Taxonomy and secondary metabolites of wood-associated fungi

edited by

Nalin Wijayawardene, Samantha Karunarathna,
Xin-Lei Fan, Qi-Rui Li

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Table of contents

1 Typification of the name *Arthopyrenia parolinii* Beltr. (Ascomycota, Dothideomycetes, Pleosporales, Arthopyreniaceae)
Deborah Isocrono, Sonia Ravera

9 Exploring ascomycete diversity in Yunnan II: Introducing three novel species in the suborder Massarineae (Dothideomycetes, Pleosporales) from fern and grasses
Rungtiwa Phookamsak, Sinang Hongsanan, Darbhe Jayarama Bhat, Dhanushka N. Wanasinghe, Itthayakorn Promputtha, Nakarin Suwannarach, Jaturong Kumla, Ning Xie, Turki M. Dawoud, Peter E. Mortimer, Jianchu Xu, Saisamorn Lumyong

51 Morphology and phylogeny of *Cytospora* (Cytosporaceae, Diaporthales) species associated with plant cankers in Tibet, China
Jiangrong Li, Jieting Li, Ning Jiang

71 Three new species of *Favolaschia* (Mycenaceae, Agaricales) from South China
Qiu-Yue Zhang, Hong-Gao Liu, Wan-Ying Li, Xin Zhang, Yu-Cheng Dai, Fang Wu, Lu-Sen Bian

91 New species, new records and common species of *Pluteus* sect. *Celluloderma* from northern China
Zheng-Xiang Qi, Ke-Qing Qian, Lei Yue, Li-Bo Wang, Di-Zhe Guo, Dong-Mei Wu, Neng Gao, Bo Zhang, Yu Li

113 *Paramphibambusa bambusicola* gen. et. sp. nov., *Arecophila xishuangbannaensis* and *A. zhaotongensis* spp. nov. in Cainiaceae from Yunnan, China
Li-Su Han, Nalin N. Wijayawardene, Chao Liu, Li-Hong Han, Itthayakorn Promputtha, Qiang Li, Abdallah M. Elgorban, Salim Al-Rejaie, Kazuaki Tanaka, Dong-Qin Dai

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Research Article

Typification of the name *Arthopyrenia parolinii* Beltr. (Ascomycota, Dothideomycetes, Pleosporales, Arthopyreniaceae)

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Abstract

Arthopyrenia parolinii Beltr. is one of the few species of the lichen genus *Arthopyrenia* A. Massal. described by Italian authors of the XIX century, lacking type formal association. In this regard, the name *Arthopyrenia parolinii* is hereby lectotypified using a specimen stored in the lichen herbarium of A.B. Massalongo at VER. Additional original material was found only at M, while another specimen at MSNVE, labelled as *Spermatodium parolinii*, although referable to this species, should not be considered as original material. *Arthopyrenia parolinii* is among the least well-known species in the genus. Given the genus *Arthopyrenia* is still very poorly known, it is important to clarify the original material of the species and propose the lectotyping. The selected lectotype is the only sample among the analyzed ones reporting complete data on the locus classicus; it conforms to the characters described in the protologue and comes from the Herbarium Beltramini.

Key words: A.B. Massalongo Herbarium, Beltramini, lectotype, lichen, nomenclature

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Introduction

The lichen genera *Arthopyrenia* A. Massal. and *Naetrocymbe* Körb. both include poorly understood non-lichenized and lichenized fungi (Isocrono et al. 2021). Despite their wide distribution, species belonging to these genera are often overlooked and pose taxonomic challenges (Hongsanan et al. 2020; Thiyagaraja et al. 2021).

As part of the research carried out by the authors focusing on the occurrence of *Arthopyrenia* and *Naetrocymbe* in Italy (Ravera 2006, 2014; Puntillo and Ravera 2013; Ravera and Isocrono 2021; Isocrono and Ravera 2022) we aimed to establish the identity of *Arthopyrenia parolinii* Beltr., a neglected species not yet typified. *A. parolinii*, such as several other multiseptated species described by Italian authors of the XIX Century –e.g., *A. cembricola* (Anzi) Lettau, *A. cinerescens* A. Massal., *A. molinii* Beltr., etc.– still await a critical study. Given the challenges relating to the genus *Arthopyrenia* which according to Wijayawardene et al. (2022) includes 5 + approximately 100 orphaned species - i.e. species that have been named and formally described, but have not been updated and reassessed following a revision of the genus - it is crucial to analyze and clarify the poorly known original material of such species and propose the lectotyping.

We have therefore examined several historical lichen samples probably attributable to *Arthopyrenia parolinii* Beltr. Among the checked exsiccates, the sample of *A. parolinii* stored in the Abramo Bartolomeo Massalongo lichen herbarium in VER, revealed that it originates from the Francesco Beltramini de' Casati collection and it was collected in the location reported in the protologue, i.e. locus classicus.

Francesco Beltramini de' Casati's (1828–1903) primary botanical interest lay in lichens. In this field, he published a richly illustrated work on the lichen flora of Bassano del Grappa, Vicenza, Italy (Beltramini de' Casati 1858). Massalongo, his friend and mentor, is honored in this work.

The possibility that Beltramini's lichenological collections may have merged into the renowned lichenologist's herbarium is plausible because of Massalongo's well-known friendship with his fellow countryman and student Beltramini.

Methods

This study is based on: i) analysis of the protologue, ii) pinpointing the location of the locus classicus, iii) search for the original material, iv) examination of specimens in M (Botanische Staatssammlung München), MSNVE (Lichenotheca Veneta by Vittore Trevisan kept at Natural History Museum of Venice Giancarlo Ligabue), and VER (Herbarium of A.B. Massalongo at the Civic Natural History Museum of Verona). High-resolution digital images from MSNVE and M were also consulted.

Macroscopic and microscopic characteristics were observed in dried specimens with a Zeiss dissecting microscope equipped with a Leica camera. Microscopic characters were examined from hand-cut sections and squashes mounted in a 5% KOH solution from dried specimens with a Zeiss Axioscope optical microscope equipped with AxioCam MRc camera (Zeiss, Welwyn Garden City, UK).

Typification follows the International Code of Nomenclature for algae, fungi and plants (Turland et al. 2018).

Results and discussion

Original material of *Arthopyrenia parolinii*

In the protologue of *Arthopyrenia parolinii*, Beltramini provides a diagnosis, a more detailed description, and some drawings showing perithecium, asci, and multiseptated spores. Particularly, the description reports:

"Thallo primum hypophlaeodico, tandem epiphlaeodico, arachnoideo effuso, cinereo-fusco. Apotheciis minutis, creberrimis, subemerso-sessilibus, hemisphaerico-conoideis, atris. Ascis clavato-ventricosis, apice rotundato-truncatis, basi in petiolum rudem attenuatis, octosporis, absque paraphysibus; sporidiis ovoideo-elongatis, basi subclavatis, 6–8-locularibus, diametro quadruplo vel quintuplo longioribus" (Beltramini de' Casati 1858).

According to Beltramini's explanation in the paper, the epithet is in honor of the "Cavaliere Nob. Alberto Parolini" (1788–1867) a renowned Italian naturalist known for his rich botanical garden named Parolini Garden at Bassano del Grappa, and for his donation of a sizable collection of natural history objects to the local civic museum.

The collecting site of the sample is mentioned as "le tiglie nel passeggio di Belvedere in Bassano" [on Tilia along Belvedere stroll in Bassano].

The sample stored in the Lichenological Herbarium of A.B. Massalongo in Verona (Fig. 1A, B) is made up of a piece of bark glued to the herbarium sheet. The label reports the location "Tilia di Bassano" [Tilia of Bassano del Grappa] and the note "Herb. Beltramini". The two sentences display different handwriting: a calligraphic comparison made with original material preserved in the Library of University of Padua (see https://phaidra.cab.unipd.it/) allowed us to identify the locality, in red ink, as written by Beltramini while the name of the species and the herbarium attribution as written by Massalongo.

We also found a second undated collection of *Arthopyrenia parolinii* in Staatliche Naturwissenschaftliche Sammlungen Bayerns Herbarium (M). This sample –M-0207340– shows the same set-up and comes from Ferdinand Christian Gustav Arnold personal herbarium.

On the sheet (Fig. 2), some notes are reported: the name of the species accredited to Beltramini, the note "nov. spec ?", the substratum "ad Tiliae truncos", the location "Pr Vicez." [Province of Vicenza], the herbarium from which the sample was taken "herb. Massalongo". All these notes appear to be written by Massalongo.

Consequently, among the few original materials that currently exist, we designate the specimen stored in VER as lectotype, as this is the most complete, and informative, and in line with the protologue.

Description of the lectotype

Thallus: epiphloeodal, dark gray, thin, non-lichenized (Fig. 1B). **Ascomata:** perithecial 0.15–0.2 mm, black, subglobose, ± circular, numerous, scattered, superficial, many with depressed ostiole; ascomatal wall of textura intricata, black, not continuous below the hamathecium; involucrellum reddish brown, clypeate, amorphous pigment localized in the cell wall; excipulum colourless, scarcely discernible; the wall pigment remains brown in K (Fig. 3A, B). **Hamathecium:** moniliform pseudoparaphyses dissolving and leaving only fragments embedded in gel; periphysoids not present; asci: 60–70 × 24–27 µm, cylindric-clavate, bitunicate with a distinct apical region lacking a nasse, dehiscence typically fissitunicate. **Ascospores:** 21–22 × 4–5 µm, 8 per ascus, irregularly arranged, colourless, clavate with rounded apices, 5–7-septate, slightly constricted at the septum; perispore indistinct. **Pycnidia:** not observed. **Chemistry:** spot tests negative.

Notes

Studies on *Arthopyrenia* species with 5–7 septate spores in Europe are still lacking. Several species formally named and described by Beltramini (e.g. *Arthopyrenia molinii* Beltr.) (Isocrono and Ravera 2022) and Massalongo have not been updated or re-evaluated in recent studies. The main reason is the difficulty of working with material dating back to the 19th century, for which there are an extremely limited number of samples, which are often difficult to locate and obtain in loan, and in many cases these species are only known from the locus classicus (Nimis et al. 2018).

Among current and known species, *Arthopyrenia grisea* (Schaer.) Körb. and *Arthopyrenia platypyrenia* (Nyl.) Arnold are the most similar to *A. parolinii*.

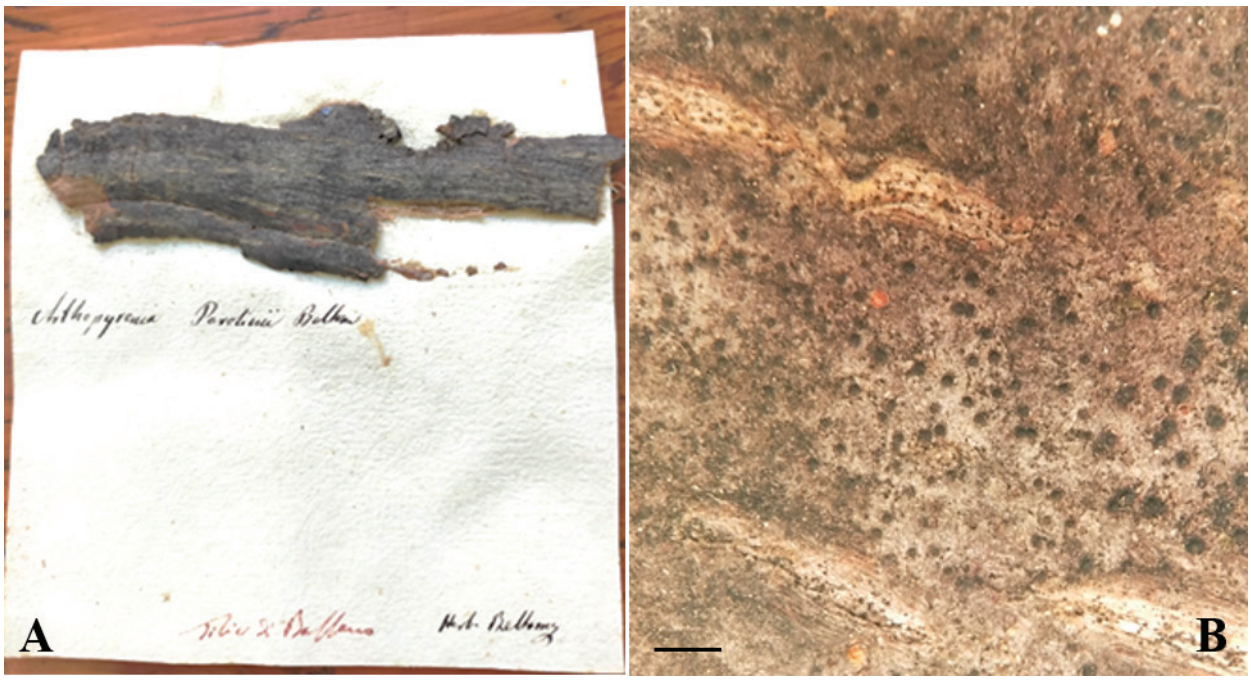

Figure 1. Exsiccata of *Arthopyrenia parolinii* Beltramini from the lichen herbarium of A.B. Massalongo in VER **A** fragment of linden bark, colonized by the lichen and glued to the herbarium sheet with the name of the species and note "Herb. Beltramini" written by Massalongo in black ink, and the locality of collection, in red ink, written by Beltramini **B** detail of thallus and perithecia in surface view. Scale bar: 1 mm.

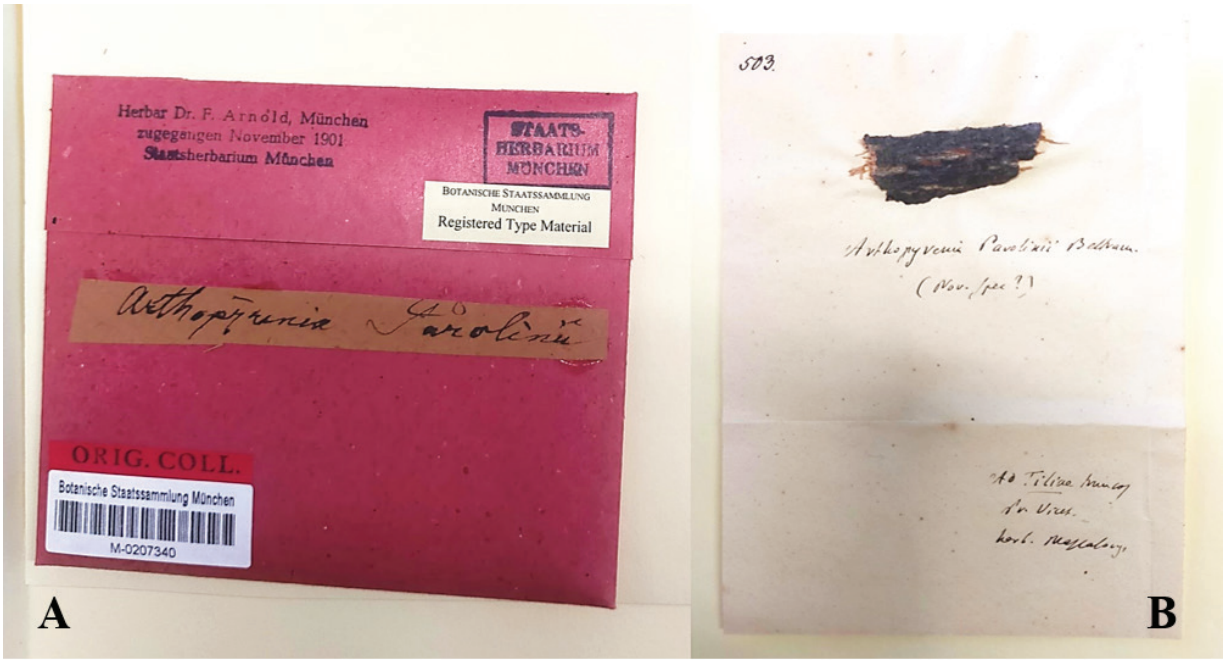

Figure 2. Exsiccata of *Arthopyrenia parolinii* Beltramini from M Lichen herbarium (M-0207340) registered as "Type Material" **A** the original label from Arnold personal herbarium **B** a fragment of linden bark, colonized by the lichen, glued to the herbarium sheet with the name of the species accredited to Beltramini and a few notes written by Massalongo.

According to Foucard (1992) *A. grisea* seems to be the most proper name for some varieties of *A. personii* Massal. with moniliform pseudoparaphyses, obpyriform asci and (3-)5(-6) septate clavate spores. The main distinction between *A. parolinii* and the varieties of *A. personii* synonymized with *A. grisea* (see e.g.

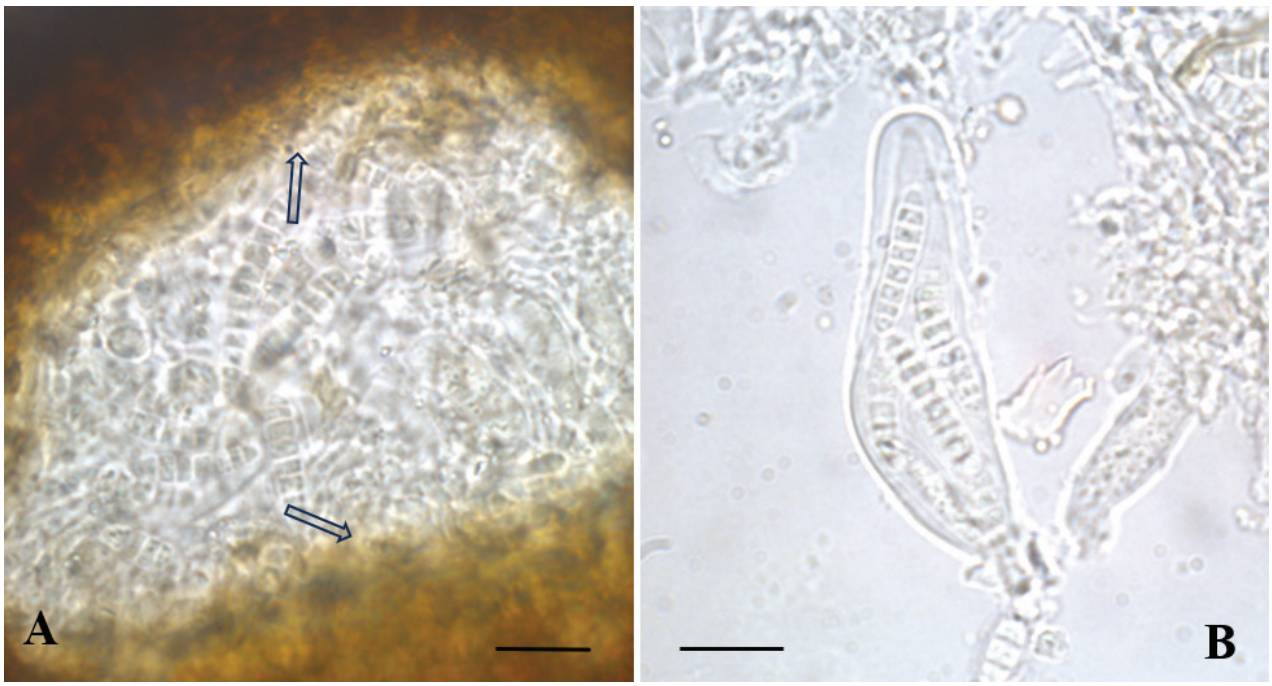

Figure 3. *Arthopyrenia parolinii* **A** vertical section through a perithecium, arrows showing hamathecium and K- excipulum **B** bitunicate asci and pluriseptate ascospores in 10% KOH. Scale bars: 15 µm.

Nimis 2023) is that *A. parolinii* permanently shows 5–7 septate spores. For its part, *A. platypyrenia*, is a rare but well-known species, usually collected on *Hedera helix* L. (Coppins and Orange 2009). It is characterized by a broad lateral ostiole and different ascus and spores. Particularly, spores are usually ellipsoidal to fusiform-ellipsoidal, 3- to 4- (to 7-) septate, constricted at all the septa, with a gelatinous sheath 2–3 µm thick and larger (24–30 × 8–10) than the ones of *A. parolinii*.

The locus classicus

In Bassano del Grappa, an Italian town located in the province of Vicenza, Italy, the avenue once known as Viale Belvedere is now named as Viale delle Fosse. This avenue was built in 1790 when the moats surrounding the Visconti walls were covered. After this work, a double row of linden trees (Fig. 4) and various statues were added to the avenue which, starting from the Porta delle Grazie, reached the Parolini Garden. This new tree-lined avenue was called "Passeggio pubblico di Belvedere o Fosse" [Public Walk of Belvedere or Fosse], the same name reported in the *A. parolinii* protologue.

220 of the original linden trees were removed for plant health reasons during World War I, and they were replaced by American elms, effectively preventing us from searching for current material on the original phorophytes (Bordignon 2016).

Samples of *Arthopyrenia parolinii*

Vittore Benedetto Antonio Trevisan transferred the epithet to *Spermatodium* Feé (Trevisan de St-Léon 1860) on the basis of the spores' characteristics. In 1869 Trevisan issued eight fascicles of his Lichenotheca Veneta. A single specimen of *Spermatodium parolinii* is included among these exsiccates deposited at MSNVE (MSNVE-24815) (see: Lichenotheca Veneta del Conte Vittore Trevisan 2023).

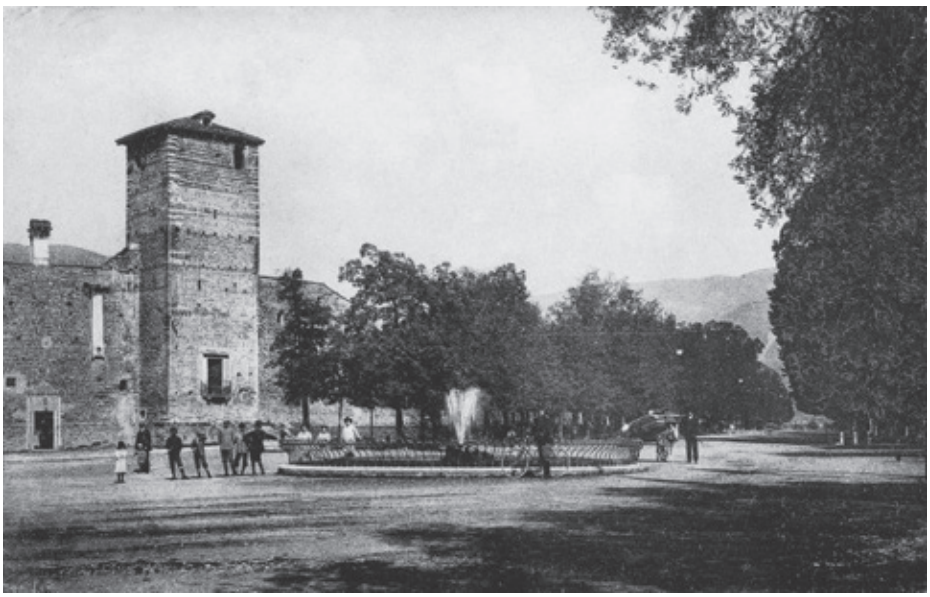

Figure 4. *Arthopyrenia parolinii* Beltr locus classicus (i.e. "Passeggio pubblico di Belvedere o Fosse") depicted in a 1917 postcard (from: Bordignon 2016). The image shows the original lime trees that are mentioned in the protologue of *A. parolinii* before their removal.

Considering the stormy relations between Trevisan and Massalongo (Nimis and Hawksworth 1994) and the absence of notes on the specimen – unlike other samples from Massalongo in MSNVE – it is conceivable that the collection was carried out by Trevisan himself and in our opinion it should be not original material.

Typification

Arthopyrenia parolinii Beltr., Lich. Bassan.: 239. 1858 ≡ *Spermatodium parolinii* (Beltr.) Trevis., Conspect. Verruc.: 11. 1860 ≡ *Santessoniolichen parolinii* (Beltr.) Tomas. & Cif., Arc. Bot. Ital.: 5. 1952 ≡ *Giacominia parolinii* Cif. & Tomas., Atti Ist. Bot. E Lab. Critt. Univ. Pavia: 256. 1954 – **Lectotype** (designated here): "*Arthopyrenia parolinii*", Herb. A.B. Massalongo (VER!). MycoBank No: 10015170

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We wish to thank Sebastiano Andreatta, curator of the herbarium VER, for his help and kind hospitality, Stefano Martellos for his support in locating samples, Raffaella Trabucco curator of the herbaria MSNVE and Andreas Beck, curator of Bryophytes and Lichens at M for the loan of samples used in this study.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Both authors equally contributed to the research, observation and selection of the source material, as well as to the conceptualization and drafting of the article.

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Data availability

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

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Research Article

Exploring ascomycete diversity in Yunnan II: Introducing three novel species in the suborder Massarineae (Dothideomycetes, Pleosporales) from fern and grasses

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Abstract

This article presents the results of an ongoing inventory of Ascomycota in Yunnan, China, carried out as part of the research project series "Exploring ascomycete diversity in Yunnan". From over 100 samples collected from diverse host substrates, microfungi have been isolated, identified and are currently being documented. The primary objective of this research is to promote the discovery of novel taxa and explore the ascomycete diversity in the region, utilising a morphology-phylogeny approach. This article represents the second series of species descriptions for the project and introduces three undocumented species found in the families Bambusicolaceae, Dictyosporiaceae and Periconiaceae, belonging to the suborder Massarineae (Pleosporales, Dothideomycetes). These novel taxa exhibit typical morphological characteristics of *Bambusicola*, *Periconia* and *Trichobotrys*, leading to their designation as *Bambusicola hongheensis*, *Periconia kunmingensis* and *Trichobotrys sinensis*. Comprehensive multigene phylogenetic analyses were conducted to validate the novelty of these species. The results revealed well-defined clades that are clearly distinct from other related species, providing robust support for their placement within their respective families. Notably, this study unveils the phylogenetic affinity of *Trichobotrys* within Dictyosporiaceae for the first time. Additionally, the synanamorphism for the genus *Trichobotrys* is also reported for the first time. Detailed descriptions, illustrations and updated phylogenies of the novel species are provided, and thus presenting a valuable resource for researchers and mycologists interested in the diversity of ascomycetes in Yunnan. By enhancing our understanding of the Ascomycota diversity in this region, this research contributes to the broader field of fungal taxonomy and their phylogenetic understanding.

Key words: Ascomycota, *Bambusicola*, *Periconia*, phylogeny, polyphasic approach, taxonomy, the Greater Mekong Subregion, *Trichobotrys*

Introduction

Pleosporales is the largest order of Dothideomycetes, comprising two main suborders (viz. Massarineae and Pleosporineae), 91 families, 653 genera (including Pleosporales genera *incertae sedis*) and a quarter of all Dothideomycetes species (Hongsanan et al. 2020; Wijayawardene et al. 2022b). The order was invalidly introduced by Luttrell (1955) and later validated by Barr (1987) and is characterised by perithecial ascomata with typically a papillate ostiole, bitunicate, fissitunicate asci and hyaline to pigmented, variedly shaped, mostly septate ascospores. The asexual morph is represented by both coelomycetes and hyphomycetes (Zhang et al. 2012; Hyde et al. 2013; Hongsanan et al. 2020). Members of Pleosporales are ecologically and morphologically diverse and also shown to be polyphyletic in various groups, as well as contained within species complexes still waiting to be resolved (Zhang et al. 2012; Hyde et al. 2013; Jaklitsch et al. 2016a; Hongsanan et al. 2020). Pleosporalean species are cosmopolitan and ubiquitous in diverse ecological niches. Their life modes include epiphytes, endophytes or parasites on living organisms, hyperparasites on fungi or insects, saprobes, pathogens and lichenised fungi (Zhang et al. 2012; Hyde et al. 2013; Tanaka et al. 2015; Jaklitsch et al. 2016a; Hongsanan et al. 2020). Of these, several genera, such as *Alternaria*, *Bipolaris*, *Didymella*, *Leptospharia*, *Parastagonospora*, *Phaeosphaeria* and *Pyrenophora*, have been reported as plant pathogens causing severe diseases on economic crops (Quaedvlieg et al. 2013; Woudenberg et al. 2013, 2014, 2015; Manamgoda et al. 2014; Ariyawansa et al. 2015a, b; Chen et al. 2015, 2017; Tanaka et al. 2015; El-Demerdash 2018; Khiralla et al. 2019; Bhunjun et al. 2020; Hongsanan et al. 2020; Backes et al. 2021; Bartosiak et al. 2021; Li et al. 2023).

A comprehensive study of the genera in Pleosporales was carried out by Zhang et al. (2012), based on morphological studies of the type specimens coupled with phylogenetic analyses. Consequently, the taxonomic treatment of numerous Pleosporales was updated by various authors, based on polyphasic taxonomic approaches, mainly using morphology-phylogeny-based taxonomy (Ariyawansa et al. 2014, 2015a, b; Phookamsak et al. 2014, 2015; Tanaka et al. 2015; Thambugala et al. 2015; Boonmee et al. 2016; Jaklitsch and Voglmayr 2016; Jaklitsch et al. 2016a, b, 2018; Su et al. 2016; Chen et al. 2017; Hashimoto et al. 2017; Wanasinghe et al. 2017a, b). Even though novel taxa of Pleosporales have been dramatically increasing over the last ten years after the taxonomic circumscription provided by Zhang et al. (2012) and Hyde et al. (2013), there is still over a quarter of the total known species lacking molecular data and/or reliable phylogenetic markers for clarifying the placements in Pleosporales.

Yunnan is known as one part of the 36 global biodiversity hotspots where over 17,000 species of vascular plants are known, including highly endemic species (Feng and Yang 2018; Cai et al. 2019). Highly diverse environments and geographical distribution, as well as flourishing vegetation, have shown the Province to be one of the richest sources of fungi, covering over 40% of the

known species in China (Feng and Yang 2018; Liu et al. 2018). Feng and Yang (2018) estimated a species number of fungi existing in Yunnan Province, based on the ratio of local vascular plants and fungi (1:6) following the suggestion of Hawksworth (2001). With this estimation, Yunnan may harbour over 104,000 fungal species; of which only 6000 described species have been reported from the Province, including approximately 3000 species of Ascomycota and Basidiomycota (Feng and Yang 2018).

Since Feng and Yang (2018) updated the status of fungal diversity in this region, the taxonomic study of ascomycetes has steadily increased and over 300 novel species have been discovered in the last five years (Luo et al. 2019; Phookamsak et al. 2019; Dong et al. 2020; Hyde et al. 2020a, b; Wanasinghe et al. 2020, 2022; Wang et al. 2020; Mortimer et al. 2021; Wijayawardene et al. 2021b, 2022a; Gu et al. 2022; Jiang et al. 2022; Yang et al. 2022a, b; Si et al. 2023). However, most studies were restricted to certain groups of ascomycetes, such as bambusicolous fungi (Jiang et al. 2019, 2021b; Dai et al. 2022; Phookamsak et al. 2022), cordycipitoid fungi (Wang et al. 2020; Fan et al. 2021; Dong et al. 2022; Tang et al. 2023), endolichenic fungi (Si et al. 2021, 2023), lignicolous freshwater fungi (Luo et al. 2018a, b, 2019; Su et al. 2018; Dong et al. 2020; Shen et al. 2022), nematode-trapping fungi (Zhang et al. 2020, 2022a, b, c, 2023; Yang et al. 2023b) and woody litter-inhabiting fungi (Mortimer et al. 2021; Wanasinghe et al. 2022), as well as fungi associated with specific host plants (e.g. *Camellia*, *Coffea*, *Magnolia*, *Mangifera* and *Rhododendron*) (Wanasinghe et al. 2020; Gu et al. 2022; Lu et al. 2022; Tibpromma et al. 2022; Wijayawardene et al. 2022a; Yang et al. 2022a, b, 2023a). Comparable with the total estimated number of species that may be found in this region, these fungal inventories are still only representing a small number of extant ascomycetes in Yunnan.

The present study aims to introduce three novel pleosporalean species from Yunnan, based on morphological characteristics and phylogenetic evidence coupled with the differences in nucleotide pairwise comparison amongst closely-related species.

Materials and methods

Sample collection, isolation, morphological examination and preservation

Samples were collected from Yunnan Province, China during 2016–2021 at three different collecting sites: Honghe (rice terraces), Kunming (botanical garden) and Xishuangbanna (secondary forest). Specimens were collected during the rainy (September) and dry seasons (January and April) and brought to the laboratory in sealed plastic Ziploc bags for further observation and examination. The samples were observed and axenic cultures, via single spore isolation, were obtained within 1–2 weeks after collection. Single spore isolation was performed using the spore suspension technique (Senanayake et al. 2020). Two sets (five spores per set) of the germinated spores were placed separately on to freshly sterilised potato dextrose agar (PDA) medium and incubated under normal day/night light conditions at room temperature (15–25 °C depending on the rainy and dry seasons). Culture characteristics, growth and sporulation *in vitro* were observed and recorded after one and four-week intervals.

Macro-morphological features, such as ascomata and fungal colonies visualised on host substrates, were observed using an Olympus SZ61 series stereomicroscope and photo-captured by a digital camera. Micro-morphological features were examined by differential interference contrast (DIC) microscopy using a Nikon ECLIPSE Ni-U compound microscope and images captured with a Nikon DS-Ri2 camera. The mucilaginous sheath that covered the ascospores was checked by staining with India Ink and the fungal centrum was stained using Congo red for checking the clearity of conidiophores and conidiogenous cells. Lactoglycerol was added to preserve important morphological features on permanent slides. All morphological features were measured using Tarosoft (R) Image FrameWork version 0.9.7. and photographic plates were edited and combined using Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA).

Axenic living cultures were preserved in PDA and sterilised double-distilled water (ddH_2O) at 4 °C for short-term storage and long-term glycerol storage at -20 °C and -80 °C, respectively. Ex-type living cultures were deposited at the collection of Rungtiwa Phookamsak housed at Honghe Center for Mountain Futures (RPC) and duplicated in the Culture Collection of the Herbarium of Cryptogams Kunming Institute of Botany, Academia Sinica (**KUNCC**), Kunming, China and Mae Fah Luang University Culture Collection (**MFLUCC**), Chiang Rai, Thailand. The type specimens were preserved with silica gel and deposited in the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (**KUN-HKAS**), China. Index Fungorum numbers (http://www.indexfungorum.org; accessed on 25 May 2023) were obtained for the newly-described taxa.

DNA extraction, PCR amplification and sequencing

Fungal genomic DNA was extracted from fresh mycelia using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, Hangzhou, China) following the procedure from the manufacturer. The genomic DNA was also extracted from ascomata using a Forensic DNA Kit (Omega, Norcross, GA, USA) in case the fungus could not be obtained from the pure culture. Amplicons were generated by polymerase chain reaction (PCR) using five phylogenetic markers, including the internal transcribed spacers region of ribosomal DNA (ITS; ITS1-5.8S-ITS2), the partial 28S large subunit nuclear ribosomal DNA (LSU), the partial 18S small subunit rDNA (SSU), the partial RNA polymerase II second largest subunit (*rpb2*) and the partial translation elongation factor 1-alpha (*tef1-α*). The ITS region was amplified with the primer pair ITS4 and ITS5 (White et al. 1990), the LSU region with LR0R and LR5 (Vilgalys and Hester 1990), the SSU region with NS1 and NS4 (White et al. 1990), the *rpb2* region with fRPB2-5F and fRPB2-7cR (Liu et al. 1999) and the *tef1-α* region with EF1-983F and EF1-2218R (Rehner and Buckley 2005). The component of PCR reaction was performed in a total volume of 25 µl, containing 2 µl DNA template (30–50 ng/µl), 1 µl of each forward and reverse primer (10 µM), 12.5 µl Master Mix (mixture of *EasyTaq*TM DNA Polymerase, dNTPs and optimised buffer; Beijing TransGen Biotech Co., Ltd., Chaoyang District, Beijing, China) and 8.5 µl of double-distilled water (ddH_2O). The thermal cycle of PCR amplification for ITS, LSU, SSU, *rpb2* and *tef1-α* was set up following Phookamsak et al. (2014, 2023). PCR products were purified and sequenced by using PCR primers at TsingKe Biological

Technology (Kunming City, Yunnan Province, China). The quality of raw sequence data was checked and trimmed of low-quality segments with BioEdit 7.1.3.0 (Hall 1999). The consensus sequences of the newly-generated strains were assembled using SeqMan Pro version 11.1.0 (DNASTAR, Inc. Madison, WI, USA) and submitted to the GenBank database to further encourage accession within the scientific community.

Sequence alignments and phylogenetic analyses

The newly-generated sequences were subjected to the nucleotide BLAST search tool on the NCBI website for checking the correctness of species identification and searching for closely-related taxa that were further included in the sequence alignment dataset. Reference sequences from relevant publications and BLAST results of the closely-related species were downloaded from GenBank to supplement the datasets (Tables 1–3). Three datasets were prepared to construct the phylogenetic trees for clarifying phylogenetic relationships of the novel taxa in Bambusicolaceae (Table 1), Dictyosporiaceae (Table 2) and Periconiaceae (Table 3). The individual gene dataset was aligned using MAFFT v.7 (Katoh et al. 2019) and improved manually where necessary in Bioedit 7.1.3.0 (Hall 1999). The alignments of individual gene datasets were prior analysed by Maximum Likelihood (ML) for checking the congruence of tree topologies and further combined into a multigene dataset. Phylogenetic analyses were performed, based on ML and Bayesian Inference (BI) analyses.

Maximum Likelihood (ML) implemented by the Randomised Axelerated Maximum Likelihood (RAxML), was performed in RAxML-HPC v.8 on the XSEDE (8.2.12) tool via the online web portal CIPRES Science Gateway v. 3.3 (Miller et al. 2010) using default settings, but adjusted with 1000 bootstrap replicates and a gamma-distributed rate variation of a general time reversible model (GTR) was applied. The BI analyses were conducted by MrBayes on XSEDE v. 3.2.7a via the same web portal as in ML, with two parallel runs. The best-fit model of nucleotide substitution was determined by MrModelTest v. 2.3 (Nylander et al. 2008). Six simultaneous Markov chains were run for 1–5 million generations, but stopped automatically when the critical value for the topological convergence diagnostic reached 0.01. Trees were sampled every 100th generation. The initial 10% of sample trees were treated as burn-in (estimated by Tracer v. 1.7; Rambaut et al. (2018)) and discarded. The remaining trees were used to calculate the posterior probabilities in the majority rule consensus tree. The phylograms were visualised using Figtree v. 1.4.0 (Rambaut and Drummond 2012) and backbone trees were laid out and edited in Adobe Illustrator version 20.0.0. software (Adobe Systems Inc., San Jose, CA, USA).

Results

Phylogenetic analyses

In this study, three phylogenetic analyses were conducted to clarify the phylogenetic placements of our new taxa within the Bambusicolaceae (Analysis 1), Dictyosporiaceae (Analysis 2) and Periconiaceae (Analysis 3), as follows:

Table 1. Species details and GenBank accession numbers used in phylogenetic analysis of *Bambusicola* species (Bambusicolaceae, Pleosporales). The new sequences are indicated in bold and the ex-type strains are indicated by superscript "T". Missing sequences are indicated by "–".

Species name	Strain/specimen no.	GenBank accession numbers				
		ITS	LSU	*rpb2*	SSU	*tef1-α*
Bambusicola aquatica[T]	MFLUCC 18-1031	MT627729	MN913710	MT878462	MT864293	MT954392
Bambusicola autumnalis[T]	CGMCC 3.24280	OQ427824	OQ427825	OQ507621	OQ427823	OQ507622
Bambusicola autumnalis	UESTCC 23.0001	OQ609612	OQ550210	OQ556791	OQ550209	OQ556792
Bambusicola bambusae[T]	MFLUCC 11-0614	JX442031	JX442035	KP761718	JX442039	KP761722
Bambusicola didymospora[T]	MFLUCC 10-0557	KU940116	KU863105	KU940163	KU872110	KU940188
Bambusicola dimorpha[T]	MFLUCC 13-0282	KY026582	KY000661	KY056663	KY038354	–
Bambusicola ficuum[T]	MFLUCC 17-0872	–	MT215580	–	MT215581	MT199326
Bambusicola fusispora[T]	MFLUCC 20-0149	MW076532	MW076531	MW034589	MW076529	–
Bambusicola guttulata[T]	CGMCC 3.20935	ON332909	ON332927	ON383985	ON332919	ON381177
***Bambusicola hongheensis*[T]**	**BN06/ KUN-HKAS 129042**	**OR233600**	**OR335804**	**OR540736**	**OR501419**	–
Bambusicola irregulispora[T]	MFLUCC 11-0437	JX442032	JX442036	KP761719	JX442040	KP761723
Bambusicola loculata[T]	MFLUCC 13-0856	KP761732	KP761729	KP761715	KP761735	KP761724
Bambusicola massarinia[T]	MFLUCC 11-0389	JX442033	JX442037	KP761716	JX442041	KP761725
Bambusicola pustulata[T]	MFLUCC 15-0190	KU940118	KU863107	KU940165	KU872112	KU940190
Bambusicola nanensis[T]	MFLUCC 21-0063	NR_176767	NG_081535	–	–	–
Bambusicola sichuanensis[T]	SICAUCC 16-0002	MK253473	MK253532	MK262830	MK253528	MK262828
Bambusicola splendida[T]	MFLUCC 11-0439	JX442034	JX442038	KP761717	JX442042	KP761726
Bambusicola subthailandica[T]	SICAU 16-0005	MK253474	MK253533	MK262831	MK253529	MK262829
Bambusicola thailandica[T]	MFLUCC 11-0147	KU940119	KU863108	KU940166	–	KU940191
Bambusicola triseptatispora[T]	MFLUCC 11-0166	KU940120	KU863109	KU940167	–	–
Corylicola italica	MFLU 19-0500	MT554925	MT554926	MT590776	MT554923	–
Corylicola italica[T]	MFLUCC 20-0111	MT633085	MT626713	MT635596	MT633084	MT590777
Leucaenicola aseptata[T]	MFLUCC 17-2423	MK347746	MK347963	MK434891	MK347853	MK360059
Leucaenicola camelliae[T]	NTUCC 18-093-4	MT112302	MT071278	MT743283	MT071229	MT374091
Leucaenicola phraeana[T]	MFLUCC 18-0472	MK347785	MK348003	MK434867	MK347892	MK360060
Occultibambusa bambusae[T]	MFLUCC 13-0855	KU940123	KU863112	KU940170	KU872116	KU940193
Occultibambusa kunmingensis[T]	KUN-HKAS 102151	MT627716	MN913733	MT878453	MT864342	MT954407
Occultibambusa sichuanensis[T]	CGMCC 3.20938	ON332913	ON332931	ON383989	–	ON381181
Palmiascoma gregariascomum[T]	MFLUCC 11-0175	KP744452	KP744495	KP998466	KP753958	–
Palmiascoma qujingense[T]	KUMCC 19-0201	MT477183	MT477185	MT495782	MT477186	–
Pseudotetraploa bambusicola[T]	CGMCC 3.20939	ON332915	ON332933	ON383991	ON332923	ON381183
Pseudotetraploa curviappendiculata[T]	JCM 12852	AB524792	AB524608	–	AB524467	–
Seriascoma bambusae[T]	KUMCC 21-0021	MZ329039	MZ329035	MZ325470	MZ329031	MZ325468
Seriascoma didymosporum[T]	MFLUCC 11-0179	KU940127	KU863116	KU940173	KU872119	KU940196
Seriascoma yunnanense[T]	MFLU 19-0690	–	MN174695	MN210324	MN174694	MN381858
Versicolorisporium triseptatum[T]	JCM 14775	AB365596	AB330081	–	AB524501	–
Versicolorisporium triseptatum	NMX1222	OL741378	OL741318	–	OL741381	–

Table 2. Species details and GenBank accession numbers used in phylogenetic analysis of taxa in Dictyosporiaceae (Pleosporales). The new sequences are indicated in bold and the ex-type strains are indicated by superscript "T". Missing sequences are indicated by "–".

Species name	Strain/ specimen no.	GenBank accession numbers			
		ITS	LSU	SSU	*tef1-α*
Anthosulcatispora subglobosa[T]	MFLUCC 17-2065/ MFLU 17-1473	MT310636	NG_073851	MT226705	MT394649
Aquadictyospora lignicola[T]	MFLUCC 17-1318	MF948621	MF948629	–	MF953164
Aquaticheirospora lignicola[T]	RK-2006a/ HKUCC10304	AY864770	AY736378	AY736377	–
Cheirosporium triseriale[T]	HMAS 180703	EU413953	EU413954	–	–
Chromolaenicola nanensis[T]	MFLUCC 17-1473	MN325015	NG_070942	MN325009	MN335648
Darksidea alpha[T]	CBS 135650	NR_137619	KP184019	KP184049	KP184166
Dendryphiella fasciculata[T]	MFLUCC 17-1074	NR_154044	NG_059177	–	–
Dendryphiella variabilis[T]	CBS 584.96	LT963453	LT963454	–	–
Dictyocheirospora bannica[T]	KH 332	LC014543	AB807513	AB797223	AB808489
Dictyocheirospora rotunda[T]	MFLUCC 14-0293b	KU179099	KU179100	–	–
Dictyosporium bulbosum	yone 221	LC014544	AB807511	AB797221	AB808487
Dictyosporium elegans[T]	NBRC 32502	DQ018087	DQ018100	DQ018079	–
Didymosphaeria rubi-ulmifolii[T]	MFLUCC 14-0023	–	KJ436586	KJ436588	–
Digitodesmium bambusicola[T]	CBS 110279	DQ018091	DQ018103	–	–
Falciformispora senegalensis[T]	CBS 196.79	MH861195	NG_057981	NG_062928	KF015687
Fuscosphaeria hungarica[T]	DSE883, CBS 147250	MW209054	MW209059	MW209065	MW238843
Gregarithecium curvisporum[T]	HHUF 30134	NR_154049	NG_059394	NG_061002	AB808523
Gregarithecium curvisporum	MS224	LC482117	–	–	–
	DCR17	MZ047572	–	–	–
Helicascus elaterascus	KT 2673/ MAFF 243867	AB809626	AB807533	AB797243	AB808508
Immotthia bambusae[T]	KUN-HKAS 112012AI	MW489455	MW489450	MW489461	MW504646
	KUN-HKAS 112012B	MW489457	MW489452	–	–
Jalapriya pulchra[T]	MFLUCC 15-0348	KU179108	KU179109	KU179110	–
Jalapriya toruloides[T]	CBS 209.65	DQ018093	DQ018104	DQ018081	–
Katumotoa bambusicola[T]	KT1517a	LC014560	AB524595	AB524454	AB539108
Lentithecium clioninum[T]	KT1149A/ HHUF:28199	NR_154137	NG_059391	NG_064845	AB808515
Lentithecium pseudoclioninum[T]	HHUF 29055	AB809633	NG_059392	NG_064847	AB808521
Loculosulcatispora thailandica[T]	KUMCC 20-0159	MT376742	MT383964	MT383968	MT380476
Magnicamarosporium iriomotense[T]	HHUF 30125/ KT 2822	NR_153445	NG_059389	NG_060999	AB808485
Montagnula cirsii[T]	MFLUCC 13-0680	KX274242	KX274249	KX274255	KX284707
Morosphaeria muthupetensis[T]	NFCCI4219	MF614795	MF614796	MF614797	MF614798
Murilentithecium clematidis[T]	MFLUCC 14-0562	KM408757	KM408759	KM408761	KM454445
Neodendryphiella mali[T]	CBS 139.95	LT906655	LT906657	EF204511	–
Neodendryphiella michoacanensis[T]	FMR 16098	NR_160583	LT906658	–	–
Neohelicascus aquaticus	MFLUCC 10-0918/ KT 1544	AB809627	AB807532	AB797242	AB808507
Paradictyocheirospora tectonae[T]	NFCCI 4878/ AMH 10301	MW854646	MW854647	–	MW854832
Phaeosphaeria oryzae[T]	CBS 110110	KF251186	KF251689	GQ387530	–
Phaeosphaeriopsis glaucopunctata[T]	MFLUCC 13-0265	KJ522473	KJ522477	KJ522481	MG520918
Pseudocoleophoma bauhiniae[T]	MFLUCC 17–2586	MK347736	MK347953	MK347844	MK360076
Pseudocoleophoma calamagrostidis[T]	KT 3284/ HHUF 30450	LC014592	LC014609	LC014604	LC014614

Species name	Strain/ specimen no.	GenBank accession numbers			
		ITS	LSU	SSU	*tef1-α*
Pseudoconiothyrium broussonetiae[T]	CBS:145036/ CPC:33570	NR_163377	NG_066331	–	MK442709
Pseudoconiothyrium typhicola[T]	MFLUCC 16-0123	KX576655	KX576656	–	–
Pseudocyclothyriella clematidis[T]	MFLUCC 17-2177A	MT310595	MT214548	–	MT394730
Pseudocyclothyriella clematidis	MFLU 16-0280	MT310596	MT214549	–	–
Pseudodictyosporium elegans[T] (=*Cheiromoniliophora elegans*)	CBS 688.93	DQ018099	DQ018106	DQ018084	–
Pseudodictyosporium thailandica[T]	MFLUCC 16-0029	NR_154347	NG_059688	NG_063611	KX259526
Sajamaea mycophila[T]	APA-2999	MK795715	MK795718	–	–
Sulcatispora acerina[T]	KT 2982	LC014597	LC014610	LC014605	LC014615
Tingoldiago graminicola[T]	KH68	LC014598	AB521743	AB521726	AB808561
Trichobotrys effusus	1179	KJ630313	–	–	–
	HNNUZCJ-94	OM281094	–	–	–
	FS524	MN545626	–	–	–
	SYSU-MS4729	MH050972	–	–	–
	DFFSCS021	JX156367	–	–	–
***Trichobotrys sinensis*[T]**	**RPC 21-007/ KUNCC 23-14554**	**OR233595**	**OR335805**	**OR501420**	**OR547995**
Trichobotrys sp. [as *Gregarithecium* sp.]	MFLUCC 13-0853	KX364281	KX364282	KX364283	–
	GMB1217	–	–	OM836759	–
Trematosphaeria pertusa[T]	CBS 122368	NR_132040	NG_057809	FJ201991	KF015701
Verrucoccum coppinsii[T]	E 00814291	MT918785	MT918770	NG_081399	–
Verrucoccum spribillei[T]	SPO 1154	MT918781	MT918764	MT918772	–
Vikalpa australiense	HKUCC 8797	DQ018092	–	–	–

Analysis 1

The *Bambusicola* species tree was constructed using a sequence dataset of the concatenated ITS, LSU, *rpb2*, SSU and *tef1-α* of all *Bambusicola* species, as well as representatives of other related genera. A total of 37 strains were included, with two strains of *Pseudotetraploa bambusicola* (CGMCC 3.20939) and *P. curviappendiculata* (JCM 12852) as the outgroup. Primarily, phylogenetic analysis of the concatenated LSU, SSU and ITS sequence dataset was conducted, based on ML and compared with the multigene phylogenetic analysis (the concatenated ITS, LSU, *rpb2*, SSU and *tef1-α* sequence dataset). Phylogenetic analysis, based on the concatenated LSU, SSU and ITS gene regions, showed a similar topology with the concatenated ITS, LSU, *rpb2*, SSU and *tef1-α* gene regions and were not significantly different (data not shown). Hence, multigene phylogenetic analysis of the concatenated ITS, LSU, *rpb2*, SSU and *tef1-α* gene regions was selected to represent the phylogenetic relationships of the new species with other closely-related species in Bambusicolaceae. The aligned dataset contained 4929 characters, including gaps. Phylogenetic relationships were inferred by conducting analyses using both ML and BI methods. The best-scoring RAxML tree was selected to represent the relationships amongst taxa, with a final likelihood value of -29592.797597 (Fig. 1). The matrix contained 1905 distinct alignment patterns, with a 22.83% proportion of gaps and completely undetermined characters. The estimated base frequencies of A = 0.243583, C = 0.258293, G = 0.271748, T = 0.226375; substitution rates AC = 1.393909, AG = 2.806593,

Table 3. Species details and GenBank accession numbers used in phylogenetic analysis of *Periconia* species (Periconiaceae, Pleosporales). The new sequences are indicated in bold and the ex-type strains are indicated by superscript "T". Missing sequences are indicated by "–".

Species	Strain No.	GenBank accession numbers			
		ITS	LSU	SSU	*tef1-α*
Flavomyces fulophazae	CBS 135664	KP184000	KP184039	KP184081	–
Flavomyces fulophazae[T]	CBS 135761	NR_137960	NG_058131	NG_061191	–
Lentithecium aquaticum[T]	CBS 123099	NR_160229	NG_064211	NG_016507	GU349068
Lentithecium clioninum[T]	KT 1149A	LC014566	AB807540	AB797250	AB808515
Lentithecium clioninum	KT 1220	LC014567	AB807541	AB797251	AB808516
Massarina cisti[T]	CBS 266.62	–	AB807539	AB797249	AB808514
Massarina eburnea	CBS 473.64	–	GU301840	GU296170	GU349040
Morosphaeria ramunculicola	KH 220	–	AB807554	AB797264	AB808530
Morosphaeria velatispora	KH 221	LC014572	AB807556	AB797266	AB808532
Periconia algeriana[T]	CBS 321.79	MH861212	MH872979	–	–
Periconia alishanica[T]	MFLUCC 19-0145	MW063165	MW063229	–	MW183790
Periconia aquatica[T]	MFLUCC 16-0912	KY794701	KY794705	–	KY814760
Periconia artemisiae[T]	KUMCC 20-0265	MW448657	MW448571	MW448658	MW460898
Periconia artemisiae	G1782	MK247789	–	–	–
Periconia atropurpurea	CBS 381.55	MH857524	MH869061	–	–
Periconia banksiae[T]	CBS 129526	JF951147	NG_064279	–	–
Periconia byssoides	KUMCC 20-0264	MW444854	MW444855	MW444856	MW460895
	MAFF 243869	LC014582	AB807569	AB797279	AB808545
	MFLUCC 17-2292	MK347751	MK347968	MK347858	MK360069
	MFLUCC 18-1553	MK347806	MK348025	MK347914	MK360068
	MFLUCC 20-0172	MW063162	MW063226	–	–
	NCYUCC 19-0314	MW063163	MW063227	–	–
Periconia caespitosa[T]	LAMIC 110 16	MH051906	MH051907	–	–
Periconia chengduensis[T]	CGMCC 3.23930	OP955987	OP956012	OP956056	OP961453
Periconia chengduensis	UESTCC 22.0140	OP955977	OP956002	OP956046	OP961443
Periconia chimonanthi[T]	KUMCC 20-0266	MW448660	MW448572	MW448656	MW460897
Periconia circinata	CBS 263.37	MW810265	MH867413	–	MW735660
Periconia citlaltepetlensis[T]	ENCB 140251 = IOM 325319.1	MH890645	MT625978	–	–
Periconia citlaltepetlensis	IOM 325319.2	MT649221	MT649216	–	–
Periconia cookei	MFLUCC 17-1399	MG333490	MG333493	–	MG438279
	MFLUCC 17-1679	–	MG333492	–	MG438278
	UESTCC 22.013	OP955968	OP955993	OP956037	–
Periconia cortaderiae[T]	MFLUCC 15-0457	KX965732	KX954401	KX986345	KY310703
Periconia cynodontis[T]	CGMCC 3.23927	OP909925	OP909921	OP909920	OP961434
Periconia cyperacearum[T]	CPC 32138	NR_160357	NG_064549	–	–
Periconia delonicis[T]	MFLUCC 17-2584	–	NG_068611	NG_065770	MK360071
Periconia didymosporum[T]	MFLU 15-0058	KP761734	KP761731	KP761738	KP761728
Periconia digitata	CBS 510.77	LC014584	AB807561	AB797271	AB808537
Periconia elaeidis[T]	MFLUCC 17-0087	MG742713	MH108552	MH108551	–
Periconia epilithographicola	MFLUCC 21–0153	OL753687	OL606155	OL606144	OL912948

Species	Strain No.	GenBank accession numbers			
		ITS	LSU	SSU	*tef1-α*
Periconia epilithographicola[T]	CBS 144017	NR_157477	–	–	–
Periconia festucae[T]	CGMCC 3.23929	OP955973	OP955998	OP956042	OP961439
Periconia genistae[T]	CBS 322.79	MH861213	MH872980	–	–
Periconia homothallica[T]	CBS 139698/ KT916	AB809645	AB807565	AB797275	AB808541
Periconia igniaria	CBS 379.86	LC014585	AB807566	AB797276	AB808542
Periconia imperatae[T]	CGMCC 3.23931	OP955984	OP956009	OP956053	OP961450
Periconia imperatae	UESTCC 22.0145	OP955979	OP956004	OP956048	OP961445
***Periconia kunmingensis*[T]**	**KUMCC 18-0173/ RPC 15-017**	**MH892346**	**MH892399**	**OR225814**	**MH908963**
Periconia lateralis	CBS 292.36	MH855804	MH867311	–	–
Periconia macrospinosa	CBS 135663	KP183999	KP184038	KP184080	–
	REF144	JN859364	JN859484	–	–
Periconia minutissima	MFLUCC 15-0245	KY794703	KY794707	–	–
	MUT 2887	MG813227	–	–	–
Periconia neobrittanica[T]	CPC 37903	NR_166344	NG_068342	–	–
Periconia palmicola[T]	MFLUCC 14-0400	–	NG_068917	MN648319	MN821070
Periconia penniseti[T]	CGMCC 3.23928	OP955971	OP955996	OP956040	OP961437
Periconia prolifica[T]	CBS 209.64	MH858422	MH870050	–	–
Periconia pseudobyssoides	KUMCC 20-0263	MW444851	MW444852	MW444853	MW460894
Periconia pseudodigitata	KT 644	MW444852	AB807562	AB797272	AB808538
Periconia pseudodigitata[T]	KT 1395	MW444853	NG_059396	NG_064850	AB808540
Periconia sahariana[T]	CBS 320.79	MW444854	MH872978	–	–
Periconia salina[T]	GJ374/ MFLU 19–1235	MW444855	MN017846	MN017912	–
Periconia spodiopogonis[T]	CGMCC 3.23932	MW444856	OP955988	OP956032	OP961429
Periconia submersa[T]	MFLUCC 16-1098	MW444857	KY794706	–	KY814761
Periconia thailandica[T]	MFLUCC 17-0065	MW444858	KY753888	KY753889	–
Periconia thysanolaenae[T]	KUMCC 20-0262	MW444859	MW444850	MW448659	MW460896
Periconia variicolor[T]	SACCR-64	MW444860	–	–	–
Periconia verrucosa[T]	MFLUCC 17-2158	MT310617	MT214572	MT226686	MT394631
Periconia verrucosa	UESTCC 22.0136	OP955966	OP955991	OP956035	OP961432
	KT 1825	–	AB807573	AB797283	AB808549
	KT 1820A	–	AB807572	AB797282	AB808548

AT = 1.064133, CG = 1.193703, CT = 6.412290, GT = 1.000000; gamma distribution shape parameter α = 0.589535; Tree-Length = 1.823129. For BI analysis, GTR + I + G was selected as the best-fit model by AIC in MrModelTest for each gene (ITS, LSU, *rpb2*, SSU and *tef1-α*). Six simultaneous Markov chains were set to run for 1,000,000 generations, but stopped at 25,000 generations because the convergence diagnostic hit the stop value, resulting in 251 total trees. The first 10% of trees were discarded as the burn-in phase of the analyses and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree, of which the final average standard deviation of split frequencies at the end of total MCMC generations was 0.005298.

Multigene phylogenetic analyses demonstrated that all genera of Bambusicolaceae formed well-resolved clades (up to 98% ML, 1.00 PP; Fig. 1) in the pres-

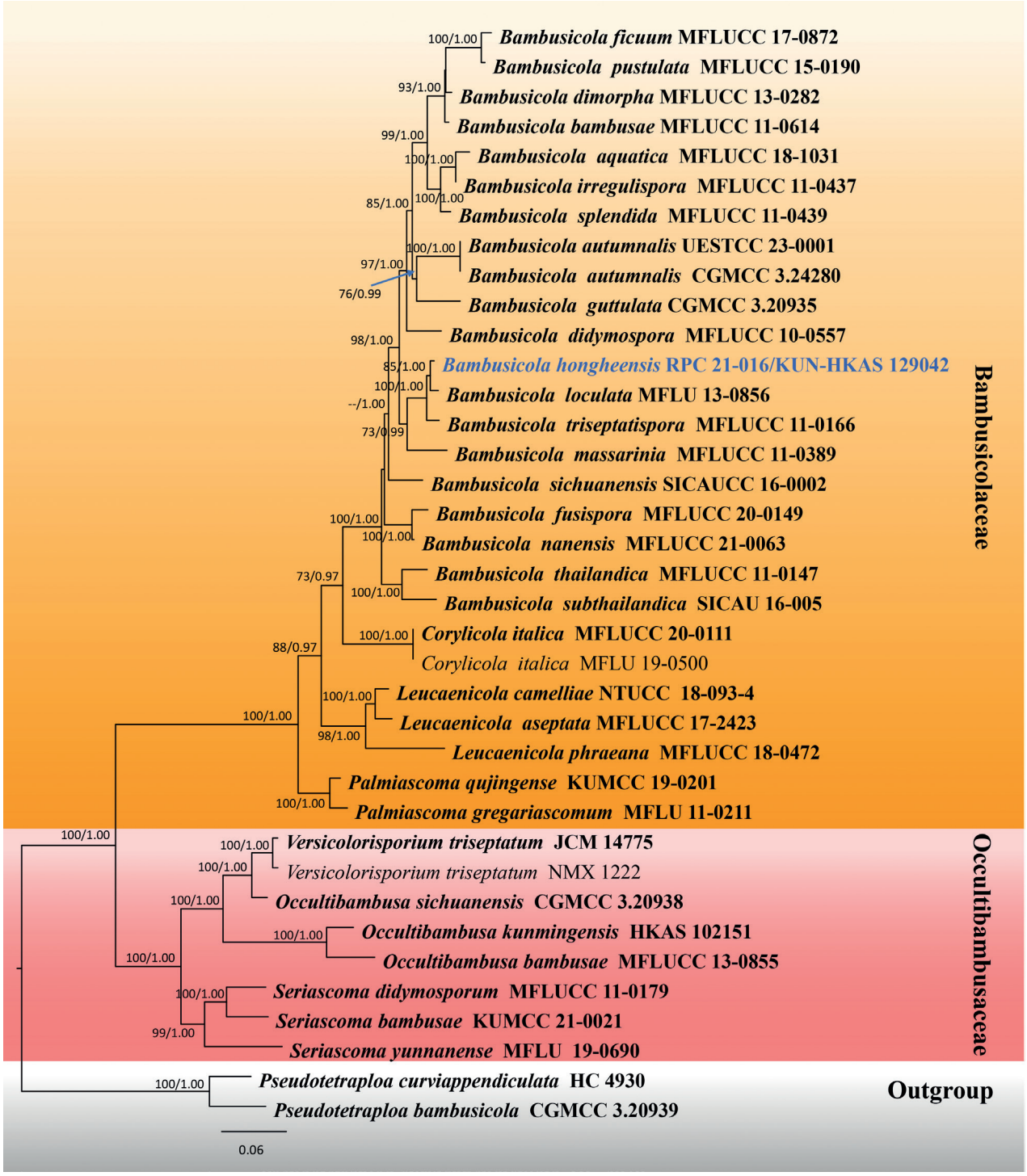

Figure 1. Phylogram of the best-scoring ML consensus tree of taxa in Bambusicolaceae and Occultibambusaceae. The new isolate is indicated in blue. Isolates from type materials are in bold. The ML ultrafast bootstrap and Bayesian PP values greater than 60% and 0.90 are shown at the nodes.

ent study. The new species, *Bambusicola hongheensis* (KUN-HKAS 129042), clustered with the clade containing *B. loculata* (MFLUCC 13-0856) (85% ML, 1.00 PP) and *B. triseptatispora* (MFLUCC 11-0166) with high statistical support (100% ML, 1.00 PP). These three species have close relationships with *B. massarinia* (MFLUCC 11-0389) (73% ML, 0.99 PP), the type genus of *Bambusicola*.

Analysis 2

The *Trichobotrys* tree was constructed using sequence data from ITS, LSU, SSU and *tef1-α*. A total of 61 strains of taxa in Dictyosporiaceae and closely-related families (viz. Didymosphaeriaceae, Lentitheciaceae, Morosphaeriaceae, Sulcatisporaceae and Trematosphaeriaceae) were included, with *Phaeosphaeria oryzae* (CBS 110110) and *Phaeosphaeriopsis glaucopunctata* (MFLUCC 13-0265) (Phaeosphaeriaceae) as the outgroup. Primarily, phylogenetic analysis of the concatenated LSU, SSU and ITS sequence dataset was conducted, based on ML and compared with phylogenetic analysis of the concatenated ITS, LSU, SSU and *tef1-α* sequence dataset. Phylogenetic analysis, based on the concatenated LSU, SSU and ITS sequence dataset, showed a similar topology with the concatenated ITS, LSU, SSU and *tef1-α* sequence dataset and were not significantly different (data not shown). Hence, multigene phylogenetic analysis of the concatenated ITS, LSU, SSU and *tef1-α* gene regions was selected to represent the phylogenetic relationships of *Trichobotrys sinensis* sp. nov. with other closely-related species in Dictyosporiaceae. The aligned dataset contained 3729 characters, including gaps. Phylogenetic relationships were inferred by conducting analyses using both ML and BI methods. The best-scoring RAxML tree was selected to represent the relationships amongst taxa, with a final likelihood value of -28366.415110 (Fig. 2). The matrix contained 1566 distinct alignment patterns, with a 39.19% proportion of gaps and completely undetermined characters. The estimated base frequencies of A = 0.239629, C = 0.244575, G = 0.269426, T = 0.246371; substitution rates AC = 1.123110, AG = 2.634717, AT = 1.787337, CG = 0.836519, CT = 6.160493, GT = 1.000000; gamma distribution shape parameter α = 0.461486; Tree-Length = 3.107341. For BI analysis, GTR + I + G was selected as the best-fit model by AIC in MrModelTest for each gene (ITS, LSU, SSU and *tef1-α*). Six simultaneous Markov chains were run for 4,085,000 generations, resulting in 40,851 total trees. The first 10% of trees were discarded as the burn-in phase of the analyses and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree, of which the final average standard deviation of split frequencies at the end of total MCMC generations was 0.009998.

Multigene phylogenetic analyses of the concatenated ITS, LSU, SSU and *tef1-α* demonstrated that all representative families formed well-resolved clades in the present study. Our new isolate grouped with two unnamed *Gregarithecium* sp. (strains GMB1217 and MFLUCC 13-0853), with high support in ML and BI analyses (99% ML, 100 PP; Fig. 2) and clustered with *Trichobotrys effusus* (strains 1179, HNNUZCJ-94, FS524, SYSU-MS4729 and DFFSCS021) with high support (100% ML, 1.00 PP; Fig. 2) in Dictyosporiaceae. *Gregarithecium* sp. (strains GMB1217 and MFLUCC 13-0853) is unpublished and showed to be conspecific with our new isolate. Therefore, our new isolate is introduced as *Trichobotrys sinensis*, based on phylogenetic evidence coupled with morphological characteristics. *Trichobotrys* formed a highly-supported subclade with *Gregarithecium* (99% ML, 1.00 PP; Fig. 2) in the present study. However, these two genera are represented by different morphs. Therefore, the congeneric status of these two genera is doubtful in the study pending future study.

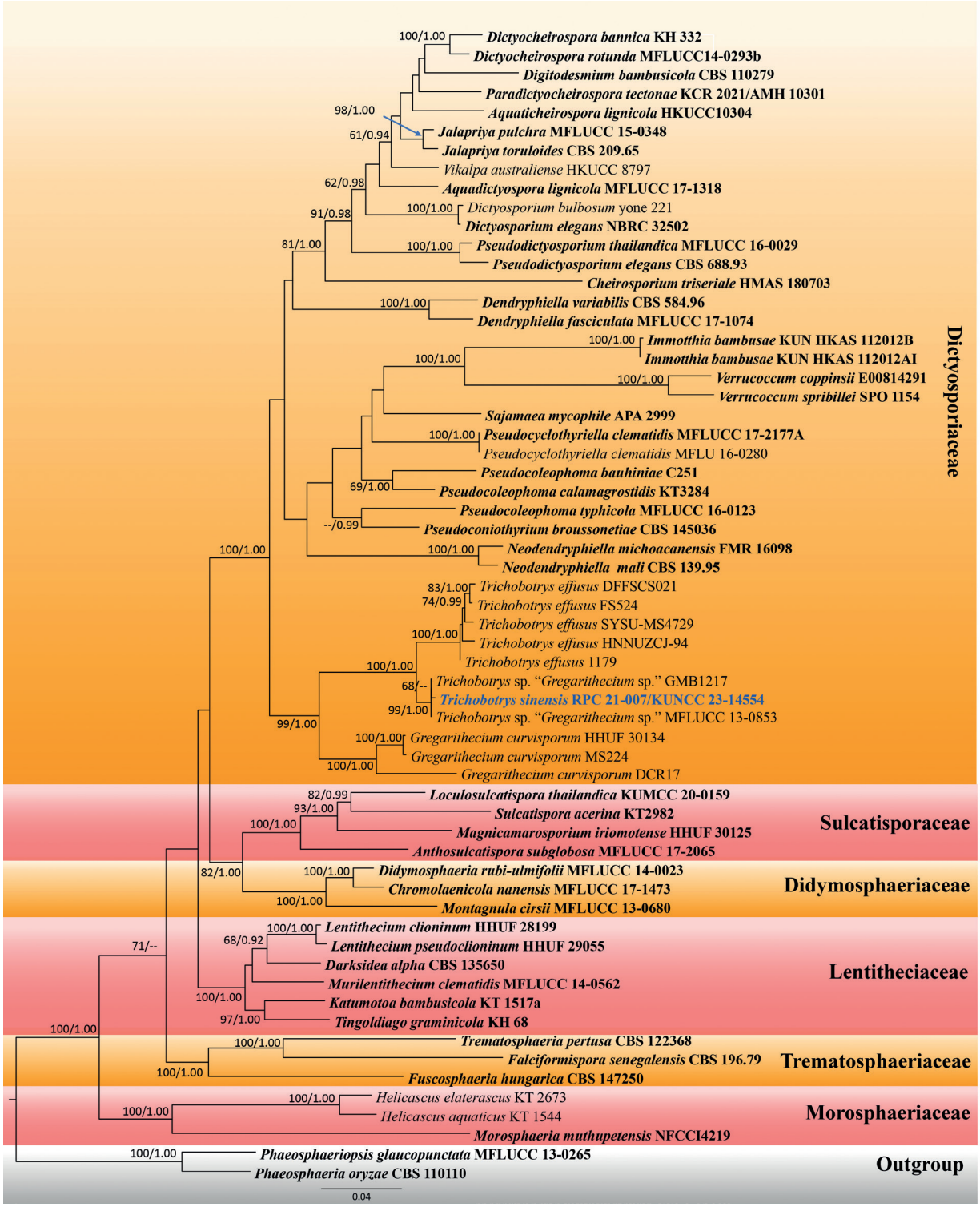

Figure 2. Phylogram of the best-scoring ML consensus tree of *Trichobotrys* species in Dictyosporiaceae and closely-related families viz. Didymosphaeriaceae, Lentitheciaceae, Morosphaeriaceae, Sulcatisporaceae and Trematosphaeriaceae. The new isolate is indicated in blue. Isolates from type materials are in bold. The ML ultrafast bootstrap and Bayesian PP values greater than 70% and 0.95 are shown at the nodes.

Analysis 3

The *Periconia* species tree was constructed using sequence data from ITS, LSU, SSU and *tef1-α* of all taxa in Periconiaceae and other related families

(viz. Lentitheciaceae, and Massarinaceae). A total of 71 strains were included, with *Morosphaeria ramunculicola* (KH 220) and *M. velatispora* (KH 221) as the outgroup. The aligned dataset contained 3646 characters, including gaps. The best-scoring RAxML tree was selected to represent the relationships amongst taxa, with a final likelihood value of -19141.848334 (Fig. 3). The matrix contained 1265 distinct alignment patterns, with a 32.87% proportion of gaps and completely undetermined characters. The estimated base frequencies of A = 0.239678, C = 0.253426, G = 0.268914, T = 0.237981; substitution rates AC = 1.751555, AG = 3.051838, AT = 1.900841, CG = 1.359429, CT = 9.411951, GT = 1.000000; gamma distribution shape parameter α = 0.505775; Tree-Length = 1.483987. For BI analysis, GTR + I + G was selected as the best-fit model by AIC in MrModelTest for each gene (ITS, LSU, SSU and *tef1-α*). Six simultaneous Markov chains were run for 555,000 generations, resulting in 5551 total trees. The first 10% of trees were discarded as the burn-in phase of the analyses and the remaining trees were used for calculating posterior probabilities in the majority rule consensus tree, of which the final average standard deviation of split frequencies at the end of total MCMC generations was 0.009941.

Multigene phylogenetic analyses demonstrated that the new species *Periconia kunmingensis* (KUMCC 18-0173) formed a distinct lineage and clustered with the clade containing *P. cookei* (MFLUCC 17-1679, MFLUCC 17-1399 and UESTCC 22.013), *P. delonicis*, (MFLUCC 17-2584), *P. elaeidis* (MFLUCC 17-0087), *P. palmicola* (MFLUCC 14-0400) and *P. verrucosa* (MFLUCC 17-2158, Lu40-1, KT1820A and KT1825), with strong statistical support (100% ML, 1.00 PP; Fig. 3).

Taxonomy

Bambusicolaceae D.Q. Dai & K.D. Hyde, Fungal Diversity 63: 49 (2013)
Index Fungorum: IF804293

Notes. Bambusicolaceae was first introduced by Hyde et al. (2013) to accommodate *Bambusicola* with *B. massarinia* being the type species. Subsequently, another three genera were accommodated in this family viz. *Corylicola* (Wijesinghe et al. 2020), *Leucaenicola* (Jayasiri et al. 2019) and *Palmiascoma* (Liu et al. 2015). Species of these genera have been reported from various hosts, such as *Camellia*, *Corylus*, *Eucalyptus*, Fagaceae sp., *Leucaena*, *Osmanthus* and palm and so far, found distributed in China (Sichuan and Yunnan), Italy and Thailand (Liu et al. 2015; Jayasiri et al. 2019; Ariyawansa et al. 2020a, b; Hongsanan et al. 2020; Wijesinghe et al. 2020; Monkai et al. 2021). Members of Bambusicolaceae are mainly saprobes; however, Ariyawansa et al. (2020a, b) reported that species of *Leucaenicola* associated with leaf spot diseases on *Camellia* and *Osmanthus* in Taiwan (China). Bambusicolaceae is a well-studied family, based on morphological characteristics of sexual-asexual morphs and multigene phylogenetic evidence. Recent taxonomic treatment carried out by Hongsanan et al. (2020) revealed that the family belongs to the suborder Massarineae, order Pleosporales of Dothideomycetes, comprising four genera and 25 species (http://www.indexfungorum.org; accessed on 25 May 2023).

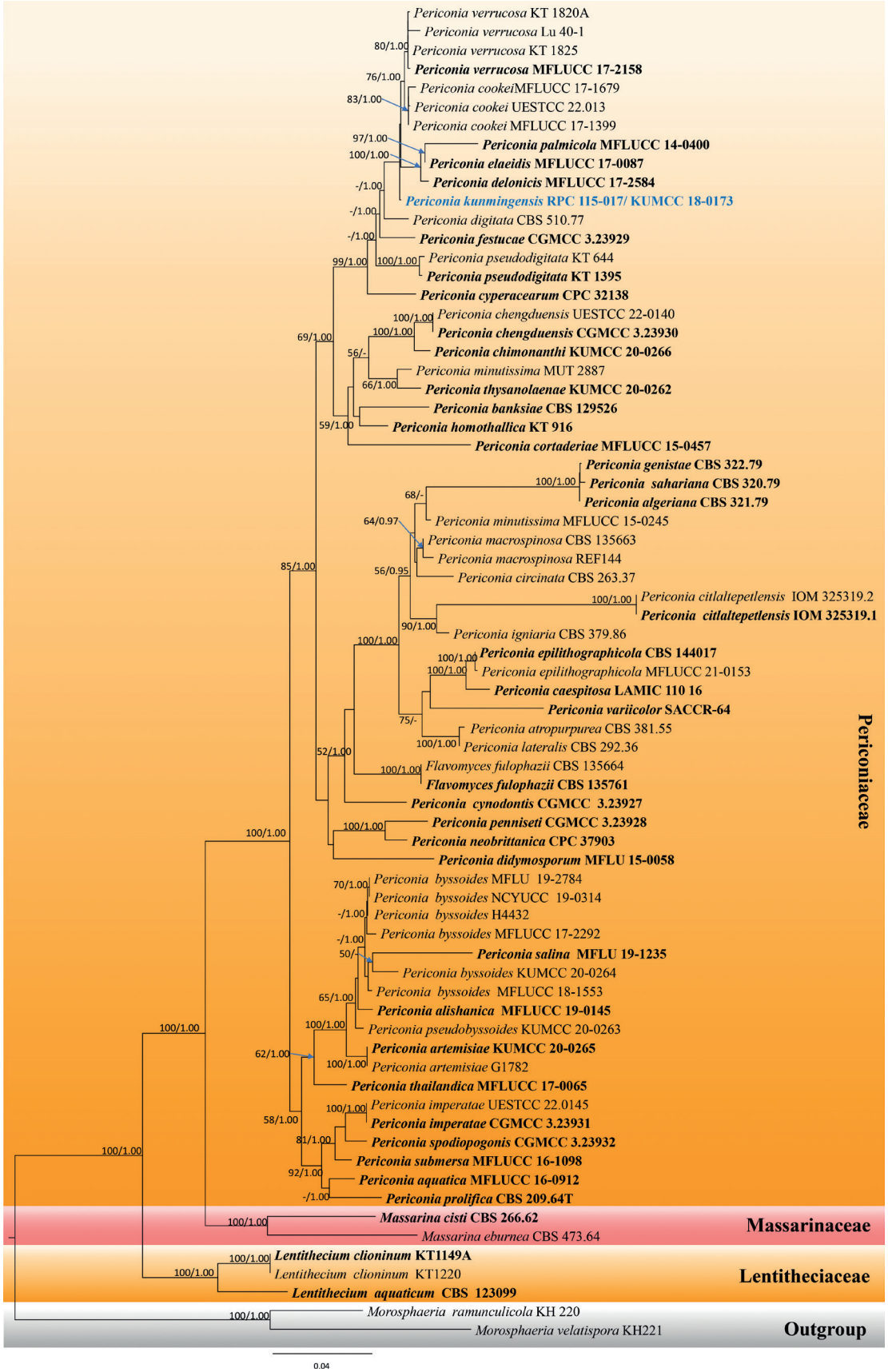

Figure 3. Phylogram of the best-scoring ML consensus tree of taxa in Periconiaceae and the closely-related families Lentitheciaceae and Massarinaceae. The new isolate is indicated in blue. Isolates from type materials are in bold. The ML ultrafast bootstrap and Bayesian PP values greater than 50% and 0.95 are shown at the nodes.

Bambusicola **D.Q. Dai & K.D. Hyde, Cryptog. Mycol. 33(3): 367 (2012)**
Index Fungorum: IF801041

Notes. *Bambusicola* was introduced by Dai et al. (2012) to accommodate four saprobic species associated with bamboo, namely *B. bambusae*, *B. irregulispora*, *B. massarinia* and *B. splendida*. Subsequently, many species were included in the genus which were mainly known as saprobes on different bamboos in terrestrial habitats (Dai et al. 2012, 2015, 2017; Thambugala et al. 2017; Monkai et al. 2021; Phukhamsakda et al. 2022; Yu et al. 2022). However, *B. sichuanensis* and *B. subthailandica* were reported as parasites on *Phyllostachys heteroclada* (Yang et al. 2019). While *B. aquatica* was reported as a saprobe submerged in freshwater (Dong et al. 2020) and *B. ficuum* was reported on dead twigs of *Ficus* (Brahmanage et al. 2020). *Bambusicola* is morphologically well-studied and appear pleomorphic. Besides, its phylogenetic affinities have been well-clarified, based on multigene phylogenetic evidence (e.g. *B. didymospora*, *B. massarinia*, *B. triseptatispora*) (Dai et al. 2012, 2017). Currently, there are 17 species accepted in the genus, mostly distributed in the Sichuan and Yunnan Provinces of China and Thailand (http://www.indexfungorum.org; accessed on 25 May 2023). In the present study, we introduce a novel species *B. hongheensis* which was collected from dead bamboo culms in Yunnan, China.

Bambusicola hongheensis **Phookamsak, Bhat & Hongsanan, sp. nov.**
Index Fungorum: IF900830
Fig. 4

Etymology. The specific epithet "*hongheensis*" refers to the locality, Honghe Hani and Yi Autonomous Prefecture (Yunnan, China), where the holotype was collected.

Holotype. KUN-HKAS 129042.

Description. Saprobic on dead culm of bamboo in terrestrial habitats, visible as black, shiny, gnarled on the host surface. ***Sexual morph*: *Ascomata*** 225–350 µm high, 340–590 µm diam., scattered, sometimes forming stroma with a clustered 1–3 ascomata, gregarious, semi-immersed, raised, becoming superficial, dark brown, dome-shaped to subconical or subglobose, glabrous, coriaceous, ostiolate with inconspicuous papilla. ***Peridium*** 40–80(–130) µm wide at sides towards the apex, 10–25 µm wide at the base, composed of several layers of small, dark brown pseudoparenchymatous cells, outer layer fused with host cells, arranged in *textura angularis* to *textura globulosa*, inner layer composed of 1–3 strata of flattened cells, of *textura globulosa* to *textura prismatica*, with thick, palisade-like cells at the sides. ***Hamathecium*** composed of 1–3 µm wide, filiform, dense, septate, branched, pseudoparaphyses, anastomosed between and above the asci, embedded in a gelatinous matrix. ***Asci*** (58–)70–90(–105)(–119) × 12–15(–17) µm ($\bar{x}$ = 80.5 × 13.5 µm, SD = ± 13.2 × 1.8, n = 25), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, shortly pedicellate, apically rounded with well-developed ocular chamber. ***Ascospores*** 22–26(–30) × 4.5–7 µm ($\bar{x}$ = 24.6 × 5.4 µm, SD = ± 2.3 × 0.5, n = 30), overlapping 1–3-seriate, hyaline, fusiform, slightly curved, 1-septate, occasionally 2–3-septate, slightly constricted at the septum, the upper cell slightly larger

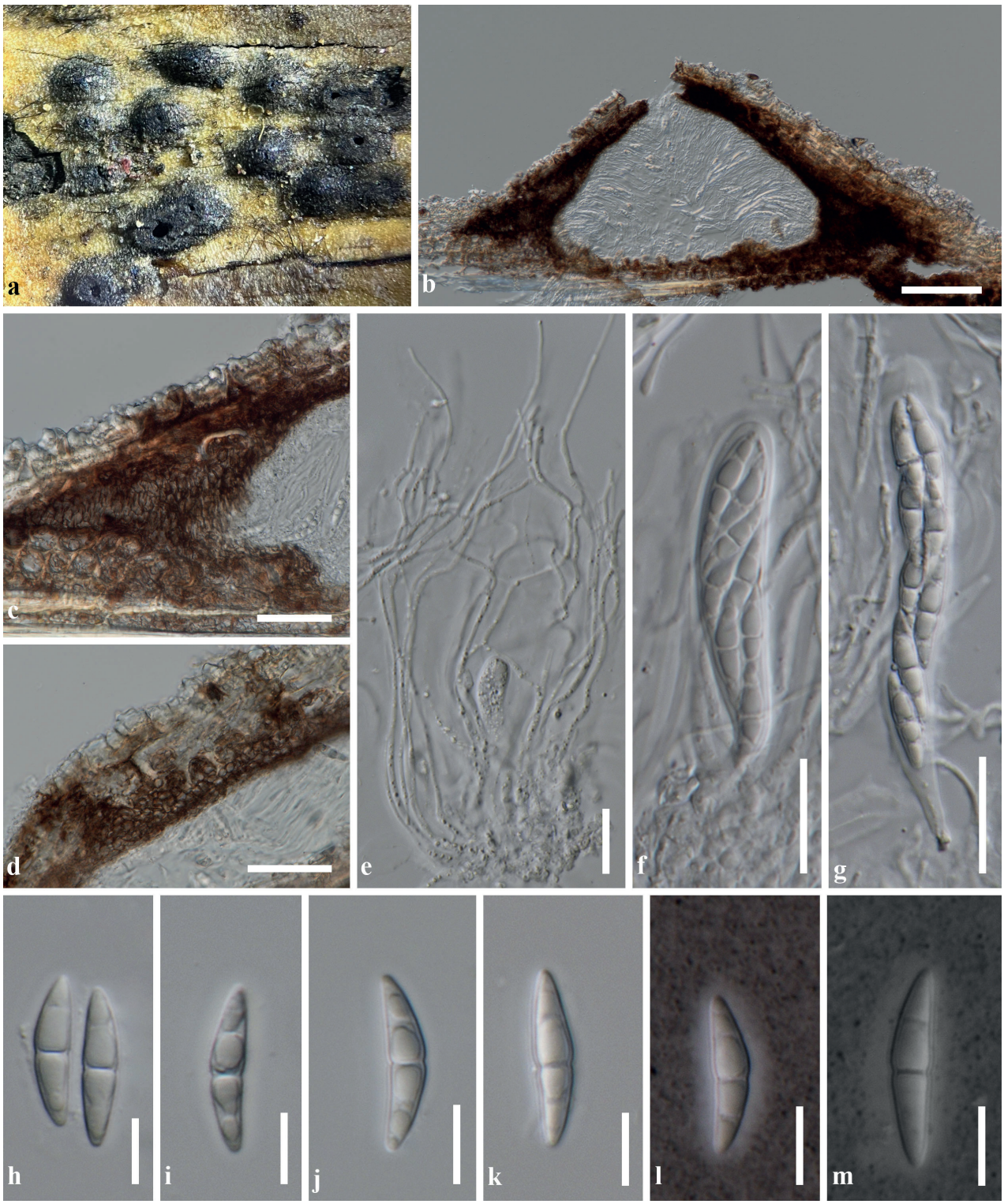

Figure 4. *Bambusicola hongheensis* (KUN-HKAS 129042, holotype) **A** the appearance of ascomata on the host surface **B** vertical section of an ascoma **C, D** peridia **E** pseudoparaphyses **F, G** asci embedded in pseudoparaphyses **H–K** ascospores **L, M** ascospores stained in India Ink show a thin mucilaginous sheath surrounding ascospores. Scale bars: 100 µm (**B**); 20 µm (**C–G**); 10 µm (**H–M**).

than the lower cell, smooth-walled, surrounded by a thin, indistinct, mucilaginous sheath. ***Asexual morph*:** Undetermined.

Distribution. China (Yunnan).

Specimen examined. China. Yunnan Province: Honghe Hani and Yi Autonomous Prefecture, Honghe County, rice terraces, on dead culm of bamboo, 26 Jan 2021, R. Phookamsak BN06 (KUN-HKAS 129042, **holotype**). **Notes**: As the axenic culture is not active, the sequences of SSU and *rpb2* were obtained from genomic DNA extracted from ascomata and dried culture.

Notes. Based on the NCBI nucleotide BLAST search of ITS sequence, *Bambusicola hongheensis* (KUN-HKAS 129042) has the closest match with *B. triseptatispora* (MFLUCC 11-0166, ex-type strain) with 98.71% similarity (Identities = 535/542 with no gap) and is similar to *B. loculata* (MFLU 15-0056, ex-type strain) with 98.69% similarity (Identities = 528/535 with 1 gap) and *B. splendida* (MFLUCC 11-0611) with 98.25% similarity (Identities = 392/399 with no gap). The NCBI nucleotide BLAST search of LSU sequence indicated that *B. hongheensis* has the closest match with *B. triseptatispora* (MFLUCC 11-0166, ex-type strain) and *B. didymospora* (MFLUCC 10-0557, ex-type strain) with 100% similarity (Identities = 802/802 with no gap) and is similar to *B. loculata* (MFLU 15-0056, ex-type strain) with 99.75% similarity (Identities = 813/815 with 2 gaps) and *B. nanensis* (MFLUCC 21-0063, ex-type strain) with 99.49% similarity (Identities = 785/789 with no gap). The NCBI nucleotide BLAST search of *rpb2* sequence indicated that *B. hongheensis* has the closest match with *B. loculata* (MFLU 15-0056, ex-type strain) with 99.90% similarity (Identities = 1042/1043 with no gaps) and is also similar to *B. triseptatispora* (MFLUCC 11-0166, ex-type strain) with 97.92% similarity (Identities = 990/1011 with no gap) and *B. massarinia* (voucher MFLU 11-0389) with 93.57% similarity (Identities = 975/1042 with 4 gaps).

Phylogenetic analyses of a concatenated ITS, LSU, *rpb2*, SSU and *tef1-α* sequence dataset demonstrated that *Bambusicola hongheensis* formed a separate branch (85% ML, 1.00 PP; Fig. 1), and clustered with *B. loculata* and *B. triseptatispora* with high support (100% ML, 1.00 PP; Fig. 1) and also clustered with the generic type of *Bambusicola*, *B. massarinia* with significant support (73% ML, 0.99 PP; Fig. 1). A nucleotide pairwise comparison of ITS sequence indicated that *B. hongheensis* differs from *B. triseptatispora* in 35/600 bp (5.83%), differs from *B. loculata* in 16/547 bp (2.92%) and differs from *B. massarinia* in 72/608 bp (11.84%). Whereas the nucleotide pairwise comparison of LSU sequence indicated that *B. hongheensis* is consistent with *B. triseptatispora* (0/802 bp) and *B. loculata* (1/816 bp), but differs from *B. massarinia* in 7/803 bp (0.87%). Furthermore, the nucleotide pairwise comparison of *rpb2* sequence indicated *B. hongheensis* is not significantly different from *B. loculata* (1/1043 bp), but differs from *B. triseptatispora* in 21/1012 bp (2.07%) and differs from *B. massarinia* in 68/1042 bp (6.52%).

Morphologically, *Bambusicola hongheensis* resembles *B. loculata* and *B. triseptatispora* in terms of the size range of ascomata, asci and ascospores. However, *B. hongheensis* has comparatively smaller ascomata (340–590 µm diam. of *B. hongheensis* vs. 350–600 µm diam. of *B. loculata* vs. 470–730 µm diam. of *B. triseptatispora*), shorter and wider asci ((58–)70–90(–105)(–119) × 12–15(–17) µm vs. 80–105 × 8–13 µm vs. (78–)80–100(–110) × 10–12(–14) µm, respectively) and sharing the size range of ascospores (22–26(–30) × 4.5–7 µm vs. 22–26.5 × 5–6 µm vs. (25–)26–30(–31) × 4–6 µm, respectively). The ascospores of *B. hongheensis* are typically hyaline, 1-septate, whereas *B. triseptatispora* has hyaline to pale brown and 3-septate ascospores (Dai et al. 2017). Distinguishing *B. loculata* from *B. hongheensis*, based on morphological characteristics alone is challenging, but *B. loculata* can be differentiated by its

larger ascomata and asci (Dai et al. 2015). However, a clear differentiation is achieved through phylogenetic evidence (Fig. 2) and nucleotide pairwise comparison of ITS gene region (2.92% difference).

Dictyosporiaceae Boonmee & K.D. Hyde, in Boonmee et al., Fungal Diversity: 10.1007/s13225-016-0363-z, [7] (2016)
Index Fungorum: IF551574

Notes. Dictyosporiaceae was introduced by Boonmee et al. (2016) to initially accommodate ten genera that were mainly represented by the hyphomycetous asexual morph, forming cheiroid, digitate, palmate and/or dictyosporous conidia. The sexual morph is scarcely known for this family, of which species of genera *Dictyosporium*, *Gregarithecium*, *Immotthia*, *Pseudocoleophoma*, *Sajamaea* and *Verrucoccum* have been represented as the sexual morph (Boonmee et al. 2016; Piątek et al. 2020; Atienza et al. 2021; Jiang et al. 2021a). Members of Dictyosporiaceae are morphologically diverse in various ecological niches, commonly known as saprobes on plant litter in terrestrial and freshwater habitats (Tanaka et al. 2015; Boonmee et al. 2016; Li et al. 2017; Crous et al. 2019; Rajeshkumar et al. 2021; Tian et al. 2022; Tennakoon et al. 2023). Besides, some genera were known as fungicolous (hyperparasites and mycoparasites) and lichenicolous fungi as well as inhabiting soil and herbivore dung (Iturrieta-González et al. 2018; Piątek et al. 2020; Atienza et al. 2021; Jiang et al. 2021a). An updated taxonomic description of Dictyosporiaceae was provided by Hongsanan et al. (2020) who listed 15 genera in this family, while Wijayawardene et al. (2022b) listed 17 genera in Dictyosporiaceae. Tennakoon et al. (2023) provided a backbone tree of Dictyosporiaceae and currently listed 20 genera in this family, namely *Aquadictyospora*, *Aquaticheirospora*, *Cheirosporium*, *Dendryphiella*, *Dictyocheirospora*, *Dictyopalmispora*, *Dictyosporium*, *Digitodesmium*, *Gregarithecium*, *Immotthia*, *Jalapriya*, *Neodendryphiella*, *Neodigitodesmium*, *Pseudocoleophoma*, *Pseudoconiothyrium*, *Pseudocyclothyriella*, *Pseudodictyosporium*, *Sajamaea*, *Verrucoccum* and *Vikalpa*.

***Trichobotrys* Penz. & Sacc., Malpighia 15(7–9): 245 (1902) [1901]**
Index Fungorum: IF10275

Notes. *Trichobotrys* was introduced by Penzig and Saccardo (1902) to accommodate the type species *T. pannosus* [as '*pannosa*']. The genus is scarcely known and only five species are available in Index Fungorum (http://www.indexfungorum.org; accessed on 25 May 2023), of which only *T. effusus* [as '*effusa*'] has molecular data available in GenBank. The genus is known as only a hyphomycetous asexual morph and is characterised by dark brown to black, effuse to velvety colonies, partly immersed to superficial mycelium, non-stromatic, macronematous, mononematous, dark brown to reddish-brown, verruculose or echinulate conidiophores, bearing short, smooth, fertile, often unciform lateral branches, with sterile, setiform apex, polyblastic, integrated, terminal or discrete, determinate, ellipsoidal, spherical or subspherical conidiogenous cells and catenated, in branched acropetal chains, spherical, brown, aseptate,

verruculose or minutely echinulate conidia (Ellis 1971; D'Souza and Bhat 2001). The taxonomic classification of the genus is doubtful due to the lack of molecular phylogeny. Recently, Wijayawardene et al. (2022b) treated *Trichobotrys* as Ascomycota genus *incertae sedis*, pending future study. In the present study, the novel species, *T. sinensis* is introduced and the phylogenetic analyses demonstrated the genus affinity in Dictyosporiaceae.

Trichobotrys sinensis **Phookamsak, Bhat & Hongsanan, sp. nov.**
Index Fungorum: IF900832
Fig.5

Etymology. The specific epithet "*sinensis*" refers to the country, China, where the holotype was collected.

Holotype. KUN-HKAS 129041.

Description. Saprobic on dead culm of *Brachiaria mutica*, submerged in a small stream. ***Sexual morph*****:** Undetermined. ***Asexual morph*****:** ***Colonies*** dull, black, effuse, visible as hairy fluffy on the host. ***Mycelia*** up to 1 mm long, 2–4 µm wide, superficial, composed of brown to dark brown, branched, septate, thick-walled, echinulate hyphae. ***Conidiophores*** (9–)15–40(–70) × 2–4 µm ($\bar{x}$ = 26.9 × 3.3 µm, n = 30), sometimes reduced to conidiogenous cells, macronematous, mononematous, straight or flexuous, brown to dark brown, septate, verruculose or echinulate, bearing short, lateral, unciform, fertile branches, with setiform apex. ***Conidiogenous cells*** 1–3.5 × 2.5–5 µm ($\bar{x}$ = 2.1 × 2.5 µm, n = 30), polyblastic, subhyaline to pale brown, ellipsoidal or hemispherical (2.5–5 × 3.5–6 µm), intercalary or terminal, integrated or discrete, sometimes denticulate on branches. ***Conidia*** 7–11 × 8–12 µm ($\bar{x}$ = 10 × 10 µm, n = 30) simple, solitary, brown to dark brown, spherical, aseptate, verruculose; sometimes in short acropetal chains. *In vitro* ***Conidiomata*** 280–470 µm high, 280–570 µm diam., black, pycnidial, solitary or clustered in a small group (2–4-loculate), scattered to gregarious, globose to subglobose, glabrous, covered by brown to dark brown mycelium, becoming a packed pycnidial wall, ostiolate, with inconspicuous, minute papilla. ***Pycnidial wall*** 20–35 µm wide, thick-walled of unequal thickness, thicker at the base, composed of multi-layered, dark brown to black pseudoparenchymatous cells, outer layers composed of *textura intricata*, inner layers composed of flattened cells of *textura angularis* to *textura prismatica*. ***Conidiophores*** reduced to conidiogenous cells. ***Conidiogenous cells*** (6.5–)10–16(–25) × 2–4.5 µm ($\bar{x}$ = 13.4 × 3.2 µm, n = 30), holoblastic to phialidic, hyaline, cylindrical to subcylindrical, terminal or intercalary, septate, smooth-walled, with distinct collarette. ***Conidia*** 2–3 × 1.5–2.5 µm ($\bar{x}$ = 2.8 × 2 µm, n = 30) hyaline, ellipsoidal to ovoid, aseptate, smooth-walled, with a guttulate.

Culture characteristics. Colonies on PDA reaching 25–28 mm diam. after two weeks at room temperature (20–27 °C), medium dense, circular, surface smooth with an entire edge, flattened, slightly raised, fairly fluffy to feathery; from above, initially white, with cream conidial masses, becoming white to cream at the margin, pale yellowish towards the centre with age; from below, white at the margin, dark grey to black towards the centre; pigmentation not produced in PDA. Sporulation in PDA after two weeks, initially visible as cream conidial masses, later forming black conidiomata with hyaline to cream conidial masses on colonies.

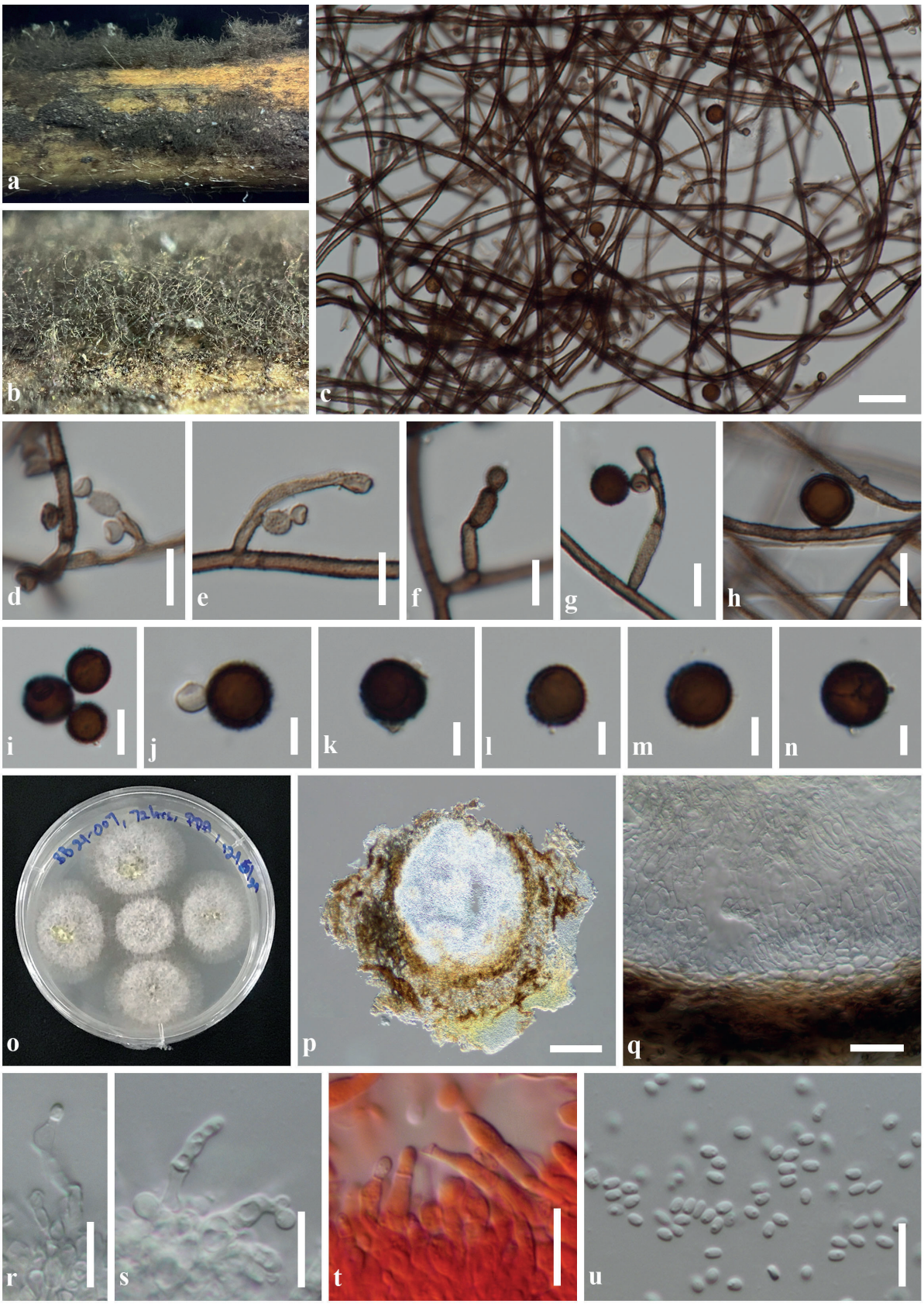

Figure 5. *Trichobotrys sinensis* (KUN-HKAS 129041, holotype) **A, B** the appearance of colonies on the host surface **C** mycelium **D–H** conidiophores bearing conidiogenous cells and conidia **I** conidia in a short acropetal chain **J–N** conidia **O** culture characteristics on PDA **P** conidioma forming on PDA after eight weeks **Q** pycnidial wall **R–T** conidiogenous cells (note: **T** = stained in Congo red) **U** conidia. Scale bars: 100 µm (**P**); 50 µm (**C**); 10 µm (**D–H, Q–U**); 5 µm (**J–N**).

Distribution. China (Yunnan).

Specimen examined. China. Yunnan Province: Xishuangbanna Dai Autonomous Prefecture, Mengla County, Bubeng, 21°36'30.13"N, 101°35'52.54"E, 664 + 5 m a.s.l., on culms of *Brachiaria mutica* submerged in a freshwater stream, 27 Apr 2021, R. Phookamsak BB21-007 (KUN-HKAS 129041, **holotype**), ex-type living culture, RPC 21-007 = KUNCC 23-14554.

Notes. Based on NCBI nucleotide BLAST search of ITS sequence, the closest hit of *Trichobotrys sinensis* (RPC 21-007/ KUNCC 23-14554) is *Gregarithecium* sp. DQD-2016a strain MFLUCC 13-0853 with 99.03% similarity (Identities = 508/513 with 2 gaps) and is similar to *Trichobotrys effusus* [as '*effusa*'] isolate 1179 (93.51% similarity, Identities = 504/539 with 13 gaps), *T. effusus* [as '*effusa*'] strain FS522 (93.35% similarity, Identities = 477/511 with 12 gaps) and *T. effusus* [as '*effusa*'] isolate HNNUZCJ-94 (93.08% similarity, Identities = 471/506 with 16 gaps). In LSU nucleotide BLAST search, the closest hit of *T. sinensis* (RPC 21-007/ KUNCC 23-14554) is *Gregarithecium* sp. DQD-2016a strain MFLUCC 13-0853 with 99.88% similarity (Identities = 848/849 with 1 gap) and is similar to *Gregarithecium* sp. isolate L13E (99.40% similarity, Identities = 830/835 with 3 gaps) and *G. curvisporum* HHUF 30134 (97.74% similarity, Identities = 822/841 with 5 gaps).

Multigene phylogenetic analyses of a concatenated ITS, LSU, SSU and *tef1-α* sequence dataset demonstrated that *Trichobotrys sinensis* (RPC 21-007/ KUNCC 23-14554) shared a branch length with *Gregarithecium* sp. DQD-2016a strain MFLUCC 13-0853 and *Gregarithecium* sp. isolate GMB 1217 and clustered with the clade of *T. effusus* (Fig. 2). However, *Gregarithecium* sp. DQD-2016a strain MFLUCC 13-0853 and *Gregarithecium* sp. isolate GMB 1217 are unpublished strains. Hence, *Trichobotrys sinensis* (RPC 21-007/ KUNCC 23-14554) is introduced herein as a new species and *Gregarithecium* sp. (strains MFLUCC 13-0853 and isolate GMB 1217) is re-identified as *T. sinensis* to avoid misidentification. Morphologically, *T. sinensis* (RPC 21-007/ KUNCC 23-14554) is typical of *Trichobotrys*, but can be distinguished from *T. effusus*, *T. pannosus*, *T. ramosus* and *T. trechisporus* in having larger conidia (2 µm diam. of *T. effusus* vs. 4 µm diam. of *T. pannosus* vs. 3–5 µm diam. of *T. ramosus* vs. 5 × 3 µm or 4 µm diam. of *T. trechisporus*) (Berkeley and Broome 1873; Penzig and Saccardo 1902; Petch 1917; Ellis 1971; D'Souza and Bhat 2001).

Periconiaceae Nann., Repert. mic. uomo: 482 (1934)
Index Fungorum: IF81124

Notes. Periconiaceae was resurrected by Tanaka et al. (2015) who provided an updated taxonomic treatment and placed the family in the suborder Massarineae (Pleosporales). Tanaka et al. (2015) accepted four genera namely, *Periconia* (Tode 1791), *Noosia* (Crous et al. 2011), *Bambusistroma* (Adamčík et al. 2015) and *Flavomyces* (Knapp et al. 2015), as well as included *Sporidesmium tengii* in the Periconiaceae. Yang et al. (2022b) re-circumscribed genera *Bambusistroma*, *Noosia* and *Periconia*, based on type studies compared with their new findings. Hence, Yang et al. (2022b) treated *Bambusistroma* and *Noosia* as synonyms of *Periconia* due to morphological resemblances and phylogenetic evidence, while the generic status of *Flavomyces* is doubted pending further studies.

***Periconia* Tode, Fung. mecklenb. sel. (Lüneburg) 2: 2 (1791)**
Index Fungorum: IF9263

Notes. *Periconia* was established by Tode (1791) to accommodate dematiaceous hyphomycetes that were unique in forming macronematous, mononematous, branched, septate, pigmented conidiophores, bearing spherical conidial heads that produced globose to ellipsoidal, aseptate, verruculose to echinulate, pigmented conidia (Tanaka et al. 2015; Hongsanan et al. 2020; Yang et al. 2022b). Species of *Periconia* are typically known by their asexual morph; only a few species have been reported with their sexual morph (Tanaka et al. 2015; Hongsanan et al. 2020; Yang et al. 2022b). *Periconia* species have been commonly reported as saprobes occurring on various host substrates in terrestrial and aquatic habitats worldwide. However, some species have been reported as endophytes, plant pathogens (e.g. *P. cicirnata*, *P. digitata* and *P. macrospinosa*) and human pathogens, as well as producing economically-important bioactive compounds (Sarkar et al. 2019; Gunasekaran et al. 2021; Hongsanan et al. 2020; Samarakoon et al. 2021; Azhari and Supratman 2021; Yang et al. 2022b; Su et al. 2023). Even though over 200 species of *Periconia* were listed in Index Fungorum (http://www.indexfungorum.org; accessed on 25 May 2023), less than half of a quarter have molecular data to clarify phylogenetic placement. Of these, the type species of *Periconia*, *P. lichenoides*, also lacks molecular data. This suggests that there is a huge research gap in the taxonomic classification of the genus *Periconia*. In the present study, we follow the latest taxonomic treatment of Yang et al. (2022b) and Su et al. (2023) and the new species *Periconia kunmingensis* occurring on fern, is introduced.

***Periconia kunmingensis* Phookamsak & Hongsanan, sp. nov.**
Index Fungorum: IF900833
Fig.6

Etymology. The specific epithet "*kunmingensis*" refers to the Kunming Institute of Botany, Kunming, Yunnan, China, where the holotype was collected.

Holotype. KUN-HKAS 102239.

Description. Saprobic on dead, standing rachis of a fern. ***Sexual morph*:** Undetermined. ***Asexual morph*: *Colonies*** on the substrates superficial, numerous, effuse, brown to dark brown, floccose. ***Mycelia*** 6–7 µm wide, partly superficial, composed of septate, branched, dark brown hyphae. ***Conidiophores*** 100–260 µm long, 7–12 µm diam., macronematous, mononematous, solitary, dark brown, 3–5-septate, unbranched below, branched only at the apex, erect, straight or slightly flexuous, sometimes swollen near the base, with 1–2 spherical guttules in each cell, forming a spherical head at the tip. ***Conidiogenous cells*** (4–)5–8(–10) × 2.5–5(–6) µm ($\bar{x}$ = 6.4 × 4 µm, n = 30) mono- to polyblastic, terminal, discrete, subspherical to fusiform, subhyaline to pale brown, verruculose. ***Conidia*** 4.5–7(–9) × 4–7(–8) µm ($\bar{x}$ = 6 × 5.9 µm, n = 50), solitary to catenate, in acropetal short chains, subglobose to globose, subhyaline to pale brown, aseptate, minutely verruculose to short-spinulose.

Culture characteristics. Colonies on PDA reaching 23–25 mm diam. after two weeks at room temperature (20–30 °C). Colony dense, circular, flattened,

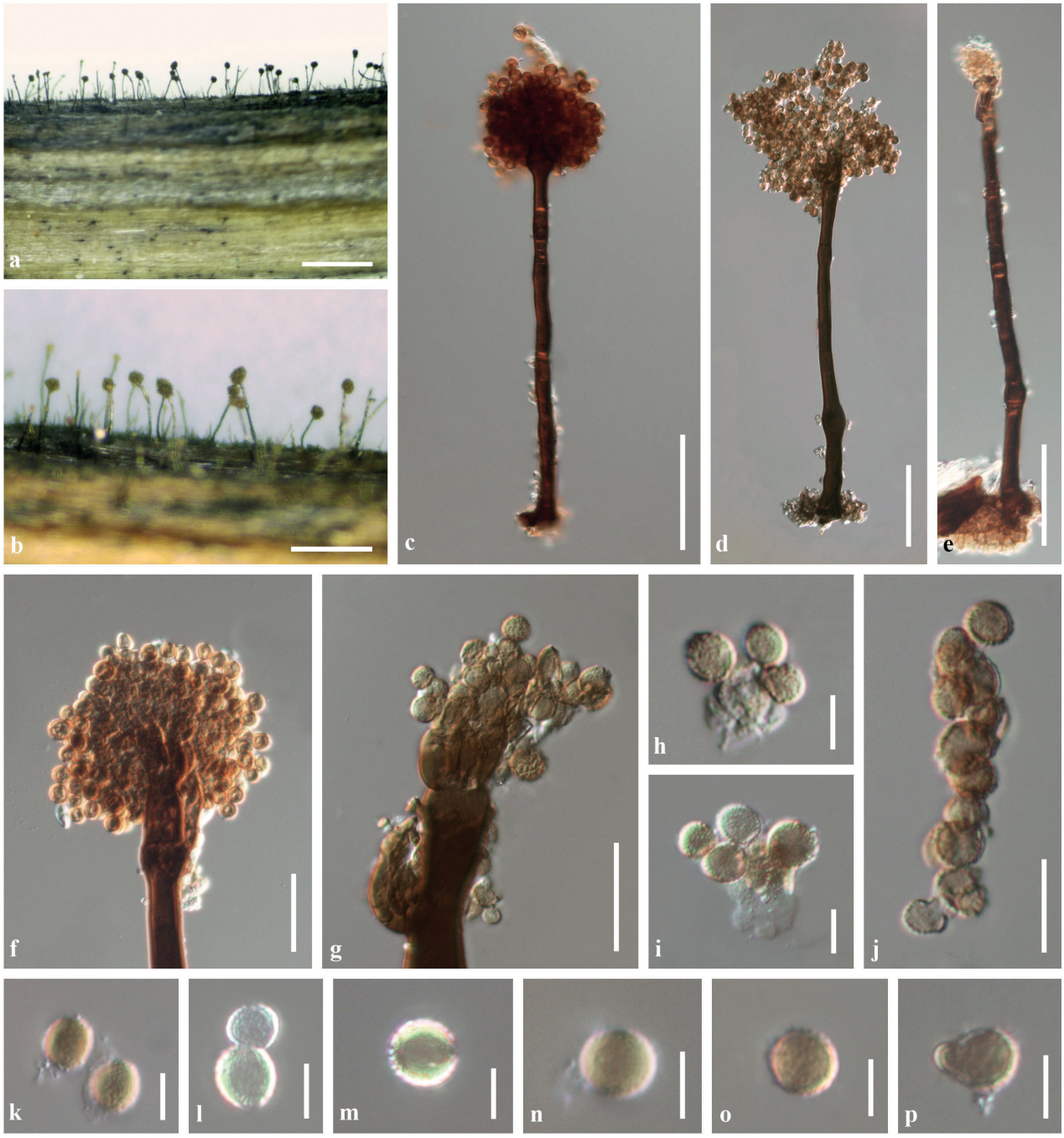

Figure 6. *Periconia kunmingensis* (KUN-HKAS102239, holotype) **A, B** the appearance of fungal colonies on host substrate **C–E** conidiophores **F, G** closed-up conidiophores with spherical heads **H, I** conidiogenous cells bearing conidia **J** conidia catenate in acropetal short chain **K–P** conidia. Scale bars: 500 µm (**A, B**); 50 µm (**C–E**); 20 µm (**F**, **G**); 10 µm (**J**); 5 µm (**H, I, K–P**).

slightly raised, surface smooth, edge fimbriate, velvety, with fairly fluffy at the margin; colony from above, white to white-grey, separated from the centre by greenish-grey radiating near the margin; colony from below, pale yellowish to cream at the margin, deep green near the margin, with dark green concentric ring, separating the margin from greenish-grey to dark green centre; slightly produced light yellowish pigment tinted agar.

Distribution. China (Yunnan).

Specimen examined. China. Yunnan Province: Kunming, Kunming Institute of Botany, on dead, standing rachis of a fern, 23 Sep 2016, R. Phookamsak KIB004 (KUN-HKAS 102239, **holotype**), ex-type living culture RPC 15-017 = KUMCC 18-0173 = MFLUCC 18-0679. Addition GenBank no: *rpb2* = OR547996.

Notes. Based on the NCBI nucleotide BLAST search of ITS sequence, the closest hits of *Periconia kunmingensis* are *Periconia* sp. strain 8R5B1-3 and *Periconia* sp. isolate LS77 with 99.80% similarity (Identities = 507/508 and 498/499 with no gap, respectively) and is similar to *P. verrucosa* isolate HNNU0545 with 99.60% similarity (Identities = 502/504 with 1 gap), *Periconia* sp. strain MFLUCC 17-0087 with 99.59% similarity (Identities = 482/484 with 1 gap) and *P. elaeidis* isolate PT49 with 99.57% similarity (Identities = 464/466 with 1 gap). In the LSU nucleotide BLAST search, *P. kunmingensis* is similar to *P. verrucosa* isolate MFLUCC 17-2158 (Identities = 847/847 with no gap), *Periconia* sp. KT 1825 (Identities = 843/843 with no gap), *P. elaeidis* strain GZCC19-0435 (Identities = 842/842 with no gap), *P. cookei* strain IHEM:28143 (Identities = 826/826 with no gap), Pleosporales sp. A1039 (Identities = 815/815 with no gap) and *P. verrucosa* isolate w232_2 (Identities = 812/812 with no gap), isolate Lu53_1 (Identities = 807/807 with no gap) and isolate Lu98_2 (Identities = 796/796 with no gap), with 100% similarities. In the *tef1-α* nucleotide BLAST search, the closest hit of *P. kunmingensis* is *Periconia* sp. KT 1820A (Identities = 745/747 with no gap) and *P. delonicis* voucher MFLU 20-0696 (Identities = 736/738 with no gap) with 99.73% similarity. *Periconia kunmingensis* is also similar to *P. delonicis* strain MFLUCC 17-2584 and *P. verrucosa* isolate MFLUCC 17-2158 with 99.60% similarity (Identities = 744/747 with no gap).

Phylogenetic analyses of the concatenated ITS, LSU, SSU and *tef1-α* sequence data showed that *Periconia kunmingensis* formed a distinct branch basally to *P. verrucosa*, *P. cookei*, *P. palmicola*, *P. elaeidis* and *P. delonicis*, respectively (Fig. 3). The ITS nucleotide pairwise comparison indicated that *P. kunmingensis* differs from *P. verrucosa* (MFLUCC 17–2158, ex-type strain) in 3/512 bp (0.59%), differs from *P. cookei* in 2/465 bp (0.43%) of MFLUCC 17–1399 and 3/465 bp (0.65%) of UESTCC 22.0134 and differs from *P. elaeidis* (MFLUCC 17–0087, ex-type strain) in 14/518 bp (2.70%). The *rpb2* nucleotide pairwise comparison indicated that *P. kunmingensis* differs from *P. verrucosa* (UESTCC 22.0136) in 35/849 bp (4.12%), differs from *P. cookei* (UESTCC 22.0134) in 30/819 bp (3.66%) and differs from *P. delonicis* (MFLUCC 17–2584, ex-type strain) in 54/1073 bp (5.03%). The *tef1-α* nucleotide pairwise comparison indicated that *P. kunmingensis* differs from *P. verrucosa* (MFLUCC 17–2158, ex-type strain) in 108/929 bp (11.63%), differs from *P. cookei* in 4/736 bp (0.54%) of MFLUCC 17-1399 and 107/906 bp (11.81%) of MFLUCC 17-1679, differs from *P. palmicola* (MFLUCC 14-0400, ex-type strain) in 19/991 bp (1.92%) and differs from *P. delonicis* (MFLUCC 17–2584, ex-type strain) in 105/987 bp (10.64%).

Distinguishing *Periconia kunmingensis* from other *Periconia* species, based on morphological features alone, presents challenges. However, differentiation can be achieved by considering variations in the sizes of conidiophores, conidiogenous cells and conidia, as well as the number of conidiophores originating from the stromatic, swollen part of the conidiophores, septation characteristics and the occurrence and origin of the host. A comprehensive morphological comparison is provided in Table 4.

Table 4. Morphological comparison of *Periconia kunmingensis* with other related species. A novel species is indicated by black bold.

Species	Conidiophores	Conidiogenous cells	Conidia	Host occurrence	Origin	Reference
Periconia cookei (IMI 16174, holotype)	360–800 µm high, singly or in groups (up to six), 2–6-septate, swollen at the apex, dark brown at the lower part, pale brown at the upper part	7–11 µm diam., spherical, ovoid or pyriform, initially hyaline, smooth-walled, becoming brown, verrucose on age	13–16 µm diam., with the wall up to 2 µm thick, spherical, brown, verrucose, singly or in short chains of 2–3 on conidiogenous cells	On stems of *Heracleum sphondylium*	Great Britain	Mason and Ellis (1953)
Periconia delonicis (MFLU 18–2100, holotype)	360–420 µm high, singly, septate, greyish-brown to dark brown, unbranched, smooth to minutely verruculose	Monoblastic, proliferating, ovoid to globose, hyaline	5.5–7 µm diam., subglobose to globose, subhyaline to pale brown, verruculose, singly or in short chains	On pods of *Delonix regia*	Thailand	Jayasiri et al. (2019)
Periconia elaeidis (MFLU 18–0626, holotype)	200–400 µm high, singly, 4–7-septate, grayish-brown to dark brown, unbranched, smooth to minutely verruculose	Polyblastic, proliferating, ovoid to globose, pale brown, smooth	4.5–6.5 µm diam., subglobose to globose, subhyaline to pale brown, verruculose, solitary	On dead leaves of oil palm	Thailand	Hyde et al. (2018)
***Periconia kunmingensis* (KUN-HKAS 102239, holotype)**	**100–260 µm high, solitary, 3–5-septate, dark brown, unbranched below, branched only at the apex, sometimes swollen near the base**	**(4–)5–8(–10) × 2.5–5(–6) µm, mono- to polyblastic, subspherical to fusiform, subhyaline to pale brown, verruculose**	**4.5–7(–9) × 4–7(–8) µm, subglobose to globose, subhyaline to pale brown, minutely verruculose to short-spinulose, solitary to catenate, in acropetal short chains**	**On dead standing rachis of a fern**	**Yunnan, China**	**This study**
Periconia palmicola (MFLU 14-0198, holotype)	151–188 µm high, singly or in groups, septate, dark brown to black, branched at the apex	3–3.5 × 3–4.8 µm, mono- to polyblastic, globose, hyaline to subhyaline	5.1–7.4 × 4.8–6.1 µm, subglobose to globose, light brown to brown, verruculose, solitary to catenate, in acropetal short chains	On dead, fallen leaves of unidentified palm	Thailand	Hyde et al. (2020)
Periconia verrucosa (MFLU 17–1516, holotype)	170–296 µm high, singly, 2–4-septate, dark brown, with 3–4 short branches at the apex	11–26 × 6–14 µm, mono- to polyblastic, retrogressive, oblong, pale brown	7–15 µm diam., globose, dark brown to reddish-brown, verrucose, acrogenous in branched chains	On dead stems of *Clematis viticella*	Belgium	Phukhamsakda et al. (2020)

Discussion

This paper, in the series "Exploring ascomycete diversity in Yunnan", presents three novel taxa in the suborder Massarineae (Pleosporales), viz. *Bambusicola hongheensis* (Bambusicolaceae), *Periconia kunmingensis* (Periconiaceae) and *Trichobotrys sinensis* (Dictyosporiaceae). The novelties of these taxa were well-justified, based on morphological characteristics and phylogenetic evidence, as well as the differences in nucleotide pairwise comparison of reliable genes amongst closely-related taxa. This provides a better fundamental knowledge of the taxonomic framework of ascomycetes in this region.

Bambusicola hongheensis is justified, based on multigene phylogeny and the differences in nucleotide pairwise comparison of the ITS region with closely-related species. Monkai et al. (2021) mentioned that many *Bambusicola* species have similar morphology, but these species can be distinguished, based on multigene phylogeny and they also recommended the use of the *rpb2* gene for delineating species level of *Bambusicola*. Unfortunately, the *rpb2* sequence did not distinguish *B. hongheensis* from *B. loculata* in the present study; however, the ITS region of *B. hongheensis* showed > 1.5% nucleotide differences amongst the closely-related species viz. *B. loculata*, *B. massarinia* and *B. triseptatispora*. This provides adequate justification for the species' novelty following the recommendation of Jeewon and Hyde (2016).

Although many *Bambusicola* species are morphologically somewhat similar, it is notable that they can also be distinguished by their represented asexual morphs that are easily sporulated *in vitro* as well as on natural substrates. For instance, coelomycetous asexual morphs of *B. massarinia* and *B. triseptatispora* sporulated *in vitro*; of which *B. massarinia* can be distinguished from *B. triseptatispora* in having pale brown, 1-septate, cylindrical conidia (Dai et al. 2012). Whereas conidia of *B. triseptatispora* are light brown, 3-septate, cylindrical to cylindrical-clavate (Dai et al. 2017). Unfortunately, the asexual morphs of *B. hongheensis* and *B. loculata* have not yet been determined. Hence, further studies on their asexual morphs sporulated *in vitro* should be carried out for a better understanding through their sexual-asexual reproduction, as well as gaining criteria of species delineation.

Trichobotrys sinensis is morphologically typical of *Trichobotrys*. *Trichobotrys* was previously classified into Ascomycota genus *incertae sedis* (Wijayawardene et al. 2022b). Although the sequence data of the type species of *Trichobotrys* is currently unavailable, the inclusion of available sequence data along with our new species that morphologically align well with *Trichobotrys* in the phylogenetic analyses, provides compelling evidence supporting the placement of *Trichobotrys* within the Dictyosporiaceae. This information contributes to our understanding of taxonomic relationships and highlights the need for further studies to explore the molecular characteristics and genetic diversity of *Trichobotrys* species within the Dictyosporiaceae.

Synanamorph is the term of use for fungal taxa producing two or more different asexual morphs which were often linked by the sporulation in culture (Wijayawardene et al. 2021a, 2022c). Many fungal taxa have been reported for their synanamorphism, such as *Botryosphaeria* with dichomera-like *in vitro* and *Neofusicoccum* (as *Fusicoccum*) (Barber et al. 2005), *Barbatosphaeria fagi* (≡ *Calosphaeria fagi*) with ramichloridium-like and sporothrix-like asexual

morphs (Réblová et al. 2015) and *Synnemasporella* with sporodochial and pycnidial asexual morphs on natural hosts (Fan et al. 2018). The formation of two or more different morphs in a single species has led to misidentification and the distinct morphs have been somehow counted as different species (Wijayawardene et al. 2021a, 2022c). It has further caused problems in the dual nomenclature of pleomorphic fungi that proposed one name for one fungus (McNeill and Turland 2012; Rossman et al. 2015). Interestingly, *Trichobotrys sinensis* formed two different asexual morphs, one in nature (as *Trichobotrys*) and another *in vitro* (pycnidial coelomycetous asexual morph) which is the first report of the synanamorphism for the genus *Trichobotrys*. This new finding provides insight into pleomorphism which is essential in further revision of taxonomic boundaries and easing of existing complications. It is noteworthy that *Trichobotrys* formed a well-resolved clade with *Gregarithecium* in the present phylogenetic analyses. Unfortunately, the sexual morph of *Trichobotrys* has not yet been determined. Similarly, the asexual morph of *Gregarithecium* has also not yet been reported. Hence, the sexual-asexual connection between *Gregarithecium* and *Trichobotrys* is doubtful pending future study.

Periconia kunmingensis is introduced in this paper, based on its morphology and phylogeny. Morphologically, *P. kunmingensis* fits well with the generic concept of *Periconia* and its phylogenetic affinity is also well-clarified within Periconiaceae. It is noteworthy that the ITS region could not be used to separate *P. kunmingensis* from other closely-related species, including *P. cookei* and *P. verrucosa*, based on the nucleotide pairwise comparison. Whereas, the ITS sequences of *P. delonicis*, *P. elaeidis* and *P. palmicola* are unavailable. The interspecific variation amongst these species may be questionable. However, the *rpb2*, and *tef1-α* gene regions which have sufficient genetic variation can be used to distinguish these species. Nevertheless, the *rpb2* gene of most *Periconia* species is unavailable. Therefore, the sequences of protein-coding genes (e.g. *rpb2* and *tef1-α*) are acquired to offer reliable phylogenetic markers for species delineation.

Over the past five years, the number of newly-described fungal species has been rapidly increasing in Yunnan. Several novel and interesting ascomycetes were described and illustrated from various host plants and on different substrates and habitats. Many studies of ascomycetous taxonomy on specific host substrates have become essential and challenging for mycologists across the region. For instance, D.N. Wanasinghe and his colleagues (2018–2022) carried out research studies on fungal biogeography and published over 40 novel taxa of wood-inhabiting fungi, as well as other substrates in this region (Bao et al. 2019; Wanasinghe et al. 2020, 2021; Yasanthika et al. 2020; Mortimer et al. 2021; Ren et al. 2021; Wijayawardene et al. 2022a; Maharachchikumbura et al. 2022). Simultaneously, S. Tibpromma and her colleagues (2018–2022) have also carried out research studies of fungal taxonomy and diversity on various host plants, such as agarwood, coffee, *Pandanus*, para rubber and tea plants. They introduced 20 novel taxa from *Pandanus* (Tibpromma et al. 2018), while taxonomic studies on the other plants (approximately 45 novel species on agarwood, coffee and para rubber) are pending (S. Tibpromma, personal data information). A comprehensive study of freshwater Sordariomycetes in Yunnan has been carried out by Luo et al. (2018a, b, 2019) who introduced more than 50 novel taxa and reported more than 75 freshwater Sordariomycetes species

in Yunnan. Even though these studies unravelled a substantial number of ascomycetes in Yunnan, there is still a huge gap of knowledge in hitherto undescribed novel taxa in this region. If considering only the plant and fungal ratio, many of the so far fungal taxonomic studies on land plants have underestimated these in Yunnan, especially on those economic and horticulture plants. Hence, the inventory of ascomycetes on these land plants will be interesting in further research studies.

Conclusion

In conclusion, this study introduces three novel species in the suborder Massarineae (Pleosporales): *Bambusicola hongheensis*, *Periconia kunmingensis* and *Trichobotrys sinensis*. These species were found as saprobes in different habitats, with *B. hongheensis* and *P. kunmingensis* occurring in terrestrial environments, while *T. sinensis* was discovered in a freshwater stream. Notably, the presence of *Trichobotrys* in a freshwater habitat is a significant finding, as it aligns with other aquatic lignicolous species within the family Dictyosporiaceae. The novelty of *B. hongheensis* is supported by multigene phylogeny and nucleotide pairwise comparison, although further genetic analysis is recommended. Differentiation between *Bambusicola* species can also be achieved through the examination of their asexual morphs. *Trichobotrys sinensis*, morphologically typical of *Trichobotrys*, is phylogenetically placed within Dictyosporiaceae and highlights the need for additional studies on molecular characteristics and genetic diversity within the genus. The observation of synanamorphism in *T. sinensis* adds complexity to its morphological identification and taxonomic boundaries. The introduction of *Periconia kunmingensis* is supported by its morphology and phylogenetic affinity within the family Periconiaceae, although the use of protein-coding genes is recommended for reliable species delineation. This study contributes to our understanding of ascomycete diversity in Yunnan and emphasises the importance of such investigations to enhance our knowledge of newly-discovered taxa.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualisation: RP, SH. Data curation: RP. Formal analysis: DNW, SH. Funding acquisition: IP, JX, NX, SL, TMD. Investigation: DJB, DNW, RP, SH. Methodology: DNW, RP, SH. Project administration: RP, NS, JK. Supervision: SL, JX, IP, NX, PEM.: Writing – original draft: RP, SH, DNW. Writing – review and editing: DJB, NS, IP, JK, JX, NX, PEM, TMD.

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Data availability

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

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Research Article

Morphology and phylogeny of *Cytospora* (Cytosporaceae, Diaporthales) species associated with plant cankers in Tibet, China

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Abstract

During our biodiversity investigations in Tibet, China, typical Cytospora canker symptoms were observed on branches of hosts *Myricaria paniculate*, *Prunus cerasifera* and *Sibiraea angustata*. Samples were studied, based on morphological features coupled with multigene phylogenetic analyses of ITS, *act*, *rpb2*, *tef1* and *tub2* sequence data, which revealed two new species (*Cytospora myricicola* **sp. nov.** and *C. sibiraeicola* **sp. nov.**) and a known species (*C. populina*). In addition, *Cytospora populina* is newly discovered on the host *Prunus cerasifera* and in Tibet.

Key words: Ascomycota, molecular phylogeny, novel taxa, Sordariomycetes, taxonomy

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Introduction

The genus *Cytospora* (Cytosporaceae, Diaporthales, Sordariomycetes, Ascomycota) was proposed by Ehrenberg (1818) and *C. chrysosperma* was selected as the lectotype later (Donk 1964). *Cytospora* has priority over *Leucocytospora*, *Leucostoma*, *Valsa*, *Valsella* and *Valseutypella* based on the dual-nomenclature criterion (Adams et al. 2005; Rossman et al. 2015). Members of *Cytospora* are characterised by the single or labyrinthine, loculate stromata, filamentous conidiophores or asci and allantoid hyaline conidia or ascospores (Spielman 1985; Adams et al. 2005; Norphanphoun et al. 2017, 2018; Fan et al. 2020; Shang et al. 2020). Species identification in *Cytospora* was previously largely based on the host affiliation and morphological descriptions; however, molecular phylogeny combined with morphology and host affiliation have became the main approaches recently (Fan et al. 2020; Shang et al. 2020; Zhu et al. 2020). Currently, over 690 species epithets of *Cytospora* have been listed in Index Fungorum (http://www.indexfungorum.org/; 2023). However, most of these were regarded as synonyms and most descriptions were unable to identify them accurately (Adams et al. 2005; Fan et al. 2020; Pan et al. 2020).

Cytospora is distributed worldwide and often known to be associated with plant diseases (Monkai et al. 2021; Pan et al. 2021; Lin et al. 2022; Travadon et al. 2022).

For example, *C. chrysosperma* is the main canker disease pathogen of polar and willow trees in China (Fan et al. 2014; Wang et al. 2015; Lin et al. 2023); *C. kuanchengensis* and additional five species are associated with Chinese chestnut cankers (Jiang et al. 2020); Fifteen *Cytospora* species were identified from destructive canker and dieback pathogens of woody hosts in the USA (Lawrence et al. 2018).

The Tibet Tibetan Autonomous Region is located on the Qinghai-Tibet Plateau, which is known as the third pole of the earth. During our biodiversity investigations in Tibet, typical fruiting bodies of *Cytospora* were discovered from *Myricaria paniculate* (Tamaricaceae), *Prunus cerasifera* (Rosaceae) and *Sibiraea angustata* (Rosaceae). The aim of the present study was to identify *Cytospora* species from the three hosts based on morphological features and molecular phylogeny of combined sequence data.

Materials and methods

Sample collection, morphology and isolation

Our biodiversity investigations were conducted in Lhasa and Shigatse cities in Tibet Tibetan Autonomous Region, China during 2022 and 2023. Diseased branches of *Myricaria paniculate*, *Prunus cerasifera* and *Sibiraea angustata* were observed and collected, packed in paper bags and returned to the laboratory for morphological study and fungal isolation.

Observation and description of *Cytospora* species was based on fruiting bodies naturally formed on the host barks. Ascostromata and conidiomata from tree barks were sectioned by hand using a double-edged blade and structures were observed under a dissecting microscope. At least 10 conidiomata/ascostromata, 10 asci and 50 conidia/ascospores were measured to calculate the mean size and standard deviation. Measurements are reported as maximum and minimum in parentheses and the range representing the mean plus and minus the standard deviation of the number of measurements is given in parentheses. Microscopy photographs were captured with a Nikon Eclipse 80i compound microscope, equipped with a Nikon digital sight DS-Ri2 high definition colour camera, using differential interference contrast illumination.

Isolates of *Cytospora* were obtained by removing the spore masses from the fruiting bodies on to clean PDA plates and incubating at 25 °C until spores germinated. Single germinated spores were then transferred to the new PDA plates and incubated at 25 °C in the dark. The cultures were deposited in the China Forestry Culture Collection Center (CFCC, http://cfcc.caf.ac.cn/) and the specimens in the Herbarium of the Chinese Academy of Forestry (CAF, http://museum.caf.ac.cn/).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fresh fungal mycelia following the method described by Doyle and Doyle (1990). Polymerase chain reactions (PCR) were conducted to amplify the internal transcribed spacer region rDNA (ITS), the partial actin (*act*) region, RNA polymerase II second largest subunit (*rpb2*), translation elongation factor 1-alpha (*tef1*) and the partial beta-tubulin (*tub2*) gene using primers and conditions listed in Table 1. The PCR products were assayed via electrophoresis in 2% agarose gels. DNA sequencing was performed using an ABI PRISM 3730XL

Table 1. Primers and PCR protocols.

Gene Regions	Primers	PCR conditions	References
ITS	ITS5/ITS4	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 48 °C for 1 min, and 72 °C for 2 min , 72 °C for 10 min	White et al. (1990)
act	ACT512F/ACT783R	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 55 °C for 1 min, and 72 °C for 2 min , 72 °C for 10 min	Carbone and Kohn (1999)
rpb2	fRPB2-5f/fRPB2-7cR	95 °C for 5 min, 35 cycles of 95 °C for 1 min, 55 °C, 1.25 min, and 72 °C for 2 min , 72 °C for 10 min	Liu et al. (1999)
tef1	983F/2218R	94 °C for 3 min, 35 cycles of 94 °C for 30 s, 54 °C for 50 s, and 72 °C for 2 min, 72 °C for 10 min	Rehner (2001)
tub2	Bt2a/Bt2b	95 °C for 4 min, 35 cycles of 94 °C for 45 s, 54 °C for 1 min, and 72 °C for 2 min , 72 °C for 10 min	Glass and Donaldson (1995)

DNA Analyser with a BigDye Terminator Kit v.3.1 (Invitrogen, Waltham, MA, USA) at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Sequence alignment and phylogenetic analyses

The obtained sequences of ITS, *act*, *rpb2*, *tef1* and *tub2* were assembled using SeqMan software version 7.1.0 (DNASTAR Inc., WI) and subjected to BLASTn search against the GenBank nucleotide database at National Center for Biotechnology Information (NCBI) to identify closely-related sequences. Sequences data of related taxa were obtained from previous publications (Fan et al. 2020; Lin et al. 2023) and downloaded from the GenBank database (Table 2). The sequences were aligned using MAFFT v.7 online web server (http://mafft.cbrc.jp/alignment/server/index.html, Katoh et al. 2019) under default settings. The Maximum Likelihood (ML) phylogenic analysis was run in the CIPRES Science Gateway platform (Miller et al. 2010), using RAxMLHPC2 on the XSEDE (v. 8.2.10) tool under the GTR substitution model and 1000 non-parametric bootstrap replicates. Bayesian analysis was performed using MrBayes v. 3.2.6 on XSEDE at the CIPRES Science Gateway with four simultaneous Markov Chain runs for 1,000.000 generations. The resulting trees were visualised in FigTree v. 1.4.0 (Rambaut 2012).

Results

Phylogenetic analyses

The combined ITS, *act*, *rpb2*, *tef1* and *tub2* dataset consisted of 199 strains, with *Diaporthe vaccinii* (CBS 160.32) as the outgroup taxon (Table 2). The final alignment comprised 3,166 characters (ITS: 567, *act*: 323, *rpb2*: 741, *tef1*: 727, *tub2*: 808), including gaps. The final ML optimisation likelihood value of the best RAxML tree was -60353.67 and the matrix had 2069 distinct alignment patterns, with 40.24% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0. 244507, C = 0. 288246, G = 0. 237262, T = 0. 229984; substitution rates AC = 1.372283, AG = 2.995828, AT = 1.353835, CG = 0.976452, CT = 5.021434, GT = 1.0; gamma distribution shape parameter α = 0.372885. The RAxML and Bayesian analyses yielded a similar tree topology. The topology of our phylogenetic tree is nearly identical to previous publications. Six isolates from the present study formed two new clades distinct from previously-known species named *Cytospora myricicola* sp. nov. and *C. sibiraeicola* sp. nov. and a known clade named *C. populina* (Fig. 1).

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

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora ailanthicola	CFCC 89970	*Ailanthus altissima*	China	MH933618	MH933526	MH933592	MH933494	MH933565
Cytospora albodisca	CFCC 53161	*Platycladus orientalis*	China	MW418406	MW422899	MW422909	MW422921	MW422933
Cytospora albodisca	CFCC 54373	*Platycladus orientalis*	China	MW418407	MW422900	MW422910	MW422922	MW422934
Cytospora alba	CFCC 55462^{T}	*Salix matsudana*	China	NR182387	OK303457	OK303516	OK303577	OK303644
Cytospora alba	CFCC 55463	*Salix matsudana*	China	MZ702596	OK303458	OK303517	OK303578	OK303645
Cytospora ampulliformis	MFLUCC 16-0583^{T}	*Sorbus intermedia*	Russia	KY417726	KY417692	KY417794	NA	NA
Cytospora ampulliformis	MFLUCC 16-0629	*Acer platanoides*	Russia	KY417727	KY417693	KY417795	NA	NA
Cytospora amygdali	CBS 144233^{T}	*Prunus dulcis*	USA	MG971853	MG972002	NA	MG971659	NA
Cytospora atrocirrhata	CFCC 89615	*Juglans regia*	China	KR045618	KF498673	KU710946	KP310858	KR045659
Cytospora atrocirrhata	CFCC 89616	*Juglans regia*	China	KR045619	KF498674	KU710947	KP310859	KR045660
Cytospora beilinensis	CFCC 50493^{T}	*Pinus armandii*	China	MH933619	MH933527	NA	MH933495	MH933561
Cytospora beilinensis	CFCC 50494	*Pinus armandii*	China	MH933620	MH933528	NA	MH933496	MH933562
Cytospora berberidis	CFCC 89927^{T}	*Berberis dasystachya*	China	KR045620	KU710990	KU710948	KU710913	KR045661
Cytospora berberidis	CFCC 89933	*Berberis dasystachya*	China	KR045621	KU710991	KU710949	KU710914	KR045662
Cytospora bungeana	CFCC 50495^{T}	*Pinus bungeana*	China	MH933621	MH933529	MH933593	MH933497	MH933563
Cytospora bungeana	CFCC 50496	*Pinus bungeana*	China	MH933622	MH933530	MH933594	MH933498	MH933564
Cytospora californica	CBS 144234^{T}	*Juglans regia*	USA	MG971935	MG972083	NA	MG971645	NA
Cytospora carbonacea	CFCC 89947	*Ulmus pumila*	China	KR045622	KP310842	KU710950	KP310855	KP310825
Cytospora carpobroti	CMW48981^{T}	*Carpobrotus edulis*	South Africa	MH382812	NA	NA	MH411212	MH411207
Cytospora celtidicola	CFCC 50497^{T}	*Celtis sinensis*	China	MH933623	MH933531	MH933595	MH933499	MH933566
Cytospora celtidicola	CFCC 50498	*Celtis sinensis*	Anhui, China	MH933624	MH933532	MH933596	MH933500	MH933567
Cytospora centrivillosa	MFLUCC 16-1206^{T}	*Sorbus domestica*	Italy	MF190122	NA	MF377600	NA	NA
Cytospora centrivillosa	MFLUCC 17-1660	*Sorbus domestica*	Italy	MF190123	NA	MF377601	NA	NA
Cytospora ceratosperma	CFCC 89624	*Juglans regia*	China	KR045645	NA	KU710976	KP310860	KR045686
Cytospora ceratosperma	CFCC 89625	*Juglans regia*	China	KR045646	NA	KU710977	KP310861	KR045687
Cytospora ceratospermopsis	CFCC 89626^{T}	*Juglans regia*	China	KR045647	KU711011	KU710978	KU710934	KR045688
Cytospora ceratospermopsis	CFCC 89627	*Juglans regia*	China	KR045648	KU711012	KU710979	KU710935	KR045689
Cytospora chrysosperma	CFCC 89629	*Salix psammophila*	China	KF765673	NA	KF765705	NA	NA
Cytospora chrysosperma	CFCC 89981	*Populus alba*	China	MH933625	MH933533	MH933597	MH933501	MH933568
Cytospora chrysosperma	CFCC 89982	*Ulmus pumila*	China	KP281261	KP310835	NA	KP310848	KP310818
Cytospora cinnamomea	CFCC 53178^{T}	*Prunus armeniaca*	China	MK673054	MK673024	NA	NA	MK672970
Cytospora coryli	CFCC 53162^{T}	*Corylus mandshurica*	China	MN854450	NA	MN850751	MN850758	MN861120
Cytospora corylina	CFCC 54684^{T}	*Corylus heterophylla*	China	MW839861	MW815937	MW815951	MW815886	MW883969
Cytospora corylina	CFCC 54685	*Corylus heterophylla*	China	MW839862	MW815938	MW815952	MW815887	MW883970
Cytospora cotini	MFLUCC 14-1050^{T}	*Cotinus coggygria*	Russia	KX430142	NA	KX430144	NA	NA
Cytospora cotoneastricola	CF 20197027	*Cotoneaster* sp.	China	MK673072	MK673042	MK673012	MK672958	MK672988
Cytospora cotoneastricola	CF 20197028	*Cotoneaster* sp.	China	MK673073	MK673043	MK673013	MK672959	MK672989
Cytospora curvata	MFLUCC 15-0865^{T}	*Salix alba*	Russia	KY417728	KY417694	NA	NA	NA
Cytospora curvispora	CFCC 54000^{T}	*Corylus heterophylla*	China	MW839851	MW815931	MW815945	MW815880	MW883963
Cytospora curvispora	CFCC 54001	*Corylus heterophylla*	China	MW839853	MW815932	MW815946	MW815881	MW883964
Cytospora davidiana	CXY 1350^{T}	*Populus davidiana*	China	KM034870	NA	NA	NA	NA
Cytospora diopuiensis	CFCC 55479	*Euonymus japonicus*	China	OQ344753	OQ410625	OQ398735	OQ398762	OQ398791

Species	Strain	Host	Origin	GenBank accession numbers				
				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora diopuiensis	CFCC 55527	*Euonymus japonicus*	China	OQ344754	OQ410626	OQ398736	OQ398763	OQ398792
Cytospora discotoma	CFCC 53137 T	*Platycladus orientalis*	China	MW418404	MW422897	MW422907	MW422919	MW422931
Cytospora discotoma	CFCC 54368	*Platycladus orientalis*	China	MW418405	MW422898	MW422908	MW422920	MW422932
Cytospora donetzica	MFLUCC 15-0864	*Crataegus monogyna*	Russia	KY417729	KY417695	KY417797	NA	NA
Cytospora donetzica	MFLUCC 16-0574T	*Crataegus monogyna*	Russia	KY417731	KY417697	KY417799	NA	NA
Cytospora donglingensis	CFCC 53159 T	*Platycladus orientalis*	China	MW418412	MW422903	MW422915	MW422927	MW422939
Cytospora donglingensis	CFCC 53160	*Platycladus orientalis*	China	MW418414	MW422905	MW422917	MW422929	MW422941
Cytospora elaeagni	CFCC 89632	*Elaeagnus angustifolia*	China	KR045626	KU710995	KU710955	KU710918	KR045667
Cytospora elaeagni	CFCC 89633	*Elaeagnus angustifolia*	China	KF765677	KU710996	KU710956	KU710919	KR045668
Cytospora elaeagnicola	CFCC 52882T	*Elaeagnus angustifolia*	China	MK732341	MK732344	MK732347	NA	NA
Cytospora elaeagnicola	CFCC 52883	*Elaeagnus angustifolia*	China	MK732342	MK732345	MK732348	NA	NA
Cytospora erumpens	CFCC 50022	*Prunus padus*	China	MH933627	MH933534	NA	MH933502	MH933569
Cytospora erumpens	CFCC 53163	*Prunus padus*	China	MK673059	MK673029	MK673000	MK672948	MK672975
Cytospora eucalypti	CBS 144241	*Eucalyptus globulus*	USA	MG971907	MG972056	NA	MG971617	NA
Cytospora euonymicola	CFCC 50499T	*Euonymus kiautschovicus*	China	MH933628	MH933535	MH933598	MH933503	MH933570
Cytospora euonymicola	CFCC 50500	*Euonymus kiautschovicus*	China	MH933629	MH933536	MH933599	MH933504	MH933571
Cytospora euonymina	CFCC 89993T	*Euonymus kiautschovicus*	China	MH933630	MH933537	MH933600	MH933505	MH933590
Cytospora euonymina	CFCC 89999	*Euonymus kiautschovicus*	China	MH933631	MH933538	MH933601	MH933506	MH933591
Cytospora fraxinigena	MFLU 17-0880 T	*Fraxinus ornus*	Italy	NR154859	NA	NA	NA	NA
Cytospora fugax	CXY 1371	*Populus simonii*	China	KM034852	NA	NA	NA	KM034891
Cytospora fugax	CXY 1381	*Populus ussuriensis*	China	KM034853	NA	NA	NA	KM034890
Cytospora fusispora	NFCCI 4372	NA	India	MN227694	NA	NA	NA	NA
Cytospora galegicola	MFLUCC 18-1199T	*Galega officinalis*	Italy	MK912128	MN685810	MN685820	NA	NA
Cytospora gigalocus	CFCC 89620T	*Juglans regia*	China	KR045628	KU710997	KU710957	KU710920	KR045669
Cytospora gigalocus	CFCC 89621	*Juglans regia*	China	KR045629	KU710998	KU710958	KU710921	KR045670
Cytospora gigaspora	CFCC 50014	*Juniperus procumbens*	China	KR045630	KU710999	KU710959	KU710922	KR045671
Cytospora gigaspora	CFCC 89634T	*Salix psammophila*	China	KF765671	KU711000	KU710960	KU710923	KR045672
Cytospora globosa	MFLU 16-2054T	*Abies alba*	Italy	MT177935	NA	MT432212	MT454016	NA
Cytospora granati	CBS 144237T	*Punica granatum*	USA	MG971799	MG971949	NA	MG971514	NA
Cytospora haidianensis	CFCC 54056	*Euonymus alatus*	China	MT360041	MT363978	MT363987	MT363997	MT364007
Cytospora haidianensis	CFCC 54057T	*Euonymus alatus*	China	MT360042	MT363979	MT363988	MT363998	MT364008
Cytospora hippophaës	CFCC 89639	*Hippophaë rhamnoides*	China	KR045632	KU711001	KU710961	KU710924	KR045673
Cytospora hippophaës	CFCC 89640	*Hippophaë rhamnoides*	China	KF765682	KF765730	KU710962	KP310865	KR045674
Cytospora japonica	CFCC 89956	*Prunus cerasifera*	China	KR045624	KU710993	KU710953	KU710916	KR045665
Cytospora japonica	CFCC 89960	*Prunus cerasifera*	China	KR045625	KU710994	KU710954	KU710917	KR045666
Cytospora joaquinensis	CBS 144235	*Populus deltoides*	USA	MG971895	MG972044	NA	MG971605	NA
Cytospora junipericola	MFLU 17-0882T	*Juniperus communis*	Italy	MF190125	NA	NA	MF377580	NA
Cytospora juniperina	CFCC 50501T	*Juniperus przewalskii*	China	MH933632	MH933539	MH933602	MH933507	NA

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				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora juniperina	CFCC 50502	*Juniperus przewalskii*	China	MH933633	MH933540	MH933603	MH933508	MH933572
Cytospora kantschavelii	CXY 1383	*Populus maximowiczii*	China	KM034867	NA	NA	NA	NA
Cytospora kuanchengensis	CFCC 52464^{T}	*Castanea mollissima*	China	MK432616	MK442940	MK578076	NA	NA
Cytospora kuanchengensis	CFCC 52465	*Castanea mollissima*	China	MK432617	MK442941	MK578077	NA	NA
Cytospora leucosperma	CFCC 89622	*Pyrus bretschneideri*	China	KR045616	KU710988	KU710944	KU710911	KR045657
Cytospora leucosperma	CFCC 89894	*Pyrus bretschneideri*	China	KR045617	KU710989	KU710945	KU710912	KR045658
Cytospora longispora	CBS 144236^{T}	*Prunus domestica*	USA	MG971905	MG972054	NA	MG971615	NA
Cytospora longistiolata	MFLUCC 16-0628	*Salix × fragilis*	Russia	KY417734	KY417700	KY417802	NA	NA
Cytospora lumnitzericola	MFLUCC 17-0508^{T}	*Lumnitzera racernosa*	Tailand	MG975778	MH253457	MH253461	NA	NA
Cytospora mali	CFCC 50028	*Malus pumila*	China	MH933641	MH933548	MH933606	MH933513	MH933577
Cytospora mali	CFCC 50029	*Malus pumila*	China	MH933642	MH933549	MH933607	MH933514	MH933578
Cytospora mali-spectabilis	CFCC 53181^{T}	*Malus spectabilis*	China	MK673066	MK673036	MK673006	MK672953	MK672982
Cytospora melnikii	CFCC 89984	*Rhus typhina*	China	MH933644	MH933551	MH933609	MH933515	MH933580
Cytospora myricicola	**CFCC 59323^{T}**	***Myricaria paniculate***	**China**	**OR769868**	**OR767324**	**OR767338**	**OR767364**	**OR767351**
Cytospora myricicola	**CFCC 59324**	***Myricaria paniculate***	**China**	**OR769869**	**OR767325**	**OR767339**	**OR767365**	**OR767352**
Cytospora myricicola	**CFCC 59325**	***Myricaria paniculate***	**China**	**OR769870**	**OR767326**	**OR767340**	**OR767366**	**OR767353**
Cytospora myrtagena	CFCC 52454	*Castanea mollissima*	China	MK432614	MK442938	MK578074	NA	NA
Cytospora myrtagena	CFCC 52455	*Castanea mollissima*	China	MK432615	MK442939	MK578075	NA	NA
Cytospora nivea	MFLUCC 15-0860	*Salix acutifolia*	Russia	KY417737	KY417703	KY417805	NA	NA
Cytospora nivea	CFCC 89641	*Elaeagnus angustifolia*	China	KF765683	KU711006	KU710967	KU710929	KR045679
Cytospora notastroma	NE_TFR5	*Populus tremuloides*	USA	JX438632	NA	NA	JX438543	NA
Cytospora notastroma	NE_TFR8	*Populus tremuloides*	USA	JX438633	NA	NA	JX438542	NA
Cytospora ochracea	CFCC 53164^{T}	*Cotoneaster* sp.	China	MK673060	MK673030	MK673001	MK672949	MK672976
Cytospora oleicola	CBS 144248^{T}	*Olea europaea*	USA	MG971944	MG972098	NA	MG971660	NA
Cytospora olivacea	CFCC 53174	*Prunus cerasifera*	China	MK673058	MK673028	MK672999	NA	MK672974
Cytospora olivacea	CFCC 53175	*Prunus dulcis*	China	MK673062	MK673032	MK673003	NA	MK672978
Cytospora palm	CXY 1276	*Cotinus coggygria*	China	JN402990	NA	NA	KJ781296	NA
Cytospora palm	CXY 1280^{T}	*Cotinus coggygria*	China	JN411939	NA	NA	KJ781297	NA
Cytospora paracinnamomea	CFCC 55453^{T}	*Salix matsudana*	China	MZ702594	OK303456	OK303515	OK303576	OK303643
Cytospora paracinnamomea	CFCC 55455^{T}	*Salix matsudana*	China	MZ702598	OK303460	OK303519	OK303580	OK303647
Cytospora parakantschavelii	MFLUCC 15-0857^{T}	*Populus × sibirica*	Russia	KY417738	KY417704	KY417806	NA	NA
Cytospora parapistaciae	CBS 144506^{T}	*Pistacia vera*	USA	MG971804	MG971954	NA	MG971519	NA
Cytospora paraplurivora	FDS-439	*Prunus armeniaca*	Canada	OL640182	OL631586	NA	OL631589	NA
Cytospora paraplurivora	FDS-564^{T}	*Prunus persica* var. *nucipersica*	Canada	OL640183	OL631587	NA	OL631590	NA
Cytospora parasitica	CFCC 53173	*Berberis* sp.	China	MK673070	MK673040	MK673010	MK672957	MK672986
Cytospora paratranslucens	MFLUCC 15-0506^{T}	*Populus alba* var. *bolleana*	Russia	KY417741	KY417707	KY417809	NA	NA
Cytospora paratranslucens	MFLUCC 16-0627	*Populus alba*	Russia	KY417742	KY417708	KY417810	NA	NA
Cytospora phialidica	MFLUCC 17-2498	*Alnus glutinosa*	Italy	MT177932	NA	MT432209	MT454014	NA
Cytospora piceae	CFCC 52841^{T}	*Picea crassifolia*	China	MH820398	MH820406	MH820395	MH820402	MH820387
Cytospora piceae	CFCC 52842	*Picea crassifolia*	China	MH820399	MH820407	MH820396	MH820403	MH820388
Cytospora pingbianensis	MFLUCC 18-1204^{T}	Undefined wood	China	MK912135	MN685817	MN685826	NA	NA
Cytospora pistaciae	CBS 144238^{T}	*Pistacia vera*	USA	MG971802	MG971952	NA	MG971517	NA

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				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora platycladi	CFCC 50504^{T}	*Platycladus orientalis*	China	MH933645	MH933552	MH933610	MH933516	MH933581
Cytospora platycladi	CFCC 50505	*Platycladus orientalis*	China	MH933646	MH933553	MH933611	MH933517	MH933582
Cytospora platycladicola	CFCC 50038^{T}	*Platycladus orientalis*	China	KT222840	MH933555	MH933613	MH933519	MH933584
Cytospora platycladicola	CFCC 50039	*Platycladus orientalis*	China	KR045642	KU711008	KU710973	KU710931	KR045683
Cytospora plurivora	CBS 144239^{T}	*Olea europaea*	USA	MG971861	MG972010	NA	MG971572	NA
Cytospora populi	CFCC 55472^{T}	*Populus* sp.	China	MZ702609	OK303471	OK303530	OK303591	OK303658
Cytospora populi	CFCC 55473	*Populus* sp.	China	MZ702610	OK303472	OK303531	OK303592	OK303659
Cytospora populicola	CBS 144240	*Populus deltoides*	USA	MG971891	MG972040	NA	MG971601	NA
Cytospora populina	CFCC 89644^{T}	*Salix psammophila*	China	KF765686	KU711007	KU710969	KU710930	KR045681
Cytospora populina	**CFCC 58856**	***Prunus cerasifera***	**China**	**OR769873**	**OR767329**	**OR767343**	**OR767369**	**NA**
Cytospora populinopsis	CFCC 50032^{T}	*Sorbus aucuparia*	China	MH933648	MH933556	MH933614	MH933520	MH933585
Cytospora populinopsis	CFCC 50033	*Sorbus aucuparia*	China	MH933649	MH933557	MH933615	MH933521	MH933586
Cytospora predappioensis	MFLUCC 17-2458^{T}	*Platanus hybrida*	Italy	MG873484	NA	NA	NA	NA
Cytospora predappioensis	MFLU 17-0327	*Platanus hybrida*	Italy	MH253451	MH253449	MH253450	NA	NA
Cytospora prunicola	MFLU 17-0995^{T}	*Prunus* sp.	Italy	MG742350	MG742353	MG742352	NA	NA
Cytospora pruni-mume	CFCC 53179	*Prunus armeniaca*	China	MK673057	MK673027	NA	MK672947	MK672973
Cytospora pruni-mume	CFCC 53180^{T}	*Prunus mume*	China	MK673067	MK673037	MK673007	MK672954	MK672983
Cytospora pruinopsis	CFCC 50034^{T}	*Ulmus pumila*	China	KP281259	KP310836	KU710970	KP310849	KP310819
Cytospora pruinopsis	CFCC 53153	*Ulmus pumila*	China	MN854451	MN850763	MN850752	MN850759	MN861121
Cytospora pruinosa	CFCC 50036	*Syringa oblata*	China	KP310800	KP310832	NA	KP310845	KP310815
Cytospora pruinosa	CFCC 50037	*Syringa oblata*	China	MH933650	MH933558	NA	MH933522	MH933589
Cytospora pubescentis	MFLUCC 18-1201^{T}	*Quercus pubescens*	Italy	MK912130	MN685812	MN685821	NA	NA
Cytospora punicae	CBS 144244	*Punica granatum*	USA	MG971943	MG972091	NA	MG971654	NA
Cytospora quercicola	MFLU 17-0881	*Quercus* sp.	Italy	MF190128	NA	NA	NA	NA
Cytospora ribis	CFCC 50026	*Ulmus pumila*	China	KP281267	KP310843	KU710972	KP310856	KP310826
Cytospora ribis	CFCC 50027	*Ulmus pumila*	China	KP281268	KP310844	NA	KP310857	KP310827
Cytospora rosae	MFLU 17-0885	*Rosa canina*	Italy	MF190131	NA	NA	NA	NA
Cytospora rosicola	CF 20197024^{T}	*Rosa* sp.	China	MK673079	MK673049	MK673019	MK672965	MK672995
Cytospora rosigena	MFLUCC 18-0921^{T}	*Rosa* sp.	Russia	MN879872	NA	NA	NA	NA
Cytospora rostrata	CFCC 89909	*Salix cupularis*	China	KR045643	KU711009	KU710974	KU710932	KR045684
Cytospora rostrata	CFCC 89910	*Salix cupularis*	China	KR045644	KU711010	KU710975	KU710933	NA
Cytospora rusanovii	MFLUCC 15-0853	*Populus × sibirica*	Russia	KY417743	KY417709	KY417811	NA	NA
Cytospora rusanovii	MFLUCC 15-0854^{T}	*Salix babylonica*	Russia	KY417744	KY417710	KY417812	NA	NA
Cytospora salicacearum	MFLUCC 15-0509	*Salix alba*	Russia	KY417746	KY417712	KY417814	NA	NA
Cytospora salicacearum	MFLUCC 15-0861	*Salix × fragilis*	Russia	KY417745	KY417711	KY417813	NA	NA
Cytospora salicicola	MFLUCC 14-1052^{T}	*Salix alba*	Russia	KU982636	KU982637	NA	NA	NA
Cytospora salicicola	MFLUCC 15-0866	*Salix* sp.	Thailand	KY417749	KY417715	KY417817	NA	NA
Cytospora salicina	MFLUCC 15-0862	*Salix alba*	Russia	KY417750	KY417716	KY417818	NA	NA
Cytospora salicina	MFLUCC 16-0637	*Salix × fragilis*	Russia	KY417751	KY417717	KY417819	NA	NA
Cytospora schulzeri	CFCC 50042	*Malus pumila*	China	KR045650	KU711014	KU710981	KU710937	KR045691
Cytospora sibiraeae	CFCC 50045^{T}	*Sibiraea angustata*	China	KR045651	KU711015	KU710982	KU710938	KR045692
Cytospora sibiraeae	CFCC 50046	*Sibiraea angustata*	China	KR045652	KU711016	KU710983	KU710939	KR045693
Cytospora sibiraeicola	**CFCC 59100^{T}**	***Sibiraea angustata***	**China**	**OR769871**	**OR767327**	**OR767341**	**OR767367**	**OR767354**
Cytospora sibiraeicola	**CFCC 59101**	***Sibiraea angustata***	**China**	**OR769872**	**OR767328**	**OR767342**	**OR767368**	**OR767355**
Cytospora sophorae	CFCC 50047	*Styphnolobium japonicum*	China	KR045653	KU711017	KU710984	KU710940	KR045694

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				ITS	*act*	*rpb2*	*tef1*	*tub2*
Cytospora sophorae	CFCC 89598	*Styphnolobium japonicum*	China	KR045654	KU711018	KU710985	KU710941	KR045695
Cytospora sophoricola	CFCC 89596	*Styphnolobium japonicum*	China	KR045656	KU711020	KU710987	KU710943	KR045697
Cytospora sophoricola	CFCC 89595^{T}	*Styphnolobium japonicum*	China	KR045655	KU711019	KU710986	KU710942	KR045696
Cytospora sophoriopsis	CFCC 55469	*Salix matsudana*	China	MZ702583	OK303445	OK303504	OK303565	OK303632
Cytospora sophoriopsis	CFCC 89600	*Styphnolobium japonicum*	China	KR045623	KU710992	KU710951	KU710915	KP310817
Cytospora sorbi	MFLUCC 16-0631^{T}	*Sorbus aucuparia*	Russia	KY417752	KY417718	KY417820	NA	NA
Cytospora sorbicola	MFLUCC 16-0584^{T}	*Acer pseudoplatanus*	Russia	KY417755	KY417721	KY417823	NA	NA
Cytospora sorbicola	MFLUCC 16-0633	*Cotoneaster melanocarpus*	Russia	KY417758	KY417724	KY417826	NA	NA
Cytospora sorbina	CF 20197660^{T}	*Sorbus tianschanica*	China	MK673052	MK673022	NA	MK672943	MK672968
Cytospora spiraeae	CFCC 50049^{T}	*Spiraea salicifolia*	China	MG707859	MG708196	MG708199	NA	NA
Cytospora spiraeae	CFCC 50050	*Spiraea salicifolia*	China	MG707860	MG708197	MG708200	NA	NA
Cytospora spiraeicola	CFCC 53138^{T}	*Spiraea salicifolia*	China	MN854448	NA	MN850749	MN850756	MN861118
Cytospora spiraeicola	CFCC 53139	*Tilia nobilis*	China	MN854449	NA	MN850750	MN850757	MN861119
Cytospora tamaricicola	CFCC 50507	*Rosa multifolora*	China	MH933651	MH933559	MH933616	MH933525	MH933587
Cytospora tamaricicola	CFCC 50508^{T}	*Tamarix chinensis*	China	MH933652	MH933560	MH933617	MH933523	MH933588
Cytospora tanaitica	MFLUCC 14-1057^{T}	*Betula pubescens*	Russia	KT459411	KT459413	NA	NA	NA
Cytospora thailandica	MFLUCC 17-0262^{T}	*Xylocarpus moluccensis*	Thailand	MG975776	MH253459	MH253463	NA	NA
Cytospora thailandica	MFLUCC 17-0263^{T}	*Xylocarpus moluccensis*	Thailand	MG975777	MH253460	MH253464	NA	NA
Cytospora tibetensis	CF 20197026	*Cotoneaster* sp.	China	MK673076	MK673046	MK673016	MK672962	MK672992
Cytospora tibetensis	CF 20197029	*Cotoneaster* sp.	China	MK673077	MK673047	MK673017	MK672963	MK672993
Cytospora tibouchinae	CPC 26333^{T}	*Tibouchina semidecandra*	France	KX228284	NA	NA	NA	NA
Cytospora translucens	CXY 1351	*Populus davidiana*	China	KM034874	NA	NA	NA	KM034895
Cytospora translucens	CXY 1359	*Populus* × beijingensis	China	KM034871	NA	NA	NA	KM034894
Cytospora ulmi	MFLUCC 15-0863^{T}	*Ulmus minor*	Russia	KY417759	NA	NA	NA	NA
Cytospora verrucosa	CFCC 53157^{T}	*Platycladus orientalis*	China	MW418408	NA	MW422911	MW422923	MW422935
Cytospora verrucosa	CFCC 53158	*Platycladus orientalis*	China	MW418410	MW422901	MW422913	MW422925	MW422937
Cytospora vinacea	CBS 141585^{T}	*Vitis interspecific*	USA	KX256256	NA	NA	KX256277	KX256235
Cytospora viridistroma	CBS 202.36^{T}	*Cercis canadensis*	USA	MN172408	NA	NA	MN271853	NA
Cytospora viticola	Cyt2	*Vitis interspecific*	USA	KX256238	NA	NA	KX256259	KX256217
Cytospora viticola	CBS 141586^{T}	*Vitis vinifera*	USA	KX256239	NA	NA	KX256260	KX256218
Cytospora xinjiangensis	CFCC 53182	*Rosa* sp.	China	MK673064	MK673034	MK673004	MK672951	MK672980
Cytospora xinjiangensis	CFCC 53183^{T}	*Rosa* sp.	China	MK673065	MK673035	MK673005	MK672952	MK672981
Cytospora xinglongensis	CFCC 52458	*Castanea mollissima*	China	MK432622	MK442946	MK578082	NA	NA
Cytospora xinglongensis	CFCC 52459	*Castanea mollissima*	China	MK432623	MK442947	MK578083	NA	NA
Cytospora xylocarpi	MFLUCC 17-0251^{T}	*Xylocarpus granatum*	Thailand	MG975775	MH253458	MH253462	NA	NA
Cytospora zhaitangensis	CFCC 56227^{T}	*Euonymus japonicus*	China	OQ344750	OQ410623	OQ398733	OQ398760	OQ398789
Cytospora zhaitangensis	CFCC 57537	*Euonymus japonicus*	China	OQ344751	OQ410624	OQ398734	OQ398761	OQ398790
Diaporthe vaccinii	CBS 160.32	*Vaccinium macrocarpon*	USA	KC343228	JQ807297	NA	KC343954	KC344196

Ex-type strains are indicated with (^{T}) after the collection number; "NA" indicates unavailable sequences; sequences produced in the current study are in bold.

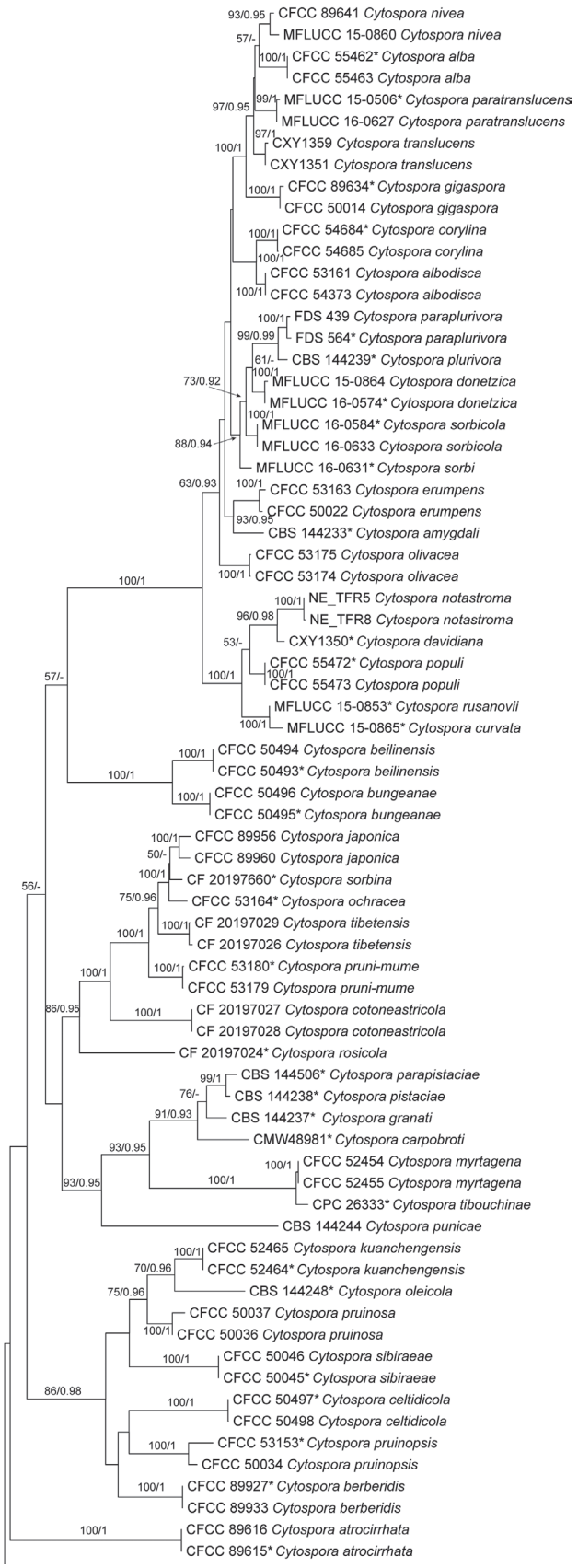

Figure 1. Maximum Likelihood tree generated from combined ITS, *act*, *rpb2*, *tef1* and *tub2* sequence data. Bootstrap support values ≥ 50% and Bayesian posterior probabilities ≥ 0.90 are demonstrated at the branches. Ex-type cultures are marked with (*).

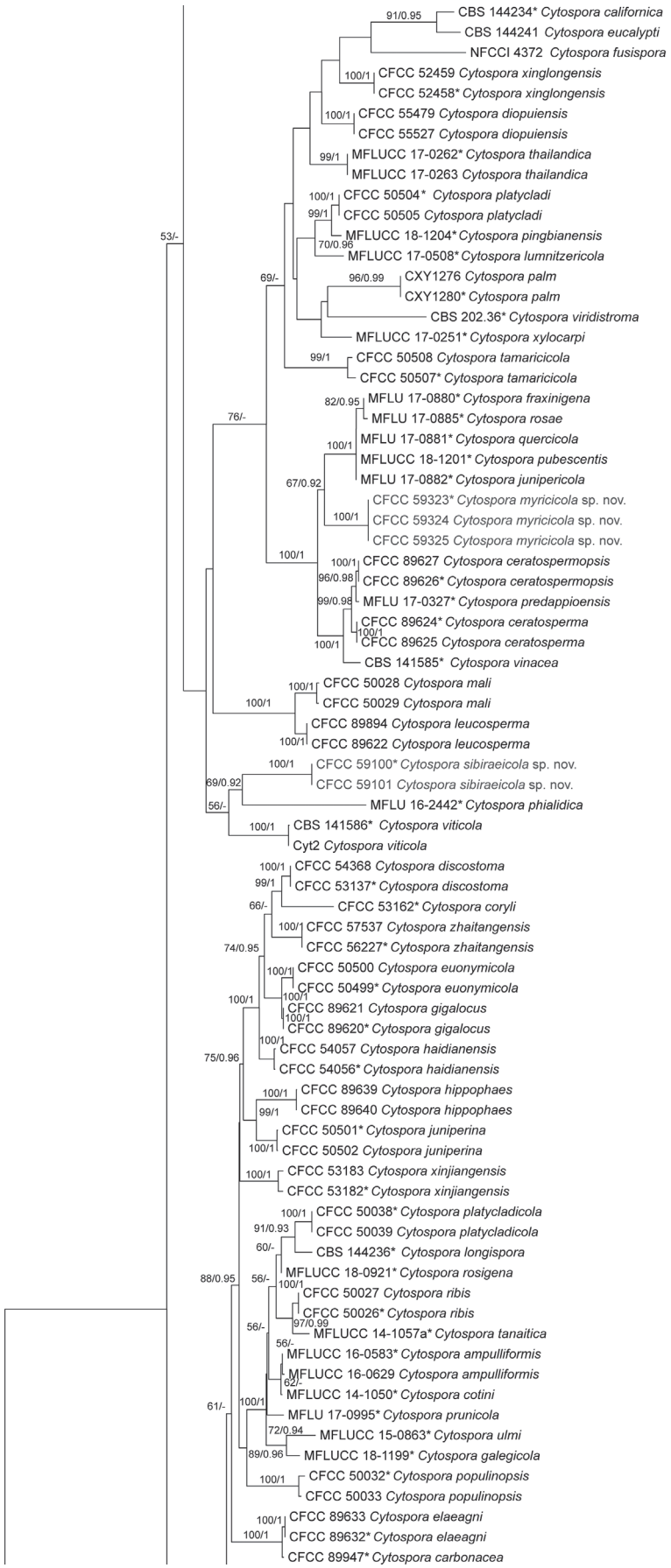

Figure 1. Continued.

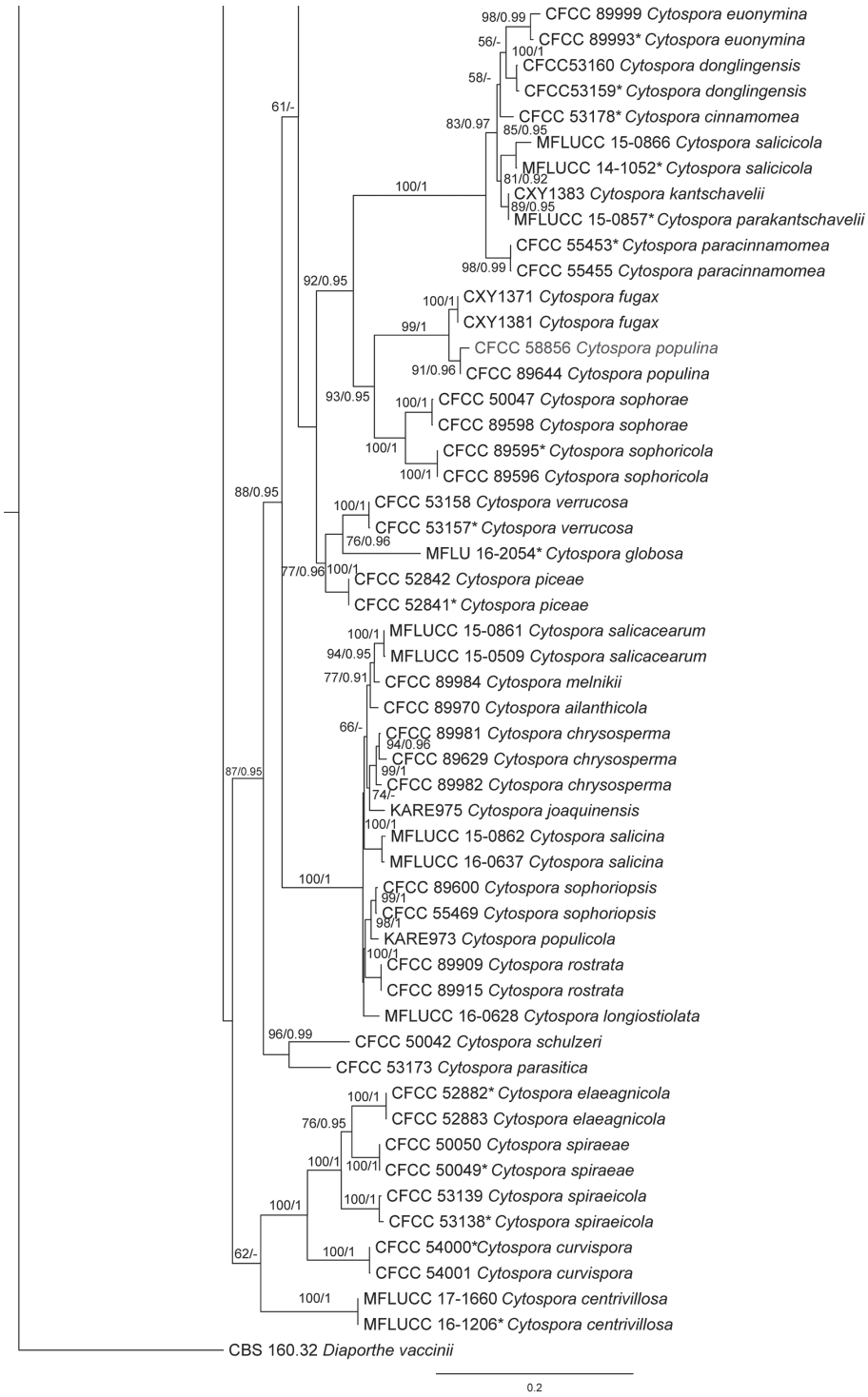

Figure 1. Continued.

Taxonomy

Cytospora myricicola **Ning Jiang, sp. nov.**
MycoBank No: 850240
Fig. 2

Etymology. "*myrici*" refers to the host genus *Myricaria* and "*-cola*" means inhabiting.

Holotype. CAF800083.

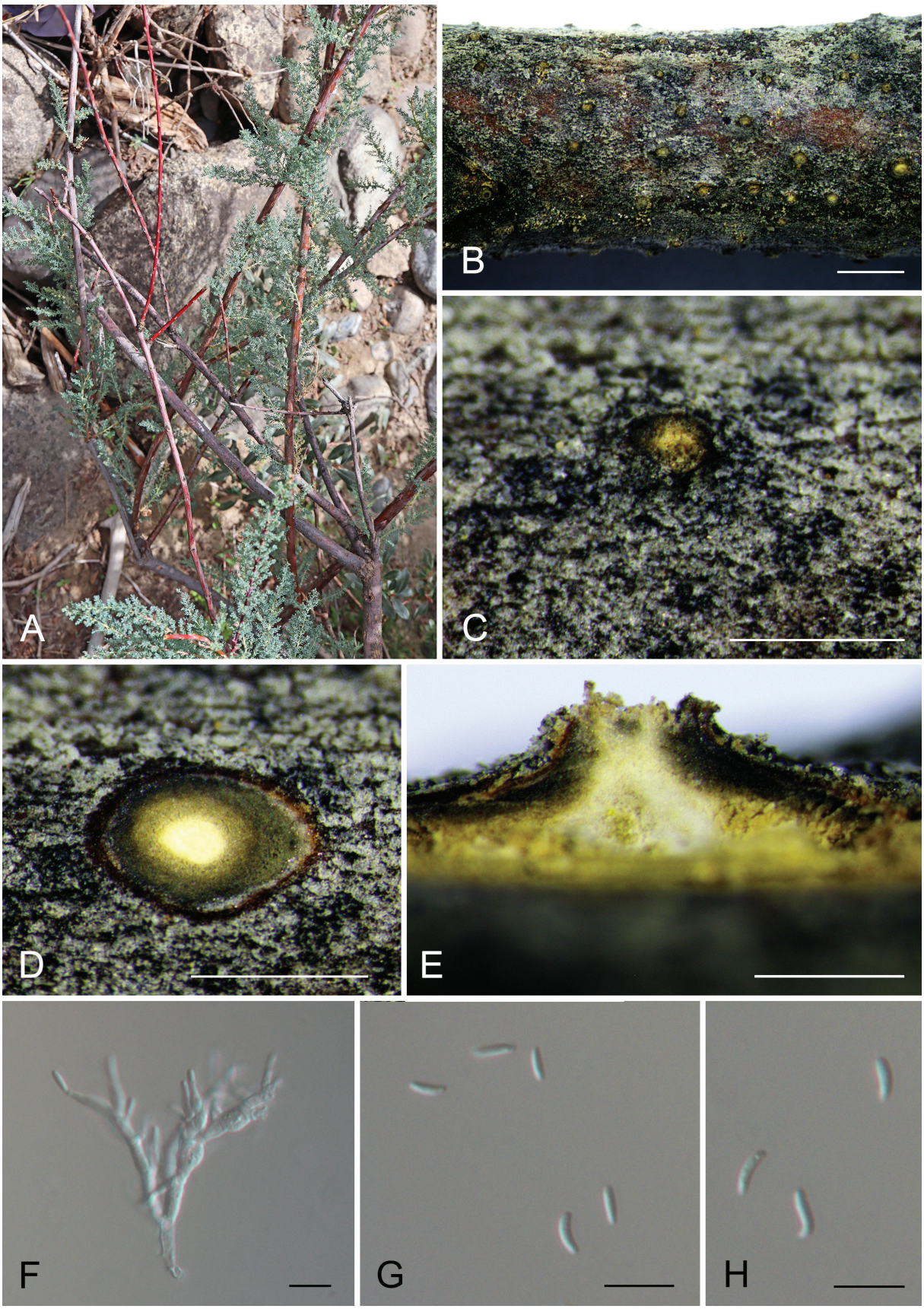

Figure 2. *Cytospora myricicola* (CAF800083, holotype) **A, B** canker disease symptom **C** conidioma **D** transverse section through a conidioma **E** longitudinal section through a conidioma **F** conidiophores and conidia **G, H** conidia. Scale bars: 2000 µm (**B**); 1000 µm (**C, D**); 500 µm (**E**); 10 µm (**F–H**).

Description. Associated with branch canker disease of *Myricaria paniculate*. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Pycnidial stromata ostiolated, semi-immersed in the host bark, scattered, discoid, with multiple locules. Conceptacle dark brown to black, circular surrounded stromata. Ectostromatic disc dark yellow, circular to ovoid, (250–)350–450(–550) µm diam., with one ostiole per disc. Ostioles dark, at the same level as the disc, (35–)55–85(–100) µm diam. Locule numerous, arranged circularly or elliptically with independent walls (245–)300–450(–550) µm diam. Peridium comprising a few layers of cells of textura angularis, with innermost layer brown, outer layer brown to dark brown. Conidiophores hyaline, branched, thin-walled, filamentous. Conidiogenous cells enteroblastic polyphialidic, 6.5–35.5 × 1.5–2.5 µm. Conidia hyaline, allantoid, smooth, aseptate, thin-walled, (4.4–)4.7–5.6(–5.8) × 1.4–1.7 µm ($\bar{x}$ = 5.2 × 1.6 µm).

Culture characteristics. Colonies on PDA flat, with flocculent aerial mycelium and entire edge, initially white, becoming dark and reaching 90 mm diameter after 10 days at 25 °C, sterile.

Materials examined. China, Tibet Tibetan Autonomous Region, Lhasa City, Mozhugongka County, Riduo Township, Zen Village, on cankered branches of *Myricaria paniculate*, 28 July 2022, Jin Peng, Liu Yuanyuan, Jiang Ning and Liu Min (CAF800083, ***holotype***); ex-type culture CFCC 59323. China, Tibet Tibetan Autonomous Region, Lhasa City, Mozhugongka County, Riduo Township, Renqinglin Village, on cankered branches of *Myricaria paniculate*, 28 July 2022, Jin Peng, Liu Yuanyuan, Jiang Ning and Liu Min (XZ010B); cultures CFCC 59324 and CFCC 59325.

Notes. Phylogenetically, *Cytospora myricicola* is close to *C. fraxinigena*, *C. junipericola*, *C. pubescentis*, *C. quercicola* and *C. rosae* (Fig. 1). Of these six species, only *C. myricicola*, *C. pubescentis* and *C. rosae* have asexual morph descriptions; *C. myricicola* (4.7–5.6 × 1.4–1.7 µm) is different from *C. pubescentis* (5.8–7.5 × 1.3–1.6 µm) by shorter conidia and from *C. rosae* (3–5 × 0.5–1 µm) by larger conidia (Senanayake et al. 2017; Shang et al. 2020). In addition, *C. myricicola* can be distinguished from the other five species by host and distribution (*C. myricicola* from *Myricaria paniculate* in China vs. *C. fraxinigena* from *Fraxinus ornus* in Italy vs. *C. junipericola* from *Juniperus communis* in Italy vs. *C. pubescentis* from *Quercus pubescens* in Italy vs. *C. quercicola* from *Quercus* sp. in Italy vs. *C.* rosae from *Rosa canina* in Italy) (Senanayake et al. 2017; Shang et al. 2020).

***Cytospora populina* (Pers.) Rabenh., Deutschl. Krypt.-Fl. (Leipzig) 1: 148. 1844**
Fig. 3

Description. Associated with branch canker disease of *Prunus cerasifera*. ***Sexual morph*:** Stromata immersed in bark. Ascostromata, erumpent through the surface of bark, lenticular, extending to a large circular area, (750–)900–1200(–1350) µm diam. Disc grey to black, circular to ovoid, (85–)100–150(–195) µm in diameter. Ostioles numerous, dark brown to black, at the same level as the disc, (25–)31–46(–52) µm diam. Locules dark brown, arranged circularly, flask-shaped to spherical, (180–)195–285(–340) µm diam. Asci clavate to elongate obovoid, (45–)55.5–62.5(–67) × (6.5–)8–12(–16) µm, 4-spored. Ascospores

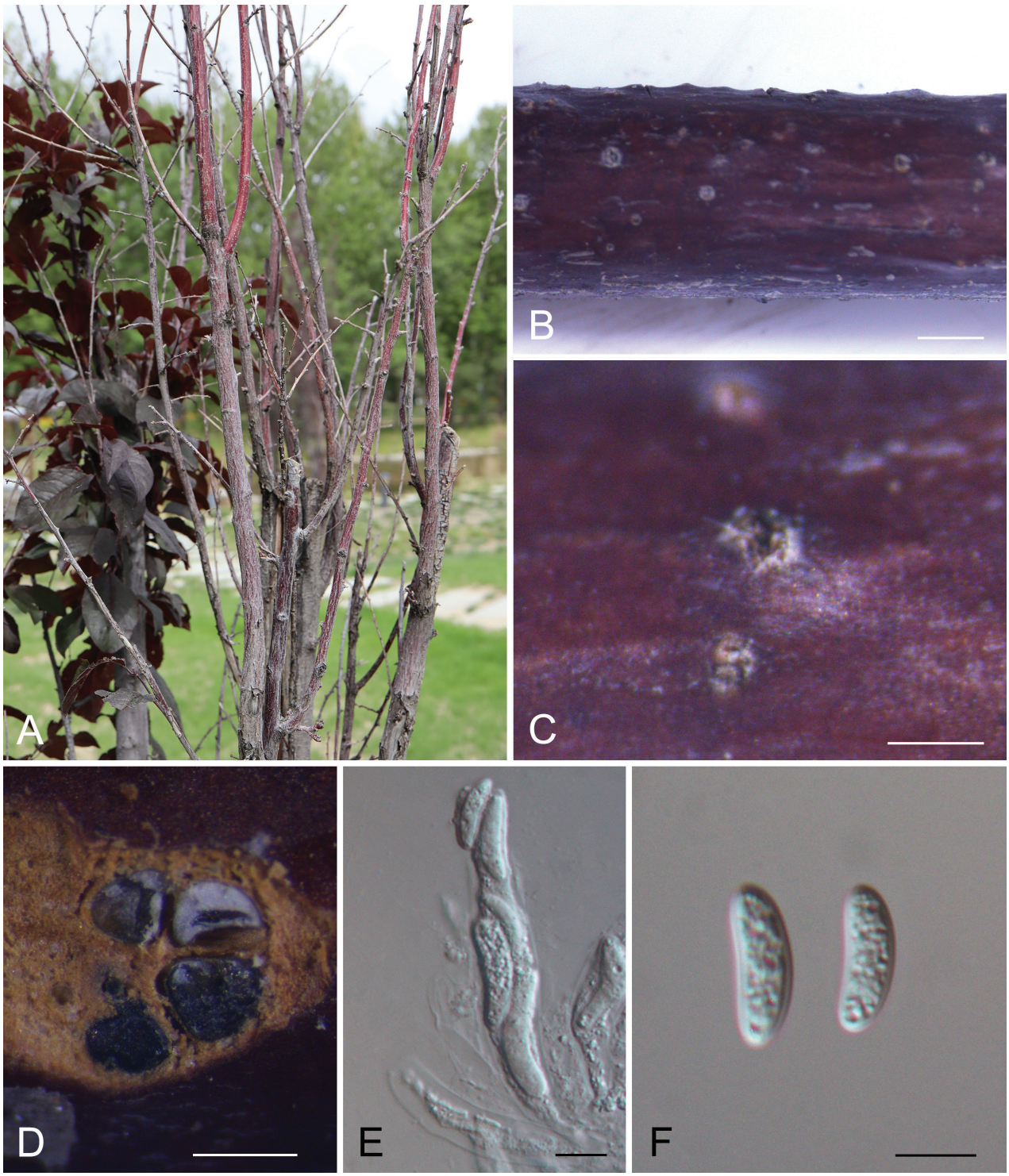

Figure 3. *Cytospora populina* (CAF800085) **A, B** canker disease symptom **C** ascostromata **D** transverse section through an ascostroma **E** asci and Ascospores **F** ascospores. Scale bars: 2000 µm (**B**); 500 µm (**C**); 200 µm (**D**); = 10 µm (**E–F**).

biseriate, elongate-allantoid, thin-walled, hyaline, aseptate, (15–)18.5–23.5(–25.5) × (4–)4.5–5.5(–6.5) µm ($\bar{x}$ = 20.4 × 5.1 µm). ***Asexual morph*:** Undetermined.

Culture characteristics. Colonies on PDA flat, with flocculent aerial mycelium and entire edge, initially white, becoming luteous and reaching 80 mm diameter after 10 days at 25 °C, sterile.

Materials examined. China, Tibet Tibetan Autonomous Region, Shigatse City, Sangzhuzi District, Gongjuelinka Park, on cankered branches of *Prunus cerasifera*, 2 August 2022, Jin Peng, Jiang Ning and Liu Min (XZ063); culture CFCC 58856.

Notes. *Cytospora populina* has been reported from *Populus canadensis* in Argentina, *Salix psammophila* in Shaanxi Province of China and *Acer pubescens Rubus* sp. in Uzbekistan (Farr 1973; Fan et al. 2015; Gafforov 2017). This fungus is distinguished from the other *Cytospora* species by its 4-ascospored asci and undiscovered asexual state (Fan et al. 2015). In the present study, we firstly found this fungus causing cankered branches of *Prunus cerasifera* in Tibet, China.

Cytospora sibiraeicola **Ning Jiang, sp. nov.**
MycoBank No: 850241
Fig. 4

Etymology. "*sibiraei*" refers to the host genus *Sibiraea* and "*-cola*" means inhabiting.

Holotype. CAF800084.

Description. Associated with branch canker disease of *Sibiraea angustata*. ***Sexual morph*****:** Undetermined. ***Asexual morph*****:** Pycnidial stromata ostiolated, immersed or semi-immersed in the host bark, scattered, discoid, with multiple locules. Conceptacle black, circular surrounded stromata. Ectostromatic disc black, circular to ovoid, (200–)300–450(–500) µm diam., with one ostiole per disc. Ostioles dark, at the same level as the disc, (30–)60–80(–95) µm diam. Locule numerous, arranged circularly or elliptically with independent walls, (200–)250–380(–500) µm diam. Peridium comprising few layers of cells of textura angularis, with innermost layer brown, outer layer brown to dark brown. Conidiophores hyaline, unbranched, thin-walled, filamentous. Conidiogenous cells enteroblastic polyphialidic, 12.5–32.5 × 2–3.5 µm. Conidia hyaline, allantoid, smooth, aseptate, thin-walled, (3.3–)3.4–4.3(–4.5) × 1.2–1.6 µm ($\bar{x}$ = 3.9 × 1.5 µm).

Culture characteristics. Colonies on PDA flat, with flocculent aerial mycelium and undulate margin, initially white, becoming olivaceous grey and reaching 90 mm diameter after 10 days at 25 °C, sterile.

Materials examined. China, Tibet Tibetan Autonomous Region, Lhasa City, Mozhugongka County, Riduo Township, Zen Village, on cankered branches of *Sibiraea angustata*, 28 July 2022, Jin Peng, Liu Yuanyuan, Jiang Ning and Liu Min (CAF800083, holotype); ex-type cultures CFCC 59100 and CFCC 59101.

Notes. *Cytospora sibiraeicola* is phylogenetically close to *C. phialidica* and *C. viticola* (Fig. 1). Morphologically, *C. sibiraeicola* (3.4–4.3 × 1.2–1.6 µm) and *C. phialidica* (3.5–5 × 1–2 µm) have much shorter conidia than *C. viticola* (5.2–7 × 0.9–1.6 µm) (Lawrence et al. 2017; Li et al. 2020). In addition, these three species can be distinguished by the host and distribution (*C. sibiraeicola* from *Sibiraea angustata* in China vs. *C. phialidica* from *Alnus glutinosa* in Italy vs. *C. viticola* from *Vitis vinifera* in the USA) (Lawrence et al. 2017; Li et al. 2020).

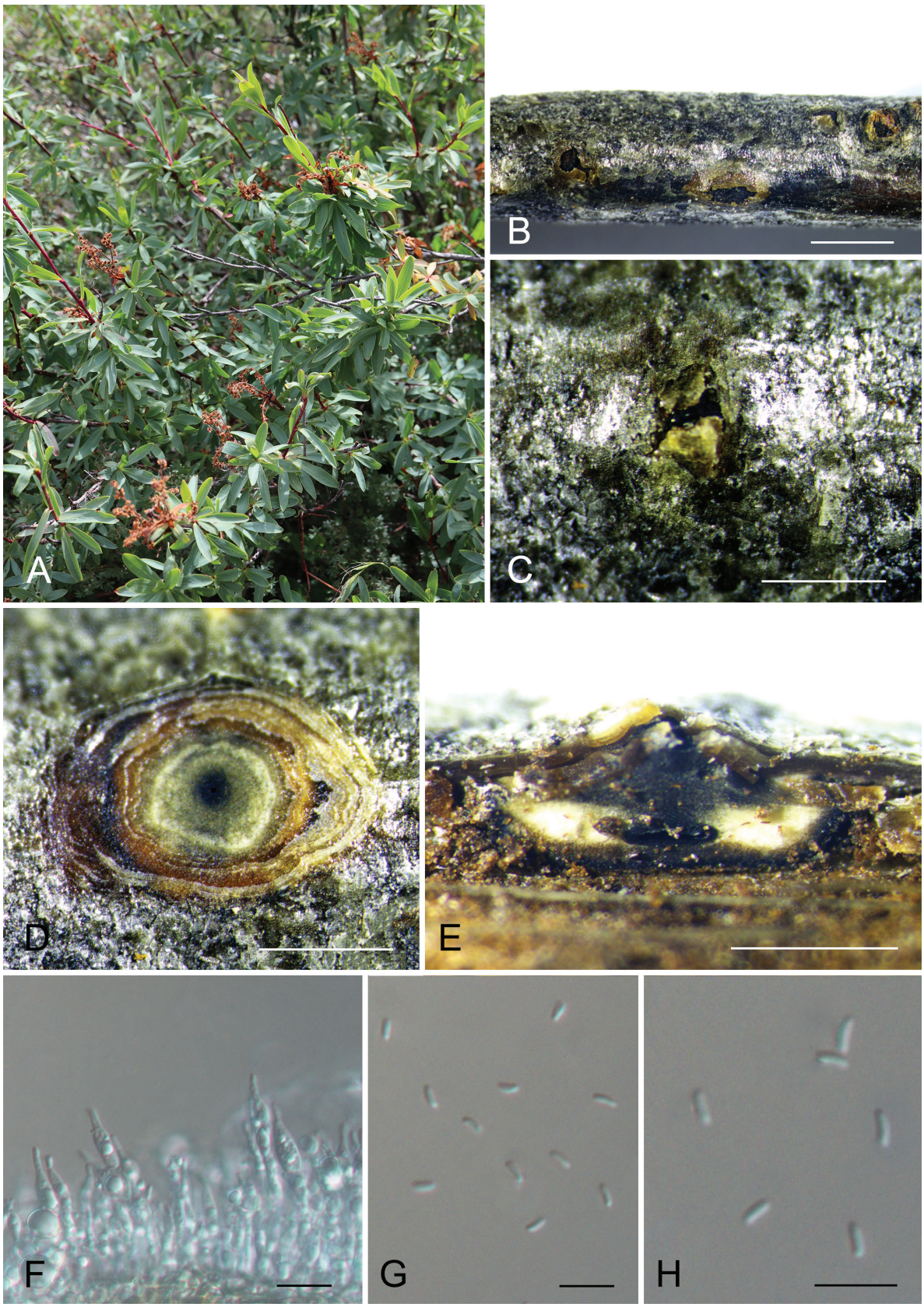

Figure 4. *Cytospora sibiraeicola* (CAF800084, holotype) **A, B** canker disease symptom **C** conidioma **D** transverse section through a conidioma **E** longitudinal section through a conidioma **F** conidiophores and conidia **G, H** conidia. Scale bars: 2000 µm (**B**); 1000 µm (**C–E**); 10 µm (**F–H**).

Discussion

Cytospora is a species-rich genus occurring on various plant hosts (Fotouhifar et al. 2010; Aiello et al. 2019; Jayawardena et al. 2019; Úrbez-Torres et al. 2020; Hanifeh et al. 2022). However, in the third pole of the Earth named Qinghai-Tibet Plateau, canker pathogens, such as *Cytospora*, have been seldom surveyed previously. In the comprehensive study on the genus *Cytospora* in China, only one species *C. chrysosperma* was recorded from Ulmus pumila in Tibet (Fan et al. 2020). Subsequently, *Cytospora cotoneastricola* and *C. tibetensis* from *Cotoneaster* sp. and *Cytospora rosicola* from *Rosa* sp. were discovered in Tibet (Pan et al. 2020). The current study introduces two new species named *C. myricicola* from *Myricaria paniculate* and *C. sibiraeicola* from *Sibiraea angustata* in Tibet, China. In addition, a new host record of *C. populina* on *Prunus cerasifera* was discovered.

To our knowledge, *Cytospora myricicola* is the first species of *Cytospora* discoved on the host genus *Myricaria* (Fan et al. 2020). *Cytospora sibiraeicola* and *C. sibiraeae* have been recorded from the host species *Sibiraea angustata* (Liu et al. 2015). *Cytospora sibiraeae* was described, based only on the sexual morph and is currently impossible to be distinguished from *C. sibiraeicola* morphologically (Liu et al. 2015). However, these two species occurring on *Sibiraea angustata* are phylogenetically obviously distinct (Fig. 1).

Species of *Cytospora* are known as opportunistic pathogens mainly infecting woody hosts and some of the species occur on a wide host range (Adams et al. 2005; Fan et al. 2020). The *Cytospora* species and their host association have been revealed in this study; however, further studies are required to confirm the fungal pathogenicity.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: JRL, JTL, NJ. Methodology: NJ. Formal analysis: JRL. Investigation: JRL, JTL, NJ. Resources: JRL, JTL, NJ. Data Curation: JRL, JTL. Writing - Original draft: JRL. Writing - Review and Editing: NJ. Visualization: JTL, NJ.

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Data availability

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

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Research Article

Three new species of *Favolaschia* (Mycenaceae, Agaricales) from South China

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Abstract

The genus *Favolaschia* within the family Mycenaceae is characterised by the gelatinous basidiomata with poroid hymenophore and most species inhabit monocotyledonous plants. In this study, many samples covering a wide geographic range in China were examined morphologically and phylogenetically using concatenated ITS1-5.8S-ITS2-nLSU sequence data. Three new species clustering in *Favolaschia* sect. *Anechinus*, namely *Favolaschia imbricata*, *F. miscanthi* and *F. sinarundinariae*, are described. *Favolaschia imbricata* is characterised by imbricate basidiomata with pale grey to greyish colour when fresh and broadly ellipsoid basidiospores measuring 7–9 × 5–6.8 µm; *F. miscanthi* is characterised by satin white basidiomata when fresh, broadly ellipsoid basidiospores measuring 7.5–10 × 5.5–7 µm and inhabit rotten *Miscanthus*; *F. sinarundinariae* is characterised by greyish-white basidiomata when fresh, dark grey near the base upon drying, broadly ellipsoid to subglobose basidiospores measuring 7–9 × 5–7 µm and inhabit dead *Sinarundinaria*. The differences amongst the new species and their morphologically similar and phylogenetically related species are discussed. In addition, an updated key to 19 species of *Favolaschia* found in China is provided.

Key words: New taxa, phylogeny, taxonomy, wood-decaying fungi

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Introduction

The genus *Favolaschia* (Pat.) Pat., typified by *F. gaillardia* (Pat.) Pat. (Patouillard 1887), belongs to the Mycenaceae Overeem (Agaricales) (Moncalvo et al. 2002; Bodensteiner et al. 2004). It is characterised by gelatinous basidiomata with poroid hymenophore, a monomitic hyphal system, a gelatinous hyphal structure, the presence of gloeocystidia and acanthocystida which are terminal, mostly swollen tips of hyphae covered by outgrowths (Clémençon 1977) and amyloid basidiospores (Singer 1945, 1974; Gillen et al. 2012; Capelari et al. 2013; Magnago et al. 2013; Zhang and Dai 2021; Zhang et al. 2023). *Favolaschia* species have a worldwide distribution and prefer humid climates with the highest species diversity in subtropical to tropical zones (Singer 1974; Gillen et al.

2012; Magnago et al. 2013). The basidiomata are found mostly on decaying parts of monocotyledonous plants, as well as ferns and cause white rot (Singer 1974; Gillen et al. 2012).

A total of 119 records of *Favolaschia* are listed in Index Fungorum (http://www.indexfungorum.org) and around 60 species are accepted (Johnston et al. 2006; Gillen et al. 2012; Magnago et al. 2013). The name "*Favolaschia*" was first introduced by Patouillard (1887) as a section of *Laschia* Fr and treated at a generic level later (Patouillard and Lagerheim 1892). The taxonomic history of the genus has been systematically compiled by Singer (1974) and Johnston et al. (2006). Singer (1974) divided *Favolaschia* into two sections, based on morphological studies, namely section *Favolaschia* Singer and section *Anechinus* Singer. Amongst them, taxa of *F.* sect. *Favolaschia* is characterised by having mostly orange, yellow, red, lateritious or pink pileus and the presence of acanthocytes in pileipellis. Inversely, taxa of *F.* sect. *Anechinus* are characterised by lacking acanthocystidia or replaced by diverticulate hyphae. Later, Johnston et al. (2006), Gillen et al. (2012) and Magnago et al. (2013) confirmed the two sections by phylogenetic analyses.

Recently, the systematic study of *Favolaschia* in China has gradually increased. Tolgor et al. (2021) investigated the phylogenetic relationships of *Favolaschia* and its related genera in Mycenaceae and reported four species distributed in China. Zhang and Dai (2021) investigated phylogenetic analysis of the genus *Favolaschia*, based on a large sample and revealed four new species in the *F. calocera* complex. Until Zhang et al. (2023) re-summarised the species diversity, phylogenetic relationships, divergence time and potential geographic distribution using a large number of samples covering a wide geographic range in China. To date, 16 species of *Favolaschia* have been reported from China, based on phylogenetic analyses, namely, *F. brevibasidiata* Q.Y. Zhang and Y.C. Dai, *F. brevistipitata* Q.Y. Zhang and Y.C. Dai, *F. longistipitata* Q.Y. Zhang and Y.C. Dai, *F. minutissima* Q.Y. Zhang and Y.C. Dai, *F. bannaensis* Q.Y. Zhang & Y.C. Dai, *F. crassipora* Q.Y. Zhang & Y.C. Dai, *F. flabelliformis* Q.Y. Zhang & Y.C. Dai, *F. manipularis* (Berk.) Teng, *F. peziziformis* (Berk. and M.A. Curtis) Kuntze, *F. pustulosa* (Jungh.) Kuntze, *F. rigida* Q.Y. Zhang & Y.C. Dai, *F. semicircularis* Q.Y. Zhang & Y.C. Dai, *F. subpustulosa* Q.Y. Zhang & Y.C. Dai, *F. tenuissima* Q.Y. Zhang & Y.C. Dai, *F. tephroleuca* Q.Y. Zhang & Y.C. Dai and *F. tonkinensis* (Pat.) Kuntze; the first four species belong to the *F. calocera* complex of the *F.* sect. *Favolaschia*; while the others belong to the *F.* sect. *Anechinus* (Zhang and Dai 2021; Guo et al. 2022; Ma et al. 2022; Wu et al. 2022a; Dong et al. 2023; Zhang et al. 2023).

During a study on wood-rotting fungi from south China, several samples belonging to *Favolaschia* were collected and three unknown species were morphologically distinguished. To confirm the affinity of the taxa, phylogenetic analysis was performed, based on a combined sequence dataset of ITS1-5.8S-ITS2-nLSU.

Materials and methods

Morphological studies

The specimens were collected from Guangxi Autonomous Region, Guangdong and Sichuan Provinces in south China and the specific locations were marked in Fig. 1. They were deposited in the Fungarium of the Institute of Microbiology, Beijing Forestry University (BJFC), Beijing, China. Macro-morphological descrip-

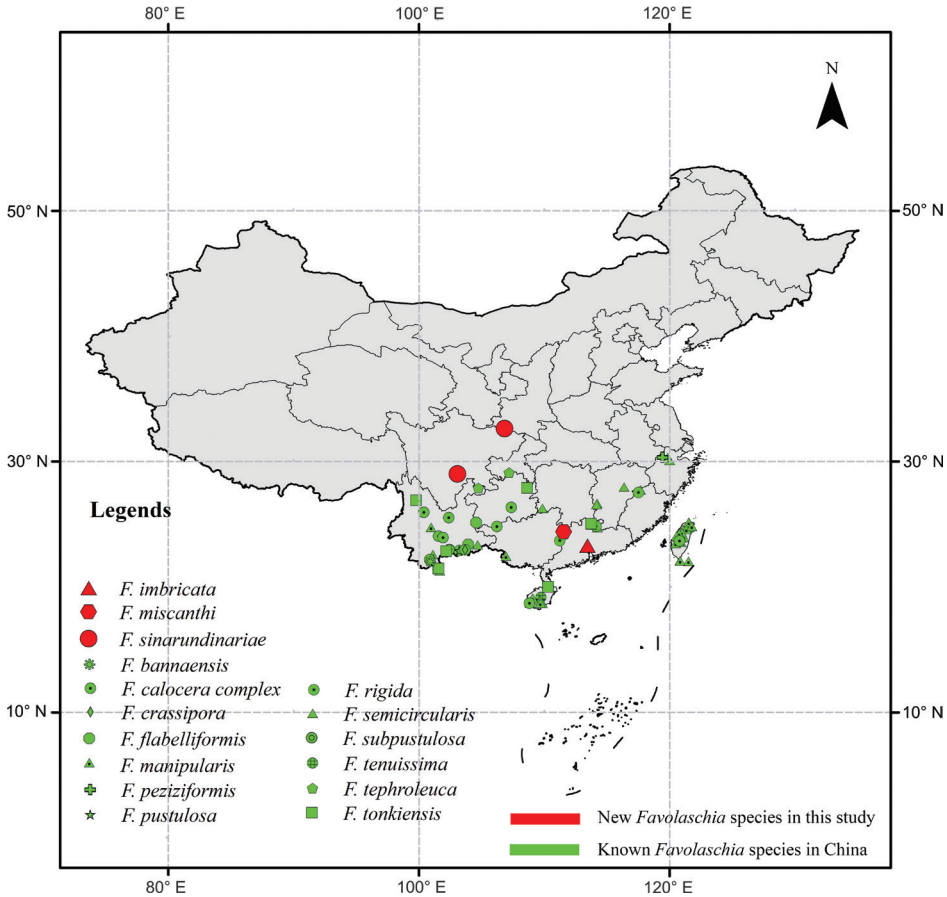

Figure 1. The geographical locations of *Favolaschia* species distributed in China (the base map of China was obtained from the Map Technology Review Center, Department of Natural Resources, http://bzdt.ch.mnr.gov.cn/).

tions were based on field notes and dried specimens. Microscopic features were examined and described in 5% KOH (potassium hydroxide) and 2% phloxine B ($C_{20}H_2Br_4Cl_4Na_2O_5$) with a magnification of up to 1,000× using a Nikon Eclipse 80i microscope and phase contrast illumination. Colour terms followed Kornerup and Wanscher (1978) and Petersen (1996). A Nikon Digital Sight DS-L3 camera was used to photograph microscopic structures. Other reagents, including Cotton Blue and Melzer's reagents were used to observe the micromorphology structures following Zhang et al. (2023). To show the variation in spore sizes, 5% of measurements were excluded from each end of the range and shown in parentheses. Thirty basidiospores from each specimen were measured. The following abbreviations are used: IKI = Melzer's reagent, IKI+ = amyloid; CB = Cotton Blue, CB– = acyanophilous in Cotton Blue; L = arithmetic average of basidiospores length, W = arithmetic average of basidiospores width, Q = L/W ratios, (n = x/y) = the number of spores (x) measured from a given number of specimens (y).

DNA extraction and sequencing

A cetyl trimethylammonium bromide (CTAB) rapid plant genome extraction kit (Aidlab Biotechnologies, Co., Ltd., Beijing, China) was used to extract DNA (Wu et al. 2020, 2022b). The ITS1-5.8S-ITS2 region was amplified with the primer pair ITS5/ITS4 (White et al. 1990) using the following protocol: initial denaturation at 95 °C for 4 min, followed by 34 cycles at 94 °C for 40 s, 54 °C for 45 s

and 72 °C for 1 min and final extension at 72 °C for 10 min. The nLSU region was amplified with the primer pair LR0R/LR7 (White et al. 1990) using the following protocol: initial denaturation at 94 °C for 1 min, followed by 34 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min and final extension at 72 °C for 10 min. The PCR products were purified and sequenced by the Beijing Genomics Institute (BGI), China with the same primers. The newly-generated sequences in this study have been deposited in GenBank and are listed in Table 1.

Phylogenetic analyses

The dataset of concatenated ITS1-5.8S-ITS2-nLSU sequences of the *Favolaschia* was analysed. Sequences of *Mycena seminau* A.L.C. Chew & Desjardin were used as outgroups (Chew et al. 2014). The newly-generated sequences in this study and additional sequences retrieved from Zhang et al. (2023) were partitioned to ITS1, 5.8S, ITS2, nrLSU and then aligned separately using MAFFT v.74 (http://mafft.cbrc.jp/alignment/server/, Katoh et al. 2017) with the G-INS-I iterative refinement algorithm and optimised manually in BioEdit v.7.0.5.3 (Hall 1999). The separate alignments were then concatenated using PhyloSuite v.1.2.2 (Zhang et al. 2020).

Maximum Likelihood (ML) analyses and Bayesian Inference (BI) were carried out by using RAxML v.8.2.10 (Stamatakis 2014) and MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003), respectively. In ML analysis, statistical support values were obtained by using rapid bootstrapping with 1000 replicates, with default settings for other parameters. For BI, the best-fit partitioning scheme and substitution model were determined by using ModelFinder (Kalyaanamoorthy et al. 2017) via the "greedy" algorithm, branch lengths estimated as "linked" and AICc. Four Markov chain Monte Carlo chains (one cold) were constructed for 5,000,000 generations, with sampling every 1000 generations. Convergence was assessed as the standard deviation of split frequencies < 0.01. The first quarter of the trees, which represented the burn-in phase of the analyses, were discarded and the remaining trees were used to calculate posterior probabilities (BPP) in the majority rule consensus tree.

Phylogenetic trees were visualised by using FigTree version 1.4.4 (Rambaut 2018). Branches that received bootstrap supports for ML (≥ 75%) and BPP (≥ 0.95) were considered as significantly supported. The best topologies from ML analyses are shown in this study and the final alignments and the retrieved topologies were deposited in TreeBASE (http://treebase.org/treebase-web/home.html), under accession ID: 30973.

Results

Phylogenetic analyses

In this study, the combined ITS1-5.8S-ITS2-nLSU dataset included sequences from 113 specimens, representing 34 species of *Favolaschia* and one species of *Mycena* (Pers.) Roussel as the outgroup (Table 1, Fig. 2). ModelFinder suggested models were HKY+F+G4 for ITS1+ITS2, K2P+G4 for 5.8s and K2P+G4 for nLSU, for the Bayesian analysis. The BI analysis resulted in a concordant topology with an average standard deviation of split frequencies of 0.008584. The ML and BI analyses resulted in nearly identical topologies and only the ML

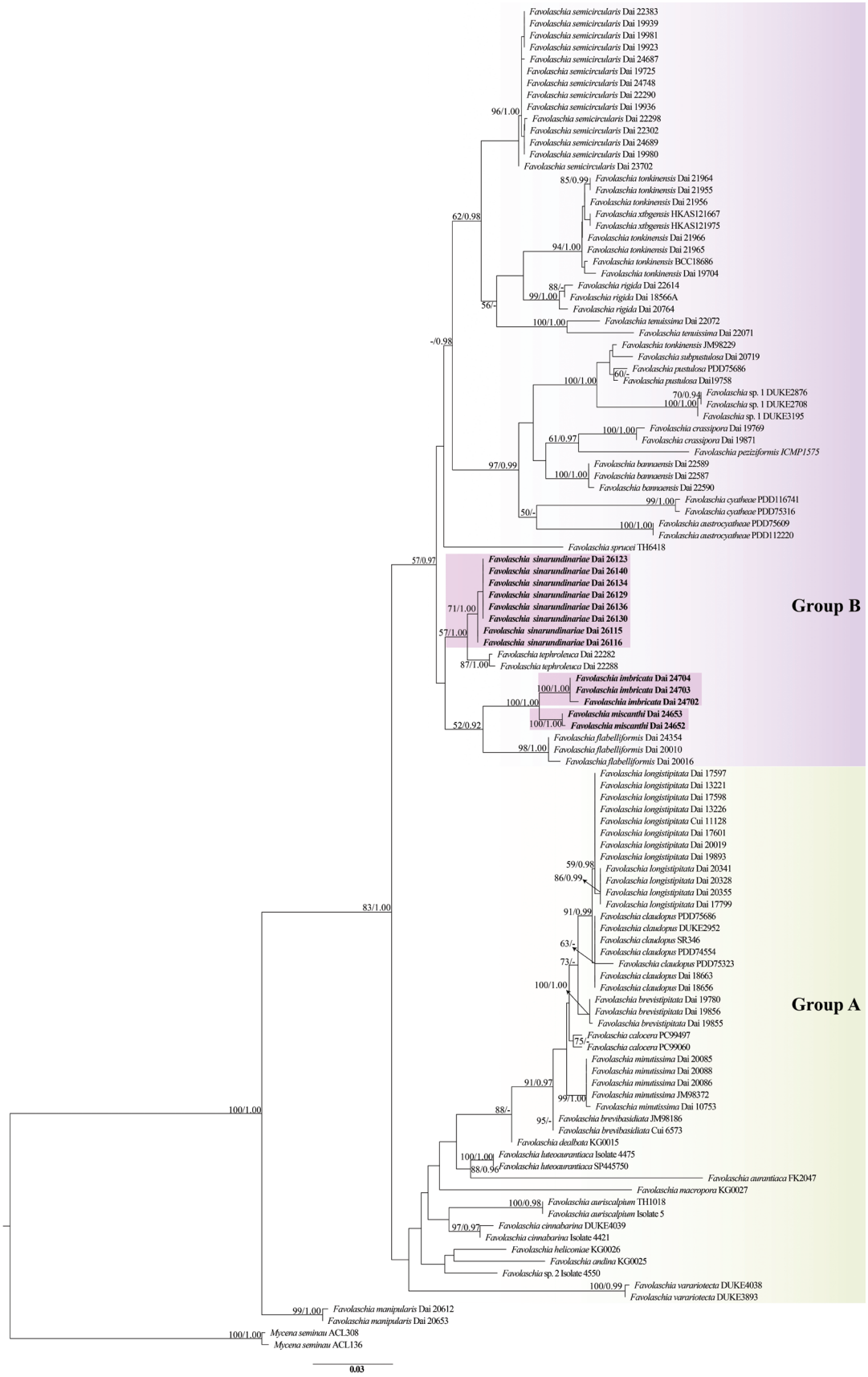

Figure 2. Maximum Likelihood (ML) tree illustrating the phylogeny of *Favolaschia*, based on a combined ITS1-5.8S-ITS2-nLSU-mtSSU-nuSSU dataset. Branches are labelled with parsimony bootstrap values (ML) higher than 50% and Bayesian Posterior Probabilities (BPPs) more than 0.90. (Group A: *Favolaschia* sect. *Favolaschia*; Group B: *Favolaschia* sect. *Anechinus*)

Table 1. Names, specimen numbers, locality and corresponding GenBank accession numbers of the taxa used in this study.

Species	Specimen no.	Locality	ITS no.	nLSU no.
Favolaschia andina	KG0025	Panama	HM246678	HM246679
F. aurantiaca	FK2047	Brazil	JX987670	–
F. aurantiaca	KG0013	Panama	–	HM246676
F. auriscalpium	Isolate 5	–	KY649461	–
F. auriscalpium	TH1018	Guyana	DQ026241	–
F. austrocyatheae	PDD75609	New Zealand	NR132809	–
F. austrocyatheae	PDD75609	New Zealand	DQ026257	–
F. bannaensis	Dai 22587	Yunnan, China	ON870497	ON870473
F. bannaensis	Dai 22589	Yunnan, China	ON870498	ON870474
F. bannaensis	Dai 22590	Yunnan, China	ON870499	ON870475
F. brevibasidiata	Cui 6573	Hainan, China	MZ661794	–
F. brevibasidiata	JM98186	Yunnan, China	DQ026239	–
F. brevistipitata	Dai 19780	Yunnan, China	MZ661772	MZ661742
F. brevistipitata	Dai 19855	Yunnan, China	MZ661773	MZ661743
F. brevistipitata	Dai 19856	Yunnan, China	MZ661774	MZ661744
F. calocera	PC99060	Madagascar	DQ26252	–
F. calocera	PC99497	Madagascar	DQ026253	–
F. cinnabarina	Isolate 4421	Brazil	JX987669	–
F. cinnabarina	RVPR82	–	–	AF261416
F. claudopus	Dai 18656	Australia	MZ661775	MZ661735
F. claudopus	Dai 18663	Australia	MZ661776	MZ661734
F. claudopus	SR346	Kenya	DQ026237	–
F. claudopus	PDD74554	New Zealand	DQ026251	–
F. claudopus	PDD75323	New Zealand	DQ026248	–
F. claudopus	PDD75686	New Zealand	DQ026249	–
F. claudopus	DUKE2952	New Zealand	DQ026238	–
F. crassipora	Dai 19769	Yunnan, China	ON870500	ON870476
F. crassipora	Dai 19871	Yunnan, China	ON870501	ON870477
F. cyatheae	PDD75316	New Zealand	NR132808	–
F. cyatheae	PDD75316	New Zealand	DQ026256	–
F. dealbata	KG0015	Panama	–	HM246677
F. flabelliformis	Dai 20010	Yunnan, China	ON870502	ON870478
F. flabelliformis	Dai 20016	Yunnan, China	ON870503	–
F. flabelliformis	Dai 24354	Yunnan, China	**OR271272**	**OR260441**
F. heliconiae	KG0026	Panama	–	HM246680
F. imbricata	**Dai 24702**	**Guangdong, China**	**OR334876**	–
F. imbricata	**Dai 24703**	**Guangdong, China**	**OR334877**	**OR334881**
F. imbricata	**Dai 24704**	**Guangdong, China**	**OR334878**	**OR334882**
F. longistipitata	Dai 13221	Yunnan, China	MZ661777	–
F. longistipitata	Dai 13226	Yunnan, China	MZ661778	–
F. longistipitata	Cui 11128	Yunnan, China	MZ661779	–
F. longistipitata	Dai 17597	Yunnan, China	MZ661780	–
F. longistipitata	Dai 17598	Yunnan, China	MZ661781	–

Species	Specimen no.	Locality	ITS no.	nLSU no.
F. longistipitata	Dai 17601	Yunnan, China	MZ661782	–
F. longistipitata	Dai 19799	Yunnan, China	MZ661784	MZ661739
F. longistipitata	Dai 19893	Yunnan, China	MZ661785	MZ661740
F. longistipitata	Dai 20019	Yunnan, China	MZ661786	MZ661741
F. longistipitata	Dai 20328	Yunnan, China	MZ661787	–
F. longistipitata	Dai 20341	Yunnan, China	MZ661788	–
F. longistipitata	Dai 20355	Yunnan, China	MZ661789	–
F. luteoaurantiaca	Isolate 4475	–	JX987667	–
F. luteoaurantiaca	SP445750	Brazil	NR132874	–
F. macropora	KG0027	Panama	NR132845	HM246682
F. manipularis	Dai 20612	Yunnan, China	MZ801776	MZ914395
F. manipularis	Dai 20653	Yunnan, China	MZ801777	–
F. minutissima	JM98372	Thailand	DQ026240	–
F. minutissima	Dai 10753	Hainan, China	MZ661790	–
F. minutissima	Dai 20085	Hainan, China	MZ661791	MZ661736
F. minutissima	Dai 20086	Hainan, China	MZ661792	MZ661737
F. minutissima	Dai 20088	Hainan, China	MZ661793	MZ661738
F. miscanthi	**Dai 24652**	**Guangxi, China**	**OR334879**	**OR334883**
F. miscanthi	**Dai 24653**	**Guangxi, China**	**OR334880**	**OR334884**
F. peziziformis	ICMP1575	Japan	DQ026255	–
F. peziziformis	PDD67440	New Zealand	–	AY572008
F. pustulosa	PDD75686	New Zealand	DQ026254	–
F. pustulosa	Dai 19758	Yunnan, China	MT292325	MT293226
F. rigida	Dai 18566A	Guangxi, China	ON870504	ON870479
F. rigida	Dai 20764	Yunnan, China	ON870505	ON870480
F. rigida	Dai 22614	Fujian, China	ON870506	–
F. semicircularis	Dai 19725	Guangdong, China	ON870507	ON870481
F. semicircularis	Dai 19923	Yunnan, China	ON870508	ON870482
F. semicircularis	Dai 19936	Yunnan, China	ON870509	ON870483
F. semicircularis	Dai 19939	Yunnan, China	ON870510	–
F. semicircularis	Dai 19980	Yunnan, China	ON870511	ON870484
F. semicircularis	Dai 19981	Yunnan, China	ON870512	ON870485
F. semicircularis	Dai 22290	Zhejiang, China	ON870513	ON870486
F. semicircularis	Dai 22298	Zhejiang, China	ON870515	ON870488
F. semicircularis	Dai 22302	Zhejiang, China	ON870516	–
F. semicircularis	Dai 23702	Hunan, China	**OR271273**	**OR260442**
F. semicircularis	Dai 24689	Guangxi, China	**OR271274**	**OR260443**
F. semicircularis	Dai 24748	Jiangxi, China	**OR271275**	**OR260444**
F. semicircularis	Dai 24687	Guangxi, China	**OR271276**	**OR260445**
F. semicircularis	Dai 22383	Fujian, China	ON870517	–
F. sinarundinariae	**Dai 26115**	**Sichuan, China**	**OR575908**	**OR575906**
F. sinarundinariae	**Dai 26116**	**Sichuan, China**	**OR575909**	**OR575907**
F. sinarundinariae	**Dai 26123**	**Sichuan, China**	**OR855969**	**OR855975**
F. sinarundinariae	**Dai 26129**	**Sichuan, China**	**OR855970**	–
F. sinarundinariae	**Dai 26130**	**Sichuan, China**	**OR855971**	–

Species	Specimen no.	Locality	ITS no.	nLSU no.
F. sinarundinariae	**Dai 26131**	**Sichuan, China**	**OR855972**	–
F. sinarundinariae	**Dai 26134**	**Sichuan, China**	**OR855973**	**OR855976**
F. sinarundinariae	**Dai 26140**	**Sichuan, China**	**OR855974**	**OR855975**
F. sp. 1	DUKE2708	Australia	DQ026234	–
F. sp. 1	DUKE2876	Australia	DQ026235	–
F. sp. 1	DUKE3195	Papua New Guinea	DQ026236	–
F. sp. 2	Isolate 4550	Panama	JX987668	–
F. sprucei	TH6418	Guyana	DQ026246	–
F. subpustulosa	Dai 20719	Yunnan, China	ON870518	ON870489
F. tenuissima	Dai 22072	Hainan, China	ON870520	ON870491
F. tenuissima	Dai 22071	Hainan, China	ON870519	ON870490
F. tephroleuca	Dai 22282	Yunnan, China	ON870521	ON870492
F. tephroleuca	Dai 22288	Chongqing, China	ON870522	ON870493
F. tonkinensis	Dai 21955	Hainan, China	ON870523	ON870494
F. tonkinensis	Dai 21956	Hainan, China	ON870524	ON870495
F. tonkinensis	Dai 21964	Hainan, China	ON870525	–
F. tonkinensis	Dai 21965	Hainan, China	ON870526	ON870496
F. tonkinensis	Dai 21966	Hainan, China	ON870527	–
F. tonkinensis	Dai 19704	Guangdong, China	**OR271277**	**OR260446**
F. tonkinensis	BCC 18686	–	MN093316	MN093317
F. tonkinensis	JM98229	Yunnan, China	DQ026247	–
F. varariotecta	DUKE3893	Puerto Rico	DQ026243	–
F. varariotecta	DUKE4038	Puerto Rico	DQ026244	–
F. xtbgensis	HKAS 121667	Yunnan, China	OL413048	OL413044
F. xtbgensis	HKAS 121975	Yunnan, China	OL413036	OL413035
Mycena seminau	ACL136	Malaysia	KF537250	KJ206952
Mycena seminau	ACL308	Malaysia	KF537252	KJ206964

Notes: New sequences are in bold; "–" represents missing data.

tree is presented with the bootstrap supports for ML and BPP not less than 50% and 0.90, respectively.

The phylogeny is similar to those of Johnston et al. (2006), Magnago et al. (2013) and Zhang et al. (2023). All *Favolaschia* samples are clustered within two groups (A and B), which correspond to the sections proposed by Singer (1974): *Favolaschia* sect. *Favolaschia* (group A) and *F.* sect. *Anechinus* (group B), except *F. manipularis*. In this study, three new lineages with high support (100/1.00, 100/1.00 and 71/1.00, respectively) nest in *F.* sect. *Anechinus* (group B). Amongst them, five specimens from Guangdong and Guangxi formed two lineages and clustered into a clade with strong support (100/1.00), namely *F. imbricata* and *F. miscanthi*. Eight specimens from Sichuan formed a support lineage, namely *F. sinarundinariae*, sister to *Favolaschia tephroleuca*. Furthermore, similarity searches with the ITS barcoding from the three new species were performed using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) from NCBI and showed less than 95% identity, respectively, in the genus *Favolaschia*, which demonstrated the obvious nucleotide differences of the three species with other species in the genus.

Taxonomy

***Favolaschia imbricata* Q.Y. Zhang, L.S. Bian, F. Wu & Y.C. Dai, sp. nov.**
MycoBank No: 851807
Figs 3A, 4

Type. China, Guangdong Province, Guangzhou, Baiyun District, Maofengshan Forest Park, dead bamboo, 15 Apr 2023, Dai 24702 (BJFC042256, holotype).

Etymology. "*imbricata*" (Lat.): refers to the species having imbricate basidiomata.

Macrostructures. Basidiomata annual, gregarious, gelatinous when fresh and dry. Pilei 2–5 × 1.5–3 mm, conchoid, semicircular to subcircular; pileal surface pale grey to greyish-white (B1) when fresh, dark grey or mouse-grey (1D1–1F1) upon drying, convex with a reticulate pattern matching the pores below, faintly pruinose when dry; margin incurved, entire; context thin. Hymenophore paler than pileal surface, greyish-white (B1) when fresh and ash-grey (1B1–1C1) when dry, poroid, about 22–55 pores per basidiomata; mature pores 0.2–0.6 mm diam., polygonal, larger near the base and smaller near the edge, the marginal pores often incomplete; tubes up to 0.3 mm long. Stipe absent.

Microstructures. Basidiospores (6.8–)7–9 × 5–6.8 µm, L = 7.92 µm, W = 5.84 µm, Q = 1.31–1.38 (*n* = 90/3), broadly ellipsoid to subglobose, hyaline, thin-walled, smooth, with some guttules, faintly IKI+, CB–. Basidia 24–30 × 7–13 µm, cylindrical or clavate with some guttules, 4–spored, sterigmata 3–6 µm long; basidioles similar in shape to the basidia, but slightly smaller. Gloeocystidia and acanthocystidia absent. Cheilocystidia 25–39 × 7–14 µm, present at dissepiment edge, cylindrical or clavate, with obtuse diverticulate projections at the apex, thin-walled. Pileipellis hyphae subparallel, smooth to diverticulate, thin-walled, 5–11 µm diam.; terminal cells cystidioid or pyriform, smooth, thin-walled. Tramal hyphae subparallel, widely spaced in a gelatinous matrix, some with dense contents, some collapsed, thin-walled, 2–5 µm diam. Clamp connections present.

Additional specimens examined. China, Guangdong Province, Guangzhou, Baiyun District, Maofengshan Forest Park, dead bamboo, 15 Apr 2023, Dai 24703 (BJFC042257, paratype), Dai 24704 (BJFC042258, paratype).

Distribution and ecology. *Favolaschia imbricata* is distributed in subtropical area of Guangdong Province, China; it grows on dead bamboo and causes a white rot.

***Favolaschia miscanthi* Q.Y. Zhang, L.S. Bian, F. Wu & Y.C. Dai, sp. nov.**
MycoBank No: 851808
Figs 3B, 5

Type. China, Guangxi Autonomous Region, Hezhou, Dazhongshan Forest Park, rotten *Miscanthus*, 19 Apr 2023, Dai 24652 (BJFC042179, holotype).

Etymology. "*miscanthi*" (Lat.): refers to the species inhabiting rotten *Miscanthus*.

Macrostructures. Basidiomata annual, gregarious, gelatinous when fresh and dry. Pilei 3–6 × 1.5–4 mm, conchoid or semicircular; pileal surface satin white (1A1–2A1) when fresh and drying, convex, transparent with a reticulate pattern matching the pores below, glabrous; margin straight, crenulate; context

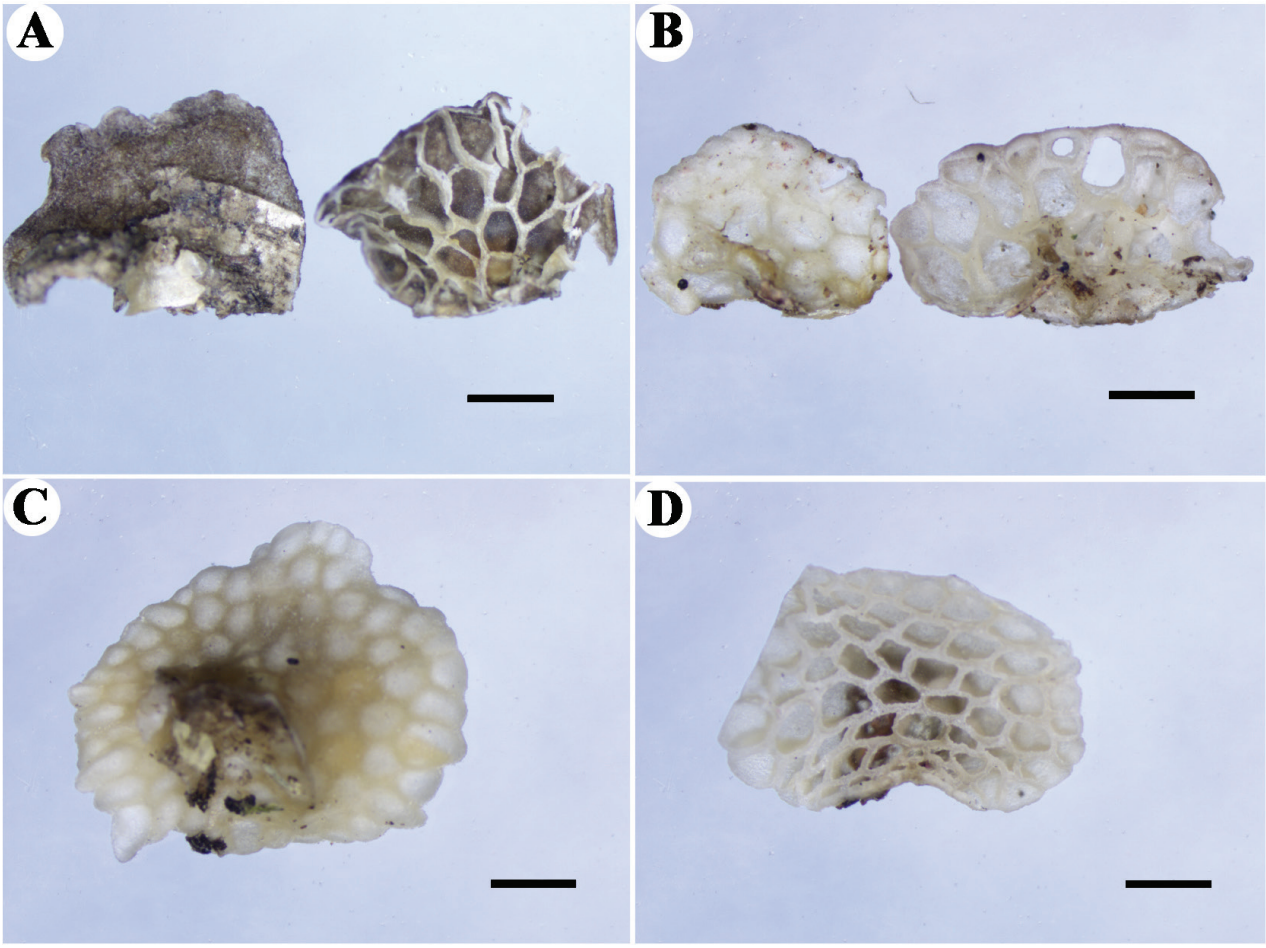

Figure 3. Dry basidiomata of *Favolaschia* species **A** *Favolaschia imbricata* **B** *Favolaschia miscanthi* **C, D** *Favolaschia sinarundinariae*. Scale bars: 1 mm.

thin, transparent. Hymenophore concolorous with pileal surface, poroid, up to 40 pores per basidiocarp; mature pores 0.5–1 mm diam., polygonal, larger near the base and smaller near the edge, the marginal pores often incomplete; tubes up to 0.4 mm long. Stipe absent.

Microstructures. Basidiospores 7.5–10 × 5.5–7(–7.5) µm, L = 8.90 µm, W = 6.28 µm, Q = 1.37–1.46 (*n* = 60/2), broadly ellipsoid to subglobose, hyaline, thin-walled, smooth, with some guttules, faintly IKI+, CB–. Basidia 32–45 × 7–10 µm, narrowly clavate, tapered towards the base, apex broadly rounded, 4–spored, sterigmata 2–8 µm long; basidioles similar in shape to the basidia, but slightly smaller. Gloeocystidia and acanthocystidia absent. Cheilocystidia 17–34 × 7–10 µm, present at dissepiment edge, broom-shaped or irregular with small diverticulate projections on the sides and at the apex, thin-walled. Pileipellis hyphae interwoven, smooth to diverticulate, thin-walled, 4–7 µm in diam.; terminal cells cylindrical or clavate, with obtuse diverticulate projections at the apex, orientated perpendicular to pileal surface. Tramal hyphae interwoven, widely spaced in a gelatinous matrix, some with dense contents, some collapsed, thin-walled, 2–6 µm in diam. Clamp connections present.

Additional specimen examined. China, Guangxi Autonomous Region, Hezhou, Dazhongshan Forest Park, rotten *Miscanthus*, 19 Apr 2023, Dai 24653 (BJFC042180, paratype).

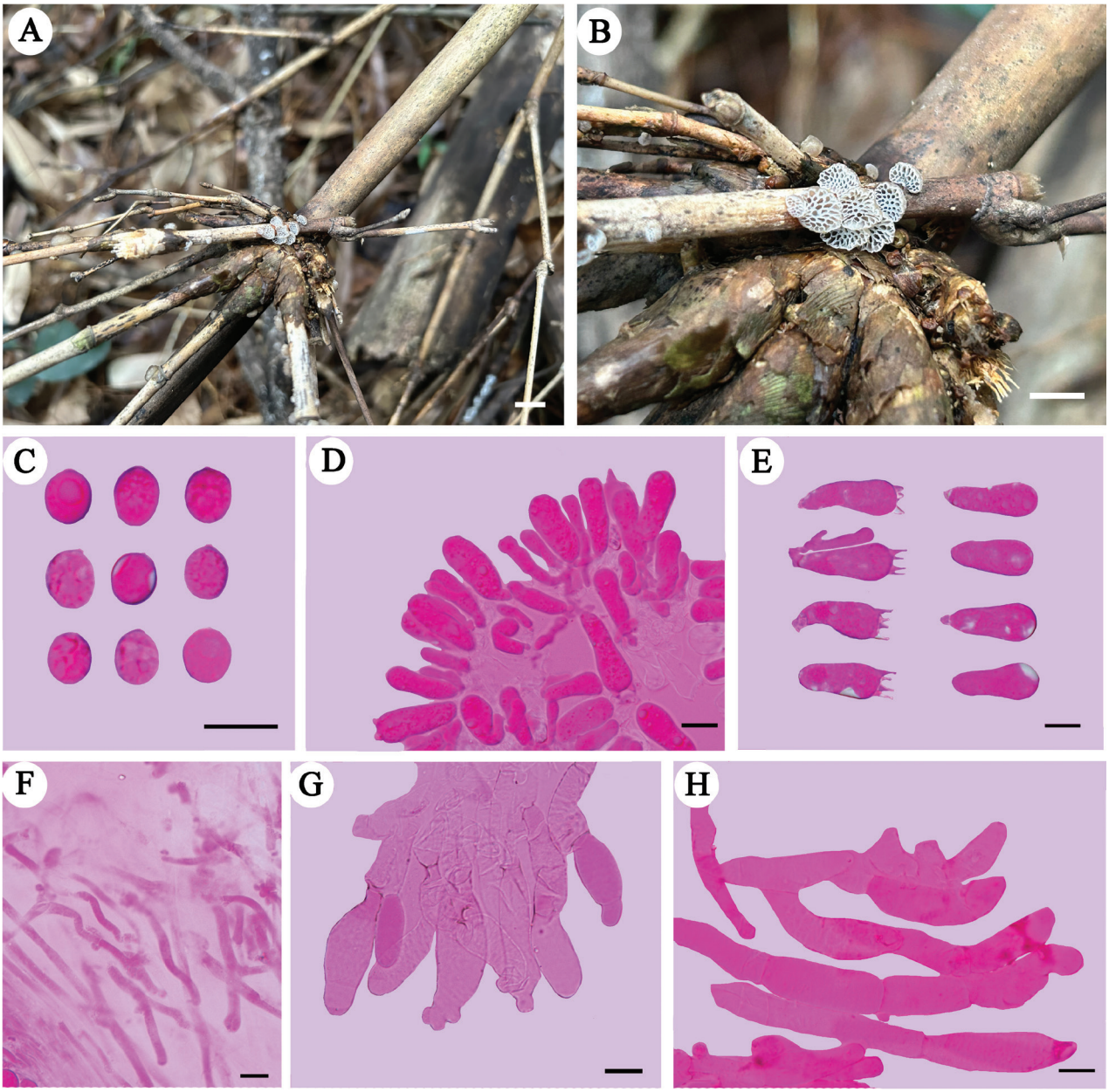

Figure 4. Basidiomata and microscopic structures of *Favolaschia imbricata* **A, B** basidiomata (Dai 24702, holotype) **C** basidiospores **D** hymenium in trama **E** basidia and basidioles **F** tramal hyphae **G** cheilocystidia at dissepiment edge **H** hyphae of pileipellis. Scale bars: 5 mm (**A, B**); 10 µm (**C–H**).

Distribution and ecology. *Favolaschia miscanthi* is distributed in the subtropical area of the Guangxi Autonomous Region, China; it grows on rotten *Miscanthus* and causes white rot.

Favolaschia sinarundinariae **Q.Y. Zhang, L.S. Bian, F. Wu & Y.C. Dai, sp. nov.**
MycoBank No: 851809
Figs 3C, D, 6

Type. China, Sichuan Province, Bazhong, Nanjiang County, Micangshan National Forest Park, 25 Sep 2023, dead *Sinarundinaria*, Dai 26123 (BJFC043673, holotype).

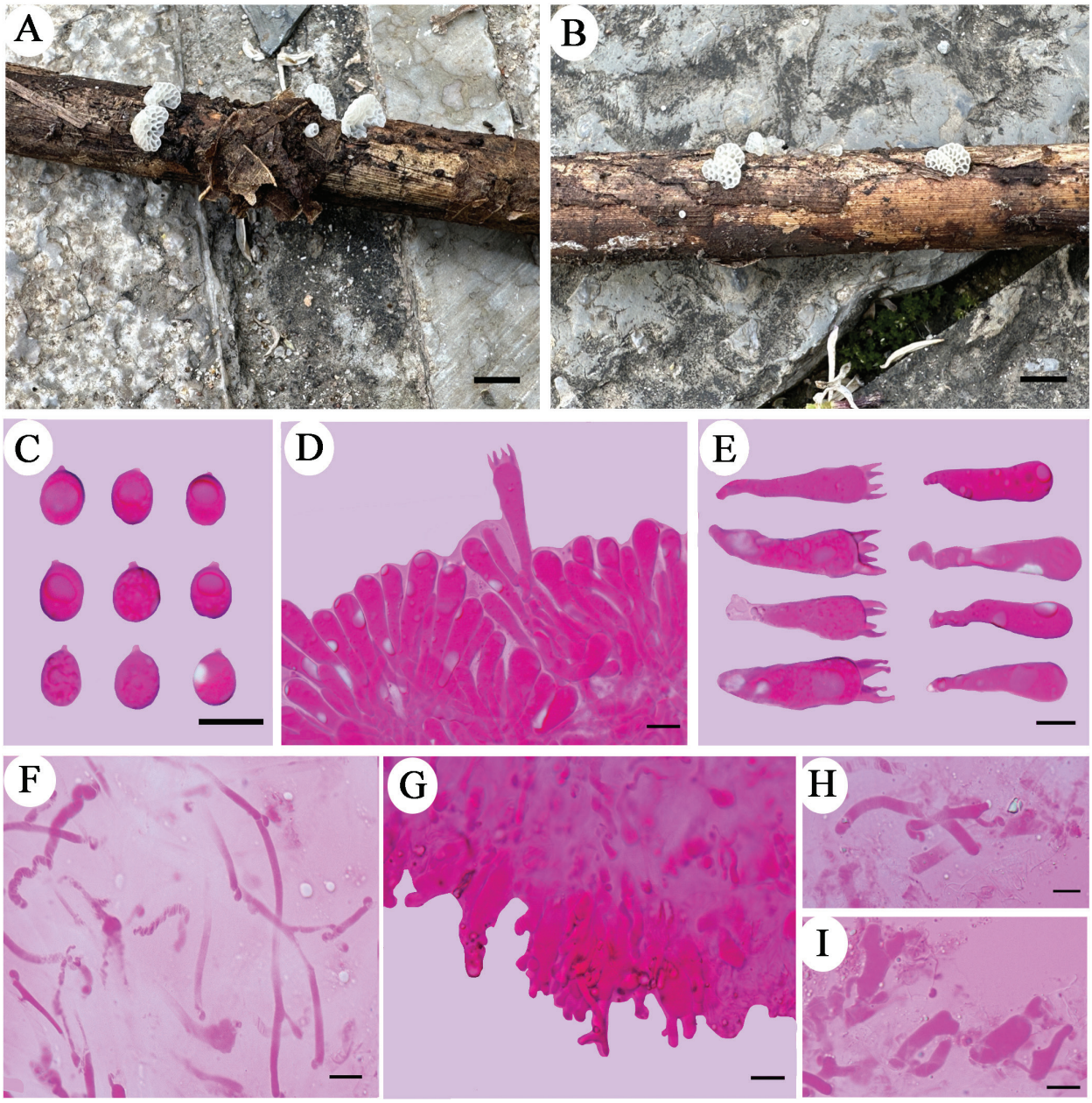

Figure 5. Basidiomata and microscopic structures of *Favolaschia miscanthi* **A, B** basidiomata (**A** Dai 24652, holotype **B** Dai 24653) **C** basidiospores **D** hymenium in trama **E** basidia and basidioles **F** tramal hyphae **G** cheilocystidia at dissepiment edge **H** hyphae of pileipellis **I** terminal cells of pileipellis. Scale bars: 5 mm (**A, B**); 10 µm (**C–I**).

Etymology. "*sinarundinariae*" (Lat.): refers to the species inhabiting dead *Sinarundinaria*.

Macrostructures. Basidiomata annual, gregarious, gelatinous when fresh and dry. Pilei 4–7 × 2–5 mm, conchoid to elliptic; pileal surface greyish-white (1B1) when fresh, dark grey (1F1) near the base upon drying, convex, transparent with a reticulate pattern matching the pores below, faintly pruinose when dry; margin straight, crenulate; context thin, opaque. Hymenophore concolorous with pileal surface, poroid, up to 80 pores per basidiomata; mature pores 0.4–1 mm diam., polygonal, the size varies irregularly, with some larger near the base or larger near the edges, the marginal pores often incomplete; tubes up to 0.5 mm long. Stipe absent.

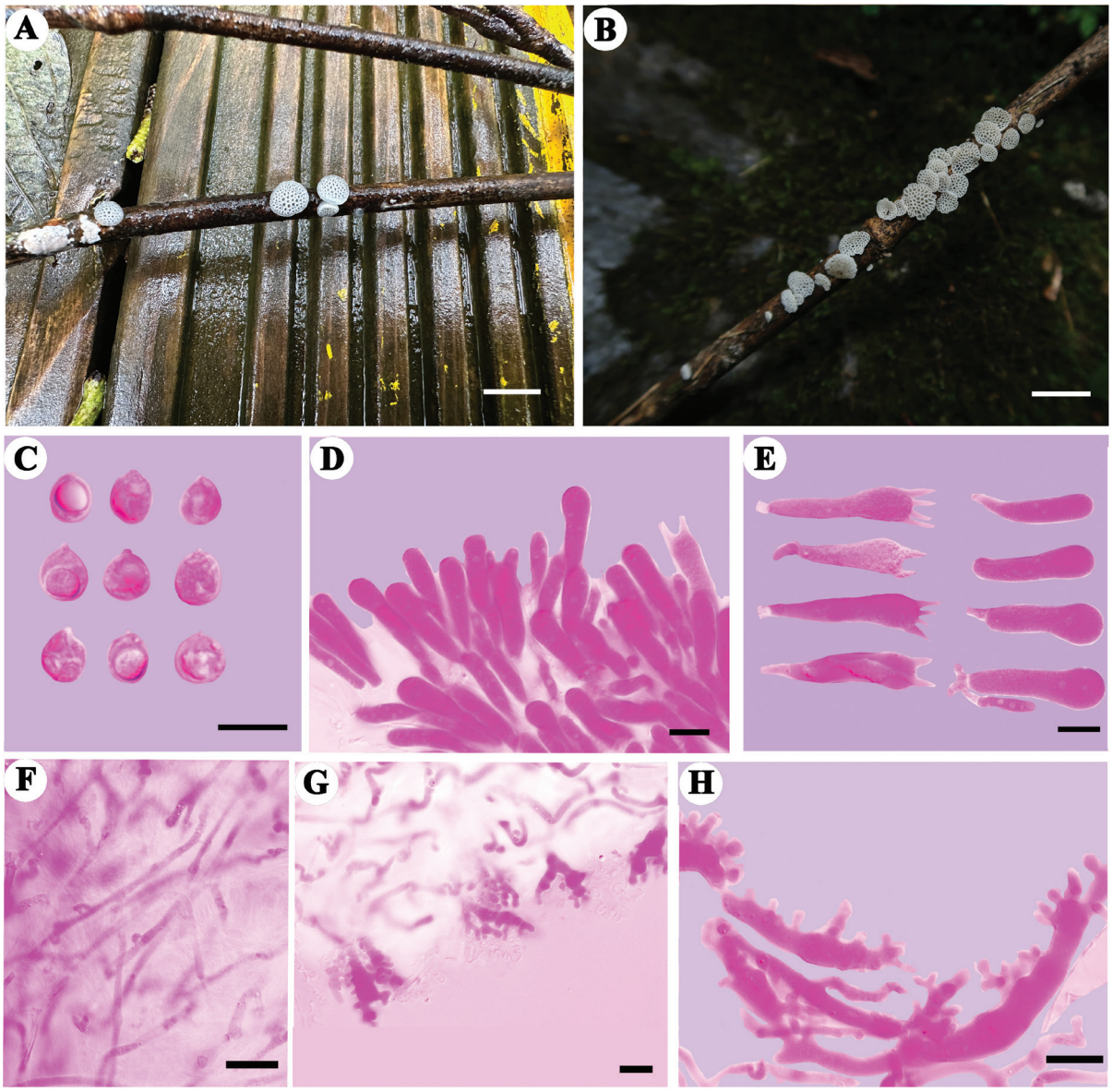

Figure 6. Basidiomata and microscopic structures of *Favolaschia sinarundinariae* **A, B** basidiomata (**A** Dai 26123, holotype **B** Dai 26116) **C** basidiospores **D** hymenium in trama **E** basidia and basidioles **F** tramal hyphae **G** cheilocystidia at dissepiment edge **H** hyphae and terminal cells of pileipellis. Scale bars: 1 cm (**A, B**); 10 µm (**C–H**).

Microstructures. Basidiospores 7–9 × 5–7(–7.5) µm, L = 7.87 µm, W = 6.08 µm, Q = 1.27–1.31 (*n* = 60/2), broadly ellipsoid to subglobose, hyaline, thin-walled, smooth, with some guttules, faintly IKI+, CB–. Basidia 40–50 × 7–9 µm, narrowly clavate, tapered towards the base, apex broadly rounded, 2 or 4–spored, sterigmata 5–10 µm long; basidioles similar in shape to the basidia, but slightly smaller. Gloeocystidia and acanthocystidia absent. Cheilocystidia 15–32 × 7–10 µm, present at dissepiment edge, antler-shaped or irregular with small diverticulate projections on the sides and at the apex, thin-walled. Pileipellis hyphae interwoven, with frequent diverticulate, thin-walled, 2–4 µm in diam., swollen to 8 µm on terminal; terminal cells irregular, with obtuse diverticulate projections at the apex, orientated perpendicular to pileal surface. Tramal hyphae interwoven, widely spaced in a gelatinous matrix, some with dense contents, some collapsed, thin-walled, 1.5–3 µm in diam. Clamp connections present.

Additional specimens examined. China, Sichuan Province, Bazhong, Nanjiang County, Micangshan National Forest Park, 25 Sep 2023, dead *Sinarundinaria*, Dai 26129 (BJFC043679, paratype), Dai 26130 (BJFC, paratype), Dai 26134 (BJFC, paratype), Dai 26136 (BJFC, paratype), Dai 26142 (BJFC043692, paratype); Leshan, Ebian County, Heizhugou, 11 Aug 2023, dead *Sinarundinaria*, Dai 26115 (BJFC043665, paratype), Dai 26116 (BJFC043666, paratype).

Distribution and ecology. *Favolaschia sinarundinariae* is distributed in the subtropical area of Sichuan Province, China; it grows on rotten *Sinarundinaria* and causes white rot.

Discussion

The earliest records of *Favolaschia* species in China date back to the 20th century (Liu 1994; Liu and Yang 1994). Recently, with the application of molecular systematics, the species of *Favolaschia* in China have been continually updated (Liu 2020; Tolgor et al. 2021; Zhang and Dai 2021; Zhang et al. 2023). Morphological examination and phylogenetic analyses identified 16 species of *Favolaschia* in China (Zhang and Dai 2021; Zhang et al. 2023). In this study, three new species of *Favolaschia* are identified in south China: *Favolaschia imbricata*, *F. miscanthi* and *F. sinarundinariae*.

Phylogenetic studies of *Favolaschia* largely support the section classification, based on morphology proposed by Singer (1974). Morphologically, the *F.* sect. *Anechinus* includes two subsections, *F.* subsect. *Rubrinae* and *F.* subsect. *Depauperatae*, the former lacks gloeocystidia and gloeoporous hyphae (with granular contents); the latter has these structures, while the two subsections are not well distinguished in the phylogenetic tree. The three new species, *Favolaschia imbricata*, *F. miscanthi* and *F. sinarundinariae*, clustered within section *Anechinus* (group B), which is characterised by the lack of acanthocysts, gloeocystidia and gloeoporous hyphae replaced by irregular cystidia at the dissepiment edge or pileipellis.

In a combined ITS1-5.8S-ITS2-nLSU dataset-based phylogeny (Fig. 2), *Favolaschia imbricata* grouped with another new species *F. miscanthi*, with strong support (100/1.00). However, *Favolaschia miscanthi* can be easily distinguished from *F. imbricata* by its larger pores (0.5–1 mm vs. 0.2–0.6 mm), white pileal surface upon drying and growing on rotten *Miscanthus*, while *F. imbricata* shares dark grey or mouse-grey pileal surface upon drying and grows on dead bamboo. Based on the sub-generic classification of *Favolaschia* by Singer (1974), *F. imbricata* belongs to *Favolaschia* sect. *Anechinus* subsect. *Rubrinae*, because it lacks acanthocystidia, gloeocystidia and gloeoplerous hyphae.

Morphologically, *Favolaschia imbricata* with *F. flabelliformis* and *F. tonkinensis* share grey to greyish-white basidiomata when fresh and occur in south China. However, *F. flabelliformis* differs from *F. imbricata* by its flabelliform pilei and smaller pores (0.2–0.25 mm vs. 0.2–0.6 mm, Zhang et al. (2023)). *F. tonkinensis* differs from *F. imbricata* by its larger basidiomata (6–24 mm vs. 2–5 mm) and larger basidiospores (8–12.5 × 7–10.5 µm vs. 7–9 × 5–6.8 µm, Singer (1974)). In addition, *F. imbricata* has numerous cheilocystidia (cylindrical or clavate, with obtuse diverticulate projections at the apex) at the dissepiment edge, while they are absent in *F. flabelliformis* and *F. tonkinensis*.

Morphologically, *Favolaschia miscanthi* is characterised by its white basidiomata, inhabiting rotten *Miscanthus*, large pores (0.5–1 mm in diam.), broom-

shaped cheilocystidia and broadly ellipsoid to subglobose basidiospores (7.5–10 × 5.5–7 μm) and belongs to *F.* sect. *Anechinus* subsect. *Rubrinae*. Four species in *F.* subsect. *Rubrinae*, viz. *F. bannaensis*, *F. rigida*, *F. semicircularis* and *F. tenuissima*, resemble *F. miscanthi* by sharing smaller (< 2 cm) and pure white to white basidiomata when fresh and occur in south China. However, *Favolaschia bannaensis* differs from *F. miscanthi* by its larger pores (up to 1.5 mm vs. up to 1 mm) and inhabit bamboo or rotten angiosperms (Zhang et al. 2023). *Favolaschia rigida* differs from *F. miscanthi* by its obvious stipe, smaller pores (0.25–0.33 mm vs. up to 0.5–1 mm) and the absence of cheilocystidia (Zhang et al. 2023). *Favolaschia semicircularis* is readily distinguished from *F. miscanthi* by its obvious stipe and shorter basidia (24–32 μm vs. 32–45 μm in length, Zhang et al. (2023)). *Favolaschia tenuissima* differs from *F. miscanthi* by its fewer pores (up to 20 vs. up to 40) and the presence of obvious stipe (Zhang et al. 2023).

Eight specimens from Heizhugou and Micangshan National Forest Park in Sichuan Province formed a support lineage (71/1.00) and there are two base pairs differences between them in the ITS regions and no differences in morphology. We described these as a new species, namely *Favolaschia sinarundinariae*. Phylogenetically, *Favolaschia sinarundinariae* and *F. tephroleuca* cluster together in a supported clade (57/1.00) within *Favolaschia* sect. *Anechinus*, with each forming highly-supported branches. However, *F. tephroleuca* is readily distinguished from *F. sinarundinariae* by its grey to dark grey pileal surface, when fresh and fewer pores (up to 40 vs. up to 80). Morphologically, *Favolaschia flabelliformis* is similar to *F. sinarundinariae* by sharing a similar pileal surface, the absence of stipe and almost the same size basidiospores (Zhang et al. 2023), but the former species has smaller basidiomata (2–3 mm vs. 4–7 mm), smaller pores (0.2–0.25 mm vs. 0.5–1 mm) and fewer pores (10–40 vs. up to 80, Zhang et al. (2023)). In addition, *F. flabelliformis* and *F. sinarundinariae* are phylogenetically distantly related.

During the investigations of *Favolaschia*, the information on distribution areas is obtained (Fig. 1). Regarding geographical distribution, *Favolaschia* is distributed in south China (the south Qinling-Huai River Line). In addition, an updated key to 19 species of *Favolaschia* is provided in the following.

A key to 19 species of *Favolaschia* found in China

1 Acanthocystidia present **2**
– Acanthocystidia absent **5**
2 Basidiomata apricot orange when fresh **3**
– Basidiomata lemon chrome when fresh **4**
3 Pilei < 5 mm ..... ***F. minutissima***
– Pilei > 5 mm ..... ***F. brevibasidiata***
4 Stipe < 5 mm in length ..... ***F. brevistipitata***
– Stipe > 5 mm in length ..... ***F. longistipitata***
5 Basidiomata campanulate ..... ***F. manipularis***
– Basidiomata conchoid, flabellate, reniform, semicircular or subcircular **6**
6 Mature pilei > 2 cm in length **7**
– Mature pilei < 2 cm in length **9**
7 Stipe present ..... ***F. tonkinensis***
– Stipe absent **8**

8 Mature pores polygonal, 3–6 mm in width ..... ***F. pustulosa***
– Mature pores round, 1–2.3 mm in width ..... ***F. subpustulosa***
9 Gloeocystidia present **10**
– Gloeocystidia absent..... **11**
10 Basidiospores 4.5–5.5 μm in width ..... ***F. peziziformis***
– Basidiospores 7–10 μm in width ..... ***F. crassipora***
11 Pilei transparent **12**
– Pilei opaque **14**
12 Stipe present, 1–3 × 0.5–1 mm ..... ***F. tenuissima***
– Stipe absent **13**
13 Basidiomata inhabiting rotten *Miscanthus* ..... ***F. miscanthi***
– Basidiomata inhabiting dead bamboo (Bambusoideae) ..... ***F. bannaensis***
14 Pilei ash grey to fuscous or dark when fresh ..... ***F. tephroleuca***
– Pilei usually white to pale white when fresh **15**
15 Cheilocystidia present at dissepiment edge **16**
– Cheilocystidia absent **17**
16 Stipe usually present ..... ***F. semicircularis***
– Stipe absent **17**
17 Mature pores 0.2–0.6 mm in width ..... ***F. imbricata***
– Mature pores up to 1 mm in width ..... ***F. sinarundinariae***
18 Stipe present, with diverticulate hyphae in pileipellis ..... ***F. rigida***
– Stipe absent, without diverticulate hyphae in pileipellis ..... ***F. flabelliformis***

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Qiu-Yue Zhang and Wan-Ying Li designed the research and contributed to data analysis and interpretation. Qiu-Yue Zhang, Xin Zhang, Yu-Cheng Dai and Hong-Gao Liu prepared the samples and drafted the manuscript. Yu-Cheng Dai, Lu-Sen Bian and Fang Wu discussed the results and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability

The sequences are deposited in the GenBank database (Table 1).

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Supplementary material 1

Three new species of *Favolaschia* (Mycenaceae, Agaricales) from South China

Authors: Qiu-Yue Zhang, Hong-Gao Liu, Wan-Ying Li, Xin Zhang, Yu-Cheng Dai, Fang Wu, Lu-Sen Bian

Data type: nex

Explanation note: In this study, the final alignments and the retrieved topologies were deposited in TreeBASE (http://treebase.org/treebase-web/home.html), under accession ID: 30973. At the same time, we upload it as an attachment to the editor.

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Research Article

New species, new records and common species of *Pluteus* sect. *Celluloderma* from northern China

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Abstract

Wood-rotting fungi are organisms that can decompose wood substrates and extract nutrients from them to support their growth. They play a crucial role in the material cycle of forest ecosystems. The genus *Pluteus* plays a significant role in wood decomposition. In this study, the morphology and molecular systematics of the sect. *Celluloderma* of the genus *Pluteus* were carried out. *Pluteus brunneodiscus* was identified as a new species, along with the discovery of two new records, *P. cystidiosus* and *P. chrysophlebius*, and a common species, *P. romellii*. *Pluteus brunneodiscus* is characterized by the brown center of the pileus that transitions to white towards the margins, with the surface cracking to form irregular granules. It is typically found in Populus forests growing on decomposing twigs or wood chips. Line drawings, color photographs, and phylogenetic analyses of related species within the genus *Pluteus* accompany the descriptions of these four species. The analyses are based on ITS + TEF1-α sequence data. Finally, a key for the twenty species within the sect. *Celluloderma* of the genus *Pluteus*, which has been documented in China, is provided.

Key words: Line drawings, morphology, phylogeny, wood-rotting fungi

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Introduction

The genus *Pluteus* Fr., which belongs to the Basidiomycota, Agaricomycetes, Agaricales, Pluteaceae, was established by Fries in 1863. The genus *Pluteus* is characterized by its free lamellae, pinkish spore print, inverse hymenophoral trama, smooth spherical to ellipsoidal basidiospores, various forms of pleurocystidia, and often cheilocystidia. It is predominantly found on decaying wood and has a global distribution (Vellinga and Schreurs 1985; Singer 1986; Justo et al. 2011a, 2011b).

The genus *Pluteus* was categorized into three sections based on the characteristics of the cystidia and pileipellis *viz*. (1) sect. *Pluteus* Fr is characterized by the existence of a cutis pileipellis and thick-walled pleurocystidia, (2) sect. *Hispidoderma* Fayod is characterized by a pileipellis that is a trichoderm composed of elongated cells and thin-walled pleurocystidia and (3) sect. *Celluloderma* Fayod is characterized by a pileipellis that is a hymeniderm or hymeniderm

with cystidioid elements, comprising of clavate to spheropedunculate cells and thin-walled pleurocystidia (Lange 1917; Imai 1938; Singer 1956). Molecular phylogenetic analysis provides support for three sections (*Pluteus* Fr, *Hispidoderma* Fayod, and *Celluloderma* Fayod) (Menolli et al. 2010; Justo et al. 2011a, 2011b).

Singer further subdivided *Pluteu*s sect. *Celluloderma* into two subsections based on the composition of the pileipellis: subsect. *Mixtini* Singer, is characterized by elongated elements, and subsect. *Eucellulodermini* Singer is characterized by the absence of such elements (Singer 1956; Singer 1958). The molecular phylogenetic studies do not divide the *Pluteus* sect. *Celluloderma* into two subsections (Justo et al. 2011b). Some species belonged to the sect. *Celluloderma* (e.g., *P. ephebeus* (Fr.) Gillet and related species). Based on their characteristics, species composed of non-metuloid cystidia and a pileipellis as cutis should belong to the sect. *Hispidoderma*. This is not consistent with the phylogenetic results. Thus, the classification of the two subsections of sect. *Celluloderma* needs to be further justified.

Vellinga and Schreurs (1985) proposed a different taxonomic system to distinguish these species (e.g., *P. ephebeus* (Fr.) Gillet and related species), dividing the *Pluteus* sect. *Celluloderma* into three subsects, *Mixtini*, *Eucelullodermini*, and *Hispidodermini* (Fayod) Vellinga and Schreurs. The latter is characterized by a trichodermal pileipellis or a euhymeniderm consisting of cylindrical to fusiform elements, which are similar to some of the characteristics of the sect. *Hispidoderma*. Additionally, Schreurs and Vellinga proposed a new group sect. *Villosi* Schreurs and Vellinga, containing species with a cutis-like pileipellis and non-metuloid (Singer 1958; Singer 1986). The proposed new sections and subsections by Singer (1958, 1986), Vellinga, and Schreurs (1985) lack support from molecular systematic studies (Justo et al. 2011a; Justo et al. 2012).

Recent studies (Minnis et al. 2006; Menolli et al. 2010; Justo et al. 2011a, 2011b; Vizzini and Ercole 2011) have indicated that sect. *Celluloderma* includes species characterized by the presence of non-metuloid pleurocystidia and a pileipellis that is either euhymeniderm or epithelioid hymeniderm, composed of short elements, which may or may not be intermixed or not with elongate cystidioid elements (corresponding to *Pluteus* sect. *Celluloderma* as defined by Singer 1956, 1958, 1986), refers to species with a cutis-like pileipellis and non-metuloid cystidia (corresponding to *Pluteus* sect. *Villosi* or *Hispidoderm*a sensu Singer p.p.).

In the current investigation, a new species, (*P. brunneodiscus*), two new records to China, (*P. chrysophlebius* and *P. cystidiosus*), and a common species, (*P. romellii*) are described. Detailed descriptions and illustrations are provided for the four species, along with clarification of the phylogenetic relationships of the identified species and related taxa from the genus *Pluteus* sect. *Celluloderma*.

Materials and methods

Morphology

In the field, photographs of fresh basidiomata were taken to scientifically and adequately reflect the growing environment and characteristics of the basidiomata, including the shape of the pileus, the color of the lamellae, and Munsell Soil Color Chart was followed for color codes (Munsell 2009). For fresh basidi-

omata, we promptly determined the size and recorded in detail the shape, size, color, odor, and other macroscopic characteristics of the basidiomata pileus, lamellae, and stipes. About 15 g of fresh context and lamellae were dried in a Ziplock bag with silica gel and returned to the lab for DNA extraction. Fresh basidiomata were dried at 40 ~ 45 °C (Hu et al. 2022), using a plant drying oven and preserved in the fungarium of Jilin Agricultural University (FJAU).

The observation of microstructural features was based on dried specimens. The dry specimens were rehydrated in 94% ethanol for microscopic examination and then mounted in 3% potassium hydroxide (KOH), 1% Congo Red, and Melzer's Reagent, using a light microscope (ZEISS, DM1000, Oberkochen, Germany). Specifically, the following symbols were used in the description: [n/m/p] indicates that 'n' randomly selected basidiospores from 'm' basidiomata of 'p' collections were measured, 'avl' means the average length of basidiospores, except for the extreme values, 'avw' refers to the average width of the basidiospores, except the extreme values, 'Q' represents the quotient of the length and width of a single basidiospore inside view, 'Qm' refers to the average Q value of all basidiospores ± standard deviation. The dimensions for basidiospores are given as (a)b–c(d). The range of b–c contains a minimum of 90% of the measured values. Extreme values (i.e., a and b) are given in parentheses (Qi et al. 2022).

Molecular phylogeny

DNA extraction, PCR amplification, and sequencing

According to the instructions, the total DNA of the specimens was extracted by the new plant genomic DNA extraction kit from Jiangsu Kangwei Century Biotechnology Limited Company, P.R. China. Subsequently, sequences of the internal transcribed spacer (ITS) region, and translation elongation factor 1-α (TEF1-α) were used for phylogenetic analyses. The amplification primers of the nr ITS: ITS1–5.8S–ITS2 regions were ITS1F and ITS4/ITS4B (White et al. 1990), and TEF1-α regions were EF1–983F and EF1–1567R (Rehner and Buckley 2005). The amplification reactions were carried out in a 25 μL system. The total amount of PCR mixed was as follows: dd H_2O 13.5 μL, 10 × Taq Buffer 5 μL, 10 mM dNTPs 1 μL, 10 mM upstream primer 1 μL, 10 mM downstream primer 1 μL, DNA sample 2 μL, 2 U/mm Taq Polymerase 1.5 μL. The cycle parameters were as follows: 5 min at 98 °C; 30 s at 98 °C, 30 s at 55 °C, 1 min at 72 °C for 40 cycles; 7 min at 72 °C; storage at 4 °C (Ševcíková et al. 2022). The PCR product was subjected to 1% agarose gel electrophoresis. The purified PCR products were sent to Sangon Biotech Limited Company, P.R. China for sequencing using the Sanger method. The sequencing results were clipped with Seqman 7.1.0 (Swindell and Plasterer 1997) and subsequently deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank).

Data analysis

The species that were morphologically similar to new species, newly recorded species, and common species, and have high sequence similarity after blast were selected (Justo et al. 2011b, 2012; Menolli et al. 2015; Desjardin and Perry 2018; Hosen et al. 2019; Hosen et al. 2021; Ševčíková et al. 2022; Qi et al. 2022;

Table 1. Names, collection numbers, reported countries and corresponding GenBank accession numbers of the taxa used in this study.

Taxon	Collection	Country	GenBank No.		Reference
			ITS	TEF1-α	
Pluteus absconditus	iNaturalist 112240775	USA (TN)	OR229047	OR242143	Ševcíková et al. 2023
P. absconditus	MO 136488	USA (TN)	KM983689	OR242144	Ševcíková et al. 2023
P. aff. *ephebeus*	BPI 882530	USA-Illinois	JQ065025	–	Menolli et al. 2015
P. aff. *ephebeus*	BPI 882531	USA-Illinois	JQ065026	–	Menolli et al. 2015
P. aff. *ephebeus*	HHB1213	USA-New Mexico	KM983670	–	Menolli et al. 2015
P. aff. *ephebeus*	AJ478	USA-Vigin Islands	KM983675	–	Menolli et al. 2015
P. aff. *ephebeus*	AJ535	Dominican Republic	KM983676	–	Menolli et al. 2015
P. aletaiensis	HMJAU 60207	China	OM991943	OP573273	Qi et al. 2022
P. aletaiensis	HMJAU 60208	China	OM992247	OP573274	Qi et al. 2022
P. aurantiorugosus	GDGM41547	China	MK791275	–	Ševcíková et al. 2022
P. aurantiorugosus	LE 312815	Russia (Europe)	ON864103	ON813296	Ševcíková et al. 2022
P. austrofulvus	AJ 857	USA, Arkansas	KM983701	ON813290	Ševcíková et al. 2022
P. austrofulvus	AJ 860	USA, Arkansas	KM983699	ON813288	Ševcíková et al. 2022
P. brunneidiscus	HMJAU 60206	China	OM991893	–	Qi et al. 2022
P. brunneidiscus	HMJAU 60210	China	OM943513	–	Qi et al. 2022
P. cervinus	REG 13641	USA	HM562152	–	Qi et al. 2022
P. cf. *nanus*	LE 213093	Russia	FJ774081	–	Justo et al. 2011
P. cf. *ephebeus*	LOU15198	Spain	KM983671	–	Menolli et al. 2015
P. cf. *ephebeus*	Shaffer4673	France	HM562080	–	Menolli et al. 2015
P. cf. *ephebeus*	Pearson sn	England	HM562198	–	Menolli et al. 2015
P. cf. *ephebeus*	9823	Italy	JF908620	–	Menolli et al. 2015
P. cf. *ephebeus*	10151	Italy	JF908621	–	Menolli et al. 2015
P. cf. *fastigiatus*	NKI12	Brazil	KM983678	–	Menolli et al. 2015
P. cf. *fuliginosus*	FK2158	Brazil	KM983677	–	Menolli et al. 2015
P. chrysophlebius	TNSF12383	Japan	HM562125	–	Justo et al. 2011a
P. chrysophlebius	SF10 (BPI)	USA (IL)	HM562180	–	Justo et al. 2011a
P. chrysophlebius	TNSF12388	Japan	HM562088	–	Justo et al. 2011a
P. chrysophlebius	SF12 (BPI)	USA (IL)	HM562182	–	Justo et al. 2011a
P. chrysophlebius	SF11 (SIU)	USA (IL)	HM562181	–	Justo et al. 2011a
P. chrysophlebius	**FJAU66561**	**China**	**OR994065**	**PP062824**	This study
P. cutefractus	BRNM825872	Spain	OR229050	OR242162	Ševcíková et al. 2023
P. cutefractus	GM 3458	Spain	OR229048	OR242165	Ševcíková et al. 2023
P. cutefractus	FG 26092015	Slovenia	OR229053	OR242164	Ševcíková et al. 2023
P. cystidiosus	LE 312852	Russia (Far East)	OR229063	OR242175	Ševcíková et al. 2023
P. cystidiosus	LE 313335	Russia (Far East)	OR229062	OR242174	Ševcíková et al. 2023
P. cystidiosus	AJ 782 (NBM-F-009790)	USA (MA)	KM983687	OR242171	Ševcíková et al. 2023
P. cystidiosus	AJ 617 (NBM-F-009788)	USA (NY)	KM983686	OR242173	Ševcíková et al. 2023
P. cystidiosus	**FJAU66556**	**China**	**OR994068**	**PP062825**	This study
P. cystidiosus	**FJAU66557**	**China**	**PP002166**	**PP062826**	This study
P. diptychocystis	NMJ184	Brazil	KM983674	–	Menolli et al. 2015
P. ephebeus	AJ234	Spain	HM562044	–	Menolli et al. 2015
P. fenzlii	TNSF12376	Japan	HM562091	–	Menolli et al. 2015
P. fenzlii	F1020647	Slovakia	HM562111	–	Menolli et al. 2015
P. fenzlii	LE 246083	Russia	FJ774082	–	Holec et al. 2017
P. fulvibadius	AJ 815	USA, California	KM983698	ON813285	Ševcíková et al. 2022
P. fulvibadius	HRL3391	Canada, Québec	ON864094	ON813287	Ševcíková et al. 2022
P. gausapatus	BRNM817745	South Korea	OR229067	OR242177	Ševcíková et al. 2023
P. gausapatus	BRNM817745	South Korea	OR229067	OR242177	Ševcíková et al. 2023

Taxon	Collection	Country	GenBank No.		Reference
			ITS	TEF1-α	
P. halonatus	FK2084	Brazil	KM983680	–	Menolli et al. 2015
P. halonatus	NKI17	Brazil	KM983679	–	Menolli et al. 2015
P. heteromarginatus	AJ172	USA	HM562058	–	Hosen et al.2019
P. hirtellus	SFSU:DED 8259	West Africa	MG968804	–	Desjardin and Perry 2018
P. inconspicuus	PDD 72485	New Zealand	MN738614	–	Ševcíková et al. 2023
P. inflatus	BRNM817761	Czech Republic	OR229033	OR242136	Ševcíková et al. 2023
P. inflatus	BRNM825836	Czech Republic	OR229035	OR242132	Ševcíková et al. 2023
P. inflatus	BRNM825837	Czech Republic	OR229036	OR242133	Ševcíková et al. 2023
P. insidiosus	15120	Italy	JF908626	–	Justo et al. 2012
P. longistriatus	Minnis309203	USA	HM562082	–	Hosen et al.2019
P. lucidus	LE F-347426	Russia	OQ732746	–	Malysheva et al. 2023
P. mammillatus	Singer244A	USA-Florida	HM562120	–	Holec et al. 2017
P. mammillatus	Minnis309202	USA-Missouri	HM562086	–	Holec et al. 2017
P. mammillatus	ASM7916	USA-Missouri	HM562119	–	Holec et al. 2017
P. brunneodiscus	**FJAU66132**	**China**	**PP002168**	**PP062821**	This study
P. brunneodiscus	**FJAU66133**	**China**	**PP002169**	**PP062822**	This study
P. brunneodiscus	**FJAU66134**	**China**	**PP002167**	**PP062823**	This study
P. parvisporus	AJ 855	USA, Arkansas	ON864099	ON813295	Ševcíková et al. 2022
P. parvisporus	iNaturalist 112236342	USA, Tennessee	ON864098	ON813294	Ševcíková et al. 2022
P. phlebophorus	AJ 81(NBM-F-009110)	Spain	HM562039	ON133554	Ševcíková et al. 2023
P. phlebophorus	AJ228 (LOU)	Spain	HM562138	–	Justo et al. 2011a
P. phlebophorus	AJ194 (LOU)	Spain	HM562137	–	Justo et al. 2011a
P. phlebophorus	AJ193 (LOU)	Spain	HM562144	–	Justo et al. 2011a
P. plautus	P59	USA-California	KF306016	–	Menolli et al. 2015
P. podospileus	LE 303682	Russia (South Siberia)	KX216331	OR242169	Ševcíková et al. 2023
P. podospileus	LE 303687	Russia (South Siberia)	KX216332	OR242168	Ševcíková et al. 2023
P. podospileus	LE 313589	Russia (South Siberia)	OR229060	OR242167	Ševcíková et al. 2023
P. riberaltensis var. *conquistensis*	FK1043	Brazil	HM562162	–	Menolli et al. 2015
P. romellii	AJ 232	Spain	HM562062	ON813280	Ševcíková et al. 2022
P. romellii	BRNM 761731	Czech Republic	ON864065	ON813278	Ševcíková et al. 2022
P. romellii	BRNM 816205	Czech Republic	ON864063	ON813276	Ševcíková et al. 2022
P. romellii	BRNM 825845	Slovakia	ON864070	ON813281	Ševcíková et al. 2022
P. romellii	**FJAU66558**	**China**	**OR994057**	**PP062827**	This study
P. romellii	**FJAU66559**	**China**	**OR994061**	**PP062828**	This study
P. rugosidiscus	BRNM761706	Slovakia	MH010876	LT991752	Ševcíková et al. 2023
P. rugosidiscus	Homola109 (MICH)	USA (MI)	HM562079	–	Justo et al. 2011a
Pluteus sp.	SP394389	USA	HM562161	–	Justo et al. 2012
Pluteus sp.	iNaturalist 27406926 (NBM-F-009806)	USA (IN)	ON006984	OR242176	Ševcíková et al. 2023
P. squarrosus	GDGM 42320	China	MK791274	–	Hosen et al.2019
P. squarrosus	GDGM 42302	China	MK791273	–	Hosen et al.2019
P. thomsonii	LE 303662	Russia	KX216329	–	Justo et al. 2012
P. tomentosulus	MO163564	USA-Pennsylvania	KM983673	–	Menolli et al. 2015
P. tomentosulus	MO93719	USA-Oregon	KM983672	–	Menolli et al. 2015
V. michiganensis	HMJAU-CR45	China	MW242665	–	Qi et al. 2022
Volvopluteus michiganensis	HMJAU-CR43	China	MW242664	–	Qi et al. 2022

Bold fonts are the sequences to be determined in this study.

Malysheva et al. 2023; Ševcíková et al. 2023; Xu et al. 2023), and details of the ITS and TEF1-α sequences of these species are shown in Table 1. The ITS and TEF1-α dataset comprised 134 representative sequences that exhibited the highest similarity to *Pluteus* spp., and two sequences of *Volvopluteus michiganensis* (A.H. Sm.) Justo and Minnis. as an outgroup.

For obtaining ITS + TEF1-α datasets of related species, sequence alignment was initially performed for ITS and TEF1-α using the "automatic" strategy and normal alignment mode of MACSE V2.03 (Ranwez et al. 2018) and MAFFT (Katoh and Standley 2013), respectively. Subsequently, the alignments were manually adjusted in BioEdit v7.1.3 (Hall 1999). Afterward, ITS and TEF1-α sequences were aligned and combined using Phylosuit V1.2.2 (Zhang et al. 2020). Then, ModelFinder (Kalyaanamoorthy et al. 2017) was used to select the best-fit models using the Bayesian information criterion (BIC). In this case, the Maximum likelihood (ML) analyses were performed in IQTree 1.6.8 (Nguyen et al. 2015), and the Bayesian inference phylogenies were performed in MrBayes 3.2.6 (Ronquist et al. 2012) (two parallel runs, 2,000,000 generations), in which the initial 25% of sampled data were discarded as burn-in. The above software was integrated into PhyloSuite 1.2.2 (Zhang et al. 2020). The ML phylogenetic tree was evaluated using the bootstrap method with a bootstrap value of 1,000 replicates; BI determined that the analysis reached smoothness with a variance of less than 0.01 and terminated the calculation. Finally, the evolutionary tree was followed up with Figtree v1.4.

Results

Phylogenetic analyses

This study's nrITS dataset comprises 93 sequences and 650 characters (gaps included). The TEF1-α dataset comprises 41 sequences and 530 characters (gaps included). The combined nrITS + TEF1-α dataset consists of 134 sequences and 1180 characters, including gaps. Of these, 16 sequences (8 nrITS and 8 TEF1-α) were newly generated in this study (Table 1). The overall topologies of the ML and BI trees were nearly identical for all datasets.

For clarity and brevity, we use the term "strongly supported" for a clade/relation that receives a bootstrap (BS) 90 and a posterior probability (PP) = 1, and "well supported" if it receives a BS 70 and a PP of 0.95. The individual support values are shown in Fig. 1.

Within the sect. *Celluloderma*, six strongly supported clades are recovered in the combined nrITS + TEF1-α dataset:

i. Clade I: This includes the clade we consider to represent *P. mammillatus* (Longyear) Minnis, Sundb. & Methven from the USA, *P. fenzlii* (Schulzer) Corriol & P.-A. Moreau from Japan, Slovakia, and Russia, *P. halonatus* from Brazil.
ii. Clade II: Includes only the newly described *P. brunneodiscus* from China. This also includes the clade we consider to represent *P. squarrosus* Hosen & T.H. Li from China, *P. hirtellus* Desjardin & B.A. Perry from West Africa, *P. plautus* (Weinm.) Gillet from the USA, *P. tomentosulus* Peck from the USA, *P. diptychocystis* Singer from Brazil, and *P. riberaltensis* var. *conquistensis* from Brazil, while *P. ephebeus* from Spain, France, England, and Italy

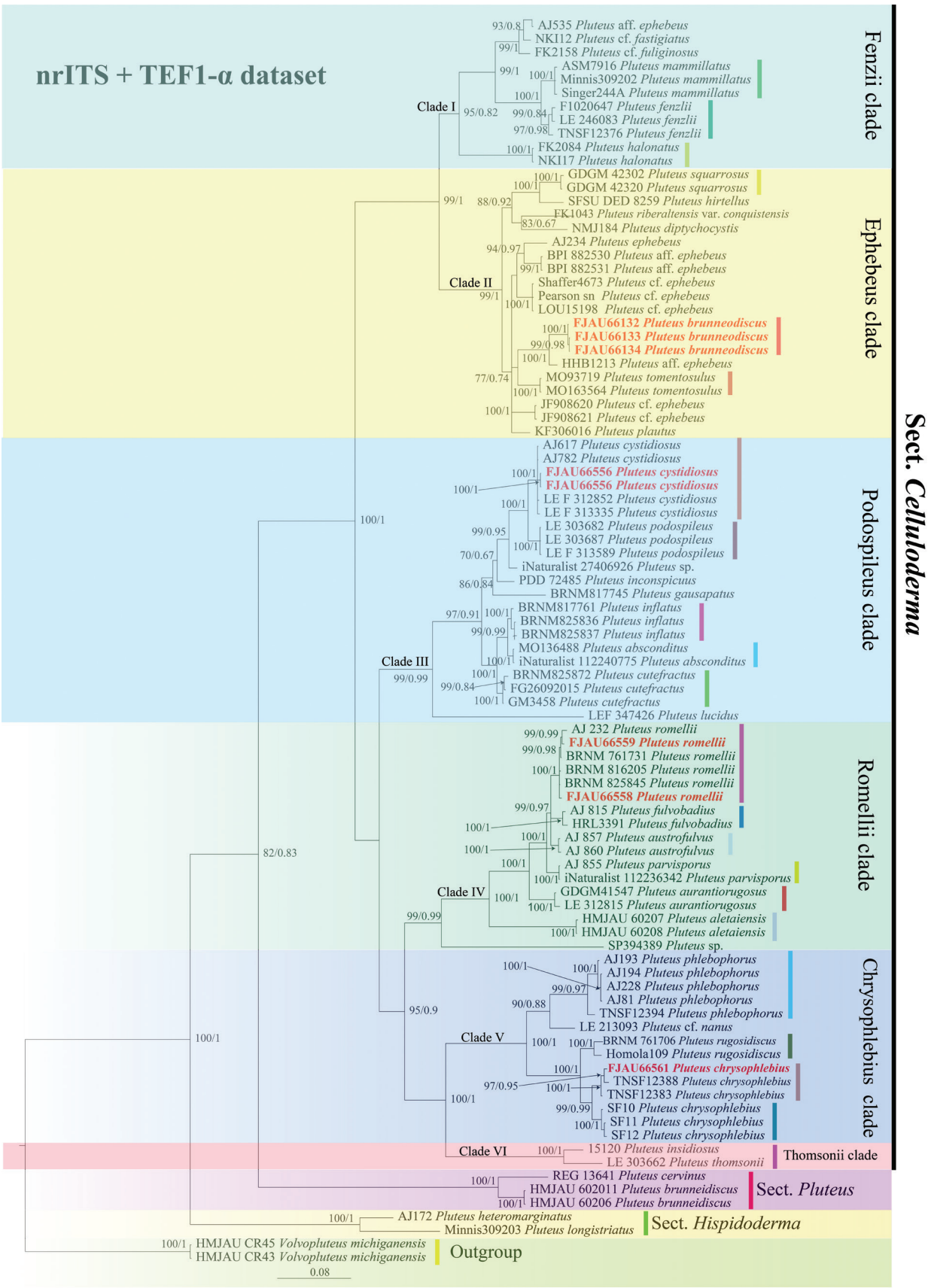

Figure 1. Phylogenetic tree of the sect. *Celluloderma* of the genus *Pluteus*. The best tree from the ML and BI analysis of the nrITS + TEF1-α dataset. The two values of internal nodes respectively represent the maximum likelihood bootstrap (MLBP)/Bayesian posterior probability (BIPP). This study species is in bold and red font.

(*P.* cf. *ephebeus* and *P.* aff. *ephebeus*), *P. fuliginosus* Murrill from Brazil (*P.* cf. *fuliginosus*), *P. fastigiatus* Singer from Brazil (*P.* cf. *fastigiatus*).

iii. Clade III: Includes the newly described *P. cystidiosus* (China). This clade also includes the clade we consider to represent *P. podospileus* Sacc. & Cub. (Russia), *P. cutefractus* Ferisin, Dovana & Justo (Spain, Slovenia), *P. inflatus* Velen (Czech Republic), *P. inconspicuus* E. Horak (New Zealand); three recently described species, *P. cystidiosus* (Russia, USA), *P. absconditus* Justo, Kalichman & S.D. Russell (USA), and *P. gausapatus* Ševčíková & Antonín (South Korea), and one likely undescribed species from the USA (iNaturalist 27406926).

iv. Clade IV: Includes the newly described *P. romellii* (China). It also includes *P. fulvibadius* Murrill (USA and Canada), *P. aurantiorugosus* (Trog) Sacc (China and Russia). Three recently described species, *P. austrofulvus* Justo, Minnis, S.D. Russell & Kalichman (USA), *P. parvisporus* Justo, Kalichman & S.D. Russell (USA) and *P. aletaiensis* Z.X. Qi, B. Zhang and Yu Li (China).

v. Clade V: Includes the newly described *P. chrysophlebius* (China). This clade also includes the clade we consider to represent *P. chrysophlebius* (Japan, USA, Japan), *P. phlebophorus* (Ditmar) P. Kumm (Spain), and *P. rugosidiscus* Murrill (Slovakia, USA).

vi. Clade VI: This clade includes the clade that we consider to represent *P. insidiosus* Vellinga & Schreurs (Italy) and *P. thomsonii* (Berk. & Broome) Dennis (Russia).

Taxonomy

***Pluteus brunneodiscus* Z.X. QI, B. Zhang & Y. Li, sp. nov.**
MycoBank No: 851479
Figs 2A–B, 3

Typification. China. Xinjiang Uygur Autonomous Region, Ili Kazakh Autonomous Prefecture, Tekes County, Aktamu Wetland, 43°15'22.61"N, 81°75'90.21"E, alt. 1243 m, 6 July 2022, Z.X. Qi (FJAU 66134, holotype!).

Sequences holotype. ITS: PP002167, TEF1-α: PP062823.

Etymology. "brunneo-": brown, "-discus": pileus disc. The species epithet "brunneodiscus" (Lat.) refers to the brown of the middle part of the pileus disc.

Diagnosis. *Pluteus brunneodiscus* differs from *P. tomentosulus* by its brown pileus in the middle, transitioning to white toward the margins, and the surface cracks to form irregular granules. It grows in poplar forests (*Populus alba* var. *pyramidalis* Bge) with decaying wood branches or chips.

Description. Basidiomata medium to large. Pileus 39–71 mm in diam, initially compressed hemispherical, surface with dense brown irregular granules (5.0YR 5/2), dirty white (5.0YR 9/2), middle brown (5.0YR 4/4), margin entire, gradually spreading at maturity, pileus middle dark brown (5.0YR 3/6), margin irregularly dehiscent at maturity or after hygrophanous. Context whitish (5.0YR 9/2), odorless, 3–6 mm thick. Lamellae initially dirty white (5.0YR 9/2), becoming flesh-brown to earth-brown at maturity (5.0YR 8/4- 5.0YR 6/4), free, dense, thick, unequal, slightly ventricose, 6–7 mm wide. Stipe 37–55 mm long, 8–11 mm wide, dirty white (5.0YR 9/2), cylindrical, slightly thicker at the base, fibrous, with white longitudinal stripes on the surface. Odorless. Spore prints pink.

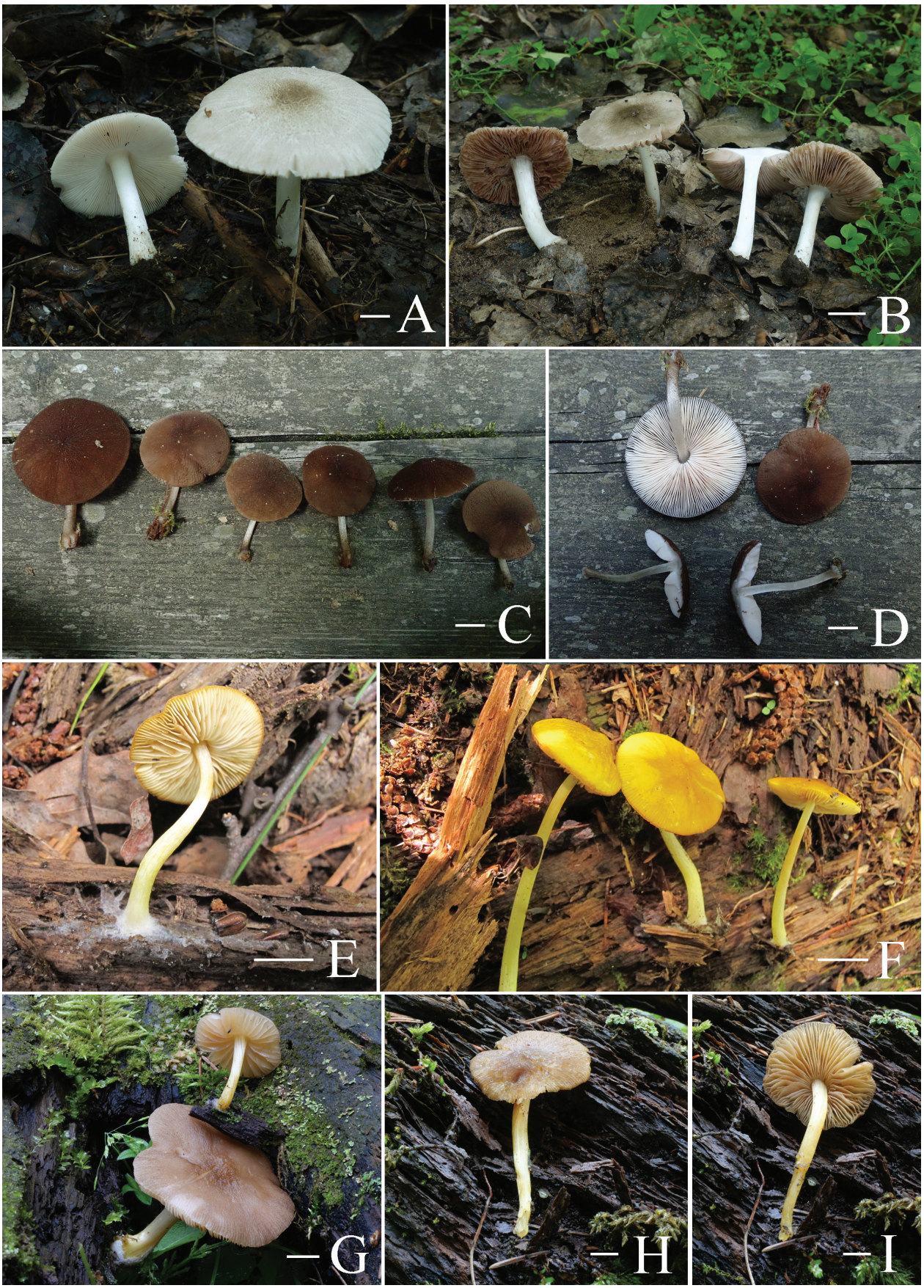

Figure 2. Basidiomata features **A–B** *Pluteus brunneodiscus* **C–D** *P. cystidiosus* **E–F** *P. chrysophlebius* **G–I** *P. romellii*. Photos by Zheng-xiang Qi (**A–B, G–I**). Photos by Di-zhe Guo (**C–F**). Scale bars: 1 cm.

Basidiospores [120, 12, 3] (–6.5) 7.0–7.5 (–8.0) × 5.0–6.0 (–6.5) µm, avL × avW = 7.0 × 6.0 µm, Q = 1.16–1.30–1.45 µm, avQ = 1.16 µm, globose, subglobose, slightly pink, smooth, thin-walled, non-dextrinoid, partially containing one droplet or irregular inclusions. Basidia 25–32 × 7–11 µm, fusiform to clavate, thin-walled, 4–sterigmate, and hyaline in KOH. Pleurocystidia abundant, scattered, 55–102 × 22–36 µm, vesicular to narrowly vesicular, or clavate, thin-walled, smooth, and hyaline in KOH. Cheilocystidia abundant, clustered, 41–79 × 18–29 µm, subfusiform to fusiform, or ventrally bulbous, apically broadly digitate 15–23 µm long, thin-walled, hyaline. Lamellar trama divergent. Pileipellis a cutis to trichodermium, hyphae 4–10 µm diam, cylindrical, hyaline, non-gelatinous; terminal cells inflated, 62–91 × 22–31 µm, obtusely rounded or pointed apically, thin-walled, with brown cytoplasmic pigments. Stipitipellis a cutis, hyphae 5–9 µm diam, cylindrical, hyaline, non-incrusted, non-gelatinous, thin-walled. Caulocystidia absent. Clamp connections absent in all tissues.

Ecology and distribution. Solitary to scattered on the ground in the broad-leaved forests (*Populus alba* var. *pyramidalis* Bge) with decaying wood branches or wood chips. Known from Xinjiang Uygur Autonomous Region of China.

Additional specimens examined. China. Xinjiang Uygur Autonomous Region, Ili Kazakh Autonomous Prefecture, Tekes County, Aktamu Wetland, 43°15'22.61"N, 81°75'90.21"E, alt. 1243 m, 6 July 2022, Z.X. Qi, D.M. Wu, N. Gao and B.K. Cui, FJAU 66132 (ITS: PP002168, TEF1-α: PP062821). China. Xinjiang Uygur Autonomous Region, Ili Kazakh Autonomous Prefecture, Tekes County, Aktamu Wetland, 43°15'22.61"N, 81°75'90.21"E, alt. 1243 m, 6 July 2022, Z.X. Qi, FJAU 66133 (ITS: PP002169, TEF1-α: PP062822).

Notes. Morphologically, *Pluteus brunneodiscus* is very similar to *P. tomentosulus* in having a white pileus. The difference lies in the surface texture, as *P. tomentosulus* has a very finely granular-tomentose surface that becomes bald at maturity, while *P. brunneodiscus* features a brown center of the pileus, transitioning to white toward the margins, with the surface cracking to form irregular granules (Vellinga and Schreurs 1985; Orton 1986; Vellinga 1990; Desjardin and Perry 2018).

In phylogenetic analyses, *P. brunneodiscus* clusters in the ephebeus clade as a sister species to *P.* aff. *ephebeus*, and has a support ratio of 1/100. However, the pileus of *P.* aff. *ephebeus* are sooty, shield-shaped fruiting bodies with pubescent or downy surfaces. They grow on rotting wood or stumps and are widely distributed in Britain and Ireland (Orton 1986; Justo et al. 2011a; Menolli et al. 2015). These characteristics distinguish *P. brunneodiscus* from *P.* aff. *ephebeus*.

Pluteus cystidiosus **(Minnis and Sundb.) Justo, Malysheva & Lebeuf, in Ševčíková et al., Journal of Fungi 9(9, no. 898): 34 (2023)**

Figs 2C–D, 4

Pluteus seticeps var. *cystidiosus* Minnis and Sundberg N. Amer. Fung. 5(1): 13 (2010). Syn.

Description. Basidiomata medium to large. Pileus 25–41 mm in diam, compressed hemispherical, surface spreading when young, surface with longitudinal vein-like folds from middle to margin when mature, margin mostly trans-

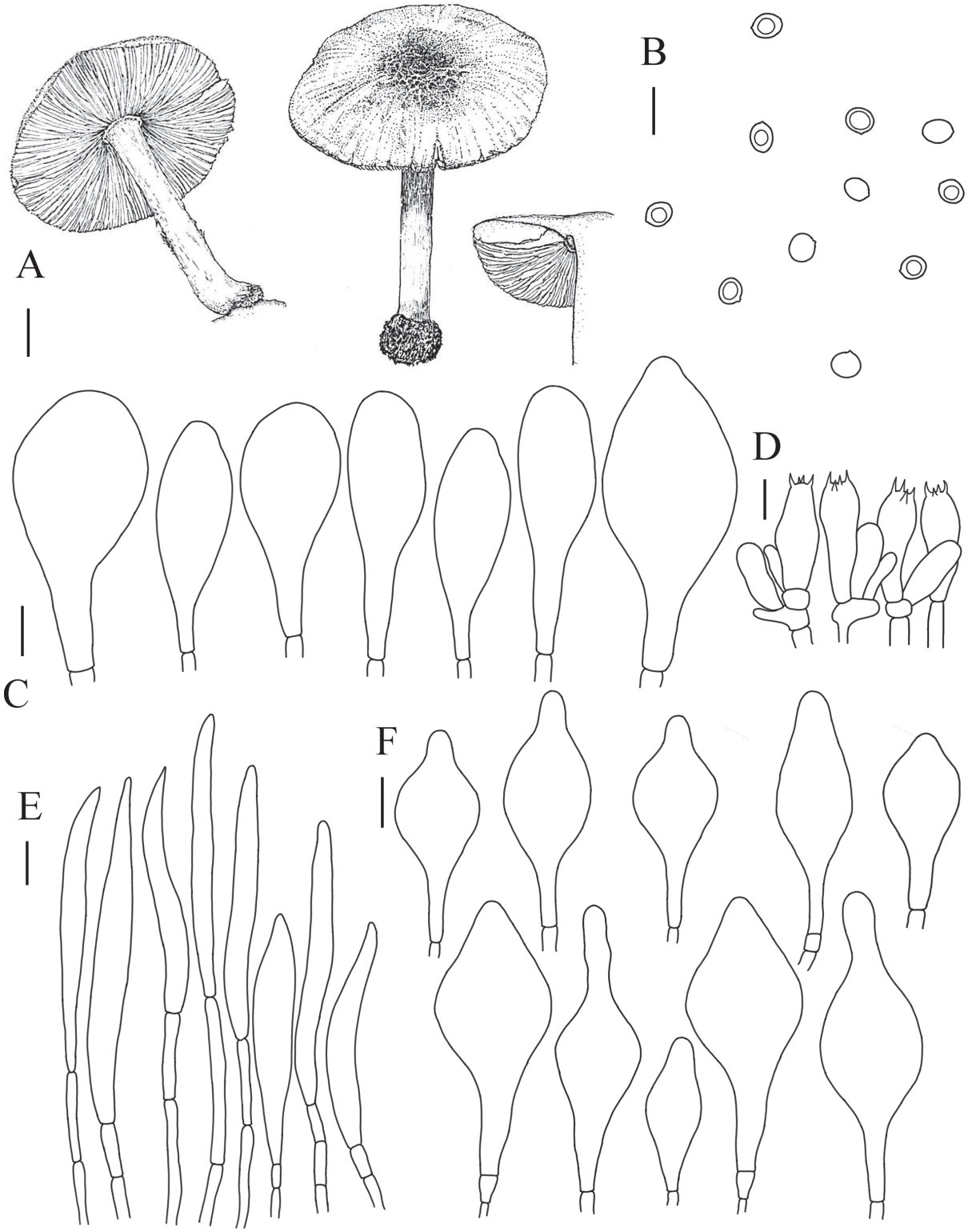

Figure 3. A Macroscopic characteristics of *Pluteus brunneodiscus* **B** basidiospores **C** pleurocystidia **D** basidia **E** pileipellis terminal cells **F** cheilocystidia. Scale bars: 1 cm (**A**); 10 µm (**B–F**).

verse folds, light brown to dark brown (5.0YR 5/6-5.0YR 4/12), margin entire. Context dirty white (2.5YR 9/4), odorless, 5–8 mm thick. Lamellae dirty white (2.5YR 9/4), free, dense, thick, unequal, ventricose, 15–18 mm wide. Stipe 30–41 mm long, 12–17 mm wide, cylindrical, slightly thicker at the base, hollow,

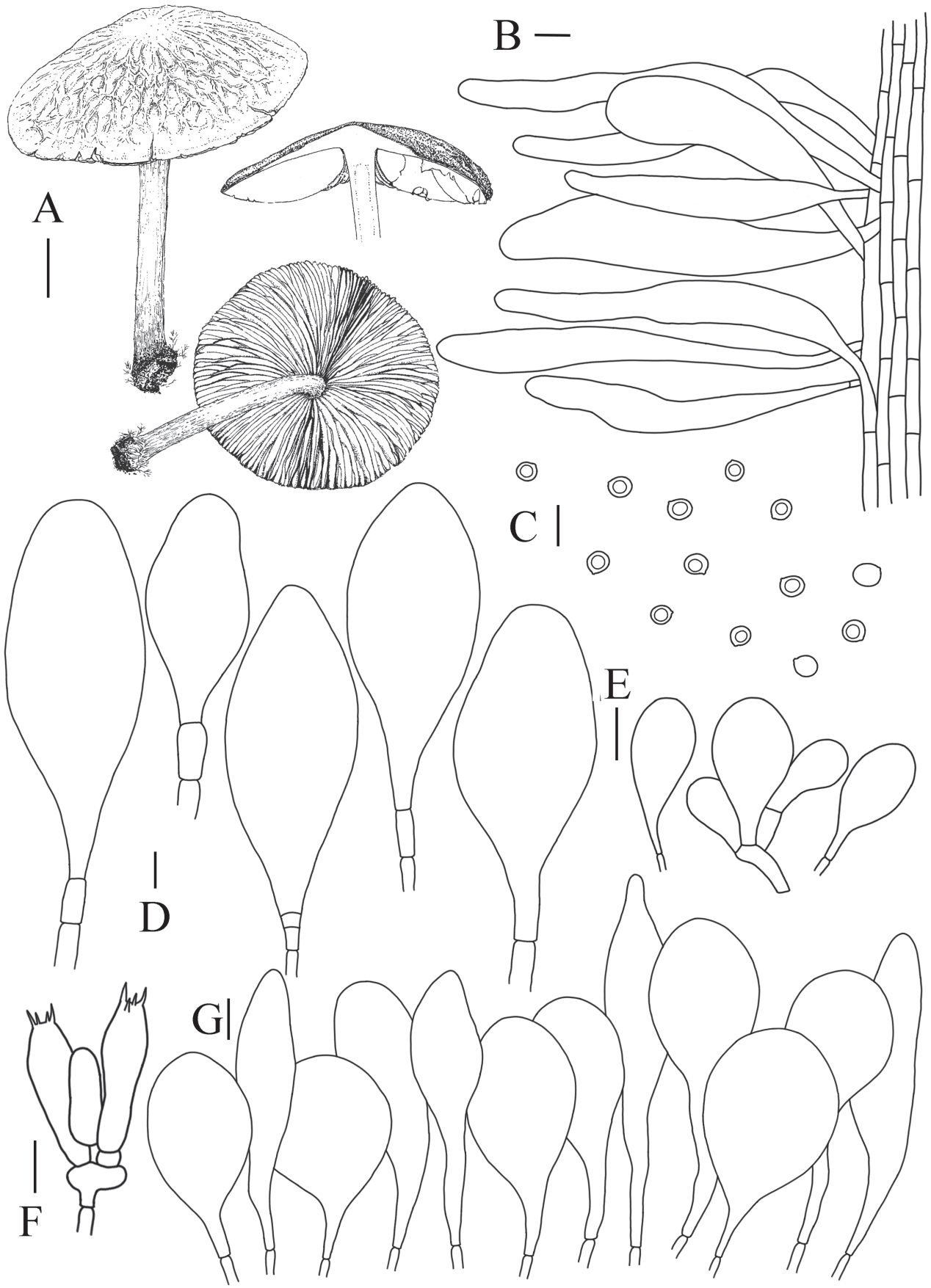

Figure 4. A Macroscopic characteristics of *Pluteus cystidiosus* **B** caulocystidia **C** basidiospores **D** pleurocystidia **E** cheilocystidia **F** basidia **G** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–G**).

fibrous, with brown serpentine or crumbly scales on the surface (2.5YR 9/2). Odorless. Spore prints pink.

Basidiospores [200, 10, 2] (–5.0) 5.5–6.0 (–6.5) × (–4.5) 5.0–5.5 µm, avL × avW = 6.0 × 5.0 µm, Q = 1.10–1.20–1.30 µm, avQ = 1.20 µm, spherical, subglobose, slightly pink, smooth, thin-walled, non-dextrinoid, partially containing one droplet or irregular inclusions. Basidia 23–31 × 7–10 µm, clavate, thin-walled, 4-sterigmate, and hyaline in KOH. Pleurocystidia abundant, scattered, 55–102 × 22–36 µm, rod-shaped or subpyriform, vesicular, thin-walled, smooth, and hyaline in KOH. Cheilocystidia abundant, clustered, 37–60 × 15–22 µm, clavate, fusiform or vesicular, thin-walled. Lamellar trama divergent. Pileipellis a hymeniderm or epithelioid hymeniderm, made up of two types of elements; spheropedunculate or pyriform, 27–55 × 24–34 µm; broadly fusiform, inflated-fusiform, lanceolate, narrowly utriform, often mucronate, 56–105 × 11–23 µm; all elements with brown intracellular pigment, often aggregated in spots, slightly thick-walled. Stipitipellis a cutis of cylindrical, hyphae 8–11 µm wide, with pale brown pigment. Caulocystidia common, often in clusters, 36–112 × 9–20 µm, cylindrical, narrowly clavate, narrowly fusiform, spheropedunculate, with brown or yellow-brown pigment. Clamp connections absent in all studied tissues.

Ecology. Scattered on decaying wood in mixed coniferous forests (*Pinus koraiensis* Siebold and Zucc).

Distribution. Canada, the USA, Japan, Russian Far East.

Additional specimens examined. China. Heilongjiang Province, Liangshui National Nature Reserve. 47°11'22.24"N, 128°47'89.11"E, 23 June 2019, D.Z. Guo, FJAU 66556 (ITS: OR994068, TEF1-α: PP062825). China. Heilongjiang Province, Liangshui National Nature Reserve. 47°11'22.24"N, 128°47'89.11"E, 28 June 2019, D.Z. Guo, FJAU 66557 (ITS: PP002166, TEF1-α: PP062826).

Note. Ševcíková et al. (2023) elevated *Pluteus seticeps* var. *cystidiosus* to *P. cystidiosus* based on specimens from the USA, Canada, Japan, and Russia. The present study reports *P. cystidiosus* as a new record in China. There was almost complete overlap in morphological variation between those reported in the present study and the holotype specimen. Both grow in temperate/cold-temperate forests. However, the basidiospores of the species in the present study were slightly larger, measuring (–5.0) 5.5–6.0 (–6.5) × (–4.5) 5.0–5.5 µm, while those of the holotype specimen were smaller, measuring 4.5–5.5 (–6.2) × 3.5–5.0 µm.

The phylogenetic tree also supports the results of our morphological study, showing that our specimens are clustered in the same branch as those from the USA and Russia, with a support ratio of 1/100.

Pluteus chrysophlebius **(Berk. & M.A. Curtis) Sacc., Syll. fung. (Abellini) 5: 678 (1887)**

Figs 2E–F, 5

Agaricus chrysophlebius Berk. and M.A. Curtis 1859. Syn.

Description. Basidiomata medium. Pileus 15–22 mm in diameter, surface not spreading, irregularly pitted, smooth, central part umbo, wrinkled or veined, yellow to bright yellow (5.0Y 9/12-5.0Y 9/20), with a hyaline stripe in the central

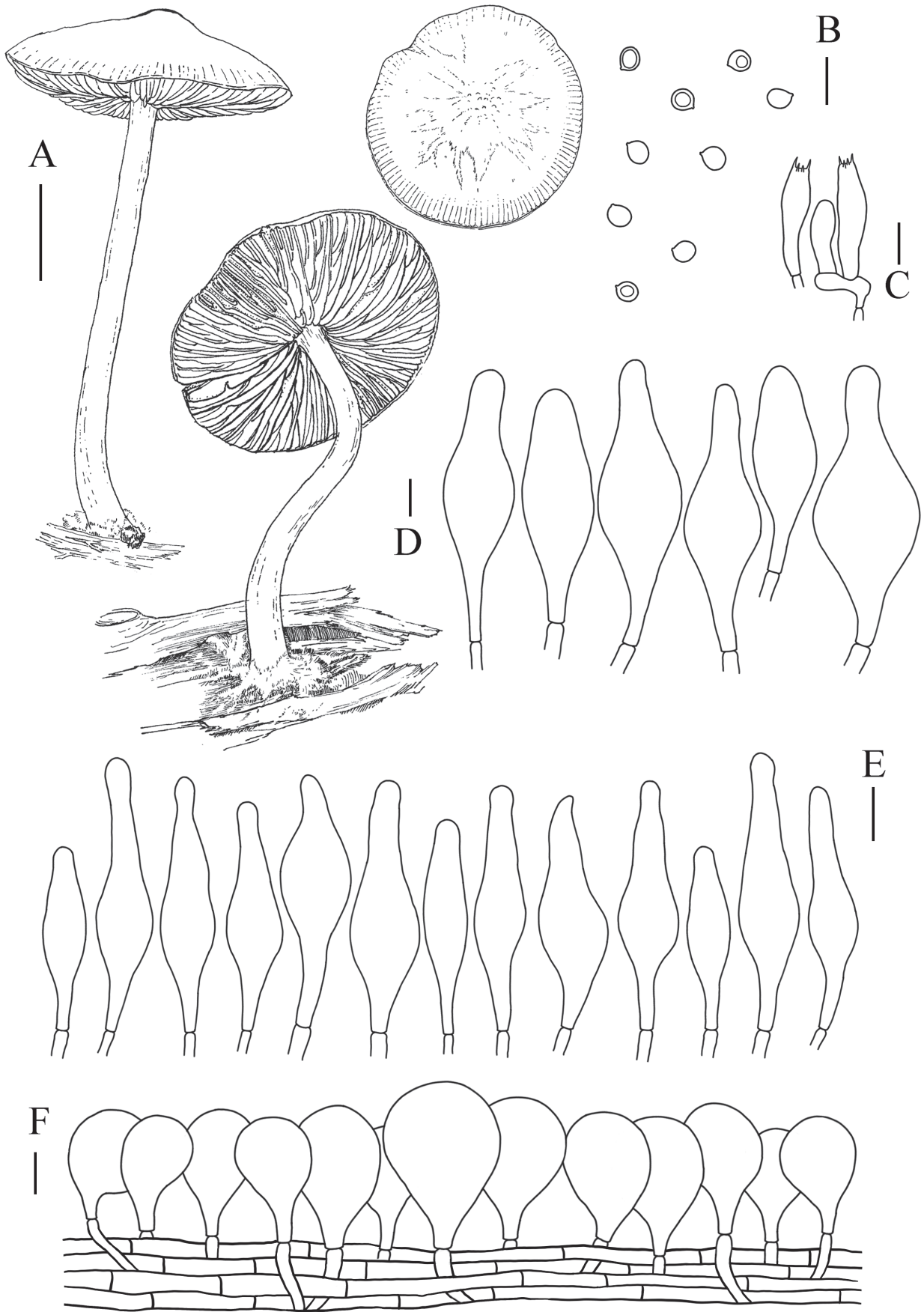

Figure 5. A macroscopic characteristics of *Pluteus chrysophlebius* **B** basidiospores **C** basidia **D** pleurocystidia **E** cheilocystidia **F** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–G**).

part 3/4 of the way toward the margin, margin entire. Context yellowish (5.0Y 9/8), odor inconspicuous. Lamellae yellow to brownish yellow (5.0Y 9/6- 5.0Y 9/8), free, dense, thick, unequal, ventricose, 6–8 mm wide. Stipe 25–42 mm long, 4–6 mm wide, cylindrical, slightly thicker at the base, fibrous, bright yellow to yellow (5.0Y 9/10-5.0Y 9/18), smooth, with white tomentose dense cilia at the base. Odorless. Spore prints pink.

Basidiospores [90, 3, 1] 5.5–6.0 × (–4.5) 5.0–5.5 µm, avL × avW = 6.0 × 5.0 µm, Q = 1.09–1.20–1.33 µm, avQ = 1.20 µm, globose, subglobose, slightly pinkish, smooth, thinly walled, non-dextrinoid, partially containing one droplet or irregular inclusions. Basidia 23–34 × 7–11 µm, clavate, thin-walled, 4-sterigmate, and hyaline in KOH. Pleurocystidia scattered, 52–78 × 15–24 µm, broad and long-necked vase-like, partly with a long neck, neck with inclusions, thin-walled, smooth, and hyaline in KOH. Chilocystidia abundant, clustered, smaller, 45–66 × 14–21 µm, similar to pleurocystidia, long-necked vase-shaped to fusiform, thin-walled. Lamellar trama divergent. Pileipellis an euhymeniderm of spheropedunculate and subglobose elements 28–67 × 18–41 µm, with brown or light brown, at the center brown to dark brown. Stipitipellis a cutis, hyphae 5–9 µm wide, hyaline, non-gelatinous, thin-walled. Caulocystidia absent. Clamp connections absent in all tissues.

Ecology. Solitary on decaying wood in mixed coniferous forests.

Distribution. North America, South America.

Additional specimens examined. China. Heilongjiang Province, Liangshui National Nature Reserve. 47°11'22.24"N, 128°47'89.11"E, 24 June 2019, D.Z. Guo, FJAU 66561 (ITS: OR994065, TEF1-α: PP062824).

Note. *Pluteus chrysophlebius* was first reported in China. It can be distinguished from other yellow-pileus species such as *P. admirabilis* (Peck) Peck, *P. aurantiacus* Murrill, *P. melleus* Murrill, and *P. rugosidiscus* Murrill by its yellowish pileus and stipe, as well as its bald pileus texture (Minnis and Sundberg 2010; Malysheva et al. 2016). The phylogenetic analysis also supports the differentiation of species.

In the phylogenetic tree, *P. chrysophlebius* formed a cluster with TNSF12383 and TNSF12388 in Asia and was sister to SF10-SF12 in the United States, with strong support for both clades.

Pluteus romellii **(Britzelm.) Lapl., Dict. iconogr. champ. sup. (Paris): 533 (1894)**
Figs 2G–I, 6

Agaricus romellii Britzelm., Hymenomyceten aus Südbayern VIII: 5 (1891). Syn.

Description. Basidiomata medium to large. Pileus 20–56 mm broad, compressed hemispherical to spreading, surface with vein-like projections extending to the pileus margin, often with striated dehiscence, with a greasy or almost waxy texture, brown to yellowish-brown (7.5YR 8/8-7.5YR 6/12), margins wavy dehiscence with translucent-striate. Context light yellow (7.5YR 8/12), odorless, 2–3 mm thick. Lamellae yellowish (10.0YR 8/10), free, medium dense, unequal, entire, ventricose, 5–7 mm wide. Stipe 26–41 mm long and 4–8 mm wide, cylindrical, slightly thicker at the base, fibrous, upper part of the stipe white to yellowish (10.0YR 9/8-10.0YR 7/12), smooth, lower part of the stipe

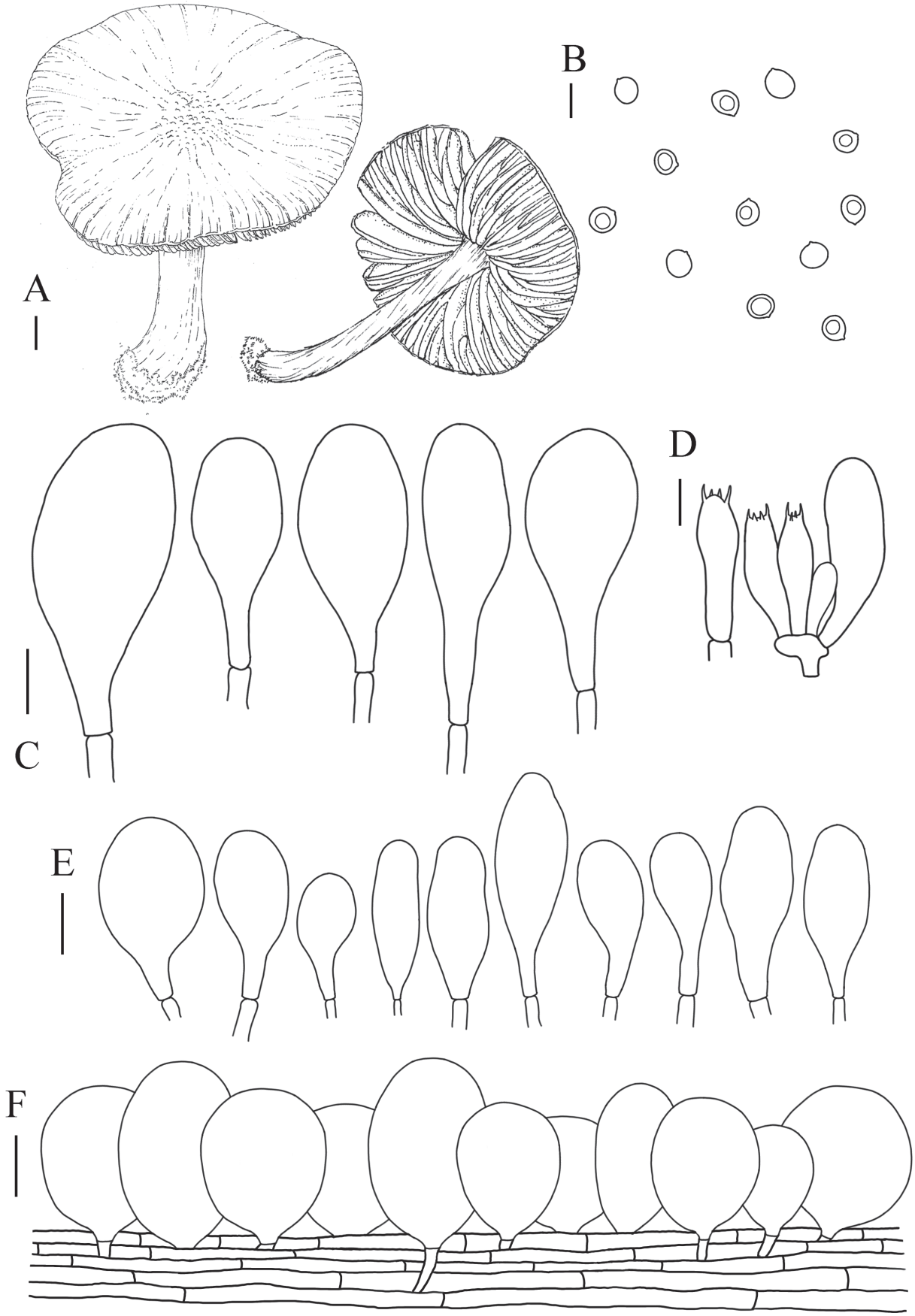

Figure 6. A macroscopic characteristics of *Pluteus romellii* **B** basidiospores **C** pleurocystidia **D** basidia **E** cheilocystidia **F** pileipellis. Scale bars: 1 cm (**A**); 10 µm (**B–E**); 20 µm (**F**).

with white tomentum, yellow to yellow-brown (10.0YR 8/8-10.0YR 8/12). Odorless. Spore print pale pink.

Basidiospores [120, 4, 2] 7.0–7.5 (–8.0) × 6.0–6.5 µm, avL × avW = 7.0 × 6.0 µm, Q = 1.07–1.25~1.33 µm, avQ = 1.16 µm, globose, subglobose to ellipsoid, transparent to slightly pinkish, smooth, and thin-walled, non-dextrinoid, partially containing one droplet or irregular inclusions. Basidia 27–32 × 8–10 µm, clavate, thin-walled, 4-sterigmate, and hyaline in KOH. Pleurocystidia abundant, scattered, 55–102 × 22–36 µm, rod-shaped or subcylindrical, fusiform, with neck and apical part broader and obtuse, thinly walled, smooth, and hyaline in KOH. Cheliocystidia abundant, clustered, 41–79 × 18–29 µm, pyriform or similarly pleurocystidia shape, thin-walled. Lamellar trama divergent. Pileipellis an euhymeniderm of spheropedunculate and subglobose elements 25–48 × 23–35 µm, with brown or light brown, at the center brown to dark brown. Stipitipellis a cutis, hyphae 6–10 µm wide, hyaline, non-gelatinous, thin-walled. Caulocystidia absent. Clamp connections absent in all tissues.

Ecology. Solitary to scattered on decaying wood in coniferous forests (*Picea schrenkiana* Fisch.).

Distribution. Europe, Americas, East Asia, Africa.

Additional specimens examined. China. Xinjiang Uygur Autonomous Region, Ili Kazakh Autonomous Prefecture, Tekes County, Jongkushtai Village, 43°12'26.61"N, 81°91'97.21"E, alt. 2139 m, 10 July 2022, Z.X. Qi, J.J. Hu, and B. Zhang, FJAU 66558 (ITS: OR994057, TEF1-α: PP062827). China. Xinjiang Uygur Autonomous Region, Ili Kazakh Autonomous Prefecture, Tekes County, Jongkushtai Village, 43°15'22.61"N, 81°75'90.21"E, alt. 2147 m, 11 July 2022, Z.X. Qi, J.J. Hu, and B. Zhang, FJAU 66559 (ITS: OR994061, TEF1-α: PP062828).

Note. Initially, the description of *Pluteus romellii* was rather vague (Britzelmayr 1891), stating that *P. romellii* was similar to *P. nanus* (Pers.) P. Kumm, with spores measuring 6–7 µm, and found growing in the soil of Bavaria. It is now widely acknowledged that *P. romellii* is characterized by a brown pileus, yellow stipe, and the absence of elongated elements in the pileipellis. This species is placed on the phylogenetic tree in subsect. *Eucellulodermini* under sect. *Celluloderma* (Orton 1986; Vellinga 1990; Ševcíková et al. 2023). Here, our description of the *P. romellii* is consistent with the commonly accepted characterization. Phylogenetic analysis shows that it clustered with the epitype (BRNM 761731) with strongly supported (99/0.98).

Key to the reported species of *Pluteus* sect. *Celluloderma* in China

1 Pileipellis consists of spheropedunculate cells and elongated cystidioid elements **2**
– Pileipellis consists of spheropedunculate cells without elongated cystidioid elements **7**
2 With caulocystidia **3**
– Without caulocystidia **6**
3 With pleurocystidia **4**
– Without pleurocystidia ..... ***Pluteus cinnabarinus***
4 Cheilocystidia with short to long mucronate at the apex ..... ***Pluteus aurantioruber***
– Cheilocystidia without short to long mucronate at the apex **5**

5 Pleurocystidia larger, measuring 35–73 (–82) × 11–31 µm ..... ***Pluteus cystidiosus***
– Pleurocystidia smaller, measuring 36–51 × 13.4–24 µm ..... ***Pluteus podospileus***
6 Pileus middle reticulate elevated, radially rugose ..... ***Pluteus thomsonii***
– Pileus brown with stripes extending to the margins ..... ***Pluteus striatus***
7 Pileipellis consists of globular, obpyriform, or spheropedunculate cells .. **8**
– Pileipellis consists of without globular, obpyriform, or spheropedunculate cells **16**
8 Grows on rotting wood **9**
– Grows on non-rotting wood ..... ***Pluteus aletaiensis***
9 Pileus, stipe bright-colored **10**
– Pileus, stipe not bright-colored **13**
10 Pileus middle folded, groove-like striate ..... ***Pluteus chrysophaeus***
– Pileus middle non-folded, groove-like striate **11**
11 Pileus bright red or orange-red ..... ***Pluteus aurantiorugosus***
– Pileus non-bright red to orange-red **12**
12 Pileus smooth, widely distributed in North America ..... ***Pluteus chrysophlebius***
– Pileus goose-yellow, margin striate ..... ***Pluteus admirabilis***
13 Basidiomata small ..... ***Pluteus nanus***
– Basidiomata non-small **14**
14 Lamellae edged with a powdery creamy material .... ***Pluteus pulverulentus***
– Lamellae edged without a powdery creamy material **15**
15 Pleurocystidia with neck and broad, blunt apex ..... ***Pluteus romellii***
– Pileus teal brown, dark cinnamon-colored, with black ribbed veins or wrinkles ..... ***Pluteus phlebophorus***
16 Grows on rotting wood **17**
– Grows on non-rotting wood ..... ***Pluteus brunneodiscus***
17 Pileus margin with hyaline stripes **18**
– Pileus margin without hyaline stripes **19**
18 Cheilocystidia with mucronate at the apex ..... ***Pluteus pallidus***
– Cheilocystidia without mucronate at the apex ..... ***Pluteus brunneoalbus***
19 Pileus with dark brown frosting powder, radially dehiscent to margins ..... ***Pluteus diettrichii***
– Pileus surface squarrose, stipe with surface covered by caulocystidia elements ..... ***Pluteus squarrosus***

Discussion

Singer (1986) and Vellinga and Schreurs (1985) classified sect. *Celluloderma* into two subsections: subsect. *Eucellulodermini* and subsect. *Mixtini*. However, subsequent systematic analyses of sect. *Celluloderma* did not have a high level of support from internal topology analysis, leading to the conclusion subsect. *Eucellulodermini* and subsect. *Mixtini* should not conform to natural taxonomy. Singer (1986) proposed that species with non-metuloid cystidia, a cutis, and trichodermal pileipellis should be classified in the sect. *Hispidoderma*. Vellinga and Schreurs (1985) proposed sect. *Villosi* on the basis of a cutis-like pileipellis

and non-metuloid cystidia. However, in the ephebeus clade, there are *P. ephebeus* from Europe and *P. riberaltensis* var. *conquistensis* from the USA. These species should be placed in sect. *Hispidoderma* and classified based on the pileipellis, but molecular results indicate that it belongs to sect. *Celluloderma.* In the phylogenetic tree, it is the sister group to *P. fenzlii*, *P. mammillatus*, and some species have a partial veil. *P. brunneodiscus* in the ephebeus clade in the present study, which has non-metuloid cystidia and pileipellis as a cutis, shares their views with Vellinga and Schreurs (1985). The phylogenetic tree also exhibits a high level of support. Further research is needed to restore these species to sect. *Villosi.*

The presence of a partial veil in *P. aurantiorugosus*, *P. aurantiorugosus* var. *aurantiovelatus*, *P. fenzlii*, and *P. mammillatus* suggests that the occurrence or nonoccurrence/ lack of the partial veil in the evolutionary history of *Pluteus* occurred independently. As stated by Singer states (Singer 1958; Minnis and Sundberg 2010; Justo et al. 2011a, 2011b; Vizzini and Ercole 2011), this characteristic is homoplasic and unsuitable for the natural classification of these fungi at the supraspecific rank.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Zheng-xiang Qi: conceptualization, writing - original draft and review and editing, data curation, formal analysis, investigation, methodology and visualization. Ke-qing Qian: Writing - review and editing. Lei Yue: formal analysis. Li-bo Wang: investigation. Di-zhe Guo: investigation. Dong-mei Wu: methodology and visualization. Neng Gao: methodology and visualization. Bo Zhang: project administration, resources and supervision. Yu Li: writing - review and editing, formal analysis.

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Data availability

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

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Research Article

Paramphibambusa bambusicola gen. et. sp. nov., *Arecophila xishuangbannaensis* and *A. zhaotongensis* spp. nov. in Cainiaceae from Yunnan, China

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Abstract

Morphological comparisons and multi locus phylogenetic analyses (base on the combined genes of ITS, LSU, *rpb*2 and *tub*) demonstrated that three new saprobic taxa isolated from bamboo belong to Cainiaceae. These taxa comprise a novel genus *Paramphibambusa* (*P. bambusicola* **sp. nov.**) and two new species, *Arecophila xishuangbannaensis* and *A. zhaotongensis*. The three new taxa belong to Cainiaceae (Xylariales, Sordariomycetes) a poorly studied family, which now comprises eight genera. *Paramphibambusa* can be distinguished from other Cainiaceae genera in having ascomata with a neck and ascospores lacking longitudinal striation, germ slits or germ pores. The two new *Arecophila* species clustered in a clade with *Arecophila* sp. and *A. bambusae*. Detailed morphological descriptions, illustrations, and an updated phylogenetic tree are provided for the new taxa.

Key words: Bambusicolous fungi, multilocus phylogeny, taxonomy, Xylariales

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Introduction

During our continuous investigation of bambusicolous fungi in Yunnan, China, we have collected one new genus and two new *Arecophila* K.D. Hyde species in Cainiaceae J.C. Krug. The family Cainiaceae (Xylariales, Sordariomycetes) was established by Krug (1978), with *Cainia* Arx & E. Müll as the type genus. Hongsanan et al. (2017) and Wijayawardene et al. (2020) accommodated Cainiaceae in Xylariomycetidae family *incertae sedis*. However, Hyde et al. (2020), Samarakoon et al. (2021), and Wijayawardene et al. (2022a) accepted Cainiaceae in Xylariales.

Maharachchikumbura et al. (2015, 2016) accepted five genera (*viz.*, *Amphibambusa* D.Q. Dai & K.D. Hyde, *Arecophila*, *Atrotorquata* Kohlm. & Volkm.-Kohlm, *Cainia*, and *Seynesia* Sacc.) in Cainiaceae based on morphology and phylogeny. Subsequently, Mapook et al. (2020) introduced *Longiappendispora*

Mapook & K.D. Hyde as a new member of Cainiaceae. Konta et al. (2021) transferred *Endocalyx* Berk. & Broome from Apiosporaceae to Cainiaceae. Li et al. (2022) revisited the monospecific genus *Alishanica* Karun et al. and synonymized it under *Arecophila*. Hence, seven genera (*Amphibambusa*, *Arecophila*, *Atrotorquata*, *Cainia*, *Endocalyx*, *Longiappendispora*, *Seynesia*) are accepted in Cainiaceae according to Hyde et al. (2020), Mapook et al. (2020), Jiang et al. (2021), and Konta et al. (2021).

Members of Cainiaceae are often found in tropical and temperate regions as saprobic fungi, which are usually associated with monocotyledons (mainly grasses) and fabaceous dicotyledons. Some *Cainia* species have been reported as causative agents of plant diseases, e.g., *C. desmazieri* C. Moreau & E. Müll (Krug 1978). Cainiaceae is morphologically characterized by immersed ascomata with a papillate ostiole, unitunicate asci, with a unique J+, apical ring or series of rings, and hyaline to pigmented, 1-septate ascospores with longitudinal striations or germ slits or germ pores, and usually surrounded by a sheath or appendages (Maharachchikumbura et al. 2016; Hyde et al. 2020). Asexual morphs of this family were reported as coelomycetous taxa, *viz*., *Cainia* and *Endocalyx*, that are characterized by black, pycnidial conidiomata, denticulate, sympodially proliferating conidiophores, branched or simple, septate, and phialidic conidiogenous cells, and hyaline, fusiform, or falcate to lunate conidia (Maharachchikumbura et al. 2016; Hyde et al. 2020; Konta et al. 2021; Wijayawardene et al. 2021a).

Arecophila was introduced by Hyde (1996) with *A. gulubiicola* K.D. Hyde as the type species. The genus *Arecophila* was initially regarded as a member of Amphisphaeriaceae G. Winter based on the morphology. Subsequently, Kang et al. (1999) accepted *Arecophila* as a member of Cainiaceae. Afterwards, the placement of *Arecophila* within the Cainiaceae has been confirmed based on analyses of partial LSU gene sequences (Jeewon et al. 2003; Senanayake et al. 2015; Li et al. 2022). Currently, 18 epithets are listed under *Arecophila* based on morpho-molecular study (Li et al. 2022; Index Fungorum 2023), and 15 epithets are listed under *Arecophila* in Species Fungorum (2023).

According to Jiang et al. (2022) and previous studies (Eriksson and Yue 1998; Hyde et al. 2002a, b; Zhou and Hyde 2002; Cai et al. 2003), only four Cainiaceae species are associated with bamboo (*Amphibambusa hongheensis* H.B. Jiang & Phookamsak, *Arecophila bambusae* Umali & K.D. Hyde, *A. coronata* (Rehm) Umali & K.D. Hyde and *A. nypae* K.D. Hyde) in China. In this study, we aim to collect bamboo samples in Yunnan, China, describe and introduce a new genus *Paramphibambusa* to accommodate *P. bambusicola*, and two new species *Arecophila xishuangbannaensis* and *A. zhaotongensis* in the family of Cainiaceae. This study enriches the species diversity of bambusicolous Cainiaceae species in China.

Materials and methods

Sample collection, single spore isolation and morphological study

Bamboo culms were collected in northeastern (Zhaotong), northwestern (Shangri-La), and southwestern (Xishuangbanna) Yunnan Province, China, stored in disposable plastic Ziplock bags and brought back to the laboratory

for examination and study. Morphological observation and single spore isolation were followed as described in Dai et al. (2017). The ascomata on the host surface were observed by Leica using a S8AP0 microscope and photographed by HDMI 200C. Micro-morphological features were observed using an Olympus BX53 compound microscope and captured with an Olympus DP74 camera (Olympus SZ61; Olympus Corporation, Tokyo, Japan). The asci were stained by Meltzer's reagent to examine the J-/J+ ring at the tip of the asci. India ink was used to stain the ascospores for checking the mucilaginous sheath. The micro-morphological features and fruiting bodies were measured by Tarosoft (R) Image FrameWork (IFW). The photo plates were created by Adobe Photoshop CS6 software (Adobe Systems Inc., San Jose, CA, USA). Herbarium material and living cultures were deposited at the Herbarium of Guizhou Medical University (GMB), Guizhou Medical University Culture Collection (GMBCC) Guiyang, Zhongkai University of Agriculture and Engineering (ZHKU), Zhongkai University of Agriculture and Engineering Culture Collection (ZHKUCC) Guangdong, China, and the Guizhou Culture Collection (GZCC), Guiyang, China. MycoBank numbers were obtained from MycoBank database (https://www.mycobank.org/; accessed on 23 January 2024) to register the newly described taxa (MycoBank 2024).

DNA extraction, PCR amplification and sequencing

Fungal genomic DNA was extracted from fresh mycelium using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux) according to the manufacturer's instructions. When culture could not be obtained, fruiting bodies were used to extract genomic DNA by using E.Z.N.A. Forensic DNA Kit (BIO-TEK) followed the protocols. Genomic DNA was conducted by polymerase chain reaction (PCR). Four phylogenetic markers, internal transcribed spacer (ITS), large-subunit ribosomal RNA (LSU), RNA polymerase II (*rpb*2), and *tub*, were amplified using primer pairs ITS4/ITS5 (White et al. 1990), LR5/LR0R (Vilgalys and Hester 1990), RPB2-5F/RPB2-7cR (Liu et al. 1999), Bt2a/Bt2b (Hsieh et al. 2005), respectively. Amplification conditions were performed according to Dai et al. (2022) and Li et al. (2022). The purified PCR fragments were sequenced at Shanghai Myobio Biomedical Technology Co. and China UW Genetics Solutions (BGI-Tech), in Shanghai, China. The newly obtained sequence data were deposited in GenBank (https://www.ncbi.nlm.nih.gov).

Sequence alignment and phylogenetic analyses

The newly generated reverse and forward sequences were assembled with Geneious (Restricted) 9.1.2 (https://www.geneious.com, accessed on 20 May 2023) and subjected to BLAST searches in GenBank (https://blast.ncbi.nlm.nih.gov/, accessed on 20 May 2023) for revealing closely matched strains (Table 1). The related sequences of families in the order Xylariales were downloaded based on the latest article Li et al. (2022). The single gene matrix was aligned via the server version of MAFFT v. 7 (Katoh and Standley 2013) (https://mafft.cbrc.jp/alignment/server). The aligned sequence datasets were trimmed by trimAl.v1.2rev59. The alignments were combined via SequenceMatrix 1.9 (Vaidya et al. 2011). The AliView 1.26 (Larsson 2014) was used to obtain phylip and nexus format files for RAxML analysis and Bayesian analysis, respectively.

Table 1. Sequences used for phylogenetic analyses in this study. The newly generated sequences are in bold. Type strains or type specimens are labelled with HT (holotype), ET (epitype), IT (isotype), and PT (paratype), T (Type), "N/A" indicates no available sequences.

Species	Strain/voucher No.	Status	GenBank accession numbers			
			ITS	LSU	*rpb2*	*tub*
Amphibambusa bambusicola	MFLUCC 11-0617	HT	KP744433	KP744474	NA	NA
Amphibambusa hongheensis	KUN-HKAS 112723	HT	MW892971	MW892969	NA	NA
Amphibambusa hongheensis	KUMCC 20-0334	HT	MW892972	MW892970	NA	NA
Amphirosellinia fushanensis	HAST 91111209	HT	GU339496	NA	GQ848339	GQ495950
Amphirosellinia nigrospora	HAST 91092308	HT	GU322457	NA	GQ848340	GQ495951
Annulohypoxylon atroroseum	ATCC 76081	–	AJ390397	KY610422	KY624233	DQ840083
Annulohypoxylon stygium	MUCL 54601	–	KY610409	KY610475	KY624292	KX271263
Apiospora arundinis	CBS 464.83	–	KF144888	KF144933	NA	KF144979
Apiospora hysteriana	ICMP 6889	–	NA	DQ368630	DQ368649	DQ368621
Apiospora kogelbergense	CBS 117206	–	KF144895	KF144941	NA	KF144987
Apiospora setosa	ATCC 58184	–	NA	AY346259	NA	NA
Arecophila australis	GZUCC0112	HT	MT742126	MT742133	NA	MT741734
Arecophila australis	GZUCC0124	PT	MT742125	MT742132	NA	NA
Arecophila bambusae	HKUCC 4794	–	NA	AF452038	NA	NA
Arecophila clypeata	GZUCC0110	HT	MT742129	MT742136	MT741732	NA
Arecophila clypeata	GZUCC0127	PT	MT742128	MT742135	NA	NA
Arecophila miscanthi	GZUCC0122	–	MT742127	MT742134	NA	NA
Arecophila miscanthi	MFLU 19-2333	HT	NR_171235	MK503827	NA	NA
Arecophila sp.	HKUCC 6487	–	NA	AF452039	NA	NA
Arecophila xishuangbannaensis	**ZHKU 23-0280**	**–**	**OR995737**	**OR995744**	**NA**	**NA**
Arecophila xishuangbannaensis	**GMB-W1283**	**HT**	**OR995736**	**OR995743**	**NA**	**NA**
Arecophila zhaotongensis	**GMBCC1145**	**HT**	**OR995740**	**OR995747**	**OR995741**	**NA**
Arecophila zhaotongensis	**ZHKU 23-0260**	**–**	**OR995738**	**OR995745**	**NA**	**NA**
Arecophila zhaotongensis	**ZHKU 23-0259**	**IT**	**OR995735**	**OR995742**	**NA**	**NA**
Astrocystis concavispora	MFLUCC 14-0174	HT	KP297404	KP340545	KP340532	KP406615
Atrotorquata lineata	HKUCC 3263	–	AF009807	NA	NA	NA
Atrotorquata spartii	MFLUCC 13-0444	HT	NA	KP325443	NA	NA
Barrmaelia rappazii	CBS 142771	HT	MF488989	MF488989	MF488998	MF489017
Barrmaelia rhamnicola	CBS 142772	ET	MF488990	MF488990	MF488999	MF489018
Cainia anthoxanthis	MFLUCC 15-0539	HT	NR_138407	KR092777	NA	NA
Cainia desmazieri	CAI	–	KT949896	KT949896	NA	NA
Cainia desmazieri	CBS 137.62	–	MH858124	MH869702	NA	NA
Cainia globosa	MFLUCC 13-0663	HT	NR_171724	KX822123	NA	NA
Cainia graminis	CBS 136.62	–	MH858123	AF431949	NA	NA
Cainia graminis	MFLUCC 15-0540	–	KR092793	KR092781	NA	NA
Cainia sp.	LSU0560	–	MT000421	MT000513	NA	NA
Camillea obularia	ATCC 28093	–	KY610384	KY610429	KY624238	KX271243
Camillea tinctor	YMJ 363	–	JX507806	NA	JX507790	JX507795
Collodiscula bambusae	GZ 62	–	KP054279	KP054280	KP276675	KP276674
Collodiscula fangjingshanensis	GZUH 0109	HT	KR002590	KR002591	KR002592	KR002589
Coniocessia maxima	CBS 593.74	HT	NR_137751	MH878275	NA	NA
Coniocessia nodulisporioides	CBS 281.77	IT	MH861061	AJ875224	NA	NA
Creosphaeria sassafras	STMA 14087	–	KY610411	KY610468	KY624265	KX271258
Daldinia bambusicola	CBS 122872	HT	KY610385	KY610431	KY624241	AY951688
Daldinia concentrica	CBS 113277	–	AY616683	KT281895	KY624243	KC977274

Species	Strain/voucher No.	Status	GenBank accession numbers			
			ITS	LSU	*rpb2*	*tub*
Endocalyx cinctus	NBRC 31306	–	MZ313191	MZ313152	NA	NA
Endocalyx cinctus	JCM 7946	–	LC228648	LC228704	NA	NA
Endocalyx grossus	JCM 5164	HT	MZ313160	MZ313138	NA	NA
Endocalyx grossus	JCM 5165	–	MZ313159	MZ313158	NA	NA
Endocalyx grossus	JCM 5166	–	MZ313179	MZ313171	NA	NA
Endocalyx indumentum	JCM 5171	HT	MZ313153	MZ313161	NA	NA
Endocalyx indumentum	JCM 8042	–	MZ313162	MZ313157	NA	NA
Endocalyx melanoxanthus	CBS147393	–	MW718204	MW718204	NA	NA
Endocalyx melanoxanthus	CBS147394	–	MW718203	MW718203	NA	NA
Endocalyx ptychospermatis	ZHKUCC 21-0008	HT	MZ493352	OK513439	NA	NA
Endocalyx ptychospermatis	ZHKUCC 21-0009	HT	MZ493353	OK513440	NA	NA
Endocalyx ptychospermatis	ZHKUCC 21-0010	HT	MZ493354	OK513441	NA	NA
Entoleuca mammata	JDR 100	–	GU300072	NA	GQ844782	GQ470230
Entonaema liquescens	ATCC 46302	–	KY610389	KY610443	KY624253	KX271248
Entosordaria perfidiosa	CBS 142773	ET	MF488993	MF488993	MF489003	MF489021
Entosordaria quercina	RQ/CBS 142774	HT	MF488994	MF488994	MF489004	MF489022
Graphostroma platystomum	CBS 270.87	HT	JX658535	AY083827	KY624296	HG934108
Hypocopra rostrata	NRRL 66178	–	KM067909	KM067909	NA	NA
Hypocrea gelatinosa	NBRC 104900	ET	JN943358	JN941453	NA	NA
Hypomontagnella barbarensis	STMA 14081	HT	MK131720	MK131718	MK135891	MK135893
Hypomontagnella monticulosa	MUCL 54604	ET	KY610404	KY610487	KY624305	KX271273
Hypoxylon fragiforme	MUCL51264	ET	KM186294	KM186295	KM186296	KX271282
Hypoxylon investiens	CBS 118185	–	KC968924	KY610451	KY624260	KC977269
Jackrogersella multiformis	CBS 119016	ET	KC477234	KT281893	KY624290	KX271262
Kretzschmaria deusta	CBS 163.93	–	KC477237	KY610458	KY624227	KX271251
Leiosphaerella chromolaenae	CBS 125586	–	JF440976	JF440976		
Longiappendispora chromolaenae	MFLUCC 17-1485	HT	NR_169723	NG_068714	NA	NA
Lopadostoma americanum	LG8	HT	KC774568	KC774568	KC774525	NA
Lopadostoma dryophilum	LG21	ET	KC774570	KC774570	KC774526	MF489023
Lopadostoma fagi	LF1	HT	KC774575	KC774574	KC774531	NA
Lopadostoma quercicola	LG27	HT	KC774610	KC774610	KC774558	NA
Lopadostoma turgidum	LT2	ET	KC774618	KC774618	KC774563	MF489024
Monographella nivalis	UPSC 3273	–	NA	AF452030	NA	NA
Nemania abortiva	BISH 467	HT	GU292816	NA	GQ844768	GQ470219
Nemania bipapillata	HAST 90080610	–	GU292818	NA	GQ844771	GQ470221
Nemania maritima	HAST 89120401	ET	GU292822	NA	GQ844775	GQ470225
Nemania primolutea	HAST 91102001	HT	EF026121	NA	GQ844767	EF025607
Obolarina dryophila	MUCL 49882	–	GQ428316	GQ428316	KY624284	GQ428322
Oxydothis frondicola	HKUCC 1001	–	NA	AY083835	NA	NA
Paramphibambusa bambusicola	**GMBCC1142**	**HT**	**OR995739**	**OR995746**	**OR995740**	**NA**
Paramphibambusa bambusicola	**ZHKUCC 23-0976**	**–**	**OR995741**	**OR995748**	**OR995739**	**NA**
Paraxylaria xylostei	MFLU 17-1636	–	MW240640	MW240570	NA	MW820914
Paraxylaria xylostei	MFLU 17-1645	–	MW240641	MW240571	NA	MW820915
Phylacia sagrana	CBS 119992	–	AM749919	NA	NA	NA
Podosordaria mexicana	WSP 176	–	GU324762	NA	GQ853039	GQ844840
Podosordaria muli	WSP 167	HT	GU324761	NA	GQ853038	GQ844839
Poronia pileiformis	WSP 88113001	ET	GU324760	NA	GQ853037	GQ502720
Poronia punctata	CBS 656.78	HT	KT281904	KY610496	KY624278	KX271281
Pyrenopolyporus nicaraguensis	CBS 117739	–	AM749922	KY610489	KY624307	KC977272

Species	Strain/voucher No.	Status	GenBank accession numbers			
			ITS	LSU	*rpb2*	*tub*
Rhopalostroma angolense	CBS 126414	–	KY610420	KY610459	KY624228	KX271277
Rosellinia aquila	MUCL 51703	–	KY610392	KY610460	KY624285	KX271253
Rosellinia corticium	MUCL 51693	–	KY610393	KY610461	KY624229	KX271254
Rostrohypoxylon terebratum	CBS 119137	HT	DQ631943	DQ840069	DQ631954	DQ840097
Ruwenzoria pseudoannulata	MUCL 51394	HT	KY610406	KY610494	KY624286	KX271278
Sarcoxylon compunctum	CBS 359.61	–	KT281903	KY610462	KY624230	KX271255
Seynesia erumpens	SMH 1291	–	NA	AF279410	AY641073	NA
Stilbohypoxylon quisquiliarum	YMJ 172	–	EF026119	NA	GQ853020	EF025605
Thamnomyces dendroideus	CBS 123578	–	FN428831	KY610467	KY624232	KY624313
Vialaea mangiferae	MFLUCC 12-0808	HT	KF724974	KF724975	NA	NA
Vialaea minutella	BRIP 56959	–	KC181926	KC181924	NA	NA
Xylaria hypoxylon	CBS 122620	ET	KY610407	KY610495	KY624231	KX271279
Zygosporium oscheoides	MFLUCC 14-0402	–	MF621585	MF621589	NA	NA

Maximum likelihood (ML) analysis was performed by RAxML-HPC2 on XSEDE (8.2.12) (Stamatakis et al. 2008; Stamatakis 2014) via the CIPRES Science Gateway V.3.3 web server (https://www.phylo.org/portal2/login!input.action) (Miller et al. 2010). The best model was GTRGAMMA, with 1000 replicates rapid bootstrapping. Bayesian inference (BI) analysis was performed by MrBayes on XSEDE (3.2.7a) in the website CIPRES Science Gateway (Ronquist et al. 2012). Markov Chain Monte Carlo (MCMC) was used to evaluate posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002). The best model test for each gene was performed via MrMTgui (Ma 2016). Six simultaneous Markov chains were run for 1000000 generations, and trees were sampled every 100th generation (resulting in 10,000 total trees). The phylogenetic trees were visualized with FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk software/figtree/) (Rambaut 2012), and edited by Adobe Illustrator CS v. 5.

Abbreviations

ATCC: American Type Culture Collection; **BISH:** Bishop Museum, Department of Natural Sciences; **CAI:** Cairo University, Botany Department; **CBS:** Culture Collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands; **GMBCC:** Guizhou Medical University Culture Collection, Guiyang, China; **GZU:** Karl-Franzens-Universitat Graz; **GZUCC:** Guizhou University Culture Collection, Guiyang, Guizhou, China; **HAST:** Research Center for Biodiversity, Academia Sinica; **HKUCC:** The University of Hong Kong Culture Collection, Hong Kong, P.R. China; **JCM:** Japan Collection of Microorganisms, Japan; **JDR:** J.D. Rogers; **KUMCC:** Kunming Institute of Botany Culture Collection; **KUN-HKAS:** Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica; **LF:** *Lopadostoma fagiL*; **LT:** *Lopadostoma turgidum*; **MFLU:** Mae Fah Luang University Herbarium; **MFLUCC:** Mae Fah Luang University Culture Collection; **MUCL:** Agro-food & Environmental Fungal Collection; **NBRC:** Biological Resource Center IFO; **NRRL:** Agricultural Research Service Culture Collection; **SMH:** Sabine M. Huhndorf; **KUN-HKAS**: Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica; **STMA:** HZI culture collection, Helmholtz Centre for Infection Research, Braunschweig, Germany; **WSP:** Washington State University, Plant Pathology Department; **YMJ:** YuMing, Ju; **ZHKUCC:** Zhongkai University of Agriculture and Engineering.

Results

Phylogenetic results

The combined dataset comprised 107 strains (Table 1). *Hypocrea gelatinosa* (NBRC 104900) was selected as the outgroup taxon. The alignment comprised 4195 bp in total (ITS 580 bp, LSU 736 bp, *rpb*2 1197 bp, and *tub* 1682 bp). The final ML optimization likelihood value of -68750.486429 and the matrix had 2603 bp distinct alignment patterns, with 45.50% of undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.240607, C = 0.260776, G = 0.259542, T = 0.239075, AC = 1.358257, AG = 3.703167, AT = 1.354909, CG = 1.087664, CT = 6.069506, GT = 1.000000; proportion of invariable sites I = 0.378984; and gamma distribution shape parameter α = 0.817253.

The final RAxML tree (Fig. 1) is based on maximum likelihood (ML), and Bayesian inference analyses with similar topology. The RAxML tree showed that *Paramphibambusa bambusicola* (GMBCC1142, ZHKUCC 23-0976) formed a distinct, stable clade basal to the other genera of Cainiaceae with high statistical support (90% ML, 1.00 PP). Moreover, *Arecophila* strains form two clades (Fig. 1), which coincide with Li et al. (2022). Our new collections cluster with *A. bambusae* Umali & K.D. Hyde (HKUCC 4794) and *Arecophila* sp. (HKUCC 6487) forming a sister branch clustered in Clade 2 (Fig. 1).

Taxonomy

Paramphibambusa **L.S. Han & D.Q. Dai, gen. nov.**
MycoBank No: MB851854

Etymology. In reference to a new genus is morphologically similar to *Amphibambusa*, but phylogenetically distinct.

Description. ***Saprobic*** on bamboo culms. **Sexual morph:** ***Ascomata*** deeply immersed beneath poorly developed clypeus, solitary, scattered, black, globose to subglobose, ostiolate, with a long neck. ***Peridium*** composed of several layers, thick-walled, hyaline to pale brown cells of textura angularis. ***Paraphyses*** hyaline, numerous, filiform to cylindrical, guttulate, branched, septate, tapering towards the apex. ***Asci*** 8-spored, rarely 6-spored, unitunicate, cylindrical, short pedicellate, straight or slightly curved, rounded at the apex, with an elliptical to trapezoidal, J+ sub-apical ring. ***Ascospores*** uniseriate or overlapping uniseriate, hyaline to golden brown, ellipsoidal, guttulate, 2–3-celled, tapering at the ends, slightly constricted at the septum, smooth-walled, surrounded by a mucilaginous sheath. **Asexual morph:** Undetermined.

Type species. *Paramphibambusa bambusicola* L.S. Han & D.Q. Dai

Notes. A monotypic genus *Paramphibambusa* is introduced based on its different morphological characteristics and the support of phylogenetic affinity with the other members in Cainiaceae. The morphological characteristics of *Paramphibambusa* resemble *Amphibambusa* in having dark clypeus, immersed, globose to subglobose ascomata, unitunicate, short pedicellate asci with a J+, and sub-apical ring, and 1-septate ascospores, surrounded by a thick mucilaginous sheath (Liu et al. 2015; Jiang et al. 2021). *Paramphibambusa* can be easily distinguished from *Amphibambusa* in having an ostiole, with a long neck, and ascospores lacking longitudinal wall ornamentations. In addition, *Paramphibambusa*

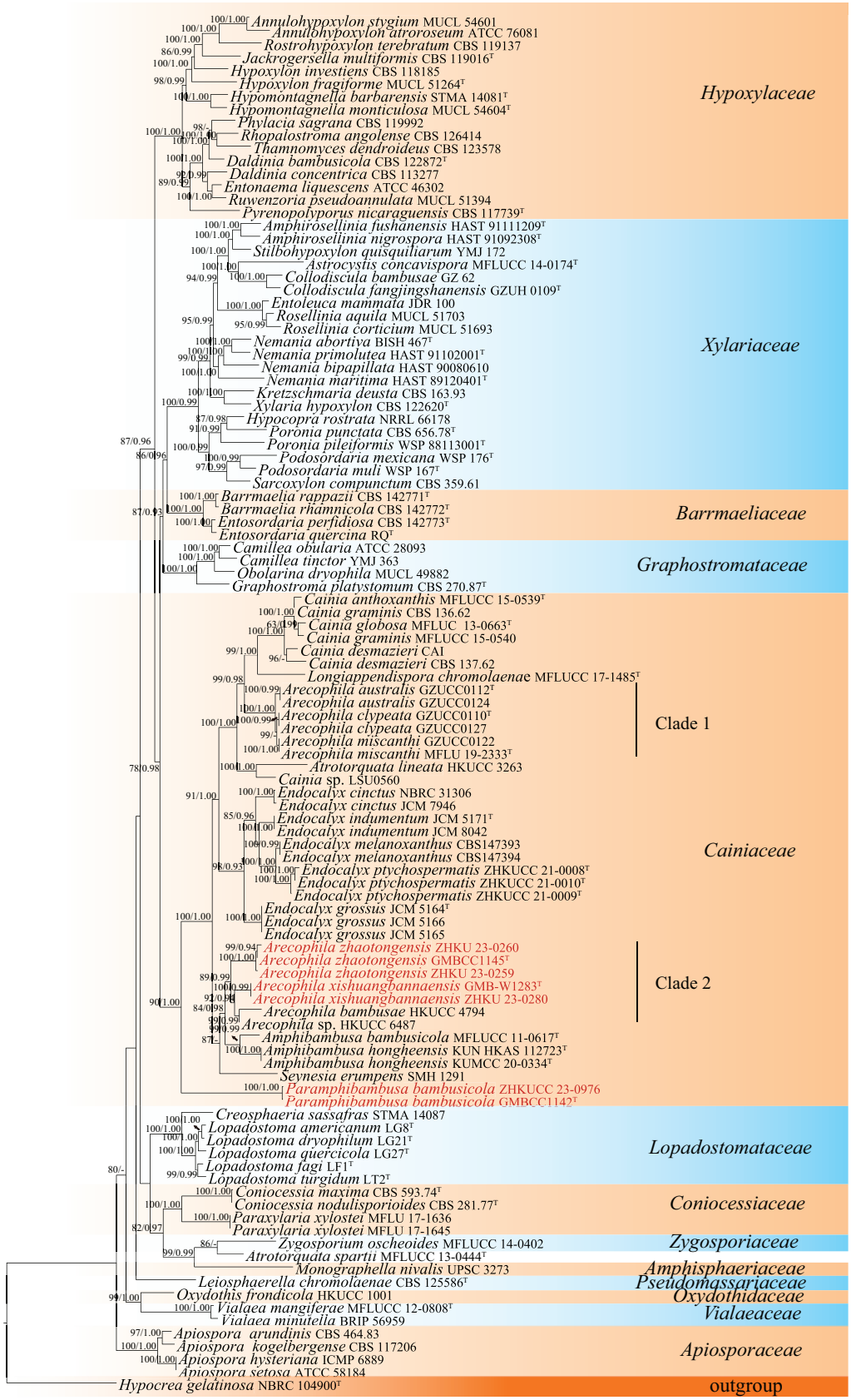

Figure 1. The RAxML tree was generated based on the combined ITS, LSU, *rpb*2, and *tub* sequence data. Bootstrap support values for ML equal to or greater than 60%, and Bayesian posterior probabilities (BYPP) equal to or higher than 0.90 are indicated above the nodes as ML/PP. Type materials are indicated by superscript "T", while the newly generated sequences are shown in red.

forms a well-separated branch basal to other cainiaceous genera with 90% ML, and 1.00 PP statistical supports (Fig. 1). *Paramphibambusa* differs from the sexual members of Cainiaceae in ascomata with a long neck leading up to the ostiole, and in that the ascospores lack longitudinal striations or germ slits or germ pores *Endocalyx* is an asexually typified genus and lacks a sexual morph to compare its morphology with *Paramphibambusa*. However, in the phylogenetic analyses, *Paramphibambusa* resides in a distinct phylogenetic lineage to *Endocalyx* (Fig. 1). Therefore, we consider *Paramphibambusa* as a distinct genus.

Paramphibambusa bambusicola **L.S. Han & D.Q. Dai, sp. nov.**
MycoBank No: MB851857
Fig. 2

Etymology. With reference to its occurrence on host bamboo.

Holotype. GMB-W1350.

Description. ***Saprobic*** on dead culms of bamboo. **Sexual morph**: ***Ascomata*** 430–580 × 500–550 µm ($\bar{x}$ = 474 × 519 µm, n = 20), deeply immersed beneath blackened poorly developed clypeus, solitary, scattered, black, globose to subglobose, ostiolate, with a long neck, 50–125 µm diam., 240–260 µm long. ***Peridium*** 15–25 µm thick, composed of several layers, thick-walled, hyaline to pale brown cells of textura angularis. ***Paraphyses*** 2–5.5 µm wide, hyaline, numerous, filiform to cylindrical, guttulate, branched, septate, tapering towards the apex. ***Asci*** 200–240 × 10–13.5 µm ($\bar{x}$ = 215 × 11.5 µm, n = 20), 8-spored, rarely 6-spored, unitunicate, cylindrical, short pedicellate, straight or slightly curved, rounded at the apex, with a 3–4 µm wide, 1.5–2 µm high ($\bar{x}$ = 3.6 × 1.7 µm, n = 20), elliptical to trapezoidal, J+, sub-apical ring. ***Ascospores*** 24–35 × 6–7.5 µm ($\bar{x}$ = 27 × 6.6 µm, n = 20), uniseriate or overlapping uniseriate, hyaline to golden brown, ellipsoidal, 2–3-celled, tapering at the ends, slightly constricted at the septum, smooth-walled, surrounded by a 9–12 µm mucilaginous sheath. **Asexual morph**: Undetermined.

Culture characters. Ascospores germinating within 24 h. Colonies reaching 45 mm diam. in 20 days under dark and at 28 °C conditions, circular, flocculent, yellowish from above and below.

Materials examined. China, Yunnan Province, Zhaotong, Zhenxiong town, 27°36'8"N, 104°56'34"E, 1673.07 m, on dead culms of bamboo, 29 July 2021, Dong-Qin Dai, Li-Su Han, DDQ02077, (GMB-W1350, holotype), GMBCC1142, ex-type; *ibid*. (ZHKU 23-0256, isotype), GZCC 23-0629, ex-isotype; Zhaotong, Zhenxiong town, Shanzhai, 27°62'52"N, 104°81'98"E, 1666.10 m, on dead culms of bamboo, 4 August 2023, Dong-Qin Dai, Li-Su Han, HLS0114 (ZHKU 23-0257), living culture ZHKUCC 23-0976.

Notes. In the phylogenetic tree, *Paramphibambusa bambusicola* formed a stable clade basal to the other species of Cainiaceae with 90% ML, and 1.00 PP statistical supports (Fig. 1). In morphology, *Paramphibambusa bambusicola* has Cainiaceae species typical characteristics that are cylindrical asci, with a J+, apical ring, and ellipsoidal ascospores surrounded by a mucilaginous sheath. However, the spores of Cainiaceae species have the ornamented walls with longitudinal striations or germ slits or germ pores. *Paramphibambusa bambusicola* differs from the current Cainiaceae species by having smooth-walled ascospores.

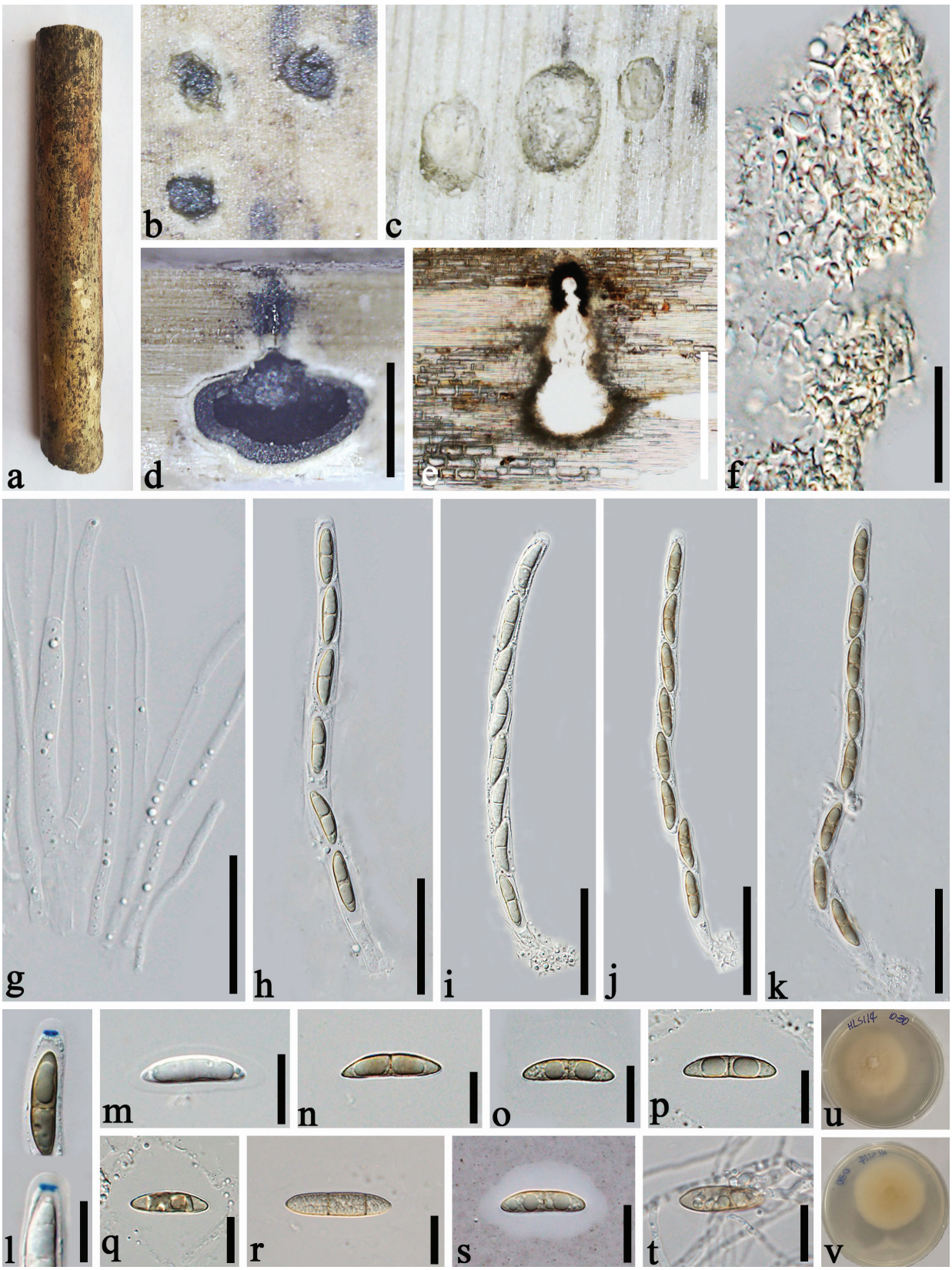

Figure 2. *Paramphibambusa bambusicola* (GMB-W1350, holotype) **a** bamboo specimen **b** black ostioles at the host surface **c** transverse section of ascomata **d, e** vertical section of ascomata with long necks and black clypeus **f** cells of peridium **g** paraphyses **h–k** asci **l** asci with J+, elliptical to trapezoidal, subapical ring (stained in Melzer's reagent) **m–s** ascospores (**s** ascospore stained in Indian ink showing mucilaginous sheath) **t** a germinating ascospore **u, v** cultures on PDA after 20 days (**u** upper, **v** reverse). Scale bars: 300 µm (**d, e**); 15 µm (**f, l–t**); 30 µm (**g**); 50 µm (**h–k**).

Therefore, based on morphological and phylogenetic studies, *P. bambusicola* is introduced hereby as a new species occurring on bamboo in Yunnan, China.

***Arecophila* K.D. Hyde, Nova Hedwigia 63(1-2): 82 (1996)**
MycoBank No: MB27653

Notes. The genus *Arecophila* is characterized by immersed ascomata, usually with a clypeus, unitunicate, cylindrical asci, commonly producing an apical ring, and ascospores with longitudinal striation or a verrucose wall, and surrounded by a mucilaginous sheath (Hyde 1996; Li et al. 2022). Li et al. (2022) provided a morphological comparison of the main characters of *Arecophila* species. The asexual morph of *Arecophila* has not been reported. According to Li et al. (2022), this genus is distributed across 12 countries and is reported from 16 host species.

***Arecophila xishuangbannaensis* L.S. Han & D.Q. Dai, sp. nov.**
MycoBank No: MB851853
Fig. 3

Etymology. Named after the location "Xishuangbanna" where the new taxon was discovered.

Holotype. GMB-W1283.

Description. ***Saprobic*** on dead culms of bamboo. **Sexual morph:** ***Ascomata*** 540–700 × 320–450 µm ($\bar{x}$ = 586 × 389 µm, n = 20), immersed beneath a black clypeus, forming white ring surrounding ostioles of ascomata, solitary or scattered, sometimes gregarious, globose to subglobose, dark brown to black. ***Ostioles*** papillate, central, black. ***Peridium*** 15–25 µm thick, comprised of several layers, thick-walled, dense, brown to hyaline, cells of textura angularis. ***Paraphyses*** 2.5–6 µm wide, hyaline, numerous, cylindrical, unbranched, septate. ***Asci*** 180–270 × 12–14 µm ($\bar{x}$ = 213 × 12.8 µm, n = 20), 8-spored, unitunicate, cylindrical, pedicellate, straight or slightly curved, apically rounded, with a 3.7–4.7 µm wide, 2.5–3 µm high ($\bar{x}$ = 4.3 × 2.7 µm, n = 20), wedge-shaped, J+, apical ring. ***Ascospores*** 23–27 × 8.5–9.5 µm ($\bar{x}$ = 24.5 × 8.8 µm, n = 20), overlapping, uniseriate, initially hyaline, pale brown to dark brown when mature, ellipsoidal, medianly 1-septate, tapering towards both ends, slightly constricted at the septum, with longitudinal striation along entire length of the ascospore, surrounded by a 3.5–5 µm thick, distinct, globose to subglobose, mucilaginous sheath. **Asexual morph:** Undetermined.

Materials examined. China, Yunnan Province, Xishuangbanna, Jinghong, Manzhang, Mengla, 21°91'97"N, 101°20'42"E, 617.14 m, on dead culms of bamboo, 16 August 2020, Dong-Qin Dai, Li-Su Han, DDQ00993, (GMB-W1283 holotype), *ibid*. (ZHKU 23-0258, isotype), *ibid*. DDQ00993-1 (ZHKU 23-0280).

Notes. In the phylogenetic tree, our new collections of *Arecophila xishuangbannaensis* (GMB-W1283, ZHKU 23-0280) formed a well-separated sister branch with *A. bambusae* (HKUCC 4794) and *Arecophila* sp. (HKUCC 6487) with 92% ML, 0.94 PP statistical supports (Fig. 1). Based on a nucleotide base pair comparison, *A. xishuangbannaensis* differs from *A. bambusae* (HKUCC 4794) in LSU gene (15/736 bp, 2%). Morphologically, *A. xishuangbannaensis* is similar

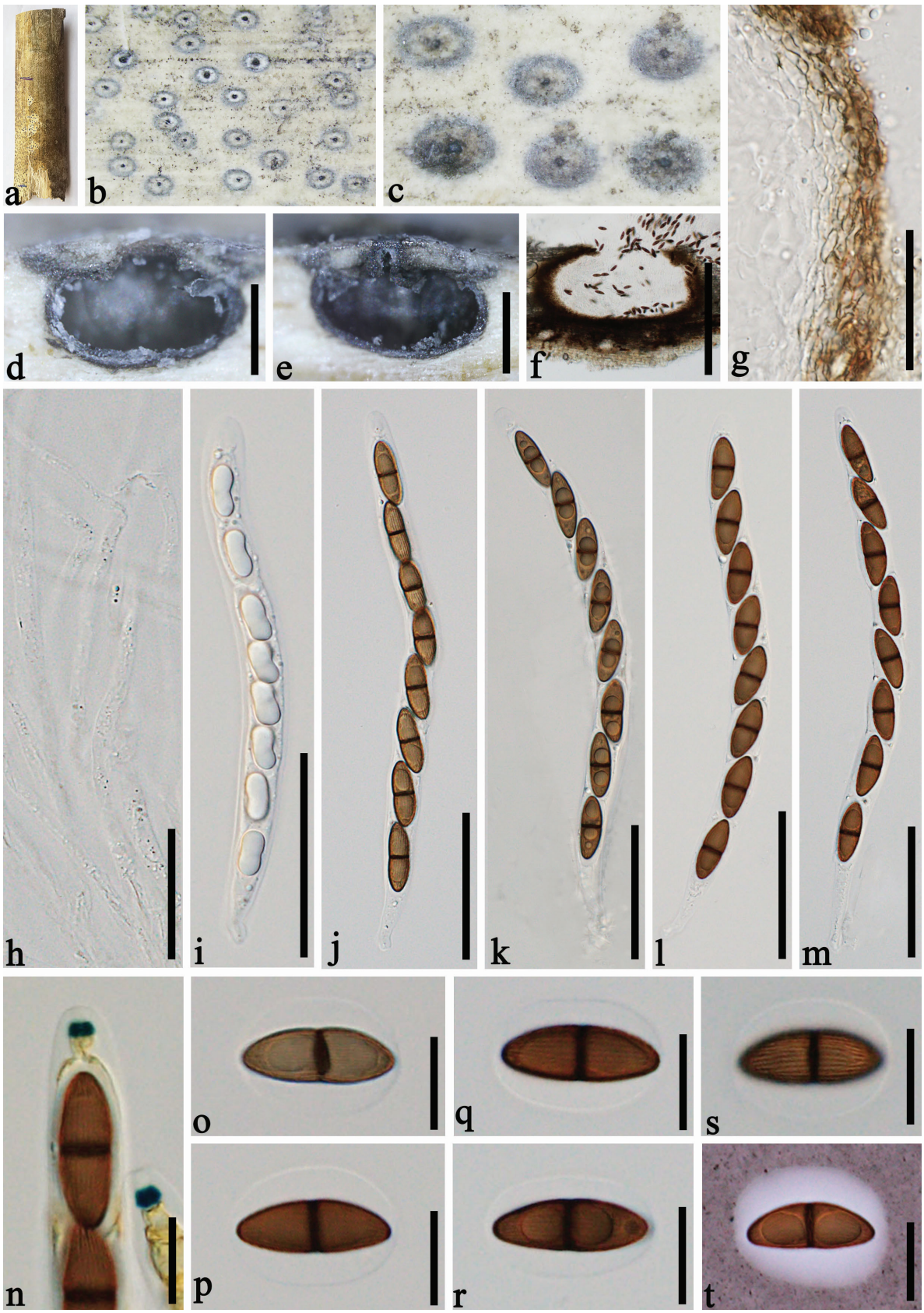

Figure 3. *Arecophila xishuangbannaensis* (GMB-W1283, holotype) **a** bamboo specimen **b, c** appearance of ostioles on host surface **d–f** vertical sections of ascomata **g** peridium **h** paraphyses **i–m** asci **n** asci with J+, wedge-shaped rings (Stained in Melzer's reagent) **o–t** ascospores (**s** showing ascospore with longitudinal striations **t** ascospore stained in Indian ink showing mucilaginous sheath). Scale bars: 300 μm (**d–f**); 20 μm (**g**); 30 μm (**h**); 50 μm (**i–m**); 15 μm (**n–t**).

to *A. bambusae*, in having cylindrical asci and ellispoidal ascospores. However, our new taxon differs *A. bambusae* by forming a white ring surrounding ostioles of ascomata and having larger asci (180–270 × 12–14 µm vs. 132.5–140 × 7.5–8 µm) and larger ascospores (23–27 × 8.5–9.5 µm vs. 19–22.5 × 5.5–7 µm) (Umali et al. 1999; Li et al. 2022). *Arecophila xishuangbannaensis* also resembles *A. notabilis* K.D. Hyde, but it has larger ascomata (586 × 389 µm vs. 400 × 360 µm) (Hyde 1996). The spores of this species did not germinate on PDA or malt extract agar (MEA) media, thus no culture is available.

Arecophila zhaotongensis **L.S. Han & D.Q. Dai, sp. nov.**
MycoBank No: MB851836
Fig. 4

Etymology. Named after the location "Zhaotong" where the new taxon was discovered.

Holotype. GMB-W1353.

Description. ***Saprobic*** on dead culms of bamboo. **Sexual morph:** ***Ascomata*** 600–960 × 450–550 µm ($\bar{x}$ = 710 × 500 µm, n = 20), immersed beneath blackened clypeus, clypeus well-developed, darkened raised discs, or as tiny ostiolar dots, solitary, scattered, sometimes gregarious, dark brown to black, globose to subglobose, papillate, with a central ostiole. ***Peridium*** 15–25 µm thick, comprising several layers, thick-walled, brown cells of textura angularis. ***Paraphyses*** 1–3 µm wide, hyaline, numerous, filiform, branched. ***Asci*** 190–240 × 10.5–14 µm ($\bar{x}$ = 215 × 11.6 µm, n = 20), 4- or 8-spored, rarely 6-spored, cylindrical, unitunicate, short pedicellate, straight or slightly curved, rounded at the apex, with a 4–4.5 µm wide, 2–2.5 µm high ($\bar{x}$ = 4.2 × 2.2 µm, n = 20), trapezoidal, J+, apical ring. ***Ascospores*** 21–30 × 6–8 µm ($\bar{x}$ = 25.5 × 7 µm, n = 20), uniseriate or overlapping uniseriate, brown, ellipsoidal, 1-septate, septate at the centre, slightly tapering at the ends, with longitudinal and sulcate striations, surrounded by a 5–10.5 µm wide, distinct, oval to spherical, mucilaginous sheath. **Asexual morph:** Undetermined.

Culture characters. Ascospores germinating within 24 h. Colonies reach 20 mm diam. in 15 days under dark and at 28 °C conditions, circular, hairy, white from above, and yellow to yellowish from below.

Materials examined. China, Yunnan Province, Diqin, Shangri-La, Bigu Mountain, on dead culms of bamboo, 22 July 2020, 27°36'56.9"N, 99°42'6.4"E, 3460 m, Dong-Qin Dai DDQ00740 (ZHKU 23-0261); Zhaotong, Zhenxiong S302, 27°36'8"N, 104°56'34"E, 1673.07 m, on dead culms of bamboo, 29 July 2021, Dong-Qin Dai, Li-Su Han, DDQ02079, (GMB-W1353, holotype), GMBCC1145, ex-type; *ibid*. (ZHKU 23-0259, isotype), ZHKUCC 23-0975, ex-isotype; *ibid*. DDQ02105 (ZHKU 23-0260).

Notes. In the phylogenetic tree, the new species *A. zhaotongensis* (GMBCC 1145, ZHKU 23-0259, ZHKU 23-0260) formed a separated sister branch to *A. bambusae* (HKUCC 4794), *Arecophila* sp. (HKUCC 6487) and *A. xishuangbannaensis* (GMB-W1283, ZHKU 23-0280) with 89% ML, 0.99 PP statistical supports (Fig. 1). Based on a nucleotide pairwise comparison, *A. zhaotongensis* differs from *A. bambusae* (HKUCC 4794) in 26/736 bp of LSU (3.5%), and differs from *A. xishuangbannaensis* (GMB-W1283, ZHKU 22-0280) in 56/563 bp of ITS

Figure 4. *Arecophila zhaotongensis* (GMB-W1353, holotype) **a** bamboo specimen **b, c** appearance of ostioles at the host surface **d, e** vertical sections of ascomata with ostioles and black clypei **f** peridium **g** paraphyses **h–m** asci **n, o** asci with a J+ trapezoidal ring (stained in Melzer's reagent) **p–t** ascospores surrounded by mucilaginous sheath (**t** ascospore with longitudinal striations) **u** a germinating ascospore **v, w** cultures on PDA after 15 days (**v** upper, **w** reverse). Scale bars: 300 µm (**d, e**); 30 µm (**f, g**); 50 µm (**h–m**); 15 µm (**n–u**).

(9.9%), 18/736 bp of LSU (2.4%). *Arecophila zhaotongensis* has larger asci than *A. bambusae* (190–240 × 10.5–14 µm vs. 132.5–140 × 7.5–8 µm) and larger ascospores (21–30 × 6–8 µm vs. 19–22.5 × 5.5–7 µm) (Umali et al. 1999). *Arecophila zhaotongensis* differs from *A. xishuangbannaensis* (GMB-W1283, ZHKU 23-0280) in having narrower ascospores (21–30 × 6–8 µm vs. 23–27 × 8.5–9.5 µm). The new species also resembles *A. muroiana* (I. Hino & Katum.) You Z. Wang et al. (Wang et al. 2004). However, *A. muroiana* lacks a clypeus absent, while a blackened clypeus was observed in *A. zhaotongensis*.

Discussion

Paramphibambusa forms deeply immersed, dark ascomata, with a long neck, J+ asci and smooth-walled ascospores. Interestingly, genera in Cainiaceae usually form ascospores with longitudinal striations or germ slits or germ pores, however, these characters were not observed in our new collection (GMB-W1350). Hence, we introduced the new genus *Paramphibambusa* in Cainiaceae based on morphological characteristics and phylogenetic analyses (Fig. 1). Moreover, we introduced two new *Arecophila* species in Cainiaceae. The establishment of *Paramphibambusa* and the introduction of two new *Arecophila* species enriches the species diversity of the family Cainiaceae and the diversity of bambusicolous fungi.

Currently, some species in the Cainiaceae are monospecific, such as *Longiappendispora* (Mapook et al. 2020), and *Paramphibambusa* (this study), while *Amphibambusa*, and *Atrotorquata* each contain only two species (Kohlmeyer and Volkmann-Kohlmeyer 1993; Liu et al. 2015; Jiang et al. 2021). Hence, more samples are needed to better understand each genus. Wijayawardene et al. (2022b) mentioned that it is essential to carry out more studies on host plants (that have been extensively studied for fungi, such as bamboo) in biodiversity-rich regions to reveal more novel species. Yunnan is exceedingly rich in fungal diversity, especially in higher level taxa, such as ascomycetes and basidiomycetes (Wijayawardene et al. 2021b; Dai et al. 2022). Hence, we believe that future studies on bamboo-associated fungi in Yunnan Province would disclose more novel taxa.

Atrotorquata was introduced as a monotypic genus by Kohlmeyer and Volkmann-Kohlmeyer (1993) to accommodate *A. lineata* Kohlm. & Volkm.-Kohlm. Subsequently, Liu et al. (2015) introduced *A. spartii* Thambug et al. as the second species. These two species share similar morphology, but their phylogenetic relationship was not well-resolved by Liu et al. (2015). Due to a lack of sequence data in GenBank, *Atrotorquata* clusters outside of Cainiaceae. More sequences especially protein genes loci are needed, to clarify its family placement.

Eighteen epithets were listed in *Arecophila* (Li et al. 2022), but only four taxa and a unnamed species have available molecular data, *viz*., *A. australis* Q.R. Li et al. (GZUCC0112, GZUCC0124), *A. bambusae* (HKUCC 4794), *A. clypeata* Q.R. Li et al. (GZUCC0110, GZUCC0127), *A. miscanthi* Q.R Li & J.C. Kang (GZUCC0122, MFLU 19-2333), and *Arecophila* sp. (HKUCC 6487). Thus, it is necessary to recollect fresh specimens and designate epitypes or reference specimens. Li et al. (2022) divided *Arecophila* into two clades based on phylogenetic analyses. We obtained the same results in our study, probably because most species of *Arecophila* lack protein genes regions in GenBank. We may need to design more suitable primers for sequencing protein genes fragments of *Arecophila* to support phylogenetic study.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Data availability

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

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