, 3 August 2019, CLZhao 18494 (SWFC).

Xylodon poroides Q. Yuan & C.L. Zhao, sp. nov.

MycoBank No: 854059 Figs 8, 9

Holotype. CHINA. Yunnan Province, Honghe, Pingbian Country, Daweishan National Nature Reserve, GPS coordinates 22°93'N, 103°69'E, altitude 1800 m, on the fallen branch of angiosperm, leg. C.L. Zhao, 1 August 2019, CLZhao 17845 (SWFC).

Etymology. poroides (Lat.): referring to the poroid hymenophore surface.

Description. Basidiomata annual, resupinate, adnate, coriaceous, without odour and taste when fresh and up to 14 cm long, 4 cm wide, 200 µm thick. Hymenial surface poroid, pores angular, 4–5 per mm, cream to pink-buff when fresh, turn to flesh-pink to pink-buff upon drying. Sterile margin slightly buff and up to 1 mm wide.

Hyphal system monomitic, generative hyphae with clamp connections, colourless, thin-walled, frequently branched, $2-3 \mu m$ in diameter; IKI–, CB–, tissues unchanged in KOH.

Cystidia of two types: (1) fusoid cystidia, colourless, thin-walled, smooth, $17.5-24.5 \times 2.5-3 \mu m$, encrusted crystals; (2) capitate cystidia, colourless, thin-walled, smooth, $11.5-15.5 \times 3.5-4.5 \mu m$; basidia clavate, with 4 sterigmata and a basal clamp connection, $15.5-19 \times 3.5-5.5 \mu m$.

Basidiospores ellipsoid, colourless, thin-walled, smooth, CB-, IKI-, (3.5-)4-5.5 × 2.5-3.5(-5) µm, L = 4.82 µm, W = 2.95 µm, Q = 1.63 (n = 30/1).

Xylodon wumengshanensis Q. Yuan & C.L. Zhao, sp. nov.

MycoBank No: 854058 Figs 10, 11

Holotype. CHINA. Yunnan Province, Zhaotong, Wumengshan National Nature Reserve, GPS coordinates 27°77'N, 104°29'E, altitude 2900 m, on the fallen branch of angiosperm, leg. C.L. Zhao, 29 August 2023, CLZhao 32517 (SWFC).

Etymology. *wumengshanensis* (Lat.): referring to the locality (Wumengshan) of the type specimen.

Description. Basidiomata annual, resupinate, adnate, coriaceous, without odour and taste when fresh and up to 4.5 cm long, 1.5 cm wide, $100-200 \mu m$ thick. Hymenial surface grandinoid, cream when fresh; turn to cream to buff upon drying. Sterile margin distinct, cream, up to 1 mm wide.



Figure 8. Basidiomata of Xylodon poroides (holotype). Scale bars: 1 cm (A); 2 mm (B).

Hyphal system monomitic, generative hyphae with clamp connections, colourless, thick-walled, rarely branched, $2-3.5\,\mu m$ in diameter; IKI–, CB–, tissues unchanged in KOH.

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Figure 9. Microscopic structures of *Xylodon poroides* (holotype): basidiospores (**A**), basidia (**B**), basidioles (**C**), fusoid cystidia (**D**), capitate cystidia (**E**), a section of hymenium (**F**). Scale bars: $10 \ \mu m$ (**A**); $20 \ \mu m$ (**B**-**F**).

Cystidia of two types: (1) capitate, colourless, thin-walled, smooth, slightly constricted at the neck, with a globose head, $24.5-29.5 \times 5-6 \mu m$; (2) fusoid, colourless, thin-walled, smooth, $14.5-22 \times 5.5-6.5 \mu m$; basidia clavate, with 4 sterigmata and a basal clamp connection, $22.5-33 \times 5-5.5 \mu m$.

Basidiospores ellipsoid, colourless, thin-walled, smooth, with one oil drop, CB-, IKI-, (4.5-) 5-6.5(-7) × 4-5.5 μ m, L = 5.55 μ m, W = 4.39 μ m, Q = 1.26 (n = 30/1).



Figure 10. Basidiomata of *Xylodon wumengshanensis* (holotype). Scale bars: 1 cm (A); 2 mm (B).





Discussion

Many recently-described wood-inhabiting fungal taxa have been reported in the subtropics and tropics, including in the genus *Xylodon* (Xiong et al. 2009; Chen et al. 2017; Kan et al. 2017a, b; Riebesehl and Langer 2017; Viner et al. 2018; Chen and Zhao 2020; Luo et al. 2021a, b, c; Luo et al. 2022; Qu and Zhao 2022; Qu et al. 2022; Viner and Miettinen 2022; Guan et al. 2023; Deng et al. 2024a, b; Zhang et al. 2024). Prior to this study, the following forty-five *Xylodon* species were report-

ed from China. The present study reports four new species in *Xylodon*, based on a combination of morphological features and molecular evidence.

Phylogenetically, based on the multiple loci in Schizoporaceae, three genera Fasciodontia, Lyomyces and Xylodon were located in this family (Wang et al. 2021). In the present study, the phylogram inferred from the ITS+nLSU data, four new species grouped into the genus Xylodon (Fig. 2). Based on ITS topology (Fig. 3), Xylodon cremeoparinaceus grouped closely with three species viz. X. detriticus, X. pruinosus and X. ussuriensis. The taxon X. luteodontioides was sister to X. nesporii. The species X. poroides was sister to X. pseudotropicus. The taxon X. wumengshanensis grouped with four taxa viz. X. patagonicus, X. radula, X. subtropicus and X. taiwanianus. However, morphologically, X. detriticus can be delimited from X. cremeoparinaceus by its smooth or warted, farinaceous hymenial surface and its wider basidia (13.1-20.0 × 3.4-5.0 µm; Viner et al. (2018)); X. pruinosus can be delimited from X. cremeoparinaceus by its grandinioid to odontoid hymenial surface and its larger basidiospores (4.5-5.9 × 3.7-4.8 µm; Viner et al. (2018)); X. ussuriensis can be delimited from X. cremeoparinaceus by its grandinioid to odontoid hymenial surface and its larger basidiospores (5.1–6.0 × 3.8–4.6 µm; Viner et al. (2018)). Xylodon nesporii can be delimited from X. Iuteodontioides by its subcylindrical basidia (15–25 × $4-5 \,\mu\text{m}$) and its longer and narrower basidiospores ($4.5-6 \times 2-2.5 \,\mu\text{m}$; Hjortstam and Ryvarden (2009)); X. pseudotropicus can be delimited from X. poroides by its shorter basidia $(9-12.5 \times 3-5 \mu m)$ and its oblong-ellipsoid basidiospores (4.3-4.9 × 2.8-3 µm; Zhao et al. (2014)); X. patagonicus can be delimited from X. wumengshanensis by its poroid to labyrinthiform hymenial surface and its narrower basidiospores $(4-5.5 \times 2.5-3.5 \mu m;$ Fernández-López et al. (2019)); X. radula can be delimited from X. wumengshanensis by its subclavate to subcylindrical basidia ($20-25 \times 4-6 \mu m$) and its longer and narrower basidiospores $(9-11 \times 3-3.5 \mu m)$; Eriksson and Ryvarden (1975)); X. subtropicus can be delimited from X. wumengshanensis by its poroid hymenial surface and its smaller basidiospores (13-18 × 4-4.5 µm; Chen et al. (2018)); X. taiwanianus can be delimited from X. wumengshanensis by its poroid hymenial surface and its smaller basidia $14-20 \times 4-5 \mu m$; Wu (2001)).

Morphologically, *Xylodon cremeoparinaceus* resembles *X. fissuratus*, *X. flocculosus*, *X. grandineus*, *X. laceratus*, and *X. wenshanensis* K.Y. Luo & C.L. Zhao by ellipsoid basidiospores. However, *X. fissuratus* differs from *X. cremeoparinaceus* due to its grandinioid hymenial surface and its shorter capitate cystidia $(11.5-16.5 \times 3-4.5 \mu m;$ Guan et al. (2023)); *X. flocculosus* differs from *X. cremeoparinaceus* due to its grandinioid hymenial surface and its barrel-shaped basidia $(11-20 \times 3.3-4.8 \mu m;$ Qu and Zhao (2022)); *X. grandineus* differs from *X. cremeoparinaceus* due to its grandinioid hymenial surface and by possessing subulate cystidia $(11-19 \times 3-5 \mu m;$ Luo et al. (2022)); *X. laceratus* differs from *X. cremeoparinaceus* due to its grandinioid hymenial surface and by possessing fusiform cystidia $(20.3-26.8 \times 5.3-6.4 \mu m;$ Qu et al. (2022)); *X. wenshanensis* differs from *X. cremeoparinaceus* due to its grandinioid hymenial surface and by possessing fusiform cystidia $(6-11 \times 3-6.5 \mu m;$ Luo et al. (2022)).

Morphologically, X. luteodontioides resembles X. fissuratus, X. laurentianus J. Fernández-López, Telleria, M. Dueñas & M.P. Martín, X. puerensis C.L. Zhao, X. subflaviporus Che C. Chen & Sheng H. Wu and X. wenshanensis due to the capitate cystidia. However, X. fissuratus differs from X. luteodontioides due to
its shorter capitate cystidia (11.5–16.5 × 3–4.5 µm) and its shorter basidia (10.5–16.5 × 2–4 µm; Guan et al. (2023)); *X. laurentianus* differs from *X. lute-odontioides* due to its poroid to labyrinthiform hymenial surface and its wider basidia (18–26 × 4.5–5.5 µm) and its longer basidiospores (5–6 × 2.5–3.5 µm; Fernández-López et al. (2019)); *X. puerensis* differs from *X. luteodontioides* due to its poroid hymenial surface and its larger basidiospores (6–7 × 4.5–5.5 µm; Guan et al. (2023)); *X. subflaviporus* differs from *X. luteodontioides* due to its poroid hymenial surface and its shorter, wider basidia (8–18 × 4–5 µm; Chen et al. (2018)).

Morphologically, X. poroides resembles X. daweishanensis, X. fissuratus, X. laceratus and X. wenshanensis by the capitate cystidia. However, X. daweishanensis differs from X. poroides due to its odontioid hymenial surface and its shorter basidia (11–15.5 × 2.5–4 μ m; Guan et al. (2023)); X. fissuratus differs from X. poroides due to its grandinioid hymenial surface; X. laceratus differs from X. poroides due to its grandinioid hymenial surface and its longer capitate cystidia (15.4–24.7 × 3.8–4.7 μ m; Qu et al. (2022)); and X. wenshanensis differs from X. poroides due to its grandinioid hymenial surface and its shorter basidia (15.4–24.7 × 3.8–4.7 μ m; Qu et al. (2022)); and X. wenshanensis differs from X. poroides due to its grandinioid hymenial surface and its shorter basidia (8–15.5 × 3–5 μ m; Luo et al. (2022)).

Morphologically, X. wumengshanensis is similar to X. asiaticus, X. laceratus, X. puerensis, X. punctus and X. wenshanensis by having the ellipsoid basidiospores. However, X. asiaticus differs from X. wumengshanensis due to its hydnoid hymenial surface and its narrower basidiospores (4–5.2 × 2.8–3.5 µm; Zhang et al. (2024)); X. laceratus differs from X. wumengshanensis due to its shorter basidia (11–17.5 × 3.2–5.5 µm) and its narrower basidiospores (3.9– $5.3 \times 2.6-4.1 \mu$ m; Qu et al. (2022)); X. puerensis differs from X. wumengshanensis due to its poroid hymenial surface and its shorter basidia (14.5–20 × $5-7 \mu$ m; Guan et al. (2023)); X. punctus differs from X. wumengshanensis due to its smooth hymenial surface and its smaller basidiospores (2–4 × 1.5–2.5 µm; Luo et al. (2022)); and X. wenshanensis differs from X. wumengshanensis due to its smaller basidia (8–15.5 × 3–5 µm) and its smaller basidiospores (3–5 × 2–3.5 µm; Luo et al. (2022)).

Wood-inhabiting fungi, a unique group of Basidiomycota, have been identified through morphological, phylogenetic and cytological studies in China (Wu et al. 2020). Currently, forty-five species of *Xylodon* have been documented in China (Riebesehl and Langer 2017; Viner et al. 2018; Riebesehl et al. 2019; Shi et al. 2019; Luo et al. 2021a; Ji et al. 2022; Guan et al. 2023; Liu et al. 2023; Zhang et al. 2024; Zhao et al. 2024). However, the species diversity of *Xylodon* in China, particularly in the subtropical and tropical regions, remains largely unexplored. This paper contributes to our understanding of fungal diversity in these areas and underscores the urgent need for further fieldwork and molecular analyses to discover new taxa.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization, C.Z.; methodology, C.Z. and Q.Y.; software, C.Z.; validation, C.Z. and Q.Y.; formal analysis, C.Z. and Q.Y.; investigation, C.Z. and Q.Y.; resources, C.Z.; writing—original draft preparation, C.Z. and Q.Y.; writing—review and editing, C.Z. and Q.Y.; visualization, C.Z. and Q.Y.; supervision, C.Z.; project administration, C.Z.; funding acquisition, C.Z. All authors have read and agreed to the published version of the manuscript.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

New species and new records of *Laccaria* (Agaricales, Basidiomycota) from Northern Thailand

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Abstract

Two new species *Laccaria pseudoalba* and *L. subroseoalbescens* are described and illustrated, based on morphological characteristics and molecular phylogenetic analysis. Two new records, *Laccaria umbilicata* and *L. yunnanensis* from Thailand, are also reported. *Laccaria subroseoalbescens* is characterized by small basidiomata, stipe equal with an enlarged base, and nearly subclavate, pale pink to light orange. *Laccaria pseudoalba* is characterized by pale orange to orange white pileus, has umbo when young on the pileus, and fistulose stipe of the pale to pastel red color. Phylogenetic analysis based on sequence data from rDNA internal transcribed spacer ITS1-5.8S-ITS2 rDNA (ITS), nuc 28S rDNA (28S), RNA polymerase II subunit 2 (*rpb2*), and translation elongation factor 1- α (*tef*1- α) are provided as further evidence. Molecular analysis confirms the phylogenetic positions of the two new species and two new records. The differences in characteristics of these two new species and closely related species are discussed herein.

Key words: 2 new taxa, Hydnangiaceae, phylogeny, taxonomy

Introduction

The genus *Laccaria* Berk. & Broome, 1883 is a group of ecologically important ectomycorrhizal fungi that inhabit the soil (He et al. 2019). *Laccaria*, along with *Hydnangium* Wallr., *Maccagnia* Mattir. and *Podohydnangium* G.W. Beaton, Pegler & T.W.K. Young, belongs to the family Hydnangiaceae within the order Agaricales, phylum Basidiomycota.

Species of *Laccaria* are characterized by collybioid to omphaloid basidiomata; echinulate, acyanophilous, and inamyloid basidiospores; and a convex, plane, or umbilicate, hygrophanous pileus. Clamps are present in all parts of the basidiomata (Singer 1986; Mueller 1992; Latha and Raj 2019). Approximately 100 species of *Laccaria* have been described worldwide (according to Index Fungorum 2024), known to form symbiotic associations with plants of more than 20 genera (including *Abies, Castanea, Fagus, Pinus, Picea, Quercus, Larix, Lithocarpus*, and others) (Wilson et al. 2017). These associations benefit plant growth and facilitate effective nutrient acquisition (Smith and Read 2008). Therefore, studying *Laccaria* diversity is crucial for understanding terrestrial ecosystems and forest management.

Laccaria species are globally distributed and have been reported on every continent except Antarctica (Kropp and Mueller 1999). They have been found in association with both angiosperms and gymnosperms worldwide (Wilson et al. 2017) and form ectomycorrhizas (ECM) with many economically important plant species (Kropp and Mueller 1999). However, due to the morphological similarity among *Laccaria* species, defining species boundaries within the genus is challenging (Sheedy et al. 2013).

Since the establishment of *Laccaria* by Berk. and Broome (1883), many mycologists have contributed to its taxonomy (Orton 1960; McNabb 1972; Mueller 1984; Wang et al. 2004; Wilson et al. 2013, 2017; Popa et al. 2014; Popa et al. 2016; Luo et al. 2016; Cho et al. 2018; Li 2020). Historically, *Laccaria* was divided into *Russuliopsis* by J. Schröt, who only included species with a white spore print in *Laccaria* (Mueller and Vellinga 1986). To date, seven sectional names have been introduced within *Laccaria* (http://www.indexfungorum.org/Names/Names.asp; accessed date: 20 June 2024), leading to much controversy in its taxonomy.

The number of *Laccaria* species described from Asia has been increasing, with more studies focusing on Basidiomycetes. Since 2013, twenty-three new species of *Laccaria* have been described in Asia (Wilson et al. 2013; Popa et al. 2014; Luo et al. 2016; Popa et al. 2016; Cho et al. 2018; Li 2020; Cui et al. 2021; Zhang et al. 2023). Nevertheless, no *Laccaria* species were reported or described in Thailand during the same period.

Thailand is renowned as one of the world's most important biodiversity hotspots with high fungal diversity (Hyde et al. 2018; Thongbai et al. 2018). During our recent investigation of *Laccaria* in Thailand, nine *Laccaria* specimens were collected. Based on morphological characteristics and phylogenetic analysis, two undescribed species and two new records have been identified. This paper provides detailed descriptions, illustrations, and phylogenetic analyses for these species.

Materials and methods

Morphological study

Specimens were collected from Chiang Mai Province, Thailand. They were photographed in the field, then separately wrapped in aluminium foil or kept in a plastic collection box. The fresh basidiomata were macro-morphologically described on the same day of collection. Colour codes were determined following Kornerup and Wanscher (1978). After being thoroughly dried at 50 °C (Hu et al. 2022) in a food drier, the specimens were stored in sealed plastic bags and deposited in Mae Fah Luang University Herbarium (**MFLU**) and Herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica (KUN-HKAS). Dried materials were sectioned under a stereo microscope, transferred onto slides, and mounted in a 5% KOH solution. For microscopic characteristics, anatomical and cytological characteristics including basidia, basidiospores, and cystidia, were observed and photographed using a Nikon Eclipse 80i microscope at magnifications up to × 1000. For SEM studies, fragments of the lamellae of the dried material were taken, sputter coated with gold, and analysis with a Hitachi S520 (Hitachi Japan). The notation [x/y/z] specifies that measurements were made on x basidiospores measured from y basidiomata of z collections. At least 50 basidiospores and 20 basidia were measured from one basidioma. Basidiospores dimensions are given as (a–) b–c (–d). Where "a" and "d" refer to the minimum and maximum values of all measurements, respectively, b–c presents the range of 95% of the measured values, and *Q* is the length/width ratio of basidiospores, Q_m is the average *Q* of all basidiospores and is given as $Q_m \pm$ standard deviation.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dried specimens using Ezup Column Fungi Genomic DNA extraction kit (Sangon China) following the manufacturer's protocol. Primer pairs for PCR were respectively ITS1/ITS4 (White et al. 1990), LR5/LR0R (Vilgalys and Hester 1990), *rpb2*-5F/*rpb2*-7cR (Liu et al. 1999), and *tef1*-983F/*tef1*-2218R (Rehner and Buckley 2005). ITS, LSU, *rpb2*, and *tef1* were amplified in 25 μ L reactions containing 12.5 μ L 2× Taq Plus Master Mix II (Vazyme Biotech Co., Ltd China), 9.5 μ L ddH₂O, 1 μ L 10 μ M of forward and reverse primers, 1 μ L DNA. PCR conditions were carried out as follows in Table 1 using a C1000 thermal cycler (Bio-Rad China). The PCR amplicons were sent to Sangon Biotech (China) for Sanger sequencing. Sequence reads were assembled in SeqMan II (DNA STAR Inc.).

Sequence alignment and phylogenetic analysis

The newly generated sequences were checked using BioEdit Sequence Alignment Editor version 7.0.4 and assembled using SeqMan (DNAstar, Madison, WI, USA). The sequences were then blasted using the Basic Local Alignment Search Tool (BLAST) against the GenBank database (Nilsson et al. 2006) to check the most closely related sequences. Reference sequences for a total of 103 specimens representing 55 species were retrieved (Table 2) and minimally adjusted by hand in BioEdit v.7.0.4 (Hall 2007) first, and then aligned using TrimAl (Salvador et al. 2009).

Maximum likelihood (ML) analysis was performed separately for each locus and the concatenated dataset using RAxML-HPC2 v. 8.2.12 (Stamatakis 2014) as implemented on the CIPRES portal (Miller et al. 2010), with the GTR+G model for both genes and 1,000 rapid bootstrap (BS) replicates. For Bayesian Inference (BI), the best substitution model for each character set was determined

Table 1. PCR primers and cycling conditions used in the study.

Locus	Primers (Reference)	PCR conditions ^a
ITS	ITS1, ITS4 (White et al. 1990)	94 °C: 30 s, 48 °C: 30 s, 72 °C: 1.00 min. (35 cycles)
LSU	LR0R, LR5 (Vilgalys and Hester 1990)	94 °C: 30 s, 48 °C: 30 s, 72 °C: 1.30 min. (35 cycles)
rpb2	f RPB2-5F, b RPB2-7cR (Liu et al. 1999)	95 °C: 30 s, 55 °C: 1 min, 72 °C: 1.30 min. (35 cycles)
tef1	983F, 2218R (Rehner and Buckley 2005)	95 °C: 30 s, 55 °C: 1 min, 72 °C: 1.30 min. (35 cycles)

° The three steps given for each primer pair were repeated for 35 cycles, preceded by an initial denaturation step of 5 min at 94 °C, and followed by a final elongation step of 10 min at 72 °C and a final hold at 4 °C.

Table 2. *Laccaria* taxa and sample IDs with geographic location and GenBank ID numbers for ITS, LSU, *rpb2*, and *tef1* sequences used in phylogenetic analysis. Sequences produced in this study are marked in bold. "*" following a species name indicates that the specimen is the holotype of that species.

Species name	Sample no.	Location	GenBank accession				
Species name			ITS	LSU	rpb2	tef1	
Laccaria acanthospora	HKAS45998	China	KU685719	KU685870	KU686069	-	
L. alba	F1121461	China	JX504129	-	_	-	
	ASIS18039	South Korea	MG519546	-	MG551620	MG551652	
	TPML20120807-69	South Korea	MG519542	MG519583	MG551616	MG551649	
L. ambigua	PDD89696*	New Zealand	KU685725	KU685876	KU686018	KU686132	
L. amethysteo-occidentalis	AWW556	America	JX504107	JX504191	KU685919	-	
-	KGP40*	America	DQ822817	-	_	-	
-	DAVFP 28205	Canada	HQ650762	-	_	-	
L. amethystina	GMM7041	Russia	KU685654	KU685797	KU685940	-	
	GMM7621	France	JX504150	JX504224	KU686046	KU686152	
L. araneosa	SFC2013091721*	South Korea	MG519549	MG519589	MG551622	MG551655	
	TPML20120912-40	South Korea	MG519548	MG519588	MG551621	MG551654	
L. aurantia	KUNF78557*	China	NR154113	-	_	-	
	MBFB001109	Japan	JQ681209	-	_	-	
L. bicolor	GMM7620	France	JX504149	JX504223	_	-	
	HKAS44062	China	JX504159	JX504235	KU686068	-	
	KA130253	South Korea	MG519524	MG519570	MG551599	MG551636	
	GM7712	USA	KM067866	-	KU686012	-	
L. bullipellis	AWW465*	China	JX504100	JX504184	KU685914	-	
L. canaliculata	GMM7267	Australia	JX504137	JX504213	KU685960	KU686093	
L. fagacicola	HKAS90435*	China	MW540806	-	_	-	
	HKAS107731	China	MW540807	-	_	-	
L. fengkaiensis	HKAS106739*	China	MN585657	MN621238	_	-	
	HKAS106741	China	MN585658	-	_	-	
L. fulvogrisea	KUN-F78556*	China	NR154114	-	_	-	
	KUN-FB-101105	China	JQ681210	-	_	-	
L. guizhouensis	HMAS352265*	China	OP244890	_	_	-	
	HMAS352266	China	OP244891	-	_	-	
L. glabripes	GMM7521	New Zealand	KU685708	KU685849	KU685991	KU686117	
L. himalayensis	AWW463	China	JX504098	JX504182	KU685913	-	
	AWW484*	China	JX504101	JX504185	KU685915	-	
L. japonica	F64167*	Japan	KU962988	-	_	-	
	SFC2012072212	South Korea	MG519518	MG519566	MG551595	MG551633	
L. longipes	F1092175	America	KU685637	KU685780	_	-	
L. laccata	GMM7615	France	JX504148	-	_	-	
L. macrocystidia	GMM7616	France	KM067850	KU685863	KU686004	-	
	GMM7612	France	KM067847	KU685861	KU686002	-	
L. miniata	GDGM76043*	China	OR689440	OR785476			
L. montana	TW0591	America	DQ149865	-	_	-	
	TW0319	America	DQ149862	-	_	-	
L. moshuijun	HKAS 93732*	China	KU962989	-	_	-	
	HKAS 123302	China	ON557378	ON556493	-	ON598893	
	HKAS 110653	China	ON557379	ON556494	-	-	
L. murina	ASIS216	South Korea	MG519553	-	-	-	
	ASIS24249	South Korea	MG519552	MG519592	MG551625	MG551658	
L. nanlingensis	GDGM 84954*	China	OR689442	OR785478	OR835199	OR826273	
	GDGM 84949	China	OR689441	OR785477	OR835198	OR826274	
L. negrimarginata	GMM7631	France	JX504152	JX504226	-	-	
	BAP360*	China	JX504120	-	-	-	

0	Sample no.	Location	GenBank accession			
Species name			ITS	LSU	rpb2	tef1
L. neovinaceoavellanea	GDGM52852*	China	OR689447	OR785479	_	-
	GDGM53063	China	OR689448	OR785480	_	-
	GDGM89621	China	OR689449	OR785481	_	-
L. nobilis	F1091206	America	KU685636	KU685779	_	-
L. oblongospora	ObiFr	France	GQ406466	-	_	-
L. ochropurpurea	PRL4777	America	KU685733	KU685883	KU686025	-
L. ohiensis	GMM7539	New Zealand	KU685712	KU685853	KU685994	KU686119
L. prava	A3394	Japan	JN942788	JN939770	JN993522	_
	ASIS19814	South Korea	MG519531	MG519575	MG551606	MG551642
	SFC2012091940*	South Korea	MG519525	_	MG551600	-
L. prava	HKAS106742*	China	MN585660	_	_	_
	HKAS106745	China	MN585661	-	-	-
L. proxima	F1081079	Argentina	KU685633	KU685777	KU685928	_
	GMM7584	Russia	KU685717	KU685858	KU685999	KU686120
L. pseudoalba	MFLU 22-0106*	Thailand	ON557377	ON556492	ON598886	-
	HKAS 110664	Thailand	ON557376	ON556491	ON598887	ON598894
L. pseudomontana	pse1625*	America	DQ149871	-	_	-
L. pumila	pum1252	America	DQ149864	-	_	-
L. roseoalbescens	LM5099*	Mexico	KJ874328	KJ874331	-	-
L. rubroalba	MS15	China	KX449358	_	_	_
	MS20	China	KX449357	-	-	-
L. rufobrunnea	GDGM82878*	China	OR689443	OR785482	OR835197	OR826272
	GDGM89627	China	OR689444	OR785483		
L. salmonicolor	GMM7596*	China	JX504143	JX504218	KU686045	KU686151
	GMM7602	China	JX504145	-	-	-
L. squarrosa	DM63*	Mexico	MF669958	MF669965	_	_
	SYC109	Panama	KP877340	_	_	-
L. subroseoalbescens	MFLU23-0339*	Thailand	PP785397	PP789598	_	_
	MFLU23-0340	Thailand	PP785398	PP789599	_	-
L. tetraspora	F1080957	Germany	KU685631	KU685775	_	-
L. torosa	SFC2015090217*	South Korea	MG519561	MG519598	MG551631	MG551664
	KA12-1306	South Korea	MG519562	-	_	_
L. tortilis	ASIS22273*	South Korea	MG519533	MG519576	MG551608	MG551644
	GMM7635	France	JX504155	KU685906	KU686053	KU686156
L. trichodermophora	F1111951	Costa Rica	KU685640	KU685784	KU686063	-
	GMM7733	America	JX504157	-	KU686013	-
L. trullisata	PRL7587	China	JX504170	JX504247	KU686047	KU686153
L. umbilicata	GDGM82883	China	OR689445	OR785485	OR835194	OR826270
	GDGM82911*	China	OR689446	OR785486	OR835192	OR826268
	MFLU 22-0105	Thailand	ON557372	ON556490	ON598888	ON598896
	HKAS 110652	Thailand	ON557371	ON556489	_	ON598895
L. versiforma	KNU2012100803	South Korea	MG519560	MG519597	MG551630	MG551663
	SFC20120926-01*	South Korea	MG519556	MG519594	MG551627	MG551660
L. vinaceoavellanea	A2986	Japan	JN942810	JN939738	JN993520	-
	A0559	Japan	JN942803	JN939756	JN993512	-
	SFC20150810-10	South Korea	MG519539	MG519580	MG551614	MG551646
L. violaceonigra	GMM7520	New Zealand	KU685707	KU685848	KU685990	-
L. violaceotincta	CAL1389*	India	MK141034	_	_	_
L. yunnanensis	KUNF78558*	China	NR154115	-	-	-
	MFLU 22-0107	Thailand	ON557374	ON556488	-	ON598892
	HKAS 110636	Thailand	ON557373	ON556487	ON598889	ON598891
	HKAS 110638	Thailand	ON557375	ON556486	ON598890	-
Mythicomyces corneines	ES11 10 2 A	Germany	KC964108	_	_	_
,	AFTOLID972	Germany	D0404393	ΔΥ745707	D0408110	D0029197
		ocimaliy	DQ-04090	AT740707	24-00110	DQ029197

with MrModeltest 2.2 (Nylander 2004) on CIPRES, using the Akaike information criterion. Bayesian analysis was performed using MrBayes ver. 3.2.7a (Ronquist et al. 2011) as implemented on CIPRES (Miller et al. 2010).

Results

Phylogenetic analyses

Thirty-three new sequences (11 of ITS, 11 of LSU, 5 of rpb2, and 6 of tef1) were generated for Laccaria species and deposited in GenBank (Table 2). The ITS dataset included 103 specimens representing 55 species, while the ITS-LSUrpb2-tef1 dataset included 71 specimens representing 42 species. Two phylogenetic analyses were conducted: one for the 5.8S, ITS1+ITS2 dataset, and the other with concatenated matrix of 5.8S+LSU, ITS1+ITS2, rpb2 codon, rpb2 introns+tef1 introns and tef1 codons (Vaidya et al. 2011). The ITS final aligned matrix contained 687 positions (170 for 5.8S, 517 for ITS1+ITS2), while the concatenated matrix contained 3,509 positions (1,054 for 5.8S+LSU, 430 for ITS1+ITS2, 1,026 for rpb2 exons, 163 for tef1 introns+rpb2 introns, 836 for tef1 exons). Based on previous phylogenies (Wilson et al. 2013, 2017; Popa et al. 2014, 2016; Luo et al. 2016; Cho et al. 2018; Li 2020; Cui et al. 2021; Zhang et al. 2023), species of the Mythicomyces corneipes (Fr.) Redhead & A.H. Sm. were selected as the outgroup. In the 5.8S-ITS1-ITS2 dataset, the following models were selected by mrModelTest: SYM for 5.8S and GTR+I+G for ITS1+ITS2. In the ITS, LSU, rpb2, and tef1 datasets, the models selected by mrModelTest were: GTR+I+G for 5.8S+LSU and tef1 codon, GTR+G for ITS1+ITS2 and rpb2 codon, GTR+G for *rpb*2 introns+*tef*1 introns.

In MrBayes analysis, two runs of five chains each were run for 2,000,000 generations and sampled every 200 generations. Convergence was further evaluated by checking that the potential scale reduction factor (PSRF) statistic was close to 1 for all parameters. Moreover, the effective sample size (ESS) was much higher than 200 for all parameters. A clade was considered to be supported if showing a bootstrap support value (BS) \geq 75% and/or a posterior probability (PP) \geq 0.90. Trees were edited in FigTree version 1.4.0 and PowerPoint.

Fig. 2 presents the phylogeny from the combined datasets. Nine specimens collected in northern Thailand formed three monophyletic clades, here described as *L. pseudoalba*, *L. subroseoalbescens*, *L. umbilicata*, and *L. yunnanensis*, respectively. Each clade was well supported by both ML and BI in the concatenated trees (Fig. 2). In our phylogenetic analysis, the four species clustered as separate clades with high support. Thus, these species are formally described in this paper.

Taxonomy

Laccaria pseudoalba S.M Tang & S.H. Li, sp. nov. MycoBank No: 844144 Figs 3, 4, 5, 12

Etymology. The epithet "pseudoalba" refers to its similarity to *L. alba* in their small basidiomata and orange-white to pale orange pileus.

Holotype. THAILAND. Chiang Mai Province: Mae On district, Huay Keaw subdistrict, Pox village, 18°43'55.6"N, 99°17'50.1"E, elevation 789 m., 6 September 2020, S. M. Tang, 2020090608 (MFLU 22-0106).

Description. Basidiomata small. Pileus 9–15 mm in diam., convex to applanate, hemispherical, applanate to plano-concave, pale orange (5A2–3, 6A2–3), orange-white (5A2–3, 6A2–3), when dry moisture loss of moisture or with age becoming whitish, clearly striate on the surface; umbo when young, becoming papilla to abrupt papilla with age; margin inflexed, sometime reflexed; context thin, 1–2 mm, pale orange (5A2–3), unchanging. Lamellae distant, arcuate, adnate with decurrent tooth, orange white (5A2–3, 6A2–3) when young, become pale orange with age, 3–4 mm in height; lamella edge even or entire, sometime undate; lamellulae in 3–4 tiers. Stipe $28.0-41.1 \times 1.8-2.7$ mm, cylindrical, central, equal with an enlarged base and nearly subclavate, pale (7–8A6) to pastel red (7A4–5, 8A4–5), smooth, basal mycelium white (1A1); stipe context stuffed, pastel red. Odor and taste not observed.

Basidia $29-38 \times 9-13 \mu m$, (mean length = 32 ± 2.5 , mean width = 11 ± 12 1.2), clavate, mostly 4-spored, rarely 2-spored, sterigmata 5-8 μm × 2-3 μm, (mean length = 6.0 ± 1.22 , mean width = 2.4 ± 0.45). Basidiospores (excluding ornamentation) [150/3/2] (6.0-) 7.1-11.0 (-12.0) × (6.5-) 7.0-10.4 $(-10.9) \mu m$, (mean length = 8.9 ± 0.83, mean width = 8.4 ± 0.71), Q = 1.00-1.36, $Q_m = 1.08 \pm 0.07$, globose to subglobose, hyaline, echinulate, spines 2–3 μ m long, ca. $1-2 \mu m$ wide at the base, crowded. Cheilocystidia $20-31 \times 6-9 \mu m$, (mean length = 25 ± 3.5 , mean width = 7 ± 1.0), narrowly clavate, thin-walled, colorless and hyaline, abundant. Pleurocystidia 15-31 × 6-8 µm, (mean length = 21 ± 4.2 , mean width = 7 ± 0.8), narrowly clavate to subclavate, flexuose or mucronate, thin-walled, hyaline, abundant. Lamellar trama 50-70 µm thick, regular, composed of slightly thick-walled, filamentous hyphae 2-8 µm wide. Lamellar edge more in number of sterile basidia. Subhymenium 7–10 µm thick, tightly interwoven, fusiform or irregular cells, $5-8 \times 3-4 \mu m$, (mean length = 7 \pm 0.8, mean width = 3.6 \pm 0.5). Pileipellis 70–100 μ m thick, orange hyaline in KOH, composed of appressed, parallel, simply septate, thin-walled, cylindrical, filamentous hyphae 4-6 µm wide, colorless and hyaline. Stipitipellis composed of appressed, parallel, simply septate, thick-walled, hyphae 3-7 um wide; stipe trama composed of longitudinally arranged, pastel red in KOH, clavate terminal cells, infrequently branching, septate, thick-walled, hyphae hyaline 3-10 µm wide. Caulocystidia not seen. Clamp present at some septa in pileipellis, lamellae and stipitipellis.

Habitat and phenology. Scattered, gregarious, or caespitose on the ground in the *Fagus* and *Dipterocarpus*.

Additional specimens examined. THAILAND. Chiang Mai Province: Mae On District, Huay Keaw Sub-district, elevation 799 m. 6 September 2020, S. M. Tang, HKAS110664; ibid., 6 September 2020, S. M. Tang, HKAS110663.

Notes. In our single gene (Fig. 1) phylogenetic analysis, the phylogenetic position of *L. fengkaiensis*, *L. prava*, *L. vinaceoavellanea*, *L. violaceotincta*, *L. umbilicata* and *L. yunnanensis*, within *L. pseudoalba* is well supported (100/1.00) as monophyletic clades. However, *L. yunnanensis* has bigger basidiomata (pileus 60–100 mm wide), brownish to flesh-colored pileus, and relatively bigger basidia (45–50 × 9–10 µm) (Popa et al. 2014). Laccaria vinaceoavellanea has vinaceous-buff pileus, and rare pileocystidia (Li 2020). Laccaria violaceotincta has



Figure 1. Maximum likelihood tree based on ITS1-5.8S-ITS2. Bootstrap support values \geq 70%. The new sequences are highlighted in red, and the holotype of each species is in bold.



Figure 1. Continued.

dark brown to reddish brown pileus and pleurocystidia absent (Latha and Raj 2019). *Laccaria fengkaiensis* has relatively larger basidiomata (pileus 50–90 mm), more obvious striate, stipitipellis hyphal ends are either ascending or aggregating into scattered clusters, smaller basidiospores ($5.2-6.3 \times 5.1-6.3 \mu m$) and narrower basidia ($30-45 \times 6-8.5 \mu m$) (Li 2020). *Laccaria prava* has larger basidiomata (pileus 30-75 mm), presence of caulocystidia, and absence of pleurocystidia (Li 2020).

Laccaria pseudoalba can be confused with Laccaria alba Zhu L. Yang & Lan Wang due to their similar orange-white to whitish basidiomata. However, L. alba has white to whitish stipe while L. pseudoalba has pale to pastel red stipe, relatively thicker pileipellis ($30-75 \mu m$), absent pleurocystidia, narrower cheilocystidia ($4-6 \mu m$), and present clavate, hyaline caulocystidia (Wang et al. 2004).



Figure 2. Maximum likelihood phylogeny using ITS1-5.8S-ITS2, LSU, *rpb2*, and *tef1* sequence data to identify species of *Laccaria* growing on roots of *Mythicomyces corneipes*. ML bootstrap (\geq 70%) and posterior probabilities (\geq 0.90) are indicated above branches or in front of the branch leading to each node. The new species and a new record are highlighted in red; the holotype of each species is in bold.



Figure 3. Fresh basidiomata of *Laccaria pseudoalba* (a holotype, MFLU 22-0106 b, e HKAS 110664 c, d HKAS 110663). Scale bars: 5 mm. Photographs by Song-Ming Tang.

Laccaria subroseoalbescens S.M. Tang & S.H. Li, sp. nov.

MycoBank No: 853964 Figs 6-8, 15

Etymology. The epithet "subroseoalbescens" refers to its similarity to *L. ro-seoalbescens* in their pale orange to greyish orange and clearly striate on the pileus surface.

Holotype. THAILAND. Chiang Rai Province: Thasud, Muang District, Mae Fah Luang University Park, elevation 488 m, dominated by *Dipterocarpus* sp., 10 August 2020, OR1663 (MFLU23-0339).

Basidiomata small. Pileus 2–8 mm in diam., plano-concave to concave, glabrous, pale yellow (4A3), light yellow (4A4), pale orange (5A3) to greyish orange (5A4), light orange at center, becoming paler towards the margin, without umbo, when loss of moisture or with age becoming whitish, clearly striate on the surface;



Figure 4. *Laccaria pseudoalba* **a** basidiospores **b** basidium and basidioles **c** basidia **d** cheilocystidia **e** pleurocystidia. Scale bars: 10 µm. Photographs by Song-Ming Tang.



Figure 5. Laccaria pseudoalba A stipitipellis B pileipellis. Scale bars: 10 µm. Photographs by Song-Ming Tang.



Figure 6. Fresh basidiomata of *Laccaria subroseoalbescens* (holotype **a**, **c** OR 1663, MFLU23-0339 **b**, **d** OR 1664, MFLU23-0340). Scale bars: 1 cm.

context thin, below 1 mm, pale orange (5A2–3), unchanging. Lamellae distant, arcuate, adnate with decurrent tooth, pale pink (6–7A2), 1–2 mm in height; lamella edge even or entire, sometime undate; lamellulae in 2–3 tiers. Lamellae pale pink (6–7A2) to bright flesh-pink, 1.2 mm diam., subdecurrent or decurrent, thick, regular, close. Stipe $5.0-13.0 \times 0.8-1.7$ mm, cylindrical, central or eccentric, equal with an enlarged base and nearly subclavate, pale pink (7A2) to light orange (6A5), concolorous with pileus, becoming whitish after loss of moisture or with age, smooth; stipe context stuffed, pale orange. Odor and taste not observed.

Basidia $30-46 \times 8-14 \mu m$, av. $38 \pm 4.1 \times 13.8 \pm 1.3 \mu m$, clavate, mostly 4spored, rarely 2-spored; sterigmata $6-14\mu m \times 2-4\mu m$, av. $8.5 \pm 2.9 \times 3.3 \pm 0.8 \mu m$. Basidiospores [78/2/2] 7.0-8.9 × 6.8-9.0 μm , av. $8.3 \pm 0.6 \times 7.8 \pm 0.6 \mu m$, $Q_m = 1-1.3$, $Q_{av} = 1.1 \pm 0.08$, globose, hyaline; echinulate spines $2-3 \times 1-2 \mu m$, crowded. Cheilocystidia $23-37 \times 4-8 \mu m$, av. $34 \pm 8.5 \times 6.5 \pm 1.5 \mu m$, narrowly clavate, thin-walled, colorless and hyaline, abundant. Pleurocystidia $36-59 \times 5-8 \mu m$, av. $48 \pm 7.6 \times 6.5 \pm 1.3 \mu m$, subclavate, narrowly clavate, flexuose or mucronate, thin-walled, hyaline hyphae. Lamellar edge more in number of ster-



Figure 7. *Laccaria subroseoalbescens* (OR1663, MFLU23-0339) **a** basidia **b** cheilocystidia **c** pleurocystidia **d** basidiospores. Scale bars: 10 μm.

ile basidia, composed of clavate, cylindrical inflated cells $11-23 \times 8-15 \mu m$, thin-walled, colorless, similar to basidioles in shape. Subhymenium $10-24 \mu m$ thick, tightly interwoven, fusiform or irregular cells, $4-7 \times 5-6 \mu m$. Lamellar trama 74–90 μm thick, regular, composed of slightly thick-walled, filamentous hyphae 2–5 μm wide. Pileipellis 60–90 μm thick, colorless hyaline in KOH,



Figure 8. *Laccaria subroseoalbescens* (OR1663, MFLU23-0339) **A** pileipellis **B** stipitipellis. Scale bars: 10 μm. Photographs by Song-Ming Tang.

composed of appressed, parallel, simply septate, thin-walled, cylindrical, filamentous hyphae 7–11 μ m wide, colorless and hyaline. Stipitipellis composed of appressed, parallel, simply septate, thick-walled, hyphae 3–5 μ m wide; stipe trama composed of longitudinally arranged, pastel red in KOH, clavate terminal cells, infrequently branching, septate. Caulocystidia abundant, flexuose, thinwalled, hyaline hyphae, 4–5 μ m wide. Clamp present at some septa in pileipellis, lamellae and stipitipellis.

Habitat and phenology. Scattered on the ground in subtropical forests of *Dipterocarpus*.

Additional specimens examined. THAILAND. Chiang Rai Province: Thasud, Muang District, Mae Fah Luang University, 10 August 2020, elev. 489 m, OR1664 (MFLU23-0340).

Notes. In single gene (Fig. 1) phylogenetic analysis, *L. subroseoalbescens* is closely related to *L. pseudoalba*. However, *L. pseudoalba* has a pale orange to orange white pileus, and larger basidiospores $7.1-11.0 \times 7.0-10.4 \mu m$, shorter basidia sterigmata ($5-8 \mu m \times 2-3 \mu m$).

Laccaria acanthospora A.W. Wilson & G.M. Muell., L. ambigua K. Hosaka, A.W. Wilson & G.M. Mueller, and L. negrimarginata A.W. Wilson & G.M. Mueller have similar small basidiomata (pileus ≤ 15 mm) as L. subroseoalbescens. However, L. acanthospora has orange pileus, relatively longer spines (2–6 µm) on the basidiospores, and longer basidia (40–56 × 10–14 µm) (Wilson et al. 2013). Laccaria ambigua has orange-brown basidiomata, without the striates on the pileus margin, and stipe orange-brown to ochraceous buff (Wilson et al. 2017). Laccaria negrimarginata has dark blackish brown to dark brown pileus and stipe, fibrillose to appressed squamulose on the pileus surface (Wilson et al. 2013).

Laccaria indohimalayana K. Das, I. Bera & Vizzini and L. roseoalbescens T.J. Baroni, Montoya & Bandala are similar to L. subroseoalbescens in their sharing a light yellow basidiomata. However, L. indohimalayana doesn't have cheilocystidia and pleurocystidia, and is clearly separated in the phylogeny (Wang et al. 2019). Laccaria roseoalbescens has larger pileus (7–29 mm), and shorter echinae (1–2.5 μ m) (Montoya et al. 2015).

Laccaria umbilicata Ming Zhang, in Zhang, Gao, Mu & Deng, Journal of Fungi 9(12, no. 1179): 16 (2023) Figs 9–11, 15

Description. Basidiomata small. Pileus 7–11 mm in diam., applanate to plano-concave, depressed to subumbilicate shape of center, light yellow (1B4), when loss of moisture or with age becoming whitish, clearly striate towards the margin on the surface, without umbo; margin straight, eroded of margin; context thin, 0.5–1 mm, light yellow (1B4). Lamellae reddish brown (8E5–8), 3–5 mm wide; lamellulae subdecurrent to decurrent, thick, regular, distant, 3–4 mm in height; lamella edge even or entire, sometime undate, lamellulae in 3–4 tiers. Stipe $11.0-18.0 \times 1.4-2.0$ mm, cylindrical, fistulose, central or eccentric, equal with an enlarged base and nearly subclavate, white (1A1), sometime pale orange, basal mycelium white (1A1); stipe context fistulose, white, sometime pale orange. Odor and taste not observed.



Figure 9. Fresh basidiomata of *Laccaria umbilicata* (**a**–**d** MFLU 22-0105, **e**, **f** HKAS 110652, **g** HKAS 110651). Scale bars: 5 mm. Photographs by Song-Ming Tang.

Basidia $30-49 \times 9-15 \mu$ m, (mean length = 39 ± 6.3 , mean width = 12 ± 1.9), clavate, hyaline, 4-spored; sterigmata $5-8 \times 2-3 \mu$ m (mean length = 6 ± 0.7 , mean width = 2.5 ± 0.23). Basidiospores (excluding ornamentation) [150/3/2] (6.4-) $7.9-11.0 (-12.0) \times (5.7-$) $7.4-9.6 (-10.8) \mu$ m, (mean length = 9.4 ± 0.76 , mean width = 8.9 ± 0.73), Q = 1.00-1.34, Q_m = 1.07 ± 0.06 , globose to



Figure 10. *Laccaria umbilicata* (MFLU 22-0105) **a** basidiospores **b−c** basidia **d** cheilocystidia **e** pleurocystidia. Scale bars: 10 µm. Photographs by Song-Ming Tang.

subglobose, hyaline, echinulate; spines 0.2–0.5 µm long, ca. 0.5–0.8 µm wide at the base, crowded. Cheilocystidia 15–20 × 3–5 µm, (mean length = 17 ± 1.7, mean width = 4 ± 0.8), subclavate, narrowly clavate, hyphae-like, flexuose or mucronate, thin-walled, hyaline, abundant. Pleurocystidia 17–25 × 4–6 µm, (mean length = 20 ± 2.6, mean width = 5 ± 0.7), subclavate, narrowly clavate, hy-



Figure 11. Laccaria umbilicata A pileipellis B stipitipellis. Scale bars: 10 µm. Photographs by Song-Ming Tang.

phae-like, flexuose or mucronate, thin-walled, hyaline, abundant. Lamellar trama regular, $50-70 \ \mu m$ wide, composed of slightly thick-walled, filamentous hyphae $2-8 \ \mu m$ wide. Subhymenium $7-11 \ \mu m$ thick, tightly interwoven, fusiform or irregular cells, $5-10 \times 2-3 \ \mu m$, (mean length = 8 ± 1.2 , mean width = 2.3 ± 0.3). Lamellar edge heteromorphous, more in number of cheilocystidia. Pileipellis $60-100 \ \mu m$ thick, composed of interwoven radiating, thin-walled, cylindrical, filamentous hyphae $3-8 \ \mu m$ wide. Stipitipellis composed of appressed, parallel, simply septate, thick-walled, hyphae $2-8 \ \mu m$ wide; stipe trama composed of longitudinally arranged, pale orange in KOH, clavate terminal cells, infrequently branching, septate, thick-walled, hyphae $8-20 \ \mu m$ wide. Caulocystidia not seen. Clamp present at some septa in pileipellis, lamellae and stipitipellis.

Habitat and phenology. Gregarious or caespitose on the ground associated with the *Fagus* and *Dipterocarpus*.

Additional specimens examined. THAILAND, Chiang Mai Province: Mae On District, Huay Keaw, Pox Village, elevation 795 m., 6 September 2020, S. M. Tang, HKAS110652; ibid., 6 September 2020, S. M. Tang, HKAS110651. Chiang Mai Province, Mae On District, Huay Keaw, elevation 812 m, 6 September 2020, S. M. Tang, 2020090626 (MFLU 22-0105)

Notes. Following BLASTn searches of NCBI GenBank, the closest matches of the ITS and LSU sequences of our new collection (HKAS110652) is *L. umbilicata* (specimen GDGM82911 (holotype) ITS 99.67% shared identity; specimen GDGM82883 LUS 99.54% shared identity). The morphology of Thai collections was fit to the original description of *L. umbilicata* from Southwest China (Yunnan) by Zhang et al. (2023), including small basidiomata (10–28 mm), pale yellow, pale orange to light orange pileus, and clavate to ellipsoid pleurocystidia. Phylogenetically, our specimens grouped with *L. umbilicata* GDGM82911 (holotype) have high support values (Fig. 1, 100). Thus, we identified this specimen as a new record from Thailand.

Laccaria yunnanensis F. Popa, Rexer, G. Kost, Mycol. Progress 13(4): 1113 (2014) Figs 12–15

Description. Basidiomata large. Pileus 50–110 mm in diam., plano-concave, concave to hemisphaericus, glabrous, without umbo, yellowish-brown (5D4–5D8), brown (6E5–6E8), dark brown (7–8F5–8), yellowish brown when young, becoming dark brown with age, clearly striate on the surface; margin inflexed, sometimes reflexed; context thin, 2–3 mm, yellowish brown (5D4). Lamellae adnate, distant, yellowish-brown (5D4–5D8), brown (6E5–6E8), dark brown (7–8F5–8), 5–8 mm in height; lamella edge even or entire, sometime undate; lamellulae subdecurrent or decurrent, thick, regular, close; lamellulae in 2–3 tiers. Stipe 24.2–77.8 × 2.1–5.6 mm, cylindrical, central or eccentric, equal, smooth, same color as the pileus, yellowish-brown (5D4–8), brown (6E5–8), dark brown (7F5–8, 8F5–8), to whitish at the base, basal mycelium white (1A1); stipe context stuffed, yellowish brown. Odor and taste not observed.

Basidia $41-53 \times 7-15 \mu$ m, (mean length = 45 ± 8.3 , mean width = 10 ± 2.3), clavate, mostly 4-spored, rarely 2-spored, sterigmata 6-9 μ m, 2-3 μ m wide at base. Basidiospores (excluding ornamentation) [250/5/2] 7.9-10.9



Figure 12. *Laccaria yunnanensis* **a**–**d** basidiomata (**a**, **b** HKAS 110638 **c** HKAS 110631 **d** HKAS 110630). Scale bars: 1 cm. Photographs by Song-Ming Tang.

× 8.0–10.9 μ m, (mean length = 9.5 ± 0.81, mean width = 9.4 ± 0.73), Q = 1.00–1.21, $Q_m = 1.12$, globose, hyaline, echinulate, spines 1–2 µm long, ca. 0.5–1.0 μ m wide at the base, crowded. Pleurocystidia 50–70 × 10–25 μ m, (mean length = 59 ± 5.4 , mean width = 18 ± 2.4), clavate to ellipsoid, thinwalled, hyaline hyphae. Cheilocystidia abundant, 25-50 × 4-8 µm, (mean length = 38 ± 3.5 , mean width = 6 ± 0.8), subclavate, narrowly clavate to cylindrical, flexuose or mucronate, thin-walled, hyaline hyphae. Lamellar trama 60-100 mm thick regular, composed of slightly thick-walled, filamentous hyphae 2-12 µm wide. Lamellar edge more in number of cheilocystidia. Subhymenium 7–10 µm thick, tightly interwoven, fusiform or irregular cells, 2–4 \times 4–5 µm, (mean length = 3 ± 0.2, mean width = 4.3 ± 0.3). Pileipellis 40–80 µm thick, yellowish brown in KOH, composed of radiating interwoven, thinwalled, cylindrical, filamentous hyphae 3-5 µm wide. Stipitipellis composed of appressed, parallel, simply septate, thick-walled, hyphae 5-20 µm wide; stipe trama composed of longitudinally arranged, pale yellowish in KOH, clavate terminal cells, infrequently branching, septate, thick-walled, hyphae 6-18



Figure 13. Laccaria yunnanensis A pileipellis B stipitipellis. Scale bars: 20 µm. Photographs by Song-Ming Tang.



Figure 14. *Laccaria yunnanensis* **a** basidiospores **b** basidia **c** cheilocystidia **d** pleurocystidia. Scale bars: 10 µm. Photographs by Song-Ming Tang.

 μm wide. Caulocystidia not seen. Clamp present at some septa in pileipellis, lamellae and stipitipellis.

Habitat and phenology. Scattered, gregarious, or caespitose on the ground in *Dipterocarpus* and *Fagus*.

Material examined. THAILAND. Chiang Mai Province: Mare Taeng District, Pha Deng Village, 14 July 2020, S. M. Tang, HKAS 110638; ibid., 14 July 2020 S. M. Tang, HKAS 110636; ibid., 11 August 2020 S. M. Tang, MFLU 22-0107; ibid., 10 September 2020 F. M. Yu, HKAS 110630.

Notes. The morphology of Thai collections fit the original description of *L. yunnanensis* from Southwest China (Yunnan) by Popa et al. (2014) including large basidiomata (pileus 50–110 mm in diam.), yellowish brown, brown, dark



Figure 15. Characteristics of basidiospores ornamentations **a**, **b** *Laccaria pseudoalba* **c**, **d** *Laccaria subroseoalbescens* **e**-**g** *Laccaria umbilicata* **h**-**i** *Laccaria yunnanensis*. Scale bars: 2 μm. Photographs by Song-Ming Tang.

brown, yellowish brown or dark brown pileus, basidia clavate, and clavate to ellipsoid pleurocystidia. Our molecular analysis also indicated that four Thai collections belong to the same species.

Discussion

With the development of molecular phylogenetic analysis, many new *Laccaria* species have been rapidly described (Wilson et al. 2013, 2017; Popa et al. 2014, 2016; Luo et al. 2016; Cho et al. 2018; Li 2020; Cui et al. 2021; Zhang et al. 2023). Morphological characteristics and systematically informative traits are few in *Laccaria*; hence, molecular analyses are important for classification and species identification. In this study, we used the molecular

phylogenetic analysis (single gene ITS1+ITS2 and 5.8 S Fig. 1), and multi-locus phylogenetic analysis (ITS, LSU, *RPB2* and *TEF1* sequences Fig. 2) to evaluate the taxonomy of *Laccaria* in Thailand. We identified 2 new species, namely *L. pseudoalba*, *L. subroseoalbescens*, and two new records, *L. umbilicata* and *L. yunnanensis*.

Before this study, five *Laccaria* species, namely *L. amethystina* Cooke, *L. laccata, L. ohiensis* (Mont.) Singer, *L. proxima* (Boud.) Pat., and *L. vinaceoavellanea* Hongo were reported to occur in Thailand based on morphological characteristics, but the specimens lacked detailed descriptions (Chandrasrikul et al. 2011). In the future, more extensive specimen collection is needed in Thailand to determine whether these species are indeed distributed there.

So far, only *Fagus* and *Dipterocarpus* have been found to host *L. pseudoalba, L. umbilicata* and *L. subroseoalbescens.* Species in *Laccaria* are similar in morphology characters, so habitat and host trees can provide important information for species identification. It is clear that several *Laccaria* species have a wide range of host trees while other species of *Laccaria* associate with a limited group or single host (Mueller 1992). For example, *L. laccata* (Scop.) Cooke (hosts: *Castanea, Quercus, Pinus*) and *L. himalayensis* A.W. Wilson & G.M. Muell. (hosts: *Abies, Pinus, Picea*) have been reported with a variety of hosts in forests; whereas *L. trichodermophora* G.M. Muell. (host: *Quercus*) and *L. masoniae* G. Stev. (host: Nothofagus) have only been found with a single host tree species (Mueller 1984).

To date, 42 species of *Laccaria* have been reported in Asia (Wilson et al. 2013, 2017; Popa et al. 2014, 2016; Luo et al. 2016; Cho et al. 2018; Li 2020; Zhang et al. 2023). These species are described in China (26 species), South Korea (12 species), Japan (seven species), India (seven species), and Thailand (four species, this study). The taxonomy of *Laccaria* species in Thailand is still poorly understood and unclear. As a result of their very similar morphological characteristics, many *Laccaria* species are misidentified as the same species. Thus, for a better understanding of the species diversity of *Laccaria* in Thailand and their relationships within the genus, additional studies and data are required.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Novel discoveries of Xylariomycetidae (Ascomycota) taxa from peat swamp forests and other terrestrial habitats in Thailand

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Abstract

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Copyright: © Omid Karimi et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). In a comprehensive survey of fungi conducted in the northern (Chiang Rai Province) and southern (Narathiwat Province) regions of Thailand, several xylariales-like specimens were discovered. Through the integration of molecular phylogeny and morphological analyses, one previously undocumented taxon, *Oxydothis narathiwatensis* **sp. nov.**, was identified, along with *Xylaria bawanglingensis* and *Hypoxylon hypomiltum* as new host and geographical records from *Afzelia xylocarpa*, and *Dalbergia cana*, respectively. In addition, *Annulohypoxylon thailandicum* was identified as a new host record from *Swietenia macrophylla* in Thailand. The morphological characters, including ascomata, asci, and ascospores, were compared with known *Oxydothis, Xylaria, Hypoxylon*, and *Annulohypoxylon* species. Multi-locus phylogenetic analyses based on ITS, LSU, and SSU (for Oxydothidaceae), ITS, *rpb2*, tub2, and *act* (for Xylariaceae), and ITS, LSU, *rpb2*, and *tub2* (for Hypoxylaceae) gene regions were carried out to refine the taxonomic classifications of these specimens further. This research contributes to understanding fungal diversity in these ecologically significant regions, highlighting insights into the relationships among xylariales-like species.

Key words: Fungal diversity, multi-gene phylogeny, novel species, Sordariomycetes, taxonomy

Introduction

Xylariomycetidae, introduced by Eriksson and Winka (1997), is one of the largest subclasses in Ascomycota and belongs to the class Sordariomycetes (Hyde et al. 2020a). This subclass encompasses three orders and more than 35 families (Wijayawardene et al. 2022). Among these, Xylariaceae and Hypoxylaceae stand out as two particularly significant families (Wendt et al. 2018; Voglmayr et al. 2019; Hyde et al. 2020a, 2020b; Sun et al. 2021; Hernandez-Restrepo et al. 2022; Sugita et al. 2022, 2024; Wijayawardene et al. 2022), while Oxydothidaceae is one of the poorly represented families in terms of sequence data, with fewer than 20 species having sequence data (Konta et al. 2016; Hyde et al. 2020b; Senanayake et al. 2023).

Xylariaceae stands out as one of the largest and most diverse families within the Xylariales, a fact highlighted by various studies (Ju and Rogers 1996; Fröhlich and Hyde 2000; Tang et al. 2009; Stadler et al. 2013; Koyani et al. 2016; Daranagama et al. 2018; Konta et al. 2020; Samarakoon et al. 2022; Li et al. 2024a, 2024b). *Xylaria* is the largest genus in Xylariaceae, with *Xylaria hypoxylon* as the type species (Peršoh et al. 2009; Wijayawardene et al. 2022). The majority of xylariaceous species function as endophytes or saprobes, thriving on fallen woods, leaves, fruits, seeds, dung, soil, and termite nests. Notably, a few are recognized as plant pathogens (Rogers 2000; Okane et al. 2008; Stadler et al. 2013; Husbands et al. 2018; Pourmoghaddam et al. 2022).

The family Hypoxylaceae, initially proposed by de Candolle (cf. de Lamarck and de Candolle 1805), was later synonymized under Xylariaceae but resurrected and emended by Wendt et al. (2018). It encompasses approximately 422 species across 19 genera, notably Hypoxylon and Annulohypoxylon (Hyde et al. 2020a; Wijayawardene et al. 2022), thriving in diverse climates and primarily associated with dead dicotyledonous wood. Hypoxylon, the type genus introduced by Bulliard (1791), consists of 235 species, often found in warmer regions, particularly the neotropics (Ju and Rogers 1996; Daranagama et al. 2018). Traditionally, Hypoxylon was characterized by nodulisporium-like anamorphs and specific stromatal features (Ju and Rogers 1996), but modern understanding integrates morphology with molecular phylogeny and stromatal pigment profile (Hsieh et al. 2005; Kuhnert et al. 2015). As a result, Hypoxylon sect. Annulata sensu evolved into the genus Annulohypoxylon following phylogenetic analyses (Hsieh et al. 2005), with subsequent revisions and species descriptions (Kuhnert et al. 2017). Annulohypoxylon, predominantly characterized by ostioles encircled by an annulated disc, comprises 69 species (https://www. speciesfungorum.org), exhibiting diverse ecological roles (Pažoutová et al. 2013; Daranagama et al. 2018; Wendt et al. 2018; Cruz et al. 2020). Both Hypoxylon and Annulohypoxylon are known for harboring a diverse array of secondary metabolites, some of which exhibit promising agricultural and medicinal properties (Scherlach et al. 2010; Helaly et al. 2018; Becker and Stadler 2021).

Oxydothidaceae was introduced by Konta et al. (2016) to accommodate *Oxydothis* species. *Oxydothis* was introduced by Penzig and Saccardo (1897) with the type species *O. grisea* and two other species, *O. nigricans* and *O. maculosa*, placed in the family Amphisphaeriaceae (sensu Eriksson and Hawksworth 1991). Hyde (1993c) reviewed the genus and proposed that *Oxydothis* should be transferred from Amphisphaeriaceae to the Hyponectriaceae based on ascus, ascospore, and peridium morphologies. He also emphasized the consistency of ascus and ascospore morphology, which is essential for identifying species, and compared it with the closely related genera *Ceriospora, Frondispora*, *Lasiobertia* and *Leiosphaerella* (Hyde 1993c). Kang et al. (1999) transferred the genus to Clypeosphaeriaceae, but Jeewon et al. (2003) suggested that it was related to *Leiosphaerella* (Xylariales, genera incertae sedis) based on DNA sequence data. Konta et al. (2016) transferred *Oxydothis* to Oxydothidaceae (Xylariales). Besides confirming *Oxydothis* placement in Oxydothidaceae, the family was accepted in Amphisphaeriales by Samarakoon et al. (2022) and Wijayawardene et al. (2022). Currently, *Oxydothis* comprises 80 species listed in the Species Fungorum (https://www.speciesfungorum.org, accessed in June 2024). *Oxydothis* species are predominantly found on bamboo, palms, and *Pandanus*, primarily as saprobes (Hyde 1993a, 1993b, 1994a, 1994b; Wang and Hyde 1999; Fröhlich and Hyde 2000; Wong and Hyde 2001; Taylor and Hyde 2003; Shenoy et al. 2005; Hidayat et al. 2006; Tibpromma et al. 2018), with occasional occurrences as pathogens (Fröhlich and Hyde 1994) and endophytes (Hyde 1994b; Taylor et al. 1999).

This paper describes one new species, *Oxydothis narathiwatensis*, and presents three new records, *Xylaria bawanglingensis*, *Hypoxylon hypomiltum*, and *Annulohypoxylon thailandicum*. Additionally, molecular sequence data and phylogenetic information are provided for these taxa.

Materials and methods

Sampling and morphological studies

Xylariaceae and Hypoxylaceae specimens were collected in 2022 from dead wood of *Afzelia xylocarpa*, *Swietenia macrophylla*, and *Dalbergia cana* at Doi Tung National Park and Mae Fah Luang University, Chiang Rai, Thailand. Oxydothidaceae specimens were collected from submerged rachis of *Eleio-doxa conferta* from a peat swamp forest in Narathiwat Province, Thailand, in 2023. Morphological observations and single spore isolations followed the procedures outlined in Senanayake et al. (2020). The samples were cultured in different media, including potato dextrose agar (PDA), malt extract agar (MEA), and oatmeal agar (OA). Herbarium specimens were deposited in the Herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand. Living cultures are deposited in the Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand. Faces of fungi and Index Fungorum numbers are registered as described in Jayasiri et al. (2015) and Index Fungorum (https://www.indexfungorum.org), respectively. Descriptions and collection details are added to the Greater Mekong Subregion database (Chaiwan et al. 2021).

DNA extraction, PCR amplification, and Phylogenetic analyses

Fresh mycelia were used to extract genomic DNA using the Mega Genomic DNA Extraction Kit, following the manufacturer's instructions (Omega Bio-tek Inc, The United States). The PCR amplifications were conducted for six loci, including Internal transcribed spacer (ITS), large subunit rDNA (LSU), nuclear small subunit rDNA (SSU), RNA polymerase II subunit (*rpb2*), beta-tubulin (*tub2*), and actin (*act*) (Table 1). Sequence data of related taxa (Xylariaceae, Hypoxylaceae, and Oxydothidaceae) were downloaded from the GenBank database (Suppl. material 1: tables S1–S3). The phylogenetic analyses based on maximum likelihood (ML) and Bayesian inferences were conducted for both the individual datasets and the concatenated dataset of six loci. The CIPRES Science Gateway platform (Miller et al. 2010) was used for performing the maximum likelihood (ML) phylogenetic analysis, utilizing the RAxMLHPC2 tool on the XSEDE (v. 8.2.10) platform (Stamatakis 2014). The analysis employed the

Gene region	Primers	PCR Condition	References
ITS	ITS5/ITS4	Initial denaturation: 94 °C (3 min); 35 cycles of denaturation: 95 °C (1 min), annealing: 53 °C (55 sec) and extension 72 °C (2 min); Final extension: 72 °C (10 min)	White et al. (1990)
LSU	LR0R/LR5	Initial denaturation: 94 °C (5 min); 35 cycles of denaturation: 94 °C (30 sec), annealing: 55 °C (50 sec) and extension 72 °C (2 min); Final extension: 72 °C (10 min)	Vilgalys and Hester (1990); Rehner and Samuels (1994)
SSU	NS1/NS4	Initial denaturation: 94 °C (3 min); 35 cycles of denaturation: 94 °C (30 sec), annealing: 55 °C (50 sec) and extension 72 °C (2 min); Final extension: 72 °C (10 min)	White et al. (1990)
rpb2	fRPB2-5F/ fRPB2-7cR	Initial denaturation: 95 °C (5 min); 35 cycles of denaturation: 95 °C (1 min), annealing: 52°C (1 min) and extension 72 °C (2 min); Final extension: 72 °C (10 min)	Liu et al. (1999)
tub2	T1/T22	Initial denaturation: 95 °C (2 min); 35 cycles of denaturation: 95 °C (1 min), annealing: 54 °C (1.5 min) and extension 72 °C (2 min); Final extension: 72 °C (10 min)	O'Donnell et al. (1998); Hsieh et al. (2005)
act	ACT-512F/ ACT-738R	Initial denaturation: 95 °C (5 min); 35 cycles of denaturation: 95 °C (1 min), annealing: 55 °C (30 sec) and extension 72 °C (1 min); Final extension: 72 °C (10 min)	Carbone and Kohn (1999)

Table 1. Primers and PCR conditions used for each gene region in the current study.

GTRGAMMA substitution model and the rapid bootstrap analysis algorithm for 1000 replicates. The Bayesian analysis was performed using MrBayes v. 3.2.6 on XSEDE at the CIPRES Science Gateway, with the analysis set to two parallel runs, four Markov chains, and run for 10,000,000 generations. The pairwise homoplasy index (PHI) test was conducted using the combined sequence dataset comprising ITS, LSU, and SSU genes of closely related species using Split Tree version 4.18.2 (Huson and Bryant 2006) to evaluate the recombination level. The generated phylograms were visualized using FigTree v. 1.4.0 (Rambaut 2015), and annotations were added using Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, United States).

Results

Phylogenetic analysis of Xylariaceae

The combined ITS, *rpb2*, *tub2*, and *act* dataset consisted of 213 isolates belonging to 151 Xylariaceae taxa, with *Hypoxylon fragiforme* (MUCL 51264), *Hypoxylon monticulosum* (MUCL 54604), and *Daldinia loculatoi-des* (CBS:113279) as outgroup taxa (Suppl. material 1: table S1). The final alignment comprised 3,726 characters (ITS: 606 bp, *rpb2*: 1,191 bp, *tub2*: 1,627 bp, *act*: 302 bp), including gaps. The final ML optimization likelihood value of the best RAxML tree (Fig. 1) was -136336.552252, and the matrix had 2,610 distinct alignment patterns, with 32.19% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.234039, C = 0.279027, G = 0.241491, T = 0.245443; substitution rates AC = 1.325774, AG = 5.176867, AT = 1.193683, CG = 1.131857, CT = 6.488876, GT = 1.000000; gamma distribution shape parameter α = 0.409124. The RAxML and Bayesian analyses yielded almost similar tree topologies. The topology of our phylogenetic tree is nearly identical to previous publications (Friebes et al.

2022; Pan et al. 2022; Ju et al. 2023; Karimi et al. 2023; Li et al. 2024b) with minor differences, which may be due to different taxon sampling, as well as different studies that have explored the taxonomy of *Xylaria* using various combinations of gene regions, such as ITS, *act*, *tub2*, and *rpb2* combination (Garcia-Aroca et al. 2021), *act* and *tub2* combination (Wangsawat et al. 2021), *tub2*, *act*, and *rpb2* combination (Hsieh et al. 2020, 2022) or ITS, *tub2*, and *rpb2* combination (Pan et al. 2022).

Taxonomy

Xylaria bawanglingensis Y.P. Wu & Q.R. Li, J. Syst. Evol. 16 (2024)

Index Fungorum: IF849696 Facesoffungi Number: FoF16035 Figs 1, 2

Description. Saprobic on dead wood of Afzelia xylocarpa (Fabaceae). Sexual morph: Stromata $0.5-2 \times 0.1-0.3$ cm ($\bar{x} = 1.2 \times 0.17$ cm, n = 15), solitary or in a small group, not branched or rarely branched at the base, cylindrical to obclavate, narrowing towards the apex, and sometimes with a tomentum; surface rough, wrinkled, slightly cracked, gray to blackish and interior yellow. Perithecia 165–380 µm diam., ($\bar{x} = 285 \mu$ m, n = 15), immersed, subglobose, ostioles inconspicuous. Paraphyses 5–10 µm width ($\bar{x} = 8 \mu$ m, n = 20), cylindrical, septate. Asci 68–153 × 4–5.5 µm ($\bar{x} = 109 \times 4.8 \mu$ m, n = 30), 8-spored, cylindrical, and spore bearing part 67–87 µm long. Apical apparatus $0.2-2 \times 1-2 \mu$ m ($\bar{x} = 1.3 \times 1.5 \mu$ m, n = 15), bluing in Melzer's iodine reagent. Ascospores $8-12 \times 2-5 \mu$ m ($\bar{x} = 10 \mu$ m × 4 µm, n = 70), uniseriate, ellipsoid, subhyaline, light brown to dark brown, smooth, guttulate, germ slit apparent, sigmoid, almost full length of spore. Asexual morph: Undetermined.

Culture characters. Colonies grown on PDA, reaching 30 mm in diameter after 15 days at 25 °C, under dark conditions, mycelium superficial to immersed, without pigment diffusion, sporulation and concentric zones, white on the top and back views.

Material examined. THAILAND, Doi Tung National Park, Chiang Rai, on dead wood of *Afzelia xylocarpa*, 27 September 2022, N. Afshari 5C3T2R2 (MFLU 24-0018), living culture MFLUCC 24-0087.

Known distribution. China (Li et al. 2024b), and Thailand (This study).

Known hosts. Afzelia xylocarpa (This study).

Notes. Our collection (MFLU 24-0018) morphologically resembles the *Xylaria bawanglingensis* (GMB1023) in having solitary, fertile inflated, roughened surface and gray stromata with interior yellow, subglobose ostiolate perithecia, cylindrical asci with a J+ apical ring and unicellular ascospores with spore-length sigmoid germ slit and almost similar sized stromata, perithecia, asci and ascospores. In the phylogenic analyses (Fig. 1), our collection (MFLUCC 24-0087) clustered with *Xylaria bawanglingensis* (GMB1023, GMB1162) with 100% ML bootstrap support and 1.00 posterior probability support. In this study, we report our isolate (MFLU 24-0018) as a new host and geographical record of *Xylaria bawanglingensis* on *Afzelia xylocarpa* from Thailand.

Omid Karimi et al.: Introduce a new species with new host and geographical records



Figure 1. RAxML tree based on the analysis of a combined ITS, *rpb2*, *tub2*, and *act* dataset. ML bootstrap supports (MLBS) equal to or higher than 65% and Bayesian posterior probabilities (BYPP) equal to or greater than 0.95 are given near the nodes. Newly generated isolate of the current study is in red, and ex-types are in bold. The tree is rooted to Daldinia loculatoides, Hypoxylon fragiforme and Hypoxylon monticulosum.

Omid Karimi et al.: Introduce a new species with new host and geographical records



Figure 1. Continued.



Figure 2. *Xylaria bawanglingensis* (MFLU 24-0018, new host and geographical record) **a** stromata on host **b**, **c** stromata **d** longitudinal section through the stroma **e**-**h** asci **i** paraphyses **j** apical ring bluing in Melzer's reagent **k**, **l** ascospores **m** upper view and reverse view of the one-week-old colony on PDA. Scale bars: 1 mm (**b**, **d**); 20 μ m (**e**-**g**); 70 μ m (**h**); 20 μ m (**i**); 5 μ m (**j**); 10 μ m (**k**, **l**).

Phylogenetic analysis of Hypoxylaceae

The combined phylogenetic dataset of ITS, LSU, rpb2, and tub2 contains 164 isolates belonging to 116 Hypoxylaceae taxa (Suppl. material 1: table S2). After trimming, the analyzed dataset comprised 3,499 characters, including gaps (ITS = 605 bp, LSU = 740 bp, rpb2= 1,082 bp, tub2 = 1,072 bp). The final ML optimization likelihood value of the best RAxML tree was -76207.411440 (Fig. 3), and the matrix had 1933 distinct alignment patterns, with 33.77% undetermined characters or gaps. In our phylogenetic tree, we have labeled the clades as follows: Annulohypoxylon: A, Daldinia: D, Entonaema: E, Hypomontagnella: Hm, Hypoxylon: Hyp, Jackrogersella: J, Pyrenopolyporus: P, Rhopalostroma: Rho, Rostrohypoxylon: Ros, Ruwenzoria: Ruw and Thamnomyces: T. The topologies of phylogenetic trees based on the combined dataset generated from ML and BI analyses were almost identical, while the statistical supports showed slight differences. Our phylogenetic tree is nearly identical to previously published studies (Wendt et al. 2018; Becker et al. 2020; Lambert et al. 2021; Ma et al. 2022; Cedeño-Sanchez et al. 2023). Hypoxylon papillatum clustered at a basal position (Hyp1) with 100% ML bootstrap support and 1.00 posterior probability support. The clade Hyp2 was segregated from the backbone of the tree with 90% ML bootstrap support and 1.00 posterior probability support. Members of Hyp3 were clustered with 69% ML bootstrap support and 0.99 posterior probability support, while the sister clade Hyp4 clustered with 67% ML bootstrap support. All the clades of Annulohypoxylon (A1-A5), Hypomontagnella, Jackrogersella, Rostrohypoxylon, Pyrenopolyporus, Entonaema, Ruwenzoria, Thamnomyces, Rhopalostroma, and Daldinia displayed well-supported segregation in the phylogeny (Fig. 3). Our strain of A. thailandicum (MFLUCC 24-0086) clustered closer to A. thailandicum (MFLUCC 13-0118) with 97% ML bootstrap support and 1.00 posterior probability support. Our collection (MFLUCC 24-0088) of H. hypomiltum clustered with H. hypomiltum (MUCL 51845) with 100% ML bootstrap support and 1.00 posterior probability support.

Annulohypoxylon thailandicum Daranag. & K.D. Hyde, Fungal Diversity 72: 53 (2015)

Index Fungorum: IF550799 Facesoffungi Number: FoF00373 Figs 3, 4

Description. Saprobic on a dead branch of Swietenia macrophylla (Meliaceae). Sexual morph: Ascostromata $0.8-2 \times 0.5-1.5 \text{ mm}$ (\bar{x} = 1.5 ×1 mm, n = 15), spherical to hemispherical, superficial, effused-pulvinate, conglomerate, solitary or catenated, with conspicuous perithecial mounds, carbonaceous, black surface, blackish granules beneath surface and between perithecia, releasing greenish-olivaceous pigments in 10% KOH. Perithecia $0.35-0.8 \times 0.4-1.0 \text{ mm}$ ($\bar{x} = 0.5 \times 0.6 \text{ mm}$, n =15), immersed, spherical to pyriform, encased in carbonaceous tissue. Ostioles coarsely papillate, truncatum-type disc, 0.25-0.4 mm diam. Paraphyses not observed. Asci 79-100 × 3.5-4.9 µm ($\bar{x} = 91 \times 4.9 \text{ µm}$, n = 20), unitunicate, cylindrical, uniseriate, rarely overlapping uniseriate, 8-spored, apical apparatus faintly bluing in Melzer's reagent.



Figure 3. RAxML tree based on the analysis of the combined ITS, LSU, *rpb2*, and *tub2* dataset. ML bootstrap supports (MLBS) equal to or higher than 65%, and the Bayesian posterior probabilities (BYPP) equal to or greater than 0.90 are given near the nodes. The ex-types are in bold. The two new sequences are shown in red font. The phylogenetic analyses constituted *Xylaria hypoxylon* (Outgroup) and 11 genera of Hypoxylaceae: *Annulohypoxylon* (A), *Daldinia* (D), *Entonaema* (E), *Hypomontagnella* (Hm), *Hypoxylon* (Hyp), *Jackrogersella* (J), *Pyrenopolyporus* (P), *Rhopalostroma* (Rho), *Rostrohypoxylon* (Ros), *Ruwenzoria* (Ruw) and *Thamnomyces* (T).



Figure 3. Continued.



Figure 4. Annulohypoxylon thailandicum (MFLU 24-0019, new host record) **a** host **b** stromatal habit on host **c** ostioles with ostiolar discs **d** stromata in horizontal section showing perithecia **e** pigments in KOH **f**-**h** asci **i** ascal apical apparatus in Melzer's reagent **j**-**m** ascospores **o**, **p** colonies on PDA after two weeks. Scale bars: 1 mm (**b**); 500 μ m (**c**, **d**); 30 μ m (**f**-**h**); 10 μ m (**i**); 5 μ m (**j**-**m**).

Ascospores $7-10 \times 3.5-5.0 \ \mu m (\bar{x} = 8.5 \times 4.5 \ \mu m, n = 30)$, unicellular, ellipsoid-inequilateral, narrowly to broadly rounded ends, hyaline at immaturity, olivaceous brown to brown at maturity, straight spore-length germ slit, epispore smooth, perispore dehiscent in 10% KOH. **Asexual morph**: Undetermined. **Culture characters.** Colonies on PDA reaching 4 cm after one week, white, effuse, erose, medium, dull, rhizoid edge, no sporulation, top white, reverse white at first, turning light brown after few days (4–5 days).

Material examined. THAILAND, Mae Fah Luang University, Chiang Rai, on a dead branch of *Swietenia macrophylla* (Meliaceae), 20 November 2022, Raheleh Asghari MOF4 (MFLU 24-0019), living culture MFLUCC 24-0086.

Known distribution. China (Zhang et al. 2023), Indonesia (Yurnaliza et al. 2021), and Thailand (Liu et al. 2015, this study).

Known hosts. *Elaeis guineensis* (Yurnaliza et al. 2021) and *Swietenia macrophylla* (This study).

Notes. Our collection (MFLU 24-0019) resembles *A. thailandicum* in having effused-pulvinate black ascostromata, releasing pigment in KOH, papillate perithecia, with truncatum-type disc, cylindrical asci with J+ apical ring, ellipsoid-inequilateral brown ascospores with germ slit and dehiscent perispore in 10% KOH as detailed in Liu et al. (2015). Our specimen differs slightly in having larger perithecia and smaller ascospores (Table 2). Sharing similar morphological characteristics, such as ascospores, ostiolar disc, and the release of pigments in KOH, *A. thailandicum* (MFLU 24-0019) can be compared to *A. archeri* and *A. microcarpum* (Liu et al. 2015). However, it is crucial to note that the latter two species exhibit reddish ascostromata with a smaller truncatum-type disc, distinguishing them from *A. thailandicum*. In the multi-loci phylogenetic tree, *A. thailandicum* isolate MFLUCC 24-0086 clustered with *A. thailandicum* (MFLUCC 13-0118) with 100% ML bootstrap support and 1.00 posterior probability support (Fig. 3). In this study, we report our collection (MFLU 24-0019) as a new host record on *Swietenia macrophylla* from Thailand.

Name	stromata size (mm)	Perithecia (mm)	Asci (µm)	Apical ring (µm)	Ascospore size (µm)	Germ slit
A. thailandicum MFLU 13-0441 (Liu et al. 2015)	$0.5-0.7 \times 0.8-1.7 \times 0.8$ ($\overline{x} = 0.7 \times 1.5 \times 0.8$)	$\begin{array}{c} 0.35 - 0.5 \times 0.3 - 0.7 \\ (\overline{x} = 0.4 \times 0.5) \end{array}$	89–100.8 × 4.5– 6.5 (x=97 × 5.6)	globose, 1.5 × 2	6−11.5 × 4−6.5 (x̄ =12.5 × 5.6)	Straight
A. thailandicum MFLU 24-0019 (This study)	$0.8-2 \times 0.5-1.5 (\bar{x} = 1.5 \times 1)$	$0.35-0.8 \times 0.4-1.0 \text{ mm}$ ($\overline{x} = 0.5 \times 0.6 \text{ mm}$)	79–100 × 3.5–4.9 μm (x̄ = 91 × 4.9 μm)	discoid, wedge, 1.4–1.8 × 0.7–1.1	7–10 × 3.5–5.0 μm (x̄ = 8.5 × 4.5 μm)	Straight
<i>A. archeri</i> (Ju and Rogers 1996)	_	0.1 mm diam	_	_	9−10.5 µm × 4−5 µm	-
A. microcarpum (Ju and Rogers 1996)	_	0.15-0.2 mm diam	_	_	7−8 × 3−4µm	-

Table 2. Synopsis of morphological characteristics of Annulohypoxylon thailandicum, A. archeri, and A. microcarpum.

Hypoxylon hypomiltum Mont., Annls Sci. Nat., Bot., sér. 2 13: 356 (1840)

Index Fungorum: IF158066 Facesoffungi Number: FoF06137 Figs 3, 5

Description. Saprobic on dead wood of *Dalbergia cana* (Fabaceae). Sexual morph: Stromata superficial, sessile, effused, pulvinate, conspicuous, hemispherical, dense, forming compact mass, discoid, black, KOH extractable pigments yellow. Perithecia $360-400 \times 300-350 \mu m$ ($\bar{x} = 350 \times 300 \mu m$, n =15),



Figure 5. *Hypoxylon hypomiltum* (MFLU 24-0043, new host and geographical record) **a** host **b** stromatal habit on host **c** stromata in vertical section showing perithecia **d**, **e** asci **f** pigments in KOH **g** ascal apical apparatus in Melzer's reagent **h**-**i** ascospore (**i** ascospore with perispore **j** ascospore with germ slit) **k** germinated ascospore **l** colony on PDA after three weeks. Scale bars: 500 μ m (**b**, **c**); 20 μ m (**d**, **e**); 5 μ m (**g**-**j**); 10 μ m (**k**).

hemispherical to spherical. **Asci** cylindrical, 8-spored, uniseriate, spore-bearing part $43-55 \times 4-6 \ \mu m \ (\bar{x} = 48 \times 5 \ \mu m, n = 20)$, and stipes $37-50 \ (\bar{x} = 45, n = 20)$, with amyloid apical apparatus bluing in Melzer's reagent, discoid, $0.4-0.6 \times 1.3-1.5 \ \mu m \ (\bar{x} = 5 \times 1.4 \ \mu m, n = 10)$. **Ascospores** $6-8 \times 3-4 \ \mu m \ (\bar{x} = 7 \times 3.5 \ \mu m \ n = 20)$, brown, ellipsoidal with rounded ends, uniseriate, aseptate, guttulate, straight germ slit. **Asexual morph**: Undetermined.

Culture characters. Colonies grown on PDA, reaching 55 mm in diameter after 20 days at 25 °C, under dark conditions, circular, flat, smooth, entire edge, medium dense, mycelium superficial to immersed, no sporulation, yellow pigment diffusion, light brown on the top and reverse pale yellowish-brown.

Material examined. THAILAND, Doi Tung National Park, Chiang Rai, on dead wood of *Dalbergia cana* (Fabaceae), 27 September 2022, N. Afshari 4C3T2R3a (MFLU 24-0043), living culture MFLUCC 24-0088.

Known distribution. French Guiana (Montagne 1840), Japan (Abe 1986), Panama (Standley 1933), Pakistan (Ahmad 1978), Sri Lanka (Palapathwala et al. 2019), Thailand (This study), USA (Ju and Rogers 1996), and India (Sarbhoy et al. 1971).

Known hosts. Fagus crenata (Abe 1986), Morus alba (Ahmad 1978), Mangifera indica (Sarbhoy et al. 1971), and Dalbergia cana (This study).

Notes. Our collection (MFLU 24-0043) shares similar characteristics with *Hypoxylon hypomiltum* (Montagne 1840), including effuse, pulvinate, conspicuous, hemispherical stromata, KOH extractable pigments, hemispherical to spherical perithecia, cylindrical, uniseriate asci, brown, ellipsoidal, unicellular ascospores with a straight germ slit, and mostly similar sized stromata, perithecia, asci and ascospores. In the phylogenic analyses (Fig. 3), our collection (MFLUCC 24-0088) clustered with *Hypoxylon hypomiltum* (MUCL52887) with 100% ML bootstrap support and 1.00 posterior probability support. In this study, we report our isolate (MFLU 24-0043) as a new host and geographical record of *Hypoxylon hypomiltum* on *Dalbergia cana* from Thailand.

Phylogenetic analysis of Oxydothidaceae

The combined ITS, LSU, and SSU dataset consisted of 20 isolates belonging to Oxydothidaceae taxa, with Vialaea mangiferae (MFLUCC 12-0808) and Vialaea minutella (BRIP 56959) as outgroup taxa (Suppl. material 1: table S3). The final alignment comprised 2,657 characters (ITS: 452 bp, LSU: 1,200 bp, SSU: 1,021 bp), including gaps. The final ML optimization likelihood value of the best RaxML tree was -9733.301849 (Fig. 6), and the matrix had 1,637 distinct alignment patterns, with 30% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.256773, C = 0.222793, G = 0.273403, T = 0.247031; substitution rates AC = 1.198924, AG = 2.053309, AT = 1.196302, CG = 1.103201, CT = 3.773909, GT = 1.000000; gamma distribution shape parameter α = 0.253842. The topology of our phylogenetic tree is identical to previous publications (Konta et al. 2016; Hu et al. 2022; Senanayake et al. 2023). Our collection of the new species Oxydothis narathiwatensis (MFLUCC 24-0085) is from a distinct clade with Oxydothis hoehnelii (HKUCC 3854) and Oxydothis sp. (IFO 32218) with 99% ML bootstrap support (Fig. 6).

Oxydothis narathiwatensis O. Karimi & K.D. Hyde, sp. nov.

Index Fungorum: IF902133 Facesoffungi Number: FoF16036 Figs 6–8

Etymology. The epithet *"narathiwatensis"* refers to Narathiwat Province, where the holotype was collected.

Holotype. MFLU 24-0044.

Description. *Saprobic* on the submerged rachis of *Eleiodoxa conferta* (Arecaceae). *Sexual morph*: *Ascomata* 170–320 µm diam., ($\bar{x} = 250$ µm diam., n = 15), mostly in small groups, immersed, erumpent, with the non-blistering area on the host, subglobose or pyriform. *Peridium* 17–30 µm ($\bar{x} = 22$ µm, n = 10), thick, dark brown to black, textura angularis. *Paraphyses* 40–80 × 3–6 µm ($\bar{x} = 62 \times 4$ µm, n = 20), cylindrical, fragmented, hyaline, branched or non-branched. *Asci* 171–257 × 7–11 µm ($\bar{x} = 225 \times 9$ µm, n = 20), 8-spored, cylindrical, unitunicate, short pedicellate, smooth-walled, with a J+, wedge-shaped, subapical ring. *Ascospores* 95–121 × 3–5 µm ($\bar{x} = 110 \times 4$ µm, n = 20), 2–3-seriate, hyaline, filiform, straight, curved or flexuous, rounded ends, centrally uniseptate, guttulate with smooth walls. *Appressoria* 10–20 × 9–10 µm ($\bar{x} = 13 \times 9.5$ µm, n = 10), irregular, hyaline to green, thick-walled, verrucose. *Asexual morph*: Undetermined.

Culture characters. Colonies on PDA, reaching 55 mm in diameter after 30 days at 25–27 °C, under dark conditions, medium dense, mycelium superficial to immersed, circular, flat, raised in the center with aerial mycelium, dull surface, entire edge, velvety, without pigment diffusion and sporulation, dark brown on the top and reverse-side black.

Material examined. THAILAND, Narathiwat, peat swamp forest, on the submerged rachis of *Eleiodoxa conferta* (Arecaceae), 3 August 2023, O. Karimi, 19-W (MFLU 24-0044, holotype); Ex-type living culture MFLUCC 24-0085.

Notes. Morphologically, Oxydothis narathiwatensis (MFLU 24-0044) shares similar characteristics with O. gigantea (BRIP 21921) and O. maquilingiana (3975) in having cylindrical asci with J+, wedge-shaped, subapical ring and filiform ascospores (Hyde 1994b). However, O. narathiwatensis (MFLU 24-0044) differs from O. gigantea (BRIP 21921) in having longer and narrower asci $(171-257 \times 7-11 \mu m vs. 240 \times 20 \mu m)$, and shorter and narrower ascospore (95–121 × 3–5 μm vs. 100–150 × 6.5–7.5 μm). Oxydothis narathiwatensis (MFLU 24-0044) differs from O. maquilingiana (3975) in having longer and narrower asci (171-257 × 7-11 µm vs. 140-150 × 12-14 µm), longer and narrower ascospore $(95-121 \times 3-5 \mu m vs. 85-95 \times 5-6 \mu m)$ and longer ascal ring $(1.5-5 \times 1-3 \mu m vs. 2.6-3.5 \times 1.6-2.4 \mu m)$. However, due to the lack of sequence data for O. gigantea and O. maquilingiana, a phylogenetic comparison with O. narathiwatensis was not possible. Phylogenetically, O. narathiwatensis (MFLUCC 24-0085) formed a robust subclade (100% ML) basal to O. hoehnelii (KDH 1837). Morphologically, O. narathiwatensis differs from O. hoehnelii in having shorter and narrower asci $(171-257 \times 7-11 \,\mu\text{m vs})$. $250-290 \times 12-14 \mu m$), fusiform ascospores against filiform ascospores in 0. narathiwatensis (MFLU 24-0044) and longer and narrower ascospores (95- $121 \times 3-5 \mu m$ vs. $72-86 \times 7-10 \mu m$). The result of the pairwise homoplasy index (PHI) test revealed no significant recombination ($\Phi w = 0.4$) between



Figure 6. RAxML tree is based on the analysis of a combined dataset of ITS, LSU, and SSU sequence data. Maximum likelihood bootstrap support (MLBS) values equal to or higher than 60%, and the Bayesian posterior probability (BYPP) equal to or greater than 0.95 are given near the nodes. The ex-types are in bold. The new sequence is shown in yellow font. The tree is rooted with *Vialaea mangiferae* and *Vialaea minutella*.

O. narathiwatensis (MFLUCC 24-0085) and its closely related species (Fig. 8). Therefore, we introduced *Oxydothis narathiwatensis* (MFLU 24-0044) as a novel species based on morphological evidence and phylogenetic analyses (Figs 6–8).



Figure 7. *Oxydothis narathiwatensis* (MFLU 24-0044, holotype) **a** host substrate **b** close up of ascomata **c** section of ascoma **d** peridium **e** paraphyses **f** j+ reaction of apical ring in Melzer's reagent **g**, **h** asci **i**–**l** ascospores **m** germinating ascospore **n** appressoria **o**, **p** colony on PDA after two weeks. Scale bars: 500 μ m (**b**); 50 μ m (**c**, **d**, **g**, **h**); 20 μ m (**e**, **m**); 5 μ m (**f**); 25 μ m (**i**–**l**); 10 μ m (**n**).



Figure 8. The split diagram resulting from the pairwise homoplasy index (PHI) test was constructed using the combined ITS, LSU, and SSU sequence data of closely related taxa. The PHI test (Φ w) < 0.05 indicates significant recombination within the dataset. The newly identified taxon is represented in blue.

Discussion

In this study, xylariales-like specimens were collected from Doi Tung National Park and Mae Fah Luang University in Chiang Rai Province, located in northern Thailand, and from Narathiwat Province in southern Thailand. Our investigation, employing a polyphasic approach that combines both morphological and molecular analyses (Figs 1–8), resulted in the identification of one novel species, *Oxydothis narathiwatensis*, and documented a new host record for *Annulohypoxylon thailandicum* from *Swietenia macrophylla* and two new host and geographical records of *Xylaria bawanglingensis* and *Hypoxylon hypomiltum* from *Afzelia xylocarpa* and *Dalbergia cana*, respectively, in Thailand.

Our *Xylaria* species were collected from the dead wood of *Afzelia xylocarpa*, and phylogenetic analysis reveals that it forms a sister clade with the wood-in-habiting species, *Xylaria bawanglingensis* (GMB1023 and GMB1162) and *Xylaria feejeensis* (HAST 92092013 and JRD 180). However, *Xylaria phyllocharis* (HAST 528), collected on fallen leaves (Dennis 1956) is separated from these two wood-inhabiting species in our phylogenetic tree; this finding agrees and is comparable with previous studies (Hsieh et al. 2010; Pan et al. 2022).

Annulohypoxylon thailandicum (MFLU 13-0441) was described by Daranagama & K.D. Hyde in Liu et al. (2015) from Chiang Mai. In the phylogenetic analyses, the closest species to our isolate (MFLUCC 24-0086) was found to be *A. thailandicum* (MFLUCC 13-0118), with slight differences in the ascostromata size. The relatedness is also statistically well-supported in our phylogenic analyses (Fig. 3). *Annulohypoxylon thailandicum* (MFLU 13-0441) has also been reported from Guizhou, China (Zhang et al. 2023), and North Sumatra, Medan City, Indonesia (Yurnaliza et al. 2021). The climate of Doi Suthep, Chiang Mai, Chiang Rai (north of Thailand), Guizhou (China), and Medan City, North Sumatra (Indonesia) are mostly similar, showing a preference for Hypoxylaceae for tropical to subtropical climates. Hence, we believe that conducting more studies in tropical to subtropical climatic areas will enhance our understanding and lead to the discovery of more undescribed species of *Annulohypoxylon*.

The delimiting characteristics of the sexual morph of Hypoxylon include never erecting unipartite stromata, with solid and homogenous basal tissue underneath the perithecial layer (Ju and Rogers 1996). Based on the morphological assessment, our collection fits well with Hypoxylon hypomiltum (Montagne 1840). In our phylogenetic analysis (Fig. 3), there is a notable clustering of all four isolates of H. griseobrunneum, which diverges from the findings in recent publications (Ma et al. 2022; Song et al. 2022; Yang et al. 2022). This discrepancy may stem from the utilization of sequences with incorrect names. For instance, Hsieh et al. (2005) provided β -tubulin and actin sequences for the Hypoxylon anthochroum isolate BCRC 34050 and incorporated them into their phylogenetic tree. However, Kuhnert et al. (2015) later updated the status of the BCRC 34050 isolate, assigning it to H. griseobrunneum after conducting morphological and phylogenetic analyses that revealed a close relationship with H. griseobrunneum isolates. Subsequently, this sequence was employed for the latter species. In contrast, recent publications (Ma et al. 2022; Song et al. 2022; Yang et al. 2022) have utilized the sequence of the BCRC 34050 isolate as H. anthochroum. Therefore, due to frequent changes in taxonomy, it is crucial to carefully choose accurate sequences and species names.

Oxydothis species belong to the family Oxydothidaceae and comprise approximately 80 species, predominantly associated with palms (Arecaceae) in various habitats, including peat swamps, marine, and terrestrial environments, where they are primarily found as saprobes (Hyde 1993a, 1993b, 1994b; Wang and Hyde 1999, 2001; Fröhlich and Hyde 2000; Taylor and Hyde 2003; Shenoy et al. 2005; Hidayat et al. 2006). Despite the substantial diversity within this genus, only 15 species have sequence data. Among these, some species lack crucial genetic markers, such as ITS sequences (e.g., O. calamicola, and O. rhapidicola) or SSU or LSU sequences (e.g., O. chinensis, O. cyrtostachicola, and O. fortunei), which impacts the accuracy of phylogenetic reconstructions (Hidayat et al. 2006; Konta et al. 2016; Hu et al. 2022; Senanavake et al. 2023). Therefore, obtaining sequence data for previously described species is imperative to enhance our understanding of the phylogenetic relationships among Oxydothis species. In our study, we proposed a new species collected from submerged rachides of Eleiodoxa conferta in Narathiwat Province. We provided sequence data (ITS, LSU, and SSU) with detailed morphological descriptions. Notably, our investigation revealed the production of appressoria by germinating ascospores in our saprobic species, O. narathiwatensis, is similar to 0. garethionesii, 0. metroxylonicola, 0. metroxylonis, and O. palmicola (Konta et al. 2016). Appressoria in saprobic fungi raises intriguing questions about their ecological role and evolutionary adaptation. Some Oxydothis species are endophytes, and several produce appressoria, indicating an endophytic lifestyle (Taylor et al. 1999; Konta et al. 2016). This may suggest that these species are likely host-specific endophytes that change their lifestyle and become early saprobes when palm fronds die (Chethana et al. 2021). This supports the hypothesis that many saprobes initially start as endophytes (Chethana et al. 2021; Bhunjun et al. 2024). Additionally, the production of appressoria can help the fungi attach to their substrate in aquatic habitats (Au et al. 1996). Further exploration into the functional role of appressoria in Oxydothis species is warranted and could vield valuable insights into their ecology, interactions with host plants, and broader evolutionary strategies. This research has the potential to significantly enhance our understanding of fungal ecology and evolution in diverse ecosystems, shedding light on the intricate relationships between their host plants.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary information

Authors: Omid Karimi, Naghmeh Afshari, Raheleh Asghari, Qirui Li, K. W. Thilini Chethana, Kevin D. Hyde, Fatimah O. Alotibi

Data type: docx

- Explanation note: table S1. List of taxa used for the phylogenetic reconstruction (Xylariaceae). GenBank accession numbers, specimen number, origin, status and reference. table S2. List of taxa used for the phylogenetic reconstruction (Hypoxylaceae). table S3. List of taxa used for the phylogenetic reconstruction (Oxydothidaceae).
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Research Article

Three new species of *Cyanosporus* (Polyporales, Basidiomycota) from China

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Abstract

Cyanosporus is a cosmopolitan genus characterized by effused-reflexed to pileate basidiomata with a bluish tint and allantoid to cylindrical basidiospores which are negative to weakly positive in Melzer's reagent and Cotton Blue, causing a brown rot. Three new species of *Cyanosporus*, namely, *C. linzhiensis*, *C. miscanthi* and *C. tabuliformis* are described and illustrated. Phylogenies on *Cyanosporus* are reconstructed with seven loci DNA sequences including ITS, nLSU, nSSU, mtSSU, RPB1, RPB2 and TEF1 based on phylogenetic analyses combined with morphological examination. The description for the new species is given. The main morphological characteristics of all 38 accepted species in *Cyanosporus* are summarized.

Key words: Brown rot, phylogeny, polypore, Postia, taxonomy, wood-decaying fungi

Introduction

The genus *Cyanosporus* McGinty (Polyporales, Basidiomycota), typified by *C. caesius* (Schrad.) McGinty, was established by Lloyd (1909). It is characterized by annual, resupinate, effused-reflexed to pileate basidiomata; white, cream, bluish gray to pinkish buff pileal surface; white, cream, bluish gray to ash gray pore surface, hyphal system monomitic with generative hyphae clamped, and allantoid to cylindrical basidiospores, thin- to slightly thick-walled, with negative to weakly positive reaction in Melzer's reagent and Cotton Blue, causing a brown rot in decayed wood (Papp 2014; Shen et al. 2019; Liu et al. 2021, 2022).

The type species of *Cyanosporus* (*P. caesius*, basionym: *Boletus caesius*) was previously treated as *Polyporus caesius* (Schrad.) Fr. (Fries 1821) and *Tyromyces caesius* (Schrad.) Murrill (Murrill 1907), the latter name was accepted by some mycologists (Donk 1960; Jahn 1963; Lowe 1975). Subsequently, the species was also transferred into *Spongiporus* Murrill, *Postia* Fr., *Oligoporus* Bref., respectively (David 1980; Jülich 1982; Gilbertson and Ryvarden 1985),

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because *Postia* has priority over *Spongiporus* and *Oligoporus*, and *Postia caesia* was widely accepted (Papp 2014).

Cyanosporus species were included in Postia Fr. typified by Postia lactea (Fr.) P. Karst. Molecular analysis (Tura et al. 2008; Pildain and Rajchenberg 2012; Ortiz-Santana et al. 2013) clustered the Postia species in two clades, one of which included Postia caesia (Schrad.) P. Karst. Several studies acknowledged the morphological variability of P. caesia, and its closely related taxa referred to as P. caesia complex (Tura et al. 2008; Pildain and Rajchenberg 2012; Papp 2014). Papp (2014) discussed the taxonomic status of the Postia caesia complex, and proposed a subgenus, Postia subg. Cyanosporus (McGinty) V. Papp for including the complex (involved five species: P. alni, P. caesia, P. luteocaesia, P. mediterraneocaesia, P. subcaesia). Miettinen et al. (2018) studied the Postia caesia complex based on phylogenetic and morphological analyses, selected a neotype of P. caesia (LY BR-6776 collected from Germany) from type locality and described ten new species in P. caesia complex. A recent molecular study on Postia s.l. and related genera in Shen et al. (2019), considered Cyanosporus and Postia were two different genera. Two separate species were addressed in the family Postiaceae (Liu et al. 2023). Morphologically both genera differ by the more or less bluish basidiocarps and weakly cyanophilous basidiospores in Cyanosporus, while in Postia, basidiocarps lack a blue tint and basidiospores are acyanophilous (Shen et al. 2019; Liu et al. 2021, 2022, 2023).

Up to now, 35 species have been accepted in *Cyanosporus*, 23 of which are distributed in China (Liu et al. 2022). During the studies on Chinese polyporoid fungi causing brown rot, some samples were collected from southwest and northern China that morphologically correspond to *Cyanosporus*. The objective of this study is to confirm the identity of these specimens, through a phylogenetic analysis based on a seven loci dataset (ITS+nLSU+mtSSU+nuSSU+RP-B1+RPB2+TEF1), and to describe and illustrate the new species.

Materials and methods

Morphological studies

The studied specimens are deposited in the Fungarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Morphological descriptions are based on field notes and voucher specimens. The microscopic analysis follows Miettinen et al. (2018) and Wu et al. (2022a). Sections were studied at a magnification of up to 1000× using a Nikon Eclipse 80i microscope and phase contrast illumination. Description of microscopic features and measurements was made from slide preparations stained with KOH, Cotton Blue and Melzer's reagent. Basidiospores were measured from sections cut from the tubes. To represent the variation in the size of spores, 5% of measurements were excluded from each end of the range and are given in parentheses. In the description: KOH = 5% potassium hydroxide, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, CB = Cotton Blue, CB– = acyanophilous in Cotton Blue, L = arithmetic average of spore length, W = arithmetic average of spore width, Q = L/W ratios, and n = number of basidiospores/measured from given number of specimens. Color terms follow Anonymous (1969) and Petersen (1996).

DNA extraction, amplification and sequencing

A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain DNA from dried specimens, followed by the polymerase chain reaction (PCR) according to the manufacturer's instructions with some modifications (Shen et al. 2019; Sun et al. 2020). The internal transcribed spacer (ITS) and large subunit nuclear ribosomal RNA gene (nLSU) were amplified using the primer pairs ITS5/ITS4 and LR0R/LR7 (White et al. 1990; Hopple and Vilgalys 1999) (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/). The small subunit mitochondrial ribosomal DNA (mtSSU) region was amplified with primer pairs MS1 and MS2 (White et al. 1990). The small subunit nuclear ribosomal RNA gene (nSSU) region was amplified with primer pairs RNA gene (nSSU) region was amplified with primer pairs RPB1-Af and RPB1-Cr (Matheny et al. 2002). The RPB2 was amplified with primer pairs fRPB2-5F and fRPB2-7CR (Matheny 2005).

The PCR procedure for ITS, mtSSU and TEF1 was as follows: initial denaturation at 95 °C for 3 min, followed by 34 cycles at 94 °C for 40 s, 54 °C for ITS, 58 °C for mtSSU, and 54 °C for TEF for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR procedure for nLSU and nSSU was as follows: initial denaturation at 94 °C for 1 min, followed by 34 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for nLSU and 52 °C for nSSU for 1 min and extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The PCR procedure for RPB1 and RPB2 was initial denaturation at 94 °C for 2 min, followed by 10 cycles at 94 °C for 45 s, 60 °C for 45 s and 72 °C for 1.5 min, then followed by 37 cycles at 94 °C for 45 s, 52 °C for 1 min and 72 °C for 1.5 min, and a final extension of 72 °C. The PCR products were purified and sequenced at the Beijing Genomics Institute (BGI), China, with the same primers as used in PCR. Newly generated sequences were deposited in GenBank. All sequences analysed in this study are listed in Table 1.

0	Specimen voucher	Country	GenBank accession NO.							
Species			ITS	LSU	mtSSU	nrSSU	RPB1	RPB2	TEF1	
Amaropostia hainanensis B.K. Cui, L.L. Shen & Y.C. Dai	Cui 13739 (holotype)	China	KX900909	KX900979	KX901053	KX901123	KX901171	KX901223	_	
A. stiptica (Pers.) B.K. Cui, L.L. Shen & Y.C. Dai	Cui 10043	China	KX900906	KX900976	KX901046	KX901119	KX901167	KX901219	_	
<i>Amylocystis Iapponica</i> (Romell) Bondartsev & Singer	HHB-13400	USA	KC585237	KC585059	_	-	_	-	_	
A. lapponica	OKM-4418	USA	KC585238	KC585060	_	-	-	-	_	
Antrodia serpens (Fr.) P. Karst.	Dai 7465	Luxemburg	KR605813	KR605752	KR606013	KR605913	-	KR610832	KR610742	
A. tanakae (Murrill) Spirin & Miettinen	Cui 9743	China	KR605814	KR605753	KR606014	KR605914	_	KR610833	KR610743	
<i>Calcipostia guttulata</i> (Sacc.) B.K. Cui, L.L. Shen & Y.C. Dai	Cui 10018	China	KF727432	KJ684978	KX901065	KX901138	KX901181	KX901236	KX901276	
C. guttulata	Cui 10028	China	KF727433	KJ684979	KX901066	KX901139	KX901182	KX901237	KX901277	

Table 1. Species, specimens, and GenBank accession number of sequences used for phylogenetic analyses in this study.

Enocioo	Specimen	Country	GenBank accession NO.						
Species	voucher	Country	ITS	LSU	mtSSU	nrSSU	RPB1	RPB2	TEF1
<i>Cyanosporus alni</i> (Niemelä & Vampola) B.K. Cui, L.L. Shen & Y.C. Dai	H 7019137 (holotype)	Slovakia	MG137026	_	_	_	_	_	_
C. alni	Cui 7185	China	KX900879	KX900949	KX901017	KX901092	KX901155	KX901202	KX901254
C. alni	Dai 14845	Poland	KX900880	KX900950	KX901018	KX901093	KX901156	KX901203	KX901255
<i>C. arbuti</i> (Spirin) B.K. Cui & Shun Liu	Spirin 8327 (holotype)	USA	MG137039	-	-	-	-	-	MG137132
C. <i>auricomus</i> (Spirin & Niemelä) B.K. Cui & Shun Liu	Cui 13518	China	KX900887	KX900957	KX901025	KX901100	_	KX901209	_
C. auricomus	Cui 13519	China	KX900888	KX900958	KX901026	KX901101	_	-	-
C. auricomus	TN 8310 (holotype)	Finland	MG137040	-	-	-	-	-	-
<i>C. bifarius</i> (Spirin) B.K. Cui & Shun Liu	Spirin 6402 (holotype)	Russia	MG137043	-	_	-	-	-	MG137133
C. bifarius	Cui 17534	China	OL423598	OL423608	OL437195	OL423620	OL444985	OL446999	OL444994
C. bifarius	Cui 16277	China	OL423599	OL423609	OL437196	OL423621	OL444986	OL447000	OL444995
<i>C. bubalinus</i> B.K. Cui & Shun Liu	Cui 16976	China	MW182172	MW182225	MW182208	MW182189	MW191547	MW191563	MW191530
C. bubalinus	Cui 16985 (holotype)	China	MW182173	MW182226	MW182209	MW182190	MW191548	MW191564	MW191531
C. caesiosimulans (G.F. Atk.) B.K. Cui & Shun Liu	Spirin 4199	Russia	MG137061	_	_	-	_	_	MG137140
C. caesiosimulans	Miettinen 16976 (holotype)	USA	MG137054	_	_	_	_	_	MG137137
<i>C. caesius</i> (Schrad.) McGinty	Schuster 51 (neotype)	Germany	MG137045	-	_	-	-	-	_
C. caesius	Miettinen 14156	Finland	MG137048	-	-	-	-	-	MG137134
C. caesius	Cui 18630	France	OL423600	OL423610	OL437197	OL423622	_	-	OL444996
C. aff. caesius	K 32713	UK	AY599576	-	-	-	-	-	-
C. aff. caesius	K 32425	UK	AY599575	-	-	-	-	-	-
C. coeruleivirens (Corner) B.K. Cui, Shun Liu & Y.C. Dai	Miettinen 12214	Indonesia	MG137063	_	_	_	_	_	_
C. coeruleivirens	Dai 19220	China	MW182174	MW182227	MW182210	MW182191	MW191549		MW191532
C. comatus (Miettinen) B.K. Cui & Shun Liu	Miettinen 14755,1 (holotype)	USA	MG137066	_	_	_	_	_	_
C. cyanescens (Miettinen) B.K. Cui & Shun Liu	Miettinen 13602 (holotype)	Finland	MG137067	_	_	-	_	-	MG137142
C. cyanescens	Miettinen 15919.2	Spain	MG137071	_	_	_	_	_	MG137144
<i>C. flavus</i> B.K. Cui & Shun Liu	Cui 18547	China	MW448564	MW448561	_	MW448557	MW452596	MW452599	MW452601
C. flavus	Cui 18562 (holotype)	China	MW448565	MW448562	-	MW448558	MW452597	MW452600	MW452602
C. fusiformis B.K. Cui, L.L. Shen & Y.C. Dai	Cui 10775	China	KX900868	KX900938	KX901006	KX901081	_	KX901191	KX901245
C. fusiformis	Dai 15036 (holotype)	China	KX900867	KX900937	KX901005	KX901080	_	KX901190	KX901244
<i>C. glaucus</i> (Spirin & Miettinen) B.K. Cui & Shun Liu	Spirin 5317	Russia	MG137078	_	_	_	_	_	_
Species	Specimen	Country			Genl	Bank accessio	n NO.		
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Species	voucher	Country	ITS	LSU	mtSSU	nrSSU	RPB1	RPB2	TEF1
C. glaucus	Spirin 6580 (holotype)	Russia	MG137081	-	-	-	_	-	MG137145
C. gossypinus (Moug. & Lév.) B.K. Cui & Shun Liu	Rivoire 6658 (topotype)	France	_	_	_	_	_	_	MG137146
C. hirsutus B.K. Cui & Shun Liu	Cui 17083 (holotype)	China	MW182179	MW182233	MW182214	MW182197	MW191554	MW191568	MW191538
C. hirsutus	Cui 17343	China	OL423601	OL423611	OL437198	OL423623	OL444987	OL447001	OL444997
C. hirsutus	Cui 17342	China	OL423602	OL423612	OL437199	OL423624	OL444988	OL447002	OL444998
C. linzhiensis	Dai 27141	China	PP479781 ^a	PP479803°	PP510196 ^a	PP488288ª	PP526258°	PP526267ª	-
C. linzhiensis	Dai 27023 (holotype)	China	PP479782ª	PP479804ª	PP510197ª	PP488289ª	PP526259 ^a	PP526268ª	-
<i>C. livens</i> (Miettinen & Vlasák) B.K. Cui & Shun Liu	Spirin 8728	USA	MG137090	_	_	-	_	-	MG137150
C. livens	Miettinen 17177 (holotype)	USA	MG137082	-	-	-	_	-	MG137147
C. luteocaesius (A. David) B.K. Cui, L.L. Shen & Y.C. Dai	Rivoire 2605 (topotype)	France	MG137091	_	_	-	_	-	_
C. <i>magnus</i> (Miettinen) B.K. Cui & Shun Liu	Dai 21105	China	OL423603	OL423613	OL437200	OL423625	OL444989	OL447003	OL444999
C. magnus	Cui 16983	China	MW182180	MW182234	MW182215	MW182198	MW191555	MW191569	MW191539
C. magnus	Miettinen 10634 (holotype)	China	KC595944	KC595944	_	_	_	_	MG137151
C. mediterraneocaesius (M. Pieri & B. Rivoire) B.K. Cui, L.L. Shen & Y.C. Dai	LY BR 4274	France	KX900886	_	KX901024	KX901099	_	_	_
C. <i>microporus</i> B.K. Cui, L.L. Shen & Y.C. Dai	Cui 11014 (holotype)	China	KX900878	KX900948	KX901016	KX901091	-	KX901201	-
C. microporus	Dai 11717	China	KX900877	KX900947	KX901015	KX901090	_	KX901200	-
C. miscanthi	Dai 26684	China	PP479784 ^a	PP479806 ^a	PP510199ª	PP488291ª	PP526261ª	PP526270ª	PP526276 ^a
C. miscanthi	Dai 26687 (holotype)	China	PP479786ª	PP479808ª	PP510201ª	PP488293ª	PP526263ª	PP526272 ^a	PP526277ª
C. miscanthi	Dai 26689	China	PP479783ª	PP479805 ^a	PP510198 ^a	PP488290 ^a	PP526260 ^a	PP526269ª	PP526275 ^a
C. miscanthi	Dai 26695	China	PP479787 ^a	PP479809 ^a	PP510202 ^a	PP488294ª	PP526264ª	PP526273 ^a	PP526278 ^a
C. miscanthi	Dai 26701	China	PP479785°	PP479807 ^a	PP510200 ^a	PP488292ª	PP526262ª	PP526271ª	_
C. nothofagicola B.K. Cui, Shun Liu & Y.C. Dai	Cui 16697 (holotype)	Australia	MW182181	MW182235	MW182216	MW182199	MW191556	MW191570	MW191540
C. nothofagicola	Dai 18765	Australia	MW182182	MW182236	MW182217	MW182200	MW191557	-	MW191541
<i>C. piceicola</i> B.K. Cui, L.L. Shen & Y.C. Dai	Cui 10626 (holotype)	China	KX900862	KX900932	KX901001	KX901075	-	KX901185	-
C. piceicola	Cui 12158	China	KX900866	KX900936	KX901004	KX901079	KX901153	KX901189	KX901243
<i>C. populi</i> (Miettinen) B.K. Cui & Shun Liu	Miettinen 17043 (holotype)	USA	MG137092	_	_	-	-	-	MG137153
C. populi	Cui 17087a	China	MW182183	MW182237	MW182218	MW182201	MW191558	MW191571	MW191542
C. populi	Dai 18934	China	OL423604	OL423614	OL437201	OL423626	OL444990	OL447004	OL445000
C. populi	Cui 17557	China	OL423605	OL423615	OL437202	OL423627	OL444991	OL447005	OL445001
C. rigidus B.K. Cui & Shun Liu	Cui 17032 (holotype)	China	OL423606	OL423617	OL437204	OL423629	OL444993	-	OL445003

Species	Specimen	Country			Gen	Bank accessio	n NO.		
Species	voucher	Country	ITS	LSU	mtSSU	nrSSU	RPB1	RPB2	TEF1
C. simulans (P. Karst.) B.K. Cui & Shun Liu	Miettinen 20422	Finland	MG137110	-	_	_	-	_	MG137160
C. simulans	TN 8846 (holotype)	Finland	MG137103	_	_	_	-	-	-
C. subcaesius (A. David) B.K. Cui, L.L. Shen & Y.C. Dai	JV 0110/24	Czechia	MG137117	_	_	_	-	-	MG137164
C. subcaesius	Alix David 652 (isotype)	France	MG137116	_	_	_	_	_	-
<i>C</i> . subhirsutus B.K. Cui, L.L. Shen & Y.C. Dai	Cui 11330	China	KX900873	KX900943	KX901011	KX901086	_	KX901196	KX901250
C. subhirsutus	Dai 14892 (holotype)	China	KX900871	KX900941	KX901009	KX901084	-	KX901194	KX901248
C. submicroporus B.K. Cui & Shun Liu	Cui 16306	China	MW182184	MW182239	MW182220	MW182203	MW191560	MW191573	MW191544
C. submicroporus	Cui 18156 (holotype)	China	MW182186	MW182241	MW182222	MW182205	-	MW191574	-
C. subungulatus B.K. Cui & Shun Liu	Cui 18046 (holotype)	China	MW448566	MW448563	MW448560	MW448559	MW452598	-	MW452603
C. subungulatus	Zhao 10833	China	MW742586	OL423616	OL437203	OL423628	OL444992	-	OL445002
C. subviridis (Ryvarden & Guzmán) B.K. Cui & Shun Liu	Spirin 8774a	USA	MG137120	_	_	_	-	-	MG137166
C. subviridis	Penttilä 14376	Finland	-	-	-	-	-	-	MG137165
C. tabuliformis	Dai 26063 (holotype)	China	PP479788ª	PP479810 ^a	PP510203ª	PP488295ª	PP526265ª	PP526274ª	PP526279ª
C. tabuliformis	Dai 26066	China	PP479789ª	PP479811ª	PP510204ª	PP488296ª	PP526266ª	-	PP526280 ^a
C. tenuicontextus B.K. Cui & Shun Liu	Cui 16280 (holotype)	China	OL423607	OL423618	OL437205	OL423630	-	-	OL445004
C. tenuicontextus	Zhao 813	China	MG231802	OL423619	OL437206	OL423631	-	-	OL445005
<i>C. tenuis</i> B.K. Cui, Shun Liu & Y.C. Dai	Cui 10788 (holotype)	China	KX900885	KX900955	KX901023	KX901098	KX901161	KX901208	-
C. tenuis	Dai 12974	China	KX900884	KX900954	KX901022	KX901097	KX901160	KX901207	KX901258
C. tricolor B.K. Cui, L.L. Shen & Y.C. Dai	Cui 12233 (holotype)	China	KX900876	KX900946	KX901014	KX901089	-	KX901199	KX901253
C. tricolor	Cui 10790	China	KX900875	KX900945	KX901013	KX901088	-	KX901198	KX901252
<i>C. ungulatus</i> B.K. Cui, L.L. Shen & Y.C. Dai	Cui 10778	China	KX900870	KX900940	KX901008	KX901083	_	KX901193	KX901247
C. ungulatus	Dai 12897 (holotype)	China	KX900869	KX900939	KX901007	KX901082	KX901154	KX901192	KX901246
<i>C. yanae</i> (Miettinen & Kotir.) B.K. Cui & Shun Liu	Kotiranta 27606	Russia	MG137122	_	_	_	-	-	MG137168
C. yanae	Kotiranta 27454 (holotype)	Russia	MG137121	_	_	_	-	-	MG137167
<i>Cystidiopostia hibernica</i> (Berk. & Broome) B.K. Cui, L.L. Shen & Y.C. Dai	Cui 2658	China	KX900905	KX900975	KX901045	KX901118	_	KX901218	-
<i>C. inocybe</i> (A. David & Malençon) B.K. Cui, L.L. Shen & Y.C. Dai	LY BR 3703	France	KX900903	KX900973	KX901044	KX901116	-	-	KX901267

Snacias	Specimen	Country			Genl	Bank accessio	n NO.		
Species	voucher	Country	ITS	LSU	mtSSU	nrSSU	RPB1	RPB2	TEF1
<i>C. pileata</i> (Parmasto) B.K. Cui, L.L. Shen & Y.C. Dai	Cui 10034	China	KX900908	KX900956	KX901050	KX901122	KX901170	KX901222	KX901269
Fuscopostia duplicate (L.L. Shen, B.K. Cui & Y.C. Dai) B.K. Cui, L.L. Shen & Y.C. Dai	Dai 13411 (holotype)	China	KF699125	KJ684976	KR606027	KR605928	KX901174	KR610845	KR610756
<i>F. fragilis</i> (Fr.) B.K. Cui, L.L. Shen & Y.C. Dai	JV 0610/8	Czechia	JF950573	-	-	_	-	-	-
F. leucomallella (Murrill) B.K. Cui, L.L. Shen & Y.C. Dai	Cui 9599	China	KF699123	KJ684983	KX901056	KX901129	KX901176	KX901228	KX901272
Jahnoporus brachiatus Spirin, Vlasák & Miettinen	X 3232	Russia	KU165781	-	-	_	-	-	-
<i>J. hirtus</i> (Cooke) Nuss	Spinosa 10 X 2014	USA	KU165784	-	-	-	KY949044	-	-
<i>J. oreinus</i> Spirin, Vlasák & Miettinen	X 3241	Russia	KU165785	-	-	_	-	-	-
<i>Oligoporus rennyi</i> (Berk. & Broome) Donk	TN-6645	Finland	KC595929	KC595929	-	_	-	-	-
<i>O. sericeomollis</i> (Romell) Bondartseva	Cui 9870	China	KX900920	KX900990	KX901068	KX901141	KX901184	-	-
Osteina obducta (Berk.) Donk	Cui 10074	China	KX900924	KX900994	KX901071	KX901144	-	KX901240	-
<i>0. undosa</i> (Peck) Zmitr.	Dai 7105	China	KX900921	KX900991	KX901069	KX901142	-	KX901238	-
Postia amurensis Y.C. Dai & Penttilä	Dai 903 (holotype)	China	KX900901	KX900971	KX901042	-	-	-	-
<i>P. hirsuta</i> L.L. Shen & B.K. Cui	Cui 11237 (holotype)	China	KJ684970	KJ684984	KX901038	KX901113	-	-	KX901266
<i>P. lactea</i> (Fr.) P. Karst.	Cui 12141	China	KX900892	KX900962	KX901029	KX901104	KX901163	KX901211	KX901260
P. lowei (Pilát) Jülich	Cui 9585	China	KX900898	KX900968	KX901035	KX901110	-	-	-
P. ochraceoalba L.L. Shen, B.K. Cui & Y.C. Dai	Cui 10802 (holotype)	China	KM107903	KM107908	KX901041	KX901115	-	KX901216	-
<i>P. sublowei</i> B.K. Cui, L.L. Shen & Y.C. Dai	Cui 9597 (holotype)	China	KX900900	KX900970	KX901037	KX901112	-	-	KX901265
P. tephroleuca (Fr.) Jülich	Dai 12610	Finland	KX900897	KX900967	KX901034	KX901109	KX901166	KX901214	KX901263
<i>Resupinopostia lateritia</i> (Renvall) B.K. Cui, L.L. Shen & Y.C. Dai	Dai 2652	China	KX900913	KX900983	-	_	-	-	-
<i>R. sublateritia</i> B.K. Cui & Shun Liu	Dai 22760	China	OQ476281	OQ476340	OQ476447	OQ476396	OQ506088	OQ511187	OQ511241
Spongiporus balsameus (Peck) A. David	Cui 9835	China	KX900916	KX900986	KX901061	KX901134	-	KX901233	_
<i>S. leucospongia</i> (Cooke & Harkn.) Murrill	JV 0709/123	USA	_	KX900988	KX901064	KX901137	-	_	KX901275
S. floriformis (Quél.) Zmitr.	Cui 10292	China	KM107899	KM107904	KX901058	KX901131	KX901178	KX901230	KX901274

^a Newly generated sequences in this study. Bold = new taxa and sequences generated in this study.

Sequence alignment

Sequences generated from this study were aligned with additional sequences downloaded from GenBank using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). The final ITS, nLSU, mtSSU, nSSU, RPB1, RPB2 and TEF1 datasets were subsequently aligned using MAFFT v.7 under the E-INS-i strategy with no cost for opening gaps and equal cost for transformations (command line: mafft –genafpair –maxiterate 1000) (Katoh and Standley 2013) and visualized in BioEdit. Alignments were spliced and transformed formats in Mesquite v.3.2. (Maddison and Maddison 2017). Multiple sequence alignments were trimmed by trimAl v.1.2 using the -htmlout-gt 0.8 -st option to deal with gaps when necessary (Capella-Gutierrez et al. 2009).

Phylogenetic analyses

In this study, a seven loci dataset (ITS+LSU+mtSSU+nrSSU+RPB1+RP-B2+TEF1) was used to reconstruct the phylogenetic position of the new species. The sequence alignment and the retrieved topology were deposited in TreeBase (http://www.treebase.org), under accession ID: 31280 (Reviewer access URL: http://purl.org/phylo/treebase/phylows/study/TB2:S31280?x-access-code=605c3765137c8814e37dd70c560cb4de&format=html). Sequences of *Antrodia serpens* (Fr.) P. Karst. and *Antrodia tanakae* (Murrill) Spirin & Miettinen, obtained from GenBank, were used as the outgroups (Liu et al. 2021). The phylogenetic analyses followed the approach of Han et al. (2016) and Zhu et al. (2019). Maximum parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses were performed based on one dataset. The best-fit evolutionary model was selected by Akaike Information Criterion (AIC) in MrModeltest 2.2 (Nylander 2004) after scoring 24 models of evolution in PAUP* version 4.0b10 (Swofford 2002).

The MP topology and bootstrap values (MP-BS) obtained from 1000 replicates were computed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were set to 5,000 branches of zero length were collapsed, and all parsimonious trees were saved. Descriptive tree statistics tree length (TL), composite consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each maximum parsimonious tree (MPT) generated. Sequences were also analysed using Maximum Likelihood (ML) with RAxML-HPC2 through the CIP-RES Science Gateway (Miller et al. 2010). Branch support (BT) for ML analysis was determined by 1 000 bootstrap replicates. Bayesian phylogenetic inference and Bayesian Posterior Probabilities (BPP) were computed with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 3.5 M generations until the split deviation frequency value was less than 0.01, and trees were sampled every 100 generations. The first 25% of the sampled trees were discarded as burn-in and the remaining ones were used to reconstruct a majority rule consensus and calculate Bayesian Posterior Probabilities (BPP) of the clades. All trees were viewed in FigTree v. 1.4.3 (http://tree.bio.ed.ac. uk/software/figtree/). Branches that received bootstrap support MP \geq 75%,

 $ML \ge 75\%$, and $BPP \ge 0.95$ were considered as significantly supported. The MP and ML bootstrap supports $\ge 50\%$ and $BBP \ge 0.90$ are presented on topologies from ML analysis, respectively.

Results

Molecular phylogeny

The combined seven loci dataset (ITS+LSU+mtSSU+nrSSU+RPB1+RPB2+TEF1) included sequences from 115 samples representing 68 species. The dataset had an aligned length of 5639 characters, of which 3800 (56%) characters are constant, 345 (9%) are variable and parsimony-uninformative and 1494 (35%) are parsimony informative. Maximum parsimony analysis yielded eleven equally-parsimonious tree (TL = 6767, CI = 0.414, RI = 0.701, RC = 0.290, HI = 0.586). The phylogenetic reconstruction performed with Maximum Likelihood (ML) and Bayesian Inference (BI) analyses showed similar topology and few differences in statistical support. The best model-fit applied in the Bayesian analysis was GTR+I+G, lset nst = 4, rates = invgamma, and prset statefreqpr = dirichlet (1, 1, 1, 1). Bayesian analysis resulted in a nearly congruent topology with an average standard deviation of split frequencies = 0.008647 to ML analysis, and thus only the ML tree is provided (Fig. 1). The phylogeny (Fig. 1) confirmed Cyanosporus and Postia as two independent and closely related clades with full support (100% MP, 100% ML, 1.00 BPP). Sequences of three new species, viz. C. linzhiensis, C. miscanthi, C. tabuliformis, were placed in three fully supported and independent lineages in Cyanosporus clade (Fig. 1). Though the Cyanosporus clade formed two subclades, the supports were at a very weak rate and species in the two subclades share similar characteristics. So the morphological characters cannot explain this separation.

Taxonomy

The main morphological characteristics of the accepted species in *Cyanosporus* are provided in Table 2.

Cyanosporus linzhiensis Y.C. Dai, Chao G. Wang, Yuan Yuan & Ghobad-Nejhad, sp. nov.

MycoBank No: 853174 Figs 2, 3

Holotype. CHINA. • Xizang Autonomous Region: Nyingchi, Zayü County, 27 Oct. 2023, on fallen branch of *Pinus yunnanensis*, Dai 27023 (BJFC 044575, GenBank: ITS PP479782, LSU PP479804, mtSSU PP510197, nrSSU PP488289, RPB1 PP526259, RPB2 PP526268).

Etymology. In reference to the species being found in Linzhi (Nyingchi) of Xizang Autonomous Region, southwest China.

Diagnosis. Cyanosporus linzhiensis is characterized by their pileate basidiomata with a bluish tint and azonate pileal surface when fresh and dry, white to pale bluish gray pore surface when fresh, pores angular to irregular, 5–6 per mm, cystidioles fusoid and basidiospores allantoid, $4-5 \times 1.2-1.5 \mu m$.



Figure 1. A Maximum Likelihood phylogenetic tree of *Cyanosporus* based on a dataset of ITS+nLSU+mtSSU+nuSSU+RP-B1+RPB2+TEF1. ML bootstrap values higher than 50% and Bayesian posterior probabilities values more than 0.90 are shown. New taxa are in bold.

Table 2. The main	morpholo	ogical characteris	tics of the accepted species	in cyanosporus.					
Species	Type locality	Basidiomata	Upper surface	Color of poroid surface	Amyloid (greenish in IKI) tramal hyphae	Shape of basidiospores	Size of Basidiospores (µm)	Cyanophilous basidiospores	References
C. alni	Slovakia	Annual, Pileate to rarely effused- reflexed	Cream, ochraceous to brownish with a bluish-grayish tint, velutinate	White to cream, in older and dry specimens with a light bluish- grayish tint	1	Allantoid to very narrow cylindrical	4.4-6 × 1.1-1.3	1	Niemelä et al. (2001); Miettinen et al. (2018)
C. arbuti	USA	Annual, Pileate to effused-reflexed	White to pale cream; glabrous	White to cream, in older and dry specimens with a light bluish- grayish tint	1	Allantoid	4.1-5.1 × 1-1.2	+	Miettinen et al. (2018)
C. auricomus	Finland	Annual, Pileate	White to cream, yellowish to bright yellow, in older specimens with pale to dark ochraceous; hirsute	Bright yellow, green when bruised, then with an ochraceous tint	+	Allantoid	4.4-5.6×1.5-1.8	+	Miettinen et al. (2018)
C. bifarius	Russia	Annual, Pileate	Light gray, then with an ochraceous tint; velutinate	White to cream, in older and dry specimens with a light ochraceous tint	1	Allantoid	3.7-4.4×1-1.2	+	Miettinen et al. (2018)
C. bubalinus	China	Annual, Pileate	White to cream when fresh, cream to pinkish buff when dry; tomentose	White to cream when fresh, straw yellow to buff when dry	1	Cylindrical, slightly curved	4.3-4.8 × 1.2-1.7	I	Liu et al. (2021)
C. caesiosimulans	USA	Annual, Pileate to effused-reflexed	White to cream, then grayish to pale ochraceous with very rarely with bluish flecks or faint zones; glabrous	White to cream, in older and dry specimens with a light bluish-grayish tint	1	Allantoid	4.2-5.5 × 1.1-1.4	+	Miettinen et al. (2018)
C. caesius	Germany	Annual, Pileate to effused-reflexed	Grayish to bluish when fresh, blue when bruised; hirsute	White to pale gray when fresh, bluish when bruised	+	Cylindrical to allantoid	4.5-6 × 1.5-2	+	Ryvarden and Gilbertson (1994)
C. coeruleivirens	Malaysia	Annual, Pileate	White to bluish green; velutinate	White when fresh, bluish green when bruised	1	Allantoid	4-5 × 1-1.3	+	Corner (1989)
C. comatus	USA	Annual, Pileate to effused-reflexed	Cream to pale ochraceous; velutinate	Cream, in older and dry specimens with a bluish- grayish tint	1	Allantoid	4.1-4.9×1.1-1.3	+	Miettinen et al. (2018)
C. cyanescens	Finland	Annual, Pileate to reraly effused- reflexed	White to pale ochraceous, then pale ochraceous, rarely with a bluish-grayish tint; glabrous	White to cream, in older and dry specimens with a light bluish-grayish tint	1	Allantoid	4.7-6.1 × 1.1-1.6	+	Miettinen et al. (2018)
C. flavus	China	Annual, Pileate	Ash-gray to light vinaceous gray when fresh, pale mouse-gray to mouse-gray when dry, hirsute	White to cream when fresh, buff to lemon-chrome when dry	1	Slim allantoid	4.6-5.2 × 0.8-1.3	I	Liu et al. (2022)
C. fusiformis	China	Annual, Pileate to effused-reflexed	White to cream, with a blue tint at the center when fresh, vinaceous gray to dark gray when dry; tomentose	White when fresh, buff to clay buff when dry	1	Slim allantoid	4.5-5.2 × 0.8-1.1	I	Shen et al. (2019)
C. glaucus	Russia	Annual, Pileate	Grayish, plumbeous to bluish gray or grayish-brown; hirsute	White to cream when fresh, in older and dry specimens with a light bluish-grayish tint	+	Allantoid	4.1-5.4×1.1-1.5	+	Miettinen et al. 2018

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Species	Type locality	Basidiomata	Upper surface	Color of poroid surface	Amyloid (greenish in IKI) tramal hyphae	Shape of basidiospores	Size of Basidiospores (µm)	Cyanophilous basidiospores	References
C. gossypinus	France	Annual, Pileate	Cream to light gray; glabrous	Cream to bluish-grayish	I	Cylindrical to allantoid	4.1-5.1 × 1.2-1.7	+	Miettinen et al. (2018)
C. hirsutus	China	Annual, Pileate	Ash-gray to light grayish brown with bluish gray zones when fresh, grayish to grayish brown when dry; hirsute	Cream when fresh, straw yellow to olivaceous buff when dry	1	Cylindrical, slightly curved	4-4.7×1.2-1.5	I	Liu et al. (2021)
C. linzhiensis	China	Annual, pileate	White with a blue tint when fresh, becoming white to pinkish buff when dry, velutinate	White to pale bluish gray when fresh, pinkish buff to honey yellow and with a blue tint when dry	1	Allantoid	4-5×1.2-1.5	I	This study
C. livens	NSA	Annual, Pileate	Cream, plumbeous to bluish gray to ochraceous; velutinate	Cream, in older and dry specimens with a light bluish-grayish tint	1	Cylindrical to allantoid	4.1-5.7 × 1.1-1.5	+	Miettinen et al. (2018)
C. Iuteocaesius	France	Annual, resupinate to effused-reflexed	White to yellow when fresh, brownish when dry, tomentose	Yellow when fresh, with a light bluish tint when bruised	+	Allantoid	5-6×2	+	Ryvarden and Gilbertson (1994)
C. magnus	China	Annual, Pileate	White when fresh, cream to light grayish and ochraceous when dry; velutinate	White when fresh, ochraceous with a bluish tint when dry	1	Allantoid	3.6-4.4×1-1.2	+	Miettinen et al. (2018)
C. mediterraneocaesius	France	Annual, effused- reflexed to resupinate	White to cream or pale ochraceous; velutinate	White to cream, in older and dry specimens pale ochraceous, with a light bluish-grayish tint	1	Cylindrical to allantoid	4.2-5.8×1.3-1.7	+	Miettinen et al. (2018)
C. microporus	China	Annual, Pileate	White to cream with blue tint when fresh, cream to pinkish-buff when dry; velutinate	White when fresh, bluish when bruised, cream to buff when dry	1	Allantoid	4.5-4.9×1-1.2	I	Shen et al. (2019)
C. miscanthi	China	Annual, Pileate to effused-reflexed	White to pale bluish gray when fresh and dry; velutinate	White to pale bluish gray when fresh, bluish gray to ash gray when dry	1	Cylindrical to allantoid	4-5×1.5-2	I	This study
C. nothofagicola	Australia	Annual, Pileate to effused-reflexed	Buff to olivaceous buff when fresh, pale mouse gray to buff yellow when dry; tomentose	White to cream when fresh, cream to buff yellow when dry	I	Cylindrical to allantoid	3.8-5 × 1-1.7	I	Liu et al. (2021)
C. piceicola	China	Annual, Pileate	Cream to clay buff, with bluish gray zones when fresh, light grayish- brown when dry, velutinate	White with a bluish tint when fresh, cream when dry	1	Allantoid	4-4.5 × 0.9-1.3	I	Shen et al. (2019)
C. populi	NSA	Annual, Pileate to effused-reflexed	White to cream, pale ochraceous to grayish, rarely with bluish flecks or indistinct zones; glabrous	White to cream when fresh, in older and dry specimens with a light bluish-grayish tint	I	Cylindrical to allantoid	4.2-5.6×1-1.3	+	Miettinen et al. (2018)
C. rigidus	China	Annual, Pileate	Buff yellow to clay buff when fresh, olivaceous buff to grayish brown when dry; glabrous	White to cream when fresh, buff yellow to pinkish buff when dry	1	Cylindrical to allantoid	3.7-4.2 × 0.9-1.3	I	Liu et al. (2022)
C. simulans	Finland	Annual, effused- reflexed to resupinate	White to cream when fresh, blue, grayish or pale ochraceous when dry, glabrous	White to cream when fresh, in older and dry specimens with a light bluish-grayish tint	+	Cylindrical to allantoid	4.4-6.3 × 1.3-1.8	+	Miettinen et al. (2018)

Species	Type locality	Basidiomata	Upper surface	Color of poroid surface	Amyloid (greenish in IKI) tramal hyphae	Shape of basidiospores	Size of Basidiospores (µm)	Cyanophilous basidiospores	References
C. subcaesius	France	Annual, Pileate to effused-reflexed	White to ochraceous with a slight grayish to bluish tint in spots and streaks; glabrous	White to pale gray	I	Allantoid	4-5×1-1.2	I	Ryvarden and Melo (2017)
C. subhirsutus	China	Annual, Pileate	Pale mouse-gray and cream zones when fresh, cream to buff when dry; hirsute	White when fresh, pinkish buff to honey yellow when dry	I	Allantoid	4-4.5 × 0.9-1.3	+	Shen et al. (2019)
C. submicroporus	China	Annual, Pileate	Cream to pinkish buff when fresh, buff to buff yellow when dry, velutinate	White to smoky gray when fresh, buff to olivaceous buff when dry	I	Allantoid	3.6-4.7 × 1-1.3	+	Liu et al. (2021)
C. subungulatus	China	Annual, Pileate	Pale mouse-gray to ash-gray when fresh, dark-gray to mouse-gray when dry; glabrous	White to cream when fresh, cream to pinkish buff when dry	I	Cylindrical to allantoid	4.5-5.2 × 1.1-1.4	I	Liu et al. (2022)
C. subviridis	Mexico	Annual, Pileate	Pale ochraceous, ochraceous to grayish; glabrous	White to cream, in older and dry specimens with a light bluish- grayish tint	I	Cylindrical to allantoid	3.8-4.5×1-1.3	+	Miettinen et al. (2018)
C. tabuliformis	China	Annual, pileate	Cream to buff at the base, grayish blue at the margin when fresh, olivaceous buff to ash gray when dry; hirsute	White to sulphur yellow when fresh, cream, pale cinnamon buff to pale mouse-gray when dry	1	Cylindrical to allantoid	4.3-5.5×1.5-2	1	This study
C. tenuicontextus	China	Annual, Pileate	Cream to pinkish buff with a little blue tint when fresh, light vinaceous gray to pale mouse-gray when dry, velutinate	White to cream when fresh, pinkish buff to buff when dry	1	Allantoid	3.8-4.3 × 0.8-1.2	1	Liu et al. (2022)
C. tenuis	China	Annual, Pileate to effused-reflexed	Buff to olivaceous buff when fresh, cream to olivaceous buff when dry tomentose	White to cream when fresh, buff yellow to pinkish buff when dry	1	Cylindrical, slightly curved	4.7-6×1.3-2	+	Liu et al. (2021)
C. tricolor	China	Annual, Pileate	Light grayish brown with bluish gray zones when fresh, grayish brown when dry, velutinate	White when fresh, cream to buff when dry	I	Allantoid	4-4.8 × 0.8-1.2	+	Shen et al. (2019)
C. ungulatus	China	Annual, Pileate	Olivaceous buff, pinkish buff, cream to ash-gray and white zones when fresh, slightly darkening when dry; glabrous	White when fresh, cream when dry	1	Allantoid	4.5-5 × 0.9-1.2	I	Shen et al. (2019)
C. yanae	Russia	Annual, effused- reflexed to resupinate	White to cream, pale ochraceous or bluish to deep brown; glabrous	White, with a light to strong bluish- grayish tint	I	Cylindrical to allantoid	4.3-5.8×1.2-1.6	+	Miettinen et al. (2018)
Bold = new taxa. Abbrev	riations user	d: + = present, - = Abs	sent.						



Figure 2. Basidiomata of Cyanosporus linzhiensis (Dai 27023, holotype). Scale bar: 1 cm.

Basidiomata annual, pileate, soft and without odor or taste when fresh, becoming soft corky to fragile upon drying; pileus flabelliform, up to 3 cm, 3.5 cm wide and 8 mm thick at the base. Pileal surface white, somewhat with a bluish tint when fresh, becoming white to pinkish buff when dry, velutinate, azonate. Hymenophore white to pale bluish gray when fresh, becoming pinkish buff to honey yellow and with a blue tint upon drying; sterile margin almost absent; pores angular to irregular, 5–6 per mm, with thin dissepiments becoming lacerate. Context white, soft corky, up to 5 mm thick. Tubes concolorous with pore surface, soft corky to fragile when dry, up to 3 mm long. Context and tubes turn dark olive green in KOH.

Hyphal system monomitic; hyphae clamped, hyaline, slightly thick-walled, with a wide lumen, smooth; in the context frequently branched, more or less flexuous, loosely interwoven, $3-5 \mu m$ in diam; in the tubes unbranched, straight, subparallel along the tubes, agglutinated, $2-3.5 \mu m$ in diam.

Cystidia absent, but cystidioles fusoid are present, thin-walled, $12-13.5 \times 3 \mu m$. **Basidia** clavate, $9-13 \times 4-5 \mu m$, with basal clamp and four sterigmata.

Basidiospores allantoid, $4-5 \times 1.2-1.5 \mu$ m, L = 4.5 μ m, W = 1.4 μ m, Q= 3.1- 3.5 (n = 60/2), thin-walled, smooth, hyaline, IKI-, CB-.

Type of rot. Brown rot.

Additional specimen examined. CHINA. • Xizang Autonomous Region: Nyingchi, Bomê County, 27 Oct. 2023, on fallen angiosperm trunk, Dai 27141 (BJFC 044575, GenBank: ITS PP479781, LSU PP479803, mtSSU PP510196, nrSSU PP488288, RPB1 PP526258, RPB2 PP526267).



Figure 3. Cyanosporus linzhiensis (Dai 27023, holotype,) **A** basidiospores **B** basidia and basidioles **C** cystidioles **D** hyphae from context **E** hyphae from trama. Scale bars: $5 \mu m$ (**A**); $10 \mu m$ (**B**-**E**).

Cyanosporus miscanthi Y.C. Dai, Chao G. Wang, Yuan Yuan & Ghobad-Nejhad, sp. nov. MycoBank No: 853175

Figs 4, 5

Holotype. CHINA. • Xizang Autonomous Region: Nyingchi, Medog County, 24 Oct. 2023, on dead *Miscanthus*, Dai 26687 (BJFC 044237, GenBank: ITS PP479786, LSU PP479808, mtSSU PP510201, nrSSU PP488293, RPB1 PP526263, RPB2 PP526272, TEF1 PP526277).

Etymology. In reference to *Miscanthus* the genus where this species was found. **Diagnosis.** *Cyanosporus miscanthi* is characterized by effused-reflexed to pileate tiny basidiomata, slightly concentrically zonate pileal surface, white to pale bluish gray pore surface when fresh, angular pores, 7–9 per mm, fusoid cystidioles and cylindrical to allantoid basidiospores, $4-5 \times 1.5-2 \mu m$.



Figure 4. Basidiomata of Cyanosporus miscanthi (Dai 26687, holotype). Scale bar: 1 cm.

Basidiomata annual, effused-reflexed to pileate, soft and without odor or taste when fresh, becoming fragile to soft corky upon drying, up to 1 cm long and 0.8 cm wide when resupinate; pileus semicircular, projecting up to 0.8 cm, 1.2 cm wide and 1.2 mm thick at the base. Pileal surface white, pale bluish gray to bluish green when fresh and dry, velutinate, slightly concentrically zonate when dry; margin sharp, slightly curved when dry. Hymenophore poroid, white to pale bluish gray when fresh, becoming bluish gray to ash gray when dry; sterile margin almost absent; pores angular, 7–9 per mm; dissepiments thin, entire to slightly lacerate. Context white, soft corky, up to 0.3 mm thick. Tubes concolorous with pore surface, fragile to soft corky when dry, up to 0.9 mm long. Context and tubes turn dark olive green in KOH.

Hyphal system monomitic; hyphae clamped, hyaline, slightly thick-walled, smooth, with a wide lumen, frequently branched, more or less flexuous, in the context loosely interwoven, $3-4.5 \,\mu$ m in diam in the tubes subparallel along the tubes, agglutinated, $2.5-3 \,\mu$ m in diam.

Cystidia absent, but cystidioles fusoid present, thin-walled, $11-15 \times 4 \mu m$.

Basidia clavate, $9-13 \times 4-5 \mu m$, with four sterigmata and a basal clamp connection.

Basidiospores cylindrical to allantoid, $4-5(-5.5) \times 1.5-2 \mu m$, L = 4.2 μm , W = 1.9 μm , Q = 2.2-2.4 (n = 120/4), hyaline, thin-walled, IKI-, CB-.

Type of rot. Brown rot.

Additional specimens examined. CHINA. • Xizang Autonomous Region: Nyingchi, Medog County, 24 Oct. 2023, on dead *Miscanthus*, Dai 26684 (BJFC044234, ITS PP479784, LSU PP479806, mtSSU PP510199, nrSSU PP488291, RPB1 PP526261, RPB2 PP526270, TEF1 PP526276); Dai 26695 (BJFC044245, ITS PP479787, LSU



Figure 5. Cyanosporus miscanthi (Dai 26687, holotype) **A** basidiospores **B** basidia and basidioles **C** cystidioles **D** hyphae from context **E** hyphae from trama. Scale bars: $5 \mu m$ (**A**); $10 \mu m$ (**B**-**E**).

PP479809, mtSSU PP510202, nrSSU PP488294, RPB1 PP526264, RPB2 PP526273, TEF1 PP526278); Dai 26689 (BJFC044239, ITS PP479783, LSU PP479805, mtSSU PP510198, nrSSU PP488290, RPB1 PP526260, RPB2 PP526269, TEF1 PP526275); Dai 26701 (BJFC044251, ITS PP479785, LSU PP479807, mtSSU PP510200, nrSSU PP488292, RPB1 PP526262, RPB2 PP526271).

Cyanosporus tabuliformis Y.C. Dai, Chao G. Wang, Yuan Yuan & Ghobad-Nejhad, sp. nov.

MycoBank No: 853176 Figs 6, 7

Holotype. CHINA. • Shanxi Province: Changzhi, Qinyuan County, Taiyueshan Forest Park, 31 Aug. 2023, on fallen branch of *Pinus tabuliformis*, Dai 26063 (BJFC

043612, Genbank: ITS PP479788, LSU PP479810, mtSSU PP510203, nrSSU PP488295, RPB1 PP526265, RPB2 PP526274, TEF1 PP526279).

Etymology. In reference to the specific epithet of the substrate, *Pinus tabuliformis* in which this species was found.

Diagnosis. Cyanosporus tabuliformis is characterized by a pileate basidiomata with cream, buff to grayish blue and hirsute azonate pileal surface when fresh, angular pores, 4-5 per mm, fusoid cystidioles, and cylindrical to allantoid basidiospores, $4.3-5.5 \times 1$. 5-2 µm.

Basidiomata annual, pileate, soft and without odor or taste when fresh, becoming more or less fragile to corky upon drying. Pileus flabelliform, projecting up to 1.5 cm, 3.5 cm wide and 5 mm thick at the base. Pileal surface cream to buff at the base, grayish blue at the margin when fresh, becoming olivaceous buff to ash gray upon drying, hirsute, azonate when dry; margin blunt. Hymenophore poroid, white to sulphur yellow when fresh, unchanged when bruised, becoming cream, pale cinnamon buff to pale mouse gray upon drying; sterile margin white when fresh, cream to buff when dry, up to 0.2 mm wide; pores angular to irregular, 4–5 per mm, with thin dissepiments, becoming lacerate. Context white, soft corky, up to 2 mm thick. Tubes concolorous with pore surface, fragile to soft corky when dry, up to 3 mm long.

Hyphal system monomitic, hyphae clamped, hyaline, slightly thick-walled with a wide lumen, in the context frequently branched, straight, distinctly interwoven, $3-4 \mu m$ in diam; in the tubes rarely branched, more or less flexuous, subparallel along the tubes, agglutinated, $2.8-3.5 \mu m$ in diam.

Cystidia absent, but cystidioles fusoid present, $10-12 \times 4 \mu m$.

Basidia clavate, $13-16 \times 4.5-5 \mu m$, with basal clamp and four sterigmata.



Figure 6. Basidiomata of Cyanosporus tabuliformis (Dai 26063, holotype). Scale bar: 1 cm.





Basidiospores cylindrical to allantoid, $4.3-5.5 \times 1.5-2 \mu m$, L = $4.8 \mu m$, W = $1.9 \mu m$, Q = 2.6 (n = 60/2), hyaline, thin-walled, sometimes with one or two small guttules, IKI-, CB-.

Type of rot. Brown rot.

Additional specimen examined. CHINA. • Shanxi: Changzhi, Qinyuan County, Taiyueshan Forest Park, 31 Aug. 2023, on fallen branch of *Pinus tabuliformis*, Dai 26066 (BJFC 043615, Genbank: ITS PP479789, LSU PP479811, mtSSU PP510204, nrSSU PP488296, RPB1 PP526266, TEF1 PP526280).

Discussion

The *Cyanosporus* was established by McGinty in1909 with one species *C. caesius*. Recently, Papp (2014) based on morphological characters, proposed the subgenus *Postia* subg. *Cyanosporus* for the *P. caesius* complex. Mi-

ettinen et al. (2018) studied the *Postia caesia* complex using sequences from two DNA loci, ITS and TEF1 selected a neotype of *P. caesia* (LY BR-6776 collected from Germany) from type locality and described ten new species in *Postia*. However, *Cyanosporus* as an independent genus was raised again in recent studies (Shen et al. 2019; Liu et al. 2021, 2022, 2023). In our study, samples of 38 *Cyanosporus* species including three new species formed a strongly supported clade distinguished from *Postia* (Fig. 1).

Cyanosporus is a cosmopolitan genus causing a brown rot in different angiosperm and gymnosperm wood. Out of 38 species, currently 26 species are recorded in China. *Cyanosporus* usually has effused-reflexed to pileate poroid basidiomata with a bluish tint and thin- to slightly thick-walled basidiospores distinguished from other genera of Postiaceae (Liu et al. 2023).

Cyanosporus linzhiensis is phylogenetically related to *C. magnus* (Miettinen) B.K. Cui & Shun Liu, and both species have pileate basidiomata with white, velutinate and azonate pileal surface, almost the same size of pores (4–5 per mm in *C. magnus* vs. 5–6 per mm in *C. linzhiensis*, Miettinen et al. 2018), and they are recorded in China. However, the latter has distinct white pileal margin and narrower basidiospores ($3.6-4.4 \times 1-1.2 \mu m vs. 4-5 \times 1.2-1.5 \mu m$, Miettinen et al. 2018). *Cyanosporus caesiosimulans* and *C. livens* are similar to *C. linzhiensis* by white velutinate pileal surface, almost the same pores (5–7 per mm in *C. caesiosimulans*; 4–6 per mm in *C. livens*; 5–6 per mm in *C. linzhiensis*, Miettinen et al. 2018) and allantoid basidiospores of about the same size (4.2- $5.5 \times 1.1-1.4 \mu m$ in *C. caesiosimulans*; $4.1-5.7 \times 1.1-1.5 \mu m$ in *C. livens*; $4-5 \times$ $1.2-1.5 \mu m$ in *C. linzhiensis*, Miettinen et al. 2018). However, the former two are not currently distributed in China, and unrelated to *C. linzhiensis* in phylogeny.

Cyanosporus miscanthi and C. rigidus B.K. Cui & Shun Liu are phylogenetically related (Fig. 1), but they are different in morphology. The latter has rigid basidiomata when dry, buff yellow to clay buff and distinct concentrically zonate pileal surface when fresh, buff-yellow to pinkish buff pore surface when dry, the absence of cystidioles and smaller basidiospores (3.7–4.2 × 0.9–1.3 μ m vs. 4–5 × 1.5–2 µm, Liu et al. 2022). Cyanosporus nothofagicola B.K. Cui, Shun Liu & Y.C. Dai, C. tenuis B.K. Cui, Shun Liu & Y.C. Dai and C. miscanthi share effused-reflexed to pileate basidiomata, soft corky to fragile when dry, angular pores, white, pale mouse gray to pale bluish gray pore surface when fresh, and fusoid cystidioles. However, C. nothofagicola has narrower basidiospores (3.8- $5 \times 1-1.7 \mu m vs. 4-5 \times 1.5-2 \mu m$, Liu et al. 2021), and is grown on Nothofagus occurring in Australia. Cyanosporus tenuis also has tiny basidiomata, but it has wider contextual hyphae (2.6-7 µm in diam. vs. 3-4.5 µm in diam.), larger basidia ($18-28 \times 3.7-6 \mu m vs. 9-13 \times 4-5 \mu m$) and relatively larger basidiospores $(4.7-6 \times 1.3-2 \mu m vs. 4-5 \times 1.5-2 \mu m$, Liu et al. 2021). In addition, they form independent lineages in the phylogeny (Fig. 1).

Cyanosporus tabuliformis and *C. auricomus* (Spirin & Niemelä) B.K. Cui & Shun Liu form a sister group without strong support. They share the pileate basidiomata with hirsute and azonate pileal surface, almost the same size of pores (4–6 per mm vs. 4–5 per mm, Miettinen et al. 2018) and basidiospores (4.4–5.6 × 1.5–1.8 µm vs. 4.3–5.5 × 1.5–2 µm, Miettinen et al. 2018), and growth on gymnosperm wood. However, the latter has bright yellow pore surface when fresh, green when bruised (Miettinen et al. 2018); moreover, their sister relationship lacks strong support hinting at them being two distinct species.

Cyanosporus cyanescens (Miettinen) B.K. Cui & Shun Liu has pileal surface with cream to pale ochraceous color at the base, grayish blue at the margin when fresh, and it is somewhat similar to *C. tabuliformis*, yet its slimmer basidio-spores ($4.7-6.1 \times 1.1-1.6 \mu m vs. 4.3-5.5 \times 1.5-2 \mu m$, Miettinen et al. 2018) make it different from *C. tabuliformis*. In addition, *C. cyanescens* is distantly related to *C. tabuliformis* in our phylogeny (Fig. 1).

Although extensive studies on Chinese polypores have been carried out recently, and more than 1000 species were reported (Dai 2009; Cui et al. 2019; Dai et al. 2021; Wu et al. 2022a, 2022b; Mao et al. 2023; Wang et al. 2023; Yuan et al. 2023; Zhang et al. 2023; Zhou et al. 2023; Zhao et al. 2024), the richness of this group fungi is still not well recognized, especially in the southwest China. In this paper two species of *Cyanosporus* are described from Xizang (Tibet, southwest China) demonstrate that more taxa will be described after further investigations in the virgin forests of Xizang.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Chao-Ge Wang, Shun Liu, Masoomeh Ghobad-Nejhad, Hong-Gao Liu, Yu-Cheng Dai and Yuan Yuan designed the research and contributed to data analysis and interpretation. Chao-Ge Wang and Shun Liu conducted the molecular experiments and analyzed the data. Chao-Ge Wang and Yu-Cheng Dai prepared the samples and drafted the manuscript. Chao-Ge Wang, Masoomeh Ghobad-Nejhad, Hong-Gao Liu, Yu-Cheng Dai and Yuan Yuan discussed the results and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability

All of the data that support the findings of this study are available in the main text.

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Research Article

Phylogenetic classification of arbuscular mycorrhizal fungi: new species and higher-ranking taxa in Glomeromycota and Mucoromycota (class Endogonomycetes)

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Abstract

Arbuscular mycorrhizal (AM) fungi - Glomeromycota and Endogonomycetes - comprise multiple species and higher-level taxa that have remained undescribed. We propose a mixed morphology- and DNA-based classification framework to promote taxonomic communication and shed light into the phylogenetic structure of these ecologically essential fungi. Based on eDNA samples and long reads as type materials, we describe 15 new species and corresponding genera (Pseudoentrophospora kesseensis, Hoforsa rebekkae, Kahvena rebeccae, Kelottijaervia shannonae, Kungsaengena shadiae, Langduoa dianae, Lehetua indrekii, Lokruma stenii, Moostea stephanieae, Nikkaluokta mahdiehiae, Parnigua craigii, Riederberga sylviae, Ruua coralieae, Tammsaarea vivikae and Unemaeea nathalieae), the genus Parvocarpum as well as 19 families (Pseudoentrophosporaceae, Hoforsaceae, Kahvenaceae, Kelottijaerviaceae, Kungsaengenaceae, Langduoaceae, Lehetuaceae, Lokrumaceae, Moosteaceae, Nikkaluoktaceae, Parniguaceae, Riederbergaceae, Ruuaceae, Tammsaareaceae, Unemaeeaceae, Bifigurataceae, Planticonsortiaceae, Jimgerdemanniaceae and Vinositunicaceae) and 17 orders (Hoforsales, Kahvenales, Kelottijaerviales, Kungsaengenales, Langduoales, Lehetuales, Lokrumales, Moosteales, Nikkaluoktales, Parniguales, Riederbergales, Ruuales, Tammsaareales, Unemaeeales, Bifiguratales and Densosporales), and propose six combinations (Diversispora bareae, Diversispora nevadensis, Fuscutata cerradensis, Fuscutata reticulata, Viscospora deserticola and Parvocarpum badium) based on phylogenetic evidence. We highlight further knowledge gaps in the phylogenetic structure of AM fungi and propose an alphanumeric coding system for preliminary communication and reference-based eDNA quality-filtering of the remaining undescribed genus- and family-level groups. Using AM fungi as examples, we hope to offer a sound, mixed framework for classification to boost research in the alpha taxonomy of fungi, especially the "dark matter fungi".

Key words: Dark taxa, DNA-based classification, holotype, molecular phylogeny, species description

Introduction

Arbuscular mycorrhizal (AM) fungi play a crucial role in mineral nutrition and stress alleviation of a vast majority of vascular plants (Brundrett and Tedersoo 2018), especially in the grassland and tropical forest ecosystems (Soudzilovskaia et al. 2019). Besides higher plants, AM fungi also associate with certain liverworts (Marchantiophyta) and hornworts (Anthocerotophyta), forming arbuscule-like structures in their thalli and improving their access to nutrients in soil (Ligrone et al. 2007; Bidartondo and Duckett 2010).

Traditionally, only members of the phylum Glomeromycota (occasionally considered as subphylum Glomeromycotina within Mucoromycota, sensu Spatafora et al. (2016)) have been recognised as AM mycobionts (Smith and Read 2008; Varma et al. 2017). However, there is strong morphological and molecular evidence for AM associations between Endogonomycetes (subphylum Mucoromycotina within Mucoromycota) and various plant groups, including hornworts, liverworts and herbaceous vascular plant species (Bidartondo et al. 2011; Desiro et al. 2013; Bonfante and Venice 2020). Further molecular evidence for endogonomycete associations in plant roots (Orchard et al. 2017b) suggests that these fungi may be as important as Glomeromycota in AM associations in evolutionary (Hoysted et al. 2018) and ecological (Orchard et al. 2017b) terms. Except for Geosiphon, taxa of Glomeromycota are recognised as obligate root symbionts, but such information is lacking for Endogonomycetes. Certain small groups of Endogonomycetes are known as ectomycorrhizal symbionts (Tedersoo and Smith 2017) or saprotrophs (Berch and Fortin 1983; Hirose et al. 2014). Members of Endogonomycetes may form macroscopic (semi)hypogeous fruiting bodies containing sexual zygospores or asexual chlamydospores. A few species of Glomeromycota form such fruiting bodies containing chlamydospores (also termed glomerospores), but most species of Glomeromycota produce single glomerospores on hyphal tips in soil. The few in vitro culturable species of Glomeromycota can be grown exclusively in co-culture (except Geosiphon) with plant roots (but see Tanaka et al. (2022)). Some saprotrophic, ectomycorrhizal and AM species of Endogonomycetes can be grown in pure culture (e.g. Field et al. (2015)).

Given the high attention on Glomeromycota as the primary AM root symbionts, their taxonomy is relatively well established, with three classes, six orders, 17 families and 52 genera accepted (Wijayawardene et al. 2022; Błaszkowski et al. 2022, 2023; da Silva et al. 2023). Conversely, the Endogonomycetes comprise a single order (Endogonales), two families and seven genera (Wijayawardene et al. 2022). The DNA samples of two genera of Endogonomycetes (*Peridiospora* and *Sclerogone*) have never been sequenced due to difficulties accessing old collections.

Much of the Glomeromycota DNA barcoding and phylogenetics research relies on the rRNA internal transcribed spacer (ITS) region and 5' quarter of the 28S gene (LSU). However, all nuclear LSU, ITS region and 18S rRNA gene (SSU) are nearly equally used for molecular identification from soil and plant roots. The geographically most inclusive studies have focused on either the SSU marker (Davison et al. 2015; Vasar et al. 2022) or the ITS region (Tedersoo et al. 2014, 2021; Kivlin 2020; Mikryukov et al. 2023). Due to the paucity of species-level reference data, the SSU-based surveys suffer relatively more

from poor species- and genus-level identification (Tedersoo et al. 2024). In Endogonomycetes, taxonomic studies have used all SSU, ITS and LSU markers. Short-read endogonomycete ITS1 and ITS2 sequences derived from general soil fungal surveys are common in the International Nucleotide Sequence Databases Consortium (INSDC), but the few endogonomycete AM-focused studies have used a long marker fragment of SSU (e.g. Bidartondo et al. (2011); Albornoz et al. (2022)). For the identification from soil or roots, sufficient coverage of both groups requires the use of specific primers. Therefore, focusing on one of the AM groups reduces the amplification of the other (Seeliger et al. 2023). Molecular identification of AM fungi has been heavily biased towards the Glomeromycota, whereas Endogonomycetes have been virtually ignored in 99% of molecular surveys of AM fungi in the last 15 years. For both groups, several undescribed family- or order-level taxa have been revealed based on eDNA, suggesting that much of the taxonomic and phylogenetic diversity remains yet to be described (Desiro et al. 2013; Öpik et al. 2014).

Historically, species of both Endogonomycetes and Glomeromycota have been described in the genus Endogone Link (erected by Link (1809)) that was used in a cross-phylum sense until 1980s, although the genus Glomus Tul. & C. Tul. was erected for G. macrocarpum nearly 150 years earlier (Tulasne and Tulasne 1844). Most of the glomeromycotan species were later transferred to Glomus under the family Glomeraceae (Pirozynski and Dalpé 1989), order Glomerales (Morton and Benny 1990), class Glomeromycetes (Cavalier-Smith 1998) and phylum Glomeromycota (Schüssler et al. 2001). In the last two decades, the initial large genera Glomus and Endogone were split into multiple smaller genera based on combined morphological and molecular analyses. Additional families and orders of Glomeromycota were described by Schüssler et al. (2001), Walker and Schüssler (2004) and Błaszkowski et al. (2021). Gigasporales and Entrophosporales were erected from Glomerales more recently (Gautam and Patel 2007; Błaszkowski et al. 2022). Paraglomerales and Archaeosporales were assigned class rank (Oehl et al. 2011). Recently, the class Endogonomycetes was erected (Doweld 2014) to include the Endogonales (Jaczewski and Jaczewski 1931), covering the mucoromycotan families Endogonaceae (Saccardo 1889) and Densosporaceae (Desiro et al. 2017) as well as two order-level clades "GS21" and "GS22" (Tedersoo et al. 2017) recognised based on soil eDNA samples.

The main purpose of this article is to develop a mixed phylogenetic classification framework that integrates environmental DNA (eDNA) sequences into a specimen-based classification system, which is particularly relevant for high-diversity and cryptic taxonomic groups, such as AM fungi with predicted richness of thousands of species. Already three decades ago, it was stated: "It is unavoidable that DNA will serve as character source for contemporary taxonomic descriptions" (cf. Reynolds and Taylor (1991:311)). Such a mixed morphology- and eDNA-based classification framework is expected to facilitate species discovery and promote work on alpha taxonomy. "Leaving this diversity unnamed or unclassified is not an option, as it would continue to be an enormous and increasing impediment to communication and research in the field" (cf. Lücking and Hawksworth (2018:146)). Fungal species with names improve our capacity to refer to particular organisms and facilitate biodiversity surveys, conservation planning and assessment of toxic, pathogenic and mutualistic organisms in a direct way (Ryberg and Nilsson 2018; Lücking et al. 2021). Furthermore, a well-structured taxonomic hierarchy would offer additional possibilities for using phylodiversity and evolutionary methods without performing phylogenetic analyses (Tedersoo et al. 2018), and it would improve taxonomy-aware chimera filtering in reference-based methods for metabarcoding analyses (Nilsson et al. 2010). The main shortfalls of sequence-based classification include eroding the concept of physical type material and parallel classifications based on specimens and sequences or using different DNA markers (Hongsanan et al. 2018; Lücking and Hawksworth 2018; Thines et al. 2018). Therefore, many leading fungal taxonomists do not approve use of DNA sequences (Thines et al. 2018; Zamora et al. 2018) or eDNA sample (Hongsanan et al. 2018) as holotypes.

Here, we use the mixed specimen-eDNA phylogenetic classification framework to shed light into the phylogenetic diversity of the two groups of AM fungi - Glomeromycota and Endogonomycetes. By using eDNA samples as holotypes (Reynolds and Taylor 1991; Renner 2016), DNA sequences as lectotypes and diagnoses based on molecular differences in ITS and LSU marker genes (Renner 2016), we first describe novel species for the highly divergent groups of AM fungi following the International Code of Nomenclature for Algae, Fungi and Plants (Turland et al. 2018; Lücking et al. 2021). Building on these species, we then introduce novel families and orders. Finally, we provide a large number of taxonomically re-annotated and novel SSU, ITS and LSU sequences, equipped with preliminary alphanumeric taxonomic identifiers, where relevant, to the scientific community.

Materials and methods

We downloaded the sequence data identified as Glomeromycota, Mucoromycota and uncultured fungi from three nucleotide sequence databases - NCBI (Sayers et al. 2024; https://www.ncbi.nlm.nih.gov/), UNITE v.9.1 (Abarenkov et al. 2024; https://unite.ut.ee/) and EUKARYOME v.1.7 (Tedersoo et al. 2024; https://eukaryome.org/). We also added rRNA gene sequences from scaffolds in the Joint Genome Institute data portal (https://genome.jgi.doe.gov/portal/). The unidentified fungi were first assigned to rough taxonomic groups based on BLASTn gueries against identified sequences in EUKARYOME v.1.7. For sequences affiliated with Glomeromycota or Endogonomycetes, we conducted phylogenetic analyses separately for the SSU, LSU and a longer fragment spanning much of SSU, ITS and LSU. A large part of the ITS region was not used for the phylogeny reconstruction because of alignment unreliability. The sequences of Glomeromycota and Endogonomycetes were aligned using MAFFT v.7 (Katoh and Standley 2013), followed by manual trimming of overarching and misaligned ends and manual correction in case of obvious misalignments using AliView v.1.26 (Larsson 2014). The alignments were further trimmed to exclude unalignable regions and subjected to ClipKIT v.1.4.0 (Steenwyk et al. 2020) to remove phylogenetically uninformative positions, including rare introns and insertions. Based on the alignments, we visually evaluated mismatches to commonly used primers targeting SSU, ITS and LSU regions.

Phylogenetic analyses were performed using IQ-TREE v.2.2.5 (Minh et al. 2020), with standard options including 1000 trees and 1000 ultrafast boot-

strap replicates. The trees were visualised and used for taxonomic re-annotation in FigTree v.1.4.4 (Rambaut 2018). Various taxa of Mucoromycota with relatively short branches were tested as potential outgroups. The first three rounds of alignments and analyses were primarily used to detect and remove low-quality reads and chimeric sequences. From the fourth round onwards, the reads were assigned to clades corresponding to putative genera, families and orders, following the monophyly criterion and accounting for the level of sequence divergence in previously described groups. We included at least one read from each described species to delimit clades and assign taxonomy. For both Glomeromycota and Endogonomycetes, we focused mainly on the long fragment covering the ITS and LSU regions because of: 1) the greatest taxonomic resolution in the ITS2 and D2 subregion of LSU, 2) the occurrence of the largest number of described species and 3) the presence of most abundant and diverse set of eDNA reads from soil and roots falling into these groups (Tedersoo et al. 2024).

Diagnoses of species were prepared based on molecular characters in the ITS and LSU regions by selecting the most characteristic short barcodes (20–30 bases) for the target species using multiple sequence alignments. The barcodes typically had no ambiguous position for the target species and had at least two differences from closely-related species. We also estimated the number of mutations (i.e. alignment mismatches) allowed for the target species to be separable from related species (typically set to 0 or 1). For the entire alignment length of ITS and LSU, we estimated the maximum proportion of differences amongst sequences corresponding to the target species (i.e. within-species variability).

For establishing higher-ranking taxa such as genera, families and orders, we used the following criteria: i) monophyly; ii) bootstrap support >95; iii) phylogenetic breadth and divergence roughly comparable to previously described taxa; and iv) minimising the number of novel taxa (i.e. preferably retaining larger groups if there were multiple alternative splitting possibilities). Based on a visual assessment of the ITS and LSU alignments and phylograms, we predicted the approximate number of (potential) species for each newly-described genus (but extrapolation to unobserved taxa was not attempted).

The eDNA samples with the highest proportions of target reads were selected as holotypes, except in the cases where long reads spanning SSU, ITS and LSU were available along with the stored DNA samples. Lectotypes were identified amongst the highest-quality sequences derived from these holotype DNA samples. Most of the type materials and additional samples were derived from composite topsoil samples (40 subsamples of 5 cm diam. to 5 cm depth from 2500-m² area) of the Global Soil Mycobiome consortium (GSMc) project (Tedersoo et al. 2021), FunAqua sediment samples (V. Prins et al., unpublished) or from various soil samples sequenced by Jamy et al. (2022). Both eDNA and corresponding substrate samples are maintained as vouchered collections in the repository of the University of Tartu (acronym TUE, with 6-digit accession numbers). The sequences were first deposited in the EUKARYOME database (denoted by "EUK" with 7-digit accession numbers) and subsequently submitted to the INSDC and UNITE databases. EUKARYOME v.1.9.2 includes 55,648 and 10,081 annotated reads of Glomeromycota and Endogonomycetes, respectively.

Results

Phylogeny of Glomeromycota

The SSU-ITS-LSU phylogram supported the separation of all described Glomeromycota orders and families, and placed these into expected positions (Fig. 1, Suppl. material 1) as in previous analyses based on rRNA gene and partial genomes (Stockinger et al. 2012; Montoliu-Nerin et al. 2021; Rosling et al. 2024). A vast majority of valid genera were separated from each other with high statistical support. As exceptions, the genera Otospora Oehl, Palenzuela & N.Ferrol (O. bareae) and Tricispora Oehl, Sieverd., G.A.Silva & Palenz. (T. nevadensis) were nested within Diversispora C.Walker & A.Schüssler, whereas species of Dentiscutata Sieverd., F.A.Souza & Oehl (D. cerradensis and D. reticulata) were placed within Fuscutata Oehl, F.A.Souza & Sieverd. The type species of Dentiscutata (D. nigra) was not sequenced for the ITS region, but an analysis of the LSU region indicated that D. nigra is placed separately from other species of Dentiscutata that clustered with Fuscutata (Suppl. material 2). Corymbiglomus corymbiforme Blaszk. & Chwat – the type species of this genus – was nested within the genus Redeckera C.Walker & A.Schüssler, whereas C. globiferum (Koske & C.Walker) Blaszk. & Chwat served as a sister group to species of Redeckera. Furthermore, the recently described genus Blaszkowskia G.A.Silva & Oehl was nested within Viscospora. Where relevant, we propose new combinations (see below).

Of previously described species, *Dominikia compressa* (Sieverd., Oehl, Palenz., Sánchez-Castro & G.A.Silva) Oehl, Palenz., Sánchez-Castro & G.A.Silva (basionym *Glomus compressum* Sieverd., Oehl, Palenz., Sánchez-Castro & G.A.Silva) formed a well-supported group in a sister position to the rest of *Dominikia* Błaszk., Chwat & Kovács, but their close relationship was poorly supported and inconsistent amongst various phylograms prepared. Thus, *D. compressa* is currently being transferred to a new genus (J. Błaszkowski et al., in prep.). Similarly, *Glomus badium* Oehl, D.Redecker & Sieverd. was placed outside the genus *Glomus* as a well-supported small clade, but its sister relationships with other genera remain unresolved. Based on both phylogenetic and morphological characters, we propose to treat *G. badium* as a new genus, herein designated as *Parvocarpum* (see below).

Our phylogenetic analysis revealed a large number of previously undescribed or unsequenced taxa. One of these taxa was located as a deep clade in the Entrophosporales, which warrants consideration as a new family outside the Entrophosporaceae. We describe the new species, genera and families based on eDNA samples and sequences. The Archaeosporaceae and Diversisporaceae families each revealed two novel genus-level taxa, whereas the Paraglomeraceae harboured one new genus-level taxon. The most prominent family – Glomeraceae – was found to include 30 novel genus-level taxa clearly distinct from current delimitations of known genera based on our criteria. We propose informal alphanumeric labels for these genera to enable their communication (see below). For the Glomeraceae, it is most likely that, upon DNA sequencing of materials belonging to unsequenced species, many will fall into these unnamed groups (like the cases of *D. compressa* and *G. badium*).



Figure 1. Phylogenetic position of genera and genus-level groups of Glomeromycota based on Maximum Likelihood analysis of SSU-ITS-LSU sequences. The clades are collapsed, with the number of sequences included in parentheses. Question marks above branches indicate low ultra-rapid bootstrap support (< 95). Orders are highlighted in different colours. For the genus *Entrophospora*, the so-called S-type reads (VanKuren et al. 2013) were included for comparison. The uncollapsed phylogram is given in Suppl. material 1.

Phylogeny of Endogonomycetes

The SSU-5.8S-LSU phylogram resolved the internal structure of Endogonomycetes reasonably well, except the order of divergence for most of the 17 main, deep-branching groups (Fig. 2, Suppl. material 3). The hitherto described and sequenced species of Endogone and Jimgerdemannia Trappe, Desirò, M.E.Sm., Bonito & Bidartondo, as well as Densospora McGee and Sphaerocreas Sacc. & Ellis, formed two relatively large order-level groups that were distantly related to each other and surrounded by eDNA sequences derived from soil. Species of Bifiguratus Torres-Cruz & Porras-Alfaro formed a small, deep-branching, order-level group, with no clear sister group. For each of the additional 14 order-level groups, we described new species based on eDNA sample and seguence information. These 14 species were further assigned to genera and families based on the internal branching structure of these orders, with other unnamed groups labelled alphanumerically. Two potentially order-level groups remain undescribed and unlabelled, because these were found from a single locality and their phylogenetic position may change with extra sequences and more precise alignments.

The additional SSU phylogram confirmed the separation of the main orders, although nearly half of them lacked SSU sequence data or were represented by a single read (Suppl. material 4). Furthermore, the SSU phylogram indicated that the vast majority of putative AM fungi were widely dispersed in the groups corresponding to Densosporales (mostly Densosporaceae and Planticonsortiaceae), Endogonales (including Endogonaceae, Jimgerdemanniaceae and other family-level taxa) and Hoforsales (formerly clade GS22), and to some extent in Bifiguratales. However, a few root-derived sequences fell outside these groups, suggesting that certain other orders lacking the SSU sequence data may also host AMF.

Taxonomic combinations, emendations and descriptions in Glomeromycota

Diversispora bareae (Palenz., N.Ferrol & Oehl) Tedersoo & Magurno, comb. nov. MycoBank No: 853545

Otospora bareae Palenz., N.Ferrol & Oehl, in Palenzuela, Ferrol, Boller, Azcón-Aguilar & Oehl, Mycologia 100(2): 298 (2008). Basionym.

Description. As presented originally in Palenzuela et al. (2008).

Diagnosis. *Diversispora bareae* differs from other species of the *Diversispora* by producing acaulosporoid (otosporoid) spores compared with diversisporoid and entrophosporoid (tricisporoid) spores in other described species. Glomerospores with inner flexible hyaline layer and pigmented sporiferous saccule. Phylogenetically belongs to *Diversispora* based on the SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1).

Notes. The new combination invites an amendment of the genus *Diversispora* to accommodate species with otosporoid spores.



Figure 2. Phylogenetic position of genera and genus-level groups of Endogonomycetes based on Maximum Likelihood analysis of SSU-5.8S-LSU sequences. The clades are collapsed, with the number of sequences included in parentheses. Question marks above branches indicate low ultra-rapid bootstrap support (< 95). Orders are highlighted in different colours. The uncollapsed phylogram is given in Suppl. material 3.

Diversispora nevadensis (Palenz., N.Ferrol, Azcón-Aguilar & Oehl) Tedersoo & Magurno, comb. nov.

MycoBank No: 853546

Entrophospora nevadensis Palenz., N.Ferrol, Azcón-Aguilar & Oehl, in Palenzuela, Barea, Ferrol & Azcón-Aguilar, Mycologia 102(3): 627 (2010). Basionym.

Description. See Palenzuela et al. (2010).

Diagnosis. *Diversispora nevadensis* differs from other species of the *Diversispora* by producing entrophosporoid (tricisporoid) spores compared with diversisporoid and acaulosporoid (otosporoid) spores in other species. Glomerospores with inner flexible hyaline wall layers without granular beaded surface and no Melzer reaction. Phylogenetically nested in *Diversispora* based on the SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1).

Notes. The new combination invites an amendment of the genus *Diversispora* to accommodate species with entrophosporoid (tricisporoid) spores.

Diversispora C.Walker & A.Schüssler emend. Tedersoo & Magurno MycoBank No: 28884

Type species. *Diversispora spurca* (C.M.Pfeiffer, C.Walker & Bloss) C.Walker & Schüssler.

Description. Spores diversisporoid, rarely otosporoid or tricisporoid. Diversisporoid spores formed singly, in clusters or in large disorganised fruiting bodies with high spore numbers. Spores with 1–4 wall layers; pores often closed with a septum. Subtending hyphal pores rarely open. Otosporoid spores formed laterally on the persistent neck of a sporiferous saccule. Tricisporoid spores with inner flexible hyaline wall layers (formed de novo) without granular beaded surface and no Melzer reaction. Spore pores generally closed by a septum at the spore base, arising from the innermost wall lamina or inner layer or from both. Forms a monophyletic group within Diversisporaceae based on the SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1).

Fuscutata cerradensis (Spain & J. Miranda) Tedersoo & Magurno, comb. nov. MycoBank No: 853547

Scutellospora cerradensis Spain & J. Miranda, Mycotaxon 60: 130 (1996). Basionym. Dentiscutata cerradensis Sieverd., F.A.Souza & Oehl, Mycotaxon 106: 342 (2009). Synonym.

Description. See Spain and Miranda (1996).

Diagnosis. Fuscutata cerradensis differs from other species of the Fuscutata by spore wall ornamentation, three-walled spores and dark-pigmented multilobed germinal shield produced in the inner wall. Phylogenetically forms a monophyletic clade with *F. heterogama* - the type species of genus - based on the SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1). **Notes.** The new combination invites an amendment of genus *Fuscutata* to accommodate species with dark, multilobed germinal shields. However, we decided not to prepare an amendment for *Fuscutata* because the genus *Dentiscutata*, their close relative, requires additional information to confirm their status, supported only in the LSU sequence of *D. nigra*.

Fuscutata reticulata (Koske, D.D.Mill. & C.Walker) Tedersoo & Magurno, comb. nov.

MycoBank No: 853548

Gigaspora reticulata Koske, D.D.Mill. & C.Walker, Mycotaxon 16(2): 429 (1983). Basionym.

Dentiscutata reticulata (Koske, D.D.Mill. & C.Walker) Sieverd., F.A.Souza & Oehl, Mycotaxon 106: 342 (2009). Synonym.

Description. See Koske et al. (1983).

Diagnosis. Fuscutata reticulata differs from other species of the Fuscutata by spore wall ornamentation, three-walled spores and dark-pigmented, multilobed germinal shield produced in the inner wall. Phylogenetically forms a monophyletic clade with *F. heterogama* - type species of genus - based on the SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1).

Notes. See note of F. cerradensis.

Viscospora deserticola (Trappe, Bloss & J.A.Menge) Tedersoo & Magurno, comb. nov.

MycoBank No: 853549

Glomus deserticola Trappe, Bloss & J.A.Menge, Mycotaxon 20 (1): 123 (1984). Basionym.

Blaszkowskia deserticola (Trappe, Bloss & J.A.Menge) Oehl & G.A.Silva, Mycol. Progr. 22 (11, no. 74): 5 (2023). Synonym.

Description. See Trappe et al. (1984).

Diagnosis. Subtending hyphae pigmented over long distances (>100 µm) unlike in other species of *Viscospora* and *Septoglomus*. Differs from other species of *Viscospora* by spore colour (da Silva et al. 2023).

Notes. Transferred from *Blaszkowskia* to *Viscospora* because of phylogenetic nestedness within *Viscospora* and recognition as a separate genus would render *Viscospora* paraphyletic and leave many orphan taxa in the *Septoglomus-Viscospora* clade (Suppl. material 1; da Silva et al. (2023)).

Parvocarpum Magurno, gen. nov. MycoBank No: 853558

Type species. Parvocarpum badium (Oehl, Redecker & Sieverd.) Magurno.

Description. Producing glomoid-like spores surrounding a central plexus of interwoven hyphae in small organised fruiting bodies, lacking a peridium. Spores with inner flexible hyaline layer and short subtending hyphae. Forms a monophyletic group within Glomeraceae based on SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1).

Notes. Based on ITS and LSU sequences, Parvocarpum includes 10-20 species.

Parvocarpum badium (Oehl, Redecker & Sieverd.) Magurno, comb. nov. MycoBank No: 853560

Glomus badium Oehl, D.Redecker & Sieverd., Angew. Botan. 79: 39 (2005). Basionym.

Funneliformis badius (Oehl, Redecker & Sieverd.) C.Walker & A.Schüssler. Synonymy.\

Description. See Oehl et al. (2005).

Etymology. *parvus* (Latin) = small; and *carpum* (Latin) = body, referring to the small size of fruiting bodies produced.

Diagnosis. *P. badium* differs from other genera of the Glomeraceae by producing glomoid-like spores surrounding a central plexus of interwoven hyphae in small organised fruiting bodies, lacking a peridium. Spores with inner flexible hyaline layer and short subtending hyphae. Phylogenetically distinct from *G. macrocarpum* and other *Glomus sens. str.* species based on the SSU-ITS-LSU phylogram (Fig. 1, Suppl. material 1).

Notes. Phylogenetic position of *P. badium* within the genus *Parvocarpum* is unresolved because of a single available short read.

Pseudoentrophosporaceae Tedersoo & Magurno, fam. nov.

MycoBank No: 853564

Type genus. Pseudoentrophospora Tedersoo & Magurno.

Description. Covers the monophyletic group in Entrophosporales (Fig. 1). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1631429, EUK1105140 and EUK0135500 (Suppl. material 1).

Notes. Recognised based on eDNA sequences only. Currently monogeneric.

Pseudoentrophospora Tedersoo & Magurno, gen. nov. MycoBank No: 853565

Type species. Pseudoentrophospora kesseensis Tedersoo & Magurno.

Description. Covers the monophyletic group in Pseudoentrophosporaceae (Fig. 1). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1631429, EUK1105140 and EUK0135500 (Suppl. material 1).

Notes. Recognised based on eDNA sequences only. There are potentially 3–6 species in *Pseudoentrophospora* based on ITS sequences, some of which are represented by sequences EUK1105140 (tropical rainforest soil in El

Yunque, Puerto Rico, 18.29°N, -65.78°E); EUK1010525 (GSMc plot S056, tropical rainforest soil in Pegaima Mountains, Guyana, 5.43567°N, -60.08825°E); and EUK0133825 (flooded grassland soil in Dijle, Belgium, 5.83°N, 4.65°E).

Pseudoentrophospora kesseensis Tedersoo & Magurno, sp. nov. MycoBank No: 853566

Diagnosis. Differs from other species of *Pseudoentrophospora* and *Entrophospora* based on the ITS region (ITS2 positions 127–146 gaaccgcaaattacgcatta, one mismatch allowed) and LSU (positions 486–515 gaacaggtcaacatcaattct-tattgccat, one mismatch allowed) as indicated in Fig. 3.

Type. Soil eDNA sample TUE101916 (*holotype*); eDNA sequence EUK1631429 (*lectotype*); GSMc plot G4940, coppiced *Juniperus-Acer* woodland (soil sample TUE001916) in Kesse Island, Estonia, 58.63443°N, 23.43938°E.

Description. Other eDNA sequences EUK1636430–EUK1636432 from the type locality.

Etymology. *pseudo* (Greek) = false; *Entrophospora* (Latin) refers to a related fungal genus; and *kesseensis* (Latin) indicates locality of the type species. The name depicts phylogenetic relatedness to *Entrophosphora* and the only locality where the type species has been recorded.

Notes. Found from a single site, with ITS and LSU sequences differing up to 0.5% and 1%, respectively. The ITS1 subregion harbours only 58 bases, being amongst the shortest across fungi (excl. microsporidians).

Taxonomic descriptions of Endogonomycetes

Endogonomycetes Doweld emend. Tedersoo

MycoBank No: 550357

Type order. Endogonales Jacz. & P.A.Jacz.

Description. Fruiting body absent, rarely present - hypogeous or on debris, globose, irregular, sometimes resupinate, 1–20 mm in diam., may be composed of aggregated zygosporangial clusters. Reproductive structures as zygosporangia (in *Endogone, Jimgerdemannia*) or chlamydospores (in *Vinositunica, Densospora*), aggregated in the fruiting body or as chlamydospores on extraradical hyphae (in *Planticonsortium*). Chlamydospore wall continuous, multilayered, with dense subtending hyphae, lacking septa. Hyphae filamentous, coenocytic, sometimes with secondary septa, rarely yeast-like (in *Bifiguratus*). Forms a monophyletic group in Mucoromycota, as the least inclusive clade covering



Figure 3. Diagnostic barcodes for Pseudoentrophospora kesseensis relative to closely-related taxa in ITS2 and LSU.

accessions UDB025468, UDB28692, EUK1201418, EUK1203196, EUK1602762, EUK1202520, EUK1203766, EUK1107335 and EUK1602357 (Suppl. material 3).

Notes. Endogonomycetes harbours currently 17 orders and two unassigned, potentially order-level groups represented by sequences EUK1604020 and EUK1603073 (GSMc plot G3308, *Juniperus communis* coppiced grassland soil in Atla, Estonia, 58.30122°N, 21.93600°E); and EUK1602478 (GSMc plot G4627, mixed forest soil in Tudusoo, Estonia, 59.11368°N, 26.75944°E).

Hoforsales Tedersoo, ord. nov.

MycoBank No: 853567

Type family. Hoforsaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1100001, EUK1602331 and EUK1602346 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Hoforsaceae and another potentially family-level group, which is represented by sequence EUK1631675 (GSMc plot G4124, *Populus tremula* forest soil in Mäla, Estonia, 58.58693°N, 23.28597°E). Hoforsales corresponds to clade GS22 (sensu Tedersoo et al. (2017)).

Hoforsaceae Tedersoo, fam. nov.

MycoBank No: 853569

Type genus. Hoforsa Tedersoo.

Description. Covers the monophyletic group in Hoforsales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1100001, EUK1107311 and EUK1602325 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently monogeneric.

Hoforsa Tedersoo, gen. nov.

MycoBank No: 853570

Type species. Hoforsa rebekkae Tedersoo.

Description. Covers the monophyletic group in Hoforsaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1100001, EUK1107311 and EUK1602325 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. There are potentially about 20 species in *Hoforsa* based on ITS and LSU sequences, with examples including taxa represented by sequences EUK1107311 (bog peat in Svartberget, Sweden, 64.24°N, 19.76°E) and AM260926 (bog peat, Scotland) first isolated by Rebekka Artz (Artz et al. 2007). Most taxa are found from various soils, but the LSU sequence AB982123 originates from an ectomycorrhizal root of Dipterocarpaceae (Lambir, Malaysia). The most common taxon at 99% LSU sequence similarity (EUK1602281) has been recorded from 31 localities in Estonia and Latvia. The genus has a global distribution and it occurs commonly in soil samples but rarely in roots.

Hoforsa rebekkae Tedersoo, sp. nov.

MycoBank No: 853571

Diagnosis. Separation from other species of *Hoforsa* based on the ITS region (ITS2 positions 108–127 ggratcycccgaggtgtgaaac; one mismatch allowed) and LSU (positions 546–565 ctcctggtgctctcacccgt; no mismatch allowed) as indicated in Fig. 4.

Type. Soil eDNA sample: TUE128830 (*holotype*); eDNA sequence EUK1100001 (*lectotype*); *Pinus sylvestris* forest near Hofors, Sweden (60.49°N, 16.30°E).

Description. Other sequences: EUK1104560 (type locality); OU004104 (San Francisco, Ecuador, root sample); and KP889387 and KP889486 (both coniferous forest soil in British Columbia, Canada).

Etymology. *Hofors* (Swedish) refers to type locality; and *Rebekka* (Scotch) refers to the first name Rebekka Artz who was the first to collect materials from this genus.

Notes. Found from three sites across three continents, with ITS sequences differing up to 3.5% and LSU sequences up to 0.5%.

Kahvenales Tedersoo, ord. nov.

MycoBank No: 853572

Type family. Kahvenaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1634339 and EUK1630771 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Kahvenaceae.

Kahvenaceae Tedersoo, fam. nov.

MycoBank No: 853573

Type genus. Kahvena Tedersoo.

Description. Covers the monophyletic group in Kahvenales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1634339 and EUK1630771 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Kahvena.



Figure 4. Diagnostic barcodes for Hoforsa rebekkae relative to closely-related taxa in ITS2 and LSU.

Kahvena Tedersoo, gen. nov.

MycoBank No: 853574

Type species. *Kahvena rebeccae* Tedersoo.

Description. Covers the monophyletic group in Kahvenaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1634339 and EUK1630771 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Based on ITS sequences, *Kahvena* is comprised of two species; the other represented by sequences EUK1630771 (GSMc plot G4185, *Picea-Pinus* forest soil in Ristipalo, Estonia, 58.10241°N, 27.47874°E) and ON963629 (*Pinus sylvestris* forest soil, Lithuania).

Kahvena rebeccae Tedersoo, sp. nov.

MycoBank No: 853575

Diagnosis. Separation from other species of *Kahvena* based on the ITS region (ITS2 positions 200–218 cattcgcaggaatagccag; one mismatch allowed) and from other species of Endogonomycetes based on LSU (positions 653–683 ac-gcaagctccagatcgaatctccgggctaa; one mismatch allowed) as indicated in Fig. 5.

Type. Soil eDNA sample TUE100738 (*holotype*); eDNA sequence EUK1634339 (*lectotype*); GSMc plot G4196, *Populus-Picea-Pinus* forest (soil sample TUE000738) in Kahvena, Estonia (58.27991°N, 25.23165°E).

Description. Other sequences: EUK1635883–EUK1635886 (type locality); EUK1631811 (GSMc plot G2767, mixed woodland soil at Mäebe, Estonia, 58.30937°N, 22.07618°E); KF618358 (*Picea mariana* forest soil, AK, USA); MT596306 (Tobiotsuka Kofun, Japan, 34.6355°N, 133.6814°E); KU062529 (unknown source); and KF565426 (Duke Forest, NC, USA, 35.97°N, -79.09°E), isolated by Rebecca C. Mueller (Mueller et al. 2014).

Etymology. *Kahvena* (Estonian) refers to type locality; and *Rebecca* (English) refers to the first name of Rebecca C. Mueller, who collected the first materials belonging to this genus and the type species.

Notes. Found from temperate and subarctic forests in Europe, Asia and North America, with ITS and LSU sequences differing up to 4% (excluding a 29-base deletion in EUK1631811 and KU062529) and 1.5%, respectively. Considered as a single species because of high intraspecific variation amongst common sequence variants in the type locality (2% in ITS and 1% in LSU, representing both indels and substitutions).

Kelottijaerviales Tedersoo, ord. nov.

MycoBank No: 853576

Type family. Kelottijaerviaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1202520 and EUK1633699 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Kelottijaerviaceae.
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Figure 5. Diagnostic barcodes for Kahvena rebeccae relative to closely-related taxa in ITS2 and LSU.

Kelottijaerviaceae Tedersoo, fam. nov.

MycoBank No: 853577

Type genus. Kelottijaervia Tedersoo.

Description. Covers the monophyletic group in Kelottijaerviales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1202520 and EUK1633699 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Kelottijaervia*.

Kelottijaervia Tedersoo, gen. nov.

MycoBank No: 853578

Type species. Kelottijaervia shannonae Tedersoo.

Description. Covers the monophyletic group in Kelottijaerviaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1202520 and EUK1633699 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Kelottijaervia* is comprised of about five species that are represented by sequences EUK1603128 (GSMc plot G2755X, *Pinus sylvestris* forest soil, Liiva-Putla, Estonia, 58.38859°N, 22.65545°E); EUK0302816 (plot G5403, mixed coniferous forest in Kõrveküla, Estonia, 58.43789°N, 26.75099°E); EUK1104755 (*Pinus sylvestris* forest soil near Hofors, Sweden, 60.49°N, 16.30°E); and KP889573 (coniferous forest soil in British Columbia, Canada). The genus seems to prefer acidic coniferous forest habitats.

Kelottijaervia shannonae Tedersoo, sp. nov.

MycoBank No: 853579

Diagnosis. Separation from other species of *Kelottijaervia* based on the ITS region (positions 212–239 taatgtgagtgcaggaaatattatgact; one mismatch allowed) and LSU (positions 600–619 ctttggggtggcggtcgctg; one mismatch allowed) as indicated in Fig. 6.

Type. eDNA sample TUE100189 (*holotype*); eDNA sequence EUK1202520 (*lectotype*); GSMc plot G2836 Finland, subpolar *Betula pubescens* forest (soil sample TUE000189) in Kelottijärvi, Finland, 68.60353°N, 21.74517°E.

Description. Other sequences: EUK1603540, (GSMc plot G4196, *Populus-Picea-Pinus* forest soil in Kahvena, Estonia, 58.27991°N, 25.23165°E);

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Figure 6. Diagnostic barcodes for Kelottijaervia shannonae relative to closely-related taxa in ITS2 and LSU.

EUK1603663 (GSMc plot G4406, mixed coniferous forest soil in Tarumaa, Estonia, 59.20745°N, 27.15333°E); EUK1602832 (GSMc plot G5828, *Malus domestica* orchard soil in Mooste, Estonia, 58.15335°N, 27.19642°E); and KP889965 (coniferous forest soil in British Columbia, Canada) that was first isolated by Shannon H.A. Guichon (Guichon 2015).

Etymology. *Kelottijärvi* (Finnish) refers to type locality; and *Shannon* (English) refers to the first name of Shannon H.A. Guichon who collected the first materials belonging to this genus.

Notes. Found in Estonia, Finland and Canada, with ITS and LSU sequences displaying up to 2% and 1% of differences, respectively.

Kungsaengenales Tedersoo, ord. nov.

MycoBank No: 853580

Type family. Kungsaengenaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603402 and EUK1602136 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Kungsaengenaceae.

Kungsaengenaceae Tedersoo, fam. nov.

MycoBank No: 853581

Type genus. Kungsaengena Tedersoo.

Description. Covers the monophyletic group in Kungsaengenales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603402 and EUK1602136 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Kungsaengena* and a genus-level unassigned species represented by sequence EUK0013897 (GSMc plot G2907, subtropical forest soil in Cuc Phuong, Viet Nam, 20.34902°N, 105.59649°E).

Kungsaengena Tedersoo, gen. nov. MycoBank No: 853582

Type species. *Kungsaengena shadiae* Tedersoo.

Description. Covers the monophyletic group in Kungsaengenaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603402 and EUK1602136 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Kungsaengena* comprises 5–6 species. Other putative species in this genus are represented by sequences EUK1603803 (GSMc plot G5906, stadium soil in Karksi-Nuia, Estonia, 58.10088°N, 25.55959°E); EUK1603124 (GSMc plot G5003, *Pinus sylvestris* forest soil in Naissaar, Estonia, 59.5634°N, 24.5451°E); EUK1217319 (FunAqua sample W0279s, lake sediment near Bezdan, Serbia, 45.82031°N, 18.9599°E); and MW215857 (forest nursery soil in Lithuania).

Kungsaengena shadiae Tedersoo, sp. nov.

MycoBank No: 853583

Diagnosis. separation from other species of *Kungsaengena* based on the ITS region (ITS2 positions 25–44 tgggaacccatttcgtcgga; one mismatch allowed) and LSU (positions 665–694 cgttggggctgggacgcccgtcgctcgcac; one mismatch allowed) as indicated in Fig. 7.

Type. eDNA sample TUE128324 (*holotype*); eDNA sequence EUK1603402 (*lectotype*); GSMc plot G5763, wet grassland (soil sample TUE028324) in Haage, Estonia, 58.35555°N, 26.61277°E).

Description. other sequences: EUK1604022 (GSMc plot G5906, football field soil in Karksi-Nuia, Estonia, 58.10088°N, 25.55959°E); EUK1604023 (GSMc plot G5844, wet pasture soil in Tuhala, Estonia, 59.23003°N, 25.00283°E); EUK1604025 (GSMc plot G4444, Estonia, mixed forest soil in Altnurga, Estonia, 58.53676°N, 26.28321°E); and OU942286 (grassland soil in Kungsängen, Sweden, 59.837°N, 17.661°E), isolated by Shadi Eshghi Sahraei (Eshghi Sahraei et al. 2022).

Etymology. *Kungsängen* (Swedish) refers to type locality; and *Shadi* (Persian) refers to the first name of Shadi Eshghi Sahraei who analysed materials collected from the type locality.

Notes. Found from the Baltic States and Sweden, with ITS and LSU sequences differing up to 15% and 1%, respectively. The ITS region is infested with microsatellite-like regions and homopolymers, and many sequence variants have long deletions in multiple positions. *K. shadiae* seems to be generalist in terms of habitat type.

Langduoales Tedersoo, ord. nov. MycoBank No: 853584





Figure 7. Diagnostic barcodes for Kungsaengena shadiae relative to closely-related taxa in ITS2 and LSU.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1107335, EUK1103607 and EUK1632831 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Langduoaceae and another potentially family-level group, which is represented by sequences EUK1632831 (GSMc plot G4104, *Salix alba* wetland forest soil in Koiva, Estonia, 57.68283°N, 26.20146°E); EUK1603795 (GSMc plot G5906, football field in Karksi-Nuia, Estonia, 58.10088°N, 25.55959°E); and EUK1602996 (GSMc plot G4171, mixed coniferous forest soil in Nõmmeotsa, Estonia, 58.48765°N, 26.22523°E).

Langduoaceae Tedersoo, fam. nov.

MycoBank No: 853585

Type genus. Langduoa Tedersoo.

Description. Covers the monophyletic group in Langduoales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1107335, EUK1103607 and EUK1632829 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently represented by *Langduoa*.

Langduoa Tedersoo, gen. nov.

MycoBank No: 853586

Type species. Langduoa dianae Tedersoo.

Description. Covers the monophyletic group in Langduoaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1107335, EUK1103607 and EUK1632829 (Suppl. material 3).

Notes. Based on ITS sequences, *Langduoa* is comprised of 40–50 species. The genus is distributed globally in multiple habitat types, but not found from roots so far. Most *Langduoa* species are poorly separable based on the LSU marker. Other putative species in *Langduoa* are represented by sequences EUK1103607 (tropical rainforest soil in El Yunque, Puerto Rico, 18.29°N, -65.78°E); EUK1631446 (GSMc plot G4189, *Populus tremula* forest soil in Tammsaare, Estonia, 57.84444°N, 27.20141°E); and MW215048 (tree nursery soil in Lithuania), which was recorded by Diana Marčiulynienė (Marčiulynienė et al. 2021).

Langduoa dianae Tedersoo, sp. nov.

MycoBank No: 853587

Diagnosis. Separation from other species of *Langduoa* based on the ITS region (positions 87–106 actgagccttgcagcaacaatctccccttt; no mismatch allowed) and LSU (positions 617–636 ccctctcggggggggggggggg; no mismatch allowed) as indicated in Fig. 8.

Type. Soil eDNA sample TUE128827 (*holotype*); eDNA sequence: EUK1107335 (*lectotype*); montane grassland in Langduo, Tibet, 29.4°N, 94.4°E.



Figure 8. Diagnostic barcodes for Langduoa dianae relative to closely-related taxa in ITS2 and LSU.

Description. Other sequences: EUK1602727 and EUK1602728 (both from GSMc plot G5906, stadium grassland soil in Karksi-Nuia, Estonia, 58.10088°N, 25.55959°E); EUK1604031 (GSMc plot G4185, *Picea-Pinus* forest soil in Ristipalo, Estonia, 58.10241°N, 27.47874°E); and EUK1604032 (GSMc plot G4766, soil of coppiced garden dominated by *Fraxinus* and *Ulmus* in Ruudiküla, Estonia, 58.33630°N, 25.78084°E).

Etymology. Langduo (Tibetan) refers to type locality; and Diana (Lithuanian) refers to the first name of Diana Marčiulynienė who was the first to record this genus.

Notes. Found from grassland soils in Estonia and Tibet, with ITS and LSU sequences differing up to 0.2%. So far, not found from the roots.

Lehetuales Tedersoo, ord. nov. MycoBank No: 853588

Type family. Lehetuaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603180, EUK1602375 and EUK1602377 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Lehetuaceae.

Lehetuaceae Tedersoo, fam. nov. MycoBank No: 853589

Type genus. Lehetua Tedersoo.

Description. Covers the monophyletic group in Lehetuales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603180, EUK1602375 and EUK1602377 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Lehetua* and another potentially genus-level group that is represented by sequences EUK1602869 (GSMc plot G4531, *Picea abies* forest soil in Selisoo, Estonia, 57.621658°N, 27.179296°E) and EUK1603296 (GSMc plot S590, *Populus tremula* forest soil in Lehetu, Estonia, 59.01857°N, 24.28041°E); and unassigned sequences EUK0025664 (GSMc plot G5536, tropical rainforest soil in Bamboesi, Suriname, 5.54086°N, -54.03131°E) and EUK0030289 (GSMc plot AV120, tropical rainforest soil in El Zafire, Colombia, -3.9997°N, 69.8947°E).

Lehetua Tedersoo, gen. nov.

MycoBank No: 853590

Type species. Lehetua indrekii Tedersoo.

Description. Covers the monophyletic group in Lehetuaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603180, EUK1602366 and EUK1602374 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Lehetua* is comprised of 8–10 species. Other putative ITS-based species in *Lehetua* are represented by sequences EUK1602811 (GSMc plot G4105, *Picea abies* forest soil in Lepa, Estonia, 57.70158°N, 26.23993°E); EUK1603124 (GSMc plot G5003, *Pinus sylvestris* forest soil in Naissaar, Estonia; 59.5634°N, 24.5451°E); and EUK0022184 (GSMc plot AV106, *Pseudomonotes tropenbosii* rainforest soil in El Zafire, Colombia, -3.995°N, -69.898°E).

Lehetua indrekii Tedersoo, sp. nov.

MycoBank No: 853591

Diagnosis. Separation from other species of *Lehetua* based on the ITS region (positions 219–248 ttataatcttacgaagtactgaggtgatta; one mismatch allowed) and LSU (positions 515–546 aactaaaggratgtggctcctcggagtgttta; one mismatch allowed) as indicated in Fig. 9.

Type. Soil eDNA sample TUE103095 (*holotype*); type sequence EUK1603180 (*lectotype*); GSMc plot S590, *Populus tremula* forest (soil sample TUE003095) in Lehetu, Estonia, 59.01857°N, 24.28041°E.

Description. Other sequences: EUK1603180 (type locality); EUK1602367 (LSU only; type locality; also found in 50 other sites in Estonia); EUK1634481 (GSMc plot G4195, *Quercus robur* woodland soil in Lustivere, Estonia, 58.66293°N, 26.08465°E); EUK1603818 (GSMc plot G5824, managed grassland soil in Kuremaa, Estonia, 58.74138°N, 26.52727°E); EUK1603131 (GSMc plot G4105, *Picea abies* forest soil in Lepa, Estonia, 57.70158°N, 26.23993°E); EUK0021956 (GSMc plot G5150, subarctic grassland soil in Kokelv, Norway, 70.61116°N, 24.62483°E); and EUK0023592 (GSMc plot S035, mixed deciduous forest soil in Kedrovaya Pad, Russia, 43.10834°N, 131.55447°E).

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Etymology. *Lehetu* (Estonian) refers to type locality (also meaning "leafless"); and *Indrek* (Estonian) refers to the first name of Indrek Hiiesalu who collected materials from the type locality.

Notes. Found in Baltic States, Scandinavia and Russia, with ITS and LSU sequences differing up to 3.5% and 0.2%, respectively. Seems to be a generalist in terms of habitat type and soil pH; so far, not found from roots.

Lokrumales Tedersoo, ord. nov.

MycoBank No: 853594

Type family. Lokrumaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1203766, EUK1600125 and EUK1600268 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Lokrumaceae and another potentially family-level taxon, represented by sequences EUK1602809 (GSMc plot G4499, rich, calcareous *Picea abies* forest soil in Kurisoo, Estonia; 59.12808°N, 25.76395°E); EUK1603041 and EUK1603145 (both GSMc plot G4185, *Picea-Pinus* forest soil in Ristipalo, Estonia, 58.10241°N, 27.47874°E).

Lokrumaceae Tedersoo, fam. nov.

MycoBank No: 853595

Type genus. Lokruma Tedersoo.

Description. Covers the monophyletic group in Lokrumales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1203766, EUK1600125 and EUK1600078 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Lokruma* and a few sequences not assigned to any genus; these include EUK0014543 and EUK0006923 (both GSMc plot G5106, subtropical forest soil in Brejo da Lapa, Brazil, -22.3582°N, -44.7383°E) and EUK1602939 (GSMc plot G4464, *Quercus robur* forest soil in Ruu, Estonia, 59.45059°N, 25.22166°E).

Lokruma Tedersoo, gen. nov. MycoBank No: 853596

Type species. Lokruma stenii Tedersoo.

Description. Covers the monophyletic group in Lokrumaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1203766, EUK1600125 and EUK1600078 (Suppl. material 3).

Notes. Based on ITS sequences, *Lokruma* is comprised of 35–40 species, some of which are represented by sequences EUK1200048 (GSMc plot G5130, grassland soil in Angera, Italy, 45.77336°N, 8.59657°E); EUK1602967 (GSMc plot G4626, *Picea-Populus* forest soil in Kõrve, Estonia, 59.07754°N, 26.76144°E); and EUK1603058 (*Picea abies* forest soil in Serga, Estonia, 57.76052°N, 27.47502°E). Given the relatively high intraspecific differences and low interspecific differences, the LSU region is not optimal for distinguishing species of *Lokruma*.

Lokruma stenii Tedersoo, sp. nov. MycoBank No: 853597

Diagnosis. Separation from other species of *Lokruma* based on the ITS region (positions 159–178 taacttaattttttcccgag; one mismatch allowed) as shown in Fig. 10. There are no short barcodes in the first 700 bp of LSU that allow distinguishing *L. stenii* all from other congeners.

	159	ITS2 subregion	178
EUK1203766 Lokruma stenii type	t a a c t	t <mark>aa</mark> ttttttcccg	a g
EUK1603283 Lokruma stenii	taact	t <mark>aa</mark> tttttt <mark>ccc</mark>	r <mark>a</mark> g
EUK1604042 Lokruma stenii	taact	t <mark>aa</mark> tttttt <mark>ccc</mark>	r <mark>a</mark> g
EUK1604041 Lokruma stenii	taact	t <mark>aa</mark> tttttt <mark>ccc</mark> g	ra g
EUK1603431 Lokruma	tactt	t <mark>a</mark> tttttttcccca	i a g
EUK1603184 Lokruma	atatt	aattttttccca	i a g
EUK1602882 Lokruma	atata	aattttttccca	, a g
EUK1603243 Lokruma	a t a t a	t <mark>at</mark> ttttttccca	a g
EUK1603569 Lokruma	taago	taattttttccca	a g
EUK1603485 Lokruma	taagt	t a a t t t t t t t c c c a	i a g
EUK1603891 Lokruma	taaac	caatttttt - ca	i a g
EUK1603386 Lokruma	ttaac	taattttttcca	. a g
EUK1603267 Lokruma	taaac	taattttttcca	, a g
EUK0010130 Lokruma	t a a a 🖸	taattttttccca	, a g
EUK1602796 Lokruma	aaaaa	tttttttttccca	a g
EUK1603322 Lokruma	aa <mark>tt</mark> a	atttttttccca	, a g
EUK1603072 Lokruma	a a a c a	ttttttt	, a g
EUK1603457 Lokruma	ttaaa	C t t t t t t t t c t c a	, a g
EUK0009902 Lokruma	taatc	taa-tetttcca	a g
EUK1603195 Lokruma	taaac	taattttttccca	a g
EUK0014543 Lokrumaceae	ccaga	aattttettaaat	t c
EUKUUU6923 Lokrumaceae	cccct	aaaaaa 🖉 aaa t	,tg
EUK1602809 Lokrumales fam01 gen01	t c a g t	aa <mark>tttttaaaccg</mark>	gg

Figure 10. Diagnostic barcodes for Lokruma stenii relative to closely-related taxa in ITS2.

Type. Soil eDNA sample TUE103193 (*holotype*); type sequence EUK1203766 (*lectotype*); GSMc plot S689, *Pinus halepensis* forest (soil sample TUE003193) in Lokrum, Croatia, 42.6223°N, 18.1241°E.

Description. Other sequences: EUK1603283 (GSMc plot G4301, *Betula pendula* forest soil in Männamaa, Estonia, 58.83258°N, 22.63346°E); EUK1604041 (GSMc plot S480, *Populus-Picea* forest soil in Käru, Estonia, 58.80407°N, 25.22249°E); EUK1604042 (GSMc plot G4734, *Populus-Alnus* forest soil in Urissaare, Estonia, 58.02673°N, 24.65739°E); and EUK1600039 (LSU: GSMc plot HB19, *Populus x wettsteinii* forest plantation soil, Oja, Estonia, 58.82747°N, 26.37799°E).

Etymology. *Lokrum* (Serbo-Croatian) refers to type locality; and *Sten* (Estonian) refers to the first name of Sten Anslan who collected the materials from the type locality.

Notes. Found in Croatia and Estonia, with ITS and LSU sequences displaying up to 1% of differences.

Moosteales Tedersoo, ord. nov.

MycoBank No: 853598

Type family. Moosteaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1604044, JQ311412 and EUK1600278 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Moosteaceae.

Moosteaceae Tedersoo, fam. nov.

MycoBank No: 853600

Type genus. Moostea Tedersoo.

Description. Covers the monophyletic group in Moosteales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1604044, JQ311412 and EUK1600278 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Moostea* and two other potential genera. One of these is represented by sequences EUK0030179 (GSMc plot G4146, mixed forest soil in High Point Reserve Park, NJ, USA, 41.31569°N, -74.66485°E); EUK1600279 (GSMc plot G5826, *Malus domestica* orchard soil in Tabivere, Estonia, 58.54286°N, 26.61575°E); and JQ311412 (microcosm soil in Los Alamos, NM, USA), isolated by Stephanie A. Eichorst (Eichorst and Kuske 2012). The other genus is represented by sequences EUK1600278 (GSMc plot S570, *Betula pubescens* wetland forest soil in Nõmme, Estonia, 58.47962°N, 22.94584°E); EUK0029679 (GSMc plot G2749, *Eucalyptus* spp. woodland soil near Lake Copperfield, Australia, -13.84191°N, 131.81858°E); and EUK0028885 (GSMc plot G5081, *Coccoloba* sp. woodland soil near Lagoa Grande, Brazil, -10.6342°N, -36.7579°E).

Moostea Tedersoo, gen. nov.

MycoBank No: 853601

Type species. Moostea stephanieae Tedersoo.

Description. Covers the monophyletic group in Moosteaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1604044, EUK1103239 and EUK1600287 (Suppl. material 3).

Notes. The ITS sequences are poorly alignable because of long deletions and inserts in certain species. Based on ITS sequences, *Moostea* is comprised of 25–30 species, some of which are represented by sequences EUK1103239 (tropical rainforest soil in El Yunque, Puerto Rico, 18.29°N, -65.78°E); EUK1603515 (GSMc plot G5835, airfield soil in Ridali, Estonia, 57.93692°N, 26.98099°E); and EUK0014332 (GSMc plot S1225, grassland soil in Ayapel, Colombia, 8.27825°N, -75.1257°E).

Moostea stephanieae Tedersoo, sp. nov.

MycoBank No: 853603

Diagnosis. Separation from other species of *Moostea* based on the ITS region (positions 68–97 gcagatgatcgtgagggagttctcttcttc; one mismatch allowed) and LSU (positions 436–455 tgggcttctgctccggcgta; one mismatch allowed) as indicated in Fig. 11.

Type. Soil eDNA sample TUE128417 (*holotype*); eDNA sequence EUK1604044 (*lectotype*); GSMc plot G5828, *Malus domestica* orchard (soil sample TUE028417) in Mooste, Estonia, 58.15335°N, 27.19642°E.

Description. Other sequences: EUK1600287 (LSU: type locality); EUK1604043 and EUK1603823 (both GSMc plot G5835, airfield soil in Ridali, Estonia, 57.93692°N, 26.98099°E).

Etymology. *Mooste* (Estonian) refers to type locality; and *Stephanie* (English) refers to the first name of Stephanie A. Eichorst who collected the first materials from the respective family.

Notes. Found in two sites in Estonia, with ITS and LSU sequences displaying up to 1% and 0.3% differences, respectively.

Nikkaluoktales Tedersoo, ord. nov. MycoBank No: 853604

Type family. Nikkaluoktaceae Tedersoo.



Figure 11. Diagnostic barcodes for Moostea stephanieae relative to closely-related taxa in ITS2 and LSU.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1203196, EUK1600291 and EUK1600248 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Nik-kaluoktaceae.

Nikkaluoktaceae Tedersoo, fam. nov.

MycoBank No: 853605

Type genus. Nikkaluokta Tedersoo.

Description. Covers the monophyletic group in Nikkaluoktales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1203196, EUK1600291 and EUK1600248 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Nikkaluokta* and another potentially genus-level group that is represented by sequences EUK1602730 (GSMc plot S554, *Betula-Quercus* woodland soil in Mädapea, Estonia, 59.32169°N, 26.2621°E); EUK1602729 (GSMc plot FF14, *Picea abies* forest soil in Kõdesi, Estonia, 58.61484°N, 27.12781°E); and EUK1600257 (GSMc plot G4464, *Quercus robur* forest soil in Ruu, Estonia, 59.45059°N, 25.22166°E).

Nikkaluokta Tedersoo, gen. nov.

MycoBank No: 853606

Type species. Nikkaluokta mahdiehiae Tedersoo.

Description. Covers the monophyletic group in Nikkaluoktales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1203196, EUK1600291, EUK1600289, EUK1600235, EUK1600225, EUK1600250 and EUK1600248 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Nikkaluokta* is comprised of 15–20 species, some of which are represented by sequences EUK1603884 (GSMc plot G4406, mixed coniferous forest soil in Tarumaa, Estonia, 59.20745°N, 27.15333°E); EUK1603411 (GSMc plot G4462, *Salix viminalis* energy plantation soil in Kambja, Estonia, 58.25166°N, 26.71276°E); and EUK0006485 (GSMc plot MX23, *Pinus hartwegii* montane forest soil in Iztaccihuatl, Mexico, 19.12622°N, -98.65972°E).

Nikkaluokta mahdiehiae Tedersoo, sp. nov.

MycoBank No: 853607

Diagnosis. Separation from other species of *Nikkaluokta* based on the ITS region (positions 97–116 cctgggcaaattttttttc; one mismatch allowed) and LSU (positions 687–717 cttggatataagaagtggaatctacacaaat; one mismatch allowed) as indicated in Fig. 12.

Type. Soil eDNA sample TUE100497 (*holotype*); eDNA sequence EUK1203196 (*lectotype*); subarctic *Pinus sylvestris* forest (soil sample TUE000497) in Nikkaluokta, Sweden, 67.85596°N, 19.47575°E.

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Figure 12. Diagnostic barcodes for Nikkaluokta mahdiehiae relative to closely-related taxa in ITS2 and LSU.

Description. Other sequences: EUK1203537 (type locality) and EUK1603797 (GSMc plot G5003, *Pinus sylvestris* forest soil in Naissaare, Estonia, 59.56340°N, 24.54510°E).

Etymology. *Nikkaluokta* (Sami) refers to type locality; and *Mahdieh* (Persian) refers to the first name of Mahdieh Hosseyni Moghaddam who sequenced the type materials using target capture protocols.

Notes. Found in Sweden and Estonia, with ITS and LSU sequences displaying up to 1% and 0.2% differences, respectively.

Parniguales Tedersoo, ord. nov.

MycoBank No: 853608

Type family. Parniguaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1635261, EUK1602353, EUK1602857 and EUK1602732 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently represented by Parniguaceae.

Parniguaceae Tedersoo, fam. nov.

MycoBank No: 853609

Type genus. Parnigua Tedersoo.

Description. Covers the monophyletic group in Parniguales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1635261, EUK1602353, EUK1602857 and EUK1602732 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently represented by *Parnigua* and another potentially genus-level group, which is characterised by sequences EUK0016514 (GSMc plot S1218, urban park soil in Qujing, China, 25.52619°N, 103.74497°E), EUK0028452 (GSMc plot G3060, *Vateria indica* forest in Hebri, India, 13.45437°N, 75.02213°E), EUK1602857 (GSMc plot G5771, grassland soil in Hino, Estonia, 57.57566°N, 27.22649°E), EUK1602732 (GSMc

plot G5777, grassland soil in Eoste, Estonia, 58.11427°N, 27.08404°E) and EUK1602733 (GSMc plot G5816, *Trifolium pratense* cropland soil in Hermani, Estonia, 58.80705°N, 25.75639°E).

Parnigua Tedersoo, gen. nov. MycoBank No: 853610

Type species. Parnigua craigii Tedersoo.

Description. Covers the monophyletic group in Parniguaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1635261 and EUK1602353 (Suppl. material 3).

Notes. Based on stringent criteria, there are around five species in this genus, but all these may represent a single variable biological species. In this genus, across and within species, the ITS region has very low variability when compared with LSU (up to 3% differences across species). Other putative species in *Parnigua* are represented by sequences EUK1602947 (GSMc plot G4444, mixed forest soil in Altnurga, Estonia, 58.53676°N, 26.28321°E); EUK1603686 (GSMc plot G5844, wet pasture land soil in Tuhala, Estonia, 59.23003°N, 25.00283°E); EUK1633696 (GSMc plot G4207 *Tilia cordata* forest soil in Ubari, Estonia, 59.492609°N, 25.285663°E); EUK1603848 (GSMc plot G5883, flooded grassland soil in Kasari, Estonia, 58.73608°N, 23.98599°E); EUK1602353 (GSMc plot G4389, *Quercus-Tilia* forest soil in Naha, Estonia, 57.520914°N, 26.601199°E); MF484762 (agricultural soil in England); and MW163928 (*Crocus sativus* cropland soil in Aosta Valley, Italy). The genus can be found from various soils but not from roots. However, SSU sequences are lacking, and links to AM fungi in SSU-based studies cannot be tested.

Parnigua craigii Tedersoo, sp. nov.

MycoBank No: 853611

Diagnosis. Separation from other species of *Parnigua* based on the ITS region (positions 51–80 actgagccttgcagcaacaatctccccttt; no mismatch allowed) and LSU (positions 444–463 ggcgggaaatcagccccct; no mismatch allowed) as indicated in Fig. 13.

Type. Soil eDNA sample TUE102228 (*holotype*); type sequence: EUK1635261 (*lectotype*); GSMc plot G5251, *Quercus robur* woodland (soil sample TUE002228) in Parnigu, Estonia, 58.64096°N, 26.38468°E.

Description. Other sequences: EUK1635874 (GSMc plot G4499, calcareous *Picea abies* forest soil in Kurisoo, Estonia; 59.12808°N, 25.76395°E); EUK1635875 (GSMc plot G4746, *Betula pendula* forest soil in Karjamõisa, Estonia, 57.59761°N, 26.35493°E); EUK1635878 (GSMc plot G4794, *Ulmus-Fraxinus* forest soil in Lõhtsuu, Estonia, 57.91781°N, 26.52069°E); EUK1603328 (GSMc plot G4167, *Salix pentandra* peat soil in Tammispää, Estonia, 58.92051°N, 27.01118°E); EUK1602985 (GSMc plot G5923, *Malus domestica* orchard soil in Kalnabeites, Latvia, 57.1333°N, 24.8566°E); OU939710 (grassland soil in Kungsängen, Sweden, 59.837°N, 17.661°E); and MH625006 (grassland soil in Wakanui, New Zealand, -43.668°N, 172.470°E), first isolated by Craig R. Anderson (Anderson et al. 2018).

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Figure 13. Diagnostic barcodes for Parnigua craigii relative to closely-related taxa in ITS2 and LSU.

Etymology. *Parnigu* (Estonian) refers to type locality; and *Craig* (English) refers to the first name of Craig R. Anderson who was the first to record this species. **Notes.** Found from Estonia, Sweden and New Zealand, with ITS and LSU se-

quences differing up to 0.5%. Found in all croplands, grasslands, deciduous and coniferous forests.

Riederbergales Tedersoo, ord. nov.

MycoBank No: 853612

Type family. Riederbergaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1602903, EUK1603115, EUK1602258, EUK1602253, EUK1602251 and EUK1104709 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Riederbergaceae and four additional potentially family-level taxa represented by sequences EUK1100540 (bog peat soil in Svartberget, Sweden, 64.24°N, 19.76°E); EUK1602254 (GSMc plot G5826, *Malus domestica* orchard in Tabivere, Estonia, 58.54286°N, 26.61575°E); EUK1602251, EUK1602253 and EUK1602257 (all GSMc plot G5828, Estonia, *Malus domestica* orchard soil in Mooste, Estonia, 58.15335°N, 27.19642°E). Sequences EUK0031975 (GSMc plot S1082, *Araucaria araucana* forest, Nahuelbuta, Chile, -37.78985°N, -73.0038°E) and EUK1217433 (GSMc plot G4777, maritime grassland (saltmarsh) soil in Härs-hämani, Estonia, 59.33103°N, 23.92720°E) represent additional, monospecific, potentially family-level groups not included in the phylograms due to the lack of LSU sequences.

Riederbergaceae Tedersoo, fam. nov.

MycoBank No: 853613

Type genus. Riederberga Tedersoo.

Description. Covers the monophyletic group in Riederbergales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1602903, EUK1602242 and EUK1602243 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Riederberga*.

Riederberga Tedersoo, gen. nov.

MycoBank No: 853614

Type species. Riederberga sylviae Tedersoo.

Description. Covers the monophyletic group in Riederbergaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1602903, EUK1602242 and EUK1602243 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Riederberga* is comprised of 5–6 species, some of which are represented by sequences EUK1602859 (GSMc plot G4770, *Populus berolinensis* dominated coppiced garden in Ubasalu, Estonia, 59.06755°N, 24.47842°E); EUK1602912 (GSMc plot G4772, *Juniperus communis* calcareous woodland soil in Kohatu, Estonia, 58.95934°N, 24.30017°E); EUK1602761 (GSMc plot G4434, mixed woodland soil in Kalli, Estonia, 58.53770°N, 24.06659°E); and EUK1603687 (GSMc plot G4229, *Quercus robur* woodland soil in Niidiaia, Estonia, 58.88603°N, 24.47280°E).

Riederberga sylviae Tedersoo, sp. nov.

MycoBank No: 853615

Diagnosis. Separation from other species of *Riederberga* based on the ITS region (ITS2 positions 186–215 gctttggacggcatgcgaatctgcatcaca; one mismatch allowed) and LSU (positions 656–685 tcaccaatcgacgtcaatcggcatgcgtct; one mismatch allowed) as indicated in Fig. 14.

Type. Soil eDNA sample TUE128372 (*holotype*); eDNA sequence: EUK1602903 (*lectotype*); GSMc plot G5783, wet grassland (soil sample TUE028372) in Altnurga, Estonia, 58.55682°N, 26.29259°E.

Description. Other sequences: EUK1604046 and EUK1604047 (both type locality); and GU055683 (ITS part considered; managed grassland soil in Riederberg, Austria, 48.25°N, 16.07°E), collected by Sylvia Klaubauf (Klaubauf et al. 2010).

Etymology. *Riederberg* (German) refers to type locality; and *Sylvia* (German) refers to the first name of Sylvia Klaubauf, who first collected the materials of type species and the entire order from the type habitat.

Notes. Found in Austria and Estonia, with ITS and LSU sequences displaying up to 1% differences.



Figure 14. Diagnostic barcodes for Riederberga sylviae relative to closely-related taxa in ITS2 and LSU.

Ruuales Tedersoo, ord. nov.

MycoBank No: 853616

Type family. Ruuaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603424, EUK1600239, EUK1600169 and EUK1600180 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Ruuaceae.

Ruuaceae Tedersoo, fam. nov.

MycoBank No: 853617

Type genus. Ruua Tedersoo.

Description. Covers the monophyletic group in Ruuales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603424, EUK1600239, EUK1600169 and EUK1600180 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Ruua* and another genus-level taxon represented by sequence EUK1602764 (GSMc plot G4189, *Populus tremula* forest soil in Tammsaare, Estonia, 57.84444°N, 27.20141°E).

Ruua Tedersoo, gen. nov. MycoBank No: 853618

Type species. Ruua coralieae Tedersoo.

Description. Covers the monophyletic group in Ruuaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1603424, EUK1600239, EUK1600169 and EUK1600180 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Ruua* is comprised of 3–4 potential species that are represented by sequences EUK1632165 (GSMc plot S510, village habitat soil in Kihnu, Estonia, 58.1282°N, 23.9815°E); EUK1603289 (GSMc plot G4450, *Fraxinus-Tilia* forest soil in Nigula, Estonia, 58.0190°N, 24.6803°E); EUK1103406 (freshwater in Skogaryd, Sweden, 58.37°N, 12.16°E); and FN610984 (*Fagus sylvatica* forest soil in Breuil-Chenue, France, 47.301°N, 4.076°E), isolated by Coralie Damon (Damon et al. 2010).

Ruua coralieae Tedersoo, sp. nov.

MycoBank No: 853619

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Figure 15. Diagnostic barcodes for Ruua coralieae relative to closely-related taxa in ITS2 and LSU.

Type. eDNA sample TUE101598 (*holotype*); eDNA sequence EUK1603424; GSMc plot G4464, *Quercus robur* forest (soil sample TUE101598) in Ruu, Estonia, 59.45059°N, 25.22166°E.

Description. Other sequences: EUK1602853 and EUK1600135 (type locality); EUK1604050 (GSMc plot G5002, *Tilia-Quercus* forest soil in Naissaar, Estonia, 59.57530°N, 24.53590°E); and EUK1604051 (GSMc plot S480, *Populus-Picea* forest soil in Käru, Estonia, 58.80407°N, 25.22249°E).

Etymology. *Ruu* (Estonian) refers to type locality; and *Coralie* (French) refers to the first name of Coralie Damon, who collected the first materials belonging to this genus.

Notes. Found from three sites in Estonia, with ITS and LSU sequences displaying up to 0.3% differences.

Tammsaareales Tedersoo, ord. nov.

MycoBank No: 853620

Type family. Tammsaareaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1602762, EUK1635767 and EUK1602763 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Tammsaareaceae.

Tammsaareaceae Tedersoo, fam. nov.

MycoBank No: 853621

Type genus. Tammsaarea Tedersoo.

Description. Covers the monophyletic group in Tammsaareales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1602762, EUK1635767 and EUK1602763 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Tammsaarea* and the sequence EUK1602763 (GSMc plot G5835, airfield soil in Ridali, Estonia, 57.93692°N, 26.98099°E).

Tammsaarea Tedersoo, gen. nov.

MycoBank No: 853622

Type species. Tammsaarea vivikae Tedersoo.

Description. Covers the monophyletic group in Tammsaareaceae (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1602762 and EUK1635767 (Suppl. material 3).

Notes. Based on ITS sequences, *Tammsaarea* is comprised of two species; the other being represented by LSU sequences EUK1601269, EUK1635767 and EUK1635768 (all GSMc plot G4185, *Picea-Pinus* forest soil in Ristipalo, Estonia, 58.10241°N, 27.47874°E).

Tammsaarea vivikae Tedersoo, sp. nov. MycoBank No: 853683

Diagnosis. Separation from other species of *Tammsaarea* and other species of Endogonomycetes based on ITS (positions 228–257 ggaccgagaaggcg-caatagttgaacaatt; one mismatch allowed) and LSU (positions 585–604 ataactatcggacaaagttt; one mismatch allowed) as indicated in Fig. 16.

Type. eDNA sample TUE100731 (*holotype*); eDNA sequence EUK1602762 (*lectotype*); GSMc plot G4189, *Populus tremula* forest (soil sample TUE000731) in Tammsaare, Estonia, 57.84444°N, 27.20141°E.

Description. Other sequences EUK1604048 and EUK1604049 (type locality).

Etymology. *Tammsaare* (Estonian) refers to the type locality and one of the most famous Estonian writers, Anton Hansen Tammsaare; and *Vivika* (Estonian) refers to the first name of Vivika Adamson who provided access to the type locality.

Notes. Found from a single locality in Estonia, with ITS and LSU sequences differing up to 0.5% and 0.3%, respectively.

Unemaeeales Tedersoo, ord. nov.

MycoBank No: 853684

Type family. Unemaeeaceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1630871 and EUK1635889 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes Unemaeeaceae.





Unemaeeaceae Tedersoo, fam. nov.

MycoBank No: 853685

Type genus. Unemaeea Tedersoo.

Description. Covers the monophyletic group in Unemaeeales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1630871 and EUK1635889 (Suppl. material 3).

Notes. Recognised based on eDNA sequences only. Currently includes *Une-maeea* and multiple poorly alignable ITS sequences with no LSU, for example EUK1217297 (FunAqua sample W0006s, lake sediment in Petrolandia, Brazil, -8.9908°N, -38.2251°E) and FJ528738 (*Araucaria* spp. plantation soil, Gadgarra, Australia, -17.1641°N, 145.6469°E) that was isolated by Nathalie J.A. Curlevski (Curlevski et al. 2010). It seems that several Unemaeeaceae spp. have preferential habitat in anoxic soils and sediments.

Unemaeea Tedersoo, gen. nov.

MycoBank No: 853686

Type species. Unemaeea nathalieae Tedersoo.

Description. Covers the monophyletic group in Unemaeeales (Fig. 2). Phylogenetically delimited as the least inclusive clade covering sequence accessions EUK1630871 and EUK1635889 (Suppl. material 3).

Notes. Based on ITS and LSU sequences, *Unemaeea* is comprised of three species; others represented by sequences EUK1217289 (freshwater lake sediment near Bezdan, Serbia, 45.82031°N, 18.9599°E) and KX196132 (deciduous forest soil in Champaign County, IL, USA).

Unemaeea nathalieae Tedersoo, sp. nov.

MycoBank No: 853687

Diagnosis. Separation from other species of *Unemaeea* based on the ITS region (5.8S positions 122–151 gtcagtgtttgccacggagtatgccggctt; no mismatch allowed) and from other species of Endogonomycetes based on LSU (positions 694–723 gggcttgtcatggcagagggacacgtcgta; no mismatch allowed) as indicated in Fig. 17.

Type. Soil eDNA sample TUE100213 (*holotype*); eDNA sequence EUK1630871 (*lectotype*); GSMc plot G3318, marshland (soil sample TUE000213) in Unemäe, Estonia, 58.28253°N, 22.46296°E.





Description. Other sequences: EUK1635887–EUK1635890 (type locality) and EUK1213720 (FunAqua sample W0581s, river sediment in Floresti, Romania, 46.75472°N, 23.49923°E).

Etymology. Unemäe (Estonian) refers to the type locality; and *Nathalie* (English) refers to the first name of Nathalie J.A. Curlevski who collected the first materials belonging to this genus.

Notes. The end of 5.8S and start of LSU are strongly diverged compared with other species of *Unemaeea* and Endogonomycetes. As no other confamilial LSU sequences are available, the diagnostic positions are compared against the most divergent, unalignable part across Endogonomycetes. Found in anoxic soil in Estonia and Romania, with ITS sequences displaying up to 4% differences.

Bifiguratales Tedersoo, ord. nov. MycoBank No: 853688

Type family. Bifigurataceae Tedersoo.

Description. Covers the monophyletic group in Endogonomycetes (Fig. 2). Cultured mycelium filamentous, aseptate, coenocytic, 2 μ m diam., mucose in appearance, commonly producing budding yeast-like cells; chlamydospores intercalary, 5–10 μ m diam., forming on hyphal tips. Phylogenetically delimited by the least inclusive clade covering sequence accessions HM123225, EUK1104879, KF568171 and KF567389.

Notes. Comprised of a single family Bifigurataceae. Order description is adapted from Torres-Cruz et al. (2017).

Bifigurataceae Tedersoo, fam. nov.

MycoBank No: 853689

Type genus. *Bifiguratus* T.J.Torres-Cruz & A.Porras-Alfaro.

Description. Cultured mycelium filamentous, aseptate, coenocytic, 2 µm diam., mucose in appearance, commonly producing budding yeast-like cells; chlamydospores intercalary, 5–10 µm diam., forming on hyphal tips. Phylogenetically delimited by the least inclusive clade covering sequence accessions HM123225, EUK1104879, KF568171 and KF567389.

Notes. Comprised of a single genus *Bifiguratus* that is commonly found in soil and occasionally in roots of non-AM plants. No sexual structures have been revealed. Family description is adapted from Torres-Cruz et al. (2017).

Densosporales Tedersoo, ord. nov.

MycoBank No: 853690

Type family. Densosporaceae Desirò, M.E.Sm., Bidartondo, Trappe & Bonito.Description. Densosporales is defined as a monophyletic group in Endogonomycetes (Fig. 2, Suppl. material 3) that corresponds to Densosporaceae sensu Desiro et al. (2017). Covers *Densosporacae* and Planticonsortiaceae and the least inclusive clade with sequence accessions UDB028692, EUK1104889, EUK1104816 and EUK1601509 (Suppl. material 3).

Notes. Densosporales harbours roughly one half of the Endogonomycetes based on LSU data. It comprises *Densosporacae*, Planticonsortiaceae and 16 additional family-level groups collectively covering >200 species. LSU has much greater phylogenetic resolution compared with SSU (Suppl. materials 3, 4), and the potential utility of the ITS region seems to vary greatly by family. Many more ITS-LSU sequences are needed to understand family- and genus-level composition of Densosporales.

Densosporaceae Desirò, M.E.Sm., Bidartondo, Trappe & Bonito, emend. Tedersoo

MycoBank No: 821851

Type genus. Densospora McGee.

Description. Phylogenetic diagnosis as in Desiro et al. (2017), but includes a more limited phylogenetic group - the least inclusive clade comprised of *Densospora* spp. (accessions JF414167 and UDB28692), *Sphaerocreas pubescens* (accession LC107618) and accession EUK1601029 (Suppl. material 3).

Notes. Based on SSU phylogeny (Suppl. material 4), one or both of the genera *Densospora* and *Sphaerocreas* are paraphyletic, and their relationships require further research. Most species in both genera remain to be sequenced, including *D. tubiformis* (P.A.Tandy) McGee - the type species of *Densospora*.

Planticonsortiaceae Tedersoo, fam. nov.

MycoBank No: 853691

Type genus. Planticonsortium C.Walker & D.Redecker.

Description. Emanating hyphae $0.5-4 \mu m$ diam., forming colourless to brown chlamydospores ($10-12 \mu m$, up to $35 \mu m$ diam.), sometimes ropelike strands; appressoria swollen, frequently with several thin hyphae giving an insect-like appearance. Intraradical mycelium $0.5-4 \mu m$ diam., smooth to angular, with (sub-)globose swellings, forming comb-like (ctenoid), fanshaped, palmate, antler-like, digitate or feather-like structures appearing clasped around epidermal and cortical cells; forming finely branched arbuscules. All hyphae stain darkly in acidic blue stains, more strongly for extraradical hyphae. Monophyletic group in Densosporales (Fig. 2, Suppl. materials 3, 4).

Notes. Planticonsortiaceae covers roughly one third of Endogonomycetes reads based on LSU (Suppl. material 3) and SSU (Suppl. material 4), but is poorly represented in the ITS dataset. This may be due to the highly divergent and relatively long ITS region (800–1200 bases). Based on the LSU phylogram, Planticonsortiaceae harbours seven genus-level groups with >100 putative species. The description is adapted from Walker et al. (2018).

Endogonales Jacz. & P.A.Jacz., emend. Tedersoo

MycoBank No: 90720

Type family. Endogonaceae Paol.

Description. Fruiting body hypogeous or on debris, globose, irregular, sometimes resupinate, 1–10 mm in diam., may be composed of aggregated zygosporangial clusters, with zygospores formed on apposed suspensors. Hyphae of fruiting body tissue coenocytic, aseptate, sometimes with secondary septa that form micropores. Reproductive structures as zygosporangia, rarely azygosporangia (co-existing with zygosporangia in *Endogone pisiformis*) or chlamydospores (in *Vinositunica*), distributed randomly or radially in fruiting bodies, 100–700 µm diam., with yellow granular contents. Zygosporangial wall comprises outer sporangiothecium with 1–4 openings and inner eusporium with no openings. Azygosporangia rare, with a single-layered wall and separated from the single suspensor by a gametangial septum. Chlamydospore wall continuous, multilayered, with dense subtending hyphae, lacking septa. Forms a monophyletic group in Endogonomycetes as the least inclusive clade covering accessions EUK1601498, EUK1100757, LC002628, LC431107, EUK1104693 and UDB025468.

Notes. Includes taxa with or without fruiting bodies and with ectomycorrhizal, arbuscular mycorrhizal and saprotrophic lifestyles. Endogonales harbours Endogonaceae, Jimgerdemanniaceae and Vinositunicaceae families, as well as seven potentially family-level taxa, collectively comprising >200 species based on ITS and LSU sequences. Order description is adapted from Morton and Benny (1990) and Yamamoto et al. (2020).

Endogonaceae Paol., emend. Tedersoo

MycoBank No: 81877

Type genus. Endogone Link.

Description. Fruiting body hypogeous or on debris, globose, irregular, sometimes resupinate, 1–10 mm diam., may be composed of aggregated zygosporangial clusters, with zygospores formed on apposed suspensors. Hyphae of fruiting body tissue coenocytic, aseptate, sometimes with secondary septa that form micropores. Reproductive structures as zygosporangia, rarely azygosporangia (co-existing with zygosporangia in *Endogone pisiformis*) distributed randomly or radially in fruiting bodies, 100–700 µm diam., with yellow granular contents. Zygosporangial wall comprises outer sporangiothecium with 1–4 openings and inner eusporium with no openings. Azygosporangia rare, with a single-layered wall, and separated from the single suspensor by a gametangial septum. Forms a monophyletic group in Endogonales as the least inclusive clade covering accessions LC002628, EUK1601764 and EUK1601442.

Notes. Covers species of *Endogone* that are saprotrophic or potentially ectomycorrhizal (/endogone2 and /endogone3 lineages, *sensu* Tedersoo and Smith (2017)) and four closely-related genus-level taxa.

Jimgerdemanniaceae Tedersoo, fam. nov.

MycoBank No: 853692

Type genus. Jimgerdemannia Trappe, Desirò, M.E.Sm., Bonito & Bidartondo.

Description. Includes *Jimgerdemannia* and closely-related genera that form a monophyletic group in Endogonales, with the least inclusive clade covering accessions KC568319, EUK1631035, JN890102, UDB025468 and OU942919 (Suppl. material 3).

Notes. Jimgerdemanniaceae covers an ectomycorrhizal genus *Jimgerde-mannia* and six genus-level taxa that are soil-inhabiting, potentially arbuscular mycorrhizal and probably not producing macroscopic fruiting bodies.

Vinositunicaceae Tedersoo, fam. nov.

MycoBank No: 853693

Type genus. Vinositunica Koh.Yamam., Degawa & A.Yamada.

Description. Fruiting bodies epigeous or semi-hypogeous, reniform or irregular, often with a short stipe-like sterile base, 2–20 mm in diam. Peridium white, partly purple, in a single layer, composed of coenocytic aseptate hyphae. Gleba pale yellow to purplish-grey, composed of numerous radially or randomly distributed chlamydospores. Chlamydospores granular, with yellow contents, broadly ellipsoid, 50–700 µm diam, terminal on single subtending hypha. Cell wall composed of purplish to vinaceous outer layer and colourless inner layer.

Notes. Vinositunicaceae includes the genus *Vinositunica*. This group has not been found from root or soil eDNA samples thus far, and ITS sequences are not available. Probably humus saprotrophs. Family description is adapted from Yamamoto et al. (2020).

Primer bias

To evaluate whether some part of the dark diversity of putative AM fungi can be accounted for by primer bias as suggested for Glomeromycota (Kohout et al. 2014; van Geel et al. 2014; Seeliger et al. 2023), we tested the commonly used SSU, ITS and LSU primers for critical mismatches based on multiple sequence alignments. The AMV4.5NF (Sato et al. 2005) and AM-Sal-F (Seeliger et al. 2023) primers, proposed to cover both AM fungal groups, exhibited several (near-)terminal mismatches to many groups of Glomeromycota and one central and one near-terminal mismatch to many groups of Endogonomycetes. The FRE-F (Seeliger et al. 2023) primer had multiple mismatches to most target Endogonomycetes groups including a terminal mismatch to some groups. The reverse SSU primers AMDGR (Sato et al. 2005) and FRE-R (Seeliger et al. 2023) matched well with Glomeromycota, but had one or more (near)-terminal mismatches to several groups of Endogonomycetes. Regarding the ITS-LSU primers, ITS1F (Gardes and Bruns 1993), ITS1 (White et al. 1990), gITS7ngs and ITS4ngsUni (Tedersoo and Lindahl 2016) had single central mismatches to a few Glomeromycota and Endogonomycetes lineages, whereas ITS9munngs

(Tedersoo and Lindahl 2016) had no mismatches. The fungi-specific primer ITS1catta (Tedersoo and Anslan 2019) had (near)-terminal mismatches to several minor lineages of both AM groups.

Of Glomeromycota-specific primers, wSSUmcf (Krüger et al. 2009) matched well to all target lineages. The primer wLSUmbr (Krüger et al. 2009) had one central mismatch to *Pervetustus* and Archaeosporales, suggesting a negligible bias.

Of Endogonomycetes-specific primers designed and tested initially, ITS3-End displayed mismatches to multiple groups, while LR3-End had 1–2 central mismatches to Jimgerdemanniaceae and terminal mismatches to Unemaeeaceae. For Endogonomycetes, we thus recommend use of universal forward primers gITS7ngs or LROR or the newly-designed LF350End (ccgatagcgaacaagtac; also amplifies many other fungi) in combination with the combination of reverse primers LR3-End2 (aycattahgycagcgacc; >99% of Endogonomycetes) and LR3-End2a (aycattahgycagccgtta; Unemaeeaceae). These primer pairs yield amplicons of 900–1200 bases, ca. 700 bases and ca. 400 bases, respectively. For simultaneous amplification of Glomeromycota and Endogonomycetes, only universal or fungal primers can be recommended (e.g., forward primers gITS7ngs, LROR and LF350 combined with a reverse primer TW13; White et al. (1990)) along with deep sequencing to 10⁵ reads.

Discussion

In this paper, we describe 15 new species of potentially AM fungi belonging to Glomeromycota and Endogonomycetes from soil eDNA samples. These new species and six re-combinations lead to 16 new genera, 19 new families and 17 new orders that are well delimited by phylogenetic analyses of rRNA genes. The high taxonomic and phylogenetic resolution at the levels of species to class render long-read rRNA gene sequences highly useful for both species delimitation and phylogeny reconstruction. Future studies using protein-encoding genes or whole-genome analyses will be useful for solving phylogenetic uncertainties related to rapid rRNA gene evolution in certain groups (e.g. Entrophosporales) and unsettled branching order (e.g. endogonomycete orders). For this study, the genomes that were available for only 13 described genera of Glomeromycota and two genera of Endogonomycetes (Rosling et al. 2024) would have added no extra value. Our phylogenies indicate that eDNA from soil and sediment habitats may substantially add to novel phylogenetic diversity in these groups, especially in Endogonomycetes. Studies combining fine root staining and DNA sequencing should improve our understanding of the symbiotic potential of these newly-described groups and the evolution of AM associations in general.

We rely on public long-read rRNA gene sequences to describe new species in previously unrecognised family- and order-level taxa, using eDNA samples as holotypes and sequences as lectotypes. Previous DNA-based taxonomic studies on fungi have described new species in well-known genera (Bridge and Hughes 2012; Kirk 2012; Kalsoom Khan et al. 2020) or families (de Beer et al. 2016; Lücking and Moncada 2017) based on typifying sequences of the ITS region. The species described here are usually represented by both ITS and LSU regions from multiple eDNA samples. This allows us to estimate rough intraspecific variation and interspecific distances, and develop continuous, 20–30-base diagnostic barcodes (see also Kalsoom Khan et al. (2020)) for ITS and LSU regions separately. This contrasts with other studies that point to single diagnostic differences scattered across the entire marker length (de Beer et al. 2016; Lücking and Moncada 2017), or provide no sequence-diagnostic features. The continuous barcodes are better findable for the human eye and software, such as custom BLAST algorithms (Camacho et al. 2009), Cutadapt (Martin 2011), CAOS-R (Bergmann 2024) and SeqKit2 (Shen et al. 2024). For nearly all species (except *Parnigua craigii*), these diagnostic barcodes are more informative for the ITS region than LSU due to greater variability and taxonomic resolution. The species of *Langduoa* and *Lokruma* have relatively lower LSU short barcode resolution compared with other taxa. Nonetheless, species from all groups can be distinguished well based on ITS1 or ITS2 sequences and usually by LSU sequences.

The newly-described species, genera, families and orders are represented exclusively by eDNA sequences supplied with metadata ranging from none to ample background information about location and environmental properties, depending on the source of reads and success in contacting the data producers or material collectors. Besides fragmented information about habitat and distribution, soil eDNA provides no information about biotic interactions or functioning. Given the paucity of data from non-soil habitats outside northern Europe, we refrain from speculating about the distribution and functional role of the described species and higher-level taxa.

The Glomeromycota SSU-ITS-LSU phylogram is congruent with previous studies at the level of families and orders (Oehl et al. 2011; Redecker et al. 2013; Blaszkowski et al. 2021, 2022; Montoliu-Nerin et al. 2021; Rosling et al. 2024). The newly-described entrophosporalean family Pseudoentrophosporaceae does not affect the overall phylogenetic structure of Glomeromycota, but expands the phylogenetic breadth of Entrophosporales. Besides this new family, we also recorded multiple potentially new genera, most of which have also been revealed in previous analyses of root and soil materials. In this paper, we refrain from formally describing these for two main reasons. First, most of these genera are relatively common, and there are high chances that the corresponding species have been described but yet to be sequenced; here, we sincerely hope that the current study motivates the publishing of these materials, kept in several research teams' drawers. Second, we are surveying hundreds of global soil and sediment samples using the ITS- and LSU-orientated Glomeromycota- and Endogonomycetes-specific primers, which will likely reveal novel diversity and improve delimiting the new putative taxa. For their short-term communication, we propose alphanumeric labels that facilitate quality-filtering, especially chimaera control, for forthcoming eDNA studies. The currently accepted genera and proposed genus-level groups for Glomeromycota and Endogonomycetes are provided in Suppl. materials 5, 6, respectively.

The Endogonomycetes SSU-5.8S-LSU phylogram only marginally reflects the SSU-focused multigene phylograms of Desiro et al. (2017) and Yamamoto et al. (2020) and the SSU-based phylogram of Albornoz et al. (2022). Here, we distinguish 17 well-supported orders in Endogonomycetes, including Densosporales and Bifiguratales (ord. nov.) and Endogonales (sens. str.), as well as entirely new groups represented by no sequenced culture, spore or fruiting body specimen. For anchoring names to these orders, we describe well-chosen representative species, genera and families based on eDNA and long-read sequences.

To communicate these groups' internal structure, we propose alphanumeric codes for putative families and genera as for the Glomeromycota. Furthermore, our analyses indicate much greater phylogenetic and species-level resolution for the LSU marker than the SSU (Suppl. materials 3, 4). The sequence data accumulated thus far also reveal much more information available for LSU compared with ITS and SSU at the level of species and orders (Desiro et al. 2017; Suppl. material 3). However, these datasets are biased for soil (ITS and LSU) or plant samples (SSU). As a downside of the ITS-LSU approach, the genus *Planticonsortium* lacks sequence data for these markers and cannot be reliably assigned to any of the multiple Planticonsortiaceae genus-level groups. However, ultra-long reads should be able to bridge the SSU and LSU and provide insights into Planticonsortiaceae soon.

We have encountered several conflicting situations by focusing on the mixed morphology- and eDNA-based classification. Undoubtedly, there is a potential risk of parallel morphological and DNA-based descriptions, especially given that nearly half of the accepted species of AM fungi are represented by no sequence data. However, the high-throughput sequencing methods have been available for >15 years, making it increasingly less likely that old spore collections from microscope slides will be successfully sequenced soon.

In addition to the parallel morphology-based and DNA-based descriptions, the focus on different morphological characters may also hamper the taxonomy of AM fungi. We find that a pair of glomeromycete genera, Redeckera and Corymbiglomus, that can be seemingly well delimited by morphological characters, are not clearly separated in phylogenetic analysis. Importantly, Redeckera spp. are described based on the small glomerospores clustering in large fruiting bodies, whereas species in Corymbiglomus are distinguished based on glomerospores on hyphal tips. The presence of spore dimorphism, as recently revealed for Entrophospora-Claroideoglomus (Blaszkowski et al. 2022), might be behind the inconsistency between phylogenetic and morphological data. Since Diversisporaceae harbours multiple described and undescribed genera, we leave the taxonomy of the Redeckera-Corymbiglomus group to be settled in further studies. Furthermore, species of Endogonomycetes have been described based on chlamydospores on hyphae (Planticonsortium), chlamydosporic fruiting bodies (Vinositunica and Densospora), zygosporangial fruiting bodies (Endogone and Jimgerdemannia) and features of pure cultures (Bifiguratus), potentially resulting in parallel classification based on different characters. Here, the massive amount of available eDNA sequences enables us to bridge a vast majority of these taxa (except Vinositunica and Planticonsortium) and translate the various types of morphological descriptions into a common DNA-based language.

Conclusions

This study offers the first example of a mixed morphological and eDNA-based classification from species to order level in the fungal kingdom. Our approach of typifying both eDNA samples and sequences and preparing diagnoses based on DNA barcodes will likely boost alpha and higher-level taxonomic research in fungi and potentially in non-fungal organisms. Such a mixed classification would help provide human-readable names to many of the "dark matter" fungi (Nilsson et al. 2016) and tremendously reduce the number of entirely

unidentified fungi. To avoid parallel DNA-based classifications, we propose that the description of new species should primarily focus on the universal fungal barcode - the ITS region, preferably supplemented with at least one additional taxonomically or phylogenetically informative marker - LSU or SSU in the case of amplicons - or a protein-encoding gene for genomes derived from tissues. Accordingly, we propose using both the ITS and LSU markers for the two groups of AM fungi, considering taxonomic resolution, availability of specific primers and the large number of previously described reference species.

Our research also points out that, in addition to registering newly-described fungal taxa, we urgently need a linked system (related to, for example, INSDC, MycoBank and/or UNITE - the leading platforms that cross-communicate fungal species and molecular sequence data) for mandatory registering of taxonomic emendations and taxonomic updates of sequences, especially when new taxa are erected based on already published sequences. Such sequence registration would minimise the risk that taxon names of the sequences in databases evolve in different directions and that new species are described several times based on the same or related sequences.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Maximum Likelihood phylogram indicating phylogenetic relationships amongst *Glomeromycota* based on SSU-ITS-LSU sequences

Authors: Leho Tedersoo, Franco Magurno, Saad Alkahtani, Vladimir Mikryukov Data type: pdf

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Supplementary material 2

Maximum Likelihood phylogram indicating phylogenetic relationships amongst *Gigasporales* based on LSU sequences

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Supplementary material 3

Maximum Likelihood phylogram indicating phylogenetic relationships amongst *Endogonomycetes* based on SSU-5.8S-LSU sequences

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Supplementary material 4

Maximum Likelihood phylogram indicating phylogenetic relationships amongst *Endogonomycetes* based on SSU sequences

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Supplementary material 5

Currently recognised orders, families and genera and proposed taxonomic groups in *Glomeromycota*

Authors: Leho Tedersoo, Franco Magurno, Saad Alkahtani, Vladimir Mikryukov Data type: pdf

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Supplementary material 6

Currently recognised orders, families and genera and proposed taxonomic groups in *Endogonomycetes*

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Research Article

Campanophyllum microsporum (Agaricales, Agaricomycetes), Calocera multiramosa, and Dacrymyces naematelioides (Dacrymycetales, Dacrymycetes), three new species from Yunnan Province, southwestern China

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Abstract

Three new species belonging to Basidiomycota from southwestern China are described based on morphological and molecular data. Campanophyllum microsporum is morphologically characterized by dorsally pseudostipitate, pale orange to brownish orange pileus, excentric to lateral pseudostipe, crowded lamellae, cylindrical-ellipsoid basidiospores $3.0-4.2 \times 1.7-2.2 \mu$ m, narrowly clavate to clavate basidia $14.5-23.0 \times 3.0-4.2 \mu$ m, and cylindrical to clavate cheilocystidia 22.0-55.0 × 5.0-10.8 µm. Calocera multiramosa is morphologically characterized by stipitate, yellowish to orange, dendroid, and dichotomously branched basidiomata, cylindrical to clavate basidia $36.5-52.5 \times 3.8-6.1 \mu m$, navicular or reniform, 1–5-septate mature basidiospores 10.4–16.7 × 5.2–7.4 μ m. Dacrymyces naematelioides is morphologically characterized by stipitate and cerebriform, orange to light brown basidiomata, cylindrical to clavate, smooth or roughened basidia 38.5-79.5 × 6.5-10.6 µm, broadly and elliptic-fusiform, 7-septate mature basidiospores $18.5-28.6 \times 8.9-13.8 \ \mu\text{m}$. These three new species are supported by the phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) analyses with combined nuclear ribosomal DNA (rDNA) internal transcribed spacer (ITS) and large ribosomal subunit (LSU) sequences. Full descriptions and photographs of these new species are provided.

Key words: Basidiomycota, new taxon, phylogenetic analyses, taxonomy

Introduction

The monotypic genus *Campanophyllum* Cifuentes & R.H. Petersen was proposed to accommodate *Lentinus proboscideus* Fr., traditionally contains dorsally pseudostipitate pileus with tricholomataceus, excentric to lateral pseudostipe, crowded lamellae, cylindrical-ellipsoid spores, cylindrical, clavate to utriform cheilocystidia, and grows on rotten wood (Cifuentes et al. 2003). *L. proboscideus* was combined into the genus *Campanophyllum* in the family Cyphellaceae, and



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Copyright: [©] Yuan-Hao Ma et al. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). designated a neotype by Cifuentes et al. (2003). In addition, the authors demonstrated that this species represents a novel species of a novel genus distinct from its closest relatives through a comprehensive analysis of morphological characteristics, molecular data, and sexual compatibility (Cifuentes et al. 2003). The species was found in montane forests in Colombia, Costa Rica, Ecuador, Mexico, and Panama, and is currently being assessed for inclusion in the IUCN red list as endangered (https://redlist.info/iucn/species_view/488791/) (Cifuentes et al. 2003; Reschke et al. 2021). Recently, researchers also collected the species of *C. proboscideum* in India and studied its fungal extracts, which are rich in natural antioxidants and highly effective antimicrobial activity (Borthakur et al. 2020). However, the specimens collected from India may not be of *C. proboscideum*, but of another species, due to the huge differences in the ITS sequences. The absence of detailed morphological descriptions precludes the ability to ascertain the specific species to which the specimen belongs.

Calocera (Fr.) Fr. and Dacrymyces Nees are the two major polyphyletic groups in the family Dacrymycetaceae, characterized by pulvinate to dendroid or cerebriform basidiomata (Shirouzu et al. 2007, 2009, 2013, 2017; Zamora and Ekman 2020; Fan et al. 2021; Lian et al 2022; Zamora et al. 2022). Their classifications in Dacrymycetes are based on morphology and have remained unaltered (McNabb 1965, 1973; Shirouzu et al. 2017; Zamora and Ekman 2020). However, this morphology-based classification has often conflicted with the results of molecular phylogenetic analyses, and Calocera, Dacrymyces, and Dacryopinax G.W. Martin have been shown to be non-monophyletic genera (Shirouzu et al. 2013, 2017; Zamora and Ekman 2020). Phylogenetic analysis shows that species of Calocera and Dacrymyces are distributed in many clades of the family Dacrymycetaceae. The three species of C. cornea (Batsch) Fr., C. lutea (Massee) McNabb, and C. fusca Lloyd were clustered into three distinct clades, rather than forming a single clade, despite belonging to the same genus, and many of the species of Dacrymyces were grouped with other genera in one clade (Shirouzu et al. 2017; Zamora and Ekman 2020).

Calocera is ecologically saprobic, causing brown rot except *C. viscosa* (Pers.) Bory, and *C. lutea* which are white rot species (Shirouzu et al. 2009, 2013). According to the Index Fungorum (https://www.indexfungorum.org) as of June 2024, 95 species names of *Calocera* are recorded. In China, only six species have been reported: *C. sinensis* McNabb, *C. hunanensis* B. Liu & K. Tao, *C. mangshanensis* B. Liu & L. Fan, *C. morchelloides* B. Liu & L. Fan, *C. bambusicola* Sheng H. Wu, and *C. tibetica* F. Wu, L.F. Fan & Y.C. Dai (McNabb 1965; Liu et al. 1988; Liu and Fan 1989, 1990; Fan et al. 2021). *Dacrymyces* described by Nees (1816) based on *D. stillatus*, is treated as a genus of saprotrophic fungi (Shirouzu et al. 2009; Zamora and Ekman 2020; Zamora et al. 2022). A total of 234 species names of *Dacrymyces* is recorded in the Index Fungorum in June 2024, and the genus appears to be the most polyphyletic in the phylogeny of the Dacrymycetales (Shirouzu et al. 2022).

The Laojun Mountain is one of the main parts of the Three Parallel Rivers of Yunnan Protected Areas (TPRYPA), the World Natural Heritage Site, in northwest Yunnan Province, southwestern China. The TPRYPA is part of the Mountains of Southwest China Biodiversity Hotspot, which includes 12,000 plant species, 29 percent of which are found nowhere else (Zhang et al. 2010; Mittermeier et al. 2011). The Laojun Mountain is located between 26°2.80'–27°36.60'N, 99°1.20'–99°54.60'E and includes four counties, including Yulong, Jianchuan, Lanping, and Weixi, with an area of about 108,500 hm² and elevations ranging from 2,100 to 4,513 m (Zhang et al. 2010). The dominant tree species in Laojun Mountain are *Abies* sp., *Acer* sp., *Betula* sp., *Cyclobalanopsis* sp., *Fargesia* sp., *Lithocarpus* sp., *Picea* sp., *Pinus* sp., *Quercus* sp., *Rhododendron* sp., and *Sorbus* sp. (Wu and Zhu 1987).

During the investigation of the diversity of macrofungi in the Laojun Mountain, a multitude of specimens, including a dozen belonging to the *Campanophyllum* genus and several belonging to the genera *Calocera* and *Dacrymyces*, were collected from July to September 2019–2023. In this study, the specimens of these three new species were collected from the same position in a deciduous forest of the Laojun Mountain. With the combination of morphological observations and phylogenetic analyses, we described three new species, namely *Campanophyllum microsporum*, *Calocera multiramosa*, and *Dacrymyces naematelioides*.

Materials and methods

Specimen collection, morphological observation, and isolation

The fungal specimens used in this study were collected from the Laojun Mountain in northwestern Yunnan Province, China. After collection, the specimens were dried in an electric drier at ca. 45 °C, and deposited in the Herbarium of Cryptogams, Kunming Institute of Botany of the Chinese Academy of Sciences (HKAS). Macromorphological characteristics and habitats were obtained from field notes and photographs. Color codes were based on Kornerup and Wanscher (1978). Micromorphological features were observed from the dried specimens and measured and photographed in 5% KOH solution (w/v) and 1% Congo Red solution (w/v) using a Leica DM6 B upright light microscope and Leica Application Suite X (LAS X, version 3.7.5). In the description of basidiospores, the abbreviations m/n/p denote m basidiospores measured from n basidiomata of p collections. The dimensions of the microscopic structures are given as (a-) b-c (-d), in which b-c contains at least 90% of the measured values, and (a-) and (-d) are the extreme values provided in parentheses. The Q value stands for the ratio of length/width of an individual basidiospore and basidium, and L_m/W_m/Q_m refers to the average length/width/Q value of all basidiospores (Na et al. 2022; Wei et al. 2024). The strains of Campanophyllum microsporum were isolated from the inner tissue of fresh basidiomata using a Yeast Extract Peptone Dextrose Agar (YPD) Medium consisting of 2 g yeast extract (Beijing Aoboxing Biotech Co., Ltd.), 2 g peptone (Beijing Aoboxing Biotech Co., Ltd.), 20 g dextrose (Tianjin Fengchuan Chemical Reagent Co., Ltd.), 13 g agar (Biosharp Life Sciences), and 1000 mL distilled water. The living cultures were preserved at the National Germplasm Bank of Edible Mushroom (Yunnan). Their isolate IDs are YAASM 7490 and 7491.

DNA extraction, PCR amplification and sequencing

The genomic DNA was extracted from the dry specimens and cultured mycelia using the Fungal gDNA kit GD2416 (Biomiga CA, USA) following the manufacturer's instructions. The entire ITS and partial LSU of the nrDNA region were

amplified from the total DNA using the primer pair ITS5/ITS4 (White et al. 1990) and LR0R/LR7 (Vilgalys and Hester 1990; Moncalvo et al. 2000), respectively, and no DNA template was used as the negative control. The PCR cycling for the amplification of both ITS and LSU was as set follows: an initial denaturation at 95 °C for 3 min, followed by 34 cycles of 95 °C for 30 s, 56 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were sequenced bi-directionally by Tsingke Biotechnology Co., Ltd. Kunming, China. Newly generated sequences of both directions were assembled using the software SeqMan version 11.1.0 (DNASTAR, Inc.), and submitted to GenBank (accession nos. ITS: PP550870–PP550882, LSU: PP550017–PP550027).

Sequence alignment and phylogenetic analyses

The sequences used in this study were those retrieved from GenBank combined with newly generated sequences. Taxon information and GenBank accession numbers of all the sequences are listed in Table 1. All sequences were aligned using the software MAFFT 7.503 (Katoh and Standley 2013) with the default settings and edited manually using BioEdit 7.2.5 (Hall 1999). After alignment, the ITS and LSU datasets were concatenated using the program SequenceMatrix 1.8.1 (Vaidya et al. 2011). Phylogenetic analyses of Cyphellaceae and Dacrymycetaceae were performed using maximum likelihood (ML) and Bayesian inference (BI) analyses based on the sequences matrix on the personal computer. The best-fit models for the concatenated ITS+LSU dataset were selected according to the Akaike Information Criterion (AIC) in jModelTest 2.1.10 (Guindon and Gascuel 2003; Darriba et al. 2012). ML analyses of the concatenated ITS+LSU dataset of Cyphellaceae and Dacrymycetaceae were performed using RAxML-NG 1.1.0 (Kozlov et al. 2019) under the GTR+I+G model with 1,000 bootstrap replicates. BI analyses of Cyphellaceae and Dacrymycetaceae were implemented using MrBayes 3.2.7 (Ronquist et al. 2012) under the GTR+I+G model. There were four independent runs, each of which had four chains for 15,000,000 generations sampling from the posterior distribution every 1000th generation. The first 25% of the sampled trees were discarded as burn-in, while the remaining trees were used to obtain the Bayesian posterior probabilities of the clades. The constructed phylogenetic trees were visualized and edited in FigTree 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and Adobe Illustrator 25.3.1. Flammulina velutipes (Curtis) Singer, was used as an outgroup in the phylogeny of Cyphellaceae (Vizzini et al. 2022), while Suillus pictus (Peck) Kuntze, and Coprinus comatus (O.F. Müll.) Pers., were used as the outgroup in the phylogeny of Dacrymycetaceae (Shirouzu et al. 2013). The final alignments and the retrieved topologies were deposited in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S31416) with submission ID 31416.

Results

Phylogenetic analyses

In the phylogeny of Cyphellaceae, 36 sequences were used for phylogenetic analyses, of which four sequences were newly generated in this study. The concatenated dataset of ITS and LSU sequences comprised a total of 1695 characters. Table 1. Taxa used in the phylogenetic analyses and their corresponding GenBank accession numbers. Newly generated sequences are in bold. Type materials are marked with 'T'.

Species	Isolate ID /Voucher	Country	GenBank Accession Numbers			
			ITS	LSU	Keterence	
Agaricomycetes						
Campanophyllum microsporum	– /HKAS 133167	China	PP550870	PP550018	this study	
C. microsporum	– /HKAS 133168	China	PP550871	PP550019	this study	
C. microsporum	– /HKAS 133169	China	PP550872	PP550020	this study	
C. microsporum	- /HKAS 133170 [™]	China	PP550873	PP550017	this study	
C. proboscideum	- /TENN56402	Mexico	AY230866	AY230866	Cifuentes et al. (2003)	
C. proboscideum	- /TENN56427	Mexico	AY230867	AY230867	Cifuentes et al. (2003)	
C. proboscideum	- /PA46	Panama	MW386067	-	Reschke et al. (2021)	
C. proboscideum	- /PAN327	Panama	MW386071	-	Reschke et al. (2021)	
C. proboscideum	- /PAN373	Panama	MW386072	-	Reschke et al. (2021)	
C. proboscideum	- /NEHU.MBSRJ. 38	India	KP843881	-	unpublished	
Chondrostereum coprosmae	– /PDD: 119544	New Zealand	OL709440	-	unpublished	
C. coprosmae	- /PDD: 89940	New Zealand	OL709437	OL709436	unpublished	
C. purpureum	HHB-13334-sp. /-	USA	AF518607	_	Hibbett and Binder (2002)	
C. purpureum	SFI-B18 /-	Ireland	MT535785	MT559785	unpublished	
C. purpureum	14-2300 /-	USA	MG774405	_	Merlet et al. (2018)	
C. purpureum	CBS 350.53 /-	France	MH857241	MH868775	Vu et al. (2019)	
C. vesiculosum	- /PDD: 119640	New Zealand	OR607672	_	unpublished	
Cunninghammyces umbonatus	– /He 5316	China	MW557955	_	unpublished	
C. umbonatus	– /He 5311	China	MW557940	MW557954	unpublished	
C. umbonatus	– /He 5313	China	MW557941	_	unpublished	
Cyphella digitalis	- /PVKU3421	Czech	OM837174	_	Holec et al. (2022)	
		Republic				
C. digitalis	Thorn-617 /-	USA	AY293175	-	Binder et al. (2005)	
C. digitalis	CBS 679.82 /-	USA	DQ486698	AY635771	Matheny et al. (2006)	
Gloeostereum incarnatum	G1905 /HCC-3	Russia	MK278092	-	Varga et al. (2019)	
G. incarnatum	- /KUC20131022-28	South Korea	KJ668540	KJ668393	Jang et al. (2015)	
G. incarnatum	3332 /-	Sweden	AF141637	-	Parmasto and Hallenberg (2000)	
G. incarnatum	- /NIFoS 1948	South Korea	MH992519	-	unpublished	
G. incarnatum	BCC 41461 /-	Thailand	KY614001	KY614002	unpublished	
G. cimri	CBS 145006 ^T /-	Netherlands	MT023735	MN266884	Ahmed et al. (2020)	
Granulobasidium vellereum	G0482 /DK 2781	Poland	MK278094	-	Varga et al. (2019)	
G. vellereum	CBS 52.84 /-	USA	AY745729	-	unpublished	
G. vellereum	– /B. Gilsenius (GB)	Sweden	DQ677490	-	Larsson (2007)	
G. vellereum	BAFCcult 4367 /-	Argentina	KC881193	-	Robles et al. (2015)	
G. vellereum	TJU_NOV19 /-	China	OM237077	-	unpublished	
Incrustocalyptella columbiana	– /K:237992	United Kingdom	MW830122	-	unpublished	
Flammulina velutipes	AFTOL-ID 558 /-	USA	AY854073	AY639883	unpublished	
Dacrymycetes						
Calocera cornea	CBS 124.84 /-	Canada	AB712437	AB472738	Shirouzu et al. (2013)	
C. cornea	ICMP 20465 /PDD 104991	New Zealand	LC131403	LC131362	Shirouzu et al. (2017)	
C. cornea	AFTOL-ID 438 /-	unknown	AY789083	AY701526	unpublished	
C. cornea	ICMP 21223 /PDD 107847	New Zealand	LC131404	LC131363	Shirouzu et al. (2017)	
C cornea	- /I IDS E-0/077/	Swedon	MN505626	MN505626	Zamora and Ekman (2020)	
	/ OF 3 F-940/ / 4		NN/101060	NIN1550020		
c. cornea	- /UWU(MYC)6922	Ukraine	IVIV/191969	IVIVI 159089	Savcnenko et al. (2021)	
C. furcata	– /H:Spirin 10949	Russia	MW191975	MW159088	Savchenko et al. (2021)	

Species	Isolate ID /Voucher	Country	GenBank Accession Numbers		
			ITS	LSU	Reterence
C. furcata	- /TU135016	Estonia	MW191958	MW159087	Savchenko et al. (2021)
C. tibetica	– /Dai20171⊺	China	MW549777	MW750403	Fan et al. (2021)
C. tibetica	- /Dai20178	China	MW549778	MW750404	Fan et al. (2021)
C. multiramosa	- / HKAS 133171 [™]	China	PP550874	PP550021	this study
C. multiramosa	- /HKAS 133172	China	PP550875	PP550022	this study
C. multiramosa	- /HKAS 133173	China	PP550876	PP550023	this study
C. viscosa	AFTOL-ID 1679 /MW 591	Germany	DQ520102	DQ520102	unpublished
C. viscosa	TUFC12873 /TNS-F-15704	Japan	AB712439	AB299048	Shirouzu et al. (2013)
C. viscosa	- /UPS F-940773	Sweden	MN595628	MN595628	Zamora and Ekman (2020)
C. viscosa	- /CWU(MYC)6937	Ukraine	MW191970	MW159090	Savchenko et al. (2021)
Cerinomyces aculeatus	- /TUMH61942 (TUFC50098) [™]	Japan	MW191955	MW159053	Savchenko et al. (2021)
C. atrans	TUFC 30545 /-	Canada	AB712443	AB712423	Shirouzu et al. (2013)
C. borealis	- /0160848™	Norway	MW191890	MW159042	Savchenko et al. (2021)
C. brevisetus	– /URM:Chikowski 1544 [⊤]	Brazil	MW191886	MW159046	Savchenko et al. (2021)
C. creber	- /UPS:F-946512 [™]	Spain	MW191985	MW191985	Savchenko et al. (2021)
C. enatus	TUFC12876 /TNS-F-21034	Japan	AB712441	AB472696	Shirouzu et al. (2013)
C. ramosissimus	CFMR:FP-150848 [™] /-	Belize	AB712446	AB712426	Shirouzu et al. (2013)
Dacrymyces burdsallii	CFMR:HHB-6908 ^T /-	USA	AB712444	AB712424	Shirouzu et al. (2013)
D. capitatus	- /Dai 20023	China	OL587808	OL546776	unpublished
D. capitatus	CBS 293.82 /-	Canada	AB712450	AB472741	Shirouzu et al. (2013)
D. ceraceus	CFMR:HHB-8969 [⊤] /-	USA	AB712442	AB712422	Shirouzu et al. (2013)
D. chrysocomus	- /UPS:F-940136	Spain	MN595629	MN595629	Zamora and Ekman (2020)
D. chrysocomus	- /UPS:F-940134	Sweden	MN595630	MN595630	Zamora and Ekman (2020)
D. chrysospermus	TUFC13115 /TNS-F-15712	Japan	AB712452	AB299073	Shirouzu et al. (2013)
D. chrysospermus	- /H:Spirin 10795	Russia	MW191974	MW159078	Savchenko et al. (2021)
D. chrysospermus	– /H:Miettinen 14818	USA	MW191961	MW159077	Savchenko et al. (2021)
D. aff. Chrysospermus	- /UPS:F-593536	Japan	MN595631	MN595631	Zamora and Ekman (2020)
D. dictyosporus	CFMR:HHB-8618 /-	USA	AB712454	AB712429	Shirouzu et al. (2013)
D. estonicus	- /UPS:F-940137	Sweden	MN595632	MN595632	Zamora and Ekman (2020)
D. estonicus	- /UPS:F-940138	Sweden	MN595633	MN595633	Zamora and Ekman (2020)
D. fennicus	– /H:Miettinen 21174	Finland	MW191957	MW159071	Savchenko et al. (2021)
D. fennicus	- /UPS:F-946596	Sweden	MZ147627	MZ147627	Savchenko et al. (2021)
D. grandinioides	- /H7008841	Kenya	MW191950	MW159076	Savchenko et al. (2021)
D. lacrymalis	TUFC13327 /TNS-F-15719	Japan	AB712456	AB299069	Shirouzu et al. (2013)
D. cf. minor	– /H:Miettinen 19137	Finland	MW191967	MW159080	Savchenko et al. (2021)
D. cf. minor	– /H:Miettinen 20591	Finland	MW191965	MW159079	Savchenko et al. (2021)
D. naematelioides	- /HKAS 133174a [⊤]	China	PP550877	PP550024	this study
D. naematelioides	– /HKAS 133174b [⊤]	China	PP550878	PP550025	this study
D. naematelioides	- /HKAS 133174c [™]	China	PP550879	PP550026	this study
D. naematelioides	- /HKAS 133174d [™]	China	PP550880	PP550027	this study
D. naematelioides	YAASM 7490 /-	China	PP550881	_	this study
D. naematelioides	YAASM 7491 /-	China	PP550882	_	this study
D. ovisporus	– /H:Miettinen 20787	Finland	MW191964	MW159074	Savchenko et al. (2021)
D. ovisporus	- /H:Spirin 11145	Norway	MW191960	MW159073	Savchenko et al. (2021)
D. pinacearum	- /UPS:F-593533	Japan	MN595637	MN595637	Zamora and Ekman (2020)
D. pinacearum	- /UPS:F-593535	Japan	MN595638	MN595638	Zamora and Ekman (2020)
D. puniceus	TUFC12833 /TNS-F-15711	Japan	AB712449	AB299057	Shirouzu et al. (2013)
D. puniceus	- /Wu180	China	OL587812	OL546780	unpublished
D. sinostenosporus	– /Dai 20003 [⊤]	China	MW540888	MW540890	Lian et al. (2022)
D. sinostenosporus	– /Dai 20008	China	MW540889	MW540891	Lian et al. (2022)
D. sobrius	CFMR:RLG-13487 ^T /-	USA	AB712445	AB712425	Shirouzu et al. (2013)

Species	Isolate ID /Voucher	Country	GenBank Accession Numbers		
			ITS	LSU	Reference
D. stenosporus	ICMP 20488 /PDD 105018 ^T	New Zealand	LC131433	LC131396	Shirouzu et al. (2017)
D. stenosporus	ICMP 21237 /PDD 107970	New Zealand	LC131434	LC131397	Shirouzu et al. (2017)
D. stillatus (anamorph)	- /UPS:F-939814	Sweden	MN595676	MN595676	Zamora and Ekman (2020)
D. stillatus (anamorph)	- /UPS:F-939816	Sweden	-	MN593494	Savchenko et al. (2021)
D. stillatus (teleomorph)	- /UPS:F-939814	Sweden	MN595677	MN595677	Zamora and Ekman (2020)
D. stillatus (teleomorph)	- /UPS:F-939816	Sweden	-	MN593495	Savchenko et al. (2021)
D. subalpinus	TUFC12834 /TNS-F-15730	Japan	AB712465	AB299060	Shirouzu et al. (2013)
D. venustus	– /0:Adane 150 [⊤]	Ethiopia	MW191949	MW159075	Savchenko et al. (2021)
Dacryonaema macnabbii	- /UPS:F-940949	Sweden	MN595650	MN595650	Zamora and Ekman (2020)
D. macnabbii	- /UPS:F-940992	Sweden	MN595653	MN595653	Zamora and Ekman (2020)
D. macrosporum	- /UPS:F-940998	Finland	MN595660	MN595660	Zamora and Ekman (2020)
D. macrosporum	- /UPS:F-941001	Finland	MN595661	MN595661	Zamora and Ekman (2020)
D. rufum	- /UPS:F-941005	Sweden	MN595646	MN595646	Zamora and Ekman (2020)
D. rufum	- /UPS:F-941012	Finland	MN595649	MN595649	Zamora and Ekman (2020)
Dacryopinax elegans	– /TENN 066927	USA	MN595640	MN595640	Zamora and Ekman (2020)
Dacryopinax sp.	– /H7008759	Kenya	MW191959	MW159091	Savchenko et al. (2021)
D. spathularia	TUFC12846 /TNS-F-21048	Japan	AB712473	AB472710	Shirouzu et al. (2013)
D. spathularia	FCME 27539 /-	Mexico	MN733711	MN733722	Castro-Santiuste et al. (2020)
D. spathularia	– /H:Miettinen 20559	Indonesia	MW191976	MW159092	Savchenko et al. (2021)
Dendrodacrys ciprense	- /UPS:F-946590 [⊤]	Cyprus	OM519385	OM519385	Zamora et al. (2022)
D. ciprense	- /UPS:F-946591	Cyprus	OM519386	OM519386	Zamora et al. (2022)
D. concrescens	- /UPS:F-946602 [⊤]	Sweden	OM519390	OM519390	Zamora et al. (2022)
D. ellipsosporum	- /UPS:F-946604 [⊤]	Spain	OM519392	OM519392	Zamora et al. (2022)
D. oblongisporum	- /UPS:F-979568 [⊤]	Spain	OM519400	OM519400	Zamora et al. (2022)
Ditiola peziziformis	– /H:Haikonen 24269	Finland	MW191972	MW159070	Savchenko et al. (2021)
D. peziziformis	– /H:Haikonen 30097	Finland	MN595642	MN595642	Zamora and Ekman (2020)
D. radicata	- /H:Miettinen 20590.2	Finland	MW191966	MW159083	Savchenko et al. (2021)
D. radicata	- /UPS:F-939957	Sweden	MN595641	MN595641	Zamora and Ekman (2020)
Guepiniopsis buccina	- /CWU(MYC)7014	Ukraine	MW191971	MW159086	Savchenko et al. (2021)
G. buccina	- /UPS:F-940947	Spain	MN595643	MN595643	Zamora and Ekman (2020)
Unilacryma unispora	- /UPS:F-941279	Sweden	MN595667	MN595667	Zamora and Ekman (2020)
U. bispora	- /UPS:F-941254	Sweden	MN595670	MN595670	Zamora and Ekman (2020)
U. bispora	- /UPS:F-941266	Sweden	MN595674	MN595674	Zamora and Ekman (2020)
Dacrymycetes sp.	NBRC 110592 /-	Japan	LC004003	LC003884	Shirouzu et al. (2016)
Coprinus comatus	AFTOL-ID 626 /-	USA	AY854066	AY635772	Shirouzu et al. (2013)
Suillus pictus	AFTOL-ID 717 /-	USA	AY854069	AY684154	Shirouzu et al. (2013)

ML and BI analyses generated similar topologies, so only the ML tree is presented along with the support values from the Maximum likelihood bootstrap (BS, >75%) values and Bayesian inference (BI) posterior probabilities (PP, >0.95) (Fig. 1). The phylogeny revealed that Cyphellaceae was divided into three clades, and these three genera of *Incrustocalyptella* Agerer, *Cyphella* Fr., and *Campanophyllum* constituted one of the three major clades with strong statistical supports (99% BS, 1.00 PP). In the ML tree, the phylogenetic results demonstrated that *Campanophyllum microsporum* formed a distinct lineage closely related to *C. proboscideum* and *Campanophyllum* sp. (Voucher NEHU.MBSRJ. 38) with strong statistical supports (98% BS, 0.99 PP). The ITS sequences from *C. microsporum* and *C. proboscideum* were markedly different, with ca. 105 different nucleobases, and the ITS sequences of *C. microsporum* and *Campanophyllum* sp. (Voucher NEHU.MBSRJ. 38) were different with about 20 different nucleobases.



Figure 1. Maximum likelihood (ML) tree of Cyphellaceae based on the combined ITS+LSU dataset. ML bootstrap values (BS > 75%) and Bayesian posterior probabilities (PP > 0.95) are shown at the nodes in the order of BS/PP. The tree is rooted with *Flammulina velutipes*. The new taxon is indicated in bold.

In the phylogeny of Dacrymycetaceae, the concatenated dataset of LSU and ITS sequences comprised a total of 1649 characters. 94 sequences were used for phylogenetic analyses, of which nine sequences were newly generated in this study. ML and BI analyses generated similar topologies, so only the ML tree is presented (Fig. 2). Within Dacrymycetes, we distinguish four main groups, Dacrymycetaceae (clade A), Cerinomycetaceae (clade B), Dacryonaemataceae (clade C), and Unilacrymaceae (clade D). The clade A included several genera and the majority of species, and formed a sister group to the clades B, C, and D with strong statistical support (95% BS, 1.00 PP). Samples of the two new species were placed in the clade A, and one of the new species of Dacrymyces naematelioides formed a non-monophyletic, strongly supported group. The new species Calocera multiramosa was found to be closely related to C. tibetica with high supports (85% BS, 1.00 PP), and the two species clustered together with C. viscosa with strong supports (91% BS, 1.00 PP). The new species D. naematelioides formed a sister lineage to D. chrysospermus with 78% bootstrap support and 1.00 posterior probability.



Figure 2. Maximum likelihood (ML) tree of Dacrymycetaceae based on the combined ITS+LSU dataset. ML bootstrap values and Bayesian posterior probabilities are shown at the nodes in the order of BS/PP. The tree is rooted with *Suillus pictus* and *Coprinus comatus*. The new taxon is indicated in bold.

Taxonomy

Campanophyllum microsporum Y.H. Ma, W.M. Chen & Y.C. Zhao, sp. nov. MycoBank No: 853503

Figs 3-5

Diagnosis. Campanophyllum microsporum is characterized by dorsally pseudostipitate pileus, excentric to lateral pseudostipe, crowded lamellae, cylindrical-ellipsoid basidiospores $(3.0-4.2 \times 1.7-2.2 \mu m)$, narrowly clavate to clavate basidia $(14.5-23.0 \times 3.0-4.0 \mu m)$, and cylindrical to clavate cheilocystidia $(22.0-55.0 \times 5.0-11.0 \mu m)$; occurrence in a deciduous forest and solitary, cespitose, scattered, or gregarious habit on rotten wood.

Type. CHINA. Yunnan Province: Jianchuan County, Laojunshan Town (26°35.85'N, 99°40.44'E, elev. 3100 m), on rotten wood, 21 September 2023, Yuan-Hao Ma, Min Zeng & Wei-Min Chen (Holotype: HKAS 133170!, ex-type: YAASM 7187).

Etymology. The epithet "microsporum" refers to the smaller basidiospores compared to *Campanophyllum proboscideum*.

Description. Basidiomata pseudostipitate, dorsally and eccentrically or laterally attached to substrate, occasionally central, pendent, broadly cyphelloid to crepidotoid, lamellate. Pileus $5.0-12.0 \times 4.0-9.0$ cm, spathulate, flabelliform to rounded-flabelliform, sometimes subcircular; plano-convex when young and applanate when older, margin inrolled, lobate when fully expanded; surface moist, initially pale orange (5A2–4), greyish orange (5B2–3), or light orange (6A2–5), then brownish orange (6C5–6), light brown (6D5–8), often with small stains of darker colors. Context thick, fleshy, whitish, and unchanging in color when injured. Lamellae extending radially from attachment point within pseudostipe, very crowded, sometimes forked, white to off-white, sometimes with small blackish stains. Pseudostipe $0.5-2.5 \times 0.4-1.0$ cm, concolorous with pileus, discolouring to blackish-ochre (6E5-7, 6F7). Spore print white. Taste mild, odor indistinct.

Basidiospores [149/7/4] (2.7–)3.0–4.2(–4.5) × (1.5–)1.7–2.2(–2.6) µm, $L_m = 3.5 \mu m$, $W_m = 1.9 \mu m$, Q = 1.4–2.5, $Q_m = 1.8$, cylindrical-ellipsoid, smooth, hyaline, thin-walled, inamyloid. Basidia (13.5–)14.5–23.0(–26.0) × (2.3–)3.0– 4.2(–4.6) µm, $L_m = 17.6 \mu m$, $W_m = 3.6 \mu m$, Q = 3.6–7.4, $Q_m = 4.9$, narrowly clavate to clavate, 4-spored, sterigma 0.9-2.2 µm. Cheilocystidia abundant, (17.5–)22.0– $55.0(-59.0) \times (4.2–)5.0–10.8(-13.9) \mu m$, $L_m = 37.0 \mu m$, $W_m = 7.2 \mu m$, hyaline, thin-walled, mostly cylindrical to clavate, sometimes lageniform, rod-like, or beaked-utriform, pedunculate (1.9–12.5 × 1.8–4.1 µm). Pleurocystidia not observed. Lamellar trama hyaline, parallel, hyphae 3.6–17.6 µm in diameter, thin- to thick-walled. Pileipellis composed of repent, parallel hyphae, 4.2–11.5 (–17.0) µm in diameter, sometimes with yellow-brown, intracellular pigments. Clamp connections present in all tissues of basidiomata.

Culture characteristics. Colonies grown on YPD reaching 40 mm radius within 20 days at 22 °C in the dark, forming abundant aerial mycelium, usually zonate. Mycelium irregularly cottony, with common clamp connections, pallid mouse gray to pale brown in aerial mycelium with age, easily forming basidiomata in the Petri plate.

Habitat and distribution. Solitary, cespitose, scattered, or gregarious on rotten wood in a deciduous forest; known from Yunnan, China.



Figure 3. Basidiomata of *Campanophyllum microsporum* in the field **A** HKAS 133170 (Holotype) **B** HKAS 133169. Photos by Y.H. Ma. Scale bars: 3 cm.



Figure 4. Morphological features of *Campanophyllum microsporum* on YPD medium after 20 days in the dark in a 9 cm Petri plate (ex-type YAASM 7187) **A** surface of colony **B** reverse of colony. Photos by Y.H. Ma. Scale bars: 2 cm.

Additional specimens examined. CHINA, Yunnan Province: Jianchuan County, Laojunshan Town, 7 July 2022, Yuan-Hao Ma, Ping Liu & Yong-Chang Zhao (HKAS 133167, HKAS 133168); 26 July 2023, Yuan-Hao Ma & Ping Liu (HKAS 133169).

Notes. *Campanophyllum microsporum* is similar to *C. proboscideum* in both macro- and micro-morphology, including broadly cyphelloid to crepidotoid basidiomata, spathulate, flabelliform to rounded-flabelliform pileus, and very crowded lamellae; cylindrical-ellipsoid basidiospores, narrowly clavate to clavate basidia. However, several other features can distinguish the two species. Morphologically, the new species have smaller basidiospores $(3.0-4.2 \times 1.7-2.2 \ \mu m \ vs. 4-4.5 \times 2-3 \ \mu m)$, slenderer and longer basidia $(14.5-23.0 \times 3.0-4.2 \ \mu m \ vs. 14-17 \times 4.5-5.0 \ \mu m)$, and larger cheilocystidia $(22.0-55.0 \times 5.0-10.8 \ \mu m \ vs. 18-25 \times 9-11 \ \mu m)$ (Cifuentes et al. 2003).



Figure 5. Microscopic structures of *Campanophyllum microsporum* (Holotype HKAS 133170) **A** basidiospores in Congo red **B** basidia in Congo red **C–H** cheilocystidia (**C–E** in KOH solution **F–H** in Congo red). Photos by Y.H. Ma. Scale bars: 10 μm.

Calocera multiramosa Y.H. Ma, W.M. Chen & Y.C. Zhao, sp. nov. MycoBank No: 853504 Figs 6-8

Diagnosis. Calocera multiramosa differs from other species of the genus by yellowish to orange basidiomata, dendroid and dichotomously branches, branched, smooth, thin-walled marginal hyphae ($2.0-4.8 \mu m$), branched, thin-

walled internal hyphae (2.9–10.0 μ m), cylindrical to clavate basidia (36.5–52.5 × 4.0–6.0 μ m), 1–5-septate, navicular or reniform basidiospores (10.4–16.7 × 5.2–7.4 μ m), occurrence in a deciduous or coniferous forest, occasionally scattered habit on standing timber.

Type. CHINA. Yunnan Province: Shangri-La County, Pudacuo National Park (27°50.61'N, 99°57.03'E, elev. 3800 m), on standing timber, 17 August 2020, Yuan-Hao Ma, Hong-Mei Chai & Wei-Min Chen (Holotype: HKAS 133171!).

Etymology. The epithet "multiramosa" refers to abundant branches of basidiomata.

Description. Basidiomata stipitate, fasciculate, usually geminate, occasionally scattered, gelatinous, 1.5-4.0 cm in height, tough, dendroid and dichotomously branched, cylindrical or flattened, surface smooth, yellowish to orange (5B8, 6A8, 6B7–8), 0.3-0.5 cm in diameter at the upper branching part. Marginal hyphae on sterile surfaces cylindrical, branched, smooth, straight or flexuous, septate, thin-walled, hyaline, 2.0-4.8 µm in diameter. Internal hyphae branched, septate, thin-walled, hyaline, 2.9-10.0 µm in diameter. Hymenium limited to the upper surface of basidomata, amphigenous, composed of basidia and simple cylindrical hyphidia; hyphidia hyaline or pale yellow, smooth, thin-walled. Subhymenial hyphae hyaline, smooth or scabrous, thin- or slightly thick-walled, 2.5-7.3 µm in diameter. Basidia cylindrical to clavate, hyaline or pale yellow, thin-walled,



Figure 6. Basidiomata of *Calocera multiramosa* **A** HKAS 133171 (Holotype) **B** HKAS 133172 **C** HKAS 133173. Photos by Y.H. Ma. Scale bars: 3 cm.



Figure 7. Microscopic structures of *Calocera multiramosa* in Congo red (Holotype HKAS 133171) **A**, **B** basidiospores **C** germinating basidiospores **D** probasidia, developing basidia and hyphidia **E** abnormal developing basidia with septa and geminations. Photos by Y.H. Ma. Scale bars: 10 μm.



Figure 8. Microscopic structures of *Calocera multiramosa* in Congo red (Holotype HKAS 133171) **A** marginal hyphae **B** internal hyphae **C** subhymenial hyphae, probasidia, developing basidia, and hyphidia. Photos by Y.H. Ma. Scale bars: 10 µm.

becoming bifurcate when mature, $(33.5-)36.5-52.5(-55.0) \times (3.5-)3.8-6.1(-6.4) \mu m$, $L_m = 45.1 \mu m$, $W_m = 4.9 \mu m$, sometimes with many septa. Basidiospores [102/3/3], navicular or reniform, straight or curved, with a small apiculum at the top, thin-walled with thin septa, hyaline to pale yellow, sometimes with oil drops when young and in the germination stage, $(6.5-)10.4-16.7(-17.0) \times (4.5-)5.2-7.4(-8.8) \mu m$, $L_m = 14.3 \mu m$, $W_m = 6.3 \mu m$, Q = (1.4-)1.6-2.7(-2.8), $Q_m = 2.3$, 1–5-septate at maturity. Germination with conidia by abnormally developing basidia with lots of septa, by hyphae with septa, or by germ tubes. Clamp connections absent in all tissues of the basidiomata.

Habitat and distribution. Geminate, occasionally scattered on standing timber in a deciduous or coniferous forest; known from Yunnan, China.

Additional specimens examined. CHINA. Yunnan Province: Shangri-La County, Pudacuo National Park, 28 August 2021, Yuan-Hao Ma, Ping Liu & Yong-Chang Zhao (HKAS 133172); Jianchuan County, Laojunshan Town, 26 July 2023, Yuan-Hao Ma & Ping Liu (HKAS 133173).

Notes. Calocera multiramosa resembles C. tibetica, C. viscosa and C. mangshanensis in dendrite basidiomata. However, C. multiramosa is distinguished from C. tibetica by larger basidiospores (10.4-16.7 × 5.2-7.4 µm vs. 9.0-13.0 × 5.0-6.0 μ m) with different septa (1-5 vs. 3-4) (Fan et al. 2021); C. multiramosa differs from C. viscosa in larger basidia (36.5-52.5 × 3.8-6.1 µm vs. $23-42 \times 3-4.5 \mu m$) and basidiospores with different septa (1-5 vs. 0-1) (McNabb 1965; Shirouzu et al. 2009). C. multiramosa can be distinguished from C. mangshanensis by larger (10.4-16.7 × 5.2-7.4 µm vs. 10.0-13.0 × $4.5-5.5 \mu$ m), more septate (1-5 vs. 0-1) basidiospores (Liu and Fan 1989). The new species grows on angiosperm and gymnosperm wood, while C. tibetica and C. viscosa only grows on gymnosperm wood and C. mangshanensis only grows on decayed angiosperm wood (McNabb 1965; Liu and Fan 1989; Oberwinkler 2014). C. multiramosa can be distinguished from C. cornea by the size of the basidiomata (1.5-4.0 cm vs. 0.1-0.5 cm high) (Shirouzu et al. 2009), and C. furcata by the mature basidiospores with different septa (1-5 vs. 1-3) (McNabb 1965). The specimen of C. multiramosa, collected from the Laojun Mountain could not be designated as the holotype because of many immature basidiospores. Therefore, the specimen of C. multiramosa collected from a coniferous forest in the Pudacuo National Park was designated as the holotype.

Dacrymyces naematelioides Y.H. Ma, W.M. Chen & Y.C. Zhao, sp. nov. MycoBank No: 853505 Figs 9–11

Diagnosis. Dacrymyces naematelioides differs from other species of the genus by stipitate and cerebriform basidiomata, smooth or roughened, simple or branched, septate marginal hyphae $(3.0-8.5 \ \mu m)$, smooth or roughened, thin-walled, branched, and septate internal hyphae $(2.3-11.0 \ \mu m)$, cylindrical to clavate, smooth or roughened basidia $(38.5-79.5 \times 6.5-10.6 \ \mu m)$, broadly elliptic-fusiform, 7-septate mature basidiospores $(18.5-28.6 \times 8.9-13.8 \ \mu m)$, the absence of clamp connections, occurrence in a deciduous forest, and fasciculate, gregarious, or scattered habit on rotten wood.



Figure 9. Basidiomata of Dacrymyces naematelioides A-D HKAS 133174 (Holotype). Photos by Y.H. Ma. Scale bars: 3 cm.



Figure 10. Microscopic structures of *Dacrymyces naematelioides* (Holotype HKAS 133174) **A** immature and mature basidiospores in Congo red and KOH solution **B** probasidia, developing basidia and hyphidia in Congo red. Photos by Y.H. Ma. Scale bars: 10 μm.



Figure 11. Microscopic structures of *Dacrymyces naematelioides* (Holotype HKAS 133174) **A** marginal hyphae **B** internal hyphae **C** subhymenial hyphae. Photos by Y.H. Ma. Scale bars: 10 µm.

Type. CHINA. Yunnan Province: Jianchuan County, Laojunshan Town (26°35.86'N, 99°40.46'E, elev. 3100 m), 21 September 2023, Yuan-Hao Ma, Min Zeng & Wei-min Chen (Holotype: HKAS 133174!).

Etymology. The epithet "naematelioides" refers to the similarity of the new species in terms of macromorphological features to *Naematelia aurantialba*.

Description. Basidiomata stipitate, fasciculate and conspicuous, gregarious or scattered, gelatinous when fresh, cerebriform, 2.5-4.5 cm high, surface smooth, orange to light brown (6A8, 6D7-8), occasionally colorless, stipe flat cylindrical, usually with white hairs. Marginal hyphae on sterile surfaces of basidiocarps cylindrical, simple or branched, smooth or roughened, straight or flexuous, septate, thick-walled, hyaline, 3.0-8.5 µm in diameter. Internal hyphae branched, septate, thin-walled, hyaline, smooth or roughened, 2.3-11.0 µm in diameter. Hymenium limited to the upper surface of the basidoma, amphigenous, composed of basidia and simple cylindrical hyphidia; hyphidia hyaline or pale yellow, smooth, thin-walled. Subhymenial hyphae, smooth or roughened, thin- to thick-walled, 2.5-5.3 µm in diameter. Basidia cylindrical to clavate, smooth or roughened, pale yellow, thin-walled, becoming bifurcate, (30.0-)38.5-79.5(-83.5) × (5.5-)6.5-10.6(-11.1) μm, L_m = 60.2 μm, W_m = 8.3 µm. Basidiospores [95/5/1], broadly and elliptic-fusiform, with a small apiculum at the base, thin-walled, pale yellow, with oil drops when young, (16.5–)18.5–28.5(–29.5) × (8.7–)8.9–13.8(–14.6) μm, L_m = 23.9 μm, W_m = 11.0 μ m, Q = (1.5–)1.8–2.4(–2.5), Q_m = 2.2, usually 7–septate, rarely 3– or 4– septate at maturity. Germination not observed. Clamp connections absent in all tissues of the basidiomata.

Habitat and distribution. Fasciculate, gregarious, or scattered habit on rotten wood, and occurrence in a deciduous forest; known from Yunnan, China.

Notes. *Dacrymyces naematelioides* resembles *D. chrysospermus* and *D. dictyosporus* in shape and size of basidiomata. Microscopically, *D. chrysospermus* differs from *D. naematelioides* by narrower basidia (4–6.5 μ m vs. 6.5–10.6 μ m in width) and smaller basidiospores (16.5–23 × 5–7.5 μ m vs. 18.5–28.6 ×8.9–13.8 μ m), and *D. dictyosporus* differs by smooth basidia and thick-walled basidiospores (Martin et al. 1958; McNabb 1973).

Discussion

In this study, we described three new species from Yunnan Province, China, based on morphological evidence and multi-locus phylogenic analyses. The identified morphological features of *Campanophyllum* include dorsally pseudostipitate pileus, excentric to lateral pseudostipe, and crowded lamellae (Cifuentes et al. 2003; Reschke et al. 2021). The specimen of *Campanophyllum* sp. (Voucher NEHU.MBSRJ. 38) reported from India formed a sister lineage to *C. microsporum* with strong supports (100% BS, 1.00 PP). The specimen (Voucher NEHU.MBSRJ. 38) is presumably a new species in the genus *Campanophyllum* based on the phylogenetic trees (Fig. 1), but it needs to be further confirmed. Meanwhile, our research indicates that more new species of this genus will be discovered in China. However, their habitat is in decline and disappearing.

The species of *Calocera* in the family Dacrymycetaceae are typically distinguished morphologically based on simple or forked clavarioid basidiocarps (Oberwinkler 2014), but the genus *Dacrymyces* includes more than 30 species with variable basidiomata including pulvinate, discoid, turbinate, spathulate, flabellate, and cylindrical forms (McNabb 1973; Shirouzu et al. 2009). Several species of *Dacrymyces* are morphologically close to *Calocera* by sharing spathulate or cylindrical basidioma, and yet they can be distinguished by some other morphological features, such as the septa of the basidiospores, morphology of the basidia, and morphology of the marginal hyphae. More appropriate genus boundaries and definitions can be obtained by studying detailed morphological and molecular data on more specimens of Dacrymycetaceae. Continuing collection efforts and herbarium searches in unidentified Dacrymycetaceae will certainly uncover more new species (Savchenko et al. 2021).

Phylogenetic analyses, based on two combined loci (ITS, LSU), as well as morphological characteristics, are important for the identification of *Calocera* and *Dacrymyces* species. The two newly proposed species formed separate branches on the phylogenetic trees with strong statistical support, and the phylogenetics for the genera presented here were found to be similar to those of previous studies (Shirouzu et al. 2013). The results of our study indicated that the specimens of *Dacrymyces naematelioides* collected from the same locality formed two distinct clades in the phylogenetic analysis (Fig. 2) with strong statistical support (100% BS, 1.00 PP), but there is no marked difference in their morphological characteristics. This suggests that there may be some variation in this species at the molecular level.

The abnormal developing basidia and probasidia with a lot of septa in specimens of *Calocera multiramosa* were also observed clearly under the microscope, and sometimes they can germinate with microconidia in the basidiomata. This microscopic feature may also be useful in identifying species in the genus *Calocera*. The surface of the basidia of the *Dacrymyces naematelioides* is smooth or roughened (Fig. 9). However, the surface features of the basidia did not seem to have been noted in much of the literature, and it is likely that in most species the surface of the basidia is smooth or has only one morphological feature.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

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Data availability

All of the data that support the findings of this study are available in the main text.

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Corrigenda

Corrigendum: Vadthanarat S, Raghoonundon B, Lumyong S, Raspé O (2024) *Rostrupomyces*, a new genus to accommodate *Xerocomus sisongkhramensis*, and a new *Hemileccinum* species (Xerocomoideae, Boletaceae) from Thailand. MycoKeys 103: 129– 165. https://doi.org/10.3897/mycokeys.103.107935

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It was kindly brought to our attention that the names *Rostrupomyces* and *Rostrupomyces sisongkhramensis* were not valid because incomplete designation of the type species of the former name and of the basionym of the latter name (Shenzhen code: Art. 40.1, see Arts 40.3 and Arts 6.3, 12.1; and Art. 41.5; Turland et al. 2018). Therefore, we would like to properly typify the genus and cite the basionym of the new combination, as follows.

Rostrupomyces Vadthanarat & Raspé, gen. nov.



Type species. *Xerocomus sisongkhramensis* Khamsuntorn, Pinruan & Luangsa-ard Persoonia 49: 295 (2022).

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Rostrupomyces sisongkhramensis (Khamsuntorn, Pinruan & Luangsa-ard) Vadthanarat, Raghoonundon & Raspé, comb. nov.

Basionym. Xerocomus sisongkhramensis Khamsuntorn, Pinruan & Luangsa-ard, Persoonia 49: 295 (2022). Citation: Vadthanarat S, Raghoonundon B, Lumyong S, Raspé O (2024) Corrigendum: Vadthanarat S, Raghoonundon B, Lumyong S, Raspé O (2024) Rostrupomyces, a new genus to accommodate Xerocomus sisongkhramensis, and a new Hemileccinum species (Xerocomoideae, Boletaceae) from Thailand. MycoKeys 103: 129-165. https://doi.org /10.3897/ mycokeys.103.107935. MycoKeys 107: 351-352. https://doi.org/10.3897/ mycokeys.107.132226

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Additional information

Conflict of interest

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Data availability

All of the data that support the findings of this study are available in the main text.