

Biodiversity of lignicolous freshwater fungi from the Nanpan River Basin in Guizhou and Guangxi Provinces, China, with descriptions of fifteen species

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Abstract

The karst-dominated Nanpan River Basin (flowing through Yunnan, Guizhou and Guangxi Provinces, China) harbors unique aquatic ecosystems, however, the freshwater fungal diversity remains poorly studied. This study aims to investigate the taxonomic composition, diversity, and distribution patterns of lignicolous freshwater fungi using an integrative approach combining morphological observation and molecular phylogenetics. A total of 352 strains of lignicolous freshwater fungi were isolated from submerged decaying wood from the Nanpan River Basin in Guizhou and Guangxi Provinces, during 21–25 Nov 2024. Molecular identification via BLASTn analysis of ITS or LSU sequences revealed 189 species across 104 genera, 70 families, 27 orders, and six classes. Guizhou Province exhibited higher species richness, with 136 species identified from 213 strains, compared to 79 species from 139 strains in Guangxi Province. Community composition analysis revealed that only a few genera (32) and species (26) overlapped between the two provinces. *Dictyocheirospora* and *Periconia* were the dominant genera common to both provinces; *Pseudohalonectria* (6.1%), and *Myrmecridium* (5.2%) dominated in Guizhou, whereas *Neokalmusia* (7.9%) and *Pseudoastrosphaeriella* (5%) were the dominant genera in Guangxi. In addition, ten new species are introduced, namely: *Apiospora submersa*, *Lolia guangxiensis*, *Neomyrmecridium submersum*, *Ophioceras lignicola*, *Pseudoteichospora aquatica*, *Tetraploa guizhouensis*, *T. nanpanjiangensis*, *T. verrucispora*, *Veronaea submersa*, and *Wongia guizhouensis* based on characteristics and phylogenetic evidence. *Wongia bandungensis* is synonymized under *W. suae* based on phylogenetic analysis and morphological characteristics. In addition, five known species were collected and are described. These findings significantly expand the known diversity of lignicolous freshwater fungi in Southwest China, highlighting the basin as a hotspot for understudied freshwater fungal taxa and providing critical baseline data for the conservation and ecological study of these ecologically important organisms.

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INTRODUCTION

Despite representing a significant portion of global biodiversity, fungi remain understudied (Hyde et al. 2024a). Estimates suggest there are 2.5 million fungal species worldwide, yet only about 160,000 have been described (<https://indexfungorum.org/Names/Names.asp>), with the vast majority from terrestrial habitats (Hawksworth & Lücking 2017, Hyde et al. 2020, 2024a, Niskanen et al. 2023). Freshwater fungi are particularly underexplored, with approximately 3,870 species formally described, accounting for 2–3% of known fungal diversity (Calabon et al. 2022, 2023). Yet, they represent a critically important ecological group in aquatic ecosystems, characterized by remarkably high diversity, distributed across 13 fungal phyla, viz. *Aphelidiomycota*, *Ascomycota*, *Basidiomycota*, *Blastocladiomycota*, *Chytridiomycota*, *Entomophthoromycota*, *Monoblepharomycota*, *Mortierellomycota*, *Mucoromycota*, *Olpidiomycota*, *Rozellomycota*, *Sanchytriomycota*, and *Zoopagomycota* (Calabon et al. 2022). Recent discoveries, however, underscore their hidden richness: between 2015 and 2020 alone, 298 new freshwater fungal species were reported from China and Thailand (Calabon et al. 2021, Bao et al. 2021). Yang et al. (2023) introduced 43 new species from karst landscapes in China and Thailand; Wang et al. (2024) reported 48 species from karst landscapes in Yunnan Province, China, of which 14 are new; Shen et al. (2024) introduced 18 new species from plateau lakes in Yunnan Province, China; and recently, Xu et al. (2025) and Shen et al. (2025) introduced 82 new species from China. These hint at the immense untapped diversity within these ecosystems.

Lignicolous freshwater fungi play pivotal roles in driving nutrient cycling and ecosystem functioning (Wong et al. 1998, Hyde et al. 2016). These fungi colonize submerged woody debris in streams, ponds, lakes, and tree hollows (Hyde et al. 2016), where they facilitate the decomposition of woody plant material, recycling critical nutrients such as carbon and nitrogen (Jones et al. 2014, Gulis et al. 2019). Beyond their role in decomposition, lignicolous freshwater fungi also contribute to habitat structuring, support aquatic food webs (Wong et al. 1998, Wurzbacher et al. 2014, Tsui et al. 2016), and may act as bioindicators of ecosystem health, particularly in response to environmental stressors like pollutants (Röhl et al. 2017, Barros et al. 2024, Siriarchawatana et al. 2024, French et al. 2024), or climate change (Hyde et al. 2016). Despite their ecological importance, our understanding of their biodiversity remains limited, with many species yet to be discovered and described.

The Nanpan River Basin, a unique and ecologically significant river basin in southwestern China, serves as a major tributary of the Pearl River. Flowing through Yunnan, Guizhou, and Guangxi provinces, it traverses diverse landscapes characterized by karst topography, subtropical monsoon climates, and lush terrestrial vegetation, while encompassing several major plateau lakes, including Fuxian Lake, Xingyun Lake, Yangzonghai Lake, and Qiluhu Lake (Fangtong et al. 2024). This region is renowned for its high biodiversity, including endemic flora and fauna, yet its freshwater fungal communities remain poorly described. Existing studies on freshwater fungi in this river basin have primarily focused on the lakes in Yunnan Province. For example, Cai et al. (2002) investigated freshwater fungal diversity in Fuxian Lake, reporting 64 species, including a new taxon. Luo et al. (2004) also studied the fungi on submerged wood in Lake Dianchi, a heavily polluted lake in Yunnan, China. However, these studies relied primarily on morphological traits to identify freshwater fungi and did not conduct molecular systematic analyses of these communities. Additionally, several studies have investigated yeast diversity in the water bodies and sediments of lakes within the Nanpan River Basin, including Yangzong Lake (Yan et al. 2013), Fuxian Lake (Guo et al. 2016), and Yilong Lake (Li et al. 2020a). In recent years, several studies have focused on plateau lakes in Yunnan Province, including several lakes of the Nanpan River Basin (e.g., Yangzonghai Lake, Fuxian Lake, Yilong Lake, Qilu Lake, and Xingyun Lake), which have reported 58 new species (Shen et al. 2024, 2025). These works highlight the rich diversity of freshwater fungi in the basin. Nevertheless, Guizhou and Guangxi provinces, which form the lower reaches of

the Nanpan River, remain understudied. Consequently, critical gaps persist in our understanding of fungal species richness, community dynamics, and their functional roles within this ecologically sensitive region.

Against this backdrop, the present study aims to comprehensively assess the biodiversity of lignicolous freshwater fungi in the Nanpan River Basin. By integrating morphological identification and molecular phylogenetics, we aim to: (1) investigate lignicolous freshwater fungi from the Nanpan River Basin with a comprehensive sampling; (2) characterize the species composition and richness of lignicolous freshwater fungi; and (3) describe novel species with detailed descriptions and illustrations. This research not only fills a critical gap in regional biodiversity records but also lays the foundation for conservation strategies to preserve freshwater fungal communities and their ecosystem services. By highlighting the unique fungal diversity of the Nanpan River Basin, we aim to underscore the importance of protecting understudied aquatic habitats in southwestern China, where biodiversity hotspots coexist with rapid anthropogenic development. Ultimately, this study contributes to global efforts to understand microbial ecology in freshwater systems and informs sustainable management of ecologically sensitive river basins worldwide.

MATERIALS AND METHODS

Sample collection

Samples of submerged decaying wood were randomly collected along the Nanpan River from 13 sites distributed in Guizhou and Guangxi provinces, China, from November 21, 2024, to November 25, 2024. Detailed information about the collections is presented in Fig. 1 and Table 1. The samples were brought to the laboratory in plastic bags and incubated in the plastic boxes lined with moisture-absorbing paper for one week.

Table 1 Details of the sampling sites.

Code	Collection Site	Province	Geographical location	Elevation (m)	Collection date
Site1	Xingyi City	Guizhou	25°2'58.2"N; 104°59'27.6"E	750	2024.11.21
Site2	Xingyi City	Guizhou	24°57'06.1"N; 105°1'29.4"E	740	2024.11.21
Site3	Xingyi City	Guizhou	25°2'15.4"N; 104°59'03.2"E	780	2024.11.22
Site4	Xingyi City	Guizhou	24°58'15.4"N; 104°55'24.3"E	1080	2024.11.22
Site5	Anlong County	Guizhou	24°56'58.0"N; 105°7'11.5"E	630	2024.11.23
Site6	Longlin County	Guangxi	24°57'57.4"N; 105°15'25.7"E	400	2024.11.23
Site7	Longlin County (Yacha Town)	Guangxi	24°55'50.0"N; 105°15'39.8"E	400	2024.11.23
Site8	Longlin County (Gebu Town)	Guangxi	24°47'19.4"N; 105°2'07.1"E	740	2024.11.24
Site9	Longlin County (Hongyan Town)	Guangxi	24°46'16.6"N; 105°7'30.6"E	760	2024.11.24
Site10	Longlin County (Jingzhongshan Town)	Guangxi	24°40'03.1"N; 104°52'04.2"E	720	2024.11.24
Site11	Longlin County (Jingzhongshan Town)	Guangxi	24°40'04.4"N; 104°52'43.9"E	720	2024.11.24
Site12	Xilin County	Guangxi	24°37'51.8"N; 104°41'16.5"E	750	2024.11.25
Site13	Xilin County	Guangxi	24°38'13.7"N; 104°35'38.3"E	770	2024.11.25

Isolation and morphological examination

Morphological studies were conducted according to the methods outlined by Luo et al.

(2018). Fungal isolates were obtained using single-spore isolation, as described by Senanayake et al. (2020). Germinating ascospores and conidia were transferred to fresh potato dextrose agar (PDA) medium and incubated at room temperature for 2–4 weeks. Cultures were then grown for 1–2 months and deposited in the Guizhou Academy of Agriculture Sciences Culture Collection (GZCC), with corresponding specimens. Specimens were deposited in the Guizhou Academy of Agriculture Sciences (Herb. GZAAS). Index Fungorum numbers and faces of fungi numbers were registered as outlined in the Index Fungorum (2025) and Jayasiri et al. (2015).

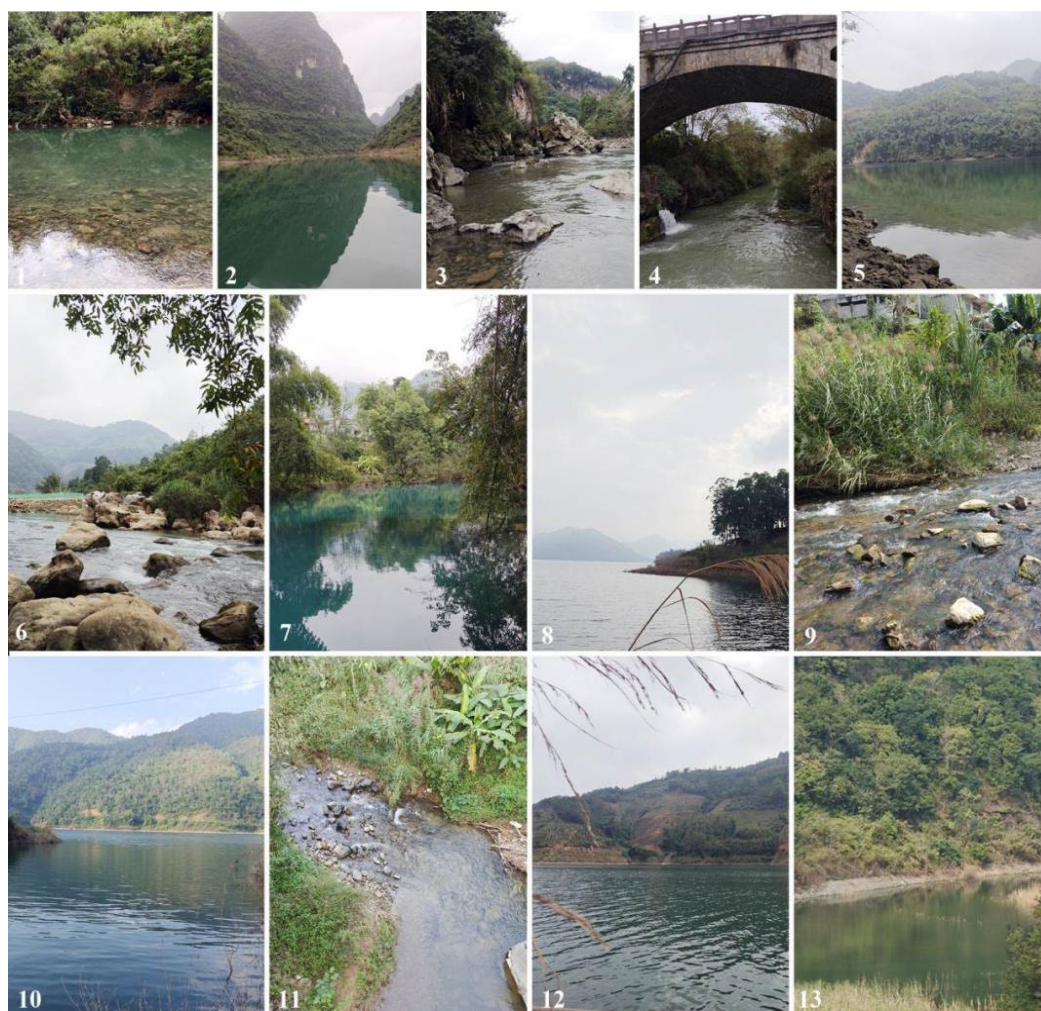


Figure 1 – The images of the 13 sample collection locations mentioned in Table 1.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted from fungal mycelium using methods described by Luo et al. (2018). The EZ gene™ Fungal gDNA Kit (GD2416) was used for DNA extraction following the manufacturer's protocol. Primer sequences are listed in Table 2. PCR amplifications were conducted in 25 µL reactions containing: 12.5 µL of 2×Power Taq PCR Master Mix (a ready-to-use solution with 0.1 Units/µL Taq DNA Polymerase, 500 µM dNTP Mixture [dATP, dCTP, dGTP, dTTP each], 20 mM Tris-HCl [pH 8.3], 100 mM KCl, 3 mM MgCl₂, stabilizers, and enhancers); 1 µL of each primer; 1 µL DNA template; and 9.5 µL nuclease-free water. This study utilized six loci: internal transcribed spacer (ITS), large subunit ribosomal RNA (LSU), small subunit ribosomal RNA (SSU), RNA polymerase II second-largest subunit (*rpb2*), translation elongation factor 1-alpha gene (*tef1-α*), and beta-tubulin gene (*tub2*). The primers for the six loci are shown in table2, and the PCR thermal cycle program for regions were as follows: initial denaturation at 98°C for 2 mins, followed by 35 cycles of denaturation at 98°C for 10 seconds, annealing at 55°C for 1 min, elongation at 72°C for 10 seconds and final extension at 72°C for 2 mins. Amplification success

was verified via 1% agarose gel electrophoresis with ethidium bromide staining. PCR product purification and sequencing were performed using the primers listed above at Chongqing Tsingke Biological Engineering Technology and Services Co., Ltd. (Chengdu, P.R. China).

Table 2 Information on loci and primers used in this study.

Loci	PCR primers (forward/reverse)	Sequences of primers	References
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
	ITS4	TCCTCCGCTTATTGATATGC	
LSU	LR0R	ACCCGCTGAACTTAAGC	Vilgalys & Hester (1990)
	LR5	TCCTGAGGGAACTTCG	
SSU	NS1	GTAGTCATATGCTTGTCTC	White et al. (1990)
	NS4	CTTCCGTCAATTCCTTTAAG	
<i>rpb2</i>	fRPB2-5f	GAYGAYMGWGATCAYTTYGG	Liu et al. (1999)
	fRPB2-7cR	CCCATRGCTTGYYTTRCCCAT	
<i>tef1-α</i>	EF-1	ATGGGTAAGGARGACAAGAC	O'Donnell et al. (2010)
	EF-2	GGARGTACCAGTSATCATG	
<i>tub2</i>	Bt2a	GGTAACCAAATCGGTGCTGCTTT	Glass & Donaldson (1995)
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	

*Abbreviations: ITS – Internal Transcribed Spacer; LSU – Large Subunit ribosomal RNA; SSU – Small Subunit Ribosomal RNA; *rpb2* – RNA polymerase II second largest subunit; *tef1-α* – translation elongation factor 1-alpha gene; *tub2* – beta-tubulin gene.

Sequence alignment and phylogenetic analyses

To identify the closest matches to the fungal isolates collected, BLAST searches were performed using the ITS or LSU regions, and selected sequences with high similarity were identified. Information on all sequences used for phylogenetic analyses is available in the supplementary materials (See Table S2). Assembled sequences and selected published sequences were initially processed using BioEdit v7.2.3 and aligned using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley 2013, Kato et al. 2019). Final adjustments to the alignments were made in BioEdit v7.2.3 as needed.

Maximum likelihood (ML) phylogenetic analyses were conducted using RAXML-HP v.8 (Stamatakis 2006, Stamatakis et al. 2008) on the XSEDE Teragrid via the CIPRES Science Gateway (Miller et al. 2010). Analyses employed the GTRGAMMA substitution model with rapid bootstrapping, followed by 1,000 bootstrap replicates to assess node support. The optimal tree was selected from suboptimal runs based on the highest likelihood score.

Bayesian inference was conducted using MrBayes v3.1.2 (Ronquist et al. 2012). The best-fit substitution model was determined using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) were calculated via Markov Chain Monte Carlo (MCMC) sampling in MrBayes v3.1.2. Six simultaneous chains ran for 1 billion generations, with trees sampled every 1,000 generations (yielding 100,000 trees). The first 20,000 trees (representing the burn-in phase) were discarded, and the remaining 80,000 trees were used to compute a majority-rule consensus tree with PP values.

Fungal community analysis

BLASTn searches in NCBI of ITS or LSU sequences for 352 lignicolous freshwater fungal strains were performed, and the results were statistically analyzed by class, order, family, genus, and species levels. Detailed information for all 352 strains is provided in the supplementary materials (see Table S1). The data were comprehensively visualized with various charts-bar, pie, chord, and Venn diagrams-using Origin 2022 (Shi 2022) and Office 365, then carefully edited with Adobe Photoshop CC 2018 to enhance clarity and presentation.

RESULTS

Diversity and community composition of lignicolous freshwater fungi

A total of 352 lignicolous freshwater fungal strains were isolated from the Nanpan River Basin in Guizhou and Guangxi provinces, China. Molecular identification via BLASTn analysis of ITS or LSU sequences revealed a taxonomic hierarchy spanning six classes (*Agaricomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Lecanoromycetes*, *Mortierellomycetes*, and *Sordariomycetes*), 27 orders, 70 families, 104 genera, and 189 species within the phylum *Ascomycota* (Table 3 and S1). *Pleosporales*, accounting for 192 isolates (55%) was the most abundant order (Fig. 2). In contrast, *Xylariales* (9.7%), *Magnaporthales* (5.7%), *Hypocreales* (4.5%), and *Myrmecridiales* (4.5%), collectively accounting for 24.4% of all isolates (Fig. 2). At the family level, *Dictyosporiaceae* (25 isolates, 7.1%), *Periconiaceae* (24 isolates, 6.8%), *Apiosporaceae* (19 isolates, 5.4%), *Occultibambusaceae* (18 isolates, 5.1%), *Didymosphaeriaceae* (16 isolates, 4.5%), and *Myrmecridiaceae* (16 isolates, 4.5%) were the most dominant families in Nanpan River Basin (Fig. 3). Notably, *Dictyocheiropsora* and *Periconia* were the most prevalent genera across the basin (Fig. 4).

Significant differences in fungal community composition were observed between the provinces of Guizhou and Guangxi. Guizhou Province exhibited higher species richness, with 136 species (213 strains) spanning 83 genera, 61 families, 24 orders, and six classes (*Agaricomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Lecanoromycetes*, and *Sordariomycetes*) (Table 3 and S1). Dominant genera including *Dictyocheiropsora* (14 strains, 6.6%), *Pseudohalonectria* (13 strains, 6.1%), *Myrmecridium* (11 strains, 5.2%), and *Periconia* (11 strains, 5.2%) (Fig. 4). Notably, *Myrmecridium schulzeri* (8 strains, 3.7%) and *Pseudohalonectria lignicola* (8 strains, 3.7%) were the most abundant species in the Guizhou Province (Table 3).

In contrast, Guangxi Province supported 79 species (139 strains) distributed across 53 genera, 37 families, 14 orders, and four classes (*Dothideomycetes*, *Eurotiomycetes*, *Mortierellomycetes*, and *Sordariomycetes*) (Table 3 and S1). Dominant genera including *Periconia* (13 strains, 9.3%), *Neokalmusia* (11 strains, 7.9%), *Dictyocheiropsora* (9 strains, 6.4%), and *Pseudoastrosphaeriella* (7 strains, 5%) (Fig. 4). Prominent species were *Neokalmusia aquibrunnea* (11 strains, 7.9%) and *Pseudoastrosphaeriella africana* (7 strains, 5%) (Table 3 and S1).

Comparative analysis revealed that *Pleosporales* emerged as the dominant order in both provinces (Fig. 5), aligning with its established global role in lignocellulose degradation. Notably, Guizhou exhibited a unique enrichment of *Xylariales* and *Magnaporthales*, while Guangxi demonstrated a more homogeneous fungal community structure across taxa. This uniformity was interrupted only by *Chaetothyriales* (7 strains), which displayed higher strain abundance. In contrast, orders such as *Magnaporthales*, *Microthyriales*, *Myrmecridiales*, *Pleurotheciales*, and *Xylariales* were represented by only five strains each in the study (Fig. 2, Table S1). The species- and genus-level comparisons between Guizhou and Guangxi provinces revealed minimal overlap, with only 26 species and 32 genera shared between the provinces. In contrast, 110 species and 51 genera were unique to Guizhou, and 53 species and 21 genera were exclusive to Guangxi (Fig. 2). These patterns underscore Guizhou's higher endemism and Guangxi's tendency to host taxa with broader geographic distributions.

Taxonomy

Based on BLASTn sequence similarity analyses, several fungal strains exhibited lower than 98% sequence similarity to previously described taxa. In this section, we performed morphological and phylogenetic analyses to identify these strains, which were classified into 15 distinct species, including 10 new species and five with new geographic or habitat records. These findings substantially advance our understanding of freshwater fungal diversity and highlight the urgent need for continued exploration and research on this ecologically and evolutionarily significant group. The latest *Outline of fungi and fungus-like taxa* (Hyde et al. 2024b) is followed in the classification used here.

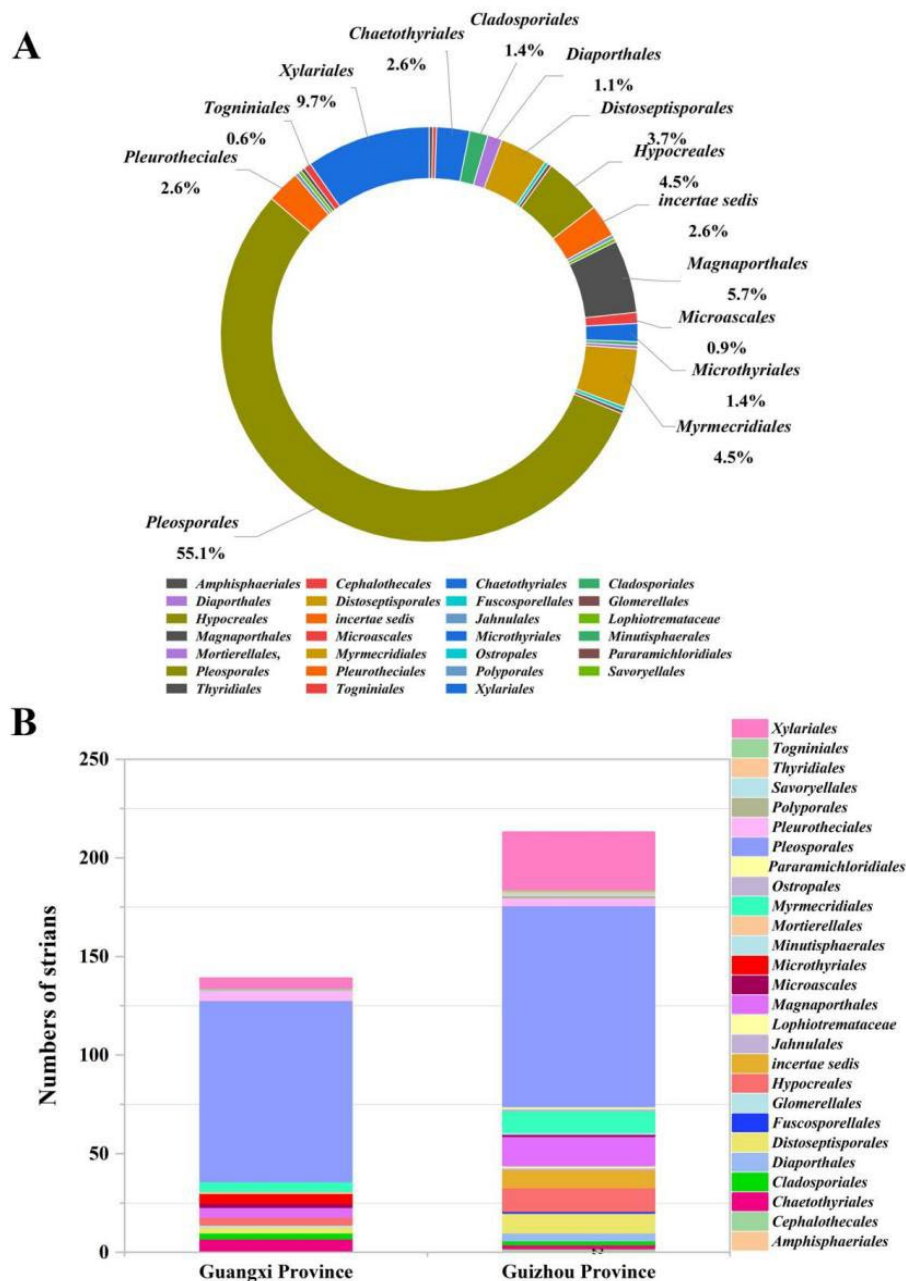


Figure 2 – Order-level distribution patterns of lignicolous freshwater fungi in the Nanpan River Basin (Guizhou and Guangxi provinces). **A** Orders of lignicolous freshwater fungi in the Nanpan River from Guizhou and Guangxi provinces. **B** Strain count distribution across lignicolous freshwater fungal orders in the Nanpan River Basin (Guizhou and Guangxi provinces).

Ascomycota Caval. Sm.

Dothideomycetes O. E. Erikss. & Winka.

Pleosporales Luttr. ex M.E. Barr

Lindgomycetaceae K. Hiray., Kaz. Tanaka & Shearer

Lolia Abdel-Aziz & Abdel-Wahab, Mycotaxon 114: 36 (2011)

Lolia was introduced by Abdel-Aziz & Abdel-Wahab (2010) to accommodate a freshwater species, *L. aquatica*, collected in Egypt, and is known only from its asexual morph. Subsequently, Abdel-Aziz (2016) revised the genus by introducing a new species *L. dictyospora*, with a sexual morph and providing the first formal description of the sexual morph for *L. aquatica*. The genus is characterized by acervular conidiomata; clavate, ellipsoidal, or cylindrical conidia with apical,

subapical, or basal appendages; immersed to erumpent, globose to subglobose ascomata; fissitunicate, cylindric-clavate asci; and fusiform, yellow to dark-brown, septate ascospores surrounded by a gelatinous sheath. Shen et al. (2024) described a new species, *L. fusiformispora*. Currently, three species are accepted in the genus, all restricted to freshwater habitats and reported only from China and Egypt.

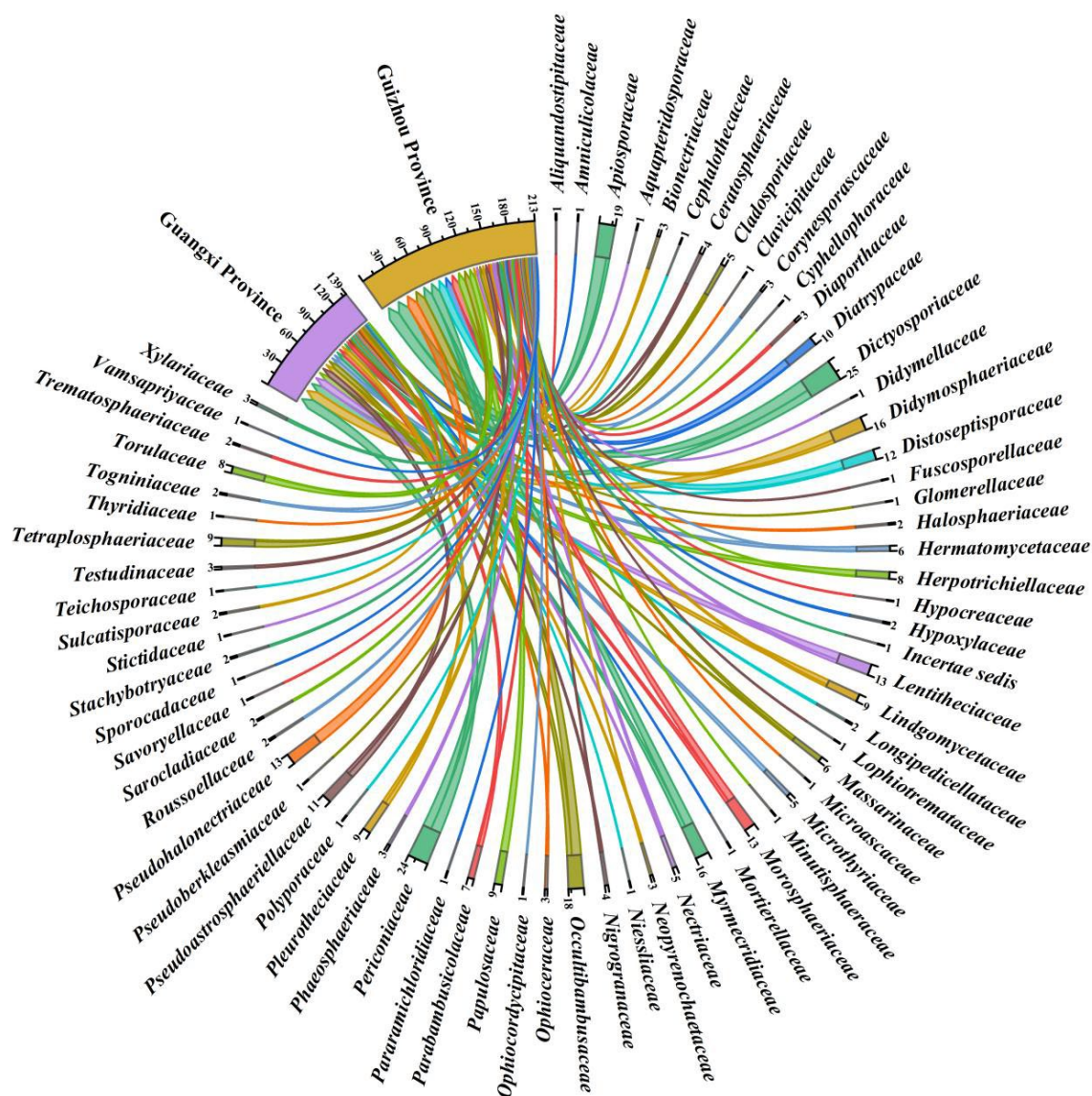


Figure 3 – Family-level distribution patterns of lignicolous freshwater fungi in the Nanpan River Basin (Guizhou and Guangxi provinces).

Lolia guangxiensis D.F.Bao, J.C Kang &Y.Z. Lu, sp. nov.

Index Fungorum number: IF 904389; Facesoffungi number: FoF 19049.

Etymology – Refers to the type locality, Guangxi Province, China.

Holotype – GZAAS 25-0729

Saprobic on submerged decaying wood. Sexual morph: *Ascomata* 245–300 µm high, 235–300 µm wide, solitary, immersed to sub-immersed, globose to subglobose, dark-brown to black, coriaceous to sub-carbonaceous, ostiolate. *Ostioles* papillate, cylindrical. *Peridium* 25–35 µm wide, two layers, outer layer dark brown, thick-walled, comprising dark brown cells of *textura prismatica* to *textura angularis*; inner layer consists of polygonal, thin-walled, hyaline to yellow-brown cells of *textura prismatica*. *Pseudoparaphyses* 1.5–2.5 µm wide, embedded in gel, enlarged at the lower parts of the ascomatal venter to form a glabrous structure, hyaline, septate, branched, smooth-

walled. *Asci* 112–140 × 13–16 μm ($\bar{x}$ = 126.3 × 14.5 μm, n = 30), 8-spored, bitunicate, fissitunicate, cylindric-clavate, pedicellate, with a minute ocular chamber. *Ascospores* 30–37.5 × 5–7 μm ($\bar{x}$ = 34 × 6 μm, n = 50), 2–3-seriate, fusiform with obtuse ends, tapering towards both ends, straight or slightly curved, yellowish brown to brown, 4–7 transverse septate, constricted at the septa, third and fourth cell enlarged, widest at the central cell, smooth-walled, guttulate, surrounded by a large gelatinous sheath.



Figure 4 – Genus-level distribution patterns of lignicolous freshwater fungi in the Nanpan River Basin (Guizhou and Guangxi provinces). **A** Strain count distribution across lignicolous freshwater fungal genera in the Nanpan River Basin (Guizhou and Guangxi provinces). **B** Strain count distribution across lignicolous freshwater fungal genera in the Guangxi section of the Nanpan

River. **C** Strain count distribution across lignicolous freshwater fungal genera in the Guizhou section of the Nanpan River.

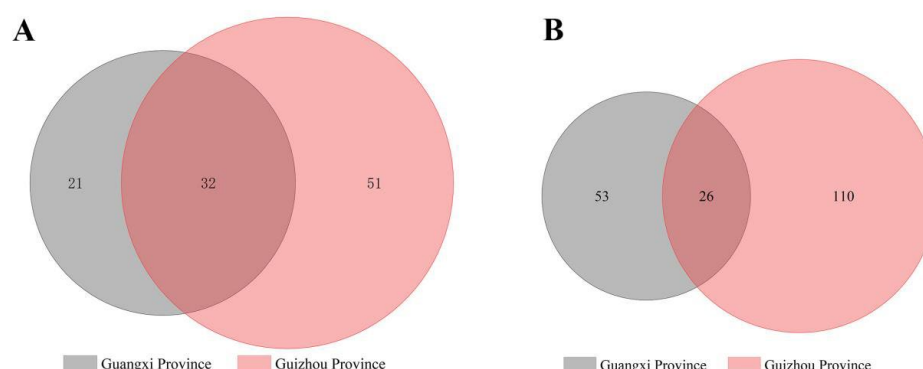


Figure 5 – Venn diagrams illustrate the distribution of lignicolous freshwater fungi in the Nanpan River from Guizhou and Guangxi provinces, depicting shared and unique taxa at species and generic levels, respectively. **A** The generic level. **B** The species level.

Table 3 The lignicolous freshwater fungi from Nanpan River Basin in Guizhou and Guangxi provinces and their relative frequency percentages (RF).

Species	Guangxi Province		Guizhou Province	
	Number of strains	RF (%)	Number of strains	RF (%)
<i>Amniculicola asexualis</i>	1	0.72	–	–
<i>Anthosulcatispora heveae</i>	1	0.72	–	–
<i>Apiospora submersa</i>	–	–	1	0.47
<i>Ap. arundinis</i>	1	0.72	–	–
<i>Ap. malaysiana</i>	1	0.72	–	–
<i>Ap. marii</i>	–	–	4	1.88
<i>Ap. paraphaeosperma</i>	–	–	6	2.82
<i>Ap. piptatheri</i>	1	0.72	–	–
<i>Ap. rasikravindrae</i>	–	–	1	0.47
<i>Aquapteridospora bambusinum</i>	1	0.72	–	–
<i>Aq. thailandica</i>	–	–	1	0.47
<i>Aq. arundinis</i>	1	0.72	–	–
<i>Aq. malaysianum</i>	–	–	2	0.94
<i>Aq. phaeospermum</i>	–	–	1	0.47
<i>Atrocalyx bambusae</i>	–	–	1	0.47
<i>Ceratosphaeria lignicol</i>	1	0.72	–	–
<i>Ce. yunnanensis</i>	1	0.72	–	–
<i>Chloridium aseptatum</i>	1	0.72	–	–
<i>Chromolaenicola lampangensis</i>	1	0.72	–	–
<i>Cladophialophora aquatica</i>	2	1.44	–	–
<i>Cladosporium cladosporioides</i>	1	0.72	1	0.47
<i>Cl. pseudocladosporioides</i>	1	0.72	–	–
<i>Cl. tenuissimum</i>	1	0.72	1	0.47
<i>Clonostachys rosea</i>	1	0.72	2	0.94

Table 3 Continued

Species	Guangxi Province		Guizhou Province	
	Number of strains	RF (%)	Number of strains	RF (%)
<i>Codinaea acaciae</i>	—	—	1	0.47
<i>Colletotrichum</i> sp.	1	0.72	—	—
<i>Corynespora cassiicola</i>	1	0.72	—	—
<i>Co. yunnanensis</i>	—	—	2	0.94
<i>Cyphellophora neerlandica</i>	—	—	1	0.47
<i>Dactylonectria torresensis</i>	—	—	1	0.47
<i>Daldinia hawksworthii</i>	—	—	2	0.94
<i>Dendryphion hydei</i>	—	—	2	0.94
<i>Dendryphion</i> sp.	—	—	2	0.94
<i>Diaporthe biconispora</i>	—	—	1	0.47
<i>Di. kochmani</i>	—	—	1	0.47
<i>Di. melonis</i>	—	—	1	0.47
<i>Diatrypella vulgaris</i>	—	—	1	0.47
<i>Dictyocheiropora aquadulcis</i>	1	0.72	1	0.47
<i>Dic. indica</i>	—	—	2	0.94
<i>Dic. multiappendiculata</i>	—	—	2	0.94
<i>Dic. nabanheensis</i>	3	2.16	1	0.47
<i>Dic. rotunda</i>	3	2.16	5	2.35
<i>Dic. submersa</i>	—	—	1	0.47
<i>Dic. vinaya</i>	2	1.44	2	0.94
<i>Dictyosporium heptasporum</i>	—	—	1	0.47
<i>Dict. thailandicum</i>	1	0.72	—	—
<i>Distoseptispora bambusae</i>	—	—	1	0.47
<i>Dis. clematidis</i>	—	—	1	0.47
<i>Dis. fusiformis</i>	—	—	1	0.47
<i>Dis. lignicola</i>	1	0.72	—	—
<i>Dis. nabanheensis</i>	—	—	1	0.47
<i>Dis. rostrata</i>	1	0.72	3	1.41
<i>Dis. suoluoensis</i>	—	—	3	1.41
<i>Epicoccum sorghinum</i>	—	—	1	0.47
<i>Eutypella citricola</i>	—	—	1	0.47
<i>Eu. scoparia</i>	1	0.72	1	0.47
<i>Exophiala pisciphila</i>	1	0.72	—	—
<i>Fusarium longifundum</i>	—	—	1	0.47
<i>Fuscosporella</i> sp.	—	—	1	0.47
<i>Halobyssothecium kunmingense</i>	1	0.72	—	—
<i>Ha. sichuanense</i>	2	1.44	1	0.47
<i>Helicascus alatus</i>	—	—	1	0.47
<i>He. aquaticus</i>	—	—	1	0.47
<i>He. elaterascus</i>	3	2.16	2	0.94

Table 3 Continued

Species	Guangxi Province		Guizhou Province	
	Number of strains	RF (%)	Number of strains	RF (%)
<i>Helminthosporium chengduense</i>	—	—	1	0.47
<i>Hermatomyces sphaericus</i>	5	3.60	1	0.47
<i>Hongkongmyces kokensis</i>	1	0.72	—	—
<i>Hongkongmyces</i> sp.	2	1.44	—	—
<i>Ho. wuhanensis</i>	—	—	1	0.47
<i>Hypoxylon monticulosum</i>	—	—	1	0.47
<i>Jahnula aquatica</i>	—	—	1	0.47
<i>Loculosulcatispora paeoniae</i>	1	0.72	—	—
<i>Lolia dictyospora</i>	1	0.72	2	0.94
<i>Lo. guangxiensis</i>	2	1.44	—	—
<i>Mariannaea pinicola</i>	2	1.44	—	—
<i>Metarhizium gaoligongense</i>	—	—	1	0.47
<i>Minimelanolocus rousselianus</i>	—	—	1	0.47
<i>Minutisphaera aspera</i>	—	—	1	0.47
<i>Mortierella exigua</i>	1	0.72	—	—
<i>Myrmecridium hydei</i>	1	0.72	1	0.47
<i>My. Schulzeri</i>	3	2.16	8	3.76
<i>My. submersum</i>	—	—	1	0.47
<i>Myrmecridium</i> sp.	—	—	1	0.47
<i>Natantispora unipolaris</i>	2	1.44	—	—
<i>Neoanthostomella fici</i>	—	—	2	0.94
<i>Neohelicascus gallicus</i>	1	0.72	2	0.94
<i>Ne. submersus</i>	—	—	2	0.94
<i>Ne. uniseptatus</i>	—	—	1	0.47
<i>Neokalmusia aquibrunnea</i>	11	7.91	4	1.88
<i>Neomultiseptospora yunnanensis</i>	2	1.44	—	—
<i>Neomyrmecridium submersum</i>	1	0.72	—	—
<i>Neopyrenochaeta annellidica</i>	2	1.44	—	—
<i>Neo. maesuayensis</i>	—	—	1	0.47
<i>Neotestudina rosatii</i>	—	—	1	0.47
<i>Neotestudina</i> sp.1	—	—	1	0.47
<i>Neotestudina</i> sp.2	—	—	1	0.47
<i>Niesslia</i> sp.	1	0.72	—	—
<i>Nigrograna aquilariae</i>	—	—	1	0.47
<i>Ni. hydei</i>	2	1.44	—	—
<i>Ni. chinensis</i>	—	—	1	0.47
<i>Occultibambusa aquatica</i>	1	0.72	—	—
<i>Oc. clavata</i>	1	0.72	—	—
<i>Oc. jonesii</i>	1	0.72	6	2.82
<i>Oc. phragmitis</i>	1	0.72	1	0.47

Table 3 Continued

Species	Guangxi Province		Guizhou Province	
	Number of strains	RF (%)	Number of strains	RF (%)
<i>Occultibambusa</i> sp.	1	0.72	—	—
<i>Ophioceras commune</i>	—	—	1	0.47
<i>Op. guizhouensis</i>	2	1.44	—	—
<i>Ophiosphaerella chiangraiensis</i>	—	—	2	0.94
<i>Oph. taiwanensis</i>	—	—	1	0.47
<i>Paraeutypella citricola</i>	—	—	2	0.94
<i>Paramicrothyrium</i> sp.	5	3.60	—	—
<i>Paramonodictys globosa</i>	2	1.44	1	0.47
<i>Pararamichloridium caricicola</i>	—	—	1	0.47
<i>Pararoussoella</i> sp.	—	—	1	0.47
<i>Parasarocladium gamsii</i>	—	—	1	0.47
<i>Paratrimmatostroma kunmingensis</i>	1	0.72	—	—
<i>Paratrimmatostroma</i> sp.	—	—	1	0.47
<i>Periconia cookei</i>	1	0.72	—	—
<i>Pe. cortaderiae</i>	1	0.72	—	—
<i>Pe. endophytica</i>	—	—	1	0.47
<i>Pe. imperatae</i>	—	—	1	0.47
<i>Periconia</i> sp.1	3	2.16	—	—
<i>Periconia</i> sp.2	2	1.44	—	—
<i>Periconia</i> sp.3	1	0.72	—	—
<i>Periconia</i> sp.4	1	0.72	1	0.47
<i>Periconia</i> sp.5	—	—	2	0.94
<i>Periconia</i> sp.6	—	—	1	0.47
<i>Periconia</i> sp.7	—	—	1	0.47
<i>Pe. thysanolaenae</i>	2	1.44	4	1.88
<i>Pe. yunnanensis</i>	2	1.44	—	—
<i>Peroneutypa mangrovei</i>	—	—	1	0.47
<i>Per. scoparia</i>	—	—	2	0.94
<i>Phaeoacremonium argentinense</i>	1	0.72	1	0.47
<i>Phaeoisaria sedimenticola</i>	3	2.16	—	—
<i>Phialemonium</i> sp.	—	—	1	0.47
<i>Pleurotheciella fusiformis</i>	—	—	1	0.47
<i>Pl. yunnanensis</i>	—	—	1	0.47
<i>Pleurothecium aquaticum</i>	—	—	1	0.47
<i>Pseudoastrosphaeriella africana</i>	7	5.04	3	1.41
<i>Ps. bambusae</i>	—	—	1	0.47
<i>Pseudoberkleasium chiangmaiense</i>	—	—	1	0.47
<i>Pseudocosmospora vilior</i>	—	—	1	0.47
<i>Pseudohalonectria aurantiaca</i>	—	—	5	2.35
<i>Pse. lignicola</i>	—	—	8	3.76

Table 3 Continued

Species	Guangxi Province		Guizhou Province	
	Number of strains	RF (%)	Number of strains	RF (%)
<i>Pse. bambusae</i>	1	0.72	—	—
<i>Pse. thailandensis</i>	—	—	1	0.47
<i>Purpureocillium lilacinum</i>	—	—	1	0.47
<i>Rhexoacrodictys erecta</i>	—	—	1	0.47
<i>Rh. fimicola</i>	1	0.72	—	—
<i>Rh. melanospora</i>	1	0.72	—	—
<i>Rostriconidium aquaticum</i>	—	—	1	0.47
<i>Roussoella uniloculata</i>	—	—	2	0.94
<i>Sarocladium glaucum</i>	—	—	1	0.47
<i>Savoryella bambusicola</i>	—	—	1	0.47
<i>Scedosporium satunense</i>	—	—	1	0.47
<i>Schizoxylon</i> sp.	—	—	1	0.47
<i>Seiridium ceratosporum</i>	—	—	1	0.47
<i>Seriascoma didymosporum</i>	—	—	1	0.47
<i>Setoseptoria guangxiensis</i>	5	3.60	3	1.41
<i>Stachybotrys chartarum</i>	—	—	1	0.47
<i>St. reniformis</i>	—	—	1	0.47
<i>Stenocarpella maydis</i>	—	—	1	0.47
<i>Submersispora variabilis</i>	2	1.44	—	—
<i>Tetraploa aristata</i>	—	—	2	0.94
<i>Te. longiappendiculata</i>	—	—	1	0.47
<i>Te. nanpanjiangensis</i>	—	—	1	0.47
<i>Te. obpyriformis</i>	—	—	1	0.47
<i>Tetraploa</i> sp.	—	—	1	0.47
<i>Te. guizhouensis</i>	1	0.72	—	—
<i>Te. verrucispora</i>	1	0.72	—	—
<i>Thyridium curvatum</i>	—	—	1	0.47
<i>Torula gaodangensis</i>	—	—	1	0.47
<i>To. polyseptata</i>	—	—	1	0.47
<i>Torula</i> sp.	1	0.72	—	—
<i>Trametes versicolor</i>	—	—	1	0.47
<i>Trematosphaeria hydrela</i>	—	—	1	0.47
<i>Tr. pertusa</i>	—	—	1	0.47
<i>Trichoderma paraviridescens</i>	—	—	1	0.47
<i>Vaginatispora lignicola</i>	—	—	1	0.47
<i>Va. microarmatispora</i>	2	1.44	2	0.94
<i>Vamsapriya uniseptata</i>	—	—	1	0.47
<i>Veronaea submersa</i>	4	2.88	—	—
<i>Versicolorisporium</i> sp.	1	0.72	—	—
<i>Versicolorisporium triseptatum</i>	—	—	3	1.41

Table 3 Continued

Species	Guangxi Province		Guizhou Province	
	Number of strains	RF (%)	Number of strains	RF (%)
<i>Ve. diffractum</i>	–	–	1	0.47
<i>Wongia aquatica</i>	–	–	4	1.88
<i>Wo. bambusae</i>	–	–	1	0.47
<i>Wo. guizhouensis</i>	–	–	3	1.41
<i>Wo. suae</i>	–	–	1	0.47

The relative frequency of isolation (RF) was calculated as the number of isolates of a given species divided by the total number of isolates, and also expressed as a percentage. – refers to no data.

Cultural characteristics – Ascospores germinating on PDA medium within 24 h. Colonies on PDA medium reaching 20–25mm diam. after 3weeks at 25°C, circular, with dense, velvety, brown mycelium on the surface; in reverse dark brown with entire margin.

Material examined – China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 8–24 (GZAAS 25–0728, holotype); ex-type living culture, GZCC 25–0698; China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 8–49 (GZAAS 25–0729), living culture, GZCC 25–0699.

GenBank numbers – GZCC:25–0698: ITS: PV982835, LSU: PV982817, SSU: PV982859, *rpb2*: PV987747. GZCC 25–0699: ITS: PV982839, LSU: PV982821, SSU: PV982858, *tefl-α*: PX312460.

Notes – Multi-locus phylogenetic analysis indicates that *L. guizhouensis* forms a sister clade to *L. aquatica* (Fig. 7). *Lolia guizhouensis* shares similar morphological characteristics with *L. aquatica* in having globose to subglobose, dark-brown to black, coriaceous to sub-carbonaceous, ostiolate ascomata; 8-spored, cylindric-clavate, pedicellate asci with a minute ocular chamber; and fusiform, straight or slightly curved, septate ascospores. However, *L. guizhouensis* differs from *L. aquatica* in having smaller ascomata (245–300 × 235–300 vs. 500–750 × 550–800 μm) and ascospores are yellow to brown, fusiform with obtuse ends, tapering at both ends, and enlarged at the 3rd or 4th cell from the apex (widest at the central cell) with smooth walls. Whereas, *L. aquatica* has fusiform ascospores with rounded ends, they are yellow to reddish-brown, enlarged at the third cell from the apex, and have a rough, ornamented surface. Additionally, ascospores of *L. guizhouensis* are narrower than those of *L. aquatica* (5–7 vs. 6–9 μm). Notably, only the LSU sequence data is available for *L. aquatica*. By comparing LSU nucleotide sequences between *L. guizhouensis* (GZCC 25–0698) and *L. aquatica* (strain MD6002T), we identified seven base pair differences. Based on morphology and phylogeny, we introduce *L. guizhouensis* as a new species.

Although *L. guizhouensis* is morphologically similar to *L. fusiformispora* in having fusiform, brown, septate ascospores enlarged at the 3rd or 4th cell from the apex and surrounded by a prominent gelatinous sheath (Shen et al. 2024). However, phylogenetically, they are distinct. *Lolia fusiformispora* clusters outside the genus, and close to *Aquimassariosphaeria typhicola* (Fig. 8). Shen et al. (2024) also showed that *L. fusiformispora* does not group within *Lolia*. These highlight the need for further clarification of its systematic position within the *Lolia* clade or potential reclassification.

Lolia dictyospora Abdel-Aziz, Phytotaxa 267: 4 (2016)

Fig. 8

Index Fungorum number: IF 551996; Facesoffungi number: FoF 19050.

Saprobic on submerged decaying wood. Sexual morph: *Ascomata* 195–287 μm high, 90–150 μm diam., immersed to erumpent, solitary, globose to subglobose with flattened base, papillate, dark-brown to black, coriaceous to sub-carbonaceous, ostiolate. *Peridium* 15–30 μm wide, two layers; outer layer consists of polygonal dark-brown cells with melanized thick-walls; inner layer

consists of polygonal flattened cells, thin-walled, hyaline to yellow-brown cells of *textura prismatica* to *textura angularis*. *Pseudoparaphyses* 1.5–2 μm wide, numerous, embedded in gel, anastomosing above asci and emerged into the ostiolar canal, branched, hyaline, guttulate. *Asci* 124–161 $\times$ 19–28 μm ($\bar{x}$ = 143.1 $\times$ 23.5 μm , n = 30), bitunicate, fissitunicate, four, six to eight-spored, clavate, short pedicellate. *Ascospores* 25–31 $\times$ 8–10 μm ($\bar{x}$ = 28.2 $\times$ 9.2 μm , n = 50), clavate, rounded and widest at the apex, obtuse at the base, tapering towards the base, 4–6-transverse septate, constricted at the septa, 0–1 longitudinal septa, occasionally with oblique septa, yellow-brown to dark-brown, guttulate, surrounded by a prominent gelatinous sheath, thick, sometimes swollen in water to form globular gelatinous structures. Asexual morph: Undetermined.

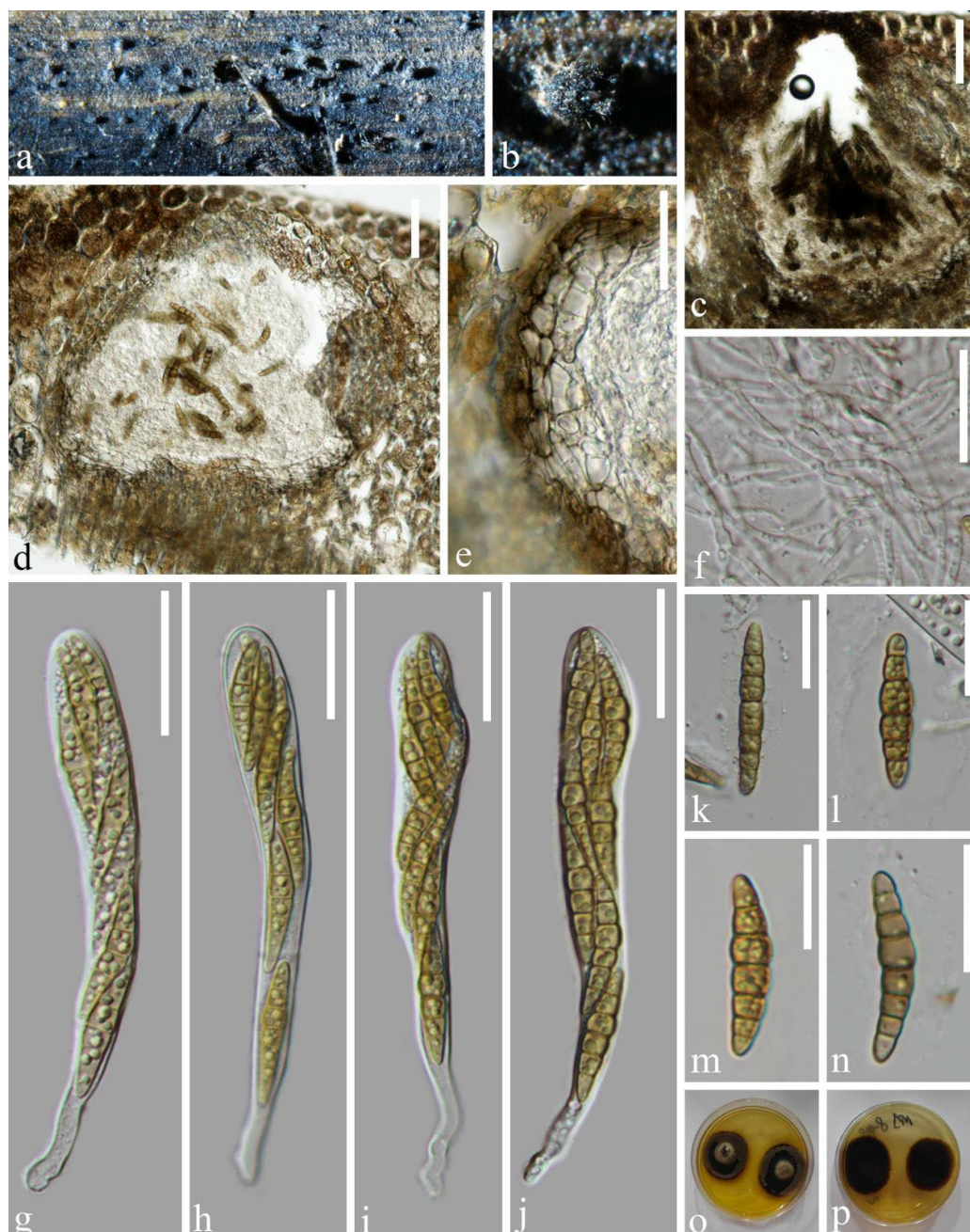


Figure 6 – *Lolia guangxiensis* (GZAAS 25–0728, holotype) a, b Appearance of ascomata on a submerged decaying wood. c, d Longitudinal sections of ascomata. e Peridium. f Pseudoparaphyses. g–j Asci. k–n Ascospores. o, p Culture on PDA from obverse and reverse. Scale bars: c, d = 50 μm , e–j = 30 μm , k–n = 20 μm .

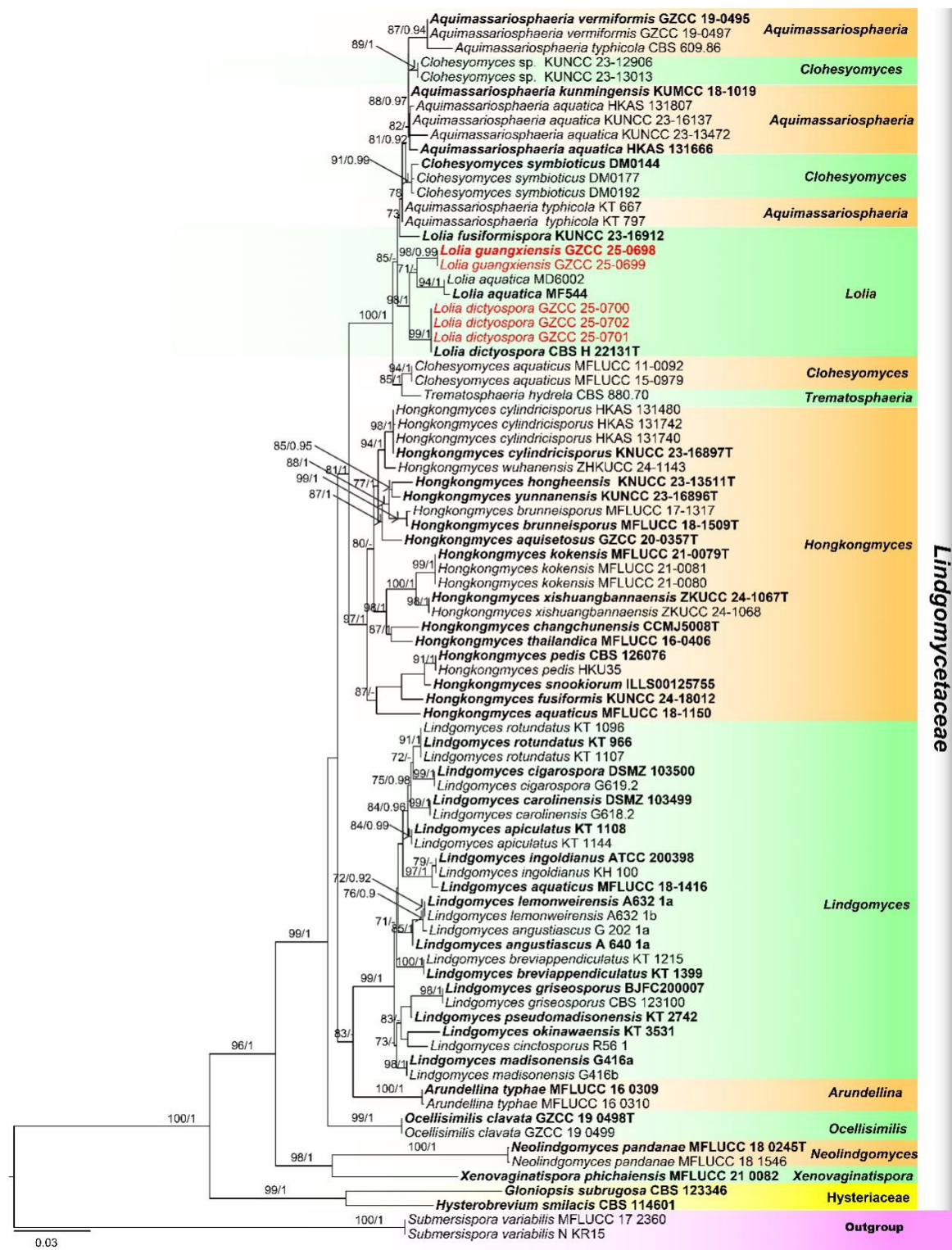


Figure 7 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated ITS, LSU, SSU, and *tef1- α* sequence datasets. A total of 85 taxa were incorporated into the combined analyses, resulting in 3,182 aligned characters after alignment. The highest-scoring RAXML tree, with a final log-likelihood value of -12371.746, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Submersispora variabilis* (MFLUCC 17–2360 and NKR15) as outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

Cultural characteristics – Ascospores germinating on PDA medium within 24 h. Colonies on

PDA medium reaching 20–25mm diam. after 3 weeks at 25°C, circular, with dense, velvety, cream to white mycelium on the surface; in reverse dark brown to black with an entire margin.

Material examined – China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 8–13 (GZAAS 25–0730), living culture, GZCC 25–0700; China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 2–98 (GZAAS 25–0731), living culture, GZCC 25–0701; China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 4–47 (GZAAS 25–0732), living culture, GZCC 25–0702.

GenBank numbers – GZCC 25–0700: ITS: PV982836, LSU: PV982818, SSU: PV982855, *tef1-α*: PX312457, *rpb2*: PV987748. GZCC 25–0701: ITS: PV982837, LSU: PV982819, SSU: PV982856, *tef1-α*: PX312458, *rpb2*: PV987749. GZCC 25–0702: ITS: PV982838, LSU: PV982820, SSU: PV982857, *tef1-α*: PX312459, *rpb2*: PV987750.

Notes – In our phylogenetic analysis, three newly obtained strains (GZCC 25–0700, GZCC 25–0701, and GZCC 25–0702) clustered with the ex-type strain of *L. dictyospora*, forming a well-supported clade (Fig. 7). Morphologically, the new isolates are nearly identical to the holotype of *L. dictyospora*, except for ascomatal, asci, and ascospores size. The new isolates have smaller ascomata (90–150 µm diam vs. 180–340 µm diam), but larger asci (124–161 × 19–28 vs. 90–124 × 12–15 µm) and ascospores (25–31 × 8–10 vs. 4–19 × 4–7 µm) compared to the holotype of *L. dictyospora* (Abdel-Aziz 2016).

Lolia dictyospora was described by Abdel-Aziz (2016) based on isolates collected from freshwater habitats in Egypt. Our new species was collected from freshwater habitats in China, and it represents the first documented occurrence of the species in this region. This geographic expansion aligns with the ecological context of the genus, as *L. dictyospora* and its relatives are thought to have evolved in freshwater environments, where they are reported exclusively to date (Abdel-Aziz & Abdel-Wahab 2010, Abdel-Aziz 2016, Shen et al. 2024).

***Teichosporaceae* M.E. Barr**

***Pseudoteichospora* X.G. Tian, K.D. Hyde & Tibpromma**

The monotypic genus *Pseudoteichospora* was introduced by Tian et al. (2024) with *P. thailandensis* designated as the type species. This species is a saprobe isolated from the dead leaves of *Ananas comosus* in Thailand. Only the asexual morphs of coelomycetes have been reported in this genus, characterized by pycnidial, dark brown to black, unilocular, globose to subglobose conidiomata, phialidic, indeterminate, cylindrical conidiogenous cells, and ellipsoidal to oval, hyaline to pale brown, aseptate conidia (Tian et al. 2024). This study introduces the second species, *P. aquatica*, collected from submerged decaying wood in China.

***Pseudoteichospora aquatica* D.F. Bao, J.C. Kang & Y.Z. Lu, sp. nov.**

Fig. 9

Index Fungorum number: IF 904391; Facesoffungi number: FoF 19051.

Etymology – Refers to the aquatic habitat of this fungus.

Holotype – GZAAS 25–0718

Saprobic on submerged decaying wood. Sexual morph: Not observed. Asexual morph: Coelomycetous. *Conidiomata* 70–175 (–219) × 79–180 (–196) µm ($\bar{x}$ = 123–129 µm, *n* = 15), pycnidial, superficial or semi-immersed, solitary, unilocular, globose to subglobose, dark brown to black. *Ostiole* central, papillate. *Conidiomatal wall* 13–28 µm wide, composed of several layers of brown cells of *textura prismatica*, with a hyaline inner lining bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5.5–13.5 × 2–3 µm ($\bar{x}$ = 9.5 × 2.3 µm, *n* = 15) phialidic, indeterminate, cylindrical, smooth-walled, hyaline. *Conidia* 5–6 × 2.5–3 µm ($\bar{x}$ = 5.5 × 2.8 µm, *n* = 40), ellipsoidal to oval with round ends, hyaline, aseptate, guttulate, mostly with two guttules at each end, smooth-walled.

Cultural characteristics – Conidia germinating on PDA medium and germ tubes produced from one end within 24 h. Colonies on PDA medium reaching 20–25 µm diam. after 30 days at 25°C; circular, entire edge, white, dense mycelium, with velutinous appearance, white on the

surface; in reverse brown to dark brown with entire margin.

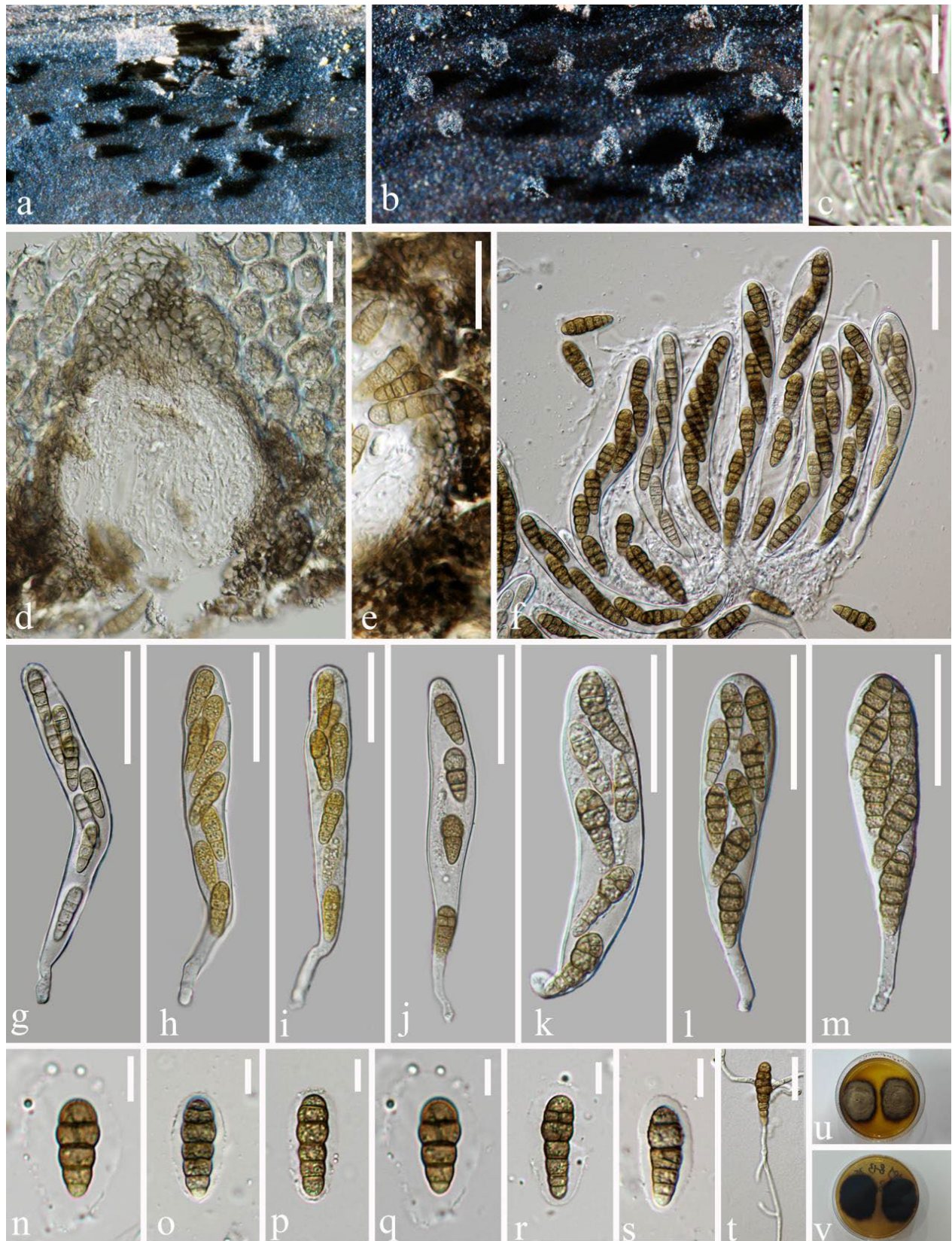


Figure 8 – *Lolita dictyospora* (GZAAS 25–0730) a, b Appearance of ascomata on a submerged decaying wood. c Pseudoparaphyses. d Longitudinal section of ascoma. e Peridium. f–m Asci. n–s Ascospores. t Germinating ascospore. u, v Culture on PDA from obverse and reverse. Scale bars: c, n–s = 10 μ m, d, e, t = 30 μ m, f–m = 50 μ m.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 1–49 (GZAAS 25–0718, holotype); ex-type living culture, GZCC 25–0688.

GenBank numbers – ITS: PV982844, LSU: PV982825, SSU: PV982861, *tef1*-α: PX312462, *rpb2*: PV987752.

Notes – Phylogenetic analysis based on combined LSU, ITS, SSU, *tef1*α, and *rpb2* sequence data revealed that *Pseudoteichospora aquatica* forms a sister clade to *P. thailandensis* with low support (Fig. 10). *Pseudoteichospora thailandensis* is the only species in the genus to date. Morphologically, *P. aquatica* shares similar morphological characteristics with *P. thailandensis*, including pycnidial, superficial or semi-immersed, dark brown to black, solitary, unilocular, globose to subglobose conidiomata; phialidic, indeterminate, hyaline conidiogenous cells; and ellipsoidal to oval, hyaline, aseptate, guttulate, smooth-walled conidia. However, critical differences in conidial morphology distinguish the two species; conidia of *P. thailandensis* are hyaline to pale brown, ellipsoidal to oval with obtuse ends, and granular to guttulate (Tian et al. 2024). In contrast, conidia of *P. aquatica* are hyaline, ellipsoidal to oval with rounded ends, and consistently guttulate with two distinct guttules at each end. These morphological distinctions, combined with phylogenetic evidence, support *P. aquatica* as a new species. This finding further expands the known habitat range of the genus *Pseudoteichospora* to include freshwater environments.

***Tetraplophaeriaceae* Kaz. Tanaka & K. Hiray**

***Tetraploa* Berk. & Broome**

Tetraploa was established by Berkeley et al. (1850), with *T. aristata* designated as the type species. This genus primarily occurs in its asexual state, characterized by cylindrical, brown, 4-celled conidia bearing 4-setae, divergent, apical appendages (Tanaka et al. 2009). Its sexual morph is characterized by immersed to erumpent, globose to subglobose, glabrous ascomata; cylindrical to clavate, short-pedicellate asci; and narrowly fusiform, septate ascospores with a narrow, mucilaginous, appendage-like sheath (Tanaka et al. 2009). Species within this genus are reported as endophytes or saprobes, inhabiting both freshwater and terrestrial habitats (Li et al. 2020b, Dong et al. 2020, Liao et al. 2022, 2024). Most records derive from bamboo, decaying wood, and other herbaceous plants, with fewer occurrences in soil (Tanaka et al. 2009, Hongsan et al. 2020, Phookamsak et al. 2022, Zhang et al. 2024a). To date, the genus comprises 48 species (<http://indexfungorum.org/names/Names.asp>, accessed 18 September 2025). In this study, we describe three new species, providing detailed morphological descriptions and illustrative plates.

***Tetraploa guizhouensis* D.F. Bao, J.C. Kang & Y.Z. Lu, sp. nov.**

Fig. 11

Index Fungorum number: IF 904392; Facesoffungi number: FoF 19052.

Etymology – Refers to the type locality, Guizhou Province, China.

Holotype – GZAAS 25–0724

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: Colonies effuse, brown or dark greyish brown. *Mycelium* mostly immersed, composed of branched, septate, brown to dark brown, smooth hyphae. *Conidiophores* absent. *Conidiogenous cells* holoblastic, monoblastic or occasionally polyblastic, integrated, determinate, terminal or intercalary, cylindrical. *Conidia* solitary, dry, straight, septate, verrucose, cylindrical to obovate, composed of a conidial body at the base and 2–4 brown to olivaceous brown apical appendages. *Conidial body* 26.5–41 × 17.5–23.5 μm ($\bar{x}$ = 33.6 × 20.6 μm, n = 30), obovoid, ovate, subcylindrical or subspherical, brown to olivaceous brown, verrucose, composed of 3–4 columns of cells, with each column 3–6-septate. *Appendages* 22–50 μm long, 2–3 μm wide at the apex, 3.5–6 μm at the base (n = 40), setose, brown, 2–5-pseudoseptate, smooth-walled.

Cultural characteristics – Colonies on PDA at 25°C, reach 20–25 mm diam. after 15 days. Colonies margin regular, circular, dry, with dense mycelium, raised, greyish brown to reddish

brown from above, dark brown from below.

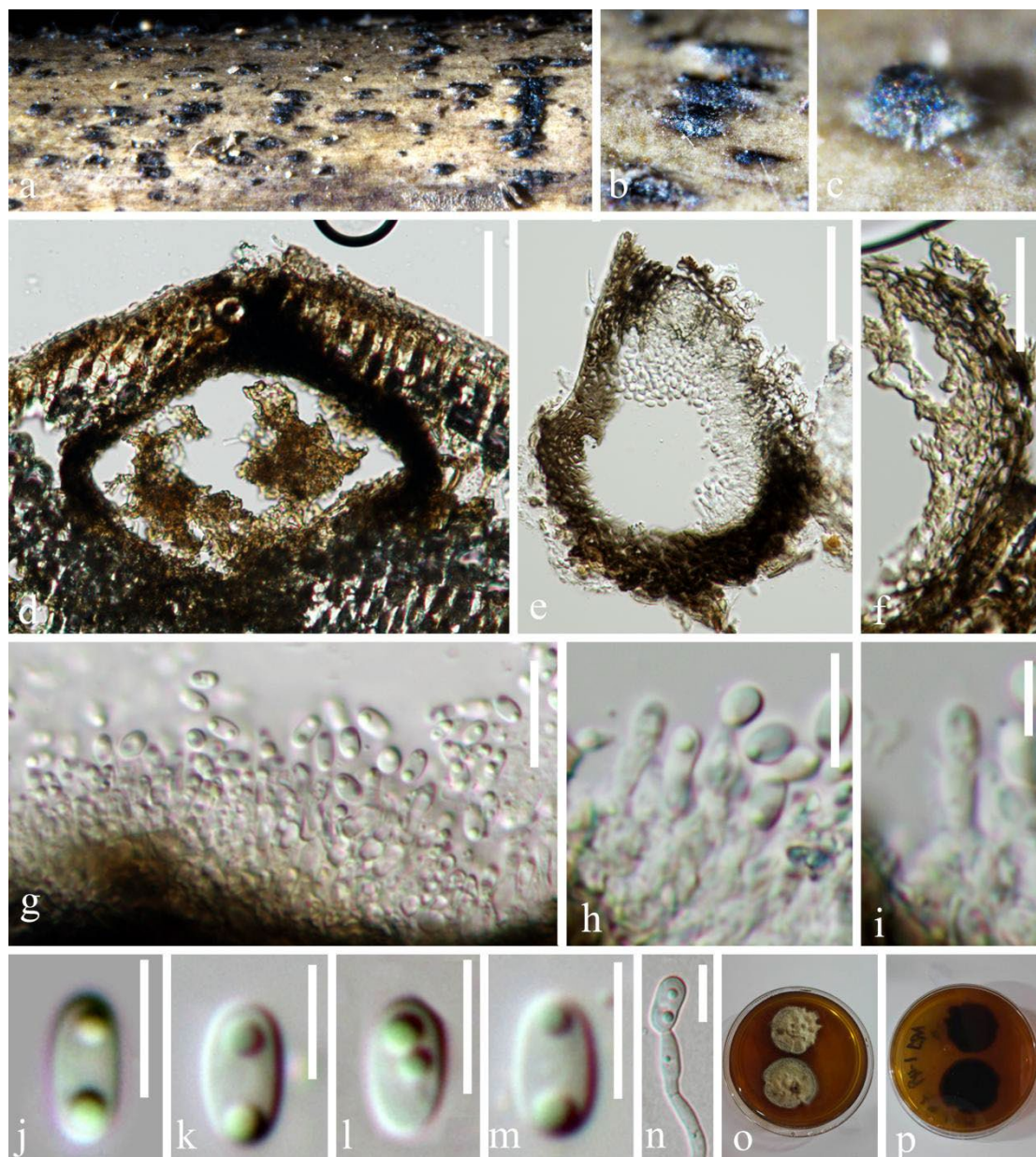


Figure 9 – *Pseudoteichospora aquatica* (GZAAS 25–0718, holotype). a–c Appearance of conidiomata on host. d, e Section through of conidiomata. f Section of peridium. g–i Conidiogenous cells and conidia. j–m Conidia. n Germinated conidium. o, p Colonies on PDA from surface and reverse. Scale bars: d, e = 50 μ m, f = 30 μ m, g = 15 μ m, h = 10 μ m, i–n = 5 μ m.

Material examined – China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 9–26 (GZAAS 25–0724, holotype); ex-type living culture, GZCC 25–0694.

GenBank numbers – ITS: PV982846, *tub2*: PV979721.

Notes – In our multi-locus phylogenetic analysis, *Tetraploa guizhounese* clustered as a sister taxon to *T. thailandica* with strong support (100% ML/1.00 PP) (Fig. 12). However, the conidial bodies of *T. guizhounese* are brown to olivaceous brown, and its appendages are 2–5-septate and shorter than those of *T. thailandica* (22–50 μ m vs. 73–136 μ m). Whereas, the appendages of *T. thailandica* are 5–10-septate (Bao et al. 2021). The nucleoside companions of the ITS locus revealed 4.07% (20/491, without gaps) differences between *T. guizhounese* and *T. thailandica* (Table 4). Thus, *T. guizhounese* is introduced as a new species.

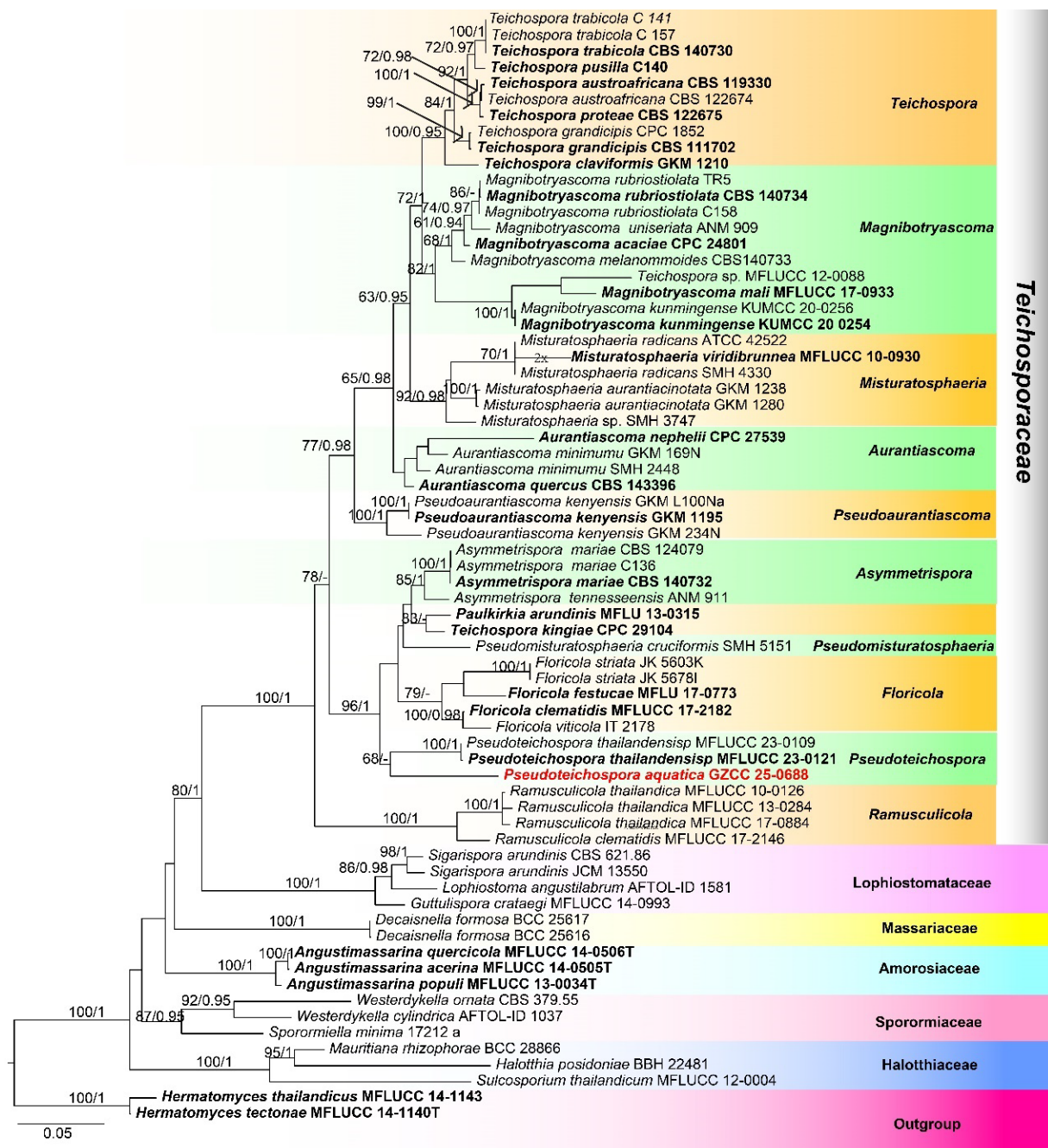


Figure 10 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated LSU, ITS, SSU, *tef1*- α , and *rpb2* sequence datasets. A total of 69 taxa were incorporated into the combined analyses, yielding 4,633 aligned characters post-alignment. The highest-scoring RAxML tree, with a final log-likelihood value of -26332.231452, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Hermatomyces thailandicus* (MFLUCC 14–1143) and *H. tectonae* (MFLUCC 14–1140) as outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

Table 4 Nucleotide differences of the ITS locus in *T. guizhouense* and *T. thailandica*.

Species	Strain	ITS
<i>T. guizhouensis</i>	GZCC 25–0694	G T T T A A C A A A T A A C G C C A G
<i>T. thailandica</i>	MFLUCC 21-0030	A C C C T G T G G G C C G G T A G G G C



Figure 11 – *Tetraploa guizhouensis* (GZAAS 25–0724, holotype). a–c Colonies on submerged decaying wood; d–p Conidia. q Germinating conidium. r, s Culture on PDA from obverse and reverse. Scale bars: d–q = 30 μ m.

Tetraploa nanpanjiangensis D.F. Bao, J.C. Kang & Y.Z. Lu, sp. nov.

Fig. 13

Index Fungorum number: IF 904393; Facesoffungi number: FoF 19053.

Etymology – Refers to the type locality, Nanpan River Basin.

Holotype – GZAAS 25–0725.

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: Colonies effuse to powdery, gregarious or cluster in small groups, brown or dark greyish brown. Mycelium mostly immersed, composed of branched, septate, hyaline, smooth hyphae.

Conidiophores absent. *Conidiogenous cells* holoblastic, monoblastic or occasionally polyblastic, integrated, determinate, terminal or intercalary, cylindrical. *Conidia* solitary, cylindrical to obovate, composed of a conidial body at the base and 1–3 brown to olivaceous brown apical appendages, septate, verrucose. *Conidial body* 24–33 × 22–29 µm ($\bar{x}$ = 28.3 × 25.7 µm, n = 30) obovoid, subcylindrical or subspherical, brown to dark brown, verrucose, composed of 2–3 closely-adhered columns of cells, with each column 2–3-septate. *Appendages* 146–264 µm long, 3–5 µm wide at the apex, 6–10 µm at the base (n = 40), setose, olivaceous brown, 5–10-septate, smooth-walled.

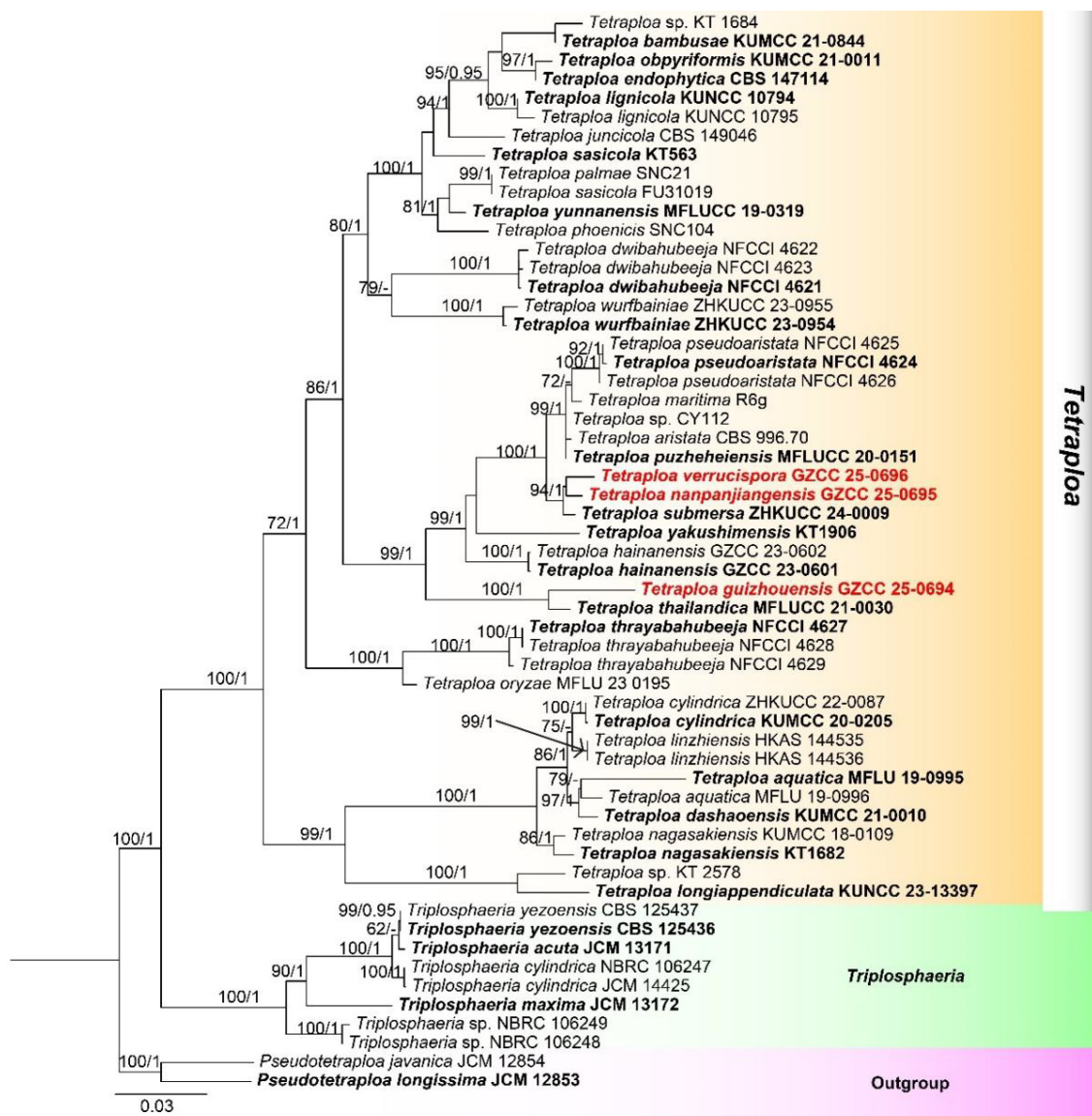


Figure 12 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated ITS, LSU, SSU, and *tub2* sequence datasets. A total of 57 taxa were incorporated into the combined analyses, yielding 3,026 aligned characters post-alignment. The highest-scoring RAxML tree, with a final log-likelihood value of -13848.900532, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Pseudotetraploa javanica* (JCM 12854) and *P. longissima* (JCM 12853) as outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

Cultural characteristics – Colonies on PDA at 25°C, reach 50–55 mm diam. after 30 days. Colonies margin regular, circular, velvety, with dense mycelium, raised, white from above, dark brown from below.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 2–80 (GZAAS 25–0725, holotype); ex-type living culture, GZCC 25–0695.

GenBank numbers – ITS: PV982847, LSU: PV982827, *tub2*: PV979722.

Notes – Phylogenetic analysis showed that *Tetraploa nanpanjiangensis* grouped with *T. submersa* and *T. verrucispora* in a well-supported clade within the genus (Fig. 12). *Tetraploa nanpanjiangensis* can be easily distinguished from *T. submersa* and *T. verrucispora* by its conidia, which are composed of 2–3 closely adhered columns; each column is 2–3-septate, with 1–3 apical appendages that are olivaceous brown and 5–10-septate. In contrast, *T. submersa* has conidia composed of 3–5 columns, each 3–5-septate, with 3–5 apical appendages, and its appendages are shorter (146–264 vs. 8–53 μm) with fewer septa (5–10 vs. 1–3 septa) compared to those of *T. nanpanjiangensis* (Zhao et al. 2024). *Tetraploa verrucispora* differs from *T. nanpanjiangensis* by the conidia composed of 3–4 columns of cells, each 3–4-septate, with 3–4 appendages that are 2–4-septate and much shorter than those of *T. nanpanjiangensis* (146–264 vs. 35–57 μm). Additionally, the conidial body of *T. nanpanjiangensis* is smaller than that of *T. verrucispora* ($24\text{--}33 \times 22\text{--}29 \mu\text{m}$ vs. $32\text{--}39 \times 20\text{--}28 \mu\text{m}$). The nucleotide comparisons of the ITS and *tub2* gene regions between *T. verrucispora* and its sister taxon *T. nanpanjiangensis*, revealed 7bp (1.4%) and 20 bp (3.2%) differences, respectively (Tables 5 and 6). Thus, based on morphological characters and phylogenetic analysis, we introduce *T. nanpanjiangensis* as a new species.

Table 5 Nucleotide differences of the ITS locus in *T. nanpanjiangensis*, *T. submersa*, and *T. verrucispora*.

Species	Strain	ITS									
<i>T. nanpanjiangensis</i>	GZCC 25–0695	T	T	A	A	A	C	A	T	A	T
<i>T. verrucispora</i>	GZCC 25–0696	T	T	G	A	G	T	G	C	A	C
<i>T. submersa</i>	ZHKUCC 24–0009	G	C	A	G	A	C	G	C	G	C

Table 6 Nucleotide differences of the *tub2* locus in *T. nanpanjiangensis*, *T. submersa*, and *T. verrucispora*.

Species	Strain	<i>tub2</i>
<i>T. nanpanjiangensis</i>	GZCC 25–0695	C G A C G C C A A C A T C C G G G A T G C T C T
<i>T. verrucispora</i>	GZCC 25–0696	T G G T T T T C G T C A C T G T G T C A T C C C
<i>T. submersa</i>	ZHKUCC 24–0009	C C A C G C C A A C A A T C T T A T T A C T T C

Tetraploa verrucispora D.F. Bao, J.C. Kang & Y.Z. Lu, sp. nov. Fig. 14

Index Fungorum number: IF 904394; Facesoffungi number: FoF 19054.

Etymology – Refers to the verrucose conidia of the new species.

Holotype – GZAAS 25–0726

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: Colonies effuse to powdery, gregarious or cluster in small groups, brown or dark greyish-brown. Mycelium mostly immersed, composed of branched, septate, hyaline, smooth hyphae. Conidiophores absent. Conidiogenous cells holoblastic, monoblastic, or occasionally polyblastic, integrated, determinate, terminal, or intercalary, cylindrical. Conidia solitary, cylindrical to obovate, straight, septate, verrucose, composed of a conidial body at the base with 3–4 apical appendages. Conidial body $32\text{--}39 \times 20\text{--}28 \mu\text{m}$ ($\bar{x} = 35.7 \times 24.3 \mu\text{m}$, $n = 30$), cylindrical to obovate, dark brown, verrucose, composed of 3–4 columns of cells, with each column 3–4-septate. Appendages 35–57 μm long, 2–3 μm wide at the apex, 3.5–5 μm at the base ($n = 40$), setose, brown to pale brown, paler towards the apex, 2–4-septate, smooth-walled.

Cultural characteristics – Colonies on PDA at 25°C, reach 50–55 mm diam. after 30 days. Colonies margin regular, circular, velvety, with dense mycelium in the margin, sparse and pale brown mycelium in the center, raised, white from above, dark brown from below.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 9–9–2 (GZAAS 25–0726, holotype); ex-type living culture, GZCC 25–0696.

GenBank numbers – ITS: PV982845, LSU: PV982826, SSU: PV982862, *tub2*: PV979720.

Notes – Phylogenetic analysis showed that *Tetraploa verrucispora* clustered with *T. nanpanjiangensis* as a sister taxon (Fig. 12). Morphologically, they are different in the number of conidial columns and appendages, including the size and numbers of septa, which we discussed above. *Tetraploa verrucispora* is also phylogenetically close to *T. submersa* (ZHKUCC 24–0009). However, *T. verrucispora* differs from *T. submersa* in that the conidia consist of 3–4 columns of cells, each 3–4-septate, with 3–4 appendages that are 2–4-septate. In contrast, the conidia of *T. submersa* are composed of 3–5 columns, each 3–5-septate, with fewer apical appendages (3–4 vs. 3–5) and fewer septa (1–3 vs. 2–4) compared to those of *T. submersa*. In addition, the conidial body of *T. nanpanjiangensis* is much smaller than that of *T. submersa* ($32\text{--}39 \times 20\text{--}28$ vs. $30\text{--}68 \times 21\text{--}53$ μm) (Zhao et al. 2024). The nucleotide of ITS and *tub2* loci between *T. verrucispora* and *T. submersa* revealed 7bp (1.4%) and 20 bp (3.2%) differences, respectively (Tables 5 and 6). Following the guidelines of Jeewon & Hyde (2016), we introduce *T. verrucispora* as a new species.

Eurotiomycetes O.E. Erikss. & Winka

Chaetothyriales M.E. Barr

Herpotrichiellaceae Munk

Veronaea Cif. & Montemart., Atti Ist. bot. Univ. Lab. crittog. Pavia, sér. 5 15: 68 (1957)

Veronaea, typified by *V. botryosa*, was established by Ciferri & Montemartini (1957). The genus is characterized by erect, mostly unbranched, pale to medium brown conidiophores, polyblastic, sympodial, terminal or intercalary conidiogenous cells, and cylindrical to pyriform, pale brown conidia that are 1- to multi-septate with smooth walls (Papendorf 1976, Arzanlou et al. 2007, Rambelli 2011, Zhang 2019, Su et al. 2023). Index Fungorum lists 32 species in *Veronaea*, of which 10 species have been transferred to other genera. Species of *Veronaea* were primarily described based solely on morphology, with only five linked to sequence data. In this study, we introduce a new species, *V. submersa*, collected from freshwater habitats in China, based on phylogenetic and morphological analyses.

Veronaea submersa D. F. Bao, J. C Kang & Y.Z. Lu, sp. nov.

Fig. 15

Index Fungorum number: IF 904395; Facesoffungi number: FoF 19055.

Etymology – Refers to the submerged habitat of the new fungus.

Holotype – GZAAS 25–0723

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Colonies effuse, brown to dark brown, hairy, dark brown to black, shiny. Mycelium in the substrate immersed or partly superficial, composed of subhyaline to pale olivaceous, smooth, hyphae. Conidiophores $53\text{--}94 \times 4\text{--}5.5$ μm ($\bar{x} = 73.5 \times 4.6$ μm , $n = 15$), macronematous, monomenatous, erect, straight or slightly flexuose, unbranched, smooth-walled, dark brown. Conidiogenous cells polyblastic, terminate, integrated, sympodial, denticulate at the upper half, cylindrical, pale brown. Conidia $8\text{--}11 \times 2.5\text{--}3.5$ μm ($\bar{x} = 9.5 \times 3.1$ μm , $n = 45$), solitary, or in chains, cylindrical to fusiform and some subclavate, tapering towards the ends, or acute at the apex, pointed at the base, subhyaline to pale brown, mostly 0 or 1-septate, rarely 2-septate, slightly constricted at the septum, with a faintly darkened, unthickened hilum, smooth-walled.



Figure 13 – *Tetraploa nanpanjiangensis* (GZAAS 25–0725, holotype). a Colonies on submerged decaying wood; b–i conidia. j Conidial body. k, l culture on PDA from the obverse and reverse. Scale bars: b–h = 50 μ m, i = 30 μ m, j = 10 μ m.



Figure 14 – *Tetraploa verrucispora* (GZAAS 25–0726, holotype). a Colonies on submerged decaying wood. b–n conidia. o Germinating conidium. p, q culture on PDA from the obverse and reverse. Scale bars: b = 50 μ m, c–e = 30 μ m, f–o = 20 μ m.

Cultural characteristics – Colonies on PDA at 25°C, reach 20–25 mm diam. after 15 days. Colonies margin regular, circular, raised, with dense mycelium, dark reddish- brown from above, dark brown to black from below.

Material examined – China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 8–54–1 (GZAAS 25–0723, holotype); ex-type living culture, GZCC 25–0693.

GenBank numbers – ITS: PV982848, LSU: PV982828, SSU: PV982863.

Notes – Phylogenetic analysis showed that *V. submersa* clustered with *V. botryose* and *V. endolichena* with 94% ML and 1.00 PP statistical support (Fig. 16). *Veronaea submersa* differs from *V. botryose* and *V. endolichena* in having solitary, or in chains, 0–2-septate, cylindrical to fusiform conidia that are tapering towards the ends, or acute at the apex and pointed at the base. In contrast, *V. endolichena* has solitary, cylindrical to pyriform, rounded at the apex, truncate at the base, 0–1-septate, with a longer and wider lower cell, and strongly constricted at the septa (Senwana et al. 2025). While *V. botryosa* has solitary, 1(–2)-septate, cylindrical to pyriform conidia, which are rounded at the apex and truncate at the base. In addition, the conidiophores and conidia of *Veronaea submersa* are shorter (53–94 vs. up to 200 µm) and larger ($8\text{--}11 \times 2.5\text{--}3.5$ vs. $(3\text{--})6.5\text{--}8.5(12) \times (1.5\text{--})2\text{--}2.5(3)$) than those of *V. botryosa* (Arzanlou et al. 2007). Based on nucleotide sequence comparisons, *V. submersa* differs from *V. botryosa* and *V. endolichena* by 5.3% and 4.3% (ITS locus) and 0.48% and 0.48% (LSU locus), respectively. Thus, *V. submersa* is hereby proposed as a new species.

Sordariomycetes

***Subclass Xylariomycetidae* O.E. Erikss & Winka**

***Amphisphaeriales* D. Hawksw. & O.E. Erikss.**

***Apiosporaceae* K.D. Hyde, J. Fröhl., Joanne E. Taylor & M.E. Barr**

***Apiospora* Sacc., Atti Soc. Veneto-Trent. Sci. Nat., Padova, Sér. 4 4: 85 (1875)**

Apiospora, a highly diverse fungal genus with a worldwide distribution, was established by Saccardo (1875), with *A. montagnei* designated as the type species. Species within this genus exhibit diverse lifestyles and have been reported as endophytes, pathogens, and saprobes, with a broad host range (Dai et al. 2016, Pintos & Alvarado 2021, Tian et al. 2021, Monkai et al. 2022, Liao et al. 2023). *Apiospora* is characterized by basauxic conidiogenesis; globose to subglobose, pale brown to brown conidia that typically appear lenticular when viewed from the side, obovoid in shape (Ai et al. 2024). Currently, 211 records are listed in Index Fungorum, most of which were reported from terrestrial habitats. In this study, we introduce a new species, *A. submersa*, from freshwater habitats.

***Apiospora submersa* D. F. Bao, J. C Kang & Y.Z. Lu, sp. nov.**

Fig. 17

Index Fungorum number: IF 904396; Facesoffungi number: FoF 19056.

Etymology – Refers to the submerged habitats of the new species.

Holotype – GZAAS 25–0727

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: Colonies on substrate dark brown to black, dull, glistening, with an abundantly sporulating centre, and conidia readily liberated when disturbed. *Mycelium* superficial, composed of branched, hyaline to pale brown, septate, smooth-walled hyphae. *Conidiophores* $11\text{--}16 \times 3.5\text{--}5$ µm ($\bar{x} = 13.3 \times 4.2$ µm, $n = 20$), micronematous, grouped together to form sporodochia, basauxic, arising from conidiophore mother cells, cylindrical, hyaline to pale brown, septate, strongly constricted at the septa, straight or flexuous, branched, smooth-walled. *Conidiogenous cells* $4.5\text{--}9 \times 3\text{--}4$ µm ($\bar{x} = 6.8 \times 3.7$ µm, $n = 15$), aggregated in clusters on hyphae, monoblastic, terminal and intercalary, ampulliform to cylindrical, hyaline to light brown, smooth-walled. *Conidia* globose to subglobose, aseptate, dark olive-green to brown, globose in surface view, $(4\text{--})6\text{--}8$ µm in diam. ($n = 40$), lenticular inside view, $6.5\text{--}8 \times 4.5\text{--}6$ µm ($\bar{x} = 7.4 \times 5.3$ µm, $n = 40$) with a straight and longitudinal germ slit spore length, smooth-walled.

Cultural characteristics – Colonies on PDA at 25°C, reach 50–55 mm diam. after 14 days. Colonies circular, margin entire, woolly appearance, grey to pale brown and colony from above; yellow to light brown from below.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 22 November 2024, D.F. Bao, NPJ 4–20 (GZAAS 25–0727, holotype); ex-type living culture, GZCC 25–0697.



Figure 15 – *Veronaea submersa* (GZAAS 25–0723, holotype). a–c Colonies on submerged decaying wood. d–h Conidiophores with conidia. i–k Conidiogenous cells with conidia. l–r Conidia. s Germinating conidium. t, u Culture on PDA from the obverse and reverse. Scale bars: d–f = 30 μ m, g, h, s = 20 μ m, i–l = 10 μ m, m–r = 5 μ m.

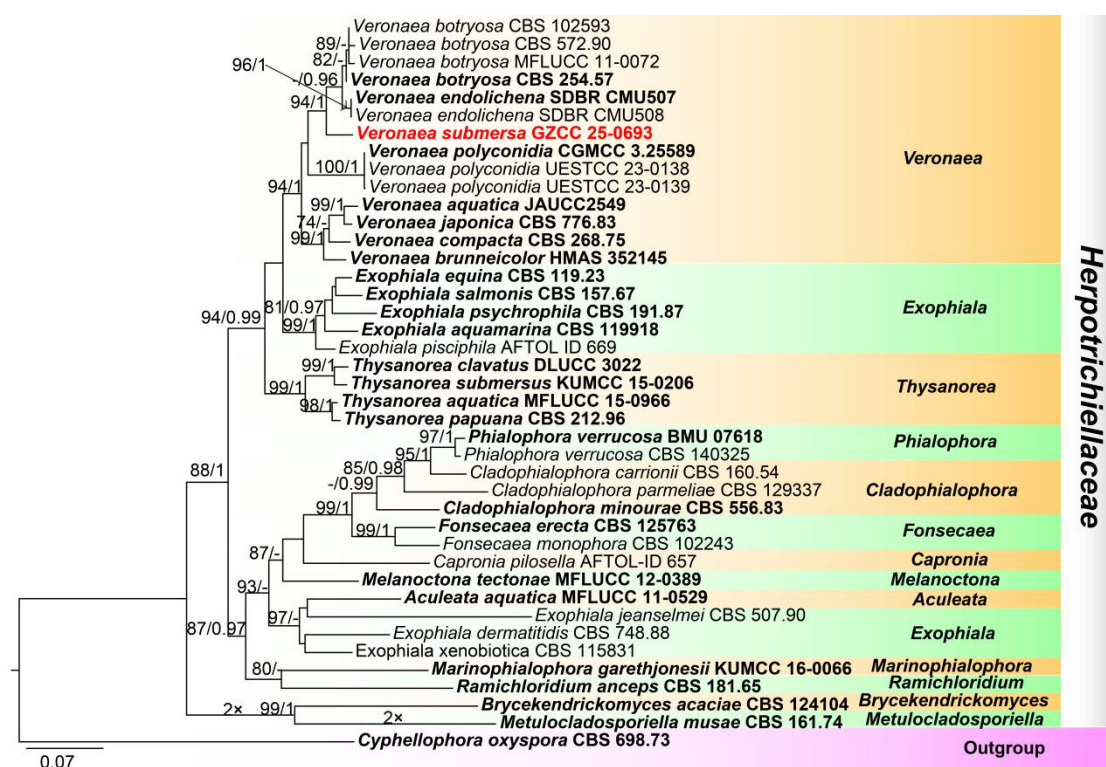


Figure 16 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated LSU, ITS, SSU, and *tub2* sequence datasets. A total of 38 taxa were incorporated into the combined analyses, yielding 2,863 aligned characters post-alignment. The highest-scoring RAxML tree, with a final log-likelihood value of -14646.5856, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Cyphellophora oxyspora* (CBS 698.73) as the outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

GenBank numbers – ITS: PV982834, LSU: PV982816, *tef1- α* : PX312456

Notes – Phylogenetic analysis revealed that *Apiospora submersa* grouped with *A. setariae* and *A. coryli* in a well-supported clade within the genus with 95% ML and 1.00 PP statistical support (Fig. 18). However, *A. submersa* differs from *A. setariae* in having smaller conidiogenous cells ($4.5\text{--}9 \times 3\text{--}4$ vs. $8\text{--}55 \times 1.35 \mu\text{m}$) and dark olive-green to brown conidia (Jiang et al. 2021). Compared to *A. coryli*, *A. submersa* is distinguished by its dark olive-green to brown, smooth-walled, and smaller conidia ($6.5\text{--}8 \times 4.5\text{--}6$ vs. $7.4\text{--}18.4 \times 6.2\text{--}12.5 \mu\text{m}$). In contrast, *A. coryli* has brown to dark brown, smooth to finely roughened conidia (Li et al. 2023). In addition, nucleoside comparisons of the ITS gene region between our new isolate and *A. setariae* and *A. coryli* revealed 31 bp (5.8%, including gaps) and 49 bp (9.2%, including gaps) differences, respectively, which strongly support our new isolate as a new species.

Incertae sedis

Papulosaceae Winka & O.E. Erikss., Mycoscience 41 (2):102 (2000)

Wongia Khemmuk, Geering & R.G. Shivas

Wongia, a well-studied genus with all species having sequence data available in GenBank, was introduced by Khemmuk et al. (2016) to accommodate two root-infecting species, *W. garrettii* and *W. griffinii*, which were initially classified in *Magnaporthe*. Currently, *Wongia* comprises 10 species (Khemruk et al. 2016, Luo et al. 2019, Bao et al. 2021, Zhang et al. 2023, Yu et al. 2024, Wang et al. 2024, Manawasinghe et al. 2025). Species in this genus are primarily reported from freshwater habitats, with the exceptions of *W. bambusae*, *W. garrettii*, *W. griffinii*, and *W. fischerai*, all other species have been reported exclusively from freshwater habitats. Among these,

W. garrettii, *W. griffinii*, and *W. fischerai* are associated with plant roots, whereas *W. bambusae* was collected from dead stems of *Bambusoideae*.

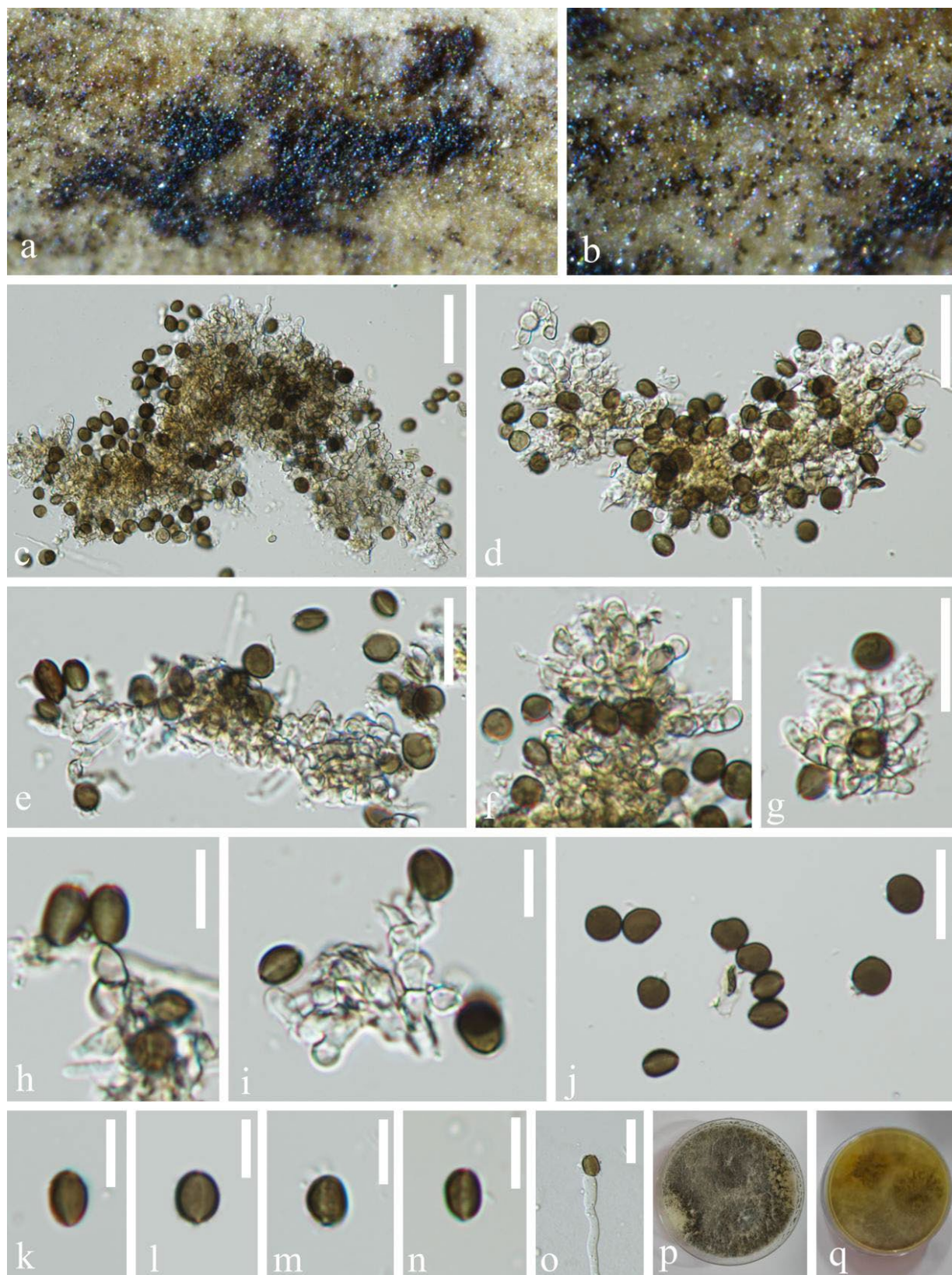


Figure 17 – *Apiospora submersa* (GZAAS 25–0727, holotype), a, b Colonies on submerged decaying wood. c–i Conidia with conidiophores. j–n Conidia. o Germinated conidium. p, n Culture on PDA from obverse and reverse. Scale bars: c, d = 30 μ m, e–g, j, o = 20 μ m, h, i, k–n = 10 μ m.

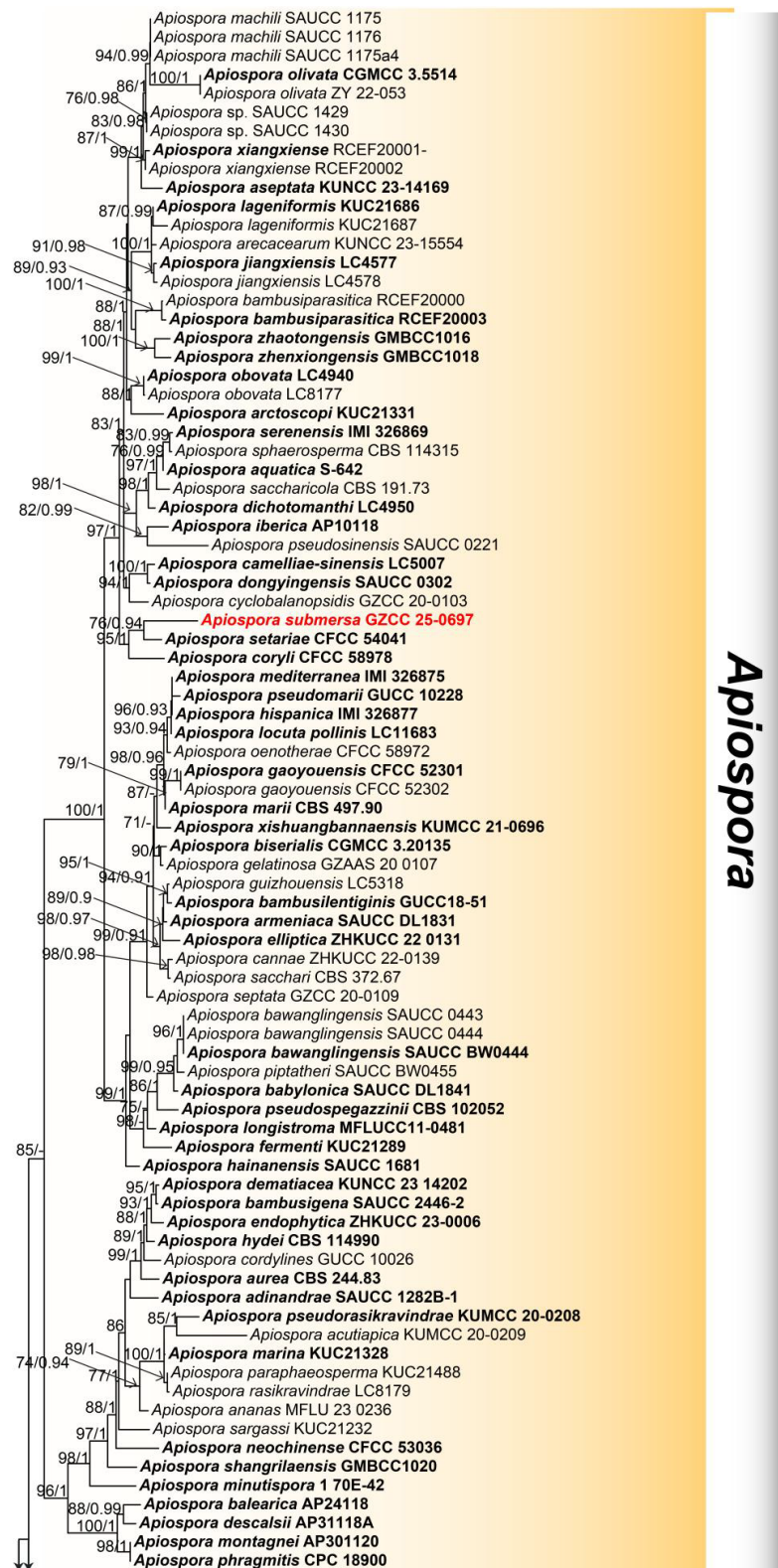


Figure 18 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated ITS, LSU, and *tef1- α* sequence datasets. A total of 153 taxa were incorporated into the combined analyses, yielding 1,916 aligned characters post-alignment. The highest-scoring RAxML tree, with a final log-likelihood value of -21773.385, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Arthrimum caricicola* (AP23518 and CBS 145903) as the outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

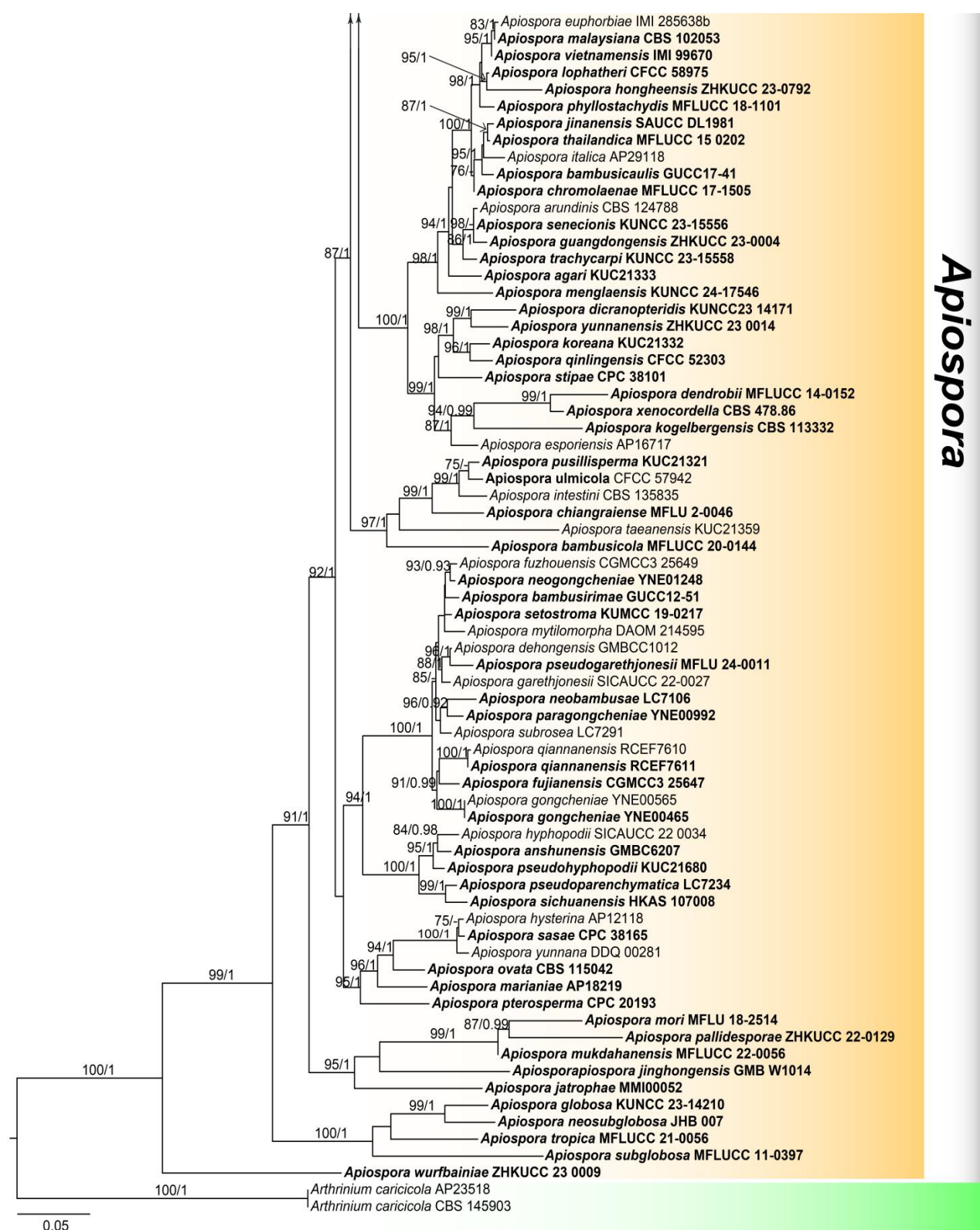


Figure 18 – Continued

Wongia is currently known only from its asexual morphs, characterized by macronematous, mononematous, solitary, unbranched, septate, dark brown, smooth conidiophores; polyblastic, denticulate, integrated, terminal conidiogenous cells; and acrogenous, clavate-to-fusiform conidia that are rounded and narrow at the apex, truncate at the base, and septate, with dark brown central cells and paler end cells (Luo et al. 2019). In this study, six fresh collections were made from freshwater habitats in China. Phylogenetic and morphological analyses identified these collections

as three species, including a new species, *W. guizhouensis*. Additionally, the known species *W. aquatica* and *W. suae* were recollected, and these species appear to be restricted to freshwater habitats, with records so far limited to China.

Wongia aquatica Z.L. Luo, K.D. Hyde & Hong Y. Su, in Luo et al., Fungal Diversity 99: 529 (2019) Fig. 19

Index Fungorum number: IF 555660; Facesoffungi number: FoF 05434.

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on substratum superficial, hairy, scattered, granulate, brown to black. *Mycelium* partly superficial and partly immersed, composed of branched, septate, smooth, brown to pale brown hyphae. *Conidiophores* 82–167 × 4–5.5 µm, ($\bar{x}$ = 124.8 × 4.7 µm, n = 30) macronematous, mononematous, simple, solitary, unbranched, slightly flexuous, septate, dark brown, paler towards apex, smooth-walled. *Conidiogenous cells* polyblastic, terminal, sympodial, denticulate, subhyaline to pale brown. *Conidia* 19.5–24 × 5–6.5 µm, ($\bar{x}$ = 21.7 × 5.8 µm, n = 40) acropleurogenous, solitary, fusiform, pointed and dark at both ends, 3-septate, brown to dark brown at central two cells, paler at end cells, guttulate, smooth-walled.

Culture characteristics – Conidia germinating on PDA medium within 24 h. Colonies on PDA medium reaching 25–30 mm in 3 weeks at 25°C in natural light, circular, with dense, grey to cream mycelium on the surface, wrinkled in the middle; in reverse dark brown to black in the middle and yellow at the entire margin.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 2–7 (GZAAS 25–0712); living culture, GZCC 25–0682. China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 2–47 (GZAAS 25–0713), living culture, GZCC 25–0683.

GenBank numbers – GZCC 25–0682: ITS: PV982851, LSU: PV982831, SSU: PV982865, *rpb2*: PV982865, *tefl-α*: PX312464. GZCC 25–0683: ITS: PV982853, *rpb2*: PV987745.

Notes – In our phylogenetic analysis, our new isolates GZCC 25–0682 and GZCC 25–0683 clustered with the ex-type strain of *W. aquatica* with 100% ML and 1.00 PP statistical support (Fig. 20). Morphologically, our new isolates are similar to the holotype of *Wongia aquatica* in having macronematous, mononematous, unbranched, septate, dark brown conidiophores, polyblastic, sympodial, denticulate conidiogenous cells, and acropleurogenous, fusiform, guttulate, 3-septate conidia with dark brown central two cells and paler end cells. Based on phylogenetic analysis and morphological characteristics, we identified our new isolates as *W. aquatica*. *Wongia aquatica* was introduced by Luo et al. (2019), collected from freshwater habitats in Yunnan Province; in contrast, our new isolates were collected from freshwater habitats in Guizhou Province. These findings suggest that *W. aquatica* is likely restricted to freshwater environments and has adapted to this habitat.

Wongia guizhouensis D.F. Bao, J.C. Kang & K.D. Hyde, sp. nov. Fig. 21

Index Fungorum number: IF 904397; Facesoffungi number: FoF 19057.

Etymology – Refers to the type locality, Guizhou Province.

Holotype – GZAAS 25–0714

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on substratum effuse, scattered, hairy, granulate, brown to black. *Mycelium* partly immersed, composed of branched, septate, smooth, brown to pale brown, smooth hyphae. *Conidiophores* 47–59 × 3.5–5 µm ($\bar{x}$ = 53.4 × 4.3 µm, n = 30) macronematous, mononematous, simple, solitary or in a small group, erect, unbranched, straight or slightly flexuous, septate, dark brown, paler towards apex, smooth-walled. *Conidiogenous cells* polyblastic, terminal, sympodial, denticulate, subhyaline to pale brown. *Conidia* 17–21 × 3.5–4.5 µm ($\bar{x}$ = 19.2 × 4.1 µm, n = 50) acropleurogenous, solitary, fusiform, truncated or pointed at the base, rounded and narrow at the apex, 3–6-septate, hyaline when young, pale brown at maturity, rarely guttulate, smooth-walled.

Culture characteristics – Conidia germinating on PDA medium within 24 h. Colonies on PDA

medium reaching 45–50 mm in 3 weeks at 25°C in natural light, circular, with brown mycelium on the surface; in reverse dark brown to black.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 1–44 (GZAAS 25–0714, holotype); ex-type living culture, GZCC 25–0684. China, Guangxi Province, Longlin City, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 9–13 (GZAAS 25–0715); living culture, GZCC 25–0685. China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 1–15 (GZAAS 25–0716); living culture, GZCC 25–0686.



Figure 19 – *Wongia aquatica* (GZAAS 25–0712). a–c Colonies on submerged decaying wood. d–h Conidiophores with conidia. i–k Conidiogenous cells with conidia. m–p conidia. q, r Culture on PDA from the above and below. Scale bars: d–h = 30 μ m, i–k, m–p = 10 μ m, l = 20 μ m.

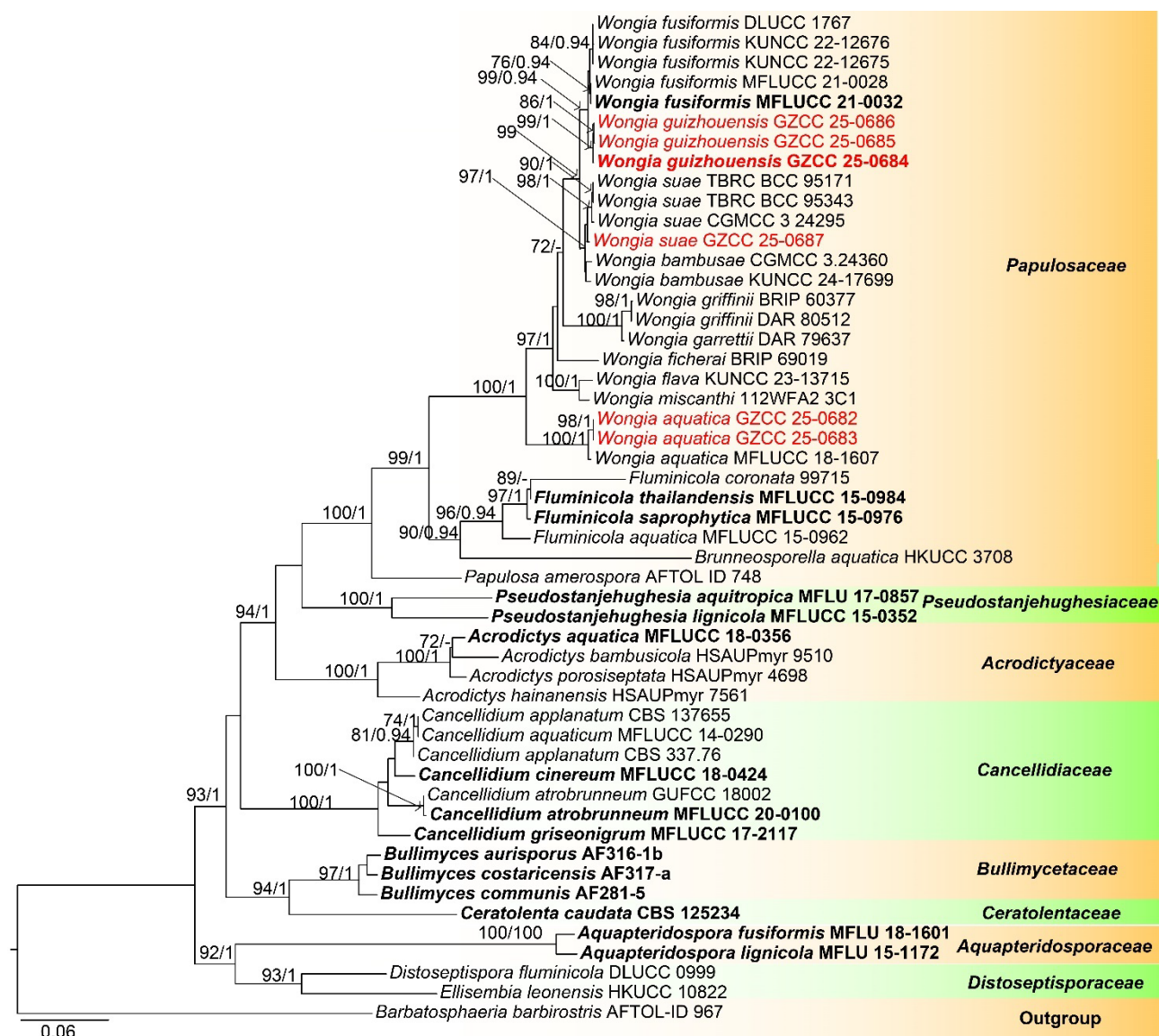


Figure 20 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated ITS, LSU, SSU, *rpb2*, and *tef1- α* sequence datasets. A total of 51 taxa were incorporated into the combined analyses, yielding 4,144 aligned characters post-alignment. The highest-scoring RAXML tree, with a final log-likelihood value of -22605.838, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Barbatosphaeria barbirostris* (AFTOL-ID 967) as outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

GenBank numbers – GZCC 25–0684: PV982850, LSU: PV982830, SSU: PV982864, *tef1- α* : PX312463, *rpb2*: PV987742. GZCC 25–0685: PV982849, LSU: PV982829, *rpb2*: PV987741. GZCC 25–0686: PV982852, LSU: PV982832, *tef1- α* : PX312465, *rpb2*: PV987744.

Notes – Phylogenetic analysis showed that *Wongia guizhouensis* (GZCC 25–0684, GZCC 25–0685 and GZCC 25–0686) formed a well-supported clade sister to *W. fusiformis* (Fig. 20). *Wongia guizhouensis* differs from *W. fusiformis* in having shorter conidiophores (47–59 vs. 97–154 μm) and 3–6-septate, pale brown, and longer conidia (17–21 vs. 14–18 μm). In contrast, the conidia of *W. fusiformis* are (1)–3-septate, pale brown to brown, slightly darker at middle cell and paler at the middle cell and paler at the end cells (Bao et al. 2021). Nucleotide comparisons of the ITS, LSU, SSU, and *tef1- α* gene regions between *W. guizhouensis* (GZCC 25–0684, ex-type) and *W. fusiformis* (MFLUCC 21–0032, ex-type) revealed 5 bp, 0 bp, 0 bp, and 6 bp differences,

respectively. The *rpb2* sequence data were unavailable for the type strain of *W. fusiformis* (MFLUCC 21–0032). We therefore compared our isolate with two other strains of *W. fusiformis* (KUNCC 22–12675 and KUNCC 22–12676) that have *rpb2* sequences, and the results revealed 20bp differences, which support our new isolates as a new species.

Wongia guizhouensis is morphologically similar to *W. suae* and *W. bambusae* in having macronematous, mononematous, simple conidiophores, polyblastic, denticulate, terminal, sympodial, and pale brown conidiogenous cells, and fusiform, clavate conidia. However, *W. guizhouensis* can be easily distinguished from *W. suae* and *W. bambusae* by the shorter conidiophores (47–59 vs. 134–172 vs. 50–90 μm), longer conidia (17–21 vs. 8–11 vs. 8–13 μm), and more conidial septa (3–6 vs. (1)–3 vs. 0–3).

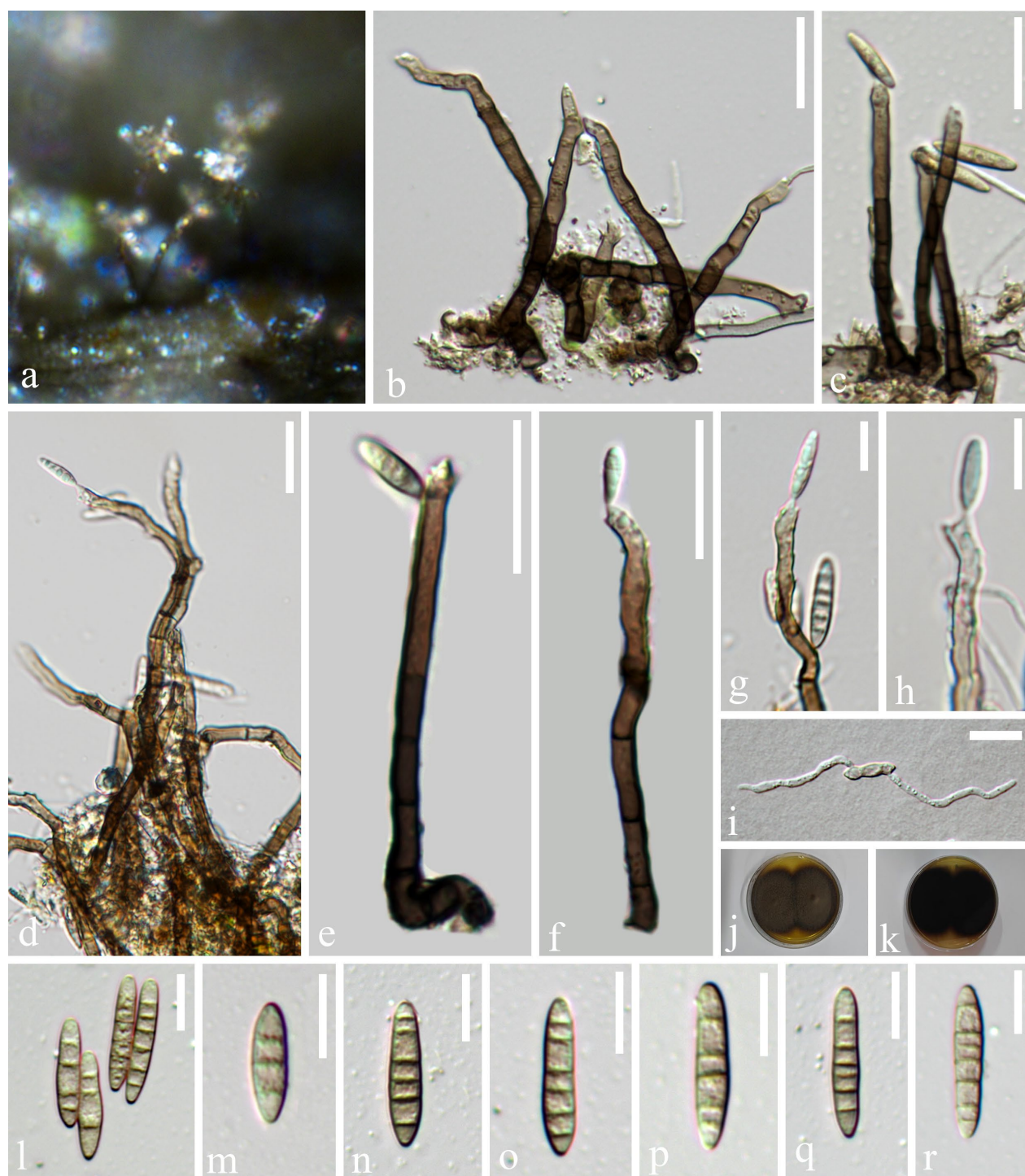


Figure 21 – *Wongia guizhouensis* (GZAAS 25–0714, holotype). a Colonies on submerged decaying

wood. b–f Conidiophores with conidia. g, h Conidiogenous cells with conidia. i Germinating conidium. j, k Culture on PDA from above and below. l–r Conidia. Scale bars: b–f, i = 20 μ m, g, h, l–r = 10 μ m.

Wongia suae Liang Zhang, H.W. Shen & Z.L. Luo, in Zhang, Shen, Bao, Li, Su & Luo, *Phytotaxa* 616(3): 265 (2023) Fig. 22

= *Wongia bandungensis* Chuaseechar., Nuankaew, Somrith. & Boonyuen, in Manawasinghe et al., *Fungal Diversity* 130: 103 (2024)

Index Fungorum number: IF 571582; Facesoffungi number: FoF 19058.

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on substratum effuse, scattered, hairy, granulate, brown to black. *Mycelium* partly immersed, composed of branched, septate, smooth, brown to pale brown, smooth hyphae. *Conidiophores* 55–95 $\times$ 3–4 μ m ($\bar{x}$ = 75.4 $\times$ 3.7 μ m, n = 25) macronematous, mononematous, simple, solitary or in a small group, erect, unbranched, straight or slightly flexuous, septate, dark brown, paler towards apex, smooth-walled. *Conidiogenous cells* polyblastic, terminal, sympodial, denticulate, subhyaline to pale brown. *Conidia* 10–14 $\times$ 3.5–4.5 μ m ($\bar{x}$ = 11.9 $\times$ 4.0 μ m, n = 35) acropleurogenous, solitary, fusiform, tapering towards both ends, truncated or pointed at the base, obtuse at the apex, 0–2-septate, light brown, guttulate, smooth-walled.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 2–43 (GZAAS 25–0717); living culture, GZCC 25–0687.

GenBank numbers – ITS: PV982854, LSU: PV982833, SSU: PV982866, *rpb2*: PV987746.

Notes – In the phylogenetic analysis, our newly obtained strain GZCC 25–0687 clustered with the sister clade consisting of *W. bandungensis* and *W. suae* (Fig. 20). *Wongia bandungensis* was recently introduced by Manawasinghe et al. (2025) based on the minor differences in conidial septation (2–3-septate in *W. suae* vs. 1–2-septate in *W. bandungensis*) and shape (clavate to fusiform with rounded at apex and acute at base vs. ellipsoidal to oblong-ellipsoidal with non-acute base and larger). Despite these morphological differences, phylogenetic reconstruction revealed no distinct clade separation between the two species. The nucleotide comparisons between *W. suae* (CGMCC 3.24295) and *W. bandungensis* (TBRC-BCC 95171, type) of ITS, SSU, *tefl- α* , and *rpb2* gene regions revealed 5bp, 0 bp, 2bp, and 6bp differences, respectively. Based on the guidelines of Jeewon & Hyde (2016), we recognized that they should be the same species, thus giving priority to *W. suae*, and synonymizing *W. bandungensis* under *W. suae*.

Our new collection is similar to *W. suae* in having macronematous, mononematous, simple, septate, dark brown conidiophores, polyblastic, terminal, sympodial, denticulate conidiogenous cells, and fusiform, rounded at apex, pointed at base, (1)–3-septate, pale brown conidia (Zhang et al. 2023). Although the conidiophores of our new collection are much shorter and conidia are slightly larger than those of *W. suae*. But phylogenetically, our new strain is closely related to *W. suae*, and there are only 8bp, 1bp, 0bp, and 6bp differences of ITS, LSU, SSU, and *rpb2* between our new collection and *W. suae* (CGMCC 3.24295), respectively, which does not support our new collection as a new species. Hence, we identified our new collection as *W. suae*. *Wongia suae* was introduced by Zhang et al. (2023) and collected from freshwater habitats in Yunnan Province, whereas our new collection was collected from Guizhou Province.

Magnaporthales Thongk., Vijaykr. & K.D. Hyde

Ophioceraeae Klaubauf, E.G. LeBrun & Crous

Ophioceras Sacc.

Ophioceras was established by Saccardo (1883), with *O. dolichostomum* as the type species. The genus is characterized by immersed, subcarbonaceous, globose ascomata with a long neck, conical to cylindrical asci, and filiform, septate hyaline ascospores (Thongkantha et al. 2009, Klaubauf et al. 2014, Yang et al. 2023). Species of *Ophioceras* typically occur as saprobes on decaying wood in both terrestrial and freshwater habitats worldwide (Luo et al. 2019, Jiang et al.

2021, Yang et al. 2023, Khalilabad & Fotouhifar 2024). To date, 52 species are listed in Index Fungorum, of which 26 have associated sequence data. This study presents a new freshwater species, *O. lignicola*, with detailed morphological descriptions and illustrations.



Figure 22 – *Wongia suae* (GZAAS 25–0717). a, b Colonies on submerged decaying wood. c, d Conidiogenous cells. e–h Conidiophores with conidia. i Conidiophores. j–o Conidia. p Germinating conidium. Scale bars: c–g, i–p = 10 µm, h = 15 µm.

Ophioceras lignicola D. F. Bao, J. C Kang & Y.Z. Lu, sp. nov.

Fig. 23

Index Fungorum number: IF 904388; Facesoffungi number: FoF 19059.

Etymology – Refers to this new taxon dwelling on wood.

Holotype – GZAAS 25–0719.

Saprobic on decaying wood, submerged wood in freshwater. Asexual morph: Undetermined.

Sexual morph: *Ascomata* 500–660 µm high, 750–850 µm diam., scattered, solitary, deeply immersed, coriaceous, black, subglobose or ellipsoidal, with a long black neck. *Ostiole* central, with a straight upright neck at one end, black, periphysate. *Peridium* 23–40 µm, composed of two layers, inner layer of hyaline to pale brown, small pseudoparenchyma cells, outer layer of pseudoparenchyma cells occluded with brown amorphous material, dark brown cells of *textura angularis*. *Paraphyses* 5–8 µm wide, hyaline, septate, constricted at septa, broader at base, tapering to the end, smooth-walled. *Asci* 90–115 × 8–11 µm ($\bar{x}$ = 101.8 × 9.4 µm, n = 30), 8-spored, unitunicate, cylindrical, hyaline, apically rounded, with a J-, thimble-shaped, subapical apparatus. *Ascospores* 66–86 × 3–4 µm ($\bar{x}$ = 76.5 × 3.5 µm, n = 30), overlapping in ascus, filiform, long-cylindrical, slightly curved, thin-walled, acute at both ends, hyaline, multi-guttulate, smooth-walled.

Cultural characteristics – Colonies on PDA at 25°C, reach 50–55 mm diam. after 15 days. Colonies circular, margin entire, woolly appearance, white to grey, and colony from above; dark brown from below.

Material examined – China, Guangxi Province, Longlin County, Yacha Town, on decaying wood submerged in Nanpan River, 23 November 2024, D.F. Bao, NPJ 6–12 (GZAAS 25–0719, holotype); ex-type living culture, GZCC 25–0689.

GenBank numbers – ITS: PV982843, LSU: PV982824, SSU: PV982860, *tef1-α*: PX312461, *rpb2*: PV987751.

Notes – The closest BLASTn matches in GenBank for the ITS sequence of our isolate GZCC 25–0689 was to *Ophioceras guizhouensis* (strain MFLU 18–2277), with 95.36% sequence identity. Multi-gene phylogenetic analysis further revealed that our new isolate GZCC 25–0689 clustered sister to *O. guizhouensis*, supported by 100% ML and 1.00 PP statistical values (Fig. 24). Morphologically, isolate GZCC 25–0689 is similar to *O. guizhouensis* in having oriate, black, subglobose or ellipsoidal ascomata with a long black neck, unitunicate, cylindrical asci with a J-, thimble-shaped, subapical apparatus and filiform, septate, hyaline, guttulate ascospores. However, the asci and ascospores dimensions were generally comparable; our new isolate exhibited larger asci (90–115 × 8–11 µm vs. (48.5–)51–56(–59) × 4.5–6 µm) and ascospores (66–86 × 3–4 µm vs. (41–)45.5–49(–53) × 1.2–1.7 µm). Notably, only ITS and LSU sequence data are available for the type strain of *O. guizhouensis*. Pairwise comparisons of ITS and LSU sequences between isolate GZCC 25–0689 and *O. guizhouensis* revealed 2.4% (10bp, without gaps) and 1.8% (15bp, without gaps) differences, respectively. Following the taxonomic guidelines of Jeewon & Hyde (2016), we recognize our new isolate as a distinct species, which we describe here as *Ophioceras lignicola*.

***Myrmecridiales* Crous**

***Myrmecridiaceae* Crous**

***Myrmecridium* Arzanlou, W. Gams & Crous 2007**

Myrmecridium was established by Arzanlou et al. (2007) for two *Ramichloridium*-like taxa, with *M. schulzeri* as the type species. Both sexual and asexual morphs are known for *Myrmecridium*; the asexual morphs are characterized by unbranched, straight or flexuose, septate conidiophores, polyblastic, integrated, cylindrical conidiogenous cells, and solitary conidia that are obovoidal or fusiform, with smooth or finely verrucose-walled surfaces (and sometimes with or without appendages) (Arzanlou et al. 2007, Jie et al. 2013, Peintner et al. 2016, Zhang et al. 2024b). While, the sexual morph is only known for *M. montsegurinum*, characterized by immersed, upright, papillate ascomata covered by a clypeus, subhyaline to weakly pigmented ascomatal wall, cylindrical, stipitate asci with a pronounced non-amyloid apical annulus and eight ellipsoidal, three-septate, delicately verrucose ascospores (Réblová et al. 2016). Species of *Myrmecridium* are commonly reported as saprobes on diverse substrates and exhibit a worldwide distribution (Crous et al. 2011, 2012, 2013, 2015, 2020, Jie et al. 2013, Peintner et al. 2016, Yang et al. 2023, Zhang et al. 2024b). To date, 29 species are accepted in this genus, all of which have been described with molecular data. In this study, we introduce two new species and a new record, based on combined phylogenetic and morphological analyses.

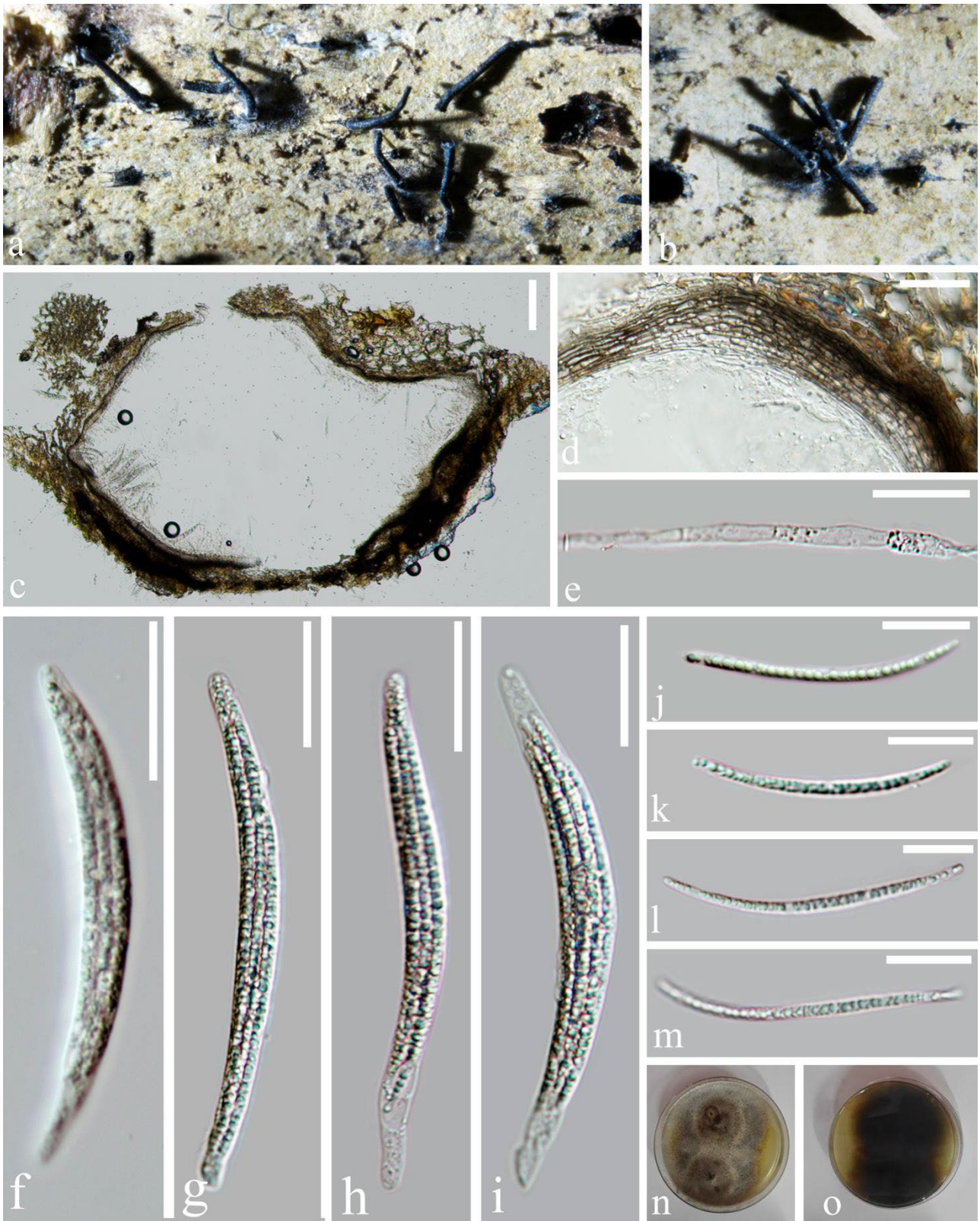


Figure 23 – *Ophioceras lignicola* (GZAAS 25–0719, holotype) a, b Appearance of ascomata on a submerged decaying wood. c Longitudinal section of ascomata. d Peridium wall. e Paraphyses. f–i Asci. j–m Ascospores. n, o Culture on PDA from obverse and reverse. Scale bars: c = 100 μ m, d–i = 30 μ m, j–m = 20 μ m.

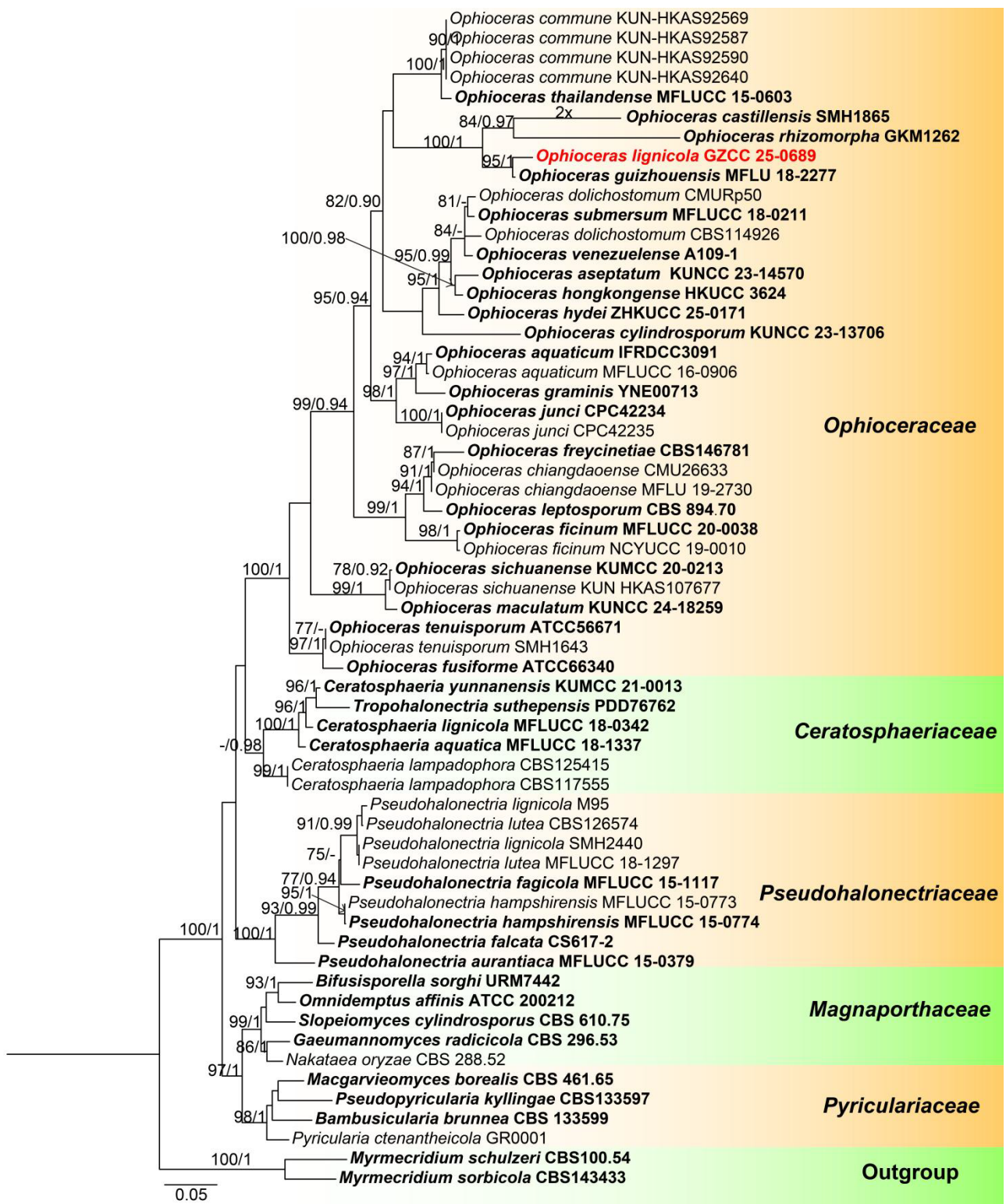


Figure 24 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated ITS, LSU, SSU, and *tef1*- α sequence datasets. A total of 60 taxa were incorporated into the combined analyses, yielding 3,404 aligned characters post-alignment. The highest-scoring RAXML tree, with a final log-likelihood value of -20591.3883, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Myrmecridium schulzeri* (CBS 100.54) and *M. sorbicola* (CBS 143433) as outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

Myrmecridium submersum L. Zhang & Z.L. Luo, in Zhang, Bao, Shen & Luo, J. Fungi 10(12,

Index Fungorum number: IF 572053; Facesoffungi number: FoF 19060.

Saprobic on decaying, submerged wood in freshwater habitats. *Sexual morph*: Undetermined. *Asexual morph*: *Colonies* on natural substrate effuse, consisting of individual or fascicular, conidiophores scattered over the substrate surface, yellowish. *Mycelium* immersed and superficial, superficial hyphae pale brown, septate, cylindrical. *Conidiophores* $111\text{--}161 \times 2.5\text{--}3.5 \mu\text{m}$ ($\bar{x} = 136.1 \times 2.8 \mu\text{m}$, $n = 22$), macronematous, mononematous, solitary or fasciated, erect, straight or slightly flexuous, cylindrical, brown to dark brown, paler towards the apex, unbranched, 1–5-septate, granulate, truncate at the apex, smooth-walled. *Conidiogenous cells* holoblastic, terminal, integrated, determinate, pale brown, cylindrical. *Conidia* $4.5\text{--}5.5 \times 1.5\text{--}2.5 \mu\text{m}$ ($\bar{x} = 4.9 \times 2 \mu\text{m}$, $n = 40$), acropleurogenous, solitary, hyaline or pale olivaceous, obovoid, pointed at the base, rounded or acute at the apex, straight, aseptate, smooth-walled. Conidial secession schizolytic.

Culture characteristics – Conidia germinating on PDA medium within 24h, and the germ tubes produced from both ends or one end. Colonies on PDA medium reaching 30–35 mm diam. after one month at 25°C in natural light. Colonies circular, velvety, with orange to yellowish and dense mycelium in the margin, sparse and orange to pink mycelium in the center, wrinkled, producing yellow pigment; in reverse orange to dark yellow with an entire margin.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 21 November 2024, D.F. Bao, NPJ 2–55 (GZAAS 25–0720); living culture, GZCC 25–0690.

GenBank numbers – ITS: PV982840.

Notes – The closest BLASTn matches in GenBank for the ITS sequence of our isolate were to *M. hydei* (strain R6B2) and *M. schulzeri* (strain 56370B), both with 97.41% sequence identity. Morphologically, *M. submersum* shares characteristics with *M. hydei* (R6B2) and *M. schulzeri* in having macronematous, mononematous, solitary or fasciculate, brown to dark brown, unbranched, septate conidiophores, holoblastic conidiogenous cells, and hyaline or pale brown, obovoid conidia. However, distinguishing these species solely on morphology is challenging, as they exhibit overlapping morphological characteristics; phylogenetic analysis is critical to confirm distinctness. Our multi-gene phylogenetic analysis placed our new isolate within the *M. submersum* clade, with 100% and 0.97 PP statistical support (Fig. 26). Morphologically, the new isolate aligned with *M. submersum*, showing conidiophore and conidial morphologies similar to those of *M. submersum*. Additionally, measurements of conidia and conidiophores were consistent with those of *M. submersum* (Zhang et al. 2024b). Pairwise comparisons of ITS sequence data revealed 5 bp differences between our isolate and *M. submersum*. Combined with morphological congruence, these genetic distinctions support the identification of our new isolate as *M. submersum*. *Myrmecridium submersum* was collected from freshwater habitats in Yunnan Province, China. In contrast, our new isolate was collected from Guizhou Province, further clarifying the geographic distribution of this species.

Myrmecridium schulzeri (Sacc.) Arzanlou, W. Gams & Crous, in Arzanlou, Groenewald, Gams, Braun, Shin & Crous, Stud. Mycol. 58: 84 (2007) Fig. 27

Index Fungorum number: IF 504560; Facesoffungi number: FoF 19061.

Saprobic on submerged decaying wood in freshwater habitats. *Sexual morph*: Undetermined. *Asexual morph*: *Colonies* on natural substrate effuse, consisting of individual or fascicular, conidiophores scattered over the substrate surface, yellowish. *Mycelium* immersed and superficial, superficial hyphae pale brown, septate, cylindrical. *Conidiophores* $80\text{--}131\text{--}(153.5) \times 2.5\text{--}4 \mu\text{m}$ ($\bar{x} = 106.1 \times 3.3 \mu\text{m}$, $n = 25$), macronematous, mononematous, solitary or fasciated, erect, straight, unbranched, cylindrical, pale brown to brown, paler towards the apex, 3–7-septate, granulate, smooth-walled. *Conidiogenous cells* holoblastic, terminal, determinate, integrated, elongating sympodially, subhyaline to pale brown, cylindrical, smooth. *Conidia* $4.5\text{--}6.5 \times 2\text{--}4 \mu\text{m}$ ($\bar{x} = 5.5 \times 3.1 \mu\text{m}$, $n = 35$), acropleurogenous, solitary, hyaline, ellipsoid to obovoid, pointed at the base, rounded at the apex, straight, aseptate, smooth-walled. Conidial secession schizolytic.



Figure 25 – *Myrmecridium submersum* (GZAAS 25–0720). a–c Colonies on submerged decaying wood. d, e Conidiogenous cells with conidia. f–j Conidiophores with conidia. k–p conidia. q Germinating conidium. r, s culture on PDA from the obverse and reverse. Scale bars: d, e, k, q = 10 μ m, f–j = 30 μ m, l–p = 5 μ m.

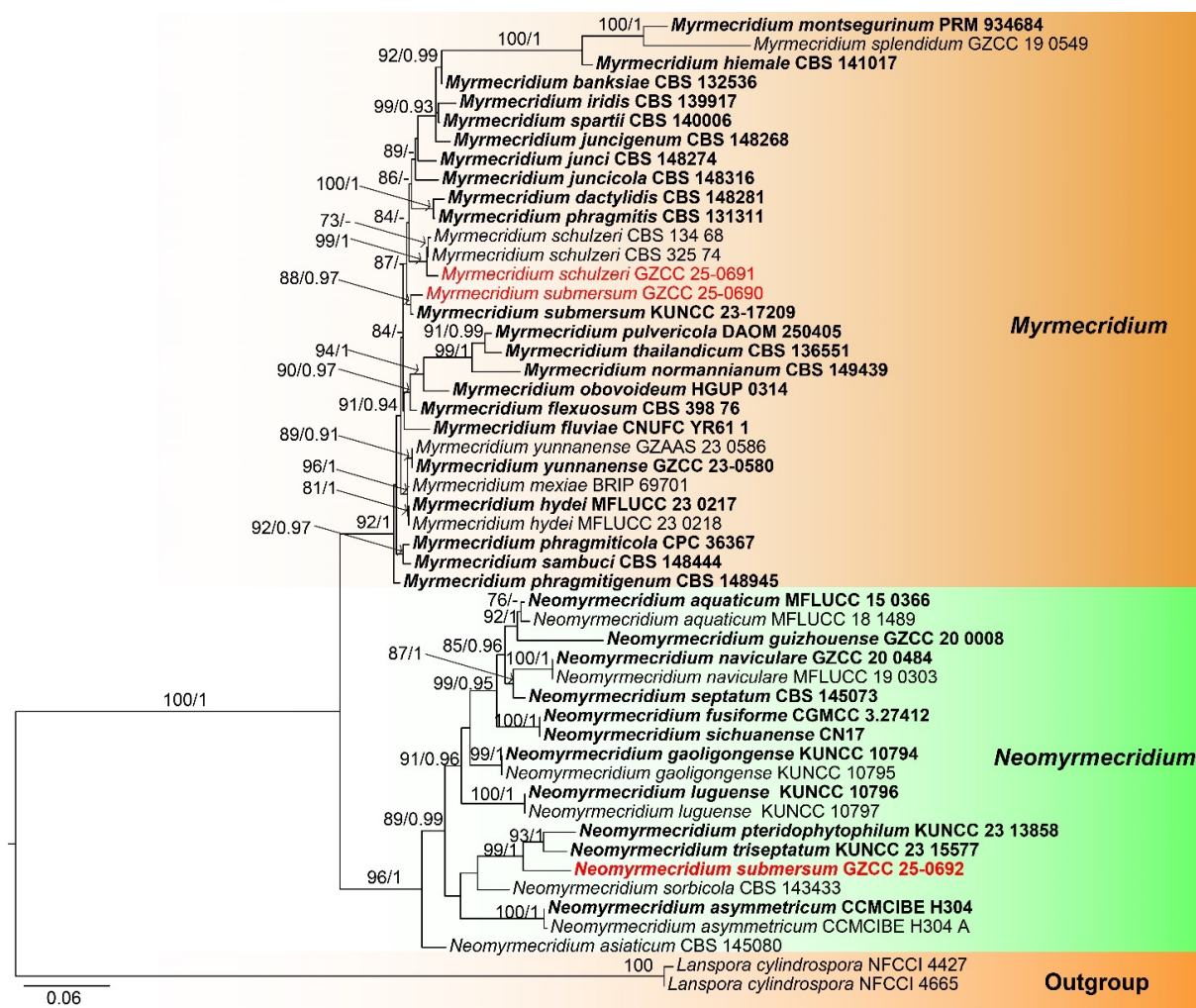


Figure 26 – Phylogram constructed from maximum likelihood (ML) analysis using concatenated ITS and LSU sequence datasets. A total of 50 taxa were incorporated into the combined analyses, resulting in 1,414 aligned characters after alignment. The highest-scoring RAXML tree, with a final log-likelihood value of -8330.1411, is presented herein. Bootstrap support values ($\geq 70\%$) for maximum likelihood (ML) and Bayesian posterior probabilities (≥ 0.90) are annotated above branches as ML BS/PP. The tree was rooted using *Lanspora cylindrospora* (NFCCI 4427 and NFCCI 4665) as the outgroup. Newly isolated strains are highlighted in red, and type strains are emphasized in bold.

Material examined – China, Guizhou Province, Xingyi City, on decaying wood submerged in Nanpan River, 22 November 2024, D.F. Bao, NPJ 4–31 (GZAAS 25–0721); living culture, GZCC 25–0691.

GenBank numbers – ITS: PV982842, LSU: PV982823.

Notes – In our phylogenetic analysis, our new isolate GZCC 25–0691 grouped with two strains of *M. schulzeri* (CBS 134.68 and CBS 325.74) (Fig. 26). Morphologically, our new isolate aligns well with the morphological characteristics of *M. schulzeri* (Arzanlou et al. 2007). Based on these combined phylogenetic and morphological data, we identify isolate GZCC 25–0691 as *M. schulzeri*.

Myrmecridium schulzeri, the type species of the genus *Myrmecridium*, is widely distributed and has been reported from diverse substrates, including *Cannomois virgata*, koring straw, *Triticum aestivum* (wheat), roots, and wheat field soil, from South Africa, Germany, Iran, and the Netherlands (Arzanlou et al. 2007; Rezakhani et al. 2019). This is the first report of this species collected from China.

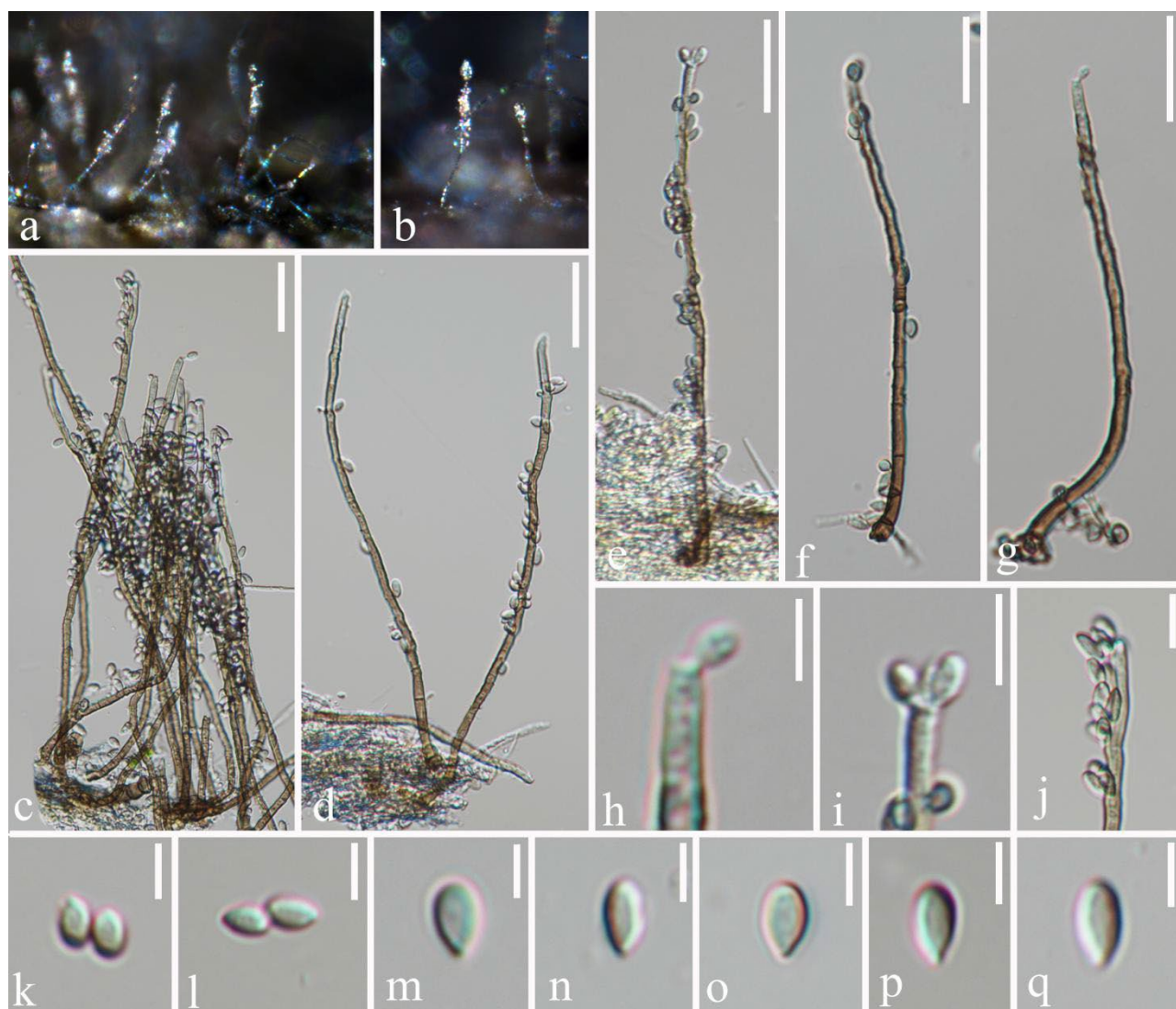


Figure 27 – *Myrmecridium schulzeri* (GZAAS 25–0721). a, b Colonies on submerged decaying wood. c–g Conidiophores with conidia. h–j Conidiogenous cells with conidia. k–q conidia. Scale bars: c–e = 30 μ m, f, g, j = 20 μ m, h, k–q = 5 μ m, i = 10 μ m.

Neomyrmecridium Crous, in Crous et al., Persoonia 41: 287 (2018)

Neomyrmecridium was established by Crous et al. (2018) to accommodate three species viz. *N. septatum*, *N. asiaticum*, and *N. sorbicola*. Since its description, additional species have been continually added to the genus based on combined phylogenetic analyses and morphological characteristics. *Neomyrmecridium* is characterized by solitary, unbranched conidiophores, polyblastic, denticulate conidiogenous cells, and fusoid-ellipsoid, septate conidia, with the upper two-thirds of the conidium cell encased in a mucoid sheath (Crous et al. 2018, Hyde et al. 2020). To date, *Neomyrmecridium* comprises 13 accepted species, with approximately half of these reported from freshwater habitats (Hyde et al. 2020, Serrano et al. 2020, Crous et al. 2021, Yang et al. 2023, Xu et al. 2023, Zhang et al. 2024b, 2025, Chen et al. 2025, Cao et al. 2025). This study introduces a new species, *N. submersum* based on morphological characteristics and phylogenetic analysis.

Neomyrmecridium submersum D. F. Bao, J. C Kang & Y.Z. Lu, sp. nov.

Index Fungorum number: IF 904387; Facesoffungi number: FoF 19062.

Etymology – Refers to the submerged habitats of the new species.

Holotype – GZAAS 25–0722

Fig. 28



Figure 28 – *Neomyrmecridium submersum* (GZAAS 25–0722, holotype). a, b Colonies on submerged decaying wood. c–g Conidiophores with conidia. h, i Conidiogenous cells with conidia. j–p conidia. q Germinating conidium. r, s culture on PDA from obverse and reverse. Scale bars: c–i = 30 μ m, j–p = 10 μ m, q = 20 μ m.

Saprobic on a decaying twig in a freshwater stream. *Colonies* on wood effuse, hairy, scattered or aggregated, dark brown, glistening. *Mycelium* mostly immersed, composed of septate, smooth, brown to hyaline hyphae. *Conidiophores* 175–332 $\times$ 4–5 μ m ($\bar{x}$ = 253.9 $\times$ 4.7 μ m, n = 20), macronematous, mononematous, erect, lower part straight, upper part flexuous, cylindrical, smooth-walled, septate, unbranched, brown, pale brown to subhyaline at the upper part.

Conidiogenous cells polyblastic, determinate, integrated, terminal, becoming intercalary, sympodial, denticulate, flexuous, brown to subhyaline. *Conidia* 15–18.5 × 5.5–6.5 μm ($\bar{x}$ = 16.7 × 6.3 μm, n = 45), acropleurogenous, fusiform, tapering towards both ends, pointed at the base with a hilum, rounded or obtuse at the apex, 0–3-septate, mostly 3-septate, smooth-walled, hyaline when young, pale brown at maturity, thin-walled.

Culture characteristics – Conidia germinating on PDA within 24 h, and germ tubes produced from both ends. Colonies growing on PDA, and after 2 weeks of incubation at room temperature, the diameter reaches about 20 mm, circular, producing yellow pigment, wrinkled, with velvety, yellowish white mycelium on the surface; in reverse dark yellow with entire margin.

Material examined – China, Guangxi Province, Gebu Town, on decaying wood submerged in Nanpan River, 24 November 2024, D.F. Bao, NPJ 8–56 (GZAAS 25–0722, holotype); ex-type living culture, GZCC 25–0692.

GenBank numbers – ITS: PV982841, LSU: PV982822.

Notes – In our multi-locus phylogenetic analysis, *Neomyrmecridium submersum* grouped with *N. pteridophytophilum*, *N. triseptatum*, *N. sorbicola*, and *N. asymmetricum* in a distinct clade within the genus, and close to *N. triseptatum* (Fig. 26). *Neomyrmecridium submersum* resembles *N. triseptatum* in having solitary, unbranched, septate conidiophores, polyblastic, terminal, denticulate conidiogenous cells and clavate to fusiform, subhyaline to pale brown, 3-septate conidia with a hilum at the base (Cao et al. 2025). However, *Neomyrmecridium submersum* has smooth-walled, smaller conidia (15–18.5 × 5.5–6.5 vs. 16–23 × 3.7–5.6 μm) and lacks a mucilaginous sheath. In contrast, the conidia of *N. triseptatum* are verrucose, with a thin mucilaginous sheath at the middle part. Nucleotide comparisons of the ITS and LSU gene regions between *N. submersum* and *N. triseptatum* revealed 44 bp and 14 bp differences, respectively, strongly supporting our new isolate as a distinct species.

Discussion

The present study represents the first comprehensive investigation of lignicolous freshwater fungi in the Nanpan River Basin (Guizhou and Guangxi provinces), a biodiversity hotspot characterized by unique karst topography and seasonal hydrological dynamics. Using integrative morphological and molecular approaches, we uncovered a remarkable diversity of 189 species across six classes, 27 orders, 70 families, and 104 genera within the *Ascomycota*, including 10 novel species (Tables 3 and S1). These findings substantially advance our understanding of freshwater fungal diversity and highlight the urgent need for continued exploration.

Fungal community composition and distribution pattern in Nanpan River Basin

Freshwater fungal communities in the Nanpan River Basin exhibit distinct compositional patterns shaped by both biotic and abiotic factors, with significant implications for understanding biodiversity and endemism in karst landscapes. All isolated strains belonged to the phylum *Ascomycota*, with *Sordariomycetes* and *Dothideomycetes* emerging as the most abundant classes, a pattern consistent with global freshwater fungal studies, where these classes dominate due to their ecological versatility in decomposing woody substrates (Luo et al. 2019, Dong et al. 2020, Calabon et al. 2022, 2023). Within the basin, *Pleosporales* was the dominant order across both Guizhou and Guangxi Provinces (Fig. 2), reinforcing its role as a key decomposer of lignocellulosic material in freshwater ecosystems. However, striking provincial disparities in species richness (136 vs. 79 species) and endemism (110 unique species in Guizhou vs. 53 in Guangxi) highlight the influence of local environmental factors. These differences align with predictions that karst regions, characterized by complex microhabitats and geological isolation, harbor cryptic biodiversity (Yang et al. 2023). Venn diagram analysis (Fig. 5) further revealed 26 shared species and 32 shared genera between provinces, indicating that while some taxa thrive broadly, others are adapted to provincial-specific conditions.

Freshwater fungal diversity and distribution are profoundly influenced by elevation,

temperature, and riparian vegetation (Wood-Eggenschwiler et al. 1983, Hyde & Goh 1999, Cai et al. 2003, Shearer et al. 1991, Tsui et al. 2001a, Unterseher et al. 2013, Shearer et al. 2015). Despite the Nanpan River serving as a provincial boundary (not a strict upstream-downstream gradient), our study revealed that Guizhou and Guangxi host distinct fungal assemblages. Guizhou's humid, mountainous karst climate with seasonal droughts and rugged terrain supports communities dominated by *Apiosporaceae*, *Dictyosporiaceae*, *Pseudohalonectriaceae*, and *Occultibambusaceae* (Fig. 3), including key genera *Apiospora*, *Dictyocheirospora*, *Myrmecridium*, *Periconia*, and *Pseudohalonectria* (Fig. 4). In contrast, Guangxi's flat, subtropical monsoon-influenced terrain with simultaneous rain-heat cycles and frequent floods hosts *Didymosphaeriaceae*, *Dictyosporiaceae*, *Lentitheciaceae*, and *Periconiaceae* (Fig. 3), where *Dictyocheirospora*, *Periconia*, *Neokalmusia*, and *Pseudohalonectria* dominate (Fig. 4). These patterns reflect divergent microhabitat preferences: Guizhou's fragmented karst microhabitats favor specialized decomposers, while Guangxi's connected floodplains support more cosmopolitan taxa.

The dominance of *Dictyocheirospora* and *Periconia* across provinces underscores their specialized niche adaptation to submerged wood habitats (Fig. 4). *Dictyocheirospora*, a globally distributed genus with over half of its described species reported from freshwater systems in southern China (Boonmee et al. 2016, Wang et al. 2016, 2024, Dong et al. 2020, Yang et al. 2018, Shen et al. 2022), thrives in the Nanpan Basin, likely due to its tolerance for karst-specific conditions. However, its long-distance dispersal mechanisms remain unclear, as no genetic or ecological studies have confirmed its dispersal pathways. In contrast, *Periconia* exhibits remarkable ecological versatility, functioning as endophyte, pathogen, and saprobe across diverse habitats. While freshwater records are rare (Hyde et al. 2017, Dong et al. 2020, Calabon et al. 2022, Shen et al. 2024, Wang et al. 2024), we isolated 13 *Periconia* strains in this study (Tables 3 and S1). We hypothesize this prevalence stems from riparian vegetation dynamics: Dominant *Gramineae* (grasses) along riverbanks provide colonization substrates, and fallen plant material introduces these fungi into aquatic habitats. This riparian-aquatic transfer likely facilitates freshwater colonization by fungi that are otherwise terrestrial-adapted (Shearer et al. 1993, Hyde et al. 2021).

At the species level, our study revealed distinct patterns of diversity and distribution. *Neokalmusia aquibrunnea* emerged as a highly widespread taxon, with 15 strains documented across both Guizhou and Guangxi provinces (Tables 3 and S1). *Neokalmusia aquibrunnea* was recently described by Yang et al. (2023) from submerged wood in Anshun City, Guizhou Province; its broad distribution across the basin underscores exceptional ecological tolerance to microhabitat variation. In Guizhou, *Pseudohalonectria lignicola* (8 strains) and *Myrmecridium schulzeri* (8 strains) dominated fungal assemblages (Table 3). *Pseudohalonectria lignicola*, historically considered cosmopolitan with records from the Seychelles (Hyde & Goh 1998), Hong Kong Province, China (Tsui et al. 2001b), Japan (Minoura & Muroi 1978), Thailand (Sivichai et al. 2000), and the United States (Shearer & von Bodman 1983, Shearer 1989), was newly confirmed in mainland China in this study. Its exclusive occurrence in freshwater habitats highlights specialized adaptation to these environments. *Myrmecridium schulzeri*, a widely distributed species previously reported from South Africa, Germany, Iran, and the Netherlands (Arzanlou et al. 2007, Rezakhani et al. 2019), represents the first confirmed record from freshwater habitats in China. This finding further reinforces Guizhou's role as a hotspot for regionally adapted taxa, where unique ecological conditions drive the emergence of both cosmopolitan and endemic fungal lineages.

These findings position the Nanpan River Basin as a critical region for studying freshwater fungal biodiversity, where karst-driven heterogeneity creates a natural laboratory for exploring ecological specialization, biogeographic patterns, and drivers of species endemism. The pronounced provincial disparities underscore the need for targeted sampling of microhabitats and expanded molecular analyses to clarify dispersal mechanisms and evolutionary histories. Additionally, investigating the functional roles of key taxa (e.g., *Dictyocheirospora* in lignin decomposition, *Periconia* in riparian-aquatic transitions) will enhance our understanding of how

these fungi contribute to ecosystem health in karst landscapes.

Diversity of freshwater fungi

The discovery of ten new species from the Nanpan River Basin represents a significant advancement in our understanding of freshwater fungal taxonomy, underscoring the region's role as a hotspot for cryptic biodiversity. For instance, *Lolia guangxiensis* exemplifies this pattern, with its distinct morphological traits, including smaller ascomata (245–300 µm) and fusiform ascospores bearing an enlarged central cell, which differentiates it from the morphologically similar species, *L. aquatica*. Similarly, *Tetraploa guizhouensis*, *T. nanpanjiangensis*, and *T. verrucisporawere* identified as novel taxa despite initial morphological resemblances, a distinction validated through phylogenetic analysis. These findings align with Hyde et al. (2021), who posited that morphologically congruent species across distinct river systems may represent cryptic lineages, thereby emphasizing the limitations of relying solely on morphology for species delimitation. Collectively, these results reinforce emerging evidence of widespread taxonomic impediments in freshwater fungi, where cryptic diversity obscures the true species richness (Hyde et al. 2021, Calabon et al. 2022).

Globally, freshwater fungi remain profoundly understudied relative to their terrestrial counterparts. Estimates suggest 2.5 million fungal species exist worldwide; yet, only ~160,000 have been formally described, of which about 3,870 (2.5%) are freshwater taxa (Hawksworth & Lücking 2017, Hyde et al. 2020, Calabon et al. 2022, Niskanen et al. 2023). This discrepancy highlights a critical gap in our understanding of freshwater fungal biodiversity. Recent expeditions in China and Thailand (2015–2020) have uncovered 298 new freshwater fungal species, underscoring the immense undiscovered diversity within these ecosystems (Calabon et al. 2021, Bao et al. 2021). This trend continues in karst landscapes, where Yang et al. (2023) reported 47 new species from Guizhou (China) and Thailand; Wang et al. (2024) documented 14 new lignicolous species from Yunnan's karst regions; and Shen et al. (2024) introduced 18 new species (21 total) from Yunnan's plateau lakes. Collectively, these studies indicated that karst regions in southern China harbor a reservoir of unexplored species diversity, driven by unique geological and ecological conditions.

The Nanpan River Basin, a karst-dominated system spanning Yunnan, Guizhou, and Guangxi Provinces, exemplifies this pattern of hyper-local endemism. Our study reveals that even minute spatial scales, specifically adjacent river segments in the lower Nanpan River, harbor strikingly distinct fungal assemblages, with 189 lignicolous species documented and minimal overlap between neighboring reaches. This microhabitat-specific diversity is attributed to localized gradients in elevation and riparian vegetation, which likely drive adaptive divergence and reproductive isolation. Such findings suggest that expanded sampling of both geographical and substrate niches will exponentially increase recorded species richness, particularly in heterogeneous karst basins where fragmented microhabitats and steep environmental gradients accelerate evolutionary innovation.

Beyond taxonomic novelty, our work illuminates the ecological and conservation significance of these understudied communities. Lignicolous freshwater fungi play pivotal roles in nutrient cycling (e.g., decomposing woody debris to recycle carbon and nitrogen) and supporting aquatic food webs (Wong et al. 1998, Jones et al. 2014, Miki et al. 2011, Hyde et al. 2016). Yet, their cryptic nature and habitat specificity render them vulnerable to environmental perturbations, including pollution and climate change (Röhl et al. 2017, Barros et al. 2024). By highlighting the Nanpan River Basin as a microcosm of freshwater fungal biodiversity, we underscore the urgency of systematic exploration and monitoring in such ecologically sensitive regions. Continued taxonomic, phylogenetic, and ecological research is essential to unravel their functional roles, inform conservation strategies, and mitigate biodiversity loss in these understudied ecosystems.

Highlights the key findings of terrestrial fungal genera in freshwater systems

Interestingly, our study revealed the presence of several typically terrestrial fungal genera,

viz. *Apiospora*, *Colletotrichum*, and *Diaporthe* in freshwater habitats of the Nanpan River Basin. These genera are well-documented as plant pathogens or endophytes in terrestrial ecosystems, with only one *Colletotrichum* species (*C. godetiae*) and three *Diaporthe* species (*D. aquatica*, *D. beckhausii*, and *D. longicolla*) having been reported from freshwater habitats (Calabon et al. 2022). Our findings challenge the conventional assumption that such plant-associated fungi are poorly adapted to colonize freshwater systems. Notably, *Apiospora* was documented for the first time in a freshwater habitat in this study. This novel occurrence, along with the detection of the other three genera, expands their known ecological niches beyond terrestrial plants to include submerged wood. While the specific ecological roles of these fungi in freshwater environments remain to be elucidated, their presence suggests potential adaptation to utilize lignocellulosic substrates (common in aquatic environments) or interactions with aquatic plants, thereby blurring the traditional boundaries between terrestrial and aquatic fungal guilds. These results underscore the need to reevaluate the habitat specificity of plant-associated fungi, as many may exhibit greater ecological flexibility than previously recognized. Further studies investigating their functional roles (e.g., decomposition, pathogenicity, or mutualism) in freshwater systems will clarify whether their occurrence represents transient colonization, niche expansion, or the evolution of distinct aquatic ecotypes.

Limitations and Future Research Directions

This study was limited to the lower reaches of the Nanpan River (in Guizhou and Guangxi), and did not cover the ecologically distinct upper reaches in Yunnan Province. Future research should expand the sampling to include Yunnan's karst-dominated headwaters, where unique, calcium-rich microhabitats are likely to harbor undocumented fungal diversity. Systematic collection across the full elevational gradient will enable comprehensive basin-wide biodiversity assessments.

Low riparian vegetation coverage at some sites restricted access to submerged woody substrates, resulting in uneven sampling. To address this, future studies should implement standardized protocols: target high-density vegetation zones, incorporate alternative substrates (e.g. leaf litter), and employ fixed-quadrat sampling to ensure spatial consistency across microhabitats.

Although this study focused solely on the lower reaches of the Nanpan River, we uncovered stark provincial divergences in freshwater fungal community structures between Guizhou and Guangxi. These pronounced differences manifested in distinct dominant taxa, endemic species distributions, and microhabitat adaptations highlight the complex interplay of karst geology, hydrological regimes, and climatic gradients even within a contiguous river segment. Therefore, future research urgently needs to conduct comprehensive sampling across the river's longitudinal gradient (from upstream to downstream) to reveal the actual patterns of change in freshwater fungal diversity and community structure along the river course, and to deeply investigate the underlying driving mechanisms.

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DECLARATION ON CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding this article.

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