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Two new species in Fuscosporellaceae from freshwater habitats in Thailand

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Abstract

A survey of freshwater fungi in Thailand resulted in the discovery of two new species in Fuscosporellaceae, herein described as *Fuscosporella aquatica* and *Mucispora phangngaensis*. *Fuscosporella aquatica* has sporodochial colonies, irregular ellipsoidal and dark brown conidia with vesicular conidiogenous cells. *Mucispora phangngaensis* has macronematous, solitary conidiophores with percurrent proliferations and dark brown obovoid conidia. Phylogenetic analysis based on combined LSU and ITS sequence data support their placement in *Fuscosporella* and *Mucispora* (Fuscosporellaceae, Fuscosporellales). Both species formed distinct clades from their type species and present as the second species in each genus. Descriptions and illustrations of the new taxa are provided. DNA sequence data of *Parafuscosporella mucosa* (from ex-type strain) are provided in this study, and confirmed its phylogenetic placement.

Key words – Asexual fungi – Phylogeny – Sordariomycetes – Taxonomy

Introduction

Fuscosporella was established as a monotypic genus for *F. pyriformis* J. Yang, J. Bhat & K.D. Hyde, and it is the type genus of Fuscosporellaceae (Yang et al. 2016). *Fuscosporella pyriformis* is morphologically similar to *Parafuscosprella moniliformis* J. Yang, J. Bhat & K.D. Hyde and *P. mucosa* J. Yang, J. Bhat & K.D. Hyde, as they share characters such as uniseptate, dark brown, obvoid to obpyriform conidia of similar size, and hyaline vesicular conidiogenous cells. *Parafuscosporella garethii* Boonyuen, Chuaseehar. & Somrith. is significantly different from the above species in its obpyramidal conidia, which are coronate at the apex with unusual conical projections (Boonyuen et al. 2016). However, molecular evidence gave a precise classification.

Mucispora was introduced by Yang et al. (2016) as a monotypic genus for *M. obscuriseptata* J. Yang, J. Bhat & K.D. Hyde. The genus is characterized by macronematous, mononematous conidiophores, monoblastic, terminal and cylindrical conidiogenous cells and ellipsoidal or obovoid dark brown conidia with three septa. The generic concept of *Mucispora* resembles *Acrogenospora*,

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Melanocephala and *Monotosporella*. *Acrogenospora* differs from *Mucispora* in having aseptate conidia (Goh et al. 1998). *Melanocephala* is specific in its cupulate proliferating conidiogenous cells and its conidia bearing a central downwardly directed collar with a fimbriate margin (Hughes 1979, Wu & Zhuang 2005). *Monotosporella* is distinguished from *Mucispora* by its cylindrical, doliiform or lageniform conidiogenous cells (Sadowski et al. 2012).

During the survey of freshwater fungi on submerged wood along a north / south gradient in the Asian / Australasian region (Hyde et al. 2016), two new freshwater taxa were collected. Phylogenetic analyses of combined LSU and ITS sequence data and morphological characters strongly support the separation of the new taxa in *Fuscosporella* and *Mucispora* respectively. We therefore introduce *Fuscosporella aquatica* and *Mucispora phangngaensis* as new species, with an illustrated account and phylogenetic evidence.

Materials & Methods

Collection and examination of specimens

Specimens of submerged, decaying wood were collected from stream in Phang Nga Province, Thailand, in December 2015. Specimens were brought to the laboratory in plastic bags and incubated in plastic boxes lined with moistened tissue paper at room temperature for one week. Morphological observations were made using a Motic SMZ 168 Series dissecting microscope for fungal structures on natural substrate. The fungal structures were collected using a syringe needle and transferred to a small drop of distilled water on a clean slide with cover glass. The fungi were examined using a Nikon ECLIPSE 80i compound microscope and photographed with a Canon 600D digital camera fitted to the microscope. Measurements were made with the Tarosoft (R) Image Frame Work program and images used for figures were processed with Adobe Photoshop CS6 software. Single spore isolations were made onto potato dextrose agar (PDA) or water agar (WA) and later transferred onto malt extract agar (MEA) or PDA following the method of Chomnunti et al. (2014). Specimens (dry wood with fungal material) are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and Guizhou Academic of Agriculture Sciences (GZAAS), China. Axenic cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Guizhou Culture Collection (GZCC). Facesoffungi and Index Fungorum numbers are registered as outlined in Jayasiri et al. (2015) and Index Fungorum (2017).

DNA extraction, PCR amplification and sequencing

Isolates were grown on PDA/MEA medium at 25 °C for one month. Fungal mycelium was scraped off and transferred to a 1.5-mL microcentrifuge tube using a sterilized lancet for genomic DNA extraction. A Biospin Fungus Genomic DNA Extraction Kit (BioFluxR, Hangzhou, P. R. China) was used to extract DNA following the manufacturer's instructions. LSU and ITS gene regions were amplified using the primer pairs LROR/LR5 and ITS5/ITS4 (White et al. 1990). The amplifications were performed in a 25 μL reaction volume containing 9.5 μL ddH_2O, 12.5 μL 2 × PCR Master Mix (TIANGEN Co., China), 1 μL of DNA template and 1 μL of each primer (10 μM). The amplification condition consisted of initial denaturation at 94 °C for 3 min; followed by 40 cycles of 45 s at 94 °C, 50 s at 56 °C and 1 min at 72 °C, and a final extension period of 10 min at 72 °C. Purification and sequencing of PCR products were carried out using the above-mentioned PCR primers at Invitrogen Biotechnology Co., China.

Phylogenetic analyses

The taxa included in the phylogenetic analyses were selected and obtained from previous studies and GenBank (Maharachchikumbura et al. 2015, 2016, Réblová et al. 2016, Yang et al. 2016). LSU and ITS gene regions were used for the combined sequence data analyses. Sequences were optimized manually to allow maximum alignment and maximum sequence similarity. The sequences were aligned using the online multiple alignment program MAFFT v.7

(http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013). The alignments were checked visually and improved manually where necessary.

Phylogenetic analysis of the sequence data consisted of maximum likelihood (ML) using RAxML-HPC v.8 (Stamatakis 2006, Stamatakis et al. 2008) on the XSEDE Teragrid of the CIPRES science Gateway (https://www.phylo.org) (Miller et al. 2010) with rapid bootstrap analysis, followed by 1000 bootstrap replicates. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTRGAMMA substitution model.

Maximum-parsimony (MP) analyses were performed using the heuristic search option with 1000 random taxa addition and tree bisection and reconnection (TBR) as the branchswapping algorithm. All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

The program MrModeltest2 v. 2.3 (Nylander 2008) was used to infer the appropriate substitution model that would best fit the model of DNA evolution for the combined datasets for Bayesian inference analysis with GTR+G+I substitution model selected. Posterior probabilities (PP) (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1 million generations, with trees sampled every 100 generations. The first 2500 trees, representing the burn-in phase of the analyses, were discarded and the remaining trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree (Larget & Simon 1999).

The resulting trees were printed with FigTree v. 1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/), and the layout was created in Microsoft PowerPoint for Mac v. 15.19.1. The alignment of phylogenetic analyses was deposited in TreeBASE (21782). Sequences generated in this study are deposited in GenBank (Table 1).

Phylogenetic results

The analyzed dataset consisted of combined LSU (1192 bp) and ITS (792 bp) sequence data (a total of 1984 characters including gaps after alignment) for 51 taxa in Sordariomycetes with *Microglossum rufum* and *Leotia lubrica* as the outgroup taxa. The best scoring RAxML tree is shown in Fig. 1.

Phylogenetic trees obtained from ML, MP and Bayesian analyses yielded trees with similar overall topology at the order and family levels which are in agreement with previous studies (Boonyuen et al. 2016, Réblová et al. 2016, Yang et al. 2016). *Fuscosporella aquatica* (MFLUCC 16-0859) clustered together with the type species *F. pyriformis* (MFLUCC 16-0570), and present as phylogenetically distinct species. *Mucispora phangngaensis* (MFLUCC 16-0865) and *M. obscuriseptata* (MFLUCC 15-0618) formed a distinct clade which represents *Mucispora* in Fuscosporellaceae, and the two isolates are phylogenetically distinct. The newly generated sequence data of *Parafuscosporella mucosa* (from ex-type strain) proved its placement in *Parafuscosporella*.

Taxonomy

Fuscosporella aquatica J. Yang & K.D. Hyde, sp. nov. Fig. 2

Index Fungorum number: IF553967; Facesoffungi number: FoF03863

Etymology – named for its aquatic habitat.

Saprobic on decaying submerged twigs. Colonies on natural substrate sporodochial, scattered, black. Mycelium partly immersed, partly superficial, composed of septate, hyaline hyphae. Conidiophores macronematous, mononematous, hyaline, smooth-walled, 25–60 × 3–7 μm. Conidiogenous cells monoblastic, integrated, terminal, globose, subglobose, ellipsoidal or clavate, hyaline, 17.5–44 × 10–19 μm ($\bar{x}$ = 28 × 14 μm, n = 15), sometimes guttulate. Conidia acrogenous,

ellipsoidal or slightly irregular shaped, 38–60 × 25.5–37 µm ($\bar{x}$ = 48.5 × 30.5 µm, n = 40), dark brown to black, smooth, with pale brown cell at the apex and the base.

Culture characteristics – Conidia germinating on PDA within 24 h. Germ tubes produced from basal cell. Colonies on PDA slow growing, reaching 10–15 mm diameter after one month at 25 °C in natural light, circular, matte, greyish-green with hyaline margin at the beginning, becoming dark brown in the inner circle, dark brown and pale brown in the middle ring, with hyaline sparse hyphae in the outer ring on the surface, with entire margin, dark brown in reverse. Sporulation absent. Vegetative hyphae are formed hyaline to mid brown, globose to ellipsoidal cells 5–12 µm diam., thick-walled, arranged in chains or moliniform.

Material examined – THAILAND, Phang Nga Province, Bann Tom Thong Khang, on decaying wood submerged in a freshwater stream, 17 December 2015, Kevin D. Hyde, Site 7-2-7 (MFLU 17-1973 **holotype**), ex-type living cultures, MFLUCC 16-0859, GZCC 17-0015.

Notes – *Fuscosporella aquatica* is the second species in the genus. *Fuscosporella aquatica* has larger conidiogenous cells (17.5–44 × 10–19 µm) and conidia (38–60 × 25.5–37 µm) compared to those in *F. pyriformis* (conidiogenous cells: 7.5–23 × 3.5–9 µm, conidia: 23.5–36 × 14–21 µm) (Yang et al. 2016). *Fuscosporella aquatica* is also distinguished by its irregular ellipsoidal conidia, while *F. pyrifomis* has obovate to obpyriform conidia. Phylogenetic analyses revealed the separation of these two species in *Fuscosporella*. Additionally, the two-species showed multiple nucleotide differences between them for LSU and ITS gene regions respectively (Table 2). In the LSU region, they differ in seven nucleotides, in the ITS region, they differ in nine nucleotides which is line with the recommendations by Jeewon & Hyde (2016).

Mucispora phangngaensis J. Yang & K.D. Hyde, sp. nov. Figs 3, 4

Index Fungorum number: IF553968, Facesoffungi number: FoF03864

Etymology – Referring to the collecting site from Phang Nga Province in Thailand.

Saprobic on decaying submerged twigs. Colonies on substrate sparse, scattered, glistening, black. Mycelium mostly immersed, consisting of septate, smooth, pale brown to hyaline hyphae. Conidiophores macronematous, mononematous, solitary, straight, erect, smooth, mid brown, paler towards the apex, 4–8-septate, 170–305 × 5–7 µm ($\bar{x}$ = 245 × 8.5 µm, n = 20), truncate at the apex, with 1–2 percurrent proliferations. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, pale brown. Conidia acrogenous, dark brown, ellipsoidal or obovoid, rarely pyriform, rounded at the apex and truncate at the base, 35–45 × 16.5–25 µm ($\bar{x}$ = 40 × 20 µm, n = 20), smooth, 3-euseptate, darkened at the upper two septa but unobservable when mature, with paler basal cell.

Culture characteristics – Conidia germinating on PDA within 24 h. Germ tubes produced from basal cell. Colonies on PDA slow growing, reaching 5–10 mm diameter after one month at 25 °C in natural light, circular, with matte dark olivaceous and dense mycelium on the surface, dark in reverse with entire margin. Hyphae subhyaline to pale brown, sometimes constricted at the septa, 3–7 µm wide. *Conidiophores* reduced to a monoblastic conidiogenous cell. *Conidiogenous cells* 8–10.5 × 3.3–4.8 µm, integrated, subhyaline to pale brown. *Conidia* 23.5–47.5 × 9.5–17.5 µm ($\bar{x}$ = 31 × 14 µm, n = 20), pale brown to mid brown, 1–4-septate, mostly 2-septate, globose to obovoid, with cells becoming bigger towards apical cell, smooth, constricted at the septa.

Material examined – THAILAND, Phang Nga Province, Bann Tom Thong Khang, on decaying wood submerged in a freshwater stream, 17 December 2015, Kevin D. Hyde, Site 7-21-2 (MFLU 17-1974 **holotype**), ex-type living cultures, MFLUCC 16-0865, GZCC 17-0020.

Notes – *Mucispora phangngaensis* is the second species in the genus. It resembles *Acrogenospora ellipsoidea* D.M. Hu, L. Cai & K.D. Hyde, *Melanocephala triseptata* (Shearer, J.L. Crane & Miller) S. Hughes and *Mucispora obscuriseptata* in having cylindrical, percurrent proliferating conidiogenous cells, with dark brown, broadly ellipsoidal to obovoid conidia (Wu & Zhuang 2005, Hu et al. 2010, Yang et al. 2016). Conidia in *A. ellipsoidea* are non-septate, while in *Me. triseptata* and *Mucispora* they are septate with obvious septa when young and obscured septa when mature. *Mucispora obscuriseptata* is distinguished from *M. phangngaensis* in having a

Table 1 Isolates used in this study.

Species name	Source	GenBank numbers		References
		LSU	ITS	
Adelosphaeria catenata	CBS 138679	KT278707	KT278721	Réblová et al. 2016
Ascotaiwania fusiformis	MFLUCC 15-0621	KX550893	**MG388215**	Yang et al. 2016
Ascotaiwania fusiformis	MFLUCC 15-0625	KX550894	**MG388216**	Yang et al. 2016
Ascotaiwania mitriformis	HKUCC 3706	AF132324	–	Ranghoo et al. 1999
Ascotaiwania sawadae	SS 00051	HQ446363	HQ446340	Boonyuen et al. 2011
Bactrodesmiastrum monilioides	FMR 10756	KF771879	KF771878	Hernández-Restrepo et al. 2015
Bactrodesmiastrum obovatum	FMR 6482	FR870266	FR870264	Hernández-Restrepo et al. 2013
Bactrodesmiastrum pyriforme	FMR 10747	FR870265	FR870263	Hernández-Restrepo et al. 2013
Bactrodesmiastrum pyriforme	FMR 11931	HE646637	HE646636	Hernández-Restrepo et al. 2013
Brachysporiella setosa	HKUCC 3713	AF132334	–	Ranghoo et al. 1999
Canalisporium exiguum	SS 00809	GQ390281	GQ390296	Sri-indrasutdhi et al. 2010
Canalisporium grenadoideum	BCC 20507	GQ390267	NR_111442	Sri-indrasutdhi et al. 2010
Canalisporium pulchrum	SS 03982	GQ390277	GQ390292	Sri-indrasutdhi et al. 2010
Conioscypha lignicola	CBS 335.93	AY484513	–	Réblová and Seifert 2004
Conioscypha minutispora	FMR 11245	KF924559	NR_137847	Crous et al. 2014
Conioscypha peruviana	ILL 41202	KF781539	–	Zelski et al. 2015
Conioscypha varia	CBS 113653	AY484512	–	Réblová & Seifert 2004
Fuscosporella aquatica	MFLUCC 16-0859	**MG388209**	**MG388212**	This study
Fuscosporella pyriformis	MFLUCC 16-0570	KX550896	**MG388217**	Yang et al. 2016
Helicoön farinosum	DAOM 241947	JQ429230	JQ429145	Réblová et al. 2012
Leotia lubrica	AFTOL-ID 1	AY544644	DQ491484	Lutzoni et al. 2004
Melanotrigonum ovale	CBS 138742	KT278708	KT278723	Réblová et al. 2016
Melanotrigonum ovale	CBS 138743	KT278709	KT278724	Réblová et al. 2016
Microglossum rufum	AFTOL-ID 1292	DQ470981	–	Spatafora et al. 2006
Mucispora obscuriseptata	MFLUCC 15-0618	KX550892	**MG388218**	Yang et al. 2016
Mucispora phangngaensis	MFLUCC 16-0865	**MG388210**	**MG388213**	This study
Parafuscosporella moniliformis	MFLUCC 15-0626	KX550895	**MG388219**	Yang et al. 2016
Parafuscosporella mucosa	MFLUCC 16-0571	**MG388211**	**MG388214**	This study
Phaeoisaria fasciculata	CBS 127885	KT278705	KT278719	Réblová et al. 2016
Phaeoisaria fasciculata	DAOM 230055	KT278706	KT278720	Réblová et al. 2016
Phaeoisaria microspora	MFLUCC 16-0033	MF167351	MF671987	Hyde et al. 2017
Phaeoisaria sedimenticola	CGMCC 3.14949	JQ031561	JQ074237	Cheng et al. 2014
Phragmocephala stemphylioides	DAOM 673211	KT278717	KT278730	Réblová et al. 2016
Plagiascoma frondosum	CBS 139031	KT278713	–	Réblová et al. 2016
Pleurotheciella centenaria	DAOM 229631	JQ429234	JQ429151	Réblová et al. 2012
Pleurotheciella rivularia	CBS 125238	JQ429232	JQ429160	Réblová et al. 2012
Pleurotheciella rivularia	CBS 125237	JQ429233	JQ429161	Réblová et al. 2012
Pleurotheciella uniseptata	DAOM 673210	KT278716	KT278729	Réblová et al. 2012
Pleurothecium floriforme	MFLUCC 15-0628	KY697277	KY697281	Hyde et al. 2017
Pleurothecium recurvatum	CBS 131272	JQ429237	JQ429149	Réblová et al. 2012
Pleurothecium recurvatum	CBS 138747	KT278714	KT278728	Réblová et al. 2016
Pleurothecium semifecundum	CBS 131271	JQ429240	JQ429159	Réblová et al. 2012
Pleurothecium semifecundum	CBS 131482	JQ429239	JQ429158	Réblová et al. 2012
Pseudoascotaiwania persoonii	A57-14C	AY094190	–	Campbell & Shearer 2004
Pseudoascotaiwania persoonii	A57-14C	AY590295	–	Campbell & Shearer 2004
Savoryella longispora	SAT 00322	HQ446380	HQ446359	Boonyuen et al. 2011
Savoryella paucispora	SAT 00866	HQ446381	HQ446360	Boonyuen et al. 2011
Savoryella verrucosa	SS 00052	HQ446374	HQ446353	Boonyuen et al. 2011
Sterigmatobotrys macrocarpa	PRM 915682	GU017317	JQ429153	Réblová and Seifert 2011, Réblová et al. 2012
Sterigmatobotrys macrocarpa	DAOM 230059 = CBS 113468	GU017316	JQ429154	Réblová & Seifert 2011, Réblová et al. 2012
Taeniolella rudis	DAOM 229838	JQ429241	JQ429152	Réblová et al. 2012

Newly generated sequences are marked in **underlined bold** font, new species are indicated in **red bold**.

Table 2 Polymorphic nucleotide in the LSU and ITS regions for *Fuscosporella aquatica* and *F. pyrifomis*.

Species	LSU							ITS								
	4	98	210	471	487	562	650	71	109	121	386	434	435	463	515	558
F. aquatica	A	C	G	T	T	T	G	T	T	–	T	A	T	A	C	T
F. pyriformis	T	T	A	C	C	C	A	C	C	C	C	G	C	G	T	C

Isolate in blue highlight is newly taxon, position of nucleotide is based on the sequence alignment of *Fuscosporella aquatica* and *F. pyrifomis*.

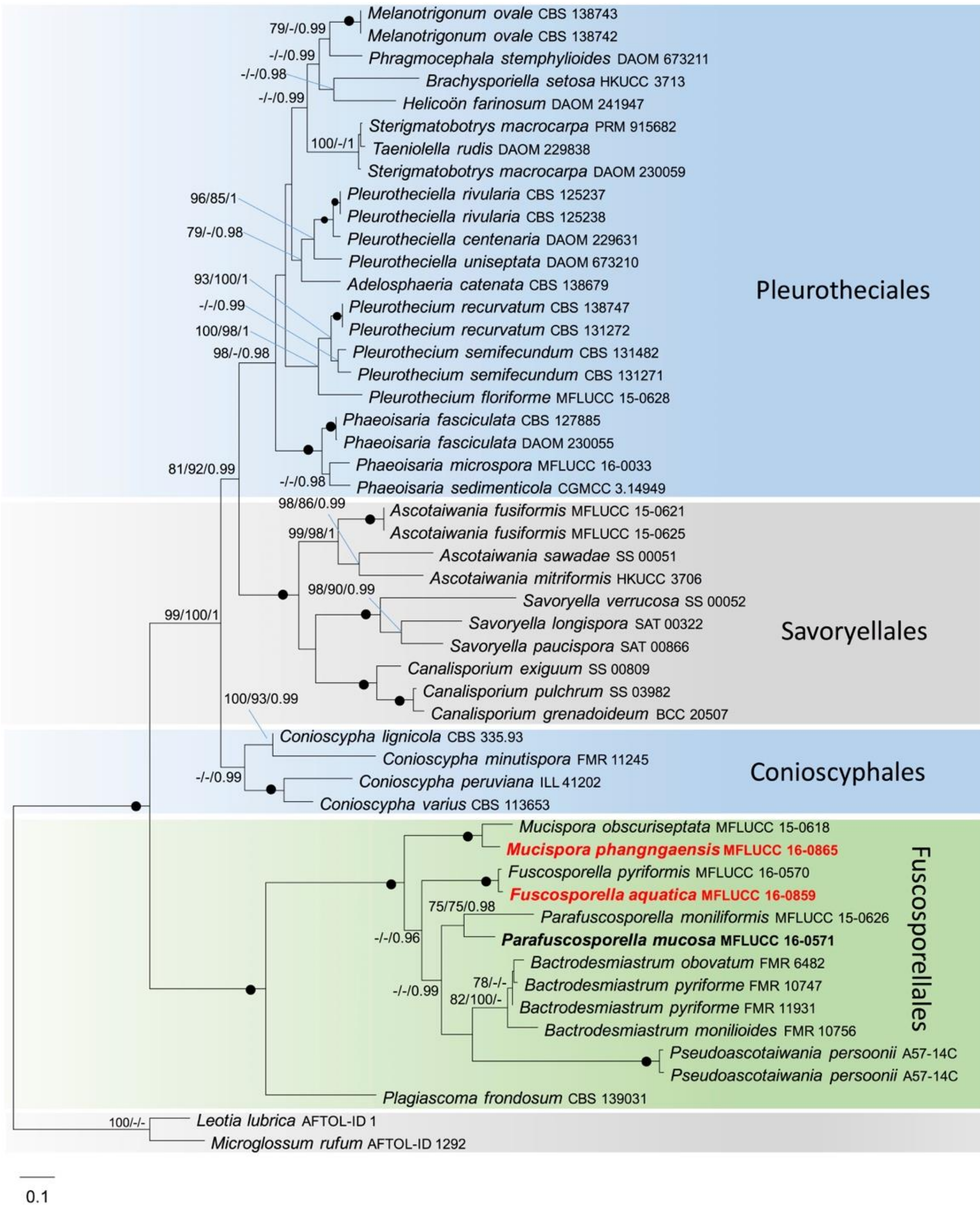

Figure 1 – Maximum likelihood majority rule consensus tree for the analyzed Hypocreomycetidae isolates based on a dataset of combined LSU and ITS sequence data. Bootstrap support values for maximum likelihood (ML) and maximum parsimony (MP) greater than 75% and Bayesian posterior probabilities greater than 0.95 are indicated above the nodes as MLBS/MPBS/PP. The scale bar represents the expected number of changes per site. The tree is rooted with *Leotia lubrica* and *Microglossum rufum*. The strain numbers are noted after the species names. The new strains are in red bold, newly generated strains are in black bold. Branches with 100% ML BS, 100% MP BS and 1.0 PP are shown as black nodes. Orders are indicated as coloured blocks.

conidial sheath. *Melanocephala triseptata* is distinctive with up to 10 proliferations. In addition, the conidiophores of *Mucispora phangngaensis* (170–305 µm long) are much longer than in *A. ellipsoidea* (87.5–162.5 µm long), *Me. triseptata* (100–250 µm long) and *M. obscuriseptata* (80–170 µm long). The phylogenetic analyses also confirmed that *Mucispora phangngaensis* and *M. obscuriseptata* are phylogenetically distinct species.

In this study, the second species in *Fuscosporella* and *Mucispora* are illustrated with morphological descriptions and phylogenetic support. Among the eleven taxa in Fuscosporellaceae, nine taxa are from aquatic habitats while two are terrestrial fungi. All the *Fuscosporella*, *Mucispora* and *Parafuscosporella* species were collected from freshwater habitats in Thailand. More detailed investigations may lead to the discovery of additional species in the order from Thailand or other countries.

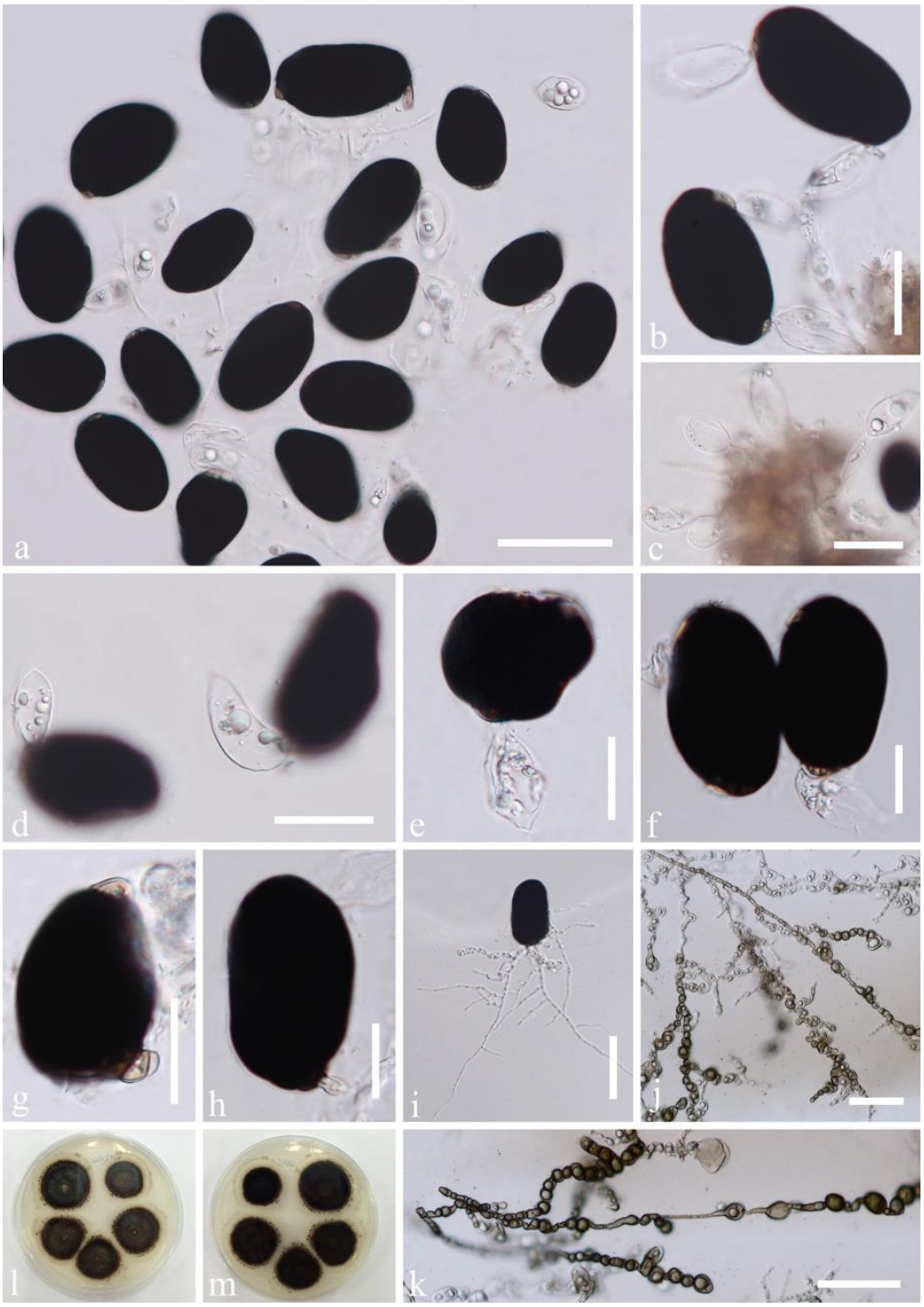

Figure 2 – *Fuscosporella aquatica* (MFLU 17-1973, holotype). a, g, h Conidia. b, c Conidiophores. d–f Conidiogenous cells with conidia. i Germinated conidium on PDA medium. j, k Hyphae on PDA medium. l, m Culture, l from above, m from below. Scale bars – a, i = 50 µm, b–d, j, k = 30 µm, e–h = 20 µm.

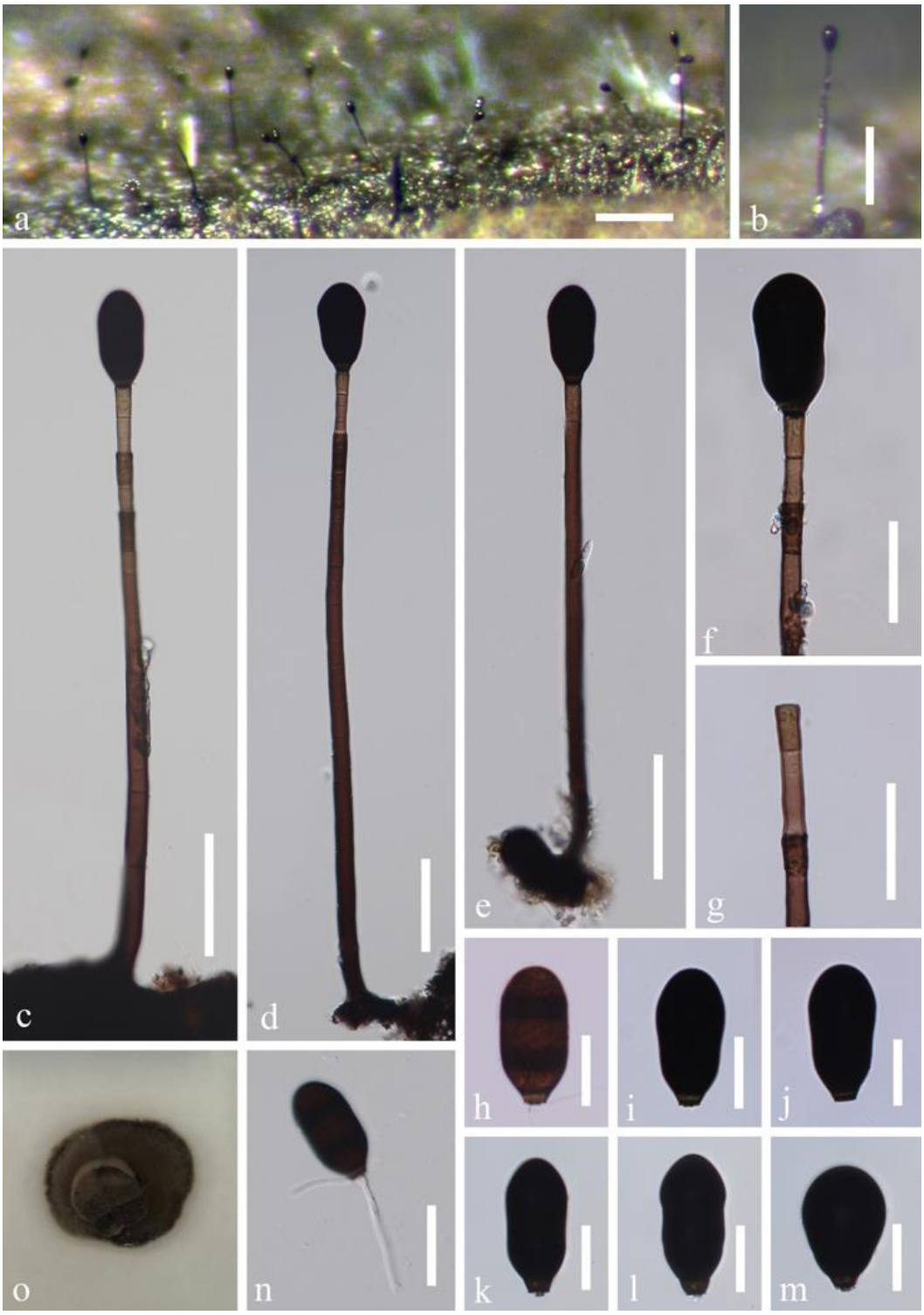

Figure 3 – *Mucispora phangngaensis* (MFLU 17-1974, holotype) a, b Colony on submerged wood. c–e Conidiophores with conidia. f Close up of the proliferation. g Apex of the conidiophore. h–m Conidia. n Germinated conidium on PDA. o Culture from above. Scale bars – a = 200 µm, b = 100 µm, c–e = 50 µm, f–g, n = 30 µm, h–m = 20 µm.

Figure 4 – Re-produced asexual morph of *Mucispora phangngaensis* on PDA medium. a, b Hyphae and conidia. c End of hyphae. d, e Conidia. Scale bars – a, b = 50 µm, c–e = 20 µm.

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