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Colletotrichum chinense sp. nov. from *Yucca gloriosa* and *C. quercicola* sp. nov. from *Quercus variabilis* in China

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Abstract

Colletotrichum is an important plant pathogenic genus causing anthracnose on a wide range of host plants. During 2019 and 2021, *Colletotrichum* isolates were obtained during surveys of anthracnose on garden plants in China. Multi-gene phylogenetic analyses of internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), chitin synthase 1 (*chs-1*), actin (*act*) and beta-tubulin (*tub2*) sequences coupled with morphological evidence support the introduction of two novel species namely *Colletotrichum chinense* sp. nov. from *Yucca gloriosa* in Beijing and *C. quercicola* sp. nov. from *Quercus variabilis* in Shaanxi Province. Phylogenetic inference revealed that two isolates of *C. chinense* belonged to the agaves species complex and were closely related to *C. agaves*, and differed from the other species within this species complex by shorter conidia and the host association. Molecular identification showed that two isolates of *C. quercicola* formed a highly supported lineage close to *C. tanaceti* in the destructivum species complex, which could be distinguished from *C. tanaceti* by straighter conidia. In pathogenicity tests, yellow spots and orange conidial masses displayed on the inoculated *Y. gloriosa* leaves and brown spots appeared on the inoculated *Q. variabilis* leaves. In addition, *C. chinense* and *C. quercicola* were re-isolated from spots of the tested leaves of *Y. gloriosa* and *Q. variabilis*.

Keywords

Ascomycota, multigene phylogeny, new species, taxonomy

Introduction

The genus *Colletotrichum* (*Glomerellaceae*, *Glomerellales*, *Sordariomycetes*) is represented by its type species *Colletotrichum lineola* (Corda 1831; Damm et al. 2009; Hyde et al. 2020). The sexual morph of *Colletotrichum*, characterized by solitary or gregarious ascomata, 8-spored asci, and one-celled hyaline ascospores, was previously known as the genera *Gnomoniopsis* and *Glomerella* (Stoneman 1898; von Schrenk and Spaulding 1903; Marin-Felix et al. 2017). The asexual morph is characterized by acervular conidiomata, often with setae, producing cylindrical or crescent-shaped conidia, and by the formation of appressoria (Sutton 1992; Marin-Felix et al. 2017). With the implementation of "one fungus one name" nomenclature, *Colletotrichum* has been chosen to represent this genus based on priority (Réblová et al. 2016).

Previously, species of *Colletotrichum* were distinguished based on host range and a suite of morphological characteristics, especially the size and shape of conidia, appressoria, and sporulating structures (von Arx 1957; Cai et al. 2009; Hyde et al. 2009a, b). However, many taxonomic problems arose, due to few reliable and often variable morphological characters among species, and uncertain or broad host relationships (Cai et al. 2009; Hyde et al. 2009a; Cannon et al. 2012; Liu et al. 2016). Thus, many species are required taxonomic revision in order to clarify their taxonomic placement (Weir et al. 2012; Damm et al. 2014; Liu et al. 2022).

To establish a stable and natural classification system, Cai et al. (2009) recommended using a polyphasic approach, emphasizing multi-locus phylogeny in conjunction with morphology, geographical and ecological information to characterize and differentiate *Colletotrichum* species. Subsequently, many *Colletotrichum* species had been successfully identified and epitypified, resulting in a much better understanding of phylogenetic relationships of this genus (Weir et al. 2012; Damm et al. 2014). Currently, more than 1000 *Colletotrichum* epithets are listed in Index Fungorum (http://www.indexfungorum.org), and at least 303 species, grouped in 16 species complexes and some singleton species (Mu et al. 2021; Alizadeh et al. 2022; Liu et al. 2022; Zheng et al. 2022).

Many species of *Colletotrichum* have been identified as plant pathogens causing anthracnose on a wide range of hosts, especially in subtropical and tropical regions, leading to significant economic losses (Hyde et al. 2009a; Cannon et al. 2012; Lima et al. 2013). In addition, *Colletotrichum* species may occur as endophytes, saprobes, or opportunistic human pathogens, sometimes as latent plant pathogens, which may switch to a pathogenic lifestyle depending on the host plant, *Colletotrichum* species, and environmental conditions. (Huang et al. 2013; Rai and Agarkar 2014; Crous et al. 2016a; De Silva et al. 2017).

In the present study, by using a nucleotide basic local alignment search tool (BLASTn) analysis (Boratyn et al. 2013) of the ITS sequences, four *Colletotrichum* isolates from *Yucca gloriosa* and *Quercus variabilis* showed highest similarity lower than 98% with species in the agaves and destructivum species complex, respectively. The agaves species complex, represented by *Colletotrichum agaves* and four closely related species, occupies a monophyletic clade within this genus (Bhunjun et al. 2021;

Talhinhas and Baroncelli 2021). The destructivum species complex is a monophyletic group of *C. destructivum* and 19 closely related species that are mainly plant pathogens (Damm et al. 2014; Bhunjun et al. 2021; Talhinhas and Baroncelli 2021). Members of this species complex are serious economic pathogens, such as *C. destructivum*, *C. lentis* and *C. higginsianum* (Damm et al. 2014; Bhadauria et al. 2019; Khodaei et al. 2019). They are characterized by conidia that are slightly curved due to their unilaterally tapering ends and by the small inconspicuous acervuli with rather effuse growth that are sometimes difficult to spot on the host plants (Damm et al. 2014).

Recently, we investigated the phylogenetic diversity of *Colletotrichum* species associated with anthracnose on garden plants in China. Four novel isolates were collected from *Y. gloriosa* and *Q. variabilis* in Beijing and Shaanxi, respectively. The aim of this study was to identify these isolates based on phylogenetic data and morphology and to confirm their pathogenicity.

Materials and methods

Sampling and fungal isolation

From 2019 to 2021, symptomatic leaves of garden plants were collected in China. Specimens were transferred to the laboratory in paper bags and stored at 4 °C until further processing. The surface of diseased leaves were sterilized with 70% ethanol and 2% NaClO for 1 min, rinsed three times with sterile water, and then samples were cut into 0.4 × 0.4 cm small pieces excised from the margins of foliar lesions, and placed on potato dextrose agar (PDA; potato extract 20 g, dextrose 20 g, agar 20 g, 1 L distilled water) plates at 25 °C in the dark. After 2–3 days, single colonies growing from the diseased tissue were transferred to new PDA plates. Single-spore cultures were obtained from the pure colonies and examined morphologically. The cultures were deposited in the China Forestry Culture Collection Center (CFCC; http://cfcc.caf.ac.cn/), and the specimens in the herbarium of the Chinese Academy of Forestry (CAF; http://museum.caf.ac.cn/).

Morphological and culture characterisation

Agar plugs (6 mm in diameter) were taken from the edge of actively growing cultures on PDA and transferred in triplicate on PDA, synthetic low-nutrient agar (SNA; Nirenberg 1976), and malt extract agar (MEA; malt extract 20 g, agar 20 g, yeast extract 2 g, sucrose 5 g, sterile deionized water 1 L) incubated in the dark at 25 °C. After 7 days, the colony characteristics, colony diameters, and pigment production on the three media were noted. Appressoria were observed on slide cultures according to Weir et al. (2012). Moreover, the shape, color and size of conidia, conidiophores, setae, conidiogenous cells and appressoria were measured and captured at least 20 measurements using a Nikon Eclipse 80i compound microscope with differential interference contrast optics.

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fungal mycelia using a CTAB DNA extraction protocol (Doyle and Doyle 1990). The internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), chitin synthase 1 (*chs-1*), actin (*act*) and beta-tubulin (*tub2*) genes were amplified and sequenced using the primer pairs ITS1/ITS4 (White et al. 1990), GDF1/GDR1 (Guerber et al. 2003), CHS-79F/CHS-345R (Carbone and Kohn 1999), ACT-512F/ACT-783R (Carbone and Kohn 1999) and T1/Bt2b (Glass and Donaldson 1995; O'Donnell and Cigelnik 1997), respectively. PCR was performed in 20 μL reaction mixtures containing 10 μL 2× Taq polymerase (Tiangen, China), which contains a premix of Taq DNA polymerase (0.1 U), dNTPs (0.5 mM), $MgCl_2$ (3 mM) Tris-HCl (20 mM), KCl (100 mM) and the appropriate buffer system, 7 μL RNase-free water, 1 μL of each primer (0.5 μM) and 1 μL of DNA template (20 ng/μl). The PCR conditions were as follows: initial heat treatment of 5 min at 94 °C, followed by 35 cycles of 30 sec at 94 °C, 30 s at 54 °C (ITS), 60 °C (*gapdh*), 59 °C (*chs-1*), 58 °C (*act*) or 55 °C (*tub2*), and 1 min at 72 °C, and a final elongation period of 7 min at 72 °C. Amplicons were purified and sequenced by ABI3730XL Gene Analyzer at the Shanghai Invitrogen Biological Technology Company Limited (Beijing, China).

Phylogenetic analyses

Newly generated sequences from the four isolates in this study were assembled using SeqMan v. 7.1.0, and the closest match using BLASTn analyses. Reference *Colletotrichum* sequences (Table 1) were downloaded from GenBank, based on recent publication (Liu et al. 2022). Multiple sequences were aligned using the MAFFT v.7.110 online programme (http://mafft.cbrc.jp/alignment/server/, Katoh et al. 2019) by default settings, and adjusted manually in MEGA v.7.0 (Kumar et al. 2016). The best-fit nucleotide substitution models for each gene were selected using jModelTest v. 2.1.7 (Darriba et al. 2012) under the Akaike information criteria (AIC).

Phylogenetic analyses using Maximum Likelihood (ML) and Bayesian Inference (BI) were performed. ML analyses were constructed on the RAxML-HPC BlackBox 8.2.10 (Stamatakis 2014) using the GTR+GAMMA model with 1000 bootstrap replicates. BI analyses were also performed using a Markov Chain Monte Carlo (MCMC) algorithm in MrBayes v. 3.2.6 (Ronquist et al. 2012). The analyses were conducted by running 5,000,000 generations in two independent runs and sampling every 100th generations. The first 25% of the trees of MCMC sampling were discarded as burn-in and posterior probabilities (PP) were determined from the remaining trees. The results were visualized in FigTree 1.4 (http://tree.bio.ed.ac.uk/software/figtree) and edited with Adobe Illustrator CS6.0.

Table 1. *Colletotrichum* spp. used for phylogenetic analyses in the study.

Species name	Accession no.[a]	GenBank Accession No.				
		ITS	***Gapdh***	***chs-1***	***act***	***tub2***
C. agaves	CBS 118190*	DQ286221	NA	NA	NA	NA
C. agaves	LC0947	MZ595831	MZ664053	MZ799266	MZ664129	MZ673955
C. americae-borealis	ATCC 11869	KM105223	KM105578	KM105293	KM105433	KM105503
C. americae-borealis	CBS 136232*	KM105224	KM105579	KM105294	KM105434	KM105504
C. antirrhinicola	CBS 102189*	KM105180	KM105531	KM105250	KM105390	KM105460
C. atractylicola	SAUCC 1307*	KR149280	KR259334	KR259333	KR132243	KU058178
C. atractylicola	SAUCC 130801	KU289192	KU289207	KU289202	KU289197	KU289212
C. boninense	CBS 123755*	JQ005153	JQ005240	JQ005327	JQ005501	JQ005588
C. brasiliense	CBS 128501*	JQ005235	JQ005322	JQ005409	JQ005583	JQ005669
C. bryoniicola	CBS 109849*	KM105181	KM105532	KM105251	KM105391	KM105461
C. chinense	**CFCC 57501***	**ON692808**	**ON755050**	**ON755046**	**ON755042**	**ON755054**
C. chinense	**CFCC 57502**	**ON692809**	**ON755051**	**ON755047**	**ON755043**	**ON755055**
C. destructivum	CBS 114801	KM105219	KM105574	KM105289	KM105429	KM105499
C. destructivum	CBS 157.83	KM105215	KM105570	KM105285	KM105425	KM105495
C. destructivum	IMI 387103	KM105221	KM105576	KM105291	KM105431	KM105501
C. destructivum	CBS 136228*	KM105207	KM105561	KM105277	KM105417	KM105487
C. euphorbiae	CBS 134725*	KF777146	KF777131	KF777128	KF777125	KF777247
C. fuscum	CBS 133701*	KM105174	KM105524	KM105244	KM105384	KM105454
C. fuscum	CBS 133702	KM105178	KM105528	KM105248	KM105388	KM105458
C. fuscum	CBS 133703	KM105175	KM105525	KM105245	KM105385	KM105455
C. fusiforme	MFLUCC 12-0437	KT290266	KT290255	KT290253	KT290251	KT290256
C. higginsianum	CPC 19379*	KM105184	KM105535	KM105254	KM105394	KM105464
C. higginsianum	CPC 19364	KM105185	KM105537	KM105255	KM105395	KM105465
C. higginsianum	CPC 19369	KM105188	KM105540	KM105258	KM105398	KM105468
C. higginsianum	CPC 19394	KM105193	KM105546	KM105263	KM105403	KM105473
C. ledebouriae	CBS 141284*	KX228254	NA	NA	KX228357	NA
C. lentis	CBS 127604*	JQ005766	KM105597	JQ005787	JQ005829	JQ005850
C. lentis	CBS 127605	KM105241	KM105598	KM105311	KM105451	KM105521
C. lini	CBS 172.51*	JQ005765	KM105581	JQ005786	JQ005828	JQ005849
C. lini	CBS 136856	KM105233	KM105589	KM105303	KM105443	KM105513
C. lini	CBS 130828	KM105234	KM105590	KM105304	KM105444	KM105514
C. neorubicola	CCR144*	MK529906	MK547520	MK547526	MK547523	MN186400
C. neorubicola	CCR145	MK529908	MK547521	MK547527	MK547524	MN186401
C. neorubicola	CCR146	MK529907	MK547522	MK547528	MK547525	MN186402
C. neosansevieriae	CBS 139918*	KR476747	KR476791	NA	KR476790	KR476797
C. ocimi	CBS 298.94*	KM105222	KM105577	KM105292	KM105432	KM105502
C. panacicola	C08048	GU935867	GU935847	NA	GU944757	NA
C. panacicola	C08061	GU935868	GU935848	NA	GU935791	NA
C. panacicola	C08087	GU935869	GU935849	NA	GU944758	NA
C. pisicola	CBS 724.97 *	KM105172	KM105522	KM105242	KM105382	KM105452
C. pleopeltidis	CBS 147082*	MW883412	NA	MW890035	MW890024	NA
C. quercicola	**CFCC 54457***	**ON692810**	**ON755052**	**ON755048**	**ON755044**	**ON755056**
C. quercicola	**CFCC 57507**	**ON692811**	**ON755053**	**ON755049**	**ON755045**	**ON755057**
C. sansevieriae	MAFF 239721*	LC179806	LC180130	LC180129	LC180127	LC180128
C. sansevieriae	BTGN2	MN386823	MN386911	NA	NA	MN386867
C. shisoi	JCM 31818*	MH660930	MH660931	MH660929	MH660928	MH660932
C. shisoi	MAFF 240106	MH660936	MH660935	MH660934	MH660933	MH660937
C. tabacum	CBS 124249	KM105206	KM105560	KM105276	KM105416	KM105486
C. tabacum	CBS 161.53	JQ005763	KM105559	JQ005784	JQ005826	JQ005847
C. tabacum	CPC 18945*	KM105204	KM105557	KM105274	KM105414	KM105484

Species name	Accession no.[a]	GenBank Accession No.				
		ITS	*Gapdh*	*chs-1*	*act*	*tub2*
C. tanaceti	BRIP 57316	JX218230	JX218245	JX259270	JX218240	JX218235
C. tanaceti	CBS 132693	JX218228	JX218243	JX259268	JX218238	JX218233
C. tanaceti	CBS 132818	JX218229	JX218244	JX259269	JX218239	JX218234
C. truncatum	IMI 135524	GU227874	GU228266	GU228364	GU227972	GU228168
C. utrechtense	CBS 130243*	KM105201	KM105554	KM105271	KM105411	KM105481
C. utrechtense	CBS 135827	KM105202	KM105555	KM105272	KM105412	KM105482
C. utrechtense	CBS 135828	KM105203	KM105556	KM105273	KM105413	KM105483
C. vignae	CBS 501.97*	KM105183	KM105534	KM105253	KM105393	KM105463
C. vignae	CPC 19383	KM105182	KM105533	KM105252	KM105392	KM105462

Notes: NA, not applicable. * ex-type strains. [a]ATCC: American Type Culture Collection, Virginia, USA; BRIP: Plant Pathology Herbarium, Department of Primary Industries, Queensland, Australia; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CFCC: China Forestry Culture Collection Center, Beijing, China; CPC: Culture collection of Pedro Crous, housed at CBS; LC: the LC Culture Collection (a personal culture collection of Lei Cai, housed in the Institute of Microbiology, Chinese Academy of Sciences); IMI: Culture collection of CABI Europe UK Centre, Egham, UK; JCM: Japan Collection of Microorganisms, RIKEN Bioresource Center, Tsukuba, Japan; MAFF: MAFF Genbank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; MFLU: Mae Fah Luang University Culture Collection, Thailand; SAUCC: Department of Plant Pathology, College of Plant Protection, Shenyang Agricultural University, China.

Pathogenicity test

The pathogenicity of two *Colletotrichum* isolates was assessed on detached healthy *Y. gloriosa* and *Q. variabilis* plants in the greenhouse. Leaves were washed in running distilled water, surface-sterilized in 70% ethanol and 2% NaClO for 1 min, then rinsed in sterile distilled water. Spores were harvested from two-week-old PDA plates with 10 ml of sterilized water with spore suspension filtered through two layers of cheesecloth to eliminate debris and mycelium. The conidial suspension was adjusted to a final inoculum concentration of 1×10^6–10^7 conidia/mL with sterile deionized water. Then 10 µL of conidial suspension was placed in the middle portion of the leaves, and inoculated sterile water in the additional leaves served as control. Each treatment had three replicates (three leaves), and the experiment was carried out twice. The inoculated leaves were placed in transparent plastic bags at 25 °C and over 90% humidity in the dark for 14 days. After appearance of symptoms, fungus isolates were re-isolated from the infected leaves and identified based on the morphological and phylogenetic analyses to fulfill Koch's postulates.

Results

Phylogenetic analyses

Closest matches in BLASTn searches with the ITS sequences, these isolates were preliminarily identified to be in the agaves and destructivum species complexes. Further, phylogenetic trees were constructed based on combined loci of ITS, *gapdh*, *act*, *chs-1* and *tub2* sequences to identify these isolates to species level.

For the agaves species complex, DNA sequences of five genes were obtained from two isolates from *Y. gloriosa* in this study, with seven reference strains of the agaves species complex, and *C. boninense* (CBS 123755, ex-type) and *C. brasiliense* (CBS

128501, ex-type) as the outgroup taxa. A total of 1649 characters including alignment gaps (578 for ITS, 94 for *gapdh*, 232 for *chs-1*, 240 for *act* and 505 for *tub2*) were included in the phylogenetic analyses. Of these characters, 1271 were constant, 162 were variable and parsimony-uninformative, and 216 were parsimony-informative. The resulting ML and BI trees had similar topologies; the ML tree (Fig. 1) was selected to represent the phylogeny with ML/BI support values. Two isolates (CFCC 57501 and CFCC 57502) formed a close clade to *C. agaves* (Fig. 1).

For the destructivum species complex, DNA sequences of five genes were obtained from two isolates from *Q. variabilis* in this study, and 44 reference strains of the destructivum species complex, and *C. truncatum* (IMI 135524) and *C. fusiforme* (MFLUCC 12-0437) as the outgroup taxa. A total of 1875 characters including gaps (560 for ITS, 236 for *gapdh*, 280 for *chs-1*, 274 for *act* and 525 for *tub2*) were obtained in the phylogenetic analyses. Of

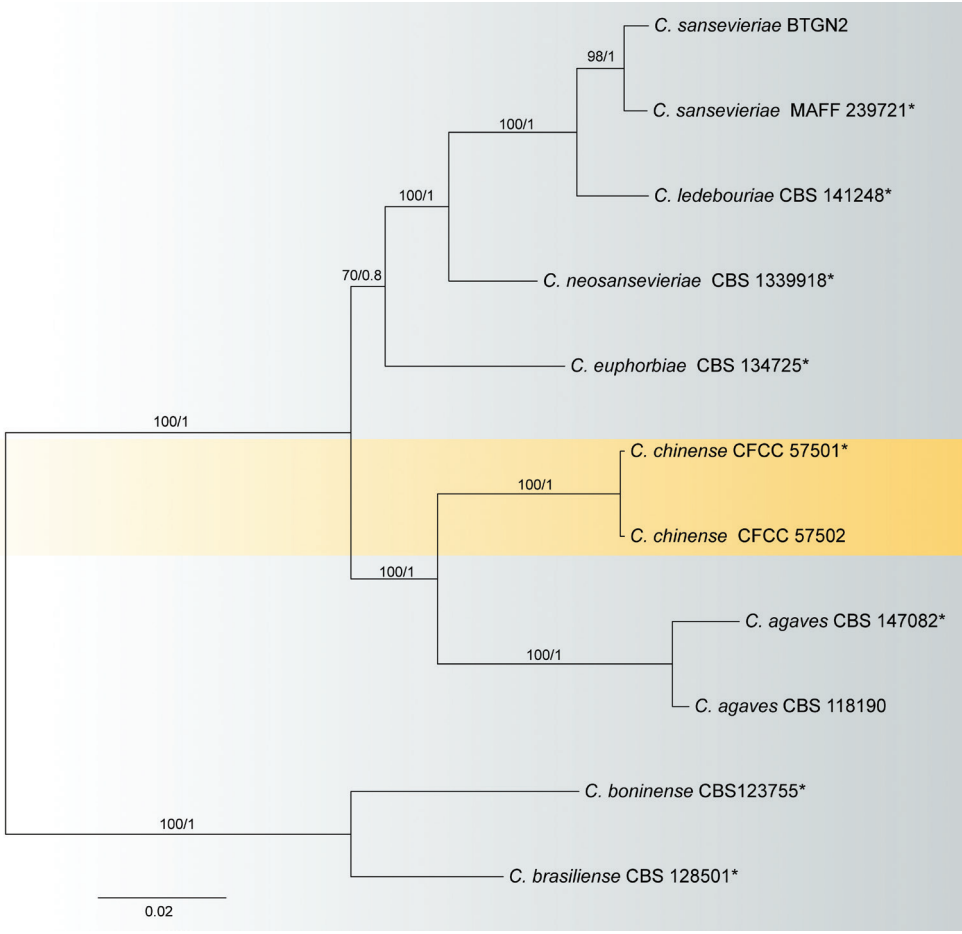

Figure 1. Phylogenetic tree obtained by Maximum likelihood analyses using the combined ITS, *gapdh*, *chs-1*, *act* and *tub2* sequence alignments of the agaves species complex. Numbers above the branches indicate ML bootstraps (left, MLBS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.7). The tree is rooted with *C. boninense* (CBS 123755, ex-type) and *C. brasiliense* (CBS 128501, ex-type).

these characters, 1292 were constant, 177 were variable and parsimony-uninformative, and 406 were parsimony-informative. The resulting ML and BI trees had similar topologies; the ML tree (Fig. 2) was selected to represent the phylogeny with ML/BI support values. Two new isolates (CFCC 54457 and CFCC 57507) formed a sister clade to *C. tanaceti* (Fig. 2).

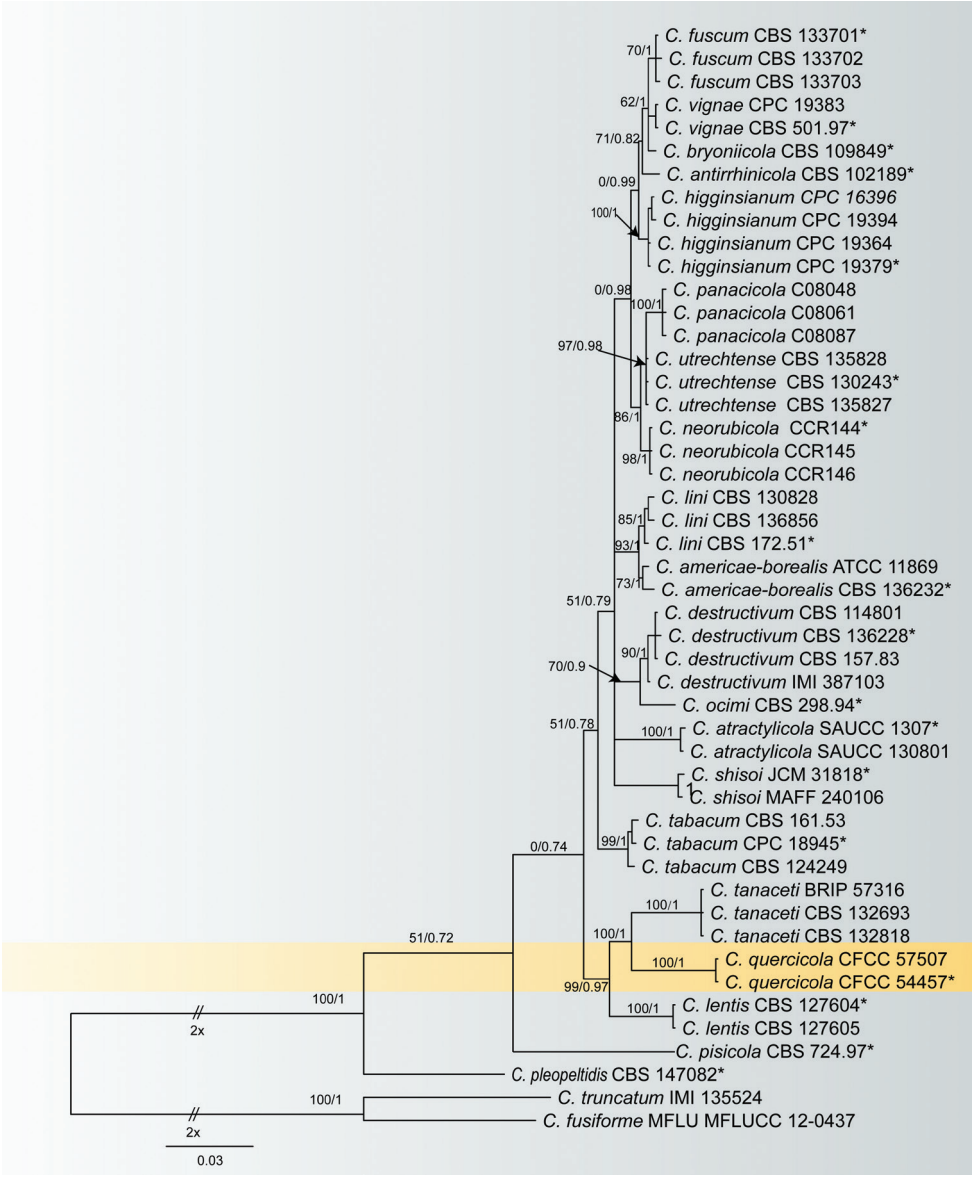

Figure 2. Phylogenetic tree obtained by Maximum likelihood analyses using the combined ITS, *gapdh*, *chs-1*, *act* and *tub2* sequence alignments of the destructivum species complex. Numbers above the branches indicate ML bootstraps (left, MLBS ≥ 50%) and Bayesian Posterior Probabilities (right, BPP ≥ 0.7). The tree is rooted with *C. fusiforme* (MFLU 12-0437CC) and *C. truncatum* (IMI 135524).

Taxonomy

Colletotrichum chinense **Ning Jiang & C.B. Wang, sp. nov.**
MycoBank No: 844527
Fig. 3

Etymology. Referring to the country, where the species was first collected.

Description. ***Sexual morph*** not observed. ***Asexual morph*** developed on PDA. ***Setae*** and ***chlamydospores*** not observed. ***Conidiomata*** acervular, abundant, pulvinate, 200–500 µm diam. ***Conidiophores*** smooth-walled, unbranched, septate, sometimes constricted at the septa, hyaline, up to 40 µm long. ***Conidiogenous cells*** 6.5–19.5 × 3–8 µm ($\overline{x}$ = 12.7 ± 2.7 × 5.3 ± 1.3 µm, n = 20), subglobose to ampulliform, smooth-walled, hyaline. ***Conidia*** 9.5–25.5 × 3.5–8.5 µm ($\overline{x}$ = 14.8 ± 1.8 × 6 ± 1 µm, n = 50), L/W ratio = 2–2.7, cylindrical, obtuse at the apex, smooth-walled, hyaline, contents granular. ***Appressoria*** not observed.

Culture characters. Colonies on PDA, flat, with an entire margin, with sparse aerial mycelium, covered with orange conidial masses, reaching 23–25 mm diam in 7 days at 25 °C. Colonies on MEA, flat, with no aerial mycelium, covered with slimy conidial masses, reaching 15–20 diam in 7 days at 25 °C. Colonies on SNA flat, sparse white hyphae, with an entire margin, reaching 12–15 diam in 7 days at 25 °C.

Specimens examined. China, Beijing City, isolated from leaf spot of *Yucca gloriosa* L., *Cheng-Bin Wang*, 15 August 2020 (holotype CAF800056; ex-type living culture: CFCC 57501); *Ibid* (living culture: CFCC 57502).

Notes. *Colletotrichum beeveri* of the boninense species complex and *C. tofieldiae* of the spaethianum species complex have been reported from *Yucca* before the present study (Liu et al. 2022). *Colletotrichum chinense* from the present study is similar to *C. beeveri* in the conidial shape, but differs in conidial size (9.5–25.5 × 3.5–8.5 µm in PDA vs. 12.5–15.5 × 5.5–6.5 µm in SNA) (Damm et al. 2012). In addition, *C. tofieldiae* differs from *C. chinense* by the falcate conidia (Damm et al. 2009). Based on phylogenetic analyses using multi-locus sequences (ITS, *gapdh*, *chs-1*, *act* and *tub2*), *C. chinense* formed a sister clade to *C. agaves* in the agaves species complex. The sequence identities between *C. chinense* CFCC 57501 and *C. agaves* LC0947 (21/578 ITS, 6/94 *gapdh*, 6/232 *chs-1*, 19/240 *act* and 26/505 *tub2*), *C. euphorbiae* CBS 134725 (31/578 ITS, 8/94 *gapdh*, 7/232 *chs-1*, 35/240 *act* and 32/505 *tub2*), *C. ledebouriae* CBS 141284 (29/578 ITS, 30/240 *act*), *C. neosansevieriae* CBS 139918 (28/578 ITS, 6/94 *gapdh*, 28/240 *act* and 27/416 *tub2*) and *C. sansevieriae* MAFF 239721 (29/578 ITS, 5/94 *gapdh*, 9/232 *chs-1*, 31/240 *act* and 44/505 *tub2*). (Nakamura et al. 2006; Crous et al. 2013, 2015, 2016b; Liu et al. 2022) The *chs-1* sequence of *C. neosansevieriae* CBS 139918 and the *gapdh*, *chs-1* and *tub2* sequences of *C. ledebouriae* CBS 141284 were missing. Morphologically, the conidia size of *C. chinense* are shorter than other species (Table 2).

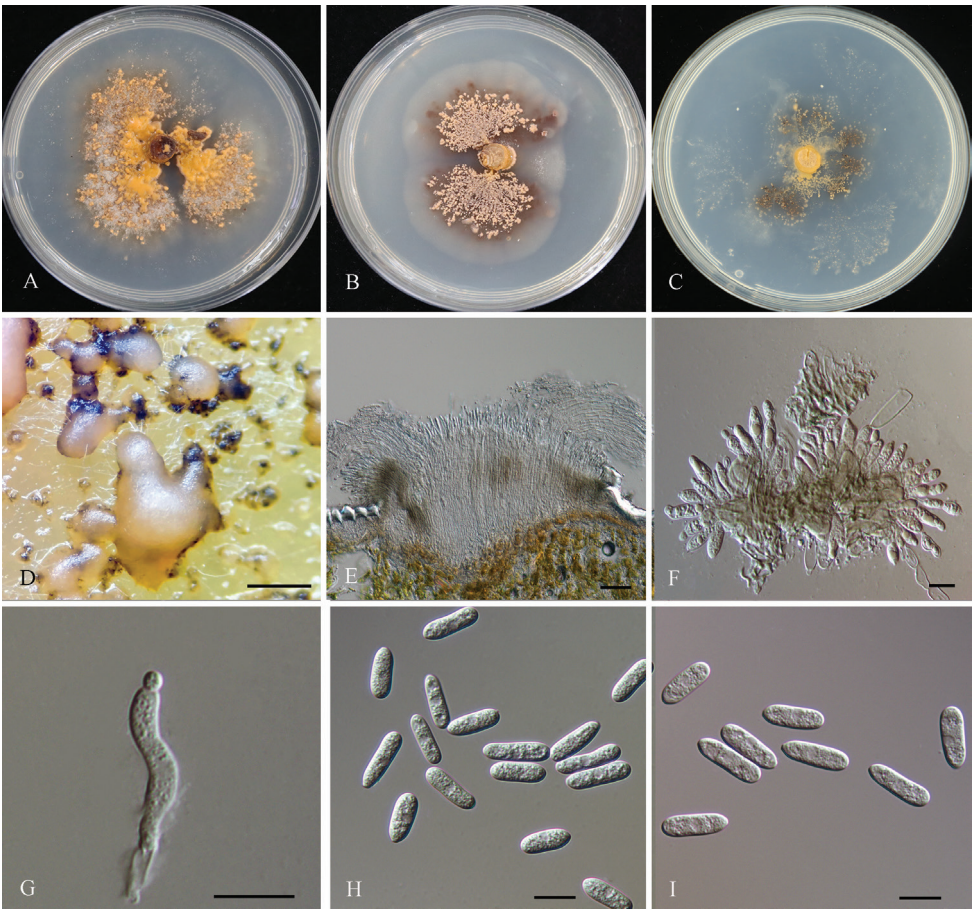

Figure 3. *Colletotrichum chinense* (CFCC 57501; ex-type) **A** colony on PDA **B** colony on MEA **C** colony on SNA **D** conidiomata formed in PDA **E** conidiophores from the host **F, G** conidiophores **H, I** conidia **F–I** from PDA. Scale bars: 200 µm (**D**); 50 µm (**E**); 20 µm (**F, G**); 10 µm (**H, I**).

Table 2. Morphological comparison of species in the agaves species complex.

Species	Type	Media for Conidia morph	Hosts	Distribution	Conidia (µm)	Appressoria (µm)	Reference
C. agaves	Epitype	PDA	*Agave* spp.	Mexico; USA; Netherlands	(17.5–)19.0–30.5(–33) × 5–8(–9.5) on	Not observed	Farr et al. (2006)
C. chinense	**Holotype**	**PDA**	***Yucca gloriosa***	**China**	**(9.5–)12.5–16.5(–25.5) × (3.5–)6.0–7.0(–8.5)**	**Not observed**	**This study**
C. euphorbiae	Holotype	SNA	*Euphorbia* sp	South Africa	(17–)23–28(–28.5) × (6–)6.5–7	(6.5–)8.5–14.5(–20.5) × (5.5–)6–10.5(–16)	Crous et al. (2013)
C. ledebouriae	Holotype	PNA	*Ledebouria floribunda*	South Africa	(15–)17–21(–22) × (5–)6	Not observed	Crous et al. (2016)
C. neosansevieriae	Holotype	SNA	*Sansevieria trifasciata*	South Africa	(16–)18–22(–25) × (4–)5–6	Not observed	Crous et al. (2015)
C. sansevieriae	Holotype	PDA	*Sansevieria* spp.	Asia; Australia; USA	12.5–(18.4)–32.5 × 3.8–(6.4)–8.8 PDA	6.3–(7.7)–8.8 × 6.3–(7.3)–7.5	Nakamura et al. (2006)

Colletotrichum quercicola **Ning Jiang & C.B. Wang, sp. nov.**
MycoBank No: 844528
Fig. 4

Etymology. Referring to the host genus, *Quercus*.

Description. ***Sexual morph*** not observed. ***Asexual morph*** developed on PDA. ***Chlamydospores*** not observed. ***Conidiomata*** acervular, abundant, globose to pulvinate, 200–400 μm diam. ***Conidiophores***, hyaline, branched, smooth-walled, up to 50 μm long. *Setae* medium brown, smooth-walled, 60–145 μm long, 1–3-septate. ***Conidiogenous cells*** 6–18 × 3–7 μm ($\overline{x}$ = 7.9 ± 3.6 × 4 ± 1.2 μm, n = 20), hyaline, smooth-walled, cylindrical to elongate ampulliform. ***Conidia*** 14.5–23 × 3–5 μm ($\overline{x}$ = 17 ± 1.7 × 3.9 ± 0.5 μm, n = 50), L/W ratio =4–5, hyaline, smooth-walled, fusiform, straight to slightly curved with both ends rounded or one end round and the other truncate. ***Appressoria*** 6–11 × 4–8 μm ($\overline{x}$ = 8.4 ± 1.4× 5 ± 1 μm, n = 50), L/W ratio = 1.5–2, single, medium brown, smooth-walled, subglobose, ovate to broadly elliptical in outline.

Culture characters. Colonies on PDA flat, with moderate aerial mycelium, margin white to light gray, gray to brown in the center, reaching 46–50 mm diam in 7 days at 25 °C. Colonies on MEA flat, covered by white aerial mycelium, white margin and light orange in the center, reaching 30–35 mm diam after 7 days at 25 °C. Colonies on SNA flat, with entire margin, covered by sparse white aerial mycelium, reaching 20 mm diam after 7 days at 25 °C.

Specimens examined. China, Shaanxi Province, Foping County, Dongshan Park, isolated from leaf spot of *Quercus variabilis* Bl., *Yong Li*, 11 September 2019 (holotype CAF800057; ex-type living culture: CFCC 54457); *Ibid* (living culture: CFCC 57507).

Notes. Four *Colletotrichum* species are presently known to occur on *Quercus* hosts, *viz*. *C. clidemiae*, *C. gloeosporioides*, *C. karstii* and *C. theobromicola* (Weir et al. 2012; Liu et al. 2021). *Colletotrichum quercicola* can be distinguished from those species based on any of the loci (ITS, *gapdh*, *chs-1*, *act* and *tub2*) and the fusiform conidia. *Colletotrichum quercicola* is a member of the destructivum species complex and near to *C. tanaceti.* Phylogenetically, this species can be distinguished from *C. tanaceti* CBS 132693 by 88 nucleotide differences in concatenated alignment (20/560 in ITS, 14/274 in *act*, 2/280 in *chs-1*, 17/236 in *gapdh*, and 33/525 in *tub2*) (Damm et al. 2014). Morphologically, *C. quercicola* CFCC 54457 conidia are straight to slightly curved, differing from distinctly curved conidia in *C. tanaceti* CBS 132693 (Damm et al. 2014).

Pathogenicity

Pathogenicity tests were conducted to confirm Koch's postulates on *Q. variabilis* leaves for *C. quercicola*, and on *Y. gloriosa* leaves for *C. chinense*. After 14 days of inoculation, necrotic lesions and typical orange conidial masses were observed from the inoculated site of *Y. gloriosa* leaves, and *Q. variabilis* leaves showed brown spot from the inoculated site, whereas all control leaves remained healthy (Fig. 5). Furthermore,

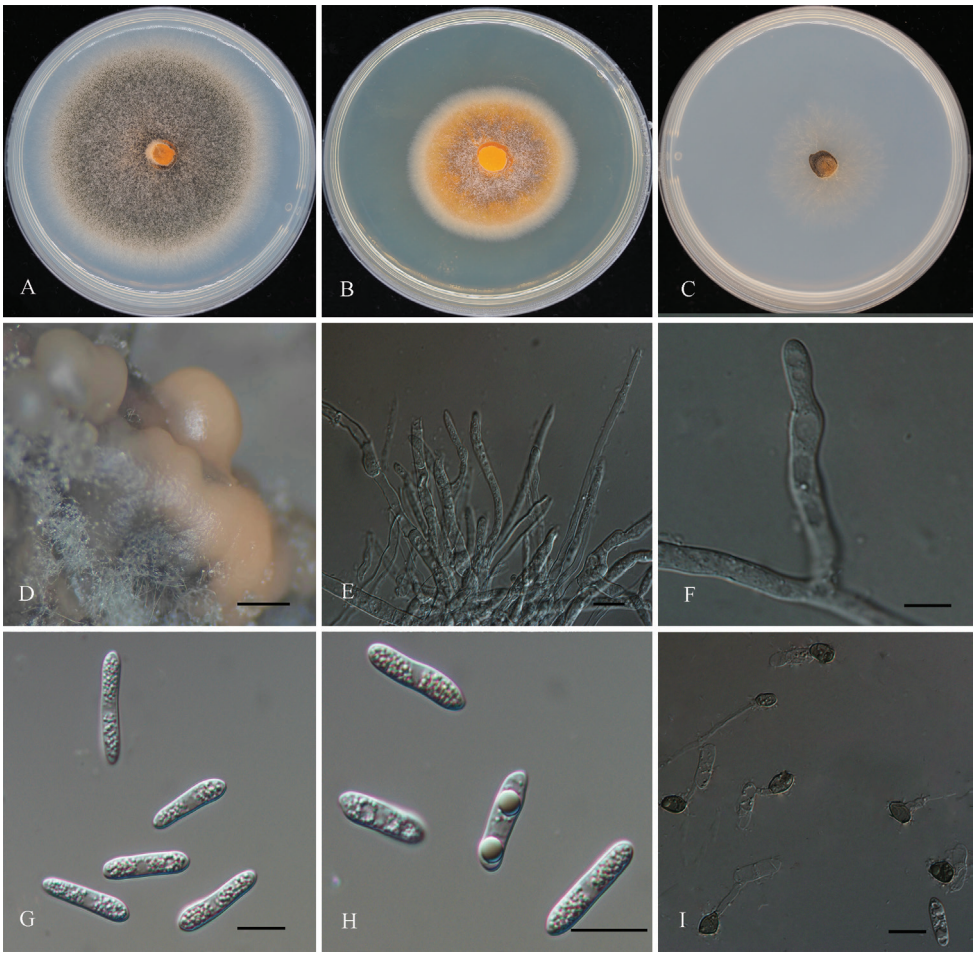

Figure 4. *Colletotrichum quercicola* (CFCC 54457; ex-type) **A** colony on PDA **B** colony on MEA **C** colony on SNA **D** conidiomata formed in PDA **E, F** conidiophores **G, H** conidia **I** appressoria were producing using a slide culture technique **E–H** from PDA. Scale bars: 200 μm (**D**); 50 μm (**E**); 20 μm (**F**); 10 μm (**G–I**).

Colletotrichum isolates could consistently be re-isolated from symptomatic lesions, but never from control leaves. And these isolates were identified as material used for inoculations based on multigene phylogenetic analyses and morphological characters, fulfilling Koch's postulates.

Discussion

In the present study, we collected garden plants with anthracnose symptoms or leaf spots in China. From these samples, the obtained *Colletotrichum* isolates were identified based on morphological features of the asexual morph obtained in culture and five combined

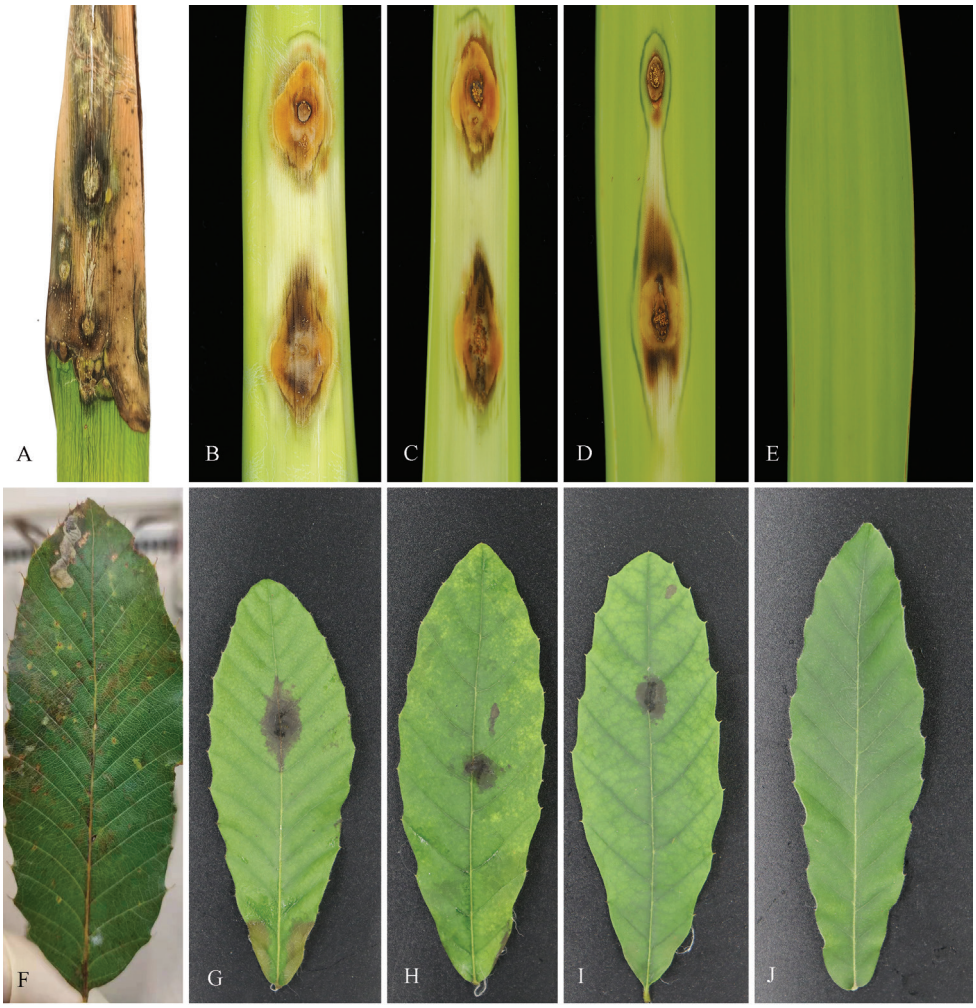

Figure 5. Typical field symptoms of disease and artificial inoculation results **A–E** *Yucca gloriosa* leaves **F–J** *Quercus variabilis* leaves **A, F** Anthracnose field symptoms **B–D** Symptoms resulting from *Colletotrichum chinense* (CFCC 57501; ex-type) after 14 days **G–I** symptoms resulting from *Colletotrichum quercicola* (CFCC 54457; ex-type) after 14 days **E, J** symptoms resulting from sterile deionized water after 14 days.

loci (ITS, *gapdh*, *chs-1*, *act* and *tub2*) phylogenies. The phylogenetic analyses revealed two novel species, *C. chinense* from *Y. gloriosa* in Beijing, and *C. quercicola* from *Q. variabilis* in the Shaanxi Province, and morphological characters can distinguish these isolates from related species. Pathogenicity test revealed *C. chinense* appearing as a causal agent of *Y. gloriosa* anthracnose and *C. quercicola* as a pathogen of *Q. variabilis* anthracnose.

ITS is evaluated as a universal DNA barcode marker for fungi (Schoch et al. 2012). However, most *Colletotrichum* species could not be distinguished based on ITS only (Cai et al. 2009; Jayawardena et al. 2016). Further, multi-locus DNA sequences, including ITS combined with supplementary barcodes, for which including some of *act*,

the intergenic region between DNA lyase and the mating-type (*mat1-2*) gene (*apMat*), DNA lyase (*apn2*), calmodulin (*cal*), *chs-1*, *gapdh*, glutamine synthetase (*gs*), superoxide dismutase (*sod2*) or *tub2* genes for species delimitation (Cannon et al. 2012; Silva et al. 2012a, b; Weir et al. 2012; Vieira et al. 2020). Generally, ITS, *act*, *chs-1*, *gapdh* and *tub2* gene regions have provided adequate resolution to differentiate species within this genus (Bhunjun et al. 2021; Jayawardena et al. 2021; Talhinhas and Baroncelli 2021). In this study, phylogenetic analyses based on five combined loci (ITS, *gapdh*, *chs-1*, *act* and *tub2*) supported that these isolates clustered in a well-supported clade in the agaves and destructivum species complexes with high confidence.

The agaves species complex groups *Colletotrichum agaves*, and four related species, *C. ledebouriae*, *C. neosansevieriae*, *C. euphorbiae* and *C. sansevieriae* (Bhunjun et al. 2021; Talhinhas and Baroncelli 2021). They are unable to be distinguished based on conidial dimensions alone (Table 2). Members of this species complex were assumed to have host specificity (Nakamura et al. 2006; Jayawardena et al. 2021; Talhinhas and Baroncelli 2021). However, three species (*C. ledebouriae*, *C. neosansevieriae* and *C. euphorbiae*) were found only once from its type strain (Crous et al. 2013, 2015, 2016b). Four species, *C. agaves* on *Agave* spp., *C. sansevieriae* on *Sansevieria* sp., *C. ledebouriae* on *Ledebouria floridunda*, *C. neosansevieriae* on *Sansevieria trifasciata*, have only been recorded from *Asparagaceae* (Talhinhas and Baroncelli 2021). In this study, *C. chinense* was isolated from symptomatic leaves of *Y. gloriosa*, belonging to the family *Asparagaceae*.

Species in the destructivum species complex are serious pathogens undergoing a hemibiotrophic lifestyle and have been associated with 49 plant species belonging to 41 genera (Damm et al. 2014; Jayawardena et al. 2021; Talhinhas and Baroncelli 2021). Many species appear to have a wide host range, while some species may affect single host species or genera (Damm et al. 2014; Talhinhas and Baroncelli 2021). Typical characteristics of species in this species complex are characterized by the presence of straight or slightly curved conidia with obtuse apices (Bhunjun et al. 2021; Jayawardena et al. 2021). Morphological differences in the size of conidia and appressoria were observed between this species complex (Table 3). The morphological approach alone makes it difficult to distinguish in this complex due to few and variable morphological characteristics.

Although morphological characters may not prove taxonomically informative for species differentiation within species complex, they are considered as a basis to taxonomic segregation for distinguishing species between different species complexes (Cannon et al. 2012; Liu et al. 2022). A polyphasic approach, emphasizing multi-gene phylogenetic analyses combined with analyses of ecological, geographical and morphological data was essential to the identification of *Colletotrichum* species (Cai et al. 2009; Jayawardena et al. 2021; Talhinhas and Baroncelli 2021). In recent years, the classification and species concepts in *Colletotrichum* was changed according to this ideal polyphasic approach (Jayawardena et al. 2021; Talhinhas and Baroncelli 2021; Liu et al. 2022). In the present study, we described two novel species based on molecular sequence analyses and morphological characters, confirming their pathological characterization. To our knowledge, this is the first report of anthracnose on *Y. gloriosa* and *Q. variabilis.* These results may provide an important basis for the prevention and control of this disease.

Table 3. Morphological comparison of species in the destructivum species complex.

Species	Type	Media for Conidia morph	Hosts	Distribution	Conidia (μm)	Appressoria (μm)	Reference
C. americae-borealis	Holotype	SNA	*Medicago sativa*; *Glycyrrhiza uralensis*	America; China	(13.5–)15.5–18(–19) × 3.5–4	(4.5–)6–10.5(–13) × (3.5–)4–7(–10)	Damm et al. (2014)
C. antirrhinicola	Holotype	SNA	*Antirrhinum majus*	New Zealand; Japan	(14.5–)15.5–19(–23.5) × (3.5–) 4–4.5(–5)	(9–)9.5–12(–13.5) × (5–)6–8(–10)	Damm et al. (2014)
C. atractylodicola	Holotype	PDA	*Atractylodes lancea*	China	13.5–19 × 4–6.5	7.5–14 × 7–10.5	Xu et al. (2018)
C. bryoniicola	Holotype	SNA	*genera of Asteraceae, Convolvulaceae,* and *Fabaceae*; etc	Netherlands; Italy	(13.5–)15–18.5(–22) × 4–5(–5.5)	(3.5–) 4–10(–18) × (2.5–)3.5–6.5(–7.5)	Damm et al. (2014)
C. destructivum	Epitype	SNA	*Trifolium* spp.; *Bletilla ochracea*; *Phragmites* sp.; etc	worldwide	(14–)14.5–16.5(–18) × 3.5–4(–4.5)	(6.5–)10–15.5(–20.5) × (4.5–)5–8(–10.5	Damm et al. (2014)
C. fuscum	Epitype	SNA	*Digitalis* spp.; *Heracleum* sp.; *Coreopsis lanceolata*	Germany; Italy; Netherlands	(16–)16.5–20(–34) × (3.5–)4–4.5(–5.5)	(6–)8.5–14.5(–19) × (6.5–)7–10(–11.5)	Damm et al. (2014)
C. higginsianum	Epitype	SNA	*Brassicaceae*; *Campanula* sp.; *Rumex acetosa*	Italy; Japan, Korea; Trinidad; Tobago; America	(17–)19–20.5(–21) × (3–)3.5–4(–4.5)	(5.5–)10–20(–28.5) × (3.5–) 5–9(–12)	Damm et al. (2014)
C. lentis	Holotype	SNA	*Lens culinaris*; *Vicia sativa*	Canada; China; Romania	(13–)16–20(–26) × 3–4(–5)	(5–)5.5–7.5(–9) × (3.5–)4.5–6(–6.5)	Damm et al. (2014)
C. lini	Epitype	SNA	*Linum sp.*; *Nigella sp.*; *Taraxacum sp.*; etc	France; Germany; America; Ireland; Tunisia;Netherlands	(13–)15–18(–22.5) × (3–)3.5–4(–4.5)	(5–)6.5–10(–12.5) × (4–)4.5–6(–7)	Damm et al. (2014)
C. neorubicola	Holotype	PDA	*Rubus idaeus*	China	(14.8–)21.5–22.7(–23.5) × (4–)4.9–5.1(–5.6)	(4–)8.2–10.5(–17.5)× (3.6–)5.6–6.8(–11.7)	Liu et al. (2020)
C. ocimi	Holotype	SNA	*Ocimum basilicum*	Italy; Australia	14.5–15.5(–16.5) × (3.5–)4–4.5	(6.5–)7–13(–15.5) × (4–)4.5–7.5(–9)	Damm et al. (2014)
C. quercicola	Holotype	PDA	***Quercus variabilis***	**China**	**(14–)14.5–17.5(–21.5) × (3–)3.3–4.3(–4.7)**	**(5.7–)6.8–9.7(–10) × (3.2–)4–6(–8)**	**This study**
C. panacicola			*Panax* sp.	Eastern Asia	17.0–22.1 × 3.4–5.1	14–8	Takimoto (1919)
C. pleopeltidis	Holotype	SNA	*Pleopeltis* sp.	South Africa	(15–)19–23(–25) × (5–)5.5(–6)	Not described	Crous et al. (2021)
C. pisicola	Holotype	SNA	*Pisum* sp.	America	(11–)15–21(–29.5) × (3–)3.5–4	(5.5–)7–11.5(–13.5) × (4–)4.5–6(–6.5)	Damm et al. (2014)
C. shisoi	Holotype	PDA	*Perilla frutescens*	Japan	(15.0–)17–19(–27.0) × (3.0–)4.0(–5.0)	(7.0–)9.0–10.0(–11.0) × (5.0–)7.0–8.0	Gan et al. (2019)
C. tabacum	Neotype	SNA	*Nicotiana* spp., *Centella asiatica*	France; India; Germany; Madagascar; Zimbabwe	(11·5–) 19–20 (–27) × (3–) 5·5–5·8 (–7·6)	(10–) 11·5–12·5 (–14·5) × (6·5–) 8·5–9·5 (–11·5)	Damm et al. (2014)
C. tanaceti	Holotype	SNA	*Tanacetum cinerariifolium*	Australia	(13–)14.5–17.5(–19) × (3–)3.5–4(–4.5)	(5–)6.5–12(–14.6)× (3.5–)4.5–7(–10)	Damm et al. (2014)
C. utrechtense	Holotype	PDA	*Trifolium pratense*	Netherlands	17.5–20.5(–23) × 3.5–4(–4.5)	(7–)10–14.5(–15) × (5–)6.5–9.5(–10)	Barimani et al. (2013)
C. vignae	Holotype	SNA	*Vigna unguiculata*	Nigeria	(12–)14–17.5(–18.5) × (3–)3.5–4(–4.5)	(4–)4.5–8.5(–12.4)× (3.5–)4–5(–6.5)	Damm et al. (2014)

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RESEARCH ARTICLE

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Four new species of *Mycena* sect. *Calodontes* (Agaricales, Mycenaceae) from northeast China

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Abstract

Species of *Mycena* sect. *Calodontes* are representative of the *Mycena* genus as a whole and are easily recognised by the pinkish, reddish, purplish to brownish pileus and larger basidiomata. Furthermore, the colour of the pileus in the species of sect. *Calodontes* often has a transition or changes in different stages and the combination of the colour of the pileus with cystidia and basidiospores can be used to recognise taxa within this section. To date, 19 species of *Mycena* sect. *Calodontes* have been reported worldwide. Including our recent description of *M. yuezhuoi*, five species of sect. *Calodontes* have been recorded in China. During examination of specimens collected in coniferous forests or mixed broadleaf-conifer forests in temperate regions of China, additional taxa assigned to sect. *Calodontes* were identified. Four new species are recognised, based mostly on characters of the pileus and cystidia. Phylogenetic analysis of sequence data from multiple DNA regions (ITS + *rpb1* + *tef1*) supported the morphological evidence. Here, we propose *M. polycystidiata*, *M. rufobrunnea*, *M. shengshanensis* and *M. subulata* as new species in *Mycena* sect. *Calodontes*. Morphological descriptions, line drawings, habitat photos and comparisons with closely-related taxa are provided. A key to the 23 known species of sect. *Calodontes* is presented.

Keywords

coniferous forest, new taxa, phylogeny, saprobic, taxonomy

* Those authors contributed equally to this work.

Introduction

Mycena sect. *Calodontes* (Fr. ex Berk.) Quél. comprises the taxa in *Mycena* (Pers.) Roussel with a pinkish, reddish, purplish to brownish and mostly hygrophanous pileus, interveined lamellae, smooth cheilocystidia and pleurocystidia (if present) and mostly amyloid spores (Fries 1821; Berkeley 1836; Maas Geesteranus 1992a, 1992b; Harder et al. 2010). The group was initially proposed as *Agaricus* trib. *Clitocybe* subtrib. *Calodontes* Fries consisting of six species, then elevated to section rank within *Agaricus* subgen. *Clitocybe* Fr. ex Berk. and finally assigned to *Mycena* in 1872 (Fries 1821; Berkeley 1836; Quélet 1872). To date, 19 species are known, mostly from Europe and North America, but five species have been described from Asia (specifically, China, India and Peninsular Malaysia) (Smith 1947; Maas Geesteranus 1980; Perry 2002; Grgurinovic 2003; Robich 2003; Chew et al. 2014; Aravindakshan and Manimohan 2015; Aronsen and Læssøe 2016; Na 2019; Liu et al. 2021).

The circumscription of subsections within sect. *Calodontes* is problematic. Initially, Smith (1947) divided sect. *Calodontes* into two subsections, *Granulatae* and *Ciliatae*, according to whether the cheilocystidia were smooth or not, but this system was not widely adopted because most of the species have previously been classified in other sections of *Mycena* on account of the red, yellow or orange basidiomata, coloured lamellar edge and echinulate or diverticulate cheilocystidia, pleurocystidia, pileipellis or stipitipellis (Maas Geesteranus 1980, 1992a, 1992b; Perry 2002; Grgurinovic 2003; Robich 2003; Harder et al. 2010; Chew et al. 2014). Based on the lamellar edge colour, spore amyloid reaction and cystidial features, a widely accepted subsectional classification of sect. *Calodontes* was proposed by Maas Geesteranus (1980) and subsequent taxonomists (Grgurinovic 2003; Harder et al. 2010; Chew et al. 2014). The primary criteria for segregation were dominated by microcharacters: subsect. *Purae* (Konrad & Maubl.) Maas Geest. with amyloid spores and colourless pleuro- and cheilocystidia; subsect. *Violacellae* Sing. ex Maas Geest. with inamyloid spores and no pleurocystidia; and subsect. *Marginatae* J.E. Lange with amyloid spores and pleurocystidia and cheilocystidia with purplish-brown contents (Maas Geesteranus 1980; Grgurinovic 2003; Harder et al. 2010; Chew et al. 2014). Although the infrasectional classification of Maas Geesteranus (1980) is generally accepted, phylogenetic analyses have provided only weak support because subsect. *Purae* and subsect. *Violacellae* are polyphyletic (Perry 2002; Grgurinovic 2003; Robich 2003; Harder et al. 2010, 2012, 2013; Chew et al 2014; Na 2019). In studying *Mycena pura* (Pers.) P. Kumm., the type of subsect. *Purae*, Maas Geesteranus (1992a, 1992b) proposed eight forma based on pileus colour. However, 11 clades have been resolved amongst materials collected from Europe and the Americas, which suggests that there may be additional undescribed taxa in subsect. *Purae* (Harder et al. 2013).

Including our recent description of *M. yuezhuoi* Z.W. Liu, Y.P. Ge & Q. Na from Kunyushan National Nature Reserve (Yantai, Shandong Province), five species of *Mycena* sect. *Calodontes* have been previously recorded in China (Li et al. 2015; Na 2019; Liu et al. 2021). In this paper, we propose an additional four new species classified in

sect. *Calodontes* from the temperate zone of northeast China. The four new species share a unique set of striking morphological characters and contribute to an improved understanding of the classification of sect. *Calodontes*.

Materials and methods

Morphological observations

Thirteen fungal specimens were examined in this study, which were mainly collected in coniferous forests and some from mixed broadleaf-conifer forests in 2021. Macrocharacters were recorded from fresh specimens. Colour codes in descriptions follow those of Kornerup and Wanscher (1978). Microcharacters were observed from tissues sampled from dried specimens and rehydrated with 5% potassium hydroxide (KOH) and stained with Congo red (1% [w/v] aqueous solution), if necessary, using a Lab A1 microscope (Carl Zeiss AG, Jena, Germany). The amyloid reaction was tested with Melzer's Reagent (Clémençon et al. 2004; Horak 2005). Twenty basidiospores were measured per specimen. For the holotype, 40 basidiospores from different basidiomata were selected for measurement. Basidiospore statistics are expressed as (*a*/*b*/*c*) (*d*)*e*-*f*-*g*(*h*) × (*i*)*j*-*k*–*l*(*m*) µm [Q = (*n*)*o*-*p*(*q*), Q = $r \pm s$], where *a*–*c* represent *a* basidiospores of *b* basidiomata from *c* specimens measured; *d* and *h* are the minimum and maximum length (5% extremum), respectively, *e* and *g* indicate the range of values for the remaining 90% of the spores and *f* is the average length; width (*i*–*m*) and Q values (*n*–*q*) are expressed in a similar manner; and *r* and *s* are the average Q value and its standard deviation, respectively (Ge et al. 2021; Liu et al. 2021; Na et al. 2021; Na et al. 2022). The measurement of basidia, cystidia and other characters were each based on 20 observations. All specimens have been deposited in the Fungarium of the Fujian Academy of Agricultural Sciences (**FFAAS**).

DNA extraction, PCR, cloning and DNA sequencing

The Plant Genomic DNA Kit (CoWin Biosciences, Beijing, China) was used to isolate total genomic DNA from dried specimens in accordance with the manufacturer's instructions. Three nuclear loci were sequenced, comprising the internal transcribed spacer (ITS), RNA polymerase II largest subunit (*rpb1*) and translation elongation factor-1 alpha (*tef1*). The primer pairs ITS1/ITS4, *rpb1*Mp_f1/*rpb1*Mp_r1 and tEFMp_f2/ tEFMp_r2 were selected to amplify ITS, *rpb1* and *tef1*, respectively (White et al. 1990; Harder et al. 2013; Yu et al. 2020). The PCR reactions were performed in a total volume of 25 µl containing 2 µl DNA template, 1 µl for each primer, 8.5 µl nuclease-free H_2O and 12.5 µl 2× Utaq PCR MasterMix (ZomanBio, Beijing, China). The PCR protocol for amplification of the ITS region was as follows: 94 °C for 4 min, then 34 cycles of 94 °C for 45 s, 52 °C for 45 s and 72 °C for 1 min, with a final extension of 72 °C for 10 min (Na et al. 2022). The PCR protocol for amplification of

the *rpb1* and *tef1* regions followed that of Harder et al. (2013): 94 °C for 60 s, then 10 cycles of 94 °C for 35 s, 53 °C for 45 s and 72 °C for 45 s; then 25 cycles of 94 °C for 35 s, 56 °C for 45 s, 72 °C for 45 s and final extension of 72 °C for 10 min. The PCR products were purified by gel electrophoresis or filter membrane and subjected to Sanger dideoxy sequencing by the Beijing Genomics Institute (Beijing, China).

Phylogenetic analysis

A combined ITS, *rpb1* and *tef1* dataset was analysed to infer relationships of the new taxa with other members of sect. *Calodontes*. We used sequences included in previous studies of sect. *Calodontes* and from members of the most closely-related section deposited in the GenBank database, which were mainly submitted by Harder et al. (2013), Osmundson et al. (2013), Chew et al. (2014) and Liu et al. (2021). For the analysis, representative species of *Mycena* sect. *Supinae* Konrad & Maubl., which is closely related to sect. *Calodontes*, were selected as the outgroup (Osmundson et al. 2013; Na and Bau 2019). Sequences for each DNA region (ITS, *rpb1* and *tef1*) were aligned in MAFFT version 7 online and the aligned matrices were manually checked with BIOEDIT 7.2.5.0 (Hall 1999; Kuraku et al. 2013; Katoh et al. 2019). The best-fit substitution model for each gene partition was determined with MODELTEST 2.3, based on the Akaike Information Criterion (Posada and Crandall 1998). Maximum Likelihood (ML) analysis was conducted by raxmlGUI 2.09 (Edler et al. 2020). The phylogenetic analysis was performed by a single analysis with six partitions (ITS1, 5.8S, ITS2, *rpb1* exons, *tef1* exons, intron of *rpb1* + introns of *tef1*), using the GTR-GAMMA model and 1,000 rapid bootstrap (BS) replicates. For Bayesian Inference (BI), two runs of six chains were run for 15,000,000 generations and sampled every 10,000 generations by MrBayes 3.2.6. At the end of the run, the average deviation of split frequencies was 0.007821, ESS (effective sample size) was 1300.3 and the average Potential Scale Reduction Factor (PSRF) parameter values (excluding NA and > 10.0) = 1.000 and the "sump" and "sumt" commands were used to summarise sampled parameters with 25% burn-in (Ronquist and Huelsenbeck 2003).

Results

Phylogenetic relationships

The dataset consisted of 192 sequences, comprising 39 newly-generated sequences (13 ITS, 13 *rpb1* and 13 *tef1*) and 153 sequences (61 ITS, 46 *rpb1* and 46 *tef1*) downloaded from GenBank. In total, 74 accessions of 19 species were included in the dataset. Detailed information for all sequences is presented in Table 1. The aligned dataset contained 1459 nucleotide sites including gaps (229 sites for ITS1, 159 sites for 5.8S, 177 sites for ITS2, 55 sites for *rpb1* exons, 295 sites for *tef1* exons, 544 sites for intron of *rpb1* + introns of *tef1*), of which 1146 were conserved, 257 were parsimony-

Table 1. Specimens used in phylogenetic analysis and GenBank accession numbers.

No.	Species	Specimen voucher	GenBank accession numbers			Locality	Reference
			ITS	*rpb1*	*tef1*		
1.	*Mycena* aff. *pura*	TL8052	FN394623	KF723687	KF723641	Ecuador	Harder et al. (2010, 2013)
2.	*M.* aff. *pura*	TL9433	FN394622	KF723688	KF723642	Ecuador	Harder et al. (2010, 2013)
3.	*M.* aff. *pura*	TL9450	KJ144653	KF723689	KF723643	Ecuador	Harder et al. (2010, 2013)
4.	*M.* aff. *pura*	TL9678	FN394621	KF723690	KF723644	Ecuador	Harder et al. (2010, 2013)
5.	*M. arcangeliana*	252b	JF908401	–	–	Spain	Osmundson et al. (2013)
6.	*M. arcangeliana*	252f	JF908402	–	–	Spain	Osmundson et al. (2013)
7.	*M. cahaya*	ACL134	KF537248	–	–	Malaysia	Chew et al. (2014)
8.	*M.* cf. *pura* I	CBH039	FN394588	KF723680	KF723634	Denmark	Harder et al. (2010, 2013)
9.	*M.* cf. *pura* II	CBH105	FN394581	KF723671	KF723625	Denmark	Harder et al. (2010, 2013)
10.	*M.* cf. *pura* II	CBH169	FN394579	KF723672	KF723626	Denmark	Harder et al. (2010, 2013)
11.	*M.* cf. *pura* II	CBH366	FN394572	KF723673	KF723627	Denmark	Harder et al. (2010, 2013)
12.	*M.* cf. *pura* II	CBH404	FN394566	KF723674	KF723628	Denmark	Harder et al. (2010, 2013)
13.	*M.* cf. *pura* III	CBH019	FN394605	KF723675	KF723629	Denmark	Harder et al. (2010, 2013)
14.	*M.* cf. *pura* III	CBH022	FN394574	KF723676	KF723630	Denmark	Harder et al. (2010, 2013)
15.	*M.* cf. *pura* III	KK	FN394606	KF723677	KF723631	Slovakia	Harder et al. (2010, 2013)
16.	*M.* cf. *pura* IV	CBH410	FN394595	KF723667	KF723621	Denmark	Harder et al. (2010, 2013)
17.	*M.* cf. *pura* IV	JV06979	FN394585	KF723668	KF723622	Denmark	Harder et al. (2010, 2013)
18.	*M.* cf. *pura* IV	TL4571	FN394583	KF723669	KF723623	Denmark	Harder et al. (2010, 2013)
19.	*M.* cf. *pura* IV	TL12786	FN394591	KF723670	KF723624	Sweden	Harder et al. (2010, 2013)
20.	*M.* cf. *pura* V	CBH226	FN394604	KF723664	KF723618	Denmark	Harder et al. (2010, 2013)
21.	*M.* cf. *pura* V	TL5614	FN394602	KF723666	KF723620	Denmark	Harder et al. (2010, 2013)
22.	*M.* cf. *pura* VI	BAP132	FN394561	KF723660	KF723614	USA	Harder et al. (2010, 2013)
23.	*M.* cf. *pura* VIII	CBH216	FN394598	KF723662	KF723616	Denmark	Harder et al. (2010, 2013)
24.	*M.* cf. *pura* VIII	CBH402	FN394599	KF723663	KF723617	Denmark	Harder et al. (2010, 2013)
25.	*M.* cf. *pura* IX	CBH166	FN394607	KF723701	KF723655	Denmark	Harder et al. (2010, 2013)

No.	Species	Specimen voucher	GenBank accession numbers			Locality	Reference
			ITS	*rpb1*	*tef1*		
26.	*M.* cf. *pura* IX	CBH358	FN394608	KF723702	KF723656	Denmark	Harder et al. (2010, 2013)
27.	*M.* cf. *pura* IX	CBH367	KF913022	KF723703	KF723657	Denmark	Harder et al. (2013)
28.	*M.* cf. *pura* IX	CBH371	KF913023	KF723704	KF723658	Denmark	Harder et al. (2013)
29.	*M.* cf. *pura* X	BAP165A	FN394563	KF723698	KF723652	USA	Harder et al. (2010, 2013)
30.	*M.* cf. *pura* XI	CBH187	FN394564	KF723678	KF723632	Sweden	Harder et al. (2010, 2013)
31.	*M.* cf. *pura* XI	CBH386	FN394565	KF723679	KF723633	Denmark	Harder et al. (2010, 2013)
32.	*M. diosma*	CBH400	FN394617	KF723699	KF723653	Denmark	Harder et al. (2010, 2013)
33.	*M. diosma*	LK1191/2000	FN394619	KF723700	KF723654	Germany	Harder et al. (2010, 2013)
34.	*M. dura*	10315	FN394560	KF723694	KF723648	Austria	Harder et al. (2010, 2013)
35.	*M. lammiensis*	TUR165927	FN394552	KF723697	KF723651	Finland	Harder et al. (2010, 2013)
36.	*M. meliigena*	39	JF908423	–	–	Italy	Osmundson et al. (2013)
37.	*M. meliigena*	39d	JF908429	–	–	Italy	Osmundson et al. (2013)
38.	*M. pearsoniana*	CBH068	FN394614	KF723691	KF723645	Germany	Harder et al. (2010, 2013)
39.	*M. pearsoniana*	JV06890	FN394612	KF723692	KF723646	Denmark	Harder et al. (2010, 2013)
40.	*M. pearsoniana*	LK880/2002	FN394613	KF723693	KF723647	Germany	Harder et al. (2010, 2013)
41.	*M. pelianthina*	CBH015	FN394549	KF723695	KF723649	Denmark	Harder et al. (2010, 2013)
42.	*M. pelianthina*	CBH016	FN394547	KF723696	KF723650	Denmark	Harder et al. (2010, 2013)
43.	***M. polycystidiata***	**FFAAS0417 Holotype**	**ON427731**	**ON468456**	**ON468469**	**China**	**This study**
44.	***M. polycystidiata***	**FFAAS0418**	**ON427732**	**ON468457**	**ON468470**	**China**	**This study**
45.	***M. polycystidiata***	**FFAAS0421**	**ON427733**	**ON468458**	**ON468471**	**China**	**This study**
46.	***M. polycystidiata***	**FFAAS0422**	**ON427734**	**ON468459**	**ON468472**	**China**	**This study**
47.	*M. pseudocorticola*	124a	JF908386	–	–	Italy	Osmundson et al. (2013)
48.	*M. pura*	IS10/11/2000	FN394611	–	–	USA	Harder et al. (2010)
49.	*M. pura* f. *lutea*	DB2005/152	FN394603	–	–	Denmark	Harder et al. (2010)
50.	*M. rosea*	UP2	FN394550	–	–	UK	Harder et al. (2010)
51.	*M. rosea*	CBH097	FN394556	KF723681	KF723635	Denmark	Harder et al. (2010, 2013)
52.	*M. rosea*	CBH383	FN394553	KF723682	KF723636	Denmark	Harder et al. (2010, 2013)
53.	*M. rosea*	CBH409	FN394551	KF723683	KF723637	Germany	Harder et al. (2010, 2013)
54.	*M. rosea*	TL12393	FN394555	KF723684	KF723638	Denmark	Harder et al. (2010, 2013)

No.	Species	Specimen voucher	GenBank accession numbers			Locality	Reference
			ITS	*rpb1*	*tef1*		
55.	*M. rosea*	TL12409	FN394557	KF723685	KF723639	Denmark	Harder et al. (2010, 2013)
56.	***M. rufobrunnea***	**FFAAS0414**	**ON427728**	**ON468453**	**ON468466**	**China**	**This study**
57.	***M. rufobrunnea***	**FFAAS0415**	**ON427729**	**ON468454**	**ON468467**	**China**	**This study**
58.	***M. rufobrunnea***	**FFAAS0416 Holotype**	**ON427730**	**ON468455**	**ON468468**	**China**	**This study**
59.	*M. seminau*	ACL136	KF537250	–	–	Malaysia	Chew et al. (2014)
60.	*M. seminau*	ACL308	KF537252	–	–	Malaysia	Chew et al. (2014)
61.	***M. shengshanensis***	**FFAAS0424 Holotype**	**ON427739**	**ON468464**	**ON468477**	**China**	**This study**
62.	***M. shengshanensis***	**FFAAS0425**	**ON427740**	**ON468465**	**ON468478**	**China**	**This study**
63.	*M. sinar*	ACL092	KF537247	–	–	Malaysia	Chew et al. (2014)
64.	*M. sinar*	ACL135	KF537249	–	–	Malaysia	Chew et al. (2014)
65.	*M. sinar* var. *tangkaisinar*	ACL307	KF537251	–	–	Malaysia	Chew et al. (2014)
66.	***M. subulata***	**FFAAS0419**	**ON427735**	**ON468460**	**ON468473**	**China**	**This study**
67.	***M. subulata***	**FFAAS0420**	**ON427736**	**ON468461**	**ON468474**	**China**	**This study**
68.	***M. subulata***	**FFAAS0423 Holotype**	**ON427737**	**ON468462**	**ON468475**	**China**	**This study**
69.	***M. subulata***	**FFAAS0426**	**ON427738**	**ON468463**	**ON468476**	**China**	**This study**
70.	*M. supina*	128a	JF908388	–	–	Italy	Osmundson et al. (2013)
71.	*M. yuezhuoi*	FFAAS0344	MW581490	MW868166	MW882249	China	Liu et al. (2021)
72.	*M. yuezhuoi*	FFAAS0345	MW581491	MW868169	MW882250	China	Liu et al. (2021)
73.	*M. yuezhuoi*	FFAAS0346	MW581492	MW868168	MW882251	China	Liu et al. (2021)
74.	*M. yuezhuoi*	FFAAS0347	MW581493	MW868167	MW882252	China	Liu et al. (2021)

informative and 56 were variable, but parsimony-uninformative. For Bayesian Inference (BI), the selected models for each DNA region of the concatenated dataset were as follows: HKY+G for ITS1 and intron of *rpb1* + introns of *tef1*, JC for 5.8S and *rpb1* exons, HKY+I+G for ITS2 and SYM+I+G for *tef1* exons. The BI and ML analyses resulted in almost identical topologies; thus, the BI topology is presented as a master tree (Fig. 1).

The phylogenetic analysis revealed that sect. *Calodontes* was strong support (BS/Bayesian posterior probability [BPP] = 100/1.00) (Fig. 1). Fifteen species and eleven *M. pura* complex clades within sect. *Calodontes* were retrieved. Four new species were resolved as monophyletic, each with strong support: *M. polycystidiata* (BS/BPP = 100/1.00), *M. rufobrunnea* (BS/BPP = 100/1.00), *M. shengshanensis* (BS/BPP = 90/1.00) and *M. subulata* (BS/BPP = 100/1.00). A sister relationship between *M. shengshanensis* and *M. pearsoniana* Dennis ex Singer was well supported. *Mycena subulata* was resolved as sister, but genetically distant from *M. pearsoniana* and *M. shengshanensis* clade. In addition, the sister relationship of *Mycena polycystidiata* and *M. rufobrunnea* were unresolved.

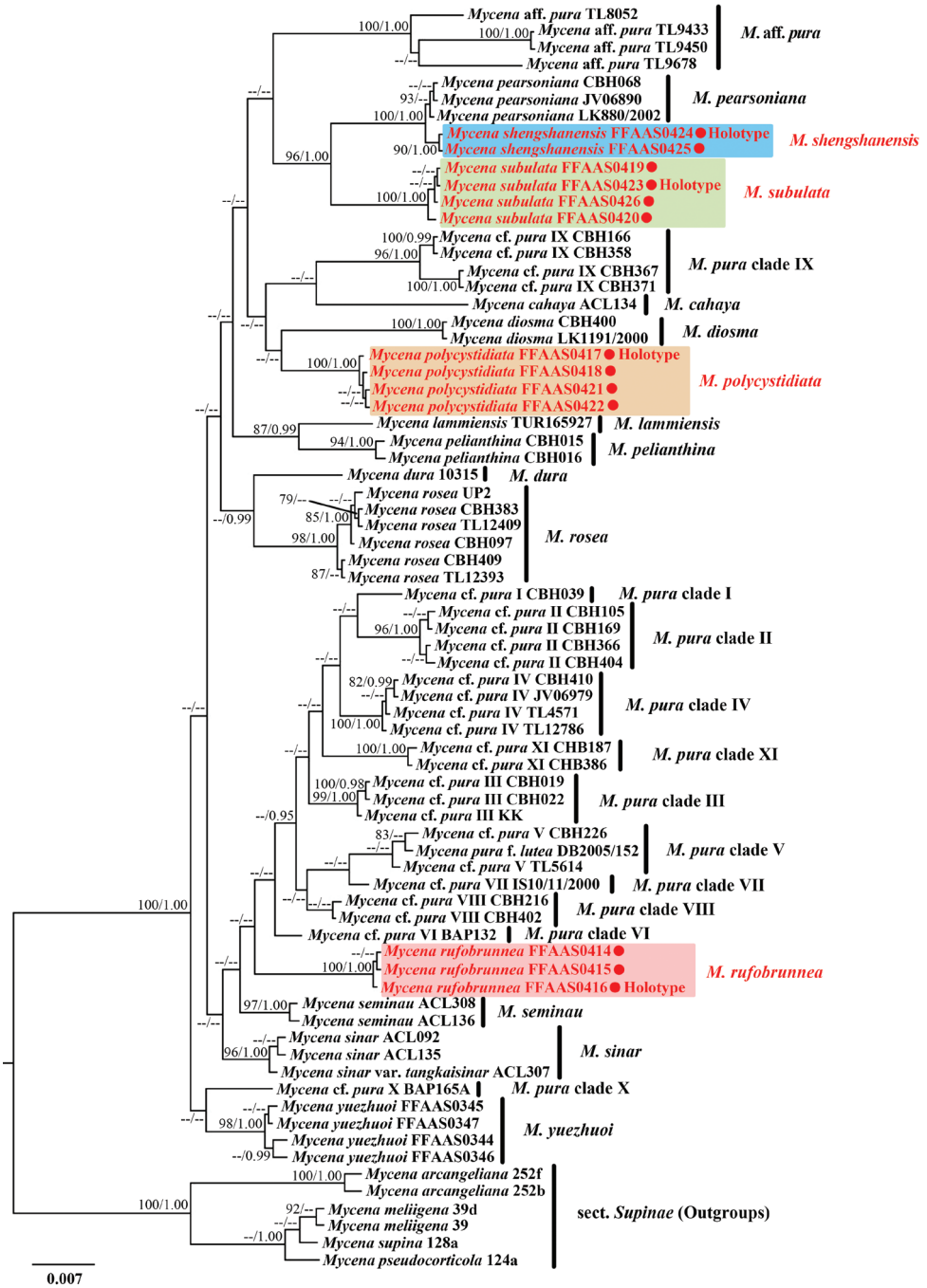

Figure 1. Bayesian Inference analysis of *Mycena* sect. *Calodontes* with ITS, *rpb1* and *tef1* sequence data. Species in *Mycena* sect. *Supinae* served as outgroup. Bootstrap values (BS) from Maximum Likelihood ≥ 75 and Bayesian posterior probabilities (BPP) ≥ 0.95 are shown on each branch (BS/BPP). The new species are marked in red.

Taxonomy

***Mycena polycystidiata* Z.W. Liu, Y.P. Ge, L. Zou & Q. Na, sp. nov.**
MycoBank No: 843977
Figs 2–5

Diagnosis. Pileus greyish-rose, umbo brownish-orange, hygrophanous. Stipe pubescent. Pleurocystidia polymorphic in shape. Stipitipellis a cutis, with numerous projecting hyphae.

Holotype. China. Heilongjiang Province: Liangshui National Nature Reserve, Yichun City, 47°12'74"N, 128°52'86"E, 20 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0417* (collection number MY0633).

Etymology. Refers to the variable shape of pleurocystidia.

Description. ***Pileus*** 14–31 mm in diam., campanulate to hemispherical when young, plano-convex with age, with obtuse umbo at centre, margin slightly revolute, at times cracked at mature; umbo brownish-orange (7C3–7C5), disc purplish-grey (13C2, 14B2, 14C2), reddish-grey (12B2, 12C2) to greyish-rose (12B3), near margin reddish-grey (12D2), greyish-ruby (12D3) or purplish-grey (13D3), margin whitish; striate none or indistinct, greyish-ruby (12E3–12E4), towards the centre up to 1/3 diam.; surface dry and rugose, hygrophanous, generally tomentose. ***Context*** white, 2 mm thick, fragile. ***Lamellae*** emarginate, slightly decurrent when old, 20–28 reaching the stipe, 1–2 tiers of lamellulae, white, irregularly intervenose, edge concolorous, wavy. ***Stipe*** 34–73 × 3–7 mm, central, cylindrical, base occasionally compressed with age; apex violet brown (11E3–11E4, 11F4), greyish-ruby (12E4), lower part brownish-grey (11D2, 11E2) to greyish-brown (11D3, 11E3) or purplish-grey (13C2), fragile, hollow; apex to middle densely pubescent, sparser towards base; whitely villose at base. ***Odour*** strongly raphanoid, ***taste*** indistinct.

Basidiospores (130/5/4) (6.4)6.7–7.4–8.3(8.8) × (3.2)3.5–3.9–4.3(4.6) µm [Q = (1.62)1.72–2.05(2.18), Q = 1.90 ± 0.11] [holotype (70/2/1) (6.7)6.9–7.6–8.5(8.7) × (3.4)3.6–4.0–4.4(4.6) µm, Q = (1.71)1.76–2.05(2.13), Q = 1.90 ± 0.09], elongated ellipsoid to cylindrical, colourless, smooth, thin-walled, amyloid. ***Basidia*** 21–31 × 6–8 µm, 4-spored, clavate, hyaline, sterigmata approximately 4 µm in length. ***Cheilocystidia*** thin-walled, hyaline, differs in two shapes, mainly utriform, 50–65 × 20–31 µm, some subclavate, 54–78 × 14–19 µm. ***Pleurocystidia*** abundant, thin-walled, hyaline, multi-shaped: lanceolate and mostly round to blunt apices, 37–81 × 12–20 µm, lanceolate and acute apices, 51–87 × 14–22 µm, elliptical, 30–86 × 12–31 µm, ovate and acute apices, 49–71 × 15–24 µm, ovate and mostly round to blunt apices, 49–73 × 16–22 µm. ***Pileipellis*** a cutis composed of four to five layers cylindrical cells, 51–81 × 4–5 µm, smooth and thin-walled; terminal cells cylindrical or fusiform, 50–69 × 3–22 µm, thin-walled, hyaline. ***Hypodermium*** formed by fusiform to subglobose hyphae, 32–69 × 18–54 µm, thin-walled, hyaline. ***Lamellar trama*** subregular, dextrinoid. ***Stipitipellis*** a cutis composed of cylindrical hyphae 5–8 µm in diam., smooth, thin-walled, with numbers of projecting hyphae 2–6 µm in diam.; ***caulocystidia*** 29–74 × 6–19 µm, clavate or fusiform, thin-walled, smooth, hyaline. ***Clamps*** present in all tissues.

Figure 2. Basidiomata of *Mycena polycystidiata* Z.W. Liu, Y.P. Ge, L. Zou & Q. Na **a, b** *FFAAS0422* **c, d** *FFAAS0417*, holotype **e–g** *FFAAS00421* **h** *FFAAS0418* Scale bars: 10 mm (**a–h**). Photographs **a–e, h** by Qin Na **f, g** by Yupeng Ge.

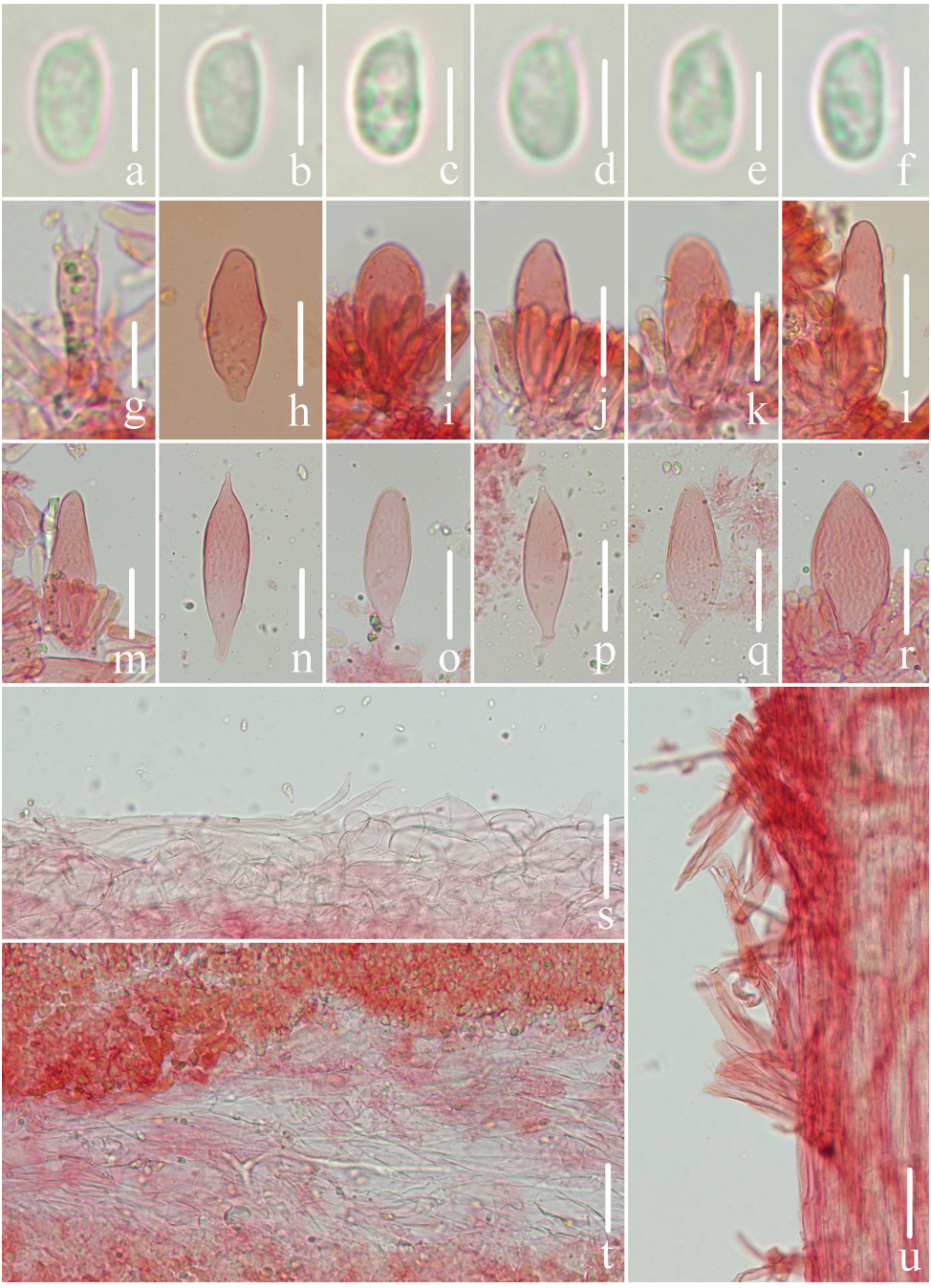

Figure 3. Microscopic features of *Mycena polycystidiata* (*FFAAS0417*, holotype) **a–f** basidiospores **g** basidia **h–l** cheilocystidia **m–r** pleurocystidia **s** pileipellis and hypodermium **t** lamellar trama **u** stipitipellis and caulocystidia. Scale bars: 5 µm (**a–f**); 10 µm (**g**); 30 µm (**h–r**); 40 µm (**s–u**).

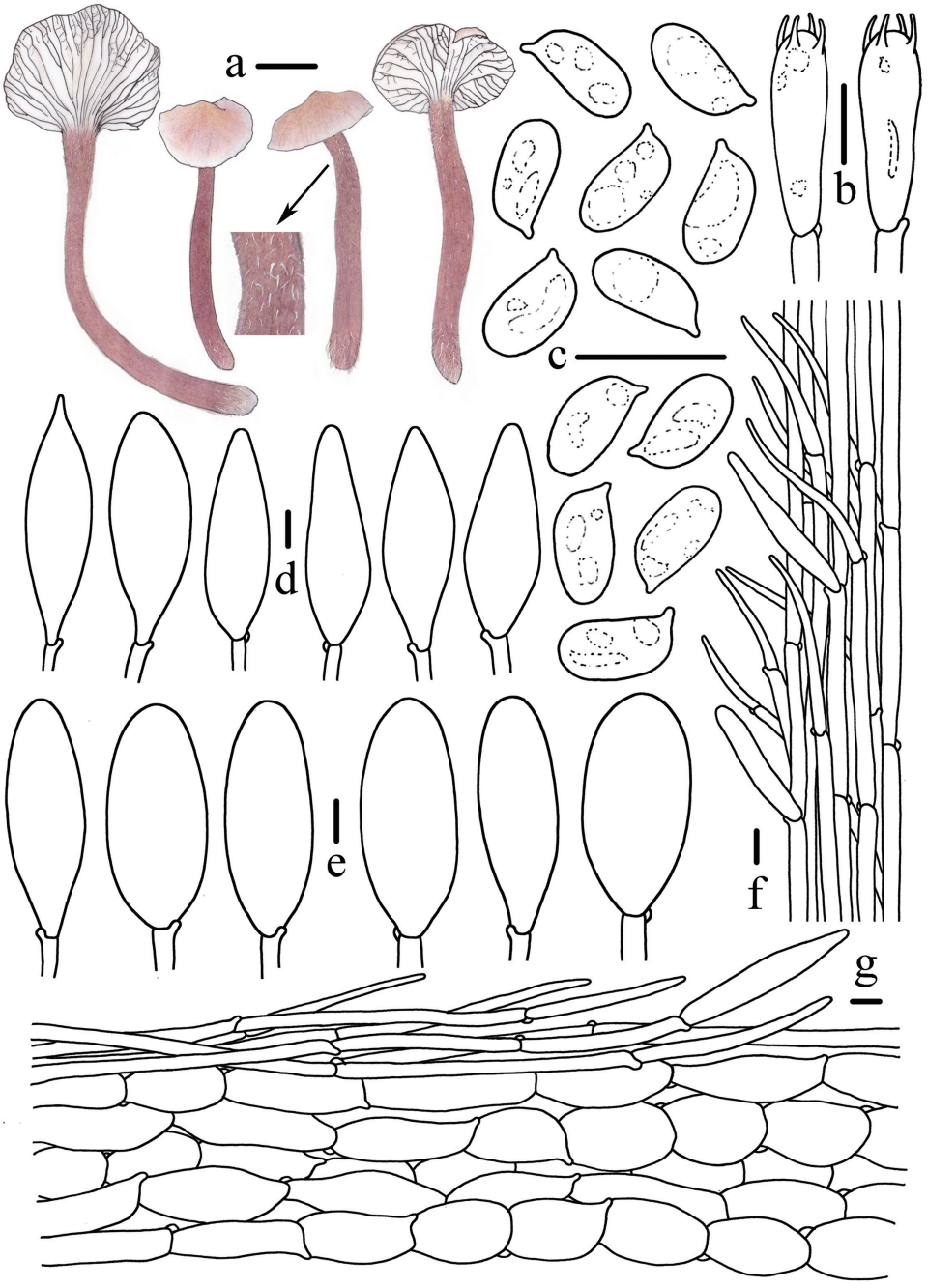

Figure 4. Morphological features of *Mycena polycystidiata* (*FFAAS0417*, holotype) **a** basidiomata **b** basidia **c** basidiospores **d** pleurocystidia **e** cheilocystidia **f** stipitipellis and caulocystidia **g** pileipellis and hypodermium. Scale bars: 10 mm (**a**); 10 μm (**b–g**). Drawings by Zewei Liu.

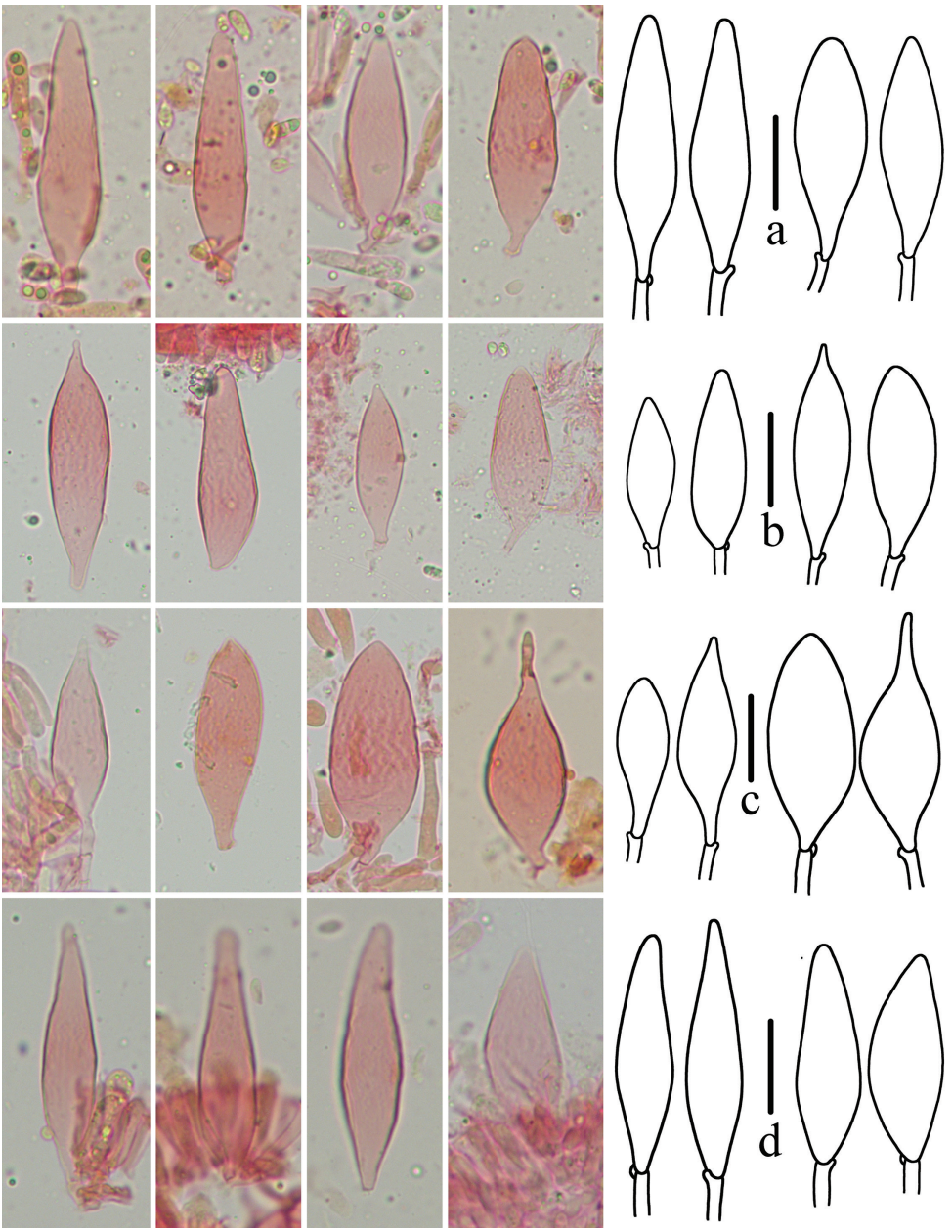

Figure 5. Pleurocystidia of *Mycena polycystidiata* **a** *FFAAS0422* **b** *FFAAS0417*, holotype **c** *FFAAS0418* **d** *FFAAS0421*. Scale bars: 25 μm (**a–d**).

Habitat. Scattered on the litter layers in *Pinus koraiensis* and *Larix gmelinii* mixed forests.

Known distribution. Heilongjiang Province, China.

Additional material examined. China. Heilongjiang Province: Liangshui National Nature Reserve, Yichun City, 47°12'82"N, 128°52'94"E, 20 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0418* (collection number MY0634); same location, 21 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0421* (collection number MY0659); same location, 21 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0422* (collection number MY0661).

Notes. Macroscopically, *Mycena luteovariegata* Harder & Læssøe and *M. pura* resemble *M. polycystidiata* in pileus colour, but the latter possesses more typically utriform cheilocystidia and uncontracted pleuro- and cheilocystidia (Perry 2002; Robich 2003; Harder et al. 2013; Aronsen and Læssøe 2016; Na 2019). *Mycena pearsoniana* also has a rose to violaceous pileus, but differs from *M. polycystidiata* in having inamyloid spores and lacking pleurocystidia (Aronsen and Læssøe 2016; Na 2019). Compared with *M. polycystidiata*, *M. sirayuktha* Aravind. & Manim. has similar cheilocystidia, but has an obviously greyish-brown striate pileus, inamyloid spores and slightly glutinous pileipellis with finger-like excrescences (Aravindakshan and Manimohan 2015).

The pleurocystidia of *M. polycystidiata* varied in shape amongst specimens (Fig. 5). In all four specimens, most pleurocystidia were lanceolate and with round to blunt apices, but pleurocystidia with lanceolate and acute apices, elliptical and ovate and acute apices were also observed in *FFAAS0417* (Holotype) and *FFAAS0418*, while elongated lageniform-lanceolate or round apices ovate were detected in *FFAAS0421* and *FFAAS0422*. The multi-shaped pleurocystidia may show a morphological continuum that changes between developmental stages. Nevertheless, the multi-shaped pleurocystidia are unquestionably diagnostic for identification of this species.

Mycena rufobrunnea Z.W. Liu, Y.P. Ge & Q. Na, sp. nov.

MycoBank No: 843978
Figs 6–8

Diagnosis. Pileus dark brown at centre, disc gradually turning paler to reddish-brown to greyish-brown, edge white. Lamellae obviously intervenose. Stipe apex to middle greyish-magenta to dull violet, lower part darker to dark purple or dark magenta. Cheilocystidia utriform, sometimes clavate. Pleurocystidia absent. Caulocystidia clavate or fusiform. Pileipellis with fusiform terminal cells.

Holotype. China. Jilin Province: Dayangcha, Erdaobaihe Town, Antu County, Yanbian Korean Autonomous Prefecture, 42°20'73"N, 127°56'06"E, 16 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0416* (collection number MY0581).

Etymology. Refers to reddish-brown pileus.

Description. ***Pileus*** 12–34 mm in diam., hemispherical to convex when young, then plano-convex, sometimes an unclear umbo at centre, margin slightly revolute, acute to subacute, at times cracked at mature; dark brown (8F6–8F8) at centre, disc gradually turning paler to reddish-brown (8D4–8D5, 8E6–8E8) to greyish-brown

Figure 6. Basidiomata of *Mycena rufobrunnea* Z.W. Liu, Y.P. Ge & Q. Na **a, b** *FFAAS0414* **c, d** *FFAAS0415* **e–h** *FFAAS0416*, holotype. Scale bars: 10 mm (**a–h**). Photographs **a–h** by Qin Na.

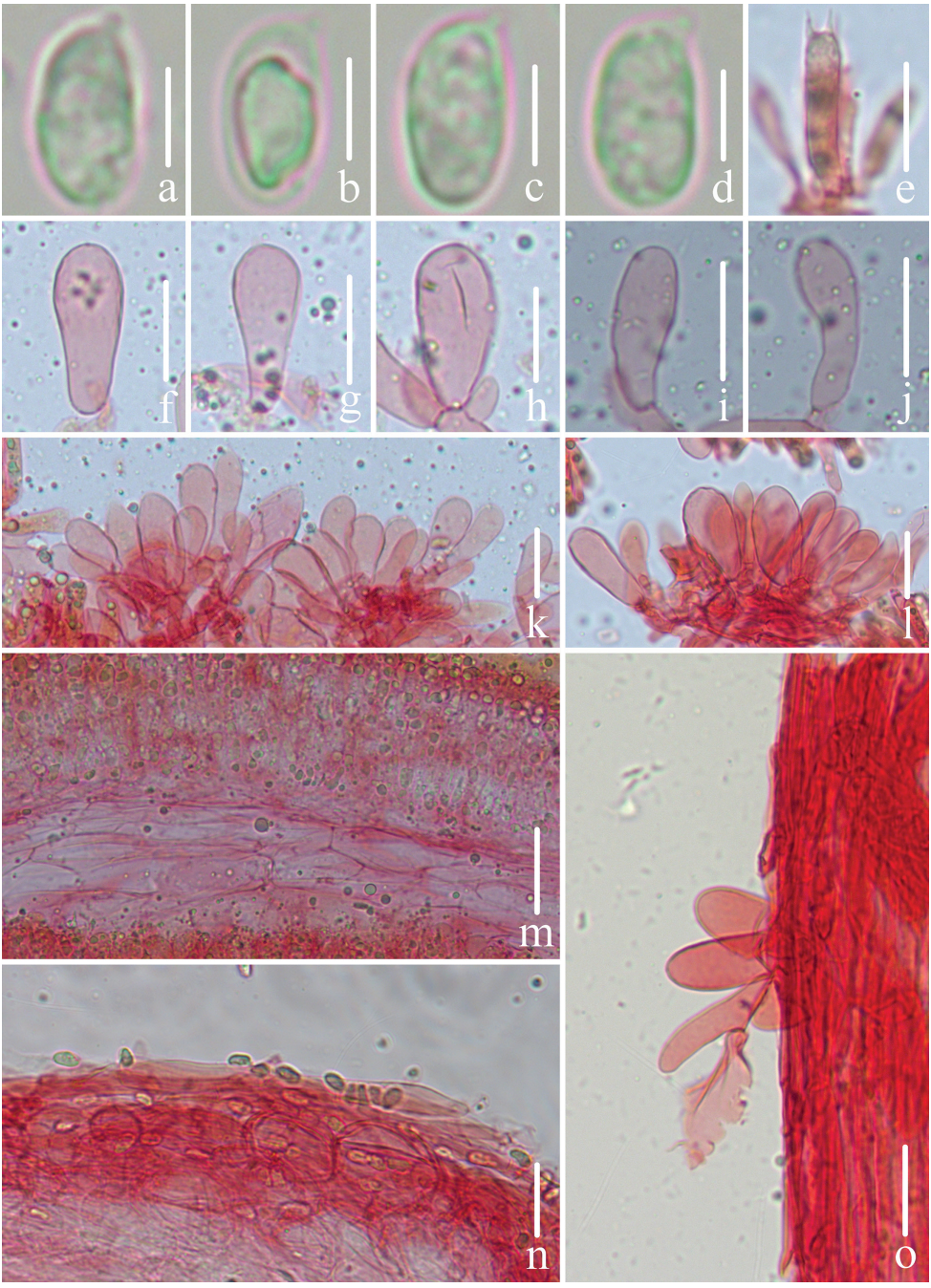

Figure 7. Microscopic features of *Mycena rufobrunnea* (*FFAAS0416*, holotype) **a–d** basidiospores **e** basidia **f–l** cheilocystidia **m** lamellar trama **n** pileipellis and *hypodermium* **o** stipitipellis and caulocystidia. Scale bars: 5 μm (**a–d**); 20 μm (**e–o**).

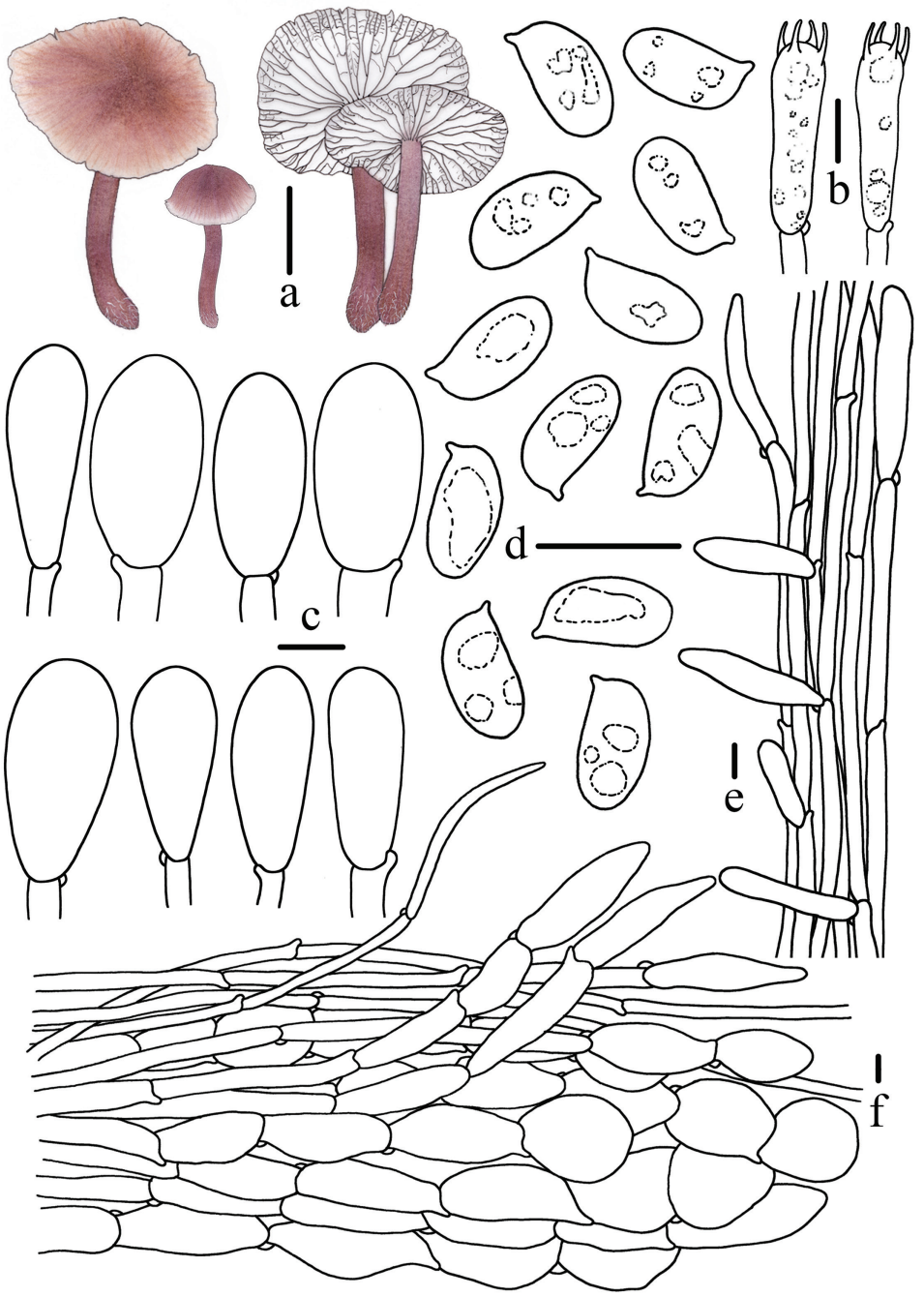

Figure 8. Morphological features of *Mycena rufobrunnea* (*FFAAS0416*, holotype) **a** basidiomata **b** basidia **c** cheilocystidia **d** basidiospores **e** stipitipellis and caulocystidia **f** pileipellis and hypodermium. Scale bars: 10 mm (**a**); 10 μm (**b–f**). Drawings by Zewei Liu.

(8D3) and turning to whitish at margin; striate reddish-brown (8D4–8D5, 8E6–8E8), towards the centre up to 1/3–1/2 diam.; surface humidus when wet. ***Context*** white, 1.5 mm thick, fragile. ***Lamellae*** adnexed to emarginate, 20–23 reaching the stipe, 1–3 tiers of lamellulae, white, irregularly intervenose, edge concolorous, slightly serrulate. ***Stipe*** 19–62 × 2–6 mm, central, cylindrical, apex to middle greyish-magenta (14E4–14E5) to dull violet (16E3–16E4), lower part darker to dark purple (14F4–14F5) or dark magenta (13F3), fragile, hollow, base slightly swollen with whitish villose. ***Odour*** raphanoid, ***taste*** indistinct.

Basidiospores (80/4/3) (7.1)7.6–8.4–9.2(9.6) × (3.8)4.0–4.5–5.0 µm [Q = (1.73)1.77–1.98(2.05), Q = 1.88 ± 0.07] [holotype (40/2/1) (7.9)8.1–8.6–9.2(9.4) × 4.2–4.6–5.0 µm, Q = (1.73)1.77–1.96(1.98), Q = 1.87 ± 0.06], elongated ellipsoid to cylindrical, colourless, smooth, thin-walled, amyloid. ***Basidia*** 24–34 × 7–10 µm, 4-spored, clavate, hyaline, sterigmata 2–3 µm in length. ***Cheilocystidia*** thin-walled, hyaline, utriform, sometimes clavate, 23–44 × 7–17 µm, abundant. ***Pleurocystidia*** absent. ***Pileipellis*** a cutis composed of four to five slightly interwoven layers of cylindrical cells, 44–70 × 4–7 µm, smooth, thin-walled; terminal cells cylindrical or fusiform, 34–65 × 4–17 µm, thin-walled, hyaline. ***Hypodermium*** formed by fusiform, subcylindrical to subglobose hyphae, 15–50 × 12–37 µm, thin-walled, hyaline. ***Lamellar trama*** subregular, dextrinoid. ***Stipitipellis*** a cutis composed of hyphae 3–9 µm in diam., smooth, thin-walled; ***caulocystidia*** common in the apex, sparse in the middle and base, 23–76 × 6–14 µm, clavate and fusiform, thin-walled, hyaline, smooth. ***Clamps*** present in all tissues.

Habitat. Scattered on the decayed logs of *Acer*, *Larix*, *Pinus*, *Populus*, *Quercus* and *Ulmus* mixed forests.

Known distribution. Jilin Province, China.

Additional material examined. China. Jilin Province: Dayangcha, Erdaobaihe Town, Antu County, Yanbian Korean Autonomous Prefecture, 42°20'72"N, 127°56'08"E, 16 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0414* (collection number MY0579); same location, 16 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0415* (collection number MY0580).

Notes. Species of sect. *Calodontes* that are macroscopically similar to *Mycena rufobrunnea* have been recorded in many regions of the world. Most taxa resemble *M. rufobrunnea* in pileus colour (Smith 1947; Maas Geesteranus 1992a, 1992b; Grgurinovic 2003; Robich 2003; Chew et al. 2014; Aronsen and Læssøe 2016). *Mycena dura* Maas Geest. & Hauskn., recorded in Europe, also has a dark brown to greyish-brown pileus, but can be distinguished from *M. rufobrunnea* in having a white stipe and having pleurocystidia (Robich 2003; Aronsen and Læssøe 2016). *Mycena kuehneriana* A.H. Sm., which is recorded from the United States and Canada, is distinguished from *M. rufobrunnea* in that its pileus is pale avellaneous with rose and lilac, almost white when faded and the spores are obviously smaller (5–6 × 2–3 µm) (Smith 1947; Maas Geesteranus 1992a, 1992b). *Mycena clarkeana* Grgur. and *M. nullawarrensis* Grgur., described from Australia, are similar to *M. rufobrunnea* in having a reddish-brown pileus, but both species have broader spores and possess pleurocystidia (Grgurinovic

2003). *Mycena cahaya* A.L.C. Chew & Desjardin, *M. seminau* A.L.C. Chew & Desjardin and *M. sinar* A.L.C. Chew & Desjardin, known from Malaysia, resemble *M. rufobrunnea* owing to the brown pileus, but differ in having adnate to subdecurrent lamellae, a yellowish-grey or brownish-orange stem, mucronate cheilocystidia and lack caulocystidia (Chew et al. 2014). Microscopically, utriform or clavate cheilocystidia and absence of pleurocystidia are key characteristics of *M. rufobrunnea*. *Mycena diosma* Krieglst. & Schwöbel has similar cheilocystidia and pleurocystidia are absent or rare, but it has a strongly hygrophanous pileus and a remarkable change in colour (Robich 2003; Aronsen and Læssøe 2016). *Mycena pura*, *M. sirayuktha* and *M. vinacea* Cleland have similar cheilocystidia, but are easily distinguished from *M. rufobrunnea* by the presence of pleurocystidia (Perry 2002; Grgurinovic 2003; Robich 2003; Aravindakshan and Manimohan 2015; Aronsen and Læssøe 2016; Na 2019).

***Mycena shengshanensis* Z.W. Liu, Y.P. Ge & Q. Na, sp. nov.**
MycoBank No: 843979
Figs 9–12

Diagnosis. Pileus brown to violet-brown at centre, disc light brown to dull red. Cheilocystidia clavate with slightly inflated apex, thick-walled. Pleurocystidia absent. Caulocystidia clavate with tapered apices, apex to middle thick-walled. Scattered to gregarious under *Larix gmelinii*.

Holotype. China. Heilongjiang Province: Shengshan National Nature Reserve, Heihe City, 49°37'45"N, 126°47'39"E, 23 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0424* (collection number MY0686).

Etymology. Refers to the type locality.

Description. ***Pileus*** 13–26 mm in diam., when young parabolic to convex, with obtuse umbo at centre, then plano-convex, margin wavy and revolute, at times cracked at mature; centre light brown (7D5–7D6), brown (7E4–7E8), dark brown (8F5–8F6), violet brown (11F4–11F6), disc paler to light brown (7D4–7D5), brown (7E5–7E6), greyish-brown (8D3), reddish-brown (8D4, 8E4), brownish-grey (11C2), dull red (11C3), margin whitish; striate indistinct, brownish-orange (7C3), greyish-brown (7D3), reddish-grey (12D2), greyish-ruby (12E3–12E5), towards the centre up to 1/3–1/2 diam.; surface slightly moist, smooth. ***Context*** white, 1–2 mm thick, fragile. ***Lamellae*** sinuate to subdecurrent, 19–25 reaching the stipe, 1–3 tiers of lamellulae, white, irregularly intervenose, edge concolorous, wavy and slightly serrulate. ***Stipe*** 26–42 × 2–4 mm, central, cylindrical, apex reddish-brown (8E4–8E5), greyish-ruby (12E4–12E5), greyish-brown (11F3), violet brown (11F4–11F5), dark ruby (12F4–12F5), lower part paler to brownish-grey (7C2), brownish-orange (7C3), greyish-brown (11E3), greyish-ruby (12E3), base darker to brown (7E5), reddish-brown (8E4–8E5), greyish-brown (11F3), violet brown (11F4–11F5), greyish-ruby (12E3), dark ruby (12F6–12F8), fragile, hollow, base swollen with white fibrils. ***Odour*** raphanoid, ***taste*** indistinct.

Figure 9. Basidiomata of *Mycena shengshanensis* Z.W. Liu, Y.P. Ge & Q. Na **a–d** *FFAAS0424*, holotype **e–h** *FFAAS0425* Scale bars: 10 mm (**a–h**). Photographs **a–c, e–g** by Yupeng Ge **d, h** by Zewei Liu.

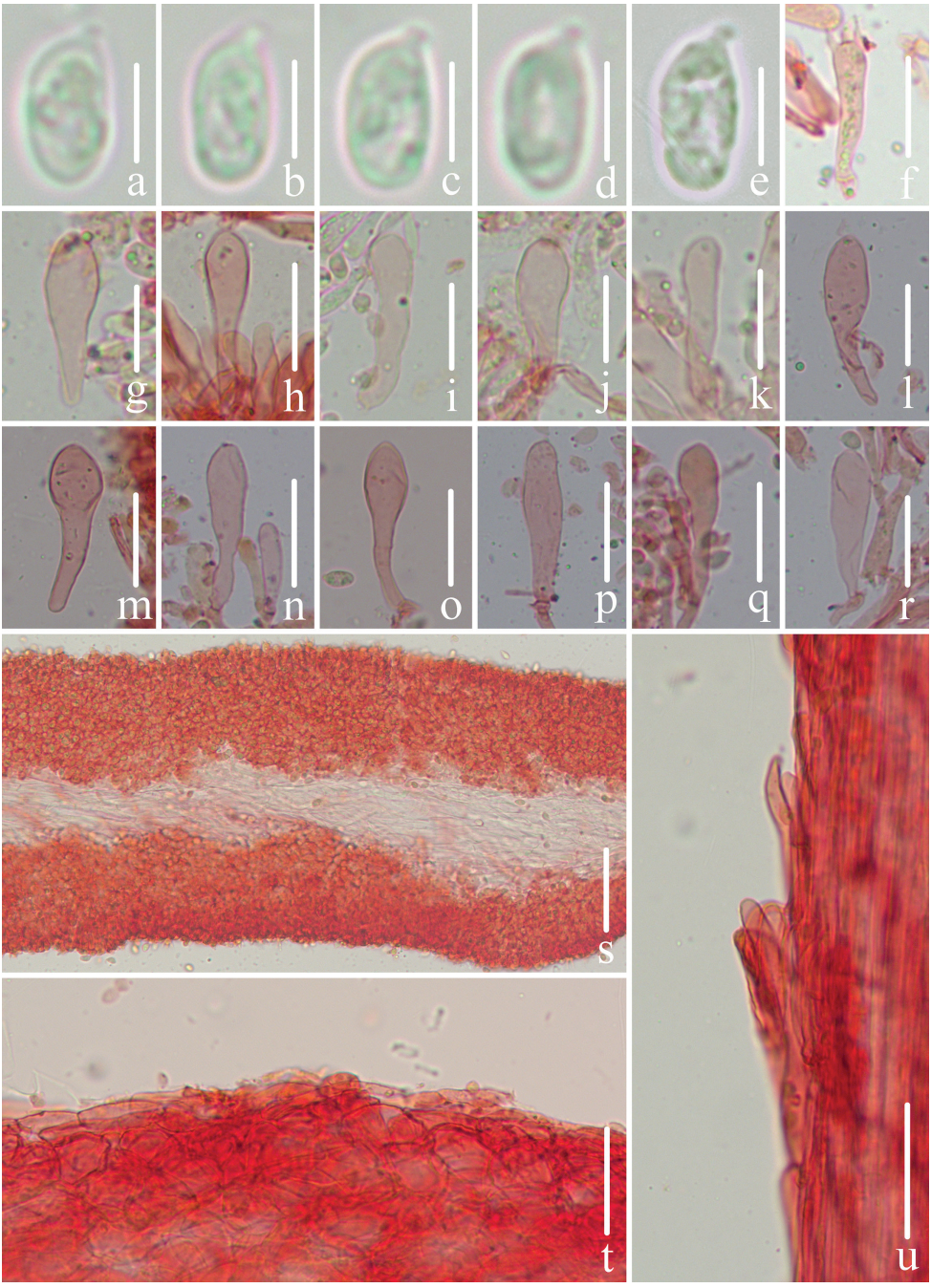

Figure 10. Microscopic features of *Mycena shengshanensis* (*FFAAS0424*, holotype) **a–e** basidiospores **f** basidia **g–r** cheilocystidia **s** lamellar trama **t** pileipellis and hypodermium **u** stipitipellis and caulocystidia. Scale bars: 5 μm (**a–e**); 20 μm (**g–r**); 40 μm (**s–u**).

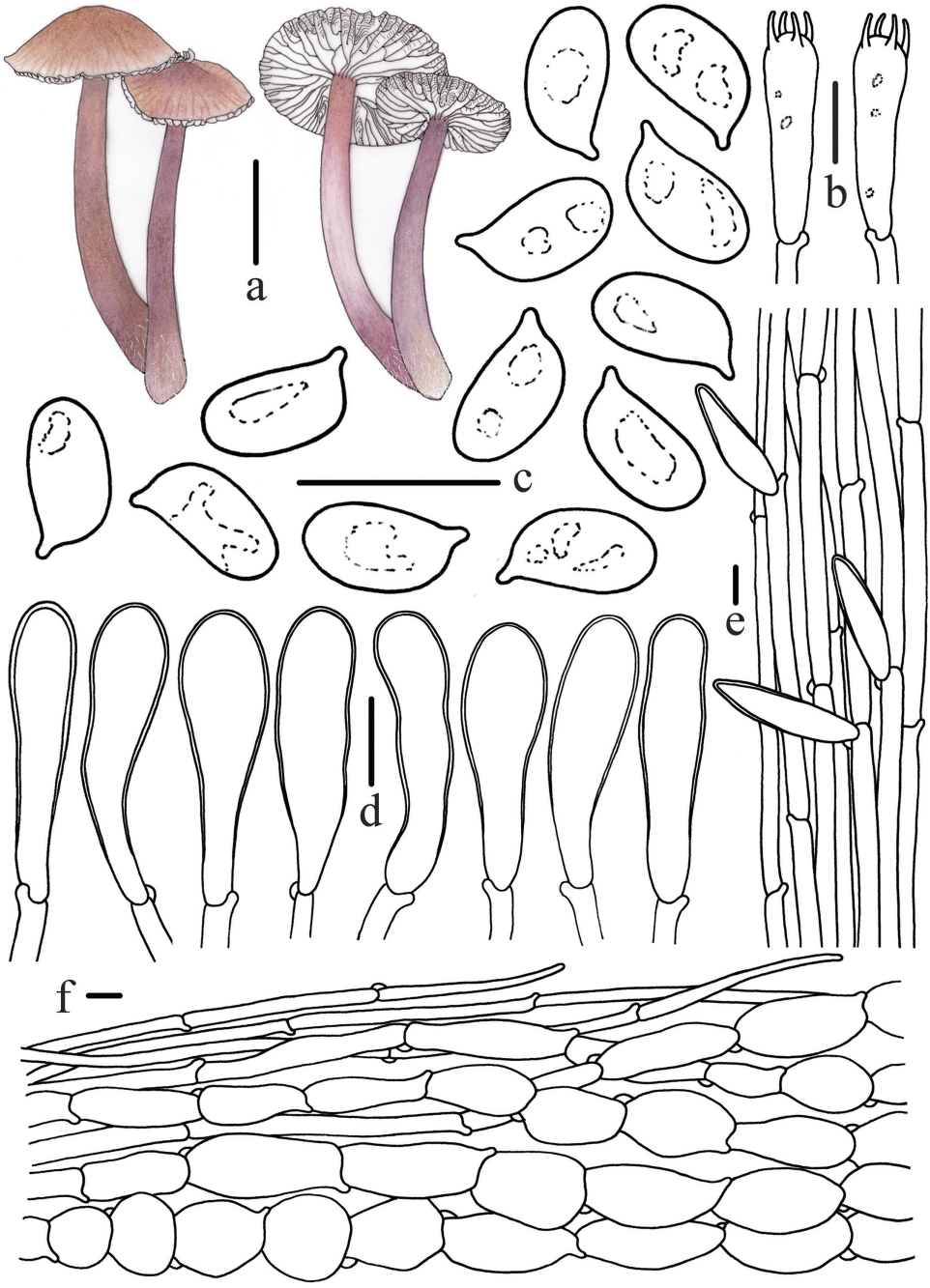

Figure 11. Morphological features of *Mycena shengshanensis* (*FFAAS0424*, holotype) **a** basidiomata **b** basidia **c** basidiospores **d** cheilocystidia **e** stipitipellis and caulocystidia **f** pileipellis and hypodermium. Scale bars: 10 mm (**a**); 10 μm (**b–f**). Drawings by Zewei Liu.

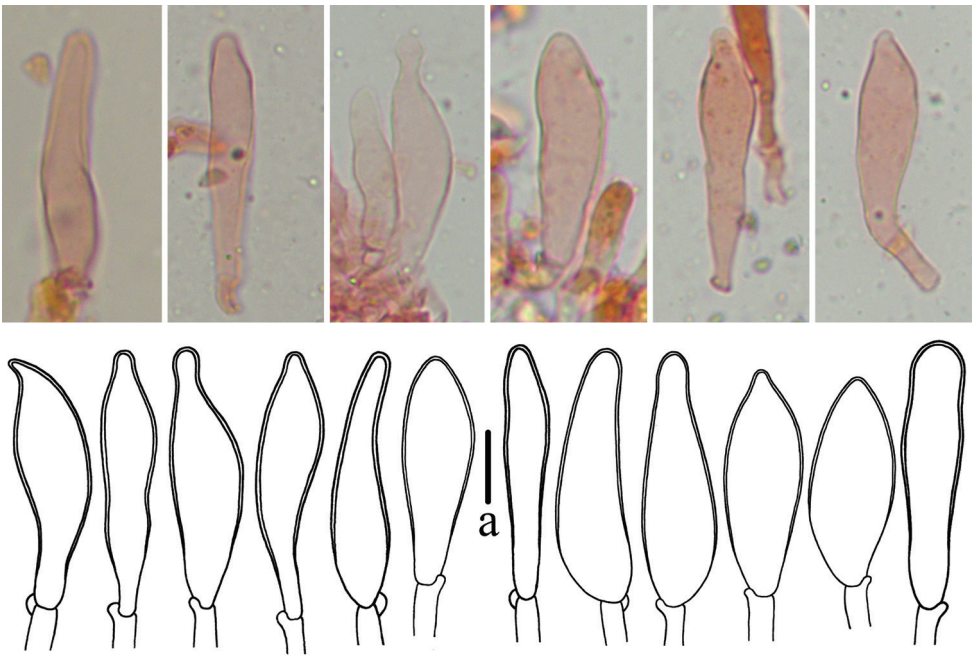

Figure 12. Cheilocystidia of *Mycena shengshanensis FFAAS0424*, holotype. Scale bars: 10 μm (**a**).

Basidiospores (60/3/2) (5.9)6.1–6.9–8.1(8.7) × 3.4–4.0–4.4(4.7) μm [Q = 1.62–1.93 (1.97), Q = 1.75 ± 0.09] [holotype (40/2/1) 6.1–7.0–8.2(8.7) × 3.4–4.0–4.4(4.7) μm, Q = (1.62)1.65–1.93(1.97), Q = 1.77 ± 0.08], elongated ellipsoid, colourless, smooth, thin-walled, amyloid. ***Basidia*** 22–32 × 6–8 μm, 4-spored, clavate, hyaline, sterigmata 3–4 μm in length. ***Cheilocystidia*** moderately thick-walled (0.5–0.6 μm), clavate with slightly inflated apex, 25–63 × 6–12 μm, hyaline. ***Pleurocystidia*** absent. ***Pileipellis*** a cutis composed of three to four layers of cylindrical cells, 24–57 × 3–5 μm, smooth and thin-walled; terminal cells cylindrical, apically narrow, 28–49 μm in length, apex 1–3 μm and base 2–5 μm in diam., thin-walled, hyaline. ***Hypodermium*** formed by fusiform to subglobose hyphae, 19–53 × 13–30 μm, thin-walled, hyaline. ***Lamellar trama*** subregular, weakly dextrinoid to dextrinoid. ***Stipitipellis*** a cutis composed of hyphae 4–9 μm in diam., smooth, thin-walled; ***caulocystidia*** 22–61 × 5–20 μm, clavate with tapered apices, apex to middle thick-walled, smooth, hyaline. ***Clamps*** present in all tissues.

Habitat. Scattered to gregarious on the litter layer in *Larix gmelinii*.

Known distribution. Heilongjiang Province, China.

Additional material examined. China. Heilongjiang Province: Shengshan National Nature Reserve, Heihe City, 23 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0425* (collection number MY0687).

Notes. In sect. *Calodontes*, *M. diosma*, *M. pearsoniana* and *M. yuezhuoi* also have clavate cheilocystidia with a slightly inflated apex and lack pleurocystidia, similar to *M. shengshanensis*, but *M. diosma* differs in pileus characters, *M. pearsoniana* has inamyloid spores and *M. yuezhuoi* has a more purple pileus and subcellular lamellar trama (Robich 2003; Aronsen and Læssøe 2016; Na 2019; Liu et al. 2021). Clavate cheilocystidia are also present in *M. luteovariegata* and *M. pura*, but these species differ in having pleurocystidia (Perry 2002; Robich 2003; Harder et al. 2013; Aronsen and Læssøe 2016; Na 2019). Macroscopically, several species in sect. *Calodontes* also have a brown with reddish or violet pileus or stipe (Smith 1947; Maas Geesteranus 1992a, 1992b; Grgurinovic 2003; Robich 2003; Aronsen and Læssøe 2016). *Mycena dura*, *M. kuehneriana* and *M. nullawarrensis* are distinguished by basidiospore size (Smith 1947; Maas Geesteranus 1992a, 1992b; Grgurinovic 2003; Robich 2003; Aronsen and Læssøe 2016). Two species, *M. seminau* and *M. sirayuktha*, reported from Southeast Asia, are similar to *M. shengshanensis* owing to the brown pileus, but they differ in having gelatinised or sometimes mucronate cheilocystidia and caulocystidia have not been observed (Chew et al. 2014; Aravindakshan and Manimohan 2015). Fusiform, obclavate, ovate and clavate cheilocystidia with a subcapitate protuberance were observed occasionally, but clavate cheilocystidia with a slightly inflated apex represented the predominant morphological type in *M. shengshanensis* (Fig. 12).

***Mycena subulata* Z.W. Liu, Y.P. Ge & Q. Na, sp. nov.**
MycoBank No: 843980
Figs 13–15

Diagnosis. Pileus reddish-grey to dull red, slightly hygrophanous. Cheilocystidia thick-walled, slenderly fusiform with distinctly long and narrow protuberance. Stipitipellis a cutis, with projecting hyphae, caulocystidia thick-walled.

Holotype. China. Heilongjiang Province: Liangshui National Nature Reserve, Yichun City, 47°13'13"N, 128°53'21"E, 21 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0423* (collection number MY0671).

Etymology. Refers to cheilocystidia with distinctly long and narrow protuberance.

Description. ***Pileus*** 9–32 mm in diam., convex to campanulate when young, hemispherical to applanate with age, margin sometimes wavy, slightly deflexed; at centre dull red (8C3), brownish-grey (8D2), greyish-brown (8D3), reddish-brown (8D4, 8E4–8E5) and dark brown (8F5), disc paler to reddish-grey (8B2, 9B2), brownish-grey (9C2), dull red (9B3, 9C3), greyish-magenta (13D3), margin light brown (7D4), brown (7E4) or dull red (9C3); striate none or indistinct, reddish-brown (8E4–8E5), towards the centre up to 1/5 diam.; surface dry, unclearly rugose or none, margin slightly hygrophanous. ***Context*** white, 1 mm thick, fragile. ***Lamellae*** sinuate to subdecurrent, 31–33 reaching the stipe, 1–3 tiers of lamellulae, white, irregularly intervenose, edge concolorous, wavy and slightly serrulate. ***Stipe*** 27–75 × 2–5 mm, central, cylindrical, apex to middle brownish-orange (7C3), dull red (8C3), brownish-grey (7D2), greyish-magenta (14D3), lower part brownish-grey

Figure 13. Basidiomata of *Mycena subulata* Z.W. Liu, Y.P. Ge & Q. Na **a, b** *FFAAS0419* **c, d** *FFAAS0420* **e–g** *FFAAS0423*, holotype **h** *FFAAS0426* Scale bars: 10 mm (**a–h**). Photographs **a, b, e, f** by Yupeng Ge **c, d** by Qin Na **g** by Zewei Liu **h** by Shixin Wang.

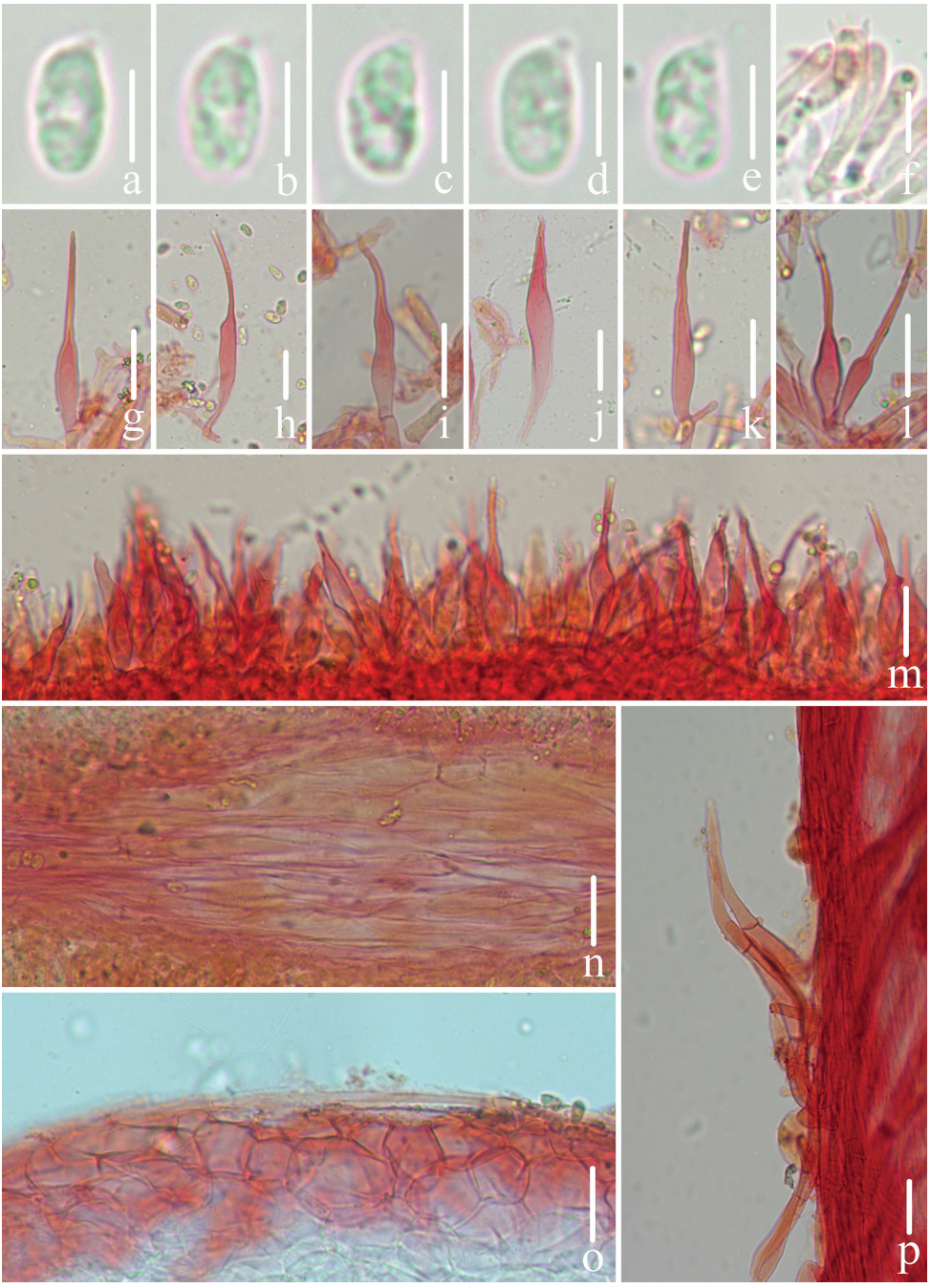

Figure 14. Microscopic features of *Mycena subulata* (*FFAAS0423*, holotype) **a–e** basidiospores **f** basidia **g–m** cheilocystidia **n** lamellar trama **o** pileipellis and hypodermium **p** stipitipellis and caulocystidia. Scale bars: 5 μm (**a–e**); 10 μm (**f**); 20 μm (**g–p**).

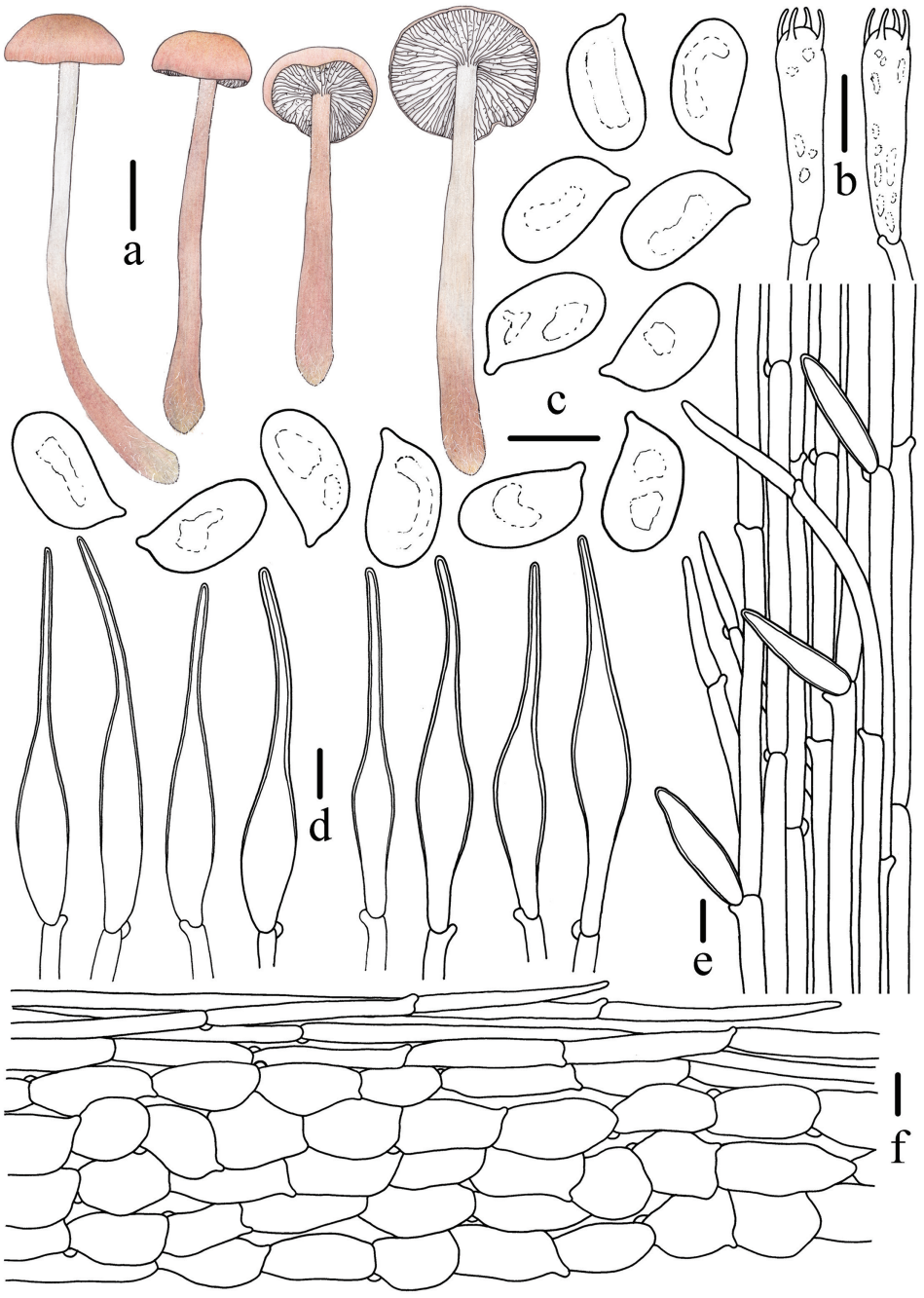

Figure 15. Morphological features of *Mycena subulata* (*FFAAS0423*, holotype) **a** basidiomata **b** basidia **c** basidiospores **d** cheilocystidia **e** stipitipellis and caulocystidia **f** pileipellis and hypodermium. Scale bars: 10 mm (**a**); 5 μm (**c**); 10 μm (**b, d–f**). Drawings by Zewei Liu.

(8C2), greyish-brown (8D3), reddish-brown (8D4), fragile, hollow, white granular near apex, base slightly swollen with white fibrils. ***Odour*** raphanoid, ***taste*** indistinct.

Basidiospores (100/5/4) 6.0–6.7–7.3(7.9) × 3.3–3.8–4.3(4.6) µm [Q = (1.61)1.65–1.87(1.90), Q = 1.76 ± 0.07] [holotype (40/2/1) (6.0)6.2–6.6–7.1 × 3.4–3.7–4.0(4.2) µm, Q = 1.65–1.87(1.90), Q = 1.78 ± 0.07], elongated ellipsoid, colourless, smooth, thin-walled, amyloid. ***Basidia*** 23–34 × 5–6 µm, 4-spored, clavate, hyaline, sterigmata 2–3 µm in length. ***Cheilocystidia*** moderately thick-walled (0.5–0.6 µm), hyaline, narrowly fusiform with long and narrow protuberance, 43–82 × 4–11 µm, protuberance 14–36 × 1–2 µm. ***Pleurocystidia*** absent. ***Pileipellis*** a cutis composed of three to four layers of cylindrical cells, 20–89 × 3–7 µm, smooth and thin-walled; terminal cells cylindrical, apically narrow, 24–61 µm in length, the apex 3–4 µm and base 4–7 µm in diam., thin-walled, hyaline. ***Hypodermium*** formed by fusiform to subglobose hyphae, 21–41 × 17–25 µm, thin-walled, hyaline. ***Lamellar trama*** subregular, dextrinoid. ***Stipitipellis*** a cutis composed of hyphae 3–8 µm in diam., smooth, thin-walled, with projecting hyphae 3–8 µm in diam.; ***caulocystidia*** 29–47 × 6–12 µm, clavate and apices tapered, thick-walled (0.5–0.6 µm), smooth. ***Clamps*** present in all tissues.

Habitat. Scattered on the litter layer in *Pinus koraiensis*, *Larix gmelinii* and *Tilia* sp. mixed forests.

Known distribution. Heilongjiang Province, China.

Additional material examined. China. Heilongjiang Province: Liangshui National Nature Reserve, Yichun City, 21 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0419* (collection number MY0654); same location, 21 August 2021, Zewei Liu, Yupeng Ge, Qin Na and Shixin Wang, *FFAAS0420* (collection number MY0657); Heilongjiang Province: Taipinggou National Nature Reserve, Hegang City, 3 September 2021, Shixin Wang, *FFAAS0426* (collection number MY0795).

Notes. Cheilocystidia with a long and narrow protuberance is the key microscopic character that distinguishes *M. subulata* and is uncommon in sect. *Calodontes* (Smith 1947; Maas Geesteranus 1992a, 1992b; Grgurinovic 2003; Robich 2003; Chew et al. 2014; Aravindakshan and Manimohan 2015; Aronsen and Læssøe 2016; Na 2019; Liu et al. 2021). *Mycena lammiensis* Harmaja and *M. pelianthina* (Fr.) Quél. have similar cheilocystidia, but differ from *M. subulata* by their broader cheilocystidia with purplish-brown contents and having pleurocystidia (Smith 1947; Robich 2003; Aronsen and Læssøe 2016). The cheilocystidia of *M. subcorticalis* (Cooke & Massee) Sacc. with a protuberance are similar to those of *M. subulata*. However, *M. subcorticalis* has larger and inamyloid spores, a gelatinised pileipellis and a stipitipellis with sparse excrescences (Grgurinovic 2003). More rarely, mucronate cheilocystidia and absence of pleurocystidia have been described for *M. pearsoniana* and its clay pink pileus is similar to that of *M. subulata*, but *M. pearsoniana* differs in having a slightly glutinous pileus when wet and inamyloid spores (Aronsen and Læssøe 2016; Na 2019). Other species that are macroscopically similar to *M. subulata*, namely *M. luteovariegata***,** *M. nullawarrensis* and *M. pura*, can be distinguished by cheilocystidia shape and presence of pleurocystidia (Perry 2002; Robich 2003; Grgurinovic 2003; Harder et al. 2013; Aronsen and Læssøe 2016; Na 2019).

Key to species of sect. *Calodontes* known worldwide

1 Stipe white **2**
– Stipe coloured **4**
2 Pileus white ..... ***Mycena subaquosa***
– Pileus coloure **3**
3 Pileus pink and lamellae emarginate, pileipellis without inflated terminal cells ..... ***Mycena rosea***
– Pileus brown and lamellae adnate, pileipellis with fusiform, subcylindrical to lageniform terminal cells ..... ***Mycena dura***
4 Lamellae edge with coloured dots **5**
– Lamellae edge white or without dots **6**
5 Caulocystidia present, spores almost broader than 4 µm ..... ***Mycena lammiensis***
– Caulocystidia absent, spores almost narrower than 4 µm ..... ***Mycena pelianthina***
6 Basidiospores inamyloid **7**
– Basidiospores amyloid **10**
7 Pleurocystidia absent **8**
– Pleurocystidia present **9**
8 Stipitipellis and caulocystidia smooth ..... ***Mycena pearsoniana***
– Stipitipellis and caulocystidia with nodulose excrescences ..... ***Mycena subcorticalis***
9 Pileipellis gelatinised, caulocystidia absent, cheilo- and pleurocytsidia base uncontracted, disc greyish-red or orange white in pileus ..... ***Mycena sirayuktha***
– Pileipellis not gelatinised, caulocystidia present, cheilo- and pleurocytsidia base contracted, disc wood brown or reddish-brown in pileus ..... ***Mycena vinacea***
10 Pleurocystidia present **11**
– Pleurocystidia absent **16**
11 Caulocystidia absent, almost cheilocystidia apically mucronate or subcapitate ..... ***Mycena cahaya***
– Caulocystidia present, almost cheilocystidia apically broadly rounded **12**
12 Caulocystidia with apical excrescences, spores more than 5.6 µm width ..... ***Mycena clarkeana***
– Caulocystidia without apical excrescences, spores less than 5.6 µm width **13**
13 Cheilocystidia base uncontracted ..... ***Mycena polycystidiata***
– Cheilocystidia base contracted **14**
14 Stipe brown to dark brown, Q_{av} = 1.5 ..... ***Mycena nullawarrensis***
– Stipe not brown to dark brown, Q_{av} > 1.5 ..... **15**

15 Pileus sulphur yellow to reddish-grey, stipe reddish-grey ..... ***Mycena luteovariegata***
– Pileus generally pinkish or purplish, stipe whitish to pinkish-purple ..... ***Mycena pura***
16 Caulocystidia absent **17**
– Caulocystidia present **18**
17 Pileus brown to dark brown, spores weakly amyloid ..... ***Mycena seminau***
– Pileus brownish-orange to greyish-yellow, spores amyloid ..... ***Mycena sinar***
18 Cheilocystidia slender fusiform, with distinctly long and narrow protuberance ..... ***Mycena subulata***
– Cheilocystidia clavate, utriform, subfusiform, or subcylindrical, with short mucronate or none **19**
19 Spores less than 6 μm length ..... ***Mycena kuehneriana***
– Spores more than 6 μm length **20**
20 Pileus more than 35 mm in diam., lamellae dark brownish-violet to reddish-violet ..... ***Mycena diosma***
– Pileus less than 35 mm in diam., lamellae white **21**
21 Lamellar trama subcellular, pileus lilac to purple ..... ***Mycena yuezhuoi***
– Lamellar trama subregular, pileus brownish **22**
22 Cheilocystidia utriform, sometimes clavate, thin-walled, lamellae adnexed to emarginate ..... ***Mycena rufobrunnea***
– Cheilocystidia clavate with slightly inflated apex, thick-walled, lamellae sinuate to subdecurrent ..... ***Mycena shengshanensis***

Discussion

Maas Geesteranus (1980) proposed that *Mycena* sect. *Calodontes* could be divided into three subsections based on the colour of the lamellar edge and the amyloid reaction of the basidiospores. Subsequently, taxonomists have followed this division, but opinions have differed on the diagnostic characters that support this classification (Grgurinovic 2003; Robich 2003; Harder et al. 2010; Chew et al. 2014). Some taxonomists classified the subsections according to the amyloid reaction of basidiospores, cheilocystidia and pleurocystidia contents and presence or absence of pleurocystidia, but the shapes of cheilocystidia and pleurocystidia were not considered (Grgurinovic 2003; Harder et al. 2010; Chew et al. 2014). Robich (2003) also did not consider the shapes of cheilocystidia and pleurocystidia, but the colour of the lamellar edge and cheilocystidia contents were emphasised to distinguish subsections. According to the historical infrasectional classification of sect. *Calodontes*, *M. polycystidiata* could be classified in subsect. *Purae*, whereas *M. rufobrunnea*, *M. shengshanensis* and *M. subulata* cannot be assigned to a subsection owing to their having amyloid spores and lacking pleurocystidia (Maas Geesteranus 1980; Harder et al. 2010).

Phylogenetic reconstructions do not fully support recognition of three subsections defined by morphological characters; notably, subsect. *Violacellae* and subsect. *Purae*

are polyphyletic in the phylogenies (Harder et al. 2010, 2012, 2013). Chew et al. (2014) supported the views of Harder et al. (2010, 2012) and the new taxa proposed by the former authors were not assigned to a subsection. Additionally, subsect. *Purae* was proved to be polyphyletic in our combined analysis of ITS, *rpb1* and *tef1* dataset, which also supported analysis, based on single gene region (Harder et al. 2013).

The five taxa of *Mycena* sect. *Calodontes* recorded from China show obvious differences in pileus colour and in the shapes of cheilocystidia and pleurocystidia (if present) (Liu et al. 2021). The colour of the pileus includes greyish-rose, reddish-grey, purple, reddish-brown and violet-brown and most show a gradual transition with age. Clavate, obclavate, utriform and fusiform cheilocystidia with a long, narrow protuberance are observed, but pleurocystidia are present only in *M. polycystidiata*. Forms and variations within *M. pura* complex had a wide range of pileus colour, but the shape of cheilocystidia was highly similar and could be clearly distinguished from the four new taxa (Robich 2003).

In our phylogenetic analysis, four new species all formed separate clades with high support and had obvious genetic distance from other species in sect. *Calodontes*. *Mycena rufobrunnea* is more closely related to the phylogenetic species within *M. pura* complex by Harder et al. (2013). While the other three new species are significantly more distant from *M. pura* complex genetically, *M. shengshanensis* and *M. subulata*, formed a sister relationship with high support from *M. pearsoniana*; *M. polycystidiata* clustered with *M. diosma*, but is poorly supported.

Based on extensive field work in China, most specimens of sect. *Calodontes* have been observed in coniferous forests or mixed coniferous-broadleaved forests in early autumn (Na 2019; Liu et al. 2021). Specimens of the four new taxa described in the present study were collected from Changbai Mountain and the Lesser Khinggan Mountains in northeast China from mixed broadleaf-Korean pine (*Pinus koraiensis*) forests (Zhao et al. 2004; Wang and Guo 2016). In particular, *M. polycystidiata* and *M. subulata* were both distributed in the Liangshui National Nature Reserve on the Lesser Khinggan Mountains, where the dominant forest species is *P. koraiensis*, mixed with fewer *Betula*, *Tilia*, *Quercus* and *Picea* individuals (She et al. 2022). Moreover, more specimens were located in the northern region of China with an average temperature not more than 20 °C in August. For example, the average temperature is 16.4 °C in Liangshui National Nature Reserve and 16.3 °C in Shengshan National Nature Reserve (Liu 2017). Therefore, we speculate that members of this section in China prefer the climate types Dwa, Dwb and Dwc according to the Köppen climate classification (Kottek et al. 2006; Wang et al. 2020).

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Lignicolous freshwater ascomycetes from Thailand: Introducing *Dematipyriforma muriformis* sp. nov., one new combination and two new records in Pleurotheciaceae

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Abstract

During the study of lignicolous freshwater fungi from Thailand, three pleurotheciaceous species were collected from freshwater habitats in Thailand. Two were identified as *Pleurothecium aquaticum* and *Rhexoacrodictys fimicola*, and the third is a new species *Dematipyriforma muriformis* sp. nov.. *Rhexoacrodictys* is accepted in Pleurotheciaceae based on phylogenetic analysis. *Rhexoacrodictys nigrospora* is transferred to *Dematipyriforma* based on phylogenetic analysis and morphological characters. *Pleurothecium aquaticum* and *Rhexoacrodictys fimicola* are reported from Thailand for the first time.

Keywords

1 new combination, 1 new taxon, freshwater fungi, phylogeny, Pleurotheciales, taxonomy

Introduction

Pleurotheciales was introduced by Réblová et al. (2016) to accommodate a single family Pleurotheciaceae. The order was originally placed in Hypocreomycetidae by Réblová et al. (2016). Hongsanan et al. (2017) showed that Pleurotheciales clustered with Conioscyphales, Fuscosporellales and Savoryellales in a monophyletic clade within Sordariomycetes. Hence, they transferred Pleurotheciales to a newly introduced subclass Savoryellomycetidae based on phylogenetic analysis and the placement has been confirmed and accepted by Dayarathne et al. (2019) and Hyde et al. (2020a).

Pleurotheciaceae was introduced by Réblová et al. (2016) with *Pleurothecium* Höhn. as the type genus. Currently, *Adelosphaeria*, *Anapleurothecium*, *Coleodictyospora*, *Dematipyriforma*, *Helicoascotaiwania*, *Melanotrigonum*, *Neomonodictys*, *Phaeoisaria*, *Phragmocephala*, *Pleurotheciella*, *Pleurothecium*, *Saprodesmium*, and *Sterigmatobotrys* are accepted in this family (Hyde et al. 2020a; Wijayawardene et al. 2020; Dong et al. 2021). The sexual morphs of Pleurotheciaceae share dark, papillate, perithecial, astromatic, immersed to superficial ascomata, unitunicate asci with a distinct non-amyloid apical annulus, and fusiform to ellipsoidal, septate, hyaline ascospores (Réblová et al. 2016; Luo et al. 2018a; Hyde et al. 2020a). The asexual morphs of Pleurotheciaceae are diverse in morphology, comprising *acrodictys*-like (*Monotosporella*), (Hyde and Yanna 2002; Sadowski et al. 2012), *helicoön*-like (*Helicoascotaiwania*, Dayarathne et al. 2019; Réblová et al. 2020), *monodictys*-like (*Neomonodictys*, Hyde et al. 2020b) and *dactylaria*-like taxa (*Pleurotheciella*, *Phaeoisaria* and *Pleurothecium*, Réblová et al. 2016; Luo et al. 2018a). Species in Pleurotheciaceae are cosmopolitan with a worldwide distribution and have been reported from both aquatic and terrestrial habitats (Réblová et al. 2016, 2020; Hernandez-Restrepo et al. 2017; Luo et al. 2018a, 2019; Hyde et al. 2020a, b).

In this study, three new collections are placed in *Dematipyriforma*, *Rhexoacrodictys* and *Pleurothecium* respectively. The monotypic genus *Dematipyriforma* was introduced to accommodate an endophytic species, *D. aquilaria* which was collected from wood of *Aquilaria crassna* (Sun et al. 2017). *Dematipyriforma* was originally placed in Savoryellales (Sun et al. 2017). However, Dong et al. (2021) showed that *Dematipyriforma* clustered within Pleurotheciales and sister to *Rhexoacrodictys* and *Saprodesmium*. In addition, the morphology of *Dematipyriforma* is similar to *Neomonodictys* in Pleurotheciales. Therefore, they transferred *Dematipyriforma* to Pleurotheciales based on phylogenetic analysis and morphological characteristics. *Rhexoacrodictys* was introduced by Baker et al. (2002) to accommodate species previously identified as *Acorcdictys* (i.e., *A. erecta*, *A. fimicola*, *A. fuliginosa* and *A. queenslandica*) and wherein *Rhexoacrodictys erecta* was designated as the type. Two additional species *R. martini* and *R. broussonetiae* were subsequently added to the genus based on morphological characteristics (Delgado 2009; Xiao et al. 2018). While *R. martini* and *R. queenslandica* were transferred to *Distoseptispora* and *Junewangia* based on phylogenetic analysis (Xia et al. 2017). Currently, four species are accepted in *Rhexoacrodictys*. *Pleurothecium* was established by Höhnel (1919) with

P. recurvatum (Morgan) Höhn as type species. *Pleurothecium* species are characterized by macronematous, mononematous, septate, brown conidiophores, polyblastic, sympodially extended, denticulate conidiogenous cells and solitary, septate, hyaline or pigmented or bicolored conidia (Goos 1969; Matsushima 1975, 1980; Subramanian and Bhat 1989; Matsushima and Matsushima 1996; Cooper 2005; Arzanlou et al. 2007; Wu and Zhang 2009; Réblová et al. 2012; Monteiro et al. 2016; Luo et al. 2018a). Presently, 11 species are accepted in the genus. Most *Pleurothecium* species are reported as saprobes from freshwater or terrestrial habitats (Wu and Zhang 2009; Réblová et al. 2012; Monteiro et al. 2016; Luo et al. 2018a).

We are currently investigating the diversity of lignicolous freshwater fungi from the Greater Mekong Subregion (Hyde et al. 2016). Thailand is an area of the Greater Mekong Subregion with rich fungal biodiversity. Freshwater fungi have been studied in Thailand over several decades initiated by Tubaki et al. (1983) who found 40 Ingoldian fungi in the stream foams. Many new freshwater taxa have since been reported in Thailand, especially a large number of lignicolous freshwater ascomycetes (Sivichai et al. 1998, 2000, 2002; Jones et al. 1999; Sivichai and Boonyene 2004; Zhang et al. 2011; Luo et al. 2019; Dong et al. 2020; Calabon et al. 2021, 2022). Until 2020, more than 302 freshwater taxa had been reported from Thailand (Zhang et al. 2011; Calabon et al. 2021). In this study, we introduce three taxa of Pleurotheciaceae, collected from freshwater habitats in Thailand. With phylogenetic analysis of ITS, LSU, SSU, RPB2 and TEF1-α sequence data, they are placed in *Dematipyriforma*, *Pleurothecium* and *Rhexoacrodictys* within Pleurotheciaceae. Of these three species, one is identified as *Pleurothecium aquaticum*, one as *Rhexoacrodictys fimicola*, and the third as a new species in *Dematipyriforma*. In addition, *Rhexoacrodictys nigrospora* is transferred to *Dematipyriforma* based on morphological and phylogenetic evidence.

Materials and methods

Collection, isolation and morphology

Submerged decaying woods were collected from the streams in Thailand. The sample incubation, examination and morphological studies were referred to the methods described by Luo et al. (2018b). Single spore isolations were followed the methods outlined by Senanayake et al. (2020). Specimens (dry wood with fungal material) were deposited in the herbarium of Mae Fah Luang University (**MFLU**), Chiang Rai, Thailand and Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (**KUNHKAS**). Pure cultures were deposited in Mae Fah Luang University Culture Collection (**MFLUCC**) and Kunming Institute of Botany culture collection (**KUNCC**). Faces of Fungi and Index Fungorum numbers were registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2022). The descriptions are added to it GMS database (Chaiwan et al. 2021).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from fungal mycelium (*Rhexoacrodictys erecta* and *Pleurothecium aquaticum*) or directly from the conidiamatal tissue thalli of fungi (*Dematipyriforma muriformis*) as outlined by Wanasinghe et al. (2018). The Ezup Column Fungi Genomic DNA Purification Kit (Sangon Biotech, China) was used to extract DNA following the manufacturer's instructions. ITS, LSU, SSU, RPB2 and TEF1-α gene regions were amplified using the primer pairs ITS5/ITS4, LR0R/LR7, NS1/NS4, fRPB2-5F/fRPB2-7cR and 983F/2218R, respectively (Vilgalys and Hester 1990; White et al. 1990; Liu et al. 1999). The amplification was performed in a 25 µl reaction volume containing 9.5 µl ddH_2O, 12.5 µl 2 × Taq PCR Master Mix with blue dye (Sangon Biotech, China), 1 µl of DNA template and 1 µl of each primer (10 µM). The amplification condition for ITS, LSU, SSU, RPB2 and TEF1-α were followed Luo et al. (2018b). DNA sequencing of PCR products were carried out using the above-mentioned PCR primers at Tsingke Biological Engineering Technology and Services Co. (Yunnan, P.R. China).

Phylogenetic analyses

The taxa used in the phylogenetic analysis were obtained from previous studies (Table 1) (Hernandez-Restrepo et al. 2017; Luo et al. 2018a, 2019; Dayarathne et al. 2019; Hyde et al. 2020b; Réblová et al. 2020; Boonmee et al. 2021; Dong et al. 2021) and downloaded from GenBank. SEQMAN v. 7.0.0 (DNASTAR, Madison, WI) was used to assemble the consensus sequences and MAFFT v.7 online program (http://mafft.cbrc.jp/alignment/server/) was used to align the sequences (Katoh et al. 2019). BioEdit was used to manually adjust the alignments and the alignment fasta file was converted to Phylip format by Alivew (Hall 2021; Larsson 2014).

Maximum likelihood (ML) analysis generated using the RAxML-HPC2 on XSEDE (v.8.2.8) in the CIPRES Science Gateway (https://www.phylo.org, Stamatakis 2006; Stamatakis et al. 2008; Miller et al. 2010) with rapid bootstrap analysis, followed by 1000 bootstrap replicates, using the GTR+I+G model of evolution.

Bayesian analysis was performed by MrBayes v. 3.2 (Ronquist et al. 2012), best-fit model of DNA evolution for the Bayesian inference analysis was estimated by MrModeltest v. 2.2 (Nylander 2004) and the GTR+I+G model was selected for LSU, ITS, RPB2 and TEF1-α, GTR+G model was selected for SSU. Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) was defined by Bayesian Markov Chain Monte Carlo (BMCMC) sampling method in MrBayes v. 3.0b4 (Huelsenbeck and Ronquist 2001). Six simultaneous Markov Chains were run for 50,000,000 generations and trees were sampled every 500th generation (resulting in 100,000 trees). The first 20% trees that represented the burn-in phase were discarded and the remaining 80% (post burn-in) trees used for calculating posterior probabilities (PP) for the majority rule consensus tree.

Phylogenetic trees were visualized with FigTree v. 1.4.2 (Rambaut 2014) and edited in Microsoft Office PowerPoint 2019 (Microsoft Inc., United States). Newly generated sequences in this study were deposited in GenBank.

Table 1. Taxa used in this study; the ex-type strains were indicated in bold, newly generated sequences are indicated by * after the species name.

Species	Strain number	GenBank accession number				
		ITS	LSU	SSU	RPB2	TEF1-α
Adelosphaeria catenata	**CBS 138679**	**KT278721**	**KT278707**	**KT278692**	**KT278743**	**–**
Anapleurothecium botulisporum	**CBS 132713**	**KY853423**	**KY853483**	**–**	**–**	**–**
Ascotaiwania lignicola	NIL00005	HQ446341	HQ446364	HQ446284	HQ446419	HQ446307
Ascotaiwania sawadae	SS00051	HQ446340	HQ446363	HQ446283	HQ446418	HQ446306
Bactrodesmiastrum obovatum	**FMR 6482**	**FR870264**	**FR870266**	**–**	**–**	**–**
Bactrodesmiastrum pyriforme	**FMR 10747**	**FR870263**	**FR870265**	**–**	**–**	**–**
Bactrodesmium abruptum	CBS 144404	MN699391	MN699408	MN699365	MN704288	MN704313
Bactrodesmium leptopus	CBS 144542	MN699388	MN699423	MN699374	MN704297	MN704321
Bactrodesmium obovatum	CBS 144077	MN699395	MN699424	MN699375	MN704298	MN704322
Canalisporium exiguum	SS00809	GQ390296	GQ390281	GQ390266	HQ446436	–
Canalisporium grenadoideum	**SS03615**	**–**	**GQ390267**	**GQ390252**	**HQ446420**	**HQ446309**
Coleodictyospora muriformis	MFLUCC 18–1243	MW981642	MW981648	MW981704	–	–
Coleodictyospora muriformis	MFLUCC 18–1279	MW981643	MW981649	MW981705	–	–
Conioscypha hoehnelii	FMR 11592	KY853437	KY853497	HF937348	–	–
Conioscypha lignicola	**CBS 335.93**	**–**	**AY484513**	**JQ437439**	**JQ429260**	**–**
Conioscypha peruviana	**ILL41202**	**–**	**KF781539**	**–**	**–**	**–**
Conioscypha pleiomorpha	**FMR 13134**	**KY853438**	**KY853498**	**–**	**–**	**–**
Dematipyriforma aquilaria	**CGMCC 3.17268**	**KJ138621**	**KJ138623**	**KJ138622**	**–**	**–**
Dematipyriforma muriformis*	**MFLU 21–0146**	**OM654773**	**OM654770**	**–**	**–**	**OM672032**
Dematipyriforma nigrospora	**MFLUCC 21-0096**	**MZ538524**	**MZ538558**	**–**	**–**	**MZ567100**
Dematipyriforma nigrospora	MFLUCC 21-0097	MZ538525	MZ538559	MZ538574	MZ567113	MZ567101
Fuscosporella pyriformis	**MFLUCC 16–0570**	**–**	**KX550896**	**KX550900**	**KX576872**	**–**
Helicoascotaiwania farinosa	**ILLS 53605**	**–**	**AY094189**	**–**	**–**	**–**
Helicoascotaiwania farinosa	DAOMC 241947	JQ429145	JQ429230	–	–	–
Helicoascotaiwania lacustris	**CBS 145963**	**–**	**MN699430**	**MN699382**	**MN704304**	**MN704329**
Helicoascotaiwania lacustris	CBS 145964	MN699400	MN699431	MN699383	MN704305	–
Helicoascotaiwania lacustris	CBS 146144	MN699401	MN699432	MN699384	MN704306	–
Leotia lubrica	AFTOL-ID1	DQ491484	AY544644	AY544746	DQ470876	DQ028596
Melanotrigonum ovale	CBS 138815	KT278722	KT278711	KT278698	KT278747	–
Microglossum rufum	AFTOL-ID 1292	–	DQ470981	DQ471033	DQ470933	DQ471104
Monotosporella setosa	HKUCC3713	–	AF132334	–	–	–
Mucispora obscuriseptata	**MFLUCC 15–0618**	**–**	**KX550892**	**KX550897**	**–**	**–**
Mucispora phangngaensis	**MFLUCC 16–0865**	**–**	**MG388210**	**MG388207**	**–**	**–**
Neomonodictys muriformis	MFLUCC 16–1136	MN644509	MN644485	–	–	MN646856
Obliquifusoideum guttulatum	**MFLUCC 18–1233**	MW981645	MW981650	MW981706	–	–
Parafuscosporella garethii	**FF00725.01**	**–**	**KX958430**	**KX958428**	**KX958432**	**–**
Parafuscosporella moniliformis	**MFLUCC 15–0626**	**–**	**KX550895**	**KX550899**	**–**	**–**
Parafuscosporella mucosa	**MFLUCC 16–0571**	**–**	**MG388211**	**MG388208**	**–**	**–**
Phaeoisaria aquatica	**MFLUCC 16–1298**	**MF399237**	**MF399254**	**–**	**MF401406**	**–**
Phaeoisaria clematidis	MFLUCC 17–1968	MG837022	MG837017	MG837027	–	–
Phaeoisaria fasciculata	**CBS 127885**	**–**	**KT278705**	**KT278693**	**KT278741**	**–**
Phaeoisaria filiformis	MFLUCC 18–0214	MK878381	MK835852	MK834785	–	MN200285
Phaeoisaria guttulata	**MFLUCC 17–1965**	**MG837021**	**MG837016**	**MG837026**	**–**	**–**
Phaeoisaria pseudoclematidis	**MFLUCC 11–0393**	**–**	**KP744501**	**KP753962**	**–**	**–**
Phaeoisaria sedimenticola	**CGMCC 3.14949**	**–**	**JQ031561**	**–**	**–**	**–**
Phaeoisaria sedimenticola	**S-908**	**MK878380**	**MK835851**	**–**	**–**	**MN200284**
Phaeoisaria sparsa	FMR11939	–	HF677185	–	–	–
Phragmocephala stemphylioides	DAOM 673211	KT278730	KT278717	–	–	–
Pleurotheciella aquatica	MFLUCC 17–0464	MF399236	MF399253	MF399220	MF401405	–
Pleurotheciella centenaria	**DAOM 229631**	**–**	**JQ429234**	**JQ429246**	**JQ429265**	**–**
Pleurotheciella fusiformis	MFLUCC 17–0115	MF399232	MF399249	MF399217	MF401402	–
Pleurotheciella guttulata	**KUMCC 15–0296**	**MF399240**	**MF399257**	**MF399223**	**MF401409**	**–**
Pleurotheciella krabiensis	**MFLUCC 18–0852**	**MG837018**	**MG837013**	**MG837023**	**–**	**–**

Species	Strain number	GenBank accession number				
		ITS	LSU	SSU	RPB2	TEF1-α
Pleurotheciella lunata	**MFLUCC 17–0111**	**MF399238**	**MF399255**	**MF399221**	**MF401407**	**–**
Pleurotheciella rivularia	**CBS 125238**	**–**	**JQ429232**	**JQ429244**	**JQ429263**	**–**
Pleurotheciella rivularia	CBS 125237	–	JQ429233	JQ429245	JQ429264	–
Pleurotheciella saprophytica	**MFLUCC 16–1251**	**MF399241**	**MF399258**	**MF399224**	**MF401410**	**–**
Pleurotheciella submersa	MFLUCC 17–1709	MF399243	MF399260	MF399226	MF401412	–
Pleurotheciella submersa	**MFLUCC 17–0456**	**MF399244**	**MF399261**	**MF399227**	**MF401413**	**–**
Pleurotheciella tropica	**MFLUCC 16–0867**	**MG837020**	**MG837015**	**MG837025**	**–**	**–**
Pleurotheciella uniseptata	**DAOM 673210**	**KT278729**	**KT278716**	**–**	**–**	**–**
Pleurothecium aquaticum	**MFLUCC 17–1331**	**MF399245**	**MF399263**	**–**	**–**	**–**
*Pleurothecium aquaticum**	KUMCC 21-0477	OM654775	OM654772	OM654807	OM672034	OM672033
Pleurothecium floriforme	**MFLUCC 15–0628**	**NR_156614**	**NG_059791**	**–**	**–**	**–**
Pleurothecium obovoideum	CBS 209.95	EU041784	EU041841	–	–	–
Pleurothecium pulneyense	MFLUCC 16–1293	–	MF399262	MF399228	MF401414	–
Pleurothecium recurvatum	CBS 138686	–	KT278715	KT278702	–	–
Pleurothecium semifecundum	**CBS 131271**	**–**	**JQ429240**	**JQ429254**	**JQ429270**	**–**
Rhexoacrodictys erecta	HSAUPmyr4622	KU999964	KX033556	KX033526	–	–
Rhexoacrodictys erecta	IFRD500–016	MT555421	MT559123	MT555735	–	–
Rhexoacrodictys erecta	HSAUP myr6489	KU999963	KX033555	KX033525	–	–
Rhexoacrodictys fimicola	HMAS 47737	KU999960	KX033553	KX033522	–	–
Rhexoacrodictys fimicola	HMAS 42882	KU999962	KX033554	KX033524	–	–
Rhexoacrodictys fimicola	HMAS 43690	KU999957	KX033550	KX033519	–	–
*Rhexoacrodictys fimicola**	MFLUCC 18–0340	OM654774	OM654771	OM654806	–	–
Saprodesmium dematiosporium	**KUMCC 18–0059**	**MW981646**	**MW981647**	**MW981707**	**–**	**–**
Savoryella aquatica	SS03801	–	HQ446372	HQ446292	HQ446405	HQ446326
Savoryella lignicola	NF00204	–	HQ446378	HQ446300	HQ446413	HQ446334
Sterigmatobotrys macrocarpa	MR2973	–	GU017317	–	–	–
Sterigmatobotrys rudis	DAOM 229838	JQ429152	JQ429241	JQ429256	JQ429272	–
Sterigmatobotrys uniseptata	**MFLUCC 15–0358**	**MK878379**	**MK835850**	**MK834784**	**–**	**–**

Results

Phylogenetic analyses

The dataset of combined ITS, LSU, SSU, RPB2 and TEF1-α sequence data comprises 81 strains with 4257 characters including gaps (ITS: 509 bp, LSU: 1006 bp, SSU: 862 bp, RPB2: 1032 bp, TEF1-α: 848 bp). *Leotia lubrica* (AFTOL-ID1) and *Microglossum rufum* (AFTOL-ID 1292) were used as outgroup taxa. RAxML and Bayesian analyses were conducted and resulted in generally congruent topologies. The best RAxML tree with a final likelihood value of –45872.924927 is presented. The matrix had 2433 distinct alignment patterns, with 44.65% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.234712, C = 0.261626, G = 0.290634, T = 0.213028; substitution rates AC = 1.347806, AG = 2.754719, AT = 1.490447, CG = 1.095887, CT = 6.696475, GT = 1.000000; gamma distribution shape parameter α = 0.316898.

In the phylogenetic analysis, *Dematipyriforma muriformis* (MFLU 21–0146) clustered with the ex-type strain of *D. aquilaria* (CGMCC 3.17268) with low support (Fig. 1). The new isolate of *Rhexoacrodictys fimicola* (MFLUCC 18–0340) clustered

with three strains of *R. fimicola* (HMAS 42882, HMAS 43690 and HMAS 47737) with 100% ML/1.00 PP support (Fig. 1). *Pleurothecium aquaticum* (KUNCC 21–0477) clustered with the ex-type strain of *P. aquaticum* (MFLUCC 17–1331) with 100% ML/1.00 PP support (Fig. 1).

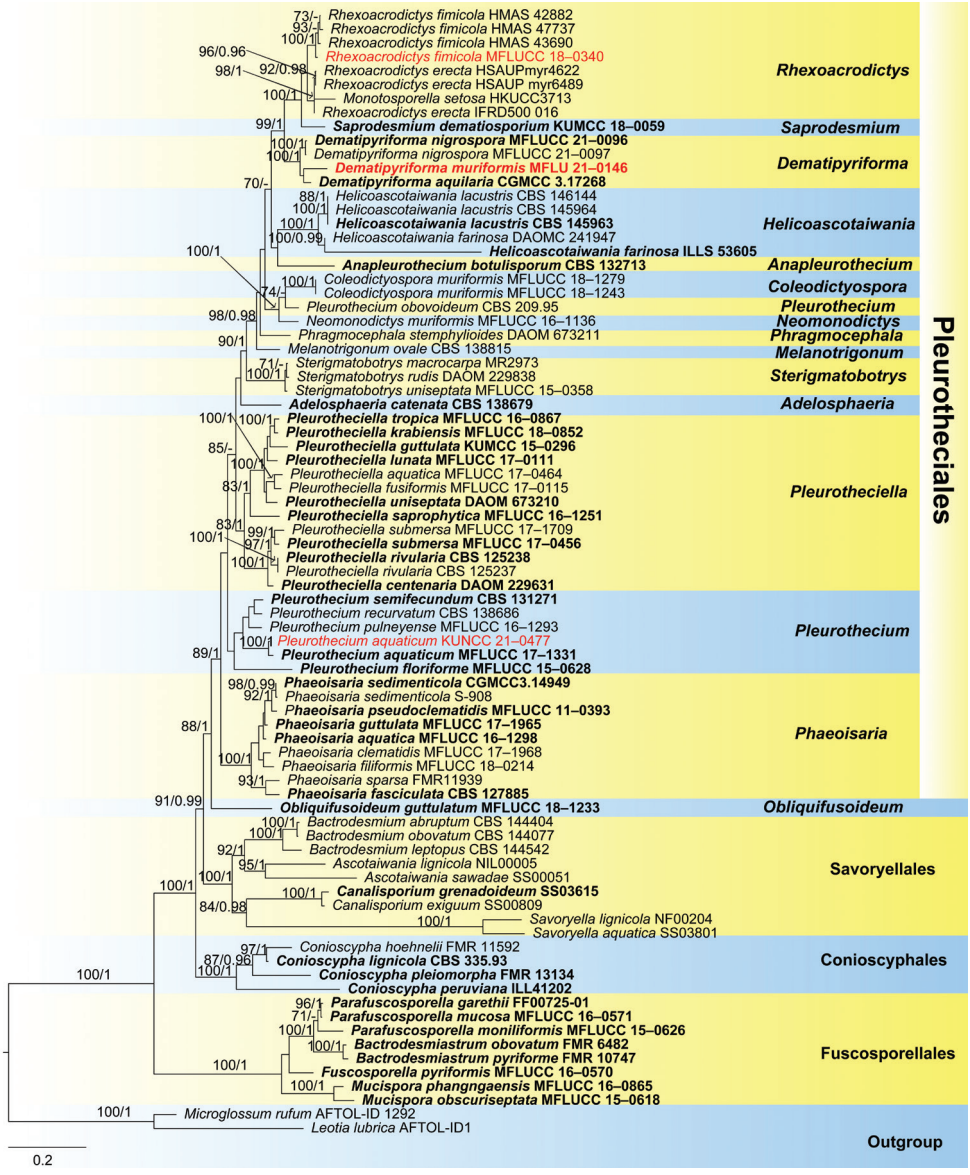

Figure 1. Phylogram based on a combined ITS, LSU SSU, RPB2 and TEF1-α sequence data of selected members of four orders of the Savoryellomycetidae. Bootstrap support values for maximum likelihood (ML) greater than 70% and Bayesian posterior probabilities (PP) greater than 0.95 are given as ML/PP above the nodes. Newly obtained sequences are indicated in red and ex-type strains are in bold.

Taxonomy

Dematipyriforma muriformis **D.F. Bao, K.D. Hyde & Z.L. Luo, sp. nov.**
Index Fungorum Number: IF553383
Facesoffungi Number: FoF10414
Fig. 2

Etymology. Referring to the muriform conidia of this species.

Holotype. MFLU 21–0146.

Description. *Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on substratum superficial, scattered, black, shining, granulate. *Mycelium* immersed, composed of hyaline, branched, septate, smooth, hyphae. *Conidiomata* sporodochial, subhyaline. *Conidiophores* 10–26.5 × 2–3 μm ($\bar{x}$ = 18.2 × 2.3 μm, n = 20), micronematous to semi-macronematous, mononematous, fasciculate, simple or branched, hyaline, cylindrical, smooth. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, hyaline, smooth. *Conidia* 23–26 × 15.5–18 μm ($\bar{x}$ = 24.6 × 16.7 μm, n = 30), acrogenous, solitary, smooth, thick-walled, ellipsoidal to obovoid, muriform, rounded at apex, pointed at base, with 3–5 transverse septa, 1-longitudinal septum in all cells and rarely in end cells, slightly constricted at septa, subhyaline to pale olivaceous when young, olive to dark brown at maturity.

Material examined. Thailand, Bangkok Province, Bang Kapi District, on decaying wood submerged in a freshwater stream, 3 October 2017, Z.L. Luo, Bsite 4–3–1 (MFLU 21–0146, holotype; KUN-HKAS 122858, isotype).

Notes. In the phylogenetic analysis, *Dematipyriforma muriformis* clustered with the ex-type strain of *D. aquilaria* (CGMCC 3.17268) within Pleurotheciaceae with low support (Fig. 1). The ITS blast result in NCBI GenBank showed that *D. muriformis* (MFLU 21–0146) is 92.36% and 91.92% similar to *D. nigrospora* (MFLUCC 21-0097) and *D. aquilaria* (CGMCC 3.17268) respectively.

Dematipyriforma muriformis resembles *D. aquilaria* in having micronematous, mononematous, smooth septate conidiophores, monoblastic, integrated, terminal, determinate conidiogenous cells and solitary, muriform conidia. However, *D. muriformis* differs from *D. aquilaria* in having hyaline conidiophores and slightly smaller conidia (23–26 × 15.5–18 vs. 25–37.5 × 15–22.5 μm). In addition, conidia of *D. muriformis* are subhyaline to pale olivaceous when young, olive to dark brown at maturity, with 3–5 transverse septa, 1-longitudinal septum in all cells and rarely in end cells. Whereas, *D. aquilaria* has pale grey olivaceous to pale brown conidia with 4–5 transverse septa and 0–2 longitudinal septa (Sun et al. 2017).

Dematipyriforma muriformis shares some similar characteristics with *Neomonodictys* taxa in Pleurotheciaceae, such as monoblastic, integrated, terminal, determinate conidiogenous cells and muriform conidia. *Neomonodictys*, however, lacks sporodochial conidiomata and conidia of *Neomonodictys* are subglobose to globose, while, *Dematipyriforma muriformis* has ellipsoidal to obovoid conidia (Hyde et al. 2020b).

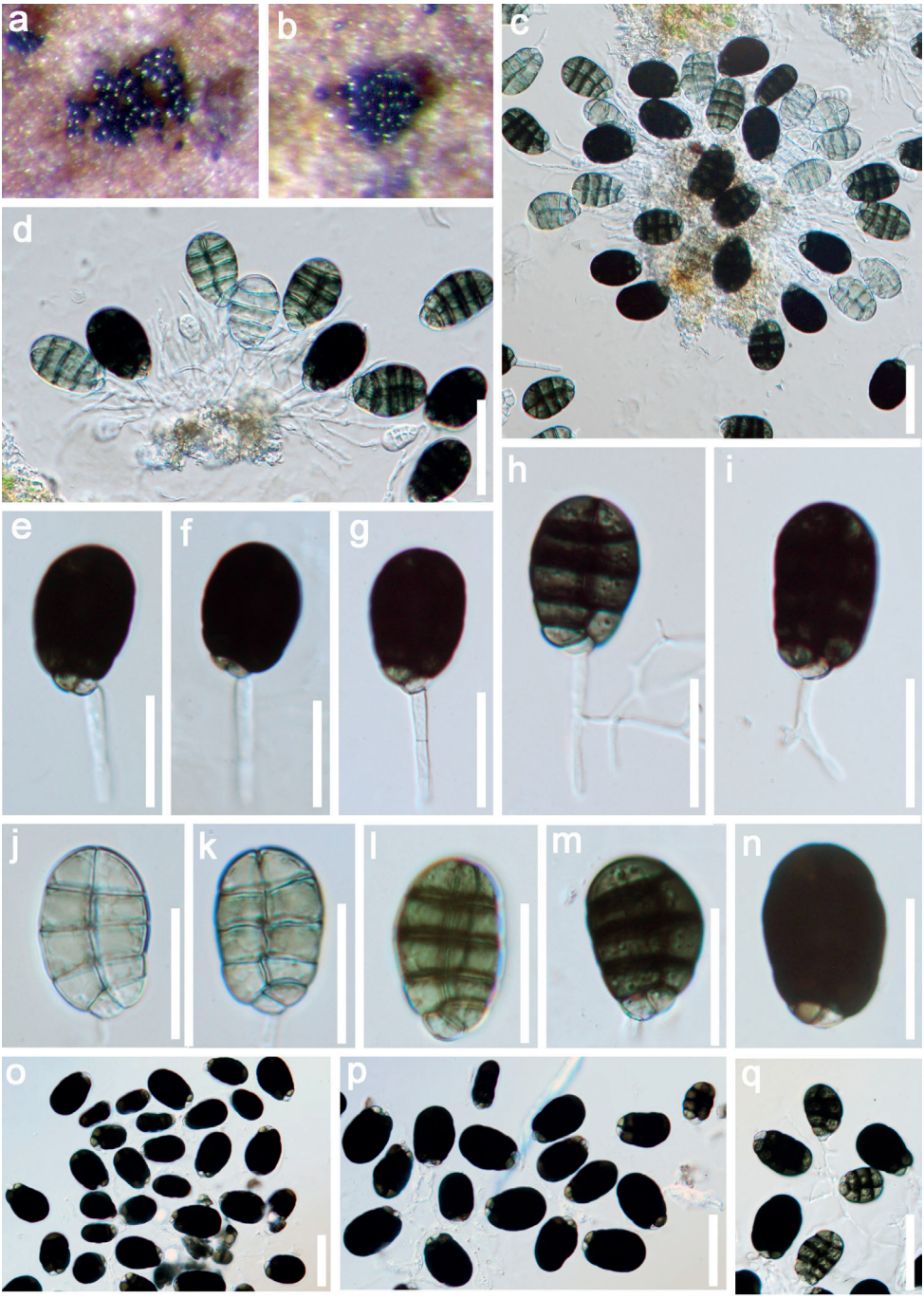

Figure 2. *Dematipyriforma muriformis* (MFLU 21–0146, holotype) **a, b** colonies on wood **c–d** conidiomata **e–i** conidiophore with conidia **j–q** conidia. Scale bars: 30 μm (**c–d, o–q**); 20 μm (**e–n**).

Dematipyriforma nigrospora **(Boonmee, D.F. Bao & K.D. Hyde) D.F. Bao, K.D. Hyde & Z.L. Luo, comb. nov.**
Index Fungorum Number: IF553384

≡ *Rhexoacrodictys nigrospora* Boonmee, D.F. Bao & K.D. Hyde, in Boonmee et al., Fungal Diversity 111: 200 (2021).

Holotype. Thailand, Phetchabun Province, on decaying bark, 25 July 2019, S. Boonmee, LSP03 (MFLU 21–0073).

Descriptions and illustrations. See Boonmee et al. (2021).

Notes. *Rhexoacrodictys nigrospora* was introduced by Boonmee et al. (2021) based on morphological characters and phylogenetic analysis. In our phylogenetic analysis, *R. nigrospora* clustered with two *Dematipyriforma* species (*D. aquilaria and D. muriformis*) in a distinct clade within Pleurotheciaceae (Fig. 1). Therefore, we transfer *Rhexoacrodictys nigrospora* to *Dematipyriforma*, as *Dematipyriforma nigrospora* comb. nov.

Dematipyriforma nigrospora resembles *D. muriformis* in having micronematous or semi-macronematous, mononematous conidiophores and monoblastic, polyblastic, integrated, terminal conidiogenous cells. However, *D. nigrospora* differs from *D. muriformis* in having brown to dark brown conidiophores and globose to subglobose, dark brown to black conidia (Boonmee et al. 2021). Conidiophores of *D. muriformis* are hyaline and conidia are ellipsoidal to obovoid, muriform, and subhyaline to pale olivaceous when young, olive to dark brown at maturity.

Rhexoacrodictys fimicola **(M.B. Ellis & Gunnell) W.A. Baker & Morgan-Jones, in Baker, Partridge & Morgan-Jones, Mycotaxon 82: 103 (2002)**
Fig. 3

Holotype. Maya, Perak, on elephant dung, September 1958, A.H.S, Onions, IMI 76413.

Description. *Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* on the substratum superficial, effuse, hairy or velvety, black. *Mycelium* mostly immersed, composed of branched, septate, smooth, pale brown hyphae. *Conidiophores* (17.5–)20–44.5 (–65.5) × 2.5–4.0 μm ($\bar{x}$ = 32.2 × 3.4 μm, n = 20), macronematous, mononematous, erect, straight or slightly flexuous, thick-walled, smooth, orange-brown or brown, 3–7-septate. *Conidiogenous cells* monoblastic, integrated, terminal. *Conidia* 16.5–24 × 11–15 μm ($\bar{x}$ = 20.3 × 13 μm, n = 30), solitary, dry, acrogenous, broadly oval to subglobose, muriform, transversely and longitudinally septate, with transverse septa typically spanning the whole conidial width, with longitudinal septa typically incomplete, short; dark-blackish brown to black, smooth, narrowly truncate at the base.

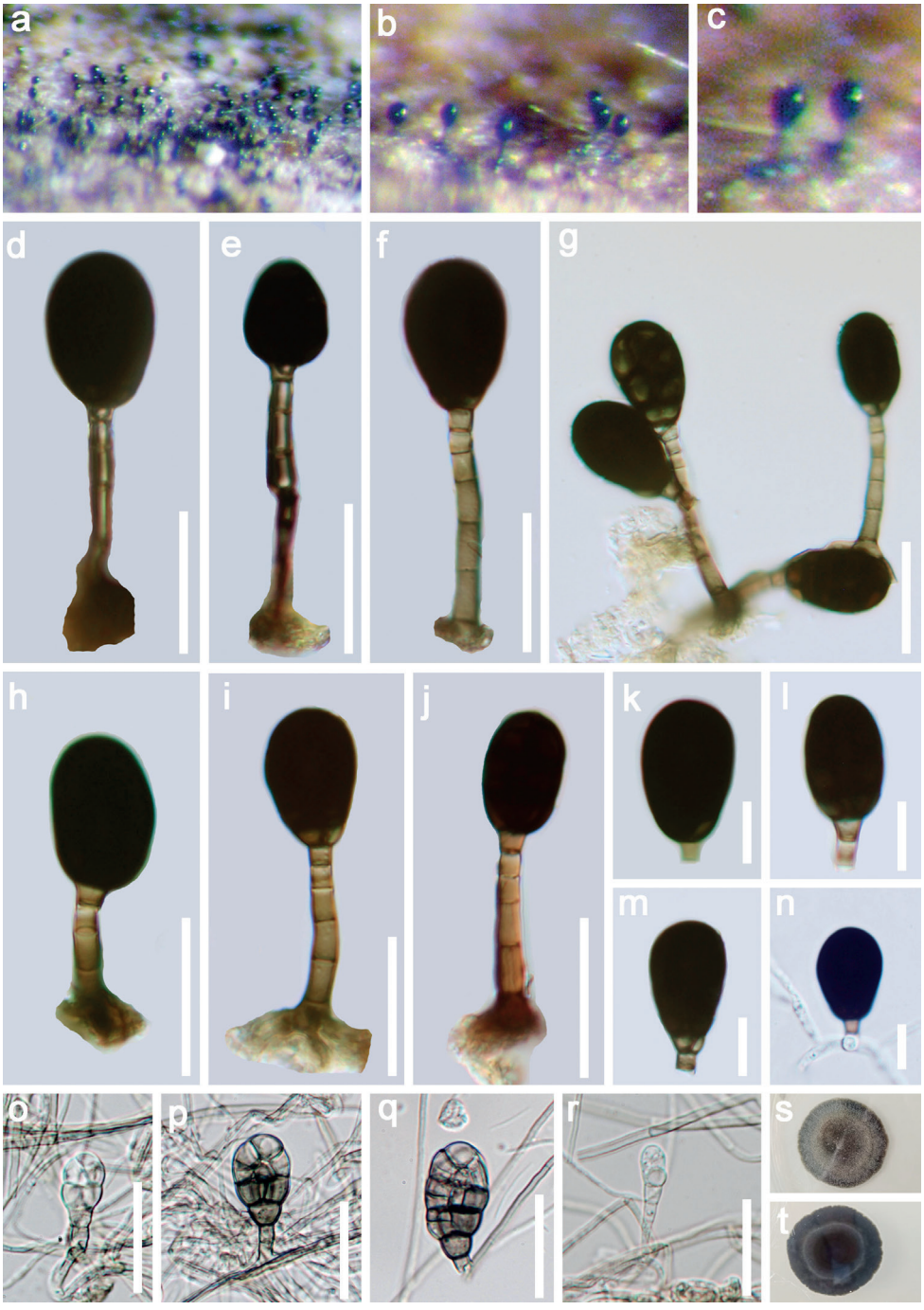

Figure 3. *Rhexoacrodictys fimicola* (MFLU 21–0147, new record) **a–c** colonies on wood **d–j** conidiophores with conidia **k–m** conidi **n** germinating conidium **o–r** re-produced asexual morph of *Rhexoacrodictys fimicola* **s–t** culture on PDA from surface and reverse. Scale bars: 20 μm (**d–j, o–r**); 10 μm (**k–n**).

Cultural characteristics. *Conidia* germinating on PDA within 24 h. Germ tubes produced from the basal cell. *Colonies* on PDA reaching 3 cm diameter in 30 days at 20–25 °C, pale brown, with dense, tight mycelia on the surface, sparse at the margin, reverse dark brown, with smooth margin. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, monoblastic, integrated, hyaline to pale brown, smooth. *Conidia* broad oval to subglobose, muriform, strongly constricted at all the septa, hyaline when young, brown to grayish-brown when aged, smooth-walled.

Material examined. Thailand, Bangkok Province, Bang Kapi District, on decaying wood submerged in a freshwater stream, 3 October 2017, Z.L. Luo, Bsite 4–3–2 (MFLU 21–0147 = KUN-HKAS 122859), living culture, MFLUCC 18–0340.

Notes. In the phylogenetic analysis, our new isolate MFLUCC 18–0340 clustered with three strains of *Rhexoacrodictys fimicola* (HMAS 42882, HMAS 43690 and HMAS 47737) with strong support (100% ML/ 1.00 PP). The nucleotide BLASTn search of ITS showed that our new strain (MFLUCC 18–0340) has 99.7%, 99.3% and 99.1% similarities with strain HMAS 43690, HMAS 47737 and HMAS 42882 of *Rhexoacrodictys fimicola*, respectively. Morphologically, our new collection is similar to *R. fimicola* in having macronematous, mononematous, indeterminate conidiophores, integrated, terminal, monoblastic, pale brown conidiogenous cells and broadly oval to subglobose, transversely and longitudinally septate, smooth, brown to black conidia, with the size of conidia and conidiophores are overlapping (Ellis 1961; Baker et al. 2002). Based on both phylogeny and morphology, we identified our species as *R. fimicola*.

Rhexoacrodictys fimicola was originally introduced by Ellis (1961) as *Acrodictys fimicola*. Baker et al. (2002) transferred *A. fimicola* to *Rhexoacrodictys* based on morphological characteristics. *Rhexoacrodictys fimicola* has been reported on *Bambusa vulgaris* and elephant dung from Africa and Malaysia respectively. Our collection, on the other hand, was collected from freshwater habitats and represents the first time it was reported from Thailand.

Pleurothecium aquaticum **Z.L. Luo, H.Y. Su & K.D. Hyde, in Luo, Hyde, Bhat, Jeewon, Maharachchikumbura, Bao, Li, Su, Yang & Su, Mycol. Prog. 17(5): 526 (2018)**

Fig. 4

Description. *Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *colonies* on substratum, effuse, shining, dark brown to black. *Mycelium* partly immersed, composed of septate, branched, smooth, dark brown hyphae. *Conidiophores* 84–110 × 3–4 µm ($\bar{x}$ = 97 × 3.4 µm, n = 10), macronematous, mononematous, erect, simple, unbranched, straight or slightly flexuous, 5–8-septate, dark brown, pale towards apex, smooth. *Conidiogenous cells* integrated, polyblastic, terminal, hyaline, denticulate, smooth. *Conidia* 18–22 × 4–5 µm ($\bar{x}$ = 20 × 4.5 µm, SD = 4 n = 30), acrogenous, solitary, clavate, mostly curved, rounded at apex, tapering at base, hyaline, 3-septate, with guttulate cells, smooth.

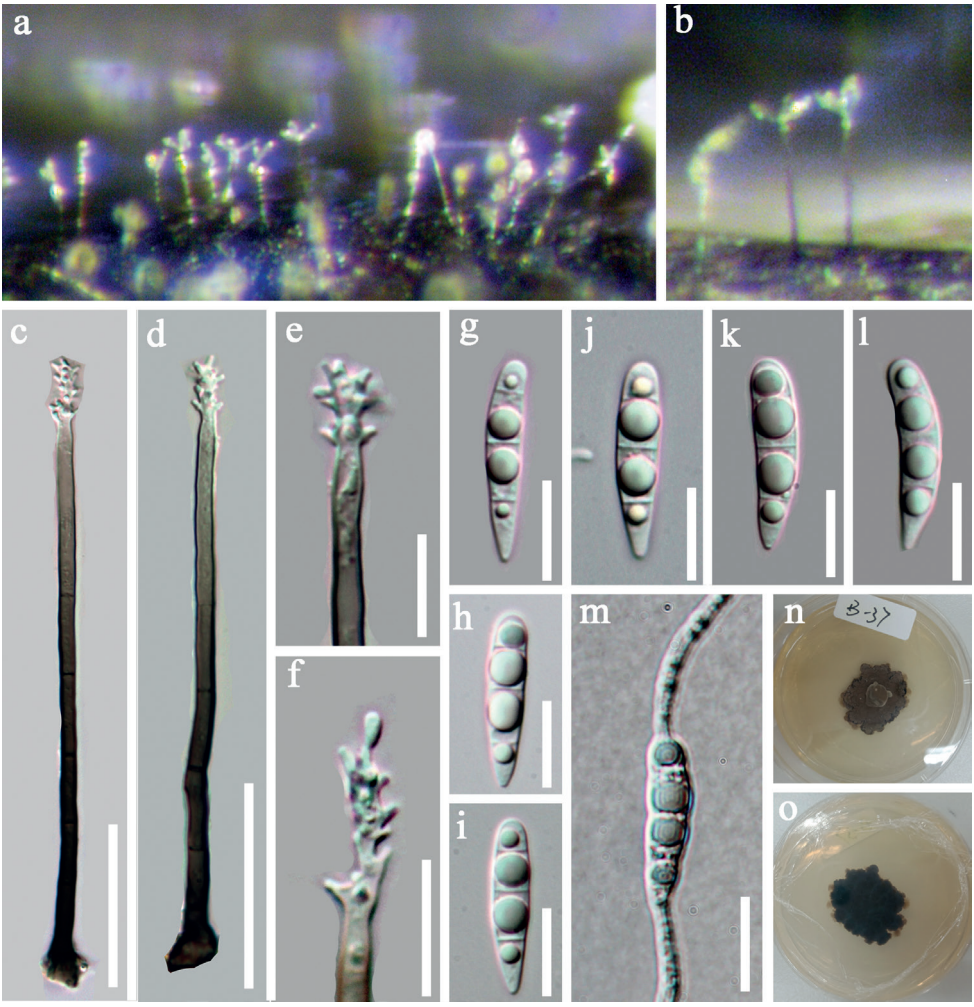

Figure 4. *Pleurothecium aquaticum* (MFLU 21–0148, new record) **a, b** colonies on wood **c, d** conidiophores **e, f** conidiogenous cells **g–i** conidia **m** germinating conidium **n, o** culture on PDA from surface and reverse. Scale bars: 30 µm (**c, d**); 10 µm (**e–m**).

Cultural characteristics. *Conidia* germinating on PDA within 24 h. Germ tubes produced from the basal and apical cells. *Colonies* on PDA reaching 2.3 cm diameter in 30 days at 20–25 °C, with dense mycelia, dry, rigid, rugose, dark brown, reverse dark brown.

Material examined. Thailand, Prachuap Khan, on submerged decaying wood, 15 August 2017, V. Kumar, site1–24–2 (MFLU 21–0148 = KUN-HKAS 122857), living culture, KUNCC 21–0477.

Notes. In the phylogenetic analysis, our new collection KUNCC 21–0477 clustered with the ex-type strain of *Pleurothecium aquaticum* (MFLUCC 17–1331) with high (100% ML/1.00 PP). In addition, the ITS and LSU BLASTn search on NCBI GenBank showed that our new strain is 99.88% and 97.45% similarities to the ex-type

of *P. aquaticum* (MFLUCC 17–1331). The new collection is morphologically similar to *P. aquaticum* in having macronematous, mononematous, septate, brown, pale brown towards the apex conidiophores, integrated, terminal, polyblastic, denticulate conidiogenous cells and hyaline, cylindrical or clavate, rounded at the apex, obtuse and tapering towards base, 3-septate conidia. We therefore identified our new collection as *P. aquaticum*. *Pleurothecium aquaticum* was introduced by Luo et al. (2018a) collected from freshwater habitats in China. Our new collection, on the other hand, was collected from Thailand and is a new record for Thailand.

Discussion

Pleurotheciaceae is a diverse family. The sexual morphs of Pleurotheciaceae are quite similar and difficult to distinguish without molecular data (Réblová et al. 2016; Hyde et al. 2020a). However, the asexual morphs in the family are morphologically diverse. Most genera have mononematous, macrounematous conidiophores (*Anapleurothecium*, *Pleurothecium*, *Pleurotheciella* and *Rhexoacrodictys*) (Réblová et al. 2016; Luo et al. 2018a, 2019; Hyde et al. 2020a), whereas some genera have synnematous conidiophores (*Phaeoisaria* and *Phragmocephala*) (Höhnel 1919; Mason and Hughes 1951; Seifert et al. 2011; Wijayawardene et al. 2012; Su et al. 2015; Réblová et al. 2016; Luo et al. 2018a), and others with micronematous or reduced conidiophores (*Neomonodictys* and *Sterigmatobotrys*). (Hyde et al. 2020b). Conidiogenous cells of *Anapleurothecium*, *Pleurothecium*, *Phaeoisaria* and *Pleurotheciella* are polyblastic and denticulate (Réblová et al. 2012, 2016; Monteiro et al. 2016; Luo et al. 2018a). *Phragmocephala* and *Monotosporella* have monoblastic conidiogenous cells (Mason and Hughes 1951; Hyde and Yanna 2002; Wijayawardene et al. 2012; Su et al. 2015). Conidia of Pleurotheciaceae are diverse in their shape, color and septation. Conidia of *Sterigmatobotrys* are fusiform and in persistent chains (Heuchert et al. 2018); *Helicoascotaiwania* has helicosporous conidia (Dayarathne et al. 2019); *Anapleurothecium*, *Melanotrigonum*, *Pleurothecium*, *Phaeoisaria* and *Pleurotheciella* have clavate, ellipsoidal, obovoidal, fusiform-cylindrical, hyaline or brown, aseptate or transversely septate conidia (Réblová et al. 2012, 2016; Monteiro et al. 2016; Hernandez-Restrepo et al. 2017; Luo et al. 2018a); *Monotosporella*, *Neomonodictys* and *Phragmocephala*have ellipsoidal or subglobose to globose conidia (Mason and Hughes 1951; Hyde and Yanna 2002;Wijayawardene et al. 2012; Su et al. 2015; Hyde et al. 2020b). However, conidia of *Neomonodictys* are muriform (Hyde et al. 2020b), whereas, *Phragmocephala* and *Monotosporella* have transversely septate conidia.

In this study, we introduced a new asexual species, *Dematipyriforma muriformis* based on both morphology and phylogeny. *Dematipyriforma* was introduced by Sun et al. (2017) with a single species *D. aquilaria* which was reported as an endophyte from *Aquilaria crassna* in China. While our new species is a saprobe isolated on submerged wood from freshwater habitats in Thailand. In addition, *Rhexoacrodictys nigrospora* is transferred to *Dematipyriforma* in this study. Currently, three species are accepted in

the genus. Morphologically, the muriform conidia of *Dematipyriforma* are similar to *Neomonodictys*, *Saprodesmium* and *Coleodictyospora*. However, *Dematipyriforma* can be distinguished from *Neomonodictys* by the shape of conidia (ellipsoidal to obovoid vs. subglobose to globose) and conidiophores (semi-micronematous to macronematous vs. micronematous or lacking conidiophores, Hyde et al. 2020b). *Dematipyriforma* differs from *Coleodictyospora* in the conidia lacking a semi-gelatinous sheath (Dong et al. 2021). *Dematipyriforma* is distinct from *Saprodesmium* by the micronematous to semi-macronematous, simple or branched, hyaline, cylindrical, conidiophores, whereas, conidiophores of *Saprodesmium* are micronematous, unbranched, consisted of 1–4 subglobose smooth, hyaline cells (Dong et al. 2021).

Rhexoacrodictys comprises six species of which four species (*R. erecta*, *R. fimicola*, *R. martini* and *R. queenslandica*) have sequence data available in the GenBank. Among them, *R. martini* and *R. queenslandica* were transferred to *Distoseptispora* and *Junewangia* based on phylogenetic analysis (Xia et al. 2017). However, sequence data of *R. martini* are doubted by several studies (Sun et al. 2020; Shen et al. 2021), as its morphology does not fit with the characters of *Distoseptispora*. *Rhexoacrodictys erecta* and *R. fimicola* clustered within Pleurotheciaceae (Luo et al. 2019; Dong et al. 2021). The placement of *Rhexoacrodictys* was questionable since it was established. Baker et al. (2002) established the genus; however, they did not mention the placement of the genus. Xia et al. (2017) firstly provided sequence data for *Rhexoacrodictys erecta* (Type species of *Rhexoacrodictys*) and *R. fimicola* based on their fresh collections, their phylogenetic analysis showed that *R.* erecta and *R. fimicola* clustered within Savoryellaceae. However, they did not include the related orders (Conioscyphales, Fuscosporellales and Pleurotheciales) in Savoryellomycetidae. Luo et al. (2019) found that *R.* erecta and *R. fimicola* grouped in Pleurotheciaceae. Recently, Dong et al. (2021) obtained the same result as Luo et al. (2019). However, Boonmee et al. (2021) and Wijayawardene et al. (2022) placed *Rhexoacrodictys* in Savoryellaceae (Savoryellales). Our result is consistent with Luo et al. (2019) and Dong et al. (2021), the two species clustered within Pleurotheciaceae (Fig. 1). On the other hand, the morphology of *Rhexoacrodictys* is similar to *Dematipyriforma*, *Neomonodictys* and *Saprodesmium*, in having muriform conidia, micronematous conidiophores and holoblastic, monoblastic conidiogenous cells. Therefore, we formally accepted *Rhexoacrodictys* in Pleurotheciaceae (Pleurotheciales) based on morphological characters and phylogenetic analysis.

In our phylogenetic analysis, *Rhexoacrodictys erecta* and *R. fimicola* clustered with *Monotosporella setosa* which is the type species of *Monotosporella*. Morphologically, *R. erecta* and *R. fimicola* fit well within the genus concept of *Monotosporella* in having macronematous, mononematous, brown, septate conidiophores, monoblastic, percurrent conidiogenous cells and acrogenous, brown septate conidia (Hughes 1958; Baker et al. 2002; Hyde and Yanna 2002). However, the strain of *Monotosporella setosa* (HKUCC 3713) lacks a morphological description. Therefore, further study is necessary to clarify the relationship between *Rhexoacrodictys* and *Monotosporella*, whether they should be combined into one genus or not. In addition, our phylogenetic analysis showed that three strains of *R. erecta* clustered with *Monotosporella setosa*. However,

M. erecta differs from *M. setosa* in having transverse and longitudinal septation, while, conidia of *M. setosa* only have transverse septa. Only LSU sequence data is available for *M. setosa*, which is not significant to distinguish in the phylogenetic tree, but morphologically they are quite distinct. Hence, we maintain them as two distinct species, however, further morphological and phylogenetic analysis is required to clarify the relationship between these two species.

In our phylogenetic analysis, *Pleurothecium obovoideum* was placed distant from *Pleurothecium* and close to *Neomonodictys muriformis* and *Coleodictyospora muriformis* which is consistent with recent studies (Luo et al. 2018a, 2019; Hyde et al. 2020b). *Pleurothecium obovoideum* was introduced by Arzanlou et al. (2007) based on morphological characters. However, their analysis showed that *P. obovoideum* clustered with *Ascotaiwania hughesii* and with more sequence data now available for *Pleurothecium* species, *P. obovoideum* is shown phylogenetically distinct from *Pleurothecium*. Morphologically, *P. obovoideum* is similar to *Pleurothecium* in having distinct brown conidiophores, polyblastic, denticulate conidiogenous cells and pale brown, ellipsoidal to obovate conidia. However, conidia of *P. obovoideum* are aseptate and solitary or in short chains whereas the conidia of *Pleurothecium* are solitary and unicellular or septate. Thus, the placement of *P. obovoideum* needs revision in the future with more evidence.

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Note on the genus *Nemania* (Xylariaceae) – first records and a new species of the genus from Iran

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Abstract

In a survey of xylarialean fungi in northern Iran, some specimens attributable to the genus *Nemania* were collected, cultured and sequenced. Morphological evidence and phylogenetic analyses of a combined ITS, LSU, *RPB2* and *TUB2* gene dataset confirmed the presence of *Nemania diffusa* and *N. serpens* in Iran for the first time. Furthermore, the new species *N. hyrcana*, which shows similarities to *N. subaenea* and its putative synonym *N. plumbea*, but significantly differs from the latter in its DNA sequences, was encountered. All species are illustrated, described and discussed. In the phylogenetic analyses, for the first time, the overlooked ex-type ITS sequences of the neotype of the generic type, *N. serpens* and that of the holotype of *N. prava*, were added to a multi-gene matrix of *Nemania*. This revealed that the two accessions of *N. serpens* (HAST 235 and CBS 679.86), for which multigene data are available in GenBank, are misidentified, while the Iranian accession of *N. serpens* has an almost identical ITS sequence to the neotype, confirming its morphological species identification. The two previously accepted species of *Euepixylon*, *E. udum* and *E. sphaeriostomum*, are embedded within *Nemania* and are revealed as close relatives of *N. serpens*, supporting the inclusion of *Euepixylon* in *Nemania*.

Keywords

Ascomycota, molecular phylogenetics, *Nemania serpens*, one new species, Sordariomycetes, taxonomy, Xylariales

Introduction

The genus *Nemania* S. F. Gray was established by Gray (1821) and has always been considered to belong to the family Xylariaceae Tul. & C. Tul., even though its species were placed in *Hypoxylon* for some time, according to the generic concepts established by Miller (1961) and other authors. The reason for this was that *Nemania* species superficially resemble those of *Hypoxylon* in having effused-pulvinate stromata on dead wood. Gray (1821) had used a somewhat ill-defined concept for this genus, which was resolved by Donk (1964) who selected *Sphaeria serpens* as the type of the genus. Later, Pouzar (1985a, 1985b) emended *Nemania* and separated the genus from *Hypoxylon* according to morphological characters and Petrini and Rogers (1986) confirmed this by studies on the cultures, pointing out the geniculosporium-like anamorph of *Nemania* species (vs. the nodulisporium-like anamorphs that are typical for *Hypoxylon* s. str.). The anamorph genus *Geniculosporium* had even eventually been erected, based on the conidial state of "*Hypoxylon*" (i.e. *Nemania*) *serpens* by Chesters and Greenhalgh (1964). This holomorphic concept has meanwhile been supported by molecular phylogenetic studies (e.g. Hsieh et al. 2010) that clearly revealed close affinities of *Nemania* to *Xylaria* and other genera with geniculosporium-like anamorphs. The most important monographs on the genus by Granmo et al. (1999) and Ju and Rogers (2002), however, still relied on morphological characters and many of the 37 taxa that were recognised by these authors have not yet been characterised by DNA sequence data.

Nemania is characterised by carbonaceous, superficial, multiperitheciate, effused-pulvinate stromata with papillate ostioles and variable presence of soft, whitish, brownish, grey or yellow internal tissue. Stromata do not release pigments in 10% potassium hydroxide (KOH). Asci are cylindrical, short or long stipitate, persistent, with an apical apparatus of various shapes, amyloid (like *N. diffusa*) or inamyloid (like *N. serpens*) in Melzer's iodine reagent. Ascospores are pale brown to dark brown or blackish-brown, ellipsoidal, cylindrical or fusoid, inequilateral, slightly inequilateral or nearly equilateral, with acute, narrowly rounded or broadly rounded ends, with a straight, conspicuous or inconspicuous germ slit of spore length to much less than spore-length. It has geniculosporium-like anamorphs (Ju and Rogers 2002; Fournier et al. 2018).

During our survey of Xylariales specimens in northern Iran, three *Nemania* taxa were recorded. Species were identified, based on morphological and molecular phylogenetic analyses. As a result, a new species and records of two further species are reported from Iran, for which detailed morphological descriptions, illustrations and phylogenetic information are here provided.

Materials and methods

Morphological observation

The fungal specimens were collected in northern Iran (Guilan, Mazandaran and Golestan Provinces). For light microscopy, fresh collections, single ascospore isolations

and cultures were examined for macro- and micromorphological characteristics, according to Ju and Rogers (2002) and Pourmoghaddam et al. (2018). Dried specimens were deposited in the University of Guilan Mycological Herbarium (**GUM**). Living cultures were deposited in the culture collection MUCL (Louvain la-Neuve, Belgium) and in the Iranian Fungal Culture Collection, Iranian Research Institute of Plant Protection, Tehran, Iran (**IRAN**).

DNA extraction, PCR and sequencing

DNA extraction of fresh cultures and amplification of the ITS (nuc rDNA internal transcribed spacer region containing ITS1-5.8S-ITS2), LSU (5' 1200 bp of the large subunit nuc 28S rDNA), *RPB2* (partial second largest subunit of the DNA-directed RNA polymerase II) and *TUB2* (partial β-tubulin) loci were carried out as described by Wendt et al. (2018).

Phylogenetic analyses

Published sequences of a single accession for each *Nemania* species served as basis for the sequence matrix. Information on all used strains, their corresponding sequences and GenBank accession numbers can be found in Table 1. In addition to the sequences retrieved from GenBank, ITS sequences of the holotype of *N. prava* and of the neotype of *N. serpens* were manually transcribed from the ITS alignment published as colour figure appendix 3 in Granmo et al. (1999), because these sequences have not been deposited in a public sequence repository. In addition, to have the ITS sequences of Granmo et al. (1999) available for further studies, the transcribed ex-type sequences were also submitted to GenBank (ex-neotype sequence of *N. colliculosa*: OP289676, ex-holotype sequence of *N. prava*: OP289674, ex-neotype sequence of *N. serpens*: OP289675). To reveal the phylogenetic position of the Iranian *Nemania* accessions, the newly-generated sequences were aligned with the GenBank sequences. All alignments were produced with the server versions of MAFFT v. 7.490 (www.ebi.ac.uk/Tools/mafft or http://mafft.cbrc.jp/alignment/server/; Katoh et al. 2019) and checked and refined using BioEdit v. 7.0.4.1 (Hall 1999).

For the phylogenetic analyses, 90 accessions of 86 species of Xylariaceae and four outgroup taxa from Graphostromataceae (*Biscogniauxia nummularia*, *Graphostroma platystomum*) and Hypoxylaceae (*Hypoxylon fragiforme*, *H. howeanum*) were included. We also included the newly-sequenced LSU, *RPB2* and *TUB2* loci of the Iranian collections of *Kretzschmaria hedjaroudei* (MUCL 57706) and *K. deusta* (MUCL 57705); for details on those accessions, see Pourmoghaddam et al. (2018). The sequence matrices of ITS, LSU, *RPB2* and *TUB2* were combined; after exclusion of ambiguously aligned and gappy regions, the resulting combined data matrix contained 4616 alignment positions from four loci (543 from ITS, 1275 from LSU, 1191 from *RPB2* and 1607 from *TUB2*).

Maximum Likelihood (ML) analyses were performed with RAxML (Stamatakis 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012) using the

Table 1. Isolation and accession numbers of sequences used in the phylogenetic analyses. Isolates/sequences in bold were isolated/sequenced in present study. N/A: not available.

Species	Strain number	Origin	Status	GenBank accession numbers				Reference
				ITS	LSU	*RPB2*	*TUB2*	
Amphirosellinia fushanensis	HAST 91111209	Taiwan	HT	GU339496	N/A	GQ848339	GQ495950	Hsieh et al. (2010)
Amphirosellinia nigrospora	HAST 91092308	Taiwan	HT	GU322457	N/A	GQ848340	GQ495951	Hsieh et al. (2010)
Anthostomelloides krabiensis	MFLUCC 15-0678	Thailand	HT	KX305927	KX305928	KX305929	N/A	Tibpromma et al. (2017)
Astrocystis concavispora	MFLUCC 14.0174	Italy		KP297404	KP340545	KP340532	KP406615	Daranagama et al. (2015)
Biscogniauxia nummularia	MUCL 51395	France	ET	KY610382	KY610427	KY624236	KX271241	Wendt et al. (2018)
Clypeosphaeria mamillana	CBS 140735	France	ET	KT949897	KT949897	MF489001	N/A	Jaklitsch et al. (2016), Voglmayr et al. (2018)
Collodiscula bambusae	GZU H0102	China		KP054279	KP054280	KP276675	KP276674	Li et al. (2015)
Collodiscula fangjingshanensis	GZU H0109	China	HT	KR002590	KR002591	KR002592	KR002589	Li et al. (2015)
Collodiscula japonica	CBS 124266	China		JF440974	JF440974	KY624273	KY624316	Jaklitsch and Voglmayr (2012), Wendt et al. (2018)
Coniolarelia limoniispora	MUCL 29409	Japan		MN984615	MN984624	MN987235	MN987240	Wittstein et al. (2020)
Dematophora bunodes	CBS 123597	Peru		MN984619	MN984625	N/A	MN987245	Wittstein et al. (2020)
Dematophora buxi	JDR 99	France		GU300070	N/A	GQ844780	GQ470228	Hsieh et al. (2010)
Dematophora necatrix	CBS 349.36	Argentina		AY909001	KF719204	KY624275	KY624310	Peláez et al. (2008), Wendt et al. (2018)
Dematophora pepo	CBS 123592	Peru		MN984620	N/A	N/A	MN987246	Wittstein et al. (2020)
Entoleuca mammata	JDR 100	France		GU300072	N/A	GQ844782	GQ470230	Hsieh et al. (2010)
Graphostroma platystomum	CBS 270.87	France	HT	JX658535	DQ836906	KY624296	HG934108	Stadler et al. (2014), Zhang et al. (2006), Wendt et al. (2018), Koukol et al. (2015)
Hypocreodendron sanguineum	JDR 169	Mexico		GU322433	N/A	GQ844819	GQ487710	Hsieh et al. (2010)
Hypoxylon fragiforme	MUCL 51264	Germany	ET	KC477229	KM186295	KM186296	KX271282	Stadler et al. (2013), Daranagama et al. (2015), Wendt et al. (2018)
Hypoxylon howeanum	MUCL 47599	Germany		AM749928	KY610448	KY624258	KC977277	Bitzer et al. (2008), Kuhnert et al. (2014), Wendt et al. (2018)
Kretzschmaria clavus	YMJ 114	French Guiana		EF026126	N/A	GQ844789	EF025611	Hsieh et al. (2010)
Kretzschmaria deusta	CBS 163.93	Germany		KC477237	KY610458	KY624227	KX271251	Stadler et al. (2013), Wendt et al. (2018)
Kretzschmaria deusta	CBS 826.72	Belgium		KU683767	KU683767	KU684309	KU684190	U'Ren et al. (2016)
Kretzschmaria deusta	**MUCL 57705**	**Iran**		MH084755	**OP359327**	**OP359596**	**OP359601**	Pourmoghaddam et al. (2018), **This study**
Kretzschmaria hedjaroudei	**MUCL 57706**	**Iran**	**HT**	MH084757	**OP359328**	**OP359597**	**OP359602**	Pourmoghaddam et al. (2018), **This study**
Kretzschmaria guyanensis	HAST 89062903	Taiwan		GU300079	N/A	GQ844792	GQ478214	Hsieh et al. (2010)
Kretzschmaria lucidula	YMJ 112	French Guiana		EF026125	N/A	GQ844790	EF025610	Hsieh et al. (2010)
Kretzschmaria megalospora	YMJ 229	Malaysia		EF026124	N/A	GQ844791	EF025609	Hsieh et al. (2010)

Species	Strain number	Origin	Status	GenBank accession numbers				Reference
				ITS	LSU	*RPB2*	*TUB2*	
Kretzschmaria neocaledonica	HAST 94031003	Taiwan		GU300078	N/A	GQ844788	GQ478213	Hsieh et al. (2010)
Kretzschmaria pavimentosa	JDR 109	Taiwan		GU300077	N/A	GQ844787	GQ478212	Hsieh et al. (2010)
Kretzschmaria sandvicensis	JDR 113	USA		GU300076	N/A	GQ844786	GQ478211	Hsieh et al. (2010)
Linosporopsis ischnotheca	CBS 145761	Switzerland	ET	MN818952	MN818952	MN820708	MN820715	Voglmayr and Beenken (2020)
Linosporopsis ochracea	CBS 145999	Germany	ET	MN818958	MN818958	MN820714	MN820721	Voglmayr and Beenken (2020)
Nemania abortiva	BISH 467	USA	HT	GU292816	N/A	GQ844768	GQ470219	Hsieh et al. (2010)
Nemania aquilariae	KUMCC 20-0268	China	HT	MW729422	MW729420	MW717891	MW881142	Tibpromma et al. (2021)
Nemania beaumontii	HAST 405	Martinique		GU292819	N/A	GQ844772	GQ470222	Hsieh et al. (2010)
Nemania bipapillata	HAST 90080610	Taiwan		GU292818	N/A	GQ844771	GQ470221	Hsieh et al. (2010)
Nemania camelliae	GMB0068	China	HT	MW851889	MW851872	MW836055	MW836029	Pi et al. (2021)
Nemania caries	GMB0070	China		MW851874	MW851857	MW836071	MW836036	Pi et al. (2021)
Nemania changningensis	GMB0056	China	HT	MW851875	MW851858	MW836061	MW836027	Pi et al. (2021)
Nemania chestersii	JF 04024	France		N/A	DQ840072	DQ631949	DQ840089	Tang et al. (2007; 2009)
Nemania cyclobalanopsina	GMB0062	China	HT	MW851883	MW851866	MW836057	MW836025	Pi et al. (2021)
Nemania delonicis	MFLU 19-2124	Thailand	HT	MW240613	MW240542	MW342617	MW775574	Samarakoon et al. (2022)
Nemania diffusa	HAST 91020401	Taiwan		GU292817	N/A	GQ844769	GQ470220	Hsieh et al. (2010)
Nemania ethancrensonii	CBS 148337	USA	HT	ON869311	ON869311	ON808489	ON808533	Voglmayr et al. (2022)
Nemania feicuiensis	GMB0059	China	HT	MW851880	MW851863	MW836063	MW836023	Pi et al. (2021)
Nemania fusoidispora	GZUH0098	China		MW851881	MW851864	MW836070	MW836037	Ariyawansa et al. (2015)
Nemania hyrcana	**MUCL 57704**	**Iran**	**HT**	**OP359332**	**OP359329**	**OP359598**	**OP359603**	**This study**
Nemania hyrcana	**MUCL 57703**	**Iran**		**OP359333**	**OP359330**	**OP359599**	**OP359604**	**This study**
Nemania illita	YMJ 236	USA		EF026122	N/A	GQ844770	EF025608	Hsieh et al. (2010)
Nemania lishuicola	GMB0065	China	HT	MW851886	MW851869	MW836065	MW836033	Pi et al. (2021)
Nemania longipedicellata	MFLU 18-0819	Thailand	HT	MW240612	MW240541	MW342616	MW775573	Samarakoon et al. (2022)
Nemania macrocarpa	WSP 265	USA	HT	GU292823	N/A	GQ844776	GQ470226	Hsieh et al. (2010)
Nemania maritima	HAST 89120401	Taiwan	ET	GU292822	N/A	GQ844775	GQ470225	Hsieh et al. (2010)
Nemania paraphysata	MFLU 19-2121	Thailand	HT	MW240609	MW240538	MW342613	N/A	Samarakoon et al. (2022)
Nemania plumbea	JF TH-04-01	Thailand	HT	DQ641634	DQ840071	DQ631952	DQ840084	Tang et al. (2007; 2009)
Nemania prava	CBS 679.86	Switzerland	PT[2]	KU683765	KU683765	KU684284	KU684188	U'Ren et al. (2016)
Nemania prava	TROM 104	Norway	HT	OP289674[3]	N/A	N/A	N/A	Granmo et al. (1999)
Nemania primolutea	HAST 91102001	Taiwan	HT	EF026121	N/A	GQ844767	EF025607	Hsieh et al. (2010)
Nemania rubi	GMB0064	China	HT	MW851885	MW851868	MW836059	MW836021	Pi et al. (2021)
Nemania serpens	TROM 174	Norway	NT	OP289675[3]	N/A	N/A	N/A	Granmo et al. (1999)
Nemania serpens	**MUCL 57702**	**Iran**		**OP359334**	**OP359331**	**OP359600**	**OP359605**	**This study**
Nemania serpens	HAST 235	Canada		GU292820	N/A	GQ844773	GQ470223	Hsieh et al. (2010)

Species	Strain number	Origin	Status	GenBank accession numbers				Reference
				ITS	LSU	*RPB2*	*TUB2*	
Nemania sphaeriostoma	JDR 261	USA		GU292821	N/A	GQ844774	GQ470224	Hsieh et al. (2010)
Nemania thailandensis	MFLU 19-2117	Thailand	HT	MW240611	MW240540	MW342615	MW775572	Samarakoon et al. (2022)
Nemania uda	CBS 148422	Austria	HT	ON869312	ON869312	ON808488	ON808532	Voglmayr et al. (2022)
Nemania yunnanensis	KUMCC 20-0267	China	HT	MW729423	MW729421	MW717892	MW881141	Tibpromma et al. (2021)
Podosordaria mexicana	WSP 176	Mexico		GU324762	N/A	GQ853039	GQ844840	Hsieh et al. (2010)
Podosordaria muli	WSP 167	Mexico	HT	GU324761	N/A	GQ853038	GQ844839	Hsieh et al. (2010)
Poronia pileiformis	WSP 88113001	Taiwan	ET	GU324760	N/A	GQ853037	GQ502720	Hsieh et al. (2010)
Poronia punctata	CBS 656.78	Australia		KT281904	KY610496	KY624278	KX271281	Senanayake et al. (2015), Wendt et al. (2018)
Rosellinia aquila	MUCL 51703	France		KY610392	KY610460	KY624285	KX271253	Wendt et al. (2018)
Rosellinia cf. *akulovii*	MUCL 57710	Iran		OL635184	OL635175	OL657210	OL657219	Pourmoghaddam et al. (2022)
Rosellinia cf. *akulovii*	MUCL 57711	Iran		OL635185	OL635176	OL657211	OL657220	Pourmoghaddam et al. (2022)
Rosellinia corticium	MUCL 51693	France		KY610393	KY610461	KY624229	KX271254	Wendt et al. (2018)
Rosellinia corticium	STMA 13324	Germany		MN984621	MN984627	MN987237	MN987241	Wittstein et al. (2020)
Rosellinia corticium	MUCL 57714	Iran		OL635180	OL635171	OL657206	OL657215	Pourmoghaddam et al. (2022)
Rosellinia nectrioides	CBS 449.89	Sweden		MN984622	MN984628	MN987239	N/A	Wittstein et al. (2020)
Sarcoxylon compunctum	CBS 359.61	South Africa		KT281903	KY610462	KY624230	KX271255	Senanayake et al. (2015), Wendt et al. (2018)
Stilbohypoxylon elaeicola	Y.M.J 173	French Guiana		EF026148	N/A	GQ844826	EF025616	Hsieh et al. (2010)
Stilbohypoxylon quisquiliarum	Y.M.J 172	French Guiana		EF026119	N/A	GQ853020	EF025605	Hsieh et al. (2010)
Xylaria acuminatilongissima	HAST 95060506	Taiwan	HT	EU178738	N/A	GQ853028	GQ502711	Hsieh et al. (2010)
Xylaria adscendens	J.D.R 865	Thailand		GU322432	N/A	GQ844818	GQ487709	Hsieh et al. (2010)
Xylaria arbuscula	CBS 126415	Germany		KY610394	KY610463	KY624287	KX271257	Fournier et al. (2011), Wendt et al. (2018)
Xylaria bambusicola	WSP 205	Taiwan	HT	EF026123	N/A	GQ844802	AY951762	Hsieh et al. (2010)
Xylaria brunneovinosa	HAST 720	Martinique	HT	EU179862	N/A	GQ853023	GQ502706	Hsieh et al. (2010)
Xylaria curta	HAST 494	Martinique		GU322444	N/A	GQ844831	GQ495937	Hsieh et al. (2010)
Xylaria discolor	HAST 131023	USA	ET	JQ087405	N/A	JQ087411	JQ087414	Hsieh et al. (2010)
Xylaria hypoxylon	CBS 122620	Sweden	ET	KY610407	KY610495	KY624231	KX271279	Sir et al. (2016), Wendt et al. (2018)
Xylaria multiplex	HAST 580	Martinique		GU300098	N/A	GQ844814	GQ487705	Hsieh et al. (2010)
Xylaria polymorpha	MUCL 49884	France		KY610408	KY610464	KY624288	KX271280	Wendt et al. (2018)

[1] ET ex-epitype, HT ex-holotype, NT ex-neotype, PT ex-paratype.

[2] ex-paratype culture of *Hypoxylon atropurpureum* var. *brevistipitatum* (= *Nemania prava*); deposited in GenBank as *Nemania serpens*.

[3] ITS sequence originally not deposited in a sequence repository, but published as colour figure (Appendix 3) in Granmo et al. (1999); manually transcribed sequence deposited in GenBank in this study.

ML + rapid bootstrap setting and the GTRGAMMA substitution model with 1000 bootstrap replicates.

Maximum Parsimony (MP) analyses were performed with PAUP v. 4.0a169 (Swofford 2002). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data; the COLLAPSE command was set to MINBRLEN. MP analysis of the combined multilocus matrix was done using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, steepest descent option not in effect). Bootstrap analyses with 1000 replicates were performed in the same way, but using 10 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate. Bootstrap values ≤ 70% are considered low, between 70 and 90% intermediate and ≥ 90% high.

Results

Molecular phylogeny

Of the 4616 characters of the combined matrix, 1884 were parsimony informative (284 in ITS, 142 in LSU, 613 in *RPB2* and 845 in *TUB2*). The phylogram of the best ML tree (lnL = – 88,062.8606) obtained by RAxML is shown as Fig. 1. The MP analysis revealed two trees of length 20,490 (not shown) that had a similar topology to the ML tree. The phylogenies reveal a monophyletic clade of *Nemania* (including *Euepixylon*), like in previous studies (Wendt et al. 2018; Pi et al. 2021; Samarakoon et al. 2022; Voglmayr et al. 2022). Within Xylariaceae, the *Nemania* clade is most closely related to the genera *Coniolariella*, *Dematophora*, *Entoleuca* and *Rosellinia*.

The genus *Nemania* (including *Euepixylon*) receives high ML (99%), but low MP (55%) support and contains three highly-supported subclades (N1-N3 in Fig. 1). The ML and MP analyses reveal the same topologies within *Nemania*, except for minor differences (not shown). As these differences are not relevant within the context of our study, they are not further considered here.

The new *Nemania* species clustered together with *N. plumbea* (JF TH-04-01) with maximum ML and MP BS support, which is a sister group to *N. delonicis*, also with maximum ML and MP BS support (Fig. 1). The ITS sequence of the Iranian collection of *N. serpens* is almost identical to the ex-neotype sequence from *N. serpens* (TROM 174) from Granmo et al. (1999) and they clustered together with maximum ML BS support. The *N. serpens* clade has a sister group relationship with *N. changningensis* with maximum ML BS support. However, another isolate deposited as *N. serpens* (HAST 235) is not contained within the *N. serpens* clade, but remotely placed as sister species to *N. chestersii*, indicating a misidentification. The ex-holotype ITS sequence of *N. prava* (TROM 104) from Granmo et al. (1999) was almost identical to *N. serpens* (CBS 679.86) and both cluster together with maximum BS support; the latter was re-identified and given in the phylogenetic tree (Fig. 1) as *N. prava* (see discussion for details). Remarkably, the

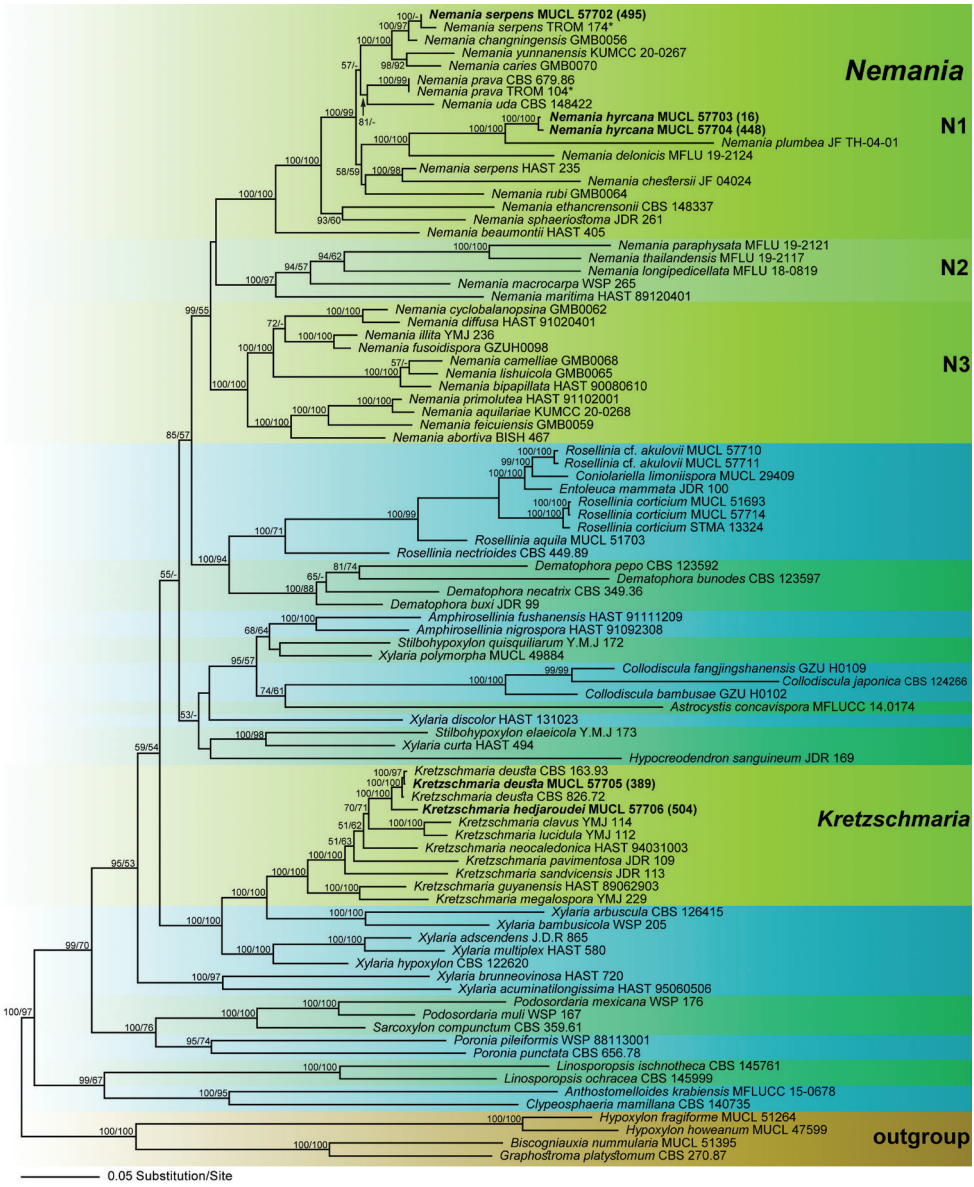

Figure 1. Phylogram of the best ML trees (lnL = –88,062.8606) revealed by RAxML from an analysis of the combined ITS-LSU-*RPB2*–*TUB2* matrix of selected Xylariaceae. Strains in bold were sequenced in the current study; for strains marked with an asterisk (*), ITS sequences were transcribed from Appendix 3 of Granmo et al. (1999). ML and MP bootstrap support above 50% are given at the first and second positions, respectively, above or below the branches.

two previously accepted species of *Euepixylon*, the European *E. udum* and the North American *E. sphaeriostomum*, are placed within *Nemania* subclade N1, but are not revealed as closest relatives (Fig. 1), supporting their classification within *Nemania*.

Taxonomy

***Nemania hyrcana* Pourmoghaddam, Voglmayr & Khodaparast, sp. nov.**
MycoBank No: 845436
Figs 2, 3

Holotype. Iran, Guilan Province, Astara County, Darband Forest, 38°21'26"N, 48°50'19"E, 17 m elev., on fallen branch of *Parrotia persica*, 7 October 2017, leg. M.J. Pourmoghaddam (GUM 1628; living culture MUCL 57704).

Etymology. The epithet is derived from "Hyrcania", an ancient biogeographical region, located in the south of the Caspian Sea where the specimens were collected.

Diagnosis. differs from *Nemania subaenea* by its smaller ascospores [12–16 × 4.5–6 vs. 14–17.5 × 6–7.5 µm].

Teleomorph. Stromata superficial, effused-pulvinate, up to 2.5 cm long, 0.2–1.4 cm wide, sessile, attachment to substrate with narrow connective; surface brown, dark brown, dark grey with a slightly shiny metallic tone, with conspicuous perithecial mounds; carbonaceous tissue immediately beneath the surface and between the perithecia; tissue beneath the perithecial layer conspicuous. Perithecia obovoid to spherical, 0.5–0.7 mm high × 0.4–0.6 mm wide; ostioles papillate to coarsely papillate. Asci cylindrical, with amyloid, urn-shaped apical apparatus, 3.5–4 µm high × 2.5–3 µm wide, stipe up to 130 µm long, spore-bearing part 60–85 × 8–12 µm. Ascospores smooth, unicellular, pale brown to brown, ellipsoid, inequilateral, with narrowly rounded ends, 12–16 × 4.5–6 µm, with straight germ slit much less than spore-length on dorsal side; perispore indehiscent in 10% KOH.

Cultures and anamorph. Colonies on OA covering a 9 cm Petri dish in 2 wk, at first white, becoming buff (45), felty, azonate; finally, attaining cream to grey after 50 days. Anamorph geniculosporium-like. Conidiophores variables in length, hyaline to light brown. Conidiogenous cells up to 50 × 2.5–3.5 µm, hyaline to light brown. Conidia hyaline, ellipsoid with truncate base, 3.5–6 × 2.5–3.5 µm (Fig. 3).

Other specimen examined. Iran, Golestan Province, Aliabad-e-Katul County, Kaboudwall Forest, 36°52'25"N, 54°53'14"E, 1076 m elev., on dead branches (host unknown), 10 November 2017, leg. M.J. Pourmoghaddam (GUM 1627; living culture MUCL 57703, IRAN 3734C).

Notes. This species resembles *Nemania subaenea* (Fig. 4), which was erected based on a single specimen from Guyana by Ju and Rogers (2002). Later, Fournier et al. (2018) reported it from Martinique and also mentioned *N. plumbea*, another single-specimen-based species from Thailand (Tang et al. 2007), which differs from *N. subaenea* only in the stromatal surface colour and in having slightly smaller ascospores (Tang et al. 2007). However, neither Ju and Rogers (2002) nor Fournier et al. (2018), who proposed that *N. plumbea* should be regarded as a synonym of *N. subaenea*, studied the cultures and anamorph of the neotropical species. The type of *N. plumbea*, on the other hand, was cultured and DNA sequences are available for comparison with the Iranian species. A comparison of these sequence data revealed significant differences

Figure 2. *Nemania hyrcana* (Holotype GUM 1628) **A, B** close-up view of stromatal surface **C** close-up view of stromatal surface showing ostioles **D, E** stroma in horizontal section showing perithecia **F** mature ascus in water with long stipe **G** immature ascus in water **H** mature ascus in water **I** mature ascus in Melzer's reagent **J** immature and mature ascospores in water **K–M** ascospores in water showing straight germ slit much less than spore-length. Scale bars: 2 mm (**A**); 0.8 mm (**B**); 0.5 mm (**C, E**); 0.4 mm (**D**); 20 μm (**F–I**); 10 μm (**J–M**).

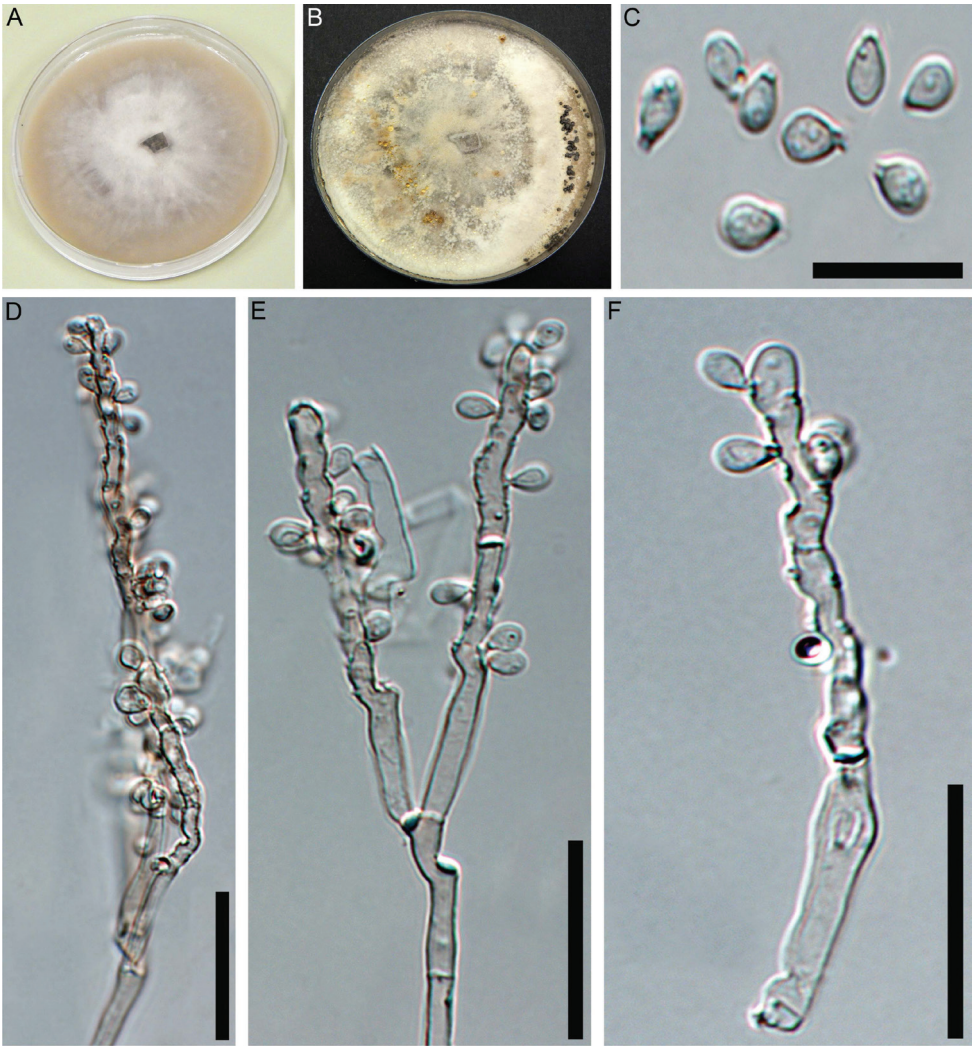

Figure 3. Culture and anamorphic structures of *Nemania hyrcana* (MUCL 57704) on OA **A, B** surface of colony after (**A**) 7 and (**B**) 50 days of incubation **C** conidia **D–F** general view of anamorph structure, conidiophores, conidiogenous cells and mature conidia of *N. hyrcana*. Scale bars: 10 µm (**C**); 20 µm (**D–F**).

between the two Iranian strains of *N. hyrcana* (MUCL 57703/ MUCL 57704) and the ex-type strain of *N. plumbea* (29/31 bp differences of 494 nucleotide characters in the ITS: 19/20 substitutions, 10/11 indels; 2 bp differences of 764 nucleotide characters in the LSU: 2 substitutions; 27 bp differences of 884 nucleotide characters in the *RPB2*: 28/27 substitutions; and 319/321 bp differences of 1422 nucleotide characters in the *TUB2*: 279/282 substitutions, 40/39 indels). This supports the erection of a new species for the Iranian fungus, for which multiple specimens and two cultures are available. Even if *N. plumbea* is not regarded as a synonym of *N. subaenea*, it should be kept in mind that both taxa are derived from tropical areas that are far away from Iran.

Figure 4. *Nemania subaenea* (isotype) **A** herbarium label **B** close-up view of stromatal surface **C** close-up view of stromatal surface showing ostioles **D, E** stroma in horizontal section showing perithecia **F** immature ascus in water **G** ascus apical plug in Melzer's reagent **H** immature and mature ascospores in water **I** ascospore in water showing straight germ slit much less than spore-length. Scale bars: 2 mm (**B**); 0.8 mm (**C**); 1 mm (**D**); 0.5 mm (**E**); 20 µm (**F**); 10 µm (**G–I**).

***Nemania serpens* (Pers.) Gray, Nat. Arr. Brit. Pl. (London) 1: 516 (1821).**
Figs 5, 6

Teleomorph. Stromata superficial, effused-pulvinate, up to 4 cm long × 0.2–1.2 cm wide, sessile, attachment to substrate with strong connective; surface dark brown to black, with conspicuous perithecial mounds, carbonaceous immediately beneath surface; tissue between and beneath perithecia black to dark brown. Perithecia obovoid, 0.35–0.65 mm high × 0.25–0.4 mm wide, ostioles papillate to coarsely papillate. Asci cylindrical, stipe up to 130 µm long, spore-bearing part 55–70 × 7–9 µm, apical apparatus not bluing in Melzer's reagent, dextrinoid (= red to red brown) in Lugol's solution. Ascospores smooth, unicellular, pale brown to brown, ellipsoid, inequilateral, with narrowly or broadly rounded ends, 10–14 × 4–5(–6) µm, with straight germ slit much less than spore-length; perispore indehiscent in 10% KOH.

Cultures and anamorph. Colonies on OA covering a 9 cm Petri dish in 18 days, at first white becoming Vinaceous (57), felty, azonate; finally, attaining Amber (47) to Honey (64) after 50 days. Anamorph geniculosporium-like. Conidiophores variables in length, hyaline to light brown. Conidiogenous cells up to 60 × 2.5–3.2 µm, hyaline to light brown. Conidia hyaline, ellipsoid with truncate base, 3–4.8 × 2–3.5 µm (Fig. 6).

Specimens examined. Iran, Mazandaran Province, Ramsar County, Safarud Forest, 36°53'49"N, 50°35'29"E, 815 m elev., on fallen branch of *Parrotia persica*, 29 October 2016, leg. M.J. Pourmoghaddam (GUM 1625; living culture MUCL 57702, IRAN 3735C); Guilan Province, Astara County, 38°23'04"N, 48°51'45.10"E, 1 m elev., on fallen branch of *Parrotia persica*, 22 October 2021, leg. M.J. Pourmoghaddam (GUM 1903).

Notes. *Nemania serpens* is a very common fungus in Europe (Petrini and Rogers 1986; http://pyrenomycetes.free.fr/, accessed 8 Aug 2022). In combination with pale olive brown ascospores with broadly rounded ends and with a short inconspicuous germ slit, *N. serpens* is characterised by a dextrinoid reaction of the ascal apical apparatus in Lugol's solution, while it does not react in Melzer's reagent, which is an exceptional combination within *Nemania* (Granmo et al. 1999; http://pyrenomycetes.free.fr/, accessed 8 Aug 2022). Most of the characters of the Iranian specimens are in accordance with the neotype specimen (Fig. 7; Ju and Rogers 2002), aside from insignificant variations in the size of ascospores. We studied the neotype material and did not observe a conspicuous ascal apical apparatus as described by Ju and Rogers (2002). Morphological species identification of the Iranian specimens is corroborated by the ITS sequence data, as the Iranian and the ex-neotype sequence of *N. serpens* are almost identical (3 substitutions, 3 gaps). Finally, we would like to mention that, for the neotype specimen, Daranagama et al. (2018) erroneously described the ascal apical apparatus as bluing (I+) in Melzer's reagent, while their fig. 7h clearly shows a not bluing (I-) ascal apical apparatus.

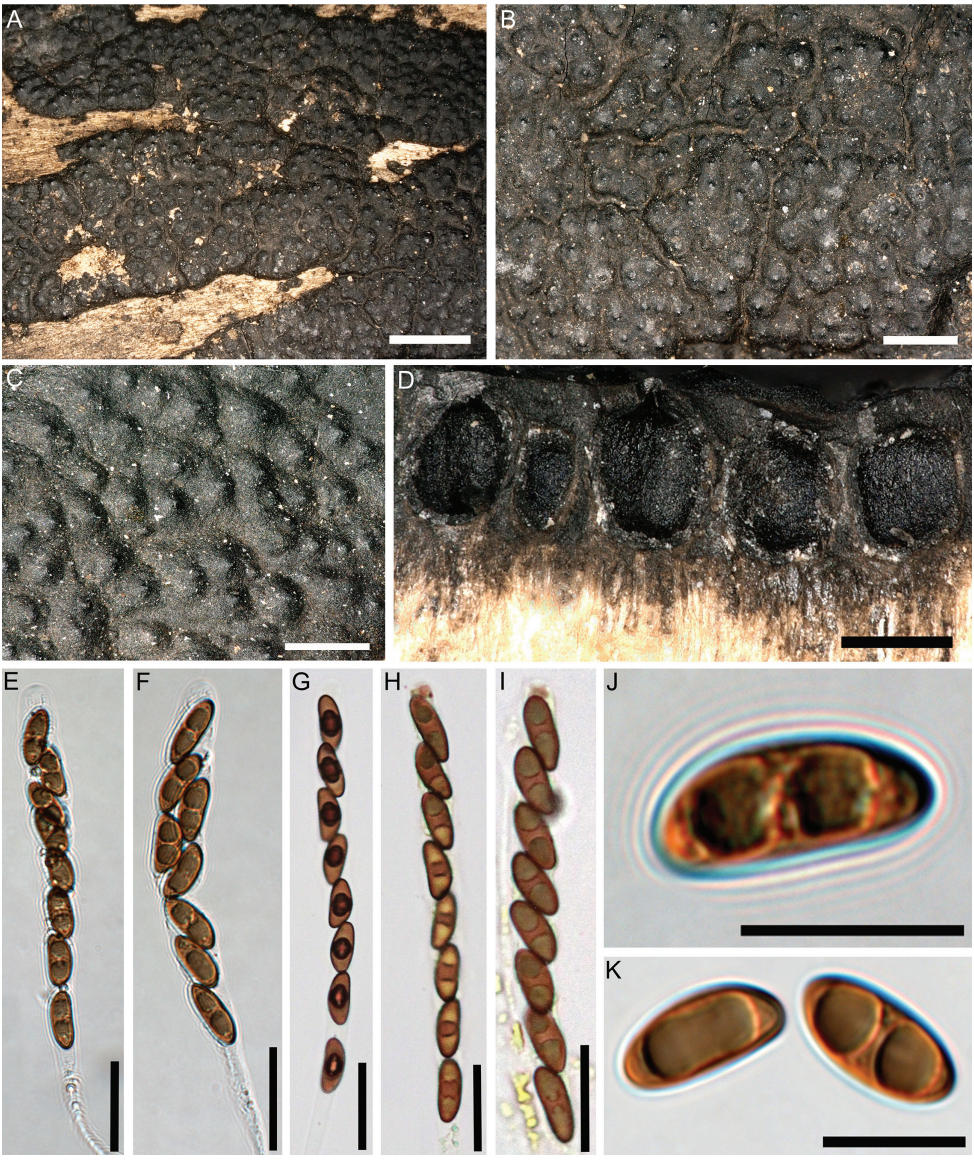

Figure 5. *Nemania serpens* (GUM 1625) **A, B** close-up view of stroma surface **C** close-up view of stroma surface showing ostioles **D** stroma in vertical section showing perithecia **E, F** mature asci in water **G** mature ascus in Melzer's reagent, showing the inamyloid (not bluing) ascal apical apparatus **H, I** mature ascus in Lugol's solution, showing the dextrinoid (= red to red brown) ascal apical apparatus **J** ascospore in water **K** ascospores in water showing straight germ slit much less than spore-length. Scale bars: 3 mm (**A**); 1 mm (**B**); 0.6 mm (**C**); 0.5 mm (**D**); 20 µm (**E–I**); 10 µm (**J, K**).

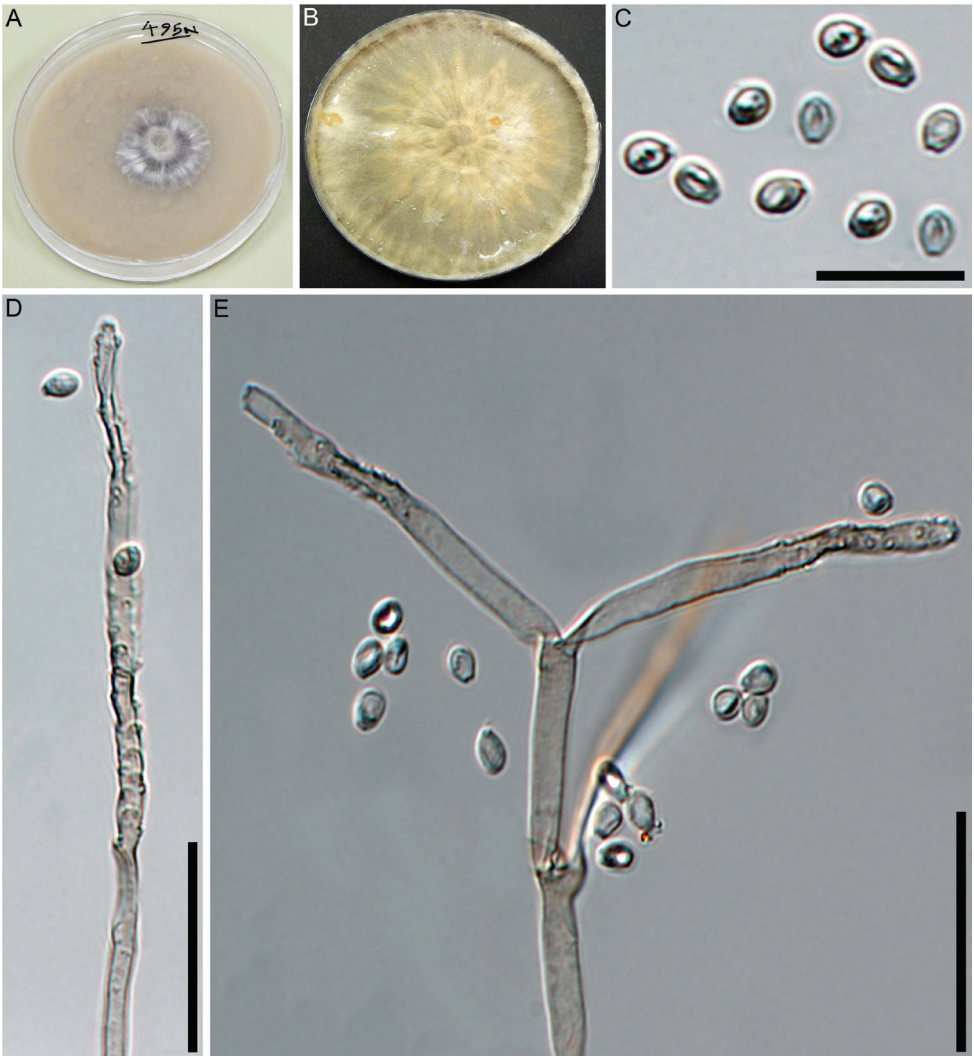

Figure 6. Culture and anamorphic structures of *Nemania serpens* (MUCL 57702) on OA **A, B** surface of colony after (**A**) 7 and (**B**) 50 days of incubation of *N. serpens* **C** conidia of *N. serpens* **D, E** general view of anamorph structure, conidiophores, conidiogenous cells and mature conidia of *N. serpens*. Scale bars: 10 µm (**C**); 20 µm (**D, E**).

***Nemania diffusa* (Sowerby) S.F. Gray, Nat. Arr. Brit. Pl. (London) 1: 517 (1821).**
Fig. 8

Teleomorph. Stromata superficial, effused-pulvinate, discoid, up to 2 cm long × 0.3–1.5 cm wide, sessile, attachment to substrate with narrow connective; surface dark brown to blackish-brown, with inconspicuous perithecial mounds, carbonaceous immediately beneath surface; tissue between and beneath perithecia black to dark brown.

Perithecia obovoid to cylindrical, 0.5–0.8 mm high × 0.3–0.5 mm wide. Ostioles papillate to coarsely papillate. Asci cylindrical, with amyloid, urn-shaped apical apparatus, 2–3 µm high × 1.5–2 µm wide, stipe up to 100 µm long, spore-bearing part 70–80 × 7–10 µm. Ascospores smooth, unicellular, brown to dark brown, ellipsoid,

Figure 7. *Nemania serpens* (neotype) **A** herbarium label **B** stromata on wood **C, D** close-up view of stroma surface **E** close-up view of stroma surface showing ostioles **F, G** mature ascus in water **H** mature ascus in Melzer's reagent, showing the inamyloid (not bluing) ascal apical apparatus **I** ascospores in water showing straight germ slit much less than spore-length **J, K** ascospores in water. Scale bars: 3 mm (**C**); 1 mm (**D**); 0.5 mm (**E**); 20 µm (**F–H**); 10 µm (**I–K**).

Figure 8. *Nemania diffusa* (GUM 1626) **A** stromatal habit **B** close-up view of stromatal surface **C, D** close-up view of stroma surface showing ostioles **E** mature ascus in water **F, G** mature asci in Melzer's reagent showing the amyloid (bluing) ascal apical apparatus **H** ascospore showing straight germ slit. Scale bars: 3 mm (**B**); 1.5 mm (**C**); 0.8 mm (**D**); 20 µm (**E–G**); 10 µm (**H**).

inequilateral, with narrowly rounded ends, 9.5–13(–14) × 4.5–6.5 µm, with straight germ slit spore-length on flattened side; perispore indehiscent in 10% KOH.

Specimen examined. Iran, Guilan Province, Rezvanshahr County, 37°37'52"N, 40°02'18"E, 7 m elev., on fallen branch of *Quercus castaneifolia*, 6 October 2016, leg.

M.J. Pourmoghaddam (GUM 1626), ITS and LSU sequences GenBank OP352258 and OP352270, respectively.

Notes. *Nemania diffusa*, originally described from England (Sowerby 1803), is a widespread and fairly common species in Europe (Fournier et al. 2018). It has also been reported from North and South America (Petrini and Rogers 1986), Papua-New Guinea (Van der Gucht 1995) and Taiwan (Ju and Rogers 1999), but it has yet to be proven whether all these morphologically identified accessions are conspecific with the European ones. The Iranian specimen is in accordance with previous descriptions by Ju and Rogers (2002). It can be differentiated from *N. albocincta* by its larger ascospores [9.5–13.5 × (4.5–)5–6 vs. 8–10 × 4–5 µm], which are also more equilateral. *Nemania obscura* also differs from it in stromatal features and smaller, strongly inequilateral ascospores (8.2–9.4 × 4.5–5.3 µm) with subacute ends. Despite several attempts, we could not achieve a living culture. Therefore, to confirm our morphological species identification, we extracted DNA from stromata and performed PCR (ITS/LSU) and sequencing according to Pourmoghaddam et al. (2018). The ITS sequence of the Iranian collection (OP352258) is completely identical to numerous sequences of European accessions of *N. diffusa*, some of which are morphologically well-documented to represent the species (e.g. MW489542 from Switzerland; Senn-Irlet et al. 2021), confirming the species identification. However, as *RPB2* and *TUB2* could not be obtained, the Iranian accession of *N. diffusa* was not added to the phylogenetic multi-locus analyses.

Discussion

In this study, we examined the phylogenetic relationships of our fresh collections with all species of *Nemania* for which multigene sequence data are available. We have performed a multigene analysis using ITS, LSU, *RPB2* and *TUB2* sequence data to determine the phylogenetic placement of these species. *Nemania* (including *Euepixylon*) clearly forms a monophyletic clade in the phylogenetic analysis which has been placed in Xylariaceae for a long time (Hyde et al. 2020). The results of our phylogenetic analyses agree well with those of Pi et al. (2021), their clade N6 corresponding to our clade N1, their clade N5 to our clade N2 and their clades N1–4 to our clade N3.

Remarkably, in the phylogenetic analyses, the two previously-accepted species of *Euepixylon* are not only contained within *Nemania*, but also do not form a monophyletic lineage, yet they are members of the same *Nemania* subclade 1 (N1; Fig. 1). Stroma morphology and the anamorph of *Euepixylon* matches *Nemania*, the main distinguishing feature being poroid (*Euepixylon*) vs. straight, conspicuous or inconspicuous germ slits of variable length (*Nemania*; Læssøe and Spooner 1993, Granmo et al. 1999). When re-establishing the genus *Euepixylon*, already Læssøe and Spooner (1993) doubted whether the genus will survive in the long run. Considering the results of the phylogenetic analyses, germ site morphology is clearly not a good character to separate *Euepixylon* from *Nemania* and the former genus should be considered as a synonym of the latter, which has already been implemented by for example, Pi et al. (2021) and Voglmayr et al. (2022)

and which we, therefore, also adopt here. Synonymy of both genera is further supported by the fact that the type species of *Euepixylon* (*E. udum*), as well as *Nemania* (*N. serpens*), are revealed to be closely related within the *Nemania* subclade 1 (N1).

Most *Nemania* species are morphologically highly similar, which makes species delimitation and identification based on morphology alone difficult and confusing (Granmo et al. 1999; Ju and Rogers 2002; Fournier et al. 2018). Recently, much progress in reliable species identification has been achieved by DNA sequence data, particularly protein-coding genes such as *RPB2* or *TUB2*, which have superior resolution compared to ITS or LSU (Lücking et al. 2020; Stadler et al. 2020). However, an obstacle for an improved species delimitation and classification is the lack of sequences of type material or well-identified reference specimens in GenBank, which is particularly important for morphologically difficult and complex lineages. *Nemania serpens*, the type species of the genus, is a good example of these problems. Until the present study, no verified sequence data were available in GenBank for *N. serpens* and the various accessions deposited under this name do not form a monophylum in phylogenetic analyses (data not shown). However, it has been widely ignored that Granmo et al. (1999), who neotypified *N. serpens* with a recent Norwegian collection (TROM 174), also generated and published an ITS sequence of their neotype. The reason for disregarding this ex-neotype ITS sequence in subsequent studies lies the fact that Granmo et al. (1999) published their sequences in their Appendix 3, a colour figure of the ITS alignment they used for their phylogenetic analyses, but they did not deposit them in a public sequence repository. The ITS sequences of Granmo et al. (1999) can, therefore, only be added to a sequence matrix if they are transcribed from this colour figure alignment, which we have done here. The addition of the ex-neotype ITS sequence of *N. serpens* from Granmo et al. (1999) to our sequence matrix revealed a high similarity to our Iranian isolate that was identified as *N. serpens* by morphological comparison with the neotype specimen. The phylogenetic analyses also revealed that another isolate (HAST 235), commonly included as *N. serpens* in phylogenies, is not closely related to the neotype, but forms a highly-supported clade with another species, *N. chestersii*, which indicates that HAST 235 does not represent *N. serpens,* but is misidentified.

A further example for incorrectly labelled sequences that could be clarified by inclusion of the ITS sequences of Granmo et al. (1999) refers to CBS 679.86, another accession erroneously deposited as *N. serpens* in GenBank. In the phylogenetic analyses, the accession CBS 679.86 has an ITS sequence almost identical to that of the ex-holotype sequence of *N. prava* from Granmo et al. (1999). However, this becomes conclusive considering that culture CBS 679.86 represents an ex-paratype culture of *Hypoxylon atropurpureum* var. *brevistipitatum* (Petrini and Rogers 1986), a synonym of *Nemania prava* (Granmo et al. 1999). Granmo et al. (1999) confirmed this synonymy by revealing identical ITS sequences for the holotype of *N. prava*, the holotype of *Hypoxylon atropurpureum* var. *brevistipitatum* and another paratype of the latter. It remains yet unclear why the sequences of culture CBS 679.86 have been deposited as *N. serpens* in GenBank. These exemplary cases once again demonstrate that species names of GenBank sequences, as well as the sources of the sequence data, need to be critically evaluated, in particular in taxonomically difficult groups.

Stromata of *Nemania* are highly carbonised and do not contain large amounts of secondary metabolites, as is the case in other phylogenetically closely-related genera, such as *Dematophora* and *Rosellinia*. Only small amounts of xylaral (in *N. diffusa*; Stadler et al. 2008) and BNT (in young *Nemania* specimens; Stadler and Hellwig 2005) have so far been detected.

Since the cultures of Xylariaceae are, in general, rich in production of secondary metabolites (Helaly et al. 2018; Becker and Stadler 2021), further analysis of *Nemania* species may be useful for a better taxonomic classification in the future. Chestersiene and furanone production have so far been described as characteristic metabolites, delimiting *Nemania* from *Hypoxylon* (Whalley and Edwards 1995). Even though this work was based on strains that are apparently not deposited in public collections, the respective compounds have, indeed, not been found in any other fungal genus. The lack of extant cultures for many described xylariaceous species, including for example, *Rosellinia* and *Dematophora* (cf. Wittstein et al. 2020), precludes comprehensive chemotaxonomic studies in the family. Recent progress in the generation of high-quality genome sequences could also enable the search for possible discriminatory biosynthetic gene clusters, as presence or absence of a cluster can serve as a predictor of the taxonomic relationship, which might be an option for future comprehensive sequencing campaigns (Wibberg et al. 2021; Kuhnert et al. 2021).

Xylariaceae is one of the most important ascomycete families found in the north of Iran which has regions with subtropical climates and houses numerous species. Until recently, studies on species biodiversity of Xylariaceae focused on the genera *Xylaria* (Hashemi et al. 2014, 2015), *Kretzschmaria* (Pourmoghaddam et al. 2018) and *Rosellinia* (Pourmoghaddam et al. 2022), which we here extend to the genus *Nemania*.

Acknowledgements

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Supplementary material 1

Alignment

Authors: Mohammad Javad Pourmoghaddam, Christopher Lambert, Hermann Voglmayr, Seyed Akbar Khodaparast, Irmgard Krisai-Greilhuber, Marc Stadler

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A new species and four new records of *Bacidia* (Lecanorales, Ramalinaceae) from South Korea, with a key to Korean species

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Abstract

A new species, *Bacidia fuscopallida* Lee & Heo and four new records, *B. ekmaniana* R. C. Harris, Ladd & Lendemer, *B. friesiana* (Hepp) Körb., *B. heterochroa* (Müll. Arg.) Zahlbr. and *B. suffusa* (Fr.) A. Schneid., are described from South Korea. *Bacidia fuscopallida* differs from *B. diffracta* S. Ekman, the most similar species, by warted but non-granular thallus, paler and smaller apothecia without pruina, proper exciple without crystals, over 11-septate ascospores and smaller pycnidia and pycnoconidia. *Bacidia ekmaniana* is recorded new to Asia, *B. heterochroa* is reported new to northeastern Asia and *B. friesiana* and *B. suffusa* are new to Korea. Molecular analyses employing internal transcribed spacer (ITS) sequences strongly support the classification of the five species of *Bacidia*. A surrogate key is provided to assist in the identification of all 19 taxa in *Bacidia* of Korea.

Keywords

biodiversity, corticolous, lichen, phylogeny, taxonomy

Introduction

Bacidia has become a species-rich genus since De Notaris' (1846) introduction. *Bacidia* (230 spp. including *Bacidiopsora*) is one of the largest genera in Ramalinaceae, with *Ramalina* (230 spp.) (Wijayawardene et al. 2020). The genus *Bacidia* was defined in a wide sense by the characteristics of crustose lichens with a chlorococcoid photobiont, biatorine or lecideine apothecia, 8-spored asci with colourless and transversely 3- or

more septate ascospores (Zahlbruckner 1905, 1921–1940). However, the traditional characterisation of the genus has been considered coarse and unnatural. The genus has been split (e.g. Santesson 1952; Vězda 1978) and particularly new taxonomic applications, based on ascus structures (Hafellner 1984), excipulum structures (Vězda 1990) and molecular results (Ekman and Wedin 2000; Ekman 2001) have reclassified the large genus into tens of different genera (e.g. Vězda 1986; Sérusiaux 1986, 1993, 1995; Lücking 1992, 1995; Aptroot and Sipman 1993; Lücking et al. 1994; Ekman 1996; Kistenich et al. 2018). Ekman (2001) represented that *Bacidia* might be delimited to the *B. rosella* (Pers.) De Not., the type species, group in a strict sense (Brand et al. 2009) and most *Bacidia* species with blue-green pigment in epihymenium are closer to *Toninia* than the type species group, based on molecular phylogeny although *B. schweinitzii* (Fr. ex E. Michener) A. Schneid.) can be an exception.

Bacidia is one of the least explored genera in Korea and the genus has just been reported since the 2010s. Since Joshi et al. (2011) introduced *B. arceutina* (Ach.) Th. Fr., *B. schweinitzii* and *B. subincompta* (Nyl.) Arnold (syn. *Toniniopsis subincompta* (Nyl.) Kistenich, Timdal, Bendiksby & S. Ekman), overall 18 species have been recorded in Korea (Zhang et al. 2012; Aptroot and Moon 2014, 2015; Kondratyuk et al. 2016, 2017, 2019a, b; Liu 2018; Yakovchenko et al. 2018). Although detected on diverse substrates (e.g. bark, moss, rock or artificial wood fence), they are mainly corticolous and were collected on deciduous, wide-leaved tree barks in humid forests.

This study describes a new species and four new records of the lichen genus *Bacidia*. Field surveys for the lichen biodiversity in the main mountains of Korea, i.e. Baekdudaegan, and several forested wetlands of South Korea were carried out during the spring to summer of 2019–2021 and 54 specimens of *Bacidia* were collected from barks of deciduous wide-leaved trees and shrubs (Fig. 1). The specimens were comprehensively analysed and identified as a new species, *B. fuscopallida*, and four new records, *B. ekmaniana*, *B. friesiana*, *B. heterochroa* and *B. suffusa*. All the collected specimens are deposited in the Herbarium of the Baekdudaegan National Arboretum (KBA), South Korea.

Materials and methods

Morphological and chemical analyses

Hand sections were prepared manually with a razor blade under a stereomicroscope (Olympus optical SZ51; Olympus, Tokyo, Japan), examined under a compound microscope (Nikon Eclipse E400; Nikon, Tokyo, Japan) and pictured using a software programme (NIS-Elements D; Nikon, Tokyo, Japan) and a DS-Fi3 camera (Nikon, Tokyo, Japan) mounted on a Nikon Eclipse Ni-U microscope (Nikon, Tokyo, Japan). The ascospores were examined at 1000× magnification in water. The length and width of the ascospores were measured and the range of spore sizes was shown with average, standard deviation (SD), length-to-width ratio and the number of measured spores. Thin-layer chromatography (TLC) was performed using solvent system C according to standard methods (Orange et al. 2001).

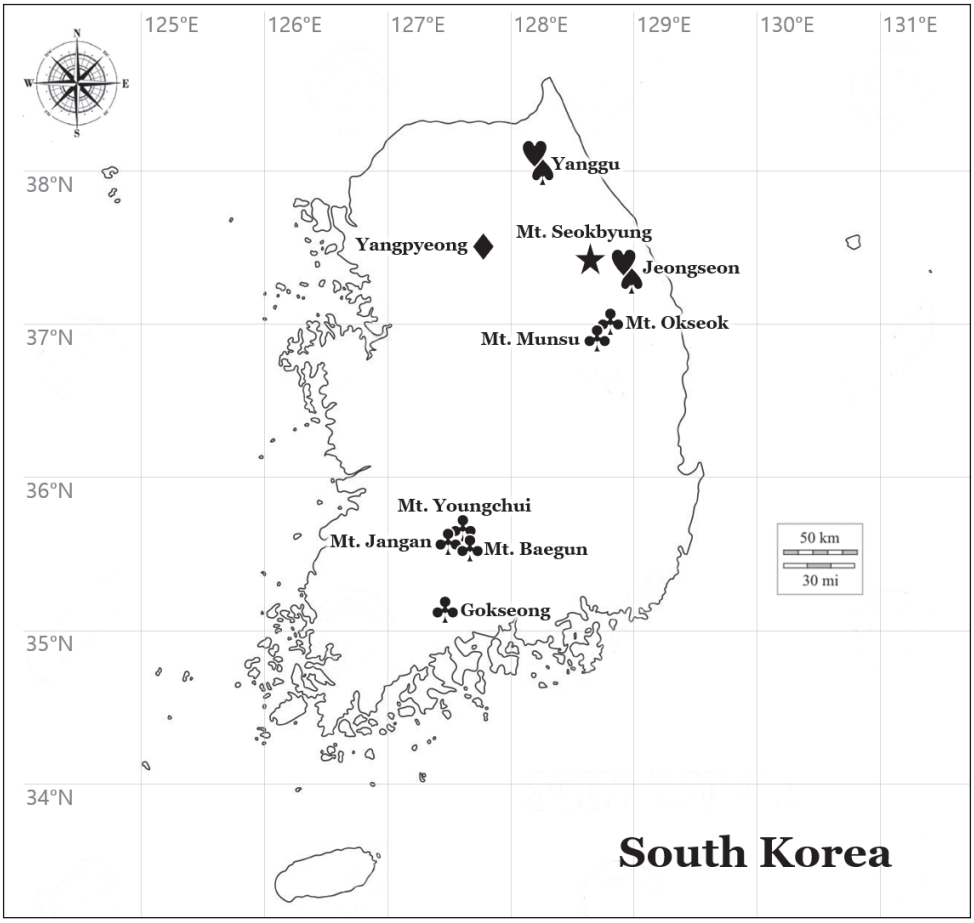


Figure 1. Specific collection sites (black symbols) for the new species *Bacidia fuscopallida* (black star) and four new records, *B. ekmaniana* (black club), *B. friesiana* (black diamond), *B. heterochroa* (black hearth) and *B. suffusa* (black spade).

Isolation, DNA extraction, amplification and sequencing

Hand-cut sections of ten to twenty ascomata per collected specimen were prepared for DNA isolation (Table 1) and DNA was extracted with a NucleoSpin Plant II Kit in line with the manufacturer's instructions (Macherey-Nagel, Düren, Germany). PCR amplifications for the internal transcribed spacer region (ITS1-5.8S-ITS2 rDNA) RNA genes were achieved using Bioneer's AccuPower PCR Premix (Bioneer, Daejeon, Korea) in 20-µl tubes with 16 µl of distilled water, 2 µl of DNA extracts and 2 µl of the primers ITS5 and ITS4 (White et al. 1990). The PCR thermal cycling parameters used were 95 °C (15 sec), followed by 35 cycles of 95 °C (45 sec), 54 °C (45 sec) and 72 °C (1 min) and a final extension at 72 °C (7 min), based on Ekman (2001). The annealing temperature was occasionally altered by ±1 degree in order to obtain a better result. PCR purification and DNA sequencing were accomplished by the genomic research company Macrogen (Seoul, Korea).

Phylogenetic analyses

An independent phylogenetic tree for the genus *Bacidia* was produced from 84 sequences from GenBank and 12 newly-generated sequences for the new species and the new records (Table 2). All ITS sequences were aligned and edited manually using ClustalW in Bioedit v.7.2.6.1 (Hall 1999). All missing and ambiguously aligned data and phylogenetically uninformative positions were removed and phylogenetically informative regions were finally analysed in MEGA X (Stecher et al. 2020). The final alignment comprised 930 bp, in which 102 variable regions were detected. The phylogenetically informative regions were 585. Phylogenetic trees with bootstrap values were obtained in RAxML GUI 2.0 beta (Edler et al. 2019) using the Maximum Likelihood method with a rapid bootstrap with 1,000 bootstrap replications and GTR GAMMA (GTR + G4) for the substitution matrix. The posterior probabilities were obtained in BEAST 2.6.4 (Bouckaert et al. 2019) using the GTR 123454 model, as the appropriate model of nucleotide substitution produced by the Bayesian model averaging methods with bModelTest (Bouckaert and Drummond 2017), empirical base frequencies, gamma for the site heterogeneity model, four categories for gamma and a 10,000,000 Markov Chain Monte Carlo chain length with a 10,000-echo state screening and 1,000 log parameters. Then, a consensus tree was constructed in TreeAnnotator 2.6.4 (Bouckaert et al. 2019) with the first 25% discard as a burn-in, no posterior probability limit, a maximum clade credibility tree for the target tree type and median node heights. All trees were displayed in FigTree 1.4.2 (Rambaut 2014) and edited in Microsoft Paint. Overall analyses in the materials and methods were undertaken based on Lee and Hur (2020).

Table 1. Hand-cut section information for DNA isolation.

Species	*Bacidia fuscopallida*	*Bacidia ekmaniana*	*Bacidia friesiana*	*Bacidia heterochroa*	*Bacidia suffusa*
Specimens	KBA-L-0001010 (isotype), KBA-L-0001037 (paratype), KBA-L-0001049 (paratype)	KBA-L-0000072, KBA-L-0002037	KBA-L-0001910, KBA-L-0001913, KBA-L-0001914, KBA-L-0001917	KBA-L-0000386, KBA-L-0002714, KBA-L-0002727, KBA-L-0002734	KBA-L-0000358, KBA-L-0000359, KBA-L-0000368, KBA-L-0002776, KBA-L-0002778, KBA-L-0002835
Ascomata sections per specimen	20	10	20	10	10
Ascomata sections per species	60	20	80	40	60

Table 2. Species list and DNA sequence information employed for phylogenetic analysis.

No.	Species	ITS	Voucher
1	*Bacidia absistens*	AF282085	Ekman 3223 (BG)
2	*Bacidia albogranulosa*	MK158340	J. Malicek 9622
3	*Bacidia albogranulosa*	MK158342	J. Vondrak 11888 (PRA)
4	*Bacidia arceutina*	AF282083	Ekman 3110 (BG)
5	*Bacidia arceutina*	JQ796851	LG DNA 579
6	*Bacidia areolata*	MH048614	M-0182592
7	*Bacidia auerswaldii*	AF282122	Johansson 20 (UPS)
8	*Bacidia bagliettoana*	AF282123	Ekman 3137 (BG)
9	*Bacidia bagliettoana*	MG838190	O-L-175215
10	*Bacidia beckhausii*	AF282071	Holien 6744 (TRH)
11	*Bacidia beckhausii*	JF714252	MSSRF Lichen Herbarium
12	*Bacidia biatorina*	AF282079	Knutsson 94–148
13	*Bacidia caligans*	AF282096	Johansson 21 (UPS)
14	*Bacidia circumspecta*	MH539764	L-13006
15	*Bacidia circumspecta*	AF282124	Ekman L1330 (LD)
16	*Bacidia cylindrophora*	MG926005	Kurokawa 1692
17	*Bacidia cylindrophora*	MG926006	Ohmura 7091 (GZU)
18	*Bacidia diffracta*	AF282090	Wetmore 26401 (MIN)
19	*Bacidia diffracta*	MH048620	Harris 46555-A
20	***Bacidia ekmaniana***	ON352611	**KBA-L-0002037**
21	*Bacidia elongata*	MH048626	M-0182571
22	*Bacidia elongata*	MH048629	M-0182627
23	*Bacidia fraxinea*	AF282088	Johansson 1620 (BG)
24	***Bacidia friesiana***	ON352609	**KBA-L-0001910**
25	***Bacidia friesiana***	ON352610	**KBA-L-0001913**
26	*Bacidia friesiana*	MH539765	L-13159
27	***Bacidia fuscopallida***	ON352607	**KBA-L-0001010**
28	***Bacidia fuscopallida***	ON352608	**KBA-L-0001049**
29	*Bacidia fuscoviridis*	AM292665	Nordin 5058 (UPS)
30	*Bacidia gigantensis*	MT425200	MCM242
31	*Bacidia hemipolia*	AF282072	Toensberg 25091 (BG)
32	***Bacidia heterochroa***	ON352606	**KBA-L-0000386**
33	***Bacidia heterochroa***	ON352612	**KBA-L-0002727**
34	***Bacidia heterochroa***	ON352613	**KBA-L-0002734**
35	*Bacidia hostheleoides*	AF282081	Seaward 108121
36	*Bacidia incompta*	AF282092	Ekman 3144 (BG)
37	*Bacidia incompta*	MG461697	KoLRI Udo-32
38	*Bacidia kurilensis*	MH048612	M-0182622
39	*Bacidia kurilensis*	MH048610	M-0182620
40	*Bacidia kurilensis*	MH048611	M-0182621
41	*Bacidia laurocerasi*	MH048609	Galanina 424
42	*Bacidia laurocerasi* subsp. *laurocerasi*	MN483106	Spribille 26334 (KLGO)
43	*Bacidia laurocerasi* subsp. *laurocerasi*	AF282078	Wetmore 74318 (MIN)
44	*Bacidia lutescens*	MG925952	Ekman 3655 (BG)
45	*Bacidia lutescens*	AF282082	Ekman L1161 (LD)
46	*Bacidia medialis*	AF282102	Ekman L1193 (LD)
47	*Bacidia polychroa*	AF282089	Knutsson 91–215

No.	Species	ITS	Voucher
48	*Bacidia rosella*	AF282086	Ekman 3117 (BG)
49	*Bacidia rubella*	AF282087	Ekman 3021 (BG)
50	*Bacidia rubella*	HQ650644	AFTOL-ID 1793
51	*Bacidia rubella*	JQ796852	LG DNA 578
52	*Bacidia rubella*	KX132984	LIFU076–16
53	*Bacidia rubella*	MG461695	AFTOL-ID 1793
54	*Bacidia rubella*	EU266078	Hur H06122
55	*Bacidia rubella*	MH048630	M-0182581
56	*Bacidia rubella*	MK158343	J. Vondrak 12200 (PRA)
57	*Bacidia sabuletorum*	AF282069	Ekman 3091 (BG)
58	*Bacidia sachalinensis*	MH048621	M-0182619
59	*Bacidia sachalinensis*	MH048625	M-0182624
60	*Bacidia schweinitzii*	AF282080	Wetmore 72619 (MIN)
61	*Bacidia schweinitzii*	KX151766	Lendemer 31230A (NY)
62	*Bacidia scopulicola*	AF282084	Ekman 3106 (BG)
63	*Bacidia sigmosporae*	MW622004	P.v.d. Boom 55090
64	*Bacidia sipmanii*	JQ796853	LG DNA 361
65	*Bacidia sorediata*	KX151772	Lendemer 33787 (NY)
66	*Bacidia sorediata*	KX151775	Barton 658 (NY)
67	*Bacidia squamulosula*	MG925955	Kalb & Kalb in Kalb, Lich. neotrop. 405
68	*Bacidia subareolata*	MK499342	MFLU 16-0573
69	*Bacidia subincompta*	AF282125	Ekman 3413 (BG)
70	*Bacidia subincompta*	KX098342	WSL DF231
71	***Bacidia suffusa***	ON352605	**KBA-L-0000359**
72	***Bacidia suffusa***	ON352614	**KBA-L-0002776**
73	***Bacidia suffusa***	ON352615	**KBA-L-0002778**
74	***Bacidia suffusa***	ON352616	**KBA-L-0002835**
75	*Bacidia suffusa*	AF282091	Wetmore 74771 (MIN)
76	*Bacidia suffusa*	AY756456	Andersen 99 (BG)
77	*Bacidia suffusa*	MH048615	M-0182601
78	*Bacidia suffusa*	MH048616	M-0182593
79	*Bacidia suffusa*	MH048617	M-0182594
80	*Bacidia suffusa*	MH048618	M-0289887
81	*Bacidia suffusa*	MH048619	M-0289888
82	*Bacidia suffusa*	MW728313	LAH 36839
83	*Bacidia suffusa*	MW788561	LAH 36838
84	*Bacidia vermifera*	AF282109	Johansson 1619 (BG)
85	*Bacidia vermifera*	KX132992	LIFU084-16 (versA)
86	*Bacidia wellingtonii*	MG925953	Ziviagina s.n.
87	*Bacidia* sp.	AY756133	KoLRI Udo-32
88	*Bacidia* sp.	KX098339	WSL DF223
89	*Bacidia* sp.	KX098340	WSL DF72
90	*Bacidia* sp.	KX098341	WSL DF80
91	*Bacidia* sp.	MG773660	Palice 19352
92	*Biatora bacidioides*	MG773663	Palice 19221
93	*Biatora bacidioides*	MG773664	Palice 19685
94	*Biatora pontica*	KF650977	C. Printzen 6114 (BG)
95	*Biatora pontica*	MK778588	J. Malicek 10212
96	*Biatora printzenii*	KF650978	C. Printzen 6837 (BG)
	Overall	**96**	

DNA sequences which were generated for the new species and the new records of *Bacidia* in this study, are presented in bold. All others were obtained from GenBank. The species names are followed by GenBank accession numbers and voucher information. ITS, internal transcribed spacer; Voucher, voucher information.

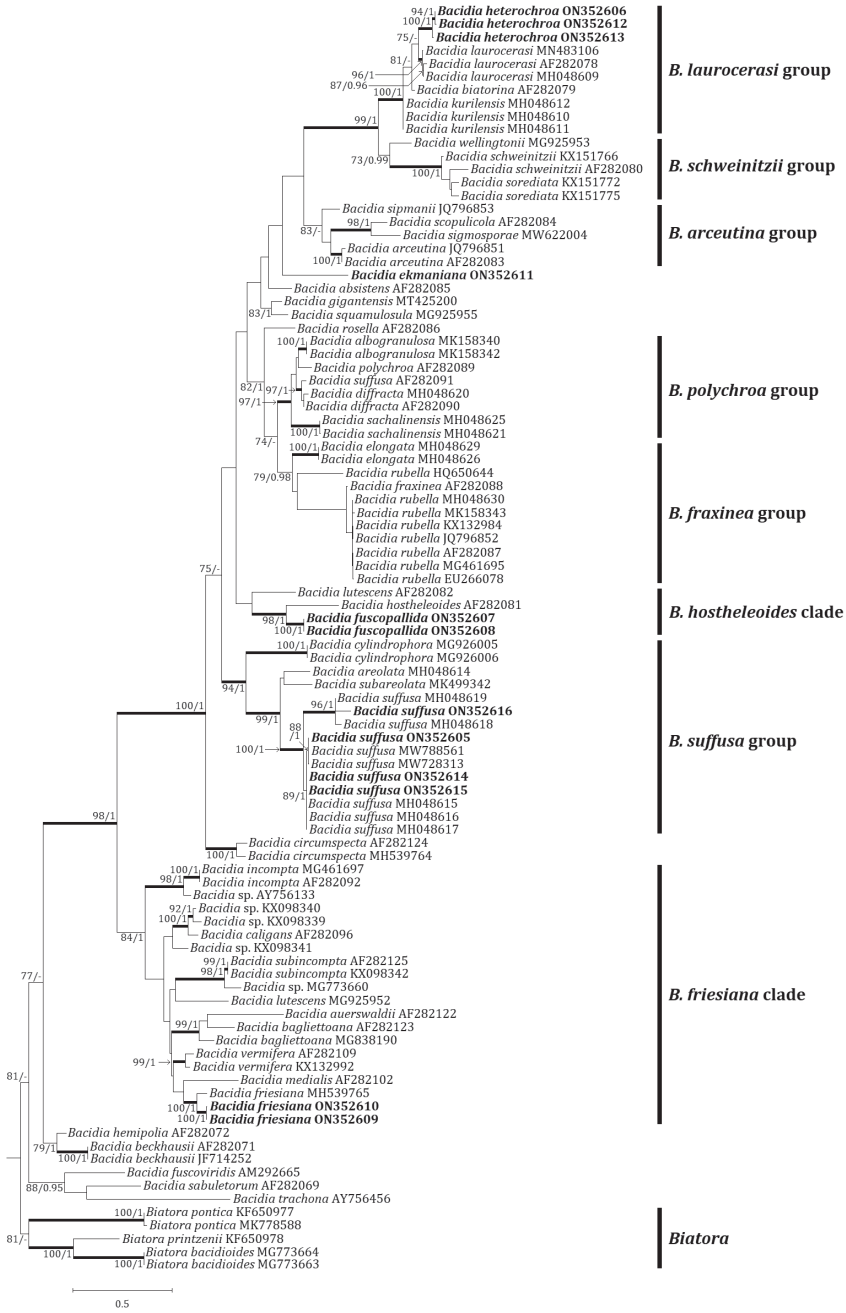


Figure 2. Phylogenetic relationships amongst available species in the genus *Bacidia*, based on a Maximum Likelihood analysis of the dataset of ITS sequences. The tree was rooted with the sequences of the genus *Biatora*, based on Gerasimova et al. (2018). Maximum Likelihood bootstrap values ≥ 70% and posterior probabilities ≥ 95% are shown above internal branches. Branches with bootstrap values ≥ 90% are shown as thick lines. New sequences produced in this study are presented in bold. All species names are followed by the GenBank accession numbers.

Results and discussion

Phylogenetic analyses

The new species is positioned in the genus *Bacidia* in the ITS tree (Fig. 2). The ITS tree describes *B. fuscopallida*, the new species, being nested with *B. hostheleoides* (Nyl.) Zahlbr., supported by a bootstrap value of 98 and a posterior probability of 1.00 for the branch. *Bacidia fuscopallida* is located in its own clade without any sequences close to it, although *B. fuscopallida* is sister to *B. hostheleoides*.

Taxonomy

***Bacidia fuscopallida* B.G. Lee & T.I. Heo, sp. nov.**
MycoBank No: 843830
Fig. 3

Diagnosis. *Bacidia fuscopallida* differs from *B. diffracta* by generally non-granular, olive-green thallus, pale yellow-orange apothecia without pruina, the absence of crystals in proper exciple, slightly narrower ascospores with up to 15-septation and smaller pycnidia and pycnoconidia.

Type. South Korea, Gangwon Province, Gangneung, Okgye-myeon, Mt. Seokbyung, 37°34.45'N, 128°55.00'E, 271 m alt., on bark of *Acer pictum* var. *mono* (Maxim.) Maxim. ex Franch., 17 June 2020, B.G. Lee & H.J. Lee 2020-000811, with *Porina hirsuta* Aptroot & K.H. Moon (holotype: KBA-L-0001011!); same locality, on bark of *Acer pictum* var. *mono*, 17 June 2020, B.G. Lee & H.J. Lee 2020-000801 (isotype: KBA-L-0001001); same locality, on bark of *Acer pictum* var. *mono*, 17 June 2020, B.G. Lee & H.J. Lee 2020-000806, with *Mikhtomia gordejevii* (Tomin) S.Y. Kondr., Kärnefelt, Elix, A. Thell, Jung Kim, A.S. Kondr. & Hur, *Straminella varia* (Hoffm.) S.Y. Kondr., Lőkös & Farkas, *Phaeophyscia limbata* (Poelt) Kashiw., *Porina hirsuta* (isotype: KBA-L-0001006); same locality, on bark of *Acer pictum* var. *mono*, 17 June 2020, B.G. Lee & H.J. Lee 2020-000810 (isotype: KBA-L-0001010; GenBank ON352607 for ITS); South Korea, Gangwon Province, Gangneung, Okgye-myeon, Mt. Seokbyung, 37°34.39' N, 128°55.01'E, 349 m alt., on bark of *Quercus mongolica* Fisch. ex Ledeb., 17 June 2020, B.G. Lee & H.J. Lee 2020-000837, with *Opeltia flavorubescens* (Huds.) S.Y. Kondr. & Hur (paratype: KBA-L-0001037); South Korea, Gangwon Province, Gangneung, Okgye-myeon, Mt. Seokbyung, 37°34.28'N, 128°54.88'E, 438 m alt., on bark of *Acer triflorum* Kom., 17 June 2020, B.G. Lee & H.J. Lee 2020-000849, with *Biatora pacifica* Printzen, Tønsberg & G. Thor (paratype: KBA-L-0001049; GenBank ON352608 for ITS).

Thallus corticolous, crustose, areoles in young stage and soon coarsely continuous or warted on aging, often overlapping for each other, rarely granular, thin when not overlapping, olivish-green, margin indeterminate, 40–90 μm thick; cortex indistinct, hyaline, up to 5 μm thick; medulla a little shown as mycelia below algal layer; photobiont chlorococcoid, cells globose to subglobose, 5–15 μm thick, algal layer

composing most part of thallus, 35–80 μm thick. Prothallus indistinct or whitish-grey and endosubstratal when present.

Apothecia numerous, solitary, marginate and flat in young stage and seeming immarginate and convex on aging (consistently marginate and flat on bark of *Acer triflorum*), 0.1–0.7 mm diam. (mean = 0.33; SD = 0.14; n = 105). Pruina absent. Disc biatorine, thalline exciple absent, pale yellow to pale orange in young stage and slightly more blackish generally around margin when mature (much more blackish on bark of *A. triflorum* and *Q. mongolica* from young stage). Proper exciple 65–80 μm wide laterally (SD = 5.7; n = 15), with radiating hyphae of 1–2.5 μm wide (SD = 0.5; n = 10) and outermost cell 2.5–4 μm wide (SD = 0.6; n = 10), hyaline to pale yellow around rim, but darker downwards (pale yellow to pale brown) and the dark colour extending to hypothecium. Epihymenium hyaline, with a little pigment of pale yellow to pale olive-brown locally, smooth and not granular, ca. 5 μm high. Hymenium hyaline, 70–100 μm high (SD = 8.9; n = 10). Hypothecium clearly pigmented, pale orange-brown to brown, prosoplectenchymatous (irregularly arranged), 70–130 μm high (SD = 18.9; n = 10). Crystals absent or a little present in upper hypothecium. Oil droplets absent. Paraphyses simple, rarely branched at tips, 1–1.5 μm wide, tips not or little swollen, not pigmented, 1.5–2 μm wide. Asci cylindrical to narrowly clavate, 8-spored, 49–72 × 11–14 μm (SD = 7.3 (L), 0.9 (W); n = 11). Ascospores 3- to 15-septate, acicular to filiform, 24–69 × 2–3.5 μm (mean = 52.8 × 2.6 μm; SD = 8.7 (L), 0.6 (W); L/W ratio = 3.8–30.5, ratio mean = 17.6, ratio SD = 5.0; n = 104). Pycnidia black, immersed and upper half only shown, globose, 60–65 μm high and 55–75 μm wide (SD = 2.4 (H), 8.2 (W); n = 5), with brownish wall, K–. Pycnoconidia hyaline, filiform, curved or almost straight, 6–17 × 0.3–0.5 μm (mean = 10.4 × 0.5 μm; SD = 2.9 (L), 0.1 (W); n = 53).

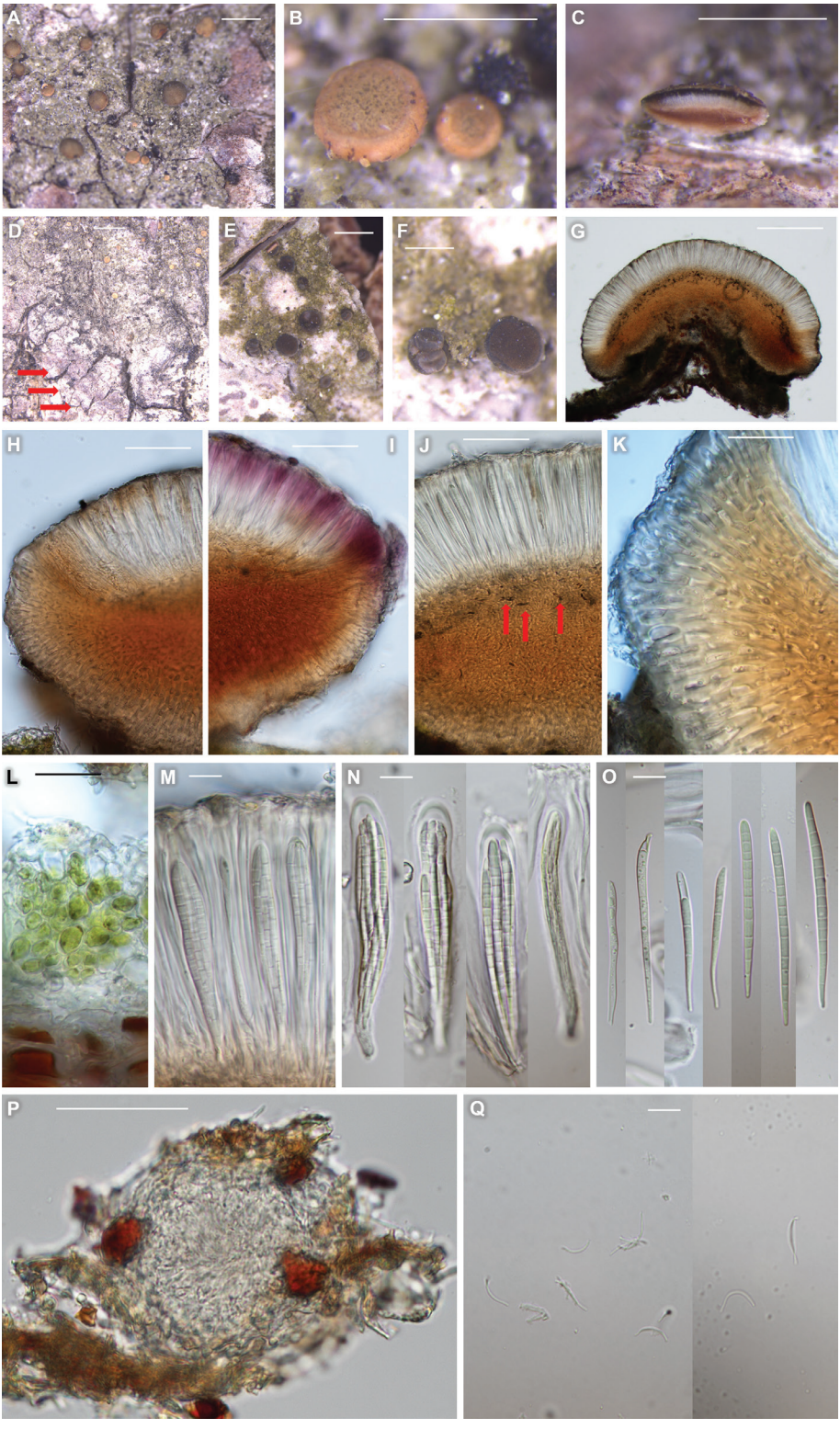
Chemistry. Thallus K– or K+ slightly yellow, KC–, C–, Pd–, UV–. Epihymenium K+ purple extending to outermost layers of proper exciple, C–. No lichen substance was detected by TLC.

Distribution and ecology. The species occurs on barks of *Acer pictum* var. *mono*, *A. triflorum* and *Quercus mongolica*. The species is currently known from the type collections.

Etymology. The species epithet indicates the pale brown colour of the lichen's apothecia.

Notes. The new species is similar to *B. diffracta* and *B. polychroa* (Th. Fr.) Körb. in having colourless epihymenium with pale orange-brown pigment and K+ purple reaction, distinctly pigmented hypothecium with yellow, orange or brown, long ascospores generally with L/W ratio over 10 amongst corticolous species. However, *B. diffracta* differs from the new species by granular thallus, darker and larger apothecia with pruina, proper exciple with radiating clusters of minute crystals, slightly wider ascospores with up to 11-septation and larger pycnidia and pynoconidia (Ekman 1996) (Table 3).

The new species is more similar to *B. polychroa* in having coarsely continuous or warted thallus. However, *B. polychroa* differs from the new species by greyish thallus, darker and larger apothecia often with pruina, proper exciple often with radiating clusters of minute crystals, wider ascospores and larger pycnidia and pycnoconidia (Ekman 1996; Smith et al. 2009) (Table 3).


A
B
C
D
E
F
G
H
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K
L
M
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Q

Table 3. Comparison of the new species with close species in the genus *Bacidia*.

Species	*Bacidia fuscopallida*	*Bacidia diffracta*	*Bacidia hostheleoides*	*Bacidia polychroa*	*Bacidia purpurans*
Thallus growth form	warted, rarely granular	finely granular	wrinkled or granular to subsquamulose	finely wrinkled to warted, sometimes areolate	areolate
Thallus colour	olivish-green	pale grey, green-grey, yellow-grey to grey	pale grey to pale green-grey	white to grey or yellow-grey	pale grey-green to dark green
Prothallus	white-grey around margin, endosubstratal	white-pale grey between granules, endosubstratal	absent	–	white, arachnoid
Apothecia (mm in diam.)	0.1–0.7	0.5–1.1	0.5–0.8	0.4–1.2	–
Disc colour	pale yellow to pale orange (young); more blackish (old)	brown-orange to dark brown	brown-orange	brown-orange to dark brown	dark purple-brown to brown
Pruina	absent	white	absent	white	absent
Crystals in proper exciple	absent	radiating clusters of minute crystals	absent	with or without radiating clusters of minute crystals	absent
Crystals in hymenium	small crystals at bottom	–	–	–	absent
Epihymenium colour	colourless with pale yellow-brown pigment	colourless with pale orange-brown pigment	very pale orange	colourless with brown-orange pigment	greyish
Hymenium height (µm)	70–100	70–100	ca. 60	55–100	ca. 100
Hypothecium colour	pale orange-brown to brown	pale brown to orange-brown	very pale orange	brown-orange to dark brown	orange-brown
Hypothecium height (µm)	70–130	–	–	–	ca. 60
Ascospore (µm)	24–69 × 2–3.5	32–69 × 1.9–4.1	16–25 × 2.9–5	31–74 × 1.9–5	50–75 × 2–4
Ascospore L/W ratio	4–31	9–27	4–9	7–30	–
Ascospore septation	3–15	3–11	3–5	2–15	3–15
Pycnidia (µm)	55–75	150	50–100	100–170	150–200
Pycnoconidia	6–17 × 0.3–0.5	10–15 × 0.5–0.6	①10–14 × 0.5 ②6–9 × 1.6–2	10–17 × 0.6–0.8	20–25 × 0.8
Substance	absent	atranorin, (trace of zeorin)	absent	(trace of atranorin)	atranorin
Reference	this study	Ekman (1996)	Ekman (1996)	Ekman (1996); Smith et al. (2009)	Lendemer et al. (2016)

The morphological and chemical characteristics of several species close to the new species are referenced from the previous literature. All information on the new species is produced from type specimens (KBA-L-0001010, KBA-L-0001011 and KBA-L-0001049) in this study.

Figure 3. *Bacidia fuscopallida* (KBA-L-0001011, holotype for **A–D, G–O** KBA-L-0001049 for **E, F** KBA-L-0001010 for **P, Q**) in morphology **A, B** habitus and apothecia on bark of *Acer pictum* var. *mono*. Olive-green thallus and pale yellow-orange apothecia **C** vertical section of apothecia **D** prothallus present around margin of habitus (red arrows) **E, F** habitus and apothecia growing on bark of *Acer triflorum* **G** apothecial section **H** epihymenium colourless or a little pigmented **I** epihymenium K+ purple **J** small crystals (red arrows) present in upper hypothecium **K** proper exciple pigmented with pale or colourless margin. Radiating hyphae wider to margin **L** photobiont composing most part of thallus **M, N** asci cylindrical to narrowly clavate. Ascospores not twisted in ascus **O** ascospores acicular to filiform up to 15-septate **P** pycnidia globose with brown wall **Q** pycnoconidia curved or almost straight. Scale bars: 1 mm (**A, E**); 500 µm (**B, C, F**); 2 mm (**D**); 200 µm (**G**); 50 µm (**H–J, P**); 20 µm (**K, L**); 10 µm (**M–O, Q**).

The new species is quite similar to *B. purpurans* R. C. Harris, Ladd & Lendemer in having greenish thallus with areoles and K+ purple reaction in epihymenium. However, *B. purpurans* differs from the new species by arachnoid prothallus, darker apothecia, green excipular rim adjacent to epihymenium, greyish epihymenium, shorter hypothecium, absence of crystals, larger ascospores and larger pycnidia and pycnoconidia (Lendemer et al. 2016) (Table 3).

The new species can be compared with *B. hostheleoides* in sharing non-pruinose apothecia and proper exciple without crystals. However, *B. hostheleoides* differs from the new species by greyish thallus, absence of prothallus, shorter hymenium, paler hypothecium and shorter ascospores with a few septa (Ekman 1996) (Table 3).

Bacidia ekmaniana R. C. Harris, Ladd & Lendemer, The Bryologist 119 (2): 154 (2016)

Fig. 4

Description. Thallus corticolous, crustose, somewhat granular when young and smoother when mature, grey, greenish-grey to pale grey, margin indeterminate. Prothallus generally not detected or whitish-grey when present.

Apothecia consistently flat or slightly convex when mature, marginate, without pruina, 0.4–1.4 mm diam. (mean = 0.75, SD = 0.23, n = 104). Disc biatorine, without thalline exciple, pale straw, light brown to brown, with a distinct proper margin which is smooth to rugose and becoming thinner but still distinct when mature. Proper exciple pale brown to red-brown, paler or colourless around rim and thicker downwards, 80–120 μm wide laterally. Epihymenium hyaline, smooth but not granular, ca. 5 μm high. Hymenium hyaline, 80–140 μm high. Hypothecium red-brown, prosoplectenchymatous (irregularly arranged), 120–250 μm high. Small crystals present a little in hypothecium, dissolving in K. Oil droplets absent. Asci narrowly clavate, 8-spored, 70–105 × 8–12 μm (n = 5). Ascospores acicular to filiform, cells near head sometimes irregularly swollen, 3- to 9-septate, 52–71 × 2–4.5 μm (n = 15). Pycnidia not detected.

Chemistry. Thallus K–, C–. Apothecial section K–, C–. No lichen substance was detected by TLC.

Notes. *Bacidia ekmaniana* is easily confused with *B. schweinitzii* under the microscope, as well as in the field because both species often share their habitat and the habiti of both species look similar particularly when the ascomata of the latter are paler. Both species are often detected from one specimen under the microscope and those were frequently regarded as one species, i.e. *B. schweinitzii*. Generally, however, *B. ekmaniana* differs from the latter by paler ascomata. *Bacidia ekmaniana* has brown but not black apothecia when mature (Lendemer et al. 2016). *Bacidia ekmaniana* differs from the latter by colourless epihymenium and paler hypothecium as well.

Bacidia ekmaniana is more similar to *B. arceutina* than *B. schweinitzii* in morphology in having pale ascomata. However, *B. ekmaniana* differs from *B. arceutina* by the colourless to pale excipular rim, colourless epihymenium and wider ascospores with

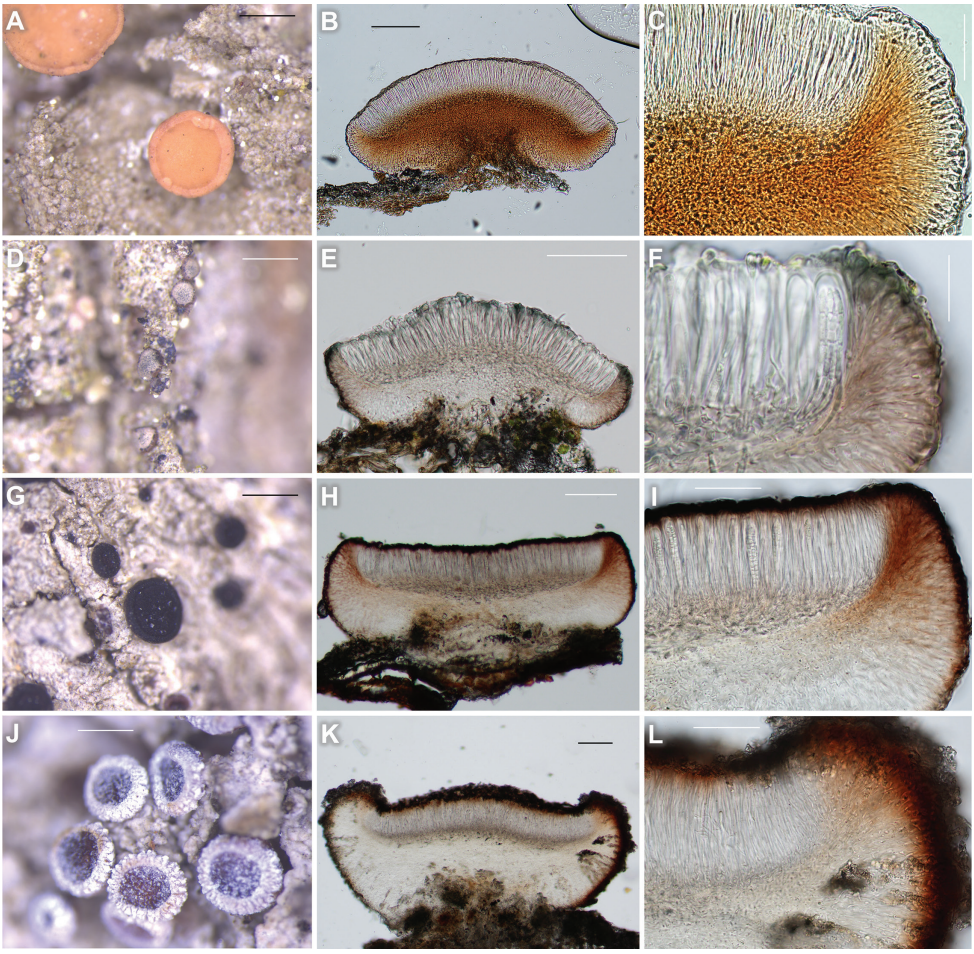

Figure 4. Four new records of *B. ekmaniana* (KBA-L-0000412 for **A–C**), *B. friesiana* (KBA-L-0001914 for **D–F**), *B. heterochroa* (KBA-L-0000386 for **G–I**) and *B. suffusa* (KBA-L-0000359 for **J–L**) in morphology **A** habitus and apothecia. Granular thallus with green-grey pigment and straw-coloured apothecia **B–C** apothecial section with colourless epihymenium, red-brown hypothecium, and pale excipulum **D** habitus and apothecia. Thallus pale grey with slightly brownish pigment and pale pink apothecia **E, F** apothecial section with greenish epihymenium **G** habitus and apothecia. Thallus pale yellowish-grey and black apothecia with red pigment **H, I** apothecial section and proper exciple with dark margin **J** habitus and apothecia. Thallus whitish pale grey and pruinose apothecia **K, L** apothecial section with radiating clusters of crystals, which produce pruina on surface. Scale bars: 500 µm (**A, D, G, J**); 100 µm (**B, E, H, K**); 50 µm (**C, I, L**); 20 µm (**F**).

more septation (Ekman 1996; also see the key couplet 23). *Bacidia ekmaniana* is new to Asia and this is the second record after North America (Lendemer et al. 2016). *Bacidia ekmaniana* is supposed to occur widespread throughout the world as the species was assumed to be *B. schweinitzii* in the past. Phylogenetic analysis resulted in *B. ekmaniana* being located in its own clade in the genus *Bacidia* (Fig. 2).

Specimens examined. South Korea, North Gyeongsang Province, Bonghwa, Chunyang-myeon, Mt. Munsu, 36°59.28'N, 128°48.17'E, 1,058 m alt., on bark of *Quercus mongolica*, 29 August 2019, B.G. Lee 2019-000072 (KBA-L-0000072); South Korea, South Jeolla Province, Gokseong, Jukgok-myeon, Taeansa Temple, 35°08.06'N, 127°23.26'E, 297 m alt., on bark of *Salix pierotii* Miq., 25 May 2020, B.G. Lee 2020-000212, with *Bacidia schweinitzii* (KBA-L-0000412); same locality, on bark of *Salix pierotii*, 25 May 2020, B.G. Lee 2020-000227, with *Bacidia schweinitzii*, *Coenogonium pineti* (Ach.) Lücking & Lumbsch, *Phaeophyscia rubropulchra* (Degel.) Moberg, *Porina melanops* Malme (KBA-L-0000427); same locality, on bark of *Idesia polycarpa* Maxim., 25 May 2020, B.G. Lee 2020-000231, with *Bacidia schweinitzii*, *Porina* aff. *melanops* (KBA-L-0000431); same locality, on bark of *Idesia polycarpa*, 25 May 2020, B.G. Lee 2020-000232 (KBA-L-0000432); same locality, on bark of *Taxicodendron vernicifluum* (Stokes) F. A. Barkley, 25 May 2020, B.G. Lee 2020-000233, with *Biatora* aff. *pacifica*, *Lecidea* sp., *Phaeophyscia rubropulchra*, *Rinodina* sp., *Traponora varians* (Ach.) J. Kalb & Kalb (KBA-L-0000433); South Korea, North Gyeongsang Province, Bonghwa, Chunyang-myeon, Mt. Okseok, 37°00.91'N, 128°46.65'E, 1,085 m alt., on bark of *Quercus mongolica*, 15 September 2020, B.G. Lee & H.J. Lee 2020-001159, with *Anisomeridium polypori* (Ellis & Everh.) M.E. Barr, *Bacidia schweinitzii*, *Rinodina* sp. (KBA-L-0001359); same locality, on bark of *Quercus mongolica*, 15 September 2020, B.G. Lee & H.J. Lee 2020-001162, with *Rinodina* sp. (KBA-L-0001362); South Korea, North Jeolla Province, Jangsu, Mt. Youngchui, 35°38.59'N, 127°37.00'E, 907 m alt., on bark of *Carpinus tschonoskii* Maxim., 08 June 2021, B.G. Lee & H.J. Lee 2021-000563, with *Lecanora megalocheila* (Hue) H. Miyaw., *Rinodina orientalis* Sheard (KBA-L-0002035); same locality, on bark of *Carpinus tschonoskii*, 08 June 2021, B.G. Lee & H.J. Lee 2021-000565, with *Arthonia apatetica* (A. Massal.) Th. Fr., *Lecidella euphorea* (Flörke) Kremp. (KBA-L-0002037; GenBank ON352611 for ITS); same locality, on bark of *Carpinus tschonoskii*, 08 June 2021, B.G. Lee & H.J. Lee 2021-000569, with *Anisomeridium polypori*, *Lecidella euphorea*, *Rinodina orientalis*, *Scoliciosporum* sp. (KBA-L-0002041); same locality, on bark of *Carpinus tschonoskii*, 08 June 2021, B.G. Lee & H.J. Lee 2021-000573, with *Arthonia apatetica*, *Lecanora* aff. *imshaugii* Brodo, *Lecidella euphorea*, *Porina hirsuta* (KBA-L-0002045); South Korea, North Jeolla Province, Jangsu, Mt. Jangan, 35°38.58'N, 127°36.96'E, 925 m alt., on bark of *Carpinus tschonoskii*, 09 June 2021, B.G. Lee & H.J. Lee 2021-000759 (KBA-L-0002231); same locality, on bark of *Carpinus tschonoskii*, 09 June 2021, B.G. Lee & H.J. Lee 2021-000760, with *Phaeophyscia adiastola* (Essl.) Essl., *Porina hirsuta*, *Rinodina orientalis*, *Scoliciosporum chlorococcum* (Graewe ex Stenh.) Vězda (KBA-L-0002232); same locality, on bark of *Carpinus tschonoskii*, 09 June 2021, B.G. Lee & H.J. Lee 2021-000766, with *Lecania* sp., *Phaeophyscia* sp., *Rinodina orientalis* (KBA-L-0002238); South Korea, North Jeolla Province, Jangsu, Mt. Baegun, 35°36.76'N, 127°36.85'E, 661 m alt., on bark of *Cornus walteri* Wangerin, 10 June 2021, B.G. Lee & H.J. Lee 2021-000926 (KBA-L-0002398); same locality, on bark of *Cornus walteri*, 10 June 2021, B.G. Lee & H.J. Lee 2021-000927 (KBA-L-0002399); same locality, on bark of *Cornus walteri*, 10 June 2021, B.G. Lee & H.J. Lee 2021-000928 (KBA-L-0002400); same locality, on bark of *Cornus wal-*

teri, 10 June 2021, B.G. Lee & H.J. Lee 2021-000929, with *Phaeophyscia adiastola* (KBA-L-0002401); same locality, on bark of *Cornus walteri*, 10 June 2021, B.G. Lee & H.J. Lee 2021-000930, with *Phaeophyscia rubropulchra* (KBA-L-0002402); same locality, on bark of *Cornus walteri*, 10 June 2021, B.G. Lee & H.J. Lee 2021-000931, with *Lecanora* sp., *Phaeophyscia adiastola* (KBA-L-0002403); same locality, on bark of *Cornus walteri*, 10 June 2021, B.G. Lee & H.J. Lee 2021-000932 (KBA-L-0002404).

***Bacidia friesiana* (Hepp) Körb., Parerga lichenol. (Breslau) 2: 133 (1860) [1865]**
Fig. 4

Description. Thallus corticolous, crustose, thin, little developed or indistinct, generally not continuous, minutely granular with contiguous granules when developed, pale grey with slightly brownish colour, margin indeterminate. Prothallus not detected.

Apothecia consistently flat or convex when mature, marginate, without pruina, 0.1–0.5 mm diam. (mean = 0.23, SD = 0.07, n = 107). Disc biatorine, without thalline exciple, pale pink to pale yellow when young and darker (particularly around margin) when mature. Proper exciple hyaline with or without pale brown pigment, the pigment slightly thicker close to hymenium or excipular rim, 40–50 μm wide laterally. Epihymenium bluish-green, ca. 5 μm high. Hymenium hyaline, 40–45 μm high. Hypothecium hyaline, 50–60 μm high; upper hypothecium paraplectenchymatous (globular to angular), lower hypothecium prosoplectenchymatous (periclinally or irregularly arranged). Crystals or oil droplets absent. Asci narrowly clavate, 8-spored, 39–41 × 10–12 μm (n = 3). Ascospores acicular to filiform, 3- or 7-septate, 28–38 × 1.5–2.5 μm (n = 14). Pycnidia not detected.

Chemistry. Epihymenium K–, C–. Hymenium K– or a few undeveloped asci K+ purplish. No lichen substance was detected by TLC.

Notes. *Bacidia friesiana* is similar to *B. circumspecta* (Norrl. & Nyl.) Malme and *B. igniarii* (Nyl.) Oxner (syn. *Scutula igniarii* (Nyl.) S. Ekman) in having epihymenium with green pigments, proper exciple without crystals and dark hypothecium amongst corticolous species. However, *B. friesiana* differs from the latter two by the excluded margin of apothecia and acicular ascospores. The latter species have a permanent margin of apothecia and bacilliform or clavate ascospores (Ekman 1996).

Phylogenetic analysis resulted in *B. friesiana* of Korea (ON352609 and ON352610) being nested with the sequences of Russia (MH539765), supported by a bootstrap value of 100 and a posterior probability of 1.00 for the branch (Fig. 2). *Bacidia friesiana* was previously reported from Europe, North America and Russian Far East (Smith et al. 2009; Gerasimova et al. 2018). This is a new record to Korea.

Specimens examined. South Korea, Gangwon Province, Yanggu, Nam-myeon, Dumu-ri, nearby a forested wetland, 38°02.12'N, 128°05.14'E, 421 m alt., on bark of *Salix pierotii*, 28 April 2020, B.G. Lee 2020-000164, with *Mikhtomia gordejevii*, *Candelaria concolor* (Dicks.) Arnold, *Phaeophyscia adiastola*, *Porina* cf. *melanops*, *Rinodina* cf. *subminuta* (KBA-L-0000364); South Korea, Gyeonggi Province, Yangpyeong, Cheongun-myeon, Dowon-ri, a forested wetland, 37°32.55'N, 127°48.60'E, 443 m alt., on bark

of *Salix pierotii*, 31 May 2021, B.G. Lee & H.J. Lee 2021-000438, with *Lecidella euphorea*, *Phaeophyscia adiastola*, *Rinodina orientalis* (KBA-L-0001910; GenBank ON352609 for ITS); same locality, on bark of *Aralia elata* (Miq.) Seem., 31 May 2021, B.G. Lee & H.J. Lee 2021-000440, with *Lecidella euphorea*, *Phaeophyscia adiastola*, *Traponora varians* (KBA-L-0001912); same locality, on bark of *Aralia elata*, 31 May 2021, B.G. Lee & H.J. Lee 2021-000441, with *Hyperphyscia adglutinata* (Flörke) H. Mayrhofer & Poelt, *Rinodina orientalis* (KBA-L-0001913; GenBank ON352610 for ITS); same locality, on bark of *Aralia elata*, 31 May 2021, B.G. Lee & H.J. Lee 2021-000442, with *Rinodina orientalis*, *Traponora varians* (KBA-L-0001914); same locality, on bark of *Aralia elata*, 31 May 2021, B.G. Lee & H.J. Lee 2021-000443, with *Hyperphyscia adglutinata*, *Rinodina orientalis*, *Traponora varians* (KBA-L-0001915); same locality, on bark of *Aralia elata*, 31 May 2021, B.G. Lee & H.J. Lee 2021-000444, with *Phaeophyscia adiastola*, *P. rubropulchra*, *Rinodina orientalis* (KBA-L-0001916); same locality, on bark of *Aralia elata*, 31 May 2021, B.G. Lee & H.J. Lee 2021-000445 (KBA-L-0001917).

Bacidia heterochroa **(Müll. Arg.) Zahlbr., Cat. Lich. Univers. 4: 204 (1926) [1927]**
Fig. 4

Description. Thallus corticolous, crustose, continuous, wrinkled, or warted, pale yellowish-grey, margin indeterminate or determinate. Prothallus generally not present or locally present as blackish bordering a different lichen.

Apothecia flat, marginate, without pruina, 0.2–0.6 mm diam. (mean = 0.33, SD = 0.11, n = 72). Disc lecideine, without thalline exciple, blackish or reddish-black. Proper exciple hyaline with pale brown pigment dispersed, pigment slightly thicker close to hymenium, 80–100 μm wide laterally. Epihymenium brown to dark brown, ca. 10 μm high. Hymenium hyaline, 80–95 μm high. Hypothecium hyaline, 80–120 μm high, with a little pale yellow pigment. Crystals or oil droplets absent. Asci narrowly clavate to cylindrical, 8-spored, 42–48 × 12–13 μm (n = 3). Ascospores acicular to filiform, 9- or 10-septate, 36–67 × 2.5–4 μm (n = 11). Pycnidia not detected.

Chemistry. Epihymenium K+ purple or intensifying, extending to excipular rim. No lichen substance was detected by TLC.

Notes. *Bacidia heterochroa* is the most similar to *B. laurocerasi* (Delise ex Duby) Zahlbr. in having smooth thallus without granules, absence of crystals in exciple, epihymenium without green pigments, pale to colourless hypothecium, K+ purple in apothecial section and narrow ascospores less than 4 μm wide amongst corticolous species. However, *B. heterochroa* differs from *B. laurocerasi* by distinctly brown-pigmented paraphysial tips, less than 16-septate ascospores which are shorter but wider (less than 80 μm long but over 3.5 μm wide) and substrate preference to deciduous trees or shrubs (Ekman 1996; Brodo 2016; also see the key couplet 21).

Phylogenetic analysis resulted in *B. heterochroa* of Korea (ON352606, ON352612 and ON352613) being nested in a sister clade to *B. laurocerasi*, supported by a bootstrap value of 75 without a posterior probability as the Maximum Likelihood analysis did not match with the Bayesian Inference for the clade. The sequences of *B. het-*

erochroa were not compared with previous records due to the lack of data (Fig. 2). *Bacidia heterochroa* was previously reported from Thailand in Asia (Aptroot et al. 2007) and this is a new record to northeastern Asia.

Specimens examined. South Korea, Gangwon Province, Yanggu, Nam-myeon, Dumu-ri, a forested wetland, 38°02.12'N, 128°05.14'E, 421 m alt., on bark of *Salix koriyanagi* Kimura ex Goerz, 28 April 2020, B.G. Lee 2020-000186 (KBA-L-0000386; GenBank ON352606 for ITS); South Korea, South Jeolla Province, Damyang, Changpyeong-myeon, Oedong-ri, a forested wetland, 35°12.00'N, 127°00.88'E, 338 m alt., on bark of *Fraxinus rhynchophylla* Hance, 12 May 2021, B.G. Lee & D.Y. Kim 2021-000214 (KBA-L-0001686); South Korea, Gangwon Province, Jeongseon, Imgye-myeon, Gamok-ri, a forested wetland, 37°32.47'N, 128°57.72'E, 760 m alt., on bark of *Acer tartaricum* subsp. *ginnala* (Maxim.) Wesm., 17 June 2021, B.G. Lee & H.J. Lee 2021-001241, with *Lecanora chionocarpa* Hue (KBA-L-0002713); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001242, with *Phaeophyscia adiastola* (KBA-L-0002714); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001255, with *Opeltia flavorubescens*, *Phaeophyscia adiastola* (KBA-L-0002727; GenBank ON352612 for ITS); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001257, with *Hyperphyscia adglutinata*, *Lecidella euphorea* (KBA-L-0002729); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001262, with *Lecidella euphorea*, *Phaeophyscia adiastola*, *Rinodina orientalis* (KBA-L-0002734; GenBank ON352613 for ITS); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001263, with *Opeltia flavorubescens*, *Phaeophyscia adiastola*, *Rinodina orientalis* (KBA-L-0002735); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001267, with *Lecidella euphorea*, *Porina hirsuta*, *Rinodina orientalis*, *Straminella varia* (KBA-L-0002739); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001269, with *Lecidella euphorea*, *Opeltia flavorubescens*, *Phaeophyscia rubropulchra*, *Rinodina orientalis* (KBA-L-0002741).

Bacidia suffusa **(Fr.) A. Schneid., Guide Study Lich.: 110 (1898)**
Fig. 4

Description. Thallus corticolous, crustose, continuous, wrinkled, warted or subsquamulose, often granular locally, whitish pale grey. Prothallus generally not present or present as dark brown to black between different colonies.

Apothecia flat, marginate, with a little or heavy white pruina, generally more pruinose at margin, 0.3–1.7 mm diam. (mean = 0.75, SD = 0.28, n = 116). Disc lecideine, without thalline exciple, brown to dark brown. Proper exciple with radiating clusters of crystals produced around hypothecium and expanding to excipular rim and finally shown as pruina on surface, hyaline downwards but brown around rim, the brown concolorous or slightly paler to epihymenium, 80–100 µm wide laterally. Epihymenium brown to dark brown, ca. 10 µm high, with pruina (ca. 10 µm high) on surface. Hymenium hyaline, 70–80 µm high. Hypothecium hyaline, 80–100 µm

high. Other small crystals present a few in upper hypothecium. Oil droplets absent. Asci cylindrical, 8-spored, 65–75 × 10–16 μm (n = 7). Ascospores acicular to filiform, up to 13-septate, 45–70 × 2.5–4.5 μm (n = 10). Pycnidia not detected.

Chemistry. Thallus K+ yellow, KC–, C–, Pd–, UV–. Epihymenium K–. Atranorin was detected by TLC.

Notes. *Bacidia suffusa* is the most similar to *B. russeola* (Kremp.) Zahlbr. in having dark apothecia, generally colourless epihymenium without green pigment, long ascospores with the L/W ratio over 11, pale or colourless hypothecium and K+ purple reaction on epihymenium and nearby excipular rim amongst corticolous species. However, *B. suffusa* differs from *B. russeola* by the presence of pruina on the disc and in proper exciple as radiating clusters of crystals and more than 10-septate ascospores (Ekman 1996).

Phylogenetic analysis resulted in *B. suffusa* of Korea (ON352605, ON352614, ON352615 and ON352616) being nested in a sister clade of the sequences of Pakistan (MW728313 and MW788561), Russia (MH048615, MH048616 and MH048617) or U.S.A. (MH048618 and MH048619). The molecular data of Korea converged into the previous data of *B. suffusa*, supported by a bootstrap value of 100 and a posterior probability of 1.00 for the branch (Fig. 2). *Bacidia suffusa* was previously detected from North America, North Caucasus, Russian Far East and Pakistan, but rare or absent in Europe (Otte 2007; Gerasimova et al. 2018, 2021; Adrees et al. 2022). This is a new record to Korea.

Specimens examined. South Korea, Gangwon Province, Yanggu, Nam-myeon, Dumu-ri, a forested wetland, 38°02.12'N, 128°05.14'E, 421 m alt., on bark of *Salix pierotii* Miq., 28 April 2020, B.G. Lee 2020-000158 (KBA-L-0000358); same locality, on bark of *Salix pierotii*, 28 April 2020, B.G. Lee 2020-000159 (KBA-L-0000359; GenBank ON352605 for ITS); same locality, on bark of *Salix pierotii*, 28 April 2020, B.G. Lee 2020-000168, with *Candelaria concolor*, *Phaeophyscia adiastola*, *Phaeophyscia hirtuosa* (Kremp.) Essl. (KBA-L-0000368); South Korea, Gangwon Province, Gangneung, Okgye-myeon, Mt. Seokbyung, 37°34.45'N, 128°55.01'E, 271 m alt., on bark of *Acer pictum* var. *mono*, 17 June 2020, B.G. Lee & H.J. Lee 2020-000799 (KBA-L-0000999); South Korea, Gangwon Province, Jeongseon, Imgye-myeon, Gamok-ri, a forested wetland, 37°32.47'N, 128°57.72'E, 760 m alt., on bark of *Fraxinus chiisanensis* Nakai, 17 June 2021, B.G. Lee & H.J. Lee 2021-001304, with *Normandina pulchella* (Borrer) Nyl., *Phaeophyscia* sp. (KBA-L-0002776; GenBank ON352614 for ITS); same locality, on bark of *Fraxinus chiisanensis*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001305, with *Anisomeridium polypori*, *Normandina pulchella*, *Phaeophyscia* sp., *Porina hirsuta* (KBA-L-0002777); same locality, on bark of *Fraxinus chiisanensis*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001306, with *Normandina pulchella*, *Opeltia flavorubescens*, *Phaeophyscia adiastola* (Essl.) Essl. (KBA-L-0002778; GenBank ON352615 for ITS); same locality, on bark of *Fraxinus chiisanensis*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001308, with *Phaeophyscia adiastola* (KBA-L-0002780); same locality, on bark of *Fraxinus chiisanensis*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001320, with *Opeltia flavorubescens* (KBA-L-0002792); same locality, on bark of *Acer tartaricum* subsp. *ginnala*, 17 June 2021, B.G. Lee & H.J. Lee 2021-001363 (KBA-L-0002835; GenBank ON352616 for ITS).

Key to the species of *Bacidia* in Korea (19 taxa)

The key is composed of all 19 species in the genus *Bacidia* of Korea, including synonyms in *Bacidina* and *Toniniopsis* species.

1 Epihymenium with green pigment **2**
– Epihymenium colourless, yellow-brown, brown to dark brown, but without green pigment **5**
2 Proper exciple with radiating clusters of coarse crystals (up to 7 µm wide); hymenium ca. 100 µm high; ascospores 40–68 × 2.5–3 µm; atranorin present ..... ***B. schweinitzii***
– Proper exciple without crystals; hymenium less than 70 µm high; ascospores less than 50 µm long; without substance **3**
3 Hypothecium colourless to pale blue-green; thallus pale grey to pale brown-grey without green colour ..... ***B. friesiana***
– Hypothecium colourless to brown, dark red-brown; thallus grey-green to green-brown **4**
4 Proper exciple with green pigment at rim, pale to colourless downwards; hypothecium K– or K+ green-brown; generally on rock or occasionally on bark or moss. ..... ***B. egenula* (*Bacidina egenula*)**
– Proper exciple colourless at rim, red-brown to black-brown downwards; hypothecium K+ purple; on bark ..... ***B. subincompta* (*Toniniopsis subincompta*)**
5 On rock **6**
– On bark or wood **12**
6 Apothecia pruinose **7**
– Apothecia not pruinose **8**
7 Thallus coarsely granular without forming soredia; apothecia 0.7–1.2 mm diam.; hymenium 70–100 µm high; hypothecium colourless to pale yellow or pale orange; ascospores 40–70 × 2.5–3 µm, 3- to 7-septate ..... ***B. rubella***
– Thallus granular with soredia; apothecia 0.3–0.7 mm diam.; hymenium 40–50 µm high; hypothecium orange-brown to dark red-brown; ascospores 24–46 × 1–2 µm, 1- to 3-septate ..... ***B. arnoldiana* (*Bacidina arnoldiana*)**
8 Disc brown, red-brown to black; hypothecium pale brown to dark brown **9**
– Disc pale yellow, pale orange to dark brown; hypothecium colourless to pale yellow or pale orange **10**
9 Proper exciple dark coloured; ascospores 25–35 × 6–10 µm, with L/W ratio less than 10 ..... ***B. hakonensis***
– Proper exciple colourless to pale brown; ascospores 24–46 × 1–2 µm, with L/W ratio over 10 ..... ***B. arnoldiana* (*Bacidina arnoldiana*)**
10 Thallus rimose, wrinkled or warted, but not granular; disc pale yellow or pale grey; epihymenium K– ..... ***B. chloroticula* (*Bacidina chloroticula*)**
– Thallus granular; disc pale to dark brown; epihymenium K+ purple **11**

11 Thallus granular forming isidia- or coral-like structures; prothallus absent; apothecia flat; ascospores 25–34 × 1.1–1.9 μm; occasionally on old wood ..... ***B. egenuloidea* (*Bacidina egenuloidea*)**

– Thallus granular-warted; white prothallus present on border; apothecia flat to convex; ascospores 24–43 × 2–2.5 μm ..... ***B. inundata* (*Bacidina inundata*)**

12 On wood. Thallus granular forming isidia- or coral-like structures; disc pale orange to dark purple-brown; proper exciple orange-brown to brown at rim; on old wood, but generally on rock..... ***B. egenuloidea* (*Bacidina egenuloidea*)**

– On bark **13**

13 Proper exciple with radiating clusters of crystals; white pruina present; atranorin present as a major compound or a trace **14**

– Proper exciple without crystals; pruina absent; without substance **17**

14 Hypothecium brown-orange to dark brown; apothecial section K+ purple-red.... ***B. polychroa***

– Hypothecium colourless to pale yellow or pale orange; apothecial section K–.... **15**

15 Thallus generally coarsely granular, pale grey to green-grey; prothallus white to pale grey when present; ascospores up to 9-septate ..... ***B. rubella***

– Thallus smooth, wrinkled, warted or granular locally, white-grey to grey; prothallus absent; ascospores up to 13-septate **16**

16 Thallus grey; disc not pruinose generally, but sometimes white-pruinose; proper exciple with radiating clusters of minute crystals (ca. 0.5 μm wide); epihymenium without distinct colour; ascospores 50–85 × 2.6–3.4 μm ..... ***B. fraxinea***

– Thallus whitish pale grey; disc light to heavily pruinose; proper exciple with radiating clusters of coarse crystals (up to 10 μm wide); epihymenium brown to dark brown; ascospores 45–70 × 2.5–4.5 μm ..... ***B. suffusa***

17 Thallus granular with soredia-like goniocysts..... **18**

– Thallus smooth, wrinkled, warted or rarely granular, but without soredia..... **19**

18 Hypothecium colourless; conidia curved without hook..... ***B. delicata* (*Bacidina delicata*)**

– Hypothecium orange-brown to dark red-brown; conidia hooked..... ***B. sulphurella* (*Bacidina sulphurella*)**

19 Disc purple-brown to black or slightly blackish when mature; epihymenium K+ purple **20**

– Disc pale yellow, pale grey or pale brown; epihymenium K– **22**

20 Proper exciple colourless to pale yellow at rim; thallus olive-green; apothecia generally pale yellow to pale orange with slightly blackish pigment; epihymenium colourless with a little pale yellow-brown pigment ..... ***B. fuscopallida***

– Proper exciple dark brown to black-brown at rim; thallus white to pale grey; apothecia purple-brown to black; epihymenium brown to dark brown **21**

21 Brown pigment of epihymenium deposited in caps of paraphysial tips; thallus wrinkled or warted, but not squamulose; prothallus blackish on border when present; ascospores 32–67 × 2.5–4.5 μm, 3- to 15-septate..... ***B. heterochroa***

– Brown pigment of epihymenium distributed in upper hymenial jelly; thallus wrinkled or warted, sometimes squamulose to varnish-like crust; prothallus

white between areoles; ascospores 45–80 × 2–3.5 µm, 7- to 28-septate .... ***B. laurocerasi***

22 Thallus rimose, wrinkled or warted; apothecia ca. 0.2 mm diam.; hypothecium colourless; ascospores 24–28 × 1–1.2 µm, 0- to 3-septate; occasionally on rock .. .. ***B. chloroticula* (*Bacidina chloroticula*)**

– Thallus granular to smooth; apothecia 0.4–1.4 mm diam.; hypothecium straw, yellow-brown to red-brown; ascospores 45–70 × 1.5–4 µm, 3- to 15-septate .. **23**

23 Proper exciple yellow-brown to brown at rim; epihymenium yellow-brown; ascospores 1.5–2.5 µm wide, 3- to 7-septate ..... ***B. arceutina***

– Proper exciple colourless to pale yellow at rim; epihymenium colourless; ascospores 2–4.5 µm wide, 3- to 15-septate ..... ***B. ekmaniana***

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A new study of *Nagrajomyces*: with two new species proposed and taxonomic status inferred by phylogenetic methods

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Abstract

Nagrajomyces (*incertae sedis*, *Ascomycota*) is a monotypic genus with a previously unknown systematic position. In this report, two new species are proposed, *Nagrajomyces fusiformis* and *Nagrajomyces laojunshanensis*. These new taxa are proposed based on morphological characteristics evident via light microscopy and molecular data. Multi-locus phylogenetic analyses (ITS rDNA, nrLSU rDNA, *RPB2*, and *TEF1-α*) show that specimens recently collected in Yunnan Province, China are closely related to *Gnomoniaceae*. Both new species and known species were discovered repeatedly in their asexual developmental form exclusively on twigs of *Rhododendron* spp. (*Ericaceae*). This indicates a host specificity of *Nagrajomyces* spp. for species of *Rhododendron*.

Keywords

host specificity, *Nagrajomyces*, new taxa, phylogeny

Introduction

Gnomoniaceae is a distinct family of *Diaporthales*, established by Winter (1886). The traditional classification of species in *Gnomoniaceae* was mainly based on morphological features, such as the morphology of ascomata and ascospores as well as the position of necks (Barr 1978; Monod 1983). Sogonov et al. (2008) used phylogenetic analyses of molecular sequence data of several genes (*TEF1-a*, nrLSU, and *RPB2*) to revise the concepts of leaf-inhabiting genera, and discovered that several traditional genera in *Gnomoniaceae* are polyphyletic. Phylogenetic analyses indicate that host specificity can be used to circumscribe genera and species of *Gnomoniaceae*. Based on phylogenetic analyses and morphological characteristics, Senanayake et al. (2017) described new taxa and excluded some genera from *Gnomoniaceae*. Since then, additional genera have been introduced for species observed in sexual developmental stages, as well as those that have only been observed as pycnidial asexual morphs, and rarely for species known in both sexual and asexual forms (Senanayake et al. 2018; Crous et al. 2019; Jiang et al. 2019; Minoshima et al. 2019; Yang et al. 2020).

Many species of *Gnomoniaceae* are important plant pathogens, such as *Apiognomonia errabunda* (Roberge ex Desm.) Höhn, which causes oak anthracnose (Sogonov et al. 2007), *Gnomoniopsis fructicola* (G. Arnaud) Sogonov, which causes strawberry stem rot (Maas 1998), and *Ophiognomonia leptostyla* (Fr.) Sogonov, which causes walnut anthracnose (Neely and Black 1976). Species of *Gnomoniaceae* can also have wide host ranges, with species on *Fagaceae*, *Onagraceae*, and *Rosaceae* being frequently infected. Species of *Rhododendron* (*Ericaceae*), which is the largest genus of woody plants in the northern hemisphere, are also known hosts of species of *Gnomoniaceae* (Monod 1983).

Nagrajomyces (*incertae sedis*, *Ascomycota*) is a monotypic genus based on *N. dictyosporus* Mel'nik (Mel'nik 1984; Nag Raj 1993). It was discovered in Russia, where it grows on twigs of *Rhododendron aureum* and develops stalked, unilocular, or plurilocular conidiomata as well as muriform conidia, and each conidium bears an apical appendage that is single, unbranched, attenuated, flexuous and can be more than 100 μm long (Mel'nik 1984; Nag Raj 1993).

In the present study, two new species were discovered on twigs of *Rhododendron* spp. in Yunnan and assigned to the genus *Nagrajomyces* based on morphological characteristics, habitat, and host. Phylogenetic analysis revealed that the proposed *Nagrajomyces* species belong to *Gnomoniaceae*.

Materials and methods

Specimen collections and isolation

Fieldwork for the discovery of fungi was conducted during June 2021 in Yunnan Province, China. Fresh pycnidia were repeatedly discovered and collected on twigs of *Rhododendron* spp. Twigs with conidiomata were packed in paper bags and transported to the laboratory

for morphological tests. Conidiomata were cut off in the laboratory using a razor blade, wrapped in paper packets, disinfected with 75% ethanol for 10 s, then 10% sodium hypochlorite for 2 min 30 s, and rinsed with distilled water three times. After absorbing the water with sterile filter paper, the conidiomata were transferred to potato dextrose agar (PDA) plates (Jiang et al. 2021) then incubated at 25 °C to obtain cultures. Dry specimens were deposited at the China Forest Biodiversity Museum of the Chinese Academy of Forestry (CAF; http://museum.caf.ac.cn) and the Herbarium of the College of Life Science, Capital Normal University (BJTC; http://smkxxy.cnu.edu.cn). Ex-type living cultures were deposited at the China Forestry Culture Collection Center (CFCC; http://cfcc.caf.ac.cn/).

Morphological analysis

Conidiomata were photographed and cut by hand using a razor blade under a Nikon SMZ-1000 stereomicroscope (Japan). Morphological characteristics of conidiomata, conidiophores, and conidia were photographed and measured with an Olympus EX-51 upright microscope (Japan), and for each structure at least 20 measurements were made. Color values were taken from ColorHexa (https://www.colorhexa.com/).

DNA extraction, polymerase chain reaction amplification, and phylogeny

Genomic DNA was extracted from specimens and cultures via the M5 Plant Genomic DNA Kit (Mei5 Biotechnology Co., Ltd., China) in accordance with the manufacturer's instructions. Table 1 summarizes the primers used to obtain sequence data for ITS rDNA, nrLSU rDNA, *RPB2*, and *TEF1-a*, and the polymerase chain reaction (PCR) amplification protocols. PCR products were analyzed in 1% electrophoretic agarose gel with a 200-bp DNA ladder, purified, and sequenced by Beijing Zhongke Xilin Biotechnology Co., Ltd. (Beijing, China). SeqMan was used to align the sequences obtained by forward and reverse primers to obtain a consensus sequence. A partition homogeneity test was performed to determine the congruence of the four datasets (Farris et al. 1994). Sequences for phylogenetic analyses were selected based on Yang et al. (2020), supplemented by sequences of *Apiosporopsis carpinea* (Fr.) Mariani, *Apiosporopsis* sp., *Juglanconis juglandina* (Kunze) Voglmayr & Jaklitsch, *Juglanconis oblonga* (Berk.) Voglmayr & Jaklitsch, and *Melanconis marginalis* (Peck) Wehm. from Senanayake et al. (2018) used as outgroup taxa. All sequences used in this study are listed in Table 2. Subsequent alignments were generated with online MAFFT tools

Table 1. Primer information and PCR amplification protocols.

Gene	Primer pairs	Reference	Amplification conditions
ITS rDNA	ITS1F/ITS4	White et al. (1990); Gardes and Bruns (1993)	Phillips et al. (2008)
LSU rDNA	LR0R/LR5	Vilgalys and Hester (1990); Rehner and Samuels (1994)	Phillips et al. (2008)
TEF1-a	EF1-728F/EF1-986R	Carbone and Kohn (1999)	Glass and Donaldson (1995)
RPB2	fRPB2-5F/fRPB2-7cR	Liu et al. (1999)	Liu et al. (1999)

Table 2. Sequences used in phylogenetic analyses. References to sequences generated in the present study are emphasized in bold.

Taxa	Voucher	ITS rDNA	LSU rDNA	*RPB2*	*TEF1-α*	References
Alnecium auctum	CBS 124263	KF570154	KF570154	KF570170	KF570200	Voglmayr and Jaklitsch (2014)
Ambarignomonia petiolorum	CBS 116866	EU199193	AY818963	EU199151	–	Mejía et al. (2008)
Ambarignomonia petiolorum	CBS 121227	EU254748	EU255070	EU219307	EU221898	Mejía et al. (2008)
Amphiporthe tiliae	CBS 119289	EU199178	EU199122	EU199137	–	Mejía et al. (2008)
Apiognomonia errabunda	AR 2813	DQ313525	–	DQ862014	DQ313565	Sogonov et al. (2007)
Apiognomonia veneta	MFLUCC 16-1193	MF190114	MF190056	–	–	Senanayake et al. (2017)
Apioplagiostoma populi	858501	KP637024	–	–	–	Wijekoon et al. (2021)
Apiosporopsis carpinea	CBS 771.79	–	AF277130	–	–	Zhang and Blackwell (2001)
Apiosporopsis sp.	Masuya 11Af2-1	–	AB669034	–	–	Osono and Masuya (2012)
Asteroma alneum	CBS 109840	EU167609	EU167609	–	–	Simon et al. (2009)
Asteroma sp.	Masuya 8Ah9-1	–	AB669035	–	–	Osono and Masuya (2012)
Cryptodiaporthe acerina	AR 3822	EU254755	EU255075	EU219253	EU221879	Sogonov et al. (2008)
Cryptodiaporthe aubertii	CBS 114196	KX929767	KX929803	KX929838	KX929732	Meyer et al. (2017)
Cryptosporella hypodermia	CBS 116866	EU199181	AF408346	EU199140	–	Mejía et al. (2008)
Ditopella biseptata	MFLU 15-2661	MF190147	MF190091	MF377616	–	Senanayake et al. (2017)
Ditopella ditopa	CBS 109748	DQ323526	EU199126	EU199145	–	Mejía et al. (2008)
Ditopellopsis sp.	CBS 121471	EU254763	EU255088	EU219254	EU221936	Sogonov et al. (2008)
Flavignomonia rhoigena	CFCC 53118	MK432674	MK429917	MK578102	–	Jiang et al. (2019)
Flavignomonia rhoigena	CFCC 53119	MK432675	MK429918	MK578103	–	Jiang et al. (2019)
Gnomonia gnomon	CBS 199.53	DQ491518	AF408361	EU219295	EU221885	Sogonov et al. (2008)
Gnomonia gnomon	CBS 829.79	AY818957	AY818964	–	EU221905	Sogonov et al. (2005)
Gnomoniella microspora	BPI 877571	EU254765	–	–	–	Sogonov et al. (2008)
Gnomoniopsis alderdunensis	CBS 125680	GU320825	–	–	–	Walker et al. (2010)
Gnomoniopsis chamaemori	CBS 803.79	EU254808	EU255107	–	–	Sogonov et al. (2008)
Gnomoniopsis racemula	AR 3892	EU254841	EU255122	EU219241	EU221889	Sogonov et al. (2008)
Juglanconis juglandina	WU 35960	KY427145	KY427145	KY427195	KY427214	Voglmayr et al. (2017)
Juglanconis oblonga	TFM FPH 2623	KY427153	KY427153	KY427203	KY427222	Voglmayr et al. (2017)

Taxa	Voucher	ITS rDNA	LSU rDNA	*RPB2*	*TEF1-α*	References
Mamianiella coryli	BPI 877578	EU254862	–	–	–	Sogonov et al. (2008)
Marsupiomyces epidermoidea	MFLU 15-2921	–	MF190058	–	–	Senanayake et al. (2017)
Marsupiomyces quercina	MFLUCC 13-0664	MF190116	MF190061	–	–	Senanayake et al. (2017)
Melanconis marginalis	BPI 748234	–	–	EU219299	EU221886	Sogonov et al. (2008)
Melanconis marginalis	BPI 748446	EU199197	AF408373	EU219301	EU221991	Sogonov et al. (2008)
Neognomoniopsis quercina	CBS 145575	MK876399	MK876440	–	–	Crous et al. (2019)
Nagrajomyces fusiformis	**CAF 800050**	**OP473599**	**OP473595**	**OP484756**	**OP484760**	**This study**
Nagrajomyces fusiformis	**BJTC 1773**	**OP473602**	**OP473598**	**–**	**OP484763**	**This study**
Nagrajomyces laojunshanensis	**CFCC 58177**	**OP456161**	**OP473594**	**OP484755**	**OP484759**	**This study**
Nagrajomyces laojunshanensis	**CAF 800049**	**OP473600**	**OP473596**	**OP484757**	**OP484761**	**This study**
Nagrajomyces laojunshanensis	**BJTC 1849**	**OP473601**	**OP473597**	**OP484758**	**OP484762**	**This study**
Occultocarpon ailaoshanense	LCM 524.01	JF779849	JF779853	JF779856	–	Mejía et al. (2011)
Occultocarpon ailaoshanense	LCM 522.01	JF779848	JF779852	JF779857	JF779862	Mejía et al. (2011)
Ophiognomonia melanostyla	LCM 389.01	JF779850	JF779854	JF779858	–	Mejía et al. (2011)
Ophiognomonia vasiljevae	AR 4298	EU254977	EU255162	EU219331	EU221999	Sogonov et al. (2008)
Phragmoporthe conformis	AR 3632	–	AF408377	–	–	Castlebury et al. (2002)
Plagiostoma aesculi	AR 3640	EU254994	EU255164	EU219269	–	Sogonov et al. (2008)
Plagiostoma rhododendri	CBS 847.79	EU255044	EU255187	EU219272	–	Sogonov et al. (2008)
Pleuroceras oregonense	AR 4333	EU255060	EU255196	EU219313	EU221931	Sogonov et al. (2008)
Pleuroceras pleurostylum	CBS 906.79	EU255061	EU255197	EU219311	EU221962	Sogonov et al. (2008)
Sirococcus conigenus	BPI 871248	EU199201	EU199134	EU199157	–	Mejía et al. (2008)
Sirococcus piceicola	BPI 871166	EU199202	EU199135	EU199158	–	Mejía et al. (2008)
Sirococcus tsugae	BPI 871167	EU199203	EU199136	EU199159	–	Mejía et al. (2008)
Sirococcus tsugae	AR 4010	EF512478	EU255207	EU219289	EU221928	Sogonov et al. (2008)
Tenuignomonia styracis	BPI 892786	–	LC379289	LC379295	LC379283	Minoshima et al. (2019)
Tenuignomonia styracis	BPI 892785	–	LC379288	LC379294	LC379282	Minoshima et al. (2019)
Valsalnicola oxystoma	AR 5137	JX519561	–	–	–	Crous et al. (2012)
Valsalnicola oxystoma	AR 4833	JX519559	JX519563	–	–	Crous et al. (2012)

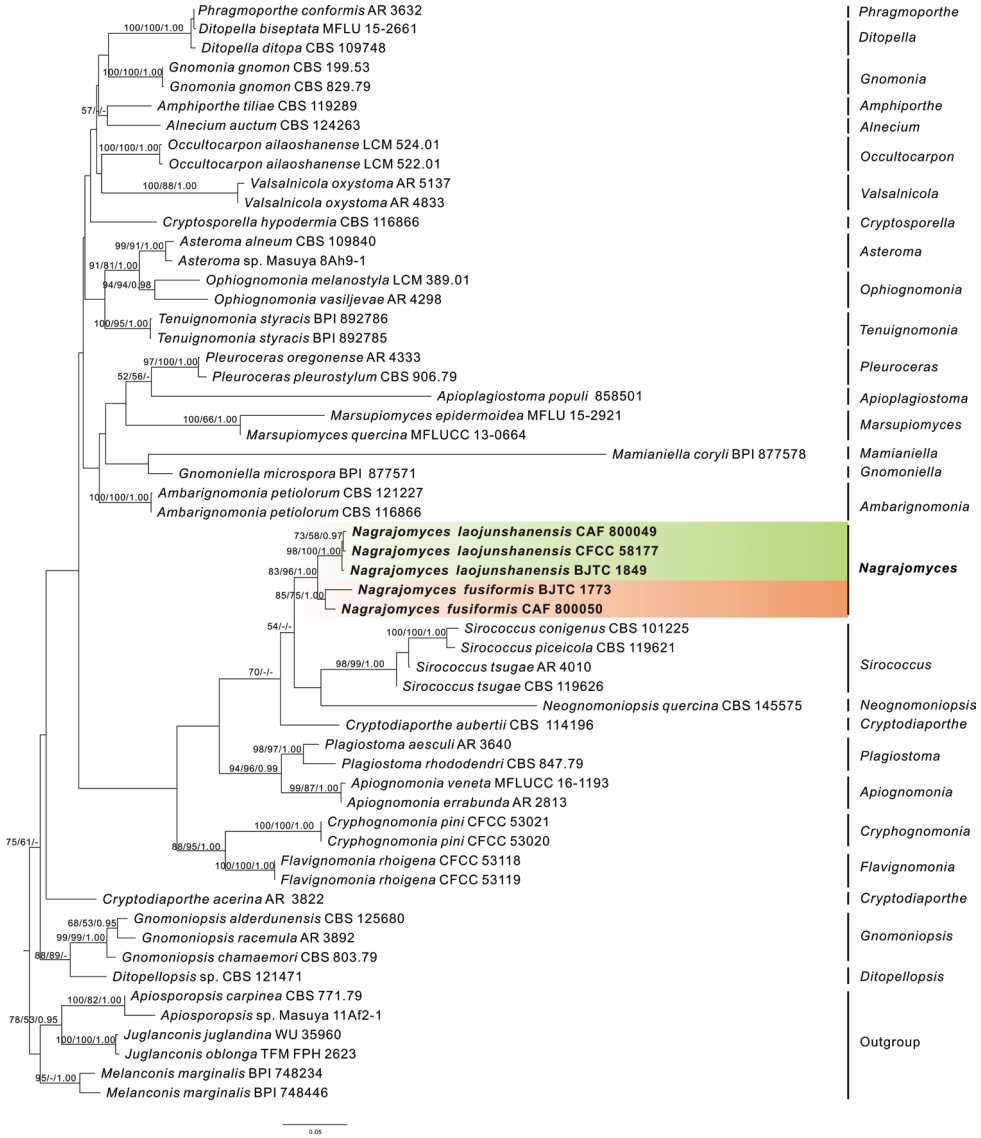

Figure 1. Phylogenetic tree based on an ML analysis of combined ITS rDNA, nrLSU rDNA, *RPB2*, and *TEF1-a* sequences of species of Gnomoniaceae. Bootstrap support values for RAxML and maximum parsimony above 50% and Bayesian posterior probability values above 0.95 are shown at the nodes. The tree is rooted with sequences of *Apiosporopsis carpinea*, *Apiosporopsis* sp., *Juglanconis juglandina*, *J. oblonga*, and *Melanconis marginalis*. References to new sequences are in bold, and the names of the two new species are highlighted by colors.

(https://www.ebi.ac.uk/Tools/msa/mafft/) and edited with Gblocks 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). The maximum likelihood (ML) tree was constructed using RAxML version 8.2.12 (Stamatakis et al. 2005;

Stamatakis 2006; Stamatakis 2014) with GTRGAMMA model and 1000 bootstrap iterations. The multi-locus Bayesian Inference (BI) tree was built by MrBayes version 3.2.6 (Ronquist and Huelsenbeck 2003). Models of nucleotide substitution for each gene used in the Bayesian analysis were determined by MrModeltest v.2.3 (Nylander 2004). Analyses of four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 100,000,000 generations, and other operational methods were applied used as described by Guo et al. (2021). The maximum parsimony (MP) tree was constructed using PAUP version 4.0 beta 10 (Swofford 2003) with 1000 random sequence additions, 1000 maxtrees were obtained, and bootstrap analysis was conducted based on 1000 replicates, with 10 replicates of random stepwise additions of taxa. For further details see Guo et al. (2021). Trees were viewed via Treeview (Page 1996).

Results

Phylogenetic analysis

Multi-locus phylogenetic analyses of species of *Gnomoniaceae* (*Diaporthales*) include sequences of 51 ingroup taxa and sequences of an outgroup formed by *Apiosporopsis carpinea*, *Apiosporopsis* sp., *Juglanconis juglandina*, *J. oblonga*, and *Melanconis marginalis* (Fig. 1). The multi-locus dataset (ITS rDNA, LSU rDNA, *RPB2* and *TEF1-a*) comprises 2875 characters, of which 945 are parsimony-informative, 200 are parsimony-uninformative and 1730 are constant. Maximum parsimony analysis of sequences resulted in one most parsimonious tree with a length (TL) of 3730 steps, a consistency index (CI) of 0.463, a retention index (RI) of 0.690, and a homoplasy index (HI) of 0.537. Bayesian and maximum likelihood trees exhibited topologies similar to this parsimony tree.

The topology of the phylogenetic tree obtained in the current study was similar to the topology presented by Yang et al. (2020). Nineteen sequences of five specimens recently collected on *Rhododendron* spp. in China form a clade with high support values. This clade is sister to sequences of species of *Siroccocus* and *Neognomoniopsis*, but with poor support values. The newly discovered clade is divided into two small subclades labeled *Nagrajomyces fusiformis* and *N. laojunshanensis*.

Taxonomy

***Nagrajomyces fusiformis* C. L. Hou & L. Zhuo, sp. nov.**
MycoBank No: 845666
Figs 2, 3

Etymology. The epithet *fusiformis* refers to fusoid conidia.

Type. China, Yunnan province, Lijiang, Yulong, 26°40'55"N, 99°54'01"E, alt. 2762 m, on dying twigs of *Rhododendron vellereum* Hutch. ex Tagg., 20 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou (holotype CAF 800050).

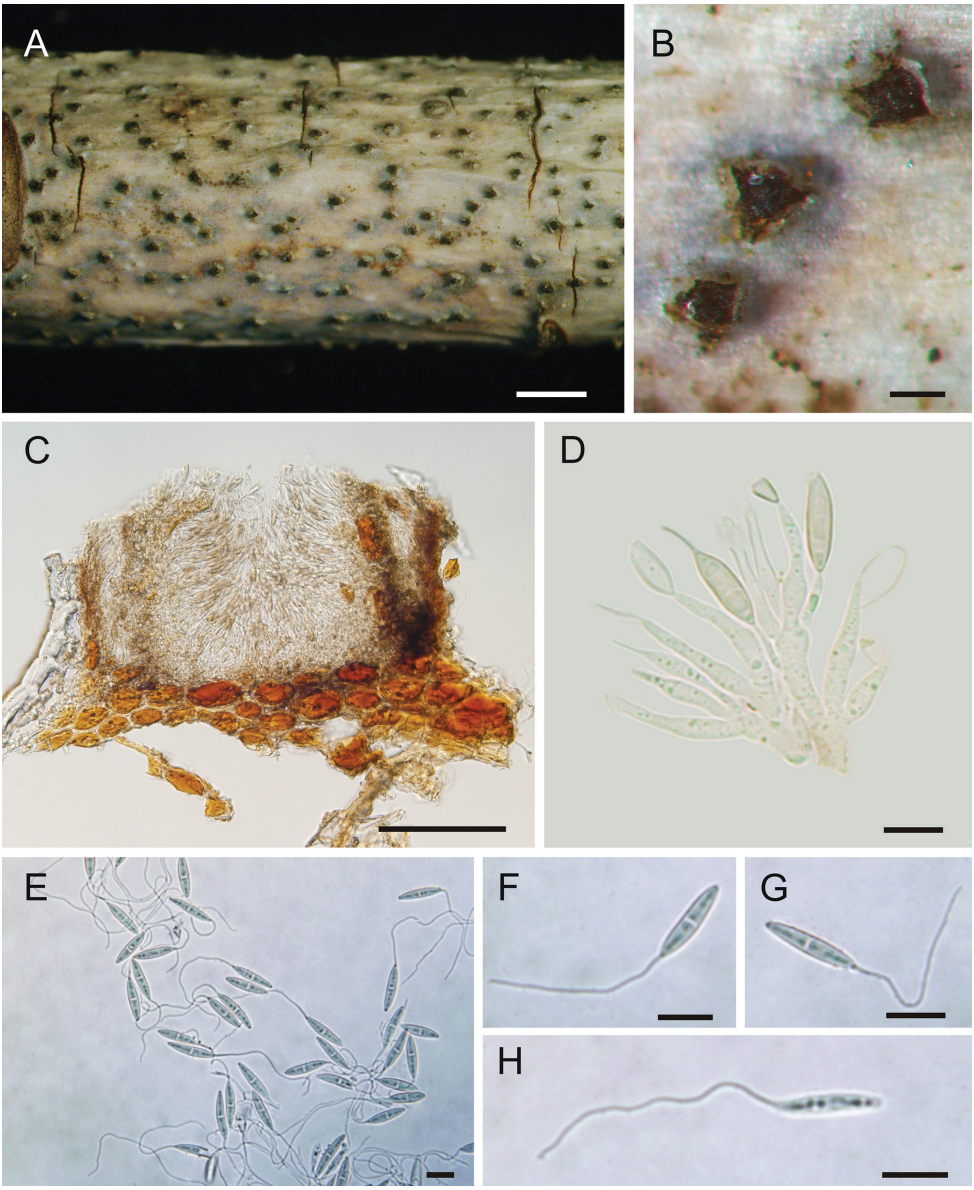

Figure 2. Micrographs of *Nagrajomyces fusiformis* (holotype CAF 800050) on twigs of *Rhododendron vellereum* **A, B** conidiomata on a dying twig **C** vertical section of a conidioma **D** conidiophores and conidia at diverse developmental stages **E–H** conidia with appendages. Scale bars: 2 mm (**A**); 200 µm (**B**); 100 µm (**C**); 10 µm (**D–H**).

Diagnosis. This new species differs from *N. dictyosporus* and *N. laojunshanensis* by fusoid to elongate-fusoid conidia with pointed ends, usually 1-septate and smaller.

Description. ***Conidiomata*** solitary, pycnidial, irregularly plurilocular, subepidermal in origin, immersed at first, then becoming erumpent through the periderm of the host, 545–554 µm diameter, 520–546 µm high, peridium

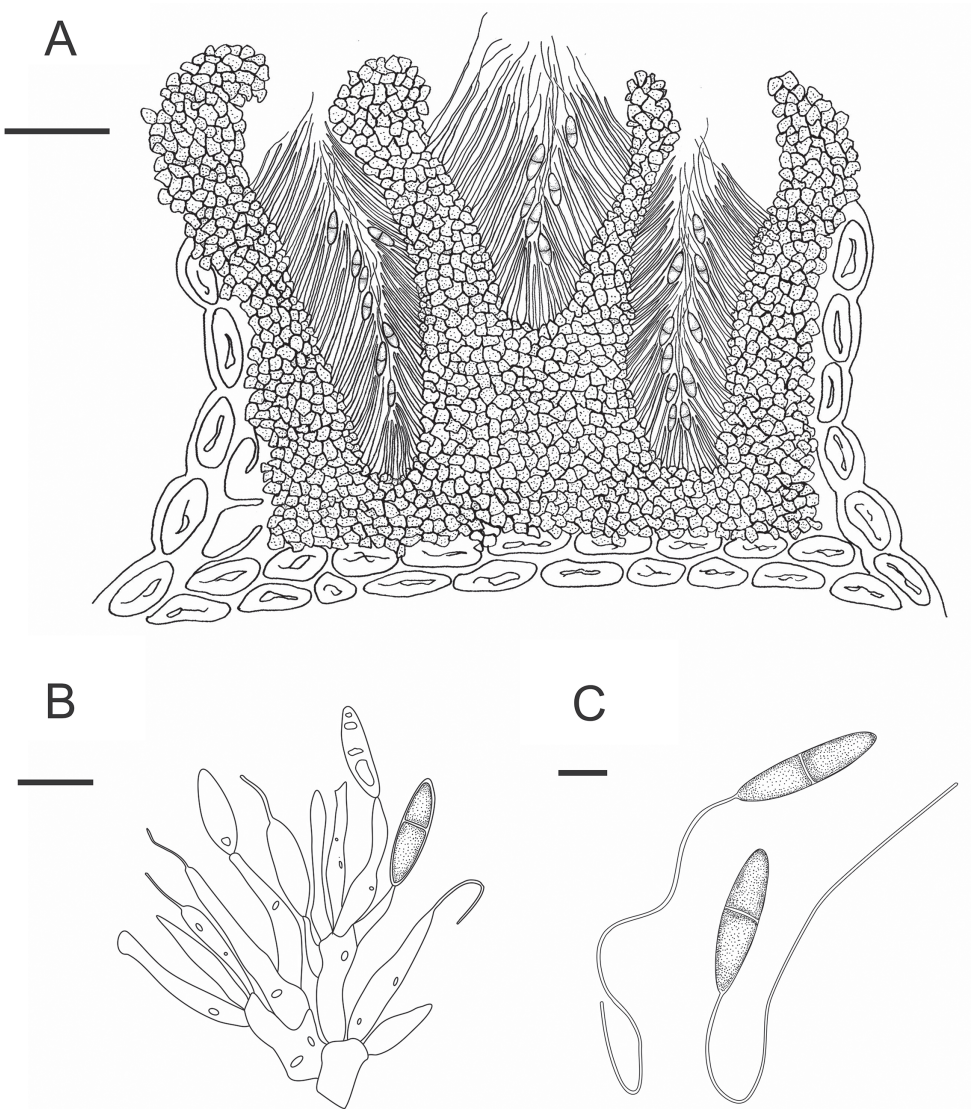

Figure 3. *Nagrajomyces fusiformis* (holotype CAF 800050) **A** vertical section of a conidioma **B** conidiophores and conidia **C** conidia with appendages. Scale bars: 100 μm (**A**); 10 μm (**B**); 5 μm (**C**).

dark brown, 47.0–67.5 μm thick. ***Conidiophores*** ampulliform, smooth, hyaline, multiguttulate, 12–29 × 2.0–3.5 μm ($\overline{x}$ = 19 × 3 μm, *n* = 20). ***Conidia*** fusoid to elongate-fusoid, 1-septate, cells equal, smooth, hyaline to pale brown, 13.5–19.0 × 3–4 μm ($\overline{x}$ = 16.5 × 3.5 μm, *n* = 20), with a whip-like appendage at the tip of each conidium, 30–77 μm ($\overline{x}$ = 51 μm, *n* = 20) in length (Fig. 4). ***Sexual morph*** not observed.

Additional specimen examined. China, Yunnan Province, Lijiang, Laojunshan, 26°37'56"N, 99°43'30"E, alt. 3873 m, on dying twigs of *Rhododendron vellereum*, 20 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou (BJTC 1773).

Notes. *Nagragomyces fusiformis* differs from other species of *Nagrajomyces* by narrower and 1-septate conidia.

***Nagrajomyces laojunshanensis* C. L. Hou & L. Zhuo, sp. nov.**
MycoBank No: 845665
Figs 4, 5

Etymology. The epithet *laojunshanensis* refers to the location where the type specimen was collected.

Type. China, Yunnan Province, Lijiang, Laojunshan, 26°39'44"N, 99°46'58"E, alt. 2910 m, on living twigs of *Rhododendron cinnabarinum* Hook. f., 20 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou (holotype CAF 800049). Ex-type culture CFCC 58177.

Diagnosis. This new species differs from *N. fusiformis* by conidia that are elongate-elliptical, blunter at both ends, and usually 3-septate and larger. *Nagrajomyces laojunshanensis* differs from *N. dictyosporus* by conidiomata that are unilocular and without stalks.

Description. ***Conidiomata*** solitary, pycnidial, unilocular, subglobose to ellipsoidal, subepidermal in origin, immersed at first, then becoming erumpent, 218–406 µm wide, 188–275 µm high, peridia black, 37–43 µm thick, opening irregularly in the upper part, with faint yellow content. ***Conidiophores*** ampulliform, smooth, hyaline, multiguttulate, 16.0–25.5 × 2–4 µm ($\overline{x}$ = 21 × 3 µm, *n* = 20). ***Conidia*** elongate-elliptical, 1–3-septate, mostly 3-septate, smooth, hyaline, 18–23 × 5.5–7.0 µm ($\overline{x}$ = 19.5 × 6.5 µm, *n* = 20), with a long, whip-like appendage at the tip of each conidium, 70–200 µm ($\overline{x}$ = 143.5 µm, *n* = 20) in length. ***Sexual morph*** not observed.

Culture characteristics. ***Cultures*** (ex-type CFCC 58177) on PDA 8 cm diameter after 1 month, with irregular margins, sparse aerial mycelium, colonies with whitish margins, with center turning black olive (#3b3c36) with increasing age. On MEA, 5.7 cm diameter after 1 month, with irregular margins, colonies with beaver (#9f8170) -colored margins, with center turning black olive (#3b3c36) with increasing age. ***Conidia*** not observed.

Additional specimen examined. China, Yunnan province, Kunming, Luquan, Jiaozixueshan, 26°05'04"N, 102°50'54"E, alt. 3823 m, on living twigs of *Rhododendron cinnabarinum* Hook. f., 23 June 2021, coll. C.L. Hou, M.J. Guo, H. Zhou, (BJTC 1849).

Notes. *Nagrajomyces laojunshanensis* differs from *N. dictyosporus* by conidia that are colorless and conidiomata that are without stalks. *Nagrajomyces laojunshanensis* differs from *N. fusiformis* by elongate-elliptical conidia with blunter ends, which are longer (18–23 µm vs. 13–19 µm) and wider (5.7–7.0 µm vs. 2.8–3.7 µm). Conidia of *N. laojunshanensis* are mostly 3-septate, whereas those of *N. fusiformis* are 1-septate. Molecular sequence data confirm the presence of two distinct species.

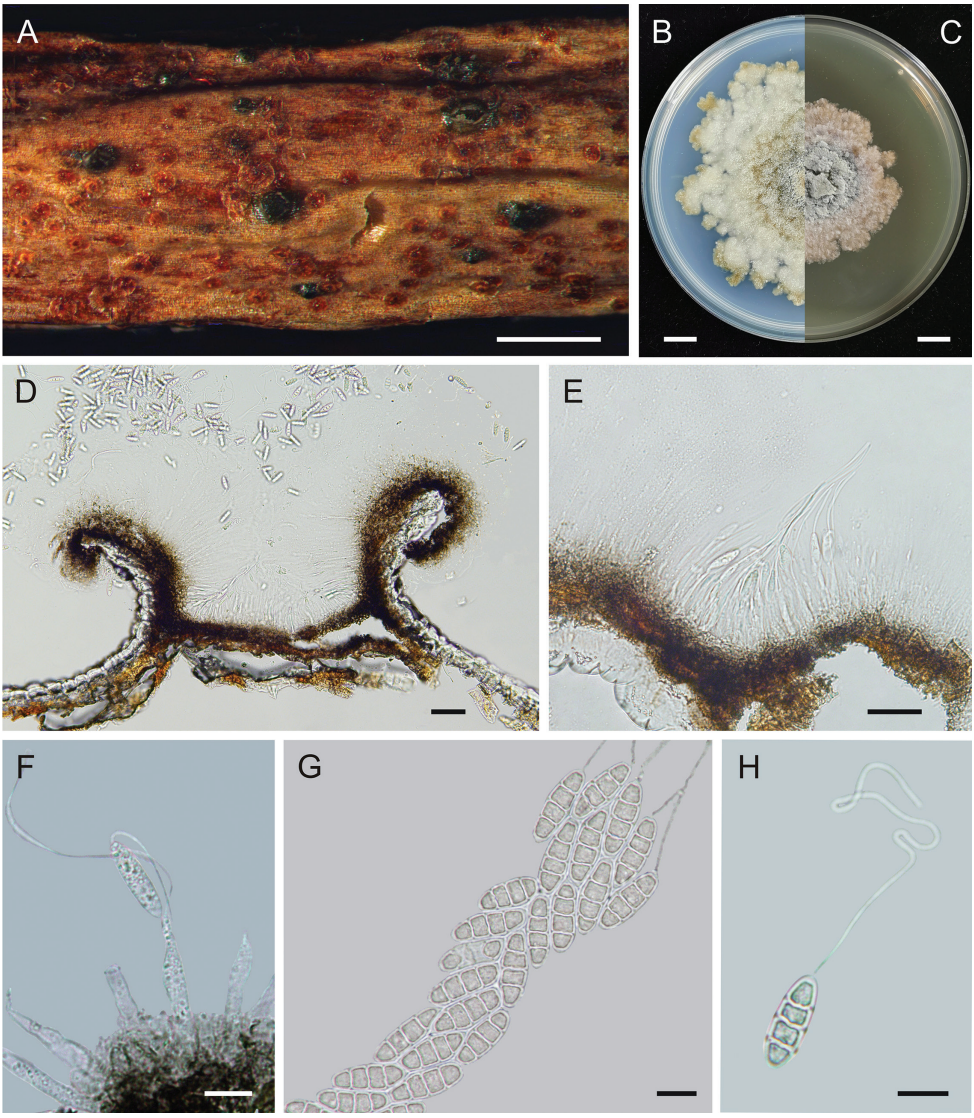

Figure 4. Micrographs of *Nagrajomyces laojunshanensis* on *Rhododendron cinnabarinum* (holotype CAF 800049) **A** conidiomata on a living twig **B** ex-type culture (CFCC 58177) on PDA after 30 days, seen from above **C** ex-type culture (CFCC 58177) on MEA after 30 days, seen from above **D** vertical section of a conidioma **E, F** conidiophores and conidia **G** conidia forming a cirrus **H** conidium with appendage. Scale bars: 1 mm (**A**); 1 cm (**B, C**); 100 µm (**D, E**); 10 µm (**F–H**).

Discussion

Morphologically, the most distinctive features of the new species of *Nagrajomyces* are septate conidia with long, single, apical appendages. The presence of this structure distinguishes them from all anamorphic genera known to belong to *Gnomoniaceae*.

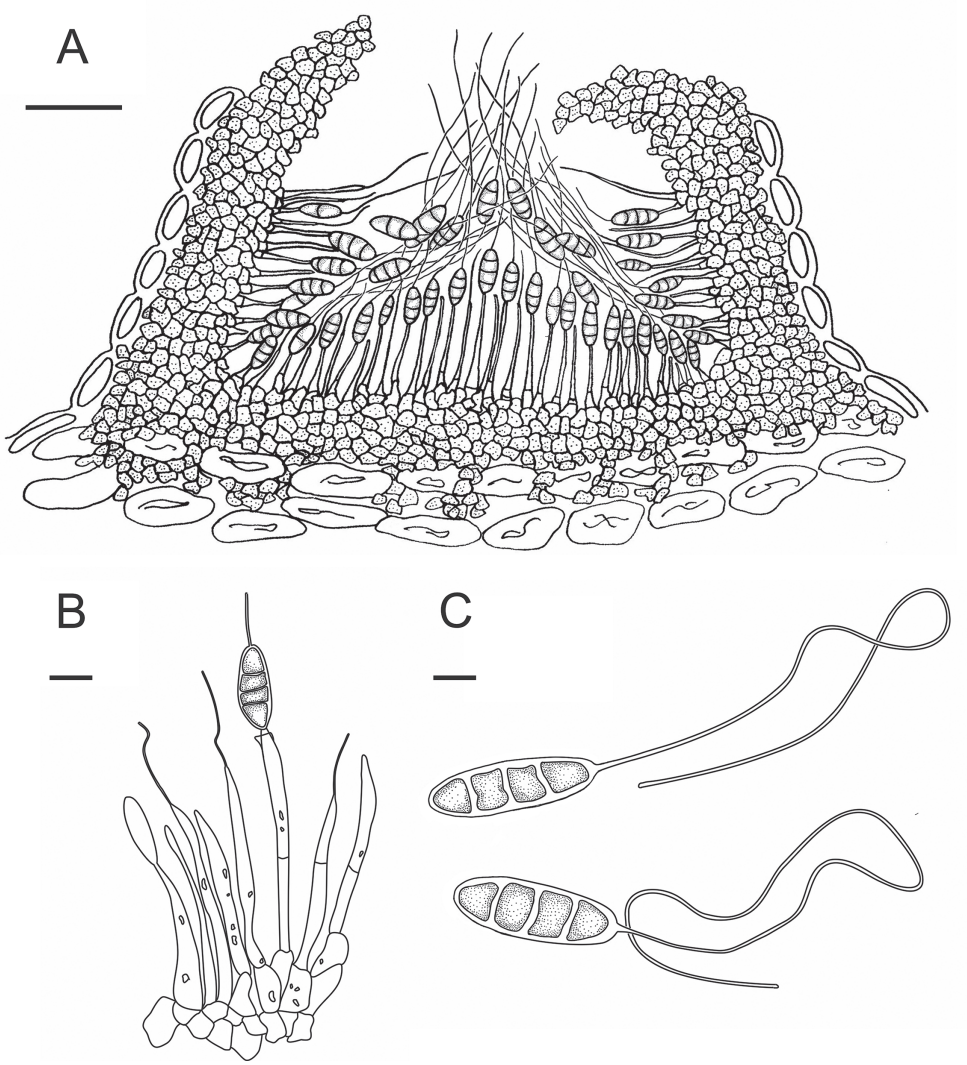

Figure 5. *Nagrajomyces laojunshanensis* (holotype CAF 800049) **A** vertical section of a conidioma **B** conidiophores and conidia **C** conidia with appendages. Scale bars: 50 μm (**A**); 10 μm (**B**); 5 μm (**C**).

Both new species proposed in the present study and the known species *N. dictyosporus* inhabit twigs of *Rhododendron*. In spite of the absence of molecular data for the type species of *Nagrajomyces*, these two new species are accommodated in *Nagrajomyces* based on significant morphological features (distinctive conidia) and identical ecology.

Many coelomycetous genera have conidia with appendages (Nag Raj 1993), and some of them share morphological characteristics with the new species proposed in this study. For example, species of *Uniseta* and *Urohendersonia* have septate conidia and a long apical appendage attached to each conidium. *Uniseta* is a monotypic genus typified by *U. flagellifera* (Ellis & Everh.) Ciccar. (Ciccarone 1947). Nag Raj (1974, 1993)

mentioned that *U. flagellifera* has a sexual morph called *Cryptodiaporthe comptoniae* (Schwein.) Barr (Barr 1991, syn. *C. aubertii* var. *comptoniae* (Schwein.) Wehm.) that is considered a synonym of *Cryptodiaporthe aubertii* (Westend.) Wehm. (Wehmeyer 1933). Conidia of this species are two-celled, hyaline, relatively inequilateral or curved, and bear a long flagellate appendage at one end (Wehmeyer 1933). *Cryptodiaporthe acerina* J. Reid & Cain and *C. aubertii* are included in the phylogenetic tree (Fig. 1) and located distant from the new species proposed herein. Furthermore, *U. flagellifera* differs from the new species proposed here by an asexual morph growing on branches of *Comptonia asplenifolia* damaged by fire (Ellis and Everhart 1889), while *Nagrajomyces* spp. develop on twigs of *Rhododendron*. Because of these differences, we consider the genus *Uniseta* to be separate from the genus *Nagrajomyces*.

Spegazzini (1902) introduced *Urohendersonia* Speg. with *Ur. platensis* Speg. as the type species. Nag Raj (1993) listed only five species in this genus, including the type species. *Urohendersonia* spp. differ from *Nagrajomyces* spp. by having globose to subglobose conidiomata immersed in host tissues, and yellowish brown to brown conidia each with an extracellular gelatinous appendage, and their host species (Nag Raj 1993). *Urohendersonia* spp. occur on diverse host species and various substrates of host, such as on leaves of *Erythrina* sp., *Manihot carthagenensis*, *Pongamia pinnnata*, *Stipa spartea*, or in the rhizospheres of *Acerva persica* and *Dactyloctenium aegyptium* (Nag Raj 1993; Wijayawardene et al. 2016). Unfortunately, there are no molecular sequence data available for any species within those genera.

In the phylogenetic analysis presented herein, the two new species, *N. fusiformis* and *N. laojunshanensis* form a clade with high support values, which is separate from other species of *Gnomoniaceae* represented by sequence data in GenBank. These two new species described in this study fill gaps in the molecular data of *Nagrajomyces* and also enable the taxonomic status of the new species to be determined.

A total of 38 genera are currently included in the family *Gnomoniaceae* based on morphological and molecular analyses (Senanayake et al. 2018; Crous et al. 2019; Jiang et al. 2019; Minoshima et al. 2019; Yang et al. 2020). Sexual morphs have been described for all but four; *Asteroma*, *Flavignomonia*, *Millerburtonia*, and *Sirococcus*. *Sirococcus* spp. are closely related to the new species described herein, whereas phylogenetic data indicate that the other three genera are distantly related to *Nagrajomyces* spp. *Asteroma* spp. have cylindrical to fusiform, acicular or broadly fusiform conidia (Senanayake et al. 2018). Conidia of *Flavignomonia* are cylindrical to oblong (Jiang et al. 2019), and conidia of *Millerburtonia* are filiform and aciculate (Ciferri 1951).

In addition to morphological characteristics and molecular sequence data, host ranges are often useful to delineate genera and species of *Gnomoniaceae* (Sogonov et al. 2008). Species of *Gnomonia*, for example, are generally associated with host plants in the *Betulaceae* family, mostly belonging to the subfamily *Coryloideae* (Sogonov et al. 2008). The two new species identified in the present study, and the known species, all develop on twigs of *Rhododendron* spp. indicating that they are specialized with respect to this host. The differences in conidiomatal structure could be explained by differences in host epidermal features or maturity. Two species of *Gnomoniaceae* are known

to inhabit *Rhododendron* spp. *Plagiostoma rhododendri* (Auersw.) Sogonov was reported on dry twigs and inflorescences of *Rhododendron hirsutum* L., and occasionally on dead leaves of *R. ferrugineum* L. (Monod, 1983). Only the sexual form of this species has been described, and phylogenetic analysis places it somewhat distant to species of *Nagrajomyces* (Fig. 1). The second species is *Gnomonia* sp., reported on rotten leaves of *R. ferrugineum* (Rehm 1906). This species lacks a specific morphological description.

Rhododendron is the largest genus of woody plants in the northern hemisphere, and its species diversity is highest in the Himalaya-Hengduan Mountains and Southeast Asia (Chamberlain et al. 1996; Shrestha et al. 2018). Considering the host preference of *Gnomoniaceae* species and the biodiversity of *Rhododendron* worldwide, additional *Gnomoniaceae* species are expected to exist on these plants.

Acknowledgements

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A new species of *Megalaria* (Ramalinaceae, Ascomycota) from Thailand, and recognition of subgenus *Catillochroma*

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Abstract

Tropical regions harbor a substantial diversity of lichenized fungi, but face numerous threats to their persistence, often even before previously unknown species have been described and their evolutionary relationships have been elucidated. *Megalaria* (Ramalinaceae) is a lichen-forming genus of fungi that produces crustose thalli, and includes a number of lineages occupying tropical rain forests; however, taxonomic and phylogenetic work on this clade is limited. Here we leverage both morphological and sequence data to describe a new species from the tropics, *M. pachaylenophila*. This taxon forms a crustose thallus, lacks secondary metabolites, and occurs in mangrove forests of Thailand. We supplemented molecular data from this species with data from other species, including two genera related to and occasionally included in *Megalaria*, namely *Catillochroma* and *Lopezaria*. Our analyses revealed *Catillochroma* species form a monophyletic group embedded within *Megalaria*, and we therefore recognize this clade at the subgeneric level. Since we only included the type species of *Lopezaria* in this study, we refrain from proposing a taxonomic conclusion for that clade at the moment. Several taxonomic combinations are made to reflect phylogenetic evidence supporting the inclusion of these species in *Megalaria*.

Keywords
Asia, lichens, mangroves, new taxa, tropical diversity

Introduction

Tropical habitats harbor a rich diversity of lichenized fungi, including numerous undescribed or unrecorded taxa (Lücking et al. 2009). The lichen biota of Thailand serves as a prime example of this trend, with the number of known species having more than doubled over the past two decades (Buaruang et al. 2017). Within Thailand, we have recently focused on the lichen biota of mangroves. Coastal forests in the tropics are species-rich (Donato et al. 2011; Friess 2016) but at great risk, with alarming rates of deforestation (Polidoro et al. 2010; Richards and Friess 2016). During our studies of crustose lichens in mangrove habitats of eastern Thailand, the first author collected a species that appeared undescribed and showed affinities with *Megalaria* Hafellner s. lat. In the family Ramalinaceae (Lücking et al. 2017), though, the circumscription and family placement of *Megalaria* have varied among authors.

Megalaria was initially circumscribed as a monospecific genus of lichen-forming fungi characterized by the formation of a crustose thallus with biatorine ascomata, a proper exciple and a pigmented epithecium, including only *M. grossa* (Pers. ex Nyl.) Hafellner at the time of its description (Hafellner 1984). Its limits were subsequently expanded to include both newly described species (Ekman and Tønsberg 1996; Fryday 2004b, 2007; Jagadeesh Ram et al. 2007; Lendemer 2007; McCarthy and Elix 2022), as well as species previously placed in *Catillaria* A. Massal. (Ekman and Tønsberg 1996; Fryday 2004a; Galloway 2004) and *Catinaria* Vain. (Schreiner and Hafellner 1992; Nimis 1993; Ekman and Tønsberg 1996). *Megalaria* was initially placed in its own family, Megalariaceae (Hafellner 1984), which was expanded to include the monospecific *Tasmidella* Kantvilas, Hafellner & Elix (Kantvilas et al. 1999). However, molecular data have since demonstrated the placement of *Megalaria* in Ramalinaceae (Ekman 2001; Miadlikowska et al. 2006, 2014; Ekman et al. 2008; Kistenich et al. 2018), while *Tasmidella* was excluded from this family (Kistenich et al. 2018).

Another genus, *Catillochroma* Kalb & Hafellner, was later described for a group of species previously placed in *Lecidea* Ach., *Lecanora* Ach., *Catinaria*, and *Megalaria*, and was distinguished from *Megalaria* on the basis of its bi-layered excipular anatomy, which included an inner layer formed of *textura intricata* with large intercellular spaces usually filled with crystals, and a uniform prosoplectenchymatous outer layer (Kalb 2007). In contrast, the exciple of *Megalaria* was regarded as being uniformly composed of prosoplectenchyma (Kalb 2007). However, historic (Galløe 1929) and modern (Fryday and Lendemer 2010) examinations of the exciple of the type species of *Megalaria*, *M. grossa*, revealed a bi-layered excipular anatomy similar to that of *Catillochroma*, but distinguished by the loose (*Catillochroma*) versus dense (*Megalaria*) spacing of hyphae in the inner layer of *textura intricata* (Fryday and Lendemer 2010). This distinction was

further clouded by the discovery of some species, such as *M. beechingii* Lendemer, with intermediate levels of spacing in the layer of *textura intricata* (Lendemer 2007; Fryday and Lendemer 2010). Consequently, excipular anatomy was regarded as insufficient for the segregation of *Catillochroma* from *Megalaria* (Fryday and Lendemer 2010).

In addition to excipular anatomy, *Catillochroma* was also distinguished from *Megalaria* through the presence of zeorin in the thallus; thus all zeorin-producing *Megalaria* species were transferred to *Catillochroma* (Kalb 2007; Lendemer and Knudsen 2008; Fryday and Lendemer 2010). For instance, the development of excipular *textura intricata* in *M. pulverea* was considered intermediate between that of *Megalaria* and *Catillochroma* (Fryday and Lendemer 2010), and its inclusion in *Catillochroma* was based on its synthesis of zeorin (Kalb 2007). However, such segregation based solely on the presence or absence of a single substance was regarded as insufficient (Fryday and Lendemer 2010). The absence of a *masse-axiale* in asci of the type species of *Catillochroma*, *C. endochroma* (Fée) Kalb, and its close relatives, was also suggested as a potential synapomorphy of this group, and it was noted that species in the *C. endochroma* group could potentially be considered distinct from *Megalaria* (Fryday and Lendemer 2010). However, the distinction of this group from *Lopezaria* Kalb & Hafellner was not clearcut (Fryday and Lendemer 2010).

Lopezaria was described as a monospecific genus for the tropical and corticolous species *Lopezaria versicolor* (Flot.) Kalb & Hafellner, distinguished mostly by its large ascospores occurring in numbers of two per ascus (Kalb 1990). Similar to *Catillochroma endochroma*, *L. versicolor* also forms a bi-layered exciple with a layer of loosely spaced *textura intricata* (Fryday and Lendemer 2010), and lacks a *masse-axiale* in the tholus (Kalb 1990). In addition, early reports suggested trace amounts of atranorin and zeorin in the thallus of *L. versicolor* (Sipman 1983), while subsequent examinations have failed to detect zeorin (Fryday and Lendemer 2010). *Lopezaria isidiza* (Makhija & Nagarkar) Aptroot & Sipman – the only other species subsequently included in *Lopezaria* (Aptroot et al. 2007) – also lacks both atranorin and zeorin (Makhija and Nagarkar 1981; Sipman 1983; Fryday and Lendemer 2010). Consequently, the distinction between *Lopezaria* from *Catillochroma*, based on the absence of zeorin and synthesis of larger ascospores (Fryday and Lendemer 2010), was regarded as insufficient (Fryday and Lendemer 2010).

Given the challenges of retaining these three genera as distinct, and in the absence of molecular evidence, all species of *Catillochroma* and *Lopezaria* were transferred to *Megalaria* (Fryday and Lendemer 2010). Thus, *Megalaria* was expanded from a monospecific genus restricted to *M. grossa* (Hafellner 1984) to include approximately 48 species globally that typically form apothecia, with some that instead form soredia and lack ascomata (McMullin and Lendemer 2016). Together, this broadly circumscribed *Megalaria* thus encompasses an ecologically broad assemblage of species that are corticolous (Ekman and Tønsberg 1996; Jagadeesh Ram et al. 2007; Fryday and Lendemer 2010; Lendemer et al. 2016; McCarthy and Elix 2016; McMullin and Lendemer 2016), bryophilous, saxicolous and terricolous, and occur in both temperate and tropical habitats (Fryday 2004b, a, 2007; Lendemer 2007; Su and Ren 2017).

Some authors have continued to recognize *Catillochroma* as a distinct genus within Ramalinaceae, instead of adopting a broadly circumscribed *Megalaria* (Lücking et al.

2017; Kalb 2022). Justification for the continued recognition of *Catillochroma* is rooted in the assumption that these species constitute a well-circumscribed group and that sampling for molecular phylogenetic analysis has remained sparse. Most recently, several *Megalaria* species used to justify the dissolution of *Catillochroma* – or described following its synonymization – were transferred from *Megalaria* to *Catillochroma* (Kalb 2022). This included *Megalaria yunnanensis*, which was described as being similar to four species, three of which were previously placed in *Catillochroma* (*M. albocincta* [Degel.] Tønsberg, *M. anaglyptica* [Kremp.] Fryday & Lendemer, *M. pulverea*), and one of which (*M. alligatorensis* Lendemer) was described following the synonymization of *Catillochroma* (Lendemer et al. 2016; Wang et al. 2019). These species share excipular features consistent with *Catillochroma* and produce atranorin, zeorin and fumarprotocetraric acid (Kalb 2007; Fryday and Lendemer 2010; Lendemer et al. 2016; Wang et al. 2019). In addition, several other species were transferred, and three species new to science were also placed in *Catillochroma* (Kalb 2022). However, the reciprocal monophyly of *Catillochroma* (and *Lopezaria*) and *Megalaria* remains to be demonstrated with broader molecular sampling.

Here we describe a new species of lichen-forming fungi from mangroves in eastern Thailand, and place it in *Megalaria* on the basis of morphological and DNA sequence data, including new sequences for an additional nine species. While we were unable to obtain sequence data from the type species of *Catillochroma*, our work still permits an evaluation of the phylogenetic relationships of species previously included in the genus *Catillochroma* (Lücking et al. 2017; Kalb 2022).

Materials and methods

Taxon selection

We sequenced fungal DNA from representatives of the new species, several taxa representing part of *Catillochroma*, as well as additional taxa potentially placed in the broadly circumscribed *Megalaria*. These data were supplemented with publicly-available sequences from additional *Megalaria* taxa (McMullin and Lendemer 2016; Kistenich et al. 2018; Wang et al. 2019), and other members of the broader Ramalinaceae clade G (Kistenich et al. 2018; van den Boom and Alvarado 2019), which includes *Megalaria*. *Biatora vernalis* was selected as the outgroup (Kistenich et al. 2018). Morphological and chemical data were obtained from recent literature and study of the examined material (Ekman and Tønsberg 1996; Kalb 2007, 2022; Lendemer 2007; Fryday and Lendemer 2010; McMullin and Lendemer 2016; Wang et al. 2019; McCarthy and Elix 2022).

Molecular methods

DNA was extracted using the Sigma REDExtract-N-Amp Plant PCR Kit (St. Louis, Missouri, U.S.A.) (Avis et al. 2003; Nelsen et al. 2009) and a 20× DNA dilution utilized in subsequent PCR reactions. Portions of the fungal internal transcribed spacer (ITS), mitochondrial small subunit (mtSSU) and nuclear ribosomal large subunit (nuLSU) were amplified using

the ITS1F (Gardes and Bruns 1993) and ITS4A (Kroken and Taylor 2001) primers for the ITS, mrSSU1 and mrSSU2R primers (Zoller et al. 1999) for the mtSSU, and the LR0R (Cubeta et al. 1991) and LR3 (Vilgalys and Hester 1990) primers for the nuLSU.

The 12.5 µL PCR reactions consisted of 5 µM of each PCR primer, 0.5 µl diluted DNA, 6.25 µl REDExtract-n-Amp PCR Ready Mix (Sigma-Aldrich, St. Louis, Missouri, U.S.A.), and 0.5–1.5 µL $MgCl_2$. The PCR cycling conditions were as follows: 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 53 °C (mtSSU), 60 °C (nuLSU) for 1 min, and 72 °C for 1 min, followed by a single 72 °C final extension for 5–10 min. Samples were visualized on a 1% ethidium bromide-stained agarose gel under UV light and cleaned with ExoSAP-IT Express (Affymetrix Inc, Santa Clara, California, U.S.A.). The 10 µl cycle sequencing reactions consisted of 0.5 µl of Big Dye version 3.1 (Applied Biosystems, Foster City, California, U.S.A.), 3.5 µl of Big Dye buffer, 1–6 µM primer, 1.5 µl of cleaned PCR product and water. Samples were sequenced with PCR primers. The cycle sequencing conditions were as follows: 96 °C for 1 minute, followed by 24 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds and 60 °C for 4 minutes. Samples were precipitated and sequenced in an Applied Biosystems 3730 DNA Analyzer (Foster City, California, U.S.A.). Sequences were assembled in Geneious Prime 2019.2.1 (https://www.geneious.com/), and submitted to GenBank (Table 1).

Table 1. Species included in the present study, collection numbers for newly sequenced specimens, GenBank accession numbers for the three loci, and internal DNA numbers for newly sequenced specimens.

Taxon	Collection (Herbarium)	Locality	ITS	mtSSU	nuLSU
Catillaria superflua	Kalb & Elix 35269 (K. Kalb)	Australia, New South Wales	–	OP689726	–
Catillochroma alleniae			KX660734	KX660733	–
Catillochroma danfordianum	Kalb & Mertens 39720 (K. Kalb)	Australia, Queensland	–	OP689730	–
Catillochroma mareebaense	Kalb & Mertens 39753 (K. Kalb)	Australia, Queensland	–	OP689728	–
Catillochroma mareebaense	K. & D. Kalb 40554 (K. & J. Kalb)	Australia, Queensland	–	OP689733	OP689723
Catillochroma phayapipakianum	J. & K. Kalb 41927 (K. & J. Kalb)	Thailand, Chiang Mai	OP698025	OP689731	–
Catillochroma phayapipakianum	J. & K. Kalb 41762 (K. & J. Kalb)	Thailand, Chiang Rai	OP698026	OP689732	OP689722
Catillochroma phayapipakianum	J. & K. Kalb 41877 (K. & J. Kalb)	Thailand, Chiang Mai	–	OP689734	OP689724
Catillochroma pulvereum			KX660735	–	–
Catillochroma yunnanense			MK348528	–	–
Cliomegalaria symmictoides			MW622003	MW622006	MW621867
Lopezaria versicolor			–	AY584622	–
Lopezaria versicolor	Mercado-Diaz 1077 (F)	Puerto Rico, Jayuya	–	OP689735	OP689719
Lopezaria versicolor	Soto 2174 (F)	Puerto Rico, Jayuya	–	OP689736	OP689721
Megalaria bengalensis	Kalb 37938 (K. Kalb)	Brazil, Sergipe,	–	OP689729	–
Megalaria columbiana			–	MN508319	–
Megalaria grossa			AF282074	MG925883	–
Megalaria grossa	Kalb & Jonitz 41079 (K. Kalb)	Ecuador, Azuay	OP698024	OP689727	OP689720

Taxon	Collection (Herbarium)	Locality	ITS	mtSSU	nuLSU
Megalaria laureri			AF282075	MG925884	–
Megalaria pachaylenophila	Phraphuchamnong (RAMK032107)	Thailand, Chumphon province	OP698023	OP689725	OP689718
Megalaria pachaylenophila	Chum 2024 (RAMK)	Thailand, Chumphon province	OP698020	–	OP689715
Megalaria pachaylenophila	Chum 2028 (RAMK)	Thailand, Chumphon province	OP698021	–	OP689716
Megalaria pachaylenophila	Chum 2072 (RAMK)	Thailand, Chumphon province	OP698022	–	OP689717
Megalaria sp.	Kalb 38739 (hb. Kalb)	China, Yunnan	OP698027	–	–
Biatora vernalis			AF282070	DQ838753	DQ838752
Niebla homalea			MG925987	MG925888	MG926085
Ramalina sinensis			MG926018	MG925921	MG926110
Tylothallia biformigera			AF282077	MG925946	MG926129

Phylogenetic analyses

Sequences for individual loci were aligned using the G-INS-i algorithm in MAFFT 7.475 (Katoh and Standley 2013) with and the "--leavegappyregion" option. Poorly aligned regions were subsequently re-aligned using the L-INS-i algorithm (MAFFT) and manual refinement in Mesquite (Maddison and Maddison 2021). Ambiguously aligned regions were then removed using GBlocks 0.91b (Castresana 2000) with a minimum block length of 5, a maximum of 10 contiguous non-conserved positions, and the minimum number of sequences required for gaps, flanking and conserved positions was set to half the number of taxa in the alignment. Alignments were concatenated, and a partitioned maximum likelihood (ML) analysis was conducted in RAxML 8.2.12 (Stamatakis 2014). The GTR+G model was applied and each locus was permitted its own parameter estimates. Support was estimated by conducting 1,000 rapid bootstrap pseudoreplicates (Stamatakis et al. 2008). The RAxML analysis was conducted using the CIPRES Science Gateway (Miller et al. 2010). Trait states for taxonomically important characters in this clade were then derived from the literature and plotted on the tips of the phylogeny.

Results

The final alignment consisted of 1727 characters (ITS: 468; mtSSU: 427; nuLSU: 832). The resulting topology (Fig. 1) revealed good support (bootstrap support ≥ 70) for the monophyly of *Megalaria* s.lat., including the type of *Megalaria* (*M. grossa*), several species of *Catillochroma*, and the type species of *Lopezaria* (*L. versicolor*). The newly discovered species from Thailand was found to be more closely related to *Megalaria versicolor* (the type of *Lopezaria*) than to the type of *Megalaria* (*M. grossa*). Species ascribed to the genus *Catillochroma* formed a strongly supported monophyletic group.

Within the *Catillochroma* clade, *C. pulvereum* (Borr.) Kalb and *C. yunnanense* (C.X. Want & L. Hu) Kalb, two fumarprotocetraric acid-containing species, formed a strongly supported, monophyletic group; however, their relationship to *C. danfordianum* Kalb

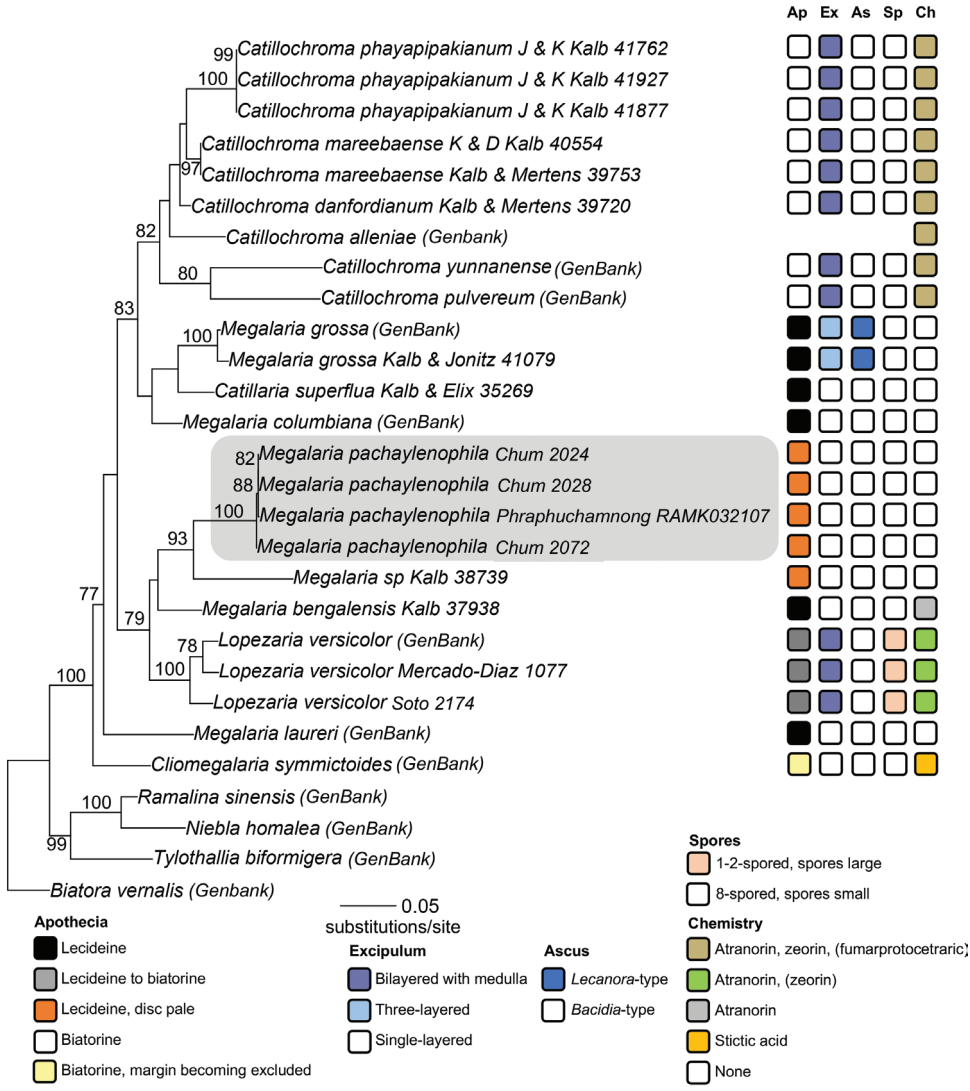

Figure 1. The ITS+mtSSU+nuLSU ML phylogeny with bootstrap values ≥ 70 shown. Newly sequenced specimens include collection info following the species name, while those derived from GenBank are indicated in parentheses. The novel species described here is highlighted in gray. Character states for selected characters are shown at the tips of the phylogeny. Ap = apothecia; Ex = exciple; As = asci; Sp = ascospores; Ch = chemistry.

and *C. phayapipakianum* Kalb – two additional fumarprotocetraric acid-producing species—remains unresolved. Zeorin producing species, which includes the entire *Catillochroma* clade, here represented by *C. yunnanense*, *C. pulvereum*, and *C. alleniae* (Lendemer and McMullin) Kalb, *C. danfordianum*, *C. mareebaense* Kalb and *M. phayapipakianum*, also formed a strongly supported monophyletic group. Species producing atranorin only were paraphyletic including *M. laureri* (Th. Fr.) Hafellner, *L. versicolor*, and *M. bengalensis* Jagadeesh Ram, Aptroot, G.P. Sinha & K.P. Singh.

The new species described here lacks substances entirely, and was embedded in a clade that includes atranorin producing species. Other sampled taxa deficient in secondary metabolites included *Catillaria superflua* (Müller Arg.) Zahlbruckner, *Megalaria columbiana* (G. Merr.) S. Ekman and *M. grossa*.

All species included were corticolous; thus it was not possible to evaluate relationships among corticolous and non-corticolous taxa. While representation was slightly skewed towards the Northern Hemisphere, species included from the Southern Hemisphere (*Catillaria superflua* [Müller Arg.] Zahlbruckner, *Catillochroma danfordianum*, *C. mareebaense*, *M. bengalensis* and *M. grossa*) did not form a monophyletic group.

Discussion

Our study provides the first, albeit limited, insight into the molecular phylogeny of *Megalaria* s. lat. and confirms that species of genera previously recognized as distinct from, or part of, *Megalaria* indeed form a monophyletic group. Sampled *Catillochroma* species were monophyletic, but nested within *Megalaria* s. lat. Hence, recognition of this zeorin-producing clade at the generic level would leave *Megalaria* paraphyletic. The resurrection of *Lopezaria* (and inclusion of the new species) and separation from *Megalaria* would still keep *Megalaria* paraphyletic, and its segregation from *Lopezaria* on the basis of morphological and chemical characters would remain challenging. Hence, we argue for the previously suggested retention of a broadly-defined *Megalaria* that includes both *Catillochroma* and *Lopezaria* (Fryday and Lendemer 2010). Given the monophyly of *Catillochroma* species examined, and the presumed close relationship of the type species to this clade (which was not sequenced here, despite several attempts), we propose to adopt an alternative classification for this morphologically recognizable clade nested with a larger genus. This approach is similar to that adopted in other groups of lichen-forming fungi, such as *Hypotrachyna* (Divakar et al. 2013). The phenotypically recognizable clade *Catillochroma* is below proposed to be recognized at the subgeneric level. This solution avoids creating a paraphyletic *Megalaria*, while also ascribing a taxonomic rank to the synapomorphies observed in species previously classified in *Catillochroma*.

Taxonomic novelties

Megalaria pachaylenophila **Phraphuchamnong, Buaruang & Lumbsch, sp. nov.**
MycoBank No: 846158
Fig. 2

Type. Thailand. Chumphon province: Pathio District; Tambon Pak Klong, 10°53.255'N, 99°28.649'E, 5 m elev., mangrove forest, on bark of *Rhizophora mucronata*, 28 March 2019; Kawinnat Buaruang et al., Chum 2771 (RAMK 034555-holotype, F-isotype).

Diagnosis. Similar to *Megalaria bengalensis*, but differs in an ochre to brownish apothecial disc (black in *M. bengalensis*) and in lacking isidia and secondary products (atranorin in *M. bengalensis*).

Etymology. The specific epithet refers to the English translation (Pāchāylen) of the Thai name for mangrove (**ป่าชายเลน**), and philos (greek) = friend, referring to the ecological preference of the new species.

Description. Thallus crustose, corticolous, gray to olive-gray or greenish gray, up to 10 cm in diameter, smooth, cracked, without soredia or isidia. Apothecia biatorine, plain and flat, becoming slightly convex with age, circular in outline or becoming deformed, sessile, 0.3–0.8 mm in diameter; margins black, shining, contrasting strongly with the coloration of the discs; discs beige to brownish, epruinose. Epihymenium 2–5 µm thick, not pigmented or light beige, K–, N–. Hymenium 75–100 µm thick, hyaline, not inspersed. Subhymenium 10–20 µm thick, hyaline. Central hypothecium 50–80 µm thick, pigmented red-brown, K+ wine-red, N–; lateral hypothecium blue to blue-black, K–, N+ purple. Excipulum 15–25 µm thick, comprised of thick, gelatinized hyaline to blue hyphae, not inspersed with crystals, K–, N+ purple. Asci cylindrical to clavate, eight-spored; ascospores narrowly ellipsoid, hyaline, one-septate (rarely simple), thin walled, not halonate, (9–)11–15 × 4–5 µm. Pycnidia not seen.

Secondary metabolites. Thallus K–, C–, and KC–, UV–, no lichen substances found using TLC.

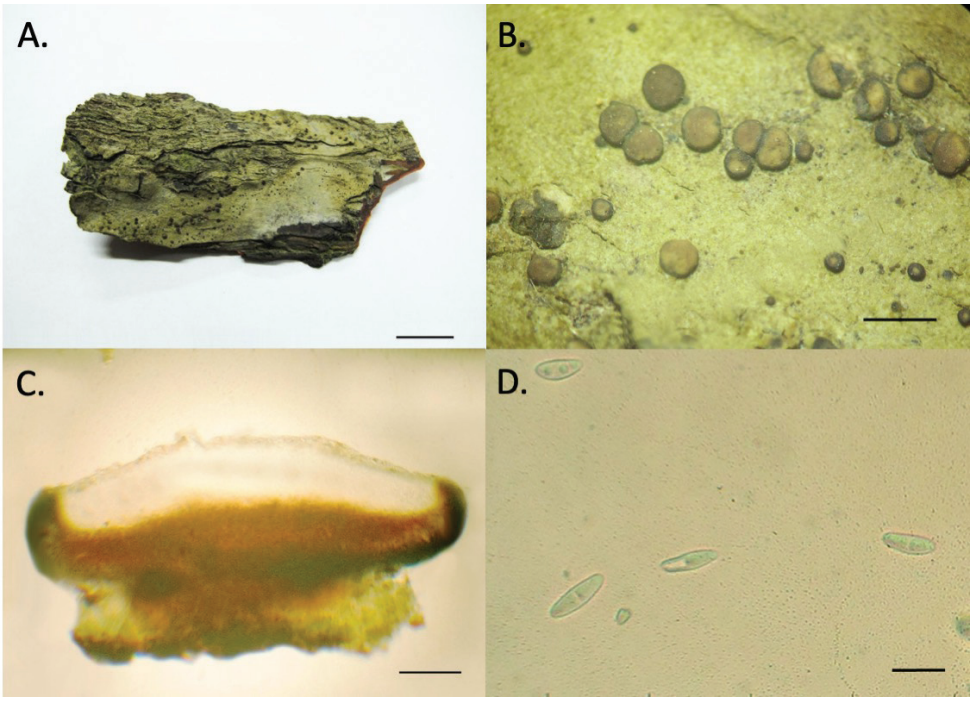

Figure 2. *Megalaria pachaylenophila* (RAMK 36122) **A** thallus **B** thallus and ascomata **C** apothecia cross section **D** ascospore. Photos by P. Phraphuchamnong. Scale bars: 1 cm (**A**); 0.5 mm (**B**); 200 µm (**C**); 10 µm (**D**).

Distribution and ecology. The new species was found in the south-eastern province of Chumphon where it was growing in old mangrove forests on the bark of *Excoecaria agallocha*, *Hibiscus tiliaceus*, *Rhizophora apiculata*, and *Rhizophora mucronata*.

Notes. In the phylogenetic tree, *Megalaria pachaylenophila* and *M. bengalensis* cluster together, and indeed, their apothecial anatomy is very similar. However, they can easily be separated by the isidiate thallus in the latter. No other species in *Megalaria* sens. lat. is known to form a beige or brownish apothecial disk. Interestingly, this can be found in some species of *Megalaria* (*Lopezaria*) *versicolor* which is the sister clade to *Megalaria pachaylenophila* and *M. bengalensis*. Additional superficially similar species include the North American *M. beechingii*, which differs in having purple-black to jet black apothecia, a margin that is concolorous with disc, and broadly ellipsoid ascospores, that are often kidney bean-shaped (Lendemer 2007). *Catillochroma phayapipakianum*, which was recently described from Thailand (Kalb 2022) and is transferred to *Megalaria* below, is readily distinguished from *M. pachaylenophila* by having larger (16–26 μm long), narrowly ellipsoid to fusiform, ascospores, and containing atranorin, zeorin, and fumarprotocetraric acid.

Additional specimens examined. Thailand. Chumphon province: Pathio District; Chum Kho sub-district, mangrove forest, on bark of *Rhizophora apiculata*, 15 Feb 2018; K. Buaruang et al., Chum 2024 (RAMK), 2028 (RAMK), 2072 (RAMK).

Below we propose new combinations to reflect the broad recognition of *Megalaria* and the recognition of the *Catillochroma* clade at subgeneric level:

***Megalaria* subgen. *Catillochroma* (Kalb) Lücking, Lumbsch & Nelsen, comb. et stat. nov.**
MycoBank No: 846159

Catillochroma Kalb, Bibl. Lichenol. 95: 298 (2007). Type species: *Catillochroma endochromum* (Fée) Kalb.

***Megalaria bicolorata* (Vain.) Lumbsch & Nelsen comb. nov.**
MycoBank No: 846160

Catillochroma bicoloratum (Vain.) Kalb., Archive for Lichenology 30: 12 (2022). – *Catillaria bicolorata* Vain. Annales Botanici Societatis Zoologicae-Botanicae Fennnicae 'Vanamo' 1: 48 (1921).

***Megalaria danfordiana* (Kalb) Lumbsch & Nelsen comb. nov.**
MycoBank No: 846161

Catillochroma danfordianum Kalb., Archive for Lichenology 30: 4–6 (2022).

***Megalaria mareebaensis* (Kalb) Lumbsch & Nelsen comb. nov.**
MycoBank No: 846162

Catillochroma mareebaense Kalb., Archive for Lichenology 30: 6–8 (2022).

***Megalaria phayapipakiana* (Kalb) Lumbsch & Nelsen comb. nov.**
MycoBank No: 846163

Catillochroma phayapipakianum Kalb., Archive for Lichenology 30: 8–10 (2022).

***Megalaria superflua* (Müll. Arg.) Kalb, Lumbsch & Nelsen comb. nov.**
MycoBank No: 846164

Catillaria superflua (Müller Arg.) Zahlbruckner., Catalogus Lichenum Universalis 4: 75 (1926). – *Patellaria superflua* Müll. Arg., Flora (Regensburg) 70: 336 (1887).

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New species and records of *Neomassaria*, *Oxydothis* and *Roussoella* (Pezizomycotina, Ascomycota) associated with palm and bamboo from China

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Abstract

Several micro fungi were gathered from bamboo and palm in Guizhou Province, China. In morphology, these taxa resemble *Neomassaria*, *Roussoella* and *Oxydothis*. Multi-gene phylogenetic analyses based on combined ITS, LSU, SSU, *rpb*2 and *tef*1 loci confirmed that two are new geographical records for China, (*viz. Roussoella siamensis*, *Neomassaria fabacearum*), while two of them are new to science (*viz. Oxydothis fortunei* sp. nov. and *Roussoella bambusarum* sp. nov.). The stromata of *Roussoella bambusarum* are similar to those of *R. thailandica*, but its ascospores are larger. In addition, multi-gene phylogenetic analyses show that *Oxydothis fortunei* is closely related to *O. inaequalis*, but the J- ascus subapical ring as well as the ascospores of *O. inaequalis* are smaller. Morphological descriptions and illustrations of all species are provided.

Keywords
2 new taxa, bambusicolous and palm fungi, phylogeny, Pleosporales, taxonomy, Xylariales

Introduction

Ascomycetous taxa on bamboo and palm are commonly observed with immersed ascomata (Dai et al. 2017). *Oxydothis* Penz. & Sacc. and *Roussoella* Sacc. are well-documented on bamboo and palms in different localities in Asia (Liu et al. 2014; Konta et al. 2016; Dai et al. 2017).

The family Oxydothidaceae S. Konta & K.D. Hyde was erected to accommodate a single genus (*Oxydothis*) by Konta et al. (2016). Species of *Oxydothis* are characterized by the cylindrical asci with a J+ (rarely J-) subapical apparatus and filiform to fusiform, hyaline,1-septate ascospores with spine-like or rounded ends (Konta et al. 2016). Anamorph is *Selenosporella* sp. (descriptions from Samuels and Rossman 1987). Eighty-five epithets of *Oxydothis* have been listed in Index Fungorum (accession date: 1 May 2022). *Oxydothis* species (such as *O. oraniopsidis* Fröhlich & Hyde, *O. cyrtostachicola* Hidayat, To-Anun & K.D. Hyde, *O. garethjonesii* Konta & Hyde) are the initial colonizers of dead palm material (Hyde 1993; Fröhlich and Hyde 1994; Hidayat et al. 2006; Konta et al. 2016).

Liu et al. (2014) introduced Roussoellaceae Jian K. Liu et al. to accommodate three genera, i.e. *Neoroussoella* Jian K. Liu et al., *Roussoella* Sacc. and *Roussoellopsis* I. Hino & Katum (Liu et al. 2014). Later, *Appendispora* K.D. Hyde, *Cytoplea* Bizz. & Sacc., *Elongatopedicellata* Jin F. Zhang et al., *Immotthia* M.E. Barr and, *Pararoussoella* Wanas et al., were added to this family (Hyde 1994; Hyde et al. 1996; Ariyawansa et al. 2015; Hyde et al. 2017; Phookamsak et al. 2019; Wijayawardene et al. 2020). Most species of Roussoellaceae were reported as saprophytic taxa on the terrestrial plants including bamboo, palms and mangroves (Liu et al. 2014; Jiang et al. 2019; Poli et al. 2020). The members of this family have 4–8 spored, and bitunicate asci with aseptate, brown to dark brown ascosporic, melanconiopsis-like or neomelanconium-like asexual morphs (Liu et al. 2014).

Hyde et al. (2016) introduced the monotypic genus *Neomassaria* Mapook et al. to accommodate *N. fabacearum* Mapooket et al. in Neomassariaceae. The *Neomassaria* is characterized by globose to subglobose ascomata with fusoid, hyaline, 1-septate ascospores, with or without a sheath but the asexual morph is undetermined (Hyde et al. 2016; Ariyawansa et al. 2018; Yang et al. 2022). Currently, only three species have been reported, *viz.*, *Neomassaria fabacearum* from the branch of *Hippocrepis emerus* (L.) Lassen (Hyde et al. 2016), *N. formosana* H.A. Ariyaw. et al. on a dead stem of *Rhododendron* sp. (Ariyawansa et al. 2018), and *N. hongheensis* E.F. Yang & Tibpromma on a decayed branch of *Mangifera indica* L. (Yang et al. 2022).

In this study, several specimens of bamboo and palm were collected from Guizhou Province. Based on their morphology and phylogeny, two new species and two new records from China are herein reported. Full descriptions, photo plates of macro-and

micro-morphological characteristics and a phylogenetic tree to show the phylogenetic placement of the new records and the new species are provided.

Materials and methods

Fungi collections, isolations and morphology

From 2021 to 2022, fresh materials were collected from bamboo and palms in forests and nature reserves of Guizhou Province, China, and returned to the lab in paper or plastic bags. Samples were treated and examined with the method described by Taylor and Hyde (2003). Morphological characteristics were examined using a Nikon SMZ 745 series stereomicroscope and photographed using a Canon 700D digital camera. Melzer's iodine reagent was used for testing the amyloid reaction of the apical apparatus structures. Micro-morphological structures were photographed using a Nikon digital camera (Canon 700D) fitted to a light microscope (Nikon Ni). At least 30 ascospores and asci of each specimen were measured using the Tarosoft image framework (v. 0.9.0.7). Photo plates were arranged and improved using Adobe Photoshop CS6 software. Specimens were kept in the Herbarium of Guizhou Medical University (**GMB**) and Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (**KUN-HKAS**).

Isolations were made by single spore isolation (Long et al. 2019) and germinated spores were transferred onto potato dextrose agar (PDA) medium for purification. The colonies grown on PDA at 25 °C were transferred to three 1.5 mL microcentrifuge tubes filled with sterile water and stored with 10% glycerol at –20 °C. Living cultures were deposited at Guizhou Medical University Culture Collection (GMBC).

DNA extraction, Polymerase chain reaction (PCR) amplification and sequencing

The OMEGA E.Z.N.A. Fungal Genomic DNA Extraction Kit (D3390, Guangzhou Feiyang Bioengineering Co., Ltd, China) was used to extract genomic DNA from fresh fungal mycelium, according to the manufacturer's instructions. The extracted DNA was stored at –20 °C.

ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990) and NS1/NS4 primers (White et al. 1990) were used for the amplification of ITS, LSU and SSU. Translation elongation factor 1-α gene region (*tef*1) and RNA polymerase II second largest subunit (*rpb2*) genes were amplified using EF1-983F and EF 1-2218R (Rehner 2001), rpb2-5f and rpb2-7cr primers (Liu et al. 1999) respectively.

PCR was carried out in a volume of 25 μL containing 9.5 μL of ddH_2O, 12.5 μL of 2× Tap PCR Master Mix (2× Tap Master Mix with dye, TIANGEN, China), 1 μL of DNA extracts and 1 μL of forward and reverse primers in each reaction. The PCR thermal cycle of ITS, LSU, SSU and *tef*1 amplification is as follows: initially 95 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 1 minute, annealing at 52 °C for 1 minute, elongation at 72 °C for 1.5 minutes, and final extension at 72

°C for 10 minutes. The PCR thermal cycle program for the partial *rpb*2 was followed as initially 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minute, annealing at 54 °C for 2 minutes, elongation at 72 °C for 1.5 minutes, and final extension at 72 °C for 10 minutes. The amplified PCR fragments were sent to Sangon Biotech (Shanghai) Co., China, for sequencing. Generated new sequences of ITS, LSU, SSU, *rpb*2 and *tef*1 regions were deposited in GenBank (Table 1).

Phylogenetic analysis

All sequences used for phylogenetic analysis were downloaded from the GenBank, based on published literature and the highest hit rate of ITS in the GenBank database. Sequence data for the construction of the phylogenetic trees are listed in Table 1. Single gene sequence alignments were generated with MAFFT v.7.110 (http://mafft.cbrc.jp/alignment/server/index.html, Katoh and Standley 2013) and multiple sequence alignments were edited manually when necessary in BioEdit v.7.0 (Hall 1999). ALTER (http://www.sing-group.org/ALTER/) was used to convert the file format (Alignment Transformation Envi-Ronment). The maximum likelihood analysis was carried out with GTR+G+I model of site substitution by using RAxML 8.2.12 BlackBox. Bayesian Inference (BI) analysis was performed with MrBayes v.3.2.7a (Huelsenbeck 2012). The branch support was evaluated with 1000 bootstrap replicates (Silvestro and Michalak 2012). Posterior probabilities (PP) were determined by Markov Chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.7a (Ronquist et al. 2012). Trees were visualized by FigTree v. 1.4.4, and additionally, layouts were done with Photoshop CS6. The alignments and respective phylogenetic trees were uploaded in TreeBASE (http://www.treebase.org. submission number: ID 29735; ID 29736; ID 29737).

Abbreviations

AFTOL-ID: Assembling the Fungal Tree of Life; **ATCC**: American Type Culture Collection; **CBS**: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; **CMW**: Tree Pathology Co-operative Program, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; **CPC**: Culture collection of Pedro Crous, housed at the Westerdijk Fungal Biodiversity Institute; **GMB**: herbarium of Guizhou Medical University; **HKAS**: herbarium of Cryptogams Kunming Institute of Botany Academia Sinica, Chinese Academy of Sciences, Kunming, China; **HKUCC**: Hong Kong University Culture Collection; **ICMP**: International Collection of Microorganisms from Plants; **IMI**: CABI Bioscience UK Centre; **JK**: J. Kohlmeyer; **KT**: K. Tanaka; **KUMCC**: Kunming Institute of Botany Culture Collection, Chinese Science Academy, Kunming, China; **MAFF**: Ministry of Agriculture, Forestry and Fisheries, Japan; **MFLU**: Mae Fah Luang University Herbarium, Chiang Rai, Thailand; **MFLUCC**: Mae Fah Luang University Culture Collection, Chiang Rai, Thailand; **MUCL**: University Catholique de Louvain; **NFCCI**: National Fungal Culture Collection of India; **SMH**: Sabine M. Huhndorf; **WU**: Fungarium of the Department of Botany and Biodiversity Research, University of Vienna; **Others**: information not available.

Table 1. Taxa of *Neomassaria*, *Roussoella*, *Oxydothis* and related genera used for phylogenetic analyses.

Species	Strain number	GenBank Accession number					References
		ITS	LSU	SSU	*rpb2*	*tef1*	
Acrocordiella occulta	RS10	KT949894	NA	NA	NA	NA	Jaklitsch et al. (2016)
Acrocordiella occulta	RS9	KT949893	NA	NA	NA	NA	Jaklitsch et al. (2016)
Aigialus grandis	JK 5244A	NA	GU301793	GU296131	GU371762	NA	Schoch et al. (2009)
Albertiniella polyporicola	CBS 457.88	NA	AF096185	AF096170	NA	NA	Suh et al. (1999)
Amniculicola lignicola	CBS 123094 (HT)	NA	EF493861	EF493863	EF493862	GU456278	Zhang et al. (2009)
Amphibambusa bambusicola	MFLUCC 11-0617	KP744433	KP744474	NA	NA	NA	Liu et al. (2015)
Amphisphaeria sorbi	MFLUCC 13 C0721	NA	KP744475	NA	NA	NA	Liu et al. (2015)
Amphisphaeria umbrina	AFTOL-ID 1229 (ET)	NA	FJ176863	FJ176809	NA	NA	Unpublished
Apiospora bambusae	ICMP 6889	NA	DQ368630	DQ368662	NA	NA	Tang et al. (2007)
Apiospora hydei	CBS 114990	KF144890	KF144936	NA	NA	NA	Crous et al. (2013)
Apiospora montagnei	AFTOL-ID 951	NA	DQ471018	NA	NA	NA	Spatafora et al. (2006)
Arecophila bambusae	HKUCC 4794	NA	AF452038	AY083802	NA	NA	Jeewon et al. (2003)
Arthopyrenia saltuensis	CBS 368.94	KF443410	AY538339	NA	KF443397	KF443404	Lumbsch et al. (2005)
Arthrinium phaeospermum	HKUCC 3395	NA	AY083832	AY083816	NA	NA	Unpublished
Astrosphaeriella aggregata	MAFF 239486 (HT)	NA	AB524591	AB524450	AB539092	AB539105	Tanaka et al. (2009)
Bartalinia robillardoides	CBS 122705 (ET)	KJ710460	KJ710438	NA	NA	NA	Crous et al. (2014)
Beltrania pseudorhombica	CBS138003	KJ869158	KJ869215	NA	NA	NA	Crous et al. (2014)
Beltraniella endiandrae	CBS137976	KJ869128	KJ869185	NA	NA	NA	Crous et al. (2014)
Broomella vitalbae	MFLUCC 15-0023	KP757755	KP757751	KP757759	NA	NA	Liu et al. (2015)
Cainia graminis	CBS 136.62 (ET)	NA	AF431949	AF431948	NA	NA	Lumbsch et al. (2002)
Cephalotheca foveolata	UAMH11631 (ET)	KC408422	KC408398	NA	NA	NA	Unpublished
Clypeosphaeria uniseptata	HKUCC6349 (ET)	NA	DQ810219	DQ810255	NA	NA	Unpublished
Colletotrichum gloeosporioides	LC0555	JN943090	JN940412	JN940356	NA	NA	Schoch et al. (2012)
Coniocessia anandra	Co108	GU553338	GU553349	NA	NA	NA	Asgari et al. (2011)
Coniocessia maxima	Co117	GU553332	GU553344	NA	NA	NA	Asgari et al. (2011)
Coniocessia nodulisporioides	Co126 (ET)	GU553333	GU553352	NA	NA	NA	Asgari et al. (2011)
Cordana abramovii	PE 0063-1a	NA	KF83336	NA	NA	NA	Zelski et al. (2014)
Cordana inaequalis	CBS 508.83	HE672146	HE672157	NA	NA	NA	Unpublished
Cordana pauciseptata	CBS 121804 (ET)	HE672149	HE672160	NA	NA	NA	Unpublished
Creosphaeria sassafras	CM AT-018	NA	DQ840056	NA	NA	NA	Unpublished
Cryptendoxyla hypophloia	WM10.89	NA	HQ014708	NA	NA	NA	Unpublished
Cycasicola goaensis	MFLU 17-0581 (HT)	NR_157510	NG_059057	NA	NA	NA	Wanasinghe et al. (2018)
Delitschia didyma	UME 31411	NA	DQ384090	AF242264	NA	NA	Kruys et al. (2006)
Delitschia winteri	AFTOL-ID 1599	NA	DQ678077	DQ678026	DQ677975	DQ677922	Schoch et al. (2006)
Diatrype disciformis	AFTOL-ID 927	NA	DQ470964	DQ471012	NA	NA	Spatafora et al. (2006)
Diatrype palmicola	MFLUCC 11-0020	KP744438	KP744482	KP753950	NA	NA	Liu et al. (2015)

Species	Strain number	GenBank Accession number					References
		ITS	LSU	SSU	*rpb2*	*tef1*	
Diatrype whitmanensis	ATCC MYA-4417	FJ746656	NA	NA	NA	NA	Unpublished
Didymella exigua	CBS 183.55 (HT)	NA	NA	GU296147	GU371764	NA	Schoch et al. (2009)
Eutypa lata	CBS 208.87	DQ006927	NA	NA	NA	NA	Rolshausen et al. (2006)
Herpotrichia juniperi	AFTOL-ID 1608	NA	DQ678080	DQ678029	DQ677978	DQ677925	Schoch et al. (2006)
Hyalotiella spartii	MFLUCC 13-0397	KP757756	KP757752	KP757760	NA	NA	Liu et al. (2015)
Hyponectria buxi	UME 31430	NA	AY083834	AF130976	NA	NA	Unpublished
Immersidiscosia eucalypti	HHUF 29920	AB594793	AB593722	AB593703	NA	NA	Tanaka et al. (2011)
Iodosphaeria tongrenensis	MFLU15-0393	KR095282	KR095283	KR095284	NA	NA	Li et al. (2015)
Lepteutypa cupressi	IMI 052255	NA	AF382379	AY083813	NA	NA	Jeewon et al. (2002)
Leptosphaerulina australis	CBS 317.83	NA	GU301830	GU296160	GU371790	GU349070	Schoch et al. (2009)
Lopadostoma turgidum	LT2	KC774618	NA	NA	NA	NA	Voglmayr et al. (2017)
Lophiostoma arundinis	AFTOL-ID 1606	NA	DQ782384	DQ782383	DQ782386	DQ782387	Schoch et al. (2006)
Lophiostoma macrostomoides	CBS 123097	NA	FJ795439	FJ795482	FJ795458	GU456277	Zhang et al. (2009)
Massaria anomia	WU 30509	NA	HQ599378	HQ599453	NA	HQ599318	Voglmayr et al. (2011)
Massaria ariae	WU 30510 (HT)	NA	HQ599381	HQ599458	NA	HQ599321	Voglmayr et al. (2011)
Massaria aucupariae	WU 30512	NA	HQ599384	HQ599455	NA	HQ599324	Voglmayr et al. (2011)
Massaria campestris	WU 30610	NA	HQ599386	NA	NA	HQ599326	Voglmayr et al. (2011)
Massaria conspurcata	WU 30519	NA	HQ599393	HQ599441	NA	HQ599333	Voglmayr et al. (2011)
Massaria gigantispora	WU 30521	NA	HQ599397	HQ599447	NA	HQ599337	Voglmayr et al. (2011)
Massaria inquinans	WU 30527	NA	HQ599402	HQ599444	HQ599460	HQ599342	Voglmayr et al. (2011)
Massaria lantanae	WU 30533 (HT)	NA	HQ599406	HQ599443	NA	HQ599346	Voglmayr et al. (2011)
Massaria macra	WU 30535 (HT)	NA	HQ599408	HQ599450	NA	HQ599348	Voglmayr et al. (2011)
Massaria mediterranea	WU 30547 (HT)	NA	HQ599414	NA	NA	HQ599354	Voglmayr et al. (2011)
Massaria parva	WU 30553	NA	HQ599418	HQ599467	NA	NA	Voglmayr et al. (2011)
Massaria platanoidea	WU 30556	NA	HQ599423	NA	NA	HQ599362	Voglmayr et al. (2011)
Massaria pyri	WU 30562 (HT)	NA	HQ599424	HQ599445	NA	HQ599363	Voglmayr et al. (2011)
Massaria ulmi	WU 30565	NA	HQ599427	NA	NA	HQ599366	Voglmayr et al. (2011)
Massaria vindobonensis	WU 30602	NA	HQ599432	NA	NA	HQ599371	Voglmayr et al. (2011)
Massaria vomitoria	WU 30606	NA	HQ599437	HQ599440	HQ599466	HQ599375	Voglmayr et al. (2011)
Massaria zanthoxyli	WU 30620	NA	HQ599439	HQ599454	NA	HQ599377	Voglmayr et al. (2011)
Massarina eburnea	CBS 473.64	NA	GU301840	GU296170	GU371732	GU349040	Schoch et al. (2009)
Massariosphaeria grandispora	CBS 613.86	NA	GU301842	GU296172	GU371725	GU349036	Schoch et al. (2009)
Melogramma campylosporum	MBU (ET)	JF440978	NA	NA	NA	NA	Jaklitsch et al. (2012)
Microdochium phragmitis	CBS 423.78 (ET)	MH861162	KP858948	NA	NA	NA	Vu et al. (2018)
Microdochium trichocladiopsis	CBS 623.77	KP858998	KP858934	NA	NA	NA	Hernandez et al. (2016)
Monosporascus cannonballus	FMR6682	NA	NA	AF340016	NA	NA	Collado et al. (2002)
Neomassaria fabacearum	MFLUCC 16-1875 (HT)	NA	KX524145	KX524147	NA	NA	Mapook et al. (2016)
Neomassaria fabacearum	**GMB0314**	**NA**	**ON4611373**	**ON461375**	**NA**	**ON505016**	**This study**

Species	Strain number	GenBank Accession number					References
		ITS	LSU	SSU	*rpb2*	*tef1*	
Neomassaria fabacearum	**GMB0388**	**NA**	**ON505052**	**ON505050**	**NA**	**ON505019**	**This study**
Neomassaria formosana	NTUCC 17-007	NA	MH714756	MH714759	NA	NA	Ariyaw et al. (2018)
Neomassaria hongheensis	KUMCC 21-0344 (HT)	NA	OL423113	OL423115	NA	NA	Yang et al. (2022)
Neoroussoella bambusae	MFLUCC 11-0124	KJ474827	KJ474839	NA	KJ474856	KJ474848	Liu et al. (2014)
Neoroussoella heveae	MFLUCC 17-1983	MH590693	MH590689	NA	NA	NA	Senwanna et al. (2018)
Neoroussoella solani	CPC 26331	KX228261	KX228312	NA	NA	NA	Crous et al. (2013)
Neottiosporina paspali	CBS 331.37	NA	EU754172	EU754073	GU371779	GU349079	Gruyter et al. (2009)
Oxydothis calamicola	MFLUCC 14-1165 (ET)	NA	KY206761	KY206767	NA	NA	Konta et al. (2016)
Oxydothis cyrtostachicola	FIH 151	DQ660334	DQ660337	NA	NA	NA	Hidayat et al. (2006)
Oxydothis fortunei	**GMB0315 (HT)**	**ON479893**	**ON479894**	**NA**	**NA**	**NA**	**This study**
Oxydothis fortunei	**GMB0389**	**ON510944**	**ON510945**	**NA**	**NA**	**NA**	**This study**
Oxydothis inaequalis	FIH 018	DQ660336	DQ660339	NA	NA	NA	Hidayat et al. (2006)
Oxydothis metroxylonicola	MFLUCC 15-0281 (ET)	KY206774	KY206763	KY206769	NA	NA	Konta et al. (2016)
Oxydothis palmicola	MFLUCC 15-0806 (ET)	KY206776	KY206765	KY206771	NA	NA	Konta et al. (2016)
Oxydothis phoenicis	MFLUCC 18-0270 (ET)	MK088066	MK088062	NA	NA	NA	Unpublished
Oxydothis rhapidicola	MFLUCC 14-0616 (ET)	NA	KY206766	KY206772	NA	NA	Konta et al. (2016)
Paramassaria samaneae	HKAS 102338	NA	NG068281	NG067686	NA	MK105748	Samarak and Hyde (2019)
Pararoussoella mangrovei	MFLU 17-1542 (HT)	MH025951	MH023318	NA	MH028250	MH028246	Wanasinghe et al. (2018)
Pararoussoella mukdahanensis	MFLU 11-0237 (HT)	NR155722	NA	NA	NA	NA	Dai et al.(2016)
Pararoussoella rosarum	MFLU 0654 (HT)	NR_157529	NG_059872	NA	NA	NA	Wanasinghe et al. (2018)
Parathyridaria percutanea	CBS 868.95	KF322118	KF366449	NA	KF366452	KF407987	Ahmed et al. (2014)
Parathyridaria ramulicola	CBS 141479 (HT)	NR_147657	NA	NG_061254	KX650584	KX650536	Jaklitsch et al. (2016)
Parathyridaria robiniae	MFLUCC 14-1119 (HT)	KY511142	KY511141	NA	NA	KY549682	Unpublished
Pestalotiopsis theae	SAJ-0021 (ET)	JN943623	JN940838	JN940785	NA	NA	Unpublished
Phialemonium atrogriseum	CBS 604.67	HE599384	HQ231981	NA	NA	NA	Summerbell et al. (2011)
Pseudomassaria chondrospora	It 1200	KR092790	KR092779	NA	NA	NA	Senanayake et al. (2015)
Pseudomassaria chondrospora	PC1 (ET)	JF440982	NA	NA	NA	NA	Jaklitsch et al. (2012)
Pseudoneoconiothyrium euonymi	CBS:143426 (HT)	MH107915	MH107961	NA	MH108007	NA	Crous et al. (2018)
Pseudoneoconiothyrium rosae	MFLU 18-0117 (HT)	NR_157523	NG_059868	NA	NA	NA	Wanasinghe et al. (2018)
Pseudoroussoella elaeicola	MFLUCC 15-15-0276a	MH742329	MH742326	NA	–	–	Unpublished
Requienella aquatic	MFLUCC 18-1040 (HT)	NR_171975	NG_073797	NA	NA	NA	Unpublished
Requienella chiangraina	MFLUCC 10-0556 (HT)	NR_155712	NG_059510	NA	NA	NA	Liu et al. (2014)
Requienella doimaesalongensis	MFLUCC 14-0584 (HT)	NR_165856	NG_068241	NA	KY678394	KY651249	Thambugala et al. (2017)

Species	Strain number	GenBank Accession number					References
		ITS	LSU	SSU	*rpb2*	*tef1*	
Requienella guttulata	MFLUCC 20-0102 (HT)	NR_172428	NG_075383	NA	NA	NA	Zhang et al. (2020)
Requienella hysterioides	MAFF 239636	NA	AB524621	AB524480	AB539101	AB539114	Schoch et al. (2009)
Requienella hysterioides	CBS 546.94	MH862484	MH874129	NA	KF443392	KF443399	Vu et al. (2018)
Requienella intermedia	CBS 170.96	KF443407	KF443382	NA	KF443394	KF443398	Ahmed et al. (2014)
Requienella japanensis	MAFF 239636 (HT)	NR_155713	NA	NA	NA	NA	Liu et al. (2014)
Requienella kunmingensis	HKAS 101773 (HT)	MH453491	MH453487	NA	MH453484	MH453480	Unpublished
Requienella magnatum	MFLUCC 15-0185 (HT)	NA	KT281980	NA	NA	NA	Unpublished
Requienella margidorensis	MUT 5329 (HT)	NR169906	MN556322	NA	MN605917	MN605897	Poli et al. (2020)
Requienella mediterranea	MUT5369 (HT)	KU314947	MN556324	NA	MN605919	MN605899	Poli et al. (2020)
Requienella mexicana	CPC 25355 (HT)	KT950848	KT950862	NA	NA	NA	Crous et al. (2014)
Requienella bambusarum	**GMB0316 (HT)**	**ON479891**	**ON479892**	**NA**	**ON505011**	**ON505015**	**This study**
Requienella bambusarum	**GMB0390**	**ON505055**	**ON505051**	**NA**	**ON505012**	**ON505017**	**This study**
Requienella neopustulans	MFLUCC 11-0609 (HT)	KJ474833	KJ474841	NA	NA	KJ474850	Liu et al. (2014)
Requienella nitidula	MFLUCC 11-0634	KJ474834	KJ474842	NA	KJ474858	KJ474851	Liu et al. (2014)
Requienella padinae	MUT 5503 (HT)	NA	MN556327	NA	MN605922	MN605902	Poli et al. (2020)
Requienella pseudohysterioides	GMBC0009 (HT)	MW881445	MW881451	NA	MW883345	NA	Unpublished
Requienella pustulans	KT 1709	NA	AB524623	NA	AB539103	AB539116	Tanaka et al. (2009)
Requienella seminuda	RS12	KT949912	NA	NA	NA	NA	Jaklitsch et al. (2016)
Requienella seminuda	RS13	KT949913	NA	NA	NA	NA	Jaklitsch et al. (2016)
Requienella siamensis	MFLUCC 0149 (HT)	KJ474837	KJ474845	NA	KJ474861	KJ474854	Liu et al. (2014)
Requienella siamensis	**GMB0317**	**ON4617749**	**ON461896**	**NA**	**ON505010**	**ON505014**	**This study**
Requienella siamensis	**GMB0391**	**ON505054**	**ON505053**	**NA**	**ON505013**	**ON505018**	**This study**
Requienella thailandica	MFLUCC 0621 (HT)	KJ474838	KJ474846	NA	NA	NA	Liu et al. (2014)
Requienella tosaensis	KT 1659	NA	AB524625	NA	AB539104	AB539117	Tanaka et al. (2009)
Requienella tuberculata	MFLUCC 0854 (HT)	KU940132	KU863121	NA	NA	KU940199	Dai et al. (2016)
Requienella verrucispora	CBS 125434 (HT)	KJ474832	NA	NA	NA	NA	Liu et al. (2014)
Requienella yunnanensis	HKAS 101762	MH453492	MH453488	NA	NA	MH453481	Unpublished
Robillarda sessilis	CBS 114312 (ET)	KR873256	KR873284	NA	NA	NA	Crous et al. (2014)
Robillarda terrae	CBS 587.71	KJ710484	KJ710459	NA	NA	NA	Crous et al. (2014)
Roussoella scabrispora	MFLUCC 14-0582	KY026583	KY000660	NA	NA	NA	Unpublished
Roussoellopsis macrospora	MFLUCC 12-0005	NA	KJ474847	NA	KJ474862	KJ474855	Liu et al. (2014)
Seiridium phylicae	CPC 19962	KC005785	KC005807	NA	NA	NA	Crous et al. (2012)
Seynesia erumpens	SMH 1291	NA	AF279410	AF279409	NA	NA	Bhattacharya et al. (2000)
Subramaniomyces fusisaprophyticus	CBS 418.95	EU040241	NA	NA	NA	NA	Crous et al. (2007)

Species	Strain number	GenBank Accession number					References
		ITS	LSU	SSU	*rpb2*	*tef*1	
Thyridaria acaciae	CBS:138873	KP004469	KP004497	NA	NA	NA	Crous et al. (2014)
Thyridaria broussonetiae	CBS 121895	KX650567	NA	NA	KX650585	KX650538	Jaklitsch et al. (2016)
Thyridariella mahakoshae	NFCCI 4215	MG020435	MG020438	NA	MG020446	MG023140	Devadatha et al. (2018)
Thyridariella mangrovei	NFCCI 4213	MG020434	MG020437	NA	MG020445	MG020443	Devadatha et al. (2018)
Torula herbarum	CBS 111855	KF443409	KF443386	NA	KF443396	KF443403	Ahmed et al. (2014)
Trematosphaeria pertusa	CBS 122371	NA	GU301876	GU348999	GU371801	GU349085	Schoch et al. (2009)
Vialaea mangiferae	MFLUCC 12-0808	KF724974	KF724975	NA	NA	NA	Senanayake et al. (2014)
Vialaea minutella	BRIP 56959 (ET)	KC181926	KC181924	NA	NA	NA	McTaggart et al. (2013)
Xylaria hypoxylon	CBS 122620 (ET)	AM993141	NA	NA	NA	NA	Persoh et al. (2009)
Xylaria polymorpha	MUCL: 49904	FN689809	NA	NA	NA	NA	Fournier et al. (2011)
Zopfia rhizophila	CBS 207.26	NA	DQ384104	L76622	NA	NA	LoBuglio et al. (1996)

Notes: Type specimens are marked with HT (holotype), ET (epitype); NA: No sequence is available in GenBank; newly generated sequences are indicated in bold.

Results

Phylogenetic analyses

Three phylogenetic trees for each genus and their related genera were provided.

The dataset for Fig. 1 consists of 40 taxa for representative strains of species in Neomassariaceae, which has 1989 characters including gaps (SSU: 1–515, *tef*1:516–1192, LSU: 1193–1989). The best scoring likelihood tree was selected with a final ML optimization likelihood value of -23512.21. *Paramassaria samaneae* Samarak & K.D. Hyde (HKAS 102338) was selected as the outgroup taxon. Strain GMB0314 gathered with *N. fabacearum* with high statistical support (100% ML, 1.00 BYPP, Fig. 1).

The dataset for Fig. 2 consists of 46 taxa for representative strains of species in Roussoellaceae with 2330 characters, including gaps (ITS: 1–375, *tef*1: 376–1063, LSU: 1064–1592, *rpb2*: 1593–2330). The final ML optimization likelihood value of the best scoring likelihood was -16254.35. *Torula herbarum* Link (CBS 111855) was selected as the outgroup taxon. Strains of the *R. bambusarum* formed a clade with *R. doimaesalongensis* Thambug. & K.D. Hyde with statistical support (26% ML, 0.97 BYPP). Strain GMB0317 gathered with *R. siamensis* Phook., Jian K. Liu & K.D. Hyde with high statistical support (100% ML, 1.00 BYPP, Fig. 2).

The alignment for Fig. 3 consists of 66 taxa for representative strains of species in Oxydothidaceae including outgroup taxa with 1630 characters (ITS: 1–307, LSU: 308–1089, SSU: 1090–1630). The best scoring likelihood tree was selected with a final ML optimization likelihood value of -19975.73. *Cordana pauciseptata* Preuss (CBS 121804) was selected as the outgroup taxon. Our strains of the new species *O. fortunei* are from a distinct clade with *O. inaequalis* Hidayat et al. (98% ML, 1 BYPP, Fig. 3).

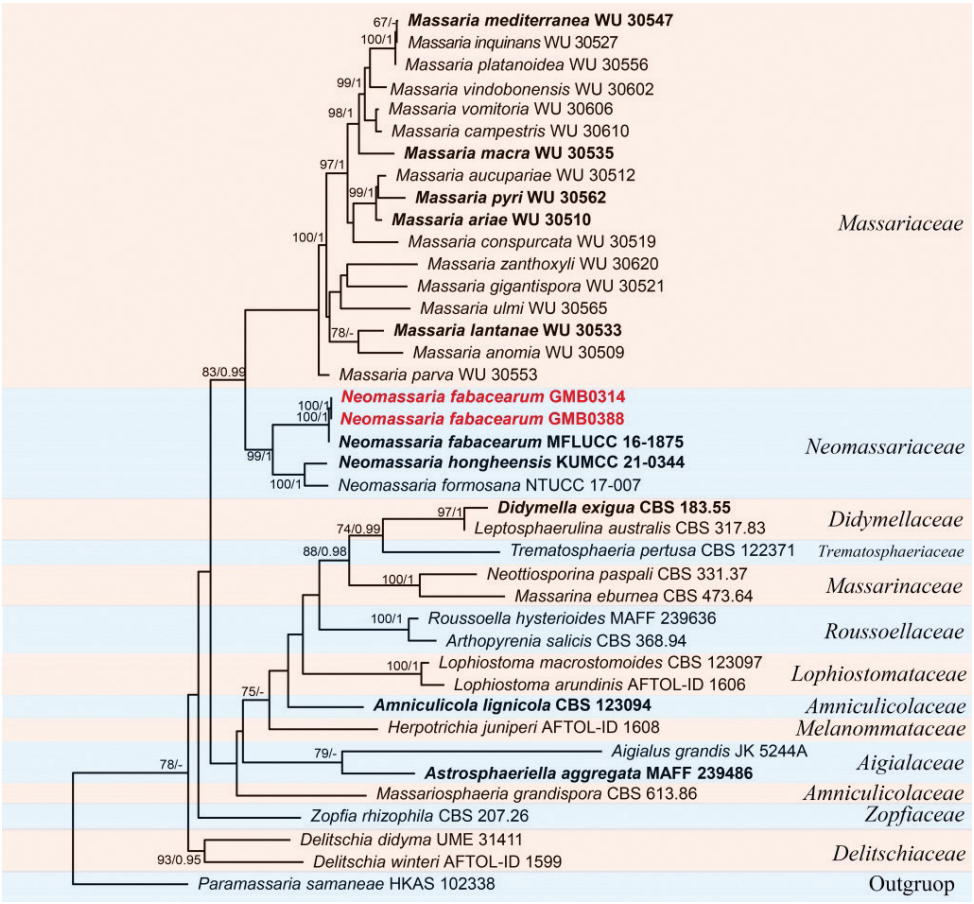

Figure 1. RAxML tree of *Neomassaria* and related genera obtained from the concatenated DNA sequence data of LSU, SSU and *tef*1 genes. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. The new collections are in red bold and type strains are in bold.

Taxonomy

The four species in this study were *Neomassaria fabacearum*, *Roussoella bambusarum*, *Roussoella siamensis*, *Oxydothis fortunei*. *Neomassaria* and *Roussoella* is a genus of ascomycete fungi in the order *Pleosporales*. *Oxydothis* is a genus of ascomycete fungi in the order *Xylariales*.

Neomassaria fabacearum **Mapook, Camporesi & K.D. Hyde, Fungal Diversity 80: 77 (2016)**

MycoBank No: 552274

Fig. 4

Descriptions. see Hyde et al. (2016).

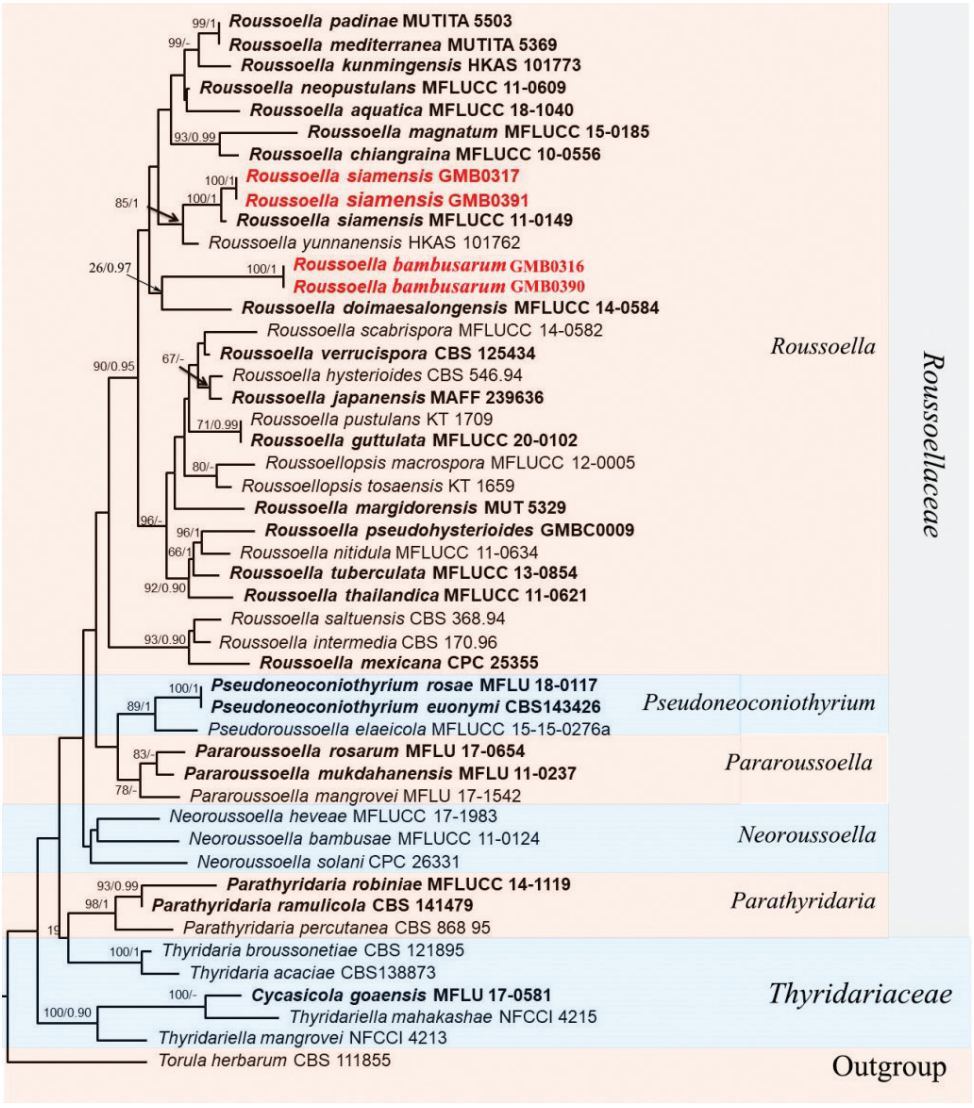

Figure 2. RAxML tree of *Roussoella* and related genera based on a combined ITS, LSU, *rpb*2 and *tef*1 sequences dataset. Bootstrap support values for ML equal to or greater than 60% and BYPP equal to or greater than 0.95 are given above the nodes. The new collections are in red bold, type strains are in bold.

Specimens examined. China, Guizhou Province, the campus of Guizhou Medical University (26°24'34.02"N, 106°45'16.22"E), on bamboo, 12 December 2021. Altitude: 1145 m, H.M. Hu, 2021GYHS23 (GMB0314; KUN-HKAS 123429; living culture GMBC0314).

Other material examined. China, Guizhou Province, the campus of Guizhou Medical University (26°24'34.01"N, 106°45'09.24"E), on bamboo, 12 December 2021. Altitude: 1135 m, H.M. Hu, 2021GYHS28 (GMB0388, living culture GMBC0388).

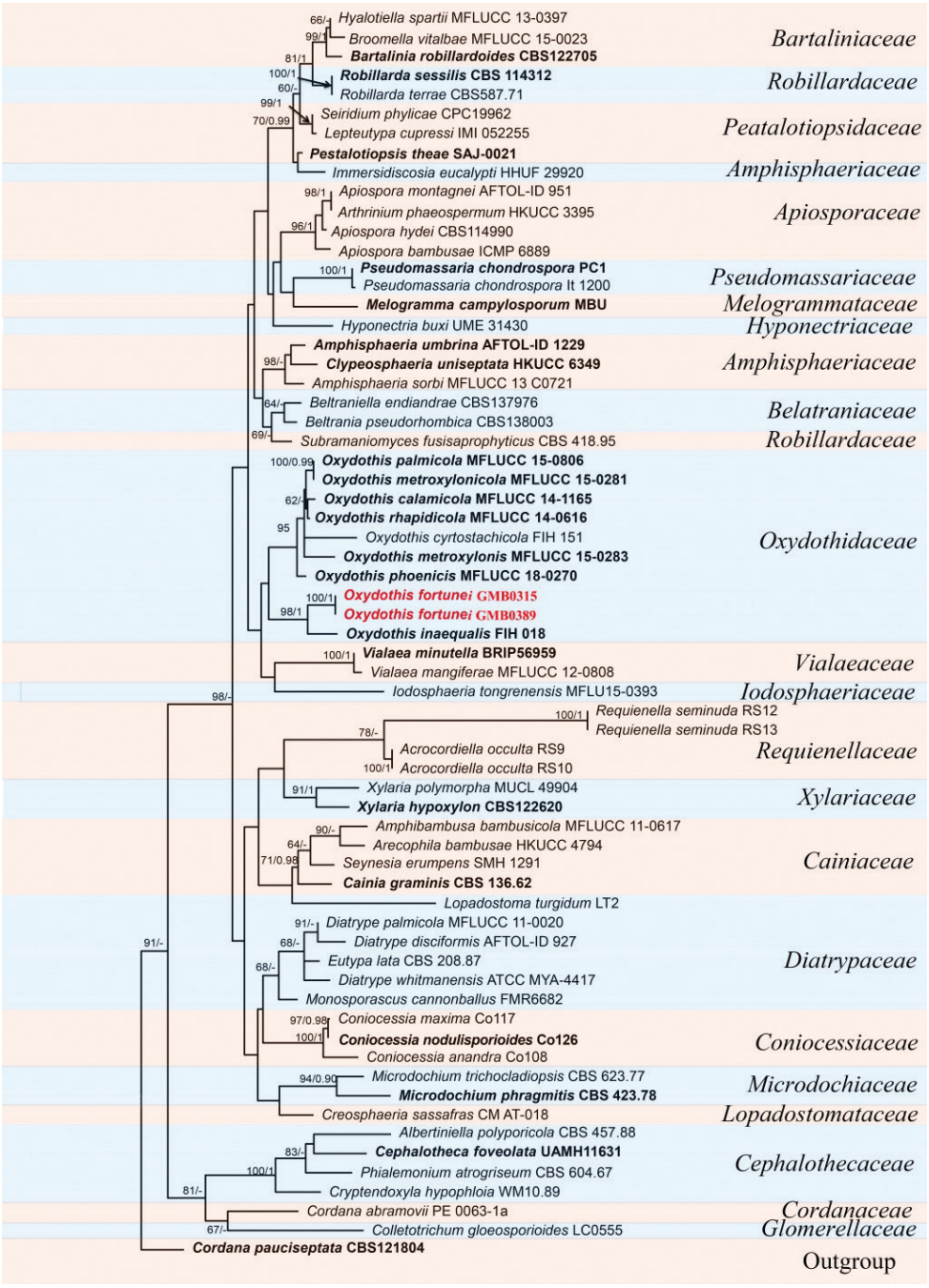

Figure 3. RAxML tree of *Oxydothis* and related genera based on a combined ITS, LSU and SSU sequences dataset. Bootstrap support values for ML equal to, or greater than, 60% and BYPP equal to or greater than 0.95 are given above the nodes. The new collections are in red bold and ex-type strains are in bold.

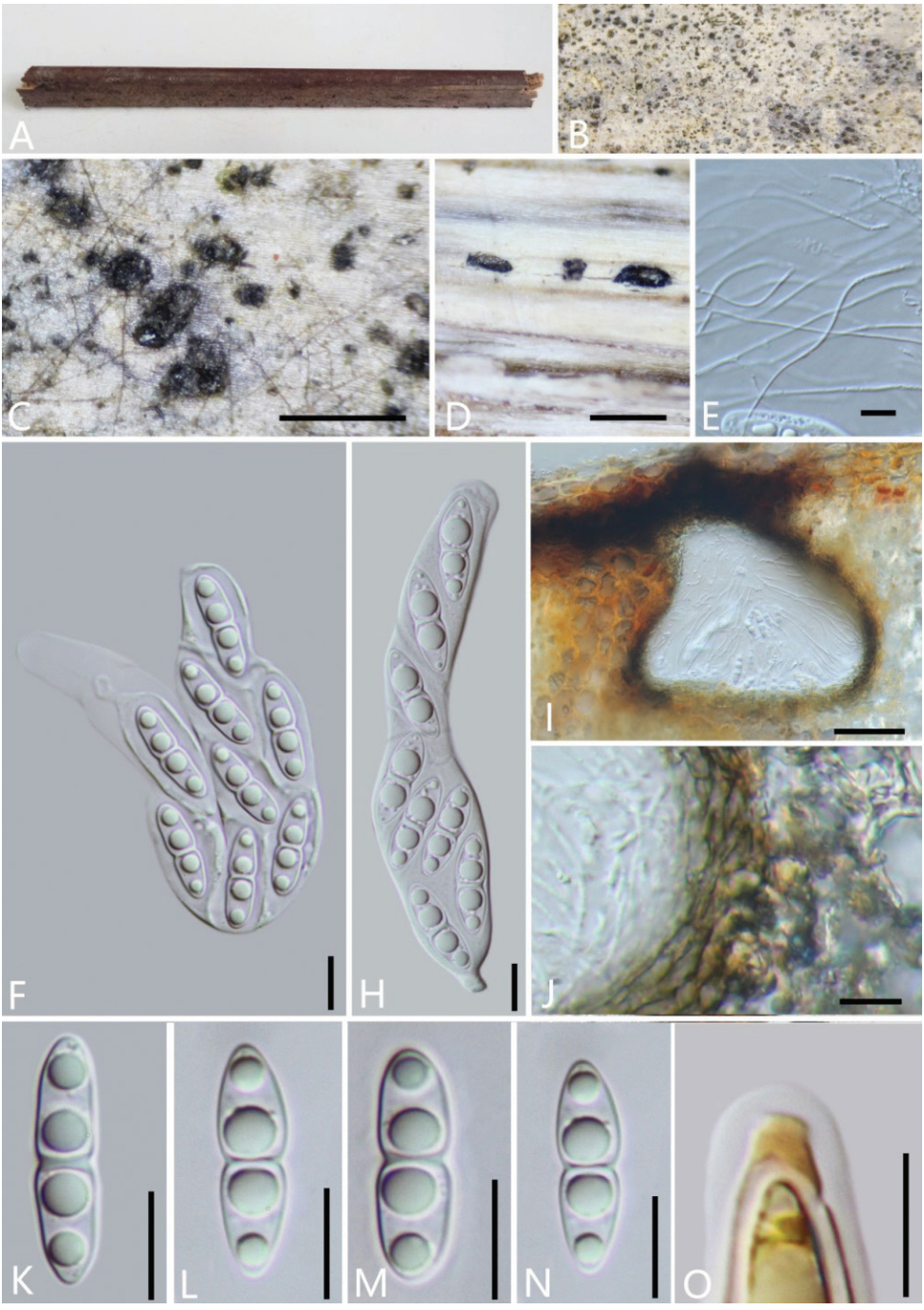

Figure 4. *Neomassaria fabacearum* (GMB0314) **A** stromata on host substrate **B, C** appearance of ascomata on substrate **D** cross section of ascomata **E** pseudoparaphyses **F, H** asci **I** longitudinal section of an ascoma **J** peridium **K–N** ascospores **O** apical apparatus (stained in Melzer's Reagent). Scale bars: 0.5 mm (**C–D**); 10 μm (**E–H, K–O**); 50 μm (**I, J**).

Notes. There are three *Neomassaria* species documented in Index Fungorum (accession date: May 1, 2022). Type species of *N. fabacearum* was originally described from Italy (Hyde et al. 2016). Subsequently, *N. formosana*, and *N. hongheensis* were introduced from Taiwan and Yunnan in China, respectively (Ariyawansa et al. 2018; Yang et al. 2022). The ascospore dimension of *N. fabacearum* is between those of *N. formosana* (20–30 × 3–7 μm) and *N. hongheensis* (14–17 × 4–8 μm) (Hyde et al. 2016; Ariyawansa et al. 2018; Yang et al. 2022). Phylogenetic analyses of the combined SSU, LSU and *tef*1 sequences dataset shows that new collections gather with *N. fabacearum* (MFLU 16–1875), the type specimen, with the high support (100% ML, 1 BYPP; Fig. 1). Morphologically, the features of GMB0314 are consistent with those of *N. fabacearum* (Hyde et al. 2016). *Neomassaria fabacearum* was first introduced to the China.

Roussoella bambusarum **H. M. Hu & Q. R. Li, sp. nov.**
MycoBank No: 844142
Fig. 5

Holotype. GMB0316.

Etymology. In reference to the host, *Bambusa bambusarum* (Lour.) Raeusch. ex Schult. 'Fernleaf' R. A. Young

Description. ***Saprobic*** on decaying culms of *B. bambusarum*. **Sexual morp:** ***Ascostromata*** 111–146 μm high, 460–560 μm diam., ($\overline{x}$ = 123 × 539 μm, n = 30), immersed under a clypeus, solitary or scattered, raised hemispherical or dome-shaped on host epidermis, black, coriaceous, glabrous, uni-loculate. ***Locules*** 335–414 μm diam., 128–212 μm high, immersed within ascostromata, black, globose to subglobose. ***Ostioles*** with minute papillate. ***Peridium*** 19–34 μm thick, composed of dark brown thin-walled cells of *textura angularis*. ***Hamathecium*** comprised of 1–2 μm wide, numerous, septate, branched, anastomosing, filiform, hyaline, pseudoparaphyses. ***Asci*** 120–143 × 8–12 μm ($\overline{x}$ = 134 × 10 μm, n = 30), 8-spored, bitunicate, cylindrical, curved, short pedicellate with knob-like pedicel, apically rounded with an indistinct ocular chamber. ***Ascospores*** 14–20 × 6–7 μm ($\overline{x}$ = 17.6 × 6.7 μm, n = 30), dark brown to brown, 1-seriate, sometimes overlapping, 2-celled, constricted at the septum, ellipsoidal to fusiform, straight, rough-walled, guttulate, conically rounded ends, with longitudinal striations. **Asexual morph:** Undetermined.

Culture characters. Ascospores germinated on PDA within 24 hours at 25 °C, colonies are reaching 5 cm diam. The colony on the surface is white, grey, circular, flocculent, dense, cottony mycelium, colony reverse is white and gray, white in the middle. Not sporulating on OA nor on PDA.

Specimens examined. China, Guizhou Province, Guiyang Huaxi National Urban Wetland Park (26°2'2.34"N, 106°34'16.22"E), on decaying culms of *B. bambusarum*, 12 October 2021. Altitude: 1130 m, Y.P Wu and H.M Hu, 2021 HXGY01 (GMB0316, holotype; KUN-HKAS 123431, isotype; GMBC0316, ex-type living culture).

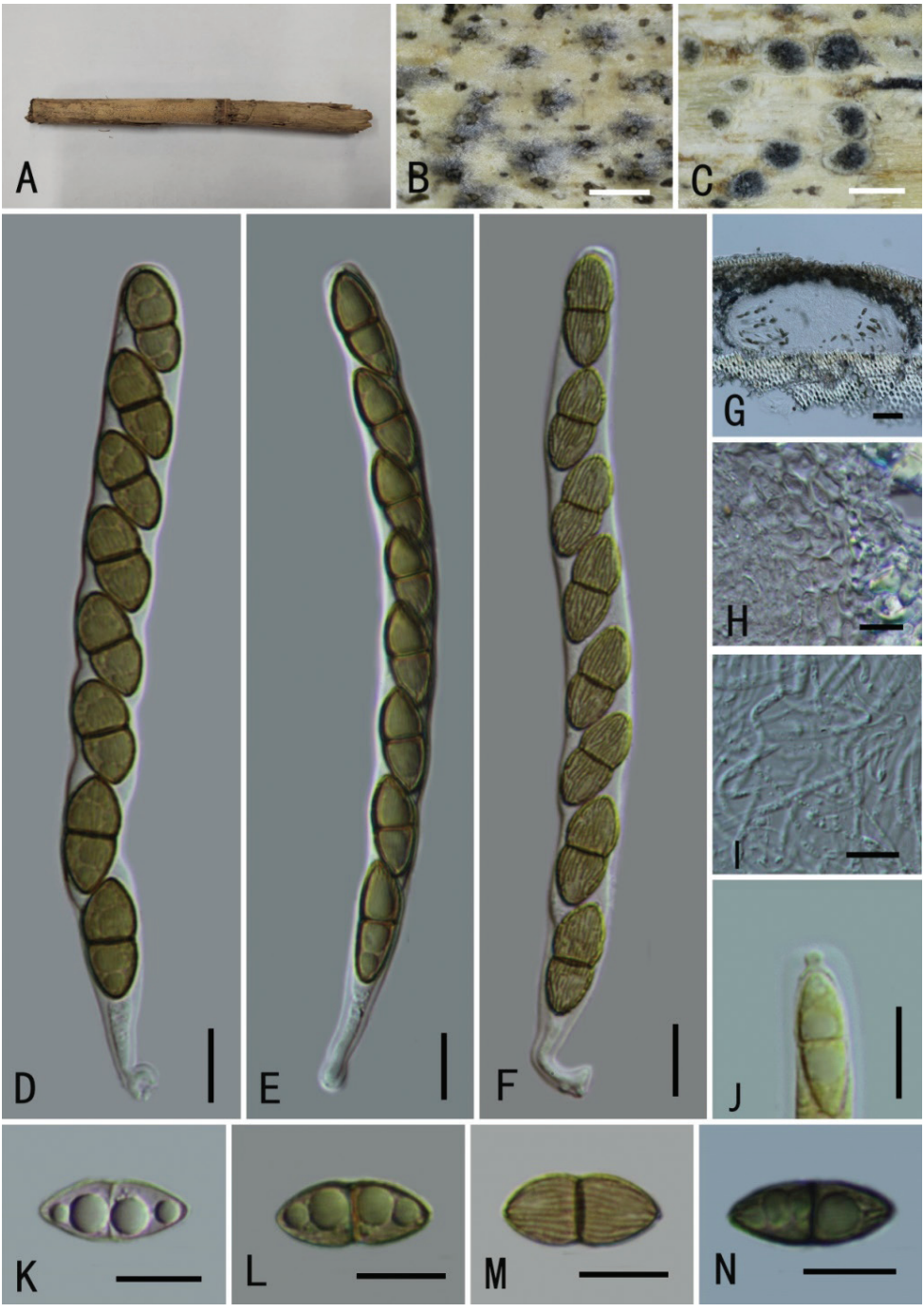

Figure 5. *Roussoella bambusarum* (Holotype, GMB0316) **A** stromata on host substrate **B** ascostromata on bamboo culm **C** cross-section of ascostromata **D–F** asci **G** longitudinal section of ascostromata **H** peridium **I** pseudoparaphyses **J** apical apparatus (stained in Melzer's Reagent) **K–N** ascospores. Scale bars: 0.5 mm (**B–C**); 10 μm (**D–F, H–N**); 50 μm (**G**).

Other examined material. China, Guizhou Province, Guiyang Huaxi National Urban Wetland Park (26°10'44.13"N, 106°43'13.12"E), on decaying culms of *B. bambusarum*, 15 October 2021. Altitude: 1201 m, Y.P Wu and H.M Hu, 2021 HXGY55 (GMB0390; GMBC0390, living culture).

Notes. Morphologically, *Roussoella bambusarum* is similar to *R. thailandica* D.Q. Dai et al., but differs from the latter by having larger ascospores (17.6 × 6.7 μm vs. 14.5 × 5.5 μm), larger upper cells, occasionally curve, narrowly at both ends, with irregular longitudinal striations. (Liu et al. 2014). Phylogenetic analysis showed that *R. bambusarum* and *R. doimaesalongensis* Thambug. & K.D. Hyde were clustered together (26% ML, 0.97 BYPP; Fig. 2) (Thambugala et al. 2017).

***Roussoella siamensis* Phook., Jian K. Liu & K.D. Hyde, Phytotaxa 181(1): 18 (2014)**
MycoBank No: 550665
Fig. 6

Descriptions. see Liu et al. (2014).

Specimens examined. China, Guizhou Province, Guiyang Huaxi National Urban Wetland Park (26°2'23.04"N, 106°34'16.22"E) on decaying culms of *B. bambusarum*, 12 October 2021. Altitude: 1130 m, Y.P. Wu and H.M. Hu, 2021 HXGY03 (GMB0317; living culture GMBC0317).

Other material examined. China, Guizhou Province, Guiyang Huaxi National Urban Wetland Park (26°2'10.10"N, 106°34'16.10"E) on decaying culms of *B. bambusarum*, 15 October 2021. Altitude: 1145 m, Y.P. Wu and H.M. Hu, 2021 HXGY70 (GMB0391; living culture GMBC0391).

Notes. Phylogenetic analyses of the alignment combining ITS, LSU, *rpb*2 and *tef*1 show that GMB0317 cluster with *R. siamensis* (MFLU 13-0639) with the high support value (100% ML, 1 BYPP; Fig. 2). Characteristics of GMB0317 are consistent with those of *R. siamensis*, which was originally introduced from decaying bamboo culms in Thailand (Liu et al. 2014) This species was first found in China.

***Oxydothis fortunei* H. M. Hu & Q. R. Li, sp. nov.**
MycoBank No: 844141
Fig. 7

Holotype. GMB0315.

Etymology. In reference to the host, *Trachycarpus fortunei* (Hook.) H. Wendl.

Description. ***Saprobic*** on surface of culms of *T. fortunei*. **Sexual morph:** ***Ascomata*** 205–317 μm diam. ($\overline{x}$ = 261 μm, n = 30), solitary or aggregated in groups, immersed, forming slightly raised as blistering areas on the host surface, long axis horizontal to that of the host, 18–41 μm high × 155–207 μm broad, in transverse section, ellipsoid, ostiolate, coriaceous, black, flat. ***Peridium*** 24–27 μm thick, composed of 2–3 several layers of flat-

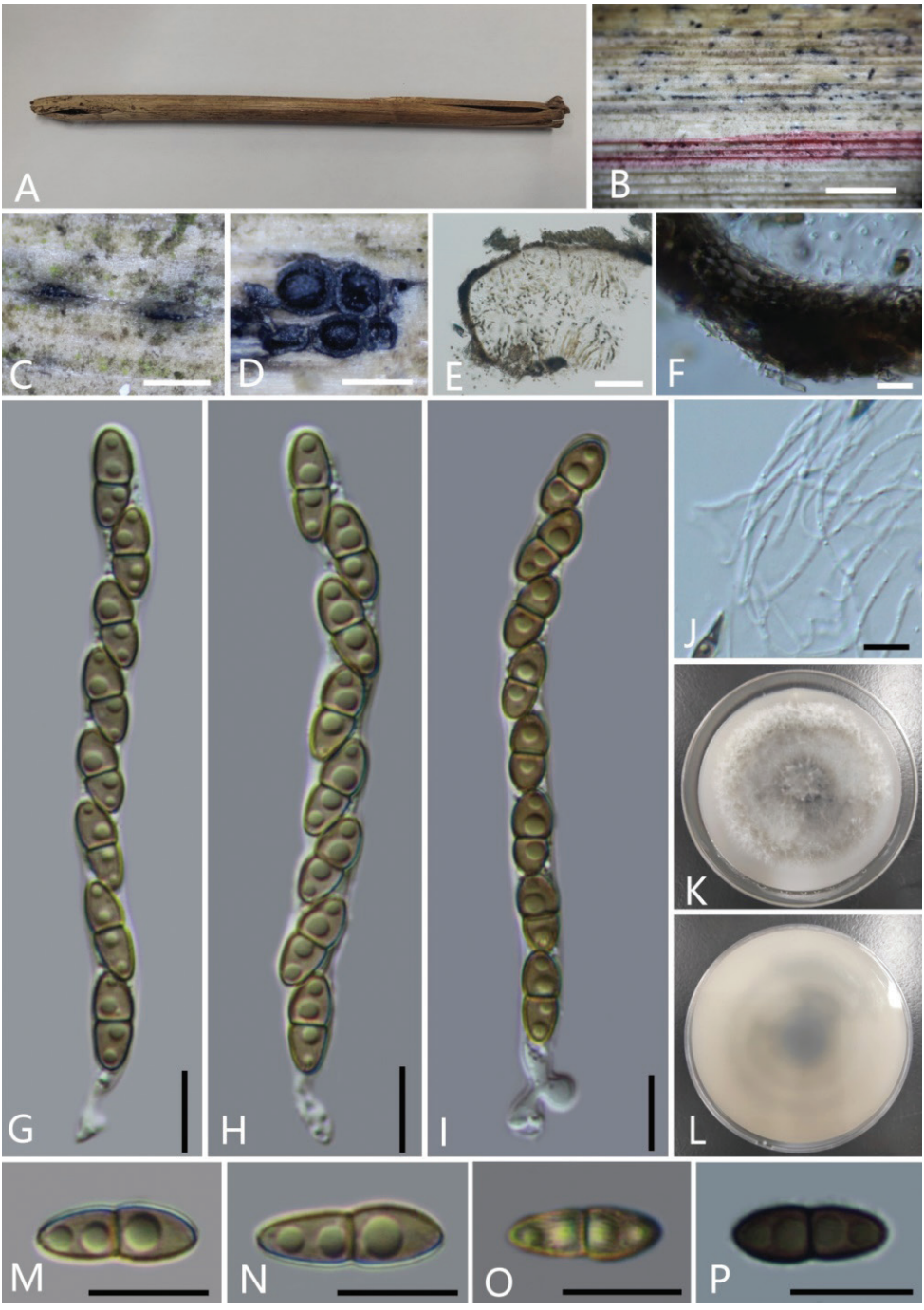

Figure 6. *Roussoella siamensis* (GMB0317) **A** stromata on host substrate **B, C** ascostromata on bamboo culm **D** cross-section of ascostromata **E** Longitudinal section of ascostromata **F** peridium **G–I** asci **J** pseudoparaphyses **K–L** culture on PDA **M–P** ascospores Scale bars: 0.5 mm (**C–D**); 50 μm (**E**); 10 μm (**F–J, M–P**).

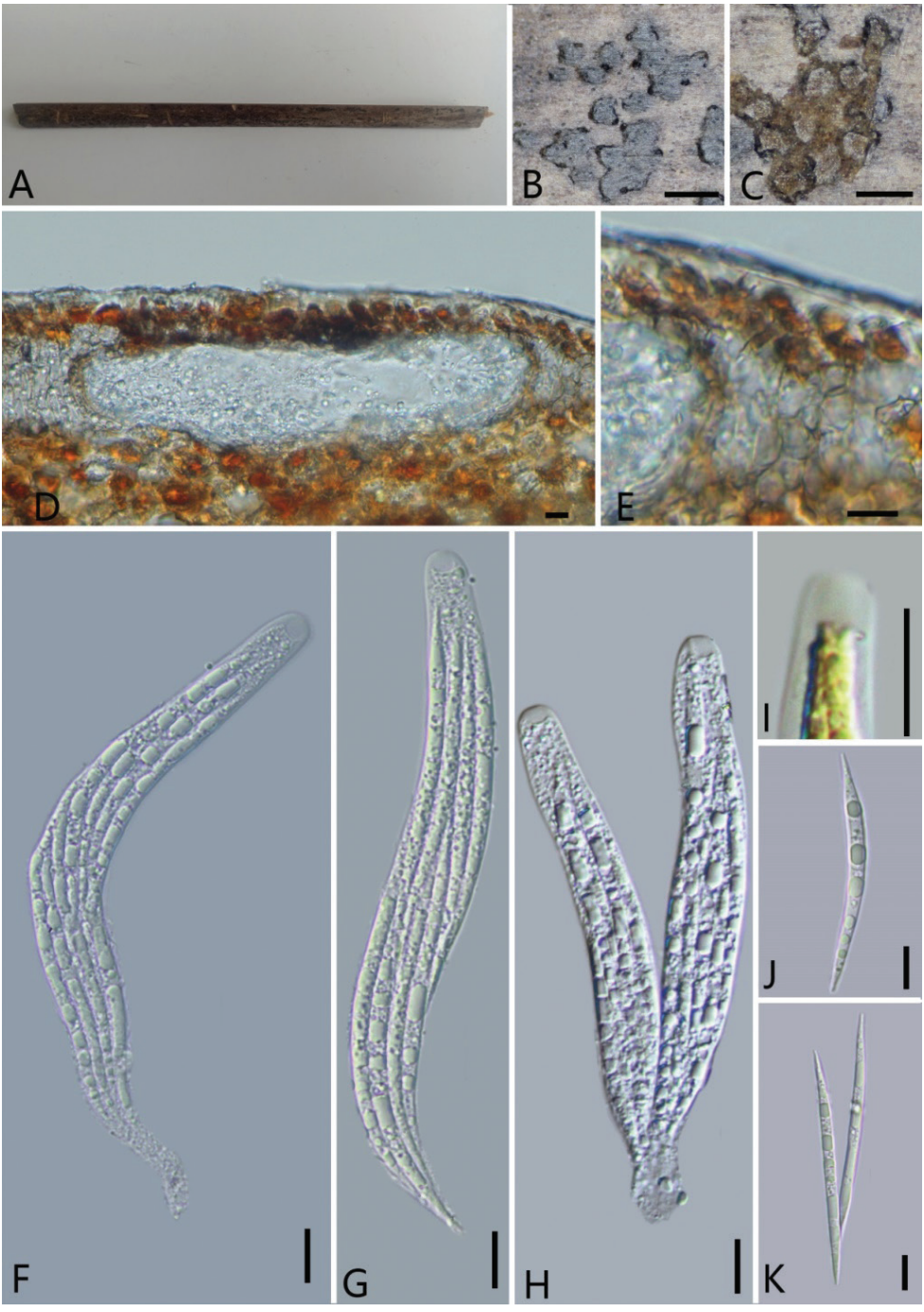

Figure 7. *Oxydothis fortunei* (Holotype, GMB0315) **A** stromata on host substrate **B** close-up of ascomata **C** cross-section of the ascomata **D** longitudinal section of an ascoma **E** peridium **F–H** asci **I** apical apparatus (stained in Melzer's Reagent) **J, K** ascospores. Scale bars: 0.5 mm (**B–C**); 10 μm (**D–K**).

tened, light-brown cells. ***Asci*** 108–121× 9–14 µm ($\bar{x}$ = 114 × 12 µm, n = 20), 8-spored, unitunicate, cylindrical, mostly straight, pedicellate, with a J-, subapical apparatus, 4.2–4.9 µm high, 5.5–6.8 µm diam. ***Ascospores*** 56–72 µm × 3–4 µm ($\bar{x}$ = 66 × 3.3 µm, n = 30), fusiform, hyaline, obliquely 1–2-seriate, tapering gradually from the center to the ends, with multi-guttules in each cell, pointed processes. **Asexual morph:** Undetermined.

Culture characteristics. Ascospores germinated on PDA within 24 hours at 25 °C, colonies are reaching 4.5 cm diam. circular, transparent, thin, colony reverse is same. Not sporulating on OA nor on PDA.

Specimen examined. China Guizhou Province, Long gong scenic spot (26°04'35.02"N, 105°52'15.04"E), on surface of culms of *T. fortunei*, 5 December 2021. Altitude: 1120m, Q.R. Li and X. Xu, 2021 LG9 (GMB0315, holotype; KUN-HKAS 123430, isotype; ex-type living culture GMBC0315).

Other examined material. China, Guizhou Province, Long gong scenic spot (26°04'47.41"N, 105°31'10.34"E), on surface of culms of palm, 7 December 2021. Altitude: 1095m, Q.R. Li and X. Xu, 2021 LG15 (GMB0389; living culture GMBC0389).

Notes. *Oxydothis fortunei* is morphologically similar to *O. nonamyloidea* K.D. Hyde and *O. rhapidicola* S. Konta & K.D. Hyde in the shape of ascospores (Hyde 1994; Hidayat et al. 2006; Konta et al. 2016). However, the ascospores of *O. fortunei* (56–72 × 3–4 µm) are shorter than those of *O. nonamyloidea* (94–115 × 3.5–4.5 µm) and *O. rhapidicola* (47–50 × 3–5 µm). Moreover, it is distinguished from *O. rhapidicola* since the latter has a blue slit-like ascus subapical apparatus in Melzer's reagent (Konta et al. 2016). *Oxydothis fortunei* showed the close kinship to *O. inaequalis* (100% ML, 1 BYPP; Fig. 3). However, *O. fortunei* differs from *O. inaequalis* by its shape of the ascospores, and the J- ascus subapical apparatus as well as the smaller ascospores (56–72 × 2.9–3.9 µm vs. 78–100 × 5–6 µm) (Hidayat et al. 2006).

Discussion

In this study, two new species and two new records associated with bamboo and palm were introduced based on phylogenetic relationships of combined ITS, LSU, SSU, *rpb*2 and *tef*1 sequences and morphological evidences.

There are a large number of fungi associated with bamboo and palm in China (Hyde et al. 2002; Phukhamsakda et al. 2022). Studies on the diversity of bamboo and palm fungi can be of economic significance and of academic value (Arnold and Lewis 2005). According to statistics, there are nearly 500 bamboo species distributed in 37 genera in China, which play an important role in human life, such as in the fields of architecture, production tools, artwork, and landscaping, etc. (Zhao and Wei 2018). In China, palms are mainly used for ornamental purposes in landscape gardens (Fetouh et al. 2018). About 2,450 species of palm plants were documented in the world, belonging to 183 genera (Qureshimatva et al. 2018). The rich and diverse ecosystems composed of these bamboo and palm resources provide good habitats for fungi to sur-

vive, creating the diversity of fungal species (Cheek et al. 2020). There are 75 genera and 189 fungal species on bamboo that have been reported in mainland China, and 79 species and 58 genera of bamboo fungi that have been reported in Hong Kong (Yong et al. 2009; Shukla et al. 2016). Many species of *Roussoella* have been introduced from the bamboo (Liu et al. 2014). New collections of *Roussoella* also were saprophyte on bamboo. Most species of *Oxydothis* were discovered on palm including *O. fortunei* (Konta et al. 2016). This is the first introduction of *Neomassaria* species associated on bamboo (Ariyawansa et al. 2018; Yang et al. 2022). In this study, four microfungi were introduced, which enriches the diversity of fungi on bamboo and palm in China. Meanwhile, all those four species are saprophyte on and accelerates the decay of bamboo or palm. As an ideal growth substrate for fungi, bamboo fungi are rich in species, and there are a large number of fungi to be discovered.

Acknowledgements

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Four new *Phragmidium* (Phragmidiaceae, Pucciniomycetes) species from Rosaceae plants in Guizhou Province of China

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Abstract

In this study, four new species of *Phragmidium* were proposed based on morphological and molecular characters. In morphology, *Phragmidium rosae-roxburghii* sp. nov. was distinguished to related taxa by its unique square to diamond-shaped urediniospores; *Ph. rubi-coreani* sp. nov. differed from *Ph. barclayi* and *Ph. cibanum* because of teliospores with fewer cells and shorter pedicels; urediniospores of *Ph. potentillae-freynianae* sp. nov. were bigger than *Ph. duchesneae-indicae*; and *Ph. rosae-laevigatae* sp. nov. produced bigger urediniospores than *Ph. jiangxiense*. The phylogenetic analyses based on the combination of two loci (ITS and LSU) also supported our morphological conclusion. In the meantime, three previously known species were also described herein.

Keywords

Basidiomycota, ITS, LSU, phylogeny, rust disease, taxonomy

Introduction

Phragmidium (Phragmidiaceae) was established by Link (1816) and characterized by laterally separated multicellular teliospores with pigmented bilaminar walls, and a thickened pedicel at the base (Wei 1988).

The genus was widely distributed around the world especially in the northern hemisphere, such as China, USA and Japan (Wei 1988; Zhuang 1989; Cummins and Hiratsuka 2003; Maier et al. 2003; Zhuang et al. 2012; Pscheidt and Rodriguez 2016; Liu et al. 2018, 2019, 2020; Zhao et al. 2021). *Phragmidium* species often caused severe rust diseases in Rosaceae plants (*Rosa*, *Rubus*, *Potentilla*, *Sanguisorba*, *Duchesnea* and *Acaena*). Species of *Phragmidium* have been reported growing on host plants of *Rosa*, *Rubus*, and *Potentilla*, with a few species on *Sanguisorba* (Cummins and Hiratsuka 2003; Maier et al. 2003; Yun et al. 2011; Pscheidt and Rodriguez 2016; Liu et al. 2018, 2019, 2020), *Duchesnea* (Zhao et al. 2021) and *Acaena* (McTaggart et al. 2016). Two species *Ph. mucronatum* (Pers.) Schltdl. and *Ph. tuberculatum* Jul. Müll., were common pathogens on ornamental roses worldwide (Wahyuno et al. 2001, 2002; Leen and Van Huylenbroeck 2007; Wilson and Aime 2014).

About 8000 species of rust fungi have been reported in the world (Zhao et al. 2021). Based on morphological features or host associations, 1200 species belonging to 71 genera of 15 families were previously reported in China. Over 70 *Phragmidium* species have been described (Cummins 1931; Arthur 1934; Zhuang et al. 1998, 2003, 2005, 2012; Wahyuno et al. 2001; Cummins and Hiratsuka 2003; Yang et al. 2015; Ali et al. 2017; Aime et al. 2018; Liu et al. 2018, 2019, 2020; Ono and Wahyuno 2019; Aime and McTaggart 2021; Zhao et al. 2021).

Traditionally, *Phragmidium* species are distinguished based on teliospores morphology (Wei 1988). According to Wahyuno et al. (2001) and Zhao et al. (2021) *Phragmidium* species are difficult to distinguish based only on morphology of asexual spore stages; thus, DNA data is essential for taxonomy and identification of *Phragmidium* species.

The combination of morphological and molecular characters has been applied in the taxonomy of rust fungi (Beenken et al. 2012; Beenken 2014; McTaggart et al. 2016, 2017; Liu et al. 2018, 2019, 2020; Ono and Wahyuno 2019; Zhao et al. 2021). *Phragmidium* includes more than 270 epithet records which are listed in MycoBank (https://www.mycobank.org) and Index Fungorum (http://www.indexfungorum.org) (accessed in October 2022). However, only 28 records were described and named by Chinese researchers, three *Phragmidium* taxa in Guizhou Province, *Ph. duchesneae-indicae*, *Ph. nonapiculatum* and *Ph. kans* were introduced by Zhao et al. (2021). In the present study, thirteen fresh rust specimens were collected on eight Rosaceae hosts, such as *Duchesnea indica*, *Potentilla freyniana*, *P. kleiniana*, *Rosa roxbunghii*, *R. laevigata*, *Rosa* sp., *Rubus coreanus* and *Ru. parrifolius* in Guizhou Province. This study aimed to determine the taxonomic status of the parasitic pecies of the Rosaceae in Guizhou Province through morphological and molecular characters. Meanwhile, we hope to contribute a significant amount of molecular data that may aid future studies and phylogenetic placement of *Phragmidium* in the Pucciniales.

Materials and methods

Sampling and microscopy observation

Thirteen fresh rust specimens were collected on branch and leaf from eight species of Rosaceae, *Duchesnea indica*, *Potentilla freyniana*, *P. kleiniana*, *Rosa roxbunghii*, *R. laevigata*, *Rosa* sp., *Rubus coreanus* and *R. parrifolius* in Guizhou Province, China. The spores from specimens were mounted in sterile water, on slides and observed using a Zeiss Scope 5 compound microscope (Axioscope 5, Jena, Germany), and photographed with an AxioCam 208 color (Jena, Germany) camera and saved as JPG files. Approximately 30 measurements were made of each feature using the ZEN 2.0 (blue edition) software. The Flora of China (http://www.efloras.org/flora_page.aspx?flora_id=4) was used to identify host plants (Liu et al. 2018). The rust specimens were deposited in the HGUP Herbarium of Department of Plant Pathology, Agricultural College, Guizhou University. Taxonomic details of our novel taxa were submitted to MycoBank (www.mycobank.org).

DNA extraction, PCR and sequencing

Rust spores were scraped from fresh plant tissues using a sterile scalpel. Total DNA of rust spores was extracted with a BIOMIGA Fungus Genomic DNA Extraction Kit (GD2416) according to the manufacturer's protocol. Targeted sequences of internal transcribed spacer of rDNA (ITS) was amplified using primers ITS4rust (5'-CAGATTACAAATTTGGGCT-3') (Beenken et al. 2012) and Rust2inv (5'-GATGAAGAACACAGTGAAA-3') (Aime 2006), and the large subunit (*LSU*) of the ribosomal RNA gene was amplified using the primers No.4 (5'-ACCCGCTG AATTTAAGCATAT-3')/No.11 (5'-CTCCTTGGTCCGTGTTTCAAGACGC-3') (Van der Auwera et al. 1994), or LR6 (5'-CGCCAGTTCTGCTTACC-3') (Vilgalys and Hester 1990), and LR0R (5'-ACCCGCTGAACTTAAGC-3') (Hopple 1994). The PCR cycling conditions were as described by Liu et al (2018). The PCR amplicons from purification and sequencing were carried out at Sangon Biotech (Chengdu, China). Newly-generated sequences were deposited in GenBank (Table 1).

Phylogenetic analyses

81 sequences, including originated from thirteen specimens and related sequences of *Phragmidium* spp. were aligned in the online version of MAFFT v. 7.307 (Katoh and Standley 2016). *Trachyspora intrusa* (BPI 843828) was selected as outgroup (Liu et al. 2020). The alignment document was edited using MEGA6 (Tamura et al. 2013) and manually adjusted when necessary.

All relevant sequences of ITS—*LSU* dataset were conducted using maximum likelihood (ML), maximum parsimony (MP) and Bayesian inference (BI) methods. ML analysis was performed using RAxML-HPC2 v.8.2.12 (Stamatakis 2014).

Table 1. Specimens and GenBank accession numbers of rust isolates included in this study.

Species	Voucher specimens	Host	Locality	ITS	*LSU*
Phragmidium andersoni	HMAS-53231 T	*Potentilla fruticosa*	Sinkiang, China	N/A	MG669120
Ph. altaicum	BJFCR03247	*Rosa albertii*	China	MH285385	MH285381
	BJFCR03246	*Rosa albertii*	China	MH285384	MH285380
	BJFCR03217 T	*Rosa albertii*	China	MH285383	MH285379
Ph. barclayi	HMAS-67281	*Rubus austrotibetanus*	Tibet, China	N/A	MG669117
Ph. barnardii	BRIP 56945	*Rubus parvifolius*	South Africa	N/A	KT199402
Ph. barnardii	**HGUP21035**	***Rubus parvifolius***	**Guizhou, China**	**OL684828**	**OL684839**
Ph. biloculare	BPI:881121	*Potentilla flabellifolia*	USA	N/A	JF907670
Ph. butleri	HMAS-67841	*Rosa macrophylla*	Tibet, China	N/A	MG669118
Ph. chayuensis	BJFC-R02532 T	*Rosa duplicata*	Tibet, China	N/A	MG669112
	BJFC-R03014 T	*Rosa duplicata*	Tibet, China	N/A	MG669113
Ph. cibanum	BJFCR02528 T	*Rubus niveus*	Tibet, China	MH128370	MG669110
	BJFCR03012 T	*Rubus niveus*	Tibet, China	MH128371	MG669111
Ph. duchesneae-indicae	**HGUP21031**	***Duchesnea indica***	**Guizhou, China**	**OL684824**	**OL684835**
	HGUP21032	***Duchesnea indica***	**Guizhou, China**	**OL684825**	**OL684836**
Ph. fragariae	WM 1317	*Potentilla sterilis*	Europe	N/A	AF426217
Ph. fusiforme	T-10	*Rosa pendulina*	Switzerland	N/A	AJ715522
Ph. fructigenum	HMUT100472	*Rosa glomerata*	Guangdong, China	N/A	KU059168
Ph. griseum	BJFCR03449	*Rubus crataegifoliu*	Beijing, China	MN264712	MN264730
	BJFCR03451	*Rubus crataegifoliu*	Beijing, China	MN264713	MN264731
	HMAS56906	*Rubus crataegifoliu*	Beijing, China	N/A	MG669115
Ph. handelii	BJFC-R01030	*Rosa webbiana*	Gansu, China	N/A	KP407631
Ph. ivesiae	BPI-877968	*Potentilla gracilis*	USA	N/A	JF907673
	BPI-863637	*Potentilla gracilis*	USA	N/A	JF907672
	BJFC-R01421	*Rosa webbiana*	Gansu, China	N/A	KP407628
Ph. japonicum	HMAS41585	*Rosa laevigata*	Fujian, China	MN264716	MN264734
	IBAR8174	*Rosa luciae*	Ibaraki, Japan	MN882389	MN848143
Ph. jiangxiense	BJFCR03452	*Rosa laevigata*	Jiangxi, China	MN264714	MN264732
	BJFCR03453 T	*Rosa laevigata*	Jiangxi, China	MN264715	MN264733
Ph. leucoaecium	BJFCR02116	*Rosa* sp.	Yunnan, China	MN264718	MN264736
	BJFCR02118 T	*Rosa* sp.	Yunnan, China	MN264719	MN264737
Ph. longissima	BJFC-R00338	*Rosa lichiangensis*	Yunnan, China	N/A	KP407633
	BJFC-R00360	*Rosa lichiangensis*	Yunnan, China	N/A	KP407634
Ph. mexicanum	BPI 843961	*Potentilla indica*	Maryland, USA	N/A	JF907660
	BPI 843829	*Potentilla indica*	Virginia, USA	N/A	JF907659
Ph. mucronatum	RUBO	*Rosa* sp.	Bochum, Germany	N/A	KU059171
	TUB 012090	*Rosa corymbifera*	Germany	N/A	AJ715520
Ph. montivagum	HMAS67176	*Rosa davurica*	China	N/A	KU059173
	FO 47828	*Rosa woodsii*	NA	N/A	AF426213
Ph. octoloculare	HMAS-140416	*Rubus biflorus*	Tibet, China	N/A	MG669119
Ph. potentillae	HMAS53236	*Potentilla virgata*	Sinkiang, China	N/A	MG669114
	BJFCR00961	*Potentilla chinensis*	Qinghai, China	MN264720	MN264738
Ph. potentillae	**HGUP21034**	***Potentilla kleiniana***	**Guizhou, China**	**OL684827**	**OL684838**
Ph. potentillae-canadensis	BPI877886	*Potentilla* sp.	North Carolina, USA	N/A	JF907667
	BPI877885	*Potentilla canadensis*	Maryland, USA	N/A	JF907668
Ph. potentillae-freynianae	**HGUP21033 T**	***Potentilla freyniana***	**Guizhou, China**	**OL684826**	**OL684837**
Ph. punjabense	BA-65A T	*Rosa brunonii*	Pakistan	N/A	KX358854
	BA-65B	*Rosa brunonii*	Pakistan	N/A	KX358855
Ph. rosae-laevigatae	**HGUP21036 T**	***Rosa laevigata***	**Guizhou, China**	**OL684829**	**OL684840**
	HGUP21037	***Rosa laevigata***	**Guizhou, China**	**OL684830**	**OL684841**

Species	Voucher specimens	Host	Locality	ITS	*LSU*
Ph. rosae-multiflorae	HMAS71053	*Rosa multiflora*	Shanxi, China	N/A	KU059174
	HMAS94924	*Rosa multiflora*	Zhejiang, China	N/A	KU059175
	BJFCR03454	*Rosa multiflora*	Jiangxi, China	MN264721	MN264739
Ph. rosae-roxburghii	**HGUP21025** [T]	***Rosa roxburghii***	**Guizhou, China**	**OL684818**	**OL684831**
	HGUP21026	***Rosa roxburghii***	**Guizhou, China**	**OL684819**	**OL684832**
	HGUP21027	***Rosa roxburghii***	**Guizhou, China**	**OL684820**	**N/A**
	HGUP21028	***Rosa* sp.**	**Guizhou, China**	**OL684821**	**OL678103**
Ph. rosae-rugosae	BJFCR03455	*Rosa rugosa*	Jiangxi, China	MN264722	MN264740
	BJFCR03456	*Rosa rugosa*	Beijing, China	MN264723	MN264741
Ph. rubi-idaei	WM 1024	*Rubus idaeus*	Europe	N/A	AF426215
	BRIP 59372	*Rubus idaeus*	Australia	N/A	MW147044
Ph. rubi-oldhami	HMAS-64306	*Rubus pungens*	Sichuan, China	N/A	MG669116
Ph. rubi-corean	**HGUP21029** [T]	***Rubus coreanus***	**Guizhou, China**	**OL684822**	**OL684833**
	HGUP21030	***Rubus coreanus***	**Guizhou, China**	**OL684823**	**OL684834**
Phragmidium sp.	HMAS41561	*Rosa multiflora*	Fujian, China	MN264717	MN264735
Ph. sanguisorbae	BPI 872232	*Sanguisorba minor*	USA	N/A	JF907674
	ML 957	*Sanguisorba minor*	Europe	N/A	AF426216
Ph. tormentillae	BPI 843392	*Potentilla* sp.	Maryland, USA	DQ354553	DQ354553
	BPI 877888	*Potentilla simplex*	Tennessee, USA	N/A	JF907669
Ph. tuberculatum	BJFCR00959	*Rosa* sp.	Qinghai, China	N/A	KP407636
	BPI 877978	*Rosa* sp.	California, USA	N/A	KJ841919
	BPI 843677	*Rosa* sp.	Argentina	N/A	KJ841921
Ph. violaceum	MCA2782	*Rubus* sp.	France	DQ142909	DQ142909
	BPI 871510	*Rubus* sp.	Oregon, USA	DQ142910	DQ142910
	BJFCR03457	*Rubus* sp.	New Zealand	MN264724	MN264742
Ph. warburgianum	BJFCR03458	*Rosa bracteata*	Japan	MN264726	MN264744
	BJFCR03459	*Rosa bracteata*	Japan	MN264727	MN264745
Ph. zangdongii	BJFCR02447 [T]	*Rosa tibetica*	Tibet, China	MH128372	MG669108
	BJFCR03013 [T]	*Rosa tibetica*	Tibet, China	MH128373	MG669109
Ph. zhouquensis	BJFCR01516 [T]	*Rosa omeiensis*	Yunnan, China	MN264728	MN264746
	BJFCR01529 [T]	*Rosa omeiensis*	Yunnan, China	MN264729	MN264747
Trachyspora intrusa	BPI 843828	*Alchemilla vulgaris*	Switzerland	DQ354550	

[T] = Type specimens. New specimens are in bold typeface.

Gaps were treated as "missing". The MP analysis of the two loci (ITS and *LSU*) was implemented with PAUP v. 4.0b10 (Swofford 2002). The phylogenetic trees were generated using the heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions. The maxtrees was set to 5000. The tree length (TL), consistency index (CI), homoplasy index (HI), retention index (RI), and rescaled consistency index (RC) were calculated. Bayesian inference analysis was inferred by MrBayes 3.2.6 (Ronquist et al. 2012). The best model for two loci (ITS and *LSU*) was determined by MrModeltest v2 (Nylander 2004), ITS: HKY+G, *LSU*: GTR+I+G. BI were performed by six Markov chain Monte Carlo. These chains were run for 5 million generations, sampling tree every 100 generations. The first 25% of resulting trees were discarded as burn-in phase of each analysis, and trees were saved every 5000 generations. Alignment matrices have been uploaded as an attachment.

Results

Phylogenetic analyses

The phylogenetic trees accommodated 82 sequences listed in Table 1. The combined alignment including ITS (493 bp) and *LSU* (544 bp) regions consisted of 1067 characters, of which 585 were constant, 89 variable characters were parsimony uninformative, and 363 were parsimony informative. We built three phylogenetic trees, ML tree, MP tree and BI tree. The MP tree was selected to represent the phylogenetic relationship of different *Phragmidium* taxa (Fig. 1). MP analysis produced the following parameters: tree length (TL) = 1011; consistency index (CI) = 0.643; homoplasy index (HI) = 0.356; retention index (RI) = 0.898; and rescaled consistency index (RC) = 0.578. *Phragmidium rubi-coreani* on *Rubus coreanus* with telial, aecial and uredinial stages formed a small branch only. *Phragmidium potentillae-freynianae* and *Ph. duchesneae-indicae* constituted a distinct subclade with high statistical support (100 ML/99 MP/1.00 PP). *Phragmidium rosae-laevigatae* was phylogenetically sister to *Ph. leucoaecium*, *Ph. japonicum*, *Ph. jiangxiense* and *Phragmidium* sp. with high support (100 ML/100 MP/1.00 PP). The four aecial-uredinial fungi on *Ro. roxburghii* kept identical base composition on ITS and *LSU* gene regions and made up a distinct subclade to *Ph. warburgianum* with high support (100 ML/99 MP/1.00 PP). Our strains represented four novel taxa, which was also supported by comparison of the DNA base pair differences between our strains and related taxa on ITS and *LSU* gene region.

The hosts of the *Phragmidium* species were mainly concentrated in *Rosa*, *Rubus* and *Potentilla* of Rosaceae (Fig. 1). Eighty-one *Phragmidium* strains clustered together as a clade, which was roughly divided into three subclades (Subclade I, Subclade II and Subclade III). For Subclade I with 16 species (*Ph. rubi-coreani*, *Ph. barclayi*, *Ph. cibanum*, *Ph. violaceum*, *Ph. barnardii*, *Ph. griseum*, *Ph. rubi-idaei*, *Ph. altaicum*, *Ph. tuberculatum*, *Ph. octoloculare*, *Ph. sanguisorbae*, *Ph. punjabense*, *Ph. rubi-oldhami*, *Ph. butleri*, *Ph. zhouquensis* and *Ph. fragariae*) (67 ML/59 MP), their hosts belonged to *Rosa*, *Rubus*, *Potentilla*, and *Sanguisorba*. *Phragmidium rubi-coreani* and *Ph. rubi-ideai* associated with host plants on the generic level had obvious genetic distance. Subclade II included 18 *Phragmidium* taxa (*Ph. biloculare*, *Ph. potentillae*, *Ph. ivesiae*, *Ph. montivagum*, *Ph. fructigenum*, *Ph. zangdongii*, *Ph. fusiforme*, *Ph. handelii*, *Ph. rosae-rugosae*, *Ph. mucronatum*, *Ph. chayuensis*, *Ph. longissima*, *Ph. rosae-multiflorae*, *Ph. mexicanum*, *Ph. potentillae-canadensis*, *Ph. potentillae-freynianae*, *Ph. duchesneae-indicae* and *Ph. tormentillae*) (95 ML), but their host plants only referred to *Rosa*, *Potentilla* and *Duchesnea*. *Phragmidium potentillae-freynianae* and *Ph. duchesneae-indicae* belonging to different generic host plants were accommodated to a branch (100 ML/99 MP/1.00 PP), but *Ph. mexicanum* and *Ph. potentillae-canadensis* formed a clade (99 ML/86 MP/1.00 PP) separated from *Ph. potentillae-freynianae* with the congeneric host plants. *Phragmidium tormentillae* associated with *Potentilla canadensis* (*P. simplex*) as its host formed an independent branch (97 ML/61 MP/0.94 PP). The *Phragmidium* host plants in Subclade III (*Ph. rosae-roxburghii*, *Ph. warburgianum*, *Ph. japonicum*,

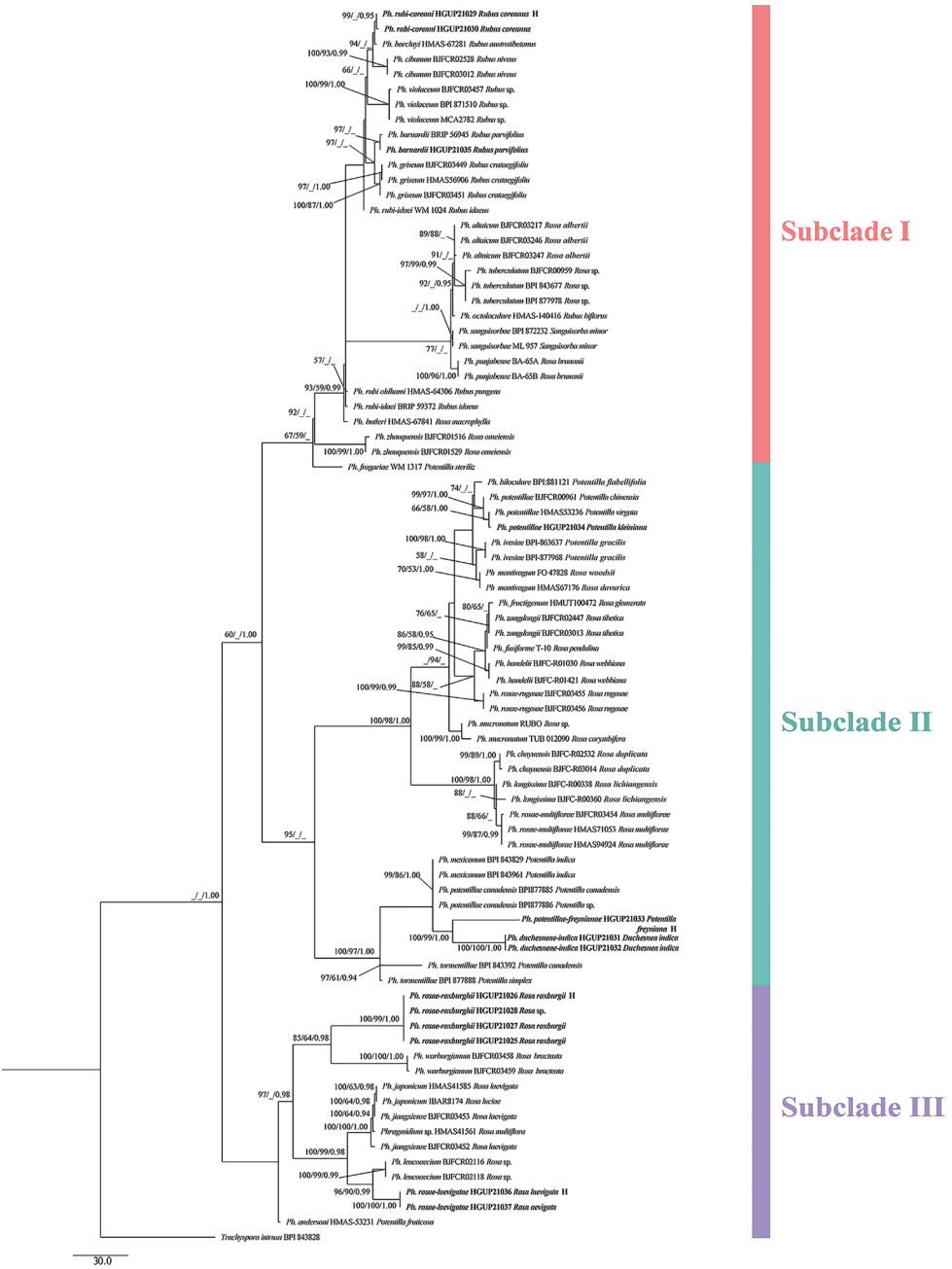

Figure 1. The maximum parsimony tree of 42 *Phragmidium* taxa based on ITS and *LSU* genes; host plants are also given.

Ph. jiangxiense, *Phragmidium* sp., *Ph. leucoaecium*, *Ph. rosae-laevigatae*, *Ph. andersoni*) belonged to *Rosa* and *Potentilla*. *Phragmidium rosae-laevigatae* and *Ph. rosae-roxburghii* with the same generic host plants did not group together (97 ML /0.98 PP).

Phragmidium japonicum, *Ph. jiangxiense* and *Phragmidium* sp. (HMAS51561) all from *Rosa* formed a branch (100 ML/100 MP/1.00 PP). *Phragmidium andersoni* collected from *Potentilla fruticosa* formed an independent branch.

RA×ML and MP bootstrap support values (MP ≥ 50%), and Bayesian posterior probability (PP ≥ 0.90) are marked on the nodes as (ML/MP/PP). Specimens from current study have put in bold and put an H in the selected holotypes. The outgroup was *Trachyspora intrusa* (BPI 843828). The scale bar indicates 30 expected changes per site.

Taxonomy

Phragmidium rosae-roxburghii **J.E. Sun & Yong Wang bis, sp. nov.**
MycoBank No: MB845041
Figs 2, 3

Diagnosis. *Phragmidium rosae-roxburghii* easily to be distinguished by its unique square to diamond-shaped urediniospores.

Holotype. China. Guizhou Province, Panzhou city, 25°89'61"N, 104°56'07"W, 750 m, 21 Mar 2021, on *Rosa roxburghii*, coll. J.E. Sun & Y.Q. Yang, HGUP21025, ITS: OL684818, LSU: OL684831.

Etymology. Referring to the host, *Rosa roxburghii*, on which the fungus was first found.

Description. ***Spermogonia*****:** unknown. ***Aecia*** formed on gold distinct, circular lesions on both sides of the stems, petioles and leaves, rarely produced on the abaxial leaf surface, scattered, flat oval to subglobose, powdery, 1.0–5.0 mm diam. Aeciospores formed in basipetal succession, oval o subglobose, 22–30 × 14–22 µm (mean 26 × 18 µm, n = 30), inclusions golden, to bright-yellow; wall 1.8–3.1 µm thick, colorless, mostly with irregularly elongated verrucae on the surface. ***Uredinia*** produced on the abaxial leaf surface, scattered to gregarious, hypophyllous, orange-colored or white, powdery, oval to rounded, 0.1–1.0 mm diam, paraphysis in the periphery of the uredinia, curved, 30–55 × 9–20 µm, colorless thin-walled. Urediniospores generally angular, square to diamond-shaped, yellowish to orange-colored, 20–30 × 16–21 µm (mean: 25 × 19 µm, n = 30), thick-walled, 0.5–2.0 µm thick, colorless, regularly echinulate with stout spines.

Rust diseases symptoms: In the early stage (March) of rust disease yellowish-orange powdery aecia formed on the stems and petioles on *Rosa roxburghii* and *Rosa* sp., the aecia were scattered, flat oval or nearly round and bordered (Fig. 2). In middle of June (Fig. 3), the upper surface of the lower leaves was turning yellow and orange spots gradually appeared on the under surface caused by uredinia, which are powdery, aggregated but without obvious boundaries.

Habitat. *Rosa roxburghii*, *Rosa* sp.

Known distribution. China, Guizhou Province.

Additional material examined. China. Guizhou Province: Duyun city, 26°45'88"N, 106°98'42"W, 820 m, 22 Jun 2021, on *Rosa roxburghii*, coll. J.E. Sun,

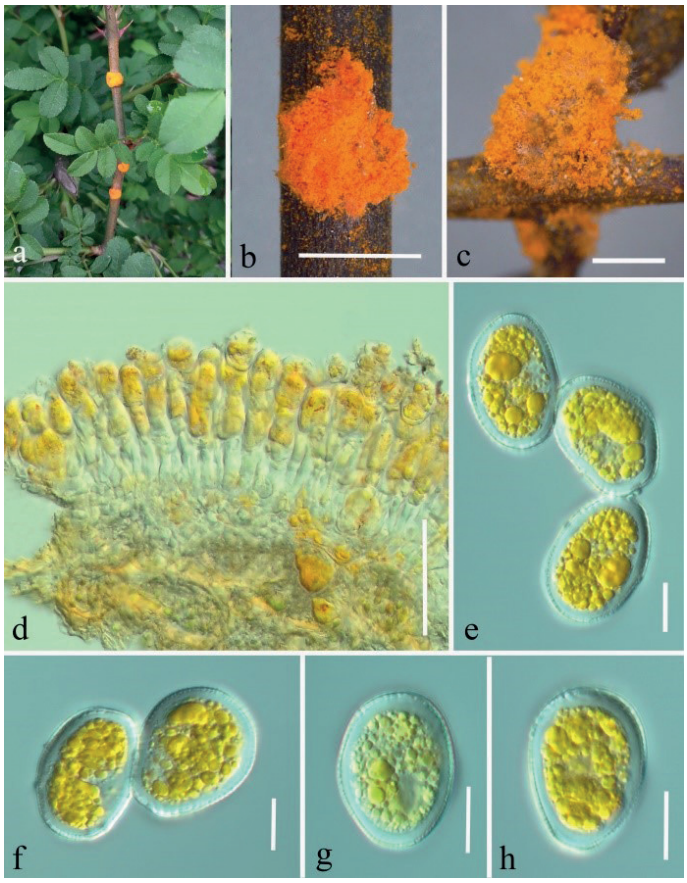

Figure 2. *Phragmidium rosae-roxburghii* sp. nov. (HGUP21025, holotype) on *Rosa roxburghii* **a–c** aecia on stem and leaf pieces. **d** longitudinal section of aecium **e–h** aeciospores. Scale bars: 2 mm (**b–c**); 50 μm (**d**); 10 μm (**e–h**).

HGUP21026; Tongren city, 28°14'09"N, 108°34'03"W, 810 m, 04 Sep 2021, on *Rosa roxburghii*, coll. J.E. Sun, HGUP21027; Guiyang city, 26°44'74"N, 106°58'67"W, 960 m, 27 Mar, 2021, on *Rosa* sp., coll. J.E. Sun, HGUP21028.

Notes. *Phragmidium rosae-roxburghii* was the first species of *Phragmidium* described on *Rosa roxburghii*. It is easily to distinguish species by its unique square to diamond-shaped urediniospores, since in other *Phragmidium* species the urediniosporas are oval to nearly spherical (Yun et al. 2011; Ono 2012; Zhuang et al. 2012; Yang et al. 2015; Liu et al. 2018, 2019, 2020; Ono and Wahyuno 2019). In phylogeny, this species only kept a close relationship to *Ph. warburgiana* (Fig. 1) but its urediniospores are yellowish to orange-colored different to *Ph. warburgiana* with colorless urediniospores (Ono 2012). We proposed *Ph. rosae-roxburghii* as a new taxon.

Figure 3. *Phragmidium rosae-roxburghii* sp. nov. (HGUP21026) on *Rosa roxburghii* **a** appearance of infected plants **b** uredinia on a leaf **c** longitudinal section of uredinium **d** paraphyses **e–i** urediniospores. Scale bars: 5 mm (**b**); 50 μm (**c**); 25 μm (**d**); 12.5 μm (**e–i**).

Phragmidium rubi-coreani **J.E. Sun & Yong Wang bis, sp. nov.**
MycoBank No: MB845042
Fig. 4

Diagnosis. *Phragmidium rubi-coreani* differs to *Ph. barclayi* by teliospores with fewer cells and shorter pedicels.

Holotype. China. Guizhou Province: Guiyang city, 26°45'86"N, 106°98'77"W, 970 m, 11 Apr, 2021, on *Rubus coreanus*, coll. J.E. Sun, HGUP21029, ITS: OL684822, LSU: OL684833.

Etymology. Referring to the host, *Rubus coreanus*, on which this species grows.

Description. ***Spermogonia*****:** unknown. ***Aecia*** golden, produced on the abaxial leaf surface, hypophyllous, and 2.5–3.5 mm diam, subglobose to globose, powdery, 2.5–3.5 mm diam. Aeciospores produced in basipetal succession, subglobose, 14–24 × 10–23 μm (mean 19 × 16 μm, n = 30), bright yellow contents, thick-walled, 1.0–4.0 μm, colorless, echinulate; paraphyses clavate, not or weakly incurved, 38–61 μm long, thick-walled, wall 2.0–2.5 μm thick. ***Telia*** hypophyllous, scattered, 0.3–0.5 mm diam, chocolate-brown. Teliospores ellipsoid to cylindrical, 3–5 celled, constricted at

the septa, bright orange, chocolate-brown to gray-brown, 29–74 × 14–37 µm (mean 50 × 25 µm, n = 30), thick-walled, wall 1.8–3.5 µm thick, colorless to chocolate-brown; pedicels not swollen at the base, 8–34 µm long, colorless. ***Uredinia*** formed on circular lesions on both sides of the leaves, powdery, yellow distinct, hypophyllous scattered, nearly oval, surrounded by host epidermis, 0.5–1.0 mm diam. Urediniospores: uredo-type, subglobose to oval, produced in basipetal succession, golden, or bright-yellow, 19–27 × 15–25 µm (mean 23 × 20 µm, n = 30), thick-walled, wall 0.8–1.5 µm thick, colorless, densely and minutely echinulate.

Rust diseases symptoms: The golden and powdery aecia were first produced on the underside of leaves. Then, scattered uredinia were formed, orange-colored and forming small round spots on the leaves. Chocolate-brown telia were produced on the leaf remnants (Fig. 4).

Habitat. *Rubus coreanus*.

Known distribution. China, Guizhou Province.

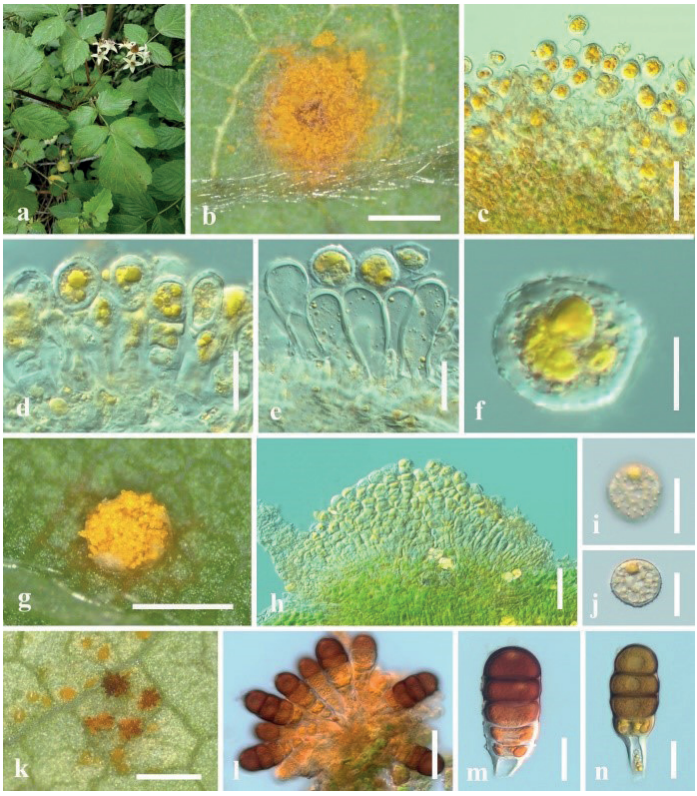

Figure 4. *Phragmidium rubi-coreani* sp. nov. (HGUP21029, holotype) on *Rubus coreanus* **a** gross features of infected leaves **b** uredinia on a leaf **c–d** longitudinal section of uredinium **e** paraphyses **f** urediniospores **g** aecia on a leaf **h** longitudinal section of aecium **i–j** aeciospores **k** telia on a leaf **l** longitudinal section of telium **m–n** Teliospores. Scale bars: 2 mm (**b**); 1 mm (**g, k**); 50 µm (**c–e, h, l**); 10 µm (**f**); 25 µm (**i–j, m–n**).

Additional material examined. China. Guizhou Province: Guiyang city, 27°10'30"N, 106°99'91"W, 830 m, 09 Apr 2021, on *Rubus coreanus*, coll. J.E. Sun, HGUP21030.

Notes. In the phylogenetic tree, *Phragmidium rubi-coreani*, *Ph. barclayi* and *Ph. cibanum* formed a branch (Fig. 1). However in morphology, teliospores of *Phragmidium rubi-coreani* have fewer septa and shorter pedicels (3–5-celled, 8–34 μm long) than *Ph. barclayi* (5–8-celled, 60–150 μm long) and *Ph. cibanum* (5–7-celled, 70–108 μm long) (Liu et al. 2018). Meanwhile, most reported *Phragmidium* taxa produce longer teliospores, such as *Ph. zangdongii* (29–74 × 14–37 μm vs. 82–110 × 23–31 μm); *Ph. kanas* (29–74 × 14–37 μm vs. 134–198 × 19–31 μm); *Ph. potentillae-canadensis* (29–74 × 14–37 μm vs. 48.1–86.8 × 30.1–33.3 μm) than the present species (Yun et al. 2011; Liu et al. 2018; Zhao et al. 2021). Thus, our fungus represented a novel taxon.

Phragmidium potentillae-freynianae **J.E. Sun & Yong Wang bis, sp. nov.**
MycoBank No: MB845043
Fig. 5

Diagnosis. Different from the related taxa by its urediniospores catenulate, such as *Ph. chayuensis*, *Ph. cibanum* and *Ph. tormentillae*.

Holotype. China. Guizhou Province;, Guiyang city, 26°44'70"N, 106°59'65"W, 801 m, 27 Mar 2021, on *Potentilla freyniana*, coll. J.E. Sun, HGUP21033, ITS: OL684826, LSU: OL684837.

Etymology. Referring to the host, *Potentilla freyniana*, on which the fungus was first found.

Description. ***Spermogonia***, ***aecia*** and ***telia*** not observed. ***Uredinia*** produced on the abaxial leaf surface, covering the entire lower surface of the leaves, hypophyllous, nearly oval, powdery, not surrounded by host epidermis, 0.1–1.0 mm diam, on densely orange spot, 0.1–1.0 mm diam. Urediniospores: uredo-type, subglobose to oval, produced in basipetal succession, 19–24 × 18–24 μm (mean 21.5 × 21 μm, n = 30), golden, or bright-yellow; thin-walled, wall 0.4–1.4 μm thick, colorless, densely and minutely echinulate.

Rust diseases symptoms: Large areas of orange powdery uredinia, covering almost the entire lower surface of the leaves, which are aggregated but without obvious boundaries (Fig. 5).

Habitat. *Potentilla freyniana*.

Known distribution. China, Guizhou Province.

Notes. In the phylogenetic tree, *Phragmidium potentillae-freynianae* formed a well-supported clade allied to *Ph. duchesneae-indicae* (Fig. 1). Morphologically, its urediniospores are bigger than *Ph. duchesneae-indicae* (21.5 × 21 μm vs. 13–19 × 11–17 μm) (Zhao et al. 2021). The comparison of DNA base composition supports the morphological separation of this fungus as a new species.

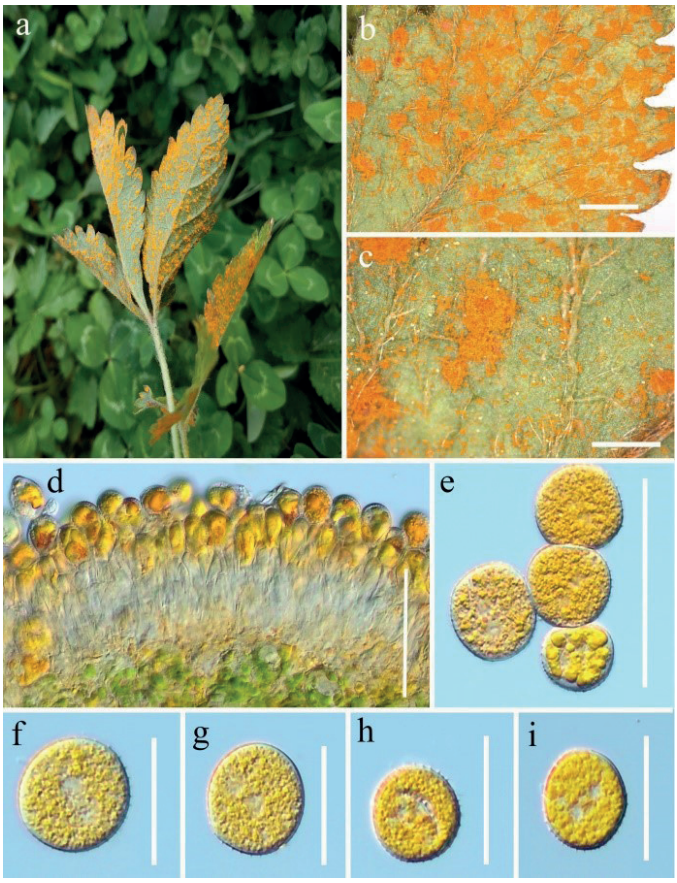

Figure 5. *Phragmidium potentillae-freynianae* sp. nov. (HGUP21033, holotype) on *Potentilla freyniana*. **a–c** uredinia on leaves **d** longitudinal section of uredinium **e–i** urediniospores. Scale bars: 2 mm (**b–c**); 50 μm (**d–e**); 25 μm (**f–i**).

Phragmidium rosae-laevigatae **J.E. Sun & Yong Wang bis, sp. nov.**
MycoBank No: MB845044
Fig. 6

Diagnosis. Different from *Ph. Jiangxiense* mainly because of bigger urediniospores.

Holotype. China. Guizhou Province: Panzhou city, 25°64'56"N, 104°84'35"W, 1800 m, 19 Jul 2021, on *Rosa laevigata*, coll. J.E. Sun, HGUP21036, ITS: OL684829, LSU: OL684840.

Etymology. Referring to the host, *Rosa laevigata*, on which the fungus was first found.

Description. ***Spermogonia*** and ***aecia*** not observed. ***Uredinia*** produced on the abaxial leaf surface, hypophyllous, subglobose to globose, powdery, 0.1–0.5 mm diam, yellow, peripherally parphyses, hyaline, 20–31 × 10–17 μm. Urediniospores square to diamond-shaped, oval to nearly spherical, 23–35 × 16–30 μm (mean 29 × 23 μm, n = 30), orange-

colored, thick-walled 0.5–2.0 μm thick, colorless, regularly echinulate with stout spines on the surface. ***Telia*** scattered compact, hypophyllous, golden, 0.1–0.5 mm diam. Teliospores (immature) oval, 24–60 × 8–20 μm (mean 50.5 × 25.5 μm, n = 30), with apical papillae (4.0–7.0 μm high, n = 10), too immature to know how many cells, orange-yellow; pedicels swollen at the base, 15–26 μm long, colorless, disconnected easily; wall 0.5–2.0 μm thick.

Rust diseases symptoms: As shown in Fig. 6, Uredinia and telia, which are bright-yellow and powdery are produced almost simultaneously on the lower surface of the yellowing and wilting leaves.

Habitat. *Rosa laevigata*.

Known distribution. China, Guizhou Province.

Additional material examined. China. Guizhou Province: Panzhou city, 25°61'81"N, 104°83'61"W, 1790 m, 19 Jul 2021, on *Rosa laevigata*, coll. J.E. Sun, HGUP21037.

Notes. Phylogenetically, *Phragmidium rosae-laevigatae* kept a close relationship to *Ph. leucoaecium*, *Ph. japonicum* and *Ph. jiangxiense* (Fig. 1). Morphologically, *Phragmidium rosae-laevigatae* has bigger urediniospores than *Ph. jiangxiense* (23–35 × 16–30 μm vs. 15–23 × 11–18 μm), but the uredinia and urediniospores of

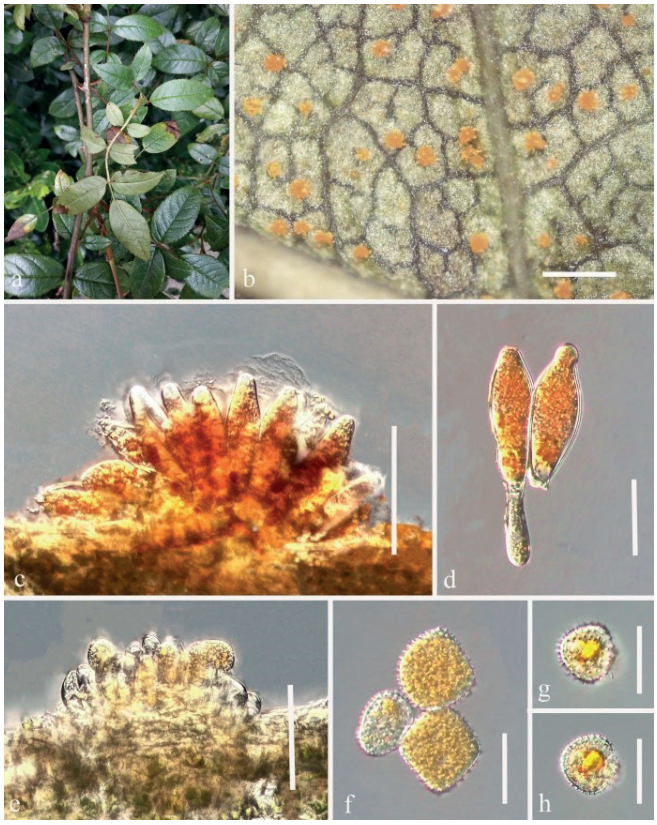

Figure 6. *Phragmidium rosae-laevigatae* sp. nov. (HGUP21036, holotype) on *Rosa laevigata* **a** gross features of infected leaves **b** uredinia and telia on a leaf **c** longitudinal section of telium **d** immature teliospores **e** longitudinal section of uredinium **f–h** urediniospores. Scale bars: 1 mm (**b**); 50 μm (**c, e**); 12.5 μm (**d, f–h**).

Ph. leucoaecium and *Ph. japonicum* were not observed (Liu et al. 2020). The comparison of DNA base composition also supported morphological conclusion. Thus, this fungus was also introduced as one novel taxon herein.

***Phragmidium duchesneae-indicae* P. Zhao & L. Cai, Fungal Diversity 5:1–58, 2021**
MycoBank No: MB557609
Fig. 7

Description. ***Spermogonia***, ***aecia*** and ***telia*** not observed. ***Uredinia*** produced on the abaxial leaf surface, hypophyllous, nearly oval, golden, densely bright orange-yellow, powdery, not surrounding by host epidermis, 0.3–1.2 mm diam, without paraphyses. Urediniospores produced in basipetal succession, mostly globose, 17–22 × 15–20 µm (mean 19.5 × 17.5 µm, n = 30), inclusions yellowish, or bright-yellow; thick-walled, wall 0.7–1.8 µm thick, colorless, densely and minutely echinulate. Telia and teliospores see Zhao et al (2021).

Habitat. *Duchesnea indica*

Known distribution. China, Guizhou Province.

Material examined. China. Guizhou Province: Guiyang city, 27°10'30"N, 106°99'91"W, 820 m, 09 Apr 2021, on *Duchesnea indica*, coll. J.E. Sun, HGUP21031;

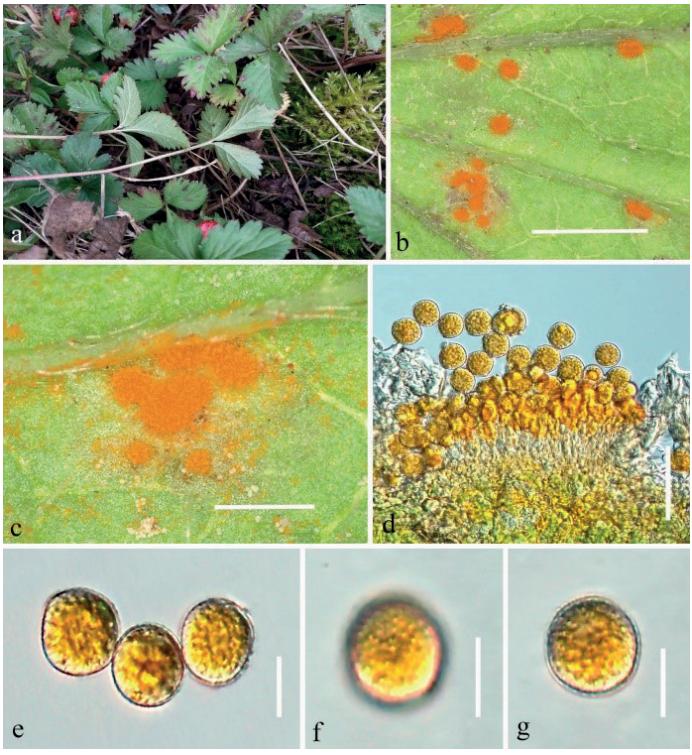

Figure 7. *Phragmidium duchesneae-indicae* (HGUP21031) on *Duchesnea indica* **a–c** uredinia on leaves **d** longitudinal section of uredinium **e–g** urediniospores. Scale bars: 2 mm (**b**); 1 mm (**c**); 50 µm (**d**); 12.5 µm (**e–g**).

Guiyang city, 27°09'26"N, 106°98'90"W, 734 m, 04 Sep 2021, on *Duchesnea indica*, coll. J.E. Sun, HGUP21032.

Notes. *Phragmidium duchesneae-indicae* was first reported on *D. indica* by Zhao et al (2021). Our specimen had similar morphology to that described by Zhao et al (2021). GenBank accession numbers (ITS and *LSU*) of *Ph. duchesneae-indicae* have not been released, and our identification is based only on a morphological comparison.

Phragmidium potentillae **(Pers.) P. Karst., Bidrag till Kännedom av Finlands Naturoch Folk, 31: 49, 1879**

MycoBank No: MB206190

Fig. 8

Description. ***Spermogonia*** and ***aecia*** not observed. ***Uredinia*** produced on the abaxial leaf surface, hypophyllous, nearly oval, powdery, densely bright orange, nearly oval, surrounding by host epidermis, 0.8–1.5 × 0.4–0.7 mm, and densely bright orange. Urediniospores angular to squarish, oval to nearly globose, produced in basipetal succession, 17–26 × 14–22 µm (mean 21.5 × 18 µm, n = 30), or bright–yellow to orange, immature urediniospores are colorless; thick-walled, wall 0.6–1.3

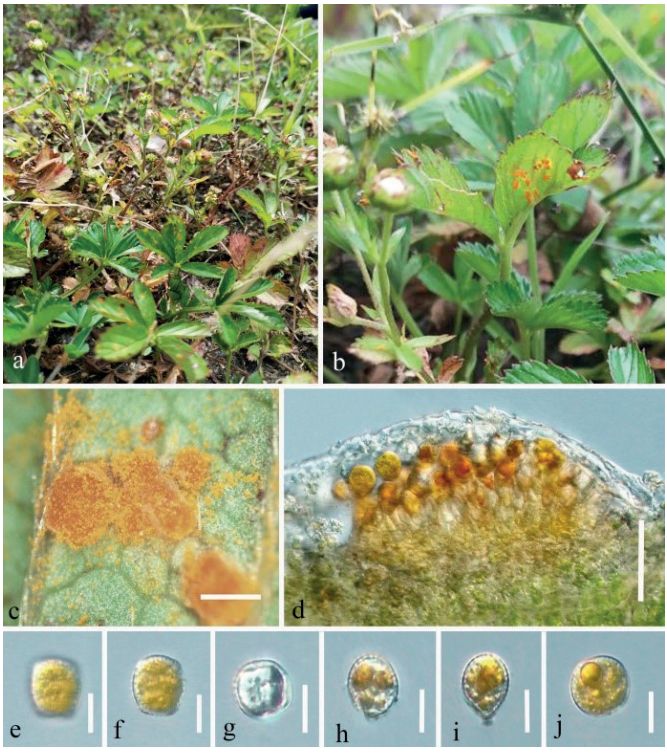

Figure 8. *Phragmidium potentillae* (HGUP21034) on *Potentilla kleiniana* **a–c** uredinia on leaves **d** longitudinal section of uredinium **e–j** urediniospores. Scale bars: 1 mm (**c**); 50 µm (**d**); 12.5 µm (**e–j**).

μm thick, colorless, densely and minutely echinulate. Telia and teliospores see Liu et al (2018).

Habitat. *Potentilla kleiniana*

Known distribution. China: Guizhou Province, Qinghai Province, Sinkiang Province; USA, the United Kingdom, Australia, Tasmania and Japan.

Material examined. China. Guizhou Province: Guiyang city, 27°09'26"N, 106°98'90"W, 730 m, 22 Jun 2021, on *Potentilla kleiniana*, coll. J.E. Sun, HGUP21034.

Notes. In the phylogenetic tree, HGUP21034 clustered with two sequences of specimens of *Phragmidium potentillae* (Fig. 1). The uredinia of *P. potentillae* described by Liu et al (2018), as 0.2–0.8 mm diam, smaller than in the specimen examined, 0.8–1.5 × 0.4–0.7 mm, the urediniospores mostly globose and echinulate, (18–25 × 15–21 μm vs. 17–26 × 14–22 μm).

Phragmidium barnardii **Plowr. & G. Winter, Revue Mycologique Toulouse 8 (32): 208 (1886)**

MycoBank No: MB249450

Fig. 9

Description. ***Spermogonia***, ***aecia*** and ***telia*** not observed. Uredinia produced on the abaxial leaf surface, hypophyllous, scattered to gregarious, oval to globose, orange,

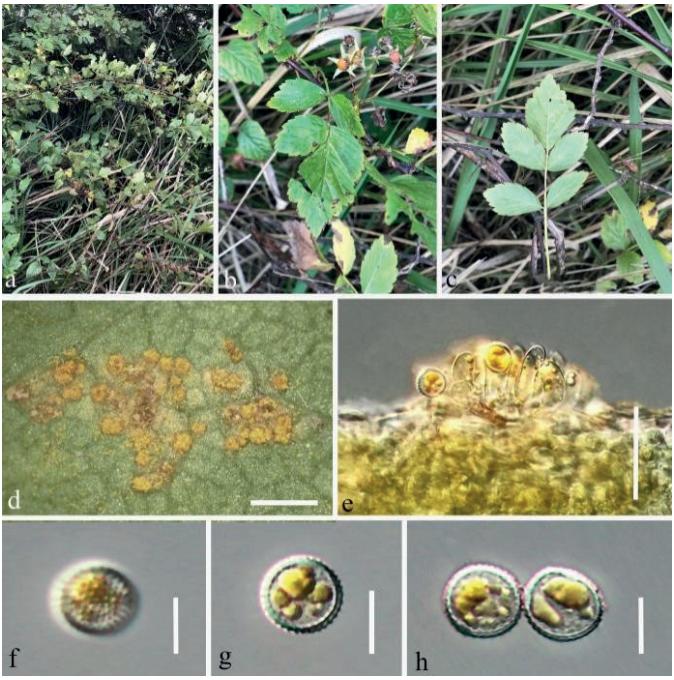

Figure 9. *Phragmidium barnardii* (HGUP21035) on *Rubus* sp. **a–d** uredinia on leaves **e** longitudinal section of uredinium **f–h** urediniospores. Scale bars: 1 mm (**d**); 50 μm (**e**); 12.5 μm (**f–h**).

powdery, 0.1–1.0 mm diam, with hyaline and curved paraphyses, 26–39 × 10–13 µm. Urediniospores orange, 16–19 × 15–18 µm (mean: 17.5 × 16.5 µm, n = 30), nearly globose; thick-walled 1.3–2.2 µm, colorless, regularly echinulate with stout spines.

Habitat. *Rubus* sp.

Known distribution. China, Guizhou Province; South Africa.

Material examined. China. Guizhou Province: Duyun city, 27°26'05"N, 107°38'91"W, 870 m, 26 Jun 2021, on *Rubus* sp., coll. J.E. Sun, HGUP21035.

Notes: *Phragmidium barnardii* was first reported on *Rubus* sp. by Winter (1886). Its DNA data was established by McTaggart et al (2016), although without description of morphological characteristics. We confirmed the specimens (HGUP21035) as *Ph. barnardii*, through phylogenetic analyse with DNA data from McTaggart et al. (2016).

Discussion

More than 70 *Phragmidium* species have been described in China, while many species without molecular data (Cummins 1931; Arthur 1934; Wahyuno et al. 2001; Cummins and Hiratsuka 2003; Zhuang et al. 2012; Yang et al. 2015; Ali et al. 2017). Recently, morphology and molecular data were gradually combined and used to describe the diversity of species in *Phragmidium* (Liu et al. 2018, 2019, 2020; Zhao et al. 2021). In the study, the four novel and three known species of *Phragmidium* were delineated based on phylogeny of the ITS and *LSU* gene regions and on morphological features.

The host plants of *Ph. punjabense*, *Ph. warburgianum*, *Ph. rosae-rugosae*, *Ph. rosae-laevigatae* and *Ph. rosae-roxburghii* all belong to *Rosa*, but *Ph. potentillae-freynianae* and *Ph. potentilla* occur on *Potentilla* sp. while *Ph. rubi-coreani* and *Ph. barnardii* occur on *Rubus* sp. However, the hosts of species with close phylogenetic relationships were not necessarily in the same genus. *Phragmidium potentilla* can be found on three plants (*P. chinensia*, *P. kleiniana* and *P. virgata*), and *Ph. rosae-roxburghii* can be parasitic on two *Rosa* plants (*Rosa roxburghii* and *Rosa* sp.). It might mean that host jumps also shaped the diversity of *Phragmidium*, like Pucciniales (McTaggart et al. 2016).

Phragmidium leucoaecium (BJFCR02118 and BJFCR02116), *Ph. japonicum* (HMAS41585), *Ph. jiangxiense* (BJFCR03452 and BJFCR03453) and *Ph. rosae-laevigatae* (HGUP21036 and HGUP21037) from *Rosa* formed a phylogenetic lineage, while three of the latter from the same host (*Rosa laevigata*) (Liu et al. 2020). This may be explained by geographical distribution, geography, climate, etc., but contradicts the concept of obligatory parasitism. We could guess that their hosts might not reflect taxonomic status of *Phragmidium*. Interestingly, *Phragmidium tibeticum*, *Ph. sikangense* and *Ph. shensianum* were named according to the collection locations (Dai 1979; Chen 2009). Their nomenclatures contradict the concept of obligatory parasitism for rust fungi, although might be easy to be understanding.

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