



<https://doi.org/10.11646/phytotaxa.416.1.1>

Murispora aquatica sp. nov. and *Murispora fagicola*, a new record from freshwater habitat in China

DAN-FENG BAO^{1,2,3}, DHANUSHKA N. WANASINGHE⁴, ZONG-LONG LUO¹, PETER E. MORTIMER^{4,5}, VINIT KUMAR^{2,3}, HONG-YAN SU^{1*} & KEVIN D. HYDE¹

¹College of Agriculture & Biological Science, Dali University, Dali 671003, Yunnan, P.R. China

²Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand

³Department of Entomology & Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

⁴Key Laboratory for Plant Biodiversity & Biogeography of East Asia (KLBP), Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, P.R. China

⁵Centre for Mountain Futures (CMF), Kunming Institute of Botany, Kunming 650201, Yunnan, P.R. China

*Corresponding author: suhongyan16@163.com

Abstract

We are currently studying lignicolous freshwater fungi in China. In this paper, we introduce two taxonomic accounts to the family Amniculicolaceae in Pleosporales viz. *Murispora aquatica* sp. nov. (from Yunnan) and a new record of *Murispora fagicola* (from Tibet). The conclusions were derived from both morphological comparisons and the results of phylogenetic analyses (concatenated ITS, LSU, SSU and TEF1- α sequence data). The new species is compared with other existing species of *Murispora* and comprehensive descriptions and micro-photographs are provided.

Keywords: 1 new species, Amniculicolaceae, phylogeny, taxonomy, freshwater fungi

Introduction

Lignicolous freshwater fungi grow on submerged woody debris in freshwater streams, ponds, lakes and tree hollows (Hyde 1995, Hyde & Goh 1998, Wong *et al.* 1998). This highly taxonomically diverse group is a specialized assemblage with the ability to decay submerged, waterlogged, woody debris. Therefore, these fungi play an important role in nutrient and carbon cycling, biological diversity and ecosystem functioning (Palmer *et al.* 1997, Hyde & Goh 1998, Wong *et al.* 1998, Cai *et al.* 2003, Hyde *et al.* 2016). Recent studies have shown these lignicolous freshwater fungi are likely to be sensitive to environmental changes and global warming (Hyde *et al.* 2016). This may lead to extinction of a number of species within this group if they cannot adapt to changes in their environment. Therefore, an improved understanding of this group of fungi is important (Hyde *et al.* 2016). If we are to surmount this challenge and limit the extent of potential loss of species, then a robust, updated fungal classification that enables clear taxonomic communication using extensive fungal collections in different geographic regions is needed.

Presently, freshwater fungi have been reported from 8 phyla: Aphelidiomycota, Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Monoblepharomycota, Mortierellomycota and Rozellomycota (Shearer *et al.* 2007, Kagami *et al.* 2012, Zhang *et al.* 2012, Jones *et al.* 2014, Wijayawardene *et al.* 2018). The most speciose phylum is Ascomycota, with freshwater representatives in 33 orders from different classes (Jones *et al.* 2009). During the last decade, studies on lignicolous freshwater fungi focused on Ascomycota (Shearer 1993, Hyde *et al.* 1996, 1998, Tsui *et al.* 2000, Cai *et al.* 2002, 2003, Ho *et al.* 2002, Luo *et al.* 2004, 2018, Jones *et al.* 2009, Boonyuen *et al.* 2011, Su *et al.* 2015, 2016, Yang *et al.* 2017), and they are highly diverse in the class Dothideomycetes and Sordariomycetes with a few members of Eurotiomycetes, Orbiliomycetes and seldom Basidiomycetes (Wijayawardene *et al.* 2017, 2018). In the Dothideomycetes there are two orders: Pleosporales and Jahnulales that support common freshwater fungi (Suetrong *et al.* 2011, Zhang *et al.* 2012) and in Sordariomycetes 14 orders have freshwater members, with Microascales and Savoryellales comprising both marine and freshwater taxa (Jones *et al.* 2009, 2019, Boonyuen *et al.* 2011, Zhang *et al.* 2017).

We are currently investigating the diversity of lignicolous freshwater fungi along a north/south gradient in the Asian/Australasian region (Hyde *et al.* 2016). Several studies on lignicolous freshwater fungi have been conducted and contribute towards our knowledge of this group of fungi (Cai *et al.* 2002, Luo *et al.* 2004, 2017, Yang *et al.* 2015, Su *et al.* 2015, 2018). In this study, we introduce two species of Amniculicolaceae which were collected from freshwater habitats in China. Amniculicolaceae is one of the families in Pleosporales that includes the majority of the familial members from freshwater habitats. This family was established by Zhang *et al.* (2009a) to accommodate freshwater fungi from Europe. Most species of this family are saprobes (Voglmayr 2004, Zhang *et al.* 2009a, b, Zhang *et al.* 2012).

Both morphological comparisons and the results of phylogenetic analyses confirmed that our new isolates belong to *Murispora*. These novelties are accounted with in-depth phylogenetic interpretations, notes, comprehensive micro-morphological illustrations and detailed descriptions.

Materials & Methods

Isolation and morphology

Specimens of submerged wood were collected from Cangshan Mountain, Yunnan Province, and Tibet province, China and brought to laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for 1 week. Specimen observations and morphological studies were conducted as following the protocols provided by Luo *et al.* (2018).

Single ascospore isolations were carried out following the method described in Chomnunti *et al.* (2014). Germinating ascospores were transferred aseptically to Potato dextrose agar (PDA) plates and grown at 16°C in the daylight. Colony colour and other characters were observed and measured after a week and again after three weeks. The specimens were deposited in the Mae Fah Luang University (MFLU) Herbarium, Chiang Rai, Thailand. Living cultures are also deposited at the Culture Collection at Mae Fah Luang University (MFLUCC). Facesoffungi numbers (FoF) were acquired as in Jayasiri *et al.* (2015) and Index Fungorum (2019).

DNA extraction, PCR amplification, and sequencing

Fungal mycelium was scraped from the surface of colonies grown on a PDA plate or MEA plate at 25°C for 4 weeks, transferred into a 1.5 mL centrifuge tube and ground using liquid nitrogen. The EZ gene™ fungal gDNA kit (GD2416) was used to extract DNA from the ground mycelium according to the manufacturer's instructions. The gene regions of the large subunit of the nuclear ribosomal DNA (LSU), the internal transcribed spacers (ITS), the small subunit of the nuclear ribosomal DNA (SSU) and the translation elongation factor (TEF1- α) RNA were amplified using the primer pairs LR0R/LR7 (Vilgalys & Hester 1990), ITS5/ITS4, NS1/ NS4 (White *et al.* 1990) and 983F/2218R (Liu *et al.* 1999) respectively. The PCR mixture was prepared as follows: 12.5 μ l of 2 \times Power Taq PCR MasterMix, 20 mM Tris-HCl pH 8.3, 100 mM KCl, 3 mM MgCl₂, stabilizer and enhancer, 1 μ l of each primer (10 μ M), 1 μ l genomic DNA extract and 9.5 μ l deionised water. The PCR of ITS, LSU, SSU and TEF1- α gene was processed as follows: 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 50 seconds, elongation at 72 °C for 1 minute and a final extension at 72 °C for 10 minutes, and finally kept at 4 °C. PCR amplification was confirmed on 1 % agarose electrophoresis gels stained with ethidium bromide. Purification and sequencing of PCR products were conducted at Shanghai Sangon Biological Engineering Technology and Services Co., Ltd (Shanghai, P.R. China).

Molecular phylogenetic analyses

Sequencing and sequence alignment

The sequence was assembled by using BioEdit, sequences with high similarity indices were determined from a BLAST search to find the closest matches with taxa in Amniculicolaceae and from recently published data (Ariyawansa *et al.* 2015, Wanasinghe *et al.* 2015, Hyde *et al.* 2019). All consensus sequences and the reference sequences were automatically aligned with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>; Katoh and Standley 2013). Aligned sequences of each gene region (ITS, LSU, SSU and TEF1- α) were combined and manually improved using BioEdit v. 7.0.5.2 (Hall 1999). Ambiguous regions were excluded from the analyses and gaps were treated as missing data. Phylogenetic analyses were obtained from Maximum Likelihood (ML) and Bayesian analysis.

Phylogenetic analyses

Maximum likelihood (ML) analysis was performed at the CIPRES Science Gateway v.3.3 (<http://www.phylo.org/portal2/>, Miller *et al.* 2010) using RAxML v. 8.2.8 as part of the “RAxML-HPC2 on XSEDE” tool (Stamatakis 2006, 2008). All model parameters were estimated by RAxML with ML estimates of 25 per site rate categories. The final ML search was conducted using the GTRGAMMA + I model which was estimated by using MrModeltest 2.2 (Nylander 2004).

Bayesian analysis was performed by using MrBayes v 3.1.2. (Ronquist *et al.* 2003) The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v.3.1.2 (Liu *et al.* 2012). Six simultaneous Markov Chains were run for one million generations, and trees were sampled every 100th generation (resulting in 10,000 trees). The first 2000 trees representing the burn-in phase of the analyses were discarded and the remaining 8000 (post burning) trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai *et al.* 2006, Liu *et al.* 2012).

Results

Phylogenetic analyses

The combined ITS, LSU, SSU, and TEF1- α gene dataset comprised 48 taxa from 6 families (Amniculicolaceae, Halotthiaceae, Lindgomycetaceae, Lophiostomaceae, Sporormiaceae and Teratospharriaceae) in Pleosporales (Table 1) with *Lentithecium fluviatile* (CBS 122367) and *Murilentithecium clematidis* (MFLUCC 14–0562) as the outgroup taxa. The RAxML analysis of the combined dataset yielded the best scoring tree (FIGURE. 1) with a final ML optimization likelihood value of -16990.520444. The alignment comprised 3143 total characters including gaps. The matrix had 975 distinct alignment patterns, with 26.56 % undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.243430, C = 0.241746, G = 0.272949, T = 0.241874; substitution rates AC = 1.301569, AG = 2.169248, AT = 1.518991, CG = 1.101597, CT = 6.160150, GT = 1.000000; gamma distribution shape parameter α = 0.169407. The Bayesian analysis resulted in a tree with the same topology and clades as the ML tree and the bootstrap values for ML above than 75% and Bayesian posterior probabilities (PP) greater than 0.95 are given at the nodes.

In our phylogenetic analyses of combined ITS, LSU, SSU and TEF1- α , Amniculicolaceae species clustered in one clade, sister to Lindgomycetaceae. All of the genera in this family were clearly separated in different clades. The two new isolates of *Murispora aquatica* and *M. fagicola* clustered within the genus *Murispora*. *Murispora aquatica* clustered with *M. hawksworthii*, however, separated from *M. hawksworthii* with significant statistical support (93 ML/1.00 PP). The new strain of *M. fagicola* (MFLUCC 19–0256) clustered with existing species *M. fagicola* (MFLUCC 13–0600) with high phylogenetic support (100 ML/1.00 PP).(FIGURE. 1)

TABLE 1. Isolates and sequences used in this study, the newly generated sequences are indicated in red and the type strains are indicated in bold.

Taxon	Strain	GenBank Accession No.			
		LSU	SSU	ITS	TEF1- α
<i>Amniculicola aquatica</i>	MFLUCC 16–1123	MK106096	–	–	MK109800
<i>A. aquatica</i>	MFLUCC 16–0915	MK106097	MK106108	–	MK109801
<i>A. guttulata</i>	MFLUCC 16–0907	MK106098	–	–	MK109802
<i>A. guttulata</i>	MFLUCC 16–1297	MK106099	–	–	–
<i>A. guttulata</i>	MFLU 18–1327	MK106100	MK106109	–	MK109803
<i>A. immersa</i>	CBS 123083	FJ795498	GU456295	–	GU456273
<i>A. lignicola</i>	CBS 123094	EF493861	EF493863	MH863274	GU456278
<i>A. parva</i>	CBS 123092	FJ795497	GU296134	MH863272	GU349065
<i>Anguillospora longissima</i>	CCM-F10304	JN673029	–	–	–

.....continued on the next page

TABLE 1. (Continued)

Taxon	Strain	GenBank Accession No.			
		LSU	SSU	ITS	TEF1- α
<i>Halothia posidoniae</i>	BBH 22481	GU479786	GU479752	–	–
<i>Lindgomyces breviappendiculata</i>	MAFF 239292	AB521749	AB521734	JQ435792	–
<i>L. cinctosporae</i>	Raja R56–1	AB522431	AB522430	JF419905	–
<i>L. ingoldianus</i>	ATCC 200398	AB521736	AB521719	NR119938	–
<i>L. rotundatus</i>	HHUF 27999	AB521740	AB521723	JQ435793	–
<i>Lophiostoma arundinis</i>	CBS 621.86	DQ782384	DQ782383	JN942965	DQ782387
<i>L. crenatum</i>	CBS 629.86	DQ678069	DQ678017	–	DQ677912
<i>L. macrostomoides</i>	CBS 123097	FJ795439	FJ795482	MH863108	GU456277
<i>L. semiliberum</i>	CBS 626.86	FJ795441	FJ795484	JN942970	–
<i>Massariosphaeria typhicola</i>	KT 797	AB521747	AB521730	JF419906	–
<i>M. typhicola</i>	KT 667	AB521746	AB521729	–	–
<i>M. typhicola</i>	CBS 609.86	EF165033	EF165037	–	–
<i>M. rhizophorae</i>	BCC 28866	GU371824	GU371832	–	GU371817
<i>Murilentithecium clematidis</i>	MFLUCC 14–0562	KM408759	KM408761	NR154174	KM454445
<i>Murispora aquatica</i>	MFLUCC 17–2221	MN325075	MN325077	MN325085	MN337969
<i>M. cardui</i>	MFLUCC 13–0761	KT709176	KT709183	KT736082	KT709190
<i>M. cicognanii</i>	MFLUCC 14–0953	KT709179	KT709186	KT736085	–
<i>M. fagicola</i>	MFLUCC 13–0600	KT709174	KT709181	NR155379	KT709188
<i>M. fagicola</i>	MFLUCC 19–0256	MN325076	–	MN325086	–
<i>M. galii</i>	MFLUCC 13–0819	KT709175	KT709182	KT736081	KT709189
<i>M. hawksworthii</i>	MFLUCC 14–0918	KT709180	KT709187	KT736086	KT709192
<i>M. medicaginicola</i>	MFLUCC 13–0762	KT709177	KT709184	KT736083	KT709191
<i>M. rubicunda</i>	IFRD 2017	FJ795507	GU456308	–	GU456289
<i>Neomassariosphaeria grandispora</i>	CBS 613.86	GU301842	GU296172	–	GU349036
<i>Preussia funiculata</i>	CBS 659.74	GU301864	GU296187	MH858746	GU349032
<i>P. lignicola</i>	CBS 264.69	GU301872	GU296197	GQ203783	GU349027
<i>P. terricola</i>	DAOM 230091	AY544686	AY544726	MH858589	DQ471063
<i>Pseudomassariosphaeria bromicola</i>	MFLUCC 15–0031	NG059595	KT305996	KT305998	KT305999
<i>Quadricrura septentrionalis</i>	CBS 125428	AB524617	AB524476	MH863683	–
<i>Repetophragma ontariense</i>	HKUCC 10830	DQ408575	–	NR154471	–
<i>Spirosphaera cupreorufescens</i>	A20	AY616236	–	–	–
<i>Sporormiella minima</i>	CBS 524.50	DQ678056	DQ678003	MH856741	DQ677897
<i>Tetraploa sasicola</i>	KT 563	AB524631	AB524490	NR119404	–
<i>Triplosphaeria maxima</i>	KT 870	AB524637	AB524496	NR119407	–
<i>Westerdykella cylindrica</i>	CBS 454.72	AY004343	AY016355	MH481315	DQ497610
<i>W. ornata</i>	CBS 379.55	GU301880	GU296208	MH857522	GU349021

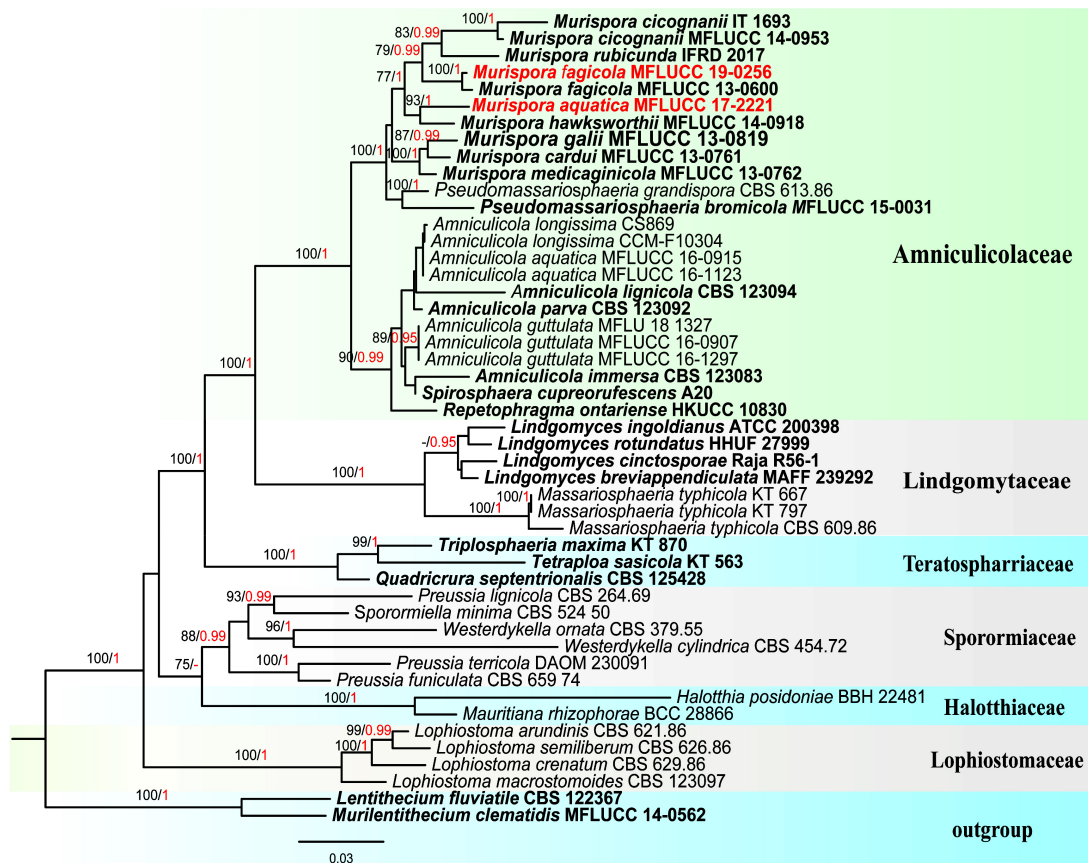


FIGURE 1. Phylogenetic tree based on RAxML analyses of a combined ITS, LSU, SSU and TEF1- α dataset. Bootstrap support values for maximum likelihood (ML, black) higher than 75% and Bayesian posterior probabilities (BYPP, red) greater than 0.95 are indicated above the nodes as ML/PP. The tree is rooted at *Lentithecium fluviatile* (CBS 122367) and *Murilentithecium clematidis* (MFLUCC 14-0562). The ex-type and reference strains are in bold and new isolates are in red.

Taxonomy

Murispora aquatica D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *sp. nov.* FIGURE. 2

Index Fungorum number: IF 556556; Facesoffungi number: FoF 06255

Etymology:—referring to the aquatic habitat of this fungus.

Holotype:—MFLU 19-0990

Saprobic on decaying submerged wood in freshwater habitats. **Sexual morph:** *Ascomata* 180–280 \times 180–250 μm (\bar{x} = 221.3 \times 213.6 μm , n = 10), carbonaceous, solitary, globose to subglobose, papillate, dark brown to black, semi-immersed to immersed, scattered, substrate stained red to purple, fused to the host tissue, ostiolate. *Ostirole* 60–85 μm high (\bar{x} = 73.2 μm , n = 5) round to papillate, short, outer cells brown to black, inner cells pale brown to hyaline. *Peridium* 18–33 μm wide comprising several layers grayish brown to black cells of *textura angularis* and *globulosa*. *Hamathecium* comprising 2–3 μm (n = 30) wide, branched, septate, hyaline, cellular pseudoparaphyses. *Asci* 123–150 \times 14–17 μm (\bar{x} = 137 \times 15.7 μm , n = 20), 8-spored, bitunicate, fission-tunicate, cylindrical-clavate, pedicellate, with a small ocular chamber. *Ascospores* 21–25 \times 8–10 μm (\bar{x} = 23 \times 9 μm , n = 30), overlapping, 1–2-seriate, ellipsoidal to fusiform, narrow and rounded at both ends, muriform, 4–6 transverse septa, 1–2 longitudinal septa in all cells and rarely in end cells, slightly constricted at transverse septa, deeply constricted at middle transverse septa, hyaline when young, becoming dark brown at mature, smooth, surrounded by a distinct mucilaginous sheath. **Asexual morph:** Undetermined.

Material examined:—CHINA, Yunnan Province, Cangshan Mountain, saprobic on decaying wood submerged in a freshwater stream. May 2017, S.M. Tang, S-853 (MFLU 19-0990, holotype), ex-type culture, MFLUCC 17-2221 = ICMP 21832.

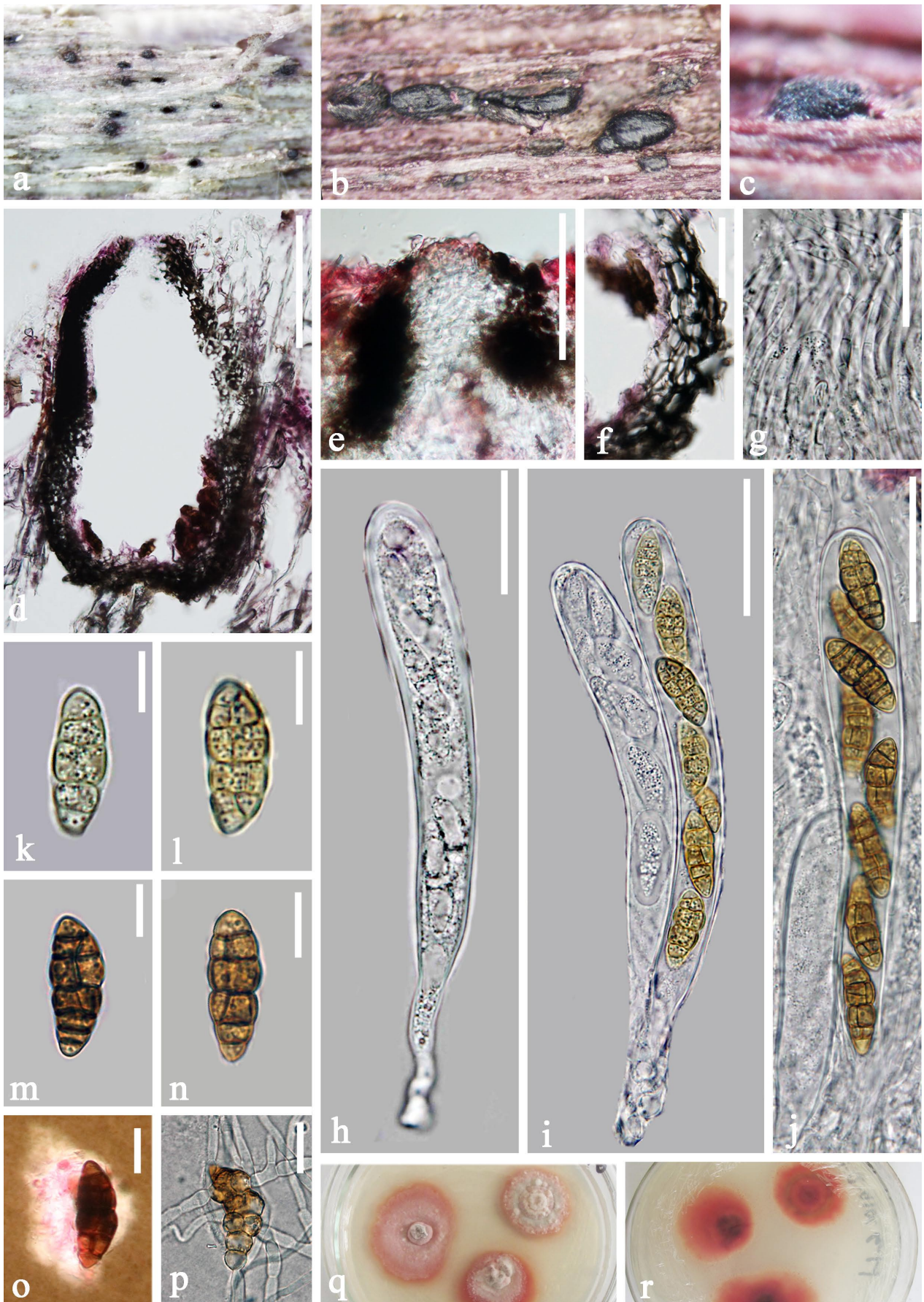


FIGURE 2. *Murispora aquatica* (MFLU 19-0990, holotype). **a–c.** Ascomata on wood. **d.** Section of ascoma. **e.** Close up of ostiole. **f.** Peridium. **g.** Pseudoparaphyses. **h–j.** Asci. **k–n.** Ascospores. **o.** Ascospore stain in Indian Ink, with mucilaginous sheath. **p.** Germinating ascospore. **q, r.** Culture on PDA from surface and reverse. Scale bars: **d** = 100 μ m, **e** = 50 μ m, **f–h** = 25 μ m, **i–j** = 35 μ m, **k–p** = 10 μ m.

Notes:—Morphologically, *Murispora aquatica* have similar shaped asci and ascospores as *M. hawksworthii* and both can stain the wood purple. However, *M. aquatica* differs from *M. hawksworthii* in having smaller asci (123–150 × 14–17 µm vs. 150–200 × 20–28 µm) and shorter ascospores (21–25 × 8–10 µm vs. 25–35 × 8–12 µm). In our phylogenetic analyses, *Murispora aquatica* clustered with *M. hawksworthii* with strong support. Moreover, we compared the basepairs of ITS and TEF1- α gene regions and there were 17 and 14 base pair differences respectively. These results strongly support our isolate to be a new species (Jeewon & Hyde 2016). Therefore, we introduce our isolate as a new species based on both phylogeny and morphological characters.

Murispora fagicola Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, in Wanasinghe *et al.* Cryptogamie, Mycologie, 36: 419–448 (2015) FIGURE. 3

Index Fungorum number: IF 551556

Saprobic on decaying submerged wood in freshwater. **Sexual morph:** *Ascomata* 180–350 × 160–315 µm (\bar{x} = 263 × 252 µm, n = 10), solitary to gregarious, semi-immersed to immersed, globose to subglobose, dark brown to black, substrate stained purple, fused to the host tissue, ostiolate. *Ostiole* 65–130 µm high (\bar{x} = 85.7 µm, n = 5), crest-like, central, papillate, dark brown to black, and occasionally with purple, opening to exterior through bark surface. *Peridium* 15–30 µm wide, comprising 3–4 layers of dark brown cells *textura angularis*. *Hamathecium* comprising 1.5–2 µm wide, filiform, branched, septate, cellular pseudoparaphyses. *Asci* 166–247 × 16–22 µm (\bar{x} = 184 × 16.8 µm, n = 30), bitunicate, fissitunicate, cylindric-clavate, short pedicellate, with a minute ocular chamber. *Ascospores* 27–31 × 11–13 µm (\bar{x} = 30.3 × 11.4 µm, n = 30), overlapping, 1–2-seriate, oval to ellipsoidal, with narrow ends, muriform, with 1–2 longitudinal septa in all cells except end cells, constricted at septa, hyaline when young, becoming dark brown at maturity, surrounded by a thin, distinct mucilaginous sheath. **Asexual morph:** Undetermined.

Material examined:—CHINA, Tibet, saprobic on decaying wood submerged in a freshwater river, May 2017, S.M. Tang, S-1290 (MFLU 19–0991), living culture, MFLUCC 19–0256.

Notes:—*Murispora fagicola* was introduced by Wanasinghe *et al.* (2015), which was collected from terrestrial habitats in Italy. In this study, our specimen was collected from freshwater habitat in Tibet, China. Morphologically, the new isolate of *Murispora fagicola* (MFLUCC 19–0256) is similar to the holotype of *M. fagicola* (MFLUCC 13–0600). However, the size of asci between these two strains MFLUCC 19–0256 and MFLUCC 13–0600 are different. Asci of MFLUCC 19–0256 are larger than MFLUCC 13–0600. We suspect it is due to the different habitats. In the phylogenetic analyses, the new isolate *M. fagicola* (MFLUCC 19–0256) clustered with *M. fagicola* (MFLUCC 13–0600) with high bootstrap support (100% ML and 1.00 BYPP) and the ITS regions of these two *M. fagicola* strains (MFLUCC 13–0600 and MFLUCC 19–0256) differ by only five base pairs (1.06%). Therefore, we identified our isolate as a new record for China based on both morphological characters and phylogenetic results.

Discussion

In our phylogenetic analyses, all of the Amniculicolaceae species clustered in a distinct clade sister to Lindgomycetaceae, which is similar to previous studies (Ariyawansa *et al.* 2015). Most Amniculicolaceae species are reported from freshwater habitats and are widely distributed in Europe, Italy, France, Denmark and Germany (Zhang *et al.* 2009a, b, Ariyawansa *et al.* 2015, Wanasinghe *et al.* 2015). However, species from *Murispora* and *Pseudomassariosphaeria* are commonly known from terrestrial habitats (Zhang *et al.* 2009a, b, Ariyawansa *et al.* 2015, Wanasinghe *et al.* 2015). In this study we introduce two species of *Murispora*, collected from China in freshwater habitats. Thus, it proves that *Murispora* species are not restricted to terrestrial ecosystems. Phylogenetically *Murispora* is a well-supported monophyletic genus, where all species have DNA based sequence data. Morphologically, they are easier to distinguish from other species in Amniculicolaceae as the spores of this genus are muriform, whereas, *Amniculicola* and *Pseudomassariosphaeria* species have fusiform spores.

In previous studies, species *Repetophragma ontariense* and *Spirosphaera cupreorufescens* were clustered with *Amniculicola* species within Amniculicolaceae (Shearer *et al.* 2009, Zhang *et al.* 2009b, Hyde *et al.* 2013, Wanasinghe *et al.* 2015) and our analyses showed the same result. However, the placement of these two species still remains uncertain, as species of *Repetophragma* are polyphyletic, they cluster in different families and orders of Sordariomycetes and Dothideomycetes (Shenoy *et al.* 2006) and most species of *Spirosphaera* were reported on Leotiomycetes. Therefore, further studies are recommended to confirm the placement of these species.

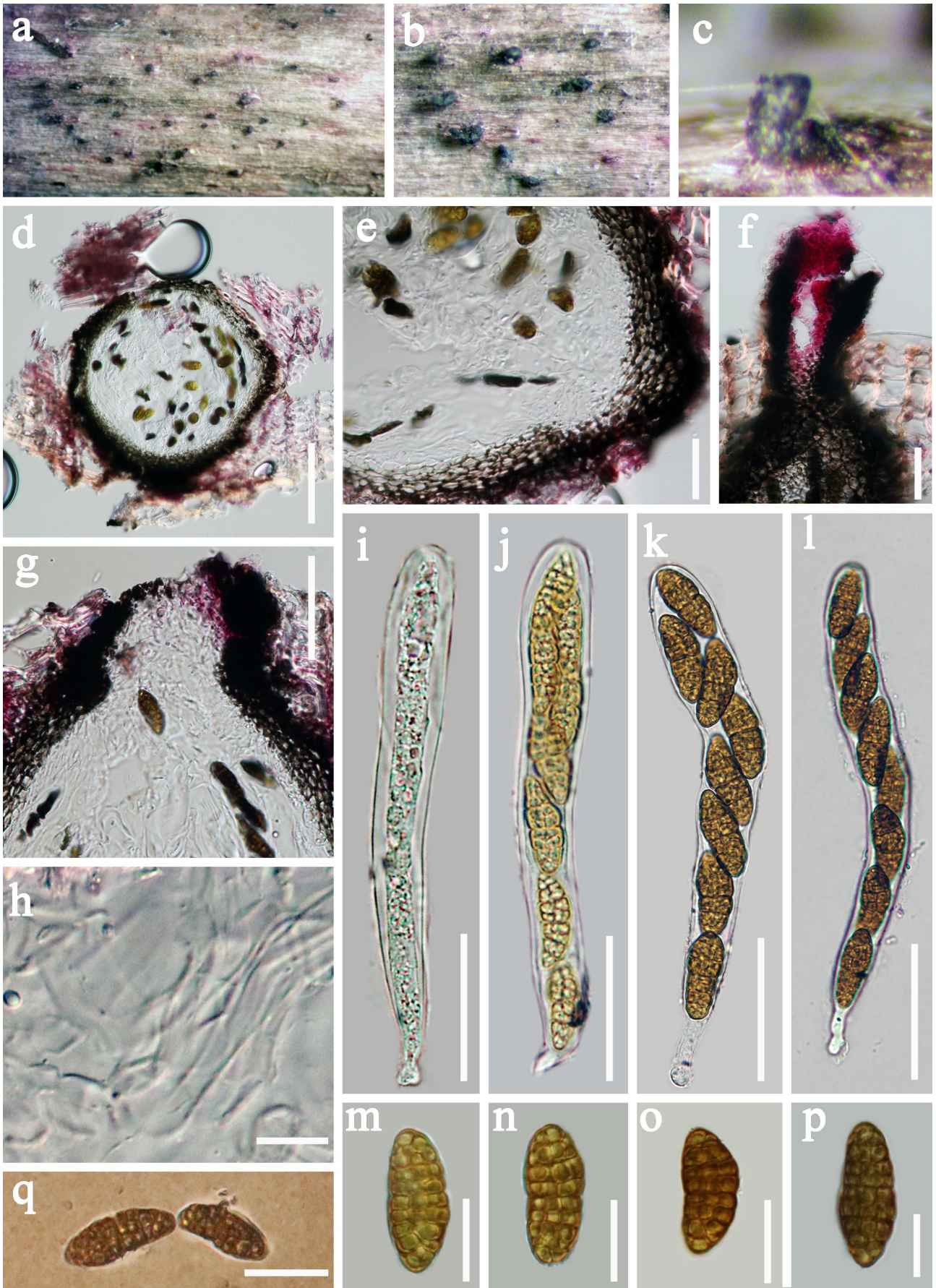


FIGURE 3. *Murispora fagicola* (MFLU 19-0991). **a–c.** Ascomata on wood. **d.** Section of ascoma. **e.** Peridium. **f, g** Ostiole. **h** Pseudoparaphyses. **i–l.** Asci. **m–p.** Ascospores. **q.** Ascospore in India Ink, with thin sheath. Scale bars: **d** = 100 μ m, **e–g** = 50 μ m, **h** = 10 μ m, **i, j** = 40 μ m, **k, l** = 45 μ m, **m–p** = 20 μ m, **q** = 25 μ m.

Ascomycetous fungi as pigment producers are often used as an alternative source for synthetic dyes, especially in the red-dye industries (Lebeau *et al.* 2017). They produce an extraordinary range of red polyketide pigments that are often more stable and soluble than plant pigments. So, fungal red polyketides, such as azaphilone, naphthoquinone, and hydroxyanthraquinone red compounds, are most promising in this respect (Hohmann *et al.* 2009, Costantino *et al.* 2009, Bonugli *et al.* 2015). Most Amniculicolaceae species can produce purple stain on the substrate (Zhang *et al.* 2008, 2009b, c, 2012, Wanasinghe *et al.* 2015). Therefore, Amniculicolaceae species are potential to be pigment producers for natural colorants.

Acknowledgements

We would like to thank the National Natural Science Foundation of China (Project ID: 31660008) and Thailand Research Fund grant “impact of climate change on fungal diversity and biogeography in the Greater Mekong Subregion (RDG6130001) for financial and laboratory support. Peter E Mortimer would like to thank the National Science Foundation of China and the South East Asian Biodiversity Resources Institute, Chinese Academy of Sciences, for financial support under the following grants: 41761144055, 41771063, Y4ZK111B01. Dan-Feng Bao thanks Shaun Pennycook from Landcare Research, Auckland, New Zealand, for advising on the taxon names, Wen-Li Li and Yan-Mei Zhang are acknowledged for their help on DNA extraction and PCR amplification and Hong-Wei Shen is acknowledged for his help on fungal measurement. Dhanushka Wanasinghe would like to thank CAS President’s International Fellowship Initiative (PIFI) for funding his postdoctoral research (number 2019PC0008).

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