MycoKeys

MycoKeys 111: 1–20 (2024)
DOI: 10.3897/mycokeys.111.137347

Research Article

Taxonomic study of *Hydnoporia* (Hymenochaetales, Hymenochaetaceae) in East Asia with two new species

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Abstract

The genus *Hydnoporia* (Hymenochaetales, Hymenochaetaceae) was first described by Murrill in 1907. However, species of *Hydnoporia* were subsequently reclassified into the genera *Hymenochaete*, *Hymenochaetopsis*, and *Pseudochaete* due to overlapping morphological characteristics, leading to multiple synonyms and confusion among researchers. Recent phylogenetic analyses based on multimarker datasets have clarified the diversity and relationships within *Hydnoporia*, but East Asian species remain underrepresented due to limited morphological data. To address this gap, we conducted a comprehensive morphological and phylogenetic analysis of East Asian *Hydnoporia* specimens using internal transcribed spacer (ITS) and translation elongation factor 1–α (*tef1*) regions. From 42 specimens, we identified six species, including two novel species, *Hydnoporia orienticorrugata* **sp. nov.** and *Hydnoporia subtabacina* **sp. nov.**, and we report *Hydnoporia rimosa* for the first time in Korea. Phylogenetic analyses also support the recombination of *Hymenochaete intricata* and *Hymenochaetopsis rigidula* as *Hydnoporia intricata* **comb. nov.** and *Hydnoporia rigidula* **comb. nov.**, respectively. By elucidating the phylogenetic relationships and morphological traits of *Hydnoporia* species from East Asia, this study contributes to a deeper understanding of the global diversity and phylogeny of the genus.

Key words: *Hymenochaete*, *Hymenochaetopsis*, novel species, phylogeny, *Pseudochaete*

OPEN ACCESS

Academic editor: R. Henrik Nilsson
Received: 18 September 2024
Accepted: 1 November 2024
Published: 25 November 2024

Citation: Cho M, Cho Y, Kwon SL, Kim D, Hosaka K, Lim YW, Kim J-J (2024) Taxonomic study of *Hydnoporia* (Hymenochaetales, Hymenochaetaceae) in East Asia with two new species. MycoKeys 111: 1–20. https://doi.org/10.3897/mycokeys.111.137347

Introduction

The genus *Hydnoporia* Murill. in the family Hymenochaetaceae (Hymenochaetales, *Basidiomycota*) was first described in 1907 and typified through *Hyd*. *olivacea* (Schwein.) Teixeira (≡*Sistotrema fuscescens*). *Hydnoporia* is characterized by brownish basidiomes of diverse shapes, ranging from effused-reflexed to pileate, cylindrical to allantoid basidiospores, and setae of various sizes (Miettinen et al. 2019). *Hydnoporia* species are found across Asia, Europe, and

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North America (He and Li 2013; Miettinen et al. 2019; Palla et al. 2023). They inhabit the branches or stumps of conifer and deciduous trees and are classified as wood-decaying corticioid fungi (Miettinen et al. 2019). Until the 1990s, many corticioid species had been delimited based on their morphological traits (Parmasto 1995; Léger 1998). Due to their overlapping morphological characteristics, *Hydnoporia* species were previously placed under the genus *Hymenochaete* Lev., which includes species characterized by brown-colored basidiomes, small cylindrical basidiospores, and the presence of setae (Parmasto et al. 2014; Miettinen et al. 2019). The lack of distinguishable characteristics between the two genera prevented a clear separation despite the efforts of some taxonomists (Parmasto 1995; Léger 1998).

Technological advancements made DNA-based analyses possible during the 1990s and onwards. This led to many taxonomic revisions, including the division of *Hymenochaete* into two separate clades: *Hymenochaete sensu stricto* and *Pseudochaete* T. Wagner & M. Fisch (Wagner and Fischer 2002). However, the genus *Pseudochaete* was present under the botanical code in the green algae *Pseudochaete* W. West & G. S. West 1903, which was considered repetitious for microorganisms despite differences in lineages. For this reason, a new name *Hymenochaetopsis* S. H. He & Jiao Yang was introduced (Yang et al. 2016). However, none of these studies considered *Hydnoporia*, which had priority. *Hymenochaetopsis* and *Pseudochaete* eventually became synonyms of *Hydnoporia* (Miettinen et al. 2019). Additionally, the clade of *Hymenochaete tabacina* (Sowerby) Lèv. and its neighboring species were encompassed within *Hydnoporia* as it formed a sister clade to the type species, *Hyd. olivacea* (Miettinen et al. 2019; Wu et al. 2022).

Currently, 14 species are accepted in *Hydnoporia*, and 20–27 more species candidates have been revealed through multimarker-based phylogenetic analysis (Miettinen et al. 2019). However, previous studies did not fully cover East Asian specimens owing to the lack of detailed morphological data, and this was problematic because East Asia is expected to be the most diverse region for *Hydnoporia* species (He and Li 2013; Miettinen et al. 2019; Palla et al. 2023). To fully understand the global diversity of *Hydnoporia*, a detailed morphological and phylogenetic analysis of East Asian specimens is essential. This study assesses *Hydnoporia* specimens from the Republic of Korea and Japan based on multigenetic marker phylogeny (ITS+*tef1*) and morphological characteristics, and it describes two new species, *Hyd. orienticorrugata* sp. nov. and *Hyd. subtabacina* sp. nov. It furthermore notes one unrecorded species from the Republic of Korea, *Hyd*. *rimosa*. Additionally, we propose synonymizing *Hymenochaete intricata* and *Hymenochaetopsis rigidula* as *Hyd*. *intricata* and *Hyd*. *rigidula*, respectively, considering the phylogenetic relationship of these species with *Hydnoporia*.

Materials and methods

Specimen collection

We studied 43 specimens labeled as *Hydnoporia*, *Hymenochaete*, *Hymenochaetopsis*, and *Pseudochaete* deposited in the Korea University Culture Collection (**KUC**), Seoul National University Fungus Collection (**SFC**), and National Institute of Biological Resources (**NIBR**) in this study. Specimens were collected nationwide from 2012 to 2021 and stored with silica gel under dry conditions to prevent contamination. A *Hyd. yasudai* specimen from **TNS** (Mycological Herbarium of the Department of Botany, National Museum of Nature and Science, Tsukuba, Japan) was also included.

Molecular identification

Genomic DNA was extracted from the dried specimens using AccuPrep® Genomic DNA Extraction Kit (Bioneer, Daejeon, Korea). Primer sets ITS1F/ITS4, ITS1F/ITS4B, ITS5/ITS4, or ITS5/LR3 were used to amplify the nuclear ribosomal internal transcribed spacer (ITS) region (White et al. 1990; Gardes and Bruns 1993). For the translation elongation factor 1–α (*tef1*) region, the primer set 983F/1567R was used (Rehner and Buckley 2005). PCR products were purified using the AccuPrep® PCR Purification Kit following the manufacturer's instructions. DNA sequencing was performed by Cosmogenetech (Seoul, Korea) using the amplified products. All sequencing results were edited using the SeqMan Lasergene package version 7.0.0 (DNAStar Inc., Madison, WI). The newly generated sequences were deposited in GenBank (Sayers et al. 2024; Table 1).

Reference ITS and *tef1* sequences for phylogenetic analysis were obtained from GenBank following Miettinen et al. (2019). All sequences were aligned by region using MAFFT v. 7.490 (Katoh and Standley 2013). Sequence alignment and concatenation were performed using Geneious Prime 2023.2.1. (https://www.geneious.com; Suppl. materials 1, 2). A phylogenetic tree was inferred on the CIPRES web portal using the concatenated ITS and *tef1* datasets with maximum likelihood (ML) and Bayesian inference (BI) methods (Miller et al. 2015). For tree inference, ITS and *tef1* sequences were partitioned into eight regions: ITS1, 5.8S, and ITS2 for the ITS region and exon 1, intron 1, exon 2, intron 2, and exon 3 for *tef1* region. jModeltest v. 2.1.10 was used to select the best-fitting substitution model for ITS and *tef1* regions (Darriba et al. 2012). The best-fitting models for ITS1, 5.8S, ITS2, exon 1, intron 1, exon 2, intron 2, and exon 3 regions were HKY+G, JC, HKY+G, K80+G, GTR+I, K80+I, HKY+I, and K80+G, respectively. The ML tree was inferred using RAxML-HPC2 in XSEDE v. 8.2.12 with 1,000 bootstrap replicates (Stamatakis 2014). BI analyses were conducted using MrBayes v. 3.2.3 on XSEDE, with the best model selected for each marker by sampling every 1,000 generations for 20 million generations (Ronquist and Huelsenbeck 2003). The phylogenetic tree was edited using FigTree v. 1.4.3 (Rambaut 2018) and Adobe Illustrator CS6 (Adobe Systems Inc., San Jose, CA, USA).

Morphological observation

Macroscopic images of each specimen were captured using a Sony α 6500 camera (Sony, Tokyo, Japan). Microscopic structures were observed using an Olympus BX51 light microscope (Olympus, Tokyo, Japan) at 40–1000 × magnification. The images were captured using a DP20 microscope (Olympus, Tokyo, Japan). At least 20 basidiospores, basidia, and setae were examined to measure the size. Specific color terms were obtained from the Munsell Soil Color Book (Color 2009). The following abbreviations were used: **L** = mean spore length; **W** = mean spore width; **Q** = L/W ratio; **x** = the number of basidiospores measured; **y** = the number of specimens; and **n** = x/y.

Table 1. List of analyzed *Hydnoporia* specimens with GenBank accession numbers of ITS and *tef1* sequences.

Species name	Specimen voucher	Country	GenBank accession no.[a]		References
			ITS	*tef1*	
Hydnoporia corrugata	Jon Klepsland 2011-7-24 (O F-247869)^{T}	Norway	MK514613	MK552138	(Miettinen et al. 2019)
	KCG001	Ireland	JQ246338	–	(Grundy et al. 2012)
	A6_wood_6	Great Britain	JN230421	–	(Grundy et al. 2012)
	B1_wood_inner	Ireland	JN230422	–	(Grundy et al. 2012)
	A3_wood_2	Great Britain	JN230419	–	(Grundy et al. 2012)
Hyd. diffissa	Otto Miettinen 19463 (H 7008917)^{T}	USA, North Carolina	MK514611	MK552136	(Miettinen et al. 2019)
	Otto Miettinen 17127.4 (H)	USA, New York	MK514598	–	(Miettinen et al. 2019)
Hyd. gigasetosa	He1461	China, Yunnan	KT828671	–	(Yang et al. 2016)
	He1442	China, Yunnan	KT828670	–	(Yang et al. 2016)
***Hyd. intricata* comb. nov.**	**KUC20121123-03**	**Korea**	**PP992254**	**PQ066850**	**This study**
	KUC20210428-20	**Korea**	**PP992255**	**PQ066851**	**This study**
	KUC20211030-01	**Korea**	**PP992256**	**PQ066852**	**This study**
	SFC20120820-11	**Korea**	**PP992257**	**–**	**This study**
	SFC20140313-01	**Korea**	**PP992258**	**PQ066853**	**This study**
	SFC20160920-36	**Korea**	**PP992259**	**PQ066854**	**This study**
	SFC20170822-68	**Korea**	**PP992260**	**PQ066855**	**This study**
	SFC20170908-28	**Korea**	**PP992261**	**PQ066856**	**This study**
	He1181	China	JQ279609	–	(He and Dai 2012)
	He412	China	JQ279608	–	(He and Dai 2012)
	He21064	China	KC505556	–	Unpublished
Hyd. lamellata	Cui7629	China	JQ279603	–	(He and Dai 2012)
	Dai10527	China	JQ279605	–	(He and Dai 2012)
Hyd. laricicola	Viacheslav Spirin 5400 (H)	Russia, Khabarovsk	MK514606	MK552132	(Miettinen et al. 2019)
	Dai13458^{T}	China, Heilongjiang	KT828672	–	(Yang et al. 2016)
	Dai11046	China, Nei Mongol	JQ279616	–	(He and Dai 2012)
	Wu 1207-122	China, Jilin	KT828673	–	(Yang et al. 2016)
Hyd. latesetosa	He492	China, Hainan	JQ716404	–	(He and Li 2013)
	He502^{T}	China, Hainan	NR_120093	–	(He and Li 2013)
Hyd. olivacea	Otto Miettinen & Kelo Käppi 16044 (H 7005770)^{T}	USA, Massachusetts	MK514612	MK552137	(Miettinen et al. 2019)
	P1201B	Peru	EU977192	–	(Smith et al. 2008)
	CMH529	USA, Missouri	KF800618	–	(Rittenour et al. 2014)
	f2Fc06	USA, Texas	GU721341	–	(Noris et al. 2011)
	319	Antarctica	KC785573	–	(Connell and Staudigel 2013)
	CFMR:DLL2011-223	USA, Wisconsin	KJ140712	–	(Brazee et al. 2014)
	CBS:126040	USA, North Carolina	MH864055	–	(Vu et al. 2019)
Hyd. rhododendri	N. Gerhold 2005-6-3	Austria	MK514593	–	(Miettinen et al. 2019)
	Viacheslav Spirin 6476 (H)	Russia, Primorsky Krai	MK514599	MK552127	(Miettinen et al. 2019)
	Viacheslav Spirin 6450 (H)	Russia, Primorsky Krai	MK514603	–	(Miettinen et al. 2019)
***Hyd. rigidula* comb. nov.**	SFC20140314-10	Korea	KX792928	–	(Kim et al. 2016)
	SFC20140411-08	Korea	KX792929	–	(Kim et al. 2016)
	SFC20140411-20	Korea	KX792930	–	(Kim et al. 2016)
	SFC20140703-24	Korea	KX792931	–	(Kim et al. 2016)
	SFC20140723-16	**Korea**	KX792932	**PQ066857**	(Kim et al. 2016), **This study**
	SFC20160713-06	**Korea**	**PP992262**	**PQ066858**	**This study**
	SFC20170324-10	**Korea**	**PP992263**	**PQ066859**	**This study**
	He379	China	JQ279613	–	(He and Dai 2012)
	He343	China	JQ279612	–	(He and Dai 2012)

Species name	Specimen voucher	Country	GenBank accession no.[a]		References
			ITS	*tef1*	
Hyd. rimosa	**KUC20121109-19**	**Korea**	**PP992264**	**–**	**This study**
	Viacheslav Spirin 5277 (H)	Russia, Khabarovsk	MK514592	MK552122	(Miettinen et al. 2019)
	Viacheslav Spirin 5678 (H)	Russia, Khabarovsk	MK514594	MK552123	(Miettinen et al. 2019)
	Viacheslav Spirin 6104 (H)	Russia, Khabarovsk	MK514595	MK552124	(Miettinen et al. 2019)
Hyd. subrigidula	He1123	China, Yunnan	JQ716402	–	(He and Li 2013)
	He1157^{T}	China, Yunnan	NR_120092	–	(He and Li 2013)
Hyd. tabacina	A. M. Ainsworth 2017-1-17 (K(M) 233332)	Great Britain	MK514614	MK890223	(Miettinen et al. 2019)
	Otto Miettinen 22126 (H)	Finland	MK782755	MK787232	(Miettinen et al. 2019)
	Viacheslav Spirin 6066a (H)	Russia, Nizhny Novgorod	MK514600	MK552128	(Miettinen et al. 2019)
Hyd. tabacinoides	CLZhao986	China, Yunnan	MG231566	–	Unpublished
	CLZhao859	China, Yunnan	MG231565	–	Unpublished
	Cui10428	China	JQ279604	–	(He and Dai 2012)
Hyd. yasudai	**KUC20100409-18**	**Korea**	**PP992265**	**PQ066860**	**This study**
	KUC20180326-05	**Korea**	**PP992266**	**PQ066861**	**This study**
	KUC20210319-14	**Korea**	**PP992267**	**PQ066862**	**This study**
	SFC20150707-58	**Korea**	**PP992268**	**PQ066863**	**This study**
	SFC20150902-19	**Korea**	**PP992269**	**–**	**This study**
	SFC20160114-04	**Korea**	**PP992270**	**PQ066864**	**This study**
	SFC20160512-38	**Korea**	**PP992271**	**–**	**This study**
	SFC20160517-06	**Korea**	**PP992272**	**PQ066865**	**This study**
	SFC20160527-41	**Korea**	**PP992273**	**PQ066866**	**This study**
	SFC20160614-52	**Korea**	**PP992274**	**–**	**This study**
	SFC20160712-18	**Korea**	**PP992275**	**PQ066867**	**This study**
	SFC20180410-24	**Korea**	**PP992276**	**PQ066868**	**This study**
	SFC20180712-04	**Korea**	**PP992277**	**PQ066869**	**This study**
	KUC11055	Korea	KJ713999	–	(Jang et al. 2015)
	KoLRI48661	Korea, Jeju	MT586954	–	Unpublished
	KoLRI_EL005212	Korea, Jeju	MN844835	–	Unpublished
	KoLRI_EL005068	Korea, Jeju	MN844834	–	Unpublished
	TNS-F78711	**Japan**	**PP992278**	**PQ066870**	**This study**
	IFO 4969	Japan	AY558598	–	(Jeong et al. 2005)
	Viacheslav Spirin 5533 (H)	Russia, Khabarovsk	MK514597	MK552126	(Miettinen et al. 2019)
	Viacheslav Spirin 6475 (H)	Russia, Primorsky Krai	MK514609	MK552135	(Miettinen et al. 2019)
	CLZhao1495	China, Yunnan	MG231611	–	Unpublished
	CLZhao1475	China, Yunnan	MG231609	–	Unpublished
	CLZhao1486	China, Yunnan	MG231610	–	Unpublished
	CLZhao1422	China, Yunnan	MG231607	–	Unpublished
	He273	China	JQ279614	–	(He and Dai 2012)
	He375	China	JQ279615	–	(He and Dai 2012)
	CLZhao867	China, Yunnan	MG231606	–	Unpublished
	CLZhao933	China, Yunnan	MH114725	–	Unpublished
	CLZhao853	China, Yunnan	MG231605	–	Unpublished
	CLZhao1549	China, Yunnan	MG231612	–	Unpublished

Species name	Specimen voucher	Country	GenBank accession no.[a]		References
			ITS	*tef1*	
***Hyd. orienticorrugata* sp. nov.**	KUC20121019-16	Korea	KJ668528	–	(Jang et al. 2016)
	KUC20121123-05	**Korea**	**PP992279**	**–**	**This study**
	KUC20131001-21	**Korea**	**PP992280**	**PQ066871**	**This study**
	SFC20140412-06	**Korea**	**PP992281**	**–**	**This study**
	SFC20150212-01	**Korea**	**PP992282**	**PQ066872**	**This study**
	SFC20150319-12	**Korea**	**PP992283**	**–**	**This study**
	SFC20150513-06	**Korea**	**PP992284**	**–**	**This study**
	SFC20151030-12[T]	**Korea**	**PP992285**	**PQ066873**	**This study**
	SFC20190619-11	**Korea**	**PP992286**	**–**	**This study**
	CLZhao938	China, Yunnan	MH114693	–	Unpublished
	He761	China	JQ279606	–	(He and Dai 2012)
	He839	China	JQ279607	–	(He and Dai 2012)
***Hyd. subtabacina* sp. nov.**	**SFC20190322-02[T]**	**Korea**	**PP992287**	**PQ066874**	**This study**
	SFC20190510-01	**Korea**	**PP992288**	**PQ066875**	**This study**
	SFC20190619-15	**Korea**	**PP992289**	**–**	**This study**
	Heikki Kotiranta 20797 (H)	Russia, Perm	MK514591	MK552121	(Miettinen et al. 2019)
	Heikki Kotiranta 25205 (H)	Russia, Kransoyarsk	MK514596	MK552125	(Miettinen et al. 2019)
	Otto Miettinen 17028.3 (H)	USA, New York	MK514601	MK552129	(Miettinen et al. 2019)
	Viacheslav Spirin 5196 (H)	Russia, Khabarovsk	MK514602	MK552130	(Miettinen et al. 2019)
	Viacheslav Spirin 6582 (H)	Russia, Khabarovsk	MK514604	–	(Miettinen et al. 2019)
	Viacheslav Spirin 6566 (H)	Russia, Khabarovsk	MK514605	MK552131	(Miettinen et al. 2019)
	Viacheslav Spirin 6520 (H)	Russia, Khabarovsk	MK514607	MK552133	(Miettinen et al. 2019)
	Viacheslav Spirin 6507 (H)	Russia, Khabarovsk	MK514608	MK552134	(Miettinen et al. 2019)
	CFMR:DLL2011-152	USA, Wisconsin	KJ140652	–	(Brazee et al. 2014)
	CFMR:DLL2011-071	USA, Wisconsin	KJ140591	–	(Brazee et al. 2014)
	CFMR:DLL2011-175	USA, Wisconsin	KJ140671	–	(Brazee et al. 2014)
	He810	China	JQ279611	–	(He and Dai 2012)
	He390	China	JQ279610	–	(He and Dai 2012)
Porodaedalea alpicola	Cui12280	China	ON358110	ON631040	(Wu et al. 2019)

[a]The sequences generated in this study are shown in bold.
[T]Indicate the type materials.

Results

Phylogenetic analyses

Based on ITS sequence analysis, the 43 assessed specimens were phylogenetically grouped within *Hydnoporia* (Fig. 1). Phylogenetic analysis of the ITS and *tef1* regions identified these specimens as six distinct Korean species (Fig. 2, Suppl. material 3). Among them, three were confirmed as previously recorded species in Republic of Korea: *Hymenochaete intricata* (Lloyd) T. Ito, *Hym. rigidula* Berk. & M.A. Curtis, and *Hydnoporia yasudai* (Imazeki) Spirin & Miettinen. One species was identified as a new record for Korea: *Hyd. rimosa* (Lloyd) Spirin & Miettinen. These four species were also well supported by morphological characteristics.

The remaining two species, previously labelled as "*Hyd. corrugata*" and "*Hyd. tabacina*", formed distinct clades from their close relatives *Hyd. corrugata*

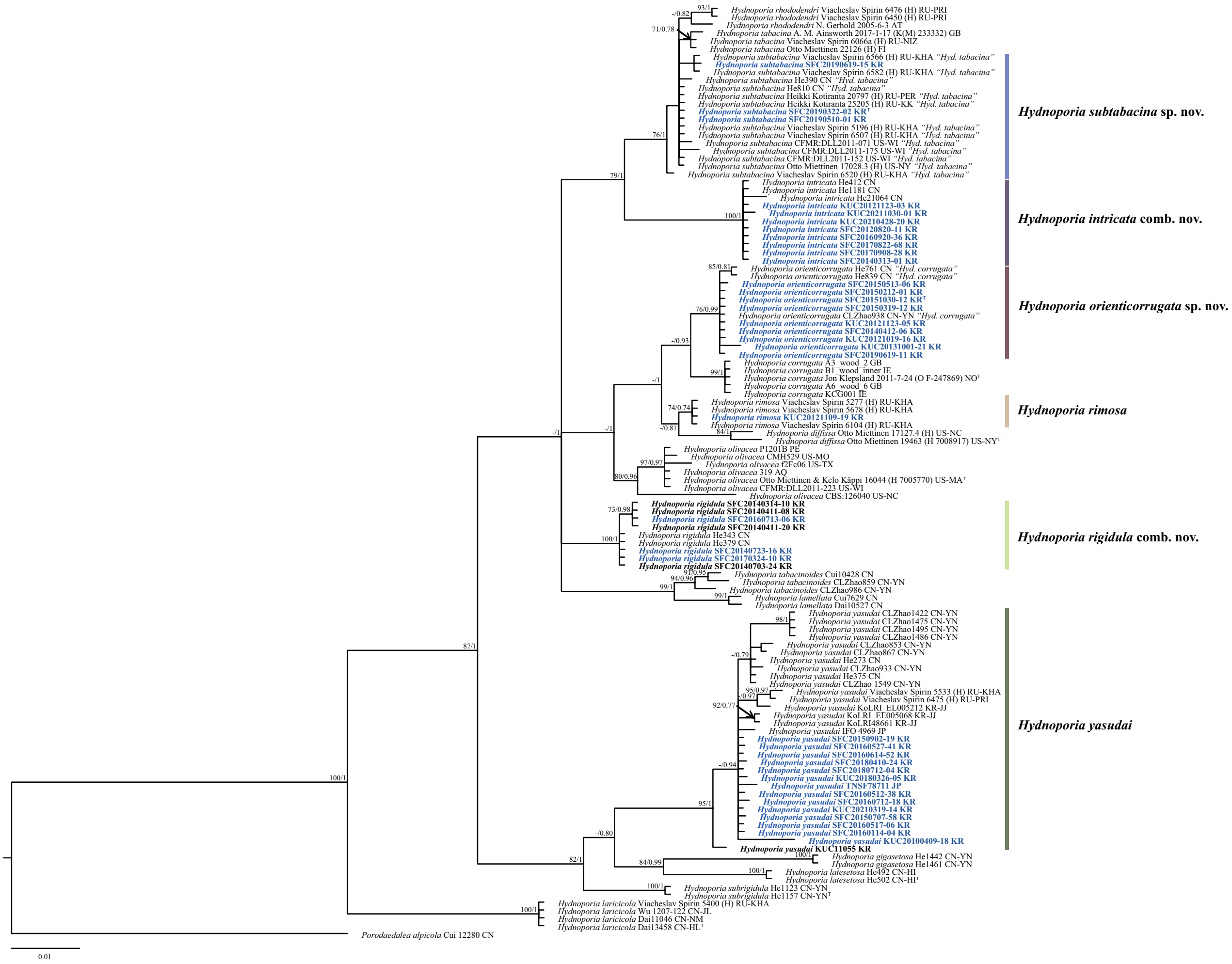

Figure 1. BI tree based on the ITS sequence datasets. The node numbers indicate the bootstrap support values (BS) above 70% and posterior probabilities (PP) over 0.7 as BS/PP. *Hydnoporia* specimens examined in this study are shown in bold. Newly generated sequences in this study are shown in blue and bold. *Porodaedalea alpicola* (Cui 12280) is used as an outgroup. Letter codes after specimen voucher indicate ISO 3166 country code followed by the origin province. Detailed information is in Table 1. Type specimens are indicated with "T".

and *Hyd. tabacina*, respectively. However, East Asian and American "*Hyd. tabacina*" were poorly differentiated from European *Hyd. tabacina* and *Hyd. rhododendri* in the ITS phylogeny. Morphological comparison and multimarker-based phylogenetic inference with other *Hydnoporia* species support the recognition of these two as new species, which we propose as *Hyd. orienticorrugata* sp. nov. and *Hyd. subtabacina* sp. nov. Morphological descriptions of the new species are provided in the Taxonomy section.

Additionally, two species previously classified as *Hymenochaete* formed strongly supported clades within *Hydnoporia*. Therefore, we propose their reclassification as *Hyd. intricata* comb. nov. and *Hyd. rigidula* comb. nov.

Taxonomy

This section includes morphological description of two new species, *Hyd. orienticorrugata* sp. nov. and *Hyd. subtabacina* sp. nov. and a previously unreported species in Korea, *Hyd. rimosa*.

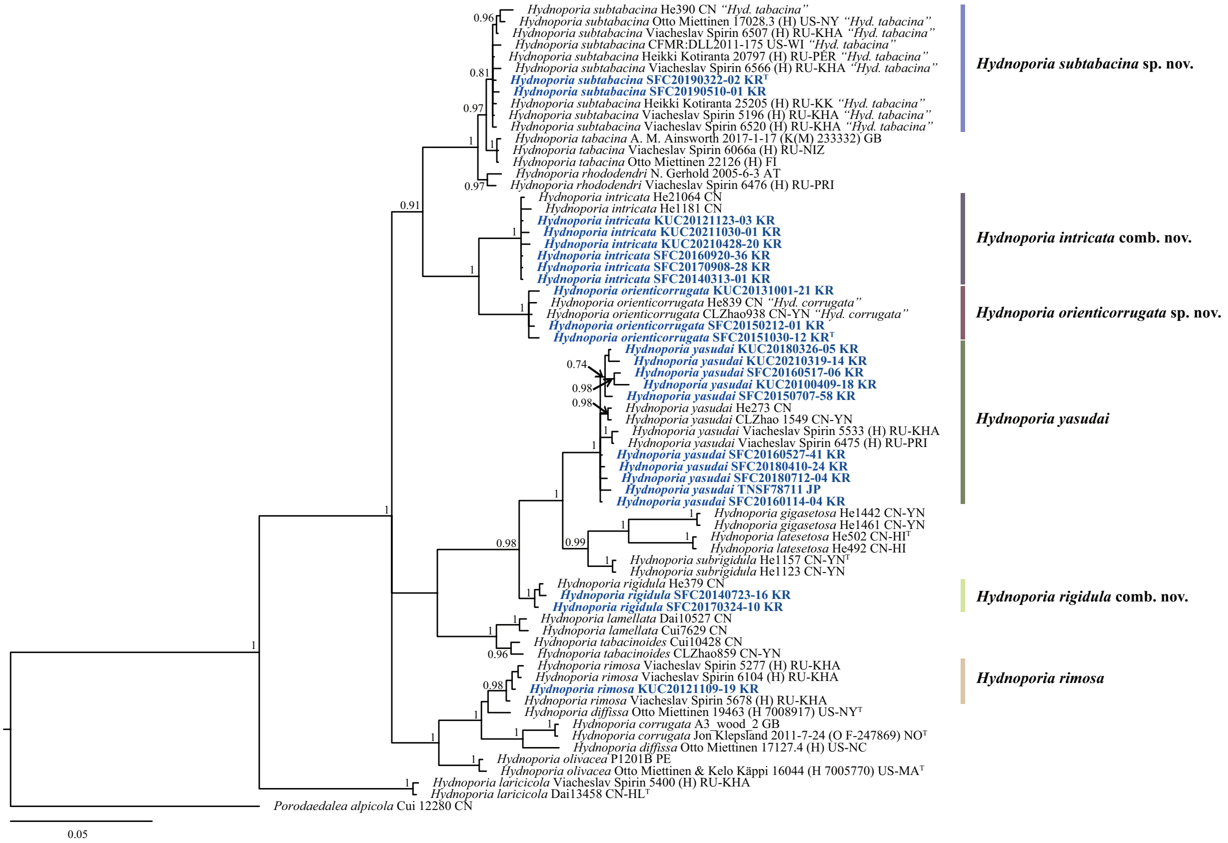

Figure 2. BI tree based on the ITS and *tef1* concatenated sequence datasets. The node numbers indicate the posterior probabilities (PP) above 0.7. *Hydnoporia* specimens examined in this study are shown in bold. Newly generated sequences in this study are shown in blue and bold. *Porodaedalea alpicola* (Cui 12280) is used as an outgroup. Letter codes after specimen voucher indicate ISO 3166 country code followed by the origin province. Detailed information is in Table 1. Type specimens are indicated with "T".

Hydnoporia orienticorrugata M.Cho, Y.Cho, Y.W.Lim & J.J.Kim, sp. nov.

MycoBank No: 854671
Fig. 3

Diagnosis. Resupinate, effused basidiome, smooth, brown to reddish brown hymenial surface, sterile margin; cylindrical basidia with 10.3–15.9 × 2.8–4.0 µm, sharp-pointed setae with widened basal part and 35.0–64.6 × 8.1–13.2 µm, narrowly cylindrical to allantoid basidiospores with 4.4–5.8 × 1.5–2.0 µm, and growing on an angiosperm branch (a few on gymnosperm branches).

Type. Korea • Gangwon-do, Pyeongchang-gun, Mt. Heungjeong, 37°65.71'N, 128°32.25'E, alt. 800 m, 30 Oct 2015, Y. W. Lim, (***holotype*:** NIBRFG0000516804; ***isotype*:** SFC20151030-12).

Description. ***Basidiome*** resupinate, effused, thin, covering up to 0.1 mm thick. ***Hymenial surface*** smooth, membranaceous, crustaceous, with many cracks, brown (7.5YR, 4/2) to reddish brown (5YR, 5/3). ***Margin*** sterile, even, concolorous with that of the center. ***Hyphal system*** dimitic; generative hyphae septate, branched, without a clamp connection, thick-walled, few thin-walled, hyaline, 2.7–3.5 µm. Skeletal hyphae aseptate, unbranched, without a clamp connection, thick-walled, reddish-yellow (5YR, 7/8) to yellow (10YR, 7/8), 3.4–4.4 µm.

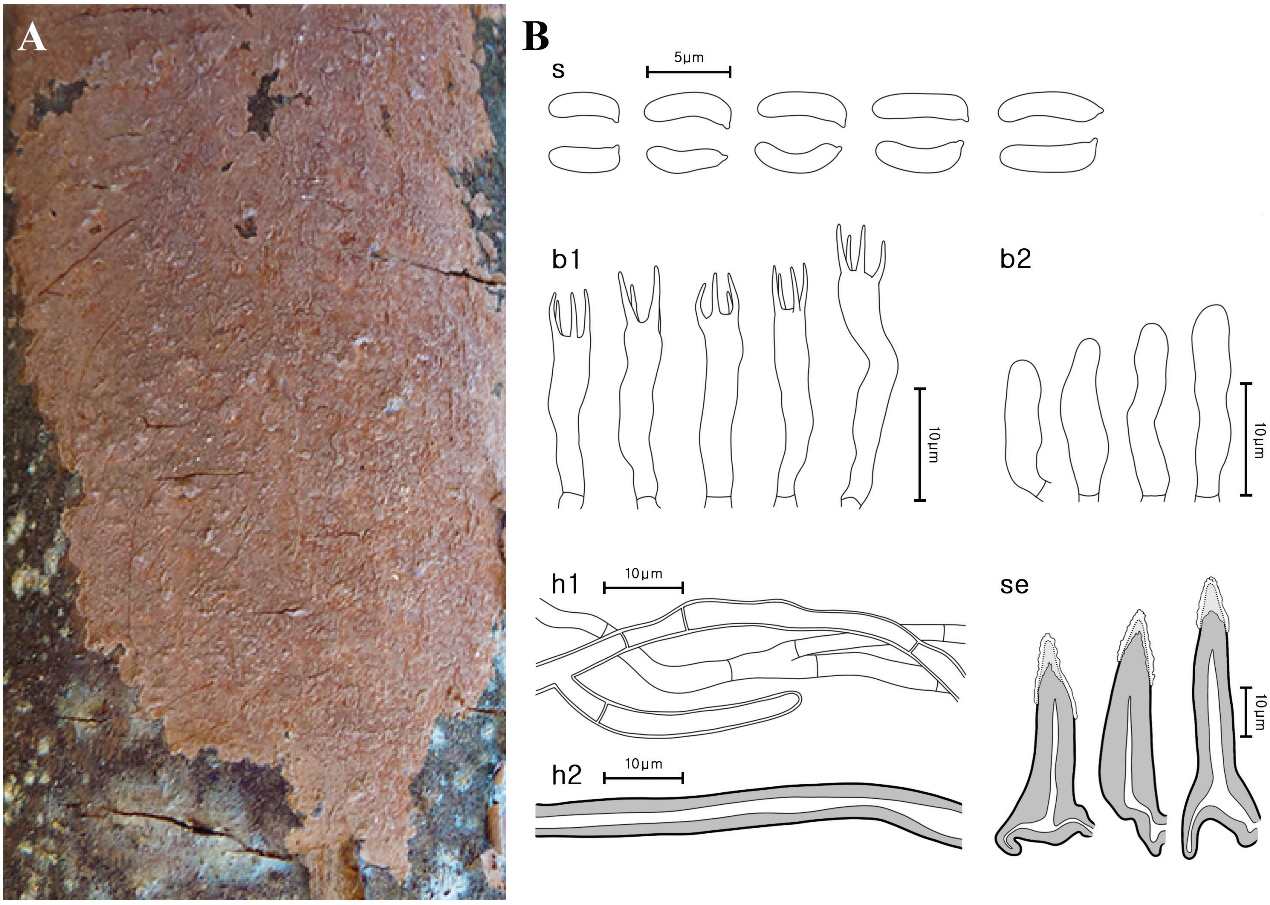

Figure 3. Morphological characteristics of *Hydnoporia orienticorrugata* (NIBRFG0000516804, holotype) **A** basidiome **B** microscopic features, **s** basidiospores; **b1** basidia; **b2** basidioles; **h1** generative hyphae; **h2** skeletal hyphae; **se** setae. Scale bars: 10 µm (**B**).

Basidia cylindrical, 4-spored, smooth, thin-walled, hyaline, 10.3–15.9(–16.6) × 2.8–4.0 µm. ***Setae*** sharp-pointed, few blunt-pointed, encrusted with crystals, distinctly widened basal part, smooth, bearing narrow lumen, thick-walled, reddish-brown (2.5YR, 4/4), 35.0–64.6 × 8.1–13.2(–14.2) µm. ***Basidiospores*** narrowly cylindrical to allantoid, smooth, slightly curved, thin-walled, hyaline, with narrow apex, a few bearing oil droplets, 4.4–5.8(–6.1) × 1.5–2.0 µm, L = 5.10 µm, W = 1.72 µm, Q = 2.53–3.44, n = 21.

Distribution. East Asia (Korea, China).

Ecology. Grew on an angiosperm branch in mixed hardwood forest, although a few grew on gymnosperm branches.

Etymology. Named after its distribution in East Asian regions and morphological similarity to *Hydnoporia corrugata*.

Additional specimens examined. Korea • Gangwon-do, Pyeongchang-gun, Odaesan National Park, 37°44.06'N, 128°35.25'E, alt. 690 m, 19 Oct 2012, Y. Jang & S. Jang, KUC20121019-16; Korea • Gangwon-do, Pyeongchang-gun, Odaesan National Park, 37°44.30'N, 128°35.03'E, alt. 660 m, 23 Nov 2012, Y. Jang & S. Jang, KUC20121123-05; Korea • Gangwon-do, Pyeongchang-gun, Odaesan National Park, 37°44.04'N, 128°35.03'E, alt. 680 m, 01 Oct 2013, Y. Jang & S. Jang, KUC20131001-21; Korea • Gangwon-do, Injae-gun, Mt. Bangtae, 37°87.53'N, 128°31.12'E, alt. 390 m, 12 Feb 2015, Y. W. Lim, SFC20150212-01.

Notes. Our specimens were phylogenetically well grouped with the Chinese specimens (He 761, He 839, and CLZhao 938), which were labeled either as *Hymenochaete corrugata*, *Hymenochaetopsis corrugata*, or *Pseudochaete corrugata* (He and Dai 2012; Yang et al. 2016) (Figs 1, 2). Other than these three specimens and KUC20121019-16 from Korea, no other records were found in East Asia, even when the other synonyms of *H. corrugata* were considered. Nevertheless, the East Asian clade formed a distinct clade from the clade with European *Hyd. corrugata*, which included the neotype specimen from Norway (O F-247869). Our findings conform to those of an earlier study, which suggested that sequences identified as *Hyd. corrugata* in Korea and China could represent a novel species (Miettinen et al. 2019). *Hydnoporia orienticorrugata* sp. nov. has micromorphological characteristics similar to those of *Hyd. corrugata*, but the latter has a grey to pale brown hymenial surface (Miettinen et al. 2019) that differs from the new species. Additionally, *Hyd. orienticorrugata* occurs on angiosperm and gymnosperm branches in Korea and China (He and Dai 2012) whereas *H. corrugata* occurs only on angiosperm branches and seems to be restricted to Europe (Austria, England, Ireland, Norway, Russia, and Sweden) (Fries 1815; Grundy et al. 2012; Miettinen et al. 2019).

Hydnoporia subtabacina **M.Cho, Y.Cho, Y.W.Lim & J.J.Kim, sp. nov.**

MycoBank No: 854672
Fig. 4

Diagnosis. Effused-reflexed, pileate basidiome, smooth, brown hymenial surface, sterile margin; cylindrical basidia with 14.6–17.9 × 2.9–3.8 µm, sharp-pointed and few elongated setae with 58.6–140.0 × 9.8–26.1 µm, narrowly cylindrical basidiospores with 4.4–5.7 × 1.6–1.9 µm, and occurs on angiosperm trees (branches and trunks).

Type. Korea • Gyeongsangbuk-do, Bonghwa-gun, Taebaeksan National Park, Baekcheon valley, 37°00.64'N, 128°98.41'E, alt. 830 m, 22 Mar 2019, Y. W. Lim & S. Yoo, (***holotype*****:** NIBRFG0000505378; ***isotype*****:** SFC20190322-02).

Description. ***Basidiome*** effused-reflexed, pileate, 0.1 mm thick. ***Hymenial surface*** smooth, membranaceous, brown (7.5YR, 5/4) to dark brown (7.5YR, 3/3). ***Margin*** sterile, slightly lighter. ***Hyphal system*** dimitic; generative hyphae septate, frequently branched, without a clamp connection, thick-walled, hyaline, 2.3–3.8 µm. Skeletal hyphae aseptate, unbranched, without a clamp connection, thick-walled, reddish-yellow (5YR, 7/8) to yellow (10YR, 7/8), 3.0–5.0 µm.

Basidia cylindrical, 4-spored, smooth, thin-walled, hyaline, (14.0–)14.6–17.9(–18.7) × 2.9–3.8 µm. ***Setae*** sharp-pointed, encrusted with crystals, cylindrical, fusiform, few elongated apex, smooth, bearing a narrow or wide lumen, thick-walled, dark reddish brown (5YR, 3/4) to dusky red (2.5YR, 3/2), 58.6–140.0 × 9.8–26.1 µm. ***Basidiospores*** narrowly cylindrical, smooth, slightly curved, thin-walled, hyaline, with narrow apex, 4.4–5.7 × 1.6–1.9 µm, L = 5.22 µm, W = 1.68 µm, Q = 2.78–3.61, n = 21.

Distribution. Korea, China, Far East Russia, and US.

Ecology. Grew on *Quercus* in angiosperm forest.

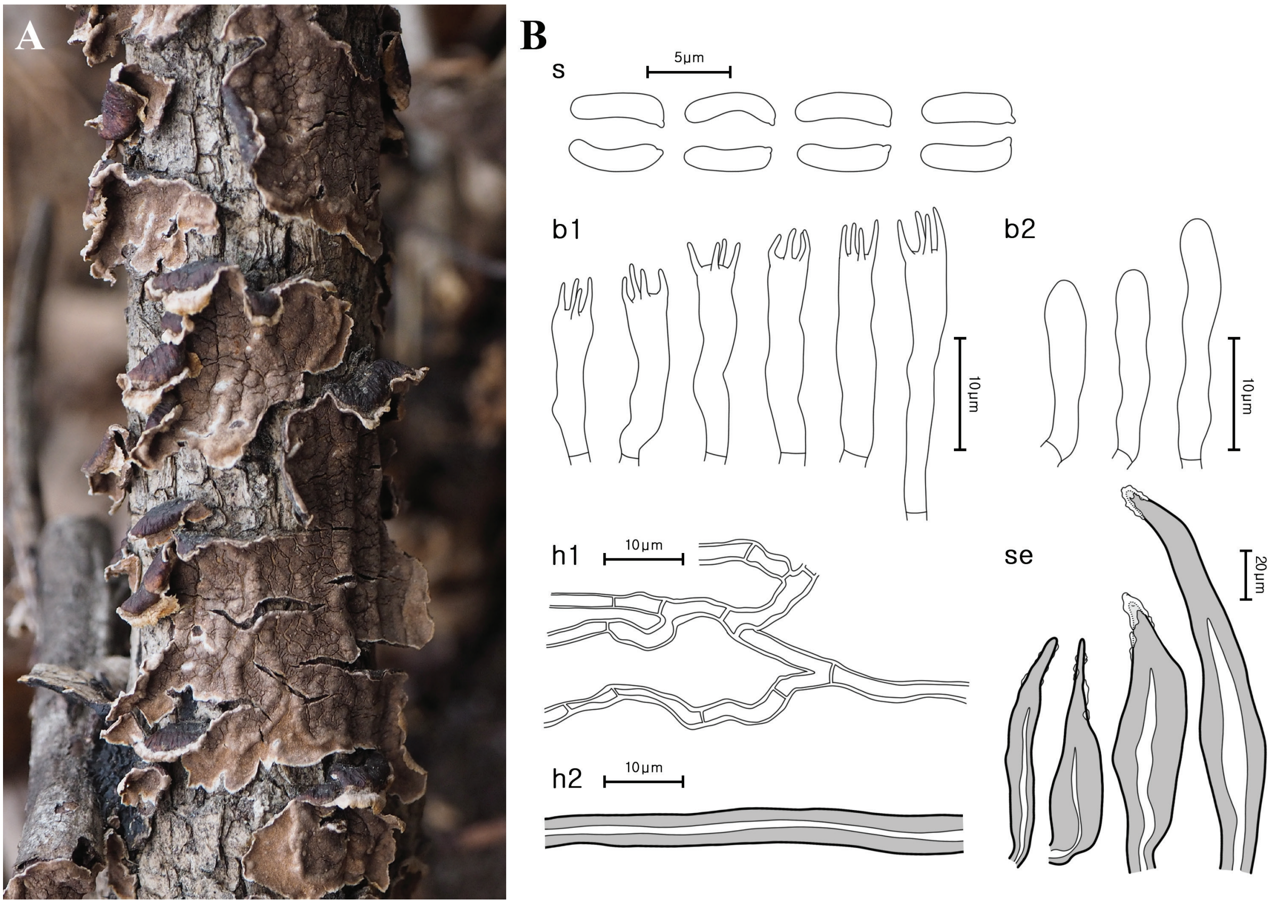

Figure 4. Morphological characteristics of *Hydnoporia subtabacina* (NIBRFG0000505378, **holotype**) **A** basidiome **B** microscopic features, **s** basidiospores; **b1** basidia; **b2** basidioles; **h1** generative hyphae; **h2** skeletal hyphae; **se** setae. Scale bars: 10 µm (**s**, **b1**, **b2**, **h1**, **h2**); 20 µm (**se**).

Etymology. Named after its morphological similarity with *Hydnoporia tabacina.*

Additional specimens examined. Korea • Gangwon-do, Taebaek-si, Taebaeksan National Park, Yuilsa Temple, 37°10.87'N, 128°91.07'E, alt. 1,250 m, 10 May 2019, Y. W. Lim & S. Yoo, SFC20190510-01.

Notes. According to our phylogenetic analysis, sequences annotated as '*Hyd. tabacina*' were divided into a European and an Asian-North American clade (Fig. 2). The holotype specimen sequence is unavailable, but it is known that the specimen (≡*Aricularia tabacina* Sowerby) locality is Britain (Sowerby 1797) and the lectotype specimen is from Sweden (Miettinen et al. 2019). Therefore, we acknowledge the European clade (Finland, Great Britain, and Western Russia) as *Hyd. tabacina* and the Asian-American clade (China, Fareast Russia, Korea, and the US) as the new species, following the results of a previous study (Miettinen et al. 2019). *Hydnoporia subtabacina* sp. nov. occurs only on angiosperm branches or trunks. The microscopic characteristics of *Hyd. subtabacina* and *Hyd. tabacina* are similar, but longer and wider basidiospores are reported in the latter species, viz. 4.58–5.9 × 1.78–2.02 µm (Miettinen et al. 2019). Further, setal measurements of our specimens had broader variation (58.6–140.0 × 9.8–26.1 µm) compared to those of the Fareast Russian (63.92–94.15 × 9.38–14.5 µm) and North American specimens (71.1–97.9 × 9.5–14.23 µm) (Miettinen et al. 2019). While there is a morphological description of Chinese '*Hyd. tabacina*', no sequence data were available for these observed specimens

(Dai 2010). Additionally, the morphological characteristics of the Chinese '*Hyd. tabacina*' with a hydnoid and yellowish basidiome (Dai 2010) differ from those of the European specimens and of *Hyd. subtabacina*. Therefore, further research is needed for an accurate identification of Chinese '*Hyd. tabacina*'.

Hydnoporia rimosa **(Lloyd) Spirin & Miettinen, Fungal Systematics and Evolution 4: 92 (2019)**

MycoBank No: 830597
Fig. 5

Diagnosis. Resupinate, effused basidiome, reddish brown hymenial surface, white to brown margin; cylindrical to narrowly clavate basidia with 10.0–14.1 × 2.7–3.4 μm, sharp to blunt pointed setae with 44.4–83.1 × 8.2–13.4 μm, narrowly cylindrical to allantoid basidiospores with 4.7–6.1 × 1.7–2.0 μm, and occurs on angiosperm branches.

Type. JAPAN • Tohoku, Sendai, 24 Oct 1920, Yasuda, (***holotype*****:** TNS-F203210; ***lectotype*****:** MBT387146).

Description. ***Basidiome*** resupinate, effused, thin, leathery, up to 0.2 mm thick. ***Hymenial surface*** smooth, membranaceous, crustaceous, with many cracks, light reddish-brown (2.5YR, 6/4) to reddish-brown (2.5YR, 5/3). ***Margin*** sterile, even, edge whitish (7.5YR, 9/2) when fresh and becomes brown (7.5YR, 5/8). ***Hyphal system*** dimitic; generative hyphae septate, branched, without a clamp connection, thick-walled, hyaline to pale brown (10YR, 6/3), 3.0–3.5 μm. Skeletal hyphae aseptate, unbranched, without a clamp connection, thick-walled, yellowish (7.5YR, 6/8) to reddish brown (2.5YR, 4/3), 3.5–4.2 μm.

Basidia cylindrical to narrowly clavate, 4-spored, smooth, thin-walled, hyaline, 10.0–14.1(–14.5) × 2.7–3.4 μm. ***Setae*** sharp- to blunt-pointed, encrusted with crystals, smooth, bearing a wide or narrow lumen, thick-walled, projecting up to 15 μm above the hymenium, dark reddish brown (2.5YR, 2.5/3), 44.4–83.1 × 8.2–13.4(–18.6) μm. ***Basidiospores*** narrowly cylindrical to allantoid, smooth, thin-walled, hyaline, with narrow apex, 4.7–6.1 × (1.5–)1.7–2.0 μm, L = 5.40 μm, W = 1.80 μm, Q = 2.64–3.30, n = 20.

Specimen examined. KOREA • Gangwon-do, Pyeongchang-gun, Odaesan National Park, 37°44.30'N, 128°35.01'E, alt. 660 m, 9 Nov 2012, Y. Jang & S. Jang, KUC20121109-19.

Notes. The observed *Hyd. rimosa* specimen from Korea has similar morphological characteristics as those of the type specimen (TNS-F203210), but the type specimen has abundant blunt-pointed setae and wider basidiospores (L = 5.22, W = 1.99, Q = 2.47–2.78) (Miettinen et al. 2019) compared to the Korean specimen. In the phylogenetic tree, *Hyd. diffissa* is closely related to *Hyd. rimosa*. These two species are practically indistinguishable in morphology, except that *Hyd. rimosa* has a wider lumen and greater size variation of setae (Miettinen et al. 2019). However, they are geographically distinct, where *Hyd. diffissa* is distributed across North and South America (Colombia, Peru, and Eastern US) (Miettinen et al. 2019), while *Hyd. rimosa* is distributed in East Asia (Japan, Korea, and Far East Russia) (Ito 1930b; Miettinen et al. 2019).

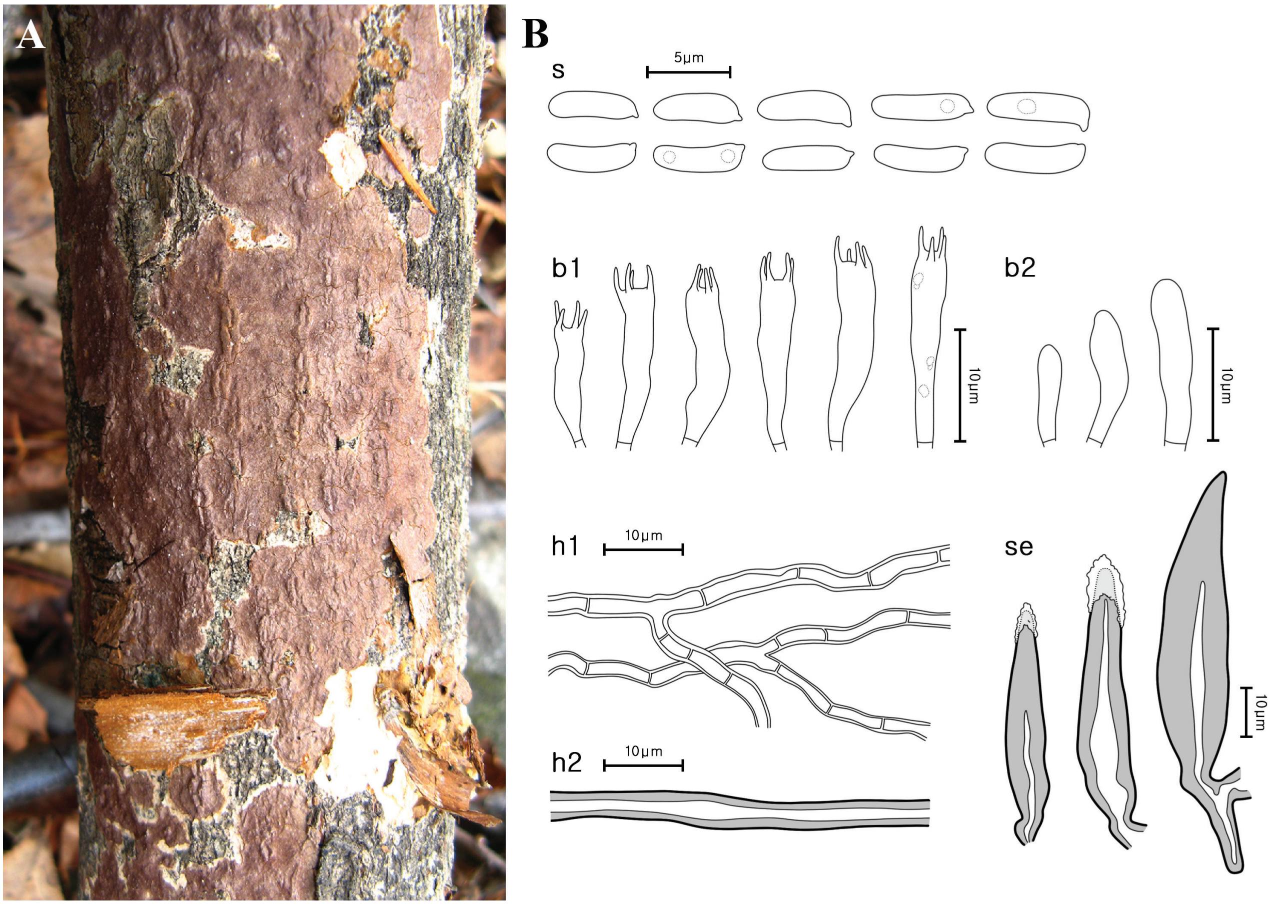

Figure 5. Morphological characteristics of *Hydnoporia rimosa* (KUC20121109-19) **A** basidiome **B** microscopic features, **s** basidiospores; **b1** basidia; **b2** basidioles; **h1** generative hyphae; **h2** skeletal hyphae; **se** setae. Scale bars: 10 μm (**B**).

***Hydnoporia intricata* (Lloyd) M.Cho, Y.Cho, Y.W.Lim & J.J.Kim, comb. nov.**
MycoBank No: 854673

Stereum intricatum Lloyd, Mycol. Writ. 7(67): 1157, 1922. Basionym.
Hymenochaete intricata (Lloyd) T.Ito, in Tokyo, Bot. Mag. 44: 156, 1930. Synonyms.
=*Pseudochaete intricata* (Lloyd) S.H.He & Y.C.Dai, Fungal Diversity, 56: 89, 2012.
=*Hymenochaetopsis intricata* (Lloyd) S.H.He & Jiao Yang, Mycol. Prog. 15(2/13): 6, 2016.

***Hydnoporia rigidula* (Berk. & M.A.Curtis) M. Cho, Y.Cho, Y.W.Lim & J.J.Kim, comb. nov.**
MycoBank No: 854674

Hymenochaete rigidula Berk. & M.A.Curtis, Journal of the Linnean Society. Botany 10: 334, 1869. Basionym.
Pseudochaete rigidula (Berk. & M.A.Curtis) S.H.He & Y.C.Dai, Fungal Diversity 56: 89, 2012. Synonyms.
=*Hymenochaetopsis rigidula* (Berk. & M.A.Curtis) S.H.He & Jiao Yang, Mycol. Prog. 15 (2/13): 6, 2016.

Taxonomic key to *Hydnoporia* in Korea

1 Basidiome resupinate **2**
– Basidiome effused-reflexed **5**
2 Basidiome margin strictly attached to substrate **3**
– Basidiome margin detached from substrate **4**
3 Setae sharp-pointed, distinctly widened base .... ***Hyd. orienticorrugata***
– Setae sharp– to blunt-pointed, narrow base .... ***Hyd. rimosa***
4 Occurs on dead angiosperm branches, setae 29.1–66.4 × 5.0–9.2 μm .... ***Hyd. rigidula***
– Occurs on dead gymnosperm branches, setae 36.0–92.7 × 9.1–19.6 μm .... ***Hyd. yasudai***
5 Basidiospores cylindrical, Q value < 3.0 .... ***Hyd. subtabacina***
– Basidiospores allantoid, Q value > 3.0 .... ***Hyd. intricata***

Discussion

Some *Hydnoporia* species are indistinguishable based on a phylogenetic tree that is inferred from ITS data alone, notably as *Hyd. rhododendri*, *Hyd. tabacina*, and *Hyd. subtabacina* (Fig. 1). This is resolved by including an additional genetic marker, *tef1*, to infer a multigenetic marker phylogeny (Fig. 2). A phylogenetic analysis solely based on ITS for fungal species identification has been criticized by taxonomists (Harder et al. 2013; Santos et al. 2017). The use of ITS alone works well for several genera (Frøslev et al. 2007; Hallenberg et al. 2007). However, it may lead to under-splitting of some taxa (Harder et al. 2013) or over-splitting of other, taxa as seen for *Hyd. yasudai* (Fig. 1). Therefore, the use of additional protein-coding genetic markers for phylogenetic analyses is essential to achieve properly resolved species clades (Fig. 2, Suppl. material 1).

To the 14 previously accepted species in *Hydnoporia*, the present study adds four ones: two new *Hydnoporia* species were described, and two other species were transferred to *Hydnoporia*. Of these 18 species, 13 have been reported in East Asia (China, Japan, Korea, and Fareast Russia), namely *Hydnoporia gigasetosa*, *Hyd. intricata*, *Hyd. lamellata*, *Hyd. laricicola*, *Hyd. latesetosa*, *Hyd. orienticorrugata* sp. nov., *Hyd. rhododendri*, *Hyd. rigidula*, *Hyd. rimosa*, *Hyd. subrigidula*, *Hyd. subtabacina* sp. nov., *Hyd. tabacinoides*, and *Hyd. yasudai* (Miettinen et al. 2019). Regarding the remaining five species, *Hyd. diffissa*, *Hyd. lenta*, and *Hyd. olivacea* have only been reported in the Americas, and *Hyd. corrugata* and *Hyd. tabacina* have only been reported in Europe (Miettinen et al. 2019). This indicates that many *Hydnoporia* species are geographically or ecologically restricted unlike many other wood-decaying fungi that are cosmopolitan and less constrained by environmental factors (Sato et al. 2012). The regional constraint for *Hydnoporia* may be the consequence of host or vector specificity.

In Korea, three previously recorded species – namely *Hydnoporia intricata*, *Hyd. rigidula*, and *Hyd. yasudai* – have been phylogenetically verified. Clades of the first two species were supported by high bootstrap values and posterior probabilities. However, *Hyd. yasudai* formed a complex, as observed by Miettinen et al. (2019), who suggested dividing the *Hyd. yasudai* complex into three to six different species. In our study, *Hyd. yasudai* specimens had large sequence variations in ITS (13 bp, 1.3%) and *tef1* (20 bp, 3.4%) but morphologically, the spore sizes were relatively constant among the Fareast Russian, Japanese, and Korean specimens (Miettinen et al. 2019). Additionally, *Hyd. yasudai* has a specific host preference for gymnosperms (*Pinus* spp., *Abies firma*, and *A. holophylla*, etc.) (Léger 1998; Dai 2010; Miettinen et al. 2019). Therefore, we proposed the *Hyd. yasudai* complex to remain as a single species, with variation based on geographical distribution. Two *Hydnoporia* species previously reported in Korea, *Hyd. corrugata* and *Hyd. tabacina*, were each represent other species. East Asian '*Hyd. corrugata*' specimens (Korean and Chinese) (He and Dai 2012) were phylogenetically separated from the European *Hyd. corrugata*, which contains the type specimen (O F-247869) (Figs 1, 2), and it was thus introduced as a new species, *Hyd. orienticorrugata* sp. nov. This result was consistent with that of Miettinen et al. (2019). Similarly, East Asian and North American '*Hyd. tabacina*' were different from the holotype – a European specimen – both phylogenetically and morphologically (Figs 2, 4), and they were introduced as a new species, *Hyd. subtabacina* sp. nov.

Two species combinations are proposed, viz. *Hyd. intricata* comb. nov. and *Hyd. rigidula* comb. nov. *Hydnoporia intricata* was first described as *Stereum intricatum* by Lloyd in 1922. It was then renamed to *Hymenochaete intricata* (Ito 1930a). After decades, He & Dai renamed it to *Pseudochaete intricata* (He and Dai 2012) and then Yang suggested *Hymenochaetopsis intricata* (Yang et al. 2016). Based on a recent study, *Pseudochaete* and *Hymenochaetopsis* are no longer valid and are considered younger synonyms of *Hydnoporia* (Miettinen et al. 2019). Based on this study, *Hymenochaete intricata* is verified to belong to *Hydnoporia*. The morphological characteristics of the Korean *Hyd. intricata* specimens studied here correspond to those of the original description. Therefore, we suggest that *Hymenochaete intricata* should be included in *Hydnoporia*. This result was further supported by phylogenetic analysis of combined sequence datasets (ITS+*tef1*) with high bootstrap support value and posterior probability (Fig. 2, Suppl. material 1).

Hydnoporia rigidula was initially reported as *Hymenochaete rigidula* Berk. & M. A. Curtis in 1868 (Berkeley and Curtis 1868). Based on multiple taxonomic revisions, it was renamed *Pseudochaete rigidula* (He and Dai 2012) and later *Hymenochaetopsis rigidula* according to (Yang et al. 2016). According to Miettinen et al. (2019) and this study, *Hymenochaete rigidula* is phylogenetically located in *Hydnoporia*. Miettinen et al. (2019) also stated that East Asian *Hyd. rigidula* may be distinct from the American *Hyd. rigidula*. However, no sequenced specimens are available from the American or Caribbean (Cuba and Jamaica) regions (He and Dai 2012; Yang et al. 2016) to verify the differences. Nevertheless, East Asian and American specimens have similar setae and basidiospore size measurements and other macro-morphological characteristics (Parmasto 2001; He and Dai 2012; Kim et al. 2016). Therefore, further assessment is required to separate the species.

In conclusion, we propose two new species and two species combinations within the genus *Hydnoporia*. Given the morphological similarities between *Hydnoporia* and *Hymenochaete*, molecular analysis is crucial for accurate species identification, ideally using multiple genetic regions. This study resolved the taxonomic confusion arising from the continuous systematic revision of some *Hydnoporia* species and emphasized the need to update old names to avoid confusion. Although *Hydnoporia* appears to be primarily distributed in East Asia, with a few species in Europe and the Americas, it remains underexplored in the Southern Hemisphere. Therefore, further investigation of the global distribution and biogeographical relationships of *Hydnoporia* is necessary to understand the true diversity of the genus and establish a stable species classification.

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 supported by the project of the National Institute of Biological Resources [NIBR202402104] under the Ministry of Environment, Republic of Korea. This study was also supported by the Korea University Grant.

Author contributions

Conceptualization: MC, YC, YWL. Data curation/collection: MC, YC, DK. Investigation: KH, YWL, JJK. Methodology: MC, YC, SLK. Project administration: YWL, JJK. Funding acquisition: JJK. Writing – original draft: MC, YC. Writing – review & editing: MC, YC, SLK, DK, YWL, JJK.

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Data availability

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

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Supplementary material 1

Data 1

Authors: Minseo Cho, Yoonhee Cho, Sun Lul Kwon, Dohye Kim, Kentaro Hosaka, Young Woon Lim, Jae-Jin Kim

Data type: fasta

Explanation note: ITS sequence alignments of all studied sequence datasets.

Link: https://doi.org/10.3897/mycokeys.111.137347.suppl1

Supplementary material 2

Data 2

Authors: Minseo Cho, Yoonhee Cho, Sun Lul Kwon, Dohye Kim, Kentaro Hosaka, Young Woon Lim, Jae-Jin Kim

Data type: fasta

Explanation note: Concatenated multimarker (ITS+*tef1*) sequence alignments of studied sequence datasets.

Link: https://doi.org/10.3897/mycokeys.111.137347.suppl2

Supplementary material 3

ML tree based on the ITS and *tef1* concatenated sequence datasets

Authors: Minseo Cho, Yoonhee Cho, Sun Lul Kwon, Dohye Kim, Kentaro Hosaka, Young Woon Lim, Jae-Jin Kim

Data type: png

Explanation note: The node numbers indicate the bootstrap support values (BS) above 70%. *Hydnoporia* specimens examined in this study are shown in bold. Newly generated sequences in this study are shown in blue and bold. *Porodaedalea alpicola* (Cui 12280) is used as an outgroup. Letter codes after specimen voucher indicate ISO 3166 country code followed by the province of origin. Type specimens are indicated with “T”.

Link: https://doi.org/10.3897/mycokeys.111.137347.suppl3

MycoKeys

MycoKeys 111: 21–40 (2024)
DOI: 10.3897/mycokeys.111.126446

Research Article

Molecular phylogeny and morphology of the genus *Fuscoporia* (Hymenochaetales, Basidiomycota) and reveal three new species of the *F. ferrea* group

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Abstract

Fuscoporia is a polypore genus of Hymenochaetaceae that causes wood decay, although some species in the genus have medicinal values. Phylogenetic analyses of concatenated ITS1-5.8S-ITS2-nLSU sequence data and morphological features identified three new species, *F. eucalypticola*, *F. resupinata* and *F. subtropica* from Australia, China and Malaysia, and these new species derived from the *Fuscoporia ferrea* group. These three species are illustrated and described. A key to resupinate species of *Fuscoporia* without mycelial setae in the world is provided.

Key words: Hymenochaetaceae, phylogeny, polypore, taxonomy

Academic editor: Alfredo Vizzini
Received: 21 May 2024
Accepted: 29 October 2024
Published: 25 November 2024

Citation: Chen Q, Chen H, Luo C-H, Lai X-H (2024) Molecular phylogeny and morphology of the genus *Fuscoporia* (Hymenochaetales, Basidiomycota) and reveal three new species of the *F. ferrea* group. MycoKeys 111: 21–40. https://doi.org/10.3897/mycokeys.111.126446

Introduction

The genus *Fuscoporia* Murrill (Hymenochaetales, Basidiomycota) with *F. ferruginosa* (Schrad.) Murrill as the type species was first described in 1907 (Murrill 1907). For a long time, it has been considered as a synonym of *Phellinus* Quél. *sensu lato* (Gilbertson 1979; Larsen and Cobb-Poulle 1990; Ryvarden and Gilbertson 1994; Ryvarden 2004). Phylogenetic studies confirmed that the currently recognized genus of *Fuscoporia* is monophyletic (Wagner and Fischer 2001, 2002; Wu et al. 2022a). *Fuscoporia* is characterized by an almost light to dark brown, resupinate, effused-reflexed to pileate basidiomata, dimitic hyphal system with generative hyphae bearing crystals, presence of hymenial setae in most species, and hyaline, thin-walled, smooth basidiospores (Fiasson and Niemelä 1984; Chen et al. 2020; Wu et al. 2022a). The species is very rich and 104 *Fuscoporia* species have been recognized (Wu et al. 2022a, b; Chen et al. 2023a, b; https://www.mycobank.org; accessed on 08-9-2024). Among them, 62 species were described during the last five years (Chen and Dai 2019; Chen et al. 2019, 2020, 2022, 2023a, b; Du et al. 2020; Tchoumi et al. 2020; Vlasák et al. 2020; Yuan et al. 2020; Raymundo 2021; Hussain et al. 2022; Wu et al. 2022a; Cho et al. 2023; Olou et al. 2023; Bittencourt et al. 2024).

Some species of *Fuscoporia* are difficult to identify because most morphological features of these species overlap. The recent studies revealed some traditional species of *Fuscoporia* are actually the species complex, such as, *Fuscoporia contigua* (Pers.) G. Cunn. (Cunningham 1948) was considered as a single species with variable basidiospores (oblong-ellipsoid or cylindric; Niemelä 2005; Dai 2010; Ryvarden and Melo 2017), but two Asian species were derived from *F. contigua* (Chen et al. 2019). A more comprehensive study (Chen et al. 2020, 2022; Wu et al. 2022a) of the genus revealed *Fuscoporia contigua* is actually the most complex speciesin that its members do not share a common geographic distribution and host preference. So far, fourteen species have been published in *F. contigua* group. The taxonomic status of some species in the genus *Fuscoporia* is in need of re-evaluation.

Fuscoporia ferrea (Pers.) G. Cunn. (1948) was characterized by resupinate, annual to perennial basidiomata, cylindric spores and distribution in the Northern Hemisphere (Cunningham 1948; Ryvarden and Gilbertson 1994; Lowe 1966). Based on time divergence, the early divergence of the *Fuscoporia* species was inferred to occur in subtropics of southern Asia with a resupinate fruiting body, and *Fuscoporia ferrea* group is the oldest lineage in the genus with stem age estimated around 49.52 Myr (Hussain et al. 2022). Some Asian specimens previously identified as *Fuscoporia ferrea* were confirmed as different species based on morphological examinations and phylogenetic analyses, and described as *F. ramulicola* Y.C. Dai & Q. Chen, *F. subferrea* Q. Chen & Y. Yuan and *F. yunnanensis* Y.C. Dai (Dai 2010; Chen and Yuan 2017; Chen and Dai 2019). In addition, *F. punctatiformis* (Murrill) Zmitr., Malysheva & Spirin was combined (Spirin et al. 2006). So five species comprise the *F. ferrea* complex and are characterized by resupinate basidiomata, absence of mycelial setae, presence of hymenial setae and cystidioles, and cylindric basidiospores (Chen et al. 2020).

In the process of exploring of wood-decaying fungi, brown and resupinate specimens were collected from southern Asia and Australia, and their morphological characteristics, taxonomic relationships and phylogenetic affinities were analyzed. Three new taxa were confirmed within *Fuscoporia ferrea* group, and they are described and illustrated. A key to resupinate and mycelial setaeless species of *Fuscoporia* in the world is provided.

Materials and methods

Morphological studies

The research specimens are conserved in the herbarium of Microbiology, Beijing Forestry University (BJFC). The macroscopic color codes follow Petersen (1996) and the microscopic analyses follow Wang et al. (2023, 2024) and Zhao et al. (2023, 2024) using a Nikon Eclipse 80i microscope with phase contrast illumination. Samples for microscopic examination and drawings were prepared from slides stained with Cotton Blue follow Zhang et al. (2023). The following abbreviations are used: **CB–** = acyanophilous, **IKI–** = neither amyloid nor dextrinoid, **L** = mean length of all spore, **W** = mean width of all spore, **Q** = L/W ratios, **n (a/b)** = number of measured spores(a) form number of specimens (b).

Molecular methods

According to the manufacturer's instructions, a CTAB rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) was used to obtain PCR products from dried samples. To generate PCR amplicons, the following primer pairs were used: ITS4 and ITS5 for the ITS1-5.8S-ITS2 region (White et al. 1990), LR0R and LR7 for the nLSU region (Vilgalys and Hester 1990). The PCR procedure was as follows: for the ITS1-5.8S-ITS2 region initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min; for nLSU, initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 1 min, 50 °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 sequenced by the Beijing Genomics Institute with the same primers. All newly generated sequences were deposited at GenBank and listed in Table 1 (http://www.ncbi.nlm.nih.gov/genbank). Besides the newly generated sequences for this study, other related sequences downloaded from GenBank based on Chen et al. (2023b) and Wu et al. (2022a, b) to explore the phylogenetic position of the newly sequenced specimens in *Fuscoporia*.

Phylogenetic analysis

The following software was used for data processing and phylogenetic analysis: BioEdit (Hall 1999), ClustalX (Thompson et al. 1997) and MAFFT (http://mafft.cbrc.jp/alignment/server/, Katoh et al. 2017) for sequences and manually adjusted, PhyloSuite v.1.2.2 (Zhang et al. 2020) for concatenated the separate alignments, PAUP* 4.0b10 (Swofford 2002) for maximum parsimony (MP) analysis, raxmlGUI 1.2 (Silvestro and Michalak 2012) for maximum likelihood (ML) analysis and MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) for Besian Inference (BI), TreeView 1.5.0 and FigTree version 1.4.4 (Rambaut 2018) to show the phylogenetic tree. The best topologies from ML analyses are shown in this study and the final alignments and the retrieved topologies has been deposited at TreeBASE (http://treebase.org/treebase-web/home.html), study ID: 31700.

In Maximum likelihood (ML) methods, statistical support values were obtained by using nonparametric bootstrapping with 1000 replicates, with default settings for all parameters. For BI analysis, the best-fit partitioning scheme and substitution model were determined by using ModelFinder (Kalyaanamoorthy et al. 2017). Tree was sampled every 1000 generations, starting from random trees with four chains for 2.5 million generations. In maximum parsimony (MP) analysis, tree was inferred using the heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions. The maxtrees parameter was set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed by a bootstrap analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics such as tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were calculated. The three phylogenetic methods produced a similar topology for each dataset, so, only the topology of the ML tree is presented along. Branches that received bootstrap support for ML and MP not less than 75% and BPP not less than 0.95 were considered as significantly supported.

Table 1. Species name, specimens, origin and GenBank accession number of sequences used in this study.

Species name	Specimens	Origin	GenBank accession no.	
			ITS	nLSU
Fuscoporia acutimarginata	Dai 15137	China	MH050751	MH050765
F. acutimarginata	Dai 16892	China	MH050752	MH050766
F. ambigua	Cui 9244	China	MN816706	MN809995
F. ambigua	JV 0509/151	USA	MN816707	MN809996
F. americana	JV 1209/3-J	USA	–	MG008466
F. americana	JV 1209/100	USA	KJ940022	MG008467
F. atlantica	SP 445618	Brazil	KP058515	KP058517
F. atlantica	SP 465829	Brazil	KP058514	KP058516
F. australasica	Dai 15625	China	MN816726	MN810018
F. australasica	Dai 15636	China	MG008397	MG008450
F. australiana	Dai 18672	Australia	MN816703	MN810014
F. australiana	Dai 18879	Australia	MN816705	MN810015
F. bambusae	Dai 16599	Thailand	MN816711	MN809999
F. bambusae	Dai 16615	Thailand	MN816715	MN810001
F. caymanensis	JV 1908/74	French Guiana	MT676832	MT676833
F. caymanensis	JV 1408/5	Costa Rica	MW009110	MW009109
F. callimorpha	Dai 17388	Brazil	MN121765	MN121824
F. callimorpha	Doll 868	Unknown	MN816701	MN809992
F. chinensis	Dai 15713	China	MN816721	MN810008
F. chinensis	Cui 11209	China	MN121767	MN121826
F. chrysea	JV 1607/106	Costa Rica	MN816736	MN810027
F. centroamericana	JV 1607/93	Costa Rica	MG008444	MG008460
F. centroamericana	O 908267	Costa Rica	MG008443	–
F. contigua	Dai 16025	USA	MG008401	MG008454
F. contigua	Dai 13567A	Romania	MG008402	MG008455
F. costaricana	JV 1407/92	Costa Rica	MG008446	MG008461
F. costaricana	JV 1504/85	Costa Rica	MG008413	MG478454
F. dhofarensis	ATN-007	Oman	OP593104	OP593105
F.dolichoseta	SFC20191015-23	Korea	ON427765	ON427795
F.dolichoseta	SFC20161006-16	Korea	ON427789	ON427817
F. eucalypti	Dai 18783	Australia	MN816730	MN810021
F. eucalypti	Dai 18792	Australia	MN816731	MN810022
F. eucalypticola	**Dai 18592A**	**Australia**	**PP732562**	**PP732631**
F. eucalypticola	**Dai 18683**	**Australia**	**PP732563**	**PP732632**
F. ferrea	MUCL 45984	France	KX961112	KY189112
F. ferrea	Cui 11801	China	KX961101	KY189101
F. ferruginosa	JV 0408/28	Czech Republic	KX961103	KY189103
F. ferruginosa	Dai 13200	France	MN816702	MN809993
F. gilva	JV 0709/75	USA	MN816720	MN810007
F. gilva	JV 1209/65	USA	MN816719	MN810006
F. gilva	URM 83957	Brazil	MH392545	MH407344
F. gilvoides	SFC2018042-12	Korea	ON427763	ON427793
F. gilvoides	MUGBt	Pakistan	ON427781	ON427810
F. hainanensis	Dai 16105	China	–	ON520809
F. hainanensis	Dai 16110	China	–	ON520810
F.hawaiana	JV 2208/H-22-J	USA	OQ817709	OQ817855
F.hawaiana	JV 2208/H-30-J	USA	OQ817710	OQ817856
F. insolita	Spirin 5251	Russia	KJ677113	–
F. insolita	Spirin 5208	Russia	MN816724	MN810016
F. karsteniana	Dai 16552	China	MN816716	MN810002
F. karsteniana	Dai 11403	China	MN816717	MN810003
F. kenyana	Dai 19205	Kenya	OP580527	OP580521
F. kenyana	Dai 19202	Kenya	OP580526	OP580520

Species name	Specimens	Origin	GenBank accession no.	
			ITS	nLSU
F. koreana	SFC20150625-05	Korea	ON427776	ON427805
F. koreana	SFC20160726-93	Korea	ON427762	ON427792
F. latispora	JV 1109/48	USA	MG008439	MG008468
F. latispora	JV 0610/VII-Kout	Mexico	MG008436	MG008469
F. licnoides	URM 84107	Brazil	MH392556	MH407355
F. licnoides	URM 83001	Brazil	MH392561	MH407357
F. marquesiana	URM 83094	Brazil	MH392544	MH407343
F. minutissima	JV 2208/H12-J	USA	OQ817711	OQ817857
F. minutissima	JV 2208/H16-J	USA	OQ817712	OQ817858
F. monticola	Dai 10909	China	MG008410	–
F. monticola	Dai 11860	China	MG008406	MG008457
F. palomari	JV 1004/5-J	USA	MN816737	–
F. palomari	JV 1305/3-J	USA	MN816738	MN810028
F. plumeriae	Dai 17814	Singapore	MN816714	MN810011
F. plumeriae	Dai 18858	Australia	MN816712	MN810010
F. punctatiformis	Dai 17443	Brazil	MH050755	MH050764
F. punctatiformis	Doll#872a	Brazil	MH050753	–
F. pulviniformis	CMW 48060	South Africa	MH599101	MH599125
F. pulviniformis	CMW 48600	South Africa	MH599102	MH599127
F. ramulicola	Dai 15723	China	MH050749	MH050762
F. ramulicola	Dai 16155	China	MH050750	MH050763
F. reticulata	SFC20121010-19	Korea	ON427766	–
F. reticulata	SFC20160115-16	Korea	ON427761	ON427791
F. rhabarbarina	Dai 16550	China	MN816744	MN810036
F. rhabarbarina	Dai 16226	China	MN816743	MN810035
F. resupinata	**Dai 20455**	**China**	**PP732567**	**PP732636**
F. resupinata	**Dai 20422**	**China**	**PP732568**	**PP732637**
F. resupinata	**Dai 21201**	**Malaysia**	**PP732569**	**PP732638**
F. roseocinerea	JV 1407/84	Costa Rica	MN816740	MN810030
F. roseocinerea	JV 1109/78-J	USA	MN816742	MN810032
F. rufitincta	JV 1008/25	USA	KJ940029	KX058575
F. rufitincta	JV 0904/142	USA	KJ940030	KX058574
F. sarcites	JV 0402/20K	Venezuela	MZ264225	MZ264218
F. scruposa	CMW 48145	South Africa	MH599105	MH599130
F. scruposa	CMW 47749	South Africa	MH599106	MH599129
F. semiarida	URM 83800	Brazil	MH392562	MH407361
F. semiarida	URM 82510	Brazil	MH392563	MH407362
F.semicephala	SFC20170524-08	Korea	ON427764	ON427794
F. semicephala	SFC20170712-20	Korea	ON427787	ON427815
F. senex	MEL 2382630	Australia	KP012992	–
F. senex	KAUNP MK41	Sri Lanka	KP794600	–
F. septoseta	Dai 12820	USA	MG008405	MN810033
F. septoseta	JV 0509/78	USA	MG008404	–
F. setifera	Dai 15710	China	MH050758	MH050767
F. setifera	Dai 15706	China	MH050759	MH050769
F. shoreae	Dai 17806	Singapore	MN816734	MN810025
F. shoreae	Dai 17818	Singapore	MN816735	MN810026
F. sinica	Dai 15468	China	MG008412	MG008459
F. sinica	Dai 15489	China	MG008407	MG008458
F. sinuosa	Dai 20498	China	MZ264226	MZ264219
F. sinuosa	Dai 20499	China	MZ264227	MZ264220
F. subchrysea	Dai 16201	China	MN816708	MN809997
F. subchrysea	Dai 17656	China	MN816709	MN809998
F. subferrea	Dai 16326	China	KX961097	KY053472
F. subferrea	Dai 16327	China	KX961098	KY053473

Species name	Specimens	Origin	GenBank accession no.	
			ITS	nLSU
F. submurina	Dai 19501	Sri Lanka	MZ264229	MZ264222
F. submurina	Dai 19655	Sri Lanka	MZ264228	MZ264221
F. subtropica	**Dai 20476**	**China**	**PP732564**	**PP732633**
F. subtropica	**Dai 19957**	**China**	**PP732565**	**PP732634**
F. subtropica	**Dai 22604**	**China**	**PP732566**	**PP732635**
F. torulosa	JV 1405/2	Czech Republic	KX961106	KY189106
F. torulosa	Dai 15518	China	MN816732	MN810023
F. viticola	JV 0911/6	Czech Republic	KX961110	–
F. viticola	He 2123	USA	MN816725	MN810017
F. wahlbergii	JV 1312/20-Kout	Spain	MN816727	MG008462
F. wahlbergii	JV 0709/169-J	USA	MN816728	–
F. yunnanensis	Cui 8182	China	MH050756	MN810029
F. yunnanensis	Dai 15637	China	MH050757	MH050768
Outgroups				
Coniferiporia weirii	CFS 504	Canada	AY829341	AY829345
Phellinidium fragrans	CBS 202.90	USA	AY558619	AY05027

Results

Molecular phylogeny

In this study, the data set of ITS and nLSU region included 118 ITS and 110 nLSU sequences from 121 samples, representing 61 species of *Fuscoporia* and *Coniferiporia weirii* (Murrill) L.W. Zhou & Y.C. Dai and *Phellinidium fragrans* (M.J. Larsen & Lombard) Nuss as the outgroups (Table 1, Fig. 1) based on previous studies (Chen and Yuan 2017). The dataset had an aligned length of 2224 characters, of which 1392 were constant, 120 variable but parsimony-uninformative, and 712 parsimony-informative. MP analysis yielded four similar topologies (TL = 3361, CI = 0.406, RI = 0.840, RC = 0.341, HI = 0.594). The BI analysis resulted in a concordant topology with an average standard deviation of split frequencies of 0.002648. The best model suggested by MrModeltest and applied in Bayesian analysis was GTR+F+I+G4 for ITS1+ITS2, K2P for 5.8s and K2P+I+G4 for nLSU. MP and BI analysis also resulted in a topology similar to that of the ML analysis. The seven specimens formed three lineages, named as *Fuscoporia eucalypticola*, *F. resupinata* and *F. subtropica*, with high support (100 in ML/1.00 in BI/100 in MP, respectively), which clustered together with *F. ferrea*, *F. punctatiformis*, *F. ramulicola*, *F. subferrea* and *F. yunnanensis*, in the *F. ferrea* clade with strongly support (100 in ML/1.00 in BI/100 in MP).

Taxonomy

Fuscoporia eucalypticola **Q. Chen, sp. nov.**
MycoBank No: 853957
Figs 2, 3

Holotype. AUSTRALIA • Victoria, Yarra Ranges National Park, on fallen branch of *Eucalyptus*, 9 May 2018, Dai 18592A (BJFC 027061).

Etymology. *Eucalypticola* (Lat.): refere to the species growing on *Eucalyptus*.

Description. ***Basidiomata*.** Annual, resupinate, inseparable from the substrate, without odor or taste and corky when fresh, rigid when dry, up to 20 cm

Figure 1. Maximum Likelihood (ML) tree illustrating the phylogeny of *Fuscoporia* and related species generated inferred from a combined ITS1-5.8S-ITS2-nLSU dataset. Statistical values (ML//BI/MP) are indicated for each node that received bootstrap support from ML and MP ≥ 75% and BPP ≥ 0.90. Names of new species are in bolds.

long, 3 cm wide and 1.5 mm thick at center. Pore olivaceous buff to greyish brown; sterile margin narrow or almost lacking, buff, up to 1 mm wide; pores irregular or sinuous, 3–5 per mm; dissepiments thin, entire, abundant setae seen in tube cavities (under lens). Subiculum clay-buff, corky, about 0.1 mm thick. Tubes olivaceous buff, up to 1 mm long.

***Hyphal structure*.** Hyphal system dimitic; generative hyphae simple septate; tissue becoming black in KOH.

***Subiculum*.** Generative hyphae infrequently, thin-walled, frequently branched, simple septate, 1.5–2.5 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a medium lumen, unbranched, aseptate, flexuous, strongly interwoven, 2–3 µm in diam.

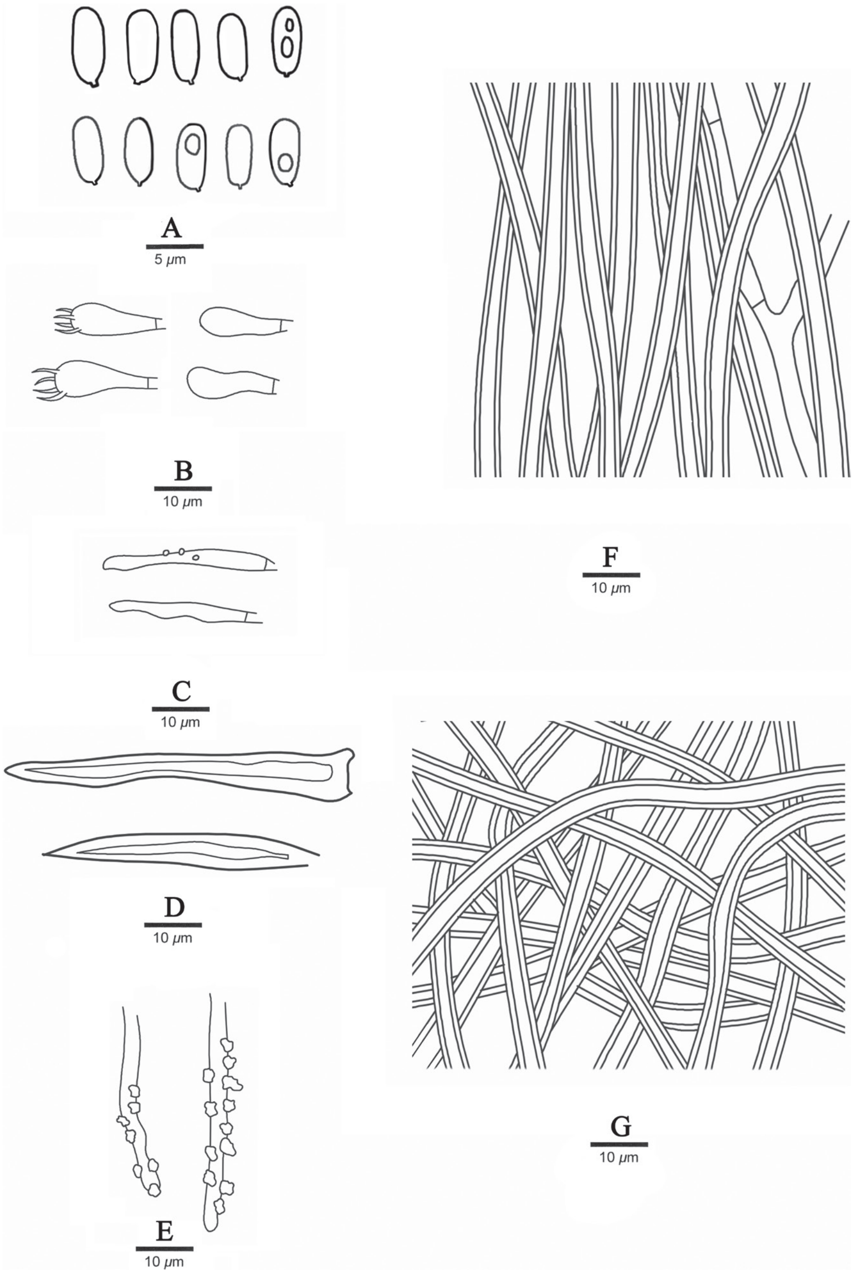

Figure 2. Microscopic structures of *Fuscoporia eucalypticola* (Dai 18592A, holotype) **A** basidiospores **B** basidia and basidioles **C** cystidioles **D** hymenial setae **E** generative hyphae at dissepiment edge **F** hyphae from tube trama **G** hyphae from subiculum.

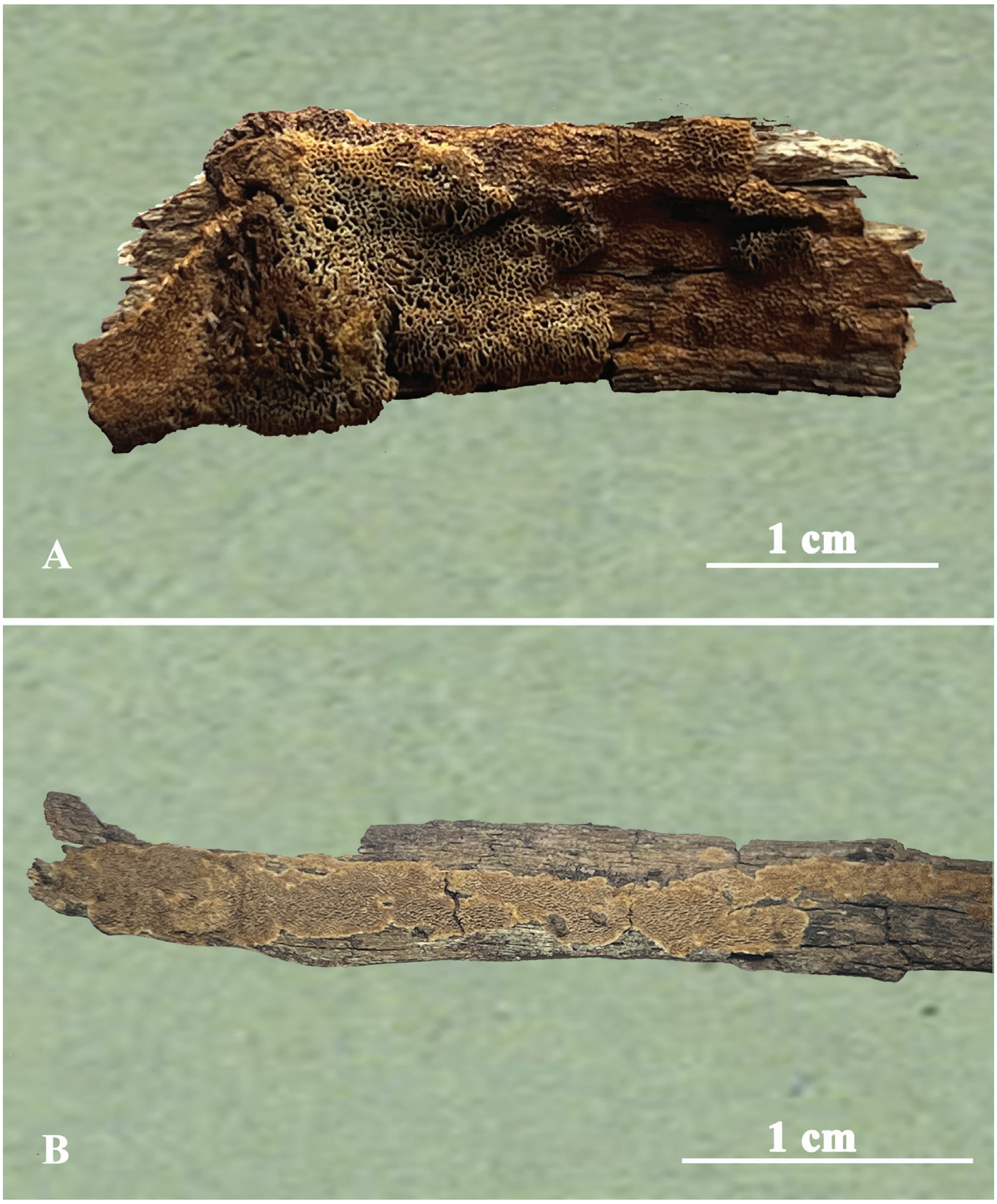

Figure 3. Basidiomata of *Fuscoporia eucalypticola* **A** Dai 18592A (holotype) **B** Dai 18683.

***Tubes*.** Generative hyphae infrequent, mostly present at subhymenium, hyaline, thin-walled, frequently branched, simple septate, 1.5–2.5 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a wide lumen, unbranched, aseptate, loosely interwoven to subparallel along the tubes, 2–3 µm in diam. Setae frequent, mostly originating from hymenium, subulate, dark brown, thick-walled, 30–60 × 4–6 µm; fusoid cystidioles frequent, hyaline and thin-walled, 25–32 × 2–4 µm; basidia barrel-shaped, with four sterigmata and a simple septum at the base, 20–25 × 4–7 µm; basidioles in shape similar to basidia, but slightly smaller.

***Basidiospores*.** Basidiospores cylindric, hyaline, thin-walled, smooth, IKI–, CB–, sometimes with a small guttule, 6.2–8 × (2–)2.1–3 µm, L = 7.03 µm, W = 2.37 µm, Q = 2.86–3.05 (n = 60/2).

Additional specimen examined. AUSTRALIA • Melbourne, Dandenong Ranges Botanical Garden, on fallen branch of *Eucalyptus*, 12 May 2018, Dai 18683 (BJFC 027152).

Fuscoporia resupinata **Q. Chen, sp. nov.**

MycoBank No: 853956
Figs 4, 5

Holotype. China • Yunnan Province, Pu'er, Taiyanghe National Forest Park, on dead angiosperm tree, 17 August 2019, Dai 20455 (BJFC032123).

Etymology. Resupinata (Lat.): refers to the species having resupinate basidiomata.

Description. ***Basidiomata*****.** Annual, resupinate, inseparable from the substrate, without odor or taste and corky when fresh, rigid when dry, up to 10.6 cm long, 4 cm wide and 1.2 mm thick at center. Pore surface fawn when fresh,

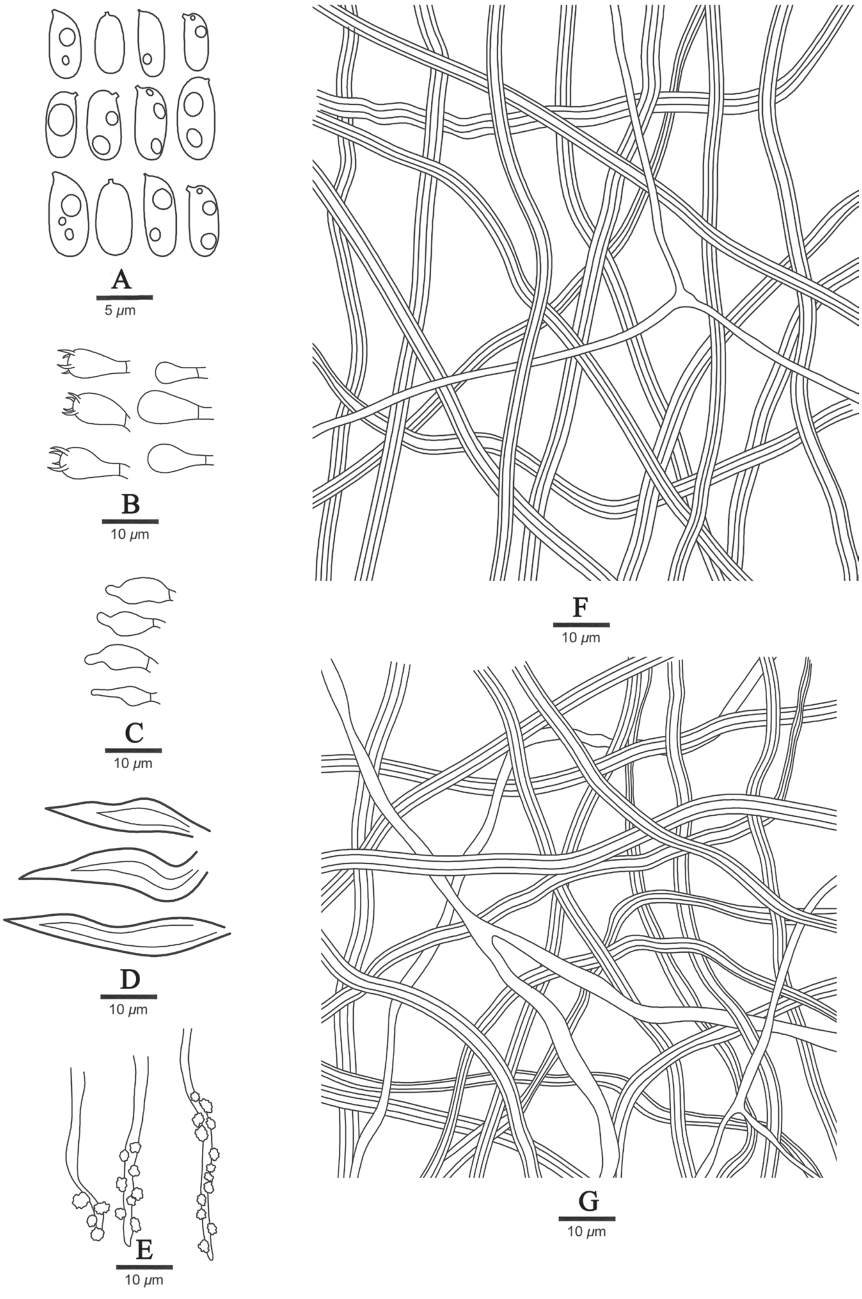

Figure 4. Microscopic structures of *Fuscoporia resupinata* (holotype, Dai 20455) **A** basidiospores **B** basidia and basidioles **C** cystidioles **D** hymenial setae **E** generative hyphae at dissepiment edge **F** hyphae from tube trama **G** hyphae from subiculum.

Figure 5. Basidiomata of *Fuscoporia resupinata* **A** Dai 20455 (holotype) **B** Dai 20422.

snuff brown when dry; sterile margin indistinct, honey-yellow when dry, up to 1 mm wide, paler than color than the pore surface; pores circular to angular, 5–7 per mm; dissepiments thin, entire, abundant setae seen in tube cavities (under lens). Subiculum honey yellow, corky, about 0.2 mm thick. Tubes grayish brown, paler contrasting with pores, rigid, up to 1 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae simple septate; tissue becoming black in KOH.

Subiculum. Generative hyphae infrequently, thin-walled, frequently branched, simple septate, 1–1.5 μm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a narrow to medium lumen, unbranched, aseptate, flexuous, strongly interwoven, 3–4 μm in diam.

***Tubes*.** Generative hyphae infrequent, mostly present at subhymenium, hyaline, thin-walled, frequently branched, simple septate, 1–2 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a narrow to medium lumen, unbranched, aseptate, loosely interwoven, 2–5 µm in diam. Setae frequent, mostly originating from hymenium, subulate, dark brown, thick-walled, 20–30 × 5–7 µm; fusoid cystidioles frequent, hyaline and thin-walled, sometimes covered with crystals, 8–12 × 3.5–5 µm; basidia barrel-shaped, with four sterigmata and a simple septum at the base, 16–20 × 6–8 µm; basidioles in shape similar to basidia, but slightly smaller.

***Basidiospores*.** Basidiospores cylindric, hyaline, thin-walled, smooth, usually glued in tetrads, IKI–, CB–, sometimes with guttules, (5.4–)5.5–7(–7.2) × (2.4–)2.5–3 µm, L = 6.38 µm, W = 2.71 µm, Q = 2.29–2.44 (n = 60/2).

Additional specimens examined. CHINA • Yunnan Province, Xinping County, Longquan Park, on fallen angiosperm branch, 16 August 2019, Dai 20422 (BJFC 032090). MALAYSIA • Selangor, Kota Damansara, Community Forest Reserve, on dead angiosperm tree, 7 December 2019, Dai 21201 (BJFC 032855).

Fuscoporia subtropica **Q. Chen, sp. nov.**

MycoBank No: 853958
Figs 6, 7

Holotype. CHINA • Yunnan Province, Wenshan Zhuang and Miao Autonomous Region, Xichou County, Xiaoqiaogou Forest Farm, on fallen angiosperm trunk, 29 June 2019, Dai 19957 (BJFC 031631).

Etymology. *Subtropica* (Lat.): refers to the species being found in subtropical area.

Description. ***Basidiomata*.** Annual, resupinate, inseparable from the substrate, without odor or taste and corky when fresh, rigid when dry, up to 15 cm long, 8 cm wide and 2.5 mm thick at center. Pore surface grayish brown to honey-yellow; sterile margin indistinct, curry-yellow, up to 1 mm wide; pores irregular to angular, sometimes sinuous, 3–5 per mm; dissepiments thin, entire, abundant setae seen in tube cavities (under lens). Subiculum clay-buff, corky, about 0.5 mm thick. Tubes olivaceous buff, up to 2 mm long.

***Hyphal structure*.** Hyphal system dimitic; generative hyphae simple septate; tissue becoming black in KOH.

***Subiculum*.** Generative hyphae infrequently, thin-walled, frequently branched, simple septate, 2–3 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a medium lumen, unbranched, aseptate, flexuous, strongly interwoven, 3–4 µm in diam.

***Tubes*.** Generative hyphae infrequent, mostly present at subhymenium, hyaline, thin-walled, frequently branched, simple septate, 2–3 µm in diam; skeletal hyphae dominant, yellowish brown, thick-walled with a narrow to medium lumen, unbranched, aseptate, loosely interwoven, 2–4 µm in diam. Setae frequent, mostly originating from hymenium, subulate, dark brown, thick-walled, 35–55 × 4–7 µm; fusoid cystidioles frequent, hyaline and thin-walled, 18–26 × 4–6 µm; basidia barrel-shaped, with four sterigmata and a simple septum at the base, 14–18 × 4–6 µm; basidioles in shape similar to basidia, but slightly smaller.

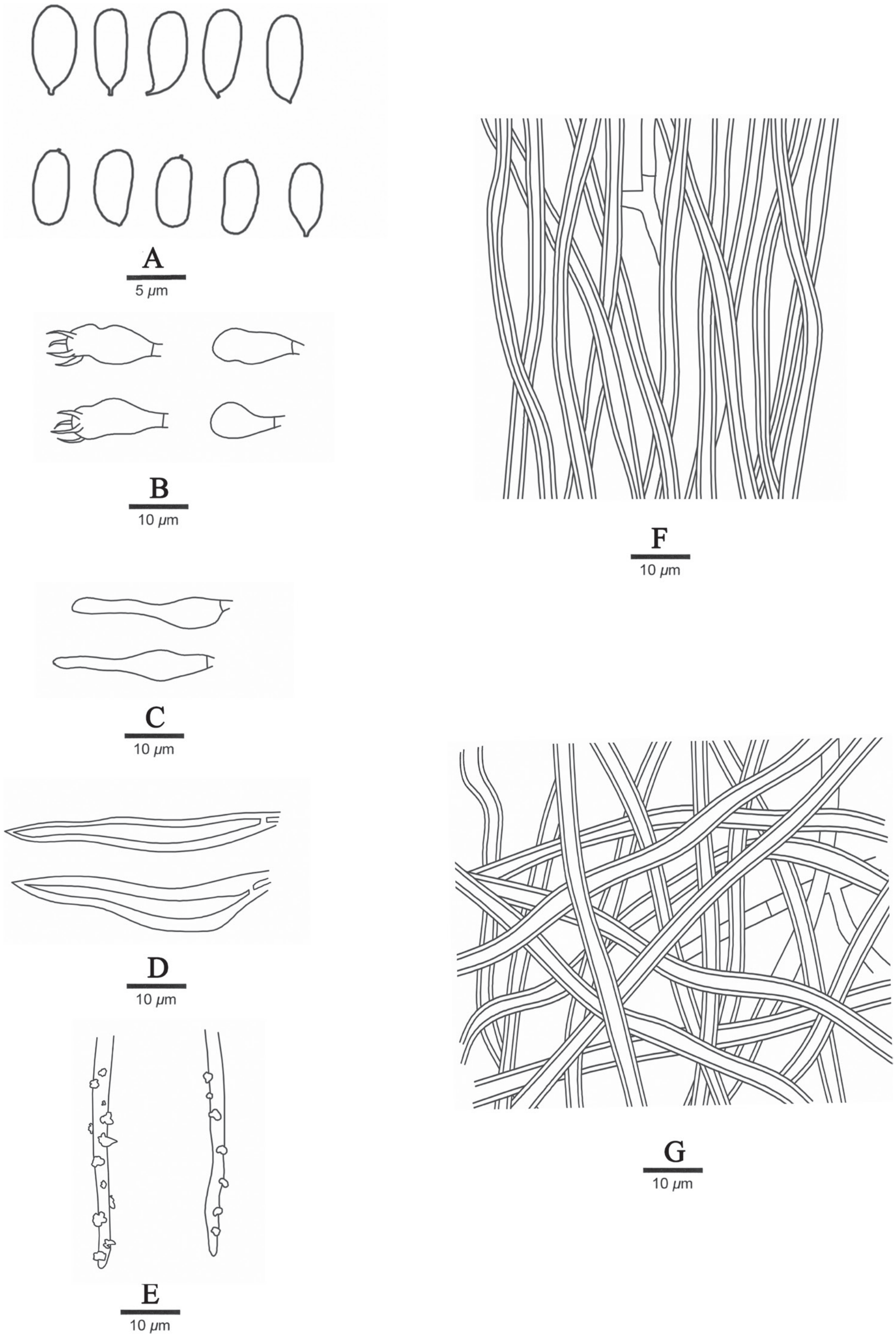

Figure 6. Microscopic structures of *Fuscoporia subtropica* (holotype, Dai 19957) **A** basidiospores **B** basidia and basidioles **C** cystidioles **D** hymenial setae **E** generative hyphae at dissepiment edge **F** hyphae from tube trama **G** hyphae from subiculum.

***Basidiospores*.** Basidiospores cylindric, hyaline, thin-walled, smooth, IKI–, CB–, (5.5–)6–7.5(–8) × 2–3(–3.2) µm, L = 6.91 µm, W = 2.66 µm, Q = 2.32–2.71 (n = 50/2).

Additional specimens examined. China • Yunnan Province, Pu’er, Pu’er Forest Park, Xiniuping Scenic Area, on fallen angiosperm branch, 17 August 2019, Dai 20476 (BJFC 032144); • Taiyanghe National Forest Park, on fallen angiosperm branch, 8 July 2021, Dai 22604 (BJFC 037178).

Figure 7. Basidiomata of *Fuscoporia subtropica* **A** Dai 19957 (holotype) **B** Dai 22604.

Discussion

Fuscoporia is a polypore genus causing wood decay, associated with angiosperms and gymnosperms (Dai et al. 2007; Wu et al. 2022a, b; Yuan et al. 2023; Zhao et al. 2024). The medicinal potential of *Fuscoporia*, such as *F. gilva* and *F. torulosa*, was confirmed by modern studies (Deveci et al. 2019; Wu et al. 2019; Duong and Dang 2022). *Fuscoporia* is widely distributed in Asia (Bakshi et al. 1970; Dai 2010; Chen and Dai 2019; Chen et al. 2019, 2020; Du et al. 2020), Africa (Reid 1975; Ryvarden and Johansen 1980; Chen et al. 2023b), Australia (Chen et al. 2020), Europe (Donk 1960; Ryvarden and Gilbertson 1994, Ryvarden and Melo 2017), South America and North America (Larsen and Cobb-Poulle 1990; Chen et al. 2019; Wu et al. 2022a, b; Chen et al. 2023a). In this study, three new species of *Fuscoporia* are described based on molecular analyses and morphological features in Australia and southern Asia.

The recent studies (Chen and Dai 2019; Chen et al. 2020) demonstrated that the species of *Fuscoporia ferrea* was a complex species. We recognized eight species in the group: *Fuscoporia ferrea* sensu stricto (Ryvarden and Gilbertson 1994; Lowe 1966) in the Northern Hemisphere, such as Northern China, Europe and North America; three new species reported in this study, *F. resupinata* and *F. subtropica* from southern Asia, *F. eucalypticola* from Australia; *F. ramulicola* (Chen and Dai 2019), *F. subferrea* (Chen and Yuan 2017) and *F. yunnanensis* (Dai 2010) also distribution in south China; *F. punctatiformis* in Neotropics (Spirin et al. 2006), such as Brazil and USA. Eight species clustered into a clade with high statistical support (100/1.00/100) in phylogenetic analysis published in this study. The members of the *Fuscoporia ferrea* group differ from other species in the genus by its resupinate basidiomata, presence of hymenial setae and cystidioles, absence of mycelial setae, and cylindric basidiospores (Dai 2010; Chen and Yuan 2017; Chen and Dai 2019).

The species in the *Fuscoporia ferrea* group have similar morphological characteristics, which sometimes may be confused. However, *F. ferrea* and *F. punctatiformis* can be segregated from the three new species by their perennial basidiomata (Lowe 1966; Spirin et al. 2006). The remaining species of the *F. ferrea* group have annual basidiomata and are distributed in southern Asia, except for *F. eucalypticola*, which is from Australia and grows on *Eucalyptus*. Furthermore, two samples of *F. eucalypticola* formed a well-supported lineage (100/1.00/100), indicating that they are phylogenetically distinct from other species in Fig. 1. *Fuscoporia eucalypticola* is closely related to *F. subtropica* in the phylogenetic tree and also has similar macromorphology in sharing annual basidiomata, irregular to angular, sometimes sinuous and bigger porse (3–5 per mm), but the latter differs in being without guttule in basidiospores and its distribution in Yunnan provinces, China.

Southern Asia is among the regions with the highest fungal biodiversity, especially in southern China (Dai et al. 2021; Zhou et al. 2023). *Fuscoporia subtropica*, *F. yunnanensis*, *F. ramulicola* and *F. resupinata*are distributed in Yunnan provinces, China, *F. resupinata*are also distributed in Malaysia, *F. subferrea* is distributed in Hainan provinces, China, which is an island. Macromorphologically the two new species, *Fuscoporia resupinata* and *F. subtropica*, are also similar to *F. subferrea*, *F. ramulicola* and *F. yunnanensis*, but *F. resupinata* differs from *F. yunnanensis* and *F. subferrea* by its medium-sized pores (5–7 per mm in *F. resupinata* vs. 3–4 per mm in *F. yunnanensis*, 7–10 per mm in *F. subferrea*; Chen and Yuan 2017); differs from *F. ramulicola* by its wider spores (2.5–3 μm, Q = 2.29–2.44 in *F. resupinata* vs. 2–2.5 μm, Q = 2.57–2.88 in *F. ramulicola*; Chen and Dai 2019). *Fuscoporia subtropica* differs from *F. ramulicola* and *F. subferrea* by its larger pores (3–5 per mm in *F. subtropica* vs. 6–7 per mm in *F. ramulicola*, 7–10 per mm in *F. subferrea*), differs from *F. yunnanensis* by its irregular pores (Dai 2010). *Fuscoporia resupinata* resembles *F. subtropica* by having annual and resupinate basidiomata, cylindric spores, but the former has smaller pores (5–7 per mm vs. 3–5 per mm), shorter fusoid cystidioles (8–12 μm vs. 18–26 μm), and its basidiospores sometimes with guttules.

A key to resupinate and mycelial setaeless species of *Fuscoporia* in the world

1 Basidiomata perennial **2**
– Basidiomata annual to biennial **4**
2 Basidiospores narrowly ovoid to narrow ellipsoid .... ***F. montana* Y.C. Dai & Niemela**
– Basidiospores cylindric to subcylindrical **3**
3 Basidiospores 4–6 × 1.5–2 μm .... ***F. punctatiformis***
– Basidiospores 6–7.8 × 2–2.5 μm .... ***F. ferrea***
4 Pores 3–5 per mm **5**
– Pores 5–10 per mm **7**
5 Pores circular, dissepiments entire and matted .... ***F. yunnanensis***
– Pores sinuous or irregular or daedaleoid, dissepiments entire and slightly lacerate with age **6**
6 Basidiospores without guttule, Q = 2.32–2.71, distribution in China .... ***F. subtropica***
– Basidiospores occasionally with a small guttule, Q = 2.86–3.05, distribution in Australia .... ***F. eucalypticola***
7 Pores 7–10 per mm; basidiospores 4.2–6.2 μm long, Q = 2.15–2.27 .... ***F. subferrea***
– Pores 5–7 per mm, basidiospores 5.5–7 μm long, Q > 2.27 .... **8**
8 Basidiospores 2.5–3 μm wide, Q = 2.29–2.44 .... ***F. resupinata***
– Basidiospores 2–2.5 μm wide, Q = 2.57–2.88 .... ***F. ramulicola***

Acknowledgements

We are grateful to Prof. Yu-Cheng Dai (Beijing Forestry University) who allowed us to study his specimens. We would like to extend our thanks to the executive editor and anonymous reviewers for their suggestions and corrections 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 research was financed by the National Natural Science Foundation of China (project no. 32100014), the Science and Technology Research Program of Chongqing Municipal Education Commission (grant no. KJQN202100737) and the Natural Science Foundation of Chongqing Science and Technology Bureau (grant no. CSTB2022NSCQ-MSX1345).

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Data availability

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

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MycoKeys 111: 41–64 (2024)
DOI: 10.3897/mycokeys.111.133093

Research Article

Four new species of *Phanerochaete* (Polyporales, Basidiomycota) from China

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Abstract

Four new wood-inhabiting fungi *viz. Phanerochaete castanea*, *P. citrinoalba*, *P. citrinorhizomorpha*, and *P. wuyiensis* **spp. nov.** – are proposed based on a combination of morphological features and molecular evidence. *Phanerochaete castanea* is characterized by soft coriaceous basidiomata detachable from the substrate, becoming reddish brown in KOH, subulate cystidia with an obtuse apex. *Phanerochaete citrinoalba* is characterized by the coriaceous basidiomata with smooth, cracking hymenial surface, sterile margins with yellowish to whitish rhizomorphs, a monomitic hyphal system, generative hyphae mostly with simple septa and occasionally with clamp connections at basal hyphae. *Phanerochaete citrinorhizomorpha* is characterized by soft coriaceous basidiomata with a salmon to peach hymenial surface, a sterile margin with yellowish rhizomorphs, simple septate generative hyphae, and clavate to subfusiform or subulate cystidia with an obtuse apex. *Phanerochaete wuyiensis* is characterized by membranaceous basidiomata with smooth or locally tuberculate hymenial surface and the whitish rhizomorphs, generative hyphae with both simple septa and clamp connections at basal hyphae, cystidia projecting above hymenium. DNA sequences of the ITS and LSU markers of the studied samples were generated, and phylogenetic analyses were performed with Maximum Likelihood and Bayesian Inference methods. The phylogenetic tree inferred from the concatenated ITS+nLSU dataset highlighted the placement of the four new species in the genus *Phanerochaete* (Phanerochaetaceae, Polyporales). Phylogenetically related and morphologically similar species to these four new species are discussed. Furthermore, an identification key to accepted species of *Phanerochaete* in China is given.

Key words: Molecular phylogeneny, Polyporales, taxonomy, wood-decaying fungi, white rot

OPEN ACCESS

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: Jennifer Luangsa-ard
Received: 25 July 2024
Accepted: 13 November 2024
Published: 3 December 2024

Citation: Luo K-Y, Zhang X, Dai Y-C, Yuan Y (2024) Four new species of *Phanerochaete* (Polyporales, Basidiomycota) from China. MycoKeys 111: 41–64. https://doi.org/10.3897/mycokeys.111.133093

Introduction

The taxa in the family Phanerochaetaceae are mostly corticioid fungi, especially *Phanerochaete*, as a major member of the family. It is a large genus with diverse morphological features, and it is widely distributed from boreal to tropical forests. It causes a white rot on all kinds of wood and plays an important role in carbon cycling (Burdsall 1985; Larsson et al. 2004; Bernicchia and Gorjón 2010; Ryvarden and Melo 2014; Ghobad-Nejhad et al. 2015; Nagy et al. 2017; Wu 2000; Xu et al. 2020; Yuan et al. 2023).

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Phanerochaete P. Karst. is typified by *P. alnea* (Fr.) P. Karst. and has a worldwide distribution (Spirin et al. 2017). It is characterized by resupinate, membranaceous basidiomata, smooth or tuberculate hymenial surface, a monomitic hyphal system, generative hyphae mostly simple septate, the presence of smooth or encrusted cystidia, thin-walled, non-amyloid, and acyanophilous basidiospores, and causing a white-rot (Wu 2000; Wu et al. 2010; Floudas and Hibbett 2015; Ghobad-Nejhad et al. 2015). Currently, MycoBank (https://www.mycobank.org/page/Simple%20names%20search) and Index Fungorum (https://www.indexfungorum.org/Names/Names.asp?pg=1) have registered 214 records and 202 records in *Phanerochaete*, respectively. About 100 species are currently accepted in *Phanerochaete* worldwide, of which 51 have been found in China (including the four new species presented here) as of June 2024 (Xu et al. 2020; Boonmee et al. 2021; Chen et al. 2021; Wang and Zhao 2021; Li et al. 2023; Yu et al. 2023; Zhang et al. 2023; Deng et al. 2024).

Taxonomists used the membranaceous nature of the basidiomata, the monomitic hyphal system, the presence of clamp connections (simple, double or multiple clamps per septum) or simple septa, and the presence of cystidia as characters for the delimitation of the genus. The simplicity of the morphological features characterise *Phanerochaete* and the existence of species with basidiomata that fulfil only some of these morphological criteria render the limits of the genus uncertain (Floudas and Hibbett 2015).

Recent phylogenetic studies show that *Phanerochaete* s.l. is polyphyletic, and several new genera have been introduced, some of them placed into different families, or even orders (Greslebin et al. 2004; Wu et al. 2010; Binder et al. 2013; Floudas and Hibbett 2015). Most *Phanerochaete* species are still retained in a monophyletic lineage (Phanerochaetaceae sensu Larsson 2007) within Polyporales, along with genera such as *Hyphodermella*, *Phlebiopsis*, and *Rhizochaete* (Larsson 2007; Wu et al. 2010; Floudas and Hibbett 2015; Miettinen et al. 2016). Most members of the genus are nested in the phlebioid clade, comprising a number of *Phanerochaete* species assembled in a highly supported clade, referred to as the core *Phanerochaete* clade (Wu et al. 2010; Floudas and Hibbett 2015; Justo et al. 2017; Chen et al. 2021).

During investigations on the wood-inhabiting fungi in the Xizang Autonomous Region, Zhejiang, and Yunnan Province of China, samples corresponding to *Phanerochaete* were collected, and four species were initially identified as potentially new by morphology. To clarify the placement and relationships of the four species, we carried out a phylogenetic and morphological studies on *Phanerochaete* in China.

Materials and methods

Morphological studies

The studied specimens were collected from wild forests and are deposited in the Fungarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Morphological descriptions are based on field notes and voucher specimens. The microscopic analysis follows Spirin et al. (2017). Freehand sections were made from dried basidiomata and mounted in 2% (w/v) potassium hydroxide (KOH) to observe color changes. 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. 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 at 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 GenBank (Sayers et al. 2024). All sequences analysed in this study are listed in Table 1. Sequences generated from this study were aligned manually with additional sequences downloaded from GenBank using AliView version 1.27 (Larsson 2014). 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 AliView. Alignments were spliced and transformed formats in Mesquite v.3.2. (Maddison and Maddison 2017). Multiple sequence alignments were trimmed by trimAI v.1.2 using the -htmlout-gt 0.8 -st option to deal with gaps, when necessary (Capella-Gutierrez et al. 2009).

Phylogenetic analyses

The two-marker DNA multiple sequence alignment (ITS+nLSU) was used to determine the phylogenetic position of the new species. The multiple sequence alignments and the retrieved topologies were deposited in Figshare (https://figshare.com/) under accession DOI: 10.6084/m9.figshare.27683265. Sequences of *Riopa metamorphosa* (Fuckel) Miettinen & Spirin and *R. pudens* Miettinen, obtained from GenBank, were used as the outgroups (Miettinen et al. 2016).

Table 1. Names, specimen numbers, references, and corresponding GenBank accession numbers of the taxa used in the phylogenetic analysis of this study. [New species are shown in bold, * type material; type specimens of other species are shown in bold].

Species name	Specimen No.	GenBank accession No.		Country	References
		ITS	LSU		
Phanerochaete aculeata	Wu 1809278	MZ422786	MZ637178	China	Chen et al. (2021)
P. aculeata	GC 1703117	MZ422785	MZ637177	China	Chen et al. (2021)
P. albida	WEI 18365	MZ422789	MZ637180	China	Chen et al. (2021)
P. albida	GC 140714	MZ422788	MZ637179	China	Chen et al. (2021)
P. alnea	K. H. Larsson 12054	KX538924	—	Norway	Spirin et al. (2017)
P. alpina	Wu 130861	MZ422790	MZ637182	China	Chen et al. (2021)
P. alpina	Wu 130877	MZ422791	MZ637183	China	Chen et al. (2021)
P. arizonica	**RLG 10248**	**KP135170**	**KP135239**	**USA**	**Floudas and Hibbett (2015)**
P. australis	He 6013	MT235656	MT248136	China	Xu et al. (2020)
P. australis	HHB 7105	KP135081	KP135240	USA	Floudas and Hibbett (2015)
P. australosanguinea	MA Fungi 91308	MH233925	MH233928	Chile	Phookamsak et al. (2019)
P. australosanguinea	**MA Fungi 91309**	**MH233926**	**MH233929**	**Chile**	**Phookamsak et al. (2019)**
P. bambusicola	He 3606	MT235657	MT248137	China	Chen et al. (2021)
P. bambusicola	**Wu 0707-2**	**MF399404**	**MF399395**	**China**	**Xu et al. (2020)**
P. brunnea	He 4192	MT235658	MT248138	China	Phookamsak et al. (2019)
P. burdsallii	RF9JR	KU668973	—	USA	Yu et al. (2023)
P. burdsallii	**He 2066**	**MT235690**	**MT248177**	**USA**	**Xu et al. (2020)**
P. burtii	HHB 4618	KP135117	KP135241	USA	Floudas and Hibbett (2015)
P. burtii	FD 171	KP135116	—	USA	Floudas and Hibbett (2015)
P. calotricha	Vanhanen 382	KP135107	—	USA	Floudas and Hibbett (2015)
P. canobrunnea	He 5726	MT235659	MT248139	Sri Lanka	Phookamsak et al. (2019)
P. canobrunnea	CHWC 150666	LC412095	LC412104	China	Phookamsak et al. (2019)
P. canolutea	LWZ 202109214a	ON897909	ON885366	China	Unpublished
P. canolutea	TNM F14823	NR175166	NG153829	China	Chen et al. (2021)
P. carnosa	He 5172	MT235660	MT248140	China	Phookamsak et al. (2019)
P. carnosa	HHB 9195	KP135129	KP135242	USA	Floudas and Hibbett (2015)
P. castanea	**Dai 24915***	**PP960566**	**PP960569**	**China**	**Present study**
P. castanea	**Dai 24916**	**PP960567**	**PP960570**	**China**	**Present study**
P. chrysosporium	He 5778	MT235661	MT248141	Sri Lanka	Phookamsak et al. (2019)
P. chrysosporium	**HHB 6251**	**KP135094**	**KP135246**	**USA**	**Floudas and Hibbett (2015)**
P. citrinoalba	**Dai 26584***	**PP779892**	**PP779887**	**China**	**Present study**
P. citrinorhizomorpha	**Dai 20753**	**PP960568**	**PP960571**	**China**	**Present study**
P. citrinorhizomorpha	**Dai 26417***	**PP779891**	**PP779886**	**China**	**Present study**
P. citrinosanguinea	FP 105385	KP135100	KP135234	USA	Floudas and Hibbett (2015)
P. concrescens	He 4657	MT235662	MT248142	China	Chen et al. (2021)
P. concrescens	**H Spirin 7322**	**KP994380**	**KP994382**	**Russia**	**Volobuev et al. (2015)**
P. conifericola	OM 8110	KP135171	—	Finland	Floudas and Hibbett (2015)
P. crystallina	Chen 3823	MZ422802	MZ637188	China	Chen et al. (2021)
P. crystallina	**Chen 3576**	**MZ422801**	**—**	**China**	**Chen et al. (2021)**
P. cumulodentata	He 2995	MT235664	MT248144	China	Xu et al. (2020)
P. cumulodentata	LERUS 298935	KP994359	KP994386	Russia	Volobuev et al. (2015)
P. cystidiata	He 4224	MT235665	MT248145	China	Phookamsak et al. (2019)
P. cystidiata	Wu 1708-326	LC412097	LC412100	China	Wu et al. (2018)
P. ericina	HHB 2288	KP135167	KP135247	USA	Floudas and Hibbett (2015)
P. ericina	He 4285	MT235666	MT248146	China	Phookamsak et al. (2019)
P. fusca	Wu 1409-163	LC412099	LC412106	China	Wu et al. (2018)
P. guangdongensis	**Wu 1809-348**	**MZ422813**	**MZ637199**	**China**	**Chen et al. (2021)**

Species name	Specimen No.	GenBank accession No.		Country	References
		ITS	LSU		
P. guangdongensis	Wu 1809-319	MZ422811	MZ637197	China	Chen et al. (2021)
P. hainanensis	**He 3562**	**MT235692**	**MT248179**	**China**	**Boonmee et al. (2021)**
P. incarnata	He 201207281	MT235669	MT248149	China	Phookamsak et al. (2019)
P. incarnata	WEI 16075	MF399406	MF399397	China	Xu et al. (2020)
P. krikophora	HHB 5796	KP135164	KP135268	USA	Floudas and Hibbett (2015)
P. laevis	He 20120917-8	MT235670	MT248150	China	Phookamsak et al. (2019)
P. laevis	HHB 15519	KP135149	KP135249	USA	Floudas and Hibbett (2015)
P. leptocystidiata	**He 5853**	**MT235685**	**MT248168**	**China**	**Xu et al. (2020)**
P. leptocystidiata	Dai 10468	MT235684	MT248167	China	Xu et al. (2020)
P. livescens	He 5010	MT235671	MT248151	China	Phookamsak et al. (2019)
P. magnoliae	He 3321	MT235672	MT248152	China	Phookamsak et al. (2019)
P. metuloidea	He 2766	MT235682	MT248164	China	Phookamsak et al. (2019)
P. minor	**He 3988**	**MT235686**	**MT248170**	**China**	**Phookamsak et al. (2019)**
P. parmastoi	He 4570	MT235673	MT248153	China	Phookamsak et al. (2019)
P. pruinosa	CLZhao 7112	MZ435346	MZ435350	China	Wang and Zhao (2021)
P. pruinosa	**CLZhao 7113**	**MZ435347**	**MZ435351**	**China**	**Wang and Zhao (2021)**
P. porostereoides	He 1902	KX212217	KX212221	China	Liu and He (2016)
P. pseudomagnoliae	**PP 25**	**KP135091**	**KP135250**	**South Africa**	**Floudas and Hibbett (2015)**
P. pseudosanguinea	**FD 244**	**KP135098**	**KP135251**	**USA**	**Floudas and Hibbett (2015)**
P. rhizomorpha	**GC 1708-335**	**MZ422824**	**MZ637208**	**China**	**Chen et al. (2021)**
P. rhizomorpha	GC 1708-354	MZ422825	MZ637209	China	Chen et al. (2021)
P. rhodella	FD 18	KP135187	KP135258	USA	Floudas and Hibbett (2015)
P. sanguineocarnosa	FD-359	KP135122	KP135245	USA	Floudas and Hibbett (2015)
P. sinensis	**He 4660**	**MT235688**	**MT248175**	**China**	**Xu et al. (2020)**
P. sinensis	GC 180956	MT235689	MT248176	China	Xu et al. (2020)
P. singularis	He1873	KX212220	KX212224	China	Liu and He (2016)
P. spadicea	**Wu 0504-15**	**MZ422837**	**MZ637219**	**China**	**Chen et al. (2021)**
P. spadicea	Wu 0504-11	MZ422836	–	China	Chen et al. (2021)
P. stereoides	He 5824	MT235677	MT248158	Sri Lanka	Phookamsak et al. (2019)
P. stereoides	He 2309	KX212219	KX212223	China	Liu and He (2016)
P. subcarnosa	**Wu 9310-3**	**MZ422841**	**GQ470642**	**China**	**Wu et al. (2010)**
P. subcarnosa	GC 1809-90	MZ422840	MZ637222	China	Chen et al. (2021)
P. subceracea	HHB-9434	KP135163	–	USA	Floudas and Hibbett (2015)
P. subrosea	**He 2421**	**MT235687**	**MT248174**	**China**	**Phookamsak et al. (2019)**
P. subsanguinea	CLZhao 10470	MZ435348	MZ435352	China	Wang and Zhao (2021)
P. subsanguinea	**CLZhao 10477**	**MZ435349**	**MZ435353**	**China**	**Wang and Zhao (2021)**
P. subtuberculata	CLZhao F5130	OP605484	OQ195088	China	Yu et al. (2023)
P. subtuberculata	**CLZhao F6838**	**OP605485**	**OQ195087**	**China**	**Yu et al. (2023)**
P. taiwaniana	He 5269	MT235680	MT248161	VietNam	Phookamsak et al. (2019)
P. taiwaniana	Wu 011213	MF399412	MF399403	China	Xu et al. (2020)
P. subtropica	CLZhao F2763	OP605518	OQ195090	China	Yu et al. (2023)
P. subtropica	**CLZhao F8716**	**OP605486**	**OQ195089**	**China**	**Yu et al. (2023)**
P. velutina	He 3079	MT235681	MT248162	China	Phookamsak et al. (2019)
P. velutina	Kotiranta 25567	KP994354	KP994387	Russia	Volobuev et al. (2015)
P. wuyiensis	**Dai 25530***	**PP779888**	**PP779883**	**China**	**Present study**
P. wuyiensis	**Dai 26246**	**PP779889**	**PP779884**	**China**	**Present study**
P. wuyiensis	**Dai 26250**	**PP779890**	**PP779885**	**China**	**Present study**
P. yunnanensis	**He 2719**	**MT235683**	**MT248166**	**China**	**Xu et al. (2020)**
Riopa metamorphosa	Viacheslav Spirin 2395	KX752601	KX752601	Russia	Miettinen et al. (2016)
R. pudens	Otto Miettinen 8772	KX752598	–	USA	Miettinen et al. (2016)

The phylogenetic analyses followed the approach of Han et al. (2015) and Zhu et al. (2019). Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were performed based on the ITS+nLSU datasets.

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 1,000 bootstrap replicates. Bayesian phylogenetic inference and Bayesian Posterior Probabilities (BPP) were computed with MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 1.6 million generations (two-marker 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 (≥ 75% (ML-BS)) and BPP (≥ 0.95 BPP) were considered as significantly supported. The ML bootstrap (ML) ≥ 50% and BBP (BPP) ≥ 0.90 are presented on topologies from ML analysis, respectively.

Results

Molecular phylogeny

The combined two-marker dataset (ITS+nLSU) included sequences from 97 samples representing 61 taxa. The phylogenetic reconstruction performed with Maximum Likelihood (ML) and Bayesian Inference (BI) analyses for the 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 = 0.014712 to ML analysis and thus, only the ML tree is shown (Fig. 1).

The phylogenetic tree inferred from the ITS+nLSU sequences indicated that the four new species belonged to *Phanerochaete* (Fig. 1). In addition, *Phanerochaete castanea* grouped together with *P. metuloidea* Y.L. Xu & S.H. He with high support (ML = 100, BPP = 1.00); *P. citrinoalba* was sister to *P. burtii* (Romell ex Burt) Parmasto, with a low support (65% BS); *P. citrinorhizomorpha* grouped together with *P. minor* Y.L. Xu & S.H. He, with a high support (ML = 100, BPP = 1.00); and *P. wuyiensis* was sister to *P. subtropica* J. Yu & C.L. Zhao with high support (ML = 100, BPP = 1.00).

Taxonomy

***Phanerochaete castanea* K.Y. Luo, Yuan Yuan, Y.C. Dai & Xin Zhang, sp. nov.**
MycoBank No: 854762
Figs 2, 3

Holotype. China • Zhejiang Province, Jinhua, Wuyi County, Niutoushan Forest Park; on a rotten bamboo; 17.VI.2023; Y.C. Dai 24915 (BJFC042468).

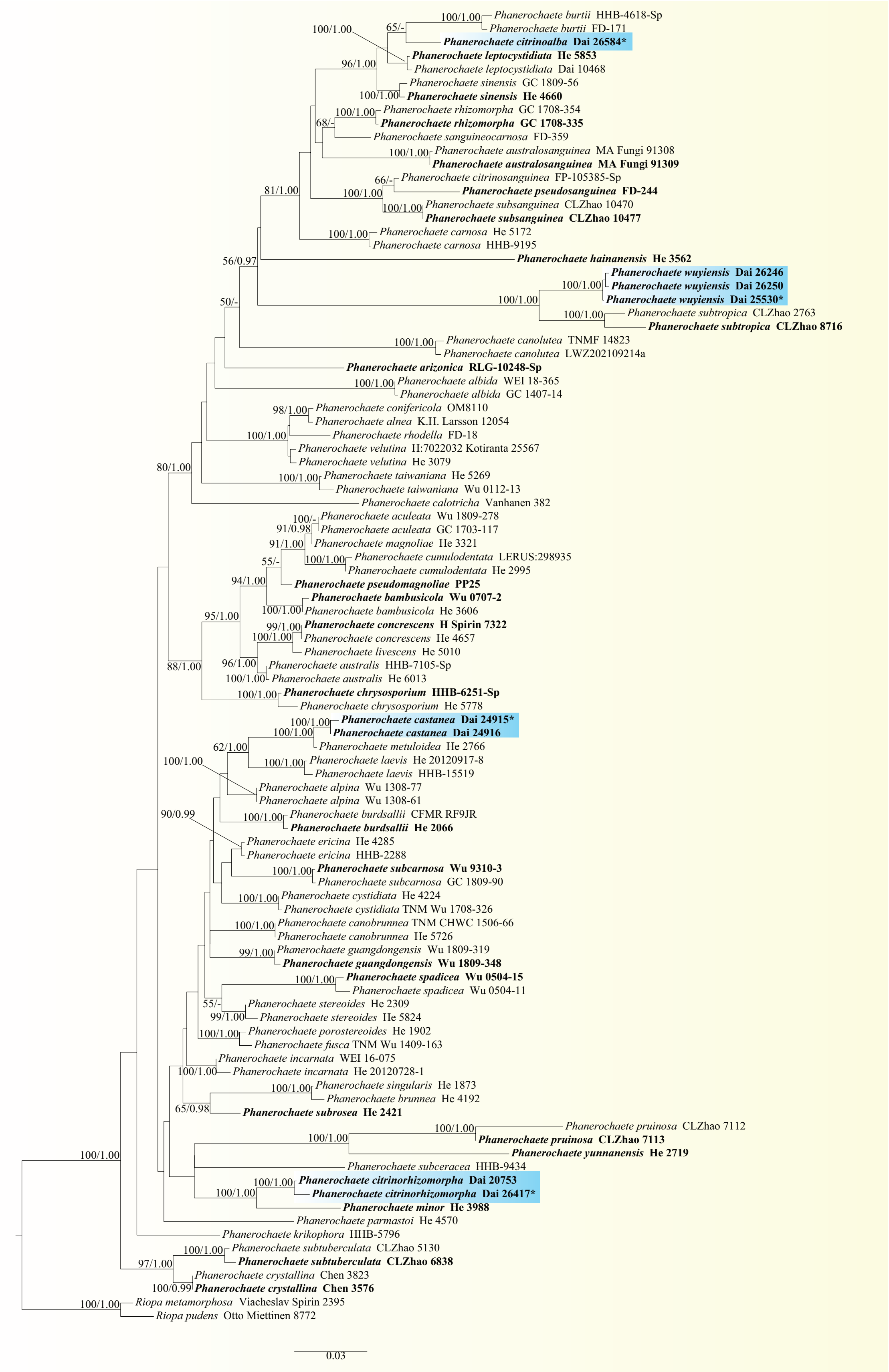

Figure 1. ML analysis of *Phanerochaete* based on dataset of ITS+nLSU. ML bootstrap values equal to or higher than 50% and Bayesian posterior probabilities values equal to or higher than 0.90 are shown. New taxa are in bold, * represents type material and in blue colour. Type specimens for all species are in bold.

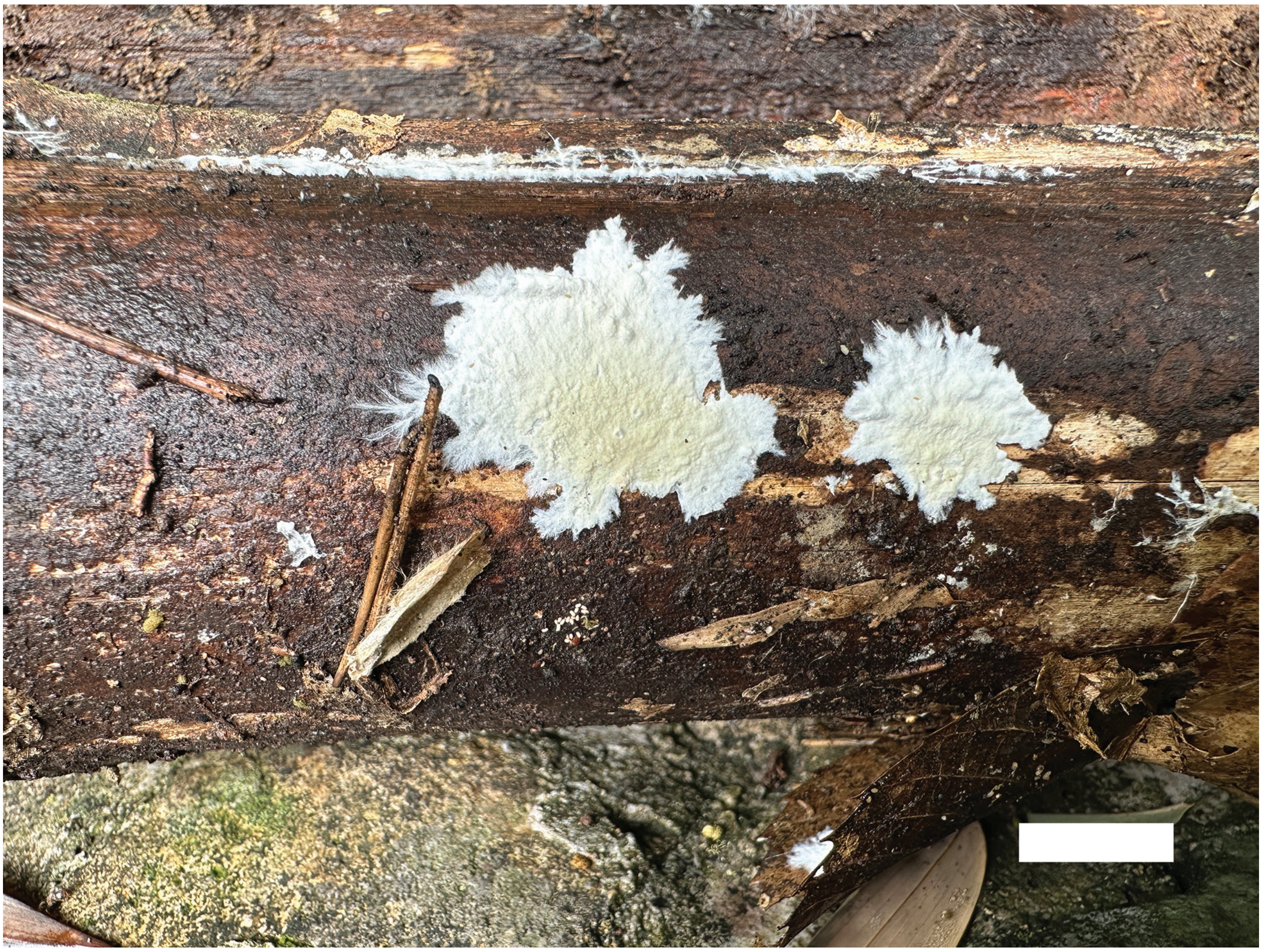

Figure 2. Basidiomata of *Phanerochaete castanea* (Holotype, Dai 24915). Scale bar: 0.5 cm.

Etymology. *Castanea* (Lat.) refers to the colour of new species' basidiomata turning reddish brown in KOH.

Description. Basidiomata annual, resupinate, adnate, soft coriaceous, without odor and taste when fresh, detachable from substrate, up to 2 cm long, 1.5 cm wide, 100–200 µm thick. Hymenial surface smooth, whitish to yellowish when fresh, yellowish brown upon drying, becoming reddish brown in KOH. Sterile margins paler than hymenial surface, thinning out, usually with whitish rhizomorphs, up to 0.2 cm.

Hyphal system monomitic, generative hyphae mostly simple septate, occasionally with clamp connections, IKI–, CB–; tissue unchanged in KOH.

Subicular hyphae hyaline, thick-walled, up to 0.1 µm thick, simple septate, occasionally bearing clamp connections, occasionally branched, parallel to interwoven, 3–5 µm in diameter. Subhymenial hyphae hyaline, thick-walled, clampless, 1.5–3 µm in diameter.

Cystidia mostly subulate with a blunt or acute apex, 45–60 × 6–8 µm, hyaline, thick-walled, up to 0.1 µm thick, with a simple septum at the base, mostly encrusted with crystal granules at apical part, some with smooth apex, projecting up to 15 µm above the hymenial layer; cystidioles absent. Basidia clavate, with four sterigmata and a basal simple septum, 19–27 × 3–5.5 µm; basidioles of similar shape to basidia, but smaller.

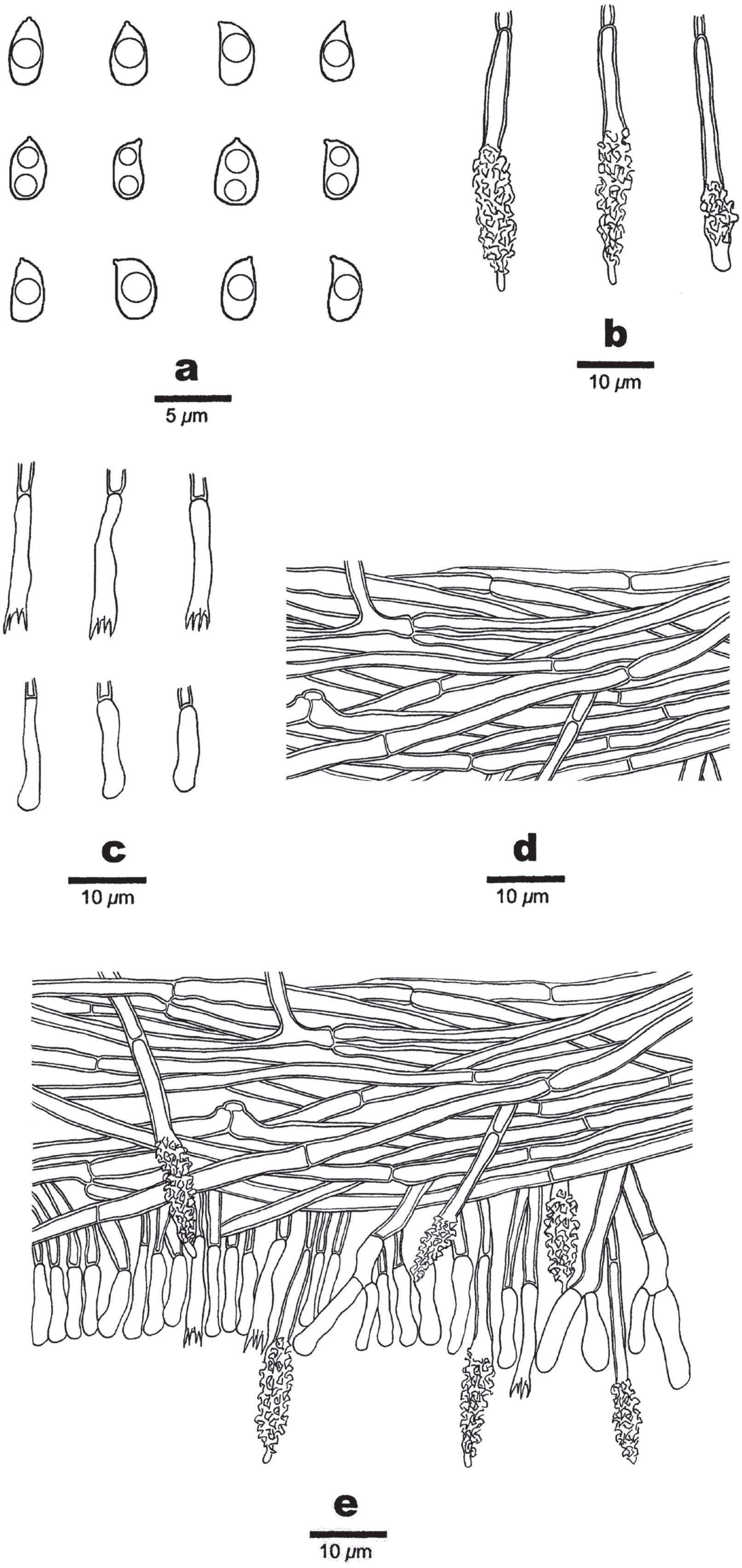

Figure 3. Microscopic structures of *Phanerochaete castanea* (Holotype, Dai 24915) **a** basidiospores **b** cystidia **c** basidia and basidioles **d** vertical section of the subiculum **e** vertical section of the hymenium and subhymenium.

Basidiospores ellipsoid, hyaline, thin-walled, smooth, usually with one or two medium guttules, IKI–, CB–, (3.7–)3.9–5.1(–5.2) × (2.2–)2.3–3.1 µm, L = 4.45 µm, W = 2.66 µm, Q = 1.67–1.68 (n=60/2).

Type of rot. White rot.

Additional specimen examined (paratype). China • Zhejiang Province, Jinhua, Wuyi County, Niutoushan Forest Park; on a rotten angiosperm wood; 17.VI.2023; Y.C. Dai 24916 (BJFC042469).

Phanerochaete citrinoalba **K.Y. Luo, Yuan Yuan, Y.C. Dai & Xin Zhang, sp. nov.**
MycoBank No: 854772
Figs 4, 5

Holotype. China • Xizang Autonomous Region, Nyingchi, Sejila Mountain; on dead bamboo; 23.X.2023; Dai 26584 (BJFC044134).

Etymology. *Citrinoalba* (Lat.) refers to the species having yellowish to whitish rhizomorphs.

Description. Basidiomata annual, resupinate, adnate, coriaceous, without odour and taste when fresh, up to 9 cm long, 1.5 cm wide, 70–130 µm thick. Hymenial surfaces smooth, cracking, white to cream when fresh, cream to slightly buff upon drying. Becoming greyish brown in KOH. Sterile margins distinct, concolorous with hymenial surface, usually with yellowish to whitish rhizomorphas, and up to 5 mm.

Figure 4. Basidiomata of *Phanerochaete citrinoalba* (Holotype, Dai 26584). Scale bar: 1 cm.

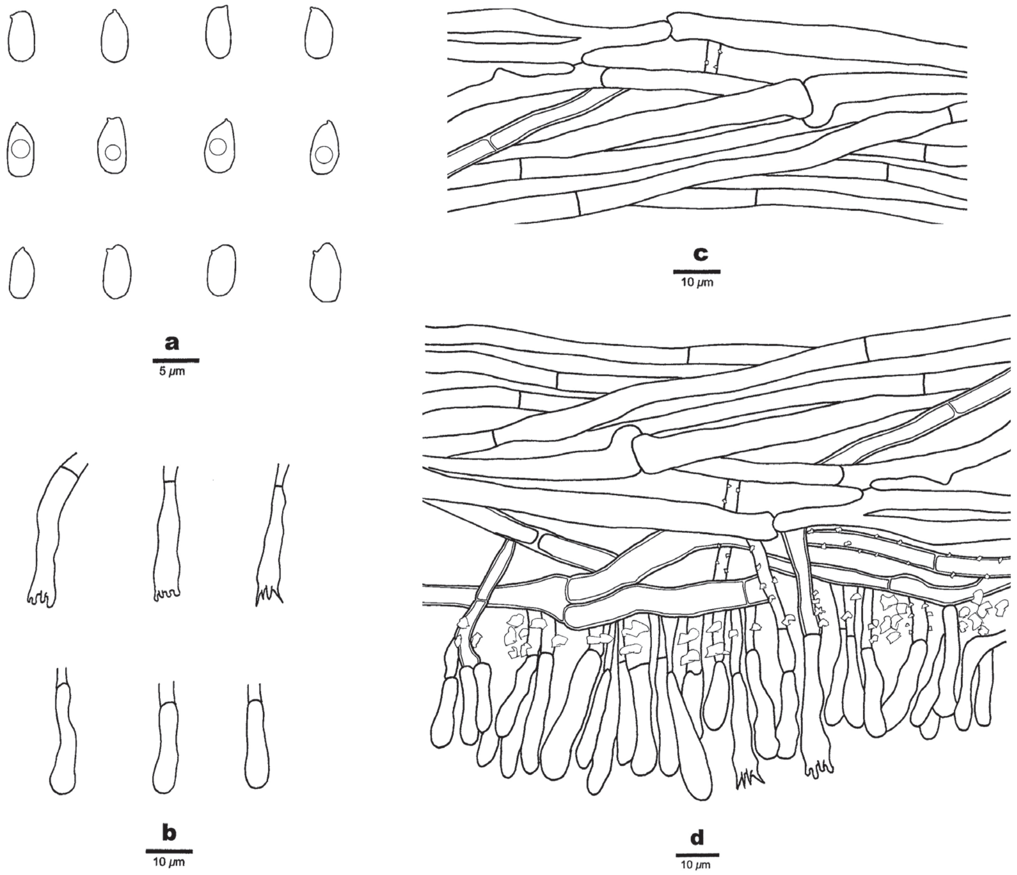

Figure 5. Microscopic structures of *Phanerochaete citrinoalba* (Holotype, Dai 26584) **a** basidiospores **b** basidia and basidioles **c** vertical section of the subiculum **d** vertical section of the hymenium and subhymenium.

Hyphal system monomitic, generative hyphae mostly with simple septa, occasionally with clamp connections in subiculum, IKI–, CB–; tissue unchanged in KOH.

Subicular hyphae hyaline, thin- to thick-walled, frequently simple septate, occasionally bearing clamp connections, branched at acute angles, subparallel to interwoven, 3–6 µm in diameter. Subhymenial hyphae hyaline, thin- to thick-walled, clampless, 2–3.5 µm in diameter, encrusted with crystal granules.

Cystidia and cystidioles absent. Subhymenium frequently with crystal granules. Basidia clavate, with four sterigmata and a basal simple septum, 17–25 × 4–7 µm; basidioles of similar shape to basidia, but smaller.

Basidiospores ellipsoid to oblong ellipsoid, hyaline, thin-walled, smooth, some with a medium guttule, IKI–, CB–, (4.7–)4.9–6.3(–6.4) × (2.2–)2.4–3(–3.1) µm, L = 5.49 µm, W = 2.66 µm, Q = 2.06 (n = 30/1).

Type of rot. White rot.

Phanerochaete citrinorhizomorpha **K.Y. Luo, Yuan Yuan, Y.C. Dai & Xin Zhang, sp. nov.**
MycoBank No: 854773
Figs 6, 7

Holotype. China • Zhejiang Province, Jinhua, Wuyi County, Xinzhai, Daozhi Village; on dead bamboo; 14.X.2023; Y.C. Dai 26417 (BJFC043967).

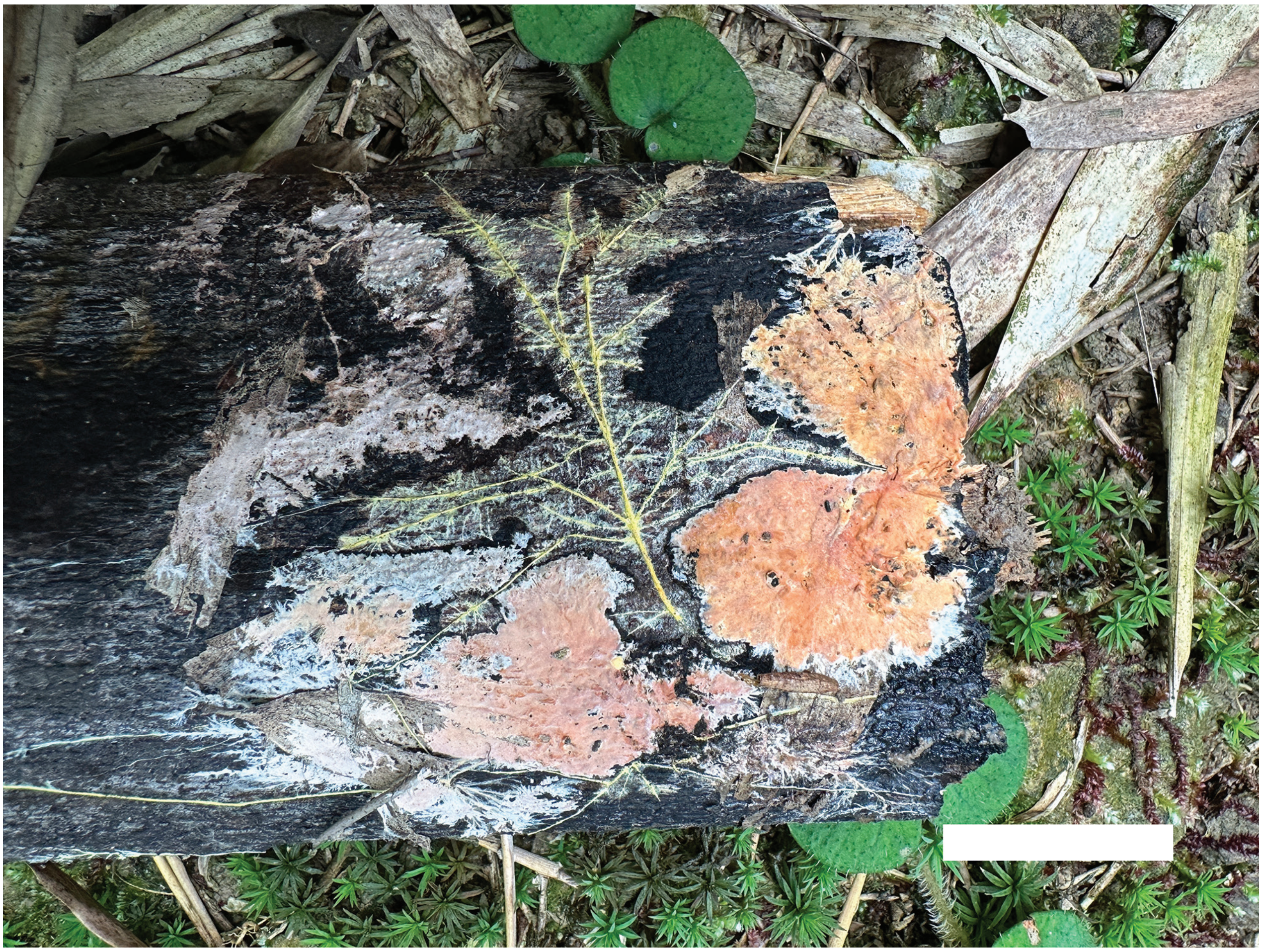

Figure 6. Basidiomata of *Phanerochaete citrinorhizomorpha* (Holotype, Dai 26417). Scale bar: 2 cm.

Etymology. *Citrinorhizomorpha* (Lat.) refers to the new species having yellowish rhizomorphs.

Description. Basidiomata annual, resupinate, adnate, soft coriaceous, without odor and taste when fresh, up to 7 cm long, 5 cm wide, 100–200 μm thick. Hymenial surfaces flesh-pink when juvenile, salmon to peach with age. Becoming purple in KOH. Sterile margins paler than hymenial surface, thinning out, usually with yellowish rhizomorphs, and up to 4 cm.

Hyphal system monomitic, generative hyphae simple septate, IKI–, CB–; tissue unchanged in KOH.

Subicular hyphae hyaline, thick-walled, up to 0.1 μm thick, simple septate, frequently branched at acute angles, interwoven, 2–6 μm in diameter. Subhymenial hyphae hyaline, thick-walled, 1.5–3 μm in diameter, encrusted with crystal granules.

Cystidia mostly subulate with an obtuse apex, hyaline, thick-walled, up to 0.1 μm thick, with a simple septum at the base, usually encrusted with crystal granules, projecting above hymenium, projecting up to 17 μm above the hymenial layer, 18–36 × 4–6 μm; cystidioles absent. Basidia clavate, with four sterigmata and a basal simple septum, 12–17 × 4–6 μm; basidioles of similar shape to basidia, but smaller.

Basidiospores ellipsoid, hyaline, thin-walled, smooth, usually with a medium guttule, IKI–, CB–, (3.3–)3.5–4.5(–4.9) × (1.8–)1.9–2.8(–3.2) μm, L = 3.94 μm, W = 2.37 μm, Q = 1.54–1.80 (n = 60/2).

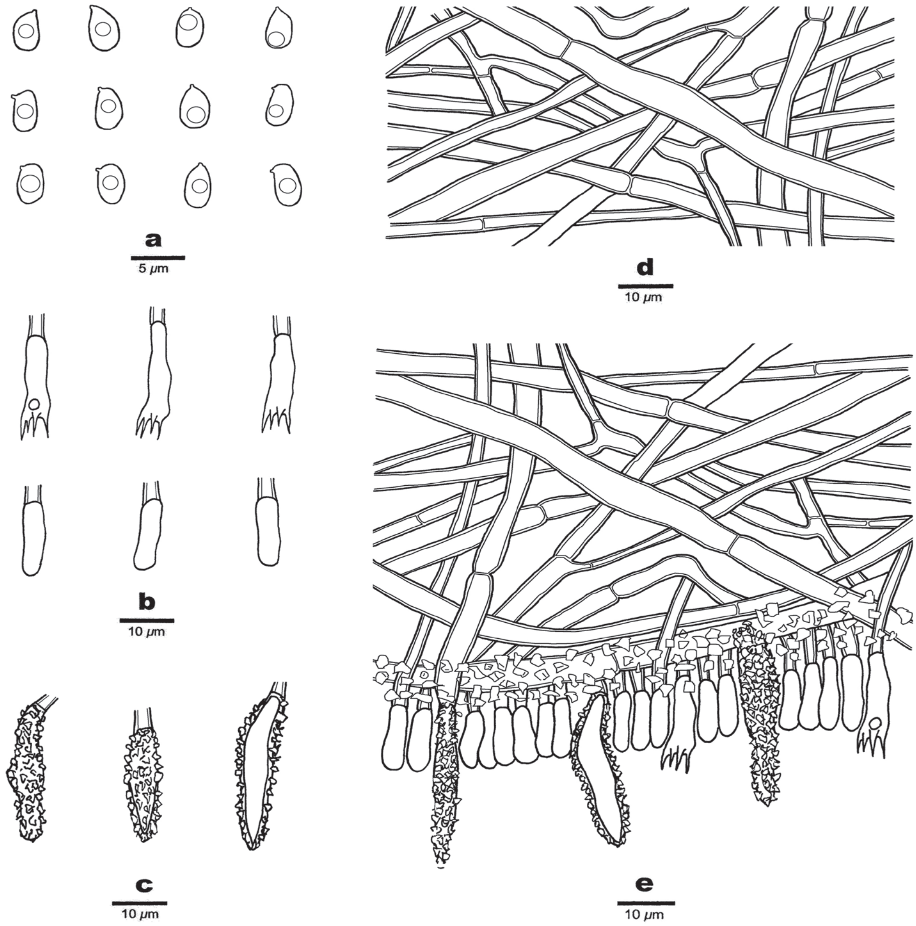

Figure 7. Microscopic structures of *Phanerochaete citrinorhizomorpha* (Holotype, Dai 26417) **a** basidiospores **b** basidia and basidioles **c** cystidia **d** vertical section of the subiculum **e** vertical section of the hymenium and subhymenium.

Type of rot. White rot.

Additional specimen examined (paratype). China • Yunnan Province, Honghe, Jinping County, Fenshuiling Nature Reserve; on a fallen angiosperm branch; 18.VIII.2019; Y.C. Dai 20753 (BJFC032420).

Phanerochaete wuyiensis **K.Y. Luo, Yuan Yuan, Y.C. Dai & Xin Zhang, sp. nov.**
MycoBank No: 854774
Figs 8, 9

Holotype. China • Zhejiang Province, Jinhua, Wuyi County, Shiehu Scenic Spot; on a fallen branch of *Pinus massoniana*; 11.VIII.2023; Y.C. Dai 25530 (BJFC043078).

Etymology. *Wuyiensis* (Lat.) refers to “Wuyi County, Zhejiang Province, East China,” where the holotype was found.

Description. Basidiomata annual, resupinate, adnate, detachable from substrate, membranaceous, without odour and taste when fresh, up to 8 cm long, 3 cm wide, 200–300 μm thick. Hymenial surfaces smooth or locally tuberculate, uncracked, whitish when fresh and upon drying. Becoming lemon-yellow in KOH. Sterile margins distinct, concolorous with hymenial surface, with whitish rhizomorphs, and up to 1 cm.

Figure 8. A basidioma of *Phanerochaete wuyiensis* (Holotype, Dai 25530). Scale bar: 2 cm.

Hyphal system monomitic, generative hyphae with simple septa and clamp connections, IKI–, CB–; tissue unchanged in KOH.

Subicular generative hyphae hyaline, thick-walled with simple septa and clamp connections, usually constricted at simple septa, sometimes with three branches at a single septum, interwoven, 3–16 µm in diameter. Subhymenial hyphae hyaline, thick-walled clampless, branched present, 2–3.5 µm in diameter.

Cystidia clavate to fusiform, hyaline, thin-walled, with a simple septum at the base, some apically encrusted with crystal granules, projecting above hymenium, 22–44 × 6–10 µm; cystidioles absent. Basidia clavate, with four sterigmata and a basal simple septum, 13–25 × 4–7 µm; basidioles of similar shape to basidia, but smaller.

Basidiospores ellipsoid, hyaline, thin-walled, smooth, with one or two medium guttules, IKI–, CB–, (3.3–)3.6–4.6(–5.4) × (1.8–)2.1–3.2(–3.4) µm, L = 4.07 µm, W = 2.48 µm, Q = 1.61–1.68 (n = 90/3).

Type of rot. White rot.

Additional specimens examined (paratype). China • Zhejiang Province, Wuyi County, Shiehu Scenic Spot; on a fallen branch of *Pinus massoniana*; 12.X.2023; Y.C. Dai 26246 (BJFC043796) • *ibid.* on rotten wood of *Pinus massoniana*; 12.X.2023; Y.C. Dai 26250 (BJFC043800).

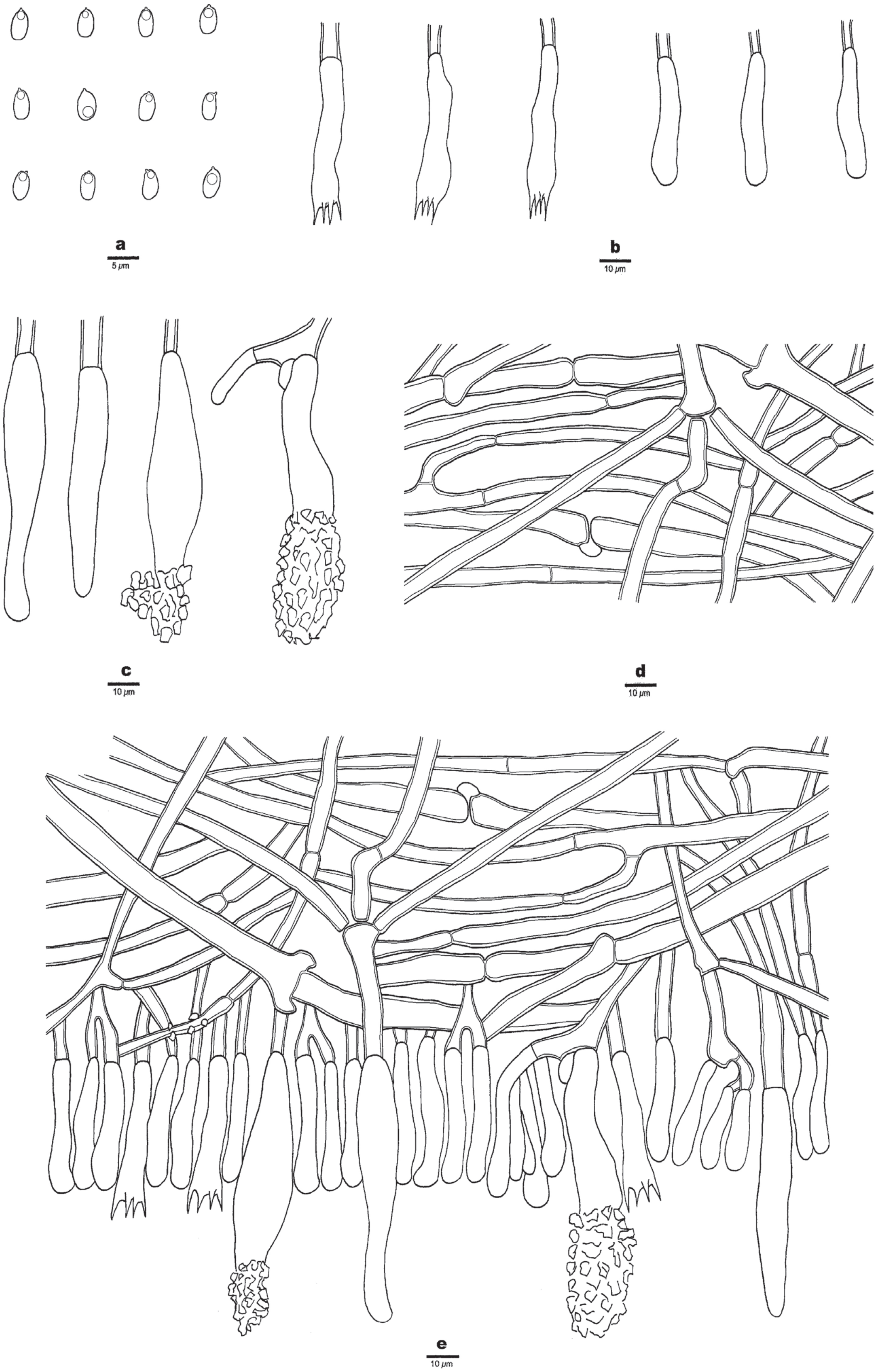

Figure 9. Microscopic structures of *Phanerochaete wuyiensis* (Holotype, Dai 25530) **a** basidiospores **b** basidia and basidioles **c** cystidia **d** vertical section of the subiculum **e** vertical section of the hymenium and subhymenium.

Discussion

Taxa of the genus *Phanerochaete* are important components of woody plant ecosystems, and they have the ability to decompose rotten wood in forest or bamboo ecosystems. Many species in the genus have been described from the subtropics and tropics in recent years (Chen et al. 2021; Li et al. 2023; Yu et al. 2023; Zhang et al. 2023; Deng et al. 2024). In the present study, four new species, *viz. Phanerochaete castanea*, *P. citrinoalba*, *P. citrinorhizomorpha* and *P. wuyiensis*, are described based on a combination of morphological features and molecular evidence.

Phylogenetically, based on ITS+nLSU topology (Fig. 1), four new species were nested in the *Phanerochaete* clade. Among them, *P. castanea* grouped together with *P. metuloidea*, however, *P. metuloidea* is delimited from *P. castanea* by having greyish orange, brownish orange to light brown hymenophore and longer basidia (40–70 × 5–8.5 µm vs. 19–27 × 3–5.5 µm, Xu et al. 2020). *Phanerochaete citrinoalba* was sister to *P. burtii*, but *P. burtii* differs from *P. citrinoalba* by having cylindric cystidia (Parmasto 1967). *P. citrinorhizomorpha* grouped together with *P. minor*, however, *P. minor* is different from *P. citrinorhizomorpha* by having membranaceous basidiomata and apically encrusted cystidia (Xu et al. 2020). *Phanerochaete wuyiensis* was sister to *P. subtropica*, but *P. subtropica* differs from *P. wuyiensis* by its coriaceous basidiomata (Yu et al. 2023).

Morphologically, *Phanerochaete castanea* resembles *P. burdsallii* Y.L. Xu et al., *P. hymenochaetoides* Y.L. Xu & S.H. He and *P. laevis* (Fr.) J. Erikss. & Ryvarden by sharing hymenophore becoming reddish brown or red in KOH. However, *P. burdsallii* is different from *P. castanea* by its membranaceous basidiomata and longer basidiospores (5.3–6 × 2.5–3 µm vs. 3.9–5.1 × 2.3–3.1 µm, Xu et al. 2020); *P. hymenochaetoides* is distinguished from *P. castanea* by its basidiomata without rhizomorphs and subicular hyphae encrusted with yellow resinous granules (Xu et al. 2020); *P. laevis* is different from *P. castanea* by its membranaceous basidiomata, ochraceous to cinnamon hymenial surface, and longer basidia (30–50 × 4–5 µm vs. 19–27 × 3–5.5 µm, Eriksson et al. 1978).

Phanerochaete citrinoalba resembles *P. daliensis* J. Yu & C.L. Zhao, *P. subtuberculata* J. Yu & C.L. Zhao and *P. tongbiguanensis* Y.L. Deng & C.L. Zhao by sharing a coriaceous basidiomata. However, *P. daliensis* is different from *P. citrinoalba* by its grandinioid hymenial surface and thick-walled basidiospores (Yu et al. 2023); *P. subtuberculata* is distinguished from *P. citrinoalba* by having tuberculate hymenial surface, generative hyphae with simple septa, and clavate cystidia (Yu et al. 2023); and *P. tongbiguanensis* is different from *P. citrinoalba* by its generative hyphae with simple septa, subclavate cystidia and bigger basidiospores (6–9 × 3–4.5 µm vs. 4.9–6.3 × 2.4–3 µm, Deng et al. 2024).

Phanerochaete citrinorhizomorpha is similar to *P. cinerea* Y.L. Xu & S.H. He, *P. guangdongensis* C.C. Chen et al., and *P. spadicea* C.C. Chen & Sheng H. Wu by sharing generative hyphae with simple septa. However, *P. cinerea* is distinguished from *P. citrinorhizomorpha* by having grey, brownish grey to greyish brown hymenophore, the absence of cystidia, and longer basidiospores (4.8–5.6 × 2–2.5 µm vs. 3.5–4.5 × 1.9–2.8 µm, Xu et al. 2020); *P. guangdongensis* is different from *P. citrinorhizomorpha* by its membranaceous to subceraceous basidiomata, buff to yellowish brown hymenial surface, and longer basidiospores

(6.9–7.8 × 2.6–3 µm vs. 3.5–4.5 × 1.9–2.8 µm, Chen et al. 2021); *P. spadicea* is distinguished from *P. citrinorhizomorpha* by having membranaceous basidiomata, buff to pale brown hymenial surface, and longer basidiospores (4.5–5 × 1.9–2.2 µm vs. 3.5–4.5 × 1.9–2.8 µm, Chen et al. 2021).

Phanerochaete wuyiensis is similar to *P. burdsallii* Y.L. Xu et al., *P. crystallina* C.C. Chen et al., and *P. subrosea* Y.L. Xu & S.H. He by sharing membranaceous basidiomata. However, *P. burdsallii* is different from *P. wuyiensis* by its hymenophore becoming reddish brown in KOH and longer basidiospores (5.3–6 × 2.5–3 µm vs. 3.6–4.6 × 2.1–3.2 µm, Xu et al. 2020); *P. crystallina* is different from *P. wuyiensis* by having cream to ochraceous-buff hymenial surface and longer basidiospores (5.1–5.7 × 2.2–2.5 µm vs. 3.6–4.6 × 2.1–3.2 µm, Chen et al. 2021); and *P. subrosea* is readily distinguished from *P. wuyiensis* by its hymenophore turning purple in KOH and longer basidiospores (5–6 × 2.5–3 µm vs. 3.6–4.6 × 2.1–3.2 µm, Xu et al. 2020).

Xizang Autonomous Region, Yunnan Province in southwest China, and Zhejiang Province in eastern China are very rich for wood-inhabiting fungi. Numerous taxa of such fungi have been described from these areas based on morphological and molecular phylogenetic analyses (Volobuev et al. 2015; Chen et al. 2018; Ordynets et al. 2018; Cui et al. 2019; Dai et al. 2021; Wu et al. 2022a, 2022b; Zhang et al. 2023; Zhou et al. 2023; Zhao et al. 2024). DNA sequence data are very useful in exploring cryptic taxa and the diversity of corticioid fungi. In the present study, four new species of *Phanerochaete* are described from these two areas, which improve our knowledge of the diversity of Chinese white rot fungi.

Key to the accepted species of *Phanerochaete* in China

1	Hymenophore poroid	***P. inflata***
–	Hymenophore non-poroid	**2**
2	Hymenophore grandinioid	**3**
–	Hymenophore smooth to raduloid	**5**
3	Basidiospores thick-walled	***P. daliensis***
–	Basidiospores thin-walled	**4**
4	Cystidia present	***P. aculeata***
–	Cystidia absent	***P. yunnanensis***
5	Hymenophore at first smooth, odontioid to raduloid when mature	**6**
–	Hymenophore smooth, more or less tuberculate	**7**
6	Distributed in northern China	***P. cumulodentata***
–	Distributed in southern China	***P. magnoliae***
7	Rhizomorpha present	**8**
–	Rhizomorpha absent	**19**
8	Hymenophore purple in KOH	**9**
–	Hymenophore unchanged, or becoming buff, grayish brown, brown, reddish brown, red or black in KOH	**10**
9	Cystidia without crystal granules	***P. subrosea***
–	Cystidia with crystal granules	***P. citrinorhizomorpha***
10	Hyphal cords reddish brown	***P. citrinosanguinea***
–	Hyphal cords white, cream, grayish or orange	**11**

11 Cystidia absent .... ***P. citrinoalba***
– Cystidia present **12**
12 Cystidia obviously encrusted with crystals .... ***P. laevis***
– Cystidia smooth or sparsely encrusted **13**
13 Generative hyphae without clamp connections in subiculum .... ***P. subsanguinea***
– Generative hyphae with clamp connections in subiculum **14**
14 Cystidia thick-walled **15**
– Cystidia thin-walled **16**
15 Cystidia with septa .... ***P. subtropica***
– Cystidia without septa .... ***P. castanea***
16 Hyphal cords turning reddish brown in KOH **17**
– Hyphal cords not turning reddish brown in KOH **18**
17 Cystidia 30–70 × 4–6 μm; basidiospores 5–6 × 2.5–3 μm .... ***P. leptocystidiata***
– Cystidia 35–50 × 4–6 μm; basidiospores 4–5 × 2–2.5 μm .... ***P. sinensis***
18 Basidiomata buff in KOH .... ***P. shenghuaii***
– Basidiomata darkening in KOH .... ***P. rhizomorpha***
19 Cystidia absent **20**
– Cystidia present **24**
20 Hymenophore brown .... ***P. porostereoides***
– Hymenophore whitish, cream, gray, grayish brown, or yellowish **21**
21 Hymenial surface lightly darkening in KOH **22**
– Hymenial surface unchanged in KOH **23**
22 Basidiomata undetachable from substrate .... ***P. pruinosa***
– Basidiomata detachable from substrate .... ***P. cinerea***
23 Basidiospores 4.2–5.2 × 1.8–2.2 μm .... ***P. spadicea***
– Basidiospores 4.2–5.1 × 2.5–3.3 μm .... ***P. brunnea***
24 Cystidia obviously encrusted **25**
– Cystidia smooth or sparsely encrusted **36**
25 Cystidia encrusted with yellow resinous granules **26**
– Cystidia encrusted without yellow resinous granules **27**
26 Hymenophore brown; quasi-binding hyphae present .... ***P. ericina***
– Hymenophore lilac pink; quasi-binding hyphae absent .... ***P. incarnata***
27 On Monocotyledons .... ***P. minor***
– On Dicotyledons **28**
28 Cystidia up to 150 μm long .... ***P. velutina***
– Cystidia up to 80 μm long **29**
29 Cystidia up to 13 μm wide **30**
– Cystidia up to 9 μm wide **31**
30 Cystidia only apically encrusted; widely distributed in China .... ***P. concrescens***
– Cystidia encrusted up to one third of the length; distributed only in southern China .... ***P. australis***
31 Hymenophore yellow to buff **32**
– Hymenophore white to cream **34**
32 Basidiomata ceraceous; basidiospores > 5.5 μm long .... ***P. livescens***
– Basidiomata membranaceous; basidiospores < 5.5 μm long **33**

33 Hymenophore yellow to yellowish brown; margin determinate .... ***P. hymenochaetoides***
– Hymenophore cream to light yellow; margin fibrillose .... ***P. cystidiata***
34 Cystidia thick-walled; basidia up to 70 µm long, 8.5 µm wide .... ***P. metuloidea***
– Cystidia thin- to slightly thick-walled; basidia up to 50 µm long, 6 µm wide.. **35**
35 Subicular hyphae thin to slightly thick-walled; cystidia subulate...... ***P. laevis***
– Subicular hyphae thick-walled; cystidia tapering but with obtuse apex .... ***P. sordida***
36 Cystidia two kinds .... ***P. robusta***
– Cystidia one kind **37**
37 Clamp connections present in subiculum **38**
– Clamp connections absent in subiculum **43**
38 Basidiospores < 4.6 µm in length **39**
– Basidiospores > 4.6 µm in length **41**
39 Cystidia < 40 µm in length .... ***P. albida***
– Cystidia > 40 µm in length **40**
40 Basidiomata turning lemon-yellow in KOH .... ***P. wuyiensis***
– Basidiomata turning greyish green in KOH .... ***P. carnosa***
41 Cystidia < 40 µm in length .... ***P. subcarnosa***
– Cystidia > 40 µm in length **42**
42 Basidiomata margin fibrillose .... ***P. affinis***
– Basidiomata margin byssoid .... ***P. alpina***
43 On bamboo **44**
– On wood **45**
44 Basidiospores > 6.2 µm in length .... ***P. bambucicola***
– Basidiospores < 6.2 µm in length .... ***P. parmastoi***
45 Basidiospores cylindrical **46**
– Basidiospores ellipsoid **48**
46 Basidiospores > 6 µm in length .... ***P. guangdongensis***
– Basidiospores < 6 µm in length **47**
47 Basidiomata coriaceous .... ***P. subtuberculata***
– Basidiomata membranaceous .... ***P. crystallina***
48 Basidiomata coriaceous to soft corky .... ***P. hainanensis***
– Basidiomata membranaceous **49**
49 Basidiospores < 6.5 µm in length .... ***P. tongbiguanensis***
– Basidiospores > 6.5 µm in length **50**
50 Basidiospores with oil-drops .... ***P. taiwaniana***
– Basidiospores without oil-drops .... ***P. stereoides***

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 (Project Nos. 32370013), the Young Elite Scientists Sponsorship Program by CAST (Project No. 2023QNRC001), Zhejiang Key Laboratory of Biological Breeding and Exploitation of Edible and Medicinal Mushrooms, and the Postdoctoral Fellowship Program (Grade C) of China Postdoctoral Science Foundation (GZC20230254).

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Data availability

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

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MycoKeys

MycoKeys 111: 65–86 (2024)
DOI: 10.3897/mycokeys.111.137898

Research Article

Melomastia (Dothideomycetes, Ascomycota) species associated with Chinese *Aquilaria* spp.

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This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

OPEN ACCESS

Academic editor: Danushka Sandaruwan Tennakoon
Received: 26 September 2024
Accepted: 13 November 2024
Published: 4 December 2024

Citation: Du T-Y, Karunarathna SC, Tibpromma S, Hyde KD, Nilthong S, Mapook A, Liu X-F, Dai D-Q, Niu C, Elgorban AM, Chukeatirote E, Wang H-H (2024) *Melomastia* (Dothideomycetes, Ascomycota) species associated with Chinese *Aquilaria* spp. MycoKeys 111: 65–86. https://doi.org/10.3897/mycokeys.111.137898

Abstract

This study is based on three terrestrial saprobic fungi associated with *Aquilaria* in Guangdong and Yunnan provinces in China. All isolated species matched with generic concepts of *Melomastia*. Detailed morphological characteristics and combined multi-gene phylogeny of LSU, SSU, and TEF revealed that the new isolates represent two new species (*Melomastia guangdongensis* and *M. yunnanensis*), and one new host and geographical record (*M. sinensis*). *Melomastia guangdongensis* is distinct from the phylogenetically closest species in having semi-immersed to immersed, globose to subglobose ascomata, and two strata of the peridium. *Melomastia yunnanensis* differs from the phylogenetically closest species in having immersed ascomata, conical ostiolar canals, and branched pseudoparaphyses. The discovery of these two new species and one new record collected expands the number of saprobic species associated with *Aquilaria* from 28 to 31. Descriptions, photo plates, and phylogenetic analyses of taxa are provided.

Key words: 2 new species, Dyfrolomycetales, new records, Pleurotremataceae, saprobes, Thymelaeaceae

Introduction

Pleurotremataceae Walt. Watson was introduced by Watson (1929) to accommodate *Pleurotrema* Müll. Arg. with *P. polysemum* (Nyl.) Müll. Arg. as the type species. The familial placement of *Pleurotrema* has been controversial, as the mature asci are neither typically unitunicate nor bitunicate (Mathiassen 1989; Hyde 1992). The placement of *Pleurotrema* has been confirmed based on the re-examined feature of the type of species *P. polysemum*, and Maharachchikumbura et al. (2016) transferred Pleurotremataceae from Sordariomycetes O.E. Erikss. & Winka to Dothideomycetes O.E. Erikss. & Winka and synonymized

Dyfrolomycetaceae K.D. Hyde, K.L. Pang, Alias, Suetrong & E.B.G. Jones under Pleurotremataceae based on morphological comparison. Currently, Pleurotremataceae is accepted as the type and only family in Dyfrolomycetales K.L. Pang, K.D. Hyde & E.B.G. Jones, with three genera, *Dyfrolomyces* K. D. Hyde, *Melomastia* Nitschke ex Sacc, and *Pleurotrema* in this family (Maharachchikumbura et al. 2016; Hongsanan et al. 2020; Wijayawardene et al. 2022; Hyde et al. 2024).

Melomastia was established by Saccardo (1875) to accommodate *M. mastoidea* (Fr.) J. Schröt. (=*Melomastia friesii* Nitschke) as the type species. Previously, relying solely on the morphological features of *Melomastia* type species, the genus was considered unresolved and classified under Ascomycota genera *incertae sedis* (Maharachchikumbura et al. 2016). Subsequently, Norphanphoun et al. (2017) assigned *Melomastia* to Pleurotremataceae based on the newly introduced taxon *M. italica* Norph., Camporesi, T.C. Wen & K.D. Hyde, supported by sequence data. Based on morphology and phylogenetic analyses, Li et al. (2022) synonymized *Dyfrolomyces* under *Melomastia* and simultaneously transferred 11 species from *Dyfrolomyces* to *Melomastia*. De Silva et al. (2022) reported two new records of *Melomastia* from Thailand. However, Kularathnage et al. (2023) maintained *Dyfrolomyces* to accommodate *D. tiomanensis* K.L. Pang, Alias, K.D. Hyde, Suetrong & E.B.G. Jones and *D. chromolaenae* Mapook & K.D. Hyde, based on morphology differences of ascospores and the phylogenetic analyses. Recently, some new taxa from Brazil, China and Thailand have been introduced, *viz. M. beihaiensis* T.Y. Du, K.D. Hyde & Tibpromma (Senanayake et al. 2023), *M. loropetalicola* Kular., W. Dong & K.D. Hyde (Dong et al. 2023), *M. puerensis* R.F. Xu & Tibpromma (Xu et al. 2024), *M. pyriformis* Kular. & Senan. (Kularathnage et al. 2023), *M. septata* J.Y. Zhang, K.D. Hyde & Y.Z. Lu (Hyde et al. 2023), and *M. septemseptata* Muxfeldt & Aptroot (Muxfeldt Naziazeno and Aptroot 2023). Currently, 66 epithets of *Melomastia* are listed in Index Fungorum (2024), while only 20 species have sequences available in GenBank.

Melomastia is characterized by immersed to semi-immersed, globose to subglobose, coriaceous to carbonaceous, ostiolate ascomata, dark brown peridium, filamentous pseudoparaphyses, bitunicate, cylindrical, 8-spored asci, and ascospores are fusiform to oblong, ovoid, or cylindrical, hyaline, 1–10-septate, with rounded or acute ends, with or without gelatinous sheath; while, the asexual morph of *Melomastia* is undetermined (Dayarathne et al. 2020; de Silva et al. 2022; Li et al. 2022; Kularathnage et al. 2023; Xu et al. 2024). Most *Melomastia* species have been recorded as saprobes from various habitats, such as terrestrial, freshwater, marine, and mangrove ecosystems (Hyde 1992; Hyde et al. 2017; Norphanphoun et al. 2017; Dayarathne et al. 2020; Phukhamsakda et al. 2020; Li et al. 2022; Hyde et al. 2023; Tian et al. 2024; Xu et al. 2024). *Melomastia* is a geographically widely distributed genus with a broad host range, which has been systematically documented in Li et al. (2022) and Kularathnage et al. (2023), *viz.* members of *Melomastia* have wide geographical distribution in Africa, Asia, Australia, Europe, and South America, while the reported hosts of *Melomastia* belong to Acanthaceae Juss., Asteraceae Bercht. & J. Presl, Euphorbiaceae Juss., Hamamelidaceae R. Br., Oleaceae Hoffmanns. & Link, Ranunculaceae Juss., Rhizophoraceae Pers., Theaceae Mirb., and Vitaceae Juss.

Aquilaria Lam. is an important agarwood resin-producing tree genus in Thymelaeaceae Juss. Agarwood resin is high-valued and very rare, and its formation is primarily due to injury, followed by microbial infection (Rasool and Mohamed 2016; Azren et al. 2018; Wang et al. 2018). So far, many reports have been published on the pathogenic and endophytic fungi associated with *Aquilaria*, while saprobic fungi have been neglected (Liu et al. 2020; Du et al. 2022a). Prior to 2022, there were only eight records of saprobic fungi associated with *Aquilaria* (Punithalingam and Gibson 1978; Subansenee et al. 1985), and molecular data and comprehensive morphological descriptions were lacking. Recently, 20 saprobic fungal species have been reported from *Aquilaria* spp. by Du et al. (2022b, 2023, 2024), Chethana et al. (2023), and Hyde et al. (2023, 2024) based on both morphological and molecular evidence. Therefore, so far only 28 records of *Aquilaria*-associated fungi have been found. This study focuses on filling the gap in research on the saprobic fungi associated with *Aquilaria*, and enriching the diversity of fungi associated with *Aquilaria*.

In this study, *Aquilaria* plant specimens with black ascomycetous fungal fruiting bodies were collected from Yunnan and Guangdong provinces in China. Based on phylogenetic and morphological analyses, these fungal collections were identified as two new species and one new record of the *Melomastia*. Full descriptions, illustrations, photo plates, and phylogenetic trees to indicate the placement of new taxa are provided.

Materials and methods

Sampling, examination, and isolation

Dead fallen branches of *Aquilaria* spp. with ascomycetous fungal fruiting bodies were collected from subtropical parts of Guangdong and Yunnan provinces in China. After recording important information (Rathnayaka et al. 2024), samples were transported to the laboratory in plastic bags. Morphological structures were examined by using an OPTEC SZ650 dissecting stereomicroscope (Chongqing, China), and an OLYMPUS DP74 (Tokyo, Japan) digital camera on an OLYMPUS optical microscope (Tokyo, Japan) was used to observe and photograph the microstructure of fungi. Micro-morphological structures were measured in Tarosoft ® Image Framework program v. 1.3, and photo plates were edited in Adobe Photoshop CS3 Extended version 22.0.0 software (Adobe Systems, California, the USA).

Fungi were isolated using single-spore isolation, as described by Senanayake et al. (2020). The fruiting bodies were cut by sterilized blades, and the ascospores were picked up by sterilized needles and cultured in potato dextrose agar (PDA) at 23–28 °C for 24–48 hours. The single germinated ascospores were picked up and transferred to PDA at 23–28 °C with recording culture characters.

Specimens were deposited at the Guizhou Medical University (GMB-W) and Mycological Herbarium of Zhongkai University of Agriculture and Engineering (MHZU), China. Living cultures are deposited in the Guizhou Medical University Culture Collection (GMBCC), Guizhou Culture Collection (GZCC), and Zhongkai University of Agriculture and Engineering Culture Collection (ZHKUCC), China. Facesoffungi (FoF) numbers were registered as described in Jayasiri et al. (2015), and MycoBank numbers (MB) were registered as outlined in MycoBank (2024).

DNA extraction, PCR amplification, and sequencing

Molecular studies were carried out according to Dissanayake et al. (2020). Total genomic DNA was extracted from one-month-old fresh fungal mycelium (grew on PDA) using a DNA Extraction Kit-BSC14S1 (BioFlux, Hangzhou, P.R. China) following the manufacturer's instructions. Polymerase chain reactions (PCR) were carried out using the following primers: 28S nrRNA gene (LSU) was amplified by using the primers LR0R and LR5 (Vilgalys and Hester 1990), 18S ribosomal RNA (SSU) was amplified using the primers NS1 and NS4 (White et al. 1990), and translation elongation factor 1-alpha (TEF) was amplified using the primers EF1-983F and EF1-2218R (Rehner 2001). The DNA amplification procedure was performed by PCR in a 25 μL containing 12.5 μL 2×Master Mix (mixture of Easy Taq TM DNA Polymerase, dNTPs, and optimized buffer (Beijing Trans Gen Biotech Co., Chaoyang District, Beijing, China)), 8.5 μL ddH_2O, 2 μL of DNA template, and 1 μL of each forward and reverse primer (10 pM). The PCR thermal cycle programs for LSU, SSU, and TEF were as follows: an initialization step of 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, an annealing step at 55 °C for 50 s, an elongation step at 72 °C for 1 min and a final extension step of 72 °C for 10 min. Purification and sequencing of PCR products were carried out by Sangon Biotech Co., Kunming, China.

Phylogenetic analyses

A combined gene dataset of LSU, SSU, and TEF was used for the phylogenetic analyses. Newly generated contigs were used to carry out the BLASTn search in NCBI to identify the most similarities taxa of our strains. The additional sequences included in the analysis were collected from previous publications (Li et al. 2022; Kularathnage et al. 2023; Xu et al. 2024) and downloaded from GenBank (Benson et al. 2014). Phylogenetic analyses were carried out with 50 sequences (Table 1). The FASTA file used for constructing the Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian Inference analyses (BI) was performed using the OFPT (Zeng et al. 2023) with the protocol. Then, the FASTA file was converted to PHYLIP and NEXUS formats for RAxML and BI phylogenetic analyses in ALTER, respectively (Glez-Peña et al. 2010).

CIPRES Science Gateway platform was used to carry out the Randomized Accelerated Maximum Likelihood (RAxML) and Bayesian Inference analyses (BI) (Miller et al. 2010). The RAxML tree analyzed with 1,000 bootstrap replicates was generated using RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis et al. 2008; Stamatakis 2014) with GTR+I+G model of evolution and bootstrap supports. The BI tree was performed with MrBayes on XSEDE (3.2.7a) (Ronquist et al. 2012) by the Markov Chain Monte Carlo (MCMC) method to evaluate posterior probabilities (BYPP) (Richard and Lippmann 1991; Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002). The best-fit nucleotide substitution models for each dataset were then selected based on the Bayesian information criterion (BIC) from twenty-two common DNA substitution models with rate heterogeneity by ModelFinder (Kalyaanamoorthy et al. 2017). The best model for LSU was TN+F+G4, TIM2e+I for SSU, and TN+F+I+G4 for TEF. Six simultaneous Markov chains were run for 2,000,000 generations, and a tree was sampled every 100th generation.

Table 1. Taxa names, strain numbers, and corresponding GenBank accession numbers of the taxa included in the present study.

Taxa Names	Strain Numbers	GenBank Accession Numbers		
		LSU	SSU	TEF
Acrospermum adeanum	M133	EU940104	EU940031	–
Anisomeridium phaeospermum	MPN539	JN887394	JN887374	JN887418
A. ubianum	MPN94	–	JN887379	JN887421
Dyfrolomyces chromolaenae	MFLUCC 17-1434 T	KY111905	MT214413	MT235800
D. tiomanensis	MFLUCC 13-0440 T	KC692156	KC692155	KC692157
Melomastia aquilariae	ZHKUCC 23-0073 T	OR807856	OR807854	OR832867
M. aquilariae	ZHKUCC 23-0088	OR807857	OR807855	OR832868
M. beihaiensis	KUMCC 21-0084 T	MZ726990	MZ727002	OK043822
M. clematidis	MFLUCC 17-2092 T	MT214607	MT226718	MT394663
M. distoseptata	MFLUCC 21-0102	MT860427	–	–
M. fulvicomae	MFLUCC 17-2083 T	MT214608	MT226719	MT394664
M. fusispora	CGMCC 3.20618 T	OK623464	OK623494	OL335189
M. fusispora	UESTCC 21.0001	OK623465	OK623495	OL335190
M. guangdongensis	**GMBCC1046 T**	**PQ530970**	**PQ530975**	**PQ559185**
M. guangdongensis	**ZHKUCC 23-0040**	**PQ530971**	**PQ530976**	**PQ559186**
M. italica	MFLUCC 15-0160 T	MG029458	MG029459	–
M. loropetalicola	ZHKUCC 22-0174 T	OP791870	OP739334	–
M. maolanensis	GZCC 16-0102 T	–	–	KY814762
M. maomingensis	ZHKUCC 23-0038 T	PP809724	PP809704	PP812255
M. maomingensis	GZCC 23-0619	PP809725	PP809705	PP812256
M. neothailandica	MFLU 17-2589 T	MN017857	–	–
M. oleae	CGMCC 3.20619 T	OK623466	OK623496	OL335191
M. oleae	UESTCC 21.0003	OK623467	OK623497	OL335192
M. oleae	UESTCC 21.0005	OK623468	OK623498	OL335193
M. oleae	UESTCC 21.0006	–	OK623499	OL335194
M. phetchaburiensis	MFLUCC 15-0951 T	MF615402	MF615403	–
M. puerensis	ZHKUCC 23-0802 T	OR922309	OR922340	OR966284
M. puerensis	ZHKUCC 23-0803	OR922310	OR922341	OR966285
M. pyriformis	ZHKUCC 22-0175 T	OP791870	OP739334	OQ718392
M. rhizophorae	BCC15481	–	KF160009	–
M. rhizophorae	JK 5456A	GU479799	–	GU479860
M. septata	MFLUCC 22-0112 T	OP749870	–	OP760198
M. sichuanensis	CGMCC 3.20620 T	OK623469	OK623500	OL335195
M. sichuanensis	UESTCC 21.0008	OK623470	OK623501	OL335196
M. sinensis	MFLUCC 17-1344 T	MG836699	MG836700	–
M. sinensis	MFLUCC 17-2606	OL782048	–	OL875098
M. sinensis	MFLU 17-0777	NG_064507	–	–
M. sinensis	**GMBCC1008**	**PQ530972**	**PQ530977**	**PQ559187**
M. thailandica	MFLU 17-2610	MN017858	MN017923	MN077069
M. thamplaensis	KUMCC 21-0671	OQ170875	OQ168226	OR613415
M. thamplaensis	MFLUCC 15-0635 T	KX925435	KX925436	KY814763

Taxa Names	Strain Numbers	GenBank Accession Numbers		
		LSU	SSU	TEF
M. winteri	CGMCC 3.20621	OK623471	OK623502	OL335197
M. yunnanensis	**GMBCC1009 ^{T}**	**PQ530973**	**PQ530978**	**PQ559188**
M. yunnanensis	**GZCC 23-0621**	**PQ530974**	**PQ530979**	**PQ559189**
Muyocopron heveae	MFLUCC 17-0066 ^{T}	MH986832	MH986828	—
Mu. lithocarpi	MFLUCC 14-1106 ^{T}	KU726967	KU726970	MT136755
Palawania thailandense	MFLU 16-1873	KY086494	—	—
P. thailandense	MFLUCC 14-1121 ^{T}	KY086493	KY086495	—
Stigmatodiscus oculatus	AP161116	—	—	MH756086
S. oculatus	AP171116	—	—	MH756087

Remarks: The newly generated sequences are indicated in bold, the superscript ^{T} indicates ex-type, and “—” indicates information unavailable.

The phylogenetic tree was visualized in FigTree v.1.4.2 (Rambaut 2012), and edited by Microsoft Office PowerPoint 2021 and Adobe Photoshop CS3 Extended version 22.0.0 software (Adobe Systems, California, the USA). All newly generated sequences in this study were deposited to the GenBank (https://www.ncbi.nlm.nih.gov/WebSub/?form=history&tool=genbank).

Results

Phylogenetic analyses

The phylogenetic trees obtained from RAxML and BI analyses provided essentially similar topologies. The RAxML analyses of the combined dataset yielded the best scoring tree (Fig. 1), which comprised 2912 base pairs of LSU = 899, SSU = 1069, and TEF = 944. The final ML optimization likelihood value was -11933.909808. The matrix had 871 distinct alignment patterns, with 23.14% being undetermined characters or gaps. Parameters for the GTR+I+G model of the combined LSU, SSU, and TEF were as follows: estimated base frequencies A = 0.239382, C = 0.262893, G = 0.291472, T = 0.206253; substitution rates AC = 0.831502, AG = 1.991603, AT = 1.062650, CG = 0.930785, CT = 8.413262, GT = 1.000000; proportion of invariable sites I = 0.495458; and gamma distribution shape parameter α = 0.612808. The final RAxML tree is shown in Fig. 1.

In this phylogenetic tree, *Melomastia* was resolved as two clades, *Melomastia sensu lato* (15 species) and *Melomastia sensu stricto* (nine species), and the results are similar to those reported by Li et al. (2022), Kularathnage et al. (2023), and Xu et al. (2024). Kularathnage et al. (2023) have separated *Melomastia* into two clades, *Melomastia sensu lato*, and *Melomastia sensu stricto*; this was done due to *Melomastia sensu stricto*’s close resemblance to the type species *M. mastoidea*, while *Melomastia sensu lato* still needs more collections, sequences, and phenotypic data to support Kularathnage et al. (2023). Our two new species, *M. guangdongensis* (GMBCC1046 and ZHKUCC 23-0040) and *M. yunnanensis* (GMBCC1009 and GZCC 23-0621), and a new record *M. sinensis* (GMBCC1008) clustered within *Melomastia sensu lato*.

New species *M. guangdongensis* (GMBCC1046 and ZHKUCC 23-0040) was well separated from *M. thamplaensis* (KUMCC 21-0671 and MFLUCC 15-0635)

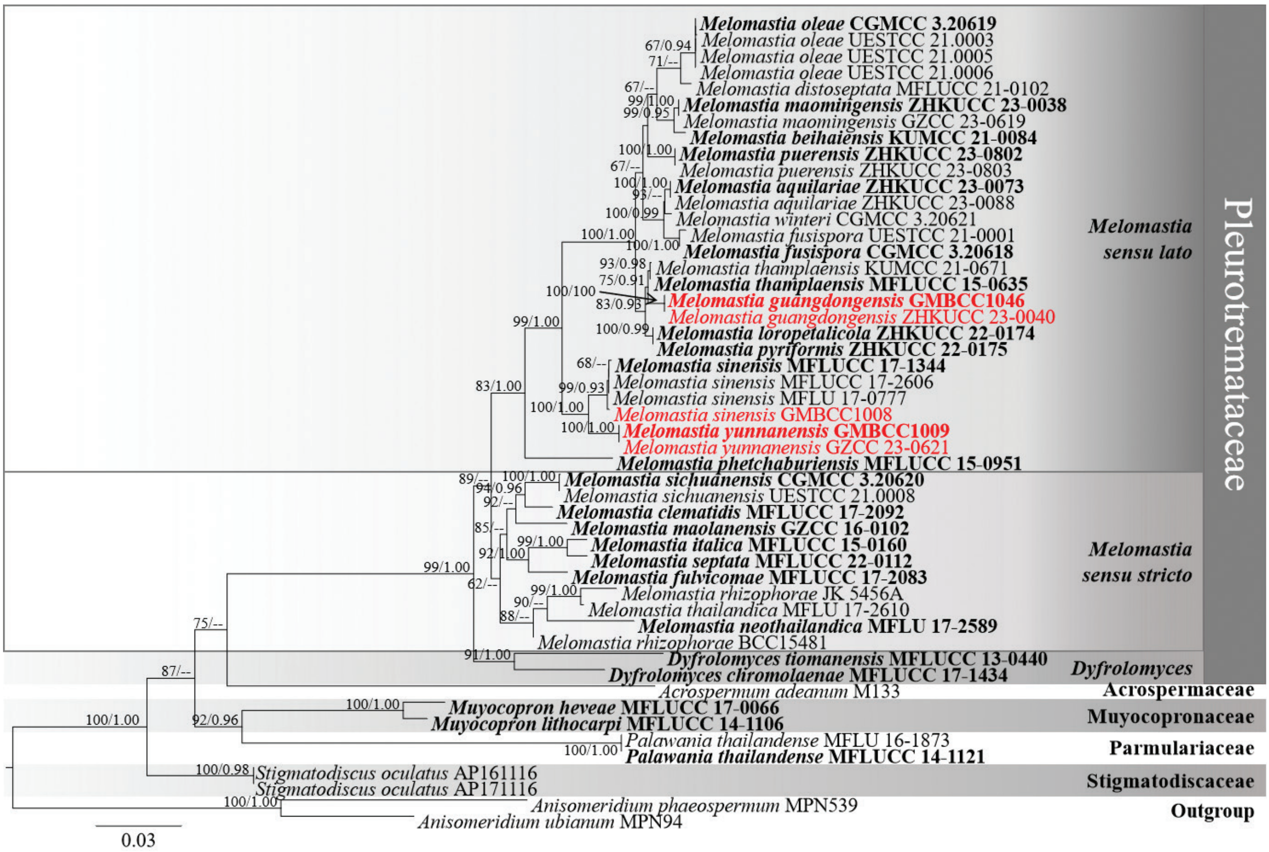

Figure 1. Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, and TEF sequence data of 50 taxa. Bootstrap support values for maximum likelihood (ML) equal to or greater than 60% and clade credibility values greater than 0.90 from Bayesian inference analysis are labelled at each node. The tree is rooted with *Anisomeridium phaeospermum* (MPN539) and *A. ubianum* (MPN94). The new isolates are indicated in red, and the ex-type strains are in bold.

in an independent lineage with 75% ML/0.91 PP statistical support; *M. yunnanensis* (GMBCC1009 and GZCC 23-0621) was well separated from *M. sinensis* (GMBCC1008, MFLU 17-0777, MFLUCC 17-1344 and MFLUCC 17-2606) in a distinct lineage with 100% ML/1.00 PP statistical support. The new record *M. sinensis* (GMBCC1008) was grouped within three strains of *M. sinensis* with 99% ML/0.93 PP statistical support.

Taxonomy

Melomastia guangdongensis **T.Y. Du, K.D. Hyde, Tibpromma & Karun., sp. nov.**
MycoBank No: 856407
Facesoffungi Number: FoF16958
Fig. 2

Etymology. Named after the type locality “Guangdong, China”.

Holotype. MHZU 23-0021

Description. ***Saprobic*** on a dead branch of *Aquilaria sinensis*. ***Sexual morph***: ***Ascomata*** (excluding neck) 180–360 µm high × 200–300 µm diam. ($\bar{x}$ = 267 × 245 µm, n = 10), visible as black dots on the host surface, black, solitary, scattered to gregarious, semi-immersed to immersed, uniloculate, globose to subglobose, coriaceous to carbonaceous, ostiolate.

Ostiolar canal 190–240 µm high × 120–160 µm wide (x̅ = 214 × 140 µm, n = 10), central, black, cylindrical, coriaceous to carbonaceous, filled with hyaline cells. ***Peridium*** 30–60 µm wide (x̅ = 40 µm, n = 20), comprising dense, several layers, outer layers brown to dark brown, thick-walled cells of ***textura angularis*** to ***textura globulosa***, inner layers hyaline, thin-walled cells of ***textura angularis*** to ***textura prismatica*****,** not fusion well with host tissue. ***Hamathecium*** comprising 1.5–3 µm wide, numerous filamentous, filiform, septate, sometimes branched, hyaline, pseudoparaphyses, attached to the base and between the asci, embedded in a gelatinous matrix. ***Asci*** 120–168 × 5.5–7.5 µm (x̅ = 144 × 6.5 µm, n = 30), bitunicate, 8-spored, cylindrical, short pedicel, rounded in apex, with an obvious ocular chamber. ***Ascospores*** (18.7–)20–26 × 5–7 µm (x̅ = 23 × 6 µm, n = 30), overlapping-uniseriate, hyaline, 3-septate at maturity, fusiform with acute ends, slightly constricted at the middle septum, smooth-walled, not surrounded by a mucilaginous sheath. ***Asexual morph*****:** Undetermined.

Culture characteristics. Ascospores germinated on PDA after 24 hours, germ tubes were produced from both ends. ***Colonies*** on PDA reaching 3 cm diam., after two weeks at 23–28 °C. Colonies obverse: dense, circular, white, velvety, slightly raised at the center, entire edge. Colonies reverse: yellow, cream at the margin.

Material examined. China • Guangdong Province, Maoming City, Dianbai District, Poxin, 21°34'28"N, 111°7'39"E, on a dead branch of *Aquilaria sinensis* (Thymelaeaceae), 3 June 2022, T.Y. Du, MMA14, (MHZU 23-0021, holotype), ex-type, GMBCC1046, other living culture, ZHKUCC 23-0040.

Notes. In the phylogenetic analyses, our new collection, *M. guangdongensis* formed a sister branch with *M. thamplaensis* strains (HKAS122773, KUMCC 21-0671, and MFLUCC 15-0635) in *Melomastia sensu lato* clade (Fig. 1) with a 75% ML/0.91 PP bootstrap support. NCBI BLASTn searches of our collection, *M. guangdongensis* showed 99.88% similarity to *M. thamplaensis* (HKAS122773) in the LSU sequence, 100% similarity to *M. thamplaensis* (AND9) in the SSU sequence, and 98.17% similarity to *M. thamplaensis* (KUMCC 21-0671) in the TEF sequence. Our new collection, *M. guangdongensis* shares similar morphology with *M. thamplaensis* in the shape of asci and ascospores. However, *M. thamplaensis* differs from *M. guangdongensis* in having clypeate, raised spots, immersed, subglobose to obpyriform, some with broad, flattened base ascomata, and three strata of peridium (Zhang et al. 2017), while *M. guangdongensis* has semi-immersed to immersed, globose to subglobose ascomata, and two strata of peridium. Base pair differences of the LSU and SSU genes between our new collection *M. guangdongensis* (GMBCC1046, ex-type) and *M. thamplaensis* (MFLUCC 15-0635, ex-type) showed that there are no nucleotide differences, while the TEF has 1.6% nucleotide differences (14/865 bp, without gaps), and a comparison of the TEF nucleotides between new collections and another strain of *M. thamplaensis* (KUMCC 21-0671) resulted in 1.7% differences (15/865 bp, without gaps) (Zhang et al. 2017; Ren et al. 2024). Therefore, we introduce our collection, *M. guangdongensis*, as a new species on a dead branch of *Aquilaria sinensis* from terrestrial habitats in China, based on both morphology and phylogenetic analyses following the guidelines of Maharachchikumbura et al. (2021).

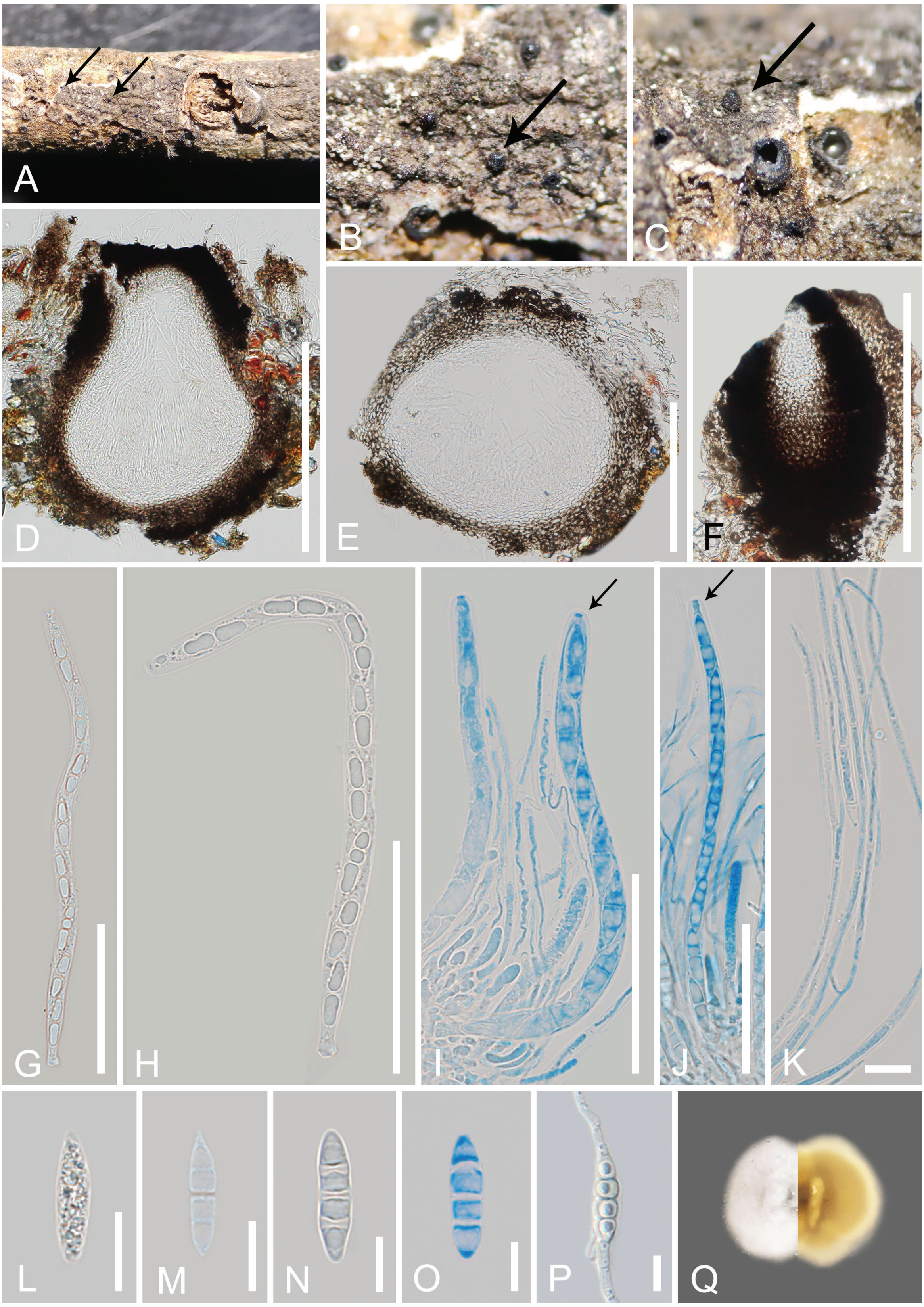

Figure 2. *Melomastia guangdongensis* (MHZU 23-0021, holotype) **A–C** appearance of ascomata on the host (the arrows indicate ascomata) **D, E** vertical sections through the ascomata **F** ostiole **G–J** asci (**I, J** asci stained with cotton blue, and arrows indicate ocular chambers) **K** pseudoparaphyses stained with cotton blue **L–O** ascospores (O ascospore stained with cotton blue) **P** germinated ascospore **Q** colony on PDA obverse and reverse view. Scale bars: 200 µm (**D–F**); 50 µm (**G–J**); 10 µm (**K–P**).

***Melomastia sinensis* (Samarak., Tennakoon & K.D. Hyde) W.L. Li, Maharachch. & Jian K. Liu (2022)**
MycoBank No: 842093
Facesoffungi Number: FoF03935
Fig. 3

Description. ***Saprobic*** on a dead branch of *Aquilaria* sp. ***Sexual morph*****:** ***Ascomata*** (excluding neck) 400–600 µm high × 430–580 µm diam. ($\bar{x}$ = 515 × 520 µm, n = 10), solitary, scattered to gregarious, semi-immersed to immersed, erumpent through host tissue, globose to subglobose, black, coriaceous to carbonaceous, ostiolate. ***Ostiolar canal*** 230–365 µm high × 200–260 µm wide ($\bar{x}$ = 303 × 230 µm, n = 10), central, black, conical, coriaceous to carbonaceous, filled with hyaline sparse periphyses. ***Peridium*** 30–120 µm wide ($\bar{x}$ = 75 µm, n = 20), comprising dense, several layers of thick-walled cells of ***textura angularis*** to ***textura prismatica***, outer layers brown to dark brown, becoming lighter inwardly. ***Hamathecium*** comprising 2.5–6.5 µm wide, numerous filamentous, filiform, septate, unbranched, hyaline pseudoparaphyses, attached to the base and between the asci, embedded in a gelatinous matrix. ***Asci*** 175–220 × 8.5–11.5 µm ($\bar{x}$ = 195 × 10.5 µm, n = 30), bitunicate, 8-spored, cylindrical, long pedicel, thickened and rounded apex, with an obvious ocular chamber. ***Ascospores*** (17.5–)20–26.5 × 7–9 µm ($\bar{x}$ = 24 × 8 µm, n = 30), overlapping-uniseriate, hyaline, when ascospores gather together, they appear light yellow, mostly 6–7-septate at maturity, cylindrical, with rounded ends, slightly constricted at the septum, often similar width of cells with several small guttules, not surrounded by a mucilaginous sheath. ***Asexual morph*****:** Undetermined.

Culture characteristics. Ascospores germinated on PDA after 24 hours, germ tubes were produced from most cells, germinated ascospores appear light yellow. ***Colonies*** on PDA reaching 3 cm diam., after two weeks at 23–28 °C. Colonies obverse: dense, circular or irregular, umbonate, cream, light yellow at the center, entire or undulate edge. Colonies reverse: dark gray, yellow at the margin.

Material examined. China • Yunnan Province, Xishuangbanna, Jinghong City, Naban River Nature Reserve, 22°7'48"N, 100°40'24"E, on a dead branch of *Aquilaria* sp. (Thymelaeaceae), 14 September 2021, Tianye Du, YNA41 (GMB-W 1006, new host and geographical record), living culture, GMBCC1008.

Host and distribution. *Aquilaria* sp. (China; this study), *Camellia sinensis* (Thailand; Hyde et al. 2018), and *Hevea brasiliensis* (Thailand; Senwanna et al. 2021).

Notes. In the phylogenetic analyses, our new collection (GMBCC1008) isolated from a dead branch of *Aquilaria* sp. grouped with *Melomastia sinensis* strains (MFLUCC 17-1344, MFLUCC 17-2606 and MFLU 17-0777) in *Melomastia sensu lato*, with a 99% ML/0.93 PP bootstrap support (Fig. 1). NCBI BLASTn searches of our collection showed 99.78% similarity to *M. sinensis* (MFLUCC 17-2606) in the LSU sequence, 99.21% similarity to *M. oleae* (UESTCC 21.0006) in the SSU sequence, and 99.67% similarity to *M. sinensis* (MFLUCC 17-2606) in the TEF sequence.

Melomastia sinensis (=*Dyfrolomyces sinensis* Samarak., Tennakoon & K.D. Hyde) was introduced by Hyde et al. (2018) as a saprobic on *Camellia sinensis* (L.) Kuntze stems. Our new collection shares a similar morphology with *M. sinensis* (MFLU 17-0777, holotype) in cylindrical ascospores with 6–7-septate ascospores. Our new collection has semi-immersed to

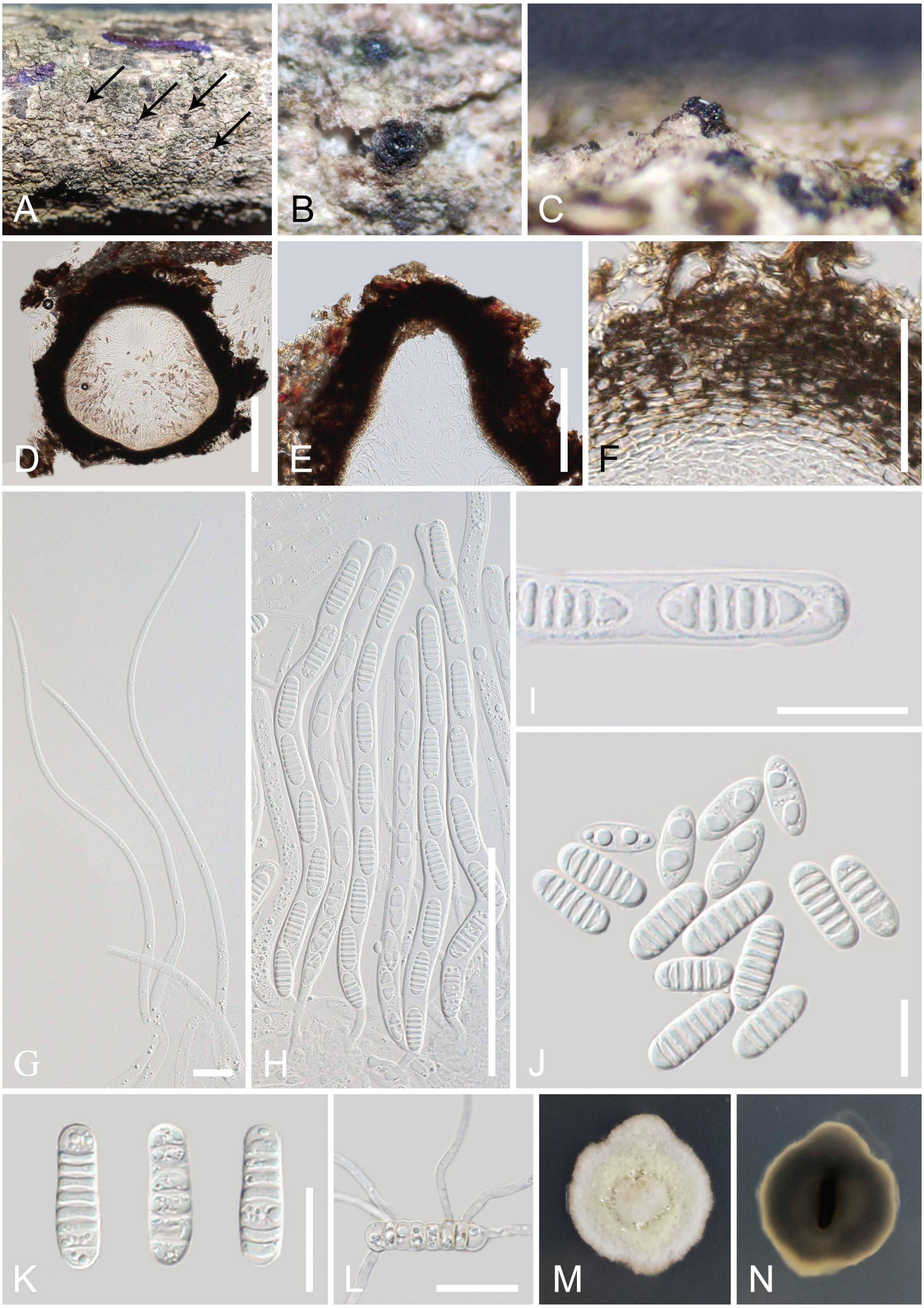

Figure 3. *Melomastia sinensis* (GMB-W 1006, new host and geographical record) **A–C** appearance of ascomata on the host (A the arrows indicate ascomata) **D** vertical sections through the ascoma **E** ostiole **F** peridium **G** pseudoparaphyses **H** asci **I** ascus with an ocular chamber **J, K** ascospores **L** germinated ascospore **M, N** colony on PDA obverse and reverse view. Scale bars: 200 µm (**D, E**); 100 µm (**H**); 50 µm (**F**); 20 µm (**I–L**); 10 µm (**G**).

immersed ascomata, differs from *M. sinensis* (MFLU 17-0777, holotype) in having superficial ascomata (Hyde et al. 2018) and differs from immersed ascomata in *M. sinensis* (MFLU 19-0232) (Senwanna et al. 2021). However, the nucleotide base pair differences between our new collection (GMBCC1008) and *M. sinensis* (MFLUCC 17-1344, ex-type) showed that the LSU and SSU gene has no nucleotide differences, while the TEF gene of *M. sinensis* (MFLUCC 17-1344, ex-type) is unavailable in NCBI (Hyde et al. 2018). The comparison of the TEF nucleotides between the new collection and another strain of *M. sinensis* (MFLUCC 17-2606) resulted in 0.3% differences (3/873 bp, without gaps) (Senwanna et al. 2021). This study first discovered *M. sinensis* on *Aquilaria* sp. in China. Therefore, we introduce our new collection as a new host and geographical record of *M. sinensis* based on both morphological study and phylogenetic analyses.

Melomastia yunnanensis **T.Y. Du, K.D. Hyde, Tibpromma & Karun., sp. nov.**
MycoBank No: 856408
Facesoffungi Number: FoF16959
Fig. 4

Etymology. Named after the type location “Yunnan, China”.

Holotype. GMB-W 1007

Description. ***Saprobic*** on a dead branch of *Aquilaria* sp. ***Sexual morph*****:** ***Ascomata*** (excluding neck) 400–500 µm high × 300–480 µm diam. ($\bar{x}$ = 458 × 395 µm, n = 10), solitary, scattered to gregarious, immersed to erumpent through host tissue, globose, black, carbonaceous, ostiolate. ***Ostiolar canal*** 100–160 µm high × 120–230 µm wide ($\bar{x}$ = 130 × 184 µm, n = 10), central, black, conical, carbonaceous, filled with hyaline sparse periphyses. ***Peridium*** 25–75 µm wide ($\bar{x}$ = 55 µm, n = 10), comprising of dense, several layers of brown to dark brown, thick-walled cells of ***textura angularis*** to ***textura prismatica***. ***Hamathecium*** comprising 2.5–7.5 µm wide, numerous filamentous, filiform, septate, sometimes branched, hyaline pseudoparaphyses, attached to the base and between the asci, embedded in a gelatinous matrix. ***Asci*** 180–220 × 7.5–10.5 µm ($\bar{x}$ = 195.5 × 9 µm, n = 30), bitunicate, 8-spored, cylindrical, short pedicel, thickened and rounded apex, with an obvious ocular chamber. ***Ascospores*** 20–24.5 × 6–8 µm ($\bar{x}$ = 22.5 × 7 µm, n = 30), overlapping-uniseriate, hyaline, when ascospores gather together, they appear light yellow, mostly 6–8-septate at maturity, mostly 7-septate, cylindrical, with rounded ends, slightly constricted at the septum, often similar width of cells with several small guttules, not surrounded by a mucilaginous sheath. ***Asexual morph*****:** Undetermined.

Culture characteristics. Ascospores germinated on PDA after 24 hours, germ tubes were produced from both ends, germinated ascospores appear light brown. ***Colonies*** on PDA reaching 2 cm diam., after two weeks at 23–28 °C. Colonies obverse: dense, circular, umbonate, gray at the center, cream, and entire edge. Colonies reverse: gray brown, light brown at the margin.

Material examined. China • Yunnan Province, Xishuangbanna, Jinghong City, Naban River Nature Reserve, 22°7'51"N, 100°40'21"E, on a dead branch of *Aquilaria* sp. (Thymelaeaceae), 14 September 2021, Tianye Du, YNA51 (GMB-W 1007, holotype), ex-type, GMBCC1009, other living culture, GZCC 23-0621.

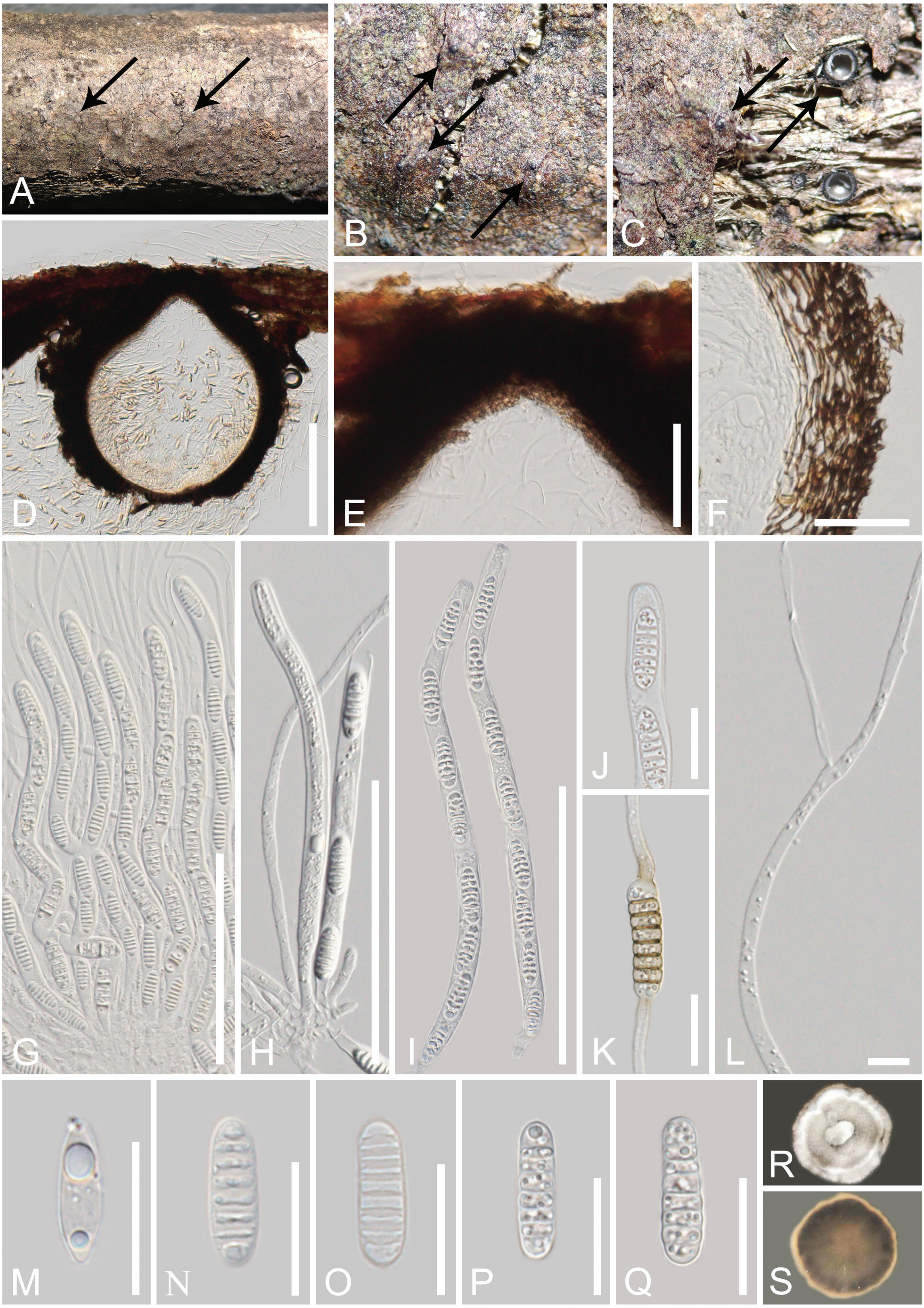

Figure 4. *Melomastia yunnanensis* (GMB-W 1007, holotype) **A–C** appearance of ascomata on the host (the arrows indicate ascomata) **D** vertical sections through the ascoma **E** ostiole **F** peridium **G–I** asci **J** asci ocular chamber **K** germinated ascospore **L** pseudoparaphyses **M–Q** ascospores **R, S** colonies on PDA obverse and reverse view. Scale bars: 200 μm (**D**); 100 μm (**G–I**); 50 μm (**E, F**); 20 μm (**J, K, M–Q**); 10 μm (**L**).

Notes. In the phylogenetic analyses, our new collection, *M. yunnanensis* formed a sister branch with *M. sinensis* (MFLUCC 17-1344, MFLUCC 17-2606, MFLU 17-0777, and GMBCC1008) in *Melomastia sensu lato* with a 100% ML/1.00 PP bootstrap support (Fig. 1). NCBI BLASTn searches of our collection *M. yunnanensis* showed 99.23% similarity to *M. sinensis* (MFLUCC 17-2606) in the LSU sequence, 98.92% similarity to *M. thamplaensis* (AND9) in the SSU sequence, and 96.34% similarity to *M. sinensis* (MFLUCC 17-2606) in the TEF sequence. Our new collection, *M. yunnanensis* shares similar morphology with *M. sinensis* in cylindrical and septate ascospores. However, *M. sinensis* differs from *M. yunnanensis* in having superficial, semi-immersed to immersed ascomata, cylindrical or conical ostiolar canal, and unbranched pseudoparaphyses (Hyde et al. 2018), while our *M. yunnanensis* has immersed ascomata, conical ostiolar canal, and pseudoparaphyses sometimes branched. In addition, the nucleotide base pair differences between our new collection *M. yunnanensis* (GMBCC1009, ex-type) and *M. sinensis* (MFLUCC 17-1344, ex-type) showed the LSU gene has 0.5% nucleotide differences (4/760 bp, without gaps), the SSU gene has 0.5% nucleotide differences (4/813 bp, without gaps), while the TEF gene of *M. sinensis* (MFLUCC 17-1344, ex-type) is unavailable (Hyde et al. 2018). We compared the TEF nucleotides between the new collection and another collection of *M. sinensis* (MFLUCC 17-2606), which resulted in 3.8% differences (33/873 bp, without gaps) (Senwanna et al. 2021). Therefore, we introduce our new collection, *M. yunnanensis*, as a new species on a dead branch of *Aquilaria* sp. from terrestrial habitats in China, based on both morphological study and phylogenetic analyses following the guidelines of Maharachchikumbura et al. (2021).

Discussion

Based on the morphological study and phylogenetic analyses, this study identifies, describes, and introduces two new species, *Melomastia guangdongensis* and *M. yunnanensis*, and a new host and geographical record of *M. sinensis* from *Aquilaria* spp. These findings significantly contribute to the understanding of the diversity and distribution of agarwood resin-producing tree-associated fungi.

Our phylogenetic analysis based on LSU, SSU, and TEF also showed that the results are similar to those of Kularathnage et al. (2023) and Xu et al. (2024), who have divided *Melomastia* into two clades, *Melomastia sensu lato* and *Melomastia sensu stricto*. However, the majority of species are clustered in *Melomastia sensu lato*, and only 20 out of 66 listed records in Index Fungorum (2024) have available sequences, posing a challenge for the study of phylogenetic analysis in this genus. To address this, we believe it is necessary to explore and collect more samples of new and known species of *Melomastia* and supplement our research with molecular studies. In addition, relevant information about *Melomastia*, such as life mode, habitat, host, geographical location, and ecological niche, must be collected and analyzed to enhance our knowledge of this genus.

Morphologically, most species in *Melomastia* have fusiform or ellipsoidal ascospores, while two species (*M. marinospora* and *M. sinensis*) show cylindrical ascospores (Li et al. 2022). Previously, the ascospores of this genus are usually reported 3-septate (e.g. *M. aquatica*, *M. clematidis*, *M. distoseptata*, *M. fusispora*, *M. maolanensis*, *M. marinospora*, *M. oleae*, *M. sichuanensis*, *M. thamplaensis*, and *M. winteri*) (Li et al. 2022). Current studies as more new taxa were introduced into this genus reveal multi-septate ascospores, while these taxa with similar characteristics do not cluster together on the phylogenetic tree (Fig. 1), such as *M. mangrovei* (7–9-septate, no molecular data available in NCBI), *M. phetchaburiensis* (1–10-septate, in *Melomastia sensu lato*), *M. rhizophorae* (4–6-septate, in *Melomastia sensu stricto*), *M. sinensis* (6–7-septate, in *Melomastia sensu lato*), and *M. thailandica* (3–5-septate, in *Melomastia sensu stricto*) (Li et al. 2022). In this study, *M. guangdongensis* shows the fusiform with 3-septate ascospores, while *M. yunnanensis* shows the cylindrical with 6–8-septate ascospores, both of these new taxa belong to *Melomastia sensu lato*. Therefore, more studies are needed to discuss the morphological and phylogenetic connections of this genus. In addition, in this study, we also found *Melomastia* from the same host genus *Aquilaria*, but when we compare ascomata, semi-immersed to immersed ascomata in *M. guangdongensis* and *M. sinensis*. In contrast, ascomata of *M. yunnanensis* are immersed to erumpent through host tissue. Further research is needed to explore whether the attachment mode of ascomata on the substrate is influenced by the host, environment, or other factors.

In recent years, many studies on saprobic fungi in economic crops, such as rice, sugarcane, rubber, coffee, mango, and macadamia nuts, have been published (Yang et al. 2022, Lu et al. 2024, Tian et al. 2024, Xu et al. 2024, Zhang et al. 2024). However, there is a noticeable lack of research on saprobic fungi in *Aquilaria* spp. This study introduces three saprobic fungal taxa, expanding the previous record of 28 saprobic fungi associated with *Aquilaria* to 31. It also highlights the urgent need for further, more in-depth investigations. We believe that future studies with a broader geographical range will be crucial in enhancing our understanding of the distribution and diversity of fungi in *Aquilaria*.

Acknowledgments

The authors are grateful to the High-Level Talent Recruitment Plan of Yunnan Province ("High-End Foreign Experts" Program and "Young Talents" Program). Tianye Du extends her heartfelt gratitude to Mae Fah Luang University for granting a tuition-fee scholarship for her Ph.D. study. Shaun Pennycook is thanked for his assistance in selecting species epithets for the new species.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The authors are grateful to the Special Basic Cooperative Research Innovation Programs of Qujing Science and Technology Bureau & Qujing Normal University (Grant No. KJL-H2022YB03), the Special Basic Cooperative Research Programs of Yunnan Provincial Undergraduate Universities (Grant No. 202101BA070001-209, 202101BA070001-279), the Yunnan Fundamental Research projects [202201AU070017], Mee-mann Chang

Academician Workstation in Yunnan Province (Grant No. 202205AF150002), and Yunnan Province Young and Middle-aged Academic and Technical Leaders Reserve Talents Program (Grant No.202305AC350252), and General Programs of the Provincial Department of Science and Technology (Grant No. 202101BA070001-076) for support. The authors also extend their appreciation to the Researchers Supporting Project number (RSP2025R56), King Saud University, Riyadh, Saudi Arabia.

Author contributions

Conceptualization: SCK, ST. Data curation: TYD. Formal analysis: SCK. Funding acquisition: DQD, ST, HHW. Investigation: SCK, ST, TYD. Methodology: SCK, ST, XFL, TYD. Project administration: ST, DQD, HHW. Resources: TYD. Software: TYD. Validation: SCK, ST, AM, EC, KDH. Visualization: TYD. Writing – original draft: TYD. Writing – review and editing: SN, KDH, AM, XFL, CN, AME, EC, SCK, ST, TYD, DQD, HHW.

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Data availability

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

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MycoKeys 111: 87–110 (2024)
DOI: 10.3897/mycokeys.111.136483

Research Article

Three new microfungi (Ascomycota) species from southern China

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This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

OPEN ACCESS

Academic editor:
Samantha C. Karunarathna
Received: 6 September 2024
Accepted: 13 November 2024
Published: 11 December 2024

Citation: Li D, Zhang M, Zhang J, Ma L, Zhang Z, Zhang J, Zhang X, Xia J (2024) Three new microfungi (Ascomycota) species from southern China. MycoKeys 111: 87–110. https://doi.org/10.3897/mycokeys.111.136483

Abstract

Apiospora, *Microdochium* and *Pestalotiopsis* have been reported as plant pathogens, endophytes or saprotrophes worldwide. Combining multiple molecular markers with morphological characteristics, this study proposes three new species, viz. *Apiospora bambusigena*, *Microdochium jianfenglingense* and *Pestalotiopsis solicola* from southern China. *Apiospora bambusigena* and *M. jianfenglingense* were collected from bamboo in Hainan Province and *P. solicola* was collected from soil in Yunnan Province. The morphologically similar and phylogenetically closely-related species were compared.

Key words: *Apiospora*, *Microdochium*, multigene phylogeny, *Pestalotiopsis*, Sordariomycetes taxonomy, three new taxa

Introduction

Bamboo, belonging to the subfamily Bambusoideae of the grass family Poaceae, is an evergreen plant with shallow roots, primarily distributed in the tropical and subtropical regions (Yeasmin et al. 2015; Dai et al. 2017). Bamboo is of high economic value; it can be used to build houses and furniture and has medicinal properties; bamboo shoots can be eaten (Jansen et al. 1995; Dransfield and Widjaja 1996; Lin 2004). Since the 18th century, when Léveillé initiated the endeavour, research into bambusicolous fungi has been conducted (Léveillé 1845). Since entering the 21st century, the diversity of bambusicolous fungi has been gradually explored (Hyde et al. 2002; Yeasmin et al. 2015; Dai et al. 2016, 2017).

Soil is an excellent culture media for the growth and development of various microorganisms, including fungi. Soil fungi play important roles in terrestrial ecosystems as decomposers in terrestrial ecosystems, participating in the carbon cycle and as pathogens and mutually beneficial symbiotic organisms of plants and animals (Taylor and Sinsabaugh 2015; Wu et al. 2024). Numerous soil-inhabiting fungi are specialised symbionts of forest trees or parasites on plant roots, but most are saprotrophs (Guarro et al. 2012).

Sordariomycetes was first introduced by Eriksson & Winka in 1997 and it was the second largest taxa in Ascomycota after Dothideomycetes, mainly characterised by non-lichenised, flask-shaped sporocarps (perithecia) and unitunicate asci (Eriksson and Winka 1997; Lumbsch 2000; Kirk et al. 2008; Hyde et al. 2013; Maharachchikumbura et al. 2015, 2016). Dai et al. (2017) reported many fungi in the taxa of Sordariomycetes on bamboo plants. Before that, reports on bambusicolous fungi were incomplete. During the experiment, various Sordariomycetes fungi were isolated from bamboo and soil, amongst which *Apiospora*, *Microdochium* and *Pestalotiopsis* accounted for a large proportion. *Apiospora* was first introduced by Saccardo in 1875 and is mainly characterised by globose to subglobose conidia, which are usually lenticular in side view, obovioid and pale-brown to brown (Hyde et al. 1998; Dai et al. 2017). *Microdochium* was first introduced by Sydow in 1924, mainly characterised by polyblastic, sympodial or annellidic conidiogenous cells with hyaline falcate conidia (Hernández-Restrepo et al. 2016). *Pestalotiopsis* was first introduced by Steyaert in 1949, mainly characterised by 5-celled conidia (Steyaert 1949). The classification and phylogenetic studies of bambusicolous fungi had important economic significance. Some bambusicolous fungi were pathogens that caused bamboo diseases, such as *Linearistroma lineare* and *Calonectria* spp., which affected the growth and development of bamboo (Dai et al. 2017). On the other hand, some bambusicolous fungi were beneficial to humans. For example, the metabolite hypocrellin produced by *Shiraia bambusicola* is of great significance in anti-cancer treatment (Dai et al. 2017).

In this study, three new species of Sordariomycetes were found amongst samples collected in the Hainan and Yunnan Provinces of China. They were identified and classified by multi-locus analysis of tandem internal transcribed spacer (ITS), 28S large subunit ribosomal RNA gene (LSU), partial RNA polymerase II second-largest subunit (RPB2), translation elongation factor 1-alpha gene (TEF1α) and beta-tubulin gene region (TUB2) datasets. The new species are described and discussed, based on their morphological characteristics along with their molecular sequence data.

Materials and methods

Sampling site

Bambusoideae plant and soil specimens were collected from Hainan and Yunnan Provinces in China and important information was noted following Rathnayaka et al. (2024). Hainan Province (18°10'–20°10'N, 108°37'–111°03'E) is located in southern China, on the northern edge of the tropics, with an abundance of tropical climate resources and fertile soil. The climate is warm and humid, which is suitable for the growth of a variety of plants, especially bamboo. Thus, Hainan Province contains abundant resources of bambusicolous fungi. Yunnan Province (21°8'–29°15'N, 97°31'–116°11'E) of China is a mountain and plateau region on the country’s south-western frontier. Yunnan boasts the most diverse array of biological resources amongst all Chinese provinces, encompassing a wide range of plants and fungi originating from tropical, subtropical, temperate and alpine growth zones.

Isolates and morphological analysis

For fresh plant tissues such as leaves, 6–8 sections (0.5 × 0.5 cm) of diseased or healthy tissues were selected and surface sterilised in 75% ethanol for 0.5 minutes, rinsed once in sterile distilled water and then immersed in a 5% sodium hypochlorite solution for 1 minute, followed by being rinsed thoroughly three times using sterile distilled water (Jiang et al. 2021a, 2021b). After rinsing three times in sterile distilled water, the tissue sections were transferred to sterilised and dried filter paper with sterilised tweezers and after the residual moisture dried, the tissue sections were spread on to potato dextrose agar (PDA: 200 g potato, 20 g dextrose, 15 g agar, sterilised distilled water added and filled to 1 litre, natural pH) medium plates and 2–4 sections were placed symmetrically on each PDA plate. For dried plant tissues such as withered twigs and other dry plant tissues, the tissues were observed under the body microscope and the single conidia were picked out with a slender picking needle and placed on to the PDA plates and 3–5 individual spores were picked out for each PDA plate. For soil samples, the dilution spreading method was adopted. A soil sample weighing 10 g was mixed with 90 ml of sterile distilled water in a conical flask. The flask was shaken at 200 rpm for 30 minutes and then allowed to settle briefly. The supernatant was extracted and diluted by 10, 100 and 1,000 times using a pipette. A volume of 100 µl of each diluted soil solution was dispensed on to PDA plates that contained streptomycin resistance materials (400 µl of 50 mg/ml streptomycin to 200 ml of PDA). A spreading rod was utilised to distribute the liquid evenly on the plates. Following a 10-minute stand, the culture plate was sealed. The prepared PDA plate was placed in a biological incubator at 25 °C for 3–4 days and then purified on a new medium plate after single colonies were grown to obtain a pure strain.

The individual colonies on the 7th and 14th days were morphologically observed and captured using a digital camera (Canon Powershot G7X). Additionally, the micromorphological characteristics of the colonies were examined with the aid of a stereomicroscope (Olympus SZX10) as well as a microscope (Olympus BX53). The two microscopes, equipped with Olympus DP80 and OPTIKA SC2000 HD colour digital cameras, observed the microscopic morphological characteristics of the structures generated during culture and captured and recorded the microscopic structure of the fungi. The pure cultured strains obtained in this experiment were cut into 0.5 × 0.5 cm pieces with a sterile scalpel and stored in a 2 ml frozen tube with 20% sterilised glycerine and 6–8 pieces were placed in each frozen tube and the frozen tube for fungal strain preservation was stored at -20 °C for further study (Wang et al. 2023; Zhang et al. 2023a).

Structural measurements were carried out using Digimizer software (v.5.6.0), with a minimum of 30 measurements for each characteristic, such as conidiophores, conidiogenous cells and conidia (Zhang et al. 2022a). The voucher specimens have been deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP) and the Herbarium Mycologicum Academiae Sinicae, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS). The ex-holotype living cultures have been archived in the Shandong Agricultural University Culture Collection (SAUCC) and the China General Microbiological Culture Collection Center (CGMCC). The taxonomic information of the new taxa has been submitted to MycoBank (http://www.mycobank.org, accessed on 29 Oct 2024).

DNA extraction, PCR amplification and sequencing

The mycelium was scraped from the growing colonies on the medium plate and the mycelium tissue was processed into a fine powder by use of a mortar or mill. The DNA of the fungal genome was extracted through the utilisation of the modified cetyltrimethylammonium bromide (CTAB) method (Guo et al. 2000) or magnetic bead kit method (OGPLF-400, GeneOnBio Corporation, Changchun, China) (Zhang et al. 2023b). Table 1 lists the five genes viz. ITS, LSU, RPB2, TEF1α and TUB2 which were used in this paper, as well as the primers and PCR reaction procedures for locus amplification.

The PCR reaction was conducted utilising an Eppendorf Master Thermocycler (Hamburg, Germany), and the detailed procedure for this reaction is provided in Table 1. Specifically, the PCR reaction was carried out in a 12 μl reaction system with the reaction composition of 6 μl 2 × Taq Master Mix (Dye Plus) (Vazyme, Nanjing, China, P112-01). The forward and reverse primers were 0.5 μl each (10 μM TsingKe, Qingdao, China), 1.5 μl template genomic DNA (about 10 ng/μl) and 3.5 μl sterilised distilled water. The resulting PCR products were examined by 1% agarose gel electrophoresis, stained with GelRed and the bands with the same size as the target fragment were observed under an ultraviolet lamp (Zhang et al. 2022b). Then the gel extraction kit (Cat: AE0101-C) (Shandong Sparkjade Biotechnology Co., Ltd.) was employed to recover the gel. The PCR amplified gene sequences were sequenced bidirectionally by Sangon Biotech Co., Ltd (Shanghai, China). Consistent sequences were obtained using MEGA v. 7.0 (Kumar et al. 2016). All sequences generated in this study have been deposited in GenBank, as detailed in Table 2.

Phylogenetic analyses

The nucleotide sequences of three new species were submitted to the NCBI's GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/, accessed on 29 Oct 2024) and the related species of all reference sequences were retrieved for phylogenetic analysis (Zhang et al. 2021). Employing the online MAFFT version 7 services and the automated policy (http://mafft.cbrc.jp/alignment/server/, accessed on 29 Oct 2024) to determine the arrangement of individual

Table 1. Gene loci and corresponding PCR primers and programmes used in this study.

Locus	PCR primers	Sequence (5' – 3')	PCR cycles	References
ITS	ITS5	GGA AGT AAA AGT CGT AAC AAG G	(94 °C: 30 s, 55 °C: 30 s, 72 °C: 45 s) × 29 cycles	(White et al. 1990)
	ITS4	TCC TCC GCT TAT TGA TAT GC		
LSU	LR0R	GTA CCC GCT GAA CTT AAG C	(94 °C: 30 s, 48 °C: 50 s, 72 °C: 1 min 30 s) × 35 cycles	(Vilgalys and Hester 1990; Rehner and Samuels 1994)
	LR5	TCC TGA GGG AAA CTT CG		
RPB2	RPB2-5F2	GGG GWG AYC AGA AGA AGG C	(94 °C: 45 s, 60 °C: 45 s, 72 °C: 2 min) × 5 cycles, (94 °C: 45 s, 54 °C: 45 s, 72 °C: 2 min) × 30 cycles	(Liu et al. 1999; Sung et al. 2007)
	RPB2-7CR	CCC ATR GCT TGY TTR CCC AT		
TEF1α	EF1	ATG GGT AAG GAR GAC AAG AC	(95 °C: 30 s, 51 °C: 30 s, 72 °C: 1 min) × 35 cycles	(O'Donnell et al. 1998)
	EF2	GGA RGT ACC AGT SAT CAT GTT		
TUB2	Bt2a	GGT AAC CAA ATC GGT GCT GCT TTC	(95 °C: 30 s, 56 °C: 30 s, 72 °C: 1 min) × 35 cycles	(Glass and Donaldson 1995)
	Bt2b	ACC CTC AGT GTA GTG ACC CTT GGC		

Table 2. Names, strain numbers, substrates, regions and corresponding GenBank accession numbers of the taxa obtained in this study.

Species	Strain No.	Substrate	Region	GenBank Accession No.				
				ITS	LSU	RPB2	TEF1α	TUB2
Apiospora bambusigena	SAUCC 2446-2^{T}	Bambusoideae sp. (leaf)	Jianfengling National Forest Park	PP702396	PP711785	–	PP716797	PP716801
	SAUCC 2446-6	Bambusoideae sp. (leaf)	Jianfengling National Forest Park	PP702397	PP711786	–	PP716798	PP716802
Microdochium jianfenglingense	SAUCC 1862-2^{T}	Bambusoideae sp. (leaf)	Jianfengling National Forest Park	PP702394	PP711783	PP716793	–	PP716799
	SAUCC 1862-5	Bambusoideae sp. (leaf)	Jianfengling National Forest Park	PP702395	PP711784	PP716794	–	PP716800
Pestalotiopsis solicola	SAUCC003804^{T}	Soil	Kunming, Fumin County	OQ692020	–	–	OQ718737	OQ718795
	SAUCC003806	Soil	Kunming, Fumin County	OQ692021	–	–	OQ718738	OQ718796
	SAUCC003807	Soil	Kunming, Fumin County	OQ692022	–	–	OQ718739	OQ718797

Notes: Ex-type strains are marked with "T".

locus, multiple sequence analysis and, if necessary, manual correction (Katoh et al. 2019). The newly-generated sequence (Table 2) is compared with related sequences (Suppl. materials 5–7). To the species level, phylogenetic analysis was performed for each locus, followed by a combined multi-locus analysis.

The phylogenetic analyses relied on Maximum Likelihood (ML) and Bayesian Inference (BI) for the multi-locus studies. To determine the optimal evolutionary model for each segment in BI, MrModelTest v. 2.3 (Nylander 2004) was utilised and the selected models were integrated into the analysis framework. Both ML and BI were executed on the CIPRES Science Gateway portal (https://www.phylo.org/, accessed on 29 Oct 2024) (Miller et al. 2012), using RaxML-HPC2 on XSEDE v. 8.2.12 (Stamatakis 2014) for ML and MrBayes on XSEDE v. 3.2.7a (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012) for BI. The default parameters were employed in the case of ML analyses, while BI was implemented with a rapid bootstrapping algorithm incorporating an automatic halt feature. The Bayesian analyses encompassed five concurrent runs spanning 5,000,000 generations, incorporating a stop rule and a sampling frequency of 50 generations. The burn-in fraction was set at 0.25 and posterior probabilities (PP) were calculated from the remaining trees. The resulting tree visualisations were generated using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, accessed on 29 Oct 2024) or ITOL: Interactive Tree of Life (https://itol.embl.de/, accessed on 29 Oct 2024) (Letunic and Bork 2021) and the final layout of the trees was refined in Adobe Illustrator CC 2019.

Results

Phylogenetic analyses

During the extensive sample collection and identification process in Hainan and Yunnan, *Apiospora*, *Microdochium* and *Pestalotiopsis* fungi exhibited high isolation frequencies, occupying a significant proportion of the total isolated fungi. Consequently, this paper describes three novel *Apiospora*, *Microdochium* and *Pestalotiopsis* species.

Apiospora bambusigena sp. nov.

Phylogenetic analysis was conducted on 101 isolates with 100 isolates of *Apiospora* species designated as the ingroup and a single strain of *Arthrinium caricicola* (CBS 145127) serving as the outgroup. The ultimate alignment encompassed 2140 concatenated characters, viz. 1–400 (ITS), 401–1200 (LSU), 1201–1600 (TEF1α) and 1601–2140 (TUB2). Amongst these, 1361 characters were constant, 234 were variable and parsimony-uninformative and 545 were parsimony-informative. The alignment comprises 918 distinct alignment patterns, with a percentage of gaps and fully undetermined characters standing at 24.62%. Estimated base frequencies were as follows: A = 0.235023, C = 0.243229, G = 0.260724, T = 0.261024; substitution rates AC = 1.392836, AG = 4.247629, AT = 1.251156, CG = 0.856792, CT = 5.000798 and GT = 1.000000; gamma distribution shape parameter α = 0.210667. Final ML Optimisation Likelihood: -17347.108598. The topology of the ML tree concurred with that derived from Bayesian Inference; thus, only the ML tree is presented. Based on the phylogeny of four genes, the 101 strains were categorised into 92 species (Suppl. material 1). The SYM+I+G model was proposed for ITS, the GTR+I+G for LSU and TUB2 and the HKY+G for TEF1α. MCMC analysis of these four tandem genes was performed over 3,535,000 generations in 70,702 trees. The initial 17,674 trees, representing the aging phase, were discarded, while the remaining trees contributed to calculating posterior probabilities in the majority rule consensus tree (Fig. 1; first value: BIPP ≥ 0.90 displayed). The alignment embodied 918 unique site patterns (ITS: 152, LSU: 182, TEF1α: 241, TUB2: 343).

Microdochium jianfenglingense sp. nov.

Phylogenetic analysis was conducted on 60 isolates comprising 58 ingroup isolates of *Microdochium* species and two outgroup strains of *Idriella lunata* (CBS 204.56, CBS 177.57). The final alignment encompassed 3034 concatenated characters, viz. 1–590 (ITS), 591–1423 (LSU), 1424–2244 (RPB2) and 2245–3034 (TUB2). Of these, 2228 were constant, 78 were variable and parsimony-uninformative and 728 were parsimony-informative. The alignment comprises 925 distinct alignment patterns, with a percentage of gaps and fully undetermined characters at 19.50%. Estimated base frequencies were as follows: A = 0.236313, C = 0.263048, G = 0.260231, T = 0.240408; substitution rates AC = 1.047671, AG = 5.296563, AT = 1.395107, CG = 0.980853, CT = 6.856348 and GT = 1.000000; gamma distribution shape parameter α = 0.125986. Final ML Optimisation Likelihood: -18130.357478. The topology of the ML tree concurred with that derived from Bayesian Inference; thus, only the Bayesian tree is presented. Based on the phylogeny of four genes, the 60 strains were categorised into 38 species (Fig. 2). The GTR+I+G model was proposed for ITS, LSU and TUB2 and the HKY+I+G for RPB2. MCMC analysis of these four tandem genes was performed over 1,885,000 generations in 56,552 trees. The initial 18,850 trees, representing the aging phase, were discarded, while the remaining trees contributed to calculating posterior probabilities in the majority rule consensus tree (Fig. 2; first value: BIPP ≥ 0.90 displayed). The alignment embodied 925 unique site patterns (ITS: 232, LSU: 92, RPB2: 336, TUB2: 265).

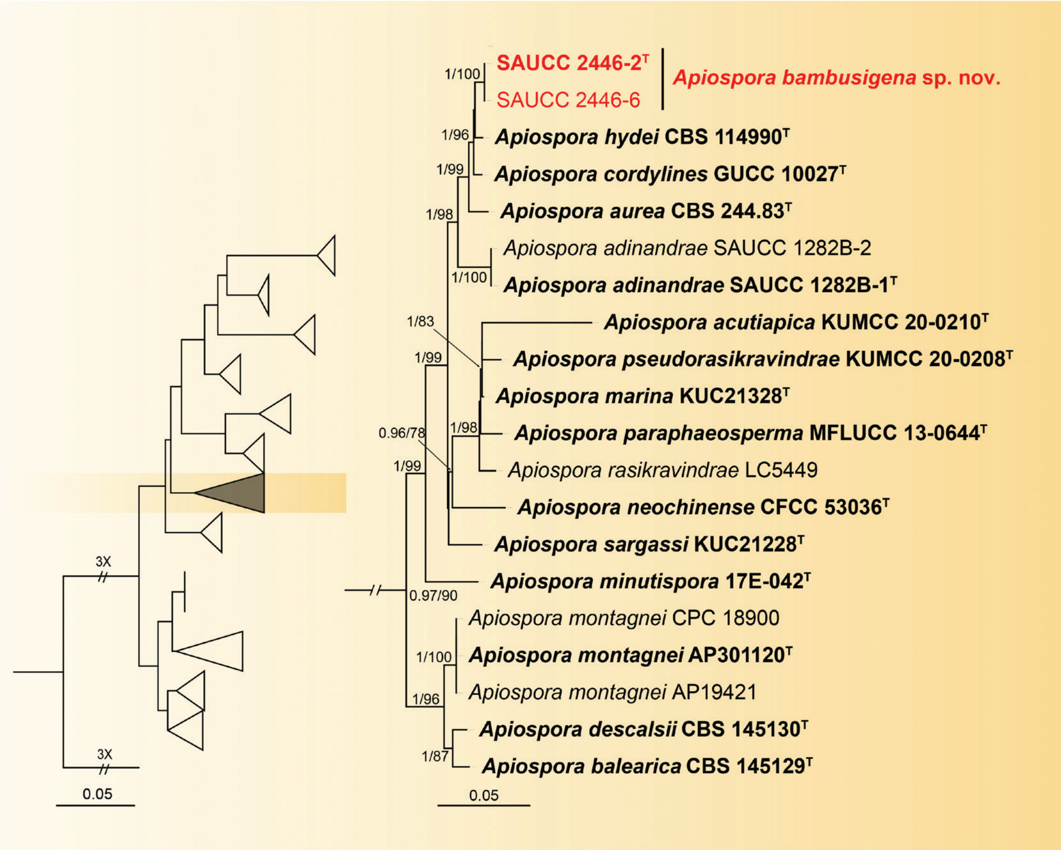

Figure 1. A Maximum Likelihood Inference tree based on a combined dataset of analysed ITS, LSU, TEF1α and TUB2 sequences. The Bayesian Inference Posterior Probability (left, BIPP ≥ 0.90) and the Maximum Likelihood Bootstrap Value (right, MLBV ≥ 75%) are shown as BIPP/MLBV above the nodes. Ex-type cultures are indicated in boldface and strains from the present study are in red. The scale bar at the bottom indicates 0.05 substitutions per site. To enhance the visual appeal of the evolutionary tree layout, certain branches are shortened by two diagonal lines ("//") with the number of times. The figure shows partial branches of the evolutionary tree related to *Apiospora bambusigena* sp. nov. and the full evolutionary tree can be found in Suppl. material 1.

Pestalotiopsis solicola sp. nov.

Phylogenetic analysis was conducted on 184 isolates with 183 isolates of *Pestalotiopsis* species designated as the ingroup and a single strain of *Neopestalotiopsis magna* (MFLUCC 12-0652) serving as the outgroup. The ultimate alignment encompassed 1738 concatenated characters, viz. 1–538 (ITS), 539–884 (TEF1α) and 885–1738 (TUB2). Amongst these, 1017 characters were constant, 241 were variable and parsimony-uninformative and 480 were parsimony-informative. The alignment has 918 distinct alignment patterns. The proportion of gaps and fully undetermined characters stands at 24.01%. Estimated base frequencies were as follows: A = 0.233313, C = 0.301076, G = 0.212813, T = 0.252798; substitution rates AC = 0.948798, AG = 3.135932, AT = 1.068842, CG = 0.904564, CT = 4.060719 and GT = 1.000000; gamma distribution shape parameter α = 0.324087. Final ML Optimisation Likelihood: -13940.781313. The topology exhibited by the ML tree verifies the corresponding topology derived from Bayesian Inference; accordingly, only the Bayesian tree is displayed. Based on the phylogenetic analysis of three genes, the 184 strains were categorised into 109 species (Suppl. material 2). The HKY+I+G model was proposed for ITS, TEF1α and TUB2. MCMC analysis of these three tandem genes was performed

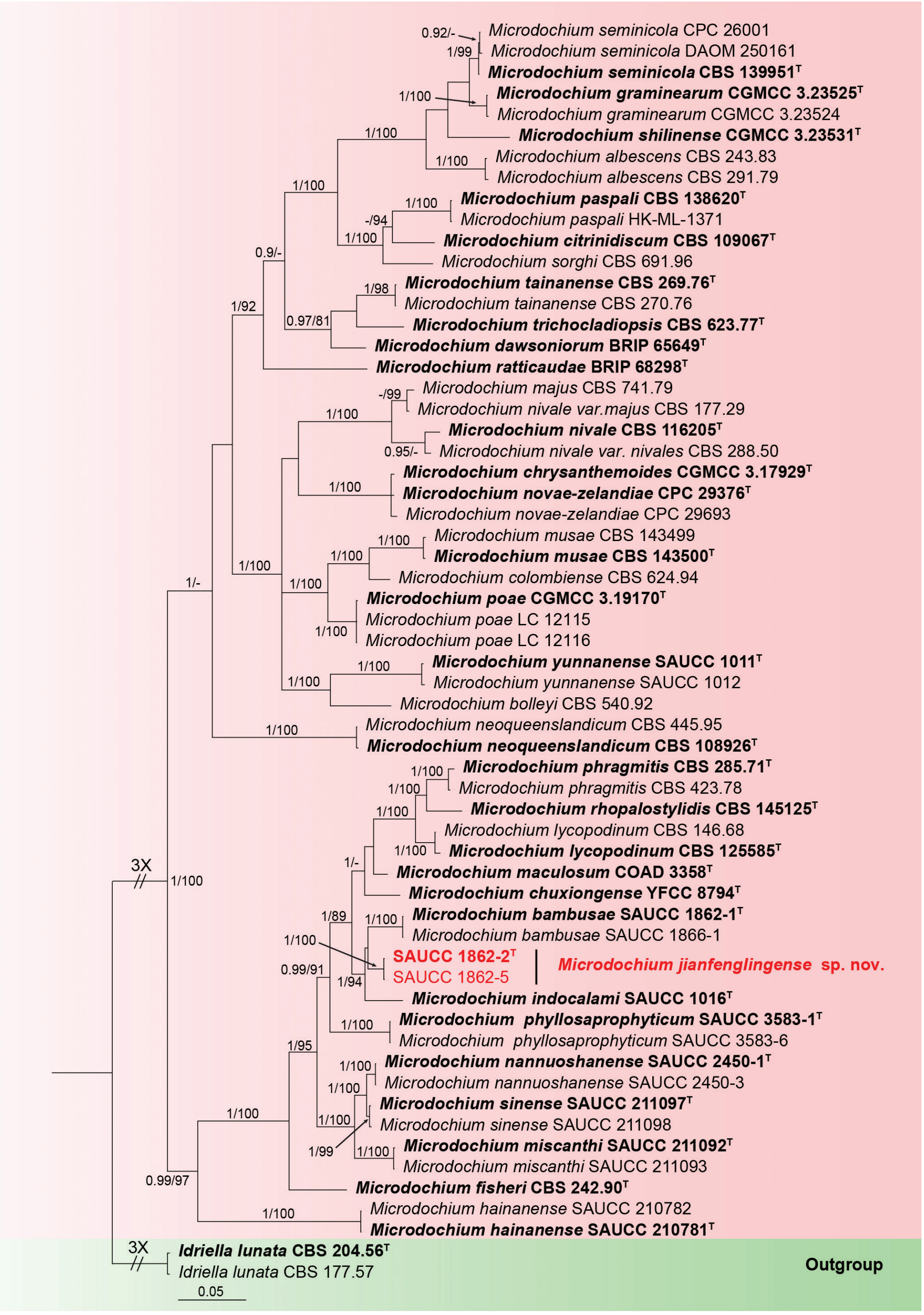

Figure 2. A Bayesian Inference tree based on a combined dataset of analysed ITS, LSU, RPB2 and TUB2 sequences. The Bayesian Inference Posterior Probability (left, BIPP ≥ 0.90) and the Maximum Likelihood Bootstrap Value (right, MLBV ≥ 75%) are shown as BIPP/MLBV above the nodes. Ex-type cultures are indicated in boldface, and strains from the present study are in red. The scale bar at the bottom indicates 0.05 substitutions per site. To enhance the visual appeal of the evolutionary tree layout, certain branches are shortened by two diagonal lines ("//") with the number of times.

over 3,325,000 generations in 49,878 trees. The initial 16,624 trees, which represent the aging phase of the analysis, are excluded, whereas the remaining trees are utilised for computing the posterior probability in the majority rule consensus tree (Fig. 3; first value: BIPP ≥ 0.80 displayed). The alignment embodied 918 unique site patterns (ITS: 177, TEF1α: 258, TUB2: 483).

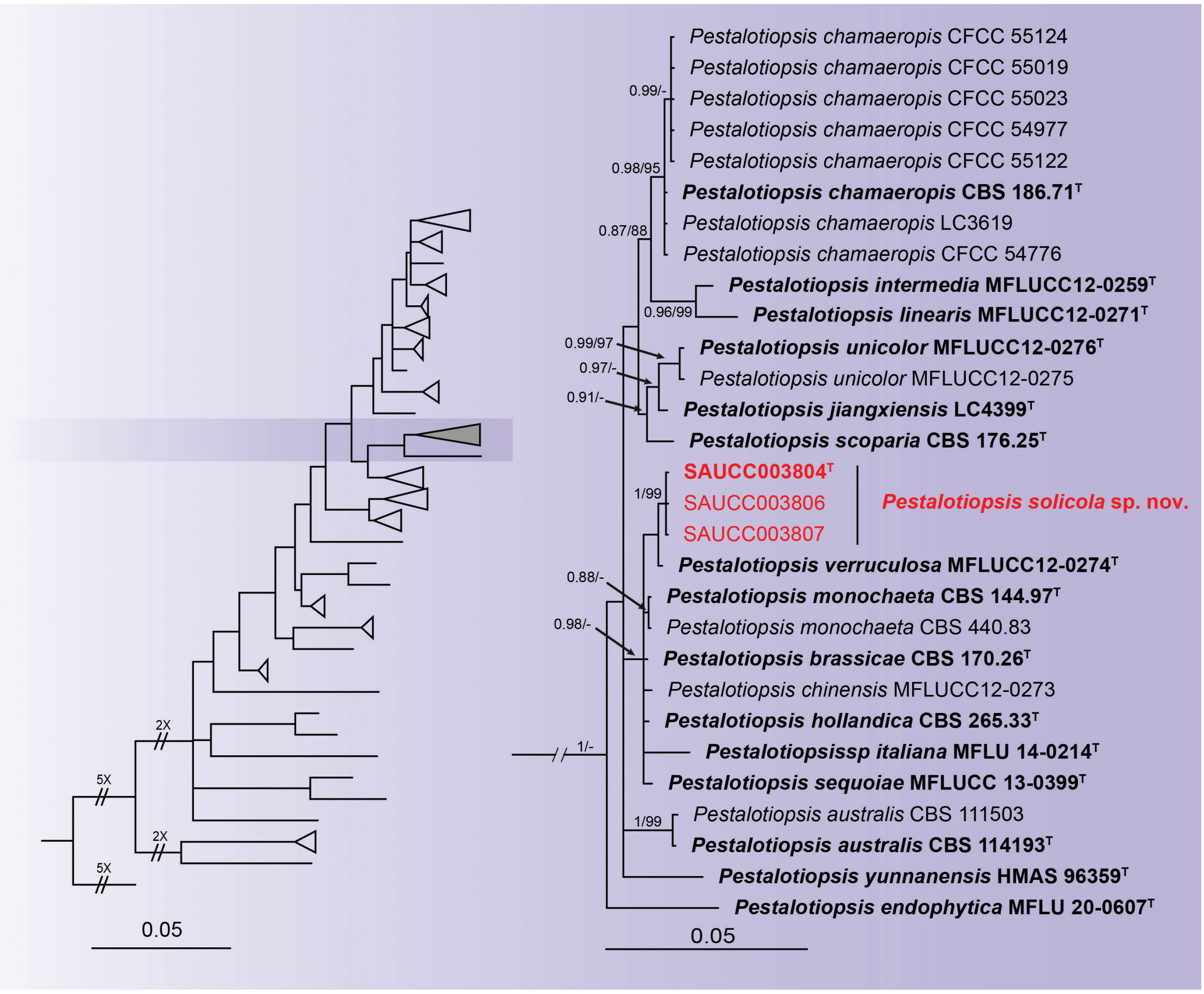

Figure 3. A Bayesian Inference tree based on a combined dataset of analysed ITS, TEF1α and TUB2 sequences. The Bayesian Inference Posterior Probability (left, BIPP ≥ 0.80) and the Maximum Likelihood Bootstrap Value (right, MLBV ≥ 75%) are shown as BIPP/MLBV above the nodes. Ex-type cultures are indicated in boldface and strains from the present study are in red. The scale bar at the bottom indicates 0.05 substitutions per site. To enhance the visual appeal of the evolutionary tree layout, certain branches are shortened by two diagonal lines ("//") with the number of times. The figure shows partial branches of the evolutionary tree related to *Pestalotiopsis solicola* sp. nov. The full evolutionary tree can be found in Suppl. material 2.

In the phylogenetic analyses of *Apiospora*, 100 isolates are clustered as a monophyletic clade (Suppl. material 1). Isolates SAUCC 2446-2 and SAUCC 2446-6 formed a new clade sister to *Apiospora hydei* (CBS 114990) shown in the phylogram. Similarly, for *Microdochium*, 58 isolates are found as a monophyletic clade (Fig. 2). Isolates SAUCC 1862-2 and SAUCC 1862-5 formed a new clade sister to *Microdochium bambusae* (SAUCC 1862-1, SAUCC 1866-1) shown in the phylogram. In the *Pestalotiopsis* phylogenetic analyses, 183 isolates are clustered as a monophyletic clade (Suppl. material 2). Isolates SAUCC003804, SAUCC003806 and SAUCC003807 formed a new clade sister to *Pestalotiopsis verruculosa* (MFLUCC12-0274) shown in the phylogram. The present research has identified three distinct new species: *Apiospora bambusigena*, *Microdochium jianfenglingense* and *Pestalotiopsis solicola*.

Taxonomy

Apiospora bambusigena **D.H. Li, Z.X. Zhang, J.W. Xia & X.G. Zhang, sp. nov.**

MycoBank No: 853701
Fig. 4

Type. China • Hainan Province: Jianfengling National Forest Park, on diseased leaves of Bambusoideae sp., 12 April 2023, D.H. Li (HMAS 352970, holotype), ex-holotype living culture SAUCC 2446-2 = CGMCC 3.27948.

Etymology. The epithet *bambusigena* refers to the fungus produced on *bambusae*.

Description. Conidiomata in culture sporodochial, aggregated or solitary, erumpent, black, surrounded by white mycelium. Conidiophores simple or confluent, hyaline, cylindrical to clavate, 7.8–18.8 × 3.7–4.6 μm, usually reduced to conidiogenous cells. Conidiogenous cells aggregative, hyaline, smooth, cylindrical, 5.2–8.8 × 3.0–4.6 μm. Conidia circular to slightly elliptical, immature conidia hyaline, rough, maturity conidia tanned to black, smooth, without a central scar, 15.0–18.0 × 14.5–17.0 μm, mean ± SD = 16.5 ± 1.0 × 16.0 ± 0.9 μm, n = 30. Sexual morph unknown.

Culture characteristics. The colonies diameter reached 80 mm after 14 days of dark culture at 25 °C on PDA, slightly rising above the surface of the substrate, non-uniform flocculent aerial mycelium and entire edge, white; reverse white.

Additional material studied. China • Hainan Province: Jianfengling National Forest Park, on diseased leaves of Bambusoideae sp., 12 April 2023, D.H. Li, HSAUP 2446-6, living culture SAUCC 2446-6.

Notes. Phylogenetic analyses of four combined sequences (ITS, LSU, TEF1α and TUB2) showed that *Apiospora bambusigena* constitutes a distinct clade, closely affiliated with *A. hydei* (CBS 114990). *Apiospora bambusigena* is distinguished from *A. hydei* by 13/598, 1/1152, 20/351 and 8/467 in ITS, LSU, TEF1α and TUB2 sequences, respectively. Morphologically, the conidia of *A. bambusigena* are narrower than *A. hydei* (15.0–18.0 × 14.5–17.0 μm vs. 15.0–17.0 × 19.0–22.0 μm) and the conidiophores of *A. bambusigena* are shorter than *A. hydei* (7.8–18.8 × 3.7–4.6 μm vs. 20–40 × 3–5 μm) (Crous and Groenewald 2013; Pintos and Alvarado 2021).

Microdochium jianfenglingense **D.H. Li, Z.X. Zhang, J.W. Xia & X.G. Zhang, sp. nov.**

MycoBank No: 853702
Fig. 5

Type. China • Hainan Province: Jianfengling National Forest Park, on diseased leaves of Bambusoideae sp., 12 April 2023, D.H. Li (HMAS 352971, holotype), ex-holotype living culture SAUCC 1862-2 = CGMCC 3.27947.

Etymology. The epithet *jianfenglingense* refers to the Jianfengling National Forest Park, where the holotype was collected.

Description. Conidiophores simple, hyaline, cylindrical to clavate, sometimes reduced to conidiogenous cells. Conidiogenous cells straight or slightly curved, 15.0–25.5 × 1.9–3.0 μm, monoblastic or polyblastic, terminal, denticulate,

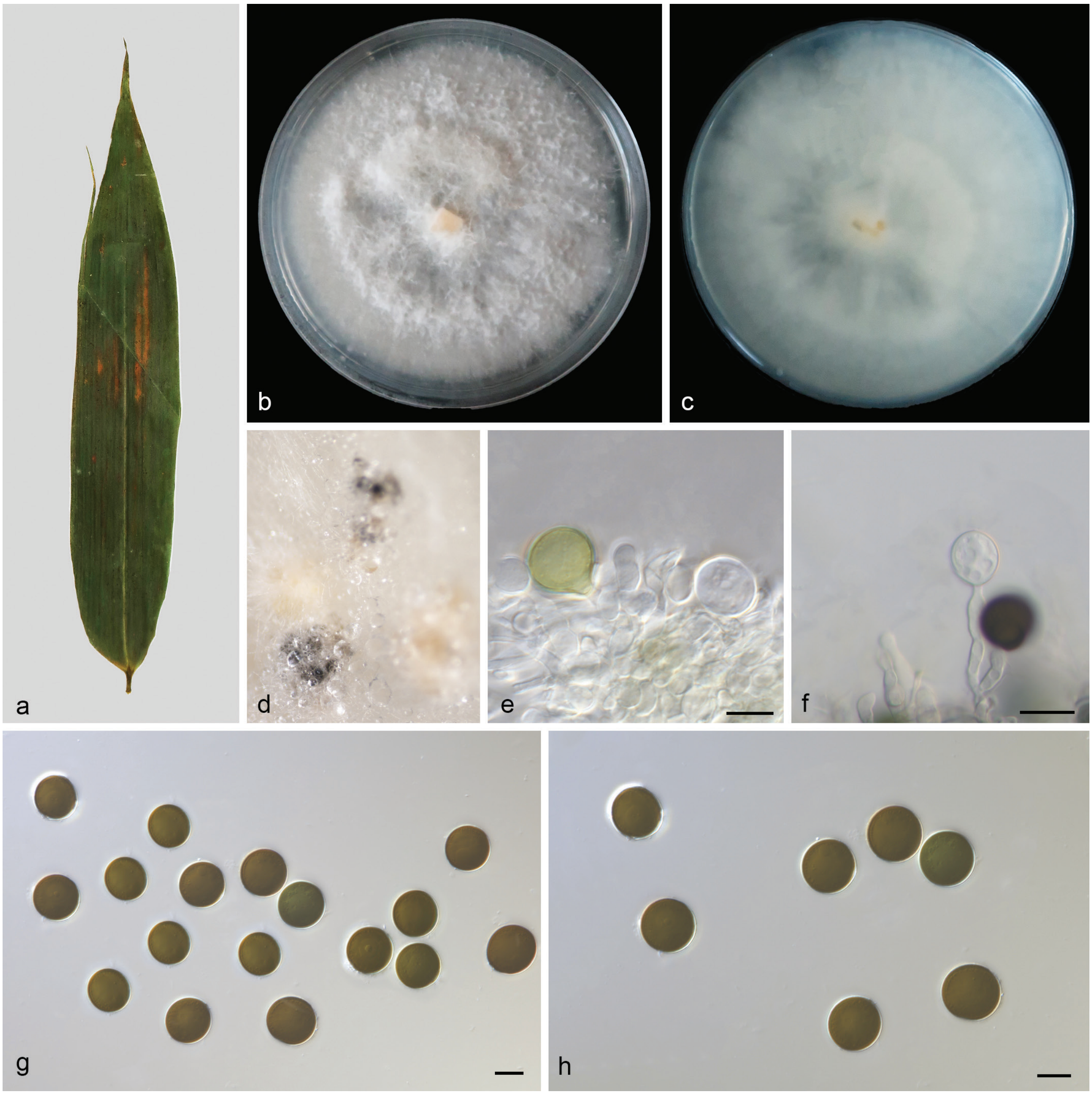

Figure 4. *Apiospora bambusigena* (HMAS 352970, holotype) **a** a leaf of Bambusoideae sp. **b, c** surface and reverse sides of colony after 14 days on PDA **d** colony overview with conidiomata **e, f** conidiogenous cells with conidia **g, h** conidia. Scale bars: 10 µm (**e–h**).

transparent, smooth, cylindrical and septate and produced on aerial mycelia. Conidia are solitary, hyaline, often 3-septate, spindle, oblong to ellipsoid, straight or curved, 13.0–24.0 × 2.5–4.5 µm, mean ± SD = 17.5 ± 2.5 × 3.4 ± 0.5 µm, n = 30, multi-guttulate and sometimes borne directly from the hyphae. No chlamydospores were observed. Sexual morph unknown.

Culture characteristics. The colonies diameter reached 69–72 mm after 14 days of dark culture at 25 °C on PDA, colonies exhibited concentric spreading, fluffy, marginal aerial mycelium white to cream, gradually turning tawny towards the centre; reverse white to tawny. The colonies diameter reached 64–74 mm after 14 days of dark culture at 25 °C on OA, colonies concentrically spreading, fluffy, aerial mycelium milky white, substrate mycelium grey in the medium; reverse white.

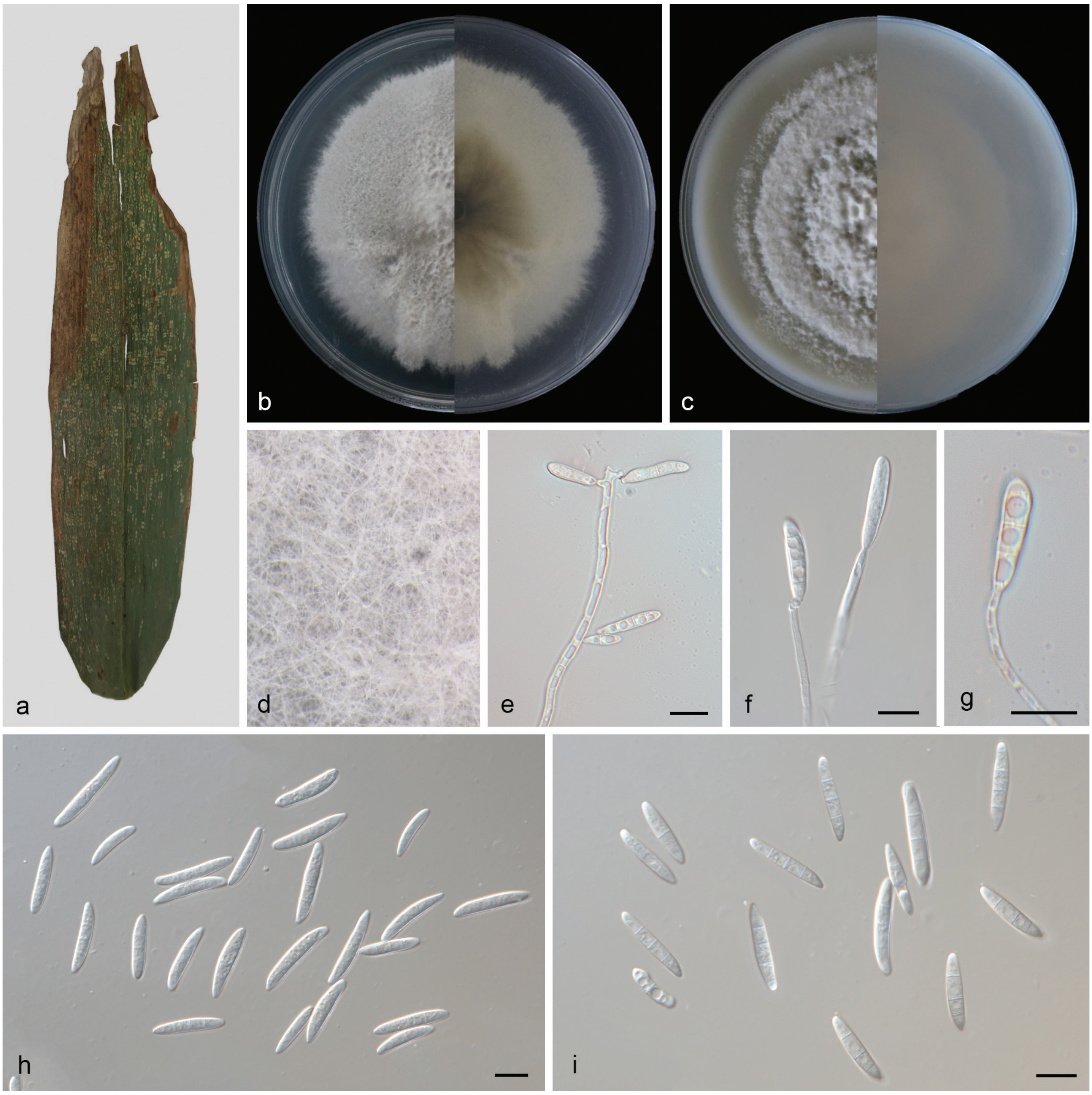

Figure 5. *Microdochium jianfenglingense* (HMAS 352971, holotype) **a** a leaf of Bambusoideae sp. **b, c** surface and reverse sides of the colony after 14 days on PDA, OA **d** colony overview **e–g** conidiogenous cells with conidia **h, i** conidia. Scale bars: 10 μm (**e–i**).

Additional material studied. China • Hainan Province: Jianfengling National Forest Park, on diseased leaves of Bambusoideae sp., 12 April 2023, D.H. Li, HSAUP 1862-5, living culture SAUCC 1862-5.

Notes. Phylogenetic analyses of four combined sequences (ITS, LSU, RPB2 and TUB2) showed that *Microdochium jianfenglingense* constitutes a distinct clade, closely affiliated with *M. bambusae* (SAUCC 1862-1 and SAUCC 1866-1) and *M. indocalami* (SAUCC 1016). *Microdochium jianfenglingense* is distinguished from *M. bambusae* (SAUCC 1866-1) by 7/535, 3/828 and 59/912 characters and from *M. indocalami* (SAUCC 1016) by 24/539, 1/832 and 48/840 characters in ITS, LSU and RPB2 sequences, respectively. Morphologically, the conidia of *M. jianfenglingense* are longer than *M. bambusae* and *M. indocalami* (13.0–24.0 × 2.5–4.5 μm vs. 13.0–17.0 × 2.5–3.5 μm vs. 13.0–15.5 × 3.5–5.5 μm). Conidiogenous cells of *M. jianfenglingense* are shorter than *M. bambusae* and *M. indocalami* (15.0–25.5 × 1.9–3.0 μm vs. 17.4–30.0 × 2.5–3.0 μm vs. 11.0–28.3 × 1.5–2.9 μm) (Huang et al. 2020; Zhang et al. 2023).

Pestalotiopsis solicola **D.H. Li, Z.X. Zhang, J.W. Xia & X.G. Zhang, sp. nov.**

MycoBank No: 854062
Fig. 6

Type. China • Yunnan Province, Kunming, Fumin County, in soil, 20 May 2023, D.H. Li (HMAS 352972, holotype), ex-holotype living culture SAUCC 003804 = CGMCC 3.22681.

Etymology. The epithet refers to the substrate “soil” from which the holotype was isolated.

Description. Conidiomata appear as sporodochial structures in culture, solitary or aggregated, black, erumpent, exuding dark conidial masses. Conidiophores simple or confluent, hyaline, cylindrical to clavate, usually reduced to conidiogenous cells. Conidiogenous cells aggregative, smooth, cylindrical to clavate, hyaline, 15.0–40.4 × 2.7–7.2 μm. Conidia fusoid, straight or slightly curved, 4-septate, smooth, slightly constricted at the septa, 24.3–32.4 × 8.0–10.0 μm; basal cell obconic with a truncate base, 2.0–5.9 μm long, thin-walled, hyaline, basal appendages single, unbranched, tubular, straight or slightly bent, 10.3–13.4 μm long; median cells 3, trapezoid or subcylindrical, thick-walled, pale brown to brown, 18.8–21.3 μm long, specifically, the first median cell from base 3.1–7.5 μm long, the second median cell 6.2–8.2 μm long, the third median cell 4.8–6.9 μm long; apical cell conic with an acute apex, hyaline, thin-walled, 2.1–5.5 μm long; apical appendages 2–5, unbranched, tubular, straight or slightly curved, 25.0–32.0 μm long. Sexual morph unknown.

Culture characteristics. The colonies diameter reached 75–80 mm after 7 days of dark culture at 25 °C on PDA, whitish, flat, with flocculent aerial mycelium forming concentric rings and entire edge; reverse white.

Additional material studied. China • Yunnan Province, Kunming, Fumin County, in soil, 20 May 2023, D.H. Li, HSAUP 003806, living culture SAUCC 003806; ibid., HSAUP 003807, living culture SAUCC 003807.

Notes. Phylogenetic analyses of three combined sequence (ITS, TEF1α and TUB2) showed that *Pestalotiopsis solicola* was found to constitute a distinct clade, closely affiliated with *P. brassicae* (CBS 170.26), *P. chinensis* (MFLUCC 12-0273), *P. hollandica* (CBS 265.33), *P. italiana* (MFLU 14-0214), *P. monochaeta* (CBS 144.97 and CBS 440.83), *P. sequoiae* (MFLUCC 13-0399) and *P. verruculosa* (MFLUCC 12-0274). *P. solicola* differs from: *P. brassicae* by 6/261 bp in TEF1α, *P. hollandica* by 6/273 bp in TEF1α and 6/769 bp in TUB2, *P. italiana* by 9/442 bp in ITS, 7/266 bp in TEF1α and 3/446 bp in TUB2, *P. monochaeta* by 15/282 bp in TEF1α, *P. verruculosa* by 1/540 bp in ITS and 2/273 bp in TEF1α. In addition, a small phylogenetic tree containing the individual genes TEF1α

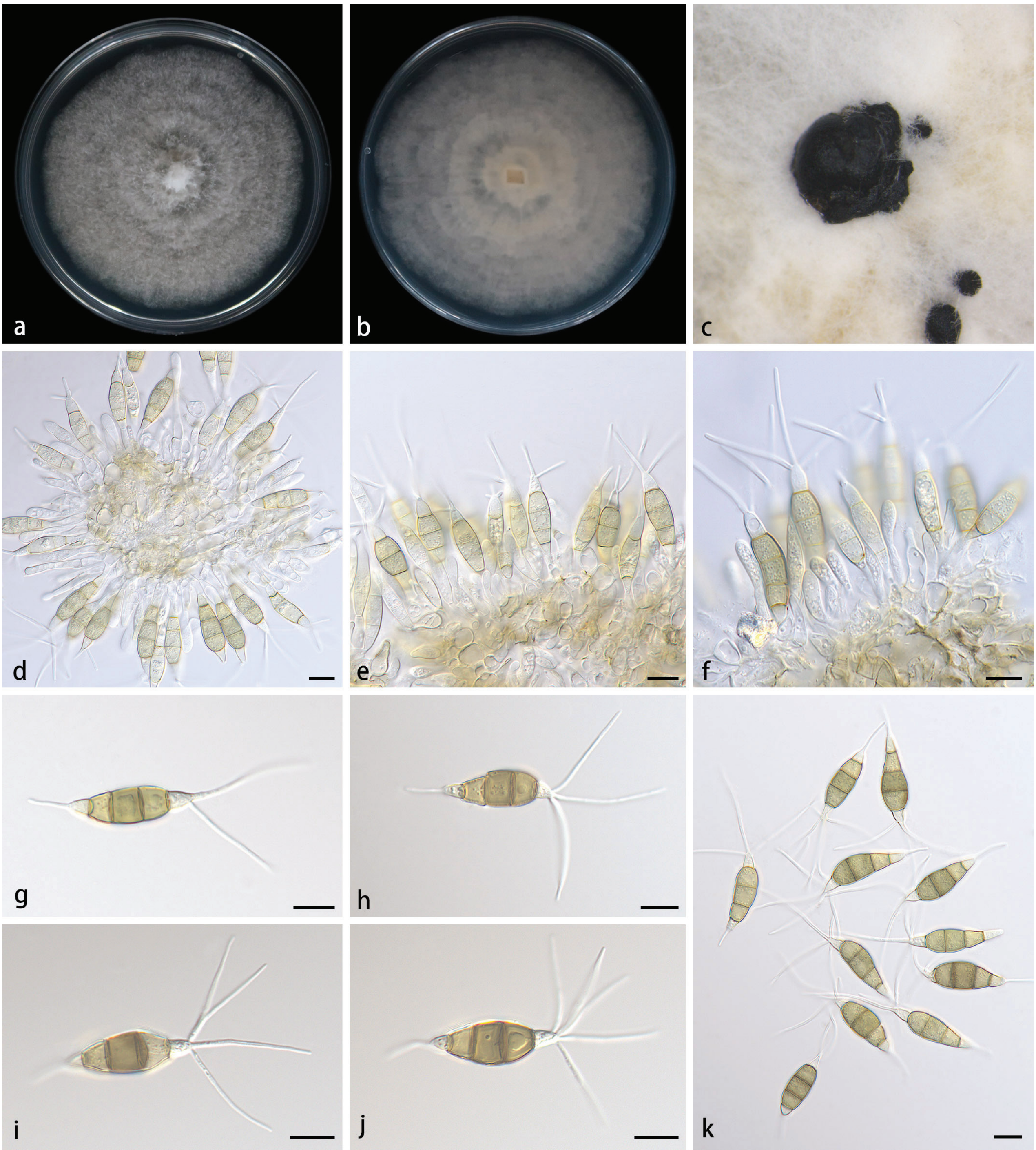

Figure 6. *Pestalotiopsis solicola* (HMAS 352972, holotype) **a, b** surface and reverse sides of colony after 7 days on PDA **c** colony overview with conidiomata **d–f** conidiogenous cells with conidia **g–k** conidia. Scale bars: 10 μm (**d–k**).

and TUB2 of these species was added (Suppl. materials 3, 4). In morphology, *P. solicola* is closely related to seven other species, but there are also differences. For more details, see the morphological comparison of the species in Table 3. The differences between *P. solicola* and other species mainly focus on the number of apical appendages, the size of conidia and the culture characteristics of the PDA medium. (Maharachchikumbura et al. 2012, 2014; Liu et al. 2015; Hyde et al. 2016).

Table 3. Morphological comparison between *P. solicola* and other closely-related species.

Species		***P. brassicae***	***P. hollandica***	***P. monochaeta***	***P. chinensis***	***P. verruculosa***	***P. italiana***	***P. sequoiae***	***P. solicola***
Culture characteristics		whitish	whitish to pale grey	whitish to pale yellow	whitish to pale yellow, reverse yellow to pale orange	whitish to pale yellow, reverse yellow to pale orange	whitish to pale grey	whitish	whitish
Conidiomata		dark brown to black	dark brown to black	dark brown to black	black	black	dark brown to black	black	black
Conidia		29–40 × 8–11.5 µm	25–34 × 8.5–10.5 µm	25–42 × 7–11.5 µm	23–32 × 7–9 µm	28–35 × 9–11 µm	26–35 × 8–11 µm	21–30 × 7.5–10 µm	24.3–32.4 × 8–10 µm
Basal cell		5–8.5 µm	5–7.5 µm	5.5–9.5 µm	5–7 µm	5–7 µm	5–7 µm	2.9–5.7 µm	2–5.9 µm
Median cells	**shape**	doliiform to subcylindrical	doliiform, verruculose	doliiform, verruculose	doliiform to cylindrical	doliiform to cylindrical, with thick verruculose walls	doliform to cylindrical, with thick verruculose walls	cylindrical	trapezoid or subcylindrical
	size	20–25 µm	16.5–24 µm	17–26 µm	20–22 µm	18–26 µm	18–28 µm	14.7–20 µm	18.8–21.3 µm
	colour	brown to olivaceous	concolourous	concolourous	concolorous, olivaceous	concolorous, olivaceous	concolorous, olivaceous	pale brown to brown and concolourous	pale brown to brown
	second cell	5.5–9 µm	5–8.5 µm	5–8.5 µm	6–7 µm	6–9 µm	5.5–8.5 µm	4.1–7 µm	3.1–7.5 µm
	third cell	7–9.5 µm	6–9 µm	7–9 µm	7–7.5 µm	6–9 µm	6–9 µm	5.4–6.9 µm	6.2–8.2 µm
	fourth cell	6–9 µm	6–8 µm	7–9 µm	6–7.5 µm	6–9 µm	6–9 µm	4.6–6.7 µm	4.8–6.9 µm
Apical cell	**shape**	cylindrical to subcylindrical	conical	conical	conical to subcylindrical	conical to subcylindrical	conical to subcylindrical	conical	conical with an acute apex
	size	3.5–7 µm	3.5–5 µm	4–6.5 µm	3–6 µm	4–6 µm	4–6.5 µm	2.9–4.8 µm	2.1–5.5 µm
Apical appendages	**quantity**	3–5 (mostly 4)	1–4	1	1–3 (mostly 3)	2–6 (mostly 3–4)	2–5 (mostly 3–4)	mostly 4	2–5
	size	27–50 µm	20–40 µm	40–75 µm	25–30 µm	25–40 µm	20–40 µm	3–17 µm	25–32 µm
Basal appendage	**size**	10–25 µm	3–9 µm	6–14 µm	7–11 µm	8–12 µm	6–10 µm	4–11 µm	10.3–13.4 µm

Discussion

Apiospora was introduced by Saccardo, with *A. montagnei* Sacc. as the type species (Saccardo 1875). Characterised by multi-locular perithecial stromata enclosing hyaline ascospores that are encompassed by a thick gelatinous sheath, the sexual morphs of *Apiospora* are distinct (Dai et al. 2017; Pintos and Alvarado 2021). Meanwhile, the asexual morphs of *Apiospora* are identified by their basauxic conidiogenesis and globose to subglobose conidia, which typically appear lenticular or obovoid in side view and range in colour from pale brown to brown (Kunze 1817; Hyde et al. 1998). *Apiospora* is similar to *Arthrinium* and *Neoarthrinium* in morphology, especially the basauxic conidiogenesis (Jiang et al. 2022; Liu et al. 2023). Most species of *Apiospora*, *Arthrinium* and *Neoarthrinium* are quite similar to each other in morphology; thus, it is difficult to distinguish them without molecular phylogenetic data.

Microdochium was established by Sydow with *M. phragmitis* as the type species (Sydow 1924). The sexual morphs of *Microdochium* are characterised by perithecial stromata with oblong to clavate asci that produce fusiform or oblong, hyaline ascospores. The asexual morphs of *Microdochium* are characterised by monoblastic or polyblastic conidiogenous cells and hyaline falcate conidia (Hernández-Restrepo et al. 2016; Liu et al. 2022; Zhang et al. 2023). *Microdo-*

chium is similar to *Idriella* in morphology; however, they can be separated by the pigmentation of their conidiogenous cells (Hernández-Restrepo et al. 2016).

Based on the conidial forms, Steyaert (1949) split *Pestalotia* into three genera, namely *Pestalotia*, *Pestalotiopsis* and *Truncatella*. Specifically, the genus *Pestalotia* was introduced for species with 6-celled conidia, *Pestalotiopsis* for species with 5-celled conidia and *Truncatella* for species with 4-celled conidia. The introduction of the genus *Pestalotiopsis* by Steyaert (1949) to accommodate the 5-celled conidial forms of *Pestalotia* resulted in appreciable controversy from Moreau and Guba (Moreau 1949; Steyaert 1949; Guba 1956, 1961). *Pestalotiopsis* species are morphologically diverse in conidial morphology and phylogenetic analyses of different gene regions have established that *Pestalotiopsis* comprises three distinct lineages (Jeewon et al. 2003; Maharachchikumbura et al. 2011, 2012). Based on these findings, Maharachchikumbura et al. (2014) divided *Pestalotiopsis* into three genera: *Pestalotiopsis*, *Neopestalotiopsis* and *Pseudopestalotiopsis*. Phenotypic analyses of conidial characters coupled with phylogenetic analyses of sequence data were used to clarify species boundaries in the three genera (Maharachchikumbura et al. 2014).

In this study, we collected parasitic or saprotrophic fungi on Bambusoideae plant or soil specimens from terrestrial habitats in Hainan and Yunnan Province, China. Based on morphological characteristics and phylogenetic data, *Apiospora bambusigena* sp. nov., *Microdochium jianfenglingense* sp. nov. and *Pestalotiopsis solicola* sp. nov. are introduced.

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 (nos. 32370001, U2002203, 32270024, 31900014), the Key Technological Innovation Program of Shandong Province, China (no. 2022CXGC020710), the Jinan City's 'New University 20 Policies' Initiative for Innovative Research Teams Project (no. 202228028) and the Innovative Agricultural Application Technology Project of Jinan City (no. CX202210).

Author contributions

Sampling, molecular biology analysis: Duhua Li and Mengyuan Zhang; fungal isolation: Jinjia Zhang; description and phylogenetic analysis: Zhaoxue Zhang; microscopy: Liguo Ma and Jie Zhang; writing-original draft preparation: Duhua Li; writing-review and editing: Jiwen Xia and Xiuguo Zhang. All authors read and approved the final manuscript.

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Data availability

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

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Supplementary material 1

A Maximum Likelihood Inference tree based on a combined dataset of analysed ITS, LSU, TEF1α and TUB2 sequences

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Explanation note: The Bayesian Inference Posterior Probability (left, BIPP ≥ 0.90) and the Maximum Likelihood Bootstrap Value (right, MLBV ≥ 75%) are shown as BIPP/MLBV above the nodes. Ex-type cultures are indicated in boldface and strains from the present study are in red. The tree was rooted in *Arthrinium caricicola* (CBS 145127). The scale bar at the bottom indicates 0.05 substitutions per site. In order to make the layout of the evolutionary tree beautiful, some branches are shortened by two diagonal lines ("//") with the number of times.

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl1

Supplementary material 2

A Bayesian Inference tree based on a combined dataset of analysed ITS, TEF1α and TUB2 sequences

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Explanation note: The Bayesian Inference Posterior Probability (left, BIPP ≥ 0.80) and the Maximum Likelihood Bootstrap Value (right, MLBV ≥ 75%) are shown as BIPP/MLBV above the nodes. Ex-type cultures are indicated in boldface and strains from the present study are in red. The tree was rooted in *Neopestalotiopsis magna* (MFLUCC 12-0652). The scale bar at the bottom indicates 0.05 substitutions per site. In order to make the layout of the evolutionary tree beautiful, some branches are shortened by two diagonal lines ("//") with the number of times.

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl2

Supplementary material 3

Phylogenetic tree of *Pestalotiopsis solicola* and related species based on TEF1α

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl3

Supplementary material 4

Phylogenetic tree of *Pestalotiopsis solicola* and related species based on TUB2

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl4

Supplementary material 5

GenBank accession numbers of the taxa used in *Apiospora* phylogenetic reconstruction

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl5

Supplementary material 6

GenBank accession numbers of the taxa used in *Microdochium* phylogenetic reconstruction

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl6

Supplementary material 7

GenBank accession numbers of the taxa used in *Pestalotiopsis* phylogenetic reconstruction

Authors: Duhua Li, Mengyuan Zhang, Jinjia Zhang, Liguo Ma, Zhaoxue Zhang, Jie Zhang, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.111.136483.suppl7

MycoKeys

MycoKeys 111: 111–127 (2024)
DOI: 10.3897/mycokeys.111.133046

Research Article

Two new species and a new record of Hypoxylaceae (Xylariales, Ascomycota) from Mexico

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Abstract

The family Hypoxylaceae has a cosmopolitan distribution with greater diversity in tropical regions, its growth habit is saprotrophic, endophytic and potentially phytopathogenic. From the revision of herbarium specimens and field collections from the Yucatan Peninsula, two new species were described: *Annulohypoxylon fusisporum*, characterized by having fusiform spores and *Hypoxylon xmatkuilense* which is distinguished by having stromata vinaceous and dark brown KOH-extractable pigments. The species are described based on morphological characters and Bayesian Inference analyses of four molecular markers (ITS, LSU, RPB2 and TUB2). In addition, one new record from Mexico is presented: *Hypoxylon bellicolor*. The presence of *Daldinia eschscholtzii*, *H. lenormandii*, *H. lividipigmentum* and *Entonaema liquescens* is confirmed with molecular data.

Key words: Diversity, Neotropics, phylogeny, Yucatan Peninsula

Academic editor: Jian-Kui Liu
Received: 25 July 2024
Accepted: 19 November 2024
Published: 17 December 2024

Citation: Reyes PE, Pinzón JP, Valenzuela R, Raymundo T, Tun-Garrido J, García-Sandoval R (2024) Two new species and a new record of Hypoxylaceae (Xylariales, Ascomycota) from Mexico. MycoKeys 111: 111–127. https://doi.org/10.3897/mycokeys.111.133046

Introduction

The family Hypoxylaceae (Xylariales, Ascomycota) contains 22 genera and 495 species (Wijayawardene et al. 2022; Bánki et al. 2024) 75 species have been cited from Mexico: *Annulohypoxylon* (6), *Daldinia* (13), *Durotheca* (1), *Entonaema* (4), *Hypomontagnella* (2), *Hypoxylon* (36), *Jackrogersella* (2), *Parahypoxylon* (1), *Phylacia* (8), *Pyrenopolyporus* (2) (CONABIO 2024).

They are characterized by having erect, glomerate, pulvinate, discoid, effused-pulvinate, hemispherical, spherical or peltate stromata; solitary or confluent, brightly colored, dark or black, pruinose or smooth, with or without extractable pigments that are evident with 10% KOH; spherical, obovoid or tubular perithecia with spherical, umbilicate or papillate ostioles, with or without discs formed by dehiscence of the surrounding tissue. They share a nodulisporium-like asexual state (which is one of the features that sets them apart from Xylariaceae) and are distributed in tropical and temperate areas of the world (Ju and Rogers 1996). Their lifestyle is mainly endophytic, saprotrophic and even associated with insect vectors (Wendt et al. 2018).

Among the most important taxonomic works that have been published about the genera that at this moment are considered as part of Hypoxylaceae, we can find the monograph of *Hypoxylon* by Ju and Rogers (1996), the revision of Child (1932) who published the first monograph of *Daldinia* with 11 species, furthermore Ju et al. (1997) described 19 species of the genus. Stadler et al. (2014a) recognized 47 taxa of *Daldinia* based on morphological, chemotaxonomic and phylogenetic evidence; Sir et al. (2016) described *D. korfii* from the Yungas region in Argentina; Chlebicki (2022) presented a review of the genus from Poland.

The genus *Entonaema*, was erected by Alfred Möller (1901) who described to *E. liquescens* and *E. mesentericum*, currently as *Xylaria mesenterica* (Stadler et al. 2008). Pošta et al. (2023) accepted six species: *E. liquescens*, *E. cinnabarinum*, *E. dengii*, *E. moluccanum*, *E. globosum*, and *E. siamensis*.

In the study by Wendt et al. (2018), based on analysis with four molecular markers (ITS, LSU, RPB2 and TUB2) Hypoxylaceae was formally recognized, clearly segregated from Xylariaceae with the following genera: *Acrostaphylus*, *Annulohypoxylon*, *Anthocanalis*, *Ascoporia*, *Chlorostroma*, *Daldinia*, *Entonaema*, *Henningsina*, *Hypoxylina*, *Hypoxylon*, *Jackrogersella*, *Phylacia*, *Pyrenomyxa*, *Pyrenopolyporus*, *Rhopalostroma*, *Rostrohypoxylon*, *Ruwenzoria*, *Sphaeria*, *Thamnomyces* and *Thuemenella*.

Lambert et al. (2019) erected *Hypomontagnella* based on a study of *Hypoxylon monticulosum* and its allies. The features that characterize them are the stromata woody to carbonaceous lacking colored granules, papillate ostioles usually with a black annulate disc, without apparent KOH-extractable pigments in mature stromata and perispores smooth or with transversally striate ornamentations.

Recently, Cedeño-Sanchez et al. (2023) proposed *Parahypoxylon* as a new genus including *P. papillatum* and *P. ruwenzoriense*; Lambert et al. (2023) studied *Phylacia* species in Argentina and found a close relationship with *Rhopalostroma*, *Thamnomyces* and *Daldinia species*, which all have similar secondary metabolites.

In Mexico, 75 species of the family are known on all types of vegetation, standing out the study of San Martín et al. (1999b) in which they reviewed the genus *Hypoxylon* including some members of Section *Annulata* (now genus *Annulohypoxylon*); San Martín and Lavin (1997) described three species of *Entonaema*; Medel et al. (2006) made a review of *Phylacia*, recording eight species; Barbosa-Reséndiz et al. (2020) reported an updated list of *Daldinia* in Mexico; Raymundo et al. (2014, 2017, 2021) and Reyes et al. (2020) have reported species of Hypoxylaceae from Protected Natural Areas: Chamela-Cuixmala, Cozumel Island, Lagunas de Chacahua National Park, Sierra de Álamos-Río Cuchujaqui and El Cielo. For this work, several specimens from herbaria and field collections in the Yucatan Peninsula have been studied taxonomically. Morphological and molecular analyses have revealed some taxonomic novelties that we report in this document.

Materials and methods

Collections sites and sampling

In the current paper, 240 specimens were studied, of which 148 are deposited in the “Alfredo Barrera Marín” Herbarium of the Universidad Autónoma de Yucatán (UADY) and the “Gastón Guzmán Huerta” Fungi Collection of the Herbarium of the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional (ENCB), while 77 were collected in the Campeche, Quintana Roo and Yucatan States between 2021–2023. In this study, nine specimens were analyzed morphologically and sequenced.

Morphological characterization

The description of macromorphological features was carried out with water and 10% KOH to examine perisporium dehiscense and to view the stromatal pigments, Melzer’s reagent to show the amyloid reaction of the ascal apical apparatus, specialized literature in each genus was reviewed (San Martín et al. 1999b; Stadler et al. 2014a; Kuhnert et al. 2016; Lambert et al. 2019; Reyes et al. 2020), colors were described according to Rayner (1970).

DNA extraction, PCR and sequencing

The stromata were macerated in liquid nitrogen, then the QIAGEN DNeasy kit (Hilden, Germany) was used according to the manufacturer’s specifications. The primers used were reported by White et al. (1990) (ITS1, ITS4), Gardes and Brunes (1993) (ITS1-F) for ITS (internal transcriber spacer gene); Vilgalys and Hester (1990) (LR07, LR0R) for LSU (large subunit ribosomal gene); Liu et al. (1999) (RPB2-5F, -7cR) for RPB2 (partial second largest subunit of the DNA-directed RNA polymerase II gene); O’Donnell and Cigelnik (1997) (T1, T22) for TUB2 (beta-tubulin gene). PCR reactions were made using 25 µl reaction:12.5 µl GoTaq® (Promega, Madison, USA) “Master Mix”, 2-3 µl DNA, 0.5 µl of each primer (100 µM), BSA 2.5 µl and 7 µl of sterilized water. The PCR conditions are detailed in Table 1. Sequencing was performed by the Sanger technique at Macrogen Inc.

Molecular phylogenetic analyses

A concatenated matrix of the four molecular markers was constructed using 274 sequences from 71 species as a reference from the GenBank (Appendix 1) and 36 obtained in the present study. Sequences were aligned using MAFFT software (Katoh and Kuma 2002) and edited in BioEdit (Hall 1999). The evolution model that best fit each group of sequences was obtained using the j Model Test 2.1.10 software (Posada and Crandall 1998) supported by Bayesian Information Criterion

Table 1. PCR conditions used in this study.

DNA locus	Initial denaturation	Cycles	Denaturation	Annealing	Elongation	Final denaturation
ITS	94 °C-5 mins	35	94 °C-1 min	55 °C-1 min	72 °C-1 min	72 °C-10 mins
LSU	94 °C-5 mins	34	94 °C- 1 min	52 °C-1 min	72 °C -2 mins	72 °C-10 mins
RPB$_2$	94 °C- 5 mins	38	94 °C-30 s	53 °C-1 min	72 °C-1.30 mins	72 °C-10 mins
TUB$_2$	94 °C-5 mins	38	94 °C -30 s	47 °C-30 s	72 °C-2.30 mins	72 °C-10 mins

(BIC). A Maximum Likelihood analysis was performed in RaxML 8.2.12 (Stamatakis 2006) with 1000 bootstrap replicates. Additionally, a Bayesian analysis was carried out with the same matrix in Mr.Bayes 3.2.6. (Ronquist and Huelsenbeck 2003) using four MCMC chains, 10000000 generations, taking samples every 1000 generations, applying a burnin of 25%, *Xylaria polymorpha* was used as an outgroup.

Results

Phylogenetic analysis

The molecular matrix of the four concatenated loci (ITS, LSU, RPB2 and TUB2) was 7283 bp in length, having 1640 for the first, 2417 for the second, 1200 for the third and 2026 for the fourth, applying the following substitution models: SYM+I+G for ITS, HKY+I+G for TUB2 and GTR+I+ G for LSU and RPB2.

The family Hypoxylaceae was represented in the phylogenetic tree by nine genera among which we can find: *Phylacia* (Phy) with two specimens, *Thamnomyces*

Figure 1. Inferred molecular phylogenetic tree obtained by Bayesian Inference using a multigene alignment (ITS, LSU, RPB2 and TUB2). The tree was rooted with *Xylaria polymorpha*. The sequences generated in the present work and the new combination are highlighted. Bayesian posterior probability values ≥ 0.98 and Bootstrap support values ≥ 70 from the Maximum Likelihood analysis are indicated on the branches.

(Tha) one specimen, *Rhopalostroma* (Rho) one specimen, *Daldinia* (D1 and D2) 14 specimens, *Ruwenzoria* (Ru) one specimen, *Pyrenopolyporus* (Py) three specimens, *Annulohypoxylon* (A) nine specimens, *Jackrogersella* three specimens, *Rostrohypoxylon* (Ro) one specimen, *Hypomontagnella* (Hyp) two specimens, *Durotheca* (Du) three specimens, *Parahypoxylon* (Pa) two specimens, *Entonaema* two specimens, *Hypoxylon* (H1, H2, H3, H4) 36 specimens (Fig. 1), which were selected by having molecular information supported by several studies (Wendt et al. 2018; Cedeño-Sanchez et al. 2023; Lambert et al. 2023; Pošta et al. 2023).

In the phylogram resulted from the Bayesian Inference analysis (Fig. 1), *Hypomontagnella*, *Jackrogersella*, *Parahypoxylon*, *Phylacia*, *Pyrenopolyporus*, *Rhopalostroma and Thamnomyces* appear as monophyletic; *Daldinia* as paraphyletic with supports in each group, D1 with 1/95 and D2 with 1/100; *Annulohypoxylon* also paraphyletic with values of 1/96; *Hypoxylon* as polyphyletic in four groups, H1 with values of 1/97, H2 with support of 1/100, H3 with 1/92, H4 + E with 1/95.

The identity of taxa previously reported for the country such as *Daldinia eschscholtzii*, *Hypoxylon lenormandii*, *H. lividipigmentum* and *Entonaema liquescens* is confirmed, of which there was limited molecular information for Mexican specimens.

According to the morphological revision of the specimens and the topology of the trees, two new species arise: *Annulohypoxylon fusisporum* and *Hypoxylon xmatkuilense*; the presence of *H. bellicolor* is confirmed in the country.

Taxonomy

New species from Mexico

***Annulohypoxylon fusisporum* P. Reyes, Pinzón, R. Valenz. & Raymundo, sp. nov.**
MycoBank No: 851003
Fig. 2

GenBank. ITS (OR807998), LSU (OR807987), RPB2 (OR825472), TUB2 (OR825468).

Diagnosis. It is characterized by having fusiform spores 10–12 × 4–5 µm, grayish green to dull green KOH-extractable pigments, ¼ perithecial mounds exposed, with straight germ slit in spore-length on the convex side.

Etymology. in reference to the fusiform spores.

Holotype. Mexico • Quintana Roo, Bacalar Experimental Station, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias; 10 March 2023; P. Reyes leg.; UADY 83.

Description. Stromata effused-pulvinate 20–70 mm long × 20–30 mm wide × 1 mm thick, surface dark brown to black (Fig. 2a) inconspicuous perithecial mounds up to 1/4 exposed (Fig. 2b) dark brown to black granules beneath surface, KOH-extractable pigments Grayish Green (50) changing to Dull Green (70) after 1 minute (Fig. 2c) perithecia 0.7–0.8 mm diam, spherical, conical-papillate ostioles surrounded with a truncatum type disc 0.2–0.25 mm diam; asci 8-spored cylindrical, the spore bearing parts 60–75 µm long × 5–7 µm wide, stipes 20–45 µm long, with amyloid, discoid apical apparatus, 1–2 µm high × 2 µm wide (Fig. 2d); ascospores 10–12 × 4–5 µm, unicellular, fusiform with narrowly rounded ends, with faint, straight germ slit along the spore on the convex side (Fig. 2e), light brown, perispore dehiscent 10% KOH (Fig. 2f).

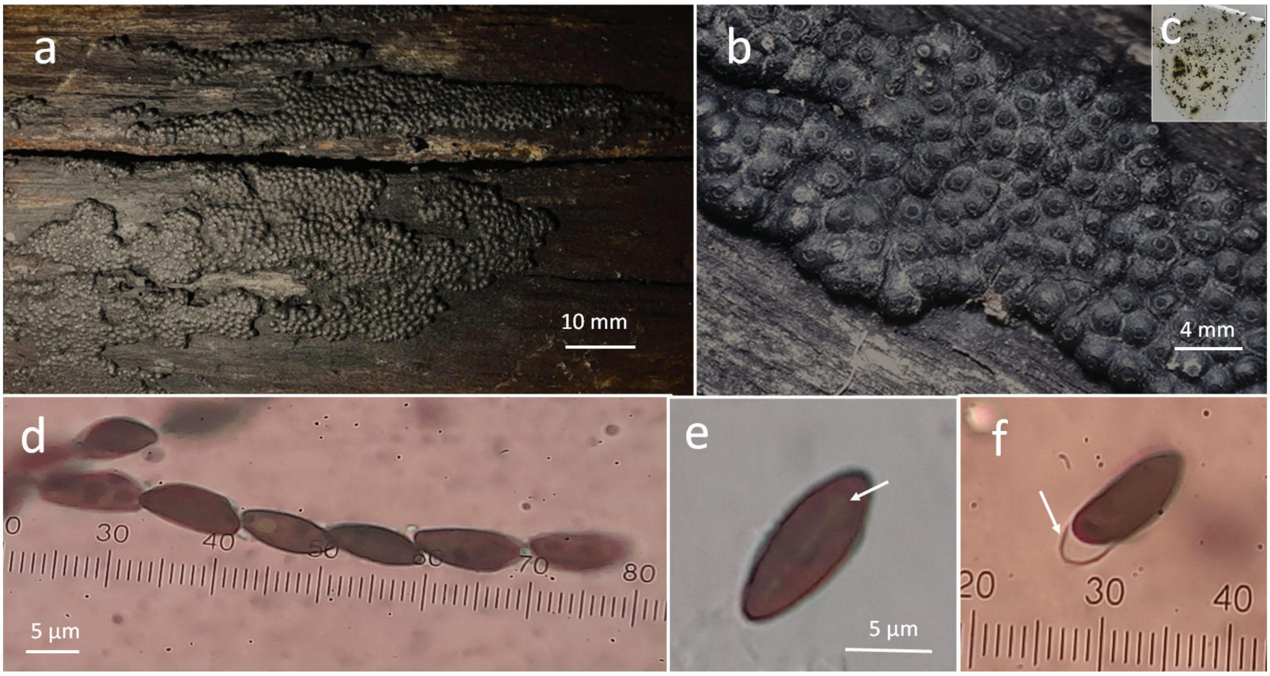

Figure 2. *Annulohypoxylon fusisporum* sp. nov. **a** general view of stromata **b** perithecia spherical with truncatum type ostiolar disc **c** KOH-extractable pigments **d** asci **e** straight germ slit **f** perispore dehiscent 10% KOH.

Host. Growing on dead wood of *Brosimum alicastrum* (Moraceae) in subevergreen forest.

Notes. It is similar to *A. subeffusum* (Hladki and Romero 2009) due to the color, shape of the stromata and the grayish green pigments, however the ascospores of this one are smaller 7–10 × 3–3.8 µm and have inconspicuous perithecial mounds. With *A. leptascum* it shares fusiform shape spores, unlike the length of the germ slit which is short, and the pigments are olive-colored, not changing after 1 minute in 10% KOH; Kuhnert et al. (2016) found BNT, truncatone A and truncatone C in aforementioned taxa.

Hypoxylon xmatkuilense P. Reyes, Pinzón, R. Valenz. & Raymundo, sp. nov.

MycoBank No: 855643
Fig. 3

Gen Bank. ITS (OR807999), LSU (OR807990), RPB2 (OR825476), TUB2 (OR825467).

Diagnosis. It characterized by having stromata effused-flattened, surface vinaceous, perithecia obovoid and dark brown KOH-extractable pigments.

Etymology. from the Mayan Xmatkuil "place where God is asked" in reference to the place where it was collected for the first time.

Holotype. Mexico • Yucatán, east from cemetery, comisaría Xmatkuil; 14 Oct 2021; P. Reyes leg.; UADY 3.

Paratypes. Mexico • ibid.; 11 July 2023; P. Reyes leg.; UADY 118 • ibid; P. Reyes leg.; UADY 119.

Description. Stromata effused-flattened, 25–100 mm long × 5–20 mm wide × 1 mm thick, surface pruinose, young stromata have a thin layer Dark Vinaceous (82), when mature this layer is lost, leaving remains that appear Brown Vinaceous (84) (Fig. 3a), subsurface blackish, composed of weakly carbonaceous tissue and

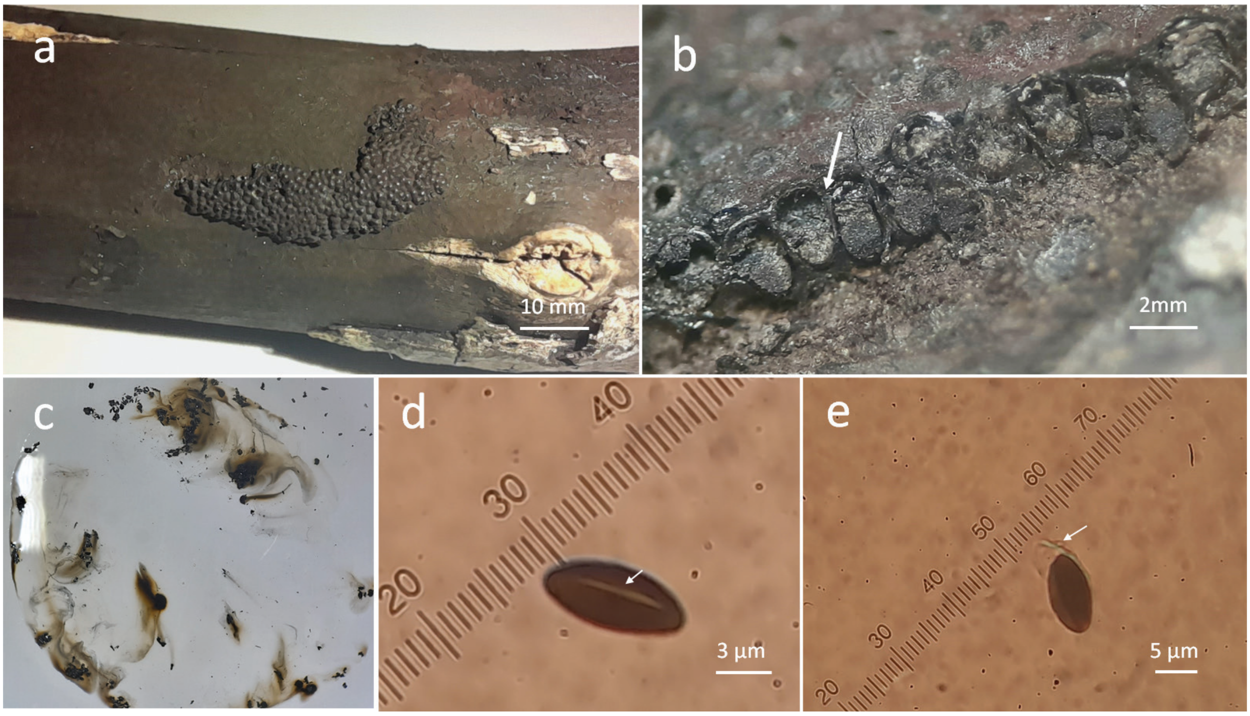

Figure 3. *Hypoxylon xmatkuilense* sp. nov **a** general view of stromata **b** perithecia obovoid **c** KOH-extractable pigments **d** straight germ slit on the convex side **e** perispore dehiscent 10% KOH.

inconspicuous brownish black granules; perithecia 1.2–1.5 mm diam, obovoid inconspicuous with umbilicate ostioles (Fig. 3b); KOH-extractable pigments dark brown (Fig. 3c); asci not seen; ascospores 10–12 × 4–5 µm, unicellular, ellipsoid, inequilateral, narrowly rounded ends, with straight 2/3 spore-length germ slit on the convex side (Fig. 3d), dark brown, perispore dehiscent in 10% KOH (Fig. 3e).

Host. Growing on dead wood of *Gymnopodium floribundum* Rolfe (Polygonaceae) in deciduous forest

Notes. It shares the effused-pulvinate to flattened stromata and vinaceous surface with *H. lateripigmentum*, *H. pulicicidum*, *H. investiens*, *H. hinnuleum*, *H. olivaceopigmentum*, it differs by having perithecia obovoid, dark brown KOH-extractable pigments and perispore dehiscent (Ju and Rogers 1996; Bills et al. 2012; Kuhnert et al. 2014a; Sir et al. 2019); Fournier et al. (2024) described *H. aeneipigmentatum* as a new taxon from Saül, French Guiana, based on ITS sequences, and noted that it belongs *H. investiens* complex; moreover reported about a basal perithecial nucleus as a new differential character in this group.

New record from Mexico

***Hypoxylon bellicolor* Cedeño-Sanchez, L. Wendt & L.C. Mejía 2020 Mycosphere 11(1): 1464 (2020).**
Fig. 4

Description. Stromata effused-pulvinate 20–90 mm long × 10–30 mm wide, Rust (39) (Fig. 4a) pruinose surface with rust granules beneath the surface (Fig. 4b), KOH-extractable pigments Luteous (12) (Fig. 4c); perithecia ovoid, umbilicate ostioles (Fig. 4d); asci 8-spored cylindrical, the spore bearing part 40–80 µm long × 5–6 µm wide, stipes 30–35 µm long, with amyloid, discoid

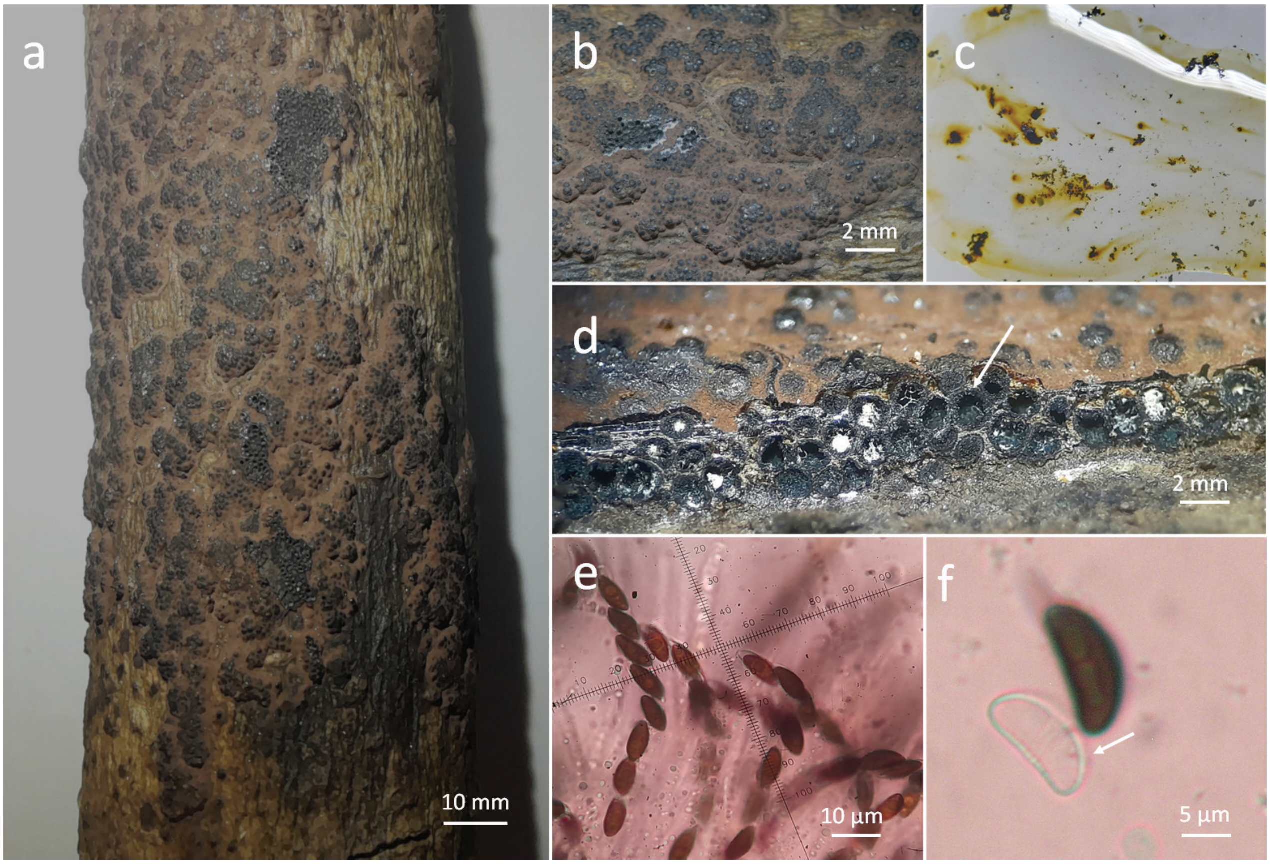

Figure 4. *Hypoxylon bellicolor* **a** general view of stromata **b** pruinose surface **c** KOH-extractable pigments **d** perithecia ovoid **e** asci **f** perispore dehiscent 10% KOH with coil-like ornamentation.

apical apparatus 2–3 µm high × 2 µm wide (Fig. 4e); ascospores 10–12 µm long × 4–6 µm wide, with germ slit straight less than spore-length on the convex side, perispore dehiscent in 10% KOH with coil-like ornamentation (Fig. 4f).

Specimens examined. Mexico • Campeche, Champotón-Campeche highway; 19 Jan 2018; P. Reyes leg.; UADY 145 • Quintana Roo, Centro de Conservación y Educación Ambiental, Cozumel; 20 Jan 2018; R. Valenzuela leg.; ENCB 17942 •Yucatán, east from the cemetery, comisaría Xmatkuil; 15 Oct 2021; P. Reyes leg.; UADY 7 • ibid.; P. Reyes leg.; UADY 9 •Southern Ecological Park, Mérida; 21 Sept 2022; P. Reyes leg.; UADY 45 • Ría Celestún Biosphere Reserve; 29 Oct 2022; P. Reyes leg.; UADY 55.

Host. Growing on dead wood of *Lysiloma latisiliquum* (L.) Benth. (Fabaceae), *Lonchocarpus* sp. (Fabaceae) in deciduous forest; on dead wood of *Conocarpus erectus* L. (Combretaceae) in coastal dune vegetation.

Known distribution. Panama (Cedeño-Sanchez et al. 2020).

Notes. It shares a stromata color and yellow pigments with *H. perforatum*, however this one is characterized by having a white disc surrounding the ostioles; in addition it has a cosmopolitan distribution (Ju and Rogers 1996).

Discussion

Since the segregation of Hypoxylaceae from Xylariaceae (Wendt et al. 2018), many questions have arisen, which have been answered over the years based on the review of groups as *Hypoxylon* that appears as polyphyletic, and from which the genera *Hypomontagnella* (Lambert et al. 2019) and *Parahypoxylon* (Cedeño-Sanchez et al. 2023) have emerged; likewise, *Daldinia* appears grouped in two different clades that are supported in the present study, so it requires a review from integral taxonomy approach to elucidate several questions that will continue to arise within the family.

In the phylogenetic tree of the present study it can be seen that most of the clades of the known genera have good statistical support, *Parahypoxylon*, *Durotheca*, *Jackrogersella*, *Pyrenopolyporus*, *Hypomontagnella*, *Rhopalostroma*, *Thamnomyces*, *Phylacia* and *Ruwenzoria* noted as monophyletic.

As for *Hypoxylon*, it appears as polyphyletic forming four groups (labeled here as H1, H2, H3, and H4, Fig. 1). Group H1 is formed by the following species: 1) *H. investiens* which has a wide distribution, mainly in a tropical climate; it is distinguished by having tubular perithecia. 2) *H. pulicicidum* segregated from the previous one by Fournier et al. (2015b) and Bills et al. (2012) who consider it a rare taxon; it is distinguished by having slightly papillated ostioles and lanceolate perithecia. 3) *H. lateripigmentum* has olive-yellow granules beneath the surface, yellowish brown pigments in 10% KOH and perispore dehiscent. 4) *H. olivaceopigmentum* is distinguished by having the largest spores in the complex 9–15.7 × 4.5–7.2 μm and has been recorded from monocotyledonous hosts. 5) *H. hinnuleum*, proposed by Sir et al. (2019) stands out by having ostioles conical black papillate. Finally, the new species *H. xmatkuilense* is characterized by having perithecia obovoid, dark brown KOH-extractable pigments and perispore dehiscent. This species is sister to *H. lateripigmentum*, which is only known from Martinique.

The members of this group have tropical distribution, *H. xmatkuilense* and *H. lateripigmentum* are Neotropical, so far reported from Caribbean area; *H. olivaceopigmentum* and *H. hinnuleum* from subtropical area, while *H. investiens* and *H. pulicicidum* are Pantropical.

H2 is made up only by *H. lividipigmentum* with two Mexican specimens; this one is characterized by having a sigmoid germ slit in the ascospores, a taxonomic character that is less frequent in the genus.

H3 where the type *H. fragiforme* stands out, so this clade stands as *Hypoxylon* sensu stricto, share distribution with *H. howeanum*, *H. ticinense* of temperate climate, meanwhile *H. crocopeplum*, *H. fendleri*, *H. lenormandii*, *H. haematostroma* and *H. rickii* have been recorded from tropical areas (Wendt et al. 2018; Cedeño-Sanchez et al. 2023).

H4 consists of species of varied distribution and specific plant associations as *H. fuscum* recorded on hosts of the family Betulaceae, in addition to *Acer* and *Salix*; *H. vogesiacum* associated with *Acer*, *H. porphyreum* with *Quercus*; regarding *H. bellicolor*, a new record from Mexico, we registered it growing on Fabaceae and Combretaceae hosts; furthermore, the sequences of the four markers were obtained from two Mexican specimens enriching the knowledge of this taxon, since they were only available ITS and TUB2 sequences (Ju and Rogers 1996; Fournier et al. 2015b; Cedeño-Sanchez et al. 2020).

The polyphyletic origin of *Hypoxylon* encourages more morphological, chemical and phylogenetic studies, including a better sampling of the species to resolve *Hypoxylon* evolutionary history and probably segregate new genera.

On the other hand, our sequences of *Entonaema liquescens* (ENCB:RV_19274) matched with those of the specimen CNF 2/11263 (Pošta et al. 2023). However, they had no similarity with those from the strain ATCC 46302 (Wendt et al. 2018). This pattern is explained by Stadler et al. (2020) and Cedeño-Sanchez et al. (2024) who point out heterogeneity and polymorphisms among multiple copies of different ITS and LSU loci.

The genus *Daldinia* resulted to be paraphyletic, in agreement with Stadler et al. (2014a), forming two groups; in D1 group, most of the species are exclusively tropical such as *D. bambusicola*, *D. eschscholtzii*, *D. placentiformis* and *D. theissenii*; the D2 group it would be the genus *sensu stricto*, this clade has a widely distribution and there is a recurrence in the hosts of Betulaceae and Lauraceae (Stadler et al. 2014a).

Annulohypoxylon also appears as paraphyletic, having *Rostrohypoxylon terebratum* nested inside the clade, which agrees with Tang et al. (2009) and Wendt et al. (2018); it is closely related to the new species *A. fusisporum*, proposed in the current study; Stadler (2011) mentioned that *R. terebratum* represents a lineage that has evolved from *Annulohypoxylon*, while differing in some morphological features and the production of unique secondary metabolites. It is worth mentioning that this taxon could be a turning point for segregating the genus, but more taxonomic and genomic samples are needed.

Conclusion

The present study is important because it provides information about the species from a tropical area, particularly from the Yucatan Peninsula, which has a valuable biogeographic history that makes the family have a great diversity, and therefore two new species are described and reports a new record from this region in Mexico; the confirmation of the identity of four species through molecular phylogenetic data is also relevant, since cryptic or semicryptic species may appear, especially in tropical areas.

The proposal of new species is fundamental for the understanding of a recently established family, in which there are still questions to be clarified. These questions will have to be supported by a polyphasic approach that provides comprehensive tools for the interpretation of the phylogenetic relationships in the group.

Acknowledgements

The authors are grateful for the facilities granted to Fernando Arellano Martín, researcher at the Chetumal Experimental Field of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). To Orlando Poot, mayor of Tres Garantías, Quintana Roo. PhD, Juan José Ancona for the identification of hosts in the municipality of Tekax, Yucatán. To the Mesoamerican Floristic Resources Laboratory and the Tropical Ecology Laboratory of the Universidad Autónoma de Yucatán for the use of the facilities and equipment and Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional for the loan of herbaria material. Reyes acknowledges the Consejo Nacional de Humanidades, Ciencia y Tecnología (CONAHCYT) for the grant awarded.

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 financed by Instituto Politécnico Nacional (SIP20230017; SIP20240029; SIP20240367).

Author contributions

Conceptualization: JPP. Project administration: RV. Supervision: JTG, TR. Visualization: RGS. Writing - original draft: PER.

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Data availability

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

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Appendix 1

Table A1. List of sequences used in phylogenetic analyses obtaneid from Gen Bank. Sequences derived from type material are marked as type (T), holotype (HT), epitype (ET) isotype (IT) and paratype (PT), the sequences obtained in this study are highlighted.

Species	Strain/voucher number	Origin and status	ITS	LSU	RPB2	TUB2	Reference
Annulohypoxylon annulatum	CBS 140775	USA (ET)	KY610418	MK287546	KY624263	KX376353	(Kuhnert et al. 2016; Wendt et al. 2018)
Annulohypoxylon atroroseum	ATCC 76081	Thailand	AJ390397	KY610422	KY624233	DQ840083	(Kuhnert et al. 2014a; Wendt et al. 2018)
***Annulohypoxylon fusisporum* sp. nov.**	**UADY:PR_83**	**Mexico (HT)**	**OR807998**	**OR807987**	**OR825472**	**OR825468**	**This study**
Annulohypoxylon leptascum	MFLUCC 13-0587	Thailand	KU604576			KU604580	Sir et al. (2016a)
Annulohypoxylon michelianum	CBS 119993	Spain	KX376320	KY610423	KY624234	KX271239	Wendt et al. (2018); Kuhnert et al. (2014a)
Annulohypoxylon moriforme	CBS 123579	Martinique	KX376321	KY610425	KY624289	KX271261	Wendt et al. (2018); Kuhnert et al. (2016)
Annulohypoxylon nitens	AXL030	Thailand	KJ934991	KJ934992	KJ934994	KJ934993	Daranagama et al. (2015)
Annulohypoxylon stygium	MUCL 54601	French Guiana	KY610409	KY610475	KY624292	KX271263	Wendt et al. (2018)
Annulohypoxylon truncatum	CBS 140778	USA (ET)	KY610419	KY610419	KY624277	KY624277	Wendt et al. (2018); Kuhnert et al. (2016)
Daldinia andina	CBS 114736	Ecuador (HT)	AM749918	KY610430	KY624239	KC977259	Bitzer et al. (2008)
Daldinia bambusicola	CBS 122872	Thailand (HT)	KY610385	KY610431	KY624241	KY624241	Wendt et al. (2018); Hsieh et al. (2005)

Species	Strain/voucher number	Origin and status	ITS	LSU	RPB2	TUB2	Reference
Daldinia caldariorum	MUCL 49211	France	AM749934	KY610433	KY624242	KC977282	Wendt et al. (2018); Kuhnert et al. (2014a); Bitzer et al. (2008)
Daldinia concentrica	CBS 113277	Germany	AY616683	KY610434	KY624243	KC977274	Wendt et al. (2018); Kuhnert et al. (2014a); Triebel et al. (2005)
Daldinia dennisii	CBS 114741	Australia (HT)	JX658477	KY610435	KY624244	KC977262	Stadler et al. (2014a); Kuhnert et al. (2014a); Wendt et al. (2018)
Daldinia eschscholtzii	MUCL 45434	Benin	JX658484	KY610437	KY624246	KC977266	Stadler et al. (2014a); Kuhnert et al. (2014a); Wendt et al. (2018)
Daldinia eschscholtzii	**UADY:PG_136**	**Mexico**	**OR808001**	**OR807988**	**OR825473**	**OR825469**	**This study**
Daldinia loculatoides	CBS 113279	UK (ET)	AF176982	KY610438	KY610438	KX271246	Johannesson et al. (2000); Wendt et al. (2018)
Daldinia macaronesica	CBS 113040	Spain (PT)	KY610398	KY610477	KY624294	KX271266	Wendt et al. (2018)
Daldinia petriniae	MUCL 49214	Austria (ET)	AM749937	KY610439	KY624248	KC977261	Bitzer et al. (2008); Kuhnert et al. (2014a); Wendt et al. (2018)
Daldinia placentiformis	MUCL 47603	Mexico	AM749921	KY610440	KY624249	KC977278	Bitzer et al. (2008); Kuhnert et al. (2014a); Wendt et al. (2018)
Daldinia steglichii	MUCL 43512	Papua New Guinea (PT)	KY610399	KY610479	KY624250	KX271269	Wendt et al. (2018)
Daldinia theissenii	CBS 113044	Argentina (PT)	KY610388	KY610441	KY624251	KX271247	Wendt et al. (2018)
Daldinia vernicosa	CBS 119316	Germany (PT)	KY610395	KY610442	KY624252	KC977260	Kuhnert et al. (2014a); Wendt et al. (2018)
Durotheca crateriformis	GMBC0205	China (T)	MH645426	MH645425	MH645427	MH049441	De Long et al. (2019)
Durotheca guizhouensis	GMBC0065	China (T)	MH645423	MH645421	MH645422	MH049439	De Long et al. (2019)
Durotheca rogersii	GMBC0204	China	MH645433	MH645434	MH645435	MH049449	De Long et al. (2019)
Entonaema liquescens	CNF 2/11263	USA	OQ869784.1	OQ865124.1	OQ877106.1	OQ877117.1	Pošta et al. (2023)
Entonaema liquescens	**ENCB:RV_19274**	**Mexico**	**OR807997**	**OR807993**	**OR825474**	**OR825466**	**This study**
Hypoxylon bellicolor	UCH9543	Panama	MN056425.1			MK908139	Cedeño-Sanchez et al. (2020)
Hypoxylon bellicolor	**UADY:PR_9**	**Mexico**	**OR808002**	**OR807989**		**OR825470**	**This study**
Hypoxylon bellicolor	**ENCB:PR_145**	**Mexico**	**OR808004**	**OR807994**	**OR825475**	**OR825471**	**This study**
Hypoxylon carneum	MUCL 54177	France	KY610400	KY610480	KY624297	KX271270	Wendt et al. (2018)
Hypoxylon cercidicola	CBS 119009	France	KC968908	KY610444	KY610444	KC977263	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon crocopeplum	CBS 119004	France	KC968907	KY610445	KY624255	KC977268	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon fendleri	MUCL 54792	French Guiana	KF234421	KY610481	KY624298	KF300547	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon fragiforme	MUCL 51264	Germany (ET)	KC477229	KM186295	KM186296	KM186296	Stadler et al. (2013); Daranagama et al. (2015); Wendt et al. (2018)
Hypoxylon fuscum	CBS 113049	Germany (ET)	KY610401	KY610482	KY624299	KX271271	Wendt et al. (2018)
Hypoxylon griseobrunneum	CBS 331.73	India (HT)	KY610402	KY610483	KY624300	KC977303	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon haematostroma	MUCL 53301	Martinique (ET)	KC968911	KY610484	KY624301	KC977291	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon hinnuleum	ATCC 36255	USA (T)	MK287537	MK287549	MK287562	MK287575	Sir et al. (2019)
Hypoxylon howeanum	MUCL 47599	Germany	AM749928	KY610448	KY624258	KC977277	Bitzer et al. (2008); Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon hypomiltum	MUCL 51845	Guadeloupe	KY610403	KY610449	KY624302	KX271249	Wendt et al. (2018)
Hypoxylon investiens	CBS 118183	Malaysia	KC968925	KY610450	KY624259	KC977270	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon investiens	TBRC 16251	Thailand			OQ108848.1	OQ144968.1	Suetrong et al. (2023)
Hypoxylon lateripigmentum	MUCL 53304	Martinique (HT)	KC968933	KY610486	KY624304	KC977290	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon lenormandii	CBS 119003	Ecuador	KC968943	KY610452	KY624261	KC977273	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon lenormandii	**UADY:PR_82**	**Mexico**	**OR808003**	**OR807991**	**OR825477**		**This study**
Hypoxylon lividipigmentum	BCRC 34077	Mexico (IT)	JN979433			AY951735	Hsieh et al. (2005)
Hypoxylon lividipigmentum	**UADY:PR_1**	**Mexico**	**OR808000**	**OR807992**	**OR825478**		**This study**
Hypomontagnella monticulosa	MUCL 54604	French Guiana (ET)	KY610404	KY610487	KY624305	KX271273	Wendt et al. (2018)
Hypoxylon musceum	MUCL 53765	Guadeloupe	KC968926	KY610488	KY624306	KC977280	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon olivaceopigmentum	DSM 107924	USA (T)	MK287530	MK287542	MK287555	MK287568	Sir et al. (2019)
Hypoxylon perforatum	CBS 115281	France	KY610391	KY610455	KY624224	KY624224	Wendt et al. (2018)
Hypoxylon petriniae	CBS 114746	France (HT)	KY610405	KY610491	KY624279	KX271274	Kuhnert et al. (2016); Wendt et al. (2018)
Hypoxylon pilgerianum	STMA 13455	Martinique	KY610412	KY610412	KY624308	KY624315	Wendt et al. (2018)
Hypoxylon porphyreum	CBS 119022	France	KC968921	KY610456	KY624225	KC977264	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon pulicicidum	CBS 122622	Martinique (HT)	JX183075	KY610492	KY624280	JX183072	Bills et al. (2012); Wendt et al. (2018)

Species	Strain/voucher number	Origin and status	ITS	LSU	RPB2	TUB2	Reference
Hypoxylon rickii	MUCL 53309	Martinique (ET)	KC968932	KY610416	KY624281	KC977288	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon rubiginosum	MUCL 52887	Germany (ET)	KC477232	KY610469	KY624266	KY624311	Stadler et al. (2013); Wendt et al. (2018)
Hypoxylon samuelsii	MUCL 51843	Guadeloupe (ET)	KC968916	KY610466	KY624269	KC977286	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypomontagnella submonticulosa	CBS 115280	France	KC968923	KY610457	KY624226	KC977267	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon ticinense	CBS 115271	France	JQ009317	KY610471	KY624272	AY951757	Hsieh et al. (2005); Wendt et al. (2018)
Hypoxylon trugodes	MUCL 54794	Sri Lanka (ET)	KF234422	KY610493	KY624282	KF300548	Kuhnert et al. (2014a); Wendt et al. (2018)
Hypoxylon vogesiacum	CBS 115273	France	KC968920	KY610417	KY624283	KX271275	Kuhnert et al. (2014a); Wendt et al. (2018)
***Hypoxylon xmatkuilense* sp.nov.**	**UADY:PR_3**	**Mexico (HT)**	**OR807999**	**OR807990**	**OR825476**	**OR825467**	**This study**
***Hypoxylon xmatkuilense* sp. nov.**	**UADY:PR_118**	**Mexico (PT)**	**OR807996**	**OR807995**	**PP239283**	**PP239284**	**This study**
Jackrogersella cohaerens	CBS 119126	Germany	KY610396	KY610497	KY624270	KY624314	Wendt et al. (2018)
Jackrogersella minutella	CBS 119015	Portugal	KY610381	KY610424	KY610424	KX271240	Kuhnert et al. (2016); Wendt et al. (2018)
Jackrogersella multiformis	CBS 119016	Germany (ET)	KC477234	KY610473	KY624290	KX271262	Kuhnert et al. (2014a); Wendt et al. (2018)
Parahypoxylon papillatum	ATCC 58729	USA (T)	KC968919	KY610454	KY624223	KC977258	Kuhnert et al. (2014a), Wendt et al. (2018)
Parahypoxylon ruwenzoriense	MUCL51392	Congo (T)	ON792786	ON954156	OP251039	ON813078	Cedeño-Sanchez et al. (2023)
Phylacia globosa	STMA 18042	Argentina	OQ437889.1	OQ437885	OQ453168	OQ453172	Lambert et al. (2023)
Phylacia lobulata	STMA 18040	Argentina	OQ437893.1	OQ437883	OQ453164	OQ453170	Lambert et al. (2023)
Pyrenopolyporus hunteri	MUCL 52673	Ivory Coast (ET)	KY610421	KY610472	KY624309	KU159530	Kuhnert et al. (2016); Wendt et al. (2018)
Pyrenopolyporus laminosus	MUCL 53305	Martinique (HT)	KC968934	KY610485	KY624303	KC977292	Kuhnert et al. (2014a); Wendt et al. (2018)
Pyrenopolyporus nicaraguensis	CBS 117739	Burkina Faso	AM749922	KY610489	KY624307	KC977272	Bitzer et al. (2008); Wendt et al. (2018)
Rhopalostroma angolense	CBS 126414	Ivory Coast	KY610420	KY610459	KY624228	KX271277	Wendt et al. (2018)
Rostrohypoxylon terebratum	CBS 119137	Thailand (HT)	DQ631943	DQ840069	DQ631954	DQ840097	Tang et al. (2007); Fournier et al. (2010)
Ruwenzoria pseudoannulata	MUCL 51394	D.R. Congo (HT)	KY610406	KY610494	KY624286	KX271278	Wendt et al. (2018)
Thamnomyces dendroidea	CBS 123578	French Guiana (HT)	FN428831	KY610467	KY624232	KY624313	Stadler et al. (2010b); Wendt et al. (2018)
Xylaria polymorpha	MUCL 49884	France	KY610408	KY610464	KY624288	KX271280	Wendt et al. (2018)

MycoKeys

MycoKeys 111: 129–146 (2024)
DOI: 10.3897/mycokeys.111.136922

Research Article

Multigene phylogeny, taxonomy, and potential biological properties of *Pseudoroussoella* and *Neoroussoella* species (Roussoellaceae, Dothideomycetes) from Asteraceae weeds in northern Thailand

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Abstract

In our study, dead stems of two Asteraceae species (weeds) were collected from northern Thailand. Both morphology and multigene phylogeny were used to determine the identity of the taxa. Maximum likelihood and Bayesian inference analyses of combined LSU, SSU, ITS, *tef1-α* and *rpb2* data revealed two new species *Pseudoroussoella bidenticola*, and *Neoroussoella chromolaenae* with one new host record of *N. entadae*. Preliminary investigation into antibacterial properties revealed that our three isolates inhibited the growth of *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus*. Additionally, we present updated phylogenetic trees for Roussoellaceae, accompanied by detailed descriptions and illustrations of the three identified species.

Key words: 2 new species, antibacterial properties, Ascomycota, *Bidens pilosa*, *Chromolaena odorata*, new host record

OPEN ACCESS

This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

Academic editor:
Danushka Sandaruwan Tennakoon
Received: 12 September 2024
Accepted: 9 November 2024
Published: 17 December 2024

Citation: Htet ZH, Hyde KD, Alotibi FO, Chethana TKW, Mapook A (2024) Multigene phylogeny, taxonomy, and potential biological properties of *Pseudoroussoella* and *Neoroussoella* species (Roussoellaceae, Dothideomycetes) from Asteraceae weeds in northern Thailand. MycoKeys 111: 129–146. https://doi.org/10.3897/mycokeys.111.136922

Introduction

Asteraceae species exhibit a widespread distribution from polar to tropical regions (Xu et al. 2017). Many of these species hold economic significance, while others are categorized as weeds (Jansen and Palmer 1987; Katinas et al. 2007; Karlsson et al. 2008). In Thailand, numerous invasive weeds have an extensive spread, with *Bidens pilosa* and *Chromolaena odorata* being prevalent at roadsides, disturbed areas, and agricultural lands (Zungsontiporn et al. 2020). Mapook et al. (2020) studied the fungal diversity in *Chromolaena odorata* and provided a global checklist of fungi associated with this plant. Moreover, the information of fungi associated with *Bidens pilosa* was provided in previous studies (Abdou et al. 2010; Guatimosim et al. 2015; Zhang et al. 2018; Li et al. 2020; Htet et al. 2024). However, more knowledge is still needed about the diversity of fungi in Asteraceae plants to understand the fungi associated with this plant family. Moreover, the diversity of fungi in these two invasive weeds in Thailand is being further explored.

Roussoellaceae was introduced by Liu et al. (2014) based on morphology and LSU, ITS, *tef1-α* and *rpb2* sequence data. Members of Roussoellaceae can be found as saprobes and human pathogens (Ahmed et al. 2014; Liu et al. 2014; Mapook et al. 2020; Hyde et al. 2023; Wu et al. 2023). Currently, there are 12 genera in Roussoellaceae, viz., *Appendispora*, *Cytoplea*, *Elongatopedicellata*, *Immorrhia*, *Neoroussoella*, *Pararoussoella*, *Pseudoneoconiothyrium*, *Pseudoroussoella*, *Roussoella*, *Roussoellopsis*, *Setoarthopyrenia*, and *Xenoroussoella* (Wijayawardene et al. 2022; Index Fungorum www.indexfungorum.org).

Neoroussoella was introduced by Liu et al. (2014) to accommodate a saprobic roussoella-like taxon with the type species *N. bambusae*. The sexual morphology of *Neoroussoella* is defined by immersed ascostromata beneath a clypeus or epidermis, appearing as black, dome-shaped, or flattened ovoid structures on the host surface. The asci are bitunicate and cylindrical, while the ascospores are brown or yellowish-brown, ellipsoidal to fusiform, and 2-celled, surrounded by a mucilaginous sheath (Liu et al. 2014). The asexual morphology of *Neoroussoella* is characterized by superficial or immersed pycnidia with annellidic, ampulliform, cylindrical conidiogenous cells, producing hyaline, pale brown, oblong to ellipsoidal conidia, each with two guttules (Liu et al. 2014; Jayasiri et al. 2019). Currently, there are 15 epithets listed in the Index Fungorum (www.indexfungorum.org), viz., *Neoroussoella alishanensis*, *N. bambusae*, *N. clematidis*, *N. chiangmaiensis*, *N. entadae*, *N. fulvicomae*, *N. heveae*, *N. lenispora*, *N. leucaenae*, *N. lignicola*, *N. magnoliae*, *N. peltophora*, *N. sedimenticola*, *N. solani*, and *N. thailandica*. Recent studies into the genus have been conducted by De Silva et al. (2022) and Hyde et al. (2023).

Pseudoroussoella was introduced by Mapook et al. (2020) based on morphology and LSU, SSU, ITS, *tef1-α* and *rpb2* sequence data. The sexual morph of *Pseudoroussoella* species is characterized by globose to subglobose, dark brown to black ascomata with an ostiole, comprised of *textura epidermoidea* to *textura angularis* or *textura intricata* cells, with septate, trabeculate pseudoparaphyses, 8-spored, bitunicate, fissitunicate, cylindrical to clavate asci with a pedicel, and uniseriate, hyaline to pale brown, oval to ellipsoid, 1-septate ascospores bearing a gelatinous sheath (Mapook et al. 2020). Asexual morphs of *Pseudoroussoella* species are distinguished by solitary, superficial, uni-loculate, globose to obpyriform, pycnidial conidiomata with an ostiole, comprised of *textura angularis* cells, annellidic, ampulliform to oblong, hyaline and unbranched conidiogenous cells and pale brown to reddish brown, aseptate conidia with guttules (Mapook et al. 2020). Currently, two species are listed in the Index Fungorum (www.indexfungorum.org).

Some genera from Roussoellaceae, like *Roussoella* and *Neoroussoella*, are recognized for their bioactive secondary metabolites (Takekawa et al. 2013; Phukhamsakda et al. 2018; Chen et al. 2021; Zhong et al. 2021; Sommart et al. 2022). Moreover, the prescreening for antibacterial activity conducted by Mapook et al. (2020) revealed that *Pseudoroussoella elaeicola* (MFLUCC 17-1483) inhibits the growth of *E. coli*, resulting in a 10 mm inhibition zone. These findings showed that the species of Roussoellaceae are potential organisms for the production of bioactive secondary metabolites.

In this study, we introduce one new species of *Pseudoroussoella* on *Bidens pilosa* (Asteraceae), and one new species with a new host record of *Neoroussoella* on *Chromolaena odorata* (Asteraceae). We also provide an updated phylogenetic tree for Roussoellaceae, based on a combined dataset of LSU, SSU, ITS, *tef1-α* and *rpb2* sequence data. Further, we explore the potential antibacterial activity of our three isolates and discuss their implications for future discoveries of bioactive compounds.

Materials and methods

Sample collection, morphological study and isolation

Dead stems from the Asteraceae plants, *Bidens pilosa* and *Chromolaena odorata*, were collected from northern Thailand. All specimens were brought to the lab in plastic bags labelled with the collection information. Single spore isolation was performed on malt extract agar (MEA) and incubated at 27 °C for 24 hours (Senanayake et al. 2020). The spores were germinated within 24 h using a Motic SMZ 168 Series microscope (Motic Asia, Hong Kong). Germinated spores were transferred to fresh MEA plates. All the detailed morphological characteristics were observed using a Nikon ECLIPSE 80i compound microscope (Nikon, Japan) fitted to a Canon 550D digital camera (Canon, Japan). Tarosoft Image Framework (v 0.9.7) was used to measure photomicrograph structures. Adobe Photoshop CS6 Extended (v 10.0.) was used to edit and prepare photo plates (Adobe system, USA). Forty-day-old cultures were used for molecular studies. Specimens were deposited at the Mae Fah Luang University Herbarium (Herb. MFLU) while living cultures were maintained at Mae Fah Luang University Culture Collection (MFLUCC). Faces of fungi (FoF) and Index Fungorum (IF) numbers were obtained as instructed by Jayasiri et al. (2015) and Index Fungorum (www.indexfungorum.org). Moreover, the species descriptions were submitted to the GMS Microfungi database (Chaiwan et al. 2021).

DNA extraction, PCR amplification and sequencing

Fifty-day-old fungal mycelium was scraped off and placed into a 1.5 ml micro-centrifuge tube using a sterile lancet. Genomic DNA extraction was done using the E.Z.N.A.® Tissue DNA Kit (Omega Biotek Inc., Georgia). Polymerase chain reaction (PCR) was used for DNA amplifications, following the protocols of Mapook et al. (2016). The details of PCR primers and protocols are shown in Table 1. The quality of PCR products was confirmed on 1% agarose gels. Purification and sequencing of PCR fragments with the primers mentioned above were carried out at a commercial sequencing provider (Solgent Co., Ltd, Thailand). The newly generated nucleotide sequences were deposited in the GenBank, and accession numbers were obtained (Table 2).

Table 1. PCR conditions used in this study.

Gene	Primers		PCR Conditions
	Forward	Reverse	
Large subunit (LSU)	LR0R	LR5	95 °C: 3 min, (94 °C: 30 s, 56 °C: 50 s, 72 °C: 1 min) × 40 cycles 72 °C: 7 min.
Small subunit (SSU)	NS1	NS4	95 °C: 3 min, (94 °C: 30 s, 55 °C: 50 s, 72 °C :1 min) × 40 cycles 72 °C: 7 min.
Internal transcribed spacer (ITS)	ITS5	ITS4	95 °C: 3 min, (94 °C: 30 s, 55 °C: 50 s, 72 °C :1 min) × 40 cycles 72 °C: 7 min.
Elongation factor-1 alpha (*tef1- α*)	EF1-983F	EF1-2218R	95 °C: 3 min, (94 °C: 30 s, 55 °C: 50 s, 72 °C: 1 min) × 40 cycles 72 °C: 7 min.
RNA polymerase II subunit (*rpb2*)	fRPB2-5F	fRPB2-7cR	95 °C: 5 min, (95 °C : 1 min, 52 °C: 2 min, 72 °C: 90 s) × 40 cycles 72 °C: 10 min

Table 2. List of taxa, specimens and sequences used in phylogenetic analyses.

Species	Strain numbers	GenBank accession numbers				
		ITS	**LSU**	**SSU**	***tef1- α***	***rpb2***
Neoroussoella alishanense	FU31016	MK503816	MK503822	MK503828	–	MN037756
N. alishanense	FU31018	MK503818	MK503824	MK503830	MK336182	MN037757
N. bambusae	MFLUCC 11-0124 T	KJ474827	KJ474839	–	KJ474848	KJ474856
***N. chromolaenae* sp. nov.**	**MFLUCC 24-0274**	**PQ226190**	**PQ226193**	**PQ226196**	**PQ240621**	**PQ240623**
N. clematidis	MFLUCC 17-2061	MT310632	MT214587	MT226700	MT394645	MT394701
N. entadae	MFLUCC 18-0243	MK347786	MK348004	MK347893	MK360065	MK434866
N. entadae	**MFLUCC 24-0275**	**PQ226191**	**PQ226194**	**PQ226197**	–	**PQ240624**
N. fulvicomae	MFLUCC 17-2073	MT310633	MT214588	MT226701	MT394646	MT394702
N. heveae	MFLUCC 17-1983	MH590693	MH590689	MH590691	–	–
N. lenispora	GZCC 16-0020 T	–	KX791431	–	–	–
N. leucaenae	MFLUCC 18-1544	MK347767	MK347984	MK347874	MK360067	MK434876
N. leucaenae	MFLUCC 17-0927	MK347733	MK347950	MK347841	MK360066	MK434896
N. lignicola	MUT 5008	MN556318	MN556320	MN556308	MN605895	MN605915
N. lignicola	MUT 5373	KU314953	MN556321	KU314954	MN605896	MN605916
N. lignicola	MUT 4904	KT699129	MN556319	MN556307	MN605894	MN605914
N. magnoliae	MFLU 18-1022	MK801232	MK801230	MK801231	MK834373	–
N. peltophora	MFLUCC 21-0071	MZ567051	MZ567206	MZ567207	MZ605441	MZ605442
N. sedimenticola	CGMCC 3.22470	OQ798949	OQ758144		OQ809046	OQ809008
N. sedimenticola	CGMCC 3.22468 T	OQ798948	OQ758143		OQ809045	OQ809007
N. solani	KT3264 T	LC195218	LC195209	LC195206	LC195212	–
N. solani	KT3265 T	LC195219	LC195210	LC195207	LC195213	LC195216
N. thailandica	MFLUCC 18-0721	OL703581	OL457704	OL764415	OM505028	ON502386
Occultibambusa bambusae	MFLUCC 11-0394	KU940124	KU863113	–	KU940194	KU940171
O. bambusae	MFLUCC 13-0855	KU940123	KU863112	KU872116	KU940193	KU940170
Pseudoneoconiothyrium rosae	MFLUCC 15-0052 T	MG828922	MG829032	MG829138	–	–
P. euonymi	CBS 143426 T	MH107915	MH107961	–	–	MH108007
P. euonymi	GLMC 1544	MT153733	MT156304	–	–	–
***Pseudoroussoella bidenticola* sp. nov.**	**MFLUCC 24-0273**	**PQ226192**	**PQ226195**	**PQ226198**	**PQ240622**	**PQ240625**
Ps. chromolaenae	MFLUCC 17-1492 T	MT214345	MT214439	MT214393	MT235769	–
Ps. elaeicola	MFLUCC 15-0276a T	MT153733	MT156304	–	–	–
Ps. elaeicola	MFLUCC 15-0276b	MH742330	MH742327	–	–	–
Ps. elaeicola	MFLUCC 17-1483	MT214348	MT214442	–	MT235772	MT235808
Pararoussoella mangrovei	MFLUCC 17-1542	MH025951	MH023318	–	MH028246	–
P. mukdahanensi	HKAS 101766	MH453489	MH453485	–	MH453478	MH453482
P. rosarum	MFLUCC 17-0796 T	MG828939	MG829048	NG_061294	MG829224	MH028250
Roussoella arundinacea	CPC 35554	MT223838	MT223928	–	MT223723	–
R. bambusarum	GMB0316(HT)	ON479891	ON479892	–	ON505015	ON505011
R. bambusarum	GMB0390	ON505055	ON505051	–	ON505017	ON505012
R. chiangraina	MFLUCC 10-0556 T	KJ474828	KJ474840	–	KJ474849	KJ474857
R. doimaesalongensis	MFLUCC 14-0584 T	KY026584	KY000659	–	KY651249	KY678394
R. hysterioides	CBS 546.94 T	KF443405	KF443381	AB524480	KF443399	KF443392
R. intermedia	CBS 170.96	KF443407	KF443382	KF443390	KF443398	KF443394
R. japanensis	MAFF 239636 T	KJ474829	AB524621	–	AB539114	AB539101
R. kunmingensis	HKAS 101773	MH453491	MH453487	–	MH453480	MH453484
R. margidorensis	MUT 5329 T	KU314944	MN556322	MN556309	MN605897	MN605917
R. mediterranea	MUT 5306	KU255054	MN556323	MN556310	MN605898	MN605918
R. mexicana	CPC25355 T	KT950848	KT950862	–	–	–
R. neopustulans	MFLUCC 11-0609 T	KJ474833	KJ474841	–	KJ474850	–
R. neopustulans	MFLUCC 12-0003 T	KU940130	KU863119	KU872122	–	–
R. nitidula	MFLUCC 11-0182 T	KJ474835	KJ474843	–	KJ474852	KJ474859
R. nitidula	MFLUCC 11-0634 T	KJ474834	KJ474842	–	KJ474851	KJ474858
R. padinae	MUT 5341	KU158153	MN556325	–	MN605900	MN605920
R. padinae	MUT 5365	KU158170	MN556326	KU158179	MN605901	MN605921
R. padinae	MUT 5503	KU314993	MN556327	MN556312	MN605902	MN605922
R. pseudohysterioides	MFLUCC 13-0852 T	KU940131	KU863120	–	KU940198	–
R. pustulans	KT 1709	–	AB524623	AB524482	AB539116	AB539103

Species	Strain numbers	GenBank accession numbers				
		ITS	LSU	SSU	*tef1- α*	*rpb2*
R. scabrispora	MFLUCC 11-0624	KJ474836	KJ474844	–	KJ474853	KJ474860
R. siamensis	GMB0317	ON4617749	ON461896	–	ON505014	ON505010
R. siamensis	GMB0391	ON505054	ON505053	–	ON505018	ON505013
R. tosaensis	KT 1659	–	AB524625	AB524484	AB539117	AB539104
R. tuberculata	MFLUCC 13-0854 T	KU940132	KU863121	–	–	–
R. verrucispora	CBS 125434 T	KJ474832	–	–	–	–
R. yunnanensis	HKAS 101762 T	MH453492	MH453488	–	MH453481	–
R. yunnanensis	MFLUCC 12-0005 T	KJ739604	KJ474847	KJ739608	KJ474855	KJ474862
Xenoroussoella triseptata	MFLUCC 17-1438	MT214343	MT214437	MT214391	MT235767	MT235804
X. triseptata	KNUF-20-NI009	LC719282	LC719283	LC723530	LC723531	LC723532

* Remarks: The letter T denotes ex-type isolates. The newly generated sequences, new species and synonymized isolates are indicated in bold font.

Sequence alignment and phylogenetic analyses

Based on the sequence data of recent publications (De Silva et al. 2020; Li et al. 2023) and BLAST search results, reference taxa were selected, and phylogenetic analyses were conducted using the combined LSU, SSU, ITS, *tef1-α* and *rpb2* sequence data. Sequence alignments were made with the MAFFT v. 7 online tool (http://mafft.cbrc.jp/alignment/server; 2016). Alignments were improved where necessary, and composite sequence alignments were obtained using MEGA v. 6.0.

RAxML and Bayesian analyses were carried out on the CIPRESS Science Gateway Portal (http://www.phylo.org) (Miller et al. 2010). Maximum likelihood analysis was performed by RAxML-HPC v.8 (Stamatakis 2014) with rapid bootstrap analysis, followed by 1000 bootstrap replicates and the GTRGAMMA substitution model. MrBayes was used to perform BI analysis on XSEDE 3.2.7 (Ronquist et al. 2012), with tree samples taken at every 1000th generation during the 5,000,000-generation run of four concurrent Markov chains. The first 25% of the trees were removed as part of the burn-in phase, and calculations for the Posterior Probability were made for the remaining 75% of the trees (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002). The phylogenetic tree was displayed using Fig Tree v1.4.0 (Rambaut 2012) and was modified in Microsoft Office PowerPoint v. 2013.

Preliminary screening for antibacterial activity

Preliminary screening for antibacterial activity was carried out following the methods of Htet et al. (2024). Antibacterial discs of ampicillin were used as a positive control for screening (Alam et al. 2019). Antibacterial activity against gram positive bacteria (*Bacillus subtilis*-TISTR 1248 and *Staphylococcus aureus*-TISTR Y4b), and gram-negative bacteria (*Escherichia coli* TISTR 527) were investigated using the agar plug diffusion method (Balouiri et al. 2016). Bacteria test organisms were obtained from Scientific and Technological Instrument Center, Mae Fah Luang University. Bacterial test organisms were sub cultured and grown on Nutrient Agar (NA) for 24 hours. After 24 hours of inoculation, 2–3 loops of the bacterial test organisms were transferred to the nutrient broth and incubated for 24 hours in a shaking incubator. Prior to adding microbial suspensions to the sterile Mueller-Hinton agar media, cell counts were performed on the suspensions (6.7×10^5 cells/mL), as detailed by Mapook et al. (2020). Fungal mycelium plugs from our isolates were transferred to a solid medium plate and allowed to grow at room temperature for 24–48 hours. Inhibition zones were measured and compared to the positive control.

Results

Phylogenetic analyses

The combined LSU, SSU, ITS *tef1-α*, and *rpb2* sequence dataset comprises 66 taxa with *Occultibambusa bambusae* strains (MFLUC 13-0855 and MFLUCC 11-0394) as the outgroup taxa. Maximum likelihood (ML) analyses and Bayesian Inference (BI) of the combined dataset were performed to determine the placement of our new isolates. Tree topologies of ML and BI criteria were similar to earlier investigations (De Silva et al. 2020; Li et al. 2023). The best-scoring RAxML tree with a final likelihood value of -28736.822321 is shown in Fig. 1. RAxML analysis yielded 1578 distinct alignment patterns,

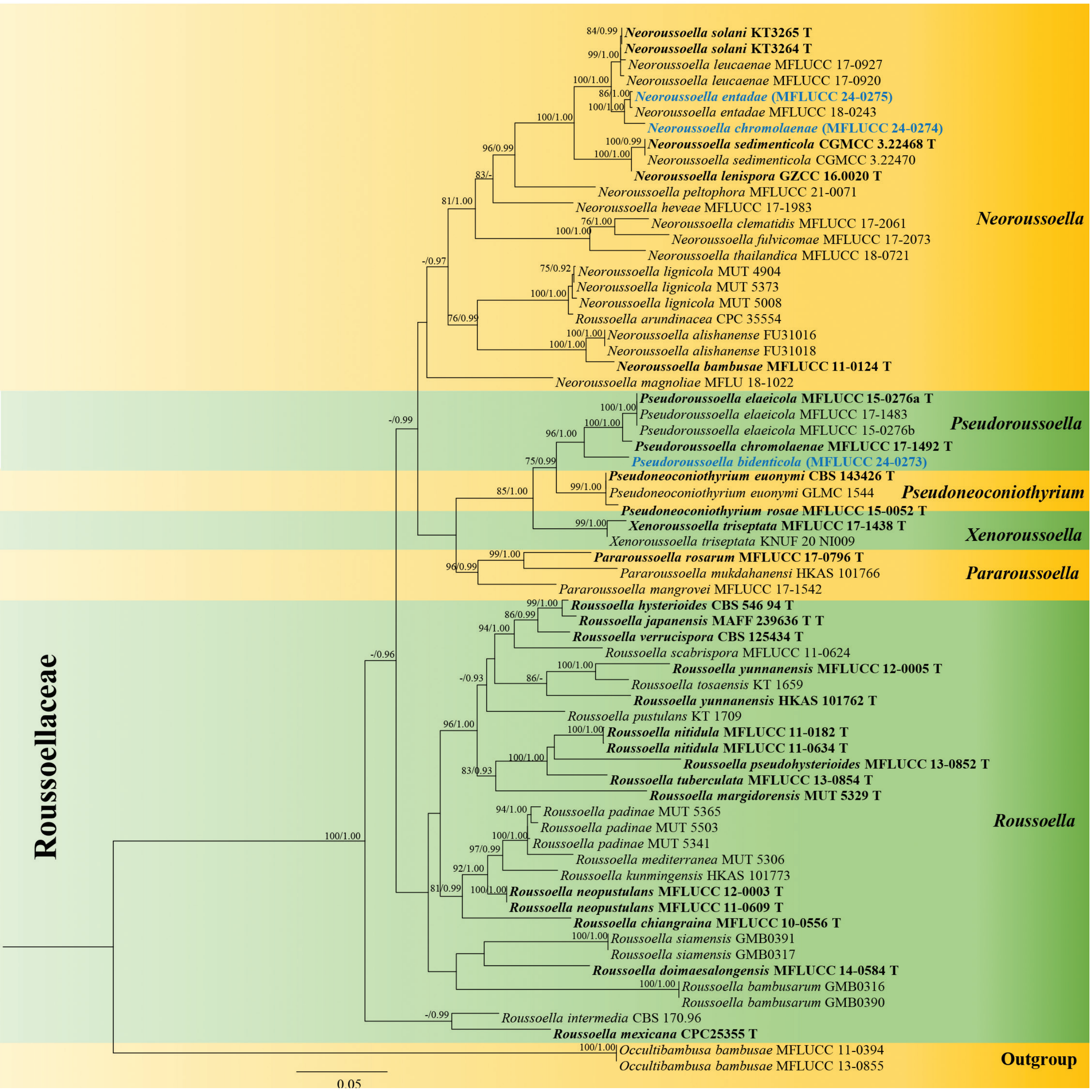

Figure 1. Phylogram generated from maximum likelihood analysis based on the combined dataset of LSU, SSU, ITS, *tef1-α* and *rpb2* sequence data. Bootstrap support values for ML equal to or greater than 75% and BYPP equal to or greater than 0.90 are given at the nodes. Newly generated sequences are in blue and type species are in bold.

with 29.39% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.245746, C = 0.258383, G = 0.266559, T = 0.229312; substitution rates: AC = 1.714222, AG = 4.957697, AT = 1.884927, CG = 1.345111, CT = 9.562411, GT = 1.000000; gamma distribution shape parameter α = 0.166563. In our phylogenetic analysis, *Neoroussoella chromolaenae* sp. nov. (MFLUCC 24-0274) clustered with *Neoroussoella entadae* (MFLUCC 18-0243 and MFLUCC 24-0275) with 100% ML and 1.00 BYPP support. Furthermore, our isolate, *Pseudoroussoella bidenticola* sp. nov. (MFLUCC 24-0273) formed a branch separated from *Ps. elaeicola* (MFLUCC 17-1483, MFLUCC 15-0276a, MFLUCC 15-0276b), and *Ps. chromolaenae* (MFLUCC 17-1492) with 96% ML and 1.00 BYPP, respectively.

Taxonomy

Neoroussoella chromolaenae **Z.H. Htet, A. Mapook & K. D. Hyde, sp. nov.**

Index Fungorum: IF902613
Facesoffungi Number: FoF16402
Fig. 2

Etymology. Name reflects the host plant *Chromolaena odorata*, from which this species was isolated.

Holotype. MFLU 24-0264.

Description. ***Saprobic*** on the dead stems of *Chromolaena odorata* (Asteraceae). ***Sexual morph***: Undetermined. ***Asexual morph***: Coelomycetous. ***Conidiomata*** 70–150 × 120–150 µm (av. 85 × 138 µm, n = 5), pycnidial, solitary, uniloculate, immersed, ostiolate. ***Ostiole*** papillate. ***Peridium*** 10–20 µm wide, comprising 2–3 layers of brown cells of ***textura angularis***. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 3–5 × 2–3.5 µm (av. 3 × 3 µm, n = 10), phialidic, ampulliform to cylindrical, hyaline. ***Conidia*** 3–6 × 2–4 µm (av. 4.4 × 3.1 µm, n = 20), hyaline, oblong to slightly ellipsoid, aseptate, with small guttules.

Culture characteristics. Conidia germinating on MEA within 24 hours, reaching 22 mm after 10 days at 27 °C, irregular, curled margin, brown in the middle and becoming pale brown on the outer parts of the culture, wrinkled on the surface; wrinkle, pale brown to brown in reverse.

Material examined. THAILAND • Chiang Rai Province, Doi Pui, 19°48'51"N, 99°52'1"E, on dead stems of *Chromolaena odorata* (Asteraceae), 14 March 2023, Zin Hnin Htet (CO-DP-3, MFLU 24-0264, holotype); ex-type culture MFLUCC 24-0274.

Notes. In a megablast search of GenBank, the closest match for the ITS sequence of our isolate was fungal sp. isolate NFC-3 (MG189955) with 99.47% similarity. The closest match for the LSU region was *N. solani* CBS 141288 (MH878207) with 100% similarity, and the closest match for the SSU region was *N. bambusae* strain GMB1295 (OM764650) with 93.99% similarity. Additionally, the closet matches for the *tef1-α* and *rpb2* gene regions were *Neoroussoella entadae* strain MFLUCC 18-0243 (MK360065) and *N. entadae* strain MFLUCC 17-0920 (MK434898) with 99.45% and 99.77% similarities, respectively.

Based on the multi-locus phylogeny (Fig. 1), our isolate (MFLUCC 24-0274) formed a separate branch from *N. entadae* with 100% ML and 1.00 BYPP.

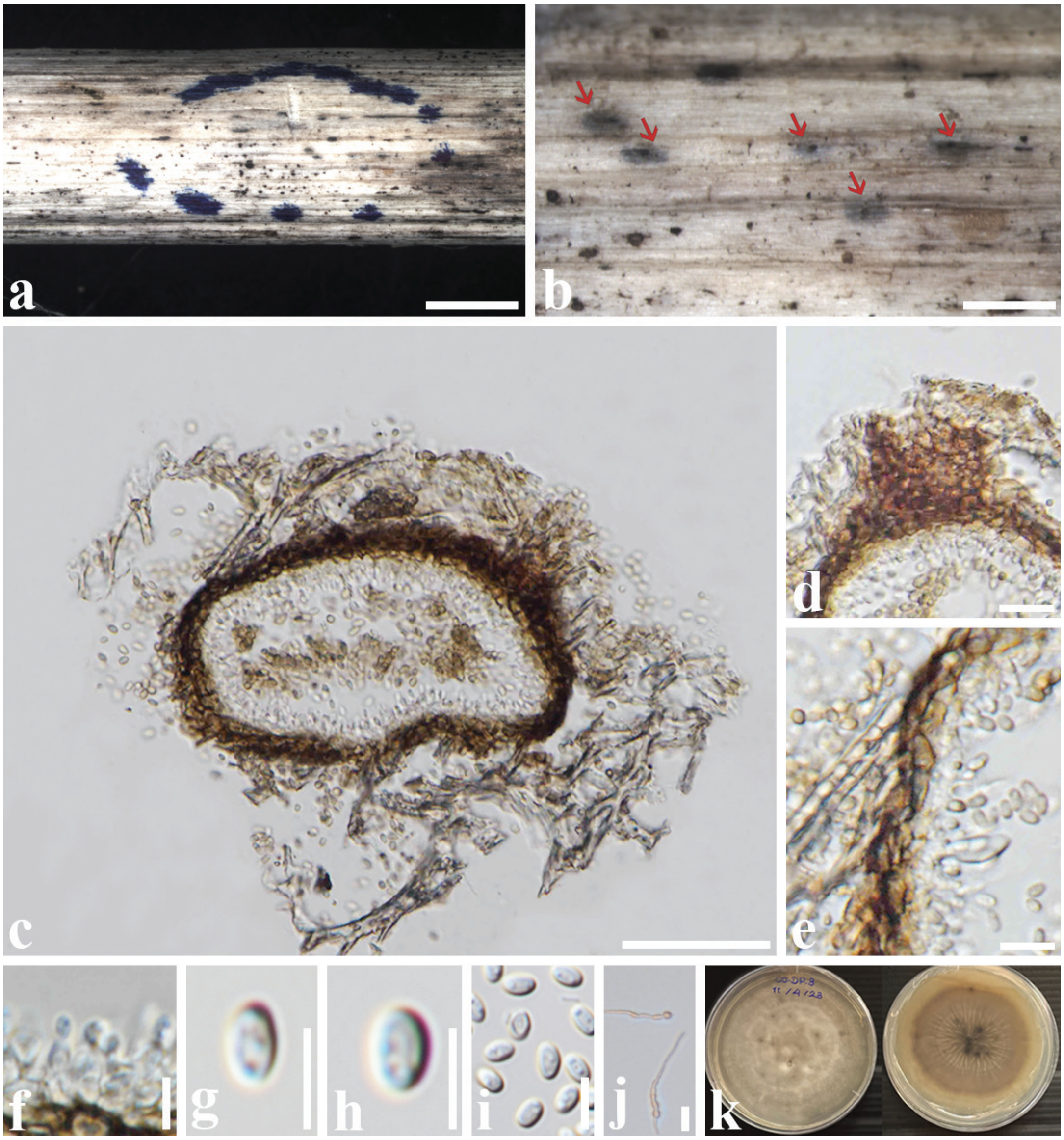

Figure 2. *Neoroussoella chromolaenae* (MFLU 24-0264, holotype) **a, b** Conidiomata on the substrate **c** a section through conidioma **d** ostiole **e** peridium **f** conidia and conidiogenous cells **g–i** conidia **j** germinating conidia **k** culture on the MEA. Scale bars: 500 µm (**a, b**); 100 µm (**c**); 20 µm (**d, e**); 10 µm (**e–j**).

A comparative analysis of base pair differences between *Neoroussoella chromolaenae* (MFLUCC 24-0274) and *Neoroussoella entadae* (MFLUCC 18-0243) revealed variations in ITS (0.6% - 3/476), LSU (0.1% - 1/838), SSU (1.9% - 14/717), *tef1-α* (0.5% - 5/902), and *rpb2* (2.0% - 18/885) without gaps, respectively. Morphologically, our collection is similar to *N. entadae* (MFLUCC 17–0920) in having solitary, unilocular, ostiolate, phialidic, ampulliform to cylindrical, hyaline conidiogenous cells, and oblong to ellipsoidal, hyaline conidia (Jayasiri et al. 2019). However, our species differs from *N. entadae* (MFLUCC 17–0920) in having smaller conidiomata (70–150 × 120–150 µm vs. 127–192 × 161–190 µm), slightly wider conidiogenous cells (2–3.5 µm vs. 0.7–1.8 µm) and larger conidia size (3–6 × 2–4 µm vs. 3–4 × 1.7–1.9 µm). Therefore, *N. chromolaenae* is described here as a new species based on phylogeny and morphological evidence. Synopsis of the asexual morph of *Neoroussoella* species is also provided in Table 3.

Table 3. Synopsis of asexual morph species in *Neoroussoella*.

Species	Conidiomata (µm)	Conidiogenous cells (µm)	Conidia (µm)	References
Neoroussoella alishanense (FU31016)	130–140, 210–225	8–14 × 2–3	3–4 × 2–3	Karunarathna et al. (2019)
Neoroussoella bambusae (MFLUCC 11-0124)	200–430 × 300–420	8–13.5 × 1.5–3	3–4 × 1.5–2	Liu et al. (2014)
Neoroussoella chromolaenae (MFLUCC 24-0274)	70–150 × 120–150	3–5 × 2–3.5	3–6 × 2–4	This study
Neoroussoella entadae (MFLUCC 17–0920)	127–192 × 161–190	3.5–5.6 × 0.7–1.8	3–4 × 1.7–1.9	Jayasiri et al. (2019)
Neoroussoella entadae (MFLUCC 24-0275)	70–120 × 100–150	3–5 × 1–3	3–5 × 2–4	This study
Neoroussoella heveae (MFLUCC 17-0338)	90–130, 115–180	3–7 × 2–5	2.5–5 × 2–4	Phookamsak et al. (2019)
Neoroussoella leucaenae (MFLUCC 18–1544)	135–175 × 120–180	5.5–9 × 3–4	3.5–4.5 × 1.9–2.6	Jayasiri et al. (2019)
Neoroussoella peltophora (MFLUCC 21-0071)	165–224 × 144–178	1–3.5 × 6.5–8	2.0–3.0 × 3.0–4.0	Pasouvang et al. (2022)
Neoroussoella solani (CPC 26331)	To 150	4–6 × 3–4	4.5–5 × 2–3	Crous et al. (2016)

Neoroussoella entadae **Jayasiri, E.B.G. Jones & K.D. Hyde, Mycosphere 10(1): 105 (2019)**

Index Fungorum: IF555568
Facesoffungi Number: FoF05275
Fig. 3

Description. ***Saprobic*** on the dead stems of *Chromolaena odorata* (Asteraceae). ***Sexual morph*:** Undetermined. ***Asexual morph*:** Coelomycetous. ***Conidiomata*** 70–120 × 100–150 µm (av. 95 × 110 µm, n = 5), pycnidial, solitary, globose to subglobose, uniloculate, immersed to semi-immersed, ostiolate. ***Ostiole*** 30–35 µm wide, with small papillate. ***Peridium*** 10–20 µm wide, comprising 2–3 layers of brown cells of ***textura angularis***. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 3–5 × 1–3 µm (av. 3.8 × 2.1 µm, n = 10), phialidic, ampulliform to cylindrical, hyaline. ***Conidia*** 3–5 × 2–4 µm (av. 3.8 × 2 µm, n = 20), hyaline, becoming pale brown when gathering, oblong to ovoid, aseptate, with small guttules.

Culture characteristics. Conidia germinating on PDA within 24 hours, reaching 24 mm after 7 days at 27 °C, irregular, entire, yellowish-brown, slightly wrinkled on the surface; pale brown to brown in reverse.

Material examined. THAILAND • Chiang Rai Province, Thoeng district, on dead stems of *Chromolaena odorata* (Asteraceae), 24 Jan 2022, A. Mapook (TCR18, MFLU 24-0265, new host record); living culture MFLUCC 24-0275.

Known host distribution. *Entada phaseoloides* (Fabaceae), *Leucaena* sp. (Fabaceae) (Jayasiri et al. 2019).

Notes. In a BLASTn search of GenBank, the closest match for the ITS sequence of our isolate was *N. solani* strain MnF107 (OQ704272) with 99.83% similarity. The closest match for the LSU region was *Roussoella* sp. strain HF3S53 (OP179275) with 99.77% similarity, and the closest match for the SSU region was Pleosporales sp. IRB20-2 (AB195632) with 100% similarity. The closest match for the *tef1-α* and *rpb2* gene region was *Neoroussoella entadae* strain MFLUCC 18-0243 (MK434866) with 99.78% and 99.53% similarity, respectively.

When we compared the morphology, our isolate is similar to *N. entadae* (MFLUCC 17–0920) in having solitary, unilocular, ostiolate conidiomata, phialidic, ampulliform to cylindrical, hyaline conidiogenous cells, and oblong to ovoid, hyaline conidia with size (3–5 × 2–4 µm vs 3–4 × 1.7–1.9 µm). However, our isolate differs from *N. entadae* (MFLUCC 17–0920) in having smaller

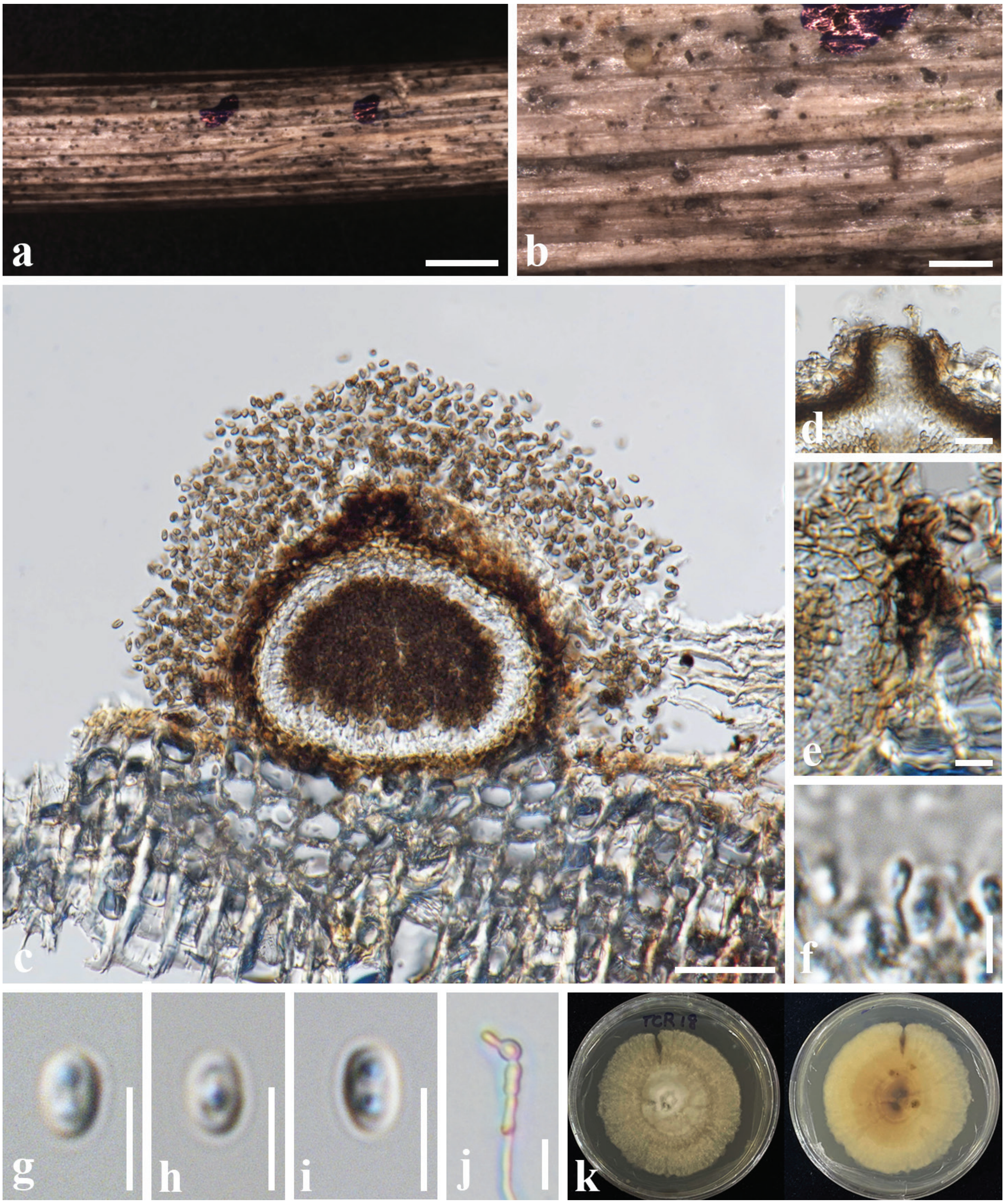

Figure 3. *Neoroussoella entadae* (MFLU 24-0265, new host record) **a, b** Conidiomata on the substrate **c** a section through conidioma **d** ostiole **e** peridium **f** conidia and conidiogenous cells **g–i** conidia **j** a germinating conidium **k** culture on the MEA. Scale bars: 500 µm (**a**); 200 µm (**b**); 50 µm (**c**); 20 µm (**d, e**); 5 µm (**f, g, h, i, j**).

conidiomata (70–120 × 100–150 µm vs. 127–192 × 161–190 µm), slightly wider conidiogenous cells (3–5 × 1–3 µm vs. 3.5–5.6 × 0.7–1.8 µm) (Table 3).

Based on the multi-locus phylogeny (Fig. 1), our isolate MFLUCC 24-0275 clustered in the same clade with *N. entadae* (MFLUCC 18-0243). Moreover, there is no significant base pair difference between MFLUCC 24-0275 and *N. entadae* (MFLUCC 18-0243). Therefore, we reported *N. entadae* as the first occurrence on *C. odorata* (Asteraceae) based on morphology and multigene phylogeny.

Pseudoroussoella bidenticola **Z.H. Htet, A. Mapook & K. D. Hyde, sp. nov.**

Index Fungorum: IF902614
Facesoffungi Number: FoF16403
Fig. 4

Etymology. Name reflects the host plant *Bidens pilosa*, from which this species was isolated.

Holotype. MFLU 24-0266.

Description. ***Saprobic*** on dead stems of *Bidens pilosa*. ***Sexual morph*****:** Undetermined. ***Asexual morph***: Coelomycetous. ***Conidiomata*** 120–150 × 150–180 µm (av. 126 × 173 µm, n = 5), pycnidial, solitary, immersed to semi-immersed, uni-loculate, brown, globose to subglobose, dark fruiting bodies on the host substrate, without an ostiole. ***Peridium*** 10–20 µm wide, comprising 2–3 layers of yellowish brown to brown cells of ***textura angularis***. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 1–2 µm long, holoblastic, short, globose to subglobose, hyaline and unbranched. ***Conidia*** 5–7.5 × 4–5.5 µm (av. 6 × 4.8 µm, n = 20), globose to subglobose, brown to reddish brown, aseptate, thick-walled with a guttule.

Culture characteristics. Conidia germinating on MEA within 24 hours, reaching 27 mm after 10 days at 27 °C, irregular, entire, concentric, opaque, flat, white to pale brown on the surface; concentric, creamy to pale brown in reverse.

Material examined. THAILAND • Chiang Rai Province, Doi Pui, 19°48'51"N, 99°52'1"E, on dead stems of *Bidens pilosa* (Asteraceae), 14 March 2023, Zin Hnin Htet (BP-DP-11, MFLU 24-0266, holotype); ex-type culture MFLUCC 24-0273.

Notes. In a BLASTn search of GenBank, the closest match for the ITS sequence of our isolate was *Roussoella elaeicola* strain MFLUCC 15-0276b (MH742330) with 94.57% similarity. The closest match for the LSU region was *Pseudoroussoella chromolaenae* isolate MFLUCC 17-2062 (MT394704) with 92.95% similarity, and the closest match for the SSU region was *Parathyridaria tyrrhenica* MUT<ITA>:5371 (KU314952) with 99.16% similarity. Additionally, the closest matches for the *tef1-α* and *rpb2* gene regions were *Pseudoroussoella elaeicola* culture MFLUCC:17-1483 (MT235772) and *Roussoella* sp. strain GMB1153 (OM755588) with 97.08% and 98.27% similarity, respectively.

Pseudoroussoella elaeicola (MFLUCC 17-1483 and MFLUCC 17–2086) was found as a sexual morph in nature (Phookamsak et al. 2019, Mapook et al. 2020); hence, we were unable to directly compare their morphology with our isolate. However, based on comparing the morphology of *Pseudorousoella bidenticola* (MFLUCC 24-0273) and *Ps. chromolaenae* (MFLUCC 17-1492), our species differs from *Ps. chromolaenae* (MFLUCC 17-1492) in having immersed to semi-immersed, globose to subglobose, brown, conidiomata without ostiole, smaller-sized (120–150 × 150–180 µm vs 130–175(–230) × 160–230 µm), holoblastic, globose to subglobose conidiogenous cells, and brown to reddish brown, globose to subglobose conidia with guttules, while *Ps. chromolaenae* (MFLUCC 17-1492) displays superficial, globose to obpyriform, yellowish brown to brown conidiomata with a central ostiole, annellidic, ampulliform to oblong conidiogenous cells, and oblong to oval, conidia that are pale brown to light brown when immature, becoming yellowish brown to reddish brown when mature (Table 4).

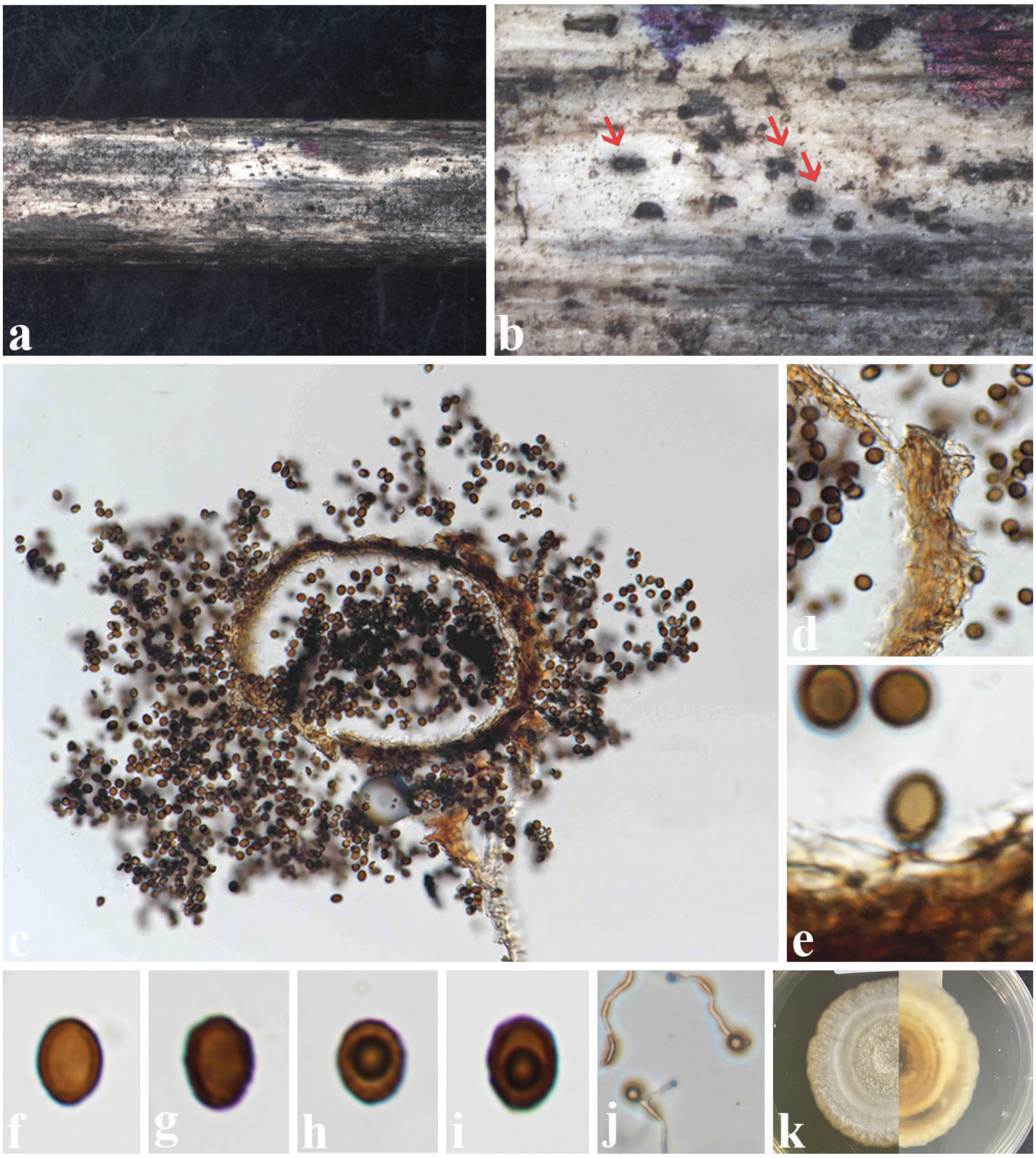

Figure 4. *Pseudoroussoella bidenticola* (MFLU 24-0266, holotype) **a**, **b** Conidiomata on the substrate **c** a section through a conidioma **d** peridium **e** conidia and conidiogenous cell **f**–**i** conidia **j** germinating conidia **k** culture on the MEA. Scale bars: 500 µm (**a, b**); 100 µm (**c**); 10 µm (**d**); 5 µm (**e–i**).

Table 4. Synopsis of sexual and asexual morph of *Chromolaenicola* species.

Species	Conidiomata (µm)	Conidiogenous cells (µm)	Conidia (µm)	References
Pseudoroussoella chromolaenae (MFLUCC 17-1492)	130–175(–230) × 160–230	–	5.5–7 × 3.5–5	Mapook et al. (2020)
Pseudoroussoella bidenticola (MFLUCC 24-0273)	120–150 × 150–180	1–2	5–7.5 × 4–5.5	This study
Species	**Ascomata (µm)**	**Asci (µm)**	**Ascospores (µm)**	**References**
Pseudoroussoella elaeicola	225–475 × 240–400	95–135 × 6–8.5	10–14 × 4.5–6	Mapook et al. (2020)

Based on the multi-locus phylogeny (Fig. 1), our isolate (MFLUCC 24-0273) formed a separate branch related to *Pseudoroussoella* species with 96% ML and 1.00 BYPP. When comparing base pair differences between *Ps. bidenticola* (MFLUCC 24-0273) and *Ps. chromolaenae* (MFLUCC 17-1492), variations

Table 5. Preliminary antibacterial activity result of this study.

Species	Zone of inhibition (mm); Ampicillin (+)		
	Bacillus subtilis	***Escherichia coli***	***Staphylococcus aureus***
Neoroussoella chromolaenae (MFLUCC 24-0274)	16 mm inhibition	11 mm inhibition	20 mm inhibition
N. entadae (MFLUCC 24-0275)	13 mm inhibition	17 mm inhibition	14 mm inhibition
Pseudoroussoella bidenticola (MFLUCC 24-0273)	18 mm inhibition	12 mm inhibition	13 mm inhibition

Abbreviation: Positive control (+).

were observed in ITS (3.6% - 23/469), LSU (0.6% - 5/799), SSU (0.6% - 4/630), *tef1-α* (2.6% - 24/891), without gaps. Therefore, we introduced our collection (MFLUCC 24-0273) as a new species based on morphology and multigene phylogeny. Moreover, this is also the first record of *Pseudoroussoella* species from *Bidens pilosa* (Asteraceae).

Preliminary screening for antibacterial activity

In our study, we explored the antibacterial activities of our three isolates against *Bacillus subtilis*, *Escherichia coli coli*, and *Staphylococcus aureus*. *Neoroussoella chromolaenae* (MFLUCC 24-0274), *N. endatae* (MFLUCC 24-0275), *Pseudoroussoella bidenticola* (MFLUCC 24-0273) exhibited antibacterial activity against all three test organisms. For *B. subtilis*, *N. chromolaenae* (MFLUCC 24-0274), *N. entadae* (MFLUCC 24-0275), and *Ps. bidenticola* (MFLUCC 24-0273) exhibited partial inhibition. Against *E. coli*, *N. chromolaenae* (MFLUCC 24-0274), *N. entadae* (MFLUCC 24-0275), and *Ps. bidenticola* (MFLUCC 24-0273) demonstrated clear inhibition. For *S. aureus*, *N. chromolaenae* (MFLUCC 24-0274) showed the most significant inhibition, followed by *N. entadae* (MFLUCC 24-0275) and *Ps. bidenticola* (MFLUCC 24-0273), with clear inhibition observed. However, none of these fungal species showed a wider inhibition zone than the positive control, ampicillin (20 mm for *B. subtilis*, 50 mm for *E. coli*, 40 mm for *S. aureus*). The measurements of clear inhibition zones produced by our new isolates are provided in Table 5.

Discussion

Our research in northern Thailand unveiled the introduction of two novel species and one new host record within the Roussoellaceae. This classification was determined through a combination of morphological analyses and a multigene phylogeny approach, adhering to the recommendations outlined by Jeewon and Hyde(2016). Mapook et al. (2020) established *Pseudoroussoella* to accommodate *Ps. chromolaenae* and *Ps. elaeicola*. Interestingly, our study revealed a third *Pseudoroussoella* strain on the dead stems of *Bidens pilosa* (Asteraceae). In our phylogenetic analyses, our isolate (MFLUCC 24-0273) formed a basal lineage to other strains (MFLUCC 17-1483; MFLUCC 15-0276a; MFLUCC 15-0276b and MFLUCC 17-1492). Our species is morphologically similar to *Ps. chromolaenae* and has a significant base pairs difference between the two, and herein, we introduced our isolate as a new species. Moreover, we found two isolates of *Neoroussoella* on the dead stems of *C. odorata* (Asteraceae). Based on the morphological similarity and multigene phylogeny, we identified *N. chromolaenae* as a new species and *N. entadae* as the first occurrence on *Chromolaena odorata*.

Following the preliminary screening for antibacterial activity, all species examined in our study demonstrated potential antibacterial properties. In a prior examination of *Pseudoroussoella* species, *Ps. chromolaena* exhibited no inhibition on *B. subtilis*, *E. coli*, and *M. plumbeus*, while *Ps. elaeicola* displayed a 10 mm inhibition zone against *E. coli* (Mapook et al. 2020). Our isolate (MFLUCC 24-0273) exhibited inhibition on all tested organisms, with inhibition zones measuring 18 mm (*B. subtilis*), 12 mm (*E. coli*), and 13 mm (*S. aureus*), respectively. These results highlight our new species, *Pseodoroussoella bidenticola*, as a particularly promising organism for further research. Additionally, *Neoroussoella chromolaenae* (MFLUCC 24-0274) and *N. entadae* (MFLUCC 24-0275) demonstrated inhibitory effects against all test organisms, and specific measurements of inhibition zones are shown in Table 4. Across the three observed species, all species exhibited antibacterial activity. The outcomes of our investigation suggest that this fungal group possesses antibacterial potential, presenting a source for the exploration of novel bioactive compounds. These findings also highlight the potential of the Roussoellaceae family for antibacterial compound discovery, a field that remains relatively underexplored.

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 Basic Research Fund support from the National Science, Research and Innovation Fund (Grant No. 652A01001, 662A01001 and 672A16001) entitled "Studies of fungi associated with Asteraceae and the discovery of biological properties", Mae Fah Luang University Grant - Reinventing University System (Grant No. 652A16049), Distinguished Scientist Fellowship Program (DSFP), King Saud University, Kingdom of Saudi Arabia.

Author contributions

The authors confirm contributions to the paper as follows: Fungal specimen collection and isolation, fungal specimen deposition and manuscript writing: Zin Hnin Htet; fungal identification and contributed in the revision of the manuscript: Zin Hnin Htet, Kevin D. Hyde, Fatimah O. Alotibi, Ausana Mapook, Thilini K. W. Chethana. All authors have read and agreed to the published version of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Data availability

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

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MycoKeys 111: 147–180 (2024)
DOI: 10.3897/mycokeys.111.135456

Research Article

Taxonomic novelty in Pleomonodictydaceae and new reports for *Ampelomyces quisqualis* (Phaeosphaeriaceae), *Melomastia maolanensis* and *M. oleae* (Pleurotremataceae)

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Abstract

This study introduces a novel genus *Robiniigena*, with its type *R. hyalinospora*. The specimen was collected on dead aerial branches of *Robinia pseudoacacia* in Italy. Based on the examination of morphology and the results of phylogenetic analyses involving nuclear 18S rDNA (SSU), nuclear 28S rDNA (LSU), nuclear rDNA ITS1-5.8S-ITS2 (ITS), translation elongation factor 1-alpha (*tef1-α*) and RNA polymerase II second largest subunit (*rpb2*) sequences, *Robiniigena* is referred to the family Pleomonodictydaceae (Pleosporales). It is characterized by immersed to erumpent, ostiolate ascomata, filiform, septate and cellular pseudoparaphyses, bitunicate, clavate to cylindric-clavate asci and fusiform, hyaline ascospores surrounded by a mucilaginous sheath. This research also establishes the taxonomic placement of the previously unclassified *Inflatispora* (Pleosporales genus *incertae sedis*) within the Pleomonodictydaceae. The sexual morph of *Ampelomyces quisqualis* (Phaeosphaeriaceae) is described for the first time and it is characterized by immersed, perithecial ascomata, a peridium comprising two layers, branched, septate and filiform pseudoparaphyses, short-pedicellate, bitunicate asci with an ocular chamber and sub-hyaline, fusiform, septate ascospores. This species, previously known only in its asexual morph, has been found as a saprobe on *Sonchus* sp. in Italy. Our identification of the sexual morph was based on LSU rDNA and ITS rDNA sequence data. *Melomastia maolanensis* (Pleurotremataceae) is reported for the first time in Thailand, collected from *Chromolaena odorata*, while *M. oleae* is documented as a new record from *Duranta erecta* in Thailand.

Key words: 2 new taxa, Dothideomycetes, Dyfrolomycetales, fungal diversity, morpho-phylogeny

This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

Academic editor:
Samantha C. Karunarathna
Received: 24 August 2024
Accepted: 26 October 2024
Published: 17 December 2024

Citation: Bundhun D, Jones EBG, Jayawardena RS, Camporesi E, Wanasinghe DN, Senanayake IC, Thiyagaraja V, Hyde KD (2024) Taxonomic novelty in Pleomonodictydaceae and new reports for *Ampelomyces quisqualis* (Phaeosphaeriaceae), *Melomastia maolanensis* and *M. oleae* (Pleurotremataceae). MycoKeys 111: 147–180. https://doi.org/10.3897/mycokeys.111.135456

Introduction

The family Pleomonodictydaceae was established to accommodate *Pleomonodictys*, along with two monodictys-like taxa, which formed a distinct clade within the suborder Massarineae, Pleosporales (Hernández-Restrepo et al. 2017). *Pleomonodictys* is characterized by mononematous, micronematous to semi-macronematous conidiophores, which are sometimes reduced to intercalary and polyblastic conidiogenous cells. The conidia, which vary in shape, are blastic, pleurogenous, muriform, and verrucose to tuberculate, and they are often solitary or occur in short chains (Hernández-Restrepo et al. 2017; Bao et al. 2021). The second genus, *Pleohelicoon* was established in the family to accommodate *Helicoon* taxa, which belonged to Pleosporales (Jayasiri et al. 2019). *Pleohelicoon* taxa are characterized by macronematous, mononematous, unbranched and hyaline to brown conidiophores, monoblastic and terminal conidiogenous cells and pale brown to dark fuscous conidia with multi-septate conidial filaments coiled 7–9 times in 3 planes, giving the conidia different shapes (Jayasiri et al. 2019). It is worth noting that no sexual morph has been reported for these genera.

Inflatispora was introduced by Zhang et al. (2011) to include the only taxon and type species, *I. pseudostromatica*, characterized by solitary to aggregated, semi-immersed or erumpent ascomata which form under a pseudostroma and open through a broad, round pore, narrow cellular pseudoparaphyses, bitunicate, cylindric-clavate asci, and hyaline, 3-septate, fusiform to cylindrical ascospores with an enlarged upper central cell and surrounded by a mucilaginous sheath (Zhang et al. 2011). *Inflatispora* shares a morphological resemblance to *Nodulosphaeria*, in terms of the swollen upper central cell of the ascospores. However, ascospores of *Nodulosphaeria* have terminal appendages, while in *Inflatispora pseudostromatica*, ascospores are surrounded by a sheath (Zhang et al. 2011). Moreover, phylogenetic analyses of nuclear 28S rDNA (LSU), nuclear 18S rDNA (SSU) and RNA polymerase II second largest subunit (*rpb2*) sequence data of *I. pseudostromatica* showed it grouped with taxa in the Massarineae, forming a separate basal lineage (Zhang et al. 2011). It was however treated as genus *incertae sedis* in the suborder owing to its uncertain familial placement (Zhang et al. 2011). A second species, *Inflatispora caryotae*, was introduced in the genus based on morpho-phylogenetic analyses (Tibpromma et al. 2017).

Ampelomyces quisqualis belongs to Phaeosphaeriaceae (Pleosporales), and is a parasite and biocontrol agent of powdery mildew (Kiss et al. 2004; Liyanage et al. 2018; Manjunatha et al. 2020). This taxon parasitizes the powdery mildew taxa by penetrating hyphae and producing pycnidia in the hyphae, conidiophores, and ascomata of the powdery mildew host, which eventually interferes with the latter's development (Kiss et al. 2004; Liyanage et al. 2018). *Ampelomyces quisqualis* is characterized by pale to dark brown, pycnidial conidiomata, with walls which are angular textured, and hyaline, ovate to elliptical, aseptate and smooth-walled conidia (Manjunatha et al. 2020). The sexual morph of the species has not been reported so far.

Melomastia, typified by *M. mastoidea* (= *M. friesii*), has been subjected to several taxonomic revisions before being finally accommodated in Dothideomycetes (Pleurotremataceae, Dyfrolomycetales) (Barr 1989, 1994; Lumbsch and Huhndorf 2010; Maharachchikumbura et al. 2015, 2016; Norphanphoun et al. 2017; Wijayawardene et al. 2017). It was earlier accommodated in Clypeosphaeriaceae

(Sordariomycetes) and was later transferred to Pleurotremataceae, the latter family then assigned to Xylariales (Barr 1989, 1994). However, the genus was then excluded from the family and classified as Ascomycota, genera *incertae sedis* (Kirk et al. 2001; Lumbsch and Huhndorf 2010). While reviewing the backbone tree for Sordariomycetes, Maharachchikumbura et al. (2015) placed *Melomastia* in Sordariomycetes genera *incertae sedis*, while Pleurotremataceae (with the single genus *Pleurotrema*) was accommodated in Chaetosphaeriaceae, Chaetosphaeriales. However, Maharachchikumbura et al. (2016) later excluded Pleurotremataceae from Sordariomycetes, since it was regarded as an earlier name of the family Dyfrolomycetaceae (Dothideomycetes) based on morphology. Meanwhile, *Melomastia* was still considered as an *incertae sedis* genus in Sordariomycetes (Maharachchikumbura et al. 2016). Norphanphoun et al. (2017) finally introduced sequence data for the *Melomastia* taxon, *M. italica* for the first time and showed that *Melomastia* belongs to Dothideomycetes, in the family Pleurotremataceae, along with *Dyfrolomyces* and *Pleurotrema*. They also synonymized *Dyfrolomyces maolanensis*, a saprobe collected from China (Zhang et al. 2017), to *Melomastia maolanensis*, owing to its closer phylogenetic affinity with *M. italica* (Norphanphoun et al. 2017). Li et al. (2022) synonymized *Dyfrolomyces* under *Melomastia* based on morphological and phylogenetic analyses. Ascospore septation, which was considered as the principal differentiating character between *Dyfrolomyces* (multi-septate) and *Melomastia* (2-septate), was no longer considered a key for delimitation of the two genera (Li et al. 2022). Kularathnage et al. (2023), however, recently reinstated *Dyfrolomyces* in Pleurotremataceae.

Aims of study

In an ongoing survey on the fungal diversity in Italy and Thailand (Chaiwan et al. 2021; Manawasinghe et al. 2022), we recovered several taxa of Dothideomycetes. We herein introduce a new genus *Robiniigena* and accommodate the *Inflatispora* genus *incertae sedis* in Pleomonodictydaceae based on morpho-phylogenetic evidence. Furthermore, the sexual morph of *Ampelomyces quisqualis* is described for the first time, based on LSU–ITS sequence data. We also introduce two new collections of *Melomastia*. *Melomastia maolanensis* is reported as a new geographical record from Thailand, while *M. oleae*, which was initially introduced from *Olea europaea* in China (Li et al. 2022), is a new host and geographical record on *Duranta erecta* in Thailand based on morphology and phylogeny. Findings herein indicate that there is still much to be discovered vis-a-vis the diversity of Dothideomycetes. Future studies in this class will undoubtedly result in further important taxonomic discoveries and advances.

Materials and methods

Sample collection, specimen examination, and isolation

Dead stems and twigs bearing fungal fruiting bodies were collected from the terrestrial environment in Italy during the dry (June) and wet (November) seasons, and in Thailand during the wet (July) and dry (November) seasons. The samples were taken to the laboratory in a plastic Ziplock bag and stored inside paper envelopes. Specimens were externally examined with a Motic SMZ 168 stereomicroscope. Free-hand sections of the sporocarps were prepared and placed on water-mounted glass slides. They were then examined using a Nikon ECLIPSE 80i compound microscope with differential interference contrast (DIC) illumination. Microscopic photography was conducted for the ascomata, peridium, pseudoparaphyses, asci, and ascospores. The images were captured with a Canon EOS 750D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work v. 0.9.7. using the necessary calibration values and images used for figures were processed with Adobe Photoshop CS6 Extended v. 13.0.1 software (Adobe Systems, San Jose, California).

Single spore isolation was carried out following the method of Senanayake et al. (2020). Ascospore suspensions were made and spread on potato dextrose agar (PDA; 39 g/L) or water agar (WA; 20 g/L). The germinated ascospores were examined after 24 h, after which they were transferred to PDA media. Cultures were incubated at 25 °C in the dark and colony color was determined according to Rayner (1970) after 2–3 weeks of growth on PDA. Herbarium specimens were deposited in Mae Fah Luang University Herbarium (MFLU) while, the living cultures at the Mae Fah Luang University Culture Collection (MFLUCC) in Thailand. Index Fungorum and Faces of Fungi numbers are provided as per Index Fungorum (https://www.indexfungorum.org) and Jayasiri et al. (2015). Taxa descriptions and notes are also uploaded on the GMS microfungi website (https://gmsmicrofungi.org/) (Chaiwan et al. 2021).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from mycelium or directly from fungal fruiting bodies. Fresh mycelium was scraped from cultures grown on PDA at 25 °C for two weeks. Around 15–20 ascomata were removed from the sterilized plant materials using fine sterile needles, while being observed through the stereomicroscope, and placed in 1.5 ml micro-centrifuge tubes (Wanasinghe et al. 2018). DNA was extracted using the Omega Bio-tek kit according to the manufacturer's instructions. The nuclear 18S rDNA (SSU), nuclear 28S rDNA (LSU), nuclear rDNA ITS1-5.8S-ITS2 (ITS), translation elongation factor 1-alpha (*tef1-α*), and the RNA polymerase II second largest subunit (*rpb2*) were amplified (Table 1). The total volume of the PCR reaction was 25 μL, and it consisted of 12.5 μL of 2× Power Taq PCR MasterMix, 1 μL of each primer, 2 μL genomic DNA extract, and 8.5 μL double-distilled water (ddH_2O). The PCR protocols were adapted accordingly (Table 1). Thirty-five cycles were used for the denaturation, annealing, and extension steps for each locus (except for *tef1-α* and *rpb2*, where 40 cycles were used). All the PCR thermal cycles included a final extension of 72 °C for 10 mins and a final hold at 4 °C.

Table 1. Loci, primers, and PCR amplification conditions used in the present study.

Loci	Primers (forward/ reverse)	Initial Denaturation	Denaturation	Annealing	Extension	References
SSU	NS1/NS4	94 °C, 3 mins	94 °C, 30s	54 °C, 50s	72 °C, 1.30 mins	White et al. (1990)
LSU	LR0R/LR5	95 °C, 5 mins	95 °C, 45s	53 °C, 45s	72 °C, 2 mins	Vilgalys and Hester (1990)
ITS	ITS5/ITS4	95 °C, 5 mins	95 °C, 45s	53 °C, 45s	72 °C, 2 mins	White et al. (1990)
tef1-α	983F/2218R	94 °C, 5 mins	94 °C, 30s	58 °C, 1.30 mins	72 °C, 1.20 mins	Carbone and Kohn (1999)
rpb2	fRPB2-5f /fRPB2-7cR	95 °C, 5 mins	95 °C, 1 min	54 °C, 2 mins	72 °C, 1.30 mins	Liu et al. (1999), Sung et al. (2007)

PCR products were verified by staining with FluoroDye™ DNA Fluorescent Loading Dye on 1% agarose electrophoresis gels. They were then purified and sequenced using the same primers at the Tsingke Biotech Co. Ltd., Kunming, China. The quality of the sequences obtained was confirmed by checking the chromatograms using BioEdit v. 7.0 (Hall 2004), after which the sequences were assembled into contigs using SeqMan v. 7.1.0. The sequence data derived in this study have been deposited in GenBank (Table 2). New species are established based on recommendations provided by Jeewon and Hyde (2016) and Maharachchikumbura et al. (2021).

Sequence alignment and phylogenetic analyses

Generated SSU, LSU, ITS, *tef1-α* and *rpb2* sequences were subjected to BLASTn searches (https://blast.ncbi.nlm.nih.gov) and similar sequences were downloaded from GenBank based on BLAST similarities and following relevant papers (Tanaka et al. 2015; Phukhamsakda et al. 2016; Hongsanan et al. 2020; Tennakoon et al. 2020; Li et al. 2022; Hyde et al. 2023; Kularathnage et al. 2023; Table 2). Individual gene matrices were aligned using the default setting in MAFFT v. 7.036 (http://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019) and manually adjusted when necessary in BioEdit v. 7.0 (Hall 2004).

Table 2. Taxa used for the phylogenetic analyses in the present study, and their corresponding GenBank accession numbers. Type strains are denoted by ^{T}. Sequences derived in this study are shown in bold black.

Taxa	Strain	GenBank accession number				
		SSU	**LSU**	**ITS**	***tef1–α***	***rpb*2**
Acrospermum adeanum	M133	EU940031	EU940104	–	–	–
Acrospermum compressum	M151	EU940012	EU940084	–	–	–
Acrospermum graminum	M152	EU940013	EU940085	–		–
Ampelomyces quisqualis	AMP	–	–	OP740825	–	–
Ampelomyces quisqualis	CBS 129.79	EU754029	EU754128	KY090653	–	–
Ampelomyces quisqualis	BRIP 72107	–	–	MZ054399	–	–
Ampelomyces quisqualis	CBS 131.79	–	MH872956	MH861188	–	–
Ampelomyces quisqualis	CBS 128.79	–	MH872954	MH861185	–	–
Ampelomyces quisqualis	CBS 133.32	–	MH866692	MH866692	–	–
Ampelomyces quisqualis	CBS 131.31	–	MH866605	AF035781	–	–
Ampelomyces quisqualis	HMLAC05119	–	OL739255	OL739255	–	–
Ampelomyces quisqualis	Chillan	–	–	MH997723	–	–
Ampelomyces quisqualis	CBS 130.79	–	–	HQ108039	–	–
Ampelomyces quisqualis	SMKC 22437	–	–	GQ324146	–	–
Ampelomyces quisqualis	SMKC 22438	–	–	GQ324128	–	–
Ampelomyces quisqualis	**MFLU 23-0142**	–	**PP751625**	**PP751509**	**PP782197**	–
Anisomeridium phaeospermum	MPN539	JN887374	JN887394	–	JN887418	–
Anisomeridium ubianum	MPN94	JN887379	–	–	JN887421	–
Anthosulcatispora subglobosa	MFLUCC 17-2065^{T}	MT226705	MT214592	MT310636	MT394649	MT394706
Aquadictyospora lignicola	MFLUCC 17-1318^{T}	–	MF948629	MF948621	MF953164	–
Aquastroma magniostiolata	HHUF 30122^{T}	AB797220	AB807510	LC014540	AB808486	–
Asteromassaria pulchra	CBS 124082	GU296137	GU301800	–	GU349066	GU371772
Bambusicola aquatica	MFLUCC 18-1031^{T}	MT864293	MN913710	MT627729	MT954392	MT878462
Brunneomurispora lonicerae	KUMCC 18-0157^{T}	MK356360	MK356346	MK356373	MK359065	–

Taxa	Strain	GenBank accession number				
		SSU	LSU	ITS	***tef1–α***	***rpb*2**
Clypeoloculus akitaensis	HHUF 27557^{T}	AB797253	AB807543	AB809631	AB808519	–
Didymosphaeria rubi-ulmifolii	MFLUCC 16-1000	MT226672	MT214555	MT310602	MT394734	–
Dyfrolomyces chromolaenae	MFLUCC 17-1434^{T}	MT214413	–	–	MT235800	–
Dyfrolomyces tiomanensis	MFLUCC 13-0440^{T}	KC692155	KC692156	–	KC692157	–
Falciformispora lignatilis	BCC 21117	GU371834	GU371826	KF432942	GU371819	–
Flavomyces fulophazii	CBS 135761^{T}	KP184082	KP184040	KP184001	–	–
Fuscostagonospora camporesii	MFLU 16-1362^{T}	MN750605	MN750590	MN750611	–	–
Fuscostagonospora cytisi	MFLUCC 16-0622^{T}	KY770977	KY770978	–	KY770979	–
Fuscostagonospora sasae	HHUF 29106^{T}	AB797258	AB807548	AB809636	AB808524	–
Halobyssothecium estuariae	MFLUCC 19-0386^{T}	MN598868	MN598871	MN598890	MN597050	–
Halomassarina thalassiae	BCC 17055	GQ925843	GQ925850	–	–	–
Helicoon sp.	JAC9590	–	–	MK432689	–	–
Helminthosporium microsorum	CBS 136910^{T}	KY984427	KY984329	KY984329	KY984448	KY984390
Inflatispora caryotae	MFLUCC 13-0825^{T}	KY264751	KY264747	KY264743	–	–
Inflatispora pseudostromatica	CBS 123110^{T}	JN231132	JN231131	–	–	JN231133
Kaseifertia cubense	CBS 680.96	AB797218	AB807508	LC014541	AB808484	–
Katumotoa bambusicola	HHUF 28661^{T}	AB524454	AB524595	LC014560	AB539108	AB539095
Kazuakitanaka yuxiensis	HKAS 122924^{T}	ON009092	ON009108	ON009124	ON009267	ON009290
Latorua caligans	CBS 576.65^{T}	–	MH870362	MH858723	–	–
Lentithecium fluviatile	CBS 122367	GU296158	GU301825	–	GU349074	–
Leptosphaeria doliolum	CBS 505.75^{T}	GU296159	GU301827	JF740205	GU349069	KY064035
Leucaenicola aseptata	MFLUCC 17-2423^{T}	MK347853	MK347963	MK347746	MK360059	MK434891
Longipedicellata megafusiformis	MFLU 21-0062^{T}	–	MZ538546	MZ538512	MZ567090	–
Macrodiplodiopsis desmazieri	CPC 24971^{T}	–	KR873272	KR873240	–	–
Massarina cisti	CBS 266.62^{T}	AB797249	AB807539	LC014568	AB808514	–
Melomastia beihaiensis	KUMCC 21-0084^{T}	MZ727002	MZ726990	–	OK043822	–
Melomastia clematidis	MFLUCC 17-2092^{T}	MT226718	MT214607	–	MT394663	–
Melomastia distoseptata	NFCCI: 4377^{T}	–	MH971236	–	–	–
Melomastia distoseptata	MFLUC 21-0102	–	MT860427	–	–	–
Melomastia fulvicomae	MFLUCC 17-2083^{T}	MT226719	MT214608	–	MT394664	–
Melomastia fusispora	CGMCC 3.20618	OK623494	OK623464	–	OL335189	–
Melomastia fusispora	UESTCC 21.0001	OK623495	OK623465	–	OL335190	–
Melomastia italica	MFLUCC 15-0160^{T}	MG029459	MG029458	–	–	–
Melomastia loropetalicola	ZHKUCC 22-0174^{T}	OQ379411	OQ379412	–	–	–
Melomastia maolanensis	GZCC 16-0102^{T}	KY111906	KY111905	–	KY814762	–
Melomastia maolanensis	**MFLU 23-0143**	**PP751954**	**PP751616**	–	**PP782198**	–
Melomastia neothailandica	MFLU 17-2589^{T}	–	MN017857	–	–	–
Melomastia oleae	CGMCC 3.20619^{T}	OK623496	OK623466	–	OL335191	–
Melomastia oleae	UESTCC 21.0003	OK623497	OK623467	–	OL335192	–
Melomastia oleae	UESTCC 21.0005	OK623498	OK623468	–	OL335193	–
Melomastia oleae	UESTCC 21.0006	OK623499	–	–	OL335194	–
Melomastia oleae	**MFLUCC 23-0086**	**PP751953**	**PP751621**	–	**PP782199**	–
Melomastia phetchaburiensis	MFLUCC 15-0951^{T}	MF615403	MF615402	–	–	–
Melomastia puerensis	ZHKUCC 23-0802^{T}	OR922340	OR922309	–	OR966284	–
Melomastia puerensis	ZHKUCC 23-0803	OR922341	OR922310	–	OR966285	–
Melomastia pyriformis	ZHKUCC 22-0175	OP739334	OP791870	–	OQ718392	–
Melomastia rhizophorae	BCC 15481	KF160009	–	–	–	–
Melomastia rhizophorae	JK 5456A	GU479766	GU479799	–	GU479860	–
Melomastia septata	MFLUCC 22-0112^{T}	–	OP749870	–	OP760198	–

Taxa	Strain	GenBank accession number				
		SSU	LSU	ITS	*tef1–α*	*rpb2*
Melomastia sichuanensis	CGMCC 3.20620	OK623500	OK623469	–	OL335195	–
Melomastia sichuanensis	UESTCC 21.0008	OK623501	OK623470	–	OL335196	–
Melomastia sinensis	MFLUCC 17-1344^{T}	MG836700	MG836699	–	–	–
Melomastia sinensis	MFLUCC 17-2606	–	OL782048	–	OL875098	–
Melomastia thailandica	MFLUCC 15-0945^{T}	KX611367	KX611366	–	–	–
Melomastia thamplaensis	AND12	OL700222	OL457709	–	–	–
Melomastia thamplaensis	MFLUCC 15-0635^{T}	KX925436	KX925435	–	KY814763	–
Melomastia winteri	CGMCC 3.20621	OK623502	OK623471	–	OL335197	–
Montagnula camporesii	MFLUCC 16-1369^{T}	MN401744	MN401742	MN401746	MN397908	MN397909
Morosphaeria velatispora	PUFD25	MK026765	MK026764	MK026766	MN532688	MN532683
Muyocopron castanopsis	MFLUCC 14-1108	KU726968	KU726965	–	MT136753	–
Muyocopron dipterocarpi	MFLU 17-2608	KU726969	KU726966	–	MT136754	–
Muyocopron heveae	MFLUCC 17-0066^{T}	MH986828	MH986832	–	–	–
Muyocopron lithocarpi	MFLUCC 14-1106^{T}	KU726970	KU726967	–	MT136755	–
Neoaquastroma bauhiniae	MFLU 17-0002^{T}	MH023315	MH023319	MH025952	MH028247	MH028251
Neohelicascus aquaticus	MFLUCC 10-0918	KC886638	KC886640	KC886639	–	–
Neokalmusia jonahhulmei	KUMCC 21-0818^{T}	ON007048	ON007039	ON007043	ON009133	ON009137
Neosetophoma lonicerae	KUMCC 18-0155^{T}	MK356363	MK356349	MK356375	MK359067	–
Palawania thailandensis	MFLICC 14-1121	–	KY086494	–	–	–
Palawania thailandensis	MFLU 16-1873	KY086495	KY086493	–	–	–
Palmiascoma gregariascomum	MFLUCC 11-0175	KP753958	KP744495	KP744452	–	KP998466
Parabambusicola bambusina	KT 2637	AB797248	AB807538	LC014580	AB808513	–
Periconia cortaderiae	MFLUCC 15-0457^{T}	KX986345	KX954401	KX965732	KY310703	–
Periconia pseudodigitata	KT1395^{T}	AB797274	AB807564	LC014591	AB808540	–
Pleohelicoon fagi	MFLUCC 17-2538	MK347925	MK348036	MK347816	–	MK434851
Pleohelicoon fagi	MFLUCC 15-0182^{T}	MK347926	MK348037	MK347817	–	MK434853
Pleohelicoon richonis	CBS 282.54	AY856952	–	MH857332	–	–
Pleomonodictys capensis	DLUCC:1323	–	MZ420757	MZ420742	–	MZ442696
Pleomonodictys capensis	HR 1	AB797261	AB807551	LC014570	AB808527	–
Pleomonodictys capensis	CBS 968.97^{T}	–	KY853521	KY853460	–	–
Pleomonodictys descalsii	FMR_12716^{T}	–	KY853522	KY853461	–	–
Pseudoasteromassaria aquatica	MFLUCC 18-1397^{T}	MT864322	MN913721	MT627674	MT954378	–
Pseudochaetosphaeronema kunmingense	KUMCC 19-0215^{T}	MN792814	MN792815	MN792812	MN794017	–
Pseudochaetosphaeronema larense	CBS 640.73^{T}	KF015652	KF015611	KF015656	KF015684	KF015706
Pseudocoleophoma calamagrostidis	KT3284^{T}	LC014604	LC014609	LC014592	LC014614	–
Pseudosplanchnonema phorcioides	CBS 122935	KY984434	KY984360	KY984360	KY984467	KY984418
Pseudoxylomyces elegans	KT 2887	AB797308	AB807598	LC014593	AB808576	–
Robiniigena hyalinospora	**MFLU 23-0141^{T}**	**PP759387**	**PP759385**	**PP759383**	**PP782200**	**PP476210**
Robiniigena hyalinospora	**MFLUCC 23-0074^{T}**	**PP759386**	**PP759384**	**PP751493**	**PP782201**	**PP476211**
Stemphylium vesicarium	CBS 191.86^{T}	DQ247812	DQ247804	MH861935	DQ471090	KC584471
Stigmatodiscus labiatus	CBS 144700^{T}	MH756065	MH756065	–	MH756083	–
Stigmatodiscus oculatus	CBS 144Z01^{T}	–	MH756069	–	MH756086	–
Stigmatodiscus pruni	CBS 142598	KX611110	KX611110	–	KX611111	–
Submersispora variabilis	MFLUCC 17-2360^{T}	MT864310	MN913682	–	–	–
Sulcatispora acerina	KT2982^{T}	LC014605	LC014610	LC014597	LC014615	–
Trematosphaeria grisea	CBS 332.50^{T}	KF015641	KF015614	KF015662	KF015698	KF015720
Triseptata sexualis	MFLUCC 11-0002^{T}	MN977850	MN977833	MN977832	–	–
Vikalpa grandispora	KUNCC 22-12425^{T}	OP526628	OP526648	OP526638	OP542240	–

Maximum likelihood (ML) and Bayesian posterior probability (BYPP) analyses were conducted using individual and combined loci datasets. The sequence alignments were converted from FASTA into PHYLIP format using the ALTER (alignment transformation environment, http://www.singgroup.org/ALTER/) bioinformatics web tool (Glez-Peña et al. 2010) before conducting the ML analyses. The latter were then performed in RAxML-HPC2 on XSEDE (v. 8.2.10) (Stamatakis 2014) with the GTRGAMMA substitution model and bootstrapping with 1000 replicates in the CIPRES Science Gateway (Miller et al. 2010) to generate ML trees.

The BYPP analyses were generated using Markov Chain Monte Carlo sampling in MrBayes v. 3.1.2 (Huelsenbeck and Ronquist 2001; Zhaxybayeva and Gogarten 2002). MrModeltest 2.3 (Nylander 2004) was initially used to estimate the best evolutionary models for each locus under the Akaike Information Criterion (AIC) implemented in PAUP v. 4.0b10 (Swofford 2002). Six simultaneous Markov chains were then run for the necessary number of generations, as mentioned under the phylogenetic trees. Trees were sampled every 1000th generation. Convergence was declared when the standard deviation of split frequencies was less than 0.01. The first 20% of generated trees, representing the burn-in phase, were discarded. The remaining 80% of trees were used to calculate the posterior probabilities in the majority rule consensus tree.

The resulting trees were viewed in FigTree v. 1.4.0 (Rambaut 2012) and modified in Microsoft PowerPoint. The ML bootstrap values (ML BS) equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above or below the branches in each phylogenetic tree. The final alignments were deposited in TreeBASE, with the submission IDs mentioned under the respective phylogenetic trees.

Results

Phylogenetic analyses

Analysis 1

The combined SSU–LSU–ITS–*tef1-α*–*rpb2* dataset for the suborder Massarineae comprised 61 strains, including the strains MFLU 23-0141 and MFLUCC 23-0074 collected in the present study and the outgroup taxa *Leptosphaeria doliolum* (CBS 505.75) and *Stemphylium vesicarium* (CBS 191.86). The concatenated alignment consisted of 5220 characters. The ML and BYPP trees were almost similar in topology and did not differ significantly. The best RAxML tree with a final likelihood value of -50756.398321 was obtained and is illustrated in Fig. 1. The alignment had 2542 distinct alignment patterns, and 39.0% of gaps or undetermined characters were present. Estimated base frequencies were as follows: A = 0.241009, C = 0.249659, G = 0.270618, T = 0.238714 and the substitution rates were: AC = 1.287337, AG = 3.234605, AT = 1.329219, CG = 1.078417, CT = 6.074542, GT = 1.0. The gamma distribution rate parameter was 0.243991, while the Tree length was 6.343450.

Robiniigena hyalinospora MFLU 23-0141 and MFLUCC 23-0074 grouped in Pleomonodictydaceae with 99% ML BS, 1.00 BYPP statistical support (Fig. 1). They were basal in the family, shared by '*Helicoon*' sp. (JAC9590), *Inflatispora caryotae*

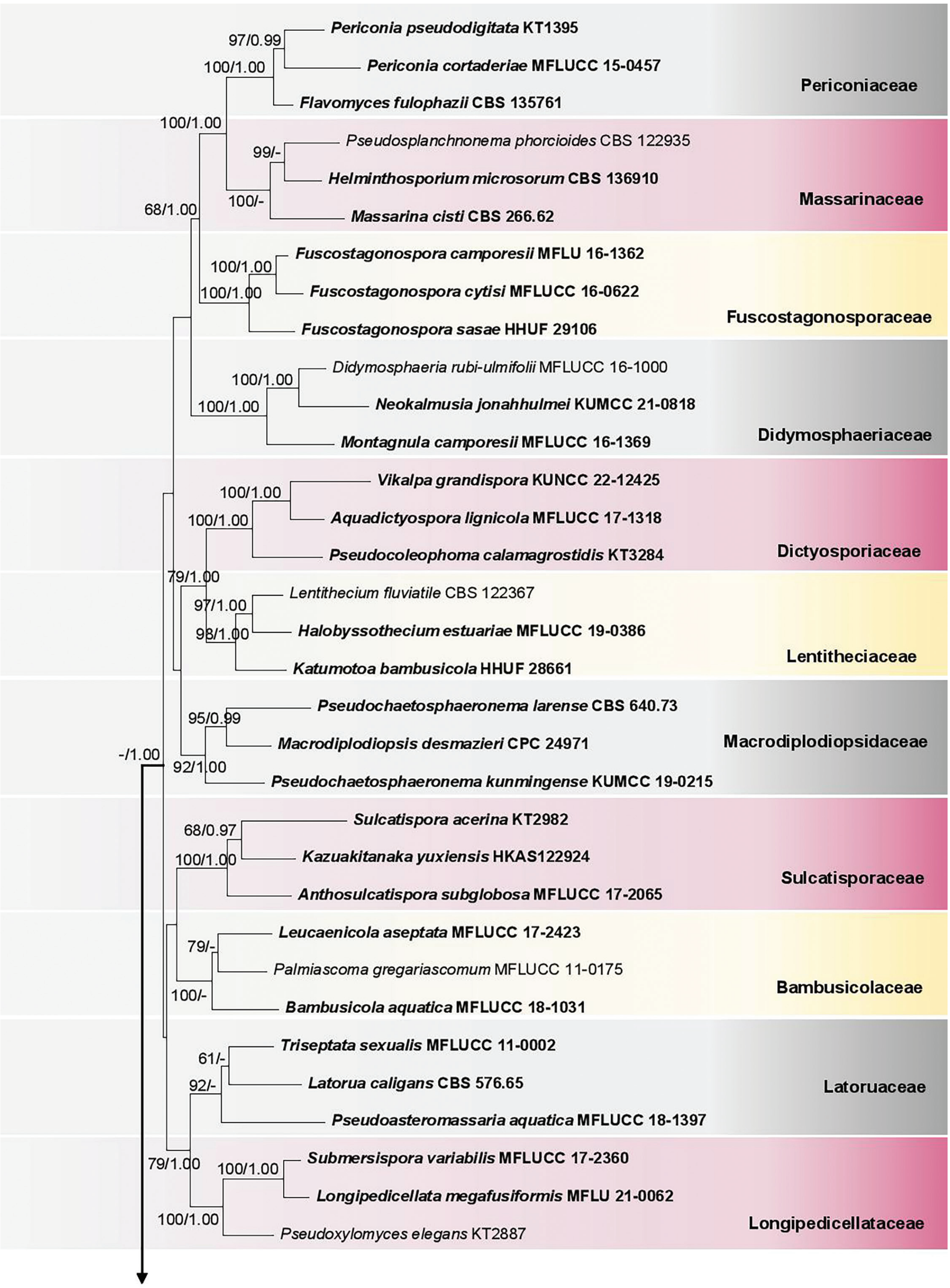

Figure 1. Phylogram generated from maximum likelihood analysis based on SSU–LSU–ITS– *tef1-α*–*rpb2* combined dataset for the families in Massarineae. The evolutionary model GTR+I+G was applied as the best-fit model for all the loci. The BYPP analysis was run for 3 million generations. The tree is rooted with *Leptosphaeria doliolum* (CBS 505.75) and *Stemphylium vesicarium* (CBS 191.86). Maximum likelihood bootstrap, ML BS (≥60%), and BYPP (≥0.95) supports are respectively shown above or below the branches. Type strains are in bold black, and the novel taxon is in bold red. (-) indicate ML BS < 60% or BYPP < 0.95. TreeBASE submission ID: 31443.

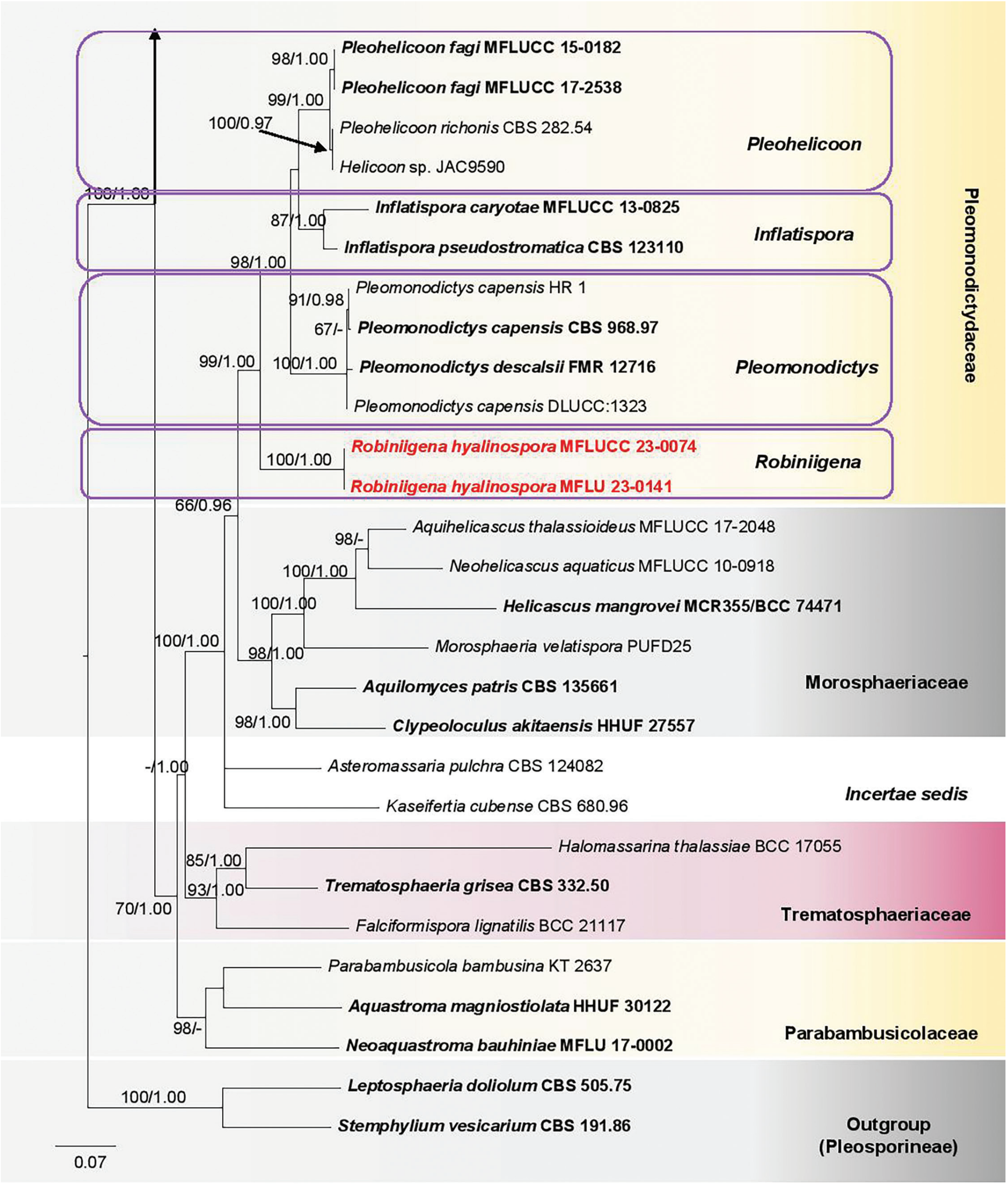

Figure 1. Continued.

(MFLUCC 13-0825), *I. pseudostromatica* (CBS 123110), *Pleohelicoon fagi* (MFLUCC 15-0182, MFLUCC 17-2538), *P. richonis* (CBS 282.54), *Pleomonodictys capensis* (CBS 968.97, DLUCC:1323, HR 1) and *Pl. descalsii* (FMR 12716). *Inflatispora caryotae* and *I. pseudostromatica* grouped in a subclade with 87% ML BS, and 1.00 BYPP support values. The two strains of *Inflatispora* are grouped with the three strains of *Pleohelicoon* and the strain '*Helicoon*' sp. (JAC9590) with 54% ML BS statistical support. The four strains of *Pleomonodictys* formed a subclade, sister to the '*Helicoon*' sp., *Inflatispora*, and *Pleohelicoon* taxa.

Analysis 2

The LSU–ITS matrix for *Ampelomyces* comprised 14 strains, including our strain MFLU 23-0142 and the outgroup taxon *Neosetophoma lonicerae* (KUMCC 18-0155). The concatenated alignment consisted of 1360 characters. The ML and BYPP trees were similar in topology and did not differ significantly. The best RAxML tree with a final optimization likelihood value of -3245.931378 was yielded and is presented below (Fig. 2). The alignment had 178 distinct patterns and 27.7% of gaps or undetermined characters. Estimated base frequencies were as follows: A = 0.247457, C = 0.216767, G = 0.254577, T = 0.281199 and the substitution rates were: AC = 4.277905, AG = 11.081952, AT = 4.397096, CG = 3.194723, CT = 27.614052, GT = 1.0. The gamma distribution rate parameter was 0.265280 while the Tree-length was 0.291945.

Strain MFLU 23-0142 nested with *Ampelomyces quisqualis* (AMP, Chillan, BRIP 72107 and CBS 133.32) in a subclade (A) with 100% ML BS, 1.00 BYPP support (Fig. 2).

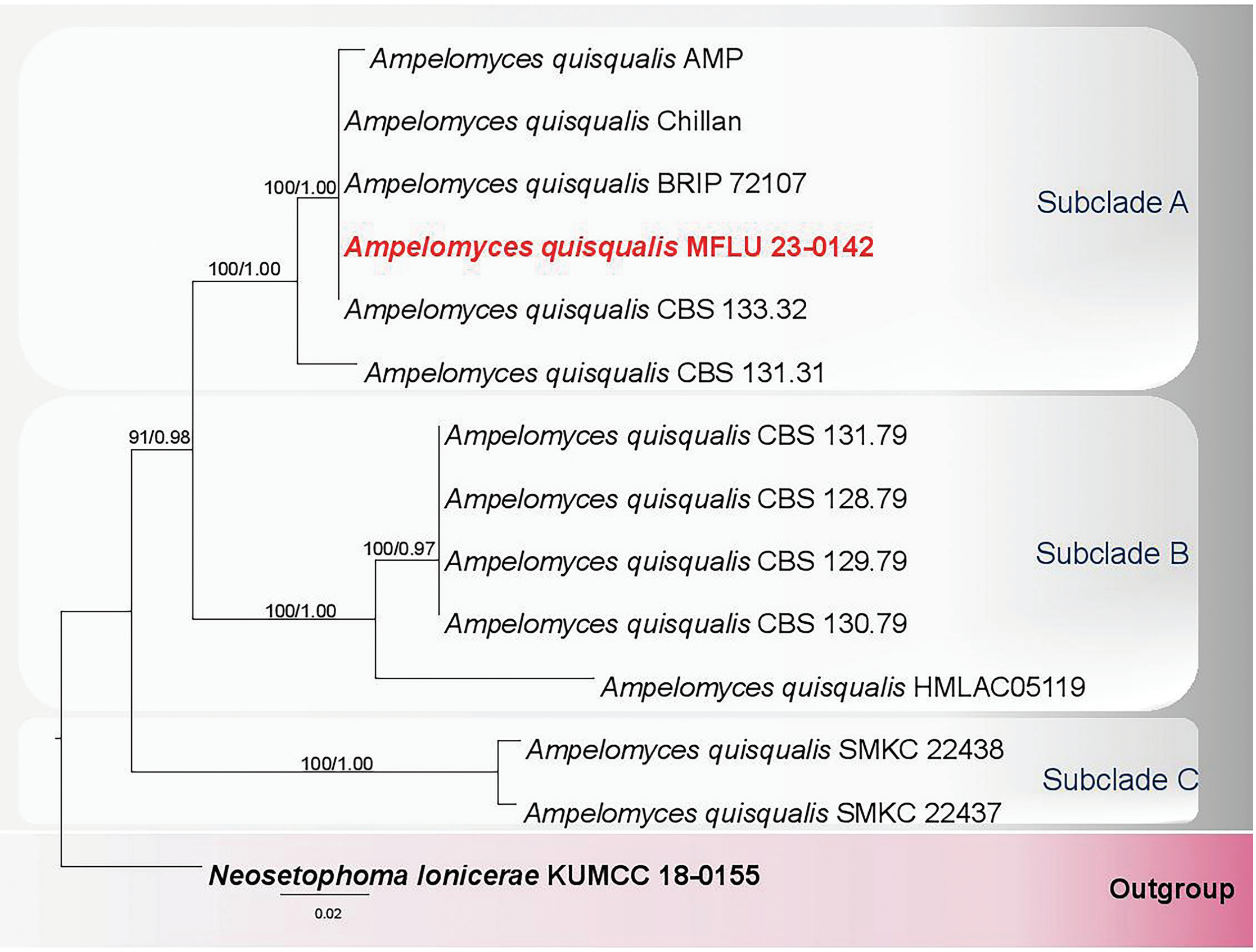

Figure 2. Phylogenetic tree generated from RaxML analysis, based on LSU–ITS matrix for *Ampelomyces*. The evolutionary model GTR+I was applied for LSU, while GTR+I+G was the best-fit model for ITS. The BYPP analysis was run for 1 million generations. The tree is rooted with *Neosetophoma lonicerae* (KUMCC 18-0155). Maximum likelihood bootstrap, ML BS (≥60%), and BYPP (≥0.95) supports are shown above the branches, respectively. The type strain is in bold, and the newly collected strain is in bold red. TreeBASE submission ID: 31444.

Analysis 3

An SSU–LSU–*tef1-α* dataset for Dyfrolomycetales and related orders and families, consisting of 48 strains, was used for analyzing the two strains MFLU 23-0143 and MFLUCC 23-0086 collected in the present study. The outgroup taxa used were *Anisomeridium phaeospermum* (MPN539) and *A. ubianum* (MPN94). The ML and BYPP trees were almost similar in topology and did not differ significantly. The best RaxML tree with a final likelihood value of -13964.812497 was yielded and is presented below (Fig. 3). The matrix had 976 distinct alignment patterns, with 23.70% of gaps or undetermined characters. Estimated base frequencies were as follows: A = 0.241398, C = 0.259224, G = 0.291514, T = 0.207864 and the substitution rates were: AC = 0.884199, AG = 2.067368, AT = 1.098051, CG = 0.895791, CT = 7.809461, GT = 1.0. The gamma distribution rate parameter was 0.210247 while the Tree-length was 1.259771.

The strain MFLU 23-0143 clustered with *Melomastia maolanensis* (GZCC 16-0102) with 100% ML BS, 1.00 BYPP statistical support. The isolate MFLUCC 23-0086 was sister to *M. oleae* (CGMCC3.20619, UESTCC 21.0003, UESTCC 21.0005, UESTCC 21.0006) with 55% ML BS and 0.67 BYPP support values (Fig. 3).

Taxonomy

In this section, we follow the classification of Wijayawardene et al. (2022) and revise it as needed. The amended description for Pleomonodictydaceae is provided, along with notes on *Inflatispora*, which is herein accommodated in the family. Descriptions, notes and illustrations are provided for: *Robiniigena hyalinospora* gen. et sp. nov., *Ampelomyces quisqualis*, *Melomastia maolanensis* and *M. oleae*.

Pleomonodictydaceae Hern.-Restr., J. Mena & Gené, Stud. Mycol. 86: 76 (2017), amended

Index Fungorum: IF820279
Facesoffungi Number: FoF08344

Description. ***Saprobic*** on woody substrates in terrestrial and aquatic habitats. **Sexual morph**: ***Ascomata*** solitary or in groups, often growing under or in a pseudostroma, immersed, semi-immersed to erumpent, perithecial, brown to dark brown, ostiolate. ***Ostiolar neck*** papillate. ***Peridium*** multi-layered, outer layer usually fusing with the stroma or host tissue. ***Pseudoparaphyses*** hyaline, filiform, cellular. ***Asci*** bitunicate, 8-spored, clavate to cylindric-clavate, pedicellate, with an ocular chamber. ***Ascospores*** 1–3-seriate, fusiform to cylindrical, hyaline, septate, surrounded by a mucilaginous sheath. **Asexual morph**: hyphomycetous. See Hernández-Restrepo et al. (2017), Jayasiri et al. (2019), Bao et al. (2021) for further details.

Type genus. *Pleomonodictys* Hern.-Restr., J. Mena & Gené

Notes. Pleomonodictydaceae has so far accommodated the two hyphomycetous genera, *Pleohelicoon* and *Pleomonodictys* (Hernández-Restrepo et al. 2017; Jayasiri et al. 2019), whose sexual morphs are still undetermined. In the present study, *Inflatispora* and the novel genus, *Robiniigena* are accepted in the family based

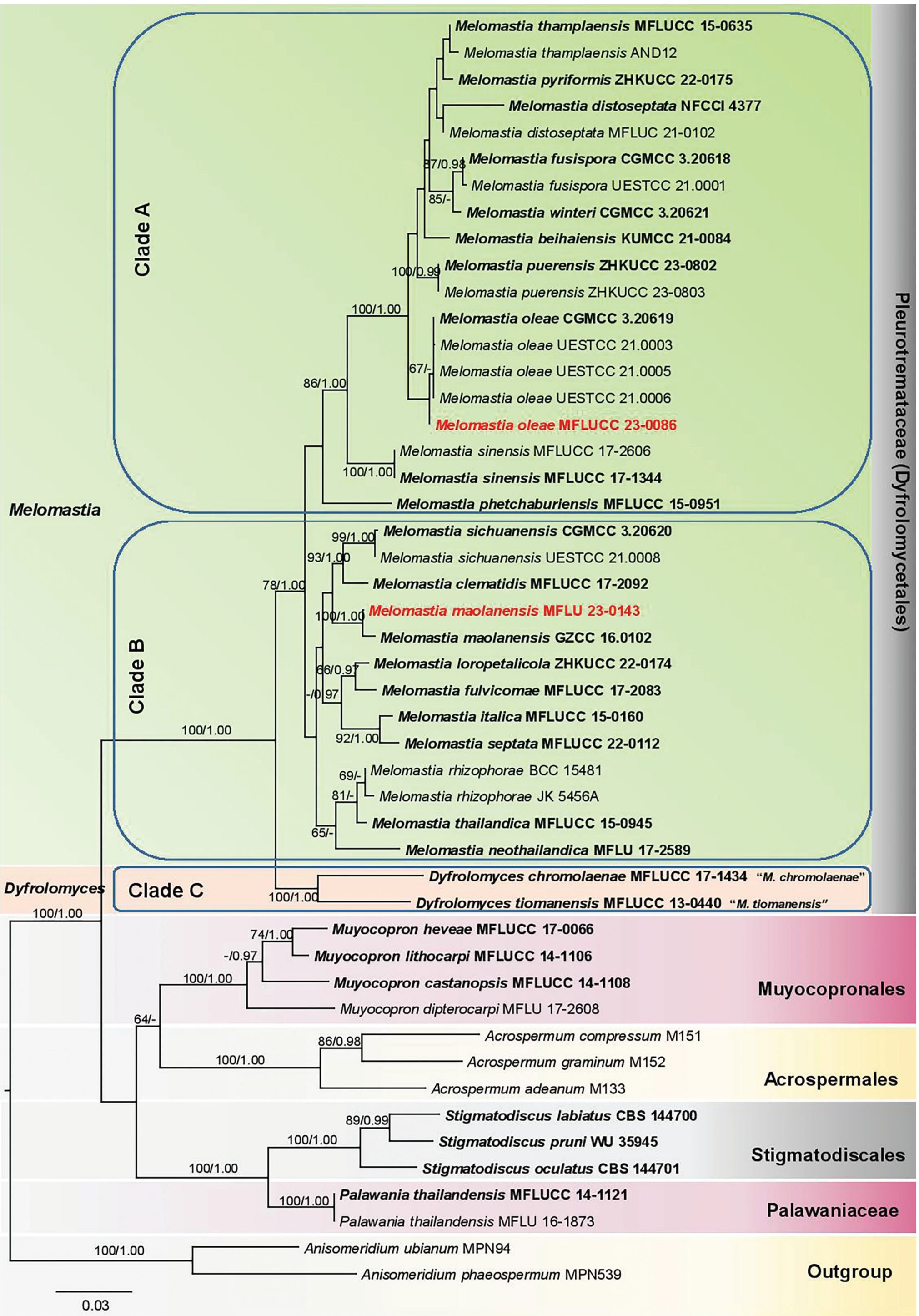

Figure 3. Phylogram generated from maximum likelihood RaxML analysis based on combined SSU, LSU, and *tef1-α* sequence data. The evolutionary model SYM+I+G was chosen as the best-fit model for SSU while GTR+I+G was applied for LSU and *tef1-α*. The BYPP analysis was run for 2 million generations. The tree is artificially rooted with *Anisomeridium phaeospermum* (MPN539) and *A. ubianum* (MPN94). Maximum likelihood bootstrap, ML BS (≥60%), and BYPP (≥0.95) are given respectively above or below the branches. Type strains are in bold. The newly generated sequences are indicated in bold red. (-) indicates ML BS < 60% or BYPP < 0.95. TreeBASE submission ID: 31445.

on the combined SSU–LSU–ITS–*tef1-α*–*rpb2* phylogenetic analyses (Fig. 1). Since both genera are known in their sexual morphs, the description of Pleomonodictydaceae is amended to include their sexual morphological characteristics.

Inflatispora **Y. Zhang ter, J. Fourn. & K.D. Hyde, Sydowia 63(2): 290 (2011)**

Index Fungorum: IF561844
Facesoffungi Number: FoF11812

Description and illustrations. see in Zhang et al. (2011), Tibpromma et al. (2017)

Type species. *Inflatispora pseudostromatica* Y. Zhang ter, J. Fourn. & K.D. Hyde

Notes. When Tanaka et al. (2015) conducted a revision of the Massarineae, *Inflatispora pseudostromatica* clustered with *Monodictys capensis* (now synonymized to *Pleomonodictys capensis*, Hernández-Restrepo et al. 2017) in a clade which was considered as *incertae sedis*. When the second taxon, *I. caryotae* was introduced, it was sister to *I. pseudostromatica*, and together both species clustered with *M. capensis* and *Asteromassaria pulchra* (fig. 39 in Tibpromma et al. 2017). None of these taxa had any familial placement and thus, *Inflatispora* was referred to Massarineae *incertae sedis* (Tibpromma et al. 2017). At the same time, in the phylogenetic analyses conducted by Hernández-Restrepo et al. (2017), *Inflatispora* (*I. pseudostromatica*) grouped with taxa of the novel genus *Pleomonodictys* (*Pleomonodictys capensis* and *Pl. descalsii*) described in that study, and those of *Bactrodesmium* (*B. cubense*), *Clypeoloculus* (*C. akitaensis*) and *Morosphaeria* (*M. velatispora*). However, *Pleomonodictys* was placed in the new family Pleomonodictydaceae, while the other genera were reported as *incertae sedis* (Hernández-Restrepo et al. 2017). Later, when Jayasiri et al. (2019) introduced *Pleohelicoon* in Pleomonodictydaceae, *Inflatispora* clustered with *Pleohelicoon* and *Pleomonodictys* in a clade, where it formed a distinct basal lineage to the two genera with low statistical support and was still considered as *incertae sedis* taxon. In the present study *Inflatispora* clusters in Pleomonodictydaceae with 99% ML BS, 1.00 BYPP statistical support (Fig. 1). *Inflatispora* resembles (in its sexual morph) *Robiniigena hyalinospora* gen. et sp. nov. in Pleomonodictydaceae and is accepted in the family based on morpho-phylogenetic evidence.

Robiniigena **Bundhun, Camporesi & K.D. Hyde, gen. nov.**

Index Fungorum: IF902255
Facesoffungi Number: FoF14884

Etymology: The name is based on the host genus *Robinia*, from which the fungus was isolated.

Description. ***Saprobic*** on woody substrates. **Sexual morph**: ***Ascomata*** immersed, with black dots present on host surface or erumpent, scattered, solitary or growing in groups in a pseudostroma, perithecial, globose to subglobose, usually unilocular, brown to dark brown, ostiolate. ***Ostiolar neck*** papillate, composed of hyaline pseudoparenchymatous cells. ***Peridium*** comprising two regions, outer region multi-layered, composed of brown to dark brown, thick-walled cells of ***textura angularis***; inner layer made up of lightly pigmented to hyaline cells. ***Hamathecium*** composed of hyaline, filiform, cellular, branched, indistinctly septate pseudoparaphyses. ***Asci*** bitunicate, fissitunicate, 8-spored, hyaline, clavate to cylindric-clavate, thin-walled, short-pedicellate, apically rounded, with an ocular chamber. ***Ascospores*** overlapping 1–2-seriate, hyaline, narrow to broadly fusiform, straight to slightly curved, euseptate, constricted at the septum, both ends conically rounded, smooth-walled, usually guttulate, surrounded by a thick or spreading mucilaginous sheath at maturity. **Asexual morph**: Undetermined.

Type species. *Robiniigena hyalinospora* Bundhun, Camporesi & K.D. Hyde

Notes. This monotypic genus forms a basal lineage in Pleomonodictydaceae with 99% ML BS and 1.00 BYPP statistical support (Fig. 1). *Robiniigena* shares several characteristics with *Inflatispora*, in terms of cellular pseudoparaphyses, clavate to cylindric-clavate, short-pedicellate asci, and hyaline, septate ascospores surrounded by a mucilaginous sheath (Zhang et al. 2011; Tibpromma et al. 2017). The ascospores of *Robiniigena* are, however, narrow to broadly fusiform and sometimes contain few large guttules, while those of *Inflatispora* are mostly narrowly fusiform to almost cylindrical and are ornamented with small guttules (Zhang et al. 2011; Tibpromma et al. 2017). The two genera are also phylogenetically distinct, with *Inflatispora* grouping with *Pleohelicoon* and *Pleomonodictys* in a clade and *Robiniigena* forming a basal separate lineage (Fig. 1).

***Robiniigena hyalinospora* Bundhun, Camporesi & K.D. Hyde, sp. nov.**

Index Fungorum: IF902256
Facesoffungi Number: FoF14885
Fig. 4

Etymology. The epithet refers to the hyaline ascospores.

Holotype. MFLU 23-0141

Description. ***Saprobic*** on *Robinia pseudoacacia*. **Sexual morph**: ***Ascomata*** 320–470 µm high, 250–600 µm diam. ($\bar{x}$ = 396 × 471 µm, n = 5), immersed, with black dots present on host surface or erumpent, visible in bark fissures, scattered, solitary or aggregated in a pseudostroma, perithecial, globose to subglobose, usually unilocular, rarely bilocular, brown to dark brown, coriaceous, ostiolate. ***Ostiolar neck*** 90–110 µm wide, papillate, comprising amorphous hyaline cells. ***Peridium*** 30–50 µm thick near the apex, 20–35 µm wide at the sides and base, comprising two regions; outer region multi-layered, composed of brown to dark brown, thick-walled cells of ***textura angularis***, fusing and becoming indistinguishable from the pseudostroma or host cells towards the outermost side; inner layer made up of lightly pigmented to hyaline cells merging with the pseudoparaphyses. ***Pseudoparaphyses*** 1.5–2.5 µm wide, numerous, filiform, cellular, branched, indistinctly septate, usually guttulate, surrounding the asci and along the innermost layer of the peridium. ***Asci*** 80–145(–160) × 15–25 µm ($\bar{x}$ = 111.5 × 20.9 µm, n = 15), bitunicate, fissitunicate, 8-spored, hyaline, clavate to cylindric-clavate, straight to slightly curved, thin-walled, short-pedicellate, apically rounded, with an ocular chamber. ***Ascospores*** (25–)30–40(–45) × 6–10(–12) µm ($\bar{x}$ = 36.8 × 8.6 µm, n = 55), overlapping 1–2-seriate, hyaline, narrow to broadly fusiform, straight to slightly curved, 1-euseptate, constricted at the septum, symmetrical or upper cell slightly longer than lower cell, wider upper cell, broad to acute and conically rounded at both ends, smooth-walled, sometimes guttulate, surrounded by a thick or spreading mucilaginous sheath when mature. **Asexual morph**: Not observed.

Culture characteristics. Ascospores germinated on WA within 24 hr. Colony on PDA, reaching 2 cm diam. after 15 days at 25 °C; above view dark grey in the middle and pale grey edges, dense, circular, umbonate, surface rough, radially furrowed, fimbriate; reverse dark brown, radiating outwardly.

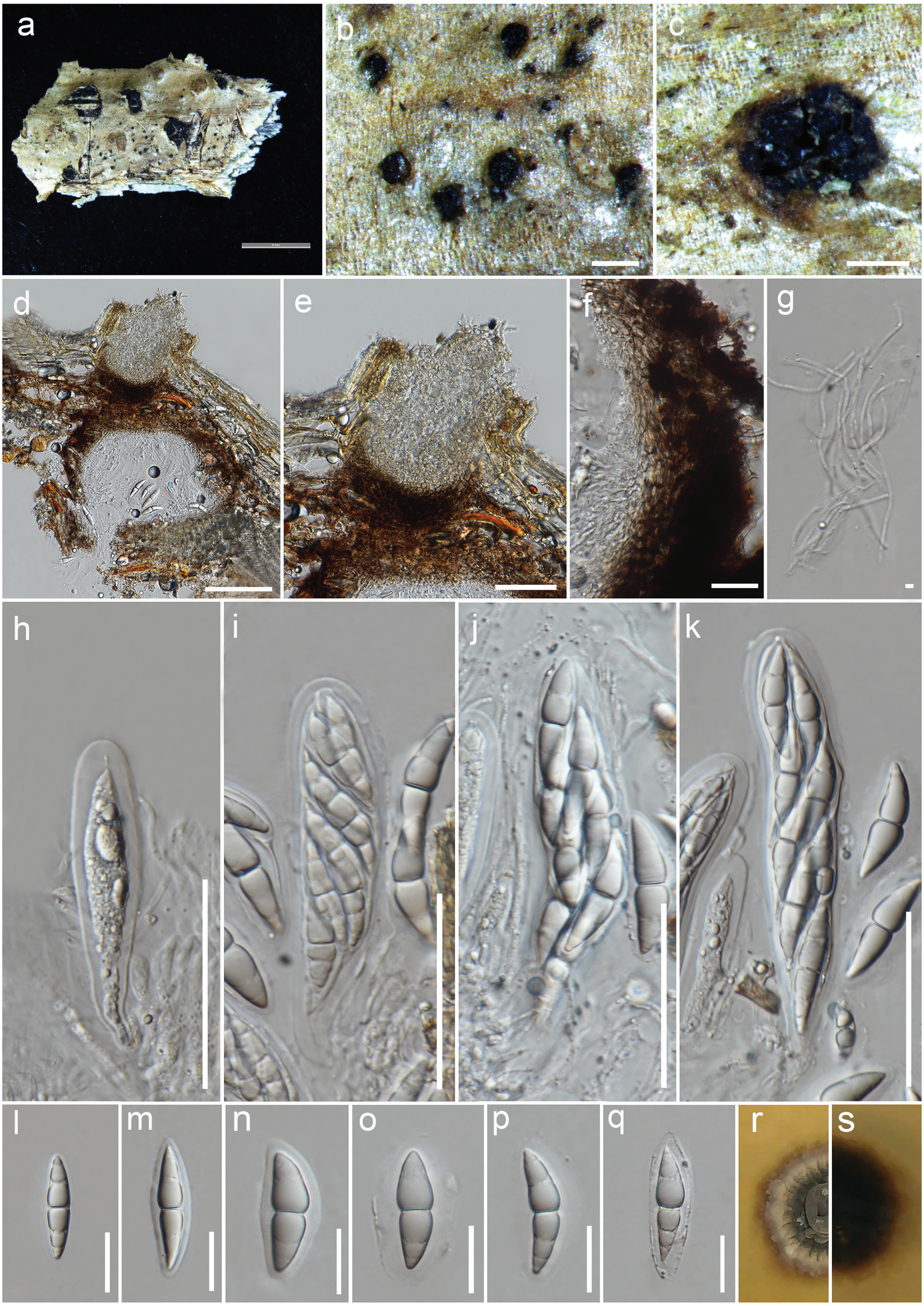

Figure 4. *Robiniigena hyalinospora* (MFLU 23-0141, holotype) **a** host substrate **b, c** ascomata on substrate **d** vertical section of an ascoma **e** section of an ostiolar neck **f** peridium **g** pseudoparaphyses **h–k** immature and mature asci **l–q** immature and mature ascospores **r** upper view of colony **s** reverse view of colony. Scale bars: 200 µm (**b**); 400 µm (**c**); 100 µm (**d**); 50 µm (**e, h–k**); 20 µm (**f, l–q**); 3 µm (**g**).

Material examined. Italy • Padova, near Torreglia; on dead aerial branches of *Robinia pseudoacacia*, 18 Nov 2021, E. Camporesi IT 4807 (holotype MFLU 23-0141), ex-type culture MFLUCC 23-0074.

Notes. *Robiniigena hyalinospora* resembles *Inflatispora pseudostromatica* by its globose to sub-globose ascomata, short-pedicellate asci, and ascospores whose upper cells are comparatively broader than the lower part (Zhang et al. 2011). The ascomata of *R. hyalinospora* are however, coriaceous and occur in a pseudostroma mainly when they are aggregated, while the ascomata of *I. pseudostromatica* are hard and form under a black pseudostroma both when the ascomata are solitary or occur in groups (Zhang et al. 2011). Furthermore, *R. hyalinospora* has narrow to broadly fusiform, 1-euseptate ascospores with conically rounded ends, whereas *I. pseudostromatica* comprises narrowly fusiform to nearly cylindrical, 3-septate ascospores with broadly or narrowly rounded ends (Zhang et al. 2011).

Robiniigena hyalinospora is similar to *I. caryotae*, in terms of 1-(eu)septate ascospores with a constricted middle septum and surrounded by a mucilaginous sheath (Tibpromma et al. 2017). However, *I. caryotae* has immersed ascomata while *R. hyalinospora* has immersed or erumpent ascomata, and the ascospores of *I. caryotae* are narrowly fusiform with acute ends while those of *R. hyalinospora* are narrow to broadly fusiform, with comparatively broader and conically rounded ends. *Robiniigena hyalinospora*, *I. caryotae* and *I. pseudostromatica*, are the only taxa in Pleomonodictydaceae with known sexual morphs.

Ampelomyces quisqualis **Ces., in Klotzsch, Bot. Ztg. 10: 301 (1852)**

Index Fungorum: IF121267
Facesoffungi Number: FoF11631
Fig. 5

Description. ***Saprobic*** on stem of *Sonchus* sp. **Sexual morph**: ***Ascomata*** 140–180 µm high, 200–260 µm diam. ($\bar{x}$ = 159 × 221 µm, n = 5), immersed, appearing as black dots on the host surface, solitary to aggregated, scattered, perithecial, unilocular, globose to subglobose, dark brown, ostiolate. ***Ostiole*** centric, comprising hyaline cells. ***Peridium*** 20–30 µm thick near the ostiole, 10–25 µm wide at the sides and 10–20 µm thick at the base, 3–4-layered, outer layer made up of thick-walled, brown cells of ***textura angularis***; inner layer made up of thin-walled, pale brown to hyaline cells of ***textura angularis***. ***Pseudoparaphyses*** 1–1.5 µm wide, numerous, hyaline, filiform, branched, septate, cellular, usually guttulate, surrounding the asci. ***Asci*** 45–65(–68) × 5–7.5 µm ($\bar{x}$ = 56.7 × 6.4 µm, n = 40), bitunicate, 8-spored, cylindrical, straight to slightly curved, thin-walled, short-pedicellate, bulbous, with an apical ocular chamber. ***Ascospores*** 12–17 × 2–4 µm ($\bar{x}$ = 15.3 × 3 µm, n = 50), overlapping uni- to bi-seriate, hyaline when immature, sub-hyaline on maturity, fusiform, straight to slightly curved, 2-celled with a median septum, symmetrical or upper cell slightly longer than lower cell, cell above septum slightly enlarged and with round or conical ends, lower cell mostly with round ends, minutely guttulate, sometimes both ends containing hyaline appendages which disappear with age. **Asexual morph**: see Manjunatha et al. (2020).

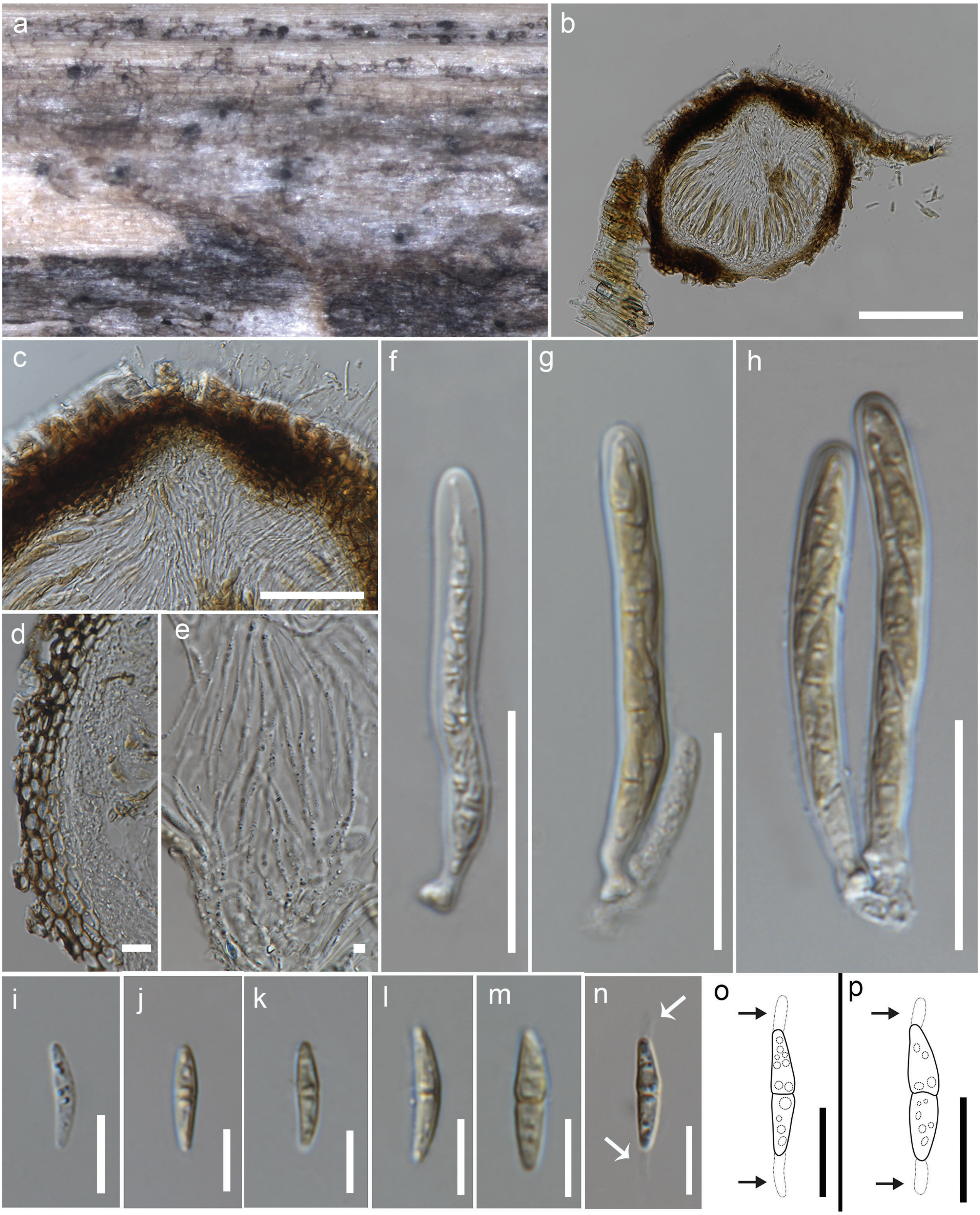

Figure 5. *Ampelomyces quisqualis* (MFLU 23-0142) **a** ascomata on host substrate **b** vertical section of an ascoma **c** section of an ostiole **d** peridium **e** pseudoparaphyses **f–h** asci **i–m** ascospores **n–p** hyaline appendages at both ends of ascospores (shown by arrows). Scale bars: 100 μm (**b**); 50 μm (**c**); 10 μm (**d, i–p**,); 3 μm (**e**); 30 μm (**f–h**).

Material examined. Italy • Forlì-Cesena, Valico Tre Faggi - Premilcuore; on dead aerial stems of *Sonchus* sp., 22 Jun 2021, E. Camporesi IT 4713, Herbarium material MFLU 23-0142.

Notes. Phylogenetic analyses based on the combined LSU–ITS dataset showed that strain MFLU 23-0142 grouped with *Ampelomyces quisqualis* (AMP, Chillan, BRIP 72107, and CBS 133.32) strains with 100% ML BS, 1.00 BYPP support (Fig. 2). While there was no base pair (bp) difference among the three strains of *A. quisqualis* (Chillan, BRIP 72107 and CBS 133.32) and MFLU 23-0142 vis-à-vis the ITS sequence (based on the aligned untrimmed dataset, including gaps), 1.2% (6/518 bp) difference between *A. quisqualis* (AMP) and strain MFLU 23-0142 was observed. Similarly, there was 0.1% bp (1/876 bp) difference between *A. quisqualis* (CBS 133.32) and MFLU 23-0142 with regards to the LSU sequence (no LSU sequence data are available for strains AMP, Chillan, and BRIP 72107 in GenBank). A morphological comparison could not be made since our strain was collected in its sexual morph while *Ampelomyces* has so far been reported in its asexual morph. Therefore, the strain MFLU 23-0142 is described as the sexual morph of *A. quisqualis* based on phylogenetic support.

Melomastia maolanensis **(Jin F. Zhang, Jian K. Liu, K.D. Hyde & Zuo Y. Liu) Norphoun, T.C. Wen & K.D. Hyde, Cryptog. Mycol. 38(4): 518 (2017)**

Index Fungorum: IF554041
Facesoffungi Number: FoF02695
Fig. 6

Description. ***Saprobic*** on dead stems of *Chromolaena odorata*. **Sexual morph**: ***Ascomata*** 420–440 µm high (including ostiole), 330–365 µm diam. ($\bar{x}$ = 426 × 351.3 µm, n = 5), visible as dark, raised, black spots on host, scattered, solitary to gregarious, immersed, with erumpent ostiole, perithecial, subglobose to obpyriform, coriaceous, dark brown to black, papillate, with a black clypeus. ***Ostiole*** 170–190 × 160–190 µm, central, oblong, dark brown to black, periphysate. ***Peridium*** 18–25 µm wide, comprising two layers; outer layer made up of thick-walled, dark brown cells fusing with host tissue, inner layer composed of thin-walled, hyaline cells. ***Pseudoparaphyses*** 1.5–3 µm wide, numerous, filiform, generally aseptate and unbranched, tapering towards the apex. ***Asci*** 80–125 × 4–7 µm ($\bar{x}$ = 102.7 × 5.5 µm, n = 20), 8-spored, non fissitunicate, long cylindrical, slightly flexuous, short-pedicellate, truncate or rounded at the apex, with a small ocular chamber. ***Ascospores*** 14–18.5 × 3–5 µm ($\bar{x}$ = 17.2 × 4.2 µm, n = 20), uniseriate, partially overlapping, hyaline, fusiform, with rounded or acute ends, 3-septate, constricted at the septa, smooth-walled, guttulate, surrounded by a mucilaginous sheath which vanishes with age. **Asexual morph**: Not observed.

Material examined. THAILAND • Chiang Mai, on dead stems of *Chromolaena odorata*, 7 Jul 2021, N.S. Wijesinghe CMN1, Herbarium material MFLU 23-0143.

Notes. In this study, strain MFLU 23-0143 clustered with *M. maolanensis* (GZCC 16-0102) with 100% ML BS, 1.00 BYPP support in the multi-locus phylogeny (Fig. 3). Nucleotide comparison of LSU and *tef1-α* revealed 0.1% (1/911 bp) and 1.3% (12/921 bp) differences between the two strains (MFLU 23-0143 and GZCC 16-0102), indicating insufficient phylogenetic differences to separate them as two different taxa (Jeewon and Hyde 2016; Maharachchikumbura et al. 2021). The two strains are also morphologically similar with ostiolate and papillate, immersed ascomata, long cylindrical and short-pedicellate asci, and ascospores that are fusiform, guttulate, and 3-septate. Therefore, strain MFLU 23-0143 is

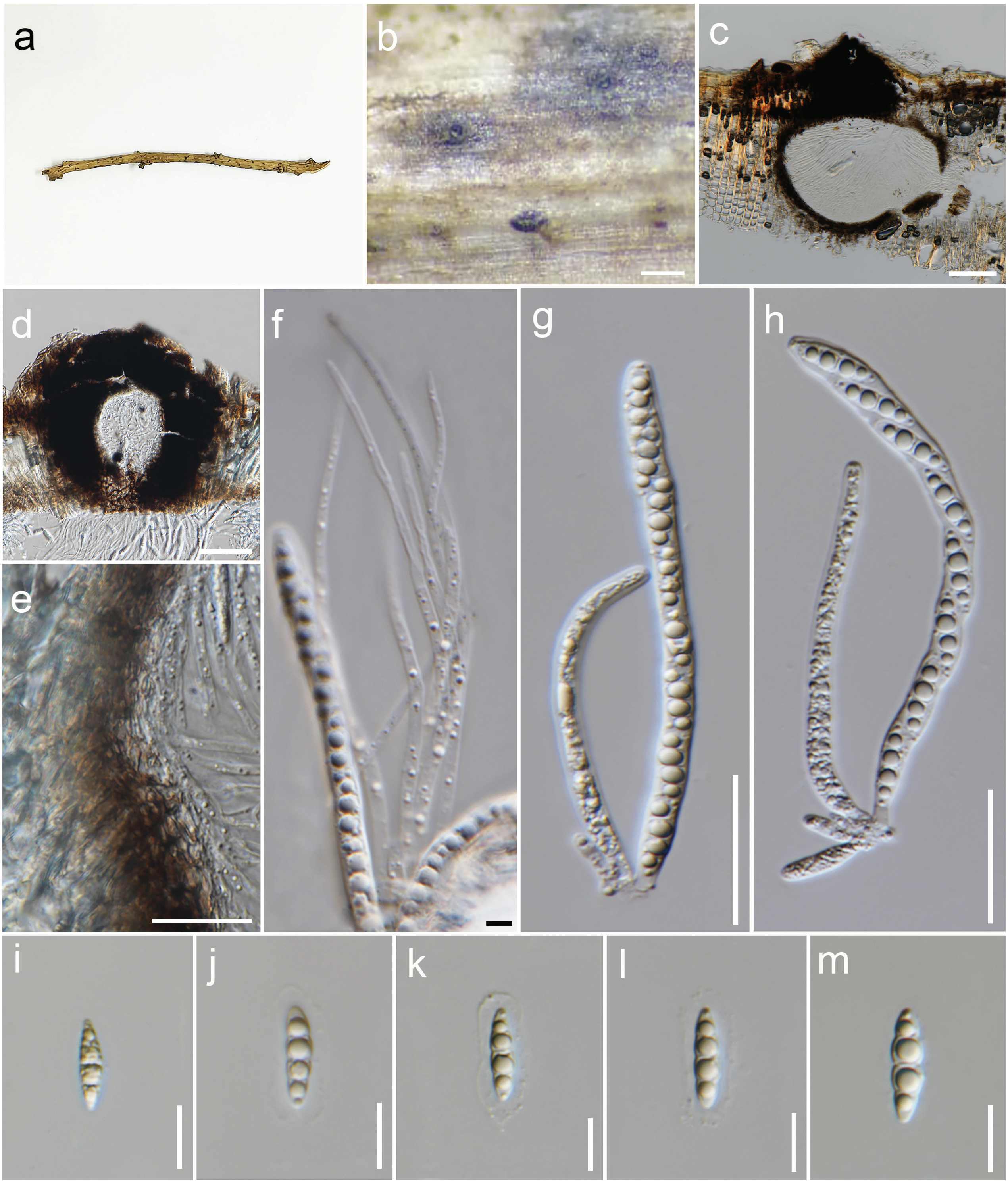

Figure 6. *Melomastia maolanensis* (MFLU 23-0143) **a** stem of *Chromolaena odorata* **b** ascomata on host **c** vertical section of an ascoma **d** ostiole **e** peridium **f** pseudoparaphyses **g, h** asci **i–m** ascospores. Scale bars: 200 μm (**b**); 100 μm (**c**); 50 μm (**d**); 20 μm (**e**); 5 μm (**f**); 30 μm (**g, h**); 10 μm (**i–m**).

identified as *M. maolanensis* based on morphological and phylogenetic evidence. The main morphological difference between the two strains is the presence of a mucilaginous sheath around the ascospores of *M. maolanensis* MFLU 23-0143, while no such report was made for the strain GZCC 16-0102 (Zhang et al. 2017). Variations in the size of the morphological characteristics between the two strains may be accounted for by environmental differences. Zhang et al. (2017) reported *M. maolanensis* from an undetermined tree branch in China. The same taxon is reported here from dead stems of *Chromolaena odorata* in Thailand.

Melomastia oleae **W.L. Li, Maharachch. & Jian K. Liu, Journal of Fungi 8(1, no. 76): 10 (2022)**

Index Fungorum: IF841500
Facesoffungi Number: FoF10534
Fig. 7

Description. ***Saprobic*** on dead branches of *Duranta erecta*. **Sexual morph**: ***Ascomata*** 415–420 µm high, 500–520 µm diam. ($\bar{x}$ = 418.9 × 511.1 µm, n = 5), visible as black, cone-shaped structures on host surface, usually solitary, scattered, semi-immersed to erumpent, globose to ampulliform, carbonaceous, dark brown to black, ostiolate. ***Ostiole*** central, dark brown to black, papillate, carbonaceous, periphysate. ***Peridium*** 18–35 µm diam., comprising two regions in vertical section; outer region carbonaceous, made up of 5–7-layered, thick-walled, brown cells of ***textura angularis*** to ***textura epidermoidea***, innermost region composed of compressed, hyaline cells. ***Pseudoparaphyses*** 1.5–2.5 µm wide, numerous, filiform, unbranched, sometimes septate. ***Asci*** 100–180 × 5.5–8 µm ($\bar{x}$ = 142.7 × 6.7 µm, n = 22), 8-spored, non fissitunicate, cylindrical, straight to flexuous, rounded at the apex, with a small ocular chamber, short-pedicellate. ***Ascospores*** 20–25 × 5–6 µm ($\bar{x}$ = 22.2 × 5.5 µm, n = 30), uniseriate, hyaline, fusiform, with rounded or obtuse ends, 3-septate, slightly constricted at the septa, guttulate, smooth-walled. **Asexual morph**: Not observed.

Culture characteristics. Colonies on PDA reaching 20 mm diam. in 3 weeks at 25 °C. Culture from above circular, regular, entire margin, dense, white; reverse pale brown.

Material examined. Thailand • Chiang Rai, Mae Fah Luang University, Mueang, Tha Sut; on dead stems and twigs of *Duranta erecta*, 30 Nov 2021, V. Thiyagraja DB 184, Herbarium material MFLU 23-0144, living culture MFLUCC 23-0086.

Notes. The isolate MFLUCC 23-0086 in the present study is basal to all the strains of *M. oleae* (Fig. 3). There was 0.2% (2/986 bp) nucleotide difference in SSU, while 0.9% (8/872 bp) in LSU and 0.1% (1/894 bp) in *tef1-α* between strain MFLUCC 23-0086 and the type strain (CGMCC3.20619) of *M. oleae*. Isolate MFLUCC 23-0086 is morphologically similar to *M. oleae* in terms of ascomata which appear as cone-shaped on the host surface, filiform and unbranched pseudoparaphyses, cylindrical, pedicellate asci and uniseriate, fusiform, 3-septate ascospores. However, the asci and ascospore sizes of the type of *M. oleae* are larger than our collection. Furthermore, the peridium of strain MFLUCC 23-0086 is observed as comprising thick-walled, brown cells of *textura angularis* to *textura epidermoidea* in the outer region and compressed, hyaline cells in the innermost region (Fig. 7). The type of *M. oleae* is reported to have a peridium with an outer thick, carbonaceous layer and the inner one made up of 5–6 layers of hyaline cells of *textura angularis* to *textura prismatica* (Li et al. 2022). Despite these morphological differences, MFLUCC 23-0086 is recognized as *M. oleae* as there is insufficient genetic variation to distinguish it as a different species (Maharachchikumbura et al. 2021; Pem et al. 2021). Furthermore,

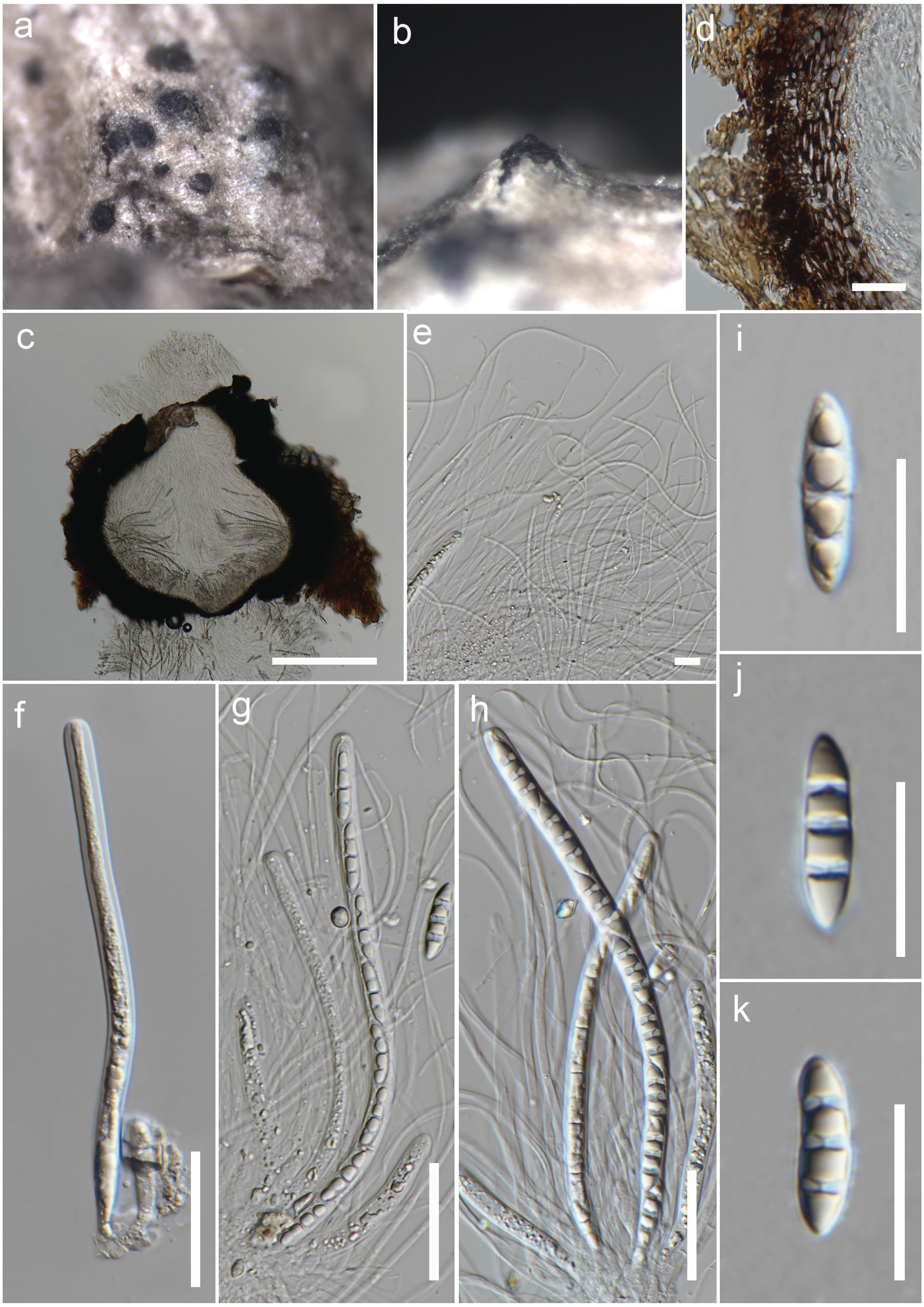

Figure 7. *Melomastia oleae* (MFLU 23-0144) **a** ascomata on the stem of *Duranta erecta* **b** an ascoma erumpent through the host tissue **c** vertical section of an ascoma **d** peridium **e** pseudoparaphyses **f–h** asci **i–k** ascospores. Scale bars: 200 μm (**c**); 20 μm (**d, i–k**); 5 μm (**e**); 30 μm (**f–h**).

the morphological differences may be due to different hosts and environmental conditions. Therefore, *M. oleae* is herein introduced as a new record from *Duranta erecta* in Thailand.

Discussion

In the present study, the familial description of the sexual morph of taxa in Pleomonodictydaceae is emended to include *Robiniigena* gen. nov. and *Inflatispora*, as both share several common morphological characteristics. *Robiniigena* also shares similar features with taxa belonging to the sister clade Morosphaeriaceae (Hongsanan et al. 2020). *Robiniigena* has cellular pseudoparaphyses, similar to *Aquihelicascus* and *Neohelicascus* taxa (Dong et al. 2020) and short-pedicellate asci resembling those of *Aquilomyces* and *Clypeoloculus* (Tanaka et al. 2015). *Robiniigena* also has hyaline ascospores with a gelatinous sheath similar to species of *Aquilomyces*, *Clypeoloculus*, and *Morosphaeria* (Suetrong et al. 2009; Tanaka et al. 2015; Devadatha et al. 2018). *Robiniigena* differs from Morosphaeriaceae species in that its ascomata are not covered with brown hyphae, as in *Aquilomyces* and *Clypeoloculus* (Tanaka et al. 2015). It has short-pedicellate asci while taxa of *Aquihelicascus* comprise asci with long pedicels (Dong et al. 2020). Ascospores are hyaline in *Robiniigena* while those of several *Helicascus* and *Neohelicascus* taxa are brown (Preedanon et al. 2017; Zeng et al. 2018; Dong et al. 2020). The introduction of *Robiniigena* and the inclusion of the *incertae sedis Inflatispora* in Pleomonodictydaceae indicates that the family is under constant taxonomic review. Further taxon sampling will undoubtedly give a better insight into the family as well as its relationship with other families in Massarineae.

Robiniigena hyalinospora was isolated from dead branches of *Robinia pseudoacacia* in Italy. Similarly, other fungi have been reported from the same host in Italy. *Cladosporium nigrellum* and *Camarosporidiella mirabellensis*, for instance, were isolated from dead or decorticated branches of *R. pseudoacacia* (Bensch et al. 2012; Wanasinghe et al. 2017). *Dothidotthia robiniae* was also retrieved from the same host as a saprobe, but from the Russian Federation (Senwanna et al. 2019). *Camarosporidiella elongata* (syn. *Cucurbitaria elongata*) and *Massaria anomia* (syn. *Aglaospora profusa*) were pathogenic on *R. pseudoacacia* in Greece (Michalopoulos-Skarmoutsos and Skarmoutsos 1999). Other fungi from the class Sordariomycetes have also been recorded from this host. For instance, *Colletotrichum nymphaeae* was identified as a pathogen of *R. pseudoacacia* in Japan (Yamagishi et al. 2016), while *Diaporthe oncostoma* was isolated from dead twigs of *R. pseudoacacia* (even though the species was also reported as a weak parasite) in Bulgaria (Stoykov 2012). These findings indicate that *Robinia pseudoacacia* hosts a diverse range of fungi with different lifestyles, across a large geographical area.

In this study, we have also discovered and reported the previously unknown sexual morph of *Ampelomyces quisqualis* (Wijayawardene et al. 2021). *Ampelomyces* comprises 16 species (Species Fungorum 2024), but sequence data are available for only *Ampelomyces quisqualis*. The inclusion of the genus in Phaeosphaeriaceae has been accepted by Phookamsak et al. (2014) based on the type species *A. quisqualis* (CBS 129.79). However, the latter strain is not the type or a verified strain of *A. quisqualis* (de Gruyter et al. 2009; Phookamsak et al. 2014; Vu et al. 2019; Hongsanan et al. 2020). Herein, we have included several representative strains of *A. quisqualis* (from NCBI) in our phylogeny, and they all cluster in a clade, but with three subclades (Fig. 2). Our collection MFLU 23-0142 grouped with *A. quisqualis* strains (AMP, Chillan, BRIP 72107, and CBS 133.32) in subclade A. A recent study by Huth et al. (2021) compared the draft genome assemblies for *A. quisqualis* strains (BRIP 72107 and HMLAC 05119; NCBI names) and revealed that the two strains are not conspecific. In the present study, strain HMLAC 05119 clustered with other *A. quisqualis* strains in subclade B, while *A. quisqualis* BRIP 72107 is nested in subclade A (Fig. 2). The presence of the subclades potentially indicates that all the strains may not actually be *A. quisqualis*; they probably belong to several different lineages in *Ampelomyces*. Several previous studies have also made such observations (Lee et al. 2007; Park et al. 2010; Angeli et al. 2011; Liyanage et al. 2018). Therefore, it is vital that *A. quisqualis* is recollected and the type sequenced to resolve these ambiguities. Until this taxonomic uncertainty is resolved, strain MFLU 23-0142, collected in the present study, is referred to *A. quisqualis*, as the sexual morph. In another scenario, *Ampelomyces* has been reported to cluster with *Neosetophoma* taxa in other phylogenetic studies (Phookamsak et al. 2014; Tibpromma et al. 2017; Hyde et al. 2018; Hongsanan et al. 2020). Until *Ampelomyces* is typified, its generic status, as well as its phylogenetic placement (including that of '*A. quisqualis*' strains), remain uncertain.

Melomastia maolanensis was originally described as a saprobe on dead branches of an undetermined host from Guizhou, China (Zhang et al. 2017), while *M. oleae* was collected from dead branches of *Olea europaea* at the foot of a mountain or mountainside in Sichuan, China (Li et al. 2022). As mentioned above, the strains collected in this study were similarly obtained from terrestrial habitats, although from different geographical locations and hosts. Some *Melomastia* taxa, such as *M. aquatica*, *M. neothailandica*, and *M. thailandica* have been collected from aquatic habitats as saprobes (Hyde 1992; Hyde et al. 2016; Dayarathne et al. 2020). *Melomastia septemseptata*, a recently introduced novel taxon based on morphological support, and characterized by dark green ascomata and multi-septate ascospores, has been found to occur on living tree bark (corticolous) in the terrestrial environment (Naziazeno and Aptroot 2023). *Melomastia distoseptata* has been reported from both terrestrial and freshwater habitats (Hongsanan et al. 2020; Boonmee et al. 2021). It is valuable to document new records of existing species, either from new geographical locations, habitats, hosts, or with different lifestyles; this enables a better understanding of the diversity and ecology of the fungal species, host jumping, and adaptations of fungi to various environmental conditions (Hyde et al. 2020; Chethana et al. 2021).

The phylogeny of the genera *Dyfrolomyces* and *Melomastia* has been confusing and not truly resolved. *Dyfrolomyces* was introduced by Pang et al. (2013) as part of a study on marine *Saccardoella* species, a genus variously classified in Clypeosphaeriaceae, Xylariales (Barr 1989), Pleurotremataceae, Xylariales (Barr 1994), and 'Unitunicate Ascomycota genera *incertae sedis* (Jones et al. 2009). Suetrong et al. (2009) earlier established that *Saccardoella rhizophorae* did not show any affinities to members of the Sordariomycetes (Maharachchikumbura et al. 2015), but grouped within the Dothideomycetes, although it did not cluster with any known families or orders in the class. Pang et al. (2013) confirmed the monophyly of *S. rhizophorae* and the new marine fungus *S. tiomanensis*, in a sister clade to Acrospermaceae (Dothideomycetes) and separately from *S. montellica*, the type species of the genus referred to Sordariomycetes at that time. Pang et al. (2013), therefore, introduced a new genus *Dyfrolomyces* to accommodate *S. rhizophorae*, *S. tiomanensis* as well as *S. mangrovei* and *S. marinospora*, and also established the family Dyfrolomycetaceae in the order Dyfrolomycetales (Hyde et al. 2013). Later, Maharachchikumbura et al. (2016) synonymized Dyfrolomycetaceae under Pleurotremataceae. Norphanphoun et al. (2017) noted that *Melomastia italica* and

Dyfrolomyces maolanensis formed a sister clade to five *Dyfrolomyces* species, and referred *D. maolanensis* to *Melomastia*. They concluded that *Dyfrolomyces*, *Pleurotrema*, and *Melomastia* belonged in the Pleurotremataceae, and shared many features in common, but considered *Melomastia* and *Dyfrolomyces* "as distinct genera". It is important to record that there were no sequences of *Melomastia* available when Pang et al. (2013) introduced the genus *Dyfrolomyces*.

In the study by Li et al. (2022), *Dyfrolomyces* and *Melomastia* species clustered together, which led to *Dyfrolomyces* and its taxa being synonymized under *Melomastia*. In the current phylogenetic study, *Melomastia* species form three clades (A, B, and C; Fig. 3). *Melomastia tiomanensis* (type species of *Dyfrolomyces*) and *M. chromolaenae* form a basal clade (clade C) with 100% ML BS, 1.00 BYPP support. Although they share common features with several *Melomastia* species, especially in their short-pedicellate, apically rounded asci, and multi-septate ascospores, they mainly differ from the other *Melomastia* taxa in their distinct spindle-shaped ascospores with tapering narrow ends (Pang et al. 2013; Mapook et al. 2020). The status of *Dyfrolomyces* is therefore justified for *M. tiomanensis* and *M. chromolaenae* based on morphological and phylogenetic evidence, as is equally reported by Kularathnage et al. (2023).

Meanwhile, the remaining *Melomastia* taxa (with molecular data available) form two clades (A and B; Fig. 3). Morphological delineation between the two clades is challenging since the taxa have overlapping features. The type species, *M. mastoidea* is characterized by 2-septate, ovoid ascospores with rounded ends, which are surrounded by a mucilaginous sheath, but it lacks sequence data. Kularathnage et al. (2023) have classified clade B as *Melomastia sensu stricto* since the taxa in that clade share most of the ascospore features with *M. mastoidea*. Clade A, which has been termed as *Melomastia sensu lato*, contains taxa whose ascospore morphology is less similar to that of *M. mastoidea*. However, this classification may not be totally accurate since *M. fusispora* in clade A also has a gelatinous sheath (Li et al. 2022). Taxa such as *M. septata*, *M. maolanensis*, and *M. sichuanensis*, which are accommodated in clade B, have 3-septate ascospores, similar to *M. oleae*, *M. distoseptata*, *M. fusispora* and *M. winteri* in clade A (Zhang et al. 2017; Hongsanan et al. 2020; Li et al. 2022; Hyde et al. 2023). Furthermore, the shape of the ascospores in both clades A and B are cylindrical, ellipsoidal to fusoid or fusiform, with rounded to acute ends. Clades A and B can indeed be indicative of two or more different genera. Moreover, *M. phetchaburiensis* and *M. sinensis*, which have been treated as uncertain species in *Melomastia* by Dong et al. (2023), may represent different genera. However, the definite classification of these taxa will be possible upon the availability of molecular data for the type species, *M. mastoidea*. Also, it could be that other characters are still underplayed. For instance, none of the taxa in either clade A or B have their asexual morphs reported so far. Therefore, with the collection of more taxa in both clades, a better resolution of their position in Pleurotremataceae will be possible. Moreover, employing more genetic markers in the phylogenetic analysis of Pleurotremataceae may provide a better and more accurate picture of the taxa in the family.

The phylogeny of *Melomastia* and *Dyfrolomyces* has long been challenging in that no sequences of *Melomastia* species were available when Pang et al. (2013) introduced *Dyfrolomyces*. Subsequent phylogenetic studies have failed to undertake an analysis of pairwise distances between the two genera or of the family Pleurotremataceae. Likewise, base pair differences between *Melomastia* species might have clarified their taxonomy if the guidelines of Jeewon and Hyde (2016) and Maharachchikumbura et al. (2021) had been followed, especially when the *Dyfrolomyces* species were synonymized by Li et al. (2022).

It is also to be noted that the phylogram in Fig. 1 involves the taxa *Pleomonodictys capensis* and *Pl. descalsii* which are clustered together but with no robust phylogenetic segregation. *Pleomonodictys descalsii* was introduced principally based on its smaller conidial size as compared to that of *Pl. capensis* (Hernández-Restrepo et al. 2017). However, Bao et al. (2021), while introducing a novel collection for *Pl. capensis*, noticed that the conidial size of their new collection closely matched that of *Pl. descalsii*, and there was also no remarkable difference vis-à-vis the other asexual features. This indicates that *Pl. capensis* could be conspecific with *Pl. descalsii* and this is equally being shown in the present phylogeny (Fig. 1). Additional morpho-phylogenetic studies are necessary to solve this taxonomic uncertainty.

Acknowledgements

Digvijayini Bundhun extends her heartfelt gratitude to Mae Fah Luang University for granting the tuition scholarship for PhD studies. She is also grateful to Professor Qi Zhao for providing experimental facilities and to Nuwanthika S. Wijesinghe for helping with specimen collection. Janith V.S. Aluthmuhandiram, Binu C. Samarakoon, and Chayanard Phukhamsakda are acknowledged for their valuable suggestions. E. B. Gareth Jones and Dhanushka N. Wanasinghe thank the King Saud University, Kingdom of Saudi Arabia for the award of a Distinguished Scientist Fellowship (DSFP). Dhanushka N. Wanasinghe thanks the Yunnan Department of Sciences and Technology of China (Grant No: 202101AS070045, 202205AM070007, 202302AE090023, 202303AP140001). The authors thank Shaun Pennycook for checking the nomenclature of the new taxa.

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 Chinese Research Fund, grant number E1644111K1, titled “Flexible introduction of the high-level expert program, Kunming Institute of Botany, Chinese Academy of Sciences”.

Author contributions

Conceptualization: DB, RSJ, KDH. Data curation: EC, DB. Formal analysis: DB, RSJ, DNW. Funding acquisition: VT, KDH. Investigation: DB, GEBJ, DNW. Methodology: DB, RSJ, DNW. Supervision: RSJ, KDH. Writing – original draft: DB, DNW. Review & editing: GEBJ, RSJ, ICS, DNW, VT, KDH.

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Data availability

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

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MycoKeys

MycoKeys 111: 181–210 (2024)
DOI: 10.3897/mycokeys.111.136202

Research Article

Assigning *Xiuguozhangia* (genus *incertae sedis*) to Pseudoberkleasmiaceae (Dothideomycetes, Pleosporomycetidae, Pleosporales) and introducing *X. broussonetiae* as a novel species

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Abstract

Xiuguozhangia species are dematiaceous hyphomycetes that are characterised by acropleurogenous, dictyoseptate, campanulate or cheiroid, and brown to dark brown conidia that are composed of several layers of cells radiating from a protuberant basal cell, and mostly seen with appendages arising from the apical cells. The genus was introduced based on morphology to accommodate five of the six *Piricaudiopsis* species that exhibited holoblastic conidial ontogeny. *Xiuguozhangia* was referred to as Ascomycota genus *incertae sedis* as it was challenging to resolve its taxonomic placement based solely on the available morphological data (no DNA sequence data was previously available). In this study, we provide DNA sequence data for LSU, ITS, SSU, *TEF1*, and *RPB2* for our isolates, collected from *Broussonetia papyrifera* (Moraceae) in northern Thailand. Based on morphology, we classify our isolates as *Xiuguozhangia*. Since they form a sister lineage to *Pseudoberkleasmium*, we place *Xiuguozhangia* in Pseudoberkleasmiaceae (Pleosporales). Within *Xiuguozhangia*, we establish these two isolates as a new taxon, *Xiuguozhangia broussonetiae*, in view of the presence of new conidiogenous cells developing from subtending cells. *Xiuguozhangia broussonetiae* differs from the extant species in the genus as it has longer conidiophores that are sometimes branched, comprising numerous septa, and its appendages are mostly untapered (sometimes tapering) towards the tips, a feature not observed in other *Xiuguozhangia* species. This is the first study to provide DNA sequence data and phylogenetic relationships for *Xiuguozhangia*. Furthermore, we analysed selected DNA sequence data and provided an updated phylogenetic tree incorporating all families (with representative genera) of Pleosporales.

Key words: 1 new taxon, hyphomycetes, morphology, phylogeny, sequence data, taxonomy

This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

Academic editor:
Samantha C. Karunarathna
Received: 3 September 2024
Accepted: 25 November 2024
Published: 20 December 2024

Citation: Gomdola D, Jeewon R, Gentekaki E, S. Jayawardena R, Hyde KD, Alotibi F, Wang Y (2024) Assigning *Xiuguozhangia* (genus *incertae sedis*) to Pseudoberkleasmiaceae (Dothideomycetes, Pleosporomycetidae, Pleosporales) and introducing *X. broussonetiae* as a novel species. MycoKeys 111: 181–210. https://doi.org/10.3897/mycokeys.111.136202

Introduction

Xiuguozhangia was introduced by Zhang et al. (2014) based on morphological characteristics and typified with *X. rosae*. The genus is characterised by effuse, hairy, and brown to dark brown colonies on natural substrates. Conidiophores are erect, straight or flexuous, multiseptate, and brown to dark brown at the base, becoming pale brown towards the apex. Conidiogenous cells are monoblastic, terminal or integrated, lageniform, and pale brown, displaying zero to several percurrent proliferations. In addition, their conidia are acropleurogenous, dictyoseptate, campanulate, cheiroid and brown, comprising multiple layers of cells, either with or without appendages (Zhang et al. 2014; Liu et al. 2024b).

Initially, five *Xiuguozhangia* species were established, all of which are combinations of *Piricaudiopsis viz*., *Xiuguozhangia appendiculata*, *X. indica*, *X. punicae*, *X. rhaphidophorae* and *X. rosae*. *Piricaudiopsis* was established by Mena and Mercado (1987) based on morphology and typified with *P. elegans*. The new combinations of *Xiuguozhangia* were proposed based on their holoblastic conidial ontogeny; *Piricaudiopsis* was reported to have enteroblastic conidial ontogeny (Zhang et al. 2014; Dubey and Jash 2024). However, this morphological difference based on conidial ontogeny is subtle, necessitating careful consideration before applying this trait in generic delineation. Given the limited taxon sampling and analysis across both genera, coupled with the lack of DNA sequence data, it is still uncertain whether *Xiuguozhangia* is actually distinct from *Piricaudiopsis*, or if they could represent the same genus. Both genera were referred to as Ascomycota genera *incertae sedis* in the latest outlines by Wijayawardene et al. (2022a) and Hyde et al. (2024c) as it was difficult to establish their precise taxonomic placement without the availability of DNA sequence data. Recently, based on morphological description, a new species was added to *Xiuguozhangia viz*., *X. macrospora*, collected from dead bamboo stems in India (Dubey and Jash 2024). To date, all extant species of *Xiuguozhangia* have been described as saprobes and collected from China and India (Zhang et al. 2014; Dubey and Jash 2024), while no studies have reported its occurrence in Thailand.

The highlights of this study are as follows: (i) We provide DNA sequence data for multiple gene regions (LSU, ITS, SSU, *TEF1*, and *RPB2*) for our isolates, collected from *Broussonetia papyrifera* (Moraceae) in northern Thailand; (ii) Based on morphology, we classify our isolates as *Xiuguozhangia*. Since they form a sister lineage to *Pseudoberkleasmium*, we place *Xiuguozhangia* in Pseudoberkleasmiaceae (Pleosporales); (iii) Within *Xiuguozhangia*, these two isolates could potentially represent a new taxon, *Xiuguozhangia broussonetiae* in view of the presence of new conidiogenous cells developing from subtending cells. This is the first study to provide DNA sequence data and phylogenetic relationships for *Xiuguozhangia*. In addition, we analysed selected sequence data from GenBank and provided an updated phylogenetic tree incorporating all families (with representative genera) of Pleosporales.

Materials and methods

Sample collection and examination, material deposition, and species delimitation methods

Decaying stems of *Broussonetia papyrifera* (Moraceae) colonised by hyphomycetes were collected from deciduous forest in the Mae Fah Luang University Botanical Garden, Chiang Rai, Thailand during the dry, hot season; collection information was noted (Rathnayaka et al. 2024) and brought to the laboratory in paper boxes for further characterisation (Senanayake et al. 2020). Morphological characteristics were observed using a Motic SMZ 168 Series stereo-microscope. Several conidia were picked from the colonies on the substrate using a sterile needle and placed directly on fresh potato dextrose agar plates (PDA, 39 g/L) containing antibiotics (Amoxicillin, MacroPhar). Conidium germination was observed within 48 hours, and pure cultures were incubated for four weeks at 25 °C under dark conditions. Digital images of micro-morphological characters were captured using a Cannon 750D camera (Canon, Tokyo, Japan) attached to a Nikon ECLIPSE E600 compound microscope (Nikon, Tokyo, Japan) based on the bright-field microscopy technique. Photo plates were assembled using Adobe Photoshop CS6 version 2020 (Adobe Systems, USA), and measurements were made using Tarosoft® Image Frame Work (version 0.97).

The holotype specimen and ex-type living culture have been deposited in the Mae Fah Luang University Herbarium (MFLU) and Mae Fah Luang University Culture Collection (MFLUCC), respectively. Faces of Fungi and Index Fungorum numbers are given for the new species (Jayasiri et al. 2015; Index Fungorum 2024). The description and illustration of the new species have also been updated in the GMS microfungi database (https://gmsmicrofungi.org/) (Chaiwan et al. 2021).

The new species is established based on the morphological species concept and complemented with the phylogenetic species concept (Maharachchikumbura et al. 2021; Pem et al. 2021). Features of *Xiuguozhangia* species are compiled, and a comparison is done to showcase the major differences across existing taxa.

DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from axenic cultures grown on PDA for 28 days using the BIOMIGA Fungus Genomic DNA Extraction Kit (BIOMIGA, San Diego, CA, USA), following the manufacturer's instructions. The internal transcribed spacer (ITS), large subunit (LSU), and small subunit (SSU), and the genes for RNA polymerase 2 (*RPB2*) and translation elongation factor 1α (*TEF1*) were amplified using the following primers: ITS1/ITS4, LR0R/LR5, NS1/NS4 for ITS, LSU, and SSU, respectively (White et al. 1990); fRpb2-5F/7CR for *RPB2* (Liu et al. 1999); and 728F/2218R for *TEF1* (O'Donnell et al. 1998; Carbone and Kohn 1999).

The polymerase chain reaction (PCR) mixture had a final volume of 20 µL, comprising 10 µL of PCR master mix, 1 µL of the forward and reverse primers each (10 µM stock concentration), 7 µL of double-distilled water, and 1 µL of the template DNA. The PCR conditions were as follows: initial denaturation at 95 °C for 3 min; denaturation at 95 °C for 45 s; annealing at 55 °C for 50 s (ITS), 52 °C for 50 s (LSU and SSU), 58 °C for 1 min 30 s (*RPB2* and *TEF1*); extension at 72 °C for 2 min; and final extension at 72 °C for 10 min (number of cycles = 40). Purification and bidirectional sequencing of PCR amplicons were carried out at Sangon Biotech (Shanghai) Co., Ltd., China.

Phylogenetic analyses

The raw reads were checked using DNA Baser Assembler, and ambiguous bases from the 5' and 3' ends were trimmed manually. Consensus sequences were generated using SeqMan (DNAStar, Madison, Wisconsin, USA). The sequences have been deposited in the NCBI GenBank database, and accession numbers for all strains are provided (Table 1). Newly obtained sequences were subjected to blast searches in NCBI, and sequences of ITS, LSU, SSU, *RPB2*, and *TEF1* from other species were retrieved from GenBank (Table 1). Two different datasets were analysed in this study. The first dataset (Dataset 1) evaluated familial relationships and was based on a larger taxon sampling, incorporating representative genera with DNA sequence data from 92 families of Pleosporales. Diademaceae and Lizoniaceae, which also belong to Pleosporales, lack molecular data. Another dataset (Dataset 2) evaluated the phylogenetic relationships within and between *Xiuguozhangia* (Pseudoberkleasmiaceae) and its phylogenetically closely related genera, *Pseudoberkleasmium* (Pseudoberkleasmiaceae) and *Hermatomyces* (Hermatomycetaceae). Dataset 2 was based on multiple strains for these genera.

Single gene datasets were aligned using MAFFT version 7 by applying the default setting (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019) and trimmed using trimAl (Capella-Gutiérrez et al. 2009). The trimmed datasets were concatenated using SequenceMatrix (Vaidya et al. 2011). Maximum likelihood (ML) phylogeny was conducted in the IQ-TREE webserver (https://iqtree.cibiv.univie.ac.at) using the default parameters and 1000 ultrafast bootstrap replicates (Nguyen et al. 2015). The nucleotide substitution model for each DNA marker was automatically generated. The Bayesian information criterion (BIC) selection results were as follows: Dataset 1 – invgamma for ITS, LSU, *RPB2* and *TEF1*, and gamma for SSU; Dataset 2 – gamma for ITS and SSU, and invgamma for LSU, *RPB2* and *TEF1*.

Bayesian inference (BI) was carried out in MrBayes on XSEDE (version 3.2.7a) in the online CIPRES Portal (https://www.phylo.org/portal2) (Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003; Miller et al. 2010). Markov chain Monte Carlo (MCMC) sampling was applied to obtain posterior probabilities (PP). Four Markov chains were run simultaneously for 50,000,000 and 5,000,000 generations for datasets 1 and 2, respectively, with trees sampled every 100th generation. Burn-in was set to 20% and the remaining 80% were used to compute the PP of the consensus trees. Phylogenetic trees were visualised in FigTree version 1.4.4 (Rambaut and Drummond 2014).

Genetic distances

To corroborate the phylogenetic placement and evolutionary relationships of the new taxon, intra- and inter-generic genetic distances were computed in MEGA-X by applying the Kimura 2-parameter substitution model, gamma distribution, and pairwise deletion options (Tamura et al. 2013).

Table 1. Names, isolate numbers, and the corresponding GenBank accession numbers of taxa used in the phylogenetic analyses. Type, ex-type, and reference strains are denoted with ^{T}. The new isolates are in bold font.

Species	Isolate/strain number	LSU	ITS	SSU	*TEF1*	*RPB2*
Acrocalymma aquatica	MFLUCC 11-0208 ^{T}	JX276952	JX276951	JX276953	–	–
Acrocalymma pterocarpi	MFLUCC 17 0926 ^{T}	MK347949	MK347732	MK347840	MK360040	–
Ageratinicola kunmingensis	KUMCC 21-0217 ^{T}	NG_243113	NR_191196	NG_242816	–	–
Aigialus grandis	BCC 20000 ^{T}	GU479775	–	GU479739	GU479839	–
Alternaria atrobrunnea	FMR 16868 ^{T}	–	LR537033	–	LR537051	LR537044
Alternaria alternata	AFTOL-ID 1610 ^{T}	DQ678082	KF465761	KC584507	KC584634	KC584375
Amniculicola aquatica	MFLUCC 16-1123 ^{T}	MK106096	–	MK106108	MK109800	–
Amorocoelophoma cassiae	MFLUCC 17-2283	MK347956	MK347739	NG_065775	MK360041	MK434894
Anastomitrabeculia didymospora	MFLU 20-0694 ^{T}	MW412978	NR_172008	NG_073568	MW411338	–
Anastomitrabeculia didymospora	MFLU 11-0236	ON077069	ON077080	ON077074	ON075063	ON075067
Angustimassarina lonicerae	MFLUCC 15-0087	KY496724	KY496759	–	–	–
Anteaglonium gordoniae	MFLUCC 17-2431 ^{T}	MK347977	MK347761	MK347867	MK360042	MK434881
Anteaglonium gordoniae	CD7	–	OK335788	–	–	–
Anteaglonium latirostrum	GKM1119 ^{T}	GQ221874	–	–	GQ221937	–
Anteaglonium latirostrum	GKML100Nb	GQ221876	–	–	GQ221938	–
Aquadictyospora clematidis	MFLUCC 17-2080 ^{T}	MT214545	MT310592	MT226664	MT394727	MT394679
Aquastroma magniostiolata	HHUF 30122 ^{T}	AB807510	LC014540	AB797220	AB808486	–
Aquasubmersa japonica	HHUF 30469 ^{T}	NG_057138	NR_154739	NG_062426	LC194384	LC194421
Aquasubmersa japonica	MFLUCC 17-2121	OP377971	OP377885	OP378047	OP473059	OP473118
Aquasubmersa japonica	MFLUCC 15-0622	OP377958	OP377872	OP378036	OP473051	OP473112
Aquasubmersa mircensis	MFLUCC 11-0401 ^{T}	NG_042699	JX276954	NG_061141	–	–
Aquihelicascus songkhlaensis	MFLUCC 18-1154 ^{T}	MN913692	MT627680	–	MT954380	–
Aquihelicascus songkhlaensis	MFLUCC 18-1273	MN913724	MT627696	MT864319	MT954369	MT878464
Aquimassariosphaeria kunmingensis	KUMCC 18-1019 ^{T}	MT627661	–	MT864312	MT954409	MT878454
Ascocylindrica marina	MD6011 ^{T}	KT252905	–	KT252907	–	–
Ascocylindrica marina	MF416	MK007123	–	MK007124	–	–
Astragalicola vasilyevae	MFLUCC 17-0832 ^{T}	MG828986	NR_157504	MG829098	MG829193	MG829248
Astrosphaeriella fusispora	MFLUCC 10-0555	KT955462	–	KT955443	KT955425	KT955413
Atrocalyx glutinosus	DAOM: 252609 ^{T}	OQ400928	OQ400918	–	OQ413076	OQ413081
Atrocalyx glutinosus	CHEM 2721	OQ400930	OQ400920	–	–	OQ413084
Bahusandhika indica	GUFCC 18001	KF460274	KF460273	–	–	–
Bambusicola bambusae	MFLUCC 11-0614 ^{T}	JX442035	JX442031	JX442039	–	KP761718
Berkleasmium aquaticum (Tubeufiales)	MFLUCC 17-0049 ^{T}	KY790432	KY790444	–	KY792608	MF535268
Berkleasmium aquaticum (Tubeufiales)	MFLUCC 17-0039	KY790431	KY790443	–	KY792607	MF535267
Berkleasmium longisporum (Tubeufiales)	MFLUCC 17-1999 ^{T}	MH558825	MH558698	–	MH550889	MH551012
Berkleasmium longisporum (Tubeufiales)	MFLUCC 17-2002	MH558826	MH558699	–	MH550890	MH551013
Bertiella fici	MFLU 19-2713 ^{T}	MW063223	–	MW079351	MW183786	–
Bertiella fici	NCYU 19-0073	MW063224	–	MW079352	MW183787	–
Bipolaris adikaramae	HSF070 ^{T}	–	MN535176	–	MT548605	–
Boeremia linicola	CBS 116.76 ^{T}	GU237938	GU237754	–	KY484705	KT389574
Boeremia linicola	CBS 248.38	KT389703	KT389486	–	–	KT389575
Biatriospora borsei	NFCCI-4245 ^{T}	MK358813	MK358818	MK358811	MK330938	–
Biatriospora marina	CY 1228	GQ925848	–	GQ925835	GU479848	GU479823
Brevicollum hyalosporum	MAFF 243400 ^{T}	LC271239	LC271242	LC271236	LC271245	LC271249
Brevicollum hyalosporum	MFLUCC 17-0071	MG602200	MG602204	MG602202	MG739516	–
Brevicollum versicolor	HHUF 30591 ^{T}	NG_058716	NR_156335	NG_065124	LC271246	LC271250
Brunneoclavispora camporesii	MFLUCC 11-0001 ^{T}	MN809328	MN809329	–	–	–
Brunneofusispora clematidis	MFLUCC 17-2070 ^{T}	MT214570	MT310615	MT226685	MT394629	MT394692
Camarosporium quaternatum	CPC 31081 ^{T}	NG_064442	NR_159756	KY929123	KY929201	–
Camarosporomyces flavigenus	CBS 314.80 ^{T}	GU238076	MH861266	NG_061093	–	–
Camarosporidiella caraganicola	MFLUCCC 14-0605 ^{T}	KP711381	KP711380	KP711382	–	–
Camarosporidiella melnikii	MFLUCC 17-0684 ^{T}	MF434250	MF434162	MF434338	MF434425	–
Capulatispora sagittiformis	HHUF 29754 ^{T}	NG_042319	NR_119393	NG_060997	LC001756	–
Caryospora aquatica	MFLU 11-1083 ^{T}	NG_059058	NR_156408	MH057850	–	–
Caryospora submersa	MFLUCC 18-1283 ^{T}	MN913720	–	–	–	–
Clematidis italica	MFLUCC 15-0084 ^{T}	KU842381	KU842380	KU842382	–	–
Coelodictyosporium rosarum	MFLUCC 17-0776 ^{T}	MG828991	MG828875	MG829102	MG829195	–
Corylicola italica	MFLU 19-0500 ^{T}	MT554926	MT554925	MT554923	–	MT590776

Species	Isolate/strain number	LSU	ITS	SSU	*TEF1*	*RPB2*
Corynespora cassiicola	CBS 100822	GU301808	–	GU296144	GU349052	GU371742
Corynespora torulosa	CPC 15989 ^{T}	KF777207	NR_145181	–	–	–
Crassimassarina macrospora	MAFF 239606 ^{T}	LC194344	LC194478	LC194302	LC194389	LC194426
Crassimassarina macrospora	HHUF 30512	LC194343	LC194477	LC194301	LC194388	LC194425
Crassiperidium octosporum	MAFF 242971 ^{T}	LC373108	LC373096	LC373084	LC373120	LC373132
Crassiperidium octosporum	MAFF 246401	LC373111	LC373099	LC373087	LC373123	LC373135
Cryptocoryneum japonicum	HHUF 30482 ^{T}	NG_059035	NR_153938	NG_065118	LC096144	LC194438
Cryptocoryneum pseudorilstonei	CBS 113641 ^{T}	NG_059036	NR_153941	LC194322	LC096152	LC194446
Cucurbitaria berberidis	MFLUCC 11-0387	KC506796	–	KC506800	–	–
Curvularia austriaca	CBS 102694 ^{T}	–	MN688802	–	MN688856	–
Curvularia eleusinicola	USJCC-0005 ^{T}	–	MT262877	–	MT432925	–
Cylindroaseptospora leucaenicola	MFLUCC 17-2424 ^{T}	MK347966	NR_163333	MK347856	–	–
Cyclothyriella rubronotata	CBS 141486 ^{T}	KX650544	NR_147651	NG_061252	KX650519	KX650574
Dacampia hookeri	GZU 73897	KT383792	–	–	–	–
Dacampia hookeri	GZU 81840	KT383795	–	–	–	–
Dacampia hookeri	GZU 75980	KT383794	–	–	–	–
Delitschia nypae	MFLUCC 17-2588 ^{T}	–	–	–	MK360049	MK434878
Dendryphion fluminicola	MFLUCC 17-1689 ^{T}	MG208141	NR_157490	–	MG207992	–
Deniquelata cassiae	CMD012A ^{T}	OR500088	OR500092	OR500090	OR501827	–
Dictyocheirospora bannica	KH 332 ^{T}	AB807513	LC014543	AB797223	AB808489	–
Didymella exigua	CBS 183.55 ^{T}	MH868977	MH857436	GU296147	–	–
Didymella rumicicola	CBS 683.79 ^{T}	MH873007	KT389503	–	–	KT389622
Dothidotthia robiniae	MFLUCC 16-1175 ^{T}	MK751817	MK751727	MK751762	MK908017	MK920237
Epicoccum duchesneae	CGMCC 3.18345 ^{T}	KY742249	KY742095	–	–	MT018115
Epicoccum duchesneae	CBS 218.81	MN973322	MN972935	–	–	MN983572
Falciformispora aquatica	MFLUCC 18-0212 ^{T}	MK063643	MK064216	–	MK099811	–
Falciformispora tompkinsii	CBS 200.79 ^{T}	MH872968	MH861199	KF015639	KF015685	KF015719
Fenestella crataegi	CBS 144857 ^{T}	–	NR_165534	–	MK357555	MK357512
Fissuroma calami	MFLUCC 13-0836 ^{T}	MF588993	–	NG_062430	MF588975	–
Flammeascoma lignicola	MFLUCC 10-0128 ^{T}	KT324583	KT324582	KT324584	KT324585	KT324586
Flavomyces fulophazii	CBS 135761 ^{T}	NG_058131	NR_137960	NG_061191	–	–
Foliophoma fallens	CBS 161.78	GU238074	KY940772	GU238215	–	KC584502
Fuscostagonospora cytisi	MFLUCC 16-0622 ^{T}	KY770978	–	KY770977	KY770979	–
Fuscostagonospora sasae	HHUF 29106 ^{T}	AB807548	AB809636	AB797258	AB808524	–
Fusculina eucalypti	CBS 120083 ^{T}	DQ923531	DQ923531	–	–	–
Fusculina eucalyptorum	CBS 145083 ^{T}	MK047499	NR_161140	–	–	–
Fusiformispora clematidis	MFLUCC 17-2077 ^{T}	MT214542	MT310589	MT226661	MT394725	MT394677
Gordonomyces mucovaginatus	CMW 22212 ^{T}	JN712552	JN712486	–	–	–
Halojulella avicenniae	BCC 20173 ^{T}	GU371822	–	GU371830	GU371815	GU371786
Helminthosporiella stilbacea	MFLUCC 15-0813 ^{T}	MT928157	MT928159	MT928161	MT928151	–
Hermatomyces amphisporus	CBS 146610 ^{T}	LR812664	LR812664	–	–	–
Hermatomyces amphisporus	CBS 146613	LR812662	LR812662	–	LR812657	LR812668
Hermatomyces amphisporus	CBS 146614	LR812666	LR812666	–	LR812660	LR812671
Hermatomyces anomianthi	MFLUCC 21-0202 ^{T}	OK655817	OL413437	–	OM117546	–
Hermatomyces bifurcatus	CCF 5900 ^{T}	LS398263	LS398263	–	LS398417	LS398344
Hermatomyces bifurcatus	CCF 5899	LS398262	LS398262	–	LS398416	LS398343
Hermatomyces clematidis	MFLUCC 17-2085 ^{T}	MT214556	MT310603	MT226673	MT394735	MT394684
Hermatomyces constrictus	CCF 5904 ^{T}	LS398264	LS398264	–	LS398418	LS398345
Hermatomyces hainanensis	GZCC 23-0592 ^{T}	OR091329	OR098708	–	–	–
Hermatomyces indicus (=*H. thailandicus*)	MFLUCC 14-1143 ^{T}	KU764692	KU144920	KU712468	–	KU712488
Hermatomyces indicus (=*H. thailandicus*)	MFLUCC 14-1144	KU764693	KU144921	KU712469	–	KU712489
Hermatomyces indicus (=*H. thailandicus*)	MFLUCC 14-1145	KU764694	KU144922	KU712470	KU872756	KU712490
Hermatomyces iriomotensis	KH 361	LC194367	LC194483	–	LC194394	LC194449
Hermatomyces jinghaensis	HKAS 112167 ^{T}	MW989519	MW989495	–	MZ042642	–
Hermatomyces krabiensis (=*H. chiangmaiensis*)	MFLUCC 16-0249 ^{T}	KX525742	KX525750	–	–	KX525754
Hermatomyces krabiensis (=*H. chiangmaiensis*)	MFLUCC 16-2819	KY559394	–	–	–	–
Hermatomyces maharashtraensis	NFCCI 4879 ^{T}	NG_241939	NR_189384	–	MZ130659	MZ130660
Hermatomyces megasporus	CCF 5898 ^{T}	LS398266	LS398266	–	LS398420	–
Hermatomyces megasporus	CCF 5897	–	LS398265	–	LS398419	LS398346
Hermatomyces nabanheensis	KUMCC 16-0149 ^{T}	KY766059	KY766058	KY766060	KY766061	–
Hermatomyces pyriformis	CGMCC 3.27462 ^{T}	PP491962	PP491964	–	PP505452	PP505454
Hermatomyces pyriformis	UESTCC 23.0441	PP491963	PP491965	–	PP505453	PP505455

Species	Isolate/strain number	LSU	ITS	SSU	*TEF1*	*RPB2*
Hermatomyces reticulatus (=*H. subiculosus*)	MFLUCC 15-0843 ^{T}	KX259523	KX259521	KX259525	KX259527	KX259529
Hermatomyces reticulatus (=*H. subiculosus*)	CCF 5893	LS398267	LS398267	–	LS398421	LS398347
Hermatomyces sphaericoides	CCF 5908 ^{T}	LS398273	LS398273	–	LS398427	LS398352
Hermatomyces sphaericoides	CCF 5895	LS398270	LS398270	–	LS398424	LS398350
Hermatomyces sphaericus	PMA 116080	LS398281	LS398281	–	LS398431	LS398356
Hermatomyces sphaericus	PMA 116081	LS398283	LS398283	–	LS398432	LS398357
Hermatomyces sphaericus	PRC 4105	–	LS398286	–	–	–
Hermatomyces sphaericus	PRC 4104	–	LS398278	–	LS398430	LS398355
Hermatomyces sphaericus	KZP 462	–	LS398287	–	LS398434	LS398359
Hermatomyces sphaericus	PRM 946201	–	LS398284	–	LS398433	LS398358
Hermatomyces sphaericus	PRC 4116	–	LS398275	–	–	–
Hermatomyces sphaericus	PRC 4100	LS398277	LS398277	–	LS398429	LS398354
Hermatomyces sphaericus	PRC 4106	LS398279	LS398279	–	–	–
Hermatomyces sphaericus	PMA 116085	–	LS398280	–	–	–
Hermatomyces sphaericus	PMA 116082	–	LS398285	–	–	–
Hermatomyces sphaericus	PRC 4117	–	LS398276	–	–	–
Hermatomyces sphaericus	MFLUCC 17-0373	OL782061	OL782144	OL780526	–	–
Hermatomyces sphaericus	HKAS 112725	MW989516	MW989492	–	MZ042639	MZ042636
Hermatomyces sphaericus	HKAS 112166	MW989517	MW989493	–	MZ042640	MZ042637
Hermatomyces sphaericus (=*H. biconisporus*)	KUMCC 17-0183	MH260296	MH275063	MH260338	MH412771	MH412755
Hermatomyces sphaericus (=*H. chromolaenae*)	MFLUCC 16-2818	KY559393	–	–	–	–
Hermatomyces sphaericus (=*H. pandanicola*)	MFLUCC 16-0251	KX525743	KX525751	KX525747	KX525759	KX525755
Hermatomyces sphaericus (=*H. saikhuensis*)	MFLUCC 16-0266	KX525740	KX525748	–	KX525756	KX525752
Hermatomyces sphaericus (=*H. saikhuensis*)	MFLUCC 16-0267	KX525741	KX525749	–	KX525757	KX525753
Hermatomyces sphaericus (=*H. tectonae*)	MFLUCC 14-1140	KU764695	–	NG_063603	KU872757	KU712486
Hermatomyces sphaericus (=*H. tectonae*)	MFLUCC 14-1141	KU764696	KU144918	KU712466	KU872758	–
Hermatomyces sphaericus (=*H. tectonae*)	MFLUCC 14-1142	KU764697	KU144919	KU712467	–	KU712487
Hermatomyces trangensis	BCC 80741 ^{T}	KY790600	KY790598	KY790602	KY790606	KY790604
Hermatomyces trangensis	BCC 80742	KY790601	KY790599	KY790603	KY790607	KY790605
Hermatomyces tucumanensis	CCF 5912	LS398288	LS398288	–	LS398435	LS398360
Hermatomyces tucumanensis	CCF 5915	LS398290	LS398290	–	LS398437	LS398362
Hermatomyces tucumanensis	CCF 5913	LS398289	LS398289	–	LS398436	LS398361
Hermatomyces turbinatus	MFLUCC 21 0038 ^{T}	MW989518	MW989494	–	MZ042641	MZ042638
Hermatomyces verrucosus	CCF 5903 ^{T}	LS398292	LS398292	–	LS398439	LS398364
Hermatomyces verrucosus	CCF 5892	LS398291	LS398291	–	LS398438	LS398363
Hongkongmyces aquaticus	MFLUCC 18-1150 ^{T}	MN913694	–	MT864302	MT954379	–
Hypsostroma caimitalense	GKM1165 ^{T}	GU385180	–	–	–	–
Hypsostroma thailandicum	MFLUCC 21-0057 ^{T}	MZ435867	MZ435865	–	–	–
Jeremyomyces labinae	CBS 144617 ^{T}	MK442529	MK442589	–	MK442695	MK442665
Juncaceicola alpina	CBS 456.84 ^{T}	MH873460	MH861761	KY090699	KF253139	KF252188
Keissleriella camporesiana	MFLUCC 15-0029 ^{T}	MN401741	MN401745	MN401743	MN397907	–
Latorua caligans	CBS 576.65 ^{T}	NG_058180	–	–	–	–
Latorua grootfonteinensis	CBS 369.72 ^{T}	NG_058181	–	–	–	–
Lentimurispora urniformis	MFLUCC 18-0497	MH179144	–	MH179160	MH188055	–
Lentithecium clioninum	HHUF 28199 ^{T}	NG_059391	NR_154137	NG_064845	AB808515	–
Lentithecium pseudoclioninum	HHUF 29055 ^{T}	NG_059392	AB809633	NG_064847	AB808521	–
Leptosphaeria chatkalica	YGS22 ^{T}	MW886099	MW886101	MW886100	MW915583	–
Leptosphaerioides guizhouensis	GZAAS 19-4017 ^{T}	OP099529	OR225067	OR134435	–	–
Leptosphaerioides guizhouensis	GZAAS 19-4018	OP099530	OR225068	OR134436	–	–
Leucaenicola phraeana	MFLUCC 18-0472 ^{T}	MK348003	MK347785	NG_065784	MK360060	MK434867
Leucaenicola aseptata	MFLUCC 17-2423 ^{T}	NG_066309	NR_163332	NG_065776	MK360059	MK434891
Libertasomyces myopori	CPC 27354 ^{T}	NG_058241	KX228281	–	–	–
Ligninsphaeria jonesii	MFLUCC 15-0641 ^{T}	KU221037	–	–	–	–
Ligninsphaeria jonesii	GZCC 15-0080	KU221038	–	–	–	–
Lindgomyces cigarospora	G619 ^{T}	KX655804	KX655794	KX655805	–	–
Lindgomyces ingoldianus	ATCC 200398 ^{T}	AB521736	NR_119938	NG_016531	–	–
Longicorpus striataspora	MFLUCC 18-0267 ^{T}	MK035988	MK035965	MK035973	MK034428	MK034436
Longiostiolum tectonae	MFLUCC 12-0562 ^{T}	KU764700	KU712447	KU712459	–	–
Longipedicellata aptrootii	MFLU 10-0297 ^{T}	KU238894	KU238893	KU238895	KU238892	KU238891
Longipedicellata megafusiformis	SJ-KR4 ^{T}	MZ538546	MZ538512	–	MZ567090	–
Lonicericola qujingensis	GMBCC1178 ^{T}	OM855602	OM855593	OM855616	OM857556	–
Lophiostoma carpini	CBS 147279 ^{T}	MW750386	NR_173000	–	MW752405	MW752384

Species	Isolate/strain number	LSU	ITS	SSU	*TEF1*	*RPB2*
Lophiostoma clavatum	MFLUCC 18-1316 ^{T}	MN274566	–	MN304835	MN328901	–
Lophiotrema eburnoides	MAFF 242970 ^{T}	LC001707	LC001709	LC001706	LC194403	LC194458
Magnibotryascoma kunmingense	HKAS 111919 ^{T}	MW424785	MW424770	MW424800	MW430106	MW430113
Magnibotryascoma rubriostiolatum	CBS 140734 ^{T}	–	KU601590	–	KU601609	KU601599
Magnicamarosporium iriomotense	HHUF 30125 ^{T}	AB807509	AB809640	AB797219	AB808485	–
Massaria inquinans	CBS 125591 ^{T}	MH875187	MH863726	HQ599442	HQ599340	–
Massarina pandanicola	MFLUCC 17-0596 ^{T}	MG646947	MG646958	MG646979	MG646986	–
Massarioramusculicola chiangraiensis	MFLUCC 17-2240 ^{T}	MH040228	MH040227	MH040229	–	–
Massariosphaeria clematidis	MFLU 16-0174 ^{T}	MT214544	MT310591	MT226663	–	–
Matsushimamyces bohaniensis	CBS 140592 ^{T}	KR350633	KP765516	–	–	–
Misturatosphaeria aurantonotata	GKM 1238 ^{T}	NG_059927	–	–	GU327761	–
Montagnula acaciae	MFLUCC 18-1636 ^{T}	ON117298	ON117280	ON117267	ON158093	–
Montagnula aquatica	MFLU 22-0171 ^{T}	OP605986	OP605992	OP600504	–	–
Montagnula aquatica	KUNCC 23-14425	OR583116	OR583097	OR583135	OR588088	OR588107
Morosphaeria muthupetensis	NFCC I4219 ^{T}	MF614796	MF614795	MF614797	MF614798	–
Multilocularia bambusae	MFLUCC 11-0180 ^{T}	KU693438	KU693446	KU693442	–	–
Multiseptospora thailandica	MFLUCC 11-0183 ^{T}	KP744490	KP744447	KP753955	KU705657	–
Murilentithecium lonicerae	MFLUCC 18-0675 ^{T}	MK214373	MK214370	MK214376	MK214379	–
Murispora galii	MFLUCC 13-0819 ^{T}	KT709175	KT736081	KT709182	KT709189	–
Muritestudina chiangraiensis	MFLUCC 17-2551 ^{T}	MG602248	MG602247	MG602249	MG602251	MG602250
Neoastrosphaeriella phoenicis	MFLUCC 18-1477 ^{T}	MN712339	MN735995	MN699324	MN744232	MN744233
Neobambusicola magnoliae	HKAS 107122 ^{T}	ON870389	ON878076	ON870914	–	–
Neocamarosporium goegapense	CPC 23676 ^{T}	KJ869220	KJ869163	–	–	–
Neocamarosporium halophilum	RSM70 ^{T}	–	OR242722	–	OR289930	–
Neocamarosporium halophilum	BBB RMS57	OR297952	OR297950	–	OR339881	–
Neohelicascus aquaticus	KUMCC 19-0107 ^{T}	MT627662	MT627719	MT864314	MT954384	–
Neolophiostoma pigmentatum	MFLUCC 10-0129 ^{T}	KT324588	KT324587	KT324589	KT324590	–
Neomassaria fabacearum	MFLUCC 16-1875 ^{T}	KX524145	–	NG_061245	KX524149	–
Neomassaria formosana	NTUCC 17-007 ^{T}	MH714756	–	MH714759	MH714762	MH714765
Neomassaria hongheensis	KUMCC 21-0344 ^{T}	OL423113	OL477614	OL423115	OL754594	OL754595
Neomassarina chromolaenae	MFLUCC 17-1480 ^{T}	MT214466	MT214372	MT214419	MT235785	MT235822
Neomassarina pandanicola	MFLUCC 16-0270 ^{T}	MG298946	MG298945	MG298947	–	–
Neomassarina thailandica	MFLU 11-0144 ^{T}	NG_059718	NR_154244	–	–	–
Neomassarina thailandica	MFLUCC 17-1432	MT214467	MT214373	MT214420	–	–
Neooccultibambusa thailandensis	MFLUCC 16-0274 ^{T}	MH260308	MH275074	MH260348	MH412780	MH412758
Neophaeosphaeria phragmiticola	KUMCC 16-0216 ^{T}	MG837009	–	NG_065735	MG838020	–
Neophaeosphaeria livistonae	NCYUCC 19-0393 ^{T}	OQ437387	OQ437390	OQ437393	–	–
Neoplatysporoides aloes	CPC 36068 ^{T}	MN567619	NR_166316	–	–	–
Neopyrenochaeta annellidica	MFLU 11-1105 ^{T}	MT183502	MT185538	–	–	–
Neopyrenochaeta cercidis	MFLUCC 18-2089 ^{T}	MK347932	MK347718	MK347823	–	MK434908
Neopyrenochaeta chiangraiensis	MFLUCC 13-0881 ^{T}	MT183503	MT185539	MT214975	MT454041	–
Neopyrenochaetopsis hominis	UTHSC: DI16-238 ^{T}	LN907381	LT592923	–	–	LT593061
Neoroussoella chiangmaiensis	MFLU 22-0205 ^{T}	OQ065735	OQ065738	OQ065736	OQ186448	OQ186450
Neothyrostroma encephalarti	CPC 35999 ^{T}	MN567613	MN562105	–	MN556831	–
Neotorula aquatica	MFLUCC 15-0342 ^{T}	KU500576	KU500569	KU500583	–	–
Neotorula submersa	KUMCC 15-0280 ^{T}	KX789217	KX789214	–	–	–
Nigrograna italica	MFLU 23-0139 ^{T}	OR538591	OR538590	–	OR531366	OR531365
Occultibambusa bambusae	MFLUCC 13-0855 ^{T}	KU863112	KU940123	–	KU940193	KU940170
Occultibambusa jonesii	GZCC 16-0117 ^{T}	KY628322	–	KY628324	KY814756	KY814758
Ochraceocephala foeniculi	CBS 145654 ^{T}	MN516774	MN516753	MN516743	MN520149	MN520145
Ohleria modesta	CBS 141480	–	KX650563	KX650513	KX650534	KX650583
Ohleria modesta	WU 36870	–	KX650562	–	KX650533	KX650582
Omania hydei	SQUCC 13750 ^{T}	MW077155	MW077146	MW077162	MW075772	MW276077
Paraconiothyrium kelleni	CBS 149290 ^{T}	NG_229027	NR_185757	OP348926	OP328919	–
Paradictyoarthrinium aquatica	MFLUCC 16-1116 ^{T}	NG_064501	NR_158861	–	–	–
Paradictyoarthrinium diffractum	MFLUCC 13-0466	KP744498	KP744455	KP753960	–	KX437764
Paradictyocheirospora tectonae	AMH 10301 ^{T}	MW854647	MW854646	–	MW854832	–
Paralophiostoma hysterioides	PUFNI 17617	MT912850	MN582758	MN582762	–	MT926117
Paraleptosphaeria polylepidis	MA 57843 ^{T}	–	NR_119469	–	–	–
Paraleptosphaeria polylepidis	APA-2999	MK795717	MK795714	MK795720	MK831009	–
Paramonodictys globosa	HKAS 129169 ^{T}	OR091331	OR139016	–	OR494045	OR494048
Parapyrenochaeta protearum	CBS 131315	JQ044453	JQ044434	–	–	LT717683

Species	Isolate/strain number	LSU	ITS	SSU	*TEF1*	*RPB2*
Periconia delonicis	MFLUCC 17-2584 ^{T}	NG_068611	–	NG_065770	–	MK434901
Periconia pseudodigitata	KT 1395 ^{T}	AB807564	LC014591	AB797274	–	–
Phaeomycocentrospora xinjangensis	CGMCC 3.20479 ^{T}	OK256190	OK256193	–	–	–
Phaeoseptum mali	MFLUCC 17-2108 ^{T}	MK625197	MK659580	–	MK647990	MK647991
Phaeoseptum terricola	MFLUCC 10-0102 ^{T}	MH105779	MH105778	MH105780	MH105781	MH105782
Phaeosphaeria oryzae	CBS 110110 ^{T}	KF251689	KF251186	GQ387530	–	KF252193
Pleomonodictys capensis	CBS 968.97 ^{T}	KY853521	MH862684	–	–	–
Pleomonodictys capensis	DLUCC 1323	MZ420757	MZ420742	–	–	MZ442696
Pleomonodictys descalsii	FMR 12716 ^{T}	KY853522	KY853461	–	–	–
Plenodomus changchunensis	CCMJ 5011 ^{T}	OL897174	OL996123	OL984031	–	–
Plenodomus changchunensis	CCMJ 5012	OL966928	OL996124	OL984032	–	OL944508
Pleopunctum clematidis	MFLUCC 17-2091 ^{T}	MT214573	MT310618	–	MT394632	MT394693
Pleopunctum thailandicum	MFLUCC 21-0039 ^{T}	MZ198896	MZ198894	–	MZ172461	–
Polyschema sclerotigenum	UTHSC DI14-305 ^{T}	KP769976	KP769975	–	–	–
Prosthemium alni	MFLUCC 17-0240 ^{T}	KY815013	KY797636	–	KY815019	–
Prosthemium intermedium	HHUF 30063 ^{T}	AB553778	AB554108	–	–	–
Pseudoasteromassaria aquatica	MFLUCC 18-1397 ^{T}	MN913721	MT627674	MT864322	MT954378	–
Pseudoastrosphaeriella longicolla	MFLUCC 11-0171 ^{T}	KT955476	–	–	KT955438	KT955420
Pseudoastrosphaeriella thailandensis	MFLUCC 11-0144 ^{T}	KT955478	–	KT955457	KT955440	KT955416
Pseudoberkleasmium acaciae	MFLUCC 17-2590 ^{T}	NG_066316	NR_163343	NG_065782	MK360073	–
Pseudoberkleasmium chiangmaiense	MFLUCC 17-1809 ^{T}	MK131260	MK131259	–	MK131261	–
Pseudoberkleasmium chiangmaiense	MFLU 21-0290	OM065940	OM066271	OM065948	OM102996	OM102997
Pseudoberkleasmium chiangmaiense	MFLUCC 17-2088	MT214585	MT310630	MT226698	MT394643	MT394699
Pseudoberkleasmium chiangmaiense	DLUCC 1655	MZ420759	MZ420744	MZ420749	MZ442693	–
Pseudoberkleasmium chiangraiense	MFLUCC 21-0154 ^{T}	OL584200	OL584189	OL606408	OL912943	OL697401
Pseudoberkleasmium chiangraiense	MFLUCC 21-0161	OL584201	OL584190	OL606409	OL912944	OL697402
Pseudoberkleasmium chiangraiense	MFLUCC 21-0162	OL584205	OL584191	OL606410	OL912945	OL697403
Pseudoberkleasmium pandanicola	KUMCC 17-0178 ^{T}	MH260304	MH275071	MH260344	–	–
Pseudochaetosphaeronema chiangraiense	MFLU 21-0083 ^{T}	MZ457922	MZ457923	–	MZ476770	–
Pseudochaetosphaeronema chiangraiense	UESTCC 23.0065	OR253260	OR253108	–	OR251160	–
Pseudochaetosphaeronema irregulare	CGMCC 3.22458 ^{T}	OQ758163	OQ798972	OQ758194	OQ809057	OQ809023
Pseudochaetosphaeronema irregulare	CGMCC 3.22461	OQ758162	OQ798971	OQ758193	OQ809056	OQ809022
Pseudocoleodictyospora sukhothaiensis	MFLUCC 12-0554 ^{T}	KU764710	KU712440	NG_062416	–	KU712493
Pseudocoleodictyospora thailandica	MFLUCC 12-0565 ^{T}	KU764701	NR_154337	NG_062417	–	KU712494
Pseudocoleophoma heteropanacicola	ZHKUCC 23-0880 ^{T}	OR365486	OR365456	–	OR700204	–
Pseudolophiotrema elymicola	HHUF 28984 ^{T}	LC194381	LC194505	LC194339	LC194418	LC194473
Pseudomassarina clematidis	MFLU 16-0493 ^{T}	MT214586	MT415397	MT226699	MT394644	MT394700
Pseudopyrenochaeta lycopersici	FMR 15746 ^{T}	EU754205	NR_103581	NG_062728	–	LT717680
Pseudopyrenochaeta terretris	FMR 15327 ^{T}	LT623216	LT623228	–	–	LT623287
Pseudotetraploa rajmachiensis	NFCCI 4618 ^{T}	MN937204	MN937222	–	–	–
Pseudoxylomyces elegans	KT 2887	AB807598	LC014593	AB797308	AB808576	–
Profundisphaeria fusiformispora	GZAAS 20-4010 ^{T}	–	–	OR134442	OR140432	OR146942
Profundisphaeria fusiformispora	GZAAS 20-4012	OR209667	–	OR134443	OR140433	–
Pyrenochaetopsis leptospora	CBS 101635 ^{T}	GQ387627	JF740262	NG_063097	MF795881	LT623282
Pyrenochaetopsis tabarestanensis	IBRC:M 30051 ^{T}	KF803343	NR_155636	NG_065034	–	–
Quadricrura bicornis	HHUF 30023 ^{T}	AB524613	AB524797	AB524472	AB524828	–
Quercicola fusiformis	MFLUCC 18-0479 ^{T}	MK348009	MK347790	MK347898	MK360085	MK434864
Quercicola guttulospora	MFLUCC 18-0481 ^{T}	MK348010	MK347791	MK347899	MK360086	–
Quixadomyces cearensis	HUEFS 238438 ^{T}	MG970695	NR_160606	–	–	–
Roussoella bambusarum	GMBC 0316 ^{T}	ON479892	ON479891	–	ON505017	ON505012
Roussoella guttulata	MFLUCC 20-0102 ^{T}	MT734818	MT734821	–	MW022188	MW022187
Rubroshiraia bambusae	HKAS 102255 ^{T}	MK804658	MK804678	MK804704	MK819218	–
Rubroshiraia bambusae	HKAS 102256	MK804659	MK804679	MK804705	MK819219	–
Salsuginea phoenicis	MFLU 19-0015 ^{T}	MK405280	–	–	MK404650	–
Salsuginea ramicola	KT 2597.2	GU479801	–	GU479768	GU479862	GU479834
Salsuginea rhizophorae	MFLU 18-0540 ^{T}	MN017851	–	MN017917	–	–
Seltsamia ulmi	CBS 143002 ^{T}	MF795794	MF795794	MF795794	MF795882	MF795836
Septoriella chlamydospora	MFLUCC 15-0177 ^{T}	KU163654	KU163658	KU163655	–	–
Septoriella hibernica	CBS 145371 ^{T}	MK540036	MK539966	–	–	MK540097
Setoarthopyrenia chromolaenae	MFLUCC 17-1444 ^{T}	MT214438	MT214344	MT214392	MT235768	MT235805
Shiraia bambusicola	GZAAS 2.0708 ^{T}	KC460982	GQ845414	–	–	–
Shiraia bambusicola	HKAS 102267	MK804657	MK804677	MK804703	MK819217	MK819237

Species	Isolate/strain number	LSU	ITS	SSU	*TEF1*	*RPB2*
Sporormurispora atraphaxidis	MFLUCC 17-0742 ^{T}	MG829083	MG828971	MG829183	–	–
Sporormurispora paulsenii	MFLUCC 17-1957 ^{T}	MK966143	–	MK963075	–	MN023029
Stagonospora forlicesenensis	MFLUCC 15-0054 ^{T}	KX655547	KX655557	KX655552	KX655562	–
Stagonospora imperaticola	MFLUCC 15-0026	KY706133	KY706143	KY706138	KY706146	KY706149
Stemphylium clematidis	MFLUCC 14-0937 ^{T}	MT214583	MT310628	MT226696	–	–
Stemphylium carpobroti	CPC 38637 ^{T}	MW175395	MW175355	–	–	–
Striatiguttula nypae	MFLUCC 18-0265 ^{T}	MK035992	MK035969	MK035977	MK034432	MK034440
Striatiguttula phoenicis	MFLUCC 18-0266 ^{T}	MK035995	MK035972	MK035980	MK034435	MK034442
Subglobosporium tectonae	MFLUCC 12-0393 ^{T}	KU764703	KU712445	KU712464	–	KU712485
Subglobosporium tectonae	MFLUCC 12-0390	KU764702	KU712446	KU712463	–	KU712495
Sublophiostoma thailandica	MFLUCC 11-0207 ^{T}	KX534212	MW136257	KX534218	KX550077	MW088714
Sublophiostoma thailandica	MFLUCC 11-0185	KX534216	MW136275	KX534222	KX550080	MW088718
Submersispora variabilis	MFLUCC 17-2360 ^{T}	MN913682	MT627683	MT864310	–	–
Submersispora variabilis	N-KR15	MZ538561	MZ538527	MZ538575	MZ567103	MZ567114
Sulcatispora acerina	HHUF 30449 ^{T}	LC014610	LC014597	LC014605	LC014615	–
Sulcatispora berchemiae	HHUF 29097 ^{T}	AB807534	AB809635	AB797244	AB808509	–
Sulcosporium thailandica	MFLUCC 12-0004	KT426563	MG520958	KT426564	–	–
Teichospora trabicola	C134 ^{T}	KU601591	KU601591	–	KU601601	KU601600
Tetraploa aquatica	MFLU 19-0995 ^{T}	MT530452	MT530448	–	–	–
Tetraploa aquatica	MFLU 19-0996	MT530453	MT530449	MT530454	–	–
Thyridaria acaciae	CBS 138873 ^{T}	NG_058127	KP004469	–	–	–
Thyridaria broussonetiae	CBS 141481 ^{T}	KX650568	KX650568	KX650515	KX650539	KX650586
Thyrostroma jaczewskii	MFLUCC 18-0787 ^{T}	MK765857	MK765856	MK765858	–	–
Torula camporesii	KUMCC 19-0112 ^{T}	MN507402	MN507400	MN507401	MN507403	MN507404
Torula pluriseptata	MFLUCC 14-0437 ^{T}	KY197855	MN061338	KY197862	KY197875	KY197869
Trematosphaeria grisea	CBS 332.50 ^{T}	NG_057979	NR_132039	NG_062930	KF015698	KF015720
Trematosphaeria pertusa	CBS 122368 ^{T}	NG_057809	NR_132040	FJ201991	KF015701	FJ795476
Tubeufia abundata (Tubeufiales)	MFLUCC 17-2024 ^{T}	MH558894	MH558769	–	MH550961	MH551095
Tubeufia aquatica (Tubeufiales)	MFLUCC 16-1249 ^{T}	KY320539	KY320522	–	KY320556	MH551142
Tubeufia hainanensis (Tubeufiales)	GZCC 22-2015 ^{T}	OR030835	OR030842	–	OR046679	OR046685
Tubeufia hainanensis (Tubeufiales)	GZCC 23-0589	OR066421	OR066414	–	OR058860	OR058853
Tzeanania taiwanensis	NTUCC 17-005 ^{T}	MH461120	MH461123	MH461126	MH461130	MH461128
Tzeanania taiwanensis	NTUCC 17-006	MH461121	MH461124	MH461127	MH461131	MH461129
Wicklowia aquatica	CBS 125634 ^{T}	MH875044	OM322822	GU266232	–	GU371813
Wicklowia fusiformispora	N-KR1 ^{T}	MZ538567	MZ538533	MZ538576	MZ567108	–
Xenomassariosphaeria clematidis	MFLUCC 14-0923 ^{T}	MT214571	MT310616	–	MT394630	–
Xenomassariosphaeria rosae	MFLUCC 15-0179 ^{T}	MG829092	–	MG829192	–	–
Xenopyrenochaetopsis pratorum	CBS 445.81 ^{T}	GU238136	MH861363	NG_062792	–	KT389671
Xiuguozhangia broussonetiae	**MFLUCC 24-0258 ^{T}**	**PQ137419**	**PQ137417**	**PQ137421**	**PQ488461**	**PQ488459**
Xiuguozhangia broussonetiae	**MFLUCC 24-0259**	**PQ137420**	**PQ137418**	**PQ137422**	–	**PQ488460**
Zopfia rosatii	CBS 427.62 ^{T}	NG_066246	NR_160090	–	–	–

– Data unavailable.

Results

Phylogenetic analyses

Blast searches of LSU, ITS, SSU, *TEF1* and *RPB2* sequences indicated that the two isolates are highly similar to various genera in Pleosporales, including *Atrocalyx*, *Hermatomyces*, *Lophiotrema* and *Pseudoberkleasmium*.

Dataset 1 consisted of 4250 characters (LSU = 1–846, ITS = 847–1355, SSU = 1356–2359, *TEF1* = 2360–3257, and *RPB2* = 3258–4250), which was analysed to depict relationships at a higher taxonomic level for *Xiuguozhangia* (Fig. 1). Outgroup taxa were selected from Tubeufiales. The log-likelihood of the consensus tree (Fig. 1) was -143487.839. The average standard deviation of split frequencies at the end of the total MCMC generations converged to 0.0092.

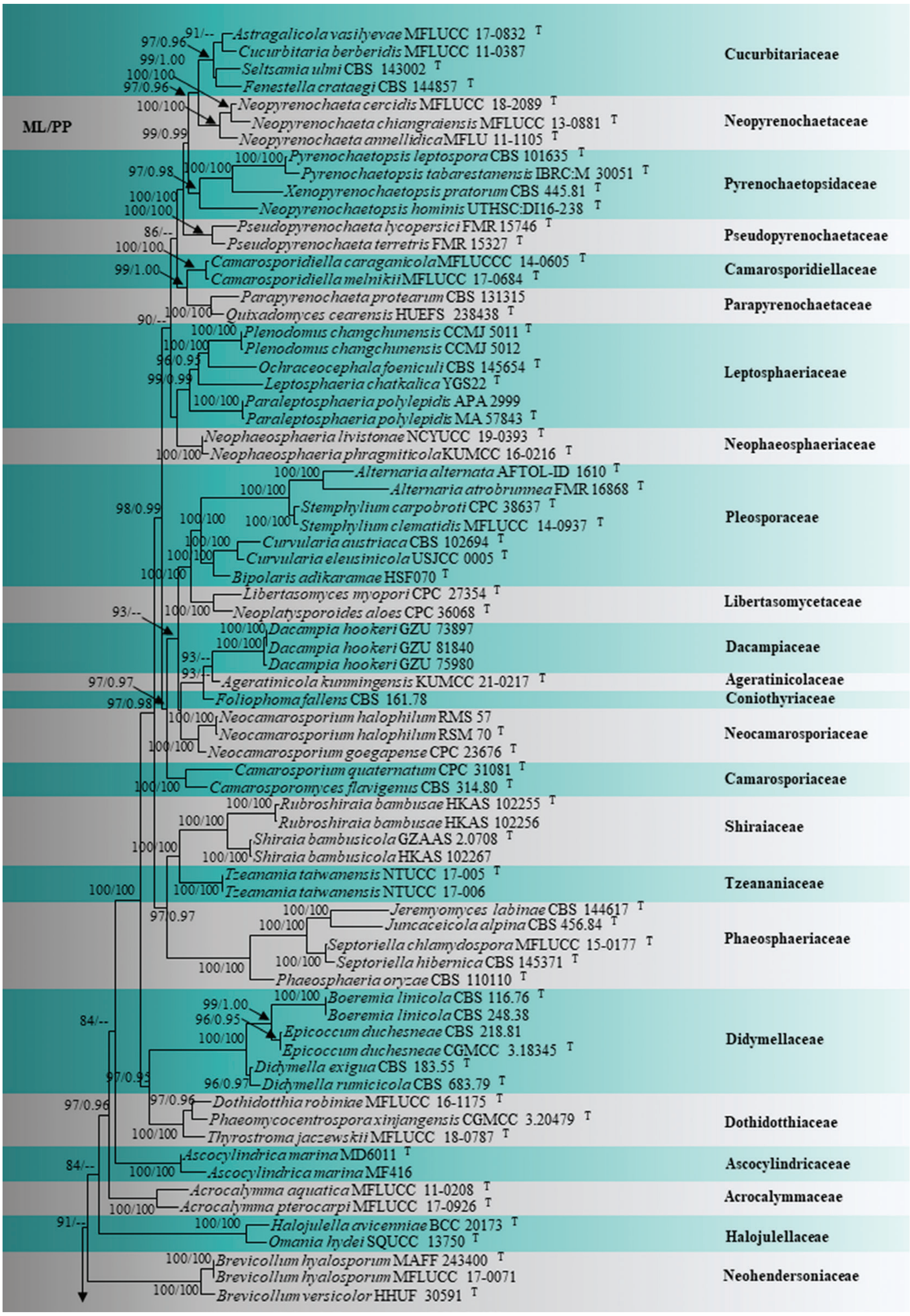

Figure 1. Maximum likelihood analysis (IQ-tree) based on a combined dataset of LSU, ITS, SSU, *TEF1*, and *RPB2* sequences of all families (with representative genera) of Pleosporales. Bootstrap support values (ML ≥ 80%) and Bayesian posterior probabilities (PP ≥ 0.95) are given above the branches or near the nodes as ML/PP. Hyphens (--) indicate bootstrap support values below 80% for ML and posterior probabilities below 0.95. The tree is rooted with *Tubeufia abundata* (MFLUCC 17-2024), *T. aquatica* (MFLUCC 16-1249), *T. hainanensis* (GZCC 22-2015 and GZCC 23-0589), *Berkleasmium aquaticum* (MFLUCC 17-0049 and MFLUCC 17-0039) and *B. longisporum* (MFLUCC 17-1999 and MFLUCC 17-2002) (Tubeufiales). Type, ex-type, and reference strains are denoted with ᵀ. The new isolates are in bold font. The different colour blocks indicate the families to which the taxa belong.

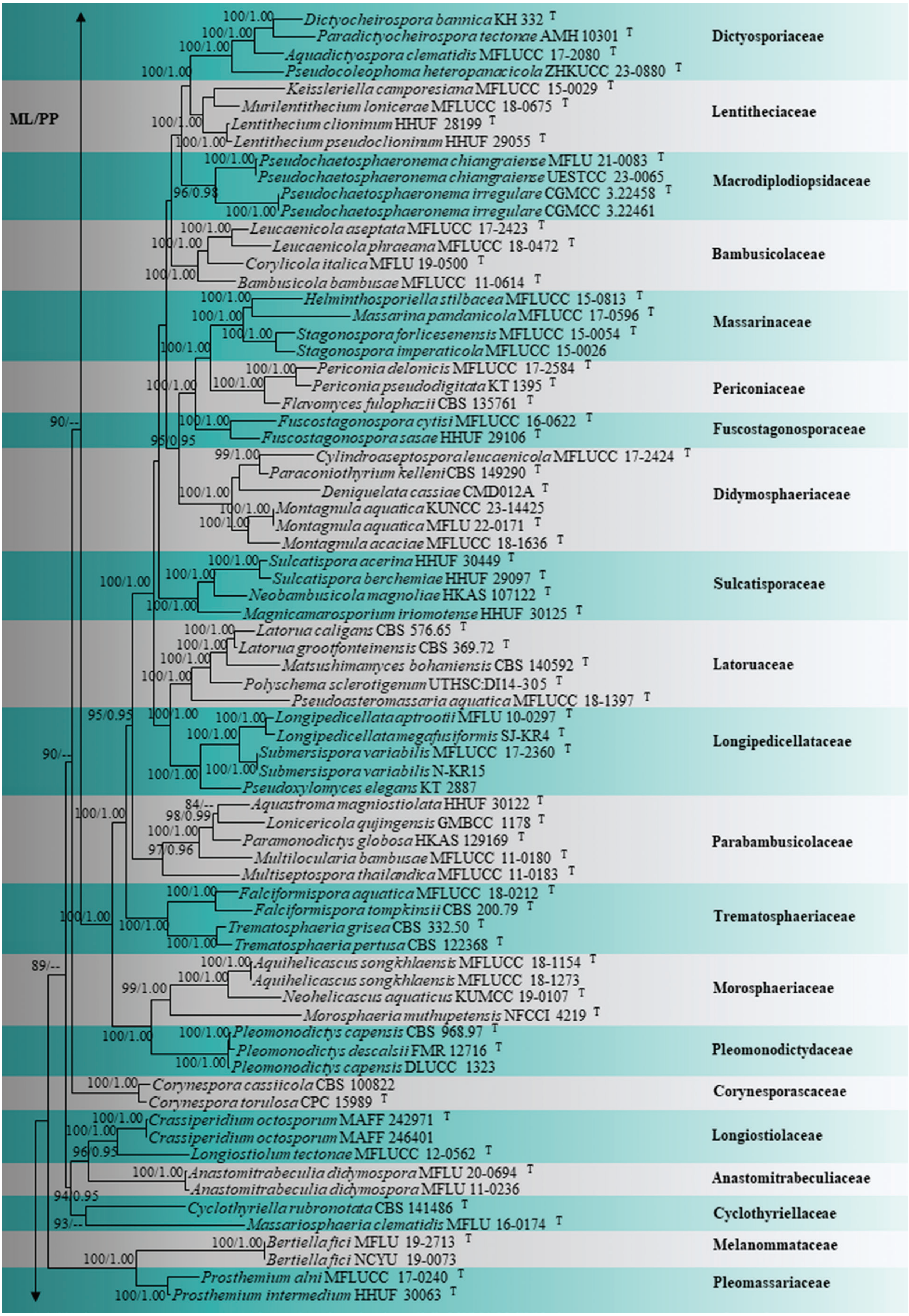

Figure 1. Continued.

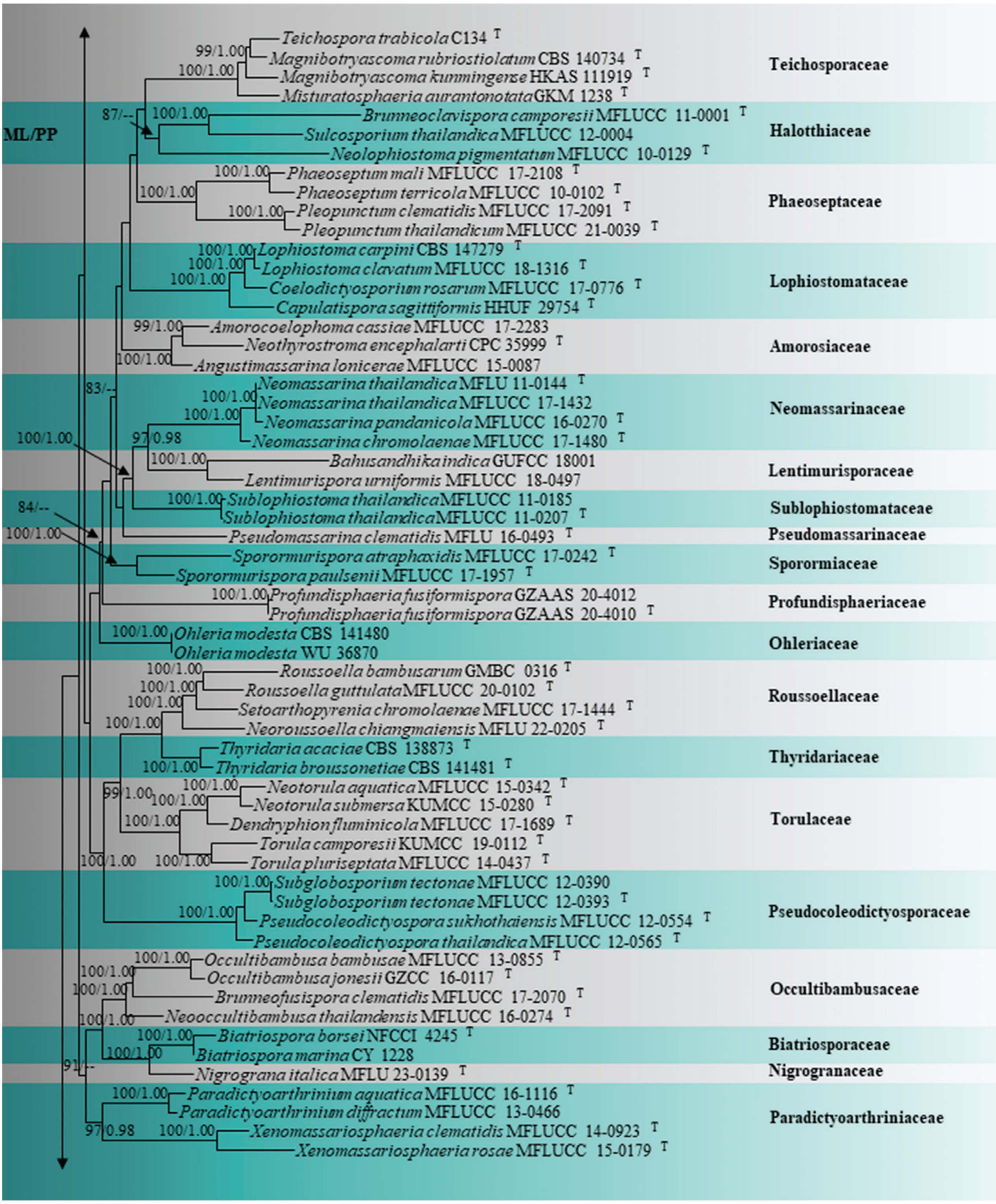

Figure 1. Continued.

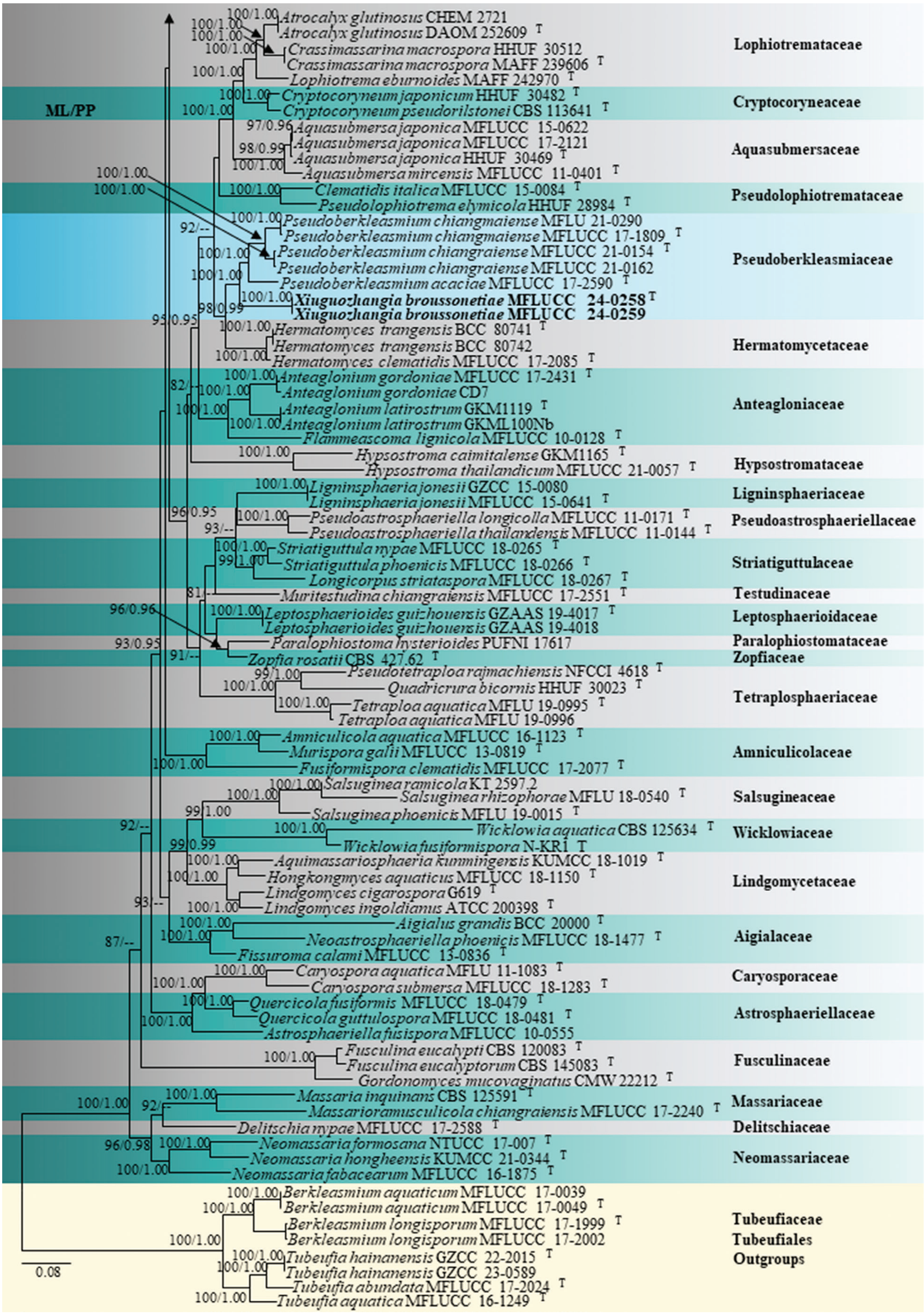

Figure 1. Continued.

Dataset 2 comprised 4285 characters (LSU = 1–833, ITS = 834–1344, SSU = 1345–2350, *TEF1* = 2351–3272, and *RPB2* = 3273–4285), and was used to infer the inter-generic relationships of *Xiuguozhangia*, which comprised multiple strains for each genus (Fig. 2). Based on the results from dataset 1, four

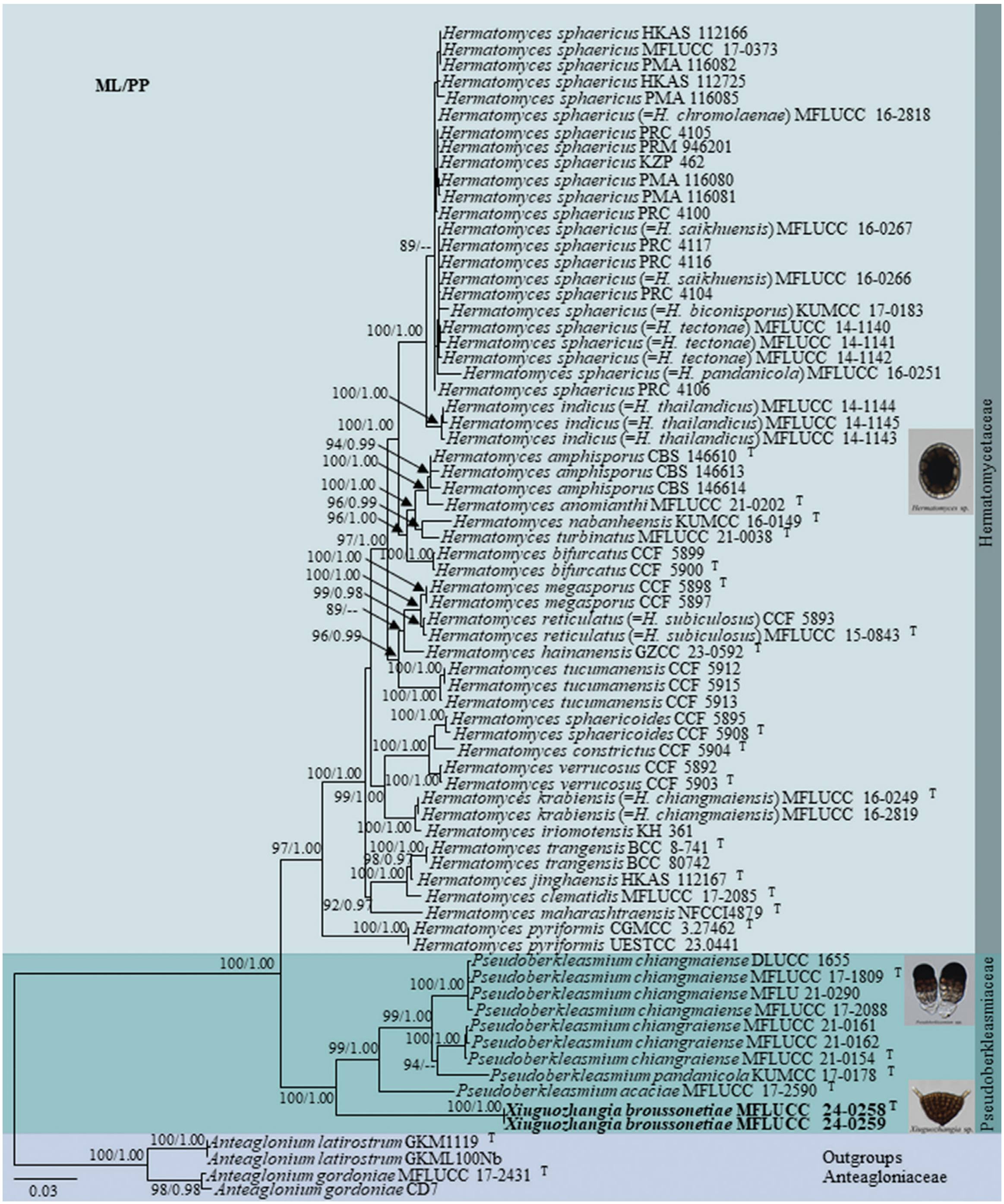

Figure 2. Maximum likelihood analysis (IQ-tree) based on the combined LSU, ITS, SSU, *TEF1* and *RPB2* sequences of *Xiuguozhangia*, *Pseudoberkleasmium* and *Hermatomyces*, generated from dataset 2. Bootstrap support values (ML ≥ 80%) and Bayesian posterior probabilities (PP ≥ 0.95) are given above the branches or near the nodes as ML/PP. Hyphens (--) indicate bootstrap support values below 80% for ML and posterior probabilities below 0.95. The tree is rooted with *Anteaglonium gordoniae* (MFLUCC 17-2431 and CD7) and *A. latirostrum* (GKM1119 and GKML100Nb) (Anteagloniaceae, Pleosporales). Type, ex-type, and reference strains are denoted with ^{T}. The new isolates are in bold font. The different colour blocks indicate the families to which the taxa belong.

taxa belonging to Anteagloniaceae (Pleosporales) were selected as outgroups as they are phylogenetically closely related to Pseudoberkleasmiaceae and Hermatomycetaceae. The log-likelihood of the consensus tree (Fig. 2) was -17829.366. The average standard deviation of split frequencies at the end of the total MCMC generations converged to 0.0031.

The two strains of *Xiuguozhangia broussonetiae* (MFLUCC 24-0258 and MFLUCC 24-0259) grouped with 100% ML and 1.00 PP support. This subclade formed a separate sister lineage to *Pseudoberkleasmium* with 100% ML and 1.00 PP support (Figs 1, 2). Single and combined gene trees from datasets 1 and 2 indicate that our species is phylogenetically most closely related to *Pseudoberkleasmium*, and its placement in Pseudoberkleasmiaceae is supported by maximum bootstrap support and posterior probability in both combined data trees.

Genetic distances

Based on the generic relationship depicted in Figs 1, 2, we computed the group mean distances between *Hermatomyces* spp. (group 1), *Pseudoberkleasmium* spp. (group 2) and *Xiuguozhangia* spp. (group 3) across ITS and LSU markers. The difference in the genetic distances across both markers is given in Table 2.

Table 2. Group mean genetic distances (%) in *Hermatomyces* spp., *Pseudoberkleasmium* spp., and *Xiuguozhangia* spp. across ITS (511 bp) and LSU (833 bp).

DNA markers	Groups	Group 1: *Hermatomyces* spp.	Group 2: *Pseudoberkleasmium* spp.
ITS	**Group 1:** *Hermatomyces* spp.	0	–
	Group 2: *Pseudoberkleasmium* spp.	9.052	0
	Group 3: *Xiuguozhangia* spp.	10.62834205	13.25008949
LSU	**Group 1:** *Hermatomyces* spp.	0	–
	Group 2: *Pseudoberkleasmium* spp.	3.1615	0
	Group 3: *Xiuguozhangia* spp.	4.1404	3.8669

Taxonomy

***Xiuguozhangia broussonetiae* Gomdola, Jayaward. & K.D. Hyde, sp. nov.**
Index Fungorum: IF901943
Facesoffungi Number: FoF16323
Fig. 3

Holotype. MFLU 24-0227.

Etymology. The specific epithet refers to the host genus, *Broussonetia*, from which the species was isolated.

Description. Saprobic on decaying stems of *Broussonetia papyrifera*. Sexual morph not observed. Asexual morph on substrate. Hyphomycetous. ***Colonies*** on the substrate effuse, hairy, olivaceous to dark brown, appearing velvety due to numerous conidiophores. ***Mycelium*** semi-immersed or immersed, composed of septate, branched, smooth, hyaline or pale brown to brown hyphae. ***Conidiophores*** (430–)550–750(–890) µm long ($\bar{x}$ = 681 µm, n = 10), 15–24(–34) µm wide ($\bar{x}$ = 21.8 µm, n = 10) at the base, 11–14(–16) µm wide ($\bar{x}$ = 12.3 µm, n = 10) in the middle, 7–10 µm(–12) wide ($\bar{x}$ = 7.8 µm, n = 10) at the apex, rarely branched, macronematous, mononematous, differentiated, smooth, thick-

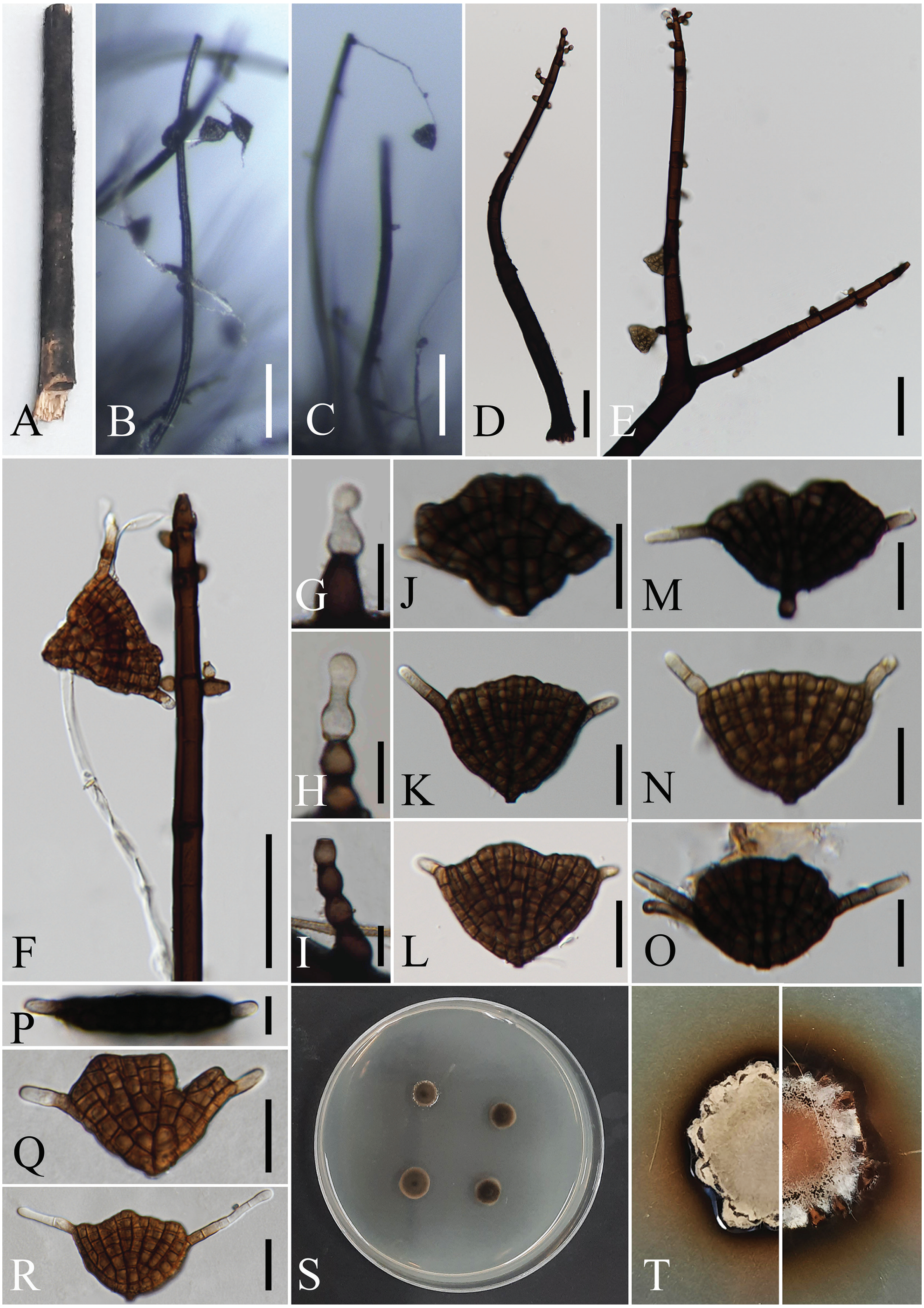

Figure 3. *Xiuguozhangia broussonetiae* (MFLUCC 24-0258) **A** substrate (*Broussonetia papyrifera*) **B, C** colonies on the substrate **D–F** conidiophores and attachment of conidia **G–I** conidiogenous cells with percurrent proliferations **J–O, Q, R** conidia bearing appendages **P** top view of a conidium **S** top view of colonies on PDA after 7 days **T** top and reverse colonies on PDA after 28 days. Scale bars: 100 µm (**B, C**); 50 µm (**D–F**); 10 µm (**G–I, P**); 20 µm (**J–O, Q, R**).

walled, erect, straight or flexuous, brown to dark brown, wider at the base and ocasionally conical at the apex, comprising 12–17 septa. ***Conidiogenous cells*** 5–12 µm long × 4–9 µm wide ($\bar{x}$ = 7.4 × 6.2 µm, n = 10), holoblastic, enteroblastic, integrated, smooth-walled, ovoid to ampulliform, hyaline or brown to dark brown, occurring terminally or intercalary on conidiophores, with up to four successive percurrent proliferations, with new conidiogenous cells developing on subtending cells. ***Conidia*** 25–40 µm long × 30–60 µm wide ($\bar{x}$ = 35 × 47 µm, n = 30), width measured between the two extremities of the apices, solitary, dictyospored and cheirospored, fan-shaped to cheiroid, lenticular in edge view, occasionally apically 2–3-lobed, dark brown to olivaceous brown, dictyoseptate, with up to 15 columns of cells radiating from a protuberant basal cell 2–3 µm wide, septa obscured by dark pigmentation, and with 1–3 apical appendages (rarely without appendages). ***Apical appendages*** (2–)6–16(–20) µm long ($\bar{x}$ = 10.9 µm, n = 10), 4–5 µm wide ($\bar{x}$ = 4.4 µm, n = 10) at the base, 4–5 µm wide ($\bar{x}$ = 4.1 µm, n = 10) at the apex, mostly short and untapered, sometimes long and tapering, arising from the sides of the outermost rows of cells of the conidium, pale brown to brown, becoming hyaline and rounded at the tips, and consisting of 1–5 septa.

Culture characteristics. On PDA, colony circular with lobate to crenated margin, reaching 25 mm diam. after 28 days incubated at 25 °C, greyish white to olivaceous brown from center to edge, fluffy, raised to convex, penetrating the media and displaying a dark brown colour around the media.

Material examined. THAILAND • Chiang Rai Province, Mae Fah Luang University Botanical Garden, on decaying stems of *Broussonetia papyrifera* (Moraceae), 19 May 2023, D. Gomdola, F2-A (MFLU 24-0227, ***holotype***), ex-type MFLUCC 24-0258.

Additional material examined. THAILAND • Chiang Rai Province, Mae Fah Luang University Botanical Garden, on decaying stems of *Broussonetia papyrifera* (Moraceae), 19 May 2023, D. Gomdola, F2-B (MFLU 24-0228), living culture MFLUCC 24-0259.

GenBank accession numbers. MFLUCC 24-0258; ITS = PQ137417, LSU = PQ137419, SSU = PQ137421, *RPB2* = PQ488459 and *TEF1* = PQ488461; MFLUCC 24-0259; ITS = PQ137418, LSU = PQ137420, SSU = PQ137422 and *RPB2* = PQ488460.

Notes. Our two isolates (MFLUCC 24-0258 and MFLUCC 24-0259) group together with 100% ML and 1.00 PP support, and this subclade is sister to *Pseudoberkleasmium* species with 100% ML and 1.00 PP support (Figs 1, 2).

A close comparison of the morphological characters across the existing *Xiuguozhangia* species is collated and presented in Table 3 to support the establishment of the new species, *X. broussonetiae*.

A summary of the main findings from the morphological assessment is presented below:

1. The conidiophores of our species, *Xiuguozhangia broussonetiae*, are longer than all other *Xiuguozhangia* taxa (up to 890 µm long), comprising numerous septa (up to 17). Furthermore, conidiophores of other *Xiuguozhangia* species are unbranched, while those of *X. broussonetiae* are sometimes branched (Fig. 3E).
2. The conidia of *Xiuguozhangia broussonetiae* (Figs 3J–O, Q, R) consist of up to 15 columns of cells radiating from a protuberant basal cell that is smaller (2–3 µm wide) compared to those of other species.

3. The appendages of *X. broussonetiae* are mostly untapered (Figs 3N, 3P–R) and sometimes taper towards the tips (Fig. 3B), whereas all other *Xiuguozhangia* species have only tapering appendages.
4. *Xiuguozhangia broussonetiae* differs from all extant species but has a close morphological overlap with *X. rosae*. However, the primary feature that demarcates *X. broussonetiae* from *X. rosae* is the number and features of the apical appendages. Up to three appendages were observed in *X. broussonetiae* (sometimes seen without appendage), while *X. rosae* has two appendages. In addition, the appendages of *X. broussonetiae* are 1–5-septate, while those of *X. rosae* display one to two septa.
5. *Xiuguozhangia broussonetiae* has percurrently proliferating, enteroblastic conidiogenous cells that sometimes produce new conidiogenous cells on subtending cells (Fig. 3D–I). This feature has not been observed in other species of the genus.

Based on these morphological differences, we conclude that our taxon is distinct from the existing *Xiuguozhangia* species.

Discussion

The number of hyphomycetes introduced over the past decade has increased substantially, indicating that their diversity is rather high (Hyde et al. 2020, 2023, 2024a, b; Bhunjun et al. 2022; Manawasinghe et al. 2022; Calabon et al. 2023; Liu et al. 2024b; Tian et al. 2024; Zhang et al. 2024). Hyphomycetes have a ubiquitous distribution in aquatic and terrestrial habitats, occurring on different substrates in tropical and subtropical regions (Seifert et al. 2011; Bao et al. 2021; Wu and Diao 2022, 2023; Dong et al. 2023; Senanayake et al. 2023; Yang et al. 2023; Liu et al. 2024a, b). Several hyphomycetes are rather speciose, for example, *Cladosporium*, *Dictyosporium*, *Helminthosporium*, and *Sporidesmium* (Shenoy et al. 2006; Hu et al. 2023; Yang et al. 2023). However, the hyphomycete genus, *Xiuguozhangia*, does not appear to be species-rich, and no studies have reported its occurrence in Thailand. This study introduces a new species, *Xiuguozhangia broussonetiae*, from *Broussonetia papyrifera* in northern Thailand.

There are more than 30,000 fungal species that exhibit the asexual morph, belonging to 2265 hyphomycetous genera (Wijayawardene et al. 2022b; Jayawardena et al. 2023; Perera et al. 2023; Ma et al. 2024; Zhang et al. 2024). The *Xiuguozhangia* species reported so far occur only in their asexual morph as saprobic hyphomycetes. A probable explanation for their asexual morph occurrence might be for survival, as conidia can be produced in large amounts and are easily released for dispersal compared to ascospores (Gilbert and Parker 2023). Their sexual morphs have not been reported yet, and no research has been conducted to link their asexual and sexual morphs, possibly due to the lack of molecular data and their availability in cultures. To date, all *Xiuguozhangia* taxa have been documented from Asia (China, India and Thailand – this study). We anticipate discovering more species in this genus, as well as the sexual morph, especially in high biodiversity areas like China and Thailand, as these taxa have not yet been extensively collected, isolated, and studied.

In the phylogenetic analysis of dataset 1, which included representative taxa from all families of Pleosporales, *Xiuguozhangia* forms a sister lineage

Table 3. Phenotypic comparison of *Xiuguozhangia* species. Features that depict *X. broussonetiae* from other *Xiuguozhangia* species are in bold.

Species and characters		*X. appendiculata*	*X. broussonetiae*	*X. indica*	*X. macrospora*	*X. punicae*	*X. rhaphidophorae*	*X. rosae*
Colonies on the natural substrates	**Features**	Effuse, hairy	Effuse, appearing velvety due to numerous conidiophores	Effuse, hairy	Effuse, hairy, growing in association with species of lichen	Effuse, hairy	Effuse, hairy	Effuse, hairy
	Colour	Olivaceous to dark brown	Olivaceous to dark brown	Olivaceous to dark brown	Olivaceous to dark brown	Olivaceous to dark brown	Olivaceous to dark brown	Olivaceous to dark brown
Conidiophores	**Size (µm)**	Up to 620 µm long, 18–35 µm wide at the base, 13–18 µm wide in the middle, 7–11.5 µm wide at the apex	(430–)550–750**(–890) µm long**, 15–24(–34) µm wide at the base, 11–14(–16) µm wide in the middle, 7–10 µm(–12) wide at the apex	Up to 530 µm long, 13–15 µm wide at the base, 10–12 µm wide in the middle, 3.6–5 µm wide at the apex	160–340(–570) µm long, 12–21 µm wide at the base, 11–14 µm wide in the middle, 6–7.5 µm wide at the apex	Up to 550 µm long, 20–30 µm wide at the base, 10–17 µm wide in the middle, 5–8 µm wide at the apex	Up to 630 µm long, 18–25 µm wide at base, 10–15 µm wide in the middle, 4–7 µm wide at apex	Up to 730 µm long, 12–20 µm wide at the base, 7–14 µm wide in the middle, 5–9 µm wide at the apex
	Features	Mononematous, erect, straight or flexuous, unbranched, smooth, thick-walled, comprising 4–8 septa	Macronematous, mononematous, erect, straight or flexuous, **rarely branched**, smooth, thick-walled, wider at the base and ocasionally conical at the apex, comprising **12–17 septa**	Mononematous, basal portion sheath-like, erect, straight, unbranched, smooth, thick-walled, comprising 4–13 septa	Mononematous, erect, straight, unbranched, smooth, thick-walled, comprising 3–8 septa	Macronematous, mononematous, erect, straight or flexuous, unbranched, smooth, thick-walled, comprising 5–11 septa	Macronematous, mononematous, erect, straight or flexuous, unbranched, smooth, thick-walled, comprising 10–15 septa	Macronematous, mononematous, erect, straight or flexuous, unbranched, smooth, thick-walled, comprising 8–14 septa
	Colour	Dark brown	Brown to dark brown	Brown, becoming paler towards the apex	Brown	Not mentioned	Not mentioned	Not mentioned
Conidiogenous cells	**Size (µm)**	Not mentioned	5–12 µm long, 4–9 µm wide	7.5–13.5 × 4.2–7.4 µm	9–19 × 7.5–10.0 µm	Not mentioned	Not mentioned	Not mentioned
	Features	Holoblastic, integrated, terminal, sometimes lateral, truncate after conidium secession	Holoblastic, enteroblastic, integrated, terminal or intercalary, smooth-walled, ovoid to ampulliform, percurrently proliferating; sometimes new condiogenous cells developing on subtending cells	Flask-shaped, integrated, terminal and lateral, truncate after conidial secession, proliferating percurrently up to 4 times	Flask-shaped, integrated, terminal, truncate after conidial secession, proliferating percurrently up to 5 times	Monotretic, integrated, terminal, sometimes lateral, with up to five successive percurrent proliferations	Monotretic, integrated, terminal and lateral	Monotretic, integrated, terminal and lateral, with up to three successive percurrent proliferations
	Colour	Not mentioned	**Hyaline** or brown to dark-brown	Brown	Brown	Not mentioned	Not mentioned	Not mentioned
Conidia	**Size (µm)**	50–80 µm long, 60–90 µm wide	25–40 µm long, 30–60 µm wide	34–44 µm long, 39–52 µm wide	(73–)81–120(–135) µm long, (51–)67–94(–106) µm wide	50–65 µm long, 58–95 µm wide	27–41 µm long, 30–43 µm wide	45–50 µm long, 53–76 µm wide
	Features	Fan-shaped, lenticular in edge view, 2–3-lobed, dictyoseptate, with rows of cells radiating from a protuberant basal cell 6–7.5 µm wide	Dictyospored and cheirospored, fan-shaped to cheiroid, lenticular in edge view, occasionally apically 2–3-lobed, dictyoseptate, with up to 15 columns of cells radiating from a protuberant basal cell **2–3 µm wide**	Fan-shaped, sometimes 2–5-lobed, lenticular in edge view, dictyoseptate, with up to 15 rows of cells, basal cell protuberant, 3.7–5 µm wide	Campanulate or fan-shaped, sometimes apically 2–3-lobed, lenticular in edge view, dictyoseptate, smooth, with up to 18 vertical rows of cells, basal cell protuberant, 7–9 µm wide	Fan-shaped, sometimes 2–3-lobed, lenticular in edge view, dictyoseptate, with up to 18 rows of cells radiating from a protuberant basal cell 4.5–6.5 µm wide	Fan-shaped, sometimes lobed, lenticular in edge view, dictyoseptate, with eight rows of cells radiating from a protuberant basal cell 2.5–5 µm wide	Fan shaped, sometimes 2–3-lobed, lenticular in edge view, dictyoseptate, with 20 rows of cells radiating from a protuberant basal cell 3–5 µm wide
	Colour	Dark brown	Dark brown to olivaceous brown	Brown	Brown	Dark brown	Dark brown	Dark brown
Apical appendage(s)	**Size (µm)**	75–120 µm long, 4–6 µm wide, tapering to 1.5–2 µm wide	(2–)6–16(–20) µm long, 4–5 µm wide at the base and apex	15–30 µm long, 3–4.4 µm wide at the base, tapering to 2.4–2.8 µm wide	(33–)52–74(–126) µm long, 6.5–8.0 µm wide at the base	24–99 µm long, 3–5.5 µm wide, tapering to 1–1.5 µm wide		15–35 µm long, 3–4 µm wide, tapering to 2–3 µm wide
	Features	2–4 appendages, tapering, 3–5-septate	Without or with 1–3 appendages, rounded at the tips, **mostly untapered**, sometimes tapering 1–5-septate	2 appendages (Up to 4), tapering, 1–2-septate	2–3 appendages (rarely up to 4), tapering, 1–3(–6)-septate	2 appendages (rarely up to 3), smooth, tapering, 0–3-septate	Appendages absent	2 appendages, tapering, 1–2-septate
	Color	Brown, and apically hyaline	Pale brown to brown, becoming hyaline at the tips	Brown, and apically hyaline	Brown, and apically hyaline	Brown, and apically hyaline	-	Brown, and apically hyaline
Host(s)		On dead twigs of an unknown host	On decaying stems of *Broussonetia papyrifera*	On dried bamboo culms	On dead bamboo stem	On dead branches of *Punica granatum*	On dead branches of *Rhaphidophora decursiva*	On dead branches of *Rosa chinensis*
Distribution(s)		India	Thailand	India	India	China	China	China
References		(Bhat and Kendrick 1993)	This study	Sureshkumar et al. (2005)	Dubey and Jash (2024)	Zhang et al. (2009), Zhang et al. (2014)	Zhang et al. (2009, 2014)	Zhang et al. (2009), Zhang et al. (2014)

to *Pseudoberkleasmium*, with maximum statistical support in both methods of analysis (Fig. 1). Pleosporales taxa were accommodated in over 90 families (with and without molecular data), and have a worldwide distribution in terrestrial and aquatic habitats (Hongsanan et al. 2020; Bhunjun et al. 2021; Jiang et al. 2021; Wijayawardene et al. 2020, 2022a; Yang et al. 2022; Tang et al. 2023; Hyde et al. 2024a, b; Pem et al. 2024; Wei et al. 2024). In this study, we maintain 95 families in this order: 92 with DNA sequence data and two without (Diademaceae and Lizoniaceae). Additionally, we exclude Mycoporaceae (*Mycoporum* spp.) from the analysis due to its divergent sequences and long branches in the preliminary phylogenetic trees. Further research is needed to determine its placement within Pleosporales. Arthopyreniaceae was classified under Pleosporales in Wijayawardene et al. (2022a) and Pem et al. (2024), but we do not retain it in this order as its taxa cluster basal to Pleosporales (results not shown), similar to the findings by Thiyagaraja et al. (2021). We also retain Sublophiostomataceae within Pleosporales (Hongsanan et al. 2021), but it was not included in Pem et al. (2024). In our phylogenetic analysis, most families in Pleosporales are monophyletic, thereby further supporting their rank. A few families [e.g., Halotthiaceae (87% ML); Pseudomassarinaceae; and Testudinaceae (81% ML)] lack strong statistical support but have consistently been recovered as distinct groups in previous studies using different datasets and/or methods of analysis (Crous et al. 2018; Yang et al. 2022), suggesting that these are separate lineages distinct from other families. However, available genetic markers and DNA sequence data may not be sufficient to resolve their familial relationships with high confidence.

Xiuguozhangia is sister to *Pseudoberkleasmium* and closely related to *Hermatomyces* (Figs 1, 2). Given that we assigned *Xiuguozhangia* in Pseudoberkleasmiaceae based on the analysis of dataset 1, we analysed dataset 2 to further validate its taxonomic placement, which was based on multiple strains representing all species of *Hermatomyces* and *Pseudoberkleasmium*. Since ITS and LSU are important genetic markers to delimit fungal genera, we computed the group mean distances across these gene regions between *Hermatomyces* spp., *Pseudoberkleasmium* spp. and *Xiuguozhangia* spp. Considering the inter-generic nucleotide differences between each group (Table 2), the establishment of *Xiuguozhangia* as a distinct genus from *Pseudoberkleasmium* is supported.

The characterisation of *Xiuguozhangia* as a separate genus from *Pseudoberkleasmium* is further corroborated based on their distinct features. *Xiuguozhangia* differs from *Pseudoberkleasmium* in their colonies on natural substrates, and conidiophore, conidiogenous cell and conidial features (Zhang et al. 2014; Tibpromma et al. 2018). *Xiuguozhangia* species are depicted by dictyoseptate, campanulate, cheiroid, and brown conidia comprising multiple layers of cells, either with or without apical appendages (Zhang et al. 2014). However, *Pseudoberkleasmium* species are characterised by broadly ellipsoidal to obovoid, flattened, one-cell thick, muriform, and brown olivaceous green conidia without any apical appendage (Tibpromma et al. 2018). Conidial images of *Pseudoberkleasmium* and *Hermatomyces* are illustrated in Fig. 2. Since other species of *Xiuguozhangia* do not have molecular data, we were unable to compare the inter-species nucleotide differences, and we had to rely solely on morphology to establish our new species, *X. broussonetiae*.

With respect to the inter-species morphological differences in the genus, the new species, *X. broussonetiae*, differs from all extant species (Table 3), but exhibits overlapping features with *X. rosae*. Nonetheless, *X. broussonetiae* differs from *X. rosae* in terms of the number and characters of the apical appendages, as outlined in the note section above. Other observed differences are given in Table 3. *Xiuguozhangia* was classified as Ascomycota genus *incertae sedis* in the latest outline by Wijayawardene et al. (2022a), as it was difficult to propose its precise familial placement without the availability of DNA sequence data. Since our study is the first to provide molecular data, we refer *Xiuguozhangia* in Pseudoberkleasmiaceae. Hyde et al. (2019) initially introduced this family as monotypic to accommodate *Pseudoberkleasmium* species based on morphology and molecular data. *Pseudoberkleasmium* was described by Tibpromma et al. (2018) based on morphology and multigene phylogenetic analyses. *Pseudoberkleasmium* taxa resemble those of *Berkleasmium*, but *Berkleasmium* belongs to Tubeufiales (Tibpromma et al. 2018; Wijayawardene et al. 2022a; this study). Besides *Pseudoberkleasmium*, we treat *Xiuguozhangia* as a member of Pseudoberkleasmiaceae based on our inferred phylogenies. Upon the addition of *X. broussonetiae* to the genus, there are seven species of *Xiuguozhangia*, sharing the same holoblastic conidial ontogeny as a generic feature.

Due to the limited taxon sampling and analysis, and the lack of comprehensive DNA sequence data, there remains uncertainty in using conidial ontogeny as a basis for distinguishing *Xiuguozhangia* from *Piricaudiopsis*. As such, we refrain from drawing further conclusions regarding their taxonomic separation at this stage. Instead, a more effective approach would involve extensive sampling across diverse localities and habitats, particularly those that support the growth of hyphomycetes. Coupled with a systematic acquisition of DNA sequence data, these efforts would provide a more robust support for classifying *Xiuguozhangia* species and other poorly studied Pleosporalean taxa (Liu et al. 2024b; Pem et al. 2024). Moreover, a thorough re-examination of the type species of each genus is essential to ascertain whether these two genera are indeed distinct or if they might represent a single taxonomic entity.

Acknowledgments

We thank Dr Shaun Pennycook for suggesting and validating the nomenclature of the new species. Deecksha Gomdola thanks the Mae Fah Luang University for providing the MFU student scholarship 2020 (reference: GR-ST-FS-63-06) to pursue to PhD program, and for allocating the thesis writing grant [reference: 7702(6)/201 (No.0275)]. Deecksha Gomdola thanks the Mushroom Research Foundation (MRF) for partly funding her project, and the staffs of the MFU botanical garden for their help in the sample collection and identification of the host. Deecksha Gomdola also thanks Prof. Dr. Yong Wang of the Guizhou University (Department of Plant Pathology) for providing funds to conduct molecular work under his NFSC grant No. 31972222. We would also like to acknowledge the grant GDPX[2019]13 for partly funding this project. We thank Miss Tang Xia and Mr. Xingguo Tian for providing conidial images of *Hermatomyces* and *Pseudoberkleasmium* to complement Fig. 2. The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R114), King Saud University, Riyadh, Saudi Arabia.

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 Guizhou University (NFSC grant No. 31972222 and GDPX[2019]13), Mae Fah Luang University, Mushroom Research Foundation and MFU student scholarship 2020 (reference: GR-ST-FS-63-06); thesis writing grant [reference: 7702(6)/201 (No.0275)]; Researchers Supporting Project number (RSP2024R114).

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Data availability

All data generated or analysed during this study are included in this published article.

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MycoKeys 111: 211–228 (2024)
DOI: 10.3897/mycokeys.111.139620

Research Article

Emended Neodactylariales (Dothideomycetes): *Szaferohypha* gen. nov. and phylogenetically related genera

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Abstract

Epiphytic fungi evolved several times in Dothideomycetes, particularly within the orders Asterinales, Capnodiales, Microthyriales, and Zeloasperisporiales, but also in other, less obvious lineages. In this study, a new genus and species, *Szaferohypha* and *S. enigmatica*, isolated from the sooty mould community on the leaves of *Symphoricarpos albus* in Poland, are described based on morphology and phylogenetic analysis using sequences of four DNA loci (LSU, ITS, SSU, and *rpb2*). Due to single isolation, it is unclear whether *Szaferohypha enigmatica* represents a very rare or accidental inhabitant of sooty mould communities. *Szaferohypha* is assigned to the poorly known family Neodactylariaceae and order Neodactylariales, together with *Beaucarneamyces*, *Neodactylaria*, and *Pseudoarthrographis*. The order and family were originally circumscribed based on the features of the genus *Neodactylaria*. Therefore, they are emended by characters of *Beaucarneamyces*, *Pseudoarthrographis*, and *Szaferohypha*.

Key words: Ascomycota, emended descriptions, one new genus, one new species, sooty mould communities, taxonomy

Academic editor: Kevin D. Hyde
Received: 17 October 2024
Accepted: 30 November 2024
Published: 27 December 2024

Citation: Piątek M, Stryjak-Bogacka M, Czachura P (2024) Emended Neodactylariales (Dothideomycetes): *Szaferohypha* gen. nov. and phylogenetically related genera. MycoKeys 111: 211–228. https://doi.org/10.3897/mycokeys.111.139620

Introduction

The class Dothideomycetes is megadiverse and the largest class of fungi in the phylum Ascomycota (Wijayawardene et al. 2014, 2020). Currently, the class is divided into 54 orders and 226 families and contains about 20,000 described species (Abdollahzadeh et al. 2020; Hongsanan et al. 2020a, 2020b; Barreto et al. 2024; Pem et al. 2024; Piątek et al. 2024). The best-known and studied orders are Capnodiales s. lato, Dothideales, Botryosphaeriales, and Pleosporales (Zhang et al. 2012; Phillips et al. 2013, 2019; Quaedvlieg et al. 2014; Thambugala et al. 2014; Yang et al. 2017; Videira et al. 2017; Abdollahzadeh et al. 2020). Other orders of Dothideomycetes are less known, primarily because their members are less common in the environment, although some of them are important for human life and the economy (Hongsanan et al. 2020b; Shen et al. 2020).

The high species diversity observed in Dothideomycetes is reflected by the wide geographical range and diversity of lifestyles of its members. They are known from all areas of the world and habitats, even capable of colonizing extreme habitats such as cold deserts (Coleine et al. 2020), deep-sea sediments, including methane sediments (Nagahama et al. 2011; Rojas-Jimenez et al. 2020), saline waters (Czachura et al. 2021), acidic environments (Kolařík et al. 2021), and resin exudates (Czachura and Janik 2024). Species of Dothideomycetes are plant pathogens, saprobes, rock-inhabiting fungi, lichens, endophytes, and epiphytes. Hongsanan et al. (2016) defined fungal epiphytes as species occurring on the living plant surfaces, especially leaves, which belong to the following orders of Dothideomycetes: Asterinales, Capnodiales, Microthyriales, Zeloasperisporiales, and Meliolales in Sordariomycetes. However, epiphytes are also known in other, less obvious lineages (Piątek et al. 2023, 2024). The special kind of epiphytes are sooty moulds, which live on leaves/needles covered with exudates of phloem-feeding insects, especially honeydew secreted by aphids (Hughes 1976).

Recently, we isolated an enigmatic fungus from a sooty mould colony on *Symphoricarpos albus* in southern Poland, which showed affinities to members of the poorly known order Neodactylariales (Qiao et al. 2020; Crous et al. 2024). In this study, a novel species accommodated in a new genus is described for this fungus. The phylogenetic placement of this new genus and its most closely related genera are analysed, and emended descriptions of the order Neodactylariales and family Neodactylariaceae are provided.

Materials and methods

Strains and morphological analyses

The strain was isolated from the sooty mould community on *Symphoricarpos albus* leaves planted in municipal greenery in southern Poland (see Piątek et al. 2023). Macroscopic features of cultures were observed and photographed using 4-week-old colonies grown on MEA and PDA at 6 °C, 15 °C, and 25 °C, as well as 15-week-old colonies grown on MEA and PDA at 15 °C. Description of culture characteristics is based on 4-week-old colonies. The morphology of colonies observed after 15 weeks is briefly mentioned in the subsection “notes”. Growth at different temperatures was assessed by measuring the colony diameter after 4 weeks. Microscopic features were analysed using colonies older than 8 weeks grown on MEA at 15 °C. Hyphae and conidia taken from the edge of the colony were mounted in lactic acid (80%) on microscope slides and examined under a Nikon Eclipse 80i light microscope. Microscopic structures were measured and photographed using NIS-Elements BR 3.0 imaging software. The holotype is a dried specimen obtained from culture and deposited in the fungal collection of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków (KRAM F). Culture is preserved in the culture collection of the Westerdijk Fungal Biodiversity Institute (CBS) and in the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków. The morphological characteristics of *Beaucarneamyces* and *Pseudoarthrographis* used for the emendation of Neodactylariales and Neodactylariaceae are taken from the literature (Crous et al. 2018, 2024).

DNA isolation, PCR, and sequencing

After growth of cultures for about one month, genomic DNA was extracted from a portion of mycelium using the DNeasy® Plant Mini Kit (Qiagen, Germany). To conduct molecular studies, four genetic loci were amplified, namely ITS1-5.8S-

ITS2 rDNA (= ITS), 28S D1–D2 rDNA (= LSU), a small subunit rDNA (= SSU), and a protein-coding gene—a partial DNA-directed RNA polymerase II second largest subunit (*rpb2*). To amplify the loci mentioned above, four different primer pairs were used: ITS1 and LR5 for a fragment containing ITS and LSU (White et al. 1990; Vilgalys and Hester 1990), NS1 and NS4 for SSU (White et al. 1990), and fRPB2-5F and fRPB2-7cR for *rpb2* (Liu et al. 1999). Polymerase chain reactions (PCR) for all loci were performed in a reaction mixture prepared as described in Piątek et al. (2023). Amplification conditions for PCR reactions of the fragment containing ITS and LSU were performed as described by Piątek et al. (2023); in turn, amplification conditions for SSU and *rpb2* were described by Piątek et al. (2024). The PCR products were checked on 1% agarose gels and enzymatically purified using Exo-BAP Mix (EURx, Gdańsk, Poland). DNA sequencing was carried out in both directions by Macrogen Europe B.V. (Amsterdam, the Netherlands). ITS was sequenced using primers ITS1 and ITS4; LSU was sequenced using primers LSU1Fd and LR5; and SSU and *rpb2* were sequenced with the same pairs of primers that were used for amplification.

Phylogenetic analysis

The affinities of obtained ITS, LSU, SSU, and *rpb2* sequences of the isolated fungus were performed in the NCBI's GenBank nucleotide database using the megablast search tool (Zhang et al. 2000). To resolve its phylogenetic position, the multilocus dataset containing LSU, ITS, SSU, and *rpb2* sequences of representatives of Dothideomycetes used by Maharachchikumbura et al. (2021, with some modifications) was obtained. The dataset was, among others, augmented by sequences of representatives of the orders Neodactylariales (*Beaucarneamyces*, *Neodactylaria*, *Pseudoarthrographis*) and Oncopodiellales (*Diplocladiella*, *Oncopodiella*) (Suppl. material 1). Sequences were separately aligned for each single-gene dataset using the MAFFT algorithm (Katoh et al. 2005) in Geneious 11.1.5 and concatenated. Phylogenetic relationships were inferred using the concatenated LSU-ITS-SSU-*rpb2* alignment by the maximum likelihood (ML) analysis using RAxML-NG v. 1.1.1 (Kozlov et al. 2019), with 1000 bootstrap replicates. The best-fit substitution models were selected with ModelTest-NG v. 0.2.0 using the Bayesian Information Criterion (BIC) (Darriba et al. 2020). The final phylogenetic tree was visualized using FigTree v1.4.3. The alignment was deposited at figshare.com (https://doi.org/10.6084/m9.figshare.27231756.v1).

Results

Phylogenetic analysis

The concatenated multilocus dataset (LSU, ITS, SSU, and *rpb2*) included sequences of 154 representatives of most of the orders of the class Dothideomycetes, including all representatives of Neodactylariales, and a member of the class Arthoniomycetes (*Schismatomma decolorans*) used as an outgroup. The concatenated alignment contained 4483 characters (LSU: 1082, ITS: 750, SSU: 1761, *rpb2*: 890, including alignment gaps). The best-fit substitution models selected for single-gene alignments were as follows: GTR+I+G4 for both ITS and LSU, TrNef+I+G4 for SSU, and TPM3uf+I+G4, TPM2uf+I+G4, and TIM2+I+G4 for *rpb2* (three codons). The phylogenetic tree resulting from maximum likelihood analysis is shown in Fig. 1. Representatives of Dothideomycetes formed lineages that correspond to the orders that were well supported, but relationships between orders were mostly not resolved. The strain of a new genus and species, *Szaferohypha enigmatica*, clustered within the lineage assigned to the order Neodactylariales, but its relationship to the remaining genera of this order (*Beaucarneamyces*, *Neodactylaria*, and *Pseudoarthrographis*) was not resolved. The clustering of genera assigned to Neodactylariales was well supported (MLB = 85%), and this order formed a strongly supported (MLB = 98%) sister group to the lineage assigned to the order Oncopodiellales that was fully supported (MLB = 100%).

Taxonomy

Neodactylariales H. Zheng & Z.F. Yu, MycoKeys 73: 74 (2020), emend. Piątek, Stryjak-Bogacka & Czachura

Description. Asexual morph from human-associated organs or saprobic on plant debris **or epiphytic on living leaves**. Conidiophores acroauxic, macronematous **or micronematous**, mononematous, branched or unbranched, **or reduced to conidiogenous cells**. Conidiogenous cells mono- and polyblastic, sympodially extended **or not**. Conidia solitary **or in branched or unbranched chains**, hyaline or pale pigmented, smooth, verrucous, or echinulate. **Chlamydospores sometimes present.** Sexual morph not observed.

Type genus. *Neodactylaria* Guevara-Suarez, Deanna A. Sutton, Wiederh. & Gené.

Notes. Because of the inclusion of genera *Beaucarneamyces*, *Pseudoarthrographis*, and *Szaferohypha* in Neodactylariales (Crous et al. 2024; this study), the emended description of this order is necessary. The original description of this order (Qiao et al. 2020) is included without changes and emended by crucial characters of these genera given in bold.

Neodactylariaceae H. Zheng & Z.F. Yu, MycoKeys 73: 75 (2020), emend. Piątek, Stryjak-Bogacka & Czachura

Description. Mycelium superficial or immersed, composed of branched, septate, hyaline to subhyaline hyphae. Conidiophores macronematous **or micronematous**, mononematous, straight or flexuous, septate, unbranched, **or reduced to conidiogenous cells**. Conidiogenous cells terminal or intercalary, **monoblastic or** polyblastic, sympodial **or not**, with short-cylindrical denticles **or without denticles**. Conidial secession schizolytic. Conidia solitary **or in branched or unbranched chains**, smooth or finely echinulate. **Chlamydospores sometimes present.** Sexual morph not observed.

Type genus. *Neodactylaria* Guevara-Suarez, Deanna A. Sutton, Wiederh. & Gené.

Notes. The order Neodactylariales contains one family, Neodactylariaceae, that is emended by features of genera *Beaucarneamyces*, *Pseudoarthrographis*, and *Szaferohypha*. The original description of this family (Qiao et al. 2020) is included without changes, and the emended part is given in bold.

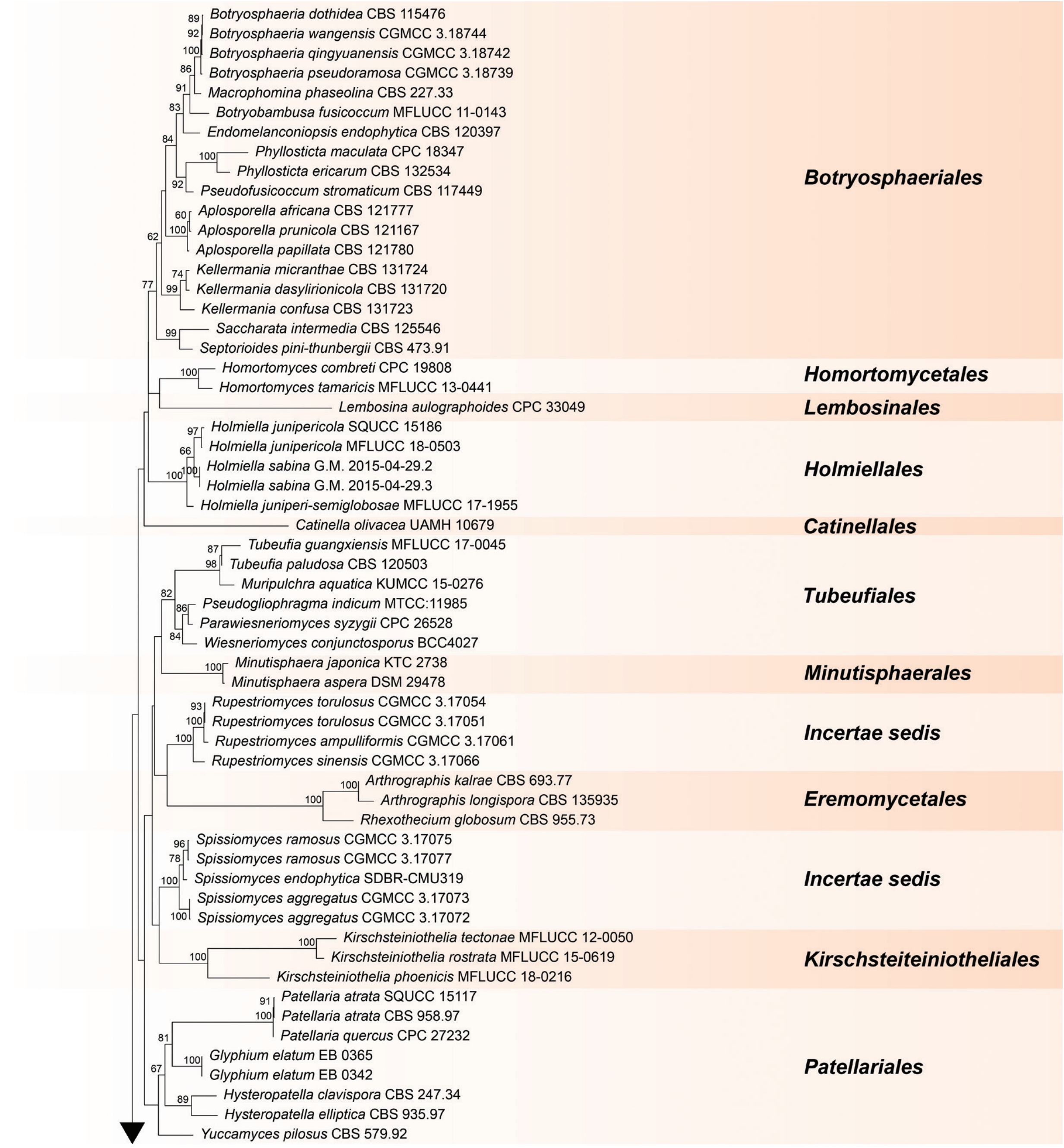

Figure 1. Phylogenetic tree of selected representatives of the orders of Dothideomycetes, including all sequenced species of Neodactylariales, obtained from a maximum likelihood analysis of the combined multi-locus alignment (LSU, ITS, SSU, *rpb2*). The position of *Szaferohypha enigmatica* is indicated in bold. Numbers above branches indicate maximum likelihood bootstrap (MLB) support values > 50%. *Schismatomma decolorans* AFTOL-ID 307 was used as an outgroup. The scale bar represents the expected number of changes per site.

Szaferohypha Piątek, Stryjak-Bogacka & Czachura, gen. nov.
MycoBank No: 856722

Etymology. Named after Polish botanist and palaeobotanist Professor Władysław Szafer (1886–1970), the first director of the W. Szafer Institute of Botany, Polish Academy of Sciences.

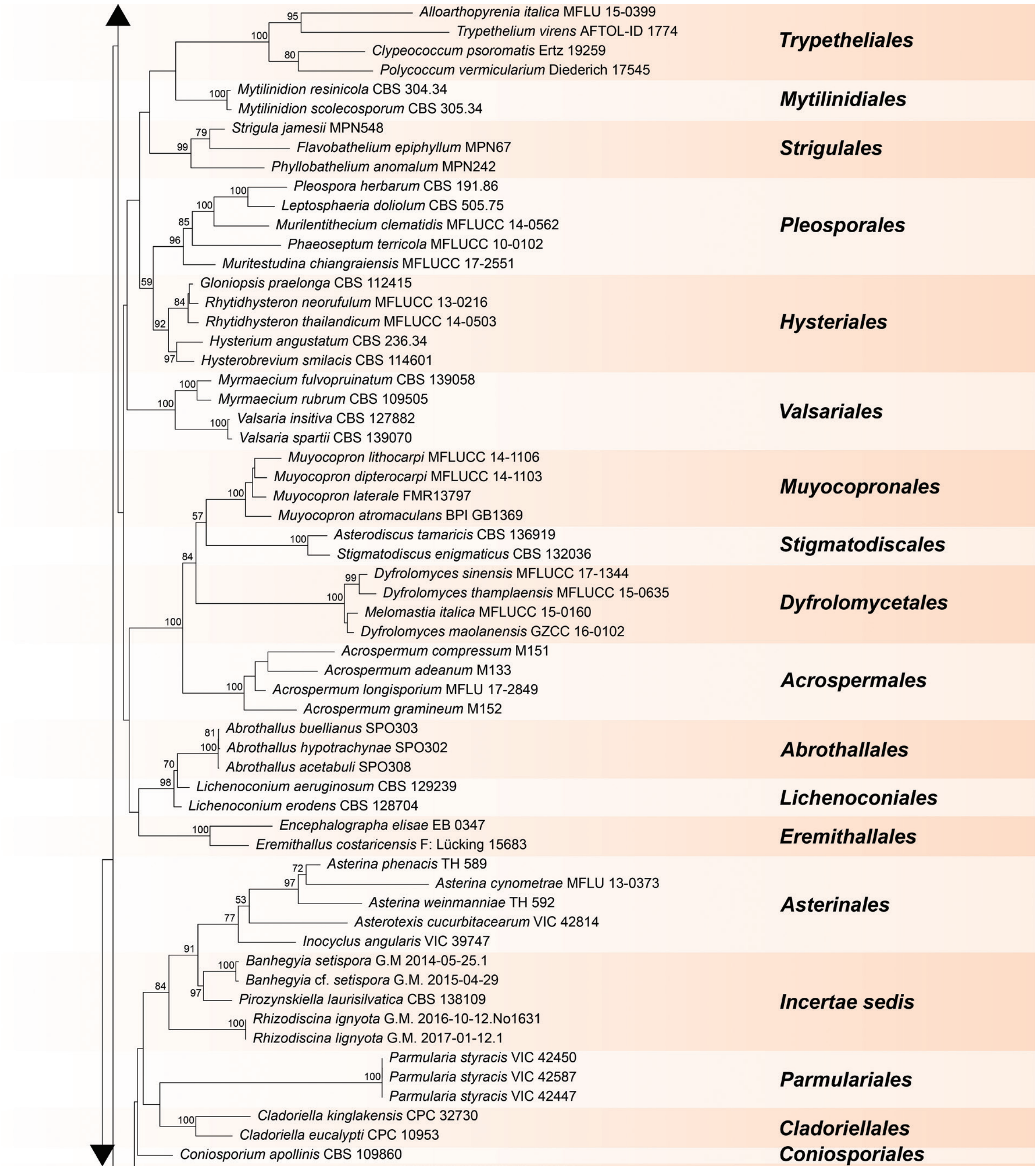

Figure 1. Continued.

Description. Colonies erumpent, spreading, umbonate, grayish-brown, with a velvety surface caused by abundant aerial mycelium, margin undulate. Mycelium composed of branched, septate, hyaline, subhyaline, pale brown, or brown, smooth or verrucose, usually thick-walled hyphae. Conidiophores micronematous, reduced to conidiogenous cells, rarely macronematous. Conidiogenous cells terminal, rarely lateral, monoblastic, hyaline, subhyaline, pale brown, or brown. Conidia globose, subglobose, rarely broadly ellipsoid, hyaline, subhyaline, or brown, aseptate, rarely with 1–2 septa or muriformly septate, smooth or finely verrucose, thick-walled, sometimes produced intercalary.

Type species. *Szaferohypha enigmatica* Piątek, Stryjak-Bogacka & Czachura.

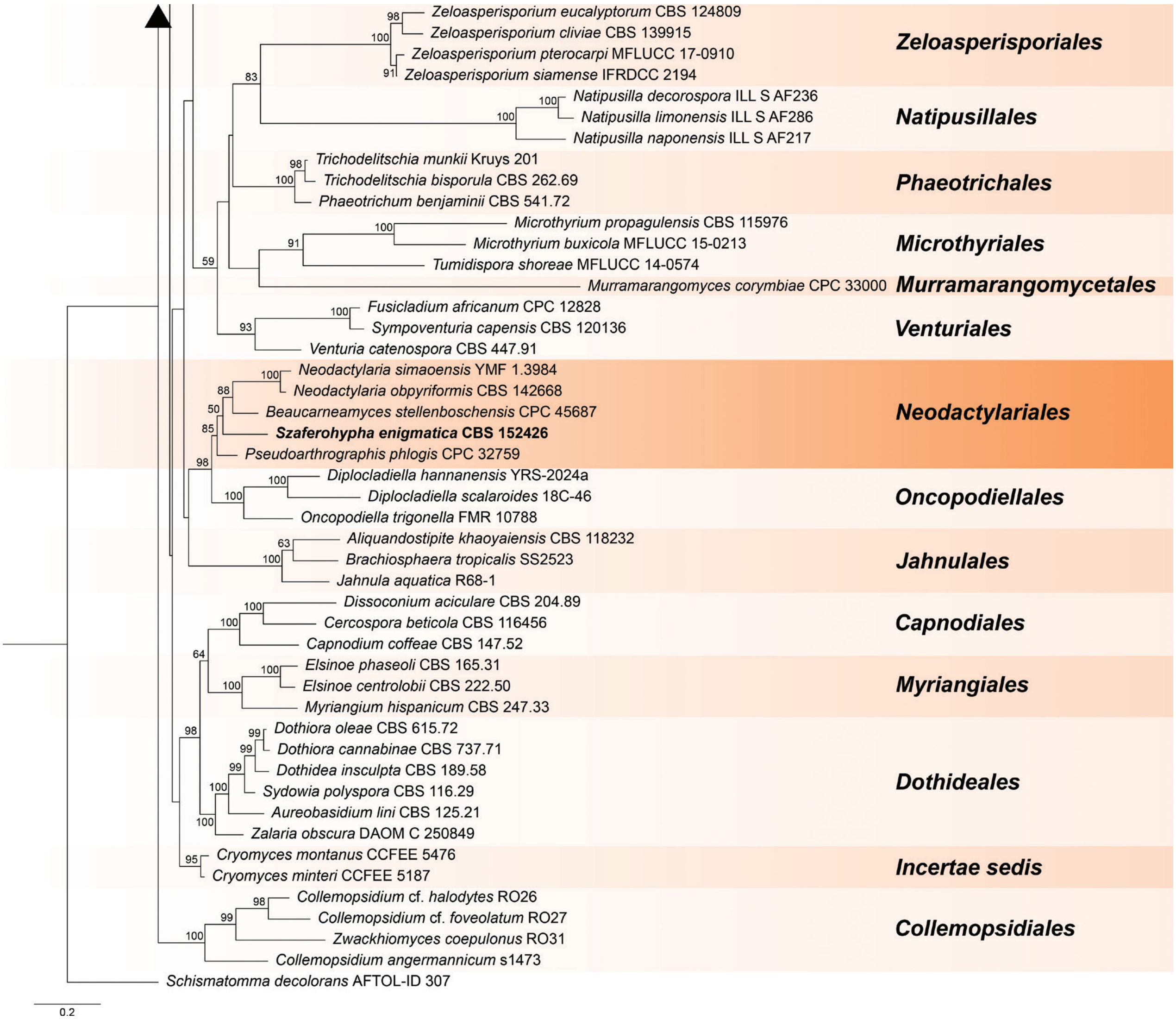

Figure 1. Continued.

Szaferohypha enigmatica **Piątek, Stryjak-Bogacka & Czachura, sp. nov.**
MycoBank No: 856723
Figs 2–4

Etymology. Refers to the uncertain taxonomic position of this fungus after the first molecular analyses.

DNA barcodes. ITS (PQ479987), LSU (PQ479989), SSU (PQ479988), *rpb2* (PQ475069).

Typus. Poland • Małopolska Province, Tarnów County: Tarnów–Piaskówka, municipal greenery, isolated from sooty mould community on *Symphoricarpos albus* leaves, 1 Oct. 2018, leg. M. Piątek, W. Bartoszek & P. Czachura (holotype KRAM F-59996; culture ex-type: G191 = CBS 152426).

Description. Mycelium composed of branched, septate, hyaline, subhyaline, pale brown, or brown, smooth or verrucose, usually thick-walled hyphae, 2–4 µm, sometimes with oil guttules; wall ca. 0.5 µm thick. Conidiophores micronematous, reduced to conidiogenous cells, rarely macronematous. Conidiogeneous cells terminal, rarely lateral, monoblastic, hyaline, subhyaline, pale brown, or brown, 3.5–13.5 × 2.5–4.5 µm. Conidia globose, subglobose, rarely broadly ellipsoid, hyaline, subhyaline, or brown, aseptate, rarely with 1–2 septa or muriformly septate, smooth or finely verrucose, thick-walled, 6.5–15 × 6–13.5 µm, sometimes germinating into hypha or produced intercalary, wall ca. 0.5–1.5 µm thick.

Culture characteristics. Colonies on MEA erumpent, spreading, umbonate, grayish, reaching 1 mm diam. after 4 weeks at 6 °C, 4 mm diam. after 4 weeks at 15 °C, and 8 mm diam. after 4 weeks at 25 °C, with a velvety surface caused by abundant aerial mycelium, margin entire. Reverse black. Colonies on PDA erumpent, spreading, umbonate, grayish-brown, reaching 3 mm diam. after 4 weeks at 6 °C, 4 mm diam. after 4 weeks at 15 °C, and 7 mm diam. after 4 weeks at 25 °C, with a velvety surface caused by abundant aerial mycelium, margin finely undulate. Reverse black.

Notes. Colonies photographed after 15 weeks of growth, depicted in Fig. 2g, h, are radially folded on MEA or folded on PDA, possess abundant aerial mycelium, grayish with white patches on MEA and brown with grayish patches on PDA, and are distinctly undulate at the margin.

Based on a megablast search of NCBI's GenBank nucleotide database, the closest hits of the named species using the ITS sequence are *Pseudoarthrographis phlogis* (strain CPC 32759, GenBank MH327796; identities = 370/414 (89%), 11 gaps (2%)), *Oncopodiella trigonella* (strain FMR 10788, GenBank KY853455; identities = 356/408 (87%), 11 gaps (2%)), and *Xylographa parallela* (voucher Z. Palice 22099 (PRM), GenBank MK778618; identities = 344/396 (87%), seven gaps (1%)). The closest hits of the named species using the LSU sequence are *Beaucarneamyces stellenboschensis* (strain CPC 45687, GenBank PP791445; identities = 826/873 (95%), four gaps (0%)), *Pseudoarthrographis phlogis* (strain CPC 32759, GenBank NG_064540; identities = 835/883 (95%), no gaps), and *Umbilicaria hypococcinea* (strain A12, GenBank JQ739991; identities = 876/927 (94%), eight gaps (1%)). The closest hits using the SSU sequence are *Cophinforma atrovirens* (strain CSM_72, GenBank MF436134; identities = 857/870 (99%), no gaps), *Gloeopycnis protuberans* (specimen DAOM 745762, GenBank NG_067652; identities = 857/870 (99%), no gaps), and *Botryosphaeria mamane* (strain CBS 117444, GenBank KF531821; identities = 857/870 (99%), no gaps). The closest hits using the *rpb2* sequence are *Shiraia bambusicola* (voucher SICAUCC 23-0005, GenBank OR424351; identities = 237/283 (84%), six gaps (2%)), *Lindra obtusa* (strain AFTOL-ID 5012, GenBank FJ238382; identities = 207/252 (82%), three gaps (1%)), and *Natonodosa speciosa* (strain CLM-RV86, GenBank MH745150; identities = 217/266 (82%), six gaps (2%)). In the case of SSU and *rpb2*, the sequences of these two regions are not available for representatives of the most closely related genera, namely *Beaucarneamyces* and *Pseudoarthrographis*.

Discussion

In this study, morphological and phylogenetic analyses were conducted to identify the enigmatic fungal strain isolated from sooty mould biofilm on the surface of *Symphoricarpos albus* leaves. In consequence, the isolated fungus is described as a new genus and species, *Szaferohypha enigmatica*, and assigned to the order Neodactylariales. Three genera were previously included in this order, namely *Beaucarneamyces*, *Neodactylaria*, and *Pseudoarthrographis* (Qiao et al. 2020; Crous et al. 2024), and *Szaferohypha* is morphologically different from all of them.

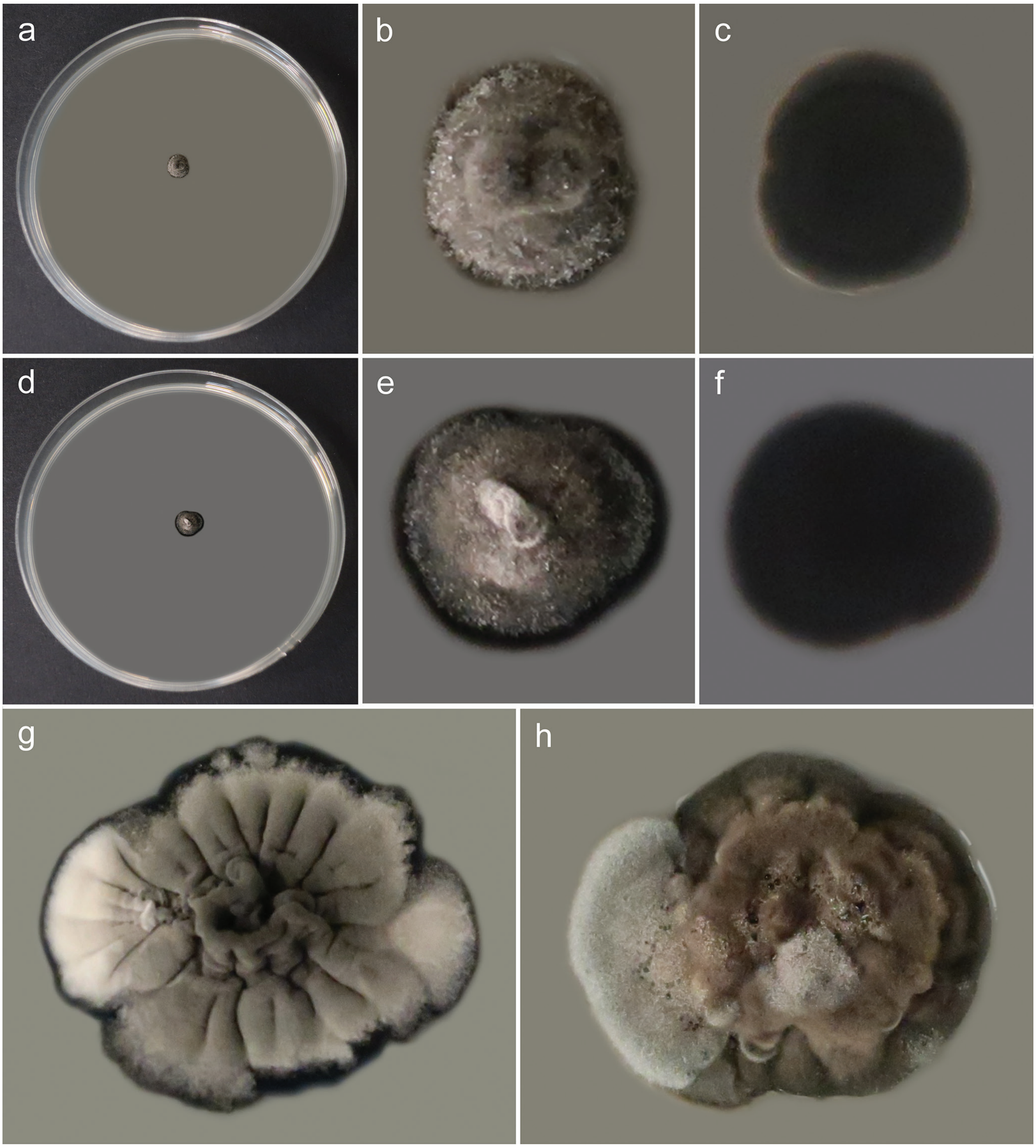

Figure 2. Morphology of cultures of *Szaferohypha enigmatica* (strain G191 = CBS 152426) after 4 weeks of growth at 25 °C **a–c** general view, upper side and reverse side of colony on MEA **d–f** general view, upper side and reverse side of colony on PDA **g** general view of 15-week-old colony on MEA (to show difference in morphology) **h** general view of 15-week-old colony on PDA (to show difference in morphology).

Beaucarneamyces, with type species *B. stellenboschensis*, was described from dead leaves of *Beaucarnea stricta* in South Africa and is characterized by hyaline conidiophores reduced to conidiogenous cells that are polyblastic with several apical denticles. Conidia in this genus are solitary, fusoid-ellipsoid, hooked, 3-septate, hyaline but with pale brown central cells (Crous et al. 2024). *Neodactylaria* is typified with *N. obpyriformis* described from human bronchoalveolar lavage in the USA (Crous et al. 2017). The second species in

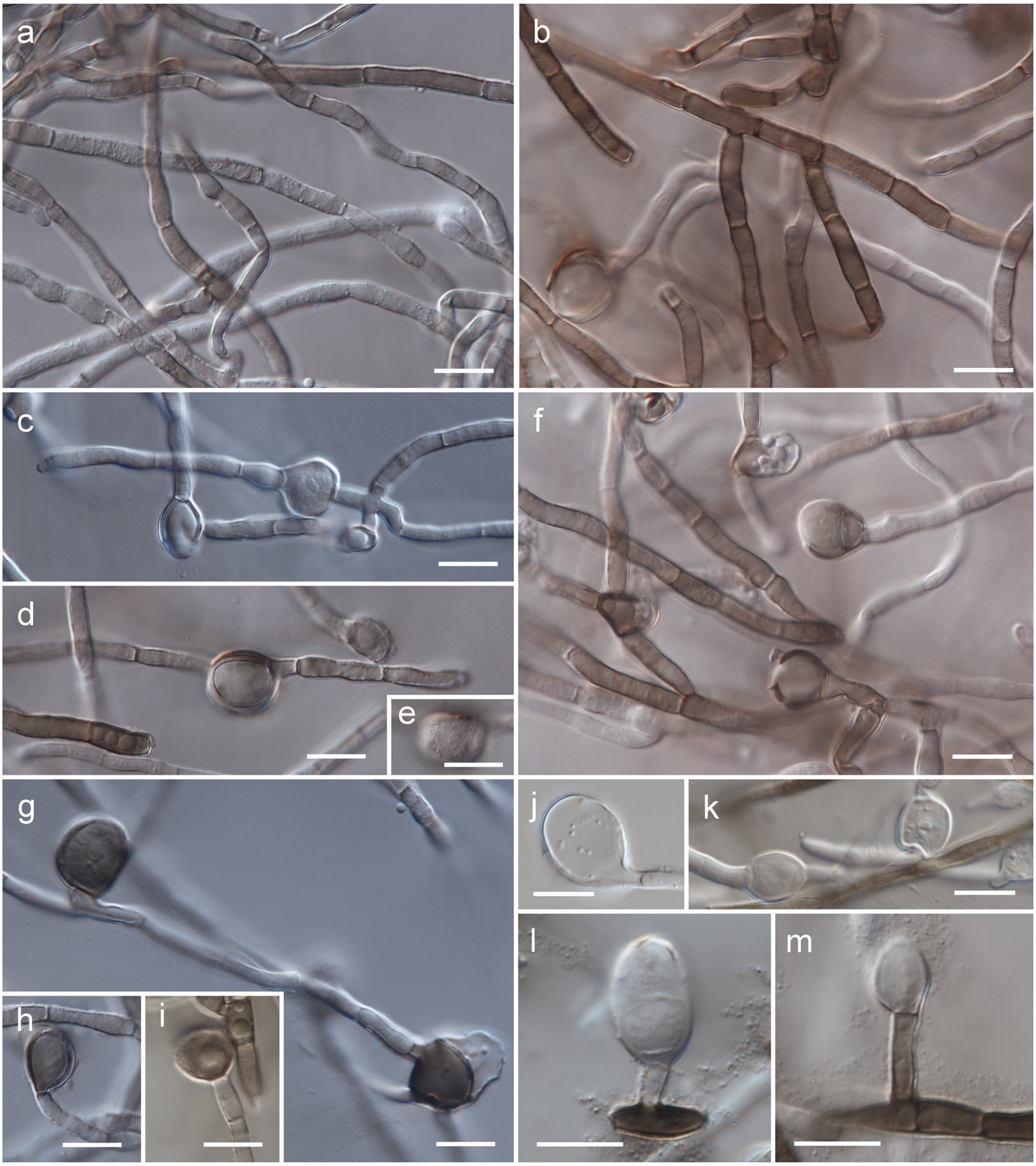

Figure 3. Morphology of *Szaferohypha enigmatica* (strain G191 = CBS 152426, all on MEA) **a, b** subhyaline or brown, smooth or verrucose hyphae **c–e** subhyaline or brown intercalary conidia (figure **e** depicts the verrucose surface of the conidium) **f** hyphae and terminal conidia **g–m** hyaline or brown, lateral or terminal conidia emerging on conidiogenous cells (figure **i** depicts the verrucose surface of the conidium). Scale bars: 10 μm.

this genus is *N. simaoensis*, described from submerged unidentified dicotyledonous leaves in China (Qiao et al. 2020). *Neodactylaria* is characterized by having straight or flexuous, brown conidiophores with polyblastic, sympodial, and denticle-like conidiogenous loci, which form solitary, obpyriform or rostrate, 0-1-septate, pale brown conidia (Crous et al. 2017). *Pseudoarthrographis*, with a type species *Pseudoarthrographis phlogis* described from *Phlox subulata*

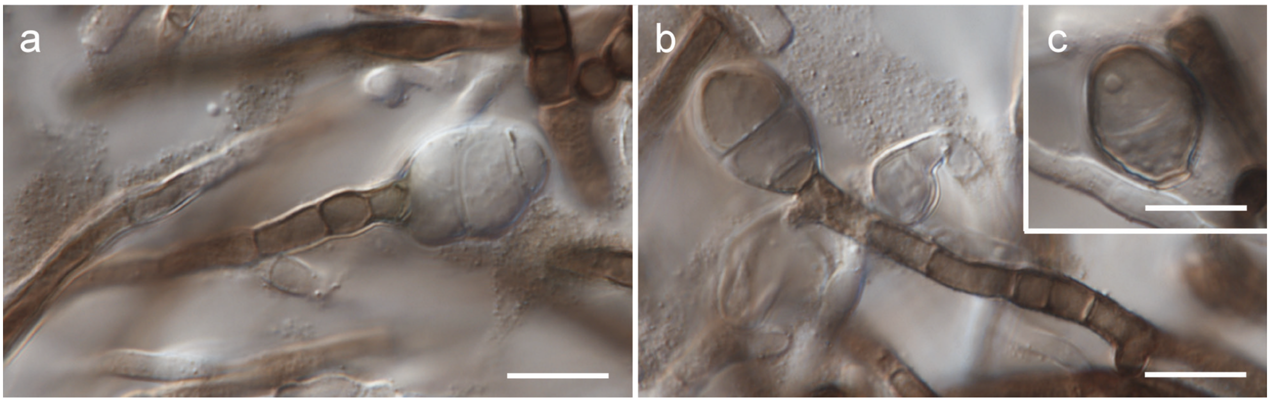

Figure 4. Morphology of *Szaferohypha enigmatica* (strain G191 = CBS 152426, all on MEA) **a, b** terminal conidia emerging on conidiogenous cells **c** detached conidium. Scale bars: 10 µm.

in New Zealand, has smooth hyphae and smooth, 0–1-septate cylindrical arthroconidia with truncate ends produced in branched or unbranched chains. In culture it also forms smooth, globose chlamydospores occurring in chains (Crous et al. 2018).

Despite numerous strains obtained during our studies of sooty mould communities, only a single strain belonging to *Szaferohypha enigmatica* was isolated. It is therefore unclear whether this species represents a very rare or accidental inhabitant of these communities and its main habitat is elsewhere.

The order Neodactylariales, with one family Neodactylariaceae, was originally described to accommodate only one genus, *Neodactylaria*, containing two species in China and the USA (Crous et al. 2017; Qiao et al. 2020). Recently, two monotypic genera, *Beaucarneamyces* and *Pseudoarthrographis*, containing species known in South Africa and New Zealand, respectively, were assigned to this order by Crous et al. (2024). Here, yet another monotypic genus, *Szaferohypha*, known from Poland, is added to Neodactylariales. Thus, currently this order includes fungi living in opposite corners of the world. The order Neodactylariales and family Neodactylariaceae were originally circumscribed based on characters known in the genus *Neodactylaria* (Qiao et al. 2020). They therefore need emendation by the features of the genera *Beaucarneamyces*, *Pseudoarthrographis*, and *Szaferohypha.* In our phylogenetic analysis, Neodactylariales is related to the order Oncopodiellales that is going to be described (Sun et al. 2024, preprint). Two genera, *Diplocladiella* and *Oncopodiella* (Hernández-Restrepo et al. 2017; Sun et al. 2024, preprint), assigned to two families, Diplocladiellaceae and Oncopodiellaceae, are included in this order (Sun et al. 2024, preprint). The relationships of Neodactylariales and Oncopodiellales are revealed here for the first time.

Sooty mould communities are an underexplored source of rare and undescribed species, of which many are probably extremophilic since they live in extreme environments (Chomnunti et al. 2014). They were previously studied using classical morphological methods (Hughes 1976) that are, however, inadequate to reveal their true diversity, and only recently started to study in modern ways using morphological and molecular methods, though mainly in tropics (Chomnunti et al. 2012, 2014) and much more rarely in temperate regions (Flessa et al. 2012, 2021). In the course of our ongoing studies of sooty mould communities covering the surface of leaves/needles of ornamental plants in

Poland, i.e., in the temperate climate, about 190 species were isolated from these communities (M. Piątek et al. unpublished data), being either as constant or accidental colonizers, of which many are undescribed. These include four new *Rachicladosporium* species (Piątek et al. 2023), a new *Lapidomyces* species (Crous et al. 2023b), a new *Xenoramularia* species (Crous et al. 2023a), a new *Pseudopezicula* species (Crous et al. 2024), and many more that are waiting for description.

Acknowledgements

We are grateful to Wacław Bartoszek (Kraków, Poland) for help in the fieldwork.

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 funded by the National Science Centre, Poland, under the project 2017/27/B/NZ9/02902, and partly through the statutory funds of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Author contributions

MP: conceptualization, investigation, formal analyses, visualisation, writing—original draft preparation; MSB: investigation, formal analyses, visualisation, writing—review and editing; PC: investigation, visualisation, writing—review and editing. All authors have read and approved the final version of the manuscript.

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Data availability

The data that support the findings of this study are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and in culture collections and fungal herbaria, as shown in Suppl. material 1 and the text.

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Supplementary material 1

List of species with strain/specimen and GenBank accession numbers used in phylogenetic analysis

Authors: Marcin Piątek, Monika Stryjak-Bogacka, Paweł Czachura

Data type: docx

Link: https://doi.org/10.3897/mycokeys.111.139620.suppl1

MycoKeys 111: 229–248 (2024)
DOI: 10.3897/mycokeys.111.125933

Research Article

Molecular phylogeny and morphology reveal three new plant pathogenic fungi species (Septobasidiales, Basidiomycota) from China

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Abstract

Three new fungal species, *Septobasidium macrobasidium*, *S. puerense* and *S. wuliangshanense*, are proposed based on a combination of the morphological features and molecular evidence. The taxon *S. macrobasidium* is characterized by the coriaceous basidiomata with a cream surface, cylindrical basidia, straight, 4-celled, subglobose or ovoid probasidia and thin-walled, narrowly cylindrical basidiospores with septa, measuring as 7–9 × 3.5–4.5 µm, the haustoria consisting of irregularly coiled hyphae; in addition, this fungus was found associated with the insect of Diaspididae. The species *S. puerense* is characterised by resupinate coriaceous basidiomata with a cinnamon brown to chestnut brown surface, cylindrical or slightly irregular basidia, 2-3-celled, slightly curved, subglobose to pyriform probasidia, probasidia cell persistent after the formation of the basidia and the haustoria with two types consisting of irregularly coiled hyphae and spindle-shape. The fungus was found associated with the insect species *Pseudaulacaspis pentagona*. The species *S. wuliangshanense* is characterised by the coriaceous basidiomata with a slightly brown surface, cylindrical or slightly irregular basidia, 2-3-celled, straight or slightly curved, pyriform, subglobose or ovoid and probasidia, haustoria consisting of irregularly coiled hyphae, associated with the insect genus *Aulacaspis*. Sequences of internal transcribed spacer region (ITS) were analysed maximum likelihood, maximum parsimony and Bayesian inference methods. The new species *S. macrobasidium* was clustered with *S. maesae*. Furthermore, *S. puerense* was retrieved as a sister to *S. carestianum*. The phylogenetic tree, inferred from the ITS sequences, highlighted that *S. wuliangshanense* was the sister to *S. aquilariae* with strong supports. Application of PHI test to the ITS tree-locus sequences revealed no recombination level within phylogenetically related species.

Key words: Forest disease, phylogenetic analyses, taxonomy, wood-inhabiting fungi, Yunnan Province

OPEN ACCESS

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: 22 April 2024
Accepted: 28 November 2024
Published: 30 December 2024

Citation: Jiang Q, Kang Z, Wang X, Zhao C (2024) Molecular phylogeny and morphology reveal three new plant pathogenic fungi species (Septobasidiales, Basidiomycota) from China. MycoKeys 111: 229–248. https://doi.org/10.3897/mycokeys.111.125933

Introduction

Fungi are a diverse, monophyletic group of eukaryotes and these organisms show immense ecological and economic impacts for playing an important role in the ecosystems as diverse as soil, trees, with hidden layers within their substrate

(James et al. 2020). Approximately 150 thousand species of fungi have been described (Cui et al. 2019; Dai et al. 2021; Wang and Zhao 2021; Sun et al. 2022; Liu et al. 2023). But the potential biodiversity of the group is likely to be 2.2–3.8 millions of species (Blackwell 2011; Taylor et al. 2014; Hibbett 2016; Dong et al. 2023). The diversity for flora of seed plants in Yunnan Province is higher than that in other areas of China. The number of discovered new fungal species totalled 1,345 from this province from 2000 to 2020 years. Endemic woody plants are rich in Yunnan, supplying rich and varied substrates for wood-decaying fungi. The pathogenic fungi with industrial, medicinal and economic value, comprise most basidiomycetes and ascomycetes growing on various kinds of living trees (Dai et al. 2015, 2021; Vinay et al. 2015; Wu et al. 2019; Luo et al. 2022).

Mutualistic and parasitic symbioses between fungi and plants are widely acknowledged to have profound influences on the evolution and ecology of terrestrial life, but less well-known are the symbioses between fungi and insects (Henk and Vilgalys 2007). The insects are protected from their enemies by the fungus and the fungus draws its nutriment from, and is distributed by, the insects. Under natural conditions, whereas the fungus cannot live without the insects, the insects are able to live without the fungus, but the life of insects unprotected by the fungus is precarious (Couch 1938).

Basidiomycete fungi have evolved many symbiotic associations with plants and animals, but fungi in the order Septobasidiales are the only large group of basidiomycetes that are obligately parasitic on insects. Understanding the evolution of insect parasitism and switches from plant parasitism in the Basidiomycota requires a phylogeny to place the order Septobasidiales within the Pucciniomycotina and to determine whether the different forms of insect parasitism in the order Septobasidiales and *Septobasidium* Pat. have a single origin. *Septobasidium* is a type of fungi that has a mutualistic relationship with insects. Although *Septobasidium* sterilize the individuals they parasitize, the fungi may protect uninfected individuals and thereby benefit the population of scale insects (Couch 1938). All fungi in the order Septobasidiales do not display this type of symbiosis. Some may be wholly parasitic because they do not form substantial protective structures. This fungus-insect symbiosis is important because of its unique altruistic and parasitic characteristics and its phylogenetic position within the Basidiomycota (Henk and Vilgalys 2007).

Septobasidium (Septobasidiaceae, Septobasidiales), erected by Patouillard (1892), typed by *Septobasidium velutinum* Pat., which is a large cosmopolitan genus characterised by perennial colonies on the surfaces of plant structures with colonies of scale insects, the basidiomata are usually white to cream, yellowish brown or brown hymenophore, rarely more brightly coloured, normally resupinate and felty in texture; their surfaces may be smooth, warty, or spiny (Patouillard 1892; Couch 1929); a monomitic hyphal system with simple septa, with or without probasidia, 2–4 celled cylindrical, curved, or straight basidia, basidiospores that are hyaline, thin-walled, smooth and cylindrical or fusiform and haustoria consisting of coiled or spindle-shaped hyphae (Ma et al. 2019). In a previous study, Couch (1938) divided it primarily based upon morphological characters related to the structure of the basidia and probasidia. Basidia may be curved or straight and may have one, two, three, or four cells. The probasidia either remains as an empty cell at the base of the mature basidia (persistent) or becomes a spore-bearing cell in the mature basidia (not persistent). Other characters used as indicators of major groups in *Septobasidium* include the layered nature of the thallus and the presence of pillar structures (Oberwinkler 1989). *Septobasidium* occurs on living leaves, stems and branches of a great variety of perennial plants, including gymnosperms, monocots and dicots. Inexperienced collectors may mistake them for corticioid basidiomycetes or even lichens (Couch 1929, 1935, 1938, 1946).

As with most basidiomycetes, basidia are produced so that they project toward the ground. Thus, the resupinate basidiomata often are found on the lower sides of branches. They occur on living rather than dead plant parts and away from the extreme tips of branches which distinguishes them from some resupinate species of Aphyllophorales (Couch 1938; Henk and Vilgalys 2007). About 305 species have been accepted into the genus worldwide (Patouillard 1892; Bresadola and Saccardo 1897; Burt 1916; Lloyd 1919; Couch 1929, 1935, 1938, 1946; Yamamoto 1956; Gómez and Henk 2004; Henk 2005; Lu and Guo 2009a, 2009b, 2010a, 2010b, 2011; Chen and Guo 2011a, 2011b, 2011c, 2012; Lu et al. 2010; Li and Guo 2013, 2014; Ma et al. 2019).

Molecular systematics has played a powerful role in inferring phylogenies within fungal groups since the early 1990s (White et al. 1990; Binder et al. 2013; Dai et al. 2015; Choi and Kim 2017). Classification of the kingdom of fungi has been updated continuously, based on the frequent inclusion of data from DNA sequences in many phylogenetic studies (Wijayawardene et al. 2020). However, molecular studies involving *Septobasidium* are rare (Henk and Vilgalys 2007; Zhao et al. 2017). One phylogenetic study of a single origin of insect symbiosis in the class Pucciniomycetes suggested that there is little or no support for *Septobasidium* as a monophyletic group (Henk and Vilgalys 2007). The previous study introduced a six-gene phylogenetic overview of Basidiomycota and allied phyla and confirmed that *S. carestianum* Bres. nested within the order Septobasidiales and grouped with *Helicobasidium mompa* Nobuj. Tanaka and *Thanatophytum crocorum* (Pers.) Nees (Zhao et al. 2017).

During investigations into the wood-inhabiting fungi in Pu’er, Yunnan of China, samples representing three additional species belonging to genus *Septobasidium* were collected. Three new *Septobasidium* taxa were found that could not be assigned to any described species. To clarify the placement and relationships of these specimens, we carried out a phylogenetic and taxonomic study based on the ITS sequences. A description, illustrations, and phylogenetic analysis results of the new species are provided.

Materials and methods

Sample collection and herbarium specimen preparation

Fresh fruiting bodies of fungi growing on angiosperm branches were collected from Pu’er of Yunnan Province, P.R. China. The samples were photographed in situ and fresh macroscopic details were recorded. Photographs were recorded using a Jianeng 80D camera (Tokyo, Japan). All of the photos were stacked and merged using Helicon Focus Pro 7.7.5 software. Specimens were dried in an electric food dehydrator at 40 °C and then sealed and stored in an envelope bag and deposited in the herbarium of the Southwest Forestry University (SWFC), Kunming, Yunnan Province, P.R. China.

Morphology

Macromorphological descriptions are based on field notes and photos captured in the field and lab. Color terminology follows Petersen (1996). Micromorphological data were obtained from the dried specimens when observed under a light microscope following the previous study (Ma et al. 2019). The following abbreviations are used: L = mean spore length (arithmetic average for all spores), W = mean spore width (arithmetic average for all spores), Q = variation in the L/W ratios between the specimens studied and n = a/b (number of spores (a) measured from given number (b) of specimens).

DNA extraction and sequencing

Genomic DNA was obtained from dried specimens using the EZNA HP Fungal DNA Kit, according to the manufacturer's instructions with some modifications. A small piece of dried fungal specimen (about 30 mg) was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer and incubated in a 65 °C water bath for 60 min. After that, 0.4 ml phenol-chloroform (24: 1) was added to each tube and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. The mixture was then transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube and 100 ml elution buffer was added to the middle of adsorbed film to elute the genomic DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White et al. 1990). The PCR procedure was as follows: initial denaturation at 95 °C for 3 min; followed by 35 cycles of 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 products were purified and directly sequenced at barium Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (https://www.ncbi.nlm.nih.gov/genbank/) (Table 1).

Phylogenetic analyses

The sequences were aligned in MAFFT version 7 using the G-INS-i strategy (Katoh et al. 2019). The alignment was manually adjusted using AliView version 1.27 (Larsson 2014). Sequences of *Helicobasidium mompa* and *Pachnocybe ferruginea* Berk. obtained from GenBank were used as an outgroup to root trees following Henk and Vilgalys (2007) in the ITS analysis (Fig. 1).

Maximum parsimony (MP), maximum likelihood (ML) and Bayesian Inference (BI) analyses were applied to the three combined datasets. The phylogenetic analysis method was adopted by previous study (Zhao and Wu 2017; Yang et al. 2023; Dong et al. 2024). MP analysis was performed in PAUP* version 4.0b10 (Swofford 2002). All of the characteristics 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

Table 1. List of species, specimens and GenBank accession numbers of sequences used in this study. [* Indicates type materials].

Species	Specimen No.	GenBank accession No.	Preference
		ITS	
Helicobasidium mompa	DAH h1	DQ241472	Henk and Vilgalys 2007
Pachnocybe ferruginea	DAH pf1	DQ241473	Henk and Vilgalys 2007
Septobasidium alni	DAH FP3	DQ241441	Henk and Vilgalys 2007
S. aquilariae	CLZhao 6610	MK804520	Ma et al. 2019
S. aquilariae	CLZhao 6611	MK804521	Ma et al. 2019
S. aquilariae	CLZhao 6612	MK804522	Ma et al. 2019
S. aquilariae	CLZhao 6613	MK804523	Ma et al. 2019
S. aquilariae	CLZhao 6614	MK804524	Ma et al. 2019
S. arachnoideum	DAH 025	DQ241443	Henk and Vilgalys 2007
S. bogoriense	998434	HM209414	Lee et al. 2023
S. broussonetiae	998436	HM209416	Unpublished
S. burtii	DAH 062	DQ241444	Henk and Vilgalys 2007
S. canescens	DAH 323	DQ241446	Henk and Vilgalys 2007
S. carestianum	DJM 644	DQ241448	Henk and Vilgalys 2007
S. castaneum	DAH 052	DQ241447	Henk and Vilgalys 2007
S. cavarae	DJM FP1	DQ241445	Henk and Vilgalys 2007
S. fumigatum	DAH 005	DQ241451	Henk and Vilgalys 2007
S. gomezii	DAH 031	DQ241462	Henk and Vilgalys 2007
S. grandisporum	DAH 065	DQ241453	Henk and Vilgalys 2007
S. griseum	DAH 016	DQ241454	Henk and Vilgalys 2007
S. hainanense	998437	HM209417	Lee et al. 2023
S. kameii	998432	HM209412	Lee et al. 2023
S. macrobasidium	**CLZhao 9624***	**PP532758**	**Present study**
S. macrobasidium	**CLZhao 9658**	**PP532759**	**Present study**
S. maesae	998433	HM209413	Unpublished
S. marianiae	LJF 7006	MK809161	Henk and Vilgalys 2007
S. marianiae	DAH 283b	DQ241456	Henk and Vilgalys 2007
S. michelianum	DAH FP5	DQ241457	Henk and Vilgalys 2007
S. pallidum	998435	HM209415	Unpublished
S. pilosum	DAH 020	DQ241458	Henk and Vilgalys 2007
S. pinicola	DAH 013	DQ241459	Henk and Vilgalys 2007
S. pseudopedicellatum	DAH 044	DQ241460	Lee et al. 2023
S. puerense	**CLZhao 9430***	**PP532760**	**Present study**
S. puerense	**CLZhao 4298**	**PP532761**	**Present study**
S. ramorum	DAH 045a	DQ241450	Lee et al. 2023
S. septobasidioides	DAH 032	DQ241461	Henk and Vilgalys 2007
S. sinuosum	DAH 036	DQ241464	Henk and Vilgalys 2007
S. taxodii	DAH 194C	DQ241466	Henk and Vilgalys 2007
S. velutinum	DAH 024	DQ241467	Lee et al. 2023
S. westonii	DAH FP2001	DQ241468	Henk and Vilgalys 2007
S. wilsonianum	DAH 037	DQ241469	Henk and Vilgalys 2007
S. wuliangshanense	**CLZhao 5809***	**PP532756**	**Present study**
S. wuliangshanense	**CLZhao 3666**	**PP532757**	**Present study**

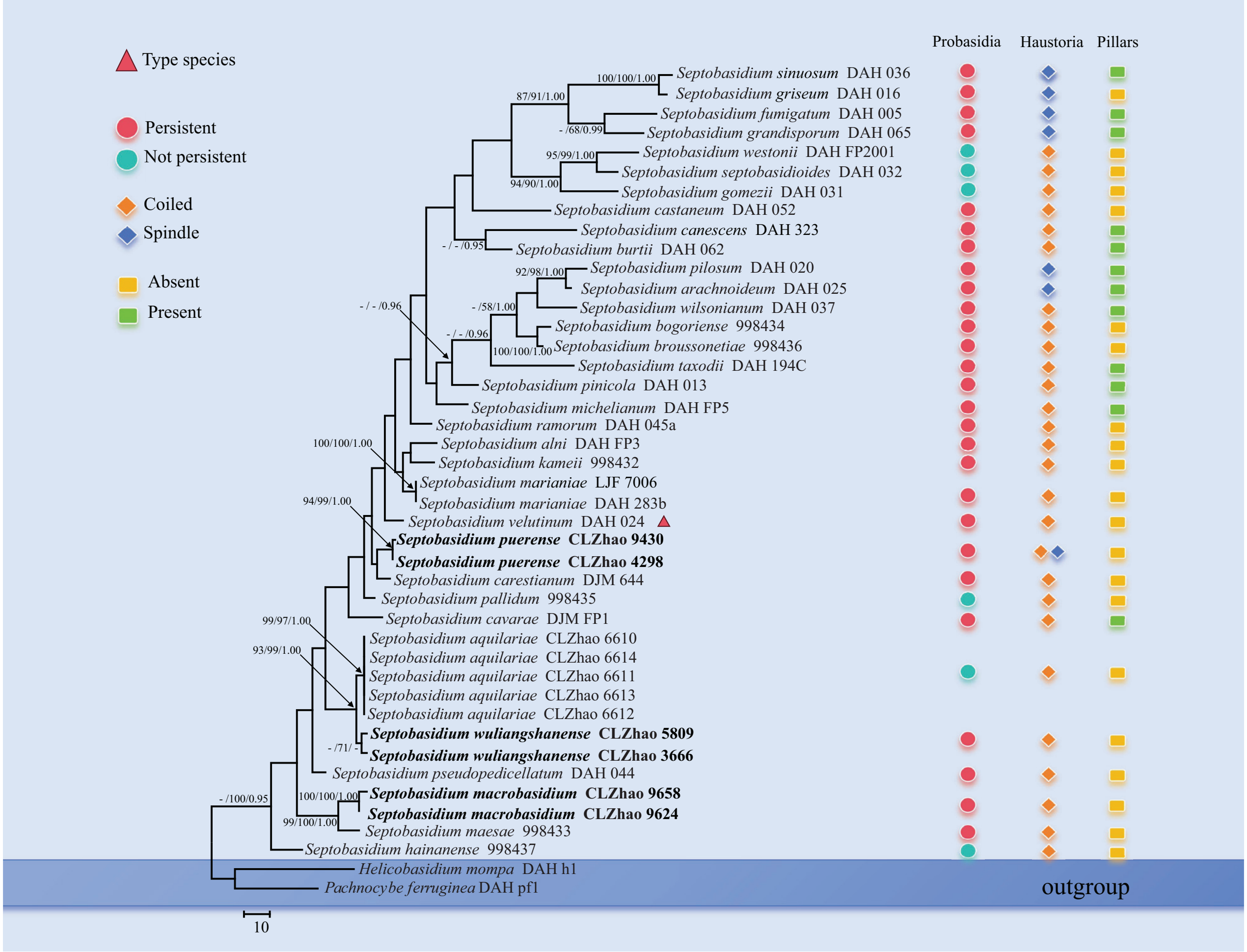

Figure 1. Maximum parsimony strict consensus tree illustrating the phylogeny of the three new species and related species in *Septobasidium*. Branches are labelled with maximum likelihood bootstrap values ≥ 70%, parsimony bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.95, respectively.

all most-parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), the consistency index (CI), the retention index (RI), the rescaled consistency index (RC) and the homoplasy index (HI) were calculated for each most-parsimonious tree generated. ML was inferred using RAxML-HPC2 through the CIPRES Science Gateway (Miller et al. 2012). Branch support (BS) for ML analysis was determined using 1,000 bootstrap replicates and evaluated under the gamma model.

MrModeltest 2.3 (Posada and Crandall 1998; Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes3.1.2 with a general time reversible (GTR+G+I) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist et al. 2012). Four Markov chains were run for 2 runs from random starting trees for 2.9 million generations (Fig. 1) and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches were considered as significantly supported if they received maximum likelihood bootstrap (ML) ≥ 70%, maximum parsimony bootstrap (MP) ≥ 50%, or Bayesian posterior probabilities (PP) ≥ 0.95.

Pairwise homoplasy test

The Genealogical concordance phylogenetic species recognition analysis (GCPSR) is a tool used to check significant recombinant events. The data were analysed using SplitsTree 4 with the pairwise homoplasy Φw, PHI test to determine the recombination level within closely related species (Bruen et al. 2006; Huson and Bryan 2006; Quaedvlieg et al. 2014). One-locus dataset (ITS) with closely related species were used for the analyses. PHI results lower than 0.05 ($\Phi w < 0.05$) indicates a significant recombination is present in the dataset. The relationships between closely related taxa were visualised by constructing split graphs from the concatenated datasets, using the LogDet transformation and splits decomposition options.

Result

Molecular phylogeny

The dataset based on ITS (Fig. 1) comprises sequences from 43 fungal specimens representing 35 species. The alignment length of this dataset is 474 characters, of which 267 characters are constant, 45 characters are variable with no information and 162 characters have no information. Maximum parsimony analysis yielded three equally parsimonious trees (TL = 967, CI = 0.3516, HI = 0.6484, RI = 0.4543, RC = 0.1597). Bayesian analysis and ML analysis resulted in a similar topology as MP analysis with an average standard deviation of split frequencies of 0.007729 (BI) and the effective sample size (ESS) across the two runs is double the average ESS (avg. ESS) = 861.

The phylogenetic tree (Fig. 1), inferred from the ITS sequences, highlighted that *Septobasidium macrobasidium* was clustered with *S. maesae* C.X. Lu & L. Guo. Furthermore, *S. puerense* was retrieved as a sister to *S. carestianum*. The new species *S. wuliangshanense* was the sister to *S. aquilariae* C.L. Zhao with strong supports.

Application of PHI test to the ITS tree-locus sequences revealed no recombination level within phylogenetically related species. The test results of ITS sequence dataset show $\Phi w = 0.5697$ ($\Phi w > 0.05$) no recombination is present in the new species *Septobasidium wuliangshanense* with *S. aquilariae*, *S. cavarae* Bres. and *S. pseudopedicellatum* Burt (Fig. 2).

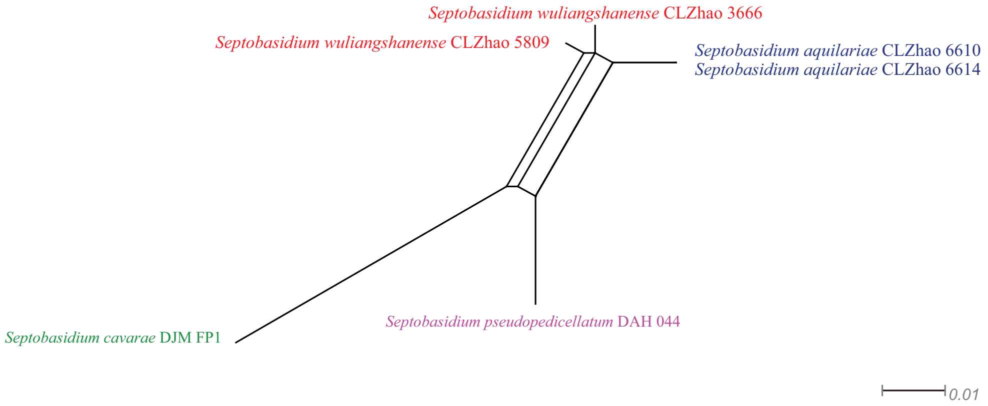

Figure 2. Split graphs showing the results of PHI test for the ITS data of *Septobasidium wuliangshanense* and closely related taxa using LogDet transformation and splits decomposition. PHI test results $\Phi w \leq 0.05$ indicate that there is significant recombination within the dataset. New taxa are in red while closely related species to new species are in other colours.

Taxonomy

Septobasidium macrobasidium **Q.Q. Jiang & C.L. Zhao, sp. nov.**

MycoBank No: 853680
Figs 3, 4

Holotype. China • Yunnan Province, Pu'er, Jingdong County, Wuliangshan National Nature Reserve, 24°29'17"N, 100°40'27"E, altitude: 1800 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 6 January 2019, CLZhao 9624 (SWFC).

Diagnosis. Differs from other *Septobasidium* species by the coriaceous basidiomata with a cream surface, cylindrical basidia (48.5–83 × 6.5–13 μm), and thin-walled, narrowly cylindrical basidiospores with septa, measuring as 7–9 × 3.5–4.5 μm.

Etymology. *Macrobasidium* (Lat.): refers to the larger basidia of the type specimen.

Description. Basidiomata perennial, resupinate, easy to separate from substrate, coriaceous upon drying, up to 9 cm long, 5 cm wide, 2 mm thick. Hymenial surface smooth, slightly cream when fresh, cream upon drying. Sterile margin narrow, white to cream, up to 0.5 mm.

Hyphal system monomitic, generative hyphae with simple septa, pale brown, thick-walled, frequently branched, interwoven. In section 850–1850 μm thick; subiculum pale brown, 30–80 μm thick; pillars brown, 350–550 μm high, 85–200 μm wide, hyphae of pillars 2–5 μm thick, hyaline or brown, forming 2–3 horizontal layers.

Basidia arising directly from the generative hyphae, basidia cylindrical, straight, 4-celled, 48.5–83 × 6.5–13 μm, colourless. Probasidia subglobose or ovoid, 13–23 × 7–20.5 μm, colourless, persistent. Haustoria consisting of irregularly coiled hyphae.

Basidiospores narrowly cylindrical, colourless, thin-walled, with septa, (6.5–)7–9(–9.5) × (3–)3.5–4.5(–5) μm, L = 8.09 μm, W = 3.94 μm, Q = 1.55–2.83 (n = 60/2).

Habitat and distribution. Growing on the plant Betulaceae Gray, associated with the insect of Diaspididae Ferris.

Additional specimen examined (*paratype*). China • Yunnan Province, Pu'er, Jingdong County, Wuliangshan National Nature Reserve, 24°29'17"N, 100°40'27"E, altitude: 1800 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 6 January 2019, CLZhao 9658 (SWFC).

Septobasidium puerense **Q.Q. Jiang & C.L. Zhao, sp. nov.**

MycoBank No: 853681
Figs 5, 6

Holotype. China • Yunnan Province, Pu'er, Jingdong County, The Forest of Pineapple, 24°30'58"N, 100°52'31"E, altitude: 2000 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 4 January 2019, CLZhao 9430 (SWFC).

Diagnosis. Differs from other *Septobasidium* species by a cinnamon brown to chestnut brown surface, subglobose to pyriform probasidia (10.5–19.5 × 5.5–9 μm), and two types of haustoria consisting of irregularly coiled hyphae and spindle-shape.

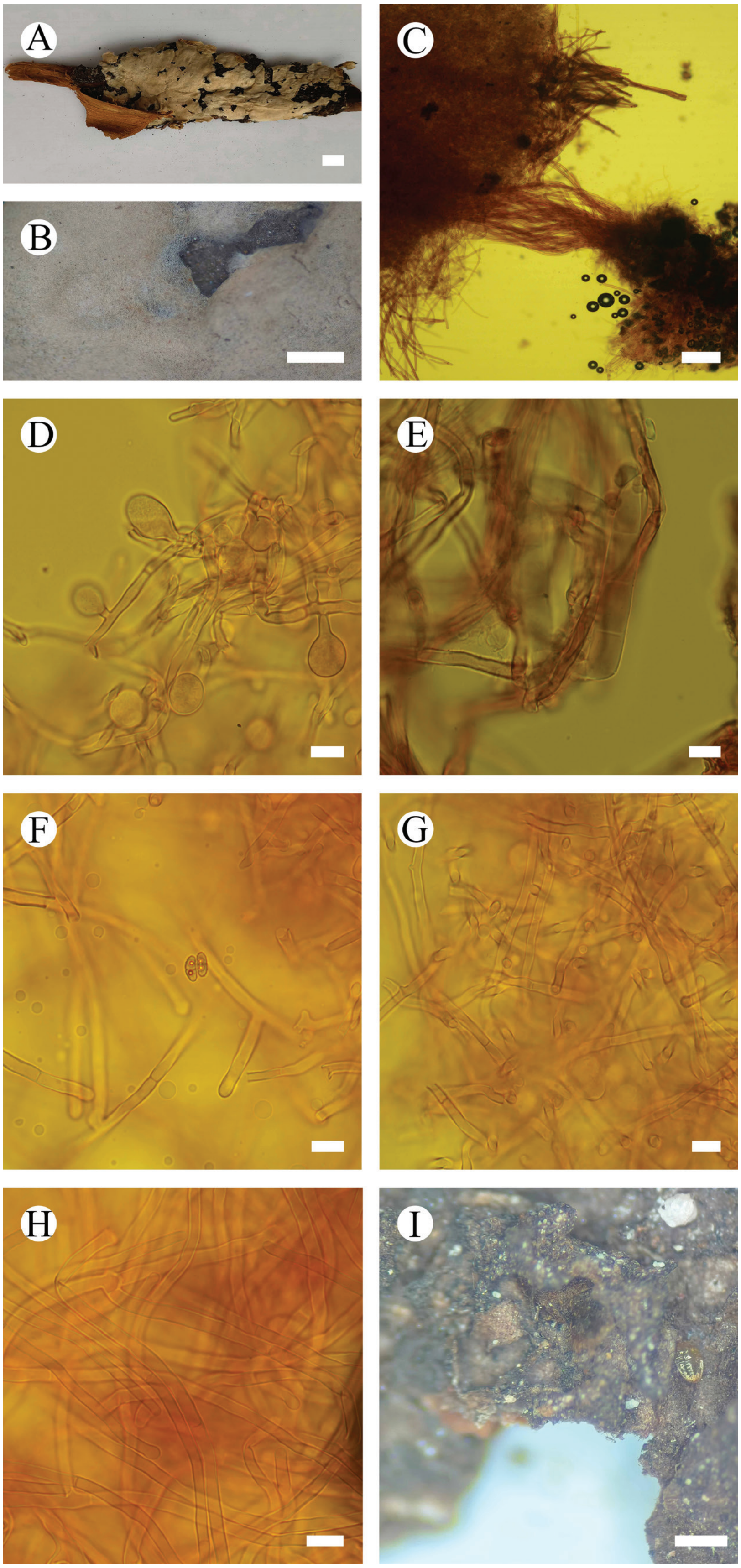

Figure 3. *Septobasidium macrobasidium* (holotype, CLZhao 9624) **A, B** basidiomata on branch **C** sections of basidiomata **D** probasidia **E** basidia **F** basidiospore **G** haustoria **H** hyphae **I** scale insect on branches. Scale bars: 1 cm (**A**); 1 mm (**B**); 100 µm (**C**); 10 µm (**D–H**); 1 mm (**I**).

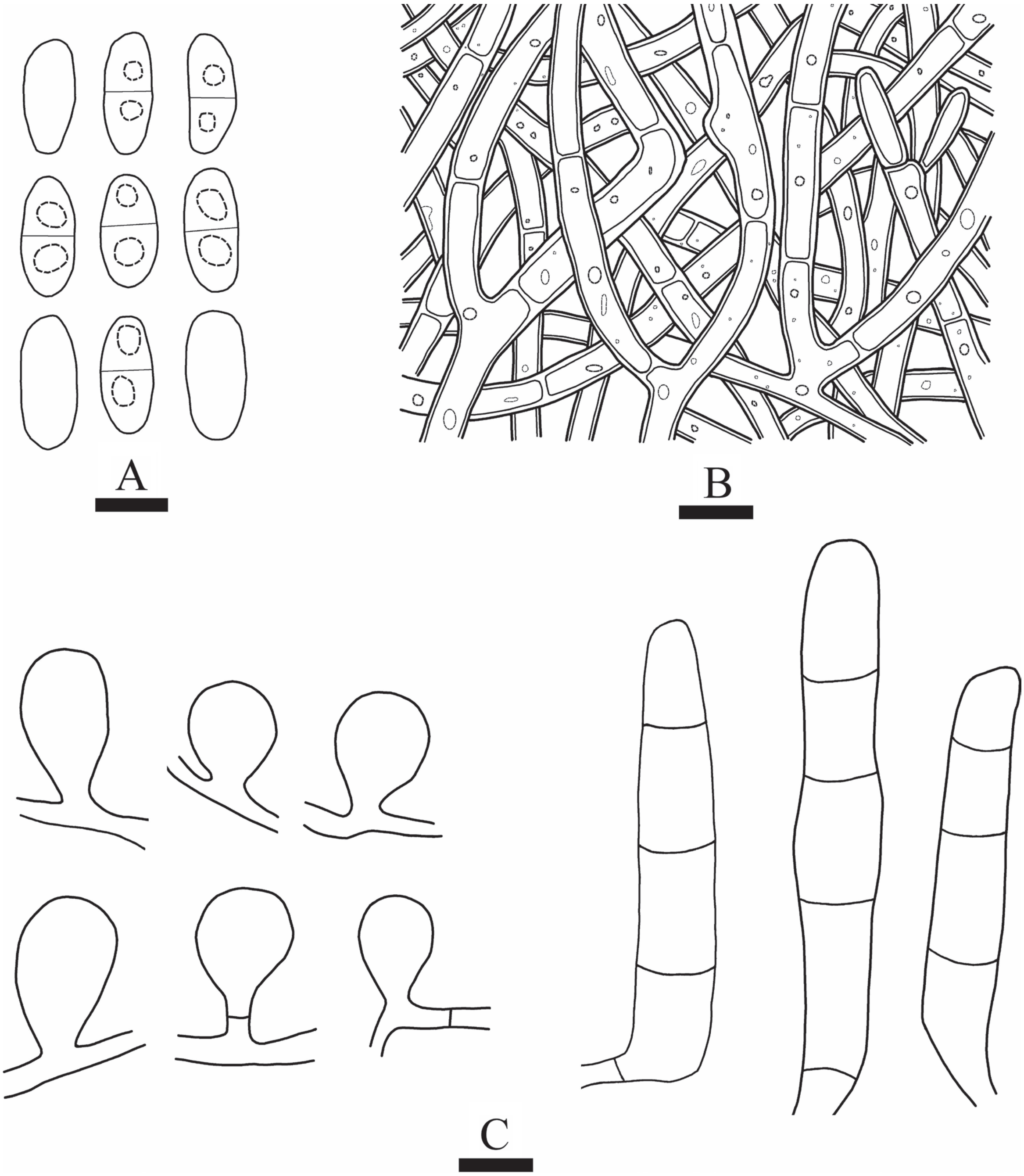

Figure 4. Microscopic structures of *Septobasidium macrobasidium* (holotype, SWFC 9624) **A** basidiospores **B** generative hyphae from hyphal layer **C** probasidia and basidia. Scale bars: 5 µm (**A**); 10 µm (**B, C**).

Etymology. *Puerense* (Lat.): refers to the locality (Pu'er) of the type specimen.

Description. Basidiomata perennial, resupinate, hard to separate from substrate, coriaceous upon drying, up to 15 cm long, 1 cm wide,1 mm thick. Hymenial surface smooth, pale brown when fresh, cinnamon brown to chestnut brown upon drying. Sterile margin slightly brown, up to 1 mm.

Hyphal system monomitic, generative hyphae with simple septa, pale brown, thick-walled. In section 380–650 µm thick; subiculum pale brown, 10–30 µm thick; pillars brown, 170–380 µm high, 40–85 µm wide, hyphae of pillars 1.5–3.5 µm thick, colorless, with closely packed parallel upright threads, forming 2–3 horizontal layers.

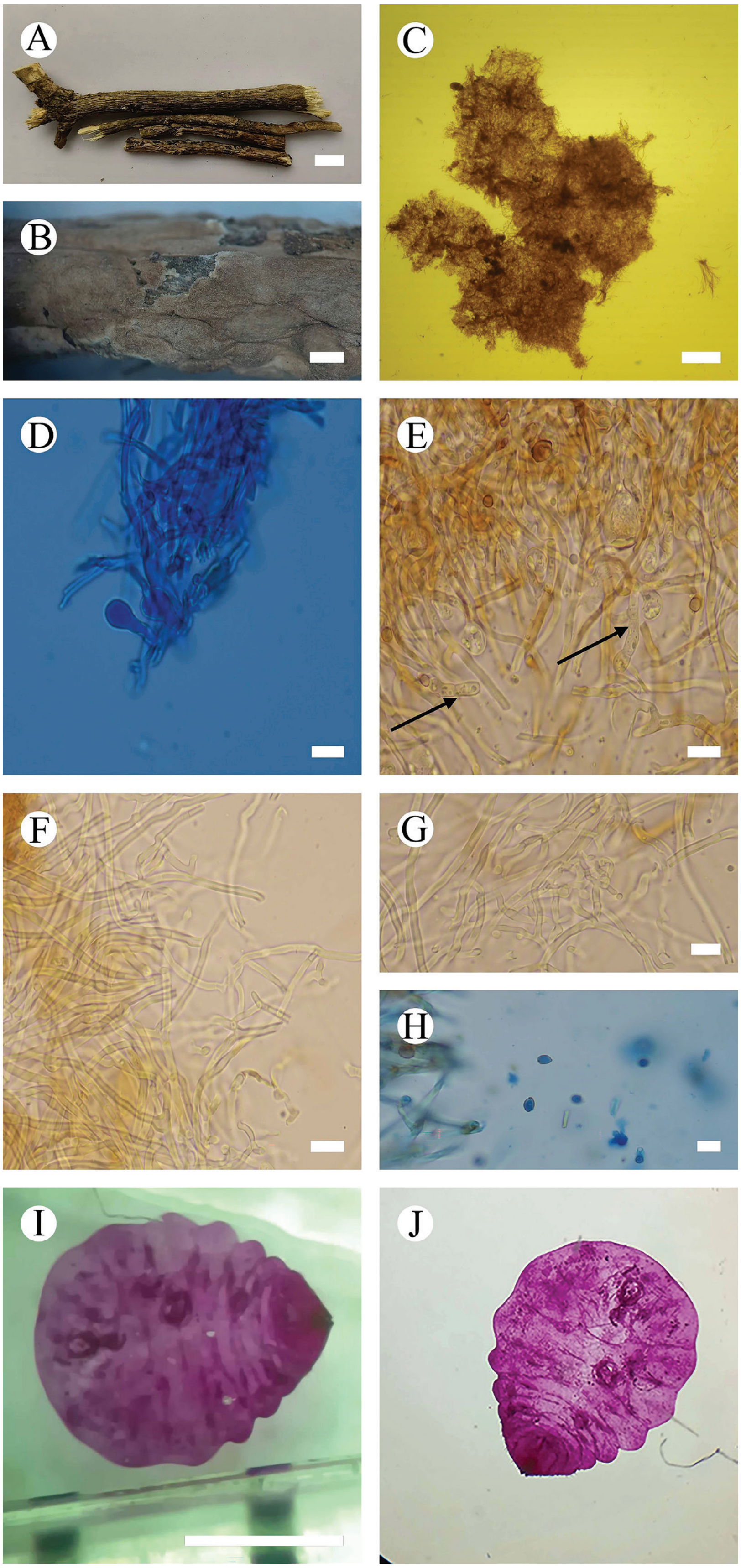

Figure 5. *Septobasidium puerense* (holotype, CLZhao 9430) **A, B** basidiomata on branch **C** sections of basidiomata **D** probasidia **E** basidia (arrow) **F** hyphae **G** haustoria consisting of irregularly coiled hyphae **H** the spindle-shaped haustoria **I, J** scale insect on branches. Scale bars: 1 cm (**A**); 1 mm (**B**); 100 μm (**C**); 10 μm (**D–H**); 1 μm (**I**).

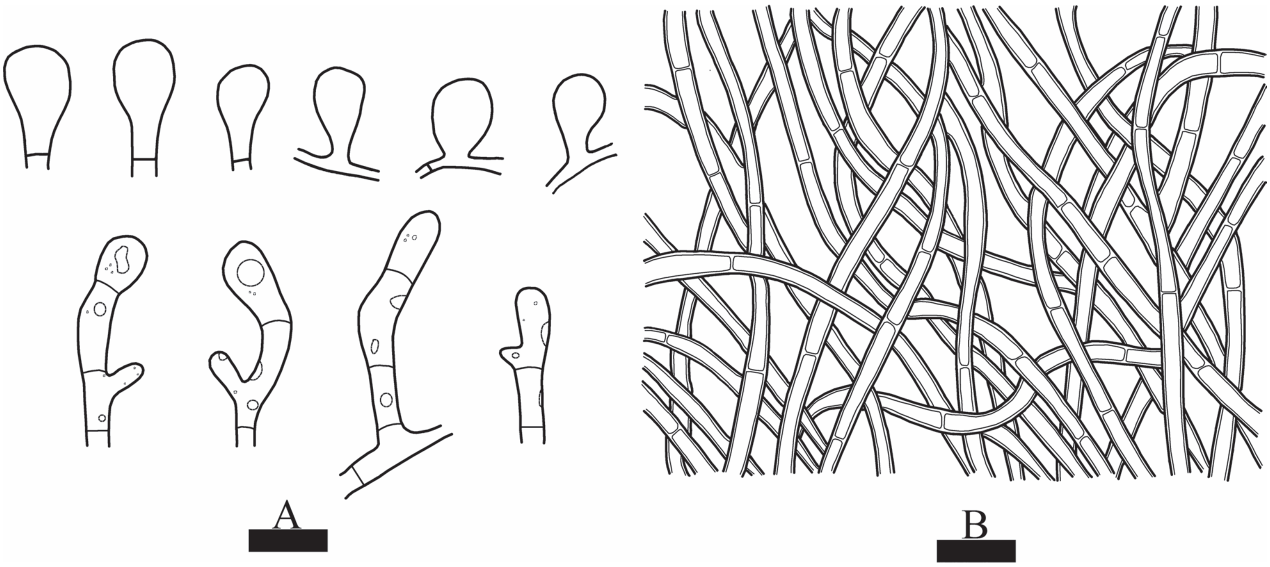

Figure 6. Microscopic structures of *Septobasidium puerense* (holotype, CLZhao 9430) **A** probasidia and basidia **B** generative hyphae from hyphal layer. Scale bars: 10 µm (**A, B**).

Basidia arising directly from the generative hyphae, cylindrical or slightly irregular, slightly curved, 2-3-celled, 17–30.5 × 3–6.5 µm, colourless. Probasidia subglobose to pyriform, 10.5–19.5 × 5.5–9 µm, colorless, probasidia cell persistent after the formation of the basidia. Basidiospores not seen. Haustoria with two types: 1) consisting of irregularly coiled hyphae; 2) spindle-shape.

Habitat and distribution. Growing on the plant Berberidaceae Juss, associated with the insect species *Pseudaulacaspis pentagona* (Targioni Tozzetti).

Additional specimen examined (*paratype*). China • Yunnan Province, Pu'er, Jingdong Country, Wuliangshan National Nature Reserve, 24°29'17"N, 100°40'27"E, altitude: 1800 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 5 October 2017, CLZhao 4298 (SWFC).

Septobasidium wuliangshanense Q.Q. Jiang & C.L. Zhao, sp. nov.

MycoBank No: 853679
Figs 7, 8

Holotype. China • Yunnan Province, Pu'er, Zhenyuan County, Heping Town, Liangzi Village, Wuliangshan National Nature Reserve, 24°29'17"N, 100°40'27"E, altitude: 1860 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 15 January 2018, CLZhao 5809 (SWFC).

Diagnosis. Differs from other *Septobasidium* species by the coriaceous basidiomata with a slightly brown surface, cylindrical or slightly irregular basidia, pyriform to subglobose or ovoid probasidia (7.5–13 × 4.5–9 µm), and the haustoria consisting of irregularly coiled hyphae.

Etymology. *Wuliangshanense* (Lat.): refers to the locality (Wuliangshan) of the type specimen.

Description. Basidiomata perennial, resupinate, easy to separate from substrate, coriaceous upon drying, up to 10 cm long, 2 cm wide, 1 mm thick. Hymenial surface smooth, cream to pale brown when fresh, slightly brown upon drying. Sterile margin cream to slightly brown, up to 2 mm.

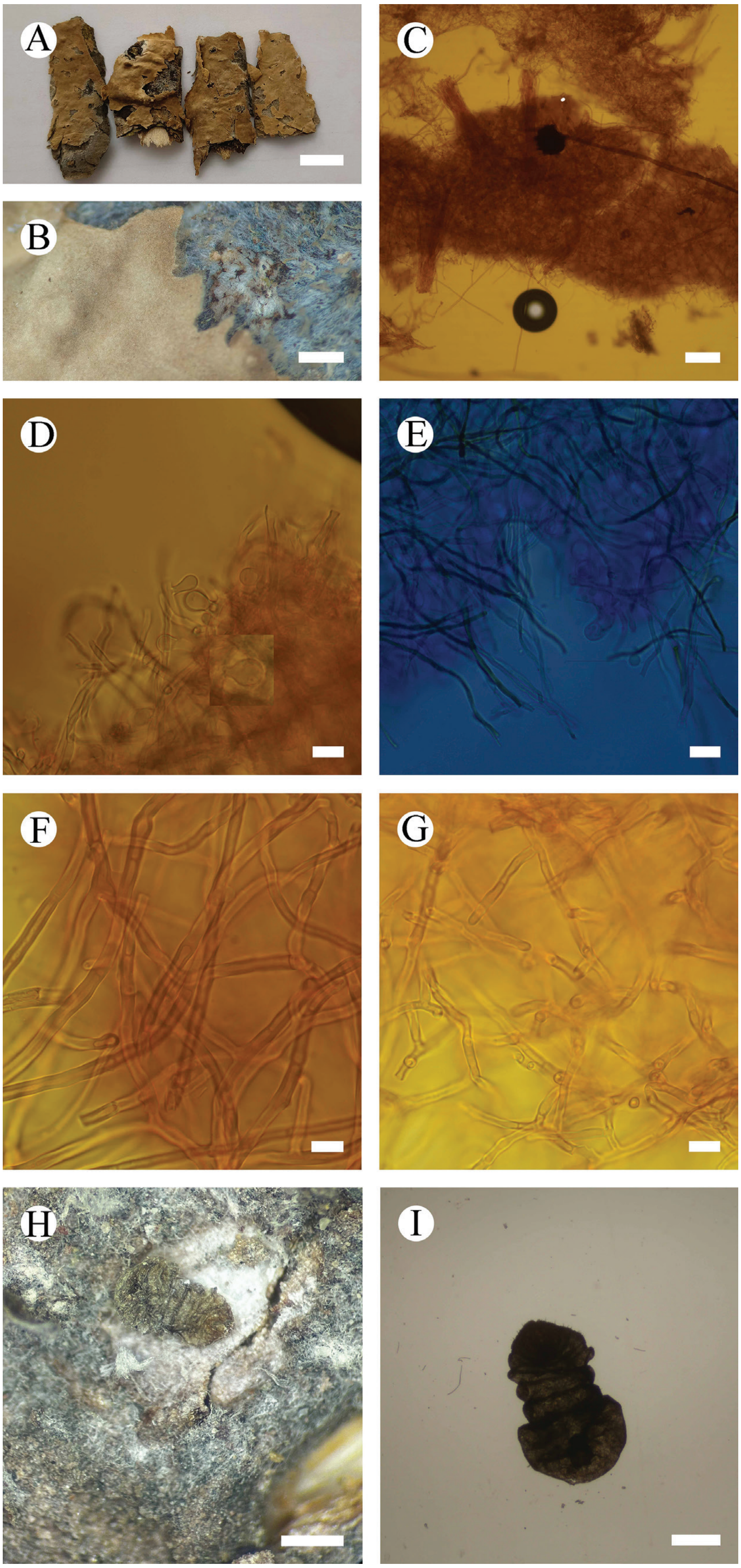

Figure 7. *Septobasidium wuliangshanense* (holotype, CLZhao 5809) **A, B** basidiomata on branch **C** sections of basidiomata **D** probasidia (arrow) **E** basidium (arrow) **F** hyphae **G** haustoria **H, I** scale insect on branches. Scale bars: 1 cm (**A**); 1 mm (**B**); 100 μm (**C**); 10 μm (**D–G**); 1 mm (**H**); 10 mm (**I**).

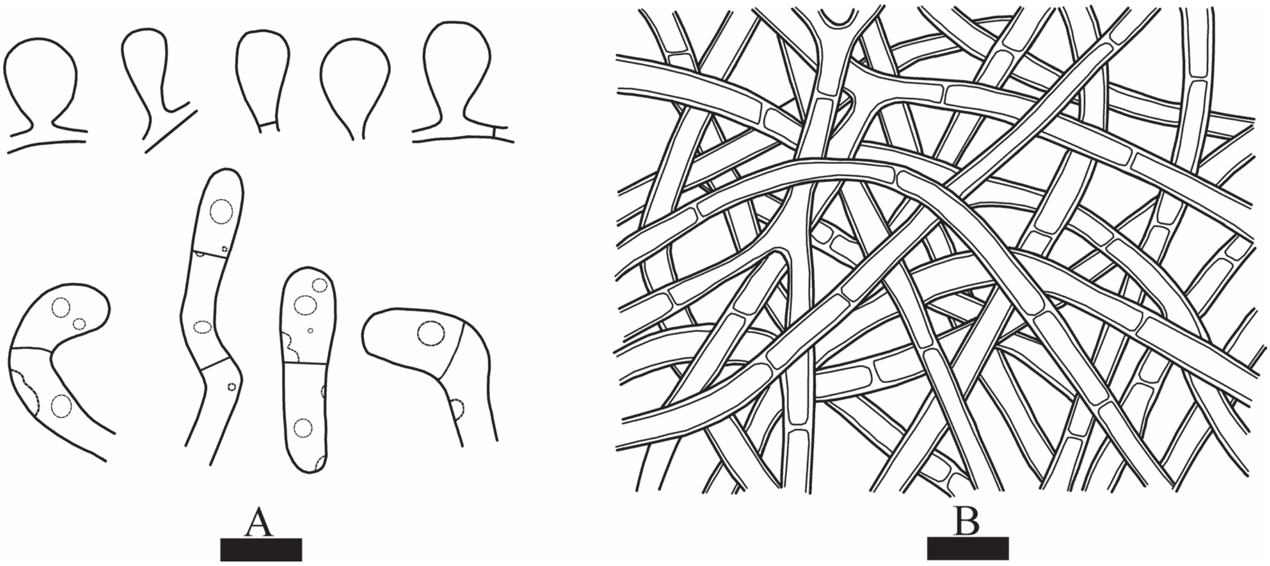

Figure 8. Microscopic structures of *Septobasidium wuliangshanense* (holotype, CLZhao 5809) **A** probasidium and basidia **B** generative hyphae from hyphal layer. Scale bars: 10 μm (**A, B**).

Hyphal system monomitic, generative hyphae with simple septa, pale brown, thick-walled. In section 660–1200 μm thick; subiculum pale brown, 20–50 μm thick; pillars brown, 150–300 μm high, 30–150 μm wide, hyphae of pillars 3–4 μm thick, brown, forming 2–3 horizontal layers.

Basidia arising directly from the generative hyphae, cylindrical or slightly irregular, colourless, straight or slightly curved, 2-3-celled, 21.5–29 × 5.5–9 μm. Probasidia pyriform, subglobose or ovoid, 7.5–13 × 4.5–9 μm, colorless, probasidia cell persistent after the formation of the basidia. Haustoria consisting of irregularly coiled hyphae. Basidiospores not seen.

Habitat and distribution. Growing on the plant Fagaceae Dumort, associated with the insect genus *Aulacaspis* Cockerell.

Additional specimen examined (*paratype*). CHINA • Yunnan Province, Pu'er, Jingdong County, Wuliangshan National Nature Reserve, in association with the genus *Aulacaspis* on Rosaceae, 24°29'17"N, 100°40'27"E, altitude: 1800 m a.s.l., on the living tree of angiosperm, leg. C.L. Zhao, 2 October 2017, CLZhao 3666 (SWFC).

Discussion

Many recently described wood-inhabiting fungal taxa have been reported worldwide, including in the genus *Septobasidium* (Patouillard 1892; Bresadola and Saccardo 1897; Burt 1916; Lloyd 1919; Couch 1929, 1935, 1938, 1946; Yamamoto 1956; Gómez and Henk 2004; Henk 2005; Chen and Guo 2009a, 2009b, 2010, 2011a, 2011b, 2011c, 2012; Lu and Guo 2009a, 2009b, 2010a, 2010b 2011, 2013, 2014; Lu et al. 2010). The diversity of *Septobasidium* is rich in China. Prior to this study, the following 58 *Septobasidium* species were reported from China, especially in subtropics and tropics (Lu and Guo 2009a, 2009b, 2010a, 2010b, 2011; Lu et al. 2010; Chen and Guo 2011a, 2011b, 2011c, 2012; Li and Guo 2013, 2014; Ma et al. 2019). Several *Septobasidium* species have been described from Yunnan Province (Lu and Guo 2010b, 2011; Ma et al. 2019). In the present study, three new species, *S. macrobasidium*, *S. puerense* and *S. wuliangshanense* are described based on phylogenetic analyses and morphological characteristics. In addition, the PHI test (Fig. 2) was carried out to confirm that there is no recombination present in the new species *S. wuliangshanense* compared with closely related taxa.

Based on ITS topology (Fig. 1), the phylogenetic tree includes the type species *Septobasidium velutinum*, which is collected from Costa Rica and most species of this genus have persistent probasidia, except for *S. aquilariae*, *S. gomezii* Henk, *S. hainanense* C.X. Lu & L. Guo, *S. pallidum* Couch, *S. septobasidioides* (Henn.) Höhn. & Litsch. and *S. westonii* Couch. Most *Septobasidium* species have irregularly coiled haustoria and the spindle-shaped haustoria of *S. fumigatum* Burt, *S. griseum* Couch, *S. grandisporum* Couch, *S. pilosum* Boedijn & B.A. Steinm and *S. sinuosum* Couch. However, *S. puerense* has two types of haustoria: 1) consisting of irregularly coiled hyphae; 2) spindle-shape. Most *Septobasidium* species have pillars, except for *S. arachnoideum* (Berk. & Broome) Bres., *S. burtii* Lloyd, *S. canescens* Burt, *S. cavarae*, *S. fumigatum*, *S. grandisporum*, *S. michelianum* (Caldesi) Pat., *S. pilosum*, *S. pinicola* Snell, *S. sinuosum*, *S. taxodii* Couch and *S. wilsonianum* L.D. Gómez & Kisim.-Hor.

Phylogenetically, based on the ITS topology (Fig. 1), *Septobasidium macrobasidium* is clustered with *S. maesae*. The new species *S. puerense* is closely related to *S. carestianum* and *S. wuliangshanense* is sister to *S. aquilariae* with strong supports. But morphologically *S. maesae* differs from *S. macrobasidium* by its greyish-brown hymenial surface and smaller basidia (28–55 × 7.5–11.5 μm), brown, fusiform basidiospore without septa (18–19.5 × 4–5 μm; Lu and Guo 2009a). The species *S. carestianum* differs from *S. puerense* by its iuventute avellaneas deinde cinnamomeas, superficiei sub lente pruinosula hymenial surface and the larger sphaerica probasidia (15.1–11.3 μm) and 4-celled, apicem acuta, longer cylindrica basidia in senectute brunnea (62–71 × 5–6.7 μm), ellipticae and flexae sporae (21–23 × 4.2–5 μm; Gómez and Henk 2004). The species *S. aquilariae* differs from *S. wuliangshanense* by its smaller basidia (15–26.5 × 4–6 μm) and without a probasidia cell and reniform basidiospores (11–19 × 4–7.5 μm), habitat and distribution growing in association with *Pseudaulacaspis* sp. on *Aquilaria sinensis* (Lour.) Spreng. (Ma et al. 2019). Further, application of PHI test to the ITS tree-locus sequences revealed no recombination level within phylogenetically in these two species (Fig. 2).

Morphologically, *Septobasidium cokeri* Couch. differs from *S. macrobasidium* by its pure white hymenial surface and restricted growth on *Quercus rubra* L. (Gómez and Henk 2004). The species *S. hainanense* differs in its purple hymenial surface and smaller basidia (25–36 × 7–13 μm; Lu and Guo 2010a). The taxon *S. maesae* differs by its perennial basidiomata peeled off after maturity and smaller basidia (28–55 × 7.5–11.5 μm; Lu and Guo 2009a).

Septobasidium guangxiense Wei Li bis & L. Guo differs from *S. puerense* in its yellowish brown hymenial surface with numerous fissures at maturity and larger basidia (27–38 × 5–10 μm; Li and Guo 2014). The species *S. hoveniae* Wei Li bis, S.Z. Chen, L. Guo & Yao Q. Ye differs in its cinnamon-brown hymenial surface and growth on *Hovenia acerba* (Li and Guo 2013; *S. polygoni* C.X. Lu & L. Guo differs in its white to cinnamon-brown hymenial surface and growth on *Polygonum campanulatum* Hook. f. (Lu and Guo 2010b); *S. reevesiae* S.Z. Chen & L. Guo differs in its thicker section (1.65–2.20 mm) and larger basidia (37–55 × 8–13 μm) and growth on *Reevesia longipetiolata* Merr. & Chun (Chen and Guo 2012) and *S. transversum* Wei Li bis & L. Guo differs in its cinnamon-brown basidiomata, its transverse layer at the pillar bases and larger basidia (42–60 × 9–16 μm; Li and Guo 2014).

Morphologically, several species of *Septobasidium broussonetiae* C.X. Lu, L. Guo & J.G. Wei, *S. brunneum* Wei Li bis & L. Guo, *S. capparis* S.Z. Chen & L. Guo, *S. euryae-groffii* C.X. Lu & L. Guo, *S. fissuratum* Wei Li bis & L. Guo and *S. gaoligongense* C.X. Lu & L. Guo are similar to *S. wuliangshanense* were found in China. However, *S. broussonetiae* is distinguished by its cracking basidiomata and growth on *Broussonetia papyrifera* (L.) L'Hér. ex Vent. (Lu et al. 2010); *S. brunneum* differs in its purple-brown hymenial surface with many cracks and growth on *Eurya* sp. (Li and Guo 2014); *S. capparis* differs by its thicker section (less than 2 mm thick) and larger basidia (45–56 × 8–12 μm; Chen and Guo 2012); *S. euryae-groffii* is distinguished by its cinnamon to chestnut brown hymenial surface and growth on *Eurya groffii* (Lu and Guo 2010b); *S. fissuratum* differs in its larger basidia (32–45 × 6–9 μm) and growth on *Castanea* sp. (Li and Guo 2013); *S. gaoligongense* differs in its dark brown hymenial surface and thinner section (260–580 μm; Lu and Guo 2010b).

Based on our phylogenetic and morphological research results, 61 species have been reported from China, including newly described in the present study and other recently published papers in this country (Gómez and Henk 2004; Lu and Guo 2009a, 2009b, 2010a, 2010b, 2011; Lu et al. 2010; Chen and Guo 2011a, 2011b, 2011c, 2012; Li and Guo 2013, 2014; Ma et al. 2019). It seems that the species diversity of *Septobasidium* is rich in China. Although the taxa of *Septobasidium* are well studied in the present paper and the species diversity, taxonomy and phylogeny of *Septobasidium* and related genera are still unresolved. A comprehensive study on this issue is urgently needed.

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. s202310677041; s202310677028).

Author contributions

Conceptualisation, CZ and QJ; methodology, CZ and QJ; software, CZ, QJ and ZK; validation, CZ and QJ; formal analysis, CZ and QJ; investigation, CZ and QJ; resources CZ; writing – original draft preparation, CZ, QJ, ZK and XW; writing – review and editing, CZ and QJ; visualisation, CZ and QJ; supervision, CZ and QJ; project administration, CZ; funding acquisition, CZ. All authors have read and agreed to the published version of the manuscript.

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Data availability

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

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