

Research Article

Two new species of *Hymenagaricus* (Agaricales, Agaricaceae) from Oman, based on morphology and molecular phylogeny

Shah Hussain^{1,2}, Moza Al-Kharousi¹, Dua'a Al-Maqbali¹, Arwa A. Al-Owaisi¹, Rethinasamy Velazhahan², Mohamed N. Al-Yahya'ei¹⁰, Abdullah M. Al-Sadi²⁰

1 Oman Animal and Plant Genetic Resources Center (Mawarid), Ministry of Higher Education, Research and Innovation, P.O. Box 515, P.C. 123, Muscat, Oman

2 Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, PO Box 34, AlKhoud 123, Oman

Corresponding authors: Mohamed N. Al-Yahya'ei (mohamed.alyahyaei@moheri.gov.om); Abdullah M. Al-Sadi (alsadi@squ.edu.om)

Abstract

Hymenagaricus has small to medium-sized mushrooms and the cap surface with squamulose pellicles, consisting of hymeniform or pseudoparenchymatous cells and yellowish-brown basidiospores. The species of *Hymenagaricus* are very similar to those of *Xanthagaricus* and it is extremely difficult to differentiate the species of both genera in the field. However, phylogenetically, both the genera are clearly distinct. In this study, we describe two new species of *Hymenagaricus*, i.e. *H. wadijarzeezicus* and *H. parvulus* from the southern part of Oman. Species descriptions are based on a combination of morphological characteristics of basidiomata and phylogenetic analyses of three gene regions: internal transcribed spacer (ITS1-5.8S-ITS2 = ITS), the large subunit of nuclear ribosomal DNA (28S) and translation elongation factor one alpha (EF-1α). Full descriptions, micrographs and illustration of anatomical features, basidiomata photos and phylogenetic analyses results of the new taxa are provided. Morphological comparisons of new taxa with similar species and a key to species included in the phylogenetic analyses are also provided.

Key words: Dhofar, diversity, taxonomy, termite mounds, two new taxa

Introduction

Three species previously in *Agaricus* subgenus *Conioagaricus*, *Agaricus hymenopileus*, *A. alphitochrous* and *A. nigrovinosus*, were placed in a new and separate genus called *Hymenagaricus* Heinem. by Heinemann in 1981. This taxonomic change was likely due to the distinct features observed in the cap of these species during different stages of their development. At the young stage, the pilei are entirely covered with a pellicle, but as they mature, the pellicle is disrupted, leaving a single large squamulose pellicle at the centre of the pileus. The squamules are composed of hymeniform or pseudoparenchymatous cells, which set these species apart from others within the genus *Agaricus* (Heinemann 1981). The genus *Hymenagaricus* was typified by *H. hymenopileus* (Heineman), belonging to the family Agaricaceae Chevall. (Heinemann 1981).

Species of *Hymenagaricus* are saprotrophic in nature and are mostly distributed in the Palaeotropical Regions. Members of this genus are recognised



Academic editor: Alfredo Vizzini Received: 2 October 2023 Accepted: 28 March 2024 Published: 17 April 2024

Citation: Hussain S, Al-Kharousi M, Al-Maqbali D, Al-Owaisi AA, Velazhahan R, Al-Yahya'ei MN, Al-Sadi AM (2024) Two new species of *Hymenagaricus* (Agaricales, Agaricaceae) from Oman, based on morphology and molecular phylogeny. MycoKeys 105: 1–19. https://doi.org/10.3897/ mycokeys.105.113591

Copyright: © Shah Hussain 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). by the squamulose pellicle on the pileus surface that mostly consists of hymeniform cells or pseudoparenchymatous tissues, yellow to yellowish-brown basidiospores and the absence of both pleurocystidia and clamp connections (Heinemann and Little Flower 1984; Reid and Eicker 1995; Little Flower et al. 1997; Hosen et al. 2017; Al-Kharousi et al. 2022a). The number of known species in the genus is 17 (Hussain et al. 2018; Kumla et al. 2021, 2023; Syed et al. 2023).

Phylogenetically, species of *Hymenagaricus* are intermixed with the monotypic genus *Heinemannomyces* Watling (Hosen et al. 2017; Hussain et al. 2018). This intermixing may be due to limited molecular data available for the previously-described species of *Hymenagaricus*. However, morphologically, both genera can be differentiated. *Heinemannomyces* with single species *H. splendidissimus* Watling, distributed in southeast Asia, has medium-sized basidiomata, with woolly fibrillose cap surface, composed of pseudoparenchymatous cells and the spore print is leaden-grey to dark blue (Watling 1998).

Four species of Agaricaceae, namely *Agaricus arabiensis* S. Hussain & Al-Sadi, *Micropsalliota ventricocystidiata* Al-Sadi & S. Hussain, *Xanthagaricus appendiculatus* Al-Sadi & S. Hussain and *X. omanicus* Al-Kharousi, Al-Sadi & S. Hussain have recently been described from Dhofar Region, Oman (Al-Kharousi et al. 2022a, 2022b; Hussain et al. 2022). However, no *Hymenagaricus* species has been reported from the country.

During the years 2022–23, macrofungal exploration missions were conducted in the Dhofar Region, in which we collected ten (10) collections of *Hymenagaricus*. Morphological characterisation and multigene (ITS, 28S, EF-1 α) phylogenetic analyses revealed that the 10 collections represent two new species, which are described in this study.

Materials and methods

Study sites and field sampling

The specimens were collected in the Dhofar Region, located in the south of the Sultanate of Oman. The region experiences a monsoon-influenced climate with a distinct wet season known as the Khareef, which occurs from June to early September (Bookhagen et al. 2005). During this time period, the moist and cool air from the Indian Ocean is drawn in by the southwest monsoon, bringing significant rainfall into the region, which is extremely rare in the rest of the Arabian Peninsula, including Oman. This seasonal variation supports a diverse ecosystem and separates Dhofar from the arid desert conditions that prevail in the Arabian Peninsula (El-Sheikh 2013). The Khareef season triggers the growth of various plants and trees, including frankincense trees, creating a lush and vibrant landscape where a number of saprotrophic mushrooms can flourish (Al-Kharousi et al. 2022a).

In the current study, mushroom specimens were collected from three localities (Wadi Naheez, Wadi Jahaneen, Wadi Jarzeez) of the Dhofar Region, in the months of August–September 2022 to 2023. The specimens were photographed in the field and field characteristics such as the shape, colour, size and smell of basidiomata were noted. The samples were dried using a fruit dehydrator with temperature adjusted at 45 °C (Hu et al. 2022). After drying, the specimens were kept in zip lock plastic bags and stored at -80 °C for two weeks to kill all the insects/eggs/larvae. After the cold temperature treatment, the samples were characterised morpho-anatomically and phylogenetically. All the samples are deposited in Oman Animal and Plant Genetic Resources Center (Mawarid), AlKhoud, Muscat, Sultanate of Oman.

Morphological investigation

For microscopic study, handmade sections were made from lamellae, cap and stipe surfaces and annulus. Thin small sections were initially mounted in 5% aqueous potassium hydroxide (KOH) (w/v) and then re-hydrated in 1% aqueous Cong red (w/v) for a more obvious appearance. Microscopic features such as the size, shape and colour of basidiospores, basidia, cheilocystidia, pellicle structure, veil and annulus morphology were studied under a compound microscope (ECLIPSE Ni-U, Nikon Co., Ltd., Japan). For size measurements of these structures, Piximetre (http://ach.log.free.fr/Piximetre/) was used. For the morphological terminology, Vellinga and Noordeloos (2001) was followed.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from dried specimens using X-AMP DNA reagent kit (Dubuque, Iowa, USA), following the manufacturer's protocol. A volume of 200 µl X-AMP DNA reagent was taken in an Eppendorf tube containing the sample (approximately 5-15 mg of gills) and incubated for 15 minutes at 70 °C. After cooling, 2 µl solution from the sample was used as a DNA template directly for polymerase chain reaction (PCR) without any further treatment. We amplified three gene regions, including the internal transcribed spacer (ITS), the large subunit of nuc rDNA (28S) and the translation elongation factor 1 alpha (EF-1a) gene. The primer combinations were: ITS1F and ITS4 for ITS (White et al. 1990; Gardes and Bruns 1993), LROR and LR5 for 28S (Vilgalys and Hester 1990; White et al. 1990), EF1-983 and EF1-1567R for EF-1a (Rehner and Buckley 2005). PuReTaqTM Ready-To-Go PCR beads (GE Healthcare UK Limited, Buckinghamshire, UK) were used for PCR amplification. We added 1.0 µl of each primer (10 µmol/l), 2 µl DNA template and 22 µl Nuclease free water to each bead. For ITS amplification, the PCR conditions were optimised as: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 54 °C for 45 s and extension at 72 °C for 1 min. For the 28S and EF-1α regions, only the annealing temperature was optimised, 52 °C for 28S and 60 °C EF-1a, respectively (Hussain et al. 2022). The PCR products were purified and then sequenced from Macrogen Inc. © (Seoul, Republic of Korea) bidirectionally using the same primers.

Sequence alignment and phylogenetic analyses

Consensus sequences were created from the forward and reverse primer reads of the newly-generated ITS, 28S and EF-1 α sequences using BioEdit v.7.0.9.0 (Hall 1999). We performed BLAST searches for the newly-generated sequences; only ITS and 28S regions showed maximum similarity with *Hy-menagaricus* species. In the case of EF-1 α sequences, the BLAST search re-

vealed *Heinemannomyces* sp. (ZRL185) is the most similar species because, in GenBank, no EF-1a sequences of *Hymenagaricus* are available. This is the reason that we used only ITS and 28S sequences in the phylogenetic analyses. A combined ITS-28S dataset was constructed from the sequences used in the recent studies of *Hymenagaricus* (Mwanga and Tibuhwa 2014; Hosen et al. 2017; Kumla et al. 2021, 2023; Syed et al. 2023). The final ITS-28S dataset was comprised of 27 specimens, including 26 ITS and 20 28S sequences (Table 1). *Agaricus campestris* L. (LAPAG370) was used as the outgroup taxon. Sequences were aligned using MAFFT v.7 (Katoh et al. 2019) and visually inspected using BioEdit v.7.0.9.0 (Hall 1999). Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used for the phylogenetic analyses. Maximum Likelihood (ML) phylogeny was performed with RAxML-HPC Black-Box, implemented on CIPRES Science Gateway (Miller et al. 2010; Stamatakis

0	Origin	Voucher number	GenBank accession		- /
Species			ITS	28S	Reference
Agaricus campestris	Spain	LAPAG370	KM657927	KP739803	Parra et al. (2016)
Heinemannomyces splendidissimus	China	Q. Zhao 2591	KY039571	KY039576	Yang and Ge (2017)
Hei. splendidissimus	China	Z.W. Ge2540	KY039570	KY039575	Yang and Ge (2017)
Hei. splendidissimus	China	GDGM46633	MF621038	MF621039	Hosen et al. (2017)
Hei. splendidissimus	China	GDGM46634	-	MF621040	Hosen et al. (2017)
Hei. splendidissimus	Thailand	ecv3586	HM488760	HM488769	Vellinga et al. (2011)
Hei. sp.	Thailand	ZRL185	KT951346	KT951527	Zhao et al. (2016)
Hymenagaricus ardosiaecolor	Togo	LAPAF9	JF727840	-	Zhao et al. (2011)
H. ardosiaecolor	Tanzania	Z4	KM360160	-	Mwanga and Tibuhwa (2014)
H. cf. kivuensis	Burundi	BR6089	KM982454	-	Mwanga and Tibuhwa (2014)
H. wadijarzeezicus	Oman	JRZ2-22-015	OR613000	OR613018	This study
H. wadijarzeezicus	Oman	JRZ2-22-013	OR612999	OR613019	This study
H. wadijarzeezicus	Oman	NHZ-22-019	OR612997	OR613020	This study
H. wadijarzeezicus	Oman	JHN-22-019	OR612998	OR613021	This study
H. wadijarzeezicus	Oman	JRZ-22-005	OR612996	OR613022	This study
H. pakistanicus	Pakistan	FAK196	OP082405	-	Syed et al. (2023)
H. pakistanicus	Pakistan	FAK195	OP082404	-	Syed et al. (2023)
H. parvulus	Oman	JRZ-22-004	OR612994	OR613017	This study
H. parvulus	Oman	JRZ2-22-002	OR612995	-	This study
H. siamensis	Thailand	SDBR-CMUWP038	OP837533	OP836600	Kumla et al. (2023)
H. siamensis	Thailand	SDBR-CMUNK1508	OP836301	OP836385	Kumla et al. (2023)
H. saisamornae	Thailand	ZRL3103	KM982450	KM982452	Kumla et al. (2021)
H. saisamornae	Thailand	SDBRCMUNKNW0474	MW349605	MW349603	Kumla et al. (2021)
H. saisamornae	Thailand	SDBR-CMUNK0369	MW345912	MW345917	Kumla et al. (2021)
H. saisamornae	Thailand	SDBR-CMUNK0567	MW349602	MW349604	Kumla et al. (2021)
H. saisamornae	Thailand	LD2012186	KM982451	KM982453	Kumla et al. (2021)
H. sp.	Pakistan	LAH35329	OQ998344	_	Usman (2018)
H. sp.	Thailand	CA833	JF727858	_	Zhao et al. (2011)

 Table 1. Taxa included in the molecular phylogenetic analyses.

2014). The best model (GTR+F+I+G4) was chosen following jModelTest2 (Darriba et al. 2012). Branch support for the ML phylogeny was executed with 1000 bootstrap replicates. For BI analyses, we used BEAST v.1.8.2 (Drummond et al. 2012). The combined ITS-28S alignment was converted to XML datafile using BEAUti v.1.8.2 (Bayesian Evolutionary Analysis Utility; Drummond et al. (2012)). A Birth-Death Incomplete Sampling speciation model (Stadler 2009) was selected. Four independent runs were performed with BEAST on XSEDE tool on the CIPRES Science Gateway (Miller et al. 2010). Resulting log files were checked in Tracer (Rambaut et al. 2014) for effective sample size (ESS) values. All ESS values were well over 200. Tree files were combined in LogCombiner v.1.8.2 (Drummond and Rambaut 2007). A maximum clade credibility (MCC) tree was obtained using the TreeAnnotator v.1.8.2 (Drummond and Rambaut 2007). The ML bootstrap (BT) percentage \geq 70 and BI posterior probabilities $(PPs) \ge 0.80$, respectively, were considered significant. For phylogenetic tree visualisation, FigTree v.1.4.2 (Rambaut 2012) was used and the tree was annotated using Adobe Illustrator CC2019. The alignment file is submitted to Tree-Base (http://purl.org/phylo/treebase/phylows/study/TB2:S30802).

Results

Phylogenetic analyses

In this study, 19 new sequences (7 ITS, 6 28S and 6 EF-1a) were generated from our collections of Hymenagaricus. There were no EF-1a sequences of the genus available in GenBank; therefore, only combined ITS-28S sequences were used in the final data matrix. The final ITS-28S dataset was comprised of 1516 characters including 1272 constant sites, 141 informative sites and 103 uninformative sites. The topology of trees revealed similar patterns in both ML and BI methods; therefore, the phylogeny inferred from ML analysis is presented here with values from both BT and PPs in Fig. 1. In both ML and BI analyses, six specimens of Heinemannomyces formed a basal group, sharing a clade with Hymenagaricus species. This clade is weakly supported in ML analyses and well supported with BI (BT 59%, PPs 0.94). However, the subclade representing Heinemannomyces specimens is strongly supported in both analyses (BT 100%, PPs 1). Species of Hymenagaricus are distributed in three clades. Clade-I with good statistical support (BT 93%, PPs 1) consisted of three species, the new species Hymenagaricus wadijarzeezicus, H. saisamornae J. Kumla & N. Suwannarach and H. cf. kivuensis Heinem. Each of these three species has its unique position, confirming their unique identity. Similarly, clade-II was strongly supported (BT 100%, PPs 1), with three taxa, H. pakistanicus M.F. Syed & M. Saba, the new species H. parvulus and an unnamed species H. sp. (LAH35329). The third is a subclade in the clade consisting of Hymenagaricus and Heinemannomyces taxa. This subclade consisted of H. siamensis J. Kumla, W. Phonrob, N. Suwannar & S., Lumyong, H. ardosiaecolor Heinem. and an unnamed species H. sp. (CA833). However, the two specimens (LAPAF9, Z4) representing *H. ardosiaecolor*, were recovered with different branch lengths. This variation in branch length could be the result of using only ITS sequences of *H. ardosiaecolor* in the phylogenetic analyses.



Figure 1. Maximum Likelihood phylogeny of *Hymenagaricus* and *Heinemannomyces*, based on combined ITS-28S sequence data, with *Agaricus campestris* as the outgroup taxon. Values above the node represent ML bootstrap percentages and BI posterior probabilities; the new species are represented in bold fonts.

Taxonomy

Hymenagaricus wadijarzeezicus Al-Sadi, Al-Yahya'ei, A. Al-Owaisi & S. Hussain, sp. nov. MycoBank No: 850249 Figs 2–4

Diagnosis. The new species *Hymenagaricus wadijarzeezicus* can be differentiated from other species of the genus by its unique whitish woolly veil, covering both the cap and the stipe surfaces.

Holotype. SULTANATE OF OMAN: Dhofar, Salalah, Wadi Jarzeez, on termite mounds, under the trees of *Anogeissus dhofarica*, 11 August 2022, S. Hussain, A. Al-Owaisi & Al-Yahya'ei, JRZ2-22-013 (holotype Mawarid-JRZ2-22-013), Gen-Bank accession: ITS = OR612999, 28S = OR613019, EF-1α = OR729599.

Etymology. The specific epithet '*wadijarzeezicus*' refers to the valley Jarzeez in the south of Oman, where the holotype was found.

Description. *Basidiomata* small to medium-sized. *Pileus* 30–80 mm in diam., at the young stage, broadly ovoid to parabolic, covered completely by a smooth, pale brownish pellicle; at mature stage, pulvinate to convex, pellicle disrupting except at the centre where it is retained as one large, smooth, brownish squamule, surface is woolly, covered with whitish, strigose to vil-

lose or floccose veil towards the margin; margin appendiculate with long, whitish, fibrils of veil. **Context** dark pinkish on cutting, 3-5 mm thick at the pileus centre. **Lamellae** free, pale pinkish at young stage, at mature stage greyish-pink to brownish, ventricose, up to 3 mm wide, densely crowded, with 1-3 series of lamellulae. **Stipe** $30-60 \times 5-10$ mm, equal, with a slightly bulbous base, with root-like rhizoid structure at the base, annulus floccose, con-



Figure 2. Basidiomata of *Hymenagaricus wadijarzeezicus* **A–C** holotype collection (JRZ2-22-013) **D, E** NHZ-22-019 **F** young fruiting bodies where the cap is entirely covered by pellicle represented by arrows (JRZ2-22-015) **G** context changed into pinkish on cutting, the arrow represents the root-like rhizoid (JRZ2-22-015). Scale bars: 20 mm.



Figure 3. Light microscopy of anatomical features of *Hymenagaricus wadijarzeezicus* (based on holotype collection JRZ2-22-013) **A** basidiospores **B**, **C** basidia **D** cheilocystidia **E** annulus **F** veil elements **G** pellicle structure. Scale bars: 10 μm (**A**); 15 μm (**B**–**D**); 15 μm (**E**–**G**).

colorous to veil; stem covered with floccose veil below the annulus, smooth above the annulus, context pinkish on cutting, fistulose. *Smell* pleasant. *Taste* not recovered.

Basidiospores (6.5)7.0-8.0(8.5) × (4.0)4.5-5.5(6.0) µm, average size 7.5 × 5.0 μ m, Q = 1.4–1.6, av. Q = 1.5; ellipsoid to broadly ellipsoid, yellowish to dark brown, smooth, thick-walled, apiculus visible, germ-pore not observed. Basidia $20-25 \times 7-9 \mu m$, on average $22.5 \times 8.0 \mu m$, clavate to cylindrical, smooth, hyaline in KOH, mostly tetrasporic, rarely bisporic. Cheilocystidia 16-23 × 7-9 µm, on average 19.5 × 8.0 µm, ellipsoid to subclavate, smooth, thin-walled, hyaline in KOH. Pleurocystidia absent. Lamellar trama regular, with 4-6.6 µm diam., cylindrical to inflated, thin-walled, hyaline hyphae. Subhymenium consisted of subglobse to irregular cells, measuring 12-18 µm diam. Pellicle is a hymeniform, consisting of chains of two or three elements, measuring 13-17 × 10-16 µm each element, globose to subglobose or ovoid, hyaline, or pale yellowish, smooth, thin-walled, these chains of elements attached to inflated hyphae with encrusted walls. Pileus veil is a cutis to ixocutis, consisting of elongated or cylindrical elements, easily detached, hyaline, thin-walled, each element measuring $13-45 \times 6-9 \mu m$. **Annulus** is an intricate trichoderm, composed of hyaline hyphae, 6-8 µm diam., cylindrical, constituted by short elements, constricted at septa, easily disarticulated. Stipe veil similar to pileus veil. Clamp connections absent in all tissues.

Habit, habitat, and distribution. Occurring in July to early September, as saprotrophic, solitary or scattered in small groups, on or near the termite mounds, under the trees of *Anogeissus dhofarica*. Currently only known from southern Oman.



Figure 4. Line drawings of anatomical features of *Hymenagaricus wadijarzeezicus* (based on holotype collection JRZ2-22-013) **A** basidiospores **B** basidia **C** cheilocystidia **D** pellicle structure **E** annulus **F** veil elements. Scale bars: 10 μ m (**A**); 15 μ m (**B**-**D**); 15 μ m (**E**, **F**).

Additional specimens examined. SULTANATE OF OMAN: Dhofar, Salalah, Wadi Naheez, on termite mounds, under the trees of Anogeissus dhofarica, 07 August 2022, S. Hussain, A. Al-Owaisi, Al-Yahya'ei & Al-Sadi, NHZ-22-019 (Mawarid-NHZ-22-019), GenBank accession: ITS = OR612997, 28S = OR613020, EF-1a = OR729602; Wadi Jarzeez, under the trees of Anogeissus dhofarica, 08 August 2022, S. Hussain, A. Al-Owaisi, Al-Yahya'ei & Al-Sadi, JRZ-22-005 (Mawarid-JRZ-22-005), GenBank accession: ITS = OR612996, 28S = OR613022, EF-1a = OR729603; Wadi Jaheen, under the trees of Anogeissus dhofarica, 10 August 2022, S. Hussain, A. Al-Owaisi, Al-Yahya'ei & Al-Sadi, JHN-22-019 (Mawarid-JHN-22-019), GenBank accession: ITS = OR612998, 28S = OR613021, EF-1a = OR729600; Wadi Jarzeez, on termite mounds, under the trees of Anogeissus dhofarica, 11 August 2022, S. Hussain, A. Al-Owaisi & Al-Yahya'ei, JRZ2-22-015 (Mawarid-JRZ2-22-015), GenBank accession: ITS = OR613000, 28S = OR613018, EF-1a = OR729601; Wadi Gogob, on termite mounds, under the trees of Anogeissus dhofarica, 22 August 2023, S. Hussain & Al-Yahya'ei, GOB-23-008 (Mawarid-GOB-23-008); Sahalanawt, on termite mounds, 27 August 2023, S. Hussain & Muhammad Salim, Sahalanawt-23-001 (Mawarid-Sahalanawt-23-001); Tetam, on termite mounds, 30 August 2023, S. Hussain & Amer Qattan, Tetam-23-001 (Mawarid-Tetam-23-001).

Notes. The new species *Hymenagaricus wadijarzeezicus* with medium-sized basidiomata, can be distinguished from the known species of the genus by its remarkable woolly cap and stipe surfaces. In *Hymenagaricus*, there are four species with a cap diameter of 50 mm or above, which are: *Hymenagaricus* cf. *kivuensis*, *H. mlimaniensis* Mwanga & Tibuhwa, *H. ardosiaecolor* and *H. alphitochrous* (Berk & Broome) Heinem. *Hymenagaricus wadijarzeezicus* is the 5th species with a cap diameter above 50 mm. None of these species has a woolly basidiomata surface, except *Hymenagaricus wadijarzeezicus*.

In ML phylogeny, the most similar species to the new species H. wadijarzeezicus is H. saisamornae. Hymenagaricus saisamornae is a recently described species from Thailand, with substantially smaller basidiomata (10-25 mm cap diam.), pileus surface covered with minute brownish squamules, stipe smooth to finely whitish squamulose and smaller basidiospores (5.5- $7.0 \times 4.0-4.5 \mu$ m; Kumla et al. (2021)). Similarly, Hymenagaricus cf. kivuensis and H. mlimaniensis, both African species, shared medium-sized pileus with H. wadijarzeezicus. Hymenagaricus cf. kivuensis has pileus of 50-100 diam., with smaller basidiospores $(4.0-6.5 \times 3.0-4.5 \mu m)$, narrower basidia $(16-20 \times 4.5-6)$ and broader hymeniform cells (Pegler 1977; Heinemann 1984). Hymenagaricus mlimaniensis has a broadly umbonate, reddish-brown disc, with sparsely squamulose surface and smaller basidiospores (4.0-7.0 × 3.5-4.5 µm; Mwanga and Tibuhwa (2014)) than H. wadijarzeezicus (7.0-8.0 × 4.5-5.5 µm). Hymenagaricus siamensis differs from H. wadijarzeezicus by its smaller basidiomata with brownish cap, measuring 22-32 mm diam., squamules consisting of pseudoparenchymatous cells (Kumla et al. 2023). Another small-sized species Hymenagaricus pakistanicus with pileus 24-30 mm diam., covered with dark brownish squamules at the cap centre, smaller basidiospores $(5.0-6.0 \times 3.5-5.0 \mu m)$ and a pseudoparenchymatous pellicle (Syed et al. 2023).

Hymenagaricus parvulus Al-Kharousi, Al-Sadi, Al-Yahya'ei, & S. Hussain, sp. nov. MycoBank No: 850248

Figs 5-7

Diagnosis. The new species Hymenagaricus parvulus can be differentiated from other species of the genus by its small-sized, creamy basidiomata, umbonate pileus covered with appressed pellicle.

Holotype. SULTANATE OF OMAN: Dhofar, Salalah, Wadi Jarzeez, on termite mounds, under the trees of Anogeissus dhofarica, 8 August 2022, S. Hussain, A. Al-Owaisi, Al-Yahya'ei & Al-Sadi, JRZ-22-004 (holotype Mawarid-JRZ-22-004), GenBank accession: ITS = OR612994, 28S = OR613017, EF-1a = OR735176.

Etymology. The specific epithet 'parvulus' refers to the small-sized basidiomata of the new species.

Description. Basidiomata small-sized. Pileus 15-25 mm in diam., at young stage globose to parabolic, surface floccose squamulose, squamules light pinkish to creamy, with appressed pellicle at the centre, margin appendiculate; at mature stage cap convex to hemispherical with the broadly umbonate disc, with appressed, pale brownish pellicle at the disc, surface finely floccose squamulose, squamules pale creamy to light greyish, margins striate, just exceeding the lamellae; context membranous, pinkish on cutting. Lamellae free, pale pinkish to brownish, ventricose, sparsely crowded, with 1-2 series of lamellulae. Stipe 25-35 × 2-5 mm, equal, annulus cortinate, concolorous to squamules; stem surface creamy, covered with finely floccose squamules below the annulus, smooth above the annulus, context pinkish on cutting, fistulose. Smell pleasant. Taste not recorded.

Basidiospores 5.0-6.5 × 4.0-4.5 µm, average size 6.0 × 4.2 µm, Q = 1.3-1.5, av. Q = 1.4; ellipsoid to broadly ellipsoid, yellowish to dark brown, smooth, thick-walled, apiculus visible, germ-pore not observed. Basidia 16.5-22.5 × $6.5-8.5 \mu m$, on average $19.0 \times 7.5 \mu m$, clavate to cylindrical, smooth, hyaline in KOH, tetrasporic. Cheilocystidia 19-25 × 9-11 μm, on average 22 × 10 μm, clavate to broadly clavate, often turning to one side, with multiseptate base, smooth, thin-walled, hyaline in KOH. Pleurocystidia absent. Subhymenium consisting of cylindrical to elongated cells, measuring 6-9 µm diam. Pellicle is a hymeniform, consisting of chains of several elements, each element measuring $14-22 \times 12-17$ µm, globose to subglobose or ovoid, hyaline or pale yellowish, smooth, thin-walled; these chains of elements attached to inflated hyphae with encrusted walls. Veil is a cutis to ixocutis, consisting of elongated or cylindrical elements, not easily detached, hyaline, thin-walled, with terminal element fusiform with papillate end, each element measuring $15-18 \times 5-7 \mu m$. Annulus is an intricate trichoderm, composed of hyaline hyphae, 4-7 µm diam., cylindrical, constituted by short elements, constricted at septa and easily disarticulated. Clamp connections absent in all tissues.

Habit, habitat and distribution. Fruiting body formation occurs in early August to early September, saprotrophic, scattered in small groups, found on termite mounds. Currently only known from southern Oman.

Additional specimens examined. SULTANATE OF OMAN: Dhofar, Salalah, Wadi Jarzeez, on termite mounds, under the trees of Anogeissus dhofarica, 11 August 2022, S. Hussain, A. Al-Owaisi, Al-Yahya'ei & Al-Sadi, JRZ2-22-002 (Mawarid-NHZ-22-002), GenBank accession: ITS = OR612995.



Figure 5. Basidiomata of *Hymenagaricus parvulus* (based on holotype collection JRZ-22-004) **A** mature and young basidiomata **B** mature basidimata.

Notes. *Hymenagaricus parvulus* is a small, cream-coloured species, differentiated from other species of the genus by its whitish to pale pinkish floccose squamules on pileus and stipe surfaces with a broadly umbonate centre. *Hymenagaricus parvulus* shares basidiomata size and basidiospores morphology with *H. pakistanicus*. However, *H. pakistanicus* can be differentiated from the new species by its caesptiose fruiting habit, pileus with pinkish to brown-



Figure 6. Light microscopy of anatomical features of *Hymenagaricus parvulus* (based on holotype collection JRZ-22-004) **A** basidiospores **B** basidia **C** cheilocystidia **D** annulus **E** pellicle structure **F** veil elements. Scale bars: 5 μm (**A**); 10 μm (**B**, **C**); 15 μm (**D**–**F**).

ish squamulose pellicle, consisting of pseudoparenchymatous cells (Syed et al. 2023). *Hymenagaricus saisamornae* differs from the new species by its smaller pileus (up to 15 mm diam. Vs. 15–25 mm of *H. parvulus*), covered with brownish pellicles and larger basidiospores ($5.5-7.0 \times 4-4.5 \mu$ m; Kumla et al. (2021)). *Hymenagaricus siamensis*, another small-sized species is distinguished from the



Figure 7. Line drawings of anatomical features of *Hymenagaricus parvulus* (based on holotype collection JRZ-22-004) A basidiospores B basidia C annulus elements D cheilocystidia E pellicle structure F veil elements. Scale bars: $5 \mu m$ (A); $10 \mu m$ (B, D); $15 \mu m$ (C, E, F).

new species by its pinkish-brown cap, pellicle comprised of pseudoparenchymatous cells and larger basidiospores ($6.5-8.0 \times 4.0-5.0 \mu m$; Kumla et al. (2023)). Similarly, *Hymenagaricus canoruber* (Berk. & Br.) Heinem. & Little Flower, known from India and Sri Lanka, is characterised by a small-sized pileus (15–25 mm diam.), with greyish-brown squamules, hymeniform pellicle and smaller basidio-

spores (4.6–5.7 3.5–4.3 µm; Heinemann and Little Flower (1984)). Hymenagaricus pallidodiscus D.A. Reid & Eicker, the smallest mushroom in the genus with pileus diam. up to 11 mm, covered with brownish squamules and smaller basidiospores (4.2–5.4 × 3.1–3.8 µm; Reid and Eicker (1999)). Hymenagaricus cylindrocystis Heinem. & Little Flower another small-sized species, has been reported in Singapore and India, with a brownish cap, larger basidiospores (6.4–8.4 × 4.5–5.6 µm) and a pseudoparenchymatous pellicle (Heinemann 1956; Heinemann and Little Flower 1984). Hymenagaricus cf. kivuensis and H. wadijarzeezicus with their medium-sized pilei can be easily differentiated from H. parvulus.

Discussion

Species of *Hymenagaricus* and *Xanthagaricus* are morphologically very similar and it is extremely difficult to differentiate the species of these genera in the field. However, in most species of *Xanthagaricus*, the cap surface is covered with small, brownish to purplish scales. These scales are concentrated at the pileus centre, while a large central, undisrupted scale at the cap centre has been observed in the most species of *Hymenagaricus*. Phylogenetically, both the genera are clearly distinct.

Phylogenetically, the species of *Hymenagaricus* are closely related to the monotypic genus *Heinemannomyces*. Morphologically, both these genera are clearly distinct. Species of *Hymenagaricus* have a squamulose cap surface and these squamules consist of hymeniform or pseudoparenchymatous cells and yellowish-brown basidiospores. The monotypic genus with single species *Heinemannomyces splendidissimus* has a brownish to greyish-red pileus, covered with a finely woolly veil and greyish to dark bluish basidiospores (Watling 1998; Hosen et al. 2017).

In our phylogenetic analyses, the specimens representing *Heinemannomyces* formed a basal group. Species of *Hymenagaricus* were recovered in three groups. One group consisted of *Hymenagaricus wadijarzeezicus*, the new species, *H. saisamornae* and *H.* cf. *kivuensis*. In this group, *Hymenagaricus saisamornae* with small-sized basidiomata intermix with *H.* cf. *kivuensis* and *H. wadijarzeezicus* both with medium-sized basidiomata. Similarly, another group consisting of *Hymenagaricus pakistanicus*, *H. parvulus* the new species and unnamed species *H.* sp. (LAH35329). All these taxa, including the unnamed species, have a small fruiting body. The third group consists of *Hymenagaricus ardosiaecolor* (medium-sized basidiomata) and *H. siamensis*, the small-sized species.

Both basidiomata size and pellicle structure are species delimitation characters in the genus *Hymenagaricus*. Based on our analyses, we can predict that these characters could be used in the future for infrageneric classification of the genus.

The two new species, *Hymenagaricus wadijarzeezicus* and *H. parvulus*, were collected in the Dhofar Region, located in the southern part of Oman. *Hymenagaricus wadijarzeezicus* is medium-sized and *H. parvulus* is a small-sized species. Both are widespread in the Region, under the trees of *Anogeissus dhofarica*. It is interesting to note that both collections of *H. parvulus* (JRZ-22-002, JRZ2-22-004) and several collections of *H. wadijarzeezicus* (NHZ-22-019, JRZ2-22-013, JRZ2-22-015, GOB-23-008, Sahalanawt-23-001, Tetam-23-001) were found on termite mounds. However, we did not find any study reporting the association of *Hymenagaricus* with termites. However, secotioid fungal ge-

nus *Podaxis* Desv. in the family Agaricaceae has an apparent relationship with termites (Conlon et al. 2016). It will be interesting to study the relationships of these mushrooms with termites.

Several species of Agaricaceae were recently reported from the Dhofar Region (Al-Kharousi et al. 2022a, 2022b; Hussain et al. 2022). It is evident that the area is rich in the diversity of Agaricaceae, including the genus *Hymenagaricus*. More new species of dark-spored agarics are likely occurring in the area.

Taxonomic key to the species of Hymenagaricus

A taxonomic key to the species of *Hymenagaricus* included in our phylogenetic analyses is presented below. This key is based on cap diameter (small-sized with cap less than 40 mm in diam. and medium-sized with cap ranging from 50-100 mm in diam.) and pellicle structure either hymeniform or pseudoparenchymatous cells.

I-sized, pileus diam. below 40 mm 2	1
um-sized, pileus diam. up to 100 mm5	-
itary or gregarious3	2
ear in cluster (casepitose), pileus 20-30 mm diam., pellicle oparenchymatous cells <i>Hymenagaricus pakistanicus</i>	-
nkish-brown, pellicle consisted of hymeniform cells4	3
ellicle consisted of pseudoparenchymatous cells	-
diam., whitish to creamy, pellicle smooth, finely ap-	4
diam., pinkish to brownish, squamulose pellicle	-
H. saisamornae	
h brownish squamules6	5
urfaces covered with whitish woolly fibrils	-
H. wadijarzeezicus	
0 mm, basidiospores 5.8–6.5 × 3.9–4.5 μm	6
100 mm, hymeniform squamules	-
100 mm, hymeniform squamules	-

Acknowledgements

We would like to thank Bader Al Quyudhi, Shamsa Al Balushi and Maryam Al Hinai for their invaluable help in the sampling trip to the Dhofar Region, which was essential to the success of our research. We would also like to thank Amer Qattan and Mohammed Al Jahwari for their guidance and support throughout the project.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was funded by the Agriculture and Fisheries Development Fund, Sultanate of Oman (grant number 104/1/1).

Author contributions

Shah Hussain and Moza Al-Kharousi: conceptualisation, writing – original draft and review and editing, data curation, formal analysis, investigation, methodology and visualisation. Dua'a Al-Maqbali and Arwa A. Al-Owaisi: sampling, data curation and investigation. Mohamed N. Al-Yahya'ei and Abdullah M. Al-Sadi: project administration, resources, supervision, writing - review and editing. Rethinasamy Velazhahan: writing - review and editing, formal analysis.

Author ORCIDs

Shah Hussain [®] https://orcid.org/0000-0002-5772-7206 Rethinasamy Velazhahan [®] https://orcid.org/0000-0002-9263-4371 Mohamed N. Al-Yahya'ei [®] https://orcid.org/0000-0002-9516-5339 Abdullah M. Al-Sadi [®] https://orcid.org/0000-0002-3419-8268

Data availability

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

References

- Al-Kharousi M, Hussain S, Al-Muharabi MA, Al-Shabibi Z, Al-Maqbali D, Al-Balushi AH, Al-Yahya'ei MN, Al-Saady N, Velazhahan R, Al-Sadi AM (2022a) The genus *Xanthagaricus*: An updated global species distribution and phylogeny with the description of two new species from Oman. Journal of Fungi 8(2): 173. https://doi.org/10.3390/ jof8020173
- Al-Kharousi M, Hussain S, Al-Muharabi MA, Al-Shabibi Z, Al-Maqbali D, Al-Balushi AH, Al-Yahya'ei MN, Al-Saady N, Velazhahan R, Al-Sadi AM (2022b) Notes on the genus *Micropsalliota* (Agaricales, Basidiomycota) and the description of a new species from Southern Oman. Phytotaxa 543(2): 113–126. https://doi.org/10.11646/phytotaxa.543.2.2
- Bookhagen B, Thiede RC, Strecker MR (2005) Abnormal monsoon years and their control on erosion and sediment flux in the high, arid northwest Himalaya. Earth and Planetary Science Letters 231(1–2): 131–146. https://doi.org/10.1016/j.epsl.2004.11.014
- Conlon BH, De Beer ZW, Henrik H, Aanen DK, Poulsen M (2016) Phylogenetic analyses of *Podaxis* specimens from Southern Africa reveal hidden diversity and new insights into associations with termites. Fungal Biology 120(9): 1065–1076. https://doi.org/10.1016/j.funbio.2016.05.011
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9(8): 772. https://doi.org/10.1038/nmeth.2109
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7(1): 214. https://doi.org/10.1186/1471-2148-7-214
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29(8): 1969–1973. https://doi.org/10.1093/molbev/mss075
- El-Sheikh MA (2013) Population structure of woody plants in the arid cloud forests of Dhofar, southern Oman. Acta Botanica Croatica 72(1): 97–111. https://doi. org/10.2478/v10184-012-0008-6

- Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetesapplication to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Heinemann P (1956) Champignons récoltés au Congo Belge par Madame M. Goossens-Fontana II. *Agaricus* Fries s.s. Bulletin du Jardin Botanique de l'État à Bruxelles 26: 1–127. https://doi.org/10.2307/3667096
- Heinemann P (1981) *Hymenagaricus* Heinem. gen. nov. (Agaricaceae). Bulletin du Jardin Botanique National de Belgique 51(3/4): 465–466. https://doi.org/10.2307/3668081
- Heinemann P (1984) *Hymenagaricus kivuensis* Heinem. sp. nov. Bulletin du Jardin Botanique National de Belgique 54(1/2): 290–291. https://doi.org/10.2307/3667880
- Heinemann P, Little Flower SR (1984) *Hymenagaricus* (Agaricaceae) de Kerala (Inde) et de Sri Lanka. Bulletin du Jardin Botanique National de Belgique 54(1/2): 151–182. https://doi.org/10.2307/3667871
- Hosen MI, Song ZP, Gates G, Karunarathna SC, Chowdhury MS, Li TH (2017) Two new species of *Xanthagaricus* and some notes on *Heinemannomyces* from Asia. MycoKeys 28: 1–18. https://doi.org/10.3897/mycokeys.28.21029
- Hu Y, Karunarathna SC, Li H, Galappaththi MC, Zhao CL, Kakumyan P, Mortimer PE (2022) The impact of drying temperature on basidiospore size. Diversity 14(4): 239. https:// doi.org/10.3390/d14040239
- Hussain S, Afshan N, Ahmad H, Sher H, Khalid AN (2018) *Xanthagaricus pakistanicus* sp. nov. (Agaricaceae): First report of the genus from Pakistan. Turkish Journal of Botany 42: 123–133. https://doi.org/10.3906/bot-1705-21
- Hussain S, Al-Kharousi M, Al-Muharabi MA, Al-Maqbali DA, Al-Shabibi Z, Al-Balushi AH, Al-Yahya'ei MN, Al Saady N, Abdel-Jalil R, Velazhahan R, Al-Sadi AM (2022) Phylogeny of *Agaricus* subgenus *Pseudochitonia* with the description of a new section and a new species from Oman. Mycological Progress 21(8): 72. https://doi.org/10.1007/s11557-022-01819-8
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Kumla J, Suwannarach N, Wannathes N (2021) Hymenagaricus saisamornae sp. nov. (Agaricales, Basidiomycota) from Northern Thailand. Warasan Khana Witthayasat Maha Witthayalai Chiang Mai 48: 827–836.
- Kumla J, Suwannarach N, Khuna S, Phonrob W, Lumyong S (2023) Hymenagaricus siamensis (Agaricaceae, Agaricales), a novel macrofungus from northern Thailand. Phytotaxa 585(2): 135–144. https://doi.org/10.11646/phytotaxa.585.2.4
- Little Flower SR, Hosagoudar VB, Abraham TK (1997) *Xanthagaricus*, a new generic name in the family Agaricaceae. New Botanist 24: 93–100.
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE), New Orleans, LA, USA, 2010, 8 pp. https://doi.org/10.1109/GCE.2010.5676129
- Mwanga ZN, Tibuhwa DD (2014) Morphology and molecular taxonomy of *Hymenagaricus mlimaniensis* species nov: A new Basidiomycota mushroom from Mlimani main campus, Tanzania. Journal of Yeast and Fungal Research 5(8): 96–102. https://doi. org/10.5897/JYFR2014.0144
- Pegler DN (1977) A preliminary agaric flora of East Africa. Kew Bulletin Additional Series 6: 1–615.

- Rambaut A (2012) FigTree Tree Figure Drawing Tool Version 131, Institute of Evolutionary 623 Biology, University of Edinburgh. http://treebioedacuk/software/figtree/ [Accessed on 30 December 2022]
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. http://beast.bio. ed.ac.uk/Tracer
- Rehner SA, Buckley E (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97(1): 84–98. https://doi.org/10.3852/mycologia.97.1.84
- Reid DA, Eicker A (1995) The genus *Hymenagaricus* Heinem. in South Africa. South African Journal of Botany 61(6): 293–297. https://doi.org/10.1016/S0254-6299(15)30549-4
- Reid DA, Eicker A (1999) South African fungi 10: New species, new records and some new observations. Mycotaxon 73: 169–197.
- Stadler T (2009) On incomplete sampling under birth-death models and connections to the sampling-based coalescent. Journal of Theoretical Biology 261(1): 58–66. https://doi.org/10.1016/j.jtbi.2009.07.018
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/ bioinformatics/btu033
- Syed MF, Saba M, Chattha SM, Hosen MI (2023) *Hymenagaricus pakistanicus* (Agaricaceae, Agaricales), a new species from Pakistan based on morphological and molecular evidence. Phytotaxa 594(4): 292–300. https://doi.org/10.11646/phytotaxa.594.4.6
- Usman M (2018) A study of macrofungi of Pabbi Forest Park, Punjab, Pakistan. Master Thesis, University of the Punjab, Lahore, Pakistan.
- Vellinga EC, Noordeloos ME (2001) *Glossary*. In: Noordeloos ME, Kuyper ThW, Vellinga EC (Eds) Flora Agaricina Neerlandica 5. Aa Balkema Publishers, Tokyo, 6–11.
- Vellinga EC, Sysouphanthong P, Hyde KD (2011) The family Agaricaceae: Phylogenies and two new white-spored genera. Mycologia 103(3): 494–509. https://doi. org/10.3852/10-204
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
- Watling R (1998) *Heinemannomyces*: A new lazuline-spored agaric genus from South East Asia. Belgian Journal of Botany 131: 133–138.
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Yang ZL, Ge ZW (2017) Six new combinations of lepiotaceous fungi from China. Mycosystema 36(5): 542–551.
- Zhao RL, Karunarathna S, Raspé O, Parra LA, Guinberteau J, Moinard M, De Kesel A, Barroso G, Courtecuisse R, Hyde KD, Guelly AK, Desjardin DE, Callac P (2011) Major clades in tropical *Agaricus*. Fungal Diversity 51(1): 279–296. https://doi.org/10.1007/ s13225-011-0136-7
- Zhao RL, Zhou JL, Chen J, Margaritescu S, Sánchez-Ramírez S, Hyde KD, Callac P, Parra LA, Li GJ, Moncalvo JM (2016) Towards standardizing taxonomic ranks using divergence times a case study for reconstruction of the *Agaricus* taxonomic system. Fungal Diversity 78(1): 239–292. https://doi.org/10.1007/s13225-016-0357-x



Research Article

Additional new species and new records of the genus Sticta (lichenised Ascomycota, lobarioid Peltigeraceae) from Bolivia

Emilia Anna Ossowska¹⁰, Bibiana Moncada^{2,3,40}, Robert Lücking^{3,40}, Adam Flakus⁵⁰, Pamela Rodriguez-Flakus⁵⁰, Sandra Olszewska⁶, Martin Kukwa¹⁰

- Department of Plant Taxonomy and Nature Conservation, Faculty of Biology, University of Gdańsk, Wita Stwosza 59, PL-80-308 Gdańsk, Poland 1
- Licenciatura en Biología, Universidad Distrital Francisco José de Caldas, Cra. 4 No. 26D-54, Torre de Laboratorios, Herbario, Bogotá D.C., Colombia 2
- 3 Research Associate, Science & Education, The Field Museum, 1400 South Lake Shore, Chicago, IL 60605, USA
- Botanischer Garten und Botanisches Museum Berlin, Freie Universität Berlin, Königin-Luise-Straße 6-8, 14195 Berlin, Germany 4
- W. Szafer Institute of Botany, Polish Academy of Sciences, Lubicz 46, PL-31-512 Kraków, Poland 5
- 6 10th High School in Gdynia, Władysława IV, PL-81-384 Gdynia, Poland

Corresponding author: Emilia Anna Ossowska (emilia.ossowska@ug.edu.pl)

Abstract

Academic editor: Thorsten Lumbsch Received: 13 February 2024 Accepted: 20 March 2024 Published: 23 April 2024

Citation: Ossowska EA, Moncada B, Lücking R, Flakus A, Rodriguez-Flakus P, Olszewska S, Kukwa M (2024) Additional new species and new records of the genus Sticta (lichenised Ascomycota, lobarioid Peltigeraceae) from Bolivia. MycoKeys 105: 21-47. https://doi.org/10.3897/ mycokeys.105.120810

Copyright: © Emilia Anna Ossowska 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). Four species of the genus Sticta are described as new from Bolivia, based on morphological examination and phylogenetic analysis of the fungal ITS barcoding marker. Additionally, two species are reported as new to Bolivia (their identification confirmed by molecular data) and one previously reported species is confirmed by molecular data for the first time. Detailed morphological and anatomical descriptions are provided for all new species. Two of the new species, S. isidiolobulata Ossowska, B. Moncada, Lücking & Kukwa and S. madidiensis Ossowska, B. Moncada, Lücking & Kukwa belong to clade I, as defined in previous studies. In contrast, S. montepunkuensis Ossowska, B. Moncada, Lücking & Kukwa and S. macrolobata Ossowska, B. Moncada, Lücking & Kukwa, also described here as new to science, belong to clade III. Sticta isidiolobulata has an irregular to suborbicular thallus of medium size, with isidia developing into spathulate lobules, cyanobacterial photobiont and apothecia with entire to weakly-crenate margins. The large irregular thallus of the cyanobacteria-associated S. macrolobata has broad lobes, apothecia with verrucous to tomentose margins and cyphellae with raised margins, whereas S. madidiensis has a medium-sized, palmate to irregular thallus with a stipe, but without vegetative propagules and apothecia. Sticta montepunkuensis has large and irregular thalli with green algae as photobiont, apothecia with crenate to verrucous margins and urceolate cyphellae with a wide pore and a scabrid basal membrane. Two species, S. beauvoisii Delise and S. riparia Merc.-Díaz are reported as new to Bolivia (the latter also as new to South America) and belong to clade III. Sticta tomentosa (Sw.) Ach., species confirmed from Bolivia by molecular data, belongs to clade II. Sticta beauvoisii is characterised by a smooth yellowish-brown upper surface with darker apices and abundant, marginal isidia and a brown lower surface with golden-chocolate brown primary tomentum and sparse, golden-brown rhizines. Sticta riparia has a strongly branched thallus, with undulate lobes and abundant, marginal, palmate, grev to dark brown phyllidia and greyish-brown lower surface with the primary tomentum absent towards the margins. Sticta tomentosa has palmate, bluish thalli with white cilia and abundant, submarginal apothecia and creamy-white lower surface with a sparse, white primary tomentum.

Key words: Diversity, lichens, molecular barcoding, new species, taxonomy

Introduction

The name Sticta was first introduced by Schreber (1791), who classified these lichens as a section within the genus Lichen. Later, Acharius (1803) raised Sticta to the rank of a genus. Sticta has a subcosmopolitan distribution and includes macrolichens with true cyphellae and tomentum present at least on the lower surface of the thalli, for example, Galloway (1994, 1995), Moncada (2012) and Moncada et al. (2018, 2020). At the beginning of the 21st century, about 120 species were known within the genus (Kirk et al. 2008), but recently, the number of known taxa has tripled (Moncada et al. 2021a). This increase is mainly related to the application of integrative taxonomy, based on molecular, morphological and anatomical data and the exploration of tropical regions, which are often habitats of unknown, endemic species (Tønsberg and Goward 2001; Moncada 2012; Lendemer and Goffinet 2015; Moncada et al. 2018, 2020; Mercado-Díaz et al. 2020; Ossowska et al. 2022a). In Colombia, for example, intensive field surveys and laboratory analyses have increased the number of identified Sticta from 42 (Sipman et al. 2008) to 150 (Moncada 2012; Moncada et al. 2013a, b, 2014). Similar explorations have been carried out in other regions of the Neotropics (Dal Forno et al. 2018; Torres et al. 2021; Ossowska 2021; Ossowska et al. 2022a, b; Crous et al. 2023), as well as in other parts of the world (McDonald et al. 2003; Simon et al. 2018; Moncada et al. 2020, 2021a; Di Meglio and Goward 2023; Kaasalainen et al. 2023). However, in many regions, the genus Sticta is still in need of revision, so the number of described species within the genus is much lower than estimated (Moncada et al. 2021a, b, c).

In Bolivia, located in the central part of the Neotropical Region of South America, research on *Sticta* has been conducted since the 19th century (Nylander 1859, 1861; Rusby 1896; Herzog 1922, 1923; Feuerer et al. 1998). As a result, eleven *Sticta* species were reported, based solely on their morphological and anatomical characters (Ossowska 2021 and literature cited therein). Recently, modern approaches in the taxonomy of *Sticta*, including molecular analyses, have been applied to Bolivian collections, resulting in recording nine additional species, including seven new to science (Moncada and Lücking 2012; Ossowska et al. 2022a, b; Crous et al. 2023). Here, we present the descriptions and records of seven additional species, including four new to science (*Sticta isidiolobulata* Ossowska, B. Moncada, Lücking & Kukwa, S. *macrolobata* Ossowska, B. Moncada, Lücking & Kukwa, S. *montepunkuensis* Ossowska, B. Moncada, Lücking & Kukwa), two new to Bolivia (*S. beauvoisii* Delise and *S. riparia* Merc.-Díaz) and the first record of *S. tomentosa* (Sw.) Ach. confirmed by molecular data.

Material and methods

Taxon sampling

The study was based on specimens collected during fieldwork in the Yungas and Tucumano-Boliviano Regions of Bolivia and deposited at KRAM, LPB and UGDA Herbaria. Morphology and anatomy were examined under stereoand compound microscopes (Nikon SMZ800N and ZEISS Axioskop). Spot test reactions were made with K (potassium hydroxide solution), C (sodium hypochlorite solution), Pd (paraphenylenediamine) and KC (K followed by C on the same thallus fragments). Secondary compounds were further analysed using thin-layer chromatography (TLC) in solvents A and C (Orange et al. 2001).

Species, which were distinguished by Moncada (2012), but have not yet been formally described are marked with quotes (e.g. 'S. *isidioimpressula*').

DNA extraction, PCR amplification and sequencing

The protocols for DNA extraction and sequencing of the nuITS rDNA marker followed Ossowska et al. (2022a).

Sequence alignment and phylogenetic analysis

The obtained sequences were aligned with available sequences of the genus *Sticta* (Suppl. material 1: table S1), using our previous alignment (Ossowska et al. 2022a) based on a recent master alignment (Moncada et al. 2020). The new sequences were added to the existing alignment using MAFFT 7.164 with the "--add" option (Katoh and Frith 2012; Katoh and Standley 2013), with subsequent manual inspection in BIOEDIT 7.0.9 (Hall 2011). Phylogenetic analysis was performed using Maximum Likelihood in RAxML 8.2.0 (Stamatakis 2014) on the CIPRES Science Gateway (Miller et al. 2010), with non-parametric bootstrapping using 400 pseudoreplicates (based on an automated saturation criterion) under the universal GTRGAMMA model. Trees were visualised in FigTree 1.4.2 (Drummond and Rambaut 2007). After initial analysis of the full taxon set containing 1,049 terminals, the alignment was reduced to a subset containing 3–10 accessions per species, for a total of 211 terminals and the phylogenetic analysis was repeated using the above approach.

Results and discussion

Seven new nulTS rDNA sequences were generated for this study. Three of these clustered into clades of previously-defined *Sticta* (McDonald et al. 2003; Moncada 2012; Widhelm et al. 2018; Mercado-Díaz et al. 2020). These are *S. beauvoisii* and *S. riparia*, which are new to Bolivia, and *S. tomentosa*, which was reported from Bolivia, but not confirmed with molecular data until now (Fig. 1). Notes on all three species are given below.

Four sequences form distinct lineages, suggesting previously undescribed taxa, are grouped within clades I (*fuliginosa* clade) and III (*weigelii* clade), as defined by Widhelm et al. (2018) (Fig. 1). Comparison of morphological and anatomical features of these specimens with similar and related taxa, as well as phylogenetic analysis, confirmed they represent species new to science. These are *Sticta isidiolobulata* sp. nov. and *S. madidiensis* sp. nov. in clade I and *S. montepunkuensis* sp. nov. and *S. macrolobata* sp. nov. placed in clade III sensu Widhelm et al. (2018) (Fig. 1). Detailed descriptions of all four new *Sticta* species are given below.

At present, the genus *Sticta* contains more than 500 species and more than one hundred morphological and sixty anatomical characters can be used for their circumscriptions (Moncada et al. 2014, 2021a; Ossowska et al. 2022a; Kaasalainen et al. 2023). These include the presence and type of vegetative



Figure 1. Best-scoring Maximum Likelihood tree of the *Sticta* target clade containing the new species from Bolivia (red) and the species new to Bolivia and phylogenetically confirmed from Bolivia (blue), based on the fungal ITS barcoding marker. Supported clades are thickened. For complete tree with individual support values, see Suppl. material 2.

propagules, such as isidia, phyllidia, soredia or lobules (Galloway 1994, 1995; Moncada 2012; Moncada et al. 2014; Ossowska et al. 2022a; Kaasalainen et al. 2023). Moreover, some species may develop two types of propagules, like the newly-introduced *S. isidiolobulata*, which has isidia and lobules or *S. cyanocaperata* Kaasalainen and *S. andina* B. Moncada, Lücking & Sérus. having isidia and phyllidia (Moncada 2012; Moncada et al. 2021b; Kaasalainen et al. 2023). Of the *Sticta* species known from Bolivia with records confirmed by molecular data, seven produce vegetative propagules and most of them have isidia: *S. andina*, *S. aymara* Ossowska et al., *S. beauvoisii*, *S. isidiokunthii* B. Moncada & Lücking and *S. weigelii* (Ach.) Vain. (Moncada et al. 2014; Ossowska 2021; Ossowska et al. 2022a). The structure of the isidia and their distribution on the thallus, as well as their colour and size, are diagnostic to distinguish species. For example, in *S. isidiokunthii*, isidia are darker and in *S. beauvoisii* lighter than the thallus; they are cylindrical to squamiform in *S. beauvoisii* and globular to cylindrical in *S. aymara*, palmate to coralloid in *S. andina* and cylindrical in other isidiate species (Moncada 2012; Moncada and Lücking 2012; Moncada et al. 2021b; Ossowska 2021; Ossowska et al. 2022a).

Phyllidia resemble isidia, but are flattened and dorsiventral (Nash 2008); they are known in two taxa from Bolivia, *S. scabrosa* B. Moncada, Merc.-Díaz & Bungartz subsp. *scabrosa* and *S. riparia*. They are dark brown in *S. riparia*, whereas of the same colour as the thallus in *S. scabrosa* subsp. *scabrosa* (Moncada 2012; Mercado-Díaz et al. 2020; Moncada et al. 2021b; Ossowska et al. 2022b).

Lobules are like small lobes, i.e. forming minute cyphellae and partly also tomentum on the lower surface (Nash 2008; Moncada 2012; Mercado-Díaz et al. 2020; Moncada et al. 2020, 2021a). To date, only a few *Sticta* species with lobules have been described, for example, *S. guilartensis* Merc.-Díaz from Puerto Rico or *S. antoniana* B. Moncada & Lücking from Hawaii (Moncada 2012; Mercado-Díaz et al. 2020; Moncada et al. 2020, 2021a). *Sticta isidiolobulata* described here is the first lobulate species from Bolivia; in this taxon, the lobules develop from isidia, especially at the edges of lobes and it is one of its diagnostic features.

Soredia are the rarest type of propagules found in *Sticta* (Moncada 2012; Moncada et al. 2013b); however, none of the known sorediate taxa has been found in Bolivia so far.

Another important diagnostic feature of Sticta is the presence of apothecia. The fertile species in Bolivia are: S. amboroensis Ossowska et al., S. bicellulata Ossowska et al., S. carrascoensis Ossowska et al., S. catharinae Ossowska et al., S. macrolobata, S. monlueckiorum Ossowska, Flakus & Rodr.Flakus, S. montepunkuensis, S. pseudoimpressula Ossowska et al. and S. tomentosa (Moncada 2012; Ossowska et al. 2022a, this paper; Crous et al. 2023). The most important differences between them are the abundance of apothecia (scarce in S. bicellulata and S. montepunkuensis or abundant in S. catharinae and S. amboroensis), their distribution (submarginal in S. tomentosa, laminal to submarginal in S. macrolobata or marginal in S. carrascoensis) or the structure of the apothecial margins (crenate to hirsute in S. amboroensis, crenate to verrucose in S. montepunkuensis, verrucous to tomentose in S. macrolobata, entire to crenate in S. pseudoimpressula, hirsute to ciliate, but in young apothecia glabrous in S. monlueckiorum) (Moncada 2012; Ossowska et al. 2022a; Crous et al. 2023). In addition, there is a group of Sticta species that have two forms: with apothecia and vegetative propagules as in S. andina, the newly introduced S. isidiolobulata and S. scabrosa subsp. scabrosa (but only in specimens from Bolivia both, phyllidia and apothecia, are present) (Moncada et al. 2014, 2021a, b; Ossowska et al. 2022b).

The cyanobacteria-associated *Sticta madidiensis* lacks both apothecia and vegetative propagules. In *Sticta*, such situations are mostly known in species possessing two photosymbiodemes, i.e. lichen thallus can be formed with a green alga or a cyanobacteria (e.g. in *S. lobarioides* B. Moncada & Coca or *S. pseudolobaria* B. Moncada & Coca). In such cases, the green algal form has abundant apothecia, whereas the cyanobacterial form usually lacks apothecia and vegetative propagules (Moncada 2012; Moncada et al. 2013a) as it is in *S. madidiensis*. Potentially *S. madidiensis* is a species that also forms photosymbiodemes, but at present, only the cyanobacterial thalli are known.

Morphodemes, which are species that are morphologically and anatomically similar, but phylogenetically distant, are common in the genus Sticta (Moncada et al. 2021b). For instance, S. andina and S. scabrosa are morphodemes of S. weigelii (Moncada et al. 2020, 2021b), whereas S. arenosella Di Meglio & Goward and S. gretae Goward & Di Meglio are morphodemes of S. fuliginosa (Di Meglio and Goward 2023). Such taxa have also been found in Bolivia and S. isidiolobulata introduced in this paper and S. pseudoimpressula described by Ossowska et al. (2022a) are morphodemes of S. impressula (Nyl.) Zahlbr. They all have a pitted to scrobiculate or rugose upper surface with apothecia and cilia (Moncada 2012; Ossowska et al. 2022a), but differ in the structure of the cyphellae and also in the colour and thickness of the primary and secondary tomentum. In addition, S. isidiolobulata also produces isidia and lobules, which are absent in S. impressula. Despite similarities to S. impressula, the new S. isidiolobulata is closely related to the still undescribed species 'S. pseudosylvatica', whereas S. pseudoimpressula forms a clade with S. bicellulata (Ossowska et al. 2022a). Sticta montepunkuensis belongs to the S. laciniata morphodeme, which has a green algal photobiont, a scrobiculate upper surface, with apothecia and without true cilia, but with a visible extension of the lower tomentum (Hooker 1822). The differences are also in the size of the thalli (in S. laciniata (Sw.) Ach., the thallus is smaller and highly branched), the distribution of the apothecia (in S. montepunkuensis, they are mainly laminal and subaggregated and in S. laciniata, submarginal and dispersed), the apothecial margins (in S. laciniata apothecia margins are tomentose and, in S. montepunkuensis crenate to verrucous) and the density of the cyphellae (S. montepunkuensis has more abundant cyphellae towards the margins and in the centre than S. laciniata).

The diversity of lichen species in Bolivia is still not fully understood; however, recent results systematically increase the number of species known from this country (Flakus et al. 2019; Guzow-Krzemińska et al. 2019; Kukwa and Ossowska 2022; Kukwa et al. 2023a, b). The knowledge on Sticta in Bolivia is also increasing and recent morphological and anatomical studies supported by phylogenetic analyses have contributed significantly to this (Ossowska 2021; Ossowska et al. 2022a, b; Crous et al. 2023). With the taxa described here, Sticta currently comprises twenty-six species in Bolivia (three other recorded species are definitely misidentifications; see Ossowska et al. (2022a)), of which twenty-one are confirmed by molecular data. A further five species remain to be verified; these are S. dilatata (Nyl.) Vain, S. fuliginosa (Dicks) Ach., S. kunthii Hook., S. laciniata and S. sinuosa Pers. (Rodriguez-Flakus et al. (2016) and literature cited therein). Literature data suggest that at least S. dilatata and S. fuliginosa can also be present in Bolivia as they have been recorded from other South American countries (Widhelm et al. 2018). Sticta fuliginosa s.str. is a subcosmopolitan species (Mc-Donald et al. 2003; Hodkinson et al. 2014; Magain and Sérusiaux 2015; Di Meglio and Goward 2023; Kaasalainen et al. 2023) and, in South America, is known only from Brazil (Magain and Sérusiaux 2015; Widhelm et al. 2018). However, numerous morphodemes of S. fuliginosa are known from the Neotropics (Moncada et al. 2015); therefore, it is likely that Bolivian material may have represented one or several of these. It is the same with S. laciniata. Records of the species may belong to other species, for example, to S. montepunkuensis, which is described as a new species in this paper and belong to the S. laciniata morphodeme. The phylogenetic positions of S. kunthii (described from Peru) and S. sinuosa (described

from Brazil) have not so far been confirmed by molecular data (Moncada 2012; Kaasalainen et al. 2023), so it may be difficult to prove their presence in Bolivia. *Sticta kunthii* was reported from Africa by Kirika et al. (2012), but Kaasalainen et al. (2023) did not find this species amongst the studied species of *Sticta*. The authors concluded that the specimens reported by Kirika et al. (2012) probably represent *S. umbilicariiformis* Hochsc. ex Flotow and/or *S. aspratilis* Kaasalainen et al. Rikkinen (Kaasalainen et al. 2023).

It is worth noting that the new *Sticta* species described here have only been found in single localities, suggesting their putative endemism. Previously, probably endemic *Sticta* species were described from Bolivia by Ossowska et al. (2022a), so if all molecularly confirmed species of *Sticta* from Bolivia are considered, 38% of them are endemic. The occurrence of endemic species of *Sticta* has been noted, for example, in Madagascar and the Mascarenes (Simon et al. 2018), as well as in Puerto Rico (Mercado-Díaz et al. 2020). However, as the genus is still not well studied in many regions, some species may appear more widespread, for example, in this paper, we report the first record of *S. riparia* from Bolivia, which has been known only from Puerto Rico so far (Mercado-Díaz et al. 2020).

The question remains open also in the case of how many species occur in Bolivia. The number of Bolivian specimens awaiting revision is still large and taking into account all data from neighbouring countries, we estimate that around 90 species of *Sticta* may occur in Bolivia.

Taxonomy

Species new to science described from Bolivia

Sticta isidiolobulata Ossowska, B. Moncada, Lücking & Kukwa, sp. nov. MycoBank No: 852904 Fig. 2

Diagnosis. Differing from *S. impressula* in the presence of isidia developing into spathulate lobules and apothecia with entire to weakly-crenate margins and the presence of sparse, secondary tomentum.

Type. BOLIVIA. Dept. Cochabamba; Prov. Carrasco, Parque Nacional Carrasco, between Meruvia and Monte Punku, 17°34'43"S, 65°15'25"W, elev. 3082 m, *Podocarpus* forest, Ceja de Monte Inferior (Altimontano), corticolous, 26 Nov. 2014, M. Kukwa 15054 (holotype UGDA, isotype LPB).

Description. Primary photobiont cyanobacterial (*Nostoc*). Stipe absent. Thallus irregular to suborbicular, subcoriaceous, up to 15 cm diam., moderately branched, with 3-5 branches per 5 cm radius, branching polytomous to anisotomic; lobes ligulate to flabellate, adjacent, plane to involute, with their apices rounded and involute and their margins entire to crenate and not thickened; lobe internodes (2-)3-5(-7) mm long, (3-)6-8(-10) mm broad. Upper surface pitted to rugose-foveolate towards the centre, beige brown with slightly darker apices when dry, shiny; surface glabrous, without papillae and pruina, with orbicular to irregular, scattered, pale beige maculae; marginal cilia absent, but extension of the lower tomentum visible. Apothecia abundant, mostly laminal or submarginal, dispersed or rarely grouped in four, subpedicellate



Figure 2. Morphology of *Sticta isidiolobulata* (holotype) **A** upper surface **B** lower surface **C** lower tomentum with cyphellae **D**, **E** Isidia developing into spathulate lobules and apothecia **F** section of apothecium. Scale bars: 1 mm (**A**–**D**, **F**, **G**); 0.5 mm (**E**); 50 μ m (**F**).

to pedicellate, without pronounced invagination on lower side, up to 2.5 mm diam.; disc orange-brown or yellow (in young apothecia), shiny, concave in young apothecia, convex in older; margin entire to weakly crenate, light brown, not visible from surface view in mature apothecia. Vegetative propagules in the form of flattened and branched isidia developing especially on margins into spathulate lobules, aggregate, branched, horizontal, up to 0.25 mm long and 0.5 mm broad, darker than the thallus, brown grey, shiny. Lower surface undulate and veined, beige to light brown towards the centre; primary tomentum dense, but absent towards the margin, thick, but thinner towards the margin,

spongy to fasciculate, soft, beige to brown in older parts; secondary tomentum present, pubescent, sparse. Rhizines absent. Cyphellae 1–20 per cm² towards the thallus centre and 21–40 per cm² towards the margin, scattered, rounded or elongated, urceolate with wide pore to cupuliform, prominent, remaining below the level of the primary tomentum, with the margin erect to raised and involute, cream to brown coloured, with tomentum; pore (0.25–)0.5–0.7 mm diam.; basal membrane scabrid, white. Medulla compact, white. Pycnidia present, immersed.

Upper cortex paraplectenchymatous, $30-75 \mu$ m thick, differentiated into two cellular layers with the upper layer consisting of 1–2 cell layers, cells $4.5-12 \times 4.5-7 \mu$ m, their walls 1–3.5 µm thick and their lumina rounded to elongated, $4-11 \times 3-6 \mu$ m. Photobiont layer 25–55 µm thick, its cells 5–10 µm diam. Medulla 50–150 µm thick, its hyphae 2–4 µm broad, without crystals. Lower cortex paraplectenchymatous, $30-60 \mu$ m thick, of 2–4 cell layers; cells $6-15 \times 6-12 \mu$ m diam., their walls 1–3 µm thick. Hairs of lower primary tomentum up to 400 µm long, in fascicles more than 20, hyphae unbranched, septate with free apices; hairs of secondary tomentum 10–18 µm long, 5–6 µm broad, consisting of two 2–4 cells. Cyphellae cavity up to 250 µm deep; cells of basal membrane with many small papillae (up to 0.5 µm high). Apothecia biatorine, up to 500 µm high, without or with distinct stipe; excipulum up to 130 µm broad, without hairs. Hymenium up to 130 µm high; epihymenium 2.5–5 µm high, yellowish, without gelatinous upper layer; epihymenium pale brown-orange. Asci 4–8-spored, ascospores fusiform, 1(–3)-septate, 25–35 × 6–8 µm.

Secondary chemistry. No lichen substances detected by TLC. All parts of thallus and apothecia K–, C–, KC–, P–.

Habitat and distribution. *Sticta isidiolobulata* is known only from the type locality in the Parque Nacional Carrasco in the Cochabamba Department. It was found on tree bark in *Podocarpus* forest.

Etymology. The epithet refers to the presence of isidia that develop into spathulate lobules, especially at the lobe margins.

Notes. *Sticta isidiolobulata* is another morph within the *S. impressula* morphodeme, like *S. pseudoimpressula* and the undescribed '*S. isidioimpressula*' (Moncada 2012; Ossowska et al. 2022a). However, the new species is the only one in this group characterised by the presence of both vegetative propagules and apothecia, isidia developing into spathulate lobules, without true cilia and with beige to brown primary tomentum, which is dense, but absent at the margins. *Sticta pseudoimpressula* lacks vegetative propagules, the tomentum is greyish-brown to black and dense at the margins (Ossowska et al. 2022a). In contrast, '*S. isidioimpressula*' produces laminal, white to grey, cylindrical isidia, instead of marginal, greyish-green and spathulate lobules observed in *S. isidioi-obulata*. Furthermore, the primary tomentum in *S. isidioimpressula* is dense and sparse towards the margins and without secondary tomentum (Moncada 2012).

The presence of propagules in the form of isidia and lobules is also characteristic of *S. macrofuliginosa* B. Moncada & Lücking from Colombia (Moncada et al. 2015) and *S. parvilobata* Merc.-Díaz from Puerto Rico (Mercado-Díaz et al. 2020). However, in *S. macrofuliginosa*, isidia are cylindrical, whereas in *S. parvilobata*, they are granular to globular. In contrast, the lobules in both species are lobuliform. These taxa also differ from *S. isidiolobulata* in the upper surface of the thallus, which is scrobiculate to foveolate with sparse laminal apothecia in the Colombian species and smooth to scrobiculate without apothecia in the Puerto Rican species. In addition, the primary tomentum is dense to the lobe margins and spongy in *S. macrofuliginosa* and sparse, but sometimes dense and hirsute to fasciculate in *S. parvilobata* (Moncada 2012; Moncada et al. 2015; Mercado-Díaz et al. 2020).

The new species is related to the Colombian 'S. *pseudosylvatica*' (Fig. 1), which still awaits formal validation. Both taxa differ in the structure of the upper surface, which is smooth to ribbed in 'S. *pseudosylvatica*' and pitted to rugose in *S. isidiolobulata*. Furthermore, 'S. *pseudosylvatica*' has abundant, laminal isidia and primary tomentum is dense to the margins (Moncada 2012). The abundance of cyphellae also varies between them and, in 'S. *pseudosylvatica*', they occur in amounts of 21–40 per cm² towards the centre and 61–100 per cm² towards the margins, while in the new species, there are 1–20 per cm² and 21–40 per cm², respectively (Moncada 2012).

Sticta macrolobata Ossowska, B. Moncada, Lücking & Kukwa, sp. nov.

MycoBank No: 852905 Fig. 3

Diagnosis. Differing from *S. laciniata* in cyanobacteria as photobiont, thallus up to 25 cm in diam., broad lobes, verrucous (rarely weakly crenate) to tomentose apothecial margins, which is often ciliate in the lower part, light to dark brown lower surface and cyphellae with elevated margins.

Type. BOLIVIA. Dept. Santa Cruz; Prov. Florida, Parque Nacional Amboró, above la Yunga Village, senda Los Helechos, 18°03'30"S, 63°54'36"W, elev. 2330 m, Yungas cloud forest, corticolous, 07 June 2011, M. Kukwa 9801 (holo-type UGDA, isotype LPB).

Description. Primary photobiont cyanobacterial (Nostoc). Stipe absent. Thallus irregular, coriaceous, up to 25 cm diam., moderately branched, with 4-5 branches per 5 cm radius, branching pleurotomous to polytomous; lobes laciniate to flabellate, plane, with their apices orbicular and involute, margins entire, not thickened, with brown marginal line; lobe internodes 7-14 mm long, 7-50 mm broad. Upper surface smooth to shallowly scrobiculate, light brown to brown with darker apices when dry, shiny; surface glabrous, without papillae and pruina, but with irregular, scattered, pale beige maculae; marginal cilia absent, but extensions of the lower tomentum visible. Apothecia abundant to sparse, principally laminal to submarginal, dispersed to aggregated, pedicellate, with pronounced invagination on the lower side, up to 5 mm diam.; disc plane, brown to chestnut-brown, shiny, epruinose to delicately pruinose; margin persistent, verrucous to tomentose, rarely weakly crenate, often ciliate in the lower part, with brown tomentum, abundant in young apothecia, sparse in old ones. Vegetative propagules absent. Lower surface plane to uneven, light towards the margins and dark brown towards the centre; primary tomentum dense, thick, but thinner towards the margin, spongy to fasciculate, golden-brown in young parts to brown in older with lighter tips; secondary tomentum present, pubescent. Rhizines present, irregularly dispersed, fasciculate to barbate, up to 6 mm, dark brown. Cyphellae 1-20 per cm² towards the thallus centre and 41-60 per cm² towards the margin, scattered, rounded to irregular, urceolate



Figure 3. Morphology of *Sticta macrolobata* (holotype) A upper surface B lower surface C apothecia with vertucous to tomentose margins D lower tomentum with cyphellae E rhizines F section of apothecium. Scale bars: 1 mm (A-E); 100 µm (F).

with wide pore, erumpent to sessile, remaining below the level of the primary tomentum, with the margin elevated and involute, brown-coloured, without tomentum or with tomentum at the base; pore (0.25-)0.5-1(-1.5) mm diam.; basal membrane scabrid, yellow. Medulla compact, yellow. Pycnidia present, sparse, immersed.

Upper cortex paraplectenchymatous, $30-40 \mu m$ thick, differentiated into two cellular layers with the upper layer consisting of 1-2 layers of small cells, cells $4-15 \times 4-10 \mu m$ diam., their walls $1-3 \mu m$ thick and their lumina rounded to isodiametric, $3-14 \mu m$ diam. Photobiont layer $45-75 \mu m$ thick, its cells $10-20 \mu m$ diam. Medulla $80-120 \mu m$ thick, its hyphae $3-4 \mu m$ broad. Lower cortex paraplectenchymatous, $30-40 \ \mu m$ thick, homogeneous, consisting of 2–3 layers of cells, cells 7–15 × 6–10 μm , their walls 2–4 μm thick. Hairs of lower primary tomentum up to 220 μm long, in fascicles of more than 20, hyphae simple or rarely branched, 6–8 μm wide with uneven walls, septate with free apices; secondary tomentum sparse, locally developed, up to 2 cells and up to 10 μm long. Cyphellae cavity up to 250 μm deep; cells of basal membrane without papillae. Apothecia biatorine, up to 1 mm high, with distinct stipe; excipulum up to 150 μm broad, laterally with projecting hairs. Hymenium up to 125 μm high; epihymenium up to 10 μm high, brown-orange, with gelatinous upper layer, covered by tiny granules. Asci 6–8-spored, ascospores fusiform, 1(–3)-septate, 25–38 × 6–8 μm .

Secondary chemistry. Unidentified substance in Rf classes A2–3 and C2. Basal membrane of cyphellae K– to K+ pale yellow, C–, KC–, P–. Medulla K+ ochraceous-yellow, C–, KC–, P–.

Habitat and distribution. *Sticta macrolobata* was found on tree bark in Yungas forest. It was collected from a single locality in the Parque Nacional Amboró in the Santa Cruz Department.

Etymology. The name refers to the presence of wide lobes, which are up to 50 mm broad.

Notes. *Sticta macrolobata* resembles *S. laciniata*, but the latter has green photobiont and the thallus is smaller, up to 10 cm broad and more branched than in the new species (Hooker 1822; Moncada 2012). Both species have apothecia with tomentose margins, but in the new species, the margins are also verrucous to rarely weakly crenate and often ciliate in the lower part, whereas in *S. laciniata*, only tomentose. In addition, in *S. macrolobata*, the apothecial discs are brown to chestnut-brown and in *S. laciniata*, orange to reddish (Hooker 1822; Moncada 2012).

The new species forms a clade with *Sticta borinquensis* Merc.-Díaz & Lücking, *S. densiphyllidiata* Merc.-Díaz & Lücking, *S. riparia* and *S. scabrosa* (Fig. 1), although with low support. All four species produce abundant propagules in the form of phyllidia which are absent in the new species (Mercado-Díaz et al. 2020; Moncada et al. 2021b). *Sticta borinquensis* and *S. densiphyllidiata* are epiphytic species known so far from Puerto-Rico (Mercado-Díaz et al. 2020) and *S. riparia* is reported here as new to Bolivia (see below). *Sticta scabrosa* subsp. *scabrosa* was recently confirmed from Bolivia (Ossowska et al. 2022b) and apothecia were observed in the Bolivian specimens for the first time.

Sticta madidiensis Ossowska, B. Moncada, Lücking & Kukwa, sp. nov. MycoBank No: 852906

Fig. 4

Diagnosis. Differing from other *Sticta* in having up to 1 cm long stipe, a palmate to irregular thalli, without vegetative propagules and apothecia, with scabrid upper surface.

Type. BOLIVIA. Dept. La Paz; Prov. Franz Tamayo, Parque Nacional y Área Natural de Manejo Integrado Madidi, below Keara Bajo, 14°41'90"S, 69°03'51"W, elev. 3060 m, open area with shrubs and scattered trees, Ceja de Monte Inferior (Altimontano), on shrubs, 18 Nov 2014, M. Kukwa 14879 (holotype UGDA, isotype LPB).



Figure 4. Morphology of *Sticta madidiensis* (holotype) **A**, **B** upper surface **C**, **D** lower surface **E** marginal cilia and extension of the lower tomentum **F** scabrid upper surface **G** lower tomentum with cyphellae **H** section of cyphella. Scale bars: 1 mm(A-G); 50 µm (H).

Description. Primary photobiont cyanobacterial (Nostoc). Stipe present, up to 1 cm long. Thallus palmate to irregular, coriaceous, up to 15 cm diam., moderately branched, with 3-5 branches per 5 cm radius, branching pleurotomous to polytomous; lobes laciniate to ligulate, imbricate, partly involute, with their apices obtuse and acute and their margins entire to sinuous, thickened; lobe internodes 4(7-)-17(-20) mm long, (5-)8-9(-12) mm broad. Upper surface smooth to slightly canaliculate, brown to brownish-grey in central part of thallus when dry, with darker apices and darker marginal line, shiny; surface slightly scrobiculate to rugose, with papillae in young parts of lobes and without pruina, but with irregular, scattered, beige maculae; marginal cilia sparse to abundant fasciculate, white to brown, up to 1 mm, in some areas extension of the lower tomentum present. Apothecia absent. Vegetative propagules absent. Lower surface smooth, yellow-beige to orange-beige; primary tomentum dense, thick, but thinner towards the margin, fasciculate to spongy, soft, whitish-yellow to dark brown in the centre; secondary tomentum present, sparse, pubescent. Rhizines absent. Cyphellae 1-10 per cm² towards the thallus centre and 21-40 per cm² towards the margin, dispersed, rounded to elongate, urceolate with wide pore, erumpent to prominent, remaining below the level of the primary tomentum, with the margin raised and involute or rarely erect, cream to dark brown-coloured, without tomentum; pore (0.25-)0.5-1(-2) mm diam.; basal membrane scabrid, white. Medulla compact, white. Apothecia not found.

Upper cortex paraplectenchymatous, up to 50 µm thick, differentiated into two cellular layers with the upper layer consisting of 1–2 layers of smaller cells, cells 5–15 × 5–10 µm, their walls 1–3 µm thick and their lumina rounded to irregular, 4–14 × 4–9 µm. Photobiont layer 30–60 µm thick, its cells 10–20 µm diam. Medulla 110–150 µm thick, its hyphae 4–5 µm broad, without crystals. Lower cortex paraplectenchymatous, 30–40 µm thick, with 2–4 cell layers; cells 7–16 µm × 6–12 diam., their walls 1–3 µm thick. Hairs of lower primary tomentum up to 500 µm long, in fascicles of more than 10, hyphae unbranched, septate with free apices; secondary tomentum sparse of up to 10 µm long. Cyphellae cavity up to 140 µm deep; cells of basal membrane without or with one papilla.

Secondary chemistry. No lichen substances detected by TLC. Basal membrane of cyphellae, K+ yellowish, C-, KC-, P-. Medulla K+ yellowish, C-, KC-, P-.

Habitat and distribution. *Sticta madidiensis* was found on shrubs in mountain vegetation with scattered trees. The species is known only from one locality in in the Madidi protected area in the La Paz Department.

Etymology. The name refers to the type locality.

Notes. The new species has a palmate thallus with a stipe, similar to *S. catharinae* recently described from Bolivia (Ossowska et al. 2022a), which is, however, not related to the new species (Fig. 1). However, cilia in the new species are sparse to abundant and white to brown, whereas in *S. catharinae*, they are abundant, agglutinated to fasciculate, dark brown with paler tips (Ossowska et al. 2022a). Furthermore, *S. catharinae* produces apothecia, which are not known in *S. madidiensis* (Ossowska et al. 2022a). Another taxon with palmate thalli is *S. neopulmonarioides* B. Moncada & Coca (a form with cyanobacteria), but it has abundant, laminal and marginal propagules (phyllidia and lobules) without apothecia (the form with green alga has apothecia, but the thallus is

larger than in *S. madidiensis* and irregular). In addition, the primary tomentum is absent towards the margins. *Sticta neopulmonaroides* is known only from Colombia (Moncada 2012; Moncada et al. 2013a).

Sticta montepunkuensis Ossowska, B. Moncada, Lücking & Kukwa, sp. nov. MycoBank No: 852907 Fig. 5

Diagnosis. Differing from other *Sticta* in the green algal photobiont, large thalli up to 30 cm diam., moderately branched, the upper surface scrobiculate to pitted or rugose, the margins of the apothecia crenate to verrucous and the presence of urceolate cyphellae with wide pores and scabrid, white to yellowish-white basal membrane.

Type. BOLIVIA. Dept. Cochabamba; Prov. Carrasco, Parque Nacional Carrasco, Korikaza close to Monte Punku, 17°33'30"S, 65°16'32"W, elev. 2880 m, lower montane Yungas cloud forest, corticolous, 27 Nov 2014, M. Kukwa 15115 (holotype UGDA, isotype LPB).

Description. Primary photobiont green alga. Stipe absent. Thallus irregular, up to 30 cm diam., moderately branched, with 3-5 branches per 5 cm radius, branching pleurotomous to polytomous; lobes ligulate to laciniate, adjacent to interspaced, plane to involute, with their apices rounded to obtuse and plane and their margins entire, slightly thickened; lobe internodes (7-)10-18(-20) mm long, (5–)10–15(–18) mm broad; thallus coriaceous. Upper surface scrobiculate, pitted or rarely rugose, yellowish-brown and darkening towards the margins when dry, with brown marginal line, shiny; surface glabrous, without papillae, pruina and maculae; marginal cilia absent, but extension of the lower tomentum visible. Apothecia sparse, principally laminal, pedicellate, without pronounced invagination on lower side, up to 0.5 mm diam.; disc brown to red-brown, shiny; margin crenate to verrucous, light cream-brown. Vegetative propagules absent. Lower surface scrobiculate to undulate or faveolate, beige to dark brown towards the centre; primary tomentum dense, thick, but thinner towards the margin, fasciculate, soft, brown often with whitish tips; secondary tomentum present, pubescent to arachnoid. Rhizines sparse, irregularly dispersed, often in groups, fasciculate to barbate, brown with paler tips, up to 1 cm long. Cyphellae 41–60 per cm² towards the thallus centre and more than 100 per cm² towards the margin, scattered, rounded to slightly elongate, urceolate with wide pore, erumpent to sessile, remaining below the level of the primary tomentum, with the margin elevated and involute, brown-coloured, without tomentum; pore (0.3–)0.5–1.8(–2.5) mm diam.; basal membrane scabrid, white to yellowish-white. Medulla compact, white. Pycnidia present.

Upper cortex paraplectenchymatous, not distinctly differentiated into layers, $50-65 \mu m$ thick, consisting of up to nine cell layers, size of cells gradually decreasing towards the upper part, cells $5-17 \times 4-14 \mu m$, their walls $1-4 \mu m$ thick and their lumina rounded to isodiametric, $4-16 \times 3-13 \mu m$. Photobiont layer $30-50 \mu m$ thick, its cells $3.5-6 \mu m$ diam. Medulla up to $160 \mu m$ thick, its hyphae $3-4.5 \mu m$ broad, without crystals. Lower cortex paraplectenchymatous, $35-50 \mu m$ thick, with 3-4 cell layers; cells $9-17 \times 8-13 \mu m$, their walls $1-3 \mu m$ thick. Hairs of lower primary tomentum up to $220 \mu m$ long, in



Figure 5. Morphology of *Sticta montepunkuensis* (holotype) **A**, **B** upper surface **C** lower surface **D** apothecia with crenate to verrucous margins **E**, **F** lower tomentum with cyphellae and rhizines. Scale bars: 1 mm.

fascicles of 6–12, simple or often branched in upper parts, septate with free or interlocked apices, up to 8 µm wide; secondary tomentum up to 25 µm long. Cyphellae cavity up to 100 µm deep; cells of basal membrane usually without or rarely with up to three papillae. Apothecia biatorine, up to 700 µm high, with very short stipe; excipulum up to 250 µm broad, laterally with projecting hairs on the lower side, simple to branched. Hymenium up to 150 µm high; epihymenium up to 10 µm high, pale orange-brown, with gelatinous upper layer, ca. 4 µm high. Asci 6–8-spored, ascospores fusiform, 1(–3)-septate, $17-32 \times 7-9$ µm.
Secondary chemistry. No lichen substances detected by TLC. All parts of thallus and apothecia K-, C-, KC-, P-.

Habitat and distribution. *Sticta montepunkuensis* is known only from the type locality in Yungas cloud forest in Nacional Parque Carrasco, where it was collected on the bark of tree, at an elevation of 2880 m.

Etymology. The name refers to the settlement Monte Punku in Parque Nacional Carrasco, near where the new species was found.

Notes. The new species is related and morphologically similar to other green algal *Sticta* species, such as *S. lobarioides* and *S. pseudolobaria* (Fig. 1). All these taxa produce apothecia, but they are aggregated, with entire to verrucose margins in *S. lobarioides*, scattered with hairy to verrucous margins in *S. pseudolobaria* and, in *S. montepunkuensis*, they are subaggregated with crenate to verrucous margins. They also differ in the presence of a stipe (absent in *S. montepunkuensis*) and the different sizes of the thalli (up to 20 cm in *S. lobarioides* and over 15 cm in *S. pseudolobaria*). The upper surface in these species is faveolate rather than scrobiculate to pitted as it is in the new species and the primary tomentum dense, secondary present in *S. montepunkuensis*) (Moncada 2012; Moncada et al. 2013a). For the differences between *S. montepunkuensis* and *S. laciniata*, see the general discussion above.

Other species known from Bolivia with green algae and large thalli include *S. amboroensis* and *S. carrascoensis*. The species differ in the structure of the tomentum. In *S. amboroensis*, it is spongy to dense, fasciculate, light to dark brown and sparse towards the margin (Ossowska et al. 2022a). *Sticta carrascoensis* has a primary tomentum that is dense towards the margin like in *S. montepunkuensis*, but it is spongy, light to dark brown, whereas in the new species, it is fasciculate, brown with white tips. *Sticta montepunkuensis* also has more abundant cyphellae, i.e. 41–60 per cm², towards the centre and more than 100 per cm² towards the margin, whereas *S. carrascoensis* has 1–10 per cm² and 21–40 per cm², respectively and *S. amboroensis* 1–20 per cm² and 21–40 per cm² (Ossowska et al. 2022a). Both, *S. amboroensis* and *S. carrascoensis*, have abundant apothecia, which are sparse in the new species and are submarginal in *S. amboroensis* and marginal to laminal in *S. carrascoensis*. Their apothecial margins are crenate to hirsute in both species, rather than crenate to verrucous as in *S. montepunkuensis* (Ossowska et al. 2022a). All three species are not closely related (Fig. 1).

Species newly reported from Bolivia

Sticta beauvoisii Delise

Fig. 6

Description. For the description, see McDonald et al. (2003) and Moncada (2012).

Habitat and distribution. The records of *S. beauvoisii* presented here are the first from Bolivia. The species was found on the bark of trees in Tucumano-Boliviano forest at elevations of 1815 m and 1900 m in the Tarija and Chuquisaca Departments. Before, *S. beauvoisii* was known from Colombia and North America: Canada and USA (McDonald et al. 2003; Moncada 2012; Moncada et al. 2020, 2021a).



Figure 6. Morphology of *Sticta beauvoisii* **A** upper surface (Kukwa 16480) **B** lower surface (Kukwa 16480) **C** marginal isidia (Kukwa 16480) **D** lower surface with tomentum, cyphellae and sparse rhizines (Kukwa 11103). Scale bars: 1 mm.

Notes. *Sticta beauvoisii* is characterised by a smooth, yellowish-brown upper surface with darker apices, without apothecia, but with abundant, marginal, cylindrical to flattened isidia, which are light to dark brown coloured, a brown lower surface, golden-chocolate brown primary tomentum which becomes thin and shorter towards the margins and a sparse, golden-brown, fibrillose to fasciculate rhizines (Delise 1825; McDonald et al. 2003; Moncada 2012).

Sticta beauvoisii belongs to clade III sensu Widhelm et al. (2018) (Fig. 1), as do, for example, S. weigelii and the undescribed 'S. luteocyphellata'. However, they differ in the colour of the upper and lower surface of the thalli, the isidia and the tomentum. In 'S. luteocyphellata' the upper surface is light brown, with a brown marginal line and dark brown isidia. The lower surface, on the other hand, is cream to dark brown, with dense in the centre, but sparse towards the margin primary tomentum and greyish-brown with paler apices (Moncada 2012). In S. weigelii, the upper surface is reddish-brown to dark brown with a black marginal line and blackish-brown isidia, while the lower surface is beige to reddish-brown with dark brown primary tomentum, dense to the margin (Moncada 2012; Ossowska 2021). Both, 'S. luteocyphellata' and S. weigelii produce abundant rhizines, which are white and fasciculate in 'S. luteocyphellata' and brownish-black and fibrillose to anziform in S. weigelii (Moncada 2012; Ossowska 2021; Torres et al. 2021). In contrast, S. beauvoisii has sparse, golden-brown, fibrillose to fasciculate rhizines (McDonald et al. 2003). Another taxon with which S. beauvoisii may be confused is the not yet formally described

'S. *pseudobeauvoisii*', but it produces narrow phyllidia, rather than isidia and the primary tomentum is light grey to brown, dense and sparse towards the margins (Moncada 2012). 'Sticta pseudobeauvoisii', like S. beauvoisii, belongs to clade III of the global Sticta phylogeny (Widhelm et al. 2018; Fig 1).

Specimens examined. BOLIVIA. Dept. Chuquisaca; Prov. Hernando Siles, 15 km west of Monte Agudo, 19°48'57"S, 64°05'60"W, elev. 1815 m, disturbed Tucumano Boliviano Forest, corticolous, 20 July 2015, M. Kukwa 16480 (LPB, UGDA). Dept. Tarija; Prov. Aniceto Arce, Papachacra, 21°41'52"S, 64°29'15"W, elev. 1900 m, Tucumano Boliviano Forest, corticolous, 8 Aug 2012, M. Kukwa 11103 (LPB, UGDA).

Sticta riparia Merc.-Díaz

Fig. 7

Description. For the description, see Mercado-Díaz et al. (2020).

Habitat and distribution. The record of *S. riparia* presented here is the first one from Bolivia and South America, as the species has been previously known only from Puerto Rico (Mercado-Díaz et al. 2020). In Bolivia, the species was found on tree bark in semi-natural Sub-Andean Amazon forest in the Cochabamba Department.



Figure 7. Morphology of *Sticta riparia* (Kukwa 18724) **A** upper surface **B** lower surface with very sparse tomentum **C**, **D** marginal phyllidia. Scale bars: 1 mm.

Notes. *Sticta riparia* has a strongly branched thallus, with undulate lobes, the margins of which are covered with branched, abundant, palmate, grey to dark brown phyllidia. The lower surface is greyish-brown, with the primary tomentum absent towards the margins. In addition, cyphellae are abundant, with a density of 41–60 per cm² towards the centre and more than 100 per cm² towards the margins (Mercado-Díaz et al. 2020). It is similar to *S. densiphyllidiata* as, in both species, the lobe margins are abundantly covered by phyllidia, but in *S. densiphyllidiata*, these are dispersed, coralloid and darker than the thallus. Furthermore, the lower surface of the latter taxon is reddish with a dense tomentum. The abundance of the cyphellae towards the margins and centre is also a feature common to both taxa. However, in *S. densiphyllidiata*, the membrane reacts with K+ weakly pink, whereas in *S. riparia*, it is K+ pale yellow (Mercado-Díaz et al. 2020). Both species belong to clade III of the *Sticta* tree (Fig. 1). *Sticta densiphyllidiata* is only known from Puerto Rico (Mercado-Díaz et al. 2020).

Recently, a new phyllidiate species, *S. cerradensis* T.D. Barbosa, J.-M. Torres, Kitaura & A.P. Loren, phylogenetically similar to *S. riparia*, has been described. However, it has larger lobes and the lower surface is light brown to dark. *Sticta cerradensis* is only known from Brazil (Torres et al. 2021).

Specimens examined. BOLIVIA. Dept. Cochabamba; Prov. Chaparre, Parque Nacional Carrasco, Guacharos, 17°03'50"S, 65°28'31"W, elev. 445 m, semi-natural Sub-Andean Amazon forest, corticolous, 10 Nov 2016, M. Kukwa 18724 (LPB, UGDA).

Species confirmed for Bolivia with molecular data

Sticta tomentosa (Sw.) Ach.

Fig. 8

Description. For a description, see Moncada (2012) and Moncada et al. (2021a).

Habitat and distribution. The record of *S. tomentosa* given here is the first from Bolivia supported by a DNA sequence. The taxon was previously reported from the country by Nylander (1859, 1861) and Herzog (1922). The specimen examined here was found on tree bark in the lower montane Yungas cloud forest in the Cochabamba Department. Outside Bolivia, *S. tomentosa* has been reported from South and North America (Galloway 1995; Moncada 2012) and Africa (Galloway 1995; Kaasalainen et al. 2023).

Notes. *Sticta tomentosa* has palmate, bluish thalli with white cilia, abundant, submarginal apothecia with entire to crenate margins; the lower surface is creamy-white with a sparse, white primary tomentum (Moncada 2012).

The palmate thallus is characteristic for newly-distinguished *S. madidiensis*; however, the taxa differ in the size of the thallus, which is smaller in *S. tomentosa* (up to 5 cm) with abundant, fasciculate cilia. In addition, *S. tomentosa* has abundant apothecia, which are absent in *S. madidiensis*. Both taxa also differ in the structure of the tomentum, which in *S. tomentosa*, is sparse and absent towards the margin and white to greyish-white towards the centre, whereas in *S. madidiensis*, the primary tomentum is dense towards the margin and whitish-yellow to dark brown in the centre (Moncada 2012; Moncada et al. 2021a). Both species are not closely related (Fig. 1).



Figure 8. Morphology of *Sticta tomentosa* (Kukwa 15138c) A upper surface B lower surface with tomentum and cyphellae C apothecia D marginal cilia. Scale bars: 1 cm (A); 1 mm (B–D).

The species may also be confused with the phylogenetically closely-related *S. leucoblepharis* (Nyl.) Tuck. (Fig. 1), but they differ in the colour of the cilia and the density of the tomentum. In *S. leucoblepharis*, the cilia are golden-brown and longer than in *S. tomentosa*, while the primary tomentum is dense and sparse towards the margins. In addition, the apothecia are laminal rather than submarginal as in *S. tomentosa* and smaller (up to 1.5 cm in diameter) and their discs are orange (Moncada 2012; Moncada et al. 2021a).

Another phylogenetically similar taxon is *S. antoniana* B. Moncada & Lücking and the two cannot be separated, based on nuITS rDNA sequences (Moncada et al. 2020; Moncada et al. 2021a). However, there are important morphological differences. *Sticta antoniana* has an irregular to orbicular and highly-branched thallus, without cilia and with abundant lobules, the primary tomentum is thick and dense, but without secondary tomentum. In *S. tomentosa*, on the other hand, the thallus is palmate to suborbicular, moderately branched, with abundant cilia and without vegetative propagules, while primary tomentum is sparse and absent towards the margin and with secondary tomentum. Both species produce apothecia, but unlike *S. tomentosa*, in *S. antoniana*, they are laminal and with crenate margins (Moncada et al. 2020; Moncada et al. 2021a).

Specimens examined. BOLIVIA. Dept. Cochabamba; Prov. Carrasco, Parque Nacional Carrasco, near Rio Ibrisu, close to Sajtarumi, 17°27'09"S, 65°16'29"W, elev. 2059 m, lower montane Yungas cloud forest, corticolous, 28 Nov 2014, M. Kukwa 15138c (LPB, UGDA).

Acknowledgements

We are grateful to the members of Herbario Nacional de Bolivia, Instituto of Herbario Nacional de Bolivia, Instituto de Ecología, Universidad Mayor de San Andrés, La Paz, for their generous cooperation. We are also greatly indebted to Emmanuël Sérusiaux, Joel A. Mercado-Díaz and anonymous reviewer for their helpful comments and Agnieszka Jabłońska and Magdalena Oset (Gdańsk, Poland) for help with the molecular work. Lichen samples were collected in Bolivia with the permission of Ministerio de Medio Ambiente y Agua and in cooperation with Herbario Nacional de Bolivia (LPB).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research received support from the SYNTHESYS Project (DE-TAF-8180) http:// www.synthesys.info/ which is financed by the European Community Research Infrastructure Action under the FP7 "Capacities" Programme and the University of Gdansk, granted to EAO.

Author contributions

Emilia Anna Ossowska: conceptualisation, descriptions of new species, determination of species, molecular laboratory work and analyses, chromatographic analyses, manuscript writing and editing; Bibiana Moncada: descriptions of new species, phylogenetic analyses, manuscript editing; Robert Lücking: phylogenetic analyses, manuscript writing and editing; Adam Flakus & Pamela Rodriguez-Flakus: photographic documentation, fieldwork, manuscript editing, Sandra Olszewska: molecular laboratory work, manuscript editing; Martin Kukwa: conceptualisation, material collecting, descriptions of new species, secondary chemistry, manuscript writing and editing.

Author ORCIDs

Emilia Anna Ossowska [©] https://orcid.org/0000-0002-1357-6071 Bibiana Moncada [©] https://orcid.org/0000-0001-9984-2918 Robert Lücking [©] https://orcid.org/0000-0002-3431-4636 Adam Flakus [©] https://orcid.org/0000-0002-0712-0529 Pamela Rodriguez-Flakus [©] https://orcid.org/0000-0001-8300-5613 Martin Kukwa [©] https://orcid.org/0000-0003-1560-909X

Data availability

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

References

Acharius E (1803) Methodus Qua Omnes Detectos Lichenes, 275-281.

Crous PW, Costa MM, Kandemir H, Vermaas M, Vu D, Zhao L, Arumugam E, Flakus A, Jurjević Ž, Kaliyaperumal M, Mahadevakumar S, Murugadoss R, Shivas RG, Tan YP, Wingfield MJ, Abell SE, Marney TS, Danteswari C, Darmostuk V, Denchev CM, Denchev TT, Etayo J, Gené J, Gunaseelan S, Hubka V, Illescas T, Jansen GM, Kezo K, Kumar S, Larsson E, Mufeeda KT, Piątek M, Rodriguez-Flakus P, Sarma PVSRN, Stryjak-Bogacka M, Torres-Garcia D, Vauras J, Acal DA, Akulov A, Alhudaib K, Asif M, Balashov S, Baral H-O, Baturo-Cieśniewska A, Begerow D, Beja-Pereira A, Bianchinotti MV, Bilański P, Chandranayaka S, Chellappan N, Cowan DA, Custódio FA, Czachura P, Delgado G, De Silva NI, Dijksterhuis J, Dueñas M, Eisvand P, Fachada V, Fournier J, Fritsche Y, Fuljer F, Ganga KGG, Guerra MP, Hansen K, Hywel-Jones N, Ismail AM, Jacobs CR, Jankowiak R, Karich A, Kemler M, Kisło K, Klofac W, Krisai-Greilhuber I, Latha KPD, Lebeuf R, Lopes ME, Lumyong S, Maciá-Vicente JG, Maggs-Kölling G, Magistà D, Manimohan P, Martín MP, Mazur E, Mehrabi-Koushki M, Miller AN, Mombert A, Ossowska EA, Patejuk K, Pereira OL, Piskorski S, Plaza M, Podile AR, Polhorský A, Pusz W, Raza M, Ruszkiewicz-Michalska M, Saba M, Sánchez RM, Singh R, Śliwa L, Smith ME, Stefenon VM, Strašiftáková D, Suwannarach N, Szczepańska K, Telleria MT, Tennakoon DS, Thines M, Thorn RG, Urbaniak J, van der Vegte M, Vasan V, Vila-Viçosa C, Voglmayr H, Wrzosek M, Zappelini J, Groenewald JZ (2023) Fungal Planet description sheets: 1550-1613. Persoonia 51(1): 280-417. https://doi.org/10.3767/persoonia.2023.51.08

- Dal Forno M, Moncada B, Lücking R (2018) *Sticta aongstroemii*, a newly recognized species in the *S. damicornis* morphodeme (Lobariaceae) potentially endemic to the Atlantic Forest in Brazil. Lichenologist (London, England) 50(6): 691–696. https://doi. org/10.1017/S0024282918000403
- Delise DF (1825) Histoire des lichens. Genre *Sticta*. Memoires de la Societe Linne'enne du Normandie 2: 1–167.
- Di Meglio JR, Goward T (2023) Resolving the *Sticta fuliginosa* morphodeme (lichenized Ascomycota: Peltigeraceae) in northwestern North America. The Bryologist 126(1): 090–110. https://doi.org/10.1639/0007-2745-126.1.090

Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7(1): e214. https://doi.org/10.1186/1471-2148-7-214

- Feuerer T, Ahti T, Vitikainen O (1998) Lichenological investigations in Bolivia. In: Marcelli MP, Seaward MRD (Eds) Lichenology in Latin America: History, current knowledge and applications. CETESB – Companhia de Tecnologia de Saneamento Ambiental – Estado de Sao Paulo, Sao Paulo, Brazil, 71–86.
- Flakus A, Etayo J, Miądlikowska J, Lutzoni F, Kukwa M, Matura N, Rodriguez-Flakus P (2019) Biodiversity assessment of ascomycetes inhabiting *Lobariella* lichens in Andean cloud forests led to one new family, three new genera and 13 new species of lichenicolous fungi. Plant and Fungal Systematics 64(2): 283–344. https://doi.org/10.2478/pfs-2019-0022
- Galloway DJ (1995) Studies on the lichen genus *Sticta* (Schreber) Ach.: III. Notes on species described by Bory de St-Vincent, William Hooker, and Delise, between 1804 and 1825. Nova Hedwigia 61: 147–188.
- Galloway DJ, Southern South American Species (1994) Studies on the lichen genus *Sticta* (Schreber) Ach.: I. Southern South American species. Lichenologist (London, England) 26(3): 223–282. https://doi.org/10.1006/lich.1994.1019

- Guzow-Krzemińska B, Flakus A, Kosecka M, Jabłońska A, Rodriguez-Flakus P, Kukwa M (2019) New species and records of lichens from Bolivia. Phytotaxa 397(4): 257–279. https://doi.org/10.11646/phytotaxa.397.4.1
- Hall T (2011) BioEdit: An important software for molecular biology. GERF Bulletin of Biosciences 2: 60–61.
- Herzog T (1922) Beitrag zur Flechtenflora von Bolivia. Hedwigia 63: 263-268.
- Herzog T (1923) Die Pflanzenwelt der bolivianischen Anden und ihres östlichen Vorlandes. In: Engler A, Drude O (Eds) Die Vegetation der Erde, volume XV. Leipzig, 258 pp.
- Hodkinson BP, Lendemer JC, McDonald T, Harris RC (2014) The status of *Sticta sylvatica*, an 'Exceedingly Rare' lichen species, in Eastern North America. Evansia 31(1): 17–24. https://doi.org/10.1639/079.031.0103
- Hooker WJ (1822) Lichenes, Achar. In: Kunth, KS, von Humboldt A, Bonpland A (Eds) Synopsis plantarum, quas in itinere ad plagam aequinoctatem orbis novi, collegerunt Al. de Humboldt et Am. Bonpland. T. 1. Parisiis: Apud F. G. Levrault, 14–39.
- Kaasalainen U, Kirika PM, Mollel NP, Hemp A, Rikkinen J (2023) The Lichen Genus Sticta (Lobariaceae, Peltigerales) in East African Montane Ecosystems. Journal of Fungi (Basel, Switzerland) 9(2): 246. https://doi.org/10.3390/jof9020246
- Katoh K, Frith MC (2012) Adding unaligned sequences into an existing alignment using MAFFT and LAST. Bioinformatics (Oxford, England) 28(23): 3144–3146. https://doi. org/10.1093/bioinformatics/bts578
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kirika P, Mugambi G, Lücking R, Lumbsch HT (2012) New Records of Lichen-Forming Fungi from Kenya. Journal of East African Natural History 101(1): 73–98. https://doi. org/10.2982/028.101.0105
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008) Dictionary of the Fungi. 10th ed. CAB International, Wallingford.
- Kukwa M, Ossowska EA (2022) New records of Parmeliaceae from Bolivia. Opuscula Philolichenum 21: 190–207.
- Kukwa M, Rodriguez-Flakus P, Aptroot A, Flakus A (2023a) Two new species of *Astrothelium* from Sud Yungas in Bolivia and the first discovery of vegetative propagules in the family Trypetheliaceae (lichen-forming Dothideomycetes, Ascomycota). MycoKeys 95: 83–100. https://doi.org/10.3897/mycokeys.95.98986
- Kukwa M, Kosecka M, Jabłońska A, Flakus A, Rodriguez-Flakus P, Guzow-Krzemińska B (2023b) *Pseudolepraria*, a new leprose genus revealed in Ramalinaceae (Ascomycota, Lecanoromycetes, Lecanorales) to accommodate *Lepraria stephaniana*. MycoKeys 96: 97–112. https://doi.org/10.3897/mycokeys.96.98029
- Lendemer JC, Goffinet B (2015) *Sticta deyana*: A New Endemic Photomorphic Lichen from the Imperiled Mid-Atlantic Coastal Plain of Eastern North America. Systematic Botany 40(4): 933–941. https://doi.org/10.1600/036364415X689979
- Magain N, Sérusiaux E (2015) Dismantling the treasured flagship lichen *Sticta fuliginosa* (Peltigerales) into four species in Western Europe. Mycological Progress 14(10): e97. https://doi.org/10.1007/s11557-015-1109-0
- McDonald T, Miadlikowska J, Lutzoni F (2003) The lichen genus *Sticta* in the Great Smoky Mountains: A phylogenetic study of morphological, chemical, and molecular data. The Bryologist 106(1): 61–79. https://doi.org/10.1639/0007-2745(2003)106[0 061:TLGSIT]2.0.CO;2

- Mercado-Díaz JA, Lücking R, Moncada B, Widhelm TJ, Lumbsch HT (2020) Elucidating species richness in lichen fungi: The genus *Sticta* (Ascomycota: Peltigeraceae) in Puerto Rico. Taxon 69(5): 851–891. https://doi.org/10.1002/tax.12320
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November, 2010, New Orleans, 8 pp. https://doi. org/10.1109/GCE.2010.5676129
- Moncada B (2012) El género *Sticta* (Schreb.) Ach. en Colombia, Taxonomía, Ecogeografía e Importancia. Doctoral thesis, Universidad Nacional de Colombia, Bogotá.
- Moncada B, Lücking R (2012) Ten new species of *Sticta* and counting: Colombia as a hot spot for unrecognized diversification in a conspicuous macrolichen genus. Phytotaxa 74(1): 1–29. https://doi.org/10.11646/phytotaxa.74.1.1
- Moncada B, Coca LF, Lücking R (2013a) Neotropical members of *Sticta* (lichenized Ascomycota: Lobariaceae) forming photosymbiodemes, with the description of seven new species. The Bryologist 116(2): 169–200. https://doi.org/10.1639/0007-2745-116.2.169
- Moncada B, Lücking R, Coca LF (2013b) Six new apotheciate species of *Sticta* (lichenized Ascomycota: Lobariaceae) from the Colombian Andes. Lichenologist (London, England) 45(5): 635–656. https://doi.org/10.1017/S0024282913000376
- Moncada B, Lücking R, Suárez A (2014) Molecular phylogeny of the genus *Sticta* (lichenized Ascomycota: Lobariaceae) in Colombia. Fungal Diversity 64(1): 205–231. https://doi.org/10.1007/s13225-013-0230-0
- Moncada B, Suárez A, Lücking R (2015) Nueve especies nuevas del género *Sticta* (Ascomycota liquenizados: Lobariaceae) del morfotipo *fuliginosa* sensu lato de Colombia. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 39(150): 50–66. https://doi.org/10.18257/raccefyn.110
- Moncada B, Mercado-Díaz JA, Lücking R (2018) The identity of Sticta damicornis (Ascomycota: Lobariaceae): a presumably widespread taxon is a Caribbean endemic. Lichenologist (London, England) 50(5): 591–597. https://doi.org/10.1017/ S0024282918000373
- Moncada B, Lücking R, Lumbsch HT (2020) Rewriting the evolutionary history of the lichen genus *Sticta* (Ascomycota: Peltigeraceae subfam. Lobarioideae) in the Hawaiian islands. Plant and Fungal Systematics 65(1): 95–119. https://doi.org/10.35535/ pfsyst-2020-0005
- Moncada B, Smith CW, Lücking R (2021a) A taxonomic reassessment of the genus *Sticta* (lichenized Ascomycota: Peltigeraceae) in the Hawaiian archipelago. Lichenologist (London, England) 53(1): 117–133. https://doi.org/10.1017/S0024282920000353
- Moncada B, Mercado-Díaz JA, Smith CW, Bungartz F, Sérusiaux E, Lumbsch HT, Lücking R (2021b) Two new common, previously unrecognized species in the *Sticta weigelii* morphodeme (Ascomycota: Peltigeraceae). Willdenowia 51(1): 35–45. https://doi. org/10.3372/wi.51.51103
- Moncada B, Mercado-Díaz JA, Magain N, Hodkinson BP, Smith CW, Bungartz F, Pérez-Pérez RE, Gumboski E, Sérusiaux E, Lumbsch HT, Lücking R (2021c) Phylogenetic diversity of two geographically overlapping lichens: Isolation by distance, environment, or fragmentation? Journal of Biogeography 48(3): 676–689. https://doi.org/10.1111/jbi.14033

Nash TH (2008) Lichen Biology (2nd edn.). Cambridge University Press, Cambridge, 486 pp. Nylander W (1859) Lichenes in regionibus exoticis quibusdam vigentes exponit synopticis enumerationibus. Annales des Sciences Naturelles, la botanique 11: 205–264.

- Nylander W (1861) Additamentum ad lichenographiam Andium Boliviensium. Annales des Sciences Naturelles, la botanique 15: 365–382.
- Orange A, James PW, White FJ (2001) Microchemical methods for the identification of lichens. British Lichen Society, London, 101 pp.
- Ossowska EA (2021) First records of *Sticta weigelii* s.str. from Bolivia confirmed by molecular data. Folia Cryptogamica Estonica 58: 65–72. https://doi.org/10.12697/ fce.2021.58.09
- Ossowska EA, Moncada B, Kukwa M, Flakus A, Rodriguez-Flakus P, Olszewska S, Lücking R (2022a) New species of *Sticta* (lichenised Ascomycota, lobarioid Peltigeraceae) from Bolivia suggest a high level of endemism in the Central Andes. MycoKeys 92: 131–160. https://doi.org/10.3897/mycokeys.92.89960
- Ossowska EA, Kosecka M, Jaskólska J, Kukwa M (2022b) Two taxa of the genus *Sticta* (Peltigerales, Ascomycota), *S. andina* and *S. scabrosa* subsp. *scabrosa*, new to Bolivia confirmed by molecular data. Plant and Fungal Systematics 67(2): 45–54. https://doi.org/10.35535/pfsyst-2022-0006
- Rodriguez-Flakus P, Kukwa M, Etayo J, Lücking R, Meneses RI, Rivas Plata E, Stanton D, Truong C, Vargas R, Flakus A (2016) Preliminary catalogue of lichens and lichenicolous fungi from Bolivia. http://bio.botany.pl/lichens-bolivia/en,strona,catalogue,5. html [Version 1.5. 31 December 2016]
- Rusby HH (1896) An enumeration of the plant collected in Bolivia by Miguel Bang, with description of new genera and species. Part III. Memoirs of the Torrey Botanical Club 6(1): 1–130.
- Schreber JCD (1791) Genera Plantarum Eorumque Characteres Naturales Secundum Numerum, Figuram, Situm, & Proportionem Omnium Fructificationis Partium. Ed. 8[a]. Vol. 2. Frankfurt am Main.
- Simon A, Goffinet B, Magain N, Sérusiaux E (2018) High diversity, high insular endemism and recent origin in the lichen genus *Sticta* (lichenized Ascomycota, Peltigerales) in Madagascar and the Mascarenes. Molecular Phylogenetics and Evolution 122: 15–28. https://doi.org/10.1016/j.ympev.2018.01.012
- Sipman HJ, Hekking W, Aguirre-C J (2008) Checklist of lichenized and lichenicolous fungi from Colombia. Instituto Ciencias Naturales. Facultad de Ciencias. Universidad Nacional de Colombia. Biblioteca José Jerónimo Triana N° 20, Bogotá D.C., 235 pp.
- Stamatakis A (2014) RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033
- Tønsberg T, Goward T (2001) *Sticta oroborealis* sp. nov. and other Pacific North American lichens forming dendriscocauloid cyanotypes. The Bryologist 104(1): 12–23. https://doi.org/10.1639/0007-2745(2001)104[0012:SOSNA0]2.0.C0;2
- Torres JM, Barbosa TD, Kitaura MJ, Spielmann AA, Lorenz AP (2021) Two new species of *Sticta* (Peltigeraceae subfam. Lobarioideae) from the Brazilian Cerrado (Brazilian savanna). The Bryologist 124(4): 506–521. https://doi.org/10.1639/0007-2745-124.4.506
- Widhelm TJ, Bertoletti FR, Asztalos MJ, Mercado-Díaz JA, Huanga J-P, Moncada B, Lücking R, Magain N, Sérusiaux E, Goffinet B, Crouch N, Mason-Gamerb R, Lumbsch HT (2018) Oligocene origin and drivers of diversification in the genus *Sticta* (Lobariaceae, Ascomycota). Molecular Phylogenetics and Evolution 126: 58–73. https://doi. org/10.1016/j.ympev.2018.04.006

Supplementary material 1

Specimens of *Sticta* used in molecular analysis with locality, voucher information, GenBank accession numbers and list of references

Authors: Emilia Anna Ossowska, Bibiana Moncada, Robert Lücking, Adam Flakus,

Pamela Rodriguez-Flakus, Sandra Olszewska, Martin Kukwa Data type: docx

Explanation note: Sequences generated for this study are in bold.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.120810.suppl1

Supplementary material 2

Best-scoring Maximum Likelihood tree of the *Sticta* target clade containing the new species from Bolivia (red) and the species new to Bolivia and phylogenetically confirmed from Bolivia (blue), based on the fungal ITS barcoding marker

Authors: Emilia Anna Ossowska, Bibiana Moncada, Robert Lücking, Adam Flakus, Pamela Rodriguez-Flakus, Sandra Olszewska, Martin Kukwa

Data type: pdf

Explanation note: Supported clades are thickened and individual support values are indicated.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.120810.suppl2



Research Article

Morphological and phylogenetic analyses reveal five new species of Porotheleaceae (Agaricales, Basidiomycota) from China

Qin Na¹⁰, Hui Zeng²⁰, Yaping Hu³⁰, Hui Ding³⁰, Binrong Ke²⁰, Zhiheng Zeng²⁰, Changjing Liu⁴, Xianhao Cheng¹⁰, Yupeng Ge^{1,20}

1 Institute of Mycological Science and Technology, School of Agriculture, Ludong University, Yantai 264025, China

- 2 Institute of Edible Fungi, Fujian Academy of Agricultural Sciences; National and Local Joint Engineering Research Center for Breeding & Cultivation of Features Edible Fungi, Fuzhou 350014, China
- 3 Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment, State Environmental Protection Scientific Observation and Research Station for Ecological Environment of Wuyi Mountains, Nanjing 210042, China

4 College of Criminal Science and Technology, Nanjing Police University, Nanjing 210042, China

Corresponding author: Yupeng Ge (gaiyupeng@126.com)

Abstract

The first occurrence of Marasmiellomycena and Pulverulina in the Chinese mycobiota are reported, M. tomentosa and P. flavoalba, two new species and M. albodescendens, a new combination, revealed by phylogenetic analyses and morphological study. These newly-recorded genera, Marasmiellomycena, which can be distinguished by their agaricoid basidiomata, dark-coloured stipe, sarcodimitic tramal structure, stipitipellis with yellow to yellowish-brown pigments and yellow-pigmented thick-walled caulocystidia and Pulverulina, which differs from other genera of Porotheleaceae by its pruinose stipe, decurrent lamellae, inamyloid basidiospores and absence of hymenial cystidia. We also formally describe three other new species of Porotheleaceae collected from Chinese temperate to subtropical zones of Fujian and Zhejiang Provinces: Clitocybula fuscostriata, Gerronema brunneosquamulosum and Leucoinocybe subglobispora. Furthermore, we include the results of a phylogenetic analysis of Porotheleaceae, based on a multi-locus (ITS, nrLSU and rpb2) dataset. According to this analysis, Chrysomycena, Clitocybula, Delicatula, Hydropodia, Hydropus, Leucoinocybe, Marasmiellomycena, Megacollybia, Pulverulina, Trogia and Vizzinia are monophyletic. However, Gerronema is identified as polyphyletic and, additionally, Porotheleum does not form a monophyletic group either because Porotheleum parvulum and Porotheleum albidum are "unassigned" in phylogenetic analysis. The results of our phylogenetic analyses, coupled with morphological observations, confirm recognition of these new taxa. Morphological descriptions, photographs, line drawings and comparisons with closely-related taxa are presented for the new species. A key to the 22 species belonging to nine genera of Porotheleaceae in China is also provided.

Key words: cyphelloid polypores, new taxon, *Porotheleum*, systematics, white-spored omphalinoid fungi



Academic editor: Maria-Alice Neves Received: 15 January 2024 Accepted: 4 April 2024 Published: 25 April 2024

Citation: Na Q, Zeng H, Hu Y, Ding H, Ke B, Zeng Z, Liu C, Cheng X, Ge Y (2024) Morphological and phylogenetic analyses reveal five new species of Porotheleaceae (Agaricales, Basidiomycota) from China. MycoKeys 105: 49–95. https://doi.org/10.3897/ mycokeys.105.118826

Copyright: © Qin Na 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)

Introduction

The family Porotheleaceae (order Agaricales), formally proposed by Murrill (1916), comprises saprotrophic, mainly wood-decaying fungi that are primarily agarics, but also include cyphelloid fungi. The type genus, Porotheleum Fr., is distinguished by fruiting in clusters of small cup-shaped to tubular cream cyphelloid basidiomes, whereas other genera are typically agaricoid (Vizzini et al. 2022). Previous taxonomic studies have included 15 genera in Porotheleaceae: Chrysomycena Vizzini, Picillo, Perrone & Dovana, Clitocybula (Singer) Singer ex Métrod, Delicatula Fayod, Gerronema Singer, Hydropodia Vizzini & Consiglio, Hydropus Kühner ex Singer, Leucoinocybe Singer ex Antonín, Borovička, Holec & Kolařík, Lignomphalia Antonín, Borovička, Holec & Kolařík, Marasmiellomycena De la Peña-Lastra, Mateos, Kolařík, Ševčíková & Antonín, Megacollybia Kotl. & Pouzar, Porotheleum, Pulverulina Matheny & K.W. Hughes, Pseudohydropus Vizzini & Consiglio, Trogia Fr. and Vizzinia Ševčíková & Kolařík (Antonín et al. 2019; Vizzini et al. 2019, 2022; Matheny et al. 2020; Senanayake et al. 2023). Most taxa, except for Porotheleum, are well characterised, based on the following features: a saprophytic habit; omphalinoid, collybioid, to clitocyboid basidiomata; partly to entirely pigmented pileus; adnexed, subdecurrent, to decurrent lamellae; smooth, thin-walled basidiospores; and the frequent presence of sarcodimitic tramal tissues (Singer 1951, 1982; Redhead 1987; Norvell et al. 1994; Hughes et al. 2007; Kumar and Manimohan 2009; Yang et al. 2012; Vizzini et al. 2019; Consiglio et al. 2022; Senanayake et al. 2023). Species of Porotheleaceae are widespread in subtropical to tropical regions and tend to be lower diversity in temperate zones (Singer 1951, 1970; Norvell et al. 1994; Antonín and Noordeloos 2004; Hughes et al. 2007; Antonín et al. 2019; Vizzini et al. 2019; Consiglio et al. 2022; Na et al. 2022a; Senanayake et al. 2023). Six new genera have recently been recognised: Chrysomycena, Hydropodia, Marasmiellomycena, Pulverulina, Pseudohydropus and Vizzinia (Vizzini et al. 2019; Matheny et al. 2020; Consiglio et al. 2022; Senanayake et al. 2023). These newly-described genera have been found in diverse regions, predominantly in Europe and North America, with some findings in Oceania, Africa and Asia, but the distribution reflects the broad yet unequal exploration of this family's species, only one species is from Japan, in Asia and none from China (Cooper 2014; Vizzini et al. 2019; Villarreal et al. 2021; Consiglio et al. 2022; Kasuya et al. 2023; Senanayake et al. 2023). While Index Fungorum (http://www.indexfungorum.org/Names/Names. asp 2023.3.16) records 670 Porotheleaceae species, only seven species are documented from China, indicating a disparity in mycological research within the region (Liu 1995; Yang et al. 2012; Liu et al. 2019; Na et al. 2022a).

A comprehensive phylogenetic analysis of Porotheleaceae has not been performed because few sequences are available. Prior to 2012, the family was informally cited in literature as the 'hydropoid' clade within the 'marasmioid' clade (Moncalvo et al. 2002; Matheny et al. 2006; Antonín et al. 2019). Many authors have since suggested that members of the hydropoid clade should be placed in the phylogenetically defined Porotheleaceae clade (Henrici 2012; Redhead 2013; Cooper 2016; Vizzini et al. 2019, 2022; Kalichman et al. 2020; Matheny et al. 2020; Senanayake et al. 2023). According to a study based on the large subunit of nuclear ribosomal DNA (nrLSU) sequences (Moncalvo et al. 2002), eight species in five genera (*Clitocybula, Gerronema, Hydropus, Megacollybia* and Porotheleum) constitute this hydropoid (/hydropoid) clade. The results of that study also support the placement of Megacollybia and Clitocybula as close relatives of Hydropus. Moncalvo et al. (2002) also proposed that Gerronema sensu Singer (1986) was polyphyletic (Lutzoni 1997; Moncalvo et al. 2000), whereas this genus as delineated by Norvell et al. (1994) was monophyletic. However, the type species of Gerronema was not included in the molecular phylogeny of Moncalvo et al. (2002). The delimitation of Gerronema by Norvell et al. (1994) was based solely on morphology in comparison to an epitype, with emphasis on the presence of sarcodimitic tissue. The hydropoid clade configuration defined by Moncalvo et al. (2002) based on ribosomal LSU is also presented in Bodensteiner et al. (2004). In a multigenic analysis (18S, 5.8S, 25S, rpb1 and rpb2) performed by Matheny et al. (2006), the hydropoid clade included Clitocybula atrialba (Murrill) Singer [currently Gerronema atrialbum (Murrill) Borovička & Kolařík], Clitocybula oculus (Peck) Singer, Henningsomyces candidus (Pers.) Kuntze, Hydnopolyporus fimbriatus (Cooke) DA Reid (currently Irpex rosettiformis C.C. Chen & Sheng H. Wu), Hydropus marginellus (Pers.) Singer, Hydropus cf. scabripes (Murrill) Singer [currently Mycopan scabripes (Murrill) Redhead, Moncalvo & Vilgalys], Megacollybia platyphylla (Pers.) Kotl. & Pouzar and several species formerly placed in Mycena (Pers.) Roussel [i.e. Mycena auricoma Har. Takah. (currently Leucoinocybe auricoma (Har. Takah.) Matheny), Mycena amabilissima (Peck) Sacc. (currently Atheniella amabilissima (Peck) Redhead, Moncalvo, Vilgalys, Desjardin & B.A. Perry) and Mycena aurantiidisca (Murrill) Murrill (currently Atheniella aurantiidisca (Murrill) Redhead, Moncalvo, Vilgalys, Desjardin & B.A. Perry)]. Henrici (2012) combined Megacollybia, Clitocybula and Hydropus, along with other genera, into the family Porotheleaceae, comprising a total of 19 genera. Redhead (2012, 2013) expanded the 'hydropoid' clade by introducing Atheniella Redhead, Moncalvo, Vilgalys, Desjardin & B.A. Perry and established the genus Phloeomana Redhead within the family Porotheleaceae. Cooper (2016) also believes that Porotheleum should belong to the Porotheleaceae family, despite the possibility of misidentification in the sequenced material of Porotheleum fimbriatum (generic type). Finally, Antonín et al. (2019) introduced the new genera Leucoinocybe and Lignomphalia, which were separated from Clitocybula. However, it should be noted that Singer (1943) originally proposed Leucoinocybe as a provisional name, rendering the use by Antonín et al. (2019) as a validation rather than the establishment of a completely new genus. In an analysis by Vizzini et al. (2019), Porotheleaceae was statistically well supported (MLB = 100%) when only Hydropus, Clitocybula, Leucoinocybe, Megacollybia, Porotheleum, Trogia and some species of Gerronema were included. In addition, Chrysomycena formed a distinct monophyletic lineage corresponding to a separate genus, sister to a clade formed by Megacollybia, Trogia and some species of Gerronema (Vizzini et al. 2019). Matheny et al. (2020) performed a phylogenetic analysis of a combined ITS-28S dataset of 73 taxa and found that Delicatula and Pulverulina (representing a new genus) are members of Porotheleaceae sensu Vizzini et al. (2019); this was in agreement with the concept of Porotheleaceae s.l. of Kalichman et al. (2020), which comprises Porotheleaceae sensu Vizzini et al. (2019), Actiniceps Berk. & Broome, Atheniella, Calyptella Quél., Chaetotyphula Corner, Hemimycena Singer, Lignomphalia, Phloeomana and Scytinotus P. Karst. Vizzini et al. (2022) considered the family Porotheleaceae to be equivalent to Porotheleaceae sensu Vizzini et

al. (2019) and included the other taxa in Porotheleaceae *s.l.* Kalichman et al. (2020) in Cyphellaceae Burnett, a sister family to Porotheleaceae. Senanayake et al. (2023) agree with the concept and composition of Porotheleaceae as defined by Vizzini et al. (2019, 2022) and proposed two new genera of the family, *Marasmiellomycena* and *Vizzinia*. Finally, *Hydropus subalpinus* (Höhn.) Singer, which was not aggregated into clade *Hydropus* with high statistical support, was recently treated as *Hydropodia subalpina* (Höhn.) Vizzini, Consiglio & M. Marchetti by Consiglio et al. (2022). In the same study, *Pseudohydropus* Vizzini & Consiglio designated as the type species, comprising a total of four species.

Seventeen species belonging to seven genera of Porotheleaceae, namely, one species of Clitocybula (Singer) Singer ex Métrod, one species of Delicatula, seven species of Gerronema, four species of Hydropus, one species of Leucoinocybe, two species of Megacollybia and one species of Trogia, have been recognised in China as of 2023 (Liu 1995; Dai et al. 2010; Yang et al. 2012; Liu et al. 2019; Wang et al. 2021; Na et al. 2022a). Progress has recently been made in clarifying the status of mycenoid and omphalinoid fungi in China, including the discovery of four new taxa from Anhui, Fujian and Zhejiang Provinces: Gerronema baishanzuense Q. Na, H. Zeng & Y.P. Ge; G. microcarpum Q. Na, H. Zeng & Y.P. Ge; G. zhujian Q. Na, H. Zeng & Y.P. Ge; and Leucoinocybe lishuiensis Q. Na, H. Zeng & Y.P. Ge (Na et al. 2021, 2022a). As part of our ongoing research on omphalinoid fungi, we uncovered the first occurrence of two newly-recorded genera, Marasmiellomycena and Pulverulina, including two new species and we incorporated one species from Porotheleum into Marasmiellomycena. We also discovered three new species belonging to Clitocybula, Gerronema and Leucoinocybe in temperate and subtropical China. We accordingly present a morphological description of the new species and provide an identification key to the 22 species of Porotheleaceae currently known from China.

Materials and methods

Specimens and morphology

Macroscopic descriptions were based on the study of fresh specimens, whereas micromorphological descriptions relied on dried materials. In our descriptions, colour abbreviations follow the colour standards and colour nomenclature of Ridgway (1912). Microscopic observations were made on dried specimens mounted in 5% potassium hydroxide (KOH) and stained with Congo red when necessary. The prepared specimens were observed under a Lab A1 microscope (Carl Zeiss AG, Jena, Germany) and photographed and recorded using ZEN 2.3 software (Carl Zeiss AG). Melzer's reagent was used to test whether spores and tissues were amyloid (Horak 2005). Twenty mature basidiospores from each basidiomata (two basidiomata per holotype) were measured in side view. Sizes of basidiospores were recorded, with the notation [a/b/c] used at the beginning of each entry in the description to indicate a basidiospores from b basidiomata of c specimens were measured. Measured sizes (including Q values) are given in the description as $(d)e-f-g(h) \times (i)j-k-l(m)$, where d is the smallest length, e-g represents the range of at least 90% of values, f is the average length and h is the largest value; width (i-m) is expressed in the same way. In addition,

Q stands for the length-width ratio of a spore and Q \pm av is the average Q of all basidiospores \pm the sample standard deviation (Ge et al. 2021; Liu et al. 2021, 2022; Na et al. 2021, 2022a, 2022b). Hyphae of the pileipellis and stipitipellis and a total of 20 basidia, cheilocystidia and caulocystidia were measured from each collection. The examined collections have been deposited in the fungarium of the Fujian Academy of Agricultural Sciences (FFAAS), China. Author abbreviations follow Index Fungorum (http://www.indexfungorum.org).

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing

Genomic DNAs of the putative new species were extracted from dried materials using a NuClean PlantGen DNA kit (Kangwei Century Biotechnology Co., Beijing, China). Gene regions were amplified using the following primer pairs: ITS1/ITS4 (White et al. 1990) for 5.8S and internal transcribed spacer ITS1 and ITS2 regions (ITS), LR0R/LR7 (Hopple and Vilgalys 1999) for the large subunit of nuclear ribosomal DNA (nrLSU) and bRPB2-6f/bRPB2-7.1R (Matheny 2005) for the second largest subunit of RNA polymerase II (rpb2). Amplifications were performed in 25 µl reaction mixtures consisting of 9.5 µl ddH₂O, 12.5 µl 2× UTaq PCR Master Mix (Zoman Biotechnology Co., Beijing, China), 1 µl of each primer (10 mM) and 1 µl DNA template. PCR amplification of the ITS region used the following protocol: initial denaturation at 95 °C for 4 min, followed by 34 cycles of 94 °C for 45 s, 52 °C for 45 s and 72 °C for 1 min and a final extension at 72 °C for 10 min. Cycling conditions used for amplification of the nrLSU were as follows: initial denaturation at 93 °C for 2 min, followed by 20 cycles of 93 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR protocol for rpb2 amplification was as follows: initial denaturation at 93 °C for 2 min, 20 cycles of 93 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min, 20 cycles of 93 °C for 1 min, 53 °C for 1 min and 72 °C for 1 min and a final extension at 72 °C for 10 min. The PCR products were subjected to Sanger dideoxy sequencing at the Beijing Genomics Institute (Beijing, China).

Phylogenetic analysis

For phylogenetic analysis, we constructed a concatenated dataset of 168 ITS, 87 nrLSU and 14 *rpb2* sequences from 58 taxa of 14 genera of Porotheleaceae. In addition, six sequences (three ITS and three nrLSU) of *Mycena purpureo-fusca* (Peck) Sacc. were included as outgroups according to the results of Na et al. (2022a). Sequences retrieved from GenBank and those obtained in this study are listed in Table 1. Alignments were performed in Mafft 7.376 (Katoh and Standley 2013). Sequence editing and necessary adjustments were carried out in BioEdit 7.0.4.1 and Clustal X 1.81 (Thompson et al. 1997; Hall 1999). Bayesian Inference (BI) and Maximum Likelihood (ML) bootstrap analyses were performed using the best-fit substitution models identified in ModelTest 3.7 (Posada and Crandall 1998). The BI analysis was carried out in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). Runs of 1,000,000 generations, with trees sampled every 100th generation, were initiated for eight heated and one cold Markov chain(s). Analyses were automatically terminated when the average standard deviation of split frequencies reached a value below 0.01 and the

No.	Таха	Voucher	Locality	ITS Sequence ID	LSU Sequence ID	rpb2 Sequence ID	Reference
1	Chrysomycena perplexa	MCVE:30184 TYPE	Italy	NR172974	NG071251	-	Vizzini et al. (2019)
2	Clitocybula albida	CUH AM064	India	MG250188	_	-	Dutta et al. (2018)
3	Clitocybula albida	CUH AM065	India	MG250189	_	-	Dutta et al. (2018)
4	Clitocybula abundans	STU:SMNS-B-FU-2017/00898	Germany	MF627833	_	-	Unpublished
5	Clitocybula familia	2319-QFB-25741	Canada	KM406970	_	-	Unpublished
6	Clitocybula familia	PRM 921866	Czech Republic	JF730327	JF730320	-	Antonín et al. (2011)
7	Clitocybula familia	BRNM 736053	Slovakia	JF730328	JF730323	-	Antonín et al. (2011)
8	Clitocybula familia	STU:SMNS-B-FU-2017/00926	Germany	MF627834	-	-	Unpublished
9	Clitocybula familia	NAMA 2017-349	USA	MH979253	-	-	Unpublished
10	Clitocybula fuscostriata	FFAAS1029	China	OR238881	OR238893	OR258374	This study
11	Clitocybula fuscostriata	FFAAS1030 Holotype	China	OR238882	OR238894	OR258375	This study
12	Clitocybula fuscostriata	FFAAS1031	China	OR238883	OR238895	OR258376	This study
13	Clitocybula lacerata	LE 6639	Russia	HM191746	-	-	Malysheva and Morozova (2011)
14	Clitocybula lacerata	LE 262744	Russia	HM191747	-	-	Malysheva and Morozova (2011)
15	Clitocybula lacerata	LE 262743	Russia	HM191748	_	-	Malysheva and Morozova (2011)
16	Clitocybula lacerata	PRM 915404	Czech Republic	LT854054	LT854030	-	Antonín et al. (2019)
17	Clitocybula lacerata	WU 19575	Austria	LT854053	LT854031	-	Antonín et al. (2019)
18	Clitocybula oculus	3512	Canada	KM406971	_	-	Unpublished
19	Clitocybula oculus	WU 20008	Canada	LT854017	LT854017	-	Antonín et al. (2019)
20	Clitocybula oculus	S.D. Russell iNaturalist # 8606755	India	MN906165	-	-	Unpublished
21	Clitocybula oculus	S.D. Russell iNaturalist # 8591258	India	MN906164	-	-	Unpublished
22	Clitocybula oculus	BIOUG24046-B03	Canada	KT695321	-	-	Telfer et al. (2015)
23	Clitocybula oculus	AFTOL-ID 1554	USA	DQ192178	DQ192178	-	Matheny et al. (2006)
24	Delicatula integrella	KA12-1305	Korea	KR673538	-	-	Kim et al. (2015)
25	Delicatula integrella	S.D. Russell MycoMap # 6067	USA	MN906231	-	-	Unpublished
26	Delicatula integrella	G0060	USA	-	MK277924	-	Varga et al. (2019)
27	Gerronema baishanzuense	FFAAS0359 Holotype	China	OL985962	OL985984	-	Na et al. (2022a)
28	Gerronema baishanzuense	FFAAS0360	China	OL985963	-	-	Na et al. (2022a)
29	Gerronema baishanzuense	FFAAS0361	China	OL985964	OL985985	-	Na et al. (2022a)
30	Gerronema baishanzuense	FFAAS0362	China	OL985965	OL985986	-	Na et al. (2022a)
31	Gerronema baishanzuense	FFAAS0363	China	OL985966	OL985987	-	Na et al. (2022a)
32	Gerronema baishanzuense	FFAAS0366	China	OL985967	OL985988	-	Na et al. (2022a)
33	Gerronema brunneosquamulosum	FFAAS1032 Holotype	China	OR238884	OR238896	OR258377	This study
34	Gerronema brunneosquamulosum	FFAAS1033	China	OR238885	OR238897	OR258378	This study
35	Gerronema indigoticum	HMJAU 47636	China	MK693727	MK693732	-	Liu et al. (2019)
36	Gerronema indigoticum	HMJAU 47942	China	MK693728	MK693733	-	Liu et al. (2019)
37	Gerronema indigoticum	HMJAU 47943	China	MK693729	MK693734	-	Liu et al. (2019)
38	Gerronema keralense	2	India	MH156555	NG_064531	-	Latha et al. (2018)
39	Gerronema keralense	BKF10263	Thailand	MZ452107	MZ452144		Direct Submission
40	Gerronema kuruvense	CAL 1665	India	NG_159831	NG_064530	-	Latha et al. (2018)
41	Gerronema kuruvense	BKF10266	Thailand	MZ452090	MZ452669	-	Direct Submission
42	Gerronema kuruvense	DCY3362(HGASMF01-15010)	Chian	MZ951144	_	-	Direct Submission
43	Gerronema microcarpum	FFAAS0365	China	_	OL985989	_	Na et al. (2022a)
44	Gerronema microcarpum	FFAAS0371	China	OL985968	OL985990	-	Na et al. (2022a)
45	Gerronema microcarpum	FFAAS0372	China	OL985969	OL985991	-	Na et al. (2022a)

Table 1. Specimens used in phylogenetic analysis, with geographic origin and GenBank accession numbers.

No.	Таха	Voucher	Locality	ITS Sequence ID	LSU Sequence ID	rpb2 Sequence ID	Reference
46	Gerronema microcarpum	FFAAS0373 Holotype	China	OL985970	OL985992	-	Na et al. (2022a)
47	Gerronema microcarpum	FFAAS0374	China	OL985971	-	-	Na et al. (2022a)
48	Gerronema microcarpum	FFAAS0375	China	OL985972	OL985993	-	Na et al. (2022a)
49	Gerronema nemorale	KACC 43599	Korea	EU883592	-	-	Unpublished
50	Gerronema nemorale	KACC 43600	Korea	EU883593	-	-	Unpublished
51	Gerronema nemorale	not indicated	Korea	EU883594	-	-	Unpublished
52	Gerronema nemorale	FA249	Pakistan	MN744686	-	-	Aqdus and Khalid (2021)
53	Gerronema nemorale	FA236	Pakistan	MN744687	-	-	Aqdus and Khalid (2021)
54	Gerronema nemorale	FA239	Pakistan	MN744688	-	-	Aqdus and Khalid (2021)
55	Gerronema strombodes	DJL05NC72	USA	EU623639	_	_	Hughes et al. (2007)
56	Gerronema strombodes	TFB12519/TENN60718	USA	EU623640	_	-	Hughes et al. (2007)
57	Gerronema strombodes	TFB12783/TENN61350	USA	EU623641	_	-	Hughes et al. (2007)
58	Gerronema strombodes	TFB11947 clone C2	USA	KY242503	_	-	Hughes et al. (2007)
59	Gerronema strombodes	TFB11947 clone C3	USA	KY242504	_	-	Hughes et al. (2007)
60	Gerronema strombodes	TFB11947 clone C5	USA	KY242506	_	-	Hughes et al. (2007)
61	Gerronema strombodes	TFB14234	USA	KY242507	_	-	Hughes et al. (2007)
62	Gerronema strombodes	TFB14514	USA	KY242509	_	-	Hughes et al. (2007)
63	Gerronema strombodes	TFB11947	USA	KY271083	-	-	from GenBank
64	Gerronema subclavatum	Redhead 5175, DAOM	not indicated	U66434	_	_	Lutzoni (1997)
65	Gerronema subclavatum	FLAS-F-60986	USA	MH016932	_	_	from GenBank
66	Gerronema subclavatum	FLAS-F-61518	USA	MH211945	_	_	from GenBank
67	Gerronema subclavatum	Smith-2018	USA	MK573888	_	_	Direct Submission
68	Gerronema subclavatum	Mushroom Observer # 243440	USA	MK607510	_	_	Direct Submission
69	Gerronema subclavatum	iNaturalist # 8545787	India	MN906021	_	_	from GenBank
70	Gerronema subclavatum	S.D. Russell MycoMap # 6854	India	MN906138	_	_	from GenBank
71	Gerronema waikanaense	PDD:87667	New Zealand	JQ694117	_	_	from GenBank
72	Gerronema wildpretii	BRNM 788347	Madeira	LT854045	LT854043	_	Antonin et al. (2019)
73	Gerronema xanthophyllum	PRM 924657	Czech Republic	LT854023	LT854023	_	Antonin et al. (2019)
74	Gerronema zhujian	FFAAS0364	China	OL985973	OL985994	-	Na et al. (2022a)
75	Gerronema zhujian	FFAAS0370	China	OL985974	OL985995	_	Na et al. (2022a)
76	Gerronema zhujian	FFAAS0376 Holotype	China	OL985975	OL985996	_	Na et al. (2022a)
77	Hydropodia subalpina (≡Hydropus subalpinus)	STU:SMNS-STU-F-0900123	Germany	MF039248	-	_	Eberhardt et al. (2018)
78	Hydropodia subalpina (≡Hydropus subalpinus)	Montri-291	not indicated	MK028414	-	-	Unpublished
79	Hydropodia subalpina (≡Hydropus subalpinus)	Montri-312	not indicated	MK028415	_	_	Unpublished
80	Hydropodia subalpina (≡Hydropus subalpinus)	Montri-323	not indicated	MK028416	-	-	Unpublished
81	Hydropodia subalpina (≡Hydropus subalpinus)	OKA-TR-K364	Turkey	MN701620	MN700170	-	Unpublished
82	Hydropodia subalpina (≡Hydropus subalpinus)	OKA-TR-K380	Turkey	MN701621	MN700171	-	Unpublished
83	Hydropodia subalpina (≡Hydropus subalpinus)	OKA-TR-B400	Turkey	MN701622	MN700172	-	Unpublished
84	Hydropus atramentosus	918	Italy	JF908050	-	-	Osmundson et al. (2013)
85	Hydropus fuliginarius	S.D. Russell ONT iNaturalist # 130794969	USA	OP643427	_	-	Unpublished
86	Hydropus fuliginarius	DAOM196062	USA	-	AF261368	-	Moncalvo et al. (2002)
87	Hydropus marginellus	AFTOL-ID 1720	not indicated	DQ490627	DQ457674	DQ472722	Matheny et al. (2006)
88	Hydropus marginellus	OSC 112834	USA	EU669314	EU852808	-	Unpublished
89	Hydropus rugosodiscus	MGW1257	USA	KY777386	-	-	Unpublished
90	Hydropus rugosodiscus	PBM4022	USA	KY777390	-	-	Unpublished
91	Hydropus rugosodiscus	Taxon 10	not indicated	MW399385	-	-	Unpublished

No.	Таха	Voucher	Locality	ITS Sequence ID	LSU Sequence ID	rpb2 Sequence ID	Reference
92	Leucoinocybe auricoma (≡Mycena auricoma)	HKAS126433	China	OQ025169	_	-	Direct Submission
93	Leucoinocybe auricoma (≡Mycena auricoma)	AFTOL-ID 1341 (specimen_ voucher HKAS41510)	China	DQ490647	-	-	Matheny et al. (2006)
94	Leucoinocybe danxiashanensis	GDGM79543	China	MZ667475	MZ667479	-	Unpublished
95	Leucoinocybe danxiashanensis	GDGM80113	China	MZ667476	MZ667480	-	Unpublished
96	Leucoinocybe danxiashanensis	GDGM80114	China	MZ667477	MZ667481	-	Unpublished
97	Leucoinocybe danxiashanensis	GDGM80184	China	MZ667478	MZ667482	-	Unpublished
98	Leucoinocybe flavoaurantia	D	Italy	HM191743	_	_	Malysheva and Morozova (2011)
99	Leucoinocybe flavoaurantia	GDOR	Italy	HM191744	_	_	Malysheva and Morozova (2011)
100	Leucoinocybe flavoaurantia	LE 262757	Russia	HM191745	_	_	Malysheva and Morozova (2011)
101	Leucoinocybe lenta	BOZ (EPITYPE)	Italy	-	LT854032	-	Antonín et al. (2019)
102	Leucoinocybe lishuiensis	FFAAS 0111 (HOLOTYPE)	China	MW424488	MW424492	-	Na et al. (2021)
103	Leucoinocybe lishuiensis	FFAAS 0112	China	MW424489	MW424493	-	Na et al. (2021)
104	Leucoinocybe lishuiensis	FFAAS 0113	China	MW424490	MW424494	-	Na et al. (2021)
105	Leucoinocybe lishuiensis	FFAAS 0115	China	MW424491	MW424495	-	Na et al. (2021)
106	Leucoinocybe sp.	KA12-0435	South Korea	KR673482	-	-	Kim et al. (2015)
107	Leucoinocybe subglobispora	FFAAS1034 Holotype	China	OR238886	OR238898	OR258379	This study
108	Leucoinocybe subglobispora	FFAAS1035	China	OR238887	OR238899	OR258380	This study
109	Leucoinocybe sulcata	CAL 1246 (HOLOTYPE)	India	KR029720	KR029721	-	Latha et al. (2015)
110	Leucoinocybe taniae	BCN-SCM B-4064	Italy	LT854057	LT854028	-	Antonín et al. (2019)
111	Marasmiellomycena albodescendens	PDD 96142	New Zealand	OL998341	OL998380	-	Consiglio et al. (2022)
112	Marasmiellomycena albodescendens	PDD 96321	New Zealand	OL998343	OL998382	-	Consiglio et al. (2022)
113	Marasmiellomycena omphaliiforme (≡Porotheleum omphaliiforme)	WU 16775	Italy	OM422777	OM423654	-	Direct Submission
114	Marasmiellomycena omphaliiforme (≡Porotheleum omphaliiforme)	LIP 0401689	France	OM422780	OM423655	-	Direct Submission
115	Marasmiellomycena omphaliiforme (≡Porotheleum omphaliiforme)	AMB 18850	France	OM422781	OM423656	-	Direct Submission
116	Marasmiellomycena omphaliiforme (≡Porotheleum omphaliiforme)	AMB 18845	France	OM422782	_	-	Direct Submission
117	Marasmiellomycena pseudoomphaliiformis	BRNM:552721	USA	OR913562	OR913566	-	Senanayake et al. (2023)
118	Marasmiellomycena pseudoomphaliiformis	BRNM:552654	USA	OR913560	OR913564	-	Senanayake et al. (2023)
119	Marasmiellomycena pseudoomphaliiformis	BRNM:552658	USA	OR913561	OR913565	-	Senanayake et al. (2023)
120	Marasmiellomycena tomentosa	FFAAS1036 Holotype	China	OR238888	OR238900	OR258381	This study
121	Marasmiellomycena tomentosa	FFAAS1037	China	OR238889	OR238901	OR258382	This study
122	Marasmiellomycena tomentosa	FFAAS1038	China	OR238890	OR238902	OR258383	This study
123	Megacollybia clitocyboidea	TFB11884/TENN60766	USA	EU623658	-	-	Hughes et al. (2007)
124	Megacollybia clitocyboidea	TENN62231	USA	EU623664	-	-	Hughes et al. (2007)
125	Megacollybia clitocyboidea	TENN62230 clone c4	USA	EU623673	_	-	Hughes et al. (2007)
126	Megacollybia clitocyboidea	TENN62230 clone c5	USA	EU623674	-	-	Hughes et al. (2007)
127	Megacollybia fallax	MICH 45002	USA	EU623714	-	-	Hughes et al. (2007)
128	Megacollybia fallax	TFB11561/TENN59447	USA	EU623723	-	-	Hughes et al. (2007)
129	Megacollybia fallax	DAOM208710	USA	EU623724	-	-	Hughes et al. (2007)
130	Megacollybia fallax	Mushroom Observer 291302	USA	MN176984	-	-	Direct Submission

No.	Таха	Voucher	Locality	ITS Sequence ID	LSU Soguenee ID	rpb2	Reference
121	Magaaalluhia fallay	Mushroom Observer 296902	1167	MT427075	Sequence in	Sequence in	Direct Submission
131	Megacollybia marginata	PRM 860926	Czech Republic	1T854022	_	_	Antonín et al. (2019)
133	Megacollybia marginata	PRM 859785	Czech Republic	17854046	1T854042	_	Antonín et al. (2019)
134	Megacollybia marginata	HR 91642	Czech Republic	1T854050	_	_	Antonín et al. (2019)
135	Megacollybia marginata	HR 91607	Czech Republic	1T854051	_	_	Antonín et al. (2019)
136	Megacollybia nlatynhylla	AFTOL-ID 560	LISA	D0249275	AY635778	D0385887	
137	Megacollybia platyphylla	BRNM 737654	Czech Republic	17854048	1T854036	_	Antonín et al. (2019)
138	Megacollybia platyphylla	BRNM 766072	Czech Republic	1785/0/0	1785/037	_	Antonín et al. (2019)
1/1	Megacollybia platyphylia	BH(100 700 72		CO207090			from ConBank
1/10	Megacollybia rodmani	DI II 527030		MW///8576	_	_	from GenBank
149	Megacollybia subfurfuracea	TEB11075/TENN50558 clone c3		FU623744	_	_	Hughes et al. (2007)
150	Megacollybia subfurfuracea	TEB11075/TENN59558 clone c3		EU622744	_		Hughes et al. (2007)
150	Megacollybia subruriuracea	DDI 7405/TENIN62059 alana a1	USA	EU622745			Hughes et al. (2007)
152	Megacollybia texensis	DPL7403/TENN02038 clone c1	USA	EU023723			Hughes et al. (2007)
153		DPL/403/TEININ02038 CIONE C2	USA	EU023720	_	_	from ConBonk
154	Megacollybla texensis	FLAS-F-01511	USA	MH211940	-	-	Trom GenBank
155	Mycena purpureofusca	HMJAU 43554	China	MG654740	MK629356	-	Na and Bau (2018)
156	Mycena purpureofusca	HMJAU 43624	China	MG654/41	MK629357	-	Na and Bau (2018)
157	Mycena purpureofusca	HMJAU 43640	China	MG654/42	MK629358	-	Na and Bau (2018)
158	Porotheleum fimbriatum	Dai 12276	China	KX081137	KX161656	-	from GenBank
159	Porotheleum fimbriatum	Dai 12289	China	KX081138	KX161654	-	from GenBank
160	Porotheleum fimbriatum	CLZhao 1120	China	MH114870	-	-	from GenBank
161	Porotheleum fimbriatum	CLZhao 2368	China	MH114871	-	-	from GenBank
162	Porotheleum fimbriatum	SWFC 006350	China	MK894078	-	-	from GenBank
163	Porotheleum fimbriatum	SWFC 006399	China	MK894079	-	-	from GenBank
164	Porotheleum parvulum	JBSD131802 Type	Dominican Republic	NR_182714	OM423657	-	Consiglio et al. (2022)
165	Pseudohydropus floccipes	AMB 18768	Spain	-	OM423637	-	Consiglio et al. (2022)
166	Pseudohydropus floccipes	BRNM 825631	Spain	OM422760	OM423636	-	Consiglio et al. (2022)
167	Pseudohydropus floccipes	BRNM 751633	Spain	OM422759	OM423635	-	Consiglio et al. (2022)
168	Pseudohydropus globosporus	BAP 661 (Holotype, SFSU)	USA	OM422758	OM423634	-	Cooper et al. (2019)
169	Pseudohydropus sp	MushroomObserver490861	Jamaica	OR879917	-	-	Direct Submission
170	Pulverulina flavoalba	FFAAS1039 Holotype	China	OR238891	OR238903	OR258384	This study
171	Pulverulina flavoalba	FFAAS1040	China	OR238892	OR238904	OR258385	This study
172	Pulverulina ulmicola	TENN 029208 Holotype	USA	NR_119887	HQ179668	-	Matheny et al. (2020)
173	Pulverulina ulmicola	TFB13871	USA	MT237476	MT237446	-	Matheny et al. (2020)
174	Pulverulina ulmicola	KUBOT-KRMK-2020-13	India	MW425325	MW425344	-	Unpublished
175	Trogia benghalensis	CUH AM031	India	KU647630	_	-	Dutta et al. (2017)
176	Trogia benghalensis	CUH AM122	India	MF967246	-	-	Dutta et al. (2017)
177	Trogia infundibuliformis	KUN_HKAS63661	China	JQ031775	JQ031780	_	Yang et al. (2012)
178	Trogia infundibuliformis	KUN_HKAS56709	China	JQ031776	JQ031781	_	Yang et al. (2012)
179	Trogia venenata	KUN_HKAS54710	China	JQ031772	JQ031778	-	Yang et al. (2012)
180	Trogia venenata	KUN_HKAS56679	China	JQ031773	JQ031779	_	Yang et al. (2012)
181	- Trogia venenata	TC2-28	China	KT968080	-	_	Mi et al. (2016)
182	Trogia venenata	MHHNU 8750	China	KX268227	_	_	Unpublished
183	Vizzinia domingensis	JBSD131801a	Dominican	OM422768	OM423646	_	Consiglio et al (2022)
104	(≡Porotheleum domingense)		Republic	OM422771	OM422640		Consiglio et al. (2022)
104	(=Porotheleum nigripes)	JEJU 131803	Republic	0101422771	0101423048	-	
Note: Newly-generated sequences are in bold.							

first 25% of trees were discarded as burn-in (Ronquist and Huelsenbeck 2003). The ML analysis was performed in RAxML GUI 2.0 using a rapid bootstrapping algorithm involving 1,000 replicates (Edler et al. 2021). The aligned datasets for Bayesian and ML analyses have been deposited in TreeBASE (submission ID 31062; study accession URL: http://purl.org/phylo/treebase/phylows/study/TB2:S31062). Phylogenetic trees were displayed using FigTree v.1.4.3.

Results

Phylogenetic analysis

A data matrix was created for 59 taxa, including 58 taxa of Porotheleaceae and, as an outgroup, one taxon of *Mycena*. Including gaps, the aligned dataset comprised 2,274 nucleotide sites: 974 for ITS, 610 for nrLSU and 690 for *rpb2* exons (all sites without introns). For the ML analysis, the best-fit substitution models selected for ITS, nrLSU and *rpb2*-exon partitions in the concatenated dataset were TPM2uf+I+G4, GTR +I+G4 and TIM2+I+G4, respectively. For the BI analysis, the best-fit substitution model selected for each of the three DNA regions (ITS, nrLSU and *rbp2* exons) was GTR+I+G. Phylogenetic reconstructions, based on BI and ML methods, yielded similar topologies. The BI topology was, therefore, selected as a representative phylogeny (Fig. 1).

In the tree shown in Fig. 1, 21 major well-supported clades are evident: Chrysomycena, Clitocybula, Delicatula, Hydropodia, Hydropus, Leucoinocybe, Marasmiellomycena, Megacollybia, Pulverulina, Pseudohydropus, Trogia and Vizzinia, all of which form monophyletic groups at the generic level. However, within Porotheleum, two species, totalling three specimens, form two unassigned clades. In addition, Marasmiellomycena, forms a well-supported (MLB = 81%; BPP = 0.90) independent clade comprising four species distinct from Vizzinia and the unassigned Porotheleum. In the phylogenetic tree, Chrysomycena, Gerronema, Hydropus, Leucoinocybe, Megacollybia, Trogia and five taxa of Clitocybula cluster together with high statistical support (MLB = 96%; BPP = 1.00), but one sequence of *Delicatula* appears outside this large clade in the Maximum Likelihood analysis. The variation in the phylogenetic analysis outcomes for Delicatula specimens can be ascribed to inconsistent sequence coverage. Of the three Delicatula specimens evaluated, two only contained ITS sequences clustered together into a clade (MLB = 99%; BPP = 0.68), suggesting some degree of relatedness. In contrast, the remaining specimen, which only included an LSU sequence, was placed differently across the analyses. Such disparities in sequence coverage are likely to be responsible for the observed discrepancies between different computational algorithms used in the phylogenetic reconstructions. Hydropodia and Pulverulinaform a large, poorly supported clade. Moreover, Hydropus (MLB = 100%; BPP = 1.00), Leucoinocybe (MLB = 99%; BPP = 1.00) and Clitocybula (MLB = 82%; BPP = 1.00) are strongly supported as distinct genera and collectively constitute a distinct clade separate from all other clades. However, Gerronema is polyphyletic (Gerronema 1 to Gerronema 6), with each individual Gerronema clade sister to Megacollybia or Trogia. Finally, Chrysomycena and Hydropodia comprise a single species each.

In the phylogenetic tree, samples of the new species and new combination are placed in *Marasmiellomycena*, where they constitute monophyletic lineag-

es, each with high statistical support (M. albodescendens: MLB = 93%, BPP = 1.00; M. tomentosa: MLB = 100%, BPP = 1.00). The four other new species are strongly supported as members of Gerronema 3, Pulverulina, Leucoinocybe and Clitocybula clades (C. fuscostriata: MLB = 92%, BPP = 1.00; G. brunneosquamulosum: MLB = 100%, BPP = 1.00; L. subglobispora: MLB = 100%, BPP = 1.00; and Pulverulina flavoalba: MLB = 100%, BPP = 1.00). Marasmiellomycena tomentosa is closely related to a clade containing two species and a new combination, M. albodescendens, M. omphaliiforme and M. pseudoomphaliiformis. Pulverulina flavoalba sp. nov. is grouped with high statistical support (MLB = 100%; BPP = 1.00) with three sequences of *Pulverulina ulmicola* (H.E. Bigelow) Matheny & K.W. Hughes from India and the USA (including holotype voucher no. TENN 029208). Within the Leucoinocybe clade, L. subglobispora constitutes a monophyletic lineage that is most closely related to Leucoinocybe lishuiensis, a new species recently described from China (Na et al. 2021). Clitocybula fuscostriata is placed along with C. lacerata (Scop.) Métrod in an unresolved lineage that is treated as C. lacerata agg. by Antonín et al. (2019) and in our studies.

Clitocybula albida A.K. Dutta, K. Acharya & Antonín, reported from India as a new species, was transferred to Porotheleum [as Porotheleum albidum (A.K. Dutta, K. Acharya & Antonín) Vizzini & Consiglio] and Porotheleum parvulum Angelini, Vizzini, Consiglio & M. Marchetti as a new species from the Dominican Republic (Dutta et al. 2018; Consiglio et al. 2022). The phylogenetic status of Clitocybula albida is currently unclear and treated as unassigned clades in the study of Senanayake et al. (2023). On the other hand, Porotheleum parvulum is known to cluster with Marasmiellomycena and Vizzinia, forming a clade. Within this clade, Porotheleum parvulum is specifically determined to be a sister group to Marasmiellomycena. In the research conducted by Senanayake et al. (2023), Pseudohydropus and Pulverulina were identified as forming a monophyletic group. Contrastingly, in our phylogenetic tree, Pseudohydropus emerges as an independent lineage, receiving robust support (MLB =100%; BPP = 1.00) and not aligning as a sister group with any other genera. The observed differences might stem from variances in sequence coverage and the evolutionary rates of the genes. While Senanayake et al. (2023) utilised ITS and LSU sequences for their phylogenetic construction, our study encompassed ITS, LSU and RPB2 in the combined phylogenetic analysis. (Fig. 1).

Taxonomy

Clitocybula fuscostriata Q.Na & Y.P.Ge, sp. nov.

MycoBank No: 849407 Figs 2-4

Diagnosis. Pileus with dark-brown striae. Differs from *C. striata* in having broader basidiospores and lacking hymenial cystidia.

Holotype. CHINA. Zhejiang Province: Baiyun National Forest Park, Liandu District, Lishui City, 2 Aug 2021, Qin Na, Yupeng Ge, Zewei Liu, Yaping Hu, Changjing Liu and Hui Ding, *FFAAS1030* (collection number MY0460).

Etymology. Name refers to the pileus with radially fuscous striae.

Description. Pileus 3.0-28.5 mm in diameter, hemispherical at first, then convex with depressed centre, expanded with age, infundibuliform with



Figure 1. Phylogenetic consensus tree inferred from the Maximum Likelihood (ML) analysis based on a concatenated ITS, nrLSU and *rpb2* dataset (MLB \geq 75%, BPP \geq 0.90 are indicated). The tree is rooted with *Mycena purpureofusca*. The new species and combination are marked by red.



deeply umbilicate at the centre when old, thin-fleshed, dry, surface innately radially Fuscous (XLVI13^{III}k) to Fuscous-Black (XLVI13^{III}m) striate, surface somewhat fibrillose, becoming glabrous, radially cracked at margin when old, Benzo Brown (XLVI13^{III}), Hair Brown (XLVI17^{III}), Fuscous (XLVI13^{III}k) to Fuscous-Black (XLVI13^{IIII}m) at the centre, Pale Smoke Grey (XLVI21^{III}f) in the margin when young, Pale Smoke Grey (XLVI21^{III}f) to Smoke Grey (XLVI21^{IIII}d) with Bone Brown (XL13^{III}m) at the centre when old. Context thin, white, fragile. Lamellae subdecurrent, white, with 1–3 tiers of lamellulae, irregularly intervenose, edges concolorous with the face. Stipe 17.0–52.0 × 1.0–2.5 mm, hollow, cylindrical, strongly and coarsely grooved, slightly bulbous at the base, fragile, finely whitish fibrillose, white in the upper part, Citrine Drab (XL21^{III}) in the base, base covered with a few white fibrils. Odour and taste inconspicuous.

Basidiospores (80/4/3) (5.2) 5.4-5.8-6.2 (6.5) × (4.2) 4.3-4.7-5.0 (5.1) µm [Q = 1.13-1.34, Q = 1.25 ± 0.050] [holotype (40/2/1) (5.3) 5.5-5.8-6.2 (6.5) × (4.2) 4.4-4.6-5.0 (5.1) µm, Q = 1.17-1.32, Q = 1.26 ± 0.040], broadly ellipsoid, hyaline in 5% KOH, smooth, thin-walled, guttulate, amyloid. Basidia $22-32 \times 5-9$ µm, 2- or 4-spored, clavate, sterigmata $2.5-4.7 \times 0.6-1.6$ µm. Hymenial cystidia absent. Lamellae edge cells scattered, cylindrical, narrowly clavate, thin-walled. Lamellar trama subregular; hyphae 3-7 µm wide, thin-walled, hy-aline, non-dextrinoid. Pileipellis hyphae 4-9 µm wide, smooth; pileocystidia $70-162 \times 7-19$ µm, cylindrical or narrowly clavate, apically obtuse, thin-walled, hy-aline, smooth. Stipitipellis a cutis made up of 3-8 µm wide hyphae, smooth, thin-walled; caulocystidia $27-63 \times 5-8$ µm, cylindrical, clavate, fusoid, apically obtuse, thin-walled base, smooth, transparent. Clamps present in all tissues.

Habit and habitat. Scattered on rotten branches or twigs in Acer, Armeniaca, Cercidiphyllum, Emmenopterys and Picea mixed forests.

Known distribution. Zhejiang Province, China.

Additional material examined. CHINA. Zhejiang Province: Baiyun National Forest Park, Liandu District, Lishui City, 2 Aug 2021, Qin Na, Yupeng Ge, Hui Zeng and Yulan Sun, *FFAAS1029* (collection number MY0459); Zhejiang Province: Baiyun National Forest Park, Liandu District, Lishui City, 2 Aug 2021, Qin Na, Yupeng Ge, Zewei Liu, Yaping Hu, Changjing Liu and Hui Ding, *FFAAS1031* (collection number MY0466).

Notes. *Clitocybula fuscostriata* is considered to be a distinct species in the genus on account of its pileus with dark-brown striae, broadly ellipsoid basidio-spores, absence of cheilocystidia and pleurocystidia and thin-walled pileipellis and stipitipellis hyphae. Five recorded species morphologically resemble this new species: *C. familia* (Peck) Singer, *C. lacerata* (Scop.) Métrod, *C. oculata* (Murrill) H.E. Bigelow, *C. striata* Dähncke, Contu & Vizzini and *C. tilieti* (Singer) Singer (Singer 1943; Romagnesi 1968; Bigelow 1973; Lennox 1979; Ludwig 2000, 2001; Dähncke et al. 2010; Antonín et al. 2011). *Clitocybula striata*, a new taxon reported from Spain, has certain morphological similarities to *C. fuscostriata*, namely, a grey-brown to brown pileus with dark-brown striae, but differs from *C. striata* in having ellipsoid basidiospores ($5-7 \times 3.5-4.8 \mum$; Q = 1.5) and presence of utriform or lageniform cheilocystidia (Dähncke et al. 2010). In contrast to *C. fuscostriata*, *C. tilieti* can be easily mistaken for *C. striata*, but the pileus of *C. tilieti* is distinctly viscid and its stipitipellis and caulo-



Figure 2. Basidiomata of *Clitocybula fuscostriata* **A**–**D** collection *FFAAS1029* **E**–**F** collection *FFAAS1030*, holotype **G**–**H** collection *FFAAS1031*. Scale bars: 10 mm (**A**–**H**).



Figure 3. Morphological features of *Clitocybula fuscostriata* (*FFAAS1030*, holotype) **A** basidiomata **B** basidiospores **C** basidia **D** caulocystidia **E** pileipellis and pileocystidia. Scale bars: 10 mm (**A**); 5 μ m (**B**); 10 μ m (**C**–**E**).



Figure 4. Microscopic features of *Clitocybula fuscostriata* (*FFAAS1030*, holotype) **A–E** basidiospores **F** basidia **G** margin of lamellae **H** lamellar trama I pileipellis and pileocystidia **J** caulocystidia. Scale bars: 5 μ m (**A–E**); 10 μ m (**F–J**). Structures were stained with 1% Congo Red aqueous solution before photographing.

cystidia are thick-walled (Singer 1943; Antonín et al. 2011). *Clitocybula lacerata* (Scop.) Métrod, the type species of *Clitocybula*, is characterised by its caespitose stipes, beige-grey to pale-grey brown pileus, presence of clavate cheilocystidia and a pileipellis with pale encrusting pigmentation, differentiating it from *C. fuscostriata* (Peck 1878; Breitenbach and Kranzlin 1991; Ludwig 2000, 2001; Antonín et al. 2019). *Clitocybula oculata* (Murrill) H.E. Bigelow and *C. familia* resemble *C. fuscostriata* in colour and size of the pileus and stipe, but can be distinguished from the new species by the size and shape of the basidiospores [*C. oculata* basidiospores $(8.5-)10-12(-13) \times 6-9 \mu m$, broadly ellipsoid or ovate; *C. familia* basidiospores $3.5-5.3(-5.5) \times 3.5-5.0 \mu m$, globose, subglobose to broadly ellipsoid] (Romagnesi 1968; Bigelow 1973; Lennox 1979; Ludwig 2000, 2001; Antonín et al. 2011).

Gerronema brunneosquamulosum Q.Na & Y.P.Ge, sp. nov.

MycoBank No: 849408 Figs 5–7

Diagnosis. Differs from *G. zhujian* in having a fuscous stipe densely covered with deep-brown pubescence or scales and by the presence of large basidiospores.

Holotype. CHINA. Zhejiang Province: Baiyun National Forest Park, Liandu District, Lishui City, 2 Aug 2021, Qin Na, Yupeng Ge, and Hui Zeng, *FFAAS1032* (collection number MY0481).

Etymology. Name refers to the pileus and stipe covered with dark-brown scales.

Description. Pileus 4.5–42.0 mm in diam., applanate and centrally depressed, subumbilicate to umbilicate when young, concave to deeply infundibulate with age, pellucid-striate or sulcate, always \pm distinctly radially striped with darkened lines, Buffy Brown (XL17"k) at the centre, Olive Buff (XL21"d) in margin when young, Olive Brown (XL17"k), Clove Brown (XL17"m), Light Greyish-Olive (XLVI21""b) in margin with age, densely covered with tiny, Warm Black-ish-Brown (XXXIX1"m) granules, pubescence or scales, slightly sparse with age, dry, lustreless, with a slightly involuted margin. Context white, thin, tough. Lamellae narrowly adnexed to subdecurrent, moderately broad, pure white, edges concolorous with the sides. Stipe $6.0-32.0 \times 1.5-2.0$ mm, central, cylindrical, almost equal above, white, densely covered with Warm Blackish-Brown (XXXIX1"m) scales, hollow, base Light Seal Brown (XXXIX9"m), slightly swollen with tiny, inconspicuous fine white hairs. Odourless. Taste mild.

Basidiospores [60/3/2] (9.0) 9.2–10.0–11.2 (12.9) × (4.9) 5.2–5.8–6.6 (7.2) μ m [Q = 1.54–1.91, Q = 1.73 ± 0.097] [holotype [40/2/1] (9.0) 9.2–10.2–11.2 (12.9) × (5.3) 5.5–5.9–6.5 (7.2) μ m, Q = 1.54–1.90, Q = 1.71 ± 0.086], ellipsoid to narrowly ellipsoid, hyaline, guttulate, thin-walled, inamyloid. Basidia 22–39 × 7–9 μ m, hyaline, clavate, 2- or 4-spored, sterigmata 2.3–6.0 × 0.8–2.2 μ m. Cheilocystidia 23–59 × 6–9 μ m, subfusiform, clavate, apex usually swollen, hyaline. Pleurocystidia absent. Lamellar trama subregular; hyphae 2–7 μ m wide, thin-walled, hyaline, inamyloid. Pileus trama subregular, sarcodimitic. Pileipellis hyphae 3–7 μ m wide, a cutis, light yellow (2B2); terminal elements clavate or utriform with rounded apex, 53–95 × 7–16 μ m, Dark Citrine (IV21m), Olive Brown (XL17"k) to Clove Brown (XL17"k) pigmented; true pileocystidia absent.



Figure 5. Basidiomata of *Gerronema brunneosquamulosum* **A**, **B** *FFAAS1032*, holotype **C**, **D** collection *FFAAS1033* **E**, **F** pileus with granules, fur or scales **G**, **H** stipe covered with dark brown scales. Scale bars: 10 mm (A–E); 5 mm (F–H).



Figure 6. Morphological features of *Gerronema brunneosquamulosum (FFAAS1032*, holotype) **A** basidiomata **B** basidia **C** basidiospores **D** cheilocystidia **E** caulocystidia **F** pileipellis. Scale bars: 10 mm (**A**); 10 µm (**B**–**F**).



Figure 7. Microscopic features of *Gerronema brunneosquamulosum* (*FFAAS1032*, holotype) **A**–**E** basidiospores **F** basidia **G**–**J** cheilocystidia **K** lamellar trama **L** pileipellis and pileocystidia **M** caulocystidia. Scale bars: 5 μm (**A**–**E**); 10 μm (structures **A**–**K**, **M** were stained with 1% Congo Red aqueous solution and **L** in 5% KOH aqueous solution before photographing).

Hyphae of the stipitipellis $5-11 \mu m$ wide, hyaline, smooth; caulocystidia long cylindrical, sometimes with rounded apex, $40-76 \times 5-12 \mu m$, hyaline, thin-walled. All tissues non-reactive in iodine. Clamps present in all tissues.

Habit and habitat. Solitary to scattered on rotten wood, branches and twigs in Acer, Ginkgo, Liriodendron, Picea and Tsuga.

Known distribution. Fujian Province, Zhejiang Province, China.

Additional material examined. CHINA. Fujian Province: Wuyi Mountain, Nanping City, 13 Aug 2021, Qin Na, Yupeng Ge, Junqing Yan, Hui Zeng, and Zewei Liu, *FFAAS1033* (collection number MY0571).

Notes. Gerronema brunneosquamulosum is unique amongst members of Gerronema on account of its fuscous pileus and stipe with dark-brown to blackish-brown pubescence or scales, larger basidiospores and a dark-pigmented pileipellis. Gerronema zhujian, reported from Anhui and Fujian Provinces in China, is the most closely allied congener of G. zhujian on the basis of the brown colouration of the umbilicus of its pileus, its whitish stipe and similarly-shaped cheilocystidia and terminal elements of the pileipellis (Na et al. 2022a). This taxon differs from G. brunneosquamulosum in having a pruinose white stipe, subdecurrent to decurrent lamellae and possessing smaller basidiospores (Na et al. 2022a). Two species of Omphalina Quél., characterised by dark pigments in the pileus, have been described from Argentina-Omphalina depauperata (Singer) Raithelh. and O. subpallida (Singer) Raithelh., formerly named Gerronema subpallidum Singer and G. depauperatum Singer, respectively. These two species most closely resemble G. brunneosquamulosum, but differ in having an unornamented stipe, ellipsoid basidiospores and no cheilocystidia (Singer 1970). Other species of Gerronema, such as G. nemorale and G. strombodes, are well characterised with a distinctly yellow, yellowish-orange, olive-yellow to yellowish-brown pileus and their micromorphological features are also different (Singer 1970; Antonín et al. 2008; Latha et al. 2018). Species of Trogia, especially Trogia fulvochracea Corner (p.31) and Trogia mycenoides (p.53) Corner, share some similarities with the new taxon (Corner 1991). Trogia fulvochracea, however, has a fulvous or cinnamon-ochraceous pileus, a smooth white stipe and smaller basidiospores (7–9.5 × 4.5–6.0 μ m). Trogia mycenoides differs in having a smaller pileus (5-30 mm in diam.), ellipsoid basidiospores and clavate to subglobose cheilocystidia; in addition, true pileocystidia are present, but are soon evanescent (Corner 1991).

Leucoinocybe subglobispora Q.Na & Y.P.Ge, sp. nov.

MycoBank No: 849409 Figs 8–10

Diagnosis. Pileus dark brown. Basidiospores subglobose to broadly ellipsoid. Pileocystidia and caulocystidia thick-walled. Differs from *L. lishuiensis* in having broader basidiospores.

Holotype. CHINA. Zhejiang Province: Tianmu Mountain, Hangzhou City, 1 Aug 2021, Qin Na, Yupeng Ge, Zewei Liu and Yulan Sun, *FFAAS1034* (collection number MY0444).

Etymology. Name refers to the subglobose to broadly ellipsoid basidiospores. **Description.** Pileus 2.5–8.0 mm in diameter, hemispherical or campanulate when young, becoming campanulate with age, umbilicate at the centre, sulcate, finely granulose all over, Dark Livid Brown (XXXIX1^{""}k), Benzo Brown (XLVI13^{""}i) to Fuscous (XLVI13^{""}k) at the centre, Pale Smoke Grey (XLVI21^{""}f) in the margin, uplifted or recurved at the margin and sometimes rimose in age, dry. Context white, thin, fragile. Lamellae adnexed to slightly subdecurrent, white, with 1–2 tiers of lamellulae, edges concolorous with the face. Stipe 9.5–14.0 × 1.0–1.5 mm, equal or slightly broadened at the base, hollow, fragile, white, sometimes inconspicuous Pale Olive-Buff (XL21^{""}d) at the base, densely pruinose, but sparsely with age, base covered with small white fibrils. Odour and taste indistinctive.

Basidiospores (60/3/2) (5.6) 5.8-6.4-7.1 (7.5) × (4.8) 5.0-5.6-6.5 (6.8) µm [Q = 1.06-1.27, Q = 1.16 ± 0.054] [holotype (40/2/1) (5.7) 5.9-6.5-7.2 (7.5) × (4.9) 5.0-5.5-6.5 (6.8) µm, Q = 1.07-1.27, Q = 1.18 ± 0.052], subglobose to broadly ellipsoid, hyaline in 5% KOH, smooth, thin-walled, guttulate, amyloid. Basidia $28-37 \times 7-9$ µm, 4-spored, clavate, sterigmata $1.4-2.7 \times 0.8-1.7$ µm. Cheilocystidia $28-62 \times 9-15$ µm, distinct, flexuose, narrowly utriform, fusoid or lageniform, subcapitate, thin-walled, hyaline. Pleurocystidia absent. Lamellae trama subregular; hyphae 2-6 µm wide, thin-walled, hyaline, amyloid. Pileipellis hyphae 2-8 µm wide, smooth; pileocystidia $62-116 \times 10-19$ µm, lageniform, subulate, apically obtuse, distinctly 0.8-1.8 µm thick-walled, with a thin-walled base, hyaline, smooth. Stipitipellis a cutis made up of 3-9 µm wide hyphae, smooth, thin-walled; caulocystidia $34-62 \times 5-10$ µm, subulate, fusoid, lageniform, sometimes clavate, always thick-walled in the middle part and with a thin-walled base, smooth, transparent. Clamps present in all tissues.

Habit and habitat. Solitary or scattered on rotten wood or branches in Acer, Armeniaca, Cercidiphyllum, Emmenopterys and Picea mixed forests.

Known distribution. Zhejiang Province, China.

Additional material examined. CHINA. Zhejiang Province: Baiyun National Forest Park, Liandu District, Lishui City, 2 Aug 2021, Qin Na, Yupeng Ge, Zewei Liu, Yaping Hu and Hui Ding, *FFAAS1035* (collection number MY0475).

Notes. Leucoinocybe subglobispora is considered to be a distinct species of Leucoinocybe on account of its subdecurrent lamellae, subglobose to broadly ellipsoid basidiospores, thick-walled pileocystidia and caulocystidia and saprophytic habitat. Leucoinocybe lenta, the type species of Leucoinocybe, also has a white stipe and lamellae, similarly-shaped cheilocystidia and thick-walled pileocystidia, but differs from the new species by the presence of a reddish-brown pileus with pinkish shades or pale pinkish-beige at the centre that fades to white towards the margin, larger basidiomata and ellipsoid basidiospores [(5.3)6.0-7.3(7.9) × (3.8)4.0-4.5(5.1) μm] (Gröger 2006; Eyssartier and Roux 2011; Antonín et al. 2019; Kaygusuz et al. 2020). Leucoinocybe taniae (= Clitocybula flavoaurantia) resembles L. subglobispora in having a brown pileus, white and decurrent lamellae and a white stipe with a brownish base, but differs in possessing the following features: a reddish-yellow pileus when old, larger and broadly amygdaliform spores $(6.2-7.8 \times 4.8-7.0 \mu m)$ and thin-walled pileocystidia and caulocystidia (Vila 2002; Contu 2003; Malysheva and Morozova 2011; Antonín et al. 2019). Leucoinocybe sulcata, recently described as a new taxon from India, is easily distinguished from the new species by the presence of greyish-orange to brown basidiomata, a larger pileus (13-52 mm in diam.), broadly ellipsoid



Figure 8. Basidiomata of *Leucoinocybe subglobispora* **A–E** collection *FFAAS1034*, holotype **F–G** collection *FFAAS1035*. Scale bars: 10 mm (**A–G**).


Figure 9. Morphological features of *Leucoinocybe subglobispora* (*FFAAS1034*, holotype) **A** basidiomata **B** basidiospores **C** cheilocystidia **D** basidia **E** caulocystidia **F** pileocystidia. Scale bars: 5 mm (**A**); 10 µm (**B**–**F**).



Figure 10. Microscopic features of *Leucoinocybe subglobispora* (*FFAAS1034*, holotype) **A–E** basidiospores **F** basidia **G–J** cheilocystidia **K** lamellar trama **L** pileipellis and pileocystidia **M** caulocystidia. Scale bars: $5 \mu m (A-E)$; $10 \mu m (F-M)$. Structures were stained with 1% Congo Red aqueous solution before photographing.

to subamygdaliform basidiospores $(5.0-6.5 \times 4.0-5.5 \mu m; Q = 1.1-1.5)$ and thin-walled caulocystidia and the absence of pileocystidia (Latha et al. 2015). *Leucoinocybe lishuiensis*, reported as a new species from south-eastern China in our previous study, can be easily mistaken for *L. subglobispora* on account of having an identical habit and habitat, a small, pure-brown pileus, slightly decurrent lamellae, similarly-shaped cheilocystidia and thick-walled pileocystidia and caulocystidia; however, the narrowly ellipsoid basidiospores and smaller pileocystidia of *L. lishuiensis* can be used to distinguish this species from *L. subglobispora* (Na et al. 2021). Another new combination of *Leucoinocybe, L. auricoma* (Har. Takah.) Matheny, originally named *Mycena auricoma* Har. Takah., is also comparable to the present species in having thick-walled pileocystidia and caulocystidia; however, *L. auricoma* has a yellowish-orange flocculent pileus and stipe, ovoid-ellipsoid to ellipsoid basidiospores ($5-7 \times 3-4 \mu m$) and pileocystidia and caulocystidia with yellow contents (Takahashi 1999; Matheny et al. 2020).

Marasmiellomycena albodescendens (J.A. Cooper) Q.Na & Y.P.Ge, comb. nov. MycoBank No: 851718

Basionym. *Porotheleum albodescendens* J.A. Cooper, in Consiglio, Vizzini, Cooper, Marchetti, Angelini, Brugaletta & Setti, Riv. Micol. 64(2): 117, 2022.

Type specimen. *Holotype*: NEW ZEALAND: North Island, Taupo, Tauhara Centre, 15 May 2011, PDD 96321.

Selected description. Consiglio et al. (2022).

Distribution. New Zealand.

Notes. *Marasmiellomycena albodescendens* has marasmielloid basidiomes, a pure-white pileus, relatively large spores, no hymenial cystidia and abundant, thick-walled pileocystidia and caulocystidia with yellowish contents. Unlike other species of *Marasmiellomycena* possessing a yellow, reddish-brown or yellowish-brown pileus, *M. albodescendens* can be easily recognised by its white pileus. The pileus of *Marasmiellomycena albodescendens* is macromorphologically more similar to some species of *Marasmiellus* Murrill (Stevenson 1964); however, its micromorphological characteristics place this species in *Marasmiellomycena*, consistent with the results of our phylogenetic analysis (Fig. 1). *Marasmiellomycena albodescendens* has been infrequently collected in New Zealand, but is probably common and widespread and grows on small, dead, fallen branches and twigs in indigenous scrub and broad-leaf forests in late summer and autumn (Consiglio et al. 2022).

Marasmiellomycena tomentosa Q.Na & Y.P.Ge, sp. nov.

MycoBank No: 851717 Figs 11-14

Diagnosis. Pileus and stipe distinctly tomentose. Pileus dark brown, subsquamulose. Basidiospores narrowly ellipsoid, slightly amyloid. Hymenial cystidia absent. Pileipellis and stipitipellis sarcodimitic, hyphae thick-walled with yellowish-brown pigments. Pileocystidia and caulocystidia thick-walled with yellow contents. Differs from *M. pseudoomphaliiformis* by possessing a distinctly tomentose, dark-brown subsquamulose pileus, narrowly ellipsoid basidiospores and absence of cheilocystidia.

Holotype. CHINA. Zhejiang Province: Tianmu Mountain, Hangzhou City, 30 Jul 2021, Qin Na, Zewei Liu, Yulan Sun and Yupeng Ge, *FFAAS1036* (collection number MY0421).

Etymology. Name refers to the tomentose to subsquamulose pileus.

Description. Pileus 0.5-18.5 mm in diameter, at first convex or campanulate, soon expanding to plano-convex, always depressed to umbilicate at the centre, surface dry, densely covered with minute white (LIII) pubescence, tomentose all over, subsquamulose, ground colour Verona Brown (XXIX13"k) to Warm Sepia (XXIX13"m), Mustard Yellow (XVI19'b), Old Gold (XVI19'i) to Buffy Citrine (XVI19'k), Saccardo's Olive (XVI19'm) at the centre, fading to Wax Yellow (XLVI21""f) when old, margin slightly sulcate, uplifted or recurved in age. Context thin, Primrose Yellow (XXX23"d). Lamellae decurrent to subdecurrent, Wax Yellow (XLVI21""f), Mustard Yellow (XVI19'b), with 1-2 tiers of lamellulae, edges concolorous with the face, slightly fimbriate edge. Stipe 7.5-21.0 × 1.0-1.6 mm, central, terete, curved, equal or slightly broadened at the base, hollow or stuffed, dry, Mustard Yellow (XVI19'b) in the upper part, Saccardo's Olive (XVI19'm), Benzo Brown (XLVI13""i), Fuscous (XLVI13""m), Deep Grevish-Olive (XLVI21""b) towards the base, densely and minutely silky-fibrillose and white (LIII) pruinose-floccose to tomentose throughout, base covered with white mycelium. Odour indistinct to fungoid, taste mild.

Basidiospores (80/4/3) (6.8) 7.2–7.6–8.2 (8.4) × (3.7) 3.9–4.1–4.5 (4.6) μ m [Q = 1.75–1.98, Q = 1.83 ± 0.052] [holotype (40/2/1) (6.8) 7.2–7.7–8.4 × 3.9–4.2–4.6 μ m, Q = 1.75–1.98, Q = 1.82 ± 0.050], narrowly ellipsoid, hyaline in 5% KOH, smooth, thin-walled, multiguttulate, slightly amyloid. Basidia 20–35 × 5–8 μ m, 2- or 4-spored, clavate, sterigmata 2.2–4.8 × 0.6–1.6 μ m. Hymenial cystidia absent. Lamellar trama subregular; hyphae 3–10 μ m wide, with 0.5–1.0 μ m thick-walled, light yellow, dextrinoid. Pileipellis hyphae 3–8 μ m wide, sarcodimitic, cutis, smooth, 0.4–1.0 μ m thick-walled, with intracellular yellowish-brown pigment; pileocystidia 38–223 × 5–12 μ m, in clusters, narrowly subulate or narrowly lageniform to fusiform with very long and tapering neck, distinctly 0.6–1.5 μ m thick-walled, yellow, smooth. Stipitipellis made up of cylindrical, 4–9 μ m wide hyphae, sarcodimitic, smooth, 0.5–1.0 μ m thick-walled, with intracellular vellour yellow shorth to the pileocystidia 45–327 × 5–9 μ m, similar to the pileocystidia, but usually longer, 0.5–1.3 μ m thick-walled, smooth, with intracellular yellowish pigment. Clamps present in all tissues.

Habit and habitat. Solitary or scattered on rotten branches, twigs and wood debris in Acer, Armeniaca, Cercidiphyllum, Emmenopterys and Picea mixed forests.

Known distribution. Zhejiang Province, China.

Additional material examined. CHINA. Zhejiang Province: Tianmu Mountain, Hangzhou City, 30 Jul 2021, Qin Na, Zewei Liu, Yulan Sun and Yupeng Ge, *FFAAS1037* (collection number MY0422); Zhejiang Province: Tianmu Mountain, Hangzhou City, 1 Aug 2021, Qin Na, Zewei Liu, Yulan Sun and Yupeng Ge, *FFAAS1038* (collection number MY0443).



Figure 11. Basidiomata of *Marasmiellomycena tomentosa* **A–D** collection *FFAAS1036*, holotype **E**, **F** collection *FFAAS1037* **G**, **H** collection *FFAAS1038*. Scale bars: 10 mm (**A–H**).



Figure 12. Morphological features of *Marasmiellomycena tomentosa* (*FFAAS1036*, holotype) **A** basidiomata **B** basidiospores **C** basidia **D** pileipellis and pileocystidia. Scale bars: 10 mm (**A**); 10 μm (**B**–**D**).



Figure 13. Morphological features of *Marasmiellomycena tomentosa* (*FFAAS1036*, holo-type) **A** stipitipellis and caulocystidia. Scale bars: $10 \mu m$ (**A**).

Notes. *Marasmiellomycena tomentosa* is a rare thermophilous species reported from south-eastern areas of China from July to August on rotten branches, twigs and woody debris of deciduous and coniferous trees (Acer, Armeniaca, Cercidiphyllum, Emmenopterys and Picea). The most distinctive characteristics of this species are a tomentose, brown subsquamulose pileus, a tomentose stipe, narrowly ellipsoid and slightly amyloid basidiospores, the absence of hymenial cystidia and thick-walled pileipellis, stipitipellis, pileocystidia and caulocystidia with yellow or brownish-orange contents. Species morphologically most closely allied to *Marasmiellomycena tomentosa* include *M. omphaliiforme*, *M. pseudoomphaliiformis* and *M. albodescendens. Marasmiellomycena pseudoomphalii*



Figure 14. Microscopic features of *Marasmiellomycena tomentosa* (*FFAAS1036*, holotype) A-E basidiospores F basidia G-J lamellae margin. Scale bars: 5 µm (A-E); 10 µm (F-J). Structures were stained in 5% KOH aqueous solution before photographing.

formis resembles M. tomentosa by the presence of a pale beige to brown pileus with finely tomentose to pubescent pileus, but differs in having white to creamwhite or beige lamellae rather than yellow, ellipsoid to ellipsoid-fusiform basidiospores $[(6.5-)7.0-9.0(-9.5) \times 4.0-5.5 \mu m]$ and clavate, fusiform to lageniform cheilocystidia (Senanayake et al. 2023). Marasmiellomycena omphaliiforme is considered to be a closely-related taxon with evident affinities to M. tomentosanot only regarding its phylogenetic placement, but also in terms of morphological features (Kühner and Romagnesi 1954; Antonín and Noordeloos 1993, 1997; Consiglio et al. 2022; Senanayake et al. 2023). The two species resemble one another in having a similarly-coloured pileus and stipe, similarly-shaped basidiospores, pileocystidia and caulocystidia and a yellowish-pigmented pileipellis and stipitipellis; however, the minutely pubescent, granulose to subsquamulose pileus, as well as the relative abundance of cheilocystidia, appear to be variable characters in M. omphaliiforme in contrast to the new species (Kühner and Romagnesi 1954; Antonín and Noordeloos 1993, 1997; Consiglio et al. 2022). According to the description of Consiglio et al. (2022), Marasmiellomycena albodescendens from New Zealand has a pure-white pileus, a thin-walled pileipellis and larger basidiospores (9.6 \pm 0.7 μ m × 5.2 \pm 0.4 μ m).

Pulverulina flavoalba Q.Na & Y.P.Ge, sp. nov.

MycoBank No: 849410 Figs 15–17

Diagnosis. Pileus white to light orange yellow. Basidiospores cylindrical. Hymenial cystidia absent. Lamellar trama, pileipellis and stipitipellis hyphae thinwalled. Differs from *Pu. ulmicola* in having larger and longer basidiospores and possessing thin-walled lamellar trama, pileipellis and stipitipellis hyphae.

Holotype. CHINA. Guangxi Zhuang Autonomous Region: Liangfengjiang National Forest Park, Nanning City, 13 Jul 2022, Yupeng Ge and Renxiu Wei, *FFAAS1039* (collection number MY0863).

Etymology. Name refers to the white to light-yellow pileus and stipe.

Description. Pileus 1.2–5.8 mm in diameter, arched or plano-convex with a slight depression at the centre when young, becoming more depressed with age; translucent striate, floccose or granulose, glabrescent when old, surface dull, dry; white (LIII) when young, aniline yellow (IV19i) or light orange-yellow (II-117d) at the centre and in the margin with age, margin decurved. Context white, thin, not fragile. Lamellae decurrent, white, orange citrine (IV19k) tinged when old, with 1–2 tiers of lamellulae, edges even, medium-broad. Stipe $1.6-14.4 \times 0.5-1.0$ mm, terete or slightly broadened at the base, curved, dry, white, with a pruinose, pubescent or fibrillose surface, sparser with age, hollow, not fragile, white, sometimes aniline yellow (IV19i), light orange-yellow (III17d) in the middle and at the base; base covered with white mycelium. Odour absent, taste mild.

Basidiospores (60/3/2) (6.8) 7.0-7.9-8.8 (9.1) × (3.3) 3.7-4.1-4.4 (4.7) µm [Q = 1.81-2.19, Q = 1.93 ± 0.099] [holotype (40/2/1) (6.8) 7.0-7.8-8.9 (9.1) × (3.3) 3.7-4.1-4.4 (4.7) µm, Q = 1.77-2.19, Q = 1.92 ± 0.084], cylindrical, hyaline in 5% KOH, smooth, thin-walled, guttulate, inamyloid, with a small, but discernible apiculus. Basidia $21-30 \times 4-6$ µm, 2- or 4-spored, clavate, sterigmata $1.9-5.6 \times 0.6-1.6$ µm. Hymenial cystidia absent. Lamellar trama subregular to interwo-



Figure 15. Basidiomata of *Pulverulina flavoalba* **A–D** collection *FFAAS1039*, holotype **E–H** collection *FFAAS1040*. Scale bars: 5 mm (**A–H**).



Figure 16. Morphological features of *Pulverulina flavoalba* (*FFAAS1039*, holotype) **A** basidiomata **B** basidia **C** basidiospores **D** caulocystidia **E** pileipellis. Scale bars: 2 mm (**A**); 10 μm (**B**, **D**, **E**); 5 μm (**C**).



Figure 17. Microscopic features of *Pulverulina flavoalba* (*FFAAS1039*, holotype) A-E basidiospores F basidia G lamellae margin H lamellar trama I pileipellis J caulocystidia. Scale bars: 5 µm (A-E); 10 µm (F-J). Structures A-F were stained in 5% KOH aqueous solution and G-J with 1% Congo Red aqueous solution before photographing.

ven; hyphae 5–15 µm wide, hyaline, thin-walled. Pileipellis a cutis of cylindrical hyphae 3–7 µm wide, smooth; end cells often protruding, $35-105 \times 3-12$ µm, cylindrical, subfusiform, apically obtuse, thin-walled, hyaline, smooth. Stipitipellis hyphae 3–8 µm wide, smooth, thin-walled; caulocystidia 19–50 × 4–9 µm, clavate, subfusiform, thin-walled, smooth, transparent. Clamps present in all tissues.

Habit and habitat. Scattered to gregarious on rotten wood, branches or fruits in mixed forests of *Acacia*, *Ficus*, *Ilex*, *Parashorea*, *Picea* and *Trachycarpus* etc. Known distribution. Guangxi Zhuang Autonomous Region, China.

Additional material examined. CHINA. Guangxi Zhuang Autonomous Region: Liangfengjiang National Forest Park, Nanning City, 13 Jul 2022, Yupeng Ge and Renxiu Wei, *FFAAS1040* (collection number MY0865).

Notes. Clitocybe ulmicola H.E. Bigelow was established by Bigelow in 1982 and published as a new combination, Pulverulina ulmicola (H.E. Bigelow) Matheny & K.W. Hughes (Matheny et al. 2020). The description of Pulverulina ulmicola modified from Bigelow (1982) includes observations based on recent American material (Matheny et al. 2020). As far as we know, only Pulverulina ulmicola has previously been included in the genus and has had morphological features described in detail (Bigelow 1982; Matheny et al. 2020). In appearance, Pulverulina ulmicola is a small, whitish, marasmioid fungus, with small basidiomata, distant decurrent lamellae, a tough texture, interwoven gill trama, long cylindrical caulocystidia and short, ellipsoid, smooth basidiospores and occurs on the bark of living Ulmus and Quercus trees. Our collections of Pulverulina flavoalba from the Guangxi Zhuang Autonomous Region represent a taxon that is distinct from Pulverulina ulmicola, as compared to the macroscopic and microscopic characters described by Matheny et al. (2020). Pulverulina ulmicola differs from P. flavoalba in having a white or whitish to very pale brown or faintly greyish pileus, broadly ellipsoid to ovoid basidiospores and lamellar trama, pileipellis and stipitipellis hyphae with thickened walls (Matheny et al. 2020). The Pulverulina genus comprises two additional species besides Pulverulina ulmicola, namely Pulverulina cyathella (J. Favre & Schweers ex Kuyper) Chalange & P.-A. Moreau and Pulverulina praticola (Kuyper, Arnolds & P.-J. Keizer) Chalange & P.-A. Morea. These two species were transferred to Pulverulina by Chalange and Moreau (2023) from their previous classification under Omphalina. Both species can be readily distinguished from Pulverulina flavoalba based on their spore size and morphology. Specifically, the spores of Pulverulina praticola [(6.0-)6.5-8.0(-8.5) × (5.0-)5.5-6.5(-7.0) µm] are noticeably wider than those of Pulverulina flavoalba, resulting in a significantly lower Q value (Q = 1.1-1.3, Q_{mean} = 1.2) compared to Pulverulina flavoalba (Kuyper et al. 1997). Similarly, Pulverulina cyathella also exhibits wider spores $[(5.5-)6.5-7.0 \times (5.0-)6.0-6.5 \mu m]$ and are (sub)globose in shape, distinguishing them from the cylindrical spores of Pulverulina flavoalba (Kuyper 1996).

Key to 22 species belonging to nine genera of Porotheleaceae in China

Delicatula integrella	Lamellae not well developed	1
	Lamellae well developed	-
	Pileocystidia present	2
9	Pileocystidia absent	-
Marasmiellomycena tomentosa	Cheilocystidia not seen	3
4	Cheilocystidia abundant	-

4	Basidiospores inamyloid <i>Megacollybia</i> 5
5	Cheilocystidia digitate, narrowly or broadly clavate or sphaeropeduncu- late rarely with short apical outgrowths
_	Cheilocystidia clavate without outgrowths Me platyphylla
6	Cheilocystidia distinctly thick-walled overall <i>Leucoinocybe</i> 7
_	Cheilocystidia thin-walled or slightly thick-walled in the base <i>Clitocybula</i> 8
7	Basidiospores narrowly ellipsoid
_	Basidiospores subglobose to broadly ellipsoidL. subglobispora
8	Basidiospores (5.2) $5.4-5.8-6.2$ (6.5) × (4.2) $4.3-4.7-5.0$ (5.1) µm, broad- ly ellipsoid
_	Basidiospores $35-53(-55) \times 35-50$ µm globose subglobose to broad-
	lv elliptic
9	Pileus trama sarcodimitic 10
_	Pileus trama not sarcodimitic18
10	Basidiospores inamyloid Trogia venenata
_	Basidiospores amyloid Gerronema11
11	Basidiomata distinctly small (Pileus < 9 mm in diam.) G. microcarpum
-	Basidiomata moderately small (Pileus > 9 mm in diam.)12
12	Pleurocystidia present G. chrysocarpum
-	Pleurocystidia absent13
13	Pileus blue G. indigoticum
-	Pileus not blue14
14	Pileus and stipe pure whiteG. albidum
_	Pileus yellow to brown, stipe white to yellowish-brown 15
15	Pileus without pubescence or scales16
-	Pileus densely covered with deep brown pubescence or scales
16	Cheilocystidia up to 48 µm long G. baisnanzuense
- 17	Stipe without fuseous pubecoppo or cooles basidiosperes (6.2) 6.7-
17	$74-80(85) \times (32) 37-41-46(48) \text{ µm}$
_	Stine with deep brown fuscous pubescence or scales basidiospores (9.0)
	$9.2-10.0-11.2 (12.9) \times (4.9) 5.2-5.8-6.6 (7.2) \ \mu m$
10	G. brunneosquamulosum
18	Chellocystidia absent
10	Dermatocystidia inconspicuous and rare Beaudobydropus flossings
-	Dermatocystidia abundant Hydronus 20
20	Carpophore blackening when touched or bruised H nigrita
_	Carpophore not blackening in any part when touched or bruised 21
21	Basidiospores ellipsoid
_	Basidiospores broadly ellipsoid
	- · · · · · · · · · · · · · · · · · · ·

Discussion

Previous molecular phylogenetic analyses of the so-called hydropoid clade and the Porotheleaceae have been conducted, based on various combinations of ITS, 28S, 18S, 5.8S, 25S, *rpb1* and *rpb2* loci (Moncalvo et al. 2002; Matheny et al. 2006, 2020; Antonín et al. 2019; Vizzini et al. 2019, 2022; Consiglio et al. 2022;

Senanayake et al. 2023). In the present study, we chose three regions, namely, ITS, nrLSU and *rpb2*, to analyse phylogenetic relationships in Porotheleaceae. Phylogenetic analyses, based on a combined dataset of these three loci, indicated that Marasmiellomycena comprising four species and Pulverulina, comprising two species, constitute monophyletic clades within Porotheleaceae. We thus report new records in China for two genera, Marasmiellomycena and Pulverulina, which cover two new species and a new combination. Marasmiellomycena now includes two new species, namely M. tomentosa and M. albodescendens. Additionally, the species previously identified as Porotheleum albodescendens has been combined as Marasmiellomycena albodescendens, representing a new combination within the Marasmiellomycena, all well characterised by having agaricoid basidiomata. On the basis of macromorphology and phylogenetic affinities, we have only retained one species in Porotheleumthe type species, Porotheleum fimbriatum (Pers.) Fr., which is distinguished by its fruiting clusters of small cup-shaped to tubular cream cyphelloid basidiomes that are densely crowded on a common membranous, resupinate subiculum/stroma with a broad rhizomorphic margin (Cooke 1989). Our results also agreed with Senanayake et al. (2023) that the genus Vizzinia contains two species V. dominingense and V. nigripes, which forms a well-supported lineage and the phylogenetic positions of Porotheleum albidum and Porotheleum parvulum are unclear.

Morphologically, Marasmiellomycena is easily recognisable as an omphalinoid mushroom in the field owing to its pileus that is depressed to umbilicate at the centre, decurrent to subdecurrent lamellae, dark-coloured stipe, sarcodimitic structure and thick-walled caulocystidia with contents. Marasmiellomycena is most similar to Vizzinia, but Vizzinia differs in basidiomata turning brownish on handling, distinctly squamulose pileus, weakly amyloid spores and absence of cheilocystidia. Pulverulina resembles Clitocybula in being an omphalinoid basidiocarps with decurrent lamellae, but can be distinguished by pruinose stipes, inamyloid basidiospores and absence of hymenial cystidia. Gerronema, Megacollybia and Trogia are more similar to Marasmiellomycena on the basis of their sarcodimitic structure. Marasmiellomycena can be readily discriminated in possessing dark-coloured stipe, inamyloid basidiospores and thick-walled caulocystidia with yellow to yellowish-brown pigments. Pulverulina species are characterised by their inamyloid basidiospores, non-sarcodimitic structure, thin-walled caulocystidia and non-pigmented pileocystidia and caulocystidia.

Our multi-gene phylogenetic analysis divided *Gerronema* into several highly-supported clades. This finding is consistent with the analyses of Antonín et al. (2019), Vizzini et al. (2019, 2022), Matheny et al. (2020) and Na et al. (2022a), who have reported that *Gerronema* is a non-monophyletic genus comprising several unrelated clades. The type of *Gerronema* has not been sequenced so it is unclear which belongs to *Gerronema* sensu stricto. Other genera in Porotheleaceae, namely, *Chrysomycena, Clitocybula, Delicatula, Hydropodia, Hydropus, Leucoinocybe, Marasmiellomycena, Megacollybia, Pulverulina, Trogia* and *Vizzinia* are monophyletic in previous phylogenetic studies as well as the present one (Matheny et al. 2020; Consiglio et al. 2022; Vizzini et al. 2022; Senanayake et al. 2023). *Hydropodia subalpina* (Höhn.) Vizzini, Consiglio & M. Marchetti, a new combination from *Hydropus*, is not related to *Hydropus* s. s.–which corresponds to the clade including the type species *Hydropus fuliginarius* (Batsch) Singer in the phylogenetic classification of Consiglio et al. (2022). In addition, Consiglio et al. (2022) consider *Hydropodia* to be sister to the *Porotheleum* clade; in our studies, however, *Hydropodia* is closer to *Pseudohydropus* and forms a sister clade.

Several species of Porotheleaceae have been reported to be edible or have toxic or ecological effects. *Megacollybia platyphylla* (Pers.) Kotl. & Pouzar (Dai et al. 2010), are known to be edible, whereas *Trogia venenata* Zhu L. Yang, Yan C. Li & L.P. Tang has caused hundreds of deaths in south-western China (Yang et al. 2012). Current evidence regarding the edibility and ecological functions of other Porotheleaceae species is insufficient. Specifically, whether they engage in symbiotic or saprophytic relationships with plants, as well as their roles within ecosystems, remains unclear. Although it is uncertain if these species exhibit symbiosis (and likely absent), future studies may uncover their capabilities to promote seed germination, similar to some *Mycena* species or possessing characteristics like bioluminescence. Further research is needed to investigate the edibility and ecological role of Porotheleaceae.

Acknowledgements

We thank Dr Junqing Yan (Jiangxi Agriculture University), Ms. Zewei Liu (Ludong University), Ms. Yulan Sun (Ludong University) and Ms. Renxiu Wei (Ludong University) for their kind help during fieldwork. We sincerely thank the reviewers for their corrections and suggestions to improve our work.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was supported by the National Natural Science Foundation of China (grant no. 32200008), the Natural Science Foundation of Shandong Province (grant no. ZR2020QC001), the 5511 Collaborative innovation project of Fujian Province (grant no. XTCXGC2021007), the Central Public-Interest Scientific Institution Basal Research Fund (grant no. GYZX200203), the East-west cooperation project, FAAS (grant no. DKBF-2022-12), the Natural Science Foundation of Fujian Province (grant no. 2023J01379), the Project of Biological Resources Survey in Wuyishan National Park (grant no. HXQT2020120701), the Project of Biodiversity Conservation in Lishui, Zhejiang Province (grant no. HXYJCP2021110648), the Biodiversity investigation, observation and assessment program of Ministry of Ecology and Environment of China (grant no. 2110404 and 2019-2023), the Shandong Agricultural Industry Technology System (2021 grant no. 26, SDAIT-07-03) and the Soft Science project of Ministry of Public Security (grant no. 2022LL75).

Author contributions

Qin Na, Xianhao Cheng, and Yupeng Ge were responsible for designing the research and contributed significantly to data analysis and interpretation. Hui Zeng, Yaping Hu, Zhiheng Zeng, Bingrong Ke, Changjing Liu, and Hui Ding actively participated in the field investigation. All authors have contributed to the manuscript and approved the version submitted for publication.

Author ORCIDs

Qin Na [®] https://orcid.org/0000-0001-8406-6389 Hui Zeng [®] https://orcid.org/0000-0003-2025-844X Yaping Hu [®] https://orcid.org/0000-0003-1242-1139 Hui Ding [®] https://orcid.org/0000-0003-4490-2105 Binrong Ke [®] https://orcid.org/0009-0008-7209-7362 Zhiheng Zeng [®] https://orcid.org/0009-0008-4208-2629 Xianhao Cheng [®] https://orcid.org/0000-0002-5922-9913 Yupeng Ge [®] https://orcid.org/0000-0001-5754-201X

Data availability

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

References

- Antonín V, Noordeloos ME (1993) A monograph of *Marasmius, Collybia* and related genera in Europe. Part 1: *Marasmius, Setulipes, and Marasmiellus*. Libri Botanici, IHW Verlag, Eching, 229 pp.
- Antonín V, Noordeloos ME (1997) A monograph of *Marasmius, Collybia* and related genera in Europe. Part 2: *Collybia, Gymnopus, Rhodocollybia, Crinipellis, Chaetocalathus,* and additions to *Marasmiellus*. Libri Botanici, IHW Verlag, Eching, 256 pp.
- Antonín V, Noordeloos ME (2004) A monograph of the genera *Hemimycena*, *Delicatula*, *Fayodia*, *Gamundia*, *Myxomphalia*, *Resinomycena*, *Rickenella*, and *Xeromphalina* (Tribus Mycenae sensu Singer, *Mycena* excluded) in Europe. Libri Botanici, IHW-Verlag, Eching, 280 pp.
- Antonín V, Ryoo R, Shin HD (2008) *Gerronema nemorale* (Basidiomycota, Agaricomycetes): Anatomic-morphological, cultivational, enzymatic and molecular characteristics and its first records in the Republic of Korea. Czech Mycology 60(2): 197–212. https://doi.org/10.33585/cmy.60204
- Antonín V, Beran M, Borovička J, Dvořák D, Holec J (2011) *Clitocybula familia* (Fungi, Agaricales) taxonomy, distribution, ecology and first records in the Czech Republic and Slovakia. Czech Mycology 63(1): 1–11. https://doi.org/10.33585/cmy.63101
- Antonín V, Borovička J, Holec J, Piltaver A, Kolařík M (2019) Taxonomic update of *Clito-cybula* sensu lato with a new generic classification. Fungal Biology 123(6): 431–447. https://doi.org/10.1016/j.funbio.2019.03.004
- Aqdus F, Khalid AN (2021) *Gerronema nemorale*: first report of the genus and species from Pakistan. Mycotaxon 136(1): 249–259. ttps://https://doi.org/10.5248/136.249
- Bigelow HE (1973) The Genus *Clitocybula*. Mycologia 65(5): 1101–1116. https://doi.org /10.1080/00275514.1973.12019530
- Bigelow HE (1982) North American species of *Clitocybe*. Part 1. Beihefte zur Nova Hedwigia 72: 5–280.

- Bodensteiner P, Binder M, Moncalvo JM, Agerer R, Hibbett DS (2004) Phylogenetic relationships of cyphelloid homobasidiomycetes. Molecular Phylogenetics and Evolution 33(2): 501–515. https://doi.org/10.1016/j.ympev.2004.06.007
- Breitenbach J, Kranzlin F (1991) Fungi of Switzerland. Vol. 3. Boletes and Agarics, 1st Part. Edition Mykologia, Lucerne, 359 pp.
- Chalange R, Moreau PA (2023) Deux taxons rares, *Omphalina praticola* et *Omphalina cyathella*, reclassés dans le genre *Pulverulina*. Bulletin de la Société Mycologique de France 139(1–2): 87–105.
- Consiglio G, Vizzini A, Cooper J, Marchetti M, Angelini C, Brugaletta E, Setti L (2022) The agaricoid members of the genus *Porotheleum* (Porotheleaceae, Agaricales), *Porotheleum* emend., *Porotheleaceae* s. stricto, and new genera for *Agaricus floccipes* and *Mycena subalpina*. Rivista di Micologia 64(2): 99–190.
- Contu M (2003) Una straordinaria nuova specie di *Pseudoomphalina* (Basidiomycetes, Leucopaxilloideae) dalla Sardegna. Micologia e Vegetazione Mediterranea 18(1): 61–68.
- Cooke WB (1989) The cyphelloid fungi of Ohio. Memoirs of the New York Botanical Garden 49: 158–172.
- Cooper JA (2014) New species and combinations of some New Zealand agarics belonging to *Clitopilus, Lyophyllum, Gerhardtia, Clitocybe, Hydnangium, Mycena, Rhodocollybia* and *Gerronema*. Mycosphere : Journal of Fungal Biology 5(2): 263–288. https://doi.org/10.5943/mycosphere/5/2/2
- Cooper JA (2016) Notes on the Porotheleaceae of New Zealand. Mycological Notes 23. Self-published.
- Cooper A, Desjardin DE, Perry BA (2019) The genus *Mycena* (Basidiomycota, Agaricales, Mycenaceae) and allied genera from Republic of São Tomé and Príncipe, West Africa. Phytotaxa 383(1): 1–47. https://doi.org/10.11646/phytotaxa.383.1.1
- Corner EJH (1991) *Trogia* (Basidiomycetes). Gardens' Bulletin Supplement, Singapore Botanic Gardens, Singapore, 100 pp. https://doi.org/10.5962/bhl.title.77527
- Dähncke RM, Contu M, Vizzini A (2010) *Clitocybula striata* sp. nov. (Basidiomycota, Agaricomycetes, Agaricales): Una nuova specie delle isole Canarie (Spagna), con note sulla diffusione del genere *Clitocybula* nell'isola di La Palma. Revista Catalana de Micologia 32: 7–12.
- Dai YC, Zhou LW, Yang ZL, Wen HA, Bau T, Li TH (2010) A revised checklist of edible fungi in China. Junwu Xuebao 29(1): 1–21. https://doi.org/10.13346/j.mycosystema.2010.01.022
- Dutta AK, Nandi S, Tarafder E, Sikder R, Roy A, Acharya K (2017) *Trogia benghalensis* (Marasmiaceae, Basidiomycota), a new species from India. Phytotaxa 331(2): 273–280. https://doi.org/10.11646/phytotaxa.331.2.11
- Dutta AK, Antonín V, Barui R, Acharya K (2018) A new species of *Clitocybula* (Marasmiaceae) from West Bengal, India. Nova Hedwigia 107(1): 195–203. https://doi. org/10.1127/nova_hedwigia/2017/0464
- Eberhardt U, Schütz N, Krause C, Beker HJ (2018) *Hebelomina* (Agaricales) revisited and abandoned. Plant Ecology and Evolution 151(1): 96–109. https://doi.org/10.5091/plecevo.2018.1361
- Edler D, Klein J, Antonelli A, Silvestro D (2021) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods in Ecology and Evolution 12(2): 373–377. https://doi.org/10.1111/2041-210X.13512
- Eyssartier G, Roux P (2011) Le guide des champignons France et Europe. Belin, Paris, 1150 pp.

- Ge YP, Liu ZW, Zeng H, Cheng XH, Na Q (2021) Updated description of *Atheniella* (Mycenaceae, Agaricales), including three new species with brightly coloured pilei from Yunnan Province, southwest China. MycoKeys 81: 139–164. https://doi.org/10.3897/mycokeys.81.67773
- Gröger F (2006) Bestimmungsschlüssel für Blätterpilze und Röhrlinge in Europa. Band 1. Regensburger Mykologische Schriften, 638 pp.
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.1021/bk-1999-0734.ch008
- Henrici A (2012) Notes & records. Field Mycology : a Magazine for the Study and Identification of Wild Fungi 13(3): 105–108. https://doi.org/10.1016/j.fldmyc.2012.06.013
- Hopple Jr JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark–spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: Divergent domains, outgroups, and monophyly. Molecular Phylogenetics and Evolution 13(1): 1–19. https://doi.org/10.1006/ mpev.1999.0634
- Horak E (2005) Röhrlinge und Blätterpilze in Europa: Bestimmungsschlüssel für Polyporales (pp), Boletales, Agaricales, Russulales. Elsevier, Spektrum Akad Verlag, 555 pp.
- Hughes KW, Petersen RH, Mata JL, Psurtseva NV, Kovalenko AE, Morozova OV, Lickey EB, Blanco JC, Lewis DP, Nagasawa E, Halling RE, Takehashi S, Aime MC, Bau T, Henkel T (2007) *Megacollybia* (Agaricales). Reports of the Tottori Mycological Institute 45: 1–57.
- Kalichman J, Kirk PM, Matheny PB (2020) A compendium of generic names of agarics and Agaricales. Taxon 69(3): 425–447. https://doi.org/10.1002/tax.12240
- Kasuya T, Kaneko R, Takehashi S, Hosaka K (2023) Hydropodia silvae-nipponicae (Porotheleaceae), a new species from evergreen broad-leaved forests in Japan. Mycoscience 64(4): 116–122. https://doi.org/10.47371/mycosci.2023.06.002
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kaygusuz O, Ševčíková H, Battistin E, Türkekul B (2020) A multi-gene molecular phylogeny regarding the two phylogenetically close genera *Hydropus* and *Leucoinocybe* (Agaricales, Basidiomycota), new for Turkey. Nova Hedwigia 111(3–4): 429–448. https://doi.org/10.1127/nova_hedwigia/2020/0601
- Kim CS, Jo JW, Kwag YN, Sung GH, Lee SG, Kim SY, Shin C-H, Han S-K (2015) Mushroom Flora of Ulleung-gun and a newly recorded Bovista species in the Republic of Korea. Mycobiology 43(3): 239–257. https://doi.org/10.5941/MYCO.2015.43.3.239
- Kühner R, Romagnesi H (1954) Compléments à la Flore analytique III. Espèces, nouvelles, critiques ou rares de Pleurotacées, Marasmiacées et Tricholomacées. Bulletin de la Société des Naturalistes d'Oyonnax 8: 73–131.
- Kumar TKA, Manimohan P (2009) Rediscovery of *Trogia cyanea* and record of *T. infundibuliformis* (Marasmiaceae, Agaricales) from Kerala State, India. Mycotaxon 109(1): 429–436. https://doi.org/10.5248/109.429
- Kuyper T (1996) Notulae ad Floram agaricinam neerlandicam–XXIV-XXVIII. Some taxonomic and nomenclatural changes in the Tricholomataceae, tribus Clitocybeae. Persoonia-Molecular Phylogeny and Evolution of Fungi 16(2): 225–232.
- Kuyper TW, Arnolds E, Keizer PJ (1997) *Omphalina praticola*, eine neue Art aus den Niederlanden. Österreichische Zeitschrift für Pilzkunde 6: 131–134.
- Latha KPD, Raj KNA, Sharafudheen SA, Manimohan P (2015) *Clitocybula sulcata*–a new species from India. Phytotaxa 208(1): 063–069. https://doi.org/10.11646/phyto-taxa.208.1.6

Latha KPD, Nanu S, Sharafudheen SA, Manimohan P (2018). Two new species of *Gerronema* (Agaricales, Basidiomycota) from Kerala State, India. Phytotaxa 364(1): 081–091. https://doi.org/10.11646/phytotaxa.364.1.5

Lennox JW (1979) Collybioid genera in the Pacific Northwest. Mycotaxon 9: 117–231.

- Liu PG (1995) Five new species of Agaricales from Southern and Southeastern Yunnan, China. Mycotaxon 6: 89–105.
- Liu LN, Mou GF, Bau T (2019) A new *Gerronema* species with striking colours from China. Phytotaxa 405(2): 074–082. https://doi.org/10.11646/phytotaxa.405.2.2
- Liu ZW, Na Q, Cheng XH, Wu XM, Ge YP (2021) *Mycena yuezhuoi* sp. nov. (Mycenaceae, Agaricales), a purple species from the peninsula areas of China. Phytotaxa 511(2): 148–162. https://doi.org/10.11646/phytotaxa.511.2.3
- Liu ZW, Ge YP, Zeng H, Cheng XH, Na Q (2022) Four new species of *Mycena* sect. *Calodontes* (Agaricales, Mycenaceae) from northeast China. MycoKeys 93: 23–56. https://doi.org/10.3897/mycokeys.93.86580
- Ludwig E (2000) Pilzkompendium. Band. 1. Abbildungen. In: Libri Botanici, IHW-Verlag, Eching, 192 pp.
- Ludwig E (2001) Pilzkompendium. Band 1. Beschreibungen. In: Libri Botanici, IHW-Verlag, Eching, 758 pp.
- Lutzoni F (1997) Phylogeny of lichen- and non-lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Systematic Biology 46(3): 373–406. https://doi.org/10.1093/sysbio/46.3.373
- Malysheva EF, Morozova OV (2011) New combinations in *Clitocybula*: A study of cystidiate *Pseudoomphalina* species (Basidiomycota, Agaricomycetes). Sydowia 63(1): 85–104.
- Matheny PB (2005) Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (*Inocybe*, Agaricales). Molecular Phylogenetics and Evolution 35(1): 1–20. https://doi.org/10.1016/j.ympev.2004.11.014
- Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang Z-L, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS (2006) Major clades of Agaricales: A multilocus phylogenetic overview. Mycologia 98(6): 982–995. https://doi.org/10.1080/15572536.2006.11832627
- Matheny PB, Hughes KW, Kalichman J, Lebeuf R (2020) *Pulverulina*, a new genus of Agaricales for *Clitocybe ulmicola*. Southeastern Naturalist (Steuben, ME) 19(3): 447–459. https://doi.org/10.1656/058.019.0301
- Mi F, Zhang Y, Yang D, Tang XZ, Wang PF, He XX, Zhang Y, Dong J, Cao Y, Liu C, Zhang K-Q, Xu J (2016) Evidence for inbreeding and genetic differentiation among geographic populations of the saprophytic mushroom *Trogia venenata* from southwestern China. PLoS ONE 11(2): 1–21. https://doi.org/10.1371/journal.pone.0149507
- Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vilgalys R (2000) Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systematic Biology 49(2): 278–305. https://doi.org/10.1093/sysbio/49.2.278
- Moncalvo JM, Vilgalys R, Redhead RA, Johnson JE, James TY, Aime MC, et al. (2002) One hundred and seventeen clades of euagarics. Molecular Phylogenetics and Evolution 23(3): 357–400. https://doi.org/10.1016/S1055-7903(02)00027-1
- Murrill WA (1916) Index to Illustrations of Fungi I-XXII. Mycologia 8(1): 47–51. https://doi. org/10.1080/00275514.1916.12018862
- Na Q, Bau T (2018) New species of *Mycena* (Mycenaceae, Agaricales) with colored lamellae and three new species records from China. Phytotaxa 361(3): 266–278. https://doi.org/10.11646/phytotaxa.361.3.2

- Na Q, Hu YP, Liu ZW, Zeng H, Qi LL, Ding H, Cheng X, Ge Y (2021) The first reported occurrence of *Leucoinocybe* (Porotheleaceae, Agaricales) in China: *Leucoinocybe lishuiensis* sp. nov. from Zhejiang Province. Nova Hedwigia 113(3–4): 453–469. https://doi. org/10.1127/nova_hedwigia/2021/0661
- Na Q, Hu YP, Zeng H, Song ZZ, Ding H, Cheng XH, Ge Y (2022a) Updated taxonomy on *Gerronema* (Porotheleaceae, Agaricales) with three new taxa and one new record from China. MycoKeys 89: 87–120. https://doi.org/10.3897/mycokeys.89.79864
- Na Q, Liu ZW, Zeng H, Ke BR, Song ZZ, Cheng XH, Ge Y (2022b) Taxonomic studies of bluish *Mycena* (Mycenaceae, Agaricales) with two new species from northern China. MycoKeys 90: 119–145. https://doi.org/10.3897/mycokeys.90.78880
- Norvell L, Redhead S, Ammirati J (1994) *Omphalina* sensu lato in North America 1-2.
 1: *Omphalina wynniae* and the genus *Chrysomphalina*. 2: *Omphalina sensu* Bigelow.
 Mycotaxon 50: 379–407.
- Osmundson TW, Robert VA, Schoch CL, Baker LJ, Smith A, Robich G, Mizzan L, Garbelotto MM (2013) Filling gaps in biodiversity knowledge for macrofungi: Contributions and assessment of an herbarium collection DNA barcode sequencing project. PLoS ONE 8(4): e62419. https://doi.org/10.1371/journal.pone.0062419
- Peck CH (1878) *Agaricus (Collybia) abundans* n. sp. Annual report on the New York State Museum of Natural History 29: 38.
- Posada D, Crandall KA (1998) Modeltest: Testing the model of DNA substitution. Bioinformatics (Oxford, England) 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817
- Redhead SA (1987) The Xerulaceae (Basidiomycetes), a family with sarcodimitic tissues. Canadian Journal of Botany 65(8): 1551–1562. https://doi.org/10.1139/b87-214
- Redhead SA (2012) Nomenclatural novelties. Index Fungorum 14: 1.
- Redhead SA (2013) Nomenclatural novelties. Index Fungorum 15: 1–2.
- Ridgway R (1912) Color standards and color nomenclature. Robert Ridgway, Washington D.C, 110 pp. https://doi.org/10.5962/bhl.title.144788
- Romagnesi H (1968) Sur un *Collybia* a spores amyloids. Collectanea Botanica 7(58): 1083–1090.
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi. org/10.1093/bioinformatics/btg180
- Senanayake C, Rossi W, Leonardi M, Weir A, McHugh MC, Rajeshkumar K, Verma RK, Karunarathna SC, Tibpromma S, Ashtekar N, Ashtamoorthy SK, Raveendran S, Kour G, Singh A, De la Peña-Lastra S, Mateos A, Kolařík M, Antonín V, Ševčíková H, Esteve-Raventós F, Larsson E, Pancorbo F, Moreno G, Altés A, Turégano Y, Du T-Y, Lu L, Li Q-R, Kang J-C, Gunaseelan S, Kezo K, Kaliyaperumal M, Fu J, Samarakoon MC, Gafforov Y, Teshaboeva S, Kunjan PC, Chamaparambath A, Flakus A, Etayo J, Rodriguez-Flakus P, Zhurbenko MP, de Silva NI, Tennakoon DS, Latha KPD, Manimohan P, Raj KNA, Calabon MS, Ahmadpour A, Heidarian Z, Alavi Z, Alavi F, Ghosta Y, Azizi R, Luo M, Zhao M-P, Kularathnage ND, Hua L, Yang Y-H, Liao C-F, Zhao H-J, Lestari AS, Jayasiri SC, Yu F-M, Lei L, Liu J-W, Karimi O, Tang S-M, Sun Y-R, Wang Y, Zeng M, Htet ZH, Linaldeddu BT, Alves A, Phillips AJL, Bregant C, Montecchio L, De Kesel A, Hustad VP, Miller AN, Fedosova AG, Kučera V, Raza M, Hussain M, Chen Y-P, Thiyagaraja V, Gomdola D, Rathnayaka AR, Dissanayake AJ, Suwannarach N, Hongsanan S, Maharachchikumbura SSN, Dissanayake LS, Wijayawardene NN, Phookamsak R, Lumyong S, Jones EBG, Yapa N, Wanasinghe DN, Xie N, Doilom M, Manawasinghe IS, Liu J-K, Zhao Q, Xu B, Hyde KD, Song J (2023) Fungal diversity notes 1611–1716:

Taxonomic and phylogenetic contributions on fungal genera and species emphasis in south China. Fungal Diversity 122(1): 161–403. https://doi.org/10.1007/s13225-023-00523-6

Singer R (1943) Das System der Agaricales III. Annales Mycologici 41(1-3): 1-189.

- Singer R (1951) New genera of fungi V. Mycologia 43(5): 598–604. https://doi.org/10.1 080/00275514.1951.12024157
- Singer R (1970) Omphalinae (Clitocybeae–Tricholomataceae, Basidiomycetes). Organization for Flora Neotropica, University of California, 131 pp.
- Singer R (1982) *Hydropus* (Basidiomycetes-Tricholomataceae-Myceneae). Flora Neotropica Monograph, New York, 154 pp.
- Singer R (1986) The Agaricales in modern taxonomy, 4th edn. Koeltz Scientific Books, Königstein, 981pp.
- Stevenson G (1964) The Agaricales of New Zealand: V. Kew Bulletin 19(1): 1–59. https://doi.org/10.2307/4108283
- Takahashi H (1999) *Mycena auricoma*, a new species of *Mycena* section *Radiatae* from Japan, and *Mycena spinosissima*, a new record in Japan. Mycoscience 40(1): 73–80. https://doi.org/10.1007/BF02465677
- Telfer A, Dewaard J, Young M, Quinn J, Perez K, Sobel C, Sones J, Levesque-Beaudin V, Derbyshire R, Fernandez-Triana J, Rougerie R, Thevanayagam A, Boskovic A, Borisenko A, Cadel A, Brown A, Pages A, Castillo A, Nicolai A, Glenn Mockford BM, Bukowski B, Wilson B, Trojahn B, Lacroix CA, Brimblecombe C, Hay C, Ho C, Steinke C, Warne C, Garrido Cortes C, Engelking D, Wright D, Lijtmaer D, Gascoigne D, Hernandez Martich D, Morningstar D, Neumann D, Steinke D, Marco DeBruin DDB, Dobias D, Sears E, Richard E, Damstra E, Zakharov E, Laberge F, Collins G, Blagoev G, Grainge G, Ansell G, Meredith G, Hogg I, McKeown J, Topan J, Bracey J, Guenther J, Sills-Gilligan J, Addesi J, Persi J, Layton K, D'Souza K, Dorji K, Grundy K, Nghidinwa K, Ronnenberg K, Lee KM, Xie L, Lu L, Penev L, Gonzalez M, Rosati M, Kekkonen M, Kuzmina M, Iskandar M, Mutanen M, Fatahi M, Pentinsaari M, Bauman M, Nikolova N, Ivanova N, Jones N, Weerasuriya N, Monkhouse N, Lavinia P, Jannetta P, Hanisch P, McMullin RT, Ojeda Flores R, Mouttet R, Vender R, Labbee R, Forsyth R, Lauder R, Dickson R, Kroft R, Miller S, MacDonald S, Panthi S, Pedersen S, Sobek-Swant S, Naik S, Lipinskaya T, Eagalle T, Decaëns T, Kosuth T, Braukmann T, Woodcock T, Roslin T, Zammit T, Campbell V, Dinca V, Peneva V, Hebert P (2015) Biodiversity inventories in high gear: DNA barcoding facilitates a rapid biotic survey of a temperate nature reserve. Biodiversity Data Journal 30(3): e6313. https://doi.org/10.3897/BDJ.3.e6313
- Thompson JD, Gibson TJ, Plewniak F (1997) The Clustal–X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 63(24): 215–228. https://doi.org/10.1093/nar/25.24.4876
- Varga T, Krizsan K, Földi C, Dima B, Sánchez-García M, Sánchez-Ramírez S, Szöllősi GJ, Szarkándi JG, Papp V, Albert L, Andreopoulos W, Angelini C, Antonín V, Barry KW, Bougher NL, Buchanan P, Buyck B, Bense V, Catcheside P, Chovatia M, Cooper J, Dämon W, Desjardin D, Finy P, Geml J, Haridas S, Hughes K, Justo A, Karasiński D, Kautmanova I, Kiss B, Kocsubé S, Kotiranta H, LaButti KM, Lechner BE, Liimatainen K, Lipzen A, Lukács Z, Mihaltcheva S, Morgado LN, Niskanen T, Noordeloos ME, Ohm RA, Ortiz-Santana B, Ovrebo C, Rácz N, Riley R, Savchenko A, Shiryaev A, Soop K, Spirin V, Szebenyi C, Tomšovský M, Tulloss RE, Uehling J, Grigoriev IV, Vágvölgyi C, Papp T, Martin FM, Miettinen O, Hibbett DS, Nagy LG (2019) Megaphylogeny resolves global patterns of mushroom evolution. Nature Ecology & Evolution 3(4): 668–678. https://doi.org/10.1038/s41559-019-0834-1

- Vila J (2002) *Clitocybula taniae* Vila, una nova espècie del litoral català. Revista Catalana de Micologia 24: 283–286.
- Villarreal M, Esteve-Raventós F, Sánchez F, Pérez-De-Gregorio MA (2021) *Chrysomycena dunicola* comb. nov. (Agaricales, Porotheleaceae), un nombre prioritario sobre *Chrysomycena perplexa*. Boletin de la Sociedad Micologica de Madrid 45: 43–52.
- Vizzini A, Picillo B, Luigi P, Dovana F (2019) *Chrysomycena perplexa* gen. et sp. nov. (Agaricales, Porotheleaceae), a new entity from the Lazio region. Rivista Micologica Romana 107(2): 96–107.
- Vizzini A, Consiglio G, Marchetti M, Borovička J, Campo E, Cooper J, Lebeuf R, Ševčíková H (2022) New data in Porotheleaceae and Cyphellaceae: Epitypification of *Prunulus* scabripes Murrill, the status of *Mycopan* Redhead, Moncalvo & Vilgalys and a new combination in *Pleurella* Horak emend. Mycological Progress 21(4): 44. https://doi. org/10.1007/s11557-022-01795-z
- Wang M, Wu D, Jiang LL, Feng Z, Xiang Z, Li P, et al. (2021) Overview on edible fungi resources in Guizhou Province. Zhongguo Shiyongjun 40(1): 7–23. https://doi. org/10.13629/j.cnki.53-1054.2021.01.002
- White TJ, Bruns TD, Lee SB, Taylor JW, Innis MA, Gelfand DH, et al. (1990) Amplification and direct sequencing of Fungal Ribosomal RNA Genes for phylogenetics. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Yang ZL, Li YC, Tang LP, Shi GQ, Zeng G (2012) *Trogia venenata* (Agaricales), a novel poisonous species which has caused hundreds of deaths in southwestern China. Mycological Progress 11(4): 937–945. https://doi.org/10.1007/s11557-012-0809-y



Research Article

Two new species of *Perenniporia* sensu lato (Polyporales, Basidiomycota) from China and two new combinations in *Crassisporus*

Chao-Ge Wang¹⁰, Jian Chen¹, Hong-Gao Liu^{2,3}, Yu-Cheng Dai¹, Yuan Yuan¹⁰

2 Yunnan Key Laboratory of Gastrodia and Fungi Symbiotic Biology, Zhaotong University, Zhaotong 657000, China

Corresponding authors: Yu-Cheng Dai (yuchengdai@bjfu.edu.cn); Yuan Yuan (yuanyuan1018@bjfu.edu.cn)

Abstract

Phylogenetic and morphological analyses on *Perenniporia* s.l. were carried out. Phylogenetic and *Perenniporia* s.l. are reconstructed with two loci DNA sequences including the internal transcribed spacer (ITS) regions and the large subunit (nLSU). Two new species from Yunnan Province, southwest China, *Perenniporia prunicola* and *P. rosicola* in *Perenniporia* s.l., are illustrated and described. *Perenniporia prunicola* is characterised by the perennial and resupinate basidiomata with a clay pink pore surface when fresh, a trimitic hyphal system, the presence of clavate to fusiform hymenial cystidia, ellipsoid to broadly ellipsoid basidiospores measuring $4.8-6.2 \times 3.6-4.5 \mu m$. *Perenniporia rosicola* is characterised by annual and resupinate basidiomata with a white pore surface when fresh, a dimitic hyphal system, the presence of dendrohyphidia, broadly ellipsoid to subglobose basidiospores measuring $5-5.8 \times 4-5.2 \mu m$. In addition, *Crassisporus* is a genus in *Perenniporia* s.l., in which two new combinations *Crassisporus minutus* and *C. mollissimus* are proposed. Main morphological characteristics of species related to new taxa are also provided.

Key words: Phylogeny, polypore, taxonomy, wood-decaying fungi



Academic editor: Kentaro Hosaka Received: 29 February 2024 Accepted: 8 April 2024 Published: 25 April 2024

Citation: Wang C-G, Chen J, Liu H-G, Dai Y-C, Yuan Y (2024) Two new species of *Perenniporia* sensu lato (Polyporales, Basidiomycota) from China and two new combinations in *Crassisporus*. MycoKeys 105: 97–118. https://doi.org/10.3897/ mycokeys.105.121858

Copyright: © Chao-Ge Wang 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).

Introduction

Perenniporia Murrill (Polyporales, Basidiomycetes) is typified by *P. medulla-panis* (Jacq.) Donk and it is one of the species-rich genera of Polyporales. Traditionally, it is characterised by annual to perennial, resupinate, effused-reflexed to pileate basidiomata with a varied coloured pore surface when fresh, a dimitic to trimitic hyphal system with generative hyphae bearing clamp connections, variably dextrinoid and cyanophilous skeletal hyphae, ellipsoid, broadly ellipsoid to subglobose, mostly thick-walled and truncate variably dextrinoid, cyano-

¹ State Key Laboratory of Efficient Production of Forest Resources, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China

³ Yunnan Engineering Research Center of Green Planting and Processing of Gastrodia, Zhaotong University, Zhaotong 657000, China

philous basidiospores and causing a white rot in dead angiosperm and gymnosperm woods (Ryvarden and Gilbertson 1994; Decock and Ryvarden 1999; Zhao et al. 2013a; Cui et al. 2019; Ji et al. 2023).

Perenniporia was established by Murrill in 1942 just with two species, P. unita (Pers.) Murrill (Basionym: Polyporus unitus Pers.) and P. nigrescens (Bres.) Murrill (Basionym: Poria nigrescens Bres.), none of which was regarded as the type species (Murrill 1942). Then P. unita was combined into different genera by other mycologists, viz. Fibuloporia unita (Pers.) Bondartsev, Fomes unitus (Pers.) J. Lowe and Fomitopsis unita (Pers.) Bondartsev (Bondartsev 1953; Lowe 1955), as well as being designated the lectotype of Perenniporia by Cooke (1953). Decock and Stalpers (2006) re-discussed the relationship and status of Polyporus unitus and Boletus medulla-panis Jacq., though they are synonymous and the latter has been normally regarded as the type species of Perenniporia in previous studies (Donk 1960; Ryvarden 1972a; Gilbertson and Ryvarden 1987; Ryvarden and Gilbertson 1994). In addition, they demonstrated Pol. unitus is not a synonym of B. medulla-panis, the latter of which was selected as the type of Perenniporia (Decock and Stalpers 2006). For now, Poria nigrescens as a synonym of Physisporinus crocatus (Pat.) F. Wu, Jia J. Chen & Y.C. Dai was described from Hungary and it has a perennial basidiomata, erubescent pores (white when fresh, then "carneo-violaceis", finally black), but no basidiospores data (Bresadola 1897).

Previous studies have shown that *Perenniporia* is a polyphyletic genus (Zhao et al. 2013a; Cui et al. 2019; Ji et al. 2023). Species in Perenniporia s.l. form seven independent clades, based on phylogenetic analysis with typical characteristics (Zhao et al. 2013a). Hornodermoporus, Perenniporiella, Truncospora, Vanderbylia etc. were derived from Perenniporia s.l. Especially, Ji et al. (2023) proposed 15 new genera previously addressed in Perenniporia s.l., based on phylogenetic and morphological analyses. Perenniporia s.s. contains three species, viz. P. hainaniana B.K. Cui & C.L. Zhao, P. medulla-panis and P. substraminea B.K. Cui & C.L. Zhao (Ji et al. 2023). Up to now, more than 120 taxa were found in Perenniporia s. l. (Ji et al. 2017; Liu et al. 2017; Shen et al. 2018; Cui et al. 2019; Zhao and Ma 2019; Ji et al. 2023). In addition, some species in Perenniporia s.l. could produce laccase (such as P. tephropora (Mont.) Ryvarden and Poriella subacida (Peck) C.L. Zhao) and carotenoid (such as Vanderbylia fraxinea (Bull.) D.A. Reid) etc. applied in both biomedical engineering and biodegradation (Si et al. 2011; Churapa and Lerluck 2016; Kim and Lee 2020).

Crassisporus B.K. Cui & Xing Ji was proposed as a new genus (Ji et al. 2019) and it has effused-reflexed to pileate basidiomata with a mostly concentrically zonate pileal surface, a trimitic hyphal system with inamyloid or non-dextrinoid skeletal hyphae, oblong to broadly ellipsoid, slightly thick-walled basidiospores (Ji et al. 2019). Four species are included in this genus currently.

During the fungal research work on polypores, the phylogeny, based on a two loci dataset (ITS+nLSU), was carried out and two unknown species of *Perenniporia* s.l. are found from southwest China and they are illustrated and described in the present paper. In addition, two new combinations in *Crassisporus* are proposed, based on phylogenetic and morphological analyses.

Materials and methods

Morphological studies

The studied specimens are deposited in the Fungarium of the Institute of Microbiology, Beijing Forestry University (BJFC) and the Institute of Applied Ecology, Chinese Academy of Sciences (IFP). Morphological descriptions are based on field notes and voucher specimens. The microscopic analysis follows Miettinen et al. (2018) and Wu et al. (2022). Sections were studied at a magnification of up to 1000× using a Nikon Eclipse 80i microscope and phase contrast illumination. Microscopic features and measurements were made from slide preparations stained with 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+ = amyloid or dextrinoid, IKI- = neither amyloid nor dextrinoid, CB = Cotton Blue, CB+ = cyanophilous in 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. Colour 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 and to perform 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 PCR procedure for ITS was as follows: initial denaturation at 95 °C for 3 min, followed by 34 cycles at 94 °C for 40 s, annealing at 54 °C for 45 s and extension 72 °C for 1 min and a final extension of 72 °C for 10 min. The PCR procedure for nLSU 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 1 min and extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. The PCR products were purified and sequenced at the Beijing Genomics Institute (BGI), China, with the same primers. DNA sequencing was performed at the Beijing Genomics Institute and the newly-generated sequences were deposited in Gen-Bank. All sequences analysed in this study are listed in Table 1. 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 and nLSU 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 visualised in BioEdit (Hall 1999). Alignments were spliced and transformed formats in Mesquite v.3.2. (Maddison and Maddison 2017). Multiple se-

Species name Sampe no. Location TS I.SU Interaction Abundisporus pubertatis Dal 11927 China JN048771 JN048790 Zhao et al (2015) Abundisporus pubertatis Dal 11277 China KC707565 KC707575 Zhao et al (2015) Abundisporus calescetosus MUCL 1438 Singapore F.4111101 F.1393867 Robledo et al (2009) Armitosporia battorii Cui 10315 China JQ861726 KX900755 Cui et al (2019) Armitosporia battorii Dal 10315 China JQ861740 JQ861726 Cui et al (2019) Aurantioporia battorii Cui 11361 China MR847215 MG847224 Cui et al (2019) Citrinoporia critinoaba Cui 11361 China MK847215 MK847224 Cui et al (2019) Citrinoporia criticola Dal 18633 Malaysia M117217 M117222 Wang et al (2020) Citrinoporia criticola Dal 1778 Singapore MT18264 Hittel al (2019) Citrinoporia criticola Dal 18256 Malaysia M117216<		0		GenBank accession No.		
Abundisporus fuscopurpurus Cuis 8638 China JN048770 JN048770 Zhao et al. (2015) Abundisporus pubertatis Dal 12140 China JN048772 JN048791 Zhao et al. (2015) Abundisporus subertatis Dal 12140 China JN048772 JN048791 Zhao et al. (2015) Abundisporus voicecurs MUCL 14381 Singapore F./111101 F./393868 Robledo et al. (2009) Amylosporia hattorii Oui 10912 China JX69779 MH875242 Cui et al. (2019) Aurantioporia atmatiaca CBS 125867 French Guiana MH663779 MH875242 Cui et al. (2019) Attrinoporia cirtinoabia Da 13643 China KX880661 Cui et al. (2019) Cirtinoporia cirtinoabia Da 13643 Malaysia MT117218 MT117223 Wang et al. (2020) Cirtinoporia corticola Da 13643 Malaysia MT117218 MT117223 Wang et al. (2020) Cirtinoporia corticola Da 13643 China MK116495 Ji et al. (2019) Cirtinoporia corticola Da 13678 China	Species name	Sample no.	Location	ITS	nLSU	References
Abundisporus pubertatis Dai 11927 China KCR27576 Zhao et al. (2015) Abundisporus pubertatis Dai 12140 China JN048772 JN048772 JN048772 JN048773 Robledo et al. (2005) Abundisporus volaceus MUCL 38017 Zimbabwe FL4111101 FL393866 Robledo et al. (2009) Anylosporia hartorii Cui 1031 China KX900675 KX900755 Cui et al. (2019) Arantioporia aurantiopai bambusicola Cui 1030 China KX900668 KX900719 Cui et al. (2019) Arantioporia aurinoalis Cui 13615 China KK9800668 KX900719 Cui et al. (2019) Citrinoporia critinoalia Dal 13643 China KK880620 KK881061 Cui et al. (2019) Citrinoporia corticola Dal 13641 Malaysia MT11721 MT11722 Wang et al. (2020) Citrinoporia corticola Dal 13641 Malaysia MT11721 MT11722 Wang et al. (2020) Citrinoporia corticola Dal 13626 Malaysia MT11721 MT11722 Wang et al. (2019) Cr	Abundisporus fuscopurpureus	Cui 8638	China	JN048771	JN048790	Zhao et al. (2015)
Abundisporus pubertatis Dal 12140 China JM048772 JM048771 Zhon48791 Zhao et al. (2015) Abundisporus volocevas MUCL 3817 Zimbabwe F.1411100 F.1333867 Robledo et al. (2009) Arm/soporia hattorii Gui 10912 China KX900755 KX900725 Cui et al. (2019) Arm/soporia hattorii Da 10315 China JQ817765 Cui et al. (2019) Aurantoporia auranticac CBS 125867 French cuiana MH663779 MH875242 Cui et al. (2019) Aurantoporia burnoabia Cui 13615 China MK880622 KX880661 Cui et al. (2019) Citrinoporia cutrinoabia Da 18631 Malaysia MT17217 MT17222 Wang et al. (2020) Citrinoporia corticola Da 18636 Malaysia MT17216 MT17223 Wang et al. (2020) Citrinoporia corticola Da 18636 Malaysia MT17216 MT17223 Wang et al. (2020) Citrinoporia corticola Da 18526 Malaysia MT17216 MT17223 Wang et al. (2020) Citrinoporia corticola	Abundisporus pubertatis	Dai 11927	China	KC787569	KC787576	Zhao et al. (2015)
Abundisporus soleceus MUIC 41438 Singapore FJ411101 FJ393867 Robiedo et al. (2009) Abundisporus violaceus MUIC 30617 Zimbabwe FJ411100 FJ393867 Robiedo et al. (2019) Arnylosporia hattorii Dai 10315 China JQ861740 JQ861756 Cui et al. (2019) Aruntioporia varnitacea CBS 125867 French Cuiuan MH86779 MH875242 Vu et al. (2019) Aurantoporia varnitacia Cui 13615 China KX800628 KX900719 Cui et al. (2019) Cittrinoporia cittinoalba Cui 13615 China KX800622 KX80661 Cui et al. (2019) Cittrinoporia corticola Dai 13643 China KX800622 KX80612 Wang et al. (2020) Citrinoporia corticola Dai 17778 Singapore MT117218 MT117223 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MT117216 MT117223 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MT117216 MT117224 Wang et al. (2019) Crassisporus matrox <td>Abundisporus pubertatis</td> <td>Dai 12140</td> <td>China</td> <td>JN048772</td> <td>JN048791</td> <td>Zhao et al. (2015)</td>	Abundisporus pubertatis	Dai 12140	China	JN048772	JN048791	Zhao et al. (2015)
Abundisporus violaceus MUIC 38617 Zimbabwe Ful1100 Fu393867 Roledo et al (2009) Amylosporia hattorii Oui 10912 China JQ661740 JQ861740 JQ8617400	Abundisporus sclerosetosus	MUCL 41438	Singapore	FJ411101	FJ393868	Robledo et al. (2009)
Amylosporia hattorii Cui 10912 China KX900675 KX900725 Cui et al. (2019) Armylosporia hattorii Dai 10315 China JQ861740 JQ861756 Cui et al. (2019) Armatioporia aurantiaca CBS 125867 French Guiana MH863779 MH875242 Vu et al. (2019) Citrinoporia citrinoalba Cui 13615 China KX800622 KX880661 Cui et al. (2019) Citrinoporia citrinoalba Dai 13643 China KX800622 KX880661 Cui et al. (2020) Citrinoporia corticola Dai 18543 Malaysia MT117218 MT117223 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MT117218 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MK116488 MK116495 Ji et al. (2019) Crassisporus microsporus Cui 16801 Australia MK116486 MK116495 Ji et al. (2019) Crassisporus microsporus Dai 16221 China MX116486 MK116495 Ji et al. (2019) Crassisporus minutus <td< td=""><td>Abundisporus violaceus</td><td>MUCL 38617</td><td>Zimbabwe</td><td>FJ411100</td><td>FJ393867</td><td>Robledo et al. (2009)</td></td<>	Abundisporus violaceus	MUCL 38617	Zimbabwe	FJ411100	FJ393867	Robledo et al. (2009)
Amylosporia hattorii Dai 10315 China JQ861740 JQ861756 Cui et al. (2019) Aurantoporia aurantiaca CBS 125867 French Guiana MH863779 MH875242 Vu et al. (2019) Citrinoporia citrinoalba Cui 13615 China KX800668 KX90079 Cui et al. (2019) Citrinoporia citrinoalba Dai 18633 Malaysia MT117217 MT117222 Wang et al. (2020) Citrinoporia corticola Dai 18633 Malaysia MT117218 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MT117219 MT117224 Wang et al. (2020) Crissisporus indicatus Dai 10788 China KX867350 KX867425 Cui et al. (2019) Crassisporus indrosporus Cui 14468 China MK116486 MK116497 Ji et al. (2019) Crassisporus minutus Zhou 120 China JX163055 JX163056 Unpublished Crassisporus minutus Cui 6575 China JX141474 MK116497 Li et al. (2019) Crassisporus minutus Cui 6575	Amylosporia hattorii	Cui 10912	China	KX900675	KX900725	Cui et al. (2019)
Aurantioporia aurantiaca CBS 125867 French Guiana MH875279 MH875242 Vu et al. (2019) Aurantioporia bambusicola Cui 11050 China KX900768 KX900719 Cui et al. (2019) Citrinoporia cirtinoalba Dai 13643 China KX880622 KX88061 Cui et al. (2019) Citrinoporia corticola Dai 18633 Malaysia MT117218 MT117222 Wang et al. (2020) Citrinoporia corticola Dai 17778 Singapore MT117218 MT117212 Wang et al. (2020) Citrinoporia corticola Dai 1778 Singapore MT117218 MT117212 Wang et al. (2020) Crassisporus inbricatus Dai 10788 China KK16465 Jet al. (2019) Crassisporus macroporus Cui 16801 Australia MK116487 MK116496 Jet al. (2019) Crassisporus minutus Zhou 120 China JX164057 JK141451 JX141451 Unpublished Crassisporus minutus Dai 22571 China JX141451 JX141451 Zhao et al. (2015) Crassisporus minutus Dai 2257<	Amylosporia hattorii	Dai 10315	China	JQ861740	JQ861756	Cui et al. (2019)
Aurantioporia bambusicola Cui 11050 China KX900668 KX900719 Cui et al. (2019) Ottrinoporia citrinoalba Cui 13615 China MG847215 MG847221 Cui et al. (2019) Citrinoporia citrinoalba Dai 13643 China KX880661 Cui et al. (2019) Citrinoporia corticola Dai 18641 Malaysia MT117217 MT117222 Wang et al. (2020) Citrinoporia corticola Dai 1778 Singapore MT117216 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 17826 Malaysia MT117216 MT117224 Wang et al. (2020) Crassisporus microtus Dai 17778 Singapore MT117216 MT117217 Wing et al. (2019) Crassisporus macroporus Cui 14468 China MK116488 MK116496 Ji et al. (2019) Crassisporus minutus Cui 6257 China JX163055 JX163056 Unpublished Crassisporus minutus Cui 6257 China JX141451 JX141451 Zhao et al. (2015) Crassisporus minutus Cui 6257 China	Aurantioporia aurantiaca	CBS 125867	French Guiana	MH863779	MH875242	Vu et al. (2019)
Citrinoporia citrinoalba Cui 13615 China MG847215 MG847224 Cui et al. (2019) Citrinoporia citrinoalba Dai 13643 China KX8806612 Cui et al. (2019) Citrinoporia corticola Dai 18641 Malaysia MT117218 MT117223 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MT117218 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 17778 Singapore MT117218 MT117224 Wang et al. (2020) Crassisporus imbricatus Dai 10788 China KC867350 KC867425 Cui et al. (2019) Crassisporus macroporus Cui 16601 Australia MK116486 MK116495 Jiet al. (2019) Crassisporus macroporus Cui 16251 China MK116487 MK116496 Jiet al. (2019) Crassisporus minutus Dai 22571 China JX163050 Unpublished Crassisporus mollssimus Dui 1024 China JX141451 JX141452 ZJA04 Cystidioporia piceicola Cui 10460 China JF10638 <t< td=""><td>Aurantioporia bambusicola</td><td>Cui 11050</td><td>China</td><td>KX900668</td><td>KX900719</td><td>Cui et al. (2019)</td></t<>	Aurantioporia bambusicola	Cui 11050	China	KX900668	KX900719	Cui et al. (2019)
Citrinoporia citrinoalba Dai 13643 China KX880622 KX880661 Cui et al. (2019) Citrinoporia corticola Dai 18633 Malaysia MT117217 MT117223 Wang et al. (2020) Citrinoporia corticola Dai 18633 Malaysia MT117219 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 1778 Singapore MT117219 MT117224 Wang et al. (2020) Crassisporus Imbricatus Dai 10788 China KC667425 Cui et al. (2019) Crassisporus macroporus Cui 14660 Australia MK116488 MK116497 Ji et al. (2019) Crassisporus minutus Cui 6595 China MX16405 Ji et al. (2019) Crassisporus minutus Cui 6557 China JX163056 Unpublished Crassisporus minutus Cui 6577 China JX141451 JX141451 Zhao et al. (2015) Crassisporus minutus Cui 6577 China JX141451 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JQ81758 Zhao an Cui (2013)	Citrinoporia citrinoalba	Cui 13615	China	MG847215	MG847224	Cui et al. (2019)
Citrinoporia corticola Dai 18633 Malaysia MT117217 MT117222 Wang et al. (2020) Citrinoporia corticola Dai 18641 Malaysia MT117218 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 18526 Malaysia MT117216 MT117224 Wang et al. (2020) Crassisporus imbricatus Dai 10788 China KC867350 KC867425 Cui et al. (2019) Crassisporus mecroprus Cui 14681 Akt116488 MK116495 Ji et al. (2019) Crassisporus microsporus Dai 16221 China MK116487 MK116495 Ji et al. (2019) Crassisporus minutus Zuo 120 China JX163055 Unpublished Crassisporus minutus Dai 10764 China JX141451 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JX141452 JX141461 Zhao et al. (2015) Crassisporus mollissimus Dai 12659 Finland KP171208 KP17230 Han et al. (2015) Crassisporus mollissimus Dai 12659 Finland KP171208 KP17838 </td <td>Citrinoporia citrinoalba</td> <td>Dai 13643</td> <td>China</td> <td>KX880622</td> <td>KX880661</td> <td>Cui et al. (2019)</td>	Citrinoporia citrinoalba	Dai 13643	China	KX880622	KX880661	Cui et al. (2019)
Citrinoporia corticola Dal 18641 Malaysia MT117218 MT117223 Wang et al. (2020) Citrinoporia corticola Dal 17778 Singapore MT117219 MT117224 Wang et al. (2020) Citrinoporia corticola Dal 18526 Malaysia MT117216 MT117224 Wang et al. (2020) Citrasisporus imbricatus Dal 10788 China KC867350 KC867425 Cui et al. (2019) Crassisporus microsporus Dal 1621 China MK116488 MK116497 Ji et al. (2019) Crassisporus microsporus Dal 1221 China MK116487 MK116497 Ji et al. (2019) Crassisporus microsporus Dal 12521 China MK116487 MK116447 Unpublished Crassisporus molitusimus Dal 12571 China JX141451 JX141461 Zhao et al. (2015) Crassisporus molitusimus Dal 10764 China JX141452 JX141461 Zhao et al. (2015) Crassisporus molitusimus Dal 10764 China JX141452 JX141461 Zhao et al. (2016) Crassisporus molitusimus D	Citrinoporia corticola	Dai 18633	Malavsia	MT117217	MT117222	Wang et al. (2020)
Chrinoporia corticola Dai 13778 Singapore MT117210 MT117224 Wang et al. (2020) Citrinoporia corticola Dai 13778 Singapore MT117216 MT117221 Wang et al. (2020) Crassisporus inbricatus Dai 10788 China KC867350 KC867425 Cui et al. (2019) Crassisporus incorporus Cui 14681 Australia MK116486 MK116497 Ji et al. (2019) Crassisporus mirotus Zui 14468 China JX163055 Jupublished Crassisporus minutus Zui 6595 China JX163055 Jupublished Crassisporus minutus Dai 12271 China JX161019 FX081142 Unpublished Crassisporus mollissimus Cui 6257 China JX141451 JX141452 JX141452 JX141452 JX141452 Zhao et al. (2015) Crassisporus mollissimus Dai 1259 Finland KP171220 Han et al. (2015) Cystidioporia piceicola Cui 10400 China JF706338 Cui and Zhao et al. (2014) Dendroporia chereofusca Dai 1259 Finland	Citrinoporia corticola	Dai 18641	Malaysia	MT117218	MT117223	Wang et al. (2020)
Chrimoponia Groticola Dai 1852 Malaysia Mility 12 Mility 21 Wang et al. (2020) Crassisporus imbricatus Dai 10788 China KC867350 KC867425 Cui et al. (2019) Crassisporus imbricatus Dai 10788 China MK116486 MK116497 Ji et al. (2019) Crassisporus macroporus Cui 1468 China MK116486 MK116497 Ji et al. (2019) Crassisporus macroporus Dai 16221 China MK116487 MK116496 Ji et al. (2019) Crassisporus minutus Dai 16221 China JX163055 JX163056 Unpublished Crassisporus minutus Dai 22571 China JX141451 JX141451 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JX141452 JX141451 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JZ861742 JQ861758 Zhao et al. (2014) Cystidioporia piceicola Dai 14181 China JZ96628 JZ706336 Cui ad 2hao (2012) Deadroporia cinereofusca Dai 12659	Citrinoporia corticola	Dai 17778	Singapore	MT117219	MT117226	Wang et al. (2020)
Dari Nopul Culture Dari 1078 China Mitti 11710 Mitti 1211 Cult 21 (2019) Crassisporus imbricatus Dai 1078 China MK116488 MK116497 Ji et al. (2019) Crassisporus microsporus Cui 16801 Australia MK116486 MK116495 Ji et al. (2019) Crassisporus microsporus Dai 16221 China MK116487 MK116495 Ji et al. (2019) Crassisporus minutus Zhou 120 China JX163055 JX163056 Unpublished Crassisporus minutus Dai 22571 China JX141451 JX141461 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JX141451 JX141462 Zhao et al. (2015) Cystidioporia piceicola Cui 10460 China JZ861782 JZao and Cui (2013a) Cystidioporia piceicola Dai 12659 Finland KP171208 KP171230 Han et al. (2015) Dendroporia cinereofusca Dai 2280 China KF568893 KF568895 Zhao et al. (2014) Pomodermoporus martius Dai 12054 China	Citrinoporia corticola	Dai 18526	Malaysia	MT117216	MT117224	Wang et al. (2020)
Crassisporus minicalusDai 10760CuimaRoborsionRoborsionCrassisporus leucoporusCui 14680AustraliaMK116486MK116495Ji et al. (2019)Crassisporus microsporusDai 16221ChinaMK116487MK116495Ji et al. (2019)Crassisporus microsporusDai 16221ChinaMK116487MK116495Ji et al. (2019)Crassisporus minutusZhou 120ChinaMK116487MK116497MK16496Crassisporus minutusDai 22571ChinaFX081142UnpublishedCrassisporus milusimusDai 10764ChinaJX141451JX141461Zhao et al. (2015)Crassisporus mollissimusDai 10764ChinaJX8141452JX141462Zhao et al. (2015)Crassisporus mollissimusDai 10764ChinaJX8141451JX141452Zhao et al. (2015)Cystidioporia piceicolaDai 12659FinlandKP171208KP171208KP171208Deaddea quercinaDai 12659ChinaKF568893KF568895Zhao et al. (2014b)Dendroporia cinereofuscaCui 2200ChinaKF568893KF568894Zhao et al. (2014b)Dendroporia laiseriousaCui 625ChinaHK26404HQ876604Zhao et al. (2014b)Dendroporus latissimusDai 12054ChinaKK2644857UnpublishedHornodermoporus martiusMUCL 41677ArgentinaF,J411093F,J333869Robledo et al. (2009)Hornodermoporus martiusMUCL 41677ArgentinaF,J411093F,J333860Robledo e	Crassisporus imbricatus	Dai 10788	China	KC867350	KC867425	Cui et al. (2010)
Crassisporus macroporus Cui 14468 China MK116496 MK116495 Jiet al. (2019) Crassisporus macroporus Dai 16221 China MK116486 MK116496 Jiet al. (2019) Crassisporus minutus Zho 120 China JX163055 JX163056 Unpublished Crassisporus minutus Dai 22571 China PP034110* Present study Crassisporus moltissimus Dai 22571 China JX141451 JX141461 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JX141451 JX141462 Zhao et al. (2015) Crystidioporia piceicola Dai 11064 China JZ861742 JQ861758 Zhao et al. (2015) Crystidioporia piceicola Dai 112659 Finland KP171208 KP171230 Han et al. (2015) Dendroporia cinereofusca Dai 12659 Finland KF568893 Khao et al. (2014b) Dendroporia cinereofusca Dai 12054 China KF568895 Zhao et al. (2014b) Pomotersis pinicola Cui 10405 China KK9768892 K2hao et al. (2014b)		Cui 16801	Australia	MK116488	MK116407	li et al. (2019)
Crassisporus microsporus Dei 16221 China IMR 110430 IMR 110430 Jet al. (2019) Crassisporus minutus Zhou 120 China JX163055 JX163056 Unpublished Crassisporus minutus Cui 6595 China KX116447 MK116447 MK116447 MK116447 MK116447 Unpublished Crassisporus minutus Dai 22571 China PP034100* PP034116* Present study Crassisporus mollissimus Dai 10764 China JX141451 JX141451 Zhao et al. (2015) Cystidioporia piceicola Dai 10764 China JX661742 JQ861758 Zhao et al. (2015) Deadalea quercina Dai 12659 Finland KP171208 KP17120 Han et al. (2015) Dendroporia cinereofusca Dai 9289 China KF568893 KF568895 Zhao et al. (2014b) Dendroporia cinereofusca Cui 5280 China KF568892 KF568895 Zhao et al. (2014b) Pomitopsis pinicola Cui 1625 China HQ876604 HQ876604 Zhao et al. (2019)	Crassisporus reacoporus	Cui 14469	China	MK116486	MK116497	li et al. (2019)
Crassisporus minutus Dial 10221 China MK116487 WK116487 UR116487 UR116487 <thur16487< th=""> UR116487 <thur16487< th=""></thur16487<></thur16487<>		Cui 14400	China	NK116407	MK116495	Ji et al. (2019)
Crassisporus minutusZindu 120ChinaJA 163035On 10 publishedCrassisporus minutusDai 22571ChinaPP034110ªPP034116ªPresent studyCrassisporus moltissimusCui 6595ChinaJX141451JX141461Zhao et al. (2015)Crassisporus mollissimusDai 10764ChinaJX141451JX141462Zhao et al. (2015)Crassisporus mollissimusDai 10764ChinaJX141452JX161363Cui and Zhao et al. (2015)Cystidioporia piceicolaDai 112659FinlandKP171208KP171230Han et al. (2015)Dendroporia cinereofuscaDai 9289ChinaKF568893KF568895Zhao et al. (2014b)Dendroporia cinereofuscaCui 1280ChinaKF568892KF568894Zhao et al. (2014b)Dendroporia sincolaCui 10405ChinaKK244852KK244857UnpublishedHornodermoporus latissimaDai 12054ChinaKX900639KY900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393850Robledo et al. (2009)Hornodermoporus martiusCui 16742AustraliaOK642221OK642275J et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642210OK642276J et al. (2023)Luteoperenniporia banaensisCui 8562ChinaJQ291727JQ291730Zhao and Cui (2013a)LuteoperenniporiaCL Zhao 5152ChinaMH784913MH784916Zhao and Cui (2013a)Luteoperenniporia		Dai 16221	China	IVIK I 10487	IV162056	Ji et al. (2019)
Crassisporus minutusCui 6393ChinaKX061079KX061142UnipublishedCrassisporus minutusDai 22571ChinaJX141451JX141461Zhao et al. (2015)Crassisporus mollissimusDai 10764ChinaJX141451JX141461Zhao et al. (2015)Cystidioporia piceicolaDai 10764ChinaJX141452JX141462Zhao et al. (2015)Cystidioporia piceicolaDai 1481ChinaJF706328JF706336Cui and Zhao (2012)Daedalea quercinaDai 12659FinlandKP171208KP171230Han et al. (2014)Dendroporia cinereofuscaDai 2289ChinaKF568893KF568895Zhao et al. (2014b)Dendroporia cinereofuscaCui 5280ChinaKF568892KF568894Zhao et al. (2014b)Pomitopsis pinicolaCui 10405ChinaKK900639KX900686Cui et al. (2014b)Hornodermoporus latissimaDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393860Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2014)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642220OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291730Zhao and Cui (2013a) <tr<< td=""><td></td><td></td><td>China</td><td>JX103055</td><td>JX103050</td><td>Unpublished</td></tr<<>			China	JX103055	JX103050	Unpublished
Crassisporus minuus Dai 22371 China PP034109 PP034109 PP034104 Crassisporus mollissimus Cui 6257 China JX141451 JX141461 Zhao et al. (2015) Crassisporus mollissimus Dai 10764 China JX141452 JX141462 Zhao et al. (2015) Cystidioporia piceicola Dai 10764 China JQ861742 JQ861758 Zhao et al. (2015) Daedalea quercina Dai 12659 Finland KP171208 KP171230 Han et al. (2014) Dendroporia cinereofusca Dai 9289 China KF568893 KF568894 Zhao et al. (2014b) Dendroporia cinereofusca Cui 10405 China KC844852 KC844857 Unpublished Hornodermoporus latissimus Dai 12054 China KX900639 KX900646 Cui et al. (2014) Hornodermoporus martius MUCL 41677 Argentina FJ411092 FJ393859 Robledo et al. (2009) Hornodermoporus martius Cui 16742 Australia OK642221 OK642275 Ji et al. (2013) Luteoperenniporia australiensis Cui 1		Cui 0595	China	KXU81079	KXU81142	Unpublished
Crassisporus moiissimusCui o237ChinaJX141451JX141461Zhao et al. (2015)Crassisporus mollissimusDai 10764ChinaJX141452JX141462Zhao et al. (2015)Cystidioporia piceicolaCui 10460ChinaJQ81742JX817462Zhao et al. (2015)Cystidioporia piceicolaDai 12659FinlandKP171208KP171230Han et al. (2012)Deadalea quercinaDai 12659FinlandKP171208KP171230Han et al. (2014)Dendroporia cinereofuscaDai 2880ChinaKF568893KF568894Zhao et al. (2014b)Dendroporia cinereofuscaCui 10405ChinaKK2844852KC844857UnpublishedHornodermoporus latissimaCui 6625ChinaHQ876604HQ876604Zhao et al. (2014a)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393850Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaJQ21727Ja et al. (2023)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 8562ChinaJQ291728JQ291729Zhao and Cui (2013a)LuteoperenniporiaCui 13627ChinaMH784913MH784916Zhao and Cui (2013a)LuteoperenniporiaCui 13625ChinaJQ291727JQ291729Zhao and Cui (2013a)LuteoperenniporiaCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)LuteoperenniporiaCu		Dai 22571	China	PP034100*	PP034116*	Present study
Crassisporus molinssimusDai 10764ChinaJX141452JX141452JX141452Zhao et al. (2015)Cystidioporia piceicolaCui 10460ChinaJQ861742JQ861758Zhao et al. (2013)Cystidioporia piceicolaDai 4181ChinaJF706328JF706336Cui and Zhao (2012)Daedalea quercinaDai 12659FinlandKP171208KP171230Han et al. (2014)Dendroporia cinereofuscaDai 9289ChinaKF56893KF568895Zhao et al. (2014b)Dendroporia cinereofuscaCui 5280ChinaKF568893KF568894Zhao et al. (2014b)Fomitopsis pinicolaCui 625ChinaHQ876604HQ876604Zhao et al. (2014b)Hornodermoporus latissimaDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393860Robledo et al. (2009)Hornodermoporus martiusMUCL 41674AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16742AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291730Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5145ChinaMH784913MH784917Zhao and Ma (2019)mopanshanensisCui 13627ChinaMH784913MH784917Zhao and Ma (2019)mopanshanensisCui 13627ChinaMH784913MH784917Zhao and Cui (2013a)Mac	Crassisporus mollissimus	Cui 6257	China	JX141451	JX141461	Zhao et al. (2015)
Cystidioporia piceicolaCui 10460ChinaJURE 1742JURE	Crassisporus mollissimus	Dai 10764	China	JX141452	JX141462	Zhao et al. (2015)
Cystidiopona piceicolaDai 12659FinlandGH/0528GJ/106336GLu and Zhao (2012)Daedalea quercinaDai 12659FinlandKP171208KP171230Han et al. (2015)Dendroporia cinereofuscaDai 9289ChinaKF568893KF568894Zhao et al. (2014b)Dendroporia cinereofuscaCui 5280ChinaKF568892KF568894Zhao et al. (2014b)Fornitopsis pinicolaCui 10405ChinaKK568892KK568895UnpublishedHornodermoporus latissimaDai 12054ChinaHQ876604HQ876604Zhao et al. (2014a)Hornodermoporus nartiusDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393850Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8562ChinaJQ291727JQ291729Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5152ChinaMH784913MH784917Zhao and Ma (2019)mopanshanensisCui 13627ChinaMH784913MH427965Cui et al. (2019)LuteoperenniporiaCui 13627ChinaJX14148JX141458Zhao and Cui (2013a)Macroporia lac	Cystidioporia piceicola	Cui 10460	China	JQ861742	JQ861758	Zhao and Cui (2013a)
Daedalea quercinaDai 12659FinlandKP1 71208KP1 71230Han et al. (2015)Dendroporia cinereofuscaDai 9289ChinaKF568893KF568895Zhao et al. (2014b)Dendroporia cinereofuscaCui 5280ChinaKF568892KF568894Zhao et al. (2014b)Dendroporia cinereofuscaCui 5280ChinaKK568892KK568894Zhao et al. (2014b)Hornodermoporus latissimaCui 6625ChinaHQ876604HQ876604Zhao et al. (2014a)Hornodermoporus martiusDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393860Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 16742AustraliaOK642210OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642211OK642276Ji et al. (2013)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5152ChinaMH784912MH784916Zhao and Ma (2019)mopanshanensisCui 13627ChinaMH427960MH427967Cui et al. (2019)yinggelingensisCui 13627ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141448JX141459Zhao and Cui (2013a)Macropor	Cystidioporia piceicola	Dai 4181	China	JF/06328	JF/06336	Cui and Zhao (2012)
Dendroporia cinereofuscaDai 9289ChinaKF568893KF568895Zhao et al. (2014b)Dendroporia cinereofuscaCui 5280ChinaKF568892KF568894Zhao et al. (2014b)Fomitopsis pinicolaCui 10405ChinaKK5684852KC844857UnpublishedHornodermoporus latissimaDai 12054ChinaHQ876604HQ876604Zhao et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393859Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia australiensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5152ChinaMH784913MH784916Zhao and Ma (2019)mopanshanensisCui 13627ChinaMH427960MH427965Cui et al. (2013a)LuteoperenniporiaCui 13627ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141448JX141459Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX861764Zhao and Cui (2013a)Macroporia subrhizomor	Daedalea quercina	Dai 12659	Finland	KP171208	KP171230	Han et al. (2015)
Dendroporia cincreofuscaCui 5280ChinaKF568892KF568894Zhao et al. (2014b)Fomitopsis pinicolaCui 10405ChinaKC844852KC844857UnpublishedHornodermoporus latissimaCui 6625ChinaHQ876604HQ876604Zhao et al. (2014a)Hornodermoporus latissimusDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393859Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2019)Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Ma (2019)mopanshanensisCL Zhao 5145ChinaMH784912MH784916Zhao and Ma (2019)LuteoperenniporiaCL Zhao 5152ChinaMH784913MH784917Zhao and Cui (2013a)LuteoperenniporiaCui 13627ChinaJX14148JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX14148JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX141448JX141458Zhao and Cui (2013a)Macropor	Dendroporia cinereofusca	Dai 9289	China	KF568893	KF568895	Zhao et al. (2014b)
Fomitopsis pinicolaCui 10405ChinaKC844852KC844857UnpublishedHornodermoporus latissimaCui 6625ChinaHQ876604HQ876604Zhao et al. (2014a)Hornodermoporus latissimusDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393859Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642221OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Ma (2019)mopanshanensisCui 13625ChinaMH784912MH784916Zhao and Ma (2019)Luteoperenniporia mopanshanensisCui 13627ChinaMH427960MH427967Cui et al. (2013a)Luteoperenniporia loggelingensisCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX861764Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX861764Zhao and Cui (2013a)M	Dendroporia cinereofusca	Cui 5280	China	KF568892	KF568894	Zhao et al. (2014b)
Hornodermoporus latissimaCui 6625ChinaHQ876604HQ876604Zhao et al. (2014a)Hornodermoporus latissimusDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393859Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411092FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)mopanshanensisCui 13625ChinaMH784913MH784917Zhao and Ma (2019)uteoperenniporiaCui 13627ChinaMH427960MH427967Cui et al. (2019)yinggelingensisCui 13627ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX86174JAo and Cui (2013a)Macroporia macroporaZhou 280ChinaJX8440MZ578444Tian et al. (2021) <tr <tr="">Macroporia nandripensis<</tr>	Fomitopsis pinicola	Cui 10405	China	KC844852	KC844857	Unpublished
Hornodermoporus latissimusDai 12054ChinaKX900639KX900686Cui et al. (2019)Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393859Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784912MH784916Zhao and Ma (2019)InopanshanensisCui 13625ChinaMH784913MH784917Zhao and Ma (2019)Iuteoperenniporia mopanshanensisCui 13627ChinaMH427960MH427967Cui et al. (2019)JinggelingensisCui 13627ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX861748JQ861764Zhao and Cui (2013a)Macroporia nancingoria nacroporia nanlingensisCui 7620ChinaMK2578440	Hornodermoporus latissima	Cui 6625	China	HQ876604	HQ876604	Zhao et al. (2014a)
Hornodermoporus martiusMUCL 41677ArgentinaFJ411092FJ393859Robledo et al. (2009)Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)mopanshanensisCui 13625ChinaMH784913MH784917Zhao and Ma (2019)uteoperenniporiaCui 13625ChinaMH427960MH427967Cui et al. (2019)uteoperenniporiaCui 13627ChinaMH427957MH427965Cui et al. (2019)yinggelingensisCui 13627ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141448JX141459Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macro	Hornodermoporus latissimus	Dai 12054	China	KX900639	KX900686	Cui et al. (2019)
Hornodermoporus martiusMUCL 41678ArgentinaFJ411093FJ393860Robledo et al. (2009)Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)LuteoperenniporiaCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)LuteoperenniporiaCL I13625ChinaMH427960Cui et al. (2019)yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macroporia naningensisCui 7620ChinaHQ84877HQ848486Zhao and Cui (2013a)	Hornodermoporus martius	MUCL 41677	Argentina	FJ411092	FJ393859	Robledo et al. (2009)
Hornodermoporus martiusCui 7992ChinaHQ876603HQ654114Zhao et al. (2014a)Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)Luteoperenniporia mopanshanensisCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)InopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCui 13625ChinaMH427960MH427967Cui et al. (2019)Luteoperenniporia mopanshanensisCui 13627ChinaMH427957MH427965Cui et al. (2019)JuggelingensisCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX861764Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia nanilingensisCui 7620ChinaMZ578440MZ578444Tian et al. (2021)	Hornodermoporus martius	MUCL 41678	Argentina	FJ411093	FJ393860	Robledo et al. (2009)
Luteoperenniporia australiensisCui 16742AustraliaOK642220OK642275Ji et al. (2023)Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)Luteoperenniporia mopanshanensisCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCui 13625ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCui 13627ChinaMH427960MH427967Cui et al. (2019)JinggelingensisCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Hornodermoporus martius	Cui 7992	China	HQ876603	HQ654114	Zhao et al. (2014a)
Luteoperenniporia australiensisCui 16743AustraliaOK642221OK642276Ji et al. (2023)Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)Luteoperenniporia mopanshanensisCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCui 13625ChinaMH427960MH427967Cui et al. (2019)JuggelingensisCui 13627ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141448JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia australiensis	Cui 16742	Australia	OK642220	OK642275	Ji et al. (2023)
Luteoperenniporia bannaensisCui 8560ChinaJQ291727JQ291729Zhao and Cui (2013a)Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)Luteoperenniporia mopanshanensisCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia yinggelingensisCui 13625ChinaMH427960MH427967Cui et al. (2019)Luteoperenniporia yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Macroporia lacerataCui 7220ChinaJX14148JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia australiensis	Cui 16743	Australia	OK642221	OK642276	Ji et al. (2023)
Luteoperenniporia bannaensisCui 8562ChinaJQ291728JQ291730Zhao and Cui (2013a)Luteoperenniporia mopanshanensisCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia yinggelingensisCui 13625ChinaMH427960MH427967Cui et al. (2019)Luteoperenniporia yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJZ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia bannaensis	Cui 8560	China	JQ291727	JQ291729	Zhao and Cui (2013a)
Luteoperenniporia mopanshanensisCLZhao 5145ChinaMH784912MH784916Zhao and Ma (2019)Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia mopanshanensisCui 13625ChinaMH427960MH427967Cui et al. (2019)Luteoperenniporia yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Luteoperenniporia yinggelingensisCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia bannaensis	Cui 8562	China	JQ291728	JQ291730	Zhao and Cui (2013a)
Luteoperenniporia mopanshanensisCL Zhao 5152ChinaMH784913MH784917Zhao and Ma (2019)Luteoperenniporia yinggelingensisCui 13625ChinaMH427960MH427967Cui et al. (2019)Luteoperenniporia yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Luteoperenniporia yinggelingensisCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataCui 7220ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia mopanshanensis	CLZhao 5145	China	MH784912	MH784916	Zhao and Ma (2019)
Luteoperenniporia yinggelingensisCui 13625ChinaMH427960MH427967Cui et al. (2019)Luteoperenniporia yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia mopanshanensis	CL Zhao 5152	China	MH784913	MH784917	Zhao and Ma (2019)
Luteoperenniporia yinggelingensisCui 13627ChinaMH427957MH427965Cui et al. (2019)Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia yinggelingensis	Cui 13625	China	MH427960	MH427967	Cui et al. (2019)
Macroporia lacerataCui 7220ChinaJX141448JX141458Zhao and Cui (2013a)Macroporia lacerataDai 11268ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Luteoperenniporia yinggelingensis	Cui 13627	China	MH427957	MH427965	Cui et al. (2019)
Macroporia lacerataDai 11268ChinaJX141449JX141459Zhao and Cui (2013a)Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Macroporia lacerata	Cui 7220	China	JX141448	JX141458	Zhao and Cui (2013a)
Macroporia macroporaZhou 280ChinaJQ861748JQ861764Zhao and Cui (2013a)Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Macroporia lacerata	Dai 11268	China	JX141449	JX141459	Zhao and Cui (2013a)
Macroporia subrhizomorphaLWZ 20190722-36ChinaMZ578440MZ578444Tian et al. (2021)Macrosporia nanlingensisCui 7620ChinaHQ848477HQ848486Zhao and Cui (2013a)	Macroporia macropora	Zhou 280	China	JQ861748	JQ861764	Zhao and Cui (2013a)
Macrosporia nanlingensis Cui 7620 China HQ848477 HQ848486 Zhao and Cui (2013a)	Macroporia subrhizomorpha	LWZ 20190722-36	China	MZ578440	MZ578444	Tian et al. (2021)
	Macrosporia nanlingensis	Cui 7620	China	HQ848477	HQ848486	Zhao and Cui (2013a)

Table 1. Information for the sequences used in this study.

On a size wome	Comula na	Lasatian	GenBank accession No.		Deferences
Species name	Sample no.	Location	ITS	nLSU	References
Macrosporia nanlingensis	Cui 7541	China	HQ848479	HQ848488	Zhao and Cui (2013a)
Microporellus subadustus	Cui 8459	China	HQ876606	HQ654113	Ji et al. (2023)
Microporellus violaceo-	MUCL 45229	Ethiopia	FJ411106	FJ393874	Robledo et al. (2009)
Minoporus minor	Cui 5782	China	H0883475		Zhao and Cui (2013a)
Minoporus minor	Dai 0108	China	KE405005	KE405016	Cui et al. (2010)
Necessia bestenensia			MC401294	MC401297	Shop at al. (2019)
Neoporia bostonensis	CL 7bao 2855	USA	MG491204	MC401285	Shen et al. (2018)
Neoporia koroono	CL 21180 2000	Voraa	V 1156212	V 1156205	
Neoporia koreana	KUC20091030-32	Korea	KJ150313	KJ150303	Jang et al. (2015)
Neoporia rhizomorpho	Cui 7507	China	ЦО654107	H0654117	Zhao and Cui (2012a)
Neoporia rhizomorpha	Doi 7249	China	HQ034107		Zhao and Cui (2013a)
Neopona mizomorpha	Dai 1248	Theiland	JF/00330	JF700348	
	Dai 16660	Theiland	K1475500	0F209291	Ji et al. (2017)
	Dai 10000	Ohima	K14/330/	0P289292	Ji et al. (2017)
Niveoporia russeimarginata	Yuan 1244	China	JU801750	JU801700	
Niveopona subrusseimarginata	Cui 16991	China	0K642224	0K642279	Ji et al. (2023)
Niveoporia subrusseimarginata	Cui 16980	China	UK042223	0K042278	Ji et al. (2023)
Perenniporia cf. dendrohyphidia	Zhou 273	China	KX900670	-	Cui et al. (2019)
Perenniporia eugeissonae	Dai 18600	Malaysia	MT232518	M1232512	Wang et al. (2020)
Perenniporia eugeissonae	Dai 18605	Malaysia	MT232519	M1232513	Wang et al. (2020)
Perenniporia hainaniana	Cui 6366	China	JQ861/45	JQ861761	Zhao and Cui (2013a)
Perenniporia hainaniana	Cui 6365	China	JQ861/44	JQ861760	Zhao and Cui (2013a)
Perenniporia luteola	Harkonen 1308a	China	JX141456	JX141466	Zhao and Cui (2013b)
Perenniporia luteola	Harkonen 1308b	China	JX141457	JX141467	Zhao and Cui (2013b)
Perenniporia medulla-panis	Cui 3274	China	JN112792	JN112793	Zhao et al. (2014a)
Perenniporia medulla-panis	MUCL 43250	Norway	FJ411087	FJ393875	Robledo et al. (2009)
Perenniporia nonggangensis	GXU 2098	China	KT894732	KT894733	Huang et al. (2017)
Perenniporia nonggangensis	Dai 17857	Singapore	MT232521	MT232515	Huang et al. (2017)
Perenniporia prunicola	Dai 24280	China	PP034101ª	PP034117ª	Present study
Perenniporia prunicola	Dai 24751	China	PP034102ª	PP034118ª	Present study
Perenniporia prunicola	Dai 24752	China	PP034103ª	_	Present study
Perenniporia pseudotephropora	Dai 17383	Brazil	MT117215	MT117220	Wang et al. (2020)
Perenniporia rosicola	Dai 22563	China	PP034110ª	PP034123ª	Present study
Perenniporia straminea	Cui 8858	China	HQ654104	JF706334	Zhao and Cui (2013a)
Perenniporia straminea	Cui 8718	China	HQ876600	HQ876600	Zhao and Cui (2013a)
Perenniporia substraminea	Cui 10191	China	JQ001853	JQ001845	Zhao et al. (2014a)
Perenniporia substraminea	Cui 10177	China	JQ001852	JQ001844	Zhao et al. (2014a)
Perenniporia subtephropora	Dai 10962	China	JQ861752	JQ861768	Zhao and Cui (2013a)
Perenniporia subtephropora	Dai 24890	China	PP034104ª	PP034119ª	Present study
Perenniporia subtephropora	Dai 25025	China	PP034105ª	PP034120ª	Present study
Perenniporia subtephropora	Dai 24871	China	PP034106ª	_	Present study
Perenniporia subtephropora	Dai 10964	China	JQ861753	JQ861769	Zhao and Cui (2013a)
Perenniporia subtephropora	Dai 24877	China	PP034107ª	PP034121ª	Present study
Perenniporia tephropora	Cui 9029	China	HQ876601	JF706339	Zhao and Cui (2013a)
Perenniporia tephropora	Cui 6331	China	HQ848473	HQ848484	Zhao and Cui (2013a)
Perenniporia tephropora	Dai 25106	China	PP034108ª	-	Present study
Perenniporia tephropora	Dai 24849	China	PP034109ª	PP034122ª	Present study
Perenniporiella chaquenia	MUCL 47647	Argentina	FJ411083	FJ393855	Robledo et al. (2009)
Perenniporiella chaquenia	MUCL 47648	Argentina	FJ411084	FJ393856	Robledo et al. (2009)
Perenniporiella micropora	MUCL 43581	Cuba	FJ411086	FJ393858	Robledo et al. (2009)
Perenniporiopsis minutissima	Cui 10979	China	KF495003	KF495013	Cui et al. (2019)
Perenniporiopsis minutissima	Dai 12457	China	KF495004	KF495014	Cui et al. (2019)
Perenniporiopsis minutissima	Dai 17383	Brazil	MT117215	MT117220	Wang et al. (2020)
Perenniporiopsis minutissima	Dai 24887	China	PP034111ª	_	Present study

Species nome	Comula na	Location	GenBank accession No.		Deferreres
Species name	Sample no.	Location	ITS	nLSU	References
Perenniporiopsis minutissima	Dai 24885	China	PP034112ª	_	Present study
Perenniporiopsis minutissima	Cui 10221	China	KX962546	KX962553	Wu et al. (2017)
Perenniporiopsis sinensis	Dai 26477	China	PP034113ª	PP034124ª	Present study
Perenniporiopsis sinensis	CLZhao 8278	China	OR149913	OR759768	Yang et al. (2024)
Poriella africana	Cui 8674	China	KF018119	KF018128	Zhao et al. (2015)
Poriella africana	Cui 8676	China	KF018120	KF018129	Zhao et al. (2015)
Poriella ellipsospora	Cui 10284	China	JQ861739	KF018133	Shen et al. (2018)
Poriella ellipsospora	Cui 10276	China	KF018124	KF018132	Shen et al. (2018)
Poriella subacida	Dai 8224	China	HQ876605	JF713024	Zhao and Cui (2013a)
Poriella valliculorum	LE 222974	Russia	KM411458	KM411474	Zmitrovich and Kovalenko (2016)
Poriella valliculorum	Cui 10053	China	KF495006	KF495017	Zhao et al. (2014a)
Rhizoperenniporia japonica	Cui 7047	China	KX900677	KX900727	Cui et al. (2019)
Sparsitubus nelumbiformis	Cui 6590	China	KX880632	KX880671	Cui et al. (2019)
Sparsitubus nelumbiformis	Cui 8497	China	KX880631	KX880670	Cui et al. (2019)
Tropicoporia aridula	Dai 12398	China	JQ001855	JQ001847	Zhao and Cui (2013a)
Tropicoporia aridula	Dai 12396	China	JQ001854	JQ001846	Zhao and Cui (2013a)
Truncatoporia pyricola	Cui 9149	China	JN048762	JN048782	Zhao and Cui (2013a)
Truncatoporia pyricola	Dai 10265	China	JN048761	JN048781	Zhao and Cui (2013a)
Truncatoporia truncatospora	Cui 6987	China	JN048778	HQ654112	Zhao and Cui (2013a)
Truncatoporia truncatospora	Dai 5125	China	HQ654098	HQ848481	Zhao and Cui (2013a)
Truncospora detrita	MUCL 42649	French Guiana	FJ411099	FJ411099	Robledo et al. (2009)
Truncospora macrospora	Cui 8106	China	JX941573	JX941596	Zhao and Cui (2013c)
Truncospora ochroleuca	MUCL 39726	China	FJ411098	FJ393865	Robledo et al. (2009)
Truncospora ochroleuca	Dai 11486	China	HQ654105	JF706349	Zhao and Cui (2012)
Truncospora ochroleuca	MUCL 39563	Australia	FJ411097	FJ393864	Robledo et al. (2009)
Truncospora ohiensis	Cui 5714	China	HQ654103	HQ654116	Cui and Zhao (2012)
Truncospora ohiensis	MUCL 41036	USA	FJ411096	FJ393863	Robledo et al. (2009)
Truncospora ornata	SP 6672	Russia	KJ410690	_	Spirin et al. (2015)
Vanderbylia delavayi	Dai 6891	China	JQ861738	_	Zhao et al. (2014a)
Vanderbylia fraxinea	Cui 8871	China	JF706329	JF706345	Zhao et al. (2014a)
Vanderbylia fraxinea	Cui 8885	China	HQ876611	JF706344	Zhao et al. (2014a)
Vanderbylia fraxinea	DP 83	Italv	AM269789	AM269853	Gualielmo et al. (2007)
Vanderbylia robiniophila	Cui 7144	China	HQ876608	JF706341	Zhao et al. (2014a)
Vanderbylia robiniophila	Cui 5644	China	H0876609	H0876609	Zhao and Cui (2013a)
Vanderbylia vicina	MUCL 44779	Ethiopia	FJ411095	FJ393862	Robledo et al. (2009)
Vanderbyliella sp.	Knudsen 04-111	China	J0861737	J0861755	Zhao and Cui (2013a)
Vanderbyliella tianmuensis	Cui 2715	China	JX141454	JX141464	Zhao and Cui (2013a)
Vanderbyliella tianmuensis	Cui 2648	China	JX141453	JX141463	Zhao and Cui (2013a)
Xanthoperenniporia maackiae	Dai 8929	China	H0654102	JF706338	Zhao and Cui (2013a)
Xanthoperenniporia maackiae	Cui 5605	China	JN048760	JN048780	Zhao et al. (2013b)
Xanthoperenniporia punctata	Dai 26121	China	PP034114ª	_	Present study
Xanthoperenniporia punctata	Dai 26120	China	PP034115ª	_	Present study
Xanthoperenniporia punctata	Dai 17916	China	MG869686	MG869688	Li et al. (2018)
Xanthoperenniporia subcorticola	Dai 7330	China	H0654094	H0654108	Zhao and Cui (2013a)
Xanthoperenniporia subcorticola	Cui 1248	China	H0848472	H0848482	Zhao and Cui (2013a)
Xanthoperenniporia subcorticola	Cui 2655	China	H0654093	H0654093	Zhao and Cui (2012)
Xanthoperenniporia tenuis	Wei 2969	China	J0001859	J0001849	Zhao and Cui (2013a)
Xanthoperenniporia tenuis	Wei 2783	China	J0001858	J0001848	Zhao and Cui (2013a)
Yuchengia kilemariensis	LE 214743	Russia	KM411457	KM411473	Zmitrovich and Kovalenko (2016)
Yuchengia narymica	Dai 10510	China	HQ654101	JF706346	Zhao et al. (2013b)

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

quence 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, one combined matrix was reconstructed for phylogenetic analyses; a two loci dataset (ITS+nLSU) was used to determine the phylogenetic position of the new species. The sequence alignments and the retrieved topologies were deposited in TreeBase (http://www.treebase.org), under accession ID: 31050 (Reviewer access URL: http://purl.org/phylo/treebase/phylows/ study/TB2:S31050?x-access-code=fa4d2a2edcdd53d63276b66a95c2058d&format=html). Sequences of *Fomitopsis pinicola* (Sw.) P. Karst. and *Daedalea quercina* (L.) Pers., obtained from GenBank, were used as the outgroups (Ji et al. 2023). The phylogenetic analyses followed the approach of Han et al. (2016) and Zhu et al. (2019). Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed, based on the two datasets. The best-fit evolutionary model was selected by Hierarchical Likelihood Ratio Tests (HLRT) and Akaike Information Criterion (AIC) in MrModelTest 2.2 (Nylander 2004) after scoring 24 models of evolution in PAUP* version 4.0b10 (Swofford 2002).

Sequences were analysed using Maximum Likelihood (ML) with RAxML-HPC2 through the CIPRES Science Gateway (www.phylo.org; Miller et al. 2010). Branch support (BT) for ML analysis was determined by 1000 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 5 M generations (two loci dataset) 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 for ML (\geq 75% (ML-BS)) and BPP (\geq 0.95 BPP) were considered as significantly supported. The ML bootstrap (ML) \geq 50% and BBP (BPP) \geq 0.90 are presented on topologies from ML analysis, respectively.

Results

Molecular phylogeny

The combined two loci dataset (ITS+nLSU) included sequences from 152 samples representing 80 taxa. The dataset had an aligned length of 2156 characters, of which 1385 (64%) characters were constant, 147 (7%) were variable and parsimony-uninformative and 624 (29%) were parsimony informative. The phylogenetic reconstruction performed with Maximum Likelihood (ML) and Bayesian Inference (BI) analyses for one combined dataset showed similar topology and few differences in statistical support. The best model-fit applied in the Bayesian analysis was GTR+I+G, lset nst = 6, 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 = 0.007133 to ML analysis and, thus, only the ML tree is provided (Fig. 1).



Figure 1. ML analysis of *Perenniporia* s.l. based on dataset of ITS+nLSU. ML bootstrap values higher than 50% and Bayesian posterior probabilities values more than 0.90 are shown. New taxa are in bold.

The phylogeny (Fig. 1) included 28 different genera in *Perenniporia* s.l., of which have eight uncertain species in regard to the generic status without typical characteristics, viz. *P. eugeissonae* P. Du & Chao G. Wang, *P. luteola* B.K. Cui & C.L. Zhao, *P. nonggangensis* F.C. Huang & Bin Liu, *P. pseudotephropora* Chao G. Wang & F. Wu, *P. rosicola*, *P. straminea* (Bres.) Ryvarden, *P. subtephropora* B.K. Cui & C.L. Zhao and *P. tephropora*. Thus, they were adopted in *Perenniporia* temporarily and distinguished from *Perenniporia* s.s.

Perenniporia prunicola nested in the Perenniporis s.s. clade and formed an independent lineage in the phylogeny (Fig. 1). In addition, it is related to *P. medulla-panis*, *P. substraminea* and *P. hainaniana*, these four species being addressed into the Perenniporia s.s. clade. Though Perenniporia rosicola grouped with four species of Perenniporia s.s. in a joint subclade, but without support. The sequences of Crassisporus minutus and C. mollissimus were obtained from holotypes and they nested in the genus Crassisporus.

ITS sequences produced significant alignments in NCBI (https://www.ncbi. nlm.nih.gov/) about *Perenniporia prunicola*, the top ten of which represent *P. medulla-panis* and the similarities of them were less than 95%. The same goes for *P. rosicola*, the similarities of the top ten ITS sequences in NCBI were less than 90% excepting one sequence tagged *P. dendrohyphidia* (Zhou 273). They are consistent with our phylogeny.

Taxonomy

Perenniporia prunicola Y.C. Dai, Yuan Yuan & Chao G. Wang, sp. nov. MycoBank No: 851532 Figs 2, 3

Holotype. China. Yunnan Province, Zhaotong, Yiliang County, Xiaocaoba Town, on living tree of *Prunus*, 2.IV.2023, Dai 24751 (BJFC040388).

Etymology. Prunicola (Lat.): refers to the species growing on Prunus.

Description. *Basidiomata.* Perennial, resupinate, corky, without odour or taste when fresh, becoming hard corky upon drying, up to 15 cm long, 5 cm wide and 16 mm thick at centre. Pore surface clay pink when fresh, becoming cream, buff yellow to fawn upon drying; sterile margin very narrow to almost absent; pores round to slightly elongated, 4–6 per mm; dissepiments slightly thick, entire. Subiculum thin, cream, corky, up to 1 mm thick. Tubes pinkish-buff to clay buff when dry, distinctly stratified, hard corky, up to 15 mm long.

Hyphal structure. Hyphal system trimitic; generative hyphae bearing clamp connections; skeletal and binding hyphae IKI-, weakly CB+; tissues becoming orange brown in KOH.

Subiculum. Generative hyphae frequent, hyaline, thin-walled, occasionally branched, more or less flexuous, $2-4 \mu m$ in diam.; skeletal hyphae dominant, hyaline, thick-walled with a wide lumen, occasionally branched, more or less flexuous, $2.5-3 \mu m$ in diam.; binding hyphae hyaline, thick-walled with a wide lumen, frequently arboriform branched, flexuous, interwoven, $1.5-2 \mu m$ in diam.

Tubes. Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, straight, $2-3 \mu m$ in diam.; skeletal hyphae dominant, hyaline, thick-walled with a medium lumen, occasionally branched, slightly flexuous, interwoven, $2-2.5 \mu m$ in diam.; binding hyphae hyaline, thick-walled with a medium



Figure 2. Basidiomata of Perenniporia prunicola (Holotype, Y.C. Dai 24751). Scale bar: 1 cm.

lumen, frequently arboriform branched, flexuous, interwoven, 1.2–1.5 µm in diam. Hymenial cystidia present, clavate to fusiform, thin-walled, smooth, 25–31 × 5–5.5 µm; cystidioles present, ventricose to fusiform, hyaline, thin-walled, 16–20 × 4.5–5 µm. Basidia clavate, with four sterigmata and a basal clamp connection, $15-22 \times 7-8$ µm; basidioles more or less pyriform, but smaller. Irregular crystals present among the hymenium.

Spores. Basidiospores ellipsoid to broadly ellipsoid, hyaline, thick-walled, smooth, usually with a medium guttule, dextrinoid, weakly CB+, $(4.5-)4.8-6.2(-6.5) \times (3.5-)3.6-4.5(-4.9) \mu$ m, L = 5.39 μ m, W = 4.07 μ m, Q = 1.29-1.37 (n = 90/3).

Type of rot. White rot.

Additional specimens examined. China. Guizhou Province, Zunyi, Suiyang County, Kuankuoshui Nature Reserve, on fallen trunk of *Prunus*, 7.VII.2022, Y.C.



Figure 3. Microscopic structures of *Perenniporia prunicola* (Holotype, Y.C. Dai 24751) **a** basidiospores **b** basidia and basidioles **c** cystidioles **d** hymenial cystidia **e** hyphae from subiculum **f** hyphae from trama.

Dai 24280 (BJFC039522); Yunnan Province, Zhaotong, Yiliang County, Xiaocaoba, on dead tree of *Prunus*, 2.IV.2023, Y.C. Dai 24752 (BJFC040389).

Notes. *Perenniporia prunicola* is characterised by perennial and resupinate basidiomata with a clay pink pore surface when fresh, round to slightly elongated pores of 4–6 per mm, a trimitic hyphal system, the presence of clavate to fusiform hymenial cystidia, ellipsoid to broadly ellipsoid and thick-walled basidiospores measuring $4.8-6.2 \times 3.6-4.5 \mu m$ and growth on *Prunus* in southwest China.

Perenniporia rosicola Y.C. Dai, Yuan Yuan & Chao G. Wang, sp. nov. MycoBank No: 851529

Figs 4, 5

Holotype. China. Yunnan Province, Mengla County, Xishuangbanna Rainforest Valley, on branch of Rosaceae, 4.VII.2021, Y.C. Dai 22563 (BJFC037137).

Etymology. Rosicola (Lat.): refers to the species growing on Rosaceae.

Description. *Basidiomata.* Annual, resupinate, soft corky, without odour or taste when fresh, becoming corky when dry, up to 2 cm long, 1.5 cm wide and 1.2 mm thick at centre. Pore surface white when fresh, becoming pale orange brown upon bruising, eventually honey yellow to clay buff upon drying; sterile margin white when fresh, becoming cream upon drying, up to 0.5 mm wide; pores round, sometimes elongated, 5–7 per mm; dissepiments thin, entire to slightly lacerate. Subiculum very thin, cream, corky, up to 0.2 mm thick. Tubes concolorous with pore surface, corky, up to 1 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae bearing clamp connections; skeletal hyphae dextrinoid, weakly CB+; tissues becoming pale olivaceous in KOH.

Subiculum. Generative hyphae infrequent, hyaline, thin-walled, occasionally branched, straight, $2-2.5 \,\mu\text{m}$ in diam.; skeletal hyphae dominant, hyaline, thick-walled with a medium to narrow lumen, frequently arboriform branched, flexuous, interwoven, $1.5-2.5 \,\mu\text{m}$ in diam.

Tubes. Generative hyphae infrequent, hyaline, thin-walled, more or less flexuous, 2–2.5 μ m in diam.; skeletal hyphae dominant, hyaline, thick-walled with a medium lumen, frequently arboriform branched, flexuous, interwoven, 1.5– 2.5 μ m in diam. Hymenial cystidia absent; cystidioles present, ventricose to fusiform, hyaline, thin-walled, 14–16 × 5–5.5 μ m. Basidia barrel-shaped, with



Figure 4. Basidiomata of Perenniporia rosicola (Holotype, Y.C. Dai 22563). Scale bar: 1 cm.




four sterigmata and a basal clamp connection, $16-20 \times 7-8 \mu m$; basidioles in shape similar to basidia, but smaller. Irregular crystals present amongst hymenia. Dendrohyphidia present.

Spores. Basidiospores broadly ellipsoid to subglobose, hyaline, thick-walled, smooth, sometimes with a medium guttule, dextrinoid, weakly CB+, 5–5.8(–6) × 4–5.2(–5.3) μ m, L = 5.39 μ m, W = 4.74 μ m, Q = 1.14 (n = 30/1). **Type of rot.** White rot.

MycoKeys 105: 97-118 (2024), DOI: 10.3897/mycokeys.105.121858

Notes. Perenniporia rosicola is characterised by annual and resupinate basidiomata with a white pore surface when fresh, round to sometimes elongated pores of 5–7 per mm, frequently arboriform branched and narrow skeletal hyphae, the presence of dendrohyphidia, broadly ellipsoid to subglobose, thickwalled basidiospores measuring 5–5.8 × 4–5.2 µm and growth on Rosaceae in southwest China.

Combinations

In our phylogenetic analyses, *Crassisporus minutus* and *C. mollissimus* form two independent lineages nested in *Crassisporus* (Fig. 1) and their characteristics fit the definition of *Crassisporus*. So, we propose the following combinations:

Crassisporus minutus (Y.C. Dai & X.S. Zhou) Y.C. Dai, Yuan Yuan & Chao G. Wang, comb. nov.

MycoBank No: 851530

Basionym. *Megasporoporia minuta* Y.C. Dai & X.S. Zhou, in Zhou & Dai, Mycological Progress 7(4): 254 (2008).

Crassisporus mollissimus (B.K. Cui & C.L. Zhao) Y.C. Dai, Yuan Yuan & Chao G. Wang, comb. nov. MycoBank No: 851531

Basionym. Abundisporus mollissimus B.K. Cui & C.L. Zhao, in Zhao, Chen, Song & Cui, Mycological Progress 14(38): 5 (2015).

Discussion

The genus *Perenniporia* s.s. clade includes four species, viz. *P. hainaniana*, *P. medulla-panis*, *P. prunicola* and *P. substraminea* and these species have the perennial and resupinate basidiomata with a cream, clay pink, buff yellow, pink-ish-buff to fawn pore surface, a dimitic to trimitic hyphal system with amyloid or dextrinoid skeletal hyphae, ellipsoid, broadly ellipsoid to subglobose and thick-walled basidiospores (Table 2).

Perenniporia prunicola is similar to *P. medulla-panis* by perennial and resupinate basidiomata with a clay pink to buff yellow pore surface, round to slightly elongated pores of 4–6 per mm, a trimitic hyphal system and ovoid to broadly ellipsoid basidiospores. In addition, both species are phylogenetically related, but the latter lacks cystidia and usually has truncate basidiospores (Ryvarden and Gilbertson 1994). Perenniporia puerensis C.L. Zhao has annual and thin basidiomata, thin dissepiments, thick-walled skeletal hyphal encrusted with pale yellow crystals, the absence of hymenial cystidia and relatively smaller basidiospores ($4.3-5.5 \times 3.7-4.7 \mu m vs. 4.8-6.2 \times 3.6-4.5 \mu m; Q = 1.14-1.21 (n = 120/4) vs. Q = 1.29-1.37 (n = 90/3), Liu et al. (2017)), which differ from$ *P. prunicola*.

Perenniporia rosicola is morphologically similar and phylogenetically related to Perenniporia cf. dendrohyphidia (Fig. 1). We studied the type of *P. dendrohyphidia*

(Rammeloo 6286) and they all have annual and resupinate basidiomata, the presence of dendrohyphidia and broadly ellipsoid to subglobose and thick-walled basidiospores. However, P. dendrohyphidia has thick and entire dissepiments, round pores of 4-6 per mm, sometimes apically truncate and relatively larger basidiospores (5.5-7 × 4.5-6 µm vs. 5-5.8 × 4-5.2 µm) and it occurs in Burundi, central Africa. Unfortunately, we did not obtain sequences from the type specimen of P. dendrohyphidia. We also studied the specimen of labelled Zhou 273 collected in China and it has thin and entire dissepiments, round to slightly elongated pores of 6-8 per mm, branched skeletal hyphae measuring 1.5-3.2 µm in diam., broadly ellipsoid to subglobose basidiospores measuring $5-6 \times 4-5 \mu m$. These characteristics are somewhat similar to P. dendrohyphidia. Thus, for the time being, we treat the specimen Zhou 273 as Perenniporia cf. dendrohyphidia. In addition, there are 20 base pairs differences between Perenniporia cf. dendrohyphidia and P. rosicola, which amounts to > 3% nucleotide differences in the ITS regions. Perenniporia subdendrohyphidia Decock was originally described by Decock from Cameroon, central Africa. However, it has smaller, oblong to oblong-ellipsoid and non-dextrinoid basidiospores (4-4.8 × 2.2-3.3 µm vs. 5-5.8 × 4-5.2, Decock (2001)). Perenniporia sinuosa Ryvarden was originally described from Amazonas, Brazil (Ryvarden 1987) and it differs from *P. rosicola* by larger pores (2–3 per mm vs. 5–7 per mm) and smaller truncate basidiospores (4–5 \times 3–4 μ m vs. 5–5.8 × 4–5.2 μm, Ryvarden (1987)). Perenniporia adnata Corner, P. albocinnamomea Corner, P. ferruginea Corner and P. penangiana Corner were all originally described from Southeast Asia and lack dendrohyphidia. In addition, the former three species above differ from *P. rosicola* by smaller basidiospores $(4-4.5 \times 3.5 \mu m \text{ in } P.$ adnate; 3.7-4.7 × 2.5-3 µm in P. albocinnamomea; 3.5-4.5 × 3-3.5 µm in P. ferruginea vs. 5–5.8 × 4–5.2 μm, Corner (1989)). Perenniporia penangiana has pileate basidiomata with a stipe, which is different from P. rosicola (Corner 1989).

All species in the *Perenniporia* s.s. clade have perennial basidiomata with a cream, clay pink, buff yellow, pinkish-buff to fawn pore surface, a dimitic to trimitic hyphal system, sometimes the presence of dendrohyphidia and truncate basidiospores. *Perenniporia* cf. *dendrohyphidia* and *P. rosicola* both have annual basidiomata with a white to cream pore surface, a dimitic hyphal system, the presence of dendrohyphidia and broadly ellipsoid to globose basidiospores without truncation. All in all, some morphological characteristics of above taxa are overlapping, but the *Perenniporia* s.s. clade is unrelated to the *Perenniporia rosicola* clade in our phylogeny (Fig. 1).

Crassisporus minutus was originally described in *Megasporoporia* by Dai and Zhou from China and it is characterised by resupinate basidiomata with a cream to pale buff pore surface when fresh, distinct sterile margin, round pores of 4–6 per mm, a dimitic hyphal system; thick-walled to subsolid skeletal hyphae, cylindrical to oblong-ellipsoid basidiospores measuring 7.7–9.7 × 3.6–4.9 µm (Zhou and Dai 2008). The type specimen of *M. minutus* Zhou 120 grouped with other samples Dai 22571 and Cui 6595 nested in *Crassisporus* in our phylogenetic analysis (Fig. 1). However, we studied the sample Dai 22571 and it has slightly thick-walled basidiospores. Thus, the new combination *Crassisporus minutus* is proposed.

Crassisporus mollissimus was originally described in *Abundisporus* by Cui and Zhao from China and it is characterised by perennial, effused-reflexed to pileate basidiomata with a concentrically zonate pileal surface, a buff to

Table 2. The list of	accepted speci	es related to new tax	a in this study.		-	-		
Species	Type locality	Basidiomata	Upper surface	Colour of poroid surface	Dendrohyphidia	Basidiospores shape	Basidiospores size (µm)	References
Crassisporus imbricatus	China: Hainan	Annual, effused- reflexed to pileate	Yellowish-brown	Buff when fresh, pale greyish-brown when dry	I	Oblong ellipsoid	10-14 × 4.5-6.2	Ji et al. (2019)
C. leucoporus	Australia: Queensland	Annual, effused- reflexed to pileate	Yellowish-brown to umber-brown	White when fresh; cream, clay buff to pale yellowish- brown when dry	1	Oblong ellipsoid	8.4-11.2 × 4.2-5.4	Ji et al. (2019)
C. macroporus	China: Guangxi	Annual, effused- reflexed to pileate	Buff to yellowish- brown when fresh, yellowish brown when dry	Cream, buff to cinnamon buff when fresh; buff, pale yellowish-brown to yellowish- brown when dry	1	Oblong ellipsoid	9.5-13.2 × 4-6.2	Ji et al. (2019)
C. microsporus	China: Yunnan	Annual, pileate	Pale yellowish- brown to yellowish- brown	Cream, buff to cinnamon buff when fresh; buff, pale yellowish-brown to yellowish- brown when dry	1	Broadly ellipsoid	4-5×3-3.7	Ji et al. (2019)
C. minutus	China: Guangxi	Annual to biennial, resupinate	I	Cream to pale buff when fresh; pale greyish when dry	I	Cylindrical to oblong ellipsoid	7.7-9.7 × 3.6-4.9	Zhou and Dai (2008)
C. mollissimus	China: Hainan	Perennial, effused- reflexed to pileate	Yellow brown to umber-brown	Buff to buff-yellow when fresh, buff-yellow when dry	I	Ellipsoid	4-4.5 × 3-3.5	Zhao et al. (2015)
Perenniporia adnata	Singapore	Perennial, resupinate	1	Ochraceous buff to pinkish ochraceous	I	Broadly ellipsoid to subglobose	4-4.5 × 3.5	Corner (1989)
P. albocinnamomea	Malaysia	Annual, effused- reflexed	Pallid buff to brownish	Light cinnamon buff	I	Ellipsoid	3.7-4.7 × 2.5-3	Corner (1989)
P. dendrohyphidia	Burundi	Annual, resupinate	1	Wood-coloured to pale isabelline	+	Broadly ellipsoid to subglobose, sometimes truncate	5.5-7 × 4.5-6	Ryvarden (1988a), this study
P. eugeissonae	Malaysia	Annual, resupinate	I	White when fresh, cream to pale straw-coloured when dry	+	Ellipsoid	5-6 × 4-5	Du et al. (2020)
P. ferruginea	Brunei	Perennial, effused- reflexed	Ferruginous brown to fuscous blackish	Pallid wood white to pale brown	1	Ellipsoid, subtriangular to subglobose	3.5-4.5 × 3-3.5	Corner (1989)
P. hainaniana	China: Hainan	Perennial, resupinate	I	Cream when fresh, cream- buff when dry	+	Broadly ellipsoid, truncate	4-4.5 × 3-4	Zhao and Cui (2013a)
P. luteola	China: Henan	Annual, resupinate	1	Cream to buff when fresh, buff to yellowish-buff when dry	1	Ellipsoid, truncate	6.1-7 × 5-5.7	Zhao and Cui (2013b)

Species	Type locality	Basidiomata	Upper surface	Colour of poroid surface	Dendrohyphidia	Basidiospores shape	Basidiospores size (µm)	References
P. medulla-panis	Australia	Annual to perennial, resupinate	1	White when fresh; white, cream, pale corky when dry; greyish-orange when bruised	1	Ellipsoid, broadly ovoid to subglobose, truncate	4.7-5.8 × 3.5-4.5	Decock and Stalpers (2006)
P. nonggangensis	China: Guangxi	Annual, resupinate to effused-reflexed	1	Cream to greyish-cream when fresh; pale yellow- orange, capucine buff to sudan brown when dry	1	Broadly ellipsoid to subglobose	3.1-4.4 × 2.7-3.6	Huang et al. (2017)
P. puerensis	China: Yunnan	Annual, resupinate	1	Cream to buff when fresh, yellow to ochraceous when dry	I	Ovoid to subglobose	4.3-5.5 × 3.7-4.7	Liu et al. (2017)
P. penangiana	Malaysia	Annual, pileate with a stipe	Pale ochraceous to brownish	Pale tan ochraceous	I	Broadly ellipsoid	5-6.5 × 4-5	Corner (1989)
P. prunicola	China: Yunnan	Perennial, resupinate	I	Clay pink when fresh; cream, buff yellow to fawn when dry	I	Ellipsoid to broadly ellipsoid	4.8-6.2 × 3.6-4.5	This study
P. pseudotephropora	Brazil	Perennial, effused- reflexed to pileate	Pinkish buff, grey to greyish -brown	Greyish to pale brown	+	Broadly ellipsoid to subglobose, truncate	4.9-5.2 × 4-4.8	Wang et al. (2020)
P. rosicola	China: Yunnan	Annual, resupinate	1	White when fresh; pale orange brown when bruised, eventually honey yellow to clay buff when dry	+	Broadly ellipsoid to subglobose	5-5.8 × 4-5.2	This study
P. sinuosa	Brazil	Annual, resupinate	I	Cream to ochraceous	1	Subglobose, truncate	4-5 × 3-4	Ryvarden (1987)
P. straminea	Philippines	Annual, resupinate	I	Straw-coloured when fresh; pale yellow brown with orange tints when dry	I	Ellipsoid	2.5-3 × 2	Ryvarden (1988b)
P. subdendrohyphidia	Cameroon	Annual to biennial, resupinate	I	White, yellowish to pale pinkish cork-coloured when bruised	÷	Oblong, Oblong ellipsoid to ellipsoid, truncate	4-4.8 × 2.8-3.3	Decock (2001)
P. substraminea	China: Zhejiang	Perennial, resupinate	1	White to cream when fresh, cream to pinkish-buff when dry	÷	Ellipsoid, truncate	3.1-3.8 × 2.4-3	Zhao et al. (2013a)
P. subtephropora	China: Guangdong	Perennial, resupinate	I	Cream when fresh; cream buff to greyish-buff when dry	I	Ellipsoid to broadly ellipsoid, truncate	4-5×3.5-4.5	Zhao and Cui (2013a)
P. tephropora	Suriname	Perennial, resupinate to rarely effused- reflexed	Dirty greyish to black	Clay buff, grey to milky coffee or pale umber	I	Broadly ellipsoid, truncate	4.5-6 × 3.5-4.5	Ryvarden (1972b)
Bold = new taxa. Abt	reviations used: +	- = Present, – = Absent.						

buff yellow pore surface when fresh, round pores of 7–8 per mm, ellipsoid and slightly thick-walled basidiospores measuring 4–4.5 × 3–3.5 μ m (Zhao et al. 2015). In addition, *Crassisporus* and *Abundisporus* are phylogenetically unrelated (Fig. 1).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The research is supported by the Research Project of Yunnan Key Laboratory of *Gastrodia* and Fungi Symbiotic Biology (TMKF2023A03) and the Yunnan Province expert workstation programme (No. 202205AF150014).

Author contributions

All authors designed the research and contributed to data analysis and interpretation, and prepared the samples and drafted the manuscript.

Author ORCIDs

Chao-Ge Wang ID https://orcid.org/0000-0003-4381-5720 Yuan Yuan ID https://orcid.org/0000-0001-6674-9848

Data availability

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

References

- Anonymous (1969) Flora of British fungi. Colour identification chart. Her Majesty's Stationery Office, London.
- Bondartsev AS (1953) The Polyporaceae of the European USSR and Caucasia, 896 pp.
- Bresadola G (1897) Hymenomycetes Hungarici Kmetiani. Atti dell'Istituto Reale dell'Accademia di Rovereto di Scienze 3: 66–120.
- Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics (Oxford, England) 25(15): 1972–1973. https://doi.org/10.1093/bioinformatics/btp348
- Churapa T, Lerluck C (2016) Physiological regulation of an ikaline-resistant laccase produced by *Perenniporia tephropora* and efficiency in biotreatment of pulp mill effluent. Mycobiology 44(4): 260–268. https://doi.org/10.5941/MYCO.2016.44.4.260
- Cooke WB (1953) The genera of Homobasidiomycetes (exclusive of the Gasteromycetes). [Special Publication]. Division of mycology and disease survey, US Department of Agriculture, Beltsville, Maryland.

Corner EJH (1989) Ad Polyporaceas 5. Beihefte zur Nova Hedwigia. 96: 1–218.

Cui BK, Zhao CL (2012) Morphological and molecular evidence for a new species of *Perenniporia* (Basidiomycota) from Tibet, southwestern China. Mycoscience 53(5): 365–372. https://doi.org/10.1007/S10267-011-0180-X

- Cui BK, Li HJ, Ji X, Zhou JL, Song J, Si J, Yang ZL, Dai YC (2019) Species diversity, taxonomy and phylogeny of Polyporaceae (Basidiomycota) in China. Fungal Diversity 97(1): 137–302. https://doi.org/10.1007/s13225-019-00427-4
- Decock C (2001) Studies in Perenniporia: African taxa I. Perenniporia dendrohyphidia and Perenniporia subdendrohyphia. Systematics and Geography of Plants 71: 45–51. https://doi.org/10.2307/3668752
- Decock C, Ryvarden L (1999) Studies in neotropical polypores. Some coloured resupinate *Perenniporia* species. Mycological Research 103(9): 1138–1144. https://doi.org/10.1017/S0953756298008284
- Decock C, Stalpers J (2006) Studies in *Perenniporia: Polyporus unitus, Boletus medullapanis*, the nomenclature of *Perenniporia, Poria* and *Physisporus*, and a note on European *Perenniporia* with a resupinate basidiome. Taxon 53(3): 759–778. https://doi. org/10.2307/25065650
- Donk MA (1960) The generic names proposed for Polyporaceae. Persoonia 1: 173-302.
- Du P, Wang CG, Tian XM (2020) *Perenniporia eugeissonae* sp. nov., a new species on palm discovered from Malaysia. Phytotaxa 449: 75–82. https://doi.org/10.11646/ phytotaxa.449.1.7
- Gilbertson RL, Ryvarden L (1987) North American polypores 2; Fungiflora, Oslo, Norway, 434–885.
- Guglielmo F, Bergemann SE, Gonthier P, Nicolotti G, Garbelotto M (2007) A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees. Journal of Applied Microbiology 103(5): 1490–1507. https://doi. org/10.1111/j.1365-2672.2007.03378.x
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Han ML, Vlasák J, Cui BK (2015) *Daedalea americana* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. Phytotaxa 204(4): 277–286. https://doi.org/10.11646/phytotaxa.204.4.4
- Han ML, Chen YY, Shen LL, Song J, Vlasák J, Dai YC, Cui BK (2016) Taxonomy and phylogeny of the brown-rot fungi: *Fomitopsis* and its related genera. Fungal Diversity 80(1): 343–373. https://doi.org/10.1007/s13225-016-0364-y
- Hopple Jr JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: Divergent domains, outgroups, and monophyly. Molecular Phylogenetics and Evolution 13(1): 1–19. https://doi.org/10.1006/ mpev.1999.0634
- Huang FC, Liu B, Wu H, Shao YY, Qin PS, Li JF (2017) Two new species of aphyllophoroid fungi (Basidiomycota) from southern China. Mycosphere: Journal of Fungal Biology 8(6): 1270–1282. https://doi.org/10.5943/mycosphere/8/6/12
- Jang Y, Jang S, Lim YW, Kim C, Kim JJ (2015) Perenniporia koreana, a new wood-rotting basidiomycete from South Korea. Mycotaxon 130(1): 173–179. https://doi. org/10.5248/130.173
- Ji XH, Thawthong A, Wu F (2017) A new species of *Perenniporia* (Polyporales, Basidiomycota) from Thailand. Mycosphere: Journal of Fungal Biology 8(8): 1102–1107. https://doi.org/10.5943/mycosphere/8/8/10
- Ji X, Wu DM, Liu S, Si J, Cui BK (2019) Crassisporus gen. nov. (Polyporaceae, Basidiomycota) evidenced by morphological characters and phylogenetic analyses with descriptions of four new species. MycoKeys 57: 61–84. https://doi.org/10.3897/mycokeys.57.38035

- Ji X, Sun YF, Wu DM, Gao N, Cui BK (2023) An updated phylogenetic assessment and taxonomic revision of *Perenniporia* sensu lato (Polyporales, Basidiomycota). Journal of Fungi (Basel, Switzerland) 9(2): 173. https://doi.org/10.3390/jof9020173
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kim J, Lee JH (2020) Development of carotenoid production process using *Perenniporia fraxinea*. Journal of Mushrooms 18: 365–371.
- Li HJ, Zhou M, Si J (2018) *Perenniporia punctata* sp. nov. (Polyporales, Basidiomycota), a new species discovered from China. Phytotaxa 360(1): 54–60. https://doi. org/10.11646/phytotaxa.360.1.5
- Liu WL, Xu T, Shen S, Liu XF, Sun Y, Zhao XL (2017) *Perenniporia puerensis* sp. nov. from southern China. Mycotaxon 132(4): 867–874. https://doi.org/10.5248/132.867
- Lowe JL (1955) Perennial polypores of North America. 3. *Fomes* with context white to rose. Mycologia 47(2): 213–224. https://doi.org/10.1080/00275514.1955.120 24445
- Maddison WP, Maddison DR (2017). Mesquite: A Modular System for Evolutionary Analysis, Version 3.2. http://mesquiteproject.org
- Miettinen O, Vlasák J, Rivoire B, Spirin V (2018) *Postia caesia* complex (Polyporales, Basidiomycota) in temperate Northern Hemisphere. Fungal Systematics and Evolution 1(1): 101–129. https://doi.org/10.3114/fuse.2018.01.05
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, 1–8. https://doi.org/10.1109/GCE.2010.5676129
- Murrill WA (1942) Florida resupinate polypores. Mycologia 34(5): 595–596. https://doi. org/10.2307/3754676
- Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Petersen JH (1996) The Danish Mycological Society's colour-chart. Foreningen til Svampekundskabens Fremme, Greve.
- Robledo GL, Amalfi M, Castillo G, Rajchenberg M, Decock C (2009) *Perenniporiella chaquenia* sp. nov. and further notes on *Perenniporiella* and its relationships with *Perenniporia* (Poriales, Basidiomycota). Mycologia 101(5): 657–673. https://doi. org/10.3852/08-040
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi. org/10.1093/bioinformatics/btg180
- Ryvarden L (1972a) Studies on the Aphyllophorales of the Canary Islands with a note on the genus *Perenniporia*. Nordic Journal of Botany 19: 139–144.
- Ryvarden L (1972b) A critical checklist of the Polyporaceae in tropical East Africa. Nordic Journal of Botany 19: 229–238.
- Ryvarden L (1987) New and noteworthy polypores from tropical America. Mycotaxon 28: 525–541.
- Ryvarden L (1988a) Two new polypores from Burundi in Africa. Mycotaxon 31: 407–409.
- Ryvarden L (1988b) Type studies in the Polyporaceae. 20. Species described by G. Bresadola. Mycotaxon 33: 303–327.
- Ryvarden L, Gilbertson RL (1994) European polypores 2. Synopsis Fungorum 7: 394-743.

- Shen S, Xu TM, Karakehian J, Zhao CL (2018) Morphological and molecular identification of a new species of *Perenniporia* (Polyporales, Basidiomycota) in North America. Phytotaxa 351(1): 63–71. https://doi.org/10.11646/phytotaxa.351.1.5
- Shen LL, Wang M, Zhou JL, Xing JH, Cui BK, Dai YC (2019) Multi-gene phylogeny and taxonomy of the brown-rot fungi: *Postia* (Polyporales, Basidiomycota) and related genera. Persoonia 42(1): 101–126. https://doi.org/10.3767/persoonia.2019.42.05
- Si J, Cui BK, He S, Dai YC (2011) Optimization of conditions for laccase production by *Perenniporia subacida* and its application in dye decolorization. Chinese Journal of Applied and Environmental Biology 17: 736–741.
- Spirin V, Kout J, Vlasák J (2015) Studies in the *Truncospora ohiensis T. ochroleuca* group (Polyporales, Basidiomycota). Nova Hedwigia 100(1–2): 159–175. https://doi. org/10.1127/nova_hedwigia/2014/0221
- Sun YF, Costa-Rezende DH, Xing JH, Zhou JL, Zhang B, Gibertoni TB, Gates G, Glen M, Dai YC, Cui BK (2020) Multi-gene phylogeny and taxonomy of *Amauroderma* s.lat. (Ganodermataceae). Persoonia 44(1): 206–239. https://doi.org/10.3767/persoonia.2020.44.08
- Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts. https://doi. org/10.1002/0471650129.dob0522
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25(24): 4876–4882. https://doi. org/10.1093/nar/25.24.4876
- Tian XM, Liu SL, Zhou LJ, Wang XW, Zhou LW (2021) *Perenniporia subrhizomorpha* sp. nov. (Polyporales, Basidiomycota) from North China. Phytotaxa 528(2): 125–132. https://doi.org/10.11646/phytotaxa.528.2.5
- Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92: 135–154. https://doi. org/10.1016/j.simyco.2018.05.001
- Wang CG, Liu SL, Wu F (2020) Two new species of *Perenniporia* (Polyporales, Basidiomycota). MycoKeys 69: 53–69. https://doi.org/10.3897/mycokeys.69.51652
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White JT (Eds) PCR Protocols: A guide to methods and applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Wu ZQ, Liu WL, Wang ZH, Zhao CL (2017) Perenniporiopsis, a new polypore genus segregated from Perenniporia (Polyporales). Cryptogamie. Mycologie 38(3): 285–299. https://doi.org/10.7872/crym/v38.iss3.2017.285
- Wu F, Zhou LW, Vlasák J, Dai YC (2022) Global diversity and systematics of Hymenochaetaceae with poroid hymenophore. Fungal Diversity 113(1): 1–192. https://doi. org/10.1007/s13225-021-00496-4
- Yang Y, Li R, Jiang QQ, Zhou HM, Muhammad A, Wang HJ, Zhao CL (2024) Phylogenetic and Taxonomic Analyses Reveal Three New Wood-Inhabiting Fungi (Polyporales, Basidiomycota) in China. Journal of Fungi (Basel, Switzerland) 10(1): 55. https://doi. org/10.3390/jof10010055

- Zhao CL, Cui BK (2012) A new species of *Perenniporia* (Polyporales, Basidiomycota) described from southern China based on morphological and molecular characters. Mycological Progress 11(2): 555–560. https://doi.org/10.1007/s11557-011-0770-1
- Zhao CL, Cui BK (2013a) Morphological and molecular identification of four new resupinate species of *Perenniporia* (Polyporales) from southern China. Mycologia 105(4): 945–958. https://doi.org/10.3852/12-201
- Zhao CL, Cui BK (2013b) Three new *Perenniporia* (Polyporales, Basidiomycota) species from China based on morphological and molecular data. Mycoscience 54(3): 231–240. https://doi.org/10.1016/j.myc.2012.09.013
- Zhao CL, Cui BK (2013c) *Truncospora macrospora* sp. nov. (Polyporales) from Southwest China based on morphological and molecular data. Phytotaxa 87(2): 30–38. https://doi.org/10.11646/phytotaxa.87.2.2
- Zhao CL, Ma X (2019) *Perenniporia mopanshanensis* sp. nov. from China. Mycotaxon 134(1): 125–137. https://doi.org/10.5248/134.125
- Zhao CL, Cui BK, Dai YC (2013a) New species and phylogeny of *Perenniporia* based on morphological and molecular characters. Fungal Diversity 58(1): 47–60. https://doi. org/10.1007/s13225-012-0177-6
- Zhao CL, Cui BK, Steffen KT (2013b) *Yuchengia*, a new polypore genus segregated from Perenniporia (Polyporales) based on morphological and molecular evidence. Nordic Journal of Botany 31(3): 331–338. https://doi.org/10.1111/j.1756-1051.2012.00003.x
- Zhao CL, He XS, Wanghe K-Y, Cui B-K, Dai Y-C (2014a) Flammeopellis bambusicola gen. et. sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. Mycological Progress 13(3): 771–780. https://doi. org/10.1007/s11557-014-0960-8
- Zhao CL, Shen LL, Cui BK (2014b) *Perenniporia cinereofusca* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. Mycoscience 55(5): 417–422. https://doi.org/10.1016/j.myc.2013.11.006
- Zhao CL, Chen H, Song J, Cui BK (2015) Phylogeny and taxonomy of the genus Abundisporus (Polyporales, Basidiomycota). Mycological Progress 14(6): 38. https://doi. org/10.1007/s11557-015-1062-y
- Zhou XS, Dai YC (2008) A new species of *Megasporoporia* (Polyporales, Basidiomycota) from China. Mycological Progress 7(4): 253–255. https://doi.org/10.1007/s11557-008-0567-z
- Zhu L, Song J, Zhou JL, Si J, Cui BK (2019) Species diversity, phylogeny, divergence time and biogeography of the genus Sanghuangporus (Basidiomycota). Frontiers in Microbiology 10: 812. https://doi.org/10.3389/fmicb.2019.00812
- Zmitrovich IV, Kovalenko AE (2016) Lentinoid and Polyporoid Fungi, Two Generic Conglomerates Containing Important Medicinal Mushrooms in Molecular Perspective. International Journal of Medicinal Mushrooms 18(1): 23–38. https://doi.org/10.1615/ IntJMedMushrooms.v18.i1.40



Research Article

Revisiting the phylogeny and taxonomy of the genus *Sidera* (Hymenochaetales, Basidiomycota) with particular emphasis on *S. vulgaris*

Vassiliki Fryssouli¹⁰, Elias Polemis¹⁰, Milton A. Typas², Georgios I. Zervakis¹⁰

1 Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, lera Odos 75, 11855 Athens, Greece

2 National and Kapodistrian University of Athens, Department of Genetics and Biotechnology, Faculty of Biology, Panepistemiopolis, Athens 15701, Greece Corresponding author: Georgios I. Zervakis (zervakis@aua.gr)

Abstract

The genus Sidera (Hymenochaetales, Basidiomycota) comprises white-rot, mono- or dimitic fungi with poroid or hydnoid hymenophore. It has a worldwide distribution albeit with fewer species present in the Southern Hemisphere. Although recent studies revealed the existence of several new Sidera species, there are still taxonomic inconsistencies and obscure phylogenetic relationships amongst certain taxa of the genus. In this work, a large number of Sidera collections were used to obtain an updated phylogeny, based on ITS and 28S rDNA sequences by including new material from Mediterranean Europe. The monophyly of the genus was strongly supported and all species with poroid hymenophore formed a highly-supported lineage with two major subclades. In total, 23 putative species were recognised. Amongst those, five are considered to possibly represent entities new to science, but further work is required since they are represented by single specimens or environmental sequences. Examined collections originally named S. lenis from southern Europe were grouped within S. vulgaris. Similarly, several collections under various names were hereby identified as S. vulgaris, including those of the recently described species S. tibetica. Furthermore, a critical discussion (based on morphoanatomical findings) is made on the key features that could be used to distinguish S. lenis from S. vulgaris.

Key words: Basidiomycetes, biodiversity, fungal phylogeny, Mediterranean Europe, mushroom, white-rot fungi

Introduction

The genus *Sidera* Miettinen & K.H. Larss. (Sideraceae, Hymenochaetales, Basidiomycota) was established to harbour four resupinate, wood-inhabiting, white-rot species, in accordance with morphological and phylogenetic evidence: *S. lenis* (P. Karst.) Miettinen (type species), *S. lowei* (Rajchenb.) Miettinen, *S. lunata* (Romell ex Bourdot & Galzin) K.H. Larsson and *S. vulgaris* (Fr.) Miettinen (Miettinen and Larsson 2011). Amongst them, *S. lowei* was originally described from Brazil (Rajchenberg 1987), while all the others were described from material collected in Europe. Recently, the diversity of the genus

Academic editor: R. Henrik Nilsson Received: 24 February 2024 Accepted: 1 April 2024 Published: 7 May 2024

Citation: Fryssouli V, Polemis E, Typas MA, Zervakis GI (2024) Revisiting the phylogeny and taxonomy of the genus *Sidera* (Hymenochaetales, Basidiomycota) with particular emphasis on *S. vulgaris*. MycoKeys 105: 119–137. https://doi. org/10.3897/mycokeys.105.121601

Copyright: © Vassiliki Fryssouli 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). was significantly expanded as a result of phylogenetic studies using nuclear ribosomal markers (ITS and LSU) to describe new taxa from boreal, temperate and tropical habitats (Du et al. 2019, 2020; Liu et al. 2021, 2022, 2023; Xu et al. 2023).

Morphologically, *Sidera* species are characterised by resupinate, whitish to cream-coloured, yellowish or buff, rarely pinkish or bluish basidiomata, poroid hymenophore with middle-sized to small pores (and, in only one case, hydnoid hymenophore; i.e. *S. lunata*), monomitic or dimitic hyphal system, generative hyphae with clamps, rather loosely arranged skeletal hyphae, presence of rosette-like crystals in subiculum and/or trama, hymenial cystidia as thin-walled hyphidia (cystidioles) and minute, allantoid to lunate, hyaline, thin-walled, negative in Melzer's reagent and acyanophilous basidiospores (Miettinen and Larsson 2011).

After re-examining the lectotype of Physisporus lenis (designated by Lowe 1956), Niemelä and Dai (1997) recognised two morphologically distinct species: one that was more common in boreal, mostly pine-inhabiting old-growth forests, i.e. Skeletocutis lenis (P. Karst.) Niemelä and another species that came across more as generalist - in terms of substrate preference - and with a more southern distribution, i.e. Sk. vulgaris (Fr.) Niemelä & Y.C. Dai. In the original description of the genus Sidera, Miettinen and Larsson (2011) used two sequences which were then considered to represent S. vulgaris, both originating from Oceania (Tasmania, Gates FF257; New Zealand, Ryvarden 37198). However, no sequences from European collections were included in their phylogenetic analysis. The sequence from Tasmania nested close to S. lowei, while the sequence from New Zealand appeared as sister to S. lenis, indicating that they possibly correspond to two distinct species. Recent phylogenetic studies in the genus Sidera repeatedly used the aforementioned two specimens (Du et al. 2019, 2020) without including any S. vulgaris material from Europe. In fact, in one of these publications (Du et al. 2020), the specimen from Tasmania was identified as S. minutipora (Rodway & Cleland) Y.C. Dai, F. Wu, G.M. Gates & Rui Du. Moreover, Liu et al. (2021) used the term "Sidera vulgaris sensu lato" to refer to the New Zealand sequence and to some newly-generated sequences from Chinese collections, which were later linked to several new species, i.e. S. americana Z.B. Liu & Yuan Yuan, S. borealis Z.B. Liu & Yuan Yuan, and S. tibetica Z.B. Liu, J. Yu & F. Wu (Liu et al. 2022, 2023). Therefore, the phylogenetic position of S. vulgaris within the genus remains obscure and controversial.

In order to resolve this issue and provide an updated phylogeny of the genus *Sidera*, several collections from Mediterranean Europe, initially identified as *Skeletocutis* sp., *Sk. lenis* and *Sk. vulgaris* from Mediterranean Europe, were included in this study, together with a large number of publicly available sequences. In addition, answers were sought to the following key questions: (a) Could available specimens confirm the presence of *S. lenis* in the Mediterranean Region? (b) Is there adequate evidence that *S. vulgaris* indeed has a cosmopolitan distribution? (c) Which *Sidera* taxa are accommodated within *S. vulgaris sensu lato* and are they related to new species correctly introduced in this genus? (d) What are the key morphological features to distinguish *S. vulgaris* from *S. lenis*?

Methods

Biological material – Morphology

Voucher specimens studied were deposited in the fungaria of the Laboratory of General and Agricultural Microbiology (Agricultural University of Athens, ACAM), the University of Oslo (O, HUBO) and the University of Salamanca (Salamanca, SALA-Fungi). Pore density was studied using a stereomicroscope (Zeiss Stemi 2000-C) at $10-20 \times$ magnification by measuring the number of pores per mm. Microscopic examination was performed with a Zeiss AxioImager A2 microscope under bright field and differential interference contrast (DIC) and microphotographs were taken with the aid of a mounted digital camera (Axiocam). Examination of microscopic features were performed in Cotton Blue, Melzer's reagent and 5% potassium hydroxide (KOH) mounting media. Measurements were taken in KOH under 1,000× magnification and DIC. For each specimen studied, a minimum number of 25 basidiospores were measured and their size (with standard deviation, SD) is provided as minimum and maximum average (average - SD and average + SD, respectively). In addition, the quotient (Q) for each basidiospore was calculated and is presented together with the respective average values (Qav). Length and width of basidia and hymenial cystidioles are also presented with the same formula: (n = x/y) refers to x measurements (of pores/mm, basidiospores, basidia and cystidioles) from y specimens. Other essential microscopical features which were also examined, including generative and skeletal hyphae from subiculum and hymenophoral trama (tubes) and the presence of stellate crystals and capitate hyphal tips.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from dried material using the Nucleospin Plant II kit (Macherey and Nagel, USA) according to manufacturer's protocol with minor modifications (Zervakis et al. 2019). The two regions of the nuclear ribosomal repeat unit - namely the ITS region and a fragment of the ribosomal large subunit gene (28S/LSU) - were amplified through the polymerase chain reaction (PCR) in a MiniAmp Plus Thermal Cycler (Applied Biosystems, CA, USA). The ITS sequences were generated using the forward and backward primers ITS1 and ITS4 (White et al. 1990) to include partial 18S, complete ITS and partial 28S rDNA. The 28S rDNA sequences included the D1/D2 domain by employing the primers LROR and LR5 (Vilgalys and Hester 1990; Stielow et al. 2015). 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 sec, 52 °C for 45 sec and 72 °C for 1 min and a final extension step of 72 °C for 10 min. The PCR procedure for 28S rDNA was as follows: initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 sec, 48 °C for 1 min and 72 °C for 1.5 min and a final extension of 72 °C for 10 min. The PCR products were purified with Pure Clean spin columns (Invitrogen, California, USA) following the manufacturer's instructions.

The PCR products were sequenced using the same forward and backward primers with the amplification procedure in an automated ABI sequencer (Life Technology) at CeMIA Inc. (Larissa, Greece). Trace files obtained from the sequencer were aligned using MEGA 11 (Tamura et al. 2021). Consensus

sequences were manually edited to remove or replace all ambiguous characters and were cross-checked against local and public databases, including the international nucleotide sequence database collaboration (INSDC, Arita et al. (2021)) and UNITE (Nilsson et al. 2019). Validated sequences were submitted to GenBank (Sayers et al. 2024) to obtain accession numbers (Table 1).

Phylogenetic analysis

Phylogenetic analysis of the genus *Sidera* was performed using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches with separate ITS and 28S rDNA datasets. In addition, a concatenated two-marker dataset was analysed by including specimens with data available for both markers. Besides the newly-generated sequences, additional reference sequences were retrieved from INSDC and UNITE using BLASTn searches. *Skvortzovia furfurella* (Bres.) Bononi & Hjortstam and *Skvortzovia furfuracea* (Bres.) G. Gruhn & Hallenberg were used as outgroups (Liu et al. 2022), while two additional Hymenochaetales species, i.e. *Alloclavaria purpurea* (O.F. Müll.) Dentinger & D.J. McLaughlin and *Rickenella mellea* (Singer & Clémençon) Lamoure, were also included in the analysis. A detailed list of specimens examined in the present study, along with corresponding information, for example, initial identification, habitat, sequence database accession number and pertinent publication (when available), is provided in Table 1.

Sequences were aligned by the online version of MAFFT v. 7 (Katoh et al. 2019) using the "G-INS-i" and "Q-INS-I" strategies for ITS for 28S rDNA barcodes, respectively. The alignments were visually inspected and manually adjusted for conspicuous errors and gapped sites in Mesquite 3.81 (Maddison and Maddison 2023). The MUMSA tool (Lassmann and Sonnhammer 2006) was used to select the best alignment.

ML analysis was performed using IQ-TREE v. 2.2.7 (Minh et al. 2022) via the CIPRES Science Gateway (Miller et al. 2015) with a random starting tree. All model parameters were estimated by the software. The best Maximum Likelihood tree was retained from all searches and the Maximum Likelihood bootstrap values (ML-BS) were determined using ultrafast bootstrapping algorithm with 10,000 replicates. BI analysis was implemented in MrBayes v.3.2.7a (Ronguist et al. 2012) employing optimal models of evolution determined for ITS and 28S rDNA with jModelTest2 v.2.1.6 (Darriba et al. 2012) under the Bayesian Information Criterion (BIC). Two parallel runs, each one consisting of four incrementally heated Monte Carlo Markov Chains, were initiated from programme-generated random trees. The analysis involved sampling every 1,000th generation until the average standard deviation of split frequency fell below 0.005. The burn-in phase (first 25% of sampled trees) was discarded. The remaining trees were used to generate a 50% majority rule consensus tree and to estimate the Bayesian posterior probabilities (BPPs). Branches with ML-BS and BPPs equal to or above 65% and 0.95, respectively, were considered as significantly supported.

The best topologies from MP analyses are presented and the final alignments and the phylograms are deposited in TreeBASE (http://www.treebase.org) under accession ID: 31153. The sequence identity was calculated using MAFFT, accessed through EMBL-EBI (https://www.ebi.ac.uk/Tools/msa/mafft/). Table 1. Biological material used in the phylogenetic analysis of genus *Sidera*. Information includes final identification of taxa (as derived from the present study; in bold typeface), initial identification of taxa as submitted in public databases or as it appeared on the material examined here for first time (when another name appears in parenthesis, it corresponds to the one subsequently used when this collection served as type material), specimen code, geographic origin, substrate and corresponding GenBank accession numbers for ITS and 28S rDNA. The reference for each entry is also provided; asterisk (*) indicates those not accompanied by a publication. Collections serving as type material are indicated with superscript letters, i.e., ^H: holotype, ^L: lectotype, and ^P: paratype; 'n.a.' denotes not available information.

Species	Collection and	Geographic	Substrate	GenBank ac	cession no.	Poforonco	
Species	Collection code	origin	Substrate	ITS	28S rDNA	Reference	
S. americana							
Sidera sp. (S. americana)	Dai 12730 ^H	USA: CT	on rotten stump of Pinus	MW198478	n.a.	Liu et al. (2021, 2023)	
S. malaysiana	Dai 19173	Canada	on rotten angiosperm wood	MW198477	MW192005	Liu et al. (2022, 2023)	
Sidera sp.	TUF101553	Estonia	Pinus sylvestris	UDB015767	n.a.	Runnel (2010)*	
S. vulgaris	Alden Dirks: ACD0413	USA: MI	n.a.	OL756000	0L742443	Dirks (2021)*	
S. borealis							
Sidera sp. (S. borealis)	Cui 11216 ^H	China: SN	fallen angiosperm trunk	MW198485	n.a.	Liu et al. (2021, 2023)	
S. cf. vulgaris	Dai 22822	China: YN	on rotten wood of <i>Picea</i>	OM974254	OM974246	Liu et al. (2022, 2023)	
Sidera sp.	TUF122801	Estonia	Pinus sylvestris	UDB023006	UDB023006	Runnel (2013)*	
S. inflata			·			'	
S. inflata	Cui 13610 ^H	China: HI	on rotten angiosperm wood	MW198480	n.a.	Liu et al. (2021)	
S. lenis					·		
S. lenis	O. Miettinen 11036.1 [∟]	Finland	n.a.	FN907914	FN907914	Miettinen and Larsson (2011)	
S. lenis	NSK 1017015	Russia	n.a.	OR364533	n.a.	Vlasenko (2023)*	
S. lenis	Dai 22834	China: YN	on rotten wood of Picea	OQ134538	n.a.	Liu et al. (2023)	
S. lenis	TUF111091	Sweden	Pinus sylvestris	UDB032409	n.a.	Sell (2015)*	
S. lowei							
S. lowei	Dollinger 922	USA: FL	Quercus	KY264044	n.a.	Dollinger and Vlasak (2016)*	
S. lowei	Ryvarden 40576	Venezuela	n.a.	FN907917	FN907917	Miettinen and Larsson (2011)	
S. lunata							
Athelopsis lunata	JS 15063 (1717)	Norway	n.a.	DQ873593	DQ873593	Larsson et al. (2006)	
S. lunata	S851	Estonia	soil	UDB0662815	n.a.	Tedersoo et al. (2018)*	
S. malaysiana							
S. malaysiana	Dai 18570 ^н	Malaysia	on rotten angiosperm wood	MW198481	MW192007	Liu et al. (2021)	
S. minutipora							
S. minutipora	Cui 16720	Australia: Tasmania	on rotten stump of Eucalyptus	MN621349	MN621348	Du et al. (2020)	
S. vulgaris	G. Gates FF257	Australia: Tasmania	n.a.	FN907922	FN907922	Miettinen and Larsson (2011)	
S. minutissima							
S. minutissima	Dai 19529 ^н	Sri Lanka	on rotten angiosperm branch	MN621352	MN621350	Du et al. (2020)	
S. minutissima	Dai 22495	China	n.a.	OM974248	OM974240	Liu et al. (2022)	
Sidera sp.	KAS: L1620	Réunion Island	n.a.	UDB024833	n.a.	Ordynets (2015)*	
Sidera sp.	TUF123971	Seychelles	n.a.	UDB039740	n.a.	Kõljalg (2018)*	

Creation	Collection code	Geographic	Cubatrata	GenBank acc	cession no.	Deference
Species	Collection code	origin	Substrate	ITS	28S rDNA	Reference
S. parallela						
S. parallela	Cui 10346 ^H	China: YN	on rotten angiosperm trunk	MK346145	n.a.	Du et al. (2020)
S. parallela	Cui 10361 ^p	China: YN	on fallen angiosperm trunk	MK346144	n.a.	Du et al. (2020)
S. parallela	Dai 22038	China	n.a.	MW477793	MW474964	Liu et al. (2022)
S. punctata			I			
S. punctata	Dai 22119 ^H	China: HI	on rotten angiosperm wood	MW418438	MW418437	Liu et al. (2021)
unc. fungus	L042880-122- 060-A02	Ocean	air filter sample	GQ999131	n.a.	Fröhlich-Nowoisky et al. (2012)
unc. fungus	L042881-122- 061-B08	Taiwan	air filter sample	GQ999432	n.a.	Fröhlich-Nowoisky et al. (2012)
S. roseo-bubalina		'	'			'
S. roseo-bubalina	Dai 11277 [⊤]	China: HA	under decay Quercus	MW198483	n.a.	Liu et al. (2021)
S. salmonea			I			
S. salmonea	Dai 23354 ^p	China: Tibet	Abies	OM974250	OM974242	Liu et al. (2022)
S. salmonea	Dai 23428	China: Tibet	Pinus armandii	OM974251	OM974243	Liu et al. (2022)
S. srilankensis		'	I			'
S. srilankensis	Dai 19654 ^н	Sri Lanka	on rotten angiosperm wood	MN621344	MN621346	Du et al. (2020)
S. srilankensis	Dai 19581 [⊳]	Sri Lanka	on rotten angiosperm wood	MN621345	MN621347	Du et al. (2020)
S. tenuis		1	I			l
S. tenuis	Dai 18697 ^н	Australia: Tasmania	on rotten stump of Eucalyptus	MK331865	MK331867	Du et al. (2020)
S. tenuis	Dai 18698 ^p	Australia	on rotten stump of Eucalyptus	MK331866	MK331868	Du et al. (2020)
S. tianshanensis						
S. tianshanensis	Cui 19143 ⁺	China: XJ	on fallen trunk of Picea schrenkiana	OP920995	OP920987	Xu et al. (2023)
S. tianshanensis	Cui 19132	China: XJ	on stump of Picea schrenkiana	OP920994	OP920986	Xu et al. (2023)
S. vesiculosa						
S. vesiculosa	BJFC025377 [⊤]	Singapore	on rotten angiosperm	MH636564	MH636566	Du et al. (2019)
S. vesiculosa	BJFC025367 ^P	Singapore	on rotten angiosperm	MH636565	MH636567	Du et al. (2019)
Sidera sp.	TUE002764	Papua New Guinea	soil	UDB07018609	n.a.	Tedersoo et al. (2020)*
S. vulgaris						
S. vulgaris	ACAM 2013-0017	Greece	Pinus halepensis	PP275215	PP275225	present work
S. vulgaris	ACAM DD2559	Greece	Abies cephalonica	PP275216	PP275226	present work
Skeletocutis vulgaris	HUBO 7745	Italy	Pinus sylvestris	PP275217	PP275227	present work
Skeletocutis lenis	HUBO 8296	Italy	Fagus	PP275218	PP275228	present work
Skeletocutis vulgaris	HUBO 8465	Italy	Pinus nigra ssp. laricio	PP275219	PP275229	present work
Skeletocutis lenis	SALA-Fungi 3749	Spain	Eucalyptus camaldulensis	PP275220	n.a.	present work
Skeletocutis lenis	SALA-Fungi 3752	Spain	Pinus pinaster	PP275221	n.a.	present work
Skeletocutis sp.	SALA-Fungi 4105	Spain	Pinus pinaster	PP275222	n.a.	present work
Skeletocutis sp.	SALA-Fungi 4111	Spain	Acer monspessulatum	PP275223	n.a.	present work
S. vulgaris	TU114503	Estonia	Populus tremula	UDB034888	n.a.	Sell (2017)*
S. vulgaris	TU135349	Estonia	Picea abies	UDB0754207	n.a.	Sell (2018)*
Sidera sp. (S. tibetica)	Dai 23648 ^н	China: Tibet	Pinus armandii	OM974253	OM974245	Liu et al. (2022)

		Geographic		GenBank ac	cession no.	5.4
Species	Collection code	origin	Substrate	ITS	28S rDNA	Reference
Sidera sp. (S. tibetica)	Dai 23407 ^P	China: Tibet	n.a.	OM974252	OM974244	Liu et al. (2022)
S. tibetica	Dai 22151	China	n.a.	MW477794	MW477794	Liu et al. (2021)
S. tibetica	Dai 21057	Belarus	on rotten wood of Picea	MW198484	MW192009	Liu et al. (2021)
S. tibetica	LE F-342597	Russia	Pinus brutia var. eldarica	OR457651	n.a.	Volobuev (2023)
Schizopora sp.	206	Spain	Castanea sativa EM root tips	MN947225	n.a.	Santolamazza-Carbone (2020)*
Schizopora sp.	DLL2009-014	USA: MN	Populus spp.	JQ673191	n.a.	Brazee et al. (2012)
Schizopora sp.	FH:BHI-F453	USA: MA	n.a.	MF161274	n.a.	Haelewaters et al. (2018)
Sidera sp.	UC2022907	USA: CA	on litter or well decayed wood in pinaceous forest	KP814250	n.a.	Rosenthal et al. (2017)
unc. Hyphodontia	1Bart548S	USA: NH	n.a.	HQ022192	n.a.	Vineis (2011)
unc. fungus	S38	Germany	air sample	FJ820526	n.a.	Fröhlich-Nowoisky et al. (2012)
Sidera sp. 1	·				'	
S. vulgaris	Ryvarden 37198	New Zealand	n.a.	FN907918	FN907918	Miettinen and Larsson (2011)
Sidera sp. 2						
Sidera sp.	UC2023008	USA: MS	decayed wood in pinaceous forest	KP814157	n.a.	Rosenthal et al. (2017)
Sidera sp. 3						
S. lowei	Ryvarden 38817	New Zealand	n.a.	FN907919	FN907919	Miettinen and Larsson (2011)
Sidera sp. 4						
unc. fungus	L042886-122- 066-F04	Taiwan	air filter	GQ999509	n.a.	Fröhlich-Nowoisky et al. (2012)
unc. fungus	L042881-122- 061-B09	Taiwan	air filter	GQ999433	n.a.	Fröhlich-Nowoisky et al. (2012)
Sidera sp. 5						
Sidera sp.	MEL:2382752	Australia: NT	n.a.	n.a.	KP012935	Bonito et al. (2014)*
Outgroups						
Alloclavaria purpurea	Miettinen 18831	ΗΠΑ: WA	old-growth forest with conifers	ON188807	ON188807	Viner (2022)*
Rickenella mellea	Lamoure 74	n.a.	n.a.	U66438	U66438	Lutzoni (1997)
Resinicium furfuraceum (Skvortzovia furfuracea)	KHL 11738	Finland	n.a.	DQ873648	DQ873648	Larsson et al. (2006)
Skvortzovia furfurella	KHL 10180	Puerto Rico	n.a.	DQ873649	DQ873649	Larsson et al. (2006)

Results

Taxonomy – Morphology

Sidera vulgaris (Fr.) Miettinen, Mycological Progress 10 (2): 136 (2011) Figs 1, 2

Polyporus vulgaris Fr., Systema Mycologicum 1: 381 (1821). Basionym.
Skeletocutis vulgaris (Fr.) Niemelä & Y.C. Dai, Annales Botanici Fennici 34 (2): 135 (1997). Synonyms.

Description. *Basidioma*—Annual to biennial, resupinate, soft when fresh and rather tough, soft-corky after drying, confluent and widely effused covering extended under-surface of decaying logs, 0.8–2.0 mm thick at the centre; pore

surface white to cream when fresh, becoming yellowish to buff when dry; sterile margin indistinct, cottony, white, thinning-out; pores very small, roundish, (5) 6-8 (10) per mm (n = 273/13); dissepiments thin, entire to slightly lacerate; subiculum very thin, cottony, concolorous with the tube layer; tubes concolorous with the poroid surface, up to 2 mm long.

Hyphal system dimitic in all parts of the basidioma; generative hyphae smooth, without encrustations, septa with clamp connections; skeletal hyphae not reacting with Cotton Blue, Melzer's reagent or KOH.

Subiculum–Hyphae interwoven, skeletal hyphae dominating, skeletals (1.7) 2-3.5 (4.0) µm in diameter, rosette-like crystal clusters rare to common.

Tubes—Hyphae subparallel to moderately interwoven. Generative hyphae, thin to slightly thick-walled, poorly branched, 1.7–3.0 µm in diameter. Skeletal hyphae, thick-walled to subsolid, hyaline, rarely branched, flexuous, 1.7–3.5 µm in diameter, with scattered swellings up to 7 µm. Dissepiment edges with both generative and skeletal hyphae that often bear a swollen, capitate apex, generative hyphae sometimes covered by a mucous droplet, rosette-like crystals frequent in mature basidiomata. Cystidioles seldom to abundant, fusoid, thin-walled, hyaline, basally swollen, with hyphoid neck and mostly obtuse or capitate tip, some bearing crystals at apex (asterocystidia), a few modified as halocystidia were also observed, (9.3) 12.4–19.9 (25.0) × (2.2) 2.8–4.0 (5.3) µm (n = 125/15). Basidia barrel-shaped to somewhat short-clavate, with four sterigmata and a basal clamp, (6.2) 6.8–9.9 (14.6) × (3.1) 3.8–4.7 (5.6) µm (n = 185/15); basidioles barrel-shaped, slightly shorter than the basidia.

Basidiospores—Cylindrical, moderately curved to lunate, thin-walled, hyaline, smooth, negative in Melzer's reagent, acyanophilous, (3.0) 3.4-3.9 (4.3) × (1.2) 1.4-1.6 (1.8) µm, Average = 3.6×1.5 µm, Q = (1.95) 2.24-2.60 (3.08) Q_{AV} = 2.41 (n = 399/15).

Distribution and hosts. The species is reported from Mediterranean Europe (e.g. Portugal, Spain, France, Italy, Croatia and Greece), Germany, Slovakia, Poland, Estonia, Sweden, Belarus, Russia, as well as from Armenia, Georgia, Iran, Kazakhstan, China, USA and Canada (Niemelä and Dai 1997; Ghobad-Nejhad 2011; Bernicchia et al. 2020; Liu et al. 2022; this work). It occurs on various broad-leaved trees of (*Alnus, Eucalyptus, Fagus, Populus, Quercus, Sorbus* and *Ulmus*), as well as on coniferous trees, i.e. *Picea, Pinus* (*P. halepensis, P. nigra* ssp. *laricio, P. pinaster, P. sylvestris*) or *Juniperus* and on *Abies cephalonica* (this work).



Figure 1. S. vulgaris specimen in situ (ACAM 2013-0017). Scale bar: 5 cm (left); 2 cm (right).



Figure 2. Micromorphological features of *S. vulgaris*; scale bar 5 μm [except of **f** and **g** 10 μm] **a** basidiospores (all specimens) **b** basidia (ACAM 2013-0017, ACAM DD2559, HUBO 7745, HUBO 8296, HUBO 8465, SALA-Fungi 3752) **c** hymenium with basidia and basidioles (HUBO 8465) **d** branched and unbranched cystidioles bearing crystals at apex (asterocystidia) (ACAM DD2559, SALA-Fungi 3752, SALA-Fungi 4111) **e** hymenial cystidioles (ACAM DD2559, HUBO 8296, SALA-Fungi 3749) **f** hyphae of the subiculum with dominating skeletals (ACAM 2013-0017) **g** dissepiment edges with skeletal and generative hyphal ends (HUBO 7745) **h** details of the rosette-like crystal clusters from tramal hyphae (HUBO 7745) **i** capitate ends of generative hyphae from dissepiments and hymenium (with mucous droplets) (HUBO 8296, SALA-Fungi 3749, SALA-Fungi 3752) **j** skeletal hyphae from dissepiments with swellings (ACAM 2013-0017, HUBO 7745, SALA-Fungi 3749, SALA-Fungi 4111).

Specimens examined. GREECE: Sterea Ellas, Fthiotida, Gardiki, on trunk of Abies cephalonica, 28 April 2007, ACAM DD2559, coll. D. Dimou. Attica, Mt. Parnitha, on trunk of P. halepensis, 30 May 2013, ACAM 2013-0017. coll. E. Polemis. ITALY: Emilia Romana, Forli, Pian del Pero Cullacea, on Ulmus glabra, 7 October 2002, HUBO 7629, coll. A. Bernicchia (as Sk. vulgaris); ibidem. on Fagus sp. 11 October 2006, HUBO 8296, coll. A. Bernicchia (as Sk. lenis); Ferrara, Bosco della Mesola, on Populus sp. 12 November 2003, HUBO 7701, coll. A. Bernicchia (as Sk. lenis); Bologna, Parko la Martina, on P. sylvestris, 16 July 2003, HUBO 7745, coll. A. Bernicchia (as Sk. vulgaris); Ravena, Pineta San Vitale, on Populus alba, 4 November 2003, HUBO 7811, coll./det. A. Bernicchia (as Sk. vulgaris). Sardinia, Tonara, Isca de sa Mela, on P. nigra ssp. laricio, 14 October 2007, HUBO 8465, coll. L. Arras (as Sk. vulgaris); Sorgono, Isca de sa Mela, on P. nigra ssp. laricio, 18 November 2009, HUBO 8522, coll. A. Bernicchia (as Sk. lenis). SPAIN: Castile-Leon, Garcibuey, on Eucalyptus camaldulensis, 7 November 2005, SALA-Fungi 3749, ibidem. on P. pinaster, 22 November 2006, SALA-Fungi 3747, coll. S.P. Gorjón (as Sk. lenis); Herguijuela de la Sierra, on P. pinaster, 18 November 2007, SALA-Fungi 3752, coll. S.P. Gorjón (as Sk. lenis); Miranda del Castañar, on P. pinaster, 22 November 2006, SALA-Fungi 4105, coll. S.P. Gorjón (as Skeletocutis sp.); San Martín del Castañar, on Acer monspessulatum, 14 October 2007, SALA-Fungi 4111, coll. S.P. Gorjón (as Skeletocutis sp.); Cepeda, on Alnus glutinosa, 29 November 2006, SALA-Fungi 3745, coll. S.P. Gorjón (as Sk. lenis).

Phylogenetic analysis

To estimate the phylogeny of the genus *Sidera*, datasets of ITS and 28S rDNA sequences were compiled, including sequences from collections with a Mediterranean distribution, as well as from pertinent specimens and environmental samples deposited in INSDC and UNITE in order to cover as much as possible the diversity and distribution of the genus. The total dataset consisted of 69 collections represented by 68 ITS and 36 28S sequences (Table 1). The material examined for the first time in the present study included nine collections from Mediterranean Europe, from which nine ITS and five 28S sequences were obtained. Additional information on the phylogenetic analyses performed for each dataset is provided in Suppl. material 1. Both applied phylogenetic strategies, ML and BI, produced phylograms characterised by a consistent topology, devoid of any supported conflicts.

The phylogenetic reconstruction, based on the ITS sequences (Fig. 3), recovered *Sidera* as a strongly-supported monophyletic clade (ML-BS 100%, BPP 1.00), which is further segregated into three well-supported main clades, A through to C. In total, 22 highly-supported terminal clades were recovered including those corresponding to the 18 formally described taxa; amongst them, 14 are represented by type sequences (plus one representing *S. tibetica*, which, however, should be considered as synonym of *S. vulgaris* as explained below). No sequences from type specimens were available for *S. lunata*, *S. vulgaris*, *S. lowei* or *S. minutipora*. In addition, four terminal clades do not correspond to the already known taxa and they could represent undescribed species. They are provisionally referred to as '*Sidera* sp. 1, 2, 3 and 4'.



Figure 3. Phylogenetic relationships within the genus *Sidera* inferred by using ML analysis on the ITS sequence dataset. ML BS \geq 65% and BPP \geq 0.95 are appended to nodes; asterisk denotes 100% ML BS and/or 1.00 BBP. Specimens studied are followed by their voucher code and geographic origin. Sequences determined in the present study appear in bold, while those representing type material are underlined. The phylogram is rooted with *Skvortzovia furfuracea* and *Skvortzovi*

Clade A (100%, 1.00) includes only *S. lunata*, which is distantly related to the rest of the *Sidera* spp. and it has a hydnoid hymenophore. All other taxa have poroid hymenophores and are grouped with significant support (95%, 0.98). They are further subdivided into clades B (100%, 1.00) and C (99%, 1.00) consisting of eight

and 13 species, respectively. Clade B includes S. lenis - the type species of the genus - represented by collections from Sweden, Finland, Russia and China, as well as a cluster composed of S. borealis and two closely-related taxonomic entities. The first of them is hereby designated 'Sidera sp. 1' (UNITE DOI: SH1110196. 09FU); it corresponds to the specimen Ryvarden 37198 from New Zealand, initially identified as S. vulgaris, but apparently not related to the real S. vulgaris, which is grouped in Clade C and includes material from the Northern Hemisphere. The second is represented by the specimen Sidera sp. UC2023008 from USA. Although closely positioned to S. borealis, it is considered as distinct from the latter species since it shows a rather low ITS sequence identity (96.5-98.4%) and distant geographic occurrence (S. borealis is reported from Europe and China). We provisionally call it 'Sidera sp. 2' (UNITE DOI: SH1110192.09FU). Moreover, clade B includes two pairs of sister species (100%, 1.00), i.e. S. tianshanensis B.K. Cui & T.M. Xu and S. salmonea Z.B. Liu, Jian Yu & F. Wu (both from Asia), as well as S. parallela Y.C. Dai, F. Wu, G.M. Gates & Rui Du and S. americana (the former originates from Asia, while the latter from North America and north Europe).

Clade C comprises the main diversity of the genus by accommodating 11 species and two entities possibly corresponding to new taxa. S. srilankensis Y.C. Dai, F. Wu, G.M. Gates & Rui Du and S. malaysiana Z.B. Liu & Y.C. Dai form a robustly-supported clade (100%, 1.00) consisting of Asian specimens. Similarly, sequences deriving from the Neotropics correspond to S. lowei. However, another collection (Ryvarden 38817) - initially identified as S. lowei from New Zealand is phylogenetically separated from the previous species and seems to represent a distinct taxon (ITS sequence identity: 83.0-83.8%), herein called 'Sidera sp. 3' (UNITE DOI: SH1110192.09FU). Furthermore, S. roseobubalina Z.B. Liu & Y.C. Dai is represented only by the holotype, originating from China. It is related to two seguences derived from environmental samples (air filters, Taiwan; ITS sequence identity to S. roseobubalina: 93.5-93.6%); hence, the latter could possibly correspond to an undescribed taxon which is provisionally named 'Sidera sp. 4' (UNITE DOI: SH1111516.09FU). The aforementioned taxa are strongly linked (96%, 1.00) with a group consisting of S. punctata Z.B. Liu & Y.C. Dai and S. vesiculosa Rui Du & M. Zhou; these four species are represented by sequences from material of Asian origin. Finally, a well-supported cluster (97%, 1.00) is composed by S. minutissima Y.C. Dai, F. Wu, G.M. Gates & Rui Du (including specimens from islands of the Indian Ocean and China), S. inflata Z.B. Liu & Y.C. Dai from China (sequence data available only from the type collection), the sister species S. minutipora (Rodway & Cleland) Y.C. Dai, F. Wu, G.M. Gates & Rui Du and S. tenuis Y.C. Dai, F. Wu, G.M. Gates & Rui Du (consisting of material from Australia) and S. vulgaris.

S. vulgaris forms a highly-supported terminal clade (100%, 1.00) composed of 22 sequences labelled with various names, for example, *S. vulgaris*, *S. lenis*, *S. tibetica*, *Sidera* sp., *Skeletocutis* sp. and *Schizopora* sp. All samples originated from the Northern Hemisphere (Europe, Asia and North America). In particular, the clade includes all material studied for the first time in the framework the present study (collected from various substrates in Spain, Italy and Greece), as well as sequences from Germany, Estonia, Belarus, Russia, China (incl. Tibet) and the USA (UNITE DOI: SH1262165.09FU).

Although represented by fewer sequences, the phylogenetic reconstructions that were based on 28S or on the concatenated ITS and 28S sequences (Suppl. material 2 and Fig. 4, respectively) provided similar topologies as the ITS tree by



Figure 4. Phylogenetic relationships within the genus *Sidera* inferred by using ML analysis on the concatenated ITS and 28S rDNA sequence dataset. ML BS \ge 65% and BPP \ge 0.95 are appended to nodes; asterisk denotes 100% ML BS and/ or 1.00 BBP. Specimens studied are followed by their voucher code and geographic origin. Sequences determined in the present study appear in bold, while those representing type material are underlined. The phylogram is rooted with *Skvortzovia furfurella*. The scale bar indicates 0.1 expected change per site per branch.

maintaining the same phylogenetic positions of *S. lunata* and of species within Clades B and C (100%, 1.00). It is also interesting that the unnamed taxa '*Sidera* sp. 1' and '*Sidera* sp. 3' seem to be well-separated from the already known species, as indicated above (Fig. 3). In addition, the specimen MEL:2382752 (Australia), originally identified as *Sidera* sp., appears to be distinct from the two most closely-related taxa, i.e. *S. srilankensis* and *S. malaysiana*; therefore, it is assigned with the provisional name '*Sidera* sp. 5' (Suppl. material 2).

Discussion

This study mainly deals with the taxonomic uncertainty associated with collections under the names *S. vulgaris* and *S. lenis*. A great deal of confusion stems from the erroneous initial identifications of such specimens and, as explained below, further obstacles were raised by the description of the (allegedly) new species *S. tibetica* (Liu et al. 2022).

Miettinen and Larsson (2011), who introduced *Sidera* as a new genus to accommodate – amongst others – the dimitic polypores *Sk. vulgaris* and *Sk. lenis*, studied the former by examining three collections: one European (Poland, Niemelä 5981) and two from the Southern Hemisphere. However, sequence data were available only from the latter two. As our results show, the collection from New Zealand (Ryvarden 37198), that was considered to represent *S. vulgaris* and was included as such in several phylogenetic studies, was assigned to clade B in the present study (as '*Sidera* sp. 1'). Furthermore, the collection from Tasmania (G. Gates FF257) was grouped in clade C and was identified as *S. minutipora* (Du et al. 2020). Therefore, it is clear that these two collections are not related to *S. vulgaris*. Moreover, several specimens included either as *S. vulgaris* or *S. vulgaris sensu lato* in the previous publications (Liu et al. 2021, 2022) were later linked to other species of this genus by the same group of authors (Liu et al. 2023), for example, to *S. americana* (Dai 12730 from USA and Dai 19173 from Canada) and *S. borealis* (Cui 11216 and Dai 22822 from China).

Most importantly, another new Sidera species was recently introduced under the name S. tibetica (Liu et al. 2022). It was described on the basis of two sequenced specimens from Tibet, but without being examined versus any real/ authentic S. vulgaris collections. In fact, the three "S. vulgaris" collections included in the aforementioned study were Ryvarden 37198 (New Zealand), Dai 19173 (Canada) and Dai 22822 (China). However, the first of these corresponds to Sidera sp. 1 (as the present work demonstrated), the second to S. americana and the third to S. borealis. In addition, all S. tibetica sequences from the work of Liu et al. (2022) [and from other recent publications where this name was also erroneously used (e.g. Volobuev (2023))] were grouped (100%, 1.00) together with a large number of S. vulgaris specimens originating from Europe (#15), Asia (#3) and USA (#4). It should additionally be noted that Liu et al. (2021) had indications that the allegedly new species occurred also in Europe (since they have examined the specimen Dai 21057, initially identified as S. vulgaris sensu lato from Belarus). However, they did not include it in the description of S. tibetica (Liu et al. 2022), whereas it was used in their more recent study under this name (Liu et al. 2023). Therefore, it is evident that S. tibetica was erroneously introduced as a new species since the respective examined material corresponds to S. vulgaris, i.e. the already existing species, previously described from specimens originating from Sweden (Niemelä and Dai 1997). Apparently, the lack of sequence data from the type material of S. vulgaris and not including correctly identified collections of the appropriate geographic origin (e.g. Europe) were the main reasons for this major issue detected in the publication of Liu et al. (2022). Hence, S. tibetica should be considered as a synonym of S. vulgaris.

The molecular evidence provided by the phylogenetic analyses (Fig. 3, Fig. 4 and Suppl. material 2) shows that *S. vulgaris* is well discriminated from *S. lenis* since the respective terminal clades are properly defined and clearly separated. On the other hand, distinguishing *S. vulgaris* from *S. lenis* morphologically remains a difficult task; this resulted in incorrect identifications of the two species in Europe and elsewhere. Until very recently, the distribution of *S. vulgaris* in south Europe was considered to be unknown because, as stated, "... it was easily confused with *S. lenis*" (Bernicchia et al. 2020). It is now clear that *S. vulgaris* is the only species of the genus which is also present in Mediterranean Europe.

Our morphological studies, in conjunction with the verified identity of specimens from DNA sequencing, revealed that the most stable and reliable character to distinguish these two species is the pore size, which is clearly smaller in *S. vulgaris*, on average, more than six pores per mm, as opposed to less than six pores per mm in *S. lenis* (Table 2). The length of the basidia, furthermore, seems to be important in this regard, as they hardly exceed 10 µm in *S. vulgaris*, while they are always longer in *S. lenis*. Regarding the size and shape of basidiospores,

 S. tibetica (Liu et al. 2022) and S. lenis (Niemelä and Dai 1997).

 S. vulgaris (this work)
 S. vulgaris (Niemelä and Dai 1997)

 S. tibetica (Liu et al. 2022)
 S. lenis (Niemelä and Dai 1997)

 Pores (per mm)
 6–8
 7–8
 4–6

Table 2. Comparison of key morphological features from collections of Sidera vulgaris (this work; Niemelä and Dai 1997),

	S. vulgaris (this work)	S. vulgaris (Niemelä and Dai 1997)	S. tibetica (Liu et al. 2022)	S. lenis (Niemelä and Dai 1997)
Pores (per mm)	6-8	6-8	7-8	4-6
Spores	3.2-4.0×1.3-1.7 μm, av. 3.64×1.51 μm Q = 2.24-2.60	2.9-3.6×0.9-1.4 μm, av. 3.14×1.08 μm Q = 2.44-3.11	2.9−3.1×1.0−1.1 µm, av. 3.01×1.05 µm Q = 2.78−2.91	3.9-4.9×1.5-2 μm, av. 4.35×1.76 μm Q = 2.29-2.74
Basidia length	6.8–11 μm	6.5-8.5 μm	8-9.5 μm	10–13.5 µm
Skeletals in KOH	1.7-3.5 μm	2.7-3.5 μm	2.0-4.0 μm	3.5-4.8 μm
Stellate crystals	frequent	very rare	frequent	frequent

our measurements indicate a much wider deviation, particularly with regards to the width, which largely affects the quotient (Q, length/width). Our work also suggests that S. vulgaris basidiospores may exceed 1.5 µm in width; this contradicts pertinent generic keys which placed a clear-cut value of 1.5 µm between these species (i.e. Ryvarden and Melo (2017); Bernicchia et al. (2020)). Apparently, this tiny value - and variations thereof - are very difficult to detect with accuracy. In contrast, the average spore length seems to be a more reliable character, since it does not exceed 4 µm in S. vulgaris, in contrast to S. lenis whose spores are usually longer. In our opinion, the presence of the stellate crystal agglomerations and the mucous deposit on the capitate generative hyphal tips are unstable characters, most likely affected by the age of basidiomata and the microscopy techniques used; thus, they are of questionable taxonomic value. In addition, the most important taxonomic features mentioned in the description of S. tibetica (Liu et al. 2022) are similar to (or not differing considerably from) those of S. vulgaris (Table 2). The deviations observed in spore size (especially) and pore density are small and they cannot be considered as significant since only three specimens of S. tibetica were used for its original description.

Conclusions

In this work, a large number of Sidera sequences (ITS and LSU rDNA) were analysed which included new material from Mediterranean Europe, as well as publicly available sequences. The monophyletic nature of the genus was strongly supported in the generated trees. Sidera lunata (characterised by a hydnoid hymenophore) was identified as the sister group to the remainder of the genus in the derived phylogeny, while species with poroid hymenophore formed a robustly supported lineage that was subdivided into two major clades. Amongst 23 species in total, five are possibly new to science, but since they are mostly represented by single collections, further work is needed before any definite conclusions could be drawn. The presence of S. lenis was assessed in north Europe, Russia and China, while examined collections from south Europe under this name were recovered within S. vulgaris. The latter species exhibits a Holarctic distribution. It occurs on dead wood of angiosperms and gymnosperms, including the regions of Eurasia where it was erroneously reported as S. tibetica. As we demonstrate, the description of this allegedly new species was based on collections that are hereby identified as S. vulgaris. This observation also emphasises the need to proceed with the epitypification of S. vulgaris since the type material maintained in Herbarium UPS may be too old for successful sequencing.

Acknowledgements

Curators of O (University of Oslo, Norway) and SALA (University of Salamanca, Spain) are thanked for loans of specimens. Our gratitude is also extended to A. Bernicchia and S.P. Gorjon for kindly providing access to herbarium collections.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

All authors have contributed equally.

Author ORCIDs

Vassiliki Fryssouli [®] https://orcid.org/0000-0002-8981-6748 Elias Polemis [®] https://orcid.org/0000-0002-2728-7350 Georgios I. Zervakis [®] https://orcid.org/0000-0002-2892-098X

Data availability

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

References

- Arita M, Karsch-Mizrachi I, Cochrane G (2021) The international nucleotide sequence database collaboration. Nucleic Acids Research 49(D1): D121–D124. https://doi. org/10.1093/nar/gkaa967
- Bernicchia A, Gorjón SP, Arras L, Facchini M, Porcu G, Trichies G (2020) Polypores of the Mediterranean region. Romar SRL, Segrate, Italy, 904.
- Brazee NJ, Lindner DL, Fraver S, D'Amato AW, Milo AM (2012) Wood-inhabiting, polyporoid fungi in aspen-dominated forests managed for biomass in the US Lake States. Fungal Ecology 5(5): 600–609. https://doi.org/10.1016/j.funeco.2012.03.002
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and high-performance computing. Nature Methods 9(8): 772. https://doi. org/10.1038/nmeth.2109
- Du R, Wang L, Zhou M, Chen J (2019) A new species of *Sidera* (Hymenochaetales, Basidiomycota) from tropical Asia. Phytotaxa 387(2): 165–171. https://doi.org/10.3390/ jof8040385
- Du R, Wu F, Gate GM, Dai YC, Tian XM (2020) Taxonomy and phylogeny of *Sidera* (Hymenochaetales, Basidiomycota): Four new species and keys to species of the genus. MycoKeys 68: 115–135. https://doi.org/10.3897/mycokeys.68.53561

- Fröhlich-Nowoisky J, Burrows SM, Xie Z, Engling G, Solomon PA, Fraser MP, Mayol-Bracero OL, Artaxo P, Begerow D, Conrad R, Andreae MO, Després VR, Pöschl U (2012) Biogeography in the air: Fungal diversity over land and oceans. Biogeosciences 9(3): 1125–1136. https://doi.org/10.5194/bg-9-1125-2012
- Ghobad-Nejhad M (2011) Updated checklist of corticioid and poroid basidiomycetes of the Caucasus region. Mycotaxon 117: 508.
- Haelewaters D, Dirks AC, Kappler LA, Mitchell JK, Quijada L, Vandegrift R, Buyck B, Pfister DH (2018) A preliminary checklist of fungi at the Boston Harbor Islands. Northeastern Naturalist 25(sp9): 45–76. https://doi.org/10.1656/045.025.s904
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Larsson KH, Parmasto E, Fischer M, Langer E, Nakasone KK, Redhead SA (2006) Hymenochaetales: A molecular phylogeny for the hymenochaetoid clade. Mycologia 98: 926–936. https://doi.org/10.3852/mycologia.98.6.926
- Lassmann T, Sonnhammer EL (2006) Kalign, Kalignvu and Mumsa: web servers for multiple sequence alignment. Nucleic Acids Research 34 (Web Server issue): W596– W599. https://doi.org/10.1093/nar/gkl191
- Liu ZB, Zhou M, Yuan Y, Dai YC (2021) Global diversity and taxonomy of *Sidera* (Hymenochaetales, Basidiomycota): Four new species and keys to species of the genus. Journal of Fungi 7(4): 251. https://doi.org/10.3390/jof7040251
- Liu ZB, Zhou M, Wu F, Yu J (2022) Two New Species of Sidera (Hymenochaetales, Basidiomycota) from Southwest China. Journal of Fungi 8(4): 385. https://doi.org/10.3390/ jof8040385
- Liu ZB, Zhou HM, Liu HG, Yuan Y (2023) Taxonomy and phylogeny of *Sidera* (Hymenochaetales, Rickenella clade) from China and North America revealing two new species. MycoKeys 96: 173–191. https://doi.org/10.3897/mycokeys.96.100743
- Lowe JL (1956) Type studies of the polypores described by Karsten. Mycologia 48: 99– 125. https://doi.org/10.1080/00275514.1956.12024520
- Lutzoni FM (1997) Phylogeny of lichen-and non-lichen-forming omphalinoid mushrooms and the utility of testing for combinability among multiple data sets. Systematic Biology 46(3): 373–406. https://doi.org/10.1093/sysbio/46.3.373
- Maddison WP, Maddison DR (2023) Mesquite: a modular system for evolutionary analysis. Version 3.81. http://www.mesquiteproject.org
- Miettinen O, Larsson KH (2011) *Sidera*, a new genus in Hymenochaetales with poroid and hydnoid species. Mycological Progress 10: 131–141. https://doi.org/10.1007/ s11557-010-0682-5
- Miller MA, Schwartz T, Pickett BE, He S, Klem EB, Scheuermann RH, Passarotti M, Kaufman S, O'Leary MA (2015) A RESTful API for Access to Phylogenetic Tools via the CIPRES Science Gateway. Evolutionary Bioinformatics Online 11: 43–48. https://doi.org/10.4137/EB0.S21501
- Minh BQ, Lanfear R, Ly-Trong N, Trifinopoulos J, Schrempf D, Schmidt HA (2022) IQ-TREE version 2.2.0: Tutorials and Manual Phylogenomic software by maximum likelihood. Nucleic Acids Research 44(W1): W232–W235. https://doi.org/10.1093/nar/ gkw1135
- Niemelä T, Dai YC (1997) Polypore *Skeletocutis lenis* and its sib *S. vulgaris*. Annales Botanici Fennici 34(2): 133–140.
- Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picard K, Glöckner FO, Tedersoo L, Saar I, Kõljalg U, Abarenkov K (2019) The

UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. Nucleic Acids Research 47(D1): D259–D264. https://doi. org/10.1093/nar/gky1022

- Rajchenberg M (1987) Type studies of Polyporaceae (Aphyllophorales) described by J. Rick. Nordic Journal of Botany 7: 553–556. https://doi.org/10.1111/j.1756-1051.1987. tb02023.x
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Rosenthal LM, Larsson KH, Branco S, Chung JA, Glassman SI, Liao HL, Peay KG, Smith DP, Talbot JM, Taylor JW, Vellinga EC, Vilgalys R, Bruns TD (2017) Survey of corticioid fungi in North American pinaceous forests reveals hyperdiversity, underpopulated sequence databases, and species that are potentially ectomycorrhizal. Mycologia 109(1): 115–127. https://doi.org/10.1080/00275514.2017.1281677
- Ryvarden L, Melo I (2017) Poroid Fungi of Europe (2nd edn.). Synopsis Fungorum Vol. 37. Fungiflora.
- Sayers EW, Cavanaugh M, Clark K, Pruitt KD, Sherry ST, Yankie L, Karsch-Mizrachi I (2024) GenBank 2024 Update. Nucleic Acids Research 52(D1): D134–D137. https://doi.org/10.1093/nar/gkad903
- Stielow JB, Lévesque CA, Seifert KA, Meyer W, Iriny L, Smits D, Renfurm R, Verkley GJ, Groenewald M, Chaduli D, Lomascolo A, Welti S, Lesage-Meessen L, Favel A, Al-Hatmi AM, Damm U, Yilmaz N, Houbraken J, Lombard L, Quaedvlieg W, Binder M, Vaas LA, Vu D, Yurkov A, Begerow D, Roehl O, Guerreiro M, Fonseca A, Samerpitak K, van Diepeningen AD, Dolatabadi S, Moreno LF, Casaregola S, Mallet S, Jacques N, Roscini L, Egidi E, Bizet C, Garcia-Hermoso D, Martín MP, Deng S, Groenewald JZ, Boekhout T, de Beer ZW, Barnes I, Duong TA, Wingfield MJ, de Hoog GS, Crous PW, Lewis CT, Hambleton S, Moussa TA, Al-Zahrani HS, Almaghrabi OA, Louis-Seize G, Assabgui R, McCormick W, Omer G, Dukik K, Cardinali G, Eberhardt U, de Vries M, Robert V (2015) One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia Molecular Phylogeny and Evolution of Fungi 35(1): 242–263. https://doi.org/10.3767/003158515X689135
- Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi. org/10.1093/molbev/msab120
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
- Vineis JH (2011) Links between the community structure of ectomycorrhizal fungi and nitrogen availability. PhD Thesis, State University of New York College of Environmental Science and Forestry.
- Volobuev SV (2023) Sidera tibetica (Hymenochaetales, Basidiomycota) A New Species to Russia. Mikologiya i Fitopatologiya. Mycology and Phytopathology 57(6): 394–400. https://doi.org/10.31857/S0026364823060156
- White TJ, Bruns T, Lee SJWT, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18(1): 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Xu TM, Sun YF, Liu S, Song CG, Gao N, Wu DM, Cui BK (2023) *Ceriporiopsis tianshanensis* (Polyporales, Agaricomycetes) and *Sidera tianshanensis* (Hymenochaetales, Agar-

icomycetes), two new species of wood-inhabiting fungi from Xinjiang, Northwest China. MycoKeys 98: 1–18. https://doi.org/10.3897/mycokeys.98.102552

Zervakis GI, Venturella G, Fryssouli V, Inglese P, Polemis E, Gargano ML (2019) *Pleurotus opuntiae* revisited – An insight to the phylogeny of dimitic *Pleurotus* species with emphasis on the *P. djamor* complex. Fungal Biology 123: 188–199. https://doi. org/10.1016/j.funbio.2018.12.00

Supplementary material 1

Detailed characteristics of the phylogenetic analysis performed for each sequence dataset used for the study of *Sidera* collections

Authors: Vassiliki Fryssouli, Elias Polemis, Milton A. Typas, Georgios I. Zervakis Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.121601.suppl1

Supplementary material 2

Phylogenetic relationships within the genus *Sidera* inferred by using ML analysis on the 28S rDNA sequence dataset

Authors: Vassiliki Fryssouli, Elias Polemis, Milton A. Typas, Georgios I. Zervakis Data type: pdf

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.121601.suppl2



Research Article

Three new species of *Teunia* (Cryptococcaceae, Tremellales) identified through phenotypic and phylogenetic analyses

Qi-Chao Guo¹⁰, Shan Liu¹⁰, Ya-Zhuo Qiao¹⁰, Feng-Li Hui^{1,20}

1 School of Life Science and Agricultural Engineering, Nanyang Normal University, Nanyang 473061, China

2 Research Center of Henan Provincial Agricultural Biomass Resource Engineering and Technology, Nanyang Normal University, Nanyang 473061, China Corresponding author: Feng-Li Hui (fenglihui@yeah.net)

Abstract

Teunia, belonging to the family Cryptococcaceae of the order Tremellales, is a genus of plant-inhabiting fungi distributed across the globe. Its members form associations with different plant parts, including flowers, fruits, leaves, seeds, and twigs. Recent efforts have aimed to explore the diversity of *Teunia* in China, however, many geographical regions have not yet been explored. In this study, we included results of five *Teunia* yeast strains that were isolated from plant materials collected in Fujian, Guizhou and Henan provinces, with descriptions, illustrations, and phylogenetic analyses of three new species: *T. acericola, T. mussaendrae* isolated from leaf surfaces in Fujian, Guizhou and Henan Provinces, and *T. qingyuanensis* obtained from rotting wood in Fujian Province.

Key words: Basidiomycota, fungal diversity, new species, plant, taxonomy

Introduction

Teunia is a recently established genus by Li et al. (2020), based on the phylogenetic analysis of a seven-gene dataset consisting of SSU rRNA, D1/D2 LSU rRNA domain, ITS region, RPB1, RPB2, TEF1-a, and CYTB. This analysis revealed a well-supported clade encompassing Cryptococcus cuniculi K.S. Shin & Y.H. Park, Fonsecazyma tronadorensis Yurkov (= Cryptococcus tronadorensis V. de García, Zalar, Brizzio, Gunde-Cim & Van Broock), Fonsecazyma betulae Yurkov, Kachalkin & Boekhout (= Kwoniella betulae K. Sylvester, Q.M. Wang & Hittinger), along with three new species T. globosa Q.M. Wang, F.Y. Bai & A.H. Li, T. helanensis Q.M. Wang, F.Y. Bai & A.H. Li, and T. korlaensis Q.M. Wang, F.Y. Bai & A.H. Li that was designated as the type species of the genus (Li et al. 2020). Since then, the increasing accessibility of sequencing services and a large quantity of available molecular data have led to a rapid expansion in the knowledge of the genus, and seven new species have been described: T. rosae Q.M. Wang, A.H. Li, G.S. Wang & Wangmu, T. rudbeckiae Q.M. Wang, A.H. Li, G.S. Wang & Wangmu (Wang et al. 2020), T. siamensis Khanam & Limtong (Khunnamwong et al. 2020), T. lichenophila Kachalkin, M.A. Tomashevskaya & T.A. Pankrato (Crous et al. 2021), T. nitrariae X.Z. Liu, F.Y. Bai & X.Y. Wei (Wei et al. 2022), and T. virginiahalliae Y.P. Tan & G.S. Pegg (Tan and Pegg 2023). In the case of



Academic editor: Margarita Dueñas Received: 8 February 2024 Accepted: 24 April 2024 Published: 15 May 2024

Citation: Guo Q-C, Liu S, Qiao Y-Z, Hui F-L (2024) Three new species of *Teunia* (Cryptococcaceae, Tremellales) identified through phenotypic and phylogenetic analyses. MycoKeys 105: 139–153. https://doi.org/10.3897/ mycokeys.105.120534

Copyright: © Qi-Chao Guo 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). *T. virginiahalliae*, which has been proposed based only on the ITS sequence, a representative reference culture has not been deposited in a culture collection, which hampers further studies on this species.

Until now, 12 species have been accepted in *Teunia* (www.indexfungorum. org/; www.mycobank.org). They all share cream to yellow-colored colonies, polar budding, non-fermentative nature, and inability to form pseudohyphae, hyphae, and ballistoconidia (Li et al. 2020). The members of Teunia have been found in diverse habitats and are frequently isolated as epiphytes from flowers (Wang et al. 2020), leaves (Sylvester al. 2015; Li et al. 2020), and tree barks (Sylvester et al. 2015), T. lichenophila was isolated as endophyte from Cladonia rangiferina and C. stellaris (Crous et al. 2021). Species of Teunia have also been isolated from soil (Khunnamwong et al. 2020; Li et al. 2020), barley from wild rabbit feces (Shin et al. 2006) and glacial biomes (de Garcia et al. 2012). Furthermore, it is hypothesized that an excess of 30 undescribed or erroneously identified strains may represent an additional 20 Teunia species (Wang et al. 2020). These potential members originate from various diverse substrates, including plant materials such as flowers (Herzberg et al. 2002; Mittelbach et al. 2015), floral nectars (Alvarez-Pérez and Herrera 2013), seeds (Fernández et al. 2012), fruits, leaves, and twigs. Others have been collected from soil (Takashima et al. 2012; Yurkov et al. 2016), coastal seawater, and extreme acidic environments (Gadanho et al. 2006). Taken together, these previous findings could be an indication that the habitat of these fungi is different plant parts.

Currently, half of the accepted species in *Teunia* were described from China, *T. globosa*, *T. helanensi*, *T. korlaensis* (Li et al. 2020), *T. rosae*, *T. rudbeckiae* (Wang et al. 2020), and *T. nitrariae* (Wei et al. 2022). However, these species have been collected from limited geographical ranges, and it is hoped that broader field investigations will reveal additional members of the genus.

During our investigation, we isolated five strains of *Teunia* from various substrates across different regions of China. Our phylogenetic analyses and examination of phenotypic features determined that the isolates represent three new species. The objective of this paper is to describe these species with morphological and molecular characters and contribute to knowledge of the diversity of *Teunia* in China.

Materials and methods

Sample collection and yeast isolation

Materials were collected from the Fujian, Guizhou, and Henan Provinces of China. One of the yeast strains was isolated from rotting wood through the enrichment method described by Shi et al. (2021). Four additional strains were harvested from leaf surfaces using the improved ballistospore-fall method described by Nakase and Takashima (1993). Based on this method, fresh leaves were cut into small pieces and adhered with a thin layer of petroleum jelly to the inner lid of a Petri dish containing yeast extract-malt extract (YM) agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, and 2% agar). The mixture was supplemented with 0.01% chloramphenicol to avoid bacterial growth. Plates were incubated at 20 °C and monitored daily for colony formation. Selected colonies were streaked onto separate YM agar plates for

purification. Following purification, strains were suspended in YM broth supplemented with 20% (v/v) glycerol and stored at -80 °C for future use. Cultures of all obtained isolates were preserved at the Microbiology Lab, Nanyang Normal University, Henan, China.

Phenotypic characterization

Morphological, physiological, and biochemical analyses were performed according to the standard methods described by Kurtzman et al. (2011). To examine the inducibility of the sexual state in each isolate, single or double strains were mixed on corn meal agar (CMA), potato dextrose agar (PDA), and V8 agar (10% V8 juice, 2% agar) at 20 °C for up to 8 weeks (Wang et al. 2020). Glucose fermentation was tested in a liquid medium with Durham fermentation tubes. Carbon and nitrogen assimilation capabilities were examined in a liquid medium, with starved inoculum used for nitrogen testing (Kurtzman et al. 2011). Growth at various temperatures (15, 20, 25, 30, 35, and 37 °C) was assessed through cultivation on YM agar plates. Cell morphology was examined with LEI-CA DM2500 cameras (LECIA Co, Wetzlar, Germany) and LASV4.13 software. All proposed names and descriptions were deposited in the MycoBank database (http://www.mycobank.org; 8 February 2024).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from each strain using the Ezup Column Yeast Genomic DNA Purification Kit, according to the manufacturer's instructions (Sangon Biotech Co., Shanghai, China). The ITS region, D1/D2 domain of the LSU rRNA, and a partial segment RPB1 were amplified with primers ITS1/ITS4 (White et al. 1990), NL1/NL4 (Kurtzman and Robnett 1998), and RPB1-Af and RPB1-Cr (Kurtzman and Robnett 2003), respectively. Amplifications were performed in a 25 µL reaction-volume tube containing 9.5 µL ddH₂O, 12.5 µL Taq 2X PCR Master Mix with blue dye (Sangon Biotech Co., Shanghai, China), 1 µL DNA template, and 1 µL of each primer. The ITS region and D1/D2 domain were amplified with an initial denaturation step of 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 51 °C, 40 s at 72 °C, and a final extension of 10 min at 72 °C (Toome et al. 2013). Amplification of the partial RPB1 gene was conducted using a touchdown PCR protocol as described by Wang et al. (2014). PCR products were then purified and sequenced by Sangon Biotech Co., Ltd (Shanghai, China) using the same primers. The identity and accuracy of each sequence were determined by comparison to sequences in GenBank. Assembly was performed with BioEdit v.7.1.3.0 (Hall 1999). All newly generated sequences were deposited in the GenBank database (https://www.ncbi. nlm.nih.gov/genbank/).

Phylogenetic analysis

Phylogenetic analyses were conducted based on LSU sequences alone and a combination of the ITS, LSU, and RPB1 dataset. *Cryptococcus amylolentus* CBS 6039^{T} and *Cryptococcus neoformans* CBS 8710^{T} were designated as outgroups (Crous et al. 2021). Individual loci sequences were aligned using

MAFFT v.7.110 (Katoh and Standley 2013) under the G-INI-I option. Poorly aligned regions were removed and adjusted manually using MEGA v.11 (Tamura et al. 2021). Aligned sequences of the different loci were concatenated with Phylosuit v.1.2.2 (Zhang et al. 2020).

Maximum likelihood (ML) analysis was conducted using RAxML v.8.2.3 with the GTRGAMMA model (Stamatakis 2014). Node ML bootstrap values (MLBS) were evaluated using 1,000 rapid replicates. The Best-fit evolution model for Bayesian inference (BI) was determined with ModelFinder (Kalyaanamoorthy et al. 2017). BI analysis was performed using MrBayes v.3.2.7a (Ronquist et al. 2012) through the CIPRES Science Gateway. Six simultaneous Markov chains were run for 50 million generations, with trees sampled every 1,000th generation. The first 25% of trees were discarded, representing the burn-in phase. Remaining trees were used to calculate the Bayesian posterior probabilities (BPP) of each clade. Trees were examined using FigTree v.1.4.3 (Andrew 2016). Branches exhibiting MLBS values ≥50% and BPP values ≥0.95 were shown at the nodes.

Results

Molecular phylogeny

A total of five yeast strains preliminarily identified as *Teunia* were studied further (Table 1). Besides the newly generated sequences, additional related sequences were also downloaded from GenBank (Table 2) for inclusion in the phylogenetic analyses.

The LSU dataset consisted of 32 sequences representing 25 species. The aligned set had a length of 603 characters, of which 480 were constant, 34 were variable and parsimony-uninformative, and 89 were parsimony-informative. The BI yielded a topology similar to the ML analysis, with an average standard deviation of split frequencies equal to 0.009938. In the LSU based phylogenetic tree (Fig. 1), five newly isolated strains formed three distinct and well-supported lineages that are distant from other *Teunia* species. Since *T. virginiahalliae* only has ITS sequence data, the phylogenetic analysis based on the ITS dataset was also performed. The phylogenetic tree (Suppl. material 1) recovered 12 known species of *Teunia*, while the newly isolated strains formed three independent lineages as in the phylogeny inferred from the LSU dataset.

The combined ITS, LSU, and RPB1 dataset encompassed sequences from 28 yeast strains representing 26 species. Including gaps, the dataset had an aligned length of 1,978 characters (549, 603, and 826 characters for ITS, LSU, and RPB1, respectively), of which 873 were constant, 381 were variable and parsimony-uninformative, and 724 were parsimony-informative. The best-fit model of the combined dataset for BI analysis was determined to be GTR+I+G, with equal nucleotide frequencies. The BI yielded a topology similar to the ML analysis, with an average standard deviation of split frequencies equal to 0.009550. The ITS, LSU, and RPB1 based phylogenetic tree (Fig. 2) produced a topology similar to that generated by the LSU based phylogenetic tree, and further confirmed the groupings of the three new species within *Teunia*.

Strains NYNU 2111141 and NYNU 2111157 were isolated from different leaves, but possess identical D1/D2 and ITS sequences. Both phylogenetic

Strain Source		Location				
Teunia acericola Y.Z. Qia	o & F.L. Hui					
NYNU 2111141 ^T	Leaf of Acer palmatum	Baotianman Nature Reserve, Neixiang County, Henan Province, China				
NYNU 2111157	Leaf of Rhus chinensis	Baotianman Nature Reserve, Neixiang County, Henan Province, China				
Teunia qingyuanensis Y.Z. Qiao & F.L. Hui						
NYNU 22475 [⊤]	Rotting wood	Qingyuan Mountain, Quanzhou City, Fujian Province, China				
Teunia mussaendrae Y.Z. Qiao & F.L. Hui						
NYNU 23232 ^T	Leaf of Mussaenda pubescens	Sifangjing Village, Pingtang County, Guizhou Province, China				
NYNU 23257	Leaf of Viburnum sp.	Sifangjing Village, Pingtang County, Guizhou Province, China				

Table 1. Yeast strains and origins investigated in this study.

 Table 2. Species name, strain/clone numbers, and GenBank accession numbers included in phylogenetic analyses. Entries in bold represent newly generated materials.

Species finalme Strain/Color Humber FTS LSU D1/D2 RPB1 Cryptococcus anylolentus CBS 6039 ^T NR_111372 KY106966 KF036342 Cryptococcus anylolentus CBS 6039 ^T NR_117785 NG_058766 KF036351 Kwoniella besitolae CBS 10118 ^T NR_111373 NG_042482 KF036351 Kwoniella dejecticola CBS 10517 ^T NR_073257 NG_058266 KF036362 Kwoniella endrophila CBS 509 ^T NR_073210 AF044742 KF036392 Kwoniella neveanensis CBS 509 ^T NR_073322 AF444742 KF036498 Kwoniella neveanensis CBS 1073 ^T NR_1174734 MK050289 MK849160 Kwoniella phi CBS 1073 ^T NR_11727 OM017170 PP236726 Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola MYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola MUCC2071 - LC715712 - Teunia acericola MUCC2071 -	Species nome	Strain/along number	GenBank accession numbers				
Cryptococcus amylolentus CBS 6039 ¹ NR_111372 KY106966 KF036342 Cryptococcus neoformans CBS 8710 ¹ NR_171785 NG_058766 KF036472 Kwoniella bestiolae CBS 10118 ¹ NR_111373 NG_042482 KF036351 Kwoniella dejecticola CBS 10117 ¹ NR_111374 NG_042483 KF036320 Kwoniella dejecticola CBS 15359 ¹ MH237945 MH237945 LS992197 Kwoniella ndrophylica CBS 569 ¹ NR_073210 AF075467 FJ534921 Kwoniella nargrovensis CBS 15359 ¹ NR_174734 MK050289 MK849160 Kwoniella nargrovensis CBS 10737 ¹ NR_111269 KY108203 KF036395 Kwoniella shivajii CBS 10737 ¹ NR_11729 MK050289 MK849160 Kwoniella shivajii CBS 10737 ¹ NR_11269 KY108203 KF036395 Kwoniella shivajii CBS 10737 ¹ NR_11269 KY108203 KF036395 Kwoniella shivajii CBS 10737 ¹ NR_169772 NG_042515 KF036401 Feunia acericola	Species name	Strain/cione number	ITS	LSU D1/D2	RPB1		
Cryptococcus neoformans CBS 8710 ¹ NR_171785 NG_058766 KF036472 Kwoniella besitolae CBS 10117 ¹ NR_111373 NG_042482 KF036351 Kwoniella dejecticola CBS 10117 ¹ NR_111374 NG_042483 KF036320 Kwoniella dendrophila CBS 6074 ¹ NR_073257 NG_058326 KF036320 Kwoniella endophytica CBS 15359 ¹ MH237945 MH237945 LS992197 Kwoniella nangrovensis CBS 569 ¹ NR_073210 AF075467 FJ534921 Kwoniella nangrovensis CBS 5057 ¹ NR_073322 AF444742 KF036498 Kwoniella nangrovensis CGBC 2.3439 ¹ NR_174734 MK050289 MK849160 Kwoniella shivajii CBS 10737 ¹ NR_165977 NG_042515 KF036401 Teunia acericola NYU 2111141 ¹ OM017170 PP236726 Teunia acericola HB1-3 - KJ507251 - Teunia acericola MUCC2071 - LC715712 - Teunia acericola F3-5 - AB618905 <t< td=""><td>Cryptococcus amylolentus</td><td>CBS 6039[™]</td><td>NR_111372</td><td>KY106966</td><td>KF036342</td></t<>	Cryptococcus amylolentus	CBS 6039 [™]	NR_111372	KY106966	KF036342		
Kwoniella bestiolae CBS 10118 ^T NR_111373 NG_042482 KF036351 Kwoniella dejecticola CBS 10117 ^T NR_111374 NG_042483 KF036362 Kwoniella endophylica CBS 6074 ^T NR_073257 NG_058326 KF036320 Kwoniella endophytica CBS 15359 ^T MH237945 LS992197 Kwoniella heveanensis CBS 569 ^T NR_073210 AF075467 FJ534921 Kwoniella nangrovensis CBS 1507 ^T NR_073322 AF444742 KF036498 Kwoniella pini CBS 10737 ^T NR_111269 KY108203 KF036395 Kwoniella shivajii CBS 10737 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111147 OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola MUCC1071 - LC71571 - Teunia acericola MUCC2071 - LC71572 - Teunia acericola F3-5 - A6161905 -	Cryptococcus neoformans	CBS 8710 [™]	NR_171785	NG_058766	KF036472		
Kwoniella dejecticola CBS 10117 ^T NR_111374 NG_042483 KF036362 Kwoniella dendrophila CBS 6074 ^T NR_073257 NG_058326 KF036320 Kwoniella endrophytica CBS 15359 ^T MH237945 MH237945 LS992197 Kwoniella heveanensis CBS 569 ^T NR_073210 AF044742 KF036498 Kwoniella mangrovensis CGMC 2.3439 ^T NR_174734 MK050289 MK849160 Kwoniella nangrovensis CGS 10737 ^T NR_111269 KY108203 KF036395 Kwoniella shivajjii CBS 10737 ^T NR_115977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP23073 PP239062 PP236727 Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola F3-5 - AB618905 - Teunia acericola GSMC 2.3648 ^T NR_174733 MK050286 </td <td>Kwoniella bestiolae</td> <td>CBS 10118[™]</td> <td>NR_111373</td> <td>NG_042482</td> <td>KF036351</td>	Kwoniella bestiolae	CBS 10118 [™]	NR_111373	NG_042482	KF036351		
Kwoniella dendrophila CBS 6074 ¹ NR_073257 NG_058326 KF036320 Kwoniella endophytica CBS 15359 ¹ MH237945 LS992197 Kwoniella heveanensis CBS 560 ¹ NR_073210 AF075467 FJ534921 Kwoniella mangrovensis CBS 650 ¹¹ NR_073322 AF444742 KF036498 Kwoniella ovata CGMCC 2.3439 ¹¹ NR_11734 MK050289 MK849160 Kwoniella ovata CGMCC 2.3439 ¹¹ NR_11729 KY08203 KF036395 Kwoniella shivajji CBS 10737 ¹¹ NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ¹¹ OM017172 OM017170 PP236726 Teunia acericola NUC 21912 - EU678944 - Teunia acericola MUCC1912 - LC115712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola F3-5 - AB618905 - Teunia acericola CGMC 2.5448 ¹¹ NR_174733 MK050288 MK849208	Kwoniella dejecticola	CBS 10117 [⊤]	NR_111374	NG_042483	KF036362		
Kwoniella endophytica CBS 15359 ^T MH237945 MH237945 LS992197 Kwoniella heveanensis CBS 569 ^T NR_073210 AF075467 FJ534921 Kwoniella mangrovensis CBS 8507 ^T NR_073322 AF444742 KF036498 Kwoniella ovata CGMCC 2.3439 ^T NR_174734 MK050289 MK849160 Kwoniella shivajii CBS 10737 ^T NR_111269 KY108203 KF036395 Kwoniella shivajii CBS 11374 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236726 Teunia acericola B1226 - EU678944 - Teunia acericola MUCC1071 - LC715712 - Teunia acericola MUCC2071 - LC715712 - Teunia acericola SGMC 2.24450 ^T KM384102 KM408130 - Teunia acericola CGMC 2.5648 ^T NR_174733 MK050288 MK849208 Teunia acericola CGMC 2.4450 ^T NR_174733 MK050287 MK849	Kwoniella dendrophila	CBS 6074 ^T	NR_073257	NG_058326	KF036320		
Kwoniella heveanensis CBS 569 ^T NR_073210 AF075467 FJ534921 Kwoniella mangrovensis CBS 8507 ^T NR_073332 AF444742 KF036498 Kwoniella ovata CGMCC 2.3439 ^T NR_174734 MK050289 MK849160 Kwoniella pini CBS 10737 ^T NR_11269 KY108203 KF036395 Kwoniella shivajii CBS 11374 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP239062 PP236727 - Teunia acericola Bl226 - EU678944 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - AB6905 - Teunia acericola F3-5 - AB6905 - Teunia acericola KM2-63732 ^T KM384102 KM408130 - Teunia acericola S-5 - AB61905 - Teunia acericola <td< td=""><td>Kwoniella endophytica</td><td>CBS 15359[™]</td><td>MH237945</td><td>MH237945</td><td>LS992197</td></td<>	Kwoniella endophytica	CBS 15359 [™]	MH237945	MH237945	LS992197		
Kwoniella mangrovensis CBS 8507 ^T NR_073332 AF444742 KF036498 Kwoniella ovata CGMCC 2.3439 ^T NR_174734 MK050289 MK849160 Kwoniella pini CBS 10737 ^T NR_111269 KY108203 KF036395 Kwoniella shivajii CBS 11374 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola Bl226 - EU678944 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola KUC2 25648 ^T NR_137887 KY106982 MN014082 Teunia deliaensis CGMCC 2.548 ^T NR_174733 MK050288 MK849208 Teunia ichenophila CBS 10309 ^T NR_174731 MK050286 MK849194 Teunia ichenophila CGMCC 2.548 ^T NR_174733 MK050286 MK8	Kwoniella heveanensis	CBS 569 [⊤]	NR_073210	AF075467	FJ534921		
Kwoniella ovata CGMCC 2.3439 ^T NR_174734 MK050289 MK849160 Kwoniella pini CBS 10737 ^T NR_111269 KY108203 KF036395 Kwoniella shivajii CBS 11374 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ^T OM017170 PP236726 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola B1226 - EU678944 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola KMC65030 ^T NR_137887 KY106982 MN014082 Teunia delaoesi CGMCC 2.5648 ^T NR_174733 MK050286 MK849208 Teunia idobosa CGMCC 2.4450 ^T NR_174731 MK050286 MK849194 Teunia ikorlaensis <td< td=""><td>Kwoniella mangrovensis</td><td>CBS 8507[⊤]</td><td>NR_073332</td><td>AF444742</td><td>KF036498</td></td<>	Kwoniella mangrovensis	CBS 8507 [⊤]	NR_073332	AF444742	KF036498		
Kwoniella pini CBS 10737 ^T NR_111269 KY108203 KF036395 Kwoniella shivajii CBS 11374 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola Bl226 - EU678944 - Teunia acericola HB31-3 - KJ507251 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - AB618905 - Teunia acericola F3-5 - AB618905 - Teunia aculuciu CBS 10309 ^T NR_13787 KY106982 MN014082 Teunia globosa CGMCC 2.4450 ^T NR_174733 MK050286 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849208 Teunia ichenophila <td< td=""><td>Kwoniella ovata</td><td>CGMCC 2.3439[™]</td><td>NR_174734</td><td>MK050289</td><td>MK849160</td></td<>	Kwoniella ovata	CGMCC 2.3439 [™]	NR_174734	MK050289	MK849160		
Kwoniella shivajii CBS 11374 ^T NR_165977 NG_042515 KF036401 Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola Bl226 - EU678944 - Teunia acericola HB31-3 - KJ507251 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - Res Med18905 - Teunia acericola F3-5 - AB618905 - - Teunia betulae NRL Y-63732 ^T KM384102 KM014082 MN014082 - Teunia duciuli CGBNC 2.648 ^T NR_174733 MK050288 MK849208 Teunia duciaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849208 Teunia intenansis CGMCC 2.6797 ^T OQ851888 OQ851887 PP236729	Kwoniella pini	CBS 10737 [™]	NR_111269	KY108203	KF036395		
Teunia acericola NYNU 2111141 ^T OM017172 OM017170 PP236726 Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola Bl226 - EU678944 - Teunia acericola HB31-3 - KJ507251 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - AB618905 - Teunia acericola KRL Y-63732 ^T KM384102 KM408130 - Teunia duciuli CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia duciuli CBS 10309 ^T NR_174733 MK050288 MK849208 Teunia korlaensis CGMCC 2.5648 ^T NR_174731 MK050286 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849208 Teunia intensis CGMCC 2.6797 ^T MN128421 MN128421 HG992858 Teunia mussaendra	Kwoniella shivajii	CBS 11374 [⊤]	NR_165977	NG_042515	KF036401		
Teunia acericola NYNU 2111157 PP239073 PP239062 PP236727 Teunia acericola Bl226 - EU678944 - Teunia acericola HB31-3 - KJ507251 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - AB618905 - Teunia acericola NRL Y-63732 ^T KM384102 KM408130 - Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia delanensis CGMCC 2.5648 ^T NR_174733 MK050286 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849208 Teunia lichenophila CBS 16716 ^T MN128421 MG992858 P236729 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia mussaendrae <td>Teunia acericola</td> <td>NYNU 2111141^T</td> <td>OM017172</td> <td>OM017170</td> <td>PP236726</td>	Teunia acericola	NYNU 2111141 ^T	OM017172	OM017170	PP236726		
Teunia acericola Bl226 - EU678944 - Teunia acericola HB31-3 - KJ507251 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - AB618905 - Teunia acericola NRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia cuniculi CBS 10309 ^T NR_174733 MK050288 MK849208 Teunia korlaensis CGMCC 2.5648 ^T NR_174731 MK050286 MK849208 Teunia korlaensis CGMCC 2.4450 ^T NR_174731 MK050286 MK849194 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849208 Teunia mussaendrae NYNU 2323 ^T OQ851888 OQ851887 PP236729 Teunia initrariae CGMCC 2.6797 ^T OM417183 - - Teunia inguguanensis <th>Teunia acericola</th> <th>NYNU 2111157</th> <th>PP239073</th> <th>PP239062</th> <th>PP236727</th>	Teunia acericola	NYNU 2111157	PP239073	PP239062	PP236727		
Teunia acericola HB31-3 - KJ507251 - Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola MUCC2071 - AB618905 - Teunia acericola NRRL Y-63732 ^T KM384102 KM408130 - Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia globsa CGMCC 2.5648 ^T NR_174733 MK050288 MK849208 Teunia helanensis CGMCC 2.4450 ^T NR_174731 MK050286 MK849194 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia ilchenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23257 PP23072 PP236729 PP236729 Teunia nitrariae CGMCC 2.5830 ^T OM417183 - - Teunia rudbe	Teunia acericola	BI226	_	EU678944	_		
Teunia acericola MUCC1912 - LC715712 - Teunia acericola MUCC2071 - LC715721 - Teunia acericola F3-5 - AB618905 - Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia globosa CGMCC 2.5648 ^T NR_174733 MK050288 MK849235 Teunia helanensis CGMCC 2.4450 ^T NR_174731 MK050286 MK849194 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23257 PP239074 PP239072 PP236729 Teunia nitrariae CGMCC 2.6797 ^T OM417183 - - Teunia nitrariae CGMCC 2.5830 ^T MK942578 MK942560 MT268696	Teunia acericola	HB31-3	_	KJ507251	_		
Teunia acericola MUCC2071 - LC715721 - Teunia acericola F3-5 - AB618905 - Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia globosa CGMCC 2.5648 ^T NR_174733 MK050288 MK849235 Teunia helanensis CGMCC 2.4450 ^T NR_174732 MK050286 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849208 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia nitrariae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rosae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia rosae CGMCC 2.5840 ^T MK942577 MK9425603 - <td>Teunia acericola</td> <td>MUCC1912</td> <td>_</td> <td>LC715712</td> <td>-</td>	Teunia acericola	MUCC1912	_	LC715712	-		
Teunia acericola F3-5 - AB618905 - Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia globosa CGMCC 2.5648 ^T NR_174733 MK050288 MK849235 Teunia helanensis CGMCC 2.4450 ^T NR_174732 MK050287 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia nitrariae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rosae CGMCC 2.5840 ^T MK942577 MK942560 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia acericola	MUCC2071	_	LC715721	-		
Teunia betulae NRRL Y-63732 ^T KM384102 KM408130 - Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia globosa CGMCC 2.5648 ^T NR_174733 MK050288 MK849235 Teunia helanensis CGMCC 2.4450 ^T NR_174732 MK050287 MK849208 Teunia helanensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia nitrariae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rosae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia rosae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 <td>Teunia acericola</td> <td>F3-5</td> <td>_</td> <td>AB618905</td> <td>-</td>	Teunia acericola	F3-5	_	AB618905	-		
Teunia cuniculi CBS 10309 ^T NR_137887 KY106982 MN014082 Teunia globosa CGMCC 2.5648 ^T NR_174733 MK050288 MK849235 Teunia helanensis CGMCC 2.4450 ^T NR_174732 MK050287 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia nitrariae CGMCC 2.6797 ^T OM417183 - - Teunia nitrariae CGMCC 2.5830 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5840 ^T MK942578 MK942560 MT268696 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia betulae	NRRL Y-63732 [⊤]	KM384102	KM408130	_		
Teunia globosa CGMCC 2.5648 ^T NR_174733 MK050288 MK849235 Teunia helanensis CGMCC 2.4450 ^T NR_174732 MK050287 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MS050286 MK849208 Teunia nussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia nussaendrae NYNU 23257 PP239074 PP239072 PP236730 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia nitrariae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rosae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia cuniculi	CBS 10309 [⊤]	NR_137887	KY106982	MN014082		
Teunia helanensis CGMCC 2.4450 ^T NR_174732 MK050287 MK849208 Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia mussaendrae NYNU 23257 PP239074 PP239072 PP236730 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia nitrariae CGMCC 2.5830 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5840 ^T MK942578 MK942560 MT268696 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia globosa	CGMCC 2.5648 [⊤]	NR_174733	MK050288	MK849235		
Teunia korlaensis CGMCC 2.3835 ^T NR_174731 MK050286 MK849194 Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia mussaendrae NYNU 23257 PP239074 PP239072 PP236730 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia qingyuanensis NYNU 22475 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia helanensis	CGMCC 2.4450 [⊤]	NR_174732	MK050287	MK849208		
Teunia lichenophila CBS 16716 ^T MN128421 MN128421 HG992858 Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia mussaendrae NYNU 23257 PP239074 PP239072 PP236730 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia qingyuanensis NYNU 22475 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia korlaensis	CGMCC 2.3835 [⊤]	NR_174731	MK050286	MK849194		
Teunia mussaendrae NYNU 23232 ^T OQ851888 OQ851887 PP236729 Teunia mussaendrae NYNU 23257 PP239074 PP239072 PP236730 Teunia nitrariae CGMCC 2.6797 ^T OM417183 OM417183 - Teunia qingyuanensis NYNU 22475 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia lichenophila	CBS 16716 [⊤]	MN128421	MN128421	HG992858		
Teunia mussaendrae NYNU 23257 PP239074 PP239072 PP236730 Teunia nitrariae CGMCC 2.6797 ⁺ OM417183 OM417183 - Teunia qingyuanensis NYNU 22475 ⁺ OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5830 ⁺ MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ⁺ MK942577 MK942595 MT268698 Teunia siamensis DMKU-XD44 ⁺ LC440108 LC420623 -	Teunia mussaendrae	NYNU 23232 ^T	OQ851888	OQ851887	PP236729		
Teunia nitrariae CGMCC 2.6797 ^T OM417183 - Teunia qingyuanensis NYNU 22475 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia mussaendrae	NYNU 23257	PP239074	PP239072	PP236730		
Teunia qingyuanensis NYNU 22475 ^T OP269841 OP269842 PP236728 Teunia rosae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ^T MK942577 MK942595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia nitrariae	CGMCC 2.6797 [⊤]	OM417183	OM417183	-		
Teunia rosae CGMCC 2.5830 ^T MK942578 MK942560 MT268696 Teunia rudbeckiae CGMCC 2.5840 ^T MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 -	Teunia qingyuanensis	NYNU 22475 [™]	OP269841	OP269842	PP236728		
Teunia rudbeckiae CGMCC 2.5840 ⁺ MK942577 MK9425595 MT268698 Teunia siamensis DMKU-XD44 ⁺ LC440108 LC420623 - Tunio tame description DDM 00004 ⁺ MT2550000 - -	Teunia rosae	CGMCC 2.5830 [⊤]	MK942578	MK942560	MT268696		
Teunia siamensis DMKU-XD44 ^T LC440108 LC420623 - Tunio tame di sunti DOM 00004 ^T ME050000 ME050000 ME050000 -	Teunia rudbeckiae	CGMCC 2.5840 [™]	MK942577	MK9425595	MT268698		
	Teunia siamensis	DMKU-XD44 ^T	LC440108	LC420623	-		
Ieunia tronadorensis DSM 26994' ME959620 ME959620 -	Teunia tronadorensis	DSM 26994 [⊤]	MF959620	MF959620	-		
Teunia virginiahalliae BRIP 64084e [™] OR660683 − −	Teunia virginiahalliae	BRIP 64084e [⊤]	OR660683	-	-		

 $^{\tau_{\!\!\!\!}}$ type strain. Species obtained in this study are in bold.





trees (Figs 1, 2) revealed that these two strains clustered with *T. korlaensis*, *T. nitrariae*, *T. rosae*, and *T. rudbeckiae*, with variations of eight to10 nt (\sim 1.3–1.7%) substitutions in the D1/D2 domain and more than 19 nt (\sim 3.7%) mismatches in the ITS region. This suggests that the strains represent a novel *Teunia* species. A search of GenBank for entries associated with our test isolates unveiled the sequences of four unpublished strains: '*Cryptococcus*' sp. BI226 (EU678944),


Figure 2. Maximum likelihood phylogenetic tree of *Teunia* generated from the combined ITS, LSU, and RPB1 sequence data. The tree is rooted with *Cryptococcus amylolentus* CBS 6039^{T} and *Cryptococcus neoformans* CBS 8710^{T} . Bootstrap values (MLBS \geq 50% and BPP \geq 0.95) are displayed near branches. Type strain sequences are marked with (T).

'Kwoniella' sp. HB31-3 (KJ507251), Teunia sp. MUCC1912 (LC715712), and Teunia sp. MUCC2071 (LC715721), along with an uncultured fungus clone F3-5 (AB618905) (Fig. 1). These sequences exhibit highly similar D1/D2 domain (0-2 nt differences) when compared with NYNU 2111141 and NYNU 2111157. This suggests they may all belong to the same novel species, for which we propose the name Teunia acericola sp. nov.

Isolated from rotting wood, strain NYNU 22475 formed a branch distant from the other *Teunia* species in the D1/D2 phylogenetic tree (Fig. 1). However, the tree based on the combined ITS, LSU, and RPB1 dataset weakly supported a cluster with *T. cuniculi* CBS 10309 (Fig. 2). The two strains differed by 16 nt (~2.9%) substitutions in the D1/D2 domain and 22 nt (~4.3%) mismatches in the ITS region, suggesting they are closely related but do not belong to the

same species. Taken together, these findings indicate that NYNU 22475 represents a novel *Teunia* species, for which we propose the name *Teunia* qingyuanensis sp. nov.

Finally, isolated from separate leaves, strains NYNU 23232 and NYNU 23257 were found to possess identical sequences and formed an independent single-species lineage in the D1/D2 phylogenetic tree (Fig. 1). The ITS, LSU, and RPB1 combined tree presented a non-supported cluster with *T. cuniculi* and the newly discovered *T. qingyuanensis* sp. nov. (Fig. 2). BLASTn searches using D1/D2 sequences indicated that novel strains were most closely related to *T. globosa*, with variations of eight nt (~1.4%) substitutions in the D1/D2 domain and 28 nt (~5%) mismatches in the ITS region. Based on the ITS region, *T. virginiahalliae* represented the closest relative, differing by 19 nt (~3.4%) substitutions. The D1/D2 sequence of *T. virginiahalliae* was not available for comparison. Thus, it was determined that NYNU 23232 and NYNU 23257 represent a novel *Teunia* species, for which we propose the name *Teunia mussaendrae* sp. nov.

Taxonomy

Teunia acericola Y.Z. Qiao & F.L. Hui, sp. nov.

MycoBank No: 852101 Fig. 3A

Etymology. Referring to *Acer*, the genus of the plant where the type strain was isolated.

Typus. CHINA. Henan Prov. Neixiang Co., Baotianman Nature Reserve; in the phylloplane of *Acer palmatum*; Nov 2021; R.R.Jia & W.T.Hu; NYNU 2111141 (holotype CICC 33544⁺, culture ex-type JCM 35732; GenBank Nos: OM017172, OM017170, PP236726).

Description. On YM agar after seven days at 20 °C, the streak culture was cream, mucoid, smooth, with entire margin. After seven days in YM broth at 20 °C, single cells were globose to ovoid, $2.5-5.5 \times 4-6 \mu m$, budding polar. After one month at 20 °C, sediment was present. In Dalmau plate culture on CMA, no hyphae or pseudohyphae were formed. Sexual structures were not observed in any of the strains or when strains are paired on PDA, CMA or V8 agar. Glucose fermentation was absent. Glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose (weak), 5-keto-D-gluconate, D-ribose, ethanol (weak), glycerol, ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, DL-lactate, succinate, D-gluconate, D-glucosamine (weak), 2-keto-D-gluconate, D-glucuronate, and glucono-1,5-lactone were assimilated as carbon sources; methanol, erythritol, and N-acetyl-D-glucosamine were not assimilated. Ethylamine and L-lysine were assimilated as nitrogen sources, nitrate, nitrite, and cadaverine were not assimilated. Maximum growth temperature was 35 °C. Growth in vitamin-free medium was negative. Growth on 50% (w/w) glucose-yeast extract agar was negative. Starch-like substances were not produced. Urease activity and Diazonium Blue B reaction were positive.

Additional strain examined. CHINA. Henan Prov. Neixiang Co., Baotianman Nature Reserve; in the phylloplane of *Rhus chinensis*; Nov 2021; R.R.Jia & W.T.Hu; NYNU 2111157 (GenBank No: PP239073, PP239062, PP236727).



Figure 3. Vegetative cells of **A** *T. acericola* sp. nov. NYNU 2111141^T **B** *T. qingyuanensis* sp. nov. NYNU 22475^T and **C** *T. mussaendrae* sp. nov. NYNU 23232^T, following 7 days of growth in YM broth at 20 °C. Scale bars: 10 μm.

Note. In the molecular analysis (Figs 1, 2), *T. acericola* sp. nov. was clustered with *T. korlaensis*, *T. nitrariae*, *T. rosae*, and *T. rudbeckiae*. *T. acericola* sp. nov. can be differentiated from those four species by its ability to assimilate raffinose and its growth capacity at 35 °C.

Teunia qingyuanensis Y.Z. Qiao & F.L. Hui, sp. nov. MycoBank No: 852102 Fig. 3B

Etymology. Referring to the locality, Qingyuan Mountain, where the type strain was collected.

Typus. CHINA. Fujian Prov. Quanzhou City, Qingyuan Mountain; in rotting wood; Mar 2022; W.T.Hu & S.B.Chu; NYNU 22475 (holotype GDMCC 2.294^T, culture ex-type PYCC 9929; GenBank Nos: OP269841, OP269842, PP236728).

Description. On YM agar after seven days at 20 °C, the streak culture was cream, mucoid and smooth, with an entire margin. After seven days in YM broth at 20 °C, single cells were ovoid to ellipsoidal, $3-7 \times 4-7.5 \mu m$, budding polar. After one month at 20 °C, sediment was present. In Dalmau plate culture on CMA, no hyphae or pseudohyphae were formed. Sexual structures were not observed on PDA, CMA or V8 agar. Glucose fermentation was absent. Glucose, inulin, sucrose, raffinose, melibiose, galactose, lactose, trehalose, maltose, melezitose, cellobiose, salicin, L-sorbose (weak), L-rhamnose, D-xylose, L-arabinose, D-arabinose, 5-keto-D-gluconate, D-ribose, ethanol, glycerol, ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, DL-lactate, succinate, D-gluconate, 2-keto-D-gluconate, D-glucuronate, and glucono-1,5-lactone were assimilated as carbon sources; methanol, erythritol, and D-glucosamine were not assimilated. Ethylamine and L-lysine were assimilated as nitrogen sources; nitrate, nitrite, and cadaverine were not assimilated. Maximum growth temperature was 30 °C. Growth in vitamin-free medium was positive. Growth on 50% (w/w) glucose-yeast extract agar was negative. Starch-like substances were not produced. Urease activity and Diazonium Blue B reaction were positive.

Note. In the molecular analysis (Fig. 2), *T. qingyuanensis* sp. nov. formed a distinct clade together with *T. cuniculi*. They can be differentiated by the ability of *T. qingyuanensis* sp. nov. to grow in vitamin-free medium and to assimilate raffinose, melibiose, and DL-Lactate.

Teunia mussaendrae Y.Z. Qiao & F.L. Hui, sp. nov.

MycoBank No: 852103 Fig. 3C

Etymology. Referring to *Mussaenda*, the genus of the plant where the type strain was isolated.

Typus. CHINA. Guizhou Prov. Pingtang Co., Sifangjing Vil.; in the phylloplane of *Mussaenda pubescens*; Feb 2023; D.Lu; NYNU 23232 (holotype CICC 33594^T, culture ex-type PYCC 9974; GenBank Nos OQ851888, OQ851887, PP236729).

Description. On YM agar after seven days at 20 °C, the streak culture was yellowish-cream, mucoid and smooth, entire margin. After seven days in YM broth at 20 °C, cells isolated were globose to ovoid, $3.5-5 \times 4.5-6 \mu m$, budding polar. After one month at 20 °C, a ring and sediment was present. In Dalmau plate culture on CMA, no hyphae or pseudohyphae were formed. Sexual structures were not observed in any of the strains or when strains were paired on PDA, CMA or V8 agar. Glucose fermentation was absent. Glucose, inulin, sucrose, galactose, lactose, trehalose, maltose, melezitose, cellobiose, salicin, L-sorbose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, 5-keto-D-gluconate, D-ribose, ethanol (weak), glycerol (weak), ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, DL-lactate, succinate, D-gluconate, D-glucosamine (weak), 2-keto-D-gluconate, D-glucuronate, and glucono-1,5-lactone were assimilated as carbon sources; raffinose, melibiose, methanol, erythritol, and N-acetyl-D-glucosamine were not assimilated. Ethylamine (delayed), L-lysine, and cadaverine (delayed) were assimilated as nitrogen sources; nitrate and nitrite were not assimilated. Maximum growth temperature was 25 °C. Starch-like substances were not produced. Urease activity and Diazonium Blue B reaction were positive.

Additional strain examined. CHINA. Guizhou Prov. Pingtang Co., Sifangjing Vil.; in the phylloplane of *Viburnum* sp.; Feb 2023; D.Lu; NYNU 23257 (GenBank Nos: PP239074, PP239072, PP236730).

Note. Based on the D1/D2 sequences, *T. mussaendrae* sp. nov. was most closely related to *T. globosa*. It can be differentiated from *T. globosa* by the ability to assimilate L-sorbose, L-arabinose, D-arabinose, ribitol, galactitol, D-glucitol, and D-gluconate. Additionally, *T. mussaendrae* sp. nov. can grow in vita-min-free medium at 25 °C, while *T. globosa* cannot.

Discussion

Our study confirms that three species with similar colors, colony morphology, and cell shapes, can be distinguished from previously described species using the polyphasic approach recommended by Li et al. (2020) and Wang et al. (2020). In this case we use physiological and biochemical characters as well as morphological and phylogenetic ones.

The genus *Teunia* is widely distributed in China, but knowledge about it is still in its infancy. The six species previously reported, come mainly from the northern regions (Li et al. 2020; Wang et al. 2020; Wei et al. 2022). The exploration of new territories, such as that carried out in the provinces of Fujian, Guizhou and Henan, is necessary to have a more exact knowledge of their distribution and ecology. The results presented in this paper increase the total number of *Teunia* species from six to nine.

Furthermore, four unpublished strains, BI226 from Brazil, HB31-3 from South Korea, MUCC1912 and MUCC2071 from Japan, as well as an uncultured fungus clone F3-5 from Japan, are conspecific with *T. acerica* sp. nov. These observations suggest that this species can have a wide distribution area. Therefore, a broader taxon sampling effort, coupled with molecular, phenotypic, physiological and biochemical data, is needed to fully understand the species diversity of *Torula* in the world.

The species of *Teunia* are frequently isolated as epiphytes from different parts of herbaceous plants, more rarely from tree barks or lichens; in this case, we isolated five yeast strains, which led to the discovery of three new species: *T. acericola* sp. nov., *T. mussaendrae* sp. nov. isolated from leaf surfaces, and *T. qingyuanensis* sp. nov. from rotting wood. We have found no previous reports of the presence of *Teunia* in rotting wood in China, hence our study is the first to report the presence of *Teunia* in rotten wood in China.

Teunia korlaensis and *T. nitrariae* are versatile extremophilic species that have been frequently found in plants inhabiting dry and alkaline environments (Wei et al. 2022), implying that these species may help plants survive in dry areas. We also isolated four strains of two novel *Teunia* species - *T. acericola* sp. nov. and *T. mussaendrae* sp. nov. - from plant leaves, and it is possible that these species provide similar ecological functions' benefits to their hosts as do *T. korlaensis* and *T. nitrariae*.

Acknowledgments

The authors express their immense gratitude to their colleagues at the School of Life Science and Agricultural Engineering, Nanyang Normal University. Special thanks to Dr. Jing-Zhao Li and Lin Zhang for their help in specimen collection and Ting Lei for assistance with phylogenetic analysis.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was funded by the National Natural Science Foundation of China (Grant No. 31570021) and Agricultural Biomass Green Conversion Technology University Scientific Innovation Team in Henan Province, China (Grant No. 24IRTSTHN036).

Author contributions

Data curation: QCG. Methodology: SL, QCG. Molecular phylogeny: QCG, YZQ. Writing - original draft: QCG. Writing - review and editing: FLH. All authors read and approved the final manuscript.

Author ORCIDs

Qi-Chao Guo [©] https://orcid.org/0009-0002-9245-479X Shan Liu [©] https://orcid.org/0009-0003-2845-1495 Ya-Zhuo Qiao [©] https://orcid.org/0009-0000-9074-2443 Feng-Li Hui [©] https://orcid.org/0000-0001-7928-3055

Data availability

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

References

- Alvarez-Pérez S, Herrera CM (2013) Composition, richness and nonrandom assembly of culturable bacterial-microfungal communities in floral nectar of Mediterranean plants. FEMS Microbiology Ecology 83(3): 685–699. https://doi.org/10.1111/1574-6941.12027
- Andrew R (2016) FigTree: Tree figure drawing tool Version 1.4.3. Institute of Evolutionary Biology, United Kingdom, University of Edinburgh Press.
- Crous PW, Cowan DA, Maggs-Kölling G, Yilmaz N, Thangavel R, Wingfield MJ, Noordeloos ME, Dima B, Brandrud TE, Jansen GM, Morozova OV, Vila J, Shivas RG, Tan YP, Bishop-Hurley S, Lacey E, Marney TS, Larsson E, Le Floch G, Lombard L, Nodet P, Hubka V, Alvarado P, Berraf-Tebbal A, Reyes JD, Delgado G, Eichmeier A, Jordal JB, Kachalkin AV, Kubátová A, Maciá-Vicente JG, Malysheva EF, Papp V, Rajeshkumar KC, Sharma A, Spetik M, Szabóová D, Tomashevskaya MA, Abad JA, Abad ZG, Alexandrova AV, Anand G, Arenas F, Ashtekar N, Balashov S, Bañares Á, Baroncelli R, Bera I, Biketova AY, Blomquist CL, Boekhout T, Boertmann D, Bulyonkova TM, Burgess TI, Carnegie AJ, Cobo-Diaz JF, Corriol G, Cunnington JH, da Cruz MO, Damm U, Davoodian N, de Santiago ALCM, Dearnaley J, de Freitas LWS, Dhileepan K, Dimitrov R, Di Piazza S, Fatima S, Fulier F, Galera H, Ghosh A, Giraldo A, Glushakova AM, Gorczak M, Gouliamova DE, Gramaje D, Groenewald M, Gunsch CK, Gutiérrez A, Holdom D, Houbraken J, Ismailov AB, Istel Ł, Iturriaga T, Jeppson M, Jurjević Ž, Kalinina LB, Kapitonov VI, Kautmanová I, Khalid AN, Kiran M, Kiss L, Kovács Á, Kurose D, Kušan I, Lad S, Læssøe T, Lee HB, Luangsa-Ard JJ, Lynch M, Mahamedi AE, Malysheva VF, Mateos A, Matočec N, Mešić A, Miller AN, Mongkolsamrit S, Moreno G, Morte A, Mostowfizadeh-Ghalamfarsa R, Naseer A, Navarro-Ródenas A, Nguyen TTT, Noisripoom W, Ntandu JE, Nuytinck J, Ostrý V, Pankratov TA, Pawłowska J, Pecenka J, Pham THG, Polhorský A, Pošta A, Raudabaugh DB, Reschke K, Rodríguez A, Romero M, Rooney-Latham S, Roux J, Sandoval-Denis M, Smith MT, Steinrucken TV, Svetasheva TY, Tkalčec Z, van der Linde EJ, V D Vegte M, Vauras J, Verbeken A, Visagie CM, Vitelli JS, Volobuev SV, Weill A, Wrzosek M, Zmitrovich IV, Zvvagina EA, Groenewald JZ (2021) Fungal Planet description sheets: 1182-1283. Persoonia 46: 313-528. https://doi.org/10.3767/persoonia.2021.46.11
- de Garcia V, Zalar P, Brizzio S, Gunde-Cimerman N, van Broock M (2012) *Cryptococcus* species (Tremellales) from glacial biomes in the southern (Patagonia) and northern

(Svalbard) hemispheres. FEMS Microbiology Ecology 82(2): 523-539. https://doi. org/10.1111/j.1574-6941.2012.01465.x

- Fernández NV, Mestre MC, Marchelli P, Fontenla SB (2012) Yeast and yeast-like fungi associated with dry indehiscent fruits of *Nothofagus nervosa* in Patagonia, Argentina. FEMS Microbiology Ecology 80(1): 179–192. https://doi.org/10.1111/j.1574-6941.2011.01287.x
- Gadanho M, Libkind D, Sampaio JP (2006) Yeast diversity in the extreme acidic environments of the Iberian Pyrite Belt. Microbial Ecology 52(3): 552–563. https://doi.org/10.1007/s00248-006-9027-y
- Hall TA (1999) Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Herzberg M, Fischer R, Titze A (2002) Conflicting results obtained by RAPD-PCR and large-subunit rDNA sequences in determining and comparing yeast strains isolated from flowers: A comparison of two methods. International Journal of Systematic and Evolutionary Microbiology 52(4): 1423–1433. https://doi.org/10.1099/00207713-52-4-1423
- Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS (2017) Modelfinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Khunnamwong P, Lertwattanasakul N, Limtong S (2020) *Teunia siamensis* f.a., sp. nov., a novel tremellaceous yeast species isolated from soil in a secondary peat swamp forest area. International Journal of Systematic and Evolutionary Microbiology 70(6): 3673–3678. https://doi.org/10.1099/ijsem.0.004219
- Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73(4): 331–371. https://doi.org/10.1023/A:1001761008817
- Kurtzman CP, Robnett CJ (2003) Phylogenetic relationships among yeasts of the 'Saccharomyces complex' determined from multigene sequence analyses. FEMS Yeast Research 3(4): 417–432. https://doi.org/10.1016/S1567-1356(03)00012-6
- Kurtzman CP, Fell JW, Boekhout T (2011) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (Eds) The Yeasts – a Taxonomic Study, 5th edn, vol. 1. Amsterdam, Elsevier, 87–110. https://doi. org/10.1016/B978-0-444-52149-1.00007-0
- Li AH, Yuan FX, Groenewald M, Bensch K, Yurkov AM, Li K, Han PJ, Guo LD, Aime MC, Sampaio JP, Jindamorakot S, Turchetti B, Inacio J, Fungsin B, Wang QM, Bai FY (2020) Diversity and phylogeny of basidiomycetous yeasts from plant leaves and soil: Proposal of two new orders, three new families, eight new genera and one hundred and seven new species. Studies in Mycology 96: 17–140. https://doi.org/10.1016/j. simyco.2020.01.002
- Mittelbach M, Yurkov AM, Nocentini D, Nepi M, Weigend M, Begerow D (2015) Nectar sugars and bird visitation define a floral niche for basidiomycetous yeast on the Canary Islands. BMC Ecology 15(1): 2. https://doi.org/10.1186/s12898-015-0036-x
- Nakase T, Takashima M (1993) A simple procedure for the high frequency isolation of new taxa of ballistosporous yeasts living on the surfaces of plants. RIKEN Review 3: 33–34.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice, across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

- Shi CF, Zhang KH, Chai CY, Yan ZL, Hui FL (2021) Diversity of the genus *Sugiyamaella* and description of two new species from rotting wood in China. MycoKeys 77: 27–39. https://doi.org/10.3897/mycokeys.77.60077
- Shin KS, Oh HM, Park YH, Lee KH, Poo H, Kwon GS, Kwon OY (2006) Cryptococcus mujuensis sp. nov. and Cryptococcus cuniculi sp. nov., basidiomycetous yeasts isolated from wild rabbit faeces. International Journal of Systematic and Evolutionary Microbiology 56(9): 2241–2244. https://doi.org/10.1099/ijs.0.64353-0
- Stamatakis A (2014) RAxML Version 8: A tool for phylogenetic analyses and post analyses of large phylogenies. Bioinformatics (Oxford, England) 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033
- Sylvester K, Wang QM, James B, Mendez R, Hulfachor AB, Hittinger CT (2015) Temperature and host preferences drive the diversification of Saccharomyces and other yeasts: A survey and the discovery of eight new yeast species. FEMS Yeast Research 15(3): fov002. https://doi.org/10.1093/femsyr/fov002
- Takashima M, Sugita T, Van BH, Nakamura M, Endoh R, Ohkuma M (2012) Taxonomic richness of yeasts in Japan within subtropical and cool temperate areas. PLoS ONE 7(11): e50784. https://doi.org/10.1371/journal.pone.0050784
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi. org/10.1093/molbev/msab120
- Tan YP, Pegg GS (2023) Nomenclatural novelties. Index of Australian Fungi 18: 1–4. https://doi.org/10.5281/zenodo.10005573
- Toome M, Roberson RW, Aime MC (2013) *Meredithblackwellia eburnean* gen. et sp. nov., Kriegeriaceae fam. nov. and Kriegeriales ord. nov. toward resolving higher-level classification in Microbotryomycetes. Mycologia 105(2): 486–495. https://doi. org/10.3852/12-251
- Wang QM, Theelen B, Groenewald M, Bai FY, Boekhout T (2014) Moniliellomycetes and Malasseziomycetes, two new classes in Ustilaginomycotina. Persoonia 33(1): 41– 47. https://doi.org/10.3767/003158514X682313
- Wang GS, Zhou Y, Xue L, Li AH, Wangmu, Wang QM (2020) Teunia rosae sp. nov. and Teunia rudbeckiae sp. nov. (Cryptococcaceae, Tremellales), two novel basidiomycetous yeast species isolated from flowers. International Journal of Systematic and Evolutionary Microbiology 70(10): 5394–5400. https://doi.org/10.1099/ijsem.0.004423
- Wei XY, Zhu HY, Song L, Zhang RP, Li AH, Niu QH, Liu XZ, Bai FY (2022) Yeast diversity in the Qaidam Basin Desert in China with the description of five new yeast species. Journal of Fungi (Basel, Switzerland) 8(8): 858. https://doi.org/10.3390/jof8080858
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Yurkov AM, Röhl O, Pontes A, Carvalho C, Maldonado C, Sampaio JP (2016) Local climatic conditions constrain soil yeast diversity patterns in Mediterranean forests, woodlands and scrub biome. FEMS Yeast Research 16(1): fov103. https://doi. org/10.1093/femsyr/fov103
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096

Supplementary material 1

Supplementary data

Authors: Qi-Chao Guo, Shan Liu, Ya-Zhuo Qiao, Feng-Li Hui

Data type: pdf

- Explanation note: **fig. S1.** Maximum likelihood phylogenetic tree of *Teunia* generated from the ITS sequence data.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.120534.suppl1



Research Article

Morphological and molecular identification for four new woodinhabiting species of *Trechispora* (Basidiomycota) from China

Kai-Yue Luo¹, Jiang-Qing Su^{1,2}, Chang-Lin Zhao^{1,2}

2 College of Forestry, Southwest Forestry University, Kunming 650224, China

Corresponding author: Chang-Lin Zhao (fungi@swfu.edu.cn)

Abstract

Four new wood-inhabiting fungi, Trechispora albofarinosa, T. bisterigmata, T. pileata and T. wenshanensis spp. nov., are proposed based on a combination of morphological features and molecular evidence. Trechispora albofarinosa is characterized by the farinose basidiomata with flocculence hymenial surface, a monomitic hyphal system with clamped generative hyphae, and ellipsoid, warted basidiospores. Trechispora bisterigmata is characterized by the membranous basidiomata with odontioid hymenial surface, rhizomorphic sterile margin, barrelled basidia and subglobose to broad ellipsoid, smooth basidiospores. Trechispora pileata is characterized by the laterally contracted base, solitary or imbricate basidiomata, fan shaped pileus, radially striate-covered surface with appressed scales, odontioid hymenophore surface, and subglobose to broad ellipsoid, thin-walled, smooth basidiospores. Trechispora wenshanensis is characterized by a cottony basidiomata with a smooth hymenial surface, and ellipsoid, thin-walled, warted basidiospores. Sequences of ITS and LSU marker of the studied samples were generated, and phylogenetic analyses were performed with the maximum likelihood, maximum parsimony, and Bayesian inference methods. The phylogenetic tree inferred from the ITS+nLSU sequences highlighted that four new species were grouped into the genus Trechispora.



Academic editor: Bao-Kai Cui Received: 7 February 2024 Accepted: 29 April 2024 Published: 15 May 2024

Citation: Luo K-Y, Su J-Q, Zhao C-L (2024) Morphological and molecular identification for four new woodinhabiting species of *Trechispora* (Basidiomycota) from China. MycoKeys 105: 155–178. https://doi. org/10.3897/mycokeys.105.120438

Copyright: [©] Kai-Yue Luo 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). Key words: East Asia, macrofungi, molecular systematics, taxonomy, 4 new taxa

Introduction

Fungi represent one of the most diverse groups of organisms on earth, with an indispensable role in the processes and functioning of ecosystems (Wu et al. 2020, 2021; Wang et al. 2021; Hyde 2022; Guan and Zhao 2023). Wood-inhabiting fungi play an important role in the carbon cycle (Dai et al. 2015; Wu et al. 2017; Chen and Zhao 2020; Guan et al. 2021; Liu et al. 2022a, b; Luo and Zhao 2022; Mardones et al. 2023; Yuan et al. 2023; Zhang et al. 2023; Zhao et al. 2023a, b; Zhou et al. 2023). The wood-inhabiting fungal order Trechisporales

¹ The Key Laboratory of Forest Resources Conservation and Utilization in the Southwest Mountains of China Ministry of Education, Key Laboratory of National Forestry and Grassland Administration on Biodiversity Conservation in Southwest China, Yunnan Provincial Key Laboratory for Conservation and Utilization of In-forest Resource, Southwest Forestry University, Kunming 650224, China

K.H. Larss. is a species-poor order, compared with most other orders within Agaricomycetes, Basidiomycota (Wijayawardene et al. 2022).

Trechispora P. Karst. (Hydnodontaceae) typified by T. onusta P. Karst., which is characterized by resupinate to effused basidiomata; a smooth to hydnoid to poroid hymenophore; ampullaceous septa; short cylindric basidia; and smooth to verrucose or aculeate basidiospores (Karsten 1890; Bernicchia and Gorjón 2010). Currently, MycoBank and Index Fungorum have registered 163 recorded and 156 recorded intraspecific names in Trechispora, respectively. About 100 species are currently accepted in Trechispora worldwide (Karsten 1890; Bondartsev and Singer 1941; Rogers and Jackson 1943; Rogers 1944; Bondartsev 1953; Liberta 1966, 1973; Parmasto 1968; Burdsall and Gilbertson 1982; Gilbertson and Budington 1970; Jülich 1975, 1976; Ryvarden 1975; Ryvarden and Liberta 1978; Hallenberg 1978, 1980; Jülich and Stalpers 1980; Rauschert 1987; Vries 1987; Larsson 1992, 1994, 1995, 1996; Hjortstam and Larsson 1995; Ryvarden 2002; Trichies and Schultheis 2002; Ryvarden et al. 2003; Miettinen and Larsson 2006; Dai 2011; Yuan and Dai 2012; Ordynets et al. 2015; Phookamsak et al. 2019; Xu et al. 2019; Chikowski et al. 2020; Haelewaters et al. 2020; Crous et al. 2021; de Meiras-Ottoni et al. 2021; Zhao and Zhao 2021; Liu et al. 2022a, b; Luo and Zhao 2022; Sommai et al. 2023), of which 38 species of the genus have been found in China (Dai 2011; Yuan and Dai 2012; Xu et al. 2019; Dai et al. 2021; Zhao and Zhao 2021; He et al. 2022; Liu et al. 2022a, b; Luo and Zhao 2022; Deng et al. 2023; Liu et al. 2024a, b).

There have been many studies on the phylogeny of this genus in recent years. A high phylogenetic diversity on the corticioid Agaricomycetes based on two genes, 5.8S and 28S showed that nine taxa of Trechispora nested into trechisporoid clade (Larsson et al. 2004). The molecular systematics suggested that Trechispora belonged to Hydnodontaceae and was related to genera Brevicellicium K.H. Larss. & Hjortstam, Porpomyces Jülich, Sistotremastrum J. Erikss., and Subulicystidium Parmasto (Telleria et al. 2013). Based on the ITS and nLSU datasets, the phylogenetic study of Trechispora reported two new Trechispora species as T. cyatheae Ordynets, Langer & K.H. Larss. and T. echinocristallina Ordynets, Langer & K.H. Larss., on La Réunion Island (Ordynets et al. 2015). The phylogeny of Trechisporales was inferred from a combined ITS-nLSU sequences, which revealed that two related genera Porpomyces, Scytinopogon Singer, grouped closely together with Trechispora and all of them nested within Hydnodontaceae (Liu et al. 2019). Based on ITS dataset, the three new species of Trechispora were described and used to evaluate the phylogenetic relationship with other species of this genus, in which T. murina was retrieved as a sister to T. bambusicola with moderate supports, and T. odontioidea formed a single lineage and then grouped with T. fimbriata and T. nivea, while T. olivacea formed a monophyletic lineage with T. farinacea, T. hondurensis, and T. mollis (Luo and Zhao 2022). Recently, based on the morphological features and molecular evidence, three new species of Trechispora have been reported from Northern and Northeastern Thailand (Sommai et al. 2023).

During investigations into the wood-inhabiting fungi in the Yunnan-Guizhou Plateau of China, samples representing four additional species belonging to genus *Trechispora* were collected. To clarify the placement and relationships of the four species, we carried out a phylogenetic and taxonomic study on *Trechispora*, based on the ITS+nLSU.

Materials and methods

Morphology

The specimens studied were deposited at the herbarium of Southwest Forestry University (SWFC), Kunming, Yunnan Province, China. The macromorphological descriptions were based on field notes and photos captured in the field and laboratory. Color, texture, taste and odor of basidiomata were mostly based on authors' field trips. Color terminology followed Kornerup and Wanscher (1978). All materials were examined under a Nikon 80i microscope. Drawings were made with the aid of a drawing tube. The measurements and drawings of the microscopic structures were made (Wu et al. 2022). The following abbreviations were used: KOH = 5% potassium hydroxide water solution, CB = cotton blue, CB- = acyanophilous, IKI = Melzer's reagent, IKI- = both inamyloid and indextrinoid, L = spore length (arithmetic average for all spores), W = spore width (arithmetic average for all spores), W = spore width (arithmetic average for all spores), W = total number of spores measured, from b = number of specimens).

Molecular phylogeny

The CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain genomic DNA from the dried specimens following the manufacturer's instructions (Zhao and Wu 2017). The nuclear ribosomal ITS region was amplified with the primers ITS5 and ITS4 (White et al. 1990). The nuclear ribosomal LSU gene was amplified with the primers LROR and LR7 (Vilgalys and Hester 1990; Rehner and Samuels 1994). 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, and a final extension of 72 °C for 10 min. 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, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company, Yunnan Province, China. All newly-generated sequences were deposited in NCBI 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). Each dataset was aligned separately at first and then the ITS+nLSU regions were combined with Mesquite version 3.51. The combined dataset was deposited in TreeBASE (submission ID 31349). Sequences of *Fibrodontia alba* Yurchenko & Sheng H. Wu and *F. brevidens* (Pat.) Hjortstam & Ryvarden retrieved from GenBank were used as an outgroup in the ITS analysis (Luo and Zhao 2022).

Maximum parsimony analysis in PAUP* version 4.0a169 (http://phylosolutions.com/paup-test/) was applied to ITS+nLSU 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 5,000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1,000 pseudo replicates (Felsenstein 1985).

Cracico nomo	Chasimon No	GenBank accession No.		Deferences	
Species name	Specifien No.	ITS	LSU	References	
Fibrodontia alba	TNM F24944	NR153983	NG060401	Yurchenko and Wu 2014	
F. brevidens	Wu 9807-16	KC928276	KC928277	Yurchenko and Wu 2014	
Trechispora alba	CH21384	OR557258	_	Liu et al. 2024a	
T. albofarinosa	CLZhao 4356	OQ241383	OQ282703	This study	
T. amianthina	CBS 202.54	-	MH868822	Vu et al. 2019	
T. araneosa	KHL 8570	AF347084	-	Larsson et al. 2004	
T. bambusicola	CLZhao 3302	MW544021	MW520171	Zhao and Zhao 2021	
T. bambusicola	CLZhao 3305	MW544022	MW520172	Zhao and Zhao 2021	
T. bispora	CBS 142.63	MH858241	MH869842	Larsson et al. 2004	
T. bisterigmata	CLZhao 2522	OQ241386	_	This study	
T. bisterigmata	CLZhao 7870	OQ241387	-	This study	
T. byssinella	UC 2023068	KP814481	-	Unpublished	
T. chartacea	FLOR56185	MK458775	-	Liu et al. 2022a	
T. clancularis	FRDBI 4426619	MW487976	-	Unpublished	
T. cohaerens	HHB-19445	MW740327	_	Unpublished	
T. copiosa	AM0427	MN701015	MN687973	de Meiras-Ottoni et al. 2021	
T. copiosa	AM0450	MN701017	MN687974	de Meiras-Ottoni et al. 2021	
T. crystallina	LWZ 20170729-2	OM523419	OM339238	Liu et al. 2022a	
T. cyatheae	FR0219443	UDB024016	UDB024017	Ordynets et al. 2015	
T. cyatheae	FR0219446	UDB024020	UDB024021	Ordynets et al. 2015	
T. dentata	Dai 22565	OK298491	OM049408	Liu et al. 2022b	
T. dimitiella	Dai 21181	OK298493	OK298949	Liu et al. 2022b	
T. dimitiella	Dai 21931	OK298492	OK298948	Liu et al. 2022b	
T. echinospora	E11/37-10	JX392850	JX392851	Telleria et al. 2013	
T. echinospora	E11/37-12	JX392853	JX392854	Telleria et al. 2013	
T. farinacea	356	AF347089	_	Larsson et al. 2004	
T. farinacea	MA-Fungi 79474	JX392855	JX392856	Telleria et al. 2013	
T. fimbriata	CLZhao 7969	MW544024	MW520174	Zhao and Zhao 2021	
T. fimbriata	CLZhao 9006	MW544025	MW520175	Zhao and Zhao 2021	
T. foetida	FLOR 56315	MK458769	_	Liu et al. 2022a	
T. fragilis	Dai 20535	OK298494	OK298950	Liu et al. 2022b	
T. gelatinosa	AM0824	MN701020	MN687977	de Meiras-Ottoni et al. 2021	
T. gelatinosa	AM01139	MN701021	MN687978	de Meiras-Ottoni et al. 2021	
T. gracilis	LWZ 20170814-17	OM523435	OM339253	Liu et al. 2022a	
T. havencampii	DED8300	NR154418	NG059993	Desjardin and Perry 2015	
T. hondurensis	HONDURAS19-F016	NR178152	NG081479	Haelewaters et al. 2020	
T. hondurensis	HONDURAS19-F016a	MT571523	MT636540	Haelewaters et al. 2020	
T. hymenocystis	KHL 8795	AF347090	-	Unpublished	
T. hymenocystis	KHL 16444	MT816397	_	Unpublished	
T. incisa	GB0090521	KU747093	KU747086	Unpublished	
T. incisa	GB0090648	KU747095	KU747087	Unpublished	
T. invisitata	5425_537	ON963772	_	Unpublished	
T. invisitata	UC2023088	KP814425	-	Unpublished	

Table 1. List of species, specimens and GenBank accession numbers of sequences used in this study.

Chaolica nomo	Cresimer No	GenBank accession No.		Defenences	
Species name	Specimen No.	ITS	LSU	References	
T. kavinioides	KGN 981002	AF347086	_	Unpublished	
T. laevispora	Dai 21655	OK298495	OM108710	Liu et al. 2022b	
T. larssonii	LWZ 20190817-11a	OM523442	OM339259	Liu et al. 2022a	
T. longiramosa	HG 140168	OM523448	OM339264	Liu et al. 2022a	
T. mellina	URM85756	_	MH280000	Unpublished	
T. microspora	FRDBI 18772216	OL828778	_	Unpublished	
T. mollis	URM85884	MK514945	MK514945	Unpublished	
T. mollis	URM85885	-	MT423667	Unpublished	
T. mollusca	iNAT 30809943	MZ269232	_	Unpublished	
T. mollusca	CFMR:DLL2011-186	KJ140681	_	Unpublished	
T. nivea	MA-Fungi 76238	JX392824	JX392825	Telleria et al. 2013	
T. nivea	MA-Fungi 76257	JX392826	JX392827	Telleria et al. 2013	
T. pallescens	FLOR56184	MK458767	-	Unpublished	
T. pallescens	FLOR56188	MK458774	-	Unpublished	
T. papillosa	AM0713	MN701022	MN687979	de Meiras-Ottoni et al. 2021	
T. papillosa	AM0795	MN701023	MN687981	de Meiras-Ottoni et al. 2021	
T. patawaensis	VPapp-GF1901	OL314550	OL314546	Unpublished	
T. perminispora	LWZ2019081639a	OM523525	OM339329	Liu et al. 2024a	
T. pileata	CLZhao 4456	OQ241388	OQ282715	This study	
T. praefocata	FRDBI 18819116	OL828784	_	Unpublished	
T. regularis	KHL 10881	AF347087	_	Unpublished	
T. rigida	URM85754	MT406381	MH279999	Unpublished	
T. sinensis	LWZ 20170816-35	OM523479	OM339287	Liu et al. 2022a	
T. stellulata	14153	MW023104	_	Unpublished	
T. stellulata	33962903	ON364078	-	Unpublished	
T. stellulata	UC2023099	KP814451	_	Unpublished	
T. stellulata	UC2023230	KP814491	_	Unpublished	
T. stevensonii	MA-Fungi 70669	JX392841	JX392842	Telleria et al. 2013	
T. stevensonii	MA-Fungi 70645	JX392843	JX392844	Telleria et al. 2013	
T. subfarinacea	LWZ2020092133a	OM523528	OM339331	Liu et al. 2024a	
T. subhelvetica	7089	JN710601	_	Unpublished	
T. subhymenocystis	LWZ 20190818-29b	OM523492	OM339299	Liu et al. 2022a	
T. subregularis	VPapp-GF2103	OL331097	OL314548	Unpublished	
T. subsinensis	LWZ 20190611-9	OM523497	OM339304	Liu et al. 2022a	
T. subsphaerospora	KHL 8511	AF347080	_	Unpublished	
T. termitophila	AM0396	MN701025	MN687983	de Meiras-Ottoni et al. 2021	
T. termitophila	AM0893	MN701026	MN687984	de Meiras-Ottoni et al. 2021	
T. torrendii	URM85886	MK515148	MH280004	Unpublished	
T. tropica	LWZ 20170613-16	OM523503	OM339311	Liu et al. 2022a	
T. tuberculata	Dai17433	OM523507	OM339314	Liu et al. 2024a	
T. wenshanensis	CLZhao 11649	OQ241389	OQ282716	This study	
T. wenshanensis	CLZhao 11715	PP712100	-	This study	
T. wenshanensis	CLZhao 22940	PP712101	-	This study	
T. yunnanensis	CLZhao 210	NR177488	MN654918	Xu et al. 2019	
T. yunnanensis	CLZhao 214	MN654922	MN654919	Xu et al. 2019	

Descriptive tree statistics - tree length (TL), composite consistency index (CI), 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 pseudoreplicates.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each dataset for the purposes of Bayesian inference (BI), Bayesian inference 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 1.7 million generations for ITS+nLSU with tree and parameters sampled every 1,000 generations. The first quarter of all of the generations were discarded as burn-ins. 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 > 70%, a maximum parsimony bootstrap support value (BT) of > 70% or a Bayesian posterior probability (BPP) of > 0.95.

Results

Molecular phylogeny

The ITS+nLSU dataset comprised sequences from 88 fungal specimens representing 64 taxa. The dataset had an aligned length of 2271 characters, of which 1376 characters were constant, 190 were variable and parsimony-uninformative and 705 were parsimony-informative. Maximum parsimony analysis yielded 300 equally parsimonious tree (TL = 5543, CI = 0.2979, HI = 0.7021, RI = 0.5278 and RC = 0.1572). 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 = 0.012925 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 389. The phylogenetic tree inferred from the ITS+nLSU sequences highlighted that four new species were grouped into the genus *Trechispora* (Fig. 1).

Taxonomy

Trechispora albofarinosa K.Y. Luo & C.L. Zhao, sp. nov. MycoBank No: 849463 Figs 2, 3

Holotype. CHINA. Yunnan Province, Pu'er, Jingdong County, Huangcaoling, Wuliangshan National Nature Reserve, 24°23'N, 100°45'E, altitude 2350 m a.s.l., on the fallen branch of *Pinus*, leg. C.L. Zhao, 5 October 2017, CLZhao 4356 (SWFC).

Etymology. *Albofarinosa* (Lat.): referring to the farinose basidiomata with white hymenial surface.



Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of the four new species and related species in *Trechispora*, based on ITS+nLSU sequences. Branches are labelled with maximum likelihood bootstrap values > 70%, parsimony bootstrap values > 50% and Bayesian posterior probabilities > 0.95, respectively.



Figure 2. Basidiomata of *Trechispora albofarinosa* (holotype) **A** the front of the basidiomata **B** characteristic hymenophore. Scale bars: 1 cm (**A**); 1 mm (**B**).



Figure 3. Microscopic structures of *Trechispora albofarinosa* (holotype) **A** basidiospores **B** basidia and basidioles **C** a cross section of basidiomata. Scale bars: $5 \mu m$ (**A**); $10 \mu m$ (**B**, **C**).

Description. Basidiomata annual, resupinate, farinose, without odor or taste when fresh, up to 3.5 cm long, 1.5 cm wide, and $300-500 \mu$ m thick. Hymenial surface flocculence, white when fresh, white to cream on drying. Sterile margin indistinct, white, and up to 0.5 mm wide.

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

Cystidia and cystidioles absent; basidia clavate, with four sterigmata and a basal clamp connection, $6.5-10 \times 3.5-5 \ \mu m$.

Basidiospores ellipsoid, colorless, thin-walled, aculeate, IKI–, CB–, 2.5–3.5 (-4) × 2–2.5 (-3.5) μ m, L = 3.18 μ m, W = 2.44 μ m, Q = 1.3 (n = 30/1).

Trechispora bisterigmata K.Y. Luo & C.L. Zhao, sp. nov. MycoBank No: 849464

Figs 4, 5

Holotype. CHINA. Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, 23°56'N, 101°29'E, altitude 2200 m a.s.l., on the trunk of *Albizia julibrissin*, leg. C.L. Zhao, 20 Aguest 2017, CLZhao 2522 (SWFC).

Etymology. *Bisterigmata* (Lat.): referring to the basidia mainly with two sterigmata.

Description. Basidiomata annual, resupinate, adnate, membranous, without odor or taste when fresh, up to 2.5 cm long, 1.5 cm wide, and 4 mm thick. Hymenial surface odontioid, cream. Sterile margin indistinct, white, rhizomorphic, up to 0.5 mm wide.

Hyphal system monomitic, generative hyphae with clamp connections, colorless, slightly thick-walled, ampullate septa frequently present in subiculum and hymenium with crystals, up to 6 μ m wide, branched, interwoven, 2.5–4 μ m in diameter; IKI–, CB–, tissues unchanged in KOH.

Cystidia and cystidioles are absent; basidia barrelled, slightly constricted, with two or four sterigmata and a basal clamp connection, $6.5-14.5 \times 3.5-5.5 \mu m$.



Figure 4. Basidiomata of *Trechispora bisterigmata* (holotype) A the front of the basidiomata B characteristic hymenophore. Scale bars: 1 cm (A); 1 mm (B).

Basidiospores subglobose to broad ellipsoid, colorless, slightly thick-walled, smooth, IKI–, CB–, (2–) $2.5-4 \times 2-3.5 \mu$ m, L = 3.03μ m, W = 2.41μ m, Q = 1.23-1.28 (n = 60/2).

Additional specimen examined (paratype). China. Yunnan Province, Yuxi, Xinping County, Mopanshan National Forestry Park, 23°56'N, 101°29'E, altitude 2200 m a.s.l., on the living angiosperm tree, leg. C.L. Zhao, 19 August 2018, CLZhao 7870 (SWFC).



Figure 5. Microscopic structures of *Trechispora bisterigmata* (holotype) **A** basidiospores **B** basidia and basidioles **C** a cross section of basidiomata. Scale bars: 5 μm (**A**); 10 μm (**B**, **C**).

Trechispora pileata K.Y. Luo & C.L. Zhao, sp. nov.

MycoBank No: 849465 Figs 6, 7

Holotype. CHINA. Yunnan Province, Pu'er, Jingdong County, Wuliangshan National Nature Reserve, 24°23'N, 100°45'E, altitude 2350 m a.s.l., on the angio-sperm trunk, leg. C.L. Zhao, 6 October 2017, CLZhao 4456 (SWFC).

Etymology. *Pileata* (Lat.): referring to the pileate basidiomata.

Description. Basidiomata annual, with a laterally contracted base, solitary or imbricate. Pileus fan shaped, cortical to corky, up to 1.5 cm long, 1 cm wide, and 2 mm thick, yellowish to yellowish brown, the surface radially striate covered with appressed scales, azonate; the hymenophore surface odontioid, yellowish brown, up to 1 mm long. Context cream, 1 mm thick. Sterile margin indistinct, slightly buff, and 0.5 mm wide.

Hyphal system monomitic, generative hyphae with clamp connections, color-less, thick-walled, frequently branched, interwoven, hyphae in spines 2.5–4 μ m in diameter, IKI–, CB–, tissues unchanged in KOH. Hyphae in context colorless, thin- to thick-walled, unbranched, interwoven, 4.5–6 μ m in diameter, IKI–, CB–, tissues unchanged in KOH.

Cystidia and cystidioles absent; basidia subcylindrical, constricted, with four sterigmata and a basal clamp connection, $5-7 \times 2.5-4 \mu m$.



Figure 6. Basidiomata of *Trechispora pileata* (holotype) **A**, **B** the front of the basidiomata **C**, **D** the back of the basidiomata. Scale bars: 0.5 cm (A); 1 mm (B); 0.5 cm (C); 1 mm (D).



Figure 7. Microscopic structures of *Trechispora pileata* (holotype) **A** basidiospores **B** basidia and basidioles **C** hyphae of context of pileus **D** a spine trama of basidiomata. Scale bars: $5 \mu m$ (**A**); $10 \mu m$ (**B**, **C**).

Basidiospores subglobose to broad ellipsoid, colorless, thin-walled, smooth, IKI-, CB-, (2.5-) 2.8-5 (-5.5) × (2.5-) 3-4.7 μ m, L = 4 μ m, W = 3.56 μ m, Q = 1.12 (n = 30/1).

Trechispora wenshanensis K.Y. Luo & C.L. Zhao, sp. nov.

MycoBank No: 849466 Figs 8, 9

Holotype. CHINA. Yunnan Province, Wenshan, Babao Town, Balao battle site, 23°22'N, 104°15'E, altitude 1300 m a.s.l., on the fallen angiosperm branch, leg. C.L. Zhao, 19 January 2019, CLZhao 11649 (SWFC).

Etymology. *Wenshanensis* (Lat.): referring to the locality (Wenshan) of the type specimen.

Description. Basidiomata annual, resupinate, adnate, cottony, easily to separate from substrate, without odor or taste when fresh, up to 5.5 cm long, 4 cm wide, and $200-400 \mu m$ thick. Hymenial surface smooth, slightly cream when fresh, cream to buff on drying. Sterile margin indistinct, cream, and 1-2 mm wide.

Hyphal system monomitic, generative hyphae with clamp connections, colorless, thin- to thick-walled, branched, interwoven, $1-2 \ \mu m$ in diameter; IKI-, CB-, tissues unchanged in KOH.

Cystidia and cystidioles are absent; basidia barrelled, with four sterigmata and a basal clamp connection, $7-10 \times 3-5 \ \mu m$.

Basidiospores ellipsoid, colorless, thin-walled, warted, IKI–, CB–, (2–) 2.5– 3.7 (–4) × (1.5–) 2–3 μ m, L = 3.02 μ m, W = 2.37 μ m, Q = 1.25–1.30 (n = 90/3).

Additional specimens examined (paratypes). China. Yunnan Province, Wenshan, Funing county, Guying village, 23°42'N, 105°53'E, altitude 1000 m a.s.l., on the fallen angiosperm branch, leg. C.L. Zhao, 20 January 2019, CLZhao 11715; Yunnan Province, Lincang, Lancangjiang Forestry Region, 25°37'N, 97°30'E, altitude 1750 m a.s.l., on the fallen angiosperm branch, leg. C.L. Zhao, 21 July 2022, CLZhao 22940 (SWFC).



Figure 8. Basidiomata of *Trechispora wenshanensis* (holotype) **A** the front of the basidiomata **B** characteristic hymenophore. Scale bars: 1 cm (**A**); 1 mm (**B**).



Figure 9. Microscopic structures of *Trechispora wenshanensis* (holotype) A basidiospores B basidia and basidioles C a cross section of basidiomata. Scale bars: $5 \mu m$ (A); 10 μm (B, C).

Discussion

Many recently described wood-inhabiting fungal taxa have been reported in the subtropics and tropics, including in the genus *Trechispora* (Ordynets et al. 2015; Phookamsak et al. 2019; Xu et al. 2019; Chikowski et al. 2020; Haelewaters et al. 2020; Crous et al. 2021; de Meiras-Ottoni et al. 2021; Zhao and Zhao 2021; Liu et al. 2022a, b; Luo and Zhao 2022; Deng et al. 2023; Sommai et al. 2023). The present study reports four new species in *Trechispora*, based on a combination of morphological features and molecular evidence.

Based on ITS+nLSU topology (Fig. 1), four new species were grouped into the genus *Trechispora*, in which *T. albofarinosa* was sister to *T. araneosa* (Höhn. & Litsch.) K.H. Larss., However, morphologically, *T. araneosa* can be delimited from *T. albofarinosa* by its odontioid to poroid hymenial surface and larger ba-

sidiospores (5–6.5 × 4–5 μ m; Larsson 1995). The second new species *T. bisterigmata* grouped closely with *T. cohaerens* (Schwein.) Jülich & Stalpers and *T. laevispora* Z.B. Liu, Y.D. Wu & Yuan Yuan. However, morphologically, *T. cohaerens* is different from *T. bisterigmata* by its thin-walled hyphal (Jülich and Stalpers 1980); *T. laevispora* can be delimited from *T. bisterigmata* by having the smooth hymenial surface, and thin-walled basidiospores (Liu et al. 2022b). The third species *T. pileata* formed a monophyletic lineage. The species *T. wenshanensis* grouped closely with *T. mellina* (Bres.) K.H. Larss. However, morphologically, *T. mellina* can be delimited from *T. wenshanensis* by having the longer basidia (15–20 × 4.5–5 µm) and smooth basidiospores (Chikowski et al. 2020).

Morphologically, Trechispora albofarinosa resembles T. olivacea K.Y. Luo & C.L. Zhao and T. yunnanensis C.L. Zhao by sharing the farinosa basidiomata. However, T. olivacea differs from T. albofarinosa by olivaceous hymenial surface and thick-walled basidiospores (Luo and Zhao 2022); T. yunnanensis can be delimited from T. albofarinosa due to its thick-walled, larger basidiospores $(7-8.5 \times 5-5.5 \mu m;$ Xu et al. 2019). The new species T. albofarinosa is similar to T. bambusicola C.L. Zhao, T. fimbriata C.L. Zhao, T. fissurata C.L. Zhao and T. murina K.Y. Luo & C.L. Zhao in its presence of ellipsoid basidiospores. T. bambusicola can be delimited from T. albofarinosa by odontioid hymenial surface with aculei cylindrical to conical (0.3-0.5 mm long), and thick-walled basidiospores (Zhao and Zhao 2021); T. fimbriata can be delimited from T. albofarinosa due to its hydnoid hymenial surface, and thick-walled basidiospores (Zhao and Zhao 2021); T. fissurata is different from T. albofarinosa by hydnoid hymenial surface and thick-walled, broadly basidiospores (3.3-4 × 2.8-3.5 µm; Zhao and Zhao 2021); T. murina can be delimited from T. albofarinosa due to its grandinioid hymenial surface and thick-walled basidiospores (Luo and Zhao 2022).

Trechispora bisterigmata is similar to *T. fastidiosa* (Pers.) Liberta by sharing the membranous basidiomata. However, *T. fastidiosa* differs from *T. bisterigmata* by smooth hymenial surface and larger basidiospores ($6-7 \times 4.5-5.5 \mu m$; Bernicchia and Gorjón 2010). *T. bisterigmata* resembles *T. bambusicola* C.L. Zhao, *T. canariensis* Ryvarden & Liberta and *T. christiansenii* (Parmasto) Liberta in its monomitic hyphal system and presence of the crystals. However, *T. bambusicola* differs from *T. bisterigmata* by its odontioid hymenial surface and ornamented basidiospores (Zhao and Zhao 2021); *T. canariensis* differs from *T. bisterigmata* due to its larger basidia ($15-20 \times 5-6 \mu m$) and thin-walled, larger basidiospores ($5-7 \times 3-3.5 \mu m$; Ryvarden and Liberta 1978); *T. christiansenii* can be delimited from *T. bisterigmata* by its larger basidia ($15-20 \times 6-7 \mu m$) and larger basidiospores ($5.5-7 \times 4-4.5 \mu m$; Liberta 1966).

Trechispora pileata is similar to *T. byssinella* (Bourdot) Liberta, *T. kavinioides* B. de Vries, *T. silvae-ryae* (J. Erikss. & Ryvarden) K.H. Larss. and *T. subsphaerospora* (Litsch.) Liberta by sharing smooth basidiospores. However, *T. byssinella* differs from *T. pileata* by having narrower ellipsoid basidiospores (Bernicchia and Gorjón 2010); *T. kavinioides* can be delimited from *T. pileata* by its odontioid hymenial surface, and narrower ellipsoid to lacrymiform basidiospores (Bernicchia and Gorjón 2010); *T. silvae-ryae* is different from *T. pileata* by dimitic hyphal system (Bernicchia and Gorjón 2010); *T. silvae-ryae* is different from *T. pileata* by dimitic hyphal system (Bernicchia and Gorjón 2010); *T. subsphaerospora* differs from *T. pileata* by having angular basidiospores (Bernicchia and Gorjón 2010). In addition, the delimitation characteristics of the genus have full resupinate basidiomata, but this new species has the pileate basidiomata with a laterally contracted base. Based on the phylogenetic

analyses, this new species groups with *Trechispora* species, therefore, we propose that the genus *Trechispora* accommodate this new species in the present study.

Trechispora wenshanensis resembles T. fastidiosa and T. laevispora Z.B. Liu, Y.D. Wu & Yuan Yuan by sharing a smooth hymenial surface. However, T. fastidiosa differs from T. wenshanensis by larger basidiospores (6-7 × 4.5-5.5 µm; Bernicchia and Gorjón 2010); T. laevispora differs from T. wenshanensis by fimbriate margin of the basidiomata and smooth basidiospores (Liu et al. 2022b). The new species T. wenshanensis is similar to T. bambusicola C.L. Zhao, T. fimbriata C.L. Zhao, T. fissurata C.L. Zhao, T. murina K.Y. Luo & C.L. Zhao and T. yunnanensis C.L. Zhao due to its ellipsoid basidiospores. However, T. bambusicola can be delimited from T. wenshanensis by odontioid hymenial surface, and thick-walled basidiospores (Zhao and Zhao 2021); T. fimbriata differs from T. wenshanensis due to its hydnoid hymenial surface, and thick-walled basidiospores (Zhao and Zhao 2021); T. fissurata is different from T. wenshanensis by hydnoid hymenial surface, and thick-walled, broadly basidiospores (3.3-4 × 2.8-3.5 µm; Zhao and Zhao 2021); T. murina can be delimited from T. wenshanensis due to its grandinioid hymenial surface, and thick-walled basidiospores (Luo and Zhao 2022); T. yunnanensis is different from T. wenshanensis by farinaceous hymenial surface and thick-walled, larger basidiospores ($7-8.5 \times 5-5.5 \mu m$; Xu et al. 2019).

Key to 42 accepted species of Trechispora in China

2	Basidiomata with clavarioid	1
6	Basidiomata without clavarioid	-
3	Basidiomata grayish brown to pale purple	2
4	Basidiomata pure white to pale yellow	-
ninal branches	Basidiomata with dense branches and long ter	3
T. longiramosa		
Т. Іаха	Basidiomata with loose branches	-
5	Basidiomata with flattened branches	4
T. tongdaoensis	Basidiomata without flattened branches	-
T. alba	Basidiomata branches polychotomous	5
T. khokpasiensis	Basidiomata branches dichotomous	-
T. pileata	Basidiomata pileate	6
7	Basidiomata resupinate to effused	-
8	Hymenophore poroid	7
andinioid, odontioid, hyd-	Hymenophore smooth, colliculose, irpicoid, g	-
	noid	
T. dimitiella	Hyphal system dimitic	8
9	Hyphal system monomitic	-
	Subicular hyphae thick-walled	9
11	Subicular hyphae thin-walled	-
T. mollusca	Ampullate septa present on subicular hyphae	10
T. suberosa	Ampullate septa absent on subicular hyphae	-
T. candidissima	Crystals in subiculum as numerous rodlets	11
arious shapes 12	Crystals in subiculum as rhomboidal plates or	-
t part of subiculum	Sphaerocysts present in cords and the adjacer	12
T. hymenocystis	- -	
	Sphaerocysts absent	_

13	Basidiospores smooth	14
-	Basidiospores ornamented	16
14	Basidiomata with rhizomorph	T. bisterigmata
-	Basidiomata without rhizomorph	15
15	Basidiospores subglobose, angular to turbinate	T. confinis
-	Basidiospores ellipsoid	T. laevispora
16	Basidiomata < 50 μm thick	17
-	Basidiomata > 50 µm thick	19
17	Crystals absent	T. gracilis
-	Crystals present	18
18	Crystals aggregated, rhomboidal fakes	. T. perminispora
-	Crystals butterfly-like, easily broken into irregular shapes	T. subaraneosa
19	Hymenophore smooth	20
-	Hymenophore colliculose, irpicoid, grandinioid, odontioid	, hydnoid 27
20	Basidiospores slightly cyanophilous	T. incisa
-	Basidiospores acyanophilous	21
21	Basidiospores > 6.5 µm long	T. yunnanensis
-	Basidiospores < 6.5 µm long	22
22	Generative hyphae < 2 μ m in diameter	T. wenshanensis
-	Generative hyphae > 2 µm in diameter	23
23	Generative hyphae thin-walled	24
-	Generative hyphae thick-walled	25
24	Hymenophore farinaceous	T. larssonii
-	Hymenophore arachnoid	. T. subfarinacea
25	Generative hyphae > $3.5 \mu m$ in diameter	T. latehypha
-	Generative hyphae < 3.5 μ m in diameter	26
26	Basidiospores ellipsoid, thin-walled	T. albofarinosa
-	Basidiospores broadly ellipsoid to globose, thick-walled	T. olivacea
27	Hymenial surface colliculose, irpicoid or grandinioid	28
-	Hymenial surface odontioid or hydnoid	
28	Generative hyphae thick-walled	T. murina
-	Generative hyphae thin-walled	
29	Growth on bamboo	T. taiwanensis
-	Growth on other plant	T. crystallina
30	Tramal hyphae thin-walled or slightly thick-walled	31
-	Tramal hyphae distinctly thick-walled	35
31	Crystals absent in trama	T. tropica
-	Crystals present in trama	32
32	Basidiospores subglobose to globose	T. odontioidea
-	Basidiospores ellipsoid or broadly ellipsoid	
33	Tramal hyphae 3–6 µm wide, spines of basidiospores co	nstricted
		T. constricta
-	Tramal hyphae 2–4 µm wide, spines of basidiospores not	constricted34
34	Cystidia present	T. chaibuxiensis
_	Cystidia absent	T. nivea
35	Hymenophore aculei > 0.4 mm long	
_	Hymenophore aculei < 0.4 mm long	
36	Margin smooth	T. fissurata
-	Margin fimbriate	37

T. dentata	Basidiomata irpicoid	37
	Basidiomata odontioid or hydnoid	_
nen fresh T. fimbriata	Hymenophore aculei sparse, cream to buff-yellow v	38
T. fragilis	Hymenophore aculei dense, white when fresh	-
T. bambusicola	Generative hyphae ampullate septa absent	39
	Generative hyphae ampullate septa present	-
T. subfissurata	Basidiospores with sharp spines	40
41	Basidiospores without sharp spines	_
	Spines of basidiospores constricted	41

- Spines of basidiospores not constricted T. sinensis

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The research was supported by the National Natural Science Foundation of China (Project Nos: 32170004, U2102220), Forestry Innovation Programs of Southwest Forestry University (Project No: LXXK-2023Z07), and the Yunnan Province College Students Innovation and Entrepreneurship Training Program (Project no. s202310677034), and the High-level Talents Program of Yunnan Province (YNQR-QNRC-2018-111).

Author contributions

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

Author ORCIDs

Jiang-Qing Su ^(b) https://orcid.org/0009-0008-5480-4502 Chang-Lin Zhao ^(b) https://orcid.org/0000-0002-8668-1075

Data availability

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

References

Bernicchia A, Gorjón SP (2010) Fungi Europaei 12: Corticiaceae s.l. Edizioni Candusso, Alassio, Italy.

Bondartsev AS (1953) The Polyporaceae of the European USSR and Caucasia. Israel Program for Scientific Translations, Jerusalem, 1–896.

- Bondartsev AS, Singer R (1941) Zur Systematik der Polyporaceen. Annales Mycologici 39(1): 43–65.
- Burdsall Jr HH, Gilbertson RL (1982) New species of Corticiaceae (Basidiomycotina, Aphyllophorales) from Arizona. Mycotaxon 15: 333–340.
- Chen JZ, Zhao CL (2020) Morphological and molecular identification of four new resupinate species of *Lyomyces* (Hymenochaetales) from southern China. MycoKeys 65: 101–118. https://doi.org/10.3897/mycokeys.65.48660
- Chikowski RS, Larsson KH, Gibertoni TP (2020) Taxonomic novelties in *Trechispora* (Trechisporales, Basidiomycota) from Brazil. Mycological Progress 19(12): 1403–1414. https://doi.org/10.1007/s11557-020-01635-y
- Crous PW, Osieck ER, Jurjević Ž (2021) Fungal Planet description sheets: 1284–1382. Persoonia 47: 178–374. https://doi.org/10.3767/persoonia.2021.47.06
- Dai YC (2011) A revised checklist of corticioid and hydnoid fungi in China for 2010. Mycoscience 52(1): 69-79. https://doi.org/10.1007/S10267-010-0068-1
- Dai YC, Cui BK, Si J, He SH, Hyde KD, Yuan HS, Liu XY, Zhou LW (2015) Dynamics of the worldwide number of fungi with emphasis on fungal diversity in China. Mycological Progress 14(8): 62. https://doi.org/10.1007/s11557-015-1084-5
- Dai YC, Yang ZL, Cui BK, Wu G, Yuan HS, Zhou LW, et al. (2021) Diversity and systematics of the important macrofungi in Chinese forests. Junwu Xuebao 40: 770–805.
- de Meiras-Ottoni A, Larsson KH, Gibertoni TB (2021) Additions to *Trechispora* and the status of *Scytinopogon* (Trechisporales, Basidiomycota). Mycological Progress 20(2): 203–222. https://doi.org/10.1007/s11557-021-01667-y
- Deng PT, Yan J, Liu XF, He ZM, Lin Y, Lu MX, Zhang P (2023) Three coralloid species of the genus *Trechispora* (Trechisporales, Basidiomycota) in China: Two newly discovered taxa and one reported for the first time. MycoKeys 99: 153–170. https://doi. org/10.3897/mycokeys.99.109375
- Desjardin DE, Perry BA (2015) A new species of *Scytinopogon* from the island of Principe, Republic of Sao Tome and Principe, West Africa. Mycosphere 6(4): 433–440. https://doi.org/10.5943/mycosphere/6/4/5
- Felsenstein J (1985) Confidence intervals on phylogenetics: An approach using bootstrap. Evolution; International Journal of Organic Evolution 39(4): 783–791. https://doi.org/10.2307/2408678

Gilbertson RL, Budington AB (1970) Three new species of wood-rotting fungi in the Corticiaceae. Mycologia 62(4): 673–678. https://doi.org/10.1080/00275514.1970.12019011

- Guan QX, Zhao CL (2023) Five new species of Schizoporaceae (Basidiomycota, Hymenochaetales) from East Asia. MycoKeys 96: 25–56. https://doi.org/10.3897/mycokeys.96.99327
- Guan QX, Li YF, Zhao CL (2021) Morphological and phylogenetic evidence for recognition of two new species of *Hyphoderma* (Basidiomycota) from southern China, with a key to all Chinese *Hyphoderma*. MycoKeys 83: 145–160. https://doi.org/10.3897/ mycokeys.83.69909
- Haelewaters D, Dima B, Abdel-Hafiz AII, Abdel-Wahab M, Abul-Ezz SR, Acar I, Aguirre-Acosta E, Aime MC, Aldemir S, Ali M, Ayala-Vásquez O, Bakhit MS, Bashir H, Battistin E, Bendiksen E, Castro-Rivera R, Çolak ÖF, De Kesel A, de la Fuente JI, Dizkırıcı A, Hussain S, Jansen GM, Kaygusuz O, Khalid AN, Khan J, Kiyashko AA, Larsson E, MartínezGonzález CR, Morozova OV, Niazi AR, Noordeloos ME, Pham THG, Popov ES, Psurtseva NV, Schoutteten N, Sher H, Türkekul I[°], Verbeken A, Ahmad H, Afshan NS, Christe P, Fiaz M, Glaizot O, Liu J, Majeed J, Markotter W, Nagy A, Nawaz H,

Papp V, Péter Á, Pfliegler WP, Qasim T, Riaz M, Sándor AD, Szentiványi T, Voglmayr H, Yousaf N, Krisai-Greilhuber I (2020) Fungal systematics and evolution: FUSE 6. Sydowia 72: 231–356.

- Hallenberg N (1978) Wood-fungi (Corticiaceae, Coniophoraceae, Lachnocladiaceae, Thelephoraceae) in N. Iran. I. Iranian Journal of Plant Pathology 14: 38–87.
- Hallenberg N (1980) New taxa of Corticiaceae from N. Iran (Basidiomycetes). Mycotaxon 11(2): 447–475.
- He MQ, Zhu XY, Li TH, Cui BK, Wang K Bau Tolgor, Zhao RL (2022) Macrofungal classification system and information platform http://www.nmdc.cn/macrofungi/ is launched. Mycosystema 41(6): 899–905.
- Hjortstam K, Larsson KH (1995) Annotated check-list to genera and species of corticioid fungi (Aphyllophorales, Basidiomycotina) with special regards to tropical and subtropical areas. Windahlia 21: 1–75.
- Hyde KD (2022) The numbers of fungi. Fungal Diversity 114(1): 1. https://doi. org/10.1007/s13225-022-00507-y
- Jülich W (1975) Studies in resupinate basidiomycetes III. Persoonia 8(3): 291-305.
- Jülich W (1976) Studies in resupinate basidiomycetes IV. Persoonia 8(4): 431–442.
- Jülich W, Stalpers JA (1980) The resupinate non-poroid Aphyllophorales of the temperate northern hemisphere. Verhandelingen Koninklijke Nederlandse Akademie van Wetenschappen 74: 1–335.
- Karsten PA (1890) Fragmenta mycologica XXIX. Hedwigia 29: 147–149.
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Kornerup A, Wanscher J (1978) Metheun Hand Book of Colour, 3rd edn. Metheun, London, 144–148.
- Larsson KH (1992) The genus *Trechispora* (Corticiaceae, Basidiomycetes). Göteborg: Dept. of Systematic Botany.
- Larsson KH (1994) Poroid species in *Trechispora* and the use of calcium oxalate crystals for species identification. Mycological Research 98(10): 1153–1172. https://doi. org/10.1016/S0953-7562(09)80200-1
- Larsson KH (1995) Taxonomy of *Trechispora farinacea* and proposed synonyms I. Species with a grandinioid or hydnoid hymenophore. Symbolae Botanicae Upsalienses 30(3): 101–118.
- Larsson KH (1996) New species and combinations in *Trechispora* (Corticiaceae, Basidiomycotina). Nordic Journal of Botany 16(1): 83–98. https://doi. org/10.1111/j.1756-1051.1996.tb00218.x
- Larsson A (2014) AliView: A fast and lightweight alignment viewer and editor for large data sets. Bioinformatics (Oxford, England) 30(22): 3276–3278. https://doi.org/10.1093/bioinformatics/btu531
- Larsson KH, Larsson E, Kõljalg U (2004) High phylogenetic diversity among corticioid homobasidiomycetes. Mycological Research 108(9): 983–1002. https://doi. org/10.1017/S0953756204000851
- Liberta AE (1966) On Trechispora. Taxon 15(8): 317-319. https://doi.org/10.2307/1216118
- Liberta AE (1973) The genus *Trechispora* (Basidiomycetes, Corticiaceae). Canadian Journal of Botany 51(10): 1871–1892. https://doi.org/10.1139/b73-240
- Liu SL, Ma HX, He SH, Dai YC (2019) Four new corticioid species in *Trechisporales* (Basidiomycota) from East Asia and notes on phylogeny of the order. MycoKeys 48: 97–113. https://doi.org/10.3897/mycokeys.48.31956

- Liu SL, He SH, Wang XW, May TW, He G, Chen SL, Zhou LW (2022a) Trechisporales emended with a segregation of Sistotremastrales ord. nov. (Basidiomycota). Mycosphere 13(1): 862–954. https://doi.org/10.5943/mycosphere/13/1/11
- Liu ZB, Wu YD, Zhao H, Lian YP, Wang YR, Wang CG, Mao W-L, Yuan Y (2022b) Outline, divergence times, and phylogenetic analyses of *Trechisporales* (Agaricomycetes, Basidiomycota). Frontiers in Microbiology 13: 818358. https://doi.org/10.3389/ fmicb.2022.818358
- Liu SL, Wang XW, Li GJ, Deng CY, Rossi W, Leonardi M, Liimatainen K, Kekki T, Niskanen T, Smith ME, Ammirati J, Bojantchev D, Abdel-Wahab MA, Zhang M, Tian E, Lu Y-Z, Zhang J-Y, Ma J, Dutta AK, Acharya K, Du T-Y, Xu J, Kim JS, Lim YW, Gerlach A, Zeng N-K, Han Y-X, Razaghi P, Raza M, Cai L, Calabon MS, Jones EBG, Saha R, Kumar TKA, Krishnapriya K, Thomas A, Kaliyaperumal M, Kezo K, Gunaseelan S, Singh SK, Singh PN, Lagashetti AC, Pawar KS, Jiang S, Zhang C, Zhang H, Qing Y, Bau T, Peng X-C, Wen T-C, Ramirez NA, Niveiro N, Li M-X, Yang ZL, Wu G, Tarafder E, Tennakoon DS, Kuo C-H, da Silva TM, Souza-Motta CM, Bezerra JDP, He G, Ji X-H, Suwannarach N, Kumla J, Lumyong S, Wannathes N, Rana S, Hyde KD, Zhou L-W (2024a) Fungal diversity notes 1717–1817: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 124(1): 1–216. https://doi.org/10.1007/s13225-023-00529-0
- Liu SL, Zhao P, Cai L, Shen S, Wei HW, Na Q, Han M, Wei R, Ge Y, Ma H, Karunarathna SC, Tibprommab S, Zhang B, Dai D, Lin L, Fan X-L, Luo Z-L, Shen H-W, Lu L, Lu W, Xu R-F, Tohtirjap A, Wu F, Zhou L-W (2024b) Catalogue of fungi in China 1. New taxa of plantinhabiting fungi. Mycology, 1–59. https://doi.org/10.1080/21501203.2024.2316066
- Luo KY, Zhao CL (2022) A molecular systematics and taxonomy research on *Trechispora ra* (Hydnodontaceae, Trechisporales): Concentrating on three new *Trechispora* species from East Asia. Journal of Fungi (Basel, Switzerland) 8(10): 1020. https://doi.org/10.3390/jof8101020
- Mardones M, Carranza-Velázquez J, Mata-Hidalgo M, Amador-Fernández X, Urbina H (2023) Taxonomy and phylogeny of the genus *Ganoderma* (Polyporales, Basidiomycota) in Costa Rica. MycoKeys 100: 5–47. https://doi.org/10.3897/mycokeys.100.106810
- Miettinen O, Larsson KH (2006) *Trechispora elongata* species nova from North Europe. Mycotaxon 96: 193–198.
- Miller MA, Pfeiffer W, Schwartz T (2012) The CIPRES Science Gateway: Enabling high-impact science for phylogenetics researchers with limited resources. Association for Computing Machinery 39: 1–8. https://doi.org/10.1145/2335755.2335836
- Nylander JAA (2004) MrModeltest v.2. Program Distributed by the Author; Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.
- Ordynets A, Larsson KH, Langer E (2015) Two new *Trechispora* species from La Réunion Island. Mycological Progress 14(11): 113. https://doi.org/10.1007/s11557-015-1133-0
- Parmasto E (1968) Conspectus Systematis Corticiacearum. Institutum Zoologicum et Botanicum Academiae Scientiarum R.P.S.S, Tartu, 1–97.
- Phookamsak R, Hyde KD, Jeewon R, Bhat DJ, Jones EBG, Maharachchikumbura SSN, Raspe O, Karunarathna SC, Wanasinghe DN, Hongsanan S, Doilom M, Tennakoon DS, Machado AR, Firmino AL, Ghosh A, Karunarathna A, Mešić A, Dutta AK, Thongbai B, Devadatha B, Norphanphoun C, Senwanna C, Wei D, Pem D, Ackah FK, Wang G-N, Jiang H-B, Madrid H, Lee HB, Goonasekara ID, Manawasinghe IS, Kušan I, Cano J, Gené J, Li J, Das K, Acharya K, Raj KNA, Latha KPD, Chethana KWT, He M-Q,

Dueñas M, Jadan M, Martín MP, Samarakoon MC, Dayarathne MC, Raza M, Park MS, Telleria MT, Chaiwan N, Matočec N, de Silva NI, Pereira OL, Singh PN, Manimohan P, Uniyal P, Shang Q-J, Bhatt RP, Perera RH, Alvarenga RLM, Nogal-Prata S, Singh SK, Vadthanarat S, Oh S-Y, Huang S-K, Rana S, Konta S, Paloi S, Jayasiri SC, Jeon SJ, Mehmood T, Gibertoni TB, Nguyen TTT, Singh U, Thiyagaraja V, Sarma VV, Dong W, Yu X-D, Lu Y-Z, Lim YW, Chen Y, Tkalčec Z, Zhang Z-F, Luo Z-L, Daranagama DA, Thambugala KM, Tibpromma S, Camporesi E, Bulgakov TS, Dissanayake AJ, Senanayake IC, Dai DQ, Tang L-Z, Khan S, Zhang H, Promputtha I, Cai L, Chomnunti P, Zhao R-L, Lumyong S, Boonmee S, Wen T-C, Mortimer PE, Xu J (2019) Fungal diversity notes 929–1035, taxonomic and phylogenetic contributions on genera and species of fungi. Fungal Diversity 95(1): 1–273. https://doi.org/10.1007/s13225-019-00421-w

- Rauschert S (1987) Nomenklatorische Studien bei Höheren Pilzen IV. Nichtblätterpilze (Aphyllophorales) mit Ausschluss der Porlinge. Feddes Repertorium 98(11–12): 657–664. https://doi.org/10.1002/fedr.19870981116
- Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98(6): 625–634. https://doi.org/10.1016/S0953-7562(09)80409-7
- Rogers DP (1944) The genera *Trechispora* and *Galzinia*. Mycologia 36(1): 70–103. https://doi.org/10.1080/00275514.1944.12017530
- Rogers DP, Jackson HS (1943) Notes on the synonymy of some North American Thelephoraceae and other resupinates. Farlowia 1(2): 263–328. https://doi. org/10.5962/p.315982
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Ryvarden L (1975) Studies in the *Aphyllophorales* of Africa 3. Three new polypores from Zaïre. Bulletin du Jardin Botanique National de Belgique 45(1/2): 197–203. https://doi.org/10.2307/3667599
- Ryvarden L (2002) A note on the genus *Hydnodon* Banker. Some neotropical wood-inhabiting fungi. Synop Fungorum 15: 31–33.
- Ryvarden L, Liberta AE (1978) Contribution to the Aphyllophoralles of the Canary Islands 4. Two new species of *Trechispora* and *Xenmastella*. Canadian Journal of Botany 56(20): 2617–2619. https://doi.org/10.1139/b78-314
- Ryvarden L, Stokland L, Larsson KH (2003) A critical checklist of Corticoid and Poroid fungi of Norway. Synop Fungorum 17: 1–209.
- Sommai S, Pinruan U, Khamsuntorn P, Lueangjaroenkit P, Somrithipol S, Luangsa-ard J (2023) Three new species of *Trechispora* from Northern and Northeastern Thailand. Mycological Progress 22(6): 42. https://doi.org/10.1007/s11557-023-01886-5
- Telleria MT, Melo I, Dueñas M, Larsson KH, Paz Martín MP (2013) Molecular analyses confirm *Brevicellicium* in Trechisporales. IMA Fungus 4(1): 21–28. https://doi. org/10.5598/imafungus.2013.04.01.03
- Trichies G, Schultheis B (2002) *Trechispora antipus* sp. nov., une seconde espèce bisporique du genre *Trechispora* (Basidiomycota, Stereales). Mycotaxon 82: 453–458.
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vries BWL de (1987) Some new corticioid fungi. Mycotaxon 28(1): 77-90.

- Vu D, Groenewald M, de Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92(1): 135–154. https://doi. org/10.1016/j.simyco.2018.05.001
- Wang CG, Vlasák J, Dai YC (2021) Phylogeny and diversity of *Bjerkandera* (Polyporales, Basidiomycota), including four new species from South America and Asia. MycoKeys 79: 149–172. https://doi.org/10.3897/mycokeys.79.63908
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR protocols: A Guide to Methods And Applications. Academic Press, San Diego, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Wijayawardene NN, Phillips AJL, Pereira DS, Dai DQ, Aptroot A, Monteiro JS, Druzhinina IS, Cai F, Fan X, Selbmann L, Coleine C, Castañeda-Ruiz RF, Kukwa M, Flakus A, Fiuza PO, Kirk PM, Kumar KCR, leperuma Arachchi IS, Suwannarach N, Tang L-Z, Boekhout T, Tan CS, Jayasinghe RPPK, Thines M (2022) Forecasting the number of species of asexually reproducing fungi (Ascomycota and Basidiomycota). Fungal Diversity 114(1): 463–490. https://doi.org/10.1007/s13225-022-00500-5
- Wu SH, Chen YP, Wei CL, Floudas D, Dai YC (2017) Two new species of *Phanerochaete* (Basidiomycota) and redescription of *P. robusta*. Mycological Progress 17(4): 425–435. https://doi.org/10.1007/s11557-017-1368-z
- Wu F, Yuan Y, Chen JJ, Cui BK, Zhou M, Dai YC (2020) Terrestriporiaceae fam. nov., a new family of Russulales (Basidiomycota). Mycosphere 11(1): 2755–2766. https://doi. org/10.5943/mycosphere/11/1/21
- Wu F, Tohtirjap A, Fan LF, Zhou LW, Alvarenga RLM, Gibertoni TB, Dai Y-C (2021) Global diversity and updated phy-logeny of *Auricularia* (Auriculariales, Basidiomycota). Journal of Fungi (Basel, Switzerland) 7(11): 933. https://doi.org/10.3390/jof7110933
- Wu F, Zhou LW, Vlasák J, Dai YC (2022) Global diversity and systematics of Hymenochaetaceae with poroid hymenophore. Fungal Diversity 113(1): 1–192. https://doi. org/10.1007/s13225-021-00496-4
- Xu TM, Chen YH, Zhao CL (2019) *Trechispora yunnanensis* sp. nov. from China. Phytotaxa 424(4): 253–261. https://doi.org/10.11646/phytotaxa.424.4.5
- Yuan HS, Dai YC (2012) Wood-inhabiting fungi in southern China. 6. Polypores from Guangxi autonomous region. Annales Botanici Fennici 49(5–6): 341–351. https://doi. org/10.5735/085.049.0605
- Yuan Y, Bian LS, Wu YD, Chen JJ, Wu F, Liu HG, Zeng GY, Dai YC (2023) Species diversity of pathogenic wood-rotting fungi (Agaricomycetes, Basidiomycota) in China. Mycology 14(3): 204–226. https://doi.org/10.1080/21501203.2023.2238779
- Yurchenko E, Wu SH (2014) Three new species of *Hyphodontia* with peg-like hyphal aggregations. Mycological Progress 13(3): 533–545. https://doi.org/10.1007/s11557-013-0935-1
- Zhang QY, Liu HG, Papp V, Zhou M, Dai YC, Yuan Y (2023) New insights into the classification and evolution of *Favolaschia* (Agaricales, Basidiomycota) and its potential distribution, with descriptions of eight new species. Mycosphere 14(1): 777–814. https://doi.org/10.5943/mycosphere/14/1/10
- Zhao CL, Wu ZQ (2017) *Ceriporiopsis kunmingensis* sp. nov. (Polyporales, Basidiomycota) evidenced by morphological characters and phylogenetic analysis. Mycological Progress 16(1): 93–100. https://doi.org/10.1007/s11557-016-1259-8

- Zhao W, Zhao CL (2021) The phylogenetic relationship revealed three new wood-inhabiting fungal species from genus *Trechispora*. Frontiers in Microbiology 12: 650195. https://doi.org/10.3389/fmicb.2021.650195
- Zhao CL, Qu MH, Huang RX, Karunarathna SC (2023a) Multi-gene phylogeny and taxonomy of the wood-rotting fungal genus *Phlebia* sensu lato (*Polyporales, Basidiomycota*). Journal of Fungi (Basel, Switzerland) 9(3): 1–41. https://doi.org/10.3390/jof9030320
- Zhao H, Dai YC, Wu F, Liu XY, Maurice S, Krutovsky KV, Pavlov IN, Lindner DL, Martin FM, Yuan Y (2023b) Insights into the ecological diversification of the Hymenochaetales based on comparative genomics and phylogenomics. Genome Biology and Evolution 15(8): 1–15. https://doi.org/10.1093/gbe/evad136
- Zhou M, Dai YC, Vlasák J, Liu HG, Yuan Y (2023) Updated systematics of *Trichaptum* s.l. (Hymenochaetales, Basidiomycota). Mycosphere 14(1): 815–917. https://doi.org/10.5943/mycosphere/14/1/11



Research Article

Morphology and phylogeny of four new species within Polycephalomycetaceae (Hypocreales) parasitising *Ophiocordyceps* species

Zuoheng Liu^{1,2*©}, Dexiang Tang^{1,2*©}, Yingling Lu^{1,2*©}, Juye Zhu^{1,2®}, Lijun Luo^{1,2®}, Tao Sun^{1,2®}, Hong Yu^{1,2®}

1 Yunnan Herbal Laboratory, College of Ecology and Environmental Sciences, Yunnan University, Kunming, 650504, China

2 The International Joint Research Center for Sustainable Utilization of Cordyceps Bioresources in China and Southeast Asia, Yunnan University, Kunming, 650504, China

Corresponding author: Hong Yu (hongyu@ynu.edu.cn, herbfish@163.com)

Abstract

Species of the family Polycephalomycetaceae grow on insects or entomopathogenic fungi and are distributed from tropical to subtropical regions. This study proposed four new species of hyperparasitic fungi from China based on six molecular markers (ITS, SSU, LSU, TEF-1a, RPB1 and RPB2) phylogenetic analyses and morphological characteristics. The four new species, i.e. Pleurocordyceps litangensis, Polycephalomyces jinghongensis, Po. multiperitheciatae and Po. myrmecophilus, were described and illustrated. Pl. litangensis, exhibiting a hyperparasitic lifestyle on Ophiocordyceps sinensis, differed from *Pleurocordyceps* other species in producing subulate β-phialides and ovoid or elliptic a-conidia. Po. jinghongensis was distinct from Polycephalomyces other species, being parasitic on Ophiocordyceps sp., as producing oval or long oval-shaped α-conidia and columns of β-conidia. Po. multiperitheciatae differed from Polycephalomyces other species as having synnemata with fertile head, linear β-conidia and parasitic on Ophiocordyceps multiperitheciata. Po. myrmecophilus was distinct from Polycephalomyces other species, being parasitic on the fungus Ophiocordyceps acroasca, as producing round or ovoid α -conidia and elliptical β -conidia without synnemata from the colonies. These four species were clearly distinguished from other species in the family Polycephalomycetaceae by phylogenetic and morphological characteristics. The morphological features were discussed and compared to relevant species in the present paper.

Key words: entomogenous fungi, hyperparasite, micromorphology, phylogenetic analyses, taxonomy

Introduction

The new family Polycephalomycetaceae was established within clavicipitoid fungi to accommodate *Perennicordyceps*, *Pleurocordyceps* and *Polycephalomyces* based on morphology and phylogenetic analyses (Xiao et al. 2023). The genus *Polycephalomyces* Kobayasi was determined to be a monotypic anamorph genus for the species *Polycephalomyces* formosus Kobayasi (Kobayasi



Academic editor: Marc Stadler Received: 31 January 2024 Accepted: 5 April 2024 Published: 16 May 2024

Citation: Liu Z, Tang D, Lu Y, Zhu J, Luo L, Sun T, Yu H (2024) Morphology and phylogeny of four new species within Polycephalomycetaceae (Hypocreales) parasitising *Ophiocordyceps* species. MycoKeys 105: 179–202. https://doi. org/10.3897/mycokeys.105.119893

Copyright: © Zuoheng Liu 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).

^{*} These authors contributed equally to this work.

1941). In the later taxonomic treatment of this genus, Seifert (1985) accepted four species, i.e. Po. formosus, Po. ramosus (Peck) Mains, Po. cylindrosporus Samson & H.C. Evans and Po. tomentosus (Schrader) Seifert. Polycephalomyces ditmarii Van Vooren & Audibert has been described as the asexual morph of Ophiocordyceps ditmarii (Quél.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora (Van Vooren and Audibert 2005). Paecilomyces sinensis C.T. Chen, S.R. Xiao & Z.Y. Shi was recombined into Polycephalomyces sinensis (Q.T. Chen, S.R. Xiao & Z.Y. Shi) W.J. Wang, X.L. Wang, Y. Li, S.R. Xiao & Y.J. Yao (Wang et al. 2012). The taxon has had a long history of being recognised as incertae sedis in Hypocreales (Kepler et al. 2013; Matočec et al. 2014). Matočec et al. (2014) established the genus Perennicordyceps and separated it from Polycephalomyces to accommodate Perennicordyceps cuboidea, Pe. paracuboidea, Pe. prolifica and Pe. ryogamiensis. Perennicordyceps was characterised by acremonium-like and hirsutella-like asexual morphs and perithecia (Xiao et al. 2023). Pleurocordyceps was established by combining the species originally assigned to the Polycephalomyces. Pl. sinensis was designated as the type species of the genus Pleurocordyceps (Wang et al. 2021).

Species of Polycephalomycetaceae grow on insects or other fungi, particularly *Ophiocordyceps* species and are distributed from tropical to subtropical regions (Bischof et al. 2003; Wang et al. 2012, 2015a, b; Matočec et al. 2014; Crous et al. 2017; Xiao et al. 2018; Poinar and Vega 2020). Several species of Polycephalomycetaceae have also been reported as hyperparasitic fungi, involving species of *Cordyceps*, *Elaphomyces*, *Hirsutella*, *Myxomycetes* and *Ophiocordyceps* (Seifert 1985; Bischof et al. 2003; Wang et al. 2012, 2015a, b).

South-western China is an area of high fungal biodiversity (Hyde et al. 2018). The rich biodiversity uncovered suggested that further collections could result in the discovery of numerous new taxa (Hyde et al. 2020a, b). In this study, the four novel species presented herein were collected from Yunnan Province and Sichuan Province in China. Morphological observations and phylogenetic analyses showed that these four species were novel and distinct from all other previously-described species in the family Polycephalomycetaceae. The four new species were discovered to be hyperparasites of *Ophiocordyceps* species. Pl. litangensis, Po. jinghongensis, Po. multiperitheciatae and Po. myrmecophilus were hyperparasitic on O. sinensis, Ophiocordyceps sp., O. multiperitheciata and O. acroasca, respectively. At present, relatively little is known about the mechanisms responsible for hyperparasitism in species of the family Polycephalomycetaceae and our findings provide ideal material for these studies. These findings have expanded the diversity of fungal species in the family Polycephalomycetaceae, providing taxonomic data to support species resource conservation and rational exploitation and utilisation of resources.

Materials and methods

Specimens and isolates

Fungal specimens parasitising *Ophiocordyceps* sp. were collected from different regions of south-western China, including Sichuan Province (Litang County) and Yunnan Province (Jinghong City, Yuanyang County, Pu'er City). The specimens were found in moist soils. Geographic information (longitude, latitude
and altitude) of collection were recorded in the field, then specimens were collected in sterilised plastic containers and transported to the laboratory. The micro-morphological characters (Synnemata) were examined using an Olympus SZ61 stereomicroscope (Olympus Corporation, Tokyo, Japan). To obtain axenic culture, the stromata was divided into 2–4 segments with sterilised blades. Each segment was immersed in hydrogen peroxide 30% (H_2O_2) for 5 min and then rinsed five times in sterile water. After drying on sterilised filter paper, these segments were inoculated on Potato Dextrose Agar (PDA) plates. The conidial masses at the apex of the stipes were picked with an inoculating needle and immersed in 5 ml of sterilised water for blending. The homogenates were then spread on PDA plates containing 0.1 g/l streptomycin and 0.05 g/l tetracycline. The plates were maintained in a culture room at 25 °C. After purification, the cultures were stored at 4 °C (Wang et al. 2015a). Dry specimens were deposited in the Yunnan Herbal Herbarium (YHH) of Yunnan University. The cultures were stored in Yunnan Fungal Culture Collection (YFCC) of Yunnan University.

Morphological studies

Cultures on potato extract agar (PDA) were incubated for 21 days at 25 °C and photographed using a Canon 750 D camera (Canon Inc., Tokyo, Japan). For asexual morphological descriptions, microscope slide cultures were prepared by placing a small amount of mycelium on 5 mm diameter PDA medium blocks that were overlaid by a cover slip (Wang et al. 2015a; Tang et al. 2023b). The observations, measurements and photographs of the phialides and conidia were made using a light microscope (Olympus BX53).

DNA extraction, PCR and sequencing

DNA templates were obtained from cultures using the CTAB method, following that described in Liu et al. (2001). The polymerase chain reaction (PCR) was used to amplify genetic markers using the following primer pairs: ITS4/ITS5 for ITS (internal transcribed spacer gene region) (White et al. 1990), NS1/NS4 for SSU (small subunit ribosomal RNA gene region) (White et al. 1990), LR0R/LR5 for LSU (large subunit rRNA gene region) (Hopple 1994) 2218R/983F for *TEF-1a* (translation elongation factor 1-alpha gene region) (Rehner and Buckley 2005), CRPB1/RPB1Croph for *RPB1* (RNA polymerase II largest subunit gene region) (Castlebury et al. 2004; Araújo et al. 2018), fRPB2-7cR/fRPB2-5F for *RPB2* (RNA polymerase II second largest subunit) (Liu et al. 1999). A total of 25 μ I PCR matrix contained PCR 2.5 μ I Buffer (Transgen Biotech, Beijing, China), 17.25 μ I sterile water, 4 μ I dNTP, 1 μ I each forward and reverse primer, 0.25 μ I Taq DNA polymerase (Transgen Biotech, Beijing, China) and 1 μ I DNA template. The matrix and reactions conditions were prepared and performed according to the methods described in previous studies (Xiao et al. 2023).

Phylogenetic analysis

In order to construct a phylogeny of the major lineages in the family Polycephalomycetaceae, most of the DNA sequences used in this work were derived from previous phylogenetic studies (Xiao et al. 2023). Phylogenetic analyses were

based on sequences of six molecular markers (ITS, SSU, LSU, TEF-1a, RPB1 and RPB2), all of which were downloaded from NCBI (https://www.ncbi.nlm.nih. gov/). Then the nucleotide sequences were combined with those generated in our study (Table 1). Sequences were aligned using ClustalX v.2.0 (Larkin et al. 2007), adjusted manually and then concatenated in BioEdit v.7.1.1 (Hall 1999). Poorly-aligned regions were removed and adjusted manually using MEGA6 (v.6.0) (Tamura et al. 2013). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fitting likelihood model for the Maximum likelihood (ML) analyses and the Bayesian inference (BI) analyses were carried out for the fungi datasets. For ML analyses, tree searches were performed in IQ-tree (v.2.1.3) (Nguyen et al. 2015), based on the best-fit model GTR+F+I+I+R3 with 5000 ultrafast bootstraps (Hoang et al. 2017) in a single run. The BI search was according to the best-fit model GTR+F+I+G4, resorting to MrBayes (v.3.2.2) for BI analysis (Ronquist et al. 2012). The phylogenetic trees constructed using the ML and the BI analyses were largely congruent and strongly supported in most branches (Fig. 1). The final phylogenetic tree was visualised with its Maximum-Likelihood bootstrap proportions (ML-BS) and Bayesian posterior probability (BI-BPP) performed using FigTree v.1.4.2 and edited via Adobe Illustrator CS6.

Table 1	. Sources	of s	selected	isolates	and	GenBank	accession	number	for	ITS	and	five	genes	of	three	genera	within
Polycep	halomyce	tace	eae were	used in	this s	study.											

Species name	Voucher	ITS	SSU	LSU	TEF-1a	RPB1	RPB2	References
Cordyceps pleuricapitata	NBRC 109979	AB925941		AB925978				Unpublished
Cordyceps pleuricapitata	NBRC 109978	AB925940		AB925977				Unpublished
Cordyceps pleuricapitata	NBRC 109977	AB925939		AB925976				Unpublished
Cordyceps pleuricapitata	NBRC 100746	JN943306	JN941749	JN941392	KF049680	JN992483	KF049668	Kepler et al. (2013)
Cordyceps pleuricapitata	NBRC 100745	JN943304	JN941750	JN941391	KF049679			Kepler et al. (2013)
Perennicordyceps elaphomyceticola	MFLU 21-0262	0Q172064	0Q172101	0Q172032	OQ459718	OQ459747	OQ459792	Xiao et al. (2023)
Perennicordyceps cuboidea	NBRC 100941	JN943329	JN941725	JN941416		JN992459		Schoch et al. (2012)
Perennicordyceps cuboidea	NBRC 103834	JN943330	JN941723	JN941418		JN992457		Schoch et al. (2012)
Perennicordyceps cuboidea	NBRC 103835	JN943333	JN941722	JN941419		JN992456		Schoch et al. (2012)
Perennicordyceps elaphomyceticola	MFLU 21-0264	OQ172067	OQ172103	OQ172035	OQ459720	OQ459749	OQ459794	Xiao et al. (2023)
Perennicordyceps elaphomyceticola	MFLU 21-0266	OQ172068	0Q172112	OQ172036	OQ459732	OQ459760	OQ459806	Xiao et al. (2023)
Perennicordyceps elaphomyceticola	MFLU 21-0263	OQ172065	0Q172102	OQ172033	OQ459719	OQ459748	OQ459793	Xiao et al. (2023)
Perennicordyceps elaphomyceticola	NTUCC 17-022	MK840824		MK840813	MK839230	MK839221	MK839212	Yang et al. (2020)
Perennicordyceps lutea	KUMCC 3004			0Q474910				Xiao et al. (2023)
Perennicordyceps paracuboidea	NBRC 100942	JN943337	JN941711	JN941430		JN992445	AB972958	Schoch et al. (2012)
Perennicordyceps prolifica	NBRC 103839	JN943342	JN941706	JN941435		JN992440		Schoch et al. (2012)
Perennicordyceps prolifica	NBRC 103838	JN943339	JN941707	JN941434		JN992441		Schoch et al. (2012)
Perennicordyceps prolifica	TNS-F-18547	KF049660	KF049613	KF049632	KF049687	KF049649	KF049670	Kepler et al. (2013)

Species name	Voucher	ITS	SSU	LSU	TEF-1a	RPB1	RPB2	References
Perennicordyceps prolifica	TNS-F-18481	KF049659	KF049612	KF049631	KF049686	KF049648		Kepler et al. (2013)
Perennicordyceps ryogamiensis	NBRC 101751	JN943343	JN941703	JN941438	KF049688	JN992437		Schoch et al. (2012)
Perennicordyceps ryogamiensis	NBRC 103837	JN943346	JN941702	JN941439		JN992436		Schoch et al. (2012)
Perennicordyceps ryogamiensis	NBRC 103842	JN943345	JN941701	JN941440		JN992435		Schoch et al. (2012)
Pleurocordyceps parvicapitata	MFLU 21-0270	0Q172082	0Q172105	0Q172054	0Q459722	0Q459751	OQ459796	Xiao et al. (2023)
Pleurocordyceps agarica	YHHPA 1305 [⊤]	KP276651	KP276655		KP276659	KP276663	KP276667	Wang et al. (2015b)
Pleurocordyceps agarica	YHCPA1307	KP276654	KP276658		KP276662	KP276666	KP276670	Wang et al. (2015b)
Pleurocordyceps agarica	YHCPA 1303	KP276653	KP276657		KP276661	KP276665	KP276669	Wang et al. (2015b)
Pleurocordyceps aurantiaca	MFLUCC 17- 2113 ^T	MG136916	MG136904	MG136910	MG136875	MG136866	MG136870	Xiao et al. (2019)
Pleurocordyceps aurantiaca	MFLUCC 17- 2114	MG136917	MG136905	MG136911	MG136874		MG136871	Xiao et al. (2019)
Pleurocordyceps aurantiaca	MFLU 17-1394	MG136918	MG136906	MG136912	MG136876	MG136867	MG136872	Xiao et al. (2019)
Pleurocordyceps aurantiaca	MFLU 17-1393 ^T		MG136907	MG136913	MG136877	MG136868	MG136873	Xiao et al. (2019)
Pleurocordyceps ramosus like	NBRC 101760	MN586827	MN586818	MN586836	MN598051	MN598042	MN598060	Wang et al. (2020)
Pleurocordyceps ramosus like	NBRC 109984	MN586828	MN586819	MN586837	MN598052	MN598043		Wang et al. (2020)
Pleurocordyceps ramosus like	NBRC 109985	MN586829	MN586820	MN586838	MN598053	MN598044		Wang et al. (2020)
Pleurocordyceps heilongtanensis	KUMCC 3008	OQ172091	0Q172111	OQ172063	OQ459731	OQ459759	OQ459805	Xiao et al. (2023)
Pleurocordyceps kanzashianus		AB027371	AB027325	AB027371				Nikoh et al. (2000)
Pleurocordyceps lanceolatus	GACP 17-2004 [⊤]	OQ172076	OQ172110	OQ172046	OQ459726	0Q459754	OQ459800	Xiao et al. (2023)
Pleurocordyceps lanceolatus	GACP 17-2005 [⊤]		OQ172109	0Q172047	0Q459727	OQ459755	OQ459801	Xiao et al. (2023)
Pleurocordyceps lianzhouensis	HIMGD20918 [⊤]	EU149921	KF226245	KF226246	KF226248	KF226247		Zhang et al. (2007)
Pleurocordyceps lianzhouensis	GIMYY9603	EU149922	KF226249	KF226250	KF226252	KF226251		Zhang et al. (2007)
Pleurocordyceps marginaliradians	MFLU 17-1582 ^T	MG136920	MG136908	MG136914	MG136878	MG136869	MG271931	Xiao et al. (2019)
Pleurocordyceps marginaliradians	MFLUCC 17- 2276 ^T	MG136921	MG136909	MG136915	MG136879		MG271930	Xiao et al. (2019)
Pleurocordyceps nipponica	BCC 1682	KF049664	KF049620	KF049638	KF049694			Kepler et al. (2013)
Pleurocordyceps nipponica	BCC 18108	KF049657	MF416624	MF416569	MF416517	MF416676	MF416462	Kepler et al. (2013)
Pleurocordyceps nipponica	NBRC 101407	JN943302	JN941752	JN941389		JN992486		Schoch et al. (2012)
Pleurocordyceps nipponica	NBRC 101405	JN943442	JN941754	JN941387		JN992488		Schoch et al. (2012)
Pleurocordyceps nipponica	BCC 2325	KF049665	KF049622	KF049640	KF049696	KF049655	KF049677	Kepler et al. (2013)
Pleurocordyceps nipponica	NHJ 4268		KF049621	KF049639	KF049695	KF049654	KF049676	Kepler et al. (2013)
Pleurocordyceps nipponica	BCC 1881		KF049618	KF049636	KF049692		KF049674	Kepler et al. (2013)
Pleurocordyceps nutansis	GACP 19-1906	OQ172079	0Q172117	OQ172049	OQ459737	OQ459763	OQ459809	Xiao et al. (2023)
Pleurocordyceps nutansis	GACP 19-1907	OQ172087	OQ172118	OQ172059	OQ459738	0Q459764	OQ459810	Xiao et al. (2023)
Pleurocordyceps nutansis	GACP 19-3019 [⊤]	OQ172086	OQ172120	OQ172058	OQ459740	OQ459766	0Q459812	Xiao et al. (2023)
Pleurocordyceps nutansis	MFLU 21-0275 [⊤]	OQ172073	0Q172119	0Q172048	OQ459739	OQ459765	OQ459811	Xiao et al. (2023)
Pleurocordyceps onorei	BRA CR23904	KU898843						Crous et al. (2017)
Pleurocordyceps onorei	BRA CR23902 [™]	KU898841						Crous et al. (2017)
Pleurocordyceps ophiocordycipiticola	MFLUCC 22- 0187	NR185465		NG229093				Wei et al. (2022)

Species name	Voucher	ITS	SSU	LSU	TEF-1a	RPB1	RPB2	References
Pleurocordyceps ophiocordycipiticola	MFLU:22-0265	0Q127364	0Q127326	0Q127397	OQ186388	OQ186435		Wei et al. (2022)
Pleurocordyceps parvicapitata	MFLU 21-0271 ^T	OQ172083	0Q172106	OQ172055	OQ459723	0Q459752	OQ459797	Xiao et al. (2019)
Pleurocordyceps parvicapitata	MFLU 21-0272	0Q172084	OQ172099	OQ172056	OQ459716	OQ459745	OQ459790	Xiao et al. (2023)
Pleurocordyceps parvicapitata	MFLU 21-0273	OQ172085	0Q172100	0Q172057	0Q459717	0Q459746	OQ459791	Xiao et al. (2023)
Pleurocordyceps phaothaiensis	BCC84553 [™]	MF959733		MF959737	MF959742	MF959745		Crous et al. (2017)
Pleurocordyceps phaothaiensis	BCC84552	MF959732		MF959736	MF959740	MF959744		Crous et al. (2017)
Pleurocordyceps phaothaiensis	BCC84551	MF959731		MF959735	MF959739	MF959743		Crous et al. (2017)
Pleurocordyceps ramosopulvinata	EFCC 5566			KF049627	KF049682	KF049645		Kepler et al. (2013)
Pleurocordyceps ramosopulvinata	SU 65			DQ118742	DQ118753	DQ127244		Chaverri et al. (2005)
Pleurocordyceps sinensis	ARSEF 1424	KF049661	KF049615	KF049634	KF049689		KF049671	Kepler et al. (2013)
Pleurocordyceps sinensis	CN 80-2 [⊤]	HQ832884	HQ832887	HQ832886	HQ832890	HQ832888	HQ832889	Wang et al. (2012)
Pleurocordyceps sinensis	HMAS 43720 [⊤]	NR119928		NG042573				Wang et al. (2012)
Pleurocordyceps sinensis	MFLU 21-0269	OQ172080	0Q172122	OQ172050	0Q459742	OQ459768		Xiao et al. (2023)
Pleurocordyceps sinensis	GACP 20-2305	0Q172075	OQ172108	0Q172045	0Q459725	0Q459753	OQ459799	Xiao et al. (2023)
Pleurocordyceps sinensis	GACP 20-2304	0Q172074	0Q172107	0Q172044	0Q459724		OQ459798	Xiao et al. (2023)
Pleurocordyceps sinensis	GZU 20-0865	OQ172071	OQ172096	0Q172043	OQ459713			Xiao et al. (2023)
Pleurocordyceps sinensis	MFLU 21-0268	OQ172070	0Q172123	0Q172052	OQ459743		OQ459815	Xiao et al. (2023)
Pleurocordyceps sinensis	MFLU 21-0267		0Q172121	OQ172051				Xiao et al. (2023)
Pleurocordyceps sinensis	MFLU 18-0162	MK863250	MK863043	MK863050	MK860188			Unpublished
Pleurocordyceps sp.	BCC 2637	KF049663		KF049637	KF049693		KF049675	Kepler et al. (2013)
Pleurocordyceps sp.	JB07.08. 16_8	KF049662	KF049616	KF049635	KF049690	KF049652	KF049672	Kepler et al. (2013)
Pleurocordyceps sp.	JB07.08.17_07b		KF049617		KF049691	KF049653	KF049673	Kepler et al. (2013)
Pleurocordyceps sp.	NBRC 109987			AB925983				Unpublished
Pleurocordyceps sp.	NBRC 109988			AB925984				Unpublished
Pleurocordyceps sp.	NBRC 109990			AB925968				Unpublished
Pleurocordyceps sp.	NBRC 110224			AB925969				Unpublished
Pleurocordyceps litangensis	YFCC 06109293	PP410597	PP541902	PP410593	PP550103	PP697751		This study
Pleurocordyceps litangensis	YFCC 06109294	PP410598	PP541903	PP410594	PP550104	PP697752	PP550107	This study
Pleurocordyceps litangensis	YFCC 06109295	PP410600	PP541905	PP410596	PP550106	PP697754		This study
Pleurocordyceps litangensis	YFCC 06109296	PP410599	PP541904	PP410595	PP550105	PP697753	PP550108	This study
Pleurocordyceps sp.	GIMCC 3.570		JX006097	JX006098	JX006100	JX006101		Zhong et al. (2016)
Pleurocordyceps tomentosus	BL4	KF049666	KF049623	KF049641	KF049697	KF049656	KF049678	Kepler et al. (2013)
Pleurocordyceps vitellina	KUMCC 3005	OQ172088		0Q172060	OQ459728	OQ459756	OQ459802	Xiao et al. (2023)
Pleurocordyceps vitellina	KUMCC 3006	OQ172089		0Q172061	OQ459729	0Q459757	OQ459803	Xiao et al. (2023)
Pleurocordyceps vitellina	KUMCC 3007	OQ172090		OQ172062	OQ459730	OQ459758	OQ459804	Xiao et al. (2023)
Pleurocordyceps yunnanensis	YHCPY1005	KF977848			KF977850	KF977852	KF977854	Wang et al. (2015a)
Pleurocordyceps yunnanensis	YHHPY1006 [™]	KF977849			KF977851	KF977853	KF977855	Wang et al. (2015a)
Polycephalomyces albiramus	GACP 21-XS08 [™]	0Q172092	0Q172115	0Q172037	OQ459735	0Q459761	OQ459807	Xiao et al. (2023)
Polycephalomyces albiramus	GACPCC 21-XS08 [⊤]	OQ172093	OQ172116	OQ172038	OQ459736	OQ459762	OQ459808	Xiao et al. (2023)
Polycephalomyces formosus	NBRC 109993 [™]	MN586833	MN586824	MN586842	MN598057	MN598048	MN598064	Wang et al. (2021)
Polycephalomyces formosus	NBRC 109994	MN586834	MN586825	MN586843	MN598058	MN598049	MN598065	Wang et al. (2021)
Polycephalomyces formosus	NBRC 109995	MN586835	MN586826	MN586844	MN598059	MN598050	MN598066	Wang et al. (2021)

Species name	Voucher	ITS	SSU	LSU	TEF-1a	RPB1	RPB2	References
Polycephalomyces formosus	GACP 21- WFKQ04	OQ172095	0Q172114	OQ172040	OQ459734			Xiao et al. (2023)
Polycephalomyces jinghongensis	YFCC 02959283	PP274089	PP274093	PP274109	PP581803	PP697747	PP581819	This study
Polycephalomyces jinghongensis	YFCC 02959284	PP274090	PP274094	PP274110	PP581804	PP697748	PP581820	This study
Polycephalomyces jinghongensis	YFCC 02959285	PP274091	PP274095	PP274111	PP581805	PP697749	PP581821	This study
Polycephalomyces jinghongensis	YFCC 02959286	PP274092	PP274096	PP274112	PP581806	PP697750	PP581822	This study
Polycephalomyces multiperitheciatae	YFCC 06149287	PP274102	PP274108	PP274118	PP581802		PP581818	This study
Polycephalomyces multiperitheciatae	YFCC 06149288	PP274098	PP274104	PP274114	PP581798	PP697743	PP581815	This study
Polycephalomyces multiperitheciatae	YFCC 06149289	PP274101	PP274107	PP274117	PP581801	PP697746	PP581817	This study
Polycephalomyces multiperitheciatae	YFCC 06149290	PP274097	PP274103	PP274113	PP581797	PP697742	PP581814	This study
Polycephalomyces multiperitheciatae	YFCC 06149291	PP274100	PP274106	PP274116	PP581800	PP697745		This study
Polycephalomyces multiperitheciatae	YFCC 06149292	PP274099	PP274105	PP274115	PP581799	PP697744	PP581816	This study
Polycephalomyces myrmecophilus	YFCC 09289443	PP410602	PP410608	PP410605	PP581795	PP697740	PP581812	This study
Polycephalomyces myrmecophilus	YFCC 09289444	PP410603	PP410609	PP410606	PP581796	PP697741	PP581813	This study
Polycephalomyces myrmecophilus	YFCC 09289445	PP410601	PP410607	PP410604	PP581794	PP697739	PP581811	This study
Tolypocladium ophioglossoides	NBRC 100998	JN943319	JN941735	JN941406	AB968602	JN992469	AB968563	Ban et al. (2015)
Tolypocladium ophioglossoides	NBRC 106330	JN943321	JN941734	JN941407	AB968603	JN992468	AB968564	Ban et al. (2015)

Results

Phylogenetic tree

Sequences of 113 samples were used for phylogenetic analysis. Tolypocladium ophioglossoides (NBRC 106330) and T. ophioglossoides (NBRC 100998) were designated as the outgroup taxa (Xiao et al. 2023). The total length of the concatenated dataset of six genes across the 113 samples was 6384 bp, including 859 bp for ITS, 1548 bp for SSU, 930 bp for LSU, 1037 bp for TEF-1a, 797 bp for RPB1 and 1213 bp for RPB2. The phylogenetic relationships show three major clades within the family Polycephalomycetaceae (Fig. 1), consisting of the clade Pleurocordyceps (16 species; BS = 100%, BPP = 1.00), the clade Perennicordyceps (6 species; BS = 100%, BPP = 1.00) and the clade Polycephalomyces (6 species; BS = 100%, BPP = 1.00). Pleurocordyceps nutansis, Pleurocordyceps sinensis (MFLU 21-0268, GZU 20-0865) are adjacent clades. Similarly, Pleurocordyceps ramosus like and Pleurocordyceps yunnanensis are contiguous branches. In addition, Pleurocordyceps kanzashianus is included in the clade Pleurocordyceps nipponica. Cordyceps pleuricapitata strains also formed a monophyletic clade (BS = 100%, BPP = 1.00). The four species collected and described in this work are clustered in the clade Pleurocordyceps (Pl. litangensis) and the clade Polycephalomyces (Po. jinghongensis, Po. multiperitheciatae and Po. myrmecophilus), respectively.



Figure 1. Phylogenetic tree of Polycephalomycetaceae, based on the concatenation of ITS, SSU, LSU, *TEF-1a*, *RPB1* and *RPB2* sequence data. The tree was generated from an alignment of 6,384 sites and 113 taxa. The phylogeny was inferred using the IQ-tree. The Maximum likelihood bootstrap values greater than 75% (on the left) and the Bayesian posterior probabilities over 0.75 (on the right) were indicated above the nodes. The new species were indicated in back bold font.

Taxonomy

Pleurocordyceps litangensis Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov.

MycoBank No: 851497 Fig. 2

Etymology. litangensis = Litang County, the epithet referred to the nature study trail in Litang County, the locality where the type specimen was collected.

Diagnosis. Pleurocordyceps litangensis and Pl. sinensis have the same host (*O. sinensis*) and β -Conidia, but the phialides (lanceolate or narrowly lageniform vs. spear point or subulate), α -conidia (Ovoid vs. Ovoid or ellipticare) are different.

Holotype. China, Sichuan Province, Ganzi Tibetan Autonomous Prefecture, Litang County, parasitic on *Ophiocordyceps sinensis* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 30°43'00"N, 100°52'00"E, alt. 4750 m, 10 June 2023, Hong Yu bis (YHH 2306055).

Sexual morph. Undetermined.

Asexual morph. Synnemata arising from the stromata of O. sinensis, solitary or alternating; clavate or spatulate, branched and unbranched, straight or sinuous. Terminal portion of a synnemata covered by a viscous mass, khaki. Colonies on PDA growing slowly, attaining a diameter of 1.4-1.6 cm in 3 weeks at 25 °C, filiform, dark yellow and reverse dry yellow. Phialides existing in two types: α - and β -phialides. Both types of phialides often reproduce new phialides at their own apices and yield catenulate β-conidia, collarettes not flared, periclinal thickening not visible. a-phialides acropleurogenous solitary on hyphae; spear point, tapering gradually from the base to the apex, 11.2–12.8 μ m long, 1.9–2.6 μ m wide at the base and 0.7–0.9 μ m wide at the apex. β-phialides terminal on solitary on hyphae; subulate, tapering abruptly from the base to the apex, 9.9-27.8 µm long, 1.6-2.5 µm wide at the base and 0.6–1.4 μ m wide at the apex. α -conidia ovoid or elliptic and occurring on the final portion of synnemata, $3.2-6.1 \times 1.8-3.9 \mu m$; β -conidia fusiform, and produce on the surface mycelium of colony, multiple, usually in chains on a phialide, 3.5–6.1 × 1.4–2.5 µm.

Host. Parasitic on Ophiocordyceps sinensis (Ophiocordycipitaceae).

Distribution. China, Sichuan Province.

Material examined. China, Sichuan Province, Ganzi Tibetan Autonomous Prefecture, Litang County, parasitic on *Ophiocordyceps sinensis* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 30°43'00"N, 100°52'00"E, alt. 4750 m, 10 June 2023, Tao Sun. Paratypes: YHH 2306058; other collections: YHH 2306059; Culture ex-type: YFCC 06109293; Other living cultures: YFCC 06109294, YFCC 06109295, YFCC 06109296.

Notes. Four strains, *Pleurocordyceps* sp. NBRC109990, *Pl.* sp. NBRC109987, *Pl.* sp. NBRC110224, *Pl.* sp. NBRC109988, were aggregated *Pl. litangensis* into one branch (Fig. 1 BS = 100%, BPP = 1.00). *Pl. litangensis* was distinct from other species of *Pleurocordyceps* by α -phialides spear point, β -phialides subulate, α -conidia ovoid or elliptic (Table 2). Thus, *Pl. litangensis* was introduced as a new species under the genus *Pleurocordyceps*.



Figure 2. Morphological features of *Pleurocordyceps litangensis* (Holotype: YHH 2306055) **a** overview of *Pleurocordyceps litangensis* and its host **b** synnemata on the insects **c**, **d** colony obverse and reverse **e**–**h**, **k** α -phialides **i** α -conidia **j** β -conidia and β -phialides. Scale Bars: 2 cm (**a**–**d**); 20 μ m (**e**–**j**); 10 μ m (**k**); 5 μ m (**g**–**i**).

Species	Host	Synnemata	Phialides	Conidia	References
Pl. agarica	<i>Ophiocordyceps</i> sp. or melolonthid larvae	Solitary, unbranched, agaricshaped; conidial mass pileus-like, light yellow to pale brown	α-phialides lanceolate; β-phialides narrowly lageniform or subulate	α-conidia globose to subglobose; β-conidia fusiform, catenate or clump together	Wang et al. (2015b)
Pl. aurantiacus	Coleoptera larvae or O. barnesii	Emerging after 30 days, solitary or not solitary, branched or unbranched, showing 1–2 radiating ring like distributions	α-phialides, narrowly lageniform. β-phialides, lanceolate or narrowly lageniform	α-conidia, globose to subglobose. β-conidia, fusiform	Xiao et al. (2018)
Pl. lanceolatus	Lepidoptera larvae	Lanceolate to corniform, solitary to crowded, stipitate, usually unbranched, rarely branched on the PDA, yellow to yellowish on the fresh specimen, covered with conidial masses, white on the PDA	α-phialides directly from hyphae, solitary, usually unbranched, subulate, at the base, tapering into a long neck; β-phialides branched into 2 or 3 phial ides, narrowly lageniform to lanceolate	α-conidia spherical, forming slimy conidial masses along the Synnemata; β-conidia fusiform	Xiao et al. (2023)
Pl. marginaliradians	Cossidae larva	Emerging after 14 days, single or branched into 2 or 3 branched, showing 1–2 radiating ring like distributions	α-phialides, elongate lageniform; β-phialides, narrow slender to narrow lageniform	α-conidia globose, catenate, one-celled, pale yellow slimy in mass. β-conidia fusiform, one-celled	Xiao et al. (2018)
Pl. parvicapitata	Perennicordyceps elaphomyceticola	Absent	Phialides, cylindrical at the base, tapering into a long neck	globose to subglobose	Xiao et al. (2023)
Pl. sinensis	Lepidoptera larvae or Ophiocordyceps sinensis	Solitary, crowded, branched or unbranched, conidial mass yellow or yellow-orange	Lanceolate or narrowly lageniform	α-conidia, ovoid; β-conidia, fusiform	Chen et al. (1984); Wang et al. (2012)
Pl. vitellina	Ophiocordyceps nigrella	Absent	α-phialides, hyaline, smooth, elongated lageniform, crowed, gathered in the middle of colony. β-phialides, hyaline, smooth, directly growing from hyphae, with or without metula at the base, solitary, lanceolate, ovate at the base, tapering into a short neck	α-conidia spherical, one- celled, smooth-walled. β-conidia fusiform, catenulate	Xiao et al. (2023)
Pl. yunnanensis	Hemiptera adults or Ophiocordyceps nutans	Solitary, caespitose or crowded, branched or unbranched; conidial mass white to yellow– brown	α-phialides cylindrical to subulate; β-phialides narrowly lageniform or subulate	α-conidia subglobose, ellipsoidal; β-conidia fusiform, catenate or clump together	Wang et al. (2015a)
Pl. nutansis	Ophiocordyceps nutans	Cylindrical, clavate, capitate, stipitate, crowded, simple, white to yellowish	Two types, both of the types observed on the same synnemata. α-phialides, gathered at the apex of the synnemata, arranged in a parallel palisade-like layer around the apex of the fertile head, hyaline, usually branched into 2–6 phialides, narrowly slender lanceolate; β-phialides , solitary, scattered along the stipe, lanceolate, ovate at the base, tapering into a long neck	α-conidia, spherical, forming slimy conidial masses on the fertile head; β-conidia fusiform, produced along stipe of the synnemata	Xiao et al. (2023)
Pl. heilongtanensis	<i>Ophiocordyceps</i> sp.	Scattered on the surface of host, cylindrical, stipitate, unbranched, white, with or without fertile head	a-phialides, hyaline, smooth, elongated lageniform, caespitose, palisade-like, crowed, gathered in the top of synnemata, mostly branched into 2-4 phialides. β-phialides hyaline, smooth, solitary, branched into 2 or 3 phial ides, with or without metula at the base, directly growing from hyphae	α-conidia, subglobose to ovoid,in yellowish slimy mass. β-conidia fusiform, one-celled	Xiao et al. (2023)
Pl. lianzhouensis	Lepidoptera larva or Ophiocordyceps crinalis	Unbranched or dichotomously branched, conidial mass not seen	In whorls or intercalary and terminal, terminally awl-shaped	Ellipsoidal, oblong to cylindrical	Wang et al. (2014)
Pl. litangensis	Ophiocordyceps sinensis	Absent	α-phialides acropleurogenous solitary on hyphae; spear point. β-phialides terminal on solitary on hyphae; subulate	α-conidia ovoid or elliptical; β-conidia fusiform	This study

Table 2. Morphological comparison of asexual morph species of Pleurocordyceps.

Polycephalomyces jinghongensis Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov. MycoBank No: 851498

Fig. 3

Etymology. jinghongensis = Jinghong City, the epithet referred to the nature study trail in Jinghong City, the locality where the type specimen was collected.

Diagnosis. Polycephalomyces jinghongensis are similar to that of Po. multiperitheciatae regarding the production of α -conidia oval, but Po. jinghongensis differ by synnemata caespitose, white to orange-yellow colour, producing cylindrical β -conidia, parasitic on Ophiocordyceps sp.

Holotype. China, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong City, parasitic on *Ophiocordyceps* sp. (Ophiocordycipitaceae), on insects buried in soil, with erect synnemata, 23°47'9"N, 102°51'41"E, alt. 2053 m, 25 September 2022, Hong Yu bis (YHH 2206047).

Sexual morph. Undetermined.

Asexual morph. Synnemata arising from the stromata of Ophiocordyceps sp., 0.8-1.6 cm long 0.1-0.3 cm thick, caespitose, unbranched or branched, white to orange-yellow colour. Colonies on PDA growing slowly, attaining a diameter of 1.3–1.7 cm in 3 weeks at 25 °C, clustered, white and reverse dry yellow. Synnemata emerging after 14 days, tufted, branched and 0.6-10 mm long, showing radiating distributions. Phialides existing in two types: a- and β-phialides. Both types of phialides often reproduce new phialides at their own apices or sides, collarettes not flared, periclinal thickening not visible. a-phialides verticillate and acropleurogenous on conidiophores and solitary on hyphae; lanceolate, tapering gradually from the base to the apex, 4.5-19.5 μ m long, 1.4–2.5 μ m wide at the base and 0.8–1.6 μ m wide at the apex. β -phialides acropleurogenous in whorls of 2-3 or intercalary and terminal on conidiophores and solitary on hyphae; diamond-shaped; tapering abruptly from the base to the apex, 10.4-17.5 µm long, 1.1-2.7 µm wide at the base, and 0.4-1.1 µm wide at the apex. α-conidia oval or long oval shape and occurring in the conidial mass on the agar or on the final portion of synnemata, 1.1-3.4 \times 0.8–1.9 µm; β -conidia columns and produced on the surface mycelium of colony, multiple, usually formed as spore balls at the phialidic apex, 2.3-3.1 × 1.2-1.3 µm.

Host. Parasitic on *Ophiocordyceps* sp. (Ophiocordycipitaceae).

Distribution. China, Yunnan Province.

Material examined. China, Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Jinghong City, parasitic on *Ophiocordyceps* sp. (Ophiocordycipitaceae), on insects buried in soil, with erect synnemata, 23°47'9"N, 102°51'41"E, alt. 2053 m, 25 September 2022, D.X. Tang. Paratypes: YHH 2206010; other collections: YHH 2207049; YHH 2206053. Culture ex-type: YFCC 02959283; Other cultures: YFCC 02959284, YFCC 02959285, YFCC 02959286.

Notes. Polycephalomyces jinghongensis was sister to Po. multiperitheciatae (Fig. 1: BS = 100%, BPP = 1.00). However, Po. multiperitheciatae differs by 6/556 bp in ITS, 3/898 bp in SSU, 2/829 bp in LSU, 23/913 bp in *TEF-1a*, 4/679 bp in *RPB2* from Po. jinghongensis. Po. jinghongensis was distinct from other species of Polycephalomyces by the white to orange-yellow colour of the caespitose synnemata (Table 3). Thus, Po. jinghongensis was introduced as a new species under the genus Polycephalomyces.



Figure 3. Morphological features of *Polycephalomyces jinghongensis* (Holotype: YHH 2209031) **a** overview of *Polycephalomyces jinghongensis* and its host **b** synnemata on the insect **c**, **d** colony obverse and reverse **e**–**g** β -phialides **h** β -conidia **i**, **k**, **l** α -phialides **j** α -conidia. Scale Bars: 2 cm (**a**, **c**, **d**); 0.5 cm (**b**); 20 µm (**e**–**h**, **j**); 10 µm (**i**, **k**. **l**).

Polycephalomyces multiperitheciatae Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov. MycoBank No: 851499 Fig. 4

Etymology. The species name referred to the host species, *Ophiocordyceps multiperitheciata*.

Diagnosis. Polycephalomyces multiperitheciatae are similar to that of Po. *jinghongensis* regarding the production of α-conidia oval, but Po. *jinghongensis*



Figure 4. Morphological features of *Polycephalomyces multiperitheciatae* (Holotype: YHH 2206047) **a** overview of *Polycephalomyces multiperitheciatae* and its host **b** Synnemata on the insect **c**, **d** colony obverse and reverse **g**, **j** α -phialides **e**, **f**, **h**, **i** β -phialides **k** α -conidia **l** β -conidia. Scale Bars: 2 cm (**a**, **c**, **d**); 0.6 cm (**b**); 20 µm (**e**-**i**, **k**); 50 µm (**j**); 10 µm (**l**).

differ by being parasitic on *O. multiperitheciata*, synnemata clustered, white, β-conidia, linear.

Holotype. China, Yunnan Province, Honghe Hani and Yi Autonomous Prefecture, Yuanyang County, parasitic on *Ophiocordyceps multiperitheciata* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 22°1'51"N, 100°52'42"E, alt. 703 m, 25 September 2022, Hong Yu bis (YHH 2206031).

Sexual morph. Undetermined.

Asexual morph. Synnemata arising from the stromata of *Ophiocordyceps multiperitheciata*, 0.8–1.8 cm long 0.2–0.5 cm thick, clustered, white to pale yellow, numerous, branched, with fertile head. Colonies on PDA growing slowly,

attaining a diameter of 1.8–2.1 cm in 3 weeks at 25 °C, clustered, white and reverse dry yellow. Synnemata emerging after 15 days, solitary, branched and 0.8–2.1 cm long, showing radiating distributions. Phialides existing in two types: α - and β -phialides. Both types of phialides often reproduce new conidia at their own apices or sides, collarettes not flared, periclinal thickening not visible. α -phialides verticillate and acropleurogenous on conidiophores and solitary on hyphae; spear point, tapering gradually from the base to the apex, 10.5–18.7 µm long, 1.1–1.9 µm wide at the base and 0.4–0.6 µm wide at the apex. β -phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; subulate, tapering abruptly from the base to the apex, 11.3–28.8 µm long, 1.2–2.5 µm wide at the base and 0.5–1.1 µm wide at the apex. α -conidia,oval and occurring in the conidial mass on the agar or on the final portion of synnemata, 0.6–1.1 × 0.3–0.6 µm; β -conidia, linear and produced on the surface mycelium of colony, multiple, usually formed as spore balls at the phialidic apex, 0.8–1.3 × 0.3–0.7 µm.

Host. Parasitic on *Ophiocordyceps multiperitheciata* (Ophiocordycipitaceae). **Distribution.** China, Yunnan Province.

Material examined. China, Yunnan Province, Honghe Hani and Yi Autonomous Prefecture, Yuanyang County, parasitic on *Ophiocordyceps multiperitheciata* (Ophiocordycipitaceae), on insects buried in soil, with erect stromata, 22°1'51"N, 100°52'42"E, alt. 703 m, 25 September 2022, D.X. Tang. Paratypes: YHH 2209032; other collections: YHH 2209033; YHH 2209034. Culture extype: YFCC 06149287; Other cultures: YFCC 06149288, YFCC 06149289, YFCC 06149290, YFCC 06149291, YFCC 06149292.

Notes. Polycephalomyces multiperitheciatae is sister to Po. jinghongensis (Fig. 1: BS = 100%, BPP = 1.00). Po. multiperitheciatae is distinct from other species of Polycephalomyces, parasitising Ophiocordyceps multiperitheciata synnemata clustered, with fertile head, β -conidia, linear (Table 3). Thus, Po. multiperitheciatae was introduced as a new species under the genus Polycephalomyces.

Polycephalomyces myrmecophilus Hong Yu bis, Z.H. Liu & D.X. Tang, sp. nov. MycoBank No: 851500 Fig. 5

Etymology. myrmecophilus = myrmecophilous, the epithet referred to the species parasitising myrmecophilous *Ophiocordyceps* species.

Diagnosis. Polycephalomyces myrmecophilus are similar to that of Po. ramosus regarding the production of two types of conidia, but Po. myrmecophilus differ by α -conidia round or ovoid, β -conidia elliptical.

Holotype. China, Yunnan Province, Pu'er City, The Sun River National Forest Park, parasitic on *Ophiocordyceps acroasca* (Ophiocordycipitaceae), on insects underside of leaves, with erect stromata, 30°34'34"N, 101°6'24"E, alt. 1095 m, 28 September 2020, Hong Yu bis (YHH 2009001);

Sexual morph. Undetermined.

Asexual morph. Synnemata arising from the *Ophiocordyceps acroasca* or *Colobopsis* sp. corpses, tomentose, white. Colonies on PDA growing slowly, attaining a diameter of 1.7-2.1 cm in 3 weeks at 25 °C, villous, cinerous, and reverse black yellow. Phialides existing in two types: α - and β -phialides. Both types of phialides



Figure 5. Morphological features of *Polycephalomyces myrmecophilus* (Holotype: YHH 2009001) **a** overview of *Polycephalomyces myrmecophilus* and its host **b**, **c** colony obverse and reverse **d** α -phialides **f**-**h** β -phialides **e** α -conidia **i** β -conidia. Scale Bars: 2 cm (**a**-**c**); 20 µm (**d**-**h**); 10 µm (**i**).

often reproduce new phialides at their own apices, collarettes not flared, periclinal thickening not visible. α -phialides verticillate and acropleurogenous on conidiophores and solitary on hyphae; lanceolate, tapering gradually from the base to the apex, 6.1–14.5 µm long, 1.4–2.3 µm wide at the base and 0.8–1.8 µm wide at the apex. β -phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; sickle-shaped, tapering abruptly from the base to the apex, 9.8–17.6 µm long, 0.9–1.6 µm wide at the base and 0.4–1.1 µm wide at the apex. α -conidia round or ovoid, and occurring in the conidial mass on the agar or on the final portion of synnemata, 0.4–0.9 × 0.3–0.9 µm; β -conidia elliptical and produced on the surface mycelium of colony, single or multiple, usually in the form of spore balls at the phialidic apex, 0.6–1.3 × 0.3–0.8 µm.

Species	Host	Synnemata	Phialides	Conidia	References
Do olhiromuo	Caullotolog.op	Symemata Stinitoto gothorod	Phielidea parrowly subulate owl shaped	Conidio ovlindrigal to	Vice et el
ro. aibitattius	(Orthoptera, Gryllotalpidae)	branched, white to pale yellow, numerous, cylindrical and tapering at the apex, without fertile head	Fritanues nariowy subulate, awrshapeu	obovoid or subglobose	(2023)
Po. baltica	Nymph or short- winged female bark louse (Psocoptera: Troctopsocidae)	Synnemata, simple, roundish	Phialides, light colored, micronematous, flask-shaped	Conidia globose, catenulate	Poinar and Vega (2020)
Po. cylindrosporus	Coleoptera, Formicidae and Pentatomidae	Synnemata cylindrical to capitate, stipitate, slender, branched	Phialides on verticils and/or acropleurogenously forming loosely arranged flared hymenia	Conidia one-type, cylindrical to bacilliform	Matočec et al. (2014)
Po. ditmarii	Paravespula vulgaris (Wasp)	Synnemata 2 to 3 distinct branches, yellowish to white, darkening at the base; surmounted by a small subsurface capitulum, dotted with numerous small blisters of orange-yellow colour	Phialides elongate, cylindrical, attenuating at the apex	globose to subglobose	Van Vooren and Audibert (2005)
Po. formosus (Type)	Coleoptera larvae or Ophiocordyceps barnesii	Synnemata 2 long, gathered, branched, with cylindrical stipe, with fertile head, spherical, white	cylindrical, tapering gradually	Conidia one-type, ellipsoid or ovoid	Kobayasi (1941)
	In culture (PDA)	Synnemata 2–3 branches,arising as several radiating rings on the colony	Phialides terminal parts of Synnemata, cylindrical to subulate at the base;	Conidia of one type, one- celled, smooth-walled, ellipsoid to ovoid, arising in a conidial mass on the agar or on the terminal portions of synnemata	Wang et al. (2021)
	In slide culture		Phialides monothetic and solitary or acropleurogenous in the whorls of 1–4, narrowly lageniform or subulate	Conidia obovoid to oblong ellipsoidal or cylindrical, forming irregular spore balls near the apex of phialides	Wang et al. (2021)
Po. ramosus	Lepidoptera larvae or Hirsutella guignardii	Synnemata solitary, crowded or caespitose, unbranched or branched, conidial mass yellow to orange-yellow	α-phialides cylindrical to narrowly lageniform; β-phialides narrowly lageniform or subulate	α-conidia, ovoid; β-conidia, fusiform	Seifert (1985); Bischof et al. (2003)
Po. paludosus	Lepidoptera larva	Capitate, cinnamon brown, branched, the branches at right angles	Subulate, phialides occurring scattered on the branches below the heads, ventricose, occasionally stellate above	Conidia produced singly, hyaline, obovoid, covered by agglutinated mucus	Mains (1948)
Po. tomentosus	Myxomycetes	Fructification a synnemata		Conidia three-type, globose or ellipsoidal or cylindrical	Seifert (1985)
Po. jinghongensis	Ophiocordyceps sp. (Ophiocordycipitaceae)	Synnemata caespitose, unbranched or branched, white to orange-yellow colour	 α-phialides verticillate and acropleurogenous on conidiophores, and solitary on hyphae; lanceolate. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; diamond-shaped. 	α-conidia oval or long oval shape, β-conidia cylindrical	This study
Po. multiperitheciatae	Ophiocordyceps multiperitheciata	Synnemata white to pale yellow, numerous, branched, with fertile head	a-phialides verticillate and acropleurogenous on conidiophores, and solitary on hyphae; spear point. β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; subulate.	α-conidia oval β-conidia linear	This study
Po. myrmecophilus	Ophiocordyceps acroasca and Ophiocordyceps sp.	Absent	a-phialides verticillate and acropleurogenous on conidiophores, and solitary on hyphae; lanceolate, β-phialides acropleurogenous in whorls of 2–3 or intercalary and terminal on conidiophores and solitary on hyphae; sickle shape.	α-conidia round or ovoid; β-conidia, elliptical	This study

Table 3. Morphology of asexual morph species of the genus Polycephalomyces.

Host. Parasitic on *Ophiocordyceps acroasca* and *Ophiocordyceps* sp. **Distribution.** China, Yunnan Province.

Material examined. China, Yunnan Province, Pu'er City, The Sun River National Forest Park, parasitic on *Ophiocordyceps acroasca* (Ophiocordycipitaceae), on insects underside of leaves, with erect stromata, 30°34'34"N, 101°6'24"E, alt. 1095 m, 28 September 2020, D.X. Tang. Paratype: YHH 2006020. Culture ex-type: YFCC 09289443; Other cultures: YFCC 09289444.

Notes. Polycephalomyces myrmecophilus was sister to Cordyceps pleuricapitata (Fig.1: BS = 100%, BPP = 1.00). Po. myrmecophilus was distinct from other species of Polycephalomyces, being parasitic on Ophiocordyceps acroasca and Ophiocordyceps sp. and producing β -phialides sickle-shaped, α -conidia round or ovoid, β -conidia elliptical (Table 3). Thus, Po. myrmecophilus was introduced as a new species under the genus of Polycephalomyces.

Discussion

Our taxonomic investigations revealed four new species of the family Polycephalomycetaceae, Pl. litangensis, Po. jinghongensis, Po. multiperitheciatae and Po. myrmecophilus. Morphological observations suggested that four species have sufficient morphological differences to justify their segregation into four species. A new species, Pl. litangensis, was described in the genus Pleurocordyceps. Pleurocordyceps litangensis was similar to Pl. agaricus, Pl. aurantiacus, Pl. lanceolatus, Pl. marginaliradians, Pl. sinensis, Pl. vitellina, Pl. yunnanensis, Pl. nutansis and Pl. heilongtanensis, by producing two types of conidia, while Pl. Parvicapitata and Pl. lianzhouensis had only one type of conidia. Pl. litangensis was distinct from other species of *Pleurocordyceps*, with having α -phialides spear point, β -phialides subulate, a-conidia ovoid or elliptic. Moreover, Pl. litangensis and Pl. sinensis both had the same host (O. sinensis) and β -Conidia, but their phialides, α -conidia size and shape were different (Table 2). Herein, we described three new species, namely, Po. jinghongensis, Po. multiperitheciatae and Po. myrmecophilus, enriching the species diversity in the genus Polycephalomyces. Six additional species are included in this genus (Table 1): Polycephalomyces baltica (Poinar and Vega 2020), Po. cylindrosporus (Matočec et al. 2014), Po. ditmarii (Van Vooren and Audibert, 2005), Po. paludosus (Mains 1948), Po. ramosus (Seifert 1985; Bischof et al. 2003) and Po. tomentosus (Seifert 1985). These species either lacked molecular data or their updated strain descriptions did not match those of the protologue (Wang et al. 2021). These three new species were similar to Po. ramosus, producing two types of conidia, while Po. baltica, Po. cylindrosporus, Po. ditmarii, Po. paludosus and Po. albiramus (Xiao et al. 2023) had only one type of conidia. Po. jinghongensis was distinct from Po. ramosus, being parasitic on Ophiocordyceps sp. producing longer a-conidia oval or long oval shape and β-conidia columns. Po. multiperitheciatae differed from Po. ramosus, being parasitic on O. multiperitheciata, having synnemata with fertile head and β -conidia linear. Po. myrmecophilus was distinguished from Po. ramosus, being parasitic on the fungus O. acroasca, producing synnemata, α -conidia round or ovoid, and β -conidia elliptical, without producing synnemata from the colonies, whereas Po. ramosus was parasitic on Lepidoptera larvae or *Hirsutella quignardii*, with α -conidia ovoid and β -conidia fusiform (Table 3).

Some species of the family Polycephalomycetaceae have been reported from more than one host, indicating their non-host specific nature (Bischof et al. 2003; Wang et al. 2012, 2015a, b; Matočec et al. 2014; Crous et al. 2017; Xiao et al. 2018). *Pl. lianzhouensis* (Wang et al. 2014) was found to parasitise insects along with the species of the genus *Ophiocordyceps*. The field investigation and studies showed that *Pl. litangensis* also parasitised *O. sinensis*, a phenomenon known as hyperparasitism. Most species of the genus *Polycephalomyces* parasitise insects in the orders Coleoptera and Hemiptera, and we have already discovered that *Po. jinghongensis*, *Po. multiperitheciatae* and *Po. myrmecophilus* are hyperparasitic on the species of *Ophiocordyceps*, expanding the diversity of hosts in *Polycephalomyces*. In subsequent studies, we should delve deeper into the ecological habits and hyperparasitic phenomena of the family Polycephalomycetaceae, explore the evolutionary relationship between hyperparasitic species and entomophytic fungi and promote their development and utilisation.

Xiao et al. (2023) introduced Pl. nutansis as a new species under the genus Pleurocordyceps. However, Pl. sinensis and Pl. nutansis were found to be grouped together in the phylogenetic tree, which may be the reason and they are sister taxa to each other. Similarly, molecular phylogenetic analysis has shown that Pl. nipponica and Pl. kanzashianus are clustered together. Nevertheless, Wang et al. (2021) pointed out that they were distinct species, based on their sexual morphology characteristics. In addition, Wang et al. (2021) noted the description of the spore type of Pl. lianzhouensis was not clear and future research should strengthen the observation of its asexual morphology to determine its more accurate classification position. Cordyceps pleuricapitata has formed a monophyletic branch in the genus Polycephalomyces. Xiao et al. (2023) noted the paratype of C. pleuricapitata lacks molecular data and the two strains (NBRC 100745, NBRC 100746) named C. pleuricapitata for which there are molecular data lack morphological information. Hence, it was not possible to clarify the precise position of C. pleuricapitata and its classification at this time. These classifications issues require further research. Phylogeny based on our concatenated data also supported that our four new species belonged to the family Polycephalomycetaceae and were distinct from each other (Fig. 1). Four strains, namely, Pleurocordyceps sp. NBRC109990, Pleurocordyceps sp. NBRC109987, Pleurocordyceps sp. NBRC110224 and Pleurocordyceps sp. NBRC109988 and Pl. litangensis were aggregated into one branch. However, the four strains had only LSU sequences in the NCBI database and were classified as undefined species in Pleurocordyceps incertae sedis. Future research will require additional morphological and phylogenetic work to clarify their taxonomic status.

Acknowledgements

We thank the National Natural Science Foundation of China (No. 31760011). We thank all those who have provided assistance for this work. Participation and sponsorship of the Yunnan University Professional Degree Graduate Practice Innovation Fund Program (ZC-22222937).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by National Natural Science Foundation of China (No.31760011). Participation and sponsorship of the Yunnan University Professional Degree Graduate Practice Innovation Fund Program (ZC-22222937).

Author contributions

Zuoheng Liu: Mainly responsible for article conception, writing and editing and also mainly responsible for species identification (contributed equally to this work); Dexiang Tang: Mainly responsible for article conception writing and editing, morphological analysis and phylogenetic analysis(contributed equally to this work); Yingling Lu: Mainly responsible for article conception, and also responsible for experimental guidance and design; Responsible for the language polishing and format modification.Juye Zhu: Collecting the information of specimens and GenBank entry number required for research. Lijun Luo; Tao Sun: Responsible for picture editing and processing. Hong Yu: Investigation, responsible for the review and modification of the article, and conducting project administration and supervision.

Author ORCIDs

Zuoheng Liu [®] https://orcid.org/0000-0003-4118-3694 Dexiang Tang [®] https://orcid.org/0000-0002-7662-224X Yingling Lu [®] https://orcid.org/0009-0008-8119-1975 Juye Zhu [®] https://orcid.org/0000-0002-4184-5646 Lijun Luo [®] https://orcid.org/0000-0002-1709-0781 Tao Sun [®] https://orcid.org/0000-0001-7837-2101 Hong Yu [®] https://orcid.org/0000-0002-2149-5714

Data availability

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

References

- Araújo JPM, Evans HC, Kepler R, Hughes DP (2018) Zombie-ant fungi across continents: 15 new species and new combinations within *Ophiocordyceps* I. *Myrmecophilous hirsutelloid* species. Studies in Mycology 90(1): 119–160. https://doi.org/10.1016/j. simyco.2017.12.002
- Ban S, Sakane T, Nakagiri A (2015) Three new species of *Ophiocordyceps* and overview of anamorph types in the genus and the family *Ophiocordyceptaceae*. Mycological Progress 14(1): 1017. https://doi.org/10.1007/s11557-014-1017-8
- Bischof JF, Sullivan RF, Hywel-Jones NL, White JF (2003) Resurrection of Blistum tomentosum and its exclusion from Polycephalomyces (Hyphomycetes, Deuteromycota) based on 28S rDNA sequence data. Mycotaxon 86: 433–444. https://doi. org/10.1007/s00572-003-0226-9
- Castlebury LA, Rossman AY, Sung GH, Hyten AS, Spatafora JW (2004) Multigene phylogeny reveals new lineage for *Stachybotrys chartarum*, the indoor air fungus. Mycological Research 108(8): 864–872. https://doi.org/10.1017/S0953756204000607

- Chen QT, Xiao SR, Shi ZY (1984) *Paecilomyces sinensis* sp. nov. and its connection with *Cordyceps sinensis*. Acta Mycologica Sinica 3(1): 24–28. https://api.semanticscholar.org/CorpusID:87314004
- Chaverri P, Bischof JF, Evans HC, Hodge KT (2005) *Regiocrella*, a new entmopathogenic genus with a pycnidial anamorph and its phylogenetic placement in the Clavicipitaceae. Mycologia 97(6): 1225–1237. https://doi:10.3852/mycologia.97.6.1225
- Crous PW, Wingfeld MJ, Burgess TI, Carnegie AJ, Hardy GESJ, Smith D, Summerel BA, Cano-Lira JF, Guarro J, Houbraken J, Lombard L, Martín MP, Sandoval-Denis M, Alexandrova AV, Barnes CW, Baseia IG, Bezerra JDP, Guarnaccia V, May TW, Hernández-Restrepo M, Stchigel AM, Miller AN, Ordoñez ME, Abreu VP, Accioly T, Agnello C, Agustin Colmán A, Albuquerque CC, Alfredo DS, Alvarado P, Araújo-Magalhães GR, Arauzo S, Atkinson T, Barili A, Barreto RW, Bezerra JL, Cabral TS, Camello Rodríguez F, Cruz RHSF, Daniëls PP, da Silva BDB, de Almeida DAC, de Carvalho Júnior AA, Decock CA, Delgat L, Denman S, Dimitrov RA, Edwards J, Fedosova AG, Ferreira RJ, Firmino AL, Flores JA, García D, Gené J, Góis JS, Gomes AAM, Gonçalves CM, Gouliamova DE, Groenewald M, Guéorguiev BV, Guevara-Suarez M, Gusmão LFP, Hosaka K, Hubka V, Huhndorf SM, Jadan M, Jurjević Ž, Kraak B, Kučera V, Kumar TKA, Kušan I, Lacerda SR, Lamlertthon S, Lisboa WS, Loizides M, Luangsa-ard JJ, Lysková P, Mac Cormack WP, Macedo DM, Machado AR, Malysheva EF, Marinho P, Matočec N, Meijer M, Mešić A, Mongkolsamrit S, Moreira KA, Morozova OV, Nair KU, Nakamura N, Noisripoom W, Olariaga I, Oliveira RJV, Paiva LM, Pawar P, Pereira OL, Peterson SW, Prieto M (2017) Fungal Planet description sheets: 625-715. Persoonia 39: 270-467. https://doi. org/10.3767/persoonia.2017.39.11
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41(41): 95–98. https://doi.org/10.1021/bk-1999-0734.ch008
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518– 522. https://doi.org/10.1093/molbev/msx281
- Hyde KD, Norphanphoun C, Chen J, Dissanayake AJ, Doilom M, Hongsanan S, Jayawardena RS, Jeewon R, Perera RH, Thongbai B, Wanasinghe DN, Wisitrassameewong K,Tibpromma S, Stadler M (2018) Thailand's amazing diversity: up to 96% of fungi in northern Thailand may be novel. Fungal Diversity 93: 215–239. https://link.springer. com/article/10.1007/s13225-018-0415-7
- Hopple JS (1994) Phylogenetic investigations in the genus *Coprinus* based on morphological and molecular characters. Ph.D. Dissertation, Duke University, Durham, NC, USA.
- Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ, Jones EBG, Bundhun D, Chen YJ, Bao DF, Boonmee S, Calabon MS, Chaiwan N, Chethana KWT, Dai DQ, Dayarathne MC, Devadatha B, Dissanayake AJ, Dissanayake LS, Doilom M, Dong W, Fan XL, Goonasekara ID, Hongsanan S, Huang SK, Jayawardena RS, Jeewon R, Karunarathna A, Konta S, Kumar V, Lin CG, Liu JK, Liu NG, Luangsa-ard J, Lumyong S, Luo ZL, Marasinghe DS, McKenzie EHC, Niego AGT, Niranjan M, Perera RH, Phukhamsakda C, Rathnayaka AR, Samarakoon MC, Samarakoon SMBC, Sarma SIC, Shang QJ, Stadler M, Tibpromma S, Wanasinghe DN, Wei DP, Wijayawardene NN, Xiao YP, Yang J, Zeng XY, Zhang SN, Xiang MM (2020a) Refined families of Sordariomycetes. Mycosphere 11(1): 305–1059. https://doi.org/10.5943/mycosphere/11/1/7
- Hyde KD, Dong Y, Phookamsak R, Jeewon R, Bhat JD, Jones EBG, Liu NG, Abeywickrama PD, Mapook A, Wei DP, Perera RH, Manawasinghe IS, Pem D, Bundhun D, Karunarathna

A, Ekanayaka AH, Bao DF, Li JF, Samarakoon MC, Napalai C, Li GJ, Phutthacharoen K, Zhang SN, Senanayake IC, Goonasekara ID, Thambugala KM, Phukhamsakda C, Tennakoon DS, Jiang HB, Yang J, Zeng M, Huanraluek N, Liu JK, Wijesinghe SN, Tian Q, Tibpromma S, Brahmanage RS, Boonmee S, Huang SK, Thiyagaraja Vi Lu YZ, Jayawardena RS, Dong W, Yang EF, Singh SK, Singh SM, Rana S, Lad SS, Anand G, Bandarupalli D, Niranjan M, Sarma VV, Liimatainen K, Aguirre-Hudson B, Niskanen T, Overall A, Alvarenga RLM, Gibertoni TB, Pfiegler WP, Horváth E, Imre A, Alves AL, da Silva Santos AC, Tiago PV, Bulgakov TS, Wanasinghe DN, Bahkali AH, Doilom M, Elgorban AM, Maharachchikumbura SSN, Rajeshkumar KC, Haelewaters D, Mortimer PE, Zhao Q, Lumyong S, Xu JC, Sheng J (2020b) Fungal diversity notes 1151–1276: Taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Diversity 100(1): 5–277. https://doi.org/10.1007/s13225-020-00439-5

- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Kepler R, Ban S, Nakagiri A, Bischof J, Hywel-Jones N, Owensby CA, Spatafora JW (2013) The phylogenetic placement of hypocrealean insect pathogens in the genus *Polycephalomyces*: An application of One Fungus One Name. Fungal Biology 117(9): 611–622. https://doi.org/10.1016/j.funbio.2013.06.002
- Kobayasi Y (1941) The genus *Cordyceps* and its allies. Science Reports of the Tokyo Bunrika Daigaku 5: 53–260.
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics (Oxford, England) 23(21): 2947–2948. https://doi.org/10.1093/bioinformatics/btm404
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Molecular biology and Evolution 16: 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092
- Liu ZY, Liang ZQ, Whalley AJS, Yao YJ, Liu AY (2001) *Cordyceps brittlebankisoides*, a new pathogen of grubs and its anamorph, *Metarhizium anisopliae* var. majus. Journal of Invertebrate Pathology 78(3): 178–182. https://doi.org/10.1006/jipa.2001.5039
- Mains EB (1948) Entomogenous fungi. Mycologia 40(4): 402–416. https://doi.org/10.1 080/00275514.1944.12017718
- Matočec N, Kušan I, Ozimec R (2014) The genus *Polycephalomyces* (Hypocreales) in the frame of monitoring Veternica cave (Croatia) with a new segregate genus *Perennicordyceps*. Ascomycete.Org : Revue Internationale pour la Taxinomie des Ascomycota 6(5): 125–133.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Nikoh N, Fukatsu T (2000) Interkingdom host jumping underground: Phylogenetic analysis of entomoparasitic fungi of the genus *Cordyceps*. Molecular Biology and Evolution 17(4): 629–638. https://doi.org/10.1093/oxfordjournals.molbev.a026341
- Poinar G, Vega FE (2020) Entomopathogenic fungi (Hypocreales: Ophiocordycipitaceae) infecting bark lice (Psocoptera) in Dominican and Baltic amber. Mycology 11(1): 71–77. https://doi.org/10.1080/21501203.2019.1706657
- Rehner SA, Buckley E (2005) A *beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversifcation and links to *Cordyceps teleomorphs*. Mycologia 97(1): 84–98. https://doi.org/10.3852/mycologia.97.1.84

- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, Miller AN, Wingfield MJ, Aime MC, An K-D, Bai F-Y, Barreto RW, Begerow D, Bergeron M-J, Blackwell M, Boekhout T, Bogale M, Boonyuen N, Burgaz AR, Buyck B, Cai L, Cai Q, Cardinali G, Chaverri P, Coppins BJ, Crespo A, Cubas P, Cummings C, Damm U, de Beer ZW, de Hoog GS, Del-Prado R, Dentinger B, Diéguez-Uribeondo J, Divakar PK, Douglas B, Dueñas M, Duong TA, Eberhardt U, Edwards JE, Elshahed MS, Fliegerova K, Furtado M, García MA, Ge Z-W, Griffith GW, Griffiths K, Groenewald JZ, Groenewald M, Grube M, Gryzenhout M, Guo L-D, Hagen F, Hambleton S, Hamelin RC, Hansen K, Harrold P, Heller G, Herrera C, Hirayama K, Hirooka Y, Ho H-M, Hoffmann K, Hofstetter V, Högnabba F, Hollingsworth PM, Hong S-B, Hosaka K, Houbraken J, Hughes K, Huhtinen S, Hyde KD, James T, Johnson EM, Johnson JE, Johnston PR, Jones EBG, Kelly LJ, Kirk PM, Knapp DG, Kõljalg U, Kovács GM, Kurtzman CP, Landvik S, Leavitt SD, Liggenstoffer AS, Liimatainen K, Lombard L, Luangsa-ard JJ, Lumbsch HT, Maganti H, Maharachchikumbura SSN, Martin MP, May TW, McTaggart AR, Methven AS, Meyer W, Moncalvo J-M, Mongkolsamrit S, Nagy LG, Nilsson RH, Niskanen T, Nyilasi I, Okada G, Okane I, Olariaga I, Otte J, Papp T, Park D, Petkovits T, Pino-Bodas R, Quaedvlieg W, Raja HA, Redecker D, Rintoul TL, Ruibal C, Sarmiento-Ramírez JM, Schmitt I, Schüßler A, Shearer C, Sotome K, Stefani FOP, Stenroos S, Stielow B, Stockinger H, Suetrong S, Suh S-O, Sung G-H, Suzuki M, Tanaka K, Tedersoo L, Telleria MT, Tretter E, Untereiner WA, Urbina H, Vágvölgyi C, Vialle A, Vu TD, Walther G, Wang Q-M, Wang Y, Weir BS, Weiß M, White MM, Xu J, Yahr R, Yang ZL, Yurkov A, Zamora J-C, Zhang N, Zhuang W-Y, Schindel D (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America 109(16): 6241-6246. https://doi.org/10.1073/pnas.1117018109
- Seifert KA (1985) A monograph of *Stilbella* and some allied *Hyphomycetes*. Studies in Mycology 27: 1–235. https://doi.org/10.2307/3807446
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197
- Tang DX, Huang O, Zou WQ, Wang YB, Wang Y, Dong QY, Sun T, Yang G, Yu H (2023b) Six new species of zombie-ant fungi from Yunnan in China. IMA Fungus 14(1): 1–9. https://doi.org/10.1186/s43008-023-00114-9
- Van Vooren N, Audibert C (2005) Révision du complexe Cordyceps sphecocephala 1re partie: Les guêpes végétales. Bulletin Mensuel de la Societe Linneenne de Lyon 74(7): 221–254. https://doi.org/10.3406/linly.2005.13604
- Wang WJ, Wang XL, Li Y, Xiao SR, Kepler RM, Yao YJ (2012) Molecular and morphological studies of *Paecilomyces sinensis* reveal a new clade in clavicipitaceous fungi and its new systematic position. Systematics and Biodiversity 10(2): 221–232. https://doi.org/10.1080/14772000.2012.690784
- Wang L, Li HH, Chen YQ, Zhang WM, Qu LH (2014) Polycephalomyces lianzhouensis sp. nov., a new species, co-occurs with Ophiocordyceps crinalis. Mycological Progress 13(4): 1089–1096. https://doi.org/10.1007/s11557-014-0996-9
- Wang YB, Yu H, Dai YD, Chen ZH, Zeng WB, Yuan F, Liang ZQ (2015a) Polycephalomyces yunnanensis (Hypocreales), a new species of Polycephalomyces parasitiz-

ing *Ophiocordyceps nutans* and stink bugs (hemipteran adults). Phytotaxa 208(1): 34–44. https://doi.org/10.11646/phytotaxa.208.1.3

- Wang YB, Yu H, Dai YD, Wu CK, Zeng WB, Yuan F, Liang ZQ (2015b) *Polycephalomyces agaricus*, a new hyperparasite of *Ophiocordyceps* sp. infecting melolonthid larvae in southwestern China. Mycological Progress 14(9): 1–9. https://doi.org/10.1007/s11557-015-1090-7
- Wang YB, Wang Y, Fan Q, Duan DE, Zhang GD, Dai RQ, Dai YD, Zeng WB, Chen ZH, Li DD, Tang DX, Xu ZH, Sun T, Nguyen TT, Tran NL, Dao VM, Zhang LD, Liu YJ, Zhang XM, Yang DR, Sanjuan T, Liu XZ, Yang ZL, Yu H (2020) Multigene phylogeny of the family *Cordycipitaceae* (*Hypocreales*): New taxa and the new systematic position of the Chinese cordycipitoid fungus *Paecilomyces hepiali*. Fungal Diversity 103(1): 1–46. https://doi.org/10.1007/s13225-020-00457-3
- Wang YH, Sayaka B, Wang WJ, Li Y, Wang K, Kirk PM, Bushley KE, Dong CH, Hawksworth DL, Yao YJ (2021) *Pleurocordyceps* gen. nov. for a clade of fungi previously included in *Polycephalomyces* based on molecular phylogeny and morphology. Journal of Systematics and Evolution 59(5): 1065–1080. https://doi.org/10.1111/jse.12705
- Wei DP, Gentekaki E, Wanasinghe DN, Tang SM, Hyde KD (2022) Diversity, molecular dating and ancestral characters state reconstruction of entomopathogenic fungi in Hypocreales. Mycosphere : Journal of Fungal Biology 13(2): 281–351. https://doi. org/10.5943/mycosphere/si/1f/8
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: a Guide to methods and applications 18: 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Xiao YP, Wen TC, Hongsanan S, Jeewon R, Luangsa-ard JJ, Brooks S, Wanasinghe DN, Long FY, Hyde KD (2018) Multigene phylogenetics of *Polycephalomyces* (Ophiocordycipitaceae, Hypocreales), with two new species from Thailand. Scientific Reports 8(1): 18087. https://doi.org/10.1038/s41598-018-36792-4
- Xiao YP, Hongsanan S, Hyde KD, Brooks S, Xie N, Long FY, Wen TC (2019) Two new entomopathogenic species of *Ophiocordyceps* in Thailand. MycoKeys 47: 53–74. https://doi.org/10.3897/mycokeys.47.29898
- Xiao YP, Wang YB, Hyde KD, Eleni G, Sun JZ, Yang Y, Meng J, Yu H, Wen TC (2023) Polycephalomycetaceae, a new family of clavicipitoid fungi segregates from Ophiocordycipitaceae. Fungal Diversity 120(2): 1–76. https://doi.org/10.1007/s13225-023-00517-4
- Yang JI, Stadler M, Chuang WY, Wu S, Ariyawansa HA (2020) In vitro inferred interactions of selected entomopathogenic fungi from Taiwan and eggs of *Meloidogyne gram-inicola*. Mycological Progress 19(1): 97–109. https://doi.org/10.1007/s11557-019-01546-7
- Zhong X, Li SS, Peng QY, Zhang JS, Kan XT, Zhang J, Kan X, Liu X (2016) A *Polycephalomyces* hyperparasite of *Ophiocordyceps sinensis* leads to shortened duration of production and reduced numbers of host ascospores. Fungal Ecology 21: 24–31. https://doi.org/10.1016/j.funeco.2016.03.002
- Zhang WM, Wang L, Tao MH, Chen YQ, Qu LH (2007) Two species of *Cordyceps* simultaneously parasitic on a larva of Lepidoptera. Mycosystema 26: 7–21.



Research Article

Unveiling species diversity within the family Conidiobolaceae (Entomophthorales) in China: Descriptions of two new species and reassessment of the taxonomic position of *Conidiobolus polyspermus*

Yong Nie^{1,2}, Ying Yin¹, Heng Zhao³, XiaoYong Liu⁴, Bo Huang¹

1 Anhui Provincial Key Laboratory for Microbial Pest Control, Anhui Agricultural University, Hefei 230036, China

2 School of Civil Engineering and Architecture, Anhui University of Technology, Ma, anshan 243002, China

3 Institute of Microbiology, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China

4 College of Life Sciences, Shandong Normal University, Jinan 250014, China

Corresponding authors: Bo Huang (bhuang@ahau.edu.cn); XiaoYong Liu (liuxy@sdnu.edu.cn)

Abstract

In the present study, two new *Conidiobolus* s.s. species were described relying on the morphological studies and phylogenetic analysis utilizing nuclear large subunit of rDNA (nucLSU), mitochondrial small subunit of rDNA (mtSSU), and elongation-factor-like gene (*EFL*) sequences. *Conidiobolus jiangxiensis* **sp. nov.** is distinguished by its short primary conidiophores, a feature not commonly observed in other *Conidiobolus* s.s. species. Conversely, *Conidiobolus marcoconidius* **sp. nov.** is characterized by larger primary conidia and the emergence of 2–5 secondary conidia from each branched secondary conidiophores. Additionally, the taxonomic reassessment of *C. polyspermus* confirms its distinct status within the genus *Conidiobolus* s.s. Moreover, molecular analyses, incorporating the nucLSU, mtSSU, and *EFL* sequences, provide robust support for the phylogenetic placement of the two newly described species and the taxonomic identity of *C. polyspermus*. This investigation contributes valuable insights into the species diversity of *Conidiobola-ceae* in China, enhancing our understanding of the taxonomy within this fungal family.

Key words: Basal fungi, EFL, mtSSU, new species, nucLSU, taxonomic position

Introduction

The reclassification of conidiobolus-like fungi into three families based on phylogenetic and morphological evidence led to the establishment of the family *Conidiobolaceae* (*Entomophthorales*), housing three genera i.e. *Azygosporus* B. Huang & Y. Nie, *Conidiobolus* s.s. B. Huang & Y. Nie, and *Microconidiobolus* B. Huang & Y. Nie (Gryganskyi et al. 2022).Members of this family primarily exhibit a saprobic lifestyle, thriving in soil and plant debris. However, exceptions exist, with *C. coronatus* distinguished for its capacity to infect both insects and humans, and *C. lunulus* being isolated from leafcutter ants in Argentina (Goffre et al. 2020; Möckel et al. 2022).



Academic editor: Kerstin Voigt Received: 27 December 2023 Accepted: 30 April 2024 Published: 22 May 2024

Citation: Nie Y, Yin Y, Zhao H, Liu X, Huang B (2024) Unveiling species diversity within the family Conidiobolaceae (Entomophthorales) in China: Descriptions of two new species and reassessment of the taxonomic position of *Conidiobolus polyspermus*. MycoKeys 105: 203–216. https://doi.org/10.3897/ mycokeys.105.117871

Copyright: © Yong Nie 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). A comprehensive taxonomy of conidiobolus-like fungi has identified *Conidiobolus* s.s. as distinct from four other genera (Nie et al. 2020a, b, 2023; Cai et al. 2021). However, the synonymy of *C. megalotocus* (= *C. polyspermus*, = *C. eurypus*), introduced by King (1976b), remains unexplored, and requiring re-evaluation. Additionally, our previous study confirmed the synonymy of *C. firmipilleus* (= *C. chlamydosporus*). Thus, this study aims to add taxonomic clarity by re-evaluating the aforementioned synonym and introducing additional taxonomic taxa.

Previous phylogenetic analyses revealed two main clades within *Conidiobolus* s.s. when Capillidiaceae and Neoconidiobolaceae species were used as outgroups (Nie et al. 2020a, b; Gryganskyi et al. 2022). However, distinctive traits within each clade were not elucidated. Notably, both clades included species lacking microspore observations, such as *C. iuxtagenitus*, *C. margaritatus*, *C. lichenicolus*, *C. taihushanensis*, *C. dabieshanensis*, and *C. longiconidiophorus* (Srinivasan and Thirumalachar 1968; Waters and Callaghan 1989; Huang et al. 2007; Nie et al. 2017, 2020b, 2023). Particularly, *C. iuxtagenitus*, potentially forming a separate lineage, produces fusiform secondary conidia, and its zygospores form via a short beak near lateral conjugation (Waters and Callaghan 1989; Nie et al. 2023). Regrettably, molecular data for *C. margaritatus* are currently unavailable, leaving unanswered questions about the relationships among these morphologically distinct species and the possibility of undiscovered lineages within this fungal group. Resolving these issues requires additional members for phylogenetic and morphological studies.

Over the last decades, only six new *Conidiobolus* s.s. species and three new records were reported from China (Wang et al. 2010; Nie et al. 2017, 2020b, 2023). Additionally, the understanding of phylogenetic relationships within the accepted species of *Azygosporus* and *Microconidiobolus* remains limited. Consequently, a comprehensive exploration of the species diversity within *Conidiobolaceae* is imperative to unravel the relationships within this intricate fungal group. This article aims to identify two new *Conidiobolus* s.s. species using morphological characters and phylogenetic analyses of nucLSU, mtSSU, and *EFL* sequences. Simultaneously, the taxonomic status of *C. polyspermus* will be clarified.

Materials and methods

Isolation and morphology

Plant debris and soil samples were collected from Dashushan and Binhu National Forest Park, Hefei City, Anhui Province, and Aixihu Forest Wetland Park, Nanchang City, Jiangxi Province, during 2022. For isolation of conidiobolus-like fungi, we are following the previous described methods (King 1976a; Nie et al. 2012). All samples were preserved in sterilized plastic bags and transported to the laboratory as soon as possible. Plant debris samples were cut into several approximately 2 cm sized fragments and placed evenly on the Petri dishes cover. Then, using a Petri dish with potato dextrose agar (PDA; potato 200 g, dextrose 20 g, agar 20 g, H_2O 1 L) inverted over the treated samples to obtain discharged conidia, and incubating at 21 °C for daily examining by a stereomicroscope (SMZ1500, Nikon Corporation, Japan) for 7 days. When conidiobolus-like fungi observed, they were transferred to new PDA plate for purification and morphological observation.

The micro-morphological structure of mycelium, primary conidia and conidiophores, secondary conidia, and resting spores at 400× magnification was observed under a BX51 microscope (Olympus Corporation, Tokyo, Japan) and imaged using a DP25 microscope-camera system (Olympus Corporation, Tokyo, Japan) under differential interference contrast (DIC) condition. Each character was made more than 35 measurements and the description was made with the method by King (1976a). The purification isolates were deposited at the Engineering Research Center of Biofilm Water Purification and Utilization Technology of Ministry of Education at Anhui University of Technology, Anhui Province, China (BWPU), and duplicated at Research Center for Entomogenous Fungi at Anhui Agricultural University, Anhui Province, China (RCEF). In addition, 14 ex-types of *Conidiobolus* s.l. were purchased from the American Type Culture Collection, Manassas, VA, USA (ATCC).

DNA extraction, PCR amplification and sequencing

Pure cultures were grown on PDA for 7 days at 21 °C. Fresh fungal mycelia were scraped from the surface of PDA and transferred to Eppendorf tubes. Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Watanabe et al. 2010). Primers used for PCR amplification of the nucLSU (LR0R/LR5), mtSSU (mtSSU1/mtSSU2R), and *EFL* (EF983/EF1aZ-1R) genes were followed as described previously (Vilgalys and Hester 1990; Zoller et al. 1999; Nie et al. 2012).

DNA amplification was performed in a 50 μ l reaction volume which contained 1 μ L dNTPs (200 μ M), 1 μ L MgCl₂ (2.5 mM), 10 μ L Phusion HF buffer (5×), 1 μ L primers each (0.5 μ M), 100 ng genomic DNA, and 0.5 μ L Taq polymerase (0.04 Unit/L, Super Pfx DNA Polymerase, Cowinbioscience Co. Ltd., Shanghai, China). PCR amplificated program followed Nie et al. (2020a). Sequencing was generated by Shanghai Genecore Biotechnologies Company (Shanghai, China), and were processed with Geneious 9.0.2 (Kearse et al. 2012) to obtain consensus sequences. All sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

DNA sequences of three loci (nucLSU, mtSSU, and EFL) originated from Conidiobolus s.s. species were downloaded from GenBank database (Table 1) with two Azygosporus and three Microconidiobolus species served as outgroups. Sequence alignment of each locus was performed with MUSCLE 3.8.31 (Edgar 2004). Then, the alignments were checked and manually adjusted in BioEdit 7.0.1 (Hall 1999). The concatenated matrices were assembled by SequenceMatrix 1.7.8 (Vaidya et al. 2011). The obtained matrix was deposited in TreeBase (https://treebase.org) with the submission ID 31051. The best-fit likelihood models were estimated for each partition with MrModeltest v.2.3 (Nylander 2004). Maximum likelihood (ML) analyses were conducted with RAxML 8.1.17 using 1000 bootstrap replicates (Stamatakis 2014). Bayesian Inference (BI) analyses were calculated with MrBayes 3.2 (Ronguist and Huelsenbeck 2003). Bayesian posterior probabilities (PP) were estimated by the Metropolis-coupled Markov chain Monte Carlo method (Geyer 1991). Four simultaneous Markov chains were run starting from random trees for 1 million generations, keeping one tree every 100th generation until the average standard deviation of split frequencies was below 0.01. The value of burn-in was set to discard 25% of trees and posterior probabilities (PP) were determined from the remaining trees. The phylogenetic tree was visualized in FigTree 1.4 (Rambaut 2012) and improved with Adobe Illustrator CS6.0.

Species	Stroino*	GenBank accession numbers					
Species	Strains	nucLSU	EFL	mtSSU			
Azygosporus macropapillatus	CGMCC 3.16068 (T)	MZ542006	MZ555650	MZ542279			
A. parvus	ATCC 14634 (T)	KX752051	KY402207	MK301192			
Conidiobolus bifurcatus	CGMCC 3.15889 (T)	MN061285	MN061482	MN061288			
C. brefeldianus	ARSEF 452 (T)	EF392382	_	EF392495			
C. chlamydosporus	ATCC 12242 (T)	JF816212	JF816234	MK301178			
C. coronatus	NRRL 28638	AY546691	DQ275337	-			
C. coronatus	RCEF 4518	JN131537	JN131543	-			
C. dabieshanensis	CGMCC 3.15763 (T)	KY398125	KY402206	MK301180			
C. firmipilleus	ARSEF 6384	JX242592	-	JX242632			
C. gonimodes	ATCC 14445 (T)	JF816221	JF816226	MK301182			
C. humicolus	ATCC 28849 (T)	JF816220	JF816231	MK301184			
C. incongruus	NRRL 28636 (T)	AF113457	_	-			
C. iuxtagenitus	ARSEF 6378 (T)	KC788410	_	-			
C. iuxtagenitus	RCEF 4445	JX946695	JX946700	MK333391			
C. jiangxiensis sp.nov.	RCEF 7484 (T)	PP034291	PP035215	PP034295			
C. jiangxiensis sp.nov.	RCEF 7485	PP034292	PP035216	PP034296			
C. khandalensis	ATCC 15162 (T)	KX686994	KY402204	MK301185			
C. lichenicolus	ATCC 16200 (T)	JF816216	JF816232	MK301186			
C. longiconidiophorus	RCEF 6563 (T)	OQ540746	OQ550509	OQ540744			
C. marcoconidius sp.nov.	RCEF 6918 (T)	PP034289	PP035213	PP034293			
C. marcoconidius sp.nov.	RCEF 7412	PP034290	PP035214	PP034294			
C. marcosporus	ATCC 16578 (T)	KY398124	KY402209	MK301188			
C. megalotocus	ATCC 28854 (T)	MF616383	MF616385	MK301189			
C. mycophagus	ATCC 16201 (T)	JX946694	JX946698	MK301190			
C. mycophilus	ATCC 16199 (T)	KX686995	KY402205	MK301191			
C. polyspermus	ATCC 14444 (T)	MF616382	MF616384	MK301193			
C. polysporus	RCEF 7058 (T)	OQ540747	OQ550510	OQ540745			
C. polytocus	ATCC 12244 (T)	JF816213	JF816227	MK301194			
C. taihushanensis	CGMCC 3.15900 (T)	MT250086	MT274290	MT250088			
C. variabilis	CGMCC 3.15901 (T)	MT250085	MT274289	MT250087			
Microconidiobolus nodosus	ATCC 16577 (T)	JF816217	JF816235	MK333388			
M. paulus	ARSEF 450 (T)	KC788409	_	-			
M. terrestris	ATCC 16198 (T)	KX752050	KY402208	MK301199			

T I I A TI	•	1 .			
lane i ine	SNACIAS	lisediiin	nnvioden	etic anal	VSAS
	opeoieo	aocaam	phylogen	cuo unui	,000

*ARSEF, ARS Entomopathogenic Fungus Collection (Ithaca, U.S.A.). ATCC, American Type Culture Collection (Manassas, U.S.A). CGMCC, China General Microbiological Culture Collection Center (Beijing, China). NRRL, ARS Culture Collection (Peoria, U.S.A). RCEF, Research Center for Entomogenous Fungi (Hefei, China). T = ex-type. The new species reported in this study are indicated in bold.

Results

Phylogenetic analyses

The concatenated dataset comprised 1883 nucleotide sites, with specific contributions of 981 for nucLSU, 501 for SSU, and 401 for *EFL*. Within this dataset, 964 characters remained constant, 656 were parsimony-informative, and 308 were parsimony-uninformative. Model selection for individual data from each partition in both ML and BI phylogenetic analyses resulted in the application of the GTR+I+G model. The ML optimization likelihood reached a final value of -13813.01, and the average standard deviation of the split frequencies at the end of the analyses was 0.00619. The resulting phylogram from the ML analysis is depicted in Fig. 1.



Figure 1. Maximum likelihood (ML) tree obtained by phylogenetic analyses of the combined nucLSU, *EFL* and mtSSU sequences. Two *Azygosporus* and three *Microconidiobolus* species were served as outgroups. The proposed new species is in boldface. Maximum Likelihood bootstrap values (\geq 70%) / Bayesian posterior probabilities (\geq 0.95) of clades are provided alongside the branches. The scale bar at the bottom left indicates substitutions per site.

Contrary to previous studies (Nie et al. 2020a, b; Gryganskyi et al. 2022), the current phylogenetic tree did not exhibit the grouping of all *Conidiobolus* s.s. members into two main clades. Notably, the phylogeny revealed that the four newly isolated strains (RCEF 6918, RCEF 7412, RCEF 7484, and RCEF 7485) were situated within the genus *Conidiobolus* s.s. Specifically, strains RCEF 7484 and RCEF 7485 clustered with *C. mycophilus*, garnering high to full support (100/1.00), while strains RCEF 6918 and RCEF 7412 formed a distinct clade with full support (100/1.00). Additionally, a subclade was formed by *C. polyspermus*, *C. mycophilus*, *C. gonimodes*, RCEF 7484, and RCEF 7485. However, *C. polyspermus* exhibited a distinct genetic distance from the other three species.

Taxonomy

Conidiobolus jiangxiensis B. Huang & Y. Nie, sp. nov. MycoBank No: 851495 Fig. 2

Etymology. *jiangxiensis* (Lat.), referring to the region where the fungus was isolated. **Known distribution.** Jiangxi Province, China.



Figure 2. Conidiobolus jiangxiensis RCEF 7484 **a** colony on PDA after 3 d at 21 °C **b** mycelia unbranched at the edge of the colony **c** hyphal segments **d**–**g** primary conidiophores bearing a single primary conidia **h**–**k** primary conidia **l**, **m** primary conidia bearing a single secondary conidium **n**, **o** zygospores formed between adjacent segments of the same hypha **p** young zygospores **q** mature zygospores. Scale bars: 100 μ m (**b**); 20 μ m (**d**–**q**).

Typification. CHINA, Jiangxi Province, Nanchang City, Aixihu Forest Wetland Park, 28°69'N, 115°99'E, from soil, 7 Dec. 2022, Y. *Nie*, holotype BWPU 221207. Ex-type culture RCEF 7484. GenBank: nucLSU = PP034291; *EFL* = PP035215; mtSSU = PP034295.

Additional specimens examined. CHINA, Jiangxi Province, Nanchang City, Aixihu Forest Wetland Park, 28°69'N, 115°99'E, from soil, 7 Dec. 2022, *Y. Nie* culture RCEF 7485. GenBank: nucLSU = PP034292; *EFL* = PP035216; mtSSU = PP034296.

Description. Colonies on PDA at 21 °C after 3 d, white, reaching ca 11 mm in diameter. Mycelia white, 8–15 µm wide, often unbranched at the edge of colony, non-septate when young, and distended to segment after 7 d. Primary conidiophores often arising from hyphae, short, $30-95 \times 7-10$ µm, unbranched and producing a single primary conidium, without widening upward near the tip. Primary conidia forcibly discharged, globose to subglobose, $30-41 \times 24-36$ µm, papilla bluntly-round, 8–13 µm wide, 3.5-9 µm long. Secondary conidiophores arising from primary conidia. Microspores not observed on the PDA culture and on the 2% water agar. Zygospores formed in axial alignment with conjugating segments after 10 days, mature zygospores smooth, usually globose, sometimes subglobose, 20-30 µm in diameter, with a 2–3 µm thick wall.

Notes. Conidiobolus jiangxiensis, C. polyspermus and C. mycophilus exhibit close phylogenetic relatedness. However, the primary conidia and zygospores of C. jiangxiensis are smaller than those of C. polyspermus, and C. jiangxiensis is further set apart from C. mycophilus by its longer primary conidiophores and larger primary conidia (Drechsler 1961; Srinivasan and Thirumalachar 1965). Despite the high similarities in nucLSU and EFL between C. jiangxiensis and C. polyspermus, their differentiation becomes evident through morphological traits. Similar instances of this phenomenon are observed in C. coronatus and C. megalotocus, as well as C. mycophagus and C. lichenicolus. Morphologically, C. jiangxiensis presents shorter primary conidiophores (no more than 95 µm) compared to the majority of other Conidiobolus s.s. members. It closely resembles C. marcosporus (50-100 μm), C. lichenicolus (30-100 μm), and C. gonimodes (20-80 μm) according to the length of primary conidiophores. Distinguishing features include its smaller primary conidia and zygospores in comparison to C. marcosporus (Srinivasan & Thirumalachar, 1967) and larger primary conidia, as well as the absence of primary conidia arising as upward branches from hyphal knots, distinguishing it from C. lichenicolus (Srinivasan and Thirumalachar 1968). Notably, C. *jiangxiensis* aligns with C. gonimodes based on primary conidia size, yet it differs by the distinct width of mycelia and the presence of unbranched primary conidiophores (Drechsler 1961). Furthermore, in the phylogenetic tree, C. jiangxiensis is distantly related to C. gonimodes. (Fig. 1)

Conidiobolus marcoconidius B. Huang & Y. Nie, sp. nov. MycoBank No: 851496 Fig. 3

Etymology. *marcoconidius* (Lat.), referring to its large primary conidia. **Known distribution.** Anhui Provinces, China.



Figure 3. Conidiobolus marcoconidius RCEF 6918 **a** colony on PDA after 3 d at 21 °C **b** mycelia unbranched at the edge of the colony **c** hyphal segments **d**–**f** primary conidiophores **g**–**j** primary conidia **k**, **l** primary conidia bearing a single secondary conidium **m** secondary conidiophore branched at the base and bearing two secondary conidia at each tip **n** secondary conidiophore branched at the tip **o** secondary conidiophore branched at the base bearing 2-5 secondary conidia at each branch **p**, **q** zygospores formed between adjacent segments of the same hypha **r** mature zygospores. Scale bars: 100 µm (**b**, **n**); 20 µm (**c**–**m**, **o**–**r**).

Typification. CHINA, Anhui Province, Hefei City, Dashushan National Forest Park, 31°84'N, 117°17'E, from plant debris, 15 Mar. 2022, *Y. Yin*, holotype DSS 20220315. Ex-type culture RCEF 6918. GenBank: nucLSU = PP034289; *EFL* = PP035213; mtSSU = PP034293.

Additional specimens examined. CHINA, Anhui Province, Hefei City, Binhu National Forest Park, 31°73'N, 117°38'E, from plant debris, 10 May 2022, Y. Yin,, culture RCEF 7412. GenBank: GenBank: nucLSU = PP034290; *EFL* = PP035214; mtSSU = PP034294.

Description. Colonies on PDA at 21 °C after 3 d white, reaching ca 8 mm in diameter. Mycelia colorless, unbranched at the edge of colony, distended to a width of 9–20 µm segment after 5 d. Primary conidiophores unbranched, slightly curved at the tip, producing a single primary conidium, without widening upward near the tip, $105-230 \times 10-16$ µm. Primary conidia forcibly discharged, mostly globose, sometimes obovoid, $45-67 \times 42-58$ µm, with a sharp or round papilla, 13-22 µm wide, 4-13 µm long. Secondary conidia arising from primary conidia, with a short or long secondary condiophore, similar and smaller to the primary conidia. Secondary conidiophores branched at the base or tip, thus bearing 2 secondary conidia at each tip. Sometimes form 2–5 secondary conidia like "tomatoes on sticks" from small to large at each branch. Microconidia not observed on the PDA culture and on the 2% water agar. Zygospores formed between adjacent segments after 7 days, smooth, globose, 30–45 µm in diameter, with a 2–4 µm thick wall.

Notes. *Conidiobolus marcoconidius* is distinguished morphologically by its larger primary conidia compared to other *Conidiobolus* s.s. species, with the exception of *C. coronatus* (King 1977). Notably, it can be readily differentiated from *C. coronatus* by the absence of villose spores (Batko 1964). Additionally, *C. marcoconidius* is characterized by secondary conidiophores that branch at the base, giving rise to 2–5 secondary conidia resembling "tomatoes on sticks" at each branch, varying in size from small to large. In the phylogenetic tree, it forms a discrete clade, setting it apart from other *Conidiobolus* s.s. species.

Conidiobolus polyspermus Drechsler, Mycologia, 53: 279. 1961. MycoBank No: 328763

Specimens examined. United States, Maryland, 26 July 1955, Drechsler, ATCC 14444.

Description. Refer to Drechsler (1961).

Notes. In accordance with King's numerical taxonomy of *Conidiobolus* (King 1976b), *C. polyspermus* was initially identified as a synonym of *C. megalotocus*. However, upon a thorough comparison of morphological traits based on the original descriptions, it became evident that *C. polyspermus* ($15-55 \times 12-48 \mu m$) produces larger conidia than those of *C. megalotocus* ($12-44 \times 10-42 \mu m$). Notably, *C. polyspermus* is not reported to form microconidia, distinguishing it from *C. megalotocus* (Drechsler 1956; 1961). Furthermore, the phylogenetic tree (Fig. 1) revealed a distinct relationship between *C. polyspermus* and *C. megalotocus* tocus. In light of these findings, we propose the separation of *C. polyspermus* from *C. megalotocus*, affirming its taxonomic status at the species level.

Discussion

Over an extended period, DNA-based techniques have played a pivotal role in uncovering both inter- and intra-species phylogenetic variations, essential for describing new species (Kidd et al. 2023). While the ITS region stands as a universal barcode marker for fungal identification, its applicability to entomophthoroid fungi is hindered by high intragenomic variation (Schoch et al. 2012; Hyde et al. 2023). Fortunately, the development of the full ribosomal operon and additional gene loci encoding proteins as fungal barcodes has addressed some of these challenges (James et al. 2006; Wurzbacher et al. 2019; Voigt et al. 2021; Zhao et al. 2023). In understanding the phylogeny of entomophthoroid fungi, reclassifications based on molecular sequences of nucLSU-SSU, mtSSU, and RPB2 have led to an updated taxonomic system proposed by Humber (2012), building upon the work of Gryganskyi et al. (2012, 2013). However, with only 31 fungal taxa having molecular data, constituting a mere fraction of entomophthoroid fungi, further phylogenetic analyses are imperative for a comprehensive understanding.

In light of the aforementioned phylogenetic framework, our study employed the same loci (excluding *EFL* instead of RPB2 for ease of amplification) to investigate the phylogeny of conidiobolus-like fungi. This endeavor resulted in the establishment of four new genera, i.e. *Azygosporus* B. Huang & Y. Nie, *Capillidium* B. Huang & Y. Nie, *Microconidiobolus* B. Huang & Y. Nie, and *Neoconidiobolus* B. Huang & Y. Nie, through a combination of molecular and morphological evidence. Additionally, *Conidiobolus* s.s. was proposed to accommodate members in the subgenus *Delacroixia* (Nie et al. 2020a; Cai et al. 2021). The evolution of phylogenomic studies in various fungal groups (Spatafora et al. 2016; Vandepol et al. 2020; Li et al. 2021) has further revealed a polyphyletic relationship among conidiobolus-like fungi, leading to the introduction of three new families, i.e. *Capillidiaceae* Y. Nie, Stajich & K.T. Hodge, *Conidiobolaceae* B. Huang, Stajich & K.T. Hodge, and *Neoconidiobolaceae* X.Y. Liu, Stajich & K.T. Hodge, grounded in morphological evidence and ancestral lifestyle considerations (Gryganskyi et al. 2022).

Furthermore, our molecular analyses underscored the high sensitivity of both nucLSU and *EFL* sequences in delineating conidiobolus-like fungi (Nie et al. 2012). Considering the balance between amplification efficiency, data integrity, and diversity, three loci of nucLSU, mtSSU, and *EFL* were selected for species recognition within *Conidiobolus* s.s. (Nie et al. 2020b, 2023).

In this study, we recovered the species status of *C. polyspermus*, while *C. eurypus* was synonymized with *C. megalotocus*. This synonymy will be subject to re-evaluation with the inclusion of molecular data for *C. eurypus*. Notably, *C. polyspermus* was also not reported to produce microconidia, a trait shared with six other *Conidiobolus* s.s. species. With the addition of descriptions for two new species in this manuscript, the count of *Conidiobolus* s.s. species lacking observation of microconidia has risen to nine. The morphological variation or genetic mutation behind this phenomenon remains a question that could be addressed not only through phylogenomic analyses but also by conducting comparative genomics analyses within a broader spectrum of *Conidiobolus* s.s. species.

With the introduction of two new *Conidiobolus* s.s. species, namely *C. jiangx-iensis* and *C. marcoconidius* in the family *Conidiobolaceae* herein, the number of known *Conidiobolus* s.s. species are up to 22. However, limited reports of new species within the genera *Azygosporus* and *Microconidiobolus* in China underscore the need for an in-depth exploration of advanced species diversity within *Conidiobolaceae* from China in our future studies.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was supported by the National Natural Science Foundation of China (No. 32370007, 31900008).

Author contributions

Conceptualization: BH. Data curation: HZ. Formal analysis: HZ. Funding acquisition: YN. Methodology: YY, YN. Resources: YY. Supervision: BH. Visualization: XL. Writing - original draft: YN. Writing - review and editing: BH, XL.

Author ORCIDs

Yong Nie b https://orcid.org/0000-0001-8964-1661 Heng Zhao b https://orcid.org/0000-0003-2938-5613 Bo Huang b https://orcid.org/0000-0001-6032-7396

Data availability

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

References

- Batko A (1964) Notes on entomophthoraceous fungi in Poland Entomophaga. Mémoires hors série 2: 129–131.
- Cai Y, Nie Y, Zhao H, Wang ZM, Zhou ZY, Liu XY, Huang B (2021) *Azygosporus* gen. nov., a synapmorphic clade in the family Ancylistaceae. MycoKeys 85: 161–172. https://doi. org/10.3897/mycokeys.85.73405
- Drechsler C (1956) Two new species of *Conidiobolus*. American Journal of Botany 43(10): 778–787. https://doi.org/10.1002/j.1537-2197.1956.tb11168.x
- Drechsler C (1961) Two species of *Conidiobolus* often forming zygospores adjacent to antheridium-like distentions. Mycologia 53(3): 278–303. https://doi.org/10.1080/00 275514.1961.12017960
- Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340
- Geyer CJ (1991) Markov chain Monte Carlo maximum likelihood. In: Keramidas EM (Ed.) Computing Science and Statistics. Proceedings of the 23rd Symposium on the Interface. Fairfax Station: Interface Foundation, USA, 156–163.
- Goffre D, Jensen AB, Lopez Lastra CC, Humber RA, Folgarait PJ (2020) *Conidiobolus lunulus*, a new entomophthoralean species isolated from leafcutter ants. Mycologia 113(1): 56–64. https://doi.org/10.1080/00275514.2020.1816387
- Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J, Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R (2012) Molecular phylogeny of the Entomophthoromycota.

Molecular Phylogenetics and Evolution 65(2): 682–694. https://doi.org/10.1016/j. ympev.2012.07.026

- Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R (2013) Phylogenetic lineages in Entomophthoromycota. Persoonia 30(1): 94–105. https://doi.org/10.3767/003158513X666330
- Gryganskyi AP, Nie Y, Hajek AE, Hodge KT, Liu XY, Aadland K, Voigt K, Anishchenko IM, Kutovenko VB, Kava L, Vuek A, Vilgalys R, Huang B, Stajich JE (2022) The early terrestrial fungal lineage of *Conidiobolus*–Transition from saprotroph to parasitic lifestyle. Journal of Fungi (Basel, Switzerland) 8(8): 789. https://doi.org/10.3390/jof8080789
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Huang B, Humber RA, Hodge KT (2007) A new species of *Conidiobolus* from Great Smoky Mountains National Park. Mycotaxon 100: 227–233.
- Humber RA (2012) Entomophthoromycota: A new phylum and reclassification for entomophthoroid fungi. Mycotaxon 120(1): 477–2492. https://doi.org/10.5248/120.477
- Hyde KD, Abdel-Wahab MA, Abdollahzadeh J, Abeywickrama PD, Absalan S, et al. (2023) Global consortium for the classification of fungi and fungus-like taxa. Mycosphere : Journal of Fungal Biology 14(1): 1960–2012. https://doi.org/10.5943/mycosphere/14/1/23
- James TY, Kau F, Schoch C, Matheny PB, Valerie H, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schüssler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lücking R, Büdel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443(7113): 818–822. https://doi.org/10.1038/nature05110
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–1649. https://doi. org/10.1093/bioinformatics/bts199
- Kidd SE, Abdolrasouli A, Hagen F (2023) Fungal nomenclature: Managing change is the name of the game. Open Forum Infectious Diseases 10(1): ofac559. https://doi. org/10.1093/ofid/ofac559
- King DS (1976a) Systematics of Conidiobolus (Entomophthorales) using numerical taxonomy I. Biology and cluster analysis. Canadian Journal of Botany 54(1–2): 45–65. https://doi.org/10.1139/b76-008
- King DS (1976b) Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy II. Taxonomic considerations. Canadian Journal of Botany 54(12): 1285–1296. https://doi.org/10.1139/b76-141
- King DS (1977) Systematics of *Conidiobolus* (Entomophthorales) using numerical taxonomy III. Descriptions of recognized species. Canadian Journal of Botany 55(6): 718–729. https://doi.org/10.1139/b77-086

- Li Y, Steenwyk JL, Chang Y, Wang Y, James TY, Stajich JE, Spatafora JW, Groenewald M, Dunn CW, Hittinger CT, Shen X-X, Rokas A (2021) A genome-scale phylogeny of the kingdom Fungi. Current Biology 31(8): 1653–1665. https://doi.org/10.1016/j. cub.2021.01.074
- Möckel L, Meusemann K, Misof B, Schwartze VU, Licht HHDF, Voigt K, Stielow B, de Hoog S, Beutel RG, Buellesbach J (2022) Phylogenetic revision and patterns of host specificity in the fungal Subphylum Entomophthoromycotina. Microorganisms 10(2): 256. https://doi.org/10.3390/microorganisms10020256
- Nie Y, Yu CZ, Liu XY, Huang B (2012) A new species of *Conidiobolus* (*Ancylistaceae*) from Anhui, China. Mycotaxon 120(1): 427–435. https://doi.org/10.5248/120.427
- Nie Y, Tang XX, Liu XY, Huang B (2017) A new species of *Conidiobolus* with chlamydospores from Dabie Mountains, eastern China. Mycosphere 8(7): 809–816. https://doi.org/10.5943/mycosphere/8/7/1
- Nie Y, Yu DS, Wang CF, Liu XY, Huang B (2020a) A taxonomic revision of the genus Conidiobolus (Ancylistaceae, Entomophthorales): Four clades including three new genera. MycoKeys 66: 55–81. https://doi.org/10.3897/mycokeys.66.46575
- Nie Y, Cai Y, Gao Y, Yu DS, Wang ZM, Liu XY, Huang B (2020b) Three new species of *Conidiobolus* sensu stricto from plant debris in eastern China. MycoKeys 73: 133–149. https://doi.org/10.3897/mycokeys.73.56905
- Nie Y, Cai Y, Zhao H, Zhou ZY, Zhao CW, Liu XY, Huang B (2023) Morphological and phylogenetic analyses reveal two new species in *Conidiobolus* s.s. (Conidiobolaceae, Entomophthorales) from China. MycoKeys 98: 221–232. https://doi.org/10.3897/mycokeys.98.103603
- Nylander JAA (2004) MrModeltest v.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Rambaut A (2012) FigTree version 1.4.0. http://tree.bio.ed.ac.uk/software/figtree/
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
- Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proceedings of the National Academy of Sciences of the United States of America 109(16): 6241–6246. https://doi.org/10.1073/pnas.1117018109
- Spatafora JW, Chang Y, Benny GL, Lazarus K, Smith ME, Berbee ML, Bonito G, Corradi N, Grigoriev I, Gryganskyi A, James TY, O'Donnell K, Roberson RW, Taylor TN, Uehling J, Vilgalys R, White MM, Stajich JE (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108(5): 1028–1046. https://doi.org/10.3852/16-042
- Srinivasan MC, Thirumalachar MJ (1965) Studies on species of *Conidiobolus* from India-IV. Sydowia 19: 86–91.
- Srinivasan MC, Thirumalachar MJ (1967) Evaluation of taxonomic characters in the genus *Conidiobolus* with key to known species. Mycologia 59(4): 698–713. https://doi. org/10.1080/00275514.1967.12018462
- Srinivasan MC, Thirumalachar MJ (1968) Studies on species of *Conidiobolus* from India-V. Mycopathologia 36(3-4): 341-346. https://doi.org/10.1007/BF02050380
- Stamatakis A (2014) RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/ bioinformatics/btu033

- Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27(2): 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x
- Vandepol N, Liber J, Desiro A, Na H, Kennedy M, Barry K, Grigoriev IV, Miller AN, O'Donnell K, Stajich JE, Bonito G (2020) Resolving the Mortierellaceae phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. Fungal Diversity 104(1): 267–289. https://doi.org/10.1007/s13225-020-00455-5
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
- Voigt K, James TY, Kirk PM, Santiago ACMDA, Waldman B, Griffith GW, Fu M, Radek R, Strassert JFH, Wurzbacher C, Jerônimo GH, Simmons DR, Seto K, Gentekaki E, Hurdeal VG, Hyde KD, Nguyen TTT, Lee HB (2021) Early-diverging fungal phyla: Taxonomy, species concept, ecology, distribution, anthropogenic impact, and novel phylogenetic proposals. Fungal Diversity 109(1): 59–98. https://doi.org/10.1007/s13225-021-00480-y
- Wang CF, Li KP, Liu YJ, Li ZZ, Huang B (2010) Three new Chinese records of *Conidiobolus*. Mycosystema 29: 595–599.
- Watanabe M, Lee K, Goto K, Kumagai S, Sugita-Konishi Y, Hara-Kudo Y (2010) Rapid and effective DNA extraction method with bead grinding for a large amount of fungal DNA. Journal of Food Protection 73(6): 1077–1084. https://doi.org/10.4315/0362-028X-73.6.1077
- Waters SD, Callaghan AA (1989) *Conidiobolus iuxtagenitus*, a new species with discharge delongate repetitional conidia and conjugation tubes. Mycological Research 93(2): 223–226. https://doi.org/10.1016/S0953-7562(89)80121-2
- Wurzbacher C, Larsson E, Bengtsson-Palme J, Wyngaert SVD, Svantesson S, Kristiansson E, Kagami M, Nilsson RH (2019) Introducing ribosomal tandem repeat barcoding for fungi. Molecular Ecology Resources 19(1): 118–127. https://doi.org/10.1111/1755-0998.12944
- Zhao H, Nie Y, Zong TK, Wang K, Lv ML, Cui YJ, Tohtirjap A, Chen J-J, Zhao C-L, Wu F, Cui B-K, Yuan Y, Dai Y-C, Liu X-Y (2023) Species diversity, updated classification and divergence time of the phylum Mucoromycota. Fungal Diversity 123(1): 49–157. https://doi.org/10.1007/s13225-023-00525-4
- Zoller S, Scheideggera C, Sperisena C (1999) PCR primers for the amplification of mitochondrial small subunit ribosomal DNA of lichen-forming ascomycetes. Lichenologist (London, England) 31(5): 511–516. https://doi.org/10.1006/lich.1999.0220


Research Article

Didymellaceae species associated with tea plant (*Camellia sinensis*) in China

Yuchun Wang^{1*}, Yiyi Tu^{1*}, Xueling Chen¹, Hong Jiang¹, Hengze Ren¹, Qinhua Lu², Chaoling Wei³, Wuyun Lv¹⁰

1 College of Tea Science and Tea Culture, Zhejiang A & F University, Hangzhou 311300, Zhejiang, China

2 Institute of Sericulture and Tea, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

3 State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, 130 Changjiang West Road, Hefei, 230036, Anhui, China

Corresponding author: Wuyun Lv (lvwuyun_blue@163.com); Chaoling Wei (weichl@ahau.edu.cn)

Abstract

Tea plant is one of the most important commercial crops worldwide. The Didymellaceae fungi can cause leaf blight disease of tea plant. In this study, 240 isolates were isolated from tea plant leaves of 10 provinces in China. Combined with multi-locus (ITS, LSU, RPB2 and TUB2) phylogenetic analysis and morphological characteristics, these isolates were identified as 25 species of six genera in Didymellaceae, including 19 known species Didymella coffeae-arabicae, D. pomorum, D. segeticola, D. sinensis, Epicoccum catenisporum, E. dendrobii, E. draconis, E. italicum, E. latusicollum, E. mackenzie, E. oryzae, E. poaceicola, E. rosae, E. sorghinum, E. tobaicum, Neoascochyta mortariensis, Paraboeremia litseae, Remotididymella anemophila and Stagonosporopsis caricae, of which 15 species were new record species and six novel species, named D. yunnanensis, E. anhuiense, E. jingdongense, E. puerense, N. yunnanensis and N. zhejiangensis. Amongst all isolates, D. segeticola was the most dominant species. Pathogenicity tests on tea plant leaves showed that E. anhuiense had the strongest virulence, while E. puerense had the weakest virulence. Besides, D. pomorum, D. yunnanensis, E. dendrobii, E. italicum, E. jingdongense, E. mackenziei, E. oryzae, E. rosae, E. tobaicum, N. mortariensis, N. yunnanensis, N. zhejiangensis and R. anemophila were non-pathogenic to the tea plant.

Key words: Camellia inhibiting fungi, Didymella, distribution, Epicoccum, leaf blight, Neoascochyta, new species, pathogenicity

Introduction

Pleosporales is a predominant order with a worldwide distribution in terrestrial and aquatic environments (An et al. 2022). In these environments, *Pleosporales* mainly survives as saprophytic fungi on dead leaves or stems (Kodsueb et al. 2006; Zhang et al. 2009a, 2009b). It also can be endophytes, epiphytes and parasites of green leaves or stems and lichens (Calatayud et al. 2001; Kruys et al. 2006; Huang et al. 2008). Didymellaceae is one of the largest family in *Pleosporales*, which was established by de Gruyter et al. (2009). It is widely distributed geographically, existing in different ecosystems, such as air, soil, water, house dust and coral and parasitising in other fungi and lichens (Sutton 1980; Chen



Academic editor: Rungtiwa Phookamsak Received: 4 February 2024 Accepted: 29 April 2024 Published: 29 May 2024

Citation: Wang Y, Tu Y, Chen X, Jiang H, Ren H, Lu Q, Wei C, Lv W (2024) Didymellaceae species associated with tea plant (*Camellia sinensis*) in China. MycoKeys 105: 217–251. https://doi.org/10.3897/ mycokeys.105.119536

Copyright: [©] Yuchun Wang 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).

^{*} These authors contributed equally.

et al. 2017; Wanasinghe et al. 2018a). Previous studies have reported that this family included three main genera: *Ascochyta, Didymella* and *Phoma*, as well as other allied phoma-like genera which grouped in the Didymellaceae (Chen et al. 2017). Besides, *Leptosphaerulina* and *Macroventura* were genetically closely similar and classified into Didymellaceae (Silva-Hanlin and Hanlin 1999; Kod-sueb et al. 2006; Aveskamp et al. 2010). Aveskamp et al. (2010) divided the family into at least 18 different clusters according to the sequence data obtained from 324 strains, redefining *Epicoccum, Peyronellaea* and *Stagonosporopsis* and demonstrating that *Ascochyta, Phoma* and *Didymella* were highly polyphyletic. As an extremely species-rich family, more than 5400 species belonging to 44 accepted genera have been recorded in Didymellaceae (Kularathnage et al. 2023).

Although the basic taxonomy of Didymellaceae has been established, the problem of multi-source of many genera has not been solved. Morphological characteristics, coupled with multi-gene molecular phylogeny, have developed as a more effective strategy for the identification of Didymellaceae, which has improved the understanding of the taxonomy (Hou et al. 2020a). For example, combining morphological observation and multi-locus phylogenetic analysis, based on ITS (the internal transcribed spacer region of the rDNA gene), LSU (partial large subunit nrDNA nucleotide sequences), RPB2 (the RNA polymerase II second largest subunit gene) and TUB2 (partial gene regions of β-tubulin), Chen et al. (2015a) clarified the generic delimitation in Didymellaceae. Seventeen fully-supported monophyletic branches in Didymellaceae were revealed and the generic circumscriptions of Ascochyta, Phoma and Didymella emended. Recently, 108 Didymellaceae isolates newly obtained from 40 host plant species in 27 plant families in China and other countries were investigated (Chen et al. 2017). Amongst these, 68 isolates representing 32 new taxa are recognised, based on morphological differences and the multi-locus phylogeny using sequences of ITS, LSU, RPB2 and TUB2 and a total of 19 genera are recognised in the Didymellaceae family (Chen et al. 2017). Wanasinghe et al. (2018a) isolated didymellaceous taxa from Alhagi pseudalhagi, Coronilla emerus, Cytisus sp., Elaeagnus angustifolia and Spartium junceum in Italy, Russia and Uzbekistan and present comprehensive morphological descriptions and in-depth phylogenetic investigation of five new species, including Ascochyta coronillae-emeri, Microsphaeropsis spartii-juncei, Neomicrosphaeropsis alhagi-pseudalhagi, N. cytisicola and N. elaeagni. Furthermore, as a cosmopolitan family, 1124 Didymellaceae strains globally collected from 92 countries, 121 plant families and 55 other substrates were examined via multi-locus phylogenetic analyses and detailed morphological comparisons (Hou et al. 2020b). Seven new genera, including Dimorphoma, Ectodidymella, Longididymella, Macroascochyta, Paramicrosphaeropsis, Pseudopeyronellaea and Sclerotiophoma were newly introduced in Didymellaceae (Hou et al. 2020b). In addition, 40 new species were identified combining phylogenetic analyses, based on concatenated DNA sequence dataset (ITS, LSU, RPB2 and TUB2) and morphological examination (Hou et al. 2020b). Given the above, phylogenetic analyses, based on a combined ITS-LSU- RPB2-TUB2 DNA sequence dataset, have been demonstrated as an effective method for the identification of Didymellaceae at species level (Hou et al. 2020a; Yuan et al. 2021; Kularathnage et al. 2023; Yang et al. 2023a).

Tea plant (*Camellia sinensis*) is one of the important commercial crops, which is widely cultivated in tropical and subtropical areas (Manawasinghe et al. 2021). Leaf blight disease caused by phytopathogens from Didymellaceae

threatens the healthy growth of tea plants (Chen et al. 2017; Manawasinghe et al. 2021; Kumhar et al. 2022; Huang et al. 2023). Some species of Didymellaceae, such as *Didymella segeticola*, *D. bellidis*, *Epicoccum camelliae*, *E. latusicollum*, *E. layuense* and *E. sorghinum*, were isolated from diseased tissues (Chen et al. 2017; Ren et al. 2019; Manawasinghe et al. 2021; Wang et al. 2021). However, comprehensive understanding on the biodiversity and pathogenicity of Didymellaceae on tea plants remains unknown. Thus, to systematically and comprehensively elaborate the species of Didymellaceae in tea plant can provide further insight into the understanding of pathogens causing leaf blight disease.

In this study, 240 isolates of Didymellaceae were obtained from tea plant leaves of ten provinces in China. We aimed to clarify the classification of these isolates using phylogenetic analyses, based on the multi-locus (ITS, LSU, *RPB2* and *TUB2*) DNA sequences and, thus, determined the biodiversity of Didymellaceae on tea plants. In addition, to evaluate the pathogenicity of isolates, we performed pathogenicity tests with 36 representative isolates on leaves of *C. sinensis* cv. *Longjing43* (LJ43), a relative susceptible cultivar (Wang et al. 2016). The pathogenicity results will preliminarily determine the dominant species associated with leaf blight.

Materials and methods

Collection and isolates

The isolates were collected from tea plants in 15 cities of ten provinces in China, including Hangzhou (30°18'N, 120°09'E), Lishui (28°66'N, 120°09'E) and Shaoxing (30°08'N, 120°49'E) Cities in Zhejiang Province, Huangshan (29°72'N, 118°32'E) and Anging (30°69'N, 116°40'E) Cities in Anhui Province, Yixing (31°28'N, 119°72'E) and Wuxi (31°47'N, 120°27'E) Cities in Jiangsu Province, Chengdu (30°24'N, 103°51'E) and Guangyuan (32°64'N, 105°89'E) Cities in Sichuan Province, Wuhan (30°30'N, 114°14'E) City in Hubei Province, Nanchang (28°55'N, 115°94'E) City in Jiangxi Province, Tongren (27°96'N, 109°28'E) City in Guizhou Province, Xinyang (32°12'N, 114°06'E) City in Henan Province, Yingde City (39°91'N, 116°52'E) in Guangdong Province and Puer (24°45'N, 100°83'E) City in Yunnan Province. The fungal strains were obtained by two different methods, one was tissue isolation from healthy leaves and the other was single spore isolation by scraping diseased spots from diseased leaves (Fig. 1) (Cai et al. 2009; Wang et al. 2016). For single spore isolation, spores were isolated from diseased leaves and suspended in sterilised ddH₂O under sterilised conditions, then were coated on potato dextrose agar (PDA) plates and cultured at 25 °C in the dark. For tissue isolation, healthy leaves were surface-sterilised and then cultured on PDA plates at 25 °C in the dark. After 2 days, single colonies were selected and transferred to new PDA plates for further pure cultivation. For further study, pure cultures were stored in 25% glycerol at -80 °C.

Type specimens of new species from this study were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS) and ex-type living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC). The descriptions of the novel species reported in this study were submitted to the MycoBank database (https://www.mycobank.org).



Figure 1. Disease symptoms on *Camellia sinensis* caused by Didymellaceae **A** leaf symptom **B** fungal fruitbody structures formed on leaves **C** close-up of fungal fruitbody structures **D** conidia. Scale bars: 10 μ m.

DNA extraction, PCR amplification and sequencing

Isolates were cultured at 28 °C in the dark for 7 days. Genomic DNA was extracted from fresh mycelia using Genomic DNA Purification Kit (Sangon Biotechnology (Shanghai) Co., Ltd., China). The fragments of ITS, LSU, *RPB2* and *TUB2* were amplified by PCR using the genomic DNA as the template (Chen et al. 2015a). PCR amplifications were performed in a reaction mixture consisting of 12 μ l 2× Taq Master Mix, 1 μ l 10 μ M forward primer, 1 μ l 10 μ M reverse primer, 1 μ l DNA template, adjusted to a final volume of 25 μ l with ddH₂O. Primer pairs used in this study were listed in Table 1. The PCR amplification procedures of four loci were as follows: ITS, predenaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s and extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 2 min, with the final extension at 72 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 48 °C for 45 s, annealing at 48 °C for 45 s, annealing at 48 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 45 s

Gene	Primer	Primer sequence (5'-3')					
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG					
	ITS4	TCCTCCGCTTATTGATATGC					
LSU	LROR	GTACCCGCTGAACTTAAGC					
	LR7	TACTACCACCAAGATCT					
RPB2	RPB2-5f2	GGGGWGAYCAGAAGAAGGC					
	RPB2-7cR	CCCATRGCTTGYTTRCCCAT					
TUB2	Btub2Fd	GTBCACCTYCARACCGGYCARTG					
	Btub4Rd	CCRGAYTGRCCRAARACRAAGTTGTC					

Table 1. Primer pairs used in this study.

56 °C for 80 s and extension at 72 °C for 2 min, with the final extension at 72 °C for 10 min; *TUB2*, predenaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 80 s, with the final extension at 72 °C for 10 min. The PCR products were visualised using 1% agarose electrophoresis gels. Sequencing was performed by Youkang Biotechnology (Hangzhou) Co., Ltd., China.

Phylogenetic analysis

Sequences of the ITS, LSU, *RPB2* and *TUB2* loci for all the isolates were blasted against the National Center for Biotechnology Information (NCBI) GenBank nucleotide datasets (http://www.ncbi.nlm.nih.gov/Blast.cgi) (Suppl. material 1). Alignments of ITS, LSU, *RPB2* and *TUB2* sequences were generated with MAFFT v.7.525 (Katoh et al. 2019) and MEGA v.6.0 software was used for manual correction (Tamura et al. 2013). To investigate the phylogenetic relationships between different isolates, both Bayesian Inference (BI) and Maximum Likelihood (ML) methods were used and followed by the concatenated alignments (Han et al. 2023). For BI analysis, Markov Chain Monte Carlo (MCMC) sampling was used to reconstruct phylogenies in MrBayes v.3.2 (Ronquist and Huelsenbeck 2003). For ML analysis, the substitution model (GTR + I + G model with gamma-distributed rate) were selected (Wang et al. 2016). Phylograms were created in FigTree v. 1.3.1 (Rambaut and Drummond 2008) and edited in Adobe Illustrator 2022 (available from https://www.adobe.com/cn/ creativecloud/roc/business.html).

Morphology

Isolates were grown on oatmeal agar (OA) and PDA plates and cultured at 28 °C for 7 days (Hou et al. 2020b). Colony diameters of each strain with three replicates were then measured and repeated at least three times. The morphological characteristics were determined after another 7 days (Boerema et al. 2004). The shape, colour and size of mature pycnidia and conidia were observed under light microscopy (SOPTOP-CX40RFL, China). Sizes of at least 30 conidia were measured with the light microscopy. The description of new species is mainly based on the morphology of colony, conidia and pycnidia, conidia size, colony growth rate and aerial hyphae on OA and PDA.

Pathogenicity tests

Asymptomatic leaves were collected from 5-year-old LJ43 grown in a tea garden in Hangzhou, Zhejiang Province, China. The fourth leaf of current-growth branches was cut off for the analysis. The detached leaves were surface-sterilised with 75% alcohol and washed with sterilised ddH₂O twice and air dried. A 5-mm mycelial disc cut from the edge of 7-day-old cultures was inoculated both sides of leaves after wounding with a sterilised needle (using a pattern of puncture perpendicular to the leaf to create the same number of wounds and this pattern was applied uniformly across all leaves) and cultured directly on a moist surface in the dark with 100% humidity at 28 °C for 3 days (Solarte et al. 2017). After 3 days, the lesion diameters were measured and photographed. Each strain with at least three replicates was repeated three times. Thirty-six representative isolates were selected for the pathogenicity test, including D. pomorum YCW196, D. segeticola YCW109, YCW192, YCW1135, YCW1289 and YCW2007, D. sinensis YCW1884 and YCW2118, D. yunnanensis CGMCC 3.24241 (YCW1909), E. anhuiense YCW961 and YCW1829, E. dendrobii YCW1866, E. draconis YCW101 and YCW187, E. italicum YCW2005, E. jingdongense YCW1868 and YCW1937, E. latusicollum YCW1921, E. mackenziei YCW1965 and YCW1967, E. oryzae YCW2010, E. poaceicola YCW1948 and YCW2115, E. rosae YCW331, E. poerense YCW224 and YCW2117, E. tobaicum YCW372, Neoascochyta mortariensis YCW1346, N. yunnanensis YCW1883, N. zhejiangensis YCW1361 and YCW1107, Paraboeremia litseae YCW1356 and YCW1363, Remotididymella anemophila YCW434 and Stagonosporopsis caricae YCW1928 and YCW1977.

Statistical analysis

The average value of all measurements was analysed using the SPSS Inc. software (IBM, New York, USA). The lesion sizes data were analysed with one-way ANOVA (analysis of variance) and the least significant difference (LSD) test and the values were presented as the mean \pm SE (standard error) of three repeats. A *P* value < 0.05 was considered statistically significant according to the LSD test.

Results

Isolates and phylogenetic analysis

In this study, 240 isolates were obtained from tea plant leaves of ten provinces in China. A multi-locus phylogeny was constructed, based on four loci (ITS, LSU, *RPB2* and *TUB2*). The ML tree from each alignment is presented, with bootstrap support values and Bayesian posterior values plotted at each node. All isolates were recognised and clustered into six genera in Didymellaceae, including *Didymella*, *Epicoccum*, *Neoascochyta*, *Paraboeremia*, *Remotididymella* and *Stagonosporopsis*.

For *Didymella* genus, phylogenetic analysis was performed with the combined sequence data from 227 isolates, including 45 referenced strains and 182 new-ly-sequenced strains. The 227 isolates comprised 2453 characters (ITS = 1-540 bp, LSU = 1504-2465 bp, *RPB2* = 545-1146 bp and *TUB2* = 1151-1499 bp) after alignment. *Pleiochaeta setosa* CBS 118.25 / CBS 496.63 and *Coniothyrium*

palmarum CBS 400.71 were used as the outgroup. Of the 182 new isolates, 171 isolates clustered with *D. segeticola* and retrieved 92% ML and 0.90 PP support, eight clustered with *D. sinensis* (99% in ML and 1 in PP), one clustered with *D. pomorum* (100% in ML and 1 in PP) and one clustered with *D. coffeae-arabicae* (94% in ML and 1 in PP). One isolate formed a new clade named *D. yunnanensis* (88% in ML and 0.92 in PP), which showed a close phylogenetic affinity to *D. prosopidis* (CBS 136414, CPC 21704 and BRIP 69579) (Fig. 2).



0.01

Figure 2. Phylogenetic tree generated by Maximum Likelihood analysis, based on the combined ITS, LSU, *RPB2* and *TUB2* dataset of *Didymella* species. Bootstrap support values above 50% and Bayesian posterior values above 0.75 are shown at each node (ML/PP). *Pleiochaeta setosa* CBS 118.25 / CBS 496.63 and *Coniothyrium palmarum* CBS 400.71 are used as outgroups. Ex-type strains are emphasised in bold.

For Epicoccum genus, phylogenetic analysis was performed with the combined sequence data from 114 isolates, including 68 referenced strains and 46 newly-sequenced strains. The 114 isolates comprised 2466 characters (ITS = 1-559 bp, LSU = 1516-2478 bp, RPB2 = 564-1162 bp and TUB2 = 1167-1511 bp) after alignment. Pleiochaeta setosa CBS 118.25 / CBS 496.63 and Co. palmarum CBS 400.71 were used as the outgroups. Of the 46 new isolates, seven isolates clustered with E. poaceicola (78% in ML and 0.96 in PP), three clustered with E. latusicollum (84% in ML and 1 in PP), one clustered with E. sorghinum (99% in ML and 1 in PP), one clustered with E. catenisporum (99% in ML and 1 in PP), three clustered with E. dendrobii (89% in ML and 0.95 in PP), two clustered with E. draconis (96% in ML and 0.76 in PP), five clustered with E. tobaicum (96% in ML and 0.90 in PP), three clustered with E. rosae (97% in ML and 1 in PP), two clustered with E. mackenzie (88% in ML and 0.98 in PP), one clustered with E. oryzae (99% in ML and 1 in PP), one clustered with E. italicum (100% in ML and 1 in PP) and 17 unidentified isolates did not match any known lineage of Epicoccum species. Amongst the 17 unidentified isolates, six isolates formed a new monophyletic clade named E. anhuiense with support values 96% in ML and 0.68 in PP, six formed a new clade named E. jingdongense showing a close phylogenetic affinity to E. dendrobii in the combined phylogeny with 83% ML and 0.99 PP support and five formed a new monophyletic clade named E. puerense with high support (98% in ML and 0.92 in PP) (Fig. 3).

For other genera, phylogenetic analysis was performed with the combined sequence data from 56 isolates, including 44 referenced strains and 12 newly-sequenced strains. The 56 isolates comprised 2385 characters (ITS = 1-480 bp, LSU = 1435-2397 bp, RPB2 = 485-1080 bp and TUB2 = 1085-1430 bp) after alignment. Pleiochaeta setosa CBS 118.25 / CBS 496.63 was used as the outgroup. Of the 12 new isolates, two isolates clustered with Stagonosporopsis caricae (99% in ML and 1 in PP), three clustered with Remotididymella anemophila (100% in ML and 1 in PP), two clustered with Paraboeremia litseae (94% in ML and 1 in PP) and one clustered with Neoascochyta mortariensis (100% in ML and 1 in PP). One isolate formed a new clade named N. yunnanensis and showed a close phylogenetic affinity to N. rosicola (MFLUCC 15-0048) in the combined phylogeny and this relationship retrieved 99% ML and 1 PP support. Two isolates formed a new monophyletic clade named N. zhejiangensis with high support (100% in ML and 1 in PP). Unfortunately, the non-viability of YCW1124 resulted in the failure of identification, so it was tentatively determined as unidentified species Neoascochyta sp. (Fig. 4).

Morphology and taxonomy

Based on the multi-locus phylogenetic analysis, six species are delineated as new and their morphological characteristics are described below. In addition, 15 new record species and three known species are noted.

Didymella coffeae-arabicae (M. M. Aveskamp et al.) Q. Chen et al., Studies in Mycology. 82: 175. 2015a

Description. see Aveskamp et al. (2009).



Figure 3. Phylogenetic tree generated by Maximum Likelihood analysis, based on the combined ITS, LSU, *RPB2* and *TUB2* dataset of *Epicoccum* species. Bootstrap support values above 50% and Bayesian posterior values above 0.75 are shown at each node (ML/PP). *Pleiochaeta setosa* CBS 118.25 / CBS 496.63 and *Co. palmarum* CBS 400.71 are used as outgroups. Ex-type strains are emphasised in bold.

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis* cv. *Longjing43*, 13 Jun 2020, Y. C. Wang, culture YCW1972.

Notes. *Didymella coffeae-arabicae* was introduced as *Phoma coffeae-arabicae* before the comprehensive revision of Didymellaceae (Chen et al. 2015a). The sexual morph of *D. coffeae-arabicae* was reported by Samaradiwakara et al.



Figure 4. Phylogenetic tree generated by Maximum Likelihood analysis, based on the combined ITS, LSU, *RPB2* and *TUB2* dataset of *Neoascochyta*, *Paraboeremia*, *Remotididymella* and *Stagonosporopsis* species. Bootstrap support values above 50% and Bayesian posterior values above 0.75 are shown at each node (ML/PP). *Pleiochaeta setosa* CBS 118.25 / CBS 496.63 is used as the outgroup. Ex-type strains are emphasised in bold.

(2023). It forms pseudo-sclerotioid chlamydospores and is easily recognised by its conspicuously wide ostiole and is phylogenetically related to a group that mainly comprises *Peyronellaea* species forming alternarioid-botryoid chlamydospores (Aveskamp et al. 2009). *Didymella coffeae-arabicae* caused leaf cankers of *Castanea mollissima* in China (Jiang et al. 2021). In the present study, one isolate from healthy tea plant leaves grouped with *D. coffeae-arabicae* with high statistical support (Fig. 2). This is the first report of *D. coffeae-arabicae* isolated from *C. sinensis*.

Didymella pomorum (Thüm.) Q. Chen & L. Cai, Studies in Mycology. 82: 179. 2015a

Description. see Boerema (1993).

Materials examined. CHINA, Yunnan Province, from diseased leaves of *C. sinensis* cv. *Dalicha*, 22 Jun 2019, Y. C. Wang, culture YCW196.

Notes. *Didymella pomorum* was introduced as *Phoma pomorum* before the comprehensive revision of Didymellaceae (Chen et al. 2015a). Chen et al.

(2015a) regarded four taxa of the respective *Phoma pomorum* varieties, viz. vars. *circinata* (CBS 285.76), *cyanea* (CBS 388.80) and *pomorum* (CBS 539.66) and the species *Ph. triticina* (CBS 354.52) to be conspecific and treated them as a single species *D. pomorum*. Pycnidia produced by this species are usually subglobose-ampulliform with a distinct ostiole (Boerema 1993). It can cause leaf spots on many plants (Boerema 1993; Romero et al. 2021). In the present study, one isolate from diseased tea plant leaves is closely related to *D. sinensis* with high statistical support (Fig. 2). This is the first report of *D. pomorum* isolated from *C. sinensis*.

Didymella segeticola (Q. Chen) Q. Chen et al., Studies in Mycology. 87: 138. 2017

Description. see Chen et al. (2015b).

Materials examined. CHINA, Jiangsu Province, Yixing City, Zhangzhu Town, Furong Village, from diseased leaves of *C. sinensis* cv. *Longjing43*, 19 Jun 2019, Y. C. Wang, culture YCW109. Zhejiang Province, Lishui City, from diseased leaves of *C. sinensis* cv. *Baiye1*, 22 Jun 2019, Y. C. Wang, culture YCW192. Zhejiang Province, Hangzhou City, from diseased leaves of *C. sinensis* cv. *Longjing43*, 6 Jun 2018, Y. C. Wang, culture YCW1289.

Notes. *Didymella segeticola* was introduced as *Phoma segeticola* before the comprehensive revision of Didymellaceae (Chen et al. 2015a). Under the current circumstance of Didymellaceae, it belongs to *Didymella*. *Didymella segeticola* can develop abundant aerial mycelium and black pycnidia on oatmeal agar (OA) plates (Chen et al. 2015b). Zhao et al. (2018) first reported that *D. segeticola* can cause tea leaf spot in the tea plantations in Guizhou Province, which results in leaf fall and a huge loss of tea leaves. In the present study, 171 isolates from diseased tea plant leaves formed a monophyletic subclade, closely related to *D. bellidis* with high statistical support (Fig. 2).

Didymella sinensis (Q. Chen) Q. Chen et al., Studies in Mycology. 87: 138. 2017

Description. see Chen et al. (2017).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW2118.

Notes. *Didymella sinensis* is closely related to *D. pomorum*. It can be observed from different host plants in a wide range, such as *Cerasus pseudocerasus* (Rosaceae), *Dendrobium officinale* (Orchidaceae) and Urticaceae. The sexual morph was characterised by ascomata aggregated, globose to irregular, brown, small and papillate. Asci were bitunicate, clavate to short cylindrical; Ascospores were biseriate, ellipsoidal, straight to slightly curved, hyaline, apex obtuse, medianly 1-septate (Chen et al. 2017). In the present study, eight isolates from healthy tea plant leaves phylogenetically grouped with *D. sinensis* with high statistical support (Fig. 2). This is the first report of *D. sinensis* isolated from *C. sinensis*.

Didymella yunnanensis Y. Wang, Y. Tu, X. Chen, H. Jiang, H. Ren, Q. Lu, C. Wei & W. Lv, sp. nov.

MycoBank No: 848984 Fig. 5

Etymology. Named after the location where it was collected, Yunnan Province.

Description. Sexual morph: undetermined. Asexual morph: Pycnidia smooth, subglobose to ellipsoidal, hyaline. Conidia ellipsoidal to subcylindrical, pale, smooth- and thin-walled, abundant, generated from pycnidia, aseptate, $4-6.5 \times 1.8-2.6 \mu m$ (av. = $5.2 \pm 0.5 \times 2.3 \pm 0.2 \mu m$, n = 30). Mycelia sparsely branched from subapical hyphal compartments (lateral branching), septate, hyaline.

Culture characteristics. Colonies on PDA have scarce aerial mycelium reaching 24–27 mm diam. after being cultured for 7 days at 28 °C in the dark, margin regular, olive in the centre, white edges; black on the reverse, white edges. Pycnidia and conidia produced on the colony surface after being cultured for 14 days at 28 °C in the dark. Colonies on OA reaching 18–21 mm diam. after 7 days at 28 °C in the dark, margin regular, aerial mycelium flat, black in the centre, white edges; olive on the reverse, white edges.

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis* cv. *Longjing43*, 16 Jun 2020, Y. C. Wang, Holotype HMAS 352387, culture ex-type CGMCC 3.24241 = YCW1909.

Notes. *Didymella yunnanensis* is closely related to *D. prosopidis* with high statistical support (88%/0.92, ML/PP, Fig. 2). *Didymella yunnanensis* has 85 bp differences in LSU locus from *D. prosopidis*. In addition, *D. yunnanensis* can be distinguished from *D. prosopidis* by the morphological features of conidia and the conidia size of *D. yunnanensis* (4–6.5 × 1.8–2.6 µm) is smaller than that of *D. prosopidis* (5–7 × 2.5–3.5 µm). In the present study, *Didymella yunnanensis* was isolated from healthy tea plant leaves.

Epicoccum anhuiense Y. Wang, Y. Tu, X. Chen, H. Jiang, H. Ren, Q. Lu, C. Wei & W. Lv, sp. nov.

MycoBank No: 848998 Fig. 6

Etymology. Named after the location where it was collected, Anhui Province.

Description. Sexual morph: undetermined. Asexual morph: Pycnidia smooth, subglobose to ellipsoidal, pale brown, attached to mycelium. Conidia ellipsoidal to subcylindrical, pale yellow to green, smooth- and thin-walled, abundant, generated from pycnidia, composed of a single cell, $10.5-16 \times 4.5-8 \mu m$ (av. = $13.4 \pm 1.4 \times 6.3 \pm 0.7 \mu m$, n = 30). Mycelia lateral branching, septate, hyaline.

Culture characteristics. Colonies on PDA reaching 75–79 mm diam. after 7 days at 28 °C in the dark, margin regular, covered by floccose aerial mycelium, greyish; reverse pale brown to pale buff, white edges. Pycnidia and conidia produced on the colony surface after being cultured for 14 days at 28 °C in the dark. Colonies on OA reaching 81–85 mm diam. after 7 days at 28 °C in the dark, margin irregular, aerial mycelium flat, whitish; reverse concolorous.

Materials examined. CHINA, Anhui Province, Anqing City, from diseased leaves of *C. sinensis* cv. *Longjingchangye*, 16 Nov 2019, Y. C. Wang, Holotype



Figure 5. *Didymella yunnanensis* CGMCC 3.24241 (YCW1909) **A**, **B** colony on PDA (front and reverse) **C**, **D** colony on OA (front and reverse) **E** myceli **F** pycnidia forming on PDA **G** conidia. Scale bars: 10 µm (**E**–**G**).



Figure 6. *Epicoccum anhuiense* YCW961 **A**, **B** colony on PDA (front and reverse) **C**, **D** colony on OA (front and reverse) **E** mycelia **F** pycnidia forming on PDA **G** conidia. Scale bars: 10 μm (**E**–**G**).

HMAS 352388, culture ex-type CGMCC 3.24242 = YCW961. Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from health leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture ex-type CGMCC 3.24246 = YCW1829.

Notes. Epicoccum anhuiense is closely related to *E. latusicollum* with high statistical support (Fig. 3). Epicoccum anhuiense has 5 bp differences in the *TUB2* sequence from *E. latusicollum*. In addition, *E. anhuiense* can be distinguished from *E. latusicollum* by the morphological features of its conidia and the conidia size of *E. anhuiense* ($10.5-16 \times 4.5-8 \mu m$) is larger than that of *D. prosopidis* ($4-6.5 \times 2-3 \mu m$). In the present study, eight strains were isolated from healthy or diseased tea plant leaves.

Epicoccum catenisporum N. Valenzuela-Lopez et al., Studies in Mycology. 90: 30. 2018

Description. see Valenzuela-Lopez et al. (2018).

Materials examined. CHINA, Jiangxi Province, Nanchang City, from diseased leaves of *C. sinensis* cv. *Zhenong139*, 22 Jun 2019, Y. C. Wang, culture YCW142.

Notes. *Epicoccum catenisporum* was introduced as *Phoma catenisporum* before the comprehensive revision of *Epicoccum* (Chen et al. 2015a). It was first isolated from a leaf spot of *Oryza sativa* in Guinea-Bissau and morphologically characterised by the production of pycnidia as observed in several other members of *Epicoccum* (Valenzuela-Lopez et al. 2018). Conidiogenous cells were phialidic, hyaline, doliiform or ampulliform and conidia were aseptate, hyaline ovoid or ellipsoidal and guttulate (Valenzuela-Lopez et al. 2018). In the present study, one isolate from diseased tea plant leaves grouped with *E. catenisporum* (CBS 181.80) with high statistical support (Fig. 3). This is the first report of *E. catenisporum* isolated from *C. sinensis*.

Epicoccum dendrobii Q. Chen et al., Studies in Mycology. 87: 140. 2017

Description. see Chen et al. (2017).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW1866.

Notes. *Epicoccum dendrobii* formed a distinct clade, closely related to *E. jingdongense* and *E. puerense* (Fig. 3). It produced typical epicoccoid conidia (multicellular-phragmosporous, verrucose). In the present study, three strains were isolated from healthy or diseased tea plant leaves. This is the first report of *E. dendrobii* isolated from *C. sinensis*.

Epicoccum draconis (Berk. ex Cooke) Q. Chen et al., Studies in Mycology. 82: 172. 2015b

Description. see de Gruyter et al. (1998).

Materials examined. CHINA, Jiangsu Province, Yixing City, Zhangzhu Town, Furong Village, from diseased leaves of *C. sinensis* cv. *Longjing43*, 19 Jun 2019, Y. C. Wang, culture YCW101.

Notes. *Epicoccum draconis* was introduced as *Phyllosticta draconis* and *Phoma draconis* previously (Chen et al. 2017). It formed a new combination by the ellipsoidal conidia (Chen et al. 2017). In the present study, two isolates from diseased tea plant leaves grouped with *E. draconis* with high statistical support (Fig. 3). This is the first report of *E. draconis* causing leaf blight on *C. sinensis*.

Epicoccum italicum Q. Chen et al., Studies in Mycology. 87: 144. 2017

Description. see Chen et al. (2017).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW2005.

Notes. Phylogenetically, *Epicoccum italicum* formed a distinct lineage closely related to *E. oryzae* (Fig. 3). *Epicoccum italicum* produced epicoccoid conidia and clavate conidiomata (Chen et al. 2017). It was first isolated from seedlings of *Acca sellowiana* in Italy (Chen et al. 2017) and reported in the dairy setting (Rodríguez et al. 2023). In addition, this species significantly reduced both leaf area of soybean consumed aboveground by caterpillars and number of cysts produced belowground by nematodes (Rivera-Vega et al. 2022). In the present study, one strain was isolated from healthy tea plant leaves. This is the first report of *E. italicum* isolated from *C. sinensis*.

Epicoccum jingdongense Y. Wang, Y. Tu, X. Chen, H. Jiang, H. Ren, Q. Lu, C. Wei & W. Lv, sp. nov.

MycoBank No: 849000 Fig. 7

Etymology. Named after the location where it was collected, Jingdong Yizu Autonomous County.

Description. Sexual morph: undetermined. Asexual morph: Pycnidia smooth, subglobose, pale brown. Conidia ellipsoidal to subcylindrical, pale yellow, smooth, generated from pycnidia, aseptate, $7.1-16 \times 4-9 \mu m$ (av. = $10.7 \pm 1.2 \times 5.4 \pm 0.6 \mu m$, n = 30). Mycelia extensively branched from subapical hyphal compartments, septate, hyaline.

Culture characteristics. Colonies on PDA reaching 35–42 mm diam. after 7 days at 28 °C in the dark, margin irregular, aerial mycelium flat, pale brown to rosy, white edges; reverse black to brown, pale buff edges. Pycnidia and conidia produced on the colony surface after being cultured for 14 days at 28 °C in the dark. Colonies on OA reaching 49–55 mm diam. after 7 days at 28 °C in the dark, margin regular, aerial mycelium flat, pale buff to whitish; reverse concolorous.

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, Holotype HMAS 352389, culture ex-type CGMCC 3.24247 = YCW1868. Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture ex-type CGMCC 3.24248 = YCW1937.

Notes. *Epicoccum jingdongense* is closely related to *E. dendrobii* and *E. puerense* with high statistical support (83%/0.99, ML/PP, Fig. 3). *Epicoccum puerense* differs in 1 bp in ITS and 40 bp in *TUB2* from *E. dendrobii*. The conidia size is larger than that of *E. dendrobii*. In the present study, six strains were isolated from healthy tea plant leaves. It was isolated and identified from tea plant for the first time.

Epicoccum latusicollum Q. Chen et al., Studies in Mycology. 87: 144. 2017

Description. see Chen et al. (2017).



Figure 7. *Epicoccum jingdongense* YCW1868 **A**, **B** colony on PDA (front and reverse) **C**, **D** colony on OA (front and reverse) **E** mycelia **F** pycnidia forming on PDA **G** conidia. Scale bars: 10 μm (**E**–**G**).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW1921.

Notes. Isolates of *Epicoccum latusicollum* were clustered into a sister clade to *E. poaceicola* and *E. sorghi* (Fig. 3). Pycnidia were black-brown and mostly spheroid and conidia were ellipsoidal to oblong, aseptate and hyaline (Chen et al. 2017; Li et al. 2023). It was first discovered from *Acer palmatum* (Aceraceae), *Camellia sinensis* (Theaceae), *Podocarpus macrophyllus* (Podocarpaceae) and *Vitex negundo* (Verbenaceae) (Chen et al. 2017). As a phytopathogen, it can cause leaf spot, leaf blight and stalk rot on many plants (Xu et al. 2022; Li et al. 2023; Wang et al. 2023). In the present study, three strains were isolated from healthy tea plant leaves.

Epicoccum mackenziei S. C. Jayasiri et al., Mycosphere 8: 1093. 2017

Description. see Jayasiri et al. (2017).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture extype CGMCC 3.24244 = YCW1965 and culture ex-type CGMCC 3.24245 = YCW1967.

Notes. *Epicoccum mackenziei* formed a distinct clade basal to *E. endophyticum* (Fig. 3). It was found as the sexual morph in nature and as chlamydospores in culture. Zhang et al. (2023) first reported that *E. mackenziei* caused dark brown spot of tea leaf in China. In the present study, two strains were isolated from healthy tea plant leaves.

Epicoccum oryzae S. Ito & Iwadare, Report of the Hokkaido Prefectural Agricultural Experiment Station 31: 1. 1934

Description. see Hou et al. (2020b).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW2010.

Notes. *Epicoccum oryzae* was synonymised as *E. nigrum* previously (Schol – Schwarz 1959). It was resurrected as a separate species, distant from *E. nigrum* and CBS 173.34 was proposed as the ex-neotype of *E. oryzae* (Hou et al. 2020b). *Epicoccum oryzae* is characterised by "olivaceous hyphae, globose or subglobose sporodochia and globose, subglobose or pyriform, granular, verrucose, olivaceous conidia, consisting of one to five cells" (Hou et al. 2020b). It clustered into a sister clade to *E. endophyticum* and *E. mackenziei* (Fig. 3). In the present study, one isolate from healthy tea plant leaves grouped with *E. draconis* (CBS 173.34 and CBS 174.34) with high statistical support (Fig. 3). This is the first report of *E. oryzae* isolated from *C. sinensis*.

Epicoccum poaceicola Thambugala & K.D. Hyde, Mycosphere. 8: 711. 2017

Description. see Thambugala et al. (2017).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW1948.

Notes. *Epicoccum poaceicola* is described as a new phoma-like species, based on phylogenetic analysis. It formed a distinct lineage closely related to *E. sorghi* (Fig. 3). Conidia produced by *E. poaceicola* were ellipsoidal to cylindrical and sometimes with small guttules (Thambugala et al. 2017). *Epicoccum poaceicola* can cause leaf spot in bamboo, camphor tree and eggplant (Liu et al. 2020; Li et al. 2022; Aementado and Balendres 2023). In the present study, seven strains were isolated from healthy tea plant leaves. This is the first report of *E. poaceicola* isolated from *C. sinensis*.

Epicoccum puerense Y. Wang, Y. Tu, X. Chen, H. Jiang, H. Ren, Q. Lu, C. Wei & W. Lv, sp. nov.

MycoBank No: 848999 Fig. 8

Etymology. Named after the location where it was collected, Puer City.

Description. Sexual morph: undetermined. Asexual morph: Pycnidia smooth, subglobose to ellipsoidal, hyaline. Conidia were not of uniform size, ellipsoidal to subcylindrical, pale yellow to green, smooth- and thin-walled, abundant, generated from pycnidia, aseptate, $6.8-15 \times 3.6-7.2 \mu m$ (av. = $9.7 \pm 1.9 \times 4.7 \pm 0.7 \mu m$, n = 30). Mycelia lateral branching, septate, hyaline.

Culture characteristics. Colonies on PDA reaching 32–41 mm diam. after 7 days at 28 °C in the dark, margin irregular, aerial mycelium flat, olivaceous to buff, white edges; reverse black to brown, pale buff edges. Pycnidia and conidia produced on the colony surface after cultured for 14 days at 28 °C in the dark. Colonies on OA reaching 51–58 mm diam. after 7 days at 28 °C in the dark, margin regular, aerial mycelium flat, rosy to pale green, white edges; reverse pale buff to whitish.

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from diseased leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, Holotype HMAS 352390, culture ex-type CGMCC 3.24249 = YCW2117. Yunnan Province, from healthy leaves of *C. sinensis* cv. *Dalicha*, 22 Jun 2019, Y. C. Wang, culture ex-type CGMCC 3.24243 = YCW224.

Notes. *Epicoccum puerense* is closely related to *E. dendrobii* with high statistical support (Fig. 3). *Epicoccum puerense* has 1 bp difference in ITS from *E. dendrobii*. The conidia size is larger than that of *E. dendrobii*. In the present study, five strains were isolated from healthy or diseased tea plant leaves. It was isolated and identified from tea plant for the first time.

Epicoccum rosae D. N. Wanasinghe et al., Fungal Diversity. 89: 29. 2018

Description. see Wanasinghe et al. (2018b).

Materials examined. CHINA, Hubei Province, Wuhan City, Jiangxia District, from diseased leaves of *C. sinensis* cv. *Yulv*, 10 Jul 2019, Y. C. Wang, culture YCW331.

Notes. *Epicoccum rosae* had pycnidial conidiomata with hyaline conidia and hyphomycetous dark sporodochia with branched conidiophores and verruculose, muriform chlamydospores. It formed a distinct lineage closely related to *E. toba-icum* (Fig. 3). In the present study, three strains were isolated from diseased tea plant leaves. This is the first report of *E. rosae* isolated from *C. sinensis*.

Epicoccum tobaicum (Svilv.) L.W. Hou et al., Studies in Mycology. 96: 348. 2020

Description. see von Szilvinyi (1936).

Materials examined. CHINA, Anhui Province, Huangshan City, from diseased leaves of *C. sinensis* cv. *Zhonghuang1*, 2 Jul 2019, Y.C. Wang, culture YCW372.

Notes. *Epicoccum tobaicum* was synonymised as *E. nigrum* previously (Hou et al. 2020b). It was resurrected as a separate species, distant from *E. nigrum* (Hou et al. 2020b). Conidia were globular to pear-shaped, dark, verrucose and multicellular (Han et al. 2021). It formed a distinct lineage closely related to *E. rosae* (Fig. 3). This species as a pathogen was isolated from diseased leaves showing leaf spot of flowering cherry and oat (Han et al. 2021; Jeong et al. 2022a). In the present study, five strains were isolated from diseased tea plant leaves. This is the first report of *E. tobaicum* isolated from *C. sinensis*.

Neoascochyta mortariensis L.W. Hou et al., Studies in Mycology. 96: 391. 2020

Description. see Hou et al. (2020b).

Materials examined. CHINA, Zhejiang Province, Hangzhou City, from healthy leaves of *C. sinensis* cv. *Longjing43*, 16 Nov. 2017 Y. C. Wang, culture ex-type CGMCC 3.24251 = YCW1346.

Notes. Neoascochyta mortariensis was introduced as Didymella graminicola previously. It was described as a new species in Neoascochyta, distant from the authentic culture of *D. graminicola* (currently: Neoascochyta graminicola) (Hou et al. 2020b). Neoascochyta mortariensis was first isolated from Oryza sativa in



Figure 8. *Epicoccum puerense* YCW2117 **A**, **B** colony on PDA (front and reverse) **C**, **D** colony on OA (front and reverse) **E** mycelia **F** pycnidia forming on PDA **G** conidia. Scale bars: 10 μm (**E**–**G**).

Italy and formed colonies on PDA covered by dense felty aerial mycelium (Hou et al. 2020b). It formed a distinct lineage closely related to *N. tardicrescens* (Fig. 4). In the present study, one strain was isolated from diseased tea plant leaves. This is the first report of *N. mortariensis* isolated from *C. sinensis*.

Neoascochyta yunnanensis Y. Wang, Y. Tu, X. Chen, H. Jiang, H. Ren, Q. Lu, C. Wei & W. Lv, sp. nov. MycoBank No: 849001 Fig. 9

Etymology. Named after the location where it was collected, Yunnan Province. Description. Sexual morph: undetermined. Asexual morph: Pycnidia smooth,

subglobose to ellipsoidal, hyaline. Conidia ellipsoidal to subcylindrical, pale yellow to green, smooth- and thin-walled, abundant, generated from pycnidia, aseptate, $8.5-11.7 \times 4.5-7 \mu m$ (av. = $9.9 \pm 0.9 \times 5.4 \pm 0.6 \mu m$, n = 30). Mycelia lateral branching, septate, hyaline.

Culture characteristics. Colonies on PDA reaching 42–45 mm diam. after 7 days 28 °C in the dark, margin regular, aerial mycelium flat, whitish; reverse black to pale buff. Pycnidia and conidia produced on the colony surface after being cultured for 14 days at 28 °C in the dark. Colonies on OA reaching 34 – 39 mm diam. after 7 days at 28 °C in the dark, margin irregular, aerial mycelium flat, black in the centre, white edges; reverse concolorous.

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, Holotype HMAS 352391, culture ex-type CGMCC 3.24253 = YCW1883.

Notes. Neoascochyta yunnanensis is closely related to *N. rosicola* with high statistical support (99%/1, ML/PP, Fig. 4). Neoascochyta yunnanensis has 2 bp differences in ITS from *N. rosicola*. In the present study, one strain was isolated from healthy tea plant leaves. It was isolated and identified from tea plant for the first time.



Figure 9. Neoascochyta yunnanensis YCW1883 A, B colony on PDA (front and reverse) C, D colony on OA (front and reverse) E mycelia F pycnidia forming on PDA G conidia. Scale bars: 10 µm (E–G).

Neoascochyta zhejiangensis Y. Wang, Y. Tu, X. Chen, H. Jiang, H. Ren, Q. Lu, C. Wei & W. Lv, sp. nov. MycoBank No: 849002 Fig. 10

Etymology. Named after the location where it was collected, Zhejiang Province.

Description. Sexual morph: undetermined. Asexual morph: Pycnidia smooth, subglobose to ellipsoidal, hyaline. Conidia biconical to subcylindrical, hyaline, smooth- and thin-walled, abundant, generated from pycnidia, aseptate, $4.8-6.5 \times 2.9-4.2 \ \mu m$ (av. = $5.6 \pm 0.5 \times 3.6 \pm 0.3 \ \mu m$, n = 30). Mycelia lateral branching or uniaxial branching, septate, hyaline.

Culture characteristics. Colonies on PDA reaching 65–69 mm diam. after 7 days at 28 °C in the dark, margin regular, aerial mycelium flat, whitish; reverse black, white edges. Pycnidia and conidia produced on the colony surface after being cultured for 14 days at 28 °C in the dark. Colonies on OA reaching 53–57 mm diam. after 7 days at 28 °C in the dark, margin regular, aerial mycelium flat, whitish; reverse olivaceous, white edges.

Materials examined. CHINA, Zhejiang Province, Hangzhou City, from diseased leaves of *C. sinensis* cv. *Longjing43*, Jun 2014, Y. C. Wang, Holotype HMAS 352392, culture ex-type CGMCC 3.24250 = YCW1107. Yunnan Province, from diseased leaves of *C. sinensis*, 23 Mar 2020, Y. C. Wang, culture CGMCC 3. YCW1361.

Notes. *Neoascochyta zhejiangensis* is closely related to *N. cylindrispora* with high statistical support (82%/77, ML/PP, Fig. 4). *Neoascochyta Cylindrispora* differs in 1 bp in ITS, 16 bp in *TUB2* and 95 bp in LSU from *N. zhejiangensis*. In the present study, two strains were isolated from healthy tea plant leaves.

Paraboeremia litseae J. R. Jiang et al., Mycological Progress. 16: 291. 2017

Description. see Jiang et al. (2017).



Figure 10. *Neoascochyta zhejiangensis* YCW1107 **A**, **B** colony on PDA (front and reverse) **C**, **D** colony on OA (front and reverse) **E** mycelia **F** pycnidia forming on PDA **G** conidia. Scale bars: 10 μm (**E**–**G**).

Materials examined. CHINA, Yunnan Province, from diseased leaves of *C. sinensis*, 23 Mar 2020, Y. C. Wang, culture YCW1356 and culture YCW1363.

Notes. Isolates of *Paraboeremia litseae* clustered into a sister clade to *P. se-laginellae* (Fig. 5). It was first isolated from *Litsea* sp. (Jiang et al. 2017). Conidia produced by *P. litseae* are oblong to ellipsoidal and aseptate with two large polar guttules (Jiang et al. 2017). This species as an endophytic fungus in *Coptis chinensis* exhibited obvious inhibition against methicillin-resistant *Staphylococcus aureus* (Ming et al. 2022). In the present study, two strains were isolated from diseased tea plant leaves. This is the first report of *P. litseae* causing leaf blight on *C. sinensis*.

Remotididymella anemophila A. L. Yang et al., International Journal of Systematic Evolutional Microbiology. 71: 10. 2021

Description. see Yang et al. (2021).

Materials examined. CHINA, Anhui Province, Huangshan City, from diseased leaves of *C. sinensis* cv. *Fenglixiang*, 2 Jul 2019, Y. C. Wang, culture YCW499. Zhejiang Province, Hangzhou City, from diseased leaves of *C. sinensis* cv. *Long-jing43*, Jun 2014, Y. C. Wang, culture YCW1118.

Notes. *Remotididymella anemophila* was clustered into a sister clade to *R. bauhiniae* (Fig. 4), characterised by shorter ascospores, longer asci and larger conidia. It was first isolated from canopy air of *Ageratina adenophora* (Spreng.) in China (Yang et al. 2021). In the present study, three strains were isolated from diseased tea plant leaves. This is the first report of *R. anemophila* causing leaf blight on *C. sinensis*.

Stagonosporopsis caricae (Sydow & P. Sydow) M. M. Aveskamp et al., Studies in Mycology. 65: 45. 2010

Description. see Sivanesan (1990).

Materials examined. CHINA, Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW1928. Yunnan Province, Puer City, Jingdong Yizu Autonomous County, from healthy leaves of *C. sinensis*, 13 Jun 2020, Y. C. Wang, culture YCW1977.

Notes. Stagonosporopsis caricae was synonymised as *Phoma caricae* with *Mycosphaerella caricae* previously (Sivanesan 1990). It formed a distinct lineage in *Stagonosporopsis* (Fig. 4). Zhang et al. (2022) observed its sexual morph and is characterised by ascomata pseudothecioid, subglobose, $121 \times 142 \mu m$, ostiolate, walls of brown *textura angularis* and smooth. Asci were bitunicate, cylindrical to clavate, $7 \times 90 \mu m$, 8-spored, ascospores elliptical, straight to slightly curved, $5 \times 17 \mu m$, 1-septate, constricted at the septum, sub-hyaline and smooth. As one of three *Stagonosporopsis* species, *S. caricae* caused gummy stem blight (Jeong et al. 2022b; Seblani et al. 2023). In the present study, two isolates from healthy tea plant leaves grouped with *S. caricae* with high statistical support (Fig. 4). This is the first report of *S. caricae* isolated from *C. sinensis*.

Pathogenicity tests

To determine the pathogenicity of isolates from these 22 species, 36 representative isolates were selected for the analysis on the healthy leaves of *C. sinensis* cv. *Longjing43* with the wound-inoculation method. Amongst the tested strains, the sizes of necrotic lesions caused by the strain YCW1829 of *E. anhuiense* were largest (av. 8.00 ± 0.42 mm); on the contrary, the size of that caused by the strain YCW224 of *E. puerense* was smallest (av. 1.35 ± 0.70 mm) (Figs 11, 12). *Didymella segeticola, E. draconis, E. latusicollum* and *E. poaceicola* could also cause necrotic lesions on the inoculated leaves. Furthermore, the other strains caused no necrotic lesions on tea plant leaves (Fig. 11). The results indicated that *E. anhuiense* had the strongest virulence; on the contrary, *E. puerense* displayed the weakest virulence. In addition, *D. pomorum, D. yunnanensis, E. dendrobii, E. italicum, E. mackenziei, E. oryzae, E. rosae, E. tobaicum, E. jingdongense, N. mortariensis, N. yunnanensis, N. zhejiangensis* and *R. anemophila* were not pathogenic to tea plants.

Geographical distribution

To explore the geographical distribution of Didymellaceae family strains associated with *C. sinensis* in China, we combined our data with these from Chen et al. (2017) and Ren et al. (2019) for the analysis (Table 2). Amongst the 240 isolates that we collected from ten provinces in China, most of the isolates were distributed in Yunnan Province. Amongst the 25 species, *D. segeticola* (171 isolates in this study and 14 isolates from Ren et al. (2019) had the widest geographical distribution, in nine provinces. Fourteen species, *D. coffeae-arabicae*, *D. pomorum*, *D. sinensis*, *D. yunnanensis*, *E. dendrobii*, *E. italicum*, *E. jingdongense*, *E. mackenziei*, *E. oryzae*, *E. poaceicola*, *E. puerense*, *N. rosicola*, *P. litseae* and *S. caricae*, were only distributed in Yunnan Province. One species, *E. catenisporum*, was only distributed in Jiangxi Province. These results suggest that *D. segeticola* as the most widely distributed species may be the dominant species causing leaf blight disease in tea plants.



Figure 11. Symptoms of Didymellaceae family strains on tea plant leaves at 3 days after inoculation.



Figure 12. Lesion diameters of Didymellaceae family strains on tea plant leaves at 3 days after inoculation. Error bars represent standard deviation.

Discussion

In this study, 240 isolates were obtained from tea plant leaves in ten provinces of four major tea regions (southwest China, south China, south Yangtze and north Yangtze) in China (Yang et al. 2023b). Based on the multi-locus (ITS, LSU, RPB2 and TUB2) sequences, three phylogenetic trees were constructed to identify the species of the tested isolates. Six novel species, named Didymella yunnanensis, Epicoccum anhuiense, Epicoccum jingdongense, Epicoccum puerense, Neoascochyta yunnanensis and Neoascochyta zhejiangensis, were identified and their morphological characteristics were described in detail (Figs 4-9). As one of the most species-rich genera in the Didymellaceae, Didymella was introduced by Saccardo (1880) with Didymella exigua (Niessl) Sacc. as the type species of the genus (Thambugala et al. 2017; Wang et al. 2021). Most species in the genus produced chlamydospores in culture (Chen et al. 2015a), whereas D. yunnanensis as one novel species of Didymella did not form chlamydospores on PDA or OA cultures (Fig. 5), which may be the result of suitable culture conditions in the incubator not being favourable for the production of the resting spores. Pycnidia of D. yunnanensis formed on PDA was smooth, subglobose to ellipsoidal, hyaline, which conflicts with the pigmented outer wall of pycnidia of Didymella genus (Chen et al. 2015a). However, based on multi-locus phylogenetic analyses, D. yunnanensis belonged to this genus as a novel species. We believed that multi-locus phylogenetic analyses were the more reliable method to clarify the genetic delimitation in Didymellaceae compared with the morphological observations. Here, we provide phylogenetic trees for Didymella, Epicoccum, Neoascochyta,

Species	Collecting location									
Species	AH	GD	GZ	HB	HN	JS	JX	SC	YN	ZJ
D. coffeae-arabicae									√	
D. pomorum									√	
D. segeticola	√	\checkmark	√⊥	√		√	√	√	√	√
D. sinensis									√	
D. yunnanensis									√	
E. anhuiense	√								√	
E. catenisporum							√			
E. dendrobii									√	
E. draconis						√				√
E. italicum									√	
E. jingdongense									√	
E. latusicollum							*		√	
E. mackenzie									√	
E. oryzae									√	
E. poaceicola									√	
E. puerense									√	
E. rosae	\checkmark			√		√				
E. sorghinum							*			√
E. tobaicum	\checkmark			√	√					√
N. mortariensis										√
N. rosicola									√	
N. zhejiangensis									√	√
P. litseae									√	
R. anemophila	\checkmark									√
S. caricae									√	

 Table 2. Geographical distribution of Didymellaceae family associated with C. sinensis in China.

AH: Anhui; GD: Guangdong; GZ: Guizhou; HB: Hubei; HN: Henan; JS: Jiangsu; JX: Jiangsi; SC: Sichuan; YN: Yunnan; ZJ: Zhejiang. This table includes data from Chen et al. (2017) (*), Ren et al. (2019) (\perp) , and our study (\checkmark).

Paraboeremia, Remotididymella and *Stagonosporopsis* using as much vouchered sequence data as possible. Six new species and 15 new records are proposed herein with support from our analysis of ITS, LSU, *RPB2* and *TUB2* sequences.

The genus Epicoccum is known as a hyphomycetous asexual morph in the Didymellaceae family (Hyde et al. 2013). However, it was emended with coelomycetous synanamorph by Chen et al. (2015a) and five Phoma species were recombined into the genus, based on multi-gene phylogenetic analysis (Thambugala et al. 2017). Epicoccum anhuiense is phylogenetically distinct from other Epicoccum species with close phylogenetic affinity to E. latusicollum (5 bp difference within the TUB2 sequence). Epicoccum jingdongense and E. puerense are also phylogenetically distinct from other Epicoccum species with close phylogenetic affinity to E. dendrobii (40 bp difference within the TUB2 sequence and 1 bp difference within the ITS sequence, respectively). Asexual morphs of the three novel species accommodated in Epicoccum were also determined and formed the coelomycetous asexual morphs (Figs 6–8), which is consistent with the characteristics of *Epicoccum* coelomycetous synasexual stage that is characterised by the formation of doliiform to flask-shaped conidiogenous cells that produce unicellular, hyaline conidia under culture conditions (Aveskamp et al. 2010; Jayasiri et al. 2017). Therefore, these species are introduced, based on the synasexual morphs and phylogenetic data.

In Neoascochyta, three different groups are observable based on conidial morphology: species with one-septate conidia, such as N. dactylidis, N. europaea, N. exitialis and N. graminicola; species with mainly one-septate conidia, but occasionally aseptate, such as N. argentina, N. cylindrispora, N. desmazieri, N. rosicola, N. tardicrescens and N. triticicola; and species with aseptate conidia, such as N. fuci, N. paspali and N. soli (Gonçalves et al. 2020). Two novel species, N. yunnanensis and N. zhejiangensis, produced aseptate conidia (Figs 9G, 10G), which fit within the last group. All the same, N. yunnanensis and N. zhejiangensis phylogenetically have a close relationship with N. rosicola and N. cylindrispora, respectively (Fig. 4). Conidia produced by N. zhejiangensis were hyaline, biconical to subcylindrical (Fig. 10G), keeping consistent with the conidial characteristics of Neoascochyta genus. By contrast, N. yunnanensis formed pale yellow conidia (Fig. 9G), which was a typical characteristic of Neoascochyta conidia. Besides, pycnidia of the two species formed on PDA was hyaline (Figs 9F, 10F), which is also a non-representative characteristic. This may be due to the culture conditions. The majority of Neoascochyta species was found in association with various Poaceae plant species, appearing to have some host preference (Gonçalves et al. 2020). In this study, we reported two novel species isolated from the tea plant for the first time.

Didymella and *Neoascochyta* genera have sexual morphs (Woudenberg et al. 2009; Jayasiri et al. 2017). However, sexual morphs of the isolates belonging to two genera were not observed under culture conditions and then undetermined. In the future, the detailed description of sexual morphs of the isolates, especially the three novel species *D. yunnanensis*, *N. yunnanensis* and *N. zhejiangensis*, will provide more morphological evidence for the identification of the novel species.

Amongst six new species in this study, most isolates were obtained from Yunnan Province (Table 2). Yunnan Province, as the oldest tea region in China, is rich in tea plant resources and is also the centre of fungi biodiversity. Molecular evidence suggested that many fungi belonging to the family Didymellaceae may be seedborne and can co-spread with the host through seeds (Fang et al. 2021; Yang et al. 2023a). Therefore, we speculated that Yunnan as the birthplace of tea plants has more abundant germplasm resources and is prone to fungal transmission. The remaining isolates were collected from Zhejiang and Anhui Provinces (Table 2), which provide the most suitable environment for tea plant growth. This warm and humid climate are also conducive to the rapid growth of fungi (Du et al. 2012).

More than half of the strains isolated from tea plants were clustered into *Did-ymella segeticola* species, indicating that this species in Didymellaceae family is probably more dominant in tea plants. They were isolated from diseased tea plant leaves and had strong virulence (Figs 11, 12), suggesting that *D. segetico-la* may be the causal agent of foliar diseases in tea plants. *Didymella* species have been reported to cause leaf spot on many plants, such as *Angelica dahurica* (Xu et al. 2016), *Bellis perennis* (Chen et al. 2015a), *Chrysanthemum morifolium* (Liu et al. 2019), *C. sinensis* (Ren et al. 2019; Wang et al. 2021), *Eleocharis dulcis* (Lv et al. 2011), *Lodium multiflorum* (Liu et al. 2022) and *Zanthoxylum bungeanum* (Yang et al. 2022). Especially, Ren et al. (2019) have also proved that *D. segeticola* is a causal agent of leaf spot on tea plants in China. However, the morphological characteristics of *D. segeticola* shared some similarities with those of *Discula theae-sinensis*, the causal agent of tea anthracnose

(Moriwaki and Sato 2009), especially the conidial morphology. We thus speculated that D. segeticola could also be the pathogen causing anthracnose on tea plant leaves. The pathogenicity of isolates in the Epicoccum genus is different; E. dendrobii, E. italicum, E. jingdongense, E. mackenziei, E. oryzae, E. rosae and E. tobaicum did not cause any disease symptoms, whereas E. anhuiense, E. draconis, E. latusicollum, E. poaceicola and E. puerense caused necrotic lesions on the tea plant leaves (Figs 11, 12). Epicoccum commonly display an endophytic lifestyle (Braga et al. 2018), so we speculated that the difference in pathogenicity may be due to the wound-inoculation method, which may result in the transition of some endophytes, such as E. anhuiense and E. puerense isolated from healthy leaves, to phytopathogens and the invasion of leaves from the artificial wounds. Therefore, the spray inoculation of healthy leaves in the future with conidia suspensions will help elucidate the pathogenic mechanism of all isolates. On the other hand, some Epicoccum species, such as E. draconis, E. latusicollum and E. poaceicola isolated from diseased leaves, were also reported as phytopathogens causing leaf spot on many plants, such as Eugenia involucrata (Bernardi et al. 2022), flowering cherry (Han et al. 2021), tobacco (Guo et al. 2020) and Weigela florida (Tian et al. 2021). Besides, Epicoccum species were mainly known as biocontrol agents against phytopathogens via inhibiting their growth and conidial germination (Braga et al. 2018). For example, E. nigrum limited the development of *Rhizoctonia solani* in potato plants by growing along its hyphae and inducing lysis (Lahlali and Hijri 2010). In addition, Epicoccum species as endophytes can produce antifungal compounds, such as epicolactone that exhibits an inhibitory activity against Remotididymella solani, epicoccamide D that induces morphogenesis and pigment formation in phytopathogenic fungus Phoma destructiva and flavipin that inhibits the growth of several fungal phytopathogens (Madrigal et al. 1991; Wangun et al. 2007; Fávaro et al. 2012; Talontsi et al. 2013). Therefore, endophytes isolated from tea plants, E. dendrobii, E. italicum, E. jingdongense, E. mackenziei, E. oryzae, E. rosae and E. tobaicum, may be beneficial species with biological control potential. Future studies could determine the inhibitory activity of these endophytes against the dominant pathogens in tea plants, such as Colletotrichum camelliae, C. fructicola, Didymella segeticola, Exobasidium vexans, Discula theae-sinensis and Pestalotiopsis theae and then identify the antifungal compounds.

The potential factors influencing the prevalence and pathogenicity of tested species in *Epicoccum* genus may be the different host-pathogen interaction patterns. Various infection strategies were deployed by pathogens to facilitate their own infection, such as secreting effectors, reprogramming the host transcriptome, rewiring host phytohormone signalling and disarming plant immune outputs (Wang et al. 2022). For *E. nigrum*, many strains secreted enzymes including amylases and proteases expected to participate mainly in the later stages of the infection (Ogórek et al. 2020). *Epicoccum sorghi* secreted polyglycine hydrolases to cleave the polyglycine linker of chitinases, antifungal proteins from *Zea mays* (Naumann et al. 2014; Naumann et al. 2017). To defend against diverse pathogens, plants have also evolved a robust innate immune system (Wang et al. 2022). Then, we speculated that *E. anhuiense, E. draconis, E. latusicollum, E. poaceicola* and *E. puerense* may adopt different infection strategies to invade tea plant (LJ43) leaves, resulting in the different outcome of host plant-pathogen interactions.

In summary, this study represents a comprehensive investigation of Didymellaceae family strains isolated from tea plant leaves of ten provinces in China. Combined with multi-locus (ITS, LSU, *RPB2* and *TUB2*) phylogenetic analysis and morphological characteristics, a total of 240 isolates were identified as 25 species of six genera, including 19 known species and six novel species. Amongst all isolates, *Didymella segeticola* was the most dominant species. Pathogenicity analysis showed that their virulence varied. These results help us comprehend the diversity of Didymellaceae family in tea plants and provide a reference for disease management.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the Fundamental Research Funds for the Provincial Universities of Zhejiang (2020YQ001); The Open Fund of State Key Laboratory of Tea Plant Biology and Utilization (SKLTOF20200109); Zhejiang Science and Technology Major Program on Agricultural New Variety Breeding-Tea Plant (2021C02067-7); the Scientific Research Project of Zhejiang Education Department (No. Y202250195); the Natural Science Foundation of Zhejiang Province (LY22C160001); the Scientific Research and Development Foundation of Zhejiang A & F University (2020FR016; 2021LFR046).

Author contributions

Data curation: HJ. Funding acquisition: YW, CW. Investigation: QL, XC, HR. Writing - original draft: YT. Writing - review and editing: WL.

Author ORCIDs

Wuyun Lv () https://orcid.org/0000-0003-3781-0763

Data availability

Sequence data from this study can be obtained from GenBank at https://www.ncbi.nlm. nih.gov/genbank/ with the accession numbers as listed in Suppl. material 1.

References

- Aementado HDR, Balendres MAO (2023) Identification of *Epicoccum poaceicola* causing eggplant leaf spot and its cross-infection potential to other solanaceous vegetable crops. Archiv für Phytopathologie und Pflanzenschutz 56(11): 872–888. https://doi.org/10.1080/03235408.2023.2227328
- An J, Fan C, Fu Z, Zhang H, Yang P (2022) Analyses of the complete mitochondrial genome of *Paraconiothyrium* sp. and gene rearrangement diversity in the *Pleosporales*. Diversity 14(8): 601. https://doi.org/10.3390/d14080601
- Aveskamp MM, Verkley GJM, de Gruyter J, Murace MA, Perelló A, Woudenberg JHC, Groenewald JZ, Crous PW (2009) DNA phylogeny reveals polyphyly of *Phoma*

section *Peyronellaea* and multiple taxonomic novelties. Mycologia 101(3): 363–382. https://doi.org/10.3852/08-199

- Aveskamp MM, de Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW (2010) Highlights of the Didymellaceae: A polyphasic approach to characterise *Phoma* and related *Pleosporalean* genera. Studies in Mycology 65: 1–60. https://doi.org/10.3114/ sim.2010.65.01
- Bernardi C, Rey MS, Júnior AW, Pietrobom JH, de Barros DR (2022) First report of *Epi*coccum nigrum causing leaf spot of *Eugenia involucrata* in Brazil. Plant Disease https://doi.org/10.1094/PDIS-09-21-1925-PDN
- Boerema GH (1993) Contributions towards a monograph of *Phoma* (Coelomycetes) II. Section *Peyronellaea*. Persoonia 15: 197–221.
- Boerema GH, de Gruyter J, Noordeloos ME, Hamers MEC (2004) *Phoma* identification manual: Differentiation of specific and infraspecific taxa in culture. CABI publishing, Wallingford, UK, 425 pp. https://doi.org/10.1079/9780851997438.0000
- Braga RM, Padilla G, Araújo WL (2018) The biotechnological potential of *Epicoccum* spp.: Diversity of secondary metabolites. Critical Reviews in Microbiology 44(6): 759–778. https://doi.org/10.1080/1040841X.2018.1514364
- Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller JM, Abang MM, Zhang JZ, Yang YL, Phoulivong S, Liu ZY, Prihastuti H, Shivas RG, McKenzie EHC, Johnston PR (2009) A polyphasic approach for studying *Colletotrichum*. Fungal Diversity 39: 183–204. https://doi. org/10.1016/j.riam.2009.11.001
- Calatayud V, Sanz MJ, Aptroot A (2001) *Lichenopyrenis galligena* (Pleomassariaceae), a new genus of gall-forming lichenicolous fungi on *Leptochidium*. Mycological Research 105(5): 634–637. https://doi.org/10.1017/S0953756201003963
- Chen Q, Jiang JR, Zhang GZ, Cai L, Crous PW (2015a) Resolving the *Phoma* enigma. Studies in Mycology 82(1): 137–217. https://doi.org/10.1016/j.simyco.2015.10.003
- Chen Q, Zhang K, Zhang G, Cai L (2015b) A polyphasic approach to characterise two novel species of *Phoma* (Didymellaceae) from China. Phytotaxa 197(4): 267–281. https://doi.org/10.11646/phytotaxa.197.4.4
- Chen Q, Hou LW, Duan WJ, Crous PW, Cai L (2017) Didymellaceae revisited. Studies in Mycology 87(1): 105–159. https://doi.org/10.1016/j.simyco.2017.06.002
- de Gruyter J, Noordeloos ME, Boerema GH (1998) Contributions towards a monograph of *Phoma* (*Coelomycetes*) – I. 3. Section *Phoma*: Taxa with conidia longer than 7 μm. Persoonia 16: 471–490.
- de Gruyter J, Aveskamp MM, Woudenberg JHC, Verkley GJM, Groenewald JZ, Crous PW (2009) Molecular phylogeny of *Phoma* and allied anamorph genera: Towards a reclassification of the *Phoma* complex. Mycological Research 113(4): 508–519. https://doi.org/10.1016/j.mycres.2009.01.002
- Du BX, Yan DF, Sun B, Xiangchuan L, Dao KQ, Li XQ (2012) Cunninghamia praelanceolata sp. nov. with associated epiphyllous fungi from the upper Miocene of eastern Zhejiang, S. E China and their palaeoecological implications. Review of Palaeobotany and Palynology 182: 32–43. https://doi.org/10.1016/j.revpalbo.2012.06.002
- Fang K, Zhou J, Chen L, Li YX, Yang AL, Dong XF, Zhang HB (2021) Virulence and community dynamics of fungal species with vertical and horizontal transmission on a plant with multiple infections. PLoS Pathogens 17(7): e1009769. https://doi.org/10.1371/ journal.ppat.1009769
- Fávaro LC, Sebastianes FL, Araújo WL (2012) Epicoccum nigrum P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. PLoS ONE 7(6): e36826. https://doi.org/10.1371/journal.pone.0036826

- Gonçalves MFM, Esteves AC, Alves A (2020) Revealing the hidden diversity of marine fungi in Portugal with the description of two novel species, *Neoascochyta fuci* sp. nov. and *Paraconiothyrium salinum* sp. nov. International Journal of Systematic and Evolutionary Microbiology 70(10): 5337–5354. https://doi.org/10.1099/ijsem.0.004410
- Guo Z, Xie H, Wang H, Huang Y, Chen Q, Xiang L, Yu Z, Yang X (2020) Leaf spot caused by *Didymella segeticola* on tobacco in China. Plant Disease 104(5): 1559. https://doi. org/10.1094/PDIS-11-19-2398-PDN
- Han VC, Yu NH, Yoon H, Son YK, Lee BH, Kim JC (2021) First report of *Epicoccum to-baicum* associated with leaf spot on flowering cherry in South Korea. Plant Disease 105(9): 2734. https://doi.org/10.1094/PDIS-12-20-2711-PDN
- Han SL, Wang MM, Ma ZY, Raza M, Zhao P, Liang JM, Gao M, Li YJ, Wang JW, Hu DM, Cai L (2023) *Fusarium* diversity associated with diseased cereals in China, with an updated phylogenomic assessment of the genus. Studies in Mycology 104(1): 87–148. https://doi.org/10.3114/sim.2022.104.02
- Hou L, Hernández-Restrepo M, Groenewald JZ, Cai L, Crous PW (2020a) Citizen science project reveals high diversity in Didymellaceae (*Pleosporales, Dothideomycetes*). MycoKeys 65: 49–99. https://doi.org/10.3897/mycokeys.65.47704
- Hou LW, Groenewald JZ, Pfenning LH, Yarden O, Crous PW, Cai L (2020b) The phoma-like dilemma. Studies in Mycology 96: 309–396. https://doi.org/10.1016/j.simyco.2020.05.001
- Huang WY, Cai YZ, Hyde KD, Corke H, Sun M (2008) Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Diversity 33: 75. https://doi.org/10.1002/yea.1639
- Huang H, Li D, Jiang S, Yang R, Yang Y, Xia Z, Jiang X, Zhao Y, Wang D, Song B, Chen Z (2023) Integrated transcriptome and proteome analysis reveals that the antimicrobial griseofulvin targets *Didymella segeticola* beta-tubulin to control tea leaf spot. Phytopathology 113(2): 194–205. https://doi.org/10.1094/PHYTO-02-22-0061-R
- Hyde KD, Jones EBG, Liu JK, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai DQ, Diederich P, Dissanayake A, Doilom M, Doveri F, Hongsanan S, Jyawardena R, Lawrey JD, Li YM, Liu YX, Lücking R, Monkai J, Muggia L, Nelsen MP, Pang KL, Phookamsak R, Senanayake IC, Shearer CA, Suetrong S, Tanaka K, Thambugala KM, Wijayawardene NN, Wikee S, Wu HX, Zhang Y, Aguirre-Hudson B, Alias SA, Aptroot A, Bahkali AH, Bezerra JL, Ghat DJ, Camporesi E, Chukeatirote E, Gueidan C, Hawksworth DL, Hirayama K, Hoog SD, Kang JC, Knudsen K, Li WJ, Li XH, Liu ZY, Mapook A, McKenzie EHC, Miller AN, Mortimer PE, Phillips AJL, Raja HA, Scheuer C, Schumm F, Taylor JE, Tian Q, Tibpromma S, Wanasinghe DN, Wang Y, Xu JC, Yacharoen S, Yan JY, Zhang M (2013) Families of *Dothideomycetes*. Fungal Diversity 63(1): 1–313. https://doi.org/10.1007/s13225-013-0263-4
- Jayasiri SC, Hyde KD, Jones EBG, Jeewon R, Ariyawansa HA, Bhat JD, Camporesi E, Kang JC (2017) Taxonomy and multigene phylogenetic evaluation of novel species in *Boeremia* and *Epicoccum* with new records of *Ascochyta* and *Didymella* (Didymellaceae). Mycosphere 8(8): 1080–1101. https://doi.org/10.5943/mycosphere/8/8/9
- Jeong MH, Choi ED, Park SY (2022a) First report of brown leaf spot caused by *Epicoc-cum tobaicum* on oat (*Avena sativa*) in Korea. Plant Disease https://doi.org/10.1094/PDIS-11-22-2532-PDN
- Jeong YJ, Kwon OK, Jeong AR, Lee H, Moon H, Lee ON, Hong JK, Park CJ (2022b) Population structure of *Stagonosporopsis* species associated with cucurbit gummy stem blight in Korea. The Plant Pathology Journal 38(5): 522–532. https://doi. org/10.5423/PPJ.OA.07.2022.0102

- Jiang JR, Chen Q, Cai L (2017) Polyphasic characterisation of three novel species of *Paraboeremia*. Mycological Progress 16(4): 285–295. https://doi.org/10.1007/ s11557-016-1253-1
- Jiang N, Fan X, Tian C (2021) Identification and characterization of leaf-inhabiting fungi from *Castanea* plantations in China. Journal of Fungi (Basel, Switzerland) 7(1): 64. https://doi.org/10.3390/jof7010064
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Kodsueb R, Dhanasekaran V, Aptroot A, Lumyong S, McKenzie EHC, Hyde KD, Jeewon R (2006) The family Pleosporaceae: Intergeneric relationships and phylogenetic perspectives based on sequence analyses of partial 28S rDNA. Mycologia 98(4): 571– 583. https://doi.org/10.1080/15572536.2006.11832660
- Kruys A, Eriksson OE, Wedin M (2006) Phylogenetic relationships of coprophilous Pleosporales (Dothideomycetes, Ascomycota), and the classification of some bitunicate taxa of unknown position. Mycological Research 110(5): 527–536. https://doi. org/10.1016/j.mycres.2006.03.002
- Kularathnage ND, Senanayake IC, Wanasinghe DN, Doilom M, Stephenson SL, Song J, Dong W, Xu B (2023) Plant-associated novel *Didymellaceous* taxa in the South China botanical garden (Guangzhou, China). Journal of Fungi (Basel, Switzerland) 9(2): 182. https://doi.org/10.3390/jof9020182
- Kumhar KC, Babu A, Nisha SN, Kishor Chand Kumhar (2022) Management of tea (*Camellia sinensis*) diseases with application of microbes: A review. Innovare Journal of Agricultural Science 10: 6–10. https://doi.org/10.22159/ijags.2022.v10i2.44271
- Lahlali R, Hijri M (2010) Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. FEMS Microbiology Letters 311(2): 152–159. https://doi.org/10.1111/j.1574-6968.2010.02084.x
- Li D, Zhang T, Song Q, Liu J, Zhang H, Luan F (2022) First report of leaf spot disease on *Cinnamomum camphora* (camphor tree) caused by *Epicoccum poaceicola* in China. Plant Disease 106(3): 1059. https://doi.org/10.1094/PDIS-04-21-0683-PDN
- Li Z, Hu J, Li W, Wang H, Guo Z, Cheng X, Cai L, Shui C (2023) Characteristics of *Epicoccum latusicollum* as revealed by genomic and metabolic phenomic analysis, the causal agent of tobacco *Epicoccus* leaf spot. Frontiers in Plant Science 14: 1199956. https://doi.org/10.3389/fpls.2023.1199956
- Liu YH, Zhang CQ, Dai DJ (2019) First report of leaf black spot on White Chrysanthemum (*Chrysanthemum morifolium*) caused by *Phoma bellidis* in China. Plant Disease 103(9): 2475. https://doi.org/10.1094/PDIS-03-19-0611-PDN
- Liu SY, He J, Bian JY, Qi XL, Song Q, Huang L (2020) First report of *Epicoccum poaceicola* causing leaf spot on *Phyllostachys viridis* in China. Plant Disease 104(12): 3257. https://doi.org/10.1094/PDIS-03-20-0484-PDN
- Liu J, Long Z, Xue L, Li C (2022) First report of *Didymella sinensis* causing leaf blight on Italian ryegrass in China. Plant Disease. https://doi.org/10.1094/PDIS-08-22-1831-PDN
- Lv R, Zheng L, Zhu Z, Pan L, Huang J, Hsiang T (2011) First report of stem blight of *Eleocharis dulcis* caused by *Phoma bellidis* in China. Plant Disease 95(9): 1190. https://doi.org/10.1094/PDIS-05-11-0438
- Madrigal C, Tadeo JL, Melgarejo P (1991) Relationship between flavipin production by *Epicoccum nigrum* and antagonism against *Monilinia laxa*. Mycological Research 95(12): 1375–1381. https://doi.org/10.1016/S0953-7562(09)80388-2

- Manawasinghe IS, Jayawardena RS, Li HL, Zhou YY, Phillips AJL, Wanasinghe DN, Dissanayake AJ, Li XH, Li YH, Hyde KD, Yan JY (2021) Microfungi associated with *Camellia sinensis*: A case study of leaf and shoot necrosis on tea in Fujian, China. Mycosphere 12(1): 430–518. https://doi.org/10.5943/mycosphere/12/1/6
- Ming Q, Huang X, Guo L, Liu D, Qin L, He Y, Tang Y, Liu Y, Liu J, Li P (2022) Diversity of endophytic fungi in *Coptis chinensis* Franch. and their activity against methicillin-resistance *Staphylococcus aureus*. Folia Microbiologica 67(6): 965–974. https://doi.org/10.1007/s12223-022-00994-1
- Moriwaki J, Sato T (2009) A new combination for the causal agent of tea anthracnose: *Discula theae-sinensis* (I. Miyake) Moriwaki & Toy. Sato, comb. Nov. Journal of General Plant Pathology 75(5): 359–361. https://doi.org/10.1007/s10327-009-0183-z
- Naumann TA, Wicklow DT, Price NPJ (2014) Polyglycine hydrolases secreted by Pleosporineae fungi that target the linker of plant class IV chitinases. The Biochemical Journal 460(2): 187–198. https://doi.org/10.1042/BJ20140268
- Naumann TA, Bakota EL, Price NPJ (2017) Recognition of corn defense chitinases by fungal polyglycine hydrolases. Protein Science 26(6): 1214–1223. https://doi. org/10.1002/pro.3175
- Ogórek R, Przywara K, Piecuch A, Cal M, Lejman A, Matkowski K (2020) Plant-fungal interactions: A case study of *Epicoccoum nigrum* Link. Plants 9(12): 1691. https://doi. org/10.3390/plants9121691
- Rambaut A, Drummond A (2008) FigTree: Tree figure drawing tool, version 1.2.2. Institute of Evolutionary Biology, University of Edinburgh.
- Ren Y, Li D, Zhao X, Wang Y, Bao X, Wang X, Wu X, Wang D, Song B, Chen Z (2019) Whole genome sequences of the tea leaf spot pathogen *Didymella segeticola*. Phytopathology 109(10): 1676–1678. https://doi.org/10.1094/PHYTO-02-19-0050-A
- Rivera-Vega LJ, Zhou W, Buchman LW, Valencia CU, Jack ALH, Lopez DC, Sword GA (2022) Plant-associated fungi affect above- and belowground pest responses to soybean plants. Journal of Applied Microbiology 133(2): 422–435. https://doi.org/10.1111/jam.15554
- Rodríguez J, Vázquez L, Flórez AB, Mayo B (2023) *Epicoccum* sp. as the causative agent of a reddish-brown spot defect on the surface of a hard cheese made of raw ewe milk. International Journal of Food Microbiology 406: 110401. https://doi.org/10.1016/j. ijfoodmicro.2023.110401
- Romero LR, Tacke D, Koopmann B (2021) First characterisation of the *Phoma* species complex on maize leaves in Central Europe. Pathogens (Basel, Switzerland) 10(9): 1216. https://doi.org/10.3390/pathogens10091216
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics (Oxford, England) 19(12): 1572–1574. https://doi. org/10.1093/bioinformatics/btg180

Saccardo PA (1880) Fungi Gallici lecti a cl. viris P. Brunaud, Abb. Letendre, A. Malbranche, J. Therry, vel editi in Mycotheca Gallica C. Roumeguèri. Series II. Michelia 2: 39–135.

- Samaradiwakara NP, de Farias ARG, Tennakoon DS, Bhunjun CS, Hyde KD, Lumyong S (2023) Sexual morph of *Allophoma tropica* and *Didymella coffeae-arabicae* (Didymellaceae, Pleosporales, Dothideomycetes), including novel host records from leaf litter in Thailand. New Zealand Journal of Botany, 1–24. https://doi.org/10.1080/002882 5X.2023.2272957
- Schol-Schwarz M (1959) The genus *Epicoccum* link. Transactions of the British Mycological Society 42(2): 149–173. https://doi.org/10.1016/S0007-1536(59)80024-3

- Seblani R, Keinath AP, Munkvold G (2023) Gummy stem blight: One disease, three pathogens. Molecular Plant Pathology 24(8): 825–837. https://doi.org/10.1111/ mpp.13339
- Silva-Hanlin DMW, Hanlin RT (1999) Small subunit ribosomal RNA gene phylogeny of several loculoascomycetes and its taxonomic implications. Mycological Research 103(2): 153–160. https://doi.org/10.1017/S0953756298006972
- Sivanesan A (1990) CMI descriptions of pathogenic fungi and bacteria, Set 99, Nos. 981–990. Mycopathologia 109: 41–62. https://doi.org/10.1007/BF00437005
- Solarte F, Muñoz CG, Maharachchikumbura SSN, Álvarez E (2017) Diversity of *Neopestalotiopsis* and *Pestalotiopsis* spp., causal agents of guava scab in Colombia. Plant Disease 102(1): 49–59. https://doi.org/10.1094/PDIS-01-17-0068-RE
- Sutton BC (1980) The coelomycetes. Fungi imperfecto with pycnidia, acervuli and stromata. Australasian Plant Pathology 9(4): 120–121. https://doi.org/10.1007/BF03213663
- Talontsi FM, Dittrich B, Schüffler A, Sun H, Laatsch H (2013) Epicoccolides: Antimicrobial and antifungal polyketides from an endophytic fungus *Epicoccum* sp. associated with *Theobroma cacao*. European Journal of Organic Chemistry 2013(15): 3174– 3180. https://doi.org/10.1002/ejoc.201300146
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12): 2725– 2729. https://doi.org/10.1093/molbev/mst197
- Thambugala KM, Wanasinghe DN, Phillips AJL, Camporesi E, Bulgakov TS, Phukhamsakda C, Ariyawansa HA, Goonasekara ID, Phookamsak R, Dissanayake AJ, Tennakoon DS, Tibpromma S, Chen YY, Liu ZY, Hyde KD (2017) Mycosphere notes 1–50: Grass (Poaceae) inhabiting *Dothideomycetes*. Mycosphere 8(4): 697–796. https://doi. org/10.5943/mycosphere/8/4/13
- Tian Y, Zhang Y, Qiu C, Liu Z (2021) First report of leaf spot of *Weigela florida* caused *Epicoccum layuense* in China. Plant Disease 105(8): 2243. https://doi.org/10.1094/ PDIS-07-20-1498-PDN
- Valenzuela-Lopez N, Cano-Lira JF, Guarro J, Sutton DA, Wiederhold N, Crous PW, Stchigel AM (2018) Coelomycetous *Dothideomycetes* with emphasis on the families Cucurbitariaceae and Didymellaceae. Studies in Mycology 90(1): 1–69. https://doi. org/10.1016/j.simyco.2017.11.003
- von Szilvinyi A (1936) Archiv für Hydrobiologie Supplement 14. Tropische Binnengewässer 6a: 519.
- Wanasinghe DN, Jeewon R, Peršoh D, Jones EBG, Camporesi E, Bulgakov TS, Gafforov YS, Hyde KD (2018a) Taxonomic circumscription and phylogenetics of novel didymellaceous taxa with brown muriform spores. Studies in Fungi 3(1): 152–175. https://doi.org/10.5943/sif/3/1/17
- Wanasinghe DN, Phukhamsakda C, Hyde KD, Jeewon R, Lee HB, Jones EBG, Tibpromma S, Tennakoon DS, Dissanayake AJ, Jayasiri SC, Gafforov Y, Camporesi E, Bulgakov TS, Ekanayake AH, Perera EH, Samarakoon MC, Goonasekara ID, Mapook A, Li WJ, Senanayake IC, Li J, Norphanphoun C, Doilom M, Bahkali AH, Xu J, Mortimer PE, Tinell L, Yibell S, Karunarathna SC (2018b) Fungal diversity notes 709 – 839: Taxonomic and phylogenetic contributions to fungal taxa with an emphasis on fungi on Rosaceae. Fungal Diversity 89(1): 1–236. https://doi.org/10.1007/s13225-018-0395-7
- Wang YC, Hao XY, Wang L, Xiao B, Wang XC, Yang YJ (2016) Diverse Collectorichum species cause anthracnose of tea plants (Camellia sinensis (L.) O. Kuntze) in China. Scientific Reports 6(1): 35287. https://doi.org/10.1038/srep35287

- Wang X, Wu X, Jiang S, Yin Q, Li D, Wang Y, Wang D, Chen Z (2021) Whole genome sequence and gene annotation resource for *Didymella bellidis* associated with tea leaf spot. Plant Disease 105(4): 1168–1170. https://doi.org/10.1094/PDIS-05-20-0939-A
- Wang Y, Pruitt RN, Nürnberger T, Wang Y (2022) Evasion of plant immunity by microbial pathogens. Nature Reviews. Microbiology 20(8): 449–464. https://doi.org/10.1038/ s41579-022-00710-3
- Wang H, Shi YP, Lin S, Liu Z, Guo J, Xiuming H, Liao H, Gao Q, Zhou H (2023) First report of *Epicoccum latusicollum* causing leaf blight on *Curcuma kwangsiensis* in China. Plant Disease 107(8): 2546. https://doi.org/10.1094/PDIS-11-22-2668-PDN
- Wangun HVK, Dahse HM, Hertweck C (2007) Epicoccamides B-D, glycosylated tetramic acid derivatives from an *Epicoccum* sp. associated with the tree fungus *Pholiota squarrosa*. Journal of Natural Products 70(11): 1800–1803. https://doi.org/10.1021/ np070245q
- Woudenberg JHC, Aveskamp MM, de Gruyter J, Spiers AG, Crous PW (2009) Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. Persoonia 22(1): 56–62. https://doi.org/10.3767/003158509X427808
- Xu H, Cui J, Zhou R, Fu J, Hao N (2016) First report of leaf spot disease in Angelica dahurica caused by Phoma bellidis in China. Journal of Phytopathology 164(7–8): 448–454. https://doi.org/10.1111/jph.12470
- Xu X, Zhang L, Yang X, Li J, Wang X, Zhao J, Xiang W (2022) First report of maize stalk rot caused by *Epicoccum latusicollum* on maize (*Zea mays* L.) in China. Plant Disease 106(8): 2255. https://doi.org/10.1094/PDIS-11-21-2392-PDN
- Yang AL, Chen L, Fang K, Dong XF, Li YX, Zhang HB, Yu ZF (2021) Remotididymella ageratinae sp. nov. and Remotididymella anemophila sp. nov., two novel species isolated from the invasive weed Ageratina adenophora in PR China. International Journal of Systematic and Evolutionary Microbiology 71(1): 004572. https://doi.org/10.1099/ ijsem.0.004572
- Yang J, Chen C, Yin X, Xu H, Long H, Gu G, Shu R, Yuan J, Zhou H (2022) *Didymella segeticola* is a new pathogen causing leaf spot disease on *Zanthoxylum bungeanum*. New Zealand Journal of Crop and Horticultural Science, 1–10. https://doi.org/10.1080/0 1140671.2022.2080235
- Yang AL, Chen L, Cheng L, Li JP, Zeng ZY, Zhang HB (2023a) Two novel species of Mesophoma gen. nov. from China. Current Microbiology 80(4): 129. https://doi. org/10.1007/s00284-023-03238-8
- Yang X, Yi X, Ni K, Zhang Q, Shi Y, Chen L, Zhao Y, Zhang Y, Ma Q, Cai Y, Ma L, Ruan J (2023b) Patterns and abiotic drivers of soil organic carbon in perennial tea (*Camellia sinensis* L.) plantation system of China. Environmental Research 237: 116925. https://doi.org/10.1016/j.envres.2023.116925
- Yuan J, Zeng XY, Geng K, Wijayawardene NN, Bhat JD, Wu SP, Wang Y, Yang ZF (2021) Allophoma species (Pleosporales: Didymellaceae) associated with Thunbergia grandiflora in Guangxi Province, China. Biodiversity Data Journal 9: e63643. https://doi. org/10.3897/BDJ.9.e63643
- Zhang Y, Schoch CL, Fournier J, Crous PW, de Gruyter J, Woudenberg JHC, Hirayama K, Tanaka K, Pointing SB, Spatafora JW, Hyde KD (2009a) Multi-locus phylogeny of *Pleosporales*: A taxonomic, ecological and evolutionary re-evaluation. Studies in My-cology 64: 85–102. https://doi.org/10.3114/sim.2009.64.04
- Zhang Y, Fournier J, Crous PW, Pointing SB, Hyde KD (2009b) Phylogenetic and morphological assessment of two new species of *Amniculicola* and their allies (*Pleosporales*). Persoonia 23(1): 48–54. https://doi.org/10.3767/003158509X472187

- Zhang J, Yang R, Jiang S, Li D, Li T, Yang Z, Yuan J, Zhao Y, Tan X, Wang D, Chen Z (2022) First report of *Stagonosporopsis caricae* causing chayote leaf spot in Guizhou Province, China. Plant Disease. https://doi.org/10.1094/PDIS-07-22-1703-PDN
- Zhang J, Yin QX, Jaing SL, Li DX, Tang D, Zhang Y, Ma YL, Li PY, Wang Y, Wang DL, Chen Z (2023) First report of *Epicoccum mackenziei* causing dark brown spot of tea leaf in Guizhou Province, Southwestern China. Plant Disease 107(7): 2247. https://doi. org/10.1094/PDIS-10-22-2372-PDN
- Zhao XZ, Wang Y, Li DX, Ren YF, Chen Z (2018) Morphological characterization and phylogenetic analysis of the pathogen *Phoma segeticola* var. *camelliae* causing a new tea disease. Acta Phytopathologica Sinica 48: 32556–32559. https://doi.org/10.13926/j. cnki.apps.000184

Supplementary material 1

Isolates of the Didymellaceae family in this study and GenBank accession numbers of the generated sequences

Authors: Yuchun Wang, Yiyi Tu, Xueling Chen, Hong Jiang, Hengze Ren, Qinhua Lu, Chaoling Wei, Wuyun Lv

Data type: docx

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.119536.suppl1


Short Communication

The ecology of lichenicolous lichens: a case-study in Italy

Pier Luigi Nimis¹⁰, Elena Pittao¹, Monica Caramia¹, Piero Pitacco¹⁰, Stefano Martellos¹⁰, Lucia Muggia¹⁰

1 University of Trieste, Department of Life Sciences, via Giorgieri 10, 34127 Trieste, Italy Corresponding author: Lucia Muggia (Imuggia@units.it)

Abstract

This paper, with Italy as a case-study, provides a general overview on the ecology of lichenicolous lichens, i.e. those which start their life-cycle on the thallus of other lichens. It aims at testing whether some ecological factors do exert a positive selective pressure on the lichenicolous lifestyle. The incidence of some biological traits (photobionts, growth-forms and reproductive strategies) in lichenicolous and non-lichenicolous lichens was compared, on a set of 3005 infrageneric taxa potentially occurring in Italy, 189 of which are lichenicolous. Lichenicolous lichens have a much higher incidence of coccoid (non-trentepohlioid) green algae, crustose growth-forms and sexual reproduction. A matrix of the 2762 species with phycobionts and some main ecological descriptors was subjected to ordination. Lichenicolous lichens occupy a well-defined portion of the ecological space, tending to grow on rocks in dry, well-lit habitats where a germinating spore is likely to have a short life-span, at all altitudes. This corroborates the hypothesis that at least some of them are not true "parasites", as they are often called, but gather the photobionts - which have already adapted to local ecological conditions - from their hosts, eventually developing an independent thallus.

Key words: Algal theft, host, lichenised fungi, photobiont, sexual reproduction, symbioses



Academic editor: Thorsten Lumbsch Received: 15 February 2024 Accepted: 28 March 2024 Published: 31 May 2024

Citation: Nimis PL, Pittao E, Caramia M, Pitacco P, Martellos S, Muggia L (2024) The ecology of lichenicolous lichens: a case-study in Italy. MycoKeys 105: 253–266. https://doi. org/10.3897/mycokeys.105.121001

Copyright: © Pier Luigi Nimis 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).

Introduction

Lichens are a symbiosis between a fungal partner, the mycobiont and one or more photosynthetic partners, the photobionts, which is either a cyanobacterium (cyanobiont), a green microalga (phycobiont) or both (Hawksworth 1988; Spribille et al. 2022; Sanders 2023, 2024). The photobiont is a carbon source for the heterotrophic mycobiont (Nash 2008) and a nitrogen source for cyanolichens, due to the cyanobacterium fixing the atmospheric nitrogen (Rikkinen 2002). In return, the mycobiont provides the photobiont with optimal living conditions, protecting it from high temperatures, light (UV radiation) and drought (Palmqvist et al. 2008; Grube 2018). Some authors regard lichens as an example of controlled-parasitism, since the fungus seems to obtain most of the benefits from the photobionts and to control them (Richardson 1999; Nash 2008). Many other organisms have been found dwelling on the surface of or within lichen thalli (Honegger 1992; Bates et al. 2011), such as non-photosynthetic bacteria (Grube 2018), unicellular basidiomycete yeasts (Spribille et al. 2016) and non-lichenised fungi (Hawksworth 1988; Arnold et al. 2009; Muggia et al. 2016; Diederich et al. 2018). Thus, lichens were recently re-defined as self-sustaining microecosystems (Insarova and Blagoveshchenskaya 2016; Hawksworth and Grube 2020, but see also the criticism by Sanders 2024)). Additional complexity was reported inside a single lichen thallus by the co-existence of multiple phycobionts (del Campo et al. 2012; Muggia et al. 2014; Moya et al. 2017; Moya et al. 2020) which respond differently to abiotic stressors and perhaps also of multiple mycobionts (Ament-Velásquez et al. 2021). Phycobiont co-existence is advantageous for lichens under extreme environmental conditions, in which this phenomenon seems to be common (del Hoyo et al. 2011; Casano et al. 2011, 2015). Lichens also host many lichenicolous, non-lichenised fungi which gain their nutrition from the host lichen thallus, draining it of its photosynthetic products, thus being regarded as parasitic or saprophytic (Hawksworth 1988; Rambold and Triebel 1992; Hafellner 2018) going as far as being necrotrophic when they have devastating effects on either the mycobiont (Diederich 1996; de los Rìos and Grube 2000) or the photobiont (Grube and Hafellner 1990).

A peculiar case is that of lichenicolous lichens, which regularly start their life-cycle on the thalli of other lichen species, eventually building their own lichenised thallus (Poelt 1958, 1990; Rambold and Triebel 1992, Diederich et al. 2018). Some of them are specialists, i.e. they can only grow on a certain species of lichen, others are more generalists (Moya et al. 2020). Some lichenicolous lichens simply overgrow other lichens in ecological successions because of space competition (Armstrong and Welch 2007). Others, the so-called cyanotrophic lichens, are green algal lichens that grow on free-living cyanobacteria or cyanobacterial lichens, probably to benefit from their nitrogen-fixing capability (Poelt and Mayrhofer 1988; Rikkinen 2002; Honegger 2012a). Finally, others always start the life-cycle on lichens with the same general type of photobiont. The latter, which are the object of the present study, are often referred to as "parasites" (Poelt 1958; Honegger 2012b), although according to several authors (e.g., Richardson (1999); Diederich et al. (2018); Moya et al. (2020)), they take over the photobiont from the host to avoid re-establishing the symbiosis by searching for a new photobiont of their own. Once the photobiont has been acquired, it can be maintained or be substituted with a different and often more favourable algal partner through algal switching (Friedl 1987; Piercey-Normore and De Priest 2001; Moya et al. 2020). To our knowledge, no large-scale assessment of species traits and ecology of the total lichenicolous lichen biota across a broad spectrum of ecological conditions was ever attempted. Taking advantage of the availability of ecological indicator values for all lichens of Italy (Nimis 2016), we have tried to provide such an overview at the level of a well-known, rich lichen flora encompassing several biomes, as that of Italy. The main aim of this paper is to test whether lichenicolous lichens differ from non-lichenicolous lichens in their ecology, i.e. whether some ecological factors could be detected, which may exert a positive selective pressure on the acquisition of a lichenicolous life-style.

Material and methods

The list of lichenicolous and non-lichenicolous lichens, their bio-morphological traits and their ecological descriptors were retrieved from Nimis and Martellos (2023). We have considered all lichen species reported from Italy, plus those known from neighbouring countries, whose presence in Italy is possible.

The bio-morphological traits are:

- a. *Photobionts*: Ch (phycobiont: green algae other than *Trentepohlia*), Tr (phycobiont: *Trentepohlia*), Cy.h (cyanobiont, filamentous), Cy.c (cyanobiont, coccoid);
- b. *Reproductive strategies*: A.f (mainly asexual, by thallus fragmentation),
 A.i (mainly asexual, by isidia or isidia-like structures), A.s (mainly asexual, by soredia or soredia-like structures), S (mainly sexual, meiotic spores of the mycobiont);
- c. Growth forms: Cr (crustose), Fol (foliose), Frut (fruticose), Lepr (leprose), Sq (squamulose).

The ecological descriptors are:

- d. *Substrata*: Epiph (epiphytic: on bark, leaves, lignum), **Sax** (saxicolous: on rocks), **Terr** (terricolous: on soil, terricolous mosses and plant debris);
- e. *Phytoclimatic range*: Oc (oceanic: restricted to areas with a humid-warm oceanic climate), Suboc (suboceanic: most common in areas with a humid-warm climate), Subc (subcontinental: restricted to areas with a dry-subcontinental climate);
- f. Altitudinal distribution (vegetation belts, as a proxy of temperature): A1 (eu-Mediterranean), A2 (submediterranean), A3 (montane), A4 (subalpine and oroboreal), A5 (alpine), A6 (nival);
- g. Poleotolerance (tolerance to anthropization): from Pol3 (species occurring in heavily disturbed areas) to Pol0 (species exclusively occurring on old trees in ancient, undisturbed forests);
- h. *Ecological indicator values*: these are "expert assessments" that qualitatively express the ecological range of species with respect to different factors on a 5-class ordinal scale (see Nimis (2016)). The predictivity of the values used in this study was tested against real data (Nimis and Martellos 2001) and proved to be high.
 - *pH of the substratum*: from *pH1* (very acid substrata) to *pH5* (basic substrata);
 - Light (solar irradiation): from L1 (in very shaded situations) to L5 (in sites with high direct solar irradiation);
 - Xerophytism (aridity): from X1 (hydro- and hygrophytic, in aquatic or marine situations or sites with a very high frequency of fog) to X5 (very xerophytic);
 - *Eutrophication*: from E1 (not resistant to eutrophication) to E5 (occurring in highly eutrophicated situations).

Data analysis was performed with the R 4.3.0 software (R Core Team 2023). Differences between lichenicolous and non-lichenicolous lichens were tested separately for growth forms, photobionts and reproductive strategies using Pearson's Chi-squared test in the package Rcmdr (Fox and Bouchet-Valat 2023). In order to test whether lichenicolous lichens occupy a well-delimited portion of the ecological space, as compared with non-lichenicolous lichens, the presence-absence matrix of species and ecological descriptors was subjected to Non-metric Multidimensional Scaling (NMDS) ordination after loading the vegan package (Oksanen et al. 2022). The function metaMDS, with Jaccard as dissimilarity index was used. The statistical significance of differences in ecological space occupancy was also tested on the same dissimilarity matrix used for NMDS, with an analysis of multivariate homogeneity of groups dispersions (function BetaDispersion 2.0, Bacaro et al. (2012, 2013)) and a Permutational Multivariate Analysis of Variance (function adonis2). Due to the absence of lichenicolous lichens with cyanobacteria as the main photobiont (see Results), cyanolichens were excluded from this analysis.

Results

On a total of 3005 lichenised species potentially occurring in Italy, 189 were retained as "lichenicolous". The mycobionts of the latter are phylogenetically clustered, most of the species in our dataset belonging to the *Lecanoromycetes* (84.4%), followed by the *Eurotiomycetes* (14.5%). The same applies for their hosts, which mostly belong to the *Lecanoromycetes* (95.3%), followed by the *Eurotiomycetes* (4%).

Table 1 compares the bio-morphological traits of lichenicolous and non-lichenicolous taxa. Lichenicolous lichens significantly differ from the other lichen species in growth forms, photobionts and reproductive strategies (Pearson's Chi-squared test, p < 0.001) and show the highest incidence of crustose forms reproducing sexually, most of them with a green, non-trentepohlioid photobiont.

Fig. 1 shows the NMDS ordination (stress value 0.226) of ecological descriptors (a) and species (b), limited to the 2762 phycolichens. In Fig. 1a, the first axis, from negative to positive scores, reflects a gradient of increasing aridity and solar irradiation, with epiphytic species tending to have negative scores,

Table 1. Comparison of some main biological traits between lichenicolous and non-lichenicolous lichens potentially occurring in Italy (3005 species). All differences are highly significant (p < 0.001).

	Lichen	icolous	Non-lichenicolous 2816 taxa		
Bio-morphological traits	189	taxa			
	n	%	n	%	
Crustose	182	96	2041	72	
oliose	0	0	358	13	
Fruticose	0	0	244	9	
_eprose	0	0	32	1	
Squamulose	7	4	141	5	
Cyanobacteria coccaceous	1	1	52	2	
Cyanobacteria filamentous	0	0	190	7	
Green algae(excl. Trentepohlia)	186	98	2322	82	
Frentepohlia	2	1	252	9	
Asexual (fragmentation)	0	0	39	1	
Asexual (isidia)	4	2	113	4	
Asexual (soredia)	5	3	480	17	
Asexual (other)	1	1	5	0	
Sexual	179	95	2184	78	



Figure 1. NMDS ordination of ecological descriptors (**a**) and of the 2762 species of phycolichens potentially occurring in Italy, with lichenicolous taxa flagged by larger dots (**b**). For abbreviations, see Material and methods.

saxicolous species positive scores and terricolous species occupying an intermediate position. The second axis reflects a gradient, from positive to negative scores, of increasing altitude/decreasing temperatures. Thus, the two axes in Fig. 1a describe an ecological space mainly defined by water (first axis) and temperature (second axis). Tolerance to eutrophication is most frequent amongst species growing in dry sites at low elevations, i.e. where human influence (agriculture, urbanisation) is the highest. The pH of the substrate seems to be less relevant, with a tendency for species growing on basic substrata to be most frequent on rocks in arid and well-lit situations, probably due to the prevalence of calcareous substrata throughout the country. Oceanic and suboceanic species tend to be bound, as it could be expected, to undisturbed, low-elevation, humid-shaded situations, for example, in lowland forests, while subcontinental species appear to be mostly saxicolous in dry situations. Lichenicolous lichens significantly differ (p < 0.001) from the other lichen species in ecological space occupation. Fig. 1b shows the occupancy of the ecological space depicted in Fig. 1a by phycolichens: lichenicolous taxa clearly tend to occupy a well-defined portion of the ecological space, i.e. to have positive scores on the first axis. Table 2 shows the distribution of the values of ecological descriptors in lichenicolous and non-lichenicolous phycolichens. Lichenicolous species differ from non-lichenicolous species in the higher percentage of saxicolous species and the higher values of the xerophytism index, followed by that, partly related, of solar irradiation, while the incidence of oceanic and suboceanic species is lower and that of subcontinental species is higher. Altitude/temperature, eutrophication, pH and poleophoby do not differentiate between the two groups.

Discussion

Lichenicolous lichens proved to be a biologically and ecologically very well-defined guild of species. Most of them reproduce sexually, have a crustose growth-form, a green, non-trentepohlioid photobiont and live on rocks in dry and very well-lit situations, at all altitudes.

	Lichen	icolous	Non-lich	enicolous	
Ecological descriptors	Ecological descriptors (188 taxa) n %		(2574 taxa)		
			n	%	
Epiph	7	4	978	38	
Sax	173	92	1394	54	
Terr	15	8	460	18	
Oc	0	0	48	2	
Suboc	9	5	434	17	
Subc	17	9	84	3	
A1	75	40	955	37	
A2	82	44	1248	48	
A3	101	54	1590	62	
A4	113	60	1377	53	
A5	106	56	879	34	
A6	10	5	137	5	
Pol3	3	2	99	4	
Pol2	23	12	514	20	
Pol1	186	99	2340	91	
Pol0	1	1	212	8	
pH1	50	27	969	38	
pH2	106	56	1635	64	
pH3	96	51	1281	50	
pH4	65	35	747	29	
pH5	56	30	548	21	
L1	2	1	64	2	
L2	6	3	476	18	
L3	42	22	1577	61	
L4	175	93	1927	75	
L5	109	58	831	32	
X1	4	2	359	14	
X2	10	5	1169	45	
Х3	87	46	1529	59	
X4	159	85	981	38	
X5	128	68	225	9	
E1	99	53	1902	74	
E2	121	64	1260	49	
E3	79	42	777	30	
E4	38	20	284	11	
E5	7	4	72	3	

 Table 2. Distribution of the values of ecological descriptors in lichenicolous and non-lichenicolous phycolichens.

Sexual reproduction requires the fungal hypha of the mycobiont to encounter a suitable photobiont to re-establish the symbiosis (Seymour et al. 2005). On the other hand, asexual reproduction consists of vegetative propagules, for example, isidia and soredia, which contain both the fungal and photosynthetic partner, being dispersed simultaneously and establishing a new thallus (Ott 1987). According to Poelt (1993), soredia are the smallest form of a miniaturised lichen and the most successful way to ensure co-dispersion of the two symbionts in a new site. The mycobiont is considered an obligate biont since it cannot occur free-living, because of its slow growth in isolation and incapability to compete with other fungi, while free-living photobionts may be common, especially in humid and moist terrestrial habitats (Nash 2008). One may, therefore, assume that asexual reproduction should be most common amongst lichens dwelling in dry and well-lit conditions, which may be unfavourable to a delicate germinating spore and perhaps also to free-living green algae. However, Nimis and Martellos (2003) have shown that sorediate lichens have a higher incidence in humid-shaded situations and are scarce both in dry, well-lit habitats and in periodically submerged sites, where sexual reproduction is prevalent. The very few lichenicolous lichens in our dataset which do not occur in dry sites – see Fig. 1b – are almost all hydrophytic species.

Both sexual and asexual reproduction have their disadvantages: sexual reproduction has a high metabolic cost and subjects the lichen to low biotic pressures in high-stress environments (Seymour et al. 2005); asexual reproduction implies low genetic recombination and, hence, a lower potential for evolutionary development (Nash 2008). Sexual reproduction could, thus, be essential to lichens of high-stress environments, providing enhanced genetic variability and a high chance of adaptation and survival. This implies also that the mycobiont is more flexible in creating a symbiosis with the better-adapted photobiont amongst those that are compatible. Lichenicolous mycobionts would take advantage of the algae available in the host thallus, thus avoiding the effort of finding an appropriate algal partner (Friedl 1987; Wedin et al. 2016; Moya et al. 2020) and, at the same time, being totally constrained by the photobionts associated with their host. One could object that in a highly stressful environment, such as city downtowns, species with vegetative propagules could prevail (see, for example, Gilbert (1990)). However, Nimis and Martellos (2003) showed that, at least in Italy, the prevalence of lichens with asexual reproduction in urban environments is overestimated, as it involves only very few (less than 1% of the total), abundant and common species. In this case, asexual reproduction could be an advantageous propagation strategy of a few r-selected species which can be accommodated within the strategy group of stress-tolerant ruderals (see also Rogers (1990); Jahns and Ott (1997)).

The absolute prevalence of crustose, saxicolous life-forms in lichenicolous lichens may be related to their high frequency in dry situations. Crustose lichens are the slowest growing of all lichens, which allows them to have a lower demand for nutrients than foliose or fruticose lichens, therefore enabling colonisation of harsher environments (Armstrong and Bradwell 2010). They are also intimately associated with the substratum and their metabolic growth rate relies on its water holding capacity, which is generally much lower in rocks than in bark or soil (Green and Lange 1995).

The scarcity of trentepohlioid photobionts in lichenicolous lichens is probably due to the fact that *Trentepohlia*, a genus of filamentous green algae, is bound to shaded-humid and warm conditions, where it often occurs in the free state. Trentepohlioid lichens indeed have their maximum diversity in tropical evergreen rainforests, where solar irradiance is low and air humidity is high (Friedl and Büdel 2012; Matos et al. 2015; Martellos et al. 2020). Finally, the scarcity of lichenicolous cyanolichens may be due to a different reason. Cyanobacteria dominate many extreme, arid environments, reaching temperatures up to 73 °C, thanks to their tolerance of desiccation and water stress, being abundantly available in the free state for lichen symbiosis in dry sites (Pentecost and Whitton 2000; Whitton and Potts 2000; Nimis et al. 2020). It has long been known that very dry, steeply inclined rocks surfaces host visually conspicuous cyanobacterial films ("Tintenstriche", Lüttge (1997)), with a very rich diversity in species (Pentecost and Whitton 2000; Nimis et al. 2020). Many mycobionts of cyanolichens may, therefore, not need to develop a lichenicolous lifestyle for acquiring their photobionts, as they would find ecologically adapted cyanobionts already available in the environment. There could be, however, an alternative reason for the scarcity of lichenicolous species in cyanolichens mostly belong to the Lecanoromycetes. The process of host colonisation could be related not only to the photobiont of the host, but also to certain mycobiont traits, such as biochemical defences to fungal invasion, likely having a relevant role in the distribution of lichenicolous fungi across the lichenised lineages of Ascomycota.

The ecological conditions prevailing on well-lit, dry rock surfaces with low water-holding capacity may be unfavourable for the establishment of lichens reproducing sexually. Once a spore falls in a suitable habitat it germinates, generating a delicate mycelium which eagerly looks for a compatible photosynthetic partner to re-build the lichen symbiosis before being destroyed by a hostile environment where water is scarce and temperatures may be high due to strong solar irradiation (Pyatt 1973; Ott 1987). It is not clear whether the possible scarcity of free-living algae in dry sites could also play a role in the acquisition of a lichenicolous life-style. For lichens of dry habitats, the probability for a germinating spore to find a suitable alga has been estimated to be extremely low by Scott (1971) and some authors (e.g. Guillitte (1993)) have found that free-living green algae are quite rare on dry rock surfaces. However, other authors (e.g. Yung et al. (2014)) have demonstrated the presence of algal species, able to lichenise, in dry environments where mycobiont species have not been recorded. In any case, an original solution to the difficulties in the lichenisation of sexually reproducing species in very dry sites, suggested by several authors, might be that of "stealing" the photobiont from the thalli of other species (Rambold and Triebel 1992; Richardson 1999), which would explain their lichenicolous lifestyle. Lichenicolous phycolichens are commonly referred to as "parasites" (Poelt and Doppelbaur 1956; Poelt 1958) and as such they are usually flagged in several modern floras and checklists (e.g. Clauzade and Roux (1985); Wirth et al. (2013); Nimis et al. (2018)). According to Smith and Smith (2015), a parasite is an organism which benefits from the tight and prolonged association with the host, which is progressively damaged and exploited to derive nourishment and a habitat. A parasite was also defined as an organism that lives on and at the expense of a host, implying a metabolic dependence from it (Esch and Fernandez 1993). Considering these definitions, the term "parasite" may not be appropriate for many lichenicolous lichens, since, at least in later life-stages, they derive nutrients from their own photobionts and not from the lichen host, as instead the lichenicolous, non-lichenised fungi do. The prolonged persistence upon the host was considered a characteristic of a parasite by Poelt and Doppelbaur (1956). While some lichenicolous lichens may be confined to the host thalli throughout their lifetime, others can become independent, not using the host as a lifelong habitat (Richardson 1999; Honegger 2012b; Moya et al. 2020). Moreover, the degree of colonisation and, thus, of damage to the host, also varies, as its thallus can be either locally or completely overgrown and replaced (Richardson 1999; Honegger

2012b). Hence, since the range of interactions is broad and the transitions fluid, the term "parasite" for lichenicolous phycolichens should be best reserved for those producing clear damage or even the disappearance of the host thallus.

The concept of "stealing of the phycobiont", though, should also be re-considered. Indeed, the lichenicolous mycobiont does not depredate the lichen host from its photosynthetic partner, but it takes some of the phycobiont cells to develop its own symbiosis and grow further using the thallus host as substrate. Moya et al. (2020) analysed the microalgal diversity and interaction patterns in crustose lichens and lichenicolous lichens on gypsum by amplicon sequencing analysis of the nuclear internal transcribed spacer (nrITS) region and characterised the microalgae by ultrastructure analyses. They found that three microalgal genera formed the pool of potential phycobionts and were available for the lichenicolous lichens.

Diederich et al. (2018) reported a total of 257 species of lichenicolous lichens worldwide. It is likely that, in dry sites, the strategy of "stealing" the phycobiont is more widespread than currently assumed and that the 257 species listed by Diederich et al. (2018), as the 189 Italian species considered in this study, are the most specialised and evidently lichenicolous ones, just the "tip of the iceberg" of what could be the real lichenicolous lichens biota. Further research, using DNA amplicon sequencing and metagenomics, could lead to the discovery of new lichenicolous lichens species, from obligate to occasional, the latter stealing the phycobiont only in harsh environments.

Conclusions

The results of the present study may be summarised as follows:

- 1. most lichenicolous lichens are crustose, with a non-trentepohlioid phycobiont;
- 2. they are clearly bound to sunny-dry habitats (rocks and soil);
- such habitats seem to exert a positive selective pressure towards sexual reproduction of the mycobiont;
- sexually reproducing species of dry habitats may encounter problems in the early stages of lichenisation and this has led to the evolution of "algal thieves";
- 5. the number of "algal thieves" in dry habitats may be higher than currently assumed.

Acknowledgements

We thank Giovanni Bacaro for constructive comments and suggestions on the statistical analyses.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Conceptualization: PLN. Formal analysis: EP, MC, PP. Writing - original draft: PLN. Writing - review and editing: LM, SM.

Author ORCIDs

Pier Luigi Nimis () https://orcid.org/0000-0003-3523-0183 Piero Pitacco () https://orcid.org/0009-0001-0730-1362 Stefano Martellos () https://orcid.org/0000-0001-5201-8948 Lucia Muggia () https://orcid.org/0000-0003-0390-6169

Data availability

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

References

- Ament-Velásquez SL, Tuovinen V, Bergström L, Spribille T, Vanderpool D, Nascimbene J, Yamamoto Y, Thor G, Johannesson H (2021) The plot thickens: Haploid and triploid-like thalli, hybridization, and biased mating type ratios in *Letharia*. Frontiers in Fungal Biology 2: 656386. https://doi.org/10.3389/ffunb.2021.656386
- Armstrong RA, Bradwell T (2010) Growth of crustose lichens: A review. Geografiska Annaler. Series A, Physical Geography 92(1): 3–17. https://doi.org/10.1111/j.1468-0459.2010.00374.x
- Armstrong RA, Welch AR (2007) Competition in lichen communities. Symbiosis 43(1): 1–12. https://doi.org/10.1007/s13199-011-0108-4
- Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A, Hofstetter V, Kauff F, Lutzoni F (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: Are lichens cradles of symbiotrophic fungal diversification? Systematic Biology 58(3): 283–297. https://doi.org/10.1093/sysbio/syp001
- Bacaro G, Gioria M, Ricotta C (2012) Testing for differences in beta diversity from plotto-plot dissimilarities. Ecological Research 27(2): 285–292. https://doi.org/10.1007/ s11284-011-0899-z
- Bacaro G, Gioria M, Ricotta C (2013) Beta diversity reconsidered. Ecological Research 28(3): 537–540. https://doi.org/10.1007/s11284-013-1043-z
- Bates ST, Cropsey GWG, Caporaso JG, Knight R, Fierer N (2011) Bacterial communities associated with the lichen symbiosis. Applied and Environmental Microbiology 77(4): 1309–1314. https://doi.org/10.1128/AEM.02257-10
- Casano LM, del Campo EM, García-Breijo FJ, Reig-Armiñana J, Gasulla F, del Hoyo A, Guéra A, Barreno E (2011) Two *Trebouxia* algae with different physiological performances are ever-present in lichen thalli of *Ramalina farinacea*. Coexistence versus competition? Environmental Microbiology 13(3): 806–818. https://doi.org/10.1111/j.1462-2920.2010.02386.x
- Casano LM, Braga MR, Álvarez R, del Campo EM, Barreno E (2015) Differences in the cell walls and extracellular polymers of the two *Trebouxia* microalgae coexisting in the lichen *Ramalina farinacea* are consistent with their distinct capacity to immobilize extracellular Pb. Plant Science 236: 195–204. https://doi.org/10.1016/j.plants-ci.2015.04.003

- Clauzade G, Roux C (1985) Likenoj de Okcidenta Europo. Ilustrita determinlibro. Bull. Soc. Bot. Centre-Ouest, N. Ser., N. Spec. 7. Royan, 893 pp.
- de los Rìos A, Grube M (2000) Host-parasite interfaces of some lichenicolous fungi in the Dacampiaceae (Dothideales, Ascomycota). Mycological Research 104(11): 1348–1353. https://doi.org/10.1017/S0953756200002951
- del Campo EM, Catalá S, Gimeno J, del Hoyo A, Martínez-Alberola F, Casano LM, Grube M, Barreno E (2012) The genetic structure of the cosmopolitan three-partner lichen *Ramalina farinacea* evidences the concerted diversification of symbionts. FEMS Microbiology Ecology 83(2): 310–323. https://doi.org/10.1111/j.1574-6941.2012.01474.x
- del Hoyo A, Álvarez R, del Campo EM, Gasulla F, Barreno E, Casano LM (2011) Oxidative stress induces distinct physiological responses in the two *Trebouxia* phycobionts of the lichen *Ramalina farinacea*. Annals of Botany 107(1): 109–118. https://doi. org/10.1093/aob/mcq206
- Diederich P (1996) The lichenicolous heterobasidiomycetes. Bibliotheca Lichenologica 61. Gebrüder Borntraeger Verlag, Berlin-Stuttugart, 198 pp.
- Diederich P, Lawrey JD, Ertz D (2018) The 2018 classification and checklist of lichenicolous fungi, with 2000 non-lichenized, obligately lichenicolous taxa. The Bryologist 121(3): 340–425. https://doi.org/10.1639/0007-2745-121.3.340
- Esch GW, Fernandez JC (1993) A Functional Biology of Parasitism: Ecological and evolutionary implications. Springer, Dordrecht, 337 pp.
- Fox J, Bouchet-Valat M (2023) Rcmdr: R Commander. R package version 2.9-0. https:// cran.r-project.org/package=Rcmdr
- Friedl T (1987) Thallus development and phycobionts of the parasitic lichen *Diploschistes muscorum*. Lichenologist (London, England) 19(2): 183–191. https://doi.org/10.1017/S002428298700015X
- Friedl T, Büdel B (2012) Photobionts. In: Nash III TH (Ed.) Lichen Biology. Second Edition. Cambridge University Press, Cambridge, 9–26. https://doi.org/10.1017/ CB09780511790478.003
- Gilbert OL (1990) The lichen flora of urban wastcland. Lichenologist (London, England) 22(1): 87–101. https://doi.org/10.1017/S0024282990000056
- Green TGA, Lange OL (1995) Photosynthesis in Poikilohydric Plants: A Comparison of Lichens and Bryophytes. In: Schulz, Caldwell MM (Eds) Ecophysiology of photosynthesis. Springer, Berlin, Germany, 319–341. https://doi.org/10.1007/978-3-642-79354-7_16
- Grube M (2018) The lichen thallus as a microbial habitat. In: Blanz P (Ed.) Biodiversity and Ecology of Fungi, Lichens, and Mosses, Kerner von Marilaun Workshop 2015 in memory of Josef Poelt. Verlag der Österreichischen Akademie der Wissenschaften, Vienna, Biosyst. Ecol. Ser 34, 529–546.
- Grube M, Hafellner J (1990) Studien an flechtenbewohnenden Pilzen der Sammelgattung Didymella (Ascomycetes, Dothideales). Nova Hedwigia 51(3): 283–360.
- Guillitte O (1993) Kinetics of plant colonization of composite materials. PhD Thesis. Univ. of Gembloux, Fac. of Agriculture, 249 pp.
- Hafellner J (2018) Focus on lichenicolous fungi: Diversity and taxonomy under the principle "one fungus-one name". In: Blanz P (Ed.) Biodiversity and Ecology of Fungi, Lichens, and Mosses, Kerner von Marilaun Workshop 2015 in memory of Josef Poelt. Verlag der Österreichischen Akademie der Wissenschaften, Vienna, Biosyst. Ecol. Ser 34, 227–243.
- Hawksworth D (1988) The variety of fungal-algal symbioses, their evolutionary significance, and the nature of lichens. Botanical Journal of the Linnean Society 96(1): 3–20. https://doi.org/10.1111/j.1095-8339.1988.tb00623.x

- Hawksworth D, Grube M (2020) Lichens redefined as complex ecosystems. The New Phytologist 227(5): 1281–1283. https://doi.org/10.1111/nph.16630
- Honegger R (1992) Lichens: mycobiont-photobiont relationships. In: Reisser W (Ed.) Algae and symbiosis. Bristol: Biopress, 225–275.
- Honegger R (2012a) Morphogenesis. In: Nash III TH (Ed.) Lichen Biology. Second Edition. Cambridge University Press, Cambridge, 69–93. https://doi.org/10.1017/ CB09780511790478.006
- Honegger R (2012b) Mycobionts. In: Nash III TH (Ed.) Lichen Biology. Second Edition. Cambridge University Press, Cambridge, 27–39. https://doi.org/10.1017/ CB09780511790478.004
- Insarova ID, Blagoveshchenskaya EY (2016) Lichen Symbiosis: Search and Recognition of Partners. Biology Bulletin of the Russian Academy of Sciences 43(5): 408–418. https://doi.org/10.1134/S1062359016040038
- Jahns HM, Ott S (1997) Life strategies in lichens some general considerations. Bibliotheca Lichenologica 67: 49–67.
- Lüttge U (1997) Cyanobacterial Tintenstrich communities and their ecology. Naturwissenschaften 84(12): 526–534. https://doi.org/10.1007/s001140050439
- Martellos S, d'Agostino M, Chiarucci A, Nimis PL, Nascimbene J (2020) Lichen Distribution Patterns in the Ecoregions of Italy. Diversity 12(8): 294. https://doi.org/10.3390/ d12080294
- Matos P, Pinho P, Aragon G, Martínez I, Nunes A, Soares AM, Branquinho C (2015) Lichen traits responding to aridity. Journal of Ecology 103(2): 451–458. https://doi. org/10.1111/1365-2745.12364
- Moya P, Molins A, Martínez-Alberola F, Muggia L, Barreno E (2017) Unexpected associated microalgal diversity in the lichen *Ramalina farinacea* is uncovered by pyrosequencing analyses. PLOS ONE 12(4): e0175091. https://doi.org/10.1371/journal. pone.0175091
- Moya P, Molins A, Chiva S, Bastida J, Barreno E (2020) Symbiotic microalgal diversity within lichenicolous lichens and crustose hosts on Iberian Peninsula gypsum biocrusts. Scientific Reports 10(1): 14060. https://doi.org/10.1038/s41598-020-71046-2
- Muggia L, Pérez-Ortega S, Kopun T, Zellnig G, Grube M (2014) Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. Annals of Botany 114(3): 463–475. https://doi.org/10.1093/aob/mcu146
- Muggia L, Fleischhacker A, Kopun T, Grube M (2016) Extremotolerant fungi from alpine rock lichens and their phylogenetic relationships. Fungal Diversity 76(1): 119–142. https://doi.org/10.1007/s13225-015-0343-8
- Nash III T (2008) Lichen Biology. Second Edition. Cambridge University Press, New York, 498 pp.
- Nimis PL (2016) The lichens of Italy. A Second Annotated Catalogue. EUT Edizioni Università di Trieste, 740 pp.
- Nimis PL, Martellos S (2001) Testing the predictivity of ecological indicator values. A comparison of real and virtual relevés of lichen vegetation. Plant Ecology 157(2): 165–172. https://doi.org/10.1023/A:1013919816804
- Nimis PL, Martellos S (2003) On the ecology of sorediate lichens in Italy. Bibliotheca Lichenologica 86: 393–406.
- Nimis PL, Martellos S (2023) ITALIC The information system on Italian lichens. Version 7.0. University of Trieste, Dept. of Biology. http://dryades.units.it/italic [Accessed on 6/6/2023]

- Nimis PL, Hafellner J, Roux C, Clerc P, Mayrhofer H, Martellos S, Bilovitz PO (2018) The Lichens of the Alps. An Annotated Catalogue. MycoKeys 31: 1–634. https://doi. org/10.3897/mycokeys.31.23568
- Nimis PL, Martellos S, Chiarucci A, Ongaro S, Peplis M, Pittao E, Nascimbene J (2020) Exploring the relationships between ecology and species traits in cyanolichens: A case study on Italy. Fungal Ecology 47: 100950. https://doi.org/10.1016/j.funeco.2020.100950
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022) vegan: Community EcologyPackage. R package version 2.6-4. https://CRAN.R-project.org/package=vegan
- Ott S (1987) Reproductive strategies in lichens. Bibliotheca Lichenologica 25: 81–93.
- Palmqvist K, Dahlman L, Jonsson A, Nash III TH (2008) The carbon economy of lichens. In: Nash III TH (Ed.) Lichen Biology. 2nd Edn. Cambridge University Press, Cambridge, 182–215. https://doi.org/10.1017/CB09780511790478.011
- Pentecost A, Whitton BA (2000) Limestones. In: Whitton BA, Potts M (Eds) The Ecology of Cyanobacteria. Springer, Dordrecht, Netherlands, 257–279. https://doi. org/10.1007/0-306-46855-7_9
- Piercey-Normore MD, DePriest PT (2001) Algal Switching among lichen symbioses. American Journal of Botany 88(8): 1490–1498. https://doi.org/10.2307/3558457
- Poelt J (1958) Über parasitische Flechten. II. Planta 51(3): 288-307. https://doi. org/10.1007/BF02125723
- Poelt J (1990) Parasitische Arten der Flechtengattung *Rhizocarpon*: eine weitere Übersicht. Mitt. Bot. Staatssamml. München 29: 515–538.
- Poelt J (1993) La riproduzione asessuale nei licheni. Notiziario della Società Lichenologica italiana 6: 9–28.
- Poelt J, Doppelbaur H (1956) Über parasitische Flechten. Planta 46(5): 467–480. https:// doi.org/10.1007/BF01911154
- Poelt J, Mayrhofer H (1988) Über Cyanotrophie bei Flechten. Plant Systematics and Evolution 158(2–4): 265–281. https://doi.org/10.1007/BF00936350
- Pyatt FB (1973): Lichen propagules. In: Ahmadjian V, Hale ME (Eds) The Lichens, Academic Press, New York and London, 17–145. https://doi.org/10.1016/B978-0-12-044950-7.50009-X
- R Core Team (2023) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.r-project.org/
- Rambold G, Triebel D (1992) The inter-lecanoralean associations. Bibliotheca Lichenologica 48. Gebrüder Borntraeger Verlag, Berlin-Stuttgart, 201 pp.
- Richardson DHS (1999) War in the world of lichens: Parasitism and symbiosis as exemplified by lichens and lichenicolous fungi. Mycological Research 103(6): 641–650. https://doi.org/10.1017/S0953756298008259
- Rikkinen J (2002) Cyanolichens: An Evolutionary Overview. In: Rai A, Bergman B, Rasmussen U (Eds) Cyanobacteria in Symbiosis. Springer, Dordrecht, 31–72. https://doi. org/10.1007/0-306-48005-0_4
- Rogers RW (1990) Ecological strategies of lichens. Lichenologist (London, England) 22(2): 149–162. https://doi.org/10.1017/S002428299000010X
- Sanders WB (2023) Is lichen symbiont mutualism a myth? Bioscience 73(9): 623-634. https://doi.org/10.1093/biosci/biad073

Sanders WB (2024) The disadvantages of current proposals to redefine lichens. The New Phytologist 241(3): 969–971. https://doi.org/10.1111/nph.19321

Scott GD (1971) Plant Symbiosis. 2nd Edn. Amold, London.

- Seymour F, Crittenden P, Dyer PS (2005) Sex in the extremes: Lichen-forming fungi. The Mycologist 19(2): 51–58. https://doi.org/10.1017/S0269915X05002016
- Smith TM, Smith RL (2015) Elements of Ecology. 9th Edn. Pearson, London, United Kingdom.
- Spribille T, Tuovinen V, Resl P, Vanderpool D, Wolinski H, Aime MC, Schneider K, Stabentheiner E, Toome-Heller M, Thor G, Mayrhofer H, Johannesson H, Mccutcheon JP (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. Science 353(6298): 488–492. https://doi.org/10.1126/science.aaf8287
- Spribille T, Resl P, Stanton DE, Tagirdzhanova G (2022) Evolutionary biology of lichen symbioses. The New Phytologist 234(5): 1566–1582. https://doi.org/10.1111/ nph.18048
- Wedin M, Maier S, Fernandez-Brime S, Cronholm B, Westberg M, Grube M (2016) Microbiome change by symbiotic invasion in lichens. Environmental Microbiology 18(5): 1428–1439. https://doi.org/10.1111/1462-2920.13032
- Whitton BA, Potts M (2000) Introduction to the cyanobacteria, Ecology of Cyanobacteria: Their Diversity in Time and Space. In: Whitton BA, Potts M (Eds) The Ecology of Cyanobacteria. Springer, Dordrecht, 1–11. https://doi.org/10.1007/0-306-46855-7_1
- Wirth V, Hauck M, Schultz M (2013) Die Flechten Deutschlands. Stuttgart, Ulmer. 2 vols, 1244 pp.
- Yung CCM, Chan Y, Lacap DC, Pérez-Ortega S, de Los Rios-Murillo A, Lee CK, Cary SC, Pointing SB (2014) Characterization of chasmoendolithic community in Miers Valley, McMurdo dry valleys, Antarctica. Microbial Ecology 68: 351–359. https://doi. org/10.1007/s00248-014-0412-7



Research Article

Taxonomy and phylogeny of *Panus* (Polyporales, Panaceae) in China and its relationship with allies

Lei Yue^{1,2*}, Junliang Chen^{3*}, Yonglan Tuo^{1,2,4}, Zhengxiang Qi^{1,2}, Yajie Liu^{1,2,4}, Xiao Lan He⁵, Bo Zhang^{1,2,4}, Jiajun Hu^{1,4,6}, Yu Li¹

- 1 Engineering Research Centre of Edible and Medicinal Fungi, Ministry of Education, Jilin Agricultural University, Changchun City, 130118, Jilin Province, China
- 2 College of Plant Protection, Jilin Agricultural University, Changchun City, 130118, Jilin Province, China
- 3 Science and Research Center for Edible Fungi of Qingyuan County, Lishui City, 323800, Zhejiang Province, China
- 4 Joint Laboratory of International Cooperation in Modern Agricultural Technology, Ministry of Education, Jilin Agricultural University, Changchun City, 130118, Jilin Province, China
- 5 Sichuan Institute of Edible Fungi, Chengdu City, 610066, Sichuan Province, China
- 6 School of life science, Zhejiang Normal University, Jinhua City, 321004, Zhejiang Province, China

Corresponding authors: Bo Zhang (zhangbofungi@126.com); Jiajun Hu (hujjfungi@163.com); Yu Li (fungi966@126.com)

Abstract

Panus is a typical wood-rotting fungi, which plays considerable roles in ecosystems and has significant economic value. The genus Panus currently consists of more than 100 species; however, only eight species have been reported from China. This study aims to distinguish and describe two novel species from the Panus similis complex, namely Panus minisporus and Panus baishanzuensis, one new record species from Zhejiang Province, Panus similis and three common species, Panus conchatus, Panus neostrigosus and Panus rudis, based on detailed morphological and phylogenetic studies, relying on Chinese specimens. Panus minisporus is characterised by its reddish-brown pileus, decurrent lamellae with cross-veins, slender stipe, smaller basidiospores, wider generative hyphae and absence of sclerocystidia. Panus baishanzuensis is featured by its pileus with concentric and darker ring zone, decurrent lamellae with cross-veins, shorter stipe, longer basidiospores, diverse and shorter cheilocystidia and smaller sclerocystidia. Internal transcribed spacer (ITS) regions, large subunit nuclear ribosomal RNA gene (nLSU) and translation elongation factor 1-a gene (tef-1a) were employed to perform a thorough phylogenetic analysis for genus Panus and related genera, using Bayesian Inference and Maximum Likelihood analysis. The results indicate that Panus minisporus and Panus baishanzuensis form two independent clades within the Panus similis complex themselves. Detailed descriptions, taxonomic notes, illustrations etc. were provided. In addition, a key to the reported species of Panus from China is also provided.

Key words: Hyphal system, novel species, Panus similis, phylogenetic

Introduction

The genus *Panus* Fr. is one of the wood-rotting fungi with significant economic and ecological value. Its taxonomic history has been carried out for a long time. In 1787, Bulliard (1786) described a new species *Agaricus conchatus* Bull



Academic editor: Kentaro Hosaka Received: 16 February 2024 Accepted: 26 April 2024 Published: 31 May 2024

Citation: Yue L, Chen J, Tuo Y, Qi Z, Liu Y, He XL, Zhang B, Hu J, Li Y (2024) Taxonomy and phylogeny of *Panus* (Polyporales, Panaceae) in China and its relationship with allies. MycoKeys 105: 267–294. https://doi. org/10.3897/mycokeys.105.121025

Copyright: © Lei Yue 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).

^{*} These authors contributed equally to this work.

(≡*Panus conchatus* (Bull.) Fr.). When the genus *Panus* was established, it was designated as the type species (Fries 1838). From then on, a large number of new taxa were described around the world (Berkeley and Broome 1883; Stevenson 1964; Senthilarasu 2015; Tibpromma et al. 2017). Till now, there are currently described 113 taxa of *Panus*, including 101 species, 10 varieties, and two metamorphs, according to Index Fungorum (http://www.indexfungorum.org). However, these species were mainly reported from Europe and North America, rarely described from Asia.

The taxonomic status between Panus and allies was often disputed, leading to confusion amongst these genera. The key characteristics to separate the genus Lentinus Fr. and Panus were hugely different in the history research, thus, leading to indistinct boundaries between these genera. Hymenophoral trama structures were adopted by Singer (1961a, 1961b, 1962) and Kühner (1980) to separate Panus and Lentinus. However, Pegler (1971, 1975) and Corner (1981) employed the hyphal systems as the key features to separate these two genera. These led to confusing and unstable taxonomic systems. Later, hyphal systems were proved to be the more reasonable key characteristics in distinguishing these species (Kibby et al. 1978; Corner 1981; Singer 1986) and treated Lentinus and Panus as distinctly separate genera. However, Pegler (1983) disagreed that Panus was an independent genus and treated it as a subgenus of Lentinus. That also led to mutual confusion between the Panus and Lentinus species. With the application of phylogenetic analysis in researching these groups, the view held by Corner (1981) was confirmed; furthermore, this group was divided into three separate genera, viz. Lentinus, Panus and Neolentinus Redhead & Ginns (Hibbett and Vilgalys 1991; Thorn et al. 2000). Meanwhile, the family status of Panus has been changed several times and it has been placed in Agaricaceae, Polyporaceae, Tricholomataceae and Meruliaceae (Fries 1838; Imai 1938; Singer 1961a, 1961b, 1962; Miller 1972; Douanla-Meli and Langer 2010; Zmitrovich and Kovalenko 2016). However, in recent years, the taxonomic status of Panus has been significantly changed again, due to phylogenetic studies and it has been upgraded to a separate family rank, Panaceae (Justo et al. 2017).

Now, genus Panus could be clearly distinguished from the genus Lentinus because of its skeletal hyphae (Corner 1981; Pegler 1983). However, Panus is not easy to distinguish from the genus Pleurotus (Fr.) P. Kumm. It is well known that the fungi of the genus Pleurotus have unique nematode-feeding properties (Li and Bau 2014). Its softer texture, on the other hand, was once thought to be a monomitic hyphal system with only generative hyphae. In contrast, the fungi of Panus have a firmer texture with a typical dimitic hyphal system, which can be distinguished from *Pleurotus*. However, as research continued, many species in the genus Panus were found to have nematode-predatory functions, which corresponded precisely to the characteristics of Pleurotus and, thus, a large number of species were moved into Pleurotus, such as Pleurotus giganteus (Berk.) Karun. & K.D. Hyde and Pleurotus tuber-regium (Fr.) Singer (Singer 1951; Klomklung et al. 2012). Yet these species that have migrated into Pleurotus have the same dimitic hyphal system as Panus, which is guite different from the commonly known characteristics of Pleurotus. This has led to a blurring of the line between Panus and Pleurotus, making it difficult to distinguish.

In addition, there are some species similar in appearance, for example, *Lentinus ciliatus* Lév., *Lentinus similis* Berk. & Broome and *Lentinus hookerianus* Berk. etc. These belonged to the sect. *Velutini* sensu Pegler and shared a similar appearance, because of its velutinate to strigose basidiomes and thick-walled skeletocystidia (Pegler 1983). However, later, four of them were recombined into *Panus*, due to the presence of skeletal hyphae and phylogenetic studies, leaving *L. velutinus* and *L. fasciatus* behind (Pegler 1983; May and Wood 1995), but these two species also show close affinity with genus *Panus* (Pegler 1983; Douanla-Meli and Langer 2010; Luangharn et al. 2019).

Due to the omission of the taxonomic studies, the species diversity of *Panus* is still unclear in China and lacks systematic research. Teng (1963) described three *Panus* species, viz. *Panus* torulosus (Pers.) Fr. (\equiv *P.* conchatus), *Panus* rudis Fr. and *Panus* setiger (Lév.) Teng in his book—*Fungi* of China. Later, Tai (1979) recorded four species, *P.* conchatus, *P.* rudis, *P.* setiger and *Panus* tigrinus (Bull.) Singer (\equiv Lentinus tigrinus (Bull.) Fr.), in "Sylloge Fungorum Sinicorum". Subsequently, the species diversity of *Panus* has been performed locally in China (Li et al. 1978; Du et al. 1983; Xu et al. 1986; Li and Qin 1991; Li et al. 2004; Shao and Xiang 2006). In 2014, the first monograph about pleurotoid and lentinoid fungi in China was published, in which 11 species of *Panus* were recorded (Li and Bau 2014). Then, "the Atlas of Chinese Macrofungal Resources" described six species (Li et al. 2015).

Therefore, up to now, there are only a total of eight *Panus* species that have been recorded from China, viz. *Panus ciliatus* (Lév.) T.W. May & A.E. Wood, *P. conchatus, Panus neostrigosus* Drechsler-Santos & Wartchow, *P. rudis, P. setiger, Panus similis* (Berk. & Broome) T.W. May & A.E. Wood, *Panus strigellus* (Berk.) Chardón & Toro and *Panus velutinus* (Fr.) Sacc. (=*L. velutinus*). In addition, most of them are located in southwest China, northwest China, south China, central China and Northeast China. *Panus* resources in east China and north China are in urgent need of development, especially in previously undocumented areas such as Zhejiang Province.

In this study, a combined morphological and phylogenetic study of six *Panus* species from China is carried out. Two new species, *Panus minisporus* and *Panus baishanzuensis* and a new record, *P. similis*, from Zhejiang Province are described in detail, along with illustrations and colour photographs.

Materials and methods

Sampling and morphological studies

All examined specimens in this study were collected from China. The pictures of fruit-body were taken in the field. After measuring and describing the fresh macroscopic characteristics, the specimens were dried at 40–50 °C in a dryer, then they were stored in the Fungarium of Jilin Agricultural University (FJAU).

Macroscopic characteristics were based on the field notes and the colours were described according to Küppers (2002). The descriptions of size were referred to Yue et al. (2023). Then microscopic characteristics were observed from the dried specimens using a Zeiss Axio lab. A1 light microscope. The dried specimens were rehydrated in 94% ethanol first, then mounted in 3% potassium hydroxide (KOH) to observe the colour, sealed in 1% Congo red to measure the

data and Melzer's reagent was used to check if the spores were amyloid or dextrinoid (Hu et al. 2022a, b). As to each specimen, at least 40 values were measured separately from different basidiomata for each feature. The measurements are given as (a)b-c(d), the range of b-c contains a minimum of 90% of the measured values and the extreme values (i.e. a and d) are given in parentheses. The extent for basidiospores is given as length × width (L × W), Q values equal to the ratio of length and width of each basidiospore in the side view, "n" represents the number of measured basidiospores, "Im" represents the arithmetic mean of the length, "wm" represents the arithmetic mean of the width and "q" represents the average Q value of all basidiospores \pm standard deviation.

DNA extraction, PCR amplification and sequencing

The total DNA was extracted using the new plant genomic DNA extraction kit from Jiangsu Kangwei Century Biotechnology Company Limited, following the instructions. Primer pairs ITS1-F/ITS4-B (Gardes and Bruns 1993), LR0R/LR5 (Cubeta et al. 1991) and 983F/2212R (Rehner and Buckley 2005) were used for amplifying and sequencing these sequences: internal transcribed spacer (ITS) regions, large subunit nuclear ribosomal RNA gene (nLSU) and translation elongation factor 1- α gene (*tef-1a*), respectively. The amplification reactions were carried out in a total 25 µl system, which is as follows: dd H₂O 13.5 µl, 2 × Es Taq MasterMix (Dye) 8 µl, 10 mM of each primer 1 µl and DNA solution 1.5 µl. The PCR reaction procedures were as follows: for ITS, 1) 95 °C for 2 min to initial denaturation, 2) 35 cycles of denaturation for 40 s at 94 °C, annealing for 1 min at 50 °C and extension for 2 min at 75 °C, 3) leave at 75 °C for 10 min (Zmitrovich and Kovalenko 2016); for nLSU, 1) 95 °C for 3 min to initial denaturation, 2) 30 cycles of denaturation for 30 s at 94 °C, annealing for 45 s at 47 °C and extension for 1 min and 30 s at 72 °C, 3) leave at 72 °C for 10 min (Hu et al. 2022b); and for tef-1 α , 1) 95 °C for 2 min to initial denaturation, 2) 9 cycles of denaturation for 40 s at 95 °C, annealing for 40 s at 60 °C and extension for 2 min at 70 °C, 3) then 36 cycles of denaturation for 45 s at 95 °C, annealing for 1 min and 30 s at 50 °C and extension for 2 min at 70 °C, 4) leave at 70 °C for 10 min (Zmitrovich and Kovalenko 2016). The PCR products were detected by 1.2% agarose gel electrophoresis, then Comate Bioscience (Jilin) Company Limited was employed to carry out purification and sequencing. Finally, the sequencing results were uploaded to GenBank (https://www.ncbi.nlm.nih.gov/ genbank/), Table 1.

Data analysis

By searching in GenBank, 68 ITS sequences, 46 nLSU sequences and 23 *tef-1a* sequences of related taxa were downloaded. A total of 10 ITS sequences, 10 nLSU sequences and three *tef-1a* sequences were newly obtained in this study. All sequences used in this paper are listed in Table 1. Each single-gene dataset was aligned in MAFFT 7 using the E-INS-i strategy (Katoh and Standley 2013) and manually adjusted where necessary in BioEdit 7.0.9 (Hall 1999). The datasets (ITS+nLSU+*tef-1a*) were then concatenated using PhyloSuite 1.2.3 (Zhang et al. 2020; Xiang et al. 2023) for multi-phylogenetic analyses. ModelFinder

2.2.0 (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-linked).

The datasets were analysed separately using Maximum Likelihood (ML) and Bayesian Inference (BI). For the ML analysis, it was performed using IQ-Tree 1.6.12 (Schmidt et al. 2014). The tree topology was verified under both 1000 bootstrap and 1000 replicates of the SH-aLRT branch test. For the BI analysis, it was performed using MrBayes 3.2.6 (Ronquist et al. 2012). The analysis employed a general time-reversible DNA substitution model and a gamma distribution to account for rate variation across the sites. Four Markov chains were executed for two runs, starting from random trees, the chains being terminated when the split deviation frequency value fell below 0.01. Tree samples were sampled every 1000 generations. The first 25% of the sampled trees were discarded as burn-in, while the remaining trees were used to construct a 50% majority consensus tree and to calculate the Bayesian posterior probabilities (BIPP). Then the phylogenetic trees were visualised using FigTree 1.4.3 (Andrew 2016).

Results

Phylogenetic analyses

Three gene fragments (ITS, nLSU and *tef-1a*) from representative species of five orders in the Agaricomycetes were selected to construct a phylogenetic analysis, which included 71 species belonging to 31 genera of 19 families. The aims are to explore the phylogenetic status within *Panus* and its relationship with allies. A total of 160 sequences were used for phylogenetic analysis in this study. The best-fit model is GTR+F+I+G4, TIM3+F+I+G4, TPM3+TNe+I for ITS, nLSU and *tef-1a* datasets, respectively. The topologies of ML and BI, based on the concatenated datasets, were consistent and typically increased support values; thus, only the result inferred from ML analysis was displayed (Fig. 1).

In the phylogenetic tree, four clades corresponding to Agaricales, Boletales, Polyporales and Gloeophyllales were revealed, with Hymenochaetales as outgroup (Fig. 1). As indicated in the tree, four families were selected as representatives of Agaricales. It is worth noting that Pleurotus is divided into two deeply-divergent clades. One represents the monomitic hyphal system species and the other is the dimitic hyphal system species (Fig. 1). Boletales is located between Agaricales and Polyporales. In the Polyporales clade, a total of seven families were selected to reconstruct the phylogenetic analysis, with the family Panaceae as the main group (Fig. 1). Amongst them, the genus Panus is divided into three clades, viz. clade A, clade B and P. similis complex clade. Thirty-one specimens we sampled formed two new species, Panus minisporus L. Yue, J.J. Hu, B. Zhang & Y. Li, sp. nov. and Panus baishanzuensis L. Yue, B. Zhang & Y. Li, sp. nov., one new record species from Zhejiang Province, P. similis and three common species, P. conchatus, P. neostrigosus and P. rudis, which were clustered in *Panus*. Further morphological research of other related species was consistent with supporting the classification of these two new species. Additionally, the order Gloeophyllales is located between the orders Polyporales and Hymenochaetales (Fig. 1).

 Table 1. Voucher/specimen ID, GenBank accession numbers and origin of the specimens included in this study. Sequences produced in this study are in bold.

Taxan	Voucher/	GenBar	k Accession	Number	Origin	Deference	
IdXUII	specimen ID	ITS (5.8S)	nLSU	tef-1a	Ungin	Reference	
Agaricus campestris	MA Fungi 80998	NR_151745	-	-	USA	Unpublished	
A. campestris	LAPAG370	KM657927	KR006607	KR006636	China	Zhou et al. (2016)	
Antella americana	HHB-4100-Sp	KP135316	KP135196	-	USA	Floudas and Hibbett (2015)	
Antrodiella stipitata	FD-136	KP135314	KP135197	-	USA	Floudas and Hibbett (2015)	
Aurantiporus albidus	CIEFAP-117	KY948739	KY948848	-	USA	Justo et al. (2017)	
Boletus edulis	HMJAU4637	-	KF112455	KF112202	China	Wu et al. (2014)	
Coniophora arida	FP-104367	GU187510	GU187573	GU187684	USA	Binder et al. (2010)	
Fomitiporella austroasiana	Dai 16244	MG657328	MG657320	-	China	Ji et al. (2018)	
Fulvifomes hainanensis	Dai 11573	KC879263	JX866779	-	China	Zhou (2014)	
Gloeophyllum sepiarium	Wilcox-3BB	HM536091	HM536061	HM536110	USA	Garcia-Sandoval et al. (2011)	
G. trabeum	1320	HM536094	HM536067	HM536113	USA	Garcia-Sandoval et al. (2011)	
Hydnoporia subrigidula	He1157	JQ716403	JQ716409	-	China	He and Li (2013)	
Hygrophoropsis aurantiaca	AFTOL-ID 714	AY854067	AY684156	AY883427	USA	Matheny et al. (2006)	
Hymenochaete bambusicola	He 4116	KY425674	KY425681	-	China	Nie et al. (2017)	
Hyphodermella rosae	FP-150552	KP134978	KP135223	-	USA	Floudas and Hibbett (2015)	
Imleria badius	MB 03-098a	-	KF030355	KF030423	USA	Nuhn et al. (2013)	
Irpex lacteus	FD-9	KP135026	KP135224	-	USA	Floudas and Hibbett (2015)	
Lentinula boryana	TENN58368	MW508930	-	MW553225	Brazi	Menolli et al. (2022)	
L. edodes	TMI1633	MW508938	-	MW553232	Thailand	Menolli et al. (2022)	
L. madagasikarensis	PC0142531	MW810301	MW810299	OK598120	Madagascar	Menolli et al. (2022)	
L. raphanica	TENN56555	MW508963	-	MW553250	Costa Rica	Menolli et al. (2022)	
Lentinus crinitus	SA37	OK393677	OK383448	-	Brazil	Unpublished	
L. tigrinus	LE214778	KM411459	KM411475	KM411490	Russia	Zmitrovich and Kovalenko (2016)	
Merulius hydnoidea	HHB-1993-sp	KY948778	KY948853	-	USA	Justo et al. (2017)	
Neolentinus adhaerens	DAOM 214911	HM536096	HM536071	HM536117	Canada	Garcia-Sandoval et al. (2011)	
Neolentinus kauffmanii	DAOM 214904	HM536097	HM536073	HM536118	USA	Garcia-Sandoval et al. (2011)	
Oxyporus corticola	ZRL20151459	LT716075	KY418899	-	China	Zhao et al. (2017)	
Panus baishanzuensis	FJAU67793	PP273985	PP273975	PP590553	China	This study	
P. baishanzuensis	FJAU67793	PP273986	PP273976	PP590554	China	This study	
P. baishanzuensis	FJAU67793	PP273987	PP273977	PP590555	China	This study	
P. ciliatus	SP446150	MT669118	MT669140	-	Brazil	Unpublished	
P. conchatus	FJAU67795	PP273979	PP273969	PP590545	China	This study	
P. conchatus	LE265028	KM411463	KM434323	KM411496	Russia	Zmitrovich and Kovalenko (2016)	
P. fulvus	DS1687	MT669122	MT669143	-	Brazil	Unpublished	
P. minisporus	FJAU67792	PP273980	PP273970	PP590550	China	This study	
P. minisporus	FJAU67792	PP273981	PP273971	PP590551	China	This study	
P. minisporus	FJAU67792	PP273982	PP273972	PP590552	China	This study	
P. neostrigosus	FJAU67796	PP273983	PP273973	PP590547	China	This study	
P. neostrigosus	LE5829	KM411451	KM411468	KM411483	Russia	Zmitrovich and Kovalenko (2016)	

Tavan	Voucher/	GenBar	k Accession	Number	Onigin	Deference
laxon	specimen ID	ITS (5.8S)	nLSU	tef-1a	Origin	Reference
P. parvus	URM80840	MT669125	MT669145	-	Brazil	Unpublished
P. rudis	FJAU7824	PP273988	PP273978	PP590546	China	This study
P. rudis	ZJ1005DKJ02	KU863049	OR772972	-	China	Luangharn et al. (2019)
P. similis	FJAU67794	PP273984	PP273974	PP590548	China	This study
P. similis	LE287548	KM411466	KM411482	-	Russia	Zmitrovich and Kovalenko (2016)
P. strigellus	INPA239979	JQ955724	JQ955731	-	Brazil	Vargas-Isla et al. (2015)
P. tephroleucus	CMINPA 1860	MN602052	-	-	Brazil	Unpublished
P. velutinus	VOG30	MT669139	MT669155	-	Brazil	Unpublished
Phanerochaete australis	HHB-7105-Sp	KP135081	KP135240	-	USA	Floudas and Hibbett (2015)
Pleurotus abieticola	6554	AY450348	-	-	Russia	Petersen and Hughes (1997)
Pl. australis	VT1953	AY315758	-	-	Australia	Zervakis et al. (2004)
Pl. calyptratus	HMAS 63355	AY562495	AY562496	-	China	Luangharn et al. (2019)
Pl. citrinopileatus	FSCC1 (PCY1)	JN234853	-	-	Malaysia	Avin et al. (2012)
Pl. cornucopiae	H-14	JQ837484	-	-	Russia	Shnyreva et al. (2012)
Pl. cystidiosus	D419	AY315774	-	-	USA	Zervakis et al. (2004)
Pl. djamor	CBS 665.85	EU424288	EU365645	-	China	Luangharn et al. (2019)
Pl. dryinus	ECS-1108	GU722278	-	-	Mexico	Huerta et al. (2010)
Pl. eous	P109	MG282448	-	-	South Korea	Unpublished
Pl. eryngii	LGAMP63	HM998811	-	-	Greece	Zervakis et al. (2014)
Pl. euosmus	CBS 307.29	EU424298	EU365654	-	United Kingdom	Unpublished
Pl. fossulatus	D1821	EU233946	U04136	-	USA	Vilgalys and Sun (1994)
Pl. fuscosquamulosus	LGAMP50	AY315789	-	-	Greece	Zervakis et al. (2004)
Pl. giganteus	CMU54-1	JQ724360	JQ724361	-	Thailand	Kumla et al. (2013)
Pl. nebrodensis	UPA28	HM998818	-	-	Italy	Zervakis et al. (2014)
Pl. opuntiae	SAF 251	MH620771	MK182780	-	Italy	Zervakis et al. (2019)
Pl. ostreatus	TENN 53662	AY854077	AY645052	AY883432	USA	Unpublished
Pl. placentodes	HKAS57781	KR827694	KR827696	KR827700	China	Liu et al. (2016)
PI. populinus	ATCC 90083	AY368667	-	-	USA	Zervakis et al. (2019)
Pl. pulmonarius	ICMP 18163	MH395973	MH395998	-	New Zealand	Unpublished
Pl. tuber-regium	FRI 3611	KX018290	-	-	Malaysia	Karunarathna et al. (2016)
Polyporus tuberaster	MUCL31757	-	AB368103	-	Japan	Sotome et al. (2008)
Pterula echo	DJM302S58	DQ494693	AY629315	GU187743	USA	Matheny et al. (2006)
Radlodon yunnanensis	BJFC 010487	NR_182985	-	OM982705	China	Wang and Dai (2022)
R. americanus	RLG6350	JQ070175	-	-	USA	Nakasone and Lindner (2012)
R. casearius	CLZhao 3796	MH114880	-	-	China	Unpublished
Serpula lacrymans	REG_383	GU187542	GU187596	GU187752	USA	Binder et al. (2010)
Steccherinum bourdotii	HHB-9743-sp	KY948818	-	-	USA	Justo et al. (2017)
Trametopsis cervina	AJ-185	JN165020	JN164796	-	USA	Justo and Hibbett (2011)
Tricholoma flavovirens	AP2I	EU186294	-	EU186270	Portugal	Unpublished
T. megalophgeum	WTU F:073091	NR_175704	-	-	USA	Unpublished
Veluticeps africana	CBS 403.83	MH861619	-	-	Gabon	Vu et al. (2019)
V. berkeleyi	HHB-8594-Sp	HM536099	HM536081	HM536126	USA	Garcia-Sandoval et al. (2011)



Figure 1. The 50% majority rule Maximum Likelihood analysis of *Panus* and the related groups, based on ITS, nLSU and *tef-1a* sequences, with Hymenochaetales as outgroup. Support values of internal nodes respectively represent the Maximum Likelihood bootstrap (MLBP \ge 70) and Bayesian posterior probability (BIPP \ge 70%). The Voucher or specimen ID and the country are marked after the species name and the sequence from the type specimen is also marked as "T" at the end.

Taxonomy

Panus minisporus L. Yue, J.J. Hu, B. Zhang & Y. Li, sp. nov. Fungal Names: FN 571875 Figs 2, 3

Etymology. The epithet 'minisporus' refers to the small basidiospores of the new species.

Diagnosis. This species is distinguished from closed species by the cyathiform or flared and reddish-brown ($N_{60}Y_{90}M_{60}$) pileus, white or dirty white ($N_{00}Y_{10-20}M_{00-10}$) lamellae with cross-veins and two tiers of lamellulae, slender stipe, smaller basidiospores, wider generative hyphae and absence of sclerocystidia.



Figure 2. Habitat of Panus minisporus (FJAU67792, holotype) A basidiocarp B pileus C lamellae D stipe. Scale bars: 1 cm.



Figure 3. Microscopic characteristics of *Panus minisporus* (FJAU67792, holotype) **A** basidiospores **B** cheilocystidia **C** basidia **D** generative hyphae of context **E** pileipellis hyphae **F** skeletal hyphae of context. Scale bars: 10 μm.

Holotype. CHINA. Guizhou Province: Qiannan Buyi and Miao Autonomous Prefecture, Libo County, Maolan National Nature Reserve, 25.32°N, 108.08°E, 8 August 2017, Jiajun Hu, FJAU67792 (GenBank accession no.: ITS: PP273980, PP273981, PP273982; nLSU: PP273970, PP273971, PP273972; *tef-1α*: PP590550, PP590551, PP590552).

Description. Basidiomata solitary, large. Pileus 2.5–6.5 cm in diameter, thin, coriaceous, applanate, cyathiform or flared, reddish-brown $(N_{60}Y_{90}M_{60})$, darker at the centre, covered with reddish-brown $(N_{60}Y_{90}M_{60})$ puberulent, stripe dense and slender, radially parallel distributed, margin integer and ciliate slightly dense. Lamellae decurrent, crowded, white or dirty white $(N_{00}Y_{10-20}M_{00-10})$, with cross-veins and two tiers of lamellulae, edge entire. Stipe $4.4-9 \times 0.2-0.5$ cm, inverted clavate, central, solid, coriaceous, surface reddish-brown $(N_{60}Y_{90}M_{60})$ or more often darker, with dense velutinus, slightly expanded at the base. Pseudosclerotium absent. Context thin, up to 1 mm thick, white $(N_{00}Y_{10}M_{00})$, coriaceous, consisting of a dimitic hyphal system with skeletal hyphae.

Generative hyphae $3-5(7) \mu m$ diameter, cylindrical, not inflated, hyaline, thin-walled, frequently branched, with prominent clamp connections. Skeletal hyphae $2-3 \mu m$ diameter, sinuous cylindrical, hyaline, with a thick-walled and continuous lumen, unbranched. Basidiospores $4.5-5(5.5) \times 2.5-3 \mu m$ (n = 40, lm = 5 μm , wm = 3 μm , Q = 1.5-1.83, q = 1.67), ellipsoid to oblong, smooth, hyaline, thin-walled. Basidia (16)19–26 × 5–6 μm , clavate or elongated, bearing 4 sterigmata. Lamella-edge sterile, with small cheilocystidia. Cheilocystidia crowded, $16-22 \times 5-6 \mu m$, with median constriction, nodulose-clavate, fusoid, irregular, hyaline, thin-walled. Sclerocystidia absent. Hymenophoral trama irregular, radiate construction, hyaline, similar in structure to the context. Pileipellis on epicutis, made up of thick-walled generative hyphae, $3-5.5(7) \mu m$ diameter, occasionally bunched, not inflated, light brown. Stipitipellis similar to pileipellis.

Ecology. Solitary on rotten wood in broad-leaved forest.

Distribution. China (Guizhou Province).

Notes. This species is characterised by the reddish-brown pileus and stipe, white or dirty white lamellae with cross-veins and two tiers of lamellulae, slender stipe, smaller basidiospores and absence of sclerocystidia.

Panus minisporus is close to P. velutinus and P. similis in morphology, because of the velutinate pileus and slender stipe. However, the pileus and stipe of P. minisporus are both reddish-brown, which is different from the pale greyish-cinnamon to rufous or tawny-brown tints of P. velutinus and the cinnamon-brown to dark chestnut-brown, with violaceous or purplish tints of P. similis. Meanwhile, the lamellae of P. minisporus are white or dirty white, with cross-veins and two tiers of lamellulae, but the lamellae of P. velutinus and P. similis have no cross-veins and with 3 or 4 and 5 tiers of lamellulae, respectively. In addition, the pseudosclerotium of P. minisporus is absent, but P. velutinus and P. similis often have distinct pseudosclerotium. In terms of micromorphology, P. minisporus has smaller spores and Q values, wider generative hyphae and absent sclerocystidia, all of which can be distinguished from P. velutinus and P. similis.

Panus baishanzuensis L. Yue, B. Zhang & Y. Li, sp. nov. Fungal Names: FN 571876 Figs 4, 5

Etymology. The epithet 'baishanzuensis' refers to the type locality, Baishanzu National Park, of this species.

Diagnosis. This species differs from closely-related species by pileus with concentric darker zones, crowded lamellae with cross-veins, shorter stipe, lack of pseudosclerotium, longer basidiospores, greater Q values, diverse and shorter cheilocystidia and smaller sclerocystidia.

Holotype. China. Zhejiang Province: Lishui City, Qingyuan County, Baishanzu National Park, 27.62°N, 118.92°E, 28 July 2023, Yingkun Yang & Lei Yue, FJAU67794 (GenBank accession no.: ITS: PP273985, PP273986, PP273987; nLSU: PP273975, PP273976, PP273977; *tef-1a*: PP590553, PP590554, PP590555).

Description. Basidiomata solitary, medium. Pileus 6–6.8 cm in diameter, thin, coriaceous, infundibuliformis or flared, ochre-brown $(N_{70}Y_{99}M_{60})$, with concentric darker zones, densely covered with brown $(N_{50}Y_{80}M_{30})$ farinaceus



Figure 4. Habitat of *Panus baishanzuensis* (FJAU67794, holotype) A basidiocarp B pileus C lamellae D, E stipe. Scale bars: 1 cm.



Figure 5. Microscopic characteristics of *Panus baishanzuensis* (FJAU67794, holotype) **A** basidia **B** basidiospores **C** sclerocystidia **D** cheilocystidia **E** pileipellis hyphae **F** generative hyphae of context **G** skeletal hyphae of context. Scale bars: 10 μm.

pilosus, stripe dense and slender, radially parallel distributed, margin sinuatus. Lamellae decurrent, crowded, pale yellow $(A_{60}M_{00}C_{00})$, with cross-veins and six tiers of lamellulae, edge entire. Stipe $2.3-3.1 \times 0.3-0.6$ cm, short clavate, excentric, solid, coriaceous, surface concolorous with the pileus or more often darker, with densely velutinus, slightly expanded at the apex. Pseudosclerotium absent. Context thin, up to 1 mm thick, white $(N_{00}Y_{10}M_{00})$, coriaceous, consisting of a dimitic hyphal system with skeletal hyphae.

Generative hyphae 2–3 µm diameter, cylindrical, not inflated, hyaline, thinwalled, frequently branched, with prominent clamp connections. Skeletal hyphae 2–3 µm diameter, sinuous cylindrical, hyaline, with a thick-walled and continuous lumen, unbranched. Basidiospores 7–8(9) × 3–3.5 µm (n = 40, Im = 7.6 µm, wm = 3.07 µm, Q = 2.14–2.67, q = 2.48), cylindrical, smooth, hyaline, thin-walled. Basidia (16)20–25 × 5.5–7 µm, clavate or elongated, bearing four sterigmata. Lamella-edge sterile, with short cheilocystidia. Cheilocystidia crowded, (12)14–19 × (5.5)6–7(8) µm, with median constriction, ellipsoid or utriform and apical protrusion, hyaline, thin-walled. Sclerocystidia abundant, (16.5)19–24 × (5)5.5–6.5(7) µm, clavate to irregularly fusoid, with a thickened wall, hyaline. Hymenophoral trama irregular, radiate construction, hyaline, similar with context. Pileipellis epicutis, made up of thick-walled generative hyphae, 3–5 µm diameter, occasionally brunched, not inflated, light brown. Stipitipellis similar to pileipellis.

Ecology. Solitary on rotten wood in broad-leaved forest.

Distribution. China (Zhejiang Province).

Notes. This species is characterised by the concentric darker zones pileus, decurrent lamellae with cross-veins, shorter stipe, longer basidiospores, diverse and shorter cheilocystidia and smaller sclerocystidia.

Panus baishanzuensis is close to *P. similis* and *P. velutinus* in morphology, because of the similar pileus and lamellae. However, the pileus of *P. velutinus* and *P. similis* without concentric darker zones distinguished them from *P. baishanzuensis*. Meanwhile, the stipe of *P. baishanzuensis* is extremely short, while the stipe of *P. velutinus* and *P. similis* is long and slender. In addition, the lamellae of *P. baishanzuensis* have cross-veins with six tiers of lamellulae, but *P. velutinus* and *P. similis* have no cross-veins and with three or four and five tiers of lamellulae. At the same time, *P. velutinus* and *P. similis* both have pseudosclerotia, while the pseudosclerotium of *P. baishanzuensis* is absent. Lastly and most importantly, *P. baishanzuensis* has longer basidiospores, graeter Q values, shorter cheilocystidia and smaller sclerocystidia than *P. similis* and *P. velutinus*.

New record of Zhejiang Province, China

Panus similis (Berk. & Broome) T.W. May & A.E. Wood, Mycotaxon 54: 148 (1995)

Figs 6, 7

Description. Basidiomata solitary, medium to large. Pileus 3–5.5 cm in diameter, thin, coriaceous, infundibuliform to cyathiform, cinnamon-brown or pale brown ($N_{50}Y_{40-60}M_{20-40}$), glabrous, radially plicate-sulcate with striae extending almost to the centre, without concentric zones; margin curved, ciliate not apparent. Lamellae decurrent, crowded, neither furcate nor anatomosing, buff or

pale brown (N₁₀A₅₀₋₆₀M₁₀₋₂₀), with five tiers of lamellulae, edge entire. Stipe 7.5– 9 × 0.35–0.9 cm, clavate, central, solid, coriaceous, surface chestnut brown, with velutinus, slightly expanded at base. Pseudosclerotium slightly small, irregular. Context thin, up to 1 mm thick, white (N₀₀Y₁₀M₀₀), coriaceous, consisting of a dimitic hyphal system with skeletal hyphae.

Generative hyphae 3–5.5 µm wide, cylindrical, not inflated, hyaline, thinwalled, frequently branched, with prominent clamp connections. Skeletal hyphae 2–3 µm diameter, sinuous cylindrical, with hyaline or pale brown thickwalled and continuous lumen, unbranched. Basidiospores $5.5-7 \times 3-3.5$ µm (n = 40, lm = 6.24 µm, wm = 3.03 µm, Q = 1.57-2.33, q = 2.06), cylindrical, smooth, hyaline, thin-walled. Basidia (18)20–25 × (4)5–6 µm, clavate, cylindrical, bearing four sterigmata. Lamella-edge sterile, with smaller cheilocystidia. Cheilocystidia crowded, (13)14–21 × 5.5-7 µm, nodulose-clavate, irregular, hyaline, thin-walled. Sclerocystidia abundant, $21(22)-32(35) \times 5-6(6.5)$ µm, clavate to irregularly fusoid, with a thick, hyaline or brownish wall. Hymenophoral trama irregular, of radiate construction, hyaline, similar to context. Pileipellis epicutis, made up of thick-walled generative hyphae, 5-6.5 µm wide, occasionally bunched, not inflated, light brown. Stipitipellis similar to pileipellis.

Ecology. Solitary on rotten wood in broad-leaved forest.

Distribution. Angola, Australia, Brunei, China, Congo, India, Ivory Coast, Kenya, Malay Peninsula, Papua New Guinea, Philippines, Sabah, Sarawak, Sri Lanka, Tanzania, Thailand, Uganda, Vietnam, Zanzibar.



Figure 6. Habitat of Panus similis (FJAU67793) A basidiocarps B pileus C lamellae D stipe. Scale bars: 1 cm.



Figure 7. Microscopic characteristics of *Panus similis* (FJAU67793) A basidia B basidiospores C cheilocystidia D sclerocystidia E pileipellis hyphae F skeletal hyphae of context G generative hyphae of context. Scale bars: 10 μ m.

Specimen examined. CHINA. Zhejiang Province: Lishui City, Qingyuan County, Baishanzu National Park, 27.62°N, 118.92°E, 28 July 2023, Yingkun Yang & Lei Yue, FJAU67793.

Notes. This species was originally described by Berkeley and Broome (1873) as *Lentinus similis* Berk. & Broome, then it was treated as *Panus fulvus* var. *similis* (Berk. & Broome) Corner (Corner 1981). However, Pegler (1983) disagreed with Corner and still accepted it as a member of *Lentinus*. Until 1995, it was first raised to a species rank as *P. similis* (May and Wood 1995).

Based on morphological research, there are some differences between our collected specimen and the original description.

Our specimen has distinct plicate-sulcate similar to the original description; however, its pseudosclerotium is smaller, whereas the original is very large. In addition, its hyphae structure is the same as that of the original description, but its spores are larger and its cheilocystidia are smaller compared to the latter.

Before this study, this species was not recorded from Zhejiang Province, China; thus it is the first report of *P. similis* from Zhejiang Province.

Panus conchatus (Bull.) Fr., Epicr. Syst. mycol. (Upsaliae): 396 (1838) [1836-1838]

Fig. 8A, B

Ecology. Solitary on rotten wood.

Distribution. Austria, Belgium, Bulgaria, China, Denmark, Eire, England, Estonia, Germany, India, Norway, Philippines, Russia, Scotland, Sri Lanka, Sweden and Wales.

Specimens examined. CHINA. Jilin Province: Baishan City, Fusong County, Quanyang Town, 4 July 2019, Bo Zhang & Jiajun Hu, FJAU67795; Baishan City, Fusong County, Quanyang Town, 4 July 2019, Bo Zhang & Jiajun Hu, FJAU67797; Baishan City, Fusong County, Quanyang Town, 4 July 2019, Bo Zhang & Jiajun Hu, FJAU67798; Dunhua City, Hancongling Scenic Area, 5 July 2019, Bo Zhang & Jiajun Hu, FJAU67799; Sichuan Province: Ganzi Tibetan Autonomous Prefecture, Jiulong County, Baitai Mountain, 16 July 2023, Xiaolan He, SAAS4904.

Notes. *Panus* is typified as this species and is widely distributed worldwide. It is recorded from Hainan, Hunan, Inner Mongolia etc. from China (Li and Bau 2014).

The appearance of this species is varied. The stipe is easily influenced by the environment, from short to long. Additionally, when aged, due to the appearance of skeletal hyphae, it becomes tough from the soft flesh.

Panus neostrigosus Drechsler-Santos & Wartchow, J. Torrey bot. Soc. 139(4): 438 (2012)

Fig. 8C

Ecology. Solitary on rotten wood.

Distribution. Argentina, Aru, Australia, Austria, Brazil, Bulgaria, Burma, Canada, China, Colombia, Cuba, Dominica, French Guiana, Germany, Galapagos, Hungary, Iran, India, Japan, Madagascar, Malay Peninsula, Mexico, Nepal, New Britain, Pakistan, Papua New Guinea, Philippine, Romania, Sabah, Santo Domingo, Sri Lanka, Russia, Thailand, Trinidad, Turkey, Uganda, U.S.A., Venezuela and Zaire. **Specimens examined.** CHINA. Henan Province: Zhumadian City, Biyang County, Minzhuang Forest Farm, 32.52°N, 113.36°E, 2 August 2023, Yajie Liu, FJAU67796; Zhumadian City, Biyang County, Yihezhai Forest Farm, 32.39°N, 113.34°E, 19 August 2022, Yajie Liu, FJAU67800; Sichuan Province: Ganzi Tibetan Autonomous Prefecture, Jiulong County, Baitai Mountain, 16 July 2023, Xiaolan He, FJAU67801.

Notes. This species is one of the most widely distributed in the genus *Panus*, which was originally described as *Lentinus strigosus* Fr. (Fries 1825) in North Carolina, USA and was transferred to *Panus* by Drechsler-Santos et al. (2012). Due to its densely villous to hispid-strigose pileus, this species is often confused with *P. similis* complex. However, it can be distinguished from the complex by its metuloids.

Panus rudis Fr., Epicr. Syst. mycol. (Upsaliae): 398 (1838) [1836–1838] Fig. 8D

Ecology. Solitary on rotten wood.

Distribution. Brazil, China, France and the Czech Republic (Czechia).

Specimens examined. CHINA. Inner Mongolia Autonomous Region: Xing'an League, Arshan City, Yiershi Town, 47.29°N, 119.84°E, 9 September 2002, Tolgor



Figure 8. Habitat of Panus common species A, B Panus conchatus C Panus neostrigosus D Panus rudis. Scale bars: 1 cm.

Bau, FJAU7824; Henan Province: Zhumadian City, Biyang County, Minzhuang Forest Farm, 32.52°N, 113.36°E, 2 August 2023, Yajie Liu, FJAU67802; Zhumadian City, Biyang County, Yihezhai Forest Farm, 32.39°N, 113.35°E, 31 August 2022, Yajie Liu, FJAU67803.

Notes. This species is extremely similar to *P. neostrigosus* in appearance and has been treated as the same species (e.g. Pegler 1983; Li and Bau 2014; Li et al. 2015; Vargas-Isla et al. 2015). However, in our conception, these two species should be considered as two independent species. *Panus rudis* differed from *P. neostrigosus* in morphology by shorter metuloids and wider cheilocystidia. Moreover, the phylogenetic analysis results also support this conclusion.

Key to the reported species of Panus from China

 Gloeocystidia and metuloids absent, sometimes small skeletocystidi
INDEDI
Dileve ekerthuvilleve te kierid etvisees
2 Prieus shor uy vinous to hispid-strigose
- Prieus grabrous, grabrescent or with a rew libriliose squamules
3 Cheilocystidia wide > 6 μm, metuloids projecting up to 15 μm beyond th basidia
 Cheilocystidia wide < 6 μm, metuloids projecting up to 35 μm beyond th
basidia
4 Elongate fusoid gloeocystidis present
- Clavate to lageniform metuloidal cystidia present
5 Lamellae is equal
- Lamellae is unegual
6 Lamellae with cross-veins
Lamellae without cross-veins
7 With lamellulae of two lengths P. minisporu
- With lamellulae of six lengths
8 Pileus neither strongly striate nor plicate-sulcate, at times finely striate o
weathered specimens
 Pileus radially striate, plicate-sulcate or with concentric zoning, glabres
cent
9 Lamellae densely crowded; pileus finely hispid, radially striate but not su
cateP. ciliatu
 Lamellae moderately crowded: pileus almost glabrous, strongly plicat
sulcate, stipe often verv long

Discussion

In this study, 31 specimens of *Panus* from China were carefully examined. Through the combination of morphological and phylogenetic studies, two new species, *P. minisporus* and *P. baishanzuensis* and one new record from Zhejiang Province, China, viz. *P. similis*, have been discovered and described in detail, which increased the species diversity of *Panus* and expanded the distribution range of *P. similis*. According to Pegler, species belonging to *P. similis* complex were usually found near the Equator in Africa, South America, Australasia and Southeast Asia etc. (Pegler 1983). Additionally, there are also a few records in south China and southwest China (Li and Bau 2014). In contrast, *P. baishanzuensis* is located in east China, which greatly increases the distribution range of species in the *P. similis* complex.

There are quite a few species that are confusing and extremely similar in appearance. Species belonging to sect. Velutini sensu Pegler, such as L. ciliatus, L. similis, L. hookerianus, L. tephroleucus, L. velutinus and L. fasciatus belong to the Lentinus at first, because of their velutinate to strigose basidiomes and thick-walled skeletocystidia (Pegler 1983). However, with the changes in genus conceptions (Corner 1981; May and Wood 1995) and the consistency of phylogenetic and ontogenic studies (Hibbett and Vilgalys 1991; Hibbett et al. 1993), as a result, four species were combined as Panus, viz. P. ciliatus, P. similis, Panus hookerianus (Berk.) T.W. May & A.E. Wood and Panus tephroleucus (Mont.) T.W. May & A.E. Wood, which have a dimitic hyphal system consisting of thick-walled skeletal hyphae and generative hyphae (May and Wood 1995) and leaving L. velutinus and L. fasciatus within Lentinus. According to phylogenetic analysis, L. fasciatus and P. ciliatus are clustered with P. rudis and far away from L. velutinus (Douanla-Meli and Langer 2010), which is also consistent with the morphological characters. In the light of Douanla-Meli and Langer (2010), species of the P. similis complex usually have slender stipe (which can be up to twice as long as the diameter of the pileus). At the same time, the stipes of L. fasciatus and P. ciliatus are only sometimes slender, but usually short and stocky, which coincides with the stipe characteristics of P. rudis (Pegler 1983; Douanla-Meli and Langer 2010; Li and Bau 2014). Similar results were also obtained in this study.

Furthermore, some species within this complex were full of arguments. Lentinus velutinus was proposed by Fries (1830); later, it was combined into P. velutinus (Fries 1838). Meanwhile, Berkeley (1843) described a new species, Lentinus fulvus Berk. Then, Pegler and Rayner (1969) transferred L. fulvus into Panus fulvus (Berk.) Pegler & R.W. Rayner. Afterwards, Corner described three varieties of P. fulvus from Malaysia, based on the pileus structure and dense lamellae (Corner 1981), which, together with P. velutinus, were rehabilitated by Pegler (1983) as L. velutinus. However, according to morphological studies (Pegler 1972, 1983; Corner 1981), L. velutinus does not have the skeleto-ligative hyphae typical of Lentinus, but has the skeletal hyphae typical of Panus, which coincides with the phylogenetic analysis (Douanla-Meli and Langer 2010; Luangharn et al. 2019). Thus, L. velutinus is more closely related to Panus than to Lentinus. Lentinus fasciatus was another controversial species; it was treated as a member of Panus in Singer and Pegler's conception (Singer 1962; Pegler 1965). Later, the combination was rejected by Pegler (1983). However, the presence of skeletal hyphae and phylogenetic studies indicate a close genetic relationship with Panus (Pegler 1983; Douanla-Meli and Langer 2010).

In addition, some taxonomic problems exist in researching *Panus*. Firstly, synonyms led to confusion in species identification, such as *P. ciliatus* sensu May & Wood and *Panus brunneipes* Corner. According to the description given by Corner (1981) for *P. brunneipes* and Pegler (1983) for *P. ciliatus* (= *L. ciliatus*), all these collections refer to the same species. However, based on the legitimate name, both names are accepted by Index Fungorum (http://www.indexfungorum.org) at the same time. However, the epithet '*ciliatus*' is retained

as the oldest one and has priority over 'brunneipes'. Secondly, the boundary between Pleurotus and Panus needs to be refined. Species belonging to Panus are known to have a dimitic hyphal system with skeletal hyphae (Corner 1981; Pegler 1983). However, Panus giganteus (Berk.) Corner and Panus tuber-regium (Fr.) Corner, which also have skeletal hyphal, were transferred into Pleurotus (Singer 1951; Karunarathna et al. 2012). Phylogenetic analysis, based on ITS fragments, showed that they are closely related to species of Pleurotus subg. Coremiopleurotus (Klomklung et al. 2012; Karunarathna et al. 2016). It is worth mentioning that almost all of them have skeletal hyphae. Based on phylogenetic analyses (Stajic et al. 2005; Menolli et al. 2014; Shnyreva and Shnyreva 2015), species of the monomitic hyphal system and species of the dimitic hyphal system were clustered into a single unit, respectively. Obviously, these species are intermediate between Panus and the monomitic hyphal system species in *Pleurotus* (Fig. 1). Perhaps these species are attributed to a new genus-level unit that could serve as a boundary to distinguish between Panus and the remaining monomitic hyphal system species in Pleurotus. Thirdly, some species were always problematic for identification, for example, P. neostrigosus and P. rudis (Li and Bau 2014; Li et al. 2015). There are obvious differences between these two species. The metuloids of P. neostrigosus project up to 35 µm longer than *P. rudis* and the width of cheilocystidia is thinner than the latter. Thus, it is possible to distinguish these confusing species by combing the two characteristics (Pegler 1983; Douanla-Meli and Langer 2010; Li and Bau 2014; Luangharn et al. 2019). In addition, P. neostrigosus is clearly separate from P. rudis through phylogenetic analysis (Douanla-Meli and Langer 2010; Luangharn et al. 2019). Last, but not least, taxonomic research of the genus Panus is not evenly developed in China. The taxonomic research relating to *Panus* is mainly focused on northeast, southwest and south China. Additionally, the resources of Panus are waiting to be employed, especially in east and northwest China.

To address these issues, a more comprehensive study of *Panus* species that integrates morphological and systematic approaches to elucidate the relationships between different species is necessary. Reviewing the type specimens of every species in *Panus* is an essential task. At the same time, more gene fragments need to be obtained to construct more objective phylogenetic trees. With these preliminary preparations, the evolutionary relationship between the Chinese *Panus* data and the world's species will be the subject of the next study.

Acknowledgements

The authors are very grateful to Prof. Tolgor Bau, Prof. Weiqiang Qin, Dr. Ao Ma, Mr. Dizhe Guo, Dr. Gu Rao and Mr. Yingkun Yang for the loan of the specimens studied; to Dr. Yang Wang, Dr. Xuefei Li, Miss Xinya Yang, Miss Xinyue Gui, Mr. Yiming Li, Miss Jiajia Wang, Mr. Libo Wang, Miss Tongtong Tan and Miss Donghan Zhang for their help in the experiment; and to the editors and reviewers for improving the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The study was supported by The Jilin Province Science and Technology Development Plan Project (No. 20230202119NC); Research on the Creation of Excellent Edible Mushroom Resources and High Quality & Efficient Ecological Cultivation Technology in Jiangxi Province (20212BBF61002), The Scientific and Technological Tackling Plan for the Key Fields of Xinjiang Production and Construction Corps (No. 2021AB004), "111" programme (D17014), Youth Doctoral Program of Zhejiang Normal University - Study on species diversity of macrofungi in Baishanzu National Park (2023QB043), Zhejiang Normal University Doctoral Initiation Fund, the Natural Science Foundation of China (Nos. 31970020) and Construction of edible mushroom resource bank and Fungal Resource Conservation System.

Author contributions

Conceptualization: BZ, YL. Data curation: LY. Formal analysis: JH, LY. Funding acquisition: BZ. Investigation: YT, JH, ZQ, YL, LY, JC, XLH. Methodology: JH. Project administration: JC, BZ. Resources: ZQ, JH, YT, XLH, YL, JC. Software: LY. Supervision: BZ, YL, JH. Validation: BZ. Visualization: BZ, JH. Writing - original draft: LY. Writing - review and editing: BZ, JH.

Author ORCIDs

Jiajun Hu lo https://orcid.org/0000-0002-7562-7612

Data availability

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

References

- Andrew R (2016) FigTree: tree figure drawing tool. Version 1.4.3. Institute of evolutionary biology. http://tree.bio.ed.ac.uk/software/figtree/ [Accessed on 4 October 2016]
- Avin FA, Bhassu S, Shin TY, Sabaratnam V (2012) Molecular classification and phylogenetic relationships of selected edible Basidiomycetes species. Molecular Biology Reports 39(7): 7355–7364. https://doi.org/10.1007/s11033-012-1567-2
- Berkeley MJ (1843) Notices of fungi in the Herbarium of the British Museum. Annals & Magazine of Natural History 10(sup66): 369–384. https://doi. org/10.1080/03745484309445244
- Berkeley MJ, Broome CE (1873) Enumeration of the Fungi of Ceylon. Part II., containing the remainder of the Hymenomycetes, with the remaining established tribes of Fungi (Continued.). Journal of the Linnean Society of London, Botany 14(74): 65–140. https://doi.org/10.1111/j.1095-8339.1873.tb00302.x
- Berkeley MJ, Broome CE (1883) IV. List of Fungi from Brisbane, Queensland; with descriptions of new species.—Part II. Transactions of the Linnean Society of London. 2nd Series. Botany 2(3): 53–73. https://doi.org/10.1111/j.1095-8339.1883.tb00004.x
- Binder M, Larsson KH, Matheny PB, Hibbett DS (2010) Amylocorticiales ord. nov. and Jaapiales ord. nov.: Early diverging clades of Agaricomycetidae dominated by corticioid forms. Mycologia 102(4): 865–880. https://doi.org/10.3852/09-288
Bulliard JBF (1786) Herbier de la France. Vol. 6. Didot et al., Paris, France.

- Corner EJH (1981) The agaric genera *Lentinus*, *Panus*, and *Pleurotus* with particular reference to Malaysian species. Beihefte zur Nova Hedwigia 69: 1–169.
- Cubeta MA, Echandi E, Abernethy T, Vilgalys R (1991) Characterization of anastomosis groups of binucleate rhizoctonia species using restriction analysis of an amplified ribosomal RNA gene. Phytopathology 81(11): 1395–1400. https://doi.org/10.1094/ Phyto-81-1395
- Douanla-Meli C, Langer E (2010) Reassessment of phylogenetic relationships of some lentinoid fungi with velutinate basidiomes based on partial 28S ribosomal RNA gene sequencing. Sydowia 62(1): 23–35.
- Drechsler-Santos ER, Wartchow F, Coimbra VRM, Gibertoni TB, Cavalcanti MAQ (2012) Studies on lentinoid fungi (*Lentinus* and *Panus*) from the semi-arid region of Brazil1. The Journal of the Torrey Botanical Society 139(4): 437–446. https://doi. org/10.3159/TORREY-D-12-00019.1
- Du F, Liu B, Li ZY, Yuan PG (1983) List of fungi in the department of biology, Shanxi University (Continued 2). Journal of Shanxi University (03): 75–91. https://doi. org/10.13451/j.cnki.shanxi.univ(nat.sci.).1983.03.012 [Natural Science Edition]
- Floudas D, Hibbett DS (2015) Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset and extensive ITS sampling. Fungal Biology 119(8): 679–719. https://doi.org/10.1016/j.funbio.2015.04.003
- Fries EM (1825) Systema orbis vegetabilis: Plantæ homonemeæ. Typographia Academica, Lunde, Sweden.
- Fries EM (1830) Eclogae fungorum, praecipue ex herbarus germanorum de scriptorum. Linnaea 5: 497–553.
- Fries EM (1838) Epicrisis systematis mycologici seu synopsis Hymenomycetum. Typographia Academica, Uppsala, Sweden.
- Garcia-Sandoval R, Wang Z, Binder M, Hibbett DS (2011) Molecular phylogenetics of the Gloeophyllales and relative ages of clades of Agaricomycotina producing a brown rot. Mycologia 103(3): 510–524. https://doi.org/10.3852/10-209
- Gardes M, Bruns TD (1993) Its primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. Molecular Ecology 2(2): 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuclc Acids Symposium Series 41: 95–98. https://doi.org/10.1021/bk-1999-0734.ch008
- He SH, Li HJ (2013) *Pseudochaete latesetosa* and *P. subrigidula* spp. nov. (Hymenochaetales, Basidiomycota) from China based on morphological and molecular characters. Mycological Progress 12(2): 331–339. https://doi.org/10.1007/s11557-012-0838-6
- Hibbett DS, Vilgalys R (1991) Evolutionary relationships of *Lentinus* to the Polyporaceae: Evidence from restriction analysis of enzymatically amplified ribosomal DNA. Mycologia 83(4): 425–439. https://doi.org/10.1080/00275514.1991.12026032
- Hibbett DS, Murakami S, Tsuneda A (1993) Hymenophore development and evolution in *Lentinus*. Mycologia 85(3): 428–443. https://doi.org/10.1080/00275514.1993.12 026294
- Hu JJ, Song LR, Tuo YL, Zhao GP, Yue L, Zhang B, Li Y (2022a) Multiple evidences reveal new species and a new record of smelly Gymnopus (Agaricales, Omphalotaceae) from China. Frontiers in Microbiology 13: 968617. https://doi.org/10.3389/ fmicb.2022.968617

- Hu JJ, Zhao GP, Tuo YL, Rao G, Zhang ZH, Qi ZX, Yue L, Liu YJ, Zhang T, Li Y, Zhang B (2022b) Morphological and molecular evidence reveal eight new species of *Gymnopus* from Northeast China. Journal of Fungi 8(4): 349. https://doi.org/10.3390/jof8040349
- Huerta G, Martínez-carrera D, Sánchez JE, Leal-lara H, Vilgalys R (2010) Genetic relationships between Mexican species of *Pleurotus* analyzing the ITS region from rDNA. Micología Aplicada International 22(1): 15–25.
- Imai S (1938) Studies on the Agaricaceae of Hokkaido. I. Journal of the Faculty of Agriculture 43(1): 1–178.
- Ji XH, Vlasák J, Tian XM, Dai YC (2018) Three new species of *Fomitiporella* (Hymenochaetales, Basidiomycota) based on the evidence from morphology and DNA sequence data. MycoKeys 73(30): 73–89. https://doi.org/10.3897/mycokeys.30.23109
- Justo A, Hibbett DS (2011) Phylogenetic classification of *Trametes* (Basidiomycota, Polyporales) based on a five-marker dataset. Taxon 60(6): 1567–1583. https://doi. org/10.1002/tax.606003
- Justo A, Miettinen O, Floudas D, Ortiz-Santana B, Sjökvist E, Lindner D, Nakasone K, Niemela T, Larsson K, Ryvarden L, Hibbett DS (2017) A revised family-level classification of the Polyporales (Basidiomycota). Fungal Biology 121(9): 798–824. https:// doi.org/10.1016/j.funbio.2017.05.010
- Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS (2017) Modelfinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Karunarathna SC, Mortimer PE, Li GJ, He MQ, Xu JC, Seelan JSS, Hassan BA, Hyde KD, Zhao RL (2016) Correct names of two cultivated mushrooms from the genus *Pleurotus* in China. Phytotaxa 260(1): 036–046. https://doi.org/10.11646/phyto-taxa.260.1.4
- Katoh K, Standley DM (2013) Mafft multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kibby G, Moser M, Plant S (1978) Keys to Agarics and Boleti (Polyporales, Boletules, Agaricales, Russulales). Roger Phillips, London.
- Klomklung N, Karunarathna SC, Chukeatirote E, Hyde KD (2012) Domestication of wild strain of *Pleurotus giganteus*. Sydowia 64(1): 39–53.
- Kühner R (1980) Les Hyménomycètes agaricoïdes: (Agaricales, Tricholomatales, Pluteales, Russulales): etude générale et classification. Société linnéenne de Lyon: Lyon, France.
- Kumla J, Suwannarach N, Jaiyasen A, Bussaban B, Lumyong S (2013) Development of an edible wild strain of Thai oyster mushroom for economic mushroom production. Warasan Khana Witthayasat Maha Witthayalai Chiang Mai 40(2): 161–172.
- Küppers H (2002) Atlas de los colores. Editorial Blume, Barcelona, Spain.
- Li Y, Bau T (2014) Flora fungorum sinicorum. 45. Pleurotoid-Lentioned fungi. Science Press, Beijing, China.
- Li WH, Qin SY (1991) Preliminary List of Medicinal Fungi in Sichuan Province. Resource Development & Market 04: 249–252[+219].
- Li ZY, Liu B, Du F (1978) List of Fungi in the Department of Biology, Shanxi University (Continued 1). Journal of Shanxi University (02): 111–127. https://doi.org/10.13451/j. cnki.shanxi.univ(nat.sci.).1978.02.015 [Natural Science Edition]
- Li TH, Song B, Wu XL, Deng WQ (2004) A Study on *Panus* in Yunnan, Guizhou and Guangxi, China. Guizhou Science (01): 47–53[+96].
- Li Y, Li TH, Yang ZL, Bau T, Dai YC (2015) Atlas of Chinese Macrofungal Resources. Central China Farmer's Publishing House, Zhengzhou, China.

- Liu XB, Li J, Horak E, Yang ZL (2016) *Pleurotus placentodes*, originally described from Sikkim, rediscovered after 164 years. Phytotaxa 267: 137e145. https://doi.org/10.11646/phytotaxa.267.2.6.
- Luangharn T, Karunarathna SC, Mortimer PE, Hyde KD, Xu J (2019) Morphological and molecular identification of *Panus conchatus* (Polyporaceae, Polyporales) from Yunnan Province, China. Studies in Fungi 4(1): 253–262. https://doi.org/10.5943/ sif/4/1/27
- Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS (2006) Major clades of Agaricales: A multilocus phylogenetic overview. Mycologia 98(6): 982–995. https://doi.org/10.1080/1557253 6.2006.11832627
- May TW, Wood AE (1995) Nomenclatural notes on Australian macrofungi. Mycotaxon 54: 147–150.
- Menolli Jr N, Breternitz BS, Capelari M (2014) The genus *Pleurotus* in Brazil: A molecular and taxonomic overview. Mycoscience 55(5): 378–389. https://doi.org/10.1016/j. myc.2013.12.001
- Menolli Jr N, Sánchez-Ramírez S, Sánchez-García M, Wang C, Patev S, Ishikawa NK, Mata JL, Lenz AR, Vargas-Isla R, Liderman L, Lamb M, Nuhn M, Hughes KW, Xiao Y, Hibbett DS (2022) Global phylogeny of the Shiitake mushroom and related *Lentinula* species uncovers novel diversity and suggests an origin in the Neotropics. Molecular Phylogenetics and Evolution 173: 107494. https://doi.org/10.1016/j.ympev.2022.107494

Miller OK (1972) Mushrooms of North America. E. P. Dutton and Company, New York, USA.

- Nakasone KK, Lindner DL (2012) Taxonomy of *Pseudolagarobasidium* (Polyporales, Basidiomycota). Fungal Diversity 55(1): 155–169. https://doi.org/10.1007/s13225-012-0161-1
- Nie T, Tian Y, Liu SL, Yang J, He SH (2017) Species of *Hymenochaete* (Hymenochaetales, Basidiomycota) on bamboos from East Asia, with descriptions of two new species. MycoKeys 20: 51–65. https://doi.org/10.3897/mycokeys.20.11754
- Nuhn ME, Binder M, Taylor AF, Halling RE, Hibbett DS (2013) Phylogenetic overview of the Boletineae. Fungal Biology 117(7–8): 479–511. https://doi.org/10.1016/j.funbio.2013.04.008
- Pegler DN (1965) Studies on Australasian Agaricales. Australian Journal of Botany 13(2): 323-356. https://doi.org/10.1071/BT9650323
- Pegler DN (1971) *Lentinus* Fr. and related genera from Congo-Kinshasa (Fungi). Bulletin du Jardin botanique national de Belgique/Bulletin van de Nationale Plantentuin van Belgie: 273–281. https://doi.org/10.2307/3667639.
- Pegler DN (1975) The classification of the genus *Lentinus* Fr. (Basidiomycota). Kavaka 3: 11–20.
- Pegler DN (1983) The genus *Lentinus*, a world monograph. Kew Bulletin Additional Series: London, Britain.
- Pegler DN, Rayner RW (1969) A contribution to the Agaric flora of Kenya. Kew Bulletin 23(3): 347–412. https://doi.org/10.2307/4117177
- Petersen RH, Hughes KW (1997) A new species of *Pleurotus*. Mycologia 89(1): 173–180. https://doi.org/10.1080/00275514.1997.12026768
- Rehner SA, Buckley E (2005) A Beauveria phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97(1): 84–98. https://doi.org/10.3852/mycologia.97.1.84

- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Schmidt HA, Minh BQ, Von Haeseler A, Minh BQ (2014) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Senthilarasu G (2015) The lentinoid fungi (*Lentinus* and *Panus*) from Western ghats, India. IMA Fungus 6(1): 119–128. https://doi.org/10.5598/imafungus.2015.06.01.06
- Shao LP, Xiang CT (2006) Chinese forest mushrooms. Northeast Forestry University Press, Harbin, China.
- Shnyreva AA, Shnyreva AV (2015) Phylogenetic analysis of *Pleurotus* species. Russian Journal of Genetics 51(2): 148–157. https://doi.org/10.1134/S1022795415020131
- Shnyreva AA, Sivolapova AB, Shnyreva AV (2012) The commercially cultivated edible oyster mushrooms *Pleurotus sajor-caju* and *P. pulmonarius* are two separate species, similar in morphology but reproductively isolated. Russian Journal of Genetics 48(11): 1080–1088. https://doi.org/10.1134/S1022795412110105
- Singer R (1951) The Agaricales in modern taxonomy. Lilloa 22: 5–832.
- Singer R (1961a) Type studies in Basidiomycetes. X. Persoonia-Molecular Phylogeny and Evolution of Fungi 2(1): 1–62.
- Singer R (1961b) Type studies on agarics IV. Sydowia 15: 133–151.
- Singer R (1962) The Agaricales in modern taxonomy (2nd edn.). J. Cramer, Weinheim, Germany.
- Singer R (1986) The Agaricales in modern taxonomy (4th edn.). Koeltz Scientific Books, Koenigstein, Germany.
- Sotome K, Hattori T, Ota Y, To-Anun C, Salleh B, Kakishima M (2008) Phylogenetic relationships of *Polyporus* and morphologically allied genera. Mycologia 100(4): 603– 615. https://doi.org/10.3852/07-191R
- Stajic M, Sikorski J, Wasser SP, Nevo E (2005) Genetic similarity and taxonomic relationships within the genus *Pleurotus* (higher Basidiomycetes) determined by RAPD analysis. Mycotaxon 93: 247–256.
- Stevenson G (1964) The Agaricales of New Zealand: V. Kew Bulletin 19(1): 1–59. https:// doi.org/10.2307/4108283
- Tai FL (1979) Sylloge Fungorum Sinicorum. Science Press, Beijing, China.
- Teng SC (1963) Fungi of China. Science Press, Beijing, China.
- Thorn RG, Moncalvo JM, Reddy CA, Vilgalys R (2000) Phylogenetic analyses and the distribution of nematophagy support a monophyletic Pleurotaceae within the poly-phyletic pleurotoid-lentinoid fungi. Mycologia 92(2): 241–252. https://doi.org/10.108 0/00275514.2000.12061151
- Tibpromma S, Hyde KD, Jeewon R, Maharachchikumbura SS, Liu JK, Bhat DJ, Jones EG, McKenzie EH, Camporesi E, Bulgakov TS, Doilom M, de Azevedo Santiago ALCM, Das K, Manimohan P, Gibertoni TB, Lim YW, Ekanayaka AH, Thongbai B, Lee HB, Yang JB, Kirk PM, Sysouphanthong P, Singh SK, Boonmee S, Dong W, Raj KNA, Latha KPD, Phookamsak R, Phukhamsakda C, Konta S, Jayasiri SC, Norphanphoun C, Tennakoon DS, Li J, Dayarathne MC, Perera RH, Xiao Y, Wanasinghe DN, Senanayake IC, Goonasekara ID, de Silva NI, Mapook A, Jayawardena RS, Dissanayake AJ, Manawasinghe IS, Chethana KWT, Luo Z-L, Hapuarachchi KK, Baghela A, Soares AM, Vizzini A, Meiras-Ottoni A, Mešić A, Dutta AK, de Souza CAF, Richter C, Lin CG, Chakrabarty D, Daranagama DA, Lima DX, Chakraborty D, Ercole E, Wu F, Simonini G, Vasquez G, da Silva GA, Plautz Jr

HL, Ariyawansa HA, Lee H, Kušan I, Song J, Sun J, Karmakar J, Hu K, Semwal KC, Thambugala KM, Voigt K, Acharya K, Rajeshkumar KC, Ryvarden L, Jadan M, Hosen MI, Mikšík M, Samarakoon MC, Wijayawardene NN, Kim NK, Matočec N, Singh PN, Tian Q, Bhatt RP, de Oliveira RJV, Tulloss RE, Aamir S, Kaewchai S, Marathe SD, Khan S, Hongsanan S, Adhikari S, Mehmood T, Bandyopadhyay TK, Svetasheva TY, Nguyen TTT, Antonín V, Li WJ, Wang Y, Indoliya Y, Tkalčec Z, Elgorban AM, Bahkali AH, Tang AMC, Su HY, Zhang H, Promputtha I, Luangsa-ard J, Xu J, Yan J, Kang JC, Stadler M, Mortimer PE, Chomnunti P, Zhao Q, Phillips AJL, Nontachaiyapoom S, Wen TC, Karunarathna SC (2017) Fungal diversity notes 491–602: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 83(1): 1–261. https://doi.org/10.1007/s13225-017-0378-0

- Vargas-Isla R, Capelari M, Menolli Jr N, Nagasawa E, Tokimoto K, Ishikawa NK (2015) Relationship between *Panus lecomtei* and *P. strigellus* inferred from their morphological, molecular and biological characteristics. Mycoscience 56(6): 561–571. https:// doi.org/10.1016/j.myc.2015.05.004
- Vilgalys R, Sun BL (1994) Ancient and recent patterns of geographic speciation in the oyster mushroom *Pleurotus* revealed by phylogenetic analysis of ribosomal DNA sequences. Proceedings of the National Academy of Sciences of the United States of America 91(10): 4599–4603. https://doi.org/10.1073/pnas.91.10.4599
- Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Hatmi AA, Groenewald JZ, Cardinali G, Houbraken J, Boekhout T, Crous PW, Robert V, Verkley GJM (2019) Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92(1): 135–154. https://doi. org/10.1016/j.simyco.2018.05.001
- Wang CG, Dai YC (2022) Phylogeny and taxonomy of *Spongipellis* (Polyporales, Basidiomycota) and its micromorphological similar genera. Mycological Progress 21(9): 73. https://doi.org/10.1007/s11557-022-01817-w
- Wu G, Feng B, Xu J, Zhu XT, Li YC, Zeng NK, Hosen MI, Yang ZL (2014) Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family Boletaceae. Fungal Diversity 69(1): 93–115. https://doi.org/10.1007/ s13225-014-0283-8
- Xiang CY, Gao FL, Jakovlić I, Lei HP, Hu Y, Zhang H, Zou H, Wang GT, Zhang D (2023) Using PhyloSuite for molecular phylogeny and Tree-Based analyses. iMeta 2(1): 1–42. https://doi.org/10.1002/imt2.87
- Xu TH, Liu Q, Wang CL, Ma T (1986) Investigation of Edible Mushroom Resources and Habitat in Anhui Province. Edible Fungi (03): 1–2+26.
- Yue L, Tuo YL, Qi ZX, Hu JJ, Liu YJ, Li XF, Liu MH, Zhang B, Liu SY, Li Y (2023) Morphology and molecular phylogeny of *Neolentinus* in northern China. PeerJ 11: e16470. https:// doi.org/10.7717/peerj.16470
- Zervakis GI, Moncalvo JM, Vilgalys R (2004) Molecular phylogeny, biogeography and speciation of the mushroom species *Pleurotus cystidiosus* and allied taxa. Microbiology (Reading, England) 150(3): 715–726. https://doi.org/10.1099/mic.0.26673-0
- Zervakis GI, Ntougias S, Gargano ML, Besi MI, Polemis E, Typas MA, Venturella G (2014) A reappraisal of the *Pleurotus eryngii* complex - new species and taxonomic combinations based on the application of a polyphasic approach, and an identification key to *Pleurotus* taxa associated with Apiaceae plants. Fungal Biology 118(9–10): 814–834. https://doi.org/10.1016/j.funbio.2014.07.001
- Zervakis GI, Venturella G, Fryssouli V, Inglese P, Polemis E, Gargano ML (2019) *Pleurotus opuntiae* revisited - An insight to the phylogeny of dimitic *Pleurotus* species with

emphasis on the *P. djamor* complex. Fungal Biology 123(3): 188–199. https://doi. org/10.1016/j.funbio.2018.12.005

- Zhang D, Gao FL, Jakovlić I, Zhou H, Zhang J, Li WX, Wang GT (2020) Phylosuite: Anintegrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhao RL, Li GJ, Sánchez-Ramírez S, Stata M, Yang ZL, Wu G, Dai YC, He SH, Cui BK, Zhou JL, Wu F, He MQ, Moncalvo J, Hyde KD (2017) A six-gene phylogenetic overview of Basidiomycota and allied phyla with estimated divergence times of higher taxa and a phyloproteomics perspective. Fungal Diversity 84(1): 43–74. https://doi. org/10.1007/s13225-017-0381-5
- Zhou LW (2014) *Fulvifomes hainanensis* sp. nov. and *F. indicus* comb. nov. (Hymenochaetales, Basidiomycota) evidenced by a combination of morphology and phylogeny. Mycoscience 55(1): 70–77. https://doi.org/10.1016/j.myc.2013.05.006
- Zhou JL, Su SY, Su HY, Wang B, Callac P, Guinberteau J, Hyde KD, Zhao RL (2016) A description of eleven new species of *Agaricus* sections *Xanthodermatei* and *Hondenses* collected from Tibet and the surrounding areas. Phytotaxa 257(2): 99–121. https:// doi.org/10.11646/phytotaxa.257.2.1
- Zmitrovich IV, Kovalenko AE (2016) Lentinoid and polyporoid fungi, two generic conglomerates containing important medicinal mushrooms in molecular perspective. International Journal of Medicinal Mushrooms 18(1): 23–38. https://doi.org/10.1615/ IntJMedMushrooms.v18.i1.40

Supplementary material 1

Newly obtained sequences

Author: Lei Yue

Data type: txt

- Explanation note: ere are the ITS, nLSU, and tef-1a sequences obtained in this study.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.121025.suppl1



Research Article

Three new endophytic *Apiospora* species (Apiosporaceae, Amphisphaeriales) from China

Xiao-Ni Yan¹⁰, Chu-Long Zhang¹⁰

Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Key Laboratory of Biology of Crop Pathogens and Insects of Zhejiang Province, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China

Corresponding author: Chu-Long Zhang (clzhang@zju.edu.cn)

Abstract

Apiospora species are widely distributed fungi with diverse lifestyles, primarily functioning as plant pathogens, as well as exhibiting saprophytic and endophytic behaviors. This study reports the discovery of three new species of *Apiospora*, namely *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*, isolated from healthy Poaceae plants in China. These novel species were identified through a multi-gene phylogenetic analysis. The phylogenetic analysis of the combined ITS, LSU, *tef1*, and *tub2* sequence data revealed that the three new species formed a robustly supported clade with *A. garethjonesii*, *A. neogarethjonesii*, *A. setostroma*, *A. subrosea*, *A. mytilomorpha*, and *A. neobambusae*. Detailed descriptions of the newly discovered species are provided and compared with closely related species to enhance our understanding of the genus *Apiospora*.

Key words: Apiospora, Ascomycota, endophyte, phylogeny, taxonomy

Introduction

Apiospora is an important genus of fungal Sordariomycetes, that produces a basauxic, arthrinium-like conidiogenesis (Hyde et al. 2020). The family Apiosporaceae was established to accommodate the genus *Apiospora* with the special conidiogenesis (Hyde et al. 1998). Over time, the membership of Apiosporaceae has undergone several revisions. It presently comprises several genera of fungi with similar morphology, including *Apiospora*, *Arthrinium*, *Nigrospora*, and *Neoarthrinium* (Wang et al. 2017; Pintos and Alvarado 2021; Jiang et al. 2022).

Within the family Apiosporaceae, *Apiospora* is closely related to *Arthrinium* and they were once considered as two life stages of a single taxon (Ellis 1965; Crous and Groenewald 2013; Réblová et al. 2016; Jiang et al. 2019). Morphologically, *Apiospora* and *Arthrinium* lack clear diagnostic features, although species of *Arthrinium* often produce conidia of various shapes (Minter and Cannon 2018; Pintos and Alvarado 2021), while most species of *Apiospora* have rounded lenticular conidia (Li et al. 2023; Liao et al. 2023). Ecologically, most sequenced collections of *Arthrinium* were found on Cyperaceae or Juncaceae in temperate, cold, or alpine habitats, while those of *Apiospora* were mainly collected on Poaceae, as well as various other plant host families, in a wide range of habitats, including tropical and subtropical regions (Dai et al. 2016; Jiang et



Academic editor: C. Phukhamsakda Received: 8 March 2024 Accepted: 11 May 2024 Published: 31 May 2024

Citation: Yan X-N, Zhang C-L (2024) Three new endophytic *Apiospora* species (Apiosporaceae, Amphisphaeriales) from China. MycoKeys 105: 295–316. https://doi. org/10.3897/mycokeys.105.122583

Copyright: [©] Xiao-Ni Yan & Chu-Long Zhang. This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0). al. 2018; Wang et al. 2018; Feng et al. 2021; Tian et al. 2021; Kwon et al. 2022; Monkai et al. 2022). With the addition of molecular evidence and the expansion of the sample, the latest phylogenetic analysis suggests that *Arthrinium* s. str. and *Apiospora* represent independent lineages within Apiosporaceae (Pintos and Alvarado 2021). Consequently, most species of *Arthrinium* have been reclassified under *Apiospora*. Furthermore, Pintos and Alvarado defined the exact identity of *Apiospora montagnei* (the type species of *Apiospora*) and delineated the phylogenetic boundaries of *Apiospora* (Pintos and Alvarado 2022).

Currently, there are 176 records in *Apiospora* (Index Fungorum; http://www. indexfungorum.org/; accessed on 8 Mar 2024). These fungi primarily act as plant pathogens, causing diseases in a wide range of host plants. For example, *A. arundinis* is the causal agent for several important plant diseases, such as kernel blight of barley (Martínez-Cano et al. 1992), brown culm streak of *Phyllostachys praecox* (Chen et al. 2014), moldy sugarcane (Liao et al. 2022), and leaf spot on *Polygonatum cyrtonema* (Gong et al. 2023). *A. marii* causes dieback of olive trees (Gerin et al. 2020), while *A. kogelbergense* leads to blight of *Bambusa intermedi* (Yin et al. 2020). Whereas, many *Apiospora* species are saprophytes, such as *A. acutiapica* (Senanayake et al. 2020), *A. garethjonesii* (Dai et al. 2016), *A. magnispora* (Zhao et al. 2023), *A. sasae* (Crous et al. 2021), and *A. thailandicum* (Dai et al. 2017). In addition, certain *Apiospora* species are reported as endophytes with wide host range, including bamboo (Wang et al. 2018), *Camellia sinensis* (Wang et al. 2018), *Wurfbainia villosa* (Liao et al. 2023), and even hive-stored pollen (Zhao et al. 2018).

Endophytic fungi exhibit rich diversity and play a significant role in the ecosystem. In a previous study, we collected and isolated endophytic fungi from healthy Poaceae plants in China (Liu et al. 2021). In this study, three new endophytic species of *Apiospora* were identified and described based on morphological characteristics and a multi-gene phylogenetic analysis, utilizing a dataset comprising the combined nuclear ribosomal DNA internal transcribed spacer (ITS), nuclear ribosomal DNA large subunit (LSU), the translation elongation factor 1-alpha (*tef1*), and β -tubulin (*tub2*) sequences.

Materials and methods

Fungal isolation

In the present work, Poaceae plant samples were collected from three locations in China: Xilingol Grassland National Nature Reserve in Inner Mongolia, Xishuangbanna, Naban River Watershed National Nature Reserve in Yunnan province, and Baishanzu National Nature Reserve in Zhejiang province (Liu et al. 2021). To isolate endophytic *Apiospora* strains, healthy tissues of asymptomatic plants were first disinfected for 3 min in 75% ethanol and 10 min in 1% sodium hypochlorite, followed by three washes in sterile distilled water. The disinfected tissues were excised, and then incubated on malt extract agar (MEA) medium at 25 °C. Subsequently, the growing hyphae were transferred to potato dextrose agar (PDA) medium to obtain pure cultures.

All strains of *Apiospora* were stored in the Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou, China. In addition, the holotype and ex-type culture were deposited in the Guangdong Microbial Culture Collection Center (GDMCC). Fungal names were registered in the Fungal Names, one of the recognised repositories of fungal taxonomy (https://nmdc.cn/fungalnames/).

Morphological study

Morphological descriptions were recorded on PDA and MEA. The morphological characteristics of the colonies were captured with a digital camera (Canon EOS700D). The fungal structures were observed and photographed using a stereomicroscope (Leica S9D) and a Leica DM2500 microscope equipped with differential interference contrast (DIC). Measurements of conidiogenous cells and conidia were reported as follows: a-b × c-d (mean, n), where "a" and "c" represent the minimum values, "b" and "d" represent the maximum values, and the mean value and number of measurements (n) are shown in parentheses (Wang et al. 2018).

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelia from pure cultures grown on PDA at 25 °C for 5-7 d were used for DNA extraction. Genomic DNA was extracted following the method as described in Chi et al. (2009).

Polymerase chain reaction (PCR) amplification was applied to amplify four gene fragments, including ITS, LSU, *tef1*, and *tub2*. The primer pairs were used: ITS1/ITS4 for ITS (White et al. 1990), LR0R/LR5 for LSU (Rehner and Samuels 1995), EF1-728F/EF2 for *tef1* (O'Donnell et al, 1998; Carbone and Kohn 1999), and T1/Bt2b for *tub2* (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). PCR program for ITS amplification was conducted with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. The annealing temperatures were adjusted to 56 °C for LSU, *tef1*, and *tub2*.

PCR was performed using a Veriti Thermal Cycler (Waltham, MA, USA). Amplification reactions contained 10 μ L of 2× Taq Plus Master Mix II (Vazyme, Nanjing, China), 0.8 μ L of each primer (10 μ M) (Sunya, Hangzhou, China), 0.8 μ L of DNA template, and double-distilled water to reach a total volume of 20 μ L. Purification and sequencing of PCR products were performed by Sunya Biotechnology Company (Hangzhou, China). All sequences generated in this study were deposited in GenBank (Table 1).

Phylogenetic analyses

The quality of obtained sequences was assessed using Chromas v.2.6.6 and the sequences were assembled using SeqMan v.7.1.0. The reference sequences were retrieved from GenBank. All sequences, including the reference sequences, were aligned in batches with MAFFT (Katoh and Standley 2013), manually correcting the resulting alignment by MEGA v.11.0.13 where necessary. A single alignment was made using ITS, LSU, *tef1* region including partial exon 4 and partial exon 5 (the largest exon), *tub2* region including exon 2, exon 3, and partial exon 4. Then phylogenetic analyses were conducted using partial sequences of the above four loci.

 Table 1. Species of Apiosporaceae used in the phylogenetic analyses. Notes: Strains in this study are marked in bold.

 "T" indicates a type culture. NA = not available.

Creation	Ctusin Numbers	liest and Substrates	Lecelity	GenBank acc		ession numbers		
Species	Strain Numbers	Host and Substrates	Locality	ITS	LSU	tef1	tub2	
Apiospora acutiapica	KUMCC 20-0209	Bambusa bambos	China	MT946342	MT946338	MT947359	MT947365	
Apiospora acutiapica	KUMCC 20-0210 T	Bambusa bambos	China	MT946343	MT946339	MT947360	MT947366	
Apiospora adinandrae	SAUCC 1282B-1 ^T	Diseased leaves of Adinandra glischroloma	China	OR739431	OR739572	OR753448	OR757128	
Apiospora adinandrae	SAUCC 1282B-2	Diseased leaves of Adinandra glischroloma	China	OR739432	OR739573	OR753449	OR757129	
Apiospora agari	KUC21333, SFC20161014-M18 [⊤]	Agarum cribrosum	South Korea	MH498520	MH498440	MH544663	MH498478	
Apiospora aquatic	MFLU 18-1628, S-642 [⊤]	Submerged wood	China	MK828608	MK835806	NA	NA	
Apiospora arctoscopi	KUC21331, SFC20200506-M05 [™]	Eggs of Arctoscopus japonicus	South Korea	MH498529	MH498449	MN868918	MH498487	
Apiospora arundinis	CBS 124788	Living leaves of Fagus sylvatica	Switzerland	KF144885	KF144929	KF145017	KF144975	
Apiospora arundinis	LC4951	Dichotomanthes tristaniicarpa	China	KY494698	KY494774	KY705097	KY705168	
Apiospora aseptata	KUNCC 23-14169 ^T	Living roots of Dicranopteris pedata	China	OR590341	OR590335	OR634949	OR634943	
Apiospora aurea	CBS 244.83 ^T	Air	Spain	AB220251	KF144935	KF145023	KF144981	
Apiospora balearica	CBS 145129, AP24118 [⊤]	Poaceae plant	Spain	MK014869	MK014836	MK017946	MK017975	
Apiospora bambusicola	MFLUCC 20-0144 T	Schizostachyum brachycladum	Thailand	MW173030	MW173087	MW183262	NA	
Apiospora bawanglingensis	SAUCC BW0444 ^T	Leaves of Indocalamus Iongiauritus	China	OR739429	OR739570	OR753446	OR757126	
Apiospora biserialis	CGMCC 3.20135 T	Bamboo	China	MW481708	MW478885	MW522938	MW522955	
Apiospora camelliae- sinensis	CGMCC 3.18333, LC5007 ^T	Camellia sinensis	China	KY494704	KY494780	KY705103	KY705173	
Apiospora camelliae- sinensis	LC8181	Brassica rapa	China	KY494761	KY494837	KY705157	KY705229	
Apiospora cannae	ZHKUCC 22-0139	Leaves of Canna sp.	China	OR164902	OR164949	OR166286	OR166322	
Apiospora cannae	ZHKUCC 22-0127 T	Leaves of Canna sp.	China	OR164901	OR164948	OR166285	OR166321	
Apiospora chiangraiense	MFLUCC 21-0053 T	Dead culms of bamboo	Thailand	MZ542520	MZ542524	NA	MZ546409	
Apiospora chromolaenae	MFLUCC 17-1505 ^T	Chromolaena odorata	Thailand	MT214342	MT214436	MT235802	NA	
Apiospora cordylinae	GUCC 10026	Cordyline fruticosa	China	MT040105	NA	MT040126	MT040147	
Apiospora cordylinae	GUCC 10027 T	Cordyline fruticosa	China	MT040106	NA	MT040127	MT040148	
Apiospora coryli	CFCC 58978 [⊤]	Dead plant culms of Corylus yunnanensis	China	OR125564	OR133586	OR139974	OR139978	
Apiospora coryli	CFCC 58979 ^T	Dead plant culms of Corylus yunnanensis	China	OR125565	OR133587	OR139975	OR139979	
Apiospora cyclobalanopsidis	CGMCC 3.20136 ^T	Cyclobalanopsidis glauca	China	MW481713	MW478892	MW522945	MW522962	
Apiospora cyclobalanopsidis	GZCC 20-0103	Cyclobalanopsidis glauca	China	MW481714	MW478893	MW522946	MW522963	
Apiospora dendrobii	MFLUCC 14-0152 ^T	Roots of Dendrobium harveyanum	Thailand	MZ463151	MZ463192	NA	NA	
Apiospora dematiacea	KUNCC 23-14202 ^T	Living stems of Dicranopteris ampla	China	OR590346	OR590339	OR634953	OR634948	
Apiospora descalsii	CBS 145130 ^T	Ampelodesmos mauritanicus	Spain	MK014870	MK014837	MK017947	MK017976	
Apiospora dichotomanthi	CGMCC 3.18332, LC4950 ^T	Dichotomanthes tristaniicarpa	China	KY494697	KY494773	KY705096	KY705167	
Apiospora dichotomanthi	LC8175	Dichotomanthes tristaniicarpa	China	KY494755	KY494831	KY705151	KY705223	
Apiospora dicranopteridis	KUNCC23-14171 [⊤]	Living stems of Dicranopteris pedata	China	OR590342	OR590336	OR634950	OR634944	
Apiospora dicranopteridis	KUNCC23-14177	Roots of Dicranopteris pedata	China	OR590343	OR590337	OR634951	OR634945	
Apiospora dongyingensis	SAUCC 0302 ^T	Leaves of bamboo	China	OP563375	OP572424	OP573264	OP573270	

	a			GenBank accession number		numbers	
Species	Strain Numbers	Host and Substrates	Locality	ITS	LSU	tef1	tub2
Apiospora dongyingensis	SAUCC 0303	Leaves of bamboo	China	OP563374	OP572423	OP573263	OP573269
Apiospora elliptica	ZHKUCC 22-0131 ^T	Dead stems of unknown plant	China	OR164905	OR164952	OR166284	OR166323
Apiospora elliptica	ZHKUCC 22-0140	Dead stems of unknown plant	China	OR164906	OR164953	NA	OR166324
Apiospora endophytica	ZHKUCC 23-0006 ^T	Living leaves of Wurfbainia villosa	China	OQ587996	OQ587984	OQ586062	OQ586075
Apiospora endophytica	ZHKUCC 23-0007	Living leaves of Wurfbainia villosa	China	OQ587997	OQ587985	OQ586063	OQ586076
Apiospora esporlensis	CBS 145136 ^T	Phyllostachys aurea	Spain	MK014878	MK014845	MK017954	MK017983
Apiospora esporlensis	UNIPAMPA010	Living leaves of the Antarctic Hairgrass Deschampsia antarctica	Antarctica	MN947641	genome	genome	genome
Apiospora euphorbiae	IMI 285638b	Bambusa sp.	Bangladesh	AB220241	AB220335	NA	AB220288
Apiospora fermenti	KUC21288, SFC20140423-M86	Seaweeds	South Korea	MF615230	NA	MH544668	MF615235
Apiospora fermenti	KUC21289 ^T	Seaweeds	South Korea	MF615226	MF615213	MH544667	MF615231
Apiospora gaoyouensis	CFCC 52301 [⊤]	Phragmites australis	China	MH197124	NA	MH236793	MH236789
Apiospora gaoyouensis	CFCC 52302	Phragmites australis China MH19712		MH197125	NA	MH236794	MH236790
Apiospora garethjonesii	GZCC 20-0115	Dead culms of bamboo	China	MW481715	MW478894	MW522947	NA
Apiospora garethjonesii	KUMCC 16-0202, JHB004, HKAS 96289 [⊤]	Dead culms of bamboo	China	KY356086	KY356091	NA	NA
Apiospora garethjonesii	SICAUCC 22-0027	Bamboo	China	ON228603	ON228659	NA	ON237651
Apiospora garethjonesii	SICAUCC 22-0028	Bamboo	China	ON228606	ON228662	NA	ON237654
Apiospora gelatinosa	GZAAS 20-0107	Bamboo	China	MW481707	MW478889	MW522942	MW522959
Apiospora gelatinosa	HKAS 11962 ^T	Bamboo	China	MW481706	MW478888	MW522941	MW522958
Apiospora globosa	KUNCC 23-14210 ^T	Living stems of Dicranopteris linearis	China	OR590347	OR590340	OR634954	NA
Apiospora gongcheniae	GDMCC 3.1045, YNE00465 ^T	Living stems of Oryza meyeriana subsp. granulata	China	PP033259	PP033102	PP034683	PP034691
Apiospora gongcheniae	YNE00565	Living stems of Oryza meyeriana subsp. granulata	China	PP033260	PP033103	PP034684	PP034692
Apiospora guangdongensis	ZHKUCC 23-0004 ^T	Living leaves of Wurfbainia villosa	China	OQ587994	OQ587982	OQ586060	OQ586073
Apiospora guangdongensis	ZHKUCC 23-0005	Living leaves of Wurfbainia villosa	China	OQ587995	OQ587983	OQ586061	OQ586074
Apiospora guiyangensis	HKAS 102403 ^T	Dead culms of Poaceae	China	MW240647	MW240577	MW759535	MW775604
Apiospora guiyangensis	KUNCC 22-12539	Poaceae plant	China	OQ029540	OQ029613	OQ186444	OQ186446
Apiospora guizhouensis	CGMCC 3.18334, LC5322 ^T	Air in karst cave	China	KY494709	KY494785	KY705108	KY705178
Apiospora guizhouensis	LC5318	Air in karst cave	China	KY494708	KY494784	KY705107	KY705177
Apiospora hainanensis	SAUCC 1681 ^T	Leaves of bamboo	China	OP563373	0P572422	OP573262	OP573268
Apiospora hainanensis	SAUCC 1682	Leaves of bamboo	China	OP563372	0P572421	OP573261	OP573267
Apiospora hispanica	IMI 326877 ^T	Beach sands	Spain	AB220242	AB220336	NA	AB220289
Apiospora hydei	CBS 114990 ^T	Culms of Bambusa tuldoides	China	KF144890	KF144936	KF145024	KF144982
Apiospora hydei	LC7103	Leaves of bamboo	China	KY494715	KY494791	KY705114	KY705183
Apiospora hyphopodii	JHB003, HKAS 96288	Bamboo	China	KY356088	KY356093	NA	NA
Apiospora hyphopodii	MFLUCC 15-003 ^T	Bambusa tuldoides	Thailand	KR069110	NA	NA	NA
Apiospora hyphopodii	SICAUCC 22-0034	Bamboo	China	ON228605	ON228661	NA	ON237653
Apiospora hysterina	AP12118	Phyllostachys aurea	Spain	MK014877	KM014844	MK017953	MK017982
Apiospora hysterina	AP29717	Phyllostachys aurea	Spain	MK014875	MK014842	MK017952	MK017981
Apiospora hysterina	ICPM 6889 ^T	Bamboo	New Zealand	MK014874	MK014841	MK017951	MK017980
Apiospora iberica	CBS 145137, AP10118 [⊤]	Arundo donax	Portugal	MK014879	MK014846	MK017955	MK017984
Apiospora intestine	CBS 135835	Gut of grasshopper	India	KR011352	MH877577	KR011351	KR011350
Apiospora intestine	MFLUCC 21-0052 T	Dead culms of bamboo	Thailand	MZ542521	MZ542525	MZ546406	MZ546410

0	Otracia Neurale and			GenBank accession numbers				
Species	Strain Numbers	Host and Substrates	Locality	ITS	LSU	tef1	tub2	
Apiospora italic	CBS 145138, AP221017 [⊤]	Arundo donax	Italy	MK014880	MK014847	MK017956	MK017985	
Apiospora italic	CBS 145139	Phragmites australis	Spain	MK014881	MK014848	NA	MK017986	
Apiospora jatrophae	CBS 134262, MMI00052 ^T	Living Jatropha podagrica	India	JQ246355	NA	NA	NA	
Apiospora jiangxiensis	CGMCC 3.18381, LC4577 ^T	<i>Maesa</i> sp.	China	KY494693	KY494769	KY705092	KY705163	
Apiospora jiangxiensis	LC4578	Camellia sinensis	China	KY494694	KY494770	KY705093	KY705164	
Apiospora kogelbergensis	CBS 113332	Cannomois virgata	South Africa	KF144891	KF144937	KF145025	KF144983	
Apiospora kogelbergensis	CBS 113333 ^T	Dead culms of Restionaceae	South Africa	KF144892	KF144938	KF145026	KF144984	
Apiospora koreanum	KUC21332, SFC20200506-M06 [⊤]	Eggs of Arctoscopus japonicus	South Korea	MH498524	MH498444	MH544664	MH498482	
Apiospora koreanum	KUC21348	Eggs of Arctoscopus japonicus	South Korea	MH498523	NA	MN868927	MH498481	
Apiospora lageniformis	KUC21686 ⁺	Culms of Phyllostachys nigra	Korea	ON764022	ON787761	ON806626	ON806636	
Apiospora lageniformis	KUC21687	Culms of Phyllostachys nigra	Korea	ON764023	ON787764	ON806627	ON806637	
Apiospora locuta- pollinis	LC11683 ^T	Brassica campestris	China	MF939595	NA	MF939616	MF939622	
Apiospora longistroma	MFLUCC 11-0479	Dead culms of bamboo	Thailand	KU940142	KU863130	NA	NA	
Apiospora longistroma	MFLUCC11-0481 T	Dead culms of bamboo	Thailand	KU940141	KU863129	NA	NA	
Apiospora lophatheri	CFCC 58975 ⁺	Diseased leaves of Lophatherum gracile	China	OR125566	OR133588	OR139970	OR139980	
Apiospora lophatheri	CFCC 58976 [⊤]	Diseased leaves of Lophatherum gracile	China	OR125567	OR133589	OR139971	OR139981	
Apiospora machili	SAUCC 1175A-4 ⁺	Diseased leaves of <i>Machilus</i> <i>nanmu</i> of Machilus nanmu	China	OR739433	OR739574	OR753450	OR757130	
Apiospora machili	SAUCC 1175	Diseased leaves of <i>Machilus</i> nanmu of Machilus nanmu	China	OQ592560	OQ615289	OQ613333	OQ613307	
Apiospora magnispora	ZHKUCC 22-0001 T	Dead stems of Bambusa textilis	China	OM728647	OM486971	OM543543	OM543544	
Apiospora malavsiana	CBS 102053 T	Macaranga hullettii	Malaysia	KF144896	KF144942	KF145030	KF144988	
Apiospora marianiae	AP18219 [™]	Dead stems of Phleum pratense	Spain	ON692406	ON692422	ON677180	ON677186	
Apiospora marii	CBS 497.90 ^T	Beach sands	Spain	AB220252	KF144947	KF145035	KF144993	
Apiospora marinum	KUC21328, SFC20140423-M02 [™]	Seaweeds	South Korea	MH498538	MH498458	MH544669	MH498496	
Apiospora mediterranea	IMI 326875 ^T	Air	Spain	AB220243	AB220337	NA	AB220290	
Apiospora minutispora	1.70E-042 [⊤]	Mountain soils	South Korea	LC517882	NA	LC518889	LC518888	
Apiospora montagnei	AP19421	Arundo micrantha	Spain	ON692418	ON692425	ON677183	ON677189	
Apiospora montagnei	AP301120, CBS 148707, PC:0125164 T	Arundo micrantha	Spain	ON692408	ON692424	ON677182	ON677188	
Apiospora mori	MFLUCC 20-0181 T	Dead leaves of Morus australis	China	MW114313	MW114393	NA	NA	
Apiospora mori	NCYUCC 19-0340	Dead leaves of Morus australis	China	MW114314	MW114394	NA	NA	
Apiospora mukdahanensis	MFLUCC 22-0056 ⁺	Dead leaves of bamboo	Thailand	0P377735	0P377742	NA	NA	
Apiospora multiloculata	MFLUCC 21-0023 T	Dead culms of Bambusae	Thailand	OL873137	OL873138	NA	OL874718	
Apiospora mytilomorpha	DAOM 214595 ^T	Dead blades of Andropogon sp.	India	KY494685	NA	NA	NA	
Apiospora neobambusae	CGMCC 3.18335, LC7106 ^T	Leaves of bamboo	China	KY494718	KY494794	KY806204	KY705186	
Apiospora neobambusae	LC7107	Leaves of bamboo	China	KY494719	KY494795	KY705117	KY705187	
Apiospora neobambusae	LC7124	Leaves of bamboo	China	KY494727	KY494803	KY806206	KY705195	
Apiospora neochinensis	CFCC 53036 T	Fargesia qinlingensis	China	MK819291	NA	MK818545	MK818547	
Apiospora neochinensis	CFCC 53037	Fargesia qinlingensis	China	MK819292	NA	MK818546	MK818548	
Apiospora neogarethjonesii	KUMCC 18-0192, HKAS 102408 ⁺	Dead culms of Bambusae	China	MK070897	MK070898	NA	NA	
Apiospora neogongcheniae	GDMCC 3.1047, YNE01248 ^T	Living stems of Poaceae plant	China	PP033263	PP033106	PP034687	PP034695	

- ·	o			GenBank accession numbers		s	
Species	Strain Numbers	Host and Substrates	Locality	ITS	LSU	tef1	tub2
Apiospora neogongcheniae	YNE01260	Living stems of Poaceae plant	China	PP033264	PP033107	PP034688	PP034696
Apiospora neosubglobosa	JHB 006	Bamboo	China	KY356089	KY356094	NA	NA
Apiospora neosubglobosa	JHB 007 [⊤]	Bamboo	China	KY356090	KY356095	NA	NA
Apiospora obovata	CGMCC 3.18331, LC4940 ^T	Lithocarpus sp.	China	KY494696	KY494772	KY705095	KY705166
Apiospora obovata	LC8177	Lithocarpus sp.	China	KY494757	KY494833	KY705153	KY705225
Apiospora oenotherae	CFCC 58972	Diseased leaves of Oenothera biennis	China	OR125568	OR133590	OR139972	OR139982
Apiospora oenotherae	LS 395	Diseased leaves of Oenothera biennis	China	OR125569	OR133591	OR139973	OR139983
Apiospora ovate	CBS 115042 [™]	Arundinaria hindsii	China	KF144903	KF144950	KF145037	KF144995
Apiospora pallidesporae	ZHKUCC 22-0129 ^T	Dead wood of unknown host	China	OR164903	OR164950	NA	NA
Apiospora pallidesporae	ZHKUCC 22-0142	Dead wood of unknown host	China	OR164904	OR164951	NA	NA
Apiospora paragongcheniae	GDMCC 3.1046, YNE00992 ^T	Living stems of Poaceae plant China		PP033261	PP033104	PP034685	PP034693
Apiospora paragongcheniae	YNE01259	Living stems of Poaceae plant	China	PP033262	PP033105	PP034686	PP034694
Apiospora paraphaeosperma	MFLUCC 13-0644 ^T	Dead culms of bamboo	Thailand	KX822128	KX822124	NA	NA
Apiospora paraphaeosperma	KUC21488	Culms of bamboo	Korea	ON764024	ON787763	ON806628	ON806638
Apiospora phragmitis	CPC 18900 ^T	Phragmites australis	Italy	KF144909	KF144956	KF145043	KF145001
Apiospora phyllostachydis	MFLUCC 18-1101 ^T	Phyllostachys heteroclada	China	MK351842	MH368077	MK340918	MK291949
Apiospora piptatheri	CBS 145149, AP4817A [⊤]	Piptatherum miliaceum	Spain	MK014893	MK014860	MK017969	NA
Apiospora piptatheri	SAUCC BW0455	Diseased leaves of Indocalamus Iongiauritus	China	OR739430	OR739571	OR753447	OR757127
Apiospora pseudomarii	GUCC 10228 T	Leaves of Aristolochia debilis	China	MT040124	NA	MT040145	MT040166
Apiospora pseudohyphopodii	KUC21680 ^T	Culms of Phyllostachys pubescens	Korea	ON764026	ON787765	ON806630	ON806640
Apiospora pseudohyphopodii	KUC21684	Culms of Phyllostachys pubescens	Korea	ON764027	ON787766	ON806631	ON806641
Apiospora pseudoparenchymatica	CGMCC 3.18336, LC7234 ^T	Leaves of bamboo	China	KY494743	KY494819	KY705139	KY705211
Apiospora pseudoparenchymatica	LC8173	Leaves of bamboo	China	KY494753	KY494829	KY705149	KY705221
Apiospora pseudorasikravindrae	KUMCC 20-0208 ^T	Bambusa dolichoclada	China	MT946344	NA	MT947361	MT947367
Apiospora pseudosinensis	CPC 21546 [↑]	Leaves of bamboo	Netherlands	KF144910	KF144957	KF145044	MN868936
Apiospora pseudosinensis	SAUCC 0221	Leaves of bamboo	China	OP563377	OP572426	OP573266	OP573272
Apiospora pseudospegazzinii	CBS 102052 ^T	Macaranga hullettii	Malaysia	KF144911	KF144958	KF145045	KF145002
Apiospora pterosperma	CBS 123185	Machaerina sinclairii	New Zealand	KF144912	KF144959	NA	KF145003
Apiospora pterosperma	CPC 20193, CBS 134000 [⊤]	Lepidosperma gladiatum	Australia	KF144913	KF144960	KF145046	KF145004
Apiospora pusillispermum	KUC21321 ^T	Seaweeds	South Korea	MH498533	MH498453	MN868930	MH498491
Apiospora pusillispermum	KUC21357	Seaweeds	South Korea	MH498532	NA	MN868931	MH498490
Apiospora qinlingensis	CFCC 52303 ^T	Fargesia qinlingensis	China	MH197120	NA	MH236795	MH236791
Apiospora qinlingensis	CFCC 52304	Fargesia qinlingensis	China	MH197121	NA	MH236796	MH236792
Apiospora rasikravindrae	LC8179	Brassica rapa	China	KY494759	KY494835	KY705155	KY705227
Apiospora rasikravindrae	MFLUCC 21-0051	Dead culms of bamboo	Thailand	MZ542523	MZ542527	MZ546408	MZ546412

	A			GenBank accession numbers				
Species	Strain Numbers	Host and Substrates	Locality	ITS	LSU	tef1	tub2	
Apiospora sacchari	CBS 372.67	Air	Not mentioned	KF144918	KF144964	KF145049	KF145007	
Apiospora sacchari	CBS 664.74	Soils under Calluna vulgaris	Netherlands	KF144919	KF144965	KF145050	KF145008	
Apiospora saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF144966	KF145051	KF145009	
Apiospora saccharicola	CBS 831.71	Not mentioned	Netherlands	KF144922	KF144969	KF145054	KF145012	
Apiospora sargassi	KUC21228 ^T	Sargassum fulvellum	South Korea	KT207746	KT207696	MH544677	KT207644	
Apiospora sargassi	KUC21232	Seaweeds	South Korea	KT207750	NA	MH544676	KT207648	
Apiospora sasae	CPC 38165, CBS 146808 ^T	Dead culms of Sasa veitchii	Netherlands	MW883402	MW883797	MW890104	MW890120	
Apiospora septata	CGMCC 3.20134, CS19-8 [†]	Bamboo	China	MW481711	MW478890	MW522943	MW522960	
Apiospora septata	GZCC 20-0109	Bamboo Food	China	MW481712	MW478891	MW522944	MW522961	
Apiospora serenensis	IMI 326869 ^T	Excipients, atmosphere and home dust	Spain	AB220250	AB220344	NA	AB220297	
Apiospora setariae	CFCC 54041 T	Decaying culms of Setaria viridis	China	MT492004	NA	MW118456	MT497466	
Apiospora setariae	MT492005	Setaria viridis	China	MT492005	NA	MW118457	MT497467	
Apiospora setostroma	KUMCC 19-0217	Dead branches of bamboo	China	MN528012	MN528011	MN527357	NA	
Apiospora sichuanensis	HKAS 107008 ^T	Dead culms of Poaceae	China	MW240648	MW240578	MW759536	MW775605	
Apiospora sorghi	URM 93000, URM 7417 ^T	Sorghum bicolor	Brazil	MK371706	NA	NA	MK348526	
Apiospora sphaerosperma	CBS 114314	Leaves of Hordeum vulgare	Iran	KF144904	KF144951	KF145038	KF144996	
Apiospora sphaerosperma	CBS 114315	Leaves of Hordeum vulgare	Iran	KF144905	KF144952	KF145039	KF144997	
Apiospora stipae	CPC 38101, CBS 146804 ⁺	Dead culms of Stipa gigantea	Spain	MW883403	MW883798	MW890082	MW890121	
Apiospora subglobosa	MFLUCC 11-0397 T	Dead culms of bamboo	Thailand	KR069112	KR069113	NA	NA	
Apiospora subrosea	CGMCC 3.18337, LC7292 ^T	Leaves of bamboo	China	KY494752	KY494828	KY705148	KY705220	
Apiospora subrosea	LC7291	Leaves of bamboo	China	KY494751	KY494827	KY705147	KY705219	
Apiospora taeanense	KUC21322 [™]	Seaweeds	South Korea	MH498515	NA	MH544662	MH498473	
Apiospora taeanense	KUC21359	Seaweeds	South Korea	MH498513	NA	MN868935	MH498471	
Apiospora thailandica	MFLUCC 15-0199	Dead culms of bamboo	Thailand	KU940146	KU863134	NA	NA	
Apiospora thailandica	MFLUCC 15-0202 T	Dead culms of bamboo	Thailand	KU940145	KU863133	NA	NA	
Apiospora tropica	MFLUCC 21-0056	Dead culms of Bambusoideae	Thailand	OK491657	OK491653	NA	OK560922	
Apiospora wurfbainiae	ZHKUCC 23-0008 T	Wurfbainia villosa	China	OQ587998	OQ587986	OQ586064	OQ586077	
Apiospora wurfbainiae	ZHKUCC 23-0009	Wurfbainia villosa	China	OQ587999	OQ587987	OQ586065	OQ586078	
Apiospora vietnamensis	IMI 99670 ^T	Citrus sinensis	Vietnam	KX986096	KX986111	NA	KY019466	
Apiospora xenocordella	CBS 478.86 ^T	Soils from roadway	Zimbabwe	KF144925	KF144970	KF145055	KF145013	
Apiospora xenocordella	CBS 595.66	Soils	Austria	KF144926	KF144971	NA	NA	
Apiospora xishuangbannaensis	KUMCC 21-0695 ^T	Rhinolophus pusillus	China	ON426832	OP363248	OR025969	OR025930	
Apiospora xishuangbannaensis	KUMCC 21-0696	Rhinolophus pusillus	China	ON426833	OP363249	OR025970	OR025931	
Apiospora yunnana	DDQ 00281	Phyllostachys nigra	China	KU940148	KU863136	NA	NA	
Apiospora yunnana	MFLUCC 15-1002 T	Phyllostachys nigra	China	KU940147	KU863135	NA	NA	
Apiospora yunnanensis	ZHKUCC 23-0014 T	Dead stems of grass	China	OQ588004	OQ587992	OQ586070	OQ586083	
Apiospora yunnanensis	ZHKUCC 23-0015	Dead stems of grass	China	OQ588005	OQ587993	OQ586071	OQ586084	
Arthrinium austriacum	GZU 345004	Carex pendula	Austria	MW208928	NA	NA	NA	
Arthrinium austriacum	GZU 345006	Carex pendula	Austria	MW208929	MW208860	NA	NA	
Arthrinium caricicola	CBS 145127, AP23518	Carex ericetorum	China	MK014871	MK014838	MK017948	MK017977	
Arthrinium caricicola	CBS 145903, CPC33297 ^T	Dead and attached leaves	Germany	MN313782	MN317266	NA	MN313861	
Arthrinium crenatum	AG19066, CBS 146353 [⊤]	Carex sp.	France	MW208931	MW208861	MW221917	MW221923	
Arthrinium curvatum	AP25418	Leaves of Carex sp.	China	MK014872	MK014839	MK017949	NA	
Arthrinium japonicum	IFO 30500	Carex despalata	Japan	AB220262	AB220356	NA	AB220309	
Arthrinium japonicum	IFO 31098	Leaves of Carex despalata	Japan	AB220264	AB220358	NA	AB220311	
Arthrinium luzulae	AP7619-3	Luzula sylvatica	Spain	MW208937	MW208863	MW221919	MW221925	
Arthrinium morthieri	GZU 345043	Cyperaceae carex	Austria	MW208938	MW208864	MW221920	MW221926	
Arthrinium	AP25619, CBS	Poaceae plant	Norway	MW208943	MW208865	NA	NA	
phaeospermum	146355							

Chaosian Churcin Numberry				GenBank accession numbers					
Species	Strain Numbers	Host and Substrates	Locality	ITS	LSU	tef1	tub2		
Arthrinium puccinioides	CBS 549.86	Lepidosperma gladiatum	Germany	AB220253	AB220347	NA	AB220300		
Arthrinium sporophleoides	GZU 345102	Carex firma	Austria	MW208944	MW208866	NA	MW221927		
Arthrinium sporophleum	AP21118, CBS 145154	Dead leaves of Juncus sp.	Spain	MK014898	MK014865	MK017973	MK018001		
Nigrospora guilinensis	CGMCC 3.18124, LC 3481 ^T	Camellia sinensis	China	KX985983	KX986113	KY019292	KY019459		
Nigrospora guilinensis	LC 7301	Stems of Nelumbo sp.	China	KX986063	NA	KY019404	KY019608		
Nigrospora hainanensis	CGMCC 3.18129, LC 7030 ^T	Leaves of Musa paradisiaca	China	KX986091	KX986112	KY019415	KY019464		
Nigrospora hainanensis	LC 6979	Leaves of Musa paradisiaca	China	KX986079	NA	KY019416	KY019586		
Nigrospora pyriformis	CGMCC 3.18122, LC 2045 ⁺	Citrus sinensis	China	KX985940	KX986100	KY019290	KY019457		
Nigrospora pyriformis	LC 2688	Lindera aggregata	China	KX985941	NA	KY019297	KY019468		
Nigrospora vesicularis	CGMCC 3.18128, LC 7010 ^T	Leaves of Musa paradisiaca	China	KX986088	KX986099	KY019294	KY019463		
Nigrospora vesicularis	LC 0322	Unknown host plant	Thailand	KX985939	NA	KY019296	KY019467		
Neoarthrinium lithocarpicola	CFCC 54456 [⊤]	Lithocarpus glaber	China	ON427580	ON427582	NA	ON456914		
Neoarthrinium lithocarpicola	CFCC 55883	Lithocarpus glaber	China	ON427581	ON427583	NA	ON456915		
Neoarthrinium trachycarpi	CFCC 53038	Trachycarpus fortune	China	MK301098	NA	MK303396	MK303394		
Neoarthrinium trachycarpi	CFCC 53039	Trachycarpus fortune	China	MK301099	NA	MK303397	MK303395		
Sporocadus trimorphus	CFCC 55171	Rose	China	OK655798	OK560389	OL814555	OM401677		
Sporocadus trimorphus	ROC 113	Rose	China	OK655799	OK560390	OL814556	OM401678		

The sequences were trimmed and concatenated, and subsequent phylogenetic analyses were performed in PhyloSuite platform (Zhang et al. 2020). ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit partition model (Edge-unlinked) using the BIC criterion. Maximum likelihood (ML) phylogenies were inferred using IQ-TREE (Nguyen et al. 2015) under Edge-linked partition model for 5000 ultrafast (Minh et al. 2013) bootstraps. Bayesian Inference (BI) phylogenies were inferred using MrBayes 3.2.6 (Ronquist et al. 2012) under partition model, in which the initial 27% of sampled data were discarded as burn-in. The resulting phylogenetic tree was visualized in FigTree v1.4.3. (http:/tree.bio.ed.ac.uk/software/figtree/) with maximum likelihood bootstrap proportions (MLBP) greater than 70% and Bayesian inference posterior probabilities (BIPP) greater than 0.90, as shown at the nodes. The phylogram was edited in Adobe Illustrator v.27.5 (Adobe Systems Inc., USA). All GenBank accession numbers of sequences used in this study are provided in Table 1.

Results

Phylogeny

The combined ITS, LSU, *tef1*, and *tub2* dataset encompassed 215 strains, including six newly sequenced strains, with *Sporocadus trimorphus* CFCC 55171 and ROC 113 serving as the outgroup taxa, and representative species of *Arthrinium*, *Nigrospora*, and *Neoarthrinium* as the sister groups. The multi-locus sequence dataset comprised 2,081 characters, including gaps, with the following character ranges: ITS (1-352), LSU (353-1149), *tef1* (1150-1775), and *tub2* (1776-2081). The topologies of phylogenetic trees generated by ML and BI analyses were congruent, and the BI tree with MLBP and BIPP is presented in Fig. 1.



Figure 1. Phylogenetic tree of *Apiospora* based on the combined ITS, LSU, *tef1*, and *tub2* sequences alignment. Maximum likelihood bootstrap proportions \geq 70% (left) and Bayesian inference posterior probability \geq 0.90 (right) are indicated at nodes (MLBP/BIPP). *Sporocadus trimorphus* (CFCC 55171 and ROC 113) are chosen as the outgroup taxa. The novel species from this study are highlighted in red.

Xiao-Ni Yan & Chu-Long Zhang: Three new endophytic Apiospora species





The phylogenetic analysis revealed that the species of *Apiospora*, *Arthrinium*, *Nigrospora*, and *Neoarthrinium* formed four well-supported distinct lineages. Within the genus *Apiospora*, the 187 strains, encompassing six newly sequenced strains, formed twelve well-supported major clades. The six endophytic strains clustered within one of the major clades H, along with *A. garethjonesii*, *A. neogarethjonesii*, *A. setostroma*, *A. subrosea*, *A. mytilomorpha*, and *A. neobambusae*. Concurrently, the six endophytic strains segregated into three independent clades with robust supported values, indicating the presence of three novel species. These novel taxa are formally described herein and assigned the new names *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*.

Taxonomy

Apiospora gongcheniae C. L. Zhang, sp. nov. Fungal Names: FN 571885 Fig. 2

Etymology. Named after Prof. Gongchen Wang in recognition of her significant contribution to the fields of mycology and plant pathology in China.

Type. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of *Oryza meyeriana* subsp. *granulata*, Aug 2015, J.J. Chen, YNE00465 (holotype GDMCC 3.1045, stored in a metabolically inactive state); ex-type culture YNE00465.

Description. Asexual morph: Hyphae hyaline, branched, septate, smooth, $1.1-2.6 \mu m$ diameter (mean = $1.7 \mu m$, n = 30). Conidiophores reduced to conidiogenous cells. Conidiogenous cells hyaline to pale brown, erect, vertucose, cylindrical with tiny denticles, clustered in groups, sometimes aggregated in clusters on hyphae or sporodochia, $3.5-9.4 \times 1.9-5.2 \mu m$ (mean = $5.6 \times 3.1 \mu m$, n = 30). Conidia pale brown to dark brown, smooth, granular, globose to subglobose in surface view, lenticular to side view with a pale longitudinal germ slit, with obvious central basal scar, $8.0-17.0 \times 6.8-16.1 \mu m$ (mean = $13.6 \times 11.6 \mu m$, n = 50). Sexual morph: Undetermined.

Culture characteristics. On PDA, colonies flat, cottony, dense, margin circular, greyish, reverse light orange, covering the 90 mm plate after 7 days at 25 °C. On MEA, colonies dusty pink, dense, covering the 90 mm plate after 7 days at 25 °C. Conidiomata black, globose, abundant, attach to surface of substrate, forming on PDA and MEA after 7–10 days.

Additional specimens examined. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of *Oryza meyeriana* subsp. *granulata*, Aug 2015, J.J. Chen, YNE00565.

Note. Phylogenetic analyses confirmed that *A. gongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea*. Based on a BLASTN search of the Gen-Bank database, it was found that *A. paragongcheniae* shares high similarities with the following strains: *A. garethjonesii* strain HKAS 96289 (93.76% in ITS, 99.81% in LSU), strain GZCC 20-0115 (93.76% in ITS, 99.24% in LSU, 94.06% in *tef1*), strain SICAUCC 22-0027 (93.76% in ITS, 99.81% in LSU, 94.51% in *tub2*), strain SICAUCC 22-0028 (93.76% in ITS, 99.81% in LSU, 93.63% in *tub2*); *A. subrosea* strain CGMCC 3.18337 (96.94% in ITS, 99.42% in LSU, 93.47% in *tef1*, 91.87% in *tub2*), strain LC7291 (90.09% in ITS, 99.41% in LSU, 93.47% in *tef1*, 91.87% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (92.86% in ITS, 99.82% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. gongcheniae*.

As a synopsis of the morphological characteristics presented in Table 2, A. gongcheniae differs from A. garethjonesii and A. neogarethjonesii in having smaller conidia ($8.0-17.0 \times 6.8-16.1 \mu$ m, mean = $13.6 \times 11.6 \mu$ m) compared to A. garethjonesii (surface view: $16-19 \mu$ m diam, side view: $17-22 \mu$ m diam) and A. neogarethjonesii ($20-35 \times 15-30 \mu$ m, mean = $28.5 \times 25.6 \mu$ m). Additionally, A. gongcheniae exhibits shorter conidiogenous cells ($3.5-9.4 \times 1.9 5.2 \mu$ m, mean = $5.6 \times 3.1 \mu$ m) in contrast to A. garethjonesii ($6-19 \times 3-5 \mu$ m,

Table 2. Synop	sis of morphological	characteristics of relati	ed Apiospora species. N	lotes: ND = Not detern	nined.		
Strains	Apiospora garethjonesii (D.Q. Dai & H.B. Jiang) Pintos & P. Alvarado (2021)	A. neogarethjonesii (D.Q. Dai & K.D. Hyde) Pintos & P. Alvarado (2021)	A. <i>subrosea</i> (M. Wang & L. Cai) Pintos & P. Alvarado (2021)	A. neobambusae Pintos & P. Alvarado (2021) (=Arthrinium bambusae M. Wang & L. Cai (2018))	A. gongcheniae	A. paragongcheniae	A. neogongcheniae
Host / Substrate	Dead culms of bamboo	Dead culms of bamboo	Leaves of bamboo	Leaves of bamboo	Stems of <i>Oryza meyeriana</i> subsp. <i>granulata</i>	Stems of unidentified Poaceae plant	Stems of unidentified Poaceae plant
Known lifestyle	Saprobe	Saprobe	Endophyte	Endophyte	Endophyte	Endophyte	Endophyte
Asci	$125-154 \times 35-42$ $\mu m (\overline{X} = 139 \times 38 \mu m,$ n = 20), 8-spored	95-125 × 20-25 μm (x̄ = 97.6 × 21.3 μm, n = 20), 8-spored	Q	Q	Q	Q	ND
Ascospores	$30-42 \times 11-16 \ \mu m$ $(\overline{X} = 39 \times 13 \ \mu m, n = 20), 2-seriate, 1-septate, ellipsoidal$	$25-30 \times 9.5-11 \ \mu m$ ($\overline{x} = 29.1 \times 10.3 \ \mu m$, n = 20), 2-seriate, overlapping, 1-septate, ellipsoidal, 3-10 \ \mu m wide	Q	Q	Q	Q	QN
Conidiomata	Black, with hair-like setae	Black, ellipsoid to irregular, coriaceous	Black, irregular	Black, irregular	Black, globose, abundant, attach to the surface of the substrate	Black, globose to irregular shape, sparse, semi-immersed in the substrate	ND
Conidiophores	Reduced to conidiogenous cells	$4.5-6 \times 3.5-4.5 \ \mu m$ ($\overline{x} = 5.4 \times 4.3 \ \mu m$, n = 20), cylindrical, aseptate	Hyaline to pale brown, smooth, erect or ascending, simple, flexuous, subcylindrical, clustered in groups, aggregated in brown sporodochia, up to 20 µm long, 2–4.5 µm width	Reduced to conidiogenous cells	Reduced to conidiogenous cells	Hyaline, erect, basauxic, doliiform, subspherical to barrel-shaped, aggregated in clusters on pale brown sporodochia, sometimes reduced to conidiogenous cells, 12.2–35.1 x 2.1–8.8 μ m ($\overline{x} = 24.5 \times 4.3 \ \mu$ m, n = 30)	QN
Conidiogenous cells	Hyaline to pale brown, smooth, ampulliform, aggregated in black sporodochia, $(5-) 6-19$ $(-20) \mu m \times (2-) 3-5$ $(-7) \mu m (\overline{x} = 11 \mu m \times 4\mu m, n = 20)$	Basauxic, cylindrical, discrete, smooth-walled, $10-48 \times 4-5.5 \ \mu m$ $(\overline{x} = 35.4 \times 4.3 \ \mu m, n = 20)$	Pale brown, smooth, doliiform to subcylindrical, $3.0-6.5 \times 2.0-5.0 \ \mu m$ $(\overline{X} = 4.7 \pm 1.2 \times 3.7 \pm 0.9,$ n = 30)	Hyaline to pale brown, erect, aggregated in clusters on hyphae, smooth, doliiform to ampulliform, or lageni- form, 4.0–12.0 × 3.0–7.0 μm ($\overline{x} = 6.6 \pm 1.8 \times 4.8 \pm$ 0.9, n = 30)	Hyaline to pale brown, erect, verrucose, cylindrical with tiny denticles, clustered in groups, sometimes aggregated in clusters on hyphae or sporodochia, 3.5– 9.4 × 1.9–5.2 µm ($\overline{x} = 5.6 \times$ 3.1 µm, n = 30)	Hyaline, ampulliform, doliiform to clavate, verucose, 5.0–13.1 $\times 2.1-6.0 \text{ µm} (\bar{x} = 8.2 \times 3.9 \text{ µm}, n = 30)$	QN
Conidia	(14–)16–19 (–20) µm diam, brown, smooth, granular, globose to subglobose in surface view, and (16–) 17–22 (–23) µm diam, with pale equatorial slit in side view	Dark brown, globose to subglobose, smooth-walled, with a truncate basal scar, 20–35 × 15–30 μ m (\overline{X} = 28.5 × 25.6 μ m, n = 20)	Pale brown to dark brown, smooth, globose to subglobose or ellipsoidal, 12.0-17.5 × 9.0-16.0 µm $(\overline{X} = 14.9 \pm 1.4 \times 11.8 \pm 1.8,$ n = 50)	Olivaceous to brown, smooth to finely roughened, subglobose to ellipsoid, 11.5–15.5 × 7.0–14.0 μ m (\overline{x} = 13.2 ± 0.8 × 11.4 ± 1.2, n = 50)	Pale brown to dark brown, smooth, granular, globbse to subglobose in surface view, lenticular to side view with a pale longitudinal germ slit, with obvious central basal scar, 8.0–17.0 × 6.8–16.1 μ m ($\overline{x} = 13.6 \times 11.6 \mu$ m, n = 50)	Pale brown to dark brown, smooth to granular, subglobose to oval, occasionally swollen into pyriform to reniform, with a pale longitudinal germ slit in side view, 8.2–18.7 × $6.4-13.4 \ {\rm µm}$ (${\rm \ddot{x}}$ = 12.4 × 10.0 ${\rm µm}$, n = 50)	QN
References	(Dai et al. 2016; Feng et al. 2021)	(Hyde et al. 2020)	(Wang et al. 2018)	(Wang et al. 2018)	This study	This study	This study



Figure 2. Apiospora gongcheniae (YNE00465, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 7 d at 25 °C on MEA **c** conidiomata on MEA **d-g** conidiogenous cells giving rise to conidia **h–i** conidia with pale germ slit. Scale bars: 500 μ m (**e**); 10 μ m (**f–k**).

mean = $11 \times 4 \mu$ m) and *A. neogarethjonesii* ($10-48 \times 4-5.5 \mu$ m, mean = $35.4 \times 4.3 \mu$ m). While *A. gongcheniae* shares a similar size range for conidia and conidiogenous cells with *A. subrosea*, it is distinguished by *A. gongcheniae* having conidia featuring a central basal scar and cylindrical conidiogenous cells with tiny denticles. Based on molecular and morphological evidence, we propose *A. gongcheniae* as a new species.

Apiospora paragongcheniae C. L. Zhang, sp. nov.

Fungal Names: FN 571886 Fig. 3

Etymology. Named after its phylogenetic close related to A. gongcheniae.

Type. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 22°04'N, 100°32'E, on the stems of unidentified Poaceae plant, Sep 2016, J.J. Chen, YNE00992 (Holotype GDMCC 3.1046, stored in a metabolically inactive state); ex-type culture YNE00992.

Description. *Asexual morph*: Hyphae hyaline, branched, septate, smooth, $1.1-2.2 \mu m$ diameter (mean = $1.6 \mu m$, n = 30). Conidiophores hyaline, erect, basauxic, doliiform, subspherical to barrel-shaped, aggregated in clusters on pale brown sporodochia, sometimes reduced to conidiogenous cells, $12.2-35.1 \times 2.1-8.8 \mu m$ (mean = $24.5 \times 4.3 \mu m$, n = 30). Conidiogenous cells hyaline, ampulliform, doliiform to clavate, verrucose, $5.0-13.1 \times 2.1-6.0 \mu m$ (mean = $8.2 \times 3.9 \mu m$, n = 30). Conidia pale brown to dark brown, smooth to granular, subglobose to oval, occasionally swollen into pyriform to reniform, with a pale longitudinal germ slit in side view, $8.2-18.7 \times 6.4-13.4 \mu m$ (mean = $12.4 \times 10.0 \mu m$, n = 50). *Sexual morph*: Undetermined.



Figure 3. *Apiospora paragongcheniae* (YNE00992, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 6 d at 25 °C on MEA **c** conidioma on MEA **d**-**i** conidiogenous cells giving rise to conidia **j**-**o** conidia. Scale bars: 500 µm (**c**); 10 µm (**d**-**o**).

Culture characteristics. On PDA, colonies flat, rounded, initially white, becoming yellowish-white, with sparse aerial mycelia, mycelium partly immersed in the medium, covering the 90 mm plate after 6 days at 25 °C. On MEA, colonies white, more abundant aerial mycelia, covering the 90 mm plate after 6 days at 25 °C. Conidiomata black, globose to irregular shape, sparse, solitary, semi-immersed in the substrate, observed on MEA after 21–30 days.

Additional specimens examined. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE001259.

Note. Phylogenetic analyses confirmed that *A. paragongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. subrosea*, *A. neobambusae* and *A. neogarethjonesii*. Based on a BLASTN search of the GenBank database, it was found that *A. paragongcheniae* shares high similarities to the following strains: *A. subrosea* strain CGMCC 3.18337 (98.05% in ITS, 99.23% in LSU, 95.93% in *tef1*, 93.63% in *tub2*), strain LC7291 (98.05% in ITS, 99.22% in LSU, 95.93% in *tef1*, 93.63% in *tub2*); *A. neobambusae* strain CGMCC 3.18335 (98.05% in ITS, 100% in LSU, 97.13% in *tef1*, 93.48% in *tub2*), strain LC7107 (98.03% in ITS, 100% in LSU, 94.44% in *tef1*, 93.48% in *tub2*), strain LC7124 (98.05% in ITS, 100% in LSU, 96.82% in *tef1*, 93.47% in *tub2*); and *A. neogarethjonesii* strain HKAS 102408 (95.43% in ITS, 99.63% in LSU). The *tef1* and *tub2* sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. paragongcheniae*.

As a synopsis of morphological characteristics presented in Table 2, *A. paragongcheniae* distinguishes itself from *A. neobambusae*, *A. neogarethjonesii*, and *A. subrosea* in the shapes and sizes of its conidia. The conidia of *A. paragongcheniae* range from subglobose to oval, occasionally swollen into pyriform to reniform shapes, measuring $8.2-18.7 \times 6.4-13.4 \mu m$. This contrasts with *A. neobambusae* (subglobose to ellipsoid, $11.5-15.5 \times 7.0-14.0 \mu m$), *A. neogarethjonesii* (globose to subglobose, $20-35 \times 15-30 \mu m$), and *A. subrosea* (globose to subglobose or ellipsoidal, $12.0-17.5 \times 9.0-16.0 \mu m$). Furthermore,

A. paragongcheniae exhibits elongated conidiogenous cells $(5.0-13.1 \times 2.1-6.0 \mu m, mean = 8.2 \times 3.9 \mu m)$ compared to A. neobambusae $(4.0-12.0 \times 3.0-7.0 \mu m, mean = 6.6 \times 4.8 \mu m)$ and A. subrosea $(3.0-6.5 \times 2.0-5.0 \mu m, mean = 4.7 \times 3.7 \mu m)$. Additionally, A. paragongcheniae exhibits shorter conidiogenous cells $(5.0-13.1 \times 2.1-6.0 \mu m)$ compared to A. neogarethjonesii $(10-48 \times 4-5.5 \mu m)$. Moreover, these species differ in the morphology of their conidiophores. A. paragongcheniae displays hyaline, basauxic, doliiform, subspherical to barrel-shaped conidiophores, whereas A. neogarethjonesii has shorter conidiophores, and A. subrosea has hyaline to pale brown, simple, subcylindrical conidiophores. Notably, the conidiophores of A. neobambusae have reduced to conidiogenous cells.

Apiospora neogongcheniae C. L. Zhang, sp. nov. Fungal Names: FN 571887

Fig. 4

Etymology. Named after its phylogenetic close related to A. gongcheniae.

Type. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE01248 (holotype GDMCC 3.1047, stored in a metabolically inactive state); ex-type culture YNE01248.

Description. Asexual morph: Hyphae hyaline, branched, septate, smooth, $1.0-2.5 \mu m$ diameter (mean = $1.5 \mu m$, n = 30). Conidia not observed. Chlamydospores single, terminal, globose, rare. **Sexual morph:** Undetermined.

Culture characteristics. On PDA, colonies flat, rounded, initially white, becoming yellowish-white, cottony, with moderate aerial mycelia, covering the 90 mm plate after 7 days at 25 °C. On MEA, colonies white, dense aerial mycelia, forming multiple circles around the center, covering the 90 mm plate after 7 days at 25 °C. Conidiomata were not observed.

Additional specimens examined. CHINA, Yunnan Province: Xishuangbanna, Naban River Watershed National Nature Reserve, 21°10'N, 99°55'E, on the stems of unidentified Poaceae plant, Oct 2018, X.X. Feng, YNE001260.

Note. Phylogenetic analyses confirmed that *A. neogongcheniae* formed an independent clade, exhibiting a close evolutionary relationship with *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea*. Based on a BLASTN search of the GenBank database, it was found that *A. neogongcheniae* shares high similarities with the following strains: *A. garethjonesii* strain HKAS 96289 (94.88% in ITS, 100% in LSU), strain GZCC 20-0115 (94.88% in ITS, 99.41% in LSU, 96.67% in tef1), strain SICAUCC 22-0027 (94.88% in ITS, 100% in LSU, 96.69% in tub2), strain SICAUCC 22-0028 (94.88% in ITS, 100% in LSU, 96.79% in tub2); *A. subrosea* strain CGMCC 3.18337 (98.35% in ITS, 99.80% in LSU, 94.61% in tef1, 94.99% in tub2); and *A. neogarethjonesii* strain HKAS 102408 (93.97% in ITS, 100% in LSU). The tef1 and tub2 sequence data are currently unavailable for *A. neogarethjonesii* to compare with *A. neogongcheniae*.

Due to the absence of sexual and asexual sporulation characters in *A. neogongcheniae*, a comparison of its culture characteristics with those of *A. garethjonesii*, *A. neogarethjonesii* and *A. subrosea* was conducted. On PDA,



Figure 4. Apiospora neogongcheniae (YNE01248, ex-type culture) **a** colonies after 7 d at 25 °C on PDA **b** colonies after 7 d at 25 °C on MEA **c** colonies after 7 d at 25 °C on SNA **d** colonies after 7 d at 25 °C on PDA with rice leaves **e** colonies after 7 d at 25 °C on MEA with rice leaves **f** colonies after 7 d at 25 °C on SNA with rice leaves **g**-**h** chlamydospores. Scale bars: 20 µm.

A. neogongcheniae exhibits a yellowish-white surface and reverse color, whereas A. garethjonesii displays a white surface with a reddish reverse, A. neogarethjonesii shows a white to black surface coloration, and A. subrosea presents a light pink surface with a peach-puff reverse. Phylogenetically, A. neogongcheniae strains YNE01248 and YNE01260 form a distinct branch with 99% MLBP and 0.95 BIPP. Therefore, we propose A. neogongcheniae as a novel species.

Discussion

In the present study, three new species of endophytic *Apiospora* were examined: *A. gongcheniae*, *A. paragongcheniae*, and *A. neogongcheniae*, all of them isolated from the stems of Poaceae plants in Yunnan province of China. According to morphological and molecular identification, the taxonomic position of the three new species was verified.

The generic circumscription of Apiospora was primarily defined through phylogenetic analysis, given the limited morphological characteristics of Apiospora and Arthrinium. The results of a multi-locus phylogenetic analysis in this study, utilizing a combined dataset of ITS, LSU, tef1, and tub2 sequences, supported the previous classification that Apiospora and Arthrinium are distinct lineages rather than synonyms (Pintos and Alvarado 2021). Unlike the six major clades identified in a previous study (Pintos and Alvarado 2022), the current study revealed twelve major clades with robust support through the phylogenetic analysis of 114 Apiospora species, including all known species with available sequences. Apiospora minutispora (Das et al. 2020) and Apiospora marianiae AP18219 (Pintos and Alvarado 2022) were not classified within these twelve major clades due to their representation by a single record. The delineation of most Apiospora species into major clades remained consistent across both studies. Notably, A. garethjonesii, A. neogarethjonesii, A. neobambusae, A. mytilomorpha, A. subrosea, and A. setostroma clustered together in a strongly supported major clade H, aligning with findings from previous studies (Crous et

al. 2021; Monkai et al. 2022; Pintos and Alvarado 2022; Liao et al. 2023; Liu et al. 2024). Within this major clade, three distinct clades representing three new species were identified (Fig. 1). *A. gongcheniae* is distinguished from *A. garethjonesii* by 34/545 nucleotides in the ITS sequences, from *A. neogarethjonesii* by 39/546, and from *A. subrosea* by 13/425. *A. paragongcheniae* is distinguished from *A. neogarethjonesii* by 10/512, from *A. neobambusae* by 10/512, and from *A. neogarethjonesii* by 24/525 nucleotides in the ITS sequences. *A. neogongcheniae* is distinguished from *A. garethjonesii* by 28/547, from *A. neogarethjonesii* by 34/547, and from *A. subrosea* by 7/425 nucleotides in the ITS sequences.

Apiospora exhibits ecological diversity, as evidenced by its wide host ranges. Most reported Apiospora species show a host preference within the Poaceae family, as noted by Monkai et al. (Monkai et al. 2022). Our new species were also found growing on plant hosts of the Poaceae family. Specifically, *A. gongcheniae* was discovered on the stems of *Oryza meyeriana* subsp. *granulata*, a member of the plant family Poaceae. The other two new species, *A. paragongcheniae* and *A. neogongcheniae*, were found on the stems of unidentified Poaceae plants. Their close relatives, *A. garethjonesii*, *A. neogarethjonesii*, *A. neobambusae*, and *A. subrosea*, were found on bamboo plants. Most *Apiospora* species exhibit saprobic and endophytic lifestyles, which are likely associated with the prevalence of *Apiospora* (Liao et al. 2023). Our new species occurred as endophytic fungi. Further investigation into endophytic *Apiospora* species will significantly enhance the diversity within the *Apiospora* genus.

Morphological characteristics, including asexual and sexual structures, serve as a fundamental basis for fungal systematics and phylogenetic studies, playing a vital role in the comprehensive examination of fungi. However, many endophytes do not form distinct asexual and sexual structures, as observed in *A. neogongcheniae* in this study, posing challenges in determining their taxonomic status based on morphological features. Recent advances in fungal taxonomy and phylogeny have provided new insights into many species with limited morphological features. Future taxonomic efforts necessitate the integration of morphological traits with molecular evidence to elucidate the natural and stable phylogenetic relationships among *Apiospora* species and their related *Arthrinium* species.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was financed by the National Natural Science Foundation of China (Grant No. 31870010).

Author contributions

Xiao-Ni Yan: Investigation, data curation, formal analysis and writing-original draft. Chu-Long Zhang: Conceptualization, methodology, validaiton, formal analysis, supervision, writing-review & editing, funding acquisition.

Author ORCIDs

Xiao-Ni Yan
https://orcid.org/0009-0009-9984-3617
Chu-Long Zhang
https://orcid.org/0000-0001-5180-0348

Data availability

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

References

- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/0027 5514.1999.12061051
- Chen K, Wu XQ, Huang MX, Han YY (2014) First report of brown culm streak of *Phyllostachys praecox* caused by *Arthrinium arundinis* in Nanjing, China. Plant Disease 98(9): 1274. https://doi.org/10.1094/PDIS-02-14-0165-PDN
- Chi M, Park S, Lee Y (2009) A quick and safe method for fungal DNA extraction. The Plant Pathology Journal 25(1): 108–111. https://doi.org/10.5423/PPJ.2009.25.1.108
 Crous PW, Groenewald JZ (2013) A phylogenetic re-evaluation of *Arthrinium*. IMA Fun-

gus 4(1): 133–154. https://doi.org/10.5598/imafungus.2013.04.01.13

- Crous PW, Hernández-Restrepo M, Schumacher RK, Cowan DA, Maggs-Kölling G, Marais E, Wingfield MJ, Yilmaz N, Adan OCG, Akulov A, Duarte EÁ, Berraf-Tebbal A, Bulgakov TS, Carnegie AJ, de Beer ZW, Decock C, Dijksterhuis J, Duong TA, Eichmeier A, Hien LT, Houbraken JAMP, Khanh TN, Liem NV, Lombard L, Lutzoni FM, Miadlikowska JM, Nel WJ, Pascoe IG, Roets F, Roux J, Samson RA, Shen M, Spetik M, Thangavel R, Thanh HM, Thao LD, van Nieuwenhuijzen EJ, Zhang JQ, Zhang Y, Zhao LL, Groenewald JZ (2021) New and Interesting Fungi. 4. Fungal Systematics and Evolution 7(1): 255–343. https://doi.org/10.3114/fuse.2021.07.13
- Dai DQ, Jiang HB, Tang LZ, Bhat DJ (2016) Two new species of Arthrinium (Apio-sporaceae, Xylariales) associated with bamboo from Yunnan, China. Mycosphere
 : Journal of Fungal Biology 7(9): 1332–1345. https://doi.org/10.5943/myco-sphere/7/9/7
- Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, Bhat DJ, Xu JC, Taylor JE, Hyde KD, Chukeatirote E (2017) Bambusicolous fungi. Fungal Diversity 82(1): 1–105. https:// doi.org/10.1007/s13225-016-0367-8
- Das K, Lee SY, Choi HW, Eom AH, Cho YJ, Jung HY (2020) Taxonomy of Arthrinium minutisporum sp. nov., Pezicula neosporulosa, and Acrocalymma pterocarpi: New Records from Soil in Korea. Mycobiology 48(6): 450–463. https://doi.org/10.1080/122 98093.2020.1830741
- Ellis MB (1965) Dematiaceous Hyphomycetes. VI. Mycological Papers 103(29): 1-46.
- Feng Y, Liu JK, Lin CG, Chen YY, Xiang MM, Liu ZY (2021) Additions to the genus Arthrinium (Apiosporaceae) from bamboos in China. Frontiers in Microbiology 12: 661281. https://doi.org/10.3389/fmicb.2021.661281
- Gerin D, Nigro F, Faretra F, Pollastro S (2020) Identification of *Arthrinium marii* as causal agent of olive tree dieback in Apulia (Southern Italy). Plant Disease 104(3): 694–701. https://doi.org/10.1094/PDIS-03-19-0569-RE
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61(4): 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

- Gong Z, Yang Y, Zhang L, Luo Q, Luo J, Zhang Y (2023) First report of *Apiospora arundinis* causing leaf spot on *Polygonatum cyrtonema* in China. Plant Disease 108(2): 524. https://doi.org/10.1094/PDIS-08-23-1580-PDN
- Hyde KD, Fröhlich J, Taylor JE (1998) Fungi from palms. XXXVI. Reflections on unitunicate ascomycetes with apiospores. Sydowia 50(1): 21–80.
- Hyde KD, Norphanphoun C, Maharachchikumbura S, Bhat DJ, Jones E, Bundhun D, Chen YJ, Bao DF, Boonmee S, Calabon MS, Chaiwan N, Chethana K, Dai DQ, Dayarathne MC, Devadatha B, Dissanayake AJ, Dissanayake LS, Doilom M, Dong W, Fan XL, Goonasekara ID, Hongsanan S, Huang SK, Jayawardena RS, Jeewon R, Karunarathna A, Konta S, Kumar, Lin CG, Liu JK, Liu NG, Luangsaard J, Lumyong S, Luo ZL, Marasinghe DS, McKenzie E, Niego A, Niranjan M, Perera RH, Phukhamsakda C, Rathnayaka AR, Samarakoon MC, Samarakoon S, Sarma VV, Senanayake IC, Shang QJ, Stadler M, Tibpromma S, Wanasinghe DN, Wei DP, Wijayawardene NN, Xiao YP, Yang J, Zeng XY, Zhang SN, Xiang MM (2020) Refined families of Sordariomycetes. Mycosphere 11(1): 305–1059. https://doi.org/10.5943/mycosphere/11/1/7
- Jiang N, Li J, Tian CM (2018) *Arthrinium* species associated with bamboo and reed plants in China. Fungal Systematics and Evolution 2: 1–9. https://doi.org/10.3114/ fuse.2018.02.01
- Jiang HB, Hyde KD, Doilom M, Karunarathna SC, Xu JC, Phookamsak R (2019) Arthrinium setostromum (Apiosporaceae, Xylariales), a novel species associated with dead bamboo from Yunnan, China. Asian Journal of Mycology 2(1): 254–268. https://doi. org/10.5943/ajom/2/1/16
- Jiang N, Voglmayr H, Ma CY, Xue H, Piao CG, Li Y (2022) A new *Arthrinium*-like genus of Amphisphaeriales in China. MycoKeys 92: 27–43. https://doi.org/10.3897/mycok-eys.92.86521
- Kalyaanamoorthy S, Minh BQ, Wong T, von Haeseler A, Jermiin LS (2017) ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. https://doi.org/10.1038/nmeth.4285
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010
- Kwon SL, Cho M, Lee YM, Lee H, Kim C, Kim GH, Kim JJ (2022) Diversity of the bambusicolous fungus *Apiospora* in Korea: Discovery of new Apiospora species. Mycobiology 50(5): 302–316. https://doi.org/10.1080/12298093.2022.2133808
- Li S, Peng C, Yuan R, Tian C (2023) Morphological and phylogenetic analyses reveal three new species of Apiospora in China. MycoKeys 99: 297–317. https://doi.org/10.3897/ mycokeys.99.108384
- Liao J, Jiang W, Wu X, He J, Li H, Wang T, Cheng L, Chen W, Mo L (2022) First report of Apiospora Mold on sugarcane in China caused by *Apiospora arundinis* (*Arthrinium arundinis*). Plant Disease 106(3): 1058. https://doi.org/10.1094/PDIS-02-21-0386-PDN
- Liao C, Senanayake IC, Dong W, Thilini Chethana KW, Tangtrakulwanich K, Zhang Y, Doilom M (2023) Taxonomic and phylogenetic updates on *Apiospora*: Introducing four new species from *Wurfbainia villosa* and grasses in China. Journal of Fungi (Basel, Switzerland) 9(11): 1087. https://doi.org/10.3390/jof9111087
- Liu WT, Chen JJ, Feng JW, Xia CY, Shao YX, Zhu YX, Liu F, Cai HM, Yang KB, Zhang CL (2021) Diversity of endophytic fungi associated with plants of Poaceae from Yunnan, Zhejiang and Inner Mongolia. Mycosystema 40(3): 502–513. https://doi. org/10.13346/j.mycosystema.200240

- Liu X, Zhang Z, Wang S, Zhang X (2024) Three new species of *Apiospora* (Amphisphaeriales, Apiosporaceae) on *Indocalamus longiauritus*, *Adinandra glischroloma* and *Machilus nanmu* from Hainan and Fujian, China. Journal of Fungi (Basel, Switzerland) 10(1): 74. https://doi.org/10.3390/jof10010074
- Martínez-Cano C, Grey WE, Sands DC (1992) First report of Arthrinium arundinis causing kernel blight on barley. Plant Disease 76(10): 1077B. https://doi.org/10.1094/PD-76-1077B
- Minh BQ, Nguyen MA, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular Biology and Evolution 30(5): 1188–1195. https://doi. org/10.1093/molbev/mst024
- Minter DW, Cannon PF (2018) Arthrinium minus. Descriptions of Fungi and Bacteria 2155. https://doi.org/10.1079/DFB/20183347366 [Descriptions of Fungi and Bacteria]
- Monkai J, Phookamsak R, Tennakoon DS, Bhat DJ, Xu S, Li Q, Xu J, Mortimer PE, Kumla J, Lumyong S (2022) Insight into the taxonomic Resolution of *Apiospora*: Introducing novel species and records from bamboo in China and Thailand. Diversity 14(11): 918. https://doi.org/10.3390/d14110918
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. Molecular Phylogenetics and Evolution 7(1): 103–116. https://doi.org/10.1006/mpev.1996.0376
- O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences of the United States of America 95(5): 2044–2049. https://doi.org/10.1073/ pnas.95.5.2044
- Pintos Á, Alvarado P (2021) Phylogenetic delimitation of *Apiospora* and *Arthrinium*. Fungal Systematics and Evolution 7(1): 197–221. https://doi.org/10.3114/fuse.2021.07.10
- Pintos Á, Alvarado P (2022) New studies on Apiospora (Amphisphaeriales, Apiosporaceae): Epitypification of Sphaeria apiospora, proposal of Ap. marianiae sp. nov. and description of the asexual morph of Ap. sichuanensis. MycoKeys 92: 63–78. https:// doi.org/10.3897/mycokeys.92.87593
- Réblová M, Miller AN, Rossman AY, Seifert KA, Crous PW, Hawksworth DL, Abdel-Wahab MA, Cannon PF, Daranagama DA, De Beer ZW, Huang S-K, Hyde KD, Jayawardena R, Jaklitsch W, Jones EBG, Ju Y-M, Judith C, Maharachchikumbura SSN, Pang K-L, Petrini LE, Raja HA, Romero AI, Shearer C, Senanayake IC, Voglmayr H, Weir BS, Wijayawarden NN (2016) Recommendations for competing sexual-asexually typified generic names in Sordariomycetes (except Diaporthales, Hypocreales, and Magnaporthales). IMA Fungus 7(1): 131–153. https://doi.org/10.5598/imafungus.2016.07.01.08
- Rehner SA, Samuels GJ (1995) Molecular systematics of the Hypocreales: A teleomorph gene phylogeny and the status of their anamorphs. Canadian Journal of Botany 73(S1): 816–823. https://doi.org/10.1139/b95-327
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Senanayake IC, Bhat JD, Cheewangkoon R, Xie N (2020) Bambusicolous Arthrinium species in Guangdong Province, China. Frontiers in Microbiology 11: 602773. https://doi. org/10.3389/fmicb.2020.602773

- Tian X, Karunarathna SC, Mapook A, Promputtha I, Xu J, Bao D, Tibpromma S (2021) One new species and two new host records of *Apiospora* from bamboo and maize in northern Thailand with thirteen new combinations. Life 11(10): 1071. https://doi. org/10.3390/life11101071
- Wang M, Liu F, Crous PW, Cai L (2017) Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. Persoonia 39(1): 118–142. https:// doi.org/10.3767/persoonia.2017.39.06
- Wang M, Tan XM, Liu F, Cai L (2018) Eight new *Arthrinium* species from China. MycoKeys 34: 1–24. https://doi.org/10.3897/mycokeys.34.24221
- White T, Bruns T, Lee S, Taylor J, Innis M, Gelfand D, Sninsky J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A guide to Methods and Applications 18: 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Yin C, Luo F, Zhang H, Fang X, Zhu T, Li S (2020) First report of *Arthrinium kogelbergense* causing blight disease of bambusa intermedia in Sichuan province, China. Plant Disease 105(1): 214. https://doi.org/10.1094/PDIS-06-20-1159-PDN
- Zhang D, Gao F, Jakovlić I, Zou H, Zhang J, Li WX, Wang GT (2020) PhyloSuite: An integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Molecular Ecology Resources 20(1): 348–355. https://doi.org/10.1111/1755-0998.13096
- Zhao YZ, Zhang ZF, Cai L, Peng WJ, Liu F (2018) Four new filamentous fungal species from newly-collected and hivestored bee pollen. Mycosphere : Journal of Fungal Biology 9(6): 1089–1116. https://doi.org/10.5943/mycosphere/9/6/3
- Zhao HJ, Dong W, Shu YX, Mapook A, Manawasinghe IS, Doilom M, Luo M (2023) Bambusicolous fungi in Guangdong, China: Establishing *Apiospora magnispora* sp. nov. (Apiosporaceae, Amphisphaeriales) based on morphological and molecular evidence. Current Research in Environmental & Applied Mycology 13(1): 1–15. https:// doi.org/10.5943/cream/13/1/1



Research Article

Three new species of *Neohelicomyces* (Tubeufiales, Tubeufiaceae) from freshwater and terrestrial habitats in China

Jian Ma^{1,2,3©}, Deecksha Gomdola^{2,3©}, Saranyaphat Boonmee^{2,3©}, Hong-Wei Shen^{2,3,4©}, Xia Tang^{2,3,5©}, Li-Juan Zhang^{1,2,3©}, Yong-Zhong Lu^{1©}, Kevin D. Hyde^{2,3,6,7,8©}

- 1 School of Food and Pharmaceutical Engineering, Guizhou Institute of Technology, Guiyang 550003, China
- 2 Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
- 3 School of Science, Mae Fah Luang University, Chiang Rai, 57100, Thailand
- 4 College of Agriculture and Biological Science, Dali University, Dali, China
- 5 Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang 550025, China
- 6 Innovative Institute for Plant Health / Key Laboratory of Green Prevention and Control on Fruits and Vegetables in South China, Ministry of Agriculture and Rural Affairs, Zhongkai University of Agriculture and Engineering, Guangzhou 510225, Guangdong, China
- 7 Department of Botany and Microbiology, College of Science, King Saud University, Saudi Arabia
- 8 CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming, Yunnan 650201, China Corresponding authors: Yong-Zhong Lu (yzlu@git.edu.cn); Kevin D. Hyde (kdhyde3@gmail.com)

Abstract

Neohelicomyces species are a group of helicosporous hyphomycetes with the potential to produce secondary metabolites. During our investigation of helicosporous fungi, six collections were isolated from both terrestrial and freshwater habitats in Guizhou Province, China. Based on multigene phylogenetic analysis (ITS, LSU, *tef1a* and *rpb2*), coupled with morphological data, three new *Neohelicomyces* species, viz. *N. guizhouensis*, *N. helicosporus* and *N. hydei* were established. A list of accepted *Neohelicomyces* species with molecular data was provided. The strain of *Neohelicomyces pallidus* (UAMH 10535) was synonymised under *N. denticulatus* based on molecular data.

Key words: Asexual morphs, Dothideomycetes, new taxa, phylogeny, taxonomy

Introduction

Genus *Neohelicomyces* Z.L. Luo, Bhat & K.D. Hyde (Tubeufiaceae) is a group of helicosporous hyphomycetes which are characterised by coiled and helical conidia (Luo et al. 2017; Lu et al. 2018b; Tibpromma et al. 2018; Crous et al. 2019a, 2019b; Dong et al. 2020; Hsieh et al. 2021; Lu et al. 2022; Yang et al. 2023). That genus, typified by *N. aquaticus*, was established by Luo et al. (2017), based on morphological characterisation and phylogenetic analysis of the combined ITS, LSU and *tef1a* sequence data. In their study, three new species (including the type species), *N. aquaticus*, *N. grandisporus* and *N. submersus*, were collected from submerged decaying wood substrata in Yunnan Province, China. Thereafter, Tibpromma et al. (2018) reported the fourth *Neohelicomyces* species, *N. pandanicola* from *Pandanus* sp. in the same province, China. Moreover, Lu et al. (2018b) re-assessed Tubeufiales, based on multi-locus phylogeny and mor-



Academic editor: Nattawut Boonyuen Received: 28 March 2024 Accepted: 13 May 2024 Published: 3 June 2024

Citation: Ma J, Gomdola D, Boonmee S, Shen H-W, Tang X, Zhang L-J, Lu Y-Z, Hyde KD (2024) Three new species of *Neohelicomyces* (Tubeufiales, Tubeufiaceae) from freshwater and terrestrial habitats in China. MycoKeys 105: 317–336. https://doi.org/10.3897/ mycokeys.105.124129

Copyright: © Jian 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). phology and introduced a new species, *N. hyalosporus*, but synonymised the following four strains under *Neohelicomyces pallidus*, i.e. *Helicosporium pallidum* (CBS 962.69 and UAMH 10535), *Tubeufia helicomyces* (CBS 271.52) and *T. paludosa* (CBS 245.49) (Linder 1929; Goos 1986; Tsui et al. 2006; Zhao et al. 2007; Ruibal et al. 2009). More recently, Crous et al. (2019a, 2019b) have introduced two species, *Neohelicomyces deschampsiae* and *N. melaleucae*, collected from terrestrial habitats in Europe (Germany) and North America (USA), respectively. Subsequent studies reporting novel *Neohelicomyces* species are listed chronologically as follows: *N. dehongensis* and *N. thailandicus* – collected from freshwater bodies in China and Thailand (Dong et al. 2020); *N. longisetosus* – collected from submerged decaying culm of *Miscanthus floridulus* (Poaceae) in Taiwan Province, China (Hsieh et al. 2021); *N. hainanensis* – collected from decaying wood in a terrestrial habitat in China (Lu et al. 2022); and *N. dehongensis* – collected from decaying submerged wood in China (Yang et al. 2023).

To date, Neohelicomyces comprises 13 species, all of which have molecular data (Table 2) and are distributed in Asia (mostly in China), Europe (Germany, Czechia, Italy and Netherlands) and North America (USA). They occur as saprobes on various plant litter in both freshwater and terrestrial habitats (e.g. on Deschampsia cespitosa, Fraxinus excelsior, Melaleuca styphelioides, Miscanthus floridulus, Pandanus sp. and Quercus robur), according to Linder (1929), Goos (1989), Tsui et al. (2006), Zhao et al. (2007); Luo et al. (2017), Lu et al. (2018b), Tibpromma et al. (2018), Crous et al. (2019a, 2019b), Dong et al. (2020), Hsieh et al. (2021), Lu et al. (2022) and Yang et al. (2023). All of the Neohelicomyces species that have been reported so far only occur in the asexual morph. Their sexual morph is yet to be documented. Neohelicomyces genus is characterised by gregarious colonies on natural substrates, with colours ranging from white, greyish-brown, to yellowish-green and pinkish. In addition, this genus is depicted by macronematous, branched and/or unbranched conidiophores, monoblastic to polyblastic, integrated, terminal or intercalary conidiogenous cells with lateral minute denticles and acropleurogenous or pleurogenous, helicoid conidia (Luo et al. 2017; Lu et al. 2018b; Tibpromma et al. 2018; Crous et al. 2019a, 2019b; Dong et al. 2020; Hsieh et al. 2021; Lu et al. 2022; Yang et al. 2023).

Previous studies have primarily focused on systematics and taxonomic research of helicosporous hyphomycetes (Abdel-Wahab et al. 2010; Boonmee et al. 2011, 2014; Lu et al. 2017a, 2017b, 2017c, 2018a, 2023a, 2023b; Kuo and Goh 2018; Lu and Kang 2020; Li et al. 2022a, 2022b; Ma et al. 2023a, 2023b; Xiao et al. 2023; Zhang et al. 2023). Recent studies on the natural products of some members from *Neohelicomyces* genus have shown that two compounds from *N. hyalosporus* (PF11-1) exhibited moderate cytotoxicity against human cancer cells (A549, TCA, RD) (Zheng et al. 2023). Therefore, the metabolites of *Neohelicomyces* species may be a potential source for preparing and developing drugs for human tumour prevention and management.

In this study, six helicosporous taxa were collected from both freshwater and terrestrial habitats in Zunyi City, Qianxinan Buyi and Miao Autonomous Prefecture, Guizhou Province, China. Based on morphological descriptions, illustrations and multi-gene phylogenetic analyses, three novel species are herein introduced, namely *Neohelicomyces guizhouensis*, *N. helicosporus* and *N. hydei*.

Materials and methods

Sampling of the collections, macro- and micro- morphological examinations

Specimens were collected from freshwater and terrestrial habitats from August 2021 to March 2022 in Zunyi City and Qianxinan Buyi and Miao Autonomous Prefecture, Guizhou Province, China. Specimens from freshwater habitats were cultured at room temperatures, with moisture maintained for 1–2 weeks. Fungal colonies and micromorphological structures on the surface of the natural substrates were observed using a stereomicroscope (SMZ-168, Nikon, Japan) and photographed using an ECLIPSE Ni compound microscope (Nikon, Tokyo, Japan), equipped with a Canon 90D digital camera.

Isolations and material deposition

Single spore isolations were conducted following the method described by Chomnunti et al. (2014). Subsequently, the germinating spores were aseptically transferred to fresh potato dextrose agar (PDA) plates, following the method outlined in Senanayake et al. (2020). Fungal mycelia were cultured on PDA and incubated at 25 °C for 45 to 50 days. Their colony characteristics, such as shape, colour, size, margin and elevation, were monitored and recorded.

Dried fungal specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (Herb. HKAS), Kunming, China and the Herbarium of Guizhou Academy of Agriculture Sciences (Herb. GZAAS), Guiyang, China. Cultures were deposited at the Guizhou Culture Collection (GZCC), Guiyang, China. The descriptions of the newly-introduced taxa were uploaded in the Faces of Fungi webpage following the guidelines outlined in Jayasiri et al. (2015). The new species were registered in the MycoBank database (https://www.mycobank.org/).

DNA extraction, PCR amplification and sequencing

Fresh mycelia were scraped with a sterilised toothpick and transferred to a 1.5 ml microcentrifuge tube. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, China), following the manufacturer's protocol. Primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), EF1-983F/EF1-2218R (Rehner and Buckley 2005) and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used to amplify ITS, LSU, *tef1a* and *rpb2* sequence fragments, respectively. The PCR amplification reactions were carried out in a 50 µl reaction volume, including 2 µl DNA, 2 µl of the forward and reverse primer each and 44 µl of $1.1 \times T3$ Supper PCR Mix (Qingke Biotech, Chongqing, China). The thermal-cycling parameters of the ITS, LSU, *tef1a* and *rpb2* regions were as follows: initial denaturation at 98 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 1 min, elongation at 72 °C for 10 s and final extension at 72 °C for 2 min. The PCR products were detected by 1% agarose gel electrophoresis and the sequencing results were provided by Beijing Qingke Biotechnology Co., Ltd.

Phylogenetic analyses

The sequence data of our new taxa were verified using BioEdit v. 7.0.5.3 (Hall 1999). The forward and reverse sequence data of LSU, *tef1a* and *rpb2* regions were assembled using SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA; Swindell and Plasterer (1997)). The sequences incorporated in this study were downloaded from GenBank (Table 1; https://www.ncbi.nlm.nih.gov/). The single gene datasets were aligned using MAFFT v.7.473 (https://mafft.cbrc.jp/alignment/server/, Katoh et al. (2019)) and trimmed using trimAl.v1.2rev59 software (Capella-Gutiérrez et al. 2009). The aligned datasets were concatenated (LSU-ITS-*tef1a-rpb2*) using SequenceMatrix-Windows-1.7.8 software (Vaidya et al. 2011). The Maximum Likelihood (ML) tree was performed in IQ Tree webserver (http://iqtree.cibiv.univie.ac.at/, Nguyen et al. (2015); Zeng et al. (2023)). To obtain a well-resolved taxonomic placement of *Neohelicomyces* spp., we added *Muripulchra* and a few *Tubeufia* species as ingroup taxa in our analyses. *Helicotubeufia* hydei (MFLUCC 17-1980) and *H.jonesii* (MFLUCC 17-0043) were selected as the outgroup taxa (Liu et al. 2018).

Bayesian Inference (BI) was performed using OFPT methods described by Zeng et al. (2023). The aligned Fasta file was converted to a Nexus format file for Bayesian analysis using AliView v. 1.27 (Daniel et al. 2010). The best-fit substitution model of the four gene matrices was selected using MrModelTest 2.3 under the Akaike Information Criterion (AIC) (Nylander et al. 2008).

The multi-gene phylogenetic trees were visualised using FigTree v. 1.4.4 and the final layout of the phylogram was edited using Adobe Illustrator CC 2019v. 23.1.0 (Adobe Systems, USA). Photo-plates and scale bars were processed using Adobe Photoshop CC 2019 (Adobe Systems, USA) and Tarosoft (R) Image Frame Work programme, respectively.

Phylogenetic results

The phylogenetic positions of our newly-introduced species were determined, based on multi-gene (ITS-LSU-*tef1a-rpb2*) phylogenetic analysis. The concatenated sequence matrix comprised 3,353 characters (ITS: 1-547, LSU: 548-1,405, *tef1a*: 1,406-2,308 and *rpb2*: 2,309-3,353) across 40 ingroup and two outgroup taxa (*Helicotubeufia hydei* and *H. jonesii*). Both the ML and BI analyses of the concatenated ITS, LSU, *tef1a* and *rpb2* datasets yielded similar tree topologies. Fig. 1 illustrates the best scoring ML tree, with a final likelihood value of -17,148.363. The decision to introduce new species based on a polyphasic approach follow the guidelines of Chethana et al. (2021).

With reference to the multi-gene phylogram (Fig. 1), our collections represent three distinct *Neohelicomyces* species within Tubeufiaceae. Our isolates, GZCC 23–0725 and GZCC 23–0726, cluster together with the clade comprising *N. denticulatus*, *N. deschampsiae*, *N. pallidus* and *N. pandanicola*. GZCC 23–0633 and GZCC 23–0634 group together and this clade forms a distinct lineage with *N. hyalosporus* (GZCC 16–0086) with 100% ML and 1.00 PP support. In addition, GZCC 23–0727 and GZCC 23–0728 form a clade together and are sister to *N. aquaticus* (MFLUCC 16–0993 and KUMCC 15–0463) with 100% ML and 0.95 PP support.

taxin ITS LSU tefp Helicotubeufia hydei MFLUCC 17-19043' MH290021 MH290025 MH290035 Murpulchra squatica DLUCC 0571 KY320531 KY320548 IC	Тахар	Strain				
Helacabeuña hydei MFLUCC 17-1980' MH290021 MH290025 MH290031 MH290035 H.jonesii MFLUCC 17-043' MH290025 MH290035 MH290035 Murjulcha aquatica DLUCC 050211 KY320533 KY320550 KY320564 MH551057 M.aquatica KUMCC 15-0245 KY320534 KY320564 KY320564 MH551055 M.aquatica MFLUCC 15-0249' KY320522 KY320564 KY320562 MH551055 N.aquatica MFLUCC 16-0993' KY320528 KY320564 KY320561 MH551056 N.aquaticus MFLUCC 16-0993' KY320528 KY320564 KY320561 MH551056 N. denticulatus GZCC 1-04447 OP377832 MW138355 - - N. denticulatus UAM1 10535 AY916462 AY856913 - - N. denticulatus GZCC 2-07267 PP512971 PP526727 PP526734 N.guathouensis GZCC 23-0726 PP512971 PP526731 PP526734 N.guathouensis GZCC 23-0634 PP512971 PP5	IdXUII	Strain	ITS	LSU	tef1a	rpb2
H. jonesii MH2UCC 17:0043" MH290020 MH290025 MH290030 MH290035 Murjulchra aquatica DLUCC 0571 KY320531 KY320551 KY320563 MH551057 M. aquatica KUMCC 15:0276 KY320532 KY320540 KY320554 KY320554 MH551057 M. aquatica MFLUCC 15:0249" KY320524 KY320540 KY320554 KY320554 MH551056 N. aquatica MFLUCC 15:0249" KY320528 KY320540 KY320554 MH551056 N. aquatica MFLUCC 16:0291" NR_171880 MN913709 MT954333 - N. denticulatus GZCC 19:0444" OP377832 MW133855 - - N. denticulatus GZCC 23:0725" PP512970 PP51273 PP526727 PP526737 N. denchampsize GZCC 23:0726 PP512970 PP512974 PP526728 PP526737 N. bianaensis GZCC 23:027 OP508735 OP698074 OP698086 OP698074 N. bianaensis GZCC 23:027 OP508735 OP698086 OP698075 N	Helicotubeufia hydei	MFLUCC 17-1980 [⊤]	MH290021	MH290026	MH290031	MH290036
Murpulcha aquatica DLUCC 0571 KY320531 KY320530 KY320563 M-response M. aquatica KUMCC 15 0245 KY320531 KY320551 KY320553 KY320553 KY320554 MY320552 KY320554 MY320552 KY320554 MY320552 MY320552 KY320554 MY320552 MY320554 MY320552 MY320554 MY320554 MY53055 N. aquatica MKLUCC 15-0463 KY320529 KY320546 KY320564 MY55036 MH551066 N. denticulatus GZCC 19-04441 OP377832 MW133855 N. denticulatus GZCC 23-0726' PP512970 PP526727 PP526737 N. guizhouensis GZCC 23-0726' PP512970 PP526731 PP526732 PP526731 N. guizhouensis GZCC 23-0726' PP512971 PP512971 PP526732 PP526732 PP526732 N. hainanensis GZCC 23-0025' PP512971 PP51975 OP690086 OP690975 N. hainanensis GZCC 23-0027' PP526732 PP526732 PP526732 <	H. jonesii	MFLUCC 17-0043 [⊤]	MH290020	MH290025	MH290030	MH290035
M. aquatica KUMCC 15-0245 KY320533 KY320550 KY320564 MH351067 M. aquatica KUMCC 15-0246 KY320532 KY320551 KY320564 MH351068 M. aquatica MELUCC 15-02497 KY320522 KY320545 KY320564 MH551065 N. aquaticus MELUCC 15-04937 KY320528 KY320545 KY320540 MH551066 N. denticulatus GCC 19-04447 OP377832 MW133855 - - N. denticulatus GCC 23-07251 PP512969 PP512973 PP526727 PP526733 N. dachampsiae GCC 23-07251 PP512970 PP512973 PP526727 PP526733 N. guizhouensis GCC 23-07251 PP512970 PP526733 PP526733 M52677 M550867 N. guizhouensis GCC 23-07251 PP512971 PP526737 PP526737 PP526737 N. guianaensis GZCC 23-02071 KX454173 KX454174 - M1551067 N. hainanensis GZCC 23-0237 OP508734 OP508774 OP698085 OP698075 <	Muripulchra aquatica	DLUCC 0571	KY320531	KY320548	-	_
M. aquatica KUMCC 15-0276 KY320534 KY320551 KY320564 M. Y320584 M. Y320584 M. Y320584 M. Y320584 M. Y320584 M. Y320584 KY320582 KY320582 KY320582 KY320582 KY320582 KY320582 KY320582 KY320582 KY320582 KY320583 KY320583 KY320584 KY320583 MH51065 N. aquaticus MKLUCC 16-09931 KY320582 KY320584 KY320583 - N. denticulatus GZCC 19-04447 OP377832 MW133855 - N. denticulatus UAMH 10535 AY916462 AY856913 - N. denticulatus UAMH 10535 AY91642 AY856913 - N. denticulatus GZCC 23-0725 PP512970 PP526727 PP526733 N. gluichouensis GZCC 23-0725 PP512971 PP512975 PP526728 PP526733 N. hainanensis GZCC 23-06337 PP512971 PP512975 PP526730 PP526733 N. helicosporus GZCC 23-0727 - PP5129	M. aquatica	KUMCC 15-0245	KY320533	KY320550	KY320563	MH551057
M. aquaticaMFLUCC 15-0249"KY320522KY320544GNechelicomyces aquaticusKUMCC 15-0463KY32052KY320546KY320561MH551065N. aquaticusMFLUCC 16-093"KY32052KY320532KY320561MH551065N. dehongensisMFLUCC 16-1029"NR.171880MN913709MT954393-N. denticulatusGZCC 19-0444"OP37R32MW133855N. denticulatusGZCC 19-0444"OP37R32MK42601AV86513N. denticulatusGZCC 23-0726PP512967PP512976PP52673PP526731N. guizhouensisGZCC 23-0726PP512970PP512974PP526732PP526731N. guizhouensisGZCC 22-0071OP508735OP508775OP508976OP508976N. hainanensisGZCC 23-0634PP512971PP512975PP526730PP526730N. hainaensisGZCC 23-0634PP512975PP512975PP526730PP526736N. hainaensisGZCC 23-0634PP512975PP512975PP526730PP526736N. hainaensisGZCC 23-0634PP512975PP526730PP526730PP526736N. hainaensisGZCC 23-0634PP512975PP52673PP52673PP526736N. hainaensisGZCC 23-0634PP512976PP52673PP52673PP52673N. hainaensisGZCC 23-0726-PP512976PP52673PP52673N. hainaensisGZCC 23-0726MS51361MI55036N. hydeiGZCC 23-0	M. aquatica	KUMCC 15-0276	KY320534	KY320551	KY320564	MH551058
Neohelicomyces aquaticus KUMCC 15-0463 KY320529 KY320546 KY320540 KY320540 N. aguaticus MFLUCC 16-0997 KY320528 KY320545 KY320540 KY320540 N. deniculatus GCCC 19-0444' OP377832 MW133855 - N. deniculatus UAMH 10535 AY916462 AY856913 - N. deniculatus UAMH 10535 AY916462 AY856913 - N. deniculatus UAMH 10535 AY916462 AY856913 - N. gaindisporus GZCC 23-07257 PP512970 PP512971 PP526727 PP526731 N. giandisporus GZCC 23-0726 PP512971 PP512974 PP526730 PP598075 N. hainanensis GZCC 23-0634 PP512971 PP512975 PP56730 PP526730 N. haicosporus GZCC 23-07271 PP512976 PP52673 PP526731 N. hydei GZCC 23-0728 PP512976 PP52673 PP526730 N. hydei GZCC 15-00867	M. aquatica	MFLUCC 15-0249 [⊤]	KY320532	KY320549	_	_
N. aquaticus MFLUCC 16-0993 ¹ KY320528 KY320545 KY320561 MH551066 N. dendiculatus GZCC 19-0444 ¹ OP377832 MW133855 - - N. denticulatus GZCC 19-0444 ¹ OP377832 MW133855 - - N. denticulatus UAMH 10535 AY916462 AY856913 - - N. denticulatus UAMH 10535 AY916462 AY856913 - - N. denticulatus GZCC 23-07257 PP512970 PP512973 PP52673 PP526731 N. guizhouensis GZCC 23-0726 PP512970 PP512975 PP526736 OP698074 N. hainanensis GZCC 23-0209 ¹ OP508734 OP508757 OP698085 OP698075 N. hainanensis GZCC 23-0203 ¹ PP512971 PP512975 PP526730 PP526736 N. hainanensis GZCC 23-0634 PP512972 PP512975 PP526730 PP526736 N. hainapersus GZCC 23-0728 - PP512977 PP526731 PP526736 N. hainaposus GZC	Neohelicomyces aquaticus	KUMCC 15-0463	KY320529	KY320546	KY320562	MH551065
N. dehongensis MFLUCC 18-1029 ^T NR_171880 MN913709 MT954393 - N. denticulatus GZCC 19-0444 ^I OP377832 MW133855 - - N. denticulatus UAMH 10535 AY916462 AY856913 - - N. deschampsiae CPC 33686 ^T MK442502 MK442538 PP526727 PP526737 N. guizhouensis GZCC 23-0725 PP512970 PP512974 PP526728 PP526737 N. guizhouensis GZCC 23-0726 PP512970 PP512974 PP526728 PP526731 N. guizhouensis GZCC 22-0277 OP508735 OP508774 OP698086 OP698074 N. hainanensis GZCC 23-0237 PP512971 PP512975 PP526730 PP526731 N. helicosporus GZCC 16-0086 ^I MH55875 MH558870 MH55036 MH55036 N. hydei GZCC 23-0728 - PP512978 PP526731 PP526731 N. hydicosporus GZC 23-0728 MN551051 - - - - N. hydicosporus	N. aquaticus	MFLUCC 16-0993 ^T	KY320528	KY320545	KY320561	MH551066
N. denticulatus GZCC 19-0444* OP377832 MW138855 N. denticulatus UAMH 10535 AY91642 AY856913 N. deschampsiae CPC 33686* MK44202 MK442538 N. gukhouensis GZCC 23-0725* PP512970 PP512973 PP526727 PP526737 N. gukhouensis GZCC 23-0726* PP512970 PP512974 PP526737 PP526737 N. gukhouensis GZCC 23-0726* PP512975 PP526737 PP526737 PP526737 N. hainanensis GZCC 23-003* OP508735 OP508775 OP698086 OP698075 N. hainanensis GZCC 23-003* PP512971 PP512975 PP526730 PP526730 N. hainanensis GZCC 16-0086* MH551057 MH550305 MH551061 N. hylosoprus GZCC 16-0086* MH55107 PP526730 PP526730 PP526731 N. hylosoprus GZCC 16-0086* MH551076 MH55087 MH550837 MH55083 A N. hylosoprus GZCC 23-0728 - PS26731	N. dehongensis	MFLUCC 18-1029 [™]	NR_171880	MN913709	MT954393	_
N. denticulatus UAMH 10535 AY916462 AY856913 - - N. deschampsiae CPC 336867 MK44202 MK442538 - - N. guahbouensis GZCC 23-07267 PP512970 PP52673 PP526738 PP526733 N. guahbouensis GZCC 23-07267 PP512970 PP526738 PP526738 PP526738 N. guahbouensis GZCC 23-0027 OP508734 OP508734 OP698085 OP698075 N. hainanensis GZCC 23-00337 OP512970 OP508735 OP698086 OP698075 N. hainanensis GZCC 23-00337 PP512971 PP526730 PP526730 PP526730 N. hainanensis GZCC 23-0234 PP512975 PP526731 PP526731 PP526731 N. hainanensis GZCC 23-0728 - PP512977 PP526731 PP526733 N. hydei GZCC 23-0728 - PP512976 PP526732 PP526733 N. hydei GZCC 23-0728 - PP512976 PP526733 PF526731 N. hydei GZCC 23-0728	N. denticulatus	GZCC 19-0444 [⊤]	OP377832	MW133855	-	_
N. deschampsiae CPC 33686' MK442602 MK442538 Image and the second	N. denticulatus	UAMH 10535	AY916462	AY856913	-	_
N. guizhouensis GZCC 23-0725' PP512970 PP512973 PP526728 PP526734 N. guizhouensis GZCC 23-0726 PP512970 PP512974 PP526728 PP526734 N. grandisporus KUMCC 15-0470' KX454173 KX454174 - MH551067 N. hainanensis GZCC 22-009' OP508734 OP508774 OP698086 OP698074 N. hainanensis GZCC 22-0027 OP508735 OP508775 OP698086 OP698075 N. helicosporus GZCC 23-0633' PP512971 PP512975 PP526729 PP526730 N. helicosporus GZCC 23-0624' PM55875 PM153870 MH550365 MH550365 N. hydiosporus GZCC 23-0727' - PP512977 PP526732 PP526732 N. hydio GZC 23-0728' - PP512975 PP526732 PP526733 N. hydio GZC 23-0728' - P P512975 PP526732 PP526733 N. hydiosporus GZCC 23-0727' - P - - - - -	N. deschampsiae	CPC 33686 [™]	MK442602	MK442538	_	-
N. guizhouensisGZCC 23-0726PP512970PP512974PP526728PP56773N. grandisporusKUMCC 15-0470"KX454173KX454174-MH551067N. hainanensisGZCC 22-2007"OP508734OP508774OP698085OP698075N. hainanensisGZCC 23-0633"PP512971PP512975PP526730PP526730N. helicosporusGZCC 23-0634"PP512971PP512976PP526730PP526730N. helicosporusGZCC 23-0634"PP512971PP512976PP526730PP526730N. hyalosporusGZCC 23-0727"7PP512977PP526730PP526731N. hyalosporusGZCC 23-07287PP512976PP526730PP526730N. hyalosporusGZCC 23-07287PP512977PP526730PP526730N. hydeiGZCC 23-07287PP512976PP526730PP526731N. hydeiGZCC 23-07287PP512976PP526730PP526730N. hydeiGZCC 23-07287PP512976PP526730PP526730N. hydeiGZCC 23-07287PP512976PP526730PP526730N. hydeiGZCC 23-0728MN562154MN567661MN556855N. hydiauCES 245.49MH5651057N. palidusCES 245.49MH8565107N. palidusCES 245.49AY916461AY856861N. palidusCES 245.49AY916450AY856861 <td< td=""><td>N. guizhouensis</td><td>GZCC 23-0725[™]</td><td>PP512969</td><td>PP512973</td><td>PP526727</td><td>PP526733</td></td<>	N. guizhouensis	GZCC 23-0725 [™]	PP512969	PP512973	PP526727	PP526733
N. grandisporus KUMCC 15-0470' KX454173 KX454174 Image of the state of	N. guizhouensis	GZCC 23-0726	PP512970	PP512974	PP526728	PP526734
N. hainanensis GCCC 22-2009 ⁺ OP508734 OP508734 OP698084 OP698074 N. hainanensis GCCC 22-2027 OP508735 OP508775 OP698086 OP698075 N. helicosporus GCCC 23-0633 ⁺ PP512971 PP512975 PP526739 PP526739 N. helicosporus GCCC 23-0634 PP512972 PP512976 PP526730 PP526730 N. hyalosporus GCCC 23-0634 PM512977 PP512977 PP526731 PM526731 N. hyalosporus GCCC 23-0728 ⁺ - PP512977 PP526731 PP526733 N. hydei GCCC 23-0728 ⁺ - PP512978 PP526732 PP526733 N. longisetosus NCYU-106H1-1-1 ⁺ MT939303 - - - - N. melaleucae GCC 23042 ⁺ MH551164 MN566815 MN56835 - - - N. malaleucus CCS 38042 ⁺ MH855104 AY856887 - - - - N. palidus CBS 245.49 MY916461 AY856887 -	N. grandisporus	KUMCC 15-0470 [™]	KX454173	KX454174	-	MH551067
N. hainanensis GZCC 22-2027 OP508735 OP508775 OP698086 OP698075 N. helicosporus GZCC 23-0633 ^T PP512971 PP512975 PP526730 PP526736 N. helicosporus GZCC 23-0634 PP512972 PP512976 PP526730 PP526736 N. hyalosporus GZCC 16-0086 ^T MH558745 MH558870 MH550936 MH551064 N. hydei GZCC 23-0727 ^T - PP512977 PP526731 PP526731 N. hydei GZCC 23-0728 - PP512978 PP526732 PP526738 N. longisetosus NCYU-106H1-1-1 ^T MT939303 - - - - N. melaleucae CPC 38042 ^T MN562154 MN567661 MN556835 - - N. pallidus CBS 245.49 MH855010 - <td>N. hainanensis</td> <td>GZCC 22-2009[™]</td> <td>OP508734</td> <td>OP508774</td> <td>OP698085</td> <td>OP698074</td>	N. hainanensis	GZCC 22-2009 [™]	OP508734	OP508774	OP698085	OP698074
N. helicosporus GZCC 23-0633 ^T PP512971 PP512975 PP526730 PP526730 N. helicosporus GZCC 23-0634 PP512972 PP512976 PP526730 PP526730 N. hyalosporus GZCC 23-0727 ^T MH558745 MH558870 MH550936 MH551064 N. hydei GZCC 23-0727 ^T - PP512977 PP526731 PP526732 N. hydei GZCC 23-0728 - PP512978 PP526732 PP526733 N. longisetosus NCYU-106H1-1-1 ^T MT939303 - - - N. melaleucae CPC 38042 ^T MN562154 MN56761 MN55835 - N. pallidus CBS 245.49 MH856510 - - - - N. pallidus CBS 926.69 AY916460 AY856887 - - - N. pandanicola MKLUCC16-0143 ^T MH250370 MH260377 MH412779 - N. barilidus MFLUCC11-0105 ^T KY320530 KY320547 - - Tubeufiacae sp. MTLUCC16-0104 ^T <	N. hainanensis	GZCC 22-2027	OP508735	OP508775	OP698086	OP698075
N. helicosporusGZCC 23-0634PP512972PP512976PP526730PP526731N. hyalosporusGZCC 16-0086 ^T MH558745MH558870MH550936MH551064N. hydeiGZCC 23-0727 ^T -PP512977PP526731PP526732N. hydeiGZCC 23-0728-PP512978PP526732PP526733N. longisetosusNCYU-106H1-1-1TMT939303N. melaleucaeCPC 38042 ^T MN562154MN567661MN556835-N. pallidusCBS 245.49MH856510N. pallidusCBS 271.52AY916461AY856886N. pallidusCBS 962.69AY916460AY856866N. pandanicolaKUMCC 16-0143 ^T MH275073MH260307MH412779-N. ubmersusMFLUCC 11-1005 ^T NR_171882MN913696Tubeufiaceae sp.ATCC 42524AY916458AY856911Tubeufia guttulataGZCC 23-040 ^T OR030841OR030834OR046678OR046678T. hainanensisGZC 22-2015 ^T OR030842OR030835OR046679OR046689T. krabiensisMFLUCC 16-022 ^T KY092417KY092412KY11703MH551118T. laxisporaMFLUCC 16-022 ^T KY092413KY092408KY117029MF532887T. hananensisMFLUCC 16-022 ^T KY092413KY092410KY117031MF53288T. hainanensisMFLUCC 16-022 ^T KY092413KY092408KY117031 </td <td>N. helicosporus</td> <td>GZCC 23-0633[™]</td> <td>PP512971</td> <td>PP512975</td> <td>PP526729</td> <td>PP526735</td>	N. helicosporus	GZCC 23-0633 [™]	PP512971	PP512975	PP526729	PP526735
N. hyalosporus GCCC 16-0086 ^T MH558745 MH558870 MH550936 MH551064 N. hydei GCCC 23-0727 ^T - PP512977 PP526731 PP526732 N. hydei GCCC 23-0728 - PP512978 PP526732 PP526733 N. longisetosus NCYU-106H1-1-1 ^T MT939303 - - - N. melaleucae CPC 38042 ^T MN562154 MN567661 MN556835 - N. pallidus CBS 245.49 MH856510 - - - - N. pallidus CBS 962.69 AY916461 AY856887 - - - N. pallidus CBS 962.69 AY916460 AY856886 - - - N. pandanicola KUMCC 16-0143 ^T MH275073 MH260307 MH412779 - N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 2-2015 ^T	N. helicosporus	GZCC 23-0634	PP512972	PP512976	PP526730	PP526736
N. hydei GZCC 23-0727 ^T - PP512977 PP526731 PP526731 N. hydei GZCC 23-0728 - PP512978 PP526732 PP526738 N. longisetosus NCYU-106H1-1.1T MT939303 - - - N. melaleucae CPC 38042 ^T MN562154 MN567661 MN55835 - N. pallidus CBS 245.49 MH856510 - - - - N. pallidus CBS 271.52 AY916461 AY856887 - - - N. pallidus CBS 962.69 AY916460 AY856886 - - - N. pandanicola KUMCC 16-0143 ^T MH275073 MH260307 MH412779 - N. ubmersus MFLUCC 16-1106 ^T KY320530 KY320547 - - N. thailandicus MFLUCC 16-1005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 22-015 ^T OR030841 <td>N. hyalosporus</td> <td>GZCC 16-0086[⊤]</td> <td>MH558745</td> <td>MH558870</td> <td>MH550936</td> <td>MH551064</td>	N. hyalosporus	GZCC 16-0086 [⊤]	MH558745	MH558870	MH550936	MH551064
N. hydeiGZCC 23-0728-PP512978PP526732PP526738N. longisetosusNCYU-106H1-1-1"MT939303N. melaleucaeCPC 38042"MN562154MN567661MN556835-N. pallidusCBS 245.49MH856510N. pallidusCBS 271.52AY916461AY856887N. pallidusCBS 962.69AY916400AY856886N. pandanicolaKUMCC 16-0143"MH275073MH260307MH412779-N. ubmersusMFLUCC 16-1106"KY320530KY320547-MH551068N. thailandicusMFLUCC 16-1005"NR_171882MN913696Tubeufiaceae sp.ATCC 42524AY916458AY856911Tubeufia guttulataGZCC 22-015"OR030841OR030834OR046678OR046684T. javanicaMFLUCC 16-0228"KJ880034KJ880036KJ880037-T. krabiensisMFLUCC 16-0228"KY092417KY092412KY117033MH551181T. laxisporaMFLUCC 16-0232"KY092413KY092408KY117029MF535287T. machaerinaeMFLUCC 17-0055MH558795MH558920MH550986MH55122T. machaerinaeMFLUCC 16-0228"KY092415KY092410KY117031MF535288T. machaerinaeMFLUCC 16-0229"KY092415KY092410KY117031MF535288T. marchaerinaeMFLUCC 16-0228"MH558795MH558920MH550868MH5535	N. hydei	GZCC 23-0727 [™]	-	PP512977	PP526731	PP526737
N. longisetosus N.CYU-106H1-1-1 ^T MT939303 - - - N. melaleucae CPC 38042 ^T MN562154 MN567611 MN556835 - N. pallidus CBS 245.49 MH856510 - - - - N. pallidus CBS 271.52 AY916461 AY856887 - - - N. pallidus CBS 962.69 AY916460 AY856886 - - - N. pandanicola KUMCC 16-0143 ^T MH275073 MH260307 MH412779 - N. ubmersus MFLUCC 16-1106 ^T KY320530 KY320547 - MH551068 N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-015 ^T OR030842 OR030835 OR046679 OR046685 T. krabiensis MFLUCC	N. hydei	GZCC 23-0728	-	PP512978	PP526732	PP526738
N. melaleucaeCPC 38042 ^T MN562154MN567661MN556835-N. pallidusCBS 245.49MH856510N. pallidusCBS 271.52AY916461AY856887N. pallidusCBS 962.69AY916460AY856886N. pandanicolaKUMCC 16-0143 ^T MH275073MH260307MH412779-N. ubmersusMFLUCC 16-0106 ^T KY320530KY320547-MH551068N. thailandicusMFLUCC 11-0005 ^T NR_171882MN913696Tubeufiaceae sp.ATCC 42524AY916458AY856911Tubeufia guttulataGZCC 23-040 ^T OR030841OR030834OR046678OR046684T. hainanensisGZCC 22-015 ^T OR030842OR030835OR046679OR046685T. javanicaMFLUCC 16-0228 ^T KY992417KY092412KY117033MH551118T. latisporaMFLUCC 16-0023 ^T KY092413KY092408KY117029MF535287T. machaerinaeMFLUCC 17-0055MH558795MH558920MH550988MH551122T. mackenzieiMFLUCC 16-0222 ^T KY092415KY092410KY117031MF532887T. mackenzieiMFLUCC 16-0223 ^T KY092415KY092410KY117031MF532887	N. longisetosus	NCYU-106H1-1-1 [⊤]	MT939303	_	-	_
N. pallidus CBS 245.49 MH856510 - - - N. pallidus CBS 271.52 AY916461 AY856887 - - N. pallidus CBS 962.69 AY916460 AY856886 - - N. pallidus CBS 962.69 AY916460 AY856886 - - N. pandanicola KUMCC 16-0143 ⁺ MH275073 MH260307 MH412779 - N. ubmersus MFLUCC 16-1106 ⁺ KY320530 KY320547 - MH551068 N. thailandicus MFLUCC 11-0005 ⁺ NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ⁺ OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ⁺ OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 16-0228 ⁺ KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ⁺ MH558792 MH55	N. melaleucae	CPC 38042 [™]	MN562154	MN567661	MN556835	_
N. pallidus CBS 271.52 AY916461 AY856887 - - N. pallidus CBS 962.69 AY916460 AY856886 - - N. pandanicola KUMCC 16-0143 ^T MH275073 MH260307 MH412779 - N. pandanicola MFLUCC 16-1106 ^T KY320530 KY320547 - MH551068 N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 16-0228 ^T KJ880036 KJ880037 - - T. krabiensis MFLUCC 16-0027 ^T KY092417 KY092412 KY117033 MH551128 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 16-0222 ^T	N. pallidus	CBS 245.49	MH856510	-	-	_
N. pallidus CBS 962.69 AY916460 AY856886 - - N. pandanicola KUMCC 16-0143 ^T MH275073 MH260307 MH412779 - N. ubmersus MFLUCC 16-1106 ^T KY320530 KY320547 - MH551068 N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 16-0228 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T KY092417 KY092412 KY117033 MH551118 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. mackenziei MFLUCC 16-0222 ^T KY092413 KY092410 KY117031 MF535288 T. mackenziei	N. pallidus	CBS 271.52	AY916461	AY856887	_	_
N. pandanicola KUMCC 16-0143 ^T MH275073 MH260307 MH412779 - N. ubmersus MFLUCC 16-1106 ^T KY320530 KY320547 - MH551068 N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 16-0228 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T KJ880034 KJ880036 KJ880037 - T. latispora MFLUCC 16-0227 ^T KY092417 KY092412 KY117033 MH551119 T. machaerinae MFLUCC 16-0222 ^T KY092413 KY092408 KY117031 MF535288 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. m	N. pallidus	CBS 962.69	AY916460	AY856886	_	_
N. ubmersus MFLUCC 16-1106 ^T KY320530 KY320547 - MH551068 N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 16-0228 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T KJ88034 KJ880036 KJ880037 - T. latispora MFLUCC 16-0228 ^T KY092417 KY092412 KY117033 MH551119 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 <td< td=""><td>N. pandanicola</td><td>KUMCC 16-0143[⊤]</td><td>MH275073</td><td>MH260307</td><td>MH412779</td><td>_</td></td<>	N. pandanicola	KUMCC 16-0143 [⊤]	MH275073	MH260307	MH412779	_
N. thailandicus MFLUCC 11-0005 ^T NR_171882 MN913696 - - Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 12-0545 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T MH558792 MH558917 MH550985 MH551118 T. latispora MFLUCC 16-0227 ^T KY092417 KY092412 KY117033 MH551119 T. machaerinae MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	N. ubmersus	MFLUCC 16-1106 [⊤]	KY320530	KY320547	_	MH551068
Tubeufiaceae sp. ATCC 42524 AY916458 AY856911 - - Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 12-0545 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T MH558792 MH558917 MH550985 MH551118 T. latispora MFLUCC 16-0227 ^T KY092412 KY117033 MH551129 T. nachaerinae MFLUCC 16-0222 ^T KY092413 KY092408 KY117029 MF535287 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	N. thailandicus	MFLUCC 11-0005 [™]	NR_171882	MN913696	_	_
Tubeufia guttulata GZCC 23-040 ^T OR030841 OR030834 OR046678 OR046684 T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 12-0545 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T MH558792 MH558917 MH550985 MH551118 T. latispora MFLUCC 16-0227 ^T KY092417 KY092412 KY117033 MH551119 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	Tubeufiaceae sp.	ATCC 42524	AY916458	AY856911	_	_
T. hainanensis GZCC 22-2015 ^T OR030842 OR030835 OR046679 OR046685 T. javanica MFLUCC 12-0545 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T MH558792 MH558917 MH550985 MH551118 T. latispora MFLUCC 16-0227 ^T KY092417 KY092412 KY117033 MH551119 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 16-0222 ^T KY092415 MH558920 MH550988 MH551122 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	Tubeufia guttulata	GZCC 23-040 [⊤]	OR030841	OR030834	OR046678	OR046684
T. javanica MFLUCC 12-0545 ^T KJ880034 KJ880036 KJ880037 - T. krabiensis MFLUCC 16-0228 ^T MH558792 MH558917 MH550985 MH551118 T. latispora MFLUCC 16-0227 ^T KY092417 KY092412 KY117033 MH551119 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 16-0222 ^T KY092415 KY092410 MH550988 MH551122 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. hainanensis	GZCC 22-2015 [⊤]	OR030842	OR030835	OR046679	OR046685
T. krabiensis MFLUCC 16-0228 ^T MH558792 MH558917 MH550985 MH551118 T. latispora MFLUCC 16-0027 ^T KY092417 KY092412 KY117033 MH551119 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 17-0055 MH558795 MH558920 MH550988 MH551122 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. javanica	MFLUCC 12-0545 ^T	KJ880034	KJ880036	KJ880037	_
T. latispora MFLUCC 16-0027 ^T KY092417 KY092412 KY117033 MH551119 T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 17-0055 MH558795 MH558920 MH550988 MH551122 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. krabiensis	MFLUCC 16-0228 [⊤]	MH558792	MH558917	MH550985	MH551118
T. laxispora MFLUCC 16-0232 ^T KY092413 KY092408 KY117029 MF535287 T. machaerinae MFLUCC 17-0055 MH558795 MH558920 MH550988 MH551122 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. latispora	MFLUCC 16-0027 [™]	KY092417	KY092412	KY117033	MH551119
T. machaerinae MFLUCC 17-0055 MH558795 MH558920 MH550988 MH551122 T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. laxispora	MFLUCC 16-0232 [™]	KY092413	KY092408	KY117029	MF535287
T. mackenziei MFLUCC 16-0222 ^T KY092415 KY092410 KY117031 MF535288 T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. machaerinae	MFLUCC 17-0055	MH558795	MH558920	MH550988	MH551122
T. muriformis GZCC 22-2039 ^T OR030843 OR030836 OR046680 OR046686	T. mackenziei	MFLUCC 16-0222 [⊤]	KY092415	KY092410	KY117031	MF535288
	T. muriformis	GZCC 22-2039 [™]	OR030843	OR030836	OR046680	OR046686
T. nigroseptum CGMCC 3.20430 ^T MZ092716 MZ853187 OM022002 OM022001	T. nigroseptum	CGMCC 3.20430 ^T	MZ092716	MZ853187	OM022002	OM022001
T. pandanicola MFLUCC 16-0321 ^T MH275091 MH260325	T. pandanicola	MFLUCC 16-0321 [⊤]	MH275091	MH260325	_	

Table 1 . Taxa used in this study and their GenBank accession numl	bers.
---	-------

Note: "T" indicates ex-type strains. Newly-generated sequences are typed in bold. "-" indicates the unavailable data in GenBank.



Figure 1. Phylogenetic tree generated from Maximum Likelihood (ML) analysis, based on the combined ITS, LSU, *tef1a* and *rpb2* sequence data. Bootstrap support values of ML equal to or greater than 75% and Bayesian posterior probabilities (PP) equal to or greater than 0.95 are given near the nodes as ML/PP, respectively. *Helicotubeufia hydei* (MFLUCC 17–1980) and *H. jonesii* (MFLUCC 17–0043) were selected as outgroup taxa. The new species are typed in bold red; "T" denotes ex-type strains.

Taxonomy

Neohelicomyces guizhouensis J. Ma, Y.Z. Lu & K.D. Hyde, sp. nov. MycoBank No: 901915 Facesoffungi Number: FoF15563 Fig. 2

Etymology. The epithet "guizhouensis" refers to Guizhou Province, from where the specimen was collected.

Holotype. HKAS 134924.

Description. *Saprobic* on decaying wood in a freshwater habitat. *Sexual morph* Unknown from natural habitat. *Asexual morph* Hyphomycetous, helicosporous. *Colonies* on natural substrate superficial, effuse, gregarious, white to light pink. *Mycelium* semi-immersed, hyaline to pale brown, septate, branched hyphae, smooth, comprising glistening conidial mass. *Conidiophores* 78–288 µm long, 4–6 µm wide ($\bar{x} = 179.5 \times 5 \mu$ m, n = 20), macronematous, mononematous, erect, flexuous, cylindrical, sometimes branched, septate, hyaline to pale brown, smooth, thick-walled. *Conidiogenous cells* 9–18 µm long, 2.5–4.5 µm wide ($\bar{x} = 14 \times 3.5 \mu$ m, n = 25), holoblastic, mono- to poly-blastic, integrated, sympodial, intercalary or terminal, cylindrical, with a denticulate protrusion, truncate at apex after conidial secession, hyaline to pale brown, smooth-walled. *Conidia* solitary, acropleurogenous, helicoid, rounded at the tips, 18–21.5 µm in diameter and conidial filament 2–2.7 µm wide ($\bar{x} = 20 \times 2.3 \mu$ m, n = 20), 94.5–148.5 µm long ($\bar{x} = 126.5 \mu$ m, n = 30), multi-septate, coiled $2^{3}/4$ – $3^{1}/2$ times, becoming loosely coiled in water, guttulate, hyaline, smooth-walled.

Culture characteristics. Conidia producing germ tubes on PDA within 9 hours of incubation at 25 °C. Colonies on PDA are circular with flat surface and undulate edge, reaching 40 mm diameter after 45 days of incubation at 25 °C, top view of colony pale pink to brown, reverse brown to dark brown.

Material examined. CHINA, Guizhou Province, Zunyi City, Renhuai City, Daba Town, on decaying wood in a freshwater habitat, 17 August 2021, Jian Ma, RH4 (HKAS 134924, holotype; GZAAS 23–0619, isotype), ex-type living cultures GZCC 23–0725; *Ibid.*, RH4.1 (GZAAS 23–0620, paratype), living culture GZCC 23–0726.

Notes. The newly-identified strains (GZCC 23–0725 and GZCC 23–0726) are phylogenetically grouped with *N. denticulatus*, *N. deschampsiae*, *N. pallidus* and *N. pandanicola* (Fig. 1). However, it is most closely related to *N. deschampsiae* (CPC 33686) phylogenetically and a comparison of polymorphic nucleotides across ITS and LSU sequences between GZCC 23–0725 and *N. deschampsiae* (CPC 33686) revealed nucleotide base disparities of 34/546 bp (6.3%, including fourteen gaps) and 4/860 bp (0.5%, including 0 gap), respectively. Morphologically, *N. guizhouensis* is most similar to *N. dehongensis* in having macronematous, mononematous, erect, flexuous branched conidiophores and solitary, helicoid, hyaline conidia (Dong et al. 2020). However, *N. dehongensis* can be delineated from *N. guizhouensis* by its longer conidia (145–210 µm vs. 94.5–148.5 µm) and wider conidial filaments (20–25 µm vs. 18–21.5 µm) (Dong et al. 2020). Therefore, based on the findings from both molecular and morphological evidence, we propose *N. guizhouensis* as a new species.



Figure 2. *Neohelicomyces guizhouensis* (HKAS 134924, holotype) **a**, **b** colonies on the host surface **c**, **d** conidiophores, conidiogenous cells and conidia **e**-**g** conidiogenous cells **h**-**I** conidia **m** germinated conidium **n**, **o** surface and reverse colonies on PDA after 45 days of incubation at 25 °C. Scale bars: 50 μm (**c**); 30 μm (**d**); 10 μm (**e**, **g**, **m**); 5 μm (**f**, **h**-**I**).
Neohelicomyces helicosporus J. Ma, Y.Z. Lu & K.D. Hyde, sp. nov.

MycoBank No: 901916 Facesoffungi Number: FoF15564 Fig. 3

Etymology. The epithet *"helicosporus"* refers to the helicoid form of conidia. **Holotype.** HKAS 134923.

Description. Saprobic on decaying wood in a terrestrial habitat. Sexual morph Unknown from natural habitat. Asexual morph Hyphomycetous, helicosporous. Colonies on natural substrate superficial, effuse, gregarious, white. Mycelium semi-immersed, hyaline to pale brown, septate, branched hyphae, smooth, comprising glistening conidial mass. Conidiophores 105-199 μ m long, 3–5.5 μ m wide (\bar{x} = 160.5 × 4 μ m, n = 25), macronematous, mononematous, erect, curved, flexible at the tip, cylindrical, unbranched, septate, hyaline, smooth-, thick-walled. Conidiogenous cells 13-22 µm long, 2.5–4.5 μ m wide (\bar{x} = 16 × 3.5 μ m, n = 20), holoblastic, monoblastic to polyblastic, integrated, sympodial, intercalary or terminal, cylindrical, with a denticulate protrusion, truncate at apex after conidial secession, hyaline, smooth-walled. Conidia solitary, acropleurogenous, helicoid, rounded at the tips, $15.5-18 \mu m$ in diameter and conidial filament $2.5-5 \mu m$ wide ($\bar{x} = 16.5 \times$ $3.5 \,\mu\text{m}$, n = 25), $103-170 \,\mu\text{m}$ long (\bar{x} = 130 μm , n = 30), indistinctly multi-septate, coiled up to 3³/₄ times, becoming loosely coiled in water, guttulate, hyaline, smooth-walled.

Culture characteristics. Conidia producing germ tubes on PDA within 9 hours of incubation at 25 °C. Colonies on PDA are irregular with umbonate surface and filiform edge, reaching 43 mm diameter after 48 days of incubation at 25 °C, top view of colony reddish-brown to black brown, reverse brown to black brown.

Material examined. CHINA, Guizhou Province, Qianxinan Buyi and Miao Autonomous Prefecture, Lianhuan Town, on decaying wood in a terrestrial habitat, 17 March 2022, Jian Ma, LHX8 (HKAS 134923, holotype; GZAAS 23–0623, isotype), ex-type living cultures GZCC 23–0633; *Ibid.*, LHX8.1 (GZAAS 23–0624, paratype), living culture GZCC 23–0634.

Notes. Our isolates (GZCC 23-0633 and GZCC 23-0634) are morphologically similar to Neohelicomyces hainanensis (Lu et al. 2022), but the phylogenetic analyses revealed that GZCC 23-0633 and GZCC 23-0634 cluster together and this clade is sister to N. hyalosporus (GZCC 16-0086) with 100% ML/1.00 PP support (Fig. 1). The polymorphism nucleotides comparison of ITS, LSU, tef1a and rpb2 sequence data between GZCC 23-0633 and Neohelicomyces hyalosporus (GZCC 16-0086), reveals nucleotide base differences of 29/527 bp (5.5%, including thirteen gaps), 2/844 bp (0.2%, including 0 gap), 27/892 bp (3.0%, including 0 gap) and 37/893 bp (4.1%, including 0 gap), respectively. Additionally, our species displays a reddish-brown pigmentation on PDA, but this feature was not observed in N. hainanensis and N. hyalosporus (Lu et al. 2018b, 2022). Furthermore, our species differs from N. hainanensis in having longer conidia (103–170 µm vs. up to 136 µm) and from N. hyalosporus in having shorter conidiophores (105–199 µm vs. 210–290 µm) (Lu et al. 2018b, 2022). Therefore, based on the phylogenetic and morphological differences, we introduce N. helicosporus herein as a novel species.



Figure 3. Neohelicomyces helicosporus (HKAS 134923, holotype) **a**, **b** colonies on the host surface **c**-**f** conidiophores and conidiogenous cells **g**-**j** conidiogenous cells **k** germinated conidium **I**-**s** conidia **t**, **u** surface and reverse colonies on PDA after 48 days of incubation at 25 °C. Scale bars: 50 μ m (**c**-**e**); 20 μ m (**f**-**h**, **k**-**s**); 10 μ m (**i**); 5 μ m (**j**).

Neohelicomyces hydei J. Ma, Y.Z. Lu & K.D. Hyde, sp. nov.

MycoBank No: 901917 Facesoffungi Number: FoF15565 Fig. 4

Etymology. The epithet *"hydei"* is named in honour of Prof. Kevin D. Hyde for his contributions to mycology.

Holotype. HKAS 134925.

Description. *Saprobic* on decaying wood in a freshwater habitat. *Sexual morph* Unknown from natural habitat. *Asexual morph* Hyphomycetous, helicosporous. *Colonies* on natural substrate superficial, effuse, gregarious, white to pale brown. *Mycelium* semi-immersed, hyaline to pale brown, septate, branched hyphae, smooth, comprising glistening conidial mass. *Conidiophores* 262–410 µm long, 5.5–7 µm wide ($\bar{x} = 335 \times 6 \mu m$, n = 30), macronematous, mononematous, erect, flexuous, cylindrical, branched, up to 20–septate, hyaline to pale brown, smooth, thick-walled. *Conidiogenous cells* 7.5–19.5 µm long, 3.5–6 µm wide ($\bar{x} = 16.5 \times 4 \mu m$, n = 35), holoblastic, monoblastic to polyblastic, integrated, intercalary or terminal, cylindrical, with a denticulate protrusion, truncate at apex after conidial secession, hyaline to pale brown, smooth-walled. *Conidia* solitary, acropleurogenous, helicoid, rounded at tip, up to 18.5 µm in diameter and conidial filaments 2–3 µm wide, 137.5–171.5 µm long ($\bar{x} = 158 \mu m$, n = 25), indistinctly multiseptate, coiled up to 4 times, becoming loosely coiled in water, guttulate, hyaline, smooth-walled.

Culture characteristics. Conidia producing germ tubes on PDA within 12 hours of incubation at 25 °C. Colonies on PDA are circular with umbonate surface and entire edge, reaching 42 mm in diameter after 50 days of incubation at 25 °C, top view of colony brown to black brown, reverse pale brown to black brown.

Material examined. CHINA, Guizhou Province, Qianxinan Buyi and Miao Autonomous Prefecture, Xianheping National Forest Park, 24°97'N, 105°63'E, on decaying wood in a freshwater habitat, 16 March 2022, Jian Ma, XHP1 (HKAS 134925, holotype; GZAAS 23–0621, isotype), ex-type living cultures GZCC 23–0727; *Ibid.*, XHP1.1 (GZAAS 23–0622, paratype), living culture GZCC 23–0728.

Notes. Our isolates, GZCC 23-0727 and GZCC 23-0728 cluster together and form a sister clade to N. aquaticus (MFLUCC 16-0993 and KUMCC 15-0463) with 96% ML/0.95 PP support. Upon comparison of the nucleotide bases between our isolates and Neohelicomyces aquaticus (MFLUCC 16-0993), the following differences were observed: 1/851 bp (0.1%, including 1 gap) across LSU, 13/869 bp (1.5%, including 1 gap) across tef1a and 46/945 bp (4.9%, with no gaps) across rpb2. Unfortunately, we were unable to compare the differences in nucleotide bases across ITS as our isolates (GZCC 23-0727 and GZCC 23-0728) lack ITS sequence data. Despite several trials using different PCR conditions, we were unable to amplify the ITS locus for our strain (GZCC 23-0727 and GZCC 23-0728) successfully. Morphologically, our isolates (GZAAS 23-0621 and GZAAS 23-0622) differ from N. aquaticus (MFLU 16-2543) as they have mostly branched and hyaline conidiophores, polyblastic, terminal and hyaline conidiogenous cells and acropleurogenous conidia (Luo et al. 2017). Based on phylogenetic placement and morphology, we identify GZCC 23-0727 and GZCC 23-0728 as a single species, Neohelicomyces hydei.



Figure 4. Neohelicomyces hydei (HKAS 134925, holotype) **a**, **b** colonies on the host surface **c**-**e** conidiophores and conidiogenous cells **f**-**h** conidiogenous cells **i**-**m** conidia **n** germinated conidium **o**, **p** surface and reverse colonies on PDA after 50 days of incubation at 25 °C. Scale bars: 50 μ m (**c**-**e**); 10 μ m (**f**-**n**).

Discussion

In this study, six helicosporous taxa were collected for the first time in northern and south-western regions of Guizhou Province, China. Based on multigene (ITS-LSU-*tef1a-rpb2*) phylogenetic analysis, coupled with morphological descriptions and illustrations, we establish three novel *Neohelicomyces* species, namely *N. guizhouensis*, *N. helicosporus* and *N. hydei*.

A list of accepted Neohelicomyces species with known sequence data is also provided (Table 2). There are 16 species (including three new species described in the present study) in Neohelicomyces, of which ten were found from freshwater habitats, while the remaining six ones were reported from terrestrial habitats, with 13 species collected from China (Linder 1929; Goos 1989; Tsui et al. 2006; Zhao et al. 2007; Luo et al. 2017; Lu et al. 2018b, 2022; Tibpromma et al. 2018; Crous et al. 2019a, 2019b; Dong et al. 2020; Hsieh et al. 2021; Yang et al. 2023). Neohelicomyces pallidus is the most widely distributed member of Neohelicomyces genus and has been reported from terrestrial habitats in various regions of the world, including China, Czechia, Italy, Japan, Netherlands and USA (Linder 1929; Goos 1989; Tsui et al. 2006; Zhao et al. 2007; Lu et al. 2018b). Given that most Neohelicomyces species and many helicosporous genera (Berkleasmium, Helicoma, Helicosporium, Helicotubeufia, Neohelicosporium, Parahelicomyces, Pleurohelicosporium, Pseudotubeufia and Tubeufia) in Tubeufiaceae were reported from China, we infer that China is a biodiversity hotspot for helicosporous fungi (Lu et al. 2018b; Hsieh et al. 2021; Ma et al. 2023a). Therefore, we anticipate to discover and classify more helicosporous taxa from different habitats. A plausible explanation for the prevalent number

No.	Species	Distribution	Habitat	References
1	N. aquaticus	China	Freshwater	Luo et al. (2017)
2	N. dehongensis	China	Freshwater	Dong et al. (2020)
3	N. denticulatus	China	Freshwater	Yang et al. (2023)
4	N. deschampsiae	Germany	Terrestrial	Crous et al. (2019a)
5	N. guizhouensis	China	Freshwater	In this study
6	N. grandisporus	China	Freshwater	Luo et al. (2017)
7	N. hainanensis	China	Terrestrial	Lu et al. (2022)
8	N. helicosporus	China	Terrestrial	In this study
9	N. hyalosporus	China	Freshwater	Lu et al. (2018b)
10	N. hydei	China	Freshwater	In this study
11	N. longisetosus	China	Freshwater	Hsieh et al. (2021)
12	N. melaleucae	USA	Terrestrial	Crous et al. (2019b)
13	N. pallidus	China, Czech Republic, Italy, Japan, Netherlands, USA	Terrestrial	Linder (1929); Goos (1989); Zhao et al. (2007); Lu et al. (2018b)
14	N. pandanicola	China	Terrestrial	Tibpromma et al. (2018)
15	N. submersus	China	Freshwater	Luo et al. (2017)
16	N. thailandicus	Thailand	Freshwater	Dong et al. (2020)
Note: T	he newly-isolated sp	ecies in this study are typed in bold.	·	·

Table 2. Checklist of accepted Neohelicomyces species with molecular data.

of *Neohelicomyces* species in China might be attributed to limited sampling in other areas or they probably occur in understudied hosts and substrates.

The conidial morphology of most Neohelicomyces species closely resembles those of Helicomyces and the typical helicoid Tubeufia genera (Zhao et al. 2007; Luo et al. 2017; Lu et al. 2018b; Ma et al. 2023b). However, most Neohelicomyces species can easily be distinguished by their longer, hyphae-like and conspicuous conidiophores, when compared to those of Helicomyces and Tubeufia (Morgan 1892; Linder 1929; Rao and Rao 1964; Goos 1985; Zhao et al. 2007; Hyde et al. 2016; Lu et al. 2017b, 2018b, 2023b; Kuo and Goh 2018; Tian et al. 2022; Ma et al. 2023b). Only two species, Neohelicomyces longisetosus and N. thailandicus, exhibit morphological variations in conidiophores when compared to other Neohelicomyces species. However, molecular data confirm their taxonomic placement in Neohelicomyces (Dong et al. 2020; Hsieh et al. 2021). For example, Neohelicomyces longisetosus resembles Helicosporium flavum in having shorter, unbranched and less septate conidiophores and terminal, ampulliform conidiogenous cells. Nonetheless, they are delineated, based on their distinct conidial morphology and DNA molecular data (Brahmanage et al. 2017; Hsieh et al. 2021).

Herein, based on multigene phylogenetic analyses, we reclassify Neohelicomyces pallidus (UAMH 10535) under N. denticulatus. Nevertheless, we were unable to compare its morphology as this taxon lacks morphological data (Kodsueb et al. 2006; Tsui and Berbee 2006; Tsui et al. 2006; Lu et al. 2018b). In our phylogenetic analyses, Neohelicomyces pallidus (UAMH 10535) clusters with Neohelicomyces denticulatus (GZCC 19-0444) with 93% ML and 1.00 PP support. In comparison of their sequence data, there were only four nucleotide differences across ITS and one nucleotide difference across LSU (Lu et al. 2018b; Yang et al. 2023). Additionally, our phylogenetic analyses showed that Tubeufia amazonensis (ATCC 42524) shares a sister relationship to Neohelicomyces species, which suggests that this taxon neither belongs to genus Neohelicomyces nor to genus Tubeufia. However, due to the lack of morphological data about Tubeufia amazonensis (ATCC 42524), we were unable to compare its features with other Neohelicomyces and Tubeufia species. Therefore, Tubeufia amazonensis (ATCC 42524) is re-categorised here as a member of Tubeufiaceae (ATCC 42524). Further studies focusing on the re-collections, isolations and morphological examinations of these strains are a prerequisite to having a more stable and resolved taxonomy.

Acknowledgements

We would like to thank Shaun Pennycook (Manaaki Whenua Landcare Research, New Zealand) for his valuable suggestions on the fungal nomenclature.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was funded by the National Natural Science Foundation of China (NSFC 32360011). The authors appreciate the support given by the Thesis Writing Grant of Mae Fah Luang University, Thailand, to Jian Ma. The work was also funded by the High-level Talents in Zhongkai University of Agriculture and Engineering, grant no: J2201080102 and the Innovative team programme of the Department of Education of Guangdong Province (2022KCXTD015 and 2022ZDJS020) and the Chinese Research Fund (project no E1644111K1) entitled "Flexible introduction of high-level expert programme, Kunming Institute of Botany, Chinese Academy of Sciences and Major science and technology projects and key R&D plans/programmes, Yunnan Province (202202AE090001). K.D. Hyde gratefully acknowledges the financial support of the Distinguished Scientist Fellowship Program of King Saud University, Riyadh, Saudi Arabia.

Author contributions

Morphological data, photo-plates and phylogenetic analyzes were completed by Jian Ma. The original draft was written by Jian Ma, and Deecksha Gomdola, Saranyaphat Boonmee, Hong-Wei Shen, Xia Tang, Yong-Zhong Lu and Kevin D. Hyde revised the paper.

Author ORCIDs

Jian Ma [©] https://orcid.org/0009-0008-1291-640X Deecksha Gomdola [©] https://orcid.org/0000-0002-0817-1555 Saranyaphat Boonmee [©] https://orcid.org/0000-0001-5202-2955 Hong-Wei Shen [©] https://orcid.org/0000-0003-2508-1970 Xia Tang [©] https://orcid.org/0000-0003-2705-604X Li-Juan Zhang [©] https://orcid.org/0000-0002-3234-6757 Yong-Zhong Lu [®] https://orcid.org/0000-0002-1033-5782 Kevin D. Hyde [©] https://orcid.org/0000-0002-2191-0762

Data availability

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

References

- Abdel-Wahab MA, Pang KL, Nagahama T, Abdel-Aziz FA, Jones EG (2010) Phylogenetic evaluation of anamorphic species of *Cirrenalia* and *Cumulospora* with the description of eight new genera and four new species. Mycological Progress 9(4): 537–558. https://doi.org/10.1007/s11557-010-0661-x
- Boonmee S, Zhang Y, Chomnunti P, Chukeatirote E, Tsui CKM, Bahkali AH, Hyde KD (2011) Revision of lignicolous Tubeufiaceae based on morphological reexamination and phylogenetic analysis. Fungal Diversity 51(1): 63–102. https://doi.org/10.1007/s13225-011-0147-4
- Boonmee S, Rossman AY, Liu JK, Li WJ, Dai DQ, Bhat JD, Jones EBG, McKenzie EHC, Xu JC, Hyde KD (2014) Tubeufiales, ord. nov., integrating sexual and asexual generic names. Fungal Diversity 68(1): 239–298. https://doi.org/10.1007/s13225-014-0304-7
- Brahmanage R, Lu Y, Bhat DJ, Wanasinghe D, Yan J, Hyde KD, Boonmee S (2017) Phylogenetic investigations on freshwater fungi in Tubeufiaceae (Tubeufiales) reveals the new genus *Dictyospora* and new species *Chlamydotubeufia aquatica* and *Helicosporium flavum*. Mycosphere: Journal of Fungal Biology 8(7): 917–933. https://doi. org/10.5943/mycosphere/8/7/8

- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics (Oxford, England) 25(15): 1972–1973. https://doi.org/10.1093/bioinformatics/btp348
- Chethana KT, Manawasinghe IS, Hurdeal V, Bhunjun CS, Appadoo M, Gentekaki E, Raspé O, Promputtha I, Hyde KD (2021) What are fungal species and how to delineate them? Fungal Diversity 109(1): 1–25. https://doi.org/10.1007/s13225-021-00483-9
- Chomnunti P, Hongsanan S, Aguirre-Hudson B, Tian Q, Peršoh D, Dhami MK, Alias AS, Xu J, Liu X, Stadler M, Hyde KD (2014) The sooty moulds. Fungal Diversity 66(1): 1–36. https://doi.org/10.1007/s13225-014-0278-5
- Crous P, Schumacher RK, Akulov A, Thangavel R, Hernández-Restrepo M, Carnegie A, Cheewangkoon R, Wingfield MJ, Summerell BA, Quaedvlieg W, Coutinho TA, Roux J, Wood AR, Giraldo A, Groenewald JZ (2019a) New and interesting fungi. 2. Fungal Systematics and Evolution 3(1): 57–134. https://doi.org/10.3114/fuse.2019.03.06
- Crous PW, Wingfield M, Lombard L, Roets F, Swart W, Alvarado P, Carnegie A, Moreno G, Luangsaard J, Thangavel R, Alexandrova AV, Baseia IG, Bellanger J-M, Bessette AE, Bessette AR, Delapeña-Lastra S, García D, Gené J, Pham THG, Heykoop M, Malysheva E, Malysheva V, Martín MP, Morozova OV, Noisripoom W, Overton BE, Rea AE, Sewall BJ, Smith ME, Smyth CW, Tasanathai K, Visagie CM, Adamík S, Alves A, Andrade JP, Aninat MJ, Araújo RVB, Bordallo JJ, Boufleur T, Baroncelli R, Barreto RW, Bolin J, Cabero J, Cabo M, Cafà G, Caffot MLH, Cai L, Carlavilla JR, Chávez R, Decastro RRL, Delgat L, Deschuyteneer D, Dios MM, Domínguez LS, Evans HC, Eyssartier G, Ferreira BW, Figueiredo CN, Liu F, Fournier J, Galli-Terasawa LV, Gil-Durán C, Glienke C, Gonçalves MFM, Gryta H, Guarro J, Himaman W, Hywel-Jones N, Iturrieta-González I, Ivanushkina NE, Jargeat P, Khalid AN, Khan J, Kiran M, Kiss L, Kochkina GA, Kolaík M, Kubátová A, Lodge DJ, Loizides M, Luque D, Manjón JL, Marbach PAS, Massolajr NS, Mata M, Miller AN, Mongkolsamrit S, Moreau P-A, Morte A, Mujic A, Navarro-Ródenas A, Németh MZ, Nóbrega TF, Nováková A, Olariaga I, Ozerskaya SM, Palma MA, Petters-Vandresen DAL, Piontelli E, Popov ES, Rodríguez A, Requejo Ó, Rodrigues ACM, Rong IH, Roux J, Seifert KA, Silva BDB, Sklená F, Smith JA, Sousa JO, Souza HG, Desouza JT, Vec K, Tanchaud P, Tanney JB, Terasawa F, Thanakitpipattana D, Torres-Garcia D, Vaca I, Vaghefi N, Vaniperen AL, Vasilenko OV, Verbeken A, Yilmaz N, Zamora JC, Zapata M, Jurjević Ž, Groenewald JZ (2019b) Fungal Planet description sheets: 951-1041. Persoonia. Persoonia 43(1): 223-425. https://doi.org/10.3767/ persoonia.2019.43.06
- Daniel GP, Daniel GB, Miguel RJ, Florentino FR, David P (2010) ALTER: Program-oriented conversion of DNA and protein alignments. Nucleic Acids Research 38: W14–W18. https://doi.org/10.1093/nar/gkq321
- Dong W, Wang B, Hyde KD, McKenzie EHC, Raja HA, Tanaka K, Abdel-Wahab MA, Abdel-Aziz FA, Doilom M, Phookamsak R, Hongsanan S, Wanasinghe DN, Yu XD, Wang GN, Yang H, Yang J, Thambugala KM, Tian Q, Luo ZL, Yang JB, Miller AN, Fournier J, Boonmee S, Hu DM, Nalumpang S, Zhang H (2020) Freshwater Dothideomycetes. Fungal Diversity 105(1): 319–575. https://doi.org/10.1007/s13225-020-00463-5
- Goos R (1985) A review of the anamorph genus *Helicomyces*. Mycologia 77(4): 606–618. https://doi.org/10.1080/00275514.1985.12025146
- Goos R (1986) A review of the anamorph genus *Helicoma*. Mycologia 78(5): 744–761. https://doi.org/10.1080/00275514.1986.12025318
- Goos R (1989) On the anamorph genera *Helicosporium* and *Drepanospora*. Mycologia 81(3): 356–374. https://doi.org/10.1080/00275514.1989.12025759

- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.
- Hsieh SY, Goh TK, Kuo CH (2021) New species and records of *Helicosporium* sensu lato from Taiwan, with a reflection on current generic circumscription. Mycological Progress 20(2): 169–190. https://doi.org/10.1007/s11557-020-01663-8
- Hyde KD, Hongsanan S, Jeewon R, Bhat DJ, McKenzie EHC, Jones EBG, Phookamsak R, Ariyawansa HA, Boonmee S, Zhao Q, Abdel-Aziz FA, Abdel-Wahab MA, Banmai S, Chomnunti P, Cui BK, Daranagama DA, Das K, Dayarathne MC, de Silva NI, Dissanayake AJ, Doilom M, Ekanayaka AH, Gibertoni TB, Góes-Neto A, Huang SK, Jayasiri SC, Jayawardena RS, Konta S, Lee HB, Li WJ, Lin CG, Liu JK, Lu YZ, Luo ZL, Manawasinghe IS, Manimohan P, Mapook A, Niskanen T, Norphanphoun C, Papizadeh M, Perera RH, Phukhamsakda C, Richter C, et al. (2016) Fungal diversity notes 367–490: Taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80: 1–270. https://doi.org/10.1007/s13225-016-0373-x
- Jayasiri SC, Hyde KD, Ariyawansa HA, Bhat DJ, Buyck B, Cai L, Dai YC, Abd-Elsalam KA, Ertz D, Hidayat I, Jeewon R, Jones EBG, Bahkali AH, Karunarathna SC, Liu J-K, Luangsa-ard JJ, Lumbsch HT, Maharachchikumbura SSN, McKenzie EHC, Moncalvo J-M, Ghobad-Nejhad M, Nilsson H, Pang K-L, Pereira OL, Phillips AJL, Raspé O, Rollins AW, Romero AI, Etayo J, Selçuk F, Stephenson SL, Suetrong S, Taylor JE, Tsui CKM, Vizzini A, Abdel-Wahab MA, Wen T-C, Boonmee S, Dai DQ, Daranagama DA, Dissanayake AJ, Ekanayaka AH, Fryar SC, Hongsanan S, Jayawardena RS, Li W-J, Perera RH, Phookamsak R, de Silva NI, Thambugala KM, Tian Q, Wijayawardene NN, Zhao R-L, Zhao Q, Kang J-C, Promputtha I (2015) The Faces of Fungi database: Fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74(1): 3–18. https://doi.org/10.1007/s13225-015-0351-8
- Katoh K, Rozewicki J, Yamada KD (2019) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. Briefings in Bioinformatics 20(4): 1160–1166. https://doi.org/10.1093/bib/bbx108
- Kodsueb R, Jeewon R, Vijaykrishna D, McKenzie EH, Lumyong P, Lumyong S, Hyde KD (2006) Systematic revision of Tubeufiaceae based on morphological and molecular data. Fungal Diversity 21: 105–130.
- Kuo CH, Goh TK (2018) Two new species of helicosporous hyphomycetes from Taiwan. Mycological Progress 17(5): 557–569. https://doi.org/10.1007/s11557-018-1384-7
- Li LL, Shen HW, Bao DF, Wanasinghe DN, Lu YZ, Feng Y, Luo ZL (2022a) The plethora of Tubeufiaceae in lakes of the northwestern Yunnan plateau, China. Frontiers in Microbiology 13: 1056669. https://doi.org/10.3389/fmicb.2022.1056669
- Li LL, Shen HW, Bao DF, Lu YZ, Su HY, Luo ZL (2022b) New species, *Parahelicomyces yun-nanensis* sp. nov. and *Tubeufia nigroseptum* sp. nov. from freshwater habitats in Yunnan, China. Phytotaxa 530(1): 21–37. https://doi.org/10.11646/phytotaxa.530.1.2
- Linder DH (1929) A monograph of the helicosporous fungi imperfecti. Annals of the Missouri Botanical Garden 16(3): 227–388. https://doi.org/10.2307/2394038
- Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16(12): 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092
- Liu JK, Lu YZ, Cheewangkoon R, To-Anun C (2018) Phylogeny and morphology of *Heli-cotubeufia* gen. nov., with three new species in Tubeufiaceae from aquatic habitats. Mycosphere: Journal of Fungal Biology 9(3): 495–509. https://doi.org/10.5943/my-cosphere/9/3/4

- Lu YZ, Kang JC (2020) Research progress on helicosporous hyphomycetes. Journal of Fungal Research 18: 304–314. https://doi.org/10.13341/j.jfr.2020.8012
- Lu YZ, Boonmee S, Bhat DJ, Hyde KD, Kang JC (2017a) *Helicosporium luteosporum* sp. nov. and *Acanthohelicospora aurea* (Tubeufiaceae, Tubeufiales) from terrestrial habitats. Phytotaxa 319(3): 241–253. https://doi.org/10.11646/phytotaxa.319.3.3
- Lu YZ, Boonmee S, Dai DQ, Liu JK, Hyde KD, Bhat DJ, Ariyawansa H, Kang JC (2017b) Four new species of *Tubeufia* (Tubeufiaceae, Tubeufiales) from Thailand. Mycological Progress 16(4): 403–417. https://doi.org/10.1007/s11557-017-1280-6
- Lu YZ, Boonmee S, Liu JK, Hyde KD, Bhat DJ, Eungwanichayapant PD, Kang JC (2017c) Novel *Neoacanthostigma* species from aquatic habitats. Cryptogamie. Mycologie 38(2): 169–190. https://doi.org/10.7872/crym/v38.iss2.2017.169
- Lu YZ, Boonmee S, Liu JK, Hyde KD, McKenzie EHC, Eungwanichayapant PD, Kang JC (2018a) Multi-gene phylogenetic analyses reveals *Neohelicosporium* gen. nov. and five new species of helicosporous hyphomycetes from aquatic habitats. Mycological Progress 17(5): 631–646. https://doi.org/10.1007/s11557-017-1366-1
- Lu YZ, Liu JK, Hyde KD, Jeewon R, Kang JC, Fan C, Boonmee S, Bhat DJ, Luo ZL, Lin CG, Eungwanichayapant PD (2018b) A taxonomic reassessment of Tubeufiales based on multi-locus phylogeny and morphology. Fungal Diversity 92(1): 131–344. https://doi. org/10.1007/s13225-018-0411-y
- Lu YZ, Ma J, Xiao XJ, Zhang LJ, Xiao YP, Kang JC (2022) Four new species and three new records of helicosporous hyphomycetes from China and their multi-gene phylogenies. Frontiers in Microbiology 13: 1053849. https://doi.org/10.3389/fmicb.2022.1053849
- Lu YZ, Ma J, Xiao XJ, Zhang LJ, Ma XY, Xiao YP, Kang JC (2023a) Two novel species and one new record of *Helicoma* from tropical China. Mycosystema 42(1): 263–277. https://doi.org/10.13346/j.mycosystema.220445
- Lu YZ, Ma J, Xiao XJ, Zhang LJ, Kang JC (2023b) Morphology and phylogeny of *Tubeufia liyui* sp. nov. Journal of Fungal Research 21: 14–23. https://doi.org/10.13341/j.jfr.2023.1582
- Luo ZL, Bhat DJ, Jeewon R, Boonmee S, Bao DF, Zhao YC, Chai HM, Su HY, Su XJ, Hyde KD (2017) Molecular phylogeny and morphological characterization of asexual fungi (Tubeufiaceae) from freshwater habitats in Yunnan, China. Cryptogamie. Mycologie 38(1): 27–53. https://doi.org/10.7872/crym/v38.iss1.2017.27
- Ma J, Xiao XJ, Liu NG, Boonmee S, Xiao YP, Lu YZ (2023a) Morphological and multigene phylogenetic analyses reveal *Pseudotubeufia* gen. nov. and two new species in Tubeufiaceae from China. Journal of Fungi (Basel, Switzerland) 9(7): 742. https://doi. org/10.3390/jof9070742
- Ma J, Zhang LJ, Boonmee S, Xiao XJ, Liu NG, Xiao YP, Luo ZL, Lu YZ (2023b) Morphological and phylogenetic analyses reveal three new species and one new record of *Tubeufia* (Tubeufiales, Tubeufiaceae) from southern China. MycoKeys 99: 87–108. https://doi.org/10.3897/mycokeys.99.107606
- Morgan AP (1892) North American Helicosporae. Journal of the Cincinnati Society of Natural History 15: 39–52.
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300
- Nylander JAA, Zoology S, Posada D, Mrmodeltest R, Os F (2008) MrModeltest2 v. 2.3 (Program for Selecting DNA Substitution Models Using PAUP*); Evolutionary Biology Centre. Uppsala, Sweden.
- Rao PR, Rao D (1964) Some helicosporae from Hyderabad-II. Mycopathologia 24(1): 27–34. https://doi.org/10.1007/BF02049433

- Rehner SA, Buckley E (2005) A beauveria phylogeny inferred from nuclear ITS and EF1-α sequences: Evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97(1): 84–98. https://doi.org/10.3852/mycologia.97.1.84
- Ruibal C, Gueidan C, Selbmann L, Gorbushina AA, Crous PW, Groenewald J, Muggia L, Grube M, Isola D, Schoch CL, Staley JT, Lutzoni F, de Hoog GS (2009) Phylogeny of rock-inhabiting fungi related to Dothideomycetes. Studies in Mycology 64: 123–133. https://doi.org/10.3114/sim.2009.64.06
- Senanayake IC, Rathnayaka AR, Marasinghe DS, Calabon MS, Gentekaki E, Lee HB, Hurdeal VG, Pem D, Dissanayake LS, Wijesinghe SN, Bundhun D, Nguyen TT, Goonasekara ID, Abeywickrama PD, Bhunjun CS, Jayawardena RS, Wanasinghe DN, Jeewon R, Bhat DJ, Xiang MM (2020) Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. Mycosphere: Journal of Fungal Biology 11(1): 2678–2754. https://doi.org/10.5943/mycosphere/11/1/20
- Swindell SR, Plasterer TN (1997) Seqman. Sequence data analysis guidebook. Springer, 75–89. https://doi.org/10.1385/0-89603-358-9:75
- Tian XG, Karunarathna SC, Xu RJ, Lu YZ, Suwannarach N, Mapook A, Bao DF, Xu JC, Tibpromma S (2022) Three new species, two new records and four new collections of Tubeufiaceae from Thailand and China. Journal of Fungi (Basel, Switzerland) 8(2): 206. https://doi.org/10.3390/jof8020206
- Tibpromma S, Hyde KD, McKenzie EHC, Bhat DJ, Phillips AJL, Wanasinghe DN, Samarakoon MC, Jayawardena RS, Dissanayake AJ, Tennakoon DS, Doilom M, Phookamsak R, Tang AMC, Xu JC, Mortimer PE, Promputtha I, Maharachchikumbura SSN, Khan S, Karunarathna SC (2018) Fungal diversity notes 840–928: Micro-fungi associated with Pandanaceae. Fungal Diversity 93(1): 1–160. https://doi.org/10.1007/s13225-018-0408-6
- Tsui CKM, Berbee ML (2006) Phylogenetic relationships and convergence of helicosporous fungi inferred from ribosomal DNA sequences. Molecular Phylogenetics and Evolution 39(3): 587–597. https://doi.org/10.1016/j.ympev.2006.01.025
- Tsui CK, Sivichai S, Berbee ML (2006) Molecular systematics of *Helicoma*, *Helicomyces* and *Helicosporium* and their teleomorphs inferred from rDNA sequences. Mycologia 98(1): 94–104. https://doi.org/10.1080/15572536.2006.11832715
- Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27(2): 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Xiao XJ, Ma J, Zhang LJ, Liu NG, Xiao YP, Tian XG, Luo ZL, Lu YZ (2023) Additions to the Genus *Helicosporium* (Tubeufiaceae, Tubeufiales) from China with an Identification Key to *Helicosporium* Taxa. Journal of Fungi (Basel, Switzerland) 2023(9): 775. https://doi.org/10.3390/jof9070775
- Yang J, Liu LL, Jones EBG, Hyde KD, Liu ZY, Bao DF, Liu NG, Li WL, Shen HW, Yu XD, Liu J-K (2023) Freshwater fungi from karst landscapes in China and Thailand. Fungal Diversity 119(1): 1–212. https://doi.org/10.1007/s13225-023-00514-7
- Zeng XY, Tan TJ, Tian FH, Wang Y, Wen TC (2023) OFPT: A one-stop software for fungal phylogeny. Mycosphere: Journal of Fungal Biology 14(1): 1730–1741. https://doi. org/10.5943/mycosphere/14/1/20

- Zhang LJ, Ma J, Ma XY, Feng X, Bai XS, Huang YT, Jayawardena RS, Mapook A, Kang JC, Lu YZ (2023) A new record of *Neohelicosporium guangxiense* and its secondary metabolites. Warasan Khana Witthayasat Maha Witthayalai Chiang Mai 50(2): 1–12. https://doi.org/10.12982/CMJS.2023.010
- Zhao GZ, Liu XZ, Wu WP (2007) Helicosporous hyphomycetes from China. Fungal Diversity 26: 313–524.
- Zheng W, Han L, He ZJ, Kang JC (2023) A new alkaloid derivative from the saprophytic fungus *Neohelicomyces hyalosporus* PF11-1. Natural Product Research: 1–5. https://doi.org/10.1080/14786419.2023.2167202



Research Article

Two novel species and a new host record of *Alternaria* (Pleosporales, Pleosporaceae) from sunflower (Compositae) in Myanmar

Zin Mar Nwe^{1,2®}, Khin Nayyi Htut^{3®}, Sein Lai Lai Aung^{1,2®}, Ya-Nan Gou^{1®}, Cheng-Xin Huang^{1®}, Jian-Xin Deng^{1,2®}

- 1 Department of Plant Protection, College of Agriculture, Yangtze University, Jingzhou 434025, China
- 2 MARA Key Laboratory of Sustainable Crop Production in the Middle Reaches of the Yangtze River (Co-Construction by Ministry and Province), Yangtze University, Jingzhou 434025, China

3 Department of Plant Pathology, Yezin Agricultural University, Nay Pyi Taw, Myanmar

Corresponding author: Jian-Xin Deng (djxin555@yangtzeu.edu.cn)



This article is part of: Diversity, taxonomy, and systematics of macrofungi from tropical Asia Edited by Olivier Raspé, Rui-Lin Zhao, Jennifer Luangsa-ard

Academic editor: Rui-Lin Zhao Received: 23 March 2024 Accepted: 13 May 2024 Published: 7 June 2024

Citation: Nwe ZM, Htut KN, Aung SLL, Gou Y-N, Huang C-X, Deng J-X (2024) Two novel species and a new host record of *Alternaria* (Pleosporales, Pleosporaceae) from sunflower (Compositae) in Myanmar. MycoKeys 105: 337–354. https://doi. org/10.3897/mycokeys.105.123790

Copyright: © Zin Mar Nwe 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).

Abstract

Sunflower (*Helianthus annuus* L.) is a widely cultivated, fast-growing crop known for its seeds and oil, with substantial ecological and economic importance globally. However, it faces challenges from leaf diseases caused by *Alternaria* species, which threaten its yield. Three small-spored *Alternaria* species were isolated from leaf spot and blight symptoms on sunflower in Myanmar. All the species were determined based on morphological characterization and a multi-locus phylogenetic assessment of seven genes, including the internal transcribed spacer of rDNA region (ITS), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), RNA polymerase second largest subunit (*RPB2*), translation elongation factor 1- α (*TEF1*), *Alternaria* major allergen gene (*Alt a 1*), endopolygalacturonase gene (*EndoPG*), and an anonymous gene region (OPA10-2). The results introduced two new *Alternaria* species, *A. myanmarensis* **sp. nov.** and *A. yamethinensis* **sp. nov.**, and a known species of *A. burnsii*, firstly reported from sunflower.

Key words: Alternaria, morphology, new host record, novel species, phylogeny

Introduction

The genus *Alternaria* Nees, 1816, which belongs to the family Pleosporaceae, order Pleosporales, and phylum Ascomycota, is a widely distributed dematiaceous fungus frequently found in plants, soil, food, and indoor air environments (Thomma 2003). It includes more than 790 species epithets, and approximately 382 species have been accepted (Hongsanan et al. 2020; Wijayawardene et al. 2020; Gannibal et al. 2022; Li et al. 2023; Liao et al. 2023). The identification and classification of *Alternaria* commonly rely on cultural features, conidial characteristics (shape, size, septation, beak formation), sporulation patterns, and hosts (Zhang 2003; Simmons 2007; Yu 2015). Normally, *Alternaria* is categorized into two obviously distinct groups: large-spored and small-spored *Alternaria* (Simmons 2007). The conidial bodies of large-spored species typically measure 60–100 μ m in length and the small-spored species are less than 60 μ m. The morphological criteria can be influenced by growth conditions, including substrate, light, and humidity, potentially undermining their reliability in characterizing the genus (Woudenberg et al. 2013).

Nowadays, diverse molecular techniques have been utilized to clarify the variability among and within *Alternaria* species (Lawrence et al. 2016). The classification has been significantly informed through phylogenetic analysis by utilizing more than ten distinct genetic loci. These loci include the regions of rDNA (nuclear small subunit (*SSU*), large subunit (*LSU*), and internal transcribed spacer (ITS)), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), RNA polymerase second largest subunit (*RPB2*), translation elongation factor 1- α (*TEF1*), *Alternaria* major allergen (*Alt a 1*), endopolygalacturonase (*EndoPG*), an anonymous genomic region (OPA10-2), calmodulin (*CAL*), and eukaryotic orthologous groups (*KOG*) (Liu et al. 2024). The genus is found to encompass 29 sections through comprehensive multi-locus phylogenetic analyses (Ghafri et al. 2019; Gannibal et al. 2022). Among them, the section *Alternaria*, which includes members with catenate and small-spored conidia, is recognized as having only 11 phylogenetic species and one species complex (Woudenberg et al. 2015).

Leaf spot and blight disease on sunflower (*Helianthus annuus* L.) caused by *Alternaria* significantly decreases head diameters and seed production (Kgatle et al. 2020). Sunflower, belonging to the Compositae family and native to North America, is an oilseed crop cultivated worldwide, with its oil ranking as the second most important source of edible vegetable oils (Zhang et al. 2021). The plant is also commercialized for livestock feed (Yegorov et al. 2019). It was introduced to Myanmar in 1968 (Favre and Myint 2009) and covered 0.224 million hectares with a yield of 9245 kg/ha in 2022 (http://faostat.fao.org/site/567/default.aspx-#ancor). In the Central Dry Zone of Myanmar (Mandalay, Sagaing, and Magway Regions), it contributes to more than 77% of the overall oilseed crop production (DOA 2020). During the monsoon season in 2023, three small-spored *Alternaria* species were isolated from leaf symptoms of sunflower collected in a plantation in Mandalay, Myanmar. In this study, those species were meticulously identified and illustrated through morphological and phylogenetic approaches.

Materials and methods

Sample collection and fungal isolation

In August 2023, sunflower leaves displaying spot and blight symptoms were randomly collected from plantations in Myanmar, Mandalay Region, Yamethin Township, Segyi Village (30°21'28.188"N, 112°08'32.136"E). From each field, samples were randomly collected at five different points, placed in separate clean zip bags and transported to the laboratory. For fungal isolation, leaf fragments from the edges of the lesions were excised, treated with a 1% sodium hypochlorite solution for three minutes, rinsed three times with distilled water, plated on moist filter papers in Petri dishes and then incubated at 25 °C in the dark for sporulation. A single spore was picked using a sterile glass needle under a stereomicroscope and inoculated onto potato dextrose agar (PDA: Difco, Montreal, Canada). Once sufficiently grown, pure cultures were isolated by a single spore and preserved in test tube slants at 4 °C in the Fungi Herbarium at Yangtze University (YZU) in Jingzhou, Hubei, China. MycoBank numbers were obtained by following the protocols outlined on (https://www.mycobank.org/).

Morphological characterization

To study the characteristics of the culture, mycelial plugs (6 mm diameter) were extracted from the periphery of 5-day-old colonies growing on PDA, transferred to fresh 90 mm PDA plates, and incubated in darkness at 25 °C for 7 days. For the examination of conidial morphology, mycelia were cultured on V8 juice agar (V8A) and potato carrot agar (PCA) under white fluorescent light at 22 °C with an 8-hour light/16-hour dark period (Simmons 2007). After a 7-day incubation period, the sporulation patterns and conidial characteristics were determined under an ECLIPSE Ni-U microscopic system (Nikon, Japan). The conidia were observed using a lactophenol-picric acid solution. Fifty randomly selected conidia were recorded.

DNA extraction, PCR amplification, and Sequencing

Genomic DNA extraction involved scraping fresh mycelia from colonies cultivated on PDA for 5 days at 25 °C, following the method outlined by Watanabe et al. (2010). Polymerase chain reaction (PCR) amplification and sequencing targeted specific genes of the internal transcribed spacer region of rDNA (ITS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), RNA polymerase second largest subunit (RPB2), translation elongation factor 1-α (TEF1), Alternaria major allergen (Alt a 1), endopolygalacturonase gene (EndoPG), and an anonymous genomic region (OPA10-2). In the PCR processes, a 25 µL reaction mixture was prepared, consisting of 21 µL of 1.1× Tag PCR Star Mix (TSINGKE), 2 µL of template DNA, and 1 µL of each primer. The amplification reaction was performed using a Bio-Rad T100 thermocycler according to the conditions listed in (Table 1). The generated products underwent electrophoresis in a 1% agarose gel and were visualized by UV transillumination. Subsequently, the amplified products were purified and sequenced in both directions, facilitated by TSINGKE Company (Beijing, China). Initially, sequences from both ends were examined and manually edited using BioEdit v. 7.0.9 (Hall 1999). Following this, the sequences were aligned and further edited with the PHYDIT v3.2 software (Chun 1995) before being submitted to GenBank (https://www.ncbi.nlm.nih.gov/) (Table 2).

Gene regions	Primers	PCR conditions	References
ITS	ITS5/ITS4	94 °C for 3 min, 34 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 2 min, 72 °C for 10 min	White et al. 1990
GAPDH	gpd1/gpd2	95 °C for 2 min, 32 cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 42 s, 72 °C for 5 min	Berbee et al. 1999
RPB2	RPB2-5F/ RPB2-7cR	94 °C for 5 min, 34 cycles of 94 °C for 45 s, 57 °C for 45 s and 72 °C for 1 min, 72 °C for 10 min	Sung et al. 2007
TEF1	EF1-728F/ EF1-986R	94 °C for 3 min, 35 cycles of 94 °C for 30 s, 55 °C for 45 s and 72 °C for 1 min, 72 °C for 10 min	Carbone and Kohn et al. 1999
Alt a 1	Alt-for/ Alt-rev	94 °C for 2 min, 33 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s, 72 °C for 10 min	Hong et al. 2005
EndoPG	PG3/ PG2b	94 °C for 3 min, 33 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 59 s, 72 °C for 5 min	Andrew et al. 2009
OPA10-2	OPA10-2L/ OPA10- 2R	94 °C for 2 min, 33 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s, 72 °C for 10 min	Andrew et al. 2009

Table 1. Primers and PCR protocols.

Table 2. The GenBank acce	ssion numbers of ⊭	A <i>lternaria</i> strains used ir								
Current of	C.	11				GenBan	k accession n	umbers		
opecies	Strain	HOST/SUDSTFATE	Country	ITS	GAPDH	TEF1	RPB2	Alt a 1	Endo-PG	OPA10-2
A. alternantherae	CBS 124392	Solanum melongena	China	KC584179	KC584096	KC584633	KC584374	KP123846	I	1
A. alternata	CBS 916.96 ^T	Arachis hypogaea	India	AF347031	AY278808	KC584634	KC584375	AY563301	JQ811978	KP124632
	CBS 102604	Minneola tangelo	Israel	KP124334	AY562410	KP125110	KP124802	AY563305	KP124035	KP124643
	CBS 102596	Citrus jambhiri	NSA	KP124328	KP124183	KP125104	KP124796	KP123877	KP124030	KP124637
	CBS 918.96	Dianthus chinensis	¥	AF347032	AY278809	KC584693	KC584435	AY563302	KP124026	KP124633
	CBS 106.34	Linum usitatissimum	Unknown	Y17071	JQ646308	KP125078	KP124771	KP123853	KP124000	KP124608
	CBS 121547	Pyrus bretschneideri	China	KP124372	KP124224	KP125150	KP124842	KP123920	KP124076	KP124685
	CBS 101.13	Peat soil	Switzerland	KP124392	KP124244	KP125170	KP124862	KP123940	KP124096	KP124705
	CBS 126.60	Mood	¥	KP124397	KP124249	KP125175	KP124867	JQ646390	KP124101	KP124710
	CBS 109730	Solanum lycopersicum	NSA	KP124399	KP124251	KP125177	KP124869	KP123946	KP124103	KP124713
	CBS 119545 ^T	Senecio skirrhodon	New Zealand	KP124409	KP124260	KP125187	KP124879	KP123956	KP124113	KP124723
A. baoshanensis	MFLUCC 21-0124 ^T	Curcubita moschata	China	MZ622003	OK236706	OK236613	OK236659	0K236760	I	I
	MFLUCC 21-0296	C. moschata	China	MZ622004	OK236707	OK236612	OK236660	0K236759	I	I
A. betae-kenyensis	CBS 118810 ^T	Beta vulgaris var. cicla	Kenya	KP124419	KP124270	KP125197	KP124888	KP123966	KP124123	KP124733
A. breviconidiophora	MFLUCC 21-0786 ^T	Digitalis sp.	Italy	MZ621997	OK236698	OK236604	OK236651	0K236751	I	I
A. burnsii	CBS 118817	Tinospora cordifolia	India	KP124424	KP124274	KP125202	KP124893	KP123971	KP124128	KP124738
	CBS 118816	Rhizophora mucronata	India	KP124423	KP124273	KP125201	KP124892	KP123970	KP124127	KP124737
	CBS 130264	Human sputum	India	KP124425	KP124275	KP125203	KP124894	KP123972	KP124129	KP124739
	CBS 879.95	Sorghum sp.	N	KP124422	KP124272	KP125200	KP124891	KP123969	KP124126	KP124736
	CBS 107.38 ^T	Cuminum cyminum	India	KP124420	JQ646305	KP125198	KP124889	KP123967	KP124124	KP124734
	CBS 108.27	Gomphrena globosa	Unknown	KC584236	KC584162	KC584727	KC584468	KP123850	KP123997	KP124605
	YZU 191042	Allium cepa	Myanmar	MN656137	MN718663	MN656147	MN656155	MN656142	I	I
	YZU 191003	A. cepa	Myanmar	MN656136	MN718662	MN656146	MN656154	MN656141	I	I
	YZU 231748	Helianthus annuus	Myanmar	OR888998	OR963608	OR979650	PP116480	OR979653	OR979659	PP034180
	YZU 231747	H. annuus	Myanmar	OR888996	OR963607	OR979649	PP116479	OR979652	OR979658	PP034179
A. falcata	MFLUCC 21-0123	Atriplex sp.	Italy	MZ621992	OK236599	OK236693	0K236649	0K236746	I	I
A. eichhorniae	CBS 489.92 ^T	Eichhornia crassipes	India	KC146356	KP124276	KP125204	KP124895	KP123973	KP124130	KP124740
A. ellipsoidialis	MFLUCC 21-0132	Eupatorium cannabinum	Italy	MZ621989	OK236596	OK236690	0K236643	0K236743	I	I
A. eupatoriicola	MFLUCC 21-0122	E. cannabinum	Italy	MZ621982	OK236683	OK236589	0K236636	0K236736	I	I
A. gaisen	CBS 632.93 ^R	Pyrus pyrifolia	Japan	KC584197	KC584116	KC584658	KC584399	KP123974	AY295033	KP124742
	CBS 118488 ^R	P. pyrifolia	Japan	KP124427	KP124278	KP125206	KP124897	KP123975	KP124132	KP124743

Crocico	Ctuoin	Laat/C. hatwate	Countra			GenBan	k accession n	umbers		
ohecies	olidiii	LIOSU/ DUDSII AIE	COUNTRY	ITS	GAPDH	TEF1	RPB2	Alt a 1	Endo-PG	0PA10-2
A. gossypina	CBS 102597	Minneola tangelo	NSA	KP124432	KP124281	KP125211	KP124902	KP123978	KP124137	KP124748
	CBS 104.32 [⊤]	Gossypium sp.	Zimbabwe	KP124430	JQ646312	KP125209	KP124900	JQ646395	KP124135	KP124746
A. iridiaustralis	CBS 118486 ^T	Iris sp.	Australia	KP124435	KP124284	KP125214	KP124905	KP123981	KP124140	KP124751
	CBS 118487	Iris sp.	Australia	KP124436	KP124285	KP125215	KP124906	KP123982	KP124141	KP124752
	YZU 161003	Iris ensata	China	MG601454	MG601454	I	MG601456	I	MG601457	I
A. jacinthicola	CBS 133751 ^T	Eichhornia crassipes	Mali	KP124438	KP124287	KP125217	KP124908	KP123984	KP124143	KP124754
	CBS 878.95	Arachis hypogaea	Mauritius	KP124437	KP124286	KP125216	KP124907	KP123983	KP124142	KP124753
A. koreana	SPL2-1 [⊤]	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 121333 ^R	Nicotiana tabacum	NSA	KP124444	KP124293	KP125223	KP124914	KP123990	KP124150	KP124761
	CBS 540.94	N. tabacum	NSA	AY278835	AY278811	KC584667	KC584409	AY563304	KP124147	KP124758
A. minimispora	MFLUCC 21-0127 ^T	Citrullus lanatus	Thailand	MZ621980	0K236587	OK236681	0K236634	0K236734	I	I
A. muriformispora	MFLUCC 21-0784 ^T	Plantago sp.	Italy	MZ621976	0K236677	OK236583	OK236630	0K236730	I	I
A. myanmarensis sp. nov.	YZU 231735	Helianthus annuus	Myanmar	OR888993	OR963611	OR979651	PP508255	OR979656	OR979662	PP034183
	YZU 231736 ^T	H. annuus	Myanmar	OR897031	OR963612	OR963615	PP508256	OR979657	OR979663	PP034184
A. orobanches	MFLUCC 21-0137 ^T	Orobanche sp.	Italy	MZ622007	0K236710	I	I	0K236763	I	I
	MFLUCC 21-0303	Orobanche sp.	Italy	MZ622008	0K236711	I	I	0K236764	I	I
A. ovoidea	MFLUCC 21-0782 ^T	Dactylis glomerata	Italy	MZ622005	OK236708	OK236614	OK236661	I	I	I
	MFLUCC 21- 0298	D. glomerata	Italy	MZ622006	0K236709	OK236615	0K236662	I	I	I
A. obpyriconidia	MFLUCC 21-0121 ^T	Vicia faba	Italy	MZ621978	OK236585	OK236680	OK236633	0K236732	I	I
A. phragmiticola	MFLUCC 21-0125 ^T	Phragmites sp.	Italy	MZ621994	OK236696	OK236602	0K236649	0K236749	I	I
A. rostroconidia	MFLUCC 21-0136 ^T	Arabis sp.	Italy	MZ621969	OK236670	OK236576	OK236623	0K236723	I	I
A. silicicola	MFLUCC 22-0072 ^T	Salix alba	Russia	MZ621999	OK236700	OK236606	OK236653	0K236753	I	I
A. tomato	CBS 114.35	Solanum lycopersicum	Unknown	KP124446	KP124295	KP125225	KP124916	KP123992	KP124152	KP124763
	CBS 103.30	S. lycopersicum	Unknown	KP124445	KP124294	KP125224	KP124915	KP123991	KP124151	KP124762
A. torilis	MFLUCC 14-0433 ^T	Torilis arvensis	Italy	MZ621988	OK236594	OK236688	0K236641	0K236741	I	I
A. yamethinensis sp. nov.	YZU 231738	Helianthus annuus	Myanmar	OR888995	OR963609	OR963613	PP179252	OR979654	OR979660	PP034181
	YZU 231739 ^T	H. annuus	Myanmar	OR889008	OR963610	OR963614	PP179253	OR979655	OR979661	PP034182
Notes: Type strains are marked 'T'. Rep	rresentative strains are ma	arked 'R'. The present strains are	e in bold.							

MycoKeys 105: 337-354 (2024), DOI: 10.3897/mycokeys.105.123790

Phylogenetic analysis

The resulting sequences were processed in the GenBank database at the National Center for Biotechnology Information (NCBI) using BLAST searches. The relevant sequences were downloaded and derived from newly reported sequences of recent publications (Woudenberg et al. 2015; Luo et al. 2018; Htun et al. 2022; Li et al. 2022, 2023; Romain et al. 2022) used in the present analysis (Table 2). The adjustments, alignments, and comparative analyses of the gene sequences were executed using ClustalX (Larkin et al. 2007) within the MEGA 11 software platform (Tamura et al. 2021) and gaps were treated as missing data. Maximum-likelihood (ML) and Bayesian inference (BI) methods were utilized to elucidate the phylogenetic relationships among Alternaria species. The ML analyses were constructed using the GTRGAMMAI model of nucleotide evolution, and 1000 bootstrap (BS) replicates were performed to assess branch support with RAxML v. 7.0.3 (Stamatakis et al. 2008). Bayesian analysis was conducted with MrBayes v.3.2.6 (Ronquist et al. 2012) with the best-fit model of nucleotide substitution, GTR+I+G, determined by MrModeltest v.2.3 (Posada and Crandall 1998) with the Akaike Information Criterion (AIC). The "MrModelblock" file in MrModeltest was run using both the PAUP path (Swofford 2002) and the MrMt path (Nylander 2004). The two simultaneous Markov Chain Monte Carlo (MCMC) algorithms were launched from random trees, covering 10⁶ generations, with data collected every 100 generations (Rannala and Yang 1996). The analysis was stopped when the standard deviation of split frequencies dropped below 0.01. A burn-in parameter of 25% was established, signifying that 75% of the trees were retained during the burn-in phase, with the remaining trees utilized for calculating the posterior probabilities in the majority-rule consensus tree. Subsequently, the phylogenetic tree was visualized and modified using Fig-Tree v. 1.4.3 (Rambaut 2016). In the phylogram, branch support is indicated by (posterior probability PP/bootstrap value BS) equal to or above 0.6/60%.

Results

Phylogenetic analyses

The combined dataset, comprising sequences from seven gene loci (ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a* 1, *EndoPG*, and OPA10-2), included 59 *Alternaria* strains, containing the present 6 strains. It had 2,722 characters with gaps, allocated as follows: 466 characters for ITS, 302 for *GAPDH*, 307 for *RPB2*, 216 for *TEF1*, 421 for *Alt a* 1, 391 for *EndoPG*, and 619 for OPA10-2. The phylogenetic tree was constructed and rooted using *Alternaria alternantherae* CBS 124392 as the outgroup. The Maximum Likelihood (ML) phylogeny was used as the foundational tree. Four strains fell into two independent clades and two, YZU 231747 and YZU 231748, were clustered with the strains of known species *A. burnsii* (Fig. 1). One of the individual clades comprising YZU 231738 and YZU 231739, with PP/BS values of 1.0/100% was found to be sister to *A. betae-kenyensis*, *A. eichhorniae*, *A. iridiaus-tralis*, and *A. salicicola*. It also fell into a subclade with *A. eichhorniae* and *A. betae-kenyensis* (PP/BS=1.0/85%). Another clade, consisting of YZU 231735 and YZU 231736, exhibited PP/BS values of 0.98/96%, falling into a group with *A. orobanches*, *A. koreana*, and *A. ovoidea*, which is highly supported by PP/BS values



Figure 1. Phylogenetic tree generated from maximum likelihood analyses using aligned ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2 gene sequences of the present *Alternaria* strains and their related species. Bootstrap support (BS) values \geq 60% and Bayesian posterior probability (PP) scores \geq 0.60 were shown at the nodes (ML/PP). *Alternaria alternantherae* CBS 124392 was used as an outgroup. Type strains are marked 'T'. Representative strains are marked 'R'. The strains from the present study are highlighted in bold.

of 1.0/94%. Additionally, the strains YZU 231747 and YZU 231748 were clustered with the previously reported *A. burnsii* strains. They also formed a subclade with a strain from Myanmar, YZU 191003, supported by PP/BS values of 0.98/65% (Fig. 1). The results indicated that the current strains represented two new species and a known species of *Alternaria*, all belonging to the section *Alternaria*.

Taxonomy

Alternaria myanmarensis M.N. Zin & J.X. Deng, sp. nov. MycoBank No: 853961 Fig. 2

Etymology. The specific epithet refers to the location, Myanmar.

Holotype. MYANMAR, Mandalay Region, Yamethin Township, Segyi Village (30°21'28.188"N, 112°08'32.136"E), collected from infected leaves of

Helianthus annuus in August 2023 by Khin Nayyi Htut (YZU-H-2023154, holotype). Ex-type culture (YZU 231736) was also obtained.

Description. Colonies on PDA are circular, light vinaceous buff with a white halo at the edge, velvety, cottony, honey to white in reverse, 68–70 mm in diameter (Fig. 2a). On PCA, conidiophores arise directly from lateral or apical aerial hyphae or medium, lightly flexuous, sometimes geniculate at the apex, 27.5–85(–90) × 2–4.5 μ m, conidia emerge from the apex or geniculate loci, short to long ellipsoid or narrow-ovoid,10–30(–42) × 7–11 μ m, 2–5 transverse septa, 2–6 units per chain, beak 3–12 μ m (Fig. 2c, e, g). On V8A, conidiophores arise from near the apex of the terminal hyphae, 24–65(–70) × 3–5 μ m, conidia 8–29(–33) × 3–14 μ m, 2–5 transverse septa, 3–6 units per chain, beak 1–9 μ m (Fig. 2b, d, f).

Additional isolate examined. MYANMAR, Mandalay Region, Yamethin Township, Segyi Village (30°21'28.188"N, 112°08'32.136"E) from the infected leaves of *Helianthus annuus*, August 2023, Khin Nayyi Htut, living cultures (YZU 231735).

Notes. This species is phylogenetically grouped with *A. koreana*, *A. orobanches*, and *A. ovoidea*, based on sequences from ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2 genes. It is distinct from *A. koreana* and *A. ovoidea* in its smaller conidial body size, particularly in width, and its sporulation patterns which produce catenulate conidia up to 6 units on PCA and V8A media, rather than those of the two closely related species (up to 2 units) on SNA and PDA (Table 3).



Figure 2. Morphology of *Alternaria myanmarensis* sp. nov. from *Helianthus annuus*: Colony on PDA for 7 days at 25 °C (**a**); Sporulation patterns on V8A (**b**) and on PCA (**c**); Conidiophores on V8A (**d**) and on PCA (**e**); Conidia on V8A (**f**) and on PCA (**g**) at 22 °C. Scale bars: 50 μ m (**b**, **c**); 25 μ m (**d**–**g**).

Species		Conidia	Medium	References			
	Shape	Body (µm)	Beak (µm)	Septa	per chain		
A. burnsii	Ovoid or ellipsoid	30-50 × 9-13	-	5-8	Short chain	Host	Simmons (2007)
	Narrow-ovoid or narrow- ellipsoid	30-40 × 8-14	Beakless	3-7	_	PCA , V8A	Simmons (2007)
	Narrow ovoid or ellipsoid	20-50 × 8-15	3-30	4-7	5-9	PCA	Htun et al. (2020)
	Ovoid or ellipsoid, tapering	16-42(-50) × 5-15	2-30(-40)	2-6	2-6	PCA	This study
	beak	9-55(-65) × 7-12	2-23(-35)	2-6	2-9	V8A	This study
A. tomato	Ellipsoid to long-ovoid	39-65 × 13-22	60-105×2	6-9	Solitary	Host	Simmons (2007)
A. myanmarensis	Short to long ellipsoid or	10-30(-42) × 7-11	3-12	2-5	2-6	PCA	This study
sp. nov.	narrow-ovoid	8-29(-33) × 3-14	1-9	2-5	3-6	V8A	This study
A. koreana	Short to long ovoid	12.9-61.2 × 8.6-20.7	-	2-7	1-2	SNA	Romain et al. (2022)
A. ovoidea	Ovoid	48-65 × 15.5-30	-	1-3	Solitary	PDA	Li et al. (2022)
A. orobanches	Obclavate to ovoid	20-50 × 10-20	-	3-6	1-2	PCA	Li et al. (2023)
A. yamethinensis	Narrow ovoid or Subellipsoid,	17-50(-65) × 8-14	5-15×2-6	2-7	2-6	PCA	This study
sp. nov.	blunt-pointed	32-57(-63) × 8-15	1.5-8×1-4	2-7	2-9	V8A	This study
A. betae- kenyensis	Ovoid or subellipsoid	20-28 × 8-10	-	5-7	15-25	PCA	Simmons (2007)
A. eichhorniae	Narrow ovoid or subellipsoid, with a blunt-pointed or rounded apical cell	50-70 × 12-18	50-150 × 4-5	7-9	1-2	V8A	Simmons (2007)
A. iridiaustralis	Ovoid and short broad ellipsoid	30-40 × 16-24	-	3-4	3-5	PCA	Simmons (2007)
	Ellipsoid or long ellipsoid	20-50 × 15-24	15-100(- 133) × 3.5-4.5	1-4	1-2	PCA	Luo et al. (2018)
A. salicicola	Straight or curved, subglobose to obclavate or obpyriform	10-50 × 12-38	_	1-6	At least 2	PCA	Li et al. (2023)

Table 3. Mo	rphological	comparison of	fthe	present	Alternaria	and their	relevant	species

Alternaria yamethinensis M.N. Zin & J.X. Deng, sp. nov.

MycoBank No: 851391 Fig. 3

Etymology. The epithet designation is attributed to the Yamethin township, which was the location where the holotype was originally collected.

Holotype. MYANMAR, Mandalay Region, Yamethin Township, Segyi Village (30°21'28.188"N, 112°08'32.136"E) on infected leaves of *Helianthus annuus*, August 2023, Khin Nayyi Htut, (YZU-H-2023154, holotype), ex-type culture (YZU 231739).

Description. Colonies on PDA are light yellow in the center, white at the edge, with flocculent hyphae, and sulfur yellow to pure yellow in reverse, 38–50 mm in diameter (Fig. 3a). On PCA, conidiophores arise from the substrate, are simple, straight or flexuous, septate, light to brown, 19-85 (-95) × 3-6.5 µm. Conidia arise from the apex or near the apex of the conidiophores, rarely from lateral hyphae, and are narrow ovoid or subellipsoid, blunt-pointed, 17-50 (-65) × 8-14 µm, with 2-7 transverse septa and 2-6 units per chain with a beak 5-15 µm (Fig. 3c, e, g). On V8A, conidiophores are 17-65 (-85.5) × 2-5.5 µm, and conidia are 32-57 (-63) × 8-15 µm with 2-7 transverse septa, 2-9 units per chain and a beak 1.5-8 µm (Fig. 3b, d, f).



Figure 3. Morphology of Alternaria yamethinensis sp. nov. from Helianthus annuus: Colony on PDA for 7 days at 25 °C (**a**); Sporulation patterns on V8A (**b**) and on PCA (**c**); Conidiophores on V8A (**d**) and on PCA (**e**); Conidia on V8A (**f**) and on PCA (**g**) at 22 °C. Scale bars: 50 μ m (**b**, **c**); 25 μ m (**d**–**g**).

Additional isolate examined. MYANMAR, Mandalay Region, Yamethin Township, Segyi Village (30°21'28.188"N, 112°08'32.136"E) on infected leaves of *Helianthus annuus*, August 2023, Khin Nayyi Htut, living culture (YZU 231738).

Notes. Phylogenetic analysis based on combined gene regions of ITS, *GAP-DH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2, along with morphological characteristics, clearly separates this species from others. It can be differentiated from *A. betae-kenyensis* ($20-28 \times 8-10 \mu m$) by conidial size, *A. eichhorniae* ($50-150 \times 4-5 \mu m$) and *A. iridiaustralis* ($15-100(-133) \times 3.5-4.5 \mu m$) by conidial beak, and *A. salicicola* ($12-38 \mu m$) by conidial body width. Moreover, it is significantly distinct from those four species by conidial units per chain (Table 3).

Alternaria burnsii Uppal, Patel & Kamat, Indian J.Agric.Sci.8:61 (1938) MycoBank No: 259164 Fig. 4

Description. Colonies on PDA are dark, surface buff to honey, cottony to vinaceous buff, with a united margin, measuring 62–64 mm in diameter (Fig. 4a). On PCA, conidiophores are single, arising laterally from hyphae, and are either straight or curved, $15-110(-115) \times (3-5.5) \mu m$. Conidia emerge from the apex and are ovoid or ellipsoid with a tapering beak, $16-42(-50) \times 5-15 \mu m$, with 2–6



Figure 4. Morphology of Alternaria burnsii from Helianthus annuus: Colony on PDA for 7 days at 25 °C (**a**); Sporulation patterns on V8A (**b**) and on PCA (**c**); Conidiophores on V8A (**d**) and on PCA (**e**); Conidia on V8A (**f**) and on PCA (**g**) at 22 °C. Scale bars: 50 μ m (**b**, **c**); 25 μ m (**d**-**g**).

transverse septa, 2–6 in a chain, and beaks are $2-30(-40) \mu m$ (Fig. 4c, e, g). On V8A, conidiophores $12-95(-103) \times (2-4) \mu m$, conidia $9-55(-65) \times 7-12 \mu m$, and 2–6 transverse septa, 2–9 in a chain, beaks $2-23(-35) \mu m$ (Fig. 4b, d, f).

Additional isolate examined. In MYANMAR, Mandalay Region, Yamethin Township, Segyi Village (30°21'28.188"N, 112°08'32.136"E), samples showing disease symptoms on *Helianthus annuus* were collected in August 2023 by Khin Nayyi Htut. The living culture is designated as YZU 231747.

Notes. *A. burnsii* has been found in many countries on different hosts and substrates. The host range of *A. burnsii* is reported to include Apiaceae: *Cuminum cyminum* (Uppal et al. 1938), *Bunium persicum* (Mondal et al. 2002), *Apium graveolens* (Zhang 2003; Zhuang 2005), Cumin (Shekhawat et al. 2013); Cucurbitaceae: *Cucurbita maxima* (Paul et al. 2015), *Triticum aestivum* and *Phoenix dactylifera* (Al-Nadabi et al. 2018), Coconut (Sunpapao et al. 2022), *Phoenix dactylifera* (Al-Nadabi et al. 2020); Liliaceae: *Allium cepa* (Htun et al. 2022), and Orchidaceae: *Bletilla striata* (Yin et al. 2023). In the present study, *A. burnsii* was firstly reported from *Helianthus annuus* in Myanmar. Phylogenetically, the present strains fall into a sub-branch with *A. burnsii* YZU 191003 from *Allium cepa* reported in Myanmar with consistent morphology and nucleotide sequences of ITS, *GAPDH*, *RPB2*, *TEF1*, and *Alt a 1*, gene regions (Htun et al. 2022) (Fig. 1).

Discussion

In this study, two new small-spored species, Alternaria myanmarensis sp. nov. and A. yamethinensis sp. nov., and a known species of A. burnsii were identified and illustrated based on morphology and phylogenetic analyses. Molecular research has demonstrated significant separation between large- and smallspored Alternaria species (Peever et al. 2004; Hong et al. 2005). The taxonomy of small-spored Alternaria species has faced controversies because they exhibit similar morphological characteristics (Wang et al. 2021). Molecular-based assays could facilitate the correct identification alongside morphological traits (Woudenberg et al. 2015; Lawrence et al. 2016). However, molecular analysis has encountered difficulties because the section Alternaria could not be clearly determined using standard markers due to minimal or no variation (Andrew et al. 2009; Prencipe et al. 2023). Previous studies indicated that the identifying criteria of small-spored Alternaria become significant only when utilizing a combination of different genes (Woudenberg et al. 2015; Zhang et al. 2023). Agreeing with Romain et al. (2022), the present species are also clearly distinguished based on a multigene sequence analysis, indicating which species belong to the section Alternaria. To date, this section includes more than 91 species, according to recent publications (Gannibal and Lawrence (2016); Nishikawa and Nakashima (2020); Gou et al. 2022, 2023; Li et al. 2023; Liao et al. 2023; He et al. 2024).

Phylogenetically, A. myanmarensis sp. nov. and A. yamethinensis sp. nov. fall into individual lineages representing new taxa. A. myanmarensis sp. nov. is characterized by small conidial body $(10-30(-42) \times 7-11 \mu m)$ catenating in a longer chain (2 to 6 units), compared with its relevant species (solitary or 2 conidia in a chain), A. koreana from Atractylodes ovata in Korea (Romain et al. 2022), A. orobanches from Orobanche sp. in Italy (Li et al. 2023) and A. ovoidea from Dactylis glomerata in Italy (Li et al. 2022). Morphologically, A. yamethinensis sp. nov. (conidial width 8–14 µm and 2–6 conidial units per chain) is quite different from its closely related species of A. *iridiaustralis* (conidial width 15–24 µm) from Iris spp. (Luo et al. 2018), A. salicicola (conidial width 12–38 µm) from Salix alba in Russia (Li et al. 2023), A. betae-kenyensis (15 to 25 conidial units per chain) from Beta vulgaris in Kenya (Simmons 2007) and A. eichhorniae (solitary or two conidia in a chain) from Eichhornia crassipes in India (Simmons 2007). Additionally, either RPB2 or OPA10-2 region serves as great marker for the delimitation of the above species.

The genus Alternaria ranks 10th among fungal genera for infecting over 4,000 plant species (Thomma 2003). The first record of Alternaria helianthi (named Helminthosporium helianthi) on sunflower in Uganda was done by Hansford (1943). Later, 12 more species were found in various sunflower-growing countries globally, including A. helianthinficiens (Simmons 1986), A. leucanthemi (Carson 1987), A. longissima (Prathuangwong et al. 1991), A. carthami (Chowdhury 1994), A. zinniae (Bhutta et al. 1997), A. alternata (Lagopodi and Thanassoulopoulos 1998), A. protenta (Cho and Yu 2000), A. heliophytonis (Simmons 2007), A. roseogrisea (Roberts 2008), A. helianthicola (Rajender et al. 2016), A. tenuissima (Wang et al. 2019), and A. solani and A. tomatophila (Zhang et al. 2021). However, it has been established that Alternaria helianthi, which is the synonym of Alternariaster helianthi, was based on morphology and phylogeny

(Simmons 2007; Wei et al. 2022). In this study, three *Alternaria* species associated with sunflower in Myanmar have been identified, and pathogenicity tests reveal that these present *Alternaria* species are causal pathogens for sunflower, of which *A. yamethinensis* sp. nov. is identified as the most pathogenic one (Suppl. material 1). This discovery underscores the importance of Alternaria leaf spot and blight on sunflower and helps in disease management in Myanmar.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The study was funded by the National Natural Science Foundation of China (32270022).

Author contributions

The conception and design of the study were a joint effort by all authors involved. Sample collection was done by Khin Nayyi Htut. Jian-Xin Deng provided crucial scientific oversight throughout both the laboratory and fieldwork. Zin Mar Nwe initiated the fungal isolation and led the research, with support in data analysis from Sein Lai Lai Aung, Ya-Nan Gou, and Cheng-Xin Huang. Zin Mar Nwe drafted the manuscript, which was refined through critical feedback from all authors. Jian-Xin Deng played a pivotal role in supervising the finalization of the manuscript, with all authors giving their approval to the completed work.

Author ORCIDs

Zin Mar Nwe © https://orcid.org/0009-0000-6376-8306 Khin Nayyi Htut © https://orcid.org/0009-0009-9498-8040 Sein Lai Lai Aung © https://orcid.org/0009-0006-2738-5598 Ya-Nan Gou © https://orcid.org/0009-0005-1740-4065 Cheng-Xin Huang © https://orcid.org/0000-0001-7770-5242 Jian-Xin Deng © https://orcid.org/0000-0001-7304-5603

Data availability

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

References

- Al-Nadabi HH, Maharachchikumbura SSN, Agrama H, Al-Azri M, Nasehi A, Al-Sadi AM (2018) Molecular characterization and pathogenicity of *Alternaria* species on wheat and date palms in Oman. European Journal of Plant Pathology 152(3): 577–588. https://doi.org/10.1007/s10658-018-1550-4
- Al-Nadabi HH, Maharachchikumbura SSN, Al-Gahaffi ZS, Al-Hasani AS, Velazhahan R, Al-Sadi AM, Hamed Al-Nadabi (2020) Molecular identification of fungal pathogens associated with leaf spot disease of date palms (*Phoenix dactylifera*). All Life 13(1): 587–597. https://doi.org/10.1080/26895293.2020.1835740

- Andrew M, Peever TL, Pryor BM (2009) An expanded multilocus phylogeny does not resolve morphological species within the small-spored *Alternaria* species complex. Mycologia 101(1): 95–109. https://doi.org/10.3852/08-135
- Berbee ML, Pirseyedi M, Hubbard S (1999) Cochliobolus phylogenetics and the origin of known, highly virulent pathogens, inferred from ITS and glyceraldehyde-3-phosphate dehydrogenase gene sequences. Mycologia 91(6): 964–977. https://doi.org/10.108 0/00275514.1999.12061106
- Bhutta AR, Bhatti MHR, Ahmad I (1997) Study on pathogenicity of seed-borne fungi of sunflower in Pakistan. Helia 20(27): 57–66.
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3): 553–556. https://doi.org/10.1080/0027 5514.1999.12061051
- Carson ML (1987) Effect of two foliar pathogens on seed yield of sunflower. Plant Disease 71(6): 549-551. https://doi.org/10.1094/PD-71-0549
- Cho HS, Yu SH (2000) Three Alternaria species pathogenic to sunflower. The Plant Pathology Journal 16(6): 331–334.
- Chowdhury S (1944) An Alternaria disease of safflower. Journal of the Indian Botanical Society 23(2): 59–65.
- Chun J (1995) Computer assisted classification and identification of Actinomycetes. Doctoral dissertation, Newcastle University.
- DOA (2020) Data Records from Department of Agriculture (DOA) Ministry of Agriculture, Livestock and Irrigation, Nay Pyi Taw, Myanmar.
- Favre R, Myint K (2009) An analysis of the Myanmar edible oil crops sub-sector. Rural Infrastructure and Agro-Industries Division, Food and Agriculture Organization of the United Nations Food and Agriculture Organization of the United Nations (FAO). FAOSTAT statistical database, Rome. http://faostat.fao.org/site/567/default.aspx-#ancor
- Gannibal PB, Lawrence DP (2016) Distribution of *Alternaria* species among sections. 3. Sections *Infectoriae* and *Pseudoalternaria*. Mycotaxon 131(4): 781–790. https://doi. org/10.5248/131.781
- Gannibal PB, Orina AS, Gasich EL (2022) A new section for *Alternaria helianthiinficiens* found on sunflower and new asteraceous hosts in Russia. Mycological Progress 21(2): 34. https://doi.org/10.1007/s11557-022-01780-6
- Ghafri AA, Maharachchikumbura SSN, Hyde KD, Al-Saady NA, Al-Sadi AM (2019) A new section and a new species of *Alternaria* encountered from Oman. Phytotaxa 405(6): 279–289. https://doi.org/10.11646/phytotaxa.405.6.1
- Gou YN, Aung SLL, Htun AA, Huang CX, Deng JX (2022) *Alternaria* species in section *Alternaria* associated with Iris plants in China. Frontiers in Microbiology 13: 1036950. https://doi.org/10.3389/fmicb.2022.1036950
- Gou YN, Aung SLL, Guo Z, Li Z, Shen S, Deng JX (2023) Four new species of small-spored *Alternaria* isolated from *Solanum tuberosum* and *S. lycopersicum* in China. Journal of Fungi 9(9): 880. https://doi.org/10.3390/jof9090880
- Hall TA (1999) Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98. https://doi.org/10.14601/Phytopathol_Mediterr-14998u1.29
- Hansford CG (1943) Contributions towards the fungus flora of Uganda, V. Fungi imperfecti. Proceedings of the Linnean Society of London 155(1): 34–67. https://doi.org/10.1111/j.1095-8312.1943.tb00340.x

- He J, Li DW, Cui WL, Huang L (2024) Seven new species of *Alternaria* (Pleosporales, Pleosporaceae) associated with Chinese fir, based on morphological and molecular evidence. MycoKeys 101: 1–44. https://doi.org/10.3897/mycokeys.101.115370
- Hong SG, Cramer RA, Lawrence CB, Pryor BM (2005) *Alt a 1* allergen homologs from *Alternaria* and related taxa: Analysis of phylogenetic content and secondary structure. Fungal Genetics and Biology 42(2): 119–129. https://doi.org/10.1016/j. fgb.2004.10.009
- Hongsanan S, Hyde KD, Phookamsak R, Wanasinghe DN, McKenzie EHC, Sarma VV, Boonmee S, Lücking R, Bhat DJ, Liu NG, Tennakoon DS (2020) Refined families of Dothideomycetes: Orders and families incertae sedis in Dothideomycetes. Fungal Diversity 105(1): 17–318. https://doi.org/10.1007/s13225-020-00462-6
- Htun AA, Liu HF, He L, Xia ZZ, Aung SLL, Deng JX (2022) New species and new record of *Alternaria* from onion leaf blight in Myanmar. Mycological Progress 21(1): 59–69. https://doi.org/10.1007/s11557-021-01765-x
- Kgatle MG, Flett B, Truter M, Aveling TA (2020) Control of Alternaria leaf blight caused by *Alternaria alternata* on sunflower using fungicides and *Bacillus amyloliquefaciens*. Crop Protection 132: 105–146. https://doi.org/10.1016/j.cropro.2020.105146
- Lagopodi AL, Thanassoulopoulos CC (1998) Effect of a leaf spot disease caused by *Alternaria alternata* on yield of sunflower in Greece. Plant Disease 82(1): 41–44. https:// doi.org/10.1094/PDIS.1998.82.1.41
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibso TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21): 2947–2948. https://doi. org/10.1093/bioinformatics/btm404
- Lawrence DP, Rotondo F, Gannibal PB (2016) Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. Mycological Progress 15(1): 1–22. https://doi.org/10.1007/ s11557-015-1144-x
- Li J, Phookamsak R, Jiang H, Bhat DJ, Camporesi E, Lumyong S, Kumla J, Hongsanan S, Mortimer PE, Xu J, Suwannarach N (2022) Additions to the Inventory of the Genus *Alternaria* section *Alternaria* (Pleosporaceae, Pleosporales) in Italy. Journal of Fungi 8(9): 898. https://doi.org/10.3390/jof8090898
- Li JF, Jiang HB, Jeewon R, Hongsanan S, Bhat DJ, Tang SM, Mortimer PE, Xu JC, Camporesi E, Bulgakov TS, Zhao GJ, Suwannarach N, Phookamsak R (2023) *Alternaria*: Update on species limits, evolution, multi-locus phylogeny, and classification. Studies in Fungi 8(1): 1–61. https://doi.org/10.48130/SIF-2023-0001
- Liao YCZ, Cao YJ, Wan Y, Li H, Li DW, Zhu LH (2023) *Alternaria arborescens* and *A. italica* causing leaf blotch on *Celtis julianae* in China. Plants 12(17): 3113. https://doi. org/10.3390/plants12173113
- Liu JW, Senanayake IC, Zhou T, Cen BY, Dong ZY, Luo M (2024) Alternaria tomentosae (Pleosporaceae, Dothideomycetes); novel endophytic species from *Citrus grandis* cv. 'Tomentosa' fruits in China. New Zealand Journal of Botany 31: 1–4. https://doi. org/10.1080/0028825X. 2023.2297830
- Luo H, Tao YQ, Fan XY, Oh SK, Lu HX, Deng JX (2018) Identification and Characterization of Alternaria iridiaustralis Causing Leaf Spot on Iris ensata in China. Mycobiology 46(2): 168–171. https://doi.org/10.1080/12298093.2018.1454007
- Mondal KK, Rana SS, Sood P, Singh Y (2002) Kalazira: A new host for *Alternaria burnsii*. Indian Phytopathology 55(4): 532–533.

- Nishikawa J, Nakashima C (2020) Japanese species of *Alternaria* and their species boundaries based on host range. Fungal Systematics and Evolution 5(1): 197–281. https://doi.org/10.3114/fuse.2020.05.13
- Nylander JAA (2004) MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.
- Paul NC, Deng JX, Lee HB, Yu SH (2015) Characterization and pathogenicity of Alternaria burnsii from seeds of Cucurbita maxima (Cucurbitaceae) in Bangladesh. Mycobiology 43(4): 384–391. https://doi.org/10.5941/MYCO. 2015.43.4.384
- Peever TL, Su G, Carpenter-Boggs L, Timmer LW (2004) Molecular systematics of citrus-associated Alternaria species. Mycologia 96(1): 119–134. https://doi.org/10.108 0/15572536.2005.11833002
- Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics 14(9): 817–818. https://doi.org/10.1093/bioinformatics/14.9.817
- Prathuangwong S, Kao SW, Sommartya T, Sinchaisri P (1991) Role of four Alternaria spp. causing leaf and stem blight of sunflower in Thailand and their chemical controls. Kasetsart Journal of National Science 25: 112–124. https://li01.tci-thaijo.org/index. php/anres/article/view/241911
- Prencipe S, Meloni GR, Nari L, Schiavon G, Spadaro D (2023) Molecular Characterization, and mycotoxigenic potential of *Alternaria* spp. Agents of Black Spots on Fruit and Leaves of *Pyrus communis* in Italy. Phytopathology 113(2): 309–320. https://doi. org/10.1094/PHYTO-03-22-0103-R
- Rajender J, Pushpavathi B, Santha MSL, Sumathi S (2016) Biochemical characterization of isolates of *Alternaria helianthi* (Hansf.) Tubaki and Nishihara causing sunflower blight. International Journal of Plant Sciences 11(2): 249–254. https://doi. org/10.15740/HAS/IJPS/11.2/249-254
- Rambaut A (2016) FigTree v1.4.3 2006–2016. Tree figure drawing Tool. Online publication. Institute of Evolutionary Biology, University of Edinburgh.
- Rannala B, Yang Z (1996) Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. Journal of Molecular Evolution 43(3): 304–311. https://doi.org/10.1007/BF02338839
- Roberts RG (2008) Alternaria roseogrisea, a new species from achenes of Helianthus annuus L. (Sunflower). Mycotaxon 103(5): 21–26. https://doi.org/10.1111/j.1439-0507.2008.01498.x
- Romain BBND, Hassan O, Kim JS, Chang T (2022) Alternaria koreana sp. nov., a new pathogen isolated from leaf spot of ovate-leaf Atractylodes in South Korea. Molecular Biology Reports 49(1): 413–420. https://doi.org/10.1007/s11033-021-06887-9
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across alarge model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029
- Shekhawat N, Trivedi A, Kumar A, Sharma SK (2013) Management of *Alternaria burnsii* causing blight of cumin. International Plant Protection 6(2): 280–284.
- Simmons EG (1986) Alternaria themes and variations (22–26). Mycotaxon 25(1): 287–308.
 Simmons EG (2007) Alternaria an Identification Manual, fully illustrated and with catalogue raisonné 1796–2007. CBS Fungal Biodiversity Centre, Utrercht, The Netherlands.
- Stamatakis A, Hoover P, Rougemont J (2008) A rapid bootstrap algorithm for the RAxML web servers. Systematic Biology 57(5): 758–771. https://doi. org/10.1080/10635150802429642

- Sung GH, Sung JM, Hywel Jones NL, Spatafora JW (2007) A multi-gene phylogeny of Clavicipitaceae (Ascomycota, Fungi): Identification of localized incongruence using a combinational bootstrap approach. Molecular Phylogenetics and Evolution 44(3): 1204–1223. https://doi.org/10.1016/j.ympev.2007.03.011
- Sunpapao A, Suwannarach N, Kumla J, Dumhai R, Riangwong K, Sanguansub S, Wanchana S, Arikit S (2022) Morphological and molecular identification of plant pathogenic fungi associated with dirty panicle disease in coconuts (*Cocos nucifera*) in Thailand. Journal of Fungi 8(4): 335. https://doi.org/10.3390/jof8040335
- Swofford DL (2002) PAUP, Phylogenetic analysis using parsimony (and other methods). Version 4.0b10. Sinauer Associates, Sunderland, MA.
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38(7): 3022–3027. https://doi. org/10.1093/molbev/msab120
- Thomma BPHJ (2003) *Alternaria* spp.: From general saprophyte to specific parasite. Molecular Plant Pathology 4(4): 225–236. https://doi.org/10.1046/j.1364-3703.2003.00173.x
- Uppal BN, Patel MK, Kamat MN (1938) *Alternaria* blight of cumin. Indian Journal of Agricultural Science 8(1): 49–62. https://eurekamag.com/research/013/304/013304137
- Wang T, Zhao J, Ma G, Bao SW, Wu XH (2019) Leaf blight of sunflower caused by *Alternaria tenuissima* and *A. alternata* in Beijing, China. Canadian Journal of Plant Pathology 41(3): 372–378. https://doi.org/10.1080/07060661.2019.1583288
- Wang F, Saito S, Michailides TJ, Xiao CL (2021) Phylogenetic, morphological, and pathogenic characterization of *Alternaria* species associated with fruit rot of Mandarin in California. Plant Disease 105(9): 2606–2617. https://doi.org/10.1094/PDIS-10-20-2145-RE
- Watanabe M, Lee K, Goto K, Kumagai S, Sugita-Konishi Y, Hara-Kudo Y (2010) Rapid and effective DNA extraction method with bead grinding for a large amount of fungal DNA. Journal of Food Protection 73(6): 1077–1084. https://doi.org/10.4315/0362-028X-73.6.1077
- Wei X, Zha QC, Yang SQ, Liu FY, Zhao L, Deng JX (2022) Taxonomic study of Alternaria helianthi. Plant Protection 48(4): 188–195. http://dx.doi.org/10.16688/j.zwbh.2021564
- White TJ, Bruns T, Lee SJ, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A guide to methods and applications 18(1): 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1
- Wijayawardene NN, Hyde KD, Al-Ani LKT, Tedersoo L, Haelewaters D, Rajeshkumar KC, Zhao R-L, Aptroot A, Leontyev DV, Saxena RK, Tokarev YS (2020) Outline of Fungi and fungus-like taxa. Mycosphere 11(1): 1060–1456. https://doi.org/10.5943/mycosphere/11/1/8
- Woudenberg JHC, Groenewald JZ, Binder M, Crous PW (2013) *Alternaria* redefined. Studies in Mycology 75(1): 171–212. https://doi.org/10.3114/sim0015
- Woudenberg JH, Seidl MF, Groenewald JZ, de Vries M, Stielow JB, Thomma BP, Crous PW (2015) *Alternaria* section *Alternaria*: Species, formae speciales or pathotypes. Studies in Mycology 82: 1–21. https://doi.org/10.1016/j.simyco.2015.07.001
- Yegorov B, Turpurova T, Sharabaeva E, Bondar Y (2019) Prospects of using by-products of sunflower oil production in compound feed industry. Journal of Food Science and Technology 13(1): 106–113. https://doi.org/10.15673/fst.v13i1.1337
- Yin FQ, Ma WL, Dan YR, Tang ZX, Song Z, Xu Q, Liu M (2023) First report of Alternaria burnsii causing leaf spot on Bletilla striata in China. Plant Disease 107(10): 3296. https://doi.org/10.1094/PDIS-04-23-0754-PDN

Yu SH (2015) Alternaria species and allied genera. Incheon. National Institute of Biological Resources Ministry of Environment 1(2): 5–156.

Zhang TY (2003) Flora Fungorum Sinicorum, Alternaria, Vol. 16. Beijing, China, 55-61.

- Zhang Y, Yu Y, Jia R, Liu L, Zhao J (2021) Occurrence of Alternaria leaf blight of sunflower caused by two closely related species *Alternaria solani* and *A. tomatophila* in Inner Mongolia. Oil Crop Science 6(2): 74–80. https://doi.org/10.1016/j.ocsci.2021.04.006
- Zhang M-J, Zheng X-R, Li H, Chen F-M (2023) *Alternaria alternata*, the Causal agent of a new needle blight disease on *Pinus bungeana*. Journal of Fungi 9(1): 71. https://doi. org/10.3390/jof9010071

Zhuang WY [Ed.] (2005) Fungi of Northwestern China. Mycotaxon Ltd., Ithaca.

Supplementary material 1

Diseased symptoms of Helianthus annuus caused by Alternaria spp.

Authors: Zin Mar Nwe, Khin Nayyi Htut, Sein Lai Aung, Ya-Nan Gou, Cheng-Xin Huang, Jian-Xin Deng

Data type: doc

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.105.123790.suppl1