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Morpho-phylogenetic evidence reveals four novel species of *Coniella* (Diaporthales, Schizoparmaceae) from southern China

Duhua Li[1,2], Zixu Dong[2], Qiyun Liu[2], Yaling Wang[2], Zhaoxue Zhang[2], Xiuguo Zhang[2], Jiwen Xia[1]

1 College of Agriculture and Forestry, Linyi University, Linyi, Shandong, 276000, China
2 College of Plant Protection, Shandong Agricultural University, Taian, Shandong, 271018, China
Corresponding author: Jiwen Xia (xiajiwen@lyu.edu.cn)

Abstract

Coniella species are distributed worldwide and have been reported as plant pathogens, endophytes, or saprobes. In our ongoing survey of terrestrial plant fungi in southern China, we obtained *Coniella* isolates from diseased plant leaf tissues in Fujian, Hainan, and Yunnan provinces. Maximum likelihood and Bayesian inference based on four loci (ITS, LSU, *rpb2*, and *tef1-α*) were used to clarify the taxonomic placement of the species. We confirmed that they represent four new species, namely *Coniella diaoluoshanensis*, *C. dongshanlingensis*, *C. grossedentatae*, and *C. veri* based on both morphology and phylogeny support. The new species are compared with other *Coniella* species, comprehensive descriptions and micrographs are provided.

Key words: Morphology, multigene phylogeny, new taxa, taxonomy

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Introduction

Coniella was formally introduced by Von Höhnel (1918) with *C. pulchella* (= *C. fragariae* (Oudem.) B. Sutton) as the type species (Von Höhnel 1918; Sutton 1977; Crous et al. 2014a). Samuels et al. (1993) initially recognized the uniqueness of *Schizoparme* and its relationship to *Coniella* and *Pilidiella*, these were initially placed in the Melanconidaceae. Both Castlebury et al. (2002) and Van Niekerk et al. (2004) revealed that these species within the Diaporthales, which they collectively designated as the *Schizoparme* complex. Rossman et al. (2007) introduced a new family, Schizoparmaceae, which comprises the distinctive teleomorph genus *Schizoparme*, its asexual state *Pilidiella*, and the closely related anamorph genus *Coniella*. These genera are cosmopolitan fungal pathogens associated with foliar, fruit, stem, and root diseases on a wide variety of hosts, including some economically important hosts (Van Niekerk et al. 2004; Alvarez et al. 2016). They occur as parasites on unrelated dicotyledonous hosts (Samuels et al. 1993) or sometimes as secondary invaders of injured plant tissues (Ferreira et al. 1997).

Coniella has undergone comprehensive morpho-molecular studies and experienced several taxonomic adjustments over the years. Petrak and Sydow (1927) classified *Coniella* into two subgenera: *Euconiella* (dark conidia),

typified by *C. pulchella*, and *Pseudoconiella* (hyaline to pale conidia), typified by *C. granati*. Von Arx (1973, 1981) classified *Coniella* and *Pilidiella* as distinct genera, with *Coniella* characterized by dark brown conidia and *Pilidiella* by hyaline conidia that darken to a pale brown when mature. Nonetheless, Sutton (1980) and Nag Raj (1993) disregarded conidial pigmentation as a defining trait and still opted to employ the earlier name *Coniella*. Samuels et al. (1993) stated *Schizoparme* as the sexual morph and positioned it in Melanconidaceae. Castlebury et al. (2002) classified *Pilidiella* and *Coniella* as members of the *Schizoparme* complex. Van Niekerk et al. (2004) demonstrated that these taxa form a distinct evolutionary lineage within the Diaporthales based on ITS, LSU, and *tef1-α* sequences. Subsequently, Rossman et al. (2007) established a new family, Schizoparmaceae, including the above three genera, *viz.* *Coniella*, *Pilidiella*, and *Schizoparme*. Alvarez et al. (2016) demonstrated that *Coniella*, *Pilidiella*, and *Schizoparme* formed a monophyletic clade in Schizoparmaceae and suggested adopting *Coniella* (the older asexual typified name) instead of *Pilidiella* and *Schizoparme*, in accordance with Article 59.1 of the International Code of Nomenclature for Algae, Fungi, and Plants (ICN, Melbourne Code; McNeill et al. 2012). Additionally, due to the many numbers of species and the similarity in morphological characteristics, they suggested that the identification of new species within *Coniella* must be based on a combination of DNA sequence data and morphological characteristics. Chethana et al. (2017) used a combination of morphological analysis and multigene phylogeny with the genealogical concordance phylogenetic species recognition (GCPSR) method to delineate species boundaries. Hyde et al. (2020) and Tennakoon et al. (2021) conducted the recent phylogenetic analyses for *Coniella* species within the Schizoparmaceae. Currently, there are 66 accepted *Coniella* species (Index Fungorum: https://indexfungorum.org; MycoBank: http://www.mycobank.org; Mu et al. 2024).

In this study, we conducted extensive sample collection in southern China, primarily collecting plant leaves with obvious fungal necrosis or typical blight spot symptoms. Several *Coniella* fungi were collected from the diseased leaves of *Ampelopsis grossedentata*, *Cinnamomum verum*, *Kadsura longipedunculata*, and *Lygodium circinnatum*. Based on morphological and multi-locus analysis employing internal transcribed spacer (ITS), 28S large subunit ribosomal RNA gene (LSU), partial RNA polymerase II second largest subunit (*rpb2*), and translation elongation factor 1-alpha gene (*tef1-α*), four new *Coniella* species, namely *C. diaoluoshanensis*, *C. dongshanlingensis*, *C. grossedentatae*, and *C. veri*, were proposed.

Materials and methods

Sample collection and isolation

During 2022 to 2024, a large number of plant leaves that exhibited obvious signs of fungal necrosis or typical blight spot symptoms were collected from Fujian, Hainan, and Yunnan provinces in China. This study used tissue isolation methods to isolate fungi (Li et al. 2024). These diseased leaves were cut into small pieces of about 25 mm^2 and surface sterilized by immersion in a 75% ethanol solution for 60 s, washed one time in sterile deionized water

for 20 s, transferred to 5% sodium hypochlorite (NaOCl) for 90 s, and then washed three times in sterile deionized water for 60 s, subsequently dried on sterilized filter paper. The tissue pieces were transferred to the potato dextrose agar (PDA, 200 g potato, 20 g dextrose, 20 g agar, add deionized water and fill to 1000 mL, natural pH) plates and placed in a biological incubator at 25 °C for 3–4 days. The hyphal tips of individual colonies were transferred to new PDA plates to obtain pure cultures, which were then cut into 25 mm^2 pieces using a sterile scalpel and stored in 2 mL frozen tubes containing 20% sterilized glycerin, with 8–10 pieces placed in each tube, for fungal strain preservation at -20 °C for further study.

Morphological and cultural characterization

The culture characteristics of the colonies were observed and photographed using a Sony Alpha 6400L digital camera (Sony Group Corporation, Tokyo, Japan) on 7 and 14 days, respectively. The micromorphological characteristics of the colonies were observed with the Olympus SZX10 stereomicroscope and Olympus BX53 microscope (Olympus Corporation, Tokyo, Japan), along with the BioHD-A20c color digital camera (FluoCa Scientific, China, Shanghai). Structural measurements were carried out using Digimizer software (v5.6.0) with a minimum of 30 measurements taken for each structure, such as conidiophores, conidiogenous cells, and conidia. The voucher specimens have been deposited in the Herbarium of the Department of Plant Pathology, Shandong Agricultural University, Taian, China (HSAUP). Additionally, the ex-type living cultures were deposited in the Shandong Agricultural University Culture Collection (SAUCC) and the China General Microbiological Culture Collection Center (CGMCC). The taxonomic information of the new taxa were submitted to MycoBank (http://www.mycobank.org, accessed on 2 Jan. 2025).

DNA extraction, PCR amplification, and sequencing

The DNA of the fungal genome was extracted using the modified cetyltrimethylammonium bromide (CTAB) method (Guo et al. 2000; Wang et al. 2023) or the magnetic bead kit method (OGPLF-400, GeneOnBio Corporation, Changchun, China) (Zhang et al. 2023). PCR amplifications of four genes (ITS, LSU, *rpb2*, and *tef1-α*) were done, and the corresponding primer pairs and PCR conditions were listed in Table 1. The PCR reaction was conducted in a 12 μL reaction volume, with a composition of 6 μL of 2 × Hieff Canace® Plus PCR Master Mix (with dye) (Cat. No. 10154ES03, Yeasen Biotechnology, Shanghai, China), 0.5 μL each of forward and reverse primer (10 μM TsingKe, Qingdao, China), and 0.5 μL of template genomic DNA (about 10 ng/μL), with the volume adjusted to 12 μL using distilled deionized water. PCR products were separated using 1% agarose gel and GelRed (TsingKe, Qingdao, China). Gel extraction was purified using a Gel Extraction Kit (Cat. No. AE0101-C, Shandong Sparkjade Biotechnology Co., Ltd., Jinan, China). The purified PCR products were subjected to bidirectional sequencing by Sangon Biotech Company Limited (Shanghai, China). The raw data were analyzed using MEGA v. 7.0 to obtain consistent sequences (Kumar et al. 2016). The sequence data have been deposited in GenBank, and their accession numbers were listed in Table 2.

Table 1. The primer sequences and PCR programs in this study.

Locus	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		
tef1-α	EF1-728F	CAT CGA GAA GTT CGA GAA GG	(95 °C: 30 s, 51 °C: 30 s, 72 °C: 1 min) × 35 cycles	O'Donnell et al. 1998; Carbone and Kohn 1999
	EF2	GGA RGT ACC AGT SAT CAT GTT		

Table 2. Species names, strain numbers, hosts or substrates, regions, and corresponding GenBank accession numbers of DNA sequences used in this study.

Species	Strain numbers	Host/Substrate	Region	GenBank accession numbers				References
				ITS	LSU	*rpb2*	*tef1-α*	
Coniella africana	CBS 114133* = CPC405	*Eucalyptus nitens*	South Africa	AY339344	AY339293	KX833421	KX833600	Van Niekerk et al. 2004; Alvarez et al. 2016
Coniella castanea	SAUCC200313*	*Castanea mollissima*	China	OL757537	OL757563	OL770463	OL780610	Wang et al. 2022
	SAUCC200314	*Castanea mollissima*	China	OL757538	OL757564	OL770464	OL780611	Wang et al. 2022
Coniella cili	GUCC 194020.1	*Rosa roxburghii*	China	ON791171	ON791212	ON815908	ON815944	Zhang et al. 2024b
	GUCC 196007.1*	*Rosa roxburghii*	China	ON791172	ON791213	ON815909	ON815945	Zhang et al. 2024b
Coniella crousii	NFCCI 2213	*Terminalia chebula*	India	HQ264189	NA	NA	NA	Rajeshkumar et al. 2011
Coniella diaoluoshanensis	**CGMCC3.27786* = SAUCC 7481-1**	**Kadsura longipedunculata**	**China**	**PQ357094**	**PQ357134**	**PQ361030**	**PQ404804**	**This study**
	SAUCC 7481-4	**Kadsura longipedunculata**	**China**	**PQ357095**	**PQ357135**	**PQ361031**	**PQ404805**	**This study**
Coniella diospyri	CBS 145071* = CPC 34674	*Diospyros mespiliformis*	South Africa	MK047439	MK047489	MK047543	MK047562	Crous et al. 2018
Coniella diplodiella	CBS 111858* = CPC3708	*Vitis vinifera*	France	AY339323	KX833335	KX833423	KX833603	Van Niekerk et al. 2004; Alvarez et al. 2016
	CBS 112729 = CPC3927	*Vitis vinifera*	South Africa	KX833520	KX833345	KX833433	KX833613	Alvarez et al. 2016
Coniella diplodiopsis	CBS 109.23 = CPC 3933	*Vitis vinifera*	Switzerland	NA	AY339287	KX833440	KX833624	Van Niekerk et al. 2004; Alvarez et al. 2016
	CBS 590.84* = CPC 3940	*Vitis vinifera*	Italy	AY339334	AY339288	NA	NA	Van Niekerk et al. 2004
	CBS 116310 = CPC 3793	*Vitis vinifera*	Italy	KX833532	KX833357	KX833443	KX833627	Alvarez et al. 2016
Coniella dongshanlingensis	**CGMCC3.27785* = SAUCC 7265-5**	**Lygodium circinnatum**	**China**	**PQ357090**	**PQ357130**	**PQ361026**	**PQ404800**	**This study**
	SAUCC 7265-6	**Lygodium circinnatum**	**China**	**PQ357091**	**PQ357131**	**PQ361027**	**PQ404801**	**This study**
Coniella duckerae	CBS 142045*= VPRI 13689	*Lepidospermum concavum*	Australia	KY924929	NA	NA	NA	Marin-Felix et al. 2017
Coniella erumpens	CBS 523.78*	Rotten wood	Chile	KX833535	KX833361	KX833446	KX833630	Alvarez et al. 2016
Coniella eucalyptigena	CBS 139893* = CPC 24793	*Eucalyptus brassiana*	Malaysia	KR476725	KR476760	NA	NA	Crous et al. 2015a
Coniella eucalyptorum	CBS 112640* = CPC 3904 = DFR 100185	*Eucalyptus grandis × E. tereticornis*	Australia	AY339338	AY339290	KX833452	KX833637	Van Niekerk et al. 2004; Alvarez et al. 2016
	CBS 114852	*Eucalyptus* sp.	Australia	KX833556	KX833380	KX833464	KX833652	Alvarez et al. 2016

Species	Strain numbers	Host/Substrate	Region	GenBank accession numbers				References
				ITS	LSU	*rpb2*	*tef1-α*	
Coniella fici	MFLU 18-2578*	*Ficus septica*	China	MW114356	MW114417	NA	NA	Tennakoon et al. 2021
Coniella fragariae	CBS 172.49* = CPC 3930	*Fragaria* sp.	Belgium	AY339317	AY339282	KX833472	KX833663	Van Niekerk et al. 2004; Alvarez et al. 2016
	CBS 454.68	*Malus sylvestris*	Denmark	KX833571	KX833393	KX833477	KX833670	Alvarez et al. 2016
Coniella fujianensis	CGMCC3.25353	*Canarium album*	China	OR623057	OR623054	OR637413	OR637415	Mu et al. 2024
	CGMCC3.25354*	*Canarium album*	China	OR623058	OR623055	OR637414	OR637416	Mu et al. 2024
Coniella fusiformis	CBS 141596* = CPC 19722	*Eucalyptus* sp.	Indonesia	KX833576	KX833397	KX833481	KX833674	Alvarez et al. 2016
	CBS 114850	*Eucalyptus pellita*	Australia	KX833574	KX833395	KX833479	KX833672	Alvarez et al. 2016
Coniella granati	CBS 132860	*Punica granatum*	Turkey	KX833577	KX833400	KX833484	KX833677	Alvarez et al. 2016
	CBS 252.38 = ATCC 12685 = CPC 3714	*Vitis vinifera*	Italy	KX833581	AY339291	KX833488	KX833681	Van Niekerk et al. 2004; Alvarez et al. 2016
Coniella grossedentatae	**SAUCC 1354-1**	**Ampelopsis grossedentata**	**China**	**PQ357062**	**PQ357102**	**PQ361000**	**PQ404774**	**This study**
	CGMCC3.27783*= SAUCC 1354-3	**Ampelopsis grossedentata**	**China**	**PQ357063**	**PQ357103**	**PQ361001**	**PQ404775**	**This study**
Coniella heterospora	CBS 143031* = FMR 15231	Herbivorous dung	Spain	LT800501	LT800500	LT800502	LT800503	Crous et al. 2017
Coniella hibisci	CBS 109757* = AR 3534	*Hibiscus* sp.	Africa	KX833589	AF408337	NA	KX833689	Castlebury et al. 2002; Marin-Felix et al. 2017
Coniella javanica	CBS 455.68*	*Hibiscus sabdariffai*	Indonesia	KX833583	KX833403	KX833489	KX833683	Alvarez et al. 2016
Coniella koreana	CBS 143.97*	NA	South Korea	KX833584	AF408378	KX833490	KX833684	Alvarez et al. 2016
Coniella lanneae	CBS 141597* = CPC 22200	*Lannea* sp.	Zambia	KX833585	KX833404	KX833491	KX833685	Alvarez et al. 2016
Coniella limoniformis	CBS 111021* = PPRI 3870 = CPC 3828	*Fragaria* sp.	South Africa	KX833586	KX833405	KX833492	KX833686	Alvarez et al. 2016
Coniella lustricola	DAOMC 251731*	NA	America	MF631778	MF631799	MF651900	MF651899	Raudabaugh et al. 2018
	DAOMC 251732	NA	America	MF631779	MF631800	NA	NA	Raudabaugh et al. 2018
	DAOMC 251733	NA	America	MF631780	MF631801	NA	NA	Raudabaugh et al. 2018
	DAOMC 251734	NA	America	MF631781	MF631802	NA	NA	Raudabaugh et al. 2018
Coniella macrospora	CBS 524.73* = CPC 3935	*Terminalia ivoriensisstem*	Ivory Coast	KX833587	AY339292	KX833493	KX833687	Alvarez et al. 2016
Coniella malaysiana	CBS 141598* = CPC 16659	*Corymbia torelliana*	Malaysia	KX833588	KX833406	KX833494	KX833688	Alvarez et al. 2016
Coniella nicotianae	CBS 875.72* = PD 72/793	*Nicotiana tabacum*	Jamaica	KX833590	KX833407	KX833495	KX833690	Alvarez et al. 2016
Coniella nigra	CBS 165.60* = IMI 181519 = IMI 181599 = CPC 4198	Soil	India	AY339319	KX833408	KX833496	KX833691	Van Niekerk et al. 2004; Alvarez et al. 2016
Coniella obovata	CBS 111025 = CPC 4196 = IMI 261318	Leaves	South Africa	AY339313	KX833409	KX833497	KX833692	Van Niekerk et al. 2004; Alvarez et al. 2016
Coniella paracastaneicola	CBS 141292* = CPC 20146	*Eucalyptus* sp.	Australia	KX833591	KX833410	KX833498	KX833693	Alvarez et al. 2016
Coniella peruensis	CBS 110394* = RMF 74.01	Soil of rain forest	Peru	KJ710463	KJ710441	KX833499	KX833695	Crous et al. 2015b; Alvarez et al. 2016
Coniella pseudodiospyri	CBS 145540* = CPC 35725	*Eucalyptus microcorys*	Australia	MK876381	MK876422	MK876479	MK876493	Crous et al. 2019
Coniella pseudogranati	CBS 137980* = CPC 22545	*Terminalia stuhlmannii*	Zambia	KJ869132	KJ869189	NA	NA	Crous et al. 2014b

Species	Strain numbers	Host/Substrate	Region	GenBank accession numbers				References
				ITS	LSU	*rpb2*	*tef1-α*	
Coniella pseudokoreana	MFLU 13-0282* = MFLUCC 12-0427	Leaves	Thailand	MF190145	NA	NA	NA	Senanayake et al. 2017
Coniella pseudostraminea	CBS 112624* = IMI 233050	*Fragaria* sp.	South Africa	KX833593	KX833412	KX833500	KX833696	Alvarez et al. 2016
Coniella quercicola	CBS 283.76	Excrements of *Glomerus*, which had eaten forest soil	The Netherlands	KX833594	KX833413	KX833501	KX833697	Alvarez et al. 2016
	CBS 904.69*	*Quercus robur*	The Netherlands	KX833595	KX833414	KX833502	KX833698	Alvarez et al. 2016
Coniella solicola	CBS 766.71*	Soil	South Africa	KX833597	KX833416	KX833505	KX833701	Alvarez et al. 2016
Coniella straminea	CBS 149.22 = CPC 3932	*Fragaria* sp.	USA	AY339348	AY339296	KX833506	KX833704	Van Niekerk et al. 2004; Alvarez et al. 2016
Coniella tibouchinae	CBS 131594* = CPC 18511	*Tibouchina granulosa*	Brazil	JQ281774	KX833418	KX833507	JQ281778	Miranda et al. 2012; Alvarez et al. 2016
Coniella veri	**CGMCC3.27787* = SAUCC 8877-4**	***Cinnamomum verum***	**China**	**PQ357098**	**PQ357138**	**PQ361034**	**PQ404810**	**This study**
	SAUCC 8877-7	***Cinnamomum verum***	**China**	**PQ357099**	**PQ357139**	**PQ361035**	**PQ404811**	**This study**
Coniella vitis	MFLUCC 16-1399* = JZB3700001	*Vitis vinifera*	China	KX890008	KX890083	NA	KX890058	Chethana et al. 2017
Coniella wangiensis	CBS 132530* = CPC 19397	*Eucalyptus* sp.	Australia	JX069873	JX069857	KX833509	KX833705	Crous et al. 2012; Alvarez et al. 2016
Dwiroopa lythri	CBS 109755* = AR 3383	*Lythrum salicaria*	USA	MN172410	MN172389	MN271801	MN271859	Jiang et al. 2020

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

Sequence alignment and phylogenetic analyses

The nucleotide sequences of four new species were submitted to the NCBI's GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/, accessed on 2 Jan. 2025), and all related species were retrieved for phylogenetic analysis. Multiple sequences were aligned using MAFFT version 7 (http://mafft.cbrc.jp/alignment/server/index.html, accessed on 2 Jan. 2025) with default settings, and manual correction was applied if necessary (Katoh et al. 2019). For phylogenetic analyses, single and concatenated sequences were subjected to analysis by Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms, respectively. Both ML and BI were executed on the CIPRES Science Gateway portal (https://www.phylo.org/, accessed on 2 Jan. 2025) or offline software (ML was executed in RaxML-HPC2 on XSEDE v8.2.12 and BI analysis was executed in MrBayes v3.2.7a with 64 threads on Linux) (Miller et al. 2012; Ronquist et al. 2012; Stamatakis 2014). For the ML analysis, the default parameters were used, and 1,000 rapid bootstrap replicates were run with the GTR+G+I model of nucleotide evolution; for BI, it was performed using a rapid bootstrapping algorithm with an automatic stop option and utilized MrModeltest v.2.3 to determine the best evolutionary model for each partition (Nylander 2004; Zhang et al. 2024a). Bayesian Inference posterior probabilities (BIPP) were evaluated by Markov Chain Monte Carlo (MCMC) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002). The BI analyses encompassed two parallel runs spanning 5,000,000 generations, with a stop rule incorporated and a sampling frequency of 50 generations. The burn-in fraction was set at 0.25, and posterior probabilities were calculated

from the remaining trees. The resulting trees were generated using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, accessed on 2 Jan. 2025) or ITOL: Interactive Tree of Life (https://itol.embl.de/, accessed on 2 Jan. 2025) (Letunic and Bork 2021), and the final layout of the trees was refined in Adobe Illustrator CC 2019. The names of the isolates in this study are marked in red in the phylogenetic tree.

Results

Molecular phylogeny

Initially, based on the ITS sequence data, we preliminarily determined that the eight strains belong to *Coniella*. Subsequently, based on ML and BI methods, we conducted a combined analysis of ITS, LSU, *rpb2*, and *tef1-α* gene data to construct phylogenetic trees for further determination of the phylogenetic position of these strains. The phylogenetic analysis of *Coniella* strains included 63 sequences, with *Dwiroopa lythri* (CBS 109755) serving as the outgroup. The final alignment comprised 2800 concatenated characters, *viz.* 1−600 (ITS), 601−1380 (LSU), 1381−2140 (*rpb2*), and 2141−2800 (*tef1-α*). The ML optimization likelihood was calculated to be -23461.791405. The matrix exhibited 1116 distinct alignment patterns, with 18.42% of characters or gaps remaining undetermined. The optimal models, evaluated by MrModeltest and selected in the BI, are as follows: the SYM+I+G model for ITS and the GTR+I+G model for LSU, *rpb2*, and *tef1-α*. The alignment exhibited a total of 1121 unique site patterns (ITS: 211, LSU: 78, *rpb2*: 322, *tef1-α*: 510). The topology of the ML tree concurred with that derived from BI; thus, only the ML tree is presented (Fig. 1). Combining morphological characteristics and molecular phylogenetic analyses, the eight strains in this study were introduced as four new species, namely *Coniella diaoluoshanensis*, *C. dongshanlingensis*, *C. grossedentatae*, and *C. veri.*

Taxonomy

***Coniella diaoluoshanensis* D.H. Li, J.W. Xia & X.G. Zhang, sp. nov.**
MycoBank No: 856520
Fig. 2

Holotype. Cʜɪɴᴀ • Hainan Province: Diaoluoshan National Forest Park, on diseased leaves of *Kadsura longipedunculata* (Schisandraceae), 18.660546°N, 109.936445°E, 94.1 m asl., 27 Mar. 2024, D.H. Li, holotype HSAUP 7481-1, ex-type living culture SAUCC 7481-1 = CGMCC3.27786.

Etymology. Named after the collection site of the type specimen, Diaoluoshan National Forest Park.

Description. *Hypha* immersed, 1.9−6.5 μm wide, branched, multi-septate, enlarged towards septum and terminal, hyaline. Asexual morph: ***Conidiomata*** nearly spherical, separate, scarce, immersed or superficial, surface uneven, sizes inconsistent, black. ***Conidiophores*** cylindrical, aseptate, straight or slightly curved, densely aggregated, simple, smooth, usually reduced to conidiogenous cells. ***Conidiogenous cells*** phialidic, simple, aggregative, hyaline, smooth, 8.1−11 × 1.4−2.6 μm (mean ± SD = 9.6 ± 0.8 × 2.1 ± 0.4 μm, n = 30), with apical periclinal
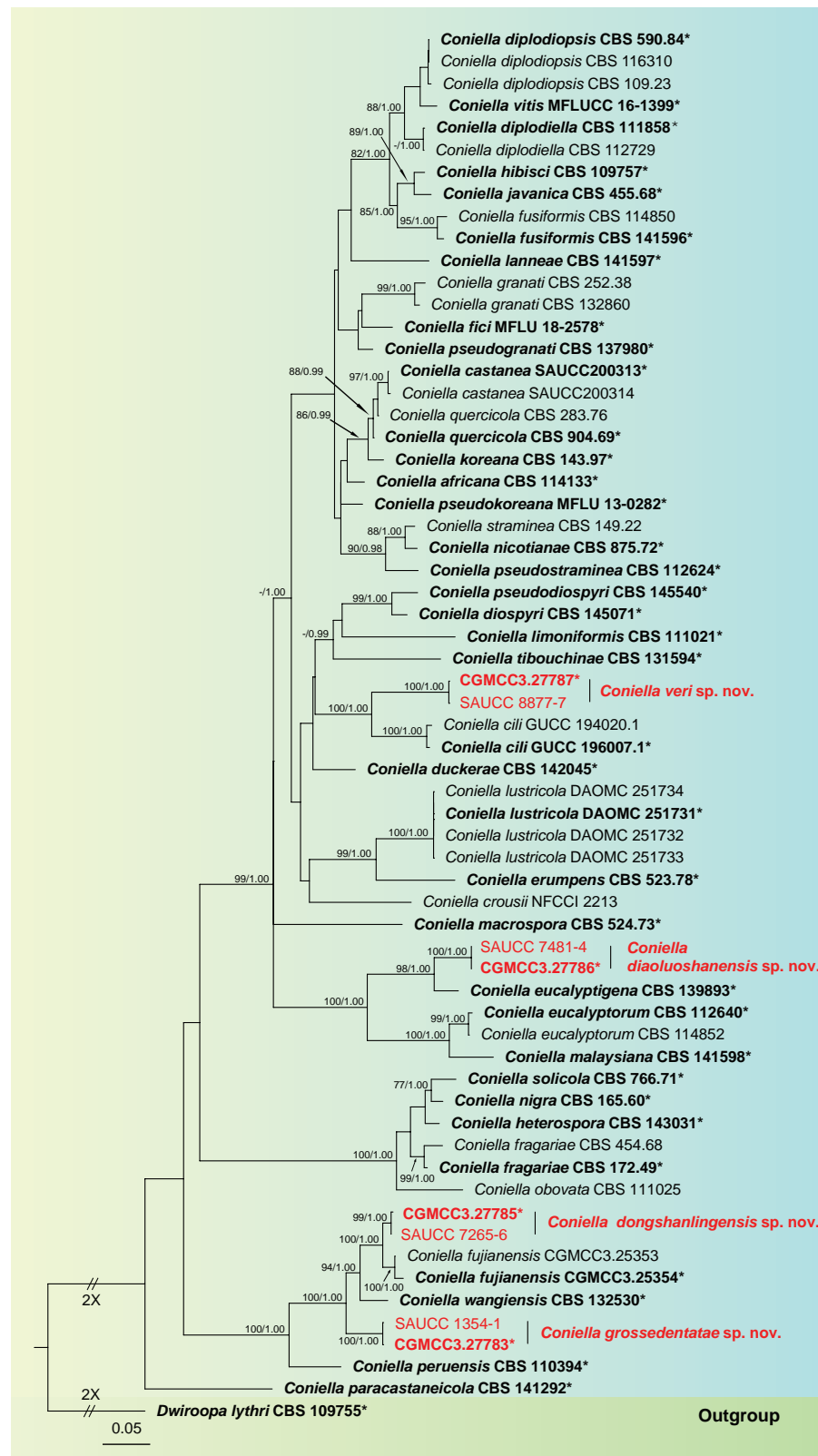
Figure 1. Phylogenetic relationship of *Coniella* based on concatenated sequences of ITS, LSU, *rpb2*, and *tef1-α* sequence data with *Dwiroopa lythri* (CBS 109755) as the outgroup. The Maximum Likelihood Bootstrap Value (left, MLBV ≥ 75%) and the Bayesian Inference Posterior Probability (right, BIPP ≥ 0.90) are shown as MLBV/BIPP above the nodes. The ex-type strains are marked with "*" and indicated in boldface. Strains from this study are shown in red. The scale bar at the bottom left represents 0.05 substitutions per site. Some branches are shortened according to the indicated multipliers to fit the page size, and these are indicated by the symbol (//).
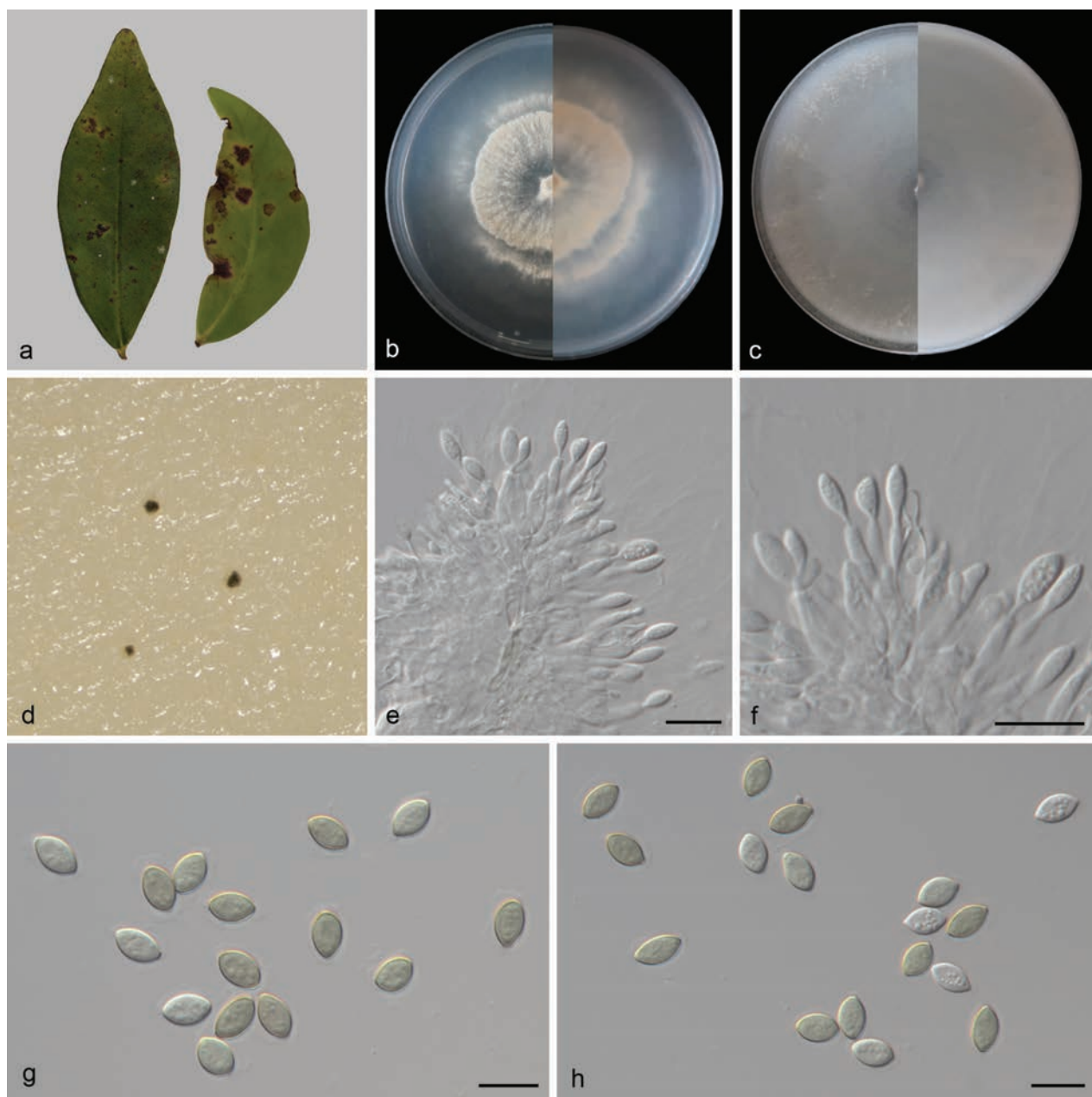
Figure 2. *Coniella diaoluoshanensis* (CGMCC3.27786) **a** leaves of *Kadsura longipedunculata* **b, c** surface and reverse sides of colony after 14 days on PDA (**b**) and OA (**c**) **d** conidiomata forming on OA **e, f** conidiophores and conidiogenous cells with developing conidia **g, h** conidia. Scale bars: 10 μm (**e–h**).

thickening, blastospore at the apex. **Conidia** elliptical or fusiform, apices tapering, subobtuse, apically rounded, widest at the middle, bases tapering to a truncate hilum, multi-guttulate, immature conidia hyaline, mature conidia pale olivaceous, wall darker than pale olivaceous body of conidium, smooth, 7.5−9.3 × 4.7−5.5 μm (mean ± SD = 8.4 ± 0.5 × 5.1 ± 0.3 μm, n = 30). Sexual morph unknown.

Culture characteristics. Colonies on PDA after 14 days of cultivation in the dark at 25 °C, reaching 75−77 mm in diam., with a growth rate of 5.4−5.5 mm/day; from above: white to cream-colored with age, sparse aerial mycelium at the center, irregularly circular, slightly low; peripheral mycelium dense, concentric rings, flat; colony edge irregular, sparse aerial mycelium, dispersed, striped; reverse: similar in color. Colonies on OA covering entire plate after 14 days of cultivation in

the dark at 25 °C; from above: white, devoid of aerial mycelium at the center, with dispersed and sparse aerial mycelium at the edges; reverse: even white texture.

Additional material studied. Cʜɪɴᴀ • Hainan Province: Diaoluoshan National Forest Park, on diseased leaves of *Kadsura longipedunculata* (Schisandraceae), 18.660546°N, 109.936445°E, 94.1 m asl., 27 Mar. 2024, D.H. Li, HSAUP 7481-4, living culture SAUCC 7481-4.

Notes. Phylogenetic analyses showed that *Coniella diaoluoshanensis* formed an independent clade (Fig. 1) and was closely related to *C. eucalyptigena* (CBS 139893), *C. eucalyptorum* (CBS 112640 and CBS 114852), and *C. malaysiana* (CBS 141598). *Coniella diaoluoshanensis* was distinguished from *C. eucalyptigena* by 4/573 and 7/791 base-pair differences in ITS and LSU sequences, from *C. eucalyptorum* (CBS 112640) by 19/565, 7/793, 68/765, and 164/539 base-pair differences in ITS, LSU, *rpb2*, and *tef1-α* sequences, and from *C. malaysiana* by 16/553, 7/783, 67/767, and 154/488 base-pair differences in ITS, LSU, *rpb2*, and *tef1-α* sequences, respectively. Morphologically, *C. eucalyptigena* lacks asexual sporulation description, making it impossible to compare microscopic structures with *C. diaoluoshanensis*. However, their macroscopic colony colors differ greatly: on PDA, *C. diaoluoshanensis* is cream-colored while *C. eucalyptigena* is salmon; on OA, *C. diaoluoshanensis* is white on the surface, whereas *C. eucalyptigena* is rosy buff. Morphologically, since *C. eucalyptigena* only had a description of sexual morphology, it could not be directly compared with the asexual morphology in this study. Then, *C. eucalyptorum* and *C. malaysiana*, which were closely related on the evolutionary tree, were selected for comparison. The conidiogenous cells of *C. diaoluoshanensis* (8.1−11 × 1.4−2.6 μm) shorter than those of *C. eucalyptorum* (10−17 × 3−3.5 μm) and *C. malaysiana* (8.5−18 × 1.5−3.5 μm); the conidia of *C. diaoluoshanensis* (7.5−9.3 × 4.7−5.5 μm) shorter than those of *C. eucalyptorum* (9−14 × 6−8 μm) and *C. malaysiana* (8−11.5 × 3−5 μm); and the mature conidial color of *C. diaoluoshanensis* (pale olivaceous) was lighter than that of *C. eucalyptorum* (medium to dark red-brown) and *C. malaysiana* (pale brown) (Van Niekerk et al. 2004; Crous et al. 2015a; Alvarez et al. 2016; Zhang et al. 2024b). Therefore, we describe our collection as a novel species.

Coniella dongshanlingensis **D.H. Li, J.W. Xia & X.G. Zhang, sp. nov.**
MycoBank No: 856519
Fig. 3

Holotype. Cʜɪɴᴀ • Hainan Province: Dongshanling Scenic Area, on diseased leaves of *Lygodium circinnatum* (Lygodiaceae), 18.802153°N, 110.421473°E, 18.8 m asl., 26 Mar. 2024, D.H. Li, holotype HSAUP 7265-5, ex-type living culture SAUCC 7265-5 = CGMCC3.27785.

Etymology. Named after the collection site of the type specimen, Dongshanling Scenic Area.

Description. *Hypha* superficial, 1.1−3.2 μm wide, less branched, multi-septate, hyaline to pale yellow. Asexual morph: *Conidiomata* pycnidial to nearly spherical, separate, superficial, surface enveloped in a gelatinous sheath, sizes inconsistent, initially appearing hyaline, becoming black with mature. *Conidiophores* cylindrical, aseptate, straight or slightly curved, densely aggregated, simple, smooth, usually reduced to conidiogenous cells. *Conidiogenous cells* phialidic,
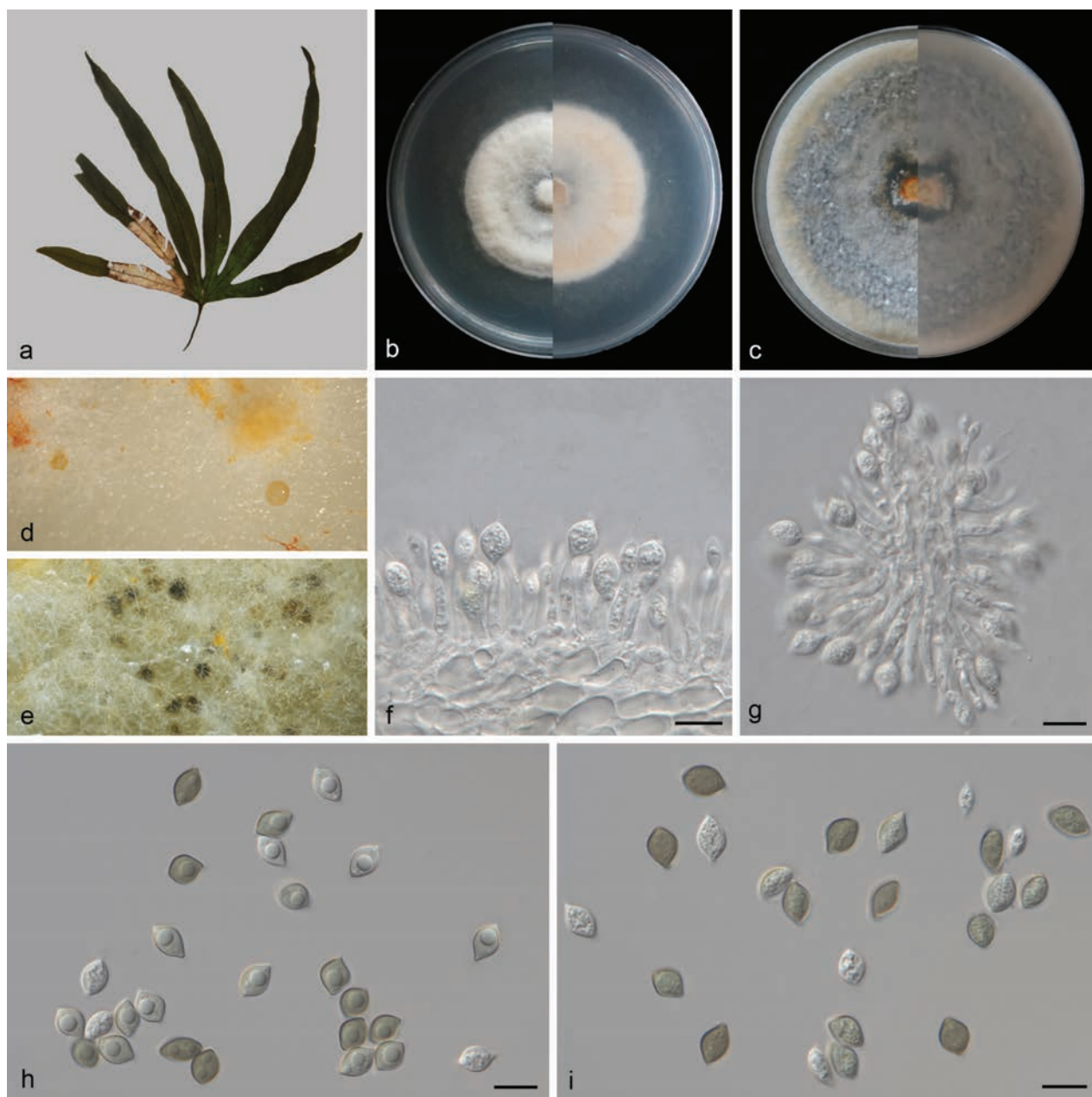
Figure 3. *Coniella dongshanlingensis* (CGMCC3.27785) **a** a leaf of *Lygodium circinnatum* **b, c** surface and reverse sides of colony after 14 days on PDA (**b**) and OA (**c**) **d, e** conidiomata forming on PDA **f, g** conidiophores and conidiogenous cells with developing conidia **h, i** conidia. Scale bars: 10 μm (**f–i**).

simple, aggregative, hyaline, smooth, 7.3−19.2 × 1.5−3.3 μm (mean ± SD = 12.6 ± 2.6 × 2.4 ± 0.5 μm, n = 30), with apical periclinal thickening, blastospore at the apex. **Conidia** elliptical to fusiform, apices tapering, subobtuse, apically rounded, bases tapering to a truncate hilum, immature conidia hyaline, multi-guttulate, mature conidia olivaceous, 1−2 guttulate, wall darker than olivaceous body of conidium, smooth, 7.8−10 × 5.1−7 μm (mean ± SD = 8.7 ± 0.6 × 6.2 ± 0.4 μm, n = 30). Sexual morph unknown.

Culture characteristics. Colonies on PDA after 14 days of cultivation in the dark at 25 °C, reaching 47−50 mm in diam., with a growth rate of 3.4−3.6 mm/day; from above: white to pale orange with age, medium aerial mycelium, circular, slightly low at the center, slightly higher at the edges; reverse: similar in color.

Colonies on OA covering entire plate after 14 days of cultivation in the dark at 25 °C; from above: pale orange, interspersed with extensive black pycnidia, medium aerial mycelium, flat; reverse: similar in color.

Additional material studied. Cʜɪɴᴀ • Hainan Province: Dongshanling Scenic Area, on diseased leaves of *Lygodium circinnatum* (Lygodiaceae), 18.802153°N, 110.421473°E, 18.8 m asl., 26 Mar. 2024, D.H. Li, HSAUP 7265-6, living culture SAUCC 7265-6.

Notes. Phylogenetic analyses showed that *Coniella dongshanlingensis* formed an independent clade (Fig. 1) and was closely related to *C. fujianensis* (CGMCC3.25353 and CGMCC3.25354). *Coniella dongshanlingensis* was distinguished from *C. fujianensis* (CGMCC3.25354) by 5/589, 9/657, and 19/306 base-pair differences in ITS, *rpb2*, and *tef1-α* sequences, respectively. Morphologically, the conidiogenous cells of *C. dongshanlingensis* (7.3−19.2 × 1.5−3.3 μm) are longer than those of *C. fujianensis* (3.5−8 × 2.5−3.5 μm); the conidia of *C. dongshanlingensis* (7.8−10 × 5.1−7 μm) slightly shorter than those of *C. fujianensis* (8−10.5 × 5.5−7.5 μm), and the mature conidial color of *C. dongshanlingensis* (olivaceous) is lighter than that of *C. fujianensis* (brown) (Mu et al. 2024). Therefore, we describe our collection as a novel species.

Coniella grossedentatae D.H. Li, J.W. Xia & X.G. Zhang, sp. nov.
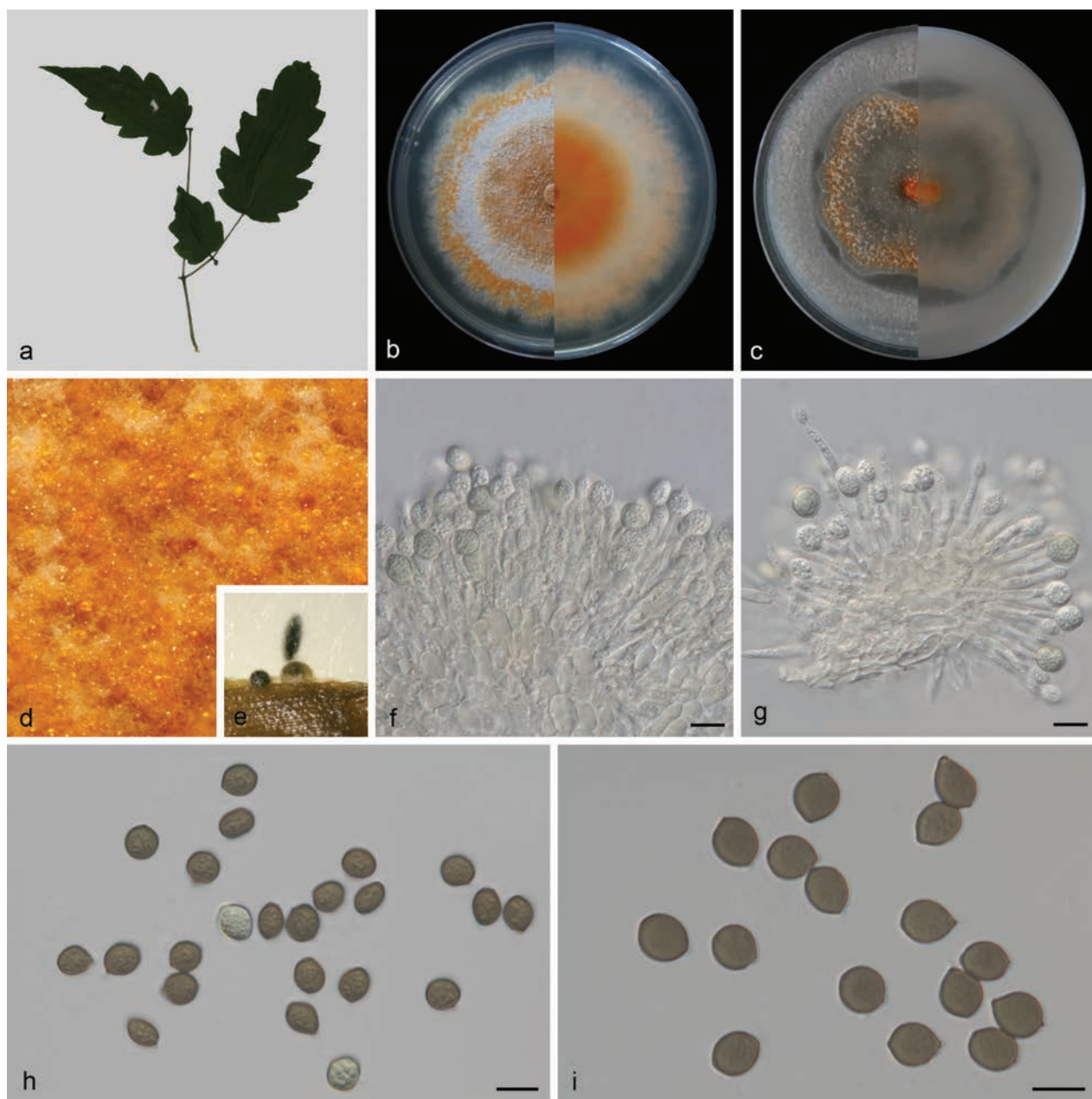
MycoBank No: 856518

Fig. 4

Holotype. Cʜɪɴᴀ • Fujian Province: Wuyishan City, Xingcun Town, on diseased leaves of *Ampelopsis grossedentata* (Vitaceae), 27.749556°N, 117.679038°E, 751.68 m asl., 15 Oct. 2022, D.H. Li, holotype HSAUP 1354-3, ex-type living culture SAUCC 1354-3 = CGMCC3.27783.

Etymology. Named after the species epithet of the host plant, *Ampelopsis grossedentata*.

Description. *Hypha* superficial, 1.3−3.5 μm wide, branched, multi-septate, hyaline to pale orange. Asexual morph: *Conidiomata* spherical or narrowly ellipsoid, separate, immersed or superficial, some surfaces enveloped in a gelatinous sheath, some surface uneven, sizes inconsistent, black. *Conidiophores* cylindrical, aseptate, straight or slightly curved, densely aggregated, simple, usually reduced to conidiogenous cells. *Conidiogenous cells* phialidic, simple, aggregative, hyaline, smooth, 10.6−23.1 × 1.7−3.8 μm (mean ± SD = 16.8 ± 3 × 2.5 ± 0.6 μm, n = 30), with apical periclinal thickening, blastospore at the apex. *Conidia* nearly spherical, apices acute, widest at the middle, bases tapering to a truncate hilum, multi-guttulate, immature conidia hyaline, mature conidia medium brown, wall darker than medium brown body of conidium, smooth, 8−10.5 × 7.5−9.5 μm (mean ± SD = 9.4 ± 0.6 × 8.4 ± 0.5 μm, n = 30). Sexual morph unknown.

Culture characteristics. Colonies on PDA after 14 days of cultivation in the dark at 25 °C, reaching 86−90 mm in diam., with a growth rate of 6.1−6.4 mm/day; from above: orange in the middle and edges, with white in between, medium aerial mycelium, granular, circular, flat; reverse: similar in color. Colonies on OA covering entire plate after 14 days of cultivation in the dark at 25 °C; from above: white in the middle and edges, with orange in between, sparse aerial mycelium, flat; reverse: similar in color.

Figure 4. *Coniella grossedentatae* (CGMCC3.27783) **a** leaves of *Ampelopsis grossedentata* **b, c** surface and reverse sides of colony after 14 days on PDA (**b**) and OA (**c**) **d** colony on PDA **e** conidiomata forming on pine needle **f, g** conidiophores and conidiogenous cells with developing conidia **h, i** conidia. Scale bars: 10 μm (**f–i**).

Additional material studied. CHINA • Fujian Province: Wuyishan City, Xingcun Town, on diseased leaves of *Ampelopsis grossedentata* (Vitaceae), 27.749556°N, 117.679038°E, 751.68 m asl., 15 Oct. 2022, D.H. Li, HSAUP 1354-1, living culture SAUCC 1354-1.

Notes. Phylogenetic analyses showed that *Coniella grossedentatae* formed an independent clade (Fig. 1) basal to *C. dongshanlingensis* (CGMCC3.27785, SAUCC 7265-6), *C. fujianensis* (CGMCC 3.25353, CGMCC 3.25354), and *C. wangiensis* (CBS 132530). *Coniella grossedentatae* can be distinguished from *C. dongshanlingensis* by 4/604, 1/793, 52/902, and 80/532 base-pair differences in ITS, LSU, *rpb2*, and *tef1-α* sequences, and from *C. fujianensis* by 8/588, 1/798, 34/657, and 64/313 base-pair differences in ITS, LSU, *rpb2*, and *tef1-α*

sequences, and from *C. wangiensis* by 2/603, 5/798, 35/767, and 79/329 base-pair differences in ITS, LSU, *rpb2*, and *tef1-α* sequences, respectively. Morphologically, the conidiogenous cells of *C. grossedentatae* (10.6−23.1 × 1.7−3.8 μm) are longer than those of *C. dongshanlingensis* (7.3−19.2 × 1.5−3.3 μm), *C. fujianensis* (3.5−8 × 2.5−3.5 μm), and *C. wangiensis* (15−20 × 3−4 μm); the conidia of *C. grossedentatae* (8−10.5 × 7.5−9.5 μm) are wider than those of *C. dongshanlingensis* (7.8−10 × 5.1−7 μm) and *C. fujianensis* (8−10.5 × 5.5−7.5 μm), and shorter than those of *C. wangiensis* (9−13 × 7−10 μm) (Crous et al. 2012; Alvarez et al. 2016). Therefore, we describe our collection as a novel species.

Coniella veri D.H. Li, J.W. Xia & X.G. Zhang, sp. nov.
MycoBank No: 856521
Fig. 5

Holotype. CHINA • Yunnan Province: Pu'er City, Yixiang Town, Pu'er Sun River Forest Park, on diseased leaves of *Cinnamomum verum* (Lauraceae), 22.593953°N, 101.086217°E, 1596.44 m asl., 15 May 2024, D.H. Li, holotype HSAUP 8877-4, ex-type living culture SAUCC 8877-4 = CGMCC3.27787.

**Etymology.** Named after the species epithet of the host plant, *Cinnamomum verum*.

Description. *Hypha* superficial, 1.3−3.3 μm wide, branched, multi-septate, hyaline. Asexual morph: *Conidiomata* spherical, aggregated or solitary, immersed or superficial, some surfaces enveloped in a gelatinous sheath, some surface uneven, sizes inconsistent, initially appearing hyaline, becoming black with mature. *Conidiophores* cylindrical, septate, branched, straight or slightly curved, densely aggregated, simple, usually reduced to conidiogenous cells. *Conidiogenous cells* phialidic, simple, aggregative, or solitary, hyaline, smooth, 9.5−17.5 × 1.2−2.5 μm (mean ± SD = 12.5 ± 1.5 × 1.8 ± 0.4 μm, n = 30), with apical periclinal thickening, blastospore at the apex. *Conidia* elliptical to fusiform, apices acute, widest at the middle, bases tapering to a truncate hilum, multi-guttulate gather at both ends, hyaline, thick-walled, smooth, 6.2−8.8 × 3.6−4.7 μm (mean ± SD = 7.7 ± 0.6 × 4 ± 0.3 μm, n = 30). Sexual morph unknown.

Culture characteristics. Colonies on PDA after 14 days of cultivation in the dark at 25 °C, reaching 81−85 mm in diam., with a growth rate of 5.8−6.1 mm/day; from above: white, medium aerial mycelium, slightly higher at the center, circular, radial, flat; reverse: pale orange in the middle, orange in the edges. Colonies on OA after 14 days of cultivation in the dark at 25 °C, reaching 72−77 mm in diam., had a growth rate of 5.1−5.5 mm/day; from above: white, sparse aerial mycelium, black pycnidia formed in the center, flat; reverse: similar in color.

Additional material studied. CHINA • Yunnan Province: Pu'er City, Yixiang Town, Pu'er Sun River Forest Park, on diseased leaves of *Cinnamomum verum* (Lauraceae), 22.593953°N, 101.086217°E, 1596.44 m asl., 15 May 2024, D.H. Li, HSAUP 8877-7, living culture SAUCC 8877-7.

Notes. Phylogenetic analyses showed that *Coniella veri* formed an independent clade (Fig. 1) and was closely related to *C. cili* (GUCC 194020.1 and GUCC 196007.1). *Coniella veri* can be distinguished from *C. cili* (GUCC 196007.1) by 31/597, 8/791, 52/869, and 125/516 base-pair differences in ITS, LSU, *rpb2*, and *tef1-α* sequences, respectively. Morphologically, the conidiogenous cells
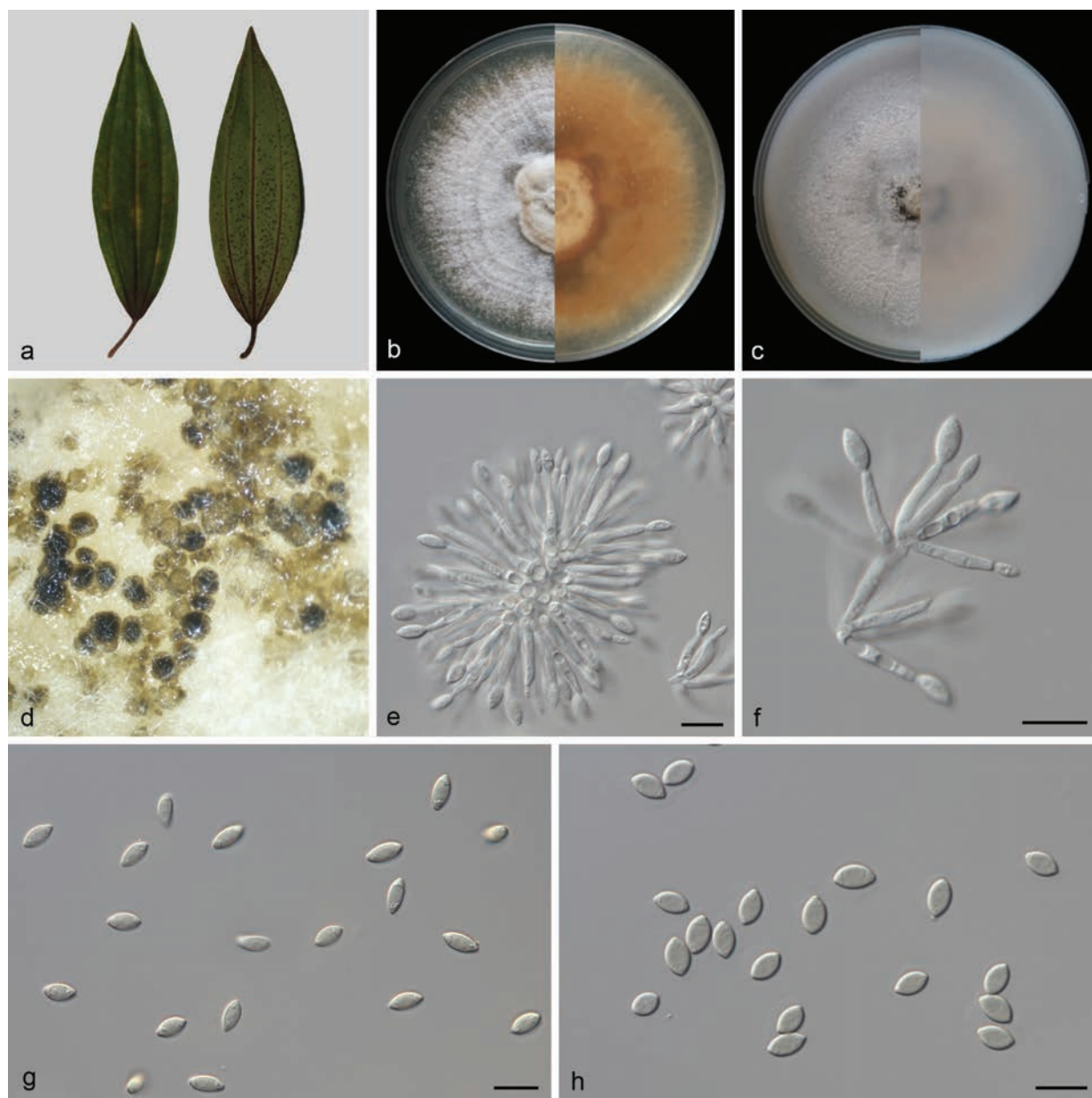
Figure 5. *Coniella veri* (CGMCC3.27787) **a** leaves of *Cinnamomum verum* **b, c** surface and reverse sides of colony after 14 days on PDA (**b**) and OA (**c**) **d** conidiomata forming on OA **e, f** conidiophores and conidiogenous cells with developing conidia **g, h** conidia. Scale bars: 10 μm (**e–h**).

of *C. veri* (9.5–17.5 × 1.2–2.5 μm) are shorter than those of *C. cili* (13–23.5 × 1–2 μm); the conidia of *C. veri* (6.2–8.8 × 3.6–4.7 μm) are shorter than those of *C. cili* (5.5–17.5 × 2.5–5 μm); the conidial shape of *C. veri* is elliptical to fusiform, whereas the conidial size and shape of *C. cili* exhibit considerable variation, including limoniform, fusoid, clavate, cylindrical, and elongated elliptical forms (Zhang et al. 2024b). Therefore, we describe our collection as a novel species.

Discussion

Coniella species have a worldwide distribution, reported in countries across all continents (Van Niekerk et al. 2004; Alvarez et al. 2016). They have been found in Asia (e.g., China, India, Indonesia, Malaysia, South Korea, and Thailand), Europe (e.g.,

Belgium, Denmark, France, Italy, the Netherlands, Switzerland, and Spain), Africa (e.g., Ivory Coast, South Africa, and Zambia), the Americas (e.g., the United States, Brazil, Peru, Jamaica, and Chile), and Oceania (e.g., Australia). These countries, ranging from landlocked nations such as Zambia and Switzerland to coastal countries like China, Brazil, and Australia, as well as island nations including Jamaica and Indonesia, are geographically diverse. They are distributed on both sides of the equator and span multiple climatic zones, from tropical to frigid, coastal to inland, and plain to mountain, encompassing diverse climate types such as tropical, temperate, and alpine. Many countries, including most of Africa, northern Brazil, Indonesia, and Malaysia, have tropical climates with high temperatures and abundant precipitation year-round. China, with its vast territory, large latitudinal span, wide longitudinal extent, and complex and diverse topography, nearly covers all major climate types, providing favorable conditions for the formation of *Coniella* species diversity (Castlebury et al. 2002; Van Niekerk et al. 2004; Alvarez et al. 2016; Raudabaugh et al. 2018; Wang et al. 2022; Mu et al. 2024; Zhang et al. 2024b).

Currently, *Coniella* has accepted 66 species, many of which were introduced solely based on morphological studies (Index Fungorum: https://indexfungorum.org; MycoBank: http://www.mycobank.org; Alvarez et al. 2016; Mu et al. 2024). Morphological characteristics of some conidia are highly similar and can be classified into two categories: one comprises olivaceous brown to brown conidia that are ellipsoid or globose, while the other category consists of hyaline conidia that are fusiform or clavate, often with very similar shapes and sizes. Rendering precise identification of *Coniella* species difficult solely on morphological characteristics (Crous et al. 2014a). Consequently, there is a strong current trend towards integrating morphological and molecular methods to assess or clarify the taxonomic placement and phylogenetic relationships of *Coniella* species (Alvarez et al. 2016). Based on phylogenetic analyses of ITS, LSU, and *tef1-α* sequence data, Van Niekerk et al. (2004) demonstrated that *Coniella* represents a distinct evolutionary lineage within the Diaporthales (Van Niekerk et al. 2004). Based on phylogenetic analyses of ITS, LSU, *rpb2*, and *tef1-α* sequence data, Alvarez et al. (2016) conducted a taxonomic revision of the genus. Since then, phylogenetic analyses of *Coniella* have largely continued to use these four genetic loci (Alvarez et al. 2016).

According to previous studies, *Coniella* species have been recorded as plant pathogens, endophytes, and saprobes (Samuels et al. 1993; Ferreira et al. 1997; Alvarez et al. 2016; Chethana et al. 2017). Their hosts encompass multiple categories, including plants (such as trees, shrubs, herbs, and ferns), animal excreta, and soils (Crous et al. 2015b; Alvarez et al. 2016). In recent years, several *Coniella* species have been reported and described in China. For example, Fröhlich and Hyde (2000) discovered *C. calamicola* on both living and dead leaves of *Daemonorops margaritae* in Hong Kong. Chen et al. (2014) first reported that *C. granati* can cause fruit rot and twig blight in pomegranate (*Punica granatum*) in Anhui Province. Chethana et al. (2017) reported that *C. vitis* is the pathogenic fungus causing white rot in grapes (*Vitis vinifera*) in Beijing Municipality, Guangxi, Hebei, Henan, and Jilin Provinces. Tennakoon et al. (2021) isolated a new species, *C. fici*, from dead leaves of *Ficus septica* (Moraceae) on the island of Taiwan. Wang et al. (2022) isolated a new species, *C. castanea*, from symptomatic leaves of *Castanea mollissima* (Fagaceae) in an orchard in Shandong Province. Mu et al. (2024) isolated a new species, *C. fujianensis*, from dis-

eased leaves of *Canarium album* (Burseraceae) in Fujian Province. Zhang et al. (2024b) isolated the endophytic species *C. cili* from healthy fruits and seeds of *Rosa roxburghii* (Rosaceae) in Guizhou Province.

During a continuous survey of terrestrial plant fungi in certain regions of southern China, four new species of *Coniella* were discovered from diseased leaf tissues of infected plants in Fujian, Hainan, and Yunnan provinces. These new species are named *Coniella diaoluoshanensis*, *C. dongshanlingensis*, *C. grossedentatae*, and *C. veri*. Among them, *C. grossedentatae* utilizes *Ampelopsis grossedentata* (Vitaceae) as its host. Van Niekerk et al. (2004) have previously reported species of *C. diplodiopsis* isolated from *Vitis vinifera* (Vitaceae) collected in Italy. In contrast, *C. diaoluoshanensis*, *C. dongshanlingensis*, and *C. veri* are the first reports that are associated with the hosts *Kadsura longipedunculata*, *Lygodium circinnatum*, and *Cinnamomum verum*, respectively. This will further broaden the host range of *Coniella* species and contribute to the fields of plant pathology and fungal taxonomy. With the increasing number of *Coniella* species, we believe that comprehensive research on this genus will uncover more hidden *Coniella* species from terrestrial plants.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Sampling, molecular biology analysis: Duhua Li and Zixu Dong; fungal isolation: Qiyun Liu and Yaling Wang; description and phylogenetic analysis: Duhua Li and Zhaoxue Zhang; microscopy: Duhua Li and Jiwen Xia; writing-original draft preparation: Duhua Li; writing-review and editing: Xiuguo Zhang and Jiwen Xia. All authors read and approved the final manuscript.

Author ORCIDs

Duhua Li https://orcid.org/0009-0006-5200-2034
Qiyun Liu https://orcid.org/0009-0009-9545-7962
Zhaoxue Zhang https://orcid.org/0000-0002-4824-9716
Xiuguo Zhang https://orcid.org/0000-0001-9733-8494
Jiwen Xia https://orcid.org/0000-0002-7436-7249

Data availability

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

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New fungal genus, three novel species and one new record from mangroves, with reclassification of *Melanconiella* (Melanconiellaceae) species

Carlo Chris S. Apurillo[1,2,3,4], Chayanard Phukhamsakda[3], Kevin D. Hyde[1,3], Vinodhini Thiyagaraja[1], E. B. Gareth Jones[5,6]

1 Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

2 School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

3 Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

4 Department of Science and Technology-Center for Research in Science and Technology (CReST), Philippine Science High School-Eastern Visayas Campus, Palo, Leyte 6501 Philippines

5 Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh, 11451, Saudi Arabia

6 Nantgaredig, 33B St Edwards Road, Southsea, Hants., UK PO5 3DH, UK

Corresponding authors: E. B. Gareth Jones (torperadgj@gmail.com); Kevin D. Hyde (kdhyde3@gmail.com)

Abstract

Mangrove ecosystems, located in the land-sea interface, host a diverse array of fungi. In this paper, we introduce a novel genus, three novel species and one new record of fungi collected from mangrove environments in Pranburi, Prachuap Khiri Khan, Thailand. We establish *Pseudomelanconiella* as a new genus in Melanconiellaceae, to accommodate *Pseudomelanconiella mangrovei*, a saprobe from submerged decomposing wood of *Avicennia marina*. Phylogenetic analysis indicates its close relation with *Septomelanconiella*, but they differ in the morphology of the conidia. Additionally, our analysis of Melanconiellaceae led to the reclassification of *Melanconiella loropetali* to *Sinodiscula loropetali* and synonymizing *Sinodiscula camellicola* and *Melanconiella camelliae*. This paper also introduces two other novel species: *Peroneutypa hibisci*, a saprobe found on *Hibiscus tiliaceus* and *Pseudochaetosphaeronema bruguierae* from *Bruguiera cylindrica*, the first species in this genus reported as a mangrove fungus. A new record of *Rimora mangrovei* from *Ceriops tagal* is also reported. These discoveries emphasize the rich fungal diversity in mangrove ecosystems supporting further exploration of this unique environment.

Key words: Fungi, mangrove, novel species, Pranburi, *Pseudomelanconiella*, saprobic fungi, taxonomy

Introduction

Mangroves, found in unique intertidal habitats, are vital ecosystems hosting diverse flora and fauna. Though they cover only 1% of tropical forests, they support various plants and animals including mammals, birds, fish, and insects (Jia et al. 2020). Mangroves are also diverse hosts to a high number of fungal species (Norphanphoun et al. 2018; Devadatha et al. 2021).

The fungal kingdom, with an estimated 2.2–13 million species has a rich evolutionary history deeply intertwined with that of terrestrial, aquatic, and marine ecosystems (Hyde and Jones 1988; Wu et al. 2019; Hyde et al. 2020, 2024; Phukhamsakda et al. 2022). However, only about 10 percent of these have been described and reported (Hyde et al. 2024). This is the lowest percentage of described species among the eukaryotes compared to plants (83–96%) and animals (19%) (Wang et al. 2020; Bhunjun et al. 2022; Phukhamsakda et al. 2022). This highlights the need to continuously survey for fungal species, especially in unique ecological niches such as the mangrove environment.

Fungal study in mangroves began in the 1920s with the report of Stevens (1920) from mangroves in Puerto Rico, followed by the work of Cribb and Cribb (1955) in Australia. Kohlmeyer (1968) listed 75 species of mangrove fungi, noting their taxonomic diversity and host preferences. Later, Hyde and Jones (1988) documented 90 species of intertidal mangrove fungi across 26 tree species. Many species are widespread across different ocean basins, indicating their adaptability to diverse environmental conditions (Hyde and Jones 1988). Jones and Alias (1997) identified 268 mangrove fungi species, observing limited host-specificity but some host preferences, indicating a nuanced relationship between fungal communities and host plant diversity within the mangrove ecosystem. They suggested higher fungal diversity in Asian tropics due to greater host diversity, although this assertion is somewhat complicated since more studies were being conducted in Asia (Hyde and Lee 1995; Jones and Alias 1997).

Schmit and Shearer (2003) published the first checklist of mangrove fungi recognizing 625 species, including some freshwater and terrestrial taxa. Devadatha et al. (2021) updated this to 850 species, marking an increase of more than 200 taxa within the 18-year between the checklists. However, Devadatha et al. (2021) focused exclusively on marine fungi from mangrove substrates, excluding those from freshwater and terrestrial environments. Over the years, the number of fungi associated with mangroves has steadily increased due to a wider interest in their study, especially in bioprospecting for bioactive compounds useful in medicine, agriculture, and biotechnology (Tan et al. 2015; Dela Cruz et al. 2020; Cadamuro et al. 2021; Sopalun et al. 2021; Chen et al. 2022).

In this study, we introduce one new genus, three novel species and one new record of fungi from mangroves in Prachuap Khiri Khan province in Thailand.

Methods

Collection, observation, and isolation

Mangrove samples were collected from Pranburi Forest Park (No. 0907.4/23579) and the Pranburi River area in Prachuap Khiri Khan, Thailand. The samples included dead branches attached to mangroves and decomposing branches submerged in brackish water. They were sealed in plastic bags and transported to the laboratory at the Center of Excellence in Fungal Research, Mae Fah Luang University in Chiang Rai, Thailand.

Fungi on the samples were examined using a stereomicroscope, and their morphological features were documented with a Nikon Eclipse Ni compound microscope equipped with a Nikon DS-Ri2 camera (Nikon, Japan). Measurements were taken using Tarosoft® Image Framework software calibrated for the microscope. Photo plates were created using Adobe Photoshop 24.0 (Adobe Systems, USA).

To cultivate the fungi, single-spore isolation technique was employed (Chomnunti et al. 2014; Senanayake et al. 2020). Cultures were incubated at room temperature for 2–4 weeks and then used for DNA extraction. These cultures were deposited in the Mae Fah Luang University Culture Collection (MFLUCC). Additionally, herbarium samples were deposited at the Mae Fah Luang University Herbarium (MFLU). Novel fungal species were registered in Index Fungorum (2024) and Faces of Fungi (Jayasiri et al. 2015).

DNA extraction and PCR

DNA extraction from mycelia of pure fungal cultures was carried out using the E.Z.N.A® Tissue DNA kit (Omega Biotek, USA) according to the manufacturer's instructions. Initial tissue lysis was performed using the Qiagen Tissuelyzer (Qiagen, Netherlands), with the samples mixed with lysis solution. Subsequent steps followed the protocol outlined in the extraction kit.

Multi-locus amplification was conducted on all isolates. PCR reactions were prepared in 25 µl volumes, comprising 21 µl of Vazyme® Rapid Taq Master Mix, 1 µl each of forward and reverse primer, and 2 µl of template DNA. The internal transcribed spacer region (ITS) and different loci were amplified for the isolates using distinct primers (Table 1). For *Pseudomelanconiella mangrovei*, nuclear large subunit ribosomal DNA (LSU), RNA polymerase II second largest subunit (*rpb2*), and translation elongation factor 1-alpha (*tef1-α*) were amplified with *tef1-α* using EF1-728F/TEF1-LLeReV primers. For *Peroneutypa hibisci*, beta-tubulin (*tub2*) was also amplified. For *Pseudochaetosphaeronema bruguierae* and *Rimora mangrovei*, LSU, 18s small subunit ribosomal gene (SSU), and *tef1-α* were amplified, using EF1-983F and EF1-2218R primers for *tef1-α*. For *R. mangrovei*, only LSU, SSU and TEF were used for the phylogenetic analysis, as detailed in the results section. The list of primers and PCR conditions for each primer pair is provided in Table 1.

Following PCR, the resulting products were electrophoresed on a 1% agarose gel to verify the sizes of the amplicons. If single bands matching the expected sizes were observed in the gel, the PCR products were forwarded for Sanger sequencing at Sangon Biotech (China).

Phylogenetic analysis

The sequences were assembled using Seqman II v. 5.0 to produce a contig. Subsequently, the contigs were searched in the Basic Local Alignment Search Tool (Madden 2003) to check for closely related sequences. Based on the results, related sequences to the isolates were retrieved based on previous studies. The National Center of Biotechnology Information (NCBI) Nucleotide database was checked to ensure the inclusion of all related species with molecular data in the analysis. The accession numbers of the sequences used in this study are shown in Suppl. material.

Table 1. Primers and PCR conditions used in the study.

Locus	Primers	Sequence (5'-3')	Reference	PCR Conditions
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1989)	95 °C, 5 min; 35 cycles of 95 °C 45 s, 53 °C 45 s, 72 °C 2 min; 72 °C 5 min
	ITS4	TCCTCCGCTTATTGATATGC		
LSU	LR0R	ACCCGCTGAACTTAAGC	Vilgalys and Hester (1990)	94 °C 3 min;; 35 cycles of 94 °C 1 min, 52 °C 50 s, 72 °C 1 min; 72 °C 10 min
	LR5	ATCCTGAGGGAAACTTC		
rpb2	fRPB2-5F	GAYGAYMGWGATCAYTTYGG	Liu et al. (1999)	94 °C 3 min;; 35 cycles of 94 °C 1 min, 52 °C 50 s, 72 °C 1 min; 72 °C 10 min
	fRPB2-7CR	CCCATRGCTTGYTTRCCCAT		
SSU	NS1	GTAGTCATATGCTTGTCTC	White et al. (1989)	95 °C 2 min; 35 cycles of 95 °C 30 s, 55 °C 50 s, 72 °C 1 min; 72 °C 10 min
	NS4	CTTCCGTCAATTCCTTTAAG		
tef1-α	EF1-728F	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn (1999)	95 °C 2 min; 35 cycles of 95 °C 30 s, 56 °C 45 s, 72 °C 1 min; 72 °C 8 min
	TEF1-LLeReV	AACTTGCAGGCAATGTGG	Jaklitsch et al. (2005)	
	EF1-983f	GCYCCYGGHCAYCGTGAYTTYAT	Rehner and Buckley (2005)	95 °C 2 min; 35 cycles of 95 °C 30s, 55 °C 50s, 72 °C 1 min; 72 °C 10 min
	EF1-2218R	CCRAACRGCRACRGTYYGTCTCAT		
tub2	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	Glass and Donaldson (1995)	95 °C 5 min; 35 cycles of 94 °C 30 s, 54 °C 30 s, 72 °C 1 min; 72 °C 8 min
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		

The sequences of the isolates and related species were aligned using Multiple Alignment using Fast Fourier Transform (MAFFT) version 7.4 with strategy set to auto, a gap extend penalty of 0.123, a gap opening penalty of 1.53, with adjust direction selected (Katoh and Standley 2013). The alignment was cleaned up by Block Mapping and Gathering with Entropy (BMGE; Criscuolo and Gribaldo 2010), with the following settings: DNAPAM matrix, a sliding window size of 3, gap rate cut-off of 0.5, maximum entropy threshold of 0.5, and minimum block size of 5. MAFFT and BMGE were performed on the NGPhylogeny site (Lemoine et al. 2019).

The aligned sequences were then subjected to maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) analyses. For ML, RAxML-HPC2 on ACCESS v. 8.2.12 (Stamatakis 2014) was utilized, using the default GTRGAMMA model and 1,000 bootstrap iterations. For MP, PAUP on XSEDE v.4.168 (Swofford 2002) set to 1,000 bootstrap iterations was employed.

Model testing was conducted prior to Bayesian analysis using MEGA v. 11 (Tamura et al. 2021), and specific models were applied during the analysis. For the multi-locus analysis, specific models for each partition of the concatenated sequences were specified in the command block. Bayesian posterior probability analysis was conducted using Markov chain Monte Carlo (MCMC) sampling in MrBayes v.3.2 on XSEDE (Ronquist et al. 2012), with four simultaneous Markov chains run for 10,000,000 generations, sampling every 1,000th generation. The run discarded 25% of the trees as the burn-in phase, using the remaining trees to compute the posterior probability (PP) in the consensus tree. Trace files of individual and combined runs were assessed using Tracer v.1.7.2 (Rambaut et al. 2018).

Phylogenetic analyses were initially performed on individual loci before multi-locus analyses were conducted. Trees generated from individual locus and multi-locus analyses for each taxon were compared, and only reported if they exhibited similar topologies. Trees were visualized using FigTree v.14.4 (Rambaut 2018), and then edited using Adobe Illustrator v.27.0 (Adobe

Systems, USA), combining the bootstrap values from ML and MP with the posterior probabilities (PP) from Bayesian analysis. The decisions as to whether to introduce new genera or species follow Jeewon and Hyde (2016) and Maharachchikumbura et al. (2021).

Results

Sordariomycetes O.E. Erikss. & Winka
Diaporthales Nannf.
Melanconiellaceae Senan., Maharachch. & K.D. Hyde

Phylogenetic analysis for *Pseudomelanconiella* isolates was performed using ITS, LSU, *rpb2* and *tef1-α* loci. The ML, MP and Bayesian multi-locus analysis consisted of a total of 3,957 characters, including gaps in the concatenated sequence. The lengths of each region were as follows: ITS (1-592), LSU (593-1,469), *rpb2* (1,470-2,631), *tef*1-α (2,632-3,957).

For ML analysis, the alignment had 1,527 distinct alignment patterns with 23.52% gaps and undetermined characters. The best-scoring tree (shown in Fig. 1) had a final optimization likelihood of -25,438.87, tree length of 1.744101 and alpha of 0.239245. The base frequencies are: A = 0.227229, C = 0.273128, G = 0.273187 and T = 0.226456, with the following substitution rates: AC = 1.270018, AG = 3.717788, AT = 1.313118, CT = 5.037061, and GT = 1.00000.

The MP analysis of the multi-locus data included 3,957 characters, with 2,470 constant characters, 1,318 parsimony-informative and 169 parsimony-uninformative characters. The most parsimonious tree had a similar topology to the maximum likelihood tree (Fig. 1), with the *Pseudomelanconiella* clade in the same position on the tree.

For the Bayesian analysis, different evolutionary models were used for each locus based on the results from MEGA: Kimura 2-parameter with gamma distribution and proportion of invariable sites (K2P+G+I) for ITS, General Time Reversible model with gamma distribution (GTR+G) for LSU and General Time Reversible model with gamma distribution and proportion of invariable sites (GTR+G+I) for *rpb2* and *tef1-α*. The final average standard deviation of split frequencies after the total MCMC generations is 0.002019. Effective sample size (ESS) values for all factors of the combined trace files ranged from 7,577 to 15,002 with the trace plots showing two independent runs have converged. The topology of the tree from the Bayesian analysis is similar to the one obtained from the maximum likelihood analysis.

Based on the ML, MP, and BI analyses, which included sequences from species belonging to Melanconiellaceae, *Pseudomelanconiella* isolates (MFLU 24-0189, MFLU 24-0190) formed a distinct clade closely related to *Septomelanconiella* (Fig. 1). Although the node that separates *Pseudomelanconiella* and *Septomelanconiella* has low support in ML and MP, it has a good support for BI and is consistently recovered in all trees generated in the three analyses. Furthermore, the base differences of ITS, LSU and *rpb2* between *Septomelanconiella* and *Pseudomelanconiella* are higher than the least base difference between genera in Melanconiellaceae. Morphologically, *Pseudomelanconiella* differs from *Septomelanconiella*, as detailed in the taxonomy notes. *Pseudomelanconiella* is also phylogenetically distant from *Sinodiscula*, the most recent genus to be added to Melanconiellaceae.
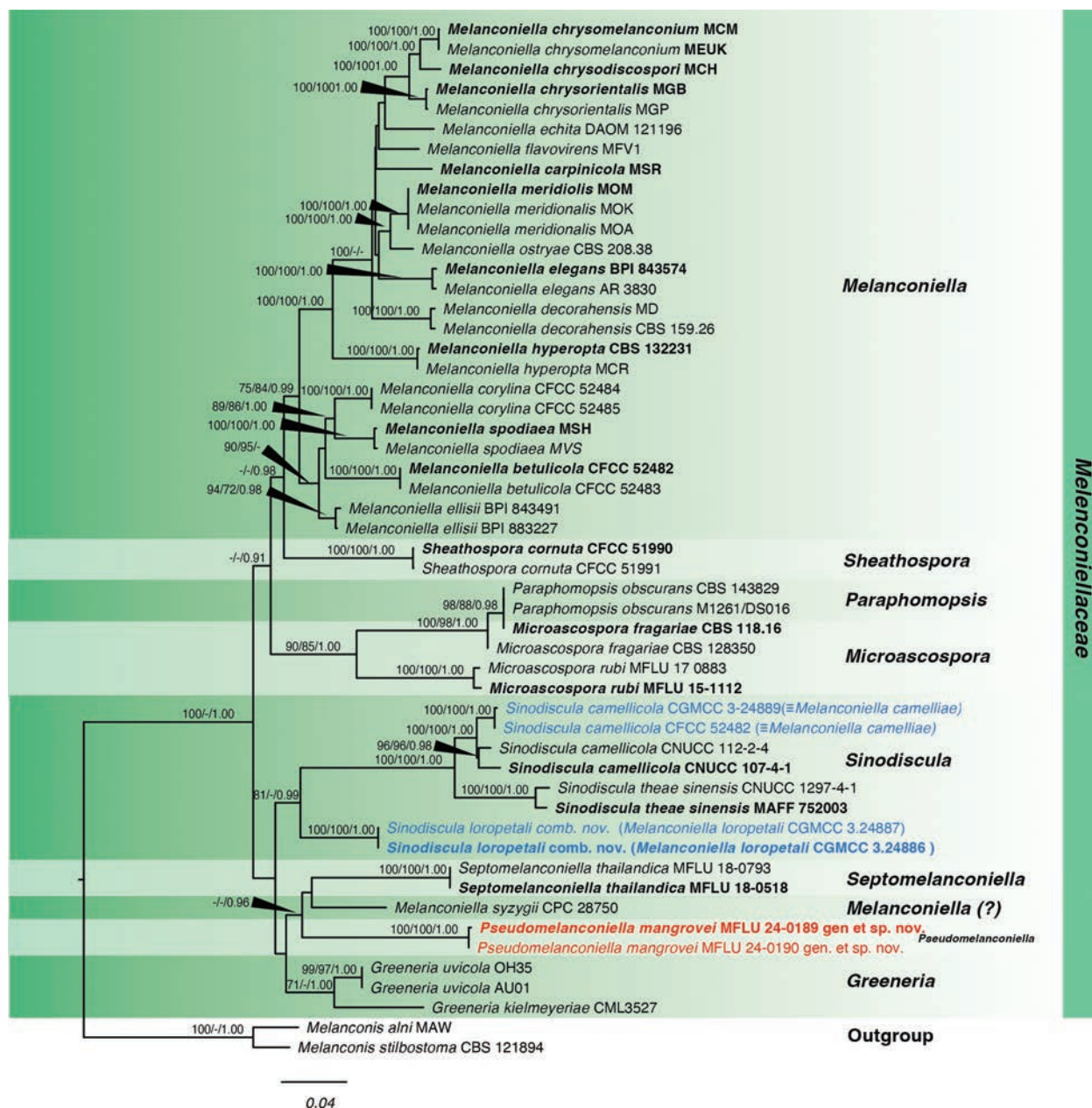
Figure 1. Phylogram of Melanconiellaceae based on a combined analysis of ITS, LSU, *rpb2*, and *tef1-α*. Values above the branches indicate bootstrap support from maximum likelihood (ML) and maximum parsimony (MP) equal to or above 70 and Bayesian posterior probabilities (PP) equal to or above 0.90. Novel species are in red, and species for synonymization and reclassification are in blue. Sequences from type species are indicated in bold. The tree is rooted with *Melanconis alni* MAW and *Melanconis stilbostoma* CBS 121894.

Our analysis shows that three *Melanconiella* species do not group within the *Melanconiella* clade. Thus, *Melanconiella camelliae* is synonymized with *Sinodiscula camellicola* while *Melanconiella loropetali* is reclassified as *Sinodiscula loropetali*. The third species, *Melanconiella syzygii*, forms a lineage with *Septomelanconiella*. However, the phylogenetic position of this isolate is not yet well-supported in the present analysis. Additional data are required to provide a more stable phylogenetic position, possibly leading to its reclassification under *Septomelanconiella*. The morphology of *M. syzygii* is similar to *Septomelanconiella* as they both possess septate conidia.

Pseudomelanconiella Apurillo, Phukhams., E.B.G. Jones & K.D. Hyde, gen. nov.
Index Fungorum: IF902576
Facesoffungi Number: FoF16488

Etymology. From the Greek "pseudo" meaning false, due to the close morphologic similarity of isolates with *Melanconiella* species.

Type species. *Pseudomelanconiella mangrovei*.

Description. Sexual morph: Undetermined. **Asexual morph: *Conidiomata*** globose to subglobose, immersed to erumpent, develop under a clypeus with variable stromata, confluent, mostly with a long, conical, central ostiole, black. ***Ostiole*** present. ***Peridium*** light brown to brown, composed of textura angularis cells. ***Conidiophores*** hyaline, septate. ***Conidiogenous cells*** hyaline, phialidic. ***Conidia*** oblong to ellipsoid, light-brown, unicellular, aseptate, verrucose, without gelatinous sheath.

Notes. Combined phylogenetic analysis of ITS, LSU, *rpb2* and *tef1-α* sequences reveal that *Pseudomelanconiella* forms a distinct, well-supported clade separate from other genera in Melanconiellaceae. The sister taxon is *Septomelanconiella*, however, this monotypic genus is distinguished by its septate, laminate conidia, which is not observed in *Pseudomelanconiella* (Phookamsak et al. 2019). Both *Pseudomelanconiella* and *Septomelanconiella* are coelomycetous and have no reported sexual morphs (Phookamsak et al. 2019). Pairwise differences between the *Septomelanconiella* and *Pseudomelanconiella* in ITS (13.2%), LSU (1.6%), *rpb2* (16.1%) are all higher than the lowest base difference observed between genera in Melanconiellaceae. *Septomelanconiella* was isolated as a saprobe from *Syzygium samarangense* in a terrestrial environment while *Pseudomelanconiella* was isolated from mangroves in a brackish water environment (Phookamsak et al. 2019). These support the establishment of a new genus with *Pseudomelanconiella mangrovei* as the type species.

Pseudomelanconiella mangrovei Apurillo, Phukhams., E.B.G. Jones & K.D. Hyde, sp. nov.
Index Fungorum: IF902577
Facesoffungi Number: FoF16489
Fig. 2

Etymology. Based on its mangrove host.

Holotype. MFLU 24-0189.

Description. *Saprobic* on decomposing branch of ***Avicennia marina*** submerged in brackish water. **Sexual morph**: Undetermined. **Asexual morph: *Conidiomata*** 100−590 µm × 200−815 µm ($\bar{x}$ = 386.3 × 566.2 µm, n = 10), globose to subglobose, immersed to erumpent, black, confluent, mostly with a central stromatic column. ***Peridium*** light-brown to brown, made of cells of textura angularis. ***Conidiophores*** 8−20 × 1−2 µm ($\bar{x}$ = 14.6 × 1.8 µm, n = 30), mostly straight, hyaline, septate, smooth unbranched. ***Conidiogenous cells*** 2−7 × 1−2 µm ($\bar{x}$ = 4.2 × 1.8 µm, n = 30), monophialidic, determinate, discrete, cylindrical to subcylindrical, smooth-walled, hyaline, arising from inner layers of conidioma. ***Conidia*** 11−14 × 3−4 µm ($\bar{x}$ = 11.4 × 3.3 µm,

Figure 2. *Pseudomelanconiella mangrovei* (MFLU 24-0189, holotype). **a** host **b, c** conidiomata on host **d** section of peridium **e** squash mount showing conidiophores and conidia **f** conidiophores, conidiogenous cells with attached conidia **g–j** conidia **k** germinated conidium **l** culture on MEA. Scale bars: 500 µm (**b**); 100 µm (**c**); 50 µm (**e, f**); 20 µm (**d,g**); 10 µm (**h–k**).

n = 50), oblong to ellipsoid, hyaline to light-brown, unicellular, aseptate, verrucose, without gelatinous sheath.

Known distribution. Thailand.

Culture characteristics. Conidia germinate in malt extract agar (MEA) within 24 hours, with germ tubes arising from one end of the conidia. Colonies on MEA grow up to 14 cm after 7 days of incubation at room temperature, circular, mostly flat or effuse with a raised ring near the center, undulate, white, translucent; reverse does not exhibit pigments.

Material examined. Thailand • Prachuap Khiri Khan Province, Pranburi District, 12°23'9"N, 99°56'51"E, on decomposing branch of *Avicennia marina* L.

(Acanthaceae) submerged in brackish water, 4 February 2023, Carlo Chris S. Apurillo, P30201 (MFLU 24-0189, *holotype*); • ibid., P30301 (MFLU 24-0190); ex-type living culture MFLUCC 24-0512 = MFLUCC 24-0513 .

GenBank numbers. MFLU 24-0189 = ITS: PP989291, LSU: PP989287, *rpb2*: PP993004, *tef1-α*: PP993001; MFLU 24-0190 = ITS: PP989292, LSU: PP989288, *tef1-α*: PP993002.

Notes. Based on combined analysis of ITS, LSU, *rpb2*, and *tef1-α* sequences, *Pseudomelanconiella mangrovei* formed a distinct clade within Melanconiellaceae, with *Septomelanconiella thailandica* as the closest taxon. *Pseudomelanconiella mangrovei* differs from *Septomelanconiella thailandica* based on the appearance of the conidia. The distinguishing characteristic of *S. thailandica* is its septate conidia (Phookamsak et al. 2019). In contrast, *Pseudomelanconiella mangrovei* conidia are aseptate. While the morphology of *Pseudomelanconiella mangrovei* is similar to *Melanconiella* species, particularly the appearance of conidiomata, conidiogenous cells and the shape of the conidia, its phylogenetic position is distinct from other *Melanconiella* species (Voglmayr et al. 2012). Thus, this isolate is classified under a novel genus, *Pseudomelanconiella*.

Sinodiscula M.J. Guo & C.L. Hou

***Sinodiscula loropetali* (T.C. Mu & Jun Z. Qiu) Apurillo, Phukhams., K.D. Hyde & E.B.G. Jones, comb. nov.**
MycoBank No: 855635
Facesoffungi Number: FoF16612

Melanconiella loropetali T.C. Mu & Jun Z. Qiu, Front. Microbiol. 14(1229705):3 (2023). Basionym. MycoBank No: 848666.

Description. Sexual morph: Undetermined. **Asexual morph**: Descriptions and illustrations refer to Mu et al. (2023).

Notes. *Melanconiella loropetali* was introduced in *Melanconiella* by Mu et al. (2023), isolated from diseased *Loropetalum sinense* in China. *Melanconiella loropetali*, *Melanconiella camelliae* and *Melanconiella syzygii*, formed a basal clade distinct from other *Melanconiella* species in Mu et al. (2023). However, only *Melanconiella* species were used in the analysis, leading to their classification as *Melanconiella*. We included other genera in Melanconiellaceae in our analysis and *M. camelliae*, *M. loropetali* and *M. syzygii* are phylogenetically distant from *Melanconiella*. The phylogenetic tree of the combined ITS, LSU, *rpb2* and *tef1-α* sequences showed that *Melanconiella loropetali* formed a sister clade to *Sinodiscula* species. Thus, we reclassified *M. loropetali* as *Sinodiscula loropetali*. *Melanconiella loropetali* is similar to *Sinodiscula* species. It fits the generic description of *Sinodiscula*, and like other *Sinodiscula* species, *Melanconiella loropetali* was isolated as a plant pathogen (Mu et al. 2023; Guo et al. 2024). Given the similarities in morphology and ecology of *M. loropetali* and *Sinodiscula* species, and the well-supported phylogenetic position of *Melanconiella loropetali*, we reclassify it as *Sinodiscula loropetali*.

Sinodiscula camellicola S.Y. Zhao, M.J. Guo & C.L. Hou, Journal of Fungi 10 (2, no. 141): 9 (2024)
Index Fungorum: IF851775
MycoBank No: 851775

≡ *Melanconiella camelliae* T.C. Mu & Jun Z. Qiu, Front. Microbiol. 14(1229705):6 (2023). MycoBank No: 848667.

Description. Descriptions and illustrations refer to Mu et al. (2023) and Guo et al. (2024).

Notes. In the present analysis, *Melanconiella camelliae* formed a well-supported clade with *Sinodiscula camellicola*. Detailed morphological comparison reveals that these two species are similar with no notable differences. Both species were isolated as pathogens from *Camellia sinensis*, from Fujian Province (China) for *Melanconiella camelliae* and from Anhui Province (China) for *Sinodiscula camellicola* (Guo et al. 2024). Moreover, the ITS sequences of *Melanconiella camelliae* and *Sinodiscula camellicola* differ by only 1%. Due to these similarities in morphology, ecology and molecular data of the two species, we propose to synonymize *M. camelliae* and *S. camellicola* under *Sinodiscula* (Melanconiellaceae).

Xylariales Nannf.
Diatrypaceae Nitschke
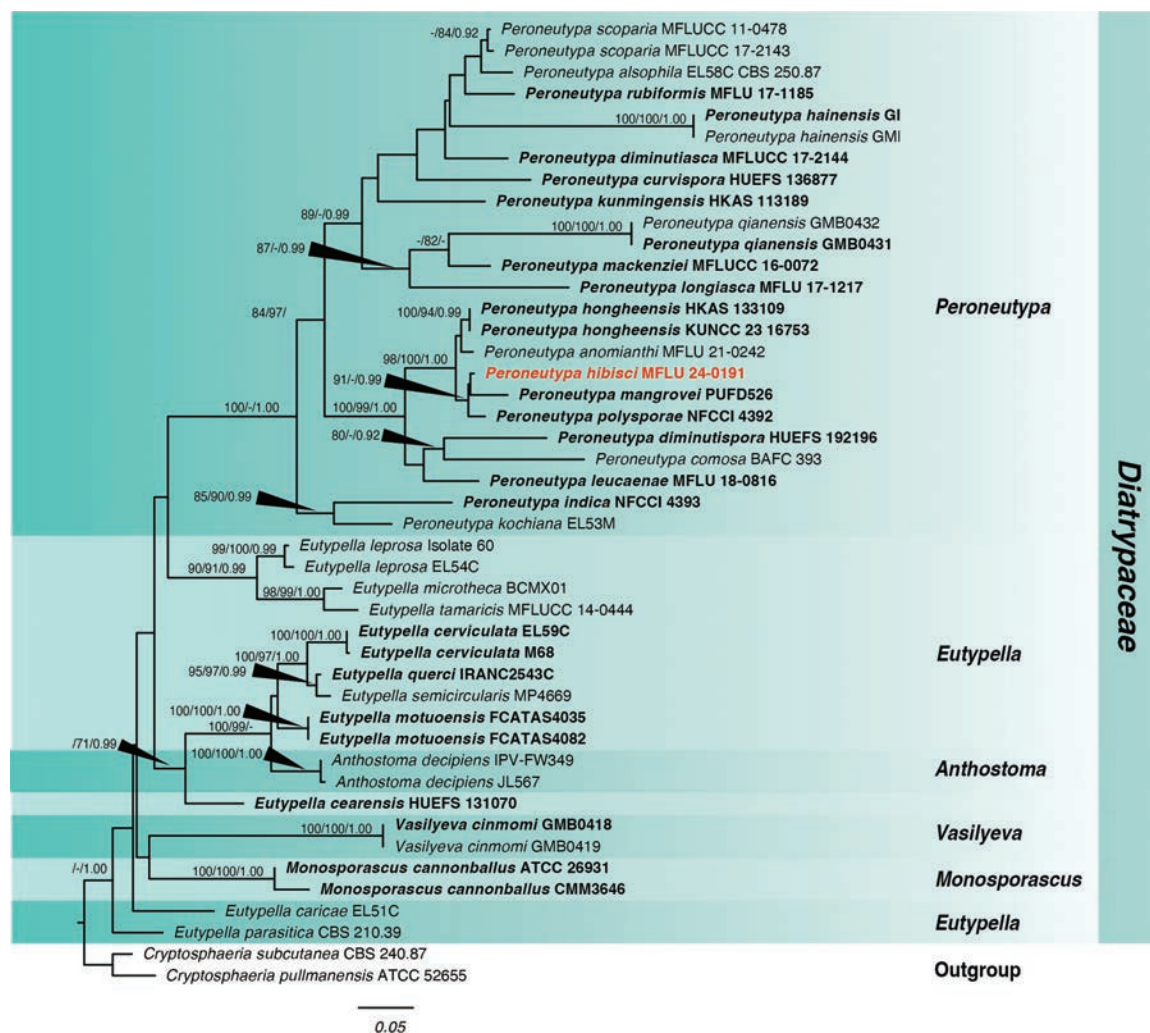**Peroneutypa Berl.**

Phylogenetic analysis of *Peroneutypa* was based on the combined ITS and *tub2* sequence data. The combined multi-locus analysis consisted of 927 characters, including gaps with the following lengths: ITS (1-531) and *tub2* (532-927). ML, MP, and BI analyses were done using single and concatenated sequences.

For ML, there were 540 distinct alignment patterns with 31.95% gaps and undetermined characters in the concatenated sequences. The maximum likelihood tree (Fig. 3) has a final ML optimization likelihood of -8,464.76, tree length of 3.225995 and alpha of 0.362736. The base frequencies are: A = 0.222507, C = 0.268440, G = 0.239063 and T = 0.269990. The rates of substitution are as follows: AC = 0.825859, AG = 2.247793, AT = 1.238868, CG = 0.697931, CT = 3.237131 and GT = 1.00000.

The MP analysis consisted of 927 total characters, 452 of which were constant, 396 parsimony-informative and 79 parsimony-uninformative. The most parsimonious tree had a similar topology as the best-scoring ML tree (Fig. 3).

For the Bayesian analysis, the model used for ITS was K2P+G and Hasegawa-Kishino-Yano with gamma distribution (HKY+G) for *tub2*. After the total MCMC generations, the average standard deviation of split frequencies is 0.002628. Analysis in Tracer showed that the two independent runs have converged based on the trace plots with the ESS values of all factors for the combined runs ranging from 4,877 to 14,599. The topology of the Bayesian tree is similar to the best-scoring tree in ML shown in Fig. 3, especially with respect to the position of *Peroneutypa hibisci*.

Figure 3. Phylogram of the combined ITS and *tub2* analysis of *Peroneutypa* and related genera in Diatrypaceae. Values above the branches indicate bootstrap support values from maximum likelihood (ML) and maximum parsimony (MP) equal to or above 0.70 and Bayesian posterior probability (PP) equal to or above 0.90. The novel species is indicated in red bold. Sequences from type species are indicated in bold. The tree is rooted with *Cryptoshaeria subcutanea* CBS 240.87 *and Cryptosphaeria pullmanensis* ATCC 52655.

The ML, MP, BI analyses showed that *Peroneutypa hibisci* formed a distinct lineage with *Peroneutypa mangrovei*, the latter showing a longer branch length (Fig. 3). Although the two species were isolated from mangroves, they differ in morphology as discussed in the taxonomy notes. Furthermore, the base pair difference in ITS and *tub2* are greater than 1.5%.

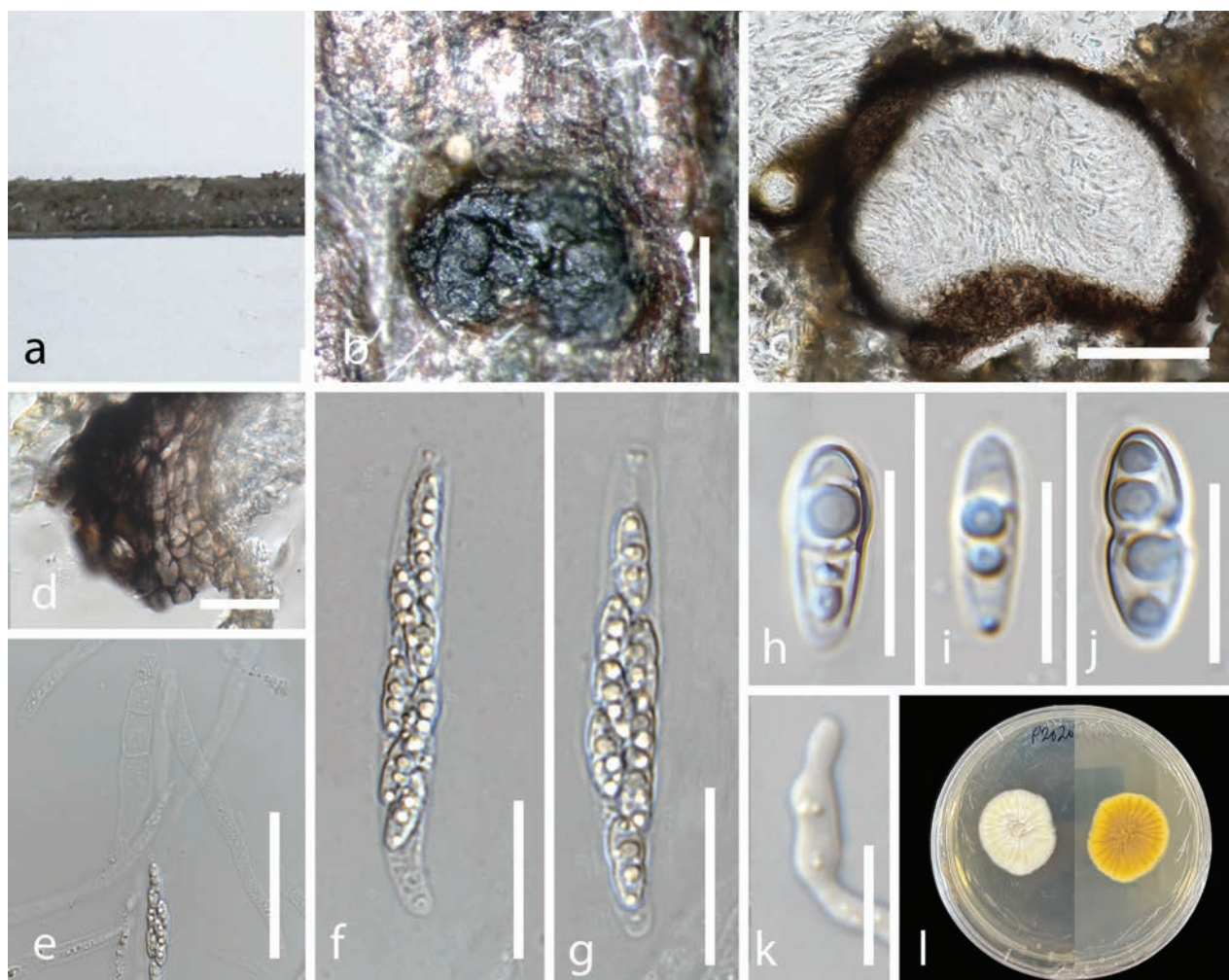***Peroneutypa hibisci* Apurillo, Phukhams., E.B.G. Jones & K.D. Hyde, sp. nov.**
Index Fungorum: IF902578
Facesoffungi Number: FoF16490
Fig. 4

Etymology. Based on the host, *Hibiscus tiliaceus*.
    Holotype. MFLU 24-0191.

Figure 4. *Peroneutypa hibisci* (MFLU 24-0191, holotype). **a** host **b** ascoma on host **c** section of ascoma **d** peridium **e** ascus with hamathecium **f, g** asci **h–j** ascospores **k** germinated ascospore **l** culture on MEA. Scale bars: 200 μm (**b**); 100 μm (**c**); 50 μm (**d**); 20 μm (**e, f**); 10 μm (**g–j**).

Description. Saprobic on decomposing branches of **Hibiscus tiliaceus** submerged in brackish water. **Sexual morph: Stromata** 1.0–1.5 mm ($\bar{x}$ = 1.2, n = 5), poorly developed, non-sulcate, solitary to gregarious, immersed to erumpent, dark brown to black. **Perithecia** 200–300 μm ($\bar{x}$ = 238, n = 5) in diameter, immersed to erumpent, globose, brown to black, ostiolate. **Peridium** 20–45 μm ($\bar{x}$ = 32, n = 5) wide, composed of two layers, outer layer dark brown to black comprising of textura angularis cells, inner layer of textura angularis cells, brown to dark-brown. **Hamathecium** 90–125 μm × 3–4 μm ($\bar{x}$ = 104 × 3.4 μm, n = 5) wide, hyaline, aseptate, unbranched. **Asci** 57–68 μm × 6–8 μm ($\bar{x}$ = 59.4 × 7.6 μm, n = 5), 8-spored, clavate, unitunicate, short stipitate, with inamyloid apical rings. **Ascospores** 8–12 μm × 3–4 μm ($\bar{x}$ = 10.7 × 3.6 μm, n = 45), hyaline, ellipsoid, with constricted median septum when mature, 2–4 guttules. **Asexual morph:** Undetermined.

Known distribution. Thailand.

Culture characteristics. Ascospores germinated in MEA within 24 hours, the germ tubes arising from both ends of the ascospore. Colonies on MEA grow up to 10 cm after 7 days of incubation at room temperature, white, filamentous, with aerial mycelium, reverse with gray pigment toward the center.

Material examined. THAILAND • Prachuap Khiri Khan: Pranburi District, 12°23'8.74"N, 99°56'51.47"E, on decomposing branches of *Hibiscus tiliaceus* submerged in brackish water. 4 February 2023, Carlo Chris S. Apurillo, P20201 (MFLU 24-0191, **holotype**), ex-type living culture, MFLUCC 24-0514.

GenBank Numbers. ITS: PP989294, *tub2* = PP993003.

Notes. *Peroneutypa hibisci* formed a lineage with *Peroneutypa mangrovei*, the latter showing a longer branch length than *Peroneutypa hibisci*. Although this clade has low support, this was consistently observed in ML, MP and BI trees. Comparison of base pair differences between these two closely related species revealed a difference of 23 out of 459 bases (5.0%) in ITS and 7 out of 349 bases (2.0%) in *tub2* sequences. Morphologically, they differ significantly: *P. hibisci* has larger, ellipsoid, guttulate ascospores (8−12 × 3−4 μm), while *P. mangrovei* has smaller, cylindrical to clavate ascospores (3−5 × 1−1.5 μm) without guttules. The asci of *P. hibisci* (57−68 × 6−8 μm) are also much larger than those of *P. mangrovei* (14−20 × 3−4 μm) (Phookamsak et al. 2019). Compared to another related species, *P. polysporae*, *P. hibisci* differs by producing median-septate, ellipsoid ascospores, whereas *P. polysporae* has smaller, unicellular, allantoid spores. Additionally, *P. polysporae* has larger asci (110−155 × 5−7.5 μm) than *P. hibisci* (Dayarathne et al. 2020). Morphological and sequence data support the introduction of *Peroneutypa hibisci* as a novel species based on the guidelines of Jeewon and Hyde (2016) and Maharachchikumbura et al. (2021).

Dothideomycetes O.E. Erikss. & Winka
Pleosporales Luttr. Ex M.E. Barr
**Macrodiplodiopsidaceae Voglmayr, Jaklitsch & Crous,**
Pseudochaetosphaeronema Punith.

The ML, MP, and BI phylogenetic analyses of *Pseudochaetosphaeronema* utilized a combined multi-locus phylogeny including ITS, LSU, SSU, and *tef1-α* sequences. The alignment had a total of 3,313 characters, including gaps, with the lengths of the regions as follows: ITS (1-523), LSU (524-1,384), SSU (1,385-2,414), and *tef*1-α (2,415-3,313). For ML, the alignment has 607 distinct patterns with 20.91% gaps and completely undetermined characters. The best-scoring tree (Fig. 5) had a final optimization likelihood of -10,102.09 with tree length of 0.507278 and alpha = 0.103258. The base frequencies are as follows: A = 0.237153, C = 0.250803, G = 0.270592, T = 0.241452 and the following substitution rates: AC = 1.724291, AG = 4.038401, AT = 2.171255, CG = 1.954009, CT = 11.161818, GT = 1.00000.

For MP, out of the 3,313 characters in the dataset, 2,715 were constant, 368 were parsimony-informative while 230 were parsimony-uninformative. The topology of the ML tree from the combined analysis was similar to that of the best-scoring tree.

In the Bayesian analysis, the models used for the different loci were as follows: K2P+G for ITS and SSU, K2P+G+I for LSU and GTR+G+I for *tef1-α*. The average standard deviation of split frequencies after the total MCMC runs is 0.001383. Evaluation of the trace files in Tracer showed ESS values for all factors ranging 4,963 to 13,845. The plots showed that the two independent runs have converged. The topology of the BI tree was similar to the best-scoring ML tree, especially the position of *Pseudochaetosphaeronema bruguierae*.
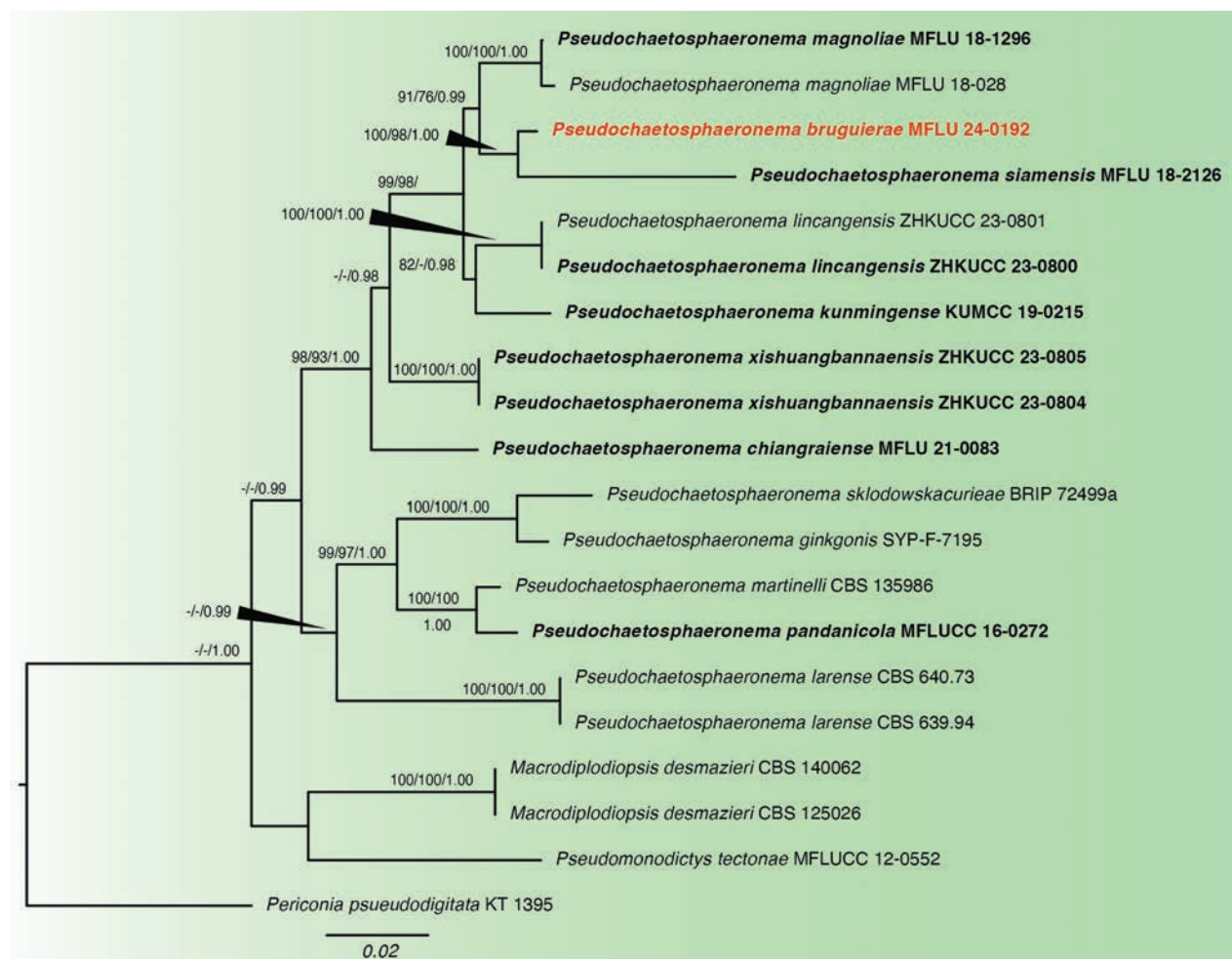
Figure 5. Phylogram of the combined ITS, LSU, SSU and *tef1-α* analysis of *Pseudochaetosphaeronema* and related genera. Values above the branches indicate bootstrap support values from maximum likelihood (ML) and maximum parsimony (MP) equal to or above 0.70 and Bayesian posterior probability (PP) equal to or above 0.90. The novel species is indicated in bold red. Sequences from type species are indicated in bold. The tree is rooted with *Periconia pseudodigitata*.

Based on the ML, MP, and BI analyses, *Pseudochaetosphaeronema bruguierae* is found in a distinct, well-supported clade with *Pseudochaetosphaeronema siamensis* as the sister taxon (Fig. 5). Notably, *P. siamensis* has a significantly longer branch length compared to *P. bruguierae*. Base differences in their sequences are also higher than 1.5%. In addition, *P. bruguierae* and *P. siamensis* differ in morphology of their conidiogenous cells and conidia, as discussed in the taxonomy notes.

***Pseudochaetosphaeronema bruguierae* Apurillo, Phukhams., E.B.G Jones & K.D. Hyde, sp. nov.**
Index Fungorum: IF902579
Facesoffungi Number: FoF16491
Fig. 6

Etymology. Based on the host *Bruguiera cylindrica*.
　Holotype. MFLU 24-0192.

Description. *Saprobic* on aerial dead branch of ***Bruguiera cylindrica***. **Sexual morph**: Undetermined. **Asexual morph**: Coelomycetous. ***Conidiomata*** 230−400 × 300−370 μm diameter ($\overline{x}$ = 344.5 × 339.0 μm, n = 10), dark brown to black, immersed to erumpent, solitary to gregarious, globose to subglobose, without ostiole. ***Conidiomata wall*** 22−66 μm, composed of dark brown, thick-walled cells. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** 7−10 μm × 1−2.7 μm ($\overline{x}$ = 10.2 × 1.7 μm, n = 20), hyaline, cylindrical or ampulliform, monophialidic, smooth. ***Conidia*** 5−10 × 2−3 μm ($\overline{x}$ = 6.5 × 2.2 μm, n = 30), hyaline to pale brown, fusiform, 2−3 septate, with constriction in the septa, with 26 guttules.

Known distribution. Thailand.

Culture characteristics. Conidia germinate in MEA within 24 hours, with germ tubes arising from one end of the conidia. Colonies on MEA grow up to 30 cm after 3 weeks of incubation at room temperature, circular, white, with light green pigment, flat or effuse, entire edge, reverse exhibits a dark green to pale brown pigmentation.

Material examined. THAILAND • Prachuap Khiri Khan Province: Pranburi District, 12°24'48"N, 99°56'51"E, on aerial dead branch of *Bruguiera cylindrica* (Rhizophoraceae), 25 October 2022, Carlo Chris S. Apurillo, P11601 (MFLU 24-0192, ***holotype***), ex-type living culture MFLUCC 24-0515.

Genbank Numbers. ITS: PP989295, LSU: PP989290, SSU: PP989296, *tef*1-α: PQ273803.

Notes. Based on a combined phylogenetic analysis of ITS, LSU, SSU, and *tef1-α* sequence data, *Pseudochaetosphaeronema bruguierae* formed a clade with *Pseudochaetosphaeronema siamensis*, with the latter showing longer branch length. Base pair comparison of ITS and *tef*1-α sequences revealed a 1.8% and 15% difference, respectively. The most significant difference between the two closely related species is their conidial morphology. *Pseudochaetosphaeronema bruguierae* has larger conidia (6.5 × 2.2 μm) which are fusiform, septate, with multiple guttules. In contrast, *Pseudochaetosphaeronema siamensis* has smaller conidia (3 × 2 μm) which are subglobose to oval without septation (Jayasiri et al. 2019). Furthermore, the conidiogenous cells of *Pseudochaetosphaeronema siamensis* are cylindrical with collarettes at the tips, while some appear flask-shaped with no collarettes for *Pseudochaetosphaeronema bruguierae* (Jayasiri et al. 2019). The conidiomata of *Pseudochaetosphaeronema bruguierae* is also larger than that of *Pseudochaetosphaeronema siamensis*. *Pseudochaetosphaeronema bruguierae* is similar to *Pseudochaetosphaeronema magnoliae* but they are phylogenetically distinct, with *P. magnoliae* forming a sister clade to *P. bruguierae* and *P. siamensis* (De Silva et al. 2022). Furthermore, *P. bruguierae* and *P. magnoliae* have a higher pairwise difference in the ITS sequences (9.3%). Although the shape of conidiogenous cells and the conidia of *P. bruguierae* and *P. magnoliae* are similar, they differ in terms of size, with *P. bruguierae* having larger conidiogenous cells and smaller conidia. Additionally, the conidia of *P. bruguierae* are septate with pronounced multiple guttules, which are not observed in the conidia of *P. magnoliae*. These support the introduction of *Pseudochaetosphaeronema bruguierae* as a novel species in *Pseudochaetosphaeronema* following the guidelines of Jeewon and Hyde (2016) and Maharachchikumbura et al. (2021).
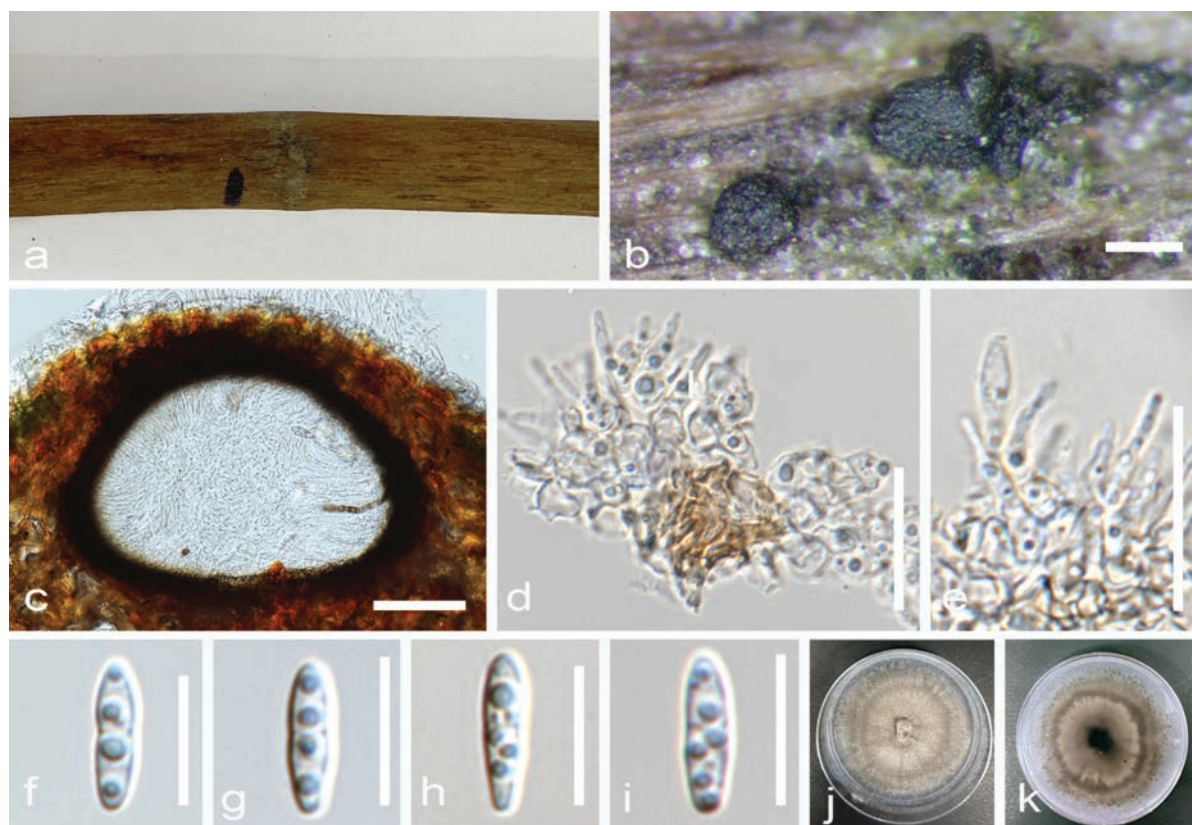
Figure 6. *Pseudochaetosphaeronema bruguierae* (MFLU 24-0192, *holotype*). **a** host **b** conidiomata on host **c** section of conidioma **d, e** conidiogenous cells **f–i** conidia **j** culture on MEA (obverse) **k** culture on MEA (reverse). Scale bars: 500 μm (**b**); 100 μm (**c**); 50 μm (**d, e**); 20 μm (**f**); 10 μm (**g–i**).

Pleosporales Luttr. ex M.E. Barr
Aigialaceae Suetrong, Sakay., E.B.G. Jones, Kohlm., Volkm.-Kohlm. & C.L. Schoch
Rimora Kohlm., Volkm.-Kohlm., Suetrong, Sakay. & E.B.G. Jones

Multi-locus analysis of *Rimora* was done using LSU, SSU and *tef1-α*. The alignment of concatenated sequences had a total length of 2,797 characters, including gaps. The specific lengths of each locus are as follows: LSU (1-853), SSU (854-1,876), *tef1-α* (1,877-2,797). In ML, the alignment has 720 distinct alignment patterns with 13.99% gaps and completely undetermined characters. After the analysis, the best-scoring ML tree (Fig. 5) had a final optimization value of -10,511.38, alpha = 0.162554 and tree length of 0.579899. The base frequencies are: A = 0.246335, C = 0.243918, G = 0.280712, and T = 0.229035 with the following substitution rates: AC = 1.97470, AG = 3.553768, AT = 1.000390, CG = 1.272768, CT = 13.114709 and GT = 1.00000.

For the BI analysis, different models were applied for each locus as follows: GTR+G for LSU and *tef1-α* and K2P+G+I for SSU. After the total MCMC generations, the average standard deviation of split frequencies is 0.003756. Analysis of trace files in Tracer showed ESS values ranging from 8,004 to 15,002 for the combined runs with the two independent runs showing convergence based on the plots. The topology of the BI tree was similar to the best-scoring ML tree (Fig. 5).

Based on the ML and BI multi-locus analyses, *Rimora mangrovei* MFLU 24-0193 is in a distinct clade with good support together with the holotype and other strains of *Rimora mangrovei* (Fig. 7). This strain, isolated from *Ceriops tagal*, represents a new host record for *Rimora mangrovei*.
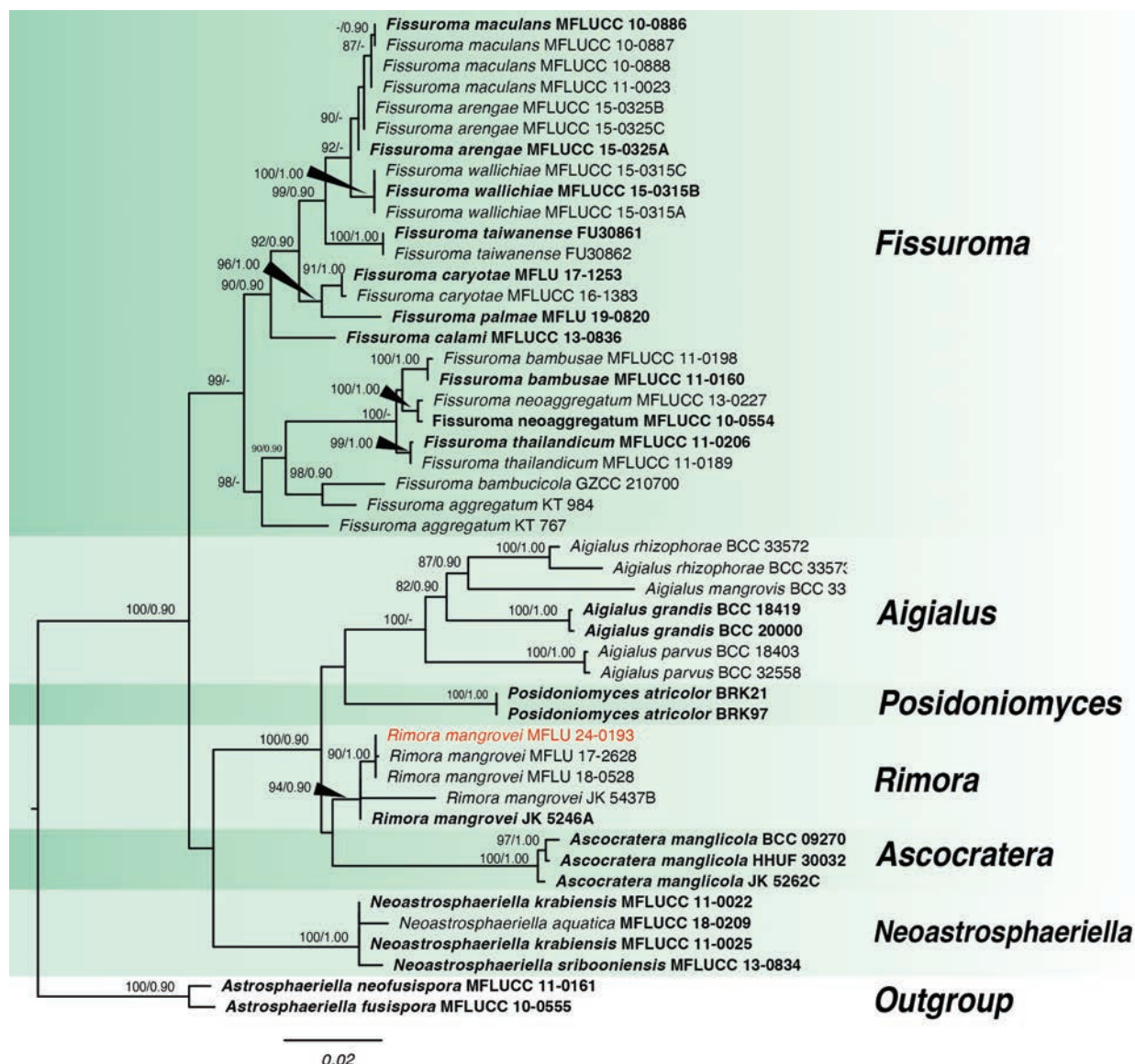
Figure 7. Phylogram of *Rimora* and closely-related genera based on a combined dataset of LSU, SSU and *tef1-α*. Values above the branches indicate bootstrap support from Maximum Likelihood (ML) and Bayesian posterior probability (PP). New record is indicated in red. Sequences from type species are indicated in bold. The tree is rooted with *Astroasphaeriella* species.

***Rimora mangrovei* (Kohlm. & Vittal) Kohlm., Volkm.-Kohlm., Suetrong, Sakay. & E.B.G. Jones**
Index Fungorum: IF515959
MycoBank No: 515959
Facesoffungi Number: FoF08152
Fig. 8

Description. *Saprobic* on decomposing wood of ***Ceriops tagal*** submerged in brackish water. **Sexual morph**: *Ascomata* 336−634 µm × 200−483 ($\bar{x}$ = 494 µm × 329 µm, n = 10) wide, globose to subglobose, black, carbonaceous, solitary to gregarious, immersed at first then later erumpent, with cleft-like ostiole, epapillate. *Peridium* 73−162 µm ($\bar{x}$ = 112 µm, n = 10) thick, cells forming textura angularis. *Pseudoparaphyses* up to 2 µm, trabeculate (*sensu* Liew et al. 2000), branched, numerous. *Asci* 175−181 × 9−15 µm ($\bar{x}$ = 178.8 × 12.4 µm,
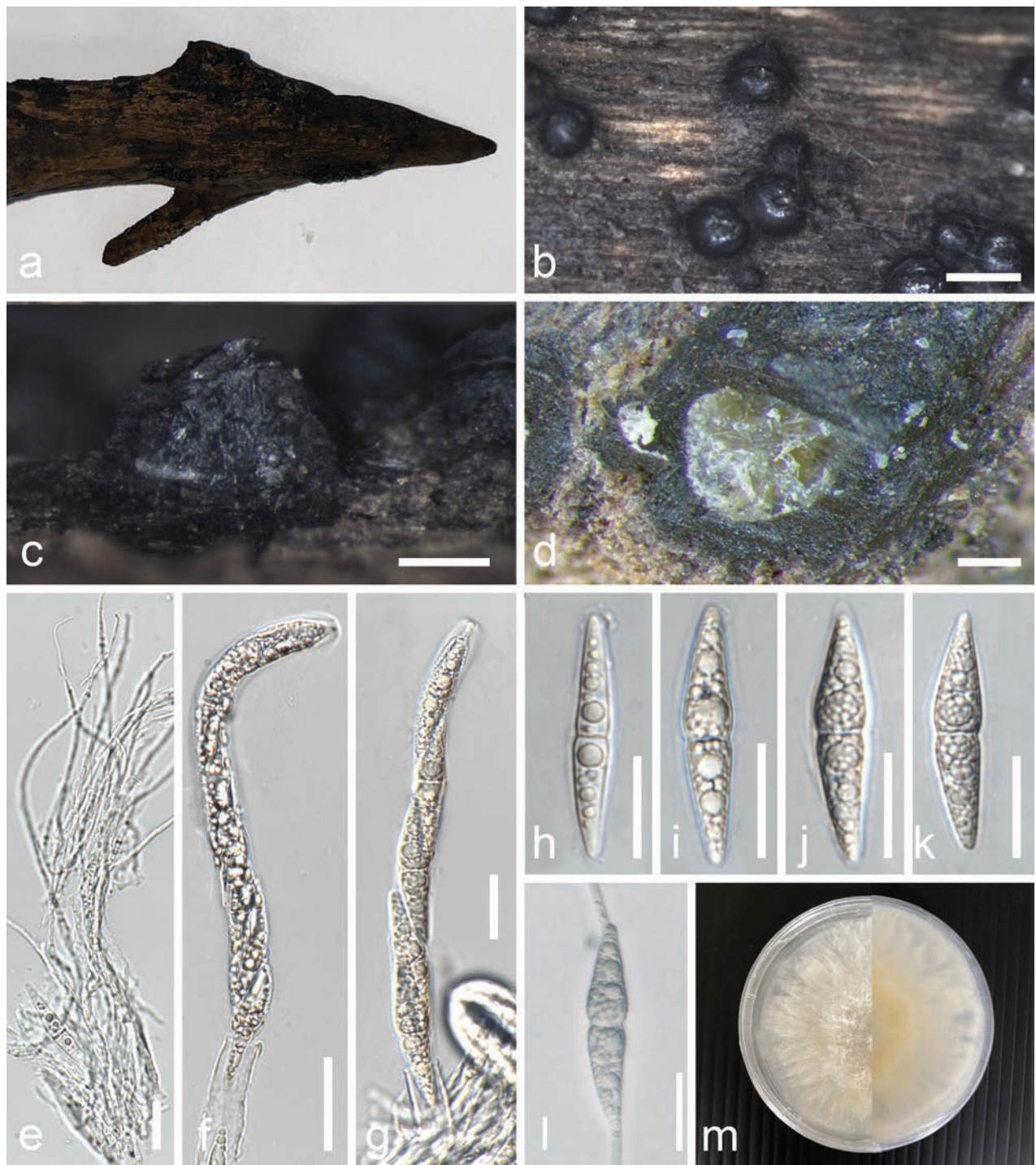
Figure 8. *Rimora mangrovei* (MFLU 24-0193). **a** host **b, c** ascomata on host **d** section of ascoma on host **e** paraphyses **f** immature ascus **g** mature ascus **h–k** ascospores **l** Germinated ascospore. **m** Colony on PDA. Scale bars: 500 µm (**b**); 200 µm (**c**); 250 µm (**d**); 20 µm (**e–l**).

n = 10), 8-spored, bitunicate, cylindrical, short pedicellate, with an apical apparatus. ***Ascospores*** 40−47 µm × 7−11 µm (x̄ = 46.7 × 8.9 µm, n = 20), fusiform, hyaline, biseriate, 1-septate, constricted at the septum, with multiple prominent guttules. **Asexual morph**: Undetermined.

Known distribution. Thailand.

Culture characteristics. Ascospores germinate in MEA within 24 hours, with germ tubes arising from one end of the ascospore. Colonies on MEA grow up to 6 cm after 14 days of incubation at room temperature, white, raised, fimbriated edge, reverse with light yellow pigment at the center which does not spread to the periphery.

Material examined. Thailand • Prachuap Khiri Khan Province: Pranburi District, 12°24'48.672"N, 99°56'51.47"E, on decomposing wood of *Ceriops tagal* (Rhizophoraceae) submerged in brackish water, 25 October 2022, by Carlo Chris S. Apurillo, P10705 (MFLU 24-0193), ex-type living culture MFLUCC 24-0516.

GenBank Numbers. ITS: PP989293, LSU: PP989289, SSU: PP989297.

Notes. Based on a combined analysis of LSU, SSU and *tef1-α* sequences, *Rimora mangrovei* MFLU 24-0193 formed a well-supported clade with the holotype and other strains of *Rimora mangrovei* in Aigialaceae. This study identifies new characteristics not previously described for this species, including the presence of an apical apparatus in the asci and 1-septate ascospores with multiple prominent guttules. *Rimora mangrovei* has been reported from various mangrove hosts such as *Avicennia* species, *Bruguiera gymnorrhiza*, *Ceriops decandra*, *Nypa fruticans*, *Rhizophora* species, *and Sonneratia* species across countries in the Atlantic, Indian and Pacific regions (Devadatha et al. 2021). This is the first record of isolation of *Rimora mangrovei* from *Ceriops tagal* in Prachuap Khiri Khan, Thailand.

Discussion

In this study, we introduce one novel genus, three novel species and one new record of fungi isolated from mangroves in Thailand. Two of these, *Pseudomelanconiella mangrovei* and *Pseudochaetosphaeronema bruguierae*, belong to genera not previously reported from mangroves. Based on morphological and molecular data, we establish *Pseudomelanconiella* as a novel genus in Melanconiellaceae (Diaporthales). This family is typified by *Melanconiella*, and also includes *Dicarpella*, *Greeneria*, *Melanconiella*, and *Microascospora* (Senanayake et al. 2017). Later additions included *Sheathospora* (Fan et al. 2018), *Septomelanconiella* (Phookamsak et al. 2019), *Paraphomopsis* (Udayanga et al. 2021), and most recently, *Sinodiscula* (Guo et al. 2024). Except for *Septomelanconiella*, all genera in this family contain species that cause plant diseases such as canker, dieback, leaf blight, and anthracnose (Du et al. 2017; Fan et al. 2018; Udayanga et al. 2021; Guo et al. 2024). However, *Pseudomelanconiella* is unique in being a saprobe in mangrove environments rather than a pathogen. While it groups with *Septomelanconiella*, it is distinct both morphologically and ecologically. Notably, it thrives in intertidal zones—an environment not previously reported for the family Melanconiellaceae.

Melanconiella loropetali is reclassified as *Sinodiscula loropetali* and *Melanconiella camelliae* was synonymized with *Sinodiscula camellicola*. *Melanconiella* species, typically found in Europe and North America on Betulaceae, have a narrow host and geographical range (Voglmayr et al. 2012). However, *M. syzygii* was reported from *Syzygium* sp. in Malaysia, the first time this genus was isolated outside Europe and North America from a different host (Crous et al. 2016). *Melanconiella loropetali* and *M. camelliae* were also introduced to expand the host

range of the genus (Mu et al. 2023). Our analysis confirms that *M. loropetali*, *M. camelliae* and *M. syzygii* do not belong to *Melanconiella*, thereby restricting the genus again to Betulaceae hosts (Voglmayr et al. 2012; Fan et al. 2018).

Peroneutypa hibisci, a novel species, is also introduced in a genus with 43 known species (Index Fungorum 2024). Only two *Peroneutypa* species are from mangroves: *P. mangrovei* and *P. scoparia* while another two were reported from *Suaeda* plants in saline habitats: *P. indica* and *P. polysporae* (Jones et al. 2019; Devadatha et al. 2021). Our analysis groups *P. hibisci*, *P. mangrovei*, and *P. polysporae* together, suggesting that *Peroneutypa* species adapted to brackish or marine environments may have diverged separately from fungi in other ecological niches. For instance, these species may exhibit specialized traits such as salt tolerance or unique metabolic pathways for nutrient acquisition in saline conditions, distinguishing them from terrestrial *Peroneutypa* species that thrive in freshwater or soil habitats.

Pseudochaetosphaeronema bruguierae is also a novel species, the first in the genus to be reported from mangroves. *Pseudochaetosphaeronema* was established in 1979 to accommodate *P. larense* (≡ *Chaetosphaeronema larense*), a pathogenic fungus isolated from human foot (Punithalingam 1979). This remained a monotypic genus until 2015 when Ahmed et al. (2015) introduced *Pseudochaetosphaeronema martinelli*, isolated as a pathogen in immuno-suppressed humans. Currently, there are 12 species recorded for this genus, many of which have been isolated as saprobes of terrestrial plants and soil (Phookamsak et al. 2019; Boonmee et al. 2021; Tan and Shivas 2022; Xu et al. 2024). Although *Pseudochaetosphaeronema* is known as a human pathogen due to the first two species introduced, the other members of this genus are saprobes. Notably, none have been isolated from mangroves. In this study, *Pseudochaetosphaeronema bruguierae* was isolated from the dead branch of a mangrove that was not submerged in brackish water, thus, it is not considered a marine fungus. Currently, there is no evidence that the isolate can survive in a saline environment. Initially classified as *incertae sedis* in Pleosporales (Dothideomycetes), *Pseudochaetosphaeronema* was later accepted under Macrodiplodiopsidaceae (Pleosporales) by Wijayawardene et al. (2022).

Rimora mangrovei is introduced with *Ceriops tagal* as a new host record. *Rimora* is a monotypic genus typified by *Rimora mangrovei* (Suetrong et al. 2009). *Rimora* is one of three genera in Aigialaceae alongside *Aigialus* and *Ascocratera*, whose type species were first isolated from mangroves (Kohlmeyer and Schatz 1985; Kohlmeyer 1986). Another species in Aigialaceae, *Posidoniomyces atricolor*, the only species in the genus, was isolated as an endophyte of seagrass (Vohník et al. 2019). These examples highlight that many members of Aigialaceae can survive in marine environment, establishing it as a well-known marine family. The addition of a new record from mangroves in this family further confirms the ecological niche of Aigialaceae.

In conclusion, this study underscores the vital importance of ongoing exploration in mangrove ecosystems to uncover the diverse fungal species and their significance (Hyde and Lee 1995; Rampadarath et al. 2018). Despite considerable research, numerous fungal species in mangroves remain undiscovered. The discovery of novel genus and species, such as *Pseudomelanconiella*, *Peroneutypa*, *Pseudochaetosphaeronema*, highlights the species richness of these environments. Further exploration is crucial not only for taxonomic understanding

but also for discovering potential bioactive compounds with pharmaceutical and biotechnological applications. Indeed, many fungi from mangroves, including endophytes, have been reported to possess bioactive compounds with antibacterial, antimutagenic, and antioxidant properties among others (Dela Cruz et al. 2020; Cadamuro et al. 2021; Sopalun et al. 2021). This study emphasizes the necessity of sustained efforts in studying mangrove fungi, both as a means of understanding ecological interactions and for potential beneficial discoveries.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: KDH, CCSA, EBGJ. Data curation: CCSA. Formal analysis: CCSA. Funding acquisition: CP, VT, KDH, EBGJ. Investigation: EBGJ, CCSA, CP. Methodology: CCSA. Project administration: KDH, VT. Supervision: EBGJ, KDH, CP, VT. Validation: EBGJ, VT, CP, KDH, CCSA. Visualization: CCSA. Writing - original draft: CCSA. Writing - review and editing: KDH, CP, CCSA, VT, EBGJ.

Author ORCIDs

Carlo Chris S. Apurillo ⓘ https://orcid.org/0000-0003-4348-0887
Chayanard Phukhamsakda ⓘ https://orcid.org/0000-0002-1033-937X
Kevin D. Hyde ⓘ https://orcid.org/0000-0002-2191-0762
Vinodhini Thiyagaraja ⓘ https://orcid.org/0000-0002-8091-4579
E. B. Gareth Jones ⓘ https://orcid.org/0000-0002-7286-5471

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

Additional information

Authors: Carlo Chris S. Apurillo, Chayanard Phukhamsakda, Kevin D. Hyde, Vinodhini Thiyagaraja, E. B. Gareth Jones

Data type: xlsx

Explanation note: **table S1.** Genbank accession codes for the sequences of Melanconiellaceae species and outgroup used in this study. **table S2.** Genbank accession numbers of *Peroneutypa* and related genera used in this study. **table S3.** Genbank accession numbers of Pseudochaetosphaeronema and related genera used in this study. **table S4.** Genbank accession numbers of Aigialaceae strains used in this study.

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MycoKeys

Research Article

Morphological and phylogenetic analyses reveal new species and records of *Fusarium* (Nectriaceae, Hypocreales) from China

Congcong Ai[1], Qiyun Liu[1], Yaling Wang[1], Zhaoxue Zhang[1], Duhua Li[1], Yun Geng[2], Xiuguo Zhang[1], Jiwen Xia[1,3]

1   *Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Taian, 271018, China*

2   *Institute of Crop Germplasm Resources, Shandong Academy of Agricultural Sciences, Jinan, 250100, China*

3   *College of Agriculture and Forestry, Linyi University, Linyi, Shandong, 276000, China*

Corresponding authors: Qiyun Liu (*liuqiyun0416@163.com*); Jiwen Xia (*xiajiwen1@126.com*)

Abstract

Species of *Fusarium* are important phytopathogens, saprobes, and endophytes around the world. Some species can affect plant health and cause yield loss of economic plants. *Fusarium* species are widely distributed in China, and many species were found from different plant hosts. The *Fusarium incarnatum-equiseti* species complex (FIESC) is one of the most significant species complexes within the genus. Based on morphological and three-gene (*cal*, *rpb2*, and *tef1*) phylogenetic analyses, two new species are in the Incarnatum clade, and two new host records are identified and described, viz. *Fusarium fici* **sp. nov.**, *Fusarium xylosmatis* **sp. nov.**, *Fusarium fecundum*, and *Fusarium weifangense*.

Key words: *Fusarium incarnatum-equiseti* species complex, multigene phylogeny, new taxa

Introduction

Johann Heinrich Friedrich Link first proposed the genus *Fusarium* (Nectriaceae, Hypocreales) in 1809 and typified it with *Fusarium roseum* (= *F. sambucinum*), with falcate or banana-shaped macroconidia and oval, subglobose, or kidney-shaped microconidia (Link 1809; Gams et al. 1997; Leslie and Summerell 2006; Liu et al. 2023; Zhang et al. 2023a). *Fusarium* is one of the most renowned and extensively spread genera in the Kingdom Fungi because of its morphological and phylogenetic diversity (Leslie and Summerell 2006; Sandoval-Denis et al. 2018; Crous et al. 2021). *Fusarium* species are known as plant pathogens, endophytes, and saprophytes (Leslie et al. 1990; Bacon and Yates 2006; Maryani et al. 2019; He et al. 2024). More than 1800 epithets of *Fusarium* have been listed in Index Fungorum (https://www.indexfungorum.org), but many species of *Fusarium* were identified solely based on morphological studies. Excessive overlap of conidial characteristics makes it difficult to morphologically distinguish *Fusarium* species. Currently, *Fusarium* taxonomy is dominated by morphological and molecular phylogenetic studies (Crous et al. 2021; He et al. 2024).

At present, *Fusarium* contains 23 monophyletic species complexes and several single-species lineages (Xia et al. 2019; O'Donnell et al. 2020; Geiser et al.

2021; He et al. 2024). The FIESC includes over 30 recognized phylogenetic species (O'Donnell et al. 2009; Villani et al. 2016; Maryani et al. 2019; Santos et al. 2019; Wang et al. 2019; Xia et al. 2019). Based on the haplotype nomenclature system, O'Donnell et al. (2009) implemented an informal classification system for FIESC and introduced the Equiseti and Incarnatum clades. The *Fusarium camptoceras* species complex (FCAMSC) was proposed for three lineages that are sister clades to the FIESC by phylogenetic studies by Xia et al. (2019). However, Han et al. (2023) included the FCAMSC in FIESC as the Camptoceras clade because the FCAMSC and FIESC clearly represent a distinct evolutionary lineage that is strongly supported by the phylogenomic tree. Thus, FIESC comprises three clades, viz. Camptoceras, Equiseti, and Incarnatum clades.

In this study, samples were collected from Hainan, Sichuan, and Yunnan Provinces of China. Two new species and two new host records were identified and classified by multi-locus analysis of calmodulin (*cal*), RNA polymerase II second largest subunit (*rpb2*), and translations elongation factor 1-alpha (*tef1*) datasets. They were described and discussed based on their morphological characteristics along with their molecular sequence data.

Materials and methods

Strain isolation and preservation

Plant specimens with necrotic spots were collected from three provinces (Hainan, Sichuan, and Yunnan) of China in 2023. Pure colonies were obtained by tissue isolation techniques (Zhang et al. 2024). Fragments (25 mm^2) were cut from the edges of diseased tissues, immersed in a 75% ethanol solution for 1 min, then rinsed in sterile water for 30 s and 10% sodium hypochlorite solution for 1 min. Fragments were rinsed three times with sterile water for 30 s, then using sterilized filter paper to absorb dry, placed on PDA for incubation at 25 °C for 3 days. The strains were preserved in 10% sterilized glycerol and stored them at 4 °C for future detailed studies. Specimens were 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 living ex-type cultures were deposited in the Shandong Agricultural University Culture Collection (SAUCC) and the China General Microbiological Culture Collection Center (CGMCC).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from fresh fungal mycelia grown on potato dextrose agar (PDA) after 7 days using the genomic DNA purification kit (OG-PLF-400, GeneOnBio Corporation, Changchun, China) according to the product manual. The calmodulin (*cal*), RNA polymerase second largest subunit (*rpb2*), and translation elongation factor 1-alpha (*tef1*) gene loci were amplified using the primer pairs listed in Table 1 (Xia et al. 2019; Han et al. 2023). The reaction was performed in a 25 μL reaction volume, consisting of 12.5 μL of 2 × Hieff Canace® Plus PCR Master Mix (Cat. No. 10154ES03, Yeasen Biotechnology, Shanghai, China), 1 μL each of forward and reverse primer (TsingKe, Qingdao, China), and 1 μL of template genomic DNA, and at last replenished the total

Table 1. Molecular markers and their PCR primers and programs used in this study.

Loci	PCR Primers	Sequence (5'→3')	PCR Cycles	References
cal	CL1	GARTWCAAGGAGGCCTTCTC	(94 °C: 30 s, 55 °C: 30 s, 72 °C: 15 s) × 35 cycles	O'Donnell et al. (2020)
	CL2A	TTTTTGCATCATGAGTTGGAC		
rpb2	5f2	GGGGWGAYCAGAAGAAGGC	(94 °C: 45 s, 57 °C: 45 s, 72 °C: 15 s) × 35 cycles	Liu et al. (1999)
	7cr	CCCATRGCTTGYTTRCCCAT		
tef1	EF-1	ATGGGTAAGGARGACAAGAC	(94 °C: 45 s, 55 °C: 45 s, 72 °C: 15 s) × 35 cycles	O'Donnell et al. (1998)
	EF-2	GGARGTACCAGTSATCATG		

volume to 25 μL with double distilled water. PCR products were separated and purified using 1% agarose gel and Safe Red (RM02852 and RM19009 ABclonal, Wuhan, China) and UV light to visualize the fragments. Gel was extracted using a gel extraction kit (Cat. No. AE0101-C, Shandong Sparkjade Biotechnology Co., Ltd., Jinan, China) (Wang et al. 2023). The purified PCR products were sequenced by Youkang Company Limited (Zhejiang, China). All sequences generated in this study were deposited in GenBank under the accession numbers provided in Suppl. material 1.

Phylogenetic analyses

The reference sequences were downloaded from NCBI's GenBank. All sequences were initially aligned with the MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server/) online service and MEGA 7.0. The concatenated aligned *cal*, *rpb2*, and *tef1* sequences were used for maximum likelihood (ML) and Bayesian inference (BI), which were run on RaxML-HPC2 with XSEDE v. 8.2.12 and MrBayes v. 3.2.7a with 64 threads on Linux (Zhang et al. 2024). For ML analyses, 100 rapid bootstrap replicates and the GTR+FO+I+G4m model as default parameters were used. For BI analyses, a fast bootstrap algorithm with an automatic stop option was performed (Zhang et al. 2023b). The SYM+G model for *cal*, the SYM+I+G model for *rpb2*, and the GTR+I+G model for *tef1* were selected and incorporated into the analyses. The Markov chain Monte Carlo (MCMC) analysis of the sequence data was performed over 5,000,000 generations, yielding 34,652 trees. Following the discard of 8,663 trees during the burn-in phase, the remaining trees were used to calculate posterior probabilities in the consensus trees.

Morphological characterization

All isolates were inoculated on potato dextrose agar (PDA) medium and oatmeal agar (OA) medium. Colony morphology, pigmentation, and growth rates were recorded. The above and reverse of the PDA and OA flat plates were captured with the Alpha 6400L digital camera (Canon Powershot G7X, Canon, Tokyo, Japan) on the 7th day. Used Carnation leaf agar (CLA; Fisher et al. 1982) medium to describe morphological features, such as shape, size, and septum number of the conidia (Wang et al. 2019). Used a stereomicroscope (Olympus SZ61, Olympus Corporation, Tokyo, Japan) and a microscope (Olympus BX53, Olympus Corporation, Tokyo, Japan) with Differential Interference Contrast (DIC) to observe the microscopic morphology. Stereomicroscope and microscope were equipped with BioHD-A20c color digital cameras (FluoCa Scientific, Shanghai, China) to capture the microscopic fungal structures. Microstructures were

randomly measured using Digimizer software v5.6.0 (https://www.digimizer.com, accessed on 18 November 2024) and calculated the mean size (av.). The "n" represents the number of measurements.

Results

Phylogenetic analyses

The combined dataset comprised 133 ingroup strains with *Fusarium concolor* (NRRL 13459) as the outgroup. The final alignment comprised 1,654 concatenated characters, spanning from positions 1 to 535 (*cal*), 536 to 1,192 (*rpb2*), and 1,193 to 1,654 (*tef1*). The ML was carried out to be -9,907.383240. MrModelTest recommended using Dirichlet base frequencies for the *cal*, *rpb2*, and *tef1* data partitions. The alignment showed a total of 563 unique site patterns (*cal*: 156, *rpb2*: 168, *tef1*: 239). Based on the three-gene (*cal*, *rpb2*, and *tef1*) phylogeny, the 134 strains were classified into 57 species. The topology of the ML tree confirmed the topology obtained from BI, with only the ML tree presented (Fig. 1). Furthermore, single gene trees were evaluated, respectively, for FIESC (Suppl. material 2).

Taxonomy

Fusarium fecundum **S.L. Han, M.M. Wang & L. Cai, Studies in Mycology 104: 87−148. 2023.**
Fig. 2

Description. On CLA, conidiophores arising from aerial mycelia, 13−71 µm long, unbranched or irregularly branched, bearing terminal or lateral phialides, often reduced to single phialides; Periclinal thickening inconspicuous; Aerial conidia hyaline, smooth, rarely ovoid to falcate, on the apical half, the dorsal side is more curved than the ventral side, and the apical cell is either blunt or hooked, basal cell barely to distinctly notched. 1-septate conidia: (16−)22−21(−27) × 4−6 µm (av. 20 × 5 µm, n = 9); 2-septate conidia: (18−)21−28(−33) × 5−7 µm (av. 26 × 6 µm, n = 9); 3-septate conidia: (32−)33−36(−41) × 5−8 µm (av. 35 × 7 µm, n = 16); 4-septate conidia: (32−)37−43(−43) × 6−9 µm (av. 39 × 7 µm, n = 18); 5-septate conidia: (41−)43−48(−53) × 7−9 µm (av. 46 × 8 µm, n = 12).

Culture characteristics. Colonies on PDA incubated at 25 °C in the dark, reaching 84−90 mm diameter in 7 d; aerial mycelia dense, white, radiate, colony margin erose; reverse surface greyish yellow in the center, odor absent. On OA in the dark, occupying an entire 90 mm diameter in 7 d; surface white and aerial mycelia scant, crateriform, reverse white, odor absent.

Materials examined. CHINA • Yunan Province, Nanuo Mountain, on leaves of *Setaria palmifolia*, 3 March 2023, Q.Y. Liu (HSAUP41424, HSAUP51424), living cultures CGMCC 3.27792 = SAUCC 2414-4, CGMCC 3.27793 = SAUCC 2414-5.

Notes. Phylogenetic analysis showed that isolates (SAUCC 2414-4 and SAUCC 2414-5) were closely related to *Fusarium fecundum* (LC15875, ex-type strain) (Fig. 1). There are no nucleotide position differences between *Fusarium fecundum* (SAUCC 2414-4) and *Fusarium fecundum* (LC15875, ex-type strain). Morphologically, *Fusarium fecundum* (SAUCC 2414-4) and *Fusarium fecundum* (LC15875, ex-type strain) are the lack of sporodochia. The aerial conidia of *Fusarium fecundum*
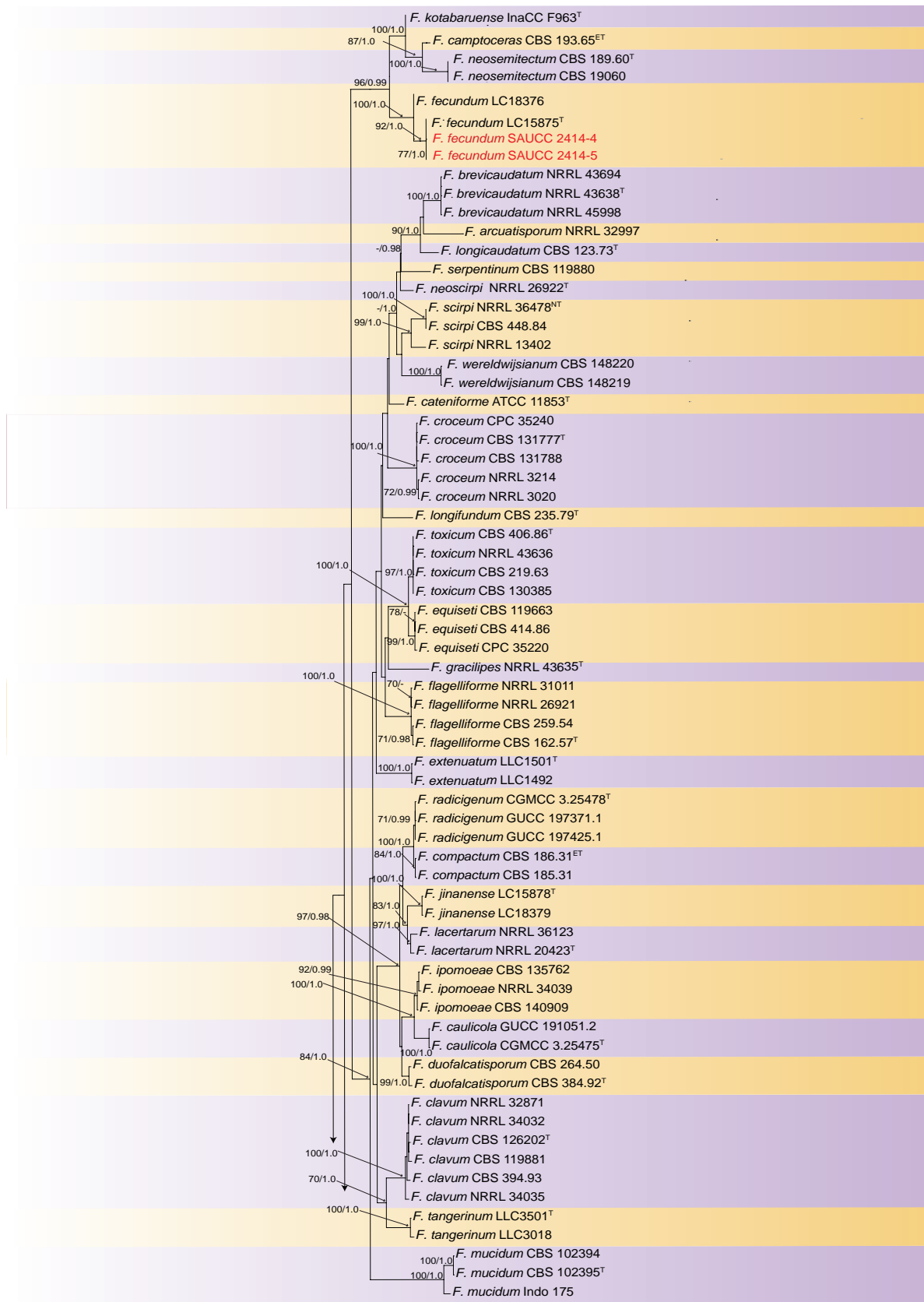
Figure 1. Phylogeny inferred based on the combined *cal-rpb2-tef1* sequence dataset of the *Fusarium incarnatum-equiseti* species complex (FIESC), with *Fusarium concolor* (NRRL 13459) as the outgroup. The RAxML Bootstrap support values (MLBS ≥ 70%) and Bayesian inference posterior probabilities (BIPP ≥ 0.90) were shown at the nodes. Ex-type, ex-epitype, and ex-neotype strains were indicated by T, ET, and NT, respectively. Strains isolated in this study were indicated in red.
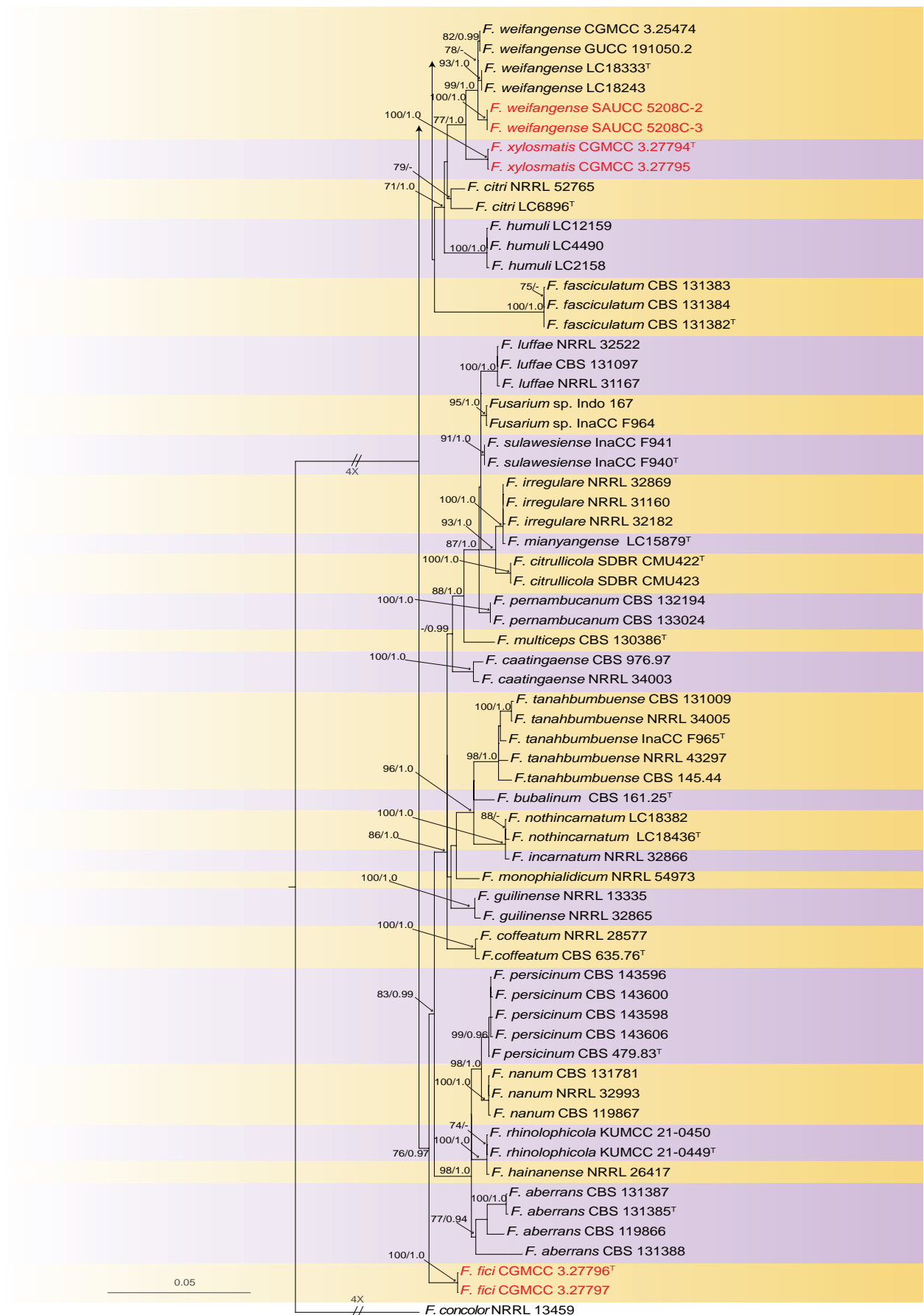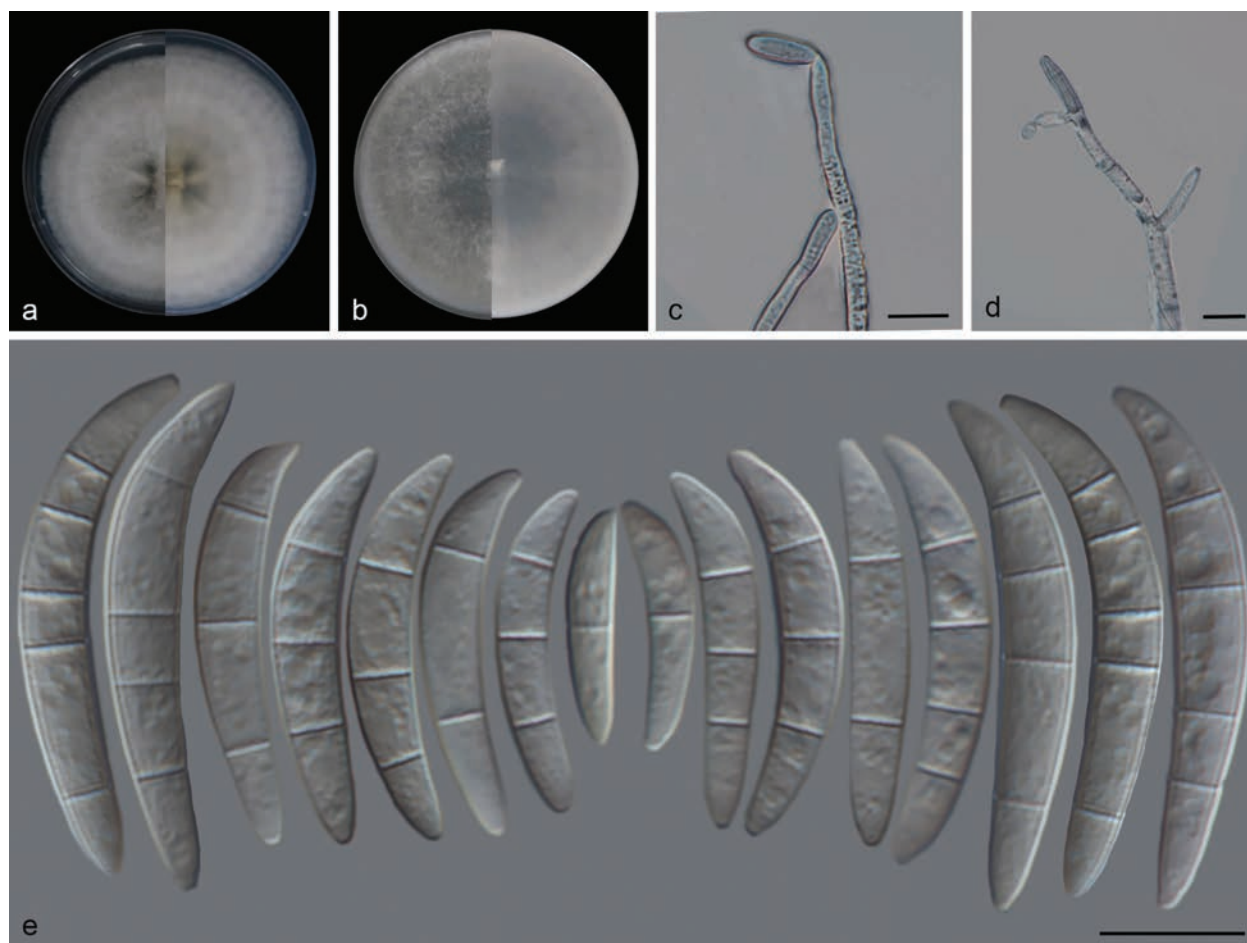
Figure 1. Continued.

Figure 2. *Fusarium fecundum* (SAUCC 2414-4) **a** colony on PDA after 7 days at 25 °C (left: above, right: reverse) **b** colony on OA after 7 days at 25 °C (left: above, right: reverse) **c, d** conidiophore on aerial mycelium with monophialides **e** aerial conidia. Scale bars: 10 μm (**c−e**).

(SAUCC 2414-4) are smaller than those of *Fusarium fecundum* (LC15875, ex-type strain). *Fusarium fecundum* was previously isolated from wheat and rice, and it has now been reported for the first time on *Setaria palmifolia* (Han et al. 2023).

Fusarium fici Q.Y. Liu, X.G. Zhang & J.W. Xia, sp. nov.
MycoBank No: 856644
Fig. 3

Etymology. Referring to the genus name of the host plant *Ficus fistulosa*.

Typus. CHINA • Hainan Province, Baoting Li and Miao Autonomous County, on leaves of *Ficus fistulosa*, 10 April 2023, Q.Y. Liu (HMAS 353395, holotype), ex-holotype culture CGMCC 3.27796 = SAUCC 3249C-3.

Description. Conidiophores arising from aerial mycelium, 17−21 μm long, unbranched, reduced to single phialidic pegs, subulate to subcylindrical; aerial conidia hyaline, smooth, and thin-walled, rarely ellipsoidal to falcate, straight to curved dorsiventrally, a blunt apical cell and barely notched basal cell, 1−3(−5)-septate; 1-septate conidia: (12−)12−16(−28) × 3−5 μm (av. 17 × 3 μm, n = 18); 2-septate conidia: (16−)17−21 (−26) × 3−5 μm (av. 19 × 4 μm, n = 17); 3-septate conidia: (20−)22−28 (−36) × 3−6 μm (av. 26 × 4 μm, n = 31); 4-septate conidia:
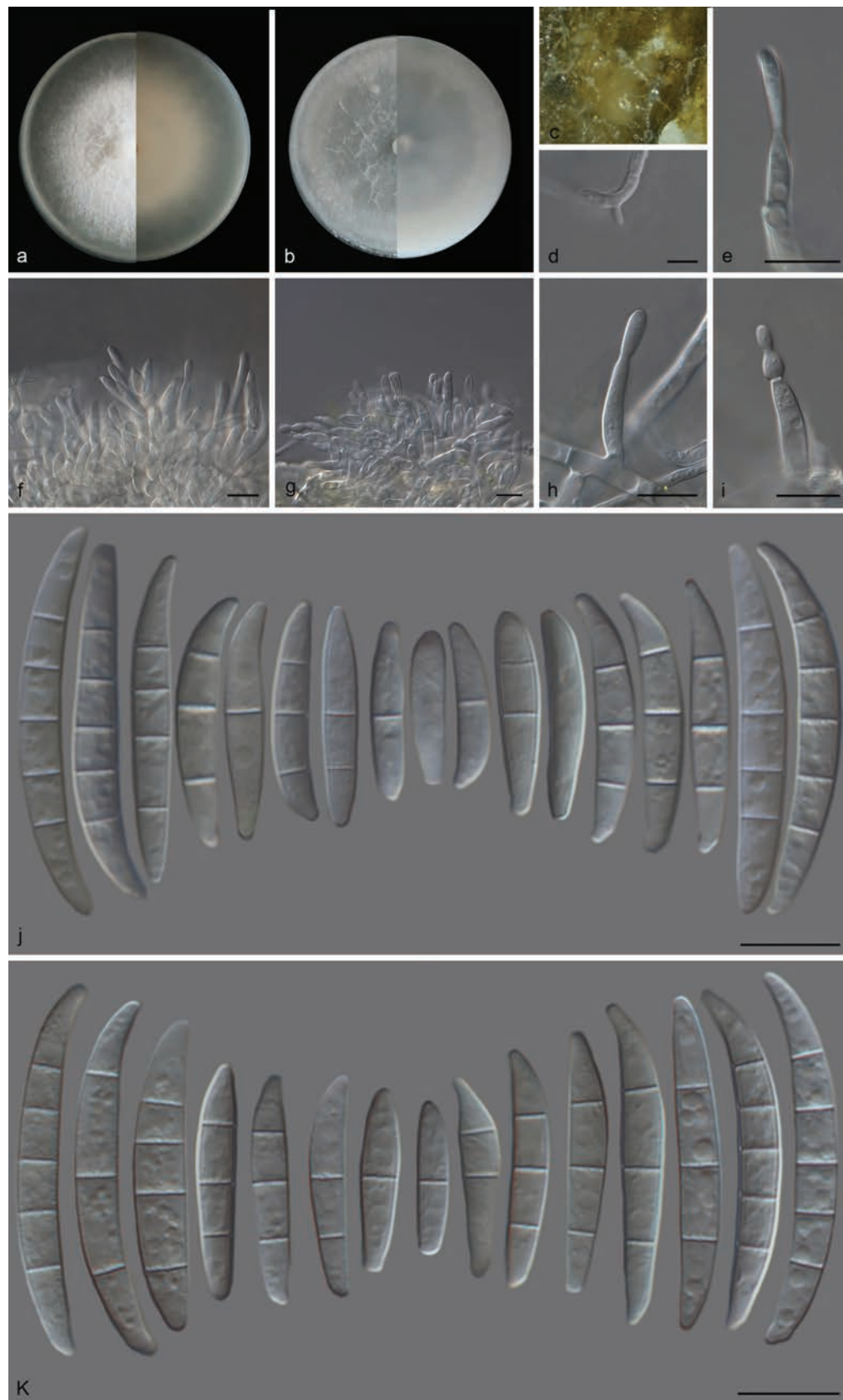
Figure 3. *Fusarium fici* (CGMCC 3.27796) **a** colony on PDA after 7 days at 25 °C (left: above, right: reverse) **b** colony on OA after 7 days at 25 °C (left: above, right: reverse) **c** sporodochia on carnation leaves **d** lateral phialidic peg on aerial mycelium **e** monophialide **f**, **g** sporodochial conidiophores **h**, **i** monophialides on aerial mycelium **j** sporodochial conidia **k** aerial conidia. Scale bars: 10 µm (**d–k**).

(28−)31−34 (−39) × 4−5 µm (av. 33 × 5 µm, n = 14); 5-septate conidia: (23−)32−33 (−36) × 4−5 µm (av. 31 × 4 µm, n = 5). Sporodochia salmon to saffron, formed abundantly on surface of carnation leaves. Sporodochial conidiophores densely and bearing apical whorls of 1 phialide; sporodochial phialides subulate to sub-cylindrical, 9−11 × 3−4 µm, smooth, thin-walled, with inconspicuous periclinal thickening; sporodochial conidia falcate, straight to curved dorsiventrally, taper-ing towards both ends, with slightly papillate, a conical to slightly papillate apical cell, a notched to foot-like basal cell, (0−)1−3(−5)-septate, hyaline, smooth, and thin-walled; 0-septate conidia: (10−)15−20(−21) × 2−4 µm (av. 16 × 3 µm, n = 9); 1-septate conidia: (13−)15−22(−25) × 2−5 µm (av. 18 × 4 µm, n = 23); 2-septate conidia: (13−)16−18(−23) × 2−5 µm (av. 18 × 3 µm, n = 23); 3-septate conidia: (19−)20−25(−29) × 3−5 µm (av. 24 × 4 µm, n = 37); 4-septate conidia: (28−)31−34(−36) × 4−5 µm (av. 33 × 4 µm, n = 12); 5-septate conidia: (34−)34−36(−38) × 3−5 µm (av. 35 × 4 µm, n = 5). Chlamydospores not observed.

Culture characteristics. Colonies on PDA incubated at 25 °C in the dark, reaching 76−80 mm diameter in 7 d, flat, convex, with abundant aerial myce-lium, colony margin lightly erose; surface white, odor absent; reverse yellowish white, odor absent. On OA in the dark, reaching 85−90 mm diameter in 7 d; aerial mycelium scant in the center forming a vacant circle, reverse white, odor absent.

Additional material studied. China • Hainan Province, Baoting Li and Miao Autonomous County, on leaves of *Ficus fistulosa*, 10 April 2023, Q.Y. Liu (HSAUP44932), living culture CGMCC 3.27797 = SAUCC 3249C-4.

Notes. Phylogenetic analyses of three combined sequences (*cal*, *rpb2*, and *tef1*) showed that *F. fici* constitutes a distinct clade, closely related to *F. aber-rans*. Between *F. fici* (CGMCC 3.27796) and *F. aberrans* (CBS 131385), there were 11/535 differences in *cal*, 13/657 in *rpb2*, and 34/462 in *tef1*. The my-celium on OA of *F. fici* (CGMCC 3.27796) is sparser than that of *F. aberrans* (CBS 131385). Morphologically, *F. fici* (CGMCC 3.27796) and *F. aberrans* (CBS 131385) have different sporodochial conidial septa (0−5-septate in *F. fici* vs. 1−3-septate in *F. aberrans*) and sporodochial phialides (1 phialide in *F. fici* vs. 2−3 phialides in *F. aberrans*). The aerial conidiophores of *F. aberrans* (16−110 µm) are longer than *F. fici* (17−21 µm) (Xia et al. 2019).

Fusarium weifangense **S.L. Han, M.M. Wang & L. Cai, Studies in Mycology 104: 87−148. 2023.**
Fig. 4

Synonym. *Fusarium caulendophyticum* H. Zhang & Y.L. Jiang, Mycosphere 14(1): 2092−2207. 2023.

Description. Conidiophores arising from aerial mycelium, 14−18 µm long, un-branched or irregularly branched, often reduced to single phialides; aerial phialides monophialidic, subulate to subcylindrical, smooth- and thin-walled, with inconspic-uous or absent periclinal thickening, 9.2−12.2 × 4.0−4.4 µm; aerial conidia hyaline, rarely ellipsoidal to falcate, slightly curved with almost parallel sides, tapering to-wards both ends, with a blunt to conical and slightly curved apical cell, blunt to bare-ly notched basal cell, smooth and thin-walled, (1−)3−5-septate; 1-septate conidia: (14−)15−19(−20) × 3−4 µm (av. 17 × 3 µm, n = 8); 2-septate conidia: (19−)19−21(−24) × 3−5 µm (av. 21 × 4 µm, n = 14); 3-septate conidia: (22−)26−31(−34) × 3−6 µm
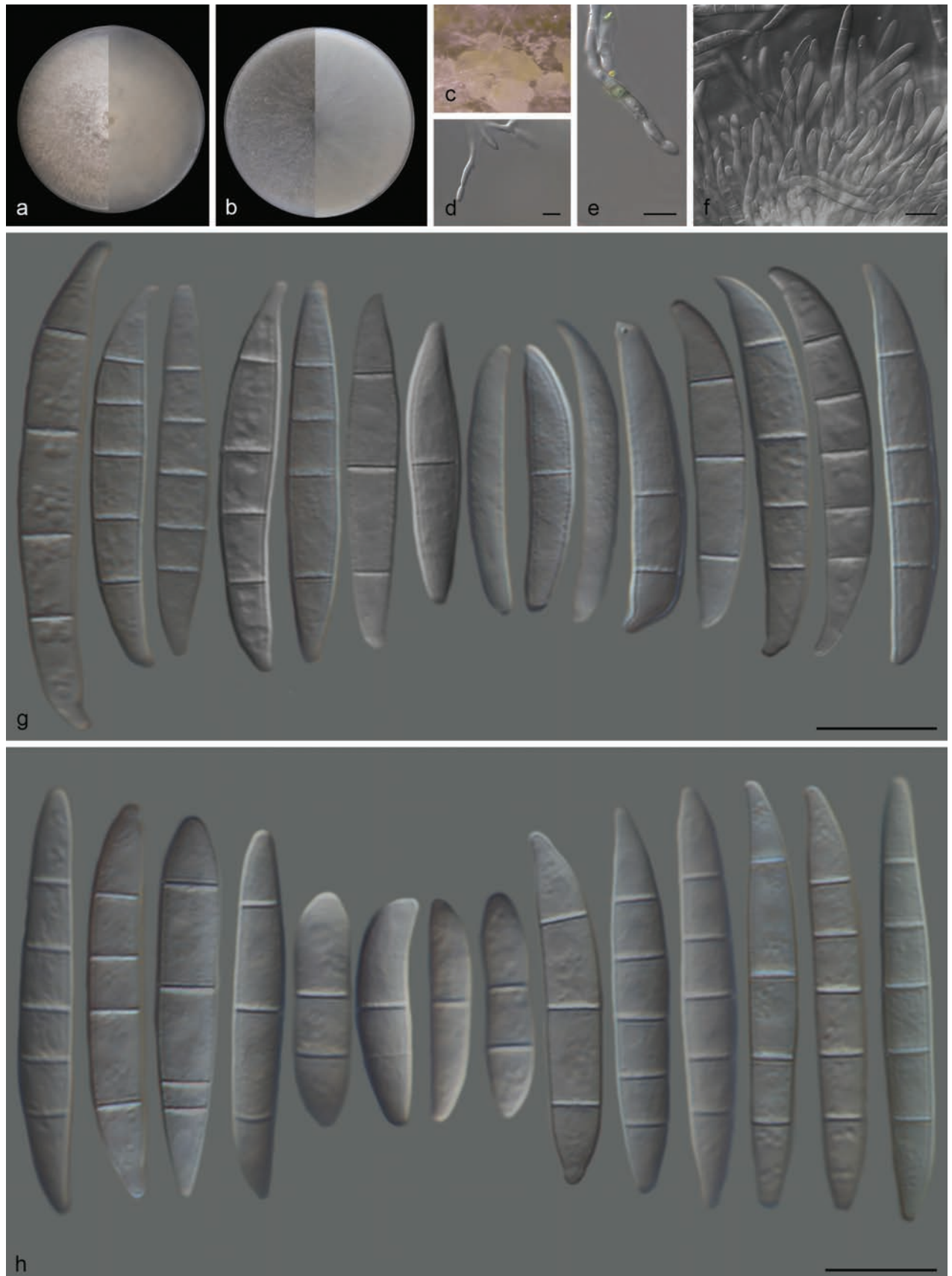
Figure 4. *Fusarium weifangense* (SAUCC 5208C-2) **a** colony on PDA after 7 days at 25 °C (left: above, right: reverse) **b** colony on OA after 7 days at 25 °C (left: above, right: reverse) **c** sporodochia on carnation leaves **d** polyphialide **e** monophialide **f** sporodochial conidiophores **g** sporodochial conidia **h** aerial conidia. Scale bars: 10 μm (**d–h**).

(av. 28 × 4 µm, n = 22); 4-septate conidia: (30−)35−36(−45) × 3−6 µm (av. 36 × 5 µm, n = 17); 5-septate conidia: (31−)34−37(−46) × 4−6 µm (av. 38 × 5 µm, n = 15). Sporodochia salmon to orange, formed abundantly on surface of carnation leaves. Sporodochial conidiophores densely, bearing apical whorls of one phialide; sporodochial phialides monophialidic, subulate to subcylindrical, 16−24 × 2−3 µm, smooth. Sporodochial conidia falcate, slightly curved, tapering towards both ends, with a slightly elongated conical or whip-like curved apical cell, a foot-like to notched basal cell, (0−)4−5-septate, hyaline, thin, and smooth-walled; 0-septate conidia: 26−28 × 3−4 µm; 1-septate conidia: (17−)26−36(−37) × 3−6 µm (av. 28 × 4 µm, n = 10); 2-septate conidia: (20−)21−37 × 3−5 µm (av. 25 × 4 µm, n = 7); 3-septate conidia: 21−33(−38) × 3−5 µm (av. 32 × 5 µm, n = 12); 4-septate conidia: (31−)32−35(−44) × 3−6 µm (av. 36 × 4 µm, n = 22); 5-septate conidia: (34−)40−45(−48) × 3−6 µm (av. 42 × 4 µm, n = 16). Chlamydospores not observed.

Culture characteristics. Colonies on PDA incubated at 25 °C in the dark, reaching 86−90 mm diameter in 7 d; surface white, flat, felty to velvety, aerial mycelia dense, colony margin entire; reverse white, odor absent. Colonies on OA incubated at 25 °C in the dark, reaching 85−89 mm diameter in 7 d; surface white and aerial mycelia scant, radiate, reverse white, radiate, odor absent.

Materials examined. CHINA • Sichuan Province, Baoting Li and Miao Autonomous County, on leaves of *Prunus salicina*, 2 July 2023, Q.Y. Liu (HSAUP20852, HSAUP30852), living cultures SAUCC 5208C-2 = CGMCC 3.27939, SAUCC 5208C-3.

Notes. *Fusarium weifangense* (LC18333, ex-type strain) was proposed by Han et al. (2023). *Fusarium caulendophyticum* (CGMCC 3.25474, ex-type strain) was proposed by Zhang et al. (2023a). *Fusarium weifangense* (LC18333, ex-type strain) was the first to be discovered. *Fusarium weifangense* (LC18333 and LC18243) are clustered with *Fusarium caulendophyticum* (CGMCC 3.25474 and GUCC 191050.2) clade in the combined phylogenetic tree (Fig. 1, Suppl. material 4). *Fusarium weifangense* (LC18333, ex-type strain) and *Fusarium caulendophyticum* (CGMCC 3.25474, ex-type strain) were similar in *cal* (0/535), *rpb2* (1/657), and *tef1* (2/462) sequences. We therefore considered the *Fusarium caulendophyticum* synonym of *Fusarium weifangense*. In this study, our strains (SAUCC 5208C-2 and SAUCC 5208C-3) are clustered with the *Fusarium weifangense* (LC18333 and LC18243) clade in the combined phylogenetic tree (Fig. 1). SAUCC 5208C-2 and SAUCC 5208C-3 were similar to the latter in *cal* (with 100% sequence identity), *rpb2* (99.85%), and *tef1* (98.70%) sequences. *Fusarium weifangense* was previously isolated from wheat, *Capsicum* sp., *Triticum* sp., *Medicago sativa*, *Lactuca sativa*, *Chenopodium quinoa*, and *Rosaceae roxburghii*, and it has now been reported for the first time on *Prunus salicina* (Wang et al. 2019; Xia et al. 2019; Yin et al. 2021; Han et al. 2023; Zhang et al. 2023a) (Suppl. material 3).

Fusarium xylosmatis Q.Y. Liu, X.G. Zhang & J.W. Xia, sp. nov.

MycoBank No: 856642

Fig. 5

Etymology. Referring to the genus name of the host plant *Xylosma congesta*.

Typus. CHINA • Yunan Province, Nanuo Mountain, on leaves of *Xylosma congesta*, 3 March 2023, Q.Y. Liu (HMAS 353394, holotype), ex-holotype culture CGMCC 3.27794 = SAUCC 2416-1.
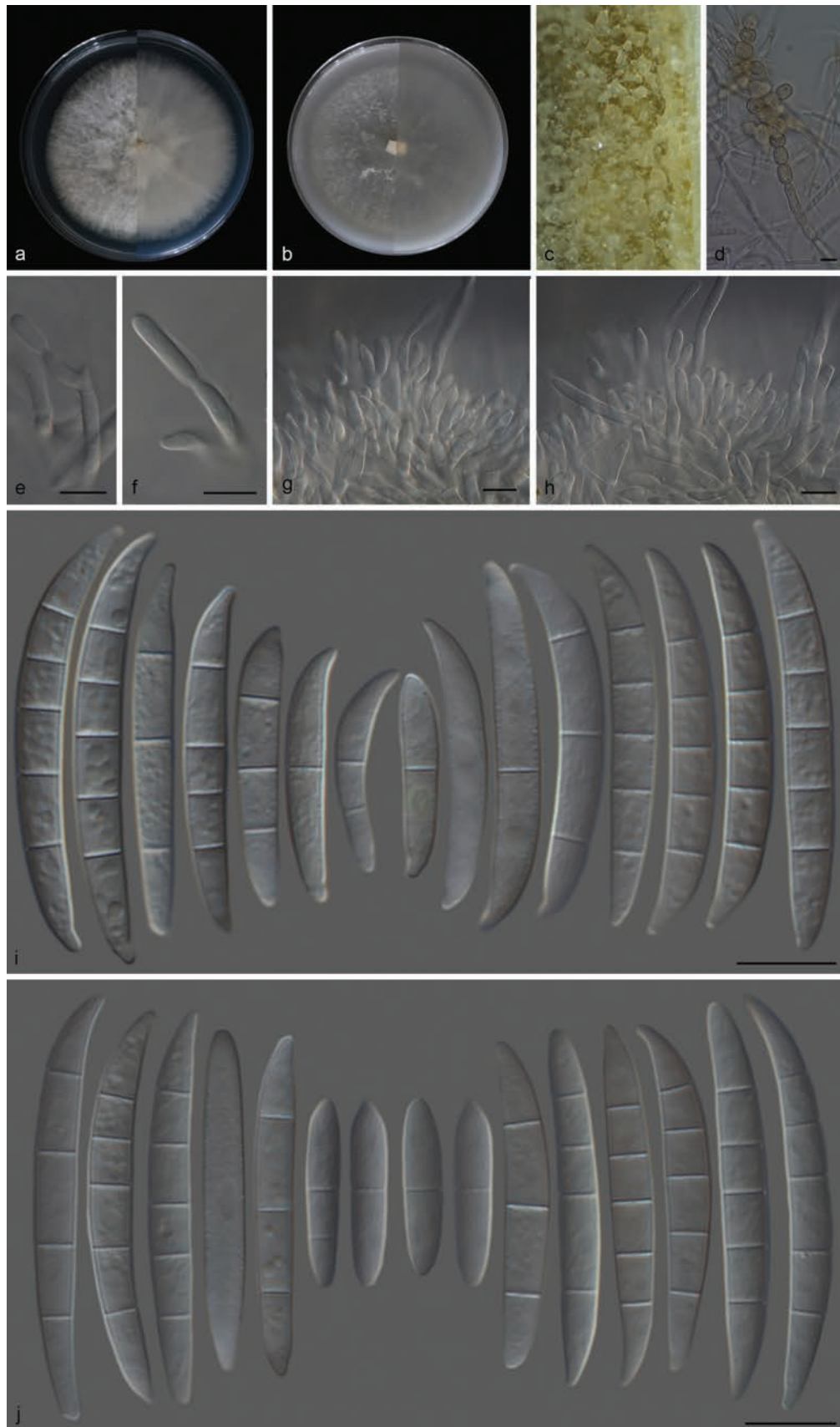
Figure 5. *Fusarium xylosmatis* (CGMCC 3.27794) **a** colony on PDA after 7 days at 25 °C (left: above, right: reverse) **b** colony on OA after 7 days at 25 °C (left: above, right: reverse) **c** sporodochia on carnation leaves **d** chlamydospores **e** polyphialide **f** monophialide **g, h** sporodochial conidiophores **i** sporodochial conidia **j** aerial conidia. Scale bars: 10 µm (**d–j**).

Description. Conidiophores arising from aerial mycelium, 25–35 μm long, unbranched or irregularly branched, often reduced to single phialides, subulate to subcylindrical, smooth, 12–15 × 4–5 μm, periclinal thickening inconspicuous; aerial conidia ellipsoidal to falcate, slightly curved, tapering towards both ends, with a blunt to conical and slightly curved apical cell and papillate basal cell, (0–)3–5-septate; 0-septate conidia: 16–20 × 3–4 μm (av. 21 × 4 μm, n = 5); 1-septate conidia: (12–)15–19(–29) × 3–4 μm (av. 18 × 4 μm, n = 33); 2-septate conidia: (16–)16–23(–29) × 3–5 μm (av. 21 × 4 μm, n = 18); 3-septate conidia: (20–)30–36(–41) × 4–5 μm (av. 31 × 5 μm, n = 45); 4-septate conidia: (31–)30–36(–34) × 4–6 μm (av. 34 × 5 μm, n = 26); 5-septate conidia: (30–)37–41(–43) × 4–6 μm (av. 38 × 5 μm, n = 26). Sporodochia pale orange, formed abundantly on surface of carnation leaves. Sporodochial conidiophores densely and irregularly branched, 15–19 × 2–3 μm, bearing apical whorls of 1–2 phialides; sporodochial phialides monophialidic, subulate to subcylindrical, 10–12 × 2–3 μm, smooth, and thin-walled; sporodochial conidia falcate, curved dorsiventrally, straight to slightly curved, tapering towards both ends, with slightly papillate, curved apical cell and a notched to foot-like basal cell, (0–)3–4(–5)-septate, hyaline, smooth, and thin-walled; 0-septate conidia: 28–30 × 3–4 μm (av. 29 × 4 μm, n = 5); 1-septate conidia: (16–)21–32(–36) × 3–5 μm (av. 27 × 4 μm, n = 11); 2-septate conidia: 22–23 × 3–4 μm (av. 23 × 4 μm, n = 4); 3-septate conidia: (22–)25–33(–41) × 3–6 μm (av. 32 × 4 μm, n = 38); 4-septate conidia: (33–)35–38(–43) × 4–6 μm (av. 37 × 5 μm, n = 26); 5-septate conidia: (36–)38–40(–44) × 4–6 μm (av. 40 × 5 μm, n = 16). Chlamydospores abundant, globose, subglobose to ellipsoid, terminal or intercalary, solitary, in pairs, or forming long chains, 8–12 μm diameter.

Culture characteristics. Colonies on PDA incubated at 25 °C in the dark, reaching 71–79 mm diameter in 7 d; aerial mycelia dense, flat, white, colony margin entire; reverse yellowish white, radiate, aerial mycelia dense, odor absent. Colonies on OA grown in the dark, reaching 69–77 mm diameter after 7 d at 25 °C, flat, aerial mycelia scant, colony margin entire, white; reverse white, odor absent.

Additional material studied. Chⁱɴᴀ • Yunan Province, Nanuo Mountain, on leaves of *Xylosma congesta*, 3 March 2023, Q.Y. Liu (HSAUP21624), living culture CGMCC 3.27795 = SAUCC 2416-2.

Notes. Phylogenetically, *F. xylosmatis* (CGMCC 3.27794) is closely related to the species *F. weifangense* (LC18333); there were 7/535 differences in *cal*, 9/657 in *rpb2*, and 8/462 in *tef1*. Morphologically, *F. xylosmatis* (CGMCC 3.27794) is distinguished from *F. weifangense* (LC18333) by the number of sporodochial conidial septa (0–5-septate in *F. xylosmatis* (CGMCC 3.27794) vs. 3–7-septate in *F. weifangense* (LC18333)) (Han et al. 2023; Zhang et al. 2023a).

Discussion

The genus and species concepts in *Fusarium* have endured significant changes (Leslie and Summerell 2006; Crous et al. 2021; He et al. 2024). Traditionally, the identification of *Fusarium* is mainly based on morphological characteristics (Wollenweber and Reinking 1935; Snyder and Hansen 1940; Toussoun and Nelson 1968; Gerlach and Nirenberg 1982; Leslie and Summerell 2006). However, identification is difficult due to the high morphological variation that complicates morphological identification among the closely related species (Leslie and Summerell 2006; Crous et al. 2021). Therefore, it is important to identify *Fusarium*

species through molecular analysis (Wang et al. 2019; Xia et al. 2019; Crous et al. 2021; Wang et al. 2022; He et al. 2024). The internal transcribed spacer (ITS), the large subunit (LSU), ATP citrate lyase (*acl1*), calmodulin (*cal*), RNA polymerase II largest subunit (*rpb1*), RNA polymerase second largest subunit (*rpb2*), translation elongation factor 1-alpha (*tef1*), and beta-tubulin (*tub2*) are used in current studies (Lombard et al. 2015; Sandoval-Denis et al. 2018; Xia et al. 2019; Crous et al. 2021; Suwannarach et al. 2023; He et al. 2024). However, the identification of *Fusarium* at the species level could not be resolved using the ribosomal DNA gene (ITS and LSU) alone (Balajee et al. 2009; O'Donnell et al. 2015; Suwannarach et al. 2023). Thus, the protein-coding genes (*acl1*, *cal*, *rpb1*, *rpb2*, *tef1*, and *tub2*) are added (Xia et al. 2019; Crous et al. 2021; Suwannarach et al. 2023; He et al. 2024). Different complexes of *Fusarium* require different gene combinations to identify.

In this study, we collected parasitic or saprotrophic fungi from terrestrial habitats in Hainan, Sichuan, and Yunnan Provinces of China on four plant specimens: *Setaria palmifolia*, *Ficus fistulosa*, *Prunus salicina*, and *Xylosma congesta*. Morphologically, these species exhibit a range of variations in spore size, shape, and ornamentation, as well as colony characteristics such as growth rate, pigmentation, and texture. We also conducted phylogenetic analyses using *cal*, *rpb2*, and *tef1* sequences and can be recognized as two new phylogenetic species (*Fusarium. fici* sp. nov. and *Fusarium xylosmatis* sp. nov.), along with two known species (*Fusarium fecundum* and *Fusarium weifangense*). The discovery of two new species underscores the rich fungal diversity in Hainan, Sichuan, and Yunnan Provinces and emphasizes the need for further exploration of understudied habitats. *Fusarium fecundum* was first reported from *Setaria palmifolia*; *Fusarium weifangense* was first reported from *Prunus salicina*. It can contribute to our knowledge of host specificity and ecological adaptation in fungal pathogens. These findings have significant implications for fungal taxonomy, ecology, and potential applications in plant pathology and biocontrol.

Additional information

Author contributions

Sampling, molecular biology analysis: Qiyun Liu and Congcong Ai; fungal isolation: Yaling Wang; description and phylogenetic analysis: Zhaoxue Zhang; microscopy: Duhua Li and Yun Geng; writing—original draft preparation: Qiyun Liu; writing—review and editing: Jiwen Xia and Xiuguo Zhang. All authors read and approved the final manuscript.

Author ORCIDs

Qiyun Liu ⓘ https://orcid.org/0009-0009-9545-7962
Zhaoxue Zhang ⓘ https://orcid.org/0000-0002-4824-9716
Duhua Li ⓘ https://orcid.org/0009-0006-5200-2034
Xiuguo Zhang ⓘ https://orcid.org/0000-0001-9733-8494
Jiwen Xia ⓘ https://orcid.org/0000-0002-7436-7249

Data availability

The sequences were deposited in the GenBank database.

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Supplementary material 1

GenBank accession numbers of the taxa used in phylogenetic reconstruction

Link: https://doi.org/10.3897/mycokeys.116.150363.suppl1

Supplementary material 2

Phylogeny of the *Fusarium incarnatum-equiseti* species complex (FIESC) inferred based on the *cal* (a), *rpb2* (b), and *tef1* (c) loci, respectively

Authors: Congcong Ai, Qiyun Liu, Yaling Wang, Zhaoxue Zhang, Duhua Li, Yun Geng, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.116.150363.suppl2

Supplementary material 3

GenBank accession numbers of the taxa used in phylogenetic reconstruction (Suppl. material 4)

Authors: Congcong Ai, Qiyun Liu, Yaling Wang, Zhaoxue Zhang, Duhua Li, Yun Geng, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.116.150363.suppl3

Supplementary material 4

Phylogeny inferred based on the combined *cal-rpb2-tef1* sequence dataset with *Fusarium concolor* (NRRL 13459) as the outgroup

Authors: Congcong Ai, Qiyun Liu, Yaling Wang, Zhaoxue Zhang, Duhua Li, Yun Geng, Xiuguo Zhang, Jiwen Xia

Data type: docx

Link: https://doi.org/10.3897/mycokeys.116.150363.suppl4

MycoKeys

Morphological and molecular identification for two new wood-inhabiting species of *Botryobasidium* (Basidiomycota) from China

Xin Li[1,2], Xin Zhang[1], Yi-Fei Sun[1], Zhen-Hao Li[3,4], An-Hong Zhu[1,5], Ying-Da Wu[2]

1 State Key Laboratory of Efficient Production of Forest Resources, School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China
2 Key Laboratory of Forest and Grassland Fire Risk Prevention, Ministry of Emergency Management, China Fire and Rescue Institute, Beijing 102202, China
3 Zhejiang Key Laboratory of Biological Breeding and Exploitation of Edible and Medicinal Mushrooms, Jinhua 321200, Zhejiang, China
4 Zhejiang Shouxiangu Pharmaceutical Co., Ltd, Jinhua 321000, Zhejiang, China
5 Coconut Research Institute, Chinese Academy of Tropical Agricultural Sciences, Wenchang, 571339, China
Corresponding authors: An-Hong Zhu (anhong.zhu@catas.cn); Ying-Da Wu (wydbjfu@163.com)

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Abstract

The wood-inhabiting fungi refer to large basidiomycetes that grow on various woody materials and are distributed in various forest ecosystems, some of which have important economic value. In the present study, two new resupinate, adnate, wood-inhabiting fungal taxa, *Botryobasidium latihyphum* and *B. zhejiangensis*, are introduced based on morphological and molecular characteristics. A molecular phylogenetic study based on sequence data from the internal transcribed spacers (ITS) and the large subunit (nLSU) regions supported the two new species in the genus *Botryobasidium*. Maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI) were employed to perform phylogenetic analyses of these datasets. The new species *B. latihyphum* is characterized by its cream hymenial surface when fresh, olivaceous buff when dry, a monomitic hyphal system with clamp connections, the presence of clavate to tubular cystidia, basidia with six sterigmata, and broadly oval basidiospores measuring 7.9–10.2 × 3.2–4.3 µm. *Botryobasidium zhejiangensis* sp. nov. is characterized by its white to buff-yellow hymenial surface when fresh, cream when dry, a monomitic hyphal system with clamp connections, lacking cystidia, basidia with six sterigmata, and broadly navicular basidiospores measuring 7.9–9.2 × 2.6–3.4 µm. The phylogenetic result inferred from ITS + nLSU sequence data revealed that *B. latihyphum* is closely related to *B. vagum*, *B. laeve*, *B. subincanum*, and *B. incanum*, while *B. zhejiangensis* is closely related to *B. leptocystidiatum*, *B. subcoronatum*, *B. xizangensis*, and *B. intertextum*.

Key words: Botryobasidiaceae, new species, phylogeny, taxonomy, wood-rotting fungi

Introduction

The wood-inhabiting fungi are large basidiomycetes that grow on various woody materials and have a global distribution (Wu et al. 2022a; Bian et al. 2023; Dong et al. 2023; Zhao et al. 2024). The wood-inhabiting fungi play an important role in maintaining the dynamic balance of energy and matter in forest ecosystems, and some of them have important economic values (Dai et al. 2007; Zhao et al. 2012; Dai et al. 2015; Wu et al. 2019; Dai et al. 2021; Yuan et al. 2023;

Zhou et al. 2023a; Dong et al. 2024a). *Botryobasidium* (Botryobasidiaceae, Basidiomycota), typified by *B. subcoronatum* (Höhn. & Litsch.) Donk, is a wood-inhabiting fungal genus with simple macro-morphology. It is characterized by annual, resupinate basidiomata with smooth, pellicular, hypochnoid, or arachnoid hymenophores; a monomitic hyphae system; generative hyphae bearing simple septa or clamp connections, branched mostly at a right angle; basidia with 2–8 sterigmata; smooth or ornamented basidiospores; and causing a white rot (Donk 1964; Langer et al. 2000b; Moncalvo et al. 2006; Xiong et al. 2009).

In the earliest classification system, *Botryobasidium* species were treated in *Corticium* Pers. based on microscopic morphological characteristics; Donk (1931) proposed *Botryobasidium* for the species with four sterigmata on basidia and basidiospores strongly ornamented with rodlets. Many species of *Botryobasidium* in the conidial state belong to the genus *Oidium* Link (Eriksson and Ryvarden 1973). Later, Langer conducted a detailed morphological study, revising the genus based on global samples and identifying 49 species within *Botryobasidium* (Langer et al. 2000a, b). Numerous *Botryobasidium* species exhibited anamorphic stages (Bernicchia and Gorjón 2010). Multiple asexual morph genera *viz.*, *Acladium* Link, *Allescheriella* Henn., *Alysidium* Kunze, *Haplotrichum* Link, *Neoacladium* P.N. Singh & S.K. Singh, *Physospora* Fr., and *Sporocephalium* Chevall. exhibit congeneric relationships with *Botryobasidium*, prompting their taxonomic reclassification under the genus *Botryobasidium* (Stalpers et al. 2021; Dong et al. 2024b).

Phylogenetically, the genus *Botryobasidium* is a well-supported monophyletic group closely related to *Tulasnella* J. Schröt., *Clavulina* J. Schröt., and *Sistotrema* Fr., but the former differed from the latter three genera by having wider hyphae and lacking oil droplets in basidia and basidiospores (Hibbett et al. 1997; Kotiranta and Saarenoksa 2005; Yuan et al. 2011).

So far, 115 species of *Botryobasidium* have been discovered worldwide (Langer 1994; Parmasto et al. 2004; Ryvarden et al. 2005; Xiong et al. 2009; Bernicchia et al. 2010; Bates et al. 2017; Ram et al. 2021; Stalpers et al. 2021; Zhou et al. 2024a), among them 17 were reported in China (Langer et al. 2000a, 2000b; Xiong et al. 2009; Dong et al. 2024b; Zhou et al. 2024a). During investigations on the diversity of wood-rotting fungi, four *Botryobasidium*-like samples were collected. Phylogenetic analyses based on the ITS and nLSU sequences were carried out to confirm their taxonomic status. Morphological and molecular evidence confirmed that the four examined specimens belong to two distinct new *Botryobasidium* species.

Materials and methods

Morphological studies

Fresh fruiting bodies of the fungi were collected from Linzhi of Xizang Autonomous Region and Jinhua of Zhejiang Province, China. After the important collection information was noted (Rathnayaka et al. 2024), the samples were taken to the laboratory at the Institute of Microbiology, Beijing Forestry University (**BJFC**), in plastic collection boxes. Specimens were dried in a mushroom dryer at 35 °C (Hu et al. 2022), then sealed and stored in an envelope bag. Examined specimens were deposited in the Fungarium of

the Institute of Microbiology, Beijing Forestry University (**BJFC**), Beijing, China. Morphological descriptions were based on field notes and dried specimens. Micro-morphological data were obtained from dried specimens and observed under a compound microscope following Dai (2010) and Li et al. (2014). Sections were studied at a magnification of 1000 × using a Nikon E80i microscope and phase contrast illumination (Nikon, Tokyo, Japan). Line drawings were made with the aid of a drawing tube.

The following abbreviations were used in the descriptions: **KOH** = 5% potassium hydroxide, **IKI** = Melzer's reagent, **IKI−** = neither amyloid nor dextrinoid, **CB** = Cotton Blue, **CB+** = cyanophilous, **CB−** = acyanophilous, **L** = mean spore length (arithmetic average of all spores), **W** = mean basidiospore width (arithmetic average of all spores), **Q** = variation in the L/W ratios between the specimens studied, **n (a/b)** = number of basidiospores (a) measured from the given number of specimens (b). In presenting basidiospore size variation, 5% of measurements were excluded from each end of the range, and these values were given in parentheses. Special color is termed follow Anonymous (1969) and Petersen (1996).

DNA extraction, polymerase chain reaction amplification, and sequencing

Total genomic DNA from the dried specimens was extracted by a Cetyltrimethyl Ammonium Bromide (**CTAB**) rapid plant genome extraction kit (Aidlab Biotechnologies Company Limited, Beijing, China) according to the manufacturer's instructions with some modifications (Du et al. 2021). ITS locus was amplified using the primer pair ITS4 (TCCTCC GCT TAT TGA TAT GC) and ITS5 (GGA AGT AAA AGT CGT AAC AAG G) (White et al. 1990), while nLSU locus was amplified with primers LR0R (ACC CGC TGA ACT TAA GC) and LR7 (TAC TAC CAC CAA GAT CT) (Vilgalys and Hester 1990).

The polymerase chain reaction (**PCR**) amplification conditions for ITS were an 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 at 72 °C for 10 min (Zhao et al. 2015), and for nLSU were initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, at 48 °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 in the Beijing Genomics Institute, China, with the same primers used in the PCR reactions.

Phylogenetic analyses

The species, specimens, and GenBank accession numbers of the sequences used in this study are shown in Table 1.

For the phylogenetic analyses, the combined two-marker dataset (ITS+nLSU) included sequences from 57 samples representing 20 taxa. *Lyomyces allantosporus* Riebesehl et al. and *Lyomyces pruni* (Lasch) Riebesehl & Langer were chosen as the outgroups (Spirin et al. 2015; Riebesehl and Langer 2017; Chen and Zhao 2020). Sequences generated from this study were aligned with additional sequences downloaded from GenBank using BioEdit (Hall 1999) and ClustalX (Thompson et al. 1997). The final ITS and nLSU datasets were subsequently aligned using MAFFT v.7 under the E-INS-i

Table 1. List of species, specimens, and GenBank accession numbers of the sequences used in this study. New species are in bold, * indicates type material, holotype, and - refers to the data unavailability.

Species name	Samples	Country	GenBank Accession no.	
			ITS	nLSU
Botryobasidium acanthosporum	Yuan 17989	China	PP229511	-
B. acanthosporum	Yuan 18083*	China	PP229512	PP218361
B. acanthosporum	Yuan 18128	China	PP229517	-
B. acanthosporum	Yuan 16326	China	PP229497	-
B. asperulum	RAS552	USA	OR471090	OR470959
B. asperulum	RAS578	USA	OR471100	OR470964
B. aureum	RAS571 SV2	USA	OR471099	-
B. aureum	RAS571 SV1	USA	OR471098	-
B. bambusinum	CLZhao 29938	China	PQ539059	PQ539062
B. bambusinum	CLZhao 29936	China	PQ539058	PQ539061
B. bambusinum	CLZhao 29916*	China	PQ539057	PQ539060
B. botryosum	AFTOL-ID 604	USA	DQ267124	DQ089013
B. candicans	UC2022891	USA	KP814227	-
B. candicans	UC2022893	USA	KP814200	-
B. candicans	HFRG_LG230226_1_FRDBI_29580226	UK	OR896129	-
B. coniferarum	LWZ20210928-3*	China	OR557259	OR527282
B. coniferarum	LWZ20171016-15	China	OR557262	OR527286
B. conspersum	AFTOL-ID 1766	USA	DQ911612	DQ521414
B. conspersum	RAS259	USA	OR471145	-
B. gossypirubiginosum	CLZhao 26052*	China	OR668924	OR708665
B. gossypirubiginosum	Dai 26208	China	PQ285750	-
B. incanum	Dai 25375	China	PQ285751	PQ28566
B. incanum	CLZhao 26697	China	OR668923	OR708664
B. indicum	Yuan 18434	China	PP209217	PP218365
B. indicum	hr5326	China	OP806032	-
B. intertextum	UC2022959 18S	USA	KP814540	-
B. laeve	RAS762	USA	OR471128	PP959648
B. latihyphum	**Dai 26858***	**China**	**PQ279526**	**PQ282521**
B. latihyphum	**Yuan 16496**	**China**	**PP331854**	**PP218153**
B. leptocystidiatum	Yuan 17706	China	PP209200	PP218353
B. leptocystidiatum	Yuan 17708*	China	PP209197	PP218354
B. robustius	CBS:945.69	Czech	MH859491	MH871272
B. robustius	iNaturalist 162067551	USA	PP436446	-
B. rubiginosum	RAS776 taxon1	USA	OR471136	-
B. simile	RAS793	USA	OR471147	-
B. simile	RAS794	USA	OR471146	-
B. subcoronatum	RAS770 SV1	USA	OR471132	-
B. subcoronatum	RAS770 SV2	USA	OR471133	-
B. subovalibasidium	Yuan 16439	China	PP209199	PP218152
B. subovalibasidium	Yuan 18179*	China	PP209196	PP218362
B. subincanum	LWZ20230417-17b	China	PP959661	PP959649
B. subincanum	LWZ20230417-41a	China	PP959660	-
B. tubulicystidium	DK14 139	USA	OL436769	-
B. vagum	LWZ20191016-22	USA	PP959659	PP959648
B. xizangense	LWZ20230722-25a*	China	PP959663	PP959650
B. xizangense	LWZ20230722-16a	China	PP959662	-
B. yunnanense	CLZhao 24877*	China	OR708666	OR668925
B. zhejiangensis	**Dai 25056***	**China**	**PQ279530**	**PQ282525**
B. zhejiangensis	**Dai 24851**	**China**	**PQ279529**	**PQ282524**
Lyomyces allantosporus	FR 0249548	France	NR_154135	-
L. pruni	GEL2327	Germany	DQ340312	-

strategy with no cost for opening gaps and equal cost for transformations (command line: mafft −genafpair −maxiterate 1,000) (Katoh and Standley 2013) and visualized in BioEdit. Alignments were spliced and transformed into formats in Mesquite v.3.2 (Maddison and Maddison 2017). Sequence alignments were deposited at TreeBASE (submission ID 32008, www.treebase.org). The best-fit evolutionary model was estimated using MrModeltest v.2.3 (Posada and Crandall 1998) as GTR + I + G for the combined dataset.

Maximum likelihood (ML) analyses were conducted using RAxML-HPC2 via the CIPRES Science Gateway (www.phylo.org; Miller et al. 2010). Branch support (BT) for ML analysis was determined by 1,000 bootstrap replicates. The maximum parsimony (MP) analysis was applied to the ITS+nLSU dataset sequences. The construction was performed in PAUP* v. 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1,000 random sequence additions. Max-trees was set to 5,000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed by a bootstrap (BT) analysis with 1,000 replicates (Felsenstein 1985). Descriptive tree statistics, i.e., tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were calculated for each maximum parsimonious tree (MPT) generated. The BI analysis was calculated with MrBayes v.3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist and Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 2 million generations, and trees were sampled every 100 generations. The first 25% of sampled trees were set as burn-in. A majority rule consensus tree of all remaining trees was calculated. The maximum likelihood bootstrap support value (BS), the maximum parsimony bootstrap support value (BT), and Bayesian posterior probabilities (BPP) simultaneously not less than 50%, 75%, and 0.95, respectively, were shown at the nodes.

Results

Phylogenetic analyses

The combined dataset of ITS+nLSU contained sequences from 47 fungal specimens representing 25 *Botryobasidium* taxa (2 new species and another 23 taxa). The combined dataset has an aligned length of 2,027 characters, of which 1,431 characters are constant, 126 are variable and parsimony uninformative, and 470 are parsimony informative. The MP analysis yielded two equally most parsimonious trees (TL = 1,672, CI = 0.587, RI = 0.856, RC = 0.502, HI = 0.413). The Bayesian analysis and MP analysis resulted in a similar topology as the ML analysis. The ML tree is provided in Fig. 1. The average SD of split frequencies in BI analyses is 0.002852 (BI). Two new species, *B. latihyphum* and *B. zhejiangensis*, were proposed based on examining type materials and phylogenetic analyses (Fig. 1).

The top five BLAST results for the ITS of *Botryobasidium latihyphum* on NCBI are *Botryobasidium* sp. (PP229498), *Botryobasidium* sp. (KP814226),
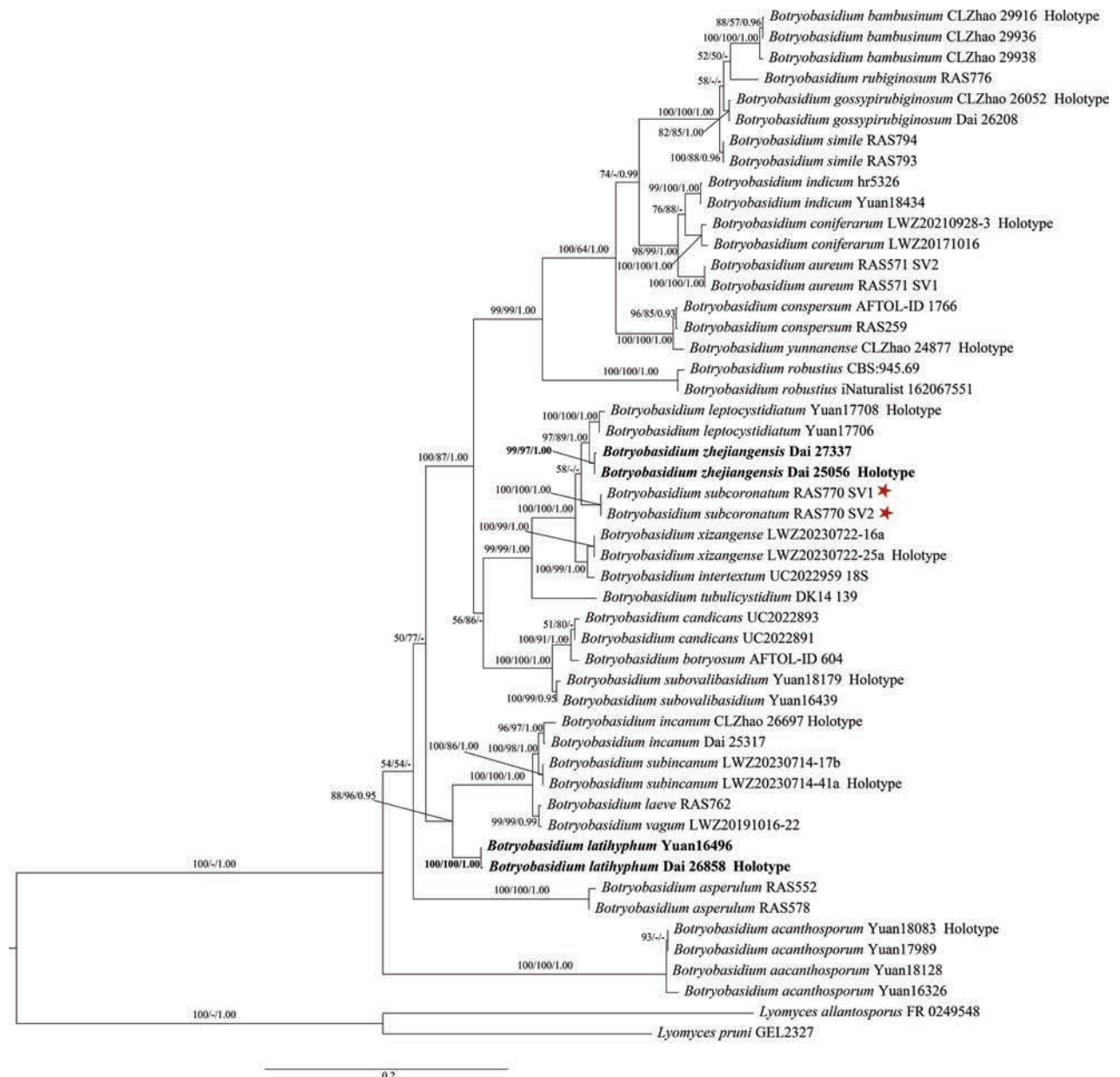
Figure 1. Maximum Likelihood tree illustrating the phylogeny of *Botryobasidium* based on combined ITS + nLSU sequence data. Branches are labeled with maximum likelihood bootstrap proportions equal to or higher than 50%, maximum parsimony bootstrap equal to or higher than 75%, and Bayesian posterior probabilities equal to or higher than 0.95. The red star represents the type species. The new species are in bold black.

uncultured Corticiales (FJ475677), *Botryobasidium* sp. (KP814344), and *Botryobasidium* sp (KP814346); the top five BLAST results for the nLSU of *B. latihyphum* on NCBI are *Botryobasidium* sp. (PP218153), *Botryobasidium* sp. (OR470952), *B. incanum* (OR708664), *B. vagum* (OR470970), and *Botryobasidium* sp. (OR470958); the top five BLAST results for the ITS of *Botryobasidium zhejiangensis* on NCBI are *Botryobasidium* sp. (OR471085), *B. vagum* (OR471082), *B. vagum* (MK809424), *B. subcoronatum* (MK809424), and *B. subcoronatum* (MK795129); and the top five BLAST results for the nLSU of *B. zhejiangensis* on NCBI are *B. subcoronatum* (OM083971), *B. vagum* (OR470953), *B. subcoronatum* (OR470950), *B. subcoronatum* (EU909344), and *B. subcoronatum* (OR470954).

Taxonomy

Botryobasidium latihyphum Xin Li, Y.J. Cui & Y.D. Wu, sp. nov.

MycoBank No: 856838

Figs 2, 3

Holotype. CHINA • Xizang Autonomous Region., Linzhi, Metuo County, the road 219 from Metuo to Bome, on fallen trunk of *Abies*, 25 October 2023, Dai 26858 (BJFC044409).

Etymology. *Latihyphum* refers to the characteristic wide subicular hyphae of the new species.

Description. *Basidiomata*: Annual, resupinate, adnate, hypochnoid, difficult to separate from substrate, up to 10 cm long, 4 cm wide, 1 mm thick at center, without odor and taste when fresh and dry; hymenophore white to cream when fresh, smooth, uncracked, cream to olivaceous buff when dry; sterile margin indistinct, thinning out, concolorous with hymenophore.

Hyphal system: Monomitic, clamp connections present, generative hyphae CB+, IKI−; tissues unchanged in KOH; subhymenial hyphae slightly thick-walled, smooth, frequently branched at right angles, loosely interwoven, 5−7 µm in diam.; subicular hyphae thick-walled, smooth, frequently branched, 7−10 µm in diam.

Hymenium: Cystidia clavate to tubular, infrequent, smooth, thin-walled, colorless, with a basal clamp connection, aseptate, CB+, IKI−, unchanged in KOH, 56−105 × 7−10 µm; basidia slightly barrel-shaped, thin-walled, with six sterigmata and a clamp connection at the base, 17−25 × 6−8.5 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores: Basidiospores oval, hyaline, thin-walled, smooth, CB+, IKI−, (7.0−)7.9−10.2(−10.3) × (3.1−)3.2−4.3(−4.4) um, L = 8.78 um, W = 3.64 um, Q = 2.41 (n = 60/2).

Figure 2. A basidioma of *Botryobasidium latihyphum* (Dai 26858). Scale bar: 1 cm.
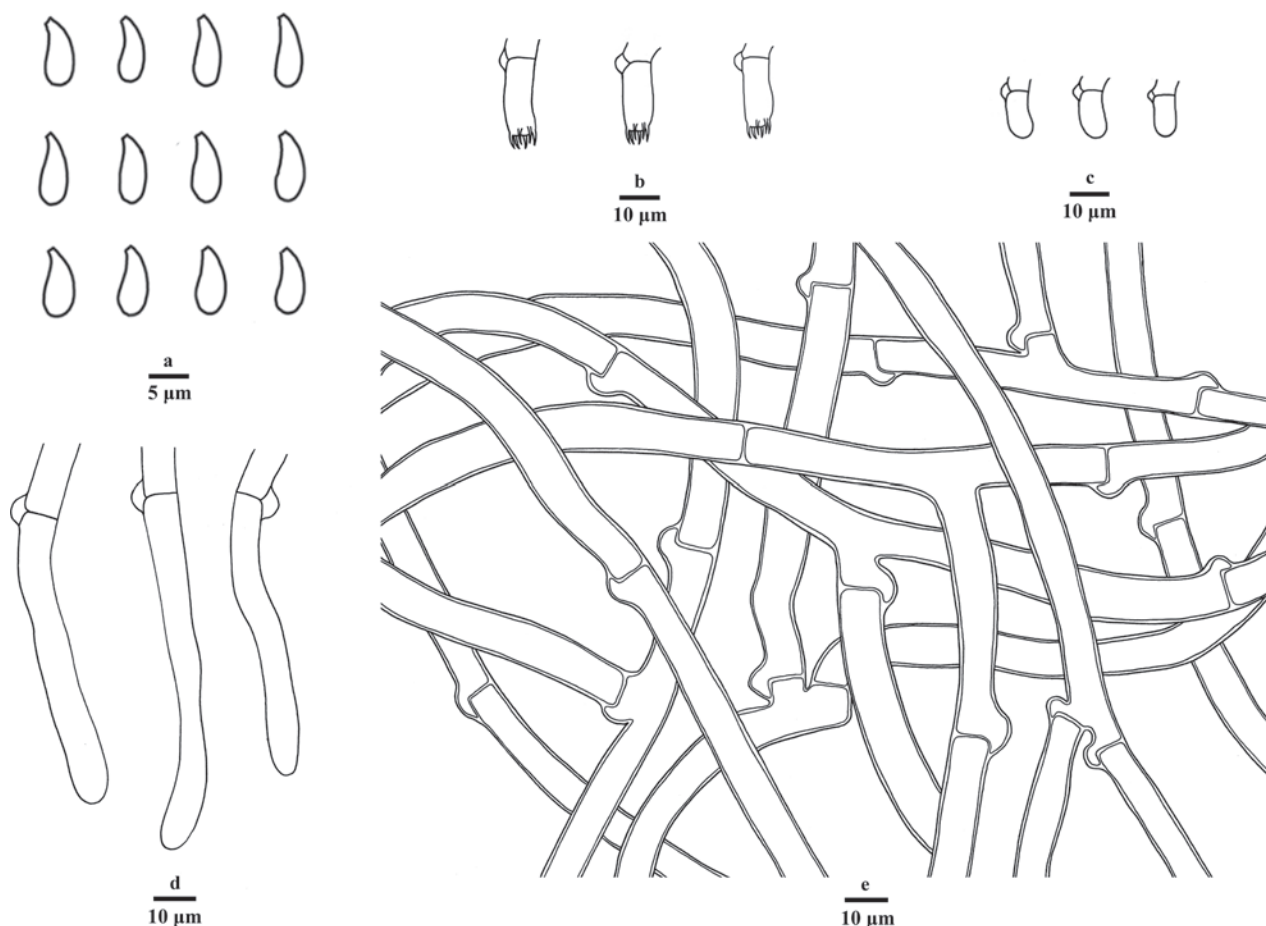
Figure 3. Microscopic structures of *Botryobasidium latihyphum* (drawn from the holotype Dai 26858) **a** basidiospores **b** basidia **c** basidioles **d** cystidia **e** subicular hyphae. Scale bars: 5 μm (**a**); 10 μm (**b–e**).

Botryobasidium zhejiangensis **Xin Li, A.H. Zhu, Yuan Yuan & Y.D. Wu, sp. nov.**
MycoBank No: 856839
Figs 4, 5

Holotype. CHINA • Zhejiang Province, Jinhua, Wuyi County, Guodong Village, on rotten wood of *Pinus massoniana*, 18 June 2023, Dai 25056 (BJFC 042609).

Etymology. *Zhejiangensis* refers to the type location, Zhejiang Province, East China.

Description. *Basidiomata*: Annual, resupinate, adnate, pellicular, difficult to separate from substrate, up to 11 cm long, 7 cm wide, 1 mm thick, without odor and taste when fresh; hymenophore white to cream, smooth, uncracked, cream to slightly buff when dry; sterile margin indistinct, thinning out, concolorous with hymenophore.

***Hyphal system*:** Monomitic, generative hyphae with clamp connections, CB+, IKI−; tissues unchanged in KOH. Subhymenial hyphae hyaline, thin-walled, smooth, frequently branched at right angles, loosely interwoven, 4−6 μm in diam.; subicular hyphae hyaline, slightly thick-walled, smooth, frequently branched, 6−8 μm in diam.

***Hymenium*:** Basidia slightly barrel-shaped, hyaline, thin-walled, with six sterigmata and a basal clamp connection, 15−19 × 5−6 μm; basidioles in shape similar to basidia, but smaller.

Figure 4. Basidiomata of *Botryobasidium zhejiangensis* (Dai 25056). Scale bar: 1 cm.

Spores: Basidiospores more or less navicular, hyaline, thin-walled, smooth, CB+, IKI−, (7.8−)7.9−9.2(−9.5) × (2.5−)2.6−3.4(−3.5) μm, L = 8.47 μm, W = 3.05 μm, Q = 2.78 (n = 60/2).

Discussion

Prior to this study, 17 *Botryobasidium* species, *viz.*, *B. acanthosporum* L.J. Zhou & H.S. Yuan, *B. arachnoideum* G. Langer, *B. asterosporum*, G. Langer, *B. coniferarum* S.L. Liu & L.W. Zhou, *B. gossypirubiginosum* Qian Zhou & C.L. Zhao, *B. grandisporum* G. Langer, *B. incanum* Qian Zhou & C.L. Zhao, *B. leptocystidiatum* L.J. Zhou & H.S. Yuan, *B. longisporum* G. Langer, *B. musisporum* G. Langer, *B. subincanum* S.L. Liu & L.W. Zhou, *B. sublaeve* G. Langer, *B. subovalibasidium* L.J. Zhou & H.S. Yuan, *B. tuberculisporum* G. Langer, *B. tubulicystidium* G. Langer, *B. xizangense* S.L. Liu & L.W. Zhou and *B.* yunnanense Qian Zhou & C.L. Zhao were reported from China (Lentz 1967; Jung 1995; Kalinina et al. 2020; Cao et al. 2021; Zhou et al. 2024a). In this study, a large number of specimens were collected from Xizang and Zhejiang provinces in China, and two new species were presented according to morphological and phylogenetic evidence, which further improved the genus diversity of *Botryobasidium* in China.

In the present study, the phylogenetic analyses using the combined ITS + nLSU dataset produced a well-resolved phylogeny (Fig. 1). *Botryobasidium latihyphum* and *B. zhejiangensis* formed two well-supported lineages (100% in ML, 100% in
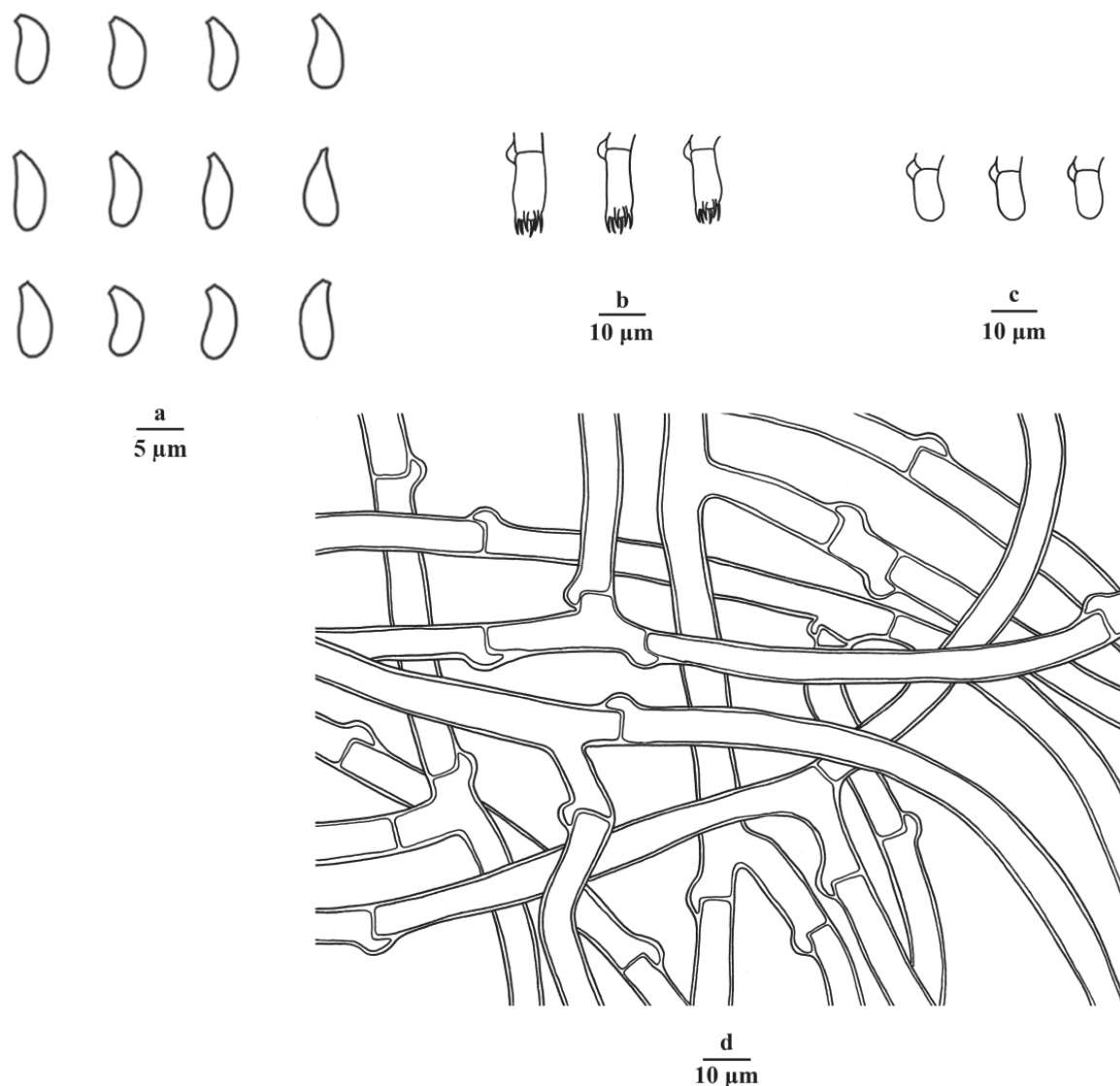
Figure 5. Microscopic structures of *Botryobasidium zhejiangensis* (drawn from the holotype Dai 24851) **a** basidiospores **b** basidia **c** basidioles **d** subicular hyphae. Scale bars: 5 μm (**a**); 10 μm (**b–d**).

MP, and 1.00 in BI; 99% in ML, 97% in MP, and 1.00 in BI). The phylogeny analyses revealed that *Botryobasidium latihyphum* is related to *B. vagum* (Berk. & M.A. Curtis) D.P. Rogers, *B. laeve* (J. Erikss.) Parmasto, *B. subincanum*, and *B. incanum* (Figure 1). However, *B. vagum* is readily distinguished from *B. latihyphum* by having reticulate to floccose hymenophore, wider basidiospores (4.5–6 μm vs. 3.2–4.3 μm, Bernicchia and Gorjón 2010), and lacking clamp connection; *B. laeve* differs from *B. latihyphum* by cylindrical basidia and shorter basidiospore (5–8 μm vs. 7.9–10.2 μm); *B. subincanum* differs from *B. latihyphum* by having simple septate hyphae, longer basidia (8–11 μm vs. 6–8.5 μm) and widder basidiospore (4–5 μm vs. 3.2–4.3 μm, Wang et al. 2024a); *B. incanum* differs from *B. latihyphum* by having arachnoid hymenophore, basidia with four sterigmata, and lacking clamp connection (Zhou et al. 2024a). Morphologically, *Botryobasidium danicum* J. Erikss & Hjortstam is similar to *B. latihyphum* by sharing a hypochnoid hymenial surface and subcylindrical basidia with six sterigmata. However, *B. danicum* differs from *B. latihyphum* by having simple septate hyphae and navicular and longer basidiospores (12–14 μm vs. 7.9–10.2 μm, Bernicchia and Gorjón 2010).

In the phylogenetic tree (Fig. 1), *Botryobasidium zhejiangensis* is related to *B. leptocystidiatum*, *B. intertextum* (Schwein.) Jülich & Stalpers, *B. subcoronatum*, and *B. xizangense* (Fig. 1). The ITS region of *B. zhejiangensis* is different from *B. leptocystidiatum* by 7.4%, but *B. leptocystidiatum* differs from *B. zhejiangensis* by having tubular cystidia and shorter basidiospores (6.5−7.8 μm vs. 7.9−9.2 μm, Zhou et al. 2024a); *B. intertextum* differs from *B. zhejiangensis* by subcylindrical basidia and wider basidiospores (1.8−2.8 μm vs. 2.6−3.4 μm); *B. subcoronatum* differs from *B. zhejiangensis* by having yellowish to ochraceous hymenial surface, bigger basidia (20−25 × 7−9 μm vs. 15−19 × 5−6 μm), shorter basidiospores (6−8 μm vs. 7.9−9.2 μm, Bernicchia and Gorjón 2010); *B. xizangense* differs from *B. zhejiangensis* by subcylindrical basidia and wider basidia (6−7 μm vs. 5−6 μm, Wang et al. 2024b). Morphologically, *Botryobasidium zhejiangensis* resembles *B. robustius* Pouzar & Hol-Jech by sharing pellicular hymenial surface and similar basidia with six sterigmata, but *B. robustius* differs from *B. zhejiangensis* by having wider basidia (8−10 μm vs. 5−6 μm, Bernicchia and Gorjón 2010) and a lack of clamp connection.

The wood-inhabiting fungi are a widely studied group of the kingdom fungi, which can promote the material circulation and energy flow of the forest ecosystem and bring great economic value. Further investigation of wood-inhabiting fungi in different forestry habitats will enrich the fungal diversity in China and the world. (Dai et al. 2009; Dai 2010; Wu et al. 2014, 2022b; Cui et al. 2019; Wu et al. 2020; Wijayawardene et al. 2022; Mao et al. 2023; Wang et al. 2023; Zhang et al. 2023; Zhao et al. 2023b; Zhou et al. 2023b; Cui et al. 2024; Qin et al. 2024; Zhou et al. 2024b).

Key to species of *Botryobasidium* in China

1	Generative hyphae with simple septa	**2**
−	Generative hyphae with clamp connections	**17**
2	Cystidia present	***B. acanthosporum***
−	Cystidia absent	**3**
3	Chlamydospores present	***B. subovalibasidium***
−	Chlamydospores absent	**4**
4	Conidia present	**5**
−	Conidia absent	**6**
5	Basidiospores > 13 μm long	***B. robustius***
−	Basidiospores < 13 μm long	***B. bambusinum***
6	Basidia with six sterigmata	**7**
−	Basidia with four sterigmata	**15**
7	Basidiospores mostly > 9 μm long	**8**
−	Basidiospores mostly < 9 μm long	**10**
8	Basidia > 20 μm long	**9**
−	Basidia < 20 μm long	***B. danicum***
9	Basidiomata reticulate to floccose	***B. vagum***
−	Basidiomata hypochnoid	***B. botryosum***
10	Basidia obovate	***B. aureum***
−	Basidia subcylindrical	**11**
11	Basidia > 8 μm wide	**12**
−	Basidia < 8 μm wide	**13**
12	Basidiomata floccose	***B. laeve***
−	Basidiomata pellicular	***B. subincanum***

13	Basidiospores navicular	**14**
–	Basidiospores subcylindrical	***B. conspersum***
14	Basal hyphae > 8 µm in diam	***B. candicans***
–	Basal hyphae < 8 µm in diam	***B. xizangense***
15	Basidiomata floccose to cotton	***B. gossypirubiginosum***
–	Basidiomata hypochnoid	**16**
16	Basidiospores > 7 µm wide	***B. isabellinum***
–	Basidiospores < 7 µm wide	***B. incanum***
17	Basidia mostly < 6 µm wide	**18**
–	Basidia mostly > 6 µm wide	**19**
18	Basidiospores > 2.5 µm wide	***B. zhejiangensis***
–	Basidiospores < 2.5 µm wide	***B. intertextum***
19	Basidiospores > 8 µm long	***B. latihyphum***
–	Basidiospores < 8 µm long	**20**
20	Cystidia absent	***B. subcoronatum***
–	Cystidia present	***B. leptocystidiatum***

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

All authors have contributed equally.

Author ORCIDs

Xin Li https://orcid.org/0009-0001-1625-8589
Xin Zhang https://orcid.org/0009-0005-8363-7852
Yi-Fei Sun https://orcid.org/0000-0003-3997-3662
Zhen-Hao Li https://orcid.org/0000-0001-8279-8981
An-Hong Zhu https://orcid.org/0000-0002-2812-8108
Ying-Da Wu https://orcid.org/0000-0003-1295-4015

Data availability

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

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Research Article

Molecular phylogeny and morphology reveal four novel species in Cordycipitaceae in China

Jing Bu[1,2,3], De-Ping Wei[2,3], Zheng-Hui Liu[1,2,3], Yang Yang[4], Zhong-Liang Liu[1,2,3], Ji-Chuan Kang[1,2,3], Xing-Can Peng[2,3,5,6], Shi-Wen Xie[1,2,3], He-Gui Zhang[4], Zhang-Jiang He[1,2,3], Shi-Ke Huang[2,3,7], Xian Zhang[2,3,5,6], Kevin D. Hyde[3,5,6], Nalin N. Wijayawardene[8], Ting-Chi Wen[1,2,3]

1 School of Pharmacy, Guizhou University, Guiyang 550025, Guizhou Province, China
2 State Key Laboratory of Green Pesticide, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, Guizhou Province, China
3 Engineering Research Center of Southwest Bio-Pharmaceutical Resources, Ministry of Education, Guizhou University, Guiyang 550025, Guizhou Province, China
4 Guizhou Guiwang Biotechnology Co., Ltd, Daozhen 563599, Guizhou Province, China
5 Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand
6 School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand
7 College of Resources and Environment, Zunyi Normal University, Zunyi 563000, Guizhou Province, China
8 Center for Yunnan Plateau Biological Resources Protection and Uilization, College of Biology and Food Engineering, Qujing Normal University, Qujing, 655011, Yunnan Province, China

Corresponding author: Ting-Chi Wen (tingchiwen@yahoo.com)

Abstract

Cordycipitaceae is a well-known family in Hypocreales, comprising numerous arthropod-pathogenic species. Many taxa in this family have been identified and described through integrated morphological and molecular analyses. In this study, phylogenetic analyses using nrLSU, ITS, nrSSU, 3P_*TEF*, *rpb1*, and *rpb2* revealed a new species, *Pleurodesmospora sanduensis*, and a new collection of *Akanthomyces baishanensis*. Additionally, a concatenated 5P_*TEF*+3P_*TEF*+*rpb1*+*MCM7* dataset was employed to clarify interspecific relationships within *Samsoniella*, identifying three new species: *Samsoniella lurida*, *S. subasiatica*, and *S. torquatistipitata*. Detailed morphological descriptions and illustrations are provided for each studied species.

Key words: Entomopathogenic fungi, four new species, morphology, phylogeny

Introduction

Cordycipitaceae belongs to Hypocreales (Hypocreomycetidae, Sordariomycetes), and currently it includes 38 genera. Their phylogenetic relationships have been confirmed through molecular and morphological studies (Sung et al. 2001, 2007; Zare and Gams 2016; Kepler et al. 2017; Zhang et al. 2017, 2021; Mongkolsamrit et al. 2018, 2020, 2021, 2022, 2023; Flakus et al. 2019; Wei et al. 2019; Thanakitpipattana et al. 2020, 2022; Wang et al. 2020; Chen et al. 2021a, 2025; Alves et al. 2022; Araújo et al. 2022; Crous et al. 2023a; Guerra-Mateo et al. 2023; Kobmoo et al. 2023; Custódio and Pereira 2024; Hyde et al. 2024; Khonsanit et al. 2024). Most Cordycipitaceae species are known as pathogens of insects and

spiders, while others are reported as hyperparasites on fungi and lichens or are isolated from soil, dung, air, and plant materials (Kepler et al. 2017; Wang et al. 2020; Wei et al. 2022). To adapt to the diverse hosts and habitats, members of Cordycipitaceae have evolved with a wide variety of teleomorphic and anamorphic characteristics (e.g., *Akanthomyces*, *Samsoniella*, and *Pleurodesmospora*).

The genus *Akanthomyces* was introduced by Lebert (1858), typifying with *A. aculeatus* (Mains 1950), and currently 60 epithets are listed in Index Fungorum (http://www.indexfungorum.org/, retrieval on 18 March 2025). Species of *Akanthomyces* are characterised by forming superficial, yellow perithecia on mycelial mat covering spider hosts and the filiform, intact ascospores (Boudier 1885; Mongkolsamrit et al. 2018). Later, the morphological diversity of *Akanthomyces* was broadened to include species with isaria-like and lecanicillium-like anamorphs based on phylogenetic evidence (Mongkolsamrit et al. 2018; Vinit et al. 2018; Chen et al. 2020a, b, 2022). The members of the genus have been reported as insect parasites, plant pathogens, fungicolous organisms, and inhabitants of peat, water, and rust (Wang et al. 2024b). Khonsanit et al. (2024) introduced four genera (i.e., *Arachnidicola*, *Lecanicillium*, *Akanthomyces*, and *Kanoksria*) to accommodate *Akanthomyces* species that are not congeneric with *Akanthomyces sensu stricto*.

Samsoniella was established by Mongkolsamrit et al. (2018) to accommodate *S. alboaurantium*, *S. aurantia*, and *S. inthanonensis* using both morphological and molecular evidence. *Samsoniella* is characterised by having yellow to orange, fleshy stromata and superficial perithecia and intact ascospores (Mongkolsamrit et al. 2018). Previous researchers have discovered 39 species that are mainly distributed in Asian countries such as China, Thailand, and Vietnam (Wang et al. 2024a). All *Samsoniella* species have been verified with molecular data, and a combination of six genes (ITS-nrSSU-nrLSU-*rpb1*-*rpb2*-3P_*TEF*) usually was used to study the interspecific relationship (Mongkolsamrit et al. 2018; Wang et al. 2023a, 2024a). However, the taxonomic classification of this genus is considered to be complex due to morphological plasticity, and there is a need to search for new genetic markers with higher resolution (Wang et al. 2023a).

The genus *Pleurodesmospora* was established based on *Pleurodesmospora coccorum*, which is featured with rostella-like phialidic conidiogenous pegs pasted in erect or procumbent conidiophores (Samson and Gams 1980). *Pleurodesmospora* species are morphologically indistinguishable, emphasising the importance of molecular analysis. Based on DNA phylogeny, Chen et al. (2021a) reported that *Pleurodesmospora* belongs to Cordycipitaceae and demonstrated that the concatenated ITS-3P_*TEF* or ITS-*rpb1*-*rpb2*-3P_*TEF* datasets were reliable in studying the interspecific relationships of this genus (Chen et al. 2021a; Yeh et al. 2021). Members of *Pleurodesmospora* are known to infect various arthropods, including Araneidae, mites, leafhoppers, and whiteflies (Samson and Gams 1980; Yeh et al. 2021). To date, only five species of this genus have been described: *Pleurodesmospora coccorum*, *P. acaricola*, *P. lemaireae*, *P. lepidopterorum*, and *P. entomophila* (Samson and Gams 1980; Chen et al. 2021a; Yeh et al. 2021; Tan and Shivas 2023, 2024). *Pleurodesmospora acaricola*, *P. coccorum*, and *P. lepidopterorum* (Chen et al. 2021a; Yeh et al. 2021) were reported from China, and *P. lemaireae* and *P. entomophila* were found in Australia (Tan and Shivas 2023, 2024).

During the surveys of entomopathogenic fungi in Guizhou, Liaoning, and Yunnan Provinces, we have collected seven insect specimens (including six Lepidoptera and one Hymenoptera) that were infected by fungi. Based on morphology, five specimens were determined as isaria-like species, one as pleurodesmospora-like, and another one as akanthomyces-like. Further morphology studies herein and molecular phylogenetic analyses revealed four novel species belonging to *Pleurodesmospora* and *Samsoniella* and one known species of *Akanthomyces*. New findings not only enrich the species diversity of these genera but also deepen our understanding of their morphology and ecology.

Materials and methods

Sample collection and isolation

A survey was conducted to collect dead insect specimens with fungal infections from Guizhou, Liaoning, and Yunnan provinces (China) from July to November 2023. The specimens were collected from the lower and upper surfaces of living leaves and leaf litter on the ground in evergreen and deciduous forests with less sunlight. The fresh specimens were documented and photographed in the fields using a camera on a mobile phone. Collected specimens were placed in plastic boxes and transported to the laboratory for further examination.

To prevent contamination of fresh specimens by opportunistic fungi in the humid plastic box, fungus isolation was performed on the same day as it was collected. The fresh fruiting bodies were examined using a stereomicroscope (Olympus SZX16). A small mass of conidia on the synnemata or sclerotium inside the insect host bodies was transferred to axenic potato dextrose agar (PDA) plates using a sterile needle. The cultures were incubated at room temperature until the colonies' size attained 2−3 cm. The pure colonies were chopped into tiny bits and stored in sterile water in a centrifuge tube and then submitted to the Kunming Institute of Botany Culture Collection (KUNCC). The fresh specimens were dried with allochroic silica gel and deposited in the Herbarium of Cryptogamic Kunming Institute of Botany Academia Sinica (HKAS), Chinese Academy of Sciences, Kunming, China.

Morphological studies

The macro-characteristics of the fresh specimens, such as hosts, colour and shape of stroma, and the orientation of perithecia, were recorded and measured using a stereomicroscope (Leica S9E). Micro-morphological characteristics, such as perithecia, asci, ascospores, phialides, and conidia, were removed from the stromata or synnemata and mounted on a glass slide with water, lactic acid cotton blue or congo red solution. A Nikon compound microscope (Nikon ECLIPSE Ni) was used to photograph the above-mentioned microstructures. The axenic PDA plates isolated from fresh specimens were cultured at room temperature for 10−14 days, and the colony characteristics (e.g., size, shape, texture and colour) were recorded. Details of the asexual morphological characteristics from cultures were also documented with a Nikon compound microscope (Nikon ECLIPSE Ni).

DNA extraction and polymerase chain reaction (PCR) amplification

Total genomic DNA was extracted from axenic living cultures and dry specimens using the DNA extraction kit (Omega Fungus Genomic DNA Extraction Kit, China), following the instructions of the manufacturer. Ten loci, including the internal transcribed spacers 1 and 2 along with the 5.8S rDNA (ITS), partial region of the nuclear ribosomal small subunit (nrSSU) and large subunit (nrLSU), and the largest and second-largest subunits of RNA polymerase II (*rpb1* and *rpb2*), were amplified. Several extra gene regions, including the partial region of the 3' and the 5' end of the translation elongation factor 1-alpha gene (3P_*TEF* and 5P_*TEF*), the replication licensing factor 7 (*MCM7*) gene, the actin beta 1 (*ACT*) gene and the beta-tubulin (*TUB*) gene, were amplified for *Samsoniella* species (Table 2). The primer pairs used for amplification were ITS 5 and ITS 4 for ITS (White et al. 1990), NS1 and NS4 for nrSSU (White et al. 1990), LROR and LR5 for nrLSU (Vilgalys and Hester 1990), 983F and 2218R for 3P_*TEF* (Rehner and Buckley 2005), EF1T and EF2T for 5P_*TEF* (Rehner and Buckley 2005; Bischoff et al. 2006), CRPB1A and RPB1Cr for *rpb1* (Castlebury et al. 2004), fRPB2-5f and fRPB2-7cR for *rpb2* (Castlebury et al. 2004), Mcm7-709 and Mcm7-1348rev for *MCM7* (Schmitt et al. 2009), Act-1 and Act-4R for *ACT* (Voigt and Wöstemeyer 2000), Bt2a and Bt1b for *TUB* (Glass and Donaldson 1995). All of the PCR was performed in a 25 µl reaction mixture consisting of 12.5 µl of the mixture, 7.5 µl of double distilled water, 1 µl of each primer, and 3 µl of DNA template, using a T100 Thermal Cycler (Bio-Rad). The PCR program for these six loci (nrLSU, ITS, nrSSU, 3P_*TEF*, *rpb1*, and *rpb2*) was outlined in Wei et al. (2021), while the PCR procedures for the 5P_*TEF* and *MCM7* genes were respectively given by Bischoff et al. (2006) and Schmitt et al. (2009). The PCR protocols for the *ACT* and *TUB* were respectively referenced from Voigt et al. (1999) and Glass and Donaldson (1995). The PCR products were purified and sequenced at Sangon Biotech Company (Shanghai, China) with the above-mentioned primers. The newly generated sequences were submitted to GenBank for assignment of accession number.

Sequence alignment and phylogenetic analyses

The quality of the sequence chromatogram generated in this study was examined using BioEdit (Hall et al. 2011). The forward and reverse sequences were assembled using Seqman (Clewley 1995) and verified with those sequence data available in GenBank through the BLAST tool. Taxa used for phylogenetic analyses of Cordycipitaceae were selected following related articles (Chen et al. 2020c; Wang et al. 2020, 2024b) and BLAST research results of the newly generated sequences (Table 1).

In order to investigate the interspecific relationship among *Samsoniella*, a separated phylogenetic analysis based on combined four-gene (5P_*TEF*+3P_ *TEF*+*rpb1*+*MCM7*) was performed with a larger taxa sampling from this genus (Table 2). The four loci were independently aligned with reference sequences using MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/). The alignments of each locus were improved using Trimal v.1.2 (Capella-Gutiérrez et al. 2009) and were concatenated using Sequence Matrix v. 1.7.8 (Vaidya et al. 2011). The final combined dataset was converted to a NEXUS file for Bayesian inference analysis and a FASTA file for maximum likelihood analysis using Aliview (Larsson 2014).

Table 1. GenBank accession numbers of the taxa used in this study.

Species	strain	nrLSU	ITS	nrSSU	3P_*TEF*	*rpb1*	*rpb2*	References
Akanthomyces aculeatus	HUA186145[T]	MF416520			MF416465			Kepler et al. 2017
A. aculeatus	HUA 772	KC519370	KC519371	KC519368	KC519366			Kepler et al. 2017
A. australiensis	BRIP 72630a	OR527524	OR527516	OR512197	OR514840		OR514848	Kepler et al. 2017
A. baishanensis	CGMCC3.25673[T]	PP179404			PP464678	PP464641	PP464655	Pu et al. 2025
A. baishanensis	CGMCC3.25674	PP179405			PP464679	PP464642	PP464656	Pu et al. 2025
A. baishanensis	**HKAS144393**	**PQ492341**	**PQ492702**	**PQ492709**	**PQ499067**	**PQ499073**	**PQ499080**	**This study**
A. bannaensis	CLZhao 34016[T]	PP571897	PP571895				PP588774	Zhang et al. 2024
A. buriramensis	BCC 45158	ON008543			ON013546	ON013561		Khonsanit et al. 2024
A. buriramensis	BCC 47939[T]	ON008545			ON013548	ON013563		Khonsanit et al. 2024
A. fusiformis	BCC 40756[T]	ON008549			ON013552	ON013567	ON013576	Khonsanit et al. 2024
A. laosensis	YFCC 1910942	OQ509511	OQ509524		OQ506287	OQ511536	OQ511550	Wang et al. 2024b
A. laosensis	YFCC 1910941[T]	OQ509510	OQ509523		OQ506286	OQ511535	OQ511549	Wang et al. 2024b
A. niveus	BCC 79887[T]	ON008551			ON013554		ON013578	Khonsanit et al. 2024
A. niveus	BCC 40747	ON008550			ON013553	ON013568	ON013577	Khonsanit et al. 2024
A. noctuidarum	BBH 16595	MT356085	MT356073		MT477979	MT477995	MT478005	Aini et al. 2020
A. noctuidarum	BCC 47498	MT356086	MT356074		MT477980	MT477996	MT477988	Aini et al. 2020
A. noctuidarum	BCC 28571	MT356087	MT356075		MT477981	MT478009	MT478006	Aini et al. 2020
A. noctuidarum	BCC 36265[T]	MT356084	MT356072		MT477978	MT477994	MT477987	Aini et al. 2020
A. phariformis	BCC 45148[T]	ON008556			ON013559		ON013583	Khonsanit et al. 2024
A. pseudonoctuidarum	YFCC 1808943[T]	OQ509512	OQ509525		OQ506288	OQ511537	OQ511551	Khonsanit et al. 2024
A. pseudonoctuidarum	YFCC 1808944	OQ509513	OQ509526		OQ506289	OQ511538	OQ511552	Khonsanit et al. 2024
A. pyralidarum	BCC 32191	MT356092	MT356081		MT477983	MT478001	MT477989	Aini et al. 2020
A. pyralidarum	BCC 40869	MT356093	MT356082		MT477984	MT478002	MT477990	Aini et al. 2020
A. pyralidarum	BCC 28816[T]	MT356091	MT356080		MT477982	MT478000	MT478007	Aini et al. 2020
Akanthomyces sp.	BCC 76537	ON008557	ON006550		ON013560		ON013584	Aini et al. 2020
A. taiwanicus	NTUPPMCC 20-060	MT974356	MT974202		MW200213	MW200221	MW200230	Chuang et al. 2024
A. tortricidarum	BCC 28583	MT356090	MT356079		MT477986	MT477999	MT477993	Aini et al. 2020
A. tortricidarum	BCC 41868	MT356089	MT356077		MT477985	MT477998	MT478008	Aini et al. 2020
A. tortricidarum	BCC 72638[T]	MT356088	MT356076		MT478004	MT477997	MT477992	Aini et al. 2020
A. tuberculatus	BCC 16819	GQ249987	GQ250012	GQ249962	GQ250037			Kepler et al. 2017
A. xixiuensis	XX21081764[T]	OP693480	OP693460	OP693478	OP838887	OP838889	OP838891	Liu et al. 2024
A. xixiuensis	HKAS125851	OP693481	OP693461	OP693479	OP838888	OP838890	OP838892	Liu et al. 2024
Arachnidicola araneicola	GY 29011		MK942435			MK955945	MK955948	Chen et al. 2019
Ara. araneogenus	GZUIF DX1		KU893152			MH978181	MH978184	Chen et al. 2018
Ara. bashanensis	CQ 05621[T]	OQ300420	OQ300412		OQ325024		OQ349684	Chen et al. 2023a
Ara. bashanensis	CQ 05622	OQ300421	OQ300411		OQ325025		OQ349685	Chen et al. 2023a
Ara. beibeiensis	CQ 05921[T]	OQ300424	OQ300415		OQ325028		OQ349688	Chen et al. 2023a
Ara. beibeiensis	CQ 05922	OQ300427	OQ300416		OQ325029		OQ349689	Chen et al. 2023a
Ara. coccidioperitheciatus	NHJ 6709	EU369042	JN049865	EU369110	EU369025	EU369067	EU369086	Kepler et al. 2017
Ara. kanyawimiae	TBRC 7242	MF140718	MF140751		MF140838	MF140784	MF140808	Mongkolsamrit et al. 2018
Ara. kanyawimiae	TBRC 7244[T]	MF140716	MF140752		MF140836			Mongkolsamrit et al. 2018
Ara. kanyawimiae	TBRC 7243	MF140717	MF140750		MF140837	MF140783	MF140807	Mongkolsamrit et al. 2018
Ara. kunmingensis	YFCC 1808940[T]	OQ509509	OQ509522		OQ506285	OQ511534	OQ511548	Wang et al. 2024b
Ara. kunmingensis	YFCC 1808939	OQ509508	OQ509521		OQ506284	OQ511533	OQ511547	Wang et al. 2024b
Ara. subaraneicola	YFCC 2107937[T]	OQ509514	OQ509527		OQ506290	OQ511539	OQ511553	Wang et al. 2024b

Species	strain	nrLSU	ITS	nrSSU	3P_TEF	rpb1	rpb2	References
Ara. subaraneicola	YFCC 2107938	OQ509515	OQ509528		OQ506291	OQ511540	OQ511554	Wang et al. 2024b
Ara. sulphureus	TBRC 7248[T]	MF140722	MF140758		MF140843	MF140787	MF140812	Mongkolsamrit et al. 2018
Ara. thailandicus	TBRC 7245[T]	MF140719	MF140754		MF140839		MF140809	Mongkolsamrit et al. 2018
Ara. tiankengensis	KY 11571[T]	ON502825	ON502848		ON525447		ON525446	Chen et al. 2023a
Ara. tiankengensis	KY 11572	ON502827	ON502821		ON525449		ON525448	Chen et al. 2023a
Ara. waltergamsii	TBRC 7252[T]	MF140714	MF140748		MF140834	MF140782	MF140806	Mongkolsamrit et al. 2018
Beauveria bassiana	ARSEF 1564		HQ880761		HQ880974	HQ880833	HQ880905	Rehner et al. 2011
B. caledonica	ARSEF 2567[T]	AF339520	HQ880817	NG064865	EF469057	EF469086	HQ880961	Rehner et al. 2011
B. medogensis	BUB 426	MG642846	MG642832	MG642889	MG642904	MG642859	MG642874	Imoulan et al. 2016
B. scarabaeidicola	ARSEF 5689	AF339524	JN049827	AF339574	DQ522335	DQ522380	DQ522431	Kepler et al. 2017
B. sinensis	BUB 504	MG642838	MG642825	MG642880	MG642895	MG642852	MG642865	Chen et al. 2013
Cordyceps amoene-rosea	CBS 107.73[T]	MF416550	MH860646	AY526464	MF416494	MF416651	MF416445	Wang et al. 2020
C. amoene-rosea	CBS 729.73	MF416551	MH860794	MF416604	MF416495	MF416652	MF416446	Wang et al. 2020
C. coleopterorum	CBS 110.73[T]	JF415988	AY624177	JF415965	JF416028	JN049903	JF416006	Kepler et al. 2017
C. farinosa	CBS 111113	MF416554	AY624181	AY526474	MF416499	MF416656	MF416450	Kepler et al. 2017
C. fumosorosea	CBS 244.31	MF416557	MH855200	MF416609	MF416503	MF416660	MF416454	Kepler et al. 2017
C. javanica	CBS 134.22	MF416558	MH854719	MF416610	MF416504	MF416661	MF416455	Kepler et al. 2017
C. militaris	OSC 93623	AY184966	JN049825	AY184977	DQ522332	DQ522377		Kepler et al. 2017
C. tenuipes	ARSEF 5135	JF415980	AY624196	MF416612	JF416020	JN049896	JF416000	Kepler et al. 2017
Kanoksria zaquensis	HMAS 246917	MT789696	MT789698	MT789700	MT797811	MT797809		Wang et al. 2023b
Kanoksria zaquensis	HMAS 246915[T]	MT789697	MT789699	MT789701	MT797812	MT797810		Wang et al. 2023b
Lecanicillium araneosus	KY 11341[T]	ON502832	ON502826		ON525443		ON525442	Chen et al. 2022
L. araneosus	KY 11342	ON502837	ON502844		ON525445		ON525444	Chen et al. 2022
L. attenuatus	CBS 402.78	AF339565	AJ292434	AF339614	EF468782	EF468888	EF468935	Kepler et al. 2017
L. lecanii	CBS 102067[T]	KM283795	MH862778	KM283771	KM283818	KM283838	KM283860	Kepler et al. 2017
L. lepidopterorum	SD05152		MT705974				MT727045	Chen et al. 2020a
L. longisporum	CBS 126.27[T]	KM283797	AJ292385		KM283820	KR064300	KM283862	Kepler et al. 2017
L. muscarius	MFLU 181145	MH497224	MH497223	MH497222	MH511807		MH511806	Kepler et al. 2017
L. neoaraneogenus	GZU1031Lea[T]			KX845705	KX845697	KX845699	KX845701	Shrestha et al. 2019
L. neocoleopterorum	GY11242		MN093297		MN097815	MN097817	MN097814	Shrestha et al. 2019
L. pissodis	CBS 118231[T]	KM283799		KM283775	KM283822	KM283842	KM283864	Chen et al. 2020a
L. sabanensis	JCh041			KC633263	KC633274			Kepler et al. 2017
Lecanicillium sp.	YFCC 945		OQ509531		OQ506294	OQ511543	OQ511557	Wang et al. 2024b
L. uredinophilum	KACC 44082[T]	KM283782		KM283758	KM283806	KM283828	KM283848	Wang et al. 2020
L. uredinophilum	KUN 101466	MG948307	MG948305	MG948309	MG948315	MG948311	MG948313	Wang et al. 2020
Pleurodesmospora acaricola	R. Kirschner 4968		MZ435417		LC629776			Yeh et al. 2021
P. coccorum	CBS 460.73	MH872455	MH860743					Yeh et al. 2021
P. entomophila	BRIP 72652a[T]	OR527526	OR527518		OR514842		OR514850	Tan and Shivas 2023
P. lemaireae	BRIP 76543a[T]	PQ792647	PQ806958					Tan and Shivas 2024
P. lepidopterorum	DY10502		MW826577		MW834319		MW834318	Chen et al. 2021a
P. lepidopterorum	DY10501[T]		MW826576		MW834317	MW834315	MW834316	Chen et al. 2021a
P. sanduensis	**HKAS144399[T]**	**PQ492342**	**PQ492703**	**PQ492710**	**PQ499068**	**PQ499074**	**PQ499081**	**This study**
Samsoniella alboaurantium	CBS 262.58[T]	MG665232	AY624179		JQ425685			Mongkolsamrit et al. 2018
S. alboaurantium	CBS 240.32	JF415979	AY624178		JF416019	JN049895	JF415999	Mongkolsamrit et al. 2018
S. alpina	YFCC 5818	MN576809		MN576753	MN576979	MN576869	MN576923	Wang et al. 2020
S. alpina	YFCC 5831	MN576810		MN576754	MN576980	MN576870	MN576924	Wang et al. 2020

Species	strain	nrLSU	ITS	nrSSU	3P_TEF	rpb1	rpb2	References
S. anhuiensis	RCEF2830[T]	OM268848		OM268843	OM483864	OM751889		Wang et al. 2024a
S. anhuiensis	RCEF2590	OR978316		OR978313	OR966516	OR989964		Wang et al. 2024a
S. antleroides	YFCC 6113	MN576804		MN576748	MN576974	MN576864	MN576918	Wang et al. 2020
S. antleroides	YFCC 6016[T]	MN576803		MN576747	MN576973	MN576863	MN576917	Wang et al. 2020
S. aranea	RCEF2831	OM268849		OM268844	OM483865	OM751882	OM802500	Wang et al. 2024a
S. aranea	RCEF2868	OM268850		OM268845	OM483866	OM751883	OM802501	Wang et al. 2024a
S. asiatica	YFCC 869[T]		OQ476473		OQ506153	OQ506195	OQ506187	Wang et al. 2023a
S. asiatica	YFCC 870		OQ476474		OQ506154	OQ506196	OQ506188	Wang et al. 2023a
S. asiatica	YFCC 871		OQ476475		OQ506155	OQ506197	OQ506189	Wang et al. 2023a
S. aurantia	TBRC 7271	MF140728	MF140764		MF140846	MF140791	MF140818	Mongkolsamrit et al. 2018
S. aurantia	TBRC 7272	MF140727	MF140763		MF140845		MF140817	Mongkolsamrit et al. 2018
S. cardinalis	YFCC 5830	MN576788		MN576732	MN576958	MN576848	MN576902	Wang et al. 2020
S. cardinalis	YFCC 6144[T]	MN576786		MN576730	MN576956	MN576846	MN576900	Wang et al. 2020
S. coccinellidicola	YFCC 8772[T]	ON621670		ON563166	ON676514	ON676502	ON568685	Wang et al. 2022
S. coccinellidicola	YFCC 8773	ON621671		ON563167	ON676515	ON676503	ON568686	Wang et al. 2022
S. coleopterorum	A19501[T]		MT626376		MN101586	MT642600	MN101585	Chen et al. 2020c
S. cristata	YFCC 6023	MN576792	OQ476480	MN576736	MN576962	MN576852	MN576906	Wang et al. 2020
S. cristata	YFCC 7004[T]	MN576793	OQ476481	MN576737	MN576963	MN576853	MN576907	Wang et al. 2020
S. duyunensis	DY09162	OQ363114	OQ379242		OQ398146			Chen et al. 2023b
S. duyunensis	DY07501	OR263307	OR263188		OR282780	OR282773	OR282776	Chen et al. 2023b
S. duyunensis	DY09502	OR263427	OR263189		OR282781		OR282777	Chen et al. 2023b
S. erucae	KY 11121[T]	ON502835	ON502828		ON525425		ON525424	Chen et al. 2022
S. erucae	KY 11122	ON502822	ON502847		ON525427		ON525426	Chen et al. 2022
S. farinospora	YFCC 8774[T]	ON621672		ON563168	ON676516	ON676504	ON568687	Wang et al. 2022
S. farinospora	YFCC 9051	ON621673		ON563169	ON676517	ON676505	ON568688	Wang et al. 2022
S. fusiformispora	RCEF5406	OM268851		OM268846		OM751890		Wang et al. 2024a
S. fusiformispora	RCEF2588[T]	OR978315		OR978312				Wang et al. 2024a
S. guizhouensis	KY 11161[T]	ON502830	ON502823		ON525429		ON525428	Chen et al. 2022
S. guizhouensis	KY 11162	ON502846	ON502845		ON525431		ON525430	Chen et al. 2022
S. haniana	YFCC 8769[T]	ON621674		ON563170	ON676518	ON676506	ON568689	Wang et al. 2022
S. haniana	YFCC 8770	ON621675		ON563171	ON676519	ON676507	ON568690	Wang et al. 2022
S. haniana	YFCC 8771	ON621676		ON563172	ON676520	ON676508	ON568691	Wang et al. 2022
S. hepiali	Cor-4	MN576799		MN576743	MN576969	MN576859	MN576913	Wang et al. 2020
S. hepiali	YFCC 661	MN576795		MN576739	MN576965	MN576855	MN576909	Wang et al. 2020
S. hepiali	ICMM 82-2[T]	MN576794		MN576738	MN576964	MN576854	MN576908	Wang et al. 2020
S. hymenopterorum	A19521		MN128224		MN101588	MT642603		Chen et al. 2020c
S. hymenopterorum	A19522[T]		MN128081		MN101591	MN101589		Chen et al. 2020c
S. inthanonensis	TBRC 7915	MF140725	MF140761		MF140849	MF140790	MF140815	Mongkolsamrit et al. 2018
S. kunmingensis	YHH 16002[T]	MN576802		MN576746	MN576972	MN576862	MN576916	Wang et al. 2020
S. lanmaoa	YFCC 6193	MN576790		MN576734	MN576960	MN576850	MN576904	Wang et al. 2020
S. lanmaoa	YFCC 6148[T]	MN576789		MN576733	MN576959	MN576849	MN576903	Wang et al. 2020
S. lasiocampidarum	NTUPPMCC 20-061	MT974364	MT974211		MW200220	MW200229		Chuang et al. 2024
S. lasiocampidarum	NTUPPMCC 20-062[T]	MT974361	MT974208		MW200218	MW200227	MW200236	Chuang et al. 2024
S. lasiocampidarum	NTUPPMCC 20-063	MT974363	MT974210		MW200219		MW200238	Chuang et al. 2024
S. lepidopterorum	DL 10071[T]		MN128076			MN101592		Chen et al. 2020c
S. lepidopterorum	DL 10072		MN128084					Chen et al. 2020c
S. lurida	**HKAS144387[T]**	**PQ492339**	**PQ492700**	**PQ492707**	**PQ499065**		**PQ499078**	**This study**
S. lurida	**HKAS144388**	**PQ492340**	**PQ492701**	**PQ492708**	**PQ499066**	**PQ499072**	**PQ499079**	**This study**

Species	strain	nrLSU	ITS	nrSSU	3P_TEF	rpb1	rpb2	References
S. neopupicola	KY 11322	ON502833	ON502834		ON525435		ON525434	Chen et al. 2022
S. neopupicola	KY 11321ᵀ	ON502839	ON502843		ON525433		ON525432	Chen et al. 2022
S. pseudogunnii	GY 407202	MZ831865	MZ831863		MZ855234		MZ855240	Chen et al. 2021b
S. pseudogunnii	GY 407201	MZ827010	MZ827470		MZ855233		MZ855239	Chen et al. 2021b
S. pseudotortricidae	YFCC 9052ᵀ	ON621677		ON563173	ON676521	ON676509	ON568692	Wang et al. 2022
S. pseudotortricidae	YFCC 9053	ON621678		ON563174	ON676522	ON676510	ON568693	Wang et al. 2022
S. pupicola	DY 101682	MZ827635	MZ827008		MZ855232		MZ855238	Chen et al. 2021b
S. pupicola	DY 101681ᵀ	MZ827009	MZ827085		MZ855231		MZ855237	Chen et al. 2021b
S. ramosa	YFCC 6020ᵀ	MN576805		MN576749	MN576975	MN576865	MN576919	Wang et al. 2020
S. sanmingense	CGMCC3.25661	PP179392		PP177395	PP482033	PP464664	PP464647	Pu et al. 2025
S. sanmingense	CGMCC3.25662 ᵀ	PP179393		PP177396	PP482034	PP464665	PP464648	Pu et al. 2025
S. sapaensis	YFCC 873ᵀ		OQ476489		OQ506152	OQ506194	OQ506186	Wang et al. 2023a
S. sapaensis	YFCC 872		OQ476488		OQ506151	OQ506193	OQ506185	Wang et al. 2023a
S. sinensis	YFCC 8766ᵀ	ON621679		ON563175	ON676523	ON676511	ON568694	Wang et al. 2022
S. sinensis	YFCC 8767	ON621680		ON563176	ON676524	ON676512	ON568695	Wang et al. 2022
S. sinensis	YFCC 8768	ON621681		ON563177	ON676525	ON676513	ON568696	Wang et al. 2022
S. subasiatica	**HKAS144400ᵀ**	**PQ492343**	**PQ492704**	**PQ492711**	**PQ499069**	**PQ499075**	**PQ499082**	**This study**
S. tiankengensis	KY 11741ᵀ	ON502838	ON502840		ON525437		ON525436	Chen et al. 2022
S. tiankengensis	KY 11742	ON502841	ON502849		ON525439		ON525438	Chen et al. 2022
S. tortricidae	YFCC 6013	MN576807		MN576751	MN576977	MN576867	MN576921	Wang et al. 2020
S. tortricidae	YFCC 6142	MN576808		MN576752	MN576978	MN576868	MN576922	Wang et al. 2020
S. tortricidae	YFCC 6131ᵀ	MN576806		MN576750	MN576976	MN576866	MN576920	Wang et al. 2020
S. torquatistipitata	**HKAS144411ᵀ**	**PQ492345**	**PQ492706**	**PQ492713**	**PQ499071**	**PQ499077**	**PQ499084**	**This study**
S. torquatistipitata	**HKAS144402**	**PQ492344**	**PQ492705**	**PQ492712**	**PQ499070**	**PQ499076**	**PQ499083**	**This study**
S. vallis	DY091092	OR263431	OR263190		OR282783			Chen et al. 2023b
S. vallis	DY091091	OR263428	OR263191		OR282782			Chen et al. 2023b
S. vallis	DY07242	OR263308	OR263186		OR282779		OR282775	Chen et al. 2023b
S. vallis	DY07241ᵀ	OR263306	OR263159		OR282778	OR282772	OR282774	Chen et al. 2023b
S. winandae	MY12469.01ᵀ	OM491231	OM491228		OM687896	OM687901	OM687899	Crous et al. 2023b
S. yuanzuiensis	NTUPPMCC 20-064ᵀ	MT974359	MT974206			MW200225	MW200234	Chuang et al. 2024
S. yuanzuiensis	NTUPPMCC 20-065	MT974360	MT974207		MW200217	MW200226	MW200235	Chuang et al. 2024
S. yunnanensis	YFCC 1527ᵀ	MN576812		MN576756	MN576982	MN576872	MN576926	Wang et al. 2020
S. yunnanensis	YFCC 1824	MN576813		MN576757	MN576983	MN576873	MN576927	Wang et al. 2020
S. yunnanensis	YFCC 7282	MN576814		MN576758	MN576984	MN576874	MN576928	Wang et al. 2020
Simplicillium lanosoniveum	CBS 101267	AF339554	AJ292395		DQ522357	DQ522405	DQ522463	Spatafora et al. 2007
Sim. lanosoniveum	CBS 704.86	AF339553			DQ522358	DQ522406	DQ522464	Spatafora et al. 2007

Note: Types are indicated by T. The newly generated sequences in this study were shown in bold.

Maximum likelihood (ML) analysis was performed using IQ-TREE 1.6.12 (Minh et al. 2020) with branch support being estimated from 1000 ultrafast bootstraps. The Bayesian inference (BI) analysis was run on MrBayes on XSEDE (3.2.7a) in the CIPRES Science Gateway. The GTR+I+G model was selected as the best-fit substitution model by MrModeltest 2.3 implemented in MrMTgui v.1.0 (Nylander 2004; Nuin 2007). Four simultaneous Markov chains were run for 100,000,000 generations, and trees were sampled every 1000 generations. Finally, phylogenetic trees were visualised using Figtree v.1.4.0 (Rambaut 2016) and edited using Adobe Illustrator 2020.

Table 2. GenBank accession numbers of the *Samsoniella* used in this study.

Species	strain	3P_*TEF*	5P_*TEF*	*rpb1*	*MCM7*	References
Samsoniella alboaurantium	CBS 240.32	JF416019		JN049895		Mongkolsamrit et al. 2018
S. alboaurantium	CBS 262.58[T]	MF416497		MF416654		Mongkolsamrit et al. 2018
S. alpina	YFCC 5818[T]	MN576979	OQ506160	MN576869	OQ506229	Wang et al. 2023a
S. alpina	YFCC 5831	MN576980	OQ506161	MN576870	OQ506230	Wang et al. 2023a
S. antleroides	YFCC 6016[T]	MN576973	OQ506162	MN576863	OQ506231	Wang et al. 2023a
S. antleroides	YFCC 6113	MN576974	OQ506163	MN576864	OQ506232	Wang et al. 2023a
S. anhuiensis	RCEF2830[T]	OM483864		OM751889		Wang et al. 2024a
S. anhuiensis	RCEF2590	OR966516		OR989964		Wang et al. 2024a
S. aranea	RCEF2831	OM483865		OM751882		Wang et al. 2024a
S. aranea	RCEF2868	OM483866		OM751883		Wang et al. 2024a
S. asiatica	YFCC 869[T]	OQ506153	OQ506164	OQ506195	OQ506233	Wang et al. 2023a
S. asiatica	YFCC 870	OQ506154	OQ506165	OQ506196	OQ506234	Wang et al. 2023a
S. asiatica	YFCC 871	OQ506155	OQ506166	OQ506197	OQ506235	Wang et al. 2023a
S. aurantia	TBRC 7271[T]	MF140846		MF140791		Mongkolsamrit et al. 2018
S. aurantia	YFCC 874	OQ506157	OQ506167	OQ506199	OQ506236	Wang et al. 2023a
S. aurantia	YFCC 880	OQ506156	OQ506168	OQ506198	OQ506237	Wang et al. 2023a
S. cardinalis	YFCC 5830	MN576958	OQ506169	MN576848	OQ506238	Wang et al. 2023a
S. cardinalis	YFCC 6144[T]	MN576956	OQ506170	MN576846	OQ506239	Wang et al. 2023a
S. coccinellidicola	YFCC 8772[T]	ON676514		ON676502		Wang et al. 2022
S. coccinellidicola	YFCC 8773	ON676515		ON676503		Wang et al. 2022
S. coleopterorum	A19501[T]	MN101586		MT642600		Chen et al. 2020c
S. cristata	YFCC 6023	MN576962	OQ506171	MN576852	OQ506240	Wang et al. 2023a
S. cristata	YFCC 7004[T]	MN576963	OQ506172	MN576853	OQ506241	Wang et al. 2023a
S. duyunensis	DY09162	OQ398146				Chen et al. 2023b
S. duyunensis	DY07501	OR282780		OR282773		Chen et al. 2023b
S. duyunensis	DY09502	OR282781				Chen et al. 2023b
S. erucae	KY11121[T]	ON525425				Chen et al. 2022
S. erucae	KY11122	ON525427				Chen et al. 2022
S. farinospora	YFCC 8774[T]	ON676516		ON676504		Wang et al. 2022
S. farinospora	YFCC 9051	ON676517		ON676505		Wang et al. 2022
S. fusiformispora	RCEF5406			OM751890		Wang et al. 2024a
S. guizhouensis	KY11161[T]	ON525429				Chen et al. 2022
S. guizhouensis	KY11162	ON525431				Chen et al. 2022
S. haniana	YFCC 8769[T]	ON676518		ON676506		Wang et al. 2022
S. haniana	YFCC 8771	ON676520		ON676508		Wang et al. 2022
S. hepiali	ICMM 82-2[T]	MN576964	OQ506173	MN576854	OQ506242	Wang et al. 2023a
S. hepiali	YFCC 868	OQ506158	OQ506175	OQ506200	OQ506244	Wang et al. 2023a
S. hepiali	YFCC 2702	MN576966	OQ506174	MN576856	OQ506243	Wang et al. 2023a
S. hymenopterorum	A19521	MN101588		MT642603		Chen et al. 2020c
S. hymenopterorum	A19522[T]	MN101591		MN101589		Chen et al. 2020c
S. inthanonensis	TBRC 7915[T]	MF140849		MF140790		Mongkolsamrit et al. 2018
S. kunmingensis	YHH 16002[T]	MN576972		MN576862		Wang et al. 2023a
S. lanmaoa	YFCC 6148[T]	MN576959	OQ506176	MN576849	OQ506245	Wang et al. 2023a
S. lanmaoa	YFCC 6193	MN576960	OQ506177	MN576850	OQ506246	Wang et al. 2023a
S. lasiocampidarum	NTUPPMCC 20-061	MW200220		MW200229		Chuang et al. 2024
S. lasiocampidarum	NTUPPMCC 20-062[T]	MW200218		MW200227		Chuang et al. 2024
S. lasiocampidarum	NTUPPMCC 20-063	MW200219				Chuang et al. 2024

| Species	strain	3P_*TEF*	5P_*TEF*	*rpb1*	*MCM7*	References
S. lepidopterorum	DL 10071[T]			MN101592		Chen et al. 2020c
S. lurida	**HKAS144387[T]**	**PQ499065**				**This study**
S. lurida	**HKAS144388**	**PQ499066**		**PQ499072**	**PV158406**	**This study**
S. neopupicola	KY11321[T]	ON525433				Chen et al. 2022
S. neopupicola	KY11322	ON525435				Chen et al. 2022
S. pseudogunii	GY407201[T]	MZ855233				Chen et al. 2021b
S. pseudogunii	GY407202	MZ855234				Chen et al. 2021b
S. pseudotortricidae	YFCC 9052[T]	ON676521		ON676509		Wang et al. 2022
S. pseudotortricidae	YFCC 9053	ON676522		ON676510		Wang et al. 2022
S. pupicola	DY101681[T]	MZ855231				Chen et al. 2021b
S. pupicola	DY101682	MZ855232				Chen et al. 2021b
S. ramosa	YFCC 6020[T]	MN576975	OQ506178	MN576865		Wang et al. 2023a
S. sanmingense	CGMCC3.25661	PP482033		PP464664		Pu et al. 2025
S. sanmingense	CGMCC3.25662	PP482034		PP464665		Pu et al. 2025
S. sapaensis	YFCC 872	OQ506151	OQ506179	OQ506193	OQ506247	Wang et al. 2023a
S. sapaensis	YFCC 873[T]	OQ506152	OQ506180	OQ506194	OQ506248	Wang et al. 2023a
S. sinensis	YFCC 8766[T]	ON676523		ON676511		Wang et al. 2022
S. sinensis	YFCC 8767	ON676524		ON676512		Wang et al. 2022
S. subasiatica	**HKAS144400[T]**	**PQ499069**	**PV158402**	**PQ499075**	**PV158407**	**This study**
S. tiankengensis	KY11741[T]	ON525437				Chen et al. 2022
S. tiankengensis	KY11742	ON525439				Chen et al. 2022
S. tortricidae	YFCC 6131[T]	MN576976	OQ506181	MN576866	OQ506249	Wang et al. 2023a
S. tortricidae	YFCC 6142	MN576978	OQ506182	MN576868	OQ506250	Wang et al. 2023a
S. torquatistipitata	**HKAS144411[T]**	**PQ499071**		**PQ499077**	**PV158408**	**This study**
S. torquatistipitata	**HKAS144402**	**PQ499070**		**PQ499076**	**PV158409**	**This study**
S. vallis	DY091092	OR282783				Chen et al. 2023b
S. vallis	DY091091	OR282782				Chen et al. 2023b
S. vallis	DY07242	OR282779				Chen et al. 2023b
S. vallis	DY07241[T]	OR282778		OR282772		Chen et al. 2023b
S. winandae	MY12469.01[T]	OM687896		OM687901		Crous et al. 2023b
S. yuanzuiensis	NTUPPMCC 20-064[T]			MW200225		Chuang et al. 2024
S. yuanzuiensis	NTUPPMCC 20-065	MW200217		MW200226		Chuang et al. 2024
S. yunnanensis	YFCC 1527[T]	MN576982	OQ506183	MN576872	OQ506251	Wang et al. 2020, 2023a
S. yunnanensis	YFCC 1824	MN576983	OQ506184	MN576873	OQ506252	Wang et al. 2020, 2023a
Akanthomyces waltergamsii	YFCC 883	OQ506159		OQ506201	OQ506253	Wang et al. 2023a

Note: Types are indicated by T. The newly generated sequences in this study were shown in bold.

Results

Phylogenetic analyses

The six-locus dataset (nrLSU, ITS, nrSSU, *3P_TEF*, *rpb1*, and *rpb2*) comprises 118 representative taxa sampled from nine genera within Cordycipitaceae, with two strains of *Simplicillium lanosoniveum* (CBS 101267 and CBS 704.86) selected as the outgroup. The ML tree inferred from the six-locus dataset is shown in Fig. 1, in which the seven strains generated in this study belong to three genera: *Akanthomyces*, *Pleurodesmospora* and *Samsoniella*. The isolate HKAS144393 clusters with *Akanthomyces baishanensis* (CGMCC3.25673 and CGMCC3.25674) with strong statistical support (100% SH-aLRT / 100% UFB / 1.00 PP, Fig. 1). The isolate HKAS144399 constitutes a distinct lineage which
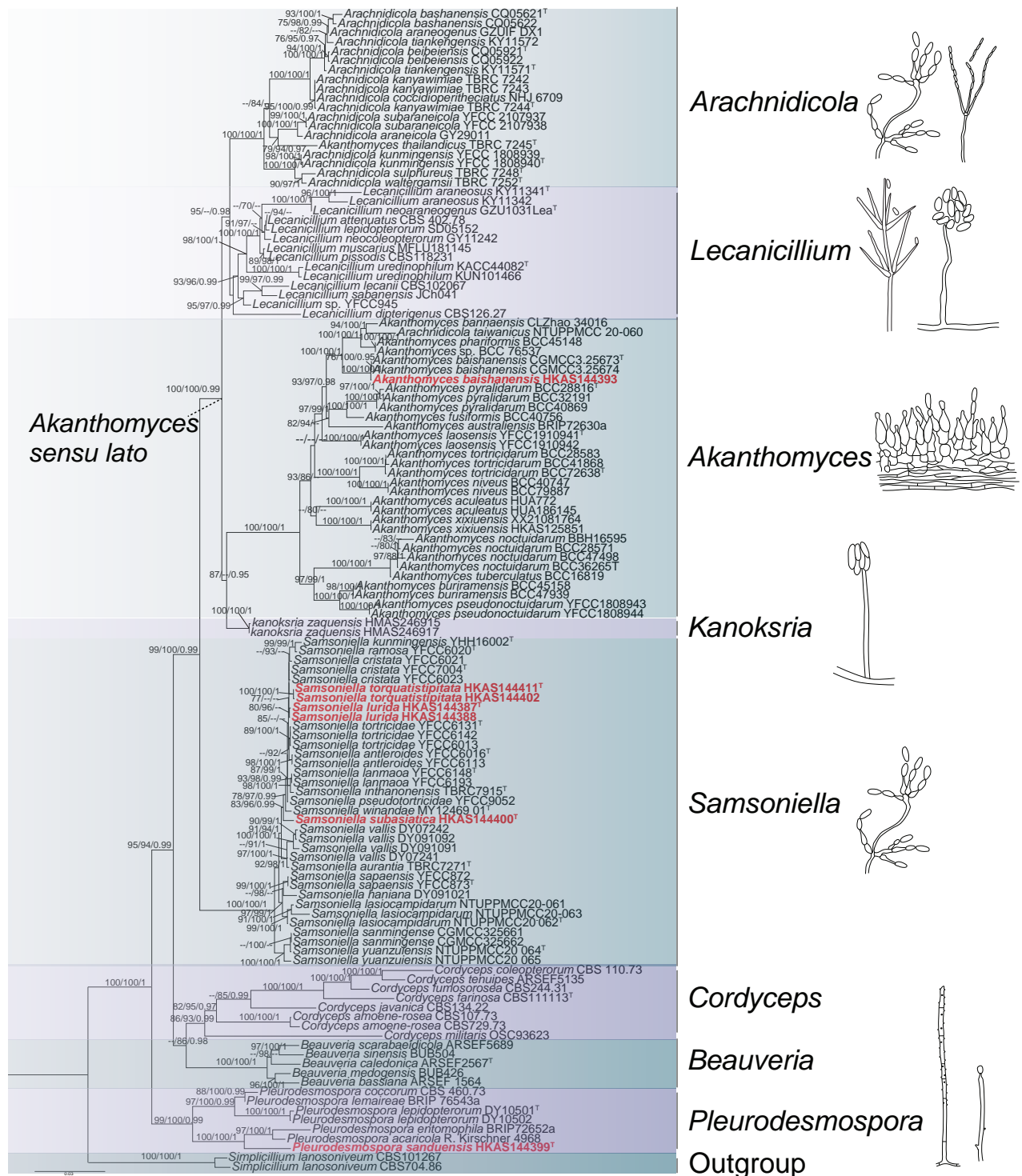
Figure 1. Phylogram generated from maximum likelihood analysis of Cordycipitaceae based on a six-locus dataset (nrLSU, ITS, nrSSU, 3P_*TEF*, *rpb1* and *rpb2*). SH-aLRT support ≥ 75%, ultrafast bootstrap support (UFB) ≥ 75%, and PP values ≥ 95% are indicated above or below branches. A hyphen (−) indicates values lower than 75% SH-aLRT, 75% UFB, and 95% PP. The isolates in this study are shown in bold red. Generic names are indicated on the right side of the tree. Ex-types are indicated by "T".

branches off the clade of *Pleurodesmospora acaricola* and *P. entomophila* with maximum support (100% SH-aLRT / 100% UFB / 1.00 PP, Fig. 1). The remaining five strains (HKAS144411, HKAS144402, HKAS144388, HKAS144402, and HKAS144400) group with species of *Samsoniella* with inadequate support.

To clarify the phylogenetic placements of the five specimens of *Samsoniella*, a separated phylogenetic tree based on four genes (5P_*TEF*+3P_*TEF*+*rpb1*+*MCM7*) was constructed with larger taxa sampling from *Samsoniella*. The four-locus dataset included 79 taxa of *Samsoniella* with 3077 bp characters (737 bp for 5P_*TEF*, 986 bp for nrSSU, 725 bp for 3P_*TEF*, 629 bp for *rpb1*). *Akanthomyces waltergamsii* YFCC 883 was designated as the out-group taxon. The ML tree (Fig. 2) shows that the isolates HKAS144387 and HKAS144388 are sisters to *S. kunmingensis* and are closely related to *S. tortricidae*, with moderate support (86% SH-aLRT / 89% UFB, Fig. 2). The isolate HKAS144400 shows a sister relationship to *Samsoniella winandae* with significant support (89% SH-aLRT / 94% UFB / 0.99 PP, Fig. 2). The isolates HKAS144411 and HKAS144402 were placed in a clade distantly related to other *Samsoniella* species with strong support (98% SH-aLRT / 100% UFB / 1.00 PP, Fig. 2). The guidelines of Maharachchikumbura et al. (2021) were followed when determining whether species were novel.

Taxonomy

Akanthomyces baishanensis **H.L. Pu & J.Z. Qiu, in Pu, Yang, Keyhani, Yang, Zheng, Qiu, Mao, Shang, Lin, Xiong, Lin, Lai, Huang, Yuan, Liang, Fan, Ma, Qiu & Qiu, J. Fungi 11(1, no. 28): 16 (2025)**
Index Fungorum: IF903210
Fig. 3

Description. Parasitic on moth (Lepidoptera). **Sexual morph.** See Pu et al. (2025). **Asexual morph.** *Synnemata* arising from the moth body, white, erect, simple, subuliform (2 × 2.7 mm) or subglobose (0.2 × 0.5 mm). *Hyphae* smooth, septate, hyaline, 1.4−2.5 μm ($\overline{x}$ = 1.8 μm, n = 30) in diam. *Conidiophores* developing from superficial hyphae of synnemata, micronematous, branched, smooth-walled, bearing solitary to clusters of phialides. *Phialides* 6−29.6 × 1.6−3.2 μm ($\overline{x}$ = 19 × 2.7 μm, n = 30), monophialidic, trimorphic, arising from anastomosing mycelia, slender filiform in shape (Fig. 3G), or arising from conidiophores, cylindrical (Fig. 3E, H, I) or subuliform (Fig. 3F) at basal portion, tapering into a thin neck. *Conidia* 3.2−4.7 × 1.8−2.8 μm ($\overline{x}$ = 3.9 × 2.2 μm, n = 50), forming on tip of phialides, hyaline, smooth-walled, fusiform, globose or broadly ovoid, gathering in chains.

Material examined. CHINA • Liaoning Province, Tieling City (42°17'22.3"N, 123°50'22.2"E), on a dead adult moth (Lepidoptera) on the stem of a plant, 25 August 2023, Ting-Chi Wen, HLJ2023082515 (HKAS144393).

Notes. Phylogenetic analysis based on six gene markers revealed that the specimen HKAS144393 and *Akanthomyces baishanensis* (CGMCC3.25673 and CGMCC3.25674) form a robustly supported monophyletic clade (100% SH-aLRT / 100% UFB / 1.00 PP, Fig. 1). Both HKAS144393 and *A. baishanensis* exhibit parasitic relationships with adult moths. Notably, HKAS144393 represents a naturally occurring asexual morph characterised by trimorphic conidiogenous structures, while the asexual morph of *A. baishanensis* described by Pu et al. (2025) was obtained from culture and displayed only a single type of conidiogenous structure. Our observations demonstrate greater morphological plasticity in this species than previously recognised.

Figure 2. Phylogram generated from maximum likelihood analysis of *Samsoniella* based on a four-locus dataset (5P_ *TEF*+3P_*TEF*+*rpb1*+*MCM7*). SH-aLRT support ≥ 75%, ultrafast bootstrap support ≥ 75%, and PP values ≥ 95% are indicated above or below branches. A hyphen (−) indicates values lower than 75% SH-aLRT, 75% UFB, and 95% PP. The isolates in this study are shown in bold red. Ex-types are indicated by "T".
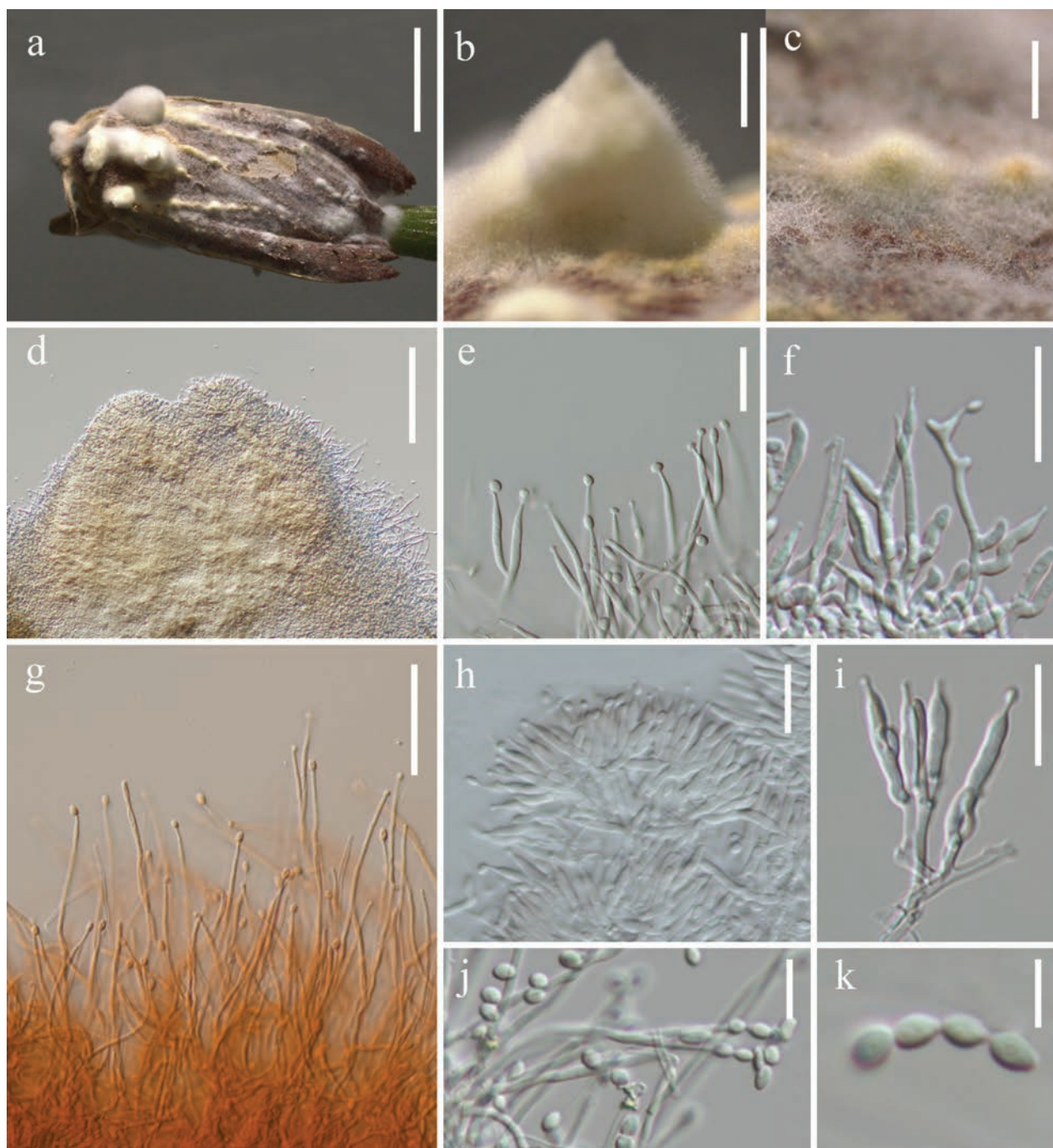
Figure 3. *Akanthomyces baishanensis* (HKAS144393) **a** fungus on an adult moth **b−d** synnemata **e−k** phialides and conidia. Scale bars: 5 mm (**a**); 1 mm (**b**); 0.5 mm (**c**); 100 μm (**d**); 30 μm (**g**); 20 μm (**e, f, h, i**); 10 μm (**j**); 5 μm (**k**).

***Pleurodesmospora sanduensis* J. Bu, K.D. Hyde & T.C. Wen, sp. nov.**
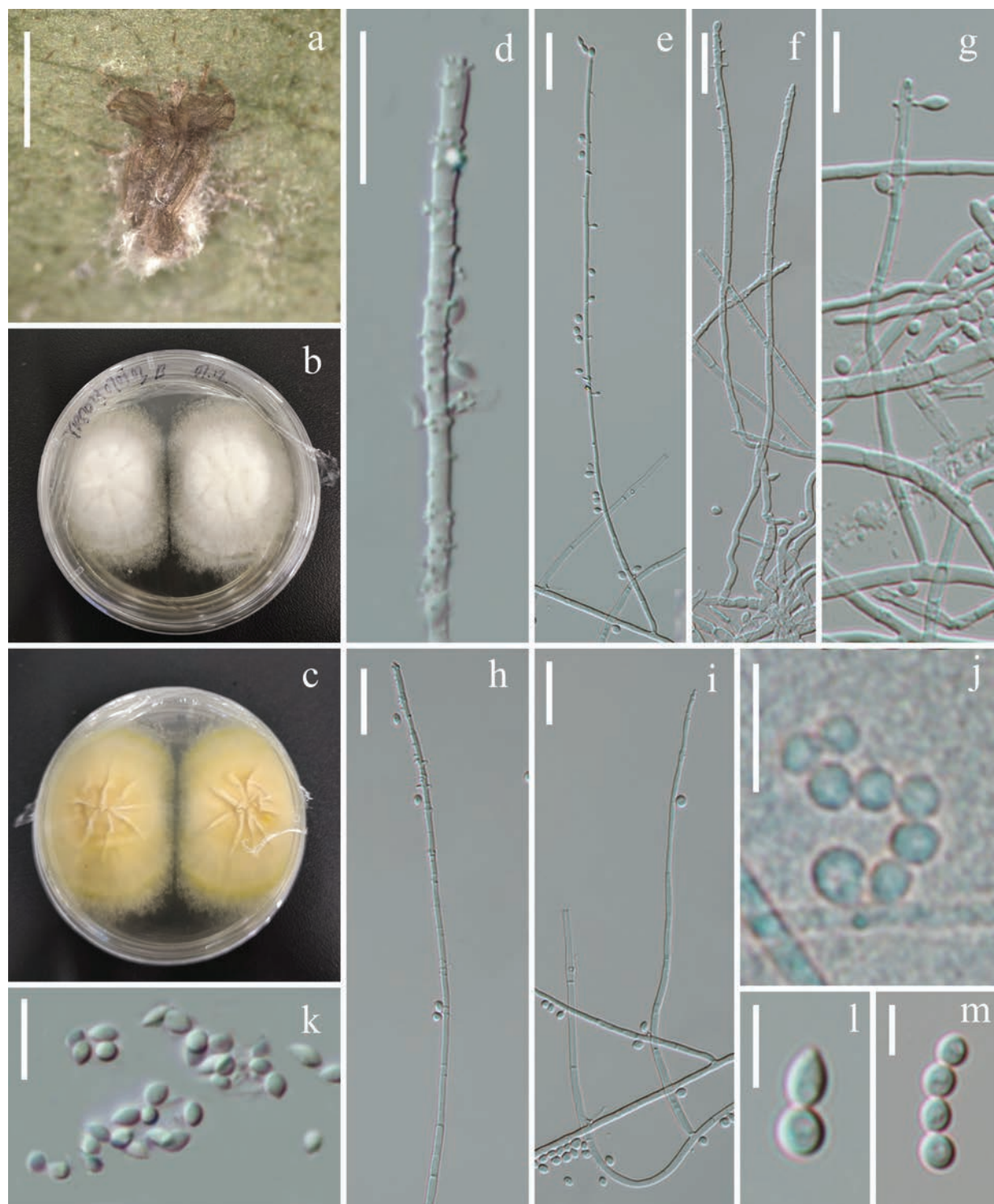
Index Fungorum: IF903211

Fig. 4

Etymology. In reference to the location of the type specimen, Sandu County of Guizhou Province, China.

   Description. Parasitic on adult Lepidoptera. **Sexual morph.** Undetermined. **Asexual morph.** *Colonies* on natural specimen white, sparse, only covering the abdomen of host. *Conidiophores* micronematous, cylindrical, erect

Figure 4. *Pleurodesmospora sanduensis* (HKAS144399) **a** fungus on host **b, c** obverse **(b)** and reverse **(c)** of colony on PDA **d−l** conidiophore and conidiogenous cells **j, k−m** conidia adhering in a chain. Scale bars: 2 mm (**a**); 20 μm (**d, e, f, h, i**); 10 μm (**g, j, k**); 5 μm (**l, m**).

or procumbent, sparsely branched, smooth, hyaline, septate, ca. 1.3−2.8 μm ($\bar{x}$ = 2 μm, n = 30) in width, from the middle part to the distal end densely covered by numerous minute, dentiform pegs, 0.7−1.8 × 0.5−0.8 μm ($\bar{x}$ = 1 × 0.7 μm, n = 25). ***Conidia*** obovoid, globose, smooth-walled, 2.7−4.8 × 1.4−2.5 μm ($\bar{x}$ = 3.7 × 2 μm, n = 30), arranged in short chains.

Culture characteristics. colonies on PDA reaching a diameter of 42 mm in three weeks at room temperature, white, circular, velvety, flat, edge entire, surface wrinkled, with radially striate, mycelia dense at centre, becoming loose outward, reverse cream-yellow.

Type. CHINA • Guizhou Province, Qiannan Buyei and Miao Autonomous Prefecture, Sandu County, the Yaoren Mountain (25°59'41"N, 107°56'41"E, alt. 987.1 m), on a dead adult of Lepidoptera on leaf litter, 08 July 2023, Jing Bu, YRS23070803B (holotype HKAS144399, ex-holotype KUNCC24-18538).

Notes. Six-locus phylogenetic analyses show that the *Pleurodesmospora sanduensis* is separated from other species of *Pleurodesmospora* with strong statistical support (100% SH-aLRT / 100% UFB / 1.00 PP, Fig. 1). *Pleurodesmospora sanduensis* is phylogenetically closely related to *P. acaricola* and *P. entomophila*. Pairwise nucleotide differences between *P. sanduensis* and *P. entomophila* (Tan and Shivas 2023) revealed 6 bp in nrLSU, 28 bp in ITS, 25 bp in *3P_TEF*, and 74 bp in *rpb2*. These molecular divergences support the recognition of *P. sanduensis* as a novel species, consistent with the taxonomic thresholds proposed by Jeewon and Hyde (2016). *Pleurodesmospora sanduensis* is similar to *P. acaricola* in producing loose and white colonies covering the host. However, *Pleurodesmospora sanduensis* differs from *P. acaricola* by its larger conidia (2.7−4.8 × 1.4−2.5 μm vs. 2.5−3 × 2 μm) in chains, but it is solitary in *P. acaricola* (Yeh et al. 2021). Additionally, chlamydospores are observed in *P. acaricola*, while it is absent in *P. sanduensis*.

Samsoniella lurida **J. Bu, K.D. Hyde & T.C. Wen, sp. nov.**
Index Fungorum: IF903212
Fig. 5

Etymology. Referring to the pale stromata arising from the host, which is different from other species in *Samsoniella*.

Description. Parasitic on cocoon of Lepidoptera. **Sexual morph.** *Stromata* 6.4−8.6 mm long, pale orange, cylindrical, unbranched or branched at base, arising from the head and end of the insect cocoon. *Stipe* cylindrical, pale orange, 0.4−0.8 mm wide. *Fertile part* clavate, pale orange, 2.5−3.1 × 0.6−1 mm, often with sterile tip (0.5−1.2 mm). The lateral sides had a longitudinal ditch without perithecia. *Perithecia* superficial, crowded, broadly ovoid, 205−455 × 144−274 μm ($\bar{x}$ = 319 × 198 μm, n = 15). *Asci* hyaline, cylindrical, 128−219 × 1.4−3.6 μm ($\bar{x}$ = 170 × 2.6 μm, n = 20). *Ascus caps* hemispherical, hyaline, 1.2−1.8 × 1.6−3 μm ($\bar{x}$ = 1.5 × 2.5 μm, n = 20). *Ascospores* filiform, hyaline, aseptate, 86−175 × 0.4−1 μm ($\bar{x}$ = 132 × 0.7 μm, n = 15) wide, do not disarticulate into part-spores. **Asexual morph.** *Synnemata* arising from the middle of the host, erect, single, 1.2 × 0.2−0.35 mm, producing a mass of floccose conidia at the apex. *Hyphae* smooth-walled, hyaline, septate, 1.5−3.6 μm ($\bar{x}$ = 2.5 μm, n = 30) wide. *Conidiophores* smooth-walled, cylindrical, verticillate, 2.3−9.1 × 1.9−2.9 μm ($\bar{x}$ = 4.9 × 2.3 μm, n = 15). *Phialides* verticillate, in whorls of two to five, lageniform, 4.2−7.3 μm ($\bar{x}$ = 5.7 μm, n = 30) long, basal portion cylindrical, tapering abruptly toward the apex, from 1.7−2.5 μm ($\bar{x}$ = 2.1 μm, n = 30) wide (base) to 0.5−0.9 μm ($\bar{x}$ = 0.7 μm, n = 30) wide (apex). *Conidia* smooth-walled, hyaline, fusiform, 1.9−2.7 × 1.1−1.9 μm ($\bar{x}$ = 2.3 × 1.4 μm, n = 30).
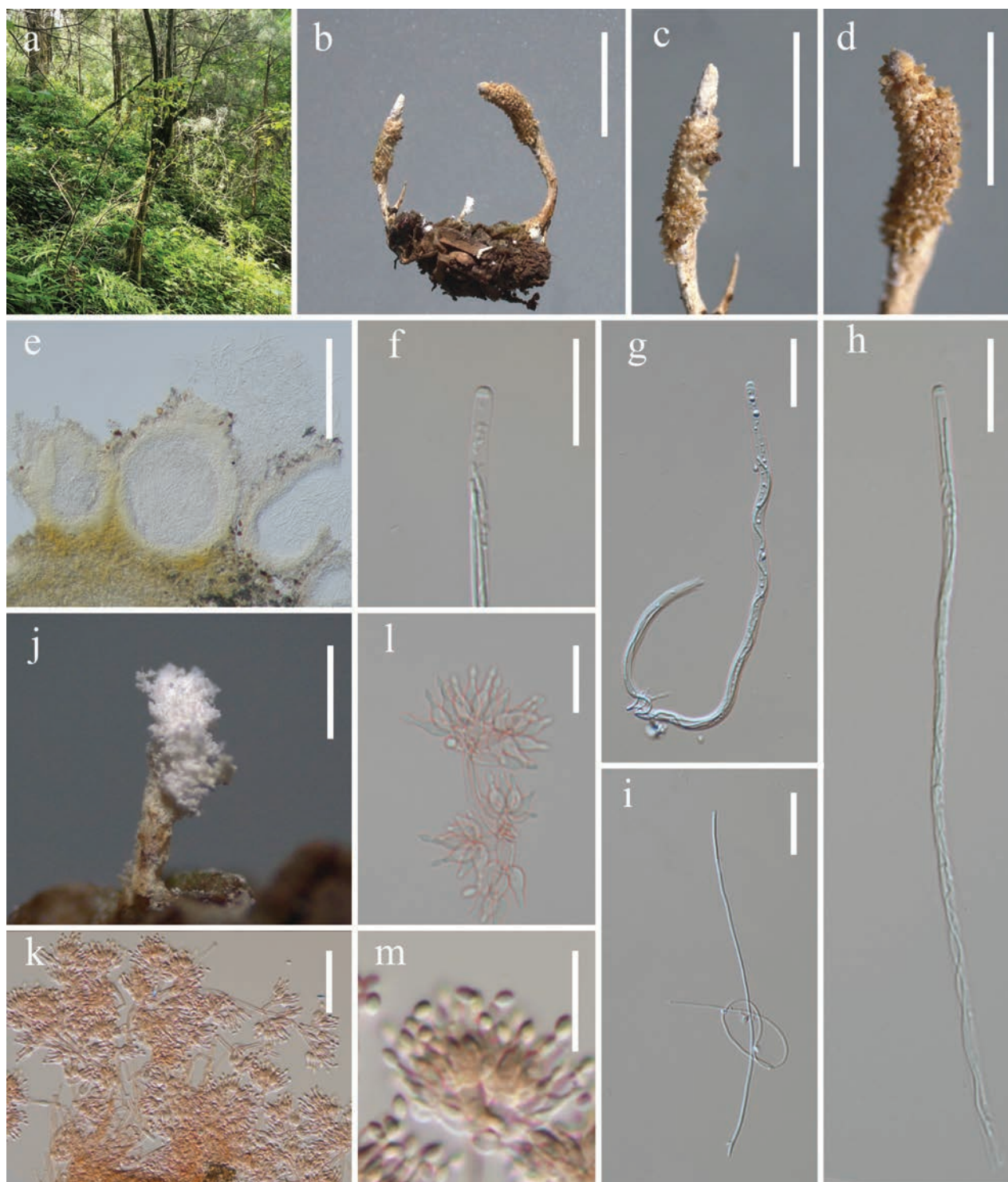
Figure 5. *Samsoniella lurida* (HKAS144387) **a** habitat **b** stromata and synnemata arising from host **c, d** fertile part with perithecia **e** vertical section of perithecia **f** ascus cap **g, h** asci **i** ascospore **j** synnema **k–m** conidiophores, phialides and conidia. Scale bars: 5 mm (**b**); 3 mm (**c, d**); 200 μm (**e**); 20 μm (**f, g, h, i**); 0.5 mm (**j**); 30 μm (**k**); 10 μm (**l, m**).

Type. CHINA • Yunnan Province, Kunming City, Panlong District, the Longchuanqiao Forest Park (25°17'05.26"N, 102°78'07.88"E, alt. 1963.9 m), on a lepidopteran cocoon buried in soil, 20 September 2023, Jing Bu, LCQ2023092034B (holotype HKAS144387).

Additional materials examined. Снima • Yunnan Province, Kunming, Xishan District, Tuanjie Country (25°08'61.38"N, 102°46'11.71"E, alt. 1971.2 m) on lepidopteran larva buried in soil, 17 October 2023, Jing Bu, MLSX2023101741B (HKAS144388, living culture KUNCC24-18534).

Notes. Phylogenetic analyses revealed that two specimens of *Samsoniella lurida* (HKAS144387 and HKAS144388) are closely related to *S. kunmingensis* and *S. tortricidae* (Fig. 2). Morphological comparisons demonstrate distinct characteristics among these species. *S. kunmingensis* and *S. tortricidae* produce larger, brightly coloured, multi-branched stromata with oblong-ovate to fusiform perithecia; *S. lurida* is characterised by pallid stromata and broadly ovoid perithecia (Table 3). Furthermore, *S. lurida* possesses a unique sterile tip, a feature not observed in other known *Samsoniella* species. Sequence comparisons between *S. lurida* and *S. kunmingensis* showed that there are 8 bp differences within 943 bp 3P_*TEF* and 12 bp differences within 979 bp *rpb2*. *S. lurida* differs from *S. tortricidae* by 10 bp within 943 bp 3P_*TEF* and 11 bp within 979 bp *rpb2*. Both morphological characters and molecular analyses support this fungus as a new species in *Samsoniella* (Jeewon and Hyde 2016).

Table 3. Comparison between the sexual morphs in *Samsoniella*. The data generated in this study are shown in bold.

Species	Host	Stromata (mm)	Fertile Part (mm)	Perithecia (µm)	Asci (µm)	Ascospores (µm)	References
S. cristata	Lepidopteran pupa	solitary or two, 25−40 long, crista-like	crista-like or subulate, 3.1−18.5 × 0.9−8.0	superficial, narrowly ovoid, 370−485 × 150−245	cylindrical,8-spored,180−356 × 3.0−4.8	bola-shaped, septate, 155−290 × 1.0−1.3	Wang et al. 2020
S. inthanonensis	Lepidopteran larva	gregarious, 20−50 long, 1−1.5 broad, cylindrical to clavate	clavate, 8−15 × 1.5−2	superficial, ovoid, 417.5−474.5 × 205−260	cylindrical, 8-spored, 300 × 2−2.5	bola-shaped, 3 or 4 septate, 221.5−267 × 0.5−1	Mongkolsamrit et al. 2018
S. kunmingensis	Lepidopteran pupa	solitary, 23 long, cylindrical to clavate	clavate, 3.3−4.2 × 0.8−1.2	superficial, narrowly ovoid to fusiform, 330−395 × 110−185	cylindrical, 8-spored, 150−297 × 3.0−4.6	bola-shaped, septate, 127−190 × 0.8−1.5	Wang et al. 2020
S. lanmaoa	Lepidopteran pupa	two to five, 38−69 long, palmately branched	clavate, 8.5−11.2 × 0.6−2.3	superficial, narrowly ovoid to fusiform, 360−467 × 124−210	cylindrical, 8-spored, 160−325 × 3.3−4.8	bola-shaped, septate, 135−260 × 0.9−1.4	Wang et al. 2020
S. lurida	**Lepidopteran pupa**	**6.4−8.6 long, cylindrical**	**clavate, 2.5−3.1 × 0.6−1.0, sterile tip 0.5−1.2 wide**	**superficial, broadly ovoid, 205−455 × 144−274**	**cylindrical, 128−219 × 1.4−3.6**	**filiform, aseptate, 86.1−174.7 × 0.4−1.0**	**This study**
S. pseudotortricidae	Lepidopteran pupa	solitary to several, 20−65 long, clavate	clavate to subulate, 10−17 × 1.5−4.2	superficial, narrowly ovoid to fusiform, 285.7−313.2 × 149.2−154.9	/	/	Wang et al. 2022
S. torquatistipitata	**Coleoptera**	**solitary, 4.4 × 0.1−0.3, clavate**	**clavate, 1.5 × 0.4**	**superficial, lageniform, 263−353 × 174−238**	**cylindrical, 8-spored, up to 114−173 × 1.6−3.3**	**filiform, 86.2−125.7 × 0.3−0.6**	**This study**
S. tortricidae	Lepidopteran cocoon	gregarious, 25−60	clavate to subulate, 5−15 × 1.2−2.3	superficial, narrowly ovoid to fusiform, 350−468 × 140−225	cylindrical, 8-spored, 170−285 × 2.8−4.0	bola-shaped, septate, 120−235 × 0.8−1.3	Wang et al. 2020
S. winandae	Lepidopteran cocoon	multiple, 8−20 long and 0.5−2 broad, cylindrical to enlarging apically	clavate, 2−8 × 2−3	superficial, narrowly ovoid, 500−570 × 135−180	cylindrical, 8-spored, 300 × 4−5	bola shaped, 3 or 5 septate, 200−265 × 0.5−1	Crous et al. 2023b

Samsoniella torquatistipitata J. Bu, K.D. Hyde & T.C. Wen, sp. nov.
Index Fungorum: IF903213
Fig. 6

Etymology. From the Latin "torqu", referring to the stipe of stroma, is torsional rather than cylindrical.

Description. Parasitic on ant (Hymenopteran). **Sexual morph.** *Stroma* arising from head of ant, orange, single, simple, 4.4 × 0.1−0.3 mm. *Stipe* fleshy, torsional, reddish-orange, up to 2.7 mm long. *Fertile part* cylindrical, becoming acuate toward the end, reddish-orange, 1.7 × 0.4 mm. *Perithecia* lageniform, superficial, 255−368 × 163−244 µm ($\bar{x}$ = 288 × 190 µm, n = 5), growing on one side of fertile part. *Asci* cylindrical, hyaline, 8-spored, 114−173 × 1.6−3.3 µm ($\bar{x}$ = 135 × 2.4 µm, n = 20), with hemispherical cap, 1.7−2.5 × 1.1−1.8 µm ($\bar{x}$ = 2.2 × 1.4 µm, n = 20). *Ascospores* filiform, aseptate, hyaline, 86−125 × 0.3−0.6 µm ($\bar{x}$ = 98.6 × 0.5 µm, n = 15), non-disarticulating. **Asexual morph**. produced on the cultures, hyphomycetous. *Hyphae* smooth, septate, hyaline, 1.2−2.0 µm ($\bar{x}$ = 1.6 µm, n = 30) in diam. *Conidiophores* smooth-walled, cylindrical or elongated ellipsoid, verticillate with phialides in whorls of two to five or singly along the hyphae, 4.4−18.4 × 1.7−3.9 µm ($\bar{x}$ = 8.4 × 2.7 µm, n = 30). *Phialides* lageniform, 6.1−10.7 µm ($\bar{x}$ = 8.0 µm, n = 30) long, basal portion inflated, 1.8−3.5 µm ($\bar{x}$ = 2.6 µm, n = 30) wide, tapering abruptly into a thin neck, 0.7−1.4 µm ($\bar{x}$ = 0.9 µm, n = 30) wide. *Conidia* subglobose, hyaline, 1.8−2.8 µm ($\bar{x}$ = 2.3 µm, n = 50) in diam.

Culture characteristics. colonies on PDA reaching 40 mm in 14 days at room temperature, circular, flat, edge entire, mycelia dense, cottony, creamy yellow at centre, becoming white outward, with concentric rings, sporulation, reverse creamy yellow, with radially striate.

Type. CHINA • Yunnan Province, Puer City, Simao District, Plum Lake Park (22°72'66.83"N, 100°97'83.57"E, alt. 1354.5 m), on an adult ant (Hymenoptera) buried in soil, 25 October 2023, Jing Bu, DSSZ20231025110B (holotype HKAS144411, ex-holotype KUNCC24-18535).

Additional materials examined. CHINA • Yunnan Province, Puer, Simao District, Plum Lake Park (22°75'14.29"N, 100°97'73.13"E, alt. 1338.8 m), on lepidopteran cocoon buried in soil, 26 October 2023, Jing Bu, MZH20231025119B (paratype HKAS144402, ex-paratype KUNCC24-18536).

Notes. The phylogenetic tree (Fig. 2) showed that *Samsoniella torquatistipitata* constitutes a distinct clade distantly related to *S. cristata*, *S. kunmingensis*, *S. lurida*, and *S. tortricidae*. A pairwise comparison of *3P_TEF*, *rpb1*, *MCM7*, and *rpb2* showed that *S. torquatistipitata* differs from *S. cristata*, *S. kunmingensis*, *S. lurida*, and *S. tortricidae* in 1−6 bp, 3−4 bp, 6−9 bp, and 4−16 bp, respectively. *Samsoniella torquatistipitata* is characterised by the small, single stroma (4.4 mm long), reddish-orange, cylindrical fertile part, superficial, lageniform perithecia, and the association with adult ants. Morphological comparisons of the novel taxa with closely related *Samsoniella* species are provided in Table 3. Both morphological characteristics and molecular analyses support this fungus as a new species in *Samsoniella* (Jeewon and Hyde 2016).
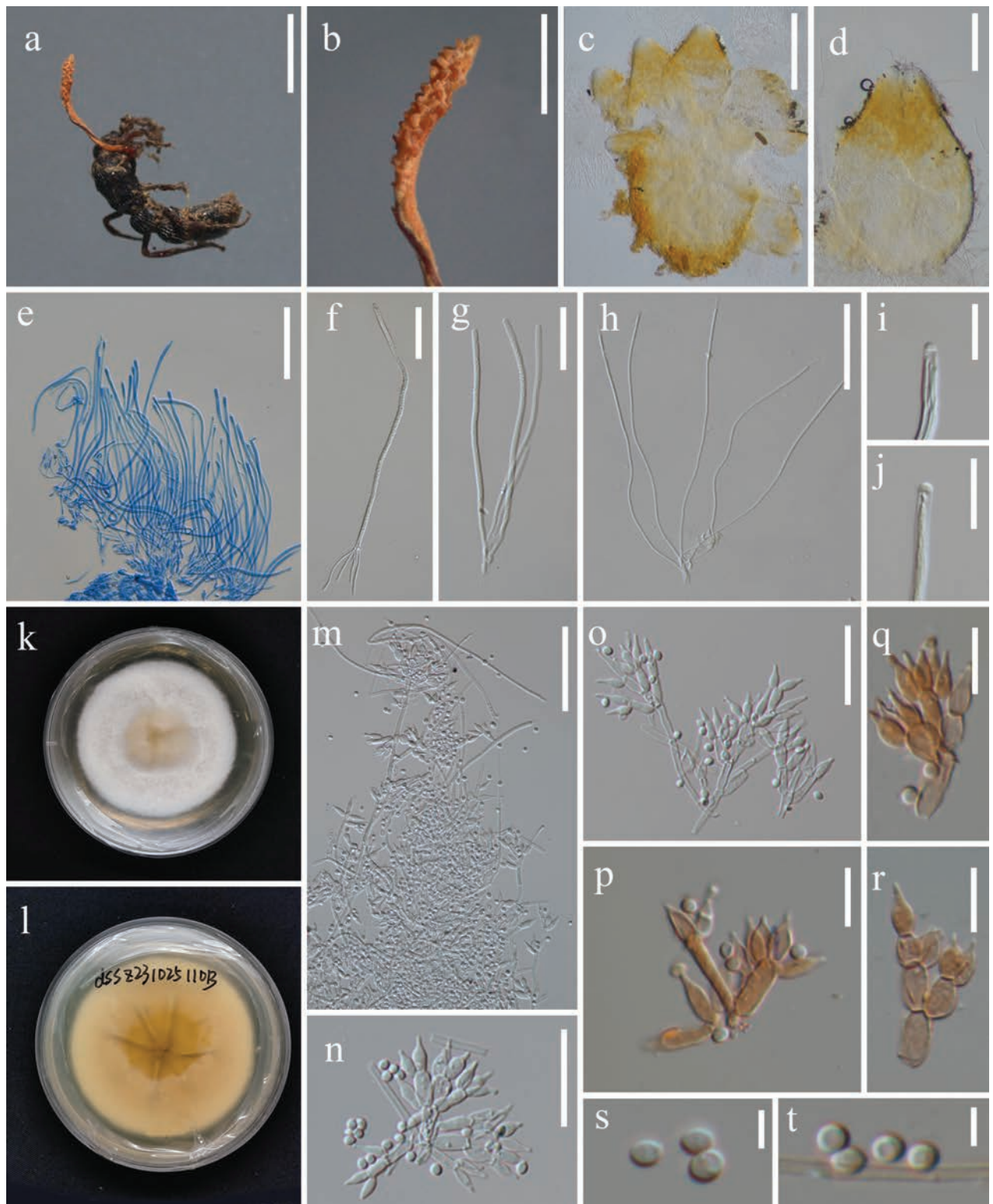
Figure 6. *Samsoniella torquatistipitata* (HKAS144411) **a** fungus on the adult ant **b** fertile part **c** vertical section of stroma **d** perithecium **e–g** asci **h** ascospore **i, j** ascus cap **k, l** obverse (**k**) and reverse (**l**) of colony on PDA; **m–r** conidiophores and phialides; **s, t** conidia. Scale bars: 3 mm (**a**); 1 mm (**b**); 200 μm (**c**); 100 μm (**d**); 50 μm (**e, m**); 30 μm (**f, g, h**); 20 μm (**n, o**); 10 μm (**i, j, p, q, r**); 3 μm (**s, t**).

Samsoniella subasiatica **J. Bu, K.D. Hyde & T.C. Wen, sp. nov.**
Index Fungorum: IF903214
Fig. 7

Etymology. Referring to the morphology similar to *Samsoniella asiatica*.

Description. Parasitic on pupa of Lepidoptera. **Sexual morph**. Undetermined. **Asexual morph**. *Synnema* arising from middle part of pupa, solitary, erect, flexuous, unbranched, 2.8 × 0.2 mm. *Stipe* cylindrical, pale orange. *Hyphae* smoothwalled, septate, hyaline 1.3−2.8 μm ($\overline{x}$ = 2.0 μm, n = 50). *Conidiophores* grouped together at the apex of synnema and the head of pupa, verticillate 3.6−7.4 × 2−3 μm ($\overline{x}$ = 5.2 × 2.4 μm, n = 20). *Phialides* lageniform, usually in whorls of two to five, 4.2−6.8 μm ($\overline{x}$ = 5.6 μm, n = 50) long, globose at basal portion, tapering gradually toward the apex, from 1.8−2.4 μm ($\overline{x}$ = 2.1 μm, n = 50) wide (base) to 0.6−1 μm ($\overline{x}$ = 0.8 μm, n = 50) wide (apex). *Conidia* single, smooth-walled, hyaline, fusiform to oval, 1.9−2.9 × 1.4−1.8 μm ($\overline{x}$ = 2.4 × 1.6 μm, n = 50).

Culture characteristics. Colonies on PDA reaching a diameter of 27−29 mm in two weeks at room temperature, white, circular, velvety, mycelia dense, becoming loose in the outmost ring, reverse brightly yellow.

Type. CHINA • Guizhou Province, Qiannan Buyei and Miao Autonomous Prefecture, Anlong County (24°99'08.43"N, 105°59'76.06"E, alt. 1395.6 m), on lepidopteran pupa on leaf litter, 07 September 2023, Jing Bu, Al2023090717B (holotype HKAS144400, ex-holotype KUNCC24-18537).

Notes. *Samsoniella subasiatica* morphologically resembles *S. asiatica* (Wang et al. 2023a) by producing a flexuous synnema, pale orange stipe, with a mass of conidia at the apex. However, *S. subasiatica* differs from *S. asiatica* in having simple synnema and larger conidia (1.9−2.9 μm vs. 1.1−1.8 μm) (Table 4). The synnema of *S. asiatica* is branched at the base (Wang et al. 2023a). Furthermore, phylogenetic analysis based on four loci revealed that *S. subasiatica* is sister to *S. winandae*, with moderate statistical support (89% SH-aLRT / 94% UFB / 0.99 PP; Fig. 2). However, *S. subasiatica* can be distinguished from *S. winandae* by its significantly smaller synnemata and phialides (4.2−6.8 × 1.8−2.4 μm vs. 5−12 × 2−3 μm) (Table 4). Additionally, a comparison of nucleotide sequences between *S. subasiatica* and *S. winandae* indicated that there are 6 bp differences in *3P_TEF*, 14 bp in *rpb1*, and 8 bp in *rpb2*. Based on the recommendations made by Jeewon and Hyde (2016), we determined this fungus as a novel species.

Discussion

Morphology-phylogeny of *Akanthomyces sensu lato*

Akanthomyces sensu lato is a monophyletic lineage, and it was segregated into four genera, including *Akanthomyces sensu stricto*, *Arachnidicola*, *Lecanicillium* and *Kanoksria*, corresponding to their morphological and ecological traits (Khonsanit et al. 2024; Wang et al. 2024b, Fig. 1). *Akanthomyces sensu stricto* comprises seventeen species pathogenic to moths, characterised by white to creamy synnemata with cylindrical, papillate phialides and catenulate conidia (Aini et al. 2020; Khonsanit et al. 2024). *Arachnidicola* comprises twelve species primarily pathogenic to spiders, displaying isaria-like anamorphs (Mongkolsamrit et al. 2018; Chen et al. 2022; 2023a; Wang et al. 2024b), except for
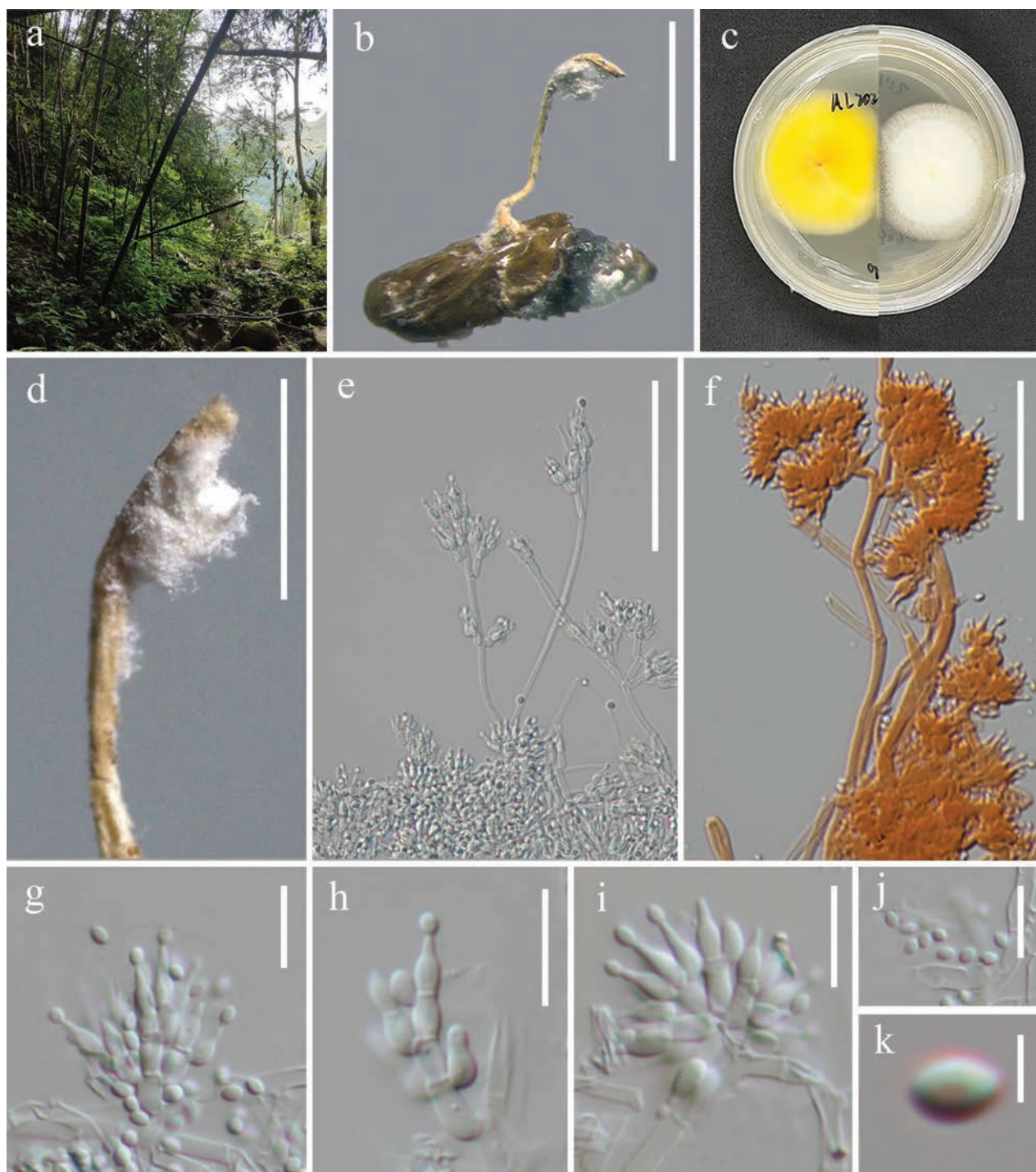
Figure 7. *Samsoniella subasiatica* (HKAS144400) **a** habitat **b** synnema arising from pupa **c** lower and upper view of the colony on PDA **d** synnema **e, f** conidiophores **g–i** phialides **j, k** conidia. Scale bars: 2 mm (**b**); 1 mm (**d**); 50 μm (**e**); 30 μm (**f**); 10 μm (**g, h, i, j**); 2 μm (**k**).

Akanthomyces thailandicus, which has a lecanicillium-like anamorph (Mongkolsamrit et al. 2018). *Lecanicillium* includes twelve species pathogenic to diverse hosts (e.g., Lepidoptera, Coleoptera, Hemiptera, spiders) with acremonium-like and verticillium-like anamorphs (Chiriví-Salomón et al. 2015; Chen et al. 2017, 2020a, 2020b, 2022; Manfrino et al. 2022). *Kanoksria*, a monotypic genus basal to the others, exhibits simplicillium-like anamorphs and is a hyperparasite on *Ophiocordyceps sinensis* (Wang et al. 2023b).

Table 4. Comparison between the asexual morphs in *Samsoniella*. The data generated in this study are shown in bold.

Species	Host	Synnemata (mm)	Conidiophores (µm)	Phialides	Phialides Size (µm)	Conidia (µm)	References
S. aurantia	Lepidopteran larva	25−75 × 1−1.5	150 × 2−3	/	(5−)7.5(−9) × 2−3	fusiform, oval with pointed ends, (2−)2.5(−3) × 1−2	Mongkolsamrit et al. 2018
S. asiatica	Lepidopteran pupa	4−26 × 0.4−1.5	4.6−10.3 × 0.8−1.9	verticillate, in whorls of two to four, or solitary on hyphae	2.7−8.6 × 0.7−1.7, 0.6−1.1 wide at apex	fusiform or oval, 1.1−1.8 × 0.8−1.2	Wang et al. 2023a
S. cristata	Lepidopteran pupa	/	3.6−11.5 × 1.7−2.5	verticillate, in whorls of two to five, or solitary on hyphae	4.5−23.2 × 1.6−2.7, 0.5−1.1 wide at apex	fusiform or oval, 2.4−3.2 × 1.6−2.3	Wang et al. 2020
S. inthanonensis	Lepidopteran larva	/	2−3 wide	verticillate, in whorls of two to five, cylindrical basal portion	basal (4−)6.5−10(−12) × (1−)1.5−2(3), neck (1−)2.5(−4) × 0.5−1	fusiform, (2−)3(−3.5) × 1.5−2	Mongkolsamrit et al. 2018
S. lanmaoa	Lepidopteran pupa	/	3.8−13.3 × 1.5−2.1	verticillate, in whorls of two to six, usually solitary on hyphae	3.5−20.7 × 1.7−2.6, 0.5−1.1 wide at apex	fusiform or oval, 1.9−2.7 × 1.4−2.0	Wang et al. 2020
S. lurida	**Lepidopteran pupa**	**1.2 × 0.2−0.35**	**2.3−9.1 × 1.9−2.9**	**verticillate, in whorls of two to five**	**4.2−7.3 × 1.7−2.5, 0.5−0.9 wide at apex**	**fusiform, 1.9−2.7 × 1.1−1.9**	**This study**
S. pseudotortricidae	Lepidopteran pupa	/	6.6−26.5 × 1.1−2.5	verticillate, in whorls of two to five, usually solitary on hyphae	5.4−6.9 × 1.0−1.6, 0.5−0.8 wide at apex	fusiform or oval, 0.9−1.5 × 0.8−1.3	Wang et al. 2022
S. subasiatica	**Lepidopteran pupa**	**2.8 × 0.2**	**3.6−7.4 × 2−3**	**verticillate, in whorls of two to five**	**4.2−6.8 × 1.8−2.4, 0.6−1.0 wide at apex**	**fusiform to oval, 1.9−2.9 × 1.4−1.8**	**This study**
S. torquatistipitata	**Coleopteran adult**	**/**	**4.4−18.4 × 1.7−3.9**	**/**	**6.1−10.7 × 1.8−3.5, 0.7−1.4 wide at apex**	**subglobose, up to 1.8−2.8 in diameter**	**This study**
S. vallis	Lepidopteran pupa	/	11.3−22.1 × 1.3−1.4	single phialide or whorls of two to four	7.2−8.1 × 2.8−3.2	fusiform to ellipsoidal, 2.3−3.1 × 1.5−2.1	Chen et al. 2023b
S. winandae	Lepidopteran pupa and cocoon	12 × 2	/	verticillate, in whorls of two to five	5−12 × 2−3	ellipsoidal, 1.5−3 × 1−2	Crous et al. 2023b

In this study, we identified a moth-pathogenic species, *Akanthomyces baishanensis*, which exhibits the typical phialide characteristics of *Akanthomyces sensu stricto*, along with previously undescribed phialide types within this clade. Although molecular data provide precise taxonomic evidence, morphological and ecological traits remain indispensable. An integrated taxonomy approach is necessary for resolving these complex fungal groups. Furthermore, ecological features may also provide valuable insights for the identification and discovery of novel *Akanthomyces* species.

The molecular phylogeny and morphology of *Samsoniella*

Sexual morphs of *Samsoniella* share similarities in producing yellowish to reddish-orange, fleshy, simple to branched stromata; superficial, ovoid to fusiform perithecia; cylindrical asci with thickened apex and filiform, multiseptate, non-disarticulating ascospores (Mongkolsamrit et al. 2018). Species of this genus are indistinguishable solely based on sexual morphology. However, they can be divided into two types based on their stroma size: **Type Ia** includes nine species with a length of stromata more than 25 mm and is pathogenic to lepidopteran hosts (Mongkolsamrit et al. 2018; Wang et al. 2020, 2022, 2023b); **Type IIa** includes six species with a length of stromata lower than 25 mm and are pathogenic to lepidopteran and hymenopteran hosts or hyperparasitic to

Cordyceps species (Wang et al. 2020; Crous et al. 2023b) (Table 5). In this study, we introduce two new species in this group, namely, *Samsoniella lurida* and *S. torquatistipitata*, based on their sexual and asexual morphs. It is worth noting that *S. torquatistipitata* is pathogenic to an adult ant and has a very small, solitary, simple, reddish-orange stroma (4.4 mm in length). This is the first time to report the sexual typified species from an adult ant and contribute to the morphological diversity of *Samsoniella*.

The asexual morphs of *Samsoniella* have been known from 39 species. Macromorphologically, they can be categorised into two types: **Type Ib** includes 16 species which have well-developed stromata and are pathogenic to Lepidoptera, Coleoptera, Hymenoptera and *Cordyceps* sp. (Mongkolsamrit et al. 2018; Wang et al. 2020, 2022, 2023a; Chen et al. 2022, 2023b; Crous et al. 2023b; Chuang et al. 2024); **Type IIb** includes 15 species which form white colonies on the host surface and are pathogenic to Lepidoptera, Coleoptera, Hymenoptera, and spiders (Chen et al. 2020c, 2021b, 2022; Wang et al. 2020, 2022, 2024a). Our new species *S. subasiatica* was known only from its asexual morphs. This species has well-developed stroma covered with a white, powdery conidia mass, extremely resembling *S. asiatica*. However, these two species are phylogenetically distant, indicating that characteristics of asexual morphs have less taxonomic significance in interspecific demarcation.

Collectively, taxonomic inferences from phylogenetic analyses do not align with the morphological categories outlined in Table 5. The morphological plasticity of *Samsoniella* species limits their utility in taxonomy, necessitating molecular analyses for accurate species delineation (Mongkolsamrit et al. 2018). The six-locus (nrLSU+ITS+nrSSU+3P_*TEF*+*rpb1*+*rpb2*) phylogeny effectively resolves genetically distant species, while it struggles with closely related taxa, particularly due to the limited resolution of the ITS regions. In contrast, the four-gene (5P_*TEF*+3P_*TEF*+*rpb1*+*MCM7*, Wang et al. 2023a) dataset provides superior resolution, highlighting its importance in refining the taxonomy of *Samsoniella*.

Table 5. Morphological synopsis of *Samsoniella* species.

Type	Species	Morphological characteristics	Host	References
Type Ia	*S. antleroides, S. aurantia, S. cristata, S. inthanonensis, S. lanmaoa, S. pseudotortricidae, S. ramosa, S. sapaensis, S. tortricidae.*	Stromata orange, fleshy, solitary to gregarious, simple or branched, more than 25 mm in length	Lepidoptera	Mongkolsamrit et al. 2018; Wang et al. 2020, 2022, 2023b
Type IIa	*S. cardinalis, S. hepiali, S. kunmingensis,* **S. lurida, S. torquatistipitata,** *S. winandae.*	Stromata orange, fleshy, solitary to gregarious, usually unbranched, less than 25 mm in length	*Cordyceps* sp., Lepidoptera	Wang et al. 2020; Crous et al. 2023b
Type Ib	*S. asiatica, S. aurantia, S. coccinellidicola, S. duyunensis, S. erucae, S. haniana, S. lasiocampidarum, S. ramosa, S. sapaensis, S. sinensis,* **S. subasiatica,** *S. tiankengensis, S. vallis, S. winandae, S. yuanzuiensis, S. yunnanensis.*	Synnemata erect, terminal irregularly branched, with conidial mass at the subterminal region of synnemata, conidial mass powdery and floccose	Lepidoptera, Coleoptera, Hymenoptera, *Cordyceps* sp.	Mongkolsamrit et al. 2018; Wang et al. 2020, 2022, 2023a; Chen et al. 2022, 2023b; Crous et al. 2023b; Chuang et al. 2024
Type IIb	*S. alpina, S. anhuiensis, S. aranea, S. coleopterorum, S. farinospora, S. formicae, S. fusiformispora, S. guizhouensis, S. hepiali, S. hymenopterorum, S. lepidopterorum, S. neopupicola, S. pupicola, S. pseudogunnii, S. sanmingense.*	White colonies surround the host surface without synnemata	Lepidoptera, Coleoptera, Hymenoptera, Spider.	Chen et al. 2020c, 2021b, 2022; Wang et al. 2020, 2022, 2024a; Pu et al. 2025

Author ORCIDs

Jing Bu https://orcid.org/0009-0006-6861-7770
De-Ping Wei https://orcid.org/0000-0002-6576-2239
Zheng-Hui Liu https://orcid.org/0000-0001-7022-4075
Zhong-Liang Liu https://orcid.org/0009-0007-9519-1418
Ji-Chuan Kang https://orcid.org/0000-0002-6294-5793
Xing-Can Peng https://orcid.org/0000-0002-7271-7639
Zhang-Jiang He https://orcid.org/0000-0002-7120-1227
Shi-Ke Huang https://orcid.org/0000-0002-2936-396X
Xian Zhang https://orcid.org/0009-0008-0919-4303
Kevin D. Hyde https://orcid.org/0000-0002-2191-0762
Nalin N. Wijayawardene https://orcid.org/0000-0003-0522-5498
Ting-Chi Wen https://orcid.org/0000-0003-1744-5869

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

Alignment of Cordycipitaceae tree-six locus

Authors: Jing Bu

Data type: fas

Explanation note: The alignment of Cordycipitaceae tree that based on six locus (nrLSU, ITS, nrSSU, tef-1α, rpb1 and rpb2).

Link: https://doi.org/10.3897/mycokeys.116.147006.suppl1

Supplementary material 2

Alignment of Samsoniella tree-five locus

Authors: Jing Bu

Data type: fas

Explanation note: The alignment of Samsoniella tree based on five locus (nrLSU, nrSSU, tef-1α, rpb1 and rpb2).

Link: https://doi.org/10.3897/mycokeys.116.147006.suppl2

Supplementary material 3

Legend for supplementary figures of single gene tree

Authors: Jing Bu

Data type: docx

Link: https://doi.org/10.3897/mycokeys.116.147006.suppl3

MycoKeys

Four new species of *Beltraniella* (Amphisphaeriales, Beltraniaceae) revealed by morphology and phylogenetic analyses from China

Wen-Wen Liu[1] , Chang-Zhun Yin[1] , Zhao-Xue Zhang[2] , Xing-Sheng Wang[1] , Zhe Meng[1], Xiu-Guo Zhang[1,2], Shi Wang[1]

1 College of Life Sciences, Shandong Normal University, Jinan, 250358, China
2 Shandong Provincial Key Laboratory for Biology of Vegetable Diseases and Insect Pests, College of Plant Protection, Shandong Agricultural University, Taian, 271018, China

Corresponding author: Shi Wang (wangssdau@126.com)

Abstract

Beltraniella is a widely-distributed genus on Earth, although its abundance is relatively limited in relation to other dematiaceous hyphomycetes. In the present study, diseased leaves of *Myristica fragrans* and decaying leaves were collected from Hainan and Sichuan Province. Fungal DNA was amplified and sequenced using two barcodes, the internal transcribed spacer (ITS) and large subunit of ribosomal RNA (LSU), and phylogenetic analyses were conducted through maximum likelihood (ML) and Bayesian inference (BI) algorithms. Four new species of *Beltraniella*, *B. dujiangyanensis*, *B. jianfengensis*, *B. myristicae*, and *B. xinglongensis* are identified through phylogenetic analyses and morphological comparison during a survey of fungal diversity in Hainan and Sichuan Provinces, China. Detailed descriptions of the morphological characteristics of these four new species are provided and illustrated with figures.

Key words: Dematiaceous hyphomycetes, novel taxa, phylogeny, Sordariomycetes, taxonomy

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Introduction

Beltraniella was proposed by Subramanian in 1952, and he selected *B. odinae* as the type species (Subramanian 1952). Currently, a total of 33 epithet records of *Beltraniella* have been documented in the Index Fungorum (http://www.indexfungorum.org/, accessed on 14 February 2025). *Beltraniella* belongs to the Sordariomycetes, Amphisphaeriales, Beltraniaceae (Hyde et al. 2024). *Beltraniella* was characterized by sterile setae, which were extensions of conidiophores, or were present among conidiophores and arising from radially lobed basal cells. Conidiophores were branched, often with setae-shaped apices, and they originated from radially lobulated basal cells. Conidiogenous cells were polyblastic and sympodial; Conidia were turbinate or biconical. The distinction between setae and conidiophores lies in their apex; setae gradually narrow to a sharp point, whereas conidiophores may sometimes be reduced to conidiogenous cells (Hyde et al. 2020b). Furthermore, *Beltraniella* is a genus of dematiaceous hyphomycetes that play a crucial ecological role in natural ecosystems

by breaking down lignin and cellulose, recycling matter and energy, and maintaining ecosystem balance.

Beltraniella typically inhabits decaying leaves and other natural substrates on the ground, maintaining the balance of natural ecosystems, and aiding in the decomposition of diseased or decaying leaves (Dighton et al. 1985). Shirouzu et al. (2010) isolated and identified *B. botryospora* Shirouzu & Tokum, a fungus frequently reported on both live and deciduous leaves of *Quercus aspera*, suggesting a close relationship with this species. Hyde et al. (2020a) reported a new species, *B. ramosiphora* C.G. Lin & K.D. Hyde, found in decomposing organic matter on decaying leaves, while Tan and Roger (2022) reported *B. hesseae* Y.P. Tan, Bishop-Hurley & R.G on the leaves of *Digitaria ciliaris*. The principal methodology employed in studying this fungus encompasses a synthesis of traditional morphological taxonomy and molecular systematics. Crous et al. (2014) isolated and characterized *B. endiandrae* Crous & Summerell, reporting that its conidia were solitary, light brown, and smooth, with hyaline transverse bands. They also demonstrated the colony color variation of *B. endiandrae* on three distinct media. The frontal and reverse sides appeared iron-gray on PDA medium; the light olive-gray patches appeared on OA medium, while the frontal side was light olive-gray and the reverse side was yellowish-brown on MEA medium. Tibpromma et al. (2018) reported two new species, *B. pandanicola* Tibpromma & K.D. Hyde and *B. thailandica* Tibpromma & K.D. Hyde [as *'thailandicus'*], and described their morphological characteristics in detail. In addition, they provided a detailed description of *Pandanus* growing on withered leaves, whereas Crous et al. (2016) employed the ITS sequence to conduct a phylogenetic analysis of *Beltraniella*. Recently, Liu et al. (2024) identified a new species named *B. jiangxiensis* P. Razaghi, Raza & L. Cai, through phylogenetic analysis based on ITS and LSU sequences, combined with morphological analysis. This method is currently widely accepted as the identification method for *Beltraniella*.

The primary objective of this study is to identify putative new strains of *Beltraniella* through morphological comparison and phylogenetic analysis. Four new species of *Beltraniella* were identified and thoroughly characterized, with their differences from closely related species compared and discussed, thereby enriching the species diversity of the genus.

Materials and methods

Sample collection and treatment

Samples of diseased or decaying leaves were collected in Hainan and Sichuan provinces from June 2023 to March 2024. Upon collection, they were numbered by time, location and plant type, and then photographed and recorded. Flatter leaves were chosen for photography. The processed samples were returned to the kraft bag for the next step. For each sample, 4–7 diseased or decaying leaves were cut into squares (5 × 5 mm) and placed in sterile containers. These were then sterilized on a clean bench. Pour in 75% alcohol, soak the leaves thoroughly for 1 minute to sterilize their surfaces. Use a disposable syringe to remove the alcohol, rinse with sterile water, then remove the sterile water and add 5% sodium hypochlorite to sterilize the leaf surface again for

30 seconds. Rinse the leaves three times with sterile water, then place them on sterilized filter paper to dry using sterilized tweezers. Once dry, clip the leaves with the diseased spot pointing downward and place 3–5 samples of them on each PDA medium (PDA: 14 g agar, 20 g dextrose, 200 g potato, 1000 mL distilled water, pH 7.0). The medium with leaves was securely wrapped with sealing film and placed in a constant temperature incubator at 25 °C for incubation. The growth of the fungus was observed every day, and after 2–3 days of incubation, the agar with fungal growth was transferred from the PDA medium to a new PDA medium for purification.

Morphological and cultural characterization

The single colonies that were isolated and purified were photographed on the 7th and 14th day of growth, using a digital camera (Canon Powershot G7X; Beijing, China), on both the surface and reverse of the PDA medium. A stereo microscope (Olympus SZX10; Beijing, China) was used to observe whether conidia were produced. If conidia were observed, a temporary mount was prepared to examine the morphology of the fungal conidia under a microscope (Olympus BX53). Subsequently, fungal structures, including conidia and conidiogenous cells, were photographed using a high-definition digital camera (Olympus DP80). All strains were stored in a 4 °C thermostat using sterilized 10% glycerol test tubes. Voucher specimens have been carefully preserved in two herbariums: the Herbarium of the Department of Plant Pathology at Shandong Agricultural University in Taian, China (HSAUP), and the Herbarium Mycologicum Academiae Sinicae at the Institute of Microbiology, Chinese Academy of Sciences in Beijing, China (HMAS). Additionally, living cultures derived from the holotype have been safeguarded in the Shandong Agricultural University Culture Collection (SAUCC). The morphological description and taxonomic characters of the new species have been uploaded to MycoBank (http://www.mycobank.org).

DNA extraction, PCR amplification, and sequencing

The method of extracting fungal DNA involves using CTAB (cetyl trimethyl ammonium bromide) (Wang et al. 2023). When the mycelium has grown to a certain degree in the PDA medium, use a sterilized scalpel to scrape approximately 0.2 g of mycelium into a 1.5 mL centrifugal tube, add precipitating CTAB lysate to the tube, pulverize the mycelium using a grinder, and then place the tube in a water bath at 65 °C for 2 hours. After pulverization, centrifuge the sample to extract the supernatant, add chloroform (and other precipitate agents) to the supernatant to isolate the genomic DNA. Further centrifuge the supernatant, and then add chloroform: isoamyl alcohol (24:1) to precipitate the DNA (Doyle and Doyle 1990; Guo et al. 2000). PCR (Polymerase Chain Reaction) amplification of the extracted fungal DNA is performed using ITS and LSU (White et al. 1990; Glass and Donaldson 1995). Each sterilized PCR tube contains a total of 25 µL reaction mixture, which includes 9.5 µL of ddH_2O, 12.5 µL of 2 × Taq Plus Master Mix (Shanghai, China) (with dye) (Yeasn Biotechnology, Shanghai, China, Cat No. 10154ES03), 1 µL of forward primer, and 1 µL of reverse primer. The products of PCR amplification were

detected by electrophoresis in a 2% agarose gel. After electrophoresis, the gel was removed and observed under UV light, where the presence of DNA was indicated by fluorescent bands (Zhang et al. 2022). PCR primer synthesis and DNA sequencing were completed by Tsingke Biotechnology Co., Ltd. (Qingdao, China). Once sequencing was completed, MAGE7 (Kumar et al. 2016) was utilized for sequence comparison and splicing of the sequencing results. The gene sequences of the four new species were uploaded to the GenBank. Subsequently, the most recent article was downloaded by searching for '*Beltraniella*' on Index Fungorum (https://indexfungorum.org/Names/Names.asp, accessed on 14 February 2025). The GenBank table mentioned in the article was found, and the results were presented in Table 1.

Phylogenetic analyses

Nucleic acid sequences of *Beltraniella* were downloaded from the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/, accessed on 14 February 2025), and GenBank accession numbers were obtained from the latest version of the article (Zhang et al. 2000). Nucleic acid sequences of the four new species were aligned with reference sequences from the literature using MAFFT 7 (http://mafft.cbrc.jp/alignment/server/, accessed on 14 February 2025) (Katoh et al. 2019). Data from the completed sequence alignments were systematically analyzed using the maximum likelihood (ML) and Bayesian inference (BI) methods. BI and ML analyses were conducted separately through registering on the CIPRES website (Miller et al. 2012). For the ML analysis, RAxML-HPC2 v.8.2.12 was used on XSEDE with 1000 rapid bootstrap replications and the GTRGAMMA model (Stamatakis 2014). MrModeltest v.2.3 (Nylander 2004) software was utilized to screen for optimal evolutionary models, while BI was conducted using MrBayes 3.2.7a (on XSEDE) (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003; Ronquist et al. 2012). FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 14 February 2025) was used to open the successfully obtained topology and reroot the tree with the outgroup. The final phylogenetic tree was created with Adobe Illustrator CC 2019. In the final phylogenetic tree output, the names and strain numbers of the new species are marked in red.

Results

Phylogenetic analyses

Interspecific relationships of the genus *Beltraniella* were identified by phylogenetic analyses. These analyses were based on downloaded sequences and newly acquired sequences of new species, using *Beltrania pseudorhombica* Crous & Y. Zhang ter CBS 138003 and *B. querna* Harkn CBS 126097 as outgroups. The concatenated sequence matrix comprised 27 sequences with a total of 1295 characters (the combined dataset: ITS: 1–502, LSU: 503–1295). There were 1192 constant characters, 33 variable but parsimony non-informative, and 70 parsimony informative characters. The topologies of the evolutionary trees obtained using the maximum likelihood (ML) and

Table 1. GenBank numbers used in the phylogenetic analysis of *Beltraniella*.

Species	Strains	Country	GenBank accession numbers	
			ITS	LSU
Beltrania querna	CBS 126097	Spain	MH864016	MH875474
Beltrania pseudorhombica	CBS 138003*	China	MH554124	NG_058667
Beltraniella acaciae	CPC 29498*	USA	NR_147685	KY173483
Beltraniella botryospora	TMQa1A18	Japan	N/A	AB496426
Beltraniella brevis	DS 2-23	China	MN252876	MN252883
Beltraniella carolinensis	9502 (IFO)	N/A	N/A	DQ810233
Beltraniella dujiangyanensis	**SAUCC427003***	**China**	**PP301351**	**PP301362**
Beltraniella dujiangyanensis	**SAUCC427004**	**China**	**PP301352**	**PP301363**
Beltraniella endiandrae	CBS 137976*	Australia	NR_148073	KJ869185
Beltraniella endiandrae	CBS 137976	Australia	KJ869128	MH878615
Beltraniella fertilis	MFLUCC 20-0119	Thailand	MT835158	MT835156
Beltraniella fertilis	MRC 3BEL	Thailand	MF580247	MF580254
Beltraniella hesseae	BRIP 72433a*	Australia	OP023124	OP023141
Beltraniella humicola	CBS 203.64	India	MH858416	MH870044
Beltraniella jianfengensis	**SAUCC639001***	**China**	**PP301353**	**PP301364**
Beltraniella jianfengensis	**SAUCC639002**	**China**	**PP301354**	**PP301365**
Beltraniella jiangxiensis	CGMCC 3.23486*	N/A	OP022178	OP022174
Beltraniella myristicae	**SAUCC638601***	**China**	**PP301355**	**PP301366**
Beltraniella myristicae	**SAUCC638602**	**China**	**PP301356**	**PP301367**
Beltraniella pandanicola	MFLUCC 18-0121*	Thailand	MH275049	MH260281
Beltraniella podocarpi	CPC 36783*	South Africa	MT373370	NG_074446
Beltraniella portoricensis	CBS 856.70	N/A	MH859981	MH871777
Beltraniella pseudoportoricensis	CBS 145547*	South Africa	NR_165552	NG_067875
Beltraniella ramosiphora	MFLU 17-2649*	Thailand	NR_171732	NG_073615
Beltraniella thailandica	MFLUCC 16-0377*	Thailand	NR_168175	NG_068824
Beltraniella xinglongensis	SAUCC737701*	China	PQ325612	PQ325618
Beltraniella xinglongensis	SAUCC737702	China	PQ325613	PQ325619

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

Bayesian inference (BI) algorithms are essentially similar. Fig. 1 shows the best-scoring maximum likelihood (ML) evolutionary tree, where maximum likelihood bootstrap analyses and Bayesian posterior probabilities (MLBS/BPP) are labeled at node positions. Eight new strains of *Beltraniella* were incorporated into the phylogenetic analysis presented in the ML tree. The eight new strains introduced in this study were divided into four monophyletic branches in the phylogenetic tree, representing four new species of *Beltraniella*, *B. dujiangyanensis*, *B. jianfengensis*, *B. myristicae*, and *B. xinglongensis*. The strains of *Beltraniella dujiangyanensis* form a distinct clade sister to *B. xinglongensis* with good bootstrap support (ML/BI = 90/0.99); *B. thailandica* forms a high-support clade (ML/BI = 89/1.00) alongside the lineage consisting of *B. dujiangyanensis* and *B. xinglongensis*; *B. myristicae* forms a high-support clade (ML/BI = 94/1.00) with *B. brevis*; and *B. jianfengensis* forms a high-support clade (ML/BI = 81/0.98) with the lineage consisting of *B. brevis* and *B. myristicae*.
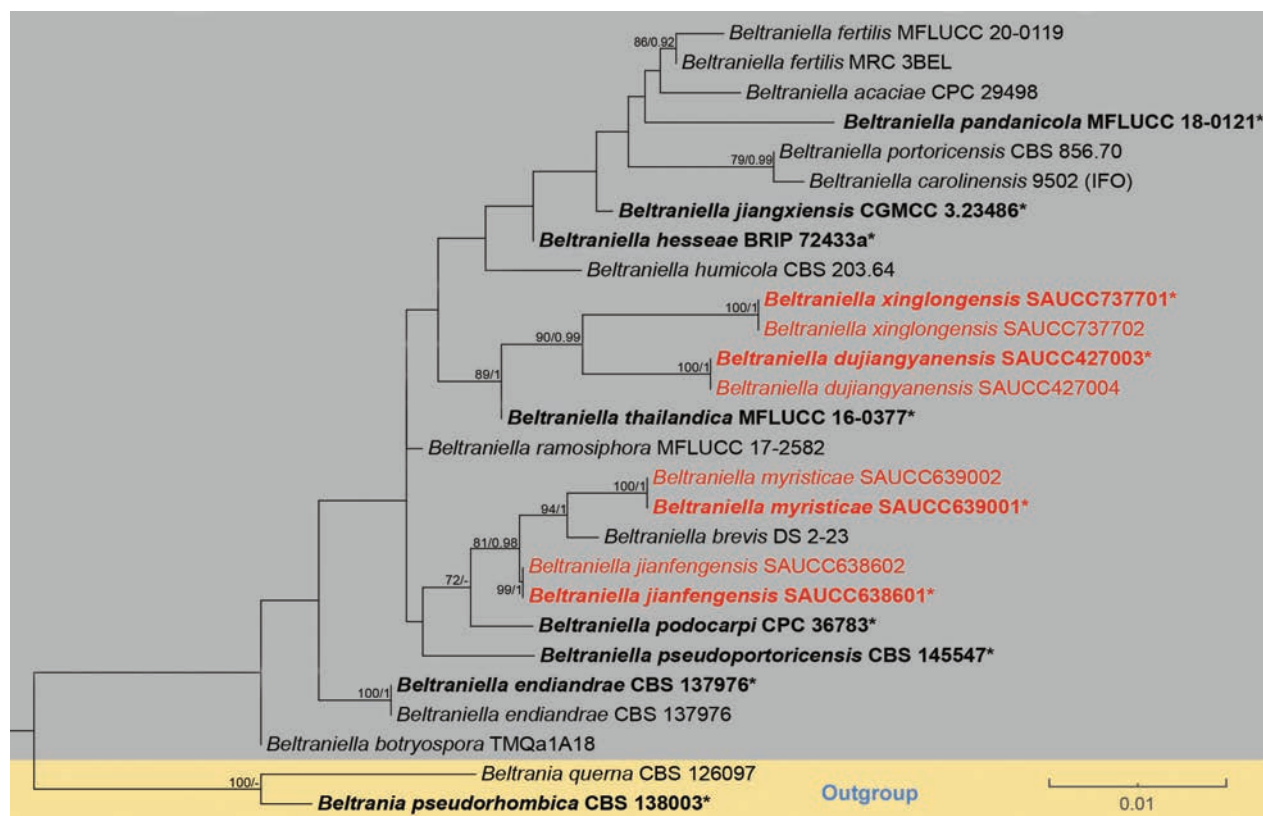
Figure 1. Phylogenetic tree of *Beltraniella* based on combined ITS and LSU sequences. Bootstrap support values exceeding 70% (ML) and 0.90 (BI) are indicated by MLBS/BPP, and new species are highlighted in red. Branches separated by gray and yellow indicate different species of *Beltraniella* and *Beltrania*. The lines in the lower right-hand corner represent changes of 0.01 nucleotides per site.

Taxonomy

Beltraniella dujiangyanensis W.W. Liu, C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov.
MycoBank No: MB853427
Fig. 2

Holotype. CHINA • Sichuan Province, Dujiangyan City, 30°57'53"N, 103°35'13"E, on decaying leaves, 24 June 2023, W.W. Liu, holotype HMAS 352921, ex-type living culture SAUCC427003.

Etymology. The epithet "*dujiangyanensis*" denotes the geographical origin of the strains, namely, Dujiangyan City.

Description. Parasitic on decaying leaves. Asexual morph: Setae unbranched, straight or flexuous, single, dark brown, subulate, thick-walled, tapering to a pointed apex, 9−10septate, verrucose, dark brown, swollen, arising from a radially lobed basal cell, 83.9−150.2 × 3.0−5.4 µm. Conidiophores hyaline, presenting two distinct forms: long and short. Long conidiophores arise from lobed basal cells, macronematous, erect, straight or slightly curved, either simple or rarely branched, septate, verrucose, dark-brown, apical part lighter, arising from basal cells of setae or from separate cells, 113.1−259.9 × 3.1−5.8 µm. Short conidiophores hyaline, septate, smooth edges, simple or branched, 13.1−31.9 × 3.2−5.7 µm. Conidiogenous cells polyblastic, integrated, determinate, cylindrical, smooth, terminal, geniculate, denticulate, hyaline

Figure 2. *Beltraniella dujiangyanensis* (holotype: HMAS 352921) **a, b** colony front and back after 7 days culture on PDA **c** setae **d** long conidiophores **e** short conidiophores **f** separating cells and conidia. Scale bars: 10 μm (**c−f**).

to subhyaline, 5.5−10.9 × 2.9−4.7 μm. Separating cells ellipsoid to subglobose, smooth, subhyaline, single, denticle at each end, 9.7−12.3 × 3.1−5.3 μm. Conidia originate directly from the conidiogenous cells in the long conidiophores and from the separating cells in the short ones. Conidia arise directly from conidiogenous cells or from separating cells, simple, teardrop-shaped, sometimes verrucose, narrow-tipped, terminal, hyaline, smooth, straight, rostrate to pointed at proximal end, truncate at distal end, 16.5−21.1 × 4.2−8.5 μm. Sexual morph: Inconclusive.

Culture characteristics. On PDA medium, after seven days of dark incubation in a 25 °C incubator, colonies reached 68 mm in diameter with a growth rate of 9.2–10.2 mm/day. Colonies on PDA medium were concentric, flatter, white, moderately dense, granular surface, sparse aerial mycelia, with mycelium in the middle portion aggregated into a circle and mycelium on the edges dispersed to form a fluffy shape; reverse, pale yellow to white, fluffy edges.

Additional material studied. China • Sichuan Province, Dujiangyan City, 30°57'53"N, 103°35'13"E, on decaying leaves, 24 June 2023, W.W. Liu, HSAUP 427004, living culture SAUCC427004.

Notes. Based on the phylogenetic tree constructed using ITS and LSU sequence data, *Beltraniella dujiangyanensis* was identified as the closest relative to *B. xinglongensis* sp. nov., with 90% MLBS and 0.99 BPP support values (Fig. 1). Additionally, there is a disparity of 16/502 bp between their ITS sequences. Morphologically, *B. dujiangyanensis* differed from *B. xinglongensis* in having shorter long conidiophores (*B. dujiangyanensis*: 113.1–259.9 × 3.1–5.8 μm vs. *B. xinglongensis*: 232.5–298.6 × 2.4–4.9 μm) and fewer septa (*B. dujiangyanensis*: 9–10 septa vs. *B. xinglongensis*: 13–15 septa), shorter in short conidiophores (*B. dujiangyanensis*: 13.1–31.9 × 3.2–5.7 μm vs. *B. xinglongensis*: 21.2–47.8 × 3.2–6.4 μm), shorter separating cells (*B. dujiangyanensis*: 9.7–12.3 × 3.1–5.3 μm vs. *B. xinglongensis*: 13.6–17.6 × 2.3–5.4 μm) and shorter conidia (*B. dujiangyanensis*: 16.5–21.1 × 4.2–8.5 μm vs. *B. xinglongensis*: 21.9–28.7 × 5.0–9.5 μm). As a result, *B. dujiangyanensis* was identified as a new species of *Beltraniella* by phylogenetic analysis and morphological comparison.

Beltraniella jianfengensis W.W. Liu, C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov.

MycoBank No: MB853429

Fig. 3

Holotype. China • Hainan Province, Ledong County, Jianfengling National Forest Park, 18°44'25"N, 108°51'32"E, on decaying leaves, 14 October 2023, W.W. Liu, holotype HMAS 352923, ex-type living culture SAUCC639001.

Etymology. The epithet "*jianfengensis*" signifies the geographical location of the holotype, specifically Jianfengling National Forest Park.

Description. Parasitic on decaying leaves. Asexual morph: Setae subulate, emerging from lobed basal cells, upright, straight or slightly curved, simple, septate, verrucose, dark-brown, swollen, radially lobed basal cell, 84.3–254.4 × 2.9–4.9 μm. Conidiophores macronematous, mononematous, occurring in two distinct forms: long and short. Long conidiophores arise from lobed basal cells and have a setiform appearance, upright, straight to slightly curved, simple or rarely branched, septate, verrucose, dark brown, swollen at the base, with a lighter apical region, arising from basal cells of setae or from separate ones, without a hyaline transverse band, 171.8–254.9 × 2.6–4.9 μm. Short conidiophores solitary or grouped, smoother, pale brown, smaller, 20.1–57.2 × 3.3–6.5 μm. Conidiogenous cells cylindrical, polyblastic, integrating sympodially, denticulate surface, 9.2–15.3 × 2.2–5.0 μm. Separating cells ellipsoid to subglobose, smooth, subhyaline, denticle at each end, 10.7–14.7 × 2.8–5.5 μm. Conidia originate directly from the conidiogenous cells on long conidiophores and from

Figure 3. *Beltraniella jianfengensis* (holotype: HMAS 352923) **a, b** colony front and back after 7 days culture on PDA **c** setae **d** long conidiophores and short conidiophores **e** conidia **f** separating cells and conidia. Scale bars: 10 μm (**c–f**).

the separating cells on short conidiophores, turbinated, obovate to obpyriform, subhyaline, simple, smooth, straight, terminal, 17.1–23.6 × 3.6–9.5 μm. Sexual morph: Inconclusive.

Culture characteristics. On PDA medium, after seven days of dark incubation in a 25 °C incubator, colonies reached 90 mm in diameter with a growth rate of 12.5–13.1 mm/day. Colonies on PDA, cottony, moderately dense, sparse aerial mycelia, steel-blue gray, granular surface, with gray exudates, flatter, smooth edge; reverse steel-blue gray, smooth edge.

Additional material studied. CHINA • Hainan Province, Ledong County, Jianfengling National Forest Park, 18°44'25"N, 108°51'32"E, on decaying leaves, 14 October 2023, W.W. Liu, HSAUP 639002, living culture SAUCC639002.

Notes. Based on the phylogenetic tree of ITS and LSU sequences, *Beltraniella jianfengensis* emerged as a cluster with *B. brevis* and *B. myristicae*. However, a significant discrepancy was noted in the ITS sequence, with a disparity of 6/502 bp between *B. jianfengensis* and *B. brevis*; and a disparity of 4/496 bp between *B. jianfengensis* and *B. myristicae*. Furthermore, a substantial difference was observed in their LSU sequences. Morphologically, *B. jianfengensis* was different from *B. brevis* by having narrower setae (*B. jianfengensis*: 84.3−254.4 × 2.9−4.9 vs. *B. brevis*: 89−251 × 4.5−10.5 µm), and shorter conidia (*B. jianfengensis*: 17.1−23.6 × 3.6−9.5 vs. *B. brevis*: 20−26.5 × 4.5−7.2 µm) (Hyde et al. 2020b), and there were also differences in conidial shape: *B. brevis* exhibited diamond-shaped conidia with a hyaline supraequatorial transverse band, whereas *B. myristicae* had teardrop-shaped conidia lacking a hyaline transverse band. Additionally, *B. jianfengensis* was different from *B. myristicae* by having longer and wider separating cells (*B. jianfengensis*: 9.2−15.3 × 2.8−5.5 vs. *B. myristicae*: 8.7−12.5 × 2.5−5.4), and there were also differences in separating cells' shape: *B. jianfengensis* features two distinct transverse projections on its surface, whereas *B. myristicae* boasts a smoother exterior devoid of such projections. Consequently, *B. jianfengensis* was classified as a new species within the genus *Beltraniella*, through a combination of phylogenetic analysis and morphological comparisons.

Beltraniella myristicae **W.W. Liu, C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov.**
MycoBank No: MB853428
Fig. 4

Holotype. CHINA • Hainan Province, Ledong County, Jianfengling National Forest Park, 18°44'25"N, 108°51'32"E, on diseased leaves of *Myristica fragrans* (Myristicaceae), 14 October 2023, W.W. Liu, holotype HMAS 352922, ex-type living culture SAUCC638601.

Etymology. The epithet "*myristicae*" is derived from the name of the host plant, *Myristica fragrans*.

Description. Associated with diseased leaves of *Myristica fragrans*, the surface of the leaf blade shows black irregular protrusions, marked with black circles and arrows in Fig. 4. Asexual morph: Setae dark-brown, simple, subulate, verrucose, 80.5−99.8 × 2.7−5.5 µm. Conidiophores present two distinct forms: long and short. Long conidiophores emerge from lobed basal cells, macronematous, setiform, upright, straight or gently curved, simple, septate, verrucose, subhyaline to pale olivaceous, swollen at the base, arising from basal cells of setae or from separate, 74.4−150.5 × 2.9−5.3 µm. Short conidiophores hyaline, septate, smooth edges, simple or branched, 18.3−45.0 × 2.7−5.8 µm. Conidiogenous cell polyblastic, ovoid, hyaline, 5.5−12.5 × 2.0−4.9 µm. Separating cells ellipsoid to subglobose, smooth, 7.0−12.8 × 2.5−5.4 µm. Conidia originate directly from the conidiogenous cells in the long conidiophores and from the separating cells in the short ones, aggregated, dry, straight, teardrop-shaped, truncate at distal end, narrow-tipped, terminal, hyaline, smooth, diaphragm, without a hyaline transverse band, 13.6−22.2 × 3.9−9.8 µm. Sexual morph: Inconclusive.

Figure 4. *Beltraniella myristicae* (holotype: HMAS 352922) **a** leaf of host plant *Myristica fragrans*, black circle and arrow indicate the location of fungal infestation **b, c** colony front and back after 7 days culture on PDA **d** setae **e** long conidiophores **f** short conidiophores **g** separating cells and conidia **h** conidia. Scale bars: 10 μm (**d–h**).

Culture characteristics. On PDA medium, after seven days of dark incubation in a 25 °C incubator, colonies reached 68 mm in diameter with a growth rate of 9.2–10.2 mm/day. Colonies on PDA raised, concentric, white, flatter, velutinous edge, with mycelium in the middle portion aggregated into a circle; reverse pale yellow, velutinous edge.

Additional material studied. CHINA • Hainan Province, Ledong County, Jianfengling National Forest Park, 18°44'25"N, 108°51'32"E, on diseased leaves of

Myristica fragrans, 14 October 2023, W.W. Liu, HSAUP 638602, living culture SAUCC638602.

Notes. Based on the phylogenetic tree constructed using ITS and LSU sequences, *Beltraniella myristicae* emerged as the closest to *B. brevis* DS 2-23 with 94% MLBS and 1.00 BPP support values (Fig. 1). However, a significant discrepancy was observed in the ITS sequence, with a disparity of 7/548 bp between *B. myristicae* and *B. brevis*. Morphologically, *B. myristicae* differed from *B. brevis* by having shorter setae (*B. myristicae*: 80.5−99.8 × 2.7−5.5 vs. *B. brevis*: 89−251 × 4.5−10.5 μm), shorter separating cells (*B. myristicae*: 7.0−12.8 × 2.5−5.4 vs. *B. brevis*: 11−18 × 3.4−4.1 μm), and shorter conidia (*B. myristicae*: 13.6−22.2 × 3.9−9.8 vs. *B. brevis*: 20−26.5 × 4.5−7.2 μm); differences in conidia (*B. brevis*: diamond-shaped, with a hyaline supraequatorial transverse band; *B. myristicae*: teardrop-shaped, without a hyaline transverse band). Consequently, *B. myristicae* was classified as a new species within the genus *Beltraniella*, based on a combination of phylogenetic analysis and morphological comparisons

Beltraniella xinglongensis W.W. Liu, C.Z. Yin, Z.X. Zhang & X.G. Zhang, sp. nov.

MycoBank No: MB856046

Fig. 5

Holotype. CHINA • Hainan Province, Wanning City, Xinglong tropical botanical garden, 18°43'59"N, 110°11'55"E, on decaying leaves, 24 April 2024, W.W. Liu, holotype HMAS 353196, ex-type living culture SAUCC737701.

Etymology. The epithet "*xinglongensis*" refers to the name of the location, Xinglong tropical botanical garden where the holotype was collected.

Description. Parasitic on decaying leaves. Asexual morph: Setae dark-brown, unbranched, tapering to a pointed apex, upright, single or in small groups, septate, straight or gently flexuous, emerging from radially lobed basal cells, 75.9−195.9 × 2.5−6.1 μm. Conidiophores septate, occasionally reduced to conidiogenous cells, smooth, swollen at the base, subhyaline to pale brown, present two distinct forms: long and short. Long conidiophores emerging from lobed basal cells, upright, straight or gently curved, simple or branched at apical regions, 13−15 septate, verrucose, swollen at the base, olivaceous to dark-brown, arising from basal cells of setae or from separate, 232.5−298.6 × 2.4−4.9 μm. Short conidiophores hyaline, septate, smooth, 21.2−47.8 × 3.2−6.4 μm. Conidiogenous cells ovoid, polyblastic, cylindrical, hyaline to subhyaline, integrated, denticulate, terminal, smooth, 6.5−9.7 × 2.8−5.4 μm. Separating cells fusiform, ellipsoid to subglobose, smooth, 13.6−17.6 × 2.3−5.4 μm. Conidia originate directly from the conidiogenous cells in the long conidiophores and from the separating cells in the short ones, teardrop-shaped, narrow-tipped, aggregated, terminal, simple, dry, straight, hyaline, smooth and integrated, without a hyaline transverse band, 21.9−28.7 × 5.0−9.5 μm. Sexual morph: Inconclusive.

Culture characteristics. On PDA medium, after seven days of dark incubation in a 25 °C incubator, colonies reached a diameter of 90 mm with a growth rate of 12.5−13.1 mm/day. Colonies on PDA raised, cottony, white, flatter, with gray exudates, sparse aerial mycelia, undulate margin; reverse white, with an undulate margin, abundant gray exudates.

Figure 5. *Beltraniella xinglongensis* (holotype: HMAS 353196) **a, b** colony front and back after 7 days culture on PDA **c** setae **d–f** long conidiophores **g** short conidiophores **h** separating cells and conidia **i** conidia. Scale bars: 10 μm (**d–i**).

Additional material studied. CHINA • Hainan Province, Wanning City, Xinglong tropical botanical garden, 18°43'59"N, 110°11'55"E, on decaying leaves, 24 April 2024, W.W. Liu, HSAUP 737701, living culture SAUCC737701.

Notes. Based on the phylogenetic tree constructed with ITS and LSU sequence, *Beltraniella dujiangyanensis* was the closest relative to *B. xinglongensis*, with a gap of 16/502 bp between their comparative ITS sequences. Morphologically, *B. xinglongensis* differed from *B. dujiangyanensis* in having longer long conidiophores (*B. xinglongensis*: 232.5–298.6 × 2.4–4.9 μm vs.

B. dujiangyanensis: 113.1–259.9 × 3.1–5.8 µm) and more septa (*B. xinglongensis*: 13–15 septa vs. *B. dujiangyanensis*: 9–10 septa), longer in short conidiophores (*B. xinglongensis*: 21.2–47.8 × 3.2–6.4 µm vs. *B. dujiangyanensis*: 13.1–31.9 × 3.2–5.7 µm), longer separating cells (*B. xinglongensis*: 13.6–17.6 × 2.3–5.4 µm vs. *B. dujiangyanensis*: 9.7–12.3 × 3.1–5.3 µm) and longer conidia (*B. xinglongensis*: 21.9–28.7 × 5.0–9.5 µm vs. *B. dujiangyanensis*: 16.5–21.1 µm). Consequently, *B. xinglongensis* was identified as a new *Beltraniella* species through phylogenetic analysis and morphological comparison

Discussion

With the increasing number of reported species within this genus, the classification of *Beltraniella* has encountered significant challenges. The observed striking similarity in spore structures, characterized by turbinate or biconic shapes, often with caudate appendages, as documented in early studies, accounts for this phenomenon (Hudson and Ellis 1972). Consequently, elucidating interspecific relationships within the genus using traditional morphological identification methods has been exceedingly challenging. We employed ITS and LSU to procure fungal DNA sequences, followed by the evaluation of phylogenetic relationships using maximum likelihood (ML) and Bayesian inference (BI) methods. To achieve a comprehensive and accurate classification, we complemented our analysis with morphological assessments (Miller et al. 2010; Stamatakis 2006; Stamatakis et al. 2008). In this investigation, four new species of fungi were identified and reported, namely *Beltraniella dujiangyanensis*, *B. jianfengensis*, *B. myristicae*, and *B. xinglongensis*. Based on DNA sequence analysis, these four new species were initially identified as *Beltraniella*. Subsequent phylogenetic analyses with other *Beltraniella* species confirmed their phylogenetic placement with high confidence (Ronquist and Huelsenbeck 2003; Crous et al. 2017; Liu et al. 2019). Subsequently, morphological assessments were conducted to discern the similarities and distinctions among *B. dujiangyanensis*, *B. jianfengensis*, *B. myristicae*, *B. xinglongensis*, and additional *Beltraniella* species within the same clade. The four species, *B. dujiangyanensis*, *B. jianfengensis*, *B. myristicae*, and *B. xinglongensis* were validated as new species within the genus *Beltraniella* based on conventional morphological analysis and molecular phylogenetic analysis. The *Beltraniella* taxon is minute, and a previous study employed phylogenetic analysis integrating ITS and LSU sequences with morphological analysis. The understanding of interspecific relationships has been enriched by the addition of supplementary morphological descriptions. Index Fungorum (https://indexfungorum.org/Names/Names. asp, accessed on 14 February 2025) listed 33 species, of which 25 were recorded in the National Center for Biotechnology Information (NCBI) (https://www. ncbi.nlm.nih.gov/, accessed on 14 February 2025). To ensure data reliability, all pertinent sequences of these 25 species were incorporated.

Beltraniella demonstrates a global distribution, supported by the GlobalFungi database (https://globalfungi.com/, accessed on 14 February 2025) comprising 1098 samples and 1626 sequences. Specifically, *Beltraniella* was detected in Asia (57.92%), South America (19.31%), North America (9.11%), Africa (7.1%), Australia (4.28%), Europe (1.73%), Pacific Ocean (0.27%), Antarctica (0.18%), and Indian Ocean (0.09%). The samples used in this study originated from

Sichuan and Hainan Provinces, which are characterized by the Central Subtropical Monsoon Humid Climate and the Tropical Rainforest Climate, respectively. These regions are characterized by abundant precipitation, a humid climate, diverse vegetation, and a rich assortment of fungi, including *Beltraniella*. In addition, *Beltraniella* is recognized as an invasive fungus affecting a broad spectrum of plants and has been observed parasitizing diverse plant leaves. For instance, two *Beltraniella* species, *B. botryospora* and *B. portoricensis*, were detected on the deciduous leaves of representative plants from the Atlantic Forest (*Inga thibaudiana*, *Myrcia splendens*, and *Pera glabra*) (dos Santos et al. 2014). Furthermore, the presence of *B. endiandrae* was verified on the fallen leaves of a Lauraceae plant in Nightcap National Park, New South Wales, Australia. (Crous et al. 2014). Our analysis of *Beltraniella*'s sequence and morphological characteristics revealed its parasitism on diseased leaves of *Myristica fragrans* and decaying leaves. Specifically, *B. dujiangyanensis*, *B. jianfengensis* and *B. xinglongensis* were identified as parasites on decaying leaves, whereas *B. myristicae* was observed to parasitize diseased leaves of *Myristica fragrans*. These findings indicate the potential existence of additional host species of *Beltraniella* among these two hosts (Hyde et al. 2007). Therefore, we expect to discover additional species of *Beltraniella* fungi through the collection of diseased leaves of *Myristica fragrans* and decaying leaves in Hainan and Sichuan Provinces. Ultimately, this research enhances our understanding of fungal diversity in Hainan and Sichuan provinces and expands the known species range of *Beltraniella* fungi.

Conclusions

In this study, a wide range of new fungal species were isolated from a large collection of diseased and decaying leaves gathered from Sichuan and Hainan provinces, China. Through rigorous phylogenetic analysis and examination of morphological characteristics, we successfully identified four new species within the genus *Beltraniella*. The pathogenicity and host associations of these newly reported *Beltraniella* fungi are relatively underexplored, necessitating further research. Building on the insights from this study, we anticipate that a more targeted collection of diseased and decaying leaves will expedite the isolation and characterization of further potential *Beltraniella* fungi.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Author ORCIDs

Wen-Wen Liu ⬤ https://orcid.org/0009-0009-3477-194X
Chang-Zhun Yin ⬤ https://orcid.org/0009-0000-0034-2199
Zhao-Xue Zhang ⬤ https://orcid.org/0000-0002-4824-9716
Xing-Sheng Wang ⬤ https://orcid.org/0009-0005-1127-7368
Shi Wang ⬤ https://orcid.org/0000-0002-7376-7638

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

DNA sequences

Authors: Wen-Wen Liu, Chang-Zhun Yin, Zhao-Xue Zhang, Xing-Sheng Wang, Zhe Meng, Xiu-Guo Zhang, Shi Wang

Data type: fas

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MycoKeys

Research Article

Five new species of *Cortinarius* (Cortinariaceae) from Yunnan, China, based on molecular and morphological evidence

Liu-Kun Jia[1,2,3*] , Zi-Rui Wang[1,2,3,4*] , Zhu-Liang Yang[1,2]

1 *State Key Laboratory of Phytochemistry and Natural Medicines, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China*

2 *Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China*

3 *College of Life Sciences, University of Chinese Academy of Sciences, Beijing, 101408, China*

4 *School of Ecology and Environmental Science, Yunnan University, Kunming, 650500, China*

Corresponding author: Zhu-Liang Yang (*fungi@mail.kib.ac.cn*)

Abstract

Cortinarius is a globally distributed, exceptionally species-rich genus of Cortinariaceae, serving as important ectomycorrhizal fungi. Yunnan province, located in southwestern China, boasts a vast array of environmental conditions and fungal resources, with numerous new *Cortinarius* species yet to be discovered. Based on morphological evidence and phylogenetic inference using a two-locus dataset, five novel species have been identified within the genus, namely *C. brunneoverrucosus*, *C. coriaceus*, *C. fuscocandidus*, *C. neodisjungendus*, and *C. sinoconfirmatus*. Notably, two of these species (*C. brunneoverrucosus* and *C. neodisjungendus*) occur in subtropical areas, while the other three species (*C. coriaceus*, *C. fuscocandidus*, and *C. sinoconfirmatus*) inhabit subalpine temperate areas. Taxonomic descriptions for these five species are provided.

Key words: Diversity, morphology, new taxa, phylogeny, taxonomy

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Introduction

Cortinarius (Pers.) Gray, belonging to the order Agaricales, is the most species-rich genus within the family Cortinariaceae. Currently, over 2,000 species have been formally described (Liimatainen et al. 2022). It is widely distributed across tropical to subpolar areas in both the Northern and Southern Hemispheres, holding irreplaceable ecological, research, and economic value (Kirk et al. 2008; Soop et al. 2019; Liimatainen et al. 2020, 2022). Based on recent genomic and multi-locus sequence data, *Cortinarius* sensu lato has been split into ten genera, with the core groups within *Cortinarius* s. l. being transferred to *Cortinarius* sensu stricto, emended in Liimatainen et al. (2022).

Cortinarius s.s., typified by *C. violaceus* (L.) Gray, has several distinguishing features. These include a pileus adorned with fibrillose squamules, a fibrillose cortina, a negative KOH reaction, and rusty brown basidiospores with weakly to strongly verrucose ornamentations. Additionally, it features a duplex pileipellis with a hypoderm that is variably developed. The basidiomata vary widely in size, ranging from very small to large, and can be dry to glutinous in texture.

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* These authors contributed equally to this work.

They exhibit a diverse array of colors, with brown being the most common (Bidaud et al. 2000, 2012; Liimatainen et al. 2015, 2017, 2020, 2022; Soop et al. 2019; Ammirati et al. 2021; Zhou et al. 2023; Long et al. 2024). Liimatainen et al. (2022) identified 11 recognized subgenera under *Cortinarius* s.s., including subgen. *Cortinarius*, subgen. *Dermocybe* (Fr.) Trog, subgen. *Illumini* Liimat., Niskanen & Kytöv, subgen. *Leprocybe* M.M. Moser, subgen. *Iodolentes* Niskanen & Liimat., subgen. *Orellani* (M.M. Moser) Gasparini, subgen. *Telamonia* (Fr.) Trog, subgen. *Infracti* Niskanen & Liimat, subgen. *Camphorati* Liimat., Niskanen & Ammirati, subgen. *Myxacium* (Fr.) Trog, and subgen. *Paramyxacium* M.M. Moser & E. Horak.

The research on the genus *Cortinarius* primarily originated and has remained concentrated in Europe, North America, and Oceania (Peintner et al. 2002a, 2002b, 2004; Garnica et al. 2003, 2005; Liimatainen et al. 2014, 2015, 2017, 2020, 2022; Niskanen 2014, 2020; Soop et al. 2019), whereas studies in East Asia are still insufficient. Since the reporting of *C. testaceus* Cooke in China by Teng and Ou (1937), numerous Chinese mycologists have described *Cortinarius* species across various areas, including northeast, north, east, south, and southwestern parts of China (Deng 1963; Li 1980; Mao and Zong 1988; Ying and Zang 1994; Zang 1996; Mao 2000, 2009). However, most of these species' names recorded by the aforementioned studies are based on those from Europe, North America, and Oceania, and their distinctiveness in China awaits confirmation through molecular evidence. Recently, based on a combination of morphological and molecular systematic evidence, 26 new *Cortinarius* species from China have been published (Wei and Yao 2013; Xie et al. 2019, 2020, 2021a, 2021b, 2022, 2023; Yuan et al. 2020; Luo and Bau 2021; Zhang et al. 2023; Zhou et al. 2023; Long et al. 2024), indicating that the species diversity of *Cortinarius* in China is high, and potential undiscovered species may exist within the genus.

In this study, five *Cortinarius* species new to science were identified in Yunnan, southwestern China. Based on a combination of morphological observations and phylogenetic analysis, we provide descriptions of these species.

Materials and methods

Specimens and morphological description

Macro-morphological characteristics were described based on fresh basidiomata, detailed field notes, and photographs taken in situ. Colors in the descriptions were coded following Kornerup and Wanscher (1981). The basidiomata size, determined by pileus width, was categorized as tiny (< 1.5 cm), small (1.5–3 cm), medium-sized (3–5 cm), or large (> 5 cm). Additionally, 'L' refers to the number of lamellae reaching the stipe, while 'l' denotes the number of lamellulae located between two lamellae.

Microscopic structures were observed with light microscopy under a ZEISS Axiostar Plus microscope. Dried specimens were sectioned and mounted in a 5% KOH solution or Melzer's reagent. Congo Red staining was applied when necessary. For observing basidiospore ornamentations, small hymenophoral fragments were taken from dried specimens, mounted on aluminum stubs with double-sided adhesive tape, coated with gold-palladium, and then observed under a ZEISS Sigma 300 scanning electron microscope (SEM) at the Kunming Institute of Botany, Chinese Academy of Sciences.

In the descriptions of basidiospores, the abbreviation [n/m/p] indicates that 'n' basidiospores were measured from 'm' basidiomata of 'p' collections. Dimensions are presented in the form (a−)b−c(−d), where the range 'b−c' includes a minimum of 90% of the measured values, with extreme values "a" or 'd' given in parentheses. The ratio of basidiospore length to width in side view is represented by Q. The mean values and average Q of basidiospores, along with standard deviations, are indicated as "av." and 'Qav.', respectively. Basidiospore shapes were determined based on descriptions by Bas (1969) and Kirk et al. (2008).

The studied collections were deposited in the Cryptogamic Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

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

Total genomic DNA was extracted from dried specimens using an Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, Shanghai, China). The ITS region was amplified using the primers ITS1F/ITS4. For older specimens, primer combinations ITS1F/ITS2 and ITS3/ITS4 were also employed (White et al. 1990; Gardes and Bruns 1993). The ribosomal large subunit 28S region (nrLSU) was amplified using the primers LROR/LR5 (Vilgalys and Hester 1990; Hopple and Vilgalys 1994).

PCR reactions were conducted using an ABI 2720 Thermal Cycler, Veriti™ Dx 96-Well Thermal Cycler, or SimpliAmp™ Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The PCR settings for the ITS1F/ITS4 were 94 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 52 °C for 40 s, and 72 °C for 1 min, with a final extension at 72 °C for 8 min. For the LROR/LR5 primers, the settings were 94 °C for 5 min, followed by 35 cycles of 94 °C for 40 s, 50 °C for 40 s, and 72 °C for 1 min, with a final extension at 72 °C for 8 min.

The PCR products were purified using a Gel Extraction and PCR Purification Combo Kit (Spin-column) (Bioteke, Beijing, China). After purification, the products were sequenced on an ABI-3730-XL DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the same primer combinations as those used for the PCR.

Phylogenetic analysis

Forward and reverse sequences were assembled and edited with SeqMan (DNA STAR package; DNAStar Inc., Madison, WI, USA). The ITS sequences were used to infer related taxa through a BLASTn search in GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The top hits in the BLASTn results confirmed that our specimens belonged to the genus *Cortinarius*. Related species were selected for the phylogenetic analyses based on BLASTn results (> 90% identity) and references from publications by Soop et al. (2019), Liimatainen et al. (2017, 2020), and Ammirati et al. (2021). A total of 64 collections representing 57 species were included in this study, with five species from the sect. *Leprocybe* selected as outgroups.

Alignments were constructed using MAFFT v7.3.10 (Katoh and Standley 2013) and optimized using BioEdit v7.2.5 (Hall 1999). The final alignments have been submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S31924).

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods, implemented in IQ-TREE v2.2.0 (Nguyen et al. 2015) and MrBayes 3.2.7 (Ronquist et al. 2012). The best-fit substitution model for ML analyses using the ITS+nrLSU matrix was determined with the '-MFP'

Table 1. DNA substitution models selected for phylogenetic analysis based on the ITS-nrLSU matrix.

Loci	Models for maximum likelihood (IQ-TREE)	Models for Bayesian inference (MrBayes)
ITS	TIM2+F+I+R2	lset applyto = (ITS) nst = 6 rates = Invgamma
nrLSU	TN+F+I	lset applyto = (nrLSU) nst = 2 rates = Propinv prset applyto = (all) statefreqpr = Dirichlet(1,1,1,1)

option in IQ-TREE v2.2.0 (Kalyaanamoorthy et al. 2017) based on the Akaike Information Criterion (AIC) (Table 1). For ML analyses, 1,000 replicates of the Shimodaira-Hasegawa-like aLRT test (SH-aLRT) (Guindon et al. 2010) and 1,000 replicates of the ultrafast bootstrap (UFB) (Hoang et al. 2018) were set. The '-p' option was used for reading the partition file (ITS: 1–744; nrLSU: 745–1627), while other parameters remained at default settings. For BI analyses, substitution models were selected based on IQ-TREE v2.2.0 outputs (Table 1). Four Markov chains were run twice from random starting trees for 10 million generations, with sampling every 100th generation. The stop value (stopval) was set to 0.001. Parameters and sampled trees were summarized after discarding the first 25% of trees as burn-in using the 'sump' and 'sumt' commands in MrBayes 3.2.7.

Results

Molecular analyses

The dataset comprised a total of 78 sequences, including 24 newly generated and 54 downloaded sequences (64 ITS, 14 nrLSU) from 64 collections representing 57 species (Table 2). The concatenated dataset (ITS-nrLSU) consisted of 1,627 positions after excluding poorly aligned regions. Accession numbers for all the sequences used for molecular analyses are provided in Table 2. Both ML and BI trees exhibited the same topology; therefore, only the ML tree, with the SH-aLRT support values, UFB values, and Bayesian posterior probabilities (BPP), is shown (Fig. 1).

Taxonomy

***Cortinarius brunneoverrucosus* Zhu L. Yang, Liu K. Jia & Zi R. Wang, sp. nov.**
MycoBank No: 857350
Fig. 2

Etymology. The epithet "*brunneoverrucosus*" (Lat.) refers to the pileus with brown verrucose squamules of this species.

Holotype. CHINA • Yunnan Province: Pu'er City, Jingdong Yi Autonomous County Ailao Mountain Subtropical Forest Ecosystem Research Station, Chinese Academy of Sciences, in a subtropical broad-leaved forest with trees of *Lithocarpus*, 24°32.57'N, 101°1.62'E, elevation 2,491 m, 23 July 2013, Yang-Yang Cui 32 (KUN-HKAS 79712). GenBank: ITS: PQ772212, nrLSU: PQ772224.

Diagnosis. *Cortinarius brunneoverrucosus* is sister to *C. corrugatus* Peck but differs by its yellowish brown to brown pileus with brown verrucose squamules, more robust stipe, relatively wider basidiospores, and exclusive occurrence in subtropical broad-leaved forest with trees of *Lithocarpus* and *Quercus* (Peck 1872; Phillips 2010; Kuo 2020).

Table 2. Voucher information, GenBank accession numbers of the samples used in the phylogenetic analysis.

Taxa	voucher	Status	Locations	Section	GenBank Accession No.		Sequence origin
					ITS	nrLSU	
Cortinarius aff. *leucophaeatus*	S: CFP536		Sweden	*Telamonia*	KC608590	in ITS	Liimatainen et al. 2020
C. aff. *tenebricus*	G: 056		France	*Verni*	MT935483	-	Liimatainen et al. 2020
C. albolens	PC: A. Bidaud 97-10-368	Holotype	France	*Hinnulei*	MT934855	in ITS	Liimatainen et al. 2020
C. ammophiloides	BP: 57443	Holotype	Hungary	*Verni*	NR_171309	-	Liimatainen et al. 2020
C. badioflavidus	WTU: JFA13668	Holotype	USA	*Hinnulei*	KU041723	in ITS	Liimatainen et al. 2020
C. boulderensis	MICH: AHS17461	Holotype	USA	*Boulderenses*	DQ499466	in ITS	Liimatainen et al. 2020
C. brunneofibrillosus	WTU: JFA13654	Holotype	USA	*Leprocybe*	MW009188	-	Ammirati et al. 2021
C. brunneoverrucosus	**KUN-HKAS 79712**	**Holotype**	**China**	***Dulciolentes***	**PQ772212**	**PQ772224**	**This study**
C. brunneoverrucosus	**KUN-HKAS 145321**		**China**	***Dulciolentes***	**PQ772211**	**PQ772223**	**This study**
C. claroplaniusculus	PC: RH2334	Holotype	France	*Disjungendi*	NR_131844	-	Liimatainen et al. 2020
C. confirmatus	PC: RH84/159		Italy	*Saturnini*	KX964440	in ITS	Liimatainen et al. 2017
C. confirmatus	PC: RH3195	Holotype	France	*Saturnini*	KX964438	in ITS	Liimatainen et al. 2017
C. coriaceus	**KUN-HKAS 145314**		**China**	***Telamonia***	**PQ772201**	**PQ772213**	**This study**
C. coriaceus	**KUN-HKAS 145315**		**China**	***Telamonia***	**PQ772203**	**PQ772215**	**This study**
C. coriaceus	**KUN-HKAS 145316**	**Holotype**	**China**	***Telamonia***	**PQ772202**	**PQ772214**	**This study**
C. corrugatus	IB: 2000544		North America	*Dulciolentens*	AF325611	in ITS	Soop et al. 2019
C. disjungendulus	H: IK98-861	Holotype	Sweden	*Disjungendi*	NR_131838	-	Liimatainen et al. 2020
C. disjungendus	H: PAK4370	Holotype	Finland	*Disjungendi*	KP013190	in ITS	Liimatainen et al. 2020
C. dulciolens	PDD: 68471	Holotype	New Zealand	*Dulciolentens*	NR_157914	-	Soop et al. 2019
C. flavifolius	EH230	Epitype	USA	*Leprocybe*	MW009217	in ITS	Ammirati et al. 2021
C. fructuodorus	H: 7001104	Holotype	USA	*Telamonia*	NR_131827	in ITS	Liimatainen et al. 2020
C. fulvopaludosus	H: 6033460	Holotype	Finland	*Hinnulei*	MG136823	in ITS	Liimatainen et al. 2020
C. fuscocandidus	**KUN-HKAS 69792**		**China**	***Hinnulei***	**PQ772209**	**PQ772221**	**This study**
C. fuscocandidus	**KUN-HKAS 70198**	**Holotype**	**China**	***Hinnulei***	**PQ772210**	**PQ772222**	**This study**
C. fuscovelatus	H: IK00-036	Holotype	Sweden	*Boulderenses*	NR_131888	-	Liimatainen et al. 2020
C. hinnuleus	TUB: 011905		Sweden	*Hinnulei*	AY669667	in ITS	Liimatainen et al. 2020
C. hughesiae	WTU: JFA13086	Holotype	USA	*Leprocybe*	MW009224	in ITS	Ammirati et al. 2021
C. imbutus	H: IK97-1162	Neotype	Finland	*Saturnini*	KX964498	in ITS	Liimatainen et al. 2017
C. ionophyllus	IB: MM1949-0052	Holotype	Austria	*Telamonia*	MT935168	-	Liimatainen et al. 2020
C. leproleptopus	PC: RH84-109	Holotype	France	*Leprocybe*	MW009226	in ITS	Ammirati et al. 2021
C. leucophaeatus	H: IK97-138		Finland	*Telamonia*	MT935196	in ITS	Liimatainen et al. 2020
C. lucorum	S: CFP490	Neotype	Norway	*Saturnini*	KX964585	in ITS	Liimatainen et al. 2017
C. malachius	G: 452		France	*Malachii*	MT934962	-	Liimatainen et al. 2020
C. melanotus	S: CFP1101	Epitype	France	*Leprocybe*	MW009230	-	Ammirati et al. 2021
C. montebelloensis	H: TN10-149	Holotype	Canada	*Disjungendi*	KP114459	in ITS	Liimatainen et al. 2020
C. neodisjungendus	**KUN-HKAS 145322**	**Holotype**	**China**	***Cinnabarini***	**PQ772207**	**PQ772219**	**This study**
C. neodisjungendus	**KUN-HKAS 145323**		**China**	***Cinnabarini***	**PQ772208**	**PQ772220**	**This study**
C. niveotraganus	H: IK98-033	Holotype	Finland	*Telamonia*	NR_131842	-	Liimatainen et al. 2020
C. odoritraganus	H: 7057490	Holotype	Canada	*Telamonia*	MT112154	-	Liimatainen et al. 2020
C. odoritraganus	MICH: 10398/G: 00121		USA	*Telamonia*	NR_170852	MK277857	Liimatainen et al. 2020
C. olididisjungendus	H: 7000854	Holotype	Canada	*Disjungendi*	NR_131839	-	Liimatainen et al. 2020
C. orasericeus	PC: RH70239	Holotype	France	*Disjungendi*	KP013203	in ITS	Liimatainen et al. 2020
C. peraurantiacus	PDD: 70818		New Zealand	*Dulciolentens*	KC520543	in ITS	Soop et al. 2019
C. piceidisjungendus	H: TN11-443	Holotype	USA	*Disjungendi*	NR_131840	-	Liimatainen et al. 2020
C. pisciodorus	PDD: 27062/JAC 13813	Holotype	New Zealand	*Dulciolentens*	MN492664	MH108417	Soop et al. 2019
C. psammocola	H: IK99-722	Holotype	Finland	*Verni*	MG136821	-	Liimatainen et al. 2020
C. pseudobovinus	IB: MM1989-0300	Holotype	USA	*Boulderenses*	DQ499465	in ITS	Liimatainen et al. 2020
C. roseonudipes	G: 37	Holotype	France	*Hinnulei*	MT935391	-	Liimatainen et al. 2020
C. rubrovioleipes	GK: 13271/635a		Switz	*Boulderenses*	MT934924	in ITS	Liimatainen et al. 2020
C. saturninus	S: CFP514	Neotype	Sweden	*Saturnini*	KX964584	in ITS	Liimatainen et al. 2017
C. semiodoratus	TUB: 011512		-	*Hinnulei*	AY669665	in ITS	Liimatainen et al. 2020
C. sinoconfirmatus	**KUN-HKAS 145318**		**China**	***Saturnini***	**PQ772206**	**PQ772218**	**This study**
C. sinoconfirmatus	**KUN-HKAS 145319**		**China**	***Saturnini***	**PQ772204**	**PQ772216**	**This study**
C. sinoconfirmatus	**KUN-HKAS 145320**	**Holotype**	**China**	***Saturnini***	**PQ772205**	**PQ772217**	**This study**
C. suberi	S: F16406	Holotype	Sweden	*Malachii*	MT935480	-	Liimatainen et al. 2020
C. suberi	S: F14331		Sweden	*Malachii*	MT934927	in ITS	Liimatainen et al. 2020

Taxa	voucher	Status	Locations	Section	GenBank Accession No.		Sequence origin
					ITS	nrLSU	
C. subionophyllus	H: TN06-050	Holotype	Norway	*Telamonia*	MF379634	-	Liimatainen et al. 2020
C. subpulchrifolius	MICH: 10419	Lectotype	USA	*Telamonia*	NR_170855	in ITS	Liimatainen et al. 2020
C. tigrinipes	G: 874	Holotype	France	*Telamonia*	MT935549	in ITS	Liimatainen et al. 2020
C. torvus	S: CFP778	Epitype	Sweden	*Telamonia*	MT935556	in ITS	Liimatainen et al. 2020
C. veneto-occidentalis	H: TN11-051	Holotype	USA	*Leprocybe*	MW009243	in ITS	Ammirati et al. 2021
C. vernus	BP:58132		Hungary	*Verni*	MT935033	in ITS	Liimatainen et al. 2020
C. vernus	CHEV 3130-T		France	*Verni*	FN429003	-	Suárez-Santiago et al. 2009
C. venustus	H: PAK3234	Holotype	Finland	*Telamonia*	MT935132	in ITS	Liimatainen et al. 2020

Newly generated sequences were marked in bold.
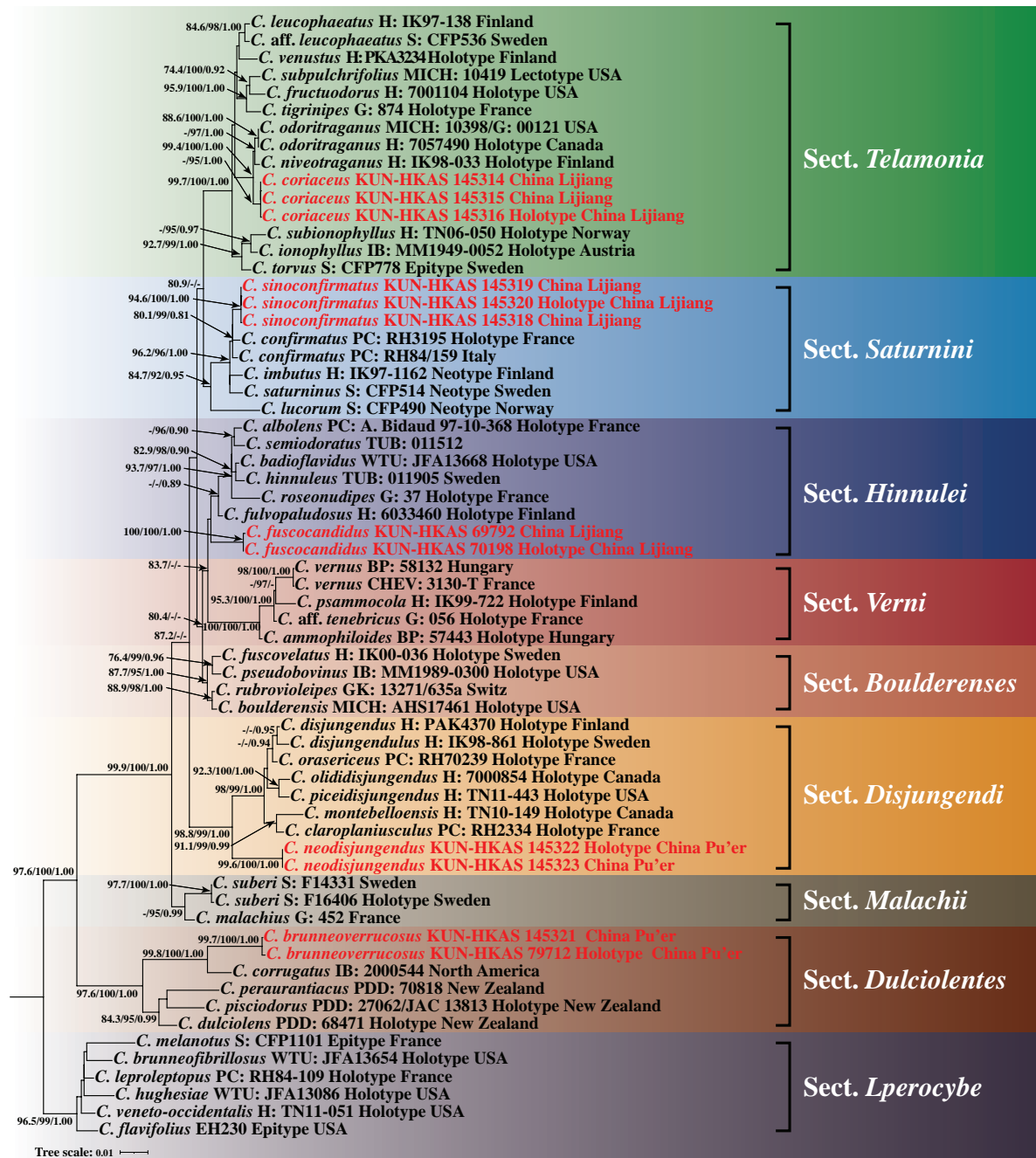
Figure 1. Maximum-likelihood phylogenetic tree of *Cortinarius* inferred from the concatenated ITS-nrLSU matrix. SH-aLRT support values ≥ 80%, UFB values ≥ 90% for ML, and BPP values ≥ 0.80 for BI are shown above the nodes as SH-aLRT/UFB/BPP. Sequences generated in this study are highlighted in red.
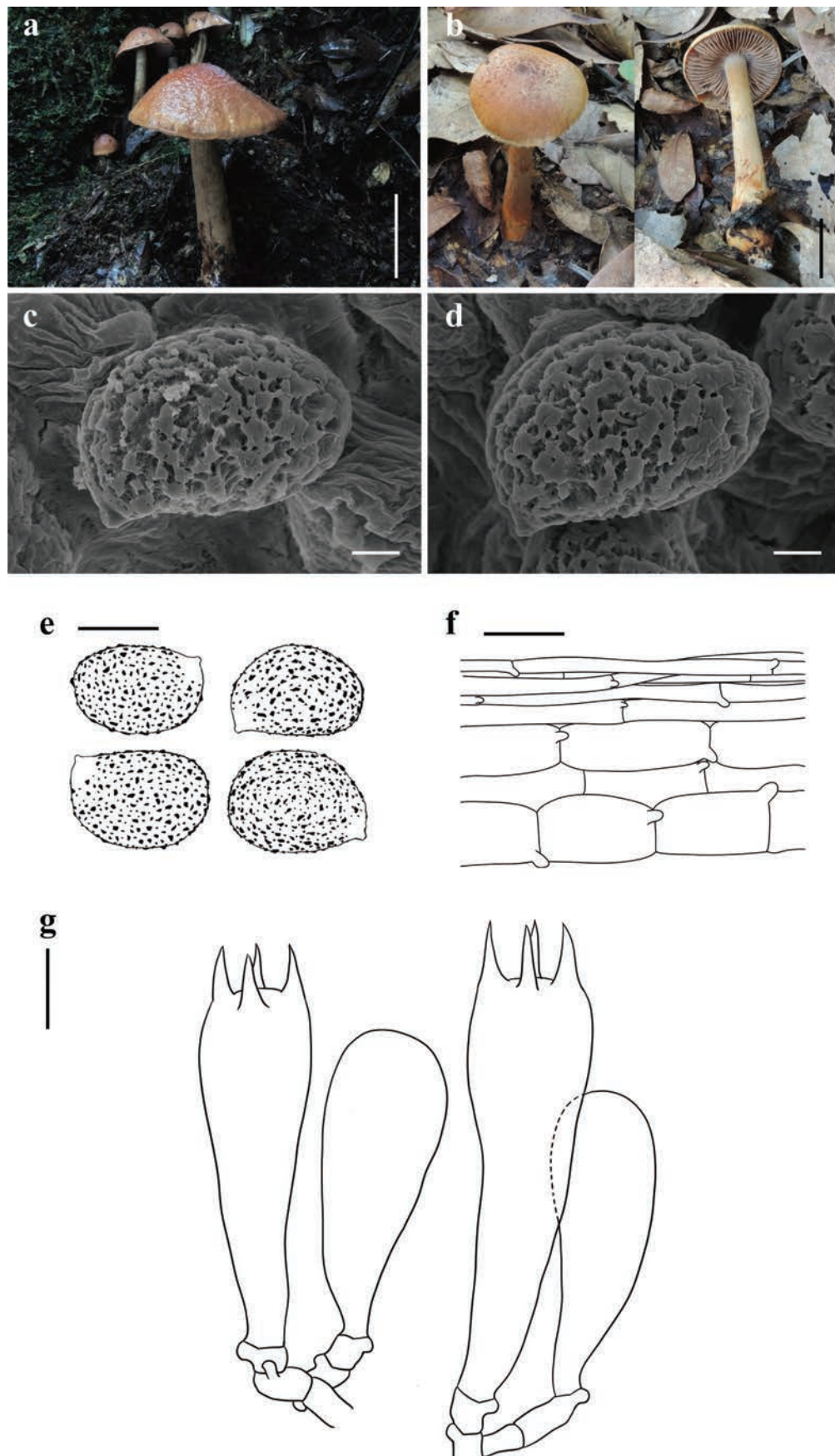
Figure 2. *Cortinarius brunneoverrucosus* (**a, c–g** KUN-HKAS 79712, Holotype **b** KUN-HKAS 145321) **a, b** basidiomata **c–e** basidiospores **f** pileipellis **g** basidia; and marginal sterile cells. Scale bars: 5 cm (**a, b**); 2 μm (**c, d**); 10 μm (**e**); 20 μm (**f, g**).

Description. Basidioma large. **Pileus** 8–10.5 cm diam, hemispherical, viscid, verrucose; yellow-brown to brown (5B7–5C7), darker towards the center (5D8), paler towards the margin (5B3–5B5); covered with brown (5C7) to dark brown (5D8–5E8) verrucose to floccose squamules; margin with innate radial stripes, occasionally with pale yellow (4A2) floccose squamules; context of pileus white (1A1). **Lamellae** adnate with decurrent tooth, crowded (L = 64–73, l = 33–38), pale brown (6A2–6A4) with a faint pale pinkish (12A2) tint. **Stipe** 8.5–18 × 1.2–2 cm, tapering upwards, pale brown (6A2–6A4) to pale yellow (3A2–3A4), covered with brown (6C4) to orange-brown (5A8) fibrillose squamules; context of stipe white (1A1); basal mycelium white (1A1) with a faint pale pinkish (12A2) tint.

Basidiospores [60/2/2] (12.5–)15–16.5(–17.5) × (10–)11.5–12.5(–15) μm, Q = 1.2–1.5(–1.75), av. = 15.64 ± 1.61 × 12.31 ± 1.48 μm, Qav. = 1.27 ± 0.12, broadly ellipsoid to broadly amygdaliform, strongly verrucose, inamyloid. **Basidia** 37.5–50 × 7.5–10 μm, 4-spored, clavate. **Trama of lamellae** regular, composed of colorless to yellowish, smooth hyphae 10–12.5 μm wide. **Cystidia** absent. **Pileipellis** duplex: epicutis weakly developed, 12–15 μm thick, composed of only 3–5 layers of interwoven to parallel, colorless to yellowish, smooth, thin-walled, long-celled hyphae 2.5–4 μm wide; hypocutis composed of parallel, colorless to yellowish brown, cylindrical, thin-walled hyphae 12.5–20 μm wide. **Clamp connections** common in all parts of basidioma.

Habitat/host. Summer to autumn. Solitary on soil in subtropical broad-leaved forests with trees of Fagaceae.

Distribution. Currently known from southwestern China.

Additional specimen examined. CHINA • Yunnan Province: Pu'er City, Jingdong Yi Autonomous County, Ailao Mountain Subtropical Forest Ecosystem Research Station, Chinese Academy of Sciences, in a subtropical broad-leaved forest with trees of *Quercus*, 24°32.57'N, 101°1.62'E, elevation 2,424 m, 8 October 2021, Jian-Wei Liu 2440 (KUN-HKAS 145321).

Notes. *Cortinarius brunneoverrucosus* is characterized by its hemispherical, viscid, verrucose pileus, pale brown lamellae with a slightly pale pinkish tint, and relatively larger, broadly ellipsoid to ellipsoid basidiospores.

Cortinarius brunneoverrucosus is sister to *C. corrugatus* Peck, originally described from the highlands in the United States, under *Aalmia latifolia*, but *C. brunneoverrucosus* is only found in subtropical China, under trees of *Lithocarpus* or *Quercus*. Moreover, *C. corrugatus* differs from *C. brunneoverrucosus* by its convex to broadly convex pileus with distinctively corrugated-wrinkled, thinner stipe, amygdaliform, relatively narrower basidiospores (12–15 × 8–10 μm) (Peck 1872; Phillips 2010; Kuo 2020).

Cortinarius brunneoverrucosus belongs to sect. *Dulciolentes* Soop, a small section that has previously included only seven species, mainly distributed in Australia, inhabiting forests with Fagaceae, Nothofagaceae, and Myrtaceae (Soop et al. 2019). However, excluding *C. corrugatus*, which is from North America and is agaricoid, as mentioned earlier, three other species from Oceania, *C. peraurantiacus* Peintner & M.M. Moser, *C. pisciodorus* (E. Horak) Peintner & M.M. Moser, and *C. dulciolens* E. Horak, M.M. Moser, Peintner & Vilgalys, are all sequestrate (Moser 1983; Peintner et al. 2002a, 2002b; Soop et al. 2019). The discovery of *C. brunneoverrucosus* represents the first species of sect. *Dulciolentes* in China and the second agaricoid taxon within the section.

Cortinarius coriaceus Zhu L. Yang, Liu K. Jia & Zi R. Wang, sp. nov.

MycoBank No: 857351

Fig. 3

Etymology. The epithet "*coriaceus*" (Lat.) refers to the brown pileus with a leathery texture of this species.

Holotype. CHINA • Yunnan Province: Lijiang City, Yulong Naxi Autonomous County, Lijiang Alpine Botanical Garden, in a subalpine temperate broad-leaved and coniferous mixed forest with trees of *Quercus* and *Pinus*, 27°0.21'N, 100°10.71'E, elevation 3,340 m, 7 August 2023, Dong-Mei Li 299 (KUN-HKAS 145316). GenBank: ITS: PQ772202, nrLSU: PQ772214.

Diagnosis. *Cortinarius coriaceus* looks like *C. odoritraganus* Niskanen, Liimat. & Ammirati, but differs in its emarginate lamellae, cylindrical stipe, and relatively larger basidiospores (Niskanen 2020).

Description. *Basidioma* medium-sized to large. *Pileus* 3 cm diam when young, 4.5–7 cm diam when mature, initially slightly campanulate, becoming plano-convex, occasionally with slightly subumbonate center, viscid, with a leathery texture; brown (6C4–6C7), paler (6A2–6A4) towards the center, covered with white (1A1) fibrillose squamules when young; pale brown to brown (6A4–6C4), pale brown (6A2), or dark brown (6D4–6D6) towards the center when mature; margin incurved, with innate radial brownish (6C2–6C3) stripes when young; context of pileus pale brown to brown (6A3–6B3, 6C6). *Lamellae* emarginate, medium-spaced (L = 38–52, l = 27–36), pale brown (6A4) with a faint pinkish (12A2) tint when young, later brown (6C4–6C7). *Stipe* 4.5–6 × 0.7–1.2 cm, cylindrical, dirty white (1A1–1B1) and pale violaceous (16A2–16A4), with more and more violaceous (16A4) tint towards the stipe apex when young, later dirty white (1A1–1B1), pale brown (6B2–6B4), covered with brown (6C6) to dark brown (6D6) fibrillose squamules; annulus cortinate; context of stipe dirty white (1A1–1B1) with brown (6C6); basal mycelium white (1A1).

Basidiospores [60/3/3] (10–)11.5–12.5(–14) × (5–)7.5–10 μm, Q = 1.25–1.43(–1.66), av. = 12.06 ± 0.85 × 8.33 ± 1.48 μm, Qav. = 1.48 ± 0.24, ellipsoid to amygdaliform, moderately to strongly verrucose, inamyloid. *Basidia* 37.5–43 × 7.5–10 μm, 4-spored, clavate. *Trama of lamellae* regular, composed of colorless to yellowish, smooth hyphae 12.5–15 μm wide. *Cystidia* absent. *Pileipellis* duplex: epicutis weakly developed, 15–20 μm thick, composed of only 2–3 layers of interwoven to parallel, colorless, smooth, thin-walled, long-celled hyphae 3–7.5 μm wide; hypocutis composed of interwoven to parallel, colorless, cylindrical, thin-walled hyphae 12.5–17.5 μm wide. *Clamp connections* common in all parts of basidioma.

Habitat/host. Summer. Solitary or gregarious on soil in subalpine temperate broad-leaved and coniferous mixed forests with trees of *Quercus* and *Pinus*.

Distribution. Currently known from southwestern China.

Additional specimens examined. CHINA • Yunnan Province: Lijiang City, Yulong Naxi Autonomous County, Lijiang Alpine Botanical Garden, in a subalpine temperate broad-leaved and coniferous mixed forest with trees of *Quercus* and *Pinus*, 27°0.21'N, 100°10.71'E, elevation 3,340 m, 7 August 2023, Guan-Rui Li 328 (KUN-HKAS 145314), same place and date, Guan-Rui Li 333 (KUN-HKAS 145315).

Notes. *Cortinarius coriaceus* is characterized by its brown, leathery-wrinkled pileus, pinkish-tinted lamellae, and relatively larger basidiospores.
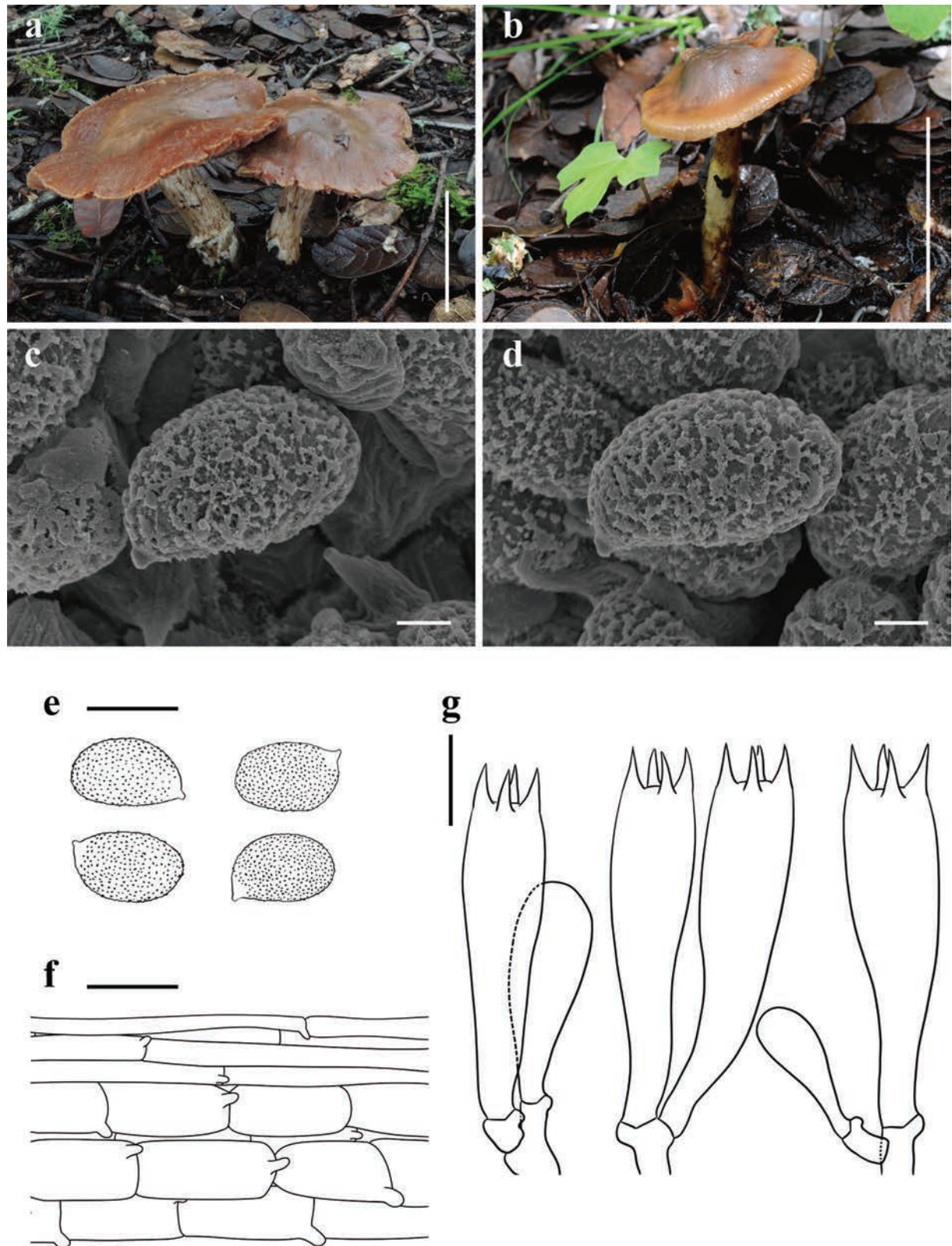
Figure 3. *Cortinarius coriaceus* (**a, c–g** KUN-HKAS 145136, Holotype **b** KUN-HKAS 145314) **a, b** basidiomata **c–e** basidiospores **f** pileipellis **g** basidia; and marginal sterile cells. Scale bars: 5 cm (**a, b**); 2 μm (**c, d**); 10 μm (**e**); 20 μm (**f, g**).

Cortinarius coriaceus is phylogenetically closely related to and morphologically similar to *C. odoritraganus*, known from Eastern North America and Costa Rica, in mixed temperate forest with *Abies* and *Betula* or mountain *Quercus* forest.

However, *C. odoritraganus* differs in its paler pileus, adnexed, purple-brown to brown lamellae, longer and thicker stipe (5–10 × 1–2 cm), and relatively smaller basidiospores (9.5–11.5 × 6–7.5 μm) (Niskanen 2020). *Cortinarius niveotraganus* Kytöv., Niskanen & Liimat., another related species, is distinguished by its hemispherical to broadly convex pileus, initially white to greyish white lamellae with bluish tints, clavate stipe, relatively smaller basidiospores (8.6–10.9 × 5.2–6.3 μm), and occurrence in planted *Betula* forests (Niskanen 2014).

Cortinarius fuscocandidus Zhu L. Yang, Liu K. Jia & Zi R. Wang, sp. nov.

MycoBank No: 857352

Fig. 4

Etymology. The epithet "*fuscocandidus*" (Lat.) refers to the dark brown pileus with a white margin of this species.

Holotype. CHINA • Yunnan Province: Lijiang City, Ninglang Yi Autonomous County, Xinyingpan Township, in a subalpine temperate broad-leaved and coniferous mixed forest with trees of *Quercus* and *Pinus*, 27°9.9'N, 100°55.63'E, elevation 2,700 m, 7 August 2011, Qing Cai 602 (KUN-HKAS 70198). GenBank: ITS: PQ772210, nrLSU: PQ772222.

Diagnosis. *Cortinarius fuscocandidus* resembles *C. fulvopaludosus* Kytov., Niskanen & Liimat. (Liimatainen 2017), but differs in its white margin, more robust stipe, and broadly ellipsoid to amygdaliform basidiospores.

Description. *Basidioma* small. *Pileus* 1.8–2 cm diam, applanate to plano-convex with a papilla, viscid; dark brown (6E7); margin white (1A1), sparsely covered with brown (6C6) fibrillose squamules; context of pileus brown (6D7). *Lamellae* emarginate with decurrent tooth, medium-spaced (L = 25–33, l = 9–12), pale brown (6B4) with a somewhat pale violaceous (16A2) tint. *Stipe* 5–7 × 0.3–0.6 cm, cylindrical, white (1A1) with a somewhat pale violaceous (16A2) tint, pale brown (6B2–6B4) when damaged; annulus cortinate; context of stipe not observed; basal mycelium white (1A1) with a somewhat pale violaceous (16A2) tint.

Basidiospores [60/2/2] 7.5–10.5 × (5–)7–10 μm, Q = 1.07–1.5(–1.65), av. = 8.19 ± 1.24 × 6.99 ± 1.26 μm, Qav. = 1.29 ± 0.18, broadly ellipsoid to amygdaliform, occasionally subglobose, strongly verrucose, inamyloid. *Basidia* 20–22.5 × 7.5–10 μm, 4-spored, clavate. *Trama of lamellae* regular, composed of colorless, smooth hyphae 7.5–10 μm wide. *Cystidia* absent. *Pileipellis* duplex: epicutis weakly developed, 11–15 μm thick, gelatinous, composed of interwoven to parallel, colorless, smooth, thin-walled, long-celled hyphae 2.5–5 μm wide, with brownish incrustation; hypocutis composed of only 3–5 layers of interwoven to parallel, colorless, cylindrical, thin-walled hyphae 7.5–15 μm wide. *Clamp connections* common in all parts of basidioma.

Habitat/host. Summer. Gregarious on soil in subalpine temperate broad-leaved and coniferous mixed forests with trees of *Quercus* and *Pinus*.

Distribution. Currently known from southwestern China.

Additional specimen examined. CHINA • Yunnan Province: Lijiang City, Gucheng District, Jinshan Township, in a subalpine temperate broad-leaved and coniferous mixed forest with trees of *Quercus* and *Pinus*, 26°54.55'N, 100°18.44'E, elevation 2,145 m, 28 July 2011, Li-Ping Tang 1331 (KUN-HKAS 69792).
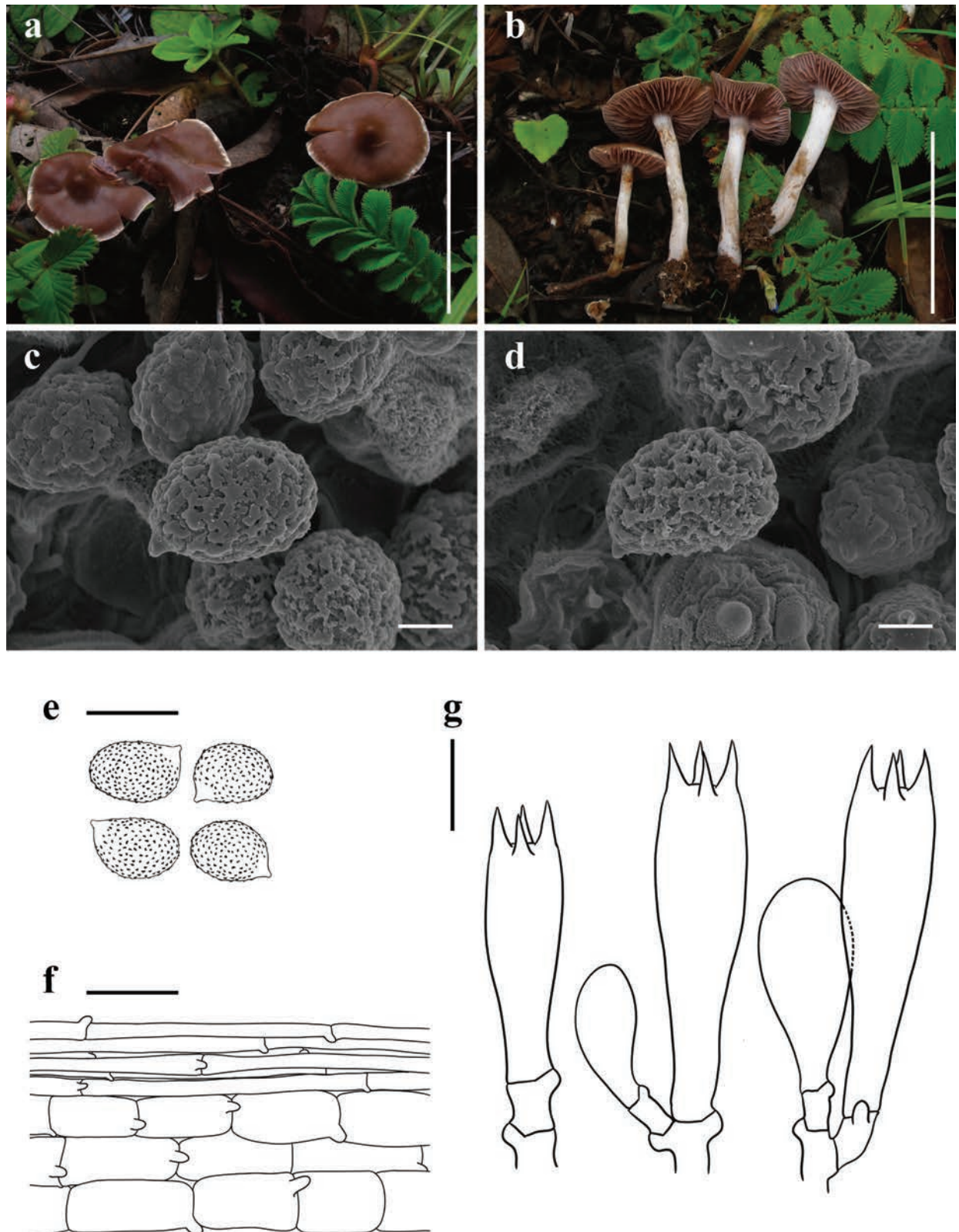
Figure 4. *Cortinarius fuscocandidus* (**a–g** KUN-HKAS 70198, Holotype) **a, b** basidiomata **c–e** basidiospores **f** pileipellis **g** basidia; and marginal sterile cells. Scale bars: 5 cm (**a, b**); 2 μm (**c, d**); 10 μm (**e**); 20 μm (**f, g**).

Notes. *Cortinarius fuscocandidus* is characterized by its dark brown, papillate pileus with a white margin, pale brown lamellae with a somewhat pale violaceous tint, and broadly ellipsoid basidiospores.

Phylogenetically, *C. fuscocandidus* belongs to sect. *Hinnulei* and is closely related to *C. fulvopaludosus*. However, the phylogenetic tree shows low support between these two similar species, which can only be distinguished by their margin coloration and basidiospore size (Liimatainen 2017).

Morphologically, *C. fuscocandidus* looks like a typical member of sect. *Hinnulei* (Fries 1838; Bidaud et al. 2012; Li et al. 2016; Liimatainen et al. 2017; 2020), where the overall coloration of the pileus is brown to dark brown. However, the white margin, somewhat pale violaceous lamellae, and broadly ellipsoid basidiospores (7.5–10.5 × (5–)7–10 μm) differentiate it from the most similar species, *C. badioflavidus* Ammirati et al., which has brown to rich brown lamellae and narrower basidiospores (8.1–10.5 × 5.8–6.5 μm) (Li et al. 2016).

Cortinarius neodisjungendus Zhu L. Yang, Liu K. Jia & Zi R. Wang, sp. nov.
MycoBank No: 857353
Fig. 5

Etymology. The epithet "*neodisjungendus*" (Lat.) refers to its similarity to *C. disjungendus*.

Holotype. CHINA • Yunnan Province: Pu'er City, Jingdong Yi Autonomous County Ailao Mountain Subtropical Forest Ecosystem Research Station, Chinese Academy of Sciences, in a subtropical broad-leaved forest with trees of *Quercus*, 24°32.57'N, 101°1.62'E, elevation 2,532 m, 8 October 2021, Jian-Wei Liu 2505 (KUN-HKAS 145322). GenBank: ITS: PQ772207, nrLSU: PQ772219.

Diagnosis. *Cortinarius neodisjungendus* differs from other species within sect. *Disjungendi* by its plano-convex pileus with an umbo, pale brown coloration, and relatively larger basidiospores (Karsten 1893; Niskanen 2014; Liimatainen et al. 2015).

Description. *Basidioma* medium-sized. ***Pileus*** 3.5–4.2 cm diam, applanate to plano-convex with an umbonate center, viscid with hygrophanous streaks; pale brown to brown (6D3–6D4), dark brown (6E6) towards the center, white (1A1) to pale brown (6B2) towards the margin, sparsely covered with white (1A1) fibrillose squamules; context not observed. ***Lamellae*** emarginate, crowded (L = 52–61, l = 48–53), pale brown (6B4) to brown (6D6). ***Stipe*** 8–10 × 0.5–0.8 cm, cylindrical with a subbulbous base 1–1.5 cm wide, white (1A1) to pale brown (6B2–6B4), base sparsely covered with brown (6C5) fibrillose squamules; basal mycelium white (1A1).

Basidiospores [60/2/2] 11–13.5(–15) × (5–)7.5–9 μm, Q = 1.43–1.71(–2), av. = 12.73 ± 0.93 × 7.52 ± 0.96 μm, Qav. = 1.71 ± 0.2, broadly amygdaliform, strongly verrucose, inamyloid. ***Basidia*** 32.5–40 × 7.5–10 μm, 4-spored, clavate. ***Trama of lamellae*** regular, composed of colorless to brownish, smooth hyphae 10–12.5 μm wide. ***Cystidia*** absent. ***Pileipellis*** duplex: epicutis weakly developed, 8.5–15 μm thick, composed of only 3–5 layers of interwoven to parallel, colorless to brownish, smooth, thin-walled, long-celled hyphae 2.5–5 μm wide; hypocutis composed of interwoven to parallel, colorless to pale brownish, cylindrical, thin-walled hyphae 12.5–15 μm wide. ***Clamp connections*** common in all parts of basidioma.

Habitat/host. Autumn. Solitary on soil in subtropical broad-leaved forests with trees of *Quercus*.

Distribution. Currently known from southwestern China.
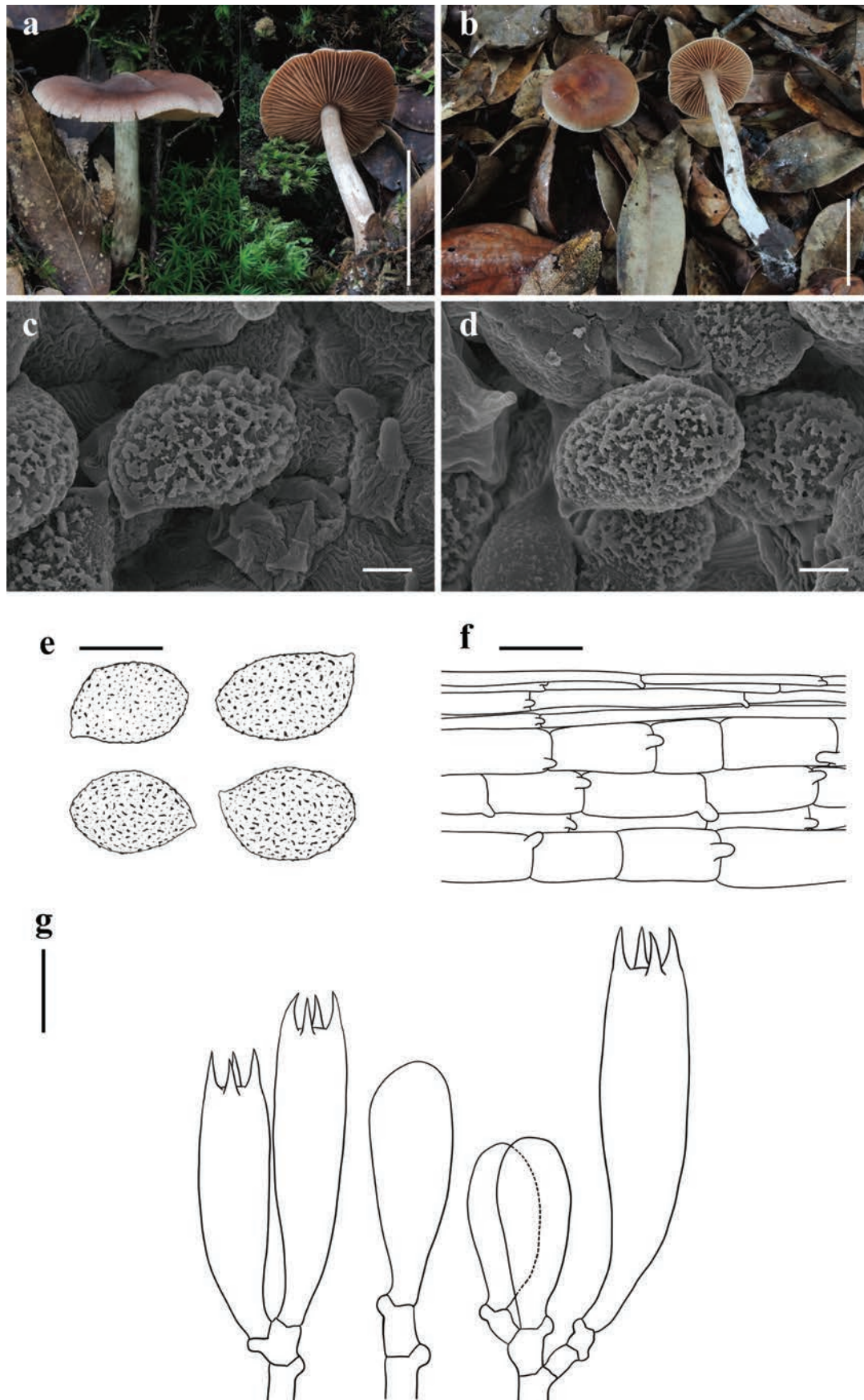
Figure 5. *Cortinarius neodisjungendus* (**a, c−g** KUN-HKAS 145322, Holotype **b** KUN-HKAS 145323) **a, b** basidiomata **c−e** basidiospores **f** pileipellis **g** basidia; and marginal sterile cells. Scale bars: 5 cm (**a, b**); 2 µm (**c, d**); 10 µm (**e**); 20 µm (**f, g**).

Additional specimen examined. CHINA • Yunnan Province: Pu'er City, Jingdong Yi Autonomous County Ailao Mountain Subtropical Forest Ecosystem Research Station, Chinese Academy of Sciences, in a subtropical broad-leaved forest with trees of *Quercus*, 24°32.57'N, 101°1.62'E, elevation 2,532 m, 8 October 2021, Jian-Wei Liu 2529 (KUN-HKAS 145323).

Notes. *Cortinarius neodisjungendus* is characterized by its hygrophanous, pale brown to brown pileus with a whitish margin, whitish stipe, and relatively larger basidiospores. All other species in sect. *Disjungendi* have a brownish pileus lacking a white margin, a brown stipe, and smaller basidiospores (range from 9–11 μm long, 6–7 μm wide) (Karsten 1893; Niskanen 2014; Liimatainen et al. 2015, 2020).

Cortinarius sinoconfirmatus Zhu L. Yang, Liu K. Jia & Zi R. Wang, sp. nov.

MycoBank No: 857354

Fig. 6

Etymology. The epithet "*sinoconfirmatus*" (Lat.) refers to the species in China that is similar to *C. confirmatus*.

Holotype. CHINA • Yunnan Province: Lijiang City, Yulong Naxi Autonomous County, Taian Township, in a subalpine temperate coniferous forest with trees of *Pinus*, 26°48.91'N, 100°5.96'E, elevation 2,633 m, 9 August 2023, Zi-Rui Wang 160 (KUN-HKAS 145320). GenBank: ITS: PQ772205, nrLSU: PQ772217.

Diagnosis. *Cortinarius sinoconfirmatus* looks like *C. confirmatus* Rob. Henry, but differs in its dark brown pileus center, more brown lamellae, thinner stipe, and larger basidiospores (Henry 1983; Mahiques et al. 2001; Ortega et al. 2007; Liimatainen et al. 2017).

Description. *Basidioma* medium-sized. *Pileus* 1.2 cm diam when young, 3–4.3 cm diam when mature, hemispherical when young, later convex, viscid; pale brown (6B2–6B4) to brown (5C6–5C7), covered with white (1A1) fibrillose squamules when young; brown (6C4–6C6), pale brown (6B2–6B4) towards the margin, dark brown (6E7) towards the center when mature; margin covered with brown (6C7) fibrillose squamules; context of pileus gelatinous, pale brown (6B2–6B4) to brown (6C7). *Lamellae* emarginate, crowded (L = 74–95, l = 46–52), pale brown (6B2–6B3) with a faint pinkish (12A2) tint when young, later brown (6B6–6C6). *Stipe* 5–7 × 0.5–0.7 cm, cylindrical, dirty white (1A1–1B1), pale brown (6B2–6B3) to brown (6C6), with a pale violaceous (16A2–16A3) tint at the stipe apex when young, later the upper 1/2 stipe dirty white, pale brown (6B2–6B3) to brown (6C6) with a pale violaceous (16A2–16A3) tint, covered with brown (7C4) fibrillose squamules, the lower 1/2 brown to dark brown (7B4–7E4); context of stipe dirty white (1A1–1B1) and brown (7C6); basal mycelium white (1A1).

Basidiospores [60/3/3] 7.5–11.5 × 4–5(6) μm, Q = (1.5–)2–3.13, av. = 9.92 ± 1.19 × 4.85 ± 0.59 μm, Qav. = 2.06 ± 0.28, ellipsoid to narrowly ellipsoid, moderately to strongly verrucose, inamyloid. *Basidia* 27.5–35 × 5–7.5 μm, 4-spored, clavate. *Trama of lamellae* regular, composed of pale yellowish, smooth hyphae 12.5–15 μm wide. *Cystidia* absent. *Pileipellis* duplex: epicutis weakly developed, 10–14 μm thick, gelatinous, composed of only 2–4 layers of interwoven to parallel, colorless to pale yellow, smooth, thin-walled, long-celled hyphae 2.5–5 μm wide; hypocutis composed of interwoven to parallel, colorless, cylindrical, thin-walled hyphae 12.5–17.5 μm wide. *Clamp connections* common in all parts of basidioma.
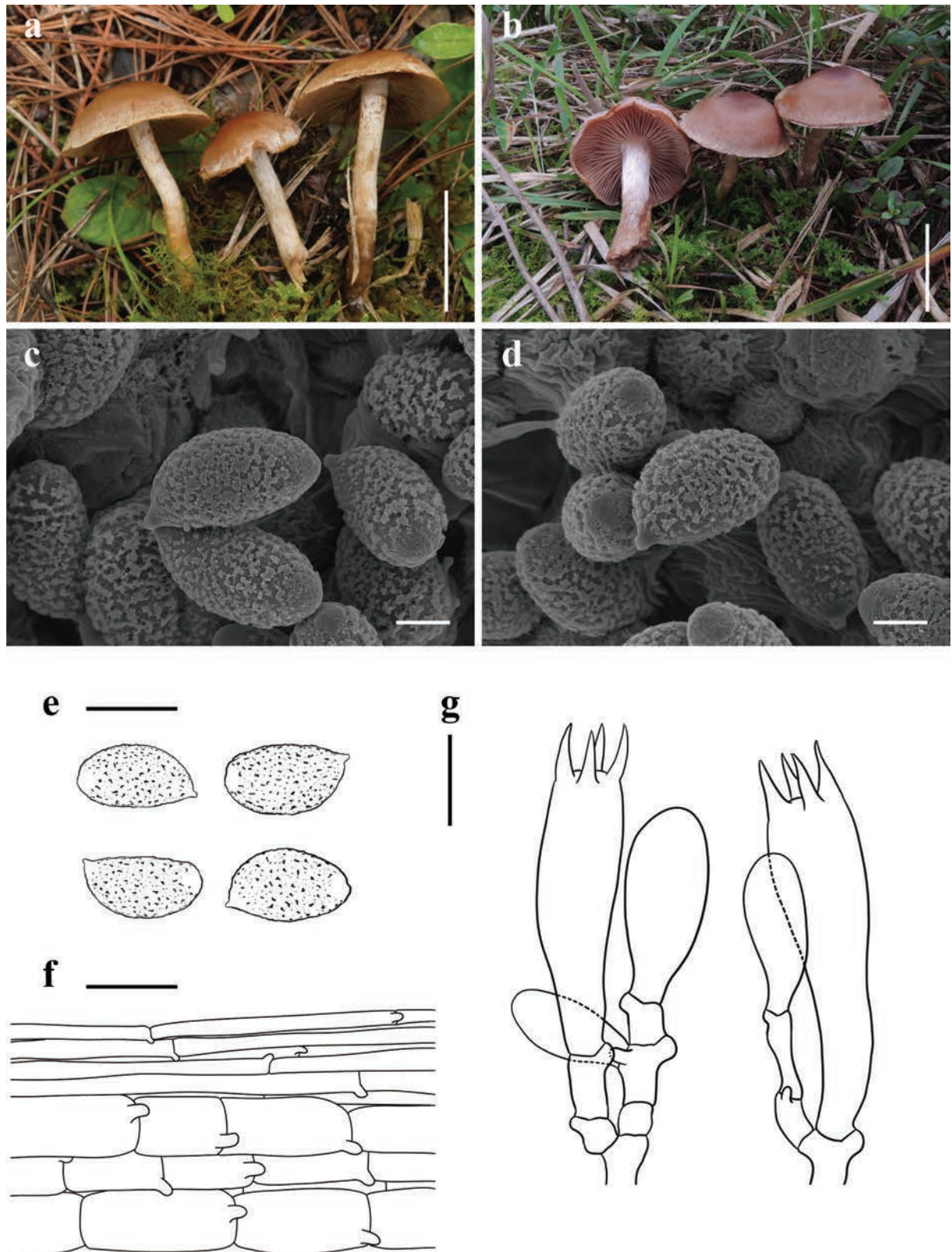
Figure 6. *Cortinarius sinoconfirmatus* (**a, c–g** KUN-HKAS 145320, Holotype **b** KUN-HKAS 145318) **a, b** basidiomata **c–e** basidiospores **f** pileipellis **g** basidia; and marginal sterile cells. Scale bars: 5 cm (**a, b**); 2 μm (**c, d**); 10 μm (**e**); 20 μm (**f, g**).

Habitat/host. Summer. Gregarious on soil in subalpine temperate coniferous forests with trees of *Pinus*.

Distribution. Currently known from southwestern China.

Additional specimens examined. CHINA • Yunnan Province: Lijiang City, Yulong Naxi Autonomous County, Taian Township, in a subalpine temperate coniferous forest with trees of *Pinus*, 26°48.91'N, 100°5.96'E, elevation 2,633 m, 9 August 2023, Zi-Rui Wang 154 (KUN-HKAS 145319); same Township and date, 26°48.32'N, 100°4.35'E, elevation 2,700 m, Dong-Mei Li 331 (KUN-HKAS 145318).

Notes. *Cortinarius sinoconfirmatus* is closely related to *C. confirmatus*, but the latter differs from the former by its paler pileus with vinaceous or violaceous tints, paler, adnate lamellae, more robust stipe, and narrower basidiospores (8.8−10 × 5.2−5.6 µm, Q = 1.55−1.9) (Henry 1983; Mahiques et al. 2001; Ortega et al. 2007; Liimatainen et al. 2017). *Cortinarius sinoconfirmatus* is also closely related to *C. imbutus* Fr. and *C. saturninus* (Fr.) Fr. However, *C. imbutus* differs from *C. sinoconfirmatus* by its pale yellow pileus and whitish stipe with somewhat violaceous tint at the stipe apex (Fries 1838), and *C. saturninus* differs from *C. sinoconfirmatus* by its dark reddish brown pileus, violet stipe with purplish red squamules (Fries 1838).

Morphologically, *C. sinoconfirmatus* looks like *C. lucorum* (Fr.) E. Berger, but the latter differs from the former by its pileus with marble-like stripes and more prominent bulbous stipe base (Bidaud et al. 2000; Matheny and Ammirati 2006).

Discussion

Phylogenetics of five new species within *Cortinarius*

In this study, five species of *Cortinarius* are described as new to science based on phylogenetic evidence and morphological characteristics. Our phylogenetic tree reveals that four of these species—*C. coriaceus*, *C. fuscocandidus*, *C. neodisjungendus*, and *C. sinoconfirmatus*—belong to subgen. *Telamonia*, while the relationships between *C. coriaceus* and *C. niveotraganus*, as well as *C. sinoconfirmatus* and *C. confirmatus*, have been resolved (Fig. 1). The phylogenetic position of *C. fuscocandidus* remains uncertain. Additionally, *C. brunneoverrucosus* is assigned to sect. *Dulciolentes* (Fig. 1), a small section not yet placed in any subgenus of *Cortinarius* (Liimatainen et al. 2022). *Cortinarius neodisjungendus* forms a strongly sister clade (98.8/99/1.00) with other species within sect. *Disjungendi*, but differs by its whitish margin and whitish stipe (Karsten 1893; Liimatainen et al. 2015, 2020).

Ecological distribution of five new species within *Cortinarius*

Ecologically, the five species fall into two categories: *Cortinarius coriaceus*, *C. fuscocandidus*, and *C. sinoconfirmatus* inhabit subalpine temperate areas, whereas *C. brunneoverrucosus* and *C. neodisjungendus* are restricted to subtropical areas. Notably, within sect. *Dulciolentes*, three sequestrate species—*C. peraurantiacus*, *C. pisciodorus*, and *C. dulciolens*—are known only from Oceania, while the agaricoid *C. corrugatus* occurs in North America (Peck 1872; Moser 1983; Peintner et al. 2002a, 2002b; Phillips 2010; Soop et al. 2019; Kuo 2020). The discovery of *C. brunneoverrucosus* in China represents the first record of sect. *Dulciolentes* in East Asia. Furthermore, the agaricoid basidioma of *C. brunneoverrucosus* provides evidence of biogeographic linkages between North America and East Asia.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Zhu L. Yang conceived and designed the study. Liu-Kun Jia and Zi-Rui Wang collected specimens from China and generated the DNA sequence data. Zhu L. Yang, Liu-Kun Jia, and Zi-Rui Wang analyzed the data and checked issues related to nomenclatural articles. Liu-Kun Jia and Zi-Rui Wang wrote the manuscript draft. Zhu L. Yang, Liu-Kun Jia, and Zi-Rui Wang revised the draft.

Author ORCIDs

Liu-Kun Jia https://orcid.org/0000-0002-3180-5077
Zi-Rui Wang https://orcid.org/0000-0003-4564-1904
Zhu-Liang Yang https://orcid.org/0000-0001-9745-8453

Data availability

Sequence alignments were deposited in TreeBASE (Study ID: TB2:S31924; URL: http://purl.org/phylo/treebase/phylows/study/TB2:S31924). DNA sequences (Table 2) are available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

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Three novel species of *Alternaria* (Pleosporales, Pleosporaceae) from cereal crops (Poaceae) in China

Hai-Feng Liu[1,2]*[iD], Feng-Yin Liu[1]*[iD], Hai-Yan Ke[1], Qing-Xiao Shi[1], Jian-Xin Deng[1][iD], Hyunkyu Sang[2][iD]

1   Department of Plant Protection, College of Agriculture, Yangtze University, Jingzhou 434025, China
2   Department of Integrative Food, Bioscience and Biotechnology, Chonnam National University, Gwangju, 61186, Republic of Korea
Corresponding authors: Jian-Xin Deng (djxin555@yangtzeu.edu.cn); Hyunkyu Sang (hksang@jnu.ac.kr)

Abstract

The genus *Alternaria* (Pleosporales, Pleosporaceae) comprises saprophytes and pathogens that are widespread around the world. Currently, more than 400 species are recognized within this genus and are classified into 29 sections. In this study, *Alternaria* strains were isolated from diseased leaves of two cereal crops, rice (*Oryza sativa*) and maize (*Zea mays*) in China. These *Alternaria* spp. were characterized by morphological characterization and phylogenetic analysis using maximum likelihood and Bayesian inference with multiple loci (ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2). Based on the above analyses, three novel species of *Alternaria* section *Alternaria* were introduced, namely *A. oryzicola* **sp. nov.**, *A. poae* **sp. nov.**, and *A. zeae* **sp. nov.** This study expands the species diversity of *Alternaria* associated with Poaceae plants in China.

Key words: Dematiaceous hyphomycetes, maize and rice diseases, morphology, new taxa, multigene phylogeny, taxonomy

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Introduction

The genus *Alternaria* consists of more than 400 species of dematiaceous hyphomycetes (Li et al. 2022, Gou et al. 2023, Liao et al. 2023, Hyde et al. 2024). Species in this genus have been mainly described as saprophytes, endophytes, or phytopathogens and currently accommodated in the family Pleosporaceae (Hyde et al. 2024). Historically, taxonomy of *Alternaria* has gone through different stages since it was first established by Nees in 1816 (Lawrence et al. 2016). In brief, *Alternaria* and related genera, especially *Macrosporum* and *Stemphylium*, were confused in earlier stages. Although attempts were made by researchers to determine their taxonomic status, issues in nomenclature and generic boundaries persisted for a long time. Afterwards, a complete revision of taxa related to *Alternaria* based on sporulation patterns and conidial morphology was undertaken by Simmons (Simmons 2007), which accelerated the establishment of order in the nomenclature of alternarioid hyphomycetes.

Nowadays, a DNA-based molecular approach has been used to better understand taxonomy of *Alternaria* (Lawrence et al. 2016). A variety of loci have been used in the classification of this genus, such as the internal transcribed spacer

*   These authors contributed equally to this work and share first authorship.

(ITS) of the rDNA region, small subunit ribosomal RNA gene (SSU), large subunit ribosomal RNA gene (LSU), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), second largest subunit of the RNA polymerase (*RPB2*), translation elongation factor 1-α (*TEF1*), *Alternaria* major allergen (*Alt a 1*), endopolygalacturonase gene (*EndoPG*), an anonymous genomic region (OPA10-2), calmodulin (*CAM*), and the plasma membrane ATPase gene (*ATP*). Based on multi-locus phylogenetic analysis, this genus consists of 29 sections (Gannibal et al. 2022, Li et al. 2023). Currently, both morphology and multi-locus phylogeny are crucial for the taxonomy of *Alternaria* spp. and have been widely employed in charactering novel species (Aung et al. 2024; He et al. 2024; Nwe et al. 2024; Bessadat et al. 2025).

Alternaria spp. have been associated with more than 4,000 host plants, ranking the genus 10th among the 100 most cited fungal genera (Lawrence et al. 2013; Woudenberg et al. 2013, 2015; Pinto and Patriarca 2017; Li et al. 2022; Bhunjun et al. 2024). Collectively, certain *Alternaria* spp. cause diseases that lead to economic losses in agricultural crops, including cereals, oil crops, fruits, and vegetables (Li et al. 2022; Haituk et al. 2023; Alasadi 2024). Cereal crops (Poaceae), such as wheat (*Triticum aestivum*), maize (*Zea mays*), and rice (*Oryza sativa*), have been widely cultivated and consumed since they are popular staple foods with most of the world's population. Infections on cereal crops caused by *Alternaria* spp. occur constantly and have attracted increasing attention worldwide (Tralamazza et al. 2018; Orina et al. 2021; Zhong et al. 2022). For instance, wheat black point is an important disease mainly caused by different *Alternaria* species (*A. alternata*, *A. infectoria*, and *A. tenuissima*), with *A. alternata* being isolated more frequently (Pinto and Patriarca 2017; Tralamazza et al. 2018). In addition, *A. tricitina* has been considered as another important pathogen causing leaf blight on wheat (Mercado Vergnes et al. 2006; Amatulli et al. 2013). Recently, *A. alternata*, *A. tenuissima*, *A. burnsii*, and an unclassified species *Alternaria* sp. were identified as causal agents of leaf blight on maize in China (Xu et al. 2022). In rice, *A. padwickii* (Syn *Trichoconis padwickii*) has been frequently detected as a seed pathogen (Gutiérrez et al. 2010). Moreover, *A. arborescens* and *A. gaisen* were also reported as leaf spot pathogens of rice in Pakistan (Akhtar et al. 2014a, 2014b).

In this study, *Alternaria* spp. were isolated from symptomatic leaves of rice and maize in Guangxi Province and in Hainan Province in China, respectively. The aim of this study was to characterize these species taxonomically using morphological traits and multi-locus phylogenetic analysis.

Materials and methods

Isolation

In 2023, diseased maize (*Zea mays*) and rice (*Oryza sativa*) leaves exhibiting leaf spot and blight symptoms were collected in Guangxi and Hainan provinces, respectively. Leaf tissues were cut into small pieces with sterile blades and placed in petri dishes containing wet filter papers. After incubation at 25 °C for 1–2 days, fungal development on tissue samples were observed with a stereo microscope. Spores of *Alternaria* spp. developed from the edge of the leave tissues were singly picked using sterile glass needles and inoculated onto PDA

(potato dextrose agar, Difco, Montreal, Canada). Pure cultures were deposited in the Fungi Herbarium of Yangtze University in Jingzhou, China. Dried cultures of the strains were also preserved in the herbarium for long-term storage.

Morphology

Colony characteristics of strains of *Alternaria* spp. were observed and recorded following 7 days of incubation at 25 °C on 90-mm PDA plates under dark conditions. To determine their conidial morphology, the strains were grown on potato carrot agar (PCA) and V8 juice agar (V8A) at 25 °C for 7 days under a photoperiod of 8 hours of light per day. Conidia of the strains were observed and imaged with an ECLIPSE Ni-U optical microscope (Nikon, Tokyo, Japan). The dimensions of the conidia were measured (n = 50). Conidial morphology was determined based on sporulation pattern and conidial characteristics.

PCR amplification

Fresh mycelia of the fungal strains grown on PDA were harvested and used for genomic DNA extraction following the procedures described by Watanabe et al. (2010). DNA solutions were then used to amplify fragments from several gene regions, including ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2. Polymerase chain reaction (PCR) amplification of the above-mentioned regions was performed by a Bio-Rad T100™ Thermal Cycler with primer pairs ITS5/ITS4 (White et al. 1990), gpd1/gpd2 (Berbee et al. 1999), EF1-728F/EF1-986R (Carbone and Kohn 1999), RPB2-5F/RPB2-7cR (Liu et al. 1999), Alt-for/Alt-rev (Hong et al. 2005), PG3/PG2b (Andrew et al. 2009), and OPA10-2L/OPA10-2R (Andrew et al. 2009), respectively. Reaction conditions for the PCR amplification were referred to previous studies (Woudenberg et al. 2015, Nwe et al. 2024). Successful amplification products were sent to TSINGKE (Beijing, China) for purification and Sanger sequencing in both directions. Sequences obtained from the company were manually examined with BioEdit v7.0.9 (Hall 1999) and then trimmed using MEGA X (Kumar et al. 2018). Consensus sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/) with accession numbers shown in Table 1. New species are established based on the recommendations outlined by Jeewon and Hyde (2016).

Phylogenetic analysis

Nucleotide sequences generated in this study were subjected to BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 31 October 2024) for similarity searches against the NCBI nucleotide database. Reference sequences of *Alternaria* spp. used for phylogenetic analysis were obtained based on recent publications (Woudenberg et al. 2015; Li et al. 2022, 2023; Romain et al. 2022; Aung et al. 2024; Nwe et al. 2024). Phylogenetic analysis was performed using the OFPT (One-click Fungal Phylogenetic Tool) program developed by Zeng et al. (2023). In brief, sequences of each genetic region were aligned by MAFFT v7.307 online version (Katoh and Standley 2013; Katoh et al. 2019) and then trimmed using TrimAI (Capella-Gutiérrez et al. 2009). Subsequently, nucleotide substitution models of each dataset were tested by ModelFinder (Kalyaanamoorthy et

Table 1. GenBank accession numbers of *Alternaria* spp. used for phylogenetic analysis.

Species	Strain	ITS	*Alt a 1*	*GAPDH*	*RPB2*	*TEF1*	*OPA10-2*	*EndoPG*
A. alstroemeriae	CBS 118808	KP124296	KP123845	KP124153	KP124764	KP125071	KP124601	KP123993
A. alstroemeriae	CBS 118809ᵀ	KP124297	–	KP124154	KP124765	KP125072	KP124602	KP123994
A. alternantherae	CBS 124392	KC584179	KP123846	KC584096	KC584374	KC584633	–	–
A. alternata	CBS 916.96ᵀ	AF347031	AY563301	AY278808	KC584375	KC584634	KP124632	JQ811978
A. alternata	CBS 112249	KP124338	KP123886	KP124192	KP124806	KP125114	KP124648	KP124039
A. arborescens	CBS 119544ᵀ	KP124408	KP123955	JQ646321	KP124878	KP125186	KP124722	KP124112
A. arborescens	CBS 102605ᵀ	AF347033	AY563303	AY278810	KC584377	KC584636	KP124712	AY295028
A. arctoseptata	MFLUCC 21-0139ᵀ	–	OK236755	OK236702	OK236655	OK236608	–	–
A. baoshanensis	MFLUCC 21-0124ᵀ	MZ622003	OK236760	OK236706	OK236659	OK236613	–	–
A. betae-kenyensis	CBS 118810ᵀ	KP124419	KP123966	KP124270	KP124888	KP125197	KP124733	KP124123
A. breviconidiophora	MFLUCC 21-0786ᵀ	MZ621997	OK236751	OK236698	OK236651	OK236604	–	–
A. burnsii	CBS 118816	KP124423	KP123970	KP124273	KP124892	KP125201	KP124737	KP124127
A. burnsii	CBS 118817	KP124424	KP123971	KP124274	KP124893	KP125202	KP124738	KP124128
A. burnsii	CBS 107.38ᵀ	KP124420	KP123967	JQ646305	KP124889	KP125198	KP124734	KP124124
A. burnsii	CBS 879.95	KP124422	KP123969	KP124272	KP124891	KP125200	KP124736	KP124126
A. burnsii	CBS 130264	KP124425	KP123972	KP124275	KP124894	KP125203	KP124739	KP124129
A. burnsii	CBS 110.50	KP124421	KP123968	KP124271	KP124890	KP125199	KP124735	KP124125
A. burnsii	CBS 108.27	KC584236	KP123850	KC584162	KC584468	KC584727	KP124605	KP123997
A. eichhorniae	CBS 489.92ᵀ	KC146356	KP123973	KP124276	KP124895	KP125204	KP124740	KP124130
A. ellipsoidialis	MFLUCC 21-0132	MZ621989	OK236743	OK236690	OK236643	OK236596	–	–
A. eupatoriicola	MFLUCC 21-0122	MZ621982	OK236736	OK236683	OK236636	OK236589	–	–
A. falcate	MFLUCC 21-0123	MZ621992	OK236746	OK236693	OK236649	OK236599	–	–
A. gaisen	CBS 118488ᴿ	KP124427	KP123975	KP124278	KP124897	KP125206	KP124743	KP124132
A. gaisen	CBS 632.93ᴿ	KC584197	KP123974	KC584116	KC584399	KC584658	KP124742	AY295033
A. gossypina	CBS 104.32ᵀ	KP124430	JQ646395	JQ646312	KP124900	KP125209	KP124746	KP124135
A. gossypina	CBS 102601	KP124433	KP123979	KP124282	KP124903	KP125212	KP124749	KP124138
A. iridiaustralis	CBS 118487	KP124436	KP123982	KP124285	KP124906	KP125215	KP124752	KP124141
A. iridiaustralis	CBS 118486ᵀ	KP124435	KP123981	KP124284	KP124905	KP125214	KP124751	KP124140
A. jacinthicola	CBS 878.95	KP124437	KP123983	KP124286	KP124907	KP125216	KP124753	KP124142
A. jacinthicola	CPC 25267	KP124439	KP123985	KP124288	KP124909	KP125218	KP124755	KP124144
A. jacinthicola	CBS 133751ᵀ	KP124438	KP123984	KP124287	KP124908	KP125217	KP124754	KP124143
A. jingzhouensis	YZU 221144ᵀ	OR883772	OR887694	OR887690	OR887688	OR887686	OR887684	OR887692
A. koreana	SPL2-1ᵀ	LC621613	LC631831	LC621647	LC621681	LC621715	LC631857	LC631844
A. lathyri	MFLUCC 21-0140ᵀ	MZ621974	OK236728	OK236675	OK236628	OK236581	–	–
A. lijiangensis	YZU 221458ᵀ	OQ679970	OQ686781	OQ686785	OQ686789	OQ686783	OQ686787	OQ686779
A. longipes	CBS 540.94ᴿ	AY278835	AY563304	AY278811	KC584409	KC584667	KP124758	KP124147
A. longipes	CBS 121332ᴿ	KP124443	KP123989	KP124292	KP124913	KP125222	KP124760	KP124149
A. longxiensis	YZU 221221ᵀ	OQ534546	OQ473629	OQ512732	OQ543009	OQ512726	OQ543003	OQ512720
A. lycopersici	YZU 221185ᵀ	OQ519795	OQ473633	OQ512736	OQ543013	OQ512730	OQ543007	OQ512724
A. macilenta	MFLUCC 21-0138ᵀ	MZ621972	OK236726	OK236673	OK236626	OK236579	–	–
A. macroconidia	MFLUCC 21-0134ᵀ	MZ622001	OK236757	OK236704	OK236657	OK236610	–	–
A. minimispora	MFLUCC 21-0127ᵀ	MZ621980	OK236734	OK236681	OK236634	OK236587	–	–
A. momordicae	YZU 161378ᵀ	OR883774	OR887695	OR887691	OR887689	OR887687	OR887685	OR887693
A. muriformispora	MFLUCC 21-0784ᵀ	MZ621976	OK236730	OK236677	OK236630	OK236583	–	–
A. myanmarensis	YZU 231736ᵀ	OR897031	OR979657	OR963612	PP508256	OR963615	PP034184	OR979663
A. oblongoellipsoidea	MFLUCC 22-0074ᵀ	MZ621967	OK236721	OK236668	OK236621	OK236574	–	–
A. obpyriconidia	MFLUCC 21-0121ᵀ	MZ621978	OK236732	OK236680	OK236633	OK236585	–	–
A. orobanches	MFLUCC 21-0137ᵀ	MZ622007	OK236763	OK236710	–	–	–	–
A. oryzicola sp. nov.	**YZU 231199ᵀ**	**PQ812549**	**PV155522**	**PV155536**	**PV155548**	**PV155528**	**PV155542**	**–**
A. ovoidea	MFLUCC 21-0782ᵀ	MZ622005		–	OK236708	OK236661	OK236614	–	–
A. phragmiticola	MFLUCC 21-0125ᵀ	MZ621994	OK236749	OK236696	OK236649	OK236602	–	–
A. poae sp. nov.	**YZU 231197ᵀ**	**PQ812551**	**PV155524**	**PV155538**	**PV155550**	**PV155530**	**PV155544**	**PV155532**
A. poae sp. nov.	**YZU 231198**	**PQ812550**	**PV155523**	**PV155537**	**PV155549**	**PV155529**	**PV155543**	**PV155531**
A. rostroconidia	MFLUCC 21-0136ᵀ	MZ621969	OK236723	OK236670	OK236623	OK236576	–	–
A. salicicola	MFLUCC 22-0072ᵀ	MZ621999	OK236753	OK236700	OK236653	OK236606	–	–
A. solanicola	YZU 221189ᵀ	OQ534548	OQ473631	OQ512734	OQ543011	OQ512728	OQ543005	OQ512722
A. tomato	CBS 103.30	KP124445	KP123991	KP124294	KP124915	KP125224	KP124762	KP124151
A. tomato	CBS 114.35	KP124446	KP123992	KP124295	KP124916	KP125225	KP124763	KP124152
A. torilis	MFLUCC 14-0433ᵀ	MZ621988	OK236741	OK236688	OK236641	OK236594	–	–
A. yamethinensis	YZU 231739ᵀ	OR889008	OR979655	OR963610	PP179253	OR963614	PP034182	OR979661
A. zeae sp. nov.	**YZU 231602ᵀ**	**PQ812548**	**PV155521**	**PV155535**	**PV155547**	**PV155527**	**PV155541**	**–**
A. zeae sp. nov.	**YZU 231638**	**PQ812547**	**PV155520**	**PV155534**	**PV155546**	**PV155526**	**PV155540**	**–**
A. zeae sp. nov.	**YZU 231640**	**PQ812546**	**PV155519**	**PV155533**	**PV155545**	**PV155525**	**PV155539**	**–**

al. 2017) and the best-fit model for each dataset was selected based on the Bayesian information criterion (BIC). All the datasets were concatenated with partition information and then used for maximum likelihood (ML) and Bayesian phylogenetic analyses with software IQ-TREE (Nguyen et al. 2015) and Mrbayes 3.2.7 (Ronquist et al. 2012), respectively. In the ML analysis, 1,000 replicates were performed using bootstrap approximation. In the Bayesian inference (BI) analysis, a Markov Chain Monte Carlo (MCMC) algorithm was employed, involving four MCMC chains running for 50,000,000 generations with sampling every 100 generations. Posterior probabilities (PP) were estimated after discarding the first 25% of sampled tree as burn-in. The consensus BI tree was generated once the average standard deviation of split frequencies fell below 0.01.

Results

Phylogenetic analysis

A total of 63 strains (including 6 strains from this study) of *Alternaria* species in section *Alternaria*, were used for phylogenetic analysis. The concatenated sequence matrix consisted of seven loci, with a total length of 3608 bp, including 514 bp from ITS, 566 bp from *GAPDH*, 753 bp from *RPB2*, 234 bp from *TEF1*, 472 bp from *Alt a 1*, 448 bp from *EndoPG*, and 621 bp from OPA10-2. The best-fit evolutionary models for each gene were as follows: JC for ITS, TNe+G4 for *RPB2*, TIMe+I for *EndoPG*, TNe+R2 for OPA10-2, and K2P+G4 for *GAPDH*, *Alt a 1* and *TEF1*. In phylogenetic analyses, similar topologies were obtained from maximum likelihood and Bayesian methods. Additionally, the six strains examined in this study were placed within *Alternaria* section *Alternaria*, clustering into three distinct clades. Specifically, strains YZU 231602, YZU 231638, and YZU 231640 (isolated from *Z. mays*) formed one clade supported with a bootstrap (BS) value of 81% and a Bayesian posterior probability (PP) of 1.00 (Fig. 1). This clade was positioned close to another clade composed of strains YZU 231197 and YZU 231198 (isolated from *O. sativa*). These two clades were relatively close to *A. burnsii* with BS/PP support values of 69%/0.73 (Fig. 1). Strain YZU 231199 isolated from *O. sativa* formed a single clade sister to strains of *A. tomato* (CBS 103.30 and CBS 114.35), supported with BS/PP values of 72%/0.80 (Fig. 1). The phylogenetic placements of these strains indicated that they represent three novel species in the genus *Alternaria* section *Alternaria*.

Taxonomy

***Alternaria oryzicola* H.F. Liu & J.X. Deng, sp. nov.**
MycoBank No: 857595
Fig. 2

Etymology. Name refers to its host *Oryza sativa*.

Type. CHINA • Hainan Province, Lingshui County, diseased leaves of *Oryza sativa*, July 2023, J.L. Yin, holotype YZU-H-2023056A (permanently preserved in a metabolically inactive state), ex-type culture YZU 231199.

Description. *Colonies* on PDA sub-circular, velvety to fluffy, white to greyish-green, darker at the center, reverse side pale yellow to light brown, 61−63 mm
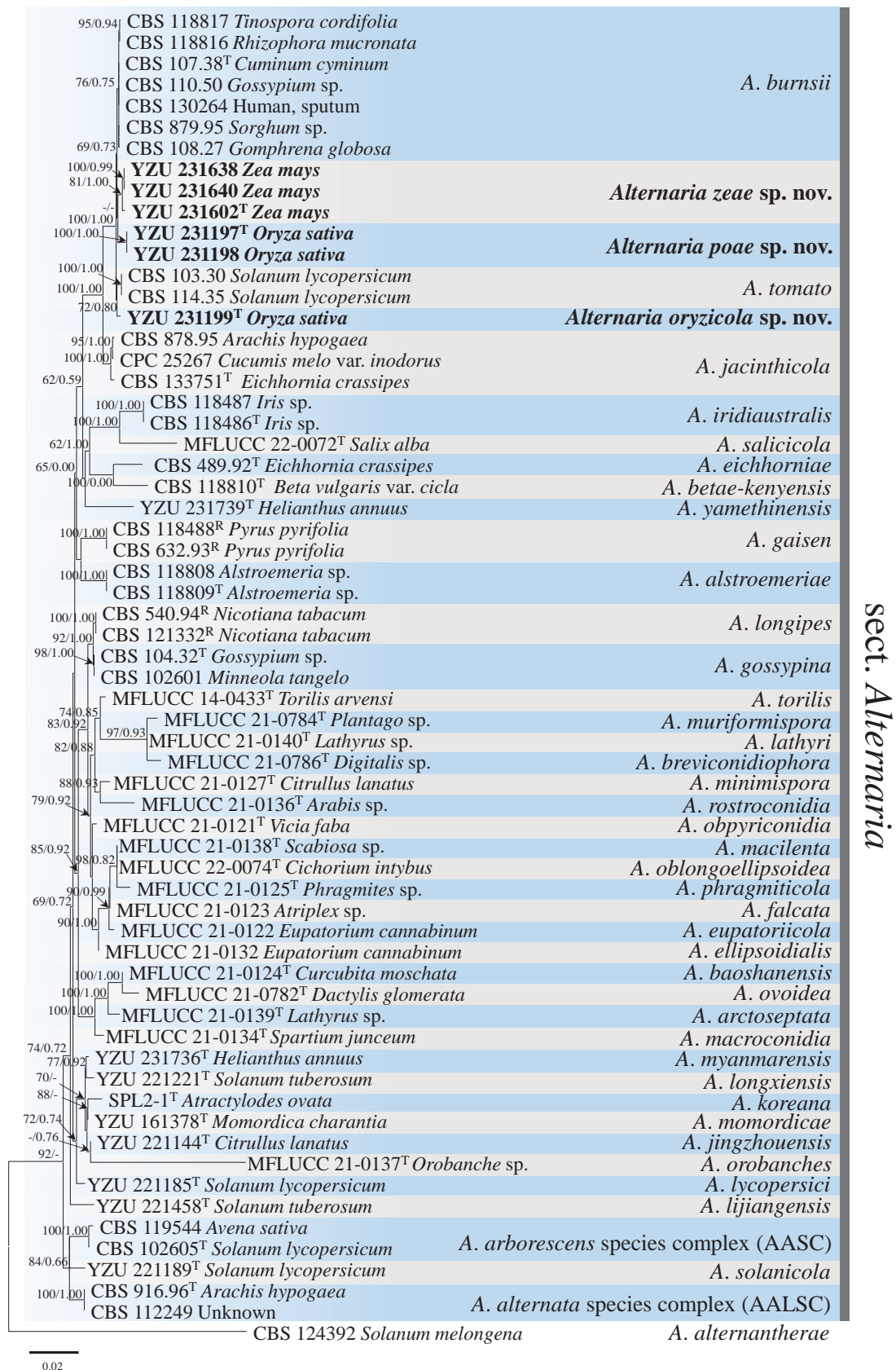
Figure 1. Phylogenetic tree constructed using the maximum likelihood method based on concatenated sequences of ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2 from *Alternaria* spp. Bootstrap support values (BS) and Bayesian posterior probability (PP) are given at the nodes (BS/PP). The strains from this study are marked in bold. Ex-type strains are indicated with 'T', representative strains are indicated with 'R'. *Alternaria alternantherae* CBS 124392 is used as the outgroup taxon.
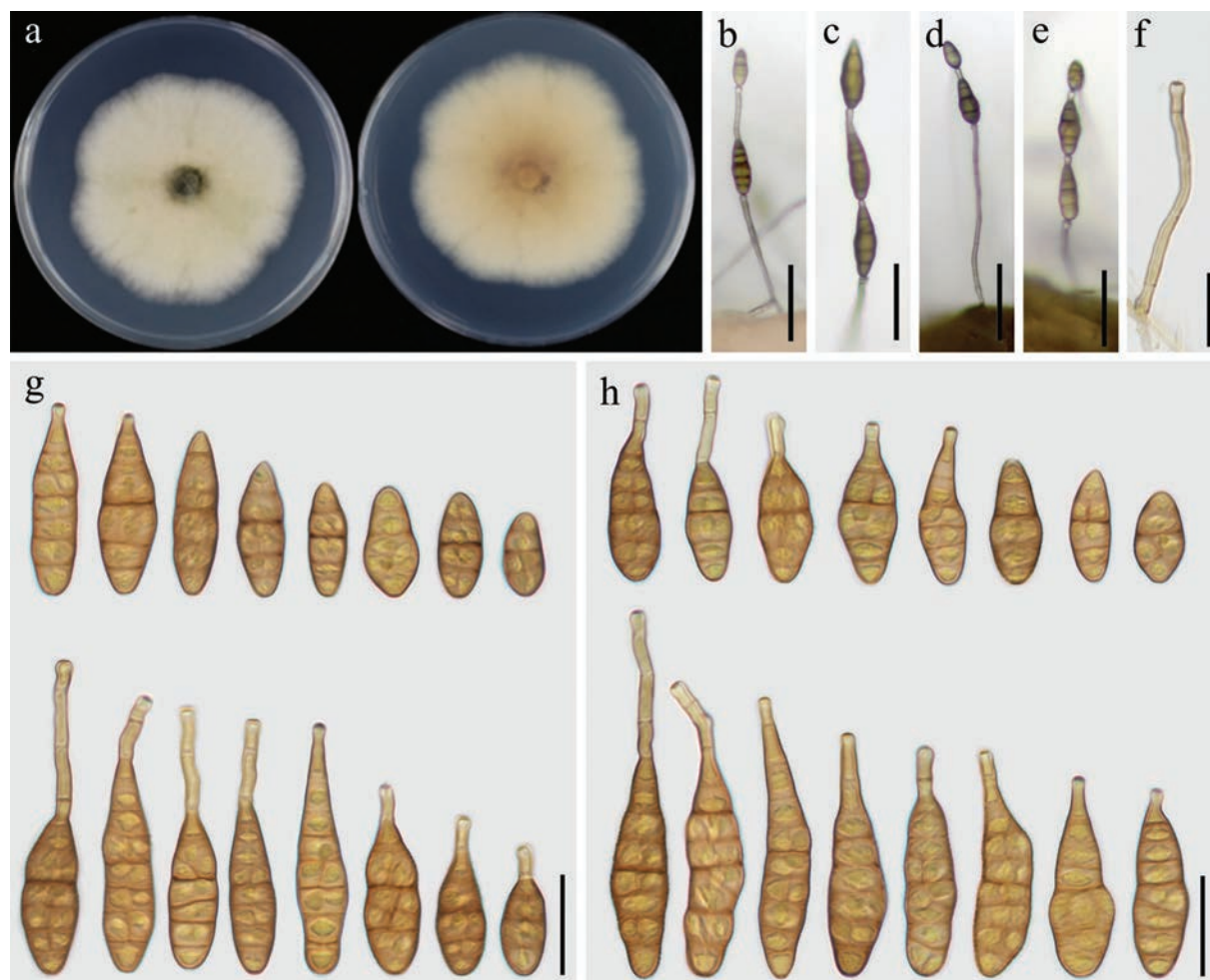
Figure 2. Morphology of *Alternaria oryzicola* sp. nov. (YZU 231199) **a** colony on PDA for 7 days at 25 °C **b, c** sporulation on PCA **d, e** sporulation on V8A **f** conidiophore and conidiogenous cell **g** conidia on PCA **h** conidia on V8A. Scale bars: 50 μm (**b, c, d, e**); 15 μm (**f**); 25 μm (**g, h**).

in diameter (Fig. 2a). On PCA, ***conidiophores***, erect or curved, unbranched, sometimes slightly expanded at the apex, 32−105 × 3−4 μm in size, with 1−6 septa (Fig. 2f). ***Conidiogenous cells*** integrated, terminal, smooth, cylindrical, apically doliiform, 5−14 × 3−4 μm, with 1 conidiogenous locus. ***Conidia*** borne in chain, 1−3 units per chain, unbranched, mostly narrow-obclavate, obclavate, or long ellipsoid, 20−48 × 9−16 μm in dimension, 1−4 transverse septa, apical beak 4−39 × 2.5−4 μm (Fig. 2b, c, g). On V8A, ***conidiophores*** unbranched, 20−67 × 3−4 μm, with 1−5 septa. ***Conidia*** solitary or in chain with 2−3 units per chain, narrow-obclavate, obclavate, or long ellipsoid, 18−56 × 9−16 μm in size, with 2−6 transverse septa, apical beak 4−39 μm in length, 3−4 μm in width (Fig. 2d, e, h).

Notes. Based on phylogenetic analysis using combined dataset of multiple regions, strain YZU 231199 was relatively close to strains of *Alternaria tomato* (CBS 103.30 and CBS 114.35). Comparative analysis of nucleotide sequences revealed that strain YZU 231199 differed from representative strain of *A. tomato* (CBS 103.30) at four regions: 3 bp differences in *GAPDH* with 1 gap; 4 bp differences in *RPB2*, 1 bp difference in *TEF1*, and 1 bp difference in OPA10-2. Morphologically, the present fungus (YZU 231199) was also different with *A. tomato* in having smaller body size, less septa, and shorter beak (Table 2). Therefore, strain YZU 231199 was introduced as a novel species *A. oryzicola* sp. nov. in this study.

**Alternaria poae H.F. Liu & J.X. Deng, sp. nov.**
MycoBank No: 857596
Fig. 3

Etymology. Name refers to its host family Poaceae.

Type. CHINA • Hainan Province, Lingshui County, diseased leaves of *Oryza sativa*, July 2023, J.L. Yin, holotype YZU-H-2023056B (permanently preserved in a metabolically inactive state), ex-type culture YZU 231197.

Description. On PDA, *colonies* sub-rounded, fluffy, cottony, white to pale green or yellow-green, reverse side pale yellow to light yellow, 55–56 mm in diameter (Fig. 3a). On PCA, *conidiophores* unbranched, curved or straight, 15–77 × 3–4 µm in size, with 1–5 septa (Fig. 3f). *Conidiogenous cells* 5–9 × 3–4 µm, integrated, terminal, cylindrical, thin-walled, smooth, apically doliiform, with 1 conidiogenous locus. *Conidia* borne single or in chain with at least 2–4 conidia per chain, unbranched, narrow-ovoid, subellipsoid, or obclavate, smooth, 20–42 × 10–19 µm, with 1–4 transverse septa. basal rounded, apical beak 6–26 × 3–4 µm (Fig. 3b, c, g). On V8A, *conidiophores* unbranched, smooth, 30–96 × 3–4 µm, with 2–7 septa. *Conidia* produced in chain with at least 2–4 conidia
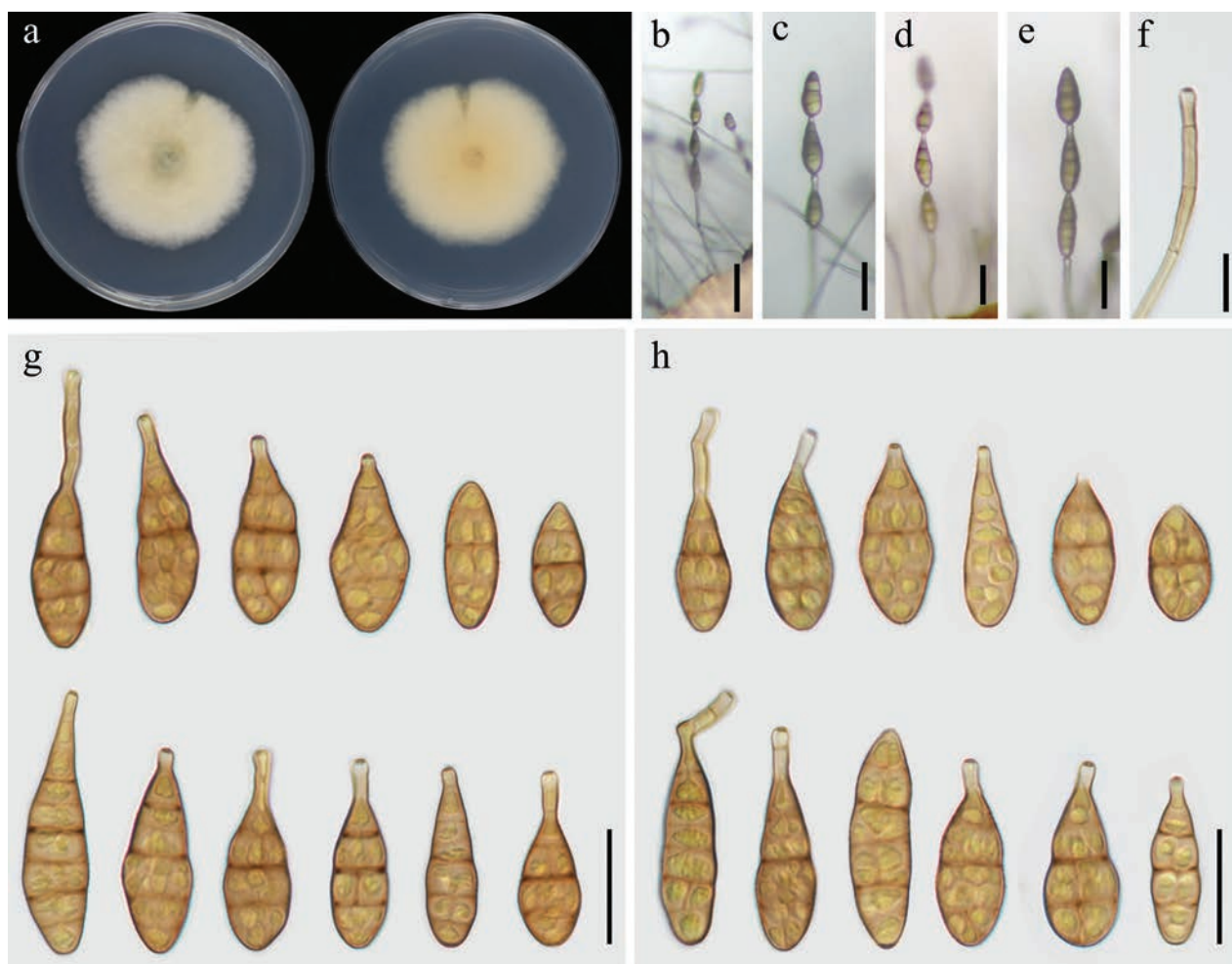
Figure 3. Morphology of *Alternaria poae* sp. nov. (YZU 231197) **a** colony on PDA for 7 days at 25 °C **b, c** sporulation on PCA **d, e** sporulation on V8A **f** conidiophore and conidiogenous cell **g** conidia on PCA **h** conidia on V8A. Scale bars: 50 µm (**b, c, d, e**); 15 µm (**f**); 25 µm (**g, h**).

per chain, subellipsoid, obclavate, or narrow-ovoid, 20–45 × 10–17 μm, 1–4 transverse septa, beak 5–17 × 3–4 μm (Fig. 3d, e, h).

Additional isolated examined. China • Hainan Province, Lingshui County, diseased leaves of *Oryza sativa*, July 2023, J.L. Yin, living culture YZU 231198.

Notes. In phylogenetic analysis using concatenated sequences of ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2, strains of *Alternaria poae* (YZU 231197 and YZU 231198) fell into a separate clade close to clades of *A. zeae* and *A. burnsii*. Based on nucleotide sequences, *A. poae* differs from *A. zeae* in five loci (3 bp in *GAPDH* with 1 gap, 5 bp in *RPB2*, 3 bp in *TEF1*, 3 bp in *Alt a 1*, and 7 bp in OPA10-2), and differs from *A. burnsii* in six loci (2 bp in *GAPDH*, 2 bp in *RPB2*, 3 bp in *TEF1*, 2 bp in *Alt a 1*, 2 bp in *EndoPG*, and 4 bp in OPA10-2). In morphology, *A. poae* can be distinguished from *A. zeae* by its shorter beak length, and from *A. burnsii* by its wider conidia bodies (Table 2).

Alternaria zeae H.F. Liu & J.X. Deng, sp. nov.
MycoBank No: 857597
Fig. 4

Etymology. Name refers to its host *Zea mays*.

Type. China • Guangxi Province, Liuzhou City, diseased leaves of *Zea mays*, September 2023, F.Y Liu, holotype YZU-H-2023150A (permanently preserved in a metabolically inactive state), ex-type culture YZU 231602.

Description. *Colonies* on PDA round, fluffy, cottony, greenish-gray, white at the margin, reverse side pale yellow, 58–59 mm in diameter (Fig. 4a). The conidial morphology on PDA and PCA was similar, with only slight differences. On PCA, *conidiophores* straight or curved, unbranched, 25–123 × 2.5–4.5 μm, with 1–8 septa (Fig. 4d). *Conidia* borne singly or in chain with 2–4 conidia per chain, ovate, ellipsoid or obclavate, with 3–6 transverse septa, 26–46 × 10–18 μm in size, mostly with septate apical beak, 9–93 × 2.5–4 μm in size (Fig. 4b, e). On V8A, *conidiophores* straight or curved, unbranched, 38–118 × 2.5–4 μm, with 1–7 septa. *Conidiogenous cells* 5–14 × 3–5 μm, integrated, apical, cylindrical, light brown, smooth, apically doliiform, with 1 conidiogenous locus. *Conidia* solitary or produced in chain with 2–4 conidia, ovate, ellipsoid or obclavate, with 3–6 transverse septa, 26–45 × 10–17 μm, apical beak 4.5–65 × 2.5–4 μm, with 0–4 septa (Fig. 4c, f).

Additional isolates examined. China • Guangxi Province, Liuzhou City, diseased leaves of *Zea mays*, September 2023, F.Y. Liu, living culture YZU 231638 and YZU 231640.

Notes. Strains of *Alternaria zeae* (YZU 231602, YZU 231638 and YZU 231640) formed a distinct clade in the multi-locus phylogenetic analysis. *Alternaria poae* and *A. burnsii* were genetically close to *A. zeae*. In nucleotide sequences, *A. zeae* differs from *A. poae* at five loci: 3 bp in *GAPDH* with 1 gap, 5 bp differences in *RPB2*, 3 bp in *TEF1*, 3 bp in *Alt a 1*, and 7 bp in OPA10-2. Nucleotide sequence differences were also observed between *A. zeae* and *A. burnsii* (3 bp in *GAPDH* with 1 gap, 2 bp in *RPB2*, 1 bp in *Alt a 1*, and 1 bp in OPA10-2). Morphologically, *A. zeae* has obviously longer beak than *A. poae* and *A. burnsii* (Table 2). In addition, conidia bodies of *A. zeae* are also wider than those of *A. burnsii* (Simmons 2007).
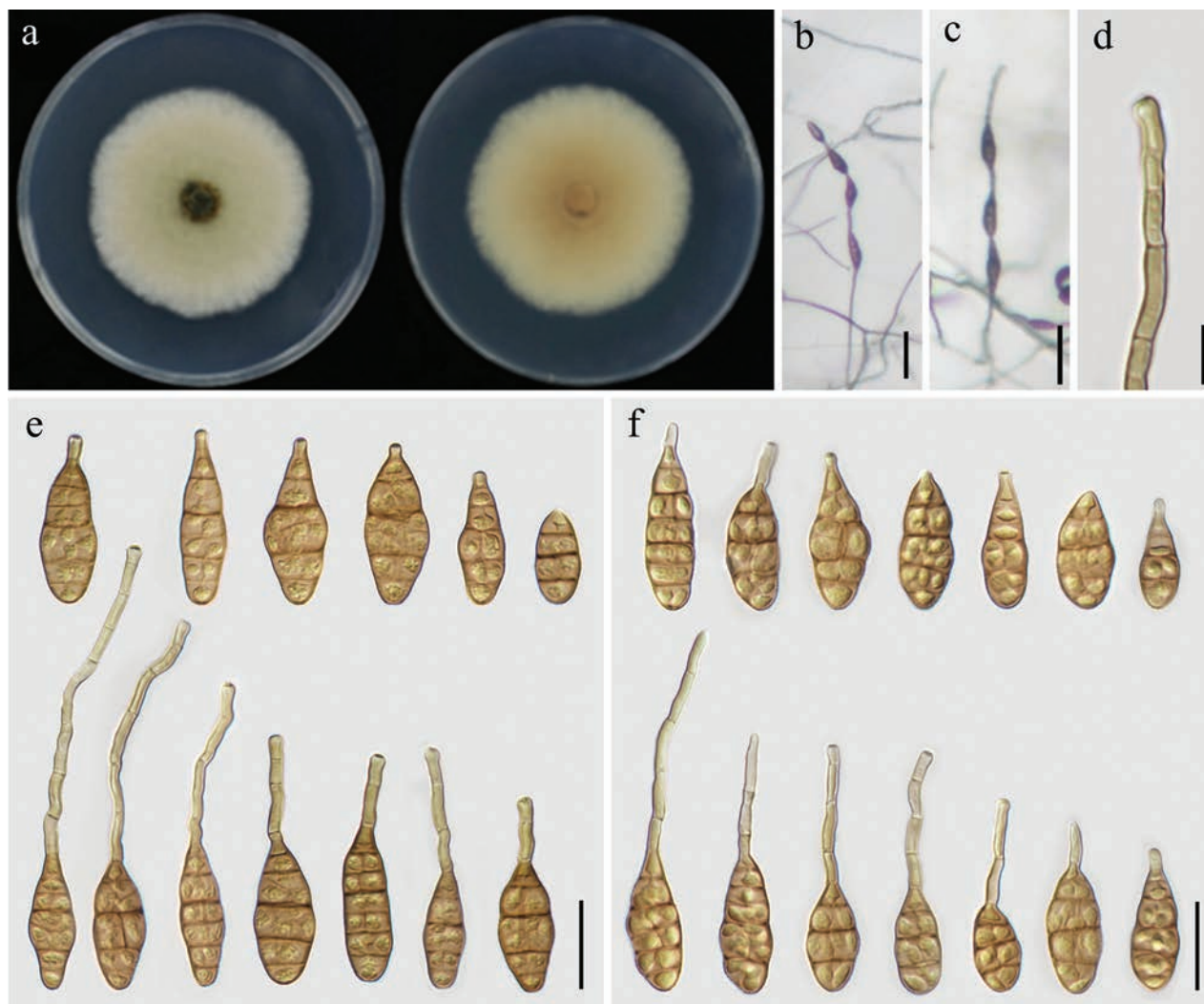
Figure 4. Morphology of *Alternaria zeae* sp. nov. (YZU 231602) **a** colony on PDA for 7 days at 25 °C **b, c** sporulation on PCA **d** conidiophore and conidiogenous cell **e** conidia on PCA **f** conidia on V8A. Scale bars: 50 μm (**b, c**); 15 μm (**d**); 25 μm (**e, f**).

Discussion

Based on integrated analyses of morphological characterization and multi-locus phylogenetic study, three novel species of *Alternaria* (*A. oryzicola* sp. nov., *A. poae* sp. nov., and *A. zeae* sp. nov.) from two different cereal crops (*O. sativa* and *Z. mays*) were described in this study. These findings contribute to the understanding of the diversity of *Alternaria* spp. on cereal crops in China.

In phylogenetic analysis using concatenated sequences of ITS, *GAPDH*, *RPB2*, *TEF1*, *Alt a 1*, *EndoPG*, and OPA10-2, all of the three novel species were assigned to distinct clades in *Alternaria* section *Alternaria*. This section contains most of the small-spored species, which include important plant, human and postharvest pathogens (Woudenberg et al. 2015). Phylogenetically, species *A. zeae* sp. nov. and *A. poae* sp. nov. were relatively close to *A. burnsii* and *A. oryzicola* sp. nov. was relatively close to *A. tomato*. These species were located at the top of the phylogenetic tree of section *Alternaria*. In terms of morphology, the three species from this study were distinguished from their related species (*A. burnsii* and *A. tomato*) based on conidial characteristics, such as conidia size, septa, and beak size, as shown in Table 2. Therefore, both

Table 2. Conidial morphology of *Alternaria* spp. from this study and previous publication.

Species	Conidia				Conidia per chain	Substrate	Reference
	Shape	Body size (μm)	Septa	Beak size (μm)			
Alternaria burnsii	ovoid or ellipsoid	30−50 × 9−13	5−8	−	Short chain	Host	Simmons (2007)
	narrow-ovoid or narrow-ellipsoid	30−40 × 8−14	3−7	−	−	PCA,V8A	Simmons (2007)
A. oryzicola sp. nov.	**narrow-obclavate, obclavate, or long ellipsoid**	**20−48 × 9−16**	**1−4**	**4−39 × 2.5−4**	**1−3**	**PCA**	**This study**
		18−56 × 9−16	**2−6**	**4−39 × 3−4**	**1−3**	**V8A**	**This study**
A. poae sp. nov.	**subellipsoid, obclavate, or narrow-ovoid**	**20−42 × 10−19**	**1−4**	**6−26 × 3−4**	**1−4**	**PCA**	**This study**
		20−45 × 10−17	**2−7**	**5−17 × 3−4**	**1−4**	**V8A**	**This study**
A. tomato	ellipsoid to long-ovoid	39−65 × 13−22	6−9	60−105 × 2	Solitary	Host	Simmons (2007)
A. zeae sp. nov.	**ovate, ellipsoid or obclavate**	**26−46 × 10−18**	**3−6**	**9−93 × 2.5−4**	**1−4**	**PCA**	**This study**
		26−45 × 10−17	**3−6**	**4.5−65 × 2.5−4**	**1−4**	**V8A**	**This study**

morphological and phylogenetic approaches provide evidence supporting the novelty of the species identified in this study.

In addition, the host is one of the important factors in the description of *Alternaria* species (Zhang 2003). According to fungus-host distribution in the USDA Fungal Databases (https://fungi.ars.usda.gov, accessed on 31 October 2024) and related publications, *A. burnsii* and *A. tomato* have been associated with different sources, but most are not from Poaceae plants. For example, *A. burnsii* was found on *Cuminum cyminum* (Simmons 2007; Woudenberg et al. 2015), *Tinospora cordifolia* (Woudenberg et al. 2015), *Rhizophora mucronata* (Woudenberg et al. 2015), *Gossypium* sp. (Woudenberg et al. 2015; El Gobashy et al. 2018), *Gomphrena globosa* (Woudenberg et al. 2015), *Sorghum* sp. (Kim et al. 2020), human sputum (Woudenberg et al. 2015), *Helianthus annuus* (Nwe et al. 2024), *Allium cepa* (Htun et al. 2022), *Apium graveolens* (Zhuang 2005), *Bunium persicum* (Mondal et al. 2002), *Cucurbita maxima* (Paul et al. 2015), *Pandanus* sp. (Hyde et al. 2018), and *Zea mays* (Xu et al. 2022). *Alternaria tomato* was reported on several plants, including *Solanum lycopersicum* (Simmons 2007), *Helianthus annuus* (Poudel et al. 2019), *Nopalea cochenillifera* (Infante et al. 2021), and *Phaseolus vulgaris* (Allen 1995). In the present study, the three novel species were isolated from two cereal crops (*Z. mays* and *O. sativa*), suggesting an increasing association of *Alternaria* species with Poaceae plants. According to previous studies, *Alternaria* spp. have been reported as predominant mycobiota in cereal grains (Kulik et al. 2015; Puvača et al. 2020; Orina et al. 2021). Most of these *Alternaria* species were predominantly classified in sections *Alternaria* and *Infectoriae* (Gannibal 2018), whereas some were sporadically found in section *Pseudoalternaria* (Gannibal 2018, Poursafar et al. 2018). Much attention has been devoted to detecting *Alternaria* spp. capable of producing mycotoxins (Orina et al. 2021). On cereal grains, several mycotoxins produced by *Alternaria* spp., such as AOH, AME, TEN, and TeA, were detected (Orina et al. 2021), posing potential risks to food safety. The ability of the three species in this study (*A. oryzicola* sp. nov., *A. poae* sp. nov., and *A. zeae* sp. nov.) to produce mycotoxins warrants further investigation. Since these three species were all isolated from diseased leaves of cereal crops, they could be potential pathogens. Furthermore, they are phylogenetically closely related to *A. burnsii* and *A. tomato*, which have recently been isolated and identified as pathogens of a cereal crop, wheat (Al-Nadabi et al. 2018).

Overall, this study characterized three novel species of *Alternaria* from two cereal crops, rice and maize, through morphological and molecular approaches. The potential interactions between these novel species and their host plants merit further investigation to uncover their ecological and agricultural impacts.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Hai-Feng Liu: methodology, data curation, writing - original draft. Feng-Yin Liu: methodology, investigation, data curation. Hai-Yan Ke: methodology. Qing-Xiao Shi: methodology. Jian-Xin Deng: conceptualization, writing – review & editing, supervision, project administration. Hyunkyu Sang: writing – review & editing, supervision.

Author ORCIDs

Hai-Feng Liu ⬤ https://orcid.org/0000-0002-9733-9240
Feng-Yin Liu ⬤ https://orcid.org/0000-0003-3114-603X
Jian-Xin Deng ⬤ https://orcid.org/0000-0001-7304-5603
Hyunkyu Sang ⬤ https://orcid.org/0000-0002-7459-5217

Data availability

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

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Research Article

Species of *Diaporthe* (Diaporthaceae, Diaporthales) associated with *Alnus nepalensis* leaf spot and branch canker diseases in Xizang, China

Jieting Li[1,2] , Yi Li[1,2] , Jiangrong Li[1,2] , Ning Jiang[3]

1 Institute of Xizang Plateau Ecology, Key Laboratory of Forest Ecology in Xizang Plateau (Xizang Agricultural and Animal Husbandry University), Ministry of Education, Linzhi, Xizang 860000, China
2 National Forest Ecosystem Observation & Research Station of Linzhi Xizang, Linzhi, Xizang 860000, China
3 Key Laboratory of Forest Protection of National Forestry and Grassland Administration, Ecology and Nature Conservation Institute, Chinese Academy of Forestry, Beijing 100091, China
Corresponding authors: Jiangrong Li (ljrong06@xza.edu.cn); Ning Jiang (n.jiang@caf.ac.cn)

Abstract

Alnus nepalensis is an important tree species in the Himalayas with significant ecological and economic roles. During disease surveys in Xizang, China, we observed leaf spot and branch canker symptoms on this tree. Fungal isolates associated with these diseases were collected and identified based on morphological characteristics and phylogenetic analysis of ITS, *cal*, *his3*, *tef1*, and *tub2* sequences. As a result, *Diaporthe alnicola* **sp. nov.** and *D. amygdali* were identified from the leaf spots, while *D. linzhiensis* was identified to be associated with the cankered branches. This study identifies pathogenic species from alder trees, providing a foundation for future disease management and forest health research.

Key words: Alder, molecular phylogeny, novel taxa, plant disease, Sordariomycetes, taxonomy

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Introduction

Alnus nepalensis (Nepalese alder) is a tree species of significant ecological and economic importance, particularly in the temperate and subtropical regions of the Himalayas, including Xizang, Nepal, and northern India (Sharma et al. 1998; Xia et al. 2023). This plant fulfills a crucial role in maintaining the ecological balance of forest ecosystems (Tobita et al. 2016; Sen et al. 2022). Beyond its ecological importance, *A. nepalensis* also has considerable economic value (Saxena et al. 2016). Given its critical role in both ecosystem function and local economies, any threats to *A. nepalensis* populations, such as leaf spot and canker diseases, could have severe consequences for forest health.

Diaporthe is a pathogenic fungal genus in the Diaporthaceae (Diaporthales, Sordariomycetes, Ascomycota) (Udayanga et al. 2012; Dissanayake et al. 2017; Jiang et al. 2025). Species of this genus are commonly associated with plant diseases, acting as pathogens, endophytes, or saprobes (Dissanayake et al. 2020; Dong et al. 2021; Jiang et al. 2021). For example, *D. hsinchuensis* and five other

species have been shown to cause leaf spots on *Camellia sinensis* (Ariyawansa et al. 2021). Several other *Diaporthe* species were shown to be associated with branch canker, dieback, and stem blight diseases (Guarnaccia et al. 2020; Guo et al. 2020; Bai et al. 2023). Additionally, *D. biconispora* and 15 other species from this genus were described as endophytes in *Citrus* (Huang et al. 2015).

Diaporthe is a species-rich genus with nearly 1,300 epithets listed in Index Fungorum (https://www.indexfungorum.org/). Over the past decade, many new species of this genus have been described based on both morphological characteristics and molecular phylogeny (Udayanga et al. 2014, 2015; Guarnaccia and Crous 2017; Yang et al. 2017, 2020, 2021; Fan et al. 2018; Manawasinghe et al. 2019; Huang et al. 2021; Sun et al. 2021; Cao et al. 2022; Lambert et al. 2023; Zhu et al. 2023, 2024; Liu et al. 2024). However, the species concepts of several taxa have been re-evaluated in recent years using the genealogical concordance phylogenetic species recognition (GCPSR) principle and coalescence-based models, such as the General Mixed Yule-Coalescent (GMYC) and Poisson Tree Processes (PTP). These re-evaluations have led to the synonymization of several species (Hilário et al. 2021a, 2021b; Dissanayake et al. 2024). For example, recent studies demonstrated that what was once thought to be a complex of nine species (*Diaporthe amygdali* species complex) is actually a single species (Hilário et al. 2021a, 2021b).

Dissanayake et al. (2024) divided the genus *Diaporthe* into seven sections and 15 species complexes based on phylogenetic analysis of all available type isolates of this genus, such as section Rudis and the *D. virgiliae* species complex. This classification has simplified phylogenetic analysis during species identification. In the present study, a survey of alder diseases was conducted in Xizang, China, with the aim of identifying the fungal species associated with leaf spots and branch cankers through a combination of morphological and molecular approaches.

Materials and methods

Sample collection, isolation, and morphology

Disease investigations were conducted from June to October in 2024 in Bayi District and Bomi County, Linzhi City, Xizang, China. Branch canker and leaf spot symptoms were observed, with canker being relatively rare and leaf spots more commonly encountered (Fig. 1). Infected branches exhibited sunken, discolored lesions, along with the presence of conidiomata of the fungal pathogen. Diseased leaves displayed small, rounded, or irregularly shaped spots, characterized by dark brown margins. Branch and leaf samples were collected and placed in paper envelopes for further analysis.

Sample branches and leaves were washed with sterile water and dried using refined absorbent cotton. Tissue fragments (5 × 5 mm) from both healthy and diseased samples were cut with a sterilized surgical knife, then immersed in 75% alcohol for 1 min, subsequently washed three times for 30 seconds each in sterile water, and dried with refined absorbent cotton. These tissue fragments were then transferred to the surface of Potato Dextrose Agar (PDA) plates. Hyphal tips grown from the tissue fragments on PDA were observed under a stereomicroscope (Discovery v8, Zeiss, Oberkochen, Germany). The fragments were then subcultured onto fresh PDA plates to obtain pure cultures.
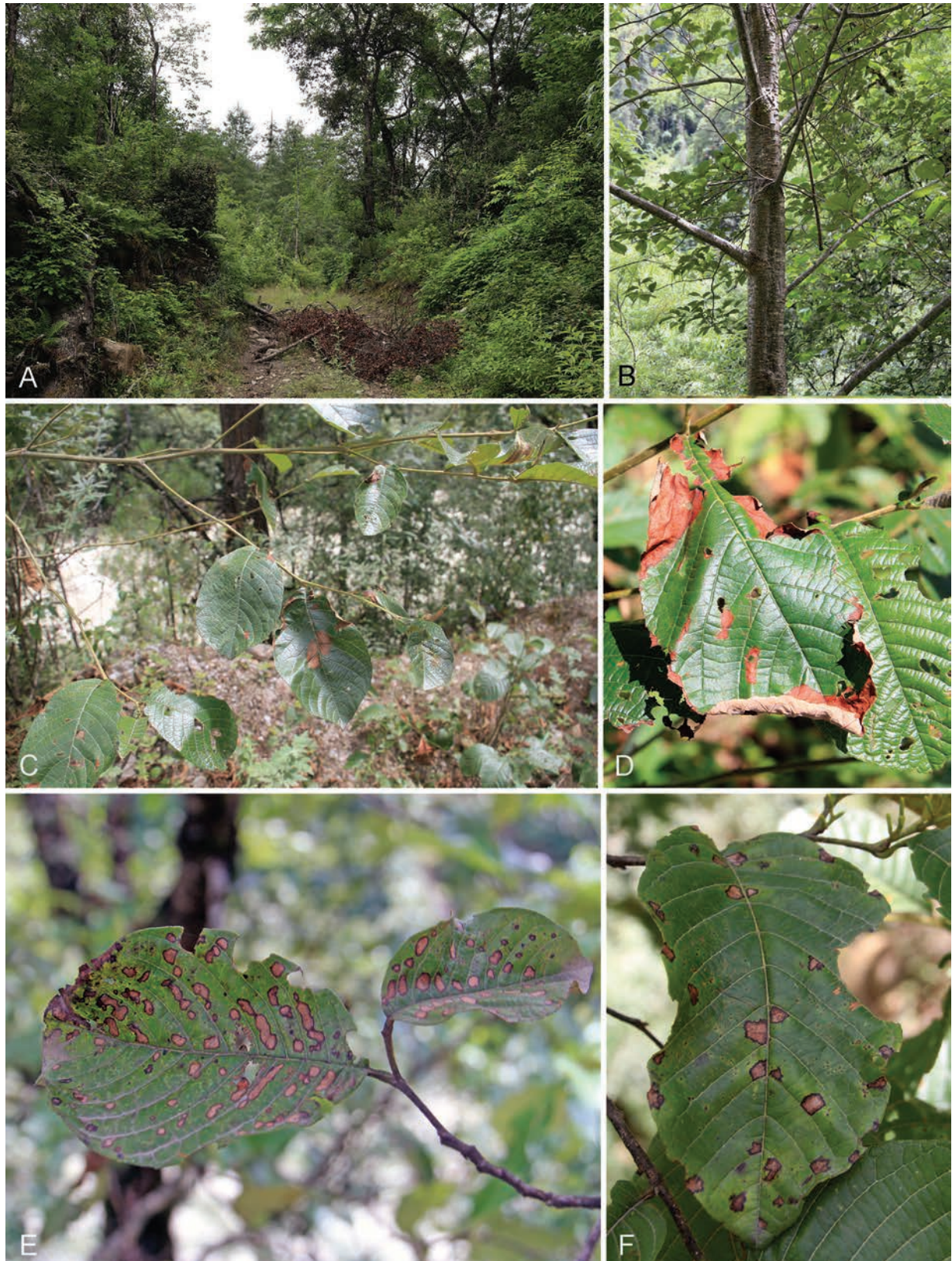
Figure 1. A, B sampling site **C–F** leaf spot symptoms of *Alnus nepalensis*.

Type specimens were deposited in the herbarium of the Chinese Academy of Forestry (CAF), and ex-type isolates were stored in the China Forestry Culture Collection Center (CFCC, https://cfcc.caf.ac.cn/).

Cultures were grown on PDA, malt extract agar (MEA), and synthetic nutrient agar (SNA) plates for observation. Conidiomata formed on the culture plates and branches were studied. The conidiomata were carefully sectioned using a double-edged blade, and fungal structures were observed under a Zeiss Discovery v8 stereomicroscope. Conidiophores, conidiogenous cells, and conidia were further examined and photographed using an Olympus BX51 microscope (Tokyo, Japan).

Phylogenetic analyses

The genomic DNA of the *Diaporthe* isolates obtained in this study was extracted from young colonies grown on PDA plates following the protocol of Doyle and Doyle (1990). The internal transcribed spacer (ITS) region of rDNA, along with fragments of the calmodulin (*cal*), histone H3 (*his3*), translation elongation factor 1-alpha (*tef1*), and partial beta-tubulin (*tub2*) genes, was amplified using the primers and protocols outlined in Table 1. The PCR products were subjected to electrophoresis on 2% agarose gels for analysis, followed by sequencing using the same primers as those employed in the PCR amplification. The sequencing service was provided by Ruibo Xingke Biotechnology Co., Ltd. (Beijing, China).

The ITS, *cal*, *his3*, *tef1*, and *tub2* gene sequences obtained in this study were queried against the GenBank nucleotide database located at the National Center for Biotechnology Information (NCBI) to identify closely related sequences and determine the associated species. Sequence data for related taxa were retrieved from Dissanayake et al. (2024) and downloaded from NCBI (Table 2). The sequences were aligned using the MAFFT v.7 online server (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2019) with default settings.

The isolates described in this study were shown to belong to the *Diaporthe* Section Rudis and the *D. virgiliae* species complex, respectively. Maximum likelihood (ML) phylogenetic analysis was conducted using the CIPRES Science Gateway platform (Miller et al. 2010), with RAxMLHPC2 on XSEDE (v. 8.2.10) under the GTR substitution model and 1000 non-parametric bootstrap replicates. Bayesian analysis was performed with MrBayes v. 3.2.6, utilizing four simultaneous Markov chain runs for 1,000,000 generations. The resulting trees were visualized using FigTree v. 1.4.0 (Rambaut 2012).

The pairwise homoplasy index test was employed to confirm the new species status using SplitsTree v.4.16.1 (Huson and Bryant 2006). Incongruence among the ITS-*cal-his3-tef1-tub2* genealogies was used as a criterion to identify hypothesized "species" and infer the occurrence of sexual recombination (Bruen et al. 2006). Results of the Φw-statistic below a 0.05 threshold (p-value < 0.05)

Table 1. Primers and PCR protocols.

Gene Regions	Primers	PCR conditions	References
ITS	ITS1/ITS4	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 48 °C for 1 min, and 72 °C for 2 min, 72 °C for 10 min	White et al. 1990
cal	CAL228F/CAL737R	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 54 °C for 1 min, and 72 °C for 2 min, 72 °C for 10 min	Carbone and Kohn 1999
his3	CYLH3F/H3-1b	95 °C for 5 min, 35 cycles of 95 °C for 1 min, 57 °C, 1.25 min, and 72 °C for 2 min, 72 °C for 10 min	Crous et al. 2004; Glass and Donaldson 1995
tef1	EF1-728F/EF1-986R	94 °C for 3 min, 35 cycles of 94 °C for 30 s, 54 °C for 50 s, and 72 °C for 2 min, 72 °C for 10 min	Carbone and Kohn 1999
tub2	T1(Bt2a)/Bt2b	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 54 °C for 1 min, and 72 °C for 2 min, 72 °C for 10 min	Glass and Donaldson 1995; O'Donnell and Cigelnik 1997

Table 2. GenBank accession numbers used in the phylogenetic analyses.

Species	Strain	GenBank accession numbers					References
		ITS	*tef1*	*tub2*	*cal*	*his3*	
Diaporthe acaciigena	CBS 129521	KC343005	KC343731	KC343973	KC343247	KC343489	Gomes et al. 2013
D. alnicola	**CFCC 70997***	**PQ636515**	**PQ635059**	**PQ635065**	**PQ635047**	**PQ635053**	**In this study**
D. alnicola	**CFCC 70998***	**PQ636516**	**PQ635060**	**PQ635066**	**PQ635048**	**PQ635054**	**In this study**
D. amygdali	CBS 126679	KC343022	KC343748	KC343990	KC343264	KC343506	Gomes et al. 2013
D. amygdali	CBS 111811	KC343019	KC343745	KC343987	KC343261	KC343503	Gomes et al. 2013
D. amygdali	CBS 115620	KC343020	KC343746	KC343988	KC343262	KC343504	Gomes et al. 2013
D. amygdali	CBS 120840	KC343021	KC343747	KC343989	KC343263	KC343505	Gomes et al. 2013
D. amygdali syn. *D. chongqingensis*	CGMCC 3.19603	MK626916	MK654866	MK691321	MK691209	MK726257	Guo et al. 2020
D. amygdali syn. *D. chongqingensis*	PSCG 435	MK626916	MK654866	MK691321	MK691209	MK726257	Guo et al. 2020
D. amygdali syn. *D. chongqingensis*	PSCG 436	MK626917	MK654867	MK691322	MK691208	MK726256	Guo et al. 2020
D. amygdali syn. *D. chongqingensis*	PSCG 436-2	MK626917	MK654867	MK691322	MK691208	MK726256	Guo et al. 2020
D. amygdali syn. *D. fusicola*	CGMCC 3.17087	KF576281	KF576256	KF576305	KF576233	NA	Gao et al. 2015
D. amygdali syn. *D. fusicola*	CGMCC 3.17088	KF576263	KF576238	KF576287	KF576221	NA	Gao et al. 2015
D. amygdali syn. *D. garethjonesii*	MFLUCC 12-0542	KT459423	KT459457	KT459441	KT459470	NA	Gao et al. 2015
D. amygdali syn. *D. kadsurae*	CFCC 52586	MH121521	MH121563	MH121600	MH121439	MH121479	Yang et al. 2018
D. amygdali syn. *D. kadsurae*	CFCC 52587	MH121522	MH121564	MH121601	MH121440	MH121480	Yang et al. 2018
D. amygdali syn. *D. mediterranea*	CBS 146754	MT007496	MT006996	MT006693	MT006768	MT007102	León et al. 2020
D. amygdali syn. *D. ovoicicola*	CGMCC 3.17092	KF576264	KF576239	KF576288	KF576222	NA	Gao et al. 2015
D. amygdali syn. *D. ovoicicola*	CGMCC 3.17093	KF576265	KF576240	KF576289	KF576223	NA	Gao et al. 2015
D. amygdali syn. *D. ovoicicola*	CGMCC 3.17094	KF576266	KF576241	KF576290	KF576224	NA	Gao et al. 2015
D. amygdali syn. *D. ovoicicola*	ACJY62	MW578711	MW597404	MW598141	MW598161	MW598183	Gao et al. 2015
D. amygdali syn. *D. sterilis*	CBS 136969	KJ160579	KJ160611	KJ160528	KJ160548	MF418350	Lombard et al. 2014
D. amygdali syn. *D. sterilis*	CPC 20580	KJ160582	KJ160614	KJ160531	KJ160551	NA	Lombard et al. 2014
D. amygdali syn. *D. ternstroemia*	CGMCC 3.15183	KC153098	KC153089	NA	NA	NA	Gao et al. 2014
D. amygdali syn. *D. ternstroemia*	CGMCC 3.15184	KC153099	KC153090	NA	NA	NA	Gao et al. 2014
D. amygdali	**CFCC 70999**	**PQ636517**	**PQ635061**	**PQ635067**	**PQ635049**	**PQ635055**	**In this study**
D. amygdali	**Q3B**	**PQ636518**	**PQ635062**	**PQ635068**	**PQ635050**	**PQ635056**	**In this study**
D. araucanorum	CBS 145285	MN509711	MN509733	MN509722	NA	NA	Zapata et al. 2020
D. araucanorum	CBS 145283	MN509709	MN509731	MN509720	NA	NA	Zapata et al. 2020
D. beckhausii	CBS 138.27	KC343041	KC343767	KC344009	KC343283	KC343525	Gomes et al. 2013
D. benedicti	BPI 893190	KM669929	KM669785	NA	KM669862		Lawrence et al. 2015
D. breviconidiophora	CGMCC 3.24298	OP056725	OP150564	OP150641	OP150718	OP150794	Dissanayake et al. 2024
D. breviconidiophora	GZCC 22-0030	OP056725	OP150564	OP150641	OP150718	OP150794	Dissanayake et al. 2024
D. cassines	CPC 21916	KF777155	KF777244	NA	NA	NA	Crous et al. 2013
D. celticola	CFCC 53074	MK573948	MK574623	MK574643	MK574587	MK574603	Cao et al. 2022
D. celticola	CFCC 53075	MK573949	MK574624	MK574644	MK574588	MK574604	Cao et al. 2022
D. crousii	CAA823	MK792311	MK828081	MK837932	MK883835	MK871450	Hilário et al. 2020
D. crousii	CAA820	MK792300	MK828072	MK837923	MK883828	MK871441	Hilário et al. 2020
D. eres	AR5193	KJ210529	KJ210550	KJ420799	KJ434999	KJ420850	Udayanga et al. 2014
D. eres	DLR12a	KJ210518	KJ210542	KJ420783	KJ434996	KJ420833	Udayanga et al. 2014
D. foikelawen	CBS 145289	MN509713	MN509735	MN509724	NA	NA	Zapata et al. 2020
D. foikelawen	CBS 145287	MN509714	MN509736	MN509725	NA	NA	Zapata et al. 2020
D. grandifori	SAUCC 194.84	MT822612	MT855924	MT855809	MT855691	MT855580	Sun et al. 2021
D. guizhouensis	GZCC 20-0338	OM060254	OL961761	OL961762	OL961763	NA	Bhunjun et al. 2022
D. guizhouensis	GZCC 22-0027	OP056683	OP150522	OP150600	OP150679	OP150754	Bhunjun et al. 2022
D. guizhouensis	GZCC 22-0045	OP056684	OP150523	OP150601	OP150680	OP150755	Bhunjun et al. 2022
D. heterophyllae	CBS 143769	MG600222	MG600224	MG600226	MG600218	MG600220	Marín-Felix et al. 2019
D. heveae	B23	KR812219	NA	NA	NA	NA	Dos Santos et al. 2015
D. linzhiensis	**CFCC 71057***	**PQ636519**	**PQ635063**	**PQ635069**	**PQ635051**	**PQ635057**	**In this study**
D. linzhiensis	**N266C***	**PQ636520**	**PQ635064**	**PQ635070**	**PQ635052**	**PQ635058**	**In this study**
D. nothofagi	BRIP 54801	JX862530	JX862536	KF170922	NA	NA	Tan et al. 2013
D. obtusifoliae	CBS 143449	MG386072	NA	NA	NA	MG386137	Crous et al. 2017
D. ocoteae	CPC 26217	KX228293	NA	KX228388	NA	NA	Crous et al. 2013

| Species	Strain	GenBank accession numbers					References
		ITS	*tef1*	*tub2*	*cal*	*his3*	
D. penetriteum	LC3353	KP714505	KP714517	KP714529	NA	KP714493	Gao et al. 2016
D. pustulata	CBS 109742	KC343185	KC343911	KC344153	KC343427	KC343669	Gomes et al. 2013
D. pustulata	CBS 109784	KC343187	KC343913	KC344155	KC343429	KC343671	Gomes et al. 2013
D. rudis	AR3422	KC843331	KC843090	KC843177	KC843146	NA	Udayanga et al. 2014
D. rudis	AR3654	KC843338	KC843097	KC843184	KC843153	NA	Udayanga et al. 2014
D. rudis	DA244	KC843334	KC843093	KC843180	KC843149	NA	Udayanga et al. 2014
D. rudis	ICMP 16419	KC145904	KC145976	NA	NA	NA	Udayanga et al. 2014
D. rudis	ICMP 7025	KC145885	KC145995	NA	NA	NA	Udayanga et al. 2014
D. rudis	CBS 113201	MH862916	KC343960	KC344202	KC343476	KC343718	Vu et al. 2019
D. rudis syn. *D. australafricana*	CBS 111886	KC343038	KC343764	KC344006	KC343280	KC343522	Gomes et al. 2013
D. rudis syn. *D. australafricana*	CBS 113487	KC343039	KC343765	KC344007	KC343281	KC343523	Gomes et al. 2013
D. rudis syn. *D. cynaroidis*	CBS 122676	KC343058	KC343784	KC344026	KC343300	KC343542	Gomes et al. 2013
D. rudis syn. *D. patagonica*	CBS 145291	MN509717	MN509739	MN509728	NA	NA	Zapata et al. 2020
D. rudis syn. *D. patagonica*	CBS 145755	MN509718	MN509740	MN509729	NA	NA	Zapata et al. 2020
D. rudis syn. *D. salicicola*	BRIP 54825	JX862531	JX862537	KF170923	NA	NA	Tan et al. 2013
D. rudis syn. *D. subcylindrospora*	KUMCC 17-0151	MG746629	MG746630	MG746631	NA	NA	Hyde et al. 2018
D. shennongjiaensis	CNUCC 201905	MN216229	MN224672	MN227012	MN224551	MN224559	Zhou and Hou 2019
D. shennongjiaensis	CNUCC 201906	MN216228	MN224673	MN227013	MN224552	MN224561	Zhou and Hou 2019
D. silvicola	CFCC 54191	MZ727041	MZ816347	MZ753491	MZ753472	MZ753481	Jiang et al. 2021
D. silvicola	M79	MZ727042	MZ816348	MZ753492	MZ753473	MZ753482	Jiang et al. 2021
D. torilicola	MFLUCC 17-1051	KY964212	KY964168	KY964096	KY964127		Dissanayake et al. 2017
D. toxica	CBS 534.93	KC343220	KC343946	KC344188	KC343462	KC343704	Gomes et al. 2013
D. toxica	CBS 546.93	KC343222	KC343948	KC344190	KC343464	KC343706	Gomes et al. 2013
D. virgiliae	CMW 40755	KP247573	NA	KP247582	NA	NA	Machingambi et al. 2015
D. virgiliae	CMW 40748	KP247566	NA	KP247575	NA	NA	Machingambi et al. 2015
D. zaofenghuang	CGMCC 3.20271	MW477883	MW480871	MW480875	MW480867	MW480863	Wang et al. 2021
D. zaofenghuang	TZFH3	MW477884	MW480872	MW480876	MW480868	MW480864	Wang et al. 2021

Note: "NA" indicates unavailable sequences; sequences produced in the current study are in bold, and * means ex-type strains from new species in this study.

indicated significant recombination. A phylogenetic network based on the combined dataset of five loci was constructed using the NeighborNet algorithm to assess the impact of recombination.

Results

Phylogenetic analyses

For the analysis of *Diaporthe* Section *Rudis*, the combined dataset of ITS, *cal*, *his3*, *tef1*, and *tub2* comprised 67 strains, with *D. eres* (AR5193 and DLR12a) used as the outgroup taxa. The final alignment included 2,691 characters (ITS: 451, *cal*: 702, *his3*: 410, *tef1*: 596, *tub2*: 532), including gaps. The final ML optimization likelihood value of the best RAxML tree was -17019.93, and the matrix contained 1,257 distinct alignment patterns, with 32.15% undetermined characters or gaps. The estimated base frequencies were A = 0.216951, C = 0.313266, G = 0.235799, T = 0.233984; substitution rates were AC = 1.028567, AG = 3.157223, AT = 1.223911, CG = 0.822997, CT = 4.362405, GT = 1.0; and the gamma distribution shape parameter α = 0.386104. Both the RAxML and Bayesian analyses produced similar tree topologies, which were consistent with those of previous studies (Norphanphoun et al. 2022; Dissanayake et al. 2024). Isolates from this study (CFCC 70999 and Q3B) clustered together with other *Diaporthe amygdali* strains, showing strong support (Fig. 2), thus confirming their identification as *D. amygdali*.
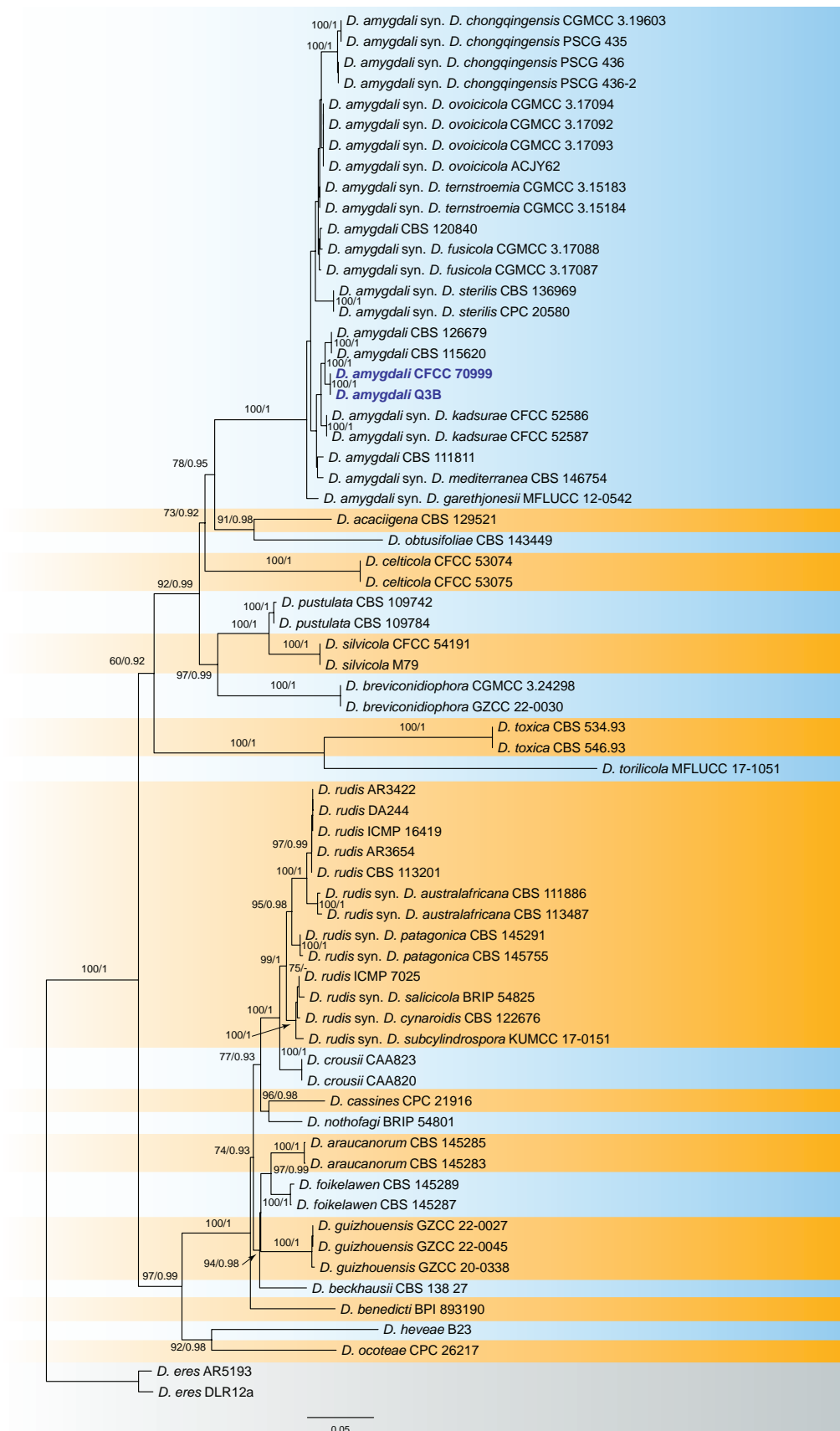
Figure 2. Maximum likelihood tree of *Diaporthe* Section Rudis generated from combined ITS, *cal*, *his3*, *tef1*, and *tub2* sequence data. Bootstrap support values ≥ 50% and Bayesian posterior probabilities ≥ 0.90 are demonstrated at the branches. Isolates from the present study are indicated in blue.

In the *Diaporthe virgiliae* species complex, the combined dataset of ITS, *cal*, *his3*, *tef1*, and *tub2* included 13 strains, with *D. shennongjiaensis* (CUNCC 201905 and CUNCC 201906) as the outgroup taxa. The final alignment contained 2,598 characters (ITS: 593, *cal*: 421, *his3*: 466, *tef1*: 331, *tub2*: 787), including gaps. The final ML optimization likelihood value of the best RAxML tree was -5834.44, and the matrix had 346 distinct alignment patterns, with 20.11% undetermined characters or gaps. The estimated base frequencies were A = 0.212455, C = 0.329026, G = 0.238268, T = 0.220251; substitution rates were AC = 1.111868, AG = 2.843163, AT = 1.775735, CG = 0.816784, CT = 3.662621, GT = 1.0; and the gamma distribution shape parameter α = 0.047755. Both RAxML and Bayesian analyses produced similar tree topologies, which closely matched those of prior publications (Norphanphoun et al. 2022; Dissanayake et al. 2024). Four isolates from this study formed two new clades distinct from any lineage and are hence accommodated as two novel species: *D. alnicola* (CFCC 70997 and CFCC 70998) and *D. linzhiensis* (CFCC 71057 and N266C).

The network relationships within the *D. virgiliae* species complex are depicted in Fig. 4, indicating no significant recombination based on the PHI test (*p* = 0.9624). Furthermore, based on the relative distances between species and the structure of the phylogenetic network, isolates within the *D. virgiliae* complex represent seven different species.

Taxonomy

Diaporthe alnicola Ning Jiang, sp. nov.
MycoBank No: 856742
Fig. 5

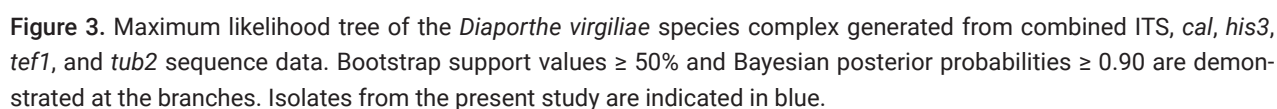**Etymology.** "*Alni*" refers to the host genus *Alnus*, and "*-cola*" means inhabiting.

Description. Associated with leaf spot disease of *Alnus nepalensis*. **Teleomorph**: Undetermined. **Anamorph**: Conidiomata formed on PDA pycnidial, scattered, erumpent, pulvinate to subglobose, dark brown, 150−350 μm diam. Conidiophores indistinct, usually reduced to conidiogenous cells. Conidiogenous cells cylindrical, attenuate towards the apex, hyaline, phialidic, 9.5−33 × 2−3 μm. Alpha conidia aseptate, hyaline, smooth, guttulate, cylindrical, straight, base truncate, (6−)6.5−7(−7.5) × (2−)2.5−3(−3.5) μm ($\bar{x}$ = 6.8 × 2.6 μm, n = 50), L/W = 2−3.4. Beta conidia aseptate, hyaline, smooth, guttulate, filiform, tapering towards both ends, curved, (13−)14.5−22(−24) × 1.5−2.5 μm ($\bar{x}$ = 18.3 × 2.1 μm, n = 50), L/W = 5.9−12.5. Gamma conidia not observed.

Culture characteristics. Colonies on PDA at 25 °C are spreading, flocculent, forming abundant aerial mycelium and an undulate margin, initially white, turning mouse gray and reaching a diameter of 90 mm after 10 d, developing dark brown conidiomata with orange conidial masses after 20 d. Colonies on MEA at 25 °C are flat, spreading, feathery, with a smooth entire margin, white, reaching a diameter of 90 mm after 15 d, sterile. Colonies on SNA at 25 °C are flat, spreading with a smooth entire margin, white, reaching 90 mm in diameter after 20 d, developing dark brown conidiomata with orange conidial masses after 30 d.

Materials examined. CHINA • Xizang Autonomous Region (Tibet), Linzhi City, Bayi District, Pailong Town, 30°4'22"N, 95°8'2"E, 2192 m, from leaf spots of

Alnus nepalensis, 9 Jul. 2024, *Ning Jiang, Jieting Li & Haoyin Zhang* (**holotype** CAF800100, ex-paratype cultures CFCC 70997 and CFCC 70998).

Notes. *Diaporthe alnicola*, identified from leaf spots on *Alnus nepalensis* in this study, is phylogenetically closely related to *D. virgiliae*, which originates from the rot root of *Virgilia oroboides* in South Africa (Fig. 3). Morphologically, *D. alnicola* is similar to *D. virgiliae* in terms of the size of alpha and beta conidia (alpha conidia: 6.5−7 × 2.5−3 μm in *D. alnicola* vs. 5.2−8 × 1.1−3.5 μm in *D. virgiliae*; beta conidia: 14.5−22 × 1.5−2.5 μm in *D. alnicola* vs. 17.1−25.4 × 1−1.8 μm in *D. virgiliae*). However, they can be distinguished by the size of their conidiogenous cells (9.5−33 × 2−3 μm in *D. alnicola* vs. 12.3−21.3 × 0.7−1.5 μm in *D. virgiliae*) (Machingambi et al. 2015). Furthermore, *D. alnicola* differs from *D. virgiliae* at the nucleotide level (ITS, 11/432; *tub2*, 7/743).

Figure 3. Maximum likelihood tree of the *Diaporthe virgiliae* species complex generated from combined ITS, *cal*, *his3*, *tef1*, and *tub2* sequence data. Bootstrap support values ≥ 50% and Bayesian posterior probabilities ≥ 0.90 are demonstrated at the branches. Isolates from the present study are indicated in blue.
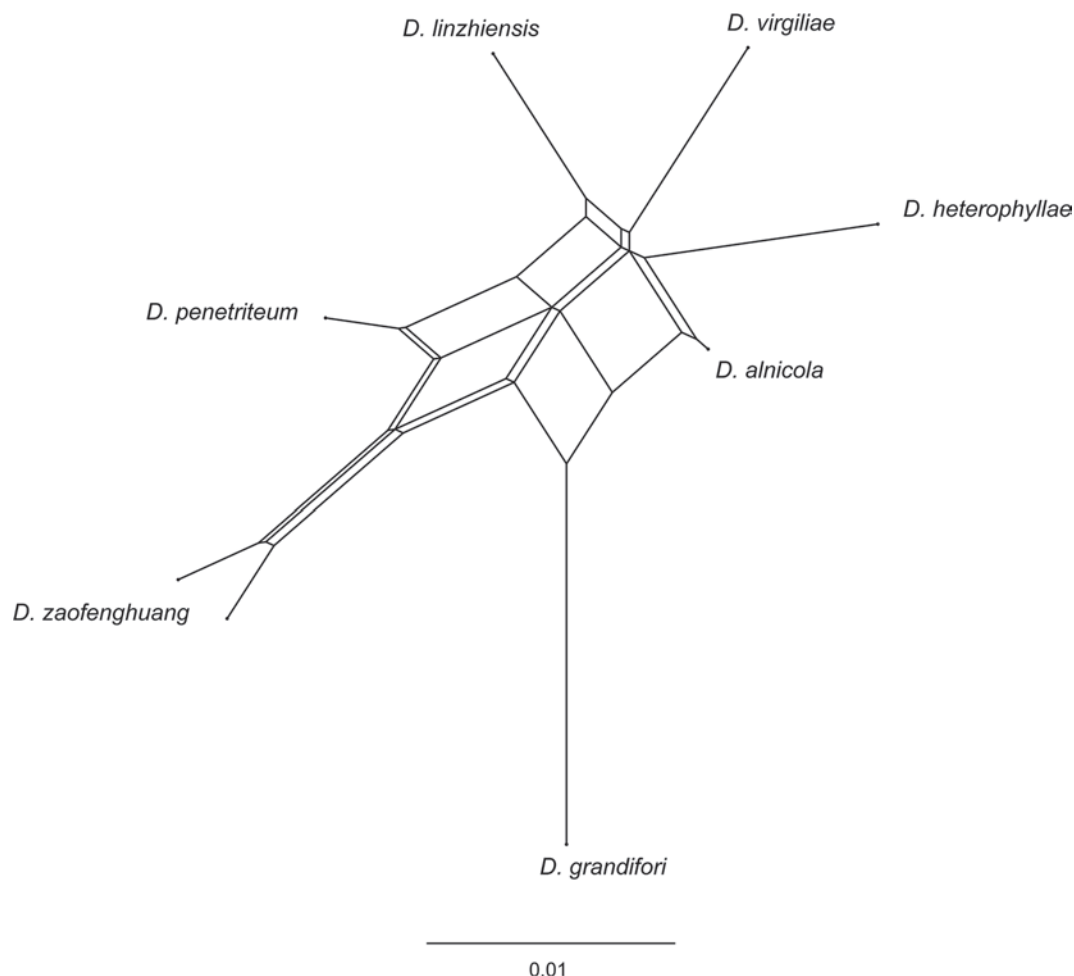
Figure 4. Phylogenetic network from concatenated data (ITS, *cal*, *his3*, *tef1*, and *tub2*) representing the structure of the *Diaporthe virgiliae* species complex, based on LogDet transformation and the NeighborNet algorithm, inferred by SplitsTree (*p* = 0.9624). The scale bar represents the expected number of substitutions per nucleotide position.

***Diaporthe amygdali* (Delacr.) Udayanga, Crous & K.D. Hyde, Fungal Diversity 56(1): 166. 2012**
Fig. 6

Description. Associated with leaf spot disease of *Alnus nepalensis*. **Teleomorph**: Undetermined. **Anamorph**: Conidiomata formed on PDA pycnidial, scattered, erumpent, subglobose, dark brown, 700–2250 µm diam. Conidiophores indistinct, usually reduced to conidiogenous cells. Conidiogenous cells cylindrical, attenuate towards the apex, hyaline, phialidic, 16.5–34 × 1.5–3 µm. Alpha conidia not observed. Beta conidia aseptate, hyaline, smooth, guttulate, filiform, tapering towards both ends, straight or slightly curved, (27.5–)30–35(–40.5) × 1.5–2 µm ($\bar{x}$ = 32.6 × 1.6 µm, n = 50), L/W = 15.8–23.1. Gamma conidia not observed.

Culture characteristics. Colonies on PDA at 25 °C are flocculent, forming concentric zones with undulate margins, initially white, turning pale brownish, and reaching a diameter of 90 mm after 10 d, developing dark brown conidiomata with white conidial masses after 25 d. Colonies on MEA at 25 °C are flat, spreading, with a smooth entire margin, white, reaching a diameter of 80 mm after 20 d, sterile. Colonies on SNA at 25 °C are flat, spreading with a feathery margin, white, reaching 80 mm in diameter after 20 d, sterile.
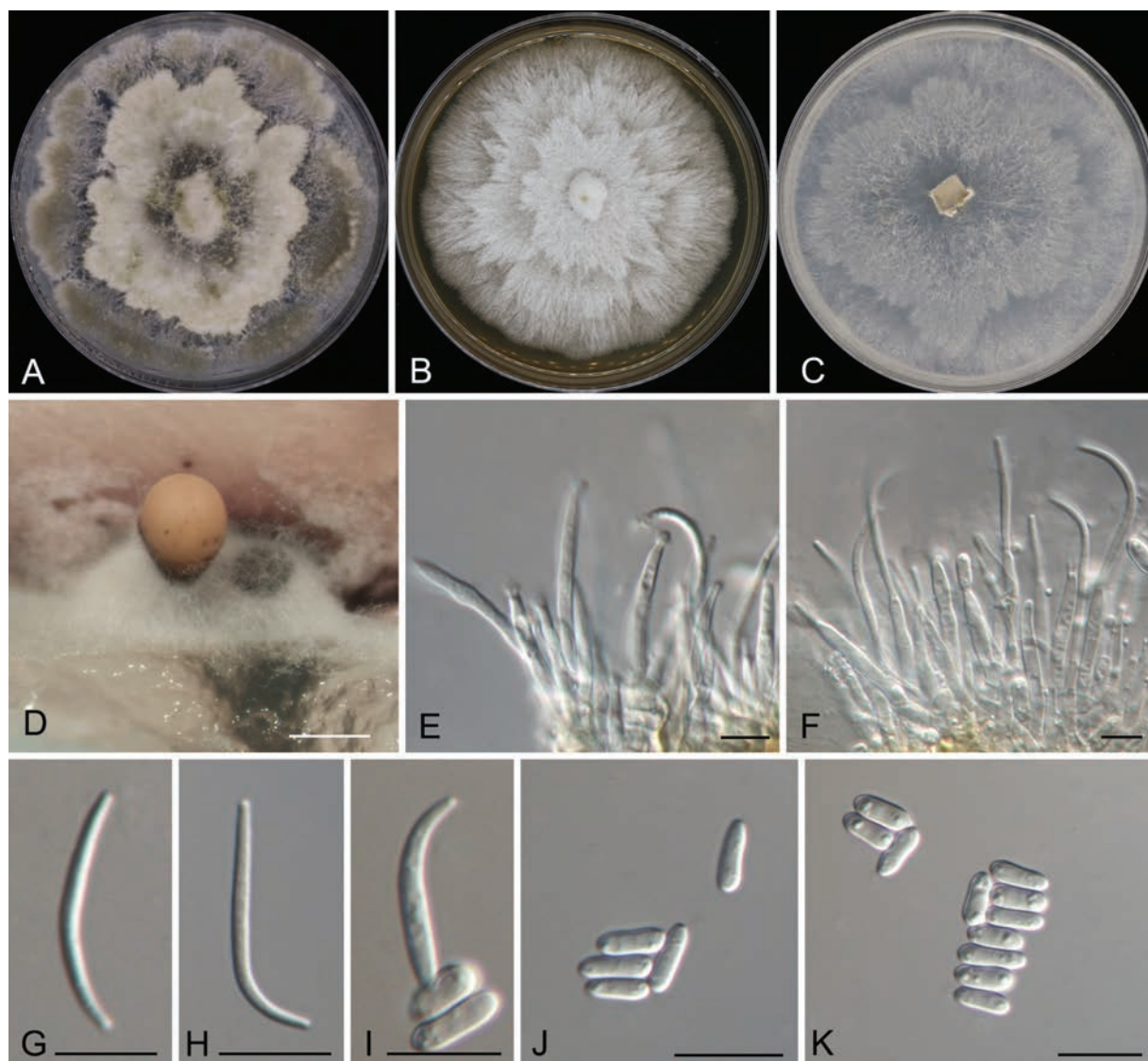
Figure 5. Morphology of *Diaporthe alnicola* **A** colony on PDA after 15 d **B** Colony on MEA after 15 d **C** colony on SNA after 15 d **D** conidioma formed on PDA **E, F** conidiogenous cells **G–K** alpha and beta conidia. Scale bars: 500 μm (**D**); 10 μm (**E–K**).

Materials examined. CHINA • Xizang Autonomous Region (Tibet), Linzhi City, Bayi District, Pailong Town, 30°4'22"N, 95°8'2"E, 2192 m, from leaf spots of *Alnus nepalensis*, 9 Jul. 2024, *Ning Jiang, Jieting Li & Haoyin Zhang* (cultures CFCC 70999 and Q3B).

Notes. The species concept of *Diaporthe amygdali* has been revised in recent studies using phylogenetic analysis, GCPSR, and coalescence-based models (Hilário et al. 2021b; Dissanayake et al. 2024). Currently, *D. amygdali* is considered synonymous with *D. chongqingensis*, *D. fusicola*, *D. garethjonesii*, *D. kadsurae*, *D. mediterranea*, *D. ovoicicola*, *D. sterilis*, and *D. ternstroemia* (Hilário et al. 2021b; Dissanayake et al. 2024). This fungus is widely distributed, inhabiting a range of plant hosts, including *Acer* spp., *Camellia sinensis*, *Lithocarpus glabra*, *Prunus dulcis*, *Prunus persica*, *Prunus salicina*, *Pyrus pyrifolia*, *Ternstroemia gymnanthera*, *Vaccinium corymbosum*, and *Vitis vinifera* (Hilário et al. 2021b). In this study, two isolates from leaf spots of *Alnus nepalensis* clustered with strains of *D. amygdali* with high support values (Fig. 2). Therefore, these two isolates were identified as *D. amygdali*, which led us to describe *Alnus nepalensis* as a new host for this fungus.
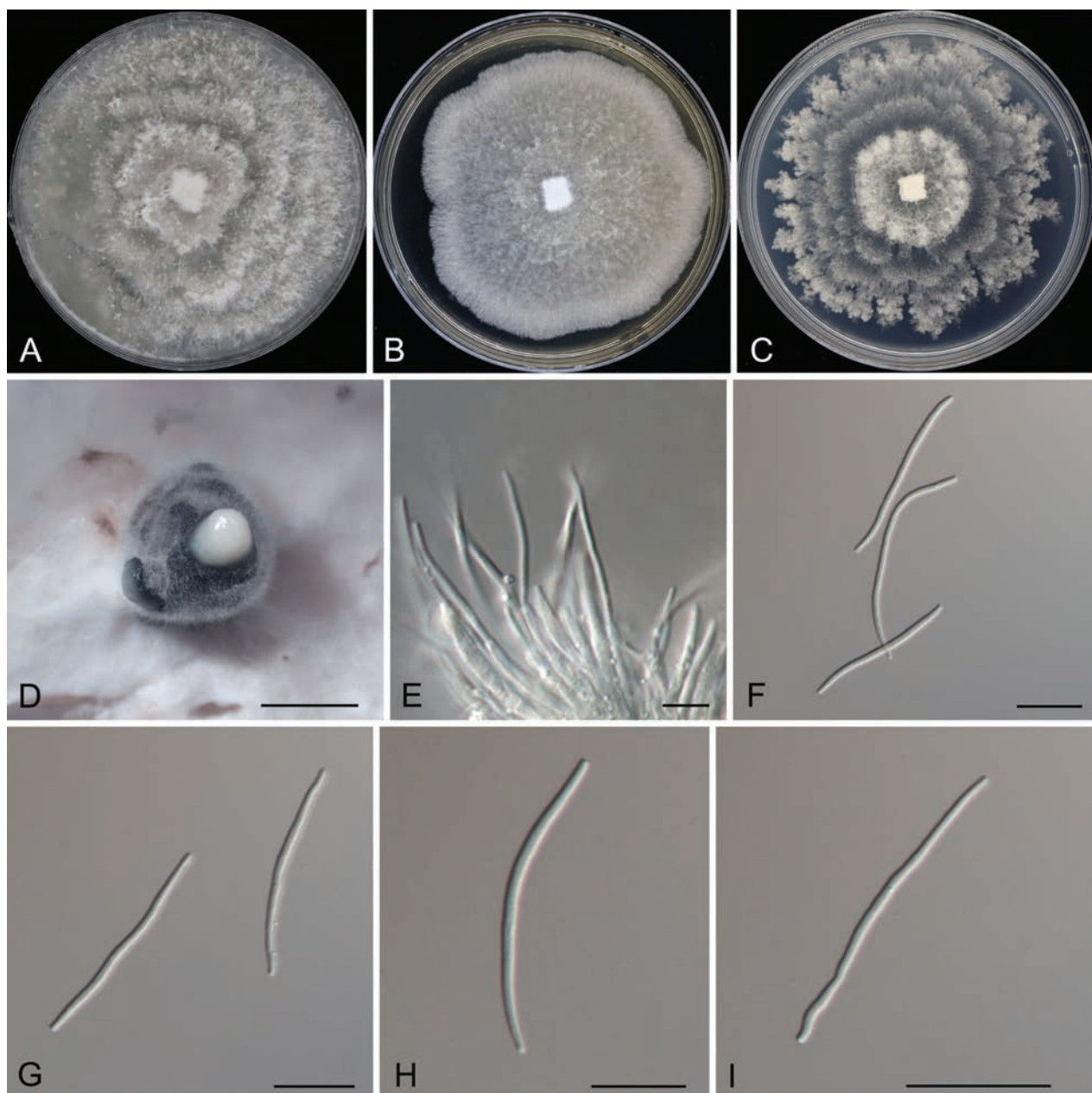
Figure 6. Morphology of *Diaporthe amygdali* **A** colony on PDA after 15 d **B** colony on MEA after 15 d **C** colony on SNA after 15 d **D** conidioma formed on PDA **E** conidiogenous cells **E–I** beta conidia. Scale bars: 800 μm (**D**); 10 μm (**E–K**).

Diaporthe linzhiensis Ning Jiang, sp. nov.

MycoBank No: 856743

Fig. 7

Etymology. Named after the collection site of the type specimen, Linzhi City.

Description. Associated with branch canker disease of *Alnus nepalensis*. **Teleomorph**: Undetermined. **Anamorph**: Conidiomata pycnidial, immersed in bark, scattered, erumpent through the bark surface, conical, with a solitary locule, 300–500 μm diam., 250–400 μm high. Conidiophores reduced to conidiogenous cells. Conidiogenous cells cylindrical, attenuate towards the apex, hyaline, phialidic, straight or slightly curved, 5.5–16 × 1.5–3 μm. Alpha conidia not
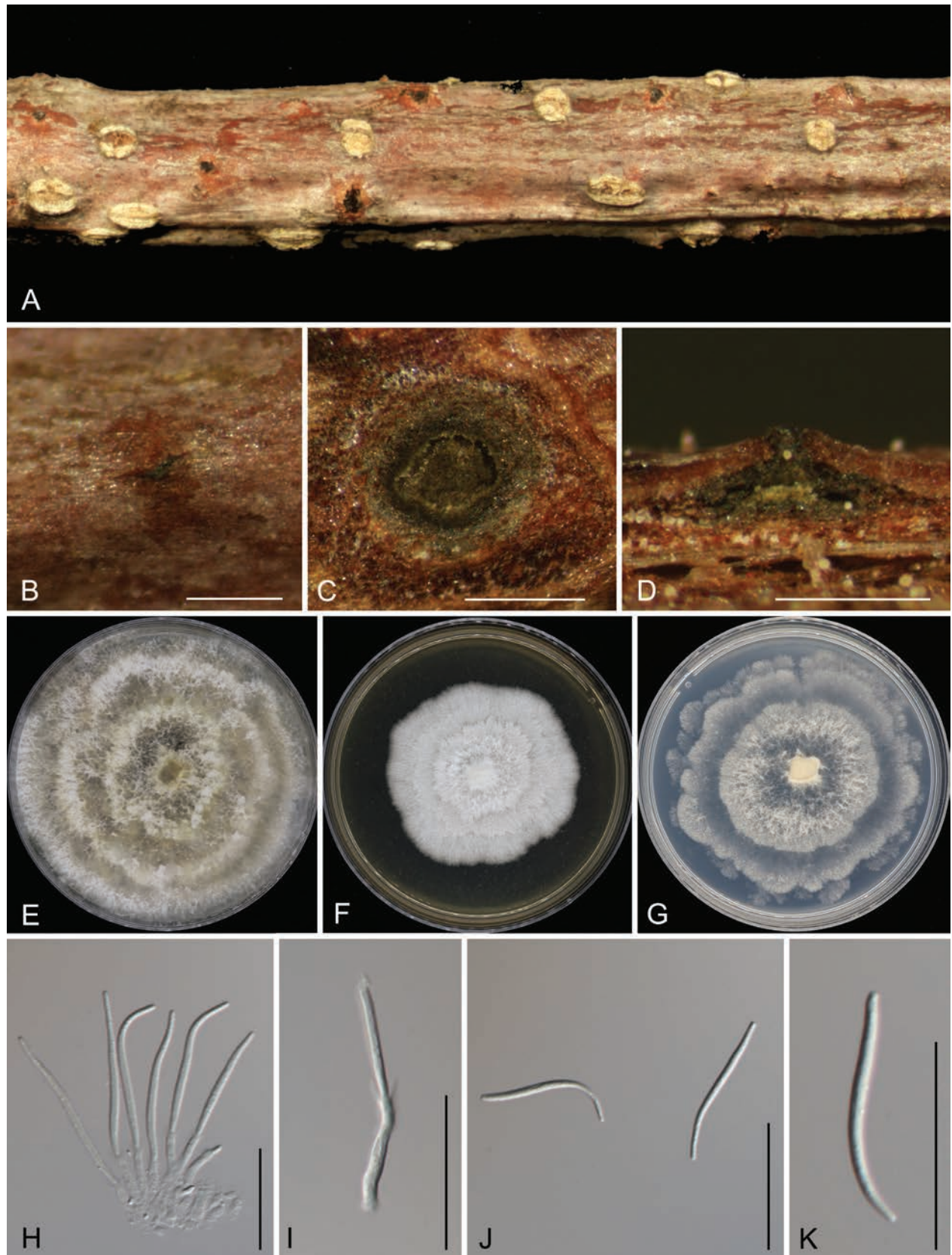
Figure 7. Morphology of *Diaporthe linzhiensis* **A, B** conidiomata formed on twigs of *Alnus nepalensis* **C** transverse section through a conidioma **D** longitudinal section through a conidioma **E** colony on PDA after 15 d **F** colony on MEA after 15 d **G** colony on SNA after 15 d **H, I** conidiogenous cells **J, K** beta conidia. Scale bars: 500 μm (**B–D**); 20 μm (**H–K**).

observed. Beta conidia aseptate, hyaline, smooth, guttulate, filiform, tapering towards both ends, straight or slightly curved, (23.5−)24.5−29(−30) × 1.5−2 μm ($\bar{x}$ = 26.6 × 1.8 μm, n = 50), L/W = 12.4−19.4. Gamma conidia not observed.

Culture characteristics. Colonies on PDA at 25 °C are spreading, flocculent, forming abundant aerial mycelium and concentric zones with an undulate margin, initially white, turning pale luteous, and reaching a diameter of 90 mm after 10 d, sterile. Colonies on MEA at 25 °C are flat, spreading, with a smooth entire margin, white, reaching a diameter of 60 mm after 20 d, sterile. Colonies on SNA at 25 °C are flat, spreading, forming concentric zones with undulate margins, white, reaching 80 mm in diameter after 20 d, sterile.

Materials examined. China • Xizang Autonomous Region (Tibet), Linzhi City, Bomi County, Tongmai Town, 30°5'53"N, 95°3'49"E, 2055 m, from branches of *Alnus nepalensis*, 9 Jul. 2024, *Ning Jiang, Jieting Li & Haoyin Zhang* (**holotype** CAF800101, ex-paratype cultures CFCC 71057 and N266C).

Notes. *Diaporthe linzhiensis* is phylogenetically closely related to *D. alnicola*, *D. heterophyllae*, and *D. virgiliae* (Fig. 2). Both *D. linzhiensis* and *D. alnicola* infect *Alnus nepalensis* in China, while *D. heterophyllae* is found on *Acacia heterophylla* in France, and *D. virgiliae* inhabits *Virgilia oroboides* in South Africa (Machingambi et al. 2015; Marín-Felix et al. 2019). Morphologically, *D. linzhiensis* shares a similar conidiogenous cell size with *D. alnicola* and *D. heterophyllae*, which is wider than that of *D. virgiliae* (5.5−16 × 1.5−3 μm in *D. linzhiensis* vs. 9.5−33 × 2−3 μm in *D. alnicola* vs. 6−9 × 1−2 μm in *D. heterophyllae* vs. 12.3−21.3 × 0.7−1.5 μm in *D. virgiliae*). Additionally, *D. linzhiensis* has longer beta conidia compared to the other species (24.5−29 × 1.5−2 μm in *D. linzhiensis* vs. 14.5−22 × 1.5−2.5 μm in *D. alnicola* vs. 17−24 × 1−2 μm in *D. heterophyllae* vs. 17.1−25.4 × 1−1.8 μm in *D. virgiliae*) (Machingambi et al. 2015; Marín-Felix et al. 2019). At the nucleotide level, *D. linzhiensis* also differs from *D. alnicola* (ITS, 23/547; *cal*, 2/382; *his3*, 6/469; *tef1*, 4/349; *tub2*, 6/778), *D. heterophyllae* (ITS, 28/560; *cal*, 11/420; *his3*, 6/447; *tef1*, 12/326; *tub2*, 3/406), and *D. virgiliae* (ITS, 16/434; *tub2*, 7/743).

Discussion

This study enhances the understanding of *Diaporthe* species on alder by revealing two previously undescribed species and a new host association, viz. *Diaporthe alnicola* sp. nov., *D. linzhiensis* sp. nov., and *D. amygdali* on *Alnus nepalensis*. *Diaporthe* is a morphologically distinct genus characterized by the production of alpha, beta, and gamma conidia. The alpha conidia are typically aseptate, hyaline, guttulate, and cylindrical to fusiform, while the beta conidia are aseptate, hyaline, and filiform (Farr et al. 2002; Udayanga et al. 2015; Guarnaccia and Crous 2017; Manawasinghe et al. 2019; Huang et al. 2021; Sun et al. 2021; Lambert et al. 2023). However, species within the genus usually share the same host genera and are morphologically similar, often exhibiting overlapping sizes of conidia or ascospores. As a result, it is relatively easy to identify specimens at the generic level, but more challenging to distinguish them at the species level (Yang et al. 2020, 2021; Zhu et al. 2023, 2024; Liu et al. 2024). In this study, we present novel findings from Xizang, China, which indicate the potential existence of numerous undescribed species in unexplored or minimally investigated regions worldwide.

Diaporthe alnicola and *D. amygdali* are here reported to be associated with leaf spot disease of *Alnus nepalensis*, which is a common disease in Linzhi,

Xizang, China. Among these pathogens, *D. alnicola* is a novel species and may be the primary pathogen associated with *A. nepalensis*. In contrast, *D. amygdali* is a generalist fungus that infects a wide range of plant hosts, including *Acer* spp., *Camellia sinensis*, *Lithocarpus glabra*, *Prunus dulcis*, *Pr. persica*, *Pr. salicina*, *Pyrus pyrifolia*, *Ternstroemia gymnanthera*, *Vaccinium corymbosum*, and *Vitis vinifera* (Hilário et al. 2021b). This suggests that *D. amygdali* may be a secondary pathogen to *A. nepalensis*. For successful disease management, it will be of paramount importance, albeit challenging, to effectively interrupt the infection cycle of *A. nepalensis* maintained by the occurrence of leaf spots caused by and due to the broad host range of *D. amygdali*. Therefore, future investigations need to focus on identifying other hosts of *D. amygdali* in Linzhi City.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: JTL, JRL, NJ. Methodology: JRL, NJ. Formal analysis: JTL, YL. Investigation: JTL, JRL, NJ. Data curation: JTL, JRL, NJ. Writing-original draft: JTL. Writing-review and editing: JRL, NJ. Visualization: NJ.

Author ORCIDs

Jieting Li https://orcid.org/0009-0001-8984-7261
Yi Li https://orcid.org/0009-0004-0656-9799
Jiangrong Li https://orcid.org/0000-0002-6679-5227
Ning Jiang https://orcid.org/0000-0002-9656-8500

Data availability

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

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MycoKeys

Three new species of *Apiospora* (Apiosporaceae, Amphisphaeriales) associated with diseased bamboo in China

Xiaoyun Chang[1], Yuanyuan Wang[1], Tao Xu[1], Guangshuo Li[2], Xianghua Yue[3], Mingjun Chen[1]

1 Anhui Province Key Laboratory of Green Control for Major Forestry Pests, Anhui Agricultural University, Hefei 230036, China
2 Key Laboratory of Biology and Sustainable Management of Plant Diseases and Pests of Anhui Higher Education Institutes, School of Plant Protection, Anhui Agricultural University, Hefei 230036, China
3 International Centre for Bamboo and Rattan, Sanya Research Base, Sanya 572000, China
Corresponding authors: Mingjun Chen (mjchen@ahau.edu.cn); Xianghua Yue (yuexianghua@icbr.ac.cn)

Abstract

Apiospora is widely distributed worldwide, primarily comprising pathogens, endophytes, and saprobes associated with plants, and most of its hosts are Poaceae. In this study, 37 pathogenic strains of *Apiospora* were isolated from diseased bamboo collected in the provinces of Hunan and Guizhou, China. Multilocus phylogenetic analysis using combined ITS, LSU, *TUB2*, and *TEF1* sequence data, along with morphological assessments, identified three new species: *A. bambusiparasitica* **sp. nov.**, *A. qiannanensis* **sp. nov.**, and *A. xiangxiense* **sp. nov.** Descriptions, illustrations, and phylogenetic trees for the newly discovered species are provided and compared with closely related *Apiospora* species to enhance our understanding of the genus *Apiospora*. The pathogenicity test results demonstrated that the three new species could cause bamboo culm diseases, providing valuable information for the diagnosis and management of bamboo culm diseases.

Key words: Bambusicolous fungi, morphology, new taxa, phylogeny, taxonomy

Introduction

The genus *Apiospora* (Amphisphaeriales, Apiosporaceae) was established and described by Saccardo in 1875, with *Apiospora montagnei* (Saccardo 1875) designated as the type species. The ongoing development of fungal taxonomy and phylogeny has led to multiple revisions of the taxonomic status of the genus *Apiospora*. Before 2021, the phylogenetic relationship between *Arthrinium* and *Apiospora* remained unclear. Molecular phylogenetic studies initially placed both genera in the family Apiosporaceae (Hyde et al. 1998). Subsequently, Crous and Groenewald (2013) proposed synonymizing *Arthrinium* with *Apiospora* and prioritized the former as per the "one fungus, one name" policy (Hawksworth et al. 2011; Réblová et al. 2016), despite a lack of data on the type species *Arthrinium caricicola*. However, Pintos and Alvarado's (2021) study showed that genetic, morphological, and ecological differences between *Apiospora* and *Arthrinium* were considered sufficient to support the taxonomic separation of the two genera. Furthermore, Pintos and Alvarado (2022) refined the identity of *Apiospora montagnei* as the type species and delineated

its phylogenetic boundaries. Additionally, many species from *Arthrinium* were transferred to *Apiospora* (Pintos and Alvarado 2021; Tian et al. 2021). As of March 2025, there are 206 epithets listed in Index Fungorum.

Morphologically, *Apiospora* and *Arthrinium* share many similarities, particularly in their asexual characteristics (Yuan et al. 2020). However, most *Apiospora* conidia are nearly spherical from the front view and lenticular from the side, while *Arthrinium* often produces conidia of various shapes, including angular, spherical, curved, boat-shaped, fusiform, and polygonal (Crous and Groenewald 2013; Pintos and Alvarado 2021, 2022; Li et al. 2023; Ai et al. 2024; Liu et al. 2024).

Ecologically, *Apiospora* is mainly associated with Poaceae or other plant hosts in tropical and subtropical regions (Liao et al. 2023; Liu et al. 2023; Zhang et al. 2023), while *Arthrinium* primarily occurs on Cyperaceae or Juncaceae hosts in temperate, cold, or alpine habitats (Sharma et al. 2014; Kwon et al. 2021; Pintos and Alvarado 2022). Current fungal taxonomy also emphasizes the correlation between host specificity and geographic location. All these pieces of evidence support the taxonomic separation of the two genera.

Bamboo is a vital non-wood bioresource, playing an irreplaceable role in economic, ecological, medicinal, and societal development (Shukla et al. 2016; Borowski et al. 2022). However, the intensification of bamboo cultivation has heightened its vulnerability to infectious diseases. Among fungal pathogens associated with bamboo, obligate pathogens such as *Phyllachora*, *Physopella*, *Puccinia*, *Stereostratum*, and *Uredo predominantly* infect bamboo leaves, while sporadic pathogens, including *Apiospora*, *Meliola*, *Fusarium*, and *Sclerotium*, target both leaves and culms (Hyde et al. 2002; Yang et al. 2019). The pathogenicity of *Apiospora* species on bamboo has garnered increasing attention. For instance, Li et al. (2016) identified *A. phaeospermum* as the causative agent of culm rot in *Phyllostachys viridis* in China. Subsequently, Yang et al. (2019) reported *A. yunnanum* as the pathogen responsible for bamboo blight in *Phyllostachys heteroclada*. More recently, Zheng et al. (2022) confirmed that *A. arundinis* caused culm rhomboid rot in Moso bamboo (*Phyllostachys edulis*). Beyond their pathogenic roles, several *Apiospora* species are recognized as endophytes, contributing to the microbial diversity of bamboo (Wang et al. 2018).

In this study, we isolated several *Arthrinium*-like taxa from diseased culms of bamboo in China. To clarify their taxonomic status, we used a dataset composed of nuclear ribosomal DNA internal transcribed spacer (ITS), large subunit ribosomal DNA (LSU), β-tubulin (*TUB2*), and translation elongation factor 1-α (*TEF1*). Based on morphological characteristics and multi-gene phylogenetic analyses, we identified and described three new *Apiospora* species.

Materials and methods

Plant material

In this study, diseased bamboo samples were collected from Jiuyi Mountain in Ningyuan County, Xiangxi Tujia and Miao Autonomous Prefecture, Hunan, and from Libo County, Qiannan Buyi and Miao Autonomous Prefecture, Guizhou, China. The International Center for Bamboo and Rattan provided the specimens. Samples were deposited in the Research Center for Entomogenous Fungi (RCEF) of Anhui Agricultural University.

Pathogen isolation

Pure cultures of all fungal isolates were obtained by the single hyphal tip isolation method. For pathogen isolation, lesion margin specimens were excised into 5 × 5 mm fragments, surface-sterilized in 2% sodium hypochlorite for 2 min, followed by immersion in 75% ethanol for 1 min, and rinsed three times consecutively with sterile water (Zheng et al. 2022). The sterilized pieces were wiped dry with sterilized filter paper and then placed into Petri dishes containing potato dextrose agar (PDA) (three pieces per dish) amended with 50 µg/mL of benzylpenicillin potassium (Cai et al. 2009). The plates were incubated at 25 °C under a 12 h light/dark photoperiod. Hyphal tips from the leading edge of fungal colonies emerging from the tissues were transferred to fresh PDA after two days to obtain pure cultures, which were subsequently maintained at 25 °C. Living cultures were stored in a metabolically inactive state at the Research Center for Entomogenous Fungi (RCEF) of Anhui Agricultural University. The MycoBank number for the newly described species is referenced as outlined in Robert et al. (2013).

Morphological characterization

For morphological identification, the purified isolated strains were incubated on PDA (fresh diced potato 200 g/L, dextrose 20 g/L, agar 20 g/L) and MEA (malt extract 20 g/L and 20 g/L agar) at 25 °C. Incubate at 25 °C in alternating light and dark (12 h for each); colony growth was observed daily, and the morphology, color, texture of colonies, and the diameter of colonies were recorded. Asexual reproductive structures were observed based on cultures on PDA, following synoptic keys for *Apiospora* species identification. In the morphological analysis, the fungi were mounted in a drop of lactophenol solution on glass slides. The microstructures, such as mycelium, conidiogenous cells, and conidia, were observed using an optical microscope (ZEISS Axiolab 5) and microphotographed. Forty conidiogenous cells and conidia were measured and examined. The colors of fresh specimens and cultures were recorded by referring to the Methuen Handbook of Color (Kornerup and Wanscher 1978).

DNA extraction and PCR amplification

The genomic DNA of the isolates was extracted from mycelium that was cultured on a PDA plate and incubated for 3–5 days at 25 °C. DNA extraction was performed according to the CTAB method (Spatafora et al. 1998).

Polymerase chain reaction (PCR) amplification was applied to amplify four gene fragments, including ITS, LSU, *TUB2*, and *TEF1*. The following primer pairs were used: ITS1/ITS4 for ITS (White et al. 1990), LR0R/LR5 for LSU (Rehner and Samuels 1995), EF1-728F/EF2 for *TEF1* (O'Donnell et al. 1998; Carbone and Kohn 1999), and T1/Bt2b for *TUB2* (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997). The PCR amplification system consisted of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 48 °C (ITS), and 45 s at 72 °C, and a final step of 2 min at 72 °C. Different annealing temperatures were used according to the genomic region to be amplified: 56 °C for *TEF1* and LSU, 58 °C for *TUB2*. The final product was detected by agarose gel electrophoresis and sent to Beijing Liuhe Huada Gene Technology Company. The resulting sequences were submitted to GenBank for sequencing.

Molecular and phylogenetic analysis

Newly generated sequences from each isolate were blasted against the GenBank database, and searches were restricted to type materials for the initial determination of the closest matching species and species complex. Related gene sequences (ITS, LSU, *TUB2*, *TEF1*) of *Apiospora* spp. from recent publications were downloaded from GenBank (Table 1) (Yan and Zhang 2024). Manual adjustments of sequences were carried out using BioEdit (Hall et al. 2011) to maximize homology.

The DNA sequences were aligned using the MAFFT v7 with the G-INS-I option (Katoh et al. 2019). Sequences were manually edited as necessary using BioEdit v7.1.9 (Hall 1999). The combined loci were analyzed using maximum likelihood (ML) and Bayesian inference (BI) methods. Combined sequences of ITS-LSU-*TUB2-TEF1* were performed in SequenceMatrix v1.7.8 (Vaidya et al. 2011). The ML analysis was conducted using the TNe+I+R4 model and 1000 bootstrap replicates. The ML analysis was designed with IQ-TREE (Trifinopoulos et al. 2016). In Bayesian inference analysis, the best-fit substitution models for different datasets were estimated using MrModeltest v2.3 based on the implementation of the Akaike information criterion (AIC) (Nylander 2004). Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (MCMC) under the estimated model of evolution (Zhaxybayeva and Gogarten 2002). Four simultaneous Markov chains were run for 20 million generations, and trees were sampled every 1000 generations. The run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. The first 25% trees, which represented the burn-in phase of the analyses, were discarded, and the remaining trees were used for calculating PP in the majority rule consensus tree. Phylogenetic trees were subsequently visualized and refined using the Interactive Tree of Life (iTOL) online platform (Letunic and Bork 2019).

Pathogenicity assay

Fresh bamboo samples were collected from the campus of Anhui Agricultural University in Anhui Province, China, to validate Koch's postulates. Bamboo culms were cut into 30 cm sections, sterilized with 75% ethanol spray, and wounded using sterile drills. Mycelial plugs (5 mm in diameter) from the edge of each isolate colony were placed onto the artificial wounds, while control pieces received PDA plugs without fungal inoculum (Zheng et al. 2022). All treated culms were placed in moisture chambers with sterile wet cotton to maintain humidity and incubated at 25 °C under a 12-hour light/12-hour dark cycle. Symptom development was observed daily, and each treatment was replicated six times.

Results

Disease symptoms and isolation of the pathogen

From the field survey, disease symptoms developed on the culm of the bamboo. The typical symptoms: (I) started with brown spots and irregular shapes that gradually enlarged, with dark edges and spread around, sometimes forming symmetrical or lobed patterns (Fig. 1A–C). (II) started with black spots and irregular shapes; each spot remained relatively small and did not expand significantly. In the later stages,

Table 1. Species of Apiosporaceae used in the phylogenetic analyses. Notes: Strains in this study are marked in bold. "T" indicates a type culture. NA = not available.

Strain	Code	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*TUB2*	*TEF1*
Apiospora acutiapica	KUMCC 20-0209	*Bambusa bambos*	China	MT946342	MT946338	MT947365	MT947359
A. adinandrae	SAUCC 1282B-1 ᵀ	Diseased leaves of *Adinandra glischroloma*	China	OR739431	OR739572	OR757128	OR753448
A. agari	KUC21333 ᵀ	*Agarum cribrosum*	South Korea	MH498520	MH498440	MH498478	MH544663
A. aquatica	S-642 ᵀ	Submerged wood	China	MK828608	MK835806	NA	NA
A. arctoscopi	KUC21331 ᵀ	Eggs of *Arctoscopus japonicus*	South Korea	MH498529	MH498449	MH498487	MN868918
A. armeniaca	SAUCC DL1831 ᵀ	Leaves of *Prunus armeniaca*	China	OQ592540	OQ615269	OQ613285	OQ613313
A. arundinis	CBS 124788	Living leaves of *Fagus sylvatica*	Switzerland	KF144885	KF144929	KF144975	KF145017
A. aseptata	KUNCC 23-14169 ᵀ	Living roots of *Dicranopteris pedata*	China	OR590341	OR590335	OR634943	OR634949
A. aurea	CBS 244.83 ᵀ	Air	Spain	AB220251	KF144935	KF144981	KF145023
A. babylonica	SAUCC DL1841 ᵀ	Diseased leaves of *Salix babylonica*	China	OQ592538	OQ615267	OQ613283	OQ613311
A. balearica	AP24118 ᵀ	Poaceae plant	Spain	MK014869	MK014836	MK017975	MK017946
A. bambusicola	MFLUCC 20-0144 ᵀ	*Schizostachyum brachycladum*	Thailand	MW173030	MW173087	NA	MW183262
A. bambusiparasitica	**RCEF20000**	**Diseased culms of bamboo**	**China**	**OR687309**	**PQ530552**	**OR712912**	**PQ538537**
A. bambusiparasitica	**RCEF20003 ᵀ**	**Diseased culms of bamboo**	**China**	**OR687306**	**PQ530551**	**OR712906**	**OR712911**
A. bawanglingensis	SAUCC BW0444 ᵀ	Leaves of *Indocalamus longiauritus*	China	OR739429	OR739570	OR757126	OR753446
A. bawanglingensis	SAUCC 0443	Diseased leaves of *Indocalamus longiauritus*	China	OQ592552	OQ615281	OQ613303	OQ613325
A. bawanglingensis	SAUCC 0444	Diseased leaves of *Indocalamus longiauritus*	China	OQ592551	OQ615280	OQ613302	OQ613324
A. biserialis	CGMCC 3.20135 ᵀ	Bamboo	China	MW481708	MW478885	MW522955	MW522938
A. camelliae-sinensis	LC5007 ᵀ	*Camellia sinensis*	China	KY494704	KY494780	KY705173	KY705103
A. cannae	ZHKUCC 22-0139	Leaves of *Canna* sp.	China	OR164902	OR164949	OR166322	OR166286
A. chiangraiense	MFLU 21-0046	Dead culms of bamboo	Thailand	MZ542520	MZ542524	MZ546409	NA
A. chromolaenae	MFLUCC 17-1505 ᵀ	*Chromolaena odorata*	Thailand	MT214342	MT214436	NA	MT235802
A. cordylines	GUCC 10026	*Cordyline fruticosa*	China	MT040105	NA	MT040147	MT040126
A. coryli	CFCC 58978 ᵀ	Dead plant culms of *Corylus yunnanensis*	China	OR125564	OR133586	OR139978	OR139974
A. cyclobalanopsidis	GZCC 20-0103	*Cyclobalanopsidis glauca*	China	MW481714	MW478893	MW522963	MW522946
A. dematiacea	KUNCC 23-14202 ᵀ	Healthy leaf *Dicranopteris ampla*	China	OR590346	OR590339	OR634948	OR634953
A. dendrobii	MFLUCC 14-0152 ᵀ	Roots of *Dendrobium harveyanum*	Thailand	MZ463151	MZ463192	NA	NA
A. descalsii	AP31118A ᵀ	*Ampelodesmos mauritanicus*	Spain	MK014870	MK014837	MK017976	MK017947
A. dichotomanthi	LC4950 ᵀ	*Dichotomanthes tristaniicarpa*	China	KY494697	KY494773	KY705167	KY705096
A. dicranopteridis	KUNCC23-14171 ᵀ	Living stems of *Dicranopteris pedata*	China	OR590342	OR590336	OR634944	OR634950
A. dongyingensis	SAUCC 0302 ᵀ	Leaves of bamboo	China	OP563375	OP572424	OP573270	OP573264
A. elliptica	ZHKUCC 22-0131 ᵀ	Dead stems of unknown plant	China	OR164905	OR164952	OR166323	OR166284
A. endophytica	ZHKUCC 23-0006 ᵀ	Living leaves of *Wurfbainia villosa*	China	OQ587996	OQ587984	OQ586075	OQ586062
A. esporiensis	AP16717	*Phyllostachys aurea*	Spain	MK014878	MK014845	MK017983	MK017954
A. euphorbiae	IMI 285638b	*Bambusa* sp.	Bangladesh	AB220241	AB220335	AB220288	NA
A. fermenti	KUC21289 ᵀ	Seaweeds	South Korea	MF615226	MF615213	MF615231	MH544667
A. gaoyouensis	CFCC 52301 ᵀ	*Phragmites australis*	China	MH197124	NA	NA	MH236793
A. gaoyouensis	CFCC 52302	*Phragmites australis*	China	MH197125	NA	NA	MH236794
A. garethjonesii	SICAUCC 22-0027	Bamboo	China	ON228603	ON228659	ON237651	NA
A. gelatinosa	GZAAS 20-0107	Bamboo	China	MW481707	MW478889	NA	MW522942
A. globosa	KUNCC 23-14210 ᵀ	Living stems of *Dicranopteris linearis*	China	OR590347	OR590340	NA	OR634954
A. gongcheniae	GDMCC 3.1045ᵀ	Stems of *Oryza meyeriana* subsp. *granulata*	China	PP033259	PP034691	PP033102	PP034683
A. gongcheniae	YNE00565	Stems of *Oryza meyeriana* subsp. *granulata*	China	PP033260	PP034692	PP033103	PP034684
A. guangdongensis	ZHKUCC 23-0004 ᵀ	*Wurfbainia villosa*	China	OQ587994	OQ587982	OQ586073	OQ586060
A. guizhouensis	LC5318	Air in karst cave	China	KY494708	KY494784	KY705177	KY705107
A. hainanensis	SAUCC 1681 ᵀ	Leaves of bamboo	China	OP563373	OP572422	OP573268	OP573262

Strain	Code	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*TUB2*	*TEF1*
A. hispanica	IMI 326877 [T]	Beach sands	Spain	AB220242	AB220336	AB220289	NA
A. hydei	CBS 114990 [T]	Culms of *Bambusa tuldoides*	China	KF144890	KF144936	KF144982	KF145024
A. hyphopodii	SICAUCC 22-0034	Bamboo	China	ON228605	ON228661	ON237653	NA
A. hysterina	AP12118	*Phyllostachys aurea*	Spain	MK014877	KM014844	MK017982	MK017953
A. iberica	AP10118 [T]	*Arundo donax*	Portugal	MK014879	MK014846	MK017984	MK017955
A. intestini	CBS 135835	Gut of grasshopper	India	KR011352	MH877577	KR011350	KR011351
A. italica	AP29118	*Arundo donax*	Italy	MK014881	MK014848	MK017986	NA
A. jatrophae	MMI00052 [T]	Living *Jatropha podagrica*	India	JQ246355	NA	NA	NA
A. jiangxiensis	LC4577 [T]	*Maesa* sp.	China	KY494693	KY494769	KY705163	KY705092
A. jiangxiensis	LC4578	Camellia sinensis	China	KY494694	KY494770	KY705164	KY705093
A. jinanensis	SAUCC DL1981 [T]	Diseased leaves of Bambusaceae sp.	China	OQ592544	OQ615273	OQ613289	OQ613317
A. kogelbergensis	CBS 113332	*Cannomois virgata*	South Africa	KF144891	KF144937	KF144983	KF145025
A. koreana	KUC21332 [T]	Eggs of *Arctoscopus japonicus*	South Korea	MH498524	MH498444	MH498482	MH544664
A. lageniformis	KUC21686 [T]	Culms of *Phyllostachys nigra*	South Korea	ON764022	ON787761	ON806636	ON806626
A. lageniformis	KUC21687	Culms of *Phyllostachys nigra*	South Korea	ON764023	ON787764	ON806637	ON806627
A. locuta-pollinis	LC11683 [T]	*Brassica campestris*	China	MF939595	NA	MF939622	MF939616
A. longistroma	MFLUCC11-0481 [T]	Dead culms of bamboo	Thailand	KU940141	KU863129	NA	NA
A. lophatheri	CFCC 58975 [T]	Diseased leaves of *Lophatherumgracile*	China	OR125566	OR133588	OR139980	OR139970
A. machili	SAUCC 1175A-4 [T]	Diseased leaves of *Machilus nanmu*	China	OR739433	OR739574	OR757130	OR753450
A. machili	SAUCC 1175	Diseased leaves of *Machilus nanmu*	China	OQ592560	OQ615289	OQ613307	OQ613333
A. machili	SAUCC 1176	Diseased leaves of *Machilus nanmu*	China	OQ592559	OQ615288	OQ613306	OQ613332
A. malaysiana	CBS 102053 [T]	*Macaranga hullettii*	Malaysia	KF144896	KF144942	KF144988	KF145030
A. marianiae	AP18219 [T]	Dead stems of *Phleum pratense*	Spain	ON692406	ON692422	ON677186	ON677180
A. marii	CBS 497.90 [T]	Beach sands	Spain	AB220252	KF144947	KF144993	KF145035
A. marina	KUC21328 [T]	Seaweeds	South Korea	MH498538	MH498458	MH498496	MH544669
A. mediterranea	IMI 326875 [T]	Air	Spain	AB220243	AB220337	AB220290	NA
A. minutispora	1.70E-42 [T]	Mountain soils	South Korea	LC517882	NA	LC518888	LC518889
A. montagnei	AP301120 [T]	*Arundo micrantha*	Spain	ON692408	ON692424	ON677188	ON677182
A. mori	MFLU 18-2514 [T]	*Morus australis*	China	MW114313	MW114393	NA	NA
A. mukdahanensis	MFLUCC 22-0056 [T]	Dead leaves of bamboo	Thailand	OP377735	OP377742	NA	NA
A. mytilomorpha	DAOM 214595	Dead blades of *Andropogon* sp.	India	KY494685	NA	NA	NA
A. neobambusae	LC7106 [T]	Leaves of bamboo	China	KY494718	KY494794	KY705186	KY806204
A. neochinense	CFCC 53036 [T]	*Fargesia qinlingensis*	China	MK819291	NA	MK818547	MK818545
A. neosubglobosa	JHB 007 [T]	Bamboo	China	KY356090	KY356095	NA	NA
A. obovata	LC4940 [T]	*Lithocarpus* sp.	China	KY494696	KY494772	KY705166	KY705095
A. obovata	LC8177	*Lithocarpus* sp.	China	KY494757	KY494833	KY705225	KY705153
A. oenotherae	CFCC 58972	Diseased leaves of *Oenothera biennis*	China	OR125568	OR133590	OR139982	OR139972
A. olivata	CGMCC 3.25514 [T]	soil	China	OR680531	OR680598	OR843234	OR858925
A. olivata	ZY 22.053	soil	China	OR680532	OR680599	OR843235	OR858926
A. ovata	CBS 115042 [T]	*Arundinaria hindsii*	China	KF144903	KF144950	KF144995	KF145037
A. pallidesporae	ZHKUCC 22-0129 [T]	Dead wood of unknown host	China	OR164903	OR164950	NA	NA
A. paraphaeosperma	KUC21488	Culms of bamboo	South Korea	ON764024	ON787763	ON806638	ON806628
A. phragmitis	CPC 18900 [T]	*Phragmites australis*	Italy	KF144909	KF144956	KF145001	KF145043
A. phyllostachydis	MFLUCC 18-1101 [T]	*Phyllostachys heteroclada*	China	MK351842	MH368077	MK291949	MK340918
A. piptatheri	SAUCC BW0455	Diseased leaves of *Indocalamus longiauritus*	China	OR739430	OR739571	OR757127	OR753447
A. pseudohyphopodii	KUC21680 [T]	Culms of *Phyllostachys pubescens*	South Korea	ON764026	ON787765	ON806640	ON806630
A. pseudomarii	GUCC 10228 [T]	Leaves of *Aristolochia debilis*	China	MT040124	NA	MT040166	MT040145
A. pseudoparenchymatica	LC7234 [T]	Leaves of bamboo	China	KY494743	KY494819	KY705211	KY705139
A. pseudorasikravindrae	KUMCC 20-0208 [T]	*Bambusa dolichoclada*	China	MT946344	NA	MT947367	MT947361
A. pseudosinensis	SAUCC 0221	Leaves of bamboo	China	OP563377	OP572426	OP573272	OP573266
A. pseudospegazzinii	CBS 102052 [T]	*Macaranga hullettii*	Malaysia	KF144911	KF144958	KF145002	KF145045
A. pterosperma	CPC 20193 [T]	*Lepidosperma gladiatum*	Australia	KF144913	KF144960	KF145004	KF145046

| Strain	Code	Host and Substrates	Locality	GenBank accession numbers			
				ITS	LSU	*TUB2*	*TEF1*
A. pusillisperma	KUC21321 ᵀ	Seaweeds	South Korea	MH498533	MH498453	MH498491	MN868930
A. qiannanensis	**RCEF7610**	**Diseased culms of bamboo**	**China**	**PQ526600**	**PQ530550**	**PQ538539**	**PQ538535**
A. qiannanensis	**RCEF7611 ᵀ**	**Diseased culms of bamboo**	**China**	**PQ526599**	**PQ530549**	**PQ538538**	**PQ538536**
A. qinlingensis	CFCC 52303 ᵀ	*Fargesia qinlingensis*	China	MH197120	NA	NA	MH236795
A. rasikravindrae	LC8179	Brassica rapa	China	KY494759	KY494835	KY705227	KY705155
A. sacchari	CBS 372.67	Air	Not mentioned	KF144918	KF144964	KF145007	KF145049
A. saccharicola	CBS 191.73	Air	Netherlands	KF144920	KF144966	KF145009	KF145051
A. sargassi	KUC21232	Seaweeds	South Korea	KT207750	NA	KT207648	MH544676
A. sasae	CPC 38165 ᵀ	Dead culms of *Sasa veitchii*	Netherlands	MW883402	MW883797	MW890120	MW890104
A. septata	GZCC 20-0109	Bamboo Food	China	MW481712	MW478891	MW522961	MW522944
A. serenensis	IMI 326869 ᵀ	Excipients, atmosphere and home dust	Spain	AB220250	AB220344	AB220297	NA
A. setariae	CFCC 54041 ᵀ	Decaying culms of *Setaria viridis*	China	MT492004	NA	MT497466	MW118456
A. setostroma	KUMCC 19-0217	Dead branches of bamboo	China	MN528012	MN528011	NA	MN527357
A. sichuanensis	HKAS 107008 ᵀ	Dead culms of Poaceae	China	MW240648	MW240578	MW775605	MW759536
Apiospora sp.	SAUCC 1429	NA	China	OQ592558	OQ615287	OQ613305	OQ613331
Apiospora sp.	SAUCC 1430	NA	China	OQ592557	OQ615286	OQ613304	OQ613330
A. sphaerosperma	CBS 114315	Leaves of *Hordeum vulgare*	Iran	KF144905	KF144952	KF144997	KF145039
A. stipae	CPC 38101 ᵀ	Dead culms of *Stipa gigantea*	Spain	MW883403	MW883798	MW890121	MW890082
A. subglobosa	MFLUCC 11-0397 ᵀ	Dead culms of bamboo	Thailand	KR069112	KR069113	NA	NA
A. subrosea	LC7291	Leaves of bamboo	China	KY494751	KY494827	KY705219	KY705147
A. taeanensis	KUC21359	Seaweeds	South Korea	MH498513	NA	MH498471	MN868935
A. thailandica	MFLUCC 15-0202 ᵀ	Dead culms of bamboo	Thailand	KU940145	KU863133	NA	NA
A. tropica	MFLUCC 21-0056	Dead culms of Bambusoideae	Thailand	OK491657	OK491653	NA	NA
A. vietnamensis	IMI 99670 ᵀ	*Citrus sinensis*	Vietnam	KX986096	KX986111	KY019466	NA
A. wurfbainiae	ZHKUCC 23-0009	*Wurfbainia villosa*	China	OQ587999	OQ587987	OQ586078	OQ586065
A. xenocordella	CBS 478.86 ᵀ	Soils from roadway	Zimbabwe	KF144925	KF144970	KF145013	KF145055
A. xiangxiense	**RCEF20001 ᵀ**	**Diseased culms of bamboo**	**China**	**OR687308**	**PQ530553**	**OR712910**	**OR712909**
A. xiangxiense	**RCEF20002**	**Diseased culms of bamboo**	**China**	**OR687307**	**PQ530548**	**OR712908**	**OR712907**
A. xishuangbannaensis	KUMCC 21-0696	*Rhinolophus pusillus*	China	ON426833	OP363249	OR025931	OR025970
A. yunnana	DDQ 00281	Phyllostachys nigra	China	KU940148	KU863136	NA	NA
A. yunnanensis	ZHKUCC 23-0014 ᵀ	Dead stems of grass	China	OQ588004	OQ587992	OQ586083	OQ586070
Arthrinium caricicola	AP23518	*Carex ericetorum*	China	MK014871	MK014838	MK017977	MK017948
Arthrinium caricicola	CBS 145903	Dead and attached leaves	Germany	MN313782	MN317266	MN313861	NA
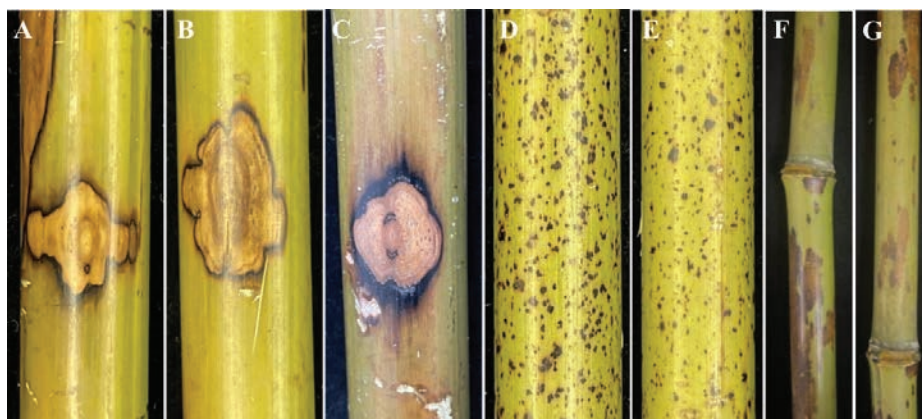
Figure 1. Symptoms of disease in naturally infected bamboo in the field.

they densely covered bamboo culm and exhibited chlorosis in the bamboo culms (Fig. 1D, E). (III) Irregular brownish lesions spread extensively, sometimes coalescing into large patches, covering a significant area of the bamboo culm (Fig. 1F, G).

A total of 37 isolates were obtained on PDA. As the colony morphology of the isolates was uniform, two representative isolates from each group were selected for further analysis: (I). RCEF20001 and RCEF20002; (II). RCEF20000 and RCEF20003; (III). RCEF7610 and RCEF7611.

Phylogenetic analysis

A comprehensive dataset integrating ITS, LSU, *TUB2*, and *TEF1* sequences was constructed from 131 strains, including six newly sequenced isolates, with *Arthrinium caricicola* (CBS 145903 and AP23518) designated as the outgroup. Multi-locus sequences contained 2,544 characters, including gaps with ITS (1-433), LSU (434-1229), *TUB2* (1230-1678), and *TEF1* (1679-2544).

The phylogenetic trees derived from ML and BI analyses exhibited consistent topologies, with the ML tree, including MLBP and BIPP values, depicted in Fig. 2. Phylogenetic analysis revealed that the six strains represented three new species lineages, which are now recognized as *A. bambusiparasitica*, *A. qiannanensis*, and *A. xiangxiense*.

Taxonomy

Apiospora bambusiparasitica X.Y. Chang & M.J. Chen, sp. nov.
MycoBank No: 851766
Fig. 3

**Etymology.** The name refers to the species that is capable of infecting the culm of bamboo.

Typification. CHINA • Hunan Province, Xiangxi Tujia and Miao Autonomous Prefecture, Ningyuan County, Jiuyi Mountain (25°24'N, 111°58'E), on diseased culms of bamboo, November 2022; X.H. Yue, holotype H5, ex-type RCEF20003.

Description. *Asexual morph*: Hyphae 1.5−5.0 μm diam, hyaline, branched, septate. *Conidiogenous cells* hyaline to pale brown, smooth, erect or flexuous, scattered or aggregated in clusters on hyphae, ampulliform to clavate, 7.0−17.0 × 2.0−4.5 μm ($\overline{x}$ = 9.6 ± 2.6 × 2.7 ± 0.7, n = 40), apical neck 6.0−10.0 μm long, basal part 3.0−6.0 μm long. *Conidia* 7.0−11.5 × 6.0−10.5 μm ($\overline{x}$ = 9.2 ± 0.9 × 8.1 ± 1.1, n = 40), brown, smooth to finely roughened, granular, globose to ellipsoid in surface view, usually with a longitudinal, hyaline, germ-slit. *Sexual morph*: Undetermined.

Culture characteristics. Colonies on PDA fluffy, spreading, margin irregular, with abundant aerial mycelia, surface and reverse white to grey, reaching 9 cm in 8 d at 25 °C. On MEA, the colony is thick in the middle and thin at the edges. The margin is irregular, the surface white, and the central color on the colony's reverse side is characterized by a deeper, brownish-yellow tone that extends towards the periphery and transitions to a lighter, pale yellow shade.

Additional specimens examined. CHINA • Hunan Province, Ningyuan County, diseased on culms of bamboo, November 2022, other living culture RCEF20000.

Note. Phylogenetic analyses confirmed that *A. bambusiparasitica* formed an independent clade (1.0 BIPP and 100% MLBS), exhibiting a close evolutionary relationship with *A. arctoscopi* and *A. obovata*. Based on a BLASTN
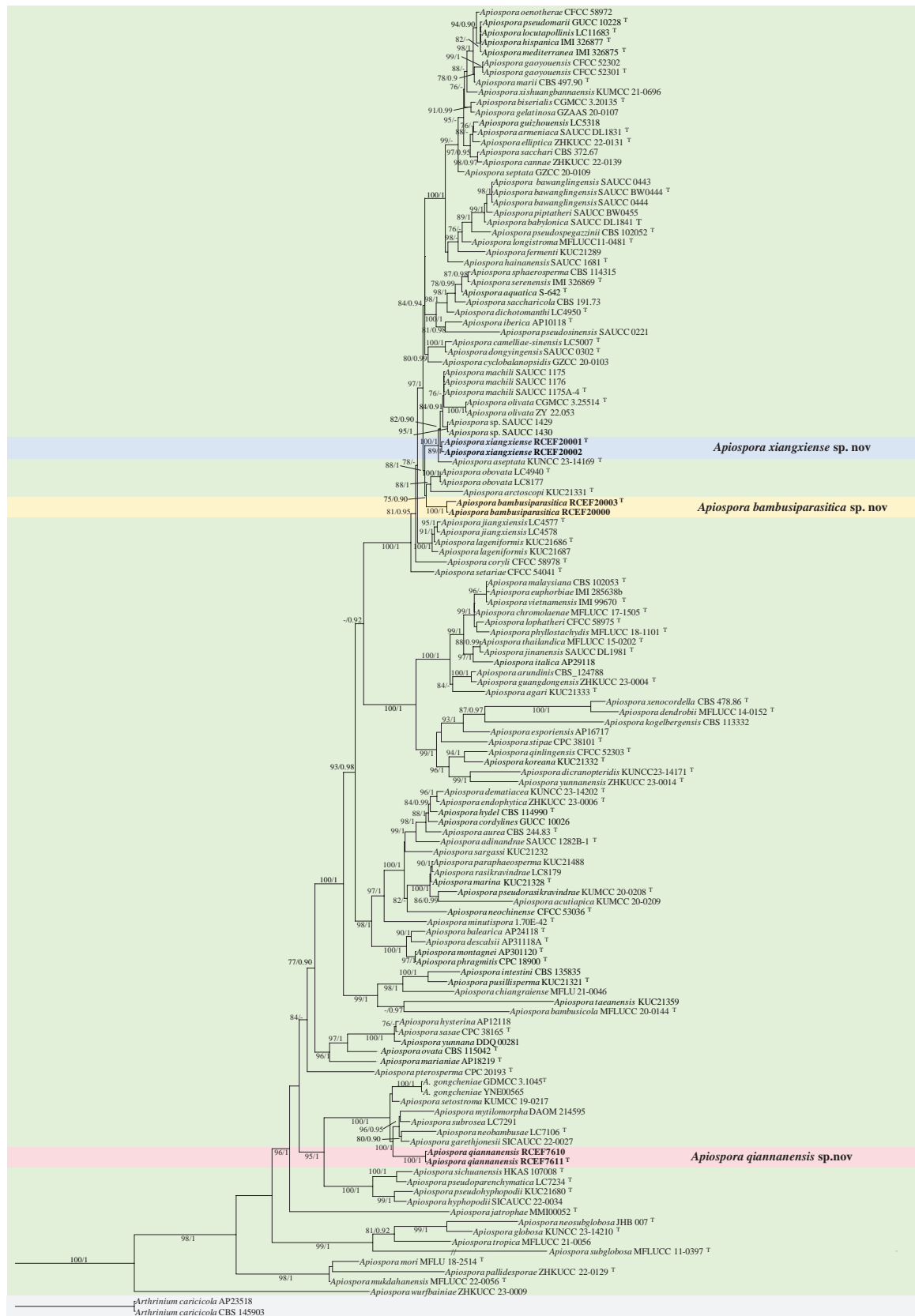
Figure 2. Phylogenetic tree of *Apiospora* based on a concatenated data matrix of ITS, LSU, *TUB2*, and *TEF1*. Bootstrap support values (> 75%) and posterior probabilities (> 0.9) are given at the nodes (ML/PP). The tree is rooted with *Arthrinium caricicola* CBS 145903 and AP23518. The novel species were highlighted. "T" indicates a type culture.
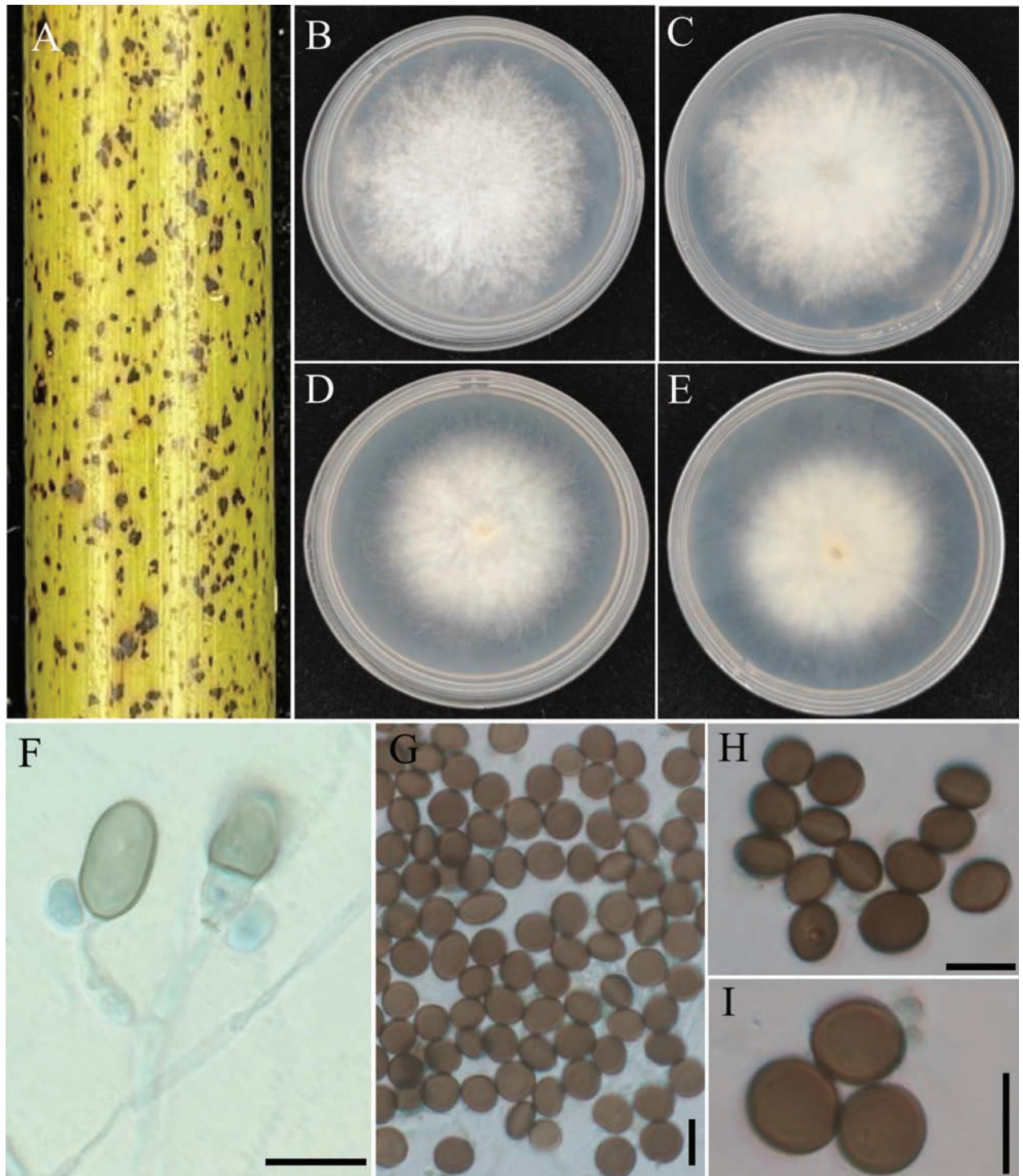
Figure 3. *Apiospora bambusiparasitica* (from ex-type living cultures RCEF20003) **A** diseased culms of bamboo **B, C** upper view and reverse view of culture on PDA **D, E** upper view and reverse view of culture on MEA **F** conidiogenous cells giving rise to conidia **G–I** conidia with pale germ slit. Scale bars: 10 µm.

search of the GenBank database, it was found that *A. bambusiparasitica* shares high similarities with the following strains: *A. arctoscopi* strain KUC21331 (86.48% in ITS, 98.9% in LSU, 92.2% in *TEF1*, 92.91% in *TUB2*); *A. obovata* strain LC4940 (90.03% in ITS, 95.77% in LSU, 93.42% in *TEF1*, 95.27% in *TUB2*); strain LC8177 (90.15% in ITS, 95.77% in LSU, 93.42% in *TEF1*, 95.27% in *TUB2*).

Table 2. Synopsis of morphological characteristics of *A. bambusiparasitica* and its closely related species.

Species	Isolation source	Country	Colony morphology (on PDA)	Conidia		References
				Shape	Diam (µm)	
A. obovata	*Lithocarpus* sp.	China	White to olivaceous-grey; Reaching 9 cm in 7 days	a. Roughened, globose to subglobose; b. obovoid, occasionally elongated to ellipsoidal.	a. 11.0−16.5; b. 16.0−31.0 × 9.0−16.0	Wang et al. (2018)
A. arctoscopi	Egg masses of *Arctoscopus japonicus*	Korea	Creamy white;5-7 cm in 5 days	globose to elongate ellipsoid	9.5−13 × 7.5−12	Kwon et al. (2021)
A. bambusiparasitica	Diseased culms of Bamboo	China	White to grey; Reaching 9 cm in 8 days	globose to elongate ellipsoid	8.6−15.4 × 6.7−10.2	**This study**

Morphologically, *A. bambusiparasitica* and *A. obovata* show distinct differences. *Apiospora obovata* forms darker colonies and produces significantly longer, ellipsoidal conidia, measuring 16.0−31.0 × 9.0−16.0 µm, whereas *A. bambusiparasitica* has spherical to oval conidia, measuring 8.6−15.4 × 6.7−10.2 µm. *Apiospora bambusiparasitica* and *A. arctoscopi* are morphologically similar, with conidia of comparable size and overlapping dimensions. However, *A. arctoscopi* forms thicker colonies with more developed hyphae. Additionally, the two species exhibit significant ecological differences in host association, as *A. arctoscopi* is associated with *Arctoscopus japonicus*, while *A. bambusiparasitica* is associated with bamboo. Current fungal taxonomy emphasizes the importance of host association. For details, see Table 2. Thus, both morphological and molecular evidence support *A. bambusiparasitica* as a new species.

Apiospora qiannanensis X.Y. Chang & M.J. Chen, sp. nov.

MycoBank No: 856457

Fig. 4

Etymology. The name refers to the locality where the type specimens were collected, Qiannan Buyi and Miao Autonomous Prefecture, Guizhou Province, China.

Typification. CHINA • Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo County (25°25'N, 107°53'E), on diseased culms of bamboo, May. 2023, X.H. Yue, holotype GZ15, ex-type RCEF7610.

Description. *Asexual morph*: Hyphae 1.5−6.0 µm diam, hyaline to pale brown, branched, septate. *Conidiophores* hyaline to pale brown, smooth, erect or ascending, simple, flexuous, subcylindrical, and grouped together. *Conidiophores* aggregated in brown sporodochia, smooth, hyaline to brown, up to 30 µm long, 3.0−4.0 µm width. *Conidiogenous cells* 9.5−23.0 × 3.0−5.5 µm ($\bar{x}$ = 15.0 ± 4.50 × 4.3 ± 0.9, n = 40), pale brown, smooth, doliiform to subcylindrical. *Conidia* 16.5−20.8 µm ($\bar{x}$ = 18.5 µm, n = 40), pale brown to dark brown, smooth, globose to subglobose. *Sexual morph*: Undetermined.

Culture characteristics. Colonies on PDA are fluffy, spreading, and circular, with moderate aerial mycelia, flocculent cotton, surface, and reverse white to grey, reaching 60 mm in 7 d at 25 °C. On MEA, surface grey-white with abundant mycelia, reverse greyish without patches.

Additional specimens examined. CHINA • Hunan Province, Ningyuan County, diseased on culms of bamboo, May 2023, other living culture RCEF7611.
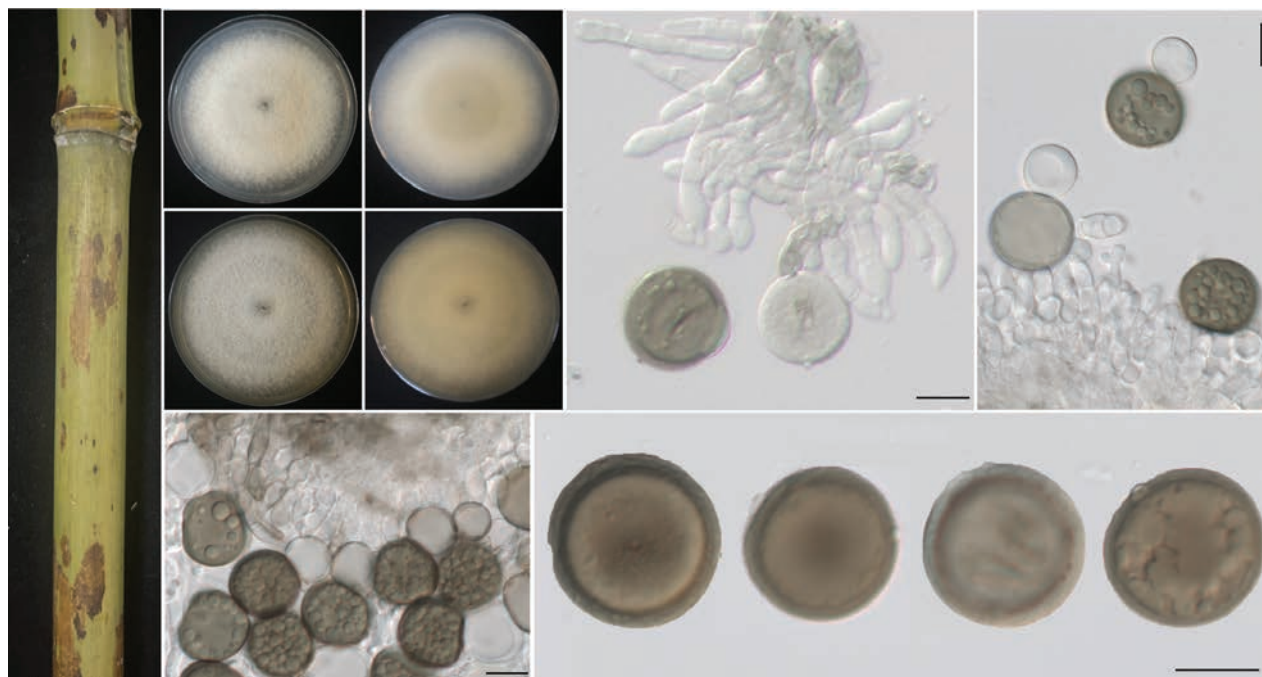
Figure 4. *Apiospora qiannanensis* (from ex-holotype strain RCEF7610) **A** diseased culms of bamboo **B, C** upper view and reverse view of culture on PDA **D, E** upper view and reverse view of culture on MEA **F–H** conidiogenous cells giving rise to conidia **I** conidia. Scale bars: 10 μm.

Note. Phylogenetic analyses confirmed that *A. qiannanensis* formed an independent clade (1.0 BIPP and 100% MLBS), exhibiting a close evolutionary relationship with *A. setostroma*, *A. mytilomorpha*, *A. subrosea*, *A. neobambusae*, and *A. garethjonesii*. Based on a BLASTN search of the GenBank database, it was found that *A. qiannanensis* exhibits some differences in the ITS, LSU, *TUB2*, and *TEF1* sequences compared to closely related species: *A. setostroma* strain KUMCC 19-0217 (92.65% in ITS, 99.16% in LSU, 95.01% in *TEF1*); *A. mytilomorpha* strain DAOM 2145955 (96.28% in ITS); *A. subrosea* strain LC7291 (90.33% in ITS, 99.02% in LSU, 94.38% in *TEF1*, 99.25% in *TUB2*); *A. neobambusae* strain LC7106 (89.16% in ITS, 99.16% in LSU, 95.22% in *TEF1*, 91.94% in *TUB2*); *A. garethjonesii* strain SICAUCC 22-0027 (93.65% in ITS, 99.29% in LSU, 94.50% in *TUB2*); *A. gongcheniae* strain GDMCC 3.1045 (95.44% in ITS, 99.41% in LSU, 93.14% in *TEF1*, 91.77% in *TUB2*).

Morphologically, colony characteristics of *A. mytilomorpha* are lacking, and the asexual morphology of *A. garethjonesii* has not been described. We compared the existing morphological data and found that these closely related species have certain differences. *A. setostroma* and *A. subrosea* produce pigments in the later stages of colonies, while the others do not. *Apiospora qiannanensis*, *A. mytilomorpha*, and *A. neobambusae* differ in conidia shape (globose to subglobose vs. fusiform or boat-shaped vs. subglobose to ellipsoid) and size (16.5–20.8 μm vs. 20–30 × 6–8.5 μm vs. 11.5–15.5 × 7.0–14.0 μm). In addition, *A. qiannanensis* differs from *A. gongcheniae* in having larger conidia (16.5–20.8 μm) compared to A. gongcheniae (8.0–17.0 × 6.8–16.1 μm). Although some morphological features overlap among these taxa, significant genetic divergence is evident, underscoring their distinct species boundaries. For details, see Table 3. Based on molecular and morphological evidence, we propose *A. qiannanensis* as a new species.

Table 3. Synopsis of morphological characteristics of *A. qiannanensis* and its closely related species.

Species	Isolation source	Country	Colony morphology (on PDA)	Conidia		References
				Shape	Diam (µm)	
A. qiannanensis	Diseased culms of bamboo	China	White to grey; Reaching 60 mm in 7 days	Globose to subglobose	16.5–20.8	**This study**
A. gongcheniae	Stems of *Oryza meyeriana* subsp. *granulata*	China	Greyish, reverse light orange; Reaching 90 mm in 7 days	Globose to subglobose	8.0–17.0 × 6.8–16.1	Yan and Zhang (2024)
A. setostroma	Dead branches of bamboo	China	Initially white, becoming greyish, reverse reddish; Reaching 35 mm in 7 days	Subglobose to obovoid, 0–1-septate	18–20 × 15–19	Jiang et al. (2019)
A. mytilomorpha	Dead blades of Andropogon	India	Undetermined	Fusiform or boat-shaped	20-30 × 6-8.5	Bhat and Kendrick (1993)
A. subrosea	Bamboo	China	Initially white, becoming light pink on surface, reverse peach-puff; Reaching 10 cm in 8 days	Globose to subglobose or ellipsoidal	12.0–17.5 × 9.0–16.0	Wang et al. (2018)
A. neobambusae	Leaf of bamboo	China	White to grey	Subglobose to ellipsoid	11.5–15.5 × 7.0–14.0	Wang et al. (2018)
A. garethjonesii	Dead culms of bamboo	China	White; Reaching 40 cm in 7 days	Undetermined	Undetermined	Dai et al. (2016)

Apiospora xiangxiense **X.Y. Chang & M.J. Chen, sp. nov.**
MycoBank No: 851765
Fig. 5

Etymology. The name refers to the locality where the type specimens were collected, Xiangxi Tujia and Miao Autonomous Prefecture, Hunan Province, China.

Typification. CHINA • Hunan Province, Xiangxi Tujia and Miao Autonomous Prefecture, Ningyuan County, Jiuyi Mountain (25°24'N, 111°58'E), on diseased culms of bamboo, November 2022, X.H. Yue, holotype H2 (stored in a metabolically inactive state), ex-type living cultures RCEF20001.

Description. *Asexual morph*: Hyphae 1.5–5.0 µm diam, hyaline, branched, septate. *Conidiogenous cells* 2.0–15.5 × 1.4–3.9 µm ($\bar{x}$ = 8.1 ± 3.9 × 2.4 ± 0.7, n = 40), aggregated in clusters on hyphae or solitary, at first hyaline, becoming pale brown, basauxic, polyblastic, sympodial, erect, cylindrical. *Conidia* 8.6–15.4 × 6.7–10.2 µm ($\bar{x}$ = 10.3 ± 1.5 × 8.3 ± 1.0, n = 40), brown, smooth to granular, globose to elongate ellipsoid in surface view, lenticular in side view, pale equatorial slit, with a central scar, 3.5 to 5.5 µm diam. Sterile cells forming on solitary loci on hyphae, brown, finely roughened, subcylindrical to clavate. *Sexual morph*: Undetermined.

Culture characteristics. Colonies on PDA are fluffy, spreading, circular, with abundant aerial mycelia, surface and reverse white to grey, sometimes with pale yellow, reaching 9 cm in 8 d at 25 °C. On MEA, slower growth, surface white, reverse white, and slightly yellowish.

Additional specimens examined. CHINA • Hunan Province, Ningyuan County, diseased on culms of bamboo, November 2022, other living culture RCEF20002.

Note. Phylogenetic analyses confirmed that *A. xiangxiense* formed an independent clade, exhibiting a close evolutionary relationship with *A. aseptata*, *A. olivata*, and *A. machili* (1.0 BIPP and 100% MLBS).

However, *A. xiangxiense* differs from *A. aseptata* in several key aspects, including conidial size (8.6–15.4 × 6.7–10.2 µm vs. 7–9.5 (–13) µm). Based on nucleotide comparisons, *A. xiangxiense* differs from *A. aseptata* by 0.69% in ITS, 0.16% in LSU, 2.36% in *TUB2*, and 0.49% in *TEF1*. *Apiospora xiangxiense* also differs from *A. machili* by having longer conidia (8.6–15.4 × 6.7–10.2 µm vs. 7.1–9.5 ×
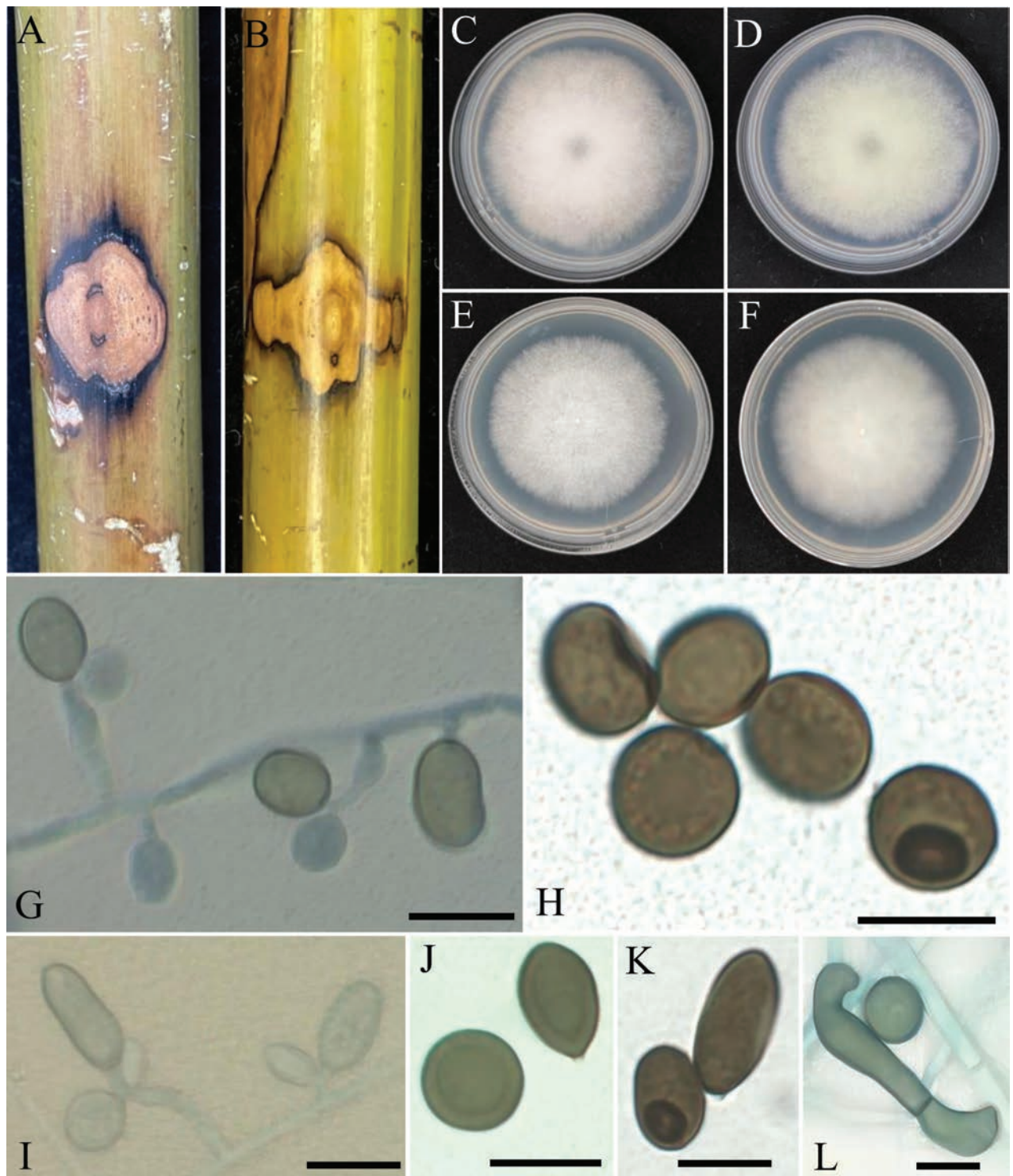
Figure 5. *Apiospora xiangxiense* (from ex-type living cultures RCEF20001) **A, B** diseased culms of bamboo **C, D** upper view and reverse view of culture on PDA **E, F** upper view and reverse view of culture on MEA **G, H** conidiogenous cells giving rise to conidia **I–K** conidia **L** sterile cells and conidia. Scale bars: 10 μm.

5.6−8.8 μm) and more elongated conidiogenous cells (2.0−15.5 × 1.4−3.9 μm vs. 6.0−8.0 × 2.5−4.0 μm). *Apiospora xiangxiense* differs from *A. olivata* by having longer conidia (8.6−15.4 × 6.7−10.2 μm) compared to *A. olivata* (8−12 × 5.5−8 μm), with sequence differences of 7.52% in ITS, 1.22% in LSU, and 1.94% in *TUB2*. Furthermore, their isolation sources are different.

Table 4. Synopsis of morphological characteristics of *A. xiangxiense* and its closely related species.

| Species	Isolation source	Country	Colony morphology (on PDA)	Conidia		References
				Shape	Diam (µm)	
A. aseptata	Healthy leaf of *Dicranopteris pedata*	China	Grey-brown; 5 cm in 10 days	Globose or sub globose	7−9.5 (−13)	Zhang et al. (2023)
A. machili	Diseased leaves of *Machilus nanmu*	China	Ivory; 69.7−78.8 mm cm in 7 days	Globose to subglobose	7.1−9.5 × 5.6−8.8	Liu et al. (2024)
A. olivata	Green belt soil	China	initially white, becoming curry on the surface; reverse pale green; more than 90 mm in 14 days	a. olivary; b. subglobose to globose	a. 8−12 × 5.5−8 µm; b. 8−11.5 µm	Zhang et al. (2024)
A. xiangxiense	Diseased culms of Bamboo	China	white to grey, sometimes with pale yellow; Reaching 9 cm in 8 days	globose to elongate ellipsoid	8.6−15.4 × 6.7−10.2	**This study**

For details, see Table 4. Thus, both morphological and molecular evidence support *A. xiangxiense* as a new species.

Pathogenicity tests

To determine the pathogenicity of the three new species isolates, three representative strains (RCEF20001, RCEF20000, and RCEF7611) were selected and inoculated onto fresh bamboo culms using a wound inoculation method. All three isolates were able to induce necrotic lesions. Inoculation with *A. xiangxiense* RCEF20001 resulted in the formation of gray-brown diamond-shaped lesions at the wound site after three days. Upon removing the epidermis, the internal lesions exhibited regular hollow black-brown diamond-shaped spots, which were larger than those observed on the surface (Fig. 6A−C). Inoculation with *A. bambusiparasitica* RCEF20000 caused rotting, diamond-shaped lesions at the wound site, with internal lesions displaying elliptical to scattered black-brown spots after the epidermis was scraped off (Fig. 6D−F). Inoculation with *A. qiannanensis* RCEF7611 resulted in gray-brown oval to diamond-shaped lesions at the wound site after three days. Scraping off the epidermis revealed hollow black-brown diamond-shaped spots, which were smaller than those seen on the surface (Fig. 6H, I). The control group was subjected to the same wound treatment as the experimental groups, but without pathogen inoculation, and no visible symptoms were observed in the control group (Fig. 6J). The symptoms observed were similar to those of naturally infected bamboo. Furthermore, the same fungus was consistently recovered from the experimentally inoculated bamboo. Deposits of the isolates are maintained at the Research Center for Entomogenous Fungi (RCEF), Anhui Agricultural University, Anhui Province, China.

Discussion

In this study, 37 isolates of *Apiospora* (Apiosporaceae, Amphisphaeriales, Sordariomycetes) were obtained from diseased culms of bamboo in China (Hunan and Guizhou Provinces). Based on morphological and culture characteristics and phylogenetic analyses of combined ITS, LSU, *TUB2*, and *TEF1* sequence data, three novel species were identified, namely *Apiospora bambusiparasitica*, *A. xiangxiense*, and *A. qiannanensis*. These findings were confirmed through both morphological and molecular characterization, verifying the taxonomic classification of the three species.
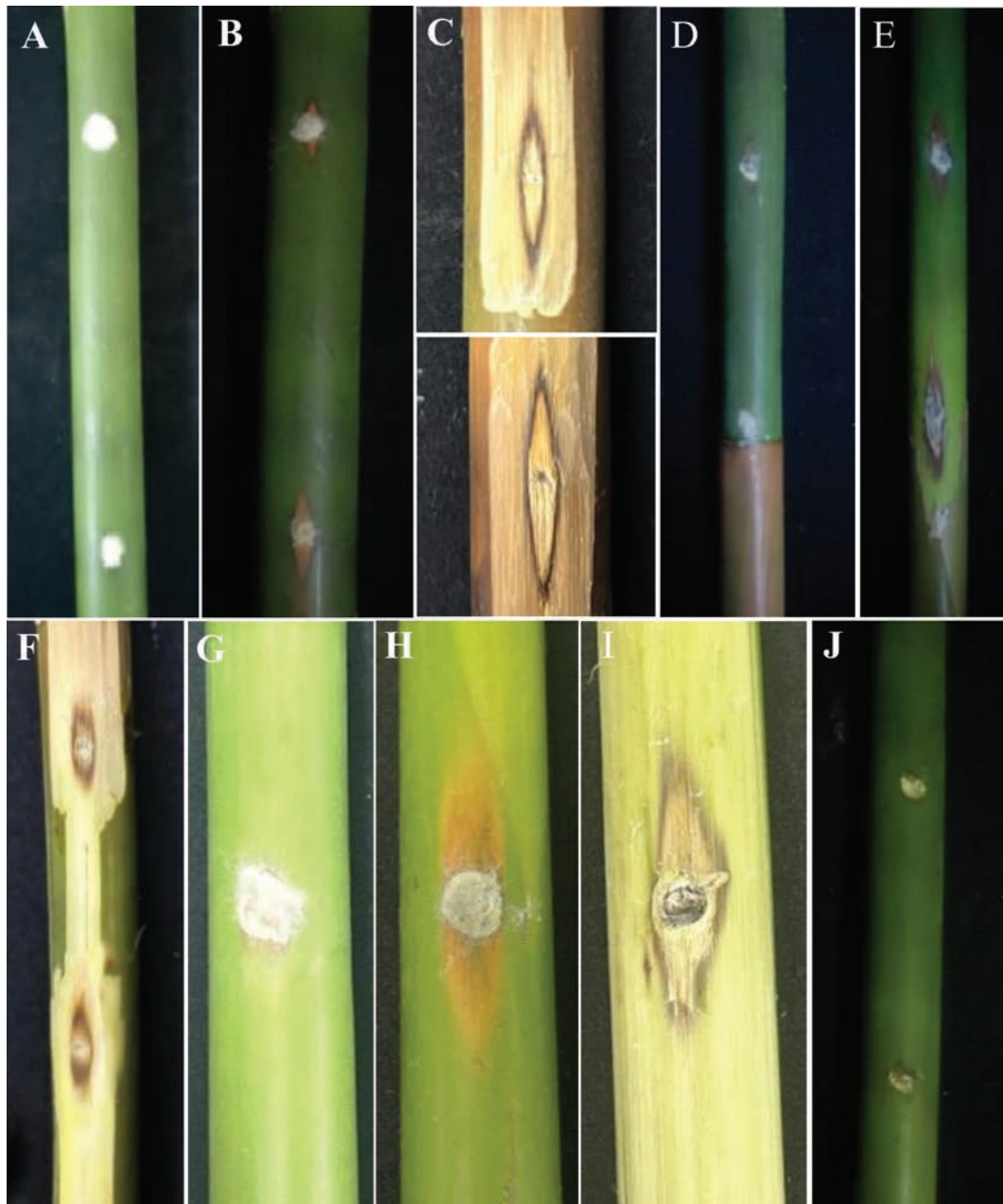
Figure 6. Pathogenicity test **A** symptoms on bamboo culm inoculated with the isolate *A. xiangxiense* RCEF20001 after 3 days **B** inoculation with RCEF20001 strain after 5 days **C** details under the diseased tissues **D** symptoms on bamboo culm inoculated with the isolate *A. bambusiparasitica* RCEF20000 after 3 days **E** inoculation with RCEF20000 strain after 5 days **F** details under the diseased tissues **G** symptoms on bamboo culm inoculated with the isolate *A. qiannanensis* RCEF7611 after 3 days **H** inoculation with RCEF7611 strain after 5 days **I** details under the diseased tissues **J** bamboo inoculated with PDA plug.

Apiospora is a cosmopolitan genus distributed across tropical, subtropical, and temperate climates, primarily associated with Poaceae, but also known to colonize a wide range of other hosts (Pintos and Alvarado 2021; Yan and Zhang 2024; Zhang et al. 2024). The strains analyzed in this study were isolated from bamboo in the subtropical regions of China (Guizhou and Hunan), further validating the previously described ecological characteristics of the genus.

According to data from Index Fungorum (accessed on October 28, 2024), the genus *Apiospora* has been recognized to have 196 species. Among them, many species of *Apiospora* are known to be associated with various living and decaying plant materials, and several *Apiospora* species act as plant pathogens. Such as *A. marii*, which causes olive tree dieback in Italy (Gerin et al. 2020); *A. phaeospermum*, which causes leaf necrosis in the olive crop in Sicily (Lo Piccolo et al. 2014); *A. arundinis*, which causes kernel blight of barley in the USA, *Phyllostachys praecox* brown culm streak disease in Nanjing, leaf blight on tea plants in China, leaf edge spot of peach in China, and culm rhomboid rot of Moso Bamboo (Martínez-Cano et al. 1992; Chen et al. 2014; Thangaraj et al. 2019; Ji et al. 2020; Zheng et al. 2022). The three species in this study were isolated from diseased culms of bamboo, and we verified their pathogenicity to bamboo under laboratory conditions. In the field, the disease symptoms caused by these fungi typically manifest as brown or black lesions of irregular shape on bamboo culms. These lesions may expand, coalesce, and in some cases, lead to extensive necrotic patches, resembling what we refer to as bamboo culm piebald-spot disease. Our pathogenicity tests confirmed that the three newly described species can induce similar symptoms under laboratory conditions, providing a theoretical basis for future research on bamboo disease management and control strategies.

In terms of biological applications, numerous *Apiospora* species produce bioactive secondary metabolites, potentially offering a promising source for pharmacological and medicinal research. For instance, *Apiospora* has shown strong antifungal activity against various plant pathogens (Hong et al. 2015). *A. saccharicola*, isolated from *Miscanthus* sp., is known to produce enzymes of industrial significance (Shrestha et al. 2015). *A. rasikravindrae*, isolated from *Coleus amboinicus*, exhibits notable cytotoxicity against WiDr cells and displays effective antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Astuti et al. 2021). Metabolites from *A. arundinis*, isolated from *Aconitum brevicalcaratum*, show cytotoxic effects on breast cancer cell lines (Shu et al. 2022), while *A. arundinis* MA30, derived from sea anemones, demonstrates significant anti-inflammatory activity (Lee et al. 2024). Whole-genome sequencing with antiSMASH analysis identified six and ten NR-PKS gene clusters in *A. malaysianum* and *A. koreana*, respectively, which may encode known or novel quinone compounds with notable biological functions (Christiansen et al. 2021). *Apiospora* holds substantial potential for synthesizing diverse secondary metabolites. However, many novel species, including new species in this study, remain underexplored. Future research necessitates further exploration of the biological applications of both known and newly discovered *Apiospora* species to comprehensively elucidate their biological properties.

In conclusion, this study provides a detailed account of three new species of *Apiospora* from China and emphasizes the importance of integrating morphological and molecular data for accurate species identification. Given their potential ecological and economic impacts on bamboo, further research is warranted. Comprehensive taxonomic and ecological investigations will offer valuable insights for potential biotechnological applications and enhance our understanding of this genus and its broader ecological and medicinal significance.

Additional information

Conflict of interest

Ethical statement

Funding

Author contributions

Conceptualization: Xiaoyun Chang and Mingjun Chen; Data curation: Xiaoyun Chang, Yuanyuan Wang, and Tao Xu; Funding acquisition: Xianghua Yue and Mingjun Chen; Investigation: Xianghua Yue; Project administration: Mingjun Chen; Resources: Xianghua Yue and Mingjun Chen; Supervision: Mingjun Chen, Xianghua Yue, and Guangshuo Li; Writing—original draft: Xiaoyun Chang; Writing—review and editing: Mingjun Chen, Guangshuo Li, Xianghua Yue, Yuanyuan Wang, and Tao Xu. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Xiaoyun Chang https://orcid.org/0000-0002-0093-9582
Yuanyuan Wang https://orcid.org/0009-0007-1801-3984
Tao Xu https://orcid.org/0009-0009-0028-6508
Guangshuo Li https://orcid.org/0000-0002-7285-0712
Xianghua Yue https://orcid.org/0000-0003-2165-7653
Mingjun Chen https://orcid.org/0000-0002-1439-7796

Data availability

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

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Biotrophic and saprophytic fungi from the *Rhodocybe-Clitopilus* clade (Entolomataceae): two new species and one new record in subtropical China

Sipeng Jian[1], Xia Chen[2], Tianwei Yang[1], Xinjing Xu[1], Feng Gao[1], Yiwei Fang[1], Jing Liu[1], Chunxia Zhang[1]

1 Yunnan Institute of Tropical Crops, Jinghong, Yunnan, 666100, China
2 The Administration Bureau of Dr. Sun Yat-sen's Mausoleum, Nanjing, Jiangsu, 210014, China
Corresponding author: Chunxia Zhang (zhangchunxia7084@163.com)

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Abstract

This study proposes two new species and a new record in the *Rhodocybe-Clitopilus* clade, based on comprehensive morphological and molecular analyses. The nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS), the large subunit ribosomal RNA gene (LSU), the RNA polymerase II second largest subunit (*RPB2*) and the translation elongation factor 1-alpha gene (*TEF1*), were employed to elucidate the relationships of *Clitopilus* and *Rhodocybe*. The first species, *Clitopilus parasiticus*, is capable of infecting the leaves of host plants in the genera *Dryopteris* and *Oplismenus*, exhibiting typical biotrophic behaviour while also demonstrating saprophytic growth on soil. Intraspecific comparisons were conducted, examining environmental factors as well as macro- and microscopic characteristics amongst individuals found on different plant hosts. Furthermore, this study reports the new saprophytic species, *Rhodocybe zijinshanensis* and provides a detailed description of *Clitopilus baronii*, a newly-recorded species in China.

Key words: Biotrophic species, Entolomataceae, morphology, multigene phylogeny, plant pathogens, taxonomy

Introduction

In nature, numerous fungi are well-known for their parasitic relationships, enabling them to thrive in dynamic environments. For example, the ergot (*Claviceps purpurea* (Fr.) Tul.) and corn smut (*Mycosarcoma maydis* (DC.) Bref.) are recognised as pathogenic fungi affecting cultivated plants (*Triticum aestivum* L. and *Zea mays* L., respectively) (Tudzynski and Scheffer 2004; McTaggart et al. 2016). Additionally, several special form genera, such as *Asterophora* Ditmar, *Squamanita* Imbach and *Hypomyces* (Fr.) Tul. & C. Tul., exhibit fungicolous parasitism or mycoparasitic behaviour (Rogerson and Samuels 2018; Elkhateeb and Daba 2021; Liu et al. 2021). However, parasitic forms are relatively rare in Agaricales Underw., particularly for biotrophic parasitism.

Saprophytic and symbiotic modes of nutrition are predominant amongst fungi in Basidiomycetes, but some fungi also employ parasitic nutrition as a strategy for survival and reproduction (Põlme et al. 2021; Shi et al. 2023). Thereinto,

biotrophic parasitism is an intriguing and unique phenomenon in fungi, defined as a nutritional strategy where fungi derive nutrients from a living host while keeping it alive, often causing restricted damage to the host plant (Luttrell 1974; Kemen and Jones 2012). Species within the Agaricales that exhibit distinct biotrophic capabilities often also possess saprophytic abilities, indicating that they are not obligate parasites. For example, Zhang et al. (2022) identified a new species, viz. *Crepidotus herbaceus* T. Bau & Y.P. Ge, which is not only parasitic on the leaves or stems of *Oreocnide frutescens* (Thunb.) Miq. and *Alpinia japonica* (Thunb.) Miq., but is also found on the plant debris or humus.

In the family Entolomataceae Kotl. & Pouzar, there are two main clades: *Entoloma* (Fr.) P. Kumm. and *Rhodocybe-Clitopilus* (Co-David et al. 2009; Baroni and Matheny 2011; Kluting et al. 2014). The *Rhodocybe-Clitopilus* clade differs from the *Entoloma* clade by its basidiospores, which are characterized by either longitudinal ridges or scattered, finely to distinctly pustulate ornamentations (Baroni 1981; Singer 1986; Kluting et al. 2014). Within the *Rhodocybe-Clitopilus* clade, most species are primarily regarded as saprophytic (Sanchez-Garcia and Matheny 2017). However, a few species, such as *Rhodophana stangliana* (Bresinsky & Pfaff) Vizzini, *Clitopilus passeckerianus* (Pilát) Sing., *C. fasciculatus* Noordel. and *C. daamsii* Noordel., also appear mycoparasite (Noordeloos 1984, 1993; Læssøe and Rosendahl 1994; Czederpiltz et al. 2001). Notably, *C. hobsonii* (Berk.) P.D. Orton has been reported to grow on stumps, fallen logs, twigs and living herbaceous leaves and stems (Orton 1960; Noordeloos 1984), highlighting its saprophytic and biotrophic capacities.

In the current study, several specimens gathered from Jiangsu Province are examined carefully. Three samples closely resembled *Pleurotus* (Fr.) P. Kumm., *Crepidotus* (Fr.) Staude and *Omphalotus* Fayod. Upon microscopic examination, they were all confirmed to the *Rhodocybe-Clitopilus* clade, respectively. Furthermore, two new species and one new record species were identified, based on the multi-gene phylogenetic tree. Therefore, all three species are described herein.

Materials and methods

Sample collections and morphological observations

The collection information of voucher specimens and the sequences used in phylogenetic analyses are shown in Table 1. The colour codes (hex triplets) from ColorHexa (https://www.colorhexa.com) were employed to depict the colour of basidiomata. These codes consist of characters ranging from a to f and 0 to 9, with each pair corresponding to the red, green and blue components of the colour. The general description of basidiomata, including both macro- and microscopic features, as well as the morphological classification rules in *Clitopilus* and *Rhodocybe* Maire were based on the work of Baroni (1981) and Jian et al. (2020a). All voucher specimens have been deposited at the Cryptogamic Herbarium of the Herbaria of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

Sections of dried basidiomata were rehydrated in purified water and 5% potassium hydroxide (KOH) and were occasionally stained with 1% Congo Red to enhance visibility. The notation "[*n/m/p*]" indicates *n* basidiospores from *m* basidiomata of *p* specimens. The measurements of basidiospores are presented in the format (a−)b−c(−d), where the range b−c includes at least 90% of the measured values, while a and d (given in parentheses) represent the extreme values.

The average length and width of basidiospore (± standard deviation) are denoted as L_m and W_m, respectively. The term Q refers to the "length/width ratio" of a basidiospore in side view, with Q_{avg} representing the average Q across all specimens (± standard deviation). Fragments isolated from specimens were attached to aluminium stubs using double-sided adhesive tape, and then coated with gold/palladium. Finally, a ZEISS EVO LS10 (Germany) scanning electron microscope (SEM) was used to observe the ornamentation of the basidiospores.

The genetic names appeared in this study are abbreviated as follows: *Clitopilus* = "*C.*", *Rhodocybe* = "*R.*".

Molecular phylogenetic analyses

In this study, we utilised two sequences of non-protein-coding and two protein-coding genes: the nuc rDNA internal transcribed spacer region ITS1-5.8S-ITS2 (ITS), the large subunit ribosomal RNA gene (LSU), the RNA polymerase II second largest subunit (*RPB2*) and the translation elongation factor 1-alpha gene (*TEF1*). The ITS and LSU genes were selected for their availability of universal primers (White et al. 1990), while the *RPB2* and *TEF1* genes were chosen due to their relatively high number of informative sites and sufficient nucleotide variation, which are essential for inferring evolutionary relationships within the Entolomataceae (Matheny et al. 2007; Co-David et al. 2009; Kluting et al. 2014). All the sequences were submitted to the National Center for Biotechnology Information (NCBI) and detailed information regarding each gene was provided in Table 1.

Genomic DNA was extracted from collected materials and herbarium specimens using the CTAB (cetyltrimethylammonium bromide) procedure outlined by Doyle and Doyle (1987). The PCR protocol followed the touchdown method described by Kluting et al. (2014), with detailed data provided in Table 2. Gel extraction and PCR (polymerase chain reaction) were conducted to purify the PCR products, which were then sequenced on an ABI-3730-XL sequence analyser (Applied Biosystems, Foster City, CA) using the same primers as in the PCR. The new sequences generated from this study are highlighted in bold in Table 1.

For the sequence alignments, Sequencher 4.1.4 (Gene Code Corp., Ann Arbor, MI) was used to concatenate sequences obtained from both direction (5'−3' & 3'−5'), to remove regions with heavy peaks and to merge degenerate bases. The sequences were then aligned using MAFFT 7.526 (Katoh et al. 2005) and manually checked in BioEdit 7.7.1 (Hall 1999). Separate single-gene analyses were performed to exclude conflicts amongst topologies using Maximum Likelihood and Bayesian Inference. Subsequently, Phyutility 2.2 (Smith and Dunn 2008) was employed to combine all the separate single-gene datasets. Any deficiencies in the DNA fragment sequences were treated as missing data in the subsequent analyses. A super-matrix was generated by combining sequences of all four loci.

Under the Akaike Information Criterion (AIC), the best-fitted substitution model for each dataset was determined with MrModelTest 2.3 (Nylander 2004). Phylogenetic analyses were conducted using Maximum Likelihood (ML) with RAxML 7.2.6 (Stamatakis 2006) and Bayesian Inference (BI) with MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). In view of the close kinship amongst *Lulesia* Singer & *Clitopilus*, as well as *Clitopilopsis* Maire & *Rhodocybe*, *Lulesia umbrinomarginata* Y.Q. Xiao et al. and *Lulesia orientalis* (S.P. Jian & Zhu L. Yang) Vizzini et al. were selected as outgroups for the phylogenetic tree of *Clitopilus*.

Table 1. Sequencing primers and the best annealing temperature for ITS, LSU, *RPB2* and *TEF1*.

Primer name	Nucleotide sequence 5'-3'	PCR annealing temperature (°C)
ITS4	TCC TCC GCT TAT TGA TAT GC	52
ITS5	GGA AGT AAA AGT CGT AAC AAG G	
LROR	ACC CGC TGA ACT TAA GC	52
LR5	TCC TGA GGG AAA CTT CG	
EF1-983F	GCY CCY GGH CAY CGT GAY TTY AT	56/touchdown*
EF1-1953R	CCR GCR ACR GTR TGT CTC AT	
bRPB2-6F	TGG GGY ATG GTN TGY CCY GC	52
bRPB2-7.1R	CCC ATR GCY TGY TTM CCC ATD GC	

Notes: * the details of the touchdown method refer to Kluting et al. (2014).

Similarly, *Clitopilopsis albida* S.P. Jian & Zhu L. Yang and *Clitopilopsis hirneola* (Fr.) Kühner were chosen as outgroups for the phylogenetic tree of *Rhodocybe*.

For ML analyses, the GTRGAMMAI model was applied to the combined dataset, with statistical support for internodes obtained through non-parametric bootstrapping with 1000 replications. For the BI analyses of the combined dataset, a partitioned mixed model was implemented, defining the sequences of ITS, LSU, *RPB2* and *TEF1* as four independent partitions, with each gene estimated using different model parameters. The best-selected model was employed and the Markov Chain Monte Carlo (MCMC) chain was run for four million generations. The STOPRULE command was set with STOPVAL = 0.01 and trees were sampled every 100 generations. We verified chain convergence using Tracer 1.5 (http://tree.bio.ed.ac.uk/software/tracer) to ensure sufficiently large effective sample size (ESS) values greater than 200. The combined tree was summarised using the sump and sumt commands with a 25% burn-in.

Results

Phylogenetic analyses

No topological inconsistency was detected between the ML and BI analyses, both for the individual genes and the multigene data. The phylogenetic tree inferred from the ML strategy is presented, with statistical results from both ML (Bootstrap Supports, BS) and BI (Posterior Probabilities, PP) displayed on the branches (see Figs 1, 2). The best-fit model for ML and BI analyses was the GTR+I+R model. In the multigene matrix for *Clitopilus*, we assembled a total of 131 sequences from four genes: 96 for ITS, 89 for LSU, 110 for *RPB2* and 78 for *TEF1*. Analogously, the multigene matrix for *Rhodocybe* included 120 sequences derived from the same four genes, with 72 for ITS, 48 for LSU, 55 for *RPB2* and 37 for *TEF1*. Finally, the dataset for *Clitopilus* included 3664 sites in total, with 788 from ITS, 957 from LSU, 784 from *RPB2* and 1135 from *TEF1*. Similarly, the dataset for *Rhodocybe* included 3881 sites, with 960 from ITS, 985 from LSU, 799 from *RPB2* and 1137 from *TEF1*.

In the phylogenetic tree of *Clitopilus* (Fig. 1), species with parasitic abilities are clustered together in *Clitopilus* sect. *Scyphoides* Singer, representing a new species. The species collected from rotten wood are positioned close to *C. baronii* Consiglio & Setti. Furthermore, the phylogenetic tree of *Rhodocybe* (Fig. 2) shows that other species collected from rotten wood are clustered within *Rhodocybe* sect. *Rufobrunnea* Singer ex T.J. Baroni, also signifying a new species.

Table 2. Collection information of voucher specimen and GenBank accession numbers for sequences used in phylogenetic analyses. H in parentheses means the holotype specimen.

Species	Collection or collector no.	Location and year	GenBank accession numbers				References
			ITS	LSU	*RPB2*	*TEF1*	
C. abprunulus	KUN-HKAS 107040[a]	Macedonia 2019	NR_172792	NG_074438	MT349666	MT349670	Jian et al. (2020b)
C. abprunulus	KUN-HKAS 107041[a]	Macedonia 2019	MT345049	MT345054	MT349667	MT349671	Jian et al. (2020b)
C. abprunulus	KUN-HKAS 107042[a]	Macedonia 2019	MT345047	MT345052	MT349665	MT349669	Jian et al. (2020b)
C. abprunulus	MEN 2003-09-14[b]	Belgium 2003	KR261096	GQ289149	GQ289221	–	Co-David et al. (2009)
C. albidus	CAL 1319[c]	Kerala State, India 2001	MF926596	MF926595	MF946579	–	Raj and Manimohan (2018)
C. amygdaliformis	KUN-HKAS 60406[a]	Yunnan, China 2008	MN061292	–	MN148120	–	Jian et al. (2020a)
C. amygdaliformis	KUN-HKAS 81125[a]	Yunnan, China 2014	NR_172768	MN065681	MN148119	MN166231	Jian et al. (2020a)
C. amygdaliformis	KUN-HKAS 87950[a]	Yunnan, China 2014	MN061290	MN065680	MN148118	MN166230	Jian et al. (2020a)
"*C.* cf. *argentinus*"	MTB 4804/2[d]	Germany 2011	–	–	KC816907	KC816823	Kluting et al. (2014)
C. austroprunulus	MEN2009001[e]	Tahune, Australia 2009	KC139084	–	–	–	Crous et al. (2012)
C. austroprunulus	MEN2009062[e]	Tasmania, Australia 2009	KC139085	–	–	–	Crous et al. (2012)
C. baronii	**KUN-HKAS 145333**	**Jiangsu, China 2023**	**PQ793166**	**PQ781610**	**PQ788395**	**PQ788402**	**This study**
C. baronii	**KUN-HKAS 145334**	**Jiangsu, China 2023**	**PQ793167**	**PQ781611**	**PQ788396**	**PQ788403**	**This study**
C. baronii	K(M)179703[f]	UK 2012	MN855362	–	MN856160	–	Consiglio and Setti (2019)
C. baronii	AMB 18359[g]	Mantova, Italy 2006	MN855365	–	MN856163	MN856174	Consiglio and Setti (2019)
C. baronii	AMB 18362[g]	Ferrara, Italy 2007	MN855368	–	MN856166	MN856176	Consiglio and Setti (2019)
C. baronii	AMB 18363[g]	Mantova, Italy 2007	NR_176131	–	MN856167	MN856177	Consiglio and Setti (2019)
C. baronii	AMB 18378[g]	Pisa, Italy 2007	MN855370	–	MN856168	MN856178	Consiglio and Setti (2019)
C. brunneiceps	KUN-HKAS 73123[a]	Yunnan, China 2011	MN061294	MN065683	MN148122	MN166233	Jian et al. (2020a)
C. brunneiceps	KUN-HKAS 80211[a]	Hubei, China 2013	MN061293	MN065682	MN148121	MN166232	Jian et al. (2020a)
C. brunneiceps	KUN-HKAS 104510[a]	Yunnan, China 2018	NR_172769	MN065684	MN148123	MN166234	Jian et al. (2020a)
C. brunneiceps	HMJAU 23509[h]	Neimenggu, China 2013	MN061296	MN065685	MN148115	–	Jian et al. (2020a)
C. chichawatniensis	LAH37431[i]	Punjab, Pakistan 2019	ON980767	ON980764	–	–	Fatima et al. (2022)
C. chichawatniensis	LAH37432[i]	Punjab, Pakistan 2020	ON980766	ON980763	–	–	Fatima et al. (2022)
C. chrischonensis	TO HG1994[j]	Basilea, Switzerland 2008	HM623128	HM623131	–	–	Vizzini et al. (2011)
"*C. cinerascens*"	8024 TJB[d]	Florida, USA 1996	–	GU384613	KC816908	KC816824	Kluting et al. (2014)
"*C. cinerascens*"	8133 TJB[d]	Louisiana, USA 1996	–	–	KC816909	KC816825	Kluting et al. (2014)
C. cretoalbus	LAH37017[i]	Punjab, Pakistan 2020	OM935685	OM934826	–	–	Izhar et al. (2023)
C. cretoalbus	LAH35709[i]	Punjab, Pakistan 2017	ON117610	ON229505	–	–	Izhar et al. (2023)
C. crispus	9982 TJB[d]	Chiang Mai, Thailand 2006	–	–	KC816910	KC816826	Kluting et al. (2014)
C. crispus	10027 TJB[d]	Chiang Mai, Thailand 2006	–	–	KC816911	KC816827	Kluting et al. (2014)
C. crispus	KUN-HKAS 84667[a]	Yunnan, China 2014	MN061314	MN065705	MN148142	MN166254	Jian et al. (2020a)
C. crispus	KUN-HKAS 87018[a]	Yunnan, China 2014	MN061315	MN065706	MN148143	MN166255	Jian et al. (2020a)
C. crispus	KUN-HKAS 90506[a]	Yunnan, China 2015	MN061312	MN065702	MN148139	MN166251	Jian et al. (2020a)
C. crispus	KUN-HKAS 90508[a]	Yunnan, China 2015	–	MN065703	MN148140	MN166252	Jian et al. (2020a)
C. crispus	KUN-HKAS 97509[a]	Yunnan, China 2016	MN061318	MN065708	MN148145	MN166258	Jian et al. (2020a)
C. crispus	KUN-HKAS 102670[a]	Yunnan, China 2017	MN061313	MN065704	MN148141	MN166253	Jian et al. (2020a)
C. crispus	KUN-HKAS 104507[a]	Yunnan, China 2017	MN061316	MN065707	MN148144	MN166256	Jian et al. (2020a)
C. cystidiatus	MEN 200350	Slovakia 2003	–	GQ289147	GQ289220	–	Co-David et al. (2009)
C. fasciculatus	MO#297071	California, USA 2017	MG551863	–	–	–	Direct submission
C. fusiformis	SAAS 1038[k]	Yunnan, China 2015	KY385634	–	KY385632	–	Wang et al. (2017)
C. fusiformis	SAAS 1892[k]	Yunnan, China 2015	NR_158328	–	KY385633	–	Wang et al. (2017)
C. fusiformis	KUN-HKAS 104513[a]	Yunnan, China 2018	MN061297	MN065686	MN148124	MN166235	Jian et al. (2020a)
C. fusiformis	KUN-HKAS 104514[a]	Yunnan, China 2018	MN061298	MN065687	MN148125	MN166236	Jian et al. (2020a)
C. fusiformis	KUN-HKAS 104515[a]	Yunnan, China 2018	MN061300	MN065690	MN148128	MN166239	Jian et al. (2020a)
C. fusiformis	KUN-HKAS 104516[a]	Yunnan, China 2018	MN061299	MN065688	MN148126	MN166237	Jian et al. (2020a)
C. fusiformis	KUN-HKAS 104517[a]	Yunnan, China 2018	–	MN065689	MN148127	MN166238	Jian et al. (2020a)
C. giovanellae	S.F.14368[l]	Trento, Italy 1888	EF413030	EF413027	–	–	Moreno et al. (2007)
C. giovanellae	AH 19780[m]	Spain 1998	–	EF413026	–	–	Moreno et al. (2007)
C. highlandensis	KUN-HKAS 68389[a]	Yunnan, China 2010	MN061310	MN065700	MN148137	MN166249	Jian et al. (2020a)
C. highlandensis	KUN-HKAS 117632[a]	Yunnan, China 2021	ON999061	ON999062	OP006563	OP006564	Jian et al. (2023)
C. hobsonii	K(M) 167650[f]	UK 2010	MN855371	–	MN856169	–	Consiglio and Setti (2019)
C. hobsonii	K(M) 122842[f]	UK 2004	NR_182819	–	MN856170	–	Consiglio and Setti (2019)
C. hobsonii	K(M) 199928[f]	UK 2015	MN855373	–	MN856171	–	Consiglio and Setti (2019)
"*C. hobsonii*"	QYL10	–	OK652826	OK655769	MN092372	MN092373	Peng et al. (2021)
"*C. hobsonii*"	DLL 9779	Queensland, Australia 2010	–	–	KC816916	KC816831	Kluting et al. (2014)

Species	Collection or collector no.	Location and year	GenBank accession numbers				References
			ITS	LSU	*RPB2*	*TEF1*	
"*C. hobsonii*"	5967 TJB[d]	New York, USA 1988	–	–	KC816917	–	Kluting et al. (2014)
"*C. hobsonii*"	DLL 9586	Queensland, Australia 2009	–	KJ021698	KC816912	KC816828	Kluting et al. (2014)
"*C. hobsonii*"	DLL 9635	Queensland, Australia 2009	–	–	KC816913	KC816829	Kluting et al. (2014)
"*C. hobsonii*"	DLL 9643	Queensland, Australia 2009	–	–	KC816914	–	Kluting et al. (2014)
"*C. hobsonii*"	DLL 9746	Queensland, Australia 2010	–	–	KC816915	KC816830	Kluting et al. (2014)
"*C. hobsonii* grp."	7051 TJB[d]	North Carolina, USA 1993	–	–	KC816918	–	Kluting et al. (2014)
C. aff. *hobsonii*	K:M195388[f]	UK 2014	MN855375	–	MN856172	MN856179	Consiglio and Setti (2019)
"*C.* aff. *hobsonii*"	UC 1860830[n]	California, USA 2011	–	–	KC816928	KC816841	Kluting et al. (2014)
C. cf. *kamaka*	KA12-0364[o]	South Korea 2012	KR673433	–	–	–	Kim et al. (2015)
C. kamaka	PDD 96106[p]	New Zealand 2010	NR_137867	–	–	–	Cooper (2014)
C. lampangensis	SDBR-CMUJK 0147[q]	Lampang, Thailand 2018	NR_175631	MK764935	MK784129	–	Kumla et al. (2019)
C. lampangensis	SDBR-CMUNK 0047[q]	Lampang, Thailand 2018	MK764934	MK773856	MK784128	–	Kumla et al. (2019)
C. orientalis	CAL 1613[c]	Kerala State, India 2011	MG345134	MG321558	MG321559	–	Raj and Manimohan (2018)
C. parasiticus	**KUN-HKAS 145335[a]**	**Jiangsu, China 2023**	**PQ793168**	**PQ781612**	**PQ788397**	**PQ788404**	**This study**
C. parasiticus (H)	**KUN-HKAS 145336[a]**	**Jiangsu, China 2024**	**PQ793169**	**PQ781613**	**PQ788398**	**–**	**This study**
C. parasiticus	**KUN-HKAS 145337[a]**	**Jiangsu, China 2024**	**PQ793170**	**PQ781614**	**PQ788399**	**PQ788405**	**This study**
C. passeckerianus	CBS:299.35[r]	Austria –	MH855682	MH867198	–	–	Vu et al. (2019)
C. passeckerianus	P73	South Korea 2015	KY962489	KY963073	–	–	Direct submission
C. passeckerianus	P78	South Korea 2015	KY962494	KY963078	–	–	Direct submission
C. passeckerianus	K:M134571[f]	UK 2005	MN855376	–	MN856173	–	Consiglio and Setti (2019)
C. paxilloides	5809 TJB[d]	California, USA 1987	–	–	KC816919	KC816832	Kluting et al. (2014)
"*C. peri*"	10040 TJB[d]	Chiang Mai, Thailand 2006	–	–	KC816921	KC816834	Kluting et al. (2014)
"*C. peri*"	10033 TJB[d]	Chiang Mai, Thailand 2006	–	–	KC816920	KC816833	Kluting et al. (2014)
"*C. peri*"	10041 TJB[d]	Chiang Mai, Thailand 2006	–	–	KC816922	KC816835	Kluting et al. (2014)
C. pinsitus	CBS 623.70[r]	England, UK –	MH859879	MH871665	–	–	Vu et al. (2019)
C. pinsitus	G. Immerzeel 1990-11	Netherlands 1990	–	GQ289148	–	–	Co-David et al. (2009)
"*C. prunulus*"	CORT:11CA012[d]	California, USA 2011	–	–	KC816926	KC816839	Kluting et al. (2014)
C. prunulus	REH8456[d]	Novgorod Region, Russa 2003	–	–	KC816923	KC816836	Kluting et al. (2014)
"*C. prunulus*"	6805 TJB[d]	New York, USA 1992	–	–	KC816924	KC816837	Kluting et al. (2014)
"*C. prunulus*"	TJB 9425[d]	Dominican Republic 2002	–	–	MN893320	MN893330	Baroni et al. (2020)
"*C. prunulus*"	AFTOL522, TJB6838[d]	USA –	DQ202272	AY700181	–	–	Direct submission
"*C. prunulus*"	TB8229[d]	New York, USA 1996	–	GU384615	GU384650	–	Baroni et al. (2011)
"*C. prunulus*"	TB9663[d]	–	–	GU384614	GU384648	–	Baroni et al. (2011)
C. prunulus	KUN-HKAS 96158[a]	Austria 2016	MN061301	MN065691	MN148129	MN166240	Jian et al. (2020a)
C. prunulus	KUN-HKAS 123138[a]	France –	OP626992	OP646418	OP939970	OP687894	He et al. (2023)
C. prunulus	HMJAU 4521[s]	Kirov, Russia 2006	MN061302	MN065692	MN148117	MN166241	Jian et al. (2020a)
C. cf. *prunulus*	KUN-HKAS 75845[a]	California, USA 2011	MN061303	MN065693	MN148130	MN166242	Jian et al. (2020a)
C. ravus	KUN-HKAS 107043[a]	Yunnan, China 2019	MT345050	MT345055	MT349668	MT349672	Jian et al. (2020b)
C. reticulosporus	WU27150[b]	Vienna, Austria 2004	KC885966	HM164412	HM164416	–	Morgado et al. (2016)
C. rugosiceps	KUN-HKAS 57003[a]	Yunnan, China 2009	MN061304	MN065694	MN148131	MN166243	Jian et al. (2020a)
C. rugosiceps	KUN-HKAS 59455[a]	Yunnan, China 2009	–	MN065696	MN148133	MN166245	Jian et al. (2020a)
C. rugosiceps	KUN-HKAS 73232[a]	Yunnan, China 2011	NR_172771	MN065695	MN148132	MN166244	Jian et al. (2020a)
C. rugosiceps	KUN-HKAS 107044[a]	Yunnan, China 2019	MT345046	MT345051	–	–	Jian et al. (2020b)
C. rugosiceps	KUN-HKAS 115921[a]	Yunnan, China 2017	MZ855871	MZ853557	MZ826364	MZ826362	He and Yang (2022)
C. scyphoides	CBS 127.47[r]	France –	MH856181	MH867707	–	–	Vu et al. (2019)
C. cf. *scyphoides*	KUN-HKAS 104511[a]	Gansu, China 2016	MN061329	MN065720	MN148157	MN166268	Jian et al. (2020a)
C. sinoapalus	KUN-HKAS 77037[a]	Jiangxi, China 2012	MN061321	MN065713	MN148149	–	Jian et al. (2020a)
C. sinoapalus	KUN-HKAS 82230[a]	Guangzhou, China 2013	MN061320	MN065712	MN148148	–	Jian et al. (2020a)
C. sinoapalus	KUN-HKAS 83831[a]	Yunnan, China 2014	–	MN065714	MN148150	–	Jian et al. (2020a)
C. sinoapalus	KUN-HKAS 101191[a]	Yunnan, China 2017	NR_172773	MN065711	MN148151	MN166261	Jian et al. (2020a)
C. sinoapalus	KUN-HKAS 102737[a]	Yunnan, China 2017	–	MN065709	MN148146	MN166259	Jian et al. (2020a)
C. sinoapalus	KUN-HKAS 102807[a]	Yunnan, China 2017	MN061319	MN065710	MN148147	MN166260	Jian et al. (2020a)
C. subalbidus	GDGM 72219[t]	Guangdong, China 2018	NR_198267	NG_243733	ON959185	ON959190	Jian et al. (2023)
C. subalbidus	GDGM 72229[t]	Guangdong, China 2018	ON963952	ON963946	ON959186	–	Jian et al. (2023)
C. subscyphoides	CAL 1325[c]	Kerala State, India 2011	MF927542	MF946580	MF946581	–	Raj and Manimohan (2018)
C. subscyphoides	GDGM 72195[t]	Guangdong, China 2018	–	–	ON959188	–	Jian et al. (2023)

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			ITS	LSU	*RPB2*	*TEF1*	
C. subscyphoides	GDGM 72683[t]	Guangdong, China 2018	ON963953	ON963947	–	–	Jian et al. (2023)
C. subscyphoides	GDGM 73056[t]	Guangdong, China 2018	ON963954	ON963948	ON959187	ON959191	Jian et al. (2023)
C. umbilicatus	KUN-HKAS 80289[a]	Hunan, China 2013	MN061323	MN065715	MN148152	MN166262	Jian et al. (2020a)
C. umbilicatus	KUN-HKAS 80310[a]	Anhui, China 2013	MN061324	MN065716	MN148153	MN166263	Jian et al. (2020a)
C. umbilicatus	KUN-HKAS 80370[a]	Fujian, China 2013	MN061325	MN065717	MN148154	MN166264	Jian et al. (2020a)
C. umbilicatus	KUN-HKAS 80945[a]	Anhui, China 2013	MN061326	MN065718	MN148155	MN166265	Jian et al. (2020a)
C. umbilicatus	KUN-HKAS 104509[a]	Yunnan, China 2017	MN061327	MN065719	MN148156	MN166266	Jian et al. (2020a)
C. velutinus	CORT 014618[d]	Dominican Republic 2015	MN784991	–	MN893321	MN893331	Baroni et al. (2020)
C. venososulcatus	8111 TJB[d]	Louisiana, USA 1996	–	–	KC816930	–	Kluting et al. (2014)
C. yunnanensis	KUN-HKAS 59712[a]	Yunnan, China 2009	MN061307	–	MN148135	–	Jian et al. (2020a)
C. yunnanensis	KUN-HKAS 82076[a]	Yunnan, China 2012	MN061306	MN065697	MN148134	MN166246	Jian et al. (2020a)
C. yunnanensis	KUN-HKAS 104518[a]	Yunnan, China 2018	MN061308	MN065698	MN148136	MN166247	Jian et al. (2020a)
C. yunnanensis	HMJAU 24677[s]	Sichuan, China 2013	MN061309	MN065699	MN148116	MN166248	Jian et al. (2020a)
"*Clitopilus* sp."	7130 TJB[d]	New York, USA 1993	–	–	KC816929	–	Kluting et al. (2014)
Clitopilus sp.	TB8067[d]	Florida, USA 1996	–	GU384612	GU384649	–	Baroni et al. (2011)
Clitopilus sp.	KUN-HKAS 104508[a]	Yunnan, China 2017	MN061311	MN065701	MN148138	MN166250	Jian et al. (2020a)
Clitopilus sp.	KUN-HKAS 104512[a]	Yunnan, China 2018	MN061330	MN065721	MN148158	MN166269	Jian et al. (2020a)
R. alutacea	5726 TJB[d]	North Carolina, USA 1987	–	–	KC816931	KC816842	Kluting et al. (2014)
R. asanii	KATO 3659[u]	Turkey 2015	KX834263	KX834264	–	–	Sesİİ and Vizzini (2017)
R. asanii	KATO 3657[u]	Turkey 2015	KX834265	–	–	–	Sesİİ and Vizzini (2017)
R. asanii	NA13102020	East Sussex, UK 2020	MW375030	–	–	–	Aplin et al. (2022)
R. asyae	KATO 3640[u]	Trabzon, Turkey 2015	KX834266	KX834267	–	–	Sesİİ and Vizzini (2017)
R. asyae	KATO 3653[u]	Trabzon, Turkey 2015	KX834268	–	–	–	Sesİİ and Vizzini (2017)
R. asyae	NA131019[v]	East Sussex, UK 2019	MN840644	–	–	–	Aplin et al. (2022)
R. aureicystidiata	PBM 1902[w]	Washington, USA –	–	AY380407	AY337412	–	Matheny (2005)
R. brunneoaurantiaca	CAL 1825[c]	West Bengal, India 2019	MW031906	MW031916	–	–	Dutta et al. (2021)
R. brunneoaurantiaca	CUH AM720[x]	West Bengal, India 2019	MW023201	MW023223	–	–	Dutta et al. (2021)
R. brunnescens	TENN 056140[y]	Tennessee, USA 1985	NR_119914	NG_058820	–	–	Baroni et al. (2011)
R. brunnescens	TENN 056140-2[y]	Tennessee, USA 1987	HQ222033	JF706313	–	–	Baroni et al. (2011)
R. byssisedoides	AG 2004-04-27	Jena, Germay 2004	–	GQ289212	GQ289279	–	Co-David et al. (2009)
R. caelata	511	Germany 2005	–	GQ289208	–	–	Co-David et al. (2009)
"*R. caelata*"	6919 TJB[d]	North Carolina, USA 1992	–	–	KC816933	KC816843	Kluting et al. (2014)
R. caelata	J. Parkin[d]	Ontario, Canada 1988	–	–	KC816934	–	Kluting et al. (2014)
R. caelata	REH3569[d]	Jurmala, Latvia 1982	–	–	KC816932	–	Kluting et al. (2014)
R. caelata	TB5890[d]	–	–	AF261282	–	–	Moncalvo et al. (2002)
"*R. caelata*"	TB6995[d]	–	–	GU384625	GU384652	–	Baroni et al. (2011)
R. cistetorum	KATO 4260[u]	Trabzon, Turkey 2019	NR_176724	MT252601	–	–	Sesİİ (2021)
R. collybioides	10417 TJB[d]	Jujuy, Argentina 2011	–	–	KC816935	KC816844	Kluting et al. (2014)
R. dominicana	ANGE 464	Dominican Republic 2014	–	–	MN893322	MN893332	Baroni et al. (2020)
R. dominicana	ANGE 473	Dominican Republic 2014	–	–	MN893323	MN893333	Baroni et al. (2020)
R. formosa	1061015-6[d]	Catalonia, Spain 2006	KU862856	–	KC816939	KC816849	Kluting et al. (2014)
R. formosa	12/198	Latium, Italy 2012	KU862857	–	–	–	Vizzini et al. (2016)
R. formosa	12/208	Latium, Italy 2012	KU862858	–	–	–	Vizzini et al. (2016)
R. formosa	1071101-4[d]	Catalonia, Spain 2007	KU862860	–	KC816947	KC816857	Kluting et al. (2014)
R. formosa	K(M): 158060[f]	England, UK 2006	MZ159381	–	KC816978	KC816885	Direct submission
R. fuliginea	E537[d]	Tasmania, Australia 1999	–	–	KC816940	KC816850	Kluting et al. (2014)
R. fumanellii	HFRG_PC200928_1	Buckinghamshire, UK 2020	MW401761	–	–	–	Aplin et al. (2022)
R. fumanellii	BOLGH_22122001	Tuscany, Italy 2022	OR831361	–	–	–	Direct submission
R. fumanellii	MCVE 29550[z]	Veneto, Italy 2017	MH399225	MH399226	–	–	Vizzini et al. (2018)
R. fusipes	DLK 587[aa]	Amazonas, Brazil 2012	MN306209	–	–	–	Silva-Filho et al. (2020)
R. fusipes	DLK 298[aa]	Amazonas, Brazil 2012	MN306210	–	–	–	Silva-Filho et al. (2020)
R. gemina	GZ 2003-09-14	Belgium 2003	–	–	GQ289277	–	Co-David et al. (2009)
"*R. gemina*"	MEN 2001119	– 2001	–	HM164411	–	–	Morgado et al. (2016)
R. gemina	CBS 604.76[f]	–	–	AF223168	–	–	Vu et al. (2019)
R. gemina	KATO 2658[u]	Turkey 2009	–	KX834269	–	–	Sesİİ and Vizzini (2017)
R. gemina	CBS 482.50[r]	–	EF421110	AF223167	EF421019	KP255478	Baroni et al. (2011)
R. griseoaurantia	CAL 1324[c]	Kerala, India 2011	NR_154435	KX083574	KX083568	–	Hyde et al. (2016)
R. griseonigrella	1081204[ab]	Barcelona, Spain 2008	KU862859	–	–	–	Vizzini et al. (2016)
R. hondensis	6103 TJB[d]	California, US 1988	–	–	KC816941	KC816851	Kluting et al. (2014)
R. incarnata	REH5369	Venezuela 1987	MT254071	–	–	–	Silva-Filho et al. (2020)

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			ITS	LSU	*RPB2*	*TEF1*	
R. indica	CAL 1323[c]	Kerala, India 2013	KX083569	NG_060166	KX083566	–	Hyde et al. (2016)
R. lateritia	Co-David 418	–	–	HM164410	–	–	Morgado et al. (2016)
R. lateritia	E1589[d]	Tasmania, Australia 2002	–	–	KC816942	KC816852	Kluting et al. (2014)
R. luteobrunnea	CAL 1322[c]	Kerala, India 2010	NR_154434	NG_060167	KX083567	–	Hyde et al. (2016)
R. luteocinnamomea	GUA241[d]	Guana Island, UK 1999	–	–	KC816943	KC816853	Kluting et al. (2014)
R. luteocinnamomea var. *fulva*	ANGE 169	Dominican Republic 2013	–	–	MN893324	MN893334	Baroni et al. (2020)
R. matesina	MCVE 29262[z]	Campania, Italy 2012	KY629961	KY629963	–	–	Crous et al. (2017)
R. matesina	MCVE 29261[z]	Campania, Italy 2016	KY629962	KY629964	–	–	Crous et al. (2017)
R. matesina	F3-2	Fnaydek, Lebanon 2018	MZ088085	–	–	–	Sleiman et al. (2021)
"*R. mellea*"	ANGE 893	Dominican Republic 2016	MN784993	–	MN893326	–	Baroni et al. (2020)
"*R. mellea*"	TJB 9823[d]	Belize 2004	MN784994	–	–	–	Baroni et al. (2020)
R. mellea	NYBG815044	Costa Rica 1986	MN784995	–	–	–	Baroni et al. (2020)
"*R. mellea*"	6883 TJB[d]	Florida, USA 1992	–	MG702608	KC816944	KC816854	Kluting et al. (2014)
"*R. mellea*"	TJB 9805[d]	Dominican Republic 2003	MN784992	–	MN893325	–	Baroni et al. (2020)
R. mellea var. *depressa*	FW 08/2019	Brazil 2019	MT408926	OL687341	–	–	Xavier et al. (2022)
R. nuciolens	WTU-F-074620	Washington, USA 2017	OP828513	–	–	–	Direct submission
R. nuciolens	TENN:076696[y]	Washington, USA 2021	ON478246	–	–	–	Direct submission
R. nuciolens	iN147673878	California, USA 2023	OR162504	–	–	–	Direct submission
R. nuciolens	iN147466901	California, USA 2023	OR168848	–	–	–	Direct submission
R. pakistanica	LAH37947[i]	Punjab, Pakistan 2022	OR606543	OR606541	–	–	Khan and Khalid (2024)
R. pakistanica	LAH37948[i]	Punjab, Pakistan 2022	OR606544	OR606542	–	–	Khan and Khalid (2024)
R. pallidogrisea	CORT 013944[d]	Australia –	NR_154437	–	–	–	Direct submission
R. pallidogrisea	118	Tasmania, Australia 2004	–	GQ289216	GQ289283	–	Co-David et al. (2009)
R. pallidogrisea	E652[d]	Tasmania, Australia 1999	–	–	KC816968	KC816875	Kluting et al. (2014)
R. paurii	JM99/233	Uttaranchal, India 1999	–	AY286004	–	–	Moncalvo et al. (2004)
R. paurii	JM99/233-2	Uttaranchal, India 1999	–	–	KC816969	KC816876	Kluting et al. (2014)
R. praesidentialis	MCVE 21991[z]	Italy –	EF679793	–	–	–	Consiglio et al. (2007)
R. pruinosostipitata	MCA1492	Guyana –	–	GU384627	GU384653	–	Baroni et al. (2011)
R. pseudoalutacea	TJB 9466[d]	Dominican Republican 2003	–	–	MN893327	MN893335	Baroni et al. (2020)
R. pseudoalutacea	TJB 9507[d]	Dominican Republican 2003	–	–	MN893328	MN893336	Baroni et al. (2020)
R. pseudopiperita	E1159[d]	Tasmania, Australia 2001	–	–	KC816979	KC816886	Kluting et al. (2014)
R. pseudopiperita	162	Tasmania, Australia 2004	–	GQ289217	GQ289284	–	Co-David et al. (2009)
R. reticulata	E2183[d]	Tasmania, Australia 2005	–	–	KC816980	KC816887	Kluting et al. (2014)
R. rhizogena	5551 TJB[d]	North Carolina, USA 1987	–	–	KC816981	KC816888	Kluting et al. (2014)
R. roseiavellanea	8130 TJB[d]	Louisiana, USA 1996	–	KR869930	KC816982	KC816889	Kluting et al. (2014)
R. roseiavellanea	PBM4056	Tennessee, USA –	MF686525	–	–	–	Direct submission
R. roseiavellanea	ANGE 947	Dominican Republic 2017	–	–	MN893329	MN893337	Baroni et al. (2020)
R. rubrobrunnea	CAL 1387[c]	Kerala, India 2014	KX951452	–	–	–	Crous et al. (2016)
Rhodocybe sp.	DLL9851	New South Wales, Australia 2010	–	–	KC816986	KC816893	Kluting et al. (2014)
Rhodocybe sp.	DLL9846	New South Wales, Australia 2010	–	–	KC816985	KC816892	Kluting et al. (2014)
Rhodocybe sp.	DLL9860	New South Wales, Australia 2010	–	–	KC816987	KC816894	Kluting et al. (2014)
Rhodocybe sp.	DLL9952	New South Wales, Australia 2010	–	–	KC816988	KC816895	Kluting et al. (2014)
Rhodocybe sp.	DLL9957	New South Wales, Australia 2010	–	–	KC816989	KC816896	Kluting et al. (2014)
Rhodocybe sp.	DLL10218	New South Wales, Australia 2011	–	–	KC816990	KC816897	Kluting et al. (2014)
Rhodocybe sp.	DLL10032	Queensland, Australia 2011	–	–	KC816991	KC816898	Kluting et al. (2014)
Rhodocybe sp.	KUN-HKAS 89081[a]	Yunnan, China 2023	MZ675559	MZ675570	MZ681892	MZ681870	He and Yang (2022)
Rhodocybe sp.	MEL:2382939	Palmerston, Australia 2014	KP012803	–	–	–	Direct submission
Rhodocybe sp.	MEL:2382705	Australia 2014	KP012885	–	–	–	Direct submission
Rhodocybe sp.	KS-RE53	New Zealand –	–	MK277733	–	–	Varga et al. (2019)
Rhodocybe sp.	Buyck 99.152	Madagascar –	–	MK278564	–	–	Varga et al. (2019)

Species	Collection or collector no.	Location and year	GenBank accession numbers				References
			ITS	LSU	*RPB2*	*TEF1*	
Rhodocybe sp.	Sulzbacher 340	Brazil –	LT594979	–	–	–	Sulzbacher et al. (2017)
Rhodocybe sp.	Sulzbacher 413	Brazil –	LT594984	–	–	–	Sulzbacher et al. (2017)
Rhodocybe sp.	HFRG_EJ171117_1	Hampshire, UK 2017	MW397197	MW397521	–	–	Aplin et al. (2022)
Rhodocybe sp.	iN130319090	Indiana, USA 2022	OP749482	–	–	–	Direct submission
Rhodocybe sp.	iN129753148	Indiana, USA 2022	OP749140	–	–	–	Direct submission
Rhodocybe sp.	iN130020200	Indiana, USA 2022	OP643320	–	–	–	Direct submission
Rhodocybe sp.	AD5 (TENN)[y]	Tennessee, USA 2011	MF773647	–	–	–	Direct submission
R. stipitata	5523 TJB[d]	Tennessee, USA 1987	–	–	KC816993	–	Kluting et al. (2014)
R. spongiosa	MCA2129		–	GU384628	GU384657	–	Baroni et al. (2011)
R. subasyae	HMJAU56921-1[s]	Jilin, China 2020	MW298803	–	–	–	Sun and Bau (2023)
R. subasyae	HMJAU56921-2[s]	Jilin, China 2020	MW298804	–	–	–	Sun and Bau (2023)
R. subasyae	HMJAU56921-3[s]	Jilin, China 2020	MW298805	–	–	–	Sun and Bau (2023)
R. tugrulii	KATO 3340[u]	Trabzon, Turkey 2014	KX271751	KX271754	–	–	Vizzini et al. (2016)
R. tugrulii	MSNG3938	Italy –	KY945354	–	–	–	Direct submission
R. tugrulii	CORT:14755[d]	New York, USA 2018	MZ322093	–	–	–	Direct submission
R. tugrulii	IMG-7316[d]	New York, USA 2017	MG050105	MG050111	–	–	Direct submission
R. tugrulii	WU-MYC 0010084[b]	Burgenland, Austria 1991	OP363995	–	–	–	Vizzini et al. (2023)
R. tugrulii	WU-MYC 0022202[b]	Niederosterreich, Austria 2002	OP363994	OP363999	OP381082	OP381084	Vizzini et al. (2023)
R. tugrulii	WU-MYC 0006178[b]	Niederosterreich, Austria 1987	–	OP364000	–	–	Vizzini et al. (2023)
R. tugrulii	WU-MYC 0006320[b]	Niederosterreich, Austria 1987	OP363992	OP363997	OP381080	OP381083	Vizzini et al. (2023)
R. tugrulii	WU-MYC 0004222[b]	Niederosterreich, Austria 1984	OP363991	–	–	–	Vizzini et al. (2023)
R. tugrulii	WU-MYC 0003753[b]	Niederosterreich, Austria 1984	OP363993	OP363998	OP381081	–	Vizzini et al. (2023)
R. tugrulii	GB-013 1395	Skaane, Sweden 1983	OP363996	OP364001	–	–	Vizzini et al. (2023)
R. zijinshanensis (H)	**KUN-HKAS 145338[a]**	**Jiangsu, China 2024**	**PQ793171**	**PQ781615**	**PQ788400**	**PQ788406**	**This study**
R. zijinshanensis	**KUN-HKAS 145339[a]**	**Jiangsu, China 2024**	**PQ793172**	**PQ781616**	**PQ788401**	**PQ788407**	**This study**
Lulesia umbrinomarginata	MHHNU 20023-2	Guangdong, China 2023	PP060632	PP059607	PP158704	PP158696	Xiao et al. (2024)
Lulesia orientalis	KUN-HKAS 75548[a]	Hubei, China 2012	MN061333	MN065727	MN148164	MN166275	Jian et al. (2020a)
Clitopilopsis albida	KUN-HKAS 104520[a]	Yunnan, China 2018	MN061336	MN065731	MN148168	MN166279	Jian et al. (2020a)
Clitopilopsis hirneola	MEN 199956	Italy –	KC710132	GQ289211	GQ289278	–	Co-David et al. (2009)

[a] The Cryptogamic Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, China (KUN-HKAS)

[b] Herbarium, Institute of Botany, University of Vienna, Austria (WU)

[c] Central National Herbarium, Kolkata, India (CAL)

[d] State University of New York College at Cortland Herbarium, Cortland, New York, USA (CORT)

[e] The Leiden Herbarium of Naturalis Biodiversity Center, Leiden, Netherland (L)

[f] The Royal Herbarium, Royal Botanic Garden, Kew, Richmond, Surrey, England, UK (KEW)

[g] Associazione Micologica Bresadola, Trento, Italy (AMB)

[h] The Herbarium of Mycology, Jilin Agricultural University, Changchun, Jilin, China (HMJAU)

[i] The Lahore, Institute of Botany, University of the Punjab, Pakistan (LAH)

[j] Herbarium generale del Dipartimento di Biologia Vegetale, Università degli Studi di Torino, Italy (TO)

[k] The Herbarium of Soil and Fertilizer Institute, Sichuan Academy of Agricultural Sciences, Sichuan, China (SAAS)

[l] The Swedish Museum of Natural History, Stockholm, Sweden (S)

[m] The Herbarium of the University of Alcalá, Madrid, Spain (AH)

[n] Jepson Herbarium, University of California, Berkeley, California, USA (JEPS)

[o] The Herbarium of Korea National Arboretum, Gyeonggi-do, Republic of Korea (KH)

[p] New Zealand Fungal Herbarium, Auckland, New Zealand (PDD)

[q] The Herbarium of the Sustainable Development of Biological Resources Laboratory, Chiang Mai University, Thailand (SDBR-CMU)

[r] Centraalbureau voor Schimmelcultures, Utrecht, Netherland (CBS)

[s] The Herbarium of Mycology, Jilin Agricultural University, Changchun, Jilin, China (HMJAU)

[t] The Mycological Herbarium of the Guangdong Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China (GDGM)

[u] Karadeniz Technical University Faculty of Forestry Herbarium, Trabzon, Turkey (KATO)

[v] United States National Arboretum, USDA-ARS, Washington, USA (NA)

[w] The Herbarium of Mahidol University, Nakhon Pathom, Thailand (PBM)

[x] The Calcatta University Herbarium, Kolkata, West Bengal, India (CUH)

[y] The Herbarium of University of Tennessee – Knoxville, Tennessee, USA (TENN)

[z] New Herbarium of Museo di Storia Naturale di Venezia, Venezia, Italy (MCVE)

[aa] Herbário of Instituto Nacional de Pesquisas da Amazônia, Amazonas, Brazil (INPA)

[ab] Herbier of Université de Lille, Lille, France (LIP)

(Newly-generated information of specimens and sequences are in bold).
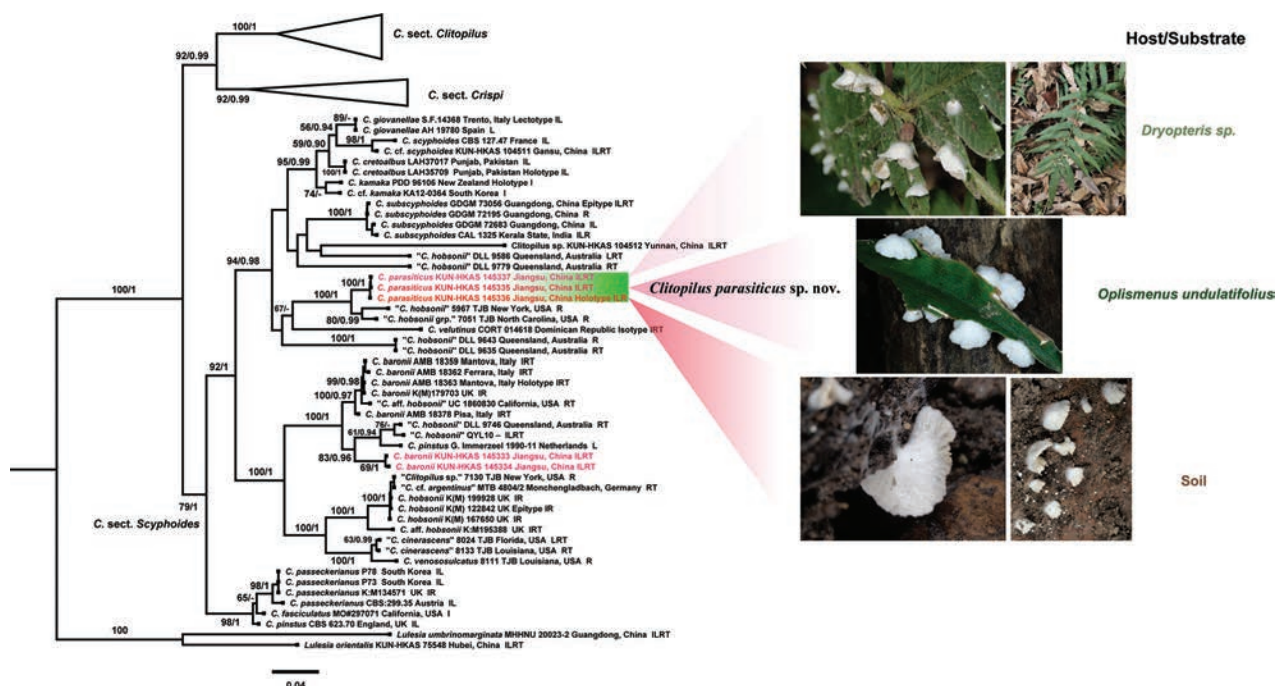
Figure 1. Phylogenetic relationships amongst representative species of *Clitopilus* were inferred from a multigene dataset (ITS-LSU-*RPB2*-*TEF1*) using both ML and BI methods (only shown the ML tree). Supported branches indicate bootstrap supports (BS > 50%) and posterior probabilities (PP > 0.90). Sequences from type specimens (holotype, epitype or isotype) are marked, while new and new record taxa are highlighted in red. The abbreviations ILRT stand for: I = ITS, L = LSU, R = *RPB2* and T = *TEF1*.

Morphological observations and SEM

The images of fresh basidiomata, substrate and habitats of the collected specimens are shown in Fig. 3. Scanning Electron Microscopy revealed that the ornamentation of basidiospores provides some extra valuable information (Fig. 4). The basidiospores of *Clitopilus* exhibit several classical longitudinal ridges (Fig. 4a–f), while those of *Rhodocybe* are characterised by undulate-pustulate walls (Fig. 4g–h). In addition, crystals on the pileus hyphae of the new species in *Clitopilus* was also identified (Fig. 4i).

Taxonomy

Clitopilus parasiticus S.P. Jian, X. Chen & Z.H. Zhang, sp. nov.
MycoBank No: 857348
Figs 1, 3e–h, 4d–f, 5a–c

Holotype. CHINA • Jiangsu Province, Nanjing City, Zijinshan, E 118.83, N 32.08, alt. 32 m, scattered on soil, in the mixed broadleaf (i.e. *Quercus variabilis*, *Robinia pseudoacacia*, *Osmanthus fragrans*, *Broussonetia papyrifera*, *Ilex latifolia* and *Yulania* sp.) forest, 15 August 2024, collected by X. Chen and Z.H. Zhang, CX 966 (KUN-HKAS 145336). GenBank: ITS = PQ793169; LSU = PQ781613; *RPB2* = PQ788398.

　　Etymology. "*parasiticus*" is proposed by its biotrophic behaviour.

　　Diagnosis. *Clitopilus parasiticus* is similar to *C. hobsonii*, but differs by the tomentose pileus, explanate margin and smaller basidiospores.
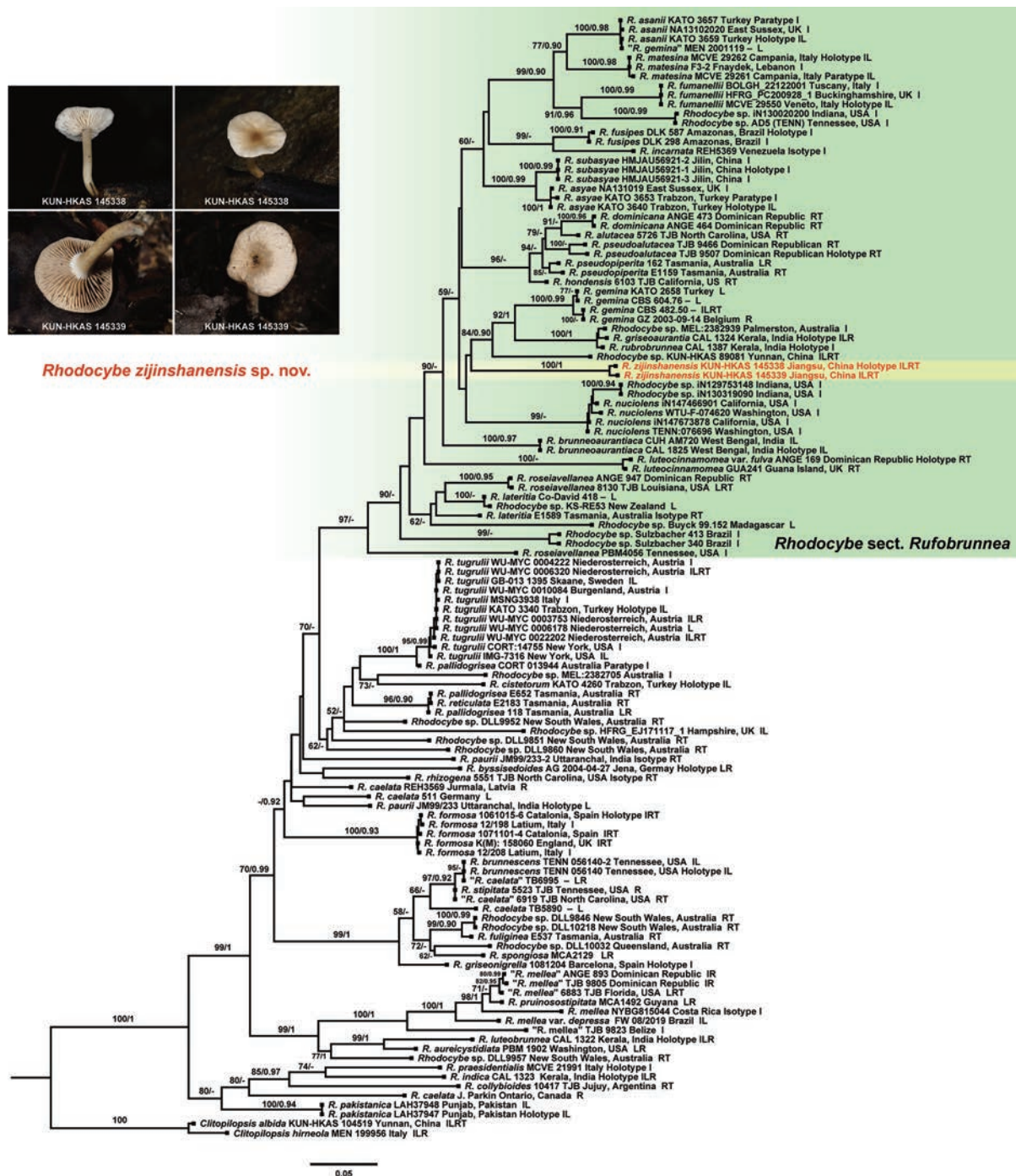
Figure 2. Phylogenetic relationships amongst representative species of *Rhodocybe* were inferred from a multigene dataset (ITS-LSU-*RPB2-TEF1*) using both ML and BI methods (only shown the ML tree). Supported branches indicate bootstrap supports (BS > 50%) and posterior probabilities (PP > 0.90). Sequences from type specimens (holotype, para-type or isotype) are marked, while new and new record taxa are highlighted in red. The abbreviations ILRT stand for: I = ITS, L = LSU, R = *RPB2* and T = *TEF1*.

Description. Basidiomata pleurotoid to conchoid, small size. Pileus 2–8 mm, convex; surface whitish (#b4c4cb) to chalk white (#e3edf3), with fine tomen-tose texture usually extending beyond the margin and densely woolly-tomen-tose at the base; margin typically applanate; context less than 1 mm thick. Lamellae meeting at an excentric point, whitish (#c6d4d3) to yellowish-white (#d3dad4) to yellowish (#dac7ac), slightly dense or crowded, edges entire and
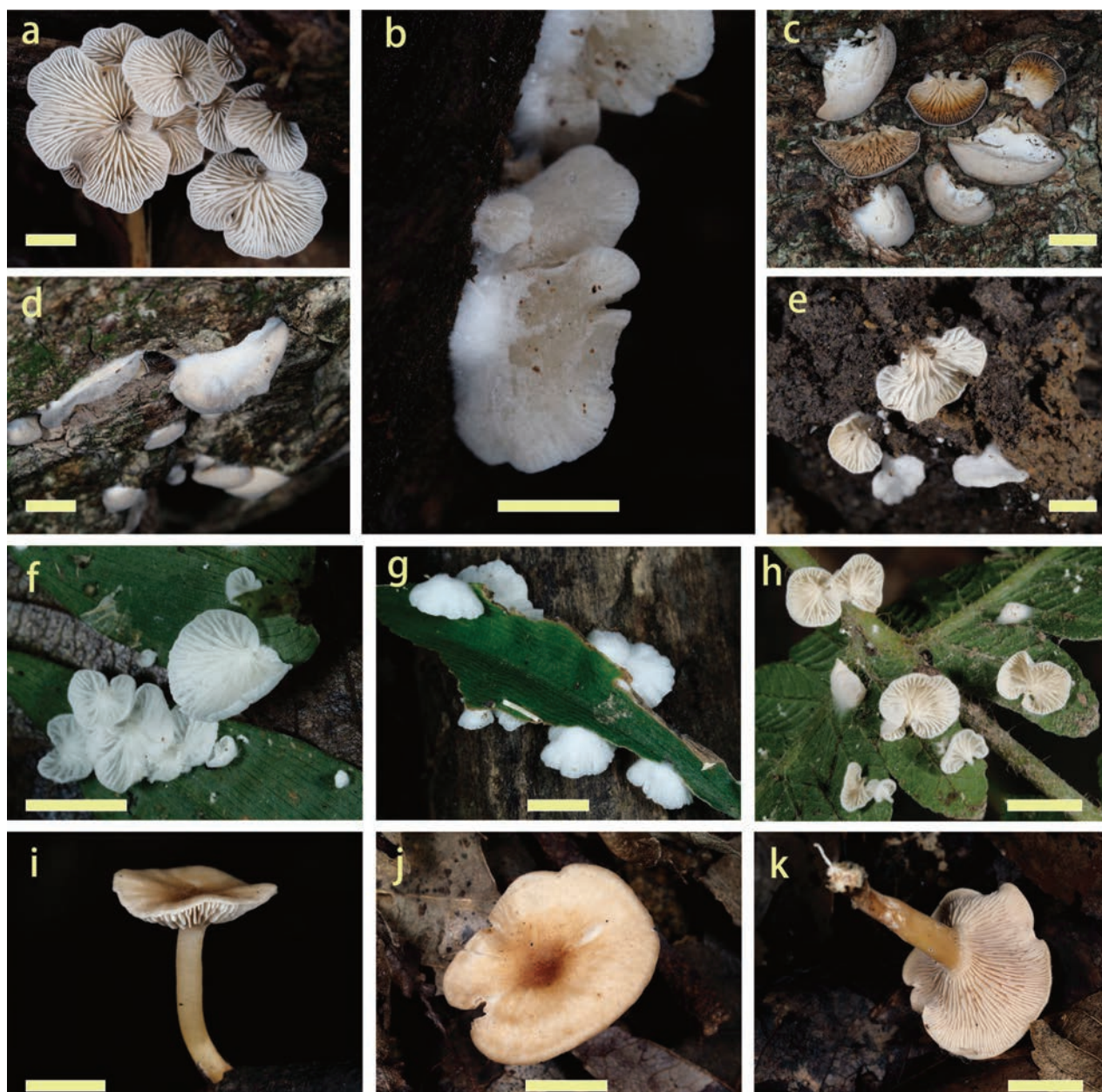
Figure 3. Basidiomata of *Clitopilus* and *Rhodocybe* **a–d** *Clitopilus baronii* (**a, b** KUN-HKAS 145333; **c, d** KUN-HKAS 145334) **e–h** *Clitopilus parasiticus* (**e** KUN-HKAS 145336, holotype; **f, g** KUN-HKAS 145335; **h** KUN-HKAS 145337) **i–k** *Rhodocybe zijinshanensis* (KUN-HKAS 145338, holotype). Scale bar: 5 mm.

concolorous, lamellulae numerous. Stipe absent or very short, eccentric to lateral, measuring 1–2 × 0.2–0.5 mm, concolorous with lamellae. The base with white (#dddddf) mycelium. Odour none.

Basidiospores (5) 5.5–8.5 × 3.5–5.0 (5.5) μm, $L_m × W_m$ = 6.6 (± 0.63) × 4.2 (± 0.34) μm, Q = 1.20–1.90 (Q_{avg} = 1.55 ± 0.13) [186/9/3], hyaline, ellipsoid to broadly fusiform, subovoid in profile and face view, slightly angled in polar view, with 7–9 inconspicuous or obscure longitudinal ridges in total. Basidia 16–23 × 6–9.5 μm, clavate, hyaline, 4-spored, rarely 2-spored; sterigmata 2–3 μm. Lamellar trama subregular, composed of thin-walled, hyaline, cylindrical hyphae with a diameter of 2.5–9 μm. Lamellae edges fertile. Pleurocystidia and cheilocystidia absent. Pileipellis a cutis composed of sparsely arranged, thin-walled,
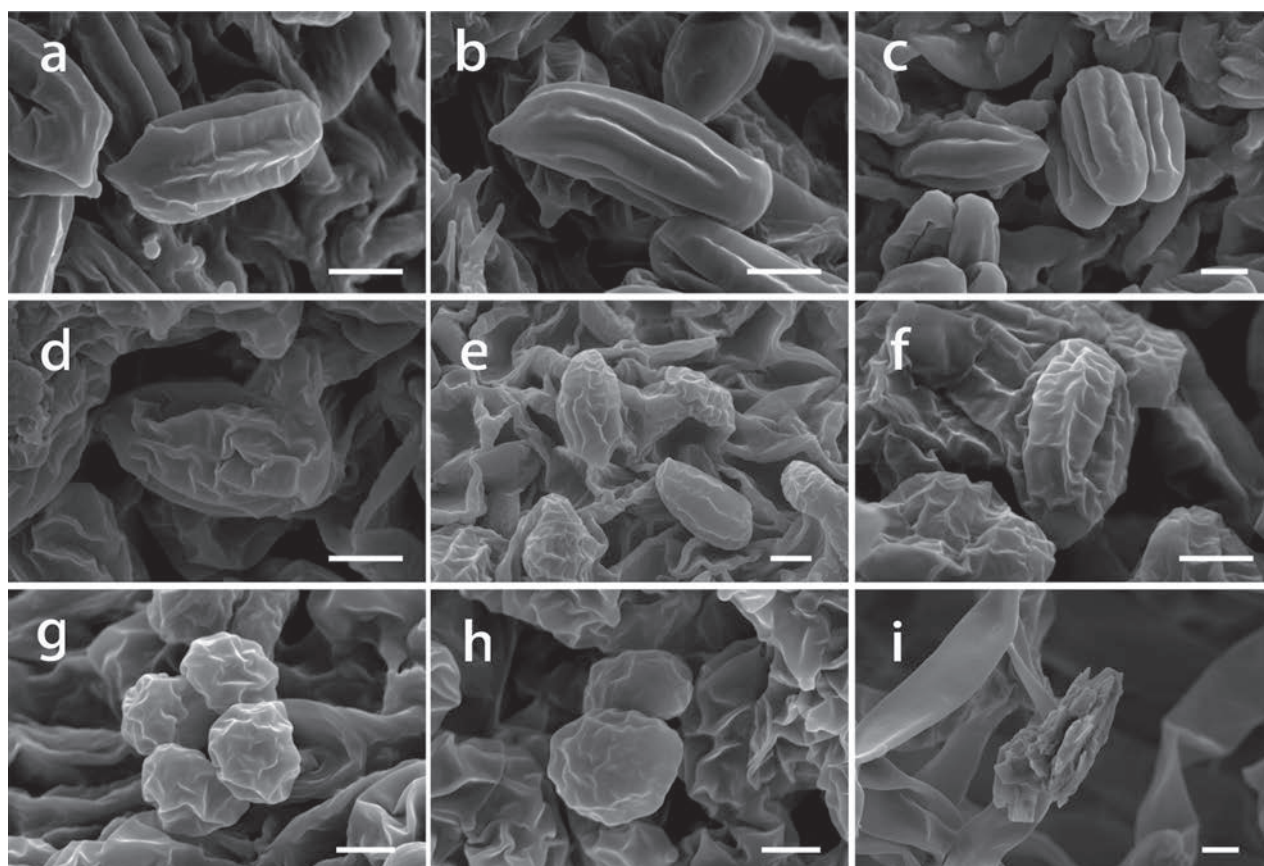
Figure 4. Basidiospores and crystals of *Clitopilus* and *Rhodocybe* reveal by SEM **a–c** *Clitopilus baronii* (KUN-HKAS 145334) **d–f** *Clitopilus parasiticus* (KUN-HKAS 145336, holotype) **g, h** *Rhodocybe zijinshanensis* (KUN-HKAS 145338, holotype) **i** the crystals around the hyphae in pileipellis of *Clitopilus parasiticus* (KUN-HKAS 145336, holotype). Scale bar: 2 μm.

hyaline, smooth, interwoven, cylindrical hyphae with a diameter of 3–5 μm, sometimes featuring erect hyphae; crystals present around the hyphae, square to subsquare, measuring 3 × 3 μm to 14 × 15 μm in area; pileal trama subregular, composed of hyaline, filamentous, thin-walled hyphae, with a diameter of 3–7.5 μm. Clamp connections absent.

Ecology and distribution. Solitary, scattered on soil, lignicolous or gregariously living on leaves of plants (*Dryopteris* sp. and *Oplismenus undulatifolius*) in the mixed broadleaf forest, distributed in Jiangsu Province, China, in August.

Additional specimens examined. CHINA • Jiangsu Province, Nanjing City, Zijinshan, alt. 48 m, dispersedly or gregariously lignicolous or living on twigs or leaves of *Oplismenus undulatifolius*, in the mixed broadleaf (i.e. *Quercus variabilis*, *Quercus aliena*, *Cunninghamia lanceolata*, *Symplocos tanakana*, *Celtis sinensis* and *Ilex cornuta*) forest, 16 August 2023, collected by X. Chen and Z.H. Zhang, CX 628 (KUN-HKAS 145335); same places, alt. 48 m, dispersedly or gregariously living on leaves of *Dryopteris* sp., 16 August 2024, collected by X. Chen and Z.H. Zhang, CX 967 (KUN-HKAS 145337).

Notes. *Clitopilus parasiticus* belongs to *Clitopilus* sect. *Scyphoides* (Fig. 1). This new taxon is similar to *C. hobsonii*, *C. daamsii*, *C. passeckerianus*, *C. pinsitus* and *C. baronii*. *Clitopilus hobsonii* was originally described from Britain and exhibits both saprophytic and parasitic abilities. It resembles *C. parasiticus* in its living habits and the shape of its basidiomata, but differs from the latter by its involute or inflexed margins of the pileus and larger basidiospores ($L_m \times W_m$ = 7.5 × 5 μm)
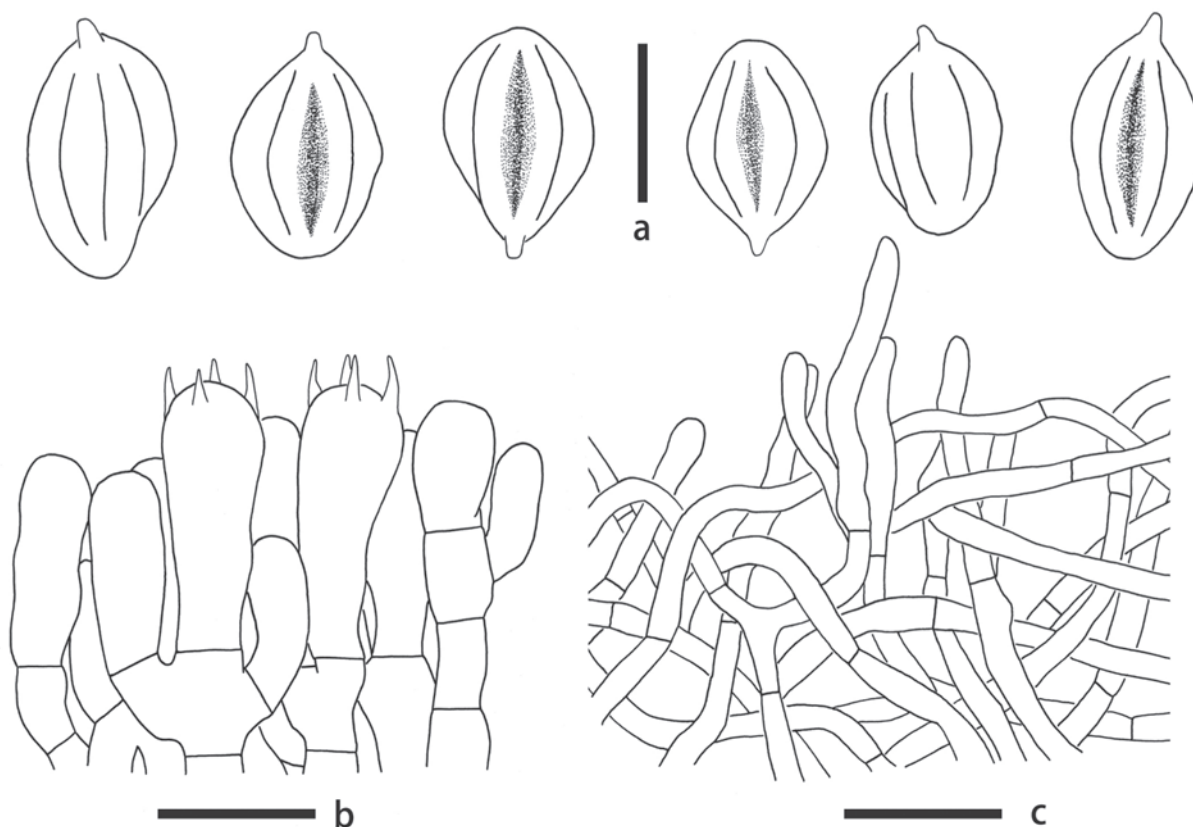
Figure 5. Microscopic features of *Clitopilus parasiticus* (KUN-HKAS 145336, holotype) **a** basidiospores **b** hymenium and subhymenium **c** pileipellis. Scale bars: 5 μm (**a**); 10 μm (**b**); 20 μm (**c**).

(Orton 1960; Noordeloos 1984; Noordeloos 1988). Meanwhile, *C. daamsii* was also similar to *C. parasiticus* in outline; however, it differs due to its xylogenous or mycoparasitic behaviour, involute margin of pileus and larger basidiospores (8–11.5 × 4.8–6.6 μm) (Noordeloos 1984). Another closely-related species is *C. passeckerianus*, which has sessile basidiomata and a white pileus. However, the habit of growing on mushroom-beds, basidiomata size (8–40 mm), the reniform to spathulate shape of the pileus and larger basidiospores (7–9 × 4–5 μm) of *C. passeckerianus* significantly differs from *C. parasiticus* (Pilát 1935; Noordeloos 1993). *Clitopilus pinsitus* was first collected from Sweden and was found growing on the trunk of *Quercus*. This species is characterised by its spatulate, white pileus (15–40 mm) and ellipsoidal to amygdaliform basidiospores (7.5–9 × 4.6–5.3 μm) with 7–8 obscure longitudinal ridges (Josserand 1937; Singer 1946a). Lastly, *C. baronii*, recently described by Consiglio and Setti (2019) in Marmirolo, Italy, resembles *C. parasiticus*, but can be differentiated by its larger pileus (5–40 mm) and basidiospores ($L_m × W_m$ = 7.6 × 5.0 μm), as well as its lageniform cheilocystidia.

***Clitopilus baronii* Consiglio & Setti, Index Fungorum 427: 1. 2019.**
Figs 3a–d, 4a–c, 6a–c

Description. Basidiomata pleurotoid to crepidotoid, small size. Pileus 3–15 mm wide, convex then expanded; surface yellowish-white (#9a8a7a), greyish (#a6a39f) to bluish-grey (#6a757b), usually subtly woolly-tomentose

at the base then reduced to border; margin slightly incurved, even, sometimes faintly striated; context less than 1 mm thick. Lamellae whitish (#a9a7a8) to yellowish (#9d896d), sometimes hygrophanous, slightly dense or crowded, edges entire and concolorous, lamellulae numerous. Stipe absent; the base with white (#e9ebed) mycelium. Odour none.

Basidiospores (6) 6.5–9.5 (11) × 4–5 (5.5) µm, $L_m × W_m$ = 7.5 (± 1.01) × 4.5 (± 0.35) µm, Q = 1.4–1.98 (Q_{avg} = 1.66 ± 0.14) [43/2/2], hyaline, ellipsoid to fusiform, subovoid in profile and face view, slightly angled in polar view with 8–10 inconspicuous or obscure longitudinal ridges in total. Basidia 17.5–24 × 6.5–9 µm, clavate, hyaline, 2- or 4-spored; sterigmata 3–5.5 µm. Lamellar trama subregular, composed of thin-walled, hyaline, cylindrical hyphae with a diameter of 2.5–9 µm. Lamellae edges fertile. Pleurocystidia and cheilocystidia absent, but occasionally forming a few cylindrical tramal hyphae with a diameter of 2–3 µm over the edge. Pileus context about 150–200 µm thick. Pileipellis a cutis composed of compactly arranged, thin-walled, hyaline, smooth, cylindrical hyphae with a diameter of 3.5–9 µm, featuring sparely arranged and erect hyphae with a diameter of 2–3 µm; pileal trama subregular or irregular, composed of hyaline, filamentous, thin-walled hyphae, with a diameter of 2.5–8.5 µm. Clamp connections absent.

Ecology and distribution. Lignicolous, scattered or gregarious on rotten wood in the mixed broadleaf forest, distributed in Jiangsu Province, China, in May.

Additional specimens examined. CHINA • Jiangsu Province, Nanjing City, Zijinshan, alt. 42 m, scattered or gregarious on rotten wood (*Quercus* sp.), in the mixed broadleaf (i.e. *Quercus acutissima*, *Quercus aliena*, *Celtis sinensis*, *Liquidambar formosana* and *Cunninghamia lanceolata*) forest, 7 May 2023, collected by X. Chen, CX 119 (KUN-HKAS 145333); same places, alt. 38 m, scattered on rotten wood (*Quercus* sp.), in the mixed broadleaf (*Quercus glauca*, *Pterocarya stenoptera*, *Ilex chinensis*, *Cunninghamia lanceolata*, *Ilex cornuta*, *Liquidambar formosana* and *Ligustrum lucidum*) forest, 9 May 2023, collected by X. Chen, CX 134 (KUN-HKAS 145334).

Notes. *Clitopilus baronii* belongs to *C.* sect. *Scyphoides* (Fig. 1). In the original description, this species was found growing on a decaying trunk of *Quercus* sp. It is characterised by its sessile basidiomata, orbicular to conchate white pileus, cream-rose lamellae, ellipsoidal to subamygdaliform basidiospores with 8–10 obscure longitudinal ridges and lageniform cheilocystidia. The macro- and microscopic features of our specimens (KUN-HKAS 145333 & 145334) closely match those described in the primary literature (Consiglio and Setti 2019). However, we did not observe any lageniform cheilocystidia in our specimens; we only identified a few thin cylindrical tramal hyphae over the edge. This observation aligns with findings by Noordeloos (1984) regarding *C. daamsii*, particularly in some older specimens. In our previous study, we also noted this phenomenon of thin cylindrical tramal hyphae at the edge in *C. crispus* Pat. However, this occurrence was generally casual and rare.

In the phylogenetic tree of *Clitopilus*, we could discover some unusual results regarding *C. baronii*. In the combined multigene analyses (ITS-LSU-*RPB2*-*TEF1*), our specimens were found to separate from the clades of *C. baronii* and grouped (BS/PP = 69/1) closer to *C. pinstus* (G. Immerzeel 1990-11). When we compared the different genes separately between our samples and holotype of *C. baronii* (AMB 18363), we found that our samples exhibited over 99% similarity in ITS region. However, the similarity was only about 90% for both *RPB2*
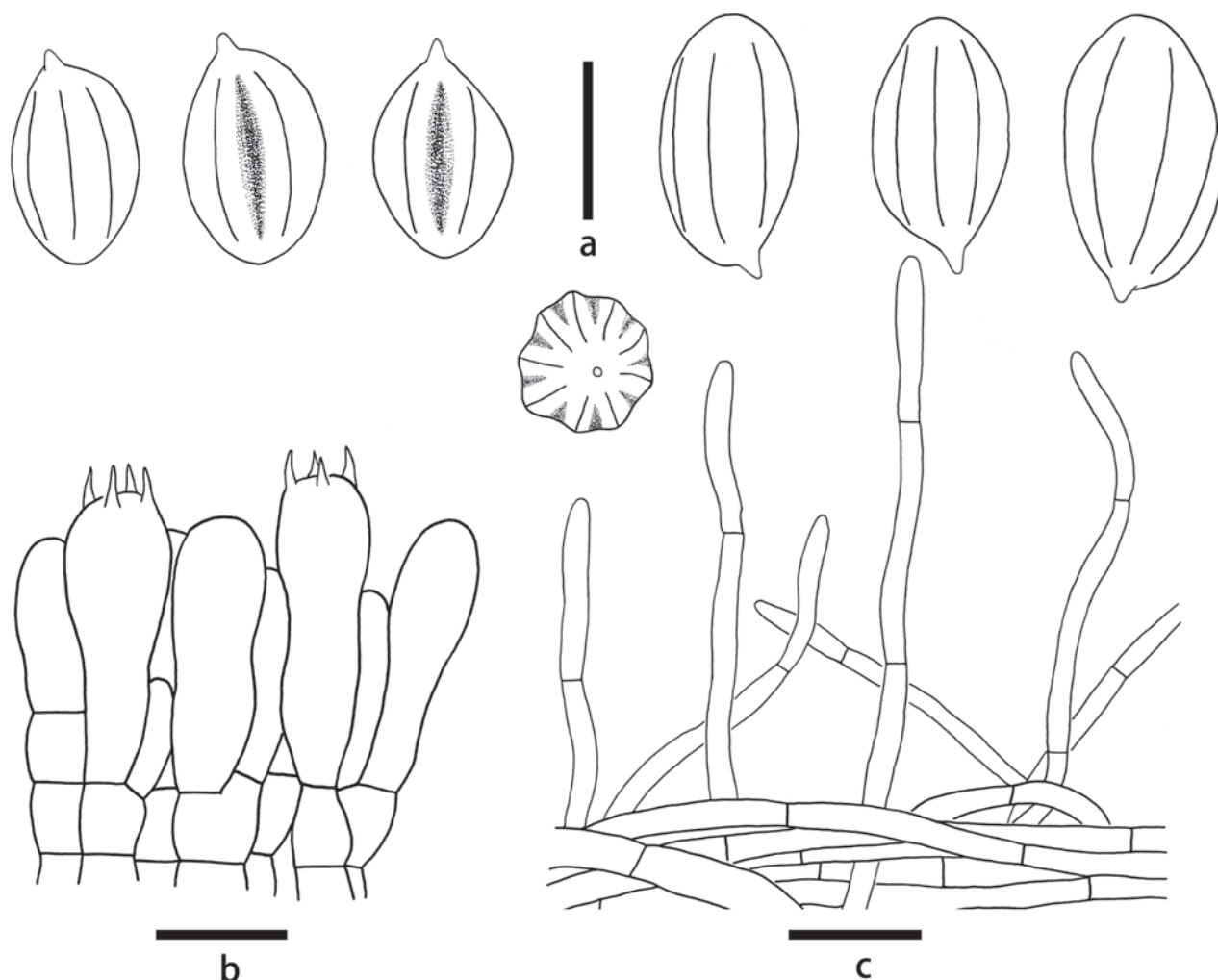
Figure 6. Microscopic features of *Clitopilus baronii* (KUN-HKAS 145334) **a** hymenium and subhymenium **b** basidiospores **c** pileipellis. Scale bars: 5 μm (**a**); 10 μm (**b**); 20 μm (**c**).

and *TEF1*. For ITS, we have tested them several times in different companies, but all yielded consistent results. Regarding *RPB2* and *TEF1*, we did not detect any issues with the original data; all sequences were bidirectionally sequenced to ensure unimodality and were matched by hand in software. Considering the macro- and microscopic features, we tentatively classified our specimens as *Clitopilus baronii*. More samples are needed to resolve our uncertainties regarding both the presence of cheilocystidia and the phylogenetic relationship.

Rhodocybe zijinshanensis S.P. Jian & X. Chen, sp. nov.

MycoBank No: 857349

Figs 2, 3i–k, 4g–h, 7a–c

Holotype. CHINA • Jiangsu Province, Nanjing City, Zijinshan, E 118.87, N 32.06, alt. 99 m, solitary on rotten wood, in mixed broadleaf (i.e. *Quercus acutissima*, *Quercus aliena*, *Aphananthe aspera*, *Osmanthus fragrans*, *Liquidambar formosana*, *Photinia serratifolia* and *Ilex chinensis*) forest, 30 August 2024, collected by X. Chen, CX 664 (KUN-HKAS 145338). GenBank: ITS = PQ793171; LSU = PQ781615; *RPB2* = PQ PQ788400; *TEF1* = PQ788406.
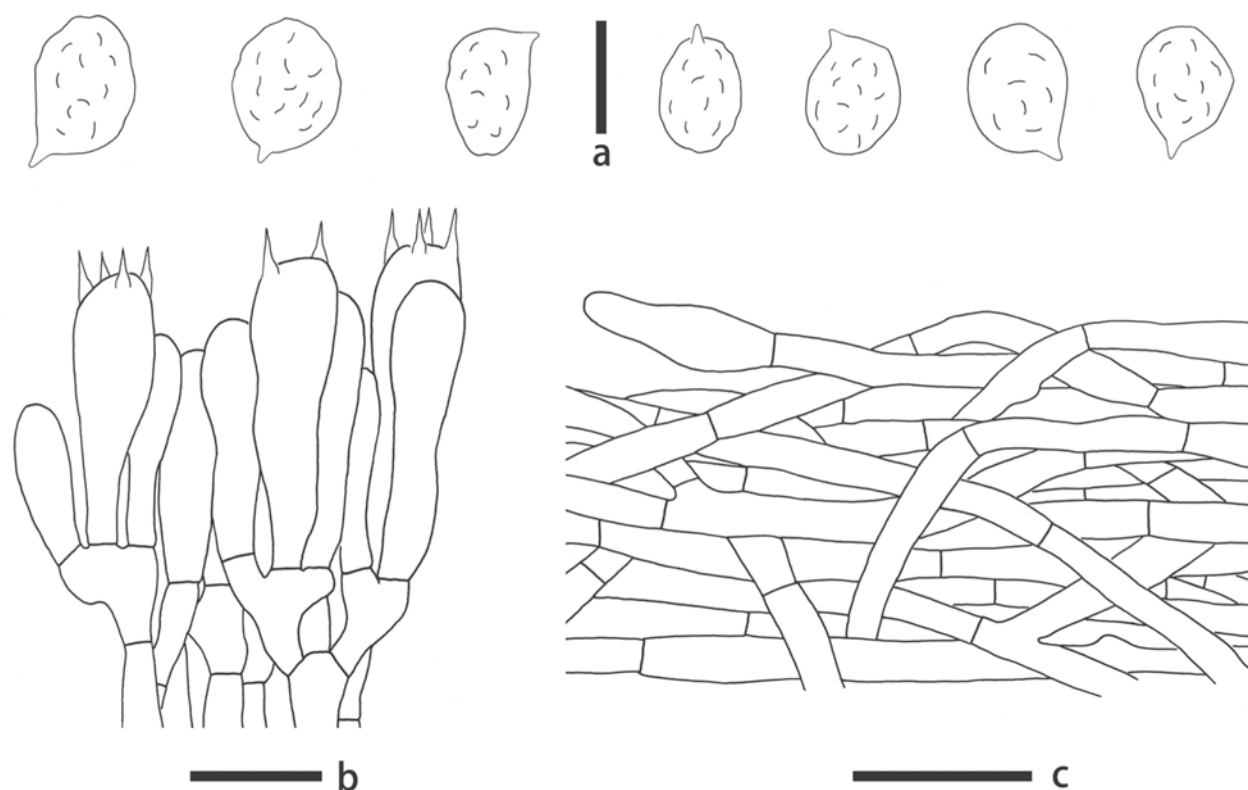
Figure 7. Microscopic features of *Rhodocybe zijinshanensis* (KUN-HKAS 145338, holotype) **a** hymenium and subhymenium **b** basidiospores. Scale bars: 5 μm (**a**); 10 μm (**b**); 20 μm (**c**).

Etymology. "*zijinshanensis*" indicates the source place, where it was located in Nanjing City, China.

Diagnosis. *Rhodocybe zijinshanensis* is similar to *R. subasyae*, but differs by its smaller yellow pileus, shorter and more slender stipes and the absence of cheilocystidia.

Description. Basidiomata omphalioid, small size. Pileus 10–15 mm wide, applanate to plano-concave; surface yellow (#eac7a2) over edge and brownish-yellow (#6c3620) over disc, distributing some radiate fibrillose, sometimes hygrophanous; margin slightly inflexed, even or undate; context about 1 mm thick. Lamellae adnate to subdecurrent, yellowish (#cdbead) to greyish-pink (#d1b4a2), dense or crowded, edges entire or undate, sometimes with transverse intervenose, concolorous with lamellae, lamellulae numerous. Stipe 7–19 × 1–2 mm, central to eccentric, cylindrical to tapering downwards, usually concolorous with pileus, densely fine scales dispersed around the top. Odour none.

Basidiospores (4.5) 5–6.5 × 3.5–5 μm, $L_m × W_m$ = 5.5 (± 0.54) × 4.3 (± 0.31) μm, Q = 1.09–1.55 (Q_{avg} = 1.28 ± 0.11) [41/2/2], hyaline, subglobose, subamygdaliform to broadly ellipsoid in profile view, ellipsoid in face view and minutely, but obviously angular in polar view (7–9 facets in total), undulate-pustulate in all views. Basidia 18.5–32 × 5.5–7.5 μm, clavate, hyaline, 2- or 4-spored; sterigmata up to 5 μm long. Lamellar trama regular, composed of 2.5–10.5 μm in diam., thin-walled, hyaline hyphae. Lamellae edges fertile. Pleurocystidia and cheilocystidia absent. Pileipellis a cutis composed of radially arranged, subregular hyphae, hyphae thin-walled, yellowish, smooth, cylindrical, 3.5–11.5 μm in diam., sometimes with oleiferous hyphae; pileal trama regular, composed of hyaline, thin-walled, cylindrical hyphae with a

diameter of 2−11 μm. Stipitipellis a cutis composed of compactly arranged, regular, thin-walled and hyaline hyphae with a diameter of 3.5−9.5 μm; Stipe trama regular, composed of thin-walled and hyaline hyphae with a diameter of 4−10.5 μm. Caulocystidia absent. Clamp connections absent.

Ecology and distribution. Solitary on rotten wood in broad-leaved forest, only found in Jiangsu Province, China, August to October.

Additional specimens examined. Cʜɪɴᴀ • Jiangsu Province, Nanjing City, Zijinshan, E 118.87, N 32.06, alt. 99 m, solitary on rotten wood, in mixed broadleaf (i.e. *Quercus acutissima*, *Quercus aliena*, *Aphananthe aspera*, *Osmanthus fragrans*, *Liquidambar formosana*, *Photinia serratifolia* and *Ilex chinensis*) forest, 30 August 2024, collected by X. Chen, CX 665 (KUN-HKAS 145339).

Notes. *Rhodocybe zijinshanensis* belongs to *R.* sect. *Rufobrunnea* (Fig. 2). Species in this section are characterised by centrally stipitate basidiomata, pilei ranged from pinkish, reddish, brown, tan to fulvous (but never greyish or white), lamellae that are adnexed to adnate or decurrent, the absence of hymenial pseudocystidia and clamp connections (Baroni 1981). *Rhodocybe zijinshanensis* is similar to several other species, including *R. asyae* Seslı & Vizzini, *R. subasyae* T. Bau & Y.L. Sun, *R. pseudoalutacea* T.J. Baroni et al. and *R. alutacea* Singer. *Rhodocybe asyae*, first recorded in Turkey, can be differentiated from *R. zijinshanensis* by its relatively larger, smooth pileus (10−30 mm), longer and thicker stipe (25−30 × 2−5 mm) and flexuous cheilocystidia (20−30 × 4−6 μm) (Seslİ and Vizzini 2017). *Rhodocybe subasyae*, a recently described species from Jilin, China, is also similar to *R. zijinshanensis*, but differs in having a smooth pileus, a longer and thicker stipe (22−37 × 5−7 mm), slightly larger basidiospores (Q_{avg} = 1.4), and cheilocystidia measuring 22.4−28.2 × 3.9−6.8 μm (Sun and Bau 2023). For *R. pseudoalutacea*, it was reported from the Dominican Republic, featured by its slightly larger pileus (10−35 mm), slender yet thick stipe (15−50 × 2−6 mm) and pileipellis composed of finely encrusted cylindrical hyphae (Baroni et al. 2020). The last species which resembled *R. zijinshanensis* was *R. alutacea*, found in Florida, USA. It is characterised by the greater pileus size (25−35 mm), a longer stipe (23−35 × 2.5−5.5 mm), and septate, flexuous cheilocystidia (20−35 × 6.5−7 μm) (Singer 1946b; Baroni 1981).

Discussion

In this study, we described two new species and documented a new record species in China: *C. parasiticus*, *R. zijinshanensis* and *C. baronii*. For the phylogenetic analysis, we utilised nearly all available sequences for the genera *Clitopilus* and *Rhodocybe*, uploaded by classified references or expert researchers (see Fig. 1). The phylogenetic tree indicates that *C. parasiticus* is closely related to *C. velutinus* T. J. Baroni & Angelini, which was discovered in the Dominican Republic. However, it can be distinguished by its larger pileus (10−25 mm), the existence of an eccentric stipe and larger basidiospores (L_m × W_m = 8.0 × 5.0 μm) with more longitudinal ridges (10−14) (Baroni et al. 2020). Similarly, *C. baronii* is closer to *C. venososulcatus* Singer, which is occurring only in Florida, USA. Nonetheless, the latter typically exhibits a larger, venose and sulcate pileus (12−23 mm) and slightly larger basidiospores (8−8.5 × 4.5−5 μm) with 6−8 obscurely longitudinal ridges (Singer 1946a). Finally, *R. zijinshanensis* approaches

to *R. nuciolens* (Murrill) Singer and *R. gemina* (Paulet) Kuyper & Noordel., but the larger size of their basidiomata (particularly in the pileus and stipe) and the presence of cheilocystidia make them easy to distinguish from the former (Baroni 1981; Seslİ and Vizzini 2017; Vizzini et al. 2018). The similar species of above species are compared in Table 3.

In the family Entolomataceae Kotl. & Pouzar, there are over 1500 described species worldwide (Co-David et al. 2009; Baroni and Matheny 2011; Karstedt et al. 2019). However, only a few species exhibit mycoparasitic capabilities, like *Entoloma abortivum* (Berk. & M.A. Curtis) Donk, *E. parasiticum* (Quél.) Kreisel, *E. pseudoparasiticum* Noordel. and *Rhodophana stangliana* (Bresinsky & Pfaff) Vizzini (Noordeloos 1988; Læssøe and Rosendahl 1994; Czederpiltz et al. 2001). Thereinto, *Entoloma abortivum* is frequently reported to co-occur with *Armillaria* (Fr.) Staude, leading to the hypothesis that *Armillaria* attacks and parasitises the basidiomata of *Entoloma abortivum* (Watling 1974). On the contrary, Czederpiltz et al. (2001) demonstrated that *E. abortivum* can actually abort the growth of Armillaria in culture media. Furthermore, Koch and Herr (2021) explained this phenomenon using transcriptomics.

Notably, some species, such as *E. clypeatum* (L.) P. Kumm., *E. niphoides* Romagn. ex Noordel., *E. saepium* (Noulet & Dass.) Richon & Roze and *E. sericeoides* (J.E. Lange) Noordel., have been reported to associate with rosaceous woody plants. However, these species are more likely to be detrimental to roots rather than forming typical mycorrhizae (Agerer and Waller 1993; Gryndler et al. 2010; Shishikura et al. 2020). In the *Rhodocybe-Clitopilus* clade, *C. daamsii* has been observed growing on *Hydnoporia tabacina* (Sowerby) Spirin et al. (previously classified as *Hymenochaete tabacina* (Sowerby) Lév.), while *C. passeckerianu* and *C. fasciculatus* have been associated with the growing-beds of cultivated *Agaricus* L. (Noordeloos 1984; Noordeloos 1988; Noordeloos 1993), although Singer questioned the mycoparasitic behaviour of *C. passeckerianu* (Singer and Harris 1987).

To investigate the saprophytic and biotrophic abilities of *C. parasiticus*, we carefully examined different specimens to identify the discrepancies between various hosts and growth on soil. The results are presented in Table 4. We found that the basidiospores from specimens KUN-HKAS 145335 and 145337, which were collected from the leaves of *Oplismenus* sp. and *Dryopteris* sp., respectively, showed no significant differences. However, there was an obvious difference with specimen KUN-HKAS 145336, where the basidiospores of *C. parasiticus* growing on soil were larger than those from specimens growing on plant leaves. Larger basidiospores often indicate more robust growth of basidiomata (Kauserud et al. 2011; Halbwachs et al. 2017), suggesting that this species may be better suited to a soil habitat than to a biotrophic lifestyle.

Furthermore, the average temperature over a fortnight in 2024 was slightly higher than in 2023, while the average precipitation during the same period was slightly lower in 2024 compared to 2023. These subtle discrepancies could influence the nutritional mode and even the choice of parasitic host. Admittedly, our judgement that this species is biotrophic on the basis of only two collections from different plant leaves, is not entirely rigorous. More experiments, including physiological and genomic analyses, are necessary for a comprehensive assessment.

Table 3. The comparison of morphological characters amongst *C. parasiticus*, *C.baronii*, *R. zijinshanensis* and similar species.

Taxa	Badisiomata	Pileus	Basidiospores (ridges)	Hymenial cystidia	Habitat	Locality	References
Clitopilus sect. *Scyphoides*							
C. baronii (Holotype)	Orbicular to conchate or spatulate, sessile	5–40 mm, white to greyish	6.9–8.4 × 4.4–5.5 µm (8–10), Q = 1.68–1.71	Cheilocystidia lageniform	On a decaying trunk of *Quercus* sp.	Italy	Consiglio and Setti (2019)
C. baronii	**Conchate, sessile**	**3–15 mm, white to greyish**	**6.5–9.5 × 4–5 µm (8–10), Q = 1.4–1.98**	**None**	**On rotten wood**	**China**	**This study**
C. daamsii (Holotype)	Orbicular to conchate, sessile	2–8 mm, white	8–11.5 × 4.8–6.6 µm (6–9), Q = 1.4–2	None	On wood or other fungi	Netherlands	Noordeloos (1984)
C. fasciculatus (Holotype)	Fasiculata, sessile	Individual 24 × 20 mm, pale brown	4.7–6.3 × 3.0–3.5 µm (3–6), Q = 1.2–1.85	None	On beds of cultivated mushrooms	Netherlands	Noordeloos (1984)
C. hobsonii (Holotype)	Orbicular or slightly reniform, sessile	5–18 mm, white to pale greyish	6.5–9 × 4–5.5 µm (7–12), Q = 1.2–2	None	On plant debris or herbaceous stems	Britain	Orton (1960)
C. parasiticus **(Holotype)**	**Conchate, sessile**	**2–8 mm, whitish to chalk white**	**5.5–8.5 × 3.5–5 µm (7–9), Q = 1.2–1.9**	**None**	**On soil, rotten wood and leaves of plants**	**China**	**This study**
C. passeckerianus (Holotype)	Reniform or resembling an ear, sessile	8–40 mm, white	7–9 × 4–5 µm (7–12), Q = 1.45–2.25	None	On mushroom-beds	Europe	Pilát (1935)
C. pinsitus (Holotype)	Spatulate, semi-cicular, sessile	15–40 mm, white to pale ochre	7–9 × 4.6–5.3 µm (7–8)	None	On trunk of *Quercus* sp.	Sweden	Josserand (1937)
C. velutinus (Holotype)	Clitocyboid	10–25 mm, pure white	7–9 × 5–6 µm (7–8), Q = 1.27–1.8	None	On soil	Dominican Republic	Baroni et al. (2020)
C. venososulcatus (Holotype)	Pleurotoid, sessile or sub sessile	12–23 mm, pallid white	8–8.5 × 4.5–5 µm (6–8)	None	On trunks or logs of Ficus aurea	USA	Singer (1946a)
Rhodocybe sect. *Rufobrunnea*							
R. alutacea (Holotype)	25–35 mm, yellowish, hygrophanous	23–35 × 2.5–5.5 mm, subequal	5.8–7.5 × 3.5–5 µm (7–9)	Cheilocystidia	On sandy soil and fallen leaves	USA	Singer (1946b)
R. asyae (Holotype)	10–30 mm, salmon pink	25–30 × 2–5 mm, tapering	5–7 × 4–5 µm, Q = 1.1–1.4	Cheilocystidia	On the grass	Turkey	Seslİ and Vizzini (2017)
R. gemina	15–80 mm, reddish incarnate	25–50 × 3–15 mm, subequal	5–6.5 × 4–5 µm	Cheilocystidia	On humus	Europe	Baroni (1981)
R. nuciolens	10–60 mm, pinkish cinnamon, hygrophanous	35–80 × 2–9 mm, equal	5.5–8 × 4–5 µm	Cheilocystidia	On humus, sandy soil or decaying wood	USA	Baroni (1981)
R. pseudoalutacea (Holotype)	10–35 mm, brown or brownish orange, hygrophanous	15–50 × 2–6 mm, equal or enlarged downwards	5.5–7 × 4–5 µm (7–10), Q = 1.2–1.6	None	On decaying humus or woody debris	Dominican Republic	Baroni et al. (2020)
R. subasyae (Holotype)	19–25 mm, beige red	22–37 × 5–7 mm, cylindrical	5.4–6.8 × 3.9–4.9 µm (6–8), Q = 1.2–1.6	Cheilocystidia	On sandy soil	China	Sun and Bau (2023)
R. zijinshanensis **(Holotype)**	**10–15 mm, yellow, hygrophanous**	**7–19 × 1–2 mm, cylindrical to tapering**	**5–6.5 × 3.5–5 µm (7–9), Q = 1.09–1.55**	**None**	**On rotten wood**	**China**	**This study**

Table 4. The intraspecies comparison of *C. parasiticus* in morphological characters and microenvironment.

Taxa	Voucher specimen	Pileus	Basidiospores (ridges)	Crystals in pileipellis	Habitate	Temp. (°C)	Prec. (mm/d)
C. parasiticus	KUN-HKAS145335 (CX628)	2–8 mm	5.5–7.0 × 4–5.5 µm, $L_m × W_m$ = 6.3 (± 0.47) × 4.24 (± 0.35) µm, Q = 1.20–1.84 (Q_{avg} = 1.49 ± 0.13) (8–9) [63/3/1]	None	On leaves of *Oplismenus undulatifolius*	29.04	5.58
C. parasiticus (Holotype)	KUN-HKAS145336 (CX966)	3–7 mm	6.0–8.5 × 4–5 µm, $L_m × W_m$ = 7.06 (± 0.6) × 4.40 (± 0.30) µm, Q = 1.40–1.81 (Q_{avg} = 1.61 ± 0.10) (7–8) [62/3/1]	Present	On soil	31.25	4.43
C. parasiticus	KUN-HKAS145337 (CX967)	3–5.5 mm	5.5–7.5 × 3.5–5 µm, $L_m × W_m$ = 6.33 (± 0.50) × 4.09 (± 0.28) µm, Q = 1.20–1.90 (Q_{avg} = 1.55 ± 0.13) (7–9) [61/3/1]	Present	On leaves of *Dryopteris* sp.	31	4.42

Notes: Temp./Prec. means the average temperatures/precipitations one week before and one week after the date of collection.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Sipeng Jian conceived, designed and completed the experiments under the guidance of Chunxia Zhang. Xia Chen, Yiwei Fang and Tianwei Yang helped to collect samples, use and adjust the microscope, with some photographs. Xinjing Xu, Jing Liu and Feng Gao assisted with extracting DNA and PCR amplification. Sipeng Jian wrote the manuscript and Chunxia Zhang revised it.

Author ORCIDs

Sipeng Jian https://orcid.org/0000-0002-2055-3169
Xia Chen https://orcid.org/0009-0002-1239-1865

Data availability

In this study, DNA sequences have been deposited in GenBank. Specimens were placed at Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN-HKAS).

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MycoKeys

Research Article

Two new species of *Penicillium* (Eurotiales, Aspergillaceae) from China based on morphological and molecular analyses

Rui-Na Liang[1,2], Xiang-Hao Lin[1], Miao-Miao An[1], Guo-Zhu Zhao[1,2]

1 College of Biological Sciences and Technology, Beijing Forestry University, Beijing 100083, China
2 National Engineering Research Center of Tree Breeding and Ecological Restoration, Beijing Forestry University, Beijing 100083, China
Corresponding author: Guo-Zhu Zhao (zhaogz@bjfu.edu.cn)

Abstract

Penicillium is a large and significant genus of fungi, exhibiting widespread distribution across diverse substrates. Ongoing taxonomic and nomenclatural revisions have led to an annual increase in the number of newly described species. This study described two new *Penicillium* species, i.e., *P. lentum* and *P. tibetense*, discovered in China. They have been identified and characterized through morphological examination and both single gene and multigene phylogenetic analyses. Based on these analyses, *P. lentum* was classified within the section *Brevicompacta*, while *P. tibetense* was placed in the section *Lanata-Divaricata*. Both species exhibited the morphological features typical of their respective sections. *Penicillium lentum* is characterized by restricted growth with dense colonies on agar media and predominantly generates terverticillate conidiophores. *Penicillium tibetense* demonstrates rapid growth on media and has vigorous growth on CYA at 30 °C, producing biverticillate conidiophores. Comprehensive descriptions and detailed illustrations of these new species were presented. A morphological comparison between the new species and their closely related taxa was provided.

Key words: Aspergillaceae, DNA barcodes, section *Brevicompacta*, section *Lanata-Divaricata*, taxonomy

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Introduction

Penicillium is widely distributed across various substrates, primarily in soil, as well as in the atmosphere, food, plant tissues, and other environments. Several species possess considerable value for human applications in food production, biocontrol, and biotechnology. For instance, *P. sclerotiorum* exhibits antagonistic activity against certain plant pathogens, demonstrating potential as a biocontrol agent (Jahan et al. 2024). The food industry utilizes *P. nalgiovense* as starter cultures for dry-fermented sausages (Ludemann et al. 2010). The capability of certain species to synthesize pigments has prompted the evaluation of these species for the production of highly stable and safe natural pigments (Morales-Oyervides et al. 2020). Nevertheless, mycotoxins generated by specific species present a significant risk to human and animal health (Nielsen et al. 2017). Notably, patulin exhibits multiple toxicities, including genotoxicity and immunotoxicity, and is predominantly produced by *P. expansum* and *P. griseofulvum* (Bandoh et al. 2009; Puel et al. 2010; Tannous et al. 2014).

Link (1809) introduced the generic name *Penicillium*, which is classified in the family Aspergillaceae. Traditional taxonomy of *Penicillium* primarily relied on morphological characters, including colony diameter, texture, conidial color, and conidiophore branching patterns. However, the variability in morphology has presented substantial challenges in accurately identifying novel species, frequently resulting in the erroneous classification of new isolates under known species (Visagie et al. 2016). Conversely, contemporary taxonomy adopts a polyphasic strategy that incorporates morphological, extrolite, genetic, and multigene phylogenetic data (Visagie et al. 2014). Houbraken et al. (2020) delivered the most comprehensive update on the genus *Penicillium* based on a phylogenetic approach combined with phenotypic, physiologic, and extrolite data. This study recognized 483 species and introduced a novel series classification, which is deemed highly predictive of potential functional traits (Houbraken et al. 2020). Subsequently, Visagie et al. (2024b) applied GCPSR (Genealogical Concordance Phylogenetic Species Recognition) and phylogenetic analyses to reassess the list of *Penicillium* species published up to 31 December 2022, resulting in an updated count of 535 species. An additional 100 species of this genus were described from 1 January 2023 to 31 December 2024 (Ansari et al. 2023; Crous et al. 2023; da Silva et al. 2023; Khuna et al. 2023; Li et al. 2023; Liu et al. 2023; Tan 2023; Tan and Shivas 2023, 2024; Tan et al. 2023, 2024a, 2024b; Wang et al. 2023; Zhang et al. 2023; Araújo et al. 2024; Crous et al. 2024; Liang et al. 2024; Lima et al. 2024; Nóbrega et al. 2024; Song et al. 2024; Visagie et al. 2024a, 2024c; Zhang et al. 2024). The increase in species numbers in recent years indicates the possibility of numerous undiscovered *Penicillium* species, and their biodiversity, ecological functions, and potential for resource development warrant further investigation.

During a comprehensive survey of *Penicillium* biodiversity in China, we found two isolates that could not be classified within existing species. In this paper, we compare these isolates with related species using multi-locus phylogenetic analyses and morphological character assessments. As a result, the isolates are described as species new to science. This study is expected to offer new perspectives on the diversity, function, ecology, and distribution of *Penicillium* members.

Materials and methods

Isolates

Soil samples were collected from the rhizosphere of plants in the Kangyu Tunnel, Tibet, China, while indoor dust samples were sourced from Beijing Forestry University, Beijing, China. To isolate the fungus, the samples were suspended in sterile water at a ratio of 1:10, vortexed to ensure homogeneity, and then diluted to 10^{-4} concentrations. Each of 100 μL from 10^{-2}, 10^{-3}, and 10^{-4} dilutions was spread on potato dextrose agar (PDA) and Martin medium with 50 ppm penicillin and 50 ppm streptomycin. The cultures were incubated at 25 °C for 5–7 days. Individual colonies were then picked from the plates and transferred to fresh PDA plates until pure cultures were obtained. Type specimens, preserved as dry cultures, were deposited in the Fungarium (HMAS), Institute of Microbiology, Chinese Academy of Sciences, while ex-type strains, maintained as living cultures, were stored at the China General Microbiological Culture Collection Centre (CGMCC).

Morphological studies

Morphological observations of colonies were conducted under strictly standardized conditions, encompassing media preparation, inoculation technique, incubation parameters, and description methods (Visagie et al. 2014). Colony characters and diameters were recorded from cultures grown on Czapek yeast autolysate agar (CYA), malt extract agar (MEA), yeast extract sucrose agar (YES), dichloran 18% glycerol agar (DG18), and creatine sucrose agar (CREA) at 25 °C for 7 days. Additional CYA plates were incubated at 30 and 37 °C. Color names and codes adhered to the book "Color Standards and Color Nomenclature" (Ridgway 1912). Ehrlich reaction was employed to assess the production of indole metabolites; a violet ring observed within ten min was deemed a positive result, while other color changes were interpreted as negative (Lund 1995; Houbraken et al. 2016).

For light microscopic observations, slides were prepared from cultures grown on MEA, and phenol glycerin solution was used as mounting fluid, with cotton blue staining if necessary. In addition, a field emission scanning electron microscope (Hitachi SU8010, Japan) was employed to examine microstructural characteristics. Agar blocks (3−4 mm × 3−4 mm) were fixed in 2.5% v/v glutaraldehyde at 4 °C for 8−12 hr, then washed three times for 10 min each with 0.1M phosphate buffer. Dehydration was performed with a gradient of ethanol (30, 50, 70, 95, and 100% v/v) for 10−20 min per step, followed by replacement with tert-butanol and ultimate vacuum freeze-dried and gold-sprayed for observation (Wei et al. 2024).

DNA extraction, sequencing, and phylogenetic analyses

Colonies were cultivated on MEA plates for 5−7 days, and DNA extraction was conducted using the E.Z.N.A.® Fungal DNA Mini Kit (Omega Bio-Tek, Inc., United States). The internal transcribed spacer (ITS), beta-tubulin (*BenA*), calmodulin (*CaM*), and RNA polymerase II second largest subunit (*RPB2*) genes were amplified using primer pairs ITS1/ITS4 (White et al. 1990), Bt2a/Bt2b (Glass and Donaldson 1995), CMD5/CMD6 (Hong et al. 2006), and RPB2-5F/RPB2-7CR (Liu et al. 1999), respectively. Polymerase chain reaction (PCR) amplification followed Visagie et al. (2014). Sequencing reactions were performed by Sangon Biotech (Shanghai) Company Limited, China. DNAMAN software (Lynnon Biosoft) was used for the assembly and trimming of the Sanger chromatograms. Sequences were submitted to GenBank (www.ncbi.nlm.nih.gov).

Sequence similarity searches were conducted using the mega BLAST program of basic local alignment search tool (BLAST) within the NCBI core nucleotide database (core_nt). Comprehensive sequence datasets were compiled containing newly generated sequences alongside reference sequences sourced from GenBank (Table 1). Sequence alignments were performed using the ClustalW algorithm and subsequently manually edited using MEGA 11 (Tamura et al. 2021). The resulting multiple sequence alignments have been deposited in TreeBASE (submission number: 31847) (www.treebase. org). Phylogenetic trees were constructed based on the ITS, *BenA*, *CaM*, and *RPB2* genes as well as the concatenated sequences of the latter three genes. Phylogenetic analyses were conducted using both maximum likelihood (ML) and Bayesian Inference (BI). ML phylogenies were performed using IQtree v. 1.6.12 (Nguyen et al. 2015), including 1000 standard non-parametric boot-

Table 1. Strains of *Penicillium* used for phylogenetic analyses.

Species	Strain	Substrate and origin	GenBank accession numbers			
			ITS	BenA	CaM	RPB2
P. abidjanum	CBS 246.67[T]	Soil, Ivory Coast	GU981582	GU981650	MN969234	JN121469
P. alagoense	URM 8086[T]	Leaves of *Miconia* sp., Brazil	MK804503	MK802333	MK802336	MK802338
P. amphipolaria	CBS 140997[T]	Soil, Antarctica	KT887872	KT887833	KT887794	MN969177
P. annulatum	CBS 135126[T]	Air sample, South Africa	JX091426	JX091514	JX141545	KF296410
P. araracuaraense	CBS 113149[T]	Leaf litter, Colombia	GU981597	GU981642	MN969237	KF296414
P. astrolabium	CBS 122427[T]	Grapes, Portugal	DQ645804	DQ645793	DQ645808	JN406634
P. ausonanum	CBS 148237[T]	Sediment of freshwater stream, Spain	LR655808	LR655809	LR655810	LR655811
P. austrosinense	CGMCC 3.18797[T]	Acidic soil, China	KY495007	KY495116	MN969328	KY495061
P. bialowiezense	CBS 227.28[T]	Soil under conifers, Poland	EU587315	AY674439	AY484828	JN406604
P. bissettii	CBS 140972[T]	Soil from spruce forest, Canada	KT887845	KT887806	KT887767	MN969178
P. brasilianum	CBS 253.55[T]	Herbarium exsiccata, Brazil	GU981577	GU981629	MN969239	KF296420
P. brevicompactum	NRRL 28139	Stroma of a wood decay fungus, USA	AY484917	DQ645795	AY484825	–
	CV1492	Unknown, South Africa	JX091398	JX091533	JX141574	–
	CBS 257.29[T]	Unknown, Belgium	AY484912	AY674437	AY484813	JN406594
P. buchwaldii	CBS 116980	Wheat, United Kingdom	JX313163	JX313181	JX313147	–
	CBS 116935	Wheat, United Kingdom	JX313156	JX313174	JX313140	–
	CBS 116929	Wheat flour, Denmark	JX313152	JX313170	JX313136	–
	CBS 117181[T]	*Hordeum vulgare*, Denmark	JX313164	MN969374	JX313148	JN406637
P. camponoti	CBS 140982[T]	Carpenter ants, Canada	KT887855	KT887816	KT887777	MN969179
P. cataractarum	CBS 140974[T]	Fallen nuts of *Carya cordiformis*, Canada	KT887847	KT887808	KT887769	MN969180
P. coffeatum	CGMCC 3.25152[T]	Soil, China	OQ870815	OR051121	OR051298	OR051466
P. daleae	CBS 211.28[T]	Soil under conifer, Poland	GU981583	GU981649	MN969251	KF296427
P. echinulonalgiovense	CBS 328.59[T]	Unknown, Japan	GU981587	GU981631	KX961269	KX961301
P. excelsum	DTO 357-D7[T]	Brazil nut shell, Brazil	KR815341	KP691061	KR815342	MN969166
	ITAL 7804	Flowers, Brazil	KT749963	KT749959	KT749962	–
P. expansum	CBS 325.48[T]	*Malus sylvestris*, USA	AY373912	AY674400	DQ911134	JF417427
P. fengjieense	CGMCC 3.25157[T]	Soil, China	OQ870765	OR051156	OR051333	OR051489
P. fennelliae	CBS 711.68[T]	Soil, Congo	JX313169	MN969382	JX313151	JN406536
P. flaviroseum	CGMCC 3.18805[T]	Acidic soil, China	KY495032	KY495141	MN969329	KY495083
P. fructuariae-cellae	CBS 145110[T]	Dried fruit of *Vitis vinifera*, Italy	MK039434	KU554679	MK045337	–
P. globosum	CBS 144639[T]	Acidic soil, China	KY495014	KY495123	MN969330	KY495067
P. griseoflavum	CGMCC 3.18799[T]	Acidic soil, China	KY495011	KY495120	MN969331	KY495064
P. griseopurpureum	CBS 406.65[T]	Soil under *Pinus* sp., United Kingdom	KF296408	KF296467	MN969261	KF296431
P. guaibinense	CCDCA 11512[T]	Soil, Brazil	MH674389	MH674391	MH674393	–
P. guangxiense	CBS 144526[T]	Soil, China	KY494986	KY495095	MN969332	KY495045
P. hainanense	CGMCC 3.18798[T]	Acidic soil, China	KY495009	KY495118	MN969333	KY495062
P. infrabuccalum	CBS 140983[T]	*Camponotus pennsylvanicus*, Canada	KT887856	KT887817	KT887778	MN969181
P. jianfenglingense	CGMCC 3.18802[T]	Acidic soil, China	KY495016	KY495125	MN969334	KY495069
P. jinyunshanicum	CGMCC 3.25162[T]	Soil, China	OQ870766	OR051157	OR051334	OR051490
P. kongii	AS3.15329[T]	leaf sample of *Cotoneaster* sp., China	KC427191	KC427171	KC427151	–
P. laevigatum	CGMCC 3.18801[T]	Acidic soil, China	KY495015	KY495124	MN969335	KY495068
P. lentum	**CGMCC 3.28596[T] = B24**	**Indoor dust, Beijing, China**	**PQ643282**	**PQ519854**	**PQ519855**	**PQ519856**
P. mariae-crucis	CBS 271.83[T]	*Secale cereale*, Spain	GU981593	GU981630	MN969275	KF296439
P. marykayhuntiae	BRIP 74934a[T]	Soil, Australia	OR271913	OR269446	–	OR269440
P. neocrassum	CBS 122428[T]	Grapes, Madeira	DQ645805	DQ645794	DQ645809	JN406633
P. newtonturnerae	BRIP 74909a[T]	Soil, Australia	OP903478	OP921964	OP921962	OP921963
P. ochrochloron	CBS 357.48[T]	Copper sulphate solution, USA	GU981604	GU981672	MN969280	KF296445
	DTO 189-A6	Unknown, Japan	KC346347	KC346324	KC346341	KC346318

Species	Strain	Substrate and origin	GenBank accession numbers			
			ITS	*BenA*	*CaM*	*RPB2*
P. olsonii	CBS 232.60ᵀ	*Musa*, France	EU587341	AY674445	DQ658165	JN121464
P. onobense	CBS 174.81ᵀ	Soil, andosol, Spain	GU981575	GU981627	MN969281	KF296447
P. panissanguineum	CBS 140989ᵀ	Soil near termite mound, Tanzania	KT887862	KT887823	KT887784	MN969182
P. paraherquei	CBS 338.59ᵀ	Soil, Japan	AF178511	KF296465	MN969285	KF296449
P. pauciramulum	CGMCC 3.25164ᵀ	Soil, associated with nest of Formicidae, China	OQ870726	OR051111	OR051288	OR051457
P. pedernalense	CBS 140770ᵀ	*Litopenaeus vannamei*, Ecuador	KU255398	KU255396	MN969322	MN969184
P. penarojense	CBS 113178ᵀ	Leaf litter, Colombia	GU981570	GU981646	MN969287	KF296450
P. piscarium	CBS 362.48ᵀ	Cod-liver oil emulsion, Germany	GU981600	GU981668	MN969288	KF296451
P. pulvillorum	CBS 280.39ᵀ	Acidic soil, United Kingdom	AF178517	GU981670	MN969289	KF296452
	CBS 275.83	Rye grain, Spain	GU981601	GU981671	KC346336	KF296423
P. rolfsii	CBS 368.48ᵀ	Fruit of *Ananas sativus*, USA	JN617705	GU981667	MN969294	KF296455
P. roodeplaatense	DTO 444-C8	Soil, South Africa	OR819195	OR820176	OR820180	OR820186
P. rotoruae	CBS 145838ᵀ	*Pinus radiata* timber stake in ground contact, New Zealand	MN315103	MN315104	MN315102	MT240842
P. rubriannulatum	CGMCC 3.18804ᵀ	Acidic soil, China	KY495029	KY495138	MN969336	KY495080
P. salamii	CBS 135391ᵀ	Salami, Italy	HG514431	HG514437	HG514432	MN969160
P. simplicissimum	CBS 372.48ᵀ	*Secale cereale*, Spain	GU981588	GU981632	MN969297	JN121507
P. singorense	CBS 138214ᵀ	House dust, Thailand	KJ775674	KJ775167	KJ775403	MN969138
P. skrjabinii	CBS 439.75ᵀ	Soil, Russia	GU981576	GU981626	MN969299	EU427252
P. soliforme	CGMCC 3.18806ᵀ	Acidic soil, China	KY495038	KY495147	MN969337	KY495047
	NN072390	Acidic soil, China	KY495019	KY495128	KY494959	KY495072
	NN072399	Acidic soil, China	KY495022	KY495131	KY494962	KY495074
P. spathulatum	CBS 117192ᵀ	Mouldy chestnut (*Castanea* sp.), France	JX313165	MN969400	JX313149	JN406636
P. spinuliferum	CBS 144483ᵀ	Acidic soil, associated with *Litchi chinensis*, China	KY495040	KY495149	MN969338	KY495090
P. stangiae	URM 8347ᵀ	Soil, Brazil	MW648590	MW646388	MW646390	MW646392
P. stolkiae	CBS 315.67ᵀ	Soil, South Africa	AF033444	JN617717	AF481135	JN121488
P. subfuscum	CBS 147455ᵀ	Soil, South Africa	MT949907	MT957412	MT957454	MT957480
P. subrubescens	CBS 132785ᵀ	Soil of *Helianthus tuberosus* field, Finland	KC346350	KC346327	KC346330	KC346306
P. subrutilans	CGMCC 3.25174ᵀ	Soil, China	OQ870816	OR051137	OR051314	OR051479
P. svalbardense	CBS 122416ᵀ	Glacial ice, Svalbard	GU981603	DQ486644	KC346338	KF296457
P. taii	CGMCC 3.25176ᵀ	Soil, China	OQ870778	OR051170	OR051347	OR051496
P. tanzanicum	CBS 140968ᵀ	Soil near termite mound, Tanzania	KT887841	KT887802	KT887763	MN969183
P. terrarumae	CBS 131811ᵀ	Soil contaminated by heavy metals, China	MN431397	KX650295	MN969323	MN969185
	CS23-08	Unknown, China	OQ870751	OR051141	OR051318	OR051481
P. tularense	CBS 430.69ᵀ	Soil under *Pinus ponderosa* and *Quercus kelloggii*, USA	AF033487	KC427175	JX313135	JN121516
	CBS 431.69	Soil under *Pinus ponderosa* and *Quercus kelloggii*, USA	JX313167	AY674433	JX313134	–
P. vanderhammenii	CBS 126216ᵀ	Leaf litter, Colombia	GU981574	GU981647	MN969308	KF296458
P. vasconiae	CBS 339.79ᵀ	Soil, Spain	GU981599	GU981653	MN969309	MN969144
P. vickeryae	BRIP 72552aᵀ	Soil, Australia	OP903479	OP921966	–	OP921965
P. viridissimum	CGMCC 3.18796ᵀ	Acidic soil, China	KY495004	KY495113	MN969339	KY495059
P. wotroi	CBS 118171ᵀ	Leaf litter, Colombia	GU981591	GU981637	MN969313	KF296460
P. tibetense	**CGMCC 3.28597ᵀ = XZ5-3**	**Rhizosphere soil, Tibet, China**	**PQ643284**	**PQ519857**	**PQ519858**	**PQ519859**
P. yuyongnianii	CGMCC 3.25187ᵀ	Soil, China	OQ870820	OR051175	OR051352	OR051499
P. zonatum	CBS 992.72ᵀ	Soil, USA	GU981581	GU981651	MN969315	KF296461

strap replicates with the best partition scheme and substitution model selected using ModelFinder (Kalyaanamoorthy et al. 2017). BI phylogenies were run in MrBayes v. 3.2.7 (Ronquist et al. 2012). Best fit models were selected according to the Akaike information criterion (AIC) using MrModeltest v. 2.4 (Nylander 2004). Posterior probabilities (PP) were estimated using Markov Chain Monte Carlo (MCMC) sampling, set to run for 1,000,000 generations with the average standard deviation of split frequencies less than 0.01 as the stopping criterion. In cases where this threshold was not achieved, the run was continued until the condition was met. Additionally, the initial 25% of the generated trees were discarded as burn-in.

Results

Morphology

Two novel species, *Penicillium lentum* and *P. tibetense*, were introduced within the sections *Brevicompacta* and *Lanata-Divaricata*, respectively, based on comprehensive phylogenetic analyses. General morphological characteristics and ecological information for the species included in these sections are provided in Table 2. Both newly described species exhibited morphological traits consistent with their respective sections. Specifically, *P. lentum* displayed limited growth with dense colonies on agar media and primarily produced terverticillate conidiophores. In contrast, *P. tibetense* demonstrated rapid growth on agar media, particularly exhibiting robust development on CYA at 30 °C, and predominantly formed biverticillate conidiophores. The morphological features of the new species and their closely related species are summarized in Table 3.

Table 2. Morphological and ecological data pertaining to the sections of the new species in this study.

Section	Morphology	Ecology	References
Brevicompacta	Colonies restricted (occasionally moderately fast), texture velutinous; conidiophores terverticillate or multiramulate branched with wide stipes, smooth-walled.	Mainly soil and foods, also on plant leaves and rotting wood.	(Houbraken and Samson 2011; Frisvad et al. 2013; Wang and Wang 2013; Houbraken et al. 2020)
Lanata-Divaricata	Colonies grow rapidly, occasionally moderately fast; conidiophores monoverticillate, biverticillate or divaricate, occasionally terverticillate.	Commonly found in soil, also on rotting leaf litter and vegetable.	(Houbraken and Samson 2011; Houbraken et al. 2020)

Table 3. Morphological features of new species and their closely related taxa.

| Species	Growth rates (mm)			Conidiophores branching	Cleistothecia /sclerotia	Conidia			Acid production on CREA
	CYA	CYA 30 °C	CYA 37 °C			Size	Shape	Roughening	
P. lentum	7–10	No growth	No growth	Terverticillate, sometimes biverticillate	Absent	2–3 × 1.5–2.5 µm	Broadly ellipsoidal	Smooth	Absent
P. tularense[a]	n.a.	n.a.	n.a.	Asymmetric and divaricate	Cleistothecia	2.2–2.6 µm	Globose to subglobose	Smooth	n.a.
P. tibetense	42–50	42–52	21–27	Biverticillate	Absent	1.5–3 µm	Globose to subglobose	Finely rough	Absent
P. excelsum[b]	35–50	n.a.	8–22	Biverticillate, sometimes terverticillate	Absent	4–5 × 2–3.2 µm	Ellipsoidal	Smooth	Absent

[a]Description based on Paden (1971), [b]Description based on Taniwaki et al. (2016).

Phylogenetic analyses

A BLAST search revealed that strain CGMCC 3.28596 is most closely related to *Penicillium tularense* (Identities: ITS: 97.52%, *BenA*: 81.13%, *CaM*: 84.91%, *RPB2*: 91.00%) within section *Brevicompacta*, and strain CGMCC 3.28597 exhibits the highest similarity to *P. excelsum* (Identities: ITS: 98.64%, *BenA*: 94.37%, *CaM*: 89.66%, *RPB2*: 94.84%) within section *Lanata-Divaricata*.
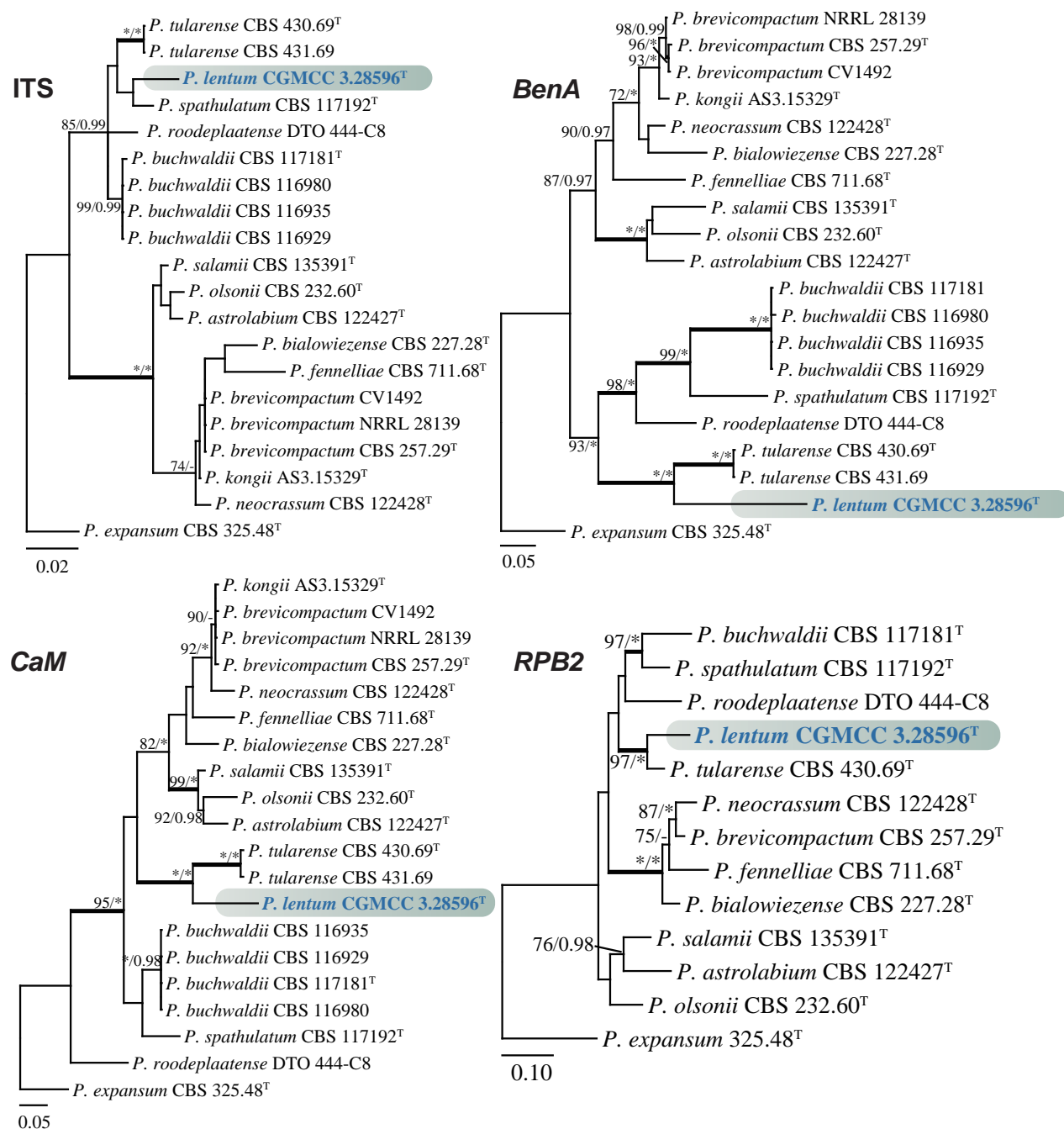
Section *Brevicompacta*

The analyses of the concatenated dataset (*BenA*, *CaM*, and *RPB2*) comprised 20 predominantly ex-type strains, each with a total sequence length of 1876 bp (*BenA*: 469 bp, *CaM*: 512 bp, *RPB2*: 895 bp). Phylogenetic analyses divided section *Brevicompacta* into four distinct clades, with the new species *Penicillium lentum* forming a robustly supported clade alongside *P. tularense* (100% bs, 1.00 pp) (Fig. 1). In the phylogenetic analyses of individual genes, the new species, together with *P. tularense*, consistently formed a well-supported clade, mostly with high support values (>97% bs, 1.00 pp), except for ITS (Fig. 2).
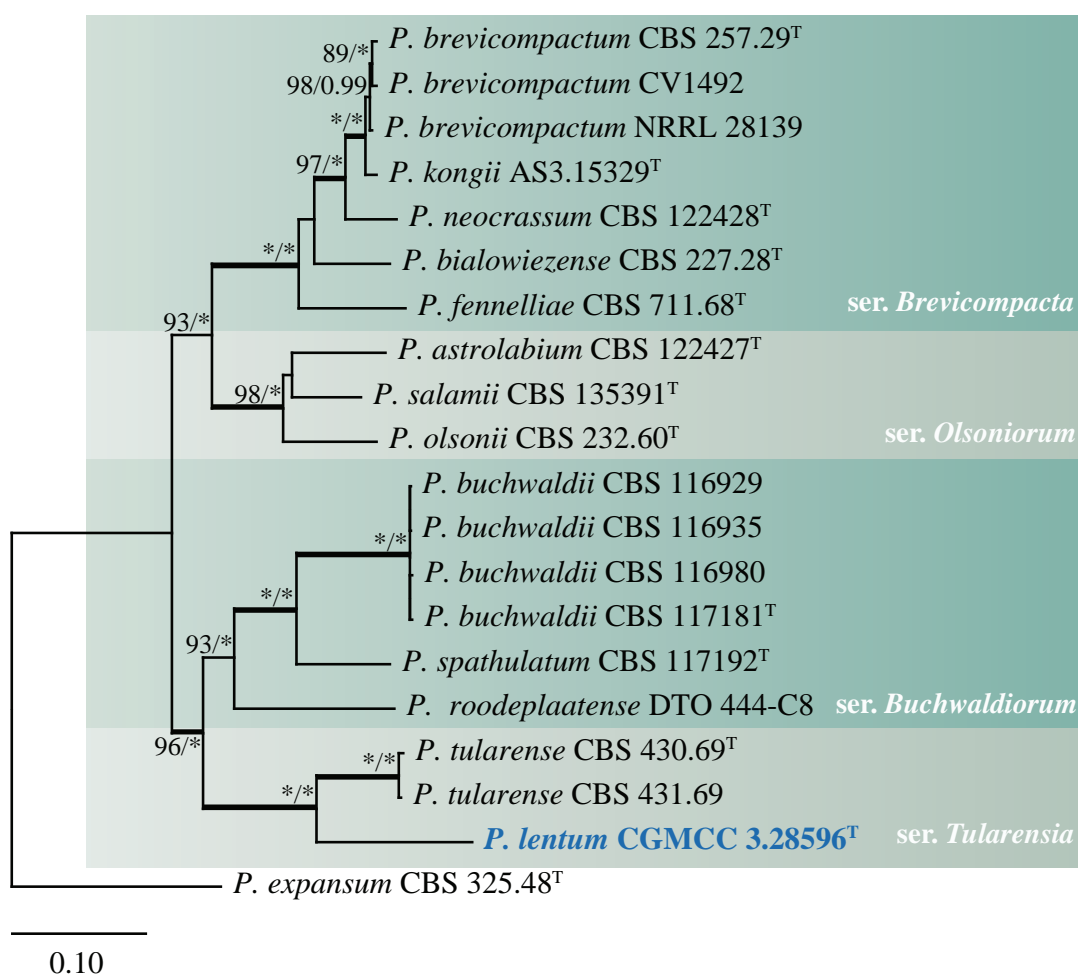
Figure 1. ML tree based on the concatenated data set (*BenA*, *CaM*, and *RPB2*) of section *Brevicompacta*. *Penicillium expansum* CBS 325.48[T] was designated as the outgroup. Nodes display bootstrap values (bs) exceeding 70% or posterior probabilities (pp) greater than 0.95. Branches with bs of 95% or higher and pp of 1.00 are depicted in bold. The strain described as the new species *P. lentum* is indicated with blue text. * Indicates bs = 100% or pp = 1.00, [T] = ex-type strain.
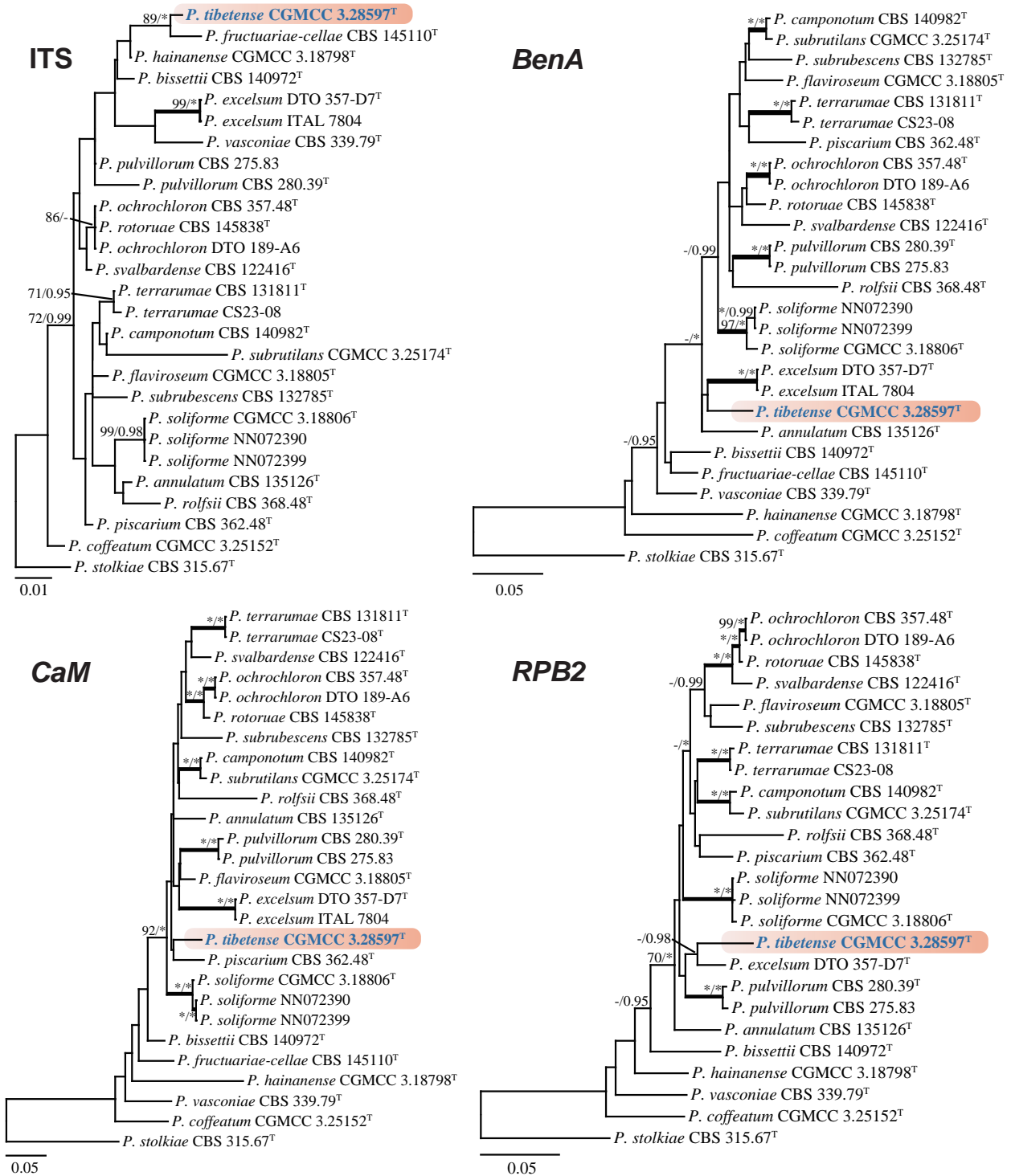
Figure 2. ML trees for section *Brevicompacta* based on ITS, *BenA*, *CaM*, and *RPB2*. *Penicillium expansum* CBS 325.48[T] was designated as the outgroup. Nodes display bootstrap values (bs) exceeding 70% or posterior probabilities (pp) greater than 0.95. Branches with bs of 95% or higher and pp of 1.00 are depicted in bold. The strain described as the new species *P. lentum* is indicated with blue text. * Indicates bs = 100% or pp = 1.00, [T] = ex-type strain.

Section *Lanata-Divaricata*

In this section, we selected the series *Simplicissima*, *Dalearum*, and *Rolfsiorum*, comprising 71 predominantly ex-type strains, for phylogenetic analyses based on the concatenated dataset totaling 1876 bp (*BenA*: 504 bp, *CaM*: 617 bp, *RPB2*: 755 bp). The resulting phylogenies revealed that *Penicillium tibetense* is closely related to *P. excelsum* (64% bs, 0.97 pp; not depicted in Fig. 3). However, the significant evolutionary divergence observed supports the recognition of
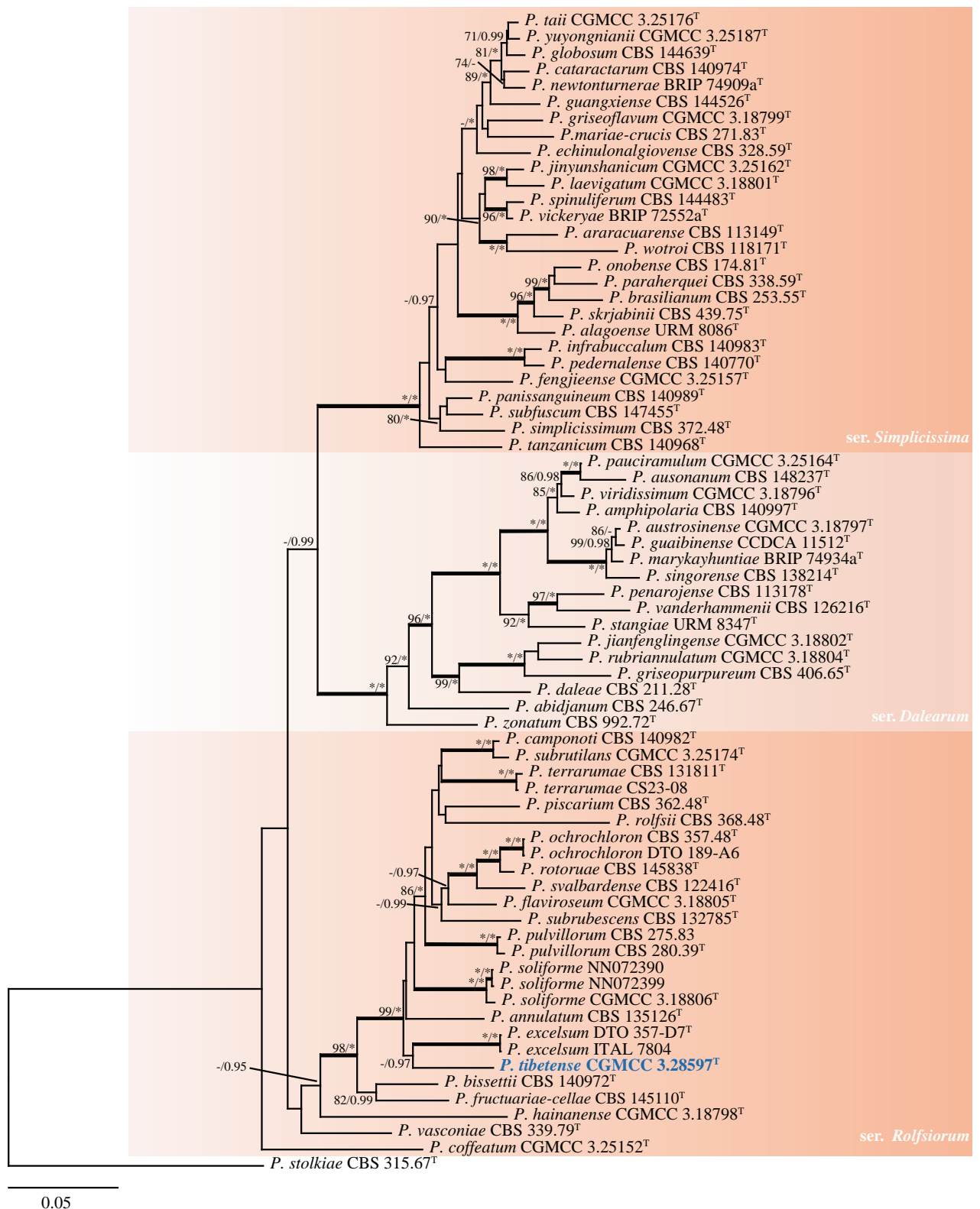
Figure 3. ML tree based on the concatenated data set (*BenA*, *CaM*, and *RPB2*) of section *Lanata-Divaricata* (series *Simplicissima*, *Dalearum*, and *Rolfsiorum*). *Penicillium stolkiae* CBS 315.67^T was designated as the outgroup. Nodes display bootstrap values (bs) exceeding 70% or posterior probabilities (pp) greater than 0.95. Branches with bs of 95% or higher and pp of 1.00 are depicted in bold. The strain described as the new species *P. tibetense* is indicated with blue text. * Indicates bs = 100% or pp = 1.00, ^{T} = ex-type strain.

P. tibetense as a distinct species (Fig. 3). Phylogenetic analyses of individual genes within series *Rolfsiorum* demonstrated generally weak clustering support, with variations observed among the ITS, *BenA*, *CaM*, and *RPB2* datasets. Furthermore, *P. ochrochloron* and *P. rotoruae* share identical ITS sequences, making them indistinguishable through ITS phylogeny alone (Fig. 4).

Figure 4. ML trees for section *Lanata-Divaricata* series *Rolfsiorum* based on ITS, *BenA*, *CaM*, and *RPB2*. *Penicillium stolkiae* CBS 315.67[T] was designated as the outgroup. Nodes display bootstrap values (bs) exceeding 70% or posterior probabilities (pp) greater than 0.95. Branches with bs of 95% or higher and pp of 1.00 are depicted in bold. The strain described as the new species *P. tibetense* is indicated with blue text. * Indicates bs = 100% or pp = 1.00, [T] = ex-type strain.

Taxonomy

Penicillium lentum R.N. Liang & G.Z. Zhao, sp. nov.

MycoBank No: 857346

Fig. 5

Infrageneric classification. Subgenus *Penicillium*, section *Brevicompacta*, series *Tularensia*.

Etymology. The specific epithet "*lentum*" is derived from lentus (Latin), reflecting the slow growth rate characteristic of this species.

Type. China • Beijing, Haidian District, Beijing Forestry University, 40°0'20"N, 116°20'51"E, from indoor dust, 1 February 2024, collected by G.Z. Zhao, B24 (holotype HMAS 353385, dried culture; culture ex-type CGMCC 3.28596).

Colony diameter after 7 d (mm). CYA 7−10; CYA 30 °C, 37 °C no growth; MEA 6−9; YES 9−13; DG18 7−11; CREA 3.5−5.

Colony characteristics (7 d). CYA at 25 °C: Colonies deep, raised at center, margins low, narrow, irregular; mycelium white; texture velutinous, floccose areas present; sporulation moderate to good, conidia antique green (R. Pl. VI); exudate clear; reverse capucine buff (R. Pl. III); soluble pigment absent. MEA at 25 °C: Colonies deep, raised at center, margins low, narrow, entire; mycelium white; texture velutinous, floccose areas present; sporulation moderate to good, conidia celandine green (R. Pl. XLVII) to deep turtle green (R. Pl. XXXII); exudate clear; reverse light orange-yellow (R. Pl. III); soluble pigment absent. YES at 25 °C: Colonies deep, radially and concentrically sulcate, raised at center, margins low, narrow, entire; mycelium white; texture velutinous and fasciculate; sporulation good to strong, conidia glaucous-green (R. Pl. XXXIII); exudate absent; reverse cinnamon (R. Pl. XXIX); soluble pigment absent. DG18 at 25 °C: Colonies low, plane, margins low, wide, entire; mycelium white; texture velutinous and fasciculate; sporulation good, conidia bluish gray-green (R. Pl. XLII); exudate absent; reverse antimony yellow (R. Pl. XV); soluble pigment absent. CREA at 25 °C: Weak growth, no acid production. Ehrlich reaction negative.

Micromorphology. *Conidiophores* biverticillate to terverticillate; ***stipes*** smooth-walled, 70−236.5 × 2.5−4.5 µm; ***rami*** two when present, 6.5−18 × 2−4 µm; ***metulae*** divergent, 2−4 per branch/ramus, 4.0−13.0 × 2.5−4.5 µm; ***phialides*** ampulliform, 3−8 per metula, 4.5−8.0 × 2−3 µm; ***conidia*** broadly ellipsoidal, smooth-walled, 2−3 × 1.5−2.5 µm.

Notes. *Penicillium lentum* belongs to section *Brevicompacta* and is most closely related to *P. tularense* (Fig. 1). *Penicillium tularense* produces light brown to pale tan cleistothecia, which are not found in the new species (Paden 1971). Additionally, *P. lentum* has broadly ellipsoidal conidia, while *P. tularense* produces globose to subglobose conidia (Table 3).
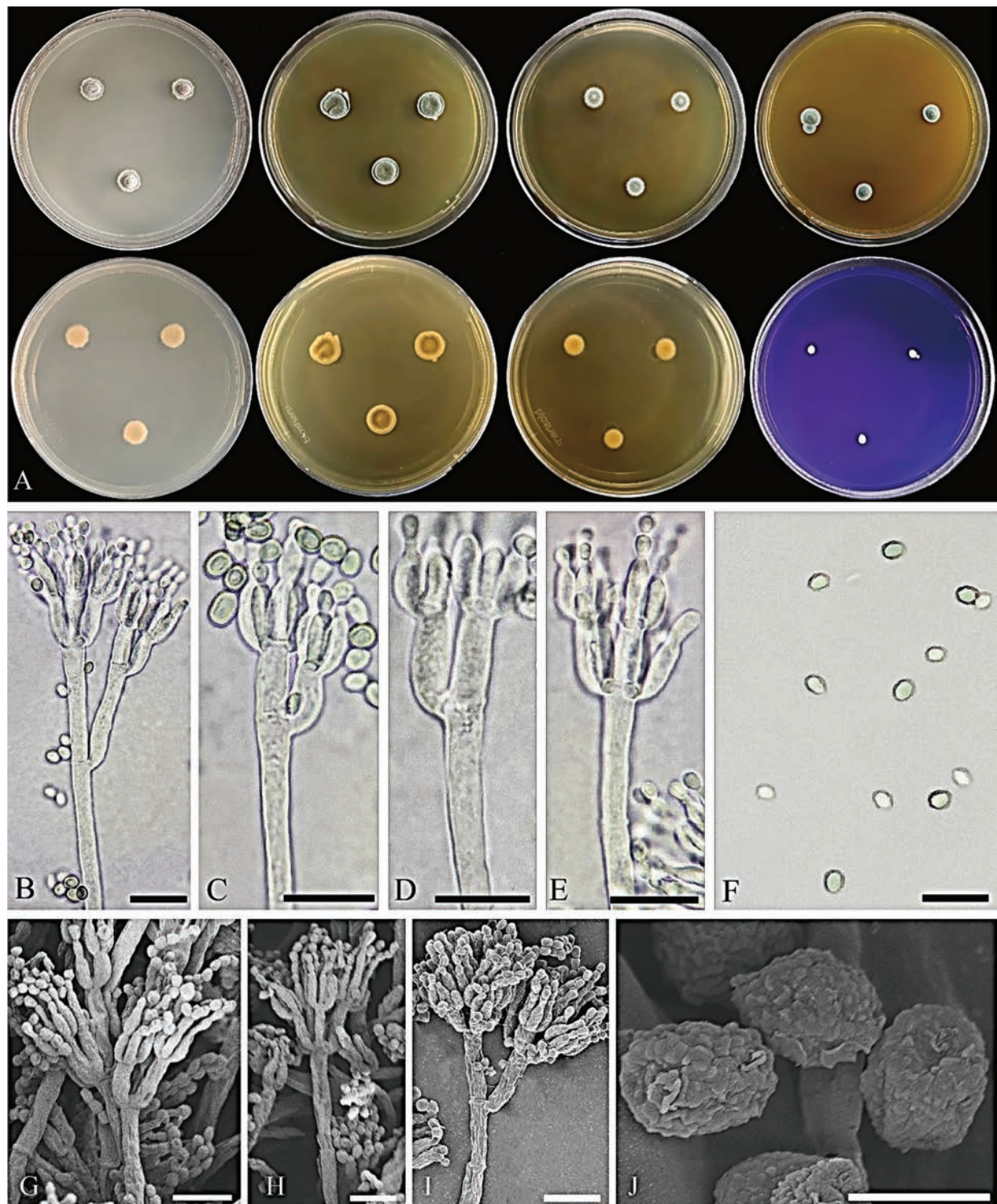
Penicillium tibetense R.N. Liang & G.Z. Zhao, sp. nov.

MycoBank No: 857347

Fig. 6

Infrageneric classification. Subgenus *Aspergilloides*, section *Lanata-Divaricata*, series *Rolfsiorum*.
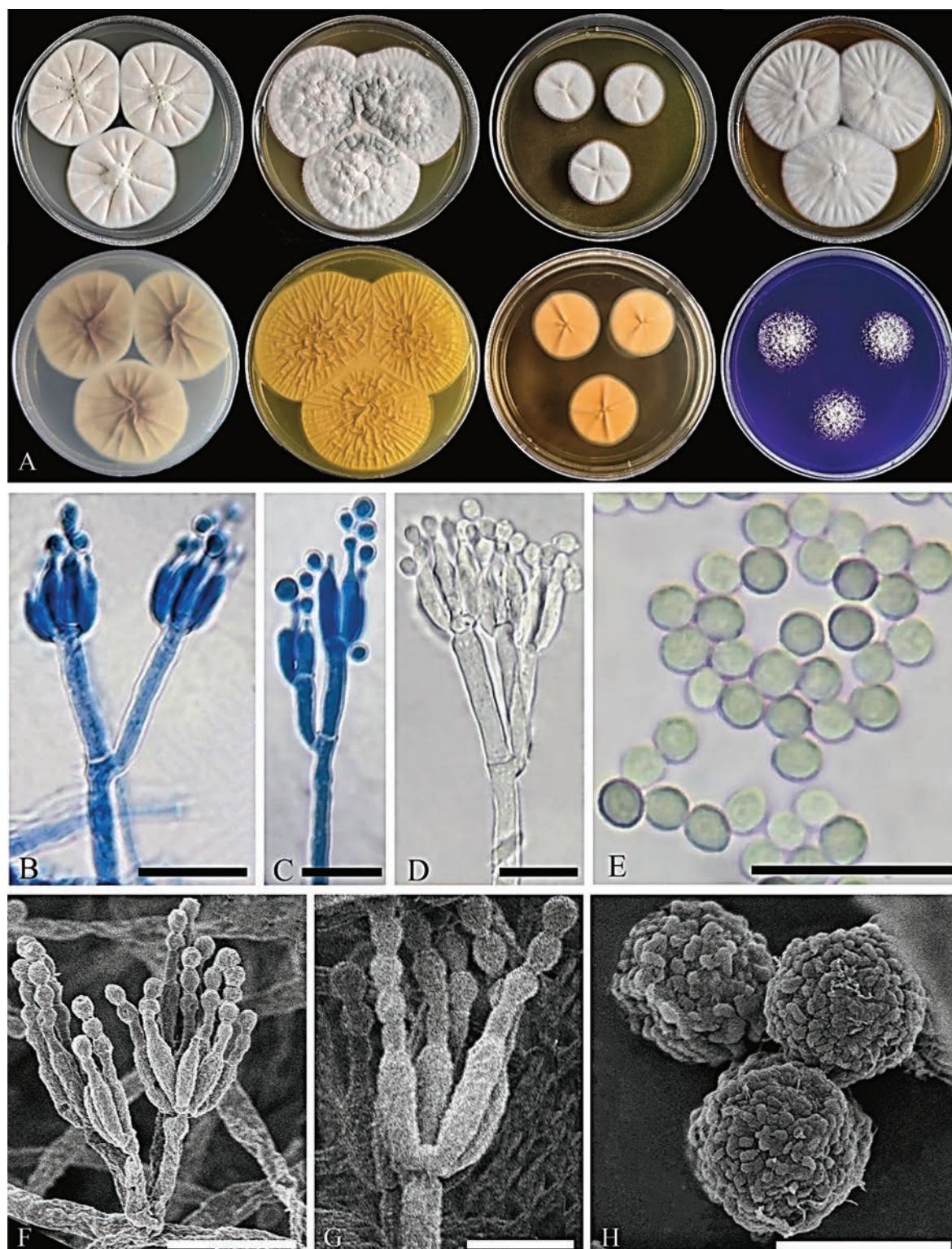
Figure 5. *Penicillium lentum* CGMCC 3.28596. **A** Colonies on medium at 25 °C for 7d (left to right, top row: CYA, YES, DG18, MEA obverse; second row: CYA reverse, YES reverse, DG18 reverse, CREA obverse) **B–E** conidiophores **F** conidia **G–I** SEM micrograph of conidiophores **J** SEM micrograph of conidia. Scale bars: 10 μm (**B–I**); 2 μm (**J**).

Etymology. The specific epithet "*tibetense*" denotes the geographical origin of the species, indicating its discovery in Tibet.

Type. CHINA • Tibet, Changdu City, Basu County, Kangyu Tunnel, 30°33'53"N, 96°15'25"E, from rhizosphere soil of grasses, 19 July 2023, collected by X.W. Peng, XZ5-3 (holotype HMAS 353386, dried culture; culture ex-type CGMCC 3.28597).

Figure 6. *Penicillium tibetense* CGMCC 3.28597. **A** Colonies on medium at 25 °C for 7d (left to right, top row: CYA, YES, DG18, MEA obverse; second row: CYA reverse, YES reverse, DG18 reverse, CREA obverse) **B–D** conidiophores **E** conidia **F, G** SEM micrograph of conidiophores **H** SEM micrograph of conidia. Scale bars: 10 μm (**B–G**); 2 μm (**H**).

Colony diameter after 7 d (mm). CYA 42–50; CYA 30 °C 42–52; CYA 37 °C 21–27; MEA 48–52; YES 46–52; DG18 20–26; CREA 24–26.

Colony characteristics (7 d). CYA at 25 °C: Colonies low to moderately deep, radially sulcate, margins low, narrow, entire; mycelium white; texture floccose;

sporulation moderate, conidia livid pink (R. Pl. XXVII); exudate clear; reverse light purple-drab (R. Pl. XLV) to avellaneous (R. Pl. XL); soluble pigment absent. CYA at 30 °C: Colonies low to moderately deep, radially sulcate, margins low, narrow, entire; mycelium white; texture floccose; sporulation moderate, conidia livid pink (R. Pl. XXVII); exudate clear; reverse brownish vinaceous (R. Pl. XXXIX); soluble pigment absent. CYA at 37 °C: Colonies moderately deep, radially sulcate, margins low, narrow, entire; mycelium white; texture floccose; sporulation sparse, conidia livid pink (R. Pl. XXVII); exudate clear; reverse light buff (R. Pl. XV); soluble pigment absent. MEA at 25 °C: Colonies low to moderately deep, radially sulcate, margins low, narrow, entire; mycelium white; texture floccose; sporulation sparse to moderate, conidia pale brownish vinaceous (R. Pl. XXXIX); exudate clear; reverse antimony yellow (R. Pl. XV); soluble pigment absent. YES at 25 °C: Colonies moderately deep, randomly sulcate, margins low, wide, entire; mycelium white; texture floccose; sporulation moderate, conidia antique green (R. Pl. VI); exudate clear; reverse antimony yellow (R. Pl. XV); soluble pigment absent. DG18 at 25 °C: Colonies low, radially sulcate, margins low, wide, entire; mycelium white; texture floccose; sporulation sparse, conidia ecru-drab (R. Pl. XLVI); exudate absent; reverse orange-pink (R. Pl. II); soluble pigment absent. CREA at 25 °C: Strong growth, no acid production. Ehrlich reaction negative.

Micromorphology. ***Conidiophores*** biverticillate; ***stipes*** finely rough-walled, 27–364.5 × 2–3 µm; ***metulae*** appressed to divergent, 2–4 per stipe, 8–15 × 1.5–3 µm; ***phialides*** ampulliform to cylindrical, 2–6 per metula, 5–10.5 × 1.5–3 µm; ***conidia*** globose to subglobose, finely rough-walled, 1.5–3 µm diam.

Notes. *Penicillium tibetense* is classified in section *Lanata-Divaricata* and exhibits a close phylogenetic relationship to *P. excelsum* (Fig. 3). This novel species generates globose to subglobose, finely rough-walled conidia that distinguish it from *P. excelsum* (Table 3). Additionally, *P. tibetense* demonstrates more robust growth on CYA at 37 °C compared to *P. excelsum* (21–27 mm vs. 8–22 mm) (Taniwaki et al. 2016).

Discussion

Penicillium, a ubiquitous and diverse fungal genus, plays pivotal roles in natural ecosystems while maintaining substantial economic importance and significant relevance to human affairs. The recent rapid increase in newly described species within this genus suggests that numerous taxa remain undiscovered. Given the extensive biotechnological applications of *Penicillium* species, accurate taxonomic identification is paramount, necessitating comprehensive species delineation through polyphasic approaches. In the present study, we introduced two novel species: one belonging to section *Brevicompacta* and the other to section *Lanata-Divaricata*.

Section *Brevicompacta* currently comprises 15 species distributed across four series (Visagie et al. 2024a, 2024b) and is represented in our findings by the newly described *P. lentum* sp. nov. This species, classified within series *Tularensia*, is characterized by predominantly terverticillate conidiophores and demonstrates a close relationship with other members of section *Brevicompacta* (Table 2). Section *Lanata-Divaricata* is characterized by its remarkable species diversity and rapid colony growth, primarily comprising soil-inhabiting

species, with over 90 taxa currently recognized across five series (Houbraken et al. 2020; Visagie et al. 2024b). The newly identified *Penicillium tibetense* assigned to series *Rolfsiorum* exhibits characteristic rapidly expanding colonies and produces biverticillate conidiophores, consistent with the morphological features typical of this series. Members of section *Lanata-Divaricata* are ecologically significant as decomposers of organic matter (Lichtner et al. 2022), with notable biotechnological potential exemplified by *P. subrubescens*, which has demonstrated efficient inulinase production (Mansouri et al. 2013).

Phylogenetic analyses of section *Brevicompacta* demonstrated that our strain *P. lentum* formed a well-supported clade with its closest relative, *P. tularense* (Fig. 1), a relationship corroborated by shared morphological characteristics such as conidiophore branching patterns and growth rates. However, our strain could be clearly distinguished from *P. tularense* based on distinct phenotypic features, including the presence or absence of cleistothecia and differences in conidial morphology (Table 3). The phylogenetic relationships within section *Lanata-Divaricata* remain unresolved, primarily due to the poor support values in certain clades (Houbraken et al. 2020), exemplified by a clade comprising *P. camponoti*, *P. piscarium*, *P. rolfsii*, *P. subrutilans*, and *P. terrarumae* (Fig. 3), which highlights the persistent challenges in resolving certain taxonomic groups even with multigene phylogenetic approaches.

To address these limitations, we recommend expanding the taxonomic sampling to include strains from diverse geographical origins and ecological niches. This strategy would not only generate additional reference sequences but also facilitate the discovery of novel species and the detection of infraspecific variation (Visagie and Houbraken 2020). Furthermore, sequencing additional gene regions represents a promising approach to enhance phylogenetic resolution (Visagie et al. 2021). The rapid development of high-throughput sequencing technologies has resulted in an accelerated increase in genomic data availability (Kapli et al. 2020), positioning phylogenomics as an essential tool in modern fungal taxonomy and systematics. By leveraging genome-scale data, phylogenomics is poised to overcome the limitations of single gene or multigene analyses, providing robust statistical support for clade resolution and enabling the reconstruction of a highly resolved fungal tree of life (Burki et al. 2020; Zhou and May 2023). These advancements underscore the transformative potential of phylogenomics in addressing long-standing taxonomic challenges and refining our understanding of fungal evolutionary relationships.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Rui-Na Liang: Formal analysis, investigation, data curation, writing – original draft preparation, visualization; Xiang-Hao Lin and Miao-Miao An: Investigation, visualization; Guo-Zhu Zhao: Conceptualization, methodology, validation, resources, writing – review and editing, supervision, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Data availability

All of the data that support the findings of this study are available in the main text. All sequences generated in this study have been submitted to GenBank.

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Two new species of *Penicillium* and a new genus in Xylariomycetidae from the forest dump-sites in Chiang Mai, Thailand

Tanapol Thitla[1*], Jutamart Monkai[2,3*], Weiqian Meng[1], Surapong Khuna[1], Ning Xie[1], Sinang Hongsanan[1,3], Saisamorn Lumyong[3,4,5]

1 Shenzhen Key Laboratory of Microbial Genetic Engineering, College of Life Science and Oceanography, Shenzhen University, Shenzhen 518060, China
2 Office of Research Administration, Chiang Mai University, Chiang Mai 50200, Thailand
3 Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
4 Center of Excellence in Microbial Diversity and Sustainable Utilization, Chiang Mai University, Chiang Mai 50200, Thailand
5 Academy of Science, The Royal Society of Thailand, Bangkok 10300, Thailand

Corresponding authors: Saisamorn Lumyong (scboi009@gmail.com); Sinang Hongsanan (sinang333@gmail.com)

Abstract

Waste accumulation in forest regions can have a severe impact on the soil mycobiome. However, research on soil fungi inhabiting forest disposal sites remains limited. Therefore, this study focused on the taxonomy and phylogeny of ascomycetes isolated from soil in forest dump-sites in Chiang Mai, Thailand. The fungal strains were identified using morphological characterisations and multigene phylogenetic reconstruction. A new genus, *Pseudoleptodontidium*, typified by *Ps. chiangmaiense* **sp. nov.** (Amphisphaeriales genera *incertae sedis*, Xylariomycetidae), along with two new species, *Penicillium chiangmaiense* (series *Janthinella*, section *Lanata-Divaricata*) and *P. terrae* (series *Erubescentia*, section *Exilicaulis*) (Aspergillaceae, Eurotiales), are described in detail and compared with closely-related species. Our discovery offers valuable insights into the soil ascomycetes associated with forest disturbances.

Key words: Eurotiomycetes, new taxa, *Pseudoleptodontidium*, soil fungi, Sordariomycetes, taxonomy

Introduction

The disposal of waste materials through open burning, landfilling and dumping in land areas or water resources contributes to environmental issues, such as air pollution ($PM_{2.5}$), as well as water and soil pollution, which can endanger the health and livelihood of humans, animals, plants and other organisms (Lin et al. 2020; Wanthongchai et al. 2021). Soil serves as a natural habitat for a wide range of fauna and flora, including fungi. Fungi are a major component of soil ecosystems, playing crucial roles in the cycling of nutrients and the decomposition of organic materials (Frąc et al. 2018; Coleine et al. 2022). The most abundant soil fungi belong to the Ascomycota, which includes the classes Arthoniomycetes, Dothideomycetes, Eurotiomycetes, Leotiomycetes and Sordariomycetes (Tedersoo et al. 2021; Gomes de Farias et al. 2023). Amongst

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* These authors have contributed equally to this work.

these, *Fusarium*, *Penicillium* and *Phoma* are the most frequently isolated genera (Tedersoo et al. 2021; Yasanthika et al. 2023). However, contamination with pollutants may adversely affect their diversity, population and ecological functions (Frąc et al. 2018; Schloter et al. 2018; Coleine et al. 2022). The ability to synthesise a wide range of enzymes for breaking down various substrates enables soil fungi to adapt and thrive in diverse environments and harsh conditions (Singh et al. 2021; Coleine et al. 2022; Sun et al. 2024).

Extensive studies have focused on isolating and characterising soil fungi from contaminated areas, landfills and urban dump-sites (Sangale et al. 2019; Verma and Gupta 2019; Ren et al. 2021; Khan et al. 2022; Gong et al. 2023; Sathiyabama et al. 2024; Sun et al. 2024). These studies have revealed diverse soil fungal communities and identified numerous new fungal taxa and strains from these polluted habitats. Moreover, they have demonstrated a significant potential for biodegradation and bioremediation. For example, Yasanthika et al. (2021) studied soil ascomycetes in China and reported a new species, *Juxtiphoma yunnanensis*, as well as two new records, *Lecanicillium dimorphum* and *Scopulariopsis brevicaulis*, from urban-industrialised soils. Ren et al. (2021) isolated 29 fungal strains from soils contaminated with explosive materials in China. Amongst them, the isolate of *Fusarium solani* demonstrated the ability to decompose alkyne-terminated polybutadiene with urethane segments (PUPB) (Ren et al. 2021). Similarly, Sangale et al. (2019) obtained 109 fungal isolates from the dumping sites of mangrove rhizosphere soil and revealed that the strains of *Aspergillus terreus* and *A. sydowii* were the most effective in breaking down polythene. Additionally, the strain of *Penicillium citrinum*, isolated from municipal landfill soils in Bhopal, India, has demonstrated efficacy in degrading low-density polyethylene (LDPE) without prior pretreatment (Khan et al. 2022).

Dump-sites, especially those located within forested areas, represent an underexplored yet ecologically significant niche. Forest dump-sites provide a distinctive habitat, characterised by decreased soil nutrients, fluctuating temperature and moisture levels and potential exposure to pollutants (Kooch et al. 2023; Sun et al. 2024). It is essential for exploring novel soil fungi from this habitat in order to determine fungal diversity and investigate their biodegradation strategies. Therefore, the present study aims to isolate and identify soil ascomycetes from disposal sites located in forests of northern Thailand. The topsoil samples from forest dump-sites in Chiang Mai Province were collected and isolated for fungi, leading to the discovery of five novel Ascomycota strains. Based on molecular analyses and morphological characteristics, two new species of *Penicillium* and a new genus in Xylariomycetidae were introduced and described.

Materials and methods

Fungal isolation

Soil samples (0–10 cm depth) were collected from three forest dump-sites in June 2024 in Chiang Mai Province, Thailand: (1) Papae, Mae Taeng District, (2) Suthep, Muang Chiang Mai District and (3) Mae Sa, Mae Rim District (Fig. 1). The collection details were noted (Rathnayaka et al. 2024) and the soil samples were placed in plastic bags and taken to the Sustainable Development of Biological Resources Laboratory (SDBR), at the Department of Biology, Faculty of Science, Chiang Mai

Figure 1. Forest dump-sites used for soil fungal isolation in this study **A** Papae, Mae Tang District, Chiang Mai Province **B** Suthep, Muang Chiang Mai District, Chiang Mai Province **C** Mae Sa, Mae Rim District, Chiang Mai Province.

University, Thailand. Upon arrival, soil fungi were isolated immediately using the serial dilution plating method with three serial dilutions in sterile water (Yasanthika et al. 2022). After dilution, 100 μl of the soil suspension was dropped and spread on potato dextrose agar (PDA; CONDALAB, Spain) supplemented with 100 μg/ml of streptomycin. The isolation plates were incubated at 25 °C in the dark for 5 days. The appearing fungal colonies were transferred to fresh PDA using the hyphal tip method (Korhonen and Hintikka 1980). The pure cultures were deposited and permanently preserved in a metabolically inactive state at the Culture Collection of Microbial Shenzhen University (MBSZU), Shenzhen University, China.

Morphological characterisation

The morphological characteristics of the obtained fungi were observed in both macro-morphology and micro-morphology, with different details depending on each fungus.

To investigate the morphology of *Penicillium* (comprising MBSZU 24-007 to MBSZU 24-010), the colony characteristics, growth rate, pigment production, sporulation or related features were investigated on Blakeslee's Malt extract agar (MEAbl), creatine sucrose agar (CREA), Czapek yeast autolysate agar with 5% NaCl (CYAS), Czapek's agar (CZ), Dichloran 18% glycerol agar (DG18), malt extract agar (MEA), oatmeal agar (OA), PDA and yeast extract sucrose agar (YES) at 25 °C in darkness for 7 days. The experiment was also performed on Czapek yeast autolysate agar (CYA) at 25, 30 and 37 °C in darkness for 7 days to characterise the macro-morphology (Visagie et al. 2014; Khuna et al. 2023). Micro-morphologically, the characteristics of conidiophores, stipes, conidiogenous cells, conidia or other structures were observed under a light microscope (Nikon DS-Ri2; Nikon, Japan), using fungal colonies grown on MEA at 25 °C in darkness for 7 days. Size data were evaluated by at least 50 measurements per structure.

The colony characteristics, growth rate and pigment production of *Pseudoleptodontidium* (MBSZU 25-005) were studied on PDA and MEA at 25 °C in darkness for 14 days. Micro-morphology was observed under a light microscope using a fungal colony grown on PDA at 25 °C in darkness for 14–21 days. The size of each morphological structure was measured at least 50 times per structure.

DNA extraction, amplification and sequencing

Fungal genomic DNA from each strain was extracted from the fungal mycelium, which had grown on PDA at 25 °C for a week, using an E.Z.N.A® Tissue DNA Kit (Omega, USA). The polymerase chain reaction (PCR) technique was used to amplify each region. Each target locus was amplified using the specific primers (Table 1). PCR amplifications were performed in 20 µl reaction mixtures, consisting of 1 µl of genomic DNA, 1 µl of each primer, 10 µl of 2× Phanta Max Master Mix (Dye Plus) (Vazyme, China) and 7 µl of deionised water. The PCR amplification was performed using a T100 Thermal Cycler (BIO-RAD, USA), with an initial denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation, annealing and elongation steps. The denaturation and elongation steps were performed at 95 °C for 30 s and 72 °C for 60 s, respectively. The annealing step was performed at different conditions depending on each target locus (Table 1). A final elongation step was performed at 72 °C for 10 minutes. The success or failure of the PCR product was determined through 1% agarose gel electrophoresis, followed by purification of the product using the E.Z.N.A® Gel Extraction Kit (Omega, USA). The quality and quantity of the purified PCR products were assessed using 1% agarose gel electrophoresis and a Nanodrop 2000 Spectrophotometer (Thermo Scientific, USA). Subsequently, the products were sequenced by BGI-Shenzhen Company (Shenzhen, Guangdong, China).

The bidirectional sequence data were assembled using the software Sequencher 5.4.6 (Nishimura 2000). The consensus sequence data were searched for sequence similarity via the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) website.

Table 1. The specific primer and annealing condition of each locus used in this study.

Loci*	Primer	Annealing condition		References
		Temperatures (°C)	Annealing period (s)	
ITS	ITS4/ITS5	52	30	White et al. (1990)
LSU	LR0R/LR5	52	30	Vilgalys and Hester (1990); Rehner and Samuels (1994)
CAM	CF1/CF4	51	60	Peterson et al. (2005)
	Cmd5/Cmd6	58	30	Hong et al. (2006)
RPB2	fRPB2-5F/ fRPB2-7cR	56	60	Liu et al. (1999)
TUB	Bt2a/Bt2b	52	30	Glass and Donaldson (1995)
	T1/Bt2b	55	45	Glass and Donaldson (1995); O'Donnell and Cigelnik (1997)

* ITS – Internal Transcribed Spacer region of the rRNA; LSU – 28S large subunit of the nuclear rRNA; *TUB* – beta-tubulin gene; *CAM* – calmodulin gene; *RPB2* – RNA polymerase II second largest subunit genes.

Phylogenetic analysis

The multi-loci phylogenetic dataset was obtained, based on previous studies of *Penicillium* section *Exilicaulis* (Ansari et al. 2023; Liu et al. 2023; Wang et al. 2023b; Visagie et al. 2024a, 2024b), *Penicillium* section *Lanata-Divaricata* (Lenz et al. 2022; Liu et al. 2023; Wang et al. 2023b; Araújo et al. 2024; Visagie et al. 2024b) and Xylariomycetidae (Samarakoon et al. 2022; Crous et al. 2023; Li et al. 2024; Samarakoon 2024) (Suppl. material 1: tables S1–S3).

The sequence data for each locus were individually aligned using MUSCLE through the software MEGA 6 (Edgar 2004) and manually adjusted in BioEdit v.7.2.5 (Hall 2004). The concatenation of the ITS, *TUB*, *CAM* and *RPB2* loci was performed for the phylogenetic analysis of *Penicillium*; in contrast, the combined ITS, LSU, *RPB2* and *TUB* loci were used for the analysis of Xylariomycetidae. Maximum Likelihood (ML) and Bayesian Inference (BI) analyses were applied to generate a phylogenetic tree. The ML analysis was conducted with 25 categories and 1,000 bootstrap (BS) replications under the GTRCAT model using RAxML-HPC2 on XSEDE (v.8.2.12) in the CIPRES web portal (Felsenstein 1985; Stamatakis 2006; Miller et al. 2009). The best-fit models of nucleotide substitution for individual locus were determined by using MrModelTest v.2.3 based on the Akaike Information Criterion (AIC) (Nylander 2004). The GTR+I+G substitution model was the best fit for all loci. The BI analysis was performed using MrBayes v.3.2.6 (Ronquist and Huelsenbeck 2003). Bayesian Posterior Probability (PP) was examined by Markov Chain Monte Carlo (MCMC) sampling. Six simultaneous Markov chains were run with random initial trees, wherein every 100th generation was sampled. The first 20% of generated trees, representing the burn-in phase of the analysis, were discarded, while the remaining trees were used to calculate PP in the majority-rule consensus tree. The tree topologies were visualised in FigTree v.1.4.0 with BS support and PP values equal to or higher than 75% and 0.95, respectively, in branches (Rambaut 2019). The final alignment and phylogram were submitted to TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S32075, accessed 19 March 2025).

Results

Phylogenetic analysis

Phylogenetic analysis of 72 taxa from *Penicillium*, section *Exilicaulis* (including *P. terrae* MBSZU 24-007 and MBSZU 24-008) was performed using a combined ITS, *TUB*, *CAM* and *RPB2* sequence dataset. *Penicillium janthinellum* CBS 340.48 and *P. limosum* CBS 339.97 were selected as the outgroup. The combined dataset comprised 2,630 characters (ITS, 1−564 bp; *TUB*, 565−1,102 bp; *CAM*, 1,103−1,701 bp; *RPB2*, 1,702−2,630 bp), including gaps. RAxML analysis of the integrated dataset yielded the best-scoring tree with a final ML optimisation likelihood value of -26380.0905. The matrix contained 1,279 distinct alignment patterns, with 13.06% of the characters being undetermined or gaps. The estimated base frequencies were recorded as follows: A = 0.2238, C = 0.2765, G = 0.2706 and T = 0.2291, while the substitution rates were as follows: AC = 1.0947, AG = 3.5202, AT = 1.1705, CG = 0.7818, CT = 5.4306 and GT = 1.0000. The gamma distribution shape parameter alpha value was equal to 0.2342, while the tree length was equal to 2.4771. The final average standard deviation of the split frequencies at the end of the total MCMC generations was computed as 0.003644 via BI analysis.

Phylogenetic analysis of 111 taxa from *Penicillium* section *Lanata-Divaricata* (including *P. chiangmaiense* MBSZU 24-009 and MBSZU 24-010) was performed using a combined ITS, *TUB*, *CAM* and *RPB2* sequence dataset.

Penicillium alogum CBS 140996 and *P. stolkiae* CBS 315.67 were selected as outgroups. The combined dataset comprised 2,549 characters (ITS, 1–563 bp; *TUB*, 564–1,114 bp; *CAM*, 1,115–1,794 bp; *RPB2*, 1,795–2,549 bp), including gaps. RAxML analysis of the integrated dataset yielded the best scoring tree with a final ML optimisation likelihood value of -35195.9174. The matrix contained 1,381 distinct alignment patterns with 12.17% undetermined characters or gaps. The estimated base frequencies were recorded as follows: A = 0.2214, C = 0.2908, G = 0.2615 and T = 0.2263, while the substitution rates were as follows: AC = 1.1361, AG = 3.5568, AT = 1.5061, CG = 0.7521, CT = 5.3860 and GT = 1.0000. The gamma distribution shape parameter alpha value was equal to 0.2744, while the tree length was equal to 3.5928. The final average standard deviation of the split frequencies at the end of the total MCMC generations was computed as 0.005628 via BI analysis.

Phylogenetic analysis of species in subclass Xylariomycetidae was performed using a combined ITS, LSU, *RPB2* and *TUB* sequence dataset of MBSZU 25-005 (proposed as *Pseudoleptodontidium chiangmaiensis*), together with 118 taxa of the subclass. *Achaetomium macrosporum* CBS 532.94, *Chaetomium elatum* CBS 374.66 and *Sordaria fimicola* CBS 723.96 were selected as outgroups. The combined dataset comprised 3,560 characters (ITS, 1–693 bp; LSU, 694–1,592 bp; *RPB2*, 1,593–2,656 bp; *TUB*, 2,657–3,560 bp), including gaps. RAxML analysis of the integrated dataset yielded the best scoring tree with a final ML optimisation likelihood value of -83630.121273. The matrix contained 2,615 distinct alignment patterns with 39.42% undetermined characters or gaps. The estimated base frequencies were recorded as follows: A = 0.256414, C = 0.231937, G = 0.280501 and T = 0.231149, while the substitution rates were as follows: AC = 0.888171, AG = 2.661198, AT = 1.161270, CG = 0.868099, CT = 3.494813 and GT = 1.000000. The gamma distribution shape parameter alpha value was equal to 0.351763, while the tree length was equal to 15.592567. The final average standard deviation of the split frequencies at the end of the total MCMC generations was computed as 0.009989 via BI analysis.

Topologically, the ML and BI phylogenetic trees of all fungal species had similar results; therefore, only the ML phylogram was demonstrated in this study. The phylogram of *Penicillium* section *Exilicaulis* showed that two new strains (MBSZU 24-007 and MBSZU 24-008) separated from other recognised species with 100% BS and 1.00 PP supports (Fig. 2). These fungal strains formed a sister clade with *P. laeve* DTO270G8 (BS 99% and PP 1.00) and belonged to the series *Erubescentia*.

While the phylogram of *Penicillium* section *Lanata-Divaricata* exhibited that MBSZU 24-009 and MBSZU 24-010 formed a distinct clade, clearly separated from other taxa with significant support (BS 100% and PP 1.00; Fig. 3). These strains also formed a sister clade with *P. brefeldianum* CBS 235.81 (BS 100% and PP 1.00) within the Series *Janthinella* clade.

The phylogram of Xylariomycetidae showed that MBSZU 25-005 clustered amongst families and taxa in Amphisphaeriales. This strain also formed a sister clade to *Neoleptodontidium aciculare* CBS 123.86 and *N. aquaticum* CBS 149455 (BS 96% and PP 1.00; Fig. 4).
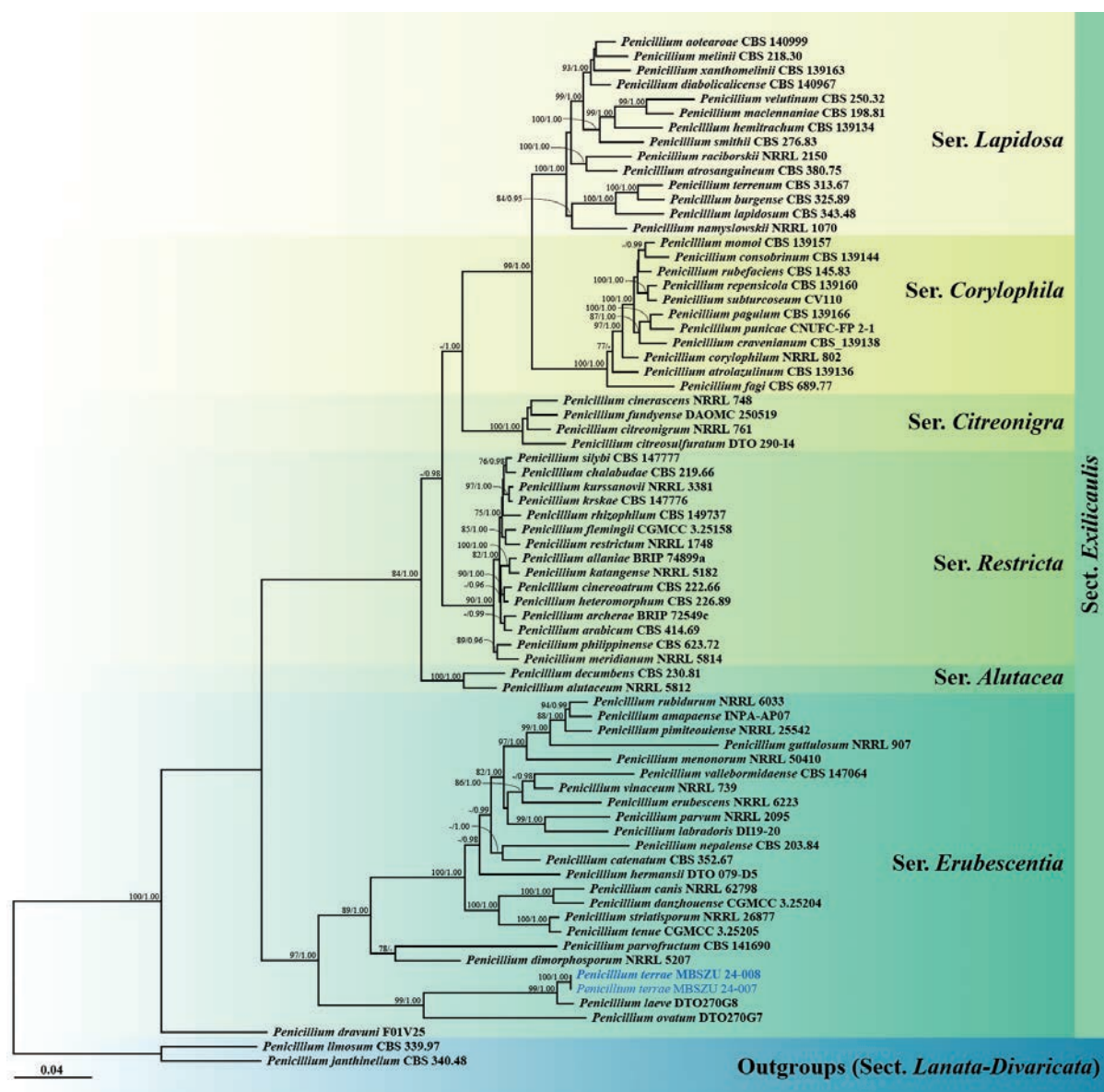
Figure 2. Phylogram generated from Maximum Likelihood analysis of 72 specimens belonging to the genus *Penicillium* section *Exilicaulis*, using the combined ITS, *TUB, CAM* and *RPB2* genes. *Penicillium janthinellum* CBS 340.48 and *P. limosum* CBS 339.97 were used as the outgroup. The numbers above branches show bootstrap percentages (left) and Bayesian Posterior Probabilities (right). Bootstrap values ≥ 75% and Bayesian Posterior Probabilities ≥ 0.95 are shown. The scale bar reflects the estimated number of nucleotide substitutions per site. The fungal strains in this study are blue. Type species are bold.

Taxonomy

Penicillium terrae Thitla, Monkai, Lumyong & Hongsanan, sp. nov.
MycoBank No: 857423

Etymology. The specific epithet *terrae* refers to the soil substrate, from which this species was isolated.

Holotype. THAILAND • Chiang Mai Province, Mae Taeng District, Papae, on soil in the forest dump-sites, 20 June 2024, T. Thitla & J. Monkai; VR040 (SZU25-005, holotype); ex-type living culture, MBSZU 24-008, dried culture permanently preserved in a metabolically inactive state, SZU25-005.
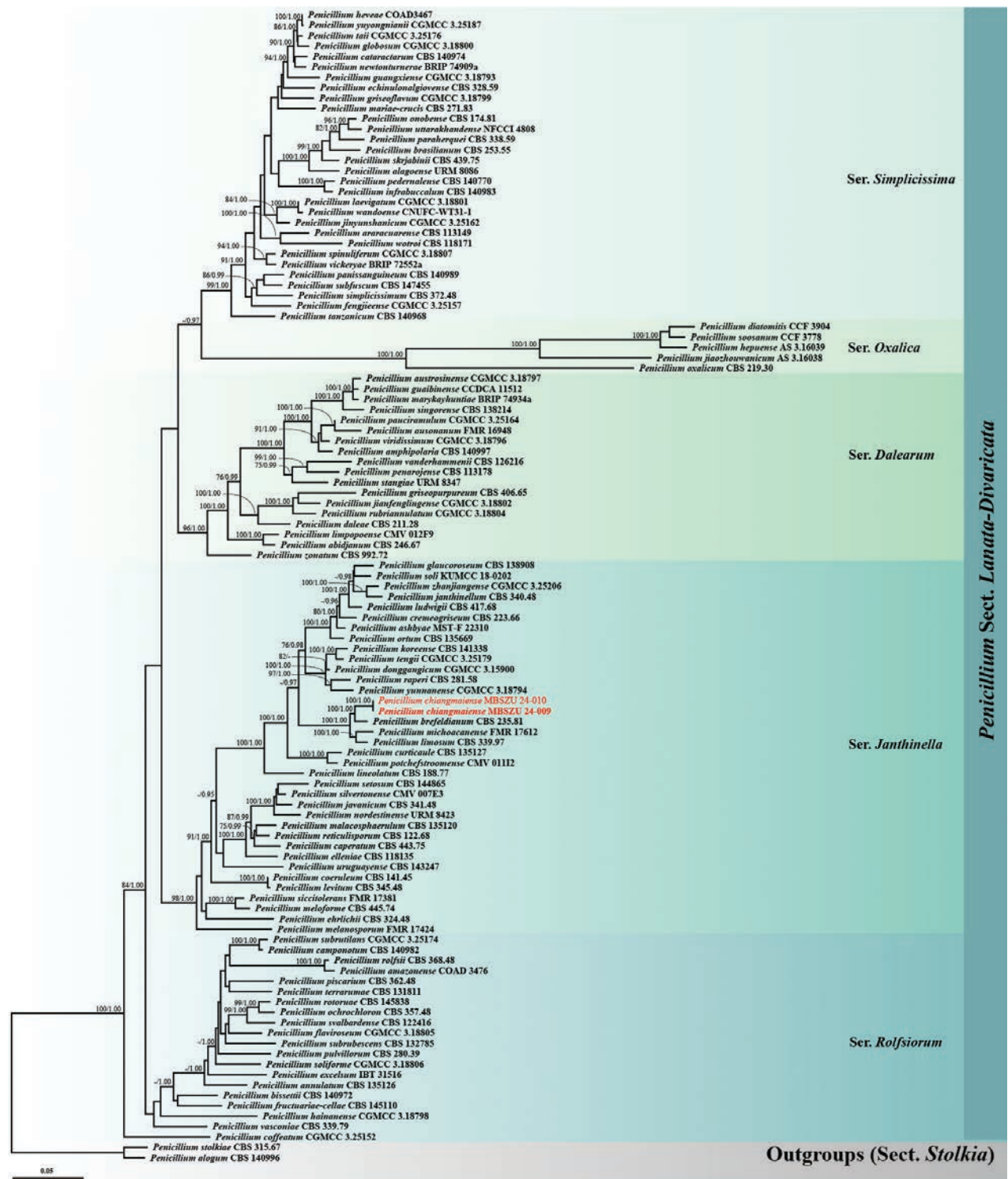
Figure 3. Phylogram generated from Maximum Likelihood analysis of 111 specimens belonging to the genus *Penicillium* section *Lanata-Divaricata* using the combined ITS, *TUB*, *CAM* and *RPB2* genes. *Penicillium alogum* CBS 140996 and *P. stolkiae* CBS 315.67 were used as the outgroup. The numbers above branches show bootstrap percentages (left) and Bayesian Posterior Probabilities (right). Bootstrap values ≥ 75% and Bayesian Posterior Probabilities ≥ 0.95 are shown. The scale bar reflects the estimated number of nucleotide substitutions per site. The fungal strains in this study are red. Type species are bold.
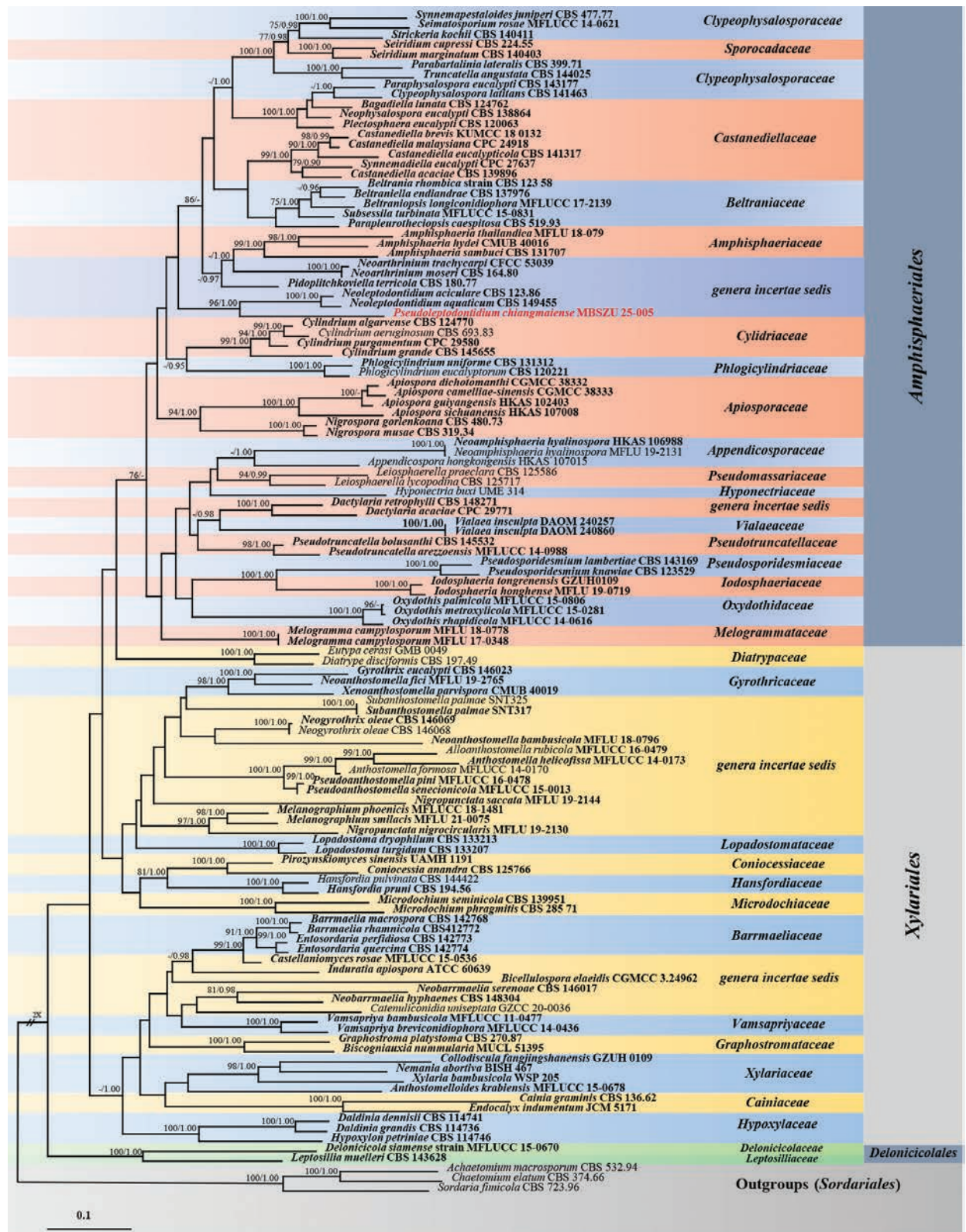
Figure 4. Phylogram generated from Maximum Likelihood analysis of 119 specimens belonging to the subclass Xylariomycetidae of the combined ITS, LSU, *RPB2* and *TUB* genes. *Achaetomium macrosporum* CBS 532.94, *Chaetomium elatum* CBS 374.66 and *Sordaria fimicola* CBS 723.96 were used as the outgroup. The numbers above branches show bootstrap percentages (left) and Bayesian Posterior Probabilities (right). Bootstrap values ≥ 75% and Bayesian Posterior Probabilities ≥ 0.95 are shown. The scale bar reflects the estimated number of nucleotide substitutions per site. The fungal strains in this study are red. Type species are bold.

Colony diam. (in mm) 7 days, 25 °C: CREA 8–11, CYA 13–18, CYAS 7–9, CZ 11–15, DG18 12–16, MEA 15–19, MEAbl 16–19, OA 13–19, PDA 12–15 and YES 9–13. 7 days, 30 °C: CYA 10–15. 7 days, 37 °C: CYA no growth.

Culture characteristics. Colonies at 25 °C for 7 days on CREA thin colonies; acid production absent (Fig. 5A). Colonies on CYA circular, convex, wrinkled texture, entire margin; white mycelia; soluble pigment absent; reverse yellowish-brown (Fig. 5B). Colonies on CYAS barely growing, circular, raised, wrinkled texture, undulate margin; white mycelia; soluble pigment absent; reverse white (Fig. 5C). On CZ thin colonies, circular, flat, entire margin; white mycelia; soluble pigment absent; reverse white (Fig. 5D). On DG18 circular, flat, wrinkled at the centre, margin smooth and entire; grey mycelia at the centre, white mycelia at the margin; soluble pigment absent; reverse greenish-grey to light yellow (Fig. 5E). Colonies on MEA circular, flat, smooth texture, entire margin; light grey mycelia; soluble pigment absent; reverse light yellow to white (Fig. 5F). On MEAbl circular, flat, wrinkled at the centre, margin smooth and entire; light grey at the centre, white at the margin; soluble pigment absent; reverse yellowish-brown (Fig. 5G). On OA circular, flat, smooth textured, entire margin; light brown mycelia at the centre, white mycelia at the margin; soluble pigment absent; reverse white (Fig. 5H). Colonies on PDA circular, flat, wrinkled texture, entire margin; white mycelia; soluble pigment absent; reverse white to light yellow (Fig. 5I). Colonies on YES circular, convex, wrinkled texture, entire margin; white mycelia; soluble pigment absent; reverse light brown (Fig. 5J). Sporulation abundantly produces on all media.

Micromorphology. Conidiophores mononematous, growing out at right angles from hyphae, unbranched, smooth, hyaline, 3–14 × 1–3 µm (Fig. 5K–P). Phialides solitary, terminal, ampulliform, smooth, hyaline, 5–12 × 1–4 µm (Fig. 5K–P). Conidia globose to subglobose, 2–4 µm diam., smooth, hyaline (Fig. 5K–N, Q). Sclerotia not observed. Sexual morph absent.

Additional strain examined. Thailand • Chiang Mai Province, Mae Taeng District, Papae, on soil in the forest dump-sites, 20 June 2024, T. Thitla & J. Monkai; CMUVR039; living culture, MBSZU 24-007, dried culture permanently preserved in a metabolically inactive state, CMUVR039.

Habitat and distribution. Soil; only known from Chiang Mai Province, Thailand.

Notes. *Penicillium* section *Exilicaulis* was first established by Pitt (1980), with *P. restrictum* as the type species. This section was initially proposed to accommodate *Penicillium* species characterised by monoverticillate conidiophores and non-vesiculated stipes. Subsequently, phylogenetic studies expanded the section to include species with bi-verticillate conidiophores and those with conidiophores bearing solitary phialides (Houbraken and Samson 2011; Visagie et al. 2016a, b; da Silva et al. 2023). Species of the sect. *Exilicaulis* have been isolated from diverse environments, including soil, marine ecosystems, air, plants and insects (Ansari et al. 2023). Currently, this section comprises over 60 species across six series: *Alutacea*, *Citreonigra*, *Corylophila*, *Erubescentia*, *Lapidosa* and *Restricta* (Ansari et al. 2023; Visagie et al. 2024a).

Penicillium terrae is classified within section *Exilicaulis*, series *Erubescentia*. Phylogenetically, this species is closely related to *P. laeve* and *P. ovatum* (Fig. 2). However, *P. laeve* and *P. ovatum* were unable to grow on CREA and CYAS media, while *P. terrae* can grow on these media. Regarding growth rates, *P. laeve* exhibited slower growth than *P. terrae*, including CYA (8–9 mm), DG18
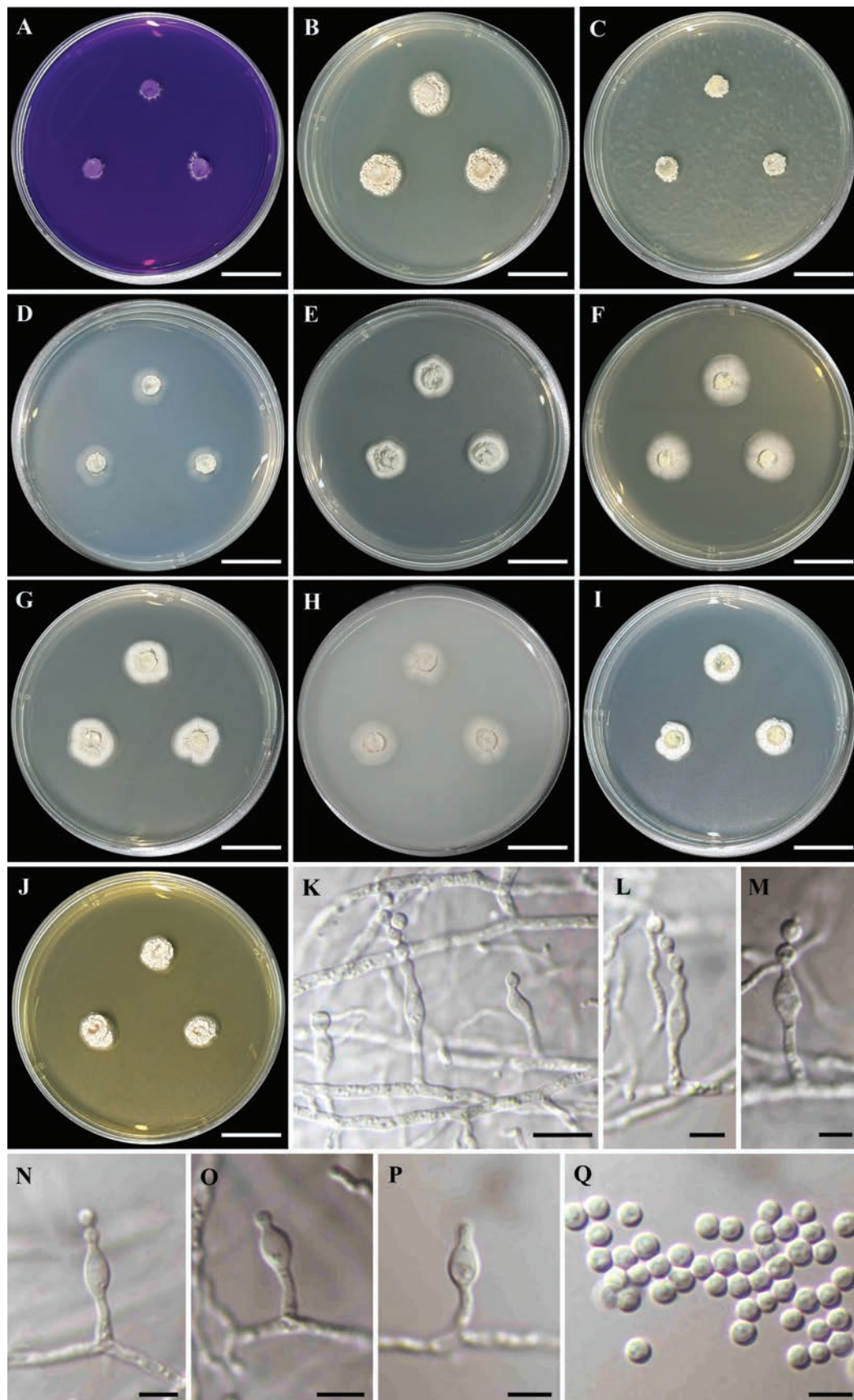
Figure 5. *Penicillium terrae* (MBSZU 24-008, ex-type living culture) **A–J** colonies at 25 °C for 7 days on CREA, CYA, CYAS, CZ, DG18, MEA, MEAbl, OA, PDA and YES, respectively **K–P** conidiophores, phialides and conidia **Q** conidia. Scale bar: 2 cm (**A–J**); 10 μm (**K**); 5 μm (**L–Q**).

(5–7 mm), OA (7–8 mm) and YES (8–9 mm) at 25 °C, as well as CYA at 30 °C (4–5 mm) (Visagie et al. 2016a). Similarly, *P. ovatum* also demonstrated slower growth compared to *P. terrae* on CYA (10–11 mm), DG18 (9–11 mm), MEA (7–8 mm) and OA (10–11 mm) at 25 °C (Visagie et al. 2016a). Micromorphologically, the phialides of *P. laeve* (4–6 μm × 2–3 μm) and *P. ovatum* (4.5–7 μm × 2–3 μm) were shorter than *P. terrae* (Visagie et al. 2016a). In terms of conidia, *P. terrae* produced globose to subglobose conidia with 2–4 μm, while *P. leave* produced globose conidia measuring 2.5–3 μm diam. and *P. ovatum* produced ellipsoidal conidia with 2–3 × 1.5–2 μm (Visagie et al. 2016a). Furthermore, a pairwise nucleotide comparison between *P. terrae* and *P. laeve* showed differences of 0.86% (5/581 bp, including gaps) in ITS, 2.87% (13/453 bp, including gaps) in *TUB*, 2.62% (13/497 bp, including gaps) in *CAM* and 1.39% (13/938 bp, including gaps) in *RPB2*. Similarly, the comparison between *P. terrae* and *P. ovatum* revealed nucleotide differences of 2.64% (15/569 bp, including gaps) in ITS, 14.41% (65/451 bp, including gaps) in *TUB*, 17.74% (91/513 bp, including gaps) in *CAM* and 12.37% (116/938 bp, including gaps) in *RPB2*.

Penicillium chiangmaiense Thitla, Monkai, Lumyong & Hongsanan, sp. nov.
MycoBank No: 857424

Etymology. The specific epithet "*chiangmaiense*" refers to the type locality "Chiang Mai Province, Thailand".

Holotype. Thailand • Chiang Mai Province, Mae Rim District, Mae Sa, on soil in the forest dump-sites, 27 June 2024, T. Thitla & J. Monkai; VR005 (SZU25-006, holotype); ex-type living culture, MBSZU 24-009, dried culture permanently preserved in a metabolically inactive state, SZU25-006.

Colony diam. (in mm) 7 days, 25 °C: CREA 40–44, CYA 50–52, CYAS 35–38, CZ 48–49, DG18 34–39, MEA 47–51, MEAbl 51–53, OA 53–54, PDA 49–50 and YES 32–38. 7 days, 30 °C: CYA 59–61. 7 days, 37 °C: CYA 55–56.

Culture characteristics. Colonies at 25 °C for 7 days on CREA thin colonies; acid production absent (Fig. 6A). Colonies on CYA and CYAS wrinkled texture, velvety, circular, flat, entire margin; white mycelia; soluble pigment absent; reverse light brown (Fig. 6B, C). On CZ, thin colonies, circular, flat, filamentous margin; white mycelia; soluble pigment absent; reverse white (Fig. 6D). On DG18, wrinkled texture, velvety, circular, flat, entire margin; white mycelia; soluble pigment absent; reverse white to pale yellow (Fig. 6E). Colonies on MEA and MEAbl smooth texture, circular, flat, entire margin; pale yellow at the centre, white at the margin; soluble pigment absent; reverse pale brown to white (Fig. 6F, G). On OA, smooth textured, velvety, circular, flat, entire margin; white mycelia; soluble pigment absent; reverse light yellow to white (Fig. 6H). Colonies on PDA circular, flat, smooth texture, entire margin; white mycelia; soluble pigment absent; reverse white to light yellow (Fig. 6I). Colonies on YES circular, flat, wrinkled texture, velvety, entire margin; white mycelia; soluble pigment absent; reverse brownish-yellow (Fig. 6J). Sporulation abundantly produces on DG18, MEA and MEAbl media. Sclerotia produces MEA, MEAbl and OA (Fig. 6P).

Micromorphology. Conidiophores monoverticillate, sometimes divaricate. Stipes hyaline, smooth–walled, 80–270 × 2–3 μm (Fig. 6K–N). Phialides

Figure 6. *Penicillium chiangmaiense* (MBSZU 24-009, ex-type living culture) **A–J** colonies at 25 °C for 7 days on CREA, CYA, CYAS, CZ, DG18, MEA, MEAbl, OA, PDA and YES, respectively **K–N** conidiophores, phialides and conidia **O** conidia **P** sclerotia produced on culture media. Scale bar: 2 cm (**A–J**); 10 μm (**K–O**); 100 μm (**P**).

terminal, ampulliform, hyaline, smooth–walled 6–17 × 2–3.5 μm (Fig. 6K–N). Conidia globose to subglobose, 2–4 μm diam., smooth, hyaline (Fig. 6K–O). Sclerotia pale brown to brown, globose to irregular, 180–260 μm diam. (Fig. 6P). Sexual morph absent.

Additional strain examined. Thailand • Chiang Mai Province, Mae Rim District, Mae Sa, on soil in the forest dump-sites, 27 June 2024, T. Thitla & J. Monkai; CMUVR005-2; living culture, MBSZU 24-010, dried culture permanently preserved in a metabolically inactive state, CMUVR005-2.

Habitat and distribution. Soil; only known from Chiang Mai Province, Thailand.

Notes. *Penicillium* section *Lanata-Divaricata* was established by Thom (1930) to include species with biverticillate conidiophores, which usually contain a main conidiophore axis and metulae that diverge (referred to as divaricate conidiophores), as well as broadly spreading colonies (Houbraken and Samson 2011; Pangging et al. 2021). Species within this section have been isolated from various sources, including soil, air, fluvial sediments and plants (Nóbrega et al. 2024). Currently, the section is divided into five series: *Dalearum*, *Janthinella*, *Oxalica*, *Rolfsiorum* and *Simplicissima* (Ansari et al. 2023; Visagie et al. 2024a).

Penicillium chiangmaiense is classified within section *Lanata-Divaricata*, series *Janthinella*. In the phylogenetic tree (Fig. 3), the new species is closely related to *P. brefeldianum*, *P. limosum* and *P. michoacanense*. However, *P. brefeldianum* produces sexual structures on cornmeal agar and *P. limosum* produces on CZ, MEA and OA, while *P. chiangmaiense* does not exhibit any sexual features (Dodge 1933; Ueda 1995). Furthermore, the growth rate of *P. limosum* on MEA (42 mm in 14 days) was slower than that of *P. chiangmaiense* (47−51 mm in 7 days) (Ueda 1995). In the case of *P. michoacanense*, the stipes (15−60 × 1−1.5 µm) and phialides (4−5 × 1.5 µm) were shorter than those of *P. chiangmaiense* (stipes 80−270 × 2−3 µm; phialides 6−17 × 2−3.5 µm) (Rodríguez-Andrade et al. 2021). Moreover, *P. michoacanense* produced weak acid on CREA, while *P. chiangmaiense* does not produce it (Rodríguez-Andrade et al. 2021). Additionally, the pairwise nucleotide comparison of *P. chiangmaiense* with related species revealed significant differences. The comparison of *P. chiangmaiense* to *P. brefeldianum* showed 0.90% (5/556 bp) difference in ITS, 4.73% (21/444 bp) in *TUB*, 4.28% (24/561 bp) in *CAM* and 1.46% (11/755 bp) in *RPB2*, including gaps. Differences in *P. chiangmaiense* and *P. limosum* were 1.09% (6/548 bp) in ITS, 5.00% (22/440 bp) in *TUB*, 6.28% (35/557 bp) in *CAM* and 0.93% (7/755 bp) in *RPB2*, including gaps. In comparison between *P. chiangmaiense* and *P. michoacanense*, the differences were 0.73% (4/548 bp) in ITS, 2.84% (11/388 bp) in *TUB*, 8.35% (34/407 bp) in *CAM* and 1.61% (12/745 bp) in *RPB2*, including gaps.

Pseudoleptodontidium Thitla, Monkai, Lumyong & Hongsanan, gen. nov.
MycoBank No: 857466

Etymology. The name refers to its morphological similarity to *Leptodontidium*.

Classification. Sordariomycetes, Xylariomycetidae, Amphisphaeriales, *incertae sedis*.

Asexual morph: Mycelium composed of hyaline to black, thin- to thick-walled, smooth, branched, septate. Conidiophores arising from hyphae, solitary, erect, cylindrical, pale brown to dark brown, thick-walled, occasionally roughened on lower part, septate. Conidiogenous cells terminal and intercalary on conidiophores, occasionally lateral on hyphae, obclavate, sympodially proliferate, denticulate, hyaline to pale brown, smooth, septate. Conidia hyaline, smooth, aseptate, subglobose to ellipsoidal, slightly curved. Chlamydospores solitary, terminal on hyphae, medium brown to dark brown, smooth, thick-walled, aseptate, subglobose. Sexual morph: absent.

Type species. *Pseudoleptodontidium chiangmaiense* Thitla, Monkai, Lumyong & Hongsanan, sp. nov.

Notes. Hernández-Restrepo et al. (2017) established *Leptodontidium* in Leptodontidiaceae (Helotiales, Leotiomycetes), characterised by erect conidiophores and conidiogenous cells with a long rachis bearing denticles, as well as the presence of a *Beauveria*-like synasexual morph. *Neoleptodontidium* was introduced by Crous et al. (2023) due to its morphological resemblance to *Leptodontidium*, but it differs in having minute, terminal and lateral exophiala-like phialides. Based on LSU phylogeny, the type species of *Neoleptodontidium* (*N. aquaticum*) clustered with *Leptodontidium aciculare* (Crous et al. 2023). Hence, Crous et al. (2023) transferred *L. aciculare* to *Neoleptodontidium* as *N. aciculare* by the morphological and phylogenetic congruence.

Pseudoleptodontidium is morphologically similar to *Neoleptodontidium*, sharing septate, subcylindrical conidiophores, terminal and lateral phialidic conidiogenous cells and aseptate subcylindrical conidia (Crous et al. 2023). However, *Pseudoleptodontidium* can be distinguished from *Neoleptodontidium* by its obclavate, sympodially proliferating, denticulate conidiogenous cells and subglobose to ellipsoidal conidia. The phylogeny, based on a combined ITS, LSU, *RPB2* and *TUB* dataset, revealed that *Pseudoleptodontidium* forms an independent lineage, sister to *Neoleptodontidium* with significant support (BS 96% ML and PP 1.00; Fig. 4). Although Crous et al. (2023) placed *Neoleptodontidium* in Xylariales genera *incertae sedis*, our phylogeny indicates that *Pseudoleptodontidium* and *Neoleptodontidium* are closely related to the Amphisphaeriaceae, Cylidriaceae, Phlogicylindriaceae and Amphisphaeriales genera *incertae sedis* (*Neoarthrinium, Pidoplitchkoviella*) (Fig. 4). Therefore, due to their distinct morphology and phylogeny, *Pseudoleptodontidium* is introduced as a genus *incertae sedis* in Amphisphaeriales, with *Ps. chiangmaiense* designated as the type species.

Pseudoleptodontidium chiangmaiense Thitla, Monkai, Lumyong & Hongsanan, sp. nov.

MycoBank No: 857467

Etymology. The specific epithet *chiangmaiense* refers to the type locality, Chiang Mai Province, Thailand.

Holotype. Thailand•Chiang Mai Province, Mueang Chiang Mai District, Su Thep, on soil in the forest dump-sites, 21 June 2024, T. Thitla & J. Monkai; VR044 (SZU25-007, holotype); ex-type living culture, MBSZU 25-005, dried culture permanently preserved in a metabolically inactive state, SZU25-007.

Colony diam. (in mm) 14 days, 25 °C: PDA 36–40 and MEA 31–38.

Culture characteristics. Colonies at 25 °C for 14 days on PDA velvety, circular, flat, entire margin; dark green at the centre, greenish-yellow at the middle, white at the margin; soluble pigment absent; reverse dark green to pale yellow, white at the margin (Fig. 7A). Colonies on MEA velvety, circular, flat, entire margin; dark green to black at the centre, yellowish-green to white at the margin; soluble pigment absent; reverse dark green at the centre, pale yellow to white at the margin (Fig. 7B).

Micromorphology. Mycelium composed of hyaline to black, thin- to thick-walled, smooth, branched, septate, 2–4.5 µm diam. hyphae (Fig. 7C–L). Conidiophores arising from hyphae, solitary, erect, cylindrical, pale brown to dark
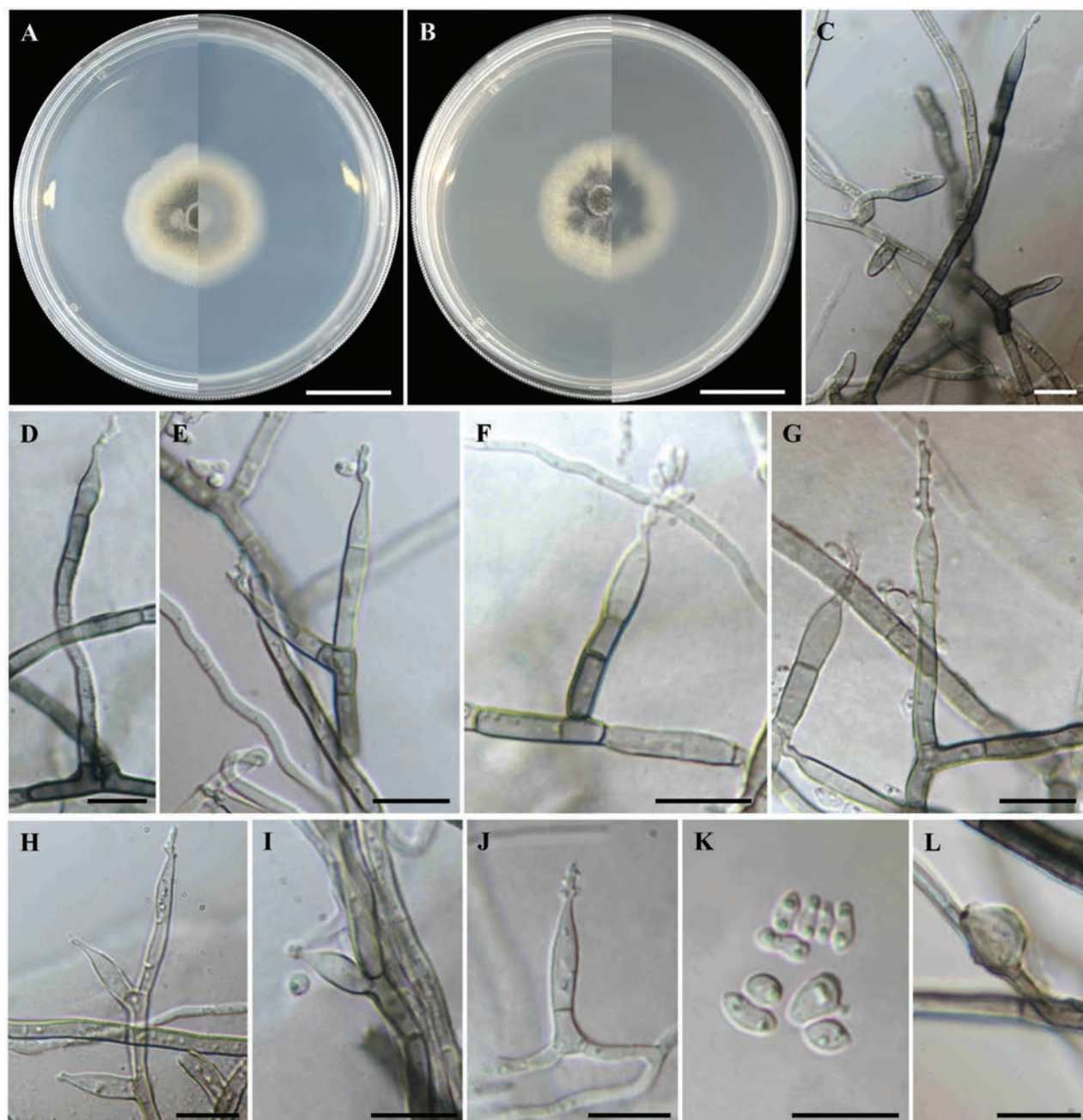
Figure 7. *Pseudoleptodontidium chiangmaiense* (MBSZU 25-005, ex-type living culture) **A, B** colonies from surface and reverse view at 25 °C for 14 days on PDA and MEA, respectively **C–J** conidiophores, conidiogenous cells and conidia **K** conidia **L** chlamydospore. Scale bar: 2 cm (**A, B**); 10 µm (**C–L**).

brown, thick-walled, occasionally roughened on lower part, septate, 7–70 × 2.5–5 µm (Fig. 7C–G). Conidiogenous cells terminal and intercalary on conidiophores, occasionally lateral on hyphae, obclavate, sympodially proliferate, denticulate, hyaline to pale brown, smooth, 0-1 septate, 7.5–26 × 3–5 µm (Fig. 7C–J). Conidia hyaline, smooth, aseptate, subglobose to ellipsoidal, slightly curved, 3–7.5 × 1.5–4 µm (Fig. 7K). Chlamydospores solitary, terminal on hyphae, medium brown to dark brown, smooth, thick-walled, aseptate, subglobose, 6–8 × 4.5–6 µm (Fig. 7L). Sexual morph absent.

Habitat and distribution. Soil; only known from Chiang Mai Province, Thailand.

Notes. *Pseudoleptodontidium chiangmaiense* has a close relationship with *Neoleptodontidium aciculare* and *N. aquaticum* (Fig. 4). However, their morphological characteristics are distinct: *Ps. chiangmaiense* has broader conidiogenous cells (7.5−26 × 3−5 µm) than *N. aciculare* (15−30 × 2−3 µm) and *N. aquaticum* (10−30 × 2−2.5 µm) and larger conidia (3−7.5 × 1.5−4 µm) than *N. aciculare* (3−4 × 1−2 µm) and *N. aquaticum* (3−4 × 1.5 µm) (Rao and De Hoog 1986; Hernández-Restrepo et al. 2017). The pairwise nucleotide comparison between *Ps. chiangmaiense* and *N. aciculare* revealed differences of 16.38% (95/580 bp, including gaps) in the ITS region and 4.92% (40/813 bp, including gaps) in the LSU region. Additionally, the comparison between *Ps. chiangmaiense* and *N. aquaticum* revealed differences of 16.23% (87/536 bp, including gaps) in the ITS region and 4.80% (39/813 bp, including gaps) in the LSU region.

Discussion

This study identifies a new genus in Xylariomycetidae, namely *Pseudoleptodontidium*, accommodating *Ps. chiangmaiense* sp. nov., along with two new species of *Penicillium*: *P. terrae* in section *Exilicaulis* and *P. chiangmaiense* in section *Lanata-Divaricata*. These species were isolated from soil collected in forest dump-sites in Chiang Mai Province, Thailand. They were characterised through morphological observations and multigene phylogenetic analyses (Figs 2−6).

Penicillium is a highly impactful genus, with species ranging from mycotoxin-producing plant pathogens and opportunistic animal and human pathogens to valuable sources of enzymes, antibiotics and bioactive compounds (Oshikata et al. 2013; Perrone and Susca 2017; Costa et al. 2019; Toghueo and Boyom 2020; Wolski 2023; Suwannarach et al. 2024). The genus was proposed by Link (1809) and currently comprises two subgenera, 34 sections, 102 series and 535 accepted species (Visagie et al. 2024a). *Penicillium* was traditionally identified, based on macro-morphology (such as colony characteristics and pigment production) and micro-morphology (including conidiophores, branches, metula, phialides and conidia) (Khuna et al. 2023). However, relying solely on morphological characteristics has proven insufficient for accurate identification. Consequently, an integrated approach combining morphology, molecular data and extrolite analysis is currently used to identify species within the genus *Penicillium* (Visagie et al. 2014; Labuda et al. 2021; Nguyen and Pham 2022; Visagie et al. 2024a, 2024b). In section *Exilicaulis*, key genetic data for species identification include the internal transcribed spacer region (ITS), beta-tubulin (*TUB*), calmodulin (*CAM*) and RNA polymerase II subunit (*RPB2*) genes (Visagie et al. 2016c). Initially, *P. laeve* and *P. ovatum* were introduced under the genus *Torulomyces* as *T. laevis* and *T. ovatus*, respectively (Ando et al. 1998). Subsequently, phylogenetic analyses using RNA polymerase II largest subunit (*RPB1*), *RPB2*, the protein required for processing of 20S pre-rRNA in the cytoplasm (*Tsr1*) and the subunit of the cytosolic chaperonin Cct ring complex (*Cct8*) led to transfer to *Penicillium* section *Torulomyces* (Houbraken and Samson 2011). Visagie et al. (2016a) reclassified these species into section *Exilicaulis* using ITS, *TUB*, *CAM* and *RPB2* sequence data. Currently, *P. laeve* and *P. ovatum* belong to the series *Erubescentia*, characterised by species with monoverticillate conidiophores, short stipes and the ability to grow at 37 °C (Houbraken et al.

2020). However, both *P. laeve* and *P. ovatum*, along with *P. terrae*, produce conidiophores with solitary phialides and were unable to grow at 37 °C. Additionally, the phylogenetic clade of these species formed a basal clade with other species in this series with strong support (BS 97% and PP 1.00) (Fig. 2). In our opinion, this distinct clade may represent a potential new series within section *Exilicaulis* and should be further studied in the future.

Prior to this study, *P. laeve* was the only species in section *Exilicaulis* reported from Thailand (Ando et al. 1998). The discovery of *P. terrae* from soil in Thailand marks the second species from this section identified in the country. Furthermore, this new species represents the 69th global species in section *Exilicaulis*, as shown in Suppl. material 1: table S1, excluding *P. janthinellum* and *P. limosum*. In addition, this study proposed a new species, *P. chiangmaiense* in section *Lanata-Divaricata*, which is the second species recorded in Thailand from this section, following the first species (*P. singorense*) described by Visagie et al. (2014b). Additionally, this new species represents the 108th global species in section *Lanata-Divaricata*, as outlined in Suppl. material 1: table S2, excluding *P. alogum* and *P. stolkiae*.

Ecologically, *Penicillium* species have been isolated from different environments (Ansari et al. 2023; Nóbrega et al. 2024). For instance, *P. chiangmaiense* and its closely-related species, including *P. brefeldianum*, *P. limosum* and *P. michoacanense*, have been found in the human digestive tract, marine sediments and soil (Dodge 1933; Ueda 1995; Rodríguez-Andrade et al. 2021). Similarly, *P. terrae* and its relatives, including *P. laeve* and *P. ovatum*, have primarily been reported from soil, with *P. laeve* specifically found in forest soils in Thailand (Ando et al. 1998). These findings highlight the ecological plasticity of *Penicillium* species, which can potentially thrive in disturbed ecosystems. Future studies examining their functional traits and metabolic profiles could further enhance better understanding of their ecological significance.

Xylariomycetidae is a large subclass within Sordariomycetes comprising numerous taxa that are polyphyletic and paraphyletic (Wendt et al. 2017; Daranagama et al. 2018; Konta et al. 2020; Samarakoon et al. 2022). The taxonomic classification of Xylariomycetidae has undergone considerable change (Maharachchikumbura et al. 2016; Samarakoon et al. 2016, 2022). Earlier, Amphisphaeriales was considered a synonym of Xylariales (Maharachchikumbura et al. 2016). However, based on morphology, molecular data, divergence estimates and ancestral state reconstruction, Samarakoon et al. (2016, 2022) subsequently reclassified Amphisphaeriales, Delonicicolales and Xylariales in Xylariomycetidae. Molecular phylogeny, based on concatenated ITS, LSU, *RPB2*, *TUB* and *TEF-1α* sequence data, demonstrated the placement of Amphisphaeriales in a sister clade to Xylariales (Samarakoon et al. 2022), which is consistent with our study (Fig. 4). However, we did not incorporate *TEF-1a* into the phylogenetic tree, as the number of taxa with available sequence data was low. The classification of taxa within Xylariomycetidae remains ambiguous, as more than 50 *incertae sedis* genera await taxonomic resolution (Samarakoon et al. 2022). Likewise, our study was unable to assign the novel genus *Pseudoleptodontidium* to any family within the Xylariomycetidae (Fig. 4). The new lineage of *Pseudoleptodontidium* and *Neoleptodontidium* also lacks significant statistical support for placement within other taxa and families in Amphisphaeriales, though it is likely linked to Amphisphaeriaceae, Cylidriaceae and Phlogicylindriaceae (Fig. 4). Further taxonomic and phylogenetic studies, including the collection of new

specimens and the examination of additional isolates, are necessary to confirm the familial placement of *Pseudoleptodontidium* and *Neoleptodontidium*.

Members of Xylariomycetidae have a worldwide distribution and occupy various ecological niches, including saprobes, endophytes and pathogens (U'Ren et al. 2016; Daranagama et al. 2018; Sugita et al. 2022; Samarakoon 2024). Recently, several new taxa have been reported as saprobes on dead plant materials from Thailand (Monkai et al. 2022; Afshari et al. 2023; Samarakoon et al. 2023; Karimi et al. 2023, 2024; Samarakoon 2024; Thakshila et al. 2024). In this study, *Pseudoleptodontidium* was isolated from soil associated with a forest dump-site in Thailand, whereas *Neoleptodontidium* species have been found in hydroponic water and decomposing wood in the USA and India (Rao and De Hoog 1986; Hernández-Restrepo et al. 2017). This demonstrates that these taxa have a broad distribution range, highlighting their adaptability in diverse environments.

These findings significantly contribute to our understanding of fungal diversity and ecology, particularly within the Ascomycota and highlight the richness and diversity of soil fungal communities in Thailand. *Penicillium* and some Xylariomycetidae taxa, such as *Amphisphaeria*, *Annulohypoxylon* and *Hypoxylon* are recognised for possessing a wide variety of secondary metabolites, which have prospective agricultural and therapeutic uses (Toghueo and Boyom 2020; Becker and Stadler 2021; Wang et al. 2023a; Wolski 2023). The discovery of novel fungi in forest dump areas presents an opportunity to explore and characterise these fungi for various applications. Therefore, further research is necessary to evaluate the capabilities of new fungal strains for extracellular enzyme production and the degradation of synthetic materials.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualisation: Tanapol Thitla, Jutamart Monkai, Sinang Hongsanan, Saisamorn Lumyong. Collection and morphological examinations: Tanapol Thitla, Jutamart Monkai, Weiqian Meng, Surapong Khuna. Molecular sequencing and phylogenetic analyses: Tanapol Thitla, Jutamart Monkai, Weiqian Meng, Surapong Khuna. Original draft preparation: Tanapol Thitla, Jutamart Monkai, Surapong Khuna. Review and editing, supervision: Tanapol Thitla, Jutamart Monkai, Weiqian Meng, Surapong Khuna, Ning Xie, Sinang Hongsanan, Saisamorn Lumyong. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Tanapol Thitla ● https://orcid.org/0000-0003-3092-0679
Jutamart Monkai ● https://orcid.org/0000-0001-6043-0625
Weiqian Meng ● https://orcid.org/0009-0006-3840-0992
Surapong Khuna ● https://orcid.org/0000-0003-1999-4001
Ning Xie ● https://orcid.org/0000-0002-5866-8535
Sinang Hongsanan ● https://orcid.org/0000-0003-0550-3152
Saisamorn Lumyong ● https://orcid.org/0000-0002-6485-414X

Data availability

The datasets generated during and/or analysed during the current study are available in the MycoBank repository (included in the manuscript) and GenBank (included in Suppl. material 1: tables S1–S3). Additionally, the datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Supplementary material 1

Additional information

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Four new species of *Entoloma* subgenus *Cyanula* (Entolomataceae, Agaricales) from subtropical regions of China

Lin-Gen Chen[1], Hong Chen[1], Ling Ding[1], Yu-Qin Xu[1], Hui Zeng[2], Sheng-Nan Wang[1,3], Jun-Qing Yan[1]

1   Jiangxi Provincial Key Laboratory of Excavation and Utilization of Agricultural Microorganisms, Jiangxi Agricultural University, Nanchang 330045, China
2   Institute of Edible mushroom, Fujian Academy of Agricultural Sciences, Fuzhou 350011, China
3   Jiangxi Provincial Key Laboratory of Subtropical Forest Resource Cultivation, College of Forestry, Jiangxi Agricultural University, Nanchang 330045, China
Corresponding author: Jun-Qing Yan (yanjunqing1990@jxau.edu.cn)

Abstract

In this study, four species of *Entoloma* subgenus *Cyanula* (*E. orientosinense*, *E. subgriseosquamulosum*, *E. subpraegracile*, and *E. wuyishanense*) from subtropical regions of China, are described as new to science based on morphological and phylogenetic analyses. Morphologically, *E. orientosinense* is characterized by the white basidiomata, relatively large basidiospores, and carneogriseum-type lamellae edge; *E. subgriseosquamulosum* is recognized by the fuscous pileus, crowded and adnate lamellae, and medium-sized basidiospores; *E. subpraegracile* is identified by the yellow pileus and intervenose lamellae with sterile or heterogeneous edge; *E. wuyishanense* is distinct by the blue basidiomata and fertile lamellae edge with slightly bluish pigmentation near the stipe. *Entoloma orientosinense* belongs to sect. *Caesiocincta*, subsect. *Queletia*, and *E. wuyishanense* belongs to sect. *Poliopodes*. The remaining two species each form independent branches and do not belong to any known sections. Detailed descriptions, color photos, and a key to related species are presented.

Key words: Basidiomycetes, new taxa, phylogeny, taxonomy

Introduction

Entoloma (Fr.) P. Kumm. is one of the most diverse genera within Agaricales, well-characterized by pink to brownish spore prints and angular basidiospores viewed in all views (Co-David et al. 2009). It shows extremely wide geographical distribution, occurring from the frigid zone to the tropics, and from alpine to basins, with most members being saprobic on shady and humid soil, mosses, or rotten wood in forests (Horak 1980; Noordeloos 1981; Largent 1994; Reschke et al. 2022b). So far, approximately 2000 *Entoloma* species have been reported in the world. In China, however, there are relatively few reports about the species of this genus, with approximately 200. Among them, some of the newly discovered species published earlier were not classified into specific subgenera (Bi et al. 1986; Zhang et al. 1994; He et al. 2011).

In the past, based on morphological taxonomy, *Cyanuli* was introduced by Romagnesi (1974) as a section within *Rhodophyllus* Quél (= *Entoloma*). The combination *Entoloma* section *Cyanula* (Romagn.) Noordeloos belonged to *Entoloma* subgenus

Leptonia of wide sense. However, the subg. *Leptonia*, traditionally divided into three sections, viz. *Leptonia*, *Cyanula*, and *Griseorubida* (Noordeloos 2004), turned out to be polyphyletic. Sect. *Leptonia* of belonged to the /*Nolanea-Claudopus* clade, and *Cyanula* and *Griseorubida* to the /*Inocephalus-Cyanula* clade (Co-David et al. 2009). Morphologically, species of sect. *Leptonia* exhibited clamp connections, whereas species of *Cyanula* lacked clamp connections. Based on these, sect. *Cyanula* was elevated to subgenus rank (Noordeloos and Gates 2012; Noordeloos et al. 2022a).

The species of *Entoloma* subg. *Cyanula* are mainly characterized by their collybioid habit, vividly colorful (often blue, violaceous to brown) and squamulose pileus, absence of clamp connections, and presence of brilliant granules and intracellular pigments in hyphae. So far, at least 500–600 species of *E.* subg. *Cyanula* have been discovered worldwide.

According to previous studies, there are 13 species belonging to *Entoloma* subg. *Cyanula* in China, 7 of which were newly described (He et al. 2011, 2012; He et al. 2017). In the past few years, during our surveys on the diversity of macrofungi in the subtropical regions of China, we have found that the species diversity under *E. subg. Cyanula* is extremely rich. In this study, four species of this subgenus are newly described based on morphological comparisons and phylogenetic analyses.

Materials and methods

Morphological studies

The collection sites of the specimens in this study were all located in the subtropical region of East China, and these specimens were deposited in the Herbarium of Fungi, Jiangxi Agricultural University (HFJAU). Fresh specimens were photographed in the field and macroscopically recorded. The color notations followed the Methuen Handbook of Colour (Kornerup and Wanscher 1978). Microscopic structures were studied under an Olympus BX53 microscope (Olympus corporation, Tokyo, Japan) by making squash preparations of sections of dried specimens. The sections were hydrated with 5% KOH solution or H_2O, and 1% Congo red was used as the staining agent when observing colorless tissues. Melzer's reagent was selected for determining whether the spores were amyloid or not (Horak 2005). At least 20 basidiospores, basidia, and cystidia were measured for each collection. The range of spore size is expressed as the form (a) b–c (d), in which "a" and "d" represent the minimum and maximum values, and 90% of the spores falling within the range "b–c". The meanings of the other spore characteristics were as follows: "Q" stood for the ratio of length and width; "av" symbolized average value; "n" meant the number of measurements; and "Qm" indicated average "Q" ± standard deviation (Bas 1969). The morphological descriptions were based on the work of Noordeloos et al. (2022a).

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from dried specimens with the NuClean Plant Genomic DNA kit (CWBIO, China) (Wang et al. 2022). The nrDNA ITS and LSU regions were amplified respectively using the primer pairs of ITS1F/ITS4, LR0R/LR5 (White et al. 1990).

PCR amplification was conducted with a 25 µL reaction system as follows: 1 µL DNA, 1 µL each for forward and reverse primers, 9.5 µL ddH$_2$O, and 12.5 µL 2 × Taq Master Mix (Dye Plus, Vazyme Biotechnology Co. Ltd., Nanjing City, China). PCR was carried out using a touchdown amplification procedure following Chen et al. (2024). The PCR products were sequenced by Qing Ke Biotechnology Co. Ltd. (Wuhan City, China).

Alignment and phylogenetic analyses

In total, 173 sequences (126 ITS sequences and 47 LSU sequences) of 126 samples were used for phylogenetic analyses based on Bayesian inference (BI) and Maximum likelihood (ML). The selection of sequences for the phylogenetic analyses was based on the results of ITS BLAST and of Noordeloos et al. (2022a) (Table 1). Some species of *Entoloma* subg. *Nolanea* were designated as outgroups. The ITS sequences and the LSU sequences were separately aligned on the MAFFT online server using the automatic selection of algorithm (Katoh et al. 2019). First, phylogenetic trees were constructed separately for ITS and LSU and their congruence was checked. BI and ML phylogenetic analyses of the concatenated sequences were run using MRBAYES v.3.2.7a (Ronquist et al. 2012) and IQTREE v.2.1.2 (Nguyen et al. 2015), respectively. For the ML analysis, models of sequence evolution were assessed in IQ-Tree prior to the analysis and allowing the partitions of sequences to have different seeds (-spp) and the results were the following: TPM2 + F + I + G4 for ITS and HKY + F + I + G4 for LSU. Ultrafast bootstrap support values were calculated from 1000 replicates. For the BI analysis, the best-fit models were determined by PARTITIONFINDER (Zhang et al. 2020) based on Bayesian information criterion (BIC) and the results were the following: GTR + F + I + G4 for ITS and HKY + F + I + G4 for LSU. The Monte Carlo Markov chains were run for 40 million generations. The first 25% of trees were discarded as burn-in. The nodes with Bayesian posterior probabilities (BI-PP) ≥ 0.95 and ML bootstrap proportions (ML-BP) ≥ 95% were considered as statistically supported. A nexus file containing sequence alignment and the original trees of ML and BI analyses are provided in Suppl. material 1.

Table 1. Details of sequences used in the phylogenetic analyses. Newly generated sequences were in bold.

Species	Location	Voucher Number	GenBank No. (ITS)	GenBank No. (LSU)	Sequence origin
Entoloma albidosimulans	Australia	MEN 2004-065, isotype	—	MK277956	Varga et al. (2019)
E. albinellum	USA	TENN:070403	KY777375	—	Unpublished in GenBank
E. argus	Vietnam	LE F-312694, holotype	OM987263	OM996175	Morozova et al. (2022)
E. argus	Vietnam	LE F-315916	OM987264	—	Morozova et al. (2022)
E. arion	Vietnam	LE F-312691, holotype	OM987259	OM996176	Morozova et al. (2022)
E. arion	Vietnam	LE F-312692	OM987260	—	Morozova et al. (2022)
E. arion	Vietnam	LE F-315917	OM987261	—	Morozova et al. (2022)
E. asprellum	Estonia	TUF106064	UDB011486	—	UNITE
E. atropapillatum	Brazil	FK0898, holotype	KF679354	KF738940	Karstedt et al. (2020)
E. azureosquamulosum	China	HKAS53408	JQ410334	JQ410326	He et al. (2012)
E. azureosquamulosum	China	GDGM29254	JQ410335	—	He et al. (2012)
E. azureosquamulosum	China	GDGM27355, holotype	NR_137086	NG_059214	He et al. (2012)
E. caespitosum	China	GDGM27564	JQ281477	JQ320130	He et al. (2012)
E. caespitosum	China	GDGM24025	JQ281490	JQ410327	He et al. (2012)
E. caespitosum	China	GDGM24026	JQ281491	JQ320133	He et al. (2012)

Species	Location	Voucher Number	GenBank No. (ITS)	GenBank No. (LSU)	Sequence origin
E. calceus	Norway	O-F-259457, holotype	NR_182489	–	Noordeloos et al. (2022b)
E. calceus	France	LIP0402265	ON008492	–	Noordeloos et al. (2022b)
E. calceus	Norway	JL12-19	ON008493	–	Noordeloos et al. (2022b)
E. callipygmaeum	Russia	LE312488	MZ145205	–	Dima et al. (2021)
E. callipygmaeum	Russia	LE312487	MZ145206	–	Dima et al. (2021)
E. callipygmaeum	Russia	LE253784, holotype	MZ145207	–	Dima et al. (2021)
E. carneogriseum	Norway	O-F-256479	UDB07673714	–	UNITE
E. cetratum	Sweden	LE311888, neotype	OL338280	–	Reschke et al. (2022a)
E. chalybeum	Russia	LE254353	KC898445	KC898500	Morozova et al. (2014)
E. chalybeum	Denmark	TUF105760	UDB034191	–	UNITE
E. consanguineum	New Zealand	PDD80751	MW775252	–	Unpublished in GenBank
E. consanguineum	New Zealand	PDD80751	MW775268	–	Unpublished in GenBank
E. coracis	Norway	O-F-256850, holotype	MW934571	MW934251	Crous et al. (2021b)
E. coracis	Norway	O-F-67255	MW934572	–	Crous et al. (2021b)
E. coracis	Norway	O-F-251952	MW934573	–	Crous et al. (2021b)
E. corvinum	France	FA4261	OR419868	–	Armada et al. (2023)
E. cyanostipitum	China	GDGM31318, holotype	KY711237	KY972694	He et al. (2017)
E. cyanostipitum	China	SAAS2239	KY711238	KY972695	He et al. (2017)
E. cyanostipitum	China	GDGM31294	KY972700	KY972693	He et al. (2017)
E. dislocatum	Spain	L0607565, holotype	ON008483	–	Noordeloos et al. (2022b)
E. dislocatum	Spain	SFC-080612-01	ON008484	–	Noordeloos et al. (2022b)
E. dislocatum	Italy	TUF105920, paratype	UDB0799300	–	UNITE
E. exile	Germany	Lueck8	KP965773	KP965791	Karich et al. (2015)
E. exile	–	KM187354	MF977976	–	Unpublished in GenBank
E. griseocyaneum	Russia	LE254351	KC898444	KC898498	Morozova et al. (2014)
E. griseocyaneum	Germany	KaiR997	MZ611684	–	Reschke et al. (2022b)
E. icarus	Vietnam	LE F-312696, holotype	OM987257	OM996174	Morozova et al. (2022)
E. icarus	Vietnam	LE F-312697	OM987258	–	Morozova et al. (2022)
E. incanum	Sweden	LE312503, neotype	OK161247	OK161275	Crous et al. (2021b)
E. incanum	Russia	LE311794	OK161249	OK161276	Crous et al. (2021b)
E. incanum	Russia	LE315858	OK161250	–	Crous et al. (2021b)
E. isborscanum	Russia	LE312486	MW934564	–	Crous et al. (2021a)
E. isborscanum	Russia	LE302088, holotype	MW934566	MW934253	Crous et al. (2021a)
E. linkii	Norway	O-F-256353	UDB07673651	–	UNITE
E. mastoideum	China	GDGM28820	JQ281476	JQ410328	He et al. (2012)
E. mastoideum	China	GDGM26597	JQ291564	JQ320126	He et al. (2012)
E. meridionale	Greece	ACAM2014-0127	OL679698	–	Lebeuf et al. (2021)
E. meridionale	Greece	ACAM2018-0152	OL679699	–	Lebeuf et al. (2021)
E. meridionale	Greece	ACAM2018-0153, holotype	OL679700	–	Lebeuf et al. (2021)
E. minutigranulosum	Russia	LE312484	MZ145210	–	Dima et al. (2021)
E. minutigranulosum	Russia	LE312483	MZ145212	–	Dima et al. (2021)
E. minutigranulosum	Russia	LE302096, holotype	MZ145214	–	Dima et al. (2021)
E. mougeotii	Estonia	TUF106917	UDB015645	–	UNITE
E. mougeotii	Estonia	TUF101633	UDB016265	–	UNITE
E. mougeotii	Estonia	TUF106505	UDB019720	–	UNITE
E. mutabilipes	Finland	TUR610/12	LN850550	–	Kokkonen (2015)
E. mutabilipes	Estonia	TUR8788	LN850551	–	Kokkonen (2015)
E. notabile	Cyprus	L-0607514, holotype	OL343537	–	Vila et al. (2021)
E. olivaceomarginatum	USA	PUL00036174	ON561593	–	Unpublished in GenBank
E. orientosinense	**China**	**HFJAU1414, holotype**	**PQ584686**	**–**	**This work**
E. orientosinense	**China**	**HFJAU1907**	**PQ584690**	**PQ584707**	**This work**
E. orientosinense	**China**	**HFJAU2616**	**PQ584687**	**–**	**This work**
E. orientosinense	**China**	**HFJAU2920**	**PQ584688**	**PQ584708**	**This work**
E. orientosinense	**China**	**HFJAU4048**	**PQ584689**	**PQ584709**	**This work**
E. pallidostriatum	Spain	L-0607566, holotype	NR_177630	–	Vila et al. (2021)

Species	Location	Voucher Number	GenBank No. (ITS)	GenBank No. (LSU)	Sequence origin
E. perasprellum	France	GC01100310, holotype	MZ145177	–	Dima et al. (2021)
E. perasprellum	Sweden	GB-0204547 / JBJ 19-107	MZ145179	–	Dima et al. (2021)
E. perasprellum	Sweden	GB-0204548 / JBJ 19-122	MZ145180	–	Dima et al. (2021)
E. perchalybeum	Sweden	GB-0209474, holotype	NR_182490	–	Noordeloos et al. (2022b)
E. perchalybeum	Finland	TUR190180	ON008495	–	Noordeloos et al. (2022b)
E. poliopus	Estonia	TUF120264	UDB024655	–	UNITE
E. praegracile	China	GDGM29251	JQ281482	JQ320129	He et al. (2013)
E. praegracile	China	GDGM29256	JQ320107	–	He et al. (2013)
E. pseudocoelestinum	Germany	Lueck10	KP965774	KP965792	Karich et al. (2015)
E. pseudocoelestinum	–	KM132400	MF977966	–	Unpublished in GenBank
E. pseudosubcorvinum	Thailand	SDBR-CMUNK0985, holotype	MZ215769	MZ203540	Bhunjun et al. (2022)
E. pseudosubcorvinum	Thailand	SDBR-CMUNK1367	MZ215770	MZ203541	Bhunjun et al. (2022)
E. pulchripes	Russia	LE312485	MZ145187	–	Dima et al. (2021)
E. pulchripes	Russia	LE311808, holotype	MZ145188	–	Dima et al. (2021)
E. pulchripes	Russia	LE311809	MZ145189	–	Dima et al. (2021)
E. queletii	Turkey	OKA-TR1002	MT741747	–	Unpublished in GenBank
E. queletii	Estonia	TUF141044	UDB07674927	–	UNITE
E. riparium	Italy	L-0607563, holotype	NR_177632	–	Vila et al. (2021)
E. riparium	Estonia	TUF120259	UDB024650	–	UNITE
E. septentrionale	Norway	O-F-254295, holotype	NR_174647	–	Noordeloos et al. (2021)
E. sericeum	Germany	KaiR237	OL338118	OL338542	Reschke et al. (2022a)
E. sericeum	–	VHAs03 2	DQ367430	DQ367423	Unpublished in GenBank
E. serrulatum	Norway	O-F-158208/DMS-730296	MZ869016	–	Reschke et al. (2022b)
E. serrulatum	Russia	LE254361	KC898447	KC898501	Morozova et al. (2014)
E. serrulatum	Iran	EnSe-1	KT833862	–	Unpublished in GenBank
E. sicoense	Portugal	PO F2244, holotype	OR026624	–	Fachada et al. (2023)
E. sicoense	Portugal	PO F2245	OR026625	–	Fachada et al. (2023)
E. subcaesiocinctum	China	SAAS103	KY711235	KY972698	He et al. (2017)
E. subcaesiocinctum	China	SAAS133, holotype	KY711236	KY972697	He et al. (2017)
E. subcorvinum	USA	MGW1494	KY744168	–	Unpublished in GenBank
E. subcorvinum	USA	SAT1518905	KY744169	–	Unpublished in GenBank
E. subgriseosquamulosum	**China**	**HFJAU3967**	**PQ584696**	**–**	**This work**
E. subgriseosquamulosum	**China**	**HFJAU3969, holotype**	**PQ584697**	**PQ584721**	**This work**
E. subpraegracile	**China**	**HFJAU1822, holotype**	**PQ584698**	**PQ584710**	**This work**
E. subpraegracile	**China**	**HFJAU3094**	**PQ584706**	**PQ584711**	**This work**
E. subpraegracile	**China**	**HFJAU3164**	**PQ584700**	**PQ584712**	**This work**
E. subpraegracile	**China**	**HFJAU3168**	**PQ584705**	**–**	**This work**
E. subpraegracile	**China**	**HFJAU5110**	**PQ584701**	**–**	**This work**
E. subpraegracile	**China**	**HFJAU5115**	**PQ584699**	**PQ584713**	**This work**
E. subpraegracile	**China**	**HFJAU5140**	**PQ584702**	**PQ584714**	**This work**
E. subpraegracile	**China**	**HFJAU5175**	**PQ584703**	**PQ584715**	**This work**
E. subpraegracile	**China**	**HFJAU5177**	**PQ584704**	**PQ584716**	**This work**
E. subserrulatum	USA	TENN:068464	KY744143	–	Unpublished in GenBank
E. subserrulatum	USA	TENN:070407	KY744177	–	Unpublished in GenBank
E. subtenuicystidiatum	China	GDGM 28459, holotype	JQ320109	JQ320116	He et al. (2013)
E. subtenuicystidiatum	China	GDGM 29246	JQ320114	JQ320132	He et al. (2013)
E. turci	Austria	WU25055	UDB0802163	–	UNITE
E. viridomarginatum	The Netherlands	JAC15761	MW775255	–	Unpublished in GenBank
E. viridomarginatum	The Netherlands	JAC12344	MW775264	–	Unpublished in GenBank
E. wuyishanense	**China**	**HFJAU3571, holotype**	**PQ584691**	**–**	**This work**
E. wuyishanense	**China**	**HFJAU3871**	**PQ584692**	**PQ584717**	**This work**
E. wuyishanense	**China**	**HFJAU3874**	**PQ584694**	**PQ584718**	**This work**
E. wuyishanense	**China**	**HFJAU3878**	**PQ584693**	**PQ584719**	**This work**
E. wuyishanense	**China**	**HFJAU3881**	**PQ584695**	**PQ584720**	**This work**

Results

Phylogenetic analysis

A total of 2136 characters were used in subsequent analyses (ITS, 841 bp; LSU, 1,295 bp), of which 1382 were constant, 670 were parsimony-informative, and 84 were singleton. For Bayesian analysis, the average standard deviation of split frequencies was less than 0.01 after 20 million generations.

The results of the phylogenetic analysis were shown in Fig. 1. The results were consistent with previous studies (Noordeloos et al. 2022a; Brandrud et al. 2023). The four new species were clustered in the subg. *Cyanula* clade and formed separate and well-supported branches, respectively. Among them, *Entoloma orientosinense* formed a separate and well-supported lineage (BI-PP = 1, ML-BP = 99%), and groups together with *E. albinellum* (Peck) Hesler and *E. queletii* (Boud.) Noordel. nested in the sect. *Caesiocincta*, subsect. *Queletia* (BI-PP = 1, ML-BP = 100%). *Entoloma subgriseosquamulosum* independently
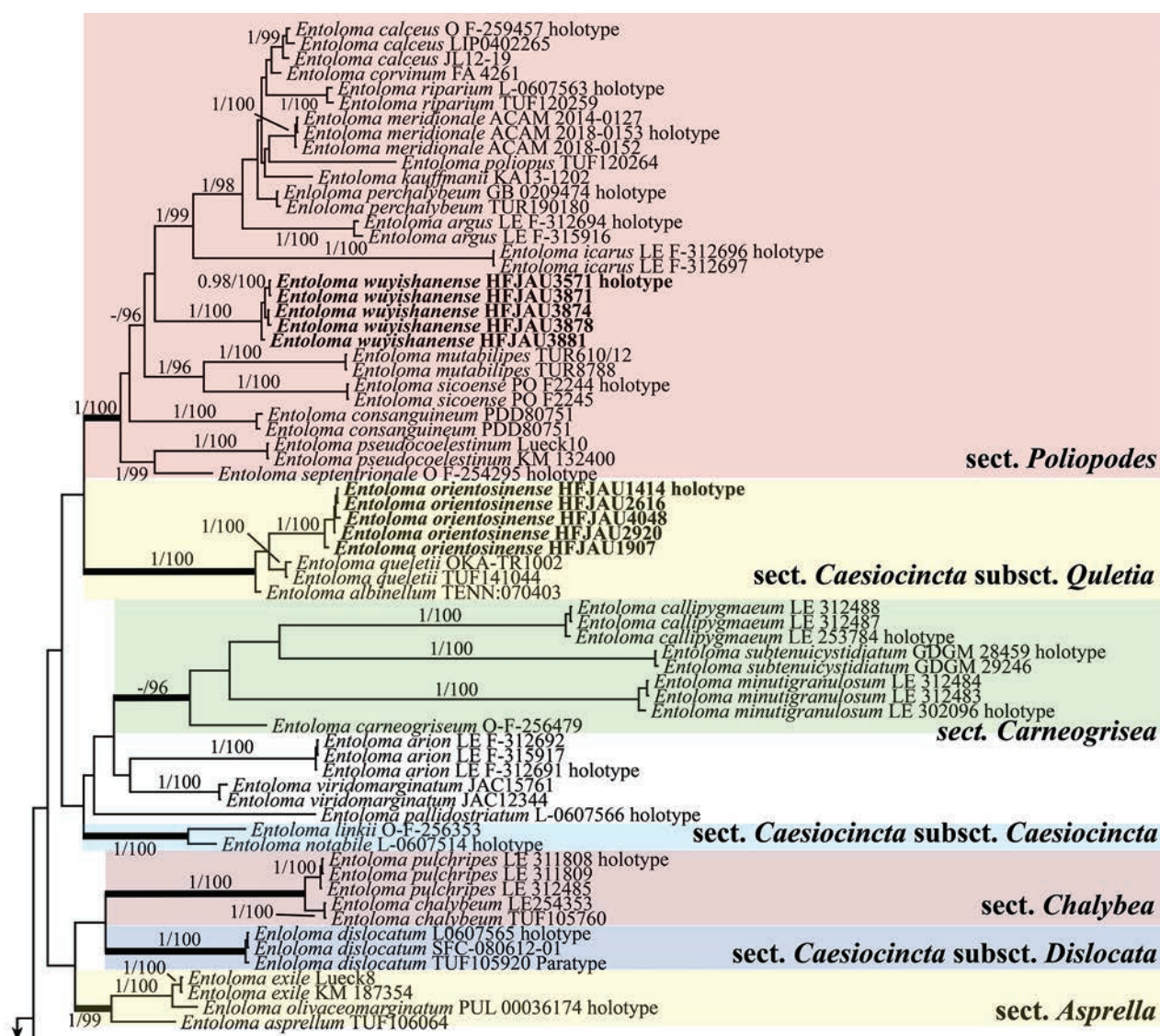
Figure 1. Phylogram of *Entoloma* subg. *Cyanula* spp. generated by Bayesian inference (BI) analysis based on ITS and LSU, rooted with *E.* subgenus *Nolanea* spp. Bayesian inference (BI-PP) ≥ 0.95 and ML bootstrap proportions (ML-BP) ≥ 95% are indicated as PP/BP. The new taxa are marked in bold.
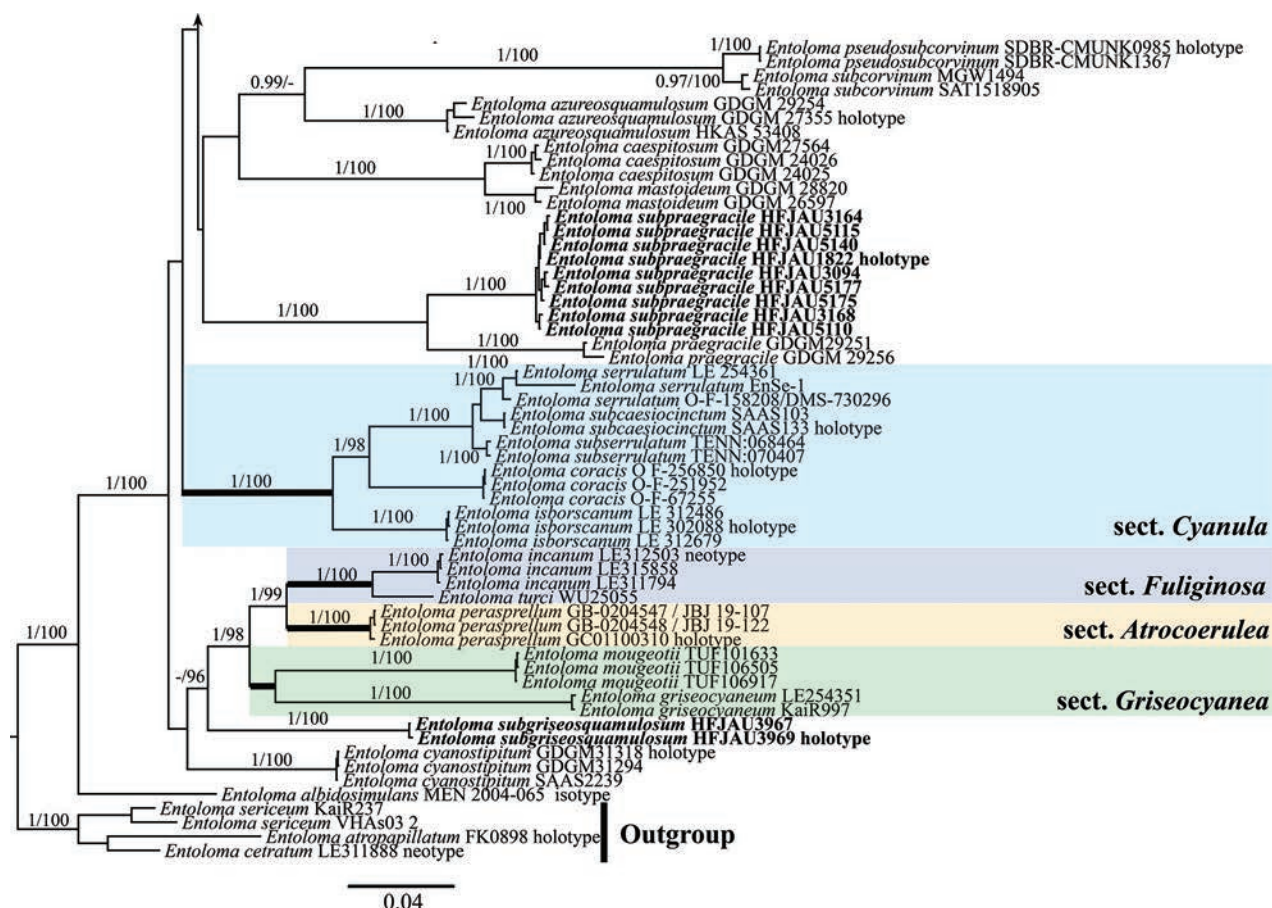
Figure 1. Continued.

formed a well-supported branch (BI-PP = 1, ML-BP = 100%). *Entoloma subprae-gracile* formed a sister lineage with *E. praegracile* Xiao L. He & T.H. Li (BI-PP = 1, ML-BP = 100%), well clustered in a small clade (BI-PP = 1, ML-BP = 100%). *Entoloma wuyishanense* formed a well-supported lineage within the sect. *Poliopodes* (BI-PP = 1, ML-BP = 100%).

Taxonomy

### *Entoloma orientosinense* J.Q. Yan, L.G. Chen & S.N. Wang, sp. nov.
MycoBank No: 858361
Fig. 2

Etymology. Refers to its type specimen originating from the eastern regions of China.

Holotype. CHINA • Anhui Province, Chizhou City, Shitan County, Guniujiang Nature Reserve, 30.0303°N, 117.5290°E, alt. 783 m, 9 October 2019, collected by Yu-Peng Ge, HFJAU1414.

Diagnosis. *Entoloma orientosinense* is mainly characterized by the white, collybioid basidiomata, fibrillous and not striate pileus, narrow, adnate to decurrent lamellae, glabrous stipe, 5–6 angled basidiospores, sterile lamellae edge of carneogriseum-type, cylindrical to subclavate cheilocystidia, absence of cell pigments and clamp connections in hyphae. It differs from *E. albinellum* by its non-striate pileus, adnate to decurrent lamellae, and smaller basidiospores.
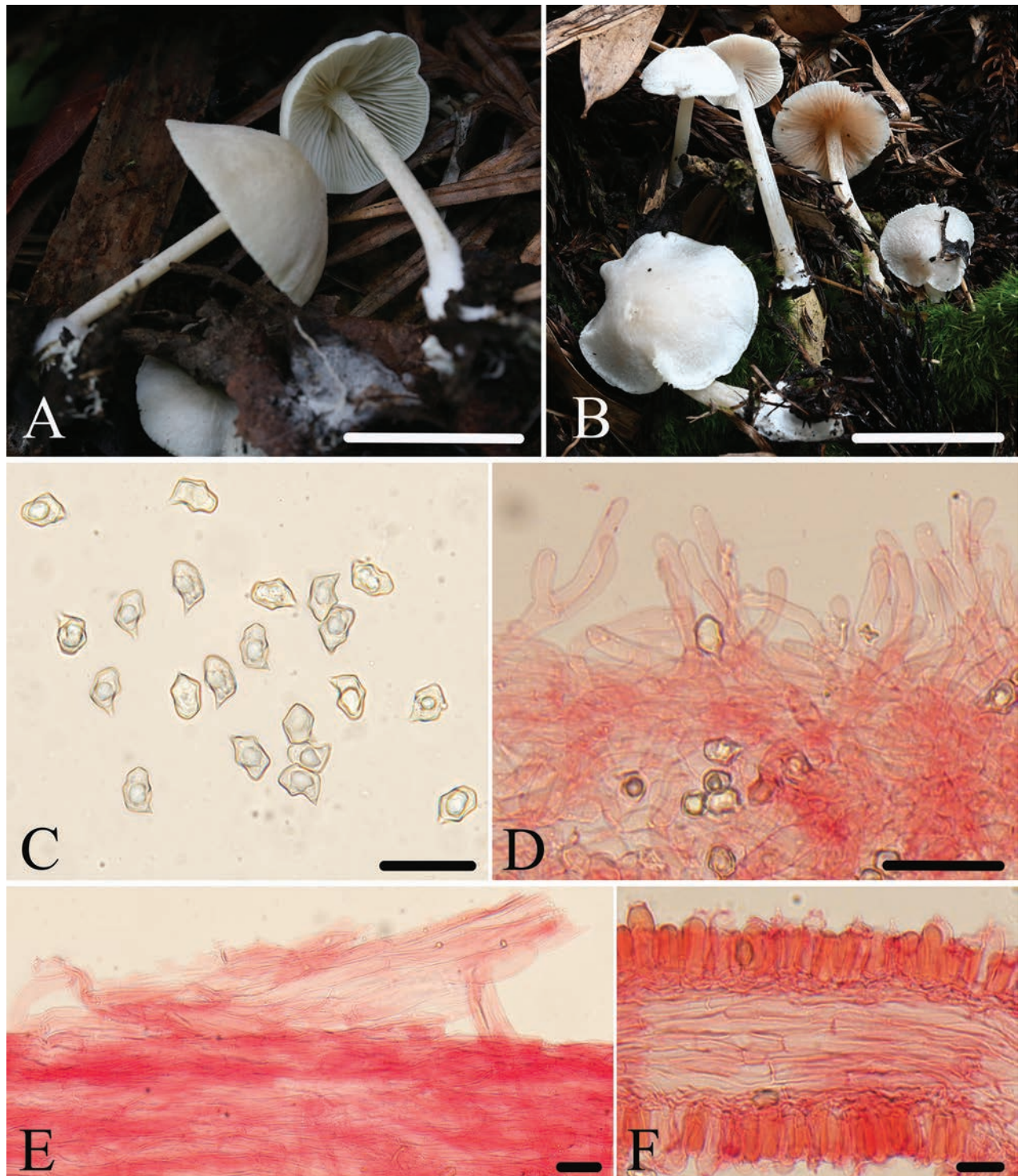
Figure 2. *E. orientosinense* **A, B** basidiomata **A** HFJAU1414, holotype **B** HFJAU2616 **C** basidiospores **D** cheilocystidia **E** pileipellis **F** lamellar trama. All microscopic structures were observed in 5% KOH, and used 1% Congo red as the stain except **C**. Scale bars: 10 mm (**A, B**); 20 μm (**C**); 30 μm (**D–F**).

Macromorphology. Basidiomata rather small, collybioid. Pileus 8–20 mm wide, convex then flattened with depressed center, with entire margin, slightly hygrophanous, fibrillous when young, then repent or raised scaly, not translucently striate, white (3A1–2). Lamellae moderately distant, 1.5–2.0 mm wide, with three types of lamellulae, adnate to decurrent, subventricose, initially white, then pink (11B4–6),

with serrulate and concolourous edge. Stipe 20–25 × 2.0–3.0 mm, central, terete, tapered upwards, hollow, concolorous or paler with the pileus, minutely tomentose in the upper part elsewhere smooth and glabrous, base with white tomentum. Context thin, concolorous to the surface. Odor indistinct, taste not tested.

Micromorphology. Basidiospores (8.5)9.3–11.0(12.5) × (6.0)6.5–8.0(9.0) μm, (av = 10.1 ×7.3 μm), Q = 1.2–1.6(1.7) (Qm = 1.4 ± 0.07, n = 200), heterodiametrical, 5–6 angles in profile view, thick-walled, inamyloid. Basidia 40–52 × 11–13 μm, clavate, 4-spored, sterigmata 5.0–10 μm long, clampless. Pleurocystidia absent. Lamellae edge sterile of carneogriseum-type. Cheilocystidia regularly dispersed in the lamellae edge, 17–47 × 4.0–7.0 μm, narrowly cylindrical to subclavate, septate, with slightly inflated apex. Lamellar trama regular, made up of cylindrical hyphae 7.0–13 μm wide. Pileipellis a cutis made up of cylindrical hyphae 8.0–11 μm broad, with transitions to a trichoderm towards the margin with clavate terminal elements 10–18 μm wide, not pigmented. Stipitipellis a cutis composed of densely arranged, cylindrical hyphae, up to 11 μm wide, slightly constricted at the septa, with acute or tapered end. Clamp connections absent.

Habitat. Solitary or scattered on soil in mixed coniferous-broad-leaved forest, or on rotten wood, soil, and moss in broadleaved forest.

Distribution. So far known from eastern China.

Additional specimens examined. China • Fujian Province, Wuyishan City, 27.7139°N, 117.6533°E, alt. 1113 m, 27 June 2022, collected by Jun-Qing Yan and Bing-Ring Ke, HFJAU4048 • Zhejiang Province, Lishui City, Suichang County, Huangtakou Village, 28.2679°N, 118.9435°E, alt. 346 m, 12 July 2020, collected by Jun-Qing Yan and Yan-Liu Chen, HFJAU1907 • Qingtian County, Shigu Lake, 28.2063°N, 120.0415°E, alt. 1130 m, 31 July 2021, collected by Jun-Qing Yan, Bing-Ring Ke, and Zhi-Heng Zeng, HFJAU2616 • Nanyang Village, 27.9603°N, 120.0020°E, alt. 522 m, 6 August 2021, collected by Yu-Peng Ge and Lan-Yu Sun, HFJAU2920.

Notes. Morphologically, *Entoloma orientosinense* has much in common with *E. albidosimulans* G.M. Gates & Noordel. and *E. albinellum* with regard to the white and collybioid basidiomata. However, *E. albidosimulans* is distinct by its broader (up to 6 mm), adnate-emarginate lamellae, and belonging to *E.* subg. *Alboleptonia* species (Gates and Noordeloos 2007). *Entoloma albinellum* differs from new species by its striate pileus, adnexed lamellae, and larger basidiospores (11–12.5 × 7.5–8.5 μm) (Hesler 1967).

In the molecular data, *E. orientosinense* fits well within subg. Cyanula, sect. *Caesiocincta*, subsect. *Queletia* including *E. albinellum* and *E. queletii*. *E. queletii* is distinguished from new species by the vinaceous-pink pileus and larger basidiospores (10–13 × 6.5–9.0 μm) (Bas et al. 1988a).

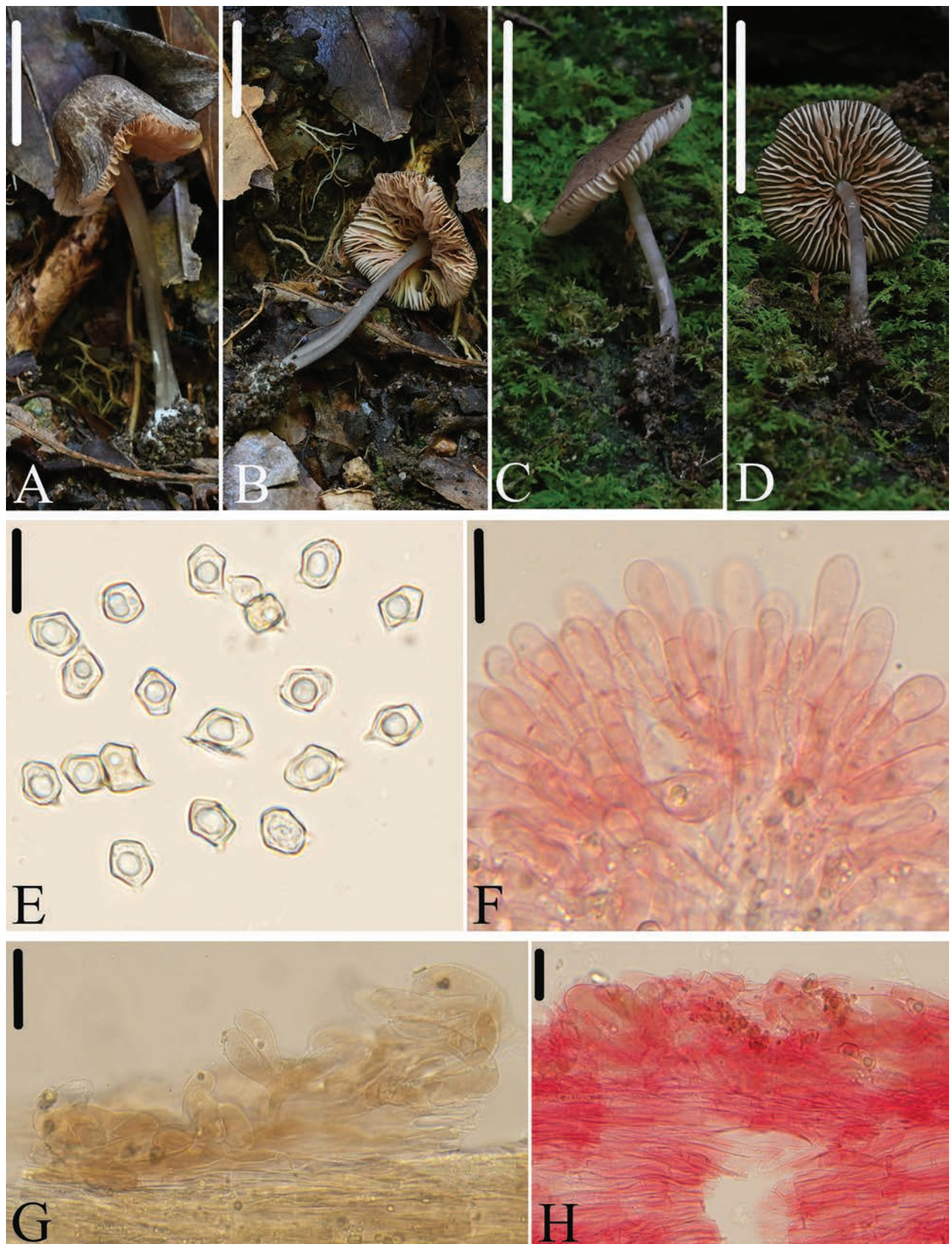*Entoloma subgriseosquamulosum* J.Q. Yan, L.G. Chen & S.N. Wang, sp. nov.
MycoBank No: 858362
Fig. 3

Etymology. Refers to its morphology similar to "*Entoloma griseosquamulosum*".

Holotype. China • Fujian Province, Wuyishan City, Yangzhuang Town, Xiyuan Village, 27.7632°N, 117.8139°E, alt. 533 m, 26 June 2022, collected by Jun-Qing Yan, Cheng-Feng Nie, and Lin-Gen Chen, HFJAU3969.

Figure 3. *E. subgriseosquamulosum* **A–D** basidiomata **A, B** HFJAU3969, holotype **C, D** HFJAU3967 **E** basidiospores **F** cheilocystidia **G** basidia **G, H** pileipellis. **G** was observed in H_2O, remaining microstructures all were observed in 5% KOH, and used 1% Congo red as the stain except **E**. Scale bars: 10 mm (**A–D**); 20 μm (**E**); 30 μm (**F–H**).

Diagnosis. *Entoloma subgriseosquamulosum* is mainly characterized by the rather small, collybioid basidiomata, fuscous pileus, crowded and adnate lamellae, glabrous stipe, medium-sized basidiospores with 5–6 angles, mostly 5 angles, and absence of clamp connections. It differs from *E. griseosquamulosum* G.M. Gates & Noordel. by its gray stipe, smaller basidiospores, and absence of brilliant granules in hyphae.

Macromorphology. Basidiomata rather small, collybioid. Pileus 11–20 mm wide, campanulate to convex with slight depressed center, with entire margin, not hygrophanous, gray hairy scaly with denser center, translucently striate almost up to 1/2 of the radius, fuscous (4D2–4F2) to dark gray (1F1–4F1), darker at center. Lamellae relatively crowded, 2.0–4.0 mm wide, with two types of lamellulae, adnate to emarginate, ventricose, initially white, then brownish-rose, with entire and concolorous edge. Stipe 20–42 × 1.5–3.0 mm, central, terete, equal, hollow, gray (1C1–1E1), darker downwards, sparsely white fibrillous in the upper part elsewhere smooth and glabrous, base with white mycelium. Context thin, white. Odor indistinct, taste not tested.

Micromorphology. Basidiospores (8.1)8.4–10.5(11) × (6.0)6.5–8.0(8.5) μm, (av = 9.3 ×7.4 μm), Q = 1.1–1.4(1.5) (Qm = 1.3 ± 0.07, n = 100), subisodiameterical or heterodiametrical, 5–6 angles, mostly 5 angles in profile view, thick-walled, inamyloid. Basidia 27–36 × 10–13 μm, clavate, 4-spored, sterigmata 6.0–9.0 μm long, clampless. Pleurocystidia absent. Lamellae edge sterile of poliopus-type. Cheilocystidia 27–64 × 9.0–14 μm, clavate. Lamellar trama regular, made up of cylindrical hyphae 7.0–12 μm wide. Pileipellis a trichoderm made up of cylindrical hyphae 6.0–12 μm broad, with clavate terminal elements and yellow-brown intracellular pigment. Stipitipellis a cutis composed of densely arranged, cylindrical hyphae, 7.0–17 μm wide, slightly constricted at the septa, with acute or attenuated end. Clamp connections absent.

Habitat. Solitary on soil or moss in broad-leaved forest.

Distribution. So far known from Fujian Province in China.

Additional specimens examined. Cʜɪɴᴀ • Fujian Province, Wuyishan City, Yangzhuang Town, Xiyuan Village, 27.7652°N, 117.8164°E, alt. 512 m, 26 June 2022, collected by Jun-Qing Yan, Cheng-Feng Nie, and Lin-Gen Chen, HFJAU3967.

Notes. Morphologically, several similar species within *Entoloma* subg. *Cyanula* that share brown to brown-gray pileus can be distinguished from the new species as follows: *E. anatinum* (Lasch) Donk is characterized by its larger basidiospores (9.0–13.5 × 7.5–9.0 μm) with 6–9 angles, and fertile lamellae edge (Donk 1949); *E. glaucobasis* Huijsman ex Noordel. has larger basidiospores (10–13.5 × 7.0–8.0 μm) (Noordeloos 1985); *E. griseosquamulosum* differs from the new species by the gray-violet stipe, larger basidiospores (9.0–12 × 7.0–9.0 μm), and presence of abundant brilliant granules in all hyphae (Noordeloos and Gates 2009); *E. phaeomarginatum* E. Horak is recognized by the fibrillose pileus, brown lamellae edge, and larger basidiospores (10–13 × 7.0–8.0 μm) (Horak 1973); *E. saponicum* G.M. Gates & Noordel. is distinct by the blackish brown lamellae edge and presence of abundant brilliant granules in all hyphae (Noordeloos and Gates 2009).

Phylogenetically, *E. cyanostipitum* Xiao L. He & W.H. Peng is closest to the new species. However, *E. cyanostipitum* is distinct by the deep blue pileus margin, lamellae edge and stipe, and the ITS region, with an 84% similarity (He et al. 2017).

Entoloma subpraegracile **J.Q. Yan, L.G. Chen & S.N. Wang, sp. nov.**
MycoBank No: 856754
Fig. 4

Etymology. Refers to its macroscopic morphology similar to "*Entoloma praegracile*"

Holotype. CHINA • Zhejiang Province, Lishui City, Qingyuan County, Bandai-houshang Village, 27.6748°N, 119.0780°E, alt. 1084 m, 7 July 2020, collected by Jun-Qing Yan and Yan-Liu Chen, HFJAU1822.

Diagnosis. *Entoloma subpraegracile* is mainly characterized by the yellow, glabrous, and striate pileus, white, adnexed to adnate lamellae with tiny lateral veins, 5–7 angled and medium-sized basidiospores, sterile or heterogeneous lamellae edge of serrulatum-type, cylindrical or clavate cheilocystidia, and absence of clamp connections. It differs from *E. praegracile* by the larger basidiomata, and sterile or heterogeneous lamellae edge.

Macromorphology. Basidiomata rather small. Pileus 10–20 mm wide, conical when young, then convex to flattened with depressed, rarely cuspidate center, with entire margin, not hygrophanous, smooth and glabrous, translucently striate almost up to the center, ochre (7B4–6), grayish yellow (1A4–5) to tawny (2C4–6), darker at center. Lamellae relatively dense, 1.5–2.0 mm wide, with tiny lateral veins and two or three types of lamellulae, adnate to adnexed, subventricose, white, with entire and concolorous edge. Stipe 25–35 × 2.0–2.5 mm, central, terete. equal, hollow, concolorous or paler with the pileus, smooth and glabrous, sometimes grooved, white tomentose at the base. Context thin, concolorous to the surface. Odor indistinct, taste not tested.

Micromorphology. Basidiospores (7.0)8.5–10.5(12) × (6.0)6.5–7.5(8.5) µm, (av = 9.6 ×7.0 µm), Q = 1.2–1.6(1.7) (Qm = 1.4 ± 0.08, n = 200), heterodiametrical, 5–7(8) angles in profile view, appearing nodulose, thick-walled, inamyloid. Basidia 27–37 × 9–12 µm, clavate, slightly constricted at middle, mainly 2-spored, sterigmata 6.0–12 µm long, clampless. Pleurocystidia absent. Lamellae edge sterile or heterogeneous of poliopus-type. Cheilocystidia dense clusters on lamellae edge, 21–53 × 7.0–14 µm, cylindrical or clavate. Lamellar trama regular, made up of cylindrical hyphae 4.0–8.0 µm wide. Pileipellis a cutis made up of cylindrical hyphae 5.0–12 µm broad, with transitions to a trichoderm towards the center with clavate terminal elements 10–16 µm wide, with tawny intracellular pigment. Stipitipellis a cutis composed of densely arranged, cylindrical hyphae, 7.0–15 µm wide, slightly constricted at the septa, with rounded end. Clamp connections absent.

Habitat. Solitary or scattered on soil in mixed coniferous-broad-leaved forest.

Distribution. So far known from eastern China.

Additional specimens examined. CHINA • Fujian Province, Wuyishan City, 27.8594°N, 117.9096°E, alt. 372 m, 12 August 2021, collected by Jun-Qing Yan and Ze-Wei Liu, HFJAU3094 • 27.8563°N, 117.8661°E, alt. 668 m, 13 August 2021, collected by Qin Na, Yu-Peng Ge, and Lan-Yu Sun, HFJAU3164, HFJAU3168 • 27.7221°N, 117.7072°E, alt. 654 m, 16 August 2023, collected by Nian-Kai Zeng, Cheng-Feng Nie, Hua-Zhi Qin, Hui Deng, Tian Jiang, and Run-Xiang Zhao, HFJAU5110, HFJAU5115, HFJAU5140, HFJAU5175, HFJAU5177.

Notes. In the phylogenetic tree, *E. subpraegracile* groups together with *E. praegracile*. *Entoloma praegracile* differs from the new species by the smaller pileus (less than 10 mm), fertile lamellae edge, and the ITS sequence with 86% similarity (He et al. 2011).
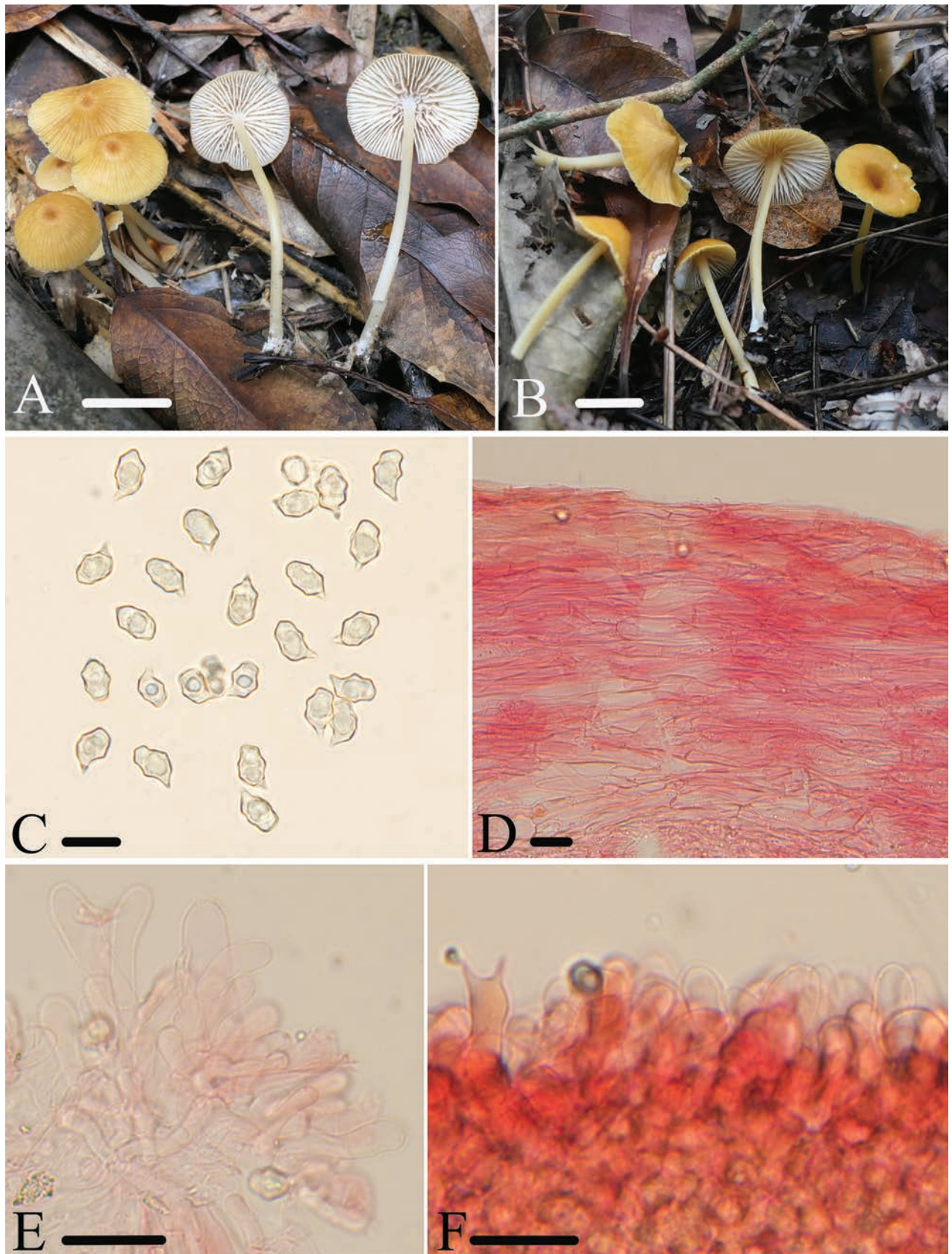
Figure 4. *E. subpraegracile* **A, B** basidiomata **A** HFJAU1822, holotype **B** HFJAU5115 **C** basidiospores **D** pileipellis **E** cheilocystidia **F** heterogeneous lamellae edge. All microscopic structures were observed in 5% KOH, and used 1% Congo red as the stain except **C**. Scale bars: 10 mm (**A, B**); 20 μm (**C**); 30 μm (**D–F**).

Some similar species with a yellow pileus within subg. *Cyanula* can be distinguished from the new species as follows: *E. chloropolium* (Fr.) M.M. Moser is recognized by the fertile to heterogeneous lamellae edge, and septate cheilocystidia (Noordeloos 2004); *E. formosum* (Fr.) Noordel. is characterized by its squamulose pileus, larger basidiospores (9.0−12.5 × 6.0−8.0 μm), and fertile or heterogeneous lamellae edge (Bas et al. 1988b). *E. luteoochraceum* Ribes & Vila is distinct by the squamous pileus, 4-spored basidia, and fertile lamellae edge (Ribes and Vila 2013); *E. pseudoturci* Noordel. has tomentose to squamous and not striate pileus, porphyrogriseum-type lamellae edge, and brilliant granules in tissue cells (Noordeloos 1984).

Entoloma wuyishanense J.Q. Yan, L.G. Chen & S.N. Wang, sp. nov.

MycoBank No: 858363

Fig. 5

Etymology. Refers to the collection locality of the holotype specimen – Wuyishan National Natural Park.

Holotype. Cʜɪɴᴀ • Fujian Province, Nanping City, Wuyishan National Natural Park, 27.5418°N, 117.4743°E, alt. 422 m, 7 June 2022, collected by Jun-Qing Yan and Lin-Gen Chen, HFJAU3571.

Diagnosis. *Entoloma wuyishanense* is mainly characterized by the rather small and blue basidiomata, squamous and striate pileus, white and adnexed lamellae with fertile edge with slightly bluish pigmentation near the stipe, relatively large basidiospores with 5−6 angles, pileipellis with fuscous intracellular pigment. It differs from *E. azureosquamulosum* Xiao L. He & T.H. Li by the striate pileus, adnexed lamellae, larger basidiospores, and fertile lamellae edge.

Macromorphology. Basidiomata rather small. Pileus 2.0−11 mm wide, conical when young, then convex to flattened with depressed center, with entire, straight or wavy margin, not hygrophanous, squamous with denser center, translucently striate almost up to the center, deep blue (20E4−7) to light gray-blue (20B2−3), darker at center. Lamellae moderately distant, 1.0−3.0 mm wide, with two types of lamellulae, adnexed, ventricose, white, with entire and bluish edge near the stipe. Stipe 9.0−26 × 1.0−2.0 mm, central, terete, equal, hollow, concolorous with pileus, paler downwards, white fibrillose, glabrescent with age, base with white mycelium. Context thin, gray-blue. Odor indistinct, taste not tested.

Micromorphology. Basidiospores (9.5)10−13.5(15) × (6.5)7.5−9.5(10) μm, (av = 11.7 ×8.5 μm), Q = 1.2−1.7(2.0) (Qm = 1.4 ± 0.11, n = 200), heterodiametrical, 5−6 angles in profile view, sometimes appearing nodulose, thick-walled, inamyloid. Basidia 25−33 × 10−13 μm, clavate, slightly constricted at middle, 4- or 2-spored, sterigmata 6.0−10 μm long, clampless. Lamellae edge fertile. Cystidia absent. Lamellar trama regular, made up of cylindrical hyphae 5.0−11 μm wide. Pileipellis a trichoderm made up of clavate to pyriform terminal cells, 41−65 × 22−36 μm. Pigment fuscous, intracellular, diffuse in pileipellis. Stipitipellis a cutis composed of densely arranged, cylindrical hyphae, 5.0−12 μm wide, with rounded end. Clamp connections absent.

Habitat. Solitary or scattered on moss in mixed coniferous-broad-leaved forest.
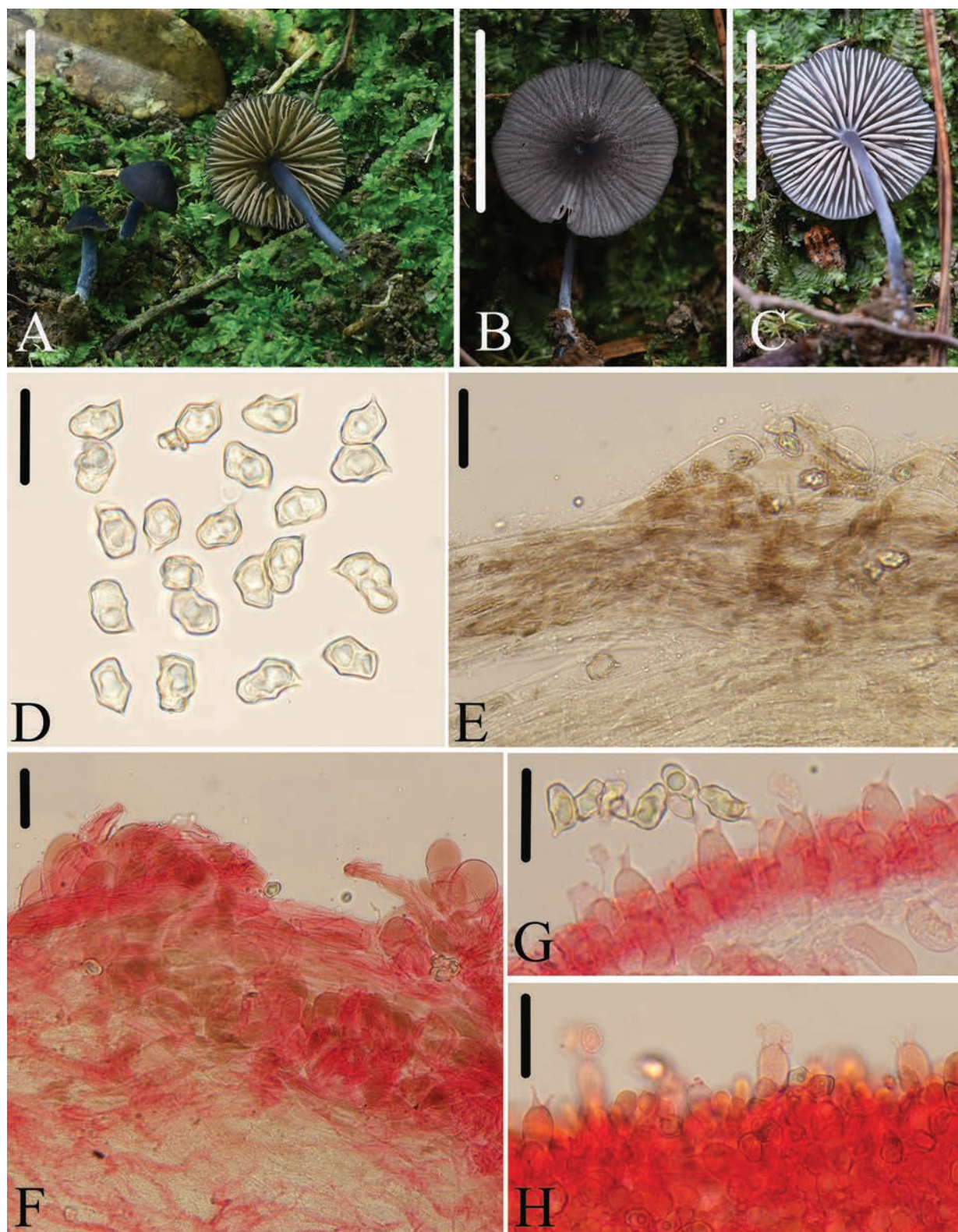
Distribution. So far known from eastern China.

Figure 5. *E. wuyishanense* **A–C** basidiomata HFJAU3571, holotype **B, C** HFJAU3871 **D** basidiospores **E, F** pileipellis **G** basidia **H** fertile lamellar edge. **E** was observed in H_2O **D, F–H** were observed in 5% KOH, and used 1% Congo red as the stain except **D**. Scale bars: 10 mm (**A–C**); 20 μm (**D**); 30 μm (**E–H**).

Additional specimens examined. CHINA • Zhejiang Province, Lishui City, Songyang County, Zicao Village, 28.4874°N, 119.5783°E, alt. 722 m, 2 July 2022, collected by Jun-Qing Yan, Cheng-Feng Nie, and Meng-Hui Han, HFJAU3871,

HFJAU3874, HFJAU3878 • Lishui City, Yunhe County, Chongtou Town, Xiayang Village, 28.0499°N, 119.4732°E, alt. 592 m, 4 July 2022, collected by Jun-Qing Yan and Cheng-Feng Nie, HFJAU3881.

Notes. Morphologically, *E. azureosquamulosum* is the most similar species with the distinction that *E. azureosquamulosum* exhibits not striate pileus, adnate lamellae, smaller basidiospores (8−10.5 × 6.5−8.0 μm), and sterile lamellae edge (He et al. 2012).

In the phylogenetic tree, *E. wuyishanense* belongs to *Cyanula* sect. *Poliopodes*, within which several species have blue pileus, including *E. argus* O.V. Morozova, E.S. Popov, A.V. Alexandrova & Noordel., *E. calceus* Noordel., Bendiksen, Brandrud, P.-A. Moreau & Vila, *E. corvinum* (Kühner) Noordel., *E. icarus* O.V. Morozova, E.S. Popov & Noordel., and *E. perchalybeum* Noordel., J.B. Jordal & Dima. However, the lamellae edge of the latter in all species is sterile. In addition, *E. argus* is characterized by the adnate lamellae and smaller basidiospores (≤10 μm) (Morozova et al. 2022); *E. calceus* shows 6−9 angled basidiospores (Noordeloos et al. 2022b); *E. corvinum* is recognized by its not striate pileus, adnate lamellae, and smaller basidiospores (8.0−11 × 6.5−7.5 μm) (Noordeloos 1982); *E. icarus* can be easily differentiated by the lateral stipe and adnate lamellae (Morozova et al. 2022); *E. perchalybeum* is distinct by the adnate lamellae and 6−7 rather bluntly angled basidiospores (Noordeloos et al. 2022b).

Discussion

Entoloma subg. *Cyanula* currently is divided into 11 sections (Noordeloos et al. 2022a; Dima et al. 2023), and which formed well-supported clades and confirmed taxonomic positions within subgenus in this study. It is worth mentioning that two of the four newly discovered species in this study, along with the majority of previously reported new taxa of subg. *Cyanula* from China, do not belong to any known sections. To address this issue, continued phylogenetic studies of subg. *Cyanula* based on both morphological characters and molecular markers for more representative specimens of this subgenus from China are necessary. This will result in a more natural classification in the future.

Notably, based on the results of this phylogenetic analysis, we have realized that the sect. *Caesiocincta* is divided into three clades. However, since none of the originating branches of these scattered clades is supported, the cause of this result cannot be determined. Additional specimen data are needed for further analysis.

The present study expands our understanding of entolomoid species by providing descriptions and phylogenetic analyses for four new species. The findings enrich our knowledge of the distribution of *E.* subg. *Cyanula* species in China and the overall diversity of *Entoloma*.

Key to *Entoloma* subg. *Cyanula* species reported in China

1 Pileus white to pink **2**
− Pileus other colored **3**
2 Pileus white, not striate, with depressed center; lamellae adnate to decurrent; basidiospores 5−6 angled; pigment not...................***E. orientosinense***
− Pileus pink, striate, with umbonate center; lamellae subfree to adnexed; basidiospores 6−8 angled; pigment yellow encrusting ....***E. mastoideum***

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization, Jun-Qing Yan; methodology, Jun-Qing Yan and Sheng-Nan Wang; software, Lin-Gen Chen, Hong Chen, Ling Ding and Yu-Qin Xu; formal analysis, Hui Zeng, Jun-Qing Yan, and Sheng-Nan Wang; investigation, Lin-Gen Chen, Hong Chen, Ling Ding, and Jun-Qing Yan; resources, Hui Zeng and Jun-Qing Yan; writing – original draft, Lin-Gen Chen; writing – review and editing, Jun-Qing Yan; visualization, Jun-Qing Yan and Sheng-Nan Wang; supervision, Jun-Qing Yan; project administration, Jun-Qing Yan; funding acquisition, Jun-Qing Yan. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Lin-Gen Chen https://orcid.org/0009-0000-2506-181X
Hong Chen https://orcid.org/0009-0004-0107-3962
Ling Ding https://orcid.org/0009-0000-5293-046X
Hui Zeng https://orcid.org/0000-0003-2025-844X
Sheng-Nan Wang https://orcid.org/0000-0003-0648-271X
Jun-Qing Yan https://orcid.org/0000-0003-1128-5171

Data availability

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

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Supplementary material 1

Supplementary data

Authors: Lin-Gen Chen, Hong Chen, Ling Ding, Yu-Qin Xu, Hui Zeng, Sheng-Nan Wang, Jun-Qing Yan

Data type: nex

Explanation note: A nexus file contains alignment sequence and original tree of ML and Bayes.

Link: https://doi.org/10.3897/mycokeys.116.145568.suppl1

MycoKeys

Biconidium sinense gen. et sp. nov. (Hypocreales, Bionectriaceae) and *Didymocyrtis shanxiensis* sp. nov. (Phaeosphaeriaceae, *Didymocyrtis*) isolated from urban soil in China

Hai-Yan Wang[1], Chunbo Dong[1], Yan-Wei Zhang[2], Wan-Hao Chen[3], Yan-Feng Han[1]

1 *Institute of Fungus Resources, Department of Ecology, College of Life Science, Guizhou University, Guiyang 550025, Guizhou, China*

2 *Key Laboratory of Development and Utilization of Biological Resources in Colleges and Universities of Guizhou Province/Key Laboratory of Ecology and Management on Forest Fire in Higher Education Institutions of Guizhou Province, Guizhou Education University, Guiyang 550018, Guizhou, China*

3 *Center for Mycomedicine Research, Basic Medical School, Guizhou University of Traditional Chinese Medicine, Guiyang 550025, Guizhou, China*

Corresponding authors: Yan-Wei Zhang (*zyw_email@163.com*); Yan-Feng Han (*swallow1128@126.com*)

Abstract

During a fungal diversity survey in various urban habitats across China, 5 fungal isolates were discovered from soil samples. Detailed morphological observations and multigene phylogenetic analyses confirmed the identification of two novel taxa: *Biconidium sinense* **gen. et sp. nov.** and *Didymocyrtis shanxiensis* **sp. nov.** These species were formally described, illustrated, and discussed, highlighting their distinct characteristics and taxonomic placement. The study expands our understanding of fungal diversity in urban environments, emphasizing the importance of combining morphological and molecular approaches for accurate species delineation and discovery.

Key words: Fungal taxonomy, mycodiversity, new taxa, phylogeny

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Introduction

Soil fungi play an important role in mediating the processes of geochemical cycling, ecosystem material cycling and energy flow. For instance, they influence soil fertility, mineral breakdown, and organic matter cycling, as well as plant health and nutrition (Guo et al. 2017; Lu 2018). Moreover, some soil fungi can produce a lot of metabolites that are essential for human life and production (Zhang et al. 2023; Wang et al. 2024). For example, among the fungal species in the soil, the strains of the genera *Aspergillus*, *Penicillium*, *Paecilomyces* and *Trichoderma* produce flavins, ankaflavin, quinones, and anthraquinone (Akilandeswari and Pradeep 2016). *Penicillium griseofulvum* can produce a range of secondary metabolites including chanoclavine I, elymoclavine, fulvic acid, and griseofulvin, all of which can be used for antimicrobial activity (Yogabaanu et al. 2017). *Acrophialophora levis* QHDZ1−2 isolated from a zoo soil can produce some compounds, such as amino acid, amines, fatty acid, and vitamins (Wang 2024). Due to a multitude of factors, it is suspected that species are disappearing before they are discovered in many habitats (Wang et al. 2018; Löbl et al. 2023; Wang et al. 2024). This implies that we still need to make

more efforts to delve deeper into soil fungi resources, contribute to the study of our Earth's fungal diversity, and provide fungal resources for social industrial production. Currently, numerous studies have investigated fungal diversity in various soil habitats across China, including caves, forests, farmland, deserts and grasslands (Guo et al. 2017; Ma et al. 2021; Ma 2023; Guo 2024; Song et al. 2024). However, the composition and diversity of soil fungi in various urban various environments appear to have been neglected.

Urbanization has been the most impactful human activity in altering landscape patterns over the past century and is widely regarded as a significant threat to global biodiversity (Grimm et al. 2008; Nugent and Allison 2022). Developing countries are experiencing the swiftest rates of urbanization, with projections indicating that approximately 68% of the global population will reside in urban areas by 2050 (Desa 2019). The process of urbanization will reshape the land landscape, impacting elements such as surface vegetation, hydrology and soil, which in turn affects biodiversity and can lead to species homogenization or even extinction of species (Buczkowski and Richmond 2012; Yan et al. 2022). Urbanization has had a profound impact on soil fungi. It fragments the original habitats, resulting in a decline in fungal diversity and the potential disappearance of some native fungal species (Zhao et al. 2012; Hou et al. 2014; Rai et al. 2018). Consequently, in the context of urbanization, the composition and distribution of soil fungi across various urban habitats should be paid more attention. In recent years, the composition and diversity of green soil fungi in different urban habitats were explored (Zhang et al. 2021, 2023, 2024; Li et al. 2022a, 2022b; Ren et al. 2022; Wang et al. 2023, 2024). Fortunately, many new species and genera have been discovered and documented in these urban settings.

Bionectriaceae Samuels & Rossman was proposed by Rossman et al. (1999) based on the sexual morph-typified genus *Bionectria* Speg. (Spegazzini 1919). It is including 26 genera. Its diagnostic characteristics are the presence of white, pale tan orange or brown, uniloculate, perithecial, rarely cleistothecial ascomata and generally not changing color in KOH.

Barr (1979) proposed the family Phaeosphaeriaceae using *Phaeosphaeria* with *Ph. oryzae* as the type species. The new genus *Diederichomyces* was described by Trakunyingcharoen et al. (2014) to include most of the lichenicolous *Phoma* species that were assigned to the Phaeosphaeriaceae by Lawrey et al. (2012). Vainio (1921) established *Didymocyrtis* Vain., based on the type species *Didymocyrtis consimilis* Vain. With the development of phylogeny, the lichenicolous species of genus *Didymocyrtis* had been assigned to *Diederichia* D. Hawksw., *Diederichomyces* Crous & Trakun., *Leptosphaeria* Pass. and *Phoma* Sacc. (Trakunyingcharoen et al. 2014). Recently, the genus *Didymocyrtis* was resurrected for these species, and the new combinations, *Didymocyrtis bryonthae* (Arnold) Hafellner, *Didymocyrtis cladoniicola* (Diederich, Kocourk. & Etayo) Ertz & Diederich, *Didymocyrtis foliaceiphila* (Diederich, Kocourk. & Etayo) Ertz & Diederich, *Didymocyrtis infestans* (Speg.) Hafellner, *Didymocyrtis kaernefeltii* (S.Y. Kondr.) Hafellner, *Didymocyrtis melanelixiae* (Brackel) Diederich, R.C. Harris & Etayo, *Didymocyrtis pseudeverniae* (Etayo & Diederich) Ertz & Diederich, *Didymocyrtis ramalinae* (Roberge ex Desm.) Ertz, Diederich & Hafellner, *Didymocyrtis slaptoniensis* (D. Hawksw.) Hafellner & Ertz, and *Didymocyrtis xanthomendozae* (Diederich & Freebury) Diederich & Freebury were created (Ertz et al. 2015). Presently, the genus *Didymocyrtis* includes twenty-nine species in the Index Fungorum.

During a continuous survey of fungal diversity exploration from different urban green soils in China, five strains from green soils of sewage treatment plant were isolated and purified. Based on the multi-gene phylogeny and morphological characteristics, these isolated strains were identified as two new taxa, *Biconidium sinense* gen. et sp. nov. and *Didymocyrtis shanxiensis* sp. nov., which are described and illustrated.

Materials and methods

Sample collection and fungal isolation

Soil samples, from 3–10 cm below the soil surface, were collected from green soil of sewage treatment plant in some cities in China. Samples were placed in sterile Ziploc plastic bags, and brought back to the laboratory. Then, the 2 g of each soil samples for fungal isolation, were placed into a sterile conical flask containing 20 mL sterile water in a 50 mL sterile conical flask, and thoroughly shaken using a Vortex vibration meter. Subsequently, the soil suspension was diluted to a concentration of 10^{-3}. Then, 1 mL of the diluted sample was transferred to a sterile Petri dish with Sabouraud's dextrose agar (SDA; peptone 10 g/L, dextrose 40 g/L, agar 20 g/L, 3.3 mL of 1% Bengal red aqueous solution) medium containing 50 mg/L penicillin and 50 mg/L streptomycin. The plates were incubated at 25 °C for 1 week, then every single colony was selected from the plates and transferred to new potato dextrose agar (PDA, potato 200 g/L, dextrose 20 g/L, agar 20 g/L) plates.

Morphological study

Strains of potentially new species were transferred to plates of malt extract agar (MEA), oatmeal agar (OA) and potato dextrose agar (PDA), and were incubated at 25 °C for examining their colony morphology and microscopic morphology. After 7 days, the colony colors according to national standard color card and diameters on the surface and reverse of inoculated Petri dishes were observed and recorded. Meanwhile, fungal hyphae and conidiogenous structures were examined, and images were captured by making direct wet mounts with 25% lactic acid on PDA, with an optical microscope (DM4 B, Leica). Strains of two novel species were deposited in the Institute of Fungus Resources, Guizhou University (GZUIFR = GZAC). Taxonomic descriptions and nomenclature of one new genus and two new species were uploaded in MycoBank (https://www.mycobank.org/).

DNA extraction, PCR amplification and sequencing

Using the BioTeke Fungus Genomic DNA Extraction kit (DP2032, BioTeke), total genomic DNA was extracted following the manufacturer's instruction. The extracted DNA was stored at −20 °C. Primer combinations: ITS1/ITS4 (White et al. 1990), LR0R/LR5 (Wang et al. 2022) and T1/TUB4Rd (O'Donnell and Cigelnik 1997; Woudenberg et al. 2009) were used for amplification of the internal transcribed spacers (ITS), the 28S nrRNA locus (LSU) and beta-tubulin gene (*tub2*), respectively. The PCR amplification conditions: ITS, 94 °C: 5 min, (94 °C: 30 s, 51 °C: 50 s, 72 °C: 45 s) × 35 cycles, 72 °C: 10 min (White et al. 1990); LSU, 94 °C:

5 min, (94 °C: 30 s, 51 °C: 1 min, 72 °C: 2 min) × 35 cycles, 72 °C: 10 min (Zhang et al. 2023); *tub2*, 94 °C: 5 min, (94 °C: 30 s, 52 °C: 30 s, 72 °C: 30 s) × 35 cycles 72 °C: 10 min (Woudenberg et al. 2009). In this study, the PCR products were sent to Quintarabio (Wuhan, China) for purification and sequencing. Strains sequences of two new species were submitted to GenBank (https://www.ncbi.nlm.nih.gov/) (Table 1 and Table 2).

Phylogenetic analysis

The relevant strains sequences were downloaded from GenBank in this paper (Table1 and Table2). *Flammocladiella decora* (Wallr.) Lechat & J. Fourn. and *Flammocladiella aceris* Crous, L. Lombard & R.K. Schumach. were used as the outgroup in phylogenetic tree 1 (Fig. 1). *Parathyridaria philadelphi* Crous & R.K. Schumach. was used as the outgroup in phylogenetic tree 2 (Fig. 3). The multiple datasets of ITS, LSU and *tub2* were aligned and trimmed in MEGA v.6.06 (Tamura et al. 2013). Using the "Concatenate Sequence" function, the concatenation of loci was conducted in PhyloSuite v.1.16 (Zhang et al. 2020). Then, the phylogenetic construction of each loci dataset was processed by both Maximum Likelihood (ML) and the Bayesian Inference (BI) methods. In ModelFinder, the Akaike Information Criterion correction (AICc) was used for the best-fit substitution model (Kalyaanamoorthy et al. 2017). With 1000 bootstrap tests using the ultrafast algorithm (Minh et al. 2013), the ML analysis was conducted in IQ-TREE v.1.6.11 (Nguyen et al. 2015). The BI analysis was performed in MrBayes v.3.2 (Ronquist et al. 2012) and Markov chain Monte Carlo (MCMC) simulations were used for 2×10^6 generations. Using FigTree version 1.4.3, the phylogenetic trees were visualized and edited in Microsoft PowerPoint.

Results

Phylogenetic analysis

In this study, using ITS sequences, our five isolates were identified and assigned to potential genera and species based on a BLASTn in NCBI. Five strains belonging to Bionectriaceae or *Didymocyrtis* were screened and tested for further identification through morphological characterization and phylogenetic analyses. Using ML and BI analyses, the two phylogenetic trees were consistent and supported strongly in branches. The ML analysis for the combined dataset provided the best scoring tree. The concatenated sequences of Fig. 1 and Fig. 3 included 90 and 16 taxa, respectively. The dataset in Fig. 1 was composed of ITS (1−382 bp) and LSU (383−782 bp) sequence data. The dataset in Fig. 3 was composed of ITS (1−402 bp) and *tub2* (403−731 bp) sequence data.

The phylogeny shows that each genus clusters into a monophyletic clade, and three strains of the genus *Biconidium* clustered in a well-separated clade, with a high support value (ML/BI 100/1) (Fig. 1). Two strains of the genus *Didymocyrtis* also clustered together, with a high support value (ML/BI 98/1) (Fig. 3). Therefore, a new genus, *Biconidium* H.Y. Wang & Y.F. Han, is introduced, and *Biconidium sinense* H.Y. Wang & Y.F. Han and *Didymocyrtis shanxiensis* H.Y. Wang & Y.F. Han as new species are proposed according to the phylogenetic analysis.

Table 1. Strains of Bionectriaceae and corresponding GenBank numbers included in phylogenetic analyses.

Species	Strains	ITS	LSU	Reference
Gliomastix murorum	CBS 154.25T	OQ429613	HQ232063	Hou et al. (2023)
Gliomastix murorum	CBS 253.79	OQ429614	OQ055521	Hou et al. (2023)
Gliomastix roseogrisea	CBS 134.56T	OQ429639	OQ055545	Hou et al. (2023)
Gliomastix tumulicola	CBS 127532T	OQ429641	OQ055547	Hou et al. (2023)
Paracylindrocarpon aloicola	CBS 141300T	KX228277	KX228328	Hou et al. (2023)
Paracylindrocarpon aloicola	CBS 135907	OQ429762	OQ055661	Hou et al. (2023)
Paracylindrocarpon aurantiacum	CBS 135909T	OQ429763	OQ055662	Hou et al. (2023)
Paracylindrocarpon multiseptatum	CBS 337.77T	OQ429768	OQ055666	Hou et al. (2023)
Fusariella curvata	MFLUCC 15-0844T	KX025152	KX025154	Hou et al. (2023)
Fusariella atrovirens	CBS 311.73	OQ429594	OR052105	Hou et al. (2023)
Fusariella arenula	CBS 330.77	OQ429592	OQ055503	Hou et al. (2023)
Fusariella arenula	CBS 329.77	OQ429593	OQ055504	Hou et al. (2023)
Selinia pulchra	A.R. 2812	HM484859	GQ505992	Hou et al. (2023)
Roumegueriella rufula	CBS 346.85	OQ429827	OQ430088	Hou et al. (2023)
Verrucostoma martinicense	CBS 138731T	OQ429934	OR052121	Hou et al. (2023)
Verrucostoma freycinetiae	MAFF 240100T	HM484866	GQ506013	Hou et al. (2023)
Synnemellisia aurantia	COAD 2070 T	KX866395	KX866396	Hou et al. (2023)
Musananaesporium tectonae	CBS 725.87T	OQ429714	OQ055615	Hou et al. (2023)
Gossypinidium sporodochiale	CBS 101694T	OQ429643	OQ055549	Hou et al. (2023)
Caespitomonium squamicola	CBS 701.73	OQ429515	OQ055426	Hou et al. (2023)
Caespitomonium squamicola	CBS 392.73	OQ429514	OQ055425	Hou et al. (2023)
Monohydropisphaera fusigera	CBS 124147T	OQ429713	OQ055614	Hou et al. (2023)
Hydropisphaera fungicola	CBS 122304T	OQ429666	OR052107	Hou et al. (2023)
Hydropisphaera suffulta	CBS 122.87	OQ429672	OQ055577	Hou et al. (2023)
Paragliomastix rosea	CBS 277.80AT	OQ429775	OQ055673	Hou et al. (2023)
Paragliomastix chiangraiensis	MFLUCC 14-0397T	MN648324	MN648329	Hou et al. (2023)
Septofusidium berolinense	CBS 731.70	OQ429859	OQ430110	Hou et al. (2023)
Pseudoacremonium sacchari	CBS 137990T	KJ869144	KJ869201	Hou et al. (2023)
Lasionectria olida	CBS 799.69T	OQ429693	OQ055598	Hou et al. (2023)
Lasionectria olida	CBS 798.69	OQ429692	OQ055597	Hou et al. (2023)
Lasionectria castaneicola	CBS 122792T	OQ429680	OQ055585	Hou et al. (2023)
Lasionectria atrorubra	CBS 123502T	OQ429674	OQ055579	Hou et al. (2023)
Verruciconidia persicina	CBS 310.59T	OQ429921	OQ430172	Hou et al. (2023)
Verruciconidia persicina	CBS 113716	OQ429922	OQ430173	Hou et al. (2023)
Verruciconidia erythroxyli	CBS 728.87T	OQ429910	OQ430161	Hou et al. (2023)
Verruciconidia infuscata	CBS 100888T	OQ429911	OQ430162	Hou et al. (2023)
Verruciconidia quercina	CBS 469.67T	OQ429925	OQ430176	Hou et al. (2023)
Verruciconidia quercina	CBS 355.77	OQ429927	OQ430178	Hou et al. (2023)
Lasionectriopsis dentifera	CBS 650.75	OQ429700	OQ055602	Hou et al. (2023)
Lasionectriopsis dentifera	CBS 574.76T	KY607540	KY607555	Hou et al. (2023)
Ochronectria thailandica	MFLUCC 15-0140T	KU564071	KU564069	Hou et al. (2023)
Lasionectriopsis germanica	CBS 143538T	OQ429701	MK276528	Hou et al. (2023)
Ochronectria calami	CBS 134535	OQ429755	OQ055654	Hou et al. (2023)
Lasionectriella arenuloides	CBS 576.76T	OQ429696	OQ055601	Hou et al. (2023)
Lasionectriella marigotensis	CBS 131606T	OQ429698	KR105613	Hou et al. (2023)
Lasionectriella rubioi	CBS 140157T	OQ429699	KU593581	Hou et al. (2023)
Ramosiphorum polyporicola	CBS 123779T	OQ429823	OQ430084	Hou et al. (2023)
Ramosiphorum polyporicola	CBS 109.87	OQ429822	OQ430083	Hou et al. (2023)
Ramosiphorum thailandicum	CBS 101914T	OQ429825	OQ430086	Hou et al. (2023)

Species	Strains	ITS	LSU	Reference
Protocreopsis rutila	CBS 396.66T	OQ429814	OQ430077	Hou et al. (2023)
Protocreopsis rutila	CBS 229.70	OQ429813	OQ430076	Hou et al. (2023)
Protocreopsis finnmarkica	CBS 147428T	OQ429803	OQ055699	Hou et al. (2023)
Protocreopsis phormiicola	CBS 567.76T	OQ429806	OQ430069	Hou et al. (2023)
Protocreopsis freycinetiae	CBS 573.76T	OQ429804	OR052113	Hou et al. (2023)
Nectriopsis lindauiana	CBS 897.70T	OQ429729	OQ055629	Hou et al. (2023)
Nectriopsis fuliginicola	CBS 400.82T	KU382175	OQ055628	Hou et al. (2023)
Nectriopsis violacea	CBS 914.70T	OQ429733	OQ055632	Hou et al. (2023)
Nectriopsis violacea	CBS 849.70	OR050510	MH871773	Hou et al. (2023)
Nectriopsis sporangiicola	CBS 166.74T	AF210661	AF210662	Hou et al. (2023)
Clonostachys spinulosispora	CBS 133762T	MH634702	KY006568	Hou et al. (2023)
Clonostachys phyllophila	CBS 921.97T	AF210664	OQ055445	Hou et al. (2023)
Stephanonectria keithii	CBS 943.72	OQ429872	OQ430121	Hou et al. (2023)
Stephanonectria keithii	CBS 100007	OQ429871	OQ430120	Hou et al. (2023)
Mycocitrus odorus	CBS 100104T	OQ429717	OQ055618	Hou et al. (2023)
Mycocitrus odorus	CBS 120610	OQ429715	OQ055616	Hou et al. (2023)
Mycocitrus zonatus	CBS 400.70	OQ429719	OQ055620	Hou et al. (2023)
Mycocitrus phyllostachydis	CBS 330.69	OQ429718	OQ055619	Hou et al. (2023)
Emericellopsis fuci	CBS 116467	OQ429564	OQ055477	Hou et al. (2023)
Emericellopsis fuci	CBS 485.92	OQ429565	OQ055478	Hou et al. (2023)
Emericellopsis maritima	CBS 491.71T	OQ429566	OQ055480	Hou et al. (2023)
Emericellopsis pallida	CBS 490.71T	OQ429574	OQ055487	Hou et al. (2023)
Emericellopsis brunneiguttula	CBS 111360T	OQ429545	OQ055457	Hou et al. (2023)
Stanjemonium grisellum	CBS 655.79T	OQ429868	OQ430117	Hou et al. (2023)
Stanjemonium ochroroseum	CBS 656.79T	OQ429869	OQ430118	Hou et al. (2023)
Proliferophialis apiculata	CBS 303.64T	OQ429796	OQ055692	Hou et al. (2023)
Proliferophialis apiculata	CBS 365.64	OQ429797	OQ055693	Hou et al. (2023)
Acremonium subulatum	CBS 588.73AT	OQ429491	OQ055402	Hou et al. (2023)
Acremonium subulatum	CBS 115996	OQ429490	OQ055401	Hou et al. (2023)
Acremonium aerium	CBS 189.70T	OQ429441	OQ055352	Hou et al. (2023)
Acremonium longiphialidicum	CBS 451.70T	OQ429475	OQ055386	Hou et al. (2023)
Acremonium purpurascens	CBS 149.62T	OQ429485	OQ055396	Hou et al. (2023)
Acremonium ellipsoideum	CBS 147433T	OQ429468	OQ055379	Hou et al. (2023)
Acremonium ellipsoideum	CBS 147434	OQ429467	OQ055378	Hou et al. (2023)
Acremonium brunneisporum	CBS 413.76T	OQ429444	OQ055355	Hou et al. (2023)
Acremonium brunneisporum	CBS 142823	OQ429445	OQ055356	Hou et al. (2023)
Acremonium multiramosum	CBS 147436T	OQ429476	OQ055387	Hou et al. (2023)
Waltergamsia pilosa	CBS 124.70T	OQ429949	OQ430199	Hou et al. (2023)
Waltergamsia pilosa	CBS 511.82	OQ429948	OQ430198	Hou et al. (2023)
Waltergamsia alkalina	CBS 741.94T	OQ429935	OQ430185	Hou et al. (2023)
Waltergamsia dimorphospora	CBS 139050T	LN810515	LN810506	Hou et al. (2023)
Geosmithia microcorthyli	CCF 3861T	NR_137566	NG_067560	Hou et al. (2023)
Geosmithia pallidum	CBS 260.33T	OQ429599	OQ055509	Hou et al. (2023)
Bulbithecium spinosum	CBS 136.33T	OQ429512	OQ055423	Hou et al. (2023)
Bulbithecium spinosum	CBS 915.85	OQ429510	OQ055421	Hou et al. (2023)
Bulbithecium arxii	CBS 737.84T	OQ429505	OQ055416	Hou et al. (2023)
Bulbithecium ellipsoideum	CBS 993.69T	OQ429507	OQ055418	Hou et al. (2023)
Ovicillium oosporum	CBS 110151T	OQ429758	OQ055657	Hou et al. (2023)
Ovicillium asperulatum	CBS 130362T	OQ429756	OQ055655	Hou et al. (2023)
Ovicillium asperulatum	CBS 426.95	KU382192	KU382233	Hou et al. (2023)

| Species	Strains	ITS	LSU	Reference
Proxiovicillium blochii	CBS 427.93T	OQ429816	OQ430079	Hou et al. (2023)
Proxiovicillium blochii	CBS 324.33	OQ429815	OQ430078	Hou et al. (2023)
Proxiovicillium lepidpterorum	CBS 101239T	OQ429817	OQ430080	Hou et al. (2023)
Hapsidospora flava	CBS 596.70T	OQ429649	OQ055555	Hou et al. (2023)
Hapsidospora flava	CBS 316.72	OQ429648	OQ055554	Hou et al. (2023)
Hapsidospora variabilis	CBS 100549T	OQ429663	OQ055569	Hou et al. (2023)
Hapsidospora stercoraria	CBS 516.70T	OQ429662	OQ055568	Hou et al. (2023)
Alloacremonium humicola	CBS 613.82T	OQ429496	OQ055407	Hou et al. (2023)
Alloacremonium ferrugineum	CBS 102877T	OQ429495	OQ055406	Hou et al. (2023)
Stilbocrea walteri	CBS 144627T	OR050519	OQ430124	Hou et al. (2023)
Stilbocrea macrostoma	CBS 114375	OQ429873	OQ430122	Hou et al. (2023)
Flammocladiella decora	CBS 142776	MF611693	MF614949	Hou et al. (2023)
Flammocladiella aceris	CBS 138906T	OQ429591	KR611901	Hou et al. (2023)
Biconidium sinense	**GZUIFR 24.013T**	**PQ595985**	**PQ595988**	**This study**
Biconidium sinense	**GZUIFR 24.014**	**PQ595986**	**PQ595989**	**This study**
Biconidium sinense	**GZUIFR 24.015**	**PQ595987**	**PQ595990**	**This study**

Note: T = Ex-type; New isolates in this study are in bold; The line "−" represents the absence of GenBank record. ITS: the internal transcribed spacer region and intervening 5.8S nrRNA; LSU: 28S large subunit.

Table 2. Strains of *Didymocyrtis* and corresponding GenBank numbers included in phylogenetic analyses.

Species	Strains	ITS	*tub2*	Reference
Didymocyrtis banksiae	CSN1049	MT813909	−	Monteiro et al. (2022)
Didymocyrtis banksiae	CSN1065	MT813919	−	Monteiro et al. (2022)
Didymocyrtis brachylaenae	CPC 32651	MH327821	MH327896	Monteiro et al. (2022)
Didymocyrtis cladoniicola	CBS 131731	KP170644	KP170694	Monteiro et al. (2022)
Didymocyrtis cladoniicola	CBS 131732	KP170645	KP170695	Monteiro et al. (2022)
Didymocyrtis consimilis	CBS 129140	MH865190	−	Monteiro et al. (2022)
Didymocyrtis consimilis	CBS 129338	MH865230	−	Monteiro et al. (2022)
Didymocyrtis epiphyscia	Freebury 1411	KT383824.1	−	Monteiro et al. (2022)
Didymocyrtis foliaceiphila	CBS 131729	KP170649	KP170699	Monteiro et al. (2022)
Didymocyrtis foliaceiphila	CBS 131730	KP170650	KP170700	Monteiro et al. (2022)
Didymocyrtis melanelixiae	Harris 57476 (NY)	KT383831	−	Monteiro et al. (2022)
Didymocyrtis melanelixiae	Harris 57475 (NY)	KT383828	−	Monteiro et al. (2022)
Didymocyrtis pini	CAA 1002 T	MW732246	MW759031	Monteiro et al. (2022)
Didymocyrtis pini	CAA 1003	MW732247	MW759030	Monteiro et al. (2022)
Didymocyrtis pseudeverniae	Diederich 17327b	KT383833	−	Monteiro et al. (2022)
Didymocyrtis pseudeverniae	Diederich 17327a	KT383832	−	Monteiro et al. (2022)
Didymocyrtis ramalinae	Paul 10i13	KT383839	−	Monteiro et al. (2022)
Didymocyrtis ramalinae	Paul 27i13	KT383836	−	Monteiro et al. (2022)
Didymocyrtis septata	KNU-JJ-1827	LC552949	−	Monteiro et al. (2022)
Didymocyrtis slaptonensis	MoraA (BR)	KT383841	−	Monteiro et al. (2022)
Didymocyrtis trassii	AB298	MG519614	−	Monteiro et al. (2022)
Didymocyrtis trassii	AB297	MG519613	−	Monteiro et al. (2022)
Didymocyrtis xanthomendozae	CBS 129666	KP170651	KP170701	Monteiro et al. (2022)
Parathyridaria philadelphi	CBS 143432	MH107905	−	Monteiro et al. (2022)
Didymocyrtis shanxiensis	**GZUIFR 24.004T**	**PQ065635**	**PQ119783**	**This study**
Didymocyrtis shanxiensis	**GZUIFR 24.005**	**PQ065636**	**PQ119784**	**This study**

Note: T = Ex-type; New isolates in this study are in bold; The line "−" represents the absence of GenBank record. ITS: the internal transcribed spacer region and intervening 5.8S nrRNA; tub2: β-tubulin.
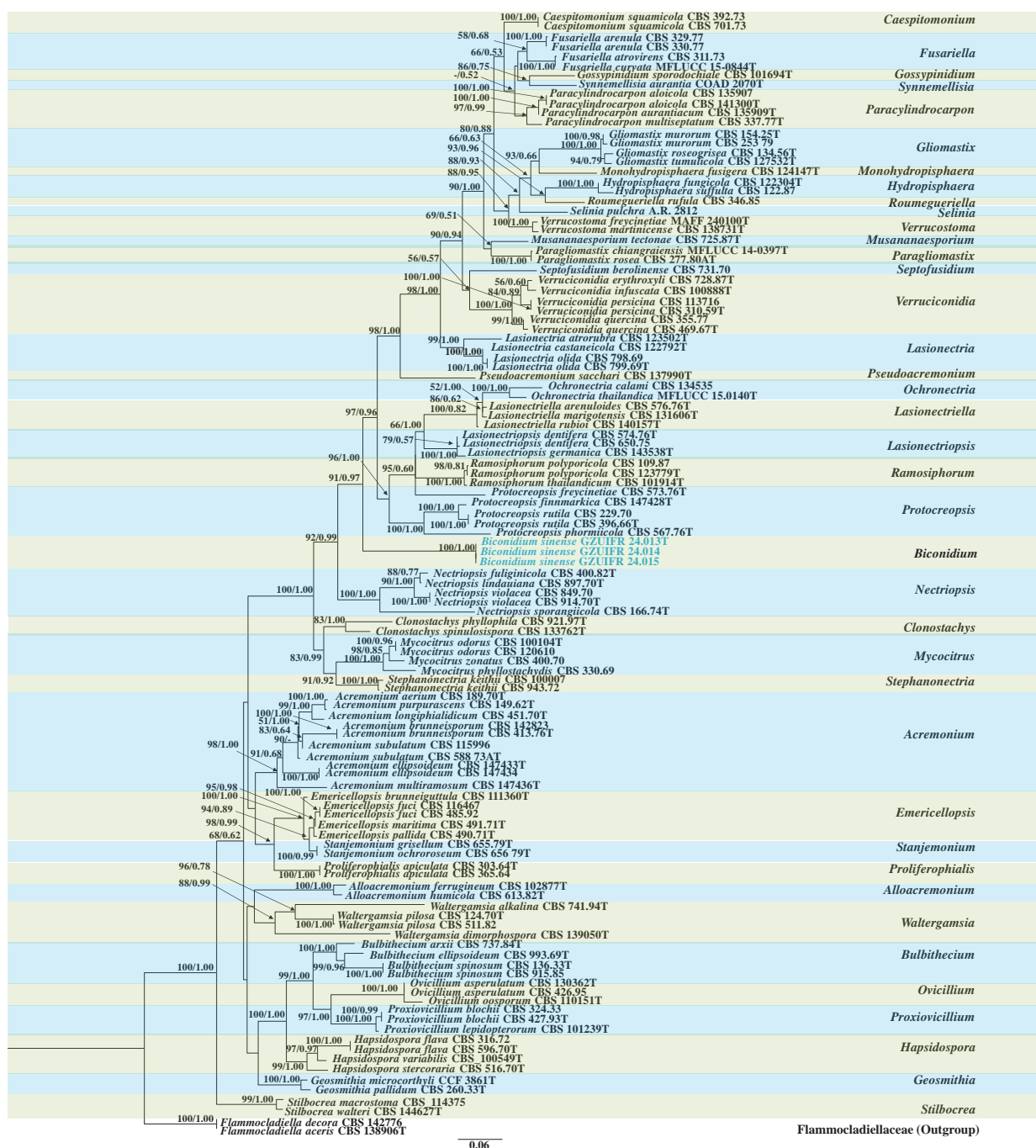
Figure 1. Phylogenetic tree of Bionectriaceae constructed from the dataset of ITS and LSU. Notes: Statistical support values (ML/BI) were shown at nodes. ML bootstrap values ≥50% and posterior probabilities ≥ 0.50 are shown above the internal branches. '−' indicates the absence of statistical support (< 50% for bootstrap proportions from ML analysis; < 0.50 for posterior probabilities from Bayesian analysis). Three new strains are shown in blue font.

Sordariomycetes O.E. Erikss. & Winka
Hypocreales Lindau
Bionectriaceae Samuels & Rossman

Biconidium **H.Y. Wang & Y.F. Han, gen. nov.**
MycoBank No: MB857281

Etymology. Referring to the bicellular conidia.

Description. *Mycelium* hyaline, septate, smooth, thin-walled. *Conidiophores* hyaline, septate, smooth-walled, solitary, straight, (sub-)erect, arising directly from hyphae, unbranched or branched, bearing 1–5 levels with 1–6 phialides per node. *Conidiogenous cells* enteroblastic, monophialidic, lateral or terminal, awl-shaped, hyaline, smooth, with globose to cylindriform thickening at conidiogenous loci. *Conidia* bicellular, podiform, unsymmetrically at both ends, hyaline, thick-walled, smooth, arranged in slimy heads. Chlamydospores and sexual morph absent.

Type species. *Biconidium sinense* H.Y. Wang & Y.F. Han

Notes. Three isolates from green soil of sewage treatment plant clearly form an independent clade on the ITS and LSU tree (Fig. 1), and are phylogenetically segregated from other genera, representing the new species with conidiogenous cells with globose to cylindriform thickening at conidiogenous loci and podiform conidia arranged in slimy heads. Therefore, we introduce *Biconidium* as a new genus to accommodate this species.

Biconidium sinense **H.Y. Wang & Y.F. Han, sp. nov.**
MycoBank No: MB857282
Fig. 2

Etymology. Referring to China where the species was isolated.

Type. China • Zhejiang Province, Hangzhou City, sewage treatment plant (30°10'53"N, 120°10'2"E), soil, August 2021, Yulian Ren, ex-type culture GZUIFR 24.013, dried holotype GZAC 24.013. ITS sequences, GenBank PQ595985; LSU sequences, GenBank PQ595988.

Description. Culture characteristics (7 days of incubation at 25 °C): Colony on PDA, 20–30 mm diam., fleshy, plicated, beige (RAL1001) at the center, villiform, traffic white (RAL 9016) at the edge, reverse, light lvory (RAL1015) at the center, cream (RAL9001) at the edge, nearly round, margin partial; Colony on MEA, 25–30 mm diam., flocculence, traffic white (RAL 9016), reverse, broom yellow (RAL1032), margin entire, round. Colony on OA, 30–35 mm diam., thin, short villous, signal white (RAL9003), reverse, cream (RAL9001), margin entire, round.

On PDA, *Mycelium* hyaline, septate, smooth, thin-walled 1.2–2.7 µm wide. *Conidiophores* hyaline, septate, smooth, solitary, straight, (sub-)erect, arising directly from hyphae, branched or unbranched, bearing 1–5 levels with 1–6 phialides, 1–3 septate at base or middle, 20–52 µm long, 1.5–2.7 µm wide at base. *Phialides* lateral or terminal, from the conidiophores or directly from the mycelia, awl-shaped, hyaline, smooth-walled, 9.5–35 µm long, 1–2.3 µm wide at base, with globose to cylindriform thickening at conidiogenous loci. polyphialides not observed. *Conidia* podiform, 1-septate, 2.5–6.0 × 1.0–3.0 µm (mean ± SD = 3.5 ± 1.0 × 2.0 ± 0.5 µm, n = 30), center-empty, unsymmetrically at both ends, apex angular, base subobtuse, hyaline, thick-, smooth-walled, arranged in slimy heads. Chlamydospores and sexual morph not observed.

Additional specimens examined. China • Zhejiang Province, Hangzhou City, sewage treatment plant (30°10'53"N, 120°10'2"E), soil, August 2021, living cultures GZUIFR 24.014 (ITS sequences, GenBank PQ595986; LSU sequences, GenBank PQ595989), GZUIFR 24.015 (ITS sequences, GenBank PQ595987; LSU sequences, GenBank PQ595990).
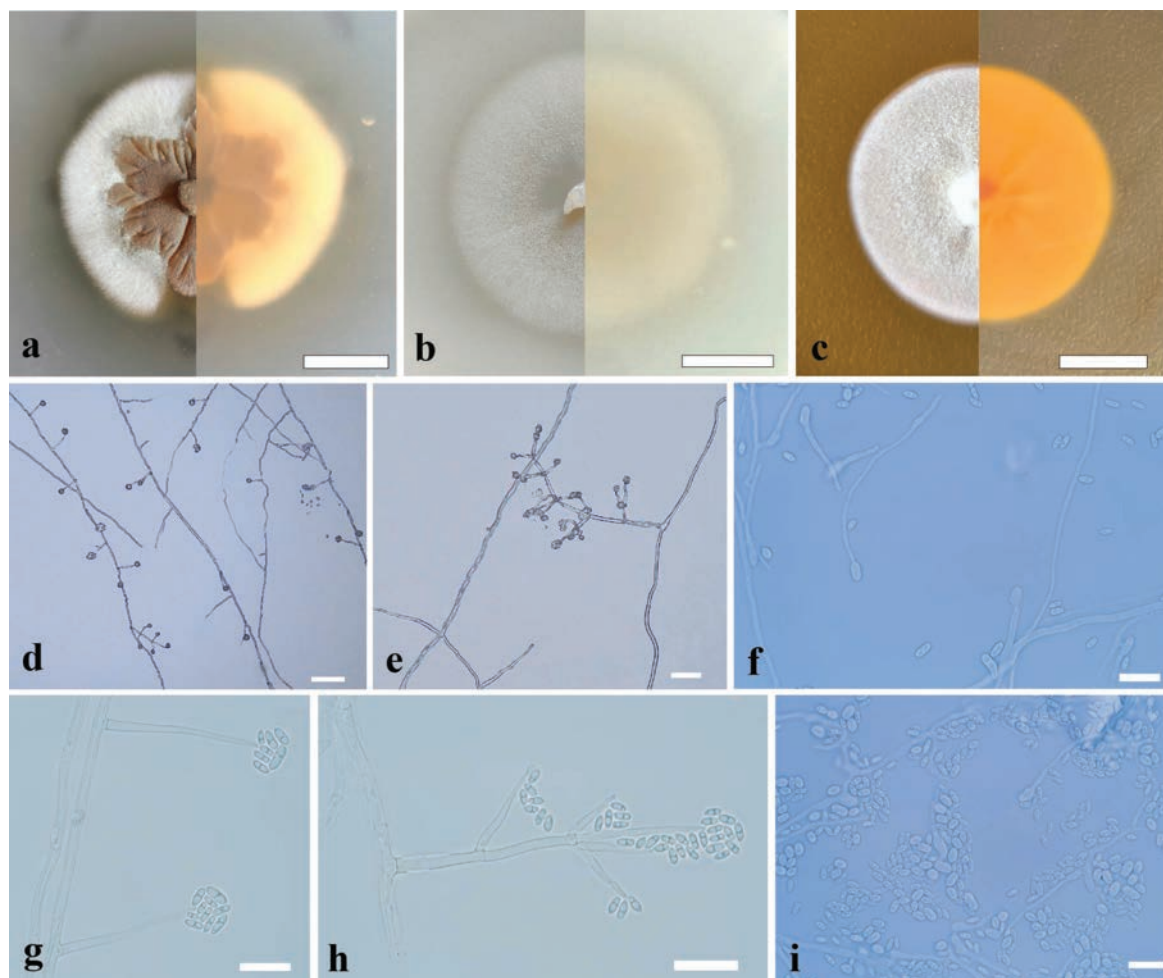
Figure 2. Morphological characteristics of *Biconidium sinense* sp. nov. **a–c** front and reverse of colony on PDA, OA and MEA after 7 days at 25 °C **d, e** conidiophores and conidial heads **f–h** conidiophores and conidia **i** conidia. Scale bars: 10 mm (**a–c**); 50 μm (**d**); 20 μm (**e**); 10 μm (**f–i**).

Notes. Phylogenetically, our three strains (GZUIFR 24.013, GZUIFR 24.014 and GZUIFR 24.015) can apparently separate with other species in Bionectriaceae, and clustered in a single clade with a high support value (BI pp = posterior probability 1, ML BS 100) (Fig. 1). *Biconidium sinense* is distinguished from other species of Bionectriaceae by conidiogenous cells with globose to cylindriform thickening at conidiogenous loci, and podiform conidia arranged in slimy heads in the morphological characteristics.

Dothideomycetes O.E. Erikss. & Winka
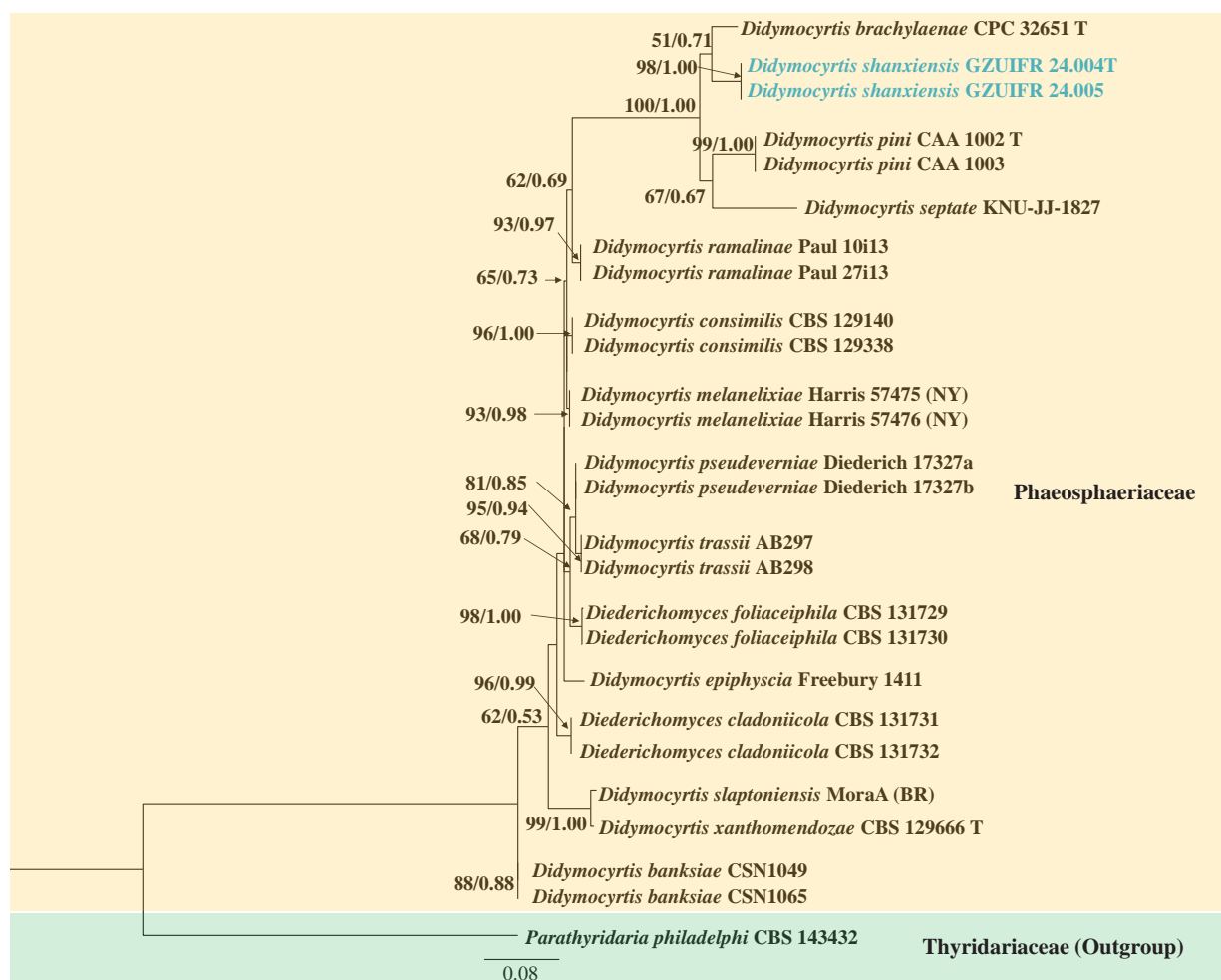**Pleosporales Luttr. ex M.E. Barr**
Phaeosphaeriaceae M.E. Barr
***Didymocyrtis* Vain.**

***Didymocyrtis shanxiensis* H.Y. Wang & Y.F. Han, sp. nov.**
MycoBank No: MB857280
Fig. 4

Etymology. shanxiensis, referring to Shanxi province where the type locality was isolated.

Figure 3. Phylogenetic tree of the genus *Didymocyrtis* constructed from the dataset of ITS and *tub2*. Notes: Statistical support values (ML/BI) were shown at nodes. ML bootstrap values ≥50% and posterior probabilities ≥ 0.50 are shown above the internal branches. Two new strains are shown in blue font.

Type. CHINA • Shanxi Province, Datong City, sewage treatment plant (40°2'42"N, 113°20'48"E), soil, August 2021, Yulian Ren, ex-type culture GZUIFR 24.004, dried holotype GZAC 24.004. ITS sequences, GenBank PQ065635; *tub2* sequences, GenBank PQ119783.

Description. Culture characteristics (7 days of incubation at 25 °C): Colony on PDA, 30−35 mm diam., thin, villiform, cream (RAL9001), reverse cream (RAL9001), regular in the margin; Colony on MEA, 20−25 mm diam., thick, villiform, light lvory (RAL1015), reverse dahlia yellow (RAL1033), regular in the margin; Colony on OA, 30−35 mm diam., texture velvety, olive yellow (RAL1020), reverse stone gray (RAL7030), regular in the margin. Black spots produced after incubating 15 days on PDA.

On PDA medium after 30 days of incubation at 25 °C, **Hyphae** septate, hyaline, smooth, thick-walled, 1.0−2.5 μm wide. **Conidiomata** submersed, brown to black, globose, 150−250 μm diam. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** globose to subglobose, also ampulliform, aseptate, hyaline, smooth, thick-walled, 4.5−10.0 × 2.0−6.0 μm (mean ± SD = 7.0 ± 1.9 × 3.5 ± 1.0 μm, n = 15). **Conidia** abundant, cymbiform mostly, brown, smooth, apex subobtuse, base truncate, 1-septate, 5.0−11.0 × 1.5−3.0 μm (mean ± SD = 7.5 ± 1.6 × 2.0 ± 0.4 μm, n = 30).
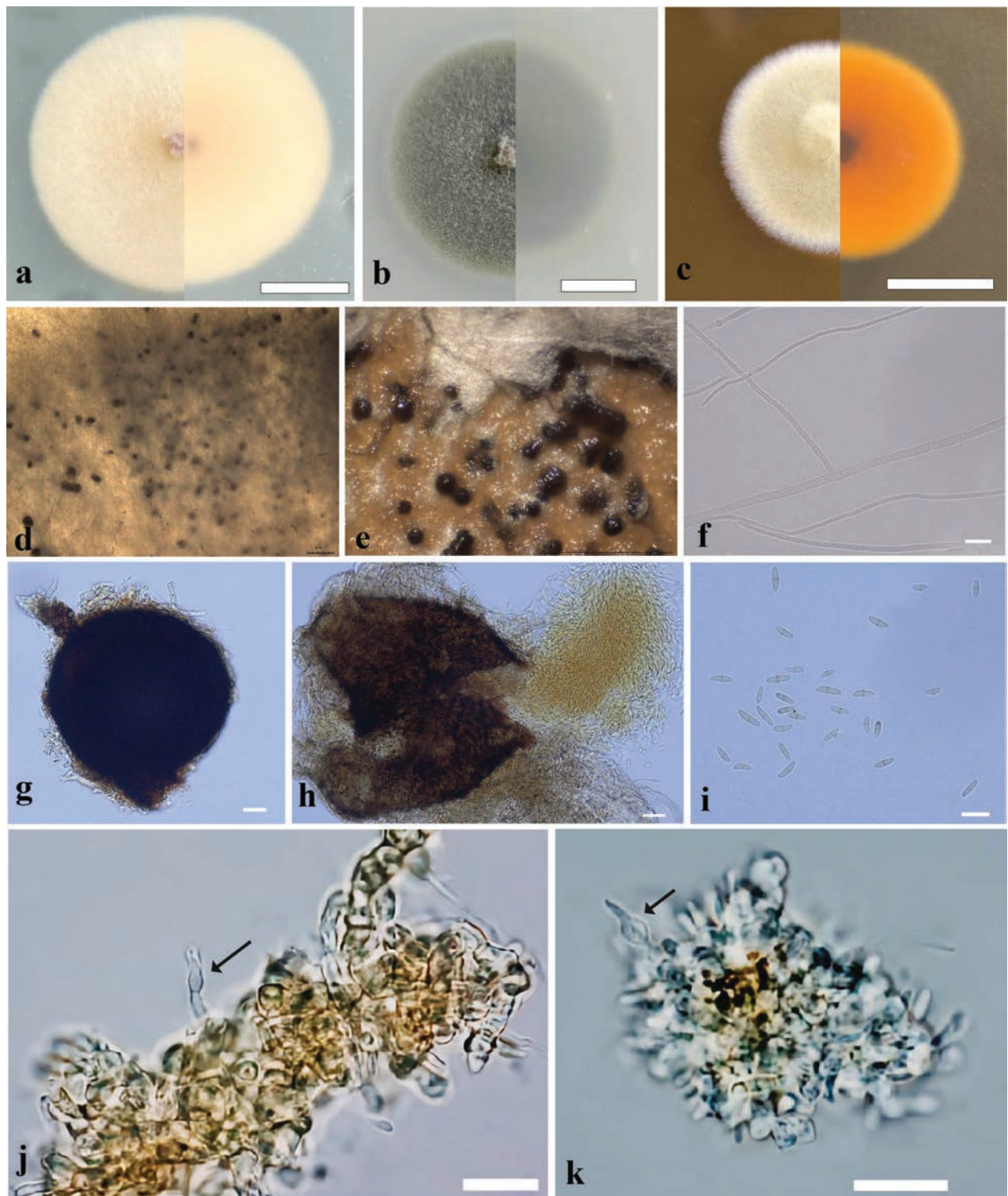
Figure 4. Morphological characteristics of *Didymocyrtis shanxiensis* sp. nov. **a–c** front and reverse of colony on PDA, OA and MEA after 7 days at 25 °C **d, e** conidiomata on culture **f** hyphae **g, h** conidiomata and ruptured conidiomata with conidia mass **i** conidia **j, k** conidiogenous cells. Scale bars: 10 mm (**a–c**); 20 μm (**g, h**); 10 μm (**i**); 20 μm (**j, k**).

Additional specimens examined. CHINA • Shanxi Province, Datong City, sewage treatment plant (40°2'42"N, 113°20'48"E), soil, August 2021, living cultures GZUIFR 24.005. ITS sequences, GenBank PQ065636; *tub2* sequences, GenBank PQ119784.

Notes. Twenty-nine species of the genus *Didymocyrtis* are recorded in the Index Fungorum. However, the DNA sequence data of fifteen species have no

records in NCBI database. Phylogenetically, our two strains (GZUIFR 24.004 and GZUIFR 24.005) clustered in a single clade with a high support value (ML/BI 98/1) (Fig. 3). In the phylogenetic tree, although our new species *D. shanxiensis* and *Didymocyrtis brachylaenae* Crous are closely related species, they were obviously different in morphological characteristics. *Didymocyrtis shanxiensis*, having conidiophores reduced to conidiogenous cells, globose to subglobose and ampulliform conidiogenous cells, and cymbiform conidia, can be distinguished from *D. brachylaenae* with subcylindrical and branched conidiophores, lining the inner cavity and ampulliform to doliiform conidiogenous cells, and fusoidellipsoid to subcylindrical conidia (Crous et al. 2018).

Discussion

Hou et al. (2023) revaluated acremonium-like fungi in Hypocreales, and found most species of *Acremonium s. lat.* grouped in genera of Bionectriaceae. Therefore, the phylogenetic tree of Bionectriaceae is provided based on multi-locus (ITS, LSU, *rpb2*, *tef-1a*) DNA sequencing analyses to accommodate 183 species and 39 genera including 10 new genera. In this study, employing ITS and LSU sequences can well distinguish the species of Bionectriaceae. From the phylogenetic tree (Fig. 1), three strains of our new species *Biconidium sinense* cluster in a well-separated clade with a high support value (ML/BI 100/1). Meanwhile, *B. sinense* having conidiogenous cells with globose to cylindriform thickening at conidiogenous loci, and podiform conidia arranged in slimy heads differs from all other species of Bionectriaceae. Therefore, *Biconidium* is introduced to accommodate a new species *B. sinense* combined with phylogenetic and morphological analyses. Bionectriaceae are including both sexual morphs and asexual taxa (Hou et al. 2023). Species of the Bionectriaceae are mostly found in terrestrial or freshwater environments, with fewer commonly found in marine habitats, and they are common coprophilous, corticolous, fungicolous, lichenicolous or herbicolous (Zhao et al. 2023). In this study, our three strains of *B. sinense* were isolated from green soils of sewage treatment plant.

In this study, although *D. shanxiensis*, *D. brachylaenae*, *D. pini* and *D. septata* clustered as the sister subclades, they were obviously different in morphological characteristics. Morphologically, the main characteristics of *D. shanxiensis* are having globose conidiomata, conidiophores reduced to conidiogenous cells, globose to subglobose and ampulliform conidiogenous cells, and the smaller size of cymbiform conidia (mean size = 7.5 × 2.0 µm). While, *D. brachylaenae* can be distinguished from *D. shanxiensis* by having subcylindrical and branched conidiophores, and fusoidellipsoid to subcylindrical conidia (Crous et al. 2018); *Didymocyrtis pini* can be distinguished from *D. shanxiensis* by having fusiform conidia (mean size = 8.5 × 2.4 µm) (Monteiro et al. 2022); *Didymocyrtis septate* differed from *D. shanxiensis* by having irregular conidiomata, and fusiform, clavate to subcylindrical conidia (mean size = 8.2 × 2.3 µm) (Das et al. 2021). At the same time, *D. shanxiensis* has a clear morphological difference from fifteen species without DNA sequence data (Joshi et al. 2024), so it is proposed as a new species in the genus *Didymocyrtis*. Up to now, the most species of *Didymocyrtis* are lichenicolous fungi living parasitic life-styleare (Ertz et al. 2015; Suija et al. 2021). Some *Didymocyrtis* spp. are pathogenic fungi and saprophytic fungi. For example, *D. brachylaenae* and *D. pini* as pathogeny

live on plant leaves (Crous et al. 2018; Monteiro et al. 2022), and *D. septata* is saprophytic in containing plant soil (Das et al. 2021). Our two strains of new species were also isolated from green land soil in this study and possible to be saprophytic. Presently, this genus includes twenty-nine species in the Index Fungorum (http://www.indexfungorum.org/Names/Names.asp, retrieval on 10 January 2025). Here, together with *D. shanxiensis*, the genus *Didymocyrtis* has a total of thirty species.

Though two new species were reported in this study, we believed that more new taxa will be found and reported from the various soil habitats, which are deserving to be explored in the future.

Acknowledgements

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Hai-Yan Wang: Writing – review & editing, Formal analysis, Project administration. Chunbo Dong, Yan-Wei Zhang and Wan-Hao Chen: Data acquisitio, Data analysis, Investigation, Data curation. Yan-Wei Zhang and Yan-Feng Han: Supervision, Project administration, Funding acquisition.

Author ORCIDs

Hai-Yan Wang https://orcid.org/0000-0001-9190-0490
Chunbo Dong https://orcid.org/0000-0001-7074-5700
Yan-Wei Zhang https://orcid.org/0000-0003-1251-5821
Wan-Hao Chen https://orcid.org/0000-0001-7240-6841
Yan-Feng Han https://orcid.org/0000-0002-8646-3975

Data availability

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

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