MycoKeys 37: 1–17 (2018)
doi: 10.3897/mycokeys.37.27265
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Tzeananiaceae, a new pleosporalean family associated with *Ophiocordyceps macroacicularis* fruiting bodies in Taiwan

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Academic editor: *T. Lumbsch* | Received 7 June 2018 | Accepted 15 July 2018 | Published 26 July 2018

Citation: Ariyawansa HA, Phillips AJL, Chuang W-Y, Tsai I (2018) Tzeananiaceae, a new pleosporalean family associated with *Ophiocordyceps macroacicularis* fruiting bodies in Taiwan. MycoKeys 37: 1–17. https://doi.org/10.3897/mycokeys.37.27265

Abstract

The order Pleosporales comprises a miscellaneous group of fungi and is considered to be the largest order of the class Dothideomycetes. The circumscription of Pleosporales has undergone numerous changes in recent years due to the addition of large numbers of families reported from various habitats and with a large amount of morphological variation. Many asexual genera have been reported in Pleosporales and can be either hyphomycetes or coelomycetes. *Phoma*-like taxa are common and have been shown to be polyphyletic within the order and allied with several sexual genera. During the exploration of biodiversity of pleosporalean fungi in Taiwan, a fungal strain was isolated from mycelium growing on the fruiting body of an *Ophiocordyceps* species. Fruiting structures that developed on PDA were morphologically similar to *Phoma* and its relatives in having pycnidial conidiomata with hyaline conidia. The fungus is characterised by holoblastic, cylindrical, aseptate conidiogenous cells and cylindrical, hyaline, aseptate, guttulated, thin-walled conidia. Phylogenetic analysis based on six genes, ITS, LSU, *rpb2*, SSU, *tef1* and *tub2*, produced a phylogenetic tree with the newly generated sequences grouping in a distinct clade separate from all of the known families. Therefore, a new pleosporalean family Tzeananiaceae is established to accommodate the monotypic genus *Tzeanania* and the species *T. taiwanensis* in Pleosporales, Dothideomycetes. The *Ophiocordyceps* species was identified as *O. macroacicularis* and this is a new record in Taiwan.

Keywords

Entomopathogenic fungi, Dothideomycetes, Multi-gene analysis, *Phoma*-like, Pleosporineae

Introduction

We have been studying the families of Pleosporales considering both morphology and molecular phylogeny with the aim of providing a natural classification of this large order (Zhang et al. 2012, Hyde et al. 2013, Ariyawansa et al. 2013, 2014, 2015). *Phoma*-like asexual morphs have been shown to be scattered within the Pleosporineae, Pleosporales (Chen et al. 2017, Valenzuela-Lopez et al. 2018). While trying to resolve the natural classification of *Phoma*-like species in Pleosporales, several new families have been introduced within the sub-order Pleosporineae by various authors (Zhang et al. 2009, 2012, Hyde et al. 2013, Ariyawansa et al. 2015, Hernández-Restrepo et al. 2017, Valenzuela-Lopez et al. 2018).

The Pleosporales is considered to be the largest and the most diverse order of the class Dothideomycetes, comprising over 4700 species classified in 53 families (Hyde et al. 2013, Ariyawansa et al. 2015, Hernández-Restrepo et al. 2017, Valenzuela-Lopez et al. 2018). Pleosporalean species are characterised by pseudothecial ascomata usually with a papilla and a peridium composed of several layers of cells (Zhang et al. 2009, 2012, Hyde et al. 2013, Jaklitsch and Voglmayr 2016, Jaklitsch et al. 2017). Asci are bitunicate, usually fissitunicate and produced within a persistent hamathecium with or without pseudoparaphyses (Ariyawansa et al. 2013, 2014, 2015, Hyde et al. 2013). Ascospores are generally septate but vary in colour and shape, with or without a gelatinous sheath (Zhang et al. 2009, 2012, Hyde et al. 2013, Jaklitsch and Voglmayr 2016, Jaklitsch et al. 2017). Asexual morphs can be coelomycetous or hyphomycetous (Zhang et al. 2009, 2012, Hyde et al. 2013, Ariyawansa et al. 2014, 2015, Hernández-Restrepo et al. 2017, Valenzuela-Lopez et al. 2018). Members of Pleosporales are ubiquitous, occurring in various habitats and can be recognised as epiphytes, endophytes or parasites of living leaves or stems, hyperparasites on fungi or insects, lichenised or saprobes of dead plant stems, leaves or bark (Zhang et al. 2012, Hyde et al. 2013, Ariyawansa et al. 2014).

Pleosporales comprises the suborders Pleosporineae and Massarineae. (Zhang et al. 2009, 2012, Hyde et al. 2013). The suborder Massarineae was proposed by Zhang et al. (2009) and currently comprises 12 families (Tanaka et al. 2015). Pleosporineae contains numerous economically important plant and human pathogens and, at present, the suborder comprises 20 families (Valenzuela-Lopez et al. 2018).

Taiwan is an island located in the western Pacific Ocean and the importance of Taiwan's rich diversity of fungal species has been often stated in Asian and global studies (Tsai et al. 2018). A number of studies have been conducted to elucidate the diversity of pleosporalean fungi associated with various hosts and habitats in Taiwan (Chang and Wang 2009, Yang et al. 2016, Tennakoon et al. 2018), but they have rarely investigated species of Pleosporales associated with entomogenous fungi. During our investigation of pleosporalean taxa in Taiwan, a *Phoma*-like fungus was isolated from mycelium growing on the fruiting body of an *Ophiocordyceps* species. The objective of the present study was to determine the taxonomic status of the isolated fungus and the *Ophiocordyceps* species, considering both morphological characters and DNA sequence data.

Materials and methods

Fungal isolation

During the course of an exploration of ascomycetous fungi in Nantou County, Taiwan (24°06'20"N, 121°11'13"E) in July 2017, fungal mycelium was observed developing on a fruiting body of an unidentified *Ophiocordyceps* species. The mycelium was transferred to and spread on a Petri-dish containing 2% water agar (WA) and incubated at 25 °C. Single conidial isolates were established from sporulating conidiomata in Petri-dishes containing WA. Germinated conidia were transferred separately to plates of PDA (Ariyawansa et al. 2016 a, b).

Sample preparation and morphological observation

Morphological descriptions were made from isolates cultured on 2% potato dextrose agar (PDA; Difco). Preparations for microscopy were mounted in distilled water, observed with an Olympus BX51 microscope with differential interference contrast (DIC) illumination and at least 30 measurements per structure were noted. Voucher specimens were deposited in the herbarium of Department of Plant Pathology and Microbiology, National Taiwan University (NTUH). Living cultures are stored at the Department of Plant Pathology and Microbiology, National Taiwan University Culture Collection (NTUCC). Taxonomic descriptions and nomenclature details were deposited in MycoBank.

DNA extraction, PCR amplification and sequencing

Single conidial isolates were grown on PDA for 28 days at 25 °C in the dark. Genomic DNA was extracted from the mycelium using the Bioman Fungus Genomic DNA Extraction Kit (Bioman) following the manufacturer's protocol (BIOMAN SCIENTIFIC CO., LTD). For *Ophiocordyceps* species, single spore isolation was not successful. Therefore DNA was extracted directly from the ascomata using a DNA extraction kit (E.Z.N.A. Forensic DNA kit, D3591-01, Omega Bio-Tek) following the protocol of Ariyawansa et al. (2014).

PCR amplification was conducted in a 50 µl reaction volume containing 5–10 ng DNA, 0.8 units Taq polymerase, 1X PCR buffer, 0.2 mM d'NTP, 0.3 µM of each primer with the addition of 1.5 mM $MgCl_2$ (Ariyawansa et al. 2014). The PCR reactions for amplification of the internal transcribed spacer regions 1 and 2 flanking the 5.8S nrRNA gene (ITS) (Schoch et al. 2012), were performed under standard conditions (White et al. 1990, Stielow et al. 2010). PCR conditions for amplification of the partial SSU (Small subunit of the nrRNA gene) and LSU (Large subunit of the nrRNA gene) followed the protocol of Ariyawansa et al. (2015). Amplification of partial β-tubulin (*tub2*), *rpb2* (partial RNA polymerase II second largest subunit gene) and *tef1* (partial translation elongation factor 1-α gene) followed the procedure

of Woudenberg et al. (2013) and Ariyawansa et al. (2014). Primer sets used for these genes were as follows: ITS: ITS5/ITS4; LSU: LR0R/LR5; SSU: NS1/NS4; *tub2*: TUB4Rd/TUB4Fd (White et al. 1990, Liu et al. 1999, Sung et al. 2007) *tef1*: EF1-728F/EF1-986R (Carbone and Kohn 1999) and *rpb2*: fRPB2-SF/ fRPB2-7cR (Woudenberg et al. 2013). The PCR products were visualised on 1.5% agarose gels stained with SYBR-safe DNA gel stain. PCR products were purified and sequenced by Genomics, New Taipei, Taiwan. DNASTAR Lasergene SeqMan Pro v.8.1.3 was used to obtain consensus sequences from sequences produced from forward and reverse primers. Newly generated sequences were deposited at NCBI GenBank under the accession numbers provided in Suppl. material 1: Table 1.

Sequence alignment and phylogenetic analysis

Multiple sequence alignments were produced with MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html). The alignments were checked visually and adjusted manually where required. Two different datasets were prepared to evaluate two phylogenies; a Pleosporales tree and a phylogeny of the genus *Ophiocordyceps*. The first tree focused on phylogenetic placement of the new family Tzeananiaceae introduced in this study in the Pleosporales and the second to determine the placement of the *Ophiocordyceps* species (NTUH 17-004) within the genus *Ophiocordyceps*. All introns and exons were aligned individually. Regions comprising various leading or trailing gaps were excluded from the ITS, LSU, *rpb2*, SSU, *tef1* and *tub2* alignments prior to tree building. All sequences obtained from GenBank and used by Hyde et al. (2013), Ariyawansa et al. (2015), Ban et al. (2015), Hernández-Restrepo et al. (2017), Wanasinghe et al. (2017), Valenzuela-Lopez et al. (2018) are listed in Suppl. material 1: Table 1. Single alignments for each locus and the combined six-gene dataset were analysed using different tree development methods.

Maximum parsimony (MP) analyses were made using PAUP v. 4.0b10 (Swofford 2002). Trees were inferred using the heuristic search option with 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple equally parsimonious trees were saved. Descriptive tree statistics for parsimony (Tree Length (TL), Consistency Index (CI), Retention Index (RI), Related Consistency Index (RC) and Homoplasy Index (HI)) were calculated.

Evolutionary models for each locus were determined individually using MrModeltest v. 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC) implemented in both PAUP v. 4.0b10 and MrBayes v. 3.

A maximum likelihood analysis (ML) was executed at the CIPRES webportal (Miller et al. 2010) using RAxML-HPC2 on XSEDE (v 8.2.8) with default parameters and bootstrapping with 1000 replicates (Stamatakis 2014). The subsequent replicates were printed on to the best scoring tree obtained previously.

Bayesian Markov Chain Monte Carlo (MCMC) analyses were conducted in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). The number of generations was set at

10 million and the run was stopped automatically when the average standard deviation of split frequencies fell below 0.01. Trees were saved each 100 generations. MCMC heated chain was set with a "temperature" value of 0.15. The distribution of log-likelihood scores was checked with Tracer v 1.5 to determine the stationary phase for each search and to decide if extra runs were required to achieve convergence (Rambaut and Drummond 2007, Ariyawansa et al. 2015). All sampled topologies below the asymptote (20%) were discarded as part of a burn-in procedure and the remaining trees were used to calculate posterior probabilities (BP) in the majority rule consensus tree.

Phylogenetic trees and data files were viewed in MEGA v. 5 (Tamura et al. 2011), TreeView v. 1.6.6 (Page 2001) and FigTree v. 1.4 (Rambaut and Drummond 2008). ML and MP bootstrap values equal to or greater than 70% and BP equal to or greater than 0.95 are given at each node in Figs 1, 2. Nodes with a posterior probability (PP) lower than 0.95 or MP and ML bootstrap support lower than 70% were considered unresolved.

Results

Phylogeny

The data for the trees conducted in the different analyses are shown below. In the multi-gene analyses, the topologies of the trees acquired for the individual loci were checked visually to confirm that the overall tree topology of the single datasets were comparable to each other and to that of the tree obtained from the combined dataset alignment. Phylogenetic trees obtained from the combined gene analyses are supplied below (Figs 1, 2). Alignments were analysed corresponding to a single gene study of ITS, LSU, *rpb2*, SSU, *tef1* and *tub2* of the two phylogenies. Comparison of the alignment properties and nucleotide substitution models are provided in Tables 1, 2.

Phylogeny of Pleosporales

The final alignment comprised 64 strains with 4558 characters (SSU 1019, LSU 877, ITS 450, *rpb2* 1013, *tef1* 902 and *tub2* 297). The maximum parsimony dataset consisted of 4558 characters of which 3226 were constant, 271 were variable and parsimony-uninformative and 1061 characters were parsimony-informative. Kishino-Hasegawa (KH) test showed length = 4234 steps, CI = 0.466, RI = 0.593, RC = 0.277 and HI = 0.534. The MCMC analysis of the six combined genes run for 66×10^4 generations resulted in 6600 trees. The first 1320 trees, representing the burn-in phase of the analyses, were discarded, while the remaining trees were used to calculate posterior probabilities in the majority rule consensus tree.

A best scoring RAxML tree is presented in Fig. 1, with the Likelihood value of -20128.721105. Phylogenetic trees generated from ML, MP and Bayesian analyses produced trees with similar overall topology at subclass and family level relationships

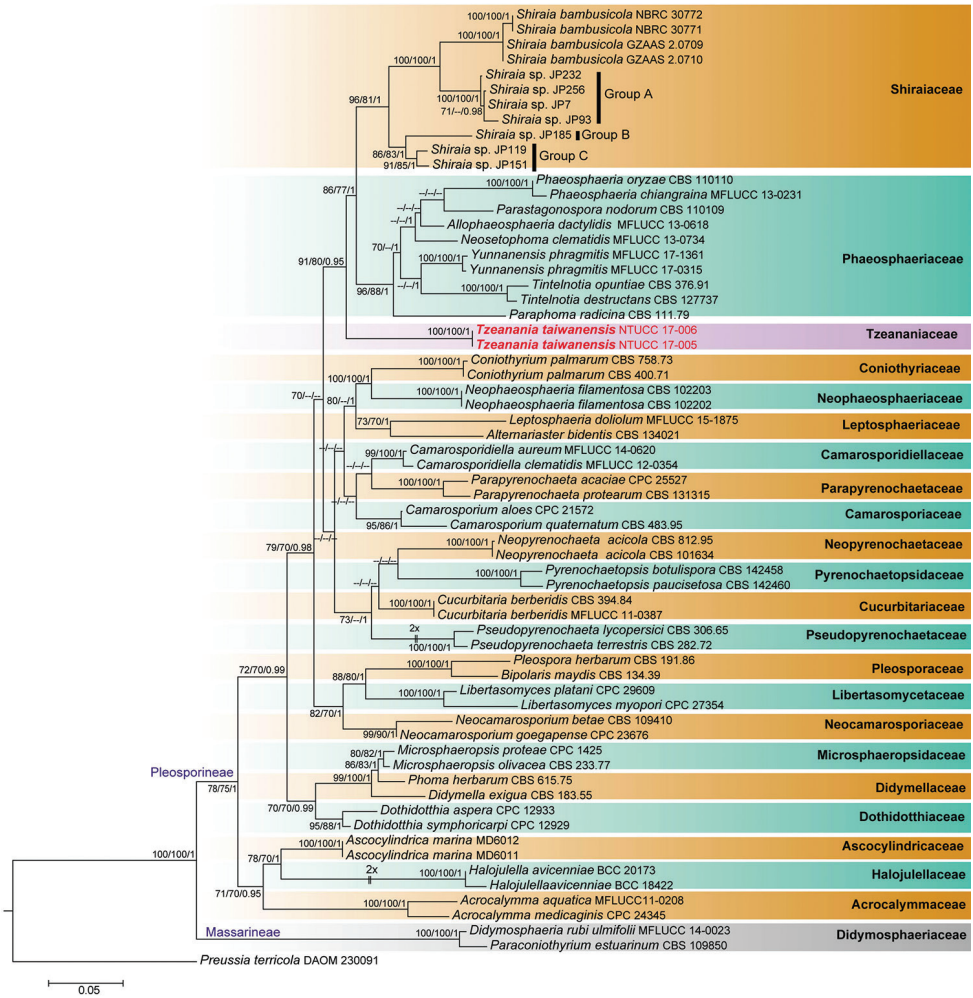

Figure 1. Phylogenetic tree (RAxML) obtained from the DNA sequence data of ITS, LSU, *rpb2*, SSU, *tef1* and *tub2* sequences of 64 strains showing taxa in suborders Massarineae and Pleosporineae within Pleosporales. The new isolates are shown in bold, red. MP and ML bootstrap values (BS) ≥70% and Bayesian posterior probabilities (PP) ≥0.95 are presented at the nodes. Several branches were shortened to facilitate presentation of the tree and this is indicated by two diagonal lines with the number of times a branch was shortened. The scale bar shows the number of estimated mutations per site. The tree was rooted to *Preussia terricola* (DAOM 230091).

Table 1. Comparison of alignment properties of genes and nucleotide substitution models used in Pleosporales phylogenetic analysis.

	LSU	SSU	*rpb2*	*tef1*	ITS	*tub2*
Alignment strategy (MAFFT v6)	G-INS-1	G-INS-1	G-INS-1 +manual	G-INS-1 +manual	G-INS-1 +manual	G-INS-1 +manual
Nucleotide substitution models for Bayesian analysis (determined by MrModeltest)	GTR+I+G	HKY+I+G	GTR+I+G	GTR+I+G	GTR+I+G	GTR+I+G

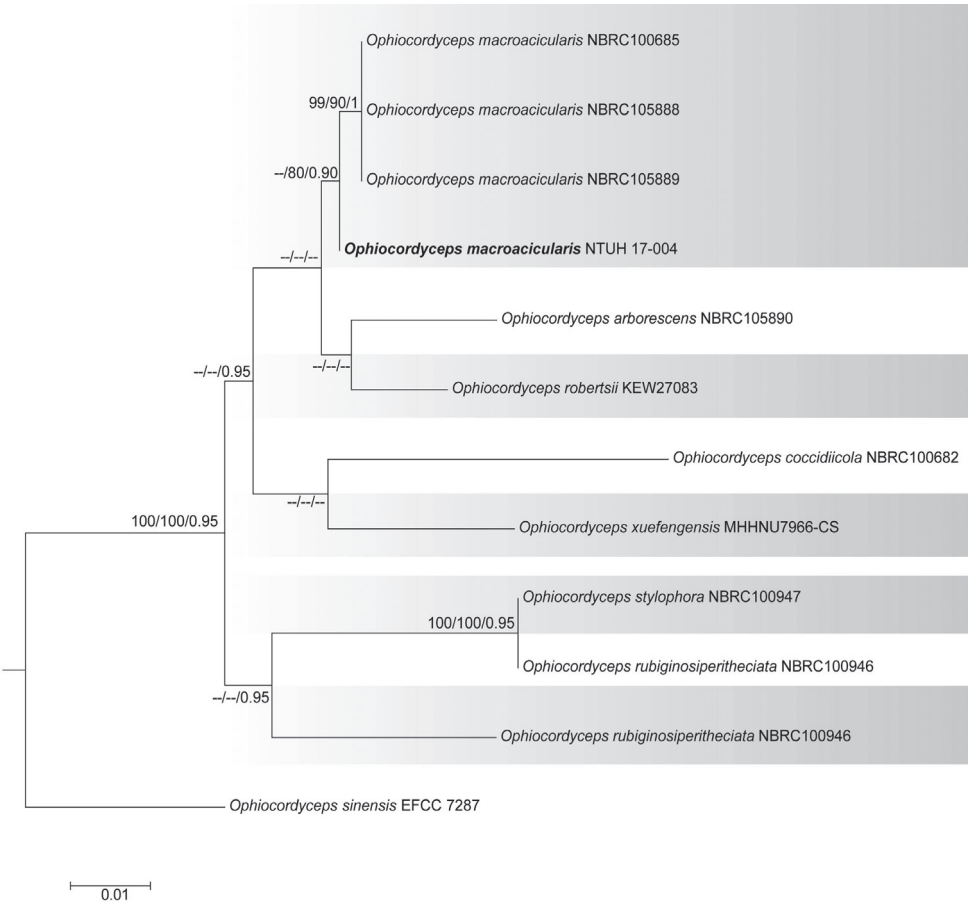

Figure 2. Phylogenetic tree (RAxML) obtained from the DNA sequence data of two loci (ITS and LSU) of *Ophiocordyceps macroacicularis* and allied taxa. The new strain is shown in bold. MP and ML bootstrap values ≥70% and Bayesian posterior probabilities ≥0.95 are presented at the nodes and the scale bar shows the number of estimated mutations per site. The tree was rooted to *Ophiocordyceps sinensis* (EFCC 7287).

Table 2. Comparison of alignment properties of genes and nucleotide substitution models used in *Ophiocordyceps* and allied species phylogenetic analysis.

	LSU	ITS
Alignment strategy (MAFFT v6)	G-INS-1	G-INS-1 +manual
Nucleotide substitution models for Bayesian analysis (determined by MrModeltest)	GTR+I	GTR+I+G

in agreement with earlier studies based on ML and Bayesian analysis (Hyde et al. 2013, Ariyawansa et al. 2015, Tanaka et al. 2015, Hernández-Restrepo et al. 2017, Wanasinghe et al. 2017, Valenzuela-Lopez et al. 2018).

The phylogenetic tree separated two distinct clades corresponding to the suborders Massarineae (represented only by the family Didymosphaeriaceae) and Pleosporineae (represented by more than 19 families). The two newly isolated strains from this study (NTUCC 17-005 and NTUCC 17-006) formed a distinct clade basal to the familial clades of *Shiraiaceae* and *Phaeosphaeriaceae* with high BS and PP support in analyses of the single locus and concatenated datasets. Hence, the novel lineage is regarded here as the new family Tzeananiaceae.

Phylogeny of *Ophiocordyceps*

The final *Ophiocordyceps* alignment comprised 12 strains. The dataset consisted of 1523 characters (LSU 899 and ITS 624). The Bayesian analysis resulted in 1×10^4 trees after 1×10^6 generations. The first 2,000 trees, showing the burn-in phase of the analyses, were discarded, while the remaining trees were used to calculate posterior probabilities in the majority rule consensus tree.

The best scoring RAxML tree is shown in Fig. 2, with the Likelihood value of -3268.294101. Phylogenetic trees acquired from ML, MP and Bayesian analysis produced trees with similar overall topology at species level relationships in agreement with a former study based on ML and Bayesian analysis (Ban et al. 2015).

Ophiocordyceps macroacicularis (NTUH 17-004), considered in this study, grouped in a well-supported clade with isolates NBRC 100685, NBRC 105888 and NBRC 105889 of *Ophiocordyceps macroacicularis* that were used by Ban et al. (2015) to introduce the species, therefore confirming the identification of the studied species.

Taxonomy

Tzeananiaceae Ariyawansa, A.J.L. Phillips & Chuang, fam. nov.
MycoBank: MB825566

Family description. *Sexual morph*: undetermined. *Asexual morph*: *Conidiomata* pycnidial, solitary or aggregated, erumpent, globose, dark brown to black. *Conidiomatal wall* of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform. *Conidia* hyaline, cylindrical, guttulate.

***Tzeanania* Ariyawansa, A.J.L. Phillips & Chuang, gen. nov.**
MycoBank: MB825567

Etymology. Named after the Taiwanese mycologist, Shean-Shong Tzean, in recognition of his extensive contributions towards the taxonomy of entomopathogenic fungi.

Type species. *Tzeanania taiwanensis* Ariyawansa, A.J.L. Phillips & Chuang.

Generic description. *Sexual morph*: undermined. *Asexual morph*: *Conidiomata* pycnidial, partially or entirely immersed in the agar, solitary or aggregated, erumpent, globose. *Conidiomatal wall* of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform. *Conidia* hyaline, smooth- and thin-walled, cylindrical, guttulate. *Chlamydospores* not observed in culture.

***Tzeanania taiwanensis* Ariyawansa, A.J.L. Phillips & Chuang, sp. nov.**
MycoBank MB825568
Fig. 3

Type. TAIWAN. Cueifong, Nantou County (24°06'20"N, 121°11'13"E), developing on a fruiting body of *Ophiocordyceps macroacicularis*, 9 July 2017, Wei-Yu Chuang, (holotype: permanently preserved in a metabolically inactive state, NTUH 17-005!; culture ex-holotype NTUCC 17-005!).

Diagnosis. Phylogeny based on ITS, LSU, *rpb2*, SSU, *tef1* and *tub2* revealed that the strains NTUCC 17-005 and NTUCC 17-006 considered in the present study formed a separate lineage sister to the familial clades of Shiraiaceae and Phaeosphaeriaceae in suborder Pleosporineae. Therefore, a new genus *Tzeanania*, a new species *T. taiwanensis* and a new family Tzeananiaceae in suborder Pleosporineae, Pleosporales are proposed here for the pycnidial coelomycete growing on the surface of the fruiting body of *Ophiocordyceps macroacicularis*.

Etymology. The epithet refers to Taiwan, where this species was collected

Description. Developing on the fruiting body of *Ophiocordyceps macroacicularis*.

Sexual morph not observed. *Asexual morph*: *Conidiomata* pycnidial, semi- or entirely immersed in the agar, solitary or aggregated, erumpent, globose, dark brown to black. *Conidiomatal wall* of *textura angularis*, 3–5 layered, composed of brown to dark brown, flattened polygonal cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, hyaline, smooth-walled, ampulliform to globose, 3–5 × 0.5–2 µm, $\bar{x}$ ± SD = 4 ± 0.7 × 1.5 ± 0.3 µm. *Conidia* hyaline, smooth-walled, thin-walled, cylindrical, guttulate, 4–6 × 1–2 µm, $\bar{x}$ ± SD = 5.3 ± 0.27 × 1.5 ± 0.08 µm. *Chlamydospores* not observed in culture.

Culture characteristics. Colonies concentric circular pattern with radial furrows, entire, whitish, grey to olivaceous, with black conidiomata clustered in circular distribution; reverse concentric circular pattern with radial furrows, beige around centre and olivaceous at edge.

Distribution. Taiwan

Additional material examined. TAIWAN. Department of Plant Pathology and Microbiology, National Taiwan University, growing on a pine needles, 10 October 2017, Wei-Yu Chuang, (paratype: NTUH 17-006!, culture ex-paratype NTUCC 17-006!).

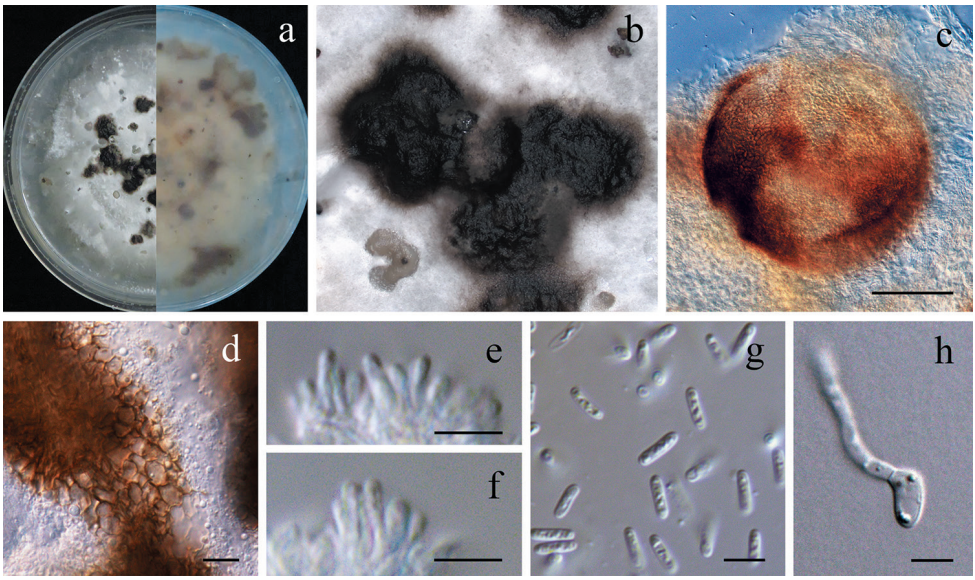

Figure 3. Morphology of *Tzeanania taiwanensis* (NTUCC 17-005) **a** Surface and lower view of colonies on PDA **b** Conidiomata sporulating on PDA **c** close-up of conidioma **d** close-up of Conidiomatal wall. **e–f** Conidiogenous cells **g** Conidia **h** Germinating conidia. Scale bars: 50µm (**c**), 10µm (**d**), 5µm (**e–h**).

Notes. *Tzeanania taiwanensis* differs from the familial type of Phaeosphaeriaceae, *Phaeosphaeria oryzae* in having erumpent, globose conidiomata, conidiomatal wall 3–5 layered, with cylindrical, aseptate, hyaline conidiogenous cells and cylindrical, hyaline, aseptate, guttulated, thin-walled conidia. *Phaeosphaeria oryzae* has immersed, uni- to multi-loculate, globose to subglobose conidiomata, conidiomatal walls comprising brown pseudoparenchymatous cells, with flattened ampulliform to doliiform, hyaline to pale brown conidiogenous cells and oblong to cylindrical, pale brown to brown, septate, smooth-walled guttulate conidia (Hyde et al. 2013).

Morphologically, *Tzeanania taiwanensis* differs from the familial type of Shiraiaceae, *Shiraia bambusicola* in having aseptate conidiogenous cells and cylindrical, hyaline, aseptate, guttulated, thin-walled conidia. *Shiraia bambusicola* has septate conidiogenous cells producing fusiform, muriform, hyaline to light brown, thick-walled conidia with irregularly arranged transverse and longitudinal septa (Hyde et al. 2013). Furthermore, *Tzeanania taiwanensis* can be clearly differentiated from *Shiraia bambusicola* by the host (*Ophiocordyceps macroacicularis* versus Bamboo) and the distribution (Taiwan versus Japan and China).

Discussion

In this study, a new family Tzeananiaceae is formally proposed in Pleosporineae, Pleosporales. This fungus was found on the surface of the fruiting bodies of *Ophiocordyceps*

macroacicularis. Phylogenetic analyses, based on DNA sequence data of ITS, LSU, *rpb2*, SSU, *tef1* and *tub2*, revealed it to form a separate lineage from all other families of Pleosporales. *Ophiocordyceps macroacicularis* is reported for the first time from Taiwan. Moreover, our study expands the base of information regarding the diversity of pleosporalean fungi associated with entomogenous taxa in Taiwan.

Molecular data play a crucial part in present-day fungal systematics, but have some limitations (Ariyawansa et al. 2014, 2015, Hyde et al. 2014, Schoch et al. 2014, Thambugala et al. 2015). The most noteworthy and disconcerting question is that the phylogeny inferred from any one gene may not disclose the evolution history of the organism (Uilenberg et al. 2004). Taylor et al. (2000) proposed operational principles for Avise and Ball's (1990) genealogical concordance species concept mainly for fungal taxa recognition. This Genealogical Concordance Phylogenetic Species Recognition (GCPSR) emphasised that species should be recognised based on genealogical concordance or genealogical non-discordance (Taylor et al. 2000, Dettman et al. 2003). This approach has been used to delineate species in several fungal groups (Udayanga et al. 2014, Manamgoda et al. 2014, Dettman et al. 2003, Ariyawansa et al. 2015). It is therefore better to integrate a polyphasic taxonomy with genotypic and phenotypic data in all forthcoming investigations (Uilenberg et al. 2004, Ariyawansa et al. 2014, 2015, Udayanga et al. 2014).

The family Shiraiaceae was introduced by Liu et al. (2013) to accommodate the bamboo parasitic genus *Shiraia* in suborder Pleosporineae. Phylogenetically, Shiraiaceae has close affinity with Phaeosphaeriaceae. Shiraiaceae species are mainly characterised by pinkish ascostromata that form on bamboo with many locules containing bitunicate asci each with six symmetrical, muriform ascospores (Hyde et al. 2013, Liu et al. 2013). The asexual morph is produced in immature ascostromata and form hyaline muriform, asymmetrical conidia (Hyde et al. 2013, Liu et al. 2013). *Shiraia* was introduced by Hennings (1900), based on *S. bambusicola*, as a monotypic genus. Later, Morakotkarn et al. (2008) reported several *Shiraia*-like strains, obtained from bamboo tissues as endophytes, which showed a close phylogenetic affinity to *Shiraia bambusicola*.

Phaeosphaeriaceae is one of the largest families in suborder Pleosporineae and includes economically important phytopathogens (Hyde et al. 2013). Species may also be found as endophytes or saprobes on different plant hosts, mainly on monocotyledons and several taxa have also been described on dicotyledons (Hyde et al. 2013). Members of Phaeosphaeriaceae are cosmopolitan and thus have been recorded from various regions around the world (Hyde et al. 2013).

Phylogenetically, *Tzeanania* has close affinity with Shiraiaceae and Phaeosphaeriaceae. To clarify the phylogeny of *Shiraia*-like fungal isolates, Morakotkarn et al. (2008) conducted a multi-gene phylogeny based on ITS, LSU and *tub2* and found three distinctive lineages, sister to *Shiraia bambusicola* clade, which were also identified with *Phoma*-like asexual morphs. Furthermore, Morakotkarn et al. (2008) concluded that *Shiraia*-like fungi Group A (Fig. 1) can be recognised as a novel species that could be allocated into a novel genus/species related to *S. bambusicola*. Single gene analysis

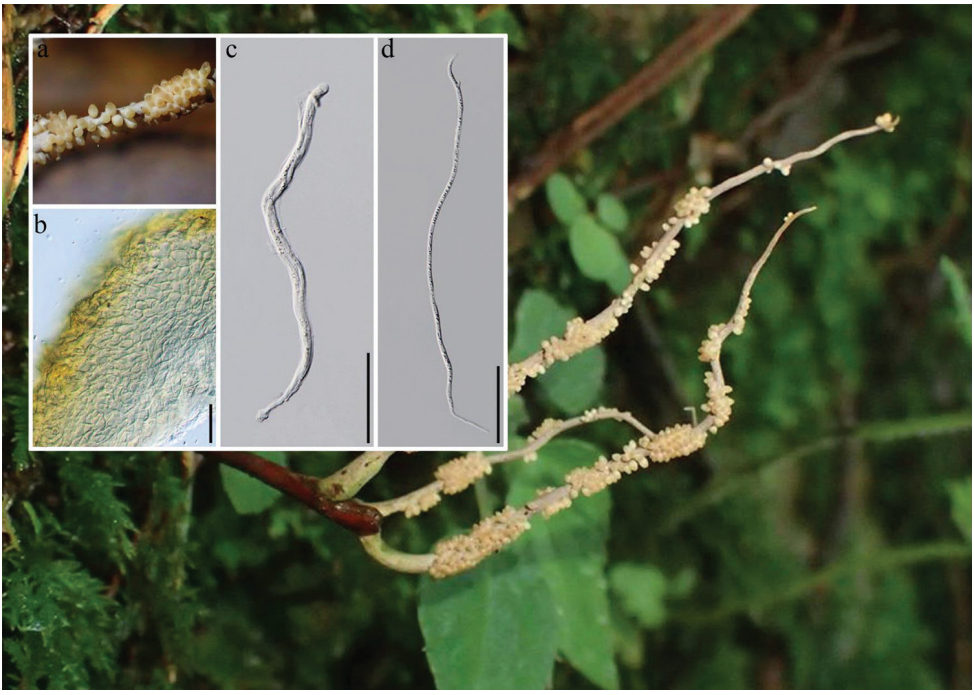

Figure 4. Morphology of *Ophiocordyceps macroacicularis* (NTUH 17-004) **a** Close-up of ascomata **b** Close-up of the peridium **c** Hyaline, cylindrical, eight-spored ascus **d** Needle-shaped, multi-septate, hyaline ascospores. Scale bars: 20 μm (**b**), 50 μm (**c–d**).

of LSU and SSU showed that our strains formed a basal lineage to the familial clade of the Shiraiaceae. Therefore to confirm phylogenetic affinity of our isolates with *S. bambusicola* and *Shiraia*-like fungi groups A, B and C, we additionally conducted a comprehensive phylogeny derived from 3 genes LSU, ITS and TUB (data not shown). We produced a tree with similar topology to the one reported by Morakotkarn et al. (2008) while our new strains formed a distinct lineage sister to the familial clades of Shiraiaceae and Phaeosphaeriaceae, which further confirms the uniqueness of the new family Tzeananiaceae in suborder Pleosporineae.

Ophiocordyceps macroacicularis S. Ban et al. was introduced by Ban et al. (2015) and was recently recorded from Thailand by Luangsa-ard et al. (2018) based on molecular phylogeny together with morphology (Figs 2, 4). To the best of our knowledge, this is the first record of *O. macroacicularis* in Taiwan. Sun et al. (2016) introduced a hyphomycetous taxon, *Calcarisporium cordycipiticola*, which was also found to infect the fruiting bodies of *Cordyceps militaris* causing significant quality and yield losses. Even though we were able to obtain a single spore culture of *T. taiwanensis* (NTUCC 17-006) using the fruiting structures formed on PDA (Fig. 3b), single spore isolation of *O. macroacicularis* was not successful. Therefore, we could not clarify the exact nutritional mode of *T. taiwanensis* or its interaction with *O. macroacicularis*. Therefore, further studies are essential to understand the interaction between this unusual fungus and its host.

Acknowledgements

This study was funded by the Ministry of Science and Technology, Taiwan (MOST project ID: 106-2621-B-002-005-MY2). Alan J.L. Phillips acknowledges the support from Biosystems and Integrative Sciences Institute (BioISI, FCT/UID/ Multi/04046/2013). We appreciate the support given by Professors CP Lin, WC Shen, TL Shen, RF Liou, Associate Professor CL Chung and Chia-Ming Hu. H. A. Ariyawansa is grateful to A.D Ariyawansa, D.M.K Ariyawansa, Ruwini Ariyawansa and Amila Gunasekara for their valuable suggestions.

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MycoKeys 37: 19–38 (2018)
doi: 10.3897/mycokeys.37.27275
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Neodendryphiella, a novel genus of the Dictyosporiaceae (Pleosporales)

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Academic editor: *M. Haase* | Received 12 April 2018 | Accepted 13 July 2018 | Published 26 July 2018

Citation: Iturrieta-González I, Gené J, Guarro J, Castañeda-Ruiz RF, García D (2018) *Neodendryphiella*, a novel genus of the Dictyosporiaceae (Pleosporales). MycoKeys 37: 19–38. https://doi.org/10.3897/mycokeys.37.27275

Abstract

In a survey of soil and herbivore dung microfungi in Mexico and Spain, several dendryphiella-like species were found. Phylogenetic analyses based on ITS and LSU sequences showed that these fungi belonged to the family Dictyosporiaceae (Pleosporales) and represent an undescribed monophyletic lineage distant from *Dendryphiella*. Therefore, the genus *Neodendryphiella* is proposed to accommodate three new species, *N. mali*, *N. michoacanensis* and *N. tarraconensis*. The novel genus shares morphological features with *Dendryphiella* such as differentiated conidiophores and polytretic integrated conidiogenous cells, that produce acropetal branched chains of conidia. *Neodendryphiella* differs in the absence of nodulose conidiophores bearing conidiogenous cells with pores surrounded by a thickened and darkened wall, typical features in the conidiogenous apparatus of *Dendryphiella*. In addition, the phylogenetic and morphological analysis of several reference strains of different *Dendryphiella* species, available for comparison, support the proposal of *D. variabilis* **sp. nov.**, which mainly differs from the other species of the genus by having conidia up to 7 septa and highlight that *D. vinosa* and *D. infuscans* are obscure species that require further taxonomic review.

Keywords

Dendryphiella, Ascomycota, Phylogeny, Taxonomy

Introduction

In an ongoing survey of asexual microfungi from soil and herbivore dung, several interesting specimens morphologically consistent with *Dendryphiella* were found from samples collected in Mexico and Spain. *Dendryphiella* is a dematiaceous hyphomycete proposed by Bubák and Ranojevič (Ranojevič 1914) and typified with *D. interseminata*, which is currently considered a synonym of *D. vinosa* (Reisinger 1968). *Dendryphiella vinosa* is a saprobic fungus commonly found on plant debris, especially on the decaying herbaceous stems of several plants (Ellis 1971, Mercado Sierra et al. 1997). The genus is characterised by pigmented conidiophores, with terminal or intercalary polytretic conidiogenous cells, with dark scarring on the nodose swellings, producing acropleurogenous, solitary or catenate conidia, which are commonly multi-septate and cylindrical with rounded ends (Ellis 1971). Although Index Fungorum and MycoBank list 17 taxa in *Dendryphiella*, a recent review of literature reported only 12 species are accepted, including the newly proposed *D. fasciculata* (Liu et al. 2017). *Dendryphiella pitsanulokensis* is the latter species added to the genus (Hyde et al. 2018). Previous phylogenetic studies, conducted mainly from sequence data of the 18S nrDNA (SSU), 28S nrDNA (LSU) and the internal transcribed spacer (ITS) nrDNA regions, showed that the marine species *D. arenariae* and *D. salina* were phylogenetically distant from the type *D. vinosa* and related to the Pleosporaceae (Gareth Jones et al. 2008, Suetrong et al. 2009). Both species were therefore moved to the genus *Paradendryphiella* (Woudenberg et al. 2013) and, more recently, *D. vinosa* was included in the family Dictyosporiaceae (Tanaka et al. 2015, Boonmee et al. 2016). However, DNA sequence data for *Dendryphiella* species is very limited to create a robust taxonomy for the genus. Only LSU and/or ITS sequences of *D. eucalyptorum*, *D. fasciculata*, *D. paravinosa*, *D. pitsanulokensis* and *D. vinosa* are available (Crous et al. 2014, 2016, Liu et al. 2017, Hyde et al. 2018). In addition, with the exception of the first four mentioned, there is no ex-type culture of other species of this genus and only reference strains of *D. vinosa* and *D. infuscans* are available in public collections for comparison.

Despite the similarity of our soil isolates to *Dendryphiella*, a preliminary study revealed that they showed a low sequence relationship with members of this genus. On the other hand, they were closely related to the strain CBS 139.95 of *Diplococcium (Di.) asperum*, which was proven to be related to the Dictyosporiaceae (Shenoy et al. 2010, Boonmee et al. 2016). It is well known that the genus *Diplococcium* is highly polyphyletic, with species distributed across different classes of the Ascomycota, with its type species, *Di. spicatum,* being related to the Helotiales in Leotiomycetes (Shenoy et al. 2010, Hernández-Restrepo et al. 2017).

The aim of the present study was to resolve the taxonomy of these dendryphiella-like fungi which, based on analysis of the ITS and LSU loci, might represent a new genus in Dictyosporiaceae.

Material and methods

Sampling and fungal strains studied

Soil and dung samples collected in different geographical regions (Mexico and Spain) were studied using the wood baiting technique, moist chambers and dilution plating method according to Calduch et al. (2004). Using the first two techniques, we found three interesting dendryphiella-like fungi, which were isolated on Potato Dextrose Agar (PDA; Pronadisa, Madrid Spain) and incubated at room temperature in the dark. Additionally, six strains from the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands (CBS), which corresponded to *D. vinosa* (CBS 117.14, CBS 118716, CBS 121797 and CBS 584.96), *D. infuscans* (CBS 381.81) and *Di. asperum* (CBS 139.95) were included in the study for morphological and sequence comparison (Table 1).

DNA extraction, sequencing and phylogenetic analysis

The isolates were cultured on PDA for 7 days at 25 °C in darkness. The DNA was extracted through the modified protocol of Werner et al. (1998). The primer pairs ITS5/ITS4 and NL1/NL4b were used to amplify ITS regions, including the 5.8S gene and the D1/D2 domain of the LSU of the nrDNA, respectively, following Cano et al. (2004). PCR products were purified and stored at -20 °C until sequencing. The same pairs of primers were used to obtain the sequences at Macrogen Europe (Macrogen Inc. Amsterdam, The Netherlands). Finally, the sequences were assembled and edited using SeqMan v. 7.0.0 (DNAStar Lasergene, Madison, WI, USA) to obtain the consensus sequences.

The sequences generated in the present study were compared with those of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Alignments for each locus were made with the MEGA (Molecular Evolutionary Genetics Analysis) software v. 6.0. (Tamura et al. 2013), using the ClustalW algorithm (Thompson et al. 1994) and refined with MUSCLE (Edgar 2004) or manually, if necessary, on the same platform. The alignment included our sequences complemented with available sequences of NCBI and NITE Biological Resource Center (NBRC) of species that conformed the different genera of the family Dictyosporiaceae (Table 1). This determined the phylogenetic position of the dendryphiella-like isolates in this group of fungi. Phylogenetic reconstructions with ITS and LSU sequences were made using Maximum Likelihood (ML) and Bayesian Inference (BI) approaches under the MEGA software v. 6.0. (Tamura et al. 2013) and MrBayes v. 3.2.6 (Ronquist et al. 2012), respectively.

For the ML phylogenetic analysis of the LSU region, the best nucleotide substitution model determined by the same programme was the Kimura 2-parameter

Table 1. Species included in this study, their origin and GenBank accession numbers.

Species	Original identification	Strain number[1]	Country	Genbank accession no.[2]	
				ITS	LSU
Aquaticheirospora lignicola		RK-2006a (T)	Thailand	AY864770	AY736378
Cheirosporium triseriale		HMAS 180703 (T)	China	EU413953	EU413954
Drechslera biseptata	***Dendryphiella vinosa***	**CBS 117.14**	**Scotland**	**LT963770**	**LT963509**
Dendryphiella eucalyptorum		CBS 137987 (T)	Spain	KJ869139	KJ869196
Dendryphiella fasciculata		MFLUCC 17-1074 (T)	Thailand	MF399213	MF399214
Dendryphiella paravinosa	***Dendryphiella vinosa***	**CBS 118716**	**New Zealand**	**LT963357**	**LT963359**
Dendryphiella paravinosa	***Dendryphiella vinosa***	**CBS 121797**	**Spain**	**LT963354**	**LT963355**
Dendryphiella paravinosa		CBS 141286 (T)	Italy	KX228257	KX228309
Dendryphiella variabilis	***Dendryphiella vinosa***	**CBS 584.96 (T)**	**Cuba**	**LT963453**	**LT963454**
Dendryphiella vinosa		NBRC 32669	Japan	DQ307316	03266901[3]
Dendryphiella vinosa		–	–	–	EU848590
Dictyocheirospora bannica		KH 332 (T)	Japan	LC014543	AB807513
Dictyocheirospora pseudomusae		KH 412	Japan	LC014549	AB807516
Dictyocheirospora rotunda		MFLUCC 14-0293b (T)	Thailand	KU179099	KU179100
Dictyosporium bulbosum		yone 221	Japan	LC014544	AB807511
Dictyosporium elegans		NBRC 32502 (T)	Japan	DQ018087	DQ018100
Dictyosporium strelitziae		CBS 123359 (T)	South Africa	FJ839618	FJ839653
Digitodesmium bambusicola		CBS 110279 (T)	Philippines	DQ018091	DQ018103
Gregarithecium curvisporum		KT 922 (T)	Japan	AB809644	AB807547
Jalapriya inflata		NTOU 3855	UK	JQ267362	JQ267363
Jalapriya pulchra		MFLUCC 15-0348 (T)	China	KU179108	KU179109
Jalapriya toruloides		CBS 209.65	–	DQ018093	DQ018104
Neodendryphiella mali	***Diplococcium asperum***	**CBS 139.95 (T)**	**Italy**	**LT906655**	**LT906657**
Neodendryphiella mali	***Dendryphiella* sp.**	**FMR 17003**	**Spain**	**LT993734**	**LT993735**
Neodendryphiella michoacanensis	***Dendryphiella* sp.**	**FMR 16098 (T)**	**Mexico**	**LT906660**	**LT906658**
Neodendryphiella tarraconensis	***Dendryphiella* sp.**	**FMR 16234 (T)**	**Spain**	**LT906659**	**LT906656**
Paradendryphiella arenaria		CBS 181.58 (T)	France	KF156010	KC793338
Paradendryphiella salina		CBS 142.60	United Kingdom	DQ411540	KC793339
Pseudocoleophoma calamagrostidis		KT 3284 (T)	Japan	LC014592	LC014609
Pseudocoleophoma polygonicola		KT 731 (T)	Japan	AB809634	AB807546
Pseudodictyosporium elegans		CBS 688.93 (T)	Taiwan	DQ018099	DQ018106
Pseudodictyosporium wauense		NBRC 30078	Japan	DQ018098	DQ018105
Torula herbarum	***Dendryphiella infuscans***	**CBS 381.81**	**Netherlands**	**LT963446**	**LT963455**

[1]CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; FMR: Facultat de Medicina, Universitat Rovira i Virgili, Reus, Spain; HMAS: The Mycological Herbarium of the Chinese Academy of Science; KH: K. Hirayama; KT: K. Tanaka; MFLUCC: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; NBRC: NITE Biological Resource Centre, Japan; NTOU: Institute of Marine Biology, National Taiwan Ocean University; RK: R. Kodsueb; yone: H. Yonezawa. (T): ex-type strain.

[2]Sequences newly generated in this study are indicated in bold.

[3]Number of sequence of the NBRC database.

with Gamma distribution and, for the ITS region, it was the General Time Reversible model with Gamma distribution. The combined analysis of these two phylogenetic markers was tested through Incongruence Length Difference (ILD) implemented in the Winclada programme (Farris et al. 1994). For the combined analysis of LSU and ITS sequences, the best nucleotide substitution model was the General Time Reversible with Gamma distribution and Invariant sites (G+I). ML bootstrap values (BML) ≥70% were considered significant.

For the BI phylogenetic analysis, the best nucleotide substitution model was determined using jModelTest (Posada 2008). For the LSU region, we used the Kimura 2-parameter with Gamma distribution (K80+G) and, for the ITS symmetrical model, we used Gamma distribution (SYM+G). The parameter settings used were two simultaneous runs of 5M generations, four Markov chains, sampled every 1000 generations. The 50% majority-rule consensus tree and posterior probability values (PP) were calculated after discarding the first 25% of the samples. A PP value of ≥0.95 was considered significant.

The DNA sequences and alignments generated in this study were deposited in GenBank (Table 1) and in TreeBASE (http://treebase.org), respectively.

Phenotypic study

The microscopic characterisation of the fungi studied was carried out according to Marin-Felix et al. (2017), using autoclaved pine twig arranged on the surface of water agar (PNA) after 7 days at 25 °C in darkness. Measurements and descriptions of the structures were taken from the specimens mounted in Shear's solution. Photomicrographs were obtained using a Zeiss Axio-Imager M1 light microscope (Zeiss, Oberkochen, Germany) with a DeltaPix Infinity × digital camera.

Macroscopic characterisation of the colonies was made on PDA, Oatmeal Agar (OA; Oatmeal 30 g, agar 13 g, distilled water 1 l), Potato Carrot Agar (PCA; potato 20 g, carrot 20 g, agar 13 g, distilled water 1 l), SNA (KH_2PO_4 1 g, KNO 1 g, $MgSO_4 \times 7H_2O$ 0.5 g, KCl 0.5 g, Glucose 0.2 g, Sucrose 0.2 g, agar 14 g, distilled water 1 l) and Malt Extract Agar (MEA; Peptone 1 g, Glucose 20 g, Malt Extract 20 g, agar 15 g, distilled water 1 l) after 14 days at 25 °C in darkness. Colony colours in descriptions were matched with Kornerup and Wanscher (1978). Cardinal temperatures for growth were obtained on PDA after 14 days in darkness.

Nomenclatural novelties and descriptions were deposited in MycoBank (Crous et al. 2004). Ex-type cultures and holotypes, which consisted of dried cultures, were deposited at the CBS. Additionally, living cultures of the new species were also preserved in the Faculty of Medicine in Reus (FMR, Spain).

Results

The BLAST query revealed that LSU sequences of our dendryphiella-like isolates (FMR 16098, FMR 16234 and FMR 17003) showed a high percentage of identity (99%) with that of the isolate CBS 139.95 of *Di. asperum* and all of them were related to the Dictyosporiaceae. However, they showed a sequence identity of between 96-97% with LSU sequences of *Dictyosporium* species and other members of this family, including several species of *Dendryphiella* deposited in the GenBank. The ITS sequences did not match significantly any of those deposited in the NCBI database.

We carried out individual and combined analyses with the LSU and ITS loci to assess relationships with members of the Dictyosporiaceae, including reference strains of *D. vinosa* and *D. infuscans* sequenced in the present study. Single phylogenies of LSU and ITS loci encompassed 31 and 30 sequences, respectively, representing 12 genera and including *Paradendryphiella arenaria* and *P. salina* (Pleosporaceae) as outgroup (Figs. S1 and S2 in the supplementary material). LSU analysis comprised 630 bp from which 111 bp were variable and 84 bp phylogenetically informative. The ITS comprised 496 bp, 266 bp being variable and 206 bp being phylogenetically informative. The topology of trees for single loci were very similar and the ILD test showed that the LSU and ITS datasets loci were congruent (P = 0.16) and could be combined. The final combined analysis encompassed 30 sequences and comprised 1126 bp (ITS 496 bp, LSU 630 bp). The ML tree showed that FMR 16098, FMR 16234, FMR 17003 and CBS 139.95 clustered together in a well-supported undescribed monophyletic lineage representing a new genus in the family (Fig. 1). The LSU and ITS sequence comparison of the four isolates revealed them as different taxa. The low identity values together with the morphological differences found amongst them allow us to propose three new species in this new genus, which are described below.

Regarding the five *Dendryphiella* strains included in this study, only three (CBS 118716, CBS 121797 and CBS 854.96) nested in the well-supported clade of *Dendryphiella* and none of them matched sequences representative of the type species of the genus *D. vinosa* (DQ 307316.1, EU848590.1 and NBRC-03266901) and used previously by other authors to establish the relationship of *D. vinosa* with the Dictyosporiaceae (Gareth Jones et al. 2008, Crous et al. 2014, 2016, Tanaka et al. 2015, Boonmee et al. 2016, Liu et al. 2017). The strains CBS 118716 and CBS 121797 matched the ex-type strain of *D. paravinosa* (CBS 141286); while CBS 584.96 nested in a terminal subclade with *D. fasciculata* and *D. paravinosa*, but it was placed in a single branch representative of a distinct taxa (Fig. 1). Its genetic difference and the production of conidia with up to 7 septa, a distinct morphological feature with respect to the accepted species of *Dendryphiella* (Liu et al. 2017, Hyde et al. 2018), justify the proposal of a new species in this genus. The other two isolates that had been received as *Dendryphiella* did not belong to this genus. The oldest reference strain of *D. vinosa* (CBS 117.14) corresponded to *Drechslera biseptata* and the strain previously identified as *D. infuscans* (CBS 381.81) matched *Torula herbarum*. The molecular identification of all the isolates included in this study is provided in Table 1.

Taxonomy

Neodendryphiella **Iturrieta-González, Dania García & Gené, gen. nov.**
MycoBank: MB824664

Etymology. The name refers to the morphological similarity with *Dendryphiella*.

Type species. *Neodendryphiella tarraconensis* Iturrieta-González, Gené & Dania García.

Description. *Conidiophores* semi-macronematous to macronematous, mononematous, erect or slightly flexuous, unbranched or branched towards the apical region, septate, subhyaline to brown, smooth to verrucose, cylindrical, some slightly swollen in the conidiogenous loci. *Conidiogenous cells* integrated, terminal or intercalary, polytretic, cylindrical or clavate, forming conidia in acropetal branched chains. *Ramoconidia* aseptate or septate, pale brown, smooth to verruculose, mostly cylindrical or subcylindrical, rounded apex and truncate base, with several pores and conidial scars often thickened and darkened. *Conidia* blastocatenate, aseptate or septate, pale brown, verruculose to verrucose, ellipsoidal, doliiform, clavate or subcylindrical, with scars thickened and darkened. *Sexual morph* not observed.

Distribution. Italy, Mexico and Spain.

Neodendryphiella mali **Iturrieta-González, Gené & Dania García, sp. nov.**
MycoBank: MB824665
Fig. 2

Etymology. Name refers to the substrate, *Malus domestica,* where the type strain of the species was collected.

Type. Italy, Dipt. Prot. Valor. Agroalimentare, from leaf of *Malus domestica*, Feb. 1995, A. Cesari (holotype CBS H-23477, culture ex-type CBS 139.95).

Description. *Mycelium* superficial and immersed, composed of septate, branched, smooth to verruculose, hyaline to pale brown hyphae of 1–3 µm wide. *Conidiophores* semi-macronematous to macronematous, mononematous, erect or slightly flexuous, branched or unbranched, up to 11-septate, cylindrical, up to 385 µm long, 3–4 µm wide, brown, usually darker toward the base, smooth to verrucose. *Conidiogenous cells* terminal and intercalary, mostly cylindrical, 8–38 × 3–4(–5) µm, with 1–4 pores. *Ramoconidia* 0–1-septate, with up to 3 terminal and lateral pores, pale brown, smooth to verruculose, mostly cylindrical, (11–)15–17(–21) × 3–4 µm. *Conidia* catenate, with up to 10 conidia in the terminal unbranched part, (0–)1-septate, usually not constricted at the septum, pale brown, verruculose to verrucose, ellipsoidal, doliiform or subcylindrical with more or less rounded ends, 4–15 × 3–5 µm.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 22 mm diam., convex, slightly convoluted at the centre, pastel grey to white (1C1/1A1), aerial mycelium scarce, with slightly fimbriate margin; reverse olive brown to yellowish-brown

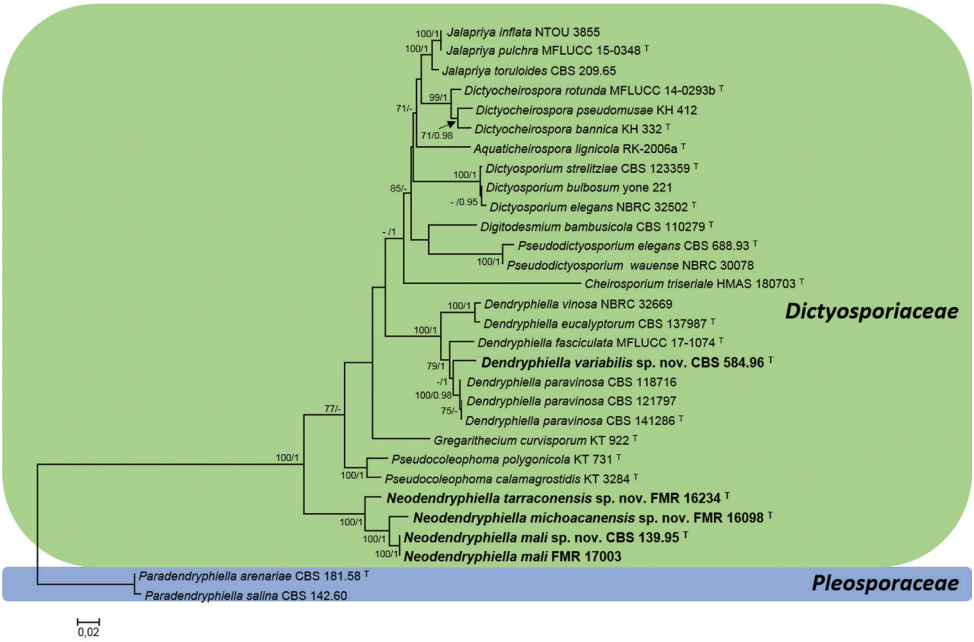

Figure 1. Maximum Likelihood (ML) tree constructed with the ITS and LSU sequences of 30 strains representatives of different taxa in the families Dictyosporiaceae and Pleosporaceae. The phylogenetic tree was rooted with *Paradendryphiella arenaria* and *P. salina*. Bootstrap support values for ML greater than 70% and Bayesian posterior probabilities greater than 0.95 are given near nodes, respectively. Names of species newly described here are indicated in bold. Branch lengths are proportional to distance. [T] Ex-type strain.

(4D3/3A2). On PCA attaining 23 mm diam., flat, olive brown to greyish-beige (4F8/4C2), aerial mycelium scarce, slightly fimbriate margin; reverse greyish-beige to brownish-grey (4C2/4D2). On OA reaching 40 mm diam., flat, granular, yellowish-brown to reddish-yellow (5E8/4B7), aerial mycelium scarce, with a regular margin; reverse olive brown to yellowish-brown (4D8/4B7). On SNA attaining 24 mm diam., flat, slightly granular, olive brown to grey (4F8/4B1), aerial mycelium scarce, with fimbriate margin; reverse yellowish-brown (5F7/5E4). On MEA reaching 11–15 mm diam., umbonate, slightly cerebriform towards the periphery, velvety, olive grey (3E2), with irregular margin; reverse olive grey (3E2).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 10 °C.

Distribution. Italy and Spain.

Additional isolates examined. Spain, Els Ports de Beseit Natural Park, Teruel, from herbivore dung, Oct. 2017, Dania García (FMR 17003)

Notes. Although LSU sequences of *N. mali* (CBS 139.95 and FMR 17003) were very similar to those of *N. michoacanensis* (FMR 16098) and *N. tarraconensis* (FMR 16234), ITS regions showed a similarity of 96.2% (identities = 441/458, gaps 2/458 (0 %)) with respect to *N. michoacanensis* and of 92.3% (identities = 423/458, gaps 1/458

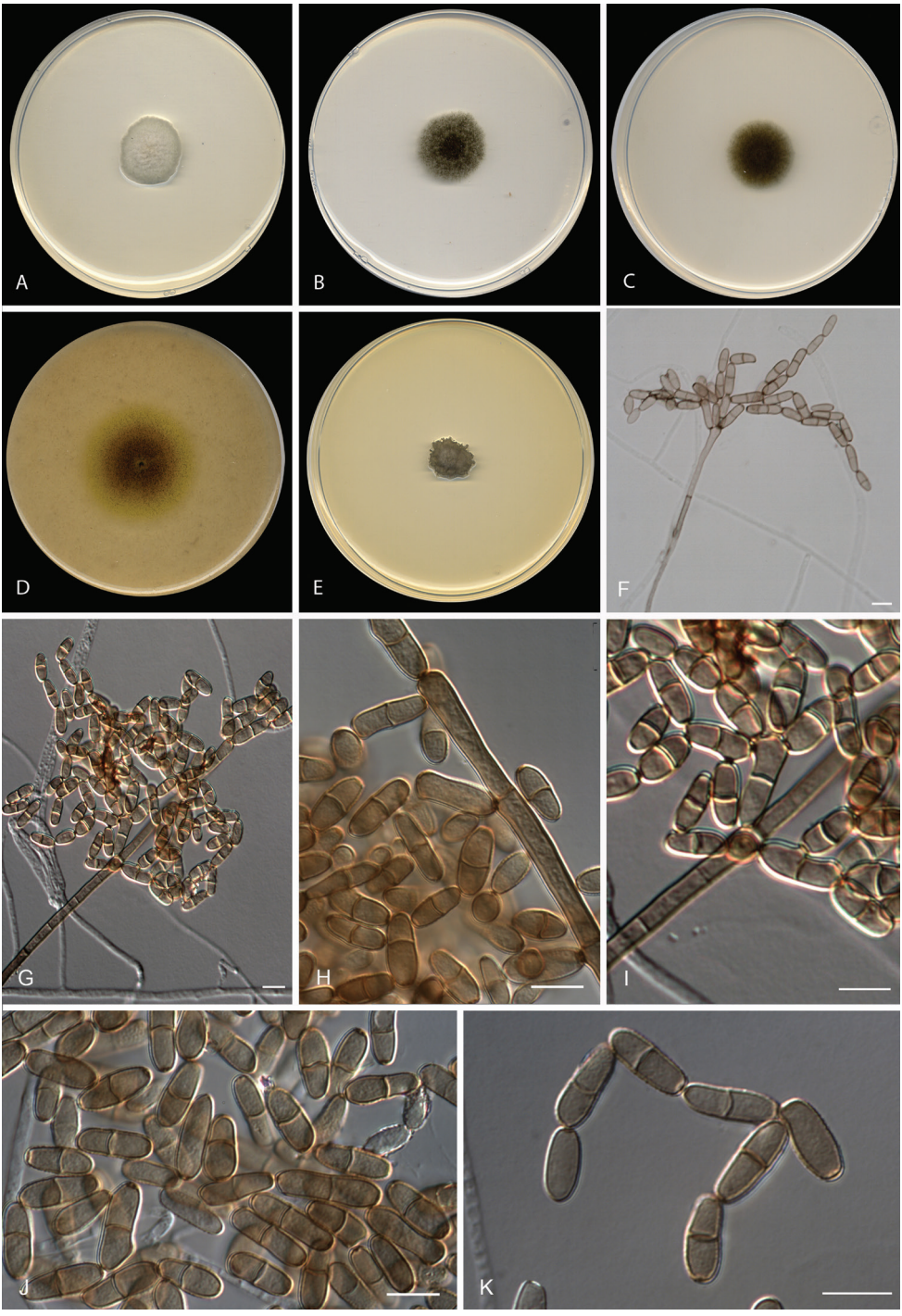

Figure 2. *Neodendryphiella mali* sp. nov. (ex-type CBS 139.95). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F–K** Conidiophores and conidia. Scale bars: 10 µm (**F–K**).

(0%)) with respect to *N. tarraconensis*. ITS sequences of the two latter species described below were 92.1% similar (identities = 422/458, gaps 0/458 (0%)).

Neodendryphilla mali is morphologically very similar to *N. michoacanensis* since both have conidia and ramoconidia 0–1-septate; however, *N. michoacanensis* has shorter conidiophores (up to 280 µm long) and terminal conidial branches with fewer conidia (up to 4 per branch), which measure 5–16(–18) × 3–6 µm. In addition, 2-septate conidia can also be present in *N. michoacanensis* and this species tends to grow faster than *N. mali* on PDA (34 mm vs 22 mm diam. after 14 d, respectively) and PCA (42 mm vs 23 mm diam. after 14 d, respectively). *Neodendryphiella mali* also resembles *D. infuscans,* but the latter exhibits longer conidiophores, up to 500 µm and smooth to minutely verruculose conidia with up to 2 septa (Ellis 1971). However, the protologue of *D. infuscans* (as *Cladosporium infuscans*; Thümen 1879), which was based on a specimen collected in Aiken (USA), describes conidia 0–1-septate, smooth-walled and up to 10 µm long. No living culture of the type specimen was preserved for further comparison.

As mentioned before, the strain CBS 139.95 was identified as *Di. asperum* and found by other authors to be related with dictyosporium-like fungi (Shenoy et al. 2010, Tanaka et al. 2015). However, the protologue of *Di. asperum* was characterised by single or fasciculate conidiophores, which were up to 250 µm long, bearing terminal or subterminal, short and unbranched chains of conidia with only 1 septum (Pirozynski 1972), morphological features that do not fit with those observed in the abovementioned strain. We therefore concluded that it was a misidentified strain and clearly represents a different species. At any rate, it is of note that the taxonomy of *Di. asperum* is controversial because of the different interpretation of the morphological features of Pirozynski's specimen (DAOM 133941c isotype). Holubová-Jechová (1982) described conidiogenous cells showing inconspicuous denticles or conidiogenous scars instead of the typical pores in conidiogenous cells of *Diplococcium* and suggested excluding this species from the genus. On the other hand, Goh and Hyde (1998) re-examined the isotype of *Di. asperum* and observed the typical pores of tretic conidiogenesis, considering it an acceptable species for *Diplococcium*. However, since only herbarium material is preserved for comparison (Pirozynski 1972), its phylogeny remains uncertain.

Neodendryphiella michoacanensis **Iturrieta-González, Dania García & Gené, sp. nov.**
MycoBank: MB824666
Fig. 3

Etymology. Name refers to Michoacán, the geographical area where the fungus was collected.

Type. Mexico, Michoacán, Villa Jiménez, from soil, Sept. 2016, E. Rodriguez-Andrade (holotype CBS H-23478; culture ex-type CBS 144323 = FMR 16098).

Description. *Mycelium* superficial and immersed, composed of septate, branched, smooth to verruculose and hyaline to pale brown hyphae of 1–3 µm wide. *Conidi-*

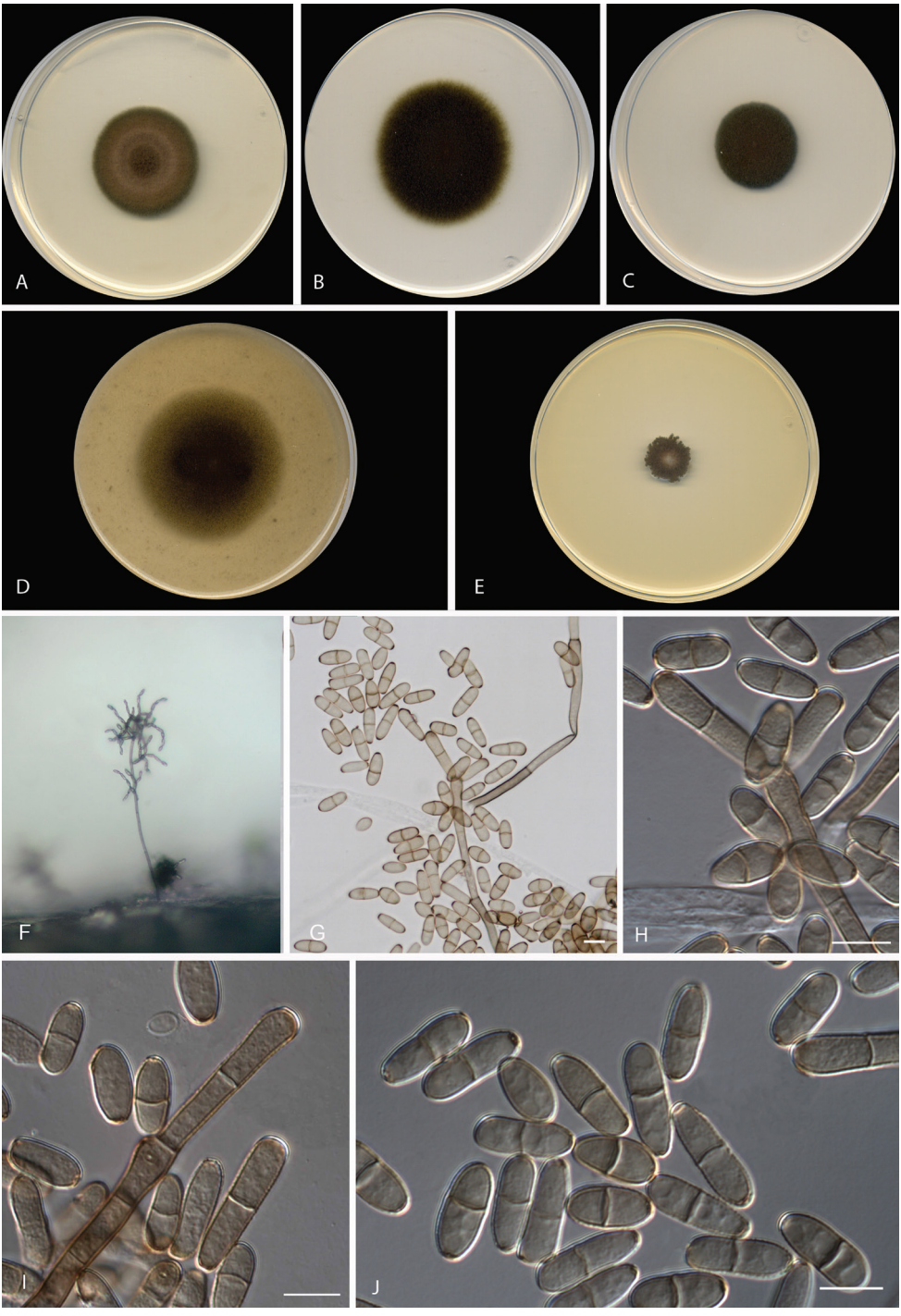

Figure 3. *Neodendryphiella michoacanensis* sp. nov. (ex-type FMR 16098). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F–J** Conidiophores and conidia. Scale bars: 10 µm (**G–J**).

ophores semi-macronematous to macronematous, mononematous, erect or slightly flexuous, slightly branched, 1–13 septate, cylindrical or slightly swollen in the conidiogenous loci, 44–280 × 2–4 µm, brown, usually darker toward the base, smooth or verruculose, verrucose at the base. *Conidiogenous cells* terminal and intercalary, cylindrical or clavate, 11–62 × 3–5 µm, with up to 3 pores. *Ramoconidia* (0–)1-septate, with up to 4 terminal or subterminal pores, pale brown, smooth to verruculose, cylindrical, subcylindrical, to slightly clavate, with more or less rounded apex and truncate base, 12–23 × 3–4(–5) µm. *Conidia* catenate, with up to 4 conidia in the terminal unbranched part, (0–)1(–2)-septate, some slightly constricted at the septum, pale brown, verruculose to verrucose, ellipsoidal or subcylindrical, 5–16(–18) × 3–6 µm.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 34 mm diam., slightly umbonate, velvety, olive brown (4F6/4E8), with slightly fimbriate margin; reverse dark green (30F8) to black. On PCA attaining 42 mm diam., flat, granular, olive brown (4F8), aerial mycelium scarce, fimbriate margin; reverse dark green to olive brown (30F8/4F8). On OA reaching 48 mm diam., flat, granular, yellowish-brown to olive (5F4/3D4), aerial mycelium scarce, with a regular margin; reverse brownish-grey to greyish-yellow (4D2/3B6). On SNA attaining 22 mm diam., flat, slightly granular, olive brown (4F8), aerial mycelium scarce, with slightly fimbriate margin; reverse dark green (30F8) to black. On MEA reaching 13–15 mm diam., slightly umbonate, flat towards the periphery, velvety, yellowish-grey to olive (3C2/3F8), with white irregular margin; reverse olive grey to dark green (3E2/30F8).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 10 °C.

Distribution. México.

Notes. *Neodendryphiella michoacanensis* morphologically resembles *N. mali*, in its conidiogenous apparatus with 0–1-septate ramoconidia, but the latter differs by having longer conidiophores (up to 385 µm), terminal conidial chains with up to 10 conidia and its conidia are 0–1-septate and smaller (4–15 × 3–5 µm). *Neodendryphiella michoacanensis* also resembles *D. uniseptata* in their conidial morphology, but ramoconidia of the latter species are often aseptate and can be up to 30 µm long (Matsushima 1971). *Dendryphiella uniseptata* is only known from the type material, which was collected in Honiara (Japan) and no ex-type culture was preserved. This species was considered a synonym of *D. infuscans* by Matsushima (1975) but not accepted by Liu et al. (2017).

Neodendryphiella tarraconensis **Iturrieta-González, Gené & Dania García, sp. nov.**
MycoBank: MB824667
Fig. 4

Etymology. Name refers to Tarragona, the geographical area where the fungus was collected.

Type. Spain, Tarragona, from garden soil, Feb. 2017, I. Iturrieta-González (holotype CBS H-23479, culture ex-type CBS 144324 = FMR 16234).

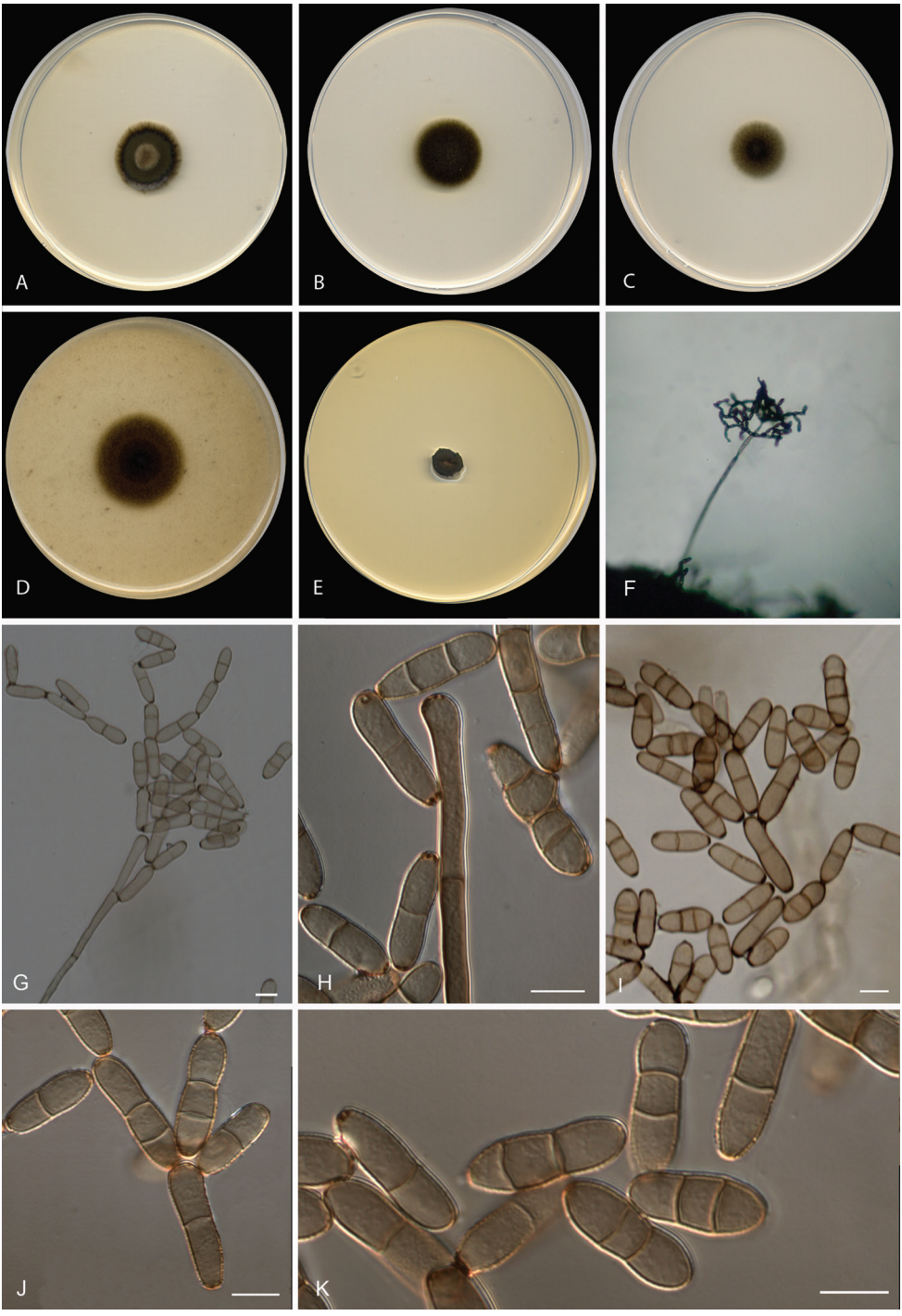

Figure 4. *Neodendryphiella tarraconensis* sp. nov. (ex-type FMR 16234). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F–K** Conidiophores and conidia. Scale bars:10 µm (**G–K**).

Description. *Mycelium* superficial and immersed abundant, composed of septate, branched, smooth to verruculose, hyaline to pale brown hyphae, 1–2 µm wide. *Conidiophores* macronematous, mononematous, erect or slightly flexuous, branched or unbranched, up to 6-septate, cylindrical, 19–185 × 2–5 µm, brown, smooth, darker and finely verrucolose towards the base. *Conidiogenous cells* terminal and intercalary, subcylindrical to clavate, 9–35 × (2–)3–4(–5) µm, with up to 2 pores. *Ramoconidia* (0–)1–2(–3)-septate, usually slightly constricted at the septa, with up to 3 terminal and subterminal pores, pale brown, smooth to verruculose, mostly cylindrical, with rounded apex and truncate base, 12–21(–23) × 4–5 µm. *Conidia* catenate, with up to 7 conidia in the terminal unbranched part, (0–)1–2-septate, pale brown, verruculose, ellipsoidal or subcylindrical with more or less rounded ends, 6–21 × 3–6(–7) µm; when 1-septate, the septum is often submedial and slightly constricted, when 2-septate, usually constricted at only one septum.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 23 mm diam., umbonate, velvety, greyish-brown to olive brown (5E3/4F8), with slightly fimbriate margin; reverse dark green (30F8) to black. On PCA attaining 24 mm diam., flat, velvety, olive brown (4F8), slightly fimbriate margin, reverse dark green to olive brown (28F5/3B2) with a pale yellow (4A3) diffusible pigment. On OA reaching 30 mm diam., flat, slightly granular, yellowish-brown to olive brown (5F8/4F4), aerial mycelium scarce, with regular margin; reverse yellowish-brown to olive brown (5F8/4F4). On SNA attaining 21 mm diam., flat, slightly granular, yellowish-brown to olive (5F4/3F5), aerial mycelium scarce, with fimbriate margin; reverse yellowish-brown to olive (5F4/3F5). On MEA reaching 8-10 mm diam., slightly elevated but depressed at the centre, radially folded, velvety, olive (2F8), with irregular margin; reverse olive (2F4).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 10 °C.

Distribution. Spain.

Notes. In addition to the genetic differences mentioned above, *N. tarraconensis* differs from the other two species in the genus by the presence of ramoconidia with up to 3 septa and conidia from terminal branches with mostly 1–2-septate. It is noteworthy that 1-septate conidia usually show a slightly longer basal cell since the septum is submedial and, when 2-septate, often only one of the septa is constricted, features not described in any species of *Dendryphiella* and *Neodendryphiella*.

Dendryphiella variabilis **Iturrieta-González, Dania García & Gené, sp. nov.**
MycoBank: MB824668
Fig. 5

Etymology. Name refers to the variable number of septa in the conidia.

Type. Cuba, from a dead leaf of a Lauraceous tree, 1996, R.F. Castañeda (holotype CBS H-23476; ex-type cultures CBS 584.96 = INIFAT C95/105-4 = MUCL 39840 = FMR 16563).

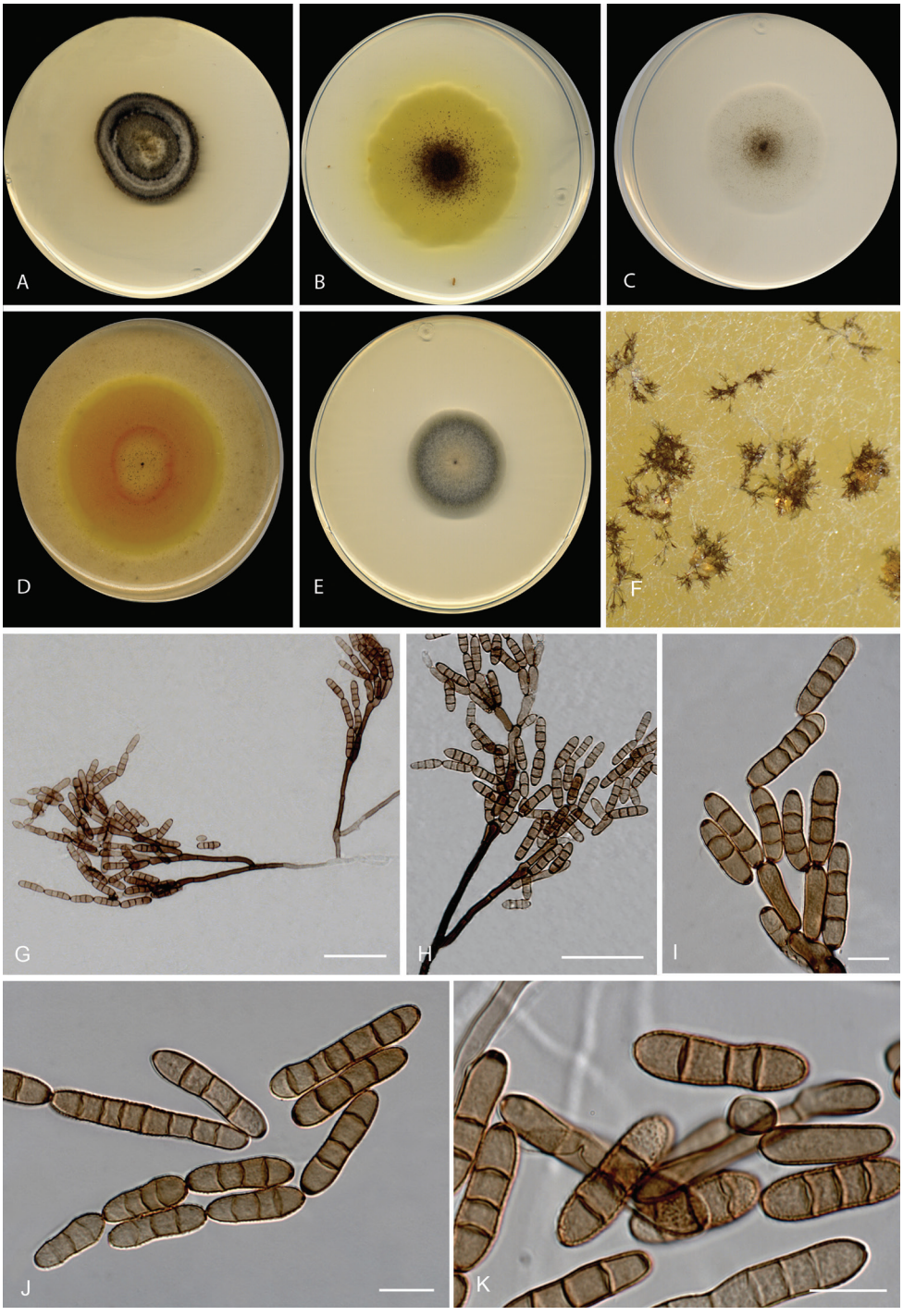

Figure 5. *Dendryphiella variabilis* sp. nov. (ex-type CBS 584.96). **A–E** Colonies on **A** PDA **B** PCA **C** SNA **D** OA **E** MEA at 25 °C after 14 d **F** Exudates and conidiophores produced on OA **G–K** Conidiophores and conidia. Scale bars: 50 µm (**G–H**), 10 µm (**I–K**).

Description. *Mycelium* superficial and immersed, composed of septate, branched, smooth to verruculose hyaline to pale brown hyphae, 1–3 µm wide. *Conidiophores* macronematous, mononematous, often arranged in loose fascicules, erect or slightly flexuous, branched, 1–8-septate, nodulose toward the apex, up to 143 µm long, 2–6 µm wide, brown, smooth to verruculose. *Conidiogenous cells* terminal and intercalary, sympodially extended towards the apex, with 1–5 pores surrounded by a thickened and darkened wall, clavate, 7–37 × 3–6(–7) µm. *Ramoconidia* (0–)2–3-septate, cylindrical to subcylindrical, with rounded ends, 16–27 × 5–6 µm, usually with 2 apical pores, conidial scars thickened and darkened. *Conidia* in short branched chains, with up to 5 conidia in the terminal unbranched part, (0–)3(–7)-septate, some constricted at the medial septum, pale brown, verruculose to verrucose, cylindrical or subcylindrical, with rounded ends, 6–44 × 4–6 µm, conidial scars often thickened and darkened. *Sexual morph* not observed.

Culture characteristics (14 d at 25 °C). Colonies on PDA reaching 30–33 mm diam., slightly umbonate, flat towards the periphery, velvety, irregularly coloured yellowish-grey to olive brown (4B2/4D3) and brownish-grey to yellowish-brown (5F2/5F4), with irregular margin; reverse yellowish-brown (5F8) to black. On PCA attaining 48 mm diam., flat, granular to velvety, yellowish-brown (5F8), aerial mycelium scarce, undulate margin; reverse olive to greyish-yellow (3F4/3B4), with a pale yellow diffusible pigment. On OA reaching 58 mm diam., flat, slightly granular, blond to reddish-yellow (5C4/4A7), light yellow (4A4) at the periphery, aerial mycelium scarce, with a regular margin, with scarce pale brown exudate; reverse same colouration with the colony surface. On SNA attaining 40 mm diam., flat, slightly granular to velvety, yellowish-brown to grey (5F7/4B1), with fimbriate margin; reverse brownish-grey to white (5D2/1A1). On MEA reaching 32 mm diam., flat, cottony, yellowish-grey to olive (4B2/3F4), yellowish-grey (3B2) at the periphery, with regular margin; reverse dark green to white (30F8/1A1).

Cardinal temperature for growth. Optimum 25 °C, maximum 30 °C, minimum 15 °C.

Distribution. Cuba.

Notes. *Dendryphiella variabilis* differs from *D. paravinosa* mainly by having longer conidia (up to 44 µm), which can have up to 7 septa. The conidia of *D. paravinosa* are up to 3-septate and measure (10–)24–27(–33) × (6–)7(–7.5) µm (Crous et al. 2016). The only species of the genus reported with conidia up to 5-septate are *D. eucalyptorum* and *D. vinosa*, but they are smaller, measuring (19–)20–23(–25) × 5(–7) µm in the former (Crous et al. 2014) and 13–39 × 4–8 µm in the latter (Ellis 1971). The other closely related species to *D. variabilis* is *D. fasciculata* (Fig. 1), but it mainly differs by the presence of fasciculate conidiophores and 3-septate conidia (Liu et al. 2017).

Discussion

The present study proposes the genus *Neodendryphiella* based on the analysis of the ITS and LSU sequences, which represented an undescribed monophyletic lineage related but phylogenetically distant from the morphologically similar genus *Dendryphiella*.

Both genera belong to the Dictyosporiaceae (Dothideomycetes) and share similar conidiophore morphology with polytretic conidiogenous cells forming usually septate conidia arranged in acropetal branched chains. *Dendryphiella* can be differentiated by the presence of nodulose conidiophores and conidiogeneous cells with pores surrounded by a thickened and darkened wall, which are absent in *Neodrendryphiella*. Other genera of the Dothideomycetes, although accommodated in different orders or families with a similar conidiogenous apparatus are *Dendryphion* (Toluraceae, Pleosporales) (Crous et al. 2014, Crous et al. 2015), *Dendryphiopsis* (Kirschsteiniotheliaceae, Kirschsteiniotheliales) (Su et al. 2016, Hernández-Restrepo et al. 2017) and *Paradendryphiella* (Pleosporaceae, Pleosporales) (Woudenberg et al. 2013). However, the genus *Diplococcium* in Leotiomycetes also shows similar asexual propagules (Shenoy et al. 2010, Hernández-Restrepo et al. 2017), which complicates the classification of these fungi based exclusively on morphological features.

Our phylogenetic study not only allowed us to distinguish very similar isolates in three distinct species, *N. mali, N. michoacanensis* and *N. tarraconensis*, but also helped us to correctly identify some strains that had previously been attributed to *Dendryphiella* (Table 1). In addition, it is of note that, considering the species accepted in *Dendryphiella* (Liu et al. 2017, Hyde et al. 2018), this genus seems to be morphologically heterogeneous and probably polyphyletic. It includes species with apparently polyblastic denticulate conidiogenous cells, such as *D. eucalypti* (Matsushima, 1983) or *D. uniseptata* (Matsushima, 1971), rather than polytretic conidiogenous cells typical of *Dendryphiella* (Rao and Narania 1974, Crous et al. 2014, 2016) or species that produce solitary conidia, such as *D. cruzalmensis* (Batista, 1946) or *D. lycopersicifolia* (Batista & Peres, 1961). In this scenario, therefore, *Dendryphiella* requires a further taxonomic re-evaluation. However, taking into account that only herbarium material is available for the type *D. vinosa* (preserved in the Kew herbarium, as *Helminthosporium vinosum*) there is a need to re-collect this species from the type locality (Cuba) for epitypification and giving nomenclature stability to the genus.

Acknowledgements

This study was supported by the Spanish Ministerio de Economía y Competitividad, Grant CGL2017-88094-P.

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Supplementary material 1

***Neodendryphiella* gen. nov. Tree LSU**

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Link: https://doi.org/10.3897/mycokeys.37.27275.suppl1

Supplementary material 2

***Neodendryphyella* gen. nov. Tree ITS**

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Link: https://doi.org/10.3897/mycokeys.37.27275.suppl2

MycoKeys 37: 39–56 (2018)
doi: 10.3897/mycokeys.37.26303
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Reclassification of *Parapterulicium* Corner (Pterulaceae, Agaricales), contributions to Lachnocladiaceae and Peniophoraceae (Russulales) and introduction of *Baltazaria* gen. nov.

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Academic editor: *M.P. Martín* | Received 14 May 2018 | Accepted 12 July 2018 | Published 31 July 2018

Citation: Leal-Dutra CA, Neves MA, Griffith GW, Reck MA, Clasen LA, Dentinger BTM (2018) Reclassification of *Parapterulicium* Corner (Pterulaceae, Agaricales), contributions to Lachnocladiaceae and Peniophoraceae (Russulales) and introduction of *Baltazaria* gen. nov.. MycoKeys 37: 39–56. https://doi.org/10.3897/mycokeys.37.26303

Abstract

The genus *Parapterulicium* was first introduced to accommodate two Brazilian species of coralloid fungi with affinities to Pterulaceae (Agaricales). Despite the coralloid habit and the presence of skeletal hyphae, other features, notably the presence of gloeocystidia, dichophyses and papillate hyphal ends, differentiate this genus from Pterulaceae *sensu stricto*. Fieldwork in Brazil resulted in the rediscovery of two coralloid fungi identifiable as *Parapterulicium*, the first verified collections of this genus since Corner's original work in the 1950s. Molecular phylogenetic analyses of nrITS and nrLSU sequences from these modern specimens revealed affinities with the /peniophorales clade in the Russulales, rather than Pterulaceae. The presence of distinctive hyphal elements, homologous to the defining features of /peniophorales, is consistent with the phylogenetic evidence and thus clearly distinguished *Parapterulicium* and its type species *P. subarbusculum* from Pterulaceae, placing this genus within /peniophorales. *Parapterulicium* was also found to be polyphyletic so *Baltazaria* gen. nov. is proposed to accommodate *P. octopodites*, *Scytinostroma galactinum*, *S. neogalactinum* and *S. eurasiaticogalactinum* also within /peniophorales.

Keywords

Molecular Phylogeny, Taxonomy, Russulales, /peniophorales, Corticioid fungi, Coralloid fungi

Introduction

Pterulaceae Corner is a diverse but poorly known family of mostly tropical coralloid fungi within order Agaricales Underw. (Dentinger and McLaughlin 2006), recognised mainly by their coralloid/filiform basidiomes with a dimitic hyphal structure (Corner 1952a, 1952b, 1957, 1970).

To date, only three of the five Pterulaceae genera have been included in molecular phylogenetic analyses, viz. *Pterula* Fr., *Deflexula* Corner and *Pterulicium* Corner (Dentinger and McLaughlin 2006, Dentinger et al. 2009). The remaining genera, *Parapterulicium* Corner and *Allantula* Corner, are known only from a few scant specimens collected by Corner as the basis of his taxonomic proposal; these are poorly preserved and impractical for molecular studies. *Allantula* (meaning 'sausage' in ancient Greek), characterised by decumbent, intercalary swellings resembling minute sausages, is known only from the type specimen (Corner 1952a) and has not been recollected despite several recent attempts at the type locality (Parque Nacional da Tijuca, Rio de Janeiro, Brazil) by the present authors. *Parapterulicium* was described for two coralloid species from Brazil that resembled Pterulaceae in their filiform statues and dimitic hyphae, but differed in the presence of gloeocystidia and dichophyses.

Corner (1952a) suggested some similarity of *Parapterulicium* to *Lachnocladium* Lév. based on the shared features of dichophyses and gloeocystidia combined with the lack of clamps. However, due to the small filiform basidiomes, branching pattern, colourless dimitic hyphae and corticioid patch, Corner referred the genus to Pterulaceae instead of Lachnocladiaceae. Corner's emphasis of skeletal hyphae as a synapomorphy for Pterulaceae has been shown previously to be incorrect with the reclassification of *Actinceps* Berk. & Broome (=*Dimorphocystis*) (Dentinger and McLaughlin 2006), although this feature remains a defining feature of Pterulaceae.

During recent field expeditions in four Brazilian states, two coralloid fungi morphologically assignable to *Parapterulicium* spp. were collected, providing fresh material for molecular phylogenetic analysis. Here we present results that show *Parapterulicium* is paraphyletic and evolutionarily related to Peniophoraceae Lotsy and Lachnocladiaceae D.A. Reid in the Russulales Kreisel ex P.M. Kirk, P.F. Cannon & J.C. David, rather than Pterulaceae in the Agaricales. We propose taxonomic changes precipitated by these results and provide a re-evaluation of distinctive morphological features, such as variations in skeletal hyphae that may be considered phylogenetically informative in light of this discovery.

Methods

Collections and morphological observations

The new collections of *Parapterulicium* are deposited at FLOR, INPA and RB. Herbarium acronyms follow Index Herbariorum (Thiers continuously updated). Macroscopic analyses were conducted following the traditional methods of Largent (1986).

Microscopic analyses were adapted from Largent et al. (1977) for pterulaceous fungi, where, instead of sectioning the basidiomes with a razor, part of the fungus was dissected with the aid of two small diameter needles. The dissections were mounted in 5% KOH, H_2O, Melzer's reagent, Congo red or 1% phloxine and then observed with an Olympus CX21 (Olympus, Tokyo, Japan) light microscope equipped with 10x, 40x and 100x objective lenses, the latter being used with immersion oil. Macro- and microscopic illustrations were based on pictures taken in the field with a Nikon D90 DSLR camera (Nikon, Tokyo, Japan) and on photos taken during microscopic observations. The colour codes follow the Munsell Soil Color Charts (Munsell 1975). Scanning electron microscopy (SEM) images were obtained using a Hitachi S-4700 field emission scanning electron microscope (Hitachi, Tokyo, Japan) and the air-dried specimens were directly stuck on the carbon tab on the stubs without any treatment. The stubs were coated with gold and platinum and examined and photographed at 5 kV.

DNA extraction, PCR amplification, cloning and sequencing

DNA was extracted from dried basidiomes by first grinding with a mortar and pestle in the presence of liquid nitrogen, followed by purification using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Partial sequences of the nuclear ribosomal internal transcribed spacers (nrITS) and nuclear ribosomal large subunit (nrLSU) were amplified by PCR using the primer pairs ITS8F-ITS6R (Dentinger et al. 2010) and LR0R-LR7 (Vilgalys and Hester 1990), respectively and following the cycling conditions in the original publications. PCR products were purified using 2 units of Exonuclease I (Thermo Fisher Scientifics) and 1 U FastAP Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientifics) per 1 µl of PCR product, incubated at 37 °C for 15 min, followed by denaturing at 85 °C for 15 min. The samples were then sent for Sanger sequencing at the IBERS Aberystwyth Translational Genomics Facility.

Sequences and chromatograms were checked, assembled and edited using GENEIOUS 10.0.2 (Kearse et al. 2012). Samples presenting indels were cloned using pGEM-T Easy Vector Systems (Promega) into Subcloning Efficiency DH5α Competent Cells (Invitrogen). Five clones from each PCR were then amplified and sequenced as above. The sequences generated in this study have been submitted to GenBank (Table 1).

Phylogenetic analysis

Prior to the inclusion in the datasets, the clones were aligned to generate one or two consensus sequences of each cloned species. Substitutions were replaced by the respective ambiguous code and, in the cases where indels were found, two different sequences were generated.

To assess the global phylogenetic position of *Parapterulicium* within Agaricomycetidae, a dataset containing the nrLSU sequences of 886 Agaricomycetidae taxa was created by adding the sequences generated in this study to the dataset of Moncalvo

Table 1. Species from clade /peniophorales and their GenBank accession numbers of ITS and nrLSU sequences. Newly generated sequences are shown in bold.

Taxa	Sample no.	Locality	GenBank Accession no.		Reference
			nrITS	nrLSU	
Asterostroma cervicolor	KHL9239	Puerto Rico	AF506408	AF506408	Larsson and Larsson (2003)
Asterostroma macrosporum	TMI 25697	Japan	NR119394	–	Suhara et al. (2010)
Asterostroma muscicola	TMI 25860	Japan	AB439551	AB439551	Suhara et al. (2010)
Baltazaria eurasiaticogalactina	CBS 666.84	France	–	AY293211	Binder et al. (2005)
Baltazaria galactina	NH4863	Sweden	AF506466	AF506466	Larsson and Larsson (2003)
Baltazaria neogalactina	CBS 758.86	France	–	–	Unpublished
Baltazaria octopodites	FLOR 56442	São Paulo – Brazil	**MH260024**	**MH260043**	This study
				MH260044	
				MH260045	
				MH260046	
Baltazaria octopodites	FLOR 56449	São Paulo – Brazil	**MH260025**	**MH260047**	This study
Baltazaria octopodites	FLOR 56460	Santa Catarina – Brazil	**MH260032**	**MH260050**	This study
Baltazaria octopodites	FLOR 63715	Paraná – Brazil	**MH260042**	**MH260060**	This study
Baltazaria octopodites	INPA 280140	Amazonas – Brazil	**MH260038**	**MH260056**	This study
			MH260039	**MH260057**	
			MH260040	**MH260058**	
			MH260041	**MH260059**	
Confertobasidium olivaceoalbum	FP90196	USA	AF511648	AF511648	Larsson and Larsson (2003)
Dendrophora albobadia	TDeAB1029	USA	AF119522	AF119522	Hsiau and Harrington (2003)
Dichostereum durum	FG1985	France	AF506429	AF506429	Larsson and Larsson (2003)
Dichostereum effuscatum	GG930915	France	AF506390	AF506390	Larsson and Larsson (2003)
Dichostereum granulosum	NH7137/696	Canada	AF506391	AF506391	Larsson and Larsson (2003)
Dichostereum pallescens	NH7046/673	Canada	AF506392	AF506392	Larsson and Larsson (2003)
Duportella lassa	SP6129	Russia	KJ509191	KJ509191	Spirin and Kout (2015)
Entomocorticium sp.	FL_19	USA	KJ620518	KJ620518	Bracewell and Six (2014)
Gloeocystidiopsis flammea	CBS 324.66	C. African Rep.	AF506437	AF506437	Larsson and Larsson (2003)
Gloiothele lamellosa	CBS404.83	Madagascar	AF506487	AF506487	Larsson and Larsson (2003)
Gloiothele torrendii	JB18615	France	AF506455	AF506455	Larsson and Larsson (2003)
Lachnocladiaceae	S1PMB7	Thailand	AB365531	AB365531	Osono et al. (2009)
Lachnocladiaceae	S335WS151	Thailand	AB365532	AB365532	Osono et al. (2009)

Taxa	Sample no.	Locality	GenBank Accession no.		Reference
			nrITS	nrLSU	
Lachnocladium cf. *brasiliense*	CALD 161213-1	Espírito Santo – Brazil	**MH260037**	**MH260055**	This study
Lachnocladium cf. *brasiliense*	KM 57848	Puerto Rico	**MH260034**	**MH260052**	This study
			MH260035	**MH260053**	
			MH260036	**MH260054**	
Lachnocladium schweinfurthianum	KM 49740	Cameroon	**MH260033**	**MH260051**	This study
Lachnocladium sp.	KHL10556	Jamaica	AF506461	AF506461	Larsson and Larsson (2003)
Lachnocladium sp.	BK171002-23	Belize	DQ154110	DQ154110	Unpublished
Metulodontia nivea	NH13108	Russia	AF506423	AF506423	Larsson and Larsson (2003)
Parapterulicium subarbusculum	FLOR 56456	Rio de Janeiro – Brazil	**MH260026**	**MH260048**	This study
Parapterulicium subarbusculum	FLOR 56459	Rio de Janeiro – Brazil	**MH260027**	**MH260049**	This study
			MH260028		
			MH260029		
			MH260030		
			MH260031		
Peniophora incarnata	NH10271	Denmark	AF506425	AF506425	Larsson and Larsson (2003)
Peniophora nuda	FPL4756	–	–	AF287880	Hibbett et al. (2000)
Scytinostroma alutum	CBS 762.81	France	–	AF393075	Binder and Hibbett (2002)
Scytinostroma caudisporum	CBS 746.86	Gabon	–	AY293210	Binder et al. (2005)
Scytinostroma portentosum	EL11-99	Sweden	AF506470	AF506470	Larsson and Larsson (2003)
Vararia insolita	CBS 667.81	Ivory Coast	–	AF518665	Hibbett and Binder (2002)
Vararia investiens	TAA161422	Norway	AF506484	AF506484	Larsson and Larsson (2003)
Vesiculomyces citrinus	EL53-97	Sweden	AF506486	AF506486	Larsson and Larsson (2003)

et al. (2002), as adapted by Dentinger and McLaughlin (2006). The analyses of this dataset demonstrated the placement of *Parapterulicium* within the Russulales. See Suppl. material 1: Agaricomycetidae analysis, for details and results of these analyses.

A more focused dataset for higher resolution phylogenetic analysis was created by removing duplicate species from the Russulales dataset of Chen et al. (2016) and adding the new sequences generated in this study alongside 29 GenBank sequences and one from CBS-KNAW database to represent all currently recognised families of Russulales, as well as all the genera of Lachnocladiaceae and Peniophoraceae with sequences available. Four sequences of *Sistostrema* Schumach. were used as outgroup. The Russulales dataset contained 135 sequences and was divided in four partitions: ITS1, 5.8S, ITS2 and nrLSU. A list of accession numbers of the sequences added to Chen et al. (2016) dataset is presented in Table 1; the complete list can be found in Suppl. material 1: SuppTable 1 and in Dentinger and McLaughlin (2006) for the Agaricomycetidae dataset.

The ITS1 and ITS2 datasets were aligned using MAFFT v7.311 (Katoh and Standley 2013) using the E-INS-i algorithm and the 5.8S and nrLSU datasets were aligned using the L-INS-i algorithm in MAFFT. The alignments were examined and adjusted manually using MEGA 7 (Kumar et al. 2016) and trimmed to remove uneven ends.

The best-fit evolutionary models were estimated for each partition separately using JMODELTEST v2.1.3 (Darriba et al. 2012; Guindon and Gascuel 2003) following the Bayesian Information Criterion (BIC). Bayesian Inference (BI) under the best-fit models was implemented using MRBAYES v3.2 (Ronquist et al. 2012) with two independent runs, each one with four chains and starting from random trees. Chains were run for 10^7 generations with tree sampling every 1000 generations. The burn-in was set to 25% and the remaining trees were used to calculate a 50% majority consensus tree and Bayesian Posterior Probability (BPP). The convergence of the runs was assessed on TRACER v1.7 (Rambaut et al. 2018) to ensure the potential scale reduction factors (PSRF) neared 1.0 and the effective sample size values (ESS) were sufficiently large.

Maximum-likelihood analysis was performed with IQTREE v1.6.3.b (Nguyen et al. 2015). The best-fit evolutionary models for this analysis were estimated by the built-in ModelFinder (option -m MF+MERGE) allowing the partitions to share the same set of branch lengths but with their own evolution rate (-spp option) (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). Branch support was assessed with 1000 replicates of ultrafast bootstrapping (Hoang et al. 2018).

Nodes with BPP ≥0.95 and/or BS ≥75 were considered strongly supported.

Alignments and phylogenetic trees are deposited in Treebase (ID: 22642).

Results

Phylogenetic analysis

A total of 37 sequences were generated in this study (19 nrITS and 18 nrLSU). The final alignment consisted of 135 sequences with 2295 characters. The BI analysis converged all runs as indicated by the effective sample sizes (ESS) of all parameters above 2000 and the potential scale reduction factors (PSRF) equal 1.000 for all the parameters. The two *Parapterulicium* species were placed with strong support into /peniophorales *sensu* Larsson and Larsson (2003) as shown in the Russulales tree (Fig. 1).

The clade /peniophorales recovered in the Russulales tree and the genera which it comprises are consistent with the neighbour-joining analyses of Larsson and Larsson (2003). However, the ML tree presented here shows better resolution of the sub-clades.

Five main clades highlighted in Fig. 1 are /lachnocladiaceae (previously /asterostromataceae), *Baltazaria*, /vararіaceae, /peniophoraceae and /metulodontia.

Clade /lachnocladiaceae (*BS=99; BPP=1*)

Lachnocladium formed a well-supported clade with *Scytinostroma*, *Vesiculomyces* E. Hagstr., *Gloiothele* Bres., *Asterostroma* Massee, *Vararia ocholeuca* (Bourdot & Galzin)

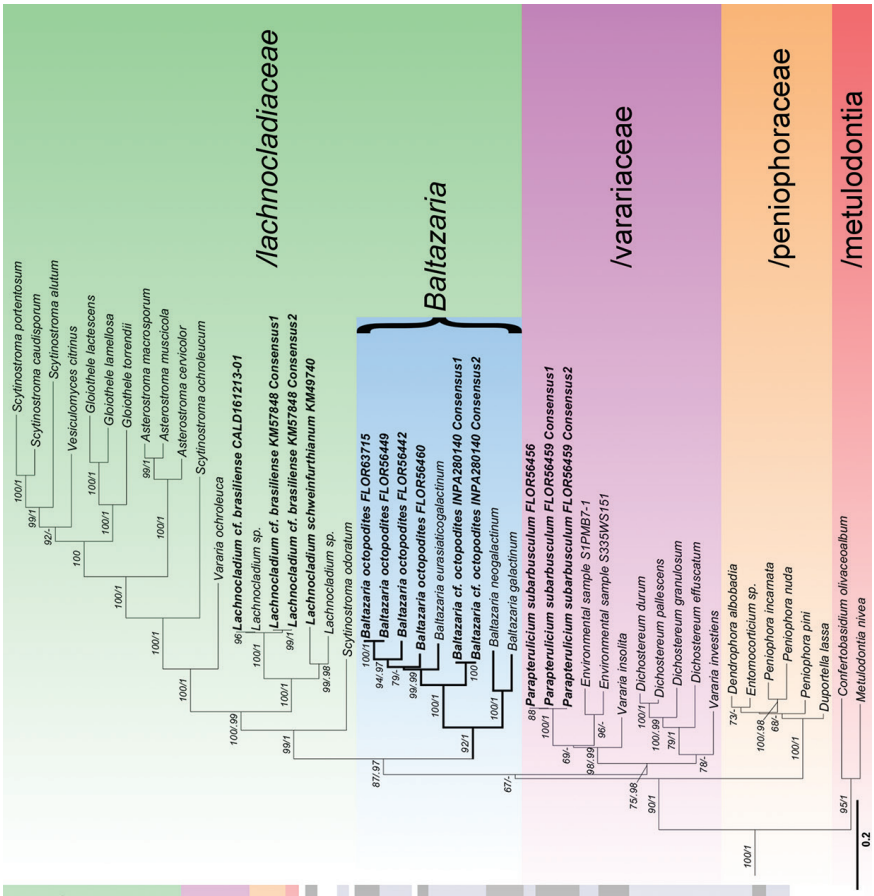

Figure 1. Maximum likelihood tree of Russulales on the left with /peniophorales amplified on the right. Support values on the branches are BS (>65) / BPP (>0.95), names in bold represent the newly generated sequences for this study and bold lines show the new genus. Details for the complete tree can be found in Suppl. material 1: SuppFigs 2, 3.

Donk, *Scytinostroma ochroleucum* Donk, *Scytinostroma odoratum* (Fr.) Donk and *Baltazaria* gen. nov. (BS=99; BPP=1).

Baltazaria (*BS=92; BPP=1*)
This clade represents the newly proposed genus (see below). It contains the sequences of *P. octopodites*, *S. eurasiaticogalactinum*, *S. neogalactinum* and *S. galactinum*. The presence of *P. octopodites* here rendered *Parapterulicium* paraphyletic necessitating reclassification.

Clade /vararíaceae (*BS=75; BPP=0.98*)
This clade includes *Parapterulicium subarbusculum*, *Dichostereum* Pilát and *Vararia* P. Karst. The inclusion of *Parapterulicium* sequences enhanced support for this clade, which was also recovered by Binder et al. (2005). The monophyly of *Dichostereum* typified by *D. durum* (Bourdot & Galzin) Pilát is strongly supported (BS=98; BPP=1). However, *Vararia* was rendered paraphyletic and will require a more thorough investigation to resolve its classification.

Parapterulicium subarbusculum, the type species of the genus, was nested within a strongly supported clade, which also contains *Vararia insolita* Boidin & Lanq. (BS=98; BPP=0.99). The reclassification of *Vararia insolita* may be warranted if future data support its placement here. *Parapterulicium subarbusculum* is also clustered with environmental sequences derived from subtropical leaf litter in Thailand (Osono et al. 2009). *Parapterulicium* spp. are not known outside of South America, but this suggests this species may be more widespread in subtropical and tropical regions than presently acknowledged.

Clade /peniophoraceae. (*BS=100; BPP=1*)
The clade /peniophoraceae includes *Peniophora*, *Duportella* Pat., *Dendrophora* (Parmasto) Chamuris and *Entomocorticium* H.S. Whitney, Bandoni & Oberw. These genera require special attention for detailed morphological and molecular studies to resolve the paraphyly of *Peniophora*, by either proposing new genera or synonymising *Dendrophora* and *Entomocorticium*. In all analyses performed in this study, there was no clear resolution for this group.

Clade /metulodontia (*BS=95; BPP=1*)
The clade contains *Metulodontia* Parmasto and *Confertobasidium* Jülich. Following Larsson and Larsson (2003), this well supported clade was recovered in all analyses performed.

Taxonomy

Parapterulicium subarbusculum **Corner, Ann. Bot., 16: 288 (1952)**
Fig. 2

Description. Basidiomes coralloid/filiform, up to 35 mm high, branched, erect, monoaxial with adventitious branches, yellow (10YR 8/6), solitary or gregarious. Stipe up

to 13 × 0.3–0.7 mm, glabrous, concolorous with the rest of the basidiomes, attached to a small resupinate base up to 3 mm wide. Branches up to 1.3 × 0.2 mm, tapering upwards, rarely with branchlets.

Habitat: On dead twigs, petioles, leaves or seeds in the forest.

Hyphal system dimitic. Generative hyphae up to 7 µm wide thin-walled, without clamps. Skeletal hyphae 2–7 µm wide, thick-walled (up to 1.3 µm), rarely branched. Abundant dextrinoid dichophyses, up to 30 µm wide, slightly thick-walled (0.5–1 µm), branching with filiform ends, tips less than 0.5 µm wide.

Resupinate patch not well-developed in the studied material but with abundant dichophyses.

Basidia not observed.

Gloeocystidia up to 65 µm long, clavate to lanceolate/subulate, thin-walled, with numerous internal droplets, IKI-.

Basidiospores (12–)13.4–16.8(–17) × 3–3.5 µm (n = 19), hyaline, smooth, elongate, subfusiform, apex obtuse, base acute with small apiculus (0.3 µm), thin-walled and slightly amyloid, scarce in all the collected samples.

Specimens examined. Brazil. Rio de Janeiro: Rio de Janeiro, Parque Nacional da Tijuca, close to Casa do Pesquisador, growing on the ground in rainforest litter, 24-25 Nov 2014, C.A. Leal-Dutra 108, 109, 117,118, 119, 120, 121, 122 (topotypes designated here: RB 639457, RB 639458, RB 639462, RB 639463, FLOR 56456, FLOR 56457, FLOR 56458, FLOR 0056459).

Distribution. Brazil. Rio de Janeiro: Rio de Janeiro (Corner 1952a, Type)

Notes. This species is recognised in the field by its characteristic resupinate disc at the base of the stipe (Fig. 2b, c). Corner (1952a) described *P. subarbusculum* from a single specimen collected in November 1948 on Corcovado in Rio de Janeiro and, based on its coralloid habit and dimitic hyphal system, placed the genus in Pterulaceae. The presence of gloeocystidia, slightly amyloid spores and dextrinoid dichophyses corroborates its placement in Russulales. It appears to be relatively common, though apparently overlooked.

***Baltazaria* C.A. Leal-Dutra, Dentinger & G.W. Griff. gen. nov.**
Mycobank No: MB825233

Etymology. In honour of Dr. Juliano Marcon Baltazar, Brazilian mycologist and authority on neotropical corticioid fungi.

Type species. *Baltazaria galactina* (Fr.) C.A. Leal-Dutra, Dentinger & G.W. Griff.

Diagnosis. Basidiomes corticioid, adherent to effused, coriaceous/membranaceous when fresh, hard when dry, usually white, cream or pale ochraceous. Context densely homogeneous with thick-walled and dextrinoid skeletal-binding hyphae, sometimes bearing rows of short papillae or skeletodendrohyphidia. Global distribution.

Notes. The diagnosis of Boidin and Lanquetin (1987) for *Scytinostroma eurasiaticogalactinum* and *S. neogalactinum* describes both species with the same morphological characters as *S. galactinum* (Fr.) Donk but with reproductive incompatibility between the

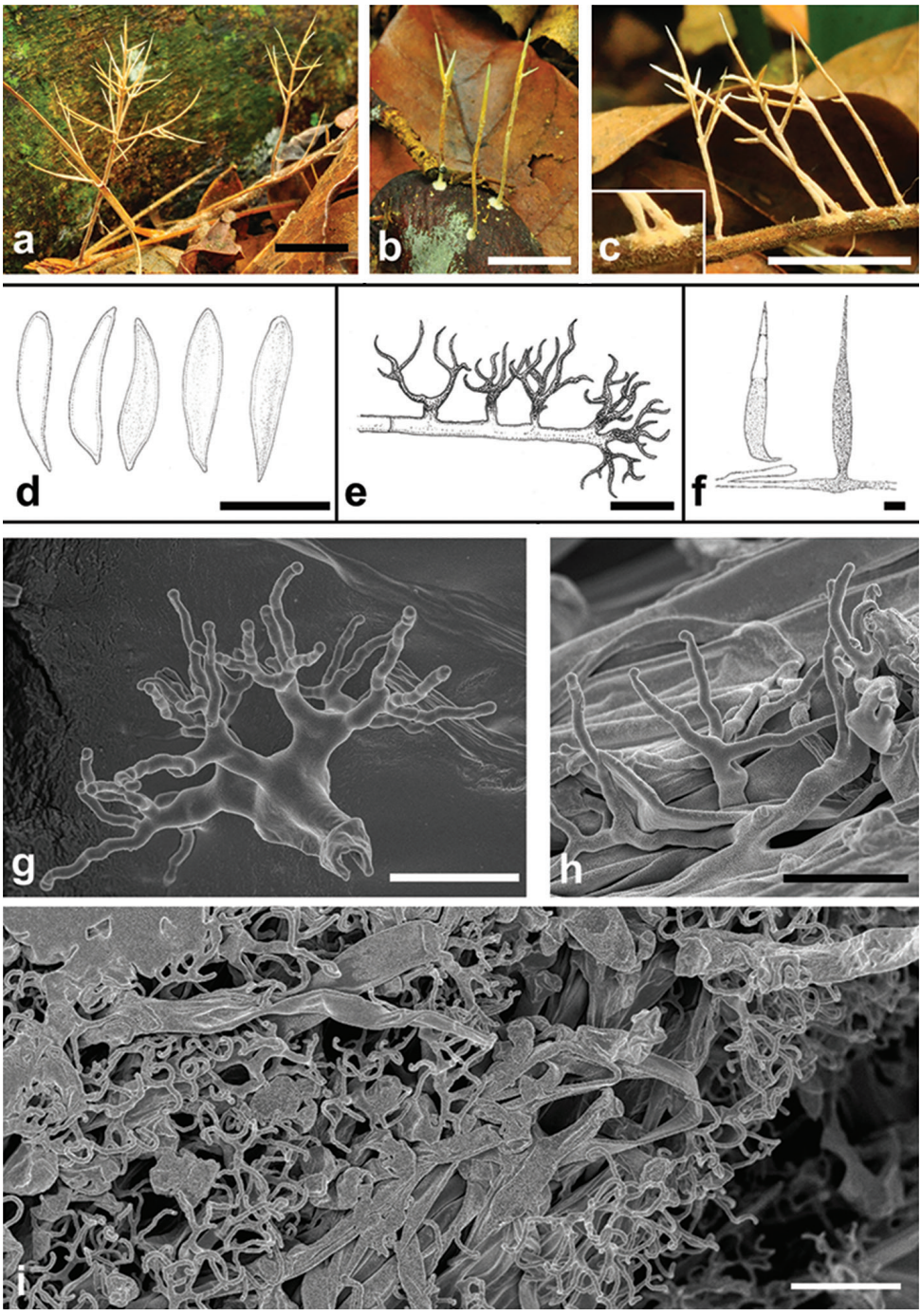

Figure 2. *Parapterulicium subarbusculum*: **a–c** basidiomes in the field. The detail in **c** shows the developing corticioid patch **d** basidiospores **e** dichophyses **f** gloeocystidia **g, h** SEM images of dichophyses; i. SEM images of basidiome surface with abundant dichophyses. Scale bars: **a–c** = 1 cm; **d–f, i** = 10 μm; **g, h** = 5 μm.

species and different distributions. In the discussion on the *S. galactinum* complex, the authors mention the branched skeletal hyphae that starts with conspicuous 2–3 branched short projections and then become longer, a feature resembling the *Parapterulicium octopodites* papillate skeletal hyphae (Fig. 3d–h). Moreover, the description of *S. galactinum* by Lentz and Burdsall (1973) mentions the hymenium with conspicuous skeletodendrohyphidia. However, Bernicchia and Gorjón (2010) claimed the species does not present dendrohyphae; instead, the authors describe the presence of skeletal-binding hyphae. It is likely that the papillate skeletal hyphae described by Corner (1952a), the short and branched projections described by Boidin and Lanquetin (1987) and the skeletodendrohyphidia described by Lentz and Burdsall (1973), are nothing more than early developmental stages of the skeletal-binding hyphae described by Bernicchia and Gorjón (2010).

Baltazaria galactina **(Fr.) C.A. Leal-Dutra, Dentinger & G.W. Griff., comb. nov.**
Mycobank No: MB825235

Basionym. *Thelephora galactina* Fr., Nova Acta R. Soc. Scient. upsal., Ser. 3 1(1): 136 (1851) [1855]. ≡ *Corticium galactinum* (Fr.) Moffatt, Bulletin of the Nat. Hist. Surv. Chicago Acad. Sci. 7(1): 137 (1909). ≡ *Scytinostroma galactinum* (Fr.) Donk, Fungus, Wageningen 26: 20 (1956).

= *Thelephora suaveolens* Moug. ex Fr., Elench. fung. (Greifswald) 1: 208 (1828).

= *Stereum suaveolens* (Moug. ex Fr.) Fr., Epicr. syst. mycol. (Upsaliae): 553 (1838) [1836-1838].

= *Xerocarpus suaveolens* (Moug. ex Fr.) P. Karst., Bidr. Känn. Finl. Nat. Folk 37: 137 (1882).

Description in Lentz and Burdsall (1973).

Baltazaria eurasiaticogalactina **(Boidin & Lanq.) C.A. Leal-Dutra, Dentinger & G.W. Griff., comb. nov.**
Mycobank No: MB825236

Basionym. *Scytinostroma eurasiaticogalactinum* Boidin & Lanq., Biblthca Mycol. 114: 57 (1987)

Description in Boidin and Lanquetin (1987).

Baltazaria neogalactina **(Boidin & Lanq.) C.A. Leal-Dutra, Dentinger & G.W. Griff., comb. nov.**
Mycobank No: MB825237

Basionym. *Scytinostroma neogalactinum* Boidin & Lanq., Biblthca Mycol. 114: 59 (1987).

Description in Boidin and Lanquetin (1987).

Baltazaria octopodites **(Corner) C.A. Leal-Dutra, Dentinger & G.W. Griff., comb. nov.**
Mycobank No: MB825234
Fig. 3

Basionym. *Parapterulicium octopodites* Corner, Ann. Bot., 16: 286 (1952)

Description. Basidiomes resupinate (Fig. 3b), 0.1–0.5 mm thick, membranaceous, covering leaves and twigs, hymenophore smooth, white (2.5Y 8/2) to pale yellow (2.5Y 8/4), forming rhizomorph-like structures up to 7 cm high and scarcely to profusely branched that may be confused with coralloid basidiomes (Fig. 3a, b).

Substrate: On dead twigs and leaves.

Hyphal system dimitic, profusely interwoven. Generative hyphae 2–5 µm wide, thin-walled, without clamps. Skeletal hyphae 2–6 µm (up to 10 µm in KOH) wide, walls dextrinoid, up to 1.5 µm thick, strongly swelling in KOH (up to 4.5 µm). Termini of hymenial skeletal hyphae papillate, presenting short protuberances 2–10 × 1.5–2.5 µm, sometimes ramified resembling skeletodendrohyphidia.

Putative hymenium with abundant basidioles up to 25 × 6 µm, clavate, growing immersed in the papillate hyphae.

Gloeocystidia up to 80 × 8–14 µm, clavate to lanceolate, thin-walled, densely multiguttulate or with abundant granular contents. Present in all parts of the basidiomes, including the corticioid form.

Basidiospores and basidia not observed.

Specimens examined. Brazil. Rio Grande do Sul: no date, J. Rick (holotype: BPI 333063). São Paulo: Apiaí, Parque Estadual Turístico do Alto Ribeira, growing on the ground in rainforest litter, 14-15 Dec. 2014, M.A. Reck 1003/14, 1069/14 (FLOR 56442, FLOR 56449). Santa Catarina: Florianópolis, UCAD, 9 Jan. 2015, G. Flores 14 (FLOR 56460). Paraná: Foz do Iguaçú, Parque Nacional do Iguaçú, Trilha da torre, 22 Jan. 2017, C.A.T. Oliveira 160 (FLOR 63715). Amazonas: Rio Preto da Eva, ARIE-PDBFF - Reserva do Km 41, 17 Mar. 2017, C.A. Leal-Dutra, L.A. Clasen, Q.V. Montoya, O. Pereira 170309-26 (INPA 280140).

Distribution. Brazil. Rio Grande do Sul: São Leopoldo (Corner, 1952a; Type). São Paulo: Apiaí. Santa Catarina: Florianópolis. Paraná: Foz do Iguaçu. Amazonas: Rio Preto da Eva (this study).

Notes. The dimitic hyphal system, the papillate surface at the ends of the skeletal hyphae and the gloeocystidia agree perfectly with Corner's original descriptions (Corner 1952a). Corner (1952a) described this species from a collection where no fertile structures were observed; the new collections were also sterile. As no spores or fertile basidia were found, the term putative hymenium is given to the region with abundant basidiole-like structures. Furthermore, the lack of sexual characters observed in *B. octopodites*, combined with the undeveloped binding-skeletal hyphae, might indicate that this species is only known by young basidiomes or non-reproductive growth forms (i.e. explorative rhizomorphs). This is the first record of *B. octopodites* from the States of Amazonas, Paraná, Santa Catarina and São Paulo.

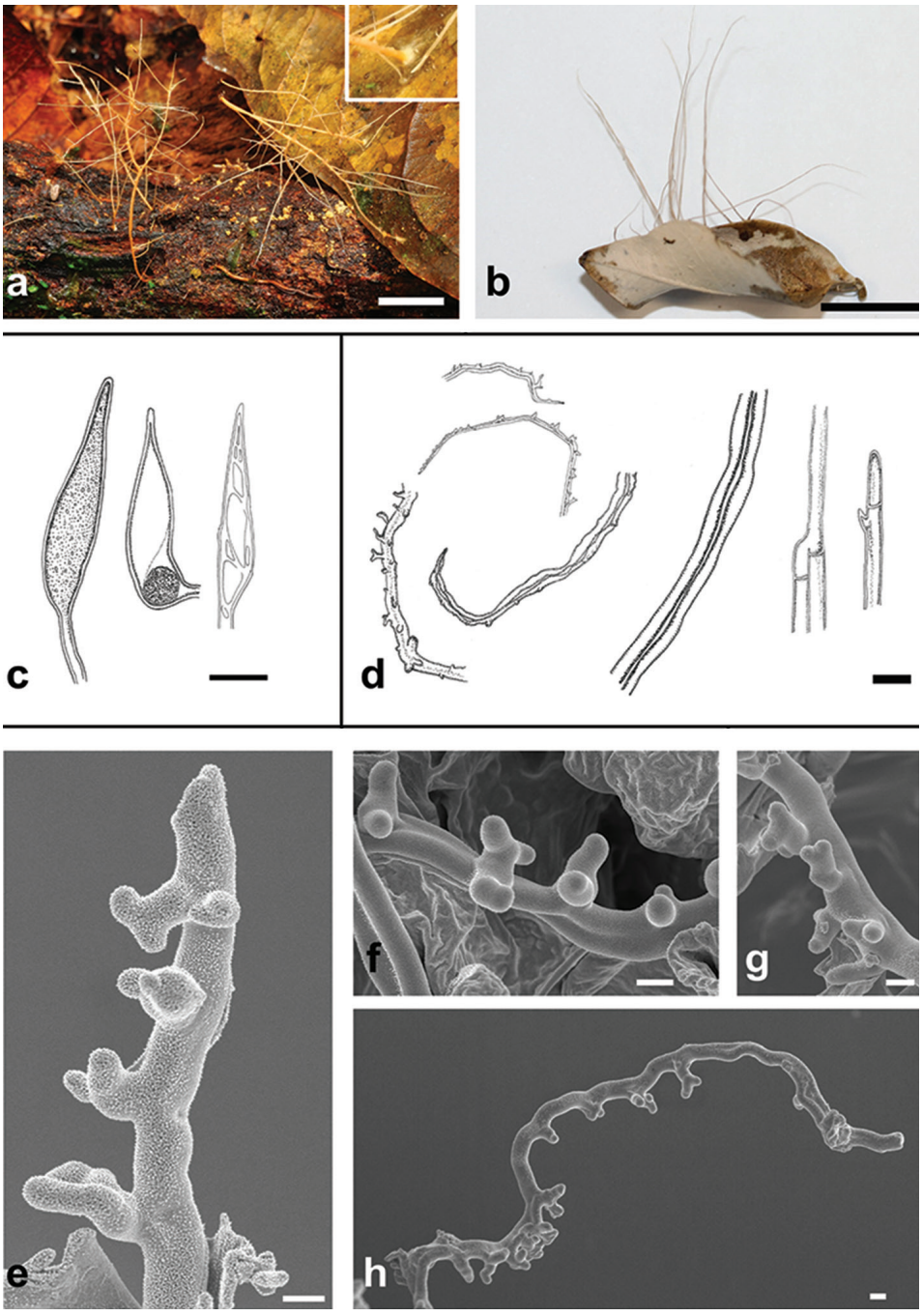

Figure 3. *Baltazaria octopodites*: **a, b** basidiomes in the field (INPA280140 and FLOR56460), the detail in **a** shows the anchorage point in the leaf, the whitish resupinate area in **b** shows the corticioid portion of the fungus **c** gloeocystidia **d** skeletal hyphae, skeletal hyphae inflated in KOH (third from the right) and generative hyphae (first and second from the right) **e–h** SEM images of papillate skeletal hyphae. Scale bars: **a–b** = 1 cm; **c–d** = 10 µm; **e–h** = 1 µm).

Discussion

The /lachnocladiaceae clade was named /asterostromataceae by Larsson and Larsson (2003), who also proposed a deeper molecular study involving *Lachnocladium* to find the exact placement of the genus. In this study, *Lachnocladium* spp., typified by *L. brasiliense* (Lév.) Pat., formed a strongly supported (BS=99; BPP=1) clade with the previously called /asterostromataceae, which includes *Scytinostroma*, *Vesiculomyces*, *Gloiothele*, *Asterostroma*, *Vararia ocholeuca*, *Scytinostroma ochroleucum*, *Scytinostroma odoratum* and the new genus *Baltazaria*. Thus, we decided to name the clade /lachnocladiaceae to suggest the need for a thorough study on the morphology of these genera to re-circumscribe Lachnocladiaceae. Binder et al. (2005) recovered this clade but did not include *Lachnocladium*. *Scytinostroma*, typified by *S. portentosum* (Berk. & M.A. Curtis) Donk, forms a clade with robust support with *S. caudisporum* Boidin, Lanq. & Gilles and *S. alutum* Lanq. (BS=99; BPP=1), meaning the other species of *Scytinostroma* sampled in this study (*S. ochroleucum*, *S. odoratum*, *S. eurasiaticogalactinum*) require reclassification. Monophyly of *Asterostroma* and *Gloiothele* is also strongly supported (BS=100; BPP=1), including the type species *A. apalum* (Berk. & Broome) Massee (= *A. muscicola*) and *Gloiothele lamellosa* (Henn.) Bres., respectively.

Future studies of Lachnocladiaceae may recommend *Baltazaria* be classified in its own family. However, we view our study as incomplete and it would therefore be premature to erect a new family at this time.

The most distinctive feature of *B. octopodites* is the papillate skeletal hyphae that form one or two rows of short, round and sometimes branched projections, similar to some skeletodendrohyphidia of *B. galactinum* (Lentz and Burdsall 1973). Another notable characteristic of this species is the hyphal swelling seen in KOH, which is also found in some species of *Peniophora* Cooke, *Dichostereum* and *Vararia* (Stalpers 1996; Stalpers and Buchanan 1991). In addition, the multigutullate gloeocystidia present in *P. subarbusculum* and *B. octopodites* might have the same origin as those in *Russula* Pers. and *Auriscalpium* Gray, which were shown by McLaughlin et al. (2008) to be a likely synapomorphy of Russulales. Taken together, alongside the molecular evidence presented in this study, these corroborating morphological features add strong support to the reclassification of these fungi and suggest that aforementioned hyphal features may be unifying characters for /peniophorales.

All collections of *B. octopodites* made to date are sterile with no spores or basidia observed. Although the hymenium might have been missed due to developmental idiosyncrasies, such as ephemeral nocturnal production (Corner 1950, McLaughlin and McLaughlin 1972), the function of the filiform projections, believed to be coralloid basidiomes, may not be for sexual reproduction. Instead, they may function as exploratory appendages, similar to mycelial cords and rhizomorphs in other fungi (e.g. *Crinipellis* Pat./*Marasmius* Fr., *Armillaria* (Fr.) Staude etc.) or as a strategy for binding substrate materials together (Cairney 1991; Hedger et al. 1993; Snaddon et al. 2011). This characteristic, combined with the fact that no spores have been reported, raises the possibility that an independent sexual form, similar to the resupinate basidiomes of

Scytinostroma, may exist. Considering these assumptions, *B. octopodites* might be more common than previously thought, since it is probably overlooked during fieldwork, mistakenly identified as a rhizomorph.

A third species of *Parapterulicium*, *P. simplex*, is still known only from type material originally collected in Argentina (Corner 1957). It would be prudent to include this species in a full revision of the genus, which would require targeted fieldwork at the type locality. We anticipate that, despite the rarity of their documentation, these filiform fungi are abundant and widespread.

Acknowledgements

CALD scholarship was provided by CAPES Foundation - Brazil, BEX 2145/15-4. Funding was provided in part from a Systematics and Taxonomy (SynTax) grant (BB-SRC, NERC) to BTMD. Parque Nacional da Tijuca (ICMBio), for logistic support and collection permits. Biological Dynamics of Forest Fragmentation Project (BDFFP) for providing logistical and field support. This is publication 743 of the BDFFP - INPA/STRI Technical Series (field reserve; location of specimen used here). We are grateful to Alan Cookson, IBERS, for assistance with Scanning electron microscopy and also to IBERS HPC and Supercomputing Wales for computing support. The Institute of Biological, Environmental, and Rural Sciences receives strategic funding from the BB-SRC. We thank Felipe Ruppenthal for the line drawings.

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Supplementary material 1

Species used in the Russulales analyses and their GenBank accession numbers of nrITS and nrLSU sequences

Authors: Caio A. Leal-Dutra, Maria Alice Neves, Gareth W. Griffith, Mateus A. Reck, Lina A. Clasen, Bryn T. M. Dentinger

Data type: species data

Link: https://doi.org/10.3897/mycokeys.37.26303.suppl1

MycoKeys 37: 57–71 (2018)
doi: 10.3897/mycokeys.37.26016
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Phylogeny and taxonomy of *Laetiporus* (Basidiomycota, Polyporales) with descriptions of two new species from western China

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Academic editor: *M-A Neves* | Received 20 April 2018 | Accepted 18 July 2018 | Published 31 July 2018

Citation: Song J, Sun Y-F, Ji X, Dai Y-C, Cui B-K (2018) Phylogeny and taxonomy of *Laetiporus* (Basidiomycota, Polyporales) with descriptions of two new species from western China. MycoKeys 37: 57–71. https://doi.org/10.3897/mycokeys.37.26016

Abstract

Laetiporus is a cosmopolitan genus of brown rot fungi. In this study, *L. medogensis* and *L. xinjiangensis* are described as new species from western China, based on morphological and molecular evidence. *L. medogensis* has only been found on gymnosperms so far and is distinguished by pinkish-buff to clay-buff pileal surface and buff-yellow pore surface, azonate to faintly zonate pileus and ellipsoid to ovoid basidiospores (5–6.2 × 4.2–5.2 µm). *L. xinjiangensis* is found on angiosperms and is characterised by pale-buff to clay-pink pileal surface, cream to light yellow pore surface, azonate to faintly zonate pileus, large pores (2–3 per mm) and small basidiospores (4.5–5 × 3–4.2 µm). The phylogeny of *Laetiporus* is reconstructed with multi-gene sequences including the internal transcribed spacer regions (ITS), the large subunit (nrLSU) and small subunit (nrSSU) of the nuclear ribosomal RNA gene, the small subunit of the mitochondrial rRNA gene (mtSSU), the translation elongation factor 1-α gene (EF-1α) and the second subunit of RNA polymerase II (RPB2). The results show that *L. medogensis* and *L. xinjiangensis* formed two distinct lineages belonging to *Laetiporus*. Illustrated descriptions of the two new species are presented. An identification key to species of *L. sulphureus* complex is provided.

Keywords

Brown-rot fungi, multi-gene phylogeny, Fomitopsidaceae, taxonomy, wood-decaying fungi

Introduction

Laetiporus Murrill (Fomitopsidaceae, Polyporales) is a cosmopolitan genus, causing brown rot on living hardwoods and conifers (Murrill 1904). Some species of the genus are known as forest pathogens and some are edible with medicinal functions (Dai et al. 2007, 2009). According to previous studies, 15 species have been accepted in the genus worldwide and 11 species have been confirmed in the *L. sulphureus* complex by phylogenetic analyses, of which six have been reported from China: *L. ailaoshanensis* B.K. Cui & J. Song, *L. cremeiporus* Y. Ota & T. Hatt., *L. montanus* Černý ex Tomšovský & Jankovský, *L. sulphureus* (Bull.) Murrill, *L. versisporus* (Lloyd) Imazeki and *L. zonatus* B.K. Cui & J. Song (Tomšovský and Jankovský 2008, Ota et al. 2009, Banik et al. 2012, Song et al. 2014, Song and Cui 2017). The species in the *L. sulphureus* complex are characterised by annual basidiocarps, soft and fleshy context and a dimitic hyphal system composed of simple septate generative hyphae and binding hyphae (Burdsall and Banik 2001, Núñez and Ryvarden 2001, Ota et al. 2009).

A molecular phylogenetic study of *Laetiporus* in Japan identified three species, viz. *L. cremeiporus*, *L. montanus* and *L. versisporus* (Ota and Hattori 2008, Ota et al. 2009). Recently, systematic studies have been carried out to define the species and explore the historical biogeography of the genus *Laetiporus* in China. Song et al. (2014) described two new *Laetiporus* species from south-western China based on morphological and molecular evidence. Further comprehensive study of Song and Cui (2017) indicated that there are two additional undescribed *Laetiporus* species.

In the present study, the two new *Laetiporus* species from western China (Clade P and Clade Q) are described based on morphological and phylogenetic analyses.

Materials and methods

Morphological studies

Morphological studies followed Han et al. (2016). The studied specimens were deposited in the herbarium of the Institute of Microbiology, Beijing Forestry University (BJFC). Macro-morphological descriptions were based on field notes. Colour terms followed Petersen (1996). Microscopic measurements and drawings were made from slide preparations of dried specimens stained with Cotton Blue and Melzer's reagent, following Han et al. (2016). Sections were studied at a magnification of 1000× using a Nikon Eclipse 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Spores were measured in tube sections. In presenting spore size variation, 5% of measurements were excluded from each end of the range and given in parentheses. The following abbreviations were used: KOH = 5% potassium hydroxide, CB = cotton blue, CB+ = cyanophilous, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, L = mean spore length (arithmetic average), W = mean spore width (arithmetic average), Q = variation in the L/W ratios between specimens studied, n (a/b) = number of spores (a) measured from a given number of specimens (b).

Molecular study and phylogenetic analysis

Genomic DNA was extracted from dried fruiting bodies using a cetyltrimethylammonium bromide rapid plant genome extraction kit (Aidlab Biotechnologies Co., Ltd., Beijing) according to the manufacturer's instructions with some modifications (Han et al. 2016). Six genetic markers were used, including ITS, nrLSU, nrSSU, EF-1α, mtSSU and RPB2. The primer pairs ITS5/4, LR0R/LR7, MS1/MS2, NS1/NS4, 983F/1567R and 6F/7R were used to amplify ITS, nrLSU, mtSSU, nrSSU, EF-1α and RPB2, respectively (http://www.biology.duke.edu/fungi/mycolab/primers.htm). A 2 × EasyTaq PCR SuperMix (Transgen Biotech, Beijing) was used to amplify the genes. The PCR procedure for ITS, EF-1α, mtSSU and RPB2 was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min and a final extension of 72 °C for 10 min. The PCR procedure for nrLSU and nrSSU was as follows: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 1 min and 72 °C for 1.5 min and a final extension of 72 °C for 10 min. The PCR products were purified using the Bioteke DNA Purification Kit (Bioteke Corporation, Beijing) and sequenced at the Beijing Genomics Institute, China, with the same primers. The basic authenticity and reliability of newly generated sequences were established based on Nilsson et al. (2012). The newly generated sequences and additional sequences downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank; Benson et al. 2017) are listed in Table 1. Sequences of ITS, nrLSU, nrSSU, EF-1α, mtSSU and RPB2 of species in *Laetiporus* and outgroups [*Antrodia serialis* (Fr.) Donk and *Fomitopsis pinicola* (Sw.) P. Karst.] were combined and aligned in MAFFT 7 (Katoh and Toh 2008; https://mafft.cbrc.jp/alignment/server/index.html) using the "G-INS-I" strategy and manually adjusted in BioEdit v7.2.6.1 (Hall 1999).

Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses were applied to the combined dataset. The best fit model of nucleotide evolution to each individual genetic marker and the combined dataset was selected with AIC (Akaike Information Criterion) using MrModeltest 2.3 (Posada and Crandall 1998, Nylander 2004). The best fit models were GTR for ITS, nrLSU, nrSSU, EF-1α, mtSSU, RPB2 and GTR+I+G for the combined dataset. The partitioned mixed model, which allows for model parameters estimated separately for each genetic marker, was used in the Bayesian analysis. BI was performed using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) with 2 independent runs, each one beginning from random trees with 4 simultaneous independent chains, performing 4,000,000 replicates, sampling one tree every 100 generations. The first 25% of the sampled trees were discarded as burn-in and the remaining ones were used to reconstruct a majority rule consensus and calculate Bayesian posterior probabilities (BPP) of the clades.

ML searches were conducted with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org) and comprised 100 ML searches under the GTR-GAMMA model, with all model parameters estimated by the programme. Only the maximum likelihood best tree from all searches was kept. In addition, 100 rapid bootstrap replicates were run with the GTRCAT model to assess the reliability of the nodes.

Table 1. A list of species, specimens and GenBank accession numbers of sequences used in this study.

Species	Collection no.	GenBank Accessions					
		ITS	nrLSU	nuSSU	mtSSU	EF-1α	RPB2
Antrodia serialis	Cui 10519	KP715307	KP715323	KR605911	KR606011	KP715337	KR610830
Fomitopsis pinicola	Cui 10405	KC844852	KC844857	KR605857	KR605961	KR610690	KR610781
Laetiporus ailaoshanensis	Dai 13567 (Paratype)	KX354470[a]	KX354498[a]	KX354535[a]	KX354577[a]	KX354623[a]	KX354665[a]
L. ailaoshanensis	Dai 13256 (Holotype)	KF951289[a]	KF951317[a]	KX354537[a]	KX354579[a]	KX354625[a]	KT894786[a]
L. caribensis	PR 6583	JN684766	-	-	-	-	-
L. caribensis	PR 914	JN684762	EU402526	-	EU402482	-	-
L. caribensis	PR 6521	JN684771	-	-	-	-	-
L. cincinnatus	Dai 12811	KF951291[a]	KF951304[a]	KX354516[a]	KX354558[a]	KX354605[a]	KT894788[a]
L. cincinnatus	DA 37	EU402557	EU402521	-	EU402485	AB472661	-
L. cincinnatus	JV 0709/168J	KF951290[a]	KF951305[a]	KX354517[a]	KX354559[a]	KX354606[a]	KX354651[a]
L. conifericola	JV 0709/81J	KF951292[a]	KF951327[a]	KX354531[a]	KX354573[a]		KX354683[a]
L. conifericola	CA 8	EU402575	EU402523	-	EU402487	AB472663	-
L. conifericola	JAM 1	EU402577	EU402524	-	EU402486	AB472664	-
L. cremeiporus	Dai 10107	KF951281[a]	KF951301[a]	KX354515[a]	KX354557[a]	KX354604[a]	KX354650[a]
L. cremeiporus	Cui 10991	KF951279[a]	KF951298[a]	-	KX354595[a]	KX354641[a]	KX354679[a]
L. cremeiporus	Cui 10586	KF951277[a]	KF951297[a]	KX354513[a]	KX354555[a]	KX354602[a]	KX354648[a]
L. gilbertsonii	JV 1109/31	KF951293[a]	KF951306[a]	KX354542[a]	KX354584[a]	KX354630[a]	KX354671[a]
L. gilbertsonii	TJV 2000/101	EU402553	EU402528	-	EU402493	AB472668	-
L. gilbertsonii	CA 13	EU402549	EU402527	-	EU402496	AB472666	-
L. huroniensis	HMC 3	EU402571	EU402540	-	-	-	-
L. huroniensis	MI 14	EU402573	EU402539	-	EU402489	AB472672	-
L. medogensis	Cui 12219 (Paratype)	KX354472[a]	KX354500[a]	KX354538[a]	KX354580[a]	KX354626[a]	KX354667[a]
L. medogensis	Cui 12240 (Holotype)	KX354473[a]	KX354501[a]	KX354539[a]	KX354581[a]	KX354627[a]	KX354668[a]
L. medogensis	Cui 12390 (Paratype)	KX354474[a]	KX354502[a]	KX354540[a]	KX354582[a]	KX354628[a]	KX354669[a]
L. montanus	Dai 15888	KX354466[a]	KX354494[a]	KX354530[a]	KX354572[a]	KX354619[a]	KX354662[a]
L. montanus	Cui 10011	KF951274[a]	KF951315[a]	KX354528[a]	KX354570[a]	KX354617[a]	KT894790[a]
L. montanus	Cui 10015	KF951273[a]	KF951311[a]	KX354529[a]	KX354571[a]	KX354618[a]	KT894791[a]
L. sp. 1	EUC 1	EU402545	EU402541	-	-	-	-
L. sp. 1	KOA 1	EU402546	EU402542	-	-	-	-
L. sp. 2	RV4A	EU840662	-	-	-	-	-
L. sp. 2	RV5A	EU840663	-	-	-	-	-
L. sp. 3	Munez 207	JN684764	-	-	-	-	-
L. sp. 4	Robledo 1122	JN684765	-	-	-	-	-
L. sulphureus	Cui 12389	KR187106[a]	KX354487[a]	KX354519[a]	KX354561[a]	KX354608[a]	KX354653[a]
L. sulphureus	Cui 12388	KR187105[a]	KX354486[a]	KX354518[a]	KX354560[a]	KX354607[a]	KX354652[a]
L. sulphureus	Dai 12154	KF951295[a]	KF951302[a]	KX354521[a]	KX354563[a]	KX354610[a]	KX354655[a]
L. sulphureus	Z.R.L. CA04	KX354479[a]	KX354506[a]	KX354545[a]	KX354587[a]	KX354633[a]	KX354674[a]
L. sulphureus	Z.R.L. CA08	KX354480[a]	KX354507[a]	KX354546[a]	KX354588[a]	KX354634[a]	KX354675[a]
L. sulphureus	DA 41	EU40256	EU402533	-	EU402481	AB472660	-
L. sulphureus	TJV 99/150	EU402567	EU402530	-	EU402492	-	-
L. sulphureus	MAS 2	EU402568	EU402531	-	EU402491	-	-
L. sulphureus	JV 1106/15	KF951296[a]	KF951303[a]	KX354520[a]	KX354562[a]	KX354609[a]	KX354654[a]
L. versisporus	Cui 7882	KF951269[a]	KF951323[a]	-	KX354596[a]	KX354642[a]	KT894783[a]
L. versisporus	Li 15071314	KX354476[a]	KX357139[a]	-	KX354598[a]	KX354644[a]	KX354680[a]
L. versisporus	Dai 13160	KF951266[a]	KF951320[a]	-	KX354597[a]	KX354643[a]	KT894785[a]
L. versisporus	Yuan 6319	KX354475[a]	KX354503[a]	KX354541[a]	KX354583[a]	KX354629[a]	KX354670[a]
L. versisporus	Dai 10992	KF951272[a]	KF951325[a]	-	KX354600[a]	KX354646[a]	KX354681[a]
L. versisporus	Dai 13052	KF951271[a]	KF951324[a]	-	KX354601[a]	KX354647[a]	KX354682[a]
L. xinjiangensis	Dai 15825 (Paratype)	KX354465[a]	KX354493[a]	KX354527[a]	KX354569[a]	KX354616[a]	KX354661[a]
L. xinjiangensis	Dai 15828 (Paratype)	KX354461[a]	KX354489[a]	KX354523[a]	KX354565[a]	KX354612[a]	KX354657[a]
L. xinjiangensis	Dai 15953 (Holotype)	KX354460[a]	KX354488[a]	KX354522[a]	KX354564[a]	KX354611[a]	KX354656[a]
L. xinjiangensis	Dai 15898A (Paratype)	KX354464[a]	KX354492[a]	KX354526[a]	KX354568[a]	KX354615[a]	KX354660[a]
L. zonatus	HKAS 71806 (Paratype)	KF951284[a]	KF951310[a]	KX354548[a]	KX354590[a]	KX354636[a]	KT894796[a]
L. zonatus	Cui 10403 (Paratype)	KF951282[a]	KF951307[a]	KX354550[a]	KX354592[a]	KX354638[a]	-
L. zonatus	Cui 10404 (Holotype)	KF951283[a]	KF951308[a]	KX354551[a]	KX354593[a]	KX354639[a]	KT894797[a]

[a] New sequences for this study.

MP analysis was applied to the combined dataset as in Song and Cui (2017). Tree construction was performed in PAUP* version 4.0b10 (Swofford 2002) with the following settings. All characters were equally weighted and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all most parsimonious trees were saved. Clade robustness was assessed using a bootstrap analysis with 1000 replicates (Felsenstein 1985). The descriptive statistics of tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI) were calculated for each most parsimonious tree generated.

Branches that received bootstrap support for maximum parsimony (MP), maximum likelihood (ML) and Bayesian posterior probabilities (BPP) greater than or equal to 75% (MP/ML) and 0.95 (BPP) were considered as significantly supported.

Results

Phylogenetic analyses

The combined dataset (ITS+nrLSU+nrSSU+mtSSU+EF-1α+RPB2) included sequences from 55 samples representing 19 taxa. *Antrodia serialis* and *Fomitopsis pinicola* were used as outgroups. The dataset had a total aligned length of 3963 characters, of which 3137 (79.2%) were constant, 301 (7.6%) were variable and parsimony uninformative and 525 (13.2%) were parsimony informative. The parsimony analysis yielded 68 equally parsimonious trees (TL = 1173, CI = 0.812, RI = 0.865, RC = 0.702, HI = 0.188). The multiple sequence alignment and tree files were deposited at TreeBase (submission ID 21249; www.treebase.org). MP analysis and BI resulted in similar topologies as the ML analysis. The consensus tree inferred from the ML analysis with MP, ML and BPP values is shown in Figure 1.

Samples of *Laetiporus* clustered together with significant support (100% MP, 100% ML and 1.00 BPP; Figure 1). Sampled specimens of the two new species *L. medogensis* and *L. xinjiangensis* formed well-supported lineages (Figure 1).

Taxonomy

Laetiporus medogensis **J. Song & B.K. Cui**
MycoBank: MB821867
Figures 2a, 3

Diagnosis. Differs from other *Laetiporus* species by its pinkish-buff to clay-buff pileal surface, buff-yellow pore surface and ellipsoid to ovoid basidiospores (5–6.2 × 4.2–5.2 μm).

Etymology. *Medogensis* (Lat.): referring to the locality (Medog County) of the type specimens.

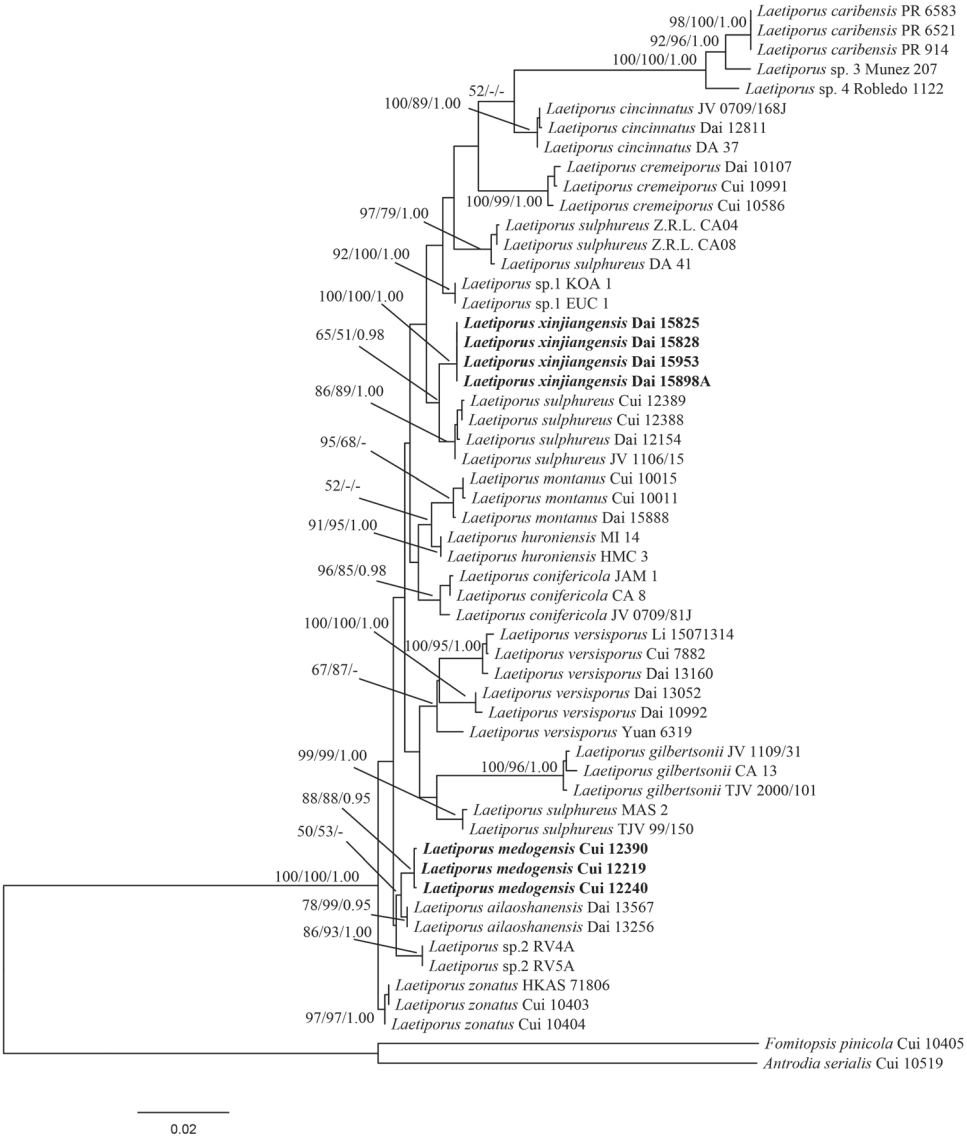

Figure 1. Strict consensus tree illustrating the phylogeny of *Laetiporus* generated by ML analysis based on ITS+nrLSU+nrSSU+mtSSU+EF-1α+RPB2 sequences. Branch support is indicated where MP/BS support is greater than 50% and collapsed below that support threshold. BPP is indicated when greater than 0.95. New species are indicated in bold.

Holotype. CHINA. Xizang Auto. Reg. (Tibet), Medog County, on living tree of *Abies*, 21 Sep 2014, Cui 12240 (BJFC 017154).

Basidiocarps. Annual, sessile to laterally substipitate, imbricate, fleshy when fresh, crumbly when dry, without odour or taste. Pileus flabelliform to dimidiate, applanate, projecting up to 9 cm, 12 cm wide and 1 cm thick. Pileal surface pinkish-buff to

clay-buff when fresh, becoming pale yellow upon drying, glabrous, azonate to faintly zonate. Margin soft and slightly viscous, fawn when juvenile, fading to reddish-brown when dry. Pore surface buff-yellow when fresh, becoming pale yellow to cream when dry; sterile margin cream when fresh, up to 3 mm wide; pores angular, 2–4 per mm; dissepiments thin, entire to lacerate. Context white when fresh, becoming cream to pale yellow when dry, up to 8.5 mm thick. Tubes concolorous with pore surface, crumbly or chalky, up to 1.5 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae simple-septate; skeletal hyphae IKI–, CB–, dissolving in KOH. Generative hyphae in context infrequent, hyaline, thin-walled, occasionally branched, up to 11 μm in diam.; skeletal hyphae in context dominant, thick-walled with a wide lumen, frequently branched and interwoven, occasionally simple-septate, hyaline, 4–11 μm in diam. Generative hyphae in tubes dominant, hyaline, thin-walled, frequently branched, simple-septate, 4–5 μm in diam.; skeletal hyphae in tubes thick-walled with a wide lumen, occasionally branched and simple-septate, subparallel along the tubes, 3–5 μm in diam.

Cystidia. Cystidia and other sterile hyphal elements absent.

Basidia. Basidia clavate, 20–25 × 8–9 μm, bearing four sterigmata and a basal simple-septum; basidioles clavate, smaller than basidia.

Spores. Basidiospores ellipsoid to ovoid, hyaline, thin-walled, smooth, IKI–, CB–, 5–6.2 × 4.2–5.2 μm, L = 5.78 μm, W = 4.73 μm, Q = 1.22–1.23 (n = 60/2).

Additional specimens (paratypes) examined. CHINA. Xizang Auto. Reg. (Tibet), Medog County, on living tree of *Abies*, 20 Sep 2014, Cui 12218 (BJFC 017132) & Cui 12219 (BJFC 017133); 21 Sep 2014, Cui 12241 (BJFC 017155); 24 Aug 2014, Cui 12390 (BJFC 017304).

***Laetiporus xinjiangensis* J. Song, Y.C. Dai & B.K. Cui**
MycoBank: MB821868
Figures 2b, 4

Diagnosis. Differs from other *Laetiporus* species by its pale-buff to clay-pink pileal surface, cream to light-yellow pore surface, large pores (2–3 per mm) and smaller basidiospores (4.5–5 × 3–4.2 μm).

Etymology. *Xinjiangensis* (Lat.): referring to the locality (Xinjiang Autonomous Region) of the type specimens.

Holotype. CHINA. Xinjiang Auto. Reg., Ili Kazak Autonomous Prefecture, Gongliu County, West Tianshan National Nature Reserve, on living tree of *Betula*, 14 Sep 2015, Dai 15953 (BJFC 020054).

Basidiocarps. Annual, sessile to laterally substipitate, imbricate, odour distinctive, taste with acid flavor, fleshy when fresh, crumbly when dry. Pilei flabelliform to dimidiate, applanate, projecting up to 15 cm, 20 cm wide and 3 cm thick. Pileal surface pale-buff to clay-pink when fresh, becoming pale-buff to cream upon drying, glabrous, azonate to faintly zonate when fresh. Margin blunt, clay-buff to greyish-

Figure 2. Basidiomata of *Laetiporus* species. **a** *L. medogensis* **b** *L. xinjiangensis*. Scale bars: **a** = 2 cm, **b** = 3 cm.

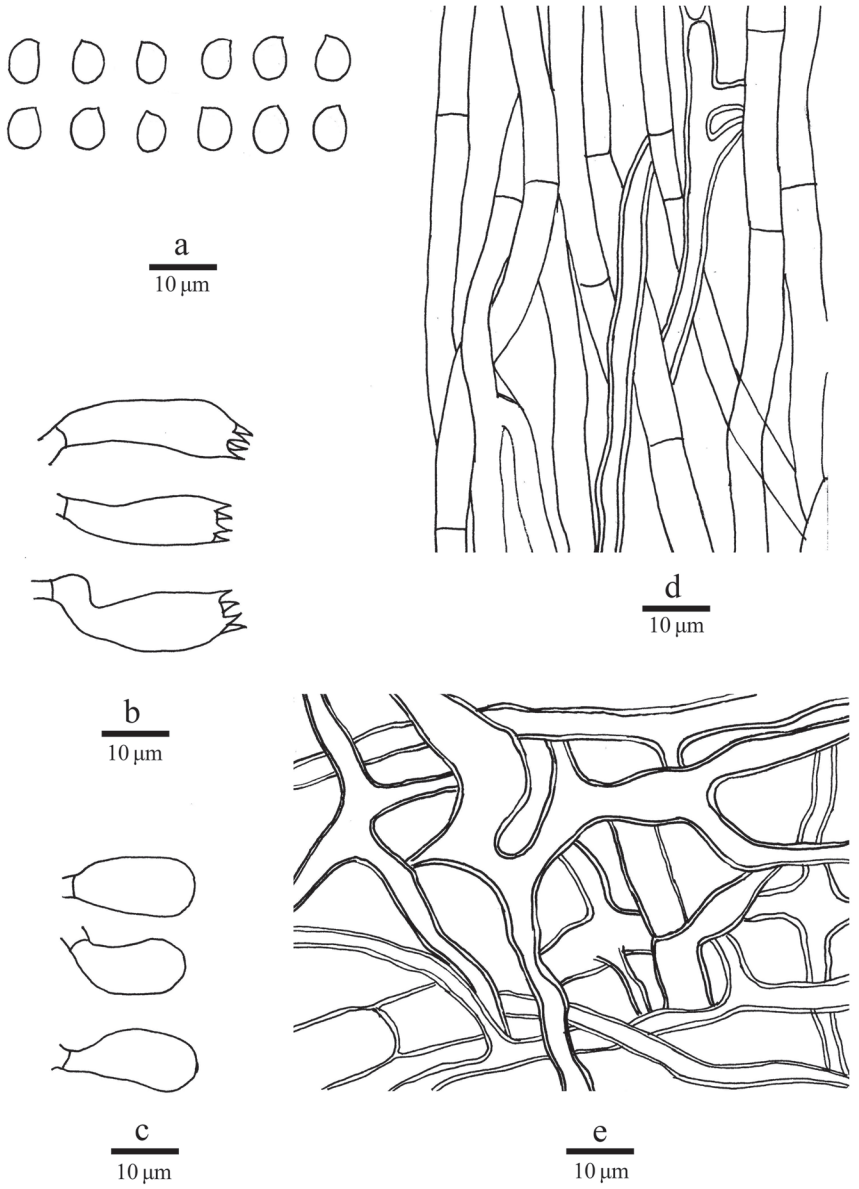

Figure 3. Microscopic structures of *Laetiporus medogensis* (drawn from the holotype). **a** Basidiospores **b** Basidia **c** Basidioles **d** Hyphae from trama **e** Hyphae from context.

brown to brown when juvenile, fading to dark brown when dry. Pore surface cream to light yellow when fresh, becoming pale yellow when dry; sterile margin pale yellow when fresh, up to 2 mm wide; pores angular, 2–3 per mm; dissepiments thin, entire to lacerate. Context white when fresh, becoming cream to pale yellow when dry, up to 2.2 cm thick. Tubes concolorous with pore surface, crumbly or chalky, up to 8 mm long.

Hyphal structure. Hyphal system dimitic; generative hyphae simple-septate; skeletal hyphae IKI–, CB–, dissolving in KOH. Generative hyphae in context infrequent, hyaline, thin-walled, occasionally branched, up to 11 μm in diam.; skeletal hyphae in context dominant, hyaline, thick-walled with a wide lumen, frequently branched and interwoven, occasionally simple-septate, 8–15 μm in diam. Generative hyphae in tubes dominant, hyaline, thin-walled, frequently branched, simple-septate, 4–6 μm in diam.; skeletal hyphae in tubes thick-walled with a wide lumen, occasionally branched and simple-septate, subparallel along the tubes or interwoven, 3–5 μm in diam.

Cystidia. Cystidia and other sterile hyphal elements absent.

Basidia. Basidia clavate, 20–25 × 6–8 μm, bearing four sterigmata and a basal simple-septum; basidioles clavate, smaller than basidia.

Spores. Basidiospores ellipsoid to ovoid, hyaline, thin-walled, smooth, IKI–, CB–, 4.5–5 × 3–4.2 μm, L = 4.87 μm, W = 3.65 μm, Q = 1.33–1.37 (n = 60/2).

Additional specimens (paratypes) examined. CHINA. Xinjiang Auto. Reg., Shihezi, on living tree of *Populus*, 9 Sep 2015, Dai 15825 (BJFC 019930) & Dai 15828 (BJFC 019931); Burqin County, on living tree of *Salix*, 9 Sep 2015, Dai 15836 (BJFC 019937) & Dai 15838 (BJFC 019939); Burqin County, Kanas Integrated Nature Landscape Protect Region, on living tree of *Salix*, 11 Sep 2015, Dai 15893 (BJFC019994); Ili Kazak Autonomous Prefecture, on living tree of *Populus*, 13 Sep 2015, Dai 15902 (BJFC 020003) & Dai 15905 (BJFC 020006); 4 Oct 2015, Dai 15898A (BJFC 019999).

Discussion

Recent studies indicated that *Laetiporus sulphureus* in East Asia is a species complex, comprising several morphologically and ecologically distinct species (Ota et al. 2009, Song et al. 2014). The current study recognised two new species, namely, *L. medogensis* and *L. xinjiangensis* and, altogether, eight *Laetiporus* species have been found in China thus far. The multi-gene phylogenetic topology showed that the new species formed two separate lineages (Figure 1).

Laetiporus medogensis and *L. ailaoshanensis* group together with moderate to low MP and ML support (50% MP and 53% ML). Both *L. medogensis* and *L. ailaoshanensis* are found in Southwest China. Morphologically, *L. ailaoshanensis* is similar to *L. medogensis* by producing orange to yellow pileal surface, white context and ellipsoid to ovoid basidiospores. However, *L. medogensis* is found on conifers and the pore surface is yellow; *L. ailaoshanensis* grows on hardwoods and has a white pore surface (Song et al. 2014). *L. sulphureus* resembles *L. medogensis* by producing yellow to orange pileal surface and yellow pore surface; however, *L. sulphureus* usually grows on hardwoods and produces thicker basidiocarps and larger basidiospores (5–7 × 4–5 μm; Ota et al. 2009). *L. versisporus* and *L. medogensis* share similar characters including yellow pileal surface, yellowish pore surface and ovoid to ellipsoid basidiospores; however, *L. versisporus* differs from *L. medogensis* in having smaller pores (2–6 per mm) and larger basidiospores (4–6.8 × 3–5.5 μm). In addition, *L. versisporus* grows on hardwoods and is mainly distributed in subtropical to tropical areas (Ota et al. 2009, Song and Cui 2017).

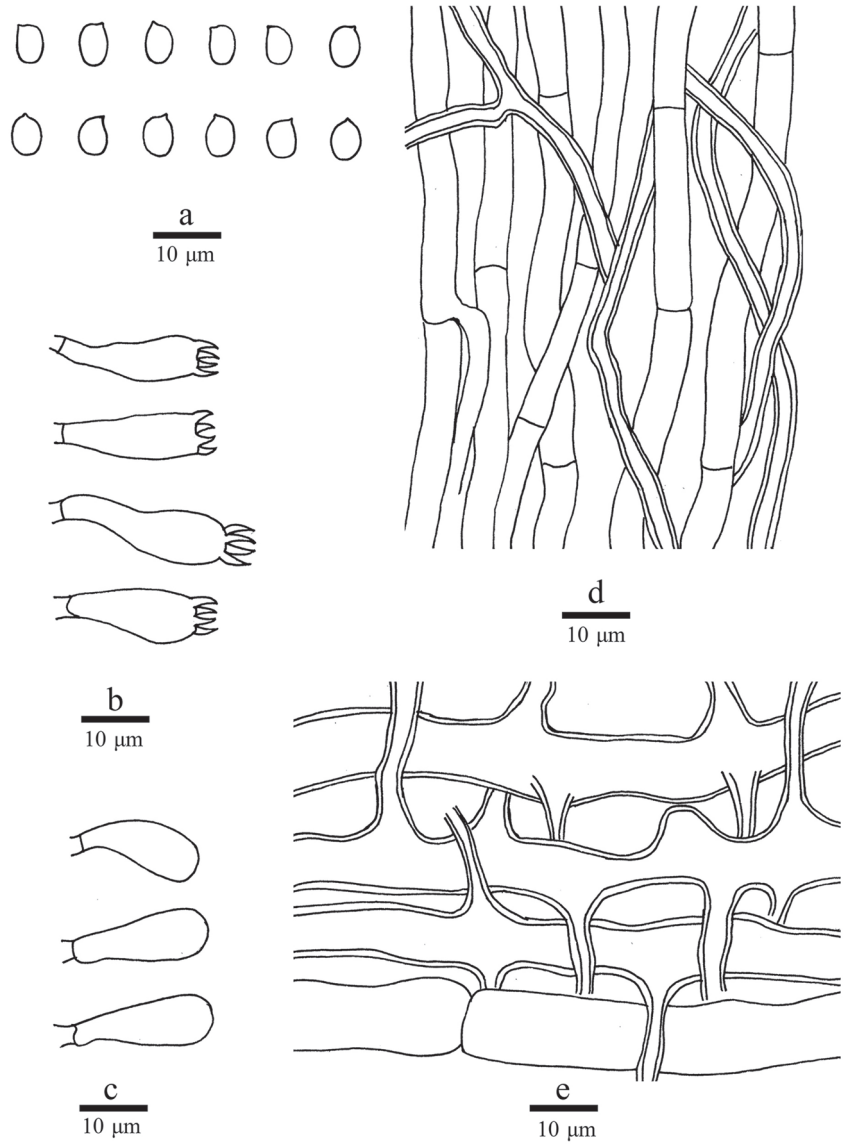

Figure 4. Microscopic structures of *Laetiporus xinjiangensis* (drawn from the holotype). **a** Basidiospores **b** Basidia **c** Basidioles **d** Hyphae from trama **e** Hyphae from context.

Laetiporus xinjiangensis, *L. sulphureus* and *L. montanus* are all common in Northwest China. Both *L. xinjiangensis* and *L. sulphureus* grow on angiosperms and group together in the phylogenetic tree with moderate MP, ML and significant BI support (65% MP, 51% ML and 0.98 BPP). Morphologically, *L. sulphureus* is similar to *L. xinjiangensis* in having yellowish pore surface and ovoid to ellipsoid basidiospores; however, *L. sulphureus* produces larger basidiospores (5–7 × 4–5 μm) and has smaller pores (2–5 per mm; Burdsall and Banik 2001). *L. montanus* is similar to *L. xinjiangensis* by

producing a burlywood pileal surface and a yellowish pore surface; however, *L. montanus* differs by producing pyriform basidiospores (6–8 × 4–5.5 µm) and by growing on gymnosperms (Tomšovský and Jankovský 2008).

Our research expanded the number of *Laetiporus* species to 17 around the world. However, studies in the Southern Hemisphere are still few and the relationships amongst *Laetiporus* species remain unresolved (Lindner and Banik 2008, Pires et al. 2016, Song and Cui 2017). More comprehensive studies on *Laetiporus* depend on more collections and data from poorly sampled areas. The main morphological characters, host trees and distribution areas of species in the *L. sulphureus* complex are provided in Table 2. An identification key to the known species of *Laetiporus* is provided.

Key to accepted species in the *Laetiporus sulphureus* complex

1 Pore surface light goldenrod to sulphur yellow or light yellow when fresh ... **2**
– Pore surface cream to white when fresh ... **10**
2 Occurring on conifers ... **3**
– Occurring on hardwoods ... **6**
3 Distributed in cool temperate to boreal zones in East Asia and Europe ... **4**
– Distributed in North America ... **5**
4 Basidiospores 6–8 × 4–5.5 µm ... ***L. montanus***
– Basidiospores 5–6.2 × 4.2–5.2 µm ... ***L. medogensis***
5 Basidiospores 5–7 × 3.8–5 µm; distributed in eastern North America ... ***L. huroniensis***
– Basidiospores 6.5–8 × 4–5 µm; distributed in far western North America ... ***L. conifericola***
6 Pores 4–5 per mm ... ***L. caribensis***
– Pores 2–4 per mm ... 7
7 Basidiocarps single, occasionally imbricate but not in large clusters; anamorphic form frequently produced ... ***L. versisporus***
– Basidiocarps imbricate, rarely single; no anamorphic form or rarely produced ... **8**
8 Basidiospores 4.5–5 × 3–4.2 µm ... ***L. xinjiangensis***
– Basidiospores 5–7 × 3–5.5 µm ... **9**
9 Distributed in temperate zones ... ***L. sulphureus***
– Distributed in temperate to tropical zones ... ***L. gilbertsonii***
10 Basidiocarps arising from soil or surface of roots near the base of living trees ... ***L. cincinnatus***
– Basidiocarp arising from trunks of standing trees or on fallen logs ... **11**
11 Distributed in mountain forests of subtropical zones ... ***L. ailaoshanensis***
– Distributed in cool temperate to boreal zones ... **12**
12 Pileal surface cream to white, pores 3–6 per mm ... ***L. zonatus***
– Pileal surface light orange to reddish-orange, pores 2–4 per mm ... ***L. cremeiporus***

Table 2. The main morphological characters, host trees and distribution areas of species in the *Laetiporus sulphureus* complex.

Species	Pileal surface	Pore surface	Pores	Basidiospores	Distribution	Host	References
L. ailaoshanensis	orange yellow to reddish orange	cream to buff	3–5/mm	ovoid to ellipsoid 5.0–6.2 × 4.0–5.0 µm	subtropical areas of south-western China	*Lithocarpus*	Song et al. 2014
L. caribensis	orange to pale orange	lemon yellow	4–5/mm	ellipsoid 4.0–4.5 × 2.7–3.6 µm	tropical zones of the Caribbean basin and central America	*Guarea guidonia*, *Dacryodes*	Banik et al. 2012
L. cincinnatus	bright salmon orange	pale cream	2–4/mm	broadly ovoid 4.5–5.5 × 3.5–4.0 µm	throughout the eastern USA except for in the states along the Gulf of Mexico, common in the Great Lakes regions	arising from the soil (*Quercus*)	Burdsall and Banik 2001
L. conifericola	bright orange to salmon orange	lemon yellow to bright creamy yellow	2–4/mm	broadly ovoid 6.5–8.0 × 4.0–5.0 µm	western North America from California to Alaska	*Tsuga*, *Picea*, *Abies*, *Pinus*	Burdsall and Banik 2001
L. cremeiporus	light orange to reddish-orange	yellowish-white to cream	2–4/mm	ovoid to ellipsoid 5.6–7.0 × 3.9–4.7 µm	cool temperate to boreal areas of East Asia	*Quercus*, *Pyrus*, *Prunus*	Ota et al. 2009
L. gilbertsonii	pale salmon orange or pale pinkish-orange	lemon yellow to pale lemon yellow (in West USA) or isabelline to nearly white (in Southeast USA)	2–4/mm	broadly ovoid 5.0–6.5 × 3.5–4.5 µm	North America, Central and South America	*Eucalyptus*, *Quercus*, *Prunus*	Burdsall and Banik 2001, Banik et al. 2012
L. huroniensis	bright orange	lemon yellow	2–4/mm	broadly ovoid 5.0–7.0 × 4.2–5.0 µm	eastern North America and in its Great Lakes areas	*Tsuga*	Burdsall and Banik 2001
L. medogensis	pinkish-buff to clay-buff	buff-yellow	2–4/mm	ellipsoid to ovoid 5–6.2 × 4.2–5.2 µm	cool temperate areas of south-western China	*Abies*	in the present study
L. montanus	light orange to reddish-orange	bright sulphurous yellow	1–4/mm	pyriform or ovoid to ellipsoid 6.0–8.0 × 4.0–5.5 µm	boreal zones in north-eastern China and in mountain areas of Japan and Central Europe	*Picea*, *Larix*, *Abies*	Tomšovský and Jankovský 2008, Ota et al. 2009
L. sulphureus	bright salmon orange	lemon yellow	2–4/mm	ovoid to ellipsoid 5.0–6.8 × 4.0–5.0 µm	North America, Europe and South America	*Acer*, *Salix*, *Gleditisa*, *Quercus*, *Fraxinus*, *Castanea*, *Salix*	Burdsall and Banik 2001
L. versisporus	whitish to sulphur yellow	usually yellow, sometimes pale yellow to nearly white	3–6/mm	ovoid to short ellipsoid 4.0–6.8 × 3.0–5.5 µm	cool temperate to tropical areas of East Asia	*Robinia*, *Castanea*, *Quercus*, *Elaeocarpus*, *Castanopsis*	Núñez and Ryvarden 2001, Ota et al. 2009
L. xinjiangensis	pale-buff to clay-pink	cream to light yellow	2–3/mm	ellipsoid to ovoid 4.5–5 × 3–4.2 µm	temperate areas of western China	*Betula*, *Populus*, *Salix*	in the present study
L. zonatus	white to cream and buff to clay-buff at base	white to cream	2–5/mm	ellipsoid to pyriform or drop-shaped 5.8–7.2 × 4.3–5.5 µm	high mountains of temperate areas of south-western China	*Quercus*	Song et al. 2014

Acknowledgements

Special thanks are due to Dr. Xiao-Lan He (Soil and Fertilizer Research Institute, Sichuan Academy of Agricultural Sciences, China) and Dr. Xiao-Yong Liu (Institute of Microbiology of the Chinese Academy of Sciences, China) for help in collecting specimens. The research is supported by the Fundamental Research Funds for the Central Universities (No. 2016ZCQ04) and the National Natural Science Foundation of China (Project Nos. 31750001, 31670016).

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MycoKeys 37: 73–92 (2018)
doi: 10.3897/mycokeys.37.26420
http://mycokeys.pensoft.net

RESEARCH ARTICLE

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Amanita tullossiana, a new species, and two new records of *Amanita* section *Lepidella* from north-western Himalaya, India

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Academic editor: *M. P. Martín* | Received 4 May 2018 | Accepted 16 July 2018 | Published 31 July 2018

Citation: Hosen MI, Mehmood T, Das K, Kudzma LV, Bhatt RP (2018) *Amanita tullossiana*, a new species, and two new records of *Amanita* section *Lepidella* from north-western Himalaya, India. MycoKeys 37: 73–92. https://doi.org/10.3897/mycokeys.37.26420

Abstract

Amanita tullossiana, a new species of *Amanita* [subgenus *Lepidella*] section *Lepidella* from India is described. The species is characterised by its ash grey to brownish-grey pileus covered with dark grey to greyish-black universal veil remnants, the upper part of its rooting stipe base covered by several rows of recurved scales, broadly ellipsoid to ellipsoid basidiospores, absence of basidial clamp connections and pileal remnants of universal veil comprising abundant, disordered inflated cells intermixed with scattered filamentous hyphae. Molecular phylogenetic analysis and morphology both support the association of *A. tullossiana* with species of Bas' stirps *Cinereoconia* – *A. cinereoconia* and *A. griseoverrucosa*. Two species, *A. griseoverrucosa* and *A. virgineoides* are reported here as new records for India.

Keywords

Amanitaceae, Basidiomycota, nrLSU, South Asian taxa, taxonomy

This article is dedicated to Dr. Rodham E. Tulloss for his contribution to mycology especially in the family Amanitaceae.

* Md. Iqbal Hosen and Tahir Mehmood have made equal contribution to this work.

Introduction

The Amanitaceae is one of the most dominant and species-rich families of Basidiomycota. Traditionally, this family is divided into three genera, namely *Amanita* Pers., *Limacella* Earle and *Catatrama* Franco-Mol. However, a recent study by Redhead et al. (2016) divided *Amanita* into two genera, *Amanita* and *Saproamanita* Redhead, Vizzini, Drehmel & Contu, the former genus including species which are mycorrhizal in nature and the latter genus including only amycorrhizal/free-living species within *Amanita*. Subsequent to their establishment of the new genus, Tulloss et al. (2016) argued against the separation of *Saproamanita* from *Amanita* because the amycorrhizal species do not form a well-supported clade and are arguably the "mother" of the genus *Amanita* rather than a sister group within it. In this study, we follow the interpretation of Tulloss et al. (2016).

The Amanitaceae is characterised by longitudinally acrophysalidic stipe tissue. The agaricoid species in the genus *Amanita* are characterised by their schizohymenial development, which is evidence in mature basidiomata by their sterile lamella margin (Bas 1969, Thongbai et al. 2016, Tulloss et al. 2016, Bhatt et al. 2017).

The genus *Amanita* is divided into two subgenera: a) *Amanita* Pers. and b) *Lepidella* (E.-J. Gilbert) Veselý based on the reaction of basidiospore walls to Melzer's reagent, the former having a negative reaction (inamyloid) and the latter having a positive reaction (amyloid) to that reagent (Corner and Bas 1962, Bas 1969, Yang 1997). The subg. *Lepidella* is further divided into four sections: i) sect. *Amidella* (J.-E Gilbert) Veselý, ii) sect. *Lepidella sensu* Bas (1969), iii) sect. *Phalloideae* (Fr.) Quél. and iv) sect. *Validae* (Fr.) Quél.

Species within *Amanita* sect. *Lepidella* are recognised by the combination of the following features: non-striate and appendiculate pileus margin and a volva that is friable, not forming an entire membranous sac (with the rare exception of a thin submembranous or membranous exterior layer). Approximately 200 taxa are listed for this section in the Amanitaceae website (http://www.amanitaceae.org/), of which 185 have been validly published (Corner and Bas 1962, Bas 1969, Tulloss and Jenkins 1985, Tulloss et al. 1992, Yang 1997, Wolfe et al. 2012, Deng et al. 2014, Cai et al. 2014, Li and Cai 2014, Hosen et al. 2015, Tulloss and Yang 2018). However, only four species, namely *A. albofloccosa* A.V. Sathe & S.D. Deshp., *A. berkeleyi* (Hooker f.) Bas, *A. eriophora* (Berk.) E.-J. Gilbert and *A. konkanensis* P.G. Sathe & S.M. Kulk. of *Amanita* sect. *Lepidella* have been reported from India so far (Bas 1969, Sathe and Daniel 1981, Kulkarni 1992).

During the course of macrofungal forays into different parts of the state of Uttarakhand, India, the second author (TM) collected several specimens of *Amanita* in broad-leaved forests. Morphological examination and molecular data indicated that the new collections herein reported represent one species new to science and two new records for India.

Materials and methods

Morphological study

Macromorphological characteristics were documented in the forest or base camp from fresh and dissected young to mature basidiomata. Photography was accomplished using a digital camera (Sony cyber-shot W730 and Cannon Power Shot SX 50). Colour codes follow Kornerup and Wanscher (1978). Samples were dried using an electric drier. Herbarium codes follow Index Herbariorum (Thiers 2018).

Micromorphological characteristics were observed with a compound microscope (Olympus CH20i) with dried material mounted in 5% KOH, 1% Phloxin, Melzer's reagent and 1% Congo red. To present basidiospore measurements, the following notation was used: "[*n*/*m*/*p*]" indicating *n* basidiospores were measured from *m* basidiomata of *p* collections with a minimum of 20 basidiospores from each collection. Biometric variables followed those in Tulloss and Lindgren (2005): **L** = the range of the average spore length computed per specimen examined. **L'** = the average spore length computed for all spores measured. **W** = the range of the average spore width computed per specimen examined. **W'** = the average spore width computed for all spores measured. **Q** = the ratio of length/breadth for a single spore and the range of the ratio of length/breadth for all spores measured. **Q** = the average value of **Q** computed for one specimen examined and the range of such averages. **Q'** = average value of **Q** computed for all spores measured. w_{cs} = the width of the central stratum of a lamella. w_{st}-*near* = the distance from an outer margin of the central stratum to the nearest base of a basidium. w_{st}-*far* = the distance from an outer margin of the central stratum to the furthest base of a basidium on the same side of the central stratum. Drawings of microscopic features were made free hand.

Molecular study

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from dry basidiomata following the modified CTAB method of Doyle and Doyle (1987). PCR was performed to amplify the partial sequence of the nuclear ribosomal large subunit (nrLSU) using universal primer pairs LR0R (GTACCCGCTGAACTTAAGC) and LR5 (ATCCTGAGGGAAACTTC) LR7 (TACTACCACCAAGATCT) (Vilgalys and Hester 1990) and the second largest subunit of RNA polymerase II (*rpb2*) using primer pair fRPB2-5F (GAYGAYMGWGATCAYTTYGG) (Liu et al. 1999) and bRPB2-7.1R (GCHATGGGKAARCARGCYATGGG) (Matheny 2005). Sequencing was performed on ABI 3730 XL DNA Analyzer (Applied Biosystems). PCR amplification (both nrLSU and

rpb2) was conducted on a thermal cycler (Eppendorf, Germany) programmed for 3 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 1 min at 55 °C, 1 min at 72 °C and a final stage of 8 min at 72 °C. The PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany). Both strands of the PCR fragment were sequenced on the 3730*xl* DNA Analyzer (Applied Biosystems, USA) using the same primer pair.

Phylogenetic analyses

In this study, a dataset of 49 nrLSU sequences of *Amanita* subg. *Lepidella* and one nrLSU sequence of *Limacella bangladeshana* Iqbal Hosen were used for phylogenetic analysis. The nrLSU sequences of Amanitaceae were selected based on BLASTn search results (Altschul et al. 1997) and availability of sequences of Amanitaceae in GenBank (Clark et al. 2016). The nrLSU dataset was then aligned with Mafft v.6.8 (Katoh et al. 2005) and manually adjusted with BioEdit v.7.0.9 (Hall 1999) using default settings. Maximum Likelihood (ML) phylogenetic analysis inferred from nrLSU sequences was performed using RAxML v.7.2.6 (Stamatakis 2006). Default settings were used for all parameters in the ML analysis and statistical support values were obtained using non-parametric bootstrapping with 1,000 replicates. Gaps in the alignment were treated as missing data in the phylogenetic analysis. *Limacella bangladeshana* was selected as the outgroup for the molecular phylogenetic analysis.

Results

Molecular phylogenetic results

In this study, five sequences (three for nrLSU and two for *rpb2*) were generated from three separate collections (RET 717-4, RET 717-9 and TM 16-1228) of *Amanita* and deposited in GenBank (Table 1). Only nrLSU sequences were used in this study to delimit the Indian *Amanita* species. The *rpb2* sequences were not used for reconstruction of molecular phylogeny because *rpb2* sequences for most of the *Amanita* species (included in the nrLSU phylogeny) are currently unavailable in GenBank for inclusion in this study. The aligned nrLSU dataset consisted of 50 sample sequences of Amanitaceae (Table 1) with 934 nucleotide sites for each sample (gaps included), of which 238 were parsimony informative characters. The resulting dataset was deposited in TreeBASE (S21668). Initial BLASTn search result of the nrLSU sequence of the Indian collection (RET 717-4) against the NCBI database exhibited 98% identity with *A. cinereopannosa* Bas (GenBank HQ539678) and 97% with *A. cinereoconia* G.F. Atk. (GenBank HQ593118). Phylogenetically, the collection RET 717-4 is grouped together with *A. cinereopannosa*, *A. cinereoconia* and *A. griseoverrucosa* Zhu L. Yang with strong bootstrap (BS) support (Fig. 1). Morpho-

Table 1. Taxa of Amanitaceae included in molecular phylogenetic analysis.

Name of the species	Herbarium voucher/collection/ collector number	Geographic location	GenBank accession number	
			nrLSU	*rpb2*
Amanita afrospinosa	RET 347-1	Zimbabwe	HQ539666	–
Amanita afrospinosa	RET 347-1	Zimbabwe	HQ539666	–
Amanita amanitoides	RET 344-9	Zambia	HQ539668	–
Amanita amerivirosa	RET 628-2	USA	KY924826	–
Amanita sp.	TM 16-1247	India	MF375478	–
Amanita armillariiformis	DAOM216919	USA	AF261436	–
Amanita atkinsoniana	RET 301-1	USA	HQ539670	–
Amanita brunnescens	BW_HP12	USA	HQ539674	–
Amanita cinereoconia	BW_PSF	USA	HQ593118	–
Amanita cinereopannosa	RET 319-8	USA	HQ539678	–
Amanita cinereovelata	HKAS 81647*	Bangladesh	KP259291	–
Amanita cokeri	BW-STF 090506-19	USA	HQ539682	–
Amanita conicoverrucosa	–	–	AY194983	–
Amanita costaricensis	RET 330-4	Costa Rica	KP258990	–
Amanita daucipes	RET 386-8	USA	HQ539688	–
Amanita eriophora	RET 350-4	Cambodia	HQ539672	–
Amanita excelsa	Ge 816	China	HQ539691	–
Amanita fritillaria	HKAS 29511	China	AF024452	–
Amanita fuliginea	HKAS 32521	China	AF024454	–
Amanita grallipes	RET 379-5	Brazil	HQ539700	–
Amanita griseoverrucosa	HKAS 38459	China	AY436495	–
Amanita griseoverrucosa	TM 16-1228	India	**MF359828**	–
Amanita heishidingensis	HKAS 76122*	China	KC429045	–
Amanita japonica	HMAS 59778	China	AF024460	–
Amanita kotohiraensis	MHHNU 6998	China	FJ011681	–
Amanita lavendula	RET 339-7	Canada	KR865979	–
Amanita longipes	RET 360-1	USA	HQ539704	–
Amanita magniverrucata	RET 594-10	USA	KR919774	–
Amanita macrocarpa	31939L	China	KC408378	–
Amanita nauseosa	DPL 6117	USA	HQ539715	–
Amanita ochrophylla	PSC1127	Australia	HQ539715	–
Amanita onusta	RET 297-3	USA	HQ539718	–
Amanita peckiana	RET 320-3	USA	HQ539720	–
Amanita phalloides	Ben Woo (WTU)	USA	AY380359	–
Amanita proxima	RET 290-10	France	HQ539728	–
Amanita polypyramis	BW_CC	USA	HQ593122	–
Amanita rufobrunnescens	GDGM 42374*	China	KT865210	–
Amanita sepiacea	HKAS 38716	China	AY436501	–
Amanita smithiana	RET 382-6	USA	HQ539740	–
Amanita solitaria	RET 298-1	France	HQ539741	–
Amanita subjunquillea	HKAS 24169	China	AF024479	–
Amanita tephrea	RET 378-9	USA	HQ539751	–
Amanita tullossiana	RET 717-4*	India	**MF945577**	**MH638335**#
Amanita vestita	HKAS 77277	China	KC429044	–
Amanita virgineoides	RET 717-9	India	**MF945578**	**MH638336**#
Amanita virgineoides	HKAS 79691	China	KJ466495	–
Amanita virgineoides	HKAS 77278	China	KC429043	–
Amanita virgineoides	HKAS 18394	China	AF024484	–
Amanita virosa	RET 291-3	USA	KY924846	–
Limacella bangladeshana	Iqbal-276*	Bangladesh	KR816668	–

Newly generated sequences are highlighted in bold. *Holotype. #sequences (*rpb2*) derived from the new collections were not used in the phylogenetic tree but provided for future references. (–) indicates information is not available or not used in this study.

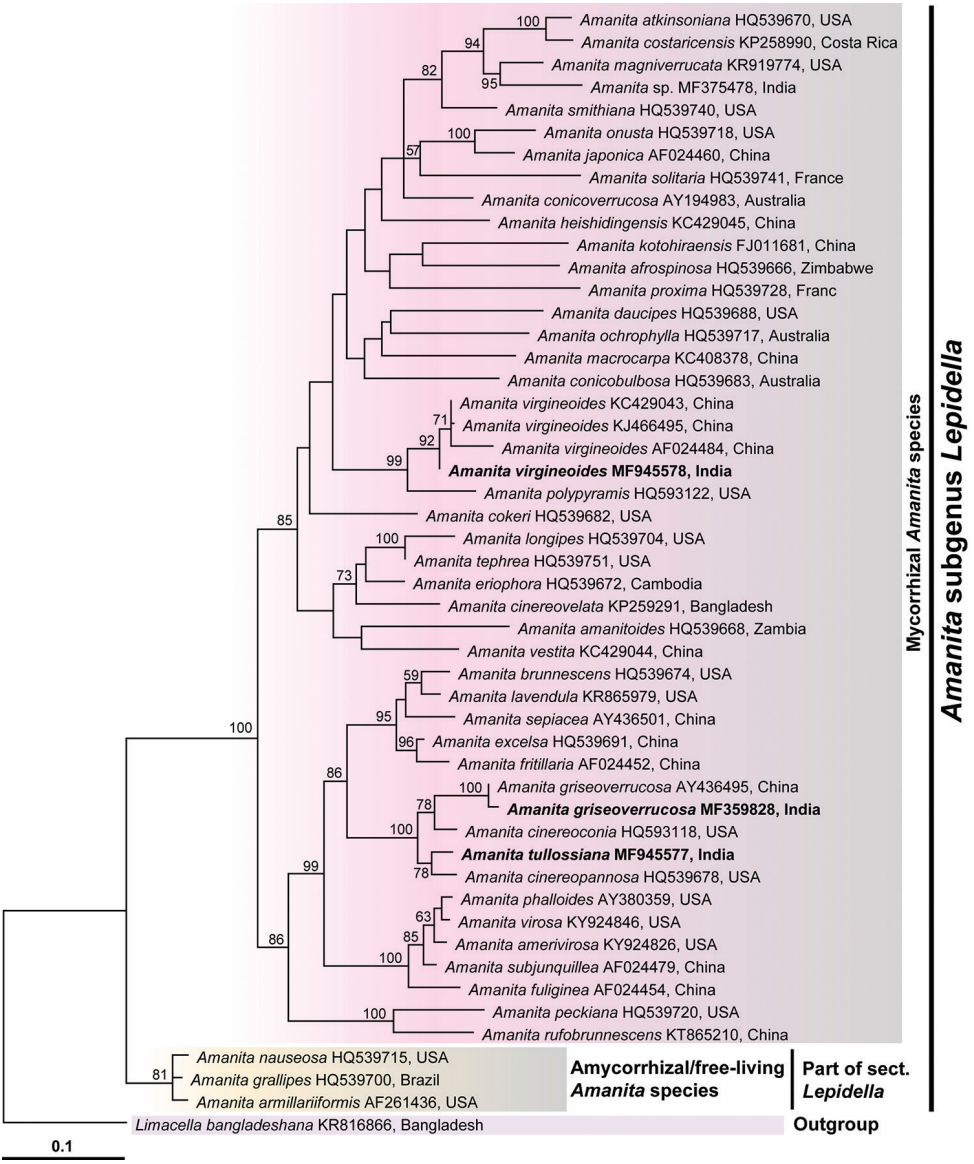

Figure 1. Phylogenetic relationships of *Amanita tullossiana*, *A. griseoverrucosa* and *A. virgineoides* inferred from nrLSU sequences using the Maximum Likelihood (ML) method. Bootstrap support values (≥50%) obtained from maximum likelihood (ML) analysis are shown above or beneath the branches at nodes. *Amanita tullossiana*, *A. virgineoides* and *A. griseoverrucosa* from India are highlighted in bold on the tree. GenBank accession numbers are provided after each species name and followed by country of origin.

logical characterisation [using the keys of Bas (1969)] and phylogenetic inference indicate the new collection (RET 717-4) is an independent species in *Amanita* [sect. *Lepidella* subsect. *Solitariae* Bas] stirps *Cinereoconia* of Bas (1969). Another two collections TM 16-1228 and RET 717-9 are reported here as *A. griseoverrucosa* and A.

virgineo*ides* Bas, respectively—new records to India. Phylogenetically, the former species is clustered with *A. cinereoconia*, *A. cinereopannosa* and *A. tullossiana* with strong support (100% ML BS); and the latter species is clustered with *A. polypyramis* (Berk. & M.A. Curtis) Sacc. (GenBank HQ593122, HQ539723) with strong support (99% ML BS) (Fig. 1).

Taxonomy

Amanita tullossiana **Mehmood, Iqbal Hosen, K. Das & R.P. Bhatt, sp. nov**.
MycoBank MB822821
Figs 2, 3

Typification. INDIA, Uttarakhand, Rudhraparyag district, Baniyakund, at 2655 m a.s.l., 30°28.998N, 79°10.658E, 26 August 2014, T. Mehmood, TM 14-475 (RET 717-4, holotype; CAL 1611, isotype).

Etymology. The epithet "*tullossiana*" (Lat., "of Tulloss") is proposed in honour of Dr. Rodham E. Tulloss for his contribution to the study of the genus *Amanita* all over the world.

Diagnosis. Distinct from all the known species of *Amanita* stirps *Cinereoconia* by the combination of the following characters: medium-sized to large basidiomata (pileus 90–170 mm wide, stipe 150–185 × 20–25 mm); brownish-grey to dark grey pileus covered with floccose to subfelted, pulverulent patches of universal veil remnants; broadly ellipsoid to ellipsoid basidiospores measuring (8.5–)9–13(–13.5) × (5.8–)6–8(–8.5) µm.

Description. *Basidiomata* medium-sized to large. *Pileus* 90–170 mm wide, initially hemispherical then convex to plano-convex and finally planar, shiny, slightly viscid when moist, ash grey (1B2), pastel grey (1C1), grey (4B1-4C1), brownish-grey, brownish-beige (6F2-3) to dark grey (1F1), slightly darker at centre; context 11–14 mm thick above stipe, white (1A1), thinning evenly toward margin, unchanging when cut or bruised. *Universal veil on pileus* as floccose to subfelted pulverulent patches, dark grey (1F1) to brownish-grey (6F2), greyish-black to dark grey (1F1), soft, up to 4 mm thick, 7–12 mm wide, irregularly distributed. *Lamellae* 6–10 mm broad, free to narrowly adnate, crowded, white (1A1), unchanging when injured; lamellulae, plentiful of several lengths, attenuate, truncate, with 8–9 lamellae per cm at margin. *Stipe* 150–185 × 20–25 mm (excluding bulb), attenuate upwards, upper part covered by dark grey (1F1) fibrils, lower part covered with recurved scales, with fibrils turn blackish when handled; context solid, white, unchanging on cutting or bruising. *Partial veil* superior, soft, cottony, white, easily collapsed or detachable. *Bulb* 70–88 × 25–41 mm, napiform to rooting, covered with brownish-grey (6F2) to dark grey (1F1) universal veil remnants, often upper part covered with grey (4B1) to dark grey (1F1) recurving scales. *Odour* indistinct, *taste* not observed. *Spore deposit* white.

Basidiospores [300/15/10] (8.5–)9–13(–13.5) × (5.8–)6–8(–8.5) µm, [**L** = 9.5–11 µm, **L'** = 10.54 µm; **W** = 6–7.5 µm, **W'** = 6.83 µm; Q = (1.29–)1.40–1.66(–1.83), **Q** = 1.38–1.59, **Q'** = 1.54], broadly ellipsoid to ellipsoid, hyaline, thin-walled, smooth, amyloid; contents monoguttulate; apiculus lateral to sublateral, up to 1 µm long. *Basidia* 45–55(–65) × 9–14 µm, 2 to 4-spored, thin-walled; sterigmata up to 4 µm long; basal clamp connections absent. *Lamellar edge tissue* sterile, mainly composed of inflated globose to subglobose cells 20–35 × 15–25 µm and clavate to subclavate cells 40–50 × 15–18 µm. *Subhymenium* 40–50 µm thick, with 3–4 layers of inflated cells, w_{st}*-near* = 35–50 µm, w_{st}*-far*= 50–70 µm, basidia arising from small inflated cells 8–15 × 6–10 µm wide. *Hymenophoral trama* bilateral, divergent; w_{cs}= 60–80 µm; well rehydrated, filamentous, undifferentiated hyphae 3–8 µm wide; with lateral stratum composed of intercalary inflated cells 66–110 × 12–19 µm wide; vascular hyphae 9–14 µm. *Pileipellis* 140–195 µm thick, in two layers, with gelatinised colourless suprapellis (45–55 µm) thick, filamentous, undifferentiated hyphae subradially arranged; subpellis (95–140 µm) thick; filamentous, undifferentiated hyphae 2–6 µm wide, densely arranged in subpellis, with yellowish-brown intracellular pigment; vascular hyphae 7–10 µm wide, infrequent. *Pileus context* filamentous, undifferentiated hyphae 2–6 µm wide, thin-walled, hyaline, interwoven; broadly clavate to ellipsoid cells 86–130 × 26–45 µm, thin-walled, hyaline. *Universal veil on pileus* disordered; filamentous, undifferentiated hyphae 2–6 µm wide, branched, thin-walled, infrequent to scattered, with pale yellow vacuolar pigments; inflated cells dominantly globose to subglobose 25–88 × 22–70 µm, infrequent broadly ellipsoid to ellipsoid or pyriform 40–60 × 10–13 µm, often in chains of 2–3, with brownish to pale yellow vacuolar pigments; vascular hyphae 6–12 µm wide, frequent. *Universal veil on stipe base* disordered; filamentous, undifferentiated hyphae 2–5 µm wide, branched, thin-walled, scattered, with pale yellow vacuolar pigments; inflated cells dominantly globose to subglobose 30–70 × 25–65 µm, infrequent broadly ellipsoid to elongated cells 30–90 × 12–18 µm, with brownish to pale yellow vacuolar pigments; vascular hyphae 10–14 µm wide, often present. *Partial veil* abundant inflated cells broadly clavate to clavate 50–120 × 16– 29 µm, thin-walled, colourless, hyaline, sometimes with yellowish-brown vacuolar pigments; filamentous, undifferentiated hyphae 3–7 µm wide, dominant, thin walled, hyaline, colourless or sometimes with yellowish-brown pigments; vascular hyphae 4–8 µm wide. *Stipe context* longitudinally acrophysalidic; filamentous, undifferentiated hyphae 5–7 µm wide; acrophysalides 150–230 × 35–56 µm, thin-walled, colourless, hyaline, vascular hyphae not found. *Clamp connections* not observed in any tissues.

Macrochemical tests on fresh basidiomata. 5% KOH - negative on pileus, 2% phenol - negative and FeSO4 crystals - negative on pileus and in stipe context.

Habitat and distribution. Solitary to subgregarious in temperate mixed forest dominated by *Quercus semicarpifolia* and *Abies pindrow*, at 2350–2655 m a.s.l. Currently only known from India.

Additional specimens examined. INDIA, Uttarakhand, Rudraparyag district, Baniyakund, 26 August 2014, T. Mehmood, TM 14-486 (GUH-M-27001); same location, 14 July 2015, T. Mehmood, TM 15-624 (GUH-M-27002); same location, 1

Figure 2. Basidiomata of *Amanita tullossiana* in natural habitat (RET 717-4, holotype; CAL 1611, isotype). **a–d** showing distinctive features of *A. tullossiana* (universal veil remnants, appendiculate pileus margin and recurved scales on the stipe surface).

August 2015, T. Mehmood, TM 15-786 (GUH-M-27003); same location, 2 August 2015, T. Mehmood, TM 15-815 (GUH-M-27004); same location, 8 August 2015, T. Mehmood, TM 15-891 (GUH-M-27005); same location, 30 August 2015, T. Mehmood, TM 15-1017 (GUH-M-27006); same location, 22 July 2016, T. Mehmood, TM 16-1123 (GUH-M-27007); same location, 26 August 2016, T. Mehmood, TM

16-1369 (GUH-M-27008); Nainital district, Mukteshwar 24 August 2016, T. Mehmood, TM 16-1338 (GUH-M-27009).

Commentary. The grey to brownish-grey universal veil, the absence of clamp connections, disordered inflated cells intermixed with scattered filamentous hyphae, together with broadly ellipsoid to cylindrical basidiospores are the key features of sect. *Lepidella* stirps *Cinereoconia* (Bas 1969). Based on the Bas' key, the new taxon could be placed in *Amanita* [sect. *Lepidella* subsect. *Solitariae*] stirps *Cinereoconia*.

In stirps *Cinereoconia*, *A. griseofarinosa* Hongo, *A. lutescens* Hongo, *A. pelioma* Bas, *A. odorata* Beeli, *A. vestita* Corner & Bas, *A. griseovelata* D.A. Reid, *A. pallidoflavescens* Dav. T. Jenkins and A. viri*dissima* Wartchow are all species that should be compared to the morphology of the present taxon. *Amanita griseofarinosa*, originally described from Japan, has a pale yellowish-grey pileus covered with dark coloured, farinose to tomentose universal veil remnants; and subglobose to broadly ellipsoid basidiospores 8.5–10 × 7–9 µm, with a lower **Q'** value = 1.2 (Bas 1969) than the basidiospores of the present taxon. *Amanita lutescens*, originally described from Japan, differs from *A. tullossiana* by its small to medium-sized basidiomata 35–60 mm broad, context turning yellowish when cut or bruised and relatively smaller basidiospores 8–10(–10.5) × 5.5–6.5 µm (Bas 1969). *Amanita pelioma*, originally described from the USA, has a greyish-olive to pale brownish pileus, distinctive brown gills, a volva that bruises a distinctive blue-green and ellipsoid to elongate basidiospores 10–12.5 × 6.5–8 µm, with a higher **Q'** value = 1.65 (Bas 1969) than in the new species. *Amanita odorata*, originally described from the Democratic Republic of Congo, has a greyish olivaceous brown pileus, pinkish-white lamellae and elongate to cylindric basidiospores 9.5–13 × 4.5–5.5 µm, with a **Q** value ranges = 1.55–2.05 (Bas 1969). *Amanita vestita*, originally described from Singapore, has a pale greyish-white pileus covered with small micaceous umber particles, broadly ellipsoid to ellipsoid basidiospores 7.5–9 × 5.5–6.5 µm, with a **Q** value ranges = 1.3–1.35 (Bas 1969) lower than in the new taxon. *Amanita griseovelata*, originally described from Victoria, Australia, has a slate-grey pileus covered pale grey, felty-pruinose universal veil remnants and subglobose to broadly ellipsoid basidiospores 7–10(–11.5) × 6.8–8.5 µm (Reid 1980). *Amanita pallidoflavescens*, originally described from the USA, has a white to silvery white pileus and bears elongate to cylindric basidiospores 8.6–10.2 × 4.7–5.5 µm (Jenkins 1980). *Amanita viridissima*, originally described from Brazil, has a green pileus and stipe, pale lamellae and elongate to cylindric basidiospores 9.8–13 × 5.7–8.3 µm, with a higher **Q'** value =1.82 (Wartchow 2016).

Amanita cinereopannosa, *A. cinereoconia* and *A. griseoverrucosa* are the phylogenetically closely related species to the new species (Fig. 1). However, all of them are distinguished morphologically. *Amanita cinereopannosa*, originally described from USA, has a white to silvery sheen pileus covered with subfelted to subpyramidal warts, abundant filamentous hyphae and ellipsoid to elongated basidiospores (8–)8.8–10(–14.1) × (4.9–)5–6.7(–8.3) µm (Tulloss and Yang 2018). Furthermore, this species is considered endemic to eastern North America and has not been recorded in other parts of the

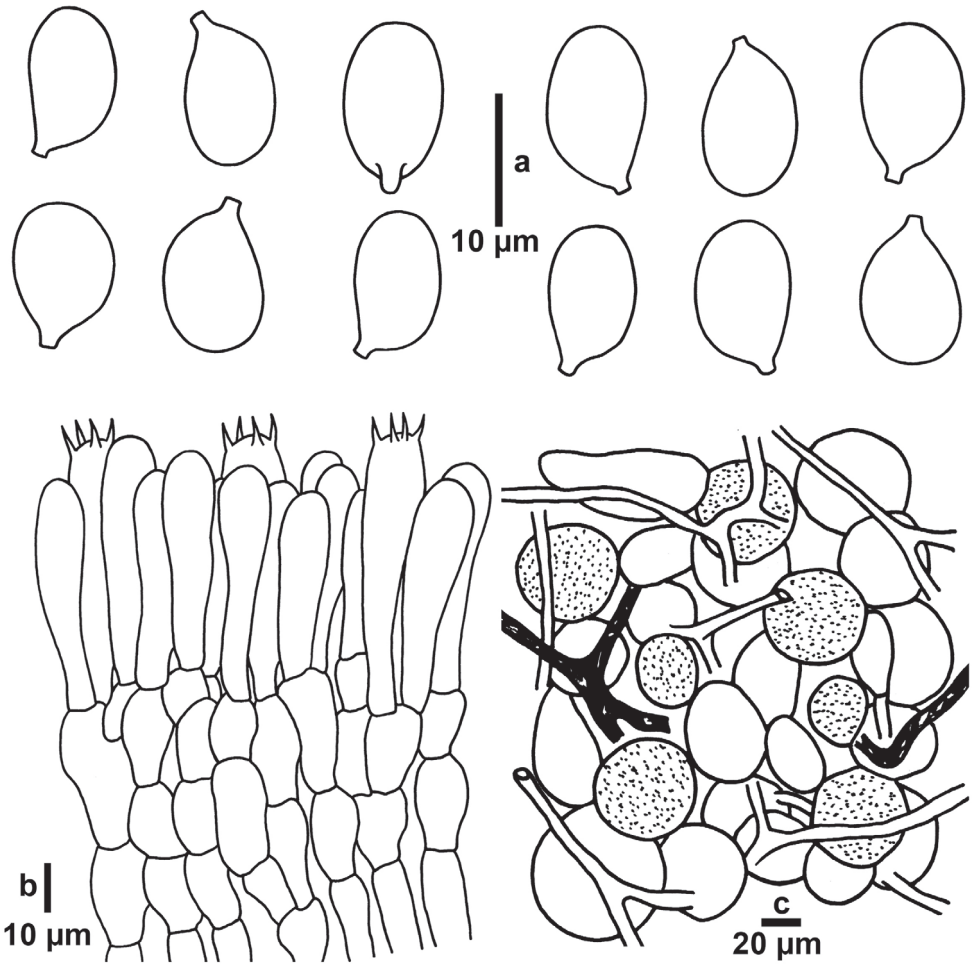

Figure 3. Microscopic features of *Amanita tullossiana* (RET 717-4, holotype; CAL 1611, isotype). **a** Basidiospores **b** Basidia at different stages of development **c** Elements of universal veil from pileus surface (vascular hyphae are dark shaded).

world (Davison et al. 2013). Bas (1969) clearly held *A. cinereopannosa* to be distinct from the species of stirps *Cinereoconia* because he placed it in his stirps *Strobiliformis*. *Amanita cinereoconia*, originally described from the USA, has a white to greyish pileus covered with grey, pulverulent to small warted universal veil remnants and bears elongate to cylindric basidiospores 7.8–10.9 × 4.7–6.2 µm, with a **Q** value = 1.72 (Jenkins 1986). In addition, *A. cinereoconia* has a peculiar smell like "chloride of lime" [meaning the smell of an outdoor pit toilet into which $CaCl_2$ has been added; hence, an odour of decaying protein] or faintly of "chlorine" (Bas 1969; Jenkins 1986). Bas proposed a variety *croceescens* of *A. cinereoconia*; however, Tulloss had the opportunity to observe the transition of a single specimen from the "type variety" to "var. *croceescens*" and attributed the yellow colouration to the *Amanita* "yellowing syndrome"

(Tulloss, pers. comm.). *Amanita griseoverrucosa*, originally described from China and reported here from India (see below), has a dirty white to greyish pileus, verrucose to conical universal remnants, a white to greyish-white stipe, a ventricose to clavate bulb and relatively smaller spores measuring 8–11 × 5.5–7 µm (Yang 2004) in comparison to *A. tullossiana* 9–13 × 6–8 µm.

***Amanita griseoverrucosa* Zhu L. Yang, Bibliotheca Mycologica 170: 155 (1997)**
Figs 4a, b, 5a, b

Description. *Basidiomata* medium-sized to large. *Pileus* 60–125 mm wide, initially hemispherical then convex to plano-convex, dry, slightly viscid when moist, whitish to greyish-white (1B1) to ash grey (1B2) to grey (1D1); context 6–11 mm thick, white (1A1), thinning evenly towards margin, unchanging when cut or bruised. *Universal veil on pileus* as felted to subconical to verrucose, brownish-grey (1D3), greyish-brown (5F3) to dark grey (1F1), soft, up to 4 mm thick, 5–8 mm wide, irregularly distributed; margin non-striate, appendiculate; *Lamellae* free to narrowly adnate, crowded, white (1A1), unchanging, 6–10 mm broad; lamellulae attenuate, plentiful, of several lengths, with 7–8 lamellae per cm at margin. *Stipe* 45–90 × 12–21 mm (excluding bulb), narrowing upwards, solid, lower part covered by light grey (1D1) fibrillose squamules, upper part covered by white farinose squamules; context white, unchanging on cutting or bruising. *Bulb* 32–62 × 19–32 mm, ventricose to clavate, white, covered with grey (1D1) to dark grey (1F1), universal veil remnants. *Partial veil* superior, soft, cottony, white, easily collapsed. *Odour* indistinct, *taste* not observed. *Spore deposit* white.

Basidiospores [80/4/2] (8–) 8.5–10(–11) × (5.5–)6 –6.5 (–7) µm, [**L** =9.05–9.17 µm, **L'** = 9.11 µm; **W** = 5.9–6.5 µm, **W'** = 6.2 µm; **Q** = (1.32–)1.42–1.5(–1.69), **Q** = 1.51–1.54, **Q'** = 1.53], ellipsoid, hyaline, thin walled, smooth, amyloid, apiculus sublateral, up to 1 µm. *Basidia* (34–)45–50(–53) × (9.5–)10–12(–14) µm, 2 to 4-spored, thin-walled, colourless, hyaline; sterigmata up to 4 µm long; basal clamp connections not observed in any tissue after extensive search. *Lamellae edge* sterile; composed of clavate or pyriform inflated cells 35–50 × 22–31 µm, thin walled, colourless, hyaline. *Subhymenium* 35–40 µm thick, w_{st}*-near* = 30–40 µm, w_{st}*-far* = 40–55 µm, basidia arising from subglobose to broadly ellipsoid cells (11–18 × 8–15 µm). *Hymenophoral trama* bilateral, divergent; w_{cs} = 40–60 µm; well rehydrated, filamentous, undifferentiated hyphae 3–8 µm wide; inflated cells ellipsoid to elongated 55–90 × 12–19 µm, diverging at an angle of approximately 40°; vascular hyphae 11–14 µm wide, infrequent. *Pileipellis* 130–150 µm thick, subradially to densely arranged, filamentous, undifferentiated hyphae 2–7 µm wide; vascular hyphae 7–10 µm wide, infrequent. *Universal veil on pileus* disordered; filamentous, undifferentiated hyphae 2–7 µm wide, scattered, branched, thin walled; inflated cells dominantly globose to subglobose 40–70 × 30–65 µm, broadly ellipsoid to ellipsoid 40–60 × 10–13 µm, often in chain of 2–3 cells, thin walled, hyaline,

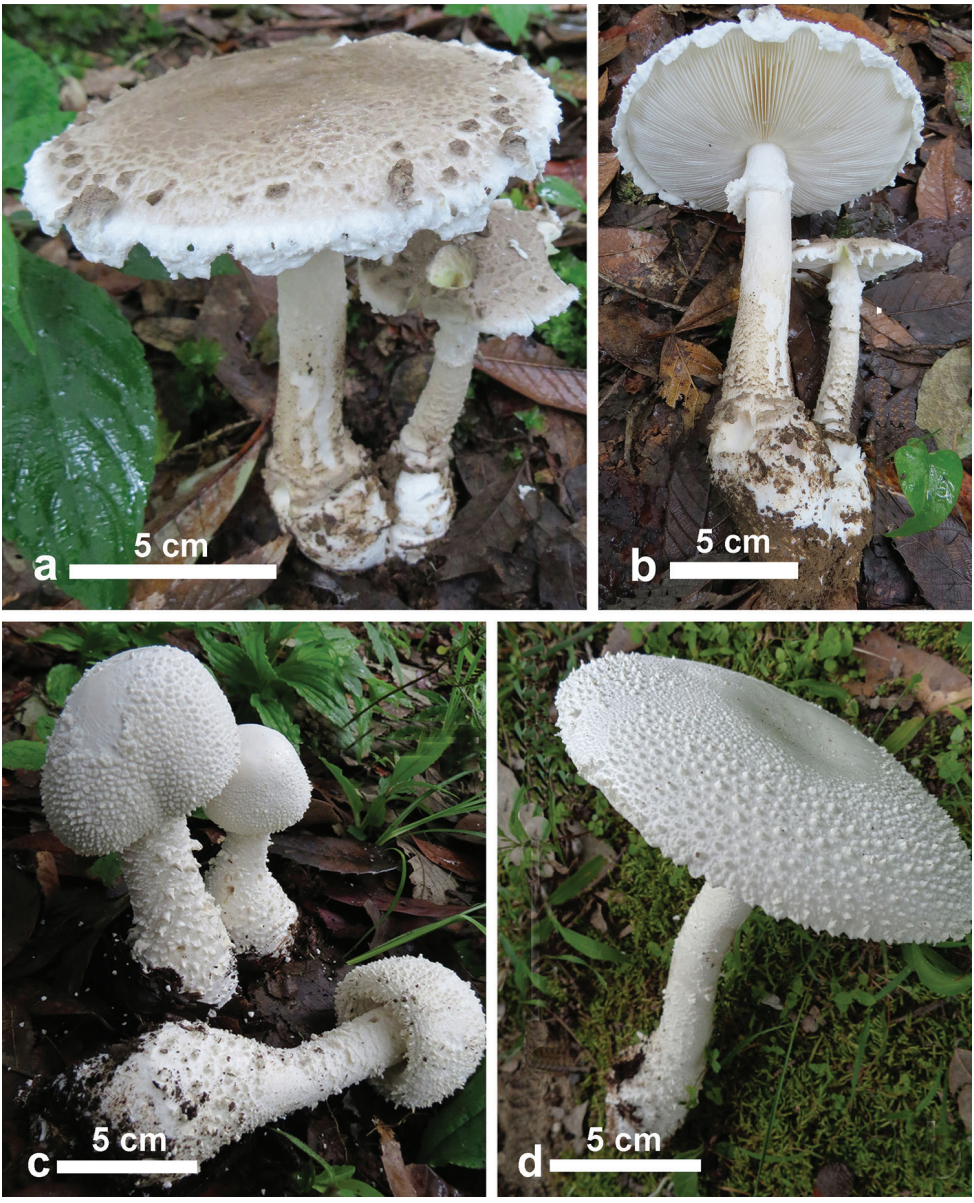

Figure 4. Basidiomata of *Amanita* species. **a, b** Basidiomata of *A. griseoverrucosa* in natural habitat (TM 16-1228) **c, b** Basidiomata of *A. virgineoides* in natural habitat (TM 14-413).

often with yellowish-brown vascular pigment. *Universal veil on base of stipe* disordered; filamentous, undifferentiated hyphae 3–8 µm wide, scattered, thin walled, branched, with brownish vacuolar pigments; inflated cells dominantly globose to subglobose 30–65 × 26–58 µm, broadly ellipsoid to ellipsoid or pyriform 26–55 × 8–13 µm, thin-walled, hyaline, with brownish vacuolar pigment. *Partial veil* abundant inflated cells clavate to broadly clavate 76–130 × 13–25 µm, thin walled,

colourless, hyaline or brownish vacuolar pigments; filamentous, undifferentiated hyphae 3–5 µm wide. *Stipe context* longitudinally acrophysalidic, filamentous, undifferentiated hyphae 5–7 µm wide; acrophysalides 220–270 × 33–45 µm, filamentous, undifferentiated hyphae 4–8 µm wide, hyaline, vascular hyphae not found. *Clamp connections* not observed in any tissue.

Habitat and distribution. Solitary to gregarious, with plants of Fagaceae, Pinaceae and Ericaceae (*Rhododendron arboretum*).

Known distribution. Currently known from China (Yang 2004, 2015) and now India.

Specimens examined. INDIA, Uttarakhand, Pauri district, Phedkhal, at 1900 m a.s.l., 30°09.728'N, 078°51.206'E, 29 July 2016, T. Mehmood, TM 16-1228 (GUH-M-27010); same location, 26 August 2015, T. Mehmood, TM-15-971 (GUH-M-27011), 1910 m a.s.l., 30°09.732'N, 078°51.214'E.

Commentary. Morphologically, the Indian collections of *A. griseoverrucosa* are characterised by a whitish to greyish-white pileus covered with easily detachable greyish-brown to dark grey, felted to verrucose universal veil remnants, a ventricose to clavate stipe base, broadly ellipsoid to ellipsoid basidiospores, universal veil on the pileus with abundant inflated cells and scattered filamentous, undifferentiated hyphae and the absence of clamp connections at bases of basidia. The characteristic features and molecular data from the Indian collections match rather well with the original description of *A. griseoverrucosa*, reported from China (Yang 2004).

The absence of clamp connections at the bases of basidia, ellipsoid to broadly ellipsoid basidiospores and abundant inflated cells with scattered hyphae in the universal veil placed this species in *Amanita* [sect. *Lepidella* subsect. *Solitariae*] stirps *Cinereoconia* (Yang 2004). Phylogenetically, both Indian (TM 16-1228) and Chinese (HKAS 38459) collections of *A. griseoverrucosa* are closely related to *A. cinereoconia* and *A. tullossiana* (Fig. 1). *Amanita cinereoconia* has a white to greyish pileus covered with pulverulent to small warted universal veil remnants and elongate to cylindric basidiospores 8.5–11.5 × 5–6.5 µm (Bas 1969, Jenkins 1986). *Amanita griseoverrucosa* is also distinguished from *Amanita tullossiana* (see above).

Amanita virgineoides Bas, Persoonia 5: 435 (1969)

Figs. 4c, d, 5c, d

Description. *Basidiomata* medium-sized to large. *Pileus* 50–140 mm wide, white to slightly yellowish-white (1A2) with age, ovoid at first, hemispherical when expanding, later convex to plano-convex to flat; slightly depressed, dry, shiny, densely covered with conical to subconcal warts; margin appendiculate, incurved; context 8–13 mm thick, thinning evenly towards margin, white, turning yellowish-white (1A2) when cut or bruised. *Universal veil on pileus* as conical, subconic to pyramidal warts, 5–10 mm thick, white, easily detachable when touched, sometimes washed away by rains, turning slightly yellowish-white (1A2) with age. *Lamellae* 12–15 mm thick, free, white

(17A1) crowded, with 8–9 lamellae per cm at margin; lamellulae attenuate, of 4–5 lengths, plentiful, white to cream. *Stipe* 75–140 × 26–22 mm (excluding bulb), white (16A1), slightly tapering upwards, the upper part covered by flocculent squamules, the lower part covered by irregularly arranged, conical to sub-conical warts; context white, solid, turning light yellowish (1A3) when cut or bruised. *Bulb* 23–29 × 23–30 mm, subglobose, ovoid to napiform, white, slightly yellowish-white with age. *Universal veil on stipe base* as white conical to subconical warts. *Partial veil* superior, white, submembranous, thick, covered with white conical warts, fragile, easily detachable when touched. *Odour* unpleasant. *Taste* not recorded. *Spore print* white.

Basidiospores [180/9/4] (7.5–)8–10.5(–11) × (5.5–)5.8–7.5 µm, [**L** = 8–10 µm, **L'** = 9.05 µm; **W** = 6.0–6.7 µm, **W'** = 6.45 µm; **Q** = (1.22–)1.33–1.55(–1.66), **Q** = 1.33–1.46, **Q'** = 1.41], colourless, hyaline, thin walled, smooth, amyloid, broadly ellipsoid to ellipsoid; apiculus lateral to sublateral, up to 1 µm long; contents monoguttulate. *Basidia* (42–)48–51(–58) × (10–)11–12(–12.5) µm, 2 to 4-spored, thin-walled, colourless, hyaline; sterigmata up to 4 µm long; basal septa often clamped. *Lamellar edge* tissue sterile, with inflated cells; subglobose to pyriform 15–25 × 8–15) µm, thin walled, colourless, hyaline, clamps present. *Subhymenium* 30 µm thick, w_{st}*-near* = 28–45 µm, w_{st}*-far*= 35–50 µm, ramose, with inflated; ovoid to ellipsoid cells 12–18 × 8–14 µm; clamp present. *Hymenophoral trama*, bilateral, divergent; w_{cs}= 40–65 µm; lateral stratum comprising of inflated intercalary segment 30–65 × 8–20 µm, common; filamentous, undifferentiated hyphae 3–9 µm wide, thin-walled, colourless, hyaline, vascular hyphae rare; clamp present. *Pileipellis* hardly differentiated; filamentous hyphae 2–7 µm wide, interwoven, non-gelatinised, thin walled, colourless, hyaline. *Universal veil on the pileus* with elements anticlinally arranged; filamentous, undifferentiated hyphae 4–8 µm wide, abundant, branched, colourless, hyaline; inflated cells dominantly subglobose to pyriform 16–46 × 14–32 µm, broadly ellipsoid to fusiform 30–66 × 10–21 µm; clamp present. *Universal veil on the stipe base* with elements anticlinally arranged; filamentous, undifferentiated hyphae 4–7 µm wide, scattered to abundant, colourless, thin walled, hyaline; inflated cells dominantly globose to subglobose 20–50 × 18– 48 µm, broadly ellipsoid to ellipsoid 45–65 × 15–20 µm, thin walled, hyaline, colourless, clamps present. *Partial veil* abundant inflated cells subglobose to ellipsoid 15–36 × 12–28 µm, thin walled, colourless, hyaline; filamentous, undifferentiated hyphae 3–8 µm wide, dominant, colourless, thin walled, clamps present. *Stipe context* longitudinally acrophysalidic; filamentous hyphae 2–13 µm wide, acrophysalides measuring 120 –181 × 20–30 µm, dominant, colourless, thin walled, hyaline, clamps present. *Clamp connections* common.

Macrochemical tests on fresh basidiomata. Chemical reactions on pileus surface: 10% NH_4OH - pinkish, 5% KOH - negative, 2% phenol - negative; and $FeSO_4$ crystals - negative on pileus and stipe context.

Habitat and distribution. Solitary to subgregarious in temperate mixed forest dominated by *Quercus leucotrichophora and Cedrus deodara* at 1850–2050 m a.s.l.

Known distribution: This species was originally described from Japan. It has also been reported from China (Yang 1997), South Korea (Kim et al. 1993), Thailand (Sanmee et al. 2008) and now India.

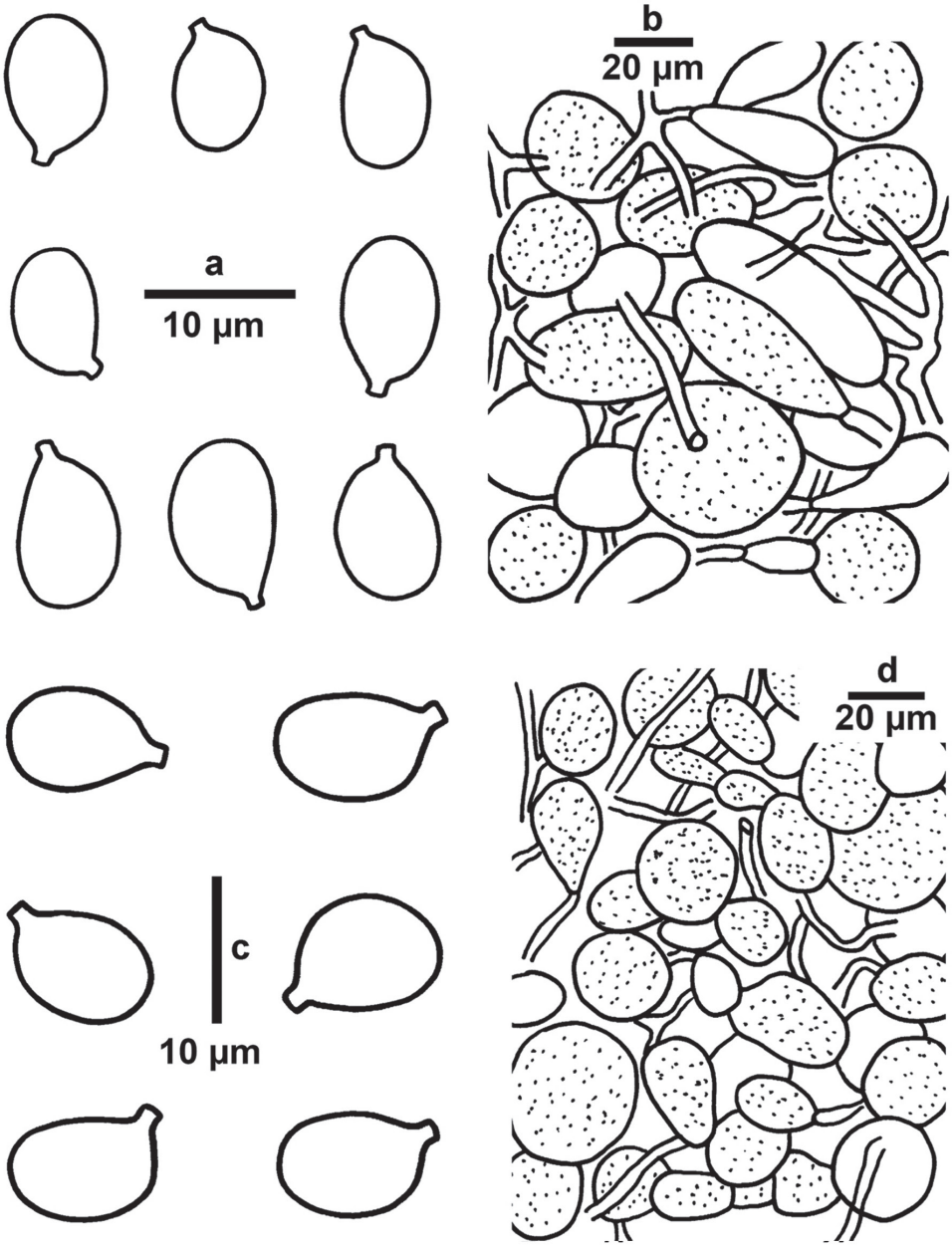

Figure 5. Microscopic features of *Amanita* species. **a, b** *Amanita griseoverrucosa* (TM 16-1247) **a** Basidiospores **b** Elements of universal veil from pileus surface **c, d** *Amanita virgineoides* (TM 14-413) **c** Basidiospores **d** Elements of universal veil from pileus surface.

Specimens examined. INDIA, Uttarakhand, Pauri district, Phedkhal, 24 August 2014, T. Mehmood, TM 14-413 (RET 717-9); same location, 12 August 2015, T. Mehmood, TM 15-917 (GUH-M-27012); same location, 16 July 2016, T. Mehmood,

TM 16-1098 (GUH-M-27013); same location, 24 July 2017, T. Mehmood, TM 17-1468 (GUH-M-27014).

Commentary. An Indian collection (RET 717-9) is grouped phylogenetically with Chinese material of *A. virgineoides* (HKAS 79691, GenBank nrLSU: KJ466495 and HKAS 77278, GenBank nrLSU: KC429043), with pairwise genetic divergence between their nrLSU sequences = 0.35% (might be intragenomic heterogeneity present amongst collections as the sequence was not clean). It is worth mentioning that there is no genetic distance between *rpb2* sequences derived from the Chinese (HKAS 79691, GenBank *rpb2*: KJ466663) and Indian (RET 717-9) collections. The evidence suggests that the two collections could be conspecific and exhibiting a minor intra-specific variability. In addition, the sample size is also small. For these reasons, we do not feel justified in erecting a new species or subspecies. Interestingly, another Chinese collection (HKAS 18394), labelled as *A. virgineoides* (GenBank nrLSU: AF024484, Weiß et al. 1998), is also grouped with the Indian collection, but the sequence derived from this collection is divergent from the two previously cited collections (Fig. 1). However, the habit and size of the basidiomata and basidiospores of the Indian collections match well with those characters in the descriptions of *A. virgineoides* provided by Bas (1969) and Yang (1997, 2015). Therefore, the Indian collection (RET 717-9) is being treated here as *A. virgineoides* – a new record for India.

Amanita virgineoides belongs to *Amanita* [sect. *Lepidella* subsect. *Solitariae*] stirps *Virgineoides* because of the presence of conical to subconical warts on the pileus surface which consist of inflated cells rather abundant hyphae, the presence of clamp connections at the bases of basidia and the broadly ellipsoid basidiospores (Bas 1969, Yang 1997). In stirps *Virgineoides*, *A. gracilior* Bas & Honrubia and *A. miculifera* Bas & Hatanaka resemble *A. virgineoides* morphologically. *Amanita gracilior*, originally described from Spain, has a white pileus turning yellowish-brown with age, a rooting base and elongate basidiospores 10–11.5 × 5.5–6.5 µm, with a higher **Q'** value = 1.8 (Bas 1969). *Amanita miculifera*, originally described from Japan, has a pearl grey pileus and a stipe with a notably radicating basal bulb (Bas and Hatanaka 1984, Yang 1997). The white basidiomata of *A. virgineoides* also resembles the basidiomata of other of Bas' stirpes. In creating these stirpes, Bas morphologically segregated these taxa from *A. virgineoides* (Bas 1969).

Acknowledgements

The authors are very grateful to Dr. Rodham E. Tulloss (USA) for his providing valuable comments on versions of this manuscript and his giving consent to propose the new species name in his honour; and to the Head, Department of Botany and Microbiology, H.N.B Garhwal University (Srinagar, Garhwal, India) for providing many facilities during the present study. The second author (TM) acknowledges the University Grants Commission (UGC, India) for providing a PhD fellowship. The third author (KD) is thankful to the Director, Botanical Survey of India, Kolkata for supporting this study. Field assistance rendered by Miss Priyanka Uniyal, Mr. Upendra Singh,

Mr. Aniket Ghosh and Mr. M.E. Hembrom are also acknowledged. This study was partially supported by the NSFC Research Fund for International Young Scientists (No. 31750110476) and 11th Special Fund of the China Postdoctoral Science Foundation (No. 2018T110854) to the first author (MIH) and the National Natural Science Foundation of China (No. 31470155).

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MycoKeys 37: 93–107 (2018)
doi: 10.3897/mycokeys.37.25901
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RESEARCH ARTICLE

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Three new species of *Aleurodiscus* s.l. (Russulales, Basidiomycota) from southern China

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Academic editor: *B. Dentinger* | Received 17 April 2018 | Accepted 27 July 2018 | Published 3 August 2018

Citation: Tian Y, Ghobad-Nejhad M, He S-H, Dai Y-C (2018) Three new species of *Aleurodiscus* s.l. (Russulales, Basidiomycota) from southern China. MycoKeys 37: 93–107. https://doi.org/10.3897/mycokeys.37.25901

Abstract

Three new species of *Aleurodiscus* s.l. with corticioid basidiomata are described and illustrated from southern China based on morphological evidence and phylogenetic analyses of ITS and nrLSU sequence data. *Aleurodiscus bambusinus* was collected from Jiangxi Province on bamboo and is distinct by having a compact texture, simple-septate generative hyphae, abundant acanthophyses, basidia with acanthophysoid appendages and smooth basidiospores. *Aleurodiscus isabellinus* was collected from Yunnan Province on both angiosperm wood and bamboo and is distinct by having soft basidiomata with yellow to yellowish-brown hymenophore, yellow acanthophyses, simple-septate generative hyphae and smooth basidiospores. *Aleurodiscus subroseus* was collected from Guangxi Autonomous Region and Guizhou Province on angiosperm wood and is distinct by having pinkish basidiomata when fresh, clamped generative hyphae, clavate acanthophyses and echinulate basidiospores. In the phylogenetic tree, *A. bambusinus* and *A. isabellinus* were nested within the *A. cerussatus* group, whilst *A. subroseus* was clustered with *A. wakefieldiae*. An identification key to 26 species of *Aleurodiscus* s.l. in China is provided.

Keywords

acanthophyses, corticioid fungi, Stereaceae, taxonomy, wood-inhabiting fungi

Introduction

Aleurodiscus s.l. is a large group of wood-inhabiting fungi with a broad morphological circumscription. It is characterised by having cupulate, effused or effused-reflexed basidiomata, a monomitic or dimitic hyphal system with simple-septate or clamped gen-

erative hyphae, smooth or ornamented, amyloid basidiospores and sterile organs such as acanthophyses, gloeocystidia and dendrohyphidia (Núñez and Ryvarden 1997). Although *Aleurodiscus* s.l. had been divided into several small genera based on different combinations of morphological characters, phylogenetic analyses did not fully support these separations (Wu et al. 2001; Dai and He 2016). Accordingly, the inter- and intrageneric phylogeny of *Aleurodiscus* s.l. in Stereaceae is still unclear and no reliable morphological characters can be used to recognise the small segregated genera. Thus, the broad sense concept of the genus has often been adopted by mycologists when describing new species (Núñez and Ryvarden 1997; Gorjón et al. 2013; Dai et al. 2017a, b).

A recent survey on *Aleurodiscus* s.l. from China (Dai and He 2016, 2017, Dai et al. 2017a, b) revealed that its species diversity is high and many species, especially those with corticioid basidiomata on both herbaceous and ligneous plants, are still undescribed. In the present study, three new species are described and illustrated from southern China, amongst which two species have abundant acanthophyses and smooth basidiospores and one species bears echinulate basidiospores. Morphological differences between new species and their relatives are discussed. Their phylogenetic positions were inferred from a combined dataset of ITS and nrLSU sequence data.

Materials and methods

Morphological studies

Voucher specimens are deposited in the herbaria of Beijing Forestry University, Beijing, China (BJFC), Centre for Forest Mycology Research, U.S. Forest Service, Madison, USA (CFMR) and Southwest Forestry University, Kunming, China (SWFC). Freehand sections were made from basidiomata and mounted in 2% (w/v) potassium hydroxide (KOH), 1% phloxine (w/v) or Melzer's reagent. Microscopic examinations were carried out with a Nikon Eclipse 80i microscope at magnifications up to 1000×. Drawings were made with the aid of a drawing tube. The following abbreviations are used: L = mean spore length, W = mean spore width, Q = L/W ratio, n (a/b) = number of spores (a) measured from number of specimens (b). Colour names and codes follow Kornerup and Wanscher (1978).

DNA extraction and sequencing

A CTAB plant genome rapid extraction kit-DN14 (Aidlab Biotechnologies Co. Ltd, Beijing) was employed for DNA extraction and PCR amplification from dried specimens. The ITS and nrLSU gene regions were amplified with primer pairs ITS5/ITS4 (White et al. 1990) and LR0R/LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm), respectively. The PCR procedures followed Dai and He (2016). DNA sequencing was performed at Beijing Genomics Institute and the sequences were deposited in GenBank.

Phylogenetic analyses

The molecular phylogeny was inferred from a combined dataset of ITS and nrLSU sequences of representative members of Stereaceae sensu Larsson (2007) (Table 1). The ingroup taxa sampling and outgroup selection followed Dai et al. (2017b). The sequences were aligned using MAFFT v.6 (Katoh and Toh 2008, http://mafft.cbrc.jp/alignment/server/). Alignments were optimised manually in BioEdit 7.0.5.3 (Hall 1999) and deposited at TreeBase (http://treebase.org/treebase-web/home.html, submission ID: 22474). Maximum Parsimony (MP), Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were performed by using PAUP* 4.0b10 (Swofford 2002), MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and RAxML 7.2.6 (Stamatakis 2006), respectively. The best models of evolution for BI were estimated by using MrModeltest 2.2 (Nylander 2004). The methods and parameter settings for the three kinds of phylogenetic analyses followed Liu et al. (2018).

Phylogeny results

The ITS-nrLSU sequences dataset contained 42 ITS and 53 nrLSU sequences from 53 samples representing 47 ingroup taxa and the outgroup (Table 1). Seven ITS and seven nrLSU sequences were generated for this study. The dataset had an aligned length of 2045 characters, of which 384 were parsimony informative. Maximum Parsimony (MP) analysis yielded 85 equally parsimonious trees. The best model estimated and applied in the Bayesian analysis was GTR+I+G. The average standard deviation of split frequencies of BI was 0.007863. ML and BI analyses resulted in almost the same tree topologies as that of MP analysis. Only the MP tree is shown in Fig. 1 with maximum likelihood and maximum parsimony bootstrap values ≥50% and BPP ≥0.95 labelled along the branches. In the tree, *A. bambusinus* and *A. isabellinus* were nested within the *A. cerussatus* (Bres.) Höhn. & Litsch. group (MP = 92%, BI = 1.00, ML = 87%). *Aleurodiscus subroseus* was clustered with *A. wakefieldiae*, but their relationship has no support in BI and ML analyses.

Taxonomy

Aleurodiscus bambusinus **S.H. He & Y.C. Dai, sp. nov.**
MycoBank: MB824755
Figs 2a–b, 3

Diagnosis. The species is distinct by having corticioid basidiomata, a compact texture, simple-septate generative hyphae, abundant acanthophyses, basidia with an acanthophysoid appendage and smooth basidiospores 7–10 × 4–6 μm and growing on bamboo.

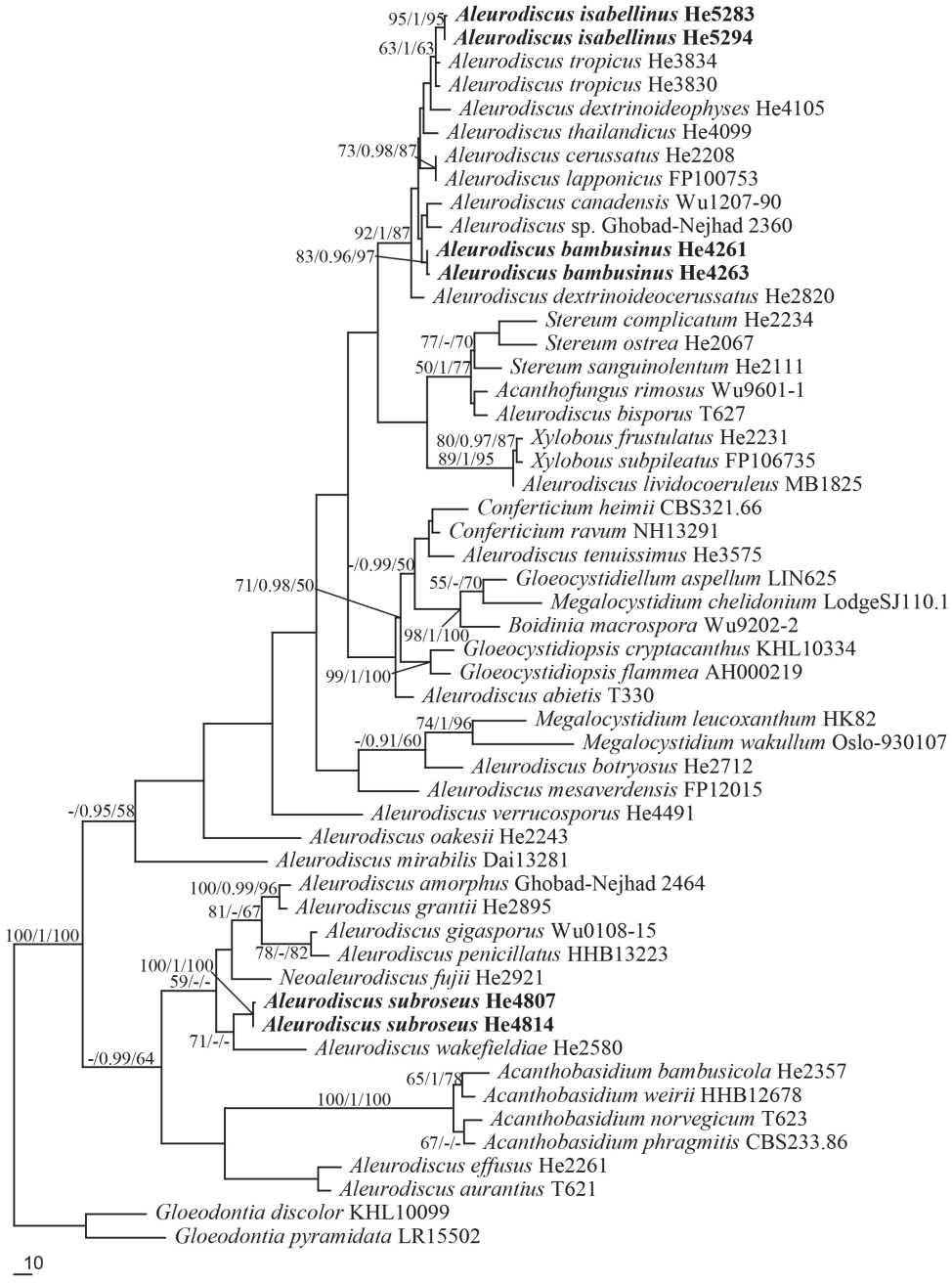

Figure 1. Maximum parsimony phylogeny of the combined ITS and nrLSU sequences data of Stereaceae. Branches are labelled with maximum parsimony and maximum likelihood bootstrap values ≥50% and Bayesian posterior probabilities ≥0.95 (MP/BI/ML).

Table 1. Species and sequences used in the phylogenetic analyses. Newly generated sequences are set in bold.

Taxa	Voucher	Locality	ITS	nrLSU
Acanthobasidium bambusicola	He 2357	China	KU559343	KU574833
A. norvegicum	T 623	France	–	AY039328
A. phragmitis	CBS 233.86	France	–	AY039305
A. weirii	HHB 12678	USA	–	AY039322
Acanthofungus rimosus	Wu 9601-1	Taiwan	–	AY039333
Aleurodiscus abietis	T 330	Canada	–	AY039324
A. amorphus	Ghobad-Nejhad 2464	China	KU559342	KU574832
A. aurantius	T 621	France	–	AY039317
A. bambusinus	**He 4261**	**China**	**KY706207**	**KY706219**
A. bambusinus	**He 4263**	**China**	**KY706208**	**KY706218**
A. bisporus	T 627	Guadeloupe	–	AY039318
A. botryosus	He 2712	China	KX306877	KY450788
A. canadensis	Wu 1207-90	China	KY706203	KY706225
A. cerussatus	He 2208	China	KX306874	KY450785
A. dextrinoideocerussatus	He 2820	China	KY706206	MH109044
A. dextrinoideophyses	He 4105	China	MH109050	KY450784
A. effusus	He 2261	China	KU559344	KU574834
A. gigasporus	Wu 0108-15	China	KY706205	KY706213
A. grantii	He 2895	China	KU559347	KU574837
A. isabellinus	**He 5283**	**China**	**MH109052**	**MH109046**
A. isabellinus	**He 5294**	**China**	**MH109053**	**MH109047**
A. lapponicus	FP 100753	USA	–	AY039320
A. lividocoeruleus	MB 1825	USA	–	AY039314
A. mesaverdensis	FP 120155	USA	KU559359	KU574817
A. mirabilis	Dai 13281	China	KU559350	KU574839
A. oakesii	He 2243	USA	KU559352	KU574840
A. penicillatus	HHB 13223	USA	–	KU574816
A. sp.	**Ghobad-Nejhad 2360**	**China**	**MH109051**	**MH109045**
A. subroseus	**He 4807**	**China**	**MH109054**	**MH109048**
A. subroseus	**He 4814**	**China**	**MH109055**	**MH109049**
A. tenuissimus	He 3575	China	KX306880	KX842529
A. thailandicus	He 4099	Thailand	KY450781	KY450782
A. tropicus	He 3830	China	KX553875	KX578720
A. tropicus	He 3834	China	KX553876	KY706221
A. verrucosporus	He 4491	China	KY450786	KY450790
A. wakefieldiae	He 2580	China	KU559353	KU874841
Boidinia macrospora	Wu 9202-2	China: Taiwan	AF506377	AF506377
Conferticium heimii	CBS 321.66	Central African Republic	AF506381	AF506381
C. ravum	NH 13291	Estonia	AF506382	AF506382
Gloeocystidiellum aspellum	LIN 625	China: Taiwan	AF506432	AF506432
Gloeocystidiopsis cryptacanthus	KHL 10334	Puerto Rico	AF506442	AF506442
G. flammea	AH 000219	La Réunion	AF506438	AF506438
Gloeodontia discolor	KHL 10099	Puerto Rico	AF506445	AF506445
G. pyramidata	LR 15502	Columbia	AF506446	AF506446
Megalocystidium chelidonium	LodgeSJ 110.1	USA	AF506441	AF506441
*M. leucoxanthu*m	HK 82	Denmark	AF506420	AF506420
M. wakullum	Oslo 930107	Tanzania	AF506443	AF506443
Neoaleurodiscus fujii	He 2921	China	KU559357	KU574845
Stereum complicatum	He 2234	USA	KU559368	KU574828
S. ostrea	He 2067	USA	KU559366	KU574826
S. sanguinolentum	He 2111	USA	KU559367	KU574827
Xylobous frustulatus	He 2231	USA	KU881905	KU574825
X. subpileatus	FP 106735	USA	–	AY039309

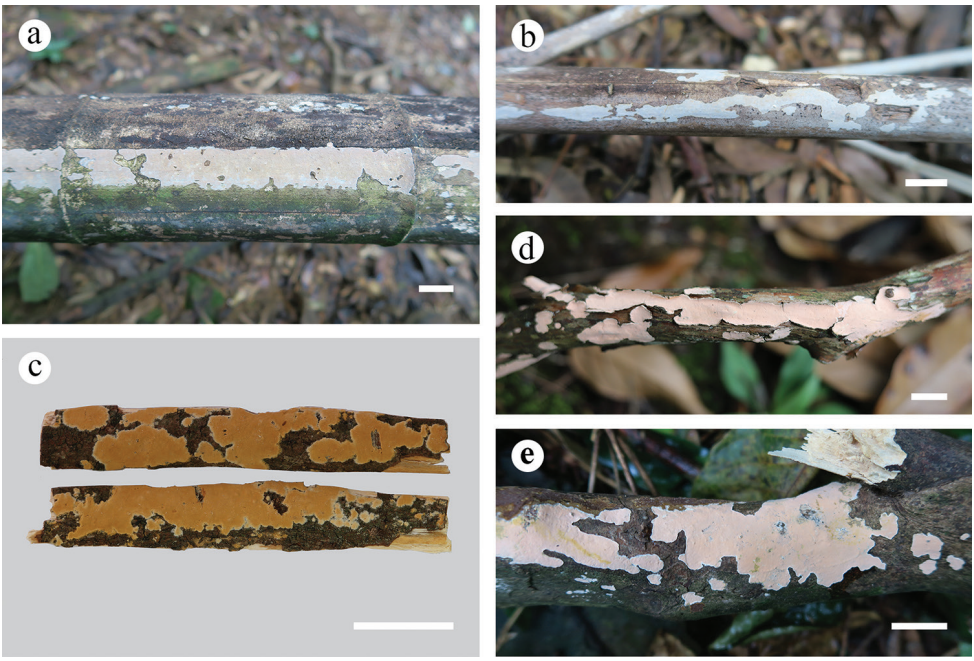

Figure 2. Basidiomata. **a–b** *Aleurodiscus bambusinus* (**a** He 4250 **b** holotype, He 4261) **c** *A. isabellinus* (holotype, KKN-2017-19) **d–e** *A. subroseus* (**d** He 5571 **e** He 4895). Scale bars: 1 cm.

Holotype. CHINA. Jiangxi Province, Yifeng County, Guanshan Nature Reserve, alt. ca. 800 m, on fallen culms and branches of bamboo, 10 Aug 2016, He 4261 (holotype, BJFC 023703).

Etymology. "*Bambusinus*" refers to the substrate of bamboo.

Basidiomata. Annual, resupinate, effused, closely adnate, inseparable from substrate, coriaceous, at first as small patches, later confluent up to 30 cm long and 2.5 cm wide, 180–300 μm thick. Hymenophore smooth, white (4A1) to yellowish-white (4A2) when young, becoming greyish-yellow [4B (3–4)] to brownish-orange [6C (5–8)] with age, uncracked or cracked with age; margin abrupt, indistinct, concolorous with hymenophore.

Microscopic structures. Hyphal system monomitic; generative hyphae simple-septate, colourless, thin- to thick-walled, scattered near the substrate, 2–4 μm in diam. Subiculum thin to indistinct. Subhymenium thick, with compact texture, composed of acanthophyses and gloeocystidia. Acanthophyses abundant, hyphoid or distinctly swollen in the middle part, colourless, thin-walled, with abundant spines in apex, 30–40 × 3–12 μm. Gloeocystidia abundant, flexuous or slightly moniliform with one to several constrictions, slightly thick-walled, negative in sulphobenzaldehyde, 30–55 × 8–13 μm. Basidia subclavate to subcylindrical, colourless, slightly thick-walled, usually with a lateral acanthophysoid appendage, with four sterigmata and a basal simple septum, 25–35 × 7–9 μm. Basidiospores ellipsoid to broadly ellipsoid, bearing a dis-

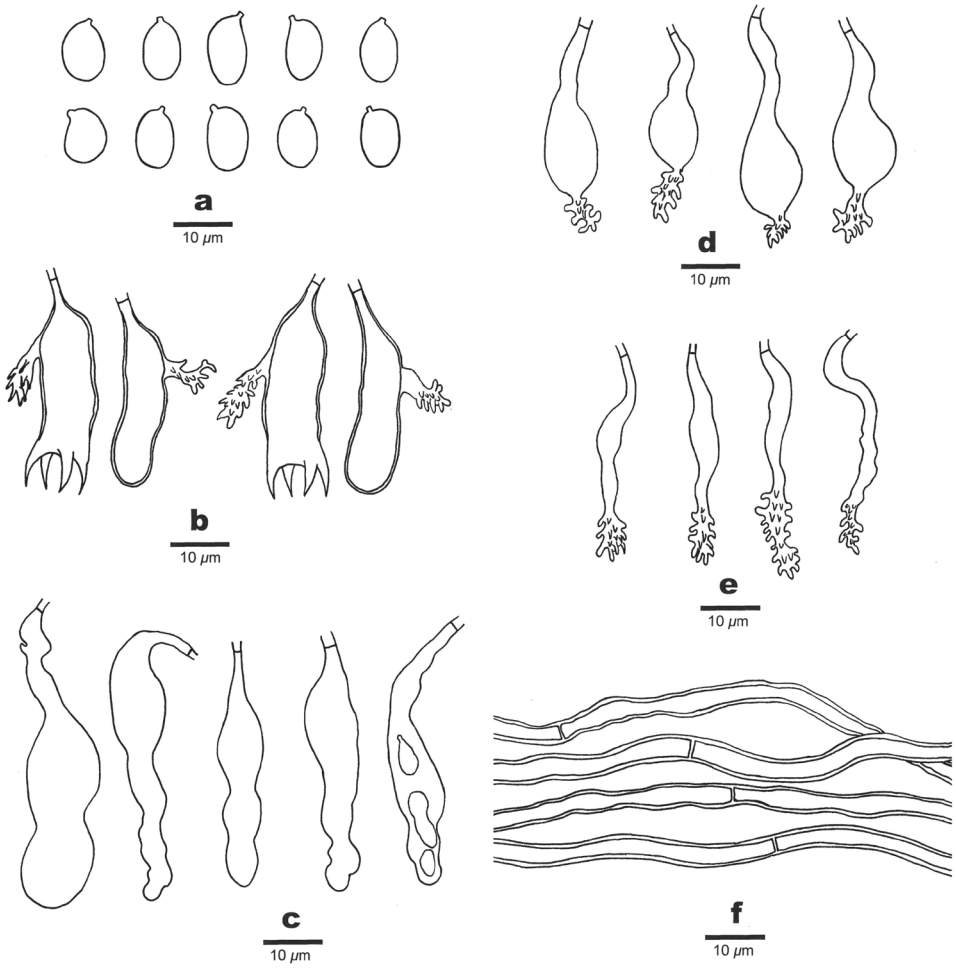

Figure 3. Microscopic structures of *Aleurodiscus bambusinus* (drawn from the holotype). **a** Basidiospores; **b** Basidia **c** Gloeocystidia **d–e** Acanthophyses **f** Generative hyphae.

tinct apiculus, colourless, thin-walled, smooth, amyloid, 7–10 × 4–6 μm, L = 8.7 μm, W = 4.9 μm, Q = 1.6–1.9 (n = 90/3).

Additional specimens examined. CHINA. Jiangxi Province, Yifeng County, Guanshan Nature Reserve, alt. ca. 800 m, on fallen culms and branches of bamboo, 10 Aug 2016, He 4250 (BJFC 023692) and He 4263 (BJFC 023705).

Remarks. *Aleurodiscus bambusinus* is morphologically similar and phylogenetically close to *A. dextrinoideophyses* S.H. He and *A. tropicus* L.D. Dai & S.H. He that also grow on bamboo in East Asia (Dai et al. 2017a, b). *Aleurodiscus dextrinoideophyses* differs from *A. bambusinus* by having apparently dextrinoid acanthophyses and smaller basidiospores (5–7 × 3–4 μm, Dai et al. 2017b). *Aleurodiscus tropicus* differs from *A. bambusinus* by having a looser texture and slightly larger basidiospores (9–12 × 5–7.5 μm, Dai et al. 2017a). The ITS similarity between *A. bambusinus* (He 4261)

and *A. dextrinoideophyses* (He 4105) is 95.6% of 434 base pairs and, between *A. bambusinus* (He 4261) and *A. tropicus* (He 3830), is 97.3% of 582 base pairs. *Aleurodiscus aberrans* G. Cunn. and *A. rimulosus* Núñez & Ryvarden are also similar to *A. bambusinus*, but they differ from this new species by having smooth basidia and growing on angiosperm wood outside of Asia (Núñez and Ryvarden 1997).

Aleurodiscus isabellinus **S.H. He & Y.C. Dai, sp. nov.**
MycoBank: MB824758
Figs 2c, 4

Diagnosis. The species is distinct by having soft, yellow to yellowish-brown and corticioid basidiomata, a loose texture, abundant yellow acanthophyses, simple-septate generative hyphae and smooth basidiospores 6–8.5 × 3–4 μm.

Holotype. CHINA. Yunnan Province, Dali County, Cangshan Nature Reserve, alt. ca. 2600 m, on fallen decorticated angiosperm branches, 27 Oct 2017, KKN-2017-19 (holotype in CFMR, isotype in BJFC).

Etymology. "*Isabellinus*" refers to the yellowish-brown basidiomata.

Basidiomata. Annual, resupinate, effused, adnate, inseparable from substrate, soft, membranaceous to coriaceous, at first as small patches, later confluent up to 15 cm long and 1 cm wide, 150–300 μm thick. Hymenophore smooth, light orange [5A(4–5)] , greyish-orange[5B(5–6)], orange [5B(7–8)] to brownish-yellow [5C(7–8)], uncracked or cracked with age; margin thinning out, fimbriate, white (5A1) when juvenile, becoming abrupt, indistinct, concolorous with hymenophore when mature.

Microscopic structures. Hyphal system monomitic, generative hyphae simple-septate, colourless, thin- to slightly thick-walled, straight, loosely interwoven, frequently branched and septate, 2–4 μm in diam. Acanthophyses abundant, colourless to yellow, thick-walled, hyphoid or arising laterally or apically from a clavate or cylindrical base 30–50 × 5–7 μm, with abundant spines in upper part, some hyphoid ones near substrate with long spines (branches) resembling binding hyphae. Gloeocystidia abundant, embedded, colourless, slightly thick-walled, subcylindrical or slightly moniliform, negative in sulphobenzaldehyde, 35–110 × 5–8 μm. Basidia clavate, colourless, thin-walled, with four sterigmata and a basal simple septum, 40–55 × 6–7 μm. Basidiospores ellipsoid to oblong ellipsoid, bearing a distinct apiculus, colourless, thin-walled, smooth, amyloid, (5.5–) 6–8.5 × (2.8–) 3–4 μm, L = 7 μm, W =3.7 μm, Q = 1.9 (n = 24/1).

Additional specimens examined. CHINA. Yunnan Province, Dali County, Cangshan Nature Reserve, alt. ca. 2600 m, on small dead bamboo, 27 Oct 2017, He 5283 (BJFC 024801) and He 5287 (BJFC 024805); on fallen angiosperm branch, 27 Oct 2017, He 5294 (BJFC 024812); Jingdong County, Ailaoshan Nature Reserve, alt. 2450 m, on fallen angiosperm branch, 4 Oct 2017, C.L. Zhao 3843 (SWFC).

Remarks. All the studied specimens of *A. isabellinus* lack a true hymenium and only the holotype has a few basidia and basidiospores. *Aleurodiscus isabellinus* was nest-

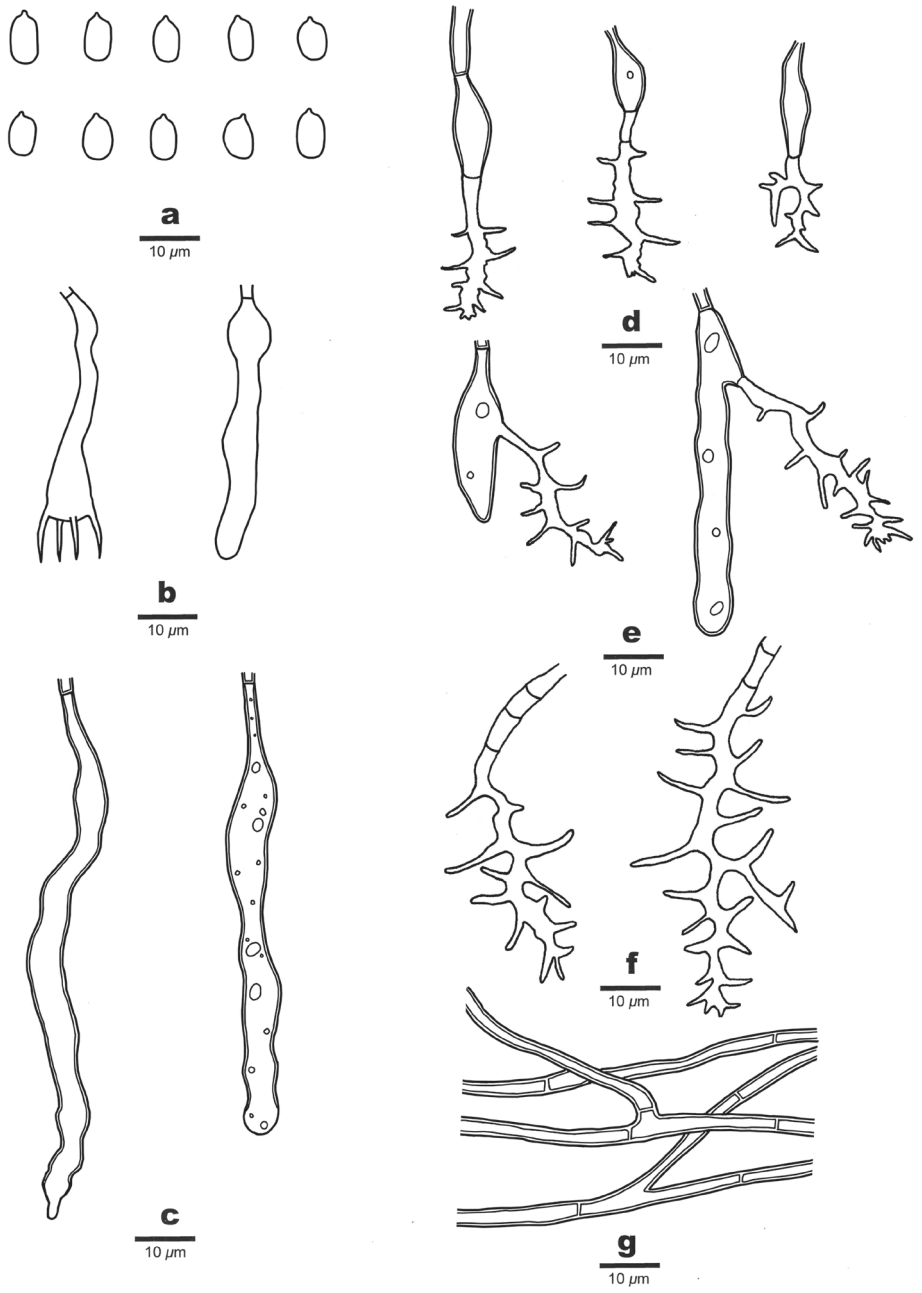

Figure 4. Microscopic structures of *Aleurodiscus isabellinus* (drawn from the isotype). **a** Basidiospores **b** A basidium and a basidiole **c** Gloeocystidia **d–f** Acanthophyses **g** Generative hyphae.

ed within the *A. cerussatus* group (Fig. 1). In this group, *Aleurodiscus thailandicus* S.H. He is similar to *A. isabellinus* by sharing the yellow basidiomata and acanthophyses, but differs by having two types of gloeocystida and acanthophyses without a clavate or

cylindrical base (Dai et al. 2017a). The ITS similarity between *A. isabellinus* (He 5283) and *A. thailandicus* (He 4099) is 93.6% of 578 base pairs. *Aleurodiscus thailandicus* was described from Thailand based on a fertile specimen on bamboo, but later several sterile specimens on bamboo from south-western China were identified as this species according to the sequence data. Morphologically, the soft and yellow to yellowish-brown basidiomata of *A. isabellinus* resemble the genus *Vararia* P. Karst. which belongs to Peniophoraceae according to phylogenetic analyses.

Aleurodiscus subroseus **S.H. He & Y.C. Dai, sp. nov.**
MycoBank: MB824757
Figs 2d–e, 5

Diagnosis. The species is distinct by having pinkish and corticioid basidiomata when fresh, clamped generative hyphae, moniliform gloeocystidia, presence of acanthophyses (acanthocystidia) and echinulate basidiospores 16–20 × 11–14 μm.

Holotype. CHINA. Guangxi Autonomous Region, Xing'an County, Mao'ershan Nature Reserve, alt. ca. 1600 m, on dead but still attached branch of living angiosperm tree, 13 Jul 2017, He 4807 (holotype, BJFC 024326).

Etymology. "*Subroseus*" (Lat.) refers to the pinkish basidiomata when fresh.

Basidiomata. Annual, resupinate, effused, closely adnate, inseparable from substrate, coriaceous, at first as small irregular patches, later confluent up to 35 cm long and 3 cm wide, up to 300 μm thick. Hymenophore smooth, pinkish-white (12A2), pink (12A3), pale orange (6A3) to light orange (6A4) when fresh, becoming pale orange (6A3), light orange [6A(4–5)], greyish-orange [6B(3–6)] to brownish-orange [6C(5–6)] when dry, uncracked; margin abrupt, white and distinct when fresh, becoming concolorous or darker than hymenophore and indistinct when dry, slightly elevated when mature.

Microscopic structures. Hyphal system monomitic, generative hyphae with clamp connections. Subiculum thin to indistinct. Subhymenium thickening with age, with embedded gloeocystidia, acanthophyses and crystals. Hyphae in this layer colourless, thin-walled, frequently branched and septate, agglutinated, 2–4 μm in diam. Gloeocystidia abundant, moniliform, with one to several constrictions, smooth, slightly thick-walled, negative in sulphobenzaldehyde, 45–70 × 6–12 μm. Acanthophyses (acanthocystidia) abundant, variable in shape and size, subclavate to subcylindrical, with few to many spines at apex, colourless, slightly thick-walled, 30–60 × 6–20 μm. Hyphidia scattered, thin-walled, colourless, rarely branched. Basidia clavate, slightly sinuous, colourless, thin-walled, smooth, with four sterigmata and a basal clamp connection, 52–80 × 13–17 μm. Basidiospores ellipsoid to broadly ellipsoid, bearing a distinct apiculus, colourless, slightly thick-walled, echinulate, strongly amyloid, 16–20 × 11–14 μm, L = 18.4 μm, W = 12.6 μm, Q = 1.5 (n = 90/3) (spines excluded).

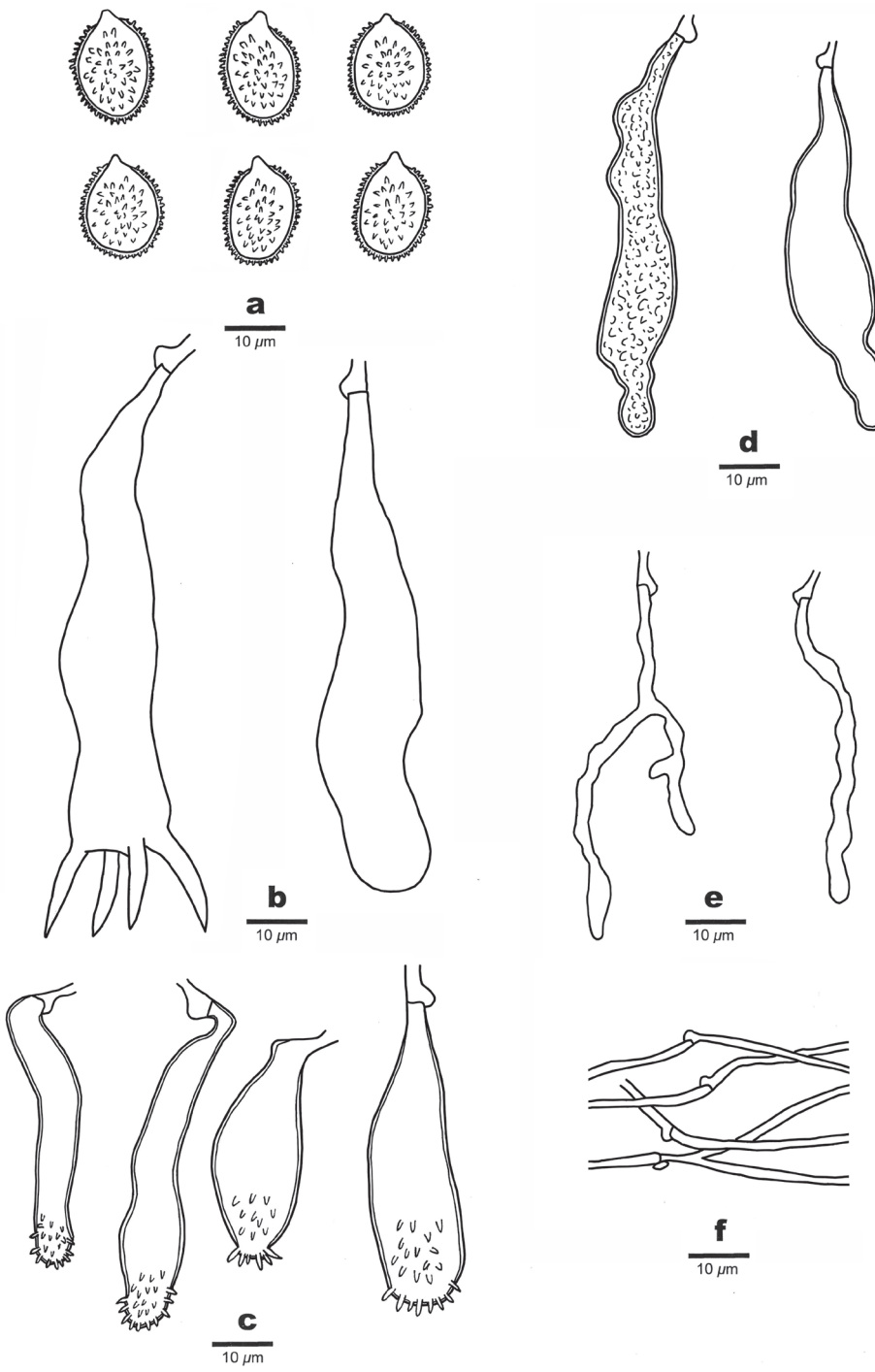

Figure 5. Microscopic structures of *Aleurodiscus subroseus* (drawn from the holotype). **a** Basidiospores; **b** A basidium and a basidiole **c** Acanthophyses **d** Gloeocystidia **e** Hyphidia **f** Generative hyphae.

Additional specimens examined. CHINA. Guangxi Autonomous Region, Xing'an County, Mao'ershan Nature Reserve, alt. ca. 1600 m, on dead but still attached branch of living angiosperm tree, 13 Jul 2017, He 4814 (BJFC 024333); Jinxiu County, Dayaoshan Nature Reserve, Yinshan Forest Park, alt. ca. 1500 m, on fallen angiosperm branch, 16 Jul 2017, He 4895 (BJFC 024414). Guizhou Province, Jiangkou County, Fanjingshan Nature Reserve, alt. 1500–2000 m, on dead but still attached branch of living angiosperm tree, 11 Jul 2018, He 5558 (BJFC); 12 Jul 2018, He 5571, He 5577, He 5581, He 5585, He 5589 and He 5593 (BJFC).

Remarks. *Aleurodiscus subroseus* is morphologically similar and phylogenetically close to *A. wakefieldiae* Boidin & Beller (Fig. 1), but the latter differs by having longer basidia (80–180 µm) and larger basidiospores (20–28 × 14–20 µm, Núñez and Ryvarden 1997). *Aleurodiscus penicillatus* Burt is similar to *A. subroseus*, but differs by growing on gymnosperm wood and having wider basidiospores (13–17 µm, Núñez and Ryvarden 1997). *Aleurodiscus mirabilis* (Berk. & M.A. Curtis) Höhn. also has pinkish fresh basidiomata and is widely distributed in southern China. However, it can be easily distinguished from *A. subroseus* by having basally warted basidia and larger basidiospores (24–28 × 14–17 µm, Núñez and Ryvarden 1997). In the phylogenetic tree (Fig. 1), *A. penicillatus* and *A. mirabilis* are distantly related to *A. subroseus*. *Aleurodiscus corticola* Gorjón et al. from Argentina on bark of living *Nothofagus dombeyi* also has moniliform gloeocystidia and similar basidiospores with *A. subroseus*, but differs by having pulvinate and tuberculate basidiomata and absence of acanthophyses (Gorjón et al. 2013).

Key to 26 species of *Aleurodiscus* s.l. in China

Acanthobasidium Oberw., *Aleurocystidiellum* P.A. Lemke and *Neoaleurodiscus* Sheng H. Wu are used for some species. Basidiospores data are from Núñez & Ryvarden (1997) or otherwise measured by the authors.

1	Basidiospores smooth	**2**
–	Basidiospores ornamented	**11**
2	Acanthophyses absent	**3**
–	Acanthophyses present	**4**
3	Basidiospores thick-walled, 23–27 × 16–21 µm; on *Rhododendron*	***Neoaleurodiscus fujii***
–	Basidiospores thin-walled, 18–23 × 14–19 µm; on *Quercus*	***A. ljubarskii***
4	Basidia with two sterigmata; basidiospores >12 µm long	***A. canadensis***
–	Basidia with four sterigmata; basidiospores <12 µm long	**5**
5	Generative hyphae simple-septate	**6**
–	Generative hyphae clamped	**10**
6	Acanthophyses apparently dextrinoid	***A. dextrinoideophyses***
–	Acanthophyses indextrinoid	**7**

7 Basidia smooth; acanthophyses yellow **8**
– Basidia with an acanthophysoid appendage; acanthophyses colourless **9**
8 Gloeocystidia of two types; acanthophyses hyphoid ..... ***A. thailandicus***
– Gloeocystidia of one type; acanthophyses hyphoid, subclavate to subcylindrical ..... ***A. isabellinus***
9 Texture loose; basidiospores 9–12 × 5–7.5 µm ..... ***A. tropicus***
– Texture compact; basidiospores 7–10 × 4–6 µm ..... ***A. bambusinus***
10 Acanthophyses apparently dextrinoid ..... ***A. dextrinoideocerussatus***
– Acanthophyses indextrinoid ..... ***A. cerussatus***
11 Acanthophyses absent **12**
– Acanthophyses present **19**
12 Generative hyphae simple-septate **13**
– Generative hyphae clamped **16**
13 Basidiomata discoid; basidiospores >20 µm long ..... ***A. amorphus***
– Basidiomata corticioid; basidiospores <20 µm long **14**
14 Basidiospores <8 µm long ..... ***A. tenuissimus***
– Basidiospores >8 µm long **15**
15 Basidiospores 12–17 × 10–15 µm; on angiosperm wood ..... ***A. ryvardenii***
– Basidiospores 8–11.5 × 6–8.5 µm; on bamboo ..... ***A. verrucosporus***
16 Basidiospores >20 µm long ..... ***A. grantii***
– Basidiospores <20 µm long ***17***
17 On *Quercus* ..... ***Aleurocystidiellum disciforme***
– On gymnosperm **18**
18 Encrusted skeletocystidia present; on *Abies* ..... ***Aleurocystidiellum subcruentatum***
– Moniliform gloeocystidia present; on *Pinus* ..... ***Aleurocystidiellum tsugae***
19 Acanthophyses amyloid ..... ***A. botryosus***
– Acanthophyses non-amyloid **20**
20 Basidiospores globose; on bamboo ..... ***Acanthobasidium bambusicola***
– Basidiospores ellipsoid; on wood **21**
21 On gymnosperm **22**
– On angiosperm **23**
22 Basidiospores 16–21 × 12–17 µm ..... ***A. effusus***
– Basidiospores 26–38 × 20–28 µm ..... ***A. gigasporus***
23 Basidiomata white when fresh; acanthophyses rare ..... ***A. microcarpus***
– Basidiomata pinkish when fresh; acanthophyses abundant **24**
24 Basidiospores 16–20 × 11–14 µm ..... ***A. subroseus***
– Basidiospores >20 µm long, >14 µm wide **25**
25 Acanthophyses hyphoid, covered with spines at whole upper part; basidia and gloeocystidia covered with spines at basal part; basidiospores usually D-shaped ..... ***A. mirabilis***
– Acanthophyses hyphoid to clavate, covered with spines only at apex; basidia and gloeocystidia smooth; basidiospores ellipsoid ..... ***A. wakefieldiae***

Acknowledgements

The authors thank Dr. Karen Nakasone (Center for Forest Mycology Research, Northern Research Station, U.S. Forest Service, Madison, USA) and Dr. Chang-Lin Zhao (Southwest Forestry University, Kunming, China) for providing their specimens. This study was supported by the National Natural Science Foundation of China (Nos. 31470144 & 31670013).

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