



Yunnan–Guizhou Plateau: a mycological hotspot

NALIN N. WIJAYAWARDENE^{1,2,3,18}, LAKMALI S. DISSANAYAKE^{4,19}, QI-RUI LI^{2,5,20*}, DONG-QI DAI^{1,21*},
 YUANPIN XIAO^{6,7,22}, TING-CHI WEN^{4,7,8,23}, SAMANTHA C. KARUNARATHNA^{9,10,11,24}, HAI-XIA WU^{12,25},
 HUANG ZHANG^{13,26}, SAOWALUCK TIBPROMMA^{9,10,11,27}, JI-CHUAN KANG^{4,28}, YONG WANG^{14,29}, XIANG-
 CHUN SHEN^{2,5,30}, LI-ZHOU TANG^{1,31}, CHUN-YING DENG^{15,32*}, YANXIA LIU^{16,33} & YINGQIAN KANG^{17,34}

¹ Center for Yunnan Plateau Biological Resources Protection and Utilization, College of Biological Resource and Food Engineering, Qujing Normal University, Qujing, Yunnan 655011, P.R. China

² State Key Laboratory of Functions and Applications of Medicinal Plants, Guizhou Medical University, Guiyang 550014, P.R. China

³ Section of Genetics, Institute for Research and Development in Health and Social Care No: 393/3, Lily Avenue, Off Robert Gunawardane Mawatha, Battaramulla 10120, Sri Lanka

⁴ The Engineering Research Center of Southwest Bio–Pharmaceutical Resources Ministry of Education, Guizhou University, Guiyang 550025, Guizhou Province, China

⁵ The Key Lab of Optimal Utilization of Natural Medicine Resources, School of Pharmaceutical Sciences, Guizhou Medical University, University Town, Gui'an New District, Guizhou 550025, P.R. China

⁶ Institute of Excellence in Fungal Research, School of Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

⁷ State Key Laboratory Breeding Base of Green Pesticide and Agricultural Bioengineering, Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Guizhou University, Guiyang 550025, China

⁸ The Mushroom Research Centre, Guizhou University, Guiyang 550025, China

⁹ Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, P.R. China

¹⁰ CIFOR-ICRAF, World Agroforestry Centre, Kunming 650201, Yunnan, P.R. China

¹¹ Centre for Mountain Futures (CMF), Kunming Institute of Botany, Kunming, Yunnan, 650201, P.R. China

¹² International Fungal Research and Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Kunming 650224, PR China

¹³ Faculty of Agriculture and Food, Kunming University of Science & Technology, Kunming 650500, People's Republic of China

¹⁴ Department of Plant Pathology, Agriculture College, Guizhou University, Guiyang 550025, P.R. China

¹⁵ Guizhou institute of biology, Guizhou academy of science, Guiyang, 550009, P.R. China

¹⁶ Guizhou Academy of Tobacco Science, No. 29, Longtanba Road, Huanshanhu District, Guiyang City, Guizhou province, P.R. China

¹⁷ Key Laboratory of Environmental Pollution Monitoring and Disease Control, Ministry of Education of Guizhou & Guizhou Talent Base for Microbiology and Human Health, Key Laboratory of Medical Microbiology and Parasitology of Education Department of Guizhou, School of Basic Medical Sciences, Guizhou Medical University, Guiyang, P.R. China

¹⁸ ✉ nalinwijayawardene@yahoo.com; <https://orcid.org/0000-0003-0522-5498>

¹⁹ ✉ dmsldlakmali.ld@gmail.com; <https://orcid.org/0000-0003-2933-3127>

²⁰ ✉ lqrnd2008@163.com; <https://orcid.org/0000-0001-8735-2890>

²¹ ✉ cicidaidongqin@gmail.com; <https://orcid.org/0000-0001-8935-8807>

²² ✉ emmaypx@gmail.com; <https://orcid.org/0000-0003-1730-3545>

²³ ✉ tingchiwen@yahoo.com; <https://orcid.org/0000-0003-1744-5869>

²⁴ ✉ samantha@mail.kib.ac.cn; <https://orcid.org/0000-0001-7080-0781>

²⁵ ✉ aileen2008haixia@gmail.com; <https://orcid.org/0000-0002-7169-6717>

²⁶ ✉ zhanghuang2002113@163.com; <https://orcid.org/0000-0002-9464-2981>

²⁷ ✉ saowaluckjai@gmail.com; <https://orcid.org/0000-0002-4706-6547>

²⁸ ✉ jckang@gzu.edu.cn; <https://orcid.org/0000-0002-6294-5793>

²⁹ ✉ yongwangbis@aliyun.com; <https://orcid.org/0000-0003-3831-2117>

³⁰ ✉ shenxiangchun@126.com; <https://orcid.org/0000-0002-4333-9106>

³¹ ✉ tanglizhou@163.com; <https://orcid.org/0000-0002-6988-1876>

³² ✉ 171934233@qq.com; <https://orcid.org/0000-0002-0960-0948>

³³ ✉ liuyanxia306@163.com; <https://orcid.org/0000-0002-2634-0068>

³⁴ ✉ 449164105@qq.com; <https://orcid.org/0000-0003-0189-9655>

*Corresponding authors: Dong-Qin Dai, ✉ cicidaidongqin@gmail.com, Qi-Rui Li, ✉ lqrnd2008@163.com, Chun-Ying Deng, ✉ 171934233@qq.com

Abstract

Guizhou and Yunnan Provinces (Yungui Plateau) in Southwestern China are well known as biodiversity hotspots. We introduce two new species in this study viz., *Mucispora hydei* (in Fuscosporellaceae, Fuscosporellales, Sordariomycetes)

and *Tolypocladium cucullae* (Ophiocordycipitaceae, Hypocreales, Sordariomycetes) and six new records based on morpho-molecular analyses. Full descriptions, color photographs and phylogenetic trees to indicate the placements of new species are provided.

Keywords: 2 new species, polyphasic approach, six new records, species diversity, taxonomy

Introduction

Guizhou and Yunnan Provinces (Yungui Plateau) in Southwestern China are known as biodiversity hotspots with high floral, faunal and microbial diversity (Xu *et al.* 2017). Due to its temperate climate, beautiful scenic spots such as waterfalls and caves, and variety of ethnic groups, Guizhou is one of the most environmentally and culturally diverse provinces in China (Liu *et al.* 2013; Chi *et al.* 2017; Liu *et al.* 2018). It is a mountainous province home to several rare animal species, like the Kuankuoshui salamander (*Pseudohynobius kuankuoshuiensis*) that is not found anywhere else in the world (Sparreboom 2014). Guizhou owns an average 61.92% karst landforms out of all the landforms and the main vegetation types in Guizhou are broadleaf and mixed forests (Liu *et al.* 2018). Domestically, Yunnan Province is contiguous with Guizhou, Sichuan, Guangxi, and Tibet in China and shares international borders with Vietnam, Laos and Myanmar (Qian *et al.* 2020). Natural resources and biodiversity in Yunnan are abundant, and approximately 19,333 plant species belong to 3,084 genera, and 440 families can be found of which 17,000 are endemic (Qian *et al.* 2020). Yunnan has three climatic areas *viz.* the tropical area at the southwest, south, and southeastern border; the subtropical zone in the west, middle and east; and the temperate zone in the high-elevation area in the northwest (Yang *et al.* 2008; Qian *et al.* 2020). Main vegetation types in Yunnan are Tropical Rainforest, Monsoon Forest, Evergreen Broadleaf Forest, Sclerophyllous Evergreen Broadleaf Forest, Deciduous Broadleaf Forest, Subtropical Needleleaf Forest, Temperate Needleleaf Forest, Bamboo Forest, Savanna-like Shrubby Grassland, Scrub and Meadow (Qian *et al.* 2020). During the last decade (2010–2020), a considerable number of mycology studies have been carried out in this region. Taxonomical studies of micro-fungi based on morpho-molecular analyses, wild mushroom cultivation and domestication, fungal secondary metabolite analyses and ethnomycological surveys are some of the major research areas.

Taxonomic research based on DNA sequences of non-pathogenic fungi is one of the popular topics among research groups on the Yungui Plateau. Researchers focus on micro-fungi based on their life modes (e.g., saprobic, epiphytic and endophytic) or habitat/niche (e.g., freshwater fungi, mycorrhizal fungi, karst fungi, air fungi). Fungal pathology (agricultural pathogens, clinical pathogens) is another well-developed field on the Yungui Plateau. Pathogens of agricultural crops, timber plants, ornamental plants and medicinal plants have been broadly studied. Entomopathogenic fungi on the Yungui Plateau is also a popular discipline.

In the course of our fieldwork on the Yungui Plateau, we encountered several interesting fungal specimens, and morpho-molecular analyses confirmed that these taxa comprise two new species and six new records (country, host or new record in Yunnan or Guizhou).

Materials and methodology

Sample collection and incubation

Living plant materials with disease symptoms and dead plant materials were randomly collected from Yunnan and Guizhou provinces. Temperature, date, time, elevation, and humidity of the collection sites were recorded. Samples were sealed in Ziploc plastic bags and returned to the laboratory. Samples were incubated using a moist chamber, sealed them and incubated at room temperature.

Isolation, morphological examination and maintain specimens and cultures

Single spore isolation was followed to isolate fungi (Senanayake *et al.* 2020). Ascomata/ conidiomata were sectioned with a razor blade, centrum tissue containing ascospores were removed using a sterile needle and placed in sterile water. A water drop, which contained the ascospore /conidial suspension, was placed on Water Agar (WA) (15 g agar, 1000 mL sterile distilled water) and incubated overnight at room temperature. Germinated spores were transferred

to potato dextrose agar (PDA Difco; 39 g/L sterile distilled water). Dried specimens were deposited at Herbaria of Guizhou Medical University, Qujing Normal University and Kunming Institute of Botany. Cultures were deposited at culture collections at Guizhou Medical University, Kunming Institute of Botany.

Morphological characteristics were captured by using a digital camera fitted on to a Nikon ECLIPSE 80i compound microscope. Squash mount preparations (Sutton 1980) were used to observe micro-morphological characteristics such as asci, ascospores and pseudoparaphyses in sexual morph; conidiophores, conidiogenous cells, conidia in asexual morphs. Free hand sections were taken to observe ascoma and peridium structures and shape of conidiomata. Melzer's reagent was used to stain the asci and apical rings, whereas Indian ink was used to stain mucilaginous sheaths surrounding the ascospores. Observed characteristics were presented as photo plates which were edited and combined using Adobe Photoshop version CS5 (Adobe Systems Inc., United States) and micro-morphological structures were measured in a Tarosoft (R) Image Frame Work version 0.9.7 program. Index Fungorum identifiers were obtained after registered new names as outlined in Index Fungorum (2021). New species are established as per guidelines established by Jeewon & Hyde (2016).

Phylogeny

DNA extraction, PCR amplification and sequencing

Genomic DNA of microfungi was extracted from fresh mycelia grown on PDA at 25–27°C using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®, Hangzhou, and P.R. China) according to the manufacturer's instructions.

DNA was also extracted from fruiting bodies of *Mucispora* sp. Surface of fruiting bodies was sterilized by 75% alcohol and rinsed three times by sterile water. Conidia and conidiophores were picked up by sterilized forceps and ground in a mortar into powder with liquid nitrogen. OMEGA E.Z.N.A. Forensic DNA Kit was used following manufacturer's instructions.

Amplification of LSU, SSU, ITS genes were performed by using LR0R/ LR5, NS1/ NS4 and ITS5/ ITS4 primers respectively (White *et al.* 1990). PCR products were sent for sequencing at Shanghai Sangon Biological Engineering Technology & Services Co. (Shanghai, P.R. China). All newly generated sequences were deposited in GenBank and accession numbers were obtained.

TABLE 1. Genes/loci used in the study with PCR primers, references and protocols.

Gene region	Primers	Thermal cycles	Reference
ITS	ITS5/ ITS4	(95 °C: 30 s, 55 °C:50 s, 72 °C: 90 s) × 35 cycles	White <i>et al.</i> (1990)
LSU	LR0R/ LR5	(95 °C: 30 s, 55 °C:50 s, 72 °C: 90 s) × 35 cycles	Vilgalys & Hester (1990), Rehner <i>et al.</i> (1994)
SSU	NS1/ NS4	(95 °C: 30 s, 55 °C:50 s, 72 °C: 90 s) × 35 cycles	White <i>et al.</i> (1990)

Phylogenetic analyses

Phylogenetic analyses were conducted based on the combined relevant genes (Table 1). Single gene alignment was carried out for prior comparable tree topologies. The combined gene sequence matrix was built based upon taxa generated in this study and related sequences retrieved from GenBank. Sequences were combined and aligned in Mega 6.0.5 (Tamura *et al.* 2013) and MAFFT: multiple sequence alignment software version 7.215 (Kato *et al.* 2019) and manually improved where necessary. Sequence alignment was converted to NEXUS file for maximum parsimony analysis using ClustalX2 v. 1.83 (Thompson *et al.* 1997) and PHYLIP-compatible for maximum likelihood analysis using ALTER (alignment transformation environment: <http://sing.ei.uvigo.es/ALTER/>). Phylogenetic analyses were performed by maximum likelihood (ML), maximum parsimony (MP) and Bayesian Inference (BI) analyses as outlined below:

a. Maximum Likelihood (ML)

Maximum likelihood analysis (ML) was performed in RaxmlGUI v.1.3 (Silvestro & Michalak 2012) with 1000 thorough bootstrap replicates. The available substitution models comprised a generalized time reversible (GTR) for nucleotides was applied with a discrete gamma distribution (Silvestro & Michalak 2012). A discrete GAMMA (Yang

1994) was complemented for each substitution model. Rapid bootstrap analysis (Stamatakis *et al.* 2014) and search for a best-scoring ML tree were applied (Silvestro & Michalak 2012).

b. Maximum Parsimony (MP)

MP analysis was carried out with stepwise additions of sequences by using PAUP v. 4.0b10 (Swofford 2002). The heuristic search option with 1000 random sequences addition and tree-bisection reconnection (TBR) of branch-swapping algorithm were performed. Maxtrees were setup at 1000. A zero of maximum branch length was collapsed and gaps were treated as missing data. Calculating of consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were included in the analysis. The robustness of the most parsimonious tree was estimated based on 1000 bootstrap replications with each 100 replicates of random stepwise addition of taxa.

c. Bayesian Inference (BI) analysis

BI analysis was performed by MrBayes v. 3.1.2 (Huelsenbeck & Ronquist 2001) with the best-fit model of sequences evolution estimated with MrModeltest 2.2 (Nylander 2004). Markov Chain Monte Carlo sampling (MCMC) was used to determine the posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1000000 to 5000000 generations based on the standard deviation of split frequencies less than 0.01. Trees were sampled every 1000th generations. First 20% trees were the burn-in phase and were discarded. Remaining trees were used to calculate the posterior probability (PP).

Taxonomy

In this section, we introduce two new species and five new records.

Karst fungi

Guizhou and Yunnan Provinces are well known destination for its karst formation. During the last five years, Chen *et al.* (2017), Zhang *et al.* (2017, 2018, 2019, 2020) broadly discussed and introduced over 50 new species from caves in Yungui Plateau. We collected a taxon inhabiting on decaying wood from a cave in Guizhou. Morphologically, it resembles *Melanocephala* and *Mucispora*. Megablast search in NCBI GenBank confirmed that it bears high sequence similarity to *Mucispora*. Morphological characteristics of the new taxon is distinct from all the other known taxa in the genus and phylogenetic analyses based on combined genes, LSU, SSU and ITS also confirmed that it is a new species of *Mucispora*.

Mucispora hydei Wijayaw., Q.R. Li, Y.C. Deng, L.S. Dissan & D-Q Dai *sp. nov.* (FIGURE 1)

Index Fungorum number: IF558463

Etymology:—Named in honour of British mycologist, K.D. Hyde for his immense contributions to mycology

Holotype:—GMB0028

Saprobic on decaying wood. **Asexual morph** Hyphomycetous. *Conidiophores* 60–110 × 8–12 μm (\bar{x} = 78.6 × 9.8 μm, n = 30), macronematous, mononematous, erect, solitary or in small groups on compactly aggregated cells, simple, cylindrical, smooth, brown, straight or slightly flexuous, percurrently proliferate 2–3 times, 1–2-septate. *Conidiogenous cells* holoblastic, integrated, terminal, cylindrical, smooth, pale brown. *Conidia* 35–50 × 20–30 μm (\bar{x} = 41.2 × 25.5 μm, n = 30), acrogenous, solitary, simple, smooth, ellipsoidal to obovoid, hyaline to subhyaline when young, dark brown when mature, with obvious septa in young conidia, paler at basal cell, truncate at base, sometimes covered by a hyaline mucilaginous sheath. **Sexual morph** Undetermined.

Material examined:—CHINA, Guizhou Province, Guiyang, Gaopo Township, Raorao village (106°48'6.54"E, 26°19'3.46"N), on decaying submerged wood, 9th December 2019, Nalin N. Wijayawardene, Q.R Li, (GMB0028, **holotype**, NNW56, **isotype**).

LSU: MW797122, SSU MW800164, ITS MW797039 (Supplementary Table 1)

Known distribution:—Guizhou Province, China

Notes:—Yang *et al.* (2016) introduced the genus *Mucispora* Jing Yang *et al.* with *M. obscuriseptata* J. Yang *et al.* as the type species. Besides the type species, the genus comprises two species *viz.* *M. phangngaensis* J. Yang & K.D. Hyde (Yang *et al.* 2017) and *M. infundibulata* J. Yang & K.D. Hyde (Hyde *et al.* 2020). All these species have been reported from submerged plant materials in Southern Thailand (Prachuap Khiri Khan Province and Phang Nga Province). In morphology, *Mucispora* closely resembles *Melanocephala* but it is specific in its cupulate proliferating conidiogenous cells and its conidia bearing a central downwardly directed collar with a fimbriate margin' (Hughes 1979; Yang *et al.* 2017).

Our new collection did not germinate in different media (WA, PDA, MEA) and in different temperatures thus we extracted DNA directly from the fruiting body (Zeng *et al.* 2018). PCR amplification of ITS (ITS4/ ITS5), LSU (primers: LR5/ LROR) and SSU (primers: NS1/NS4) were successful.

Phylogenetic analyses of combined LSU and ITS genes (Fig. 2) that our new strain is distinct from other taxa. However, the separation value is medium (69% in ML) and PP value is low. Nevertheless, morphological characters, of our collection is well-distinct from other *Mucispora* species (Table 2). Hence, we introduce the fourth species of the genus, *Mucispora hydei*. This is the first record of the genus outside Thailand.

TABLE 2. Morphological comparison of *Mucispora* species

Species	Conidiophore	Conidia	Location
<i>M. phangngaensis</i>	170–305 × 5–7	35–45 × 16.5–25	Phang Nga Province, Thailand
<i>M. obscuriseptata</i>	80–170 × 5–7.5	29–41 × 16–22	Prachuap Khiri Khan Province, Thailand
<i>M. infundibulata</i>	50–65 × 4–6	29–34 × 19–21	Phang Nga Province, Thailand
<i>M. hydei</i>	60–110 × 8–12	35–50 × 20–30	Guizhou Province, China

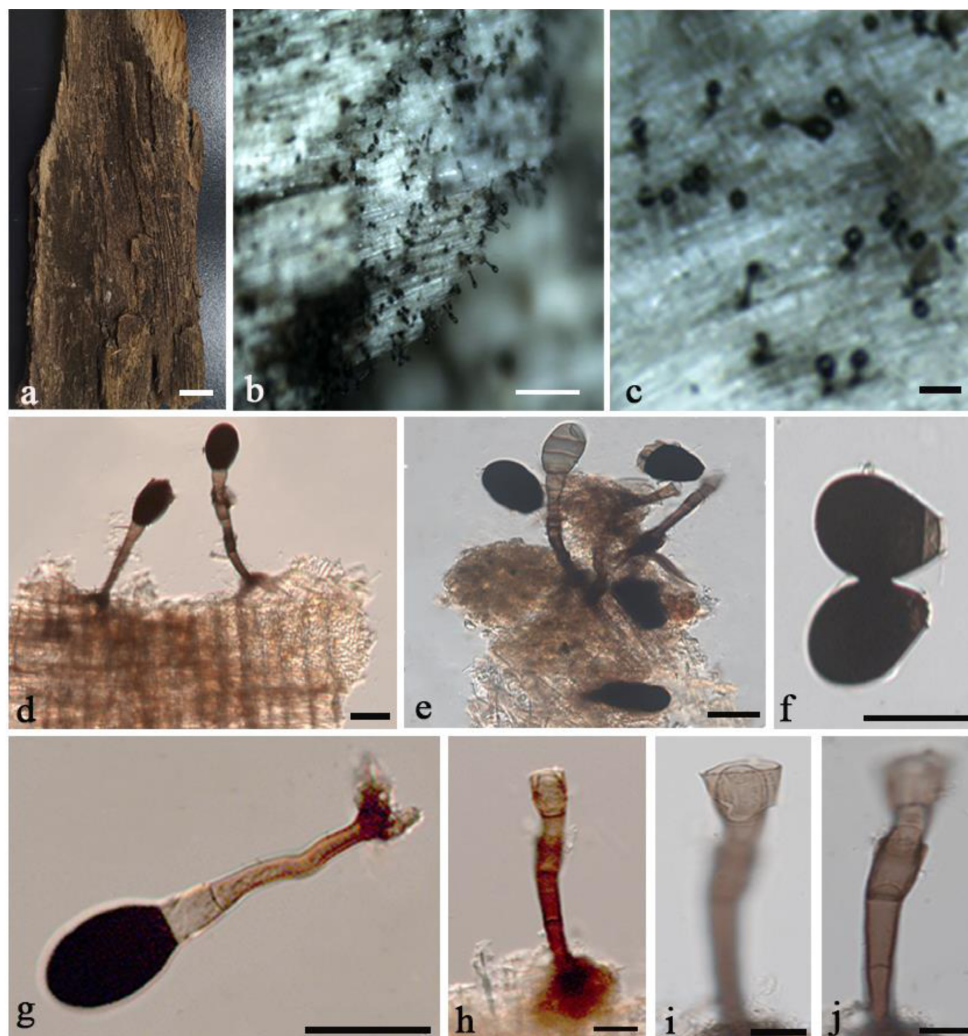


FIGURE 1. *Mucispora hydei* (GMB0028, holotype). a. Decaying wood. b, c. Colony on wood. d, e, g. Conidiophores with conidia. f. Matured conidia. h–j. Conidiophore. Scale bars: b = 100 μm, c = 200 μm, d, e, h–j = 20 μm, f, g = 40 μm.

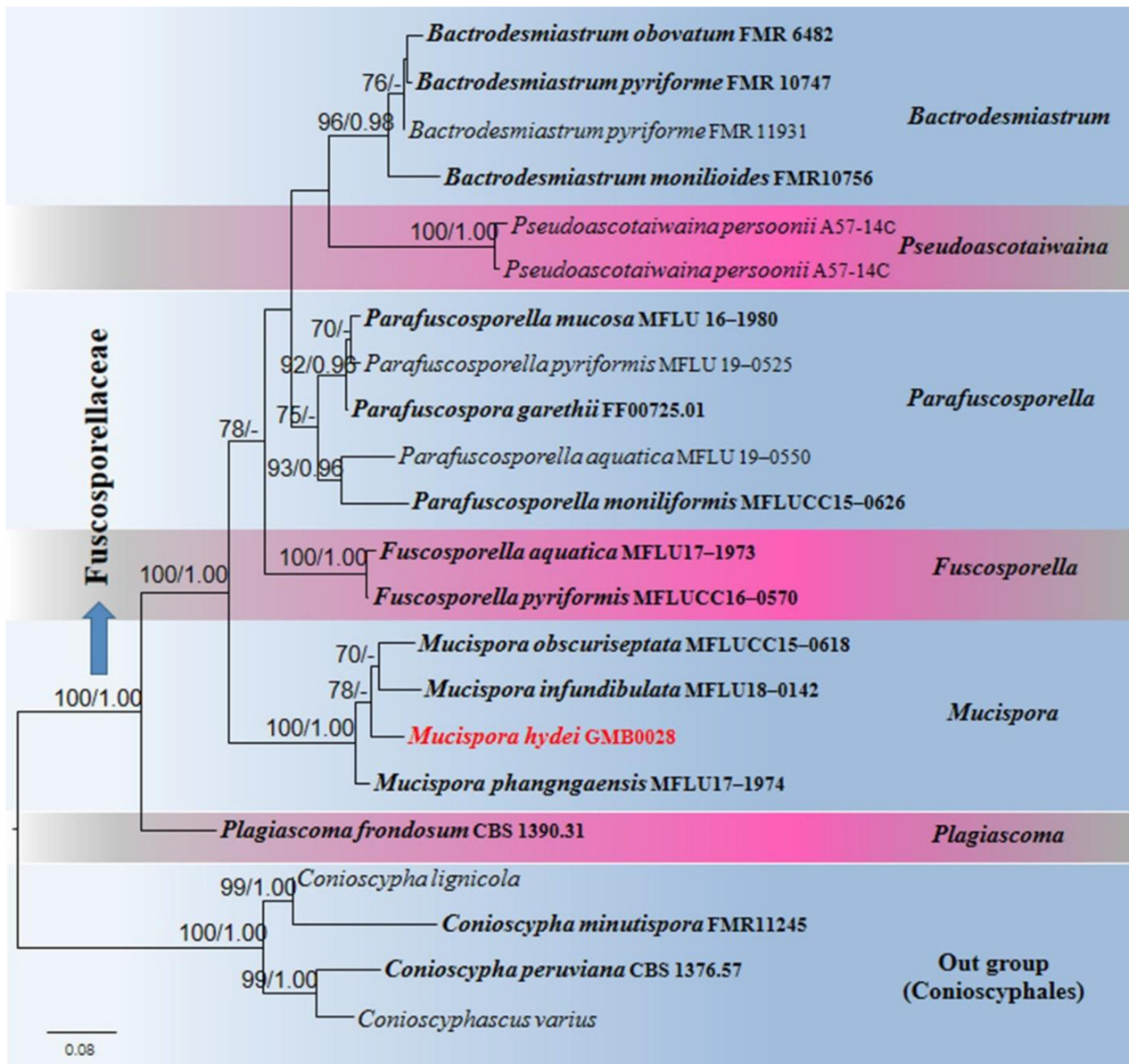


FIGURE 2. RAxML tree based on a combined dataset of partial LSU and ITS sequence analyses. Bootstrap support values for ML equal to or greater than 60 %, Bayesian posterior probabilities (BYPP) equal to or greater than 0.95 are shown as ML/ BYPP above the nodes. New isolates are in red bold. The tree is rooted to *Conioscypha lignicola* and *Conioschypha minutispora* (FMR11245) and *Conioscyphascus varius*. The scale bar represents the expected number of nucleotide substitutions per site.

Saprobic micro fungi on plants

Saprobic fungi are a vital component in the ecosystems since they are crucial in decaying organic matter, recycling minerals and nutrients (Hyde *et al.* 2018). In here, we report three new records from Yungui Plateau.

Immersidiscosia eucalypti (Pat.) Kaz. Tanaka, Okane & Hosoya, Persoonia 26: 94 (2011) (FIGURE 3)

Index Fungorum Number: IF519747

Foliicolous, the host plant is *Quercus palustris*. **Sexual morph:** Undetermined. **Asexual morph:** coelomycetous. *Conidiomata* 354–522 μm (\bar{x} = 427 μm , n = 5) diameter, 287 μm high, conspicuous, pycnidial, subglobose to sometimes lenticular in section view, semi-immersed, scattered, unilocular, with relatively thin stromatic base, black, glabrous. *Beak* of conidiomata long, 384 μm long, 13 – 61 μm wide. *Peridium* 18–42 μm wide (upper wall 25–42 μm (\bar{x} = 33 μm , n = 7) wide; basal wall 18–26 μm (\bar{x} = 27 μm , n = 7) wide), composed of 4 – 7 layers, with outer 3–5 layers light brown and inner layer hyaline, composed of thin-walled cells of *textura angularis*. *Conidiophores* up to 45 μm long,

cylindrical, branched. *Conidia* $15.4-17 \times 2.6-3.3 \mu\text{m}$ ($\bar{x} = 16.1 \times 3 \mu\text{m}$, $n = 10$), cylindrical to subcylindrical, slightly curved, 3-septate, hyaline, with an appendage at both ends; basal cell $2-2.8 \mu\text{m}$ long ($\bar{x} = 2.5 \mu\text{m}$, $n = 10$), obconic, truncate at the base; 2 median cells $10.5-12.2 \mu\text{m}$ long ($\bar{x} = 11.3 \mu\text{m}$, $n = 10$), cylindrical (second cell from the base $4.7-6.6 \mu\text{m}$ long ($\bar{x} = 5.6 \mu\text{m}$, $n = 10$), third cell $4.6-6.7 \mu\text{m}$ long ($\bar{x} = 5.7 \mu\text{m}$, $n = 10$)); apical cell $1.7-3.1 \mu\text{m}$ long ($\bar{x} = 2.7 \mu\text{m}$, $n = 10$). *Appendage* single, cellular, unbranched, filiform, flexuous or straight appendage; apical appendage $7.9-9.1 \times 0.8-1.1 \mu\text{m}$ ($\bar{x} = 8.7 \times 1 \mu\text{m}$, $n = 6$); basal appendage $7.8-9.3 \times 0.7-1.1 \mu\text{m}$ ($\bar{x} = 8.5 \times 0.9 \mu\text{m}$, $n = 6$).

Material examined:—CHINA, Yunnan Province, Dali; $25^{\circ}43'27''\text{N}$ $100^{\circ}6'54''\text{E}$, 2260 m alt.; 11 August 2019; Hai-Xia Wu leg; collected on a fallen leaf of *Quercus palustris* (IFRD 500-20) (new country record).

Known hosts and distribution (based on molecular data) :—Thailand, Yunnan China

Notes:—The genus, *Immersidiscosia* Kaz. Tanaka *et al.* (2011) was introduced by Tanaka *et al.* (2011) with *I. eucalypti* as the type species. The genus, morphologically resembles *Discosia* but phylogenetically distinct. *Immersidiscosia eucalypti* was reported from both temperate and tropical countries such as France, Italy, Japan and Tunisia (Tanaka *et al.* 2011; Hyde *et al.* 2017; Wijayawardene *et al.* 2017; Farr & Rossman 2021). This is the first report of *I. eucalypti* in China. Further collections are essentially required to study whether this taxon is pathogenic on *Quercus* species.

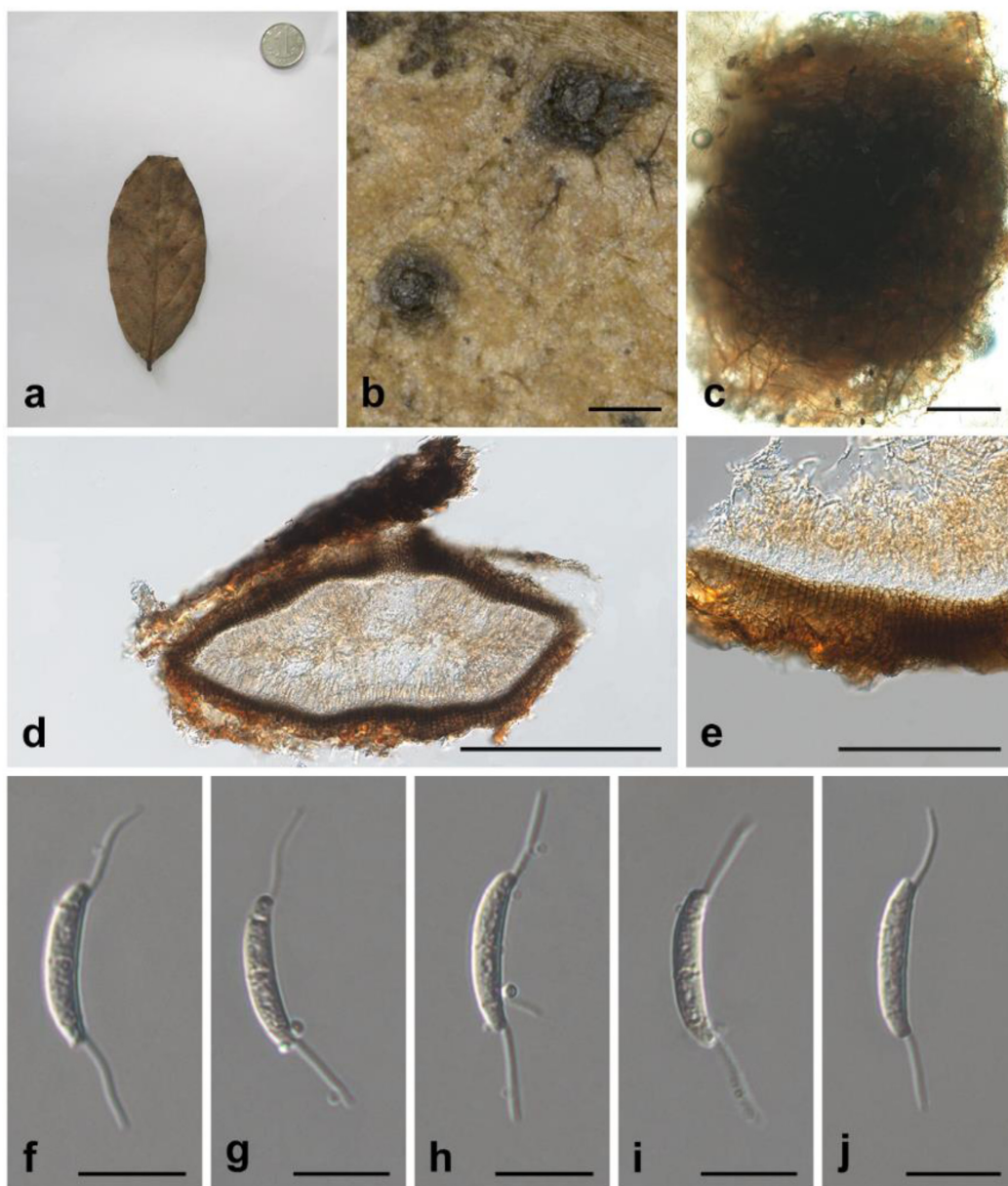


FIGURE 3. *Immersidiscosia eucalypti* (IFRD 500-20) a. Host leaves. b. Specimen with conidiomata. c. Conidiomata. d. Section of conidiomata. e. Peridium of conidiomata. f–j. Conidia. Scale bars: b = 300 μm , c, e = 100 μm , d = 200 μm , f–j = 10 μm .

Helminthosporium velutinum Link [as 'Helmisporium'], Mag. Gesell. naturf. Freunde, Berlin 3(1–2): 10, tab. 1:9 (1809) (FIGURE 4)

Index Fungorum Number: IF250075

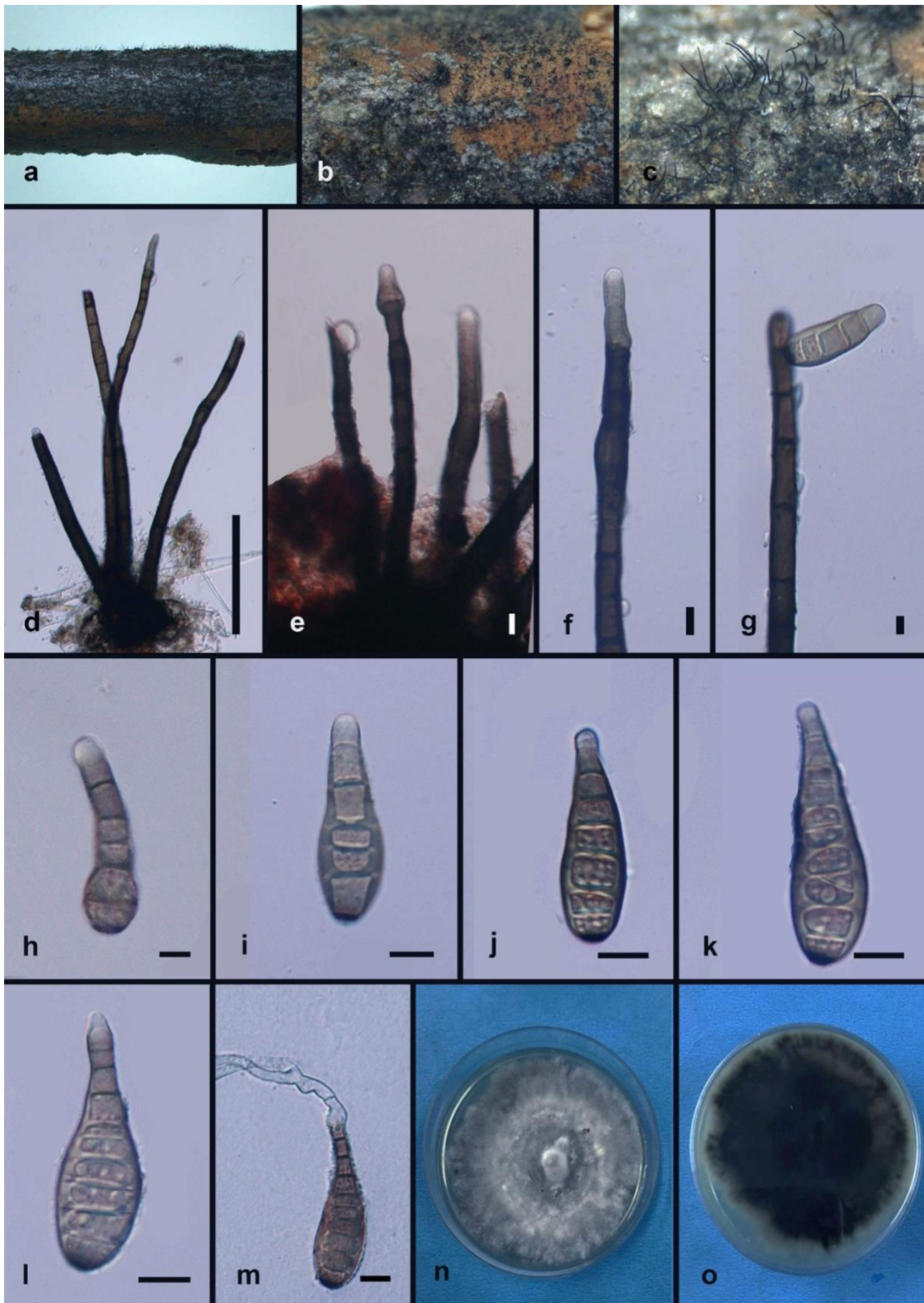


FIGURE 4. *Helminthosporium velutinum* (HKAS 107064, new host record and a new record from Guizhou Province) a–c. Colony on the substrate. d. Conidiophores. e–g. Conidiophore and conidia. h–l. Conidia. m. Germinating conidium. n, o. Culture on PDA from. n. above o. below after 4 weeks. Scale bars: d = 100 μ m, e–g = 50 μ m, h–m = 20 μ m.

Description. *Saprobic* on dead twigs, dark brown, effuse, velvety. **Sexual morph:** Undetermined. **Asexual morph:** *Mycelium* immersed, composed of branched, septate, thick-walled hyphae. *Conidiophores* mononematous, macronematous, mostly unbranched, proliferating, dark brown, $96\text{--}296 \times 5\text{--}7 \mu\text{m}$ ($\bar{x} = 153 \times 6 \mu\text{m}$, $n = 10$), 7–12 septate, erect or flexuous, tapering towards apex, bulbous at base with cells near apex of conidiophore guttulate and fertile. *Conidiogenous cells* polytretic integrated, intercalary and terminal. *Conidia* $99\text{--}131 \times 20\text{--}36 \mu\text{m}$ ($\bar{x} = 115 \times 28 \mu\text{m}$, $n = 20$) single, obclavate, pale brown to brown, 6–9 distoseptate, smooth, straight or curved, base slightly truncate, cicatrized and wider than apex, dark brown, apical cell paler than other cells, rounded at apex, guttulate when young, non-guttulate at maturity.

Culture characteristics: Colonies on PDA, reaching 21 mm diam., after 2 weeks at 20–25 °C, medium dense, circular to slightly irregular, slightly raised and cottony surface, colony from above: at first white, becoming buff; from below: blackish white at the margin, black to ash at the center; mycelium blackish.

Material examined: CHINA, Guizhou Province, Huaxi District, Guizhou university garden (South), on a dead branch of *Platanus* sp., 05 October 2019, Nalin N. Wijayawardene, NWGUP01 (HKAS 107064, **new host record, a new record from Guizhou Province**), ex-type living culture, KUMCC 20–0029

Known hosts and distribution: Guizhou province, China (this study), Yunnan Province, Dali, WanHua stream, China (Zhu *et al.* 2016).

Known hosts: *Platanus* sp. (this study), saprobic on decaying wood submerged in stream (Zhu *et al.* 2016).

GenBank Numbers: LSU: MW273148, SSU: MW273295, ITS: MW273144

Notes: *Helminthosporium velutinum*, the type species of *Helminthosporium* was re-visited by Voglmayr & Jaklitsch (2017) and designated the epitype and the ex-epitype. The genus was reported with the sexual morph however, *Helminthosporium velutinum* lacks the sexual morph (Voglmayr & Jaklitsch 2017). According to Voglmayr & Jaklitsch (2017), distribution of the species was reported as ‘Widespread and common in temperate Eurasia and America, probably almost cosmopolitan’. Zhu *et al.* (2016) reported *Helminthosporium velutinum* from submerged wood from Yunnan Province, China. In this study, we collected *Helminthosporium velutinum* on dead branches of *Platanus* sp. from Guizhou Province, China. According to Farr & Rossman (2021), a taxon named *Helminthosporium spiciferum* (Nicot 1953) (current name: *Curvularia spicifera* Index Fungorum 2021) was reported from *Platanus occidentalis*. Besides this record, as far as we know, *Helminthosporium* species have not been reported from *Platanus* species. Moreover, this is the first record of this genus from terrestrial habitats from China.

Rousoella pseudohysterioides D.Q. Dai & K.D. Hyde, Fungal Diversity 82: 37 (2016) (FIGURE 5)

Index Fungorum Number: IF552026

Saprobic on decaying bamboo culms. **Sexual morph:** *Ascostromata* forming under black area, including 3–5 locules, up to 3–5 mm long and 0.5–2 mm wide, slightly raised at maturity, irregular, black, coriaceous. *Locules* in vertical section 220–280 μm high, 180–330 μm diam., gregarious, subglobose to ellipsoidal, dark brown, with ostiolate opening. *Peridium* composed of dark brown cells comprising host and fungal tissues. *Hamathecium* comprising dense, 2–3.5 μm wide, cellular pseudoparaphyses, indistinctly septate, embedded in a gelatinous matrix. *Asci* 85–290 \times 7.5–17.5 μm ($\bar{x} = 165 \times 10.5 \mu\text{m}$, $n=30$), 8-spored, bitunicate, cylindrical, with a short furcate pedicel, with an apical ocular chamber. *Ascospores* 11–19.5 \times 4–6.5 μm ($\bar{x} = 16.5 \times 5.5 \mu\text{m}$, $n=30$), uniseriate, fusiform-ellipsoidal, 1-septate, constricted at the septum, narrow at both ends, with striate wall ornamentation, some with obvious verrucose. **Asexual morph:** Undetermined.

Material examined:—CHINA, Guizhou Province, Leigong Mountain National Nature Reserve, on dead culm of bamboo, July 2019, Q.R. Li 2019LGS13 (GMB0009), living cultures, GMBC0009 (**new country record**).

Known hosts and distribution:—Guizhou, China, Thailand

Known hosts:—Bamboo

GenBank Numbers:—ITS: MW881445; LSU: MW881451; RPB2: MW883345

Notes:—*Rousoella*, typified by *Rousoella nitidula* Sacc. & Paol. was introduced by Saccardo & Paoletti (1888). Most species of *Rousoella* were observed from monocotyledon, such as bamboo and palms (Dai *et al.* 2017; Hyde *et al.* 2018). *Rousoella pseudohysterioides* was originally introduced by Dai *et al.* (2017) isolated from Thailand. This is the first report of *Rousoella pseudohysterioides* discovered from China.

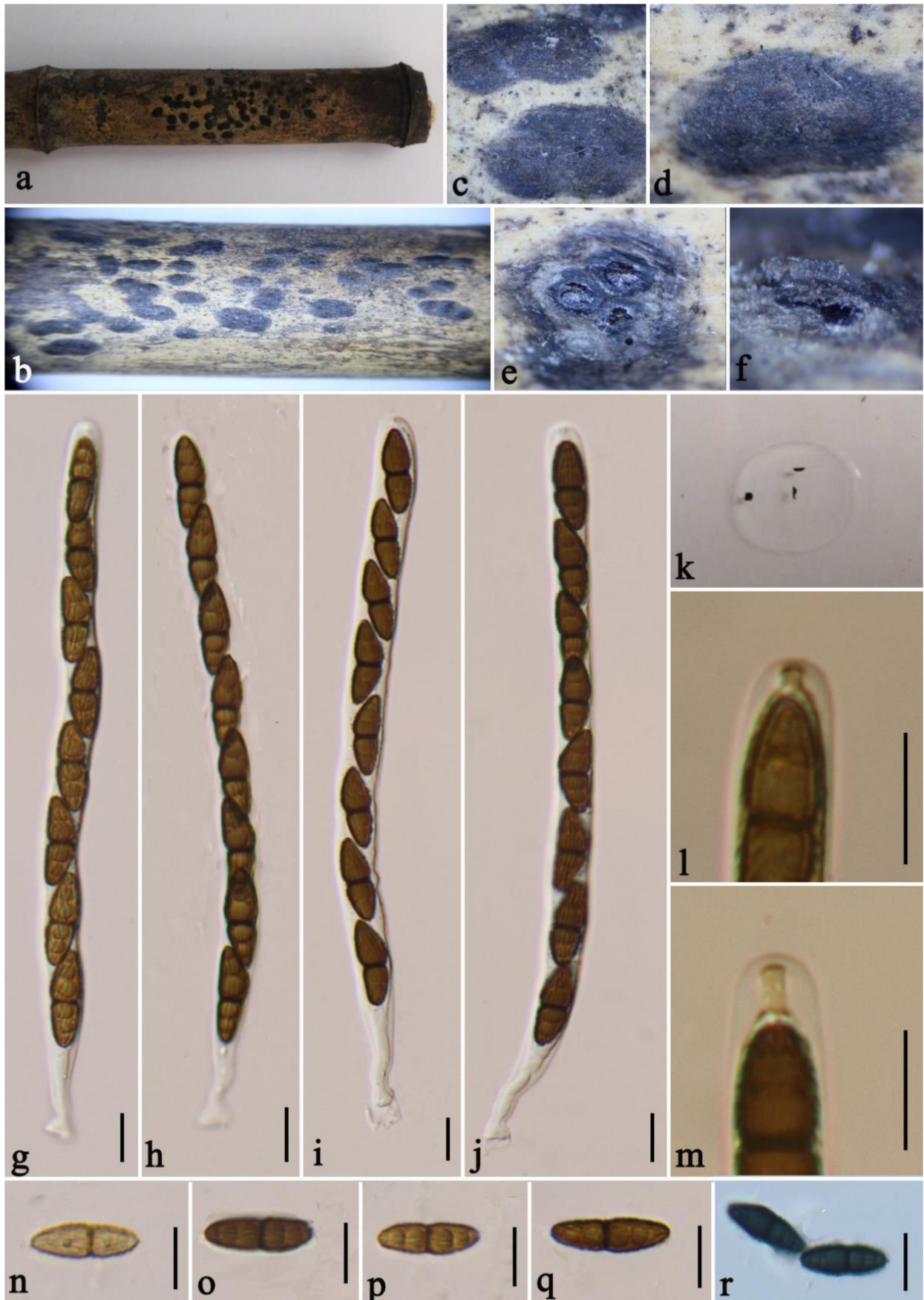


FIGURE 5. *Roussioella pseudohysterioides* (GMB0009). a–d. Ascostromata developing on bamboo culm. e, f. Vertical sections of ascostromata. g–j. Asci containing eight ascospores. k. Fragment of ascostromata in KOH without stromatal pigments. l–m. Ascus apex in Melzer’s reagent. n–r. Dark brown ascospores. Scale bars: j–r = 10 μ m.

Entomopathogenic fungi

Studying entomopathogenic fungi is one of the popular research areas in China since they are medicinal importance. *Beauveria*, *Cordyceps* and *Metarhizium* are some important genera which have widely been studied. Here, we introduce one new species and one new record of entomopathogenic fungi.

Tolypocladium W. Gams, Persoonia 6(2): 185 (1971)

Tolypocladium used to be known as an asexually genus since it was described (Gams 1971) until Hodge *et al.* (1996) linked one sexual species to this genus. This genus was transferred in the family Ophiocordycipitaceae based on phylogenetic analyses (Sung *et al.* 2007). Many species of *Elaphocordyceps* and *Chaunopycnis* have been transferred to *Tolypocladium*, which was protected in the International Code of Nomenclature for algae, fungi, and plant (Kirk *et al.* 2013, Quandt *et al.* 2014).

Tolypocladium cucullae Y.P. Xiao & T.C. Wen *sp. nov.* (FIGURE 6)

Index Fungorum Number: 558265

Etymology:—The specific epithet refers to the feature of the capitate stromata.

Holotype:—HKAS 55588

Parasitic in an unidentified host buried in the upper 1 cm of soil, forming brown to dark stromata. **Sexual morph:** *Ascomata* 8–13 cm long, 5–10 mm wide, stromatic, brown to olive when fresh, dark when dry, tough, capitate, mostly solitary, stipitate, inside hollow when mature. *Stipe* 8–12 × 0.5–0.7 cm, cylindrical, yellow to brown when fresh, dark brown when dry, with green scales on the surface when fresh, with dark furfuraceous when dry, fibrous, hollow, with stromata on the top. *Fertile head* 8–10 mm in diam, hemispherical, minutely mammilate, bracken green to dark olive when fresh, dark when dry, distinctly separated from the stipe, tough, solitary, with a cortex of closely interwoven hyaline hyphae pseudoparenchymatous in section. *Perithecia* 500–600 × 340–420 μm (\bar{x} = 560 × 380 μm, n = 30), subglobose to ovoid, immersed in stroma with slightly protruding ostiolar papilla. *Ostiole* lined with paraphyses. *Peridium* 20–25 μm (\bar{x} = 22 μm, n = 60) wide, of brown pigmented cells of *textura porrecta* to paler *textura prismatica*. *Asci* 320–400 × 10–15 μm (\bar{x} = 360 × 13 μm, n = 60), 8-spored, unitunicate, narrow cylindrical, hyaline, with thick apex. *Apical cap* 5.5–7.5 × 5–7.5 μm (\bar{x} = 6.5 × 6 μm, n = 60) μm diam, hyaline. *Ascospores* as long as asci, filiform, hyaline break into secondary spores. *Secondary spores* 25–35 × 3–4.5 μm (\bar{x} = 30 × 3.8 μm, n = 60), cylindrical to fusoid with truncated ends, smooth, hyaline, with or without septa. **Asexual morph:** Undetermined.

Material examined:—CHINA, Yunnan Province, Lijiang City, Laojun Mountain. 15 July 2008, Yun Ting Huang (HKAS 55588, **holotype**), (GZU A-77, **isotype**).

LSU: MW798786 MW798787, SSU MW798784 MW798785, ITS MW798788 MW798789 (Supplementary Table 1)

Notes:—We identified this species after we inspected the unidentified specimens in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (HKAS). According to morphology and phylogenetic analysis (Fig. 7), the new species *Tolypocladium cucullae* is close to *T. capitatum*, *T. delicatistipitatum*, *T. fumosum* and *T. longisegmentatum*. *Tolypocladium cucullae* is distinct from *T. capitatum* by producing hollow, furfuraceous stipe, smaller perithecia and smaller asci, while *T. capitatum* produces tough stipe, bigger perithecia and longer asci (Mains 1957, Table 3). *Tolypocladium cucullae* is distinct from *T. fumosum* in having larger and brown to olive when fresh, dark when dry stromata; larger, hemispherical and bracken green to dark olive when fresh, dark when dry fertile head; smaller perithecia; longer and cylindrical to fusoid secondary spores. *Tolypocladium fumosum* has smaller, pale chalcedony yellow at the base to dark gull grey at the apex stromata; ellipsoidal when young and capitate when mature fertile head; larger perithecia; shorter and cylindrical to cubic secondary spores. The phylogenetic tree also supports that *T. cucullae* is distinct from *T. capitatum* and *T. fumosum* (Fig. 7).

The morpho-characters of *T. cucullae* are similar to *T. delicatistipitatum*, but the latter has no DNA sequence data. Both of them formed stipitate stromata, subglobose to ovoid perithecia, cylindrical asci and cylindrical secondary spores with truncate ends. *Tolypocladium cucullae* is different from *Tolypocladium delicatistipitatum* in producing stromata with a hemispherical, dark (when dry) fertile part, with a thinner (5.5–6 μm in diam) apical cap and longer (25–35 μm long) secondary spores, while *T. delicatistipitatum* produces stromata with a spherical or oval fertile part, a thicker (8 μm in diam) apical cap and shorter (18–28 μm long) secondary ascospores.

Molecular data have been supplemented by four strains, including OSC 110992 (Sung *et al.* 2007), HMJAU6903 (Yan & Bau 2014), MHHNU 8699 (Chen & Zhang 2019) and 2731.S (Stensrud *et al.* 2005). Furthermore, HMJAU6903 (Yan & Bau 2014) and MHHNU 8699 (Chen & Zhang 2019) were reported molecular data with descriptions and illustrations among these four strains. *Tolypocladium cucullae* is distinct from *T. longisegmentatum* (DAOM 137162, Ginns 1988; HMJAU6903, Yan & Bau 2014; MHHNU 8699, Chen & Zhang 2019) in having a hemispherical fertile head, brown perithecia and shorter secondary spores (Table 3). Molecular data indicated that the new species has 31 bp in ITS that differ from HMJAU 6903, 36 bp in ITS that is different from MHHNU 8699, 38 bp in ITS is different from 2731.S, 26 bp in LSU that are different from OSC 110992. In conclusion, we propose *T. cucullae* as a new species.



FIGURE 6. *Tolypocladium cucullae* (HKAS 55588, holotype). a, b. Material of *Tolypocladium cucullae*. c. Ascostromata. d. Fertile head of ascostroma. e. Vertical section of stroma. f. Peridium. g–j. Asci. k. Apical cap of asci. l–o. Secondary ascospores. Scale Bars: d = 5 mm, e = 100 μ m, f = 50 μ m, g–j = 200 μ m, k, l–o = 20 μ m.

TABLE 3. Morphological comparison of closely related species of *T. cucullatae*.

Strain	Location	Stromata	Stipe (cm)	Fertile head (mm)	Perithecia (μm)	Asci (μm)	Secondary spores (μm)	Reference
DAOM 137162	America	Single, rarely double	13 \times 0.7, greyish yellow above and the lower one-third a deep yellow, cylindrical, olive, some with the basal part dark olive or black, glabrous, hollow	Broadly rounded, above the stipe apex, brown, dark brown to olive brown, glabrous, 13 diam	Imbedded, ellipsoid 500 \times 300	Cylindrical to narrowly ellipsoid 440 \times 10–15	(12–)40–65 \times 3–5	Gimms (1988)
HMJAU6903	China	Single	2.5–6.5 \times 0.2 wide, yellow brown, with black scales on the surface,	Broadly rounded, dark brown, 4–9 diam	Imbedded, ellipsoid or flask-shaped, 632–681 \times 273–292	Cylindrical, 341–428 \times 12	(12.2–) 29.3–48.8 (–73.3) \times 3.7–4.9	Yan & Bau (2014)
HKAS 55588	China	Single	8–12 \times 0.5–0.7, cylindrical, yellow to brown when fresh, dark when dry, with green scales on the surface, hollow	Hemispherical, bracken green to dark green when fresh, dark when dry, 8–10 in diam	Immersed, subglobose to ovoid, 500–600 \times 340–420	Cylindrical to fusoid 320–400 \times 10–15	25–35 \times 3–4.5	This study

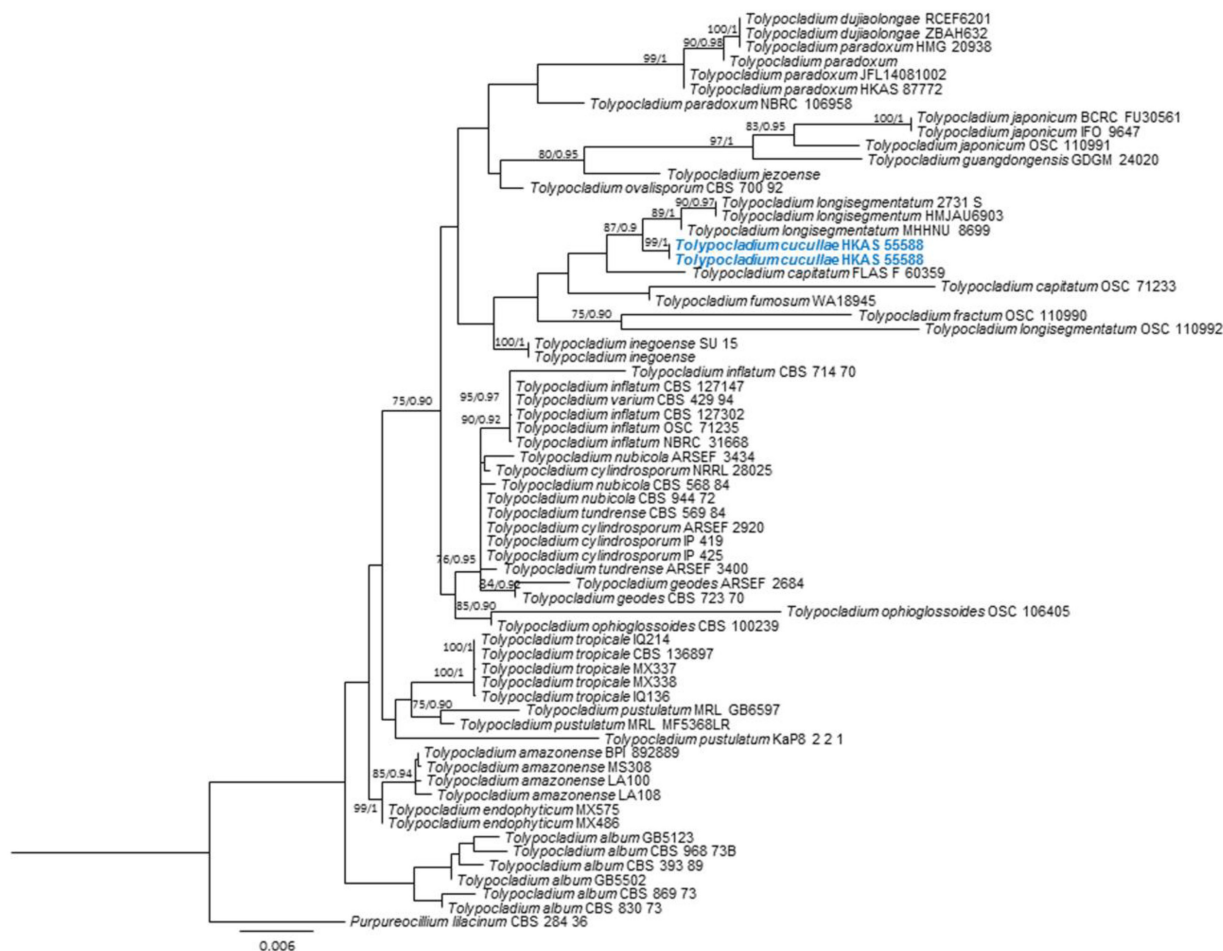


FIGURE 7. Phylogram of *Tolypocladium* generated from Maximum likelihood analysis of ITS, SSU and LSU sequence data. *Purpureocillium lilacinum* (CBS 284.36) was selected as an outgroup taxon. The tree topology of the ML analysis was similar to the BI. Maximum likelihood bootstrap values greater than 75 and Bayesian posterior probabilities over 0.90 were indicated above the nodes. The scale bar indicates 0.006 changes. The new species was in blue.

Metarhizium guizhouense Q.T. Chen & H.L. Guo, Acta Mycol. Sin. 5(3): 181 (1986) (FIGURE. 8)

Index Fungorum Number: 130206

Specimen found on stick insects (Phasmatodea). Host's internodes between abdominal segments were covered with white to pale green mycelium and sporulating conidiophores. *Conidiophores* arising from hyphae, smooth-walled. *Phialides* cylindrical, solitary, smooth-walled, 8–18 × 1–1.5 μm. *Conidia* smooth-walled, pale green to colorless (6.5–9.5 × 2.5–3 μm), cylindrical, slightly constricted in the middle, round at both ends or tapered at one end. Bi-celled conidium was not observed.

Culture characteristics:—Colonies on PDA were relatively slow-growing, fluffy, beginning to white, and the spores appear green, started to produce conidia after 3 days in culture at 25 °C in the laboratory, 17 mm diam. after 10 days. Mature conidia chains are often spread on the surface of the colony in small granular clumps. Hyphae hyaline, separated, branched, about 3 μm wide.

Material examined:—China, Guizhou Province, Guiyang, on dead stick insects, July 2019, Q.R. L., 2019GY03 (GMB0010), living cultures, GMBC0010 (**new host record**).

Known hosts and distribution:—Guizhou

Known hosts:—larvae of *Noctuidae* sp., stick insects

GenBank Numbers:—ITS: MW881444, LSU: MW881450, RPB2: MW883344

Note:—*Metarhizium guizhouense*, isolated on *Hepialus* sp. in Guizhou China, was introduced by Guo *et al.* (1986). In 1991, Liang *et al.* reported a *M. taii* Z.Q. Liang & A.Y. Liu on larvae of *Noctuidae* sp. (Lepidoptera). *Metacordyceps taii* was recognized to be the sexual morph of *M. guizhouense* by Bischoff *et al.* (2009). Qu *et al.* also

reported that *M. taii* should be treated as a synonym of *M. guizhouense* based on molecular data. This is the first report of *M. guizhouense* isolated on stick insects (Phasmatodea).

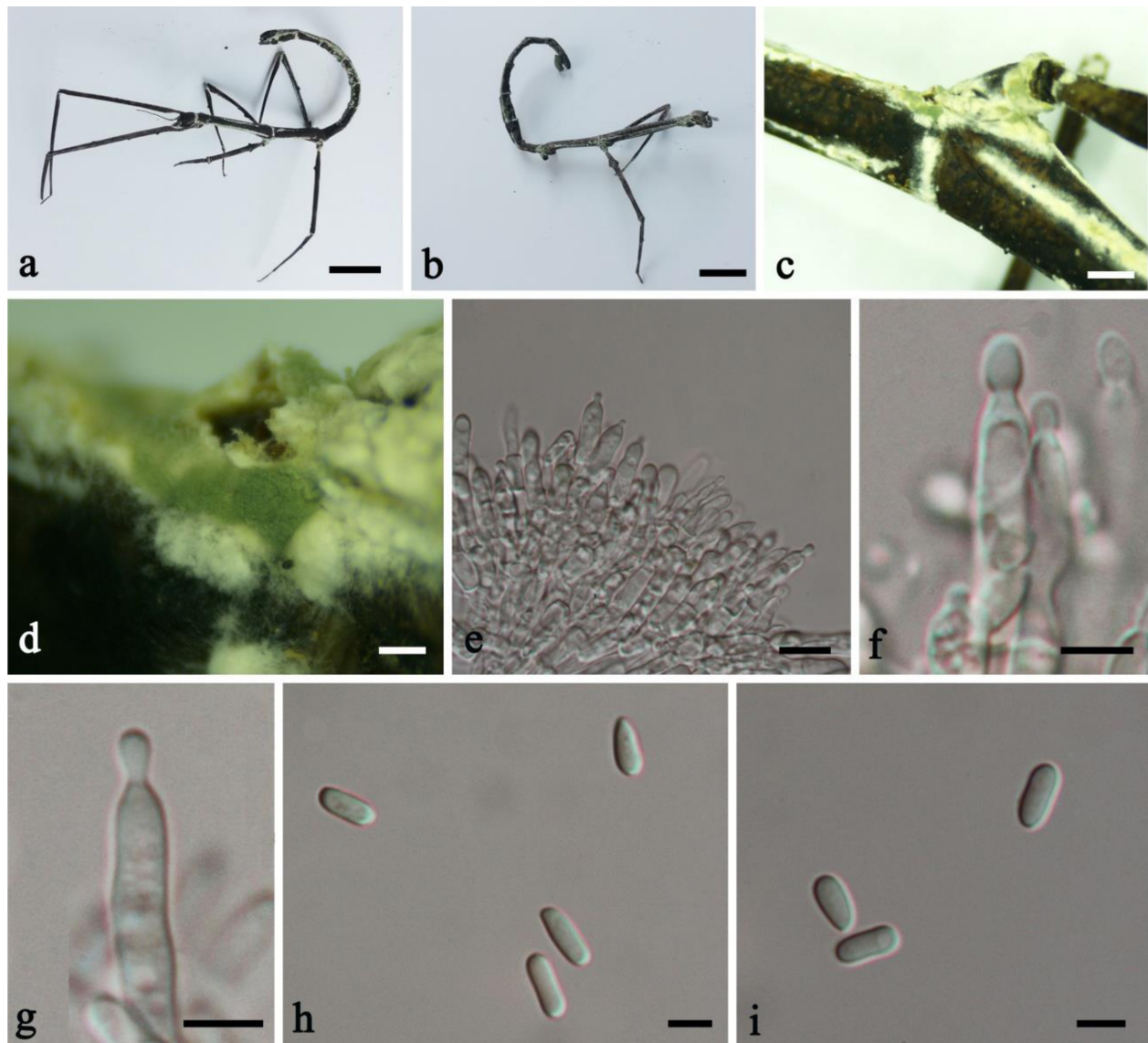


FIGURE 8. *Metarhizium guizhouense* (GMB0010) (new host record). a, b. Fungus on stick insects (Phasmatodea) c, d. Green mycelium and sporulating conidiophores covered on the surface of insect. e, f, g. Conidiophores h, i. Conidia on insect host. Scale bars: a, b = 5 mm, c = 2 mm, d = 500 μ m, j–r = 10 μ m, e–i = 5 μ m

Fresh water fungi

In Yungui region, freshwater fungi are mainly found in lotic (rivers, streams and waterfalls) habitats. They play important ecological roles since they are decomposers of submerged substrates (mainly from riparian vegetation), and participate crucial biogeochemical cycles, such as carbon cycling (Wurzbacher *et al.* 2010; Gulis & Barlocher 2017). In this study we provide a new country record of *Myrmecridium schulzeri*.

Myrmecridium schulzeri (Sacc.) Arzanlou, W. Gams & Crous, Stud. Mycol. 58: 84 (2007) (FIGURE. 9)

Index Fungorum Number: IF504560

Saprobic on submerged decaying wood. **Sexual morph** undetermined. **Asexual morph** Colonies on natural substrata effuse, superficial, scattered, hairy, solitary or in small groups, black, with a mass of visible whitish to grayish conidia on middle to upper part of conidiophores. *Mycelium* partly superficial, partly immersed. *Conidiophores* macronematous, mononematous, straight to slightly curve, unbranched, medium brown to brown at base part, pale towards top part, thin-walled, septate, 172–304 \times 2–3 μ m (\bar{x} = 212 \times 2.6 μ m, n = 15). *Conidiogenous cells* holoblastic, polyblastic,

integrated, terminal and intercalary, cylindrical, subhyaline to pale brown, forming a rachis with scattered pimple-shaped denticles which are less than 1 μm long and approx. 0.5 μm in diameter. *Conidia* solitary, fusoid or ellipsoidal to obovoidal, rounded at the apex, obtuse and tapering towards base, hyaline, aseptate, thin-walled, smooth, without guttule, some with a small protuberance, 5–6.5 \times 2.3–3.6 μm (\bar{x} = 5.8 \times 2.9 μm , n = 35).

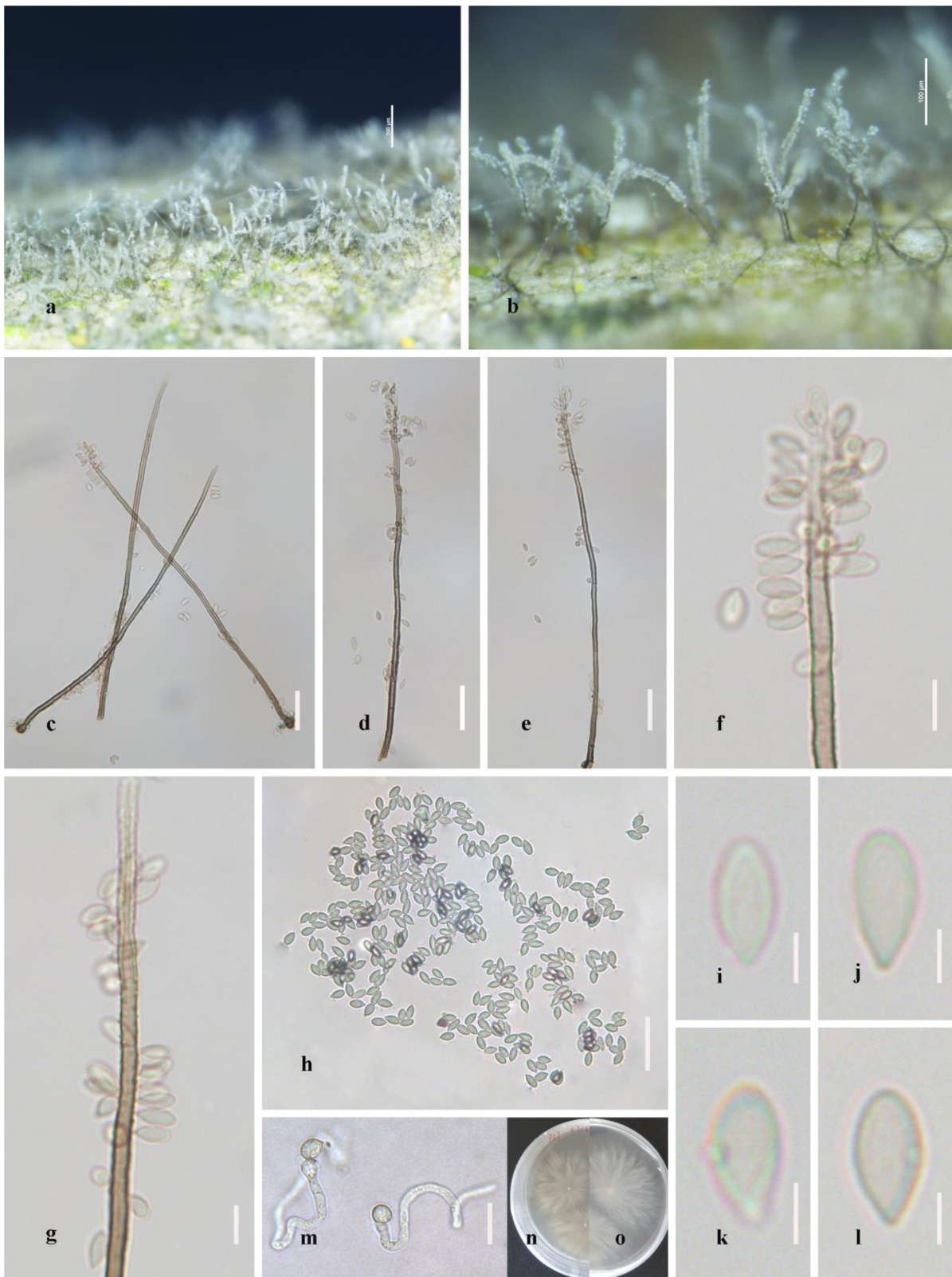


FIGURE 9. *Myrmecridium schulzeri* (IFRD500–012) a, b. Colonies on natural substrate. c–e. Conidiophores with conidia. f, g. Conidiogenous cells with conidia. h–l. Conidia. m Germinating conidia on PDA. n, o. Culture on PDA, n. from front, o. from reverse. Scale bars: c–e, h = 20 μm , m = 10 μm , f, g = 5 μm , i–l = 2 μm .

Culture characteristics:—Conidia germinating on PDA within 24h. Colonies grow on PDA attaining 38–48 mm diameter in 40d at 20–25°C in the condition of 12h-dark and 12h-light, with smooth, floccose, pale brown mycelium on the surface, reverse white, with filamentous, undulate margin.

Material examined: China, Yunnan Province, small river of Puzhehei, on dead submerged decaying wood of unidentified plants, 23 June 2018, Hao Yang, P37 (IFRD500–012), living culture = KUMCC 20–0190 (**new record from Yunnan, new habitat record**).

Known hosts and distribution: Soil (Germany, Papua New Guinea, Zaire), *Homo sapiens* (Netherlands), Wheat straw (South Africa), *Triticum aestivum* (Netherlands), *Malus sylvestris* (Switzerland), *Cannomois virgate* (South Africa)

GenBank Numbers: ITS MT559103

Notes:—*Myrmecridium* was introduced by Arzanlou *et al.* (2007) with *M. schulzeri* as type species, which was described as *Chloridium schulzerii* (Sacc.) Sacc. and *Rhinocladiella schulzeri* (Sacc.) Matsush. Our isolate fits the characters of *Myrmecridium* well in having macronematous, unbranched, septate conidiophores, polyblastic conidiogenous cells with denticles, and hyaline, thin-walled, smooth, fusoid or ellipsoidal to obovoidal conidia (Arzanlou *et al.* 2007, Jie *et al.* 2013, Peintner *et al.* 2016, Réblová *et al.* 2016). The sequence data in ITS gene region of our isolate are identical to that of *M. schulzeri*. Thus, we identified our isolate as *M. schulzeri*. Our isolate is a new geographic record in China and a new habitat record from freshwater.

Mushrooms

Panus similis Berk. & Br. In Journ. Linn. Soc., Bot. 14:43 (1873) (FIGURES 10,11)

Pileus (4.9B) 4–16 cm diameter, thin, deeply infundibuliform; surface brown to dark chestnut brown, finely velutinate at the centre, radially plicate-sulcate with the striae extending almost to the centre, margin curved downwards, ciliate. *Lamellae* decurrent, ochraceous buff, darkening at maturity, 1.5–3 mm broad, moderately spaced with lamellulae of five lengths; entire edge. *Stipe* central, 4–17 cm × 1.5–2 mm, solid, cylindric, slightly expanded at the base; surface concolorous with the pileus, uniformly velutinate and felt-like. *Context* 1–2 mm thick at the centre, coriaceous, white. *Generative hyphae* (4.7E) 2–4 µm diameter, very thin-walled, frequently branching with clamp connections. *Skeletal hyphae* (4.7E) 2–5 µm diameter, cylindric, sinuous with a thickened hyaline wall, unbranched. *Basidiospores* (4.7A) (5.5–6.5 × 2.5–3.5 (5.5 ± 0.3 × 3 ± 0.2) µm, Q = 1.83, hyaline, ellipsoid to oblong cylindric, thin-walled, with few contents. *Basidia* 17–29 × 4–5 µm, clavate, cylindric, bearing 4 sterigmata. *Lamella-edge* sterile, with small Cheilocystidia, soon collapsing. *Cheilocystidia* crowded, 17–26 × 3–6 µm, nodulose-clavate, hyaline, irregular, thin-walled. *Sclerocystidia* (4.7D) very abundant, very crowded, 19–41 × 4–9 µm, irregularly fusoid, elongate, with a thick, hyaline wall. *Hymenophoral trama* irregular of radiate construction, hyaline. *Subhymenial layer* slightly developed. *Pileipellis* on epicutis, up to 115 µm thick, of more or less repent hyaline, up to 160 µm long, 115 µm diameter, with a thickened wall of 1.5–3.5 µm. *Stipitipellis* similar to Pileipellis. Smell mushroomy, edible when it is young.

Material examined:—CHINA, Yunnan Province, Xishuangbanna, elevation 400 m, rainforest dominated by *Castanopsis* sp. and *Dipterocarpus* sp.; 4 June 2018, Samantha C. Karunarathna (HKAS 121668) (**new country record**).

Notes: *Panus similis* has a palaeotropical *Distribution* and is most commonly found in south-east Asia and Australasia, but also extends westwards across equatorial Africa. It is recognized by the excellently velutinate to glabrescent pileus with noticeable radially sulcate striate, combined with the subdistant lamellae. Large basidiocarps are frequently encountered almost always associated with a prominent pseudosclerotium. This study reports *P. similis* for the first time from China, based on both morphological characteristics (Figs. 10, 11) and phylogenetic analysis (Fig. 12).

Discussion

Why Yungui Plateau is important in Chinese mycology

Hawksworth & Lucking (2017), and Hyde *et al.* (2020) identified biodiversity-rich areas for revealing undiscovered or missing fungal species. Hence, Yungui Plateau is an important region to conduct intensive research to discover new

fungal taxa. Figure 13 shows that Yunnan and Guizhou Provinces are the leading provinces in introducing new species in China.

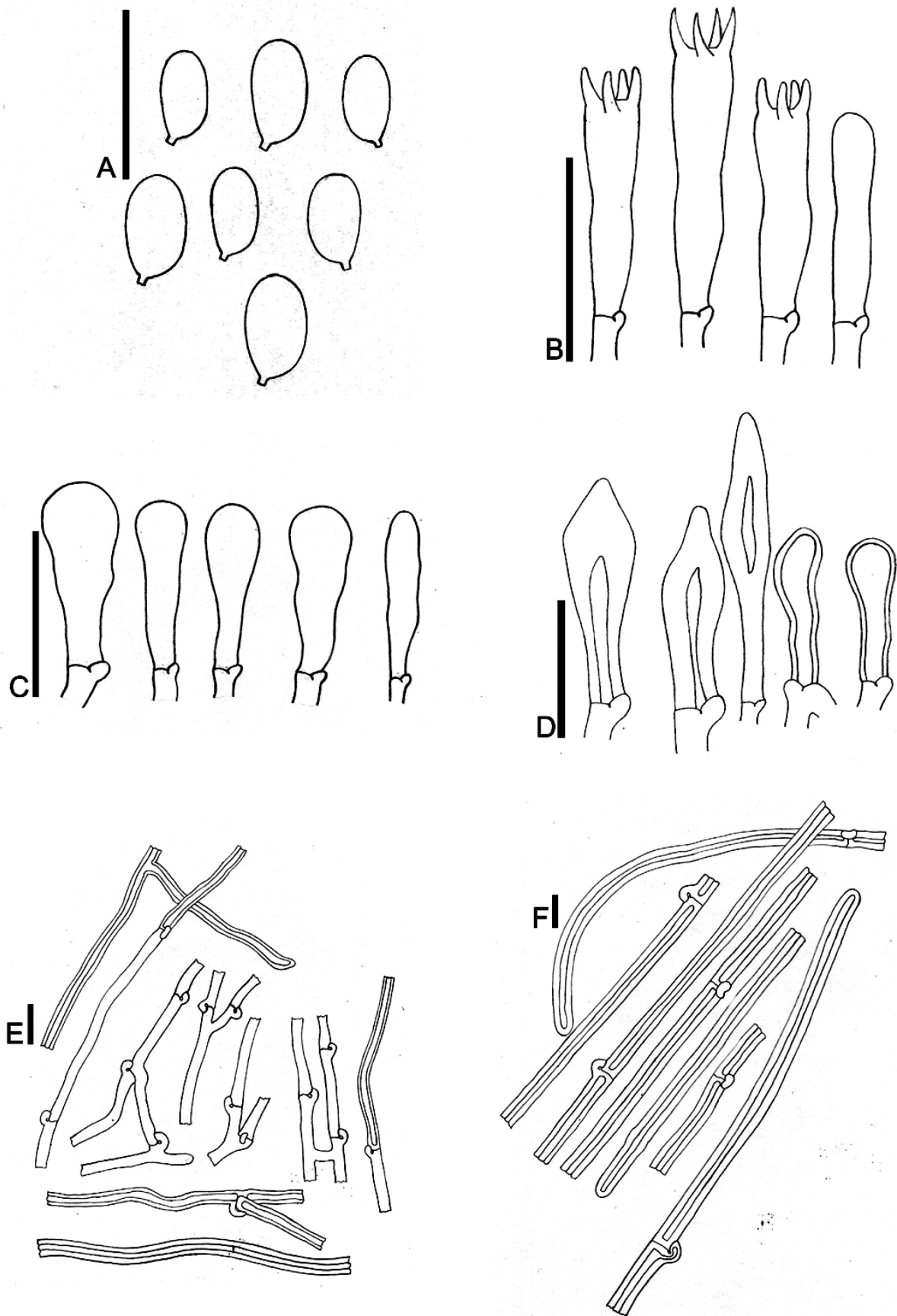


FIGURE 10. *Panus similis* a. Basidiospores, b. Basidia, c. Cheilocystidia, d. Sclerocystidia, e. Generative hyphae and Skeletal Hyphae, f. Hairs on pileus. Scale bars: b, c, d = 20 μm ; a, e, f = 10 μm



FIGURE 11. Basidiocarps of *Panus similis* (HKAS 121668) in the field.

Species prediction: host-fungi ratio, insect-fungi ratio?

Predicting species number in the Kingdom Fungi is a topic of considerable controversy. Some studies predicted global species number (e.g., Blackwell 2011, Tedersoo *et al.* 2014, Hawksworth & Lücking 2017), while some studies predicted the species number in particular geographical regions (e.g., Crous *et al.* 2006 in South Africa; Dai & Zhuang 2010 in China). However, different studies have used different techniques for calculation, leading to divergent species numbers. The most recent study carried out using high-throughput sequencing revealed 6.28 million fungal species globally (Baldrian *et al.* 2021).

Hawksworth (1991, 2001) assumed the plant:fungi ratio as 1:6; thus, based on this ratio, Feng & Yang (2018) predicted 104,000 fungal species should be present in Yunnan (number of vascular plants: 17,427 species *6 = ca. 104,000). However, Hawksworth & Lücking (2017) regarded 1:8 as a more accurate plant:fungi ratio, raising the estimation of fungal species to 139,416. However, it is also very important to consider the insect:fungi ratio. Environmental sequencing enhances species number as expected in Tedersoo *et al.* (2014). Nevertheless, Lücking & Hawksworth (2018) mentioned that these ratios might underestimate when tropical regions are taken in to account. Hyde *et al.* (2018) has also suggested ‘that a large proportion of new species awaits discovery and possibly lie in tropical regions such as Thailand’. Hence, it is extremely likely that many more fungal species exist in Yungui Plateau awaiting discovery and description.

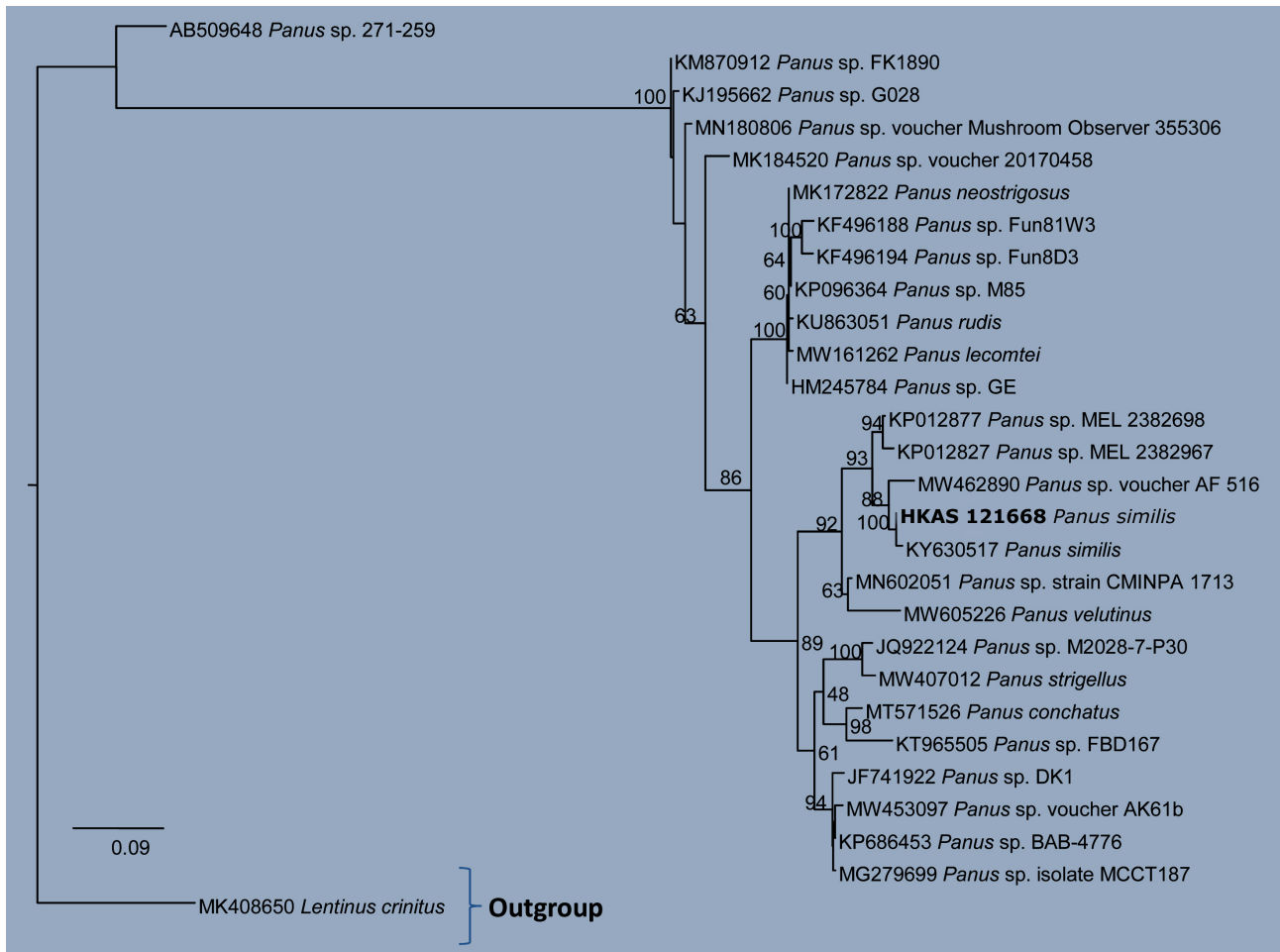


FIGURE 12. Phylogram of *Panus* generated from Maximum likelihood analysis of ITS sequence data. *Lentinus crinitus* (MK408650) was selected as the outgroup taxon. Maximum likelihood bootstrap values greater than 60% are indicated above the nodes. The new record *Panus similis* (HKAS 121668) is in black bold.

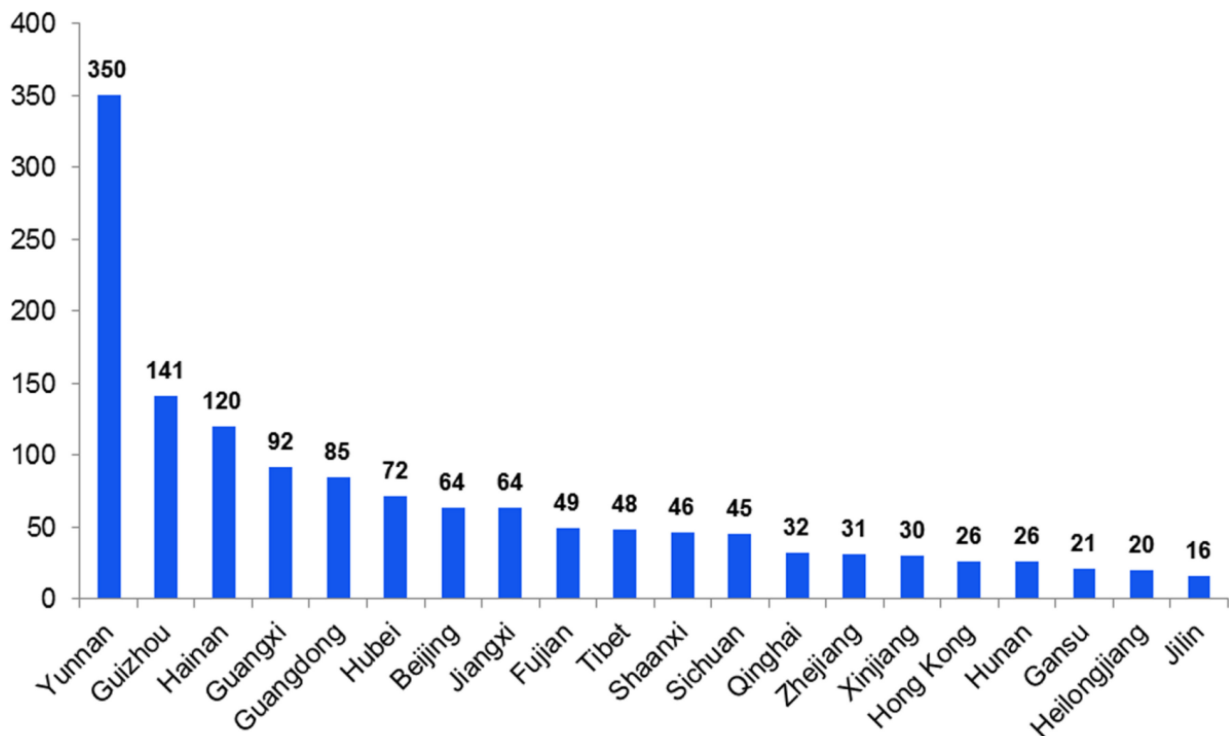


FIGURE 13. Number of species introduced from different provinces in China over the last decade (2010–2020).

Future work

It is necessary to recognize important recent changes in mycology, since outdated techniques, methodologies and literature have resulted in inaccurate calculations and conclusions. Changes in nomenclature (Hawksworth 2012; May *et al.* 2019; Wijayawardene *et al.* 2021), fungal barcoding (Schoch *et al.* 2012), genes for precise pathogenic species identification and environmental sequencing to identify unculturable taxa (Wu *et al.* 2019) are some important landmark developments in the last decade and comprise the foundational bedrock for future mycological work. Active mycology research groups in Yungui Plateau are recognized as 1) those who work on only macro fungi (i.e., mushrooms, other macrofungi and mushroom domestication); 2) those who work only on micro fungi; and 3) those who work on both micro and macro fungi. In the future, collaboration between these groups and overseas institutions will be essential to overcome limitations in data and funding.

We list potential research areas below that could be explored in the future.

Micro fungi

1. Looking for species in less-studied life modes, e.g., lichenicolous, species on rocks and karst regions (including artefacts).
2. Epitypification of old species on the Yungui Plateau.
3. Resolving species complexes of important phytopathogens using barcoding.
4. Screening for secondary metabolites of endophytic species.
5. Genomic studies of agriculturally and industrially important species.

Mushrooms

1. Domestication of new wild edible and medicinal mushrooms.
2. Checklists and guidebooks of edible, medicinal and poisonous mushrooms.

Acknowledgments

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<https://doi.org/10.11646/PHYTOTAXA.253.3.1>

Supplementary Table 1. Sources of isolates and GenBank accession numbers for the genus *Tolypocladium* and *Mucispora*.

Name	Voucher	GenBank Accession no.			References
		SSU	ITS	LSU	
<i>Adelosphaeria catenata</i>	CBS 138679	KT278692	KT278707	KT278707	Réblová <i>et al.</i> (2016)
<i>Ascotaiwania fusiformis</i>	MFLUCC 15-0621	KX550898	KX550893	KX550893	Yang <i>et al.</i> (2016)
<i>A. fusiformis</i>	MFLUCC 15-0625	KX550898	KX550894	KX550894	Yang <i>et al.</i> (2016)
<i>A. mitriformis</i>	HKUCC 3706	NA	AF132324	AF132324	Ranghoo <i>et al.</i> (1999)
<i>A. sawadae</i>	SS 00051	HQ446283	HQ446363	HQ446363	Boonyuen <i>et al.</i> (2011)
<i>Bactrodesmiastrum monilioides</i>	FMR 10756	NA	KF771879	KF771879	Hernández-Restrepo <i>et al.</i> (2015)
<i>B. obovatum</i>	FMR 6482	NA	FR870266	FR870266	Hernández-Restrepo <i>et al.</i> (2013)
<i>B. pyriforme</i>	FMR 10747	NA	FR870265	FR870265	Hernández-Restrepo <i>et al.</i> (2013)
<i>B. pyriforme</i>	FMR 11931	NA	HE646637	HE646637	Hernández-Restrepo <i>et al.</i> (2013)
<i>Brachysporiella setosa</i>	HKUCC 3713	NA	AF132334	AF132334	Ranghoo <i>et al.</i> (1999)
<i>Canalisporium exiguum</i>	SS 00809	GQ390266	GQ390281	GQ390281	Sri-indrasudhi <i>et al.</i> (2010)
<i>C. grenadoideum</i>	BCC 20507	GQ390252	GQ390267	GQ390267	Sri-indrasudhi <i>et al.</i> (2010)
<i>C. pulchrum</i>	SS 03982	GQ390262	GQ390277	GQ390277	Sri-indrasudhi <i>et al.</i> (2010)
<i>Conioscypha lignicola</i>	CBS 335.93	JQ437439	AY484513	AY484513	Réblová & Seifert (2004)
<i>C. minutispora</i>	FMR 11245	HF937347	KF924559	KF924559	Crous <i>et al.</i> (2014)
<i>C. peruviana</i>	ILL 41202	NA	KF781539	KF781539	Zelski <i>et al.</i> (2015)
<i>C. varia</i>	CBS 113653	AY484511	AY484512	AY484512	Réblová & Seifert (2004)
<i>Fuscosporella aquatica</i>	MFLUCC 16-0859	NG_062433	MG388209	MG388209	This study
<i>F. pyriformis</i>	MFLUCC 16-0570	KX550900	KX550896	KX550896	Yang <i>et al.</i> (2016)
<i>Helicoön farinosum</i>	DAOM 241947	NA	JQ429230	JQ429230	Réblová <i>et al.</i> (2012)
<i>Leotia lubrica</i>	AFTOL-ID 1	AY544746	AY544644	AY544644	Lutzoni <i>et al.</i> (2004)
<i>Melanotrigonum ovale</i>	CBS 138742	KT278695	KT278708	KT278708	Réblová <i>et al.</i> (2016)
<i>M. ovale</i>	CBS 138743	KT278696	KT278709	KT278709	Réblová <i>et al.</i> (2016)
<i>Microglossum rufum</i>	AFTOL-ID 1292	DQ471033	DQ470981	DQ470981	Spatafora <i>et al.</i> (2006)
<i>Mucispora hydei</i>	GMB0028	MW800164	MW797039	MW797122	This study
<i>M. infundibulata</i>	MFLU 18-0142	NG_073505	MH457139	MH457139	Hyde <i>et al.</i> (2020)
<i>M. obscuriseptata</i>	MFLUCC 15-0618	KX550897	KX550892	KX550892	Yang <i>et al.</i> (2016)
<i>M. phangngaensis</i>	MFLUCC 16-0865	MG388207	MG388210	MG388210	This study
<i>Parafuscosporella aquatica</i>	MFLU 19-0550	NA	MN512343	MN512343	Yang <i>et al.</i> (2020)
<i>P. garethii</i>	FF00725.01	KX958428	KX958430	KX958430	Boonyuen <i>et al.</i> (2016)
<i>P. pyriformis</i>	MFLU 19-0525	NA	MN512339	MN512339	Yang <i>et al.</i> (2020)
<i>P. moniliformis</i>	MFLUCC 15-0626	KX550899	KX550895	KX550895	Yang <i>et al.</i> (2016)
<i>P. mucosa</i>	MFLUCC 16-0571	MG388208	MG388211	MG388211	This study
<i>Phaeoisaria fasciculata</i>	CBS 127885	KT278693	KT278705	KT278705	Réblová <i>et al.</i> (2016)
<i>P. fasciculata</i>	DAOM 230055	KT278694	KT278706	KT278706	Réblová <i>et al.</i> (2016)
<i>P. microspora</i>	MFLUCC 16-0033	NA	MF167351	MF167351	Hyde <i>et al.</i> (2017)
<i>P. sedimenticola</i>	CGMCC 3.14949	NA	JQ031561	JQ031561	Cheng <i>et al.</i> (2014)
<i>Phragmocephala stemphylioides</i>	DAOM 673211	NA	KT278717	KT278717	Réblová <i>et al.</i> (2016)
<i>Plagiascoma frondosum</i>	CBS 139031	KT278701	KT278713	KT278713	Réblová <i>et al.</i> (2016)
<i>Pleurotheciella centenaria</i>	DAOM 229631	JQ429246	JQ429234	JQ429234	Réblová <i>et al.</i> (2012)
<i>P. rivularia</i>	CBS 125238	JQ429244	JQ429232	JQ429232	Réblová <i>et al.</i> (2012)
<i>P. rivularia</i>	CBS 125237	JQ429245	JQ429233	JQ429233	Réblová <i>et al.</i> (2012)
<i>P. uniseptata</i>	DAOM 673210	NA	KT278716	KT278716	Réblová <i>et al.</i> (2012)

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Supplementary Table 1. (Continued)

Name	Voucher	GenBank Accession no.			References
		SSU	ITS	LSU	
<i>Pleurothecium floriforme</i>	MFLUCC 15-0628	KY697279	KY697277	KY697277	Hyde <i>et al.</i> (2017)
<i>P. recurvatum</i>	CBS 131272	JQ429251	JQ429237	JQ429237	Réblová <i>et al.</i> (2012)
<i>P. recurvatum</i>	CBS 138747	KT278703	KT278714	KT278714	Réblová <i>et al.</i> (2016)
<i>P. semifecundum</i>	CBS 131271	JQ429254	JQ429240	JQ429240	Réblová <i>et al.</i> (2012)
<i>P. semifecundum</i>	CBS 131482	JQ429253	JQ429239	JQ429239	Réblová <i>et al.</i> (2012)
<i>Pseudoascotaiwania personii</i>	A57-14C	NA	AY094190	AY094190	Campbell & Shearer (2004)
<i>P. personii</i>	A57-14C	NA	AY590295	AY590295	Campbell & Shearer (2004)
<i>Purpureocillium lilacinum</i>	CBS 284.36	AY489689	NR_111432	NA	Luangsa-Ard <i>et al.</i> (2004)
<i>Savoryella longispora</i>	SAT 00322	HQ446302	HQ446380	HQ446380	Boonyuen <i>et al.</i> (2011)
<i>S. paucispora</i>	SAT 00866	HQ446303	HQ446381	HQ446381	Boonyuen <i>et al.</i> (2011)
<i>S. verrucosa</i>	SS 00052	HQ446296	HQ446374	HQ446374	Boonyuen <i>et al.</i> (2011)
<i>Sterigmatobotrys macrocarpa</i>	PRM 915682	NA	GU017317	GU017317	Réblová and Seifert (2011), Réblová <i>et al.</i> (2012)
<i>S. macrocarpa</i>	DAOM 230059 = CBS 113468	NA	GU017316	GU017316	Réblová & Seifert (2011), Réblová <i>et al.</i> (2012)
<i>Taeniolella rudis</i>	DAOM 229838	JQ429256	JQ429241	JQ429241	Réblová <i>et al.</i> (2012)
<i>Tolypocladium album</i>	CBS 869.73	KF747309	NR_155018	NA	Gazis <i>et al.</i> (2014)
<i>T. album</i>	CBS 393.89	NA	MH862176	MH873866	Vu <i>et al.</i> (2019)
<i>T. album</i>	CBS 968.73B	KF747314	MH860832	MH872567	Vu <i>et al.</i> (2019)
<i>T. album</i>	CBS 830.73	NG_065021	MH860811	MH872543	Vu <i>et al.</i> (2019)
<i>T. album</i>	GB5123	NA	AF389191	AF245296	Bills <i>et al.</i> (2002)
<i>T. album</i>	GB5502	AY489689	AF389192	AF245297	Bills <i>et al.</i> (2002)
<i>T. amazonense</i>	LA100	MW798784	HQ022485	KF747129	Gazis <i>et al.</i> (2014)
<i>T. amazonense</i>	LA108	MW798785	HQ022486	KF747130	Gazis <i>et al.</i> (2014)
<i>T. amazonense</i>	MS308	NA	JQ905653	KF747134	Gazis <i>et al.</i> (2014)
<i>T. amazonense</i>	BPI 892889	NA	NA	NA	Gazis <i>et al.</i> (2014)
<i>T. capitatum</i>	FLAS-F-60359	NA	MF074845	NA	Montalva <i>et al.</i> (2019)
<i>T. capitatum</i>	OSC 71233	AF049153	NA	AY489721	Quandt <i>et al.</i> (2014)
<i>T. cucullae</i>	HKAS 55588	NA	MW798788	MW798786	This study
<i>T. cucullae</i>	GZU A-77	NA	MW798789	MW798787	This study
<i>T. cylindrosporium</i>	ARSEF 2920	KF747323	MG228381	NA	Montalva <i>et al.</i> (2019)
<i>T. cylindrosporium</i>	IP 425	KF747321	MG228380	NA	Montalva <i>et al.</i> (2019)
<i>T. cylindrosporium</i>	IP 419	DQ522545	MG228379	NA	Montalva <i>et al.</i> (2019)
<i>T. cylindrosporium</i>	NRRL 28025	NA	NA	AF049173	Quandt <i>et al.</i> (2014)
<i>T. dujiaolongae</i>	RCEF6201	NA	KF696558	NA	Li <i>et al.</i> (2018)
<i>T. dujiaolongae</i>	ZBAH632	NA	KF696557	NA	Li <i>et al.</i> (2018)
<i>T. endophyticum</i>	MX575	NA	JX155949	KF747155	Gazis <i>et al.</i> (2014)
<i>T. endophyticum</i>	MX486	NA	KF747245	KF747152	Gazis <i>et al.</i> (2014)
<i>T. fractum</i>	OSC 110990	AB027322	NA	DQ518759	Sung <i>et al.</i> (2007)
<i>T. fumosum</i>	WA18945	EF469124	KU925171	KU985053	Crous <i>et al.</i> (2017)
<i>T. geodes</i>	ARSEF 2684	NA	FJ973059	NA	Ghikas <i>et al.</i> (2010)
<i>T. geodes</i>	CBS 723.70	NA	NR_164431	NA	Vu <i>et al.</i> (2019)
<i>T. guangdongensis</i>	GDGM 24020	NA	EU039881	NA	Ke & Ju (2015)
<i>T. inegoense</i>	SU-15	NA	NA	DQ118741	Chaverri <i>et al.</i> (2005)
<i>T. inegoense</i>		DQ522547	NA	AB027368	Nikoh & Fukatsu (2000)
<i>T. inflatum</i>	OSC 71235	NA	JN049844	EF469077	Sung <i>et al.</i> (2007)
<i>T. inflatum</i>	NBRC 31668	AB027320	AB103381	NA	Yokoyama <i>et al.</i> (2004)

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Supplementary Table 1. (Continued)

Name	Voucher	GenBank Accession no.			References
		SSU	ITS	LSU	
<i>T. inflatum</i>	CBS 714.70	AB027319	MH859916	MH871710	Vu <i>et al.</i> (2019)
<i>T. inflatum</i>	CBS 127302	NA	MH864514	MH875949	Vu <i>et al.</i> (2019)
<i>T. inflatum</i>	CBS 127147	NA	MH864439	MH875880	Vu <i>et al.</i> (2019)
<i>T. japonicum</i>	OSC 110991	NA	JN049824	DQ518761	Kepler <i>et al.</i> (2012)
<i>T. japonicum</i>	BCRC FU30561	NA	KT873533	NA	Ke & Ju (2015)
<i>T. japonicum</i>	IFO 9647	NA	AB027366	NA	Nikoh & Fukatsu (2000)
<i>T. jezoense</i>		NA	NA	AB027365	Nikoh & Fukatsu (2000)
<i>T. longisegmentatum</i>	OSC 110992	NA	NA	EF468816	Sung <i>et al.</i> (2007)
<i>T. longisegmentatum</i>	2731.S	AY489691	AJ786568	NA	Stensrud <i>et al.</i> (2005)
<i>T. longisegmentatum</i>	MHHNU 8699	KJ878910	MK253762	NA	Chen & Zhang (2019)
<i>T. longisegmentum</i>	HMJAU6903	NA	KJ866879	NA	Yan & Tolgor (2014)
<i>T. nubicola</i>	ARSEF 3434	NA	FJ973067	NA	Ghikas <i>et al.</i> (2010)
<i>T. nubicola</i>	CBS 944.72	NA	NA	MH878304	Vu <i>et al.</i> (2019)
<i>T. nubicola</i>	CBS 568.84	NA	NA	MH873478	Vu <i>et al.</i> (2019)
<i>T. ophioglossoides</i>	OSC 106405	JN941730	NA	AY489723	Castlebury <i>et al.</i> (2004)
<i>T. ophioglossoides</i>	CBS 100239	AB027323	KU382155	KJ878874	Quandt <i>et al.</i> (2014)
<i>T. ovalisporum</i>	CBS 700.92	NA	NR_155019	NA	Unpublished
<i>T. paradoxum</i>	JFL14081002	MF5368LR	KX017278	NA	Zha <i>et al.</i> (2018)
<i>T. paradoxum</i>	HKAS 87772	NA	KX017279	NA	Zha <i>et al.</i> (2018)
<i>T. paradoxum</i>	HMG 20938	NA	DQ901630	NA	Tian <i>et al.</i> (2010)
<i>T. paradoxum</i>	NBRC 106958	NA	JN943324	JN941411	Schoch <i>et al.</i> (2012)
<i>T. paradoxum</i>	MX338	KF747318	AB027369	AB027369	Nikoh & Fukatsu (2000)
<i>T. pustulatum</i>	MRL GB6597	KF747303	NA	AF389190	Bills <i>et al.</i> (2002)
<i>T. pustulatum</i>	MRL MF5368LR	NA	MF5368LR	AF373282	Bills <i>et al.</i> (2002)
<i>T. pustulatum</i>	KaP8.2.2.1	NA	KP698195	NA	Arhipova <i>et al.</i> (2015)
<i>T. tropicale</i>	MX337	NA	JQ905660	KF747148	Gazis <i>et al.</i> (2014)
<i>T. tropicale</i>	IQ214	NA	KF747254	NA	Gazis <i>et al.</i> (2014)
<i>T. tropicale</i>	MX338	NG_061025	KF747259	KF747149	Gazis <i>et al.</i> (2014)
<i>T. tropicale</i>	IQ136	KF747309	NA	KF747121	Gazis <i>et al.</i> (2014)
<i>T. tropicale</i>	CBS 136897	NA	NR_159005	NA	Gazis <i>et al.</i> (2014)
<i>T. tundrense</i>	ARSEF 3400	KF747314	FJ973069	NA	Ghikas <i>et al.</i> (2010)
<i>T. tundrense</i>	CBS 569.84	NG_065021	MH861781	MH873479	Vu <i>et al.</i> (2019)
<i>T. varium</i>	CBS 429.94	NA	MH862472	MH874122	Vu <i>et al.</i> (2019)

NA: Sequences not available.