



# Freshwater Dothideomycetes

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## Abstract

Freshwater Dothideomycetes are a highly diverse group of fungi, which are mostly saprobic in freshwater habitats worldwide. They are important decomposers of submerged woody debris and leaves in water. In this paper, we outline the genera of freshwater Dothideomycetes with notes and keys to species. Based on multigene analyses and morphology, we introduce nine new genera, viz. *Aquimassariosphaeria*, *Aquatospora*, *Aquihelicascus*, *Fusiformiseptata*, *Neohelicascus*, *Neojahnula*, *Pseudojahnula*, *Purpureofaciens*, *Submersispora*; 33 new species, viz. *Acrocallymma bipolare*, *Aquimassariosphaeria kunmingensis*, *Aquatospora cylindrica*, *Aquihelicascus songkhlaensis*, *A. yunnanensis*, *Ascagilis submersa*, *A. thailandensis*, *Bambusicola aquatica*, *Caryospora submersa*, *Dictyocheiropsora thailandica*, *Fusiformiseptata crocea*, *Helicosporium thailandense*, *Hongkongmyces aquaticus*, *Lentistoma aquaticum*, *Lentithecium kunmingense*, *Lindgomyces aquaticus*, *Longipedicellata aquatica*, *Neohelicascus submersus*, *Neohelicomyces dehongensis*, *N. thailandicus*, *Neohelicosporium submersum*, *Nigrograna aquatica*, *Occultibambusa kunmingensis*, *Parabambusicola aquatica*, *Pseudoasteromassaria aquatica*, *Pseudoastrophaeriella aquatica*, *Pseudoxylomyces aquaticus*, *Purpureofaciens aquatica*, *Rousoella aquatica*, *Shrungabeeja aquatica*, *Submersispora variabilis*, *Tetraploa puzheheiensis*, *T. yunnanensis*; 16 new combinations, viz. *Aquimassariosphaeria typhicola*, *Aquihelicascus thalassioideus*, *Ascagilis guttulaspora*, *A. queenslandica*, *A. seychellensis*, *A. sunyatsenii*, *Ernakulamia xishuangbannaensis*, *Neohelicascus aquaticus*, *N. chiangraiensis*, *N. egyptiacus*, *N. elaterascus*, *N. gallicus*, *N. unilocularis*, *N. uniseptatus*, *Neojahnula australiensis*, *Pseudojahnula potamophila*; 17 new geographical and habitat records, viz. *Aliquandostipite khaoyaiensis*, *Aquastroma magniostiolata*, *Caryospora aquatica*, *C. quercus*, *Dendryphiella vinosa*, *Ernakulamia cochinchinensis*, *Fissuroma neoaggregatum*, *Helicotruncatum palmigenum*, *Jahnula rostrata*, *Neoroussoella bambusae*, *N. leucaenae*, *Occultibambusa pustula*, *Paramonodictys solitarius*, *Pleopunctum pseudoellipsoideum*, *Pseudocapulatispora longiappendiculata*, *Seriascoma didymosporum*, *Shrungabeeja vadirajensis* and ten new collections from China and Thailand, viz. *Amniculicola guttulata*, *Aquaphila albicans*, *Berkleasium latisporum*, *Clohesyomyces aquaticus*, *Dictyocheiropsora rotunda*, *Flabellascoma fusiforme*, *Pseudoastrophaeriella bambusae*, *Pseudoxylomyces elegans*, *Tubeufia aquatica* and *T. cylindrothecia*. *Dendryphiella phitsanulokensis* and *Tubeufia roseohelicospora* are synonymized with *D. vinosa* and *T. tectonae*, respectively. Six orders, 43 families and 145 genera which belong to freshwater Dothideomycetes are reviewed. Of these, 46 genera occur exclusively in freshwater habitats. A world map illustrates the distribution of freshwater Dothideomycetes.

**Keywords** 42 new taxa · Freshwater distribution · Phylogeny · Submerged wood · Taxonomy

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## Introduction

Shearer (1993a) reviewed the freshwater ascomycota and provided a definition and methods to study their taxonomy, systematics, geographical distribution, ecology, and evolution. This definition and the study techniques have since commonly been used (e.g., Zhang et al. 2017a; Dong et al.

2018; Lu et al. 2018b; Luo et al. 2019; Yang et al. 2019b). Unlike other fungi, which can be often traced to a specific host, freshwater fungi have mostly been collected from “submerged wood” (Wei et al. 2018; Yu et al. 2018; Wang et al. 2019). Thus, there has been little research on the hosts of freshwater fungi. The geographical distribution of freshwater fungi is also unclear and little can be concluded as distribution patterns are still largely based on the locations of mycologists (Shearer 1993a); thus further work are needed in these areas.

Dothideomycetes and Sordariomycetes, the largest classes of Ascomycota, have been comprehensively reviewed (e.g., Hyde et al. 2013, 2020c; Maharachchikumbura et al. 2016; Hongsanan et al. 2020a, b). Some websites, e.g. (<http://fungi.life.illinois.edu/>) (Shearer and Raja 2010), (<https://www.marinefungi.org/>) (Jones et al. 2019), (<https://www.dothideomycetes.org/>) (Pem et al. 2019a) and (<https://www.freshwaterfungi.org/>) (Calabon et al. 2020), are devoted to taxonomy and classification of Dothideomycetes, provide a database of freshwater ascomycetes and online resources for marine fungi. There has been an increased interest in the taxonomy and phylogeny of freshwater fungi (Lu et al. 2018b; Yang et al. 2018; Bao et al. 2019b, c; Boonmee et al. 2019), but further research will be carried out. Luo et al. (2019) gave accounts of freshwater Sordariomycetes and provided a modified backbone tree and detailed information on distribution, holotypes, sequence data and sexual-asexual morph connections of taxa. They also introduced 47 new taxa based on fresh collections from China and Thailand, which indicated there is a high diversity of freshwater Sordariomycetes.

In this article, a comprehensive study on freshwater Dothideomycetes is carried out based on new collections from China, Egypt and Thailand, as well as studies of specimens in fungal herbaria and literature reviews. Multigene phylogenetic analyses and morphological studies have resulted in nine new genera, 33 new species, 16 new combinations and 17 new geographical and habitat records being reported. Considering that many freshwater fungi are scattered in Dothideomycetes and the study of freshwater fungi have greatly advanced, there is a need to revise all freshwater Dothideomycetes with updated knowledge, and to provide a better understanding of their morphology and phylogeny. This monograph on freshwater Dothideomycetes provides the latest generic concepts, descriptions, illustrations and keys to genera and species. Using data from this study, we present several common morphological traits that appear to be adaptations of microfungi that live in flowing freshwater. Additionally, the global distribution of freshwater

Dothideomycetes is reviewed in this study to show the poorly studied areas.

## Materials and methods

### Sample collection and specimen examination

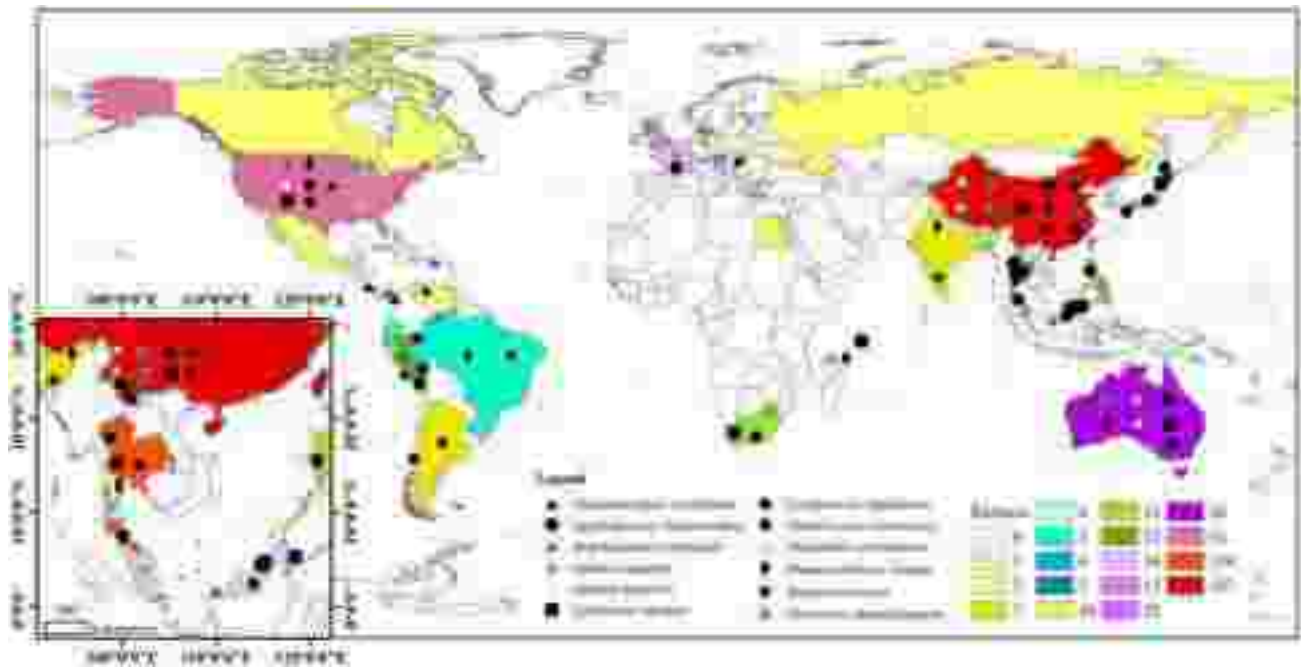
Submerged wood was mostly collected from lentic or lotic streams, lakes or rivers in China and Thailand as part of a north-south survey (Hyde et al. 2016a), and from the river Nile in Egypt between 2010 to 2019. The principle of processing samples, including collection, incubation, isolation and examination, following Shearer (1993a). The detailed procedure of single spore isolation follows Chomnunti et al. (2014). Pseudoparaphyses are observed at 100× using a Nikon ECLIPSE Ni compound microscope fitted with a Canon EOS 600D digital camera. Two types of pseudoparaphyses, cellular and trabeculate, are defined if they are clearly seen. Herbarium specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, Thailand and the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (KUN-HKAS), Kunming, China. Living cultures are deposited in the Mae Fah Luang University Culture Collection (MFLUCC), Chiang Rai, Thailand and Kunming Institute of Botany culture collection (KUMCC), Kunming, China. Facesoffungi and Index Fungorum numbers are registered as in Jayasiri et al. (2015) and Index Fungorum (2020).

### DNA extraction, PCR amplification and sequencing

Cultures were grown on PDA at room temperature (25–27 °C) and a Biospin Fungus Genomic DNA Extraction Kit (Bioer Technology Co., Hangzhou, P.R. China) was used to extract total genomic DNA from the fresh mycelium according to the manufacturer’s instructions. Fragments of five loci were amplified with polymerase chain reaction (PCR). Primer pairs LR0R/LR5, NS1/NS4, ITS5/ITS4, EF1-983F/EF1-2218R and fRPB2-5F/fRPB2-7cR are used for LSU, SSU, ITS, TEF and RPB2, respectively. The amplifications were carried out using the method described by Zhang et al. (2017a). The PCR products were viewed on 1% agarose electrophoresis gels stained with ethidium bromide. Sequencing of five loci were carried out by Shanghai Sangon Biological Engineering Technology & Services Co. Shanghai, P.R. China.

### Phylogenetic analysis

The sequences generated in this study were supplemented with other Dothideomycetous sequences obtained from GenBank, based on blast searches and relevant literatures.



**Fig. 1** World distribution of freshwater Dothideomycetes. Twelve morphologically identified freshwater species which occur worldwide are shown with different symbols on the map. The number of

freshwater dothideomycetous species in each country is shown with different colours, which indicates “richness” from zero to almost 150 species (in China)

The accession numbers used in the multigene analyses were provided in supplementary material 1 and newly obtained strains with their accession numbers were shown in supplementary material 2. Multiple sequence alignments were generated with MAFFT v. 7 (<https://mafft.cbrc.jp/alignment/server/index.html>) (Kuraku et al. 2013; Katoh et al. 2019). The alignments were visually improved with BioEdit (Hall 1999). Phylogenetic analyses of the combined aligned dataset consisted of maximum likelihood (ML) and Bayesian inference (BI). Maximum likelihood (ML) analysis was performed at the CIPRES Science Gateway v.3.3 (<https://www.phylo.org/portal2/home.action>) (Miller et al. 2010). The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR-GAMMA substitution model. The best-fit models for the Bayesian analyses were selected with MrModeltest v. 2.2 (Nylander 2004). The best-fit model GTR+I+G for LSU, ITS and RPB2, and HKY+I+G for TEF. Six simultaneous Markov chains were run for one million generations and trees were sampled every 100th generation and 10000 trees were obtained. The first 2500 trees representing the burn-in phase of the analyses were discarded, while the remaining 7500 trees were used for calculating posterior probabilities in the majority rule consensus tree. Phylogenetic tree was visualized using FigTree v1.4.0 (<http://tree.bio.ed.ac.uk/software/figtree/>, Rambaut 2012). Sequences derived in this study are deposited in GenBank.

## Results

### Phylogenetic analysis of combined LSU, ITS, TEF and RPB2 sequence data

The combined LSU, ITS, TEF and RPB2 sequence dataset were employed for species of Dothideomycetes. The alignment comprised 714 strains with an alignment length of 5882 total characters including gaps. The RAxML analysis resulted in a best scoring likelihood tree selected with a final value for the combined dataset in  $L = -216610.299349$ . The matrix has 4462 distinct alignment patterns, with 70.36% of undetermined characters or gaps. Estimated base frequencies are as follows: A = 0.238070, C = 0.259088, G = 0.278997, T = 0.223845; substitution rates AC = 1.423061, AG = 3.547230, AT = 1.643052, CG = 1.083323, CT = 7.578123, GT = 1.000000; gamma distribution shape parameter  $a = 0.439170$  (Fig. 1).

### Taxonomy

In this section, each freshwater genus is treated with a generic description and notes. A list with freshwater distribution and a key to freshwater species within each genus are provided. A key to freshwater genera within each family is also provided. An illustration of new species, new collections or line-drawings is provided after each entry, where possible. The freshwater distribution mainly follows an online resource for freshwater fungi

(<http://fungi.life.illinois.edu/>) and published papers. The species is marked with an asterisk if there are published sequences deposited in GenBank. Freshwater Dothideomycetes are distributed in six orders, viz. Jahnulales, Kirschsteinioteliales, Minutisphaerales, Natipusillales, Pleosporales and Tubeufiales; a few are scattered in Dothideomycetes families/genera *incertae sedis*. Classification and list of freshwater Dothideomycetes are shown in Table 1.

**\*Denotes species having sequence data in GenBank**

**Jahnulales** K.L. Pang, Abdel-Wahab, El-Shar., E.B.G. Jones & Sivichai, Mycol. Res. 106(9): 1033 (2002)

**Aliquandostipitaceae** Inderb., Am. J. Bot. 88(1): 54 (2001)

**Key to freshwater sexual genera of Aliquandostipitaceae**

1. Ascomata hyaline or slightly pigmented.....2
1. Ascomata dark.....3
2. Ascospores with longitudinal sulcate striations, without appendages or sheath.....*Megalohypha*
2. Ascospores lacking longitudinal sulcate striations, often with a large sheath.....*Aliquandostipite*
3. Peridium composed of single row of cells...*Neojahnula*
3. Peridium not as above.....4
4. Ascospores without sheath or appendage.....*Jahnula*
4. Ascospores with sheath or appendage.....5
5. Ascospores surrounded by a wavy mucilaginous sheath.....*Pseudojahnula*
5. Ascospores mostly with a refractive mucilaginous pad at each end.....*Ascagilis*

*Aliquandostipite* Inderbitzin, Am. J. Bot. 88(1): 54 (2001)

*Saprobic* on submerged wood or fallen, decorticated branch. **Sexual morph:** *Mycelium* visible on the substratum, of wide hyphae (> 10 µm and up to 50 µm wide), which may bear ascomata. *Ascomata* scattered to clustered or gregarious, immersed to erumpent or superficial, uniloculate, globose to broadly ellipsoidal, dimorphic, with or without stalk-like hypha attached to the base, hyaline or pale brown when young, turn to dark brown with age, membranous, papillate. *Peridium* thin, one-layered, composed of pale brown, thin-walled cells of *textura angularis* to *globosa*. *Pseudoparaphyses* numerous, cellular, sparsely branched, hyaline, septate. *Asci* 8-spored, bitunicate, fissitunicate, clavate, with thickened apical region. *Ascospores* bi- to multi-seriate, variably arranged, oval, pale brown, 1-septate, constricted at the septum, usually asymmetric, mostly with a well-developed hyaline sheath (Inderbitzin et al. 2001). **Asexual morph:** Undetermined.

*Type species: Aliquandostipite khaoyaiensis* Inderb.

*Notes:* *Aliquandostipite* was introduced to accommodate two species *A. khaoyaiensis* Inderb. and *A. sunyatsenii* Inderb., which form dimorphic ascomata (sessile and stalked) and the widest hyphae known from ascomycetes (Inderbitzin et al. 2001). However, with *Jahnula* sequences added in the phylogenetic tree, *A. sunyatsenii* clustered in *Jahnula* with high bootstrap support, which was therefore transferred to *Jahnula* based on limited data (Pang et al. 2002). Among six species recorded in Index Fungorum (2020), four have been confirmed in *Aliquandostipite* with molecular data (Huang et al. 2018; Hyde et al. 2019). *Aliquandostipite* is similar to *Jahnula*, but can be distinguished from the latter by its hyaline or less pigmented ascomata and larger asci and ascospores.

According to our observations and literature, the ascospores of *A. crystallinus* Raja et al., *A. khaoyaiensis* and *A. minuta* Raja & Shearer form acicular crystals when stored in lactic acid and glycerin (Raja et al. 2005; Raja and Shearer 2007). These three taxa, however, can be distinguished based on the gelatinous sheath and appendages on ascospores. *Aliquandostipite siamensiae* (Sivichai & E.B.G. Jones) J. Campb. et al. is the only species producing dimorphic ascospores. *Aliquandostipite manochiae* Sri-indr. et al. is distinct in that it has branched and anastomosing pseudoparaphyses, lacking arcicular crystals within the spores, and ascospore size (Liu et al. 2015). *Aliquandostipite manochiae* might be synonymous with *A. khaoyaiensis* (Inderbitzin et al. 2001) based on their similar morphological features, overlapping dimension of ascomata, asci, and ascospores, and their freshwater habitats. Molecular data are necessary to confirm *A. manochii* as a unique species (Fig. 2).

**List of freshwater Aliquandostipite species**

\**Aliquandostipite crystallinus* Raja, A. Ferrer & Shearer, Mycotaxon 91: 208 (2005); Fig. 3e, g–i

*Freshwater distribution:* Costa Rica (Raja et al. 2005), Panama (Raja et al. 2005), Peru (Shearer et al. 2015), USA (Raja et al. 2005, 2009b), Venezuela (Raja et al. 2005)

\**Aliquandostipite khaoyaiensis* Inderbitzin, Am. J. Bot. 88: 54 (2001)

*Facesoffungi number:* FoF09158; Figs. 3a–d, f, 4

*Freshwater distribution:* Costa Rica (Raja et al. 2005), USA (Raja et al. 2009b), Thailand (this study)

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* 220–240 µm high, 190–210 µm diam., hyaline to pale brown, become dark brown with age, scattered, superficial, sometimes seated in a pseudostroma, globose or subglobose, sessile, membranous, with ostiolate papilla. *Peridium* 25–40 µm thick, membranous, composed of thin-walled, pale brown, compressed cells of *textura angularis*. *Pseudoparaphyses* 2

**Table 1** Classification and list of freshwater Dothideomycetes

Order	Family	Genus (number of freshwater species)	Species treated in this study	Notes	
<b>Jahnulales</b>	<b>Aliquandostipitaceae</b>	<b>Aliquandostipite</b> (5)	<i>Al. khaoyaiensis</i>	<i>New geographical record</i>	
		<i>Ascagilis</i> ^ (7)	<i>As. guttulaspora</i>	<i>New combination</i>	
			<i>As. queenslandica</i>	<i>New combination</i>	
			<i>As. seychellensis</i>	<i>New combination</i>	
			<i>As. submersa</i>	<i>New species</i>	
			<i>As. sunyatsenii</i>	<i>New combination</i>	
			<i>As. thailandensis</i>	<i>New species</i>	
			<i>Brachiosphaera</i> ^ (2)		
			<i>Jahnula</i> ^ (11)	<i>J. rostrata</i>	<i>New geographical record</i>
			<i>Megalohypha</i> ^ (1)		
			<i>Neojahnula</i> ^ (1)		<i>New genus</i>
				<i>N. australiensis</i>	<i>New combination</i>
			<i>Pseudojahnula</i> ^ (1)		<i>new genus</i>
		<i>P. potamophila</i>	<i>new combination</i>		
		<i>Xylomyces</i> (5)			
<b>Kirschsteiniotheliales</b>	<b>Kirschsteiniotheliaceae</b>	<b>Kirschsteiniothelia</b> (6)			
<b>Minutisphaerales</b>	<b>Acrogenosporaceae</b>	<b>Acrogenospora</b> (13)			
	<b>Minutisphaeraceae</b>	<b>Minutisphaera</b> ^ (5)			
<b>Natipusillales</b>	<b>Natipusillaceae</b>	<b>Natipusilla</b> ^ (4)			
<b>Pleosporales</b>	<b>Acrocallymaceae</b>	<b>Acrocallymma</b> (2)	<i>A. bipolare</i>	<i>New species</i>	
	<b>Aigialaceae</b>	<b>Fissuroma</b> (1)	<i>F. neoaggregatum</i>	<i>New habitat record</i>	
		<b>Neoastrophaeriella</b> (1)			
		<b>Amniculicolaceae</b>	<b>Amniculicola</b> (6)	<i>A. guttulata</i>	<i>New collection</i>
		<b>Murispora</b> (4)			
		<b>Neomassariosphaeria</b> ^ (1)			
		<b>Vargamyces</b> ^ (1)			
		<b>Anteagloniaceae</b>	<b>Flammeascoma</b> (1)		
			<b>Purpureofaciens</b> ^ (1)		<i>New genus</i>
				<i>P. aquatica</i>	<i>New species</i>
		<b>Aquasubmersaceae</b>	<b>Aquasubmersa</b> (2)		
		<b>Astrophaeriellaceae</b>	<b>Aquatospora</b> ^ (1)		<i>New genus</i>
				<i>A. cylindrica</i>	<i>New species</i>
		<b>Astrophaeriella</b> (7)			
		<b>Caryospora</b> (4)	<i>C. aquatica</i>	<i>New geographical record</i>	
			<i>C. quercus</i>	<i>New habitat record</i>	
			<i>C. submersa</i>	<i>New species</i>	
		<b>Pithomyces</b> (1)			
		<b>Xenoastrophaeriella</b> (2)			
	<b>Bambusicolaceae</b>	<b>Bambusicola</b> (1)	<i>B. aquatica</i>	<i>New species</i>	
	<b>Corynesporascaceae</b>	<b>Corynespora</b> (3)			
	<b>Delitschiaceae</b>	<b>Delitschia</b> (3)			
	<b>Dictyosporiaceae</b>	<b>Aquadictyospora</b> ^ (1)			
		<b>Aquaticheirospora</b> ^ (1)			
		<b>Cheirosporium</b> ^ (2)			
		<b>Dendryphiella</b> (1)	<i>De. vinosa</i>	<i>New habitat record</i>	
				<i>New synonym</i>	
		<b>Dictyocheirospora</b> (9)	<i>Di. rotunda</i>	<i>New collection</i>	
			<i>Di. thailandica</i>	<i>New species</i>	
		<b>Dictyosporium</b> (11)			

Table 1 (continued)

Order	Family	Genus (number of freshwater species)	Species treated in this study	Notes
		<i>Digitodesmium</i> (3)		
		<i>Jalapriya</i> (1)		
		<i>Pseudocoleophoma</i> (1)		
		<i>Pseudodictyosporium</i> (1)		
		<i>Vikalpa</i> (1)		
	<b>Didymosphaeriaceae</b>	<i>Didymosphaeria</i> (1)		
		<i>Paraphaeosphaeria</i> (2)		
		<i>Paraconiothyrium</i> (1)		
		<i>Pseudotrichia</i> (1)		
	<b>Latoruaceae</b>	<i>Pseudoasteromassaria</i> (2)	<i>P. aquatica</i>	<i>New species</i>
	<b>Lentitheciaceae</b>	<i>Keissleriella</i> (1)		
		<i>Lentithecium</i> (6)	<i>L. kunmingense</i>	<i>New species</i>
		<i>Poaceascoma</i> (1)		
		<i>Setoseptoria</i> (2)		
		<i>Tingoldiogo</i> <sup>^</sup> (3)		
	<b>Lindgomycetaceae</b>	<i>Arundellina</i> (1)		
		<i>Clohesyomyces</i> <sup>^</sup> (1)	<i>C. aquaticus</i>	<i>New collection</i>
		<i>Hongkongmyces</i> (2)	<i>H. aquaticus</i>	<i>New species</i>
		<i>Aquimassariosphaeria</i> (2)		<i>New genus</i>
			<i>A. kunmingensis</i>	<i>New species</i>
			<i>A. typhicola</i>	<i>New combination</i>
		<i>Lindgomyces</i> <sup>^</sup> (14)	<i>L. aquaticus</i>	<i>New species</i>
		<i>Lolia</i> <sup>^</sup> (2)		
		<i>Neolindgomyces</i> (1)		
	<b>Longipedicellataceae</b>	<i>Longipedicellata</i> (2)	<i>L. aquatica</i>	<i>New species</i>
		<i>Pseudoxylomyces</i> <sup>^</sup> (2)	<i>P. aquaticus</i>	<i>New species</i>
			<i>P. elegans</i>	<i>New collection</i>
		<i>Submersispora</i> <sup>^</sup> (1)		<i>New genus</i>
			<i>S. variabilis</i>	<i>New species</i>
	<b>Lophiostomataceae</b>	<i>Biappendiculispora</i> (1)		
		<i>Crassiclypeus</i> <sup>^</sup> (1)		
		<i>Pseudocapulatispora</i> (1)	<i>P. longiappendiculata</i>	<i>New habitat and geographical record</i>
		<i>Flabellascoma</i> (1)	<i>F. fusiforme</i>	<i>New collection</i>
		<i>Lentistoma</i> (2)	<i>L. aquaticum</i>	<i>New species</i>
		<i>Lophiostoma</i> (6)		
		<i>Neotrematosphaeria</i> <sup>^</sup> (1)		
		<i>Neovaginatisspora</i> (1)		
		<i>Sigarispora</i> (1)		
		<i>Vaginatisspora</i> (2)		
	<b>Lophiotremataceae</b>	<i>Lophiotrema</i> (1)		
	<b>Massarinaceae</b>	<i>Byssothecium</i> (2)		
		<i>Helminthosporium</i> (2)		
	<b>Melanommataceae</b>	<i>Byssosphaeria</i> (1)		
		<i>Camposporium</i> (6)		
		<i>Herpotrichia</i> (1)		
		<i>Mamillisphaeria</i> <sup>^</sup> (1)		
		<i>Melanomma</i> (1)		
	<b>Morosphaeriaceae</b>	<i>Aquihelicascus</i> <sup>^</sup> (3)		<i>New genus</i>

**Table 1** (continued)

Order	Family	Genus (number of freshwater species)	Species treated in this study	Notes
			<i>A. songkhlaensis</i>	<i>New species</i>
			<i>A. thalassioideus</i>	<i>New combination</i>
			<i>A. yunnanensis</i>	<i>New species</i>
		<i>Neohelicascus</i> <sup>^</sup> (8)		<i>New genus</i>
			<i>Ne. aquaticus</i>	<i>New combination</i>
			<i>Ne. chiangraiensis</i>	<i>New combination</i>
			<i>Ne. egyptiacus</i>	<i>New combination</i>
			<i>Ne. elaterascus</i>	<i>New combination</i>
			<i>Ne. gallicus</i>	<i>New combination</i>
			<i>Ne. submersus</i>	<i>New species</i>
			<i>Ne. unilocularis</i>	<i>New combination</i>
			<i>Ne. uniseptatus</i>	<i>New combination</i>
	<b>Nigrogranaceae</b>	<i>Nigrograna</i> (2)	<i>Ni. aquatica</i>	<i>New species</i>
	<b>Occultibambusaceae</b>	<i>Occultibambusa</i> (3)	<i>O. kunmingensis</i>	<i>New species</i>
			<i>O. pustula</i>	<i>New habitat and geographical record</i>
		<i>Seriascoma</i> (1)	<i>S. didymosporum</i>	<i>New habitat and geographical record</i>
	<b>Parabambusicolaceae</b>	<i>Aquastroma</i> (1)	<i>A. magniostiolata</i>	<i>New geographical record</i>
		<i>Parabambusicola</i> (1)	<i>Parab. aquatica</i>	<i>New species</i>
		<i>Paramonodictys</i> (1)	<i>Param. solitarius</i>	<i>New habitat and geographical record</i>
	<b>Periconiaceae</b>	<i>Periconia</i> (6)		
	<b>Phaeoseptaceae</b>	<i>Phaeoseptum</i> (1)		
		<i>Pleopunctum</i> (1)	<i>Pl. pseudoellipsoideum</i>	<i>New habitat record</i>
	<b>Pleomassariaceae</b>	<i>Splanchnonema</i> (1)		
	<b>Pleomonodictydaceae</b>	<i>Pleomonodictys</i> (1)		
	<b>Pseudoastrospheariellaceae</b>	<i>Pseudoastrospheariella</i> (5)	<i>Ps. aquatica</i>	<i>New species</i>
			<i>Ps. bambusae</i>	<i>New collection</i>
	<b>Roussoellaceae</b>	<i>Roussoella</i> (3)	<i>R. aquatica</i>	<i>New species</i>
		<i>Neoroussoella</i> (2)	<i>N. bambusae</i>	<i>New habitat and geographical record</i>
			<i>N. leucaenae</i>	<i>New habitat and geographical record</i>
	<b>Testudinaceae</b>	<i>Angustospora</i> <sup>^</sup> (1)		
	<b>Tetraplosphaeriaceae</b>	<i>Ernakulamia</i> (2)	<i>E. cochinchensis</i>	<i>New habitat and geographical record</i>
			<i>E. xishuangbannaensis</i>	<i>New combination</i>
		<i>Shrungabeeja</i> (2)	<i>S. vadirajensis</i>	<i>New habitat and geographical record</i>
			<i>S. aquatica</i>	<i>New species</i>
		<i>Tetraploa</i> (6)	<i>T. puzheheiensis</i>	<i>New species</i>
			<i>T. yunnanensis</i>	<i>New species</i>
		<i>Triplosphaeria</i> (1)		
	<b>Torulaceae</b>	<i>Dendryphion</i> (4)		
		<i>Neotorula</i> <sup>^</sup> (2)		
		<i>Rostriconidium</i> <sup>^</sup> (1)		
		<i>Torula</i> (4)		
	<b>Trematosphaeriaceae</b>	<i>Falciformispora</i> (3)		

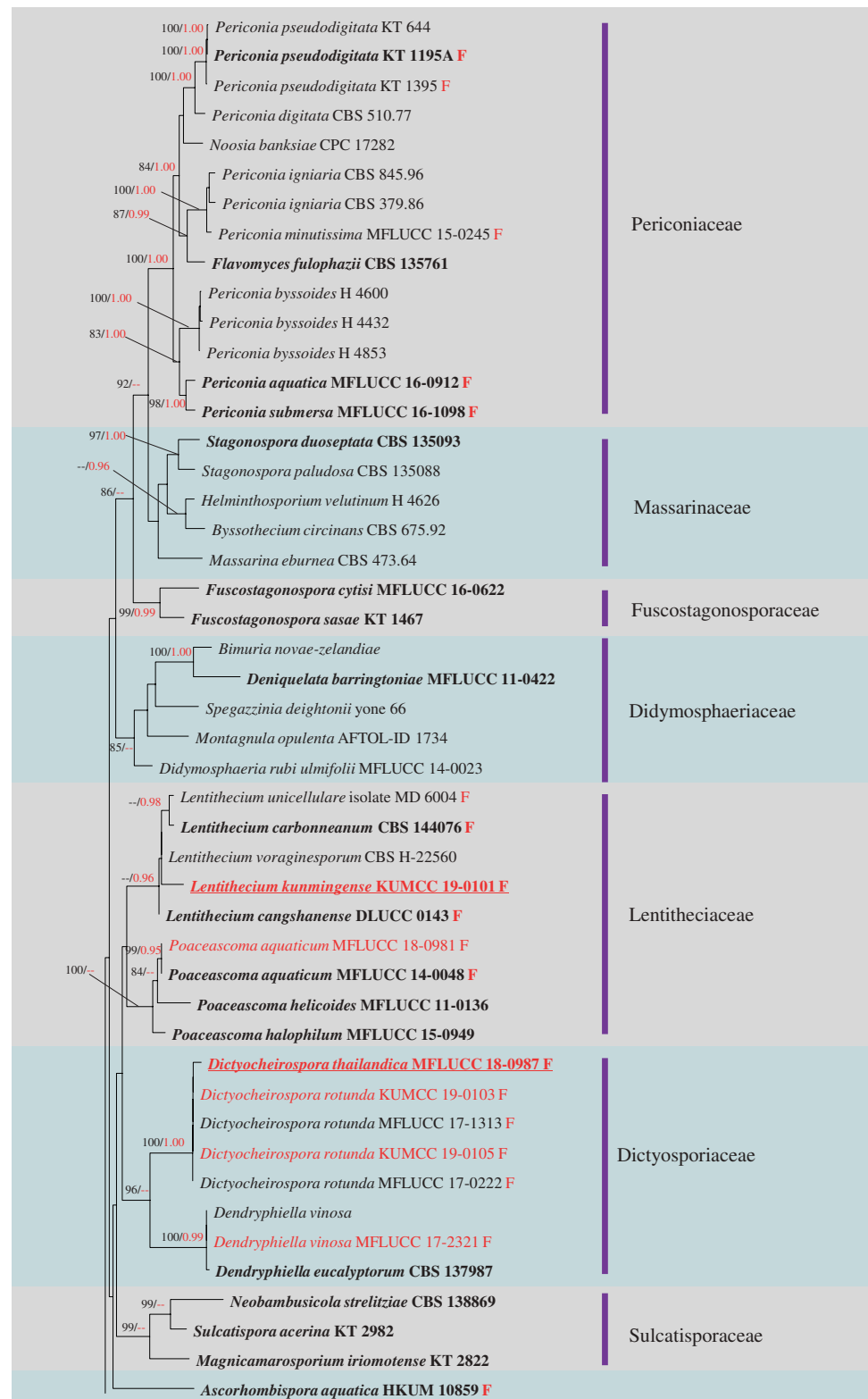
**Table 1** (continued)

Order	Family	Genus (number of freshwater species)	Species treated in this study	Notes	
Pleosporales genera <i>incertae sedis</i>	Wicklowiaceae	<i>Hadrospora</i> (1)			
		<i>Trematosphaeria</i> (4)			
		<i>Wicklowia</i> <sup>^</sup> (2)			
		<i>Ascorhombispora</i> <sup>^</sup> (1)			
		<i>Fusiformiseptata</i> <sup>^</sup> (1)	<i>F. crocea</i>	<i>New genus</i> <i>New species</i>	
Tubeufiales	Tubeufiaceae	<i>Isthmosporella</i> <sup>^</sup> (1)			
		<i>Rebentischia</i> (1)			
		<i>Aquaphila</i> (1)	<i>A. albicans</i>	<i>New collection</i>	
		<i>Berkleasmium</i> (5)	<i>B. latisporum</i>	<i>New collection</i>	
		<i>Boerlagiomyces</i> (3)			
		<i>Chlamydotubeufia</i> (4)			
		<i>Dematiohelicomycetes</i> <sup>^</sup> (1)			
		<i>Dictyospora</i> <sup>^</sup> (1)			
		<i>Helicoarctatus</i> <sup>^</sup> (1)			
		<i>Helicodochium</i> <sup>^</sup> (2)			
		<i>Helicohyalinum</i> <sup>^</sup> (2)			
		<i>Helicoma</i> (8)			
		<i>Helicomycetes</i> (5)			
		<i>Helicosporium</i> (6)	<i>Helicos. thailandense</i>	<i>New species</i>	
		<i>Helicotruncatum</i> (1)	<i>Helicot. palmigenum</i>	<i>New habitat and geographical record</i>	
		<i>Helicotubeufia</i> (2)			
		<i>Muripulchra</i> <sup>^</sup> (1)			
		<i>Neochlamydotubeufia</i> (2)			
		<i>Neohelicomyces</i> (6)	<i>Neohelicom. dehongensis</i>	<i>New species</i>	
			<i>Neohelicom. thailandicus</i>	<i>New species</i>	
		<i>Neohelicosporium</i> (11)	<i>Neohelicos. submersum</i>	<i>New species</i>	
		<i>Neotubeufia</i> <sup>^</sup> (1)			
		<i>Pseudohelicomyces</i> (3)			
<i>Pseudohelicoon</i> <sup>^</sup> (2)					
<i>Thaxteriellopsis</i> (1)					
<i>Tubeufia</i> (29)	<i>T. aquatica</i>	<i>New collection</i>			
	<i>T. cylindrothecia</i>	<i>New collection</i>			
	<i>T. tectonae</i>	<i>New synonym</i>			
Dothideomycetes families <i>incertae sedis</i>	Wiesneriomycetaceae	<i>Wiesneriomyces</i> (1)			
	Argynnaceae	<i>Lepidopterella</i> <sup>^</sup> (2)			
	Pseudorobillardaceae	<i>Pseudorobillarda</i> (1)			
	Dothideomycetes genera <i>incertae sedis</i>		<i>Alascospora</i> <sup>^</sup> (1)		
			<i>Ascominuta</i> <sup>^</sup> (2)		
			<i>Lucidascocarpa</i> <sup>^</sup> (1)		
			<i>Monodictys</i> (10)		
			<i>Ocala</i> <sup>^</sup> (1)		
			<i>Quintaria</i> (2)		
			<i>Wettsteinina</i> (2)		

<sup>^</sup>Genera known only from freshwater habitats (before this study)



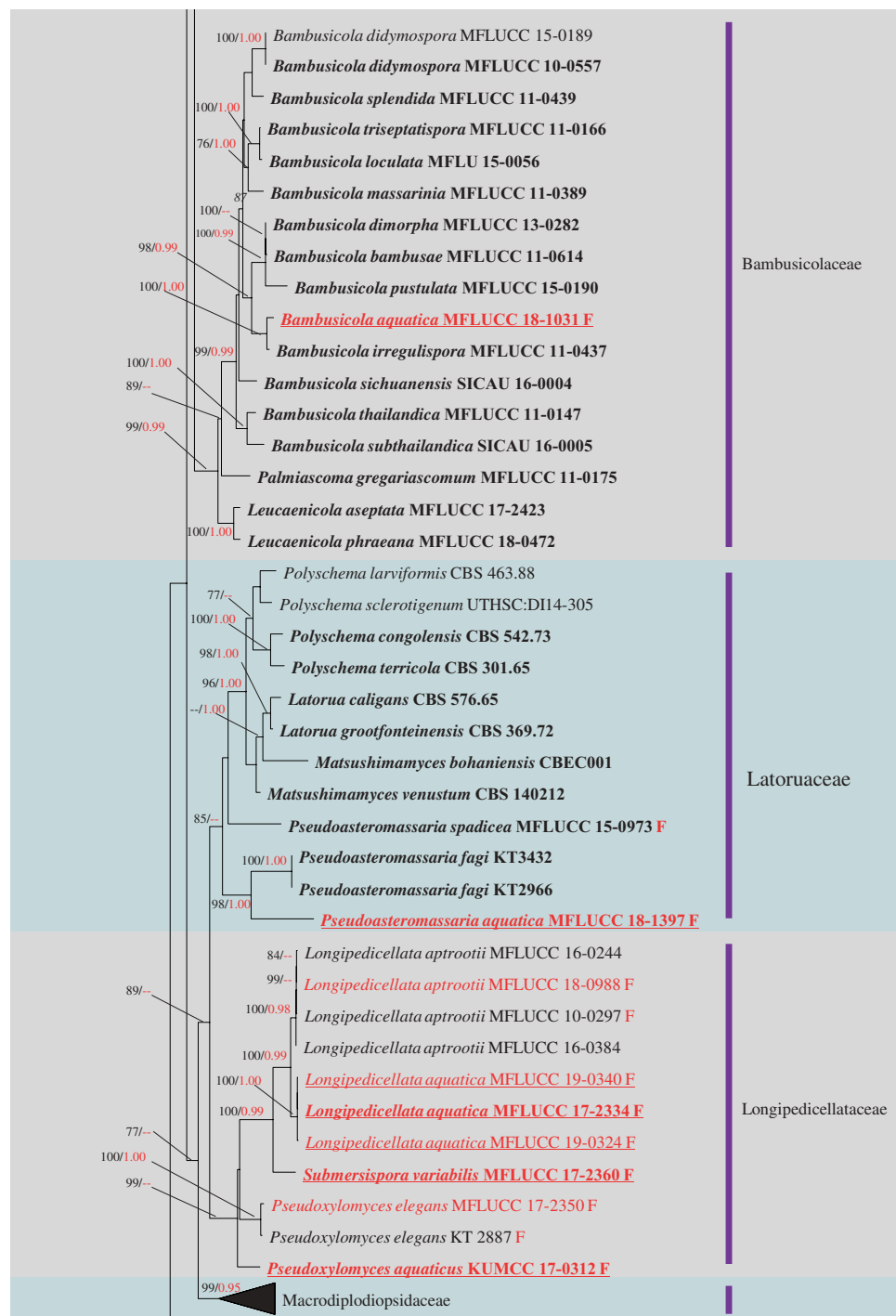
**Fig. 2** Phylogram generated from maximum likelihood analysis of combined LSU, ITS, TEF and RPB2 sequence data for species of Dothideomycetes. Bootstrap values for maximum likelihood equal to or greater than 75% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. Orders not treated in this study are compressed. The tree is rooted to *Capronia pilosella* AFTOL-ID 657 and *Endocarpon pallidulum* AFTOL-ID 661 (Eurotiomycetes)



$\mu\text{m}$  diam., numerous, cellular, sparsely branched, hyaline, septate. *Asci* 115–175  $\times$  35–65  $\mu\text{m}$  ( $\bar{x}$  = 145  $\times$  45  $\mu\text{m}$ ,  $n$  = 20), 8-spored, bitunicate, fissitunicate, clavate, thickened at the apex, sessile or short pedicellate, with a well-developed

ocular chamber. *Ascospores* 50–70  $\times$  14.5–19.5  $\mu\text{m}$  ( $\bar{x}$  = 63  $\times$  17  $\mu\text{m}$ ,  $n$  = 20), variably arranged in asci, oval, hyaline to pale brown, 1-septate, constricted at the septum, asymmetric, upper cell slightly shorter and wider than lower cell,

Fig. 2 (continued)



guttulate, straight or curved, thin-walled, smooth, sheathed. *Sheath* first appressed to the ascospore wall, expanding and detaching from the polar regions when mounted in water, becoming balloon-like at the two poles, finally surrounding the entire ascospore. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 30 mm in 45 days at 25 °C, grey from above, dark brown

from below, surface rough, with dense mycelium, mostly immersed in culture, dry, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat827-1 (MFLU 18-1573), living culture MFLUCC 18-1249; *ibid.*, hat827-2 (HKAS 105024), living culture KUMCC 19-0039; Chestnut Hill, on submerged wood in a stream, 10 May 2018, W. Dong, hat460-1 (MFLU 18-1554);

Fig. 2 (continued)

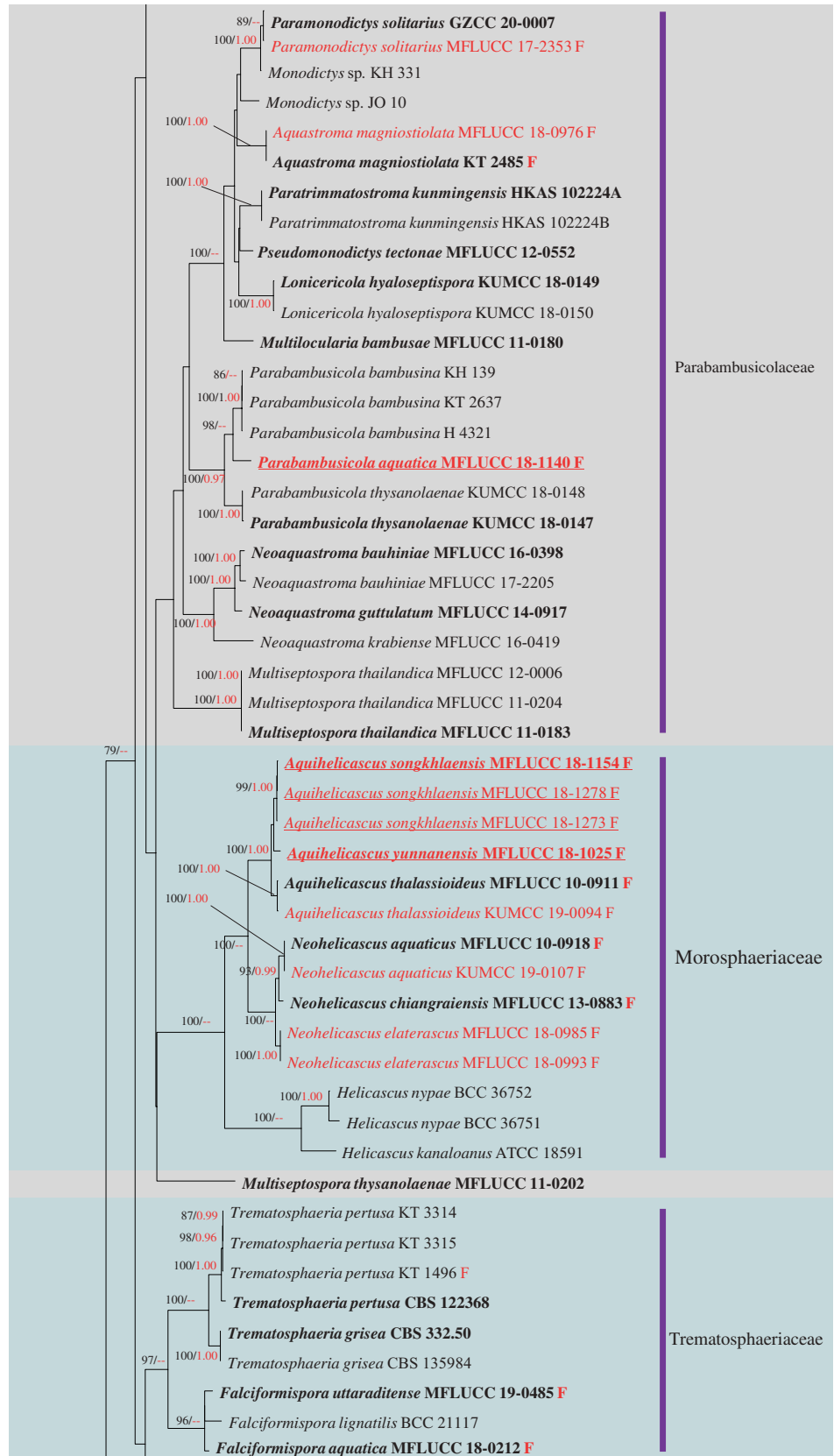


Fig. 2 (continued)

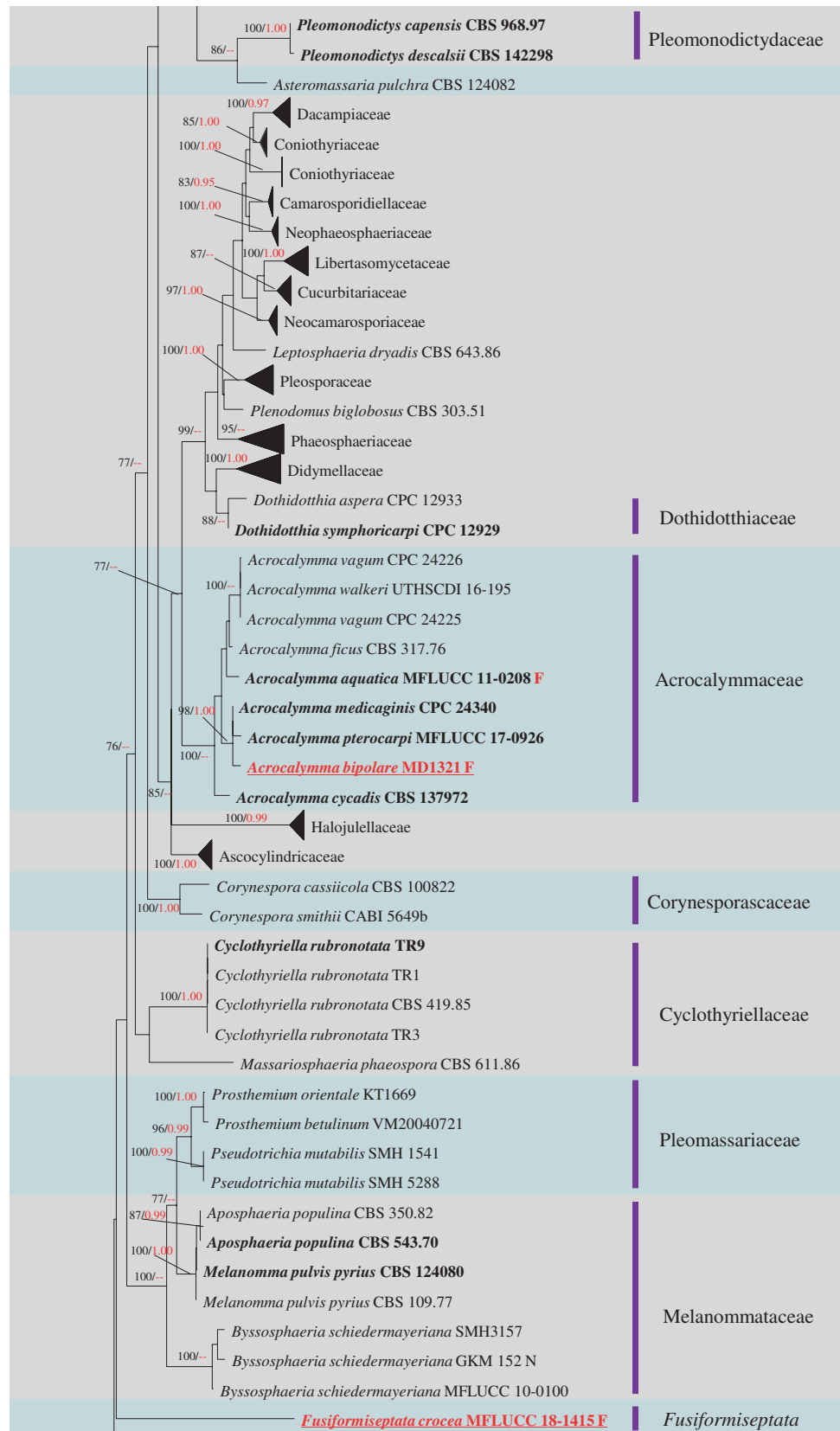
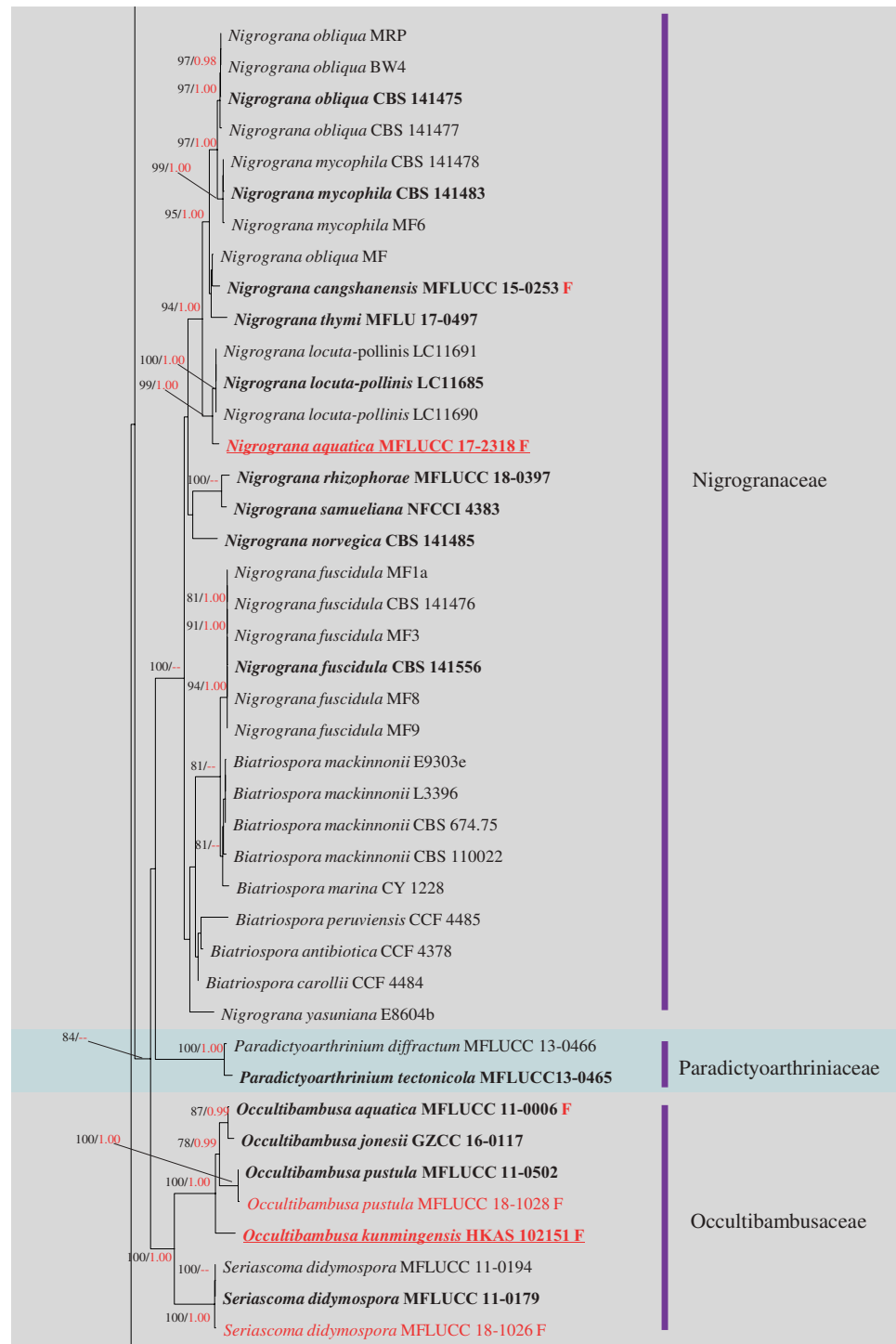


Fig. 2 (continued)

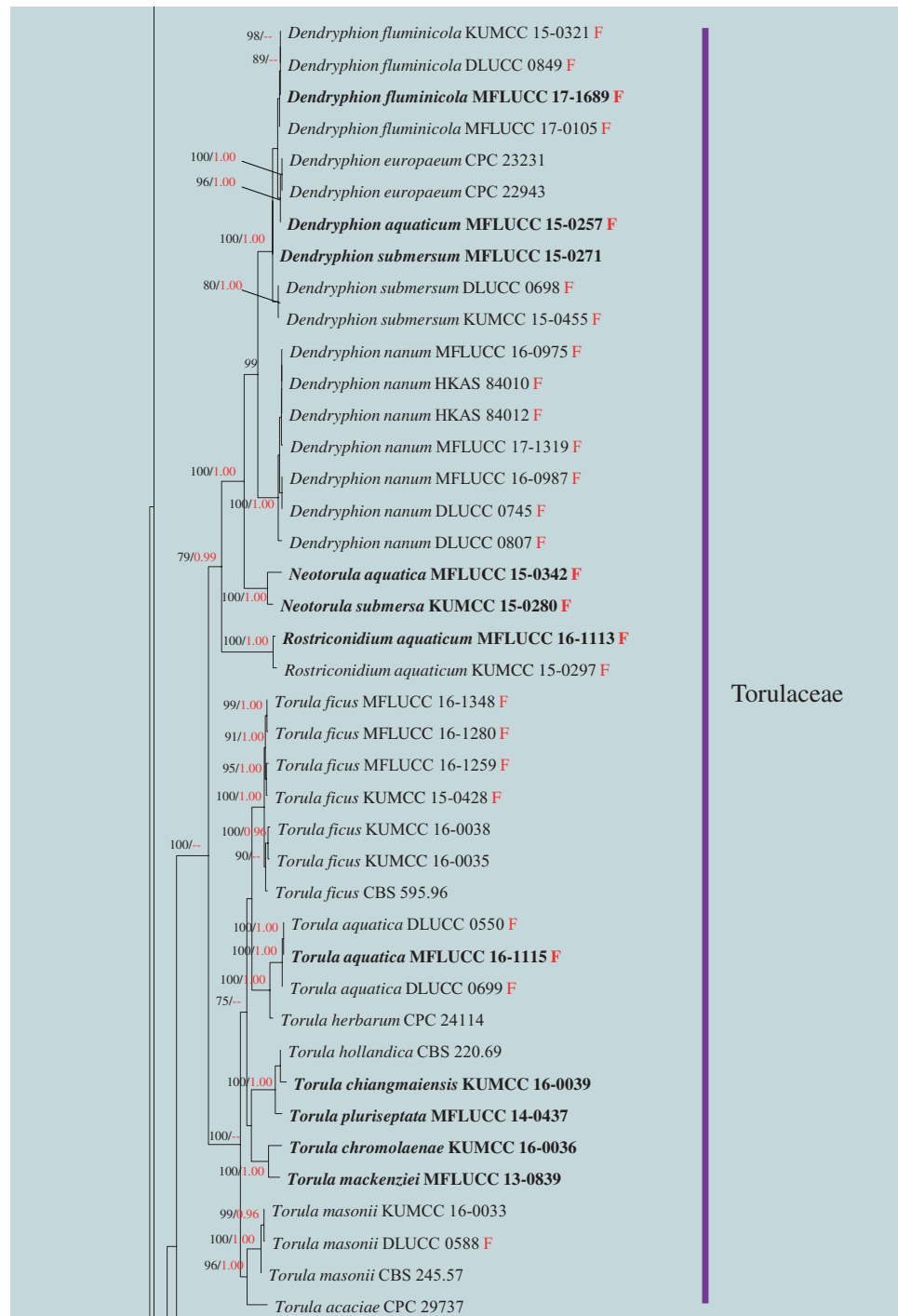


*ibid.*, hat460-2 (HKAS 105066), living culture KUMCC 19-0083.

**Notes:** Our two new isolates MFLUCC 18-1249 and KUMCC 19-0083 are identified as *Aliquandostipite khaoyaiensis* based on their identical LSU, SSU, ITS sequence data and morphology, although they lack a stalked ascumata. The new isolates cluster with *A. khaoyaiensis* and

*A. siamensis* with high bootstrap support (Fig. 14), which appears to be conspecific. However, our isolates must be *A. khaoyaiensis* because of its typical sheath. *Aliquandostipite khaoyaiensis* has monomorphic, pale brown ascospores with well-developed sheath (Inderbitzin et al. 2001). While *A. siamensis* has dimorphic, hyaline or brown ascospores without sheath (Pang et al. 2002). *Aliquandostipite siamensis*

Fig. 2 (continued)



lacks ITS sequence data and needs fresh material to confirm its phylogenetic difference with *A. khaoyaiensis*. This is a new geographical record for *A. khaoyaiensis* from parts of Thailand.

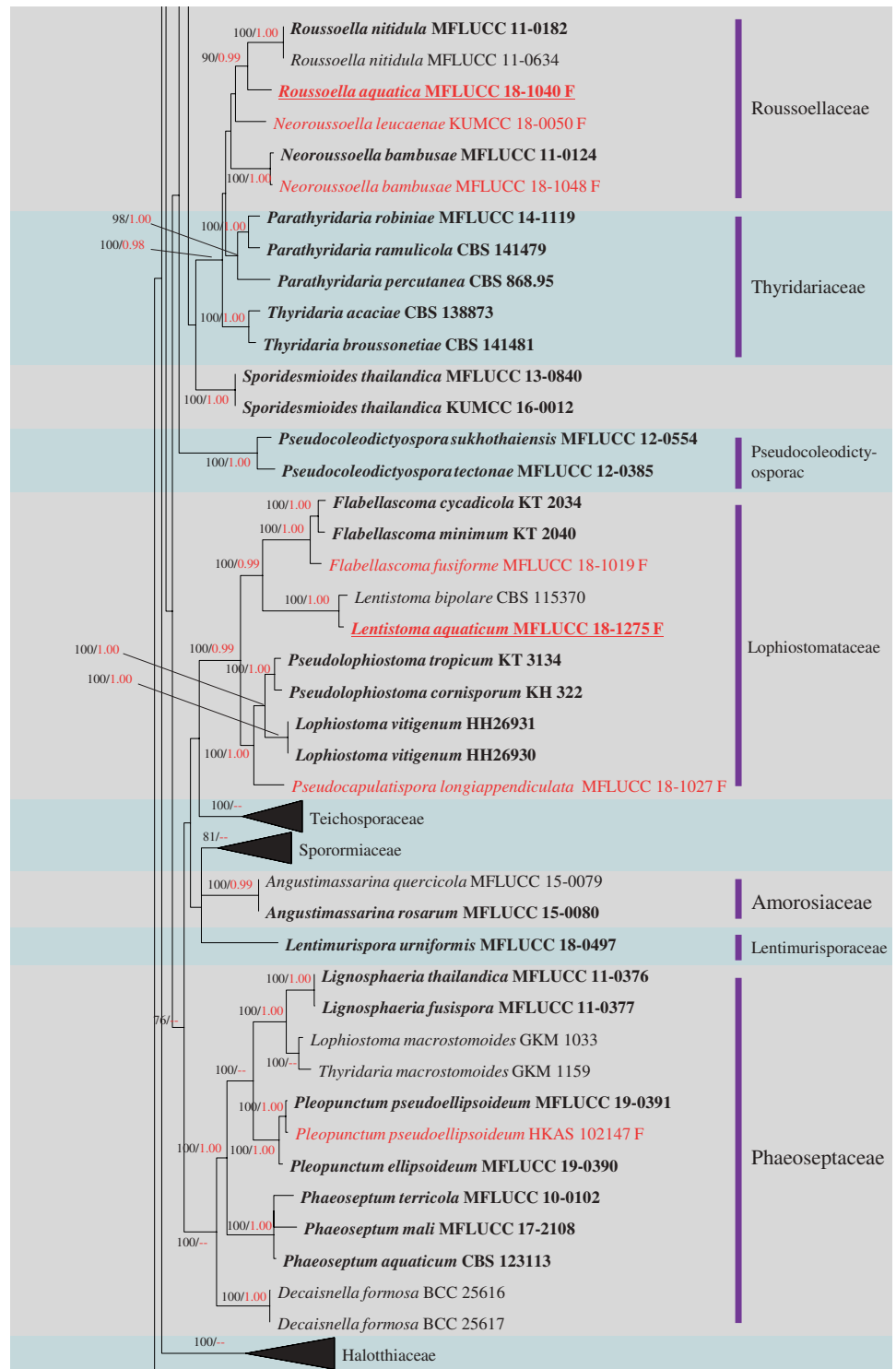
*Aliquandostipite manochii* Sri-indr., Boonyuen, Suetrong, K.L. Pang & E.B.G. Jones, Fungal Diversity 72: 103 (2015)  
Freshwater distribution: Thailand (Liu et al. 2015)

*Aliquandostipite minuta* Raja and Shearer, Mycoscience 43: 395 (2007); Fig. 5a–e

Freshwater distribution: USA (Raja and Shearer 2007; Raja et al. 2009b)

*Aliquandostipite separans* (Abdel-Wahab & El-Sharouney) J. Campb., Raja A. Ferrer, Sivichai & Shearer, Can. J. Bot. 85: 881 (2007)

Fig. 2 (continued)



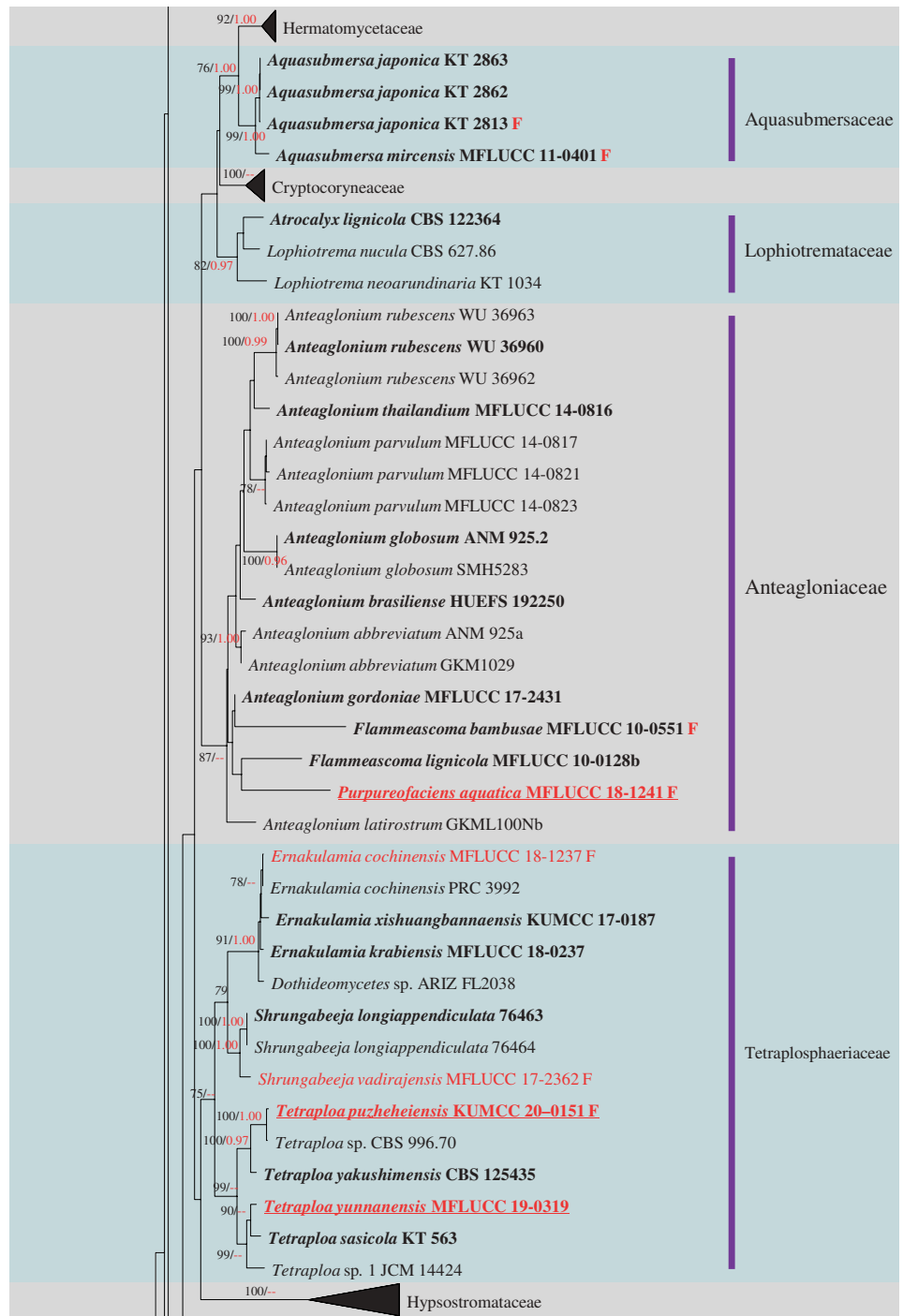
*Basionym: Patescospora separans* Abdel-Wahab & El-Sharouny, Mycol. Res. 106: 1033 (2002)

*Freshwater distribution:* Egypt (Pang et al. 2002)

\**Aliquandostipite siamensiae* (Sivichai & E.B.G. Jones) J. Campb., Raja A. Ferrer, Sivichai & Shearer, Can. J. Bot. 85: 879 (2007)

*Basionym: Jahnula siamensiae* Sivichai & E.B.G. Jones, Mycol. Res. 106: 1037 (2002)

Fig. 2 (continued)



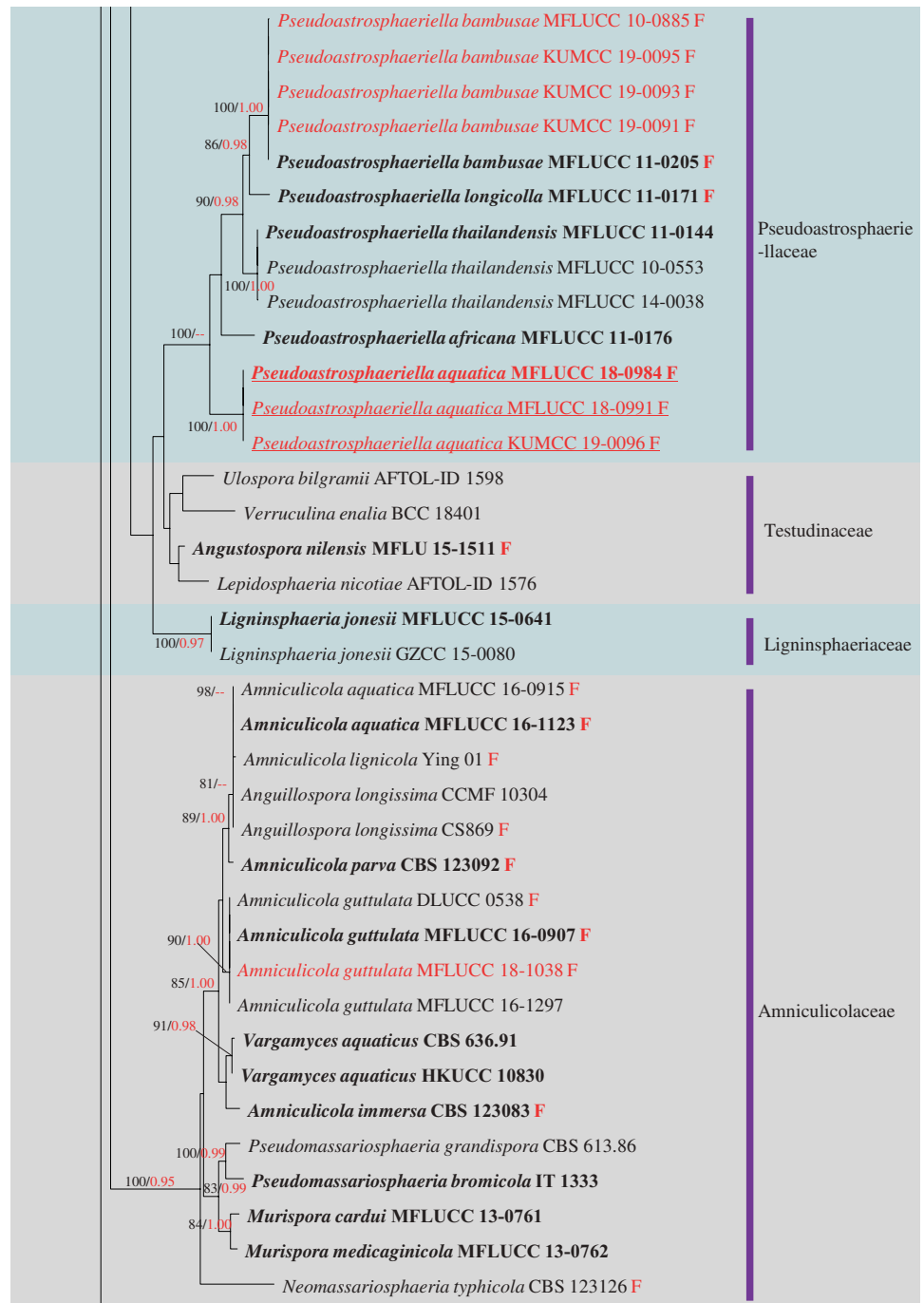
Freshwater distribution: Thailand (Pang et al. 2002), USA (Raja et al. 2009b)

**Key to freshwater *Aliquandostipite* species**

- |   |                     |   |                        |
|---|---------------------|---|------------------------|
| 1. Ascospores with sheath.....                          | 2                   | 2. Ascospores monomorphic.....                          | 3                      |
| 1. Ascospores with narrow sheath or without sheath..... |                     | 3. Ascospores without appendages.....                   | <i>A. khaoyaiensis</i> |
| .....   | <i>A. separans</i>  | 3. Ascospores with appendages.....                      | 4                      |
| 2. Ascospores dimorphic.....                            | <i>A. siamensis</i> | 4. Ascospores with small apical appendages.....         |                        |
|   |                     | .....   | <i>A. crystallinus</i> |
|   |                     | 4. Ascospores with numerous filamentous appendages..... |                        |
|   |                     | .....   | <i>A. minuta</i>       |



Fig. 2 (continued)



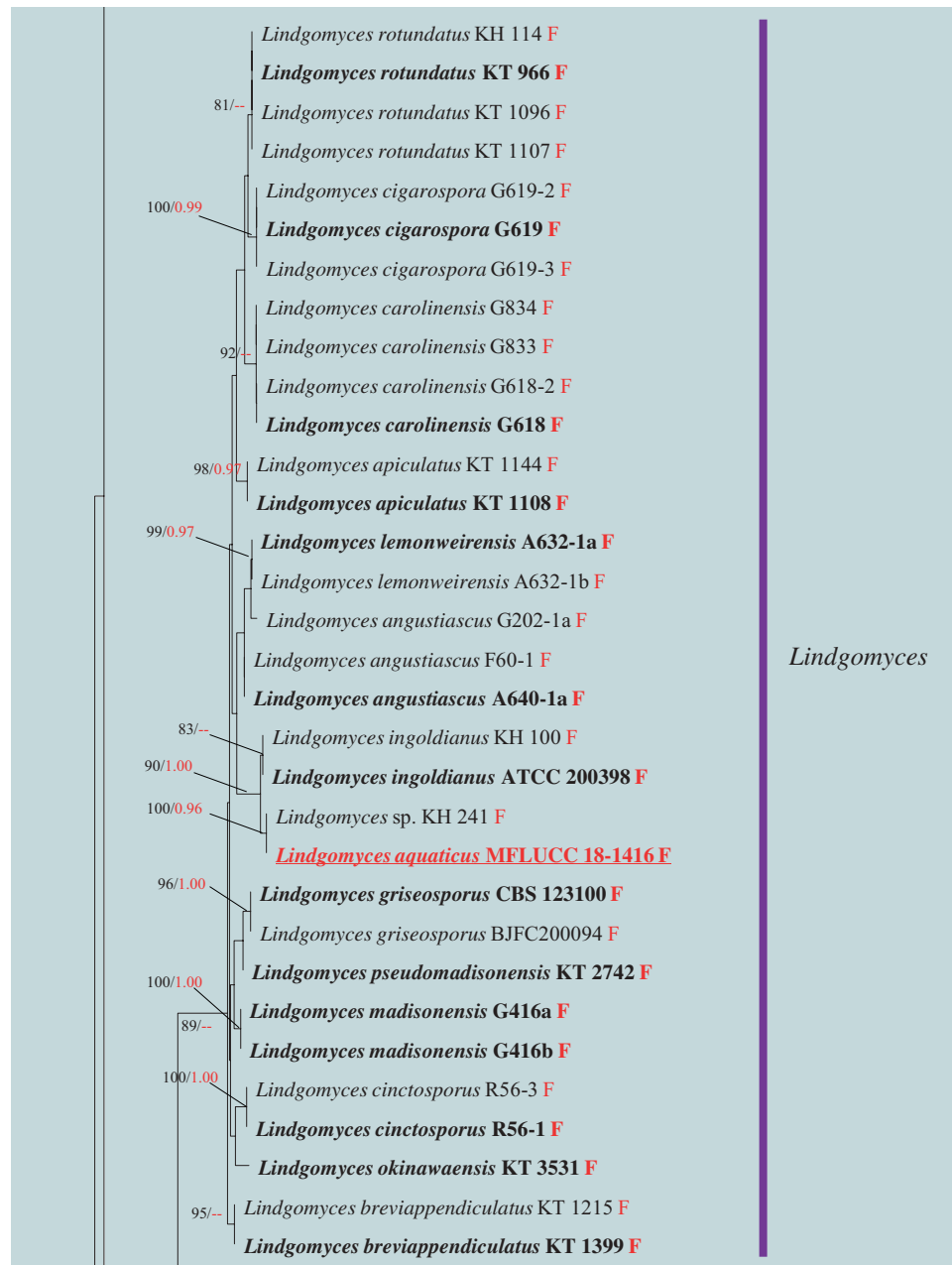
*Jahnula* Kirschst., *Annl. mycol.* 34(3): 196 (1936)

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* semi-immersed to erumpent, become superficial with base remaining immersed, solitary or clustered in small groups, globose to subglobose, unilocular, brown to dark brown, membranous, with a stalk-like strand or stoloniferous hyphae attached to the substratum, or covered by sparse hair-like projections, with ostiolate papilla. *Peridium* variable in thickness, comprising a few layers of relatively large, thin-walled, light brown cells of *textura angularis*.

*Pseudoparaphyses* cellular, persistent, filiform or hypha-like, hyaline, sparsely septate. *Asci* 8-spored, bitunicate, fissitunicate, mostly cylindrical, short-pedicellate, with an ocular chamber and sometimes with a faint ring. *Ascospores* mostly uniseriate, ellipsoid-fusiform, apical cell slightly larger, both cells tapering with rounded ends, reddish brown or dark brown, 1-septate, straight or curved (definition *sensu stricto* from Hawksworth (1984), Hyde and Goh (1999b) and Raja and Shearer (2006)). **Asexual morph:** Undetermined.

*Type species:* *Jahnula aquatica* (Kirschst.) Kirschst.

Fig. 2 (continued)



Notes: *Jahnula* is undoubtedly polyphyletic (Hyde et al. 2013, 2017, 2019; Huang et al. 2018) and *Jahnula sensu stricto* was mentioned in Huang et al. (2018), and Hyde et al. (2013). Hyde et al. (2013) suggested that taxonomic changes are needed for taxa in *Jahnula sensu lato*, but more *jahnula*-like species need to be collected and sequenced. We accept three species, *J. aquatica*, *J. granulosa* K.D. Hyde & S.W. Wong and *J. rostrata* Raja & Shearer in *Jahnula sensu stricto*, which have been collected from freshwater habitats (listed below). These three species share common characters in having dark ascomata with few appendages attached to the base, and ellipsoid-fusiform, brown,

1-septate ascospores with wider apical cell (Hawksworth 1984; Hyde and Wong 1999; Raja and Shearer 2006). Asci of *J. aquatica* are cylindrical, those of *J. granulosa* are obclavate and those of *J. rostrata* are clavate. *Jahnula aquatica* has smooth-walled ascospores without a sheath, while *J. granulosa* has granular-walled ascospores with a thin mucilaginous sheath. *Jahnula rostrata* is similar to *J. granulosa* by rough-walled ascospores, but differs in having an irregularly striated pattern. These three species formed a well-supported and stable clade in previous publications (Huang et al. 2018; Hyde et al. 2019) and in this study (Fig. 14).

Fig. 2 (continued)

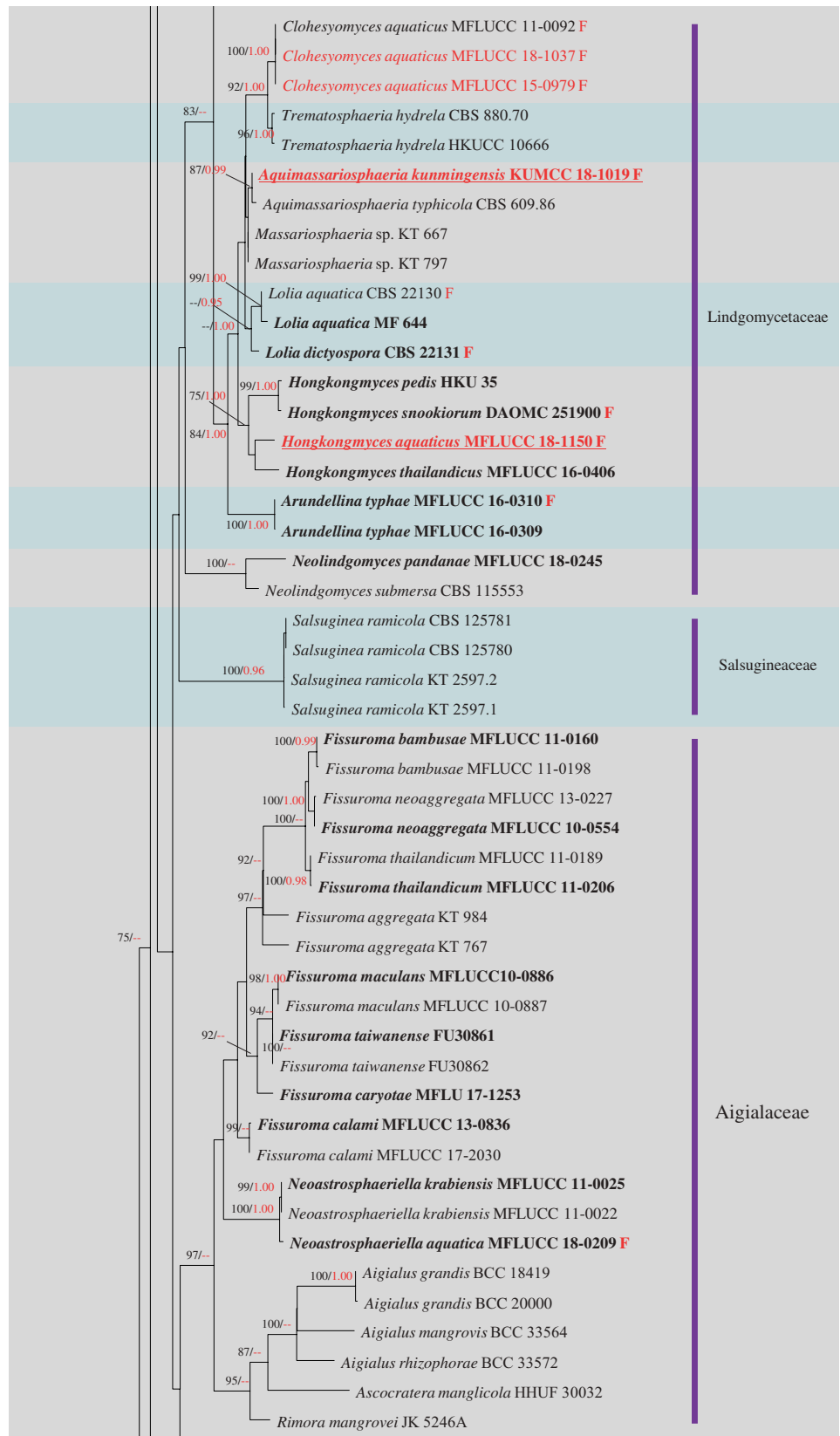


Fig. 2 (continued)

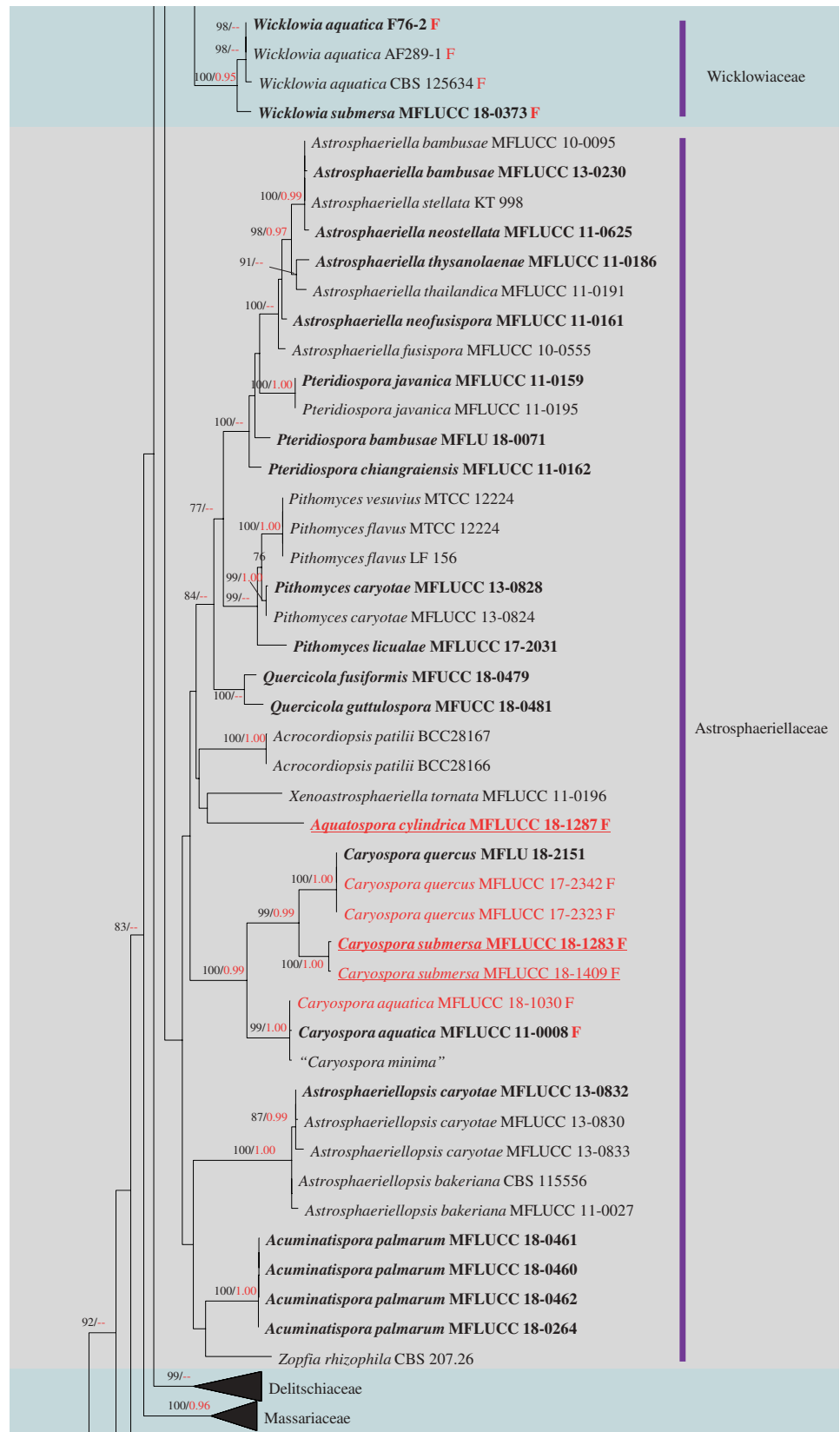
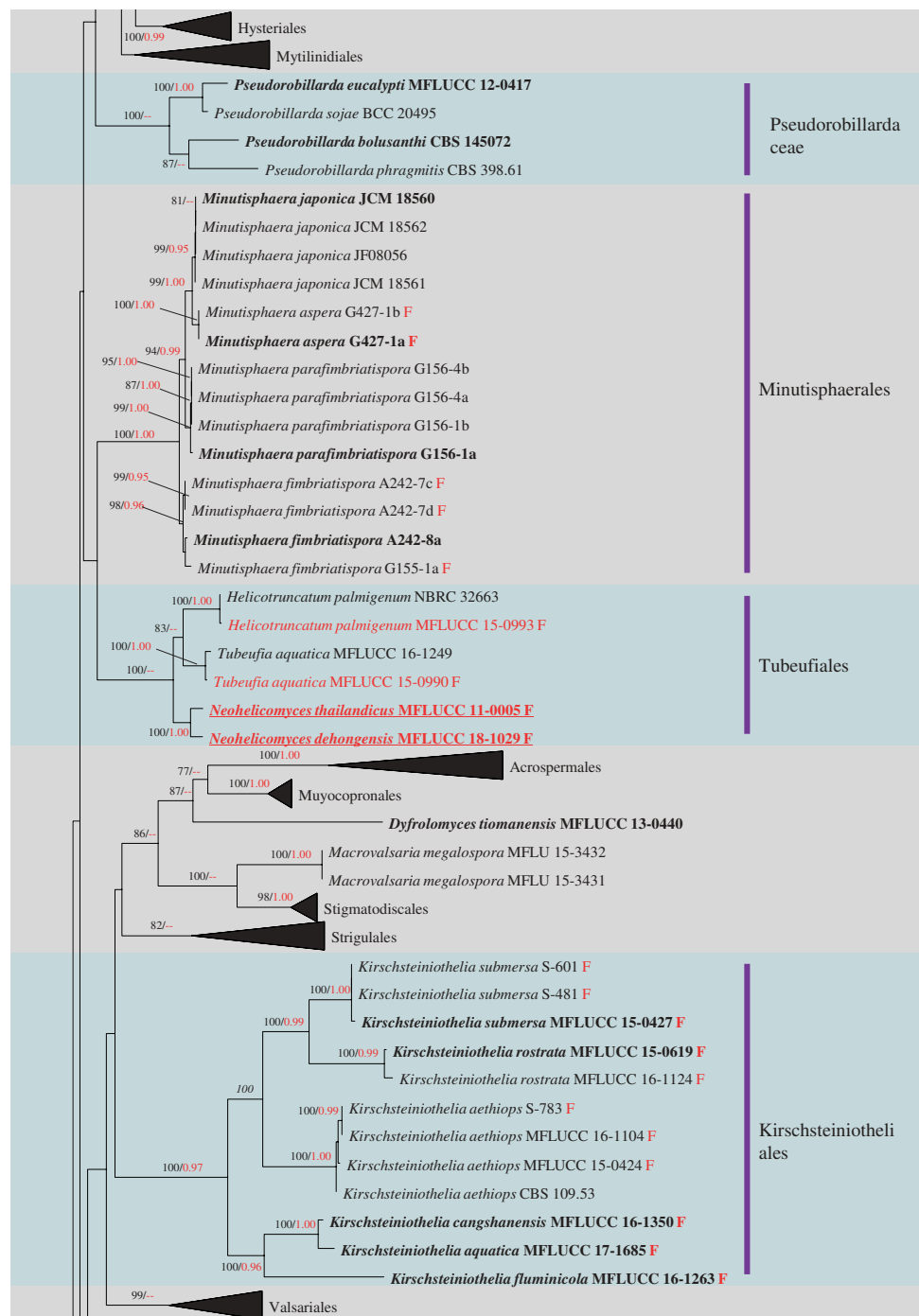


Fig. 2 (continued)

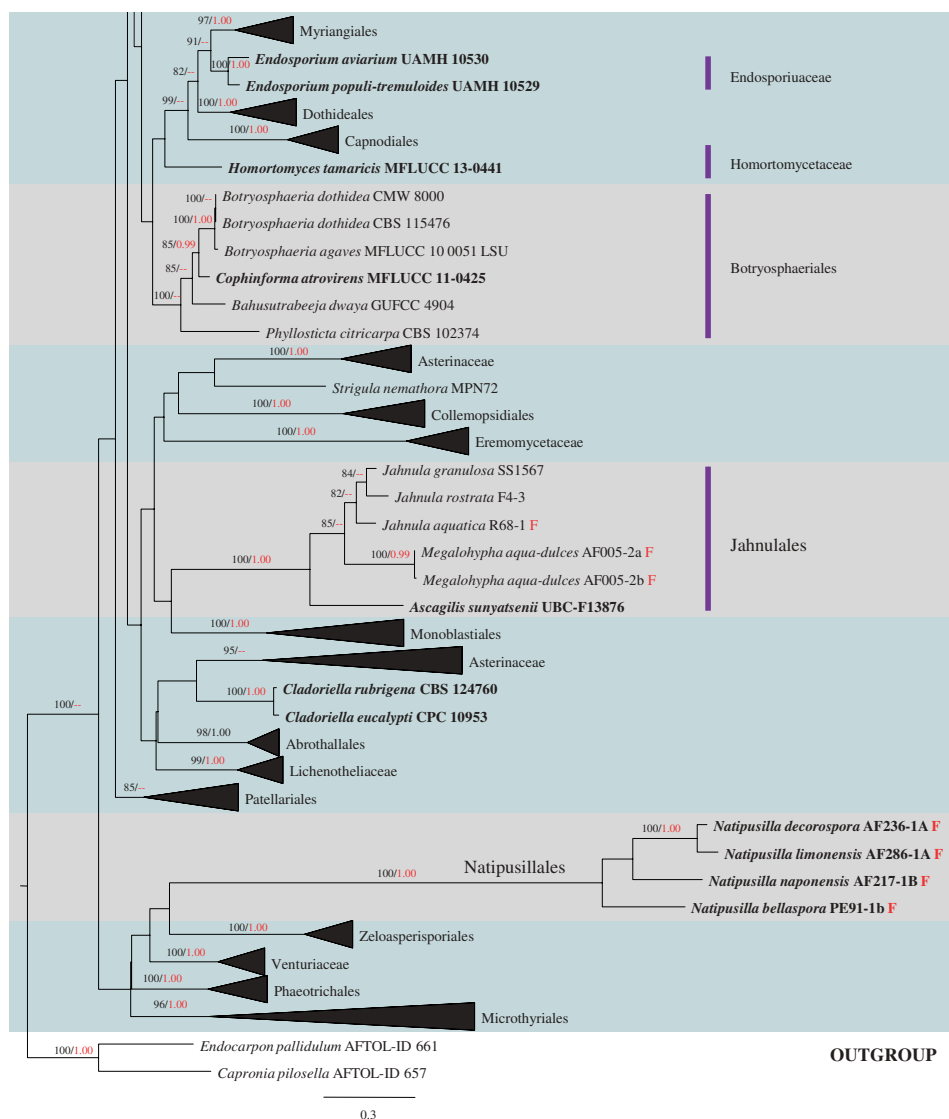


The type species, *Jahnula aquatica* commonly occurs in freshwater habitats as listed below. The lectotype of *J. aquatica* was examined, illustrated and described by Hawksworth (1984). *Jahnula aquatica* is characterized by ascogonia attached to the substratum by subiculum-like hyphae, peridium uneven in thickness, cylindrical asci, elongate-ellipsoid to very broadly fusiform, reddish brown, smooth, moderately thick-walled ascospores without a distinct gelatinous sheath (Hawksworth 1984). The sequences of

two isolates R68-1 and R68-2 (not type) were obtained by Raja and Shearer (2006), recognized and used for current phylogenetic analyses (Suetrong et al. 2011a; Huang et al. 2018; Hyde et al. 2019).

*Xylomyces chlamydosporus* Goos et al. was considered to be the asexual state of *Jahnula aquatica* based on fusiform chlamydosporous produced in culture (Sivichai et al. 2011). However, the phylogenetic analyses did not support this conclusion because *J. aquatica* and *X. chlamydosporus* scattered

Fig. 2 (continued)



in different clades within Jahnulales (Campbell et al. 2007; Suetrong et al. 2011a; this study, Fig. 14).

We introduce two new genera *Neojahnula* and *Pseudojahnula* to accommodate two known species *Jahnula australiensis* K.D. Hyde and *J. potamophila* K.D. Hyde & S.W. Wong, respectively. Seven species, i.e. *J. bipolaris*, *J. guttulaspora*, *J. queenslandica*, *J. seychellensis*, *J. submersa*, *J. sunyatsenii* and *J. thailandensis*, which are phylogenetically distant from *J. aquatica*, are transferred to *Ascagilis* K.D. Hyde. Nine species, i.e. *J. apiospora*, *J. appendiculata*, *J. bipileata*, *J. dianchia*, *J. morakotii*, *J. poonythii*, *J. purpurea*, *J. sangamonensis* and *J. systyla*, are placed in *Jahnula sensu lato*.

For convenience and practicality, we have excluded *Jahnula systyla* K.D. Hyde & S.W. Wong from the key, because we believe it may be phylogenetically related to *Aliquan-dostipite*, pending future phylogenetic studies (Fig. 6).

#### List of freshwater *Jahnula sensu stricto* species

\**Jahnula aquatica* (Plöttner & Kirschst.) Kirschst., Ann., Mycol. 34: 196 (1936); Fig. 7

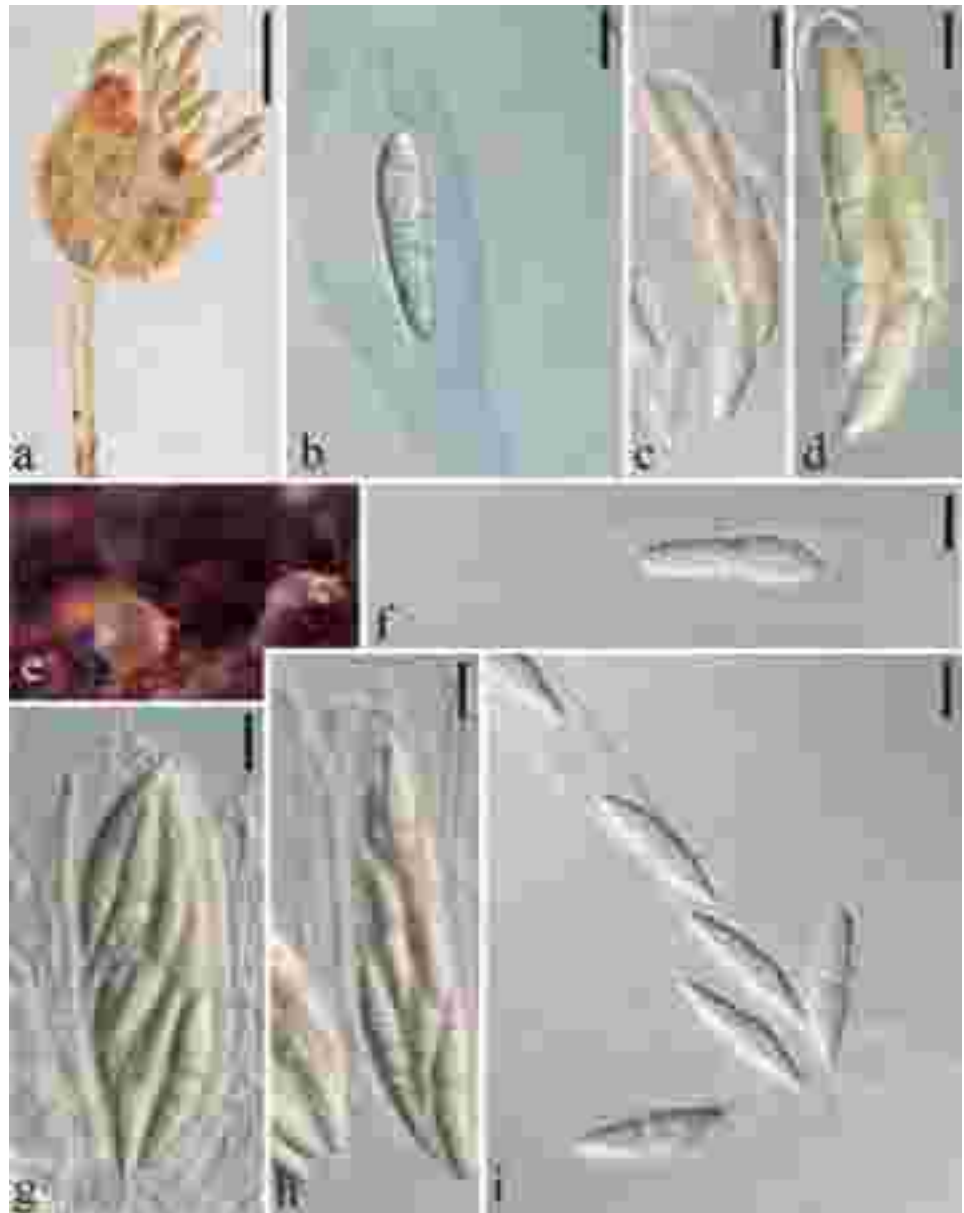
*Basionym*: *Melanopsamma aquatica* Kirschst., Krypt.-Fl. Brandenburg (Leipzig) 7(2): 226 (1911)

*Freshwater distribution*: China (Ho 1998), France (Fournier et al. 2010), Germany (Kirschstein 1936; Hawksworth 1984), Malaysia (Ho et al. 2001), South Africa (Hyde and Wong 1999), Thailand (Sivichai et al. 2011), USA (Raja and Shearer 2006; Raja et al. 2009b)

\**Jahnula granulosa* K.D. Hyde & S.W. Wong, Nova Hedwig. 68: 497 (1999)

*Freshwater distribution*: China (Cai et al. 2002a), South Africa (Hyde and Wong 1999), Thailand (Suetrong et al. 2011a)

**Fig. 3** *Aliquandostipite* spp. (Material examined: USA, Tennessee, Great Smoky Mountains National Park, Elkmont Campground, a small stream between Jakes Creek and Little River, on submerged, decorticated wood, 5 July 2002, H.A. Raja & N. Hamburger, R76-1; Florida, Big Cypress National Preserve, Cypress Swamp Loop Road, on submerged decorticated wood, 22 March 2005, H.A. Raja, J.L. Crane & A.N. Miller, F89-1). **a–d, f** *A. khaoyaiensis* (F89-1). **a** Stalked ascoma. **b** Ascospore with slug-like gelatinous sheath in nigrosin. **c, d** Asci. **f** Ascospore with thick-walled gelatinous sheath. **e, g–i** *A. crystallinus* (R76-1). **e** Ascoma. **g, h** Asci. **i** Ascospores emerging from the ascus to form a large, C-shaped to fusiform, gelatinous sheath. Scale bars: **a** = 100  $\mu$ m, **b–d** = 20  $\mu$ m, **e** = 200  $\mu$ m, **f–i** = 20  $\mu$ m



\**Jahnula rostrata* Raja & Shearer, Mycologia 98: 325 (2006)

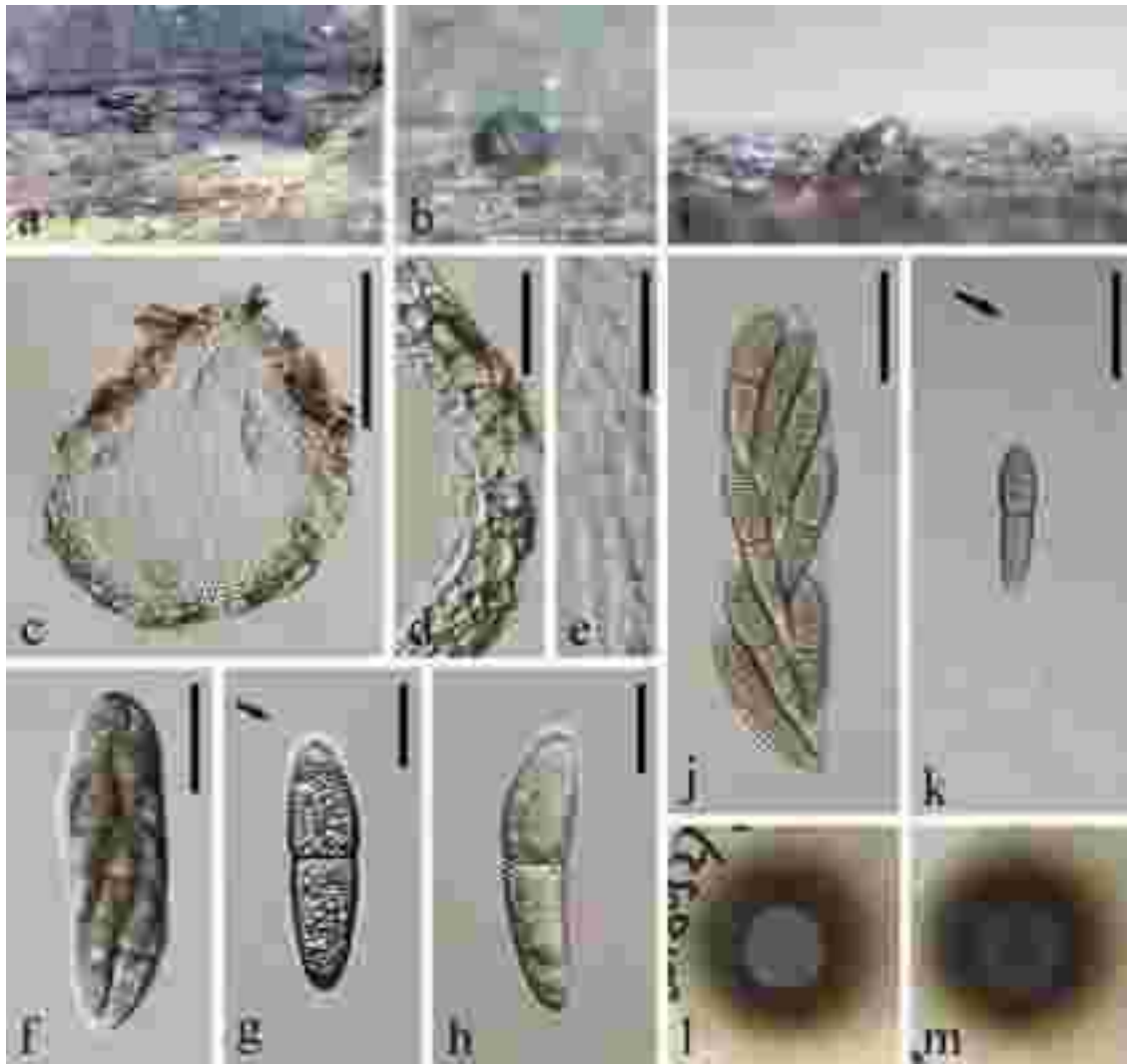
*Facesoffungi* number: FoF09237; Fig. 8

*Freshwater distribution*: China (this study), USA (Raja and Shearer 2006; Raja et al. 2009b)

*Saprobic* on submerged wood. **Sexual morph**: *Ascomata* 320–420  $\times$  310–330  $\mu$ m, superficial, solitary to scattered, subglobose or pyriform, brown to black, rough-walled, with ostiolate papilla. *Neck* 48–75  $\times$  74–98  $\mu$ m, composed of subglobose cells diverging from the ostiole. *Peridium* 25–40  $\mu$ m wide, two-layered, outer layer composed of large, dark brown, thick-walled cells of *textura angularis*, inner layer composed of compressed, hyaline, thin-walled cells of *textura angularis*. *Pseudoparaphyses* 2.5–3.5  $\mu$ m wide, cellular, hypha-like, hyaline, septate, unbranched. *Asci* 135–200

$\times$  21–32  $\mu$ m ( $\bar{x}$  = 160  $\times$  28  $\mu$ m,  $n$  = 15), 8-spored, bitunicate, fissitunicate, clavate, short pedicellate, with an ocular chamber. *Ascospores* 28–40  $\times$  11–17  $\mu$ m ( $\bar{x}$  = 35  $\times$  13.5  $\mu$ m,  $n$  = 15), overlapping biseriate, ellipsoidal or broadly fusiform, with rounded or occasionally acute ends, upper cell slightly broader than lower cell, hyaline to brown when young, dark brown at maturity, 1-septate, constricted at the septum, with thick and dark band at septum when mature, rough-walled, with minute striated pattern, guttulate, with or without sheath. **Asexual morph**: Undetermined.

*Culture characteristics*: On PDA, colonies irregular, reaching 35 mm in 30 days at 25  $^{\circ}$ C, dark from above and below, dry, hairy, dense in the centre, becoming sparse at the edge.



**Fig. 4** *Aliquandostipite khaoyaiensis* a–h, l, m (MFLU 18-1573, new geographical record), i–k (MFLU 18-1554, new geographical record). a, b, i Ascomata on host surface. c Vertical section of ascoma. d Structure of peridium. e Pseudoparaphyses. f, j Bituni-

cate asci. g, h, k Ascospores. l, m Colony on PDA (left-front, right-reverse). Scale bars: c = 100  $\mu$ m, d = 30  $\mu$ m, e, g, h = 20  $\mu$ m, f, j, k = 50  $\mu$ m

**Material examined:** CHINA, Yunnan Province, Kunming, Liangwang National Wetland Park, on submerged wood in a stream, 19 March 2019, C.X. Li, L15 (MFLU 20-0435).

**Notes:** Our collection MFLU 20-0435 is identified as *Jahnula rostrata* based on very similar morphology and only one and one nucleotide difference in LSU and SSU sequence data between MFLU 20-0435 and F4-3, respectively. They form a well-supported clade in our phylogenetic analysis (Fig. 14). MFLU 20-0435 has thinner asci than the holotype (135–200  $\times$  21–32  $\mu$ m vs. 152–190  $\times$  32–40  $\mu$ m) (Raja and Shearer 2006). Our collection is a new geographical record for *J. rostrata* from China. *Jahnula rostrata* (F4-3) lacks ITS sequence data, we supplement ITS sequence for this species in this study.

#### Key to freshwater *Jahnula sensu stricto* species

1. Ascospores smooth-walled.....*J. aquatica*
1. Ascospores rough-walled.....2
2. Ascospores granular-walled.....*J. granulosa*
2. Ascospores with an irregularly striated pattern.....  
.....*J. rostrata*

#### List of freshwater *Jahnula sensu lato* species

*Jahnula apiospora* A. Carter, Raja & Shearer, Mycoscience 49: 326 (2008); Fig. 6d, i

**Freshwater distribution:** Canada (Raja et al. 2008)





**Fig. 5** *Aliquandostipitaceae* spp. (Material examined: USA, Florida, Monroe County, freshwater swamp at Big Cypress National Preserve, on submerged decorticated wood, 17 February 2006, H.A. Raja & J.L. Crane, ILL 40108 = F117-1, **holotype**; PANAMA, Colon, Soberania National Park, Juan Grande, on submerged, decorticated wood, 12 January 2003, A. Ferrer, AF005). **a–e** *Aliquandostipite*

*minuta* (F117-1). **a** Squash mount of ascoma. **b, c** Asci. **d** Extended endoascus releasing ascospore. **e** Ascospore showing fusiform sheath and filamentous appendages. **f–j** *Megalohypha aqua-dulces* (AF005). **f** Ascoma on substrate. **g** Ascus. **h, i** Ascospores. **j** Broad hyphae in PDA. Scale bars: **a–e, g–i** = 20  $\mu$ m, **f, j** = 200  $\mu$ m

\**Jahnula appendiculata* Pinruan, K.D. Hyde & E.B.G. Jones, *Sydowia* 54: 243 (2002); Fig. 9

*Freshwater distribution*: Peru (Shearer et al. 2015), Thailand (Pinruan et al. 2002; Sivichai and Boonyuen 2010)

\**Jahnula bipileata* Raja & Shearer, *Mycologia* 98: 321 (2006); Fig. 6c, j

*Freshwater distribution*: USA (Raja and Shearer 2006)

*Jahnula morakotii* Sivichai & Boonyuen, *Mycotaxon* 112: 476 (2012)

*Freshwater distribution*: Thailand (Sivichai and Boonyuen 2010)

*Jahnula poonythii* K.D. Hyde & S.W. Wong, *Nova Hedwig*. 68: 499 (1999)

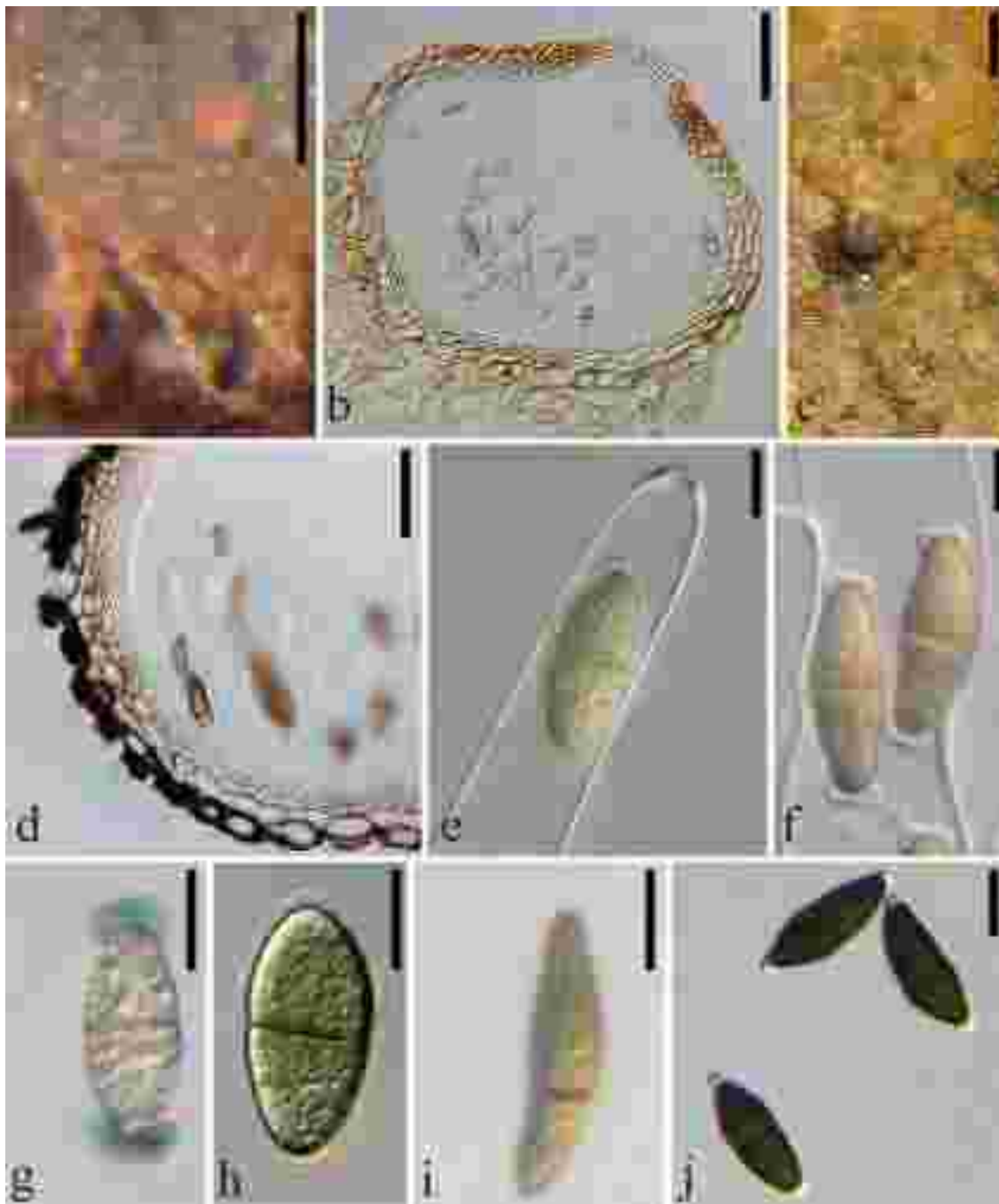
*Freshwater distribution*: China (Cai et al. 2002a; Luo et al. 2004), Mauritius (Hyde and Wong 1999), Mexico (Gonzalez and Chavarria 2005)

*Jahnula purpurea* J. Fourn., Raja & Shearer, *Myckeys* 9: 30. (2015)

*Freshwater distribution*: Martinique (Fournier et al. 2015)

\**Jahnula sangamonensis* Shearer & Raja, *Mycologia* 98: 327 (2006)

*Freshwater distribution*: USA (Raja and Shearer 2006; Raja et al. 2009b)



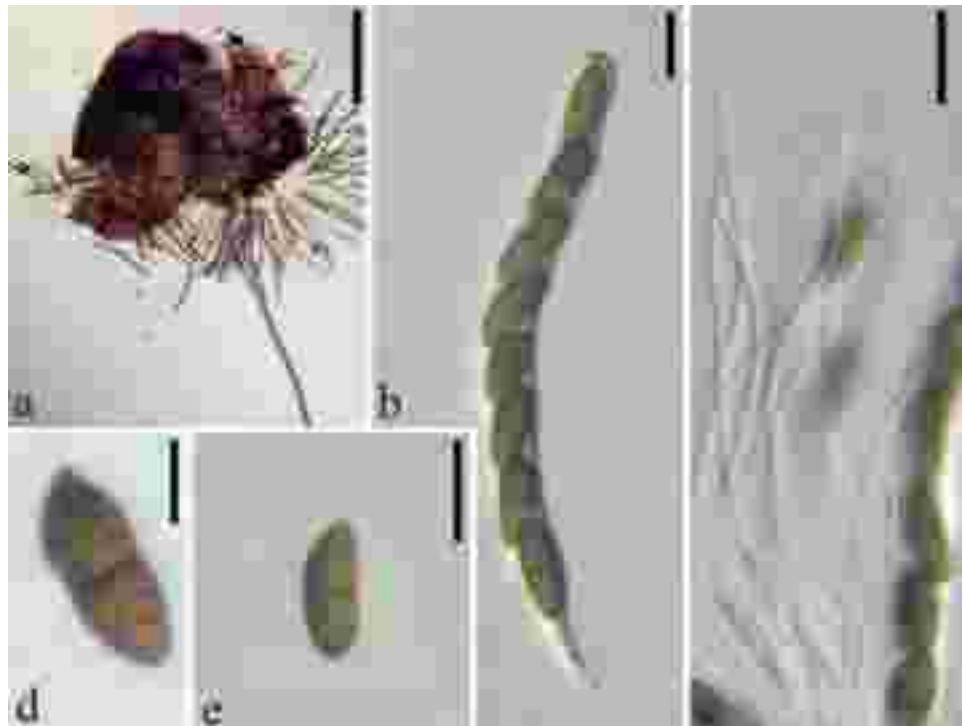
**Fig. 6** Aliquandostipitaceae spp. (Material examined: USA, Florida, on submerged wood, July 2006, J.L. Crane & H.A. Raja, F111; Costa Rica, La Selva Biological Station, Sura 60, on submerged, corticated wood, CMP, A492; Florida, Apalachicola National Forest, swampy area of Whitehead Lake, on submerged, decorticated wood, 13 July 2004, HAR and CB, F49-1 (ILL), **holotype**; CANADA, Prince Edward Island, on submerged decorticated wood, in a small creek beside dirt road near Bonshaw and Trans Canada Highway, 13 Octo-

ber 2007, A. Carter, AC-706, ILL40554, **holotype**). **a, e, h** *Pseudojahnula potamophila* (F111). **a** Ascomata attached to the wood with broad hyphae. **e, h** Ascospores. **b** Section of ascoma of *Ascagilis seychellensis* (A492). **c, j** *Jahnula bipileata* (F49-1). **c** Ascoma. **j** Ascospores. **d, i** *Jahnula apiospora* (AC-706). **d** Structure of peridium. **i** One-septate apiosporous ascospore. **f, g** Ascospores of *Ascagilis bipolaris* showing bipolar appendages. Scale bars: **a, c** = 200  $\mu$ m, **b, d–j** = 20  $\mu$ m

*Jahnula systyla* K.D. Hyde & S.W. Wong, Nova Hedwig. 68: 506 (1999)

*Freshwater distribution*: Australia (Hyde and Wong 1999), China (Ho 1998), Malaysia (Ho et al. 2001)

**Fig. 7** *Jahnula aquatica* (Material examined: USA, Illinois, Salt Fork Association Lake (SFA Lake), a manmade private lake, on submerged, decorticated wood, 15 October 2002, HAR, R68-1). **a** Squash mount of ascoma. **b** Ascus. **c** Pseudoparaphyses. **d, e** Ascospores. Scale bars: **a** = 100 µm, **b, c, e** = 20 µm, **d** = 10 µm



**Key to freshwater *Jahnula sensu lato* species**

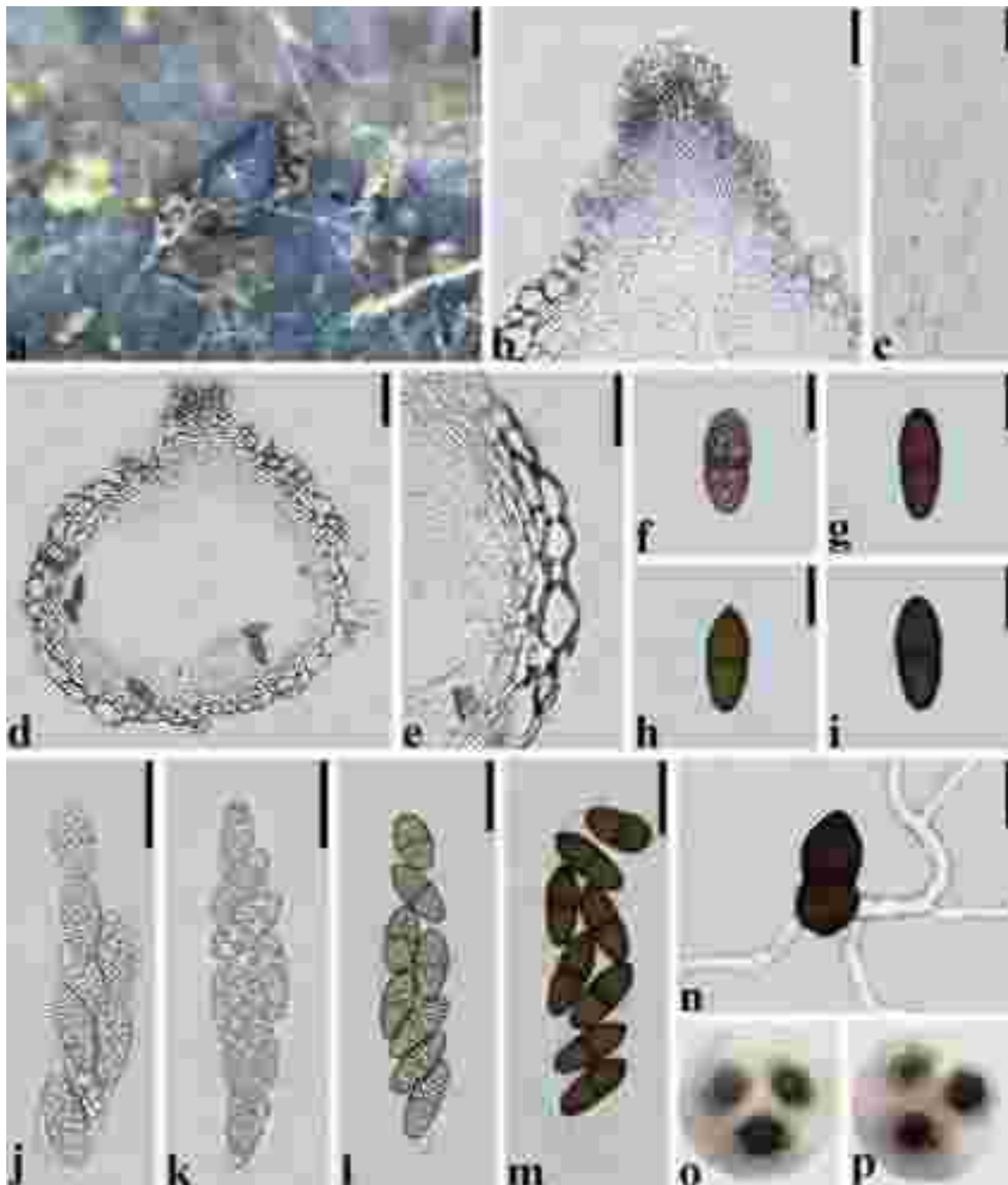
- 1. Ascospores without modification (no appendages/sheath/caps).....2
- 1. Ascospores equipped with modification (appendages/sheath/caps).....6
- 2. Ascospores asymmetric.....*J. apiospora*
- 2. Ascospores symmetric.....3
- 3. Ascomata stain substrate purple.....*J. purpurea*
- 3. Ascomata not as above.....4
- 4. Ascomata with a short but prominent neck, endoasci extend up to 500 µm long in water.....*J. sangamonensis*
- 4. Ascomata with a short papilla, endoasci not extending.....5
- 5. Ascomata with stalk-like strands attached to the base, with a few sparse hair-like projections.....*J. poonythii*
- 5. Ascomata without any appendages.....*J. dianchia*
- 6. Ascospores without unfurling bipolar appendages...*J. bipileata*
- 6. Ascospores with long unfurling bipolar appendages.....7
- 7. Ascospores 18–20 × 5–6 µm.....*J. morakotii*
- 7. Ascospores 48–55 × 23–26 µm.....*J. appendiculata*

*Ascagilis* K.D. Hyde, Aust. Syst. Bot. 5(1): 109 (1992)

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* semi-immersed or superficial, solitary, scattered or gregarious, globose or subglobose, coriaceous, black, ostiolate. *Peridium* comprising several layers of thin-walled, brown, angular cells. *Pseudoparaphyses* numerous, cellular, hyaline, thin, septate, branched. *Asci* 8-spored, clavate, obclavate, broadly or narrowly cylindrical, fissitunicate, sometimes expanding 4–5 times longer than original length, with an ocular chamber and ring. *Ascospores* bi- to tri-seriate, 1-septate, brown, mostly with a refractive mucilaginous pad at each end. **Asexual morph:** Undetermined.

*Type species:* *Ascagilis bipolaris* K.D. Hyde

*Notes:* *Ascagilis* was introduced to accommodate a jahnula-like species, *A. bipolaris*, characterized by clavate to cylindrical asci with an ocular chamber and ring and fusiform to ellipsoidal, 1-septate, brown ascospores with a pad at each end (Hyde 1992b). *Ascagilis* was suppressed since the type species *A. bipolaris* was transferred to *Jahnula* (Hyde and Wong 1999) and they thought the fusiform to ellipsoidal ascospores with bipolar pads of *Ascagilis* was insufficient to separate these two genera. *Jahnula* has been shown to be polyphyletic in previous publications (Hyde et al. 2013, 2017, 2019; Huang et al. 2018) and in this study. The clade comprising *A. bipolaris* and six other species is phylogenetically distant from *Jahnula sensu stricto* (Fig. 14). Additionally, most species including our new species, *A. submersa* and *A. thailandensis*, have a refractive mucilaginous pad at each end, which is lacking in *Jahnula sensu stricto*. All species in this clade cluster with strong bootstrap support



**Fig. 8** *Jahnula rostrata* (MFLU 20-0435, new geographical record). **a** Appearance of black ascoma on host substrate. **b** Structure of ostiole. **c** Pseudoparaphyses. **d** Vertical section of ascoma. **e** Struc-

ture of peridium. **f–i** Ascospores. **j–m** Bitunicate asci. **n** Germinated ascospore. **o, p** Colony on PDA (left-front, right-reverse). Scale bars: **b, e–n** = 30  $\mu$ m, **c** = 10  $\mu$ m, **d** = 50  $\mu$ m

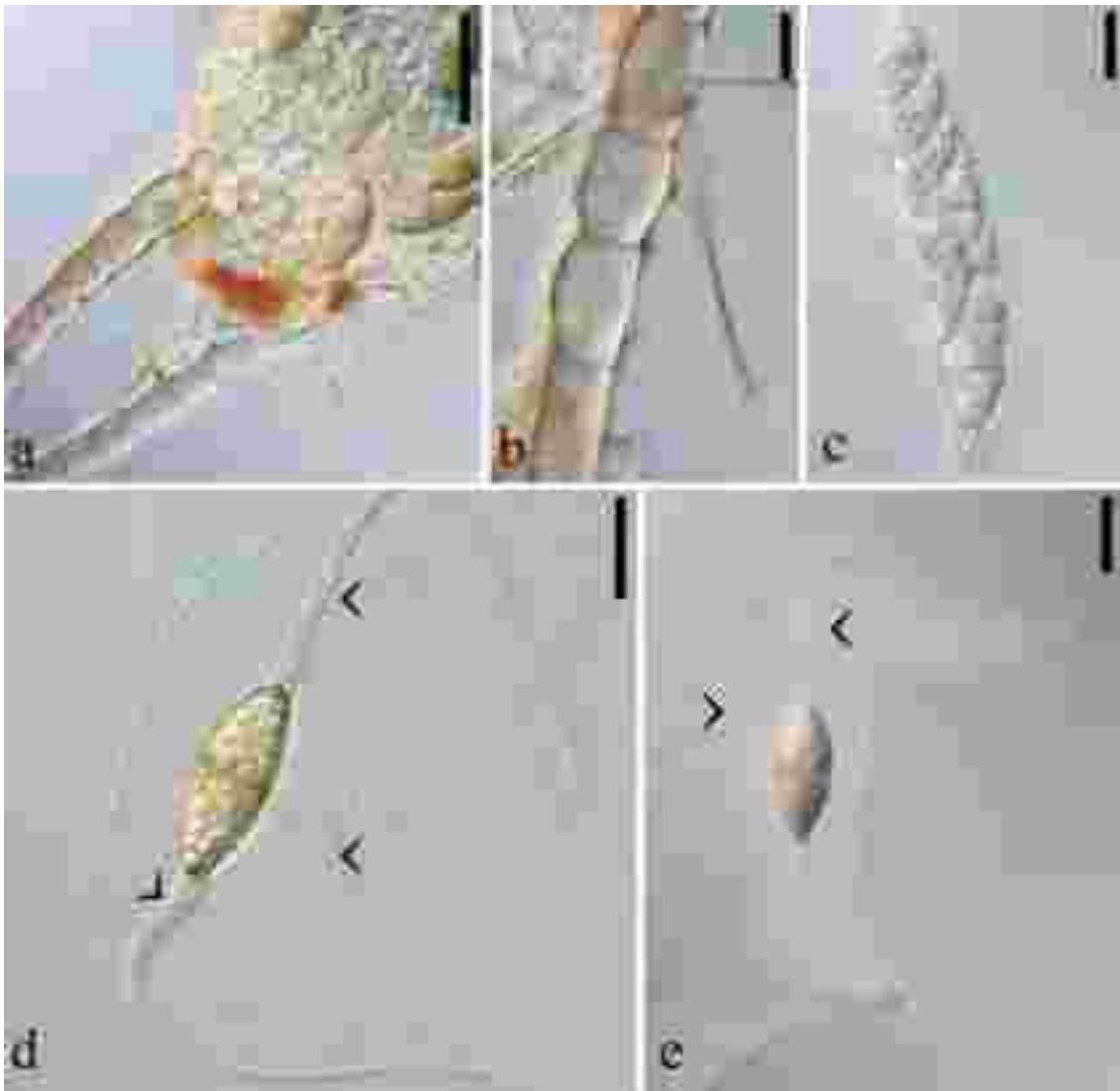
(Fig. 14). We therefore, resurrect *Ascagilis* and transfer four species and introduce two new species in the genus (see list below). The mucilaginous pad of ascospores is treated as a noticeable characteristic of *Ascagilis*.

#### List of freshwater *Ascagilis* species

\**Ascagilis bipolaris* K.D. Hyde, Aust. Syst. Bot. 5(1): 111 (1992); Fig. 6f, g

*Synonymy:* *Jahnula bipolaris* (K.D. Hyde) K.D. Hyde, Nova Hedwigia 68(3–4): 494 (1999)

*Freshwater distribution:* Australia (Hyde 1992b; Hyde and Wong 1999), China (Tsui et al. 2000; Tsui and Hyde 2004), Costa Rica (Raja and Shearer 2006), Malaysia (Ho et al. 2001), Peru (Shearer et al. 2015), Thailand (Suetrong et al. 2011a), USA (Raja et al. 2009b)



**Fig. 9** *Jahnula appendiculata* (Material examined: PERU, on submerged wood, S. Zelski & H.A. Raja, PE0010). **a** Stalked ascoma. **b** Broad hypha attached to the ascomatal sides and bases. **c** Young

ascus. **d, e** Ascospores with broad gelatinous sheath and polar appendages indicated by arrows. Scale bars: **a** = 50 µm, **b–e** = 20 µm

\**Ascagilis guttulaspora* (Qing Tian, Y.Z. Lu & K.D. Hyde) W. Dong, Doilom & K.D. Hyde, *comb. nov.*

*Index Fungorum number*: IF557893; *Facesoffungi number*: FoF09228

*Basionym*: *Jahnula guttulaspora* Qing Tian, Y.Z. Lu & K.D. Hyde, *Fungal Diversity* 87: 6 (2017)

*Freshwater distribution*: China (Hyde et al. 2017)

\**Ascagilis queenslandica* (Dayarathne, Fryar & K.D. Hyde) W. Dong, Doilom & K.D. Hyde, *comb. nov.*

*Index Fungorum number*: IF557894; *Facesoffungi number*: FoF09229

*Basionym*: *Jahnula queenslandica* Dayarathne, Fryar & K.D. Hyde, *Fungal Diversity* 96: 91 (2019)

*Freshwater distribution*: Australia (Hyde et al. 2019)

\**Ascagilis seychellensis* (K.D. Hyde & S.W. Wong) W. Dong, Doilom & K.D. Hyde, *comb. nov.*

*Index Fungorum number*: IF557895; *Facesoffungi number*: FoF09230

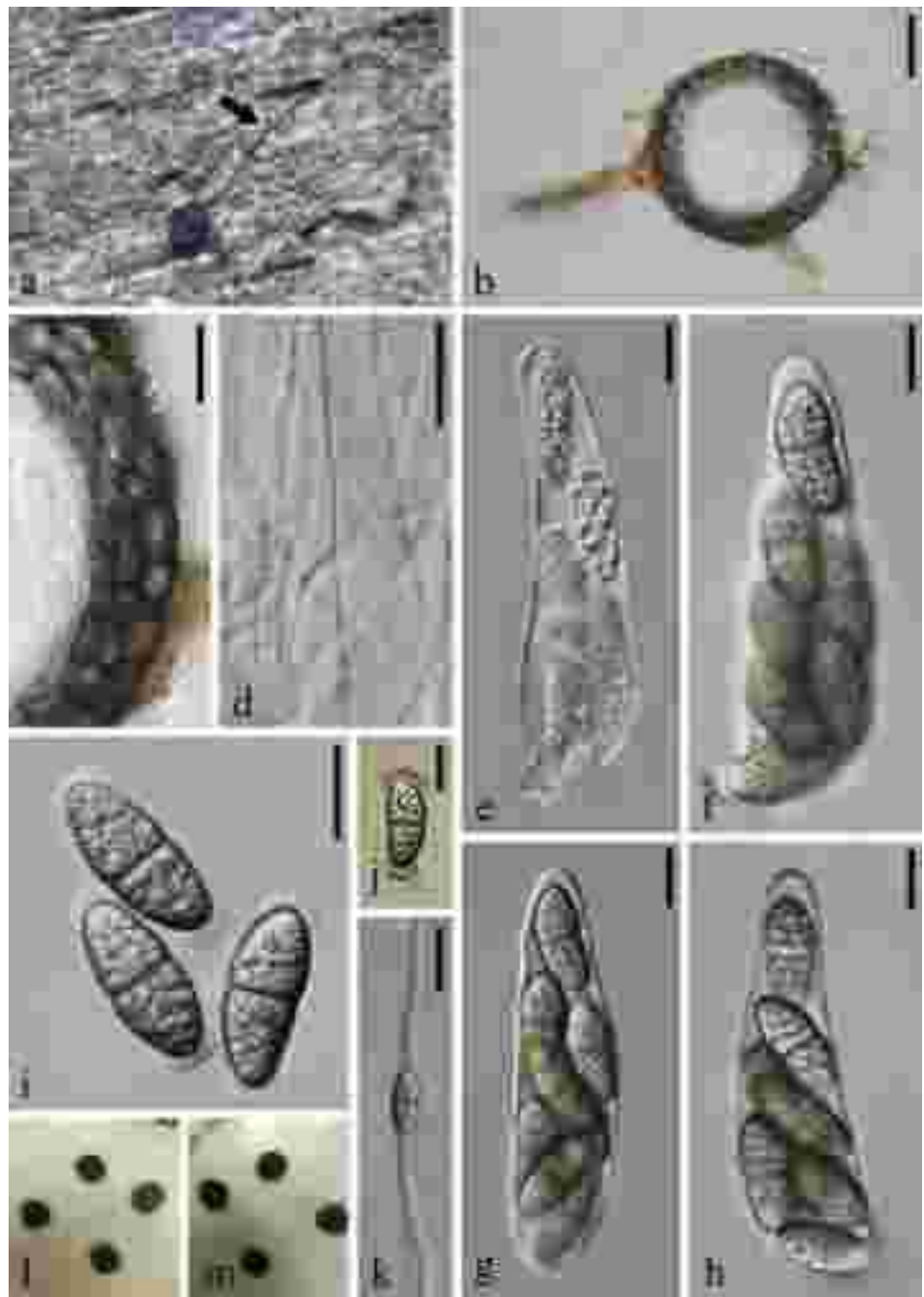
*Basionym*: *Jahnula seychellensis* K.D. Hyde & S.W. Wong, *Nova Hedwig*, 68: 504 (1999); Fig. 6b

*Freshwater distribution*: Brazil (Barbosa et al. 2013), Costa Rica (Raja and Shearer 2006), Seychelles (Hyde and Wong 1999)

\**Ascagilis submersa* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number*: IF557896; *Facesoffungi number*: FoF09238; Fig. 10

**Fig. 10** *Ascagilis submersa* (MFLU 18-1527, **holotype**). **a** Ascomata on host with associated appendages (arrow). **b** Vertical section of ascoma. **c** Structure of peridium. **d** Pseudoparaphyses. **e–h** Bitunicate asci. **i** Ascospores. **j** Ascospore in Indian Ink. **k** Germinated ascospore. **l, m** Colony on PDA (left-front, right-reverse). Scale bars: **b** = 50  $\mu$ m, **c–k** = 20  $\mu$ m



*Etymology*: in reference to the submerged habitats of the fungus

*Holotype*: MFLU 18-1527

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Ascomata* 160–250  $\mu$ m diam., black, solitary or scattered, superficial, globose or subglobose, with several long, brown to dark brown appendages which are procumbent on the substrate surface, membranous. *Peridium* 30–35  $\mu$ m thick, composed of 4–5 layers of thick-walled, olive to dark brown, compressed cells of *textura angularis*, with

2 layers of flattened, compressed, hyaline cells inwardly. *Pseudoparaphyses* 3  $\mu$ m diam., numerous, cellular, hypha-like, hyaline, septate, embedded in a gelatinous matrix. *Asci* 110–160  $\times$  35–42  $\mu$ m ( $\bar{x}$  = 135  $\times$  38  $\mu$ m,  $n$  = 10), 8-spored, bitunicate, thick-walled, broadly obclavate, with thick-walled apex and well-developed ocular chamber, completely filled by ascospores, short pedicel observed only when young. *Ascospores* 37.5–41  $\times$  17–19  $\mu$ m ( $\bar{x}$  = 40  $\times$  18  $\mu$ m,  $n$  = 20), overlapping biseriate and uniseriate near the apex, straight or slightly curved, hyaline when young, pale brown to brown

when mature, 1-septate, constricted at the septum, ellipsoidal, guttulate, thin-walled, smooth, with bipolar, helmet-shaped appendages. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 10 mm in 10 days at 25 °C, dark brown from above and below, surface rough, with sparse mycelium, dry, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, 20180513-1 (MFLU 18-1527, **holotype**), ex-type living culture MFLUCC 18-1143; *ibid.*, 20180513-2 (HKAS 104996, **isotype**), ex-type living culture KUMCC 19-0013.

**Notes:** *Ascagilis submersa* clusters with *A. queenslandica*, *A. sunyatsenii* and *A. thailandensis* (Fig. 14). *Ascagilis submersa* is similar to *A. sunyatsenii* in having ellipsoidal, pale brown ascospores with bipolar helmet-shaped appendages (Inderbitzin et al. 2001). However, *A. sunyatsenii* has saccate, ovoid to elongate asci and longer ascospores ((39–)46–52 × 16–23 µm) contrasting with broadly obclavate asci and shorter ascospores (37.5–41 × 17–19 µm) in *A. submersa* (Inderbitzin et al. 2001). *Ascagilis submersa* has olive peridium and ascospores, which are unique in the genus and different from the dark pigmented peridium and light brown ascospores of *A. sunyatsenii*. Additionally, *A. sunyatsenii* has two types of ascomata, sessile and stalked, while *A. submersa* has only sessile ascomata and with several appendages which are procumbent on the substrate surface (Inderbitzin et al. 2001). Because of scarcity of some nucleic acid genes, only ITS sequence can be compared. There are two nucleotide differences in ITS sequence data, but only crossing 207 nucleotides. The distinct morphology of *A. submersa* provides strong evidence to be a new species in *Ascagilis*

\**Ascagilis sunyatsenii* (Inderb.) W. Dong, Doilom & K.D. Hyde, **comb. nov.**

**Index Fungorum number:** IF557897; **Facesoffungi number:** FoF09239

**Basionym:** *Aliquandostipite sunyatsenii* Inderb., Am. J. Bot. 88(1): 57 (2001)

**Synonymy:** *Jahnula sunyatsenii* (Inderb.) K.L. Pang, E.B.G. Jones & Sivichai, Mycol. Res 106: 1037 (2002)

**Freshwater distribution:** China (Inderbitzin et al. 2001; Pang et al. 2002)

\**Ascagilis thailandensis* W. Dong, H. Zhang & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557898; **Facesoffungi number:** FoF09240; Fig. 11

**Etymology:** in reference to Thailand, where the holotype was collected

**Holotype:** MFLU 18-1514

**Saprobic** on decaying wood submerged in freshwater. **Sexual morph:** *Ascomata* 280–320 µm high, 200–250 µm diam., black, scattered, superficial with base immersed in host tissue, conical, membranous, coriaceous, with short ostiolate papilla. *Peridium* 30–40 µm thick, composed of 4–5 layers of thin-walled, pale brown, large cells of *textura angularis*. *Pseudoparaphyses* 3.5 µm diam., numerous, cellular, hypha-like, hyaline, septate. *Asci* 125–185 × 24–30 µm ( $\bar{x}$  = 162 × 27.5 µm, n = 5), 8-spored, bitunicate, broadly or narrowly cylindrical, with an ocular chamber, short pedicellate. *Ascospores* 33–48.5 × 11–16 µm ( $\bar{x}$  = 41 × 13 µm, n = 30), biseriate or uniseriate above ascus center and overlapping biseriate near the center or base, brown, 1-septate, slightly constricted at the septum, irregularly fusiform, mostly curved, tapering at apical cell and slightly flattened or rounded at the basal cell, guttulate, thin-walled, smooth, with small, inconspicuous pads at one or both apices. **Asexual morph:** Undetermined.

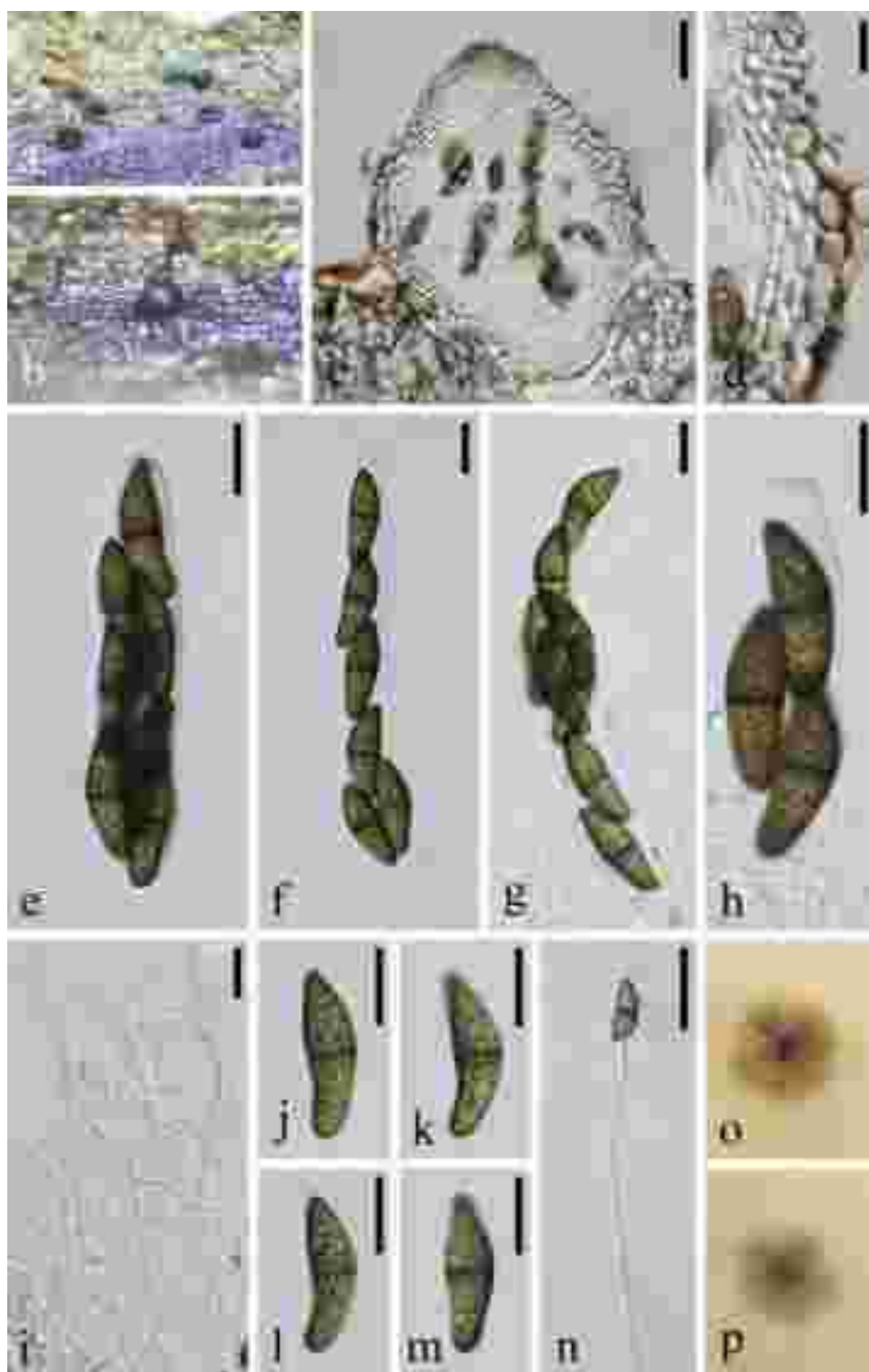
**Culture characteristics:** On PDA, colony circular, reaching 5 mm in 5 days at 25 °C, olive brown from above, brown from below, surface rough, with sparse mycelium, dry, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, 20180524 (MFLU 18-1514, **holotype**), ex-type living culture MFLUCC 18-1149; *ibid.*, Chestnut Hill, on submerged wood in a stream, 10 May 2018, W. Dong, hat454-1 (MFLU 18-1546), living culture MFLUCC 18-1247; *ibid.*, hat454-2 (HKAS 105022), living culture KUMCC 19-0037.

**Notes:** *Ascagilis thailandensis* clusters with *A. queenslandica*, *A. submersa* and *A. sunyatsenii* with low bootstrap support (Fig. 14). The phylogenetically closest species *A. sunyatsenii* only has ITS sequence data (323 nucleotides) in GenBank, which has three nucleotide differences with *A. thailandensis*. Due to the limited sequence data, *A. sunyatsenii* and *A. thailandensis* appear to represent the same species in our phylogenetic tree (Fig. 14). However, their morphological characteristics strongly support them to be the different species. *Ascagilis sunyatsenii* has two types of ascomata (sessile and stalked), saccate, ovoid to elongate asci and ellipsoidal ascospores with two conspicuous helmet-shaped appendages (Inderbitzin et al. 2001). Whereas, *A. thailandensis* has sessile ascomata, cylindrical asci and irregularly fusiform ascospores with small, inconspicuous pads. *Ascagilis thailandensis* can be easily distinguished from the other two phylogenetically related species *A. queenslandica* and *A. submersa* by ascospore shape, size and appendages (Hyde et al. 2019).

*Ascagilis thailandensis* is morphologically similar to *A. guttulaspora* in having nearly fusiform, smooth-walled, guttulate ascospores with tapering apical cell and slightly

**Fig. 11** *Ascagilis thailandensis* (MFLU 18-1514, **holotype**). **a, b** Ascomata on host tissue. **c** Vertical section of ascoma. **d** Structure of peridium. **e–h** Bitunicate asci. **i** Pseudoparaphyses. **j–m** Ascospores. **n** Germinated ascospore. **o, p** Colony on PDA (left-front, right-reverse). Scale bars: **c, n** = 50  $\mu$ m, **d–m** = 20  $\mu$ m



flattened basal cell. However, *A. guttulaspora* has smaller ascospores (29–33  $\times$  8.5–10.5  $\mu$ m vs. 33–48.5  $\times$  11–16  $\mu$ m) and without pads (Hyde et al. 2017). The ascomata of *A. guttulaspora* are attached to the substratum by wide, brown hyphae, while they are lacking in *A. thailandensis* (Hyde

et al. 2017). Our phylogenetic analysis supports them to be the different species (Fig. 14).

**Key to freshwater *Ascagilis* species**

- 1. Ascospores without mucilaginous pads.....2



1. Ascospores with mucilaginous pads at one or both ends.....3
2. Ascospores aseptate.....*A. queenslandica*
2. Ascospores 1-septate.....*A. guttulaspora*
3. Ascomata sessile.....4
3. Ascomata sessile and stalked.....6
4. Ascospores with inconspicuous pads at one or both ends.....*A. thailandensis*
4. Ascospores with conspicuous bipolar pads.....5
5. Ascospores  $37.5\text{--}41 \times 17\text{--}19 \mu\text{m}$ .....*A. submersa*
5. Ascospores  $42\text{--}52 \times 16\text{--}23 \mu\text{m}$ .....*A. bipolaris*
6. Ascospores monomorphic.....*A. sunyatsenii*
6. Ascospores dimorphic.....*A. seychellensis*

***Neojahnula*** W. Dong, H. Zhang & K.D. Hyde, *gen. nov.*

*Index Fungorum number:* IF557823; *Facesoffungi number:* FoF07688

*Etymology:* named refers to its morphological similarity to *Jahnula*

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* scattered or clustered in small groups, superficial, subglobose or obpyriform, brown to black, membranous, with scarcely projecting papilla, ostiolate, with few setae. *Peridium* thin, one-layered. *Pseudoparaphyses* numerous, cellular, hypha-like, hyaline, sparsely septate. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, sessile or short pedicellate, with an ocular chamber and faint apical ring. *Ascospores* uni- to bi-seriate, elongate, ellipsoidal, tapering towards the apices, brown, 1-septate, upper cell slightly wider than lower cell. **Asexual morph:** Undetermined.

*Type species:* *Neojahnula australiensis* (K.D. Hyde) W. Dong, H. Zhang & K.D. Hyde

*Notes:* *Jahnula australiensis* K.D. Hyde was collected from submerged wood in freshwater in Australia (Hyde 1993a). It was initially placed in *Jahnula* because the peridium comprises massive pseudoparenchymatous cells, which is the remarkable feature of *Jahnula* (Hyde 1993a). However, *J. australiensis* was shown to not belong to *Jahnula sensu stricto* and clustered basal to two asexual genera *Brachiosphaera* Nawawi and *Speiropsis* Tubaki with strong bootstrap support (Prihatini et al. 2008; Hyde et al. 2017; Huang et al. 2018; this study, Fig. 14). *Jahnula australiensis* is not supported in *Jahnula* because it has few setae surrounding the ascomata, a thin peridium comprising single row of cells, asci with an ocular chamber and faint apical ring and uni- to bi-seriate ascospores, these features are different from *Jahnula*. As suggested by Hyde et al. (2013), we transfer *J. australiensis* to a new genus, *Neojahnula* based on phylogenetic analyses and morphology.

**List of freshwater *Neojahnula* species**

\**Neojahnula australiensis* (K.D. Hyde) W. Dong, H. Zhang & K.D. Hyde, *comb. nov.*

*Index Fungorum number:* IF557899; *Facesoffungi number:* FoF09241; Fig. 12

*Basionym:* *Jahnula australiensis* K.D. Hyde, Aust. Syst. Bot. 6(2): 161 (1993)

*Freshwater distribution:* Australia (Hyde 1993a), Brunei (Ho et al. 2001), China (Ho et al. 2001), Peru (Shearer et al. 2015), Thailand (Suetrong et al. 2011a; this study) USA (Raja et al. 2009b)

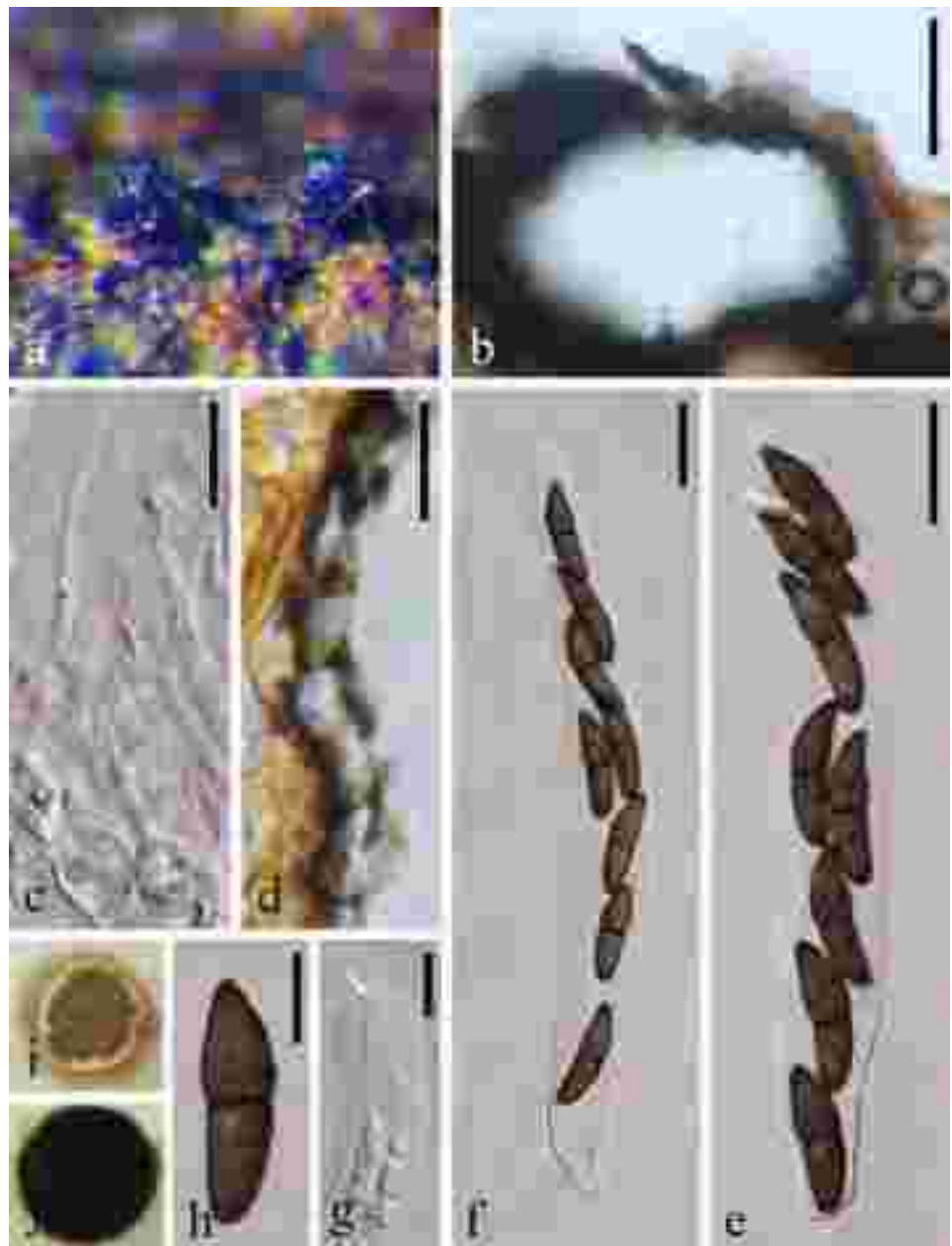
*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* 80–110  $\mu\text{m}$  high, 100–130  $\mu\text{m}$  diam., scattered or gregarious, superficial, subglobose or lenticulate when compressed, black, membranous, with scarcely projecting papilla, ostiolate, with few hyaline to pale brown hyphae. *Peridium* thin, 5–10  $\mu\text{m}$  thick, composed of one row of large, thin-walled, brown to dark brown cells of *textura angularis*. *Pseudoparaphyses* 3  $\mu\text{m}$  diam., numerous, cellular, hypha-like, hyaline, sparsely septate. *Asci* 135–175  $\times$  17–19  $\mu\text{m}$  ( $\bar{x}$  = 155  $\times$  17.5  $\mu\text{m}$ , n = 5), 8-spored, bitunicate, fissitunicate, cylindrical, sessile or short pedicellate, with an ocular chamber and faint apical ring. *Ascospores* 23–27.5  $\times$  6–7.5  $\mu\text{m}$  ( $\bar{x}$  = 25.5  $\times$  7  $\mu\text{m}$ , n = 15), uni- to bi-seriate, elongate, ellipsoidal, tapering towards the apices, rounded at the base, brown, 1-septate, constricted at the septum, upper cell slightly wider than lower cell, guttulate, mostly curved, smooth, thin-walled, without sheath. **Asexual morph:** Undetermined.

*Culture characteristics:* On PDA, colony circular, reaching 20 mm in 25 days at 25 °C, reddish brown from above, dark brown to black from below, surface rough, with dense mycelium, fluffy, raised as a annulus around the margin, dry, edge entire.

*Material examined:* THAILAND, Prachuap Khiri Khan Province, on submerged wood in a stream, 30 July 2015, K.D. Hyde, 66D (MFLU 15-2711), living culture MFLUCC 15-0975.

*Notes:* Our collection MFLUCC 15-0975 clusters with two strains of *Neojahnula australiensis* (SS3613 and SS0665) with high bootstrap support (Fig. 14). SS3613 only has ITS and SS0665 only has SSU sequence data in GenBank, which are identical with MFLUCC 15-0975. The morphology of SS3613 and SS0665 cannot be compared as they were not provided in Prihatini et al. (2008) and Pang et al. (2002), respectively. The sequence data from ex-type strain of *N. australiensis* is lacking, but our collection MFLUCC 15-0975 has identical morphological characteristics with the holotype (BRIP 19208), except for longer asci (135–175  $\times$  17–19  $\mu\text{m}$  vs. 90–140  $\times$  14–18  $\mu\text{m}$ ) (Hyde 1993a). Based on morphological characteristics and available sequence data, MFLUCC 15-0975 is identified as *N. australiensis*.

**Fig. 12** *Neojahnula australiensis* (MFLU 15-2711). **a** Ascomata on host surface. **b** Vertical section of ascoma. **c** Pseudoparaphyses. **d** Structure of peridium. **e, f** Bitunicate asci. **g** Ocular chamber and faint apical ring. **h** Ascospore. **i, j** Colony on PDA (up-front, down-reverse). Scale bars: **b, e, f** = 20  $\mu$ m, **c** = 15  $\mu$ m, **d** = 5  $\mu$ m, **g, h** = 10  $\mu$ m



***Pseudojahnula*** W. Dong, H. Zhang & K.D. Hyde, *gen. nov.*

*Index Fungorum* number: IF557824; *Facesoffungi* number: FoF07689

*Etymology*: named refers to its morphological similarity to *Jahnula*

*Saprobic* on submerged wood. **Sexual morph**: *Ascomata* solitary or gregarious, erumpent, become superficial with base remaining immersed, subglobose, obpyriform or almost conical, hyaline and then metallic grey, coriaceous, with ostiolate papilla, with algal associations. *Peridium* comprising several layers of relatively large, hyaline, thin-walled, angular cells and covered with sparse hyaline hairs. *Pseudoparaphyses* numerous, trabeculate, filamentous,

hyaline, septate, unbranched between the asci, branching and anastomosing above. *Asci* 8-spored, bitunicate, fissitunicate, obclavate, pedicellate, with an ocular chamber and faint ring. *Ascospores* bi- to tri-seriate near the base, overlapping uniseriate near the apex, 1-septate, light brown, ellipsoid-fusiform, surrounded by a mucilaginous sheath, which is wavy in outline. **Asexual morph**: Undetermined.

*Type species*: *Pseudojahnula potamophila* (K.D. Hyde & S.W. Wong) W. Dong, H. Zhang & K.D. Hyde

*Notes*: *Jahnula potamophila* is morphologically closest to *Ascagilis bipolaris* in ascospore size, but differs by narrow mucilaginous sheath which is wavy in outline (Hyde and Goh 1999b; Hyde and Wong 1999). Both species cluster

distantly in Jahnulales (Fig. 14). The phylogenetic placement of *J. potamophila* is unstable, it clustered with *Jahnula sensu stricto* clade with low bootstrap support (Huang et al. 2018), but had good affinity in Hyde et al. (2017). With *Megalohypha aqua-dulces* and our new sequences added in the order, *J. potamophila* clustered with *M. aqua-dulces* with moderate bootstrap support (Hyde et al. 2019; this study, Fig. 14).

*Jahnula potamophila* has hyaline to metallic grey ascospores, a several-layered peridium comprising hyaline cells and covered with sparse hyaline hairs, obclavate asci with an ocular chamber and faint ring, and ascospores surrounded by a wavy mucilaginous sheath (Hyde and Wong 1999). These characteristics differ from *Jahnula*, therefore, *Pseudojahnula* is introduced to accommodate *J. potamophila*.

#### List of freshwater *Pseudojahnula* species

\**Pseudojahnula potamophila* (K.D. Hyde & S.W. Wong) W. Dong, H. Zhang & K.D. Hyde, *comb. nov.*

*Index Fungorum number*: IF557900; *Facesoffungi number*: FoF09242; Fig. 6a, e, h

*Basionym*: *Jahnula potamophila* K.D. Hyde & S.W. Wong, *Nova Hedwigia* 68(3–4): 499 (1999)

*Freshwater distribution*: Australia (Hyde and Wong 1999), Costa Rica (Raja and Shearer 2006), USA (Raja et al. 2009b)

*Megalohypha* A. Ferrer & Shearer, Ferrer, Sivichai & Shearer, *Mycologia* 99: 456 (2007)

*Saprobic* on submerged wood. **Sexual morph**: *Ascomata* superficial, uniloculate, globose to obpyriform, hyaline, membranous, with ostiolate papilla, stalked or sessile. *Peridium* composed of large, hyaline, thin-walled cells. *Pseudoparaphyses* numerous, trabeculate, filamentous, hyaline, septate, branched, anastomosing above the asci. *Asci* 8-spored, bitunicate, fissitunicate, broadly clavate or fusiform, short pedicellate, with or without an ocular chamber. *Ascospores* irregularly arranged, ellipsoidal, acutely tapered at apices, straight, brown to dark brown, 1-septate, symmetrical, rough-walled, longitudinally striate, without appendages or gelatinous sheath (Ferrer et al. 2007). **Asexual morph**: Undetermined.

*Type species*: *Megalohypha aqua-dulces* A. Ferrer & Shearer

*Notes*: *Megalohypha* is monotypic with *M. aqua-dulces* occurring on submerged decorticated wood in Panama and Thailand (Ferrer et al. 2007). *Megalohypha* can be easily distinguished from *Aliquandostipite* and *Jahnula* based on its pale to dark brown, 1-septate, rough-walled ascospores with longitudinally sulcate striations and without appendages or gelatinous sheath (Ferrer et al. 2007). It formed a moderately supported clade with *Pseudojahnula* (Hyde et al. 2019) and this study (Fig. 14).

#### List of freshwater *Megalohypha* species

\**Megalohypha aqua-dulces* A. Ferrer & Shearer, *Mycologia* 99(3): 458 (2007); Fig. 5f–j

*Freshwater distribution*: Panama (Ferrer et al. 2007), Thailand (Ferrer et al. 2007)

#### Key to freshwater asexual genera of Aliquandostipitaceae

1. Chlamydo-spores long, multi-septate.....*Xylomyces*
1. Conidia tetra-radiate with 4–8 appendages.....*Brachiosphaera*

*Brachiosphaera* Nawawi, Descals, Nawawi & Webster, *Trans. Br. Mycol. Soc.* 67: 213 (1976)

*Saprobic* on submerged wood, wet wood and river foam. **Sexual morph**: Undetermined. **Asexual morph**: *Colonies* effuse, mycelium mostly immersed. *Hyphae* varying in width, septate, hyaline at first, turning olivaceous brown with age. *Conidiophores* macronematous, erect, unbranched, hyaline, septate, of variable length. *Conidiogenous cells* holoblastic, sympodial. *Conidia* acrogenous, tetra-radiate, clustered in chains, ellipsoid or round-shaped, with 4–10 conidial arms, each 1–4-septate (Nawawi and Webster 1976; Suetrong et al. 2011a).

*Type species*: *Brachiosphaera tropicalis* Nawawi

*Notes*: *Brachiosphaera* species are commonly recorded in freshwater habitats as listed below. The genus is characterized by tetra-radiate conidia with a round central cell with 4–10 arms, each of them 1–4-septate (Nawawi and Webster 1976; Suetrong et al. 2011a). The conidia of *B. tropicalis* consist of a brown, spherical body and furnished with 4–5 arms, which are longer than 1.5 times diam. of the central part (Nawawi and Webster 1976; Suetrong et al. 2011a). Currently, *B. tropicalis* is phylogenetically related to Jahnulales (Fig. 14). Sequence data for *B. jamaicensis* is necessary to shed light on its phylogenetic affinities.

#### List of freshwater *Brachiosphaera* species

*Brachiosphaera jamaicensis* (J.L. Crane & Dumont) Nawawi, *Trans. Br. mycol. Soc.* 67(2): 216 (1976)

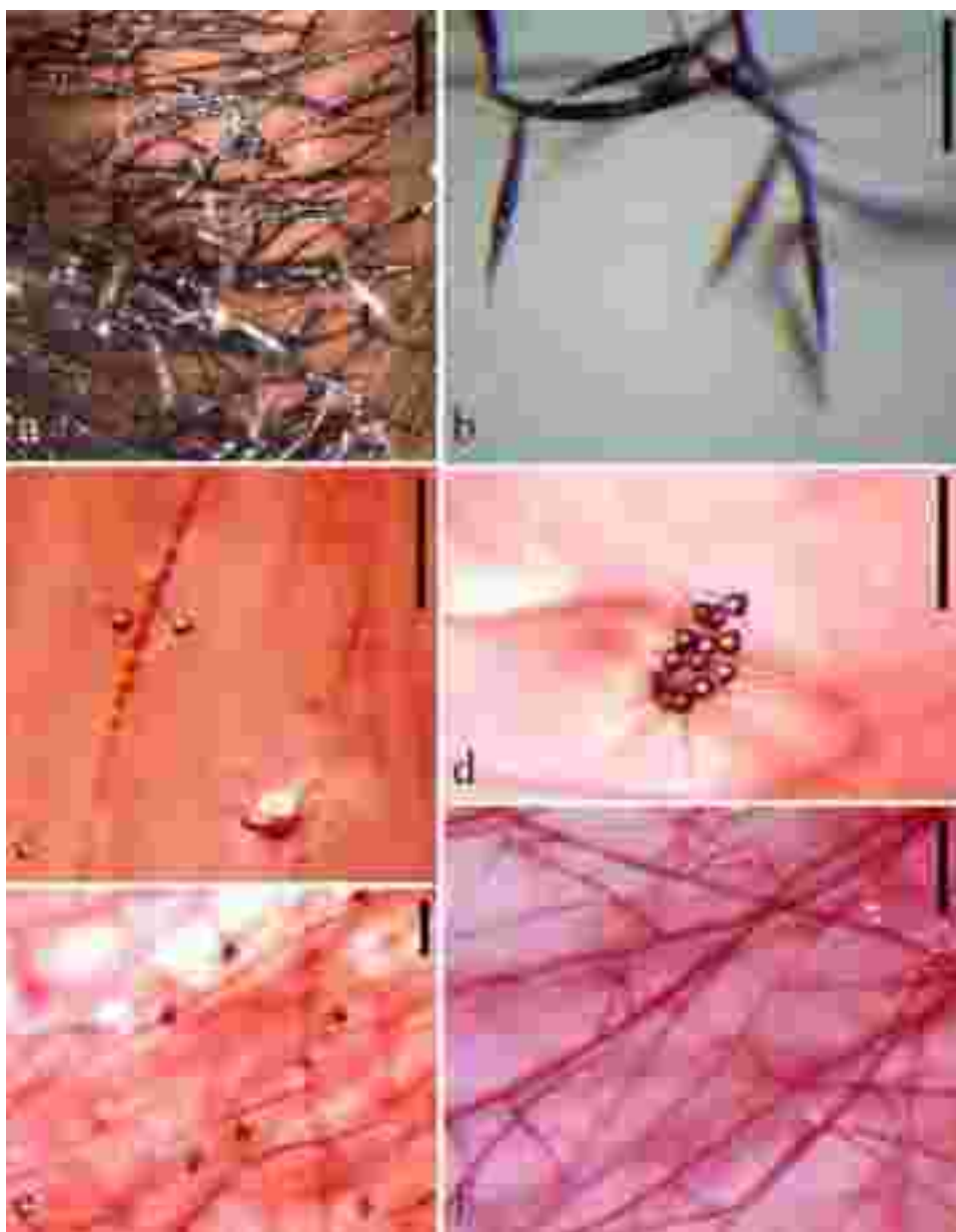
*Basionym*: *Actinospora jamaicensis* J.L. Crane & Dumont, *Can. J. Bot.* 53(9): 843 (1975)

*Freshwater distribution*: Jamaica (Crane and Dumont 1975), China (Chang 1994), Poland (Suetrong et al. 2011a)

\**Brachiosphaera tropicalis* Nawawi, *Trans. Br. mycol. Soc.* 67(2): 213 (1976); Fig. 13c–f

*Freshwater distribution*: China (Chang 1994; Cai et al. 2002a), Malaysia (Nawawi and Webster 1976), Panama (Campbell et al. 2007), Peru (Shearer et al. 2015), Puerto Rico (Nieves-Rivera and Santos-Flores 2005), Thailand (Tubaki et al. 1983; Suetrong et al. 2011a), USA (Raja et al. 2009b), Venezuela (Smits et al. 2007)

**Fig. 13 Aliquandostipitaceae spp.** (Material examined: USA, Tennessee, Great Smoky Mountains National Park, on submerged decorticated wood, 21 June 2005, H.A. Raja, A.N. Miller & E.B. Lickey, H58-4; PANAMA, Colon Province, Barro Colorado Island, small river, January 2003, on submerged decorticated wood, A. Ferrer, E192-1). **a, b** Conidia of *Xylomyces chlamyosporus* (H58-4). **c–f** *Brachiosphaera tropicalis* (E192-1). **c–e** Conidia in PDA. **f** Broad hyphae in PDA. Scale bars: **a–d** = 200  $\mu$ m, **e** = 50  $\mu$ m, **f** = 100  $\mu$ m



#### Key to freshwater *Brachiosphaera* species

1. Conidia with 4–5 arms.....*B. tropicalis*
1. Conidia with 6–10, up to 10–13 arms.....  
.....*B. jamaicensis*

*Xylomyces* Goos, Brooks & Lamore, Mycologia 69(2): 282 (1977)

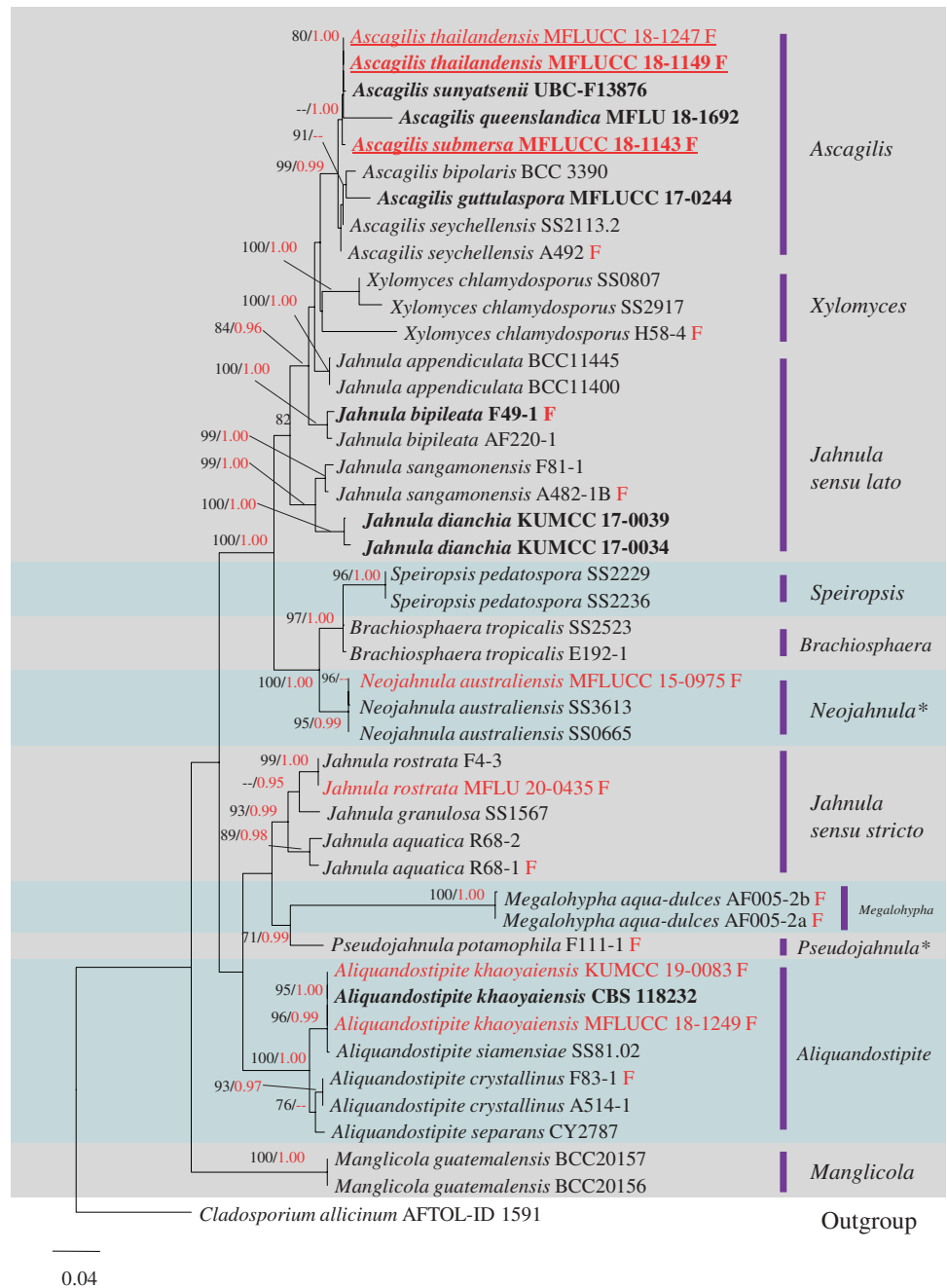
*Saprobic* on submerged wood, leaves in freshwater or rotten leaves, root in terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on natural substrate thin, effuse, reddish brown. *Mycelium* immersed and superficial, composed of branched, septate, fuscous hyphae. *Stroma* absent. *Conidiophores* and *conidia* absent. *Chlamydo-spores* abundant,

broadly fusiform, brown to blackish, intercalary, solitary or catenate, occasionally branched, straight or curved, uniform in colour or sometimes end cells paler, with thick septate, distinctly constricted at the septa, thick-walled (Goos et al. 1977).

*Type species:* *Xylomyces chlamyosporus* Goos, R.D. Brooks & Lamore

*Notes:* The chlamydo-spores of *Xylomyces* are often found on submerged wood in freshwater habitats (Goos et al. 1977; Goh et al. 1997). The type species *X. chlamyosporus* was shown to belong in Aliquandostipitaceae based on molecular data (Campbell et al. 2007; Huang et al. 2018). Freshwater species *X. aquaticus* (Dudka) K.D. Hyde & Goh and *X. elegans* Goh et al. clustered in Pleosporales, therefore they were excluded from *Xylomyces* (Prihatini et al. 2008; Suetrong et al.

**Fig. 14** Phylogram generated from maximum likelihood analysis of combined LSU, SSU and ITS sequence data for species of Jahnulales. Bootstrap values for maximum likelihood equal to or greater than 70% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. The tree is rooted to *Cladosporium allacinum* AFTOL-ID 1591 (Capnodiales)



2011a; Tanaka et al. 2015). Five species are accepted in the genus, however, only *X. chlamyosporus* has been confirmed with molecular data. The species can be distinguished by chlamyospores size, septation and ornamentation of wall (see key below). This genus is in need of additional molecular studies to establish if other described species are phylogenetically related to *X. chlamyosporus* within Jahnulales (Fig. 14).

**List of freshwater *Xylomyces* species**

*Xylomyces acerosisporus* M.S. Oliveira, Malosso & R.F. Castañeda, Mycotaxon 130: (2015)

*Freshwater distribution:* Brazil (Oliveira et al. 2015)

\**Xylomyces chlamyosporus* Goos, R.D. Brooks & Lamore [as ‘chlamyosporis’], Mycologia 69(2): 282 (1977); Fig. 13a, b

*Freshwater distribution:* Australia (Hyde and Goh 1997), Brunei (Goh et al. 1997; Fryar et al. 2004), China (Luo et al. 2004), China (Goh et al. 1997; Tsui et al. 2001c), Seychelles (Hyde and Goh 1998b), USA (Goos et al. 1977; Lamore and Goos 1978; Raja et al. 2009b)

*Xylomyces giganteus* Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101(11): 1324 (1997)

*Freshwater distribution:* Australia (Goh et al. 1997), China (Jiang et al. 2008), South Africa (Hyde et al. 1998)

*Xylomyces punctatus* Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101(11): 1328 (1997)

*Freshwater distribution:* China (Goh et al. 1997; Tsui et al. 2001c)

*Xylomyces pusillus* Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101(11): 1328 (1997)

*Freshwater distribution:* China (Goh et al. 1997; Tsui et al. 2001c; Cai et al. 2002a)

### Key to freshwater *Xylomyces* species

1. Chlamydo-spores > 25 µm wide.....2
1. Chlamydo-spores < 20 µm wide.....3
2. Chlamydo-spores 5–9-septate.....*X. chlamydo-sporis*
2. Chlamydo-spores 6–26-septate.....*X. giganteus*
3. Chlamydo-spores with distinct punctate wall.....  
.....*X. punctatus*
3. Chlamydo-spores lack distinct punctate wall.....4
4. Chlamydo-spores 42–56 × 7–11 µm.....*X. pusillus*
4. Chlamydo-spores 95–180 × 8–10 µm.....  
.....*X. acerosisporus*

**Kirschsteinietheliales** Hern.-Restr., R.F. Castañeda, Gené & Crous, Stud. Mycol. 86: 72 (2017)

**Kirschsteinietheliaceae** Boonmee & K.D. Hyde, Mycologia 104(3): 705 (2012)

*Kirschsteiniethelia* D. Hawksw., J. Linn. Soc., Bot. 91: 182 (1985)

*Synonymy:* *Dendryphiopsis* S. Hughes, Can. J. Bot. 31:655 (1953)

*Saprobic* mostly on dead wood in terrestrial or submerged wood in freshwater habitats. **Sexual morph:** *Ascomata* superficial, solitary or clustered, subglobose to globose, membranaceous, dark brown to black, with a central papilla. *Peridium* composed of several layers of cells of *textura angularis*. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline. *Asci* 8-spored, bitunicate, fission-tunicate, cylindrical-clavate, long pedicellate, apically rounded, with an ocular chamber. *Ascospores* biseriate, ellipsoidal, dull green, olive brown to dark brown at maturity, 1–2-septate, smooth-walled (Hawksworth 1985; Boonmee et al. 2012). **Asexual morph:** Hyphomycetous. *Conidiophores* macronematous, mononematous, erect, gregarious, elongate and thick-walled, straight and slightly curved, apically

branched, septate, brown to dark brown, smooth-walled. *Conidigenous cells* holoblastic, monoblastic, terminal, constricted at delimiting septa. *Conidia* broadly ellipsoid-obovoid, fusiform to obclavate, rounded at both ends, initially 1-septate, later becoming 2-septate, occasionally 3-septate, constricted and darkly pigmented at the septa, reddish brown to dark brown, grayish brown, smooth-walled (Hyde et al. 2013; Su et al. 2016b).

*Type species:* *Kirschsteiniethelia atra* (Corda) D. Hawksw.

*Notes:* *Kirschsteiniethelia* is characterized by superficial, subglobose to globose *ascomata*, cylindrical-clavate *asci* and ellipsoidal, dull green, olive-brown to dark brown *ascospores* (Boonmee et al. 2012). The asexual morph of *Kirschsteiniethelia* has been connected to *Dendryphiopsis*, typified by *D. atra* (Corda) S. Hughes with molecular data (Boonmee et al. 2012; Hyde et al. 2013; Su et al. 2016b). For convenience, Wijayawardene et al. (2014) proposed to use *Kirschsteiniethelia* over *Dendryphiopsis* and named *K. atra* as the type species. All *Kirschsteiniethelia* species formed a well-supported clade and were placed in Kirschsteinietheliaceae by Boonmee et al. (2012). Six freshwater species with molecular data have been accepted in the genus and all of which are asexual morphs (see list below). All freshwater species have unbranched conidiophores and mostly clavate conidia (slender conidia in *K. fluminicola* Z.L. Luo et al.), except *K. aethiops* (Sacc.) D. Hawksw. producing branched conidiophores and cylindrical conidia (Su et al. 2016b).

### List of freshwater *Kirschsteiniethelia* species

\**Kirschsteiniethelia aquatica* Z.L. Luo, K.D. Hyde & H.Y. Su, Mycosphere 9(4): 759 (2018)

*Freshwater distribution:* China (Bao et al. 2018)

\**Kirschsteiniethelia atra* (Corda) D. Hawksw., Fungal Diversity 69:37 (2014); Fig. 15a–h

*Basionym:* *Dendryphion atrum* Corda, Icon. fung. (Prague) 4: 33 (1840)

*Synonymy:* *Amphisphaeria aethiops* Sacc., Syll. fung. (Abellini) 1: 722 (1882)

*Dendryphiopsis atra* (Corda) S. Hughes, Can. J. Bot. 31: 655 (1953)

*Sphaeria aethiops* Berk. & Curtis, Grevillea 4: 143 (1876)

*Kirschsteiniethelia aethiops* (Sacc.) D. Hawksw., J. Linn. Soc., Bot. 91(1–2): 185 (1985)

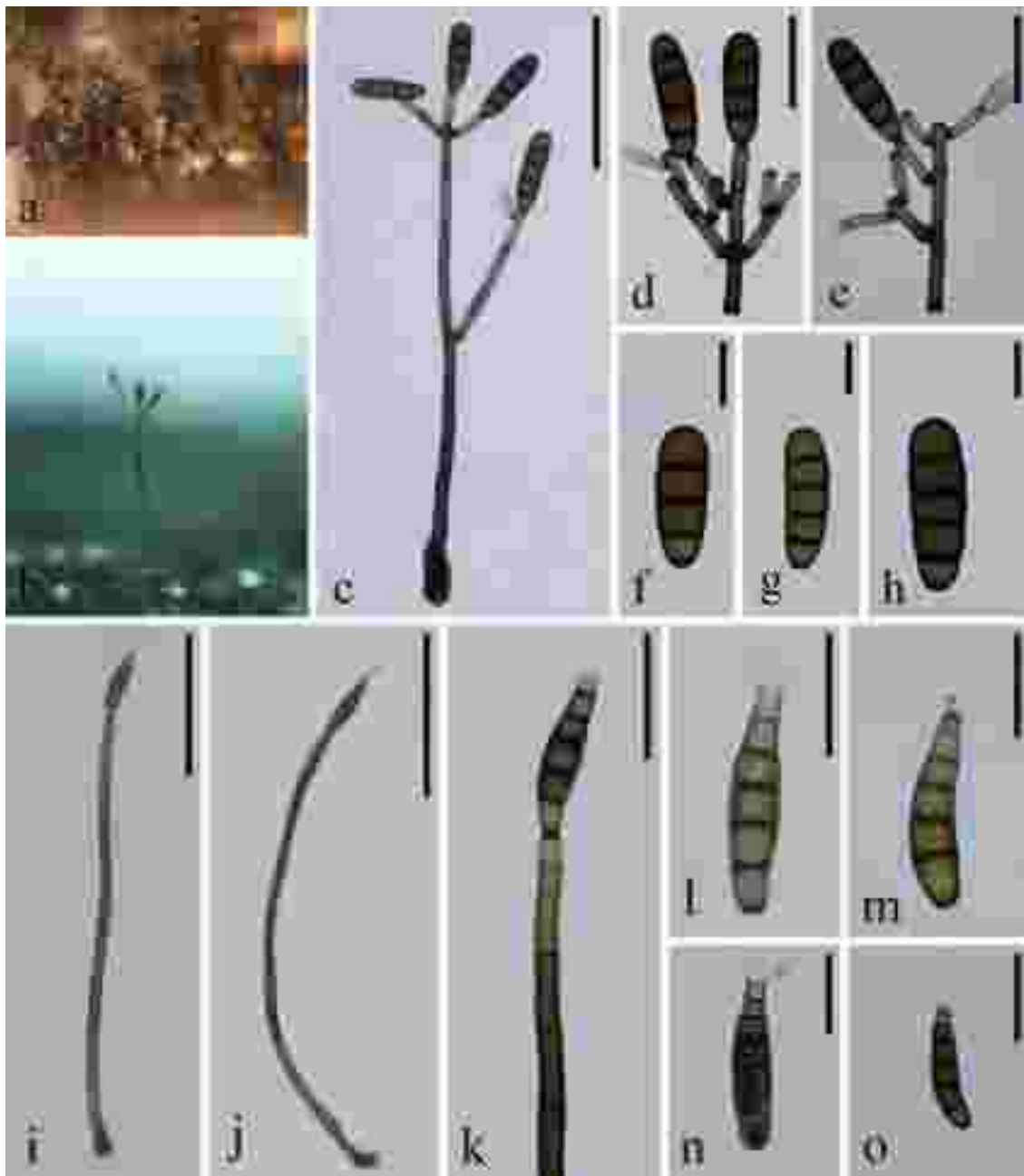
*Freshwater distribution:* China (Su et al. 2016b)

\**Kirschsteiniethelia cangshanensis* Z.L. Luo, D.F. Bao, K.D. Hyde & H.Y. Su, Mycosphere 9(4): 760 (2018)

*Freshwater distribution:* China (Bao et al. 2018)

\**Kirschsteiniethelia fluminicola* Z.L. Luo, K.D. Hyde & H.Y. Su, Mycosphere 9(4): 760 (2018)

*Freshwater distribution:* China (Bao et al. 2018)



**Fig. 15** *Kirschsteinothelia* spp. (Material examined: CHINA, Yunnan Province, Dali, Cangshan Mountain, Huadianba, saprobic on decaying wood submerged in stream, May 2014, Y. Su, S-144, HKAS 84022; *ibid.*, on decaying wood submerged in Wanhua Stream, March 2014, X.Y. Liu, S-043, HKAS 93066, **holotype**). **a–h** *Kirschsteinothelia*

*atra* (HKAS 84022). **a, b** Colonies on the substratum. **c** Conidiophores with conidia. **d, e** Conidiogenous cells and conidia. **f–h** Conidia. **i–o** *Kirschsteinothelia submersa* (HKAS 93066). **i, j** Conidiophores with conidia. **k** Conidiogenous cells with conidia. **l–o** Conidia. Scale bars: **c** = 130  $\mu$ m, **d, e** = 50  $\mu$ m, **f–h, l–o** = 20  $\mu$ m, **i, j** = 100  $\mu$ m, **k** = 30  $\mu$ m

\**Kirschsteinothelia rostrata* J Yang & K.D. Hyde, Fungal Diversity 87: 45 (2017)

*Freshwater distribution*: China (Bao et al. 2018), Thailand (Hyde et al. 2017)

\**Kirschsteinothelia submersa* H.Y. Su & K.D. Hyde, Fungal Diversity (2016); Fig. 15i–o

*Freshwater distribution*: China (Su et al. 2016b)

### Key to freshwater *Kirschsteiniothelia* species

1. Conidiophores branched.....*K. atra*
1. Conidiophores unbranched.....2
2. Conidia slender, solitary to short-catenate.....  
.....*K. fluminicola*
2. Conidia not slender, solitary.....3
3. Conidia with sheath.....4
3. Conidia without sheath.....5
4. Conidia 33–43 µm long.....*K. cangshanensis*
4. Conidia 80–150 µm long.....*K. rostrata*
5. Conidiophores 114–151 × 7–8 µm.....*K. aquatica*
5. Conidiophores 220–280 × 6–7 µm.....*K. submersa*

**Minutisphaerales** Raja, Oberlies, Shearer & A.N. Mill., *Mycologia* 107(4): 854 (2015)

**Acrogenosporaceae** Jayasiri & K.D. Hyde, *Mycosphere* 9(4): 809 (2018)

***Acrogenospora*** M.B. Ellis, *Dematiaceous Hyphomycetes* (Kew): 114 (1971)

*Saprobic* on decaying wood, bark, culms of bamboo in freshwater or terrestrial habitats. **Sexual morph:** *Hysterothecia* laterally compressed, with a prominent sunken slit, solitary to gregarious, erect and elevated, presenting an almost stipitate appearance, thick-walled. *Pseudoparaphyses* branched. *Asci* 8-spored, cylindrical, fusoid or obovate, obtuse at the apex, short pedicellate, thin-walled. *Ascospores* oval, aseptate, hyaline or moderately pigmented (Mason 1941; Jayasiri et al. 2018). **Asexual morph:** Hyphomycetous. *Colonies* effuse, dark brown to black, glistening, hairy. *Mycelium* mostly immersed, consist of septate, thin-walled, smooth, hyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, solitary, erect, subulate or cylindrical, straight or slightly flexuous, pale brown to dark brown, septate, unbranched, smooth. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal or intercalary, with percurrent proliferations, cylindrical. *Conidia* solitary, dry, acrogenous, simple, spherical, subspherical, olive to brown, aseptate, truncate at the base, smooth or verrucose (Ellis 1971; Hyde et al. 2019).

*Type species:* *Acrogenospora sphaerocephala* (Berk. & Broome) M.B. Ellis

*Notes:* *Acrogenospora* is a holomorphic genus, characterized by laterally compressed hysterothecia with a prominent sunken slit, oval, aseptate, hyaline ascospores and spherical, olive to brown conidia (Hyde et al. 2019). *Acrogenospora* is used to suppress *Farlowiella* Sacc. (Rossmann et al. 2015) and their sexual-asexual connections were linked based on molecular data (Jayasiri et al. 2018). Thus, *Acrogenospora* is the only genus in Acrogenosporaceae (Jayasiri et al. 2018; Hyde et al. 2019). The sequence data of the type species *A. sphaerocephala* were generated based on a freshwater collection from Thailand

(Hyde et al. 2019). Bao et al. (2020) introduced seven new species collected from a small area of Yunnan Province, China, which indicated *Acrogenospora* is a speciose genus. 20 species were reported in *Acrogenospora* and 13 have been confirmed with molecular data (Bao et al. 2020). Freshwater *Acrogenospora* species are morphologically very similar and the sequence data are main evidence to separate them.

### List of freshwater *Acrogenospora* species

\**Acrogenospora aquatica* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 3 (2020)  
*Freshwater distribution:* Yunnan, China (Bao et al. 2020)

\**Acrogenospora basalicellularispora* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 8 (2020)  
*Freshwater distribution:* Yunnan, China (Bao et al. 2020)

\**Acrogenospora ellipsoidea* D.M. Hu, L. Cai & K.D. Hyde, *Sydowia* 62(2): 194 (2010)  
*Freshwater distribution:* Yunnan, China (Hu et al. 2010a)

\**Acrogenospora guttulatispora* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 8 (2020)  
*Freshwater distribution:* Yunnan, China (Bao et al. 2020)

\**Acrogenospora obovoidispora* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 8 (2020)  
*Freshwater distribution:* Yunnan, China (Bao et al. 2020)

\**Acrogenospora olivaceospora* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 10 (2020)  
*Freshwater distribution:* Yunnan, China (Bao et al. 2020)

*Acrogenospora ovalis* Goh, K.D. Hyde & C.K.M. Tsui [as ‘*ovalia*’], *Mycol. Res.* 102(11): 1312 (1998)  
*Freshwater distribution:* Hong Kong (China) (Goh et al. 1998b)

\**Acrogenospora sphaerocephala* (Berk. & Broome) M.B. Ellis, *Dematiaceous Hyphomycetes* (Kew): 114 (1971)  
*Basionym:* *Monotospora sphaerocephala* Berk. & Broome, *Ann. Mag. nat. Hist.*, Ser. 3 3: 361 (1859)  
*Synonymy:* *Halysium sphaerocephalum* (Berk. & Broome) Vuill., *Bull. Séanc. Soc. Sci. Nancy, Sér. 3* 11: 167 (1911)

*Monosporella sphaerocephala* (Berk. & Broome) S. Hughes, *Can. J. Bot.* 31: 654 (1953)

*Monotosporella sphaerocephala* (Berk. & Broome) S. Hughes, *Can. J. Bot.* 36: 787 (1958)

*Freshwater distribution:* China (Goh and Hyde 1999; Tsui et al. 2000), Philippines (Cai et al. 2003a), Seychelles (Hyde and Goh 1998b), Thailand (Sivichai et al. 2000; Hyde et al. 2019), USA (Lamore and Goos 1978; Shearer and Crane 1986)



\**Acrogenospora submersa* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 12 (2020)

*Freshwater distribution*: Yunnan, China (Bao et al. 2020)

\**Acrogenospora subprolata* Goh, K.D. Hyde & C.K.M. Tsui, *Mycol. Res.* 102(11): 1314 (1998)

*Freshwater distribution*: Australia (Goh et al. 1998b), China (Goh et al. 1998b; Tsui et al. 2001c; Bao et al. 2020), Seychelles (Goh et al. 1998b), South Africa (Goh et al. 1998b), UK (Goh et al. 1998b)

\**Acrogenospora thailandica* J. Yang & K.D. Hyde, *Fungal Diversity* 96: 78 (2019)

*Freshwater distribution*: Thailand (Hyde et al. 2019)

\**Acrogenospora verrucispora* Hong Zhu, L. Cai & K.Q. Zhang [as '*verrucospora*'], *Mycotaxon* 92: 384 (2005)

*Freshwater distribution*: Yunnan, China (Zhu et al. 2005; Bao et al. 2020)

\**Acrogenospora yunnanensis* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Frontiers in Microbiology* 11: 18 (2020)

*Freshwater distribution*: Yunnan, China (Bao et al. 2020)

#### Key to freshwater *Acrogenospora* species

1. Conidiophores 850–950 × 3.5–8 µm.....  
.....*A. thailandica*
1. Conidiophores < 800 µm long.....2
2. Conidiophores have wide range of length, 100–730 × 7.2–10.5 µm.....*A. sphaerocephala*
2. Conidiophores not as above.....3
3. Conidia with a small, hyaline basal cell.....4
3. Conidia without basal cell.....5
4. Conidiophores 202–250 × 7.5–9.5 µm.....  
.....*A. aquatica*
4. Conidiophores 259–395 × 8–12 µm.....  
.....*A. basalicellularispora*
5. Conidia hyaline when young, darker when mature.....6
5. Conidia colour almost consistent.....8
6. Conidiophores 163–223 × 6.7–10 µm.....  
.....*A. submersa*
6. Conidiophores > 260 µm long.....7
7. Conidiophores 7.5–8.6 µm wide.....  
.....*A. guttulatispora*
7. Conidiophores 8.6–12 µm wide.....*A. yunnanensis*
8. Conidia verrucose.....*A. verrucispora*
8. Conidia smooth.....9
9. Conidia ellipsoidal.....*A. ellipsoidea*
9. Conidia not as above.....10
10. Conidia 24–33 × 18–22 µm.....*A. ovalia*
10. Conidia > 32 µm long.....11

11. Conidia 39–46 × 30–39 µm.....*A. subprolata*

11. Conidia < 39 µm long.....12

12. Conidiophores 209–277 × 7.5–10 µm.....  
.....*A. obovoidispora*

12. Conidiophores 102–172 × 5.8–9 µm.....  
.....*A. olivaceospora*

**Minutisphaeraceae** Raja, Oberlies, Shearer & A.N. Mill., *Mycologia* 107(4): 854 (2015)

*Minutisphaera* Shearer, A.N. Mill. & A. Ferrer, *Mycologia* 103(2): 415 (2011)

*Saprobic* on submerged wood. **Sexual morph**: *Ascomata* scattered or clustered in groups, erumpent to superficial, uniloculate, globose to subglobose, brown, with central, rounded ostiole surrounded by black hairs. *Peridium* thin, composed of *textura angularis* to *globulosa* cells. *Pseudoparaphyses* sparse in young ascomata, becoming abundant with age, cellular, hyaline, septate, simple or branched, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, ovoid to obclavate, lacking a pedicel, rounded at the apex. *Ascospores* irregularly arranged, clavate, mostly straight, hyaline to pale brown, 1(–3)-septate, constricted at the septum, multiguttulate, asymmetrical, smooth- or rough-walled when mature, with sheath and filamentous appendages (Ferrer et al. 2011). **Asexual morph**: Undetermined.

*Type species*: *Minutisphaera fimbriatispora* Shearer, A.N. Mill. & A. Ferrer

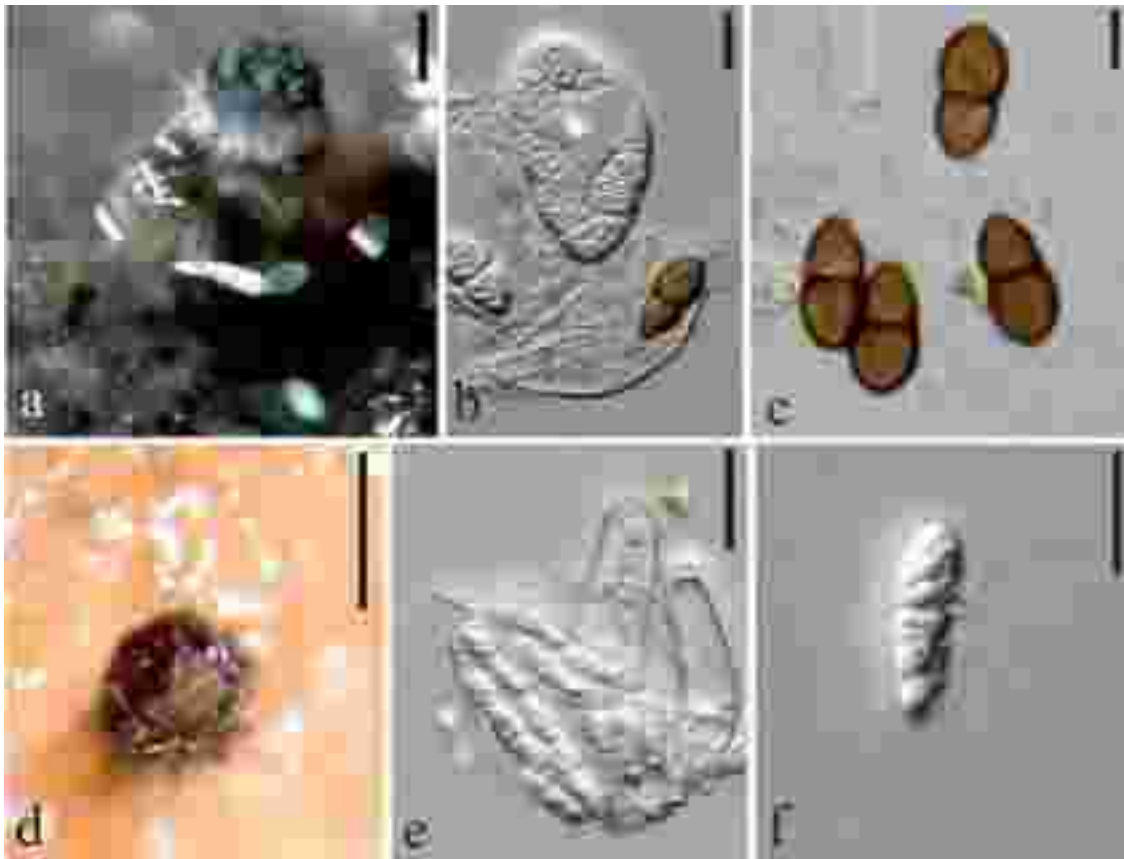
*Notes*: All species reported within *Minutisphaera* were collected from freshwater habitats (Ferrer et al. 2011; Raja et al. 2013b, 2015; Bao et al. 2019a). The ascospores of *Minutisphaera* are unusual in being asymmetrical and often equipped with a sheath and filamentous appendages (Ferrer et al. 2011; Raja et al. 2015). The shape and size of ascospores, and features of appendages and sheaths are used for distinguishing all species within the genus (see key below).

In the analysis of Raja et al. (2015) BLAST search of ITS sequences revealed that two isolates, Pleosporales sp. 39g (JX244063) and *Didymosphaeria* sp. TS\_04\_050 (HQ713763), shared 99% similarity with *Minutisphaera aspera*. Both strains were isolated as fungal endophytes in previous studies (Grünig et al. 2011). Thus, it was hypothesized that members of Minutisphaerales may lead a dual life as saprobes on submerged wood and as fungal endophytes of trees living in close proximity to rivers and streams. However, additional studies are needed to shed light on the dual ecological life style of *Minutisphaera* spp.

#### List of freshwater *Minutisphaera* species

\**Minutisphaera aquaticum* D.F. Bao, L. Xu & H.Y. Su, *Asian Journal of Mycology* 2(1): 309 (2019)

*Freshwater distribution*: Thailand (Bao et al. 2019a)



**Fig. 16** *Minutisphaera* spp. (Material examined: USA, North Carolina: Rockingham County, Piedmont Plateau, Big Beaver Island Creek, Madison, on submerged decorticated wood, 26 April 2013, H.A. Raja & N.H. Oberlies, G427-1, ILLS 72340, **holotype**; *ibid.*, G156-4, ILLS 72342,

**paratype**). **a–c** *M. aspera* (G427-1). **a** Ascoma on wood. **b** Ascus. **c** Ascospores. **d–f** *M. parafimbriatispora* (G156-4). **d** Ascoma in culture. **e** Ascus. **f** Ascospore with gelatinous sheath constricted at the mid-septum. Scale Bars: **a** = 100  $\mu$ m, **b**, **c** = 20  $\mu$ m, **d** = 50  $\mu$ m, **e**, **f** = 10  $\mu$ m

\**Minutisphaera aspera* Raja, Oberlies, Shearer & A.N. Mill, Mycologia 107(4): 854 (2015); Fig. 16a–c  
 Freshwater distribution: USA (Raja et al. 2015)

\**Minutisphaera fimbriatispora* Shearer, A.N. Mill. & A. Ferrer, Mycologia 103(2): 415 (2011)  
 Freshwater distribution: USA (Ferrer et al. 2011; Raja et al. 2013b)

\**Minutisphaera japonica* Kaz. Tanaka, Raja & Shearer, Mycologia 105(4): 966, (2013); Fig. 17  
 Freshwater distribution: Japan (Raja et al. 2013b), France (Raja et al. 2015)

\**Minutisphaera parafimbriatispora* Raja, Oberlies, Shearer & A.N. Mill, Mycologia 107(4): 855 (2015); Fig. 16d–f  
 Freshwater distribution: USA (Raja et al. 2015)

**Key to freshwater *Minutisphaera* species**

- 1. Ascospores with sheath, but lacking appendages.....2

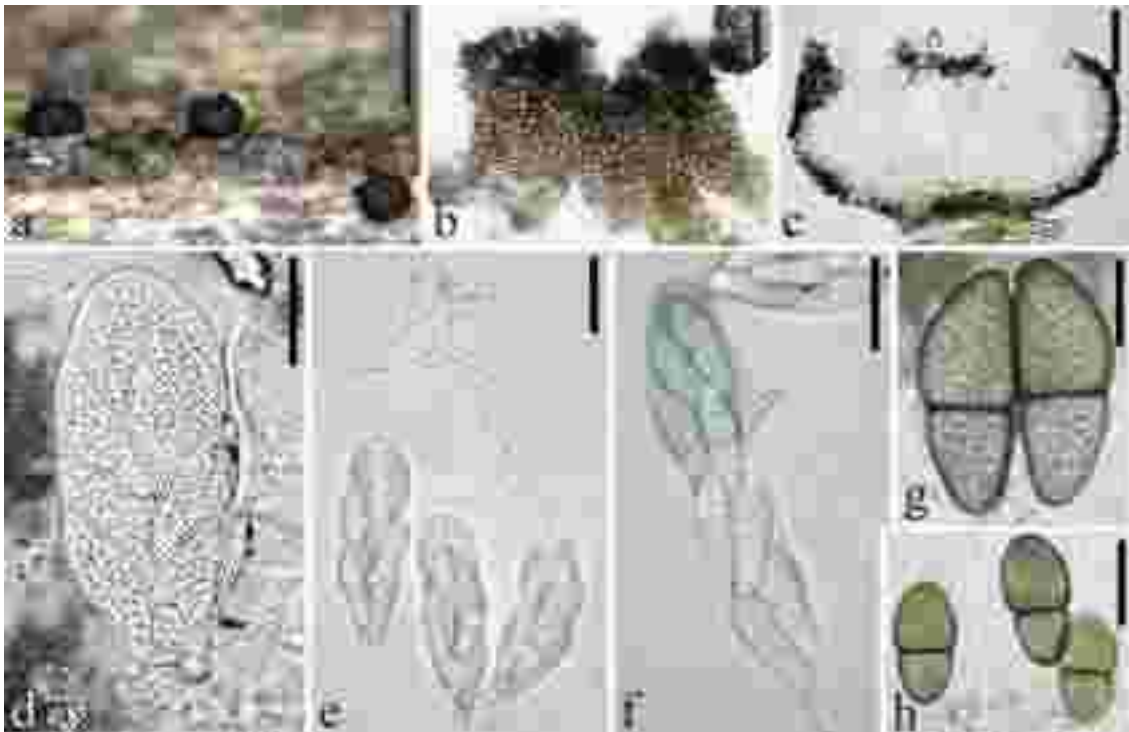
- 1. Ascospores with sheath and appendages.....4
- 2. Ascospores with rough-walled.....*M. aspera*
- 2. Ascospores smooth-walled.....3
- 3. Ascospores broadly fusiform, with acute apex.....*M. japonica*
- 3. Ascospores fusiform to clavate, with rounded apex.....*M. aquaticum*
- 4. Ascospores 24–36  $\times$  6–8  $\mu$ m.....*M. fimbriatispora*
- 4. Ascospores 18–25  $\times$  4–7  $\mu$ m.....*M. parafimbriatispora*

**Natipusillales** Raja, Shearer, A.N. Mill. & K.D. Hyde, Fungal Diversity 63: 9 (2013)

**Natipusillaceae** Raja, Shearer & A.N. Mill., Mycologia 104(2): 570 (2012)

*Natipusilla* A. Ferrer, A.N. Mill. & Shearer, Mycologia 103(2): 417 (2011)

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* scattered or aggregated, erumpent to superficial, uniloculate,



**Fig. 17** *Minutisphaera japonica* (Material examined: FRANCE, Ariège, Vernajoul, ruisseau Vernajoul, on submerged wood of *Fraxinus excelsior*, 2 July 2007, J. Fournier, JF07132, associated with *Pseudohalonectria lutea* and *Aniptodera* sp., leg det JF). **a** Ascomata

on the wood surface. **b** Squash mount of ascoma, note dark-walled hyphae on the peridial wall. **c** Longitudinal section of apothecioid ascoma. **d–f** Asci. **g, h** Ascospores. Scale bars: **a** = 200  $\mu$ m, **b, d, f, h** = 20  $\mu$ m, **c** = 50  $\mu$ m, **e** = 30  $\mu$ m, **g** = 10  $\mu$ m

globose to subglobose, hyaline to brown, lacking ostioles, occasionally with minute papilla. *Peridium* thin, membranous, glabrous, forming a *textura angularis* in surface view. *Pseudoparaphyses* sparse or absent. *Asci* 8-spored, bitunicate, fissitunicate, globose to obclavate, lacking a pedicel, with or without an ocular chamber. *Ascospores* overlapping tri- to multi-seriate, arranged in a group, fusiform or clavate, straight, the upper cell often shorter and broader than the lower one, mostly 1-septate, hyaline, occasionally becoming 3-septate, pale brown at age, guttulate when young, smooth, thin-walled, with or without a gelatinous sheath (Ferrer et al. 2011). **Asexual morph:** Undetermined.

*Type species:* *Natipusilla decorospora* A. Ferrer, A.N. Mill. & Shearer

*Notes:* All species described in this genus were reported from freshwater habitats in tropical and subtropical regions (Ferrer et al. 2011; Raja et al. 2012). Phylogenetic analysis using combined SSU and LSU sequence dataset placed this genus in a distinct clade within Dothideomycetes. Therefore, a novel family, Natipusillaceae (Raja et al. 2012), and new order Natipusillales (Hyde et al. 2013) were established for *Natipusilla*.

*Ascominuta* Ranghoo & K.D. Hyde was thought to be an earlier name of *Natipusilla* (Hyde et al. 2013) due to the bitunicate, globose asci, sheathed ascospores and freshwater

habitat (Ranghoo and Hyde 1999; Hu et al. 2010a; Ferrer et al. 2011; Raja et al. 2012). However, *Ascominuta* differs from *Natipusilla* in the numbers of ascospores per asci (4 vs. 8) and colour of ascomata (hyaline to brown vs. dark brown to black). Only LSU sequence (AF132335) of *A. lignicola* Ranghoo & K.D. Hyde (generic type) was deposited in Genbank, and it cannot be aligned with *Natipusilla*. Further molecular and morphological data are needed to clarify these two genera (Raja et al. 2012). All *Natipusilla* species are surrounded by a complex gelatinous sheath, except *N. naponensis* A. Ferrer et al. lacks a sheath (Ferrer et al. 2011; Raja et al. 2012).

#### List of freshwater *Natipusilla* species

\**Natipusilla decorospora* A. Ferrer et al., Mycologia 103(2): 417 (2011)

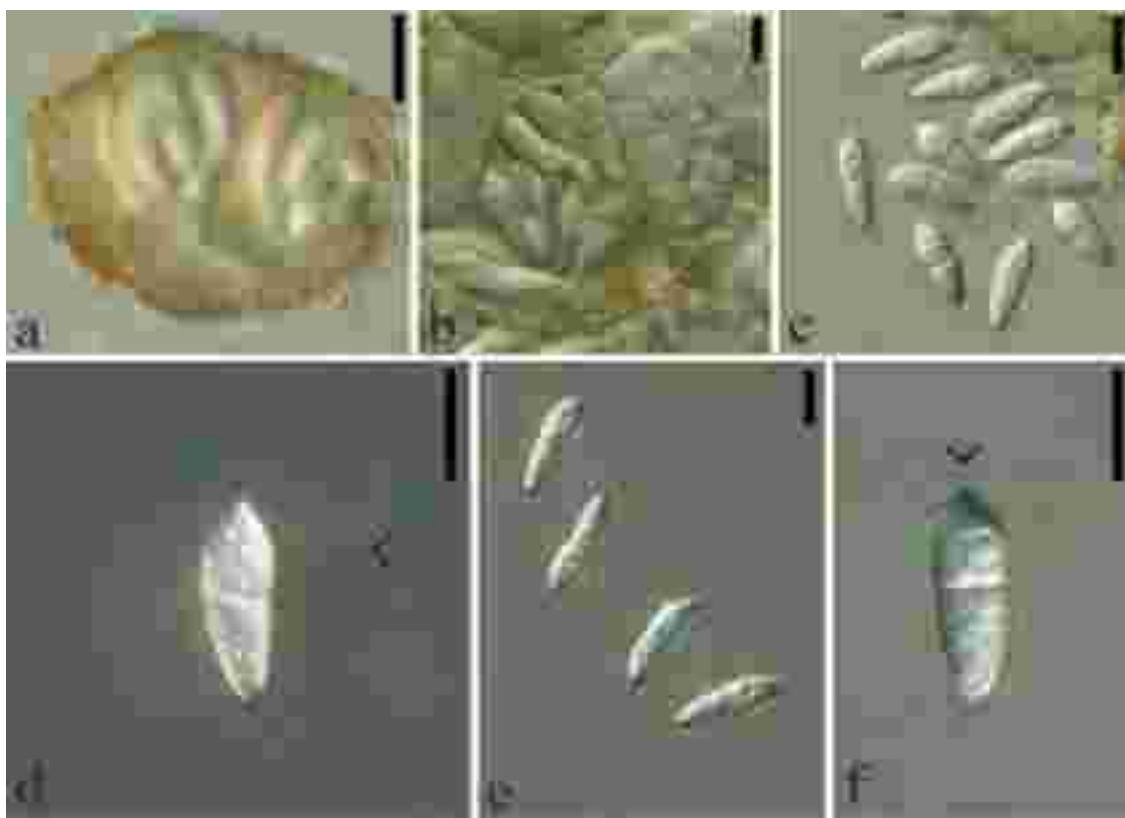
*Freshwater distribution:* Ecuador (Ferrer et al. 2011)

\**Natipusilla limonensis* A. Ferrer, A.N. Mill. & Shearer, Mycologia 103(2): 417 (2011); Fig. 18

*Freshwater distribution:* Costa Rica (Ferrer et al. 2011), Peru (Shearer et al. 2015)

\**Natipusilla naponensis* A. Ferrer, A.N. Mill. & Shearer, Mycologia 103(2): 420 (2011)

*Freshwater distribution:* Ecuador (Ferrer et al. 2011)



**Fig. 18** *Natipusilla limonensis* (Material examined: PERU, Cusco, Camanti, Stream at Quince Mil Trail 1, on submerged wood, 26 May 2010, S. Zelski & H.A. Raja, PE0003). **a** Section of ascoma. **b** Glo-

bose to subglobose asci. **c–f** Ascospores. Arrows indicating complex gelatinous sheath. Scale bars: **a** = 50  $\mu$ m, **b–f** = 20  $\mu$ m

\**Natipusilla bellaspora* Raja, Shearer & A.N. Mill., Mycologia 104(2): 570 (2012)

*Freshwater distribution:* Peru (Raja et al. 2012; Shearer et al. 2015)

#### Key to freshwater *Natipusilla* species

1. Ascospores without sheath.....*N. naponensis*
1. Ascospores with complex gelatinous sheaths.....2
2. Ascospores 40–48  $\times$  10–13  $\mu$ m.....*N. bellaspora*
2. Ascospores < 40  $\mu$ m long.....3
3. Ascospores 30–37  $\times$  6–9  $\mu$ m, with a single scythe shaped appendage at the apex.....*N. decrospora*
3. Ascospores 35–40  $\times$  9–11  $\mu$ m, without appendage...  
.....*N. limonensis*

**Pleosporales** Luttrell ex M.E. Barr

**Acrocalymmaceae** Crous & Trakun., IMA Fungus 5(2): 404 (2014)

*Acrocalymma* Alcorn & J.A.G. Irwin, Trans. Br. mycol. Soc. 88(2): 163 (1987)

*Saprobic* on submerged wood, leaf litter or other plants.

**Sexual morph:** (from culture) *Ascomata* scattered, erumpent becoming superficial, uniloculate, globose, covered with light gray, gnarled, warted hairs, black, coriaceous, with ostiolate papilla. *Neck* central, terete, composed of 2–5 layers of brown, polygonal cells around ostiole with hyaline periphyses. *Peridium* thin, composed of polygonal, brown, pseudoparenchyma cells. *Pseudoparaphyses* numerous, septate, anastomosing. *Asci* bitunicate, 8-spored, cylindrical, short pedicellate, apically rounded, with a distinct ocular chamber. *Ascospores* uni- to bi-seriate, narrowly fusiform, straight or slightly curved, hyaline first, pale reddish brown with lighter coloured end cells, 1–3-septate, constricted at first submedian septum, basically symmetrical, smooth to rough-walled, thin-walled, with 4 guttules, with a thin sheath (Shoemaker et al. 1991). **Asexual morph:** Coelomycetous. *Pycnidia* scattered or aggregated, semi-immersed to superficial, unilocular, globose, brown to black, with ostiolate papilla or rostrate. *Conidiophores* reduced. *Conidiogenous cells* holoblastic, determinate, discrete, cylindrical, lageniform to ampulliform, hyaline, smooth. *Conidia* cylindrical

to fusoid with subobtuse apex, straight, hyaline at first, becoming pigmented with age, 0–3-septate, not constricted at septa, smooth, thin-walled, with or without helmet-shaped appendages (Zhang et al. 2012a).

**Type species:** *Acrocalymma medicaginis* Alcorn & J.A.G. Irwin

**Notes:** *Acrocalymma* is well-studied because all species have sequences in GenBank. *Acrocalymma* species are easy to produce pycnidia in culture (Trakunyingcharoen et al. 2014). The sexual morph was linked to *Massarina walkeri* Shoemaker et al. which formed in the pure culture of *Acrocalymma medicaginis* (Shoemaker et al. 1991). However, these two species are phylogenetically distinct (Trakunyingcharoen et al. 2014). Therefore, Trakunyingcharoen et al. (2014) transferred *M. walkeri* to *Acrocalymma* and treated *A. medicaginis* and *A. walkeri* as distinct species. The type material of *A. medicaginis* was re-examined and described by Li et al. (2020). *Acrocalymma aquatica* is the first species reported from freshwater habitats, and it is characterized by cylindrical to fusoid, 0(–1)-septate conidia with an apical, mucilaginous, helmet-shaped appendage (Zhang et al. 2012a). In this study, we report a new freshwater species *A. bipolare*, which is collected from Egypt.

#### List of freshwater *Acrocalymma* species

\**Acrocalymma aquatica* H. Zhang & K.D. Hyde, Cryptog. Mycol. 33(3): 337 (2012); Fig. 19

**Freshwater distribution:** Thailand (Zhang et al. 2012a)

\**Acrocalymma bipolare* Abdel-Aziz & Abdel-Wahab, *sp. nov.*

**Index Fungorum number:** IF557901; **Facesoffungi number:** FoF09243; Fig. 20

**Etymology:** in reference to the polar mucoid appendages of the conidia

**Holotype:** CBS H-22673

**Saprobic** on submerged wood in freshwater habitats.

**Sexual morph:** Undetermined. **Asexual morph:** Pycnidia 175–230 µm diam., globose or subglobose, erumpent to

superficial, solitary, dark brown to black, without ostiolate papilla. *Peridium* 8–17 µm thick, composed of 8–11 layers of dark brown to black, thick-walled, cells of *textura angularis*, inwardly with narrow lumens, hyaline and thin-walled cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 2.5–7 × 2–3.5 µm, holoblastic, determinate, discrete, cylindrical or lageniform, hyaline, formed from the inner cells of the pycnidial wall. *Conidia* 9–12 × 3–5 µm ( $\bar{x}$  = 10.8 × 4.3, n = 50), cylindrical to fusiform, straight, unicellular, hyaline, thin-walled, guttulate, with rounded apex and slightly narrow, truncate base, with mucoid polar appendages that filled with oil droplets, appendages elongate in water to form filaments. *Apical appendages* 4–6 µm diam., rounded. *Lower appendages* 1–2 µm in length, sometimes split in the middle.

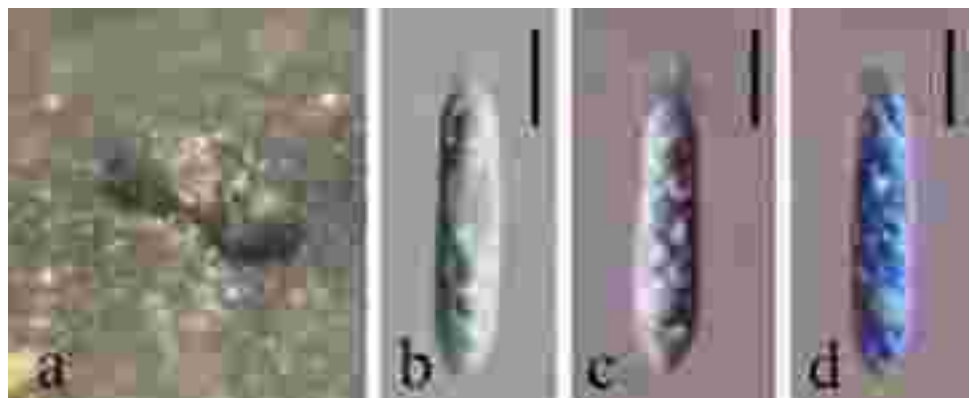
**Culture characteristics:** Colonies on PDA up to 22 mm diam. after 2 weeks at 23 °C, with grey to dark brown aerial and immersed mycelium; reverse dark–brown.

**Material examined:** EGYPT, Sohag City, River Nile, 26°33'32"N, 31°41'44"E, on decaying wood submerged in River Nile water, 14 August 2012, F.A. Abdel-Aziz, (CBS H-22673, **holotype**), ex-type living culture MD1321.

**Notes:** *Acrocalymma bipolare* clusters with the type species *A. medicaginis* and *A. pterocarpi* Jayasiri et al. with high bootstrap support (Fig. 2). Morphologically, *Acrocalymma bipolare* is different from *A. medicaginis* by its appendages that are filled with oil droplets and become filaments when mounted in water, while *A. medicaginis* processes globose to hemispherical or helmet-shaped appendages which do not form filaments in water (Alcorn and Irwin 1987). In addition, *A. bipolare* has shorter conidia than *A. medicaginis* (9–12 × 3–5 µm vs. 11–21 × 3.5–5 µm). The phylogenetically close species *A. pterocarpi* did not produce asexual morph in culture, thus their morphology can not be compared (Jayasiri et al. 2019).

*Acrocalymma bipolare* only has LSU sequence data, and it has four and six nucleotide differences with *A. medicaginis* and *A. pterocarpi*, respectively, which indicates them to be

**Fig. 19** *Acrocalymma aquatica* (Material examined: THAILAND, Chiang Mai Province, Doi Inthanon, on submerged wood, 16 November 2010, H. Zhang, d67, MFLU 11-1113, **holotype**). **a** Pycnidia on substrate surface. **b–d** Conidia with single apical appendage. Scale bars: **b–d** = 5 µm



**Fig. 20** *Acrocalymma bipolare* (CBS H-22673, holotype). **a** Vertical section of a pycnidium. **b** Section through the peridium. **c–f** Various shaped conidia with mucoid polar appendages. Note the apical appendages formed long filament (arrowed in d, stained in toluidine blue). Scale bars: **a** = 50  $\mu\text{m}$ , **b, c** = 10  $\mu\text{m}$ , **d–f** = 5  $\mu\text{m}$



different species. This is also supported by our phylogenetic analysis (Fig. 2).

*Acrocalymma bipolare* differs from the other freshwater species *A. aquatica* in having shorter conidia (9–12  $\times$  3–5  $\mu\text{m}$  vs. 12–17  $\times$  3–4  $\mu\text{m}$ ) and bipolar appendages compared with a single appendage in *A. aquatica* (Zhang et al. 2012a).

#### Key to freshwater *Acrocalymma* species

1. Conidia 12–17  $\times$  3–4  $\mu\text{m}$ , with a single, helmet-shaped appendage.....*A. aquatica*
1. Conidia 9–12  $\times$  3–5  $\mu\text{m}$ , with bipolar appendages filled with oil droplets, appendages elongate in water to form filaments.....*A. bipolare*

**Aigialaceae** Suetrong, Sakay., E.B.G. Jones, Kohlm., Volk.-Kohlm. & C.L. Schoch, Stud. Mycol. 64: 166 (2009)

#### Key to freshwater genera of Aigialaceae

1. Asci cylindro-clavate, ascospores often hyaline, smooth.....*Fissuroma*

1. Asci obclavate, ascospores often brown, verrucose.....*Neoastrophaeriella*

**Fissuroma** J.K. Liu, Phook., E.B.G. Jones & K.D. Hyde, Fungal Diversity 51(1): 145 (2011)

*Saprobic* on submerged wood, dead stem of bamboo or other plant materials. **Sexual morph:** *Ascomata* scattered, rarely clustered, solitary to gregarious, immersed beneath host epidermis, becoming raised, uniloculate, rarely biloculate joined at the base, hemispherical domes, glabrous, black, coriaceous or carbonaceous, opening with a slit-like ostiole. *Peridium* thick, uneven in thickness, poorly developed at the base, thick at sides towards the apex, composed of dark brown pseudoparenchymatous cells, with host cells, plus fungal tissue, arranged in a *textura angularis* to *textura epidermoidea*, carbonaceous at slit-like opening. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, embedded in a gelatinous matrix and anastomosing between and above the asci. *Asci* 8-spored, bitunicate, fissionate, obclavate to cylindrical, pedicellate, apically rounded, with a small ocular chamber. *Ascospores* overlapping uni- to tri-seriate, fusiform, straight to slightly curved, hyaline, 1-septate, slightly constricted at the septum, smooth, thin-walled, surrounded

by a mucilaginous sheath (Liu et al. 2011). **Asexual morph:** Coelomycetous, reported as pleurophomopsis-like from culture of *Fissuroma aggregata* (KT 767). *Conidiomata* globose to subglobose. *Conidiophores* cylindrical, simple to branched, septate. *Conidiogenous cells* phialidic. *Conidia* globose, hyaline (Tanaka and Harada 2005).

*Type species:* *Fissuroma maculans* (Rehm) J.K. Liu, E.B.G. Jones & K.D. Hyde

*Notes:* *Fissuroma* comprises 12 species in Index Fungorum (2020). Eight species have been confirmed with molecular data (Liu et al. 2011; Phookamsak et al. 2015b; Tennakoon et al. 2018; Wanasinghe et al. 2018a). However, Phookamsak et al. (2015b) indicated that *F. aggregata* (I. Hino & Katum.) Phook. et al. is a species complex which might comprise at least three distinct species based on their phylogenetic analyses. After re-examination of the holotype specimen of *F. aggregata* ( $\equiv$  *Melanopsamma aggregata*), Phookamsak et al. (2015b) considered the collection MFLU 11-0146 of Liu et al. (2011) as a distinct species from *F. aggregata*. Therefore, *F. neoaggregatum* Phook. & K.D. Hyde was introduced based on the specimen MFLU 11-0146 (Phookamsak et al. 2015b). The asexual morph was developed in the culture of *F. aggregata* (KT 767) (Tanaka and Harada 2005). However, the real identity of KT 767 needs confirmation to separate *F. aggregata* KT 984, as shown in the phylogenetic tree (Fig. 2). We provide a description and illustration for *F. neoaggregatum* collected from submerged wood in Thailand.

#### List of freshwater *Fissuroma* species

\**Fissuroma neoaggregatum* Phook. & K.D. Hyde [as ‘neoaggregata’], Fungal Diversity 74: 158 (2015); Fig. 21

*Freshwater distribution:* Thailand (this study)

*Saprobic* on submerged wood. **Sexual morph:** *Ascostromata* dark brown to black, solitary to gregarious, immersed in host epidermis, visible as raised, dome-shaped areas on the host surface, hemispherical to depressed conical, with flattened base, uniloculate, coriaceous, ostiolate, with slit-like opening. *Peridium* of unequal thickness, poorly developed at the base. *Pseudoparaphyses* dense, trabeculate, filiform, embedded in a hyaline gelatinous matrix. *Asci* 140–155  $\times$  16–21  $\mu$ m ( $\bar{x}$  = 149  $\times$  19  $\mu$ m,  $n$  = 5), 8-spored, bitunicate, obclavate, with furcate to truncate pedicel, apically rounded, with an ocular chamber. *Ascospores* 36–43  $\times$  6–8  $\mu$ m ( $\bar{x}$  = 40.5  $\times$  7  $\mu$ m,  $n$  = 10), overlapping biseriate and uniseriate at the apex, hyaline, fusiform, with acute ends, 1-septate, constricted at the septum, smooth-walled, guttulate, surrounded by a gelatinous sheath. **Asexual morph:** Undetermined.

*Material examined:* THAILAND, Chiang Rai Province, on submerged wood, 18 January 2010, H. Zhang, a45 (MFLU 11-1140), living culture MFLUCC 10-0917.

*Notes:* Our new collection MFLUCC 10-0917 is identified as *Fissuroma neoaggregatum* based on nearly identical ITS sequence data with ex-type strain MFLUCC 10-0554 (two nucleotide differences) and morphological characters. Our collection has shorter asci (140–155  $\times$  16–21  $\mu$ m vs. 155–197  $\times$  15–18  $\mu$ m) and ascospores (36–43  $\times$  6–8  $\mu$ m vs. 39–54  $\times$  7–9  $\mu$ m) than the holotype (Phookamsak et al. 2015b). Moreover, our collection shows a broad, mucilaginous sheath in Indian Ink, while the holotype has only a thin sheath (Phookamsak et al. 2015b). Other sequences are expected for MFLUCC 10-0917 to further clarify this species. This is a new habitat record for *F. neoaggregatum* from freshwater.

*Neoastrorphaeriella* J.K. Liu, E.B.G. Jones & K.D. Hyde, Fungal Diversity 51(1): 148 (2011)

*Saprobic* on decaying twigs, petiole and submerged wood in terrestrial and freshwater habitats. **Sexual morph:** *Ascomata* scattered, immersed to semi-immersed, with a slit-like ostiole, black. *Peridium* uneven in thickness, carbonaceous, composed of dark brown, thick-walled cells. *Pseudoparaphyses* trabeculate, hyaline, anastomosing, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, obclavate to cylindrical, pedicellate, with a small ocular chamber. *Ascospores* fusiform, hyaline to dark brown, mostly verrucose when mature, 1-septate, surrounded by a mucilaginous sheath (Liu et al. 2011). **Asexual morph:** Undetermined.

*Type species:* *Neoastrorphaeriella krabiensis* J.K. Liu, E.B.G. Jones & K.D. Hyde

*Notes:* *Neoastrorphaeriella* was established by Liu et al. (2011) to accommodate species having ascomata with slit-like ostioles, obclavate asci and brown, verrucose ascospores. *Neoastrorphaeriella aquatica* D.F. Bao et al. was the only species found in freshwater habitats and differed from the others by hyaline to greyish brown, verrucose ascospores and distinct molecular characters (Bao et al. 2019b). *Neoastrorphaeriella* nested well in Aigialaceae (Liu et al. 2011; Wanasinghe et al. 2018a; Bao et al. 2019b; Zhang et al. 2020; this study, Fig. 2).

#### List of freshwater *Neoastrorphaeriella* species

\**Neoastrorphaeriella aquatica* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Phytotaxa 391(3): 201 (2019)

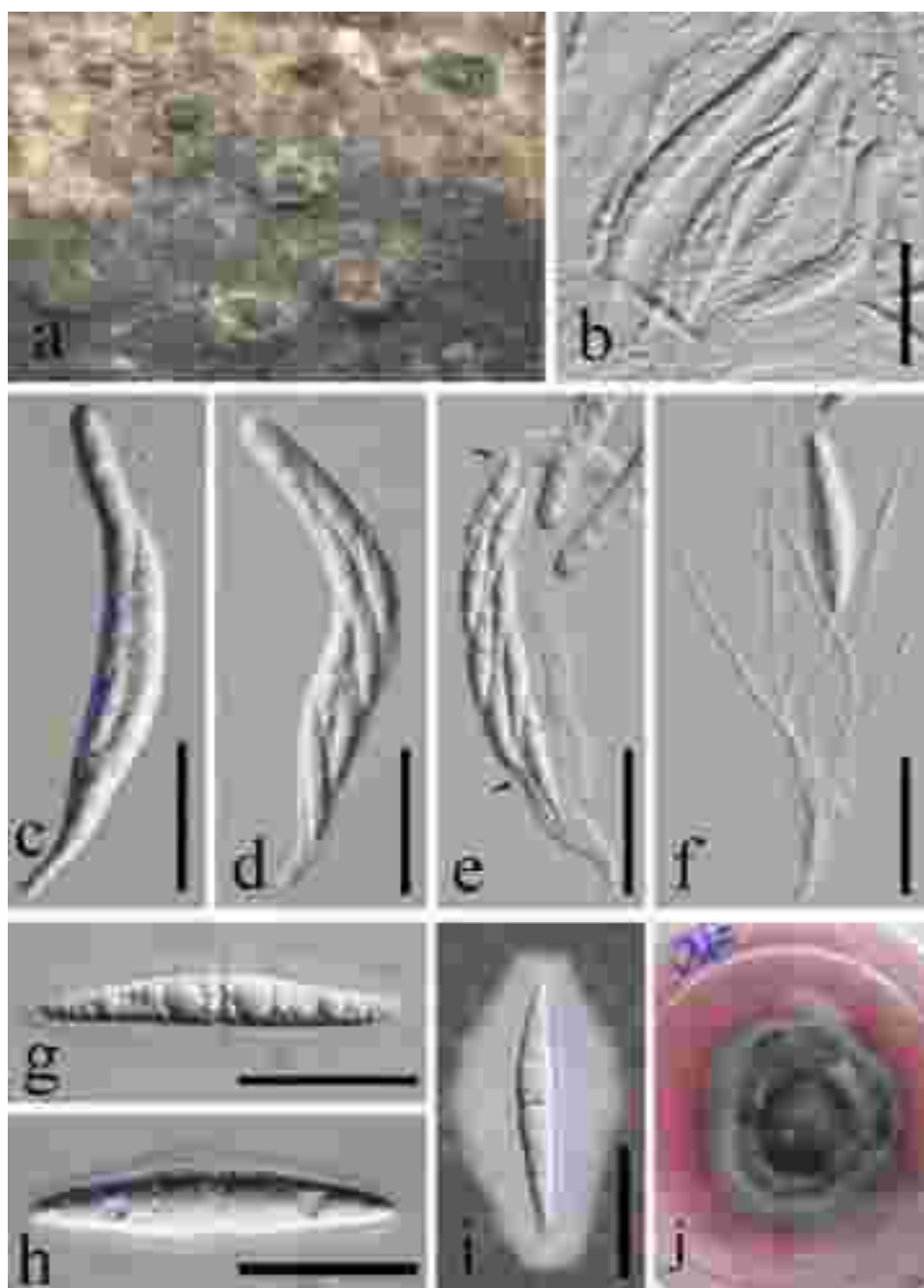
*Freshwater distribution:* Thailand (Bao et al. 2019b)

**Amniculicolaceae** Y. Zhang ter, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, Stud. Mycol. 64: 95 (2009)

#### Key to freshwater genera of Amniculicolaceae

1. Asexual morph.....*Vargamyces*
1. Sexual morph.....2

**Fig. 21** *Fissuroma neoaggregatum* (MFLU 11-1140, new habitat record). **a** Appearance of ascomata on wood. **b** Asci and pseudoparaphyses. **c–e** Asci. Note the bitunicate ascus in **e**. **f** Pseudoparaphyses. **g–i** Ascospores. (**i** in Indian Ink). **j** Front view of colony on PDA. Scale bars: **b–f** = 50  $\mu$ m, **g–i** = 20  $\mu$ m



- 2. Ascospores hyaline, 1-septate.....*Ammiculicola*
- 2. Ascospores pigmented, multi-septate.....3
- 3. Ascospores golden yellow to brown, dictyoseptate.....  
.....*Murispora*
- 3. Ascospores reddish brown, phragmoseptate.....  
.....*Neomassariosphaeria*

*Ammiculicola* Y. Zhang ter & K.D. Hyde, Mycological Research 112 (10): 1189 (2008)

*Saprobic* on submerged wood or in plant humus. **Sexual morph:** Ascomata solitary to gregarious, immersed to nearly

superficial, black, uniloculate, subglobose to conical, glabrous, ostiolate, with or without two tuberculate flared lips surrounding a slit-like ostiole, sometimes with a flattened base not easily removed from the substrate, usually staining the woody substrate purple. *Peridium* 2-layered, outer layer composed of heavily pigmented thick-walled cells of *textura angularis*, inner layer composed of hyaline thin-walled cells of *textura angularis*. *Pseudoparaphyses* dense, trabeculate, filiform, persistent, hyaline, embedded in mucilage, anastomosing between and above the asci. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to narrowly fusoid, short



pedicellate. *Ascospores* mostly uniseriate, fusoid, hyaline, septate, symmetrical, smooth, thin-walled, surrounded by a hyaline, gelatinous sheath (Zhang et al. 2008c, 2009b, 2012b). **Asexual morph:** Hyphomycetous. *Conidiophores* usually simple. *Conidia* curved or sigmoid, tapering to the end, septate, hyaline (illustration and description see Ingold (1942)).

*Type species: Amniculicola lignicola* Y. Zhang ter & K.D. Hyde

*Notes:* The freshwater genus *Amniculicola* is characterized by staining the woody substrate purple (Zhang et al. 2008c, 2009b). However, two other species, *A. aquatica* Z.L. Luo et al. and *A. guttulata* et al., did not produce pigmentation on the host substrate (Hyde et al. 2019; this study, Fig. 22). A comprehensive account of *Amniculicola* was provided by Zhang et al. (2008c, 2012b). *Amniculicola immersa* Y. Zhang ter, J. Fourn., *A. lignicola* and *A. parva* Y. Zhang ter, J. Fourn. were collected from Europe (Denmark and France), *A. longissima* (Sacc. & P. Syd.) Nadeeshan & K.D. Hyde was collected from Latin America (Costa Rica), while *A. aquatica* and *A. guttulata* were collected from Asia (China). *Amniculicola longissima* is the only asexual morphic species in the genus and confirmed by phylogenetic analyses (Hyde et al. 2019; this study, Fig. 2).

The paraphyletic nature of *Amniculicola* was shown in Wanasinghe et al. (2015), as *Amniculicola* species clustered in three different sister clades. This nature is not well resolved as one asexual genus *Vargamyces* Tóth nested within *Amniculicola* in Hyde et al. (2019) and this study (Fig. 2).

### List of freshwater *Amniculicola* species

\**Amniculicola aquatica* Z.L. Luo, K.D. Hyde & H.Y. Su, Fungal Diversity 96: 13 (2019)

*Freshwater distribution:* China (Hyde et al. 2019)

\**Amniculicola guttulata* Z.L. Luo, K.D. Hyde & H.Y. Su, Fungal Diversity 96: 13 (2019); Fig. 22

*Freshwater distribution:* China (Hyde et al. 2019, this study)

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph:** *Ascomata* 160–180 µm high, 170–190 µm diam., black, solitary, scattered or occasionally gregarious, superficial, subglobose to conical, with ostiolate papilla. *Peridium* 35–45 µm thick, comprising several layers of hyaline to pale brown, thin-walled, large cells of *textura angularis* or irregular cells. *Pseudoparaphyses* 2.2 µm diam., dense, trabeculate, filiform, persistent, hyaline, embedded in mucilage, anastomosing between and above the asci. *Asci* 105–141(–256) × 8.5–10.5 µm ( $\bar{x}$  = 158.5 × 10 µm, n = 10), 8-spored, bitunicate, fissitunicate, cylindrical to narrowly fusiform, with a twisted, bifurcate, short pedicel, 7.5–18.5 µm long, apically rounded or slightly obtuse, endoascus

elongate to 256 µm long when mature, with a small inconspicuous apical apparatus barely seen in water. *Ascospores* 21.5–27 × 5.2–6.2 µm ( $\bar{x}$  = 23.7 × 5.6 µm, n = 15), uniseriate or overlapping uni-seriate, straight or slightly curved, hyaline, 1-septate, obscure when young, visible when germinated, deeply constricted at the septa, slightly constricted at a quarter, the upper cell slightly broader than the lower one, broadly fusiform, with two prominent big guttules in the middle cell and four gradually smaller ones beside, thin-walled, smooth, with a thin, hyaline, bipolar narrowed, mucilaginous sheath which is 1–2 µm thick. **Asexual morph:** Undetermined.

*Culture characteristics:* On PDA, colony circular, reaching 25 mm diam. in 25 days at 25 °C, grey to white from above, pale grey from below, surface rough, fluffy, with dense mycelium, dry, raised, edge entire, with white margin.

*Material examined:* CHINA, Yunnan Province, Dehong, on submerged wood in a stream, 25 November 2017, G.N. Wang, H55A-1 (MFLU 18-1160), living culture MFLUCC 18-1038; *ibid.*, H55A-2 (HKAS 101736), living culture KUMCC 18-0088.

*Notes:* Our new collection MFLUCC 18-1038 is identified as *Amniculicola guttulata* based on morphological characters; identical LSU and TEF sequence data and phylogenetic analysis (Fig. 2). The asci in the holotype are up to 127 µm long, while in our collection they are up to 256 µm long when mature (Hyde et al. 2019).

\**Amniculicola immersa* Y. Zhang ter, J. Fourn., Crous & K.D. Hyde, Persoonia 23: 50 (2009)

*Freshwater distribution:* Denmark (Zhang et al. 2009b)

\**Amniculicola lignicola* Y. Zhang ter & K.D. Hyde, Mycological Research 112 (10): 1189 (2008)

*Freshwater distribution:* France (Zhang et al. 2008c)

\**Amniculicola longissima* (Sacc. & P. Syd.) Nadeeshan & K.D. Hyde, IMA Fungus 7(2): 301 (2016)

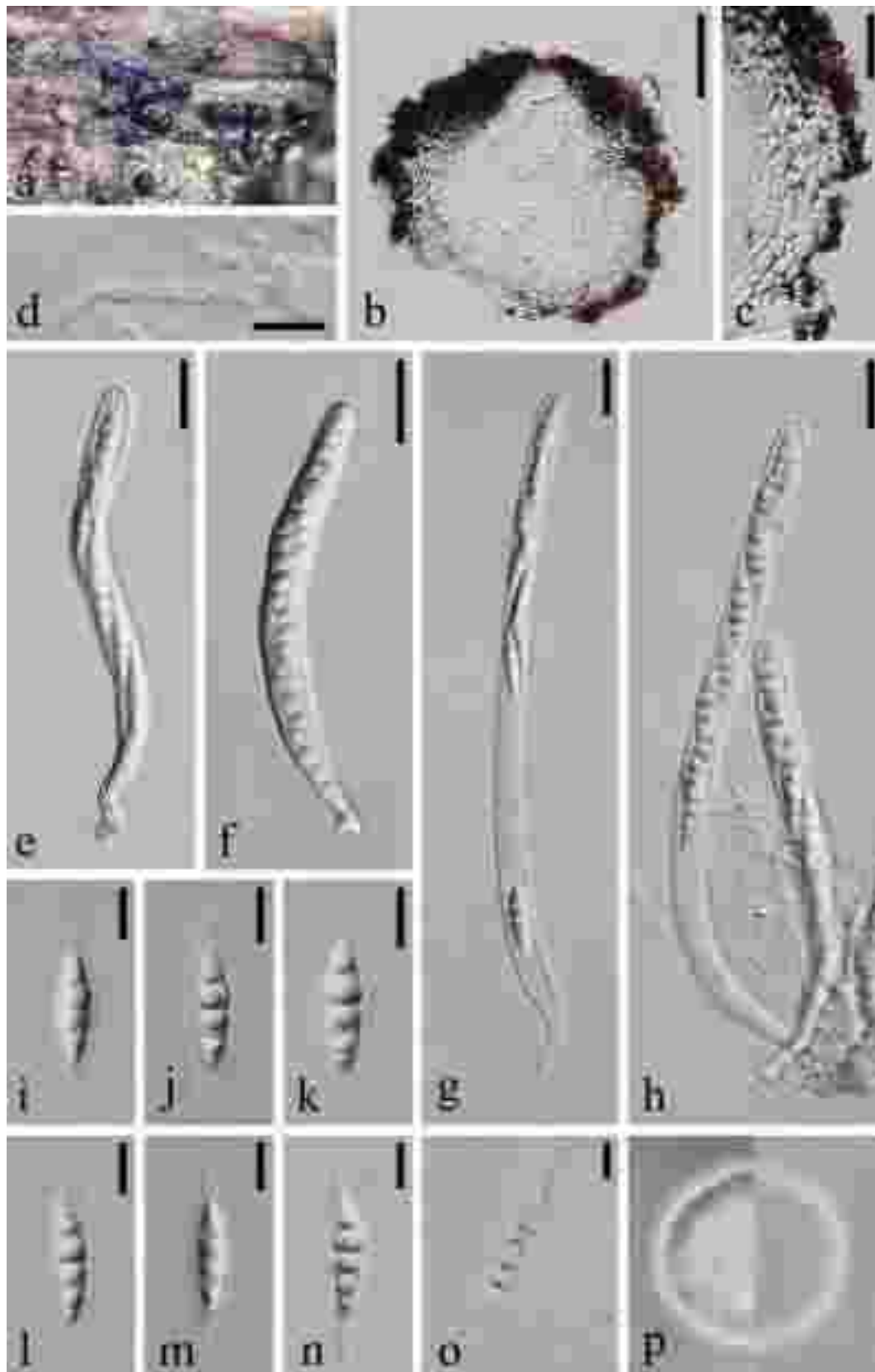
*Freshwater distribution:* Leicester, UK (Ingold 1942)

\**Amniculicola parva* Y. Zhang ter, J. Fourn., Crous & K.D. Hyde, Persoonia 23: 52 (2009)

*Freshwater distribution:* France (Zhang et al. 2009b)

### Key to freshwater *Amniculicola* species

1. Asexual morph.....*A. longissima*
1. Sexual morph.....2
2. Ascomata superficial.....3
2. Ascomata immersed.....*A. immersa*
3. Asci longer than 130 µm.....*A. lignicola*
3. Asci shorter than 130 µm.....4
4. Substrate stained purple.....*A. parva*



**Fig. 22** *Amniculicola guttulata* (MFLU 18-1160). **a** Appearance of black ascomata on host. **b** Vertical section of ascoma. **c** Structure of peridium. **d** Pseudoparaphyses. **e–h** Bitunicate asci. **i–n** Ascospores. **o** Germinated ascospore. **p** Colony on PDA (from front and reverse). Scale bars: **b** = 50  $\mu\text{m}$ , **c, e–h** = 20  $\mu\text{m}$ , **d, i–o** = 10  $\mu\text{m}$

4. Substrate natural colour.....5  
 5. Peridium 35–50  $\mu\text{m}$  thick, ascospores 24–32  $\times$  6–8  $\mu\text{m}$ .....*A. aquatica*  
 5. Peridium 27–35  $\mu\text{m}$  thick, ascospores 23–27  $\times$  5–7  $\mu\text{m}$ .....*A. guttulata*

*Murispora* Yin. Zhang, C.L. Schoch, J. Fourn., Crous & K.D. Hyde, Studies in Mycology 64: 95 (2009)

*Saprobic* on submerged wood or dead stems or branches of plant materials. **Sexual morph:** *Ascomata* scattered to gregarious, immersed, uniloculate, lenticular, slightly protruding at the apex, opening through a small rounded pore, with ostiolate papilla, stain substrate purple. *Peridium* thin, composed of a few layers cells of *textura angularis*, thicker at the apex with pseudoparenchymatous cells. *Pseudoparaphyses* cellular, embedded in mucilage. *Asci* 8-spored, bitunicate, fissitunicate, cylindro-clavate, short pedicellate. with a small ocular chamber. *Ascospores* biseriate to uniseriate near the base, fusoid with narrowly rounded ends, slightly curved, golden yellow or hyaline, turning brown when senescent, muriform, with one, rarely two longitudinal septa in all cells except end cells, 7–9-transversely septate, basically symmetrical, smooth or finely verruculose, surrounded by a wide mucilaginous sheath (Zhang et al. 2009c, 2012b). **Asexual morph:** Coelomycetous. *Pycnidia* solitary, mainly immersed, uniloculate, dark brown to black. *Peridium* reddish brown cells of *textura angularis*, with inner most layer thin, hyaline. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, determinate, hyaline, smooth, formed from the inner most layer of pycnidial wall. *Conidia* ellipsoidal, hyaline, aseptate, smooth-walled, thin-walled (Wanasinghe et al. 2015).

*Type species:* *Murispora rubicunda* (Niessl) Y. Zhang ter, J. Fourn. & K.D. Hyde

*Notes:* Zhang et al. (2009c) introduced *Murispora* based on *Pleospora rubicunda* Niessl which clustered in a robust clade with *Amniculicola* species in Amniculicolaceae. Wanasinghe et al. (2015) introduced another six species to *Murispora* from terrestrial habitats and they found the asexual morph of *M. hawksworthii* Wanas. et al. from single ascospore isolates. *Murispora* species stain substrate purple (Zhang et al. 2009c, 2012b; Wanas. et al. 2015; Bao et al. 2019d) and this character should be a common feature to the genus. Four freshwater species have been reported in *Murispora*, three of which were collected from China (see list below).

### List of freshwater *Murispora* species

\**Murispora aquatica* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Phytotaxa 416(1): 5 (2019)

*Freshwater distribution:* China (Bao et al. 2019d)

\**Murispora cicognanii* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, Cryptog. Mycol. 36(4): 437 (2015)

*Freshwater distribution:* China (Hyde et al. 2019)

\**Murispora fagicola* Wanas., Camporesi, E.B.G. Jones & K.D. Hyde, Cryptog. Mycol. 36(4): 429 (2015)

*Freshwater distribution:* China (Bao et al. 2019d)

\**Murispora rubicunda* (Niessl) Yin. Zhang, J. Fourn. & K.D. Hyde, Studies in Mycology 64: 96 (2009); Fig. 23

*Basionym:* *Pleospora rubicunda* Niessl, Verh. nat. Ver. Brünn 14: 191 (1876)

*Synonymy:* *Sphaeria rubicunda* Schwein., Transactions of the American Philosophical Society 4 (2): 222 (1832)

*Massariosphaeria rubicunda* (Niessl) Crivelli, Über die heterogene Ascomycetengattung *Pleospora* Rabh.: 144 (1983)

*Karstenula rubicunda* (Niessl) M.E. Barr, N. Amer. Fl., Ser. 2 (New York) 13: 52 (1990)

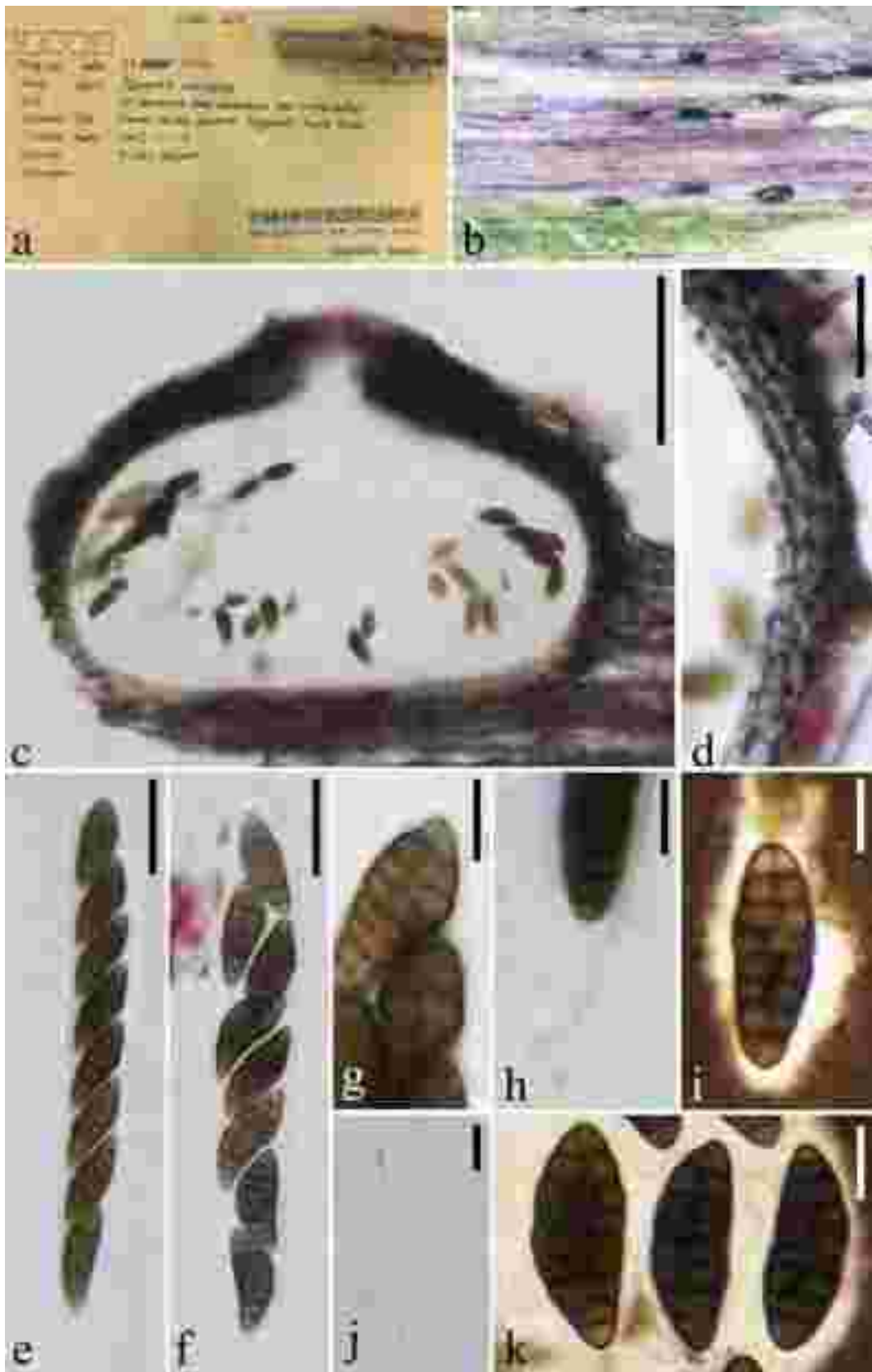
*Freshwater distribution:* Austria (Magnes and Hafellner 1991), France (Zhang et al. 2012b)

### Key to freshwater *Murispora* species

1. Ascospores > 31  $\mu\text{m}$  long.....2  
 1. Ascospores < 31  $\mu\text{m}$  long.....3  
 2. Ascospores more than 10 transverse septa.....  
 .....*M. cicognanii*  
 2. Ascospores 7–9 transverse septa.....*M. rubicunda*  
 3. Ascospores 21–25  $\times$  8–10  $\mu\text{m}$ .....*M. aquatica*  
 3. Ascospores 27–31  $\times$  11–13  $\mu\text{m}$ .....*M. fagicola*

*Neomassariosphaeria* Y. Zhang ter, J. Fourn. & K.D. Hyde, Stud. Mycol. 64: 96 (2009)

*Saprobic* on submerged culm of *Phragmites*. **Sexual morph:** *Ascomata* scattered or in small groups, deeply immersed, lenticular or depressed ellipsoidal, with ostiolate papilla, stain substrate purple, visible as black, slit-like spot on the surface of substrate. *Peridium* comprising several layers of rounded to angular, brown to dark brown, thick-walled cells. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, embedded in a matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindro-clavate to broadly clavate, with an ocular chamber and short pedicel. *Ascospores* bi- to tri-seriate, narrowly to broadly fusoid, straight or curved, multi-septate, reddish brown, minutely verruculose, usually with a thin gelatinous sheath. **Asexual morph:** Undetermined.



**Fig. 23** *Murispora rubicunda* (Material examined: FRANCE, Haute-Garonne, Avignonet, on submerged dead herbaceous stem, 16 January 2007, M. Delpont, IFRD 2017). **a** Herbarium label and specimen of *Murispora rubicunda*. **b** Appearance of ascomata on host surface. **c** Vertical section through ascoma. **d** Peridium. **e, f** Asci. **g** Apex of ascus. **h** Pedicel. **j** Pseudoparaphyses. **i, k** Ascospores with wide sheath in Indian Ink. Scale bars: **c** = 100  $\mu\text{m}$ , **d** = 20  $\mu\text{m}$ , **e, f** = 25  $\mu\text{m}$ , **g–k** = 10  $\mu\text{m}$

*Type species: Neomassariosphaeria typhicola* Y. Zhang ter, J. Fourn. & K.D. Hyde

*Notes: Neomassariosphaeria* was established in Amniculicolaceae to accommodate *Massariosphaeria typhicola* (P. Karst.) Leuchtm (Zhang et al. 2009c). Later, it was transferred to Lindgomycetaceae based on phylogenetic analysis (Ariyawansa et al. 2015). However, we found that CBS 123126 is a problematic strain, which possesses two different LSU sequences and clusters in different families, i.e. FJ795504 (Zhang et al. 2009c) in Lindgomycetaceae and GU301844 (Schoch et al. 2009) in Amniculicolaceae. We substantiate GU301844 is the real sequence of *Neomassariosphaeria typhicola* (P. Karst.) Y. Zhang ter et al., while FJ795504, listed in Zhang et al. (2009c), is invalid (pers. comm. Zhang Y.). Ariyawansa et al. (2015) only included the invalid sequence FJ795504 in their phylogenetic tree, which resulted in the classification of *Neomassariosphaeria* in Lindgomycetaceae.

*Neomassariosphaeria* often stains the woody substrate purple, and has reddish brown, multi-septate ascospores with an irregular, hyaline, gelatinous sheath, which morphologically fit well with the familial concept of Amniculicolaceae (Zhang et al. 2009c; Hyde et al. 2013). Based on morphology and our phylogenetic tree (Fig. 2), we follow Zhang et al. (2009c), to retain *Neomassariosphaeria* in Amniculicolaceae. Accession number FJ795504 bearing CBS123126 is treated as an invalid sequence.

*Neomassariosphaeria typhicola* (IFRD 2018) was considered as a synonym of *Massariosphaeria typhicola* (Zhang et al. 2009c). Although they are similar in the ascomata that staining the substrate purple, re-examination of IFRD 2018 (Fig. 24) shows that it is different from *M. typhicola* in having lenticular ascomata with an elongate papilla on the substrate, dark brown to black, reddish brown ascospores (Leuchtmann 1984). Phylogeny also placed them in different families, i.e. *N. typhicola* in Amniculicolaceae, *M. typhicola* in Lindgomycetaceae (Zhang et al. 2009c; this study, Fig. 2). We therefore, treat *Neomassariosphaeria typhicola* and *Massariosphaeria typhicola* as different species in this study.

#### List of freshwater *Neomassariosphaeria* species

\**Neomassariosphaeria typhicola* Y. Zhang ter, J. Fourn. & K.D. Hyde, Stud. Mycol. 64: 96 (2009)

*Facesoffungi* number: FoF09244; Fig. 24

*Saprobic* on submerged culm of *Phragmites*. **Sexual morph:** *Ascomata* 150–250  $\mu\text{m}$  high, 300–500  $\mu\text{m}$  diam., scattered or gregarious, immersed, lenticular or depressed ellipsoidal, with ostiolate papilla, stain the substrate purple, visible as a black, slightly protruding elongate papilla on the substrate. *Peridium* 20–35  $\mu\text{m}$  thick, comprising several layers of rounded to angular, brown to dark brown, thick-walled cells. *Pseudoparaphyses* 1–2  $\mu\text{m}$  wide, numerous, uneven in width or tubercled, trabeculate, hyaline, septate, embedded in hyaline to reddish matrix. *Asci* 90–120  $\times$  14–20  $\mu\text{m}$  ( $\bar{x}$  = 97  $\times$  16.5  $\mu\text{m}$ ,  $n$  = 10), 8-spored, bitunicate, fissitunicate, cylindro-clavate to broadly clavate, apically rounded, with a well-developed ocular chamber and short pedicel. *Ascospores* 30–40  $\times$  6–8  $\mu\text{m}$  ( $\bar{x}$  = 34.5  $\times$  6.5  $\mu\text{m}$ ,  $n$  = 20), bi- to tri-seriate, narrowly to broadly fusoid, with subacute or rounded ends, straight or curved, 7-septate, constricted at the septa, dark brown to black or reddish brown, minutely verruculose, somewhat thick-walled, usually with a thin, broadly fusoid or irregular, gelatinous sheath. **Asexual morph:** Undetermined.

*Material examined:* DENMARK, Sjaeland, Frederikskilde, Suserup Skove, Tystrup Lake, on submerged culm of *Phragmites*, 25 May 2007, leg. & det. J. Fournier (IFRD 2018).

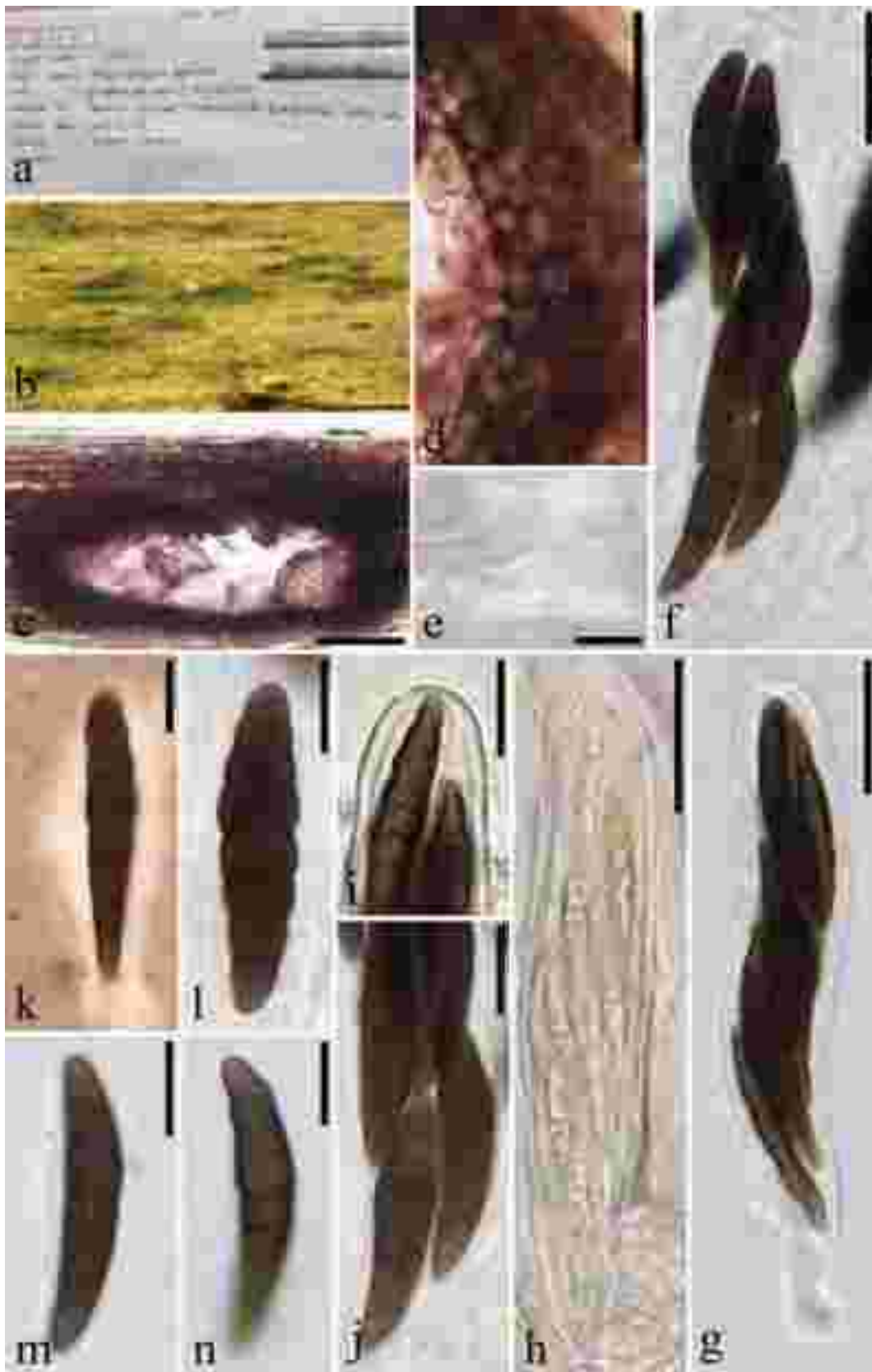
*Notes:* We re-examined the specimen *Neomassariosphaeria typhicola* IFRD 2018 collected by Zhang et al. (2009c) to confirm its generic placement in Amniculicolaceae. The sequence data (LSU: GU301844, SSU: GU296174, RPB2: GU371795) and morphological characters of *N. typhicola* support it as a distinct genus in Amniculicolaceae. The reddish brown, phragmoseptate ascospores of *Neomassariosphaeria* differs from hyaline, 1-septate ascospores of *Amniculicola* and golden yellow to brown, dictyoseptate ascospores of *Murispora* (see key to freshwater genera of Amniculicolaceae).

*Vargamyces* Tóth, Acta bot. hung. 25: 403 (1980) [1979]

*Saprobic* on submerged wood or leaves. **Sexual morph:** Undetermined. **Asexual morph:** *Mycelium* mostly immersed, composed of septate, hyaline to pale brown, smooth hyphae. *Conidiophores* micronematous to semi-macronematous, solitary, erect, straight to flexuous, cylindrical, subhyaline to pale brown, with percurrent proliferations. *Conidiogenous cells* integrated, terminal, cylindrical to cupuliforme, hyaline to pale brown. *Conidia* solitary, fusiform, truncate at the base, apically rounded, paler toward the ends, multi-septate, smooth-walled. *Microconidia* blastic growing on undifferentiated hyphae, solitary, terminal, lateral or intercalary, globose to obovoid, unicellular, hyaline, smooth (Hernández-Restrepo et al. 2017).

*Type species: Vargamyces aquaticus* (Dudka) Tóth

*Notes:* The monotypic genus *Vargamyces* was introduced by Tóth (1979) to accommodate *V. aquaticus* which was



**Fig. 24** *Neomassariosphaeria typhicola* (IFRD 2018). **a** Herbarium packet and specimen. **b** Ascomata immersed in host substrate. **c** Vertical section of ascoma. **d** Structure of peridium. **e** Pseudoparaphyses. **f, g** Mature asci. **h** Immature ascus. **i** An ocular chamber in mature ascus. **j** Verruculose ascospores. **k** Ascospore in Indian Ink. **l** Ascospore in ascus. **m** Ascospore with a thin, gelatinous sheath. **n** Mature ascospore. Scale bars: **c** = 100  $\mu\text{m}$ , **d, f–h** = 20  $\mu\text{m}$ , **e** = 3  $\mu\text{m}$ , **i–n** = 10  $\mu\text{m}$

collected from submerged leaves and wood (Dudka 1966; Hyde and Goh 1999a). *Vargamyces aquaticus* was transferred to *Xylomyces* based on detachable, fusiform, dematiaceous and multiseptate chlamydospores (Hyde and Goh 1999a). However, Hernández-Restrepo et al. (2017) showed that *V. aquaticus* clustered in Amniculicolaceae, which is phylogenetically distant from the type species of *Xylomyces*, *X. chlamydosporus* (this study, Fig. 2). Therefore, *Vargamyces* is distinct and the only asexual genus in Amniculicolaceae, as listed in Wijayawardene et al. (2018, 2020). The lectotype (Fig. 1 in Dudka (1966)) and epitype (CBS H-22992, culture ex-epitype CBS 636.91) were also designated by Hernández-Restrepo et al. (2017).

#### List of freshwater *Vargamyces* species

\**Vargamyces aquaticus* (Dudka) Tóth, Acta Mus. Silesiae, Ser. A 25(3–4): 403 (1980) [1979]

*Basionym*: *Camposporium aquaticum* Dudka, Ukr. Bot. Zh. 23:91 (1966)

*Synonymy*: *Sporidesmium ontariense* Matsush., Matsush. Mycol. Mem. 3: 16 (1983)

*Xylomyces aquaticus* (Dudka) K.D. Hyde & Goh, Mycol. Res. 103(12): 1573 (1999)

*Repetophragma ontariense* (Matsush.) W.P. Wu, Fungal Diversity Res. Ser. 15: 82 (2005)

*Freshwater distribution*: England (Hyde and Goh 1999a), Spain (Hernández-Restrepo et al. 2017), Ukraine (Dudka 1966), USA (Tóth 1979)

**Anteagloniaceae** K.D. Hyde, J.K. Liu & A. Mapook, Fungal Diversity 63: 33 (2013)

#### Key to freshwater sexual genera of Anteagloniaceae

1. Ascomata orange-pigmented at the apex, asci clavate to cylindrical-clavate, ascospores hyaline, fusiform.....*Flammeascoma*
2. Ascomata stain the substrate purple, asci cylindrical, ascospores olivaceous, ellipsoidal.....*Purpureofaciens*

**Flammeascoma** Phook. & K.D. Hyde, Fungal Diversity 72(1): 63 (2015)

*Saprobic* on submerged bamboo or dead wood. **Sexual morph**: *Ascostromata* solitary to gregarious, erumpent to superficial, visible as dull, black, dark at the basal region and orange brown at the apex, conical to lenticular, with a flattened base, uni- to bi-loculate, glabrous, coriaceous, ostiolate, with pore-like opening. *Peridium* thick-walled, of unequal thickness, poorly developed at the base, composed of several layers of dark, pseudoparenchymatous cells, with fungal tissue intermixed with the host cells. *Pseudoparaphyses* dense, trabeculate, filiform, hyaline, septate. *Asci* 8-spored, bitunicate, fissitunicate, clavate to cylindrical-clavate, short pedicellate, apically rounded with well-developed ocular chamber. *Ascospores* uni- to bi-seriate, fusiform, hyaline, becoming brown when released from asci, septate, smooth-walled, with or without mucilaginous sheath (Liu et al. 2015). **Asexual morph**: Undetermined.

*Type species*: *Flammeascoma bambusae* Phook. & K.D. Hyde

*Notes*: *Flammeascoma* was introduced to accommodate *F. bambusae*, which was collected from submerged bamboo (Liu et al. 2015). *Flammeascoma* is similar to familial type *Anteaglonium* Mugambi & Huhndorf in the shape of asci and ascospores (Mugambi and Huhndorf 2009, Liu et al. 2015). However, *Flammeascoma* is unique in producing reddish orange pigmentation at the apex of ascostromata, while *Anteaglonium* lacks this character and always sits on a thin darkened crust with or without sparse, dark brown, septate subiculum (Mugambi and Huhndorf 2009; Ariyawansa et al. 2015; Liu et al. 2015).

*Flammeascoma* appears to be polyphyletic as *F. bambusae* (type) clusters with *Anteaglonium gordoniae* Jayasiri et al. and *F. lignicola* Boonmee & K.D. Hyde clusters with our new genus *Purpureofaciens*, but without bootstrap support (Fig. 2). More species are expected to clarify whether *Flammeascoma* is polyphyletic.

#### List of freshwater *Flammeascoma* species

\**Flammeascoma bambusae* Phookamsak & K.D. Hyde, Fungal Diversity 72(1): 64 (2015); Fig. 25

*Freshwater distribution*: Thailand (Liu et al. 2015)

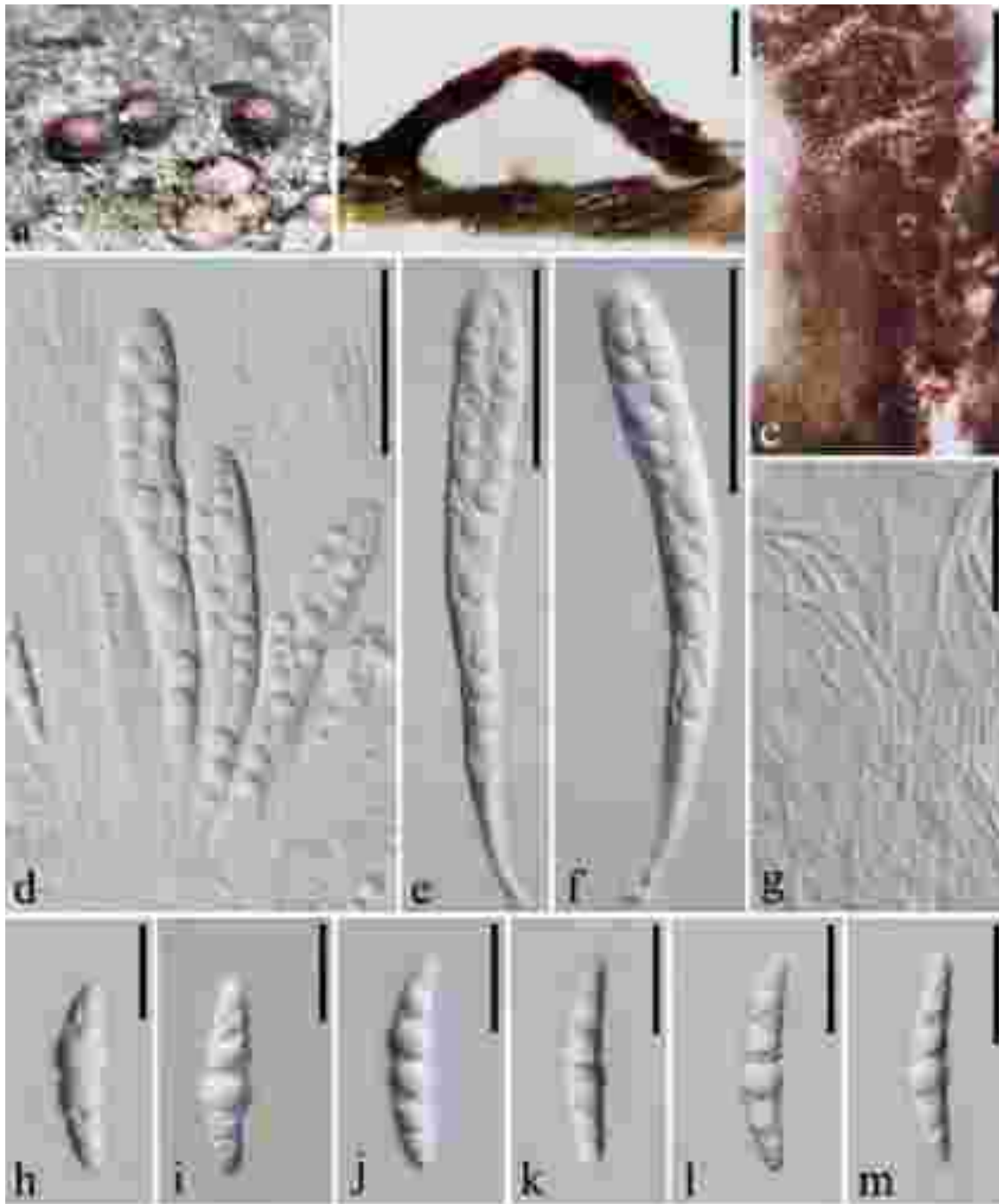
**Purpureofaciens** W. Dong, H. Zhang & K.D. Hyde, *gen. nov.*

*Index Fungorum number*: IF557804; *Facesoffungi number*: FoF08717

*Etymology*: referring to staining the substrate purple

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Ascomata* black, scattered or gregarious, semi-immersed to superficial, conical, flattened at the basal region, stain the substrate purple and produce reddish pigmentation at the apex, coriaceous, with a large, protuberant, ostiolate papilla. *Peridium* of unequal thickness, poorly developed at the base, composed of reddish brown, large



**Fig. 25** *Flammeascooma bambusae* (Material examined: THAILAND, Chiang Mai Province, Chom Tong District, Doi Inthanon, on dead stem of submerged bamboo (Bambusae), 5 September 2009, R. Phookamsak, RP0013, MFLU 11-0143, **holotype**). **a** Appearance of

ascostromata on the host surface. **b** Section through an ascostroma. **c** Peridium. **d–f** Bitunicate asci. **g** Pseudoparaphyses. **h–m** Ascospores. Scale bars: **b** = 200  $\mu$ m, **c–f** = 50  $\mu$ m, **g–m** = 20  $\mu$ m

cells of *textura angularis*. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, cylindrical, pedicellate, apically rounded. *Ascospores* uniseriate, curved, olivaceous, septate, ellipsoidal with acute ends, guttulate, thin-walled,

smooth, surrounded by a thin mucilaginous sheath. **Asexual morph:** Undetermined.

*Type species:* *Purpureofaciens aquatica* W. Dong, H. Zhang & K.D. Hyde

*Notes:* *Purpureofaciens* is unique by semi-immersed to superficial, conical ascostromata staining woody substrate



purple, cylindrical asci, ellipsoidal, curved, olivaceous ascospores with acute ends and white to reddish colonies in culture (Mugambi and Huhndorf 2009; Liu et al. 2015). The reddish culture is also observed in a strain of *Anteaglonium parvulum* (MFLUCC 14-0821), which has similar shape of ascospores, but the characters of ascomata, asci and ascospores can easily separate them (Jayasiri et al. 2016). *Flammeascoma* has reddish pigmentation at the apex of ascomata, which resembles *Purpureofaciens*. However, the conical ascomata with a large, protuberant, ostiolate papilla, cylindrical asci and ellipsoidal, olivaceous ascospores of *Purpureofaciens* are different from those of *Flammeascoma* (Liu et al. 2015). *Purpureofaciens* clusters with *Flammeascoma* species with low bootstrap support (Fig. 2) and morphology separates them as distinct genera. *Purpureofaciens* comprises one freshwater species *P. aquatica* which was collected from Thailand.

#### List of freshwater *Purpureofaciens* species

\**Purpureofaciens aquatica* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number*: IF557902; *Facesoffungi number*: FoF08718; Fig. 26

*Etymology*: in reference to aquatic habitat of the fungus

*Holotype*: MFLU 18-1569

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Ascomata* 200–300 µm high, 300–400 µm diam., dark brown to black, scattered or gregarious, semi-immersed to superficial, conical, flattened at the basal region, stain the substrate purple and produce reddish pigmentation at the apex, coriaceous, with a large, protuberant, ostiolate papilla, 110–130 µm long, 110–130 µm wide. *Peridium* unequal thickness, poorly developed at the base, up to 45 µm at the sides and 15 µm at the base, composed of reddish brown, large cells of *textura angularis*. *Pseudoparaphyses* 2 µm diam., numerous, trabeculate, filiform, hyaline, embedded in a hyaline gelatinous matrix. *Asci* 135–150 × 8.5–11 µm ( $\bar{x}$  = 143 × 9.7 µm, n = 10), 8-spored, bitunicate, cylindrical, pedicellate, apically rounded. *Ascospores* 15–22 × 7–10 µm ( $\bar{x}$  = 18.5 × 8 µm, n = 20), uniseriate, curved, olivaceous, 1-septate, constricted at the septum, ellipsoidal with acute ends, with two prominent guttules when immature, thin-walled, smooth, surrounded by a thin mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characteristics*: On PDA, colony circular, reaching 50 mm diam. in 25 days at 25 °C, annulate, white to reddish to purplish red from center to edge, surface rough, dry, raised, edge entire.

*Material examined*: THAILAND, Satun Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat266-1 (MFLU 18-1569, **holotype**), ex-type living culture MFLUCC 18-1241; *ibid.* hat266-2 (HKAS 105016, **isotype**), ex-type living culture KUMCC 19-0032.

**Aquasubmersaceae** A. Hashim. & Kaz. Tanaka, *Persoonia* 39: 56 (2017)

*Aquasubmersa* K.D. Hyde & H. Zhang, *Cryptog. Mycol.* 33(3): 340 (2012)

*Saprobic* on submerged or dead wood. **Sexual morph**: *Ascomata* scattered to grouped, immersed to semi-immersed, uniloculate, subglobose, glabrous, dark brown to black, with ostiolate papilla. *Peridium* composed of flattened, thin-walled cells of *textura angularis*. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, septate. *Asci* 8-spored, bitunicate, cylindrical, short pedicellate, apically rounded, with an ocular chamber. *Ascospores* biseriate, broadly fusiform with rounded ends, straight, hyaline, with one mostly median septum, with gelatinous sheath (Ariyawansa et al. 2015). **Asexual morph**: Coelomycetous. *Pycnidia* solitary to scattered, semi-immersed to superficial, subglobose to ellipsoidal, dark brown to black, ostiolate, minute or without papilla. *Ostiole* circular, dark-brown, central. *Peridium* composed of polygonal, hyaline to brown cells. *Conidiophores* reduced. *Conidiogenous cells* holoblastic, determinate, lageniform, hyaline, smooth. *Conidia* ellipsoidal, some with papillate base, hyaline, aseptate, smooth, thin-walled, without sheath or appendage (Zhang et al. 2012a).

*Type species*: *Aquasubmersa mircensis* H. Zhang & K.D. Hyde

*Notes*: *Aquasubmersa* was established to accommodate a freshwater coelomycetous species, *A. mircensis* (Zhang et al. 2012a). It was placed in the family Lophiotremataceae in previous studies (Ariyawansa et al. 2015; Doilom et al. 2017). A comprehensive study on Lophiotremataceae based on morphological observations and multigene analyses showed that *Aquasubmersa* should be transferred to a newly established family Aquasubmersaceae (Hashimoto et al. 2017). The second species, *A. japonica* A. Hashim. & Kaz. Tanaka with sexual morph, was also collected from freshwater habitats and conidiomata were produced in culture (Ariyawansa et al. 2015). The asexual morph of *A. japonica* matches the generic concept of *Aquasubmersa*. It differs from *A. mircensis* by pycnidia with minute papilla, thicker peridium and conidia lacking papillate bases. The type material of *A. mircensis* was re-examined by Li et al. (2020) and this study.

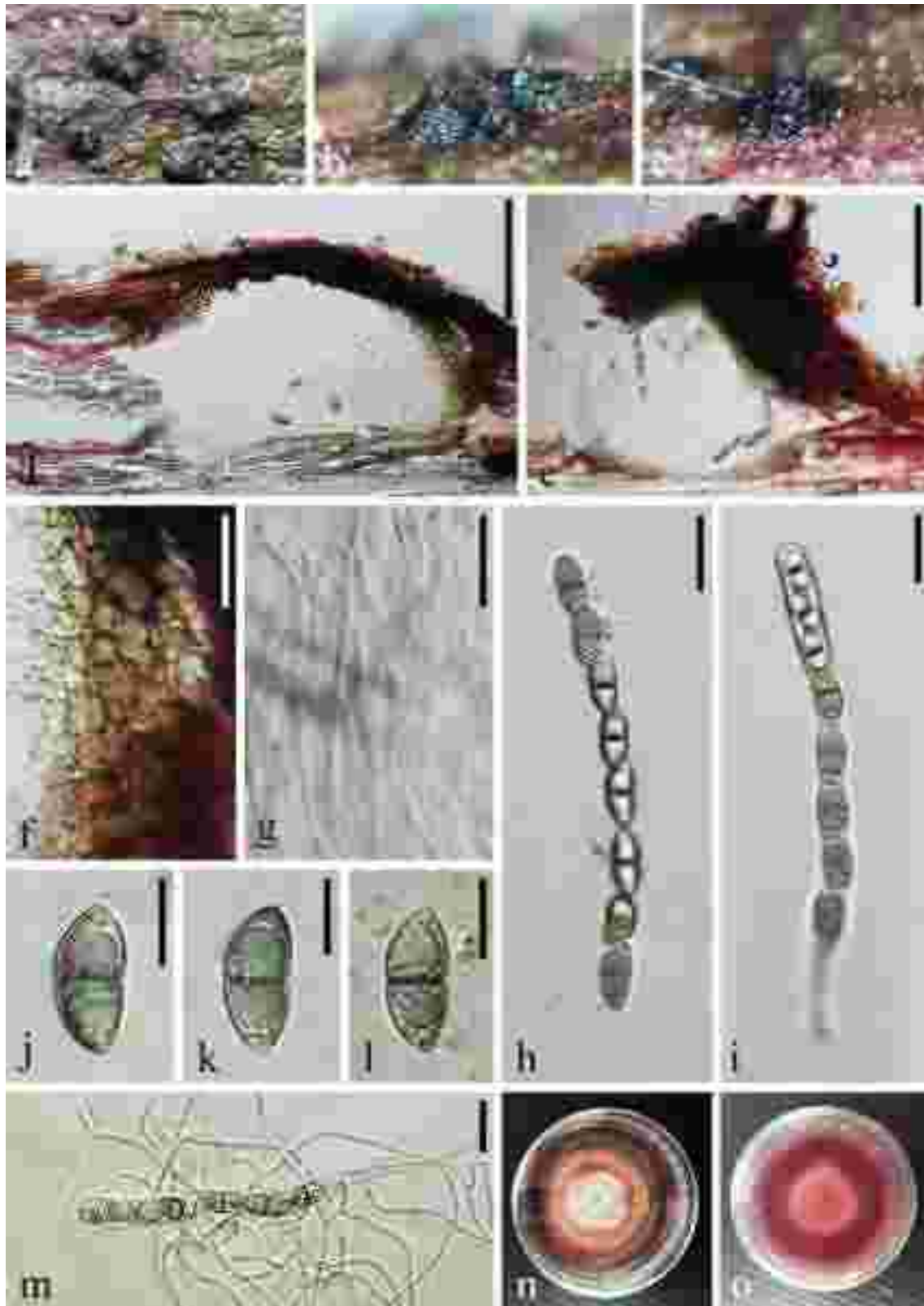
#### List of freshwater *Aquasubmersa* species

\**Aquasubmersa japonica* A. Hashim. & Kaz. Tanaka, *Fungal Diversity* 75: 87 (2015)

*Freshwater distribution*: Japan (Ariyawansa et al. 2015)

\**Aquasubmersa mircensis* H. Zhang & K.D. Hyde, *Cryptog. Mycol.* 33(3): 340 (2012); Fig. 27

*Freshwater distribution*: Thailand (Zhang et al. 2012a)



**Fig. 26** *Purpureofaciens aquatica* (MFLU 18-1569, holotype). **a–c** Appearance of black ascomata on host surface. **d, e** Vertical section of ascomata. **f** Structure of peridium. **g** Pseudoparaphyses. **h, i** Bituni-

cate asci. **j–l** Ascospores. **m** Germinated ascospores. **n, o** Colony on PDA (left-front, right-reverse). Scale bars: **d, e** = 100  $\mu$ m, **f–i, m** = 20  $\mu$ m, **j–l** = 10  $\mu$ m



**Fig. 27** *Aquasubmersa mircensis* (Material examined: THAILAND, Chiang Mai Province, Mushroom Research Centre, on submerged wood, 21 April 2011, H. Zhang, m3, MFLU 11-1001, **holotype**). **a**

Pycnidium on wood surface. **b** Section of pycnidium. **c** Peridium with conidiogenous cells. **d** Conidiogenous cells with conidia. **e, f** Conidia. Scale bars: **b** = 100  $\mu$ m, **c** = 20  $\mu$ m, **d** = 15  $\mu$ m, **e, f** = 10  $\mu$ m

**Key to freshwater *Aquasubmersa* species**

- 1. Pycnidia up to 215  $\mu$ m high, 115–195  $\mu$ m diam., with minute papilla, peridium > 20  $\mu$ m thick, conidia without papillate base.....*A. japonica*
- 1. Pycnidia 130–170  $\mu$ m high, 150–250  $\mu$ m diam., without papilla, peridium < 20  $\mu$ m thick, conidia with papillate base.....*A. mircensis*

**Astrosphaeriellaceae** Phook. & K.D. Hyde, Fungal Diversity 74: 161 (2015)

**Key to freshwater genera of Astrosphaeriellaceae**

- 1. Asci mostly broadly clavate.....2
- 1. Asci cylindrical.....3
- 2. Ascospores hyaline, cylindrical.....*Aquatospora*
- 2. Ascospores mostly dark brown to black, fusiform to broad-ellipsoid or biconic.....*Caryospora*
- 3. Producing hyphomycetous asexual morph.....*Pithomyces*
- 3. Producing coelomycetous asexual morph or unknown.....4
- 4. Ascospores brown to reddish brown, with paler end cells.....*Xenoastrosphaeriella*
- 4. Ascospores hyaline to brown, without paler end cells.....*Astrosphaeriella*

***Aquatospora*** W. Dong, H. Zhang & K.D. Hyde, *gen. nov.*  
*Index Fungorum number:* IF557903; *Facesoffungi number:* FoF09245

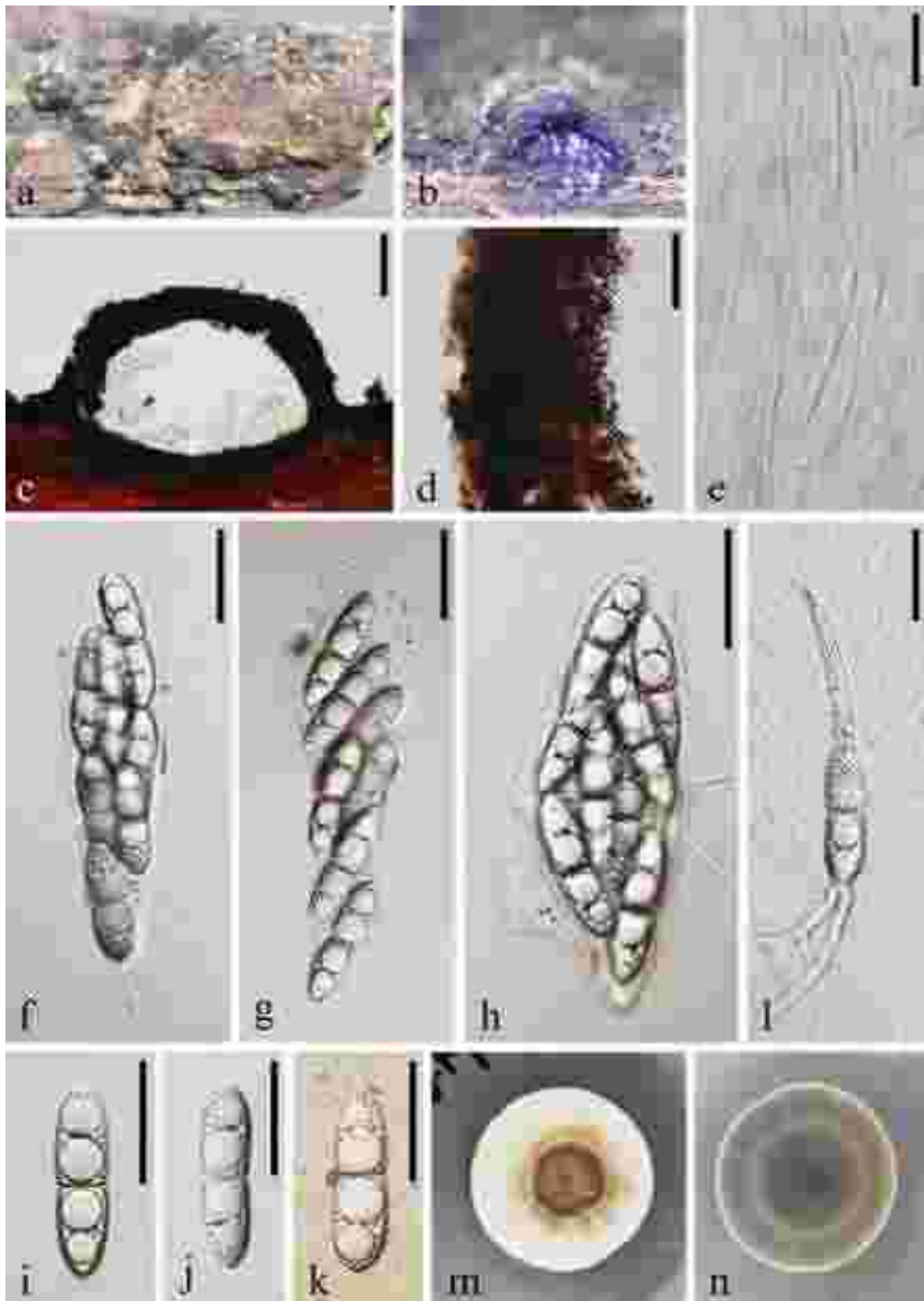
*Etymology:* referring to aquatic habitat of this fungus  
*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph:** *Ascomata* black, scattered or gregarious,

superficial, hemisphaerical to conical, base flattened, carbonaceous, with ostiolate papilla. *Ostirole* central, relatively broad, circular, black. *Peridium* uneven in width, carbonized, composed of black amorphous layer, whose cells are obscured. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, sparsely septate. *Asci* 8-spored, bitunicate, clavate to narrowly ellipsoidal, with a short pedicel, apically rounded. *Ascospores* 2–4-seriate, hyaline, septate, subcylindrical, thin-walled, smooth. **Asexual morph:** Undetermined.

*Type species:* *Aquatospora cylindrica* W. Dong, H. Zhang & K.D. Hyde

*Notes:* *Aquatospora* clusters with *Acrocordiopsis* Borse & K.D. Hyde and *Xenoastrosphaeriella* Jayasiri et al. with low bootstrap support (Fig. 2). *Acrocordiopsis* is a problematic genus, which was placed in Salsugineaceae by Hyde et al. (2013) and Caryosporaceae by Ariyawansa et al. (2015). *Acrocordiopsis* was referred to Salsugineaceae (Wijayawardene et al. 2018, 2020) based on some common morphology between *Acrocordiopsis* and *Salsuginea* K.D. Hyde (Borse and Hyde 1989; Hyde 1991). *Acrocordiopsis* clustered distantly from *Salsuginea* in the phylogenetic tree of Zhang et al. (2018) and close to *Aquatospora* in our study (Fig. 2). *Xenoastrosphaeriella* differs from *Aquatospora* in having paler end cells of ascospores (Hawksworth and Boise 1985; Phookamsak et al. 2015b; Jayasiri et al. 2019). *Aquatospora* shares similar characters with *Astrosphaeriellaceae* members in having large, erumpent to superficial, hemisphaerical or conical, carbonaceous ascomata with nearly flattened bases (Borse and Hyde 1989; Ariyawansa et al. 2015; Phookamsak et al. 2015b). However, the unique combination of morphology of clavate to narrowly ellipsoidal asci, hyaline, cylindrical ascospores and molecular characters warrant *Aquatospora* as a new genus (more details see key to freshwater genera of *Astrosphaeriellaceae*).



**Fig. 28** *Aquatospora cylindrica* (MFLU 18-1534, **holotype**). **a** Appearance of black ascomata on host. **b, c** Vertical section of ascoma. **d** Structure of peridium. **e** Pseudoparaphyses. **f–h** Bitunicate asci. **i–k** Ascospores. **k** Ascospore in Indian Ink. **l** Germinated ascospore. **m, n** Colony on PDA (left-front, right-reverse). Scale bars: **c** = 200  $\mu$ m, **d, f–l** = 50  $\mu$ m, **e** = 20  $\mu$ m

### List of freshwater *Aquatospora* species

\**Aquatospora cylindrica* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number*: IF557904; *Facesoffungi number*: FoF09246; Fig. 28

*Etymology*: referring to cylindrical ascospores of this fungus

*Holotype*: MFLU 18-1534

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Ascomata* 600–650  $\mu$ m high, 800–900  $\mu$ m diam., black, scattered or gregarious, superficial, hemispherical to conical, base flattened, carbonaceous, with ostiolate papilla. *Ostirole* central, relatively broad, circular, black. *Peridium* up to 155  $\mu$ m at the sides and 65  $\mu$ m at the base, carbonized, composed of black amorphous layer, whose cells are obscured. *Pseudoparaphyses* 0.5  $\mu$ m diam., numerous, trabeculate, filiform, hyaline, sparsely septate. *Asci* 175–270  $\times$  50–65  $\mu$ m ( $\bar{x}$  = 225  $\times$  58  $\mu$ m,  $n$  = 10), 8-spored, bitunicate, clavate to narrowly ellipsoidal, straight or slightly curved, with a short pedicel, apically rounded. *Ascospores* 70–85  $\times$  20–25  $\mu$ m ( $\bar{x}$  = 77  $\times$  23  $\mu$ m,  $n$  = 10), 2–4-seriate, cylindrical, slightly tapering at two sides, 1-septate, constricted at the septum, hyaline, straight, with four prominent big guttules, thin-walled, smooth, without sheath. **Asexual morph**: Undetermined.

*Culture characteristics*: On PDA, colony circular, reaching 40 mm in 35 days at 25 °C, brown to white from above, dark brown from below, surface rough, dry, raised, edge entire.

*Material examined*: THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat146-1 (MFLU 18-1534, **holotype**), ex-type living culture MFLUCC 18-1287; *ibid.*, hat146-2 (HKAS 105042, **isotype**), ex-type living culture KUMCC 19-0060.

*Astrosphaeriella* Syd. & P. Syd., *Annls mycol.* 11(3): 260 (1913)

*Saprobic* or *parasitic* on bamboo, palm or stout grasses in freshwater or terrestrial habitats. **Sexual morph**: *Ascostromata* solitary to gregarious, erumpent to superficial, conical or mammiform, at maturity with stellate appearance from above, dark opaque, uniloculate, glabrous, brittle, carbonaceous, ostiolate. *Peridium* relatively thick, poorly developed at the base, composed of thick, opaque and melanized cells, arranged in a *textura angularis* with palisade-like cells at the rim. *Pseudoparaphyses* dense, trabeculate, filiform, hyaline, anastomosing, embedded in a hyaline gelatinous

matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, subsessile to short-pedicellate, apically rounded with ocular chamber. *Ascospores* overlapping unito bi-seriate, hyaline to brown, fusiform with rounded to acute ends, septate, smooth, with or without appendages or mucilaginous sheaths (Phookamsak et al. 2015b). **Asexual morph**: Coelomycetous. *Conidiomata* pycnidial, scattered or solitary, immersed to superficial, conical or hemisphaerical to globose. *Pycnidial walls* composed of several layers of dark brown to black, *textura angularis* to *textura intricata* of cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic or phialidic, integrated, cylindrical or cylindrical-clavate or ampulliform, septate or aseptate, hyaline, smooth. *Conidia* globose to subglobose, or oblong, aseptate, hyaline, smooth (Phookamsak et al. 2015b).

*Type species*: *Astrosphaeriella fusispora* Syd. & P. Syd.

*Notes*: *Astrosphaeriella* is characterized by carbonaceous, conical to mammiform ascostromata with ruptured, reflexed, stellate, host remnants and hyaline to brown, fusiform ascospores (Liu et al. 2011; Phookamsak et al. 2015b). *Astrosphaeriella aquatica* K.D. Hyde, *A. seychellensis* K.D. Hyde & Goh and *A. thailandensis* J. Ren et al. were only found from freshwater habitats (Hyde 1994; Hyde and Frohlich 1998; Hyde and Goh 1998b; Ren et al. 2013). Other freshwater *Astrosphaeriella* species were found from both freshwater and terrestrial habitats (see list below). *Astrosphaeriella aquatica* resembles *A. seychellensis* and *A. thailandensis* in having hyaline, fusiform ascospores, but differs in having a wide spreading mucilaginous sheath which is drawn out at the poles.

Phookamsak et al. (2015b) transferred *Astrosphaeriella sensu lato*, which have coriaceous ascomata with short to long neck and striate ascospores, to a newly established family Pseudoastrosphaeriellaceae. *Astrosphaeriella papuana* Aptroot has striate ascospores which are typical characters of Pseudoastrosphaeriellaceae (Aptroot 1995; Hyde and Frohlich 1998; Phookamsak et al. 2015b). However, molecular data for *A. papuana* is unavailable, thus this species is retained in Astrosphaeriellaceae. *Astrosphaeriella papuana* might be transferred to Pseudoastrosphaeriellaceae when the type specimen is recollected and recircumscribed.

*Astrosphaeriella stellata* (Pat.) Sacc. was treated as an earlier name of *A. fusispora* and followed by some studies (Hyde and Frohlich 1998; Hyde et al. 2000; Tanaka et al. 2009; Liu et al. 2011). Liu et al. (2011) named one collection MFLUCC 10-0555 as *A. stellata*, which was later considered as a reference specimen of *A. fusispora* (Phookamsak et al. 2015b). Therefore, *A. stellata* isolated from freshwater habitats is probably a collection of *A. fusispora*.

The asexual morph was developed in the culture of *Astrosphaeriella bambusae* Phookamsak & K.D. Hyde (Phookamsak et al. 2015b).

**List of freshwater *Astrosphaeriella* species**

*Astrosphaeriella aquatica* K.D. Hyde, Mycol. Res. 98(7): 719 (1994)

*Freshwater distribution:* Papua New Guinea (Hyde 1994), Ecuador (Hyde and Frohlich 1998)

*Astrosphaeriella exorrhiza* Boise, Sydowia 38: 117 (1986) [1985]

*Freshwater distribution:* Ecuador (Hyde and Frohlich 1998)

*Astrosphaeriella maquilingiana* (Rehm) K.D. Hyde & J. Fröhl., Sydowia 50(1): 103 (1998)

*Basionym:* *Trematosphaeria maquilingiana* Rehm, Leaflet of Philipp. Bot. 8: 2952 (1916)

*Synonymy:* *Trematosphaeria maquilingiana* var. *schizotachyi* Rehm, Leaflet of Philipp. Bot. 8: 2952 (1916)

*Trematosphaeria maquilingiana* Rehm, Leaflet of Philipp. Bot. 8: 2952 (1916) var. *maquilingiana*

*Freshwater distribution:* Australia (Vijaykrishna and Hyde 2006)

*Astrosphaeriella papuana* Aptroot, Nova Hedwigia 60(3–4): 333 (1995)

*Freshwater distribution:* Papua New Guinea (Hyde and Frohlich 1998), Philippines (Cai et al. 2003a)

*Astrosphaeriella seychellensis* K.D. Hyde & Goh, S. Afr. J. Bot. 64: 332 (1998)

*Freshwater distribution:* Seychelles (Hyde and Goh 1998b)

\**Astrosphaeriella stellata* (Pat.) Sacc., Syll. fung. (Abellini) 24(2): 938 (1928); Fig. 29

*Basionym:* *Amphisphaeria stellata* Pat., Bull. Soc. mycol. Fr. 29: 223 (1913)

*Synonymy:* *Astrosphaeriella stellata* var. *palmicola* F. San Martín & P. Lavín, Lavín & Lavín, Acta Bot. Mexicana 46: 22 (1999)

*Astrosphaeriella bambusella* Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 129: 168 (1920)

*Astrosphaeriella fuscomaculans* W. Yamam., Sci. Rep. Hyogo Univ. Agric., Ser. 2, Agr. Biol. 1: 63 (1954)

*Microthelia fuscomaculans* (W. Yamam.) E. Müll., Beitr. Kryptfl. Schweiz 11(no. 2): 286 (1962)

*Freshwater distribution:* China (Tsui et al. 2001d; Wong and Hyde 2001; Luo et al. 2004; Hu et al. 2013), Philippines (Cai et al. 2003a)

*Astrosphaeriella thailandensis* J. Ren, C. Y. Jie, Y. L. Jiang, K. D. Hyde & Yong Wang bis, Sydowia 65(1): 33–43 (2013)

*Freshwater distribution:* Thailand (Ren et al. 2013)

**Key to freshwater *Astrosphaeriella* species**

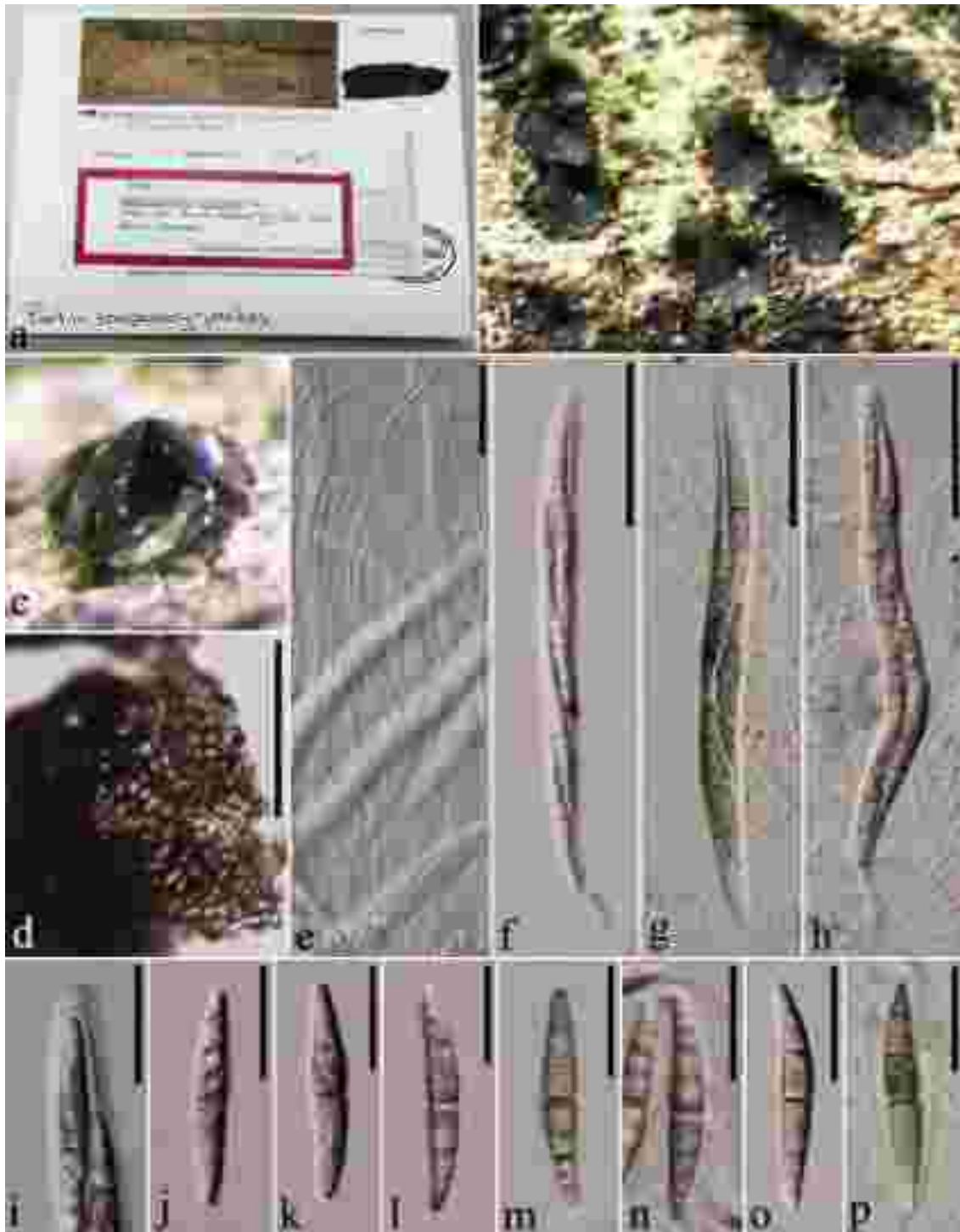
1. Ascospores surrounded by sheath or with appendage.....2
1. Ascospores without sheath or appendage.....6
2. Ascospores sometimes slightly verrucose.....  
.....*A. maquilingiana*
2. Ascospores smooth-walled.....3
3. Ascospores with minute appendages at both ends...  
.....*A. exorrhiza*
3. Ascospores surrounded by a mucilaginous sheath.....4
4. Ascospores pigmented.....*A. stellata*
4. Ascospores hyaline.....5
5. Ascospores 30–42 × 7–8 µm, sheath drawn out at the poles.....*A. aquatica*
5. Ascospores 16–22 × 4–4.5 µm, sheath appears as remnants in dried material.....*A. seychellensis*
6. Ascospores with striate ornamentation.....  
.....*A. papuana*
6. Ascospores smooth-walled.....*A. thailandensis*

*Caryospora* De Not., Micr. Ital. Novi 9: 7 (1855)

*Saprobic* on submerged wood, decaying fruit pericarp or old stone of *Amygdalus persica*. **Sexual morph:** *Ascomata* pseudothecoid, medium to large, scattered, erumpent to superficial, uniloculate, broadly conical or hemispherical, with flattened base, brown to black, carbonaceous, with ostiolate papilla. *Ostiole* relatively broad, central, circular. *Peridium* thicker at sides and thinner at base, strongly carbonized. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, sparsely septate. *Asci* (2)8-spored, bitunicate, fissitunicate, broadly cylindrical to clavate or obclavate, pedicellate. *Ascospores* large, bi- to triseriate, fusiform to broad-ellipsoid or biconic, straight, usually hyaline when young, becoming pigmented at maturity, septate, most symmetrical, smooth or rough, often with polar germ pores at each end, thin- to thick-walled, with or without sheath. **Asexual morph:** Undetermined.

*Type species:* *Caryospora putaminum* (Schwein.) Fuckel

*Notes:* *Caryospora* was placed in Caryosporaceae based on sequence data of *C. aquatica* and one putative strain of *C. minima* (Ariyawansa et al. 2015). *Caryospora* clustered with *Acrocordiopsis* with weak bootstrap support (Ariyawansa et al. 2015). Jayasiri et al. (2019) placed *Caryospora* in *Astrosphaerellaceae* based on the similarity of carbonaceous ascostromata and trabeculate pseudoparaphyses with



**Fig. 29** *Astrosphaeriella stellata* (Material examined: VIETNAM, Hanoi, on Bamboo (sur Bambou), 17 April 1911, M. Duport 451, FH 00290284, Pat 552, **holotype**). **a** Herbarium label and specimen. **b** Ascostromata on host surface. **c** Section through ascostroma. **d** Sec-

tion through peridium. **e** Pseudoparaphyses. **f–h** Bitunicate asci. **i** Ocular chamber. **j–p** Ascospores. Scale bars: **d, e, i–p** = 20  $\mu$ m, **f–h** = 50  $\mu$ m

other genera in Astrosphaeriellaceae. However, Astrosphaeriellaceae members, including *Caryospora*, did not form a well-supported clade (Jayasiri et al. 2019; this study, Fig. 2).

*Zopfia rhizophila* Rabenh. (Zopfiaceae) also clustered in Astrosphaeriellaceae, but with low bootstrap support (Jayasiri et al. 2019; this study, Fig. 2). In this study, we follow

Jayasiri et al. (2019) and accept *Caryospora* in Astrosphaeriellaceae based on morphology.

*Caryospora obclavata* was reported from freshwater habitat and characterized by obclavate asci with ellipsoidal to biconic ascospores uniseriately arranged at the apex and base (Raja and Shearer 2008). The taxonomic placement of this species needs to be confirmed with molecular data. We introduce one new species *C. submersa* based on morphology and phylogeny. Additionally, *C. aquatica* and *C. quercus* are redescribed and reported as new geographical record in Thailand and new habitat record in freshwater, respectively.

### List of freshwater *Caryospora* species

\**Caryospora aquatica* H. Zhang, K.D. Hyde & Ariyawansa, Fungal Diversity 75: 54 (2015); Figs. 30, 31

*Freshwater Distribution*: China (this study), Thailand (Ariyawansa et al. 2015)

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph**: *Ascomata* 270–310 µm high, 250–330 µm diam., black, scattered or gregarious, superficial, hemispherical, flattened at the base, carbonaceous, ostiolate, always covered with mass of black, glistening ascospores. *Peridium* 50–60 µm at the sides, poorly developed at the base, easily cracked, two-layered, outer layer strongly carbonized, amorphous, composed of several layers of black cells that cannot be differentiated and often occluded, inner layer composed of several layers of hyaline cells of *textura angularis*. *Pseudoparaphyses* 2 µm diam., numerous, trabeculate, filiform, hyaline, septate. *Asci* 160–210(–270) × 45–58 µm ( $\bar{x}$  = 190 × 52 µm, n = 10), 8-spored, bitunicate, narrowly to broadly clavate, with short pedicel, apically rounded. *Ascospores* 35–48 × 18–28 µm ( $\bar{x}$  = 42 × 22 µm, n = 40), biseriate, straight, broadly fusiform, hyaline to pale brown, 1-septate when young, becoming irregularly diamond-shaped, dark brown to black, 3-septate when mature, with rounded ends, constricted and darker at the median septum, not constricted and inconspicuous at two terminal septa, asymmetric, with two large central cells and two small end cells, guttulate, thick-walled, smooth, sometimes with polar germ pores at each end, surrounded by an irregular, mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characteristics*: On PDA, colony circular, reaching 15 mm diam. in 50 days at 25 °C, grey from above, black from below, surface rough, dry, with dense mycelium, raised, edge entire.

*Material examined*: CHINA, Yunnan Province, on submerged wood in a stream, 25 November 2017, G.N. Wang, H3A-1 (MFLU 18-1202), living culture MFLUCC 18-1030; *ibid.*, H3A-2 (HKAS 101729), living culture KUMCC 18-0079.

*Notes*: Our new collection MFLUCC 18-1030 clusters with *Caryospora aquatica* H. Zhang et al. (MFLUCC 11-0008) and one putative strain of *C. minima* Jeffers with

high bootstrap support (Fig. 2). MFLUCC 18-1030 has identical LSU sequence data with *C. aquatica* MFLUCC 11-0008 and seven (including three gaps) nucleotide differences in ITS sequence data between the two isolates, which indicate them to be the same species. Morphologically, our collection has very similar morphological characters with *C. aquatica* (Figs. 30, 31), except the slightly thinner asci (45–58 µm wide vs. 60–80 µm wide) and irregular sheaths (Ariyawansa et al. 2015). Given this, we identify our collection MFLUCC 18-1030 as *C. aquatica*. This is a new geographical record of *C. aquatica* in China.

*Caryospora minima* was initially collected from putrescent putamina of *Amygdalus persica* in Maryland, USA (Jeffers 1940). Later, several freshwater collections of *C. minima* were reported in China, Philippines and South Africa (see freshwater distribution of this species), but they were not confirmed by molecular data. Cai and Hyde (2007b) provided LSU (EU196550) and SSU (EU196551) sequence data for *C. minima* without strain code, description, illustration and herbarium information. Thus, these two sequences are doubtful. A specimen (IFRD 083-010) under name *C. minima* is illustrated in this study (Fig. 32). However, this species should be treated with caution based on the holotype BPI 71132. It would be good to establish an epitype for *C. minima* with reliable sequence data.

\**Caryospora minima* Jeffers, Mycologia 32(4): 561 (1940); Fig. 32

*Freshwater Distribution*: China (Tsui et al. 2000; Luo et al. 2004), Philippines (Cai et al. 2003a), South Africa (Hyde et al. 1998)

*Caryospora obclavata* Raja & Shearer, Mycologia 100(3): 479 (2008)

*Freshwater Distribution*: USA (Raja and Shearer 2008)

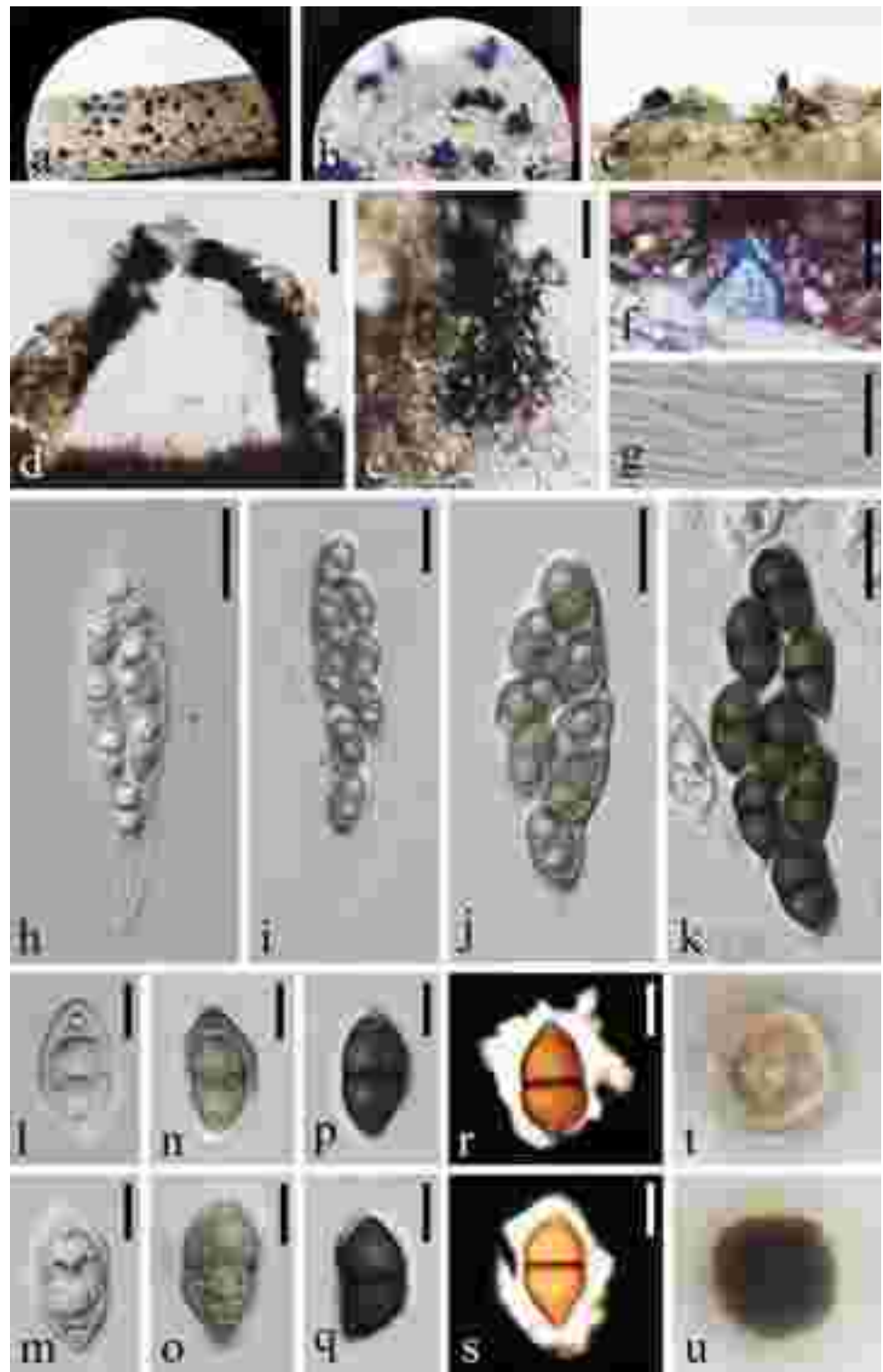
\**Caryospora quercus* Jayasiri, E.B.G. Jones & K.D. Hyde, Mycosphere 10(1): 34 (2019); Fig. 33

*Freshwater Distribution*: Thailand (this study)

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph**: *Ascomata* 400–450 µm high, 410–460 µm diam., black, scattered, semi-immersed to superficial, conical, flattened at the base, carbonaceous, ostiolate, 70 µm diam., often covered with mass of black, glistening ascospores. *Peridium* 60–70 µm at the sides, poorly developed at the base, easily cracked, two-layered, outer layer strongly carbonized, composed of several layers of black cells that cannot be differentiated and often occluded, inner layer composed of 8–10 layers of hyaline, compressed cells of *textura angularis*. *Pseudoparaphyses* 2 µm diam., numerous, trabeculate, filiform, hyaline, distantly septate. *Asci* (165–)205–270(–305) × (58–)65–80 µm ( $\bar{x}$  = 235 × 70 µm, n = 10), 8-spored, bitunicate, narrowly to broadly

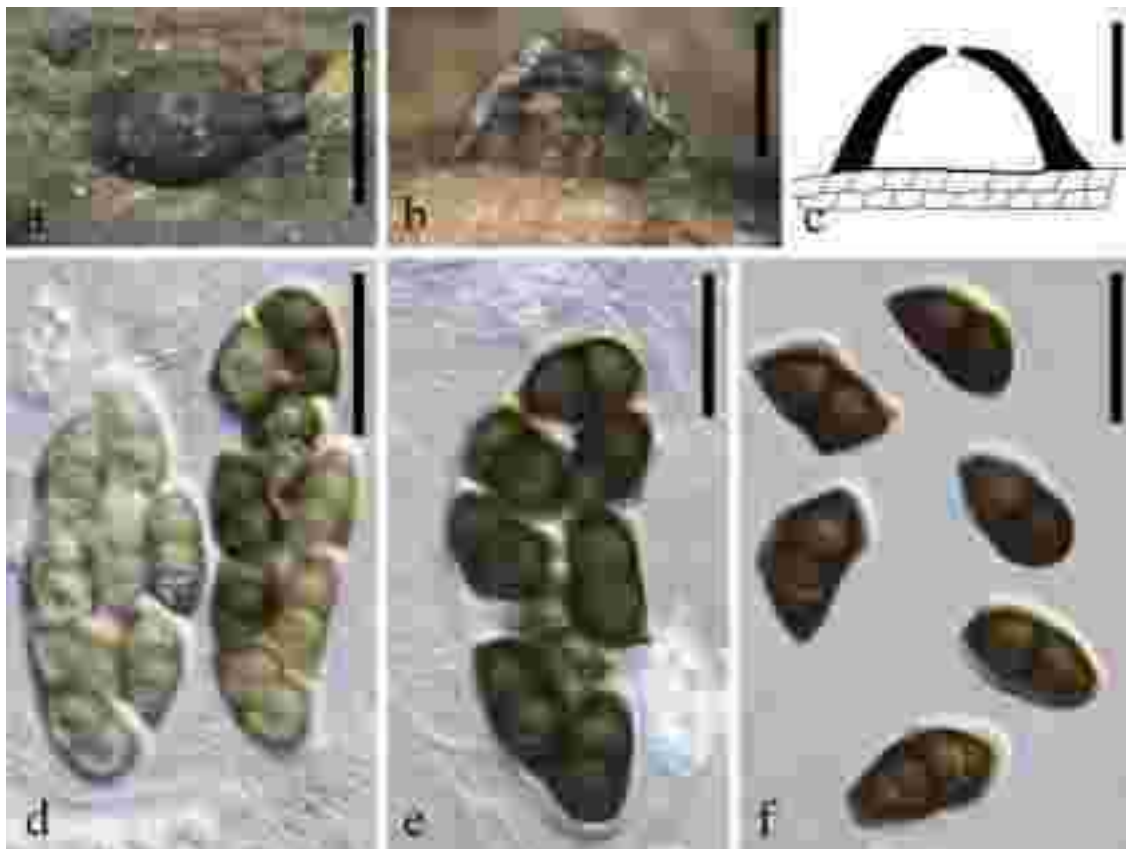


**Fig. 30** *Caryospora aquatica* (MFLU 18-1202, new geographical record). **a–c** Ascomata on host surface. **d, f** Vertical section of ascomata. **e** Structure of peridium. **g** Pseudoparaphyses. **h–k** Bitunicate asci. **l–q** Immature and mature ascospores. **r, s** Ascospores in Indian Ink. **t, u** Colony on PDA (up-front, down-reverse). Scale bars: **d** = 100  $\mu$ m, **e–g, l–s** = 20  $\mu$ m, **h–k** = 40  $\mu$ m



clavate, indistinctly pedicellate which become tapered and disappeared with age, 30–60  $\mu$ m long, apically rounded. *Ascospores* 47–60  $\times$  19–33  $\mu$ m ( $\bar{x}$  = 54  $\times$  27  $\mu$ m,  $n$  = 30), overlapping bi- to tri-seriate, straight, broadly fusiform to ellipsoidal, hyaline to pale brown, 1-septate when young, becoming irregularly diamond-shaped, dark brown to black,

3-septate when mature, with acute ends, constricted and darker at the median septum, not constricted and inconspicuous at two terminal septa, asymmetric, with two large central cells and two small end cells, thick-walled, smooth, sometimes with polar germ pores at each end, surrounded by a large, ellipsoidal, gelatinous, mucilaginous sheath, 3–37



**Fig. 31** *Caryospora aquatica* (Material examined: THAILAND, Chiang Rai Province, on submerged wood, 18 January 2010, H. Zhang, MFLU 11-1083, **holotype**). **a** Ascomata on the host surface. **b** Sec-

tion of an ascoma on wood. **c** Section of ascoma. **d, e** Different stages of asci. **f** Ascospores. Scale bars: **a** = 700  $\mu$ m, **b, c** = 350  $\mu$ m, **d, e** = 60  $\mu$ m, **f** = 50  $\mu$ m

$\mu$ m thick, sheath two-layered and large when young, becoming one-layered and smaller after mature. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 5 mm diam. in 20 days at 25 °C, grey from above and brown from below, surface rough, dry, raised, edge entire.

**Material examined:** THAILAND, Chiang Mai Province, on submerged wood in a stream, 1 September 2017, X.D. Yu, 1A (MFLU 17-1705, **holotype**), ex-type living culture MFLUCC 17-2342; *ibid.*, G.N. Wang, 4.2 (MFLU 17-1668), living culture MFLUCC 17-2323.

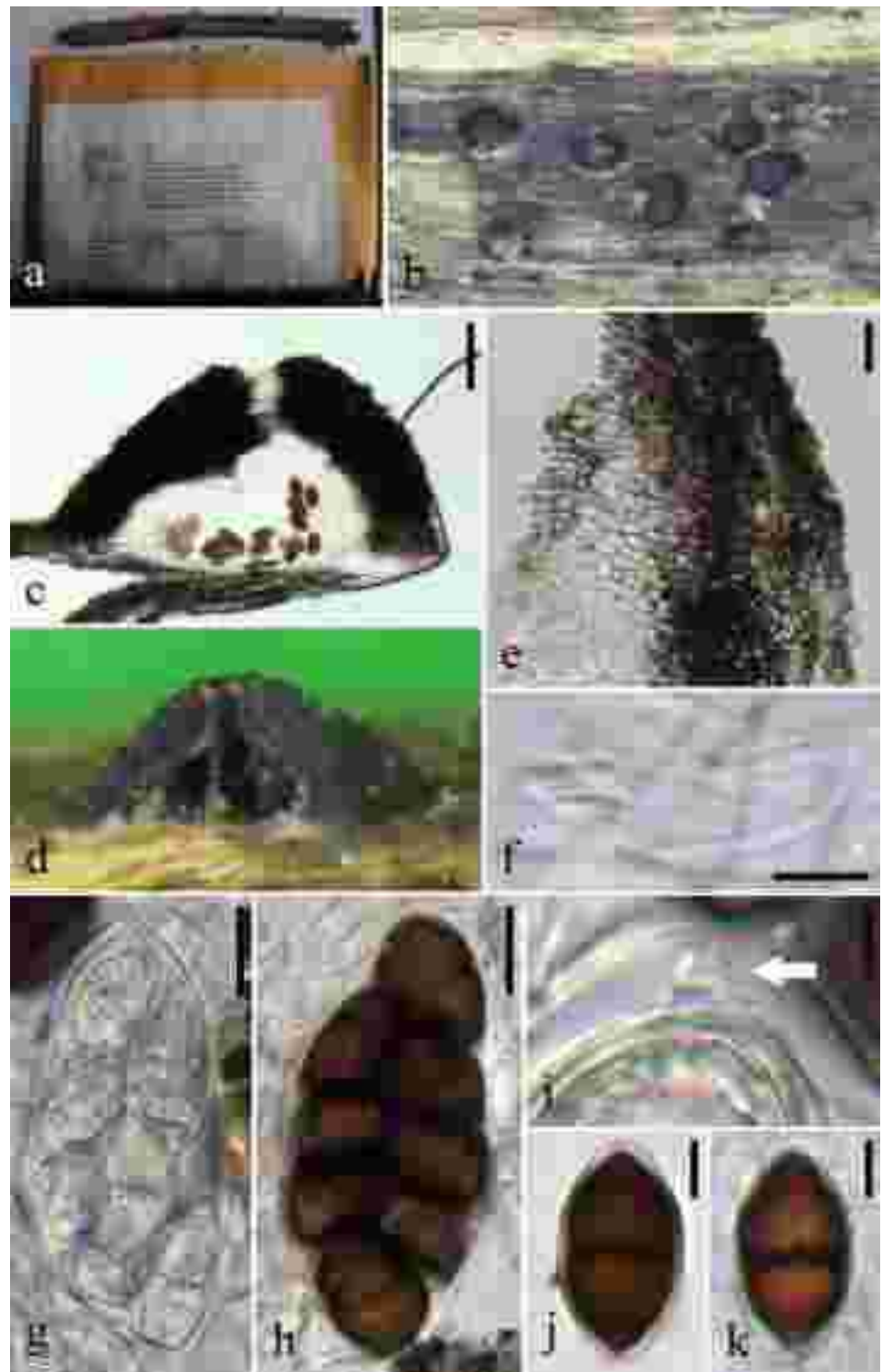
**Notes:** Our new collections MFLUCC 17-2342 and MFLUCC 17-2323 cluster with *Caryospora quercus* with strong bootstrap support (Fig. 2). They have identical LSU sequence data and there are two nucleotide differences in SSU sequence data between MFLUCC 17-2342 and MFLU 18-2151 (type). No other genes can be compared.

Our new collections have overlapping ascospore size (47–60  $\times$  19–33  $\mu$ m vs. 41–54  $\times$  18–28  $\mu$ m) and similar morphological characters with *C. quercus*, i.e. broadly fusiform to ellipsoidal and hyaline to pale brown when young, becoming irregularly diamond-shaped and dark brown to

black when mature, with acute ends, with one primary median septum and two inconspicuous septa near both ends, asymmetric, and with a sheath (Jayasiri et al. 2019). However, the ascospore sheaths in our collections are up to 37  $\mu$ m thick, while they are 5.5  $\mu$ m thick in the holotype of *C. quercus*. This might be a result of only measuring the mature ascospores in Jayasiri et al. (2019), because they are 37  $\mu$ m thick in young ascospores and 3  $\mu$ m thick in mature ascospores in our collections. Additionally, our collections have longer and wider asci ((165–)205–270(–305)  $\times$  (58–)65–80  $\mu$ m vs. (69–)110–147  $\times$  30–35  $\mu$ m) than the holotype of *C. quercus*. It is very likely that Jayasiri et al. (2019) only measured the immature asci as shown in their photo plate. We identify our collections to be *C. quercus* based on current phylogenetic analysis (Fig. 2), single gene comparison and morphology. We supplement ITS sequence data for *C. quercus*. This is a new habitat record for *C. quercus* from freshwater in Thailand.

\**Caryospora submersa* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

**Fig. 32** *Caryospora minima* (Material examined: CHINA, Hong Kong, on submerged wood in Lam Tsuen River, 1 December 1996, C. Tsui, IFRD 083-010). **a** Herbarium label and specimen of *Caryospora minima*. **b** Ascomata on host surface. **c, d** Vertical section through ascoma. **e** Structure of peridium. **f** Pseudoparaphyses. **g, h** Bitunicate asci. **i** Ocular chamber. **j** Ascospore. **k** Ascospore in Indian Ink. Scale bars: **c** = 100  $\mu$ m, **e, g, h** = 20  $\mu$ m, **f, j–k** = 10  $\mu$ m, **i** = 5  $\mu$ m



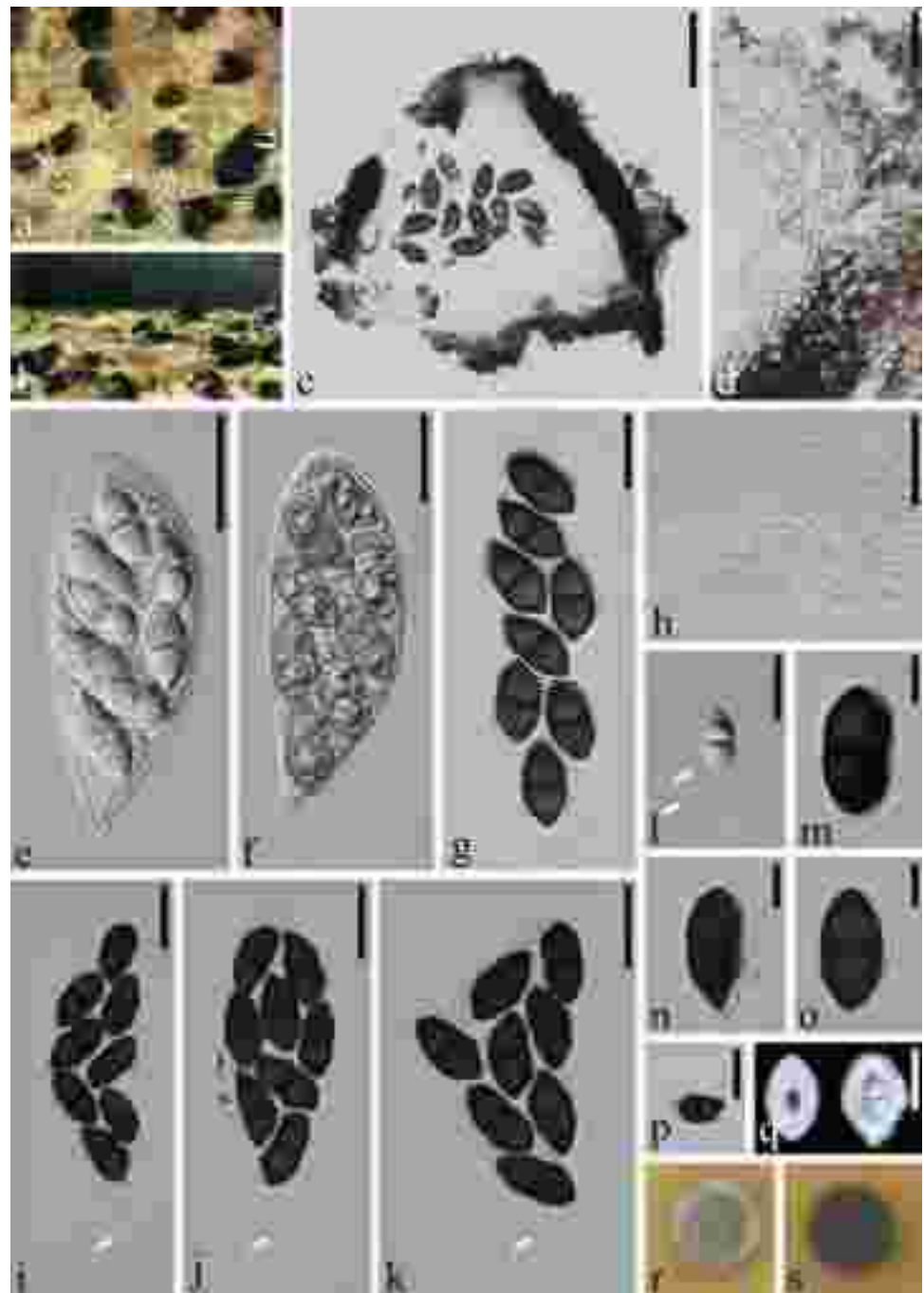
*Index Fungorum* number: IF557905; *Facesoffungi* number: FoF09248; Figs. 34, 35

*Etymology*: in reference to submerged habitat of the fungus

*Holotype*: MFLU 18-1539

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph**: *Ascomata* 400–450  $\mu$ m high, 450–540  $\mu$ m diam., black, scattered or gregarious, superficial, conical, flattened at the base, carbonaceous, with ostiolate papilla. *Peridium* 45–85  $\mu$ m at the sides, poorly developed at the base, easily cracked, strongly carbonized, composed of

**Fig. 33** *Caryospora quercus* (MFLU 17-1705, **new habitat record**). **a, b** Ascomata on host surface. **c** Vertical section of ascoma. **d** Structure of peridium. **e–g, i–k** Bitunicate asci. **h** Pseudoparaphyses. **l** Immature ascospore, with two-layered sheath (arrows). **m–o** Mature ascospores. **p** Germinated ascospore. **q** Ascospore in Indian Ink. **r, s** Colony on PDA (left-front, right-reverse). Scale bars: **c** = 100  $\mu$ m, **d, h, m–o** = 20  $\mu$ m, **e–g, i–l, p, q** = 50  $\mu$ m



several black, amorphous layers of cells that cannot be differentiated and often occluded. *Pseudoparaphyses* 2  $\mu$ m diam., numerous, trabeculate, filiform, hyaline, sparsely septate. *Asci* 130–180  $\times$  17–26  $\mu$ m ( $\bar{x}$  = 160  $\times$  21  $\mu$ m,  $n$  = 20), 8-spored, bitunicate, narrowly obclavate, with rounded apex, short pedicellate, with a well-developed ocular chamber. *Ascospores* 38–50  $\times$  8–12  $\mu$ m ( $\bar{x}$  = 44  $\times$  9  $\mu$ m,  $n$  = 20), mostly uniseriate near the apex, becoming biseriate towards the base, straight or slightly curved, hyaline, 1-septate, constricted at the septum, narrowly fusiform, guttulate,

thin-walled, smooth, surrounded by a large, oblong or ellipsoidal, mucilaginous sheath, 40–80  $\mu$ m wide, sheath becoming thin and dumbbell-like in dried specimen, 1.5–2.5  $\mu$ m wide at the sides and 5–15  $\mu$ m wide at two ends. **Asexual morph:** Undetermined.

*Culture characteristics:* On PDA, colony circular or ellipsoidal, reaching 10 mm diam. in 10 days at 25  $^{\circ}$ C, grey to black from above, dark brown from below, surface rough, dry, raised, edge entire.



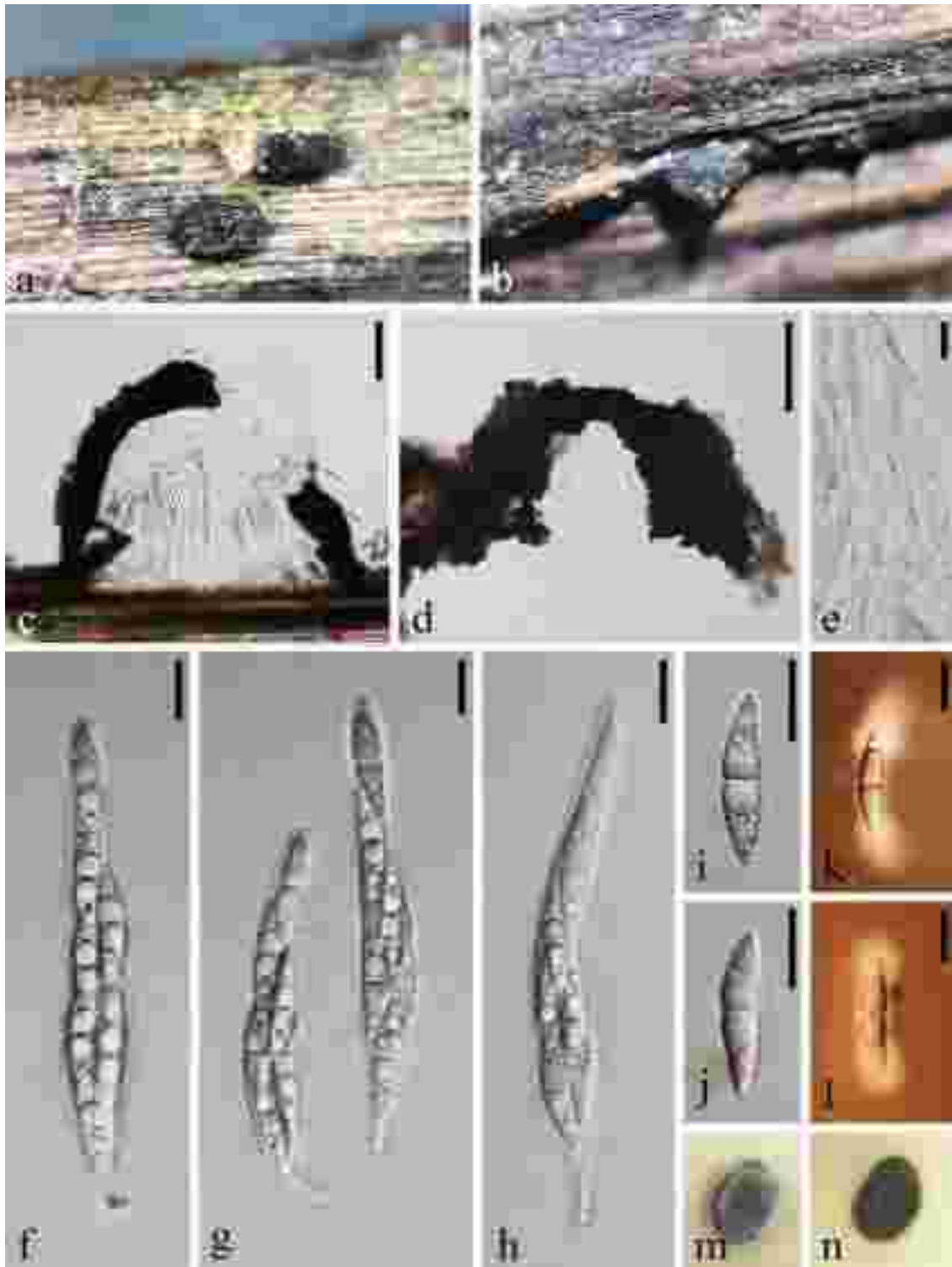
**Fig. 34** *Caryospora submersa* (MFLU 18-1539, **holotype**). **a, b** Ascomata on host surface. **c** Vertical section of ascoma. **d** Structure of peridium. **e–g** Bitunicate asci. **h** Asci embedded in pseudoparaphyses. **i–k** Ascospores. **l** Ascospore from fresh specimen mounted

in Indian Ink. **m** Germinated ascospore. **n** Colony on PDA (up-front, down-reverse). Scale bars: **c** = 100  $\mu$ m, **d, l, m** = 50  $\mu$ m, **e–k** = 20  $\mu$ m

**Material examined:** THAILAND, Songkhla Province, Chestnut hill, on submerged wood in a stream, 10 May 2018, W. Dong, hat458-1 (MFLU 18-1539, **holotype**), ex-type living culture MFLUCC 18-1283; *ibid.*, hat458-2 (HKAS 105039, **isotype**), ex-type living culture KUMCC 19-0056;

Satun Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat286-1 (MFLU 18-1541), living culture MFLUCC 18-1409; *ibid.*, hat286-2 (HKAS 105070).

**Notes:** *Caryospora submersa* is similar to other *Caryospora* species in having superficial, dark, carbonaceous



**Fig. 35** *Caryospora submersa* (MFLU 18-1541). **a, b** Ascomata on host surface. **c** Vertical section of ascoma. **d** Vertical section of ostiole papilla. **e** Pseudoparaphyses. **f–h** Bitunicate asci. **i, j** Ascospores.

**k, l** Ascospores from dried specimen mounted in Indian Ink. **m** Colony on PDA (left-front, right-reverse). Scale bars: **c** = 100  $\mu$ m, **d** = 50  $\mu$ m, **e–l** = 20  $\mu$ m

ascomata with flattened bases. *Caryospora submersa* is very different from other species by obclavate asci and narrowly fusiform, hyaline, thin-walled ascospores contrasting with broadly cylindrical to clavate asci and broadly fusiform, ellipsoidal or biconic, mostly pigmented, relatively thick-walled ascospores (Jeffers 1940; Hawksworth 1982; Abdel-Wahab and Jones 2000; Raja and Shearer 2008; Zhao and Zhao 2012; Ariyawansa et al. 2015). *Caryospora submersa* does not fit the generic concept of *Caryospora* except for the large, superficial, carbonaceous ascomata, which are similar to those in other genera within the family. However, *C. submersa* cannot be placed in other genera due to its distinct morphology and phylogenetically nests in *Caryospora* with strong bootstrap support (Fig. 2). Thus, *Caryospora submersa* sp. nov. is introduced.

### Key to freshwater *Caryospora* species

1. Ascospores rough-walled.....*C. obclavata*
1. Ascospores smooth-walled.....2
2. Ascospores thin-walled.....*C. submersa*
2. Ascospores thick-walled.....3
3. Ascospores pale brown when mature.....*C. minima*
3. Ascospores dark brown to black when mature.....4
4. Ascospores 35–48 × 18–28 µm.....*C. aquatica*
4. Ascospores 47–60 × 19–33 µm.....*C. quercus*

*Pithomyces* Berk. & Broome, J. Linn. Soc., Bot. 14(no. 74): 100 (1873) [1875]

*Saprobic* in terrestrial or freshwater habitats. **Sexual morph:** *Ascomata* dark opaque, solitary to gregarious, erumpent to superficial, mammiform to conical, with ruptured, reflexed, stellate, host remnants around the base, uniloculate, glabrous, brittle, carbonaceous, with an indistinct ostiole. *Peridium* unequal thickness, poorly developed at the base, composed of thick, dark brown to black cells. *Pseudoparaphyses* dense, trabeculate, filiform, hyaline, sparsely septate. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical, with furcate pedicel, apically rounded. *Ascospores* overlapping biseriate, pale brown to dark brown, fusiform with acute ends, septate, smooth-walled (Pratibha and Prabhugaonkar 2015; Wanasinghe et al. 2018a). **Asexual morph:** Hyphomycetous. *Conidiophores* mononematous, micronematous, mostly intercalary, sometimes denticulate, aseptate. *Conidiogenous cells* mono or polyblastic, hyaline to light brown, smooth. *Conidia* solitary, brown to black, obovate to oblong, verruculose to spinulose, transversely septate (Pratibha and Prabhugaonkar 2015; Wanasinghe et al. 2018a).

*Type species:* *Pithomyces flavus* Berk. & Broome

*Notes:* *Pithomyces* is similar to *Astrosphaeriellaceae* members in having dark, carbonaceous ascomata with a poorly developed base and fusiform ascospores (Liu et al.

2011; Phookamsak et al. 2015b; Wanasinghe et al. 2018a; Jayasiri et al. 2019). *Pithomyces* clustered in *Astrosphaeriellaceae* with low bootstrap support (Jayasiri et al. 2019; this study, Fig. 2), but morphologically belongs to *Astrosphaeriellaceae*.

Over 40 epithets are listed in Index Fungorum (2020), but only three have sequence data, viz. *Pithomyces caryotae* Wanas. et al., *P. flavus* and *P. licualae* Wanas. et al. *Pithomyces flavus* was found on the natural substratum associated with its sexual morph *Astrosphaeriella vesuvius*, which was confirmed in the phylogenetic analysis (Pratibha and Prabhugaonkar 2015). Wanasinghe et al. (2018a) found the asexual morphs of *P. caryotae* and *P. licualae* from culture, respectively. The asexual morphs of three species share similar morphological characters in having obovate, septate, pigmented and rough-walled conidia (Pratibha and Prabhugaonkar 2015; Wanasinghe et al. 2018a). *Pithomyces flavus* was recorded from freshwater habitats (Cai et al. 2002a; Hu et al. 2013), but not confirmed by molecular data.

### List of freshwater *Pithomyces* species

\**Pithomyces flavus* Berk. & Broome, J. Linn. Soc., Bot. 14(no. 74): 100 (1873) [1875]

*Basionym:* *Sphaeria vesuvius* Berk. & Broome, J. Linn. Soc., Bot. 14(no. 74): 127 (1873) [1875]

*Synonymy:* *Astrosphaeriella vesuvius* (Berk. & Broome) D. Hawksw. & Boise, Sydowia 38: 122 (1986) [1985]

*Freshwater distribution:* China (Cai et al. 2002a; Hu et al. 2013)

*Xenoastrosphaeriella* Jayasiri, E.B.G. Jones & K.D. Hyde, Mycosphere 10(1): 36 (2019)

*Saprobic* on bamboo and palms in freshwater or terrestrial habitats. **Sexual morph:** *Ascostromata* dark opaque, gregarious, erumpent to superficial, mammiform to conical with ruptured, reflexed, stellate, host remnants around the base, uniloculate, glabrous, brittle, carbonaceous, central ostiolate, with pore-like opening. *Peridium* unequal thickness, poorly developed at the base, thick at the sides towards the apex, composed of thick, opaque and melanized cells. *Pseudoparaphyses* dense, trabeculate, hyaline, distinctly septate, anastomosing between the asci, embedded in a hyaline gelatinous matrix. *Asci* 8-spored, bitunicate, cylindrical, short pedicellate, apically rounded with an ocular chamber. *Ascospores* overlapping uni- to bi-seriate, brown to reddish brown, with paler end cells, fusiform with acute ends, septate, smooth-walled (Hawksworth and Boise 1985; Phookamsak et al. 2015b; Jayasiri et al. 2019). **Asexual morph:** Undetermined.

*Type species:* *Xenoastrosphaeriella tornata* (Cooke) Jayasiri & K.D. Hyde

*Notes:* *Xenoastrosphaeriella* was established by Jayasiri et al. (2019) to accommodate *Astrosphaeriella tornata*

(Cooke) D. Hawksw. & Boise which formed a weakly supported clade with other genera in *Astrosphaeriellaceae* (Phookamsak et al. 2015b; this study, Fig. 2). *Xenoastrosphaeriella* is distinct in the family in having paler end cells of ascospores (Hawksworth and Boise 1985; Phookamsak et al. 2015b; Jayasiri et al. 2019). The second species *Xenoastrosphaeriella trochus* (D. Hawksw.) Phookamsak et al. was recently introduced based on morphology and phylogeny (Hongsanan et al. 2020a). Both species *X. tornata* and *X. trochus* were ever collected from freshwater habitats (see list below), but not confirmed by molecular data.

#### List of freshwater *Xenoastrosphaeriella* species

\**Xenoastrosphaeriella tornata* (Cooke) Jayasiri & K.D. Hyde, *Mycosphere* 10(1): 36 (2019)

*Basionym*: *Trematosphaeria tornata* Cooke, *Grevillea* 16(no. 79): 91 (1888)

*Synonymy*: *Sphaeria tornata* Berk. & M.A. Curtis, *J. Acad. nat. Sci. Philad., N.S.* 2(6): 290 (1854)

*Astrosphaeriella tornata* (Cooke) D. Hawksw. & Boise, *Sydowia* 38: 119 (1986) [1985]

*Freshwater distribution*: Philippines (Cai et al. 2003a)

\**Xenoastrosphaeriella trochus* (D. Hawksw.) Phookamsak, H.B. Jiang & K.D. Hyde, *Mycosphere* 11(1): 1762 (2020)

*Basionym*: *Melanomma trochus* Penz. & Sacc., *Malpighia* 11(9–10): 401 (1897)

*Synonymy*: *Leptosphaeria trochus* (Penz. & Sacc.) Höhn., *Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1* 118: 328 [54 repr.] (1909)

*Asterella trochus* (Penz. & Sacc.) Hara, *Nippon-gaikinguaku*: 187 (1936)

*Asterotheca trochus* (Penz. & Sacc.) I. Hino, *Bull. Miyazaki Coll. Agric. Forest.* 10: 57 (1938)

*Trematosphaeria trochus* (Penz. & Sacc.) Teng, *Sinensia, Shanghai* 9: 257 (1938)

*Astrosphaeriella trochus* (Penz. & Sacc.) D. Hawksw., *J. Linn. Soc., Bot.* 82: 46(1981)

*Freshwater distribution*: China (Tsui et al. 2000; Cai et al. 2002a, 2006b; Hu et al. 2013), Australia (Vijaykrishna and Hyde 2006), South Africa (Hyde and Frohlich 1998; Hyde et al. 1998)

#### Key to freshwater *Xenoastrosphaeriella* species

1. Ascospores 3-septate ..... *X. tornata*
1. Ascospores mostly 5-septate ..... *X. trochus*

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

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

*Saprobic* on decaying bamboo or parasitic on living plant materials in terrestrial or freshwater habitats. **Sexual morph**: *Ascostromata* solitary to gregarious, immersed or semi-immersed and becoming erumpent, variable in shape, conical, convex or dome-shaped to ampulliform, globose to subglobose, with centrally located ostiole, with or without red pigment in the ostiole, uni- to multi-loculate, coriaceous, visible as raised, dark spots or elongate structures on host surface. *Peridium* composed of dark cells of *textura angularis* or *textura epidermoidea* or pseudoparenchymatous cells, normally with the flattened base comprising of thinner, hyaline, smaller cells or intermingling with host cells. *Pseudoparaphyses* dense, trabeculate, filiform, hyaline, indistinctly septate, anastomosing and branching above the asci. *Asci* 8-spored, bitunicate, cylindrical or cylindrical-clavate, short pedicellate, with a shallow or well-developed chamber. *Ascospores* uni- to tri-seriate, fusiform, septate, mostly hyaline, rarely pale brown, mostly surrounded by a gelatinous sheath. **Asexual morph**: Coelomycetous. *Conidiomata* pycnothyrial, solitary to gregarious, scattered, immersed to semi-immersed, acerose or subglobose, pyriform or irregular, uni- to multi-loculate. *Pycnidial wall* composed of several layers, thin- to thick-walled, hyaline to dark brown cells of *textura globulosa* or *textura angularis*. *Conidiophores* indistinct. *Conidiogenous cells* holoblastic, annellidic, discrete, cylindrical, smooth. *Conidia* sometimes two types, macro and microconidia. *Macroconidia* cylindrical to ellipsoidal, pale brown to brown, septate, smooth, guttulate. *Microconidia* globose, oblong to ellipsoidal, hyaline to pale brown, aseptate, smooth, guttulate.

*Type species*: *Bambusicola massarinia* D.Q. Dai & K.D. Hyde

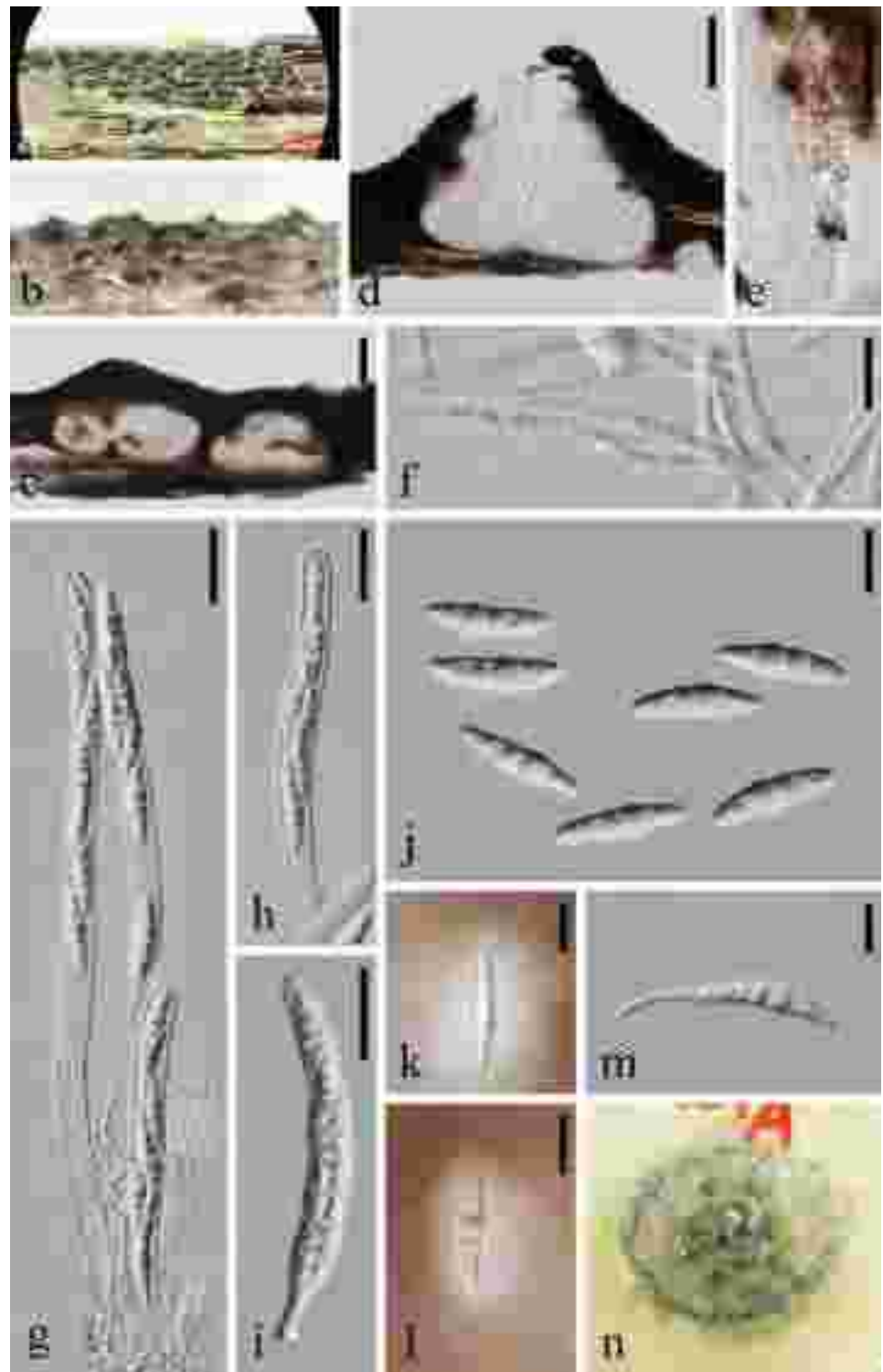
*Notes*: *Bambusicola* is well-studied because all species have sequence data in GenBank. *Bambusicola* is distinct in *Bambusicolaceae* in having fusiform, hyaline, mostly smooth-walled ascospores and cylindrical to ellipsoidal or globose conidia (Dai et al. 2012). The asexual morphs can be found in natural substrate (*Bambusicola splendida* D.Q. Dai & K.D. Hyde and *B. irregulisporea* D.Q. Dai & K.D. Hyde) or in culture (*B. massarinia* D.Q. Dai & K.D. Hyde) (Dai et al. 2012). *Bambusicola* sometimes produces two types of conidia, such as *B. dimorpha* Thambugala et al. producing macro and micro conidia (Thambugala et al. 2017). Other *Bambusicola* species only formed macroconidia characterized as cylindrical to ellipsoidal, pale brown to brown, septate conidia. *Bambusicola* species are saprobic on decaying bamboo from Thailand or parasitic on living leaves or branches of *Phyllostachys heteroclada* Oliv. from China (Dai et al. 2012, 2015, 2017; Thambugala et al. 2017; Yang et al. 2019a). Herein, we report a new *Bambusicola* species isolated from submerged wood in China.

#### List of freshwater *Bambusicola* species

\**Bambusicola aquatica* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*



**Fig. 36** *Bambusicola aquatica* (MFLU 18-1164, **holotype**). **a, b** Appearance of black ascomata on host. **c, d** Vertical sections of ascomata. **e** Structure of peridium. **f** Pseudoparaphyses. **g–i** Bitunicate asci. **j–l** Ascospores. **m** Germinated ascospore. **n** Colony on PDA (from front). Scale bars: **c, d** = 100  $\mu\text{m}$ , **e** = 50  $\mu\text{m}$ , **f, j–m** = 10  $\mu\text{m}$ , **g–i** = 20  $\mu\text{m}$



*Index Fungorum* number: IF557906; *Facesoffungi* number: FoF09249; Fig. 36

*Etymology*: referring to aquatic habitat of this fungus

*Holotype*: MFLU 18-1164

*Saprobic* on decaying bamboo submerged in freshwater.

**Sexual morph**: *Ascomata* 180–210  $\mu\text{m}$  high, 250–370  $\mu\text{m}$  diam., black, gregarious, densely clustered in a large group,

semi-immersed to superficial, conical or dome-shaped in section. *Peridium* comprising host and fungal tissues, laterally 85–95  $\mu\text{m}$  thick, composed of dark brown to black, thick-walled cells of *textura angularis* intermingled with host cells, with thinner basal part 10–35  $\mu\text{m}$  thick, composed of subhyaline, rim-like cells. *Pseudoparaphyses* 1.4–1.8  $\mu\text{m}$  diam., numerous, trabeculate, filiform, hyaline, indistinctly

septate. *Asci* 80–126.5(–202) × 7–9.5(–10.5) μm ( $\bar{x}$  = 99.5 × 9 μm, n = 15), 8-spored, bitunicate, cylindrical, apically rounded, with a shallow chamber and a short, twisted or straight pedicel, easily and rapidly elongate when mounted in water, up to 202 μm long. *Ascospores* 18–22.5 × 4–5 μm ( $\bar{x}$  = 20.5 × 4.5 μm, n = 40), overlapping biseriate or partially uniseriate, narrowly fusiform, the upper cell slightly broader than the lower one, sharply narrowed towards to ends which are narrowly rounded, mostly slightly curved, rarely straight, hyaline, 1-septate, deeply constricted at the septum, slightly constricted at a quarter, guttulate, the guttula in upper cell larger than the lower cell, thin-walled, smooth, with a thin, hyaline, inconspicuous, mucilaginous sheath which is 2 μm thick when mounted in water, but 5–10 μm thick when mounted in Indian Ink. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony irregular, reaching 30 mm diam. in 30 days at 25 °C, brown with pale green margin from above, dark brown from below, surface rough, with dense mycelium, dry, mucoid after old, edge undulate.

**Material examined:** CHINA, Yunnan Province, Dehong, on submerged wood in a stream, 25 November 2017, G.N. Wang, H29A-1 (MFLU 18-1164, **holotype**), ex-type living culture MFLUCC 18-1031; *ibid.*, H29A-2 (HKAS 101730, **isotype**), ex-type living culture KUMCC 18-0080.

**Notes:** *Bambusicola aquatica* is most similar to *B. loculata* D.Q. Dai & K.D. Hyde in having narrowly fusiform, 1-septate, hyaline ascospores with narrowly rounded ends and an inconspicuous mucilaginous sheath (Dai et al. 2015). However, they can be distinguished by their ascus characteristics (cylindric-clavate, bi- to tri-seriate, 80–105 × 8–13 μm in *B. loculata* vs. cylindrical, overlapping biseriate or partially uniseriate, 80–126.5(–202) × 7–9.5(–10.5) μm in *B. aquatica*). Additionally, the ascomata of *B. loculata* are solitary or clustered in a small group, while those of *B. aquatica* are gregarious and densely clustered in a large group.

*Bambusicola aquatica* forms a strongly supported clade with *B. irregulispora* (Fig. 2). *Bambusicola aquatica* is an ascomycetous species, while *B. irregulispora* is a coelomycetous species. There are respectively 5, 6, 30 and 14 nucleotide differences in the LSU, ITS, TEF and RPB2 sequence data between *B. aquatica* (MFLUCC 18-1031) and *B. irregulispora* (MFLUCC 11-0437), which supports them to be different species (Jeewon and Hyde 2016).

**Nigrogranaceae** Jaklitsch & Voglmayr, Stud. Mycol. 85: 54 (2016)

**Nigrograna** Gruyter, Verkley & Crous, Stud. Mycol. 75: 31 (2012) [2013]

*Saprobic* on submerged wood, decaying twigs of shrubs and trees, old fructifications of pyrenomycetes, sometimes as human pathogenic. **Sexual morph:** *Ascomata* solitary to gregarious, immersed to superficial, somewhat globose, brown to

black, ostiolate. *Peridium* comprising several layers, angular cells. *Pseudoparaphyses* numerous, trabeculate, filiform, septate, branching and anastomosing. *Asci* 8-spored, bitunicate, cylindrical to clavate, or broadly filiform, with short pedicellate and knob-like base. *Ascospores* overlapping, broadly fusiform to narrowly ellipsoid, inequilateral, septate, hyaline to brown, smooth or faintly verruculose (Jaklitsch and Voglmayr 2016; Hyde et al. 2017; Tibpromma et al. 2017). **Asexual morph:** Coelomycetous. *Pycnidia* similar to ascomata, solitary or rarely gregarious, superficial or submerged in agar, globose to subglobose or pyriform, olivaceous to olivaceous-black, with dark brown, septate mycelial outgrowths, with ostiolate papilla. *Peridium* comprising several layers of pseudoparenchymatous cells. *Conidiophores* filiform, simple to sparsely branched, with pegs along one or two sides and solitary terminal phialides, reduced in culture. *Conidiogenous cells* phialidic, discrete, ampulliform, lageniform or subcylindrical, hyaline. *Conidia* oblong, cylindrical or allantoid, sometimes ellipsoid, hyaline or subhyaline, brown in mass, aseptate, smooth (de Gruyter et al. 2013; Jaklitsch and Voglmayr 2016). Hyphomycetous. *Synnemata* superficial, effuse, gregarious, black. *Mycelium* mostly immersed, composed of septate, pale brown, smooth hyphae. *Conidiophores* macronematous, synnematus on substratum, septate, unbranched to branched, smooth, brown, compacted below half and flared at the tip. *Conidiogenous cells* polyblastic, terminal or intercalary, sympodial, smooth, swollen subcylindrical, ampulliform, denticulate, covered with several conidiogenous loci, hyaline to subhyaline. *Conidia* solitary, acrogenous, simple, dry, ellipsoidal, smooth, thin-walled, aseptate, hyaline.

**Type species:** *Nigrograna mackinnonii* (Borelli) Gruyter, Verkley & Crous

**Notes:** Most *Nigrograna* species were collected from terrestrial habitats and are morphologically very similar. Sexual and asexual morphs have been reported from natural substrates and characterized as ascomycetous and coelomycetous species (Jaklitsch and Voglmayr 2016; Tibpromma et al. 2017). Only *Nigrograna cangshanensis* was reported from freshwater habitats (China), and it differs from other species by thicker peridium and molecular characters (Tibpromma et al. 2017). We collected a hyphomycetous species from submerged wood in Thailand, which expands the known morph of this genus.

#### List of freshwater *Nigrograna* species

\**Nigrograna aquatica* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

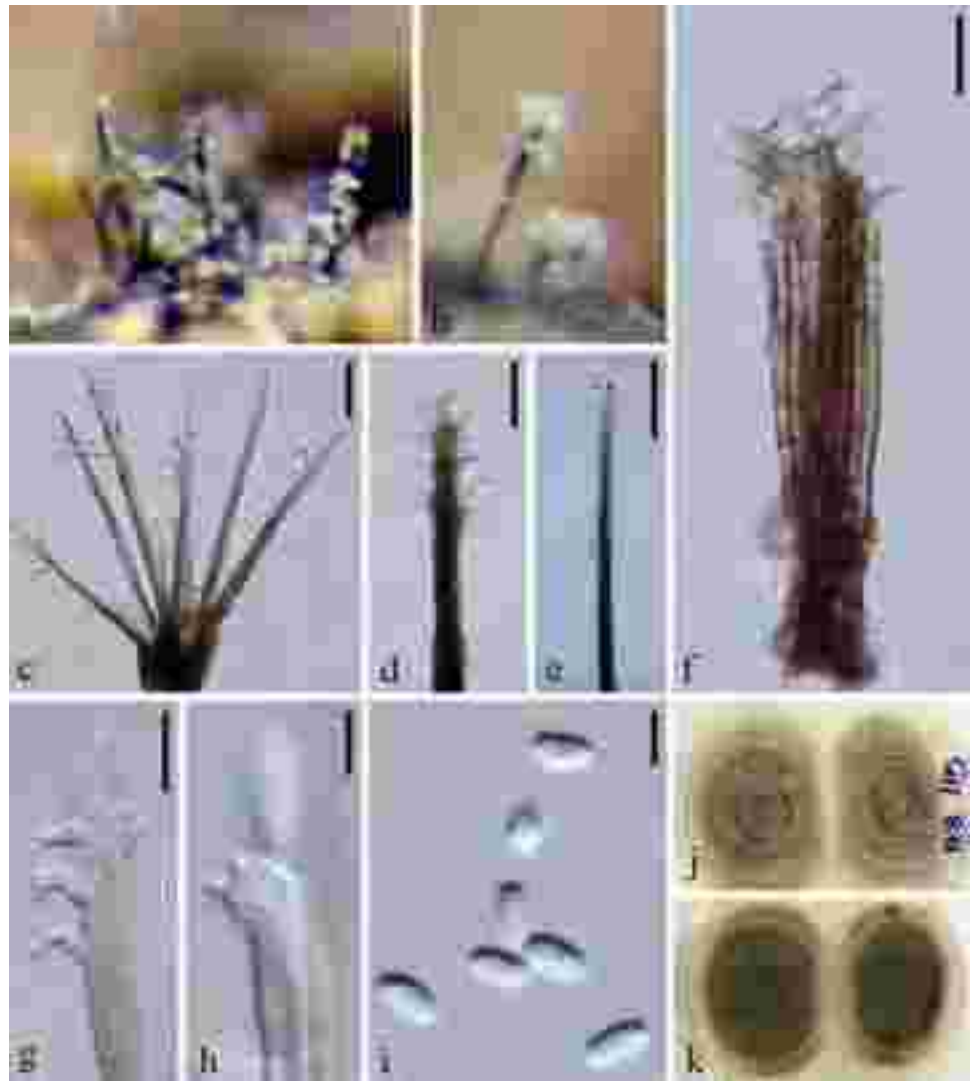
*Index Fungorum number:* IF557907; *Facesoffungi number:* FoF09250; Fig. 37

*Etymology:* in reference to aquatic habitat of this fungus

*Holotype:* MFLU 17-1661

*Saprobic* on submerged wood in freshwater. **Sexual morph:** Undetermined. **Asexual morph:** *Synnemata* 110–125 × 8.5–14.5 μm ( $\bar{x}$  = 118 × 11.8 μm, n = 5), superficial, effuse, gregarious, black, upper part covered with a white or pale

**Fig. 37** *Nigrograna aquatica* (MFLU 17-1661, **holotype**). **a, b** Colonies on submerged wood. **c–f** Conidiophores with attached conidia. **g, h** Conidiogenous cells. **i** Conidia. **j, k** Colony on PDA (up-front, down-reverse). Scale bars: **c, d** = 20  $\mu\text{m}$ , **e** = 40  $\mu\text{m}$ , **f, g** = 10  $\mu\text{m}$ , **h** = 3  $\mu\text{m}$ , **i** = 5  $\mu\text{m}$



grey powdery mass of conidia, composed of paralleled conidiophores. *Mycelium* mostly immersed, composed of septate, pale brown, smooth hyphae. *Conidiophores* 100–120  $\times$  1.5–2  $\mu\text{m}$  ( $\bar{x}$  = 100.5  $\times$  1.7  $\mu\text{m}$ ,  $n$  = 15), macronematous, synnematosus on substratum, not compacted, become divergent after mounted in water, unbranched, septate, not constricted at the septa, smooth, dark brown at the base, gradually paler towards the apex, flared in upper part. *Conidiogenous cells* 4–7.5  $\times$  1.7–2.5  $\mu\text{m}$  ( $\bar{x}$  = 5.6  $\times$  2.2  $\mu\text{m}$ ,  $n$  = 10), polyblastic, terminal or intercalary, integrated when terminal and discrete when intercalary, sympodial, smooth, swollen subcylindrical, flask-shaped, ampulliform, curved or straight, denticulate, covered with several conidiogenous loci at the upper part, hyaline to subhyaline. *Conidia* 6–7  $\times$  2–2.5  $\mu\text{m}$  ( $\bar{x}$  = 6.6  $\times$  2.2  $\mu\text{m}$ ,  $n$  = 15), solitary, acrogenous, simple, dry, ellipsoidal or cylindrical, smooth, thin-walled, aseptate, hyaline.

*Culture characteristics*: On PDA, colony circular, reaching 25 mm diam. in 30 days at 25  $^{\circ}\text{C}$ , brown to grey from

above, dark brown from below, surface rough, with dense mycelium, dry, raised, edge entire.

*Material examined*: THAILAND, Phayao Province, on submerged wood in a stream, 4 August 2017, G.N. Wang, G11 (MFLU 17-1661, **holotype**), ex-type living culture MFLUCC 17-2318.

*Notes*: *Nigrograna aquatica* is morphologically similar to *Phaeoisaria* species which are characterized by synnematosus conidiophores with polyblastic, sympodial conidiogenous cells (Cai et al. 2006a). However, they cannot be congeneric because *N. aquatica* differs in having not compacted synnemata which easily become divergent after mounted in water. In contrast, the conidiophores of *Phaeoisaria* are compacted and parallel adpressed, and never become divergent in water (Liu et al. 2015; Hyde et al. 2018a; Luo et al. 2018). The ellipsoidal or cylindrical, hyaline, aseptate, small conidia of *N. aquatica* are similar to those coelomycetous species in *Nigrograna* (de Gruyter et al. 2013; Jaklitsch and Voglmayr 2016). However, phylogenetic analyses showed

them as distinct species (Fig. 2). The phylogenetically closest species *N. locuta-pollinis* F. Liu & L. Cai was isolated from hive-stored pollen collected in Italian honey bee colonies, while *N. aquatica* was collected from submerged wood in freshwater habitats in Thailand. Morphology of *N. locuta-pollinis* was not given in protologue (Zhao et al. 2018). A comparison of their nucleotides shows that there are six and eight (including two gaps) nucleotide differences in LSU and ITS sequence data, respectively. We introduce *N. aquatica* sp. nov. based on the guideline of Jeewon and Hyde (2016) and this is the first hyphomycetous species in *Nigrograna*.

\**Nigrograna cangshanensis* Z.L. Luo, H.Y. Su & K.D. Hyde, Fungal Diversity 83: 52 (2017)

Freshwater distribution: China (Tibpromma et al. 2017)

#### Key to freshwater *Nigrograna* species

1. Sexual morph.....*N. cangshanensis*
2. Asexual morph.....*N. aquatica*

**Corynesporascaceae** Sivan., Mycol. Res. 100(7): 786 (1996)

*Corynespora* Güssow, Z. PflKrankh. 16: 13 (1906)

*Pathogenic* and saprobic on leaves or submerged wood. **Sexual morph:** *Corynesporasca*. *Ascomata* solitary to aggregated, superficial or immersed, uniloculate, globose, dark brown to black, lacking ostioles. *Peridium* comprising several layers of thin-walled, brown cells of *textura globosa* or *angularis*. *Pseudoparaphyses* cylindrical, branched, septate, apically free, disintegrate when asci mature. *Asci* 8-spored, bitunicate, obovoid, deliquescent. *Ascospores* oblong, pale to dark brown, usually asymmetric, 1-euseptate near the center, with or without indistinctly 1-distoseptate in the upper and lower cell, constricted at the central euseptum (Sivanesan 1996; Hyde et al. 2013). **Asexual morph:** Hyphomycetous. *Colonies* on the natural substrate effuse, hairy, amphigenous, brown. *Mycelium* superficial to immersed in the substrate, composed of branched, septate, thin-walled, smooth, subhyaline to brown hyphae. *Conidiophores* macronematous, mononematous, erect to procumbent, simple, none to few septate, smooth. *Conidiogenous cells* monotretic, integrated, terminal, determinate or percurrent proliferation. *Conidia* acrogenous, solitary, obclavate, wide at the truncate base, tapering towards the apex, pale brown to pale olivaceous brown, multi-distoseptate, smooth-walled, usually with a slightly melanized basal scar (Hyde et al. 2013).

*Type species:* *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei

*Notes:* *Corynespora* is characterized by obclavate, brown, multi-distoseptate conidia with slightly melanized basal scar (Hyde et al. 2013). Sivanesan (1996) linked *C. caryotae* Sivan. as the sexual morph of *Corynespora* based on cultural method. Based on the extensive use of the name *Corynespora*

for plant pathogenic fungi and its priority, Rossman et al. (2015) formally synonymized *Corynesporasca* Sivan. under *Corynespora*. *Corynespora* is therefore the only genus in Corynesporascaceae (Sivanesan 1996; Wijayawardene et al. 2018). *Corynespora* was revealed as polyphyletic (Schoch et al. 2009; Voglmayr and Jaklitsch 2017). Two freshwater species *C. lignicola* Z.L. Luo et al. and *C. submersa* Z.L. Luo et al. formed a well-supported clade with the type species *C. cassiicola*, which was represented by a strain CBS 100822 (Hyde et al. 2020a). *Corynespora aquatica* R.F. Castañeda et al. was reported from freshwater habitats, but not confirmed by molecular data (Castañeda-Ruiz et al. 2004).

#### List of freshwater *Corynespora* species

*Corynespora aquatica* R.F. Castañeda, Heredia & R.M. Arias, Mycotaxon 89: 298 (2004)

Freshwater distribution: Mexico (Castañeda-Ruiz et al. 2004)

\**Corynespora lignicola* Z.L. Luo, H.Y. Su & K.D. Hyde, Asian Journal of Mycology 3: 65 (2020)

Freshwater distribution: China (Hyde et al. 2020a)

\**Corynespora submersa* Z.L. Luo, H.Y. Su & K.D. Hyde, Asian Journal of Mycology 3: 63 (2020)

Freshwater distribution: China (Hyde et al. 2020a)

#### Key to freshwater *Corynespora* species

1. Conidia cylindrical.....*C. lignicola*
1. Conidia obclavate.....2
2. Conidia 100–150 µm long.....*C. submersa*
2. Conidia 34–46 µm long.....*C. aquatica*

**Delitschiaceae** M.E. Barr, Mycotaxon 76: 109 (2000)

*Delitschia* Auersw., Hedwigia 5: 49 (1866)

*Saprobic* on old herbivore dung or submerged wood. **Sexual morph:** *Ascomata* solitary or scattered, immersed to semi-immersed, globose to subglobose or subpyriform, often covered with dense, hyphoid hairs, semitransparent to brown or black, uniloculate, membranous to coriaceous, with a neck. *Neck* blackish, central, usually coriaceous, with a wide opening and a smooth or tuberculate to hairy surface. *Peridium* thick at the apex, thinner at the base. *Pseudoparaphyses* numerous, trabeculate, filiform, long, hyaline, septate, anastomosing and branching. *Asci* 4- to poly-spored, bitunicate, cylindrical to cylindro-clavate, short or long pedicellate. *Ascospores* partially overlapping, obliquely uniseriate, ellipsoid, reddish brown, 1-septate, cells often easily separable from each other, each cell with a full length germ slit (Luck-Allen and Cain 1975; Hyde et al. 2013). **Asexual morph:** Undetermined.

*Type species: Delitschia didyma* Auersw.

*Notes:* *Delitschia* is characterized by usually fimicolous habit, with bitunicate asci, and pigmented, 1-septate ascospores, with an elongate germ slit in each cell (Luck-Allen and Cain 1975). Most taxa of this genus are coprophilous (Luck-Allen and Cain 1975), some are wood-inhabiting (Eaton and Jones 1970; Hyde and Steinke 1996) and three species were reported from freshwater habitats (see list below). It is reasonable to infer that the lignicolous freshwater taxa might phylogenetically separate from the coprophilous taxa. This cannot be confirmed until sequences of three freshwater species are available. *Delitschia fasciatispora* K.D. Hyde and *D. palmietensis* K.D. Hyde & Steinke have 8-spored asci, while they are 2-spored in *D. bispora* Eaton & E.B.G. Jones.

**List of freshwater *Delitschia* species**

*Delitschia bispora* Eaton & E.B.G. Jones, Nova Hedwigia 19(3–4): 781 (1971) [1970]

*Freshwater distribution:* England (Eaton and Jones 1970; Eaton and Jones 1971; Eaton 1972)

*Delitschia fasciatispora* K.D. Hyde, Mycoscience 37(1): 100 (1996)

*Freshwater distribution:* Mauritius (Hyde and Steinke 1996)

*Delitschia palmietensis* K.D. Hyde & Steinke, Mycoscience 37(1): 101 (1996)

*Freshwater distribution:* Mauritius (Hyde and Steinke 1996)

**Key to freshwater *Delitschia* species**

- 1. Asci 2-spored.....*D. bispora*
- 1. Asci 8-spored.....2
- 2. Ascospores with a wide dark brown central band.....  
.....*D. fasciatispora*
- 2. Ascospores lack above characters.....*D. palmietensis*

**Dictyosporiaceae** Boonmee & K.D. Hyde, Fungal Diversity 80: 462 (2016)

**Key to sexual genera of freshwater Dictyosporiaceae species**

- 1. Ascomata somewhat soft, collapsing when dry, with hyphomycetous asexual morph.....*Dictyosporium*
- 1. Ascomata not as above, with coelomycetous asexual morph.....*Pseudocoleophoma*

**Key to asexual genera of freshwater Dictyosporiaceae species**

- 1. Coelomycetous.....*Pseudocoleophoma*
- 1. Hyphomycetous.....2
- 2. Conidiomata synnemata.....*Aquatichairospora*
- 2. Conidiomata sporodochia.....3
- 3. Conidia non-cheiroid.....*Dendryphiella*
- 3. Conidia cheiroid.....4
- 4. Conidiophores form distinct sterile branches.....  
.....*Cheirosporium*
- 4. Conidiophores with no sterile branches.....5
- 5. Conidia with divergent arms.....6
- 5. Conidia with compacted arms.....7
- 6. Conidiophores composed of moniliform hyphae.....  
.....*Digitodesmium*
- 6. Conidiophores not as above.....*Jalapriya*
- 7. Conidia complanate.....*Dictyosporium*
- 7. Conidia non-complanate.....8
- 8. Conidia with basal hyaline cell.....*Aquadictyospora*
- 8. Conidia not as above.....9
- 9. Conidial arms mostly curved towards tip.....  
.....*Dictyocheirospora*
- 9. Conidial arms mostly parallel.....10
- 10. Conidia elongated subcylindrical.....*Vikalpa*
- 10. Conidia mostly ellipsoidal.....*Pseudodictyosporium*

*Aquadictyospora* Z.L. Luo, K.D. Hyde & H.Y. Su, Mycosphere 8(10): 1590 (2017)

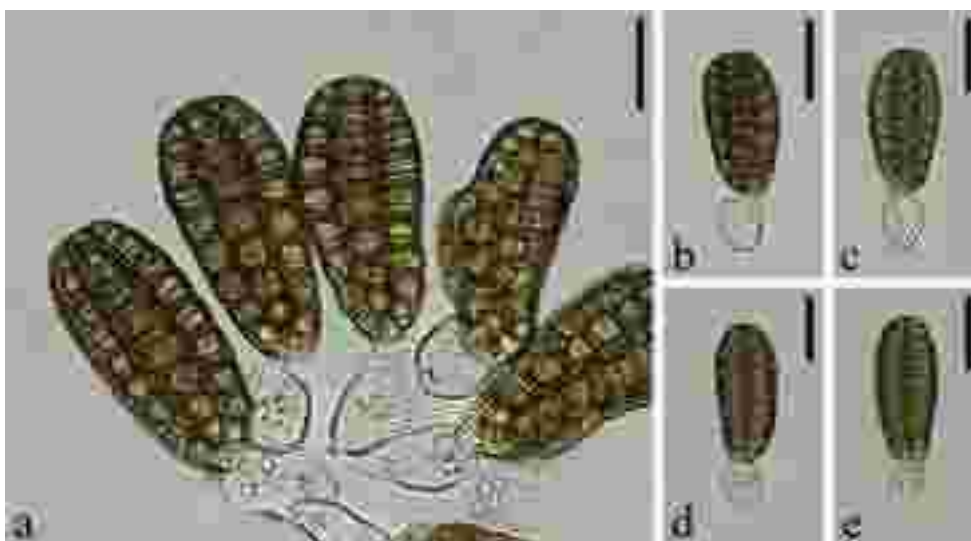
*Saprobic* on submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* on natural substratum sporodochial, superficial, compact, scattered, circular or subglobose, dark brown to black, velvety. *Mycelium* immersed, consisted of septate, hyaline hyphae. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, monoblastic. *Conidia* ellipsoidal to broadly clavate, composed of medium brown, appressed cells, rounded and brown at the apex, oval to subglobose and hyaline at the base, muriform, cheiroid, non-complanate, without appendages (Li et al. 2017a).

*Type species: Aquadictyospora lignicola* Z.L. Luo, W.L. Li, K.D. Hyde & H.Y. Su

*Notes:* The monotypic genus *Aquadictyospora* was introduced to accommodate a freshwater species *A. lignicola* collected from China (Li et al. 2017b; Bao et al. 2018). *Aquadictyospora* differs from other genera in Dictyosporiaceae by its basal hyaline cell and conidia without appendages (Li et al. 2017b; Bao et al. 2018). *Aquadictyospora* nested in Dictyosporiaceae with high bootstrap support (Li et al. 2017b; this study, Fig. 47).

**List of freshwater *Aquadictyospora* species**

**Fig. 38** *Aquadictyospora lignicola* (Material examined: CHINA, Yunnan Province, Dali, on submerged wood in a stream in Cangshan Mountain, July 2016, H. Y. Su, 4XP H 2–9–3, MFLU 17–1422, holotype). **a–e** Conidia. Scale bars: **a** = 5  $\mu$ m, **b–e** = 10  $\mu$ m



\**Aquadictyospora lignicola* Z.L. Luo, W.L. Li, K.D. Hyde & H.Y. Su, Mycosphere 8(10): 1591 (2017); Fig. 38

*Freshwater distribution:* China (Li et al. 2017)

*Aquaticheirospora* Kodsueb & W. H. Ho, J. Linn. Soc., Bot. 155: 286 (2007)

*Saprobic* on submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiomata* synnema, scattered or gregarious, erect, brown. *Mycelium* immersed in the substratum, hyaline to pale brown. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal, determinate, oblong, hyaline to pale brown. *Conidia* acrogenous, solitary, non-complanate, hyaline to brown, cheiroid, euseptate, arms vertically inserted in different planes, on a basal cell; basal cells pale brown, cuneiform, smooth, thin-walled; arms discrete, mostly divergent, cylindrical (Kodsueb et al. 2007).

*Type species:* *Aquaticheirospora lignicola* Kodsueb & W.H. Ho

*Notes:* The monotypic genus *Aquaticheirospora* is distinguished from other cheirosporous genera by synnematous conidiomata and acrogenous, euseptate, cheiroid conidia with divergent arms (Kodsueb et al. 2007). The synnematous conidiomata were thought to be not important to delimit a separated genus because the type species *A. lignicola* nested amongst *Dictyosporium* based on ITS sequence data (Cai et al. 2008). However, Boonmee et al. (2016) demonstrated that *Aquaticheirospora* was a morphologically and phylogenetically well-separated genus based on multigene phylogenetic analyses.

*Aquaticheirospora lignicola* has 3(–5) tightly packed arms on the substrate, but has (3–)5(–10) divergent arms with a brown vacuole in culture (Kodsueb et al. 2007).

#### List of freshwater *Aquaticheirospora* species

\**Aquaticheirospora lignicola* Kodsueb & W. H. Ho, J. Linn. Soc., Bot. 155: 286 (2007); Fig. 39

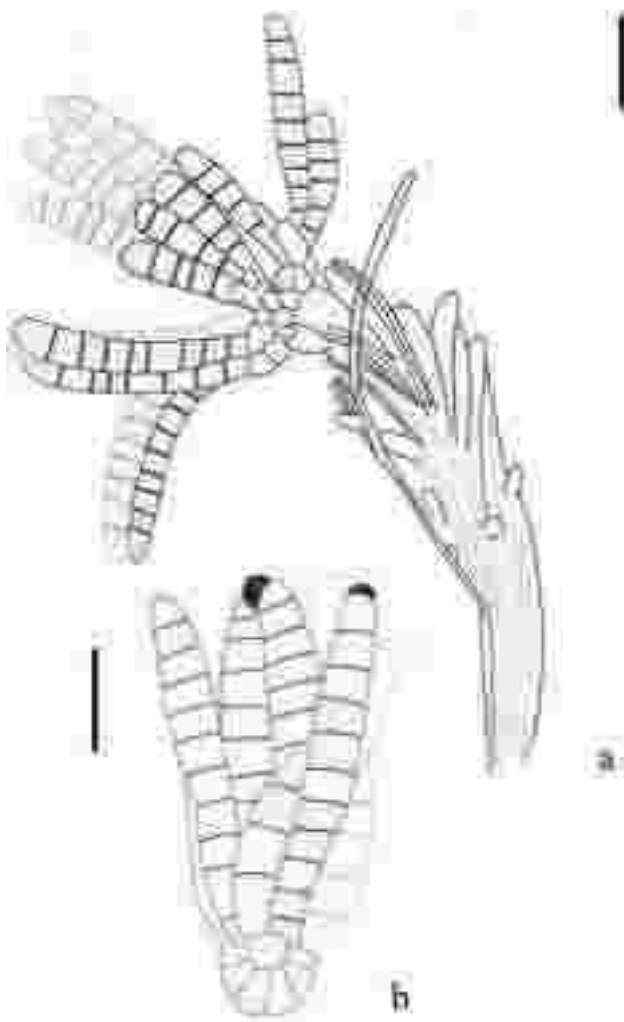
*Freshwater distribution:* Thailand (Kodsueb et al. 2007)

*Cheirosporium* L. Cai & K.D. Hyde, Persoonia 20: 55 (2008)

*Saprobic* on submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* sporodochial, scattered, punctiform, brown to black. *Mycelium* immersed or partly superficial, consisted of branched, septate, subhyaline to pale brown hyphae. *Conidiophores* semi-macronematous to macronematous, mononematous, flexuous, septate, unevenly branched, branches sterile or fertile; branches with an elongate, relatively enlarged, obclavate cell. *Conidiogenous cells* holoblastic, monoblastic, terminal, determinate. *Conidia* acrogenous, cheiroid, olivaceous to brown, solitary, dry, smooth-walled (Cai et al. 2008).

*Type species:* *Cheirosporium triseriale* L. Cai & K.D. Hyde

*Notes:* *Cheirosporium* differs from other genera in Dictyosporiaceae by its semi-macronematous to macronematous, branched conidiophores with sterile or fertile branches, on which grow an elongate, relatively enlarged, obclavate cell (Cai et al. 2008). Two species are listed in Index Fungorum (2020) and both are from freshwater habitats (Cai et al. 2008). *Cheirosporium vesiculare* Abdel-Aziz, with only LSU sequence deposited in GenBank, clustered with the type species *C. triseriale* with low bootstrap support in Abdel-Aziz (2016b) and they are unrelated in our phylogenetic analysis (data not shown). *Cheirosporium triseriale* has punctiform sporodochia, complanate conidia with 1–2 cells per row, without appendages, while *C. vesiculare* has compact sporodochia, non-complanate conidia with 9–12 cells per row and with 2 to 5 large, globose to subglobose appendages (Cai et al. 2008; Abdel-Aziz 2016b).



**Fig. 39** *Aquaticheiospora lignicola* (redrawn from Kodsueb et al. (2007), HKU(M) 17493, **holotype**). **a** Conidia produced from synnemata. **b** Conidium. Scale bars: **a** = 30  $\mu$ m, **b** = 20  $\mu$ m

The conidial length are also considerably different (see key below). The phylogenetic relationships of two species need further study.

#### List of freshwater *Cheiosporium* species

\**Cheiosporium triseriale* L. Cai & K.D. Hyde, Persoonia 20: 56 (2008)

*Freshwater distribution*: Yunnan, China (Cai et al. 2008)

\**Cheiosporium vesiculare* Abdel-Aziz, Mycosphere 7(4): 450 (2016)

*Freshwater distribution*: Egypt (Abdel-Aziz 2016b)

#### Key to freshwater *Cheiosporium* species

1. Conidia 15–25  $\times$  11–15  $\mu$ m.....*C. triseriale*
1. Conidia 42–65  $\times$  19–22  $\mu$ m.....*C. vesiculare*

*Dendryphiella* Bubák & Ranoj., Anns mycol. 12(4): 417 (1914)

*Saprobic* on dead branch, living leaves, leaf litter, dead or old herbaceous stems in terrestrial or submerged wood in freshwater habitats. **Sexual morph**: Undetermined. **Asexual morph**: Colonies effuse, rubiginous, brown or black, hairy or velvety. *Mycelium* mostly immersed. *Conidiophores* macronematous, mononematous, evenly or irregularly branched, with terminal or intercalary nodose swellings, mid to dark brown or reddish brown, smooth or verruculose. *Conidiogenous cells* polytretic, integrated, sympodial, reddish brown to pale brown, subspherical or clavate to subcylindrical, cicatrized, covered with some conidiogenous loci. *Conidia* solitary or catenate, acropleurogenous, simple, cylindrical or oblong, pale brown to mid brown, burnt sienna or olivaceous brown, septate, smooth or verruculose (Ellis 1971).

*Type species*: *Dendryphiella vinosa* (Berk. & M.A. Curtis) Reisinger

*Notes*: *Dendryphiella* was introduced by Ranojevic (1914) with the type species *D. interseminata* (Berk. & Ravenel) Bubák which was later synonymized as *D. vinosum* (Matsushima 1971). Fifteen species are accepted in the genus and six of which have sequence data (Crous et al. 2014, 2016; Liu et al. 2017c; Hyde et al. 2018a; Iturrieta-González et al. 2018). Taxonomic notes and a key to *Dendryphiella* species were provided by Liu et al. (2017c). *Dendryphiella* has non-cheiroida conidia which are similar to *Neodendryphiella* Iturrieta-González et al., but differ in having up to ten conidia in a chain. These two genera separated well in a phylogenetic tree (Iturrieta-González et al. 2018). All *Dendryphiella* species were collected from terrestrial habitats, except our new collection *D. vinosa* is collected from freshwater habitats.

#### List of freshwater *Dendryphiella* species

\**Dendryphiella vinosa* (Berk. & M.A. Curtis) Reisinger, Bull. trimest. Soc. mycol. Fr. 84(1): 27 (1968)

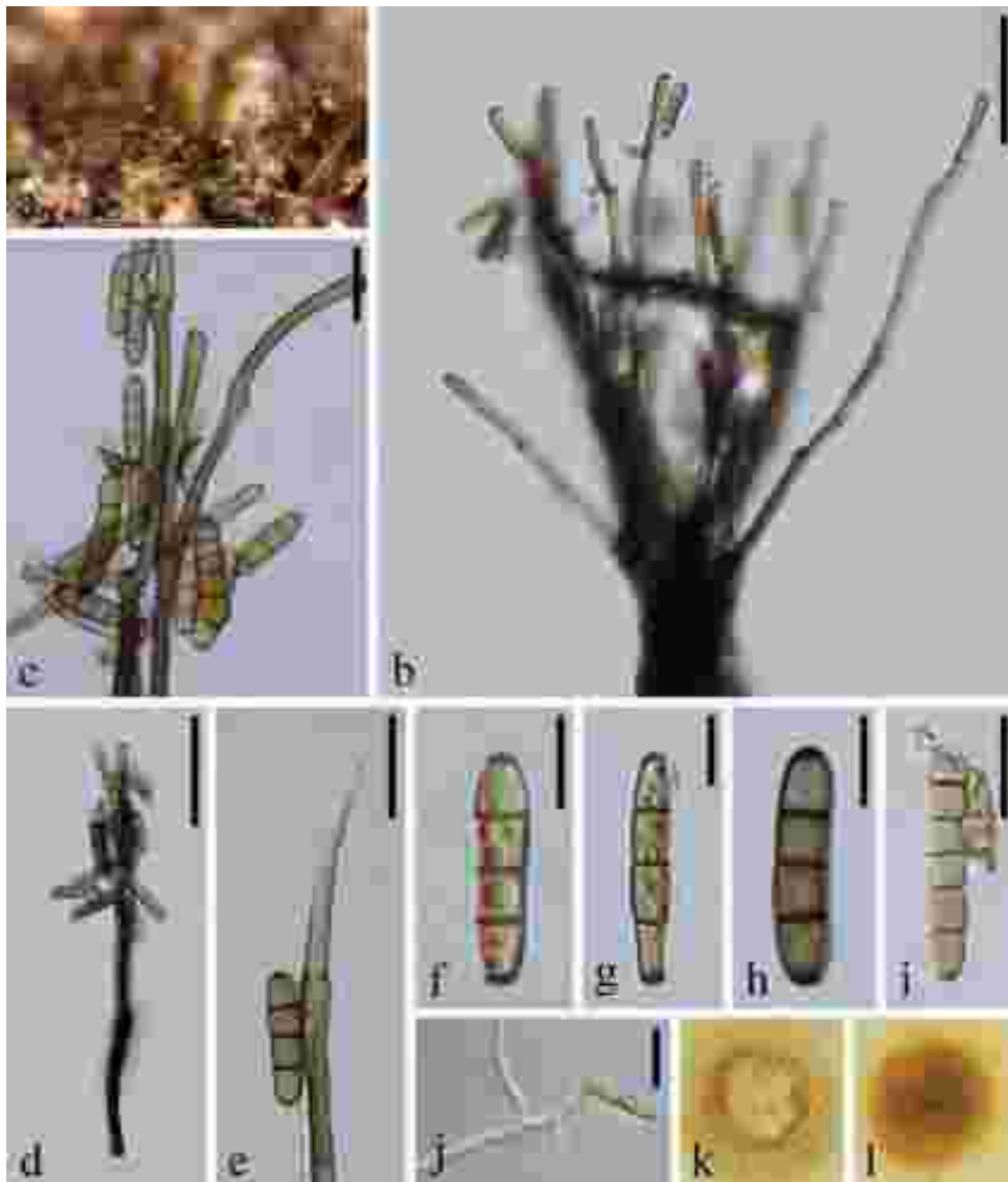
*Facesoffungi number*: FoF08673; Fig. 40

*Basionym*: *Helminthosporium vinosum* Berk. & M.A. Curtis [as ‘Helmisporium’], in Berkeley, J. Linn. Soc., Bot. 10(no. 46): 361 (1868) [1869]

*Synonymy*: *Dendryphiella phitsanulokensis* N.G. Liu & K.D. Hyde, Mycosphere 9(2): 287 (2018)

*Freshwater Distribution*: Thailand (this study)

*Saprobic* on submerged wood. **Sexual morph**: Undetermined. **Asexual morph**: Colonies on natural substrate



**Fig. 40** *Dendryphiella vinosa* (MFLU 17-1666, **new habitat record**). **a** Colonies on submerged wood. **b–d** Conidiophores with attached conidia. **e** Conidiogenous cell and conidium. **f–h** Conidia

**i** Two overlapping conidia. **j** Germinated conidium. **k, l** Colony on PDA (left-front, right-reverse). Scale bars: **b, d** = 50  $\mu\text{m}$ , **c, e, i, j** = 20  $\mu\text{m}$ , **f–h** = 10  $\mu\text{m}$

superficial, effuse, greyish-brown to black. *Mycelium* partly immersed, partly superficial, composed of septate, pale brown, smooth hyphae. *Conidiophores* uneven in width, 160–300  $\times$  4–6  $\mu\text{m}$  ( $\bar{x}$  = 235  $\times$  5  $\mu\text{m}$ ,  $n$  = 10), macronematous, mononematous, occasionally fasciculate, branched at upper part, slightly wider at nodes, 8–10  $\mu\text{m}$  diam., septate, slightly constricted at the septa, brown to reddish brown, sometimes gradually paler and tapering at tip, smooth or

delicately verruculose. *Conidiogenous cells* 35–40  $\times$  3–5  $\mu\text{m}$  ( $\bar{x}$  = 37  $\times$  4  $\mu\text{m}$ ,  $n$  = 5), polytretic, terminal or intercalary, integrated, proliferating symmetrically or asymmetrically, smooth, subcylindrical, covered with 1–2 conidiogenous loci, brown to reddish brown. *Conidia* 23–34  $\times$  5–8  $\mu\text{m}$  ( $\bar{x}$  = 27  $\times$  6.5  $\mu\text{m}$ ,  $n$  = 15), solitary, acrogenous or pleurogenous, oblong with obtuse ends, 3-septate, slightly constricted at



the septa, brown to reddish brown, darker at the hilum, simple, dry, smooth or delicately verruculose, thin-walled.

**Culture characteristics:** On PDA, colony circular, reaching 40 mm in 15 days at 25 °C, pale yellow-brown to white from above, yellow-brown from below, surface rough, with dense mycelium in the middle, sparse mycelium in outer layer, dry, raised, edge entire.

**Material examined:** THAILAND, Phayao Province, on submerged wood in a stream, 4 August 2017, G.N. Wang, G19 (MFLU 17-1666), ex-type living culture MFLUCC 17-2321.

**Notes:** *Dendryphiella vinosa* was initially collected from dead stems of Congo Bean in Cuba (Berkeley and Curtis 1869). Ellis (1971) redescribed and illustrated *D. vinosa* with reddish brown, verruculose conidiophores and 3-septate, verruculose, pale brown to reddish brown conidia. *Dendryphiella vinosa* only has LSU (EU848590) and ITS (DQ307316) sequence data in GenBank, which have been proved in following phylogenetic analyses (Crous et al. 2016, 2019; Liu et al. 2017c; Hyde et al. 2018a). Hyde et al. (2018a) introduced a new species *D. phitsanulokensis* N.G. Liu & K.D. Hyde, which differs from *D. vinosa* in having shorter and unbranched conidiophores. However, they have identical LSU sequence data and there are only one nucleotide differences in ITS sequence data between *D. phitsanulokensis* (MFLUCC 17-2513) and *D. vinosa* (NBRC 32669). Further morphological comparison shows that *D. phitsanulokensis* has identical characters with *D. vinosa* in all structures (Ellis 1971; Hyde et al. 2018a), and we consider that the conidiophore length and branch of *D. vinosa* can be variable in different living habitats. Based on single gene comparison, phylogenetic analysis (Fig. 47) and morphological comparison, we synonymize *D. phitsanulokensis* with *D. vinosa*.

Our new collection MFLUCC 17-2321 clusters with *D. vinosa* in our multigene phylogenetic analysis (Fig. 47). They have identical LSU sequence data and there are only one nucleotide differences in ITS sequence data between MFLUCC 17-2321 and *D. vinosa* MFLUCC 17-2513. Our collection MFLUCC 17-2321 is very similar to *D. vinosa* MFLUCC 17-2513 in all morphological characters and they have overlapping size of conidiophores (160–300 µm long vs. 130–260 µm long) and conidia (23–34 × 5–8 µm vs. 16–26 × 2.5–4.4 µm) (Hyde et al. 2018a). We observed branched conidiophores in our collection, which are present in Ellis (1971) but absent in Hyde et al. (2018a). We identify MFLUCC 17-2321 as *D. vinosa* based on phylogenetic analysis (Fig. 47) and their very similar morphology. This is a new habitat record of *D. vinosa* from freshwater in Thailand.

***Dictyocheirosora*** M.J. D'souza, Boonmee & K.D. Hyde, Fungal Diversity 80: 465 (2016)

**Saprobic** on decaying wood in freshwater and terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. **Conidiomata** on natural substrate sporodochial, punctiform, dark brown. **Mycelium** immersed, consisting of branched, septate, subhyaline to pale brown hyphae. **Conidiophores** micronematous or semi-macronematous, septate, hyaline to pale brown, smooth. **Conidiogenous cells** holoblastic, integrated, terminal, determinate, doliiform to cylindrical. **Conidia** acrogenous, solitary, dry, cheiroid, pale brown, complanate or non-complanate, euseptate or distoseptate, smooth-walled (Boonmee et al. 2016).

**Type species:** *Dictyocheirosora rotunda* M.J. D'souza, Bhat & K.D. Hyde

**Notes:** *Dictyocheirosora* was introduced for the species producing dark sporodochial colonies with aeroaquatic cheiroid dictyospores (Boonmee et al. 2016). Many new species and combinations were reported in the genus (Yang et al. 2018), and 21 species are listed in Index Fungorum (2020). Nine of them are from freshwater habitats, mostly in China and Thailand (see list below).

#### List of freshwater *Dictyocheirosora* species

\****Dictyocheirosora aquadulcis*** Sorvongxay, S. Boonmee & K.D. Hyde, Fungal Diversity 96: 23 (2019)

**Freshwater distribution:** Thailand (Hyde et al. 2019)

\****Dictyocheirosora aquatica*** Z.L. Luo, Bhat & K.D. Hyde, Mycosphere 7(9): 1361 (2017)

**Freshwater distribution:** China (Wang et al. 2016)

\****Dictyocheirosora garethjonesii*** Z.L. Luo, H.Y. Su & K.D. Hyde, Mycosphere 7(9): 1361 (2017)

**Freshwater distribution:** China (Wang et al. 2016)

\****Dictyocheirosora gigantea*** (Goh & K.D. Hyde) M.J. D'souza, Boonmee & K.D. Hyde, Fungal Diversity 80: 469 (2016)

**Freshwater distribution:** KwaZulu-Natal (Goh et al. 1999)

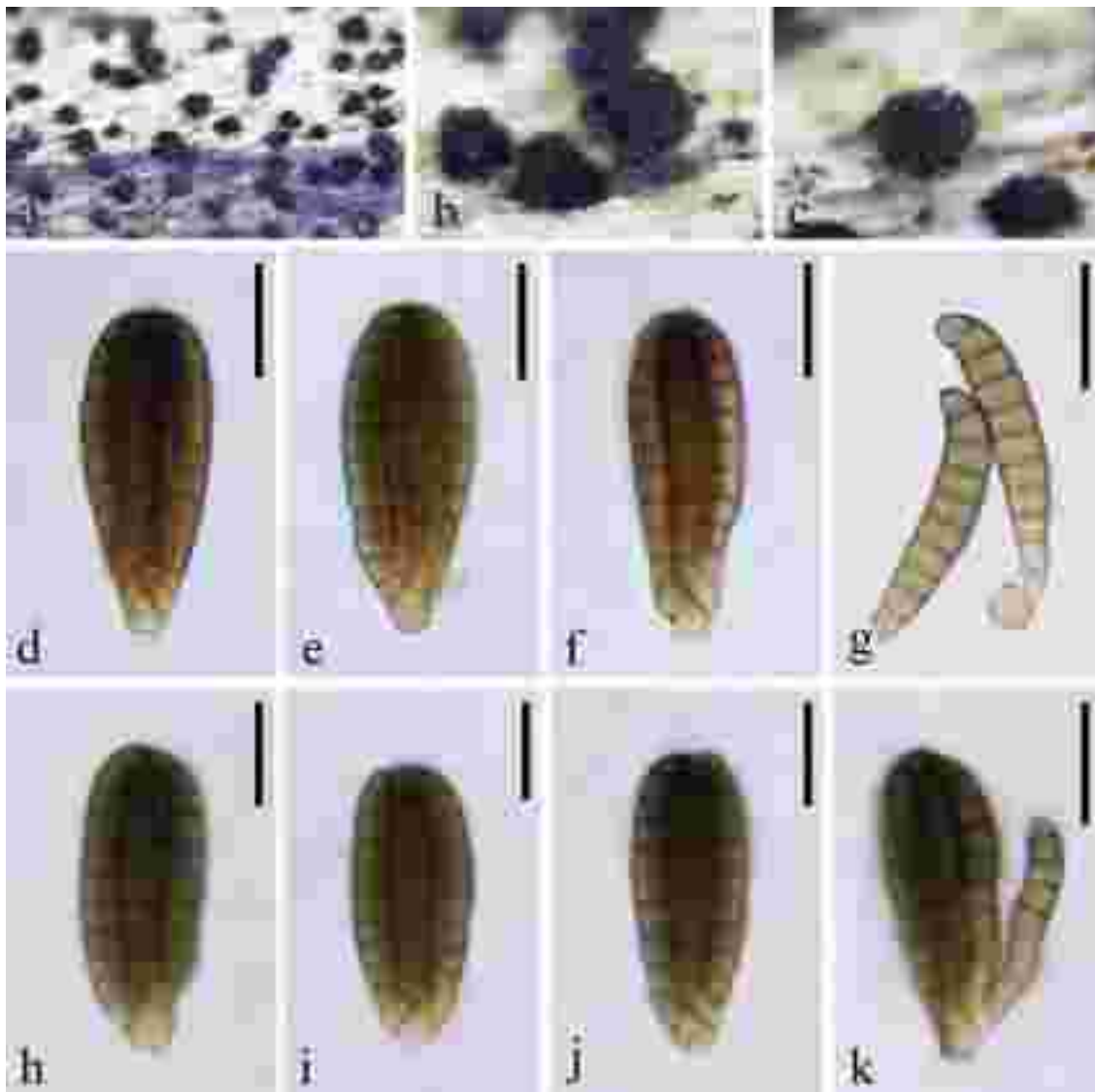
\****Dictyocheirosora indica*** (I.B. Prasher & R.K. Verma) J. Yang & K.D. Hyde, MycoKeys 36: 90 (2018)

**Freshwater distribution:** Thailand (Yang et al. 2018)

\****Dictyocheirosora rotunda*** M.J. D'souza, Bhat & K.D. Hyde, Fungal Diversity 80: 465 (2016); Fig. 41

**Freshwater distribution:** China (Wang et al. 2016; this study), Thailand (Boonmee et al. 2016)

**Saprobic** on submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. **Conidiomata** sporodochial, punctiform, velvety, black. **Mycelium** mostly immersed. **Conidiophores** micronematous, reduced. **Conidiogenous cells**



**Fig. 41** *Dictyocheirospora rotunda* a–g HKAS 102146. h–k HKAS 102143. a–c Colonies on submerged wood. d–k Conidia. Scale bars: d–k = 20  $\mu$ m

holoblastic, monoblastic, integrated, terminal, determinate, pale brown, smooth-walled. *Conidia* 50–62  $\times$  19–25  $\mu$ m ( $\bar{x}$  = 56  $\times$  22  $\mu$ m, n = 15), solitary, acrogenous, cheiroid, pale brown to dark brown, consisting of 4–6 rows of cells, rows digitate, cylindrical to narrowly clavate, inwardly curved at the apex, with a subglobose, cuneiform basal cell, each arm composed of 10–12 cells, septate, constricted at septa.

**Culture characteristics:** On PDA, colony circular, reaching 50 mm in 25 days at 25  $^{\circ}$ C, grey to brown from above, black from below, surface rough, with dense mycelium, produced conidia in culture after 10 days, dry, raised, edge entire.

**Material examined:** CHINA, Yunnan Province, Kunming University of Science & Technology, on submerged wood in a stream, 10 May 2017, C.X. Liu, L13 (HKAS

102143), living culture KUMCC 19-0103; *ibid.*, Qujing, on submerged wood in a stream, 10 May 2017, W. Dong, L40 (HKAS 102146), living culture KUMCC 19-0105.

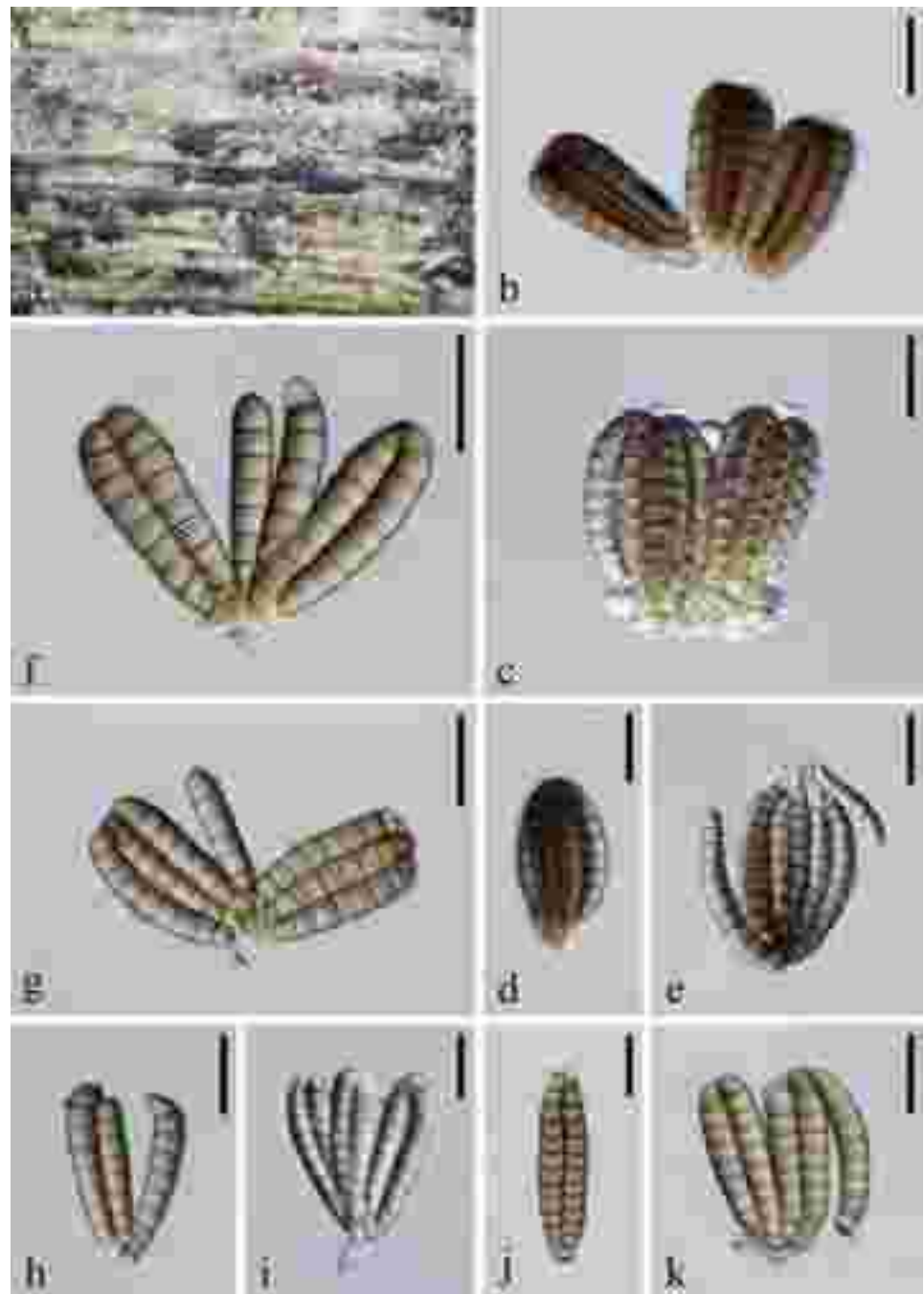
**Notes:** Our two new collections KUMCC 19-0103 and KUMCC 19-0105 have slightly thinner conidia (50–62  $\times$  19–25  $\mu$ m vs. 42–58  $\times$  19–38  $\mu$ m) than the holotype in Thailand (Boonmee et al. 2016). Two collections cluster with *D. rotunda* with high bootstrap support (Fig. 47).

\**Dictyocheirospora tetraploides* (L. Cai & K.D. Hyde) J. Yang & K.D. Hyde, MycoKeys 36: 93 (2018)

**Freshwater distribution:** China (Cai et al. 2003b)

\**Dictyocheirospora thailandica* X.D. Yu, W. Dong & H. Zhang, *sp. nov.*

**Fig. 42** *Dictyocheirospora thailandica* (MFLU 18-0999, **holotype**). **a** Colonies on submerged wood. **b–k** Conidia. **i** Conidiogenous cell with conidium. Scale bars: **b–k** = 20  $\mu$ m



*Index Fungorum number*: IF557908; *Facesoffungi number*: FoF09251; Fig. 42

*Etymology*: referring to Thailand, where the holotype was collected

*Holotype*: MFLU 18-0999

*Saprobic* on submerged wood. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. *Conidiomata* sporodochial, punctiform, black. *Mycelium* mostly immersed, composed of pale brown, thin-walled hyphae. *Conidiophores* micronematous, reduced. *Conidiogenous*

*cells* holoblastic, monoblastic, integrated, terminal, determinate, hyaline to subhyaline, smooth-walled. *Conidia* 42–65  $\times$  20–45  $\mu$ m ( $\bar{x}$  = 51  $\times$  27  $\mu$ m,  $n$  = 15), solitary, acrogenous, cheiroid, non-complanate, pale brown to dark brown, consisting of 6–7 rows of cells, rows compacted, inwardly curved at the apex, easily become divergent and digitate when in water, broadly clavate, with a subglobose or cuneiform basal cell, each arm subcylindrical to narrowly clavate, 7–7.5  $\mu$ m wide, composed of 9–12 cells, 8–11-septate, constricted at the septa, without appendages or sheaths.

**Culture characteristics:** On PDA, colony circular, reaching 40 mm in 45 days at 25 °C, pale yellow to grey from above, dark brown from below, surface rough, with dense mycelium, dry, raised, edge undulate to filiform.

**Material examined:** THAILAND, Phayao Province, on submerged wood in a stream, 23 February 2018, X.D. Yu, Y21 (MFLU 18-0999, **holotype**), ex-type living culture MFLUCC 18-0987.

**Notes:** *Dictyocheirosora thailandica* clusters with *D. taiwanense* Tennakoon et al. with low bootstrap support (Fig. 47). Morphologically, they are entirely different species. *Dictyocheirosora thailandica* has broadly clavate conidia which easily become divergent and digitate when in water, while *D. taiwanense* has narrowly cylindrical conidia comprising of tightly compacted arms (Hyde et al. 2019). In addition, *D. thailandica* has shorter and wider conidia (42–65 × 20–45 µm vs. (72–)74–84(–86) × 16–20(–24) µm) with a higher number of conidial rows (6–7 vs. 5) than *D. taiwanense* (Hyde et al. 2019). Unfortunately, *D. taiwanense* only has LSU and ITS sequence data in GenBank, which show two and three nucleotide differences between *D. thailandica* (MFLUCC 18-0987) and *D. taiwanense* (MFLUCC 17-2654), respectively. No other genes can be compared. Based on the very distinct morphology, we introduce *D. thailandica* as a new species in *Dictyocheirosora*. We believe that *D. thailandica* and *D. taiwanense* will be separated clearly in the phylogenetic tree with protein coding genes added in future.

\**Dictyocheirosora vinaya* M.J. D'souza, Bhat & K.D. Hyde, Fungal Diversity 80: 467 (2016)

**Freshwater distribution:** Thailand (Boonmee et al. 2016)

#### Key to freshwater *Dictyocheirosora* species

1. Conidia with appendage.....2
1. Conidia without appendages.....3
2. Conidial appendage 5–13 × 5–7 µm.....*D. indica*
2. Conidial appendage 10–25 × 5–10 µm.....*D. tetraploides*
3. Conidial rows closely compacted, become divergent when squashed.....4
3. Conidial rows not as above.....5
4. Conidia 58–67 × 15.5–26.5 µm.....*D. vinaya*
4. Conidia 42–65 × 20–45 µm.....*D. thailandica*
5. Conidial rows composed of > 20 cells.....*D. gigantea*
5. Conidial rows composed of < 20 cells.....6
6. Conidia > 60 µm long.....*D. aquaduleis*
6. Conidia < 60 µm long.....7
7. Conidial arm composed of up to 12 cells.....*D. rotunda*
7. Conidial arm composed of 7–10 cells.....8
8. Conidia 34–42 × 12.5–19.5 µm.....*D. aquatica*
8. Conidia 45.5–54.5 × 15.5–24.5 µm.....*D. garethjonesii*

*Dictyosporium* Corda, Weitenweber's Beitr. Nat.: 87 (1836)

**Saprobic** on decaying wood and plant debris in terrestrial and freshwater habitats. **Sexual morph:** *Ascomata* perithecial, superficial, solitary or scattered, globose to subglobose, dark brown to black, collapsed when dry, ostiolate. *Peridium* membranaceous, composed of several layers of dark brown, small cells of *textura angularis* or *textura epidermoidea*. *Pseudoparaphyses* numerous, cellular, subcylindrical, hyaline, septate. *Asci* bitunicate, fissitunicate, clavate to cylindrical or saccate, pedicellate, apically rounded with an ocular chamber. *Ascospores* biseriate, fusiform, elongated-ellipsoid, septate, hyaline, with or without mucilaginous sheath (Boonmee et al. 2016). **Asexual morph:** Hyphomycetous. *Colonies* punctiform, sporodochial, effuse or compact, olive, brown to black, glistening, with immersed mycelium. *Conidiophores* micronematous, mononematous, flexuous, irregularly branched, hyaline to brown, smooth, sometimes reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal or sometimes intercalary, determinate, cylindrical, doliiform, spherical or subspherical, hyaline to pale brown. *Conidia* solitary, dry, acrogenous or sometimes pleurogenous, cheiroid, complanate, olive to brown, smooth, multiseptate (Boonmee et al. 2016).

**Type species:** *Dictyosporium elegans* Corda

**Notes:** *Dictyosporium* was introduced for asexual species producing punctiform, sporodochial colonies and cheiroid, complanate conidia. The sexual morph *D. meiosporum* Boonmee & K.D. Hyde and *D. sexualis* Boonmee & K.D. Hyde were reported based on phylogenetic analysis (Liu et al. 2015). Nineteen freshwater *Dictyosporium* species are accepted in the genus, and 11 species are confirmed with molecular data. *Dictyosporium* species can be distinguished by the conidial size, appendage, sheath and rows (see key below).

#### List of freshwater *Dictyosporium* species

\**Dictyosporium aquaticum* Abdel-Aziz, Fungal Diversity 72: 72 (2015)

**Freshwater distribution:** Egypt (Liu et al. 2015)

*Dictyosporium biseriale* D.M. Hu, L. Cai & K.D. Hyde, Sydowia 62(2): 197 (2010)

**Freshwater distribution:** Yunnan, China (Hu et al. 2010a)

*Dictyosporium canisporum* L. Cai & K.D. Hyde, Sydowia 55(2): 130 (2003)

**Freshwater distribution:** Yunnan, China (Cai et al. 2003b)

\**Dictyosporium digitatum* J.L. Chen, C.H. Hwang & Tzean, Mycol. Res. 95(9): 1145 (1991)

*Freshwater distribution*: Australia (Goh et al. 1999), Brunei (Goh et al. 1999), China (Goh et al. 1999; Tsui et al. 2000, 2001b), Thailand (Sivichai et al. 2002)

***Dictyosporium lakefuxianense*** L. Cai, K.D. Hyde & McKenzie [as ‘lakefuxianensis’], Cryptog. Mycol. 24(1): 5 (2003)

*Freshwater distribution*: Yunnan, China (Cai et al. 2003c)

\****Dictyosporium nigroapice*** Goh, W.H. Ho & K.D. Hyde, in Goh, Hyde & Yanna, Fungal Diversity 103(1): 83 (1999)

*Freshwater distribution*: Hong Kong, China (Goh et al. 1999; Sivichai et al. 2002; Yang et al. 2018)

\****Dictyosporium olivaceosporum*** Kaz. Tanaka, K. Hiray., Boonmee & K.D. Hyde, Fungal Diversity 80: 474 (2016)

*Freshwater distribution*: Japan (Boonmee et al. 2016)

\****Dictyosporium palmae*** Abdel-Aziz, Mycosphere 7(4): [453] (2016)

*Freshwater distribution*: Egypt (Abdel-Aziz 2016b)

***Dictyosporium pelagicum*** (Linder) G.C. Hughes ex E.B.G. Jones [as ‘pelagica’], Trans. Br. mycol. Soc. 46(1): 137 (1963)

*Freshwater distribution*: USA (Shearer 1972)

***Dictyosporium polystichum*** (Höhn.) Damon, Lloydia 15: 118 (1952)

*Freshwater distribution*: Yunnan, China (Luo et al. 2004)

\****Dictyosporium stellatum*** G.P. White & Seifert, Persoonia 26: 156 (2011)

*Freshwater distribution*: Ontario (Crous et al. 2011)

***Dictyosporium tetraseriale*** Goh, Yanna & K.D. Hyde, Fungal Diversity 103(1): 87 (1999)

*Freshwater distribution*: Brunei (Goh et al. 1999), Hong Kong, China (Goh et al. 1999)

\****Dictyosporium tetrasporum*** L. Cai & K.D. Hyde, Mycoscience 48(5): 290 (2007)

*Freshwater distribution*: Yunnan, China (Cai and Hyde 2007a)

\****Dictyosporium thailandicum*** M.J. D’souza, Bhat & K.D. Hyde, Fungal Diversity 72: 78 (2015)

*Freshwater distribution*: Thailand (Liu et al. 2015)

\****Dictyosporium tratense*** J. Yang & K.D. Hyde, MycoKeys 36: 96 (2018)

*Freshwater distribution*: Thailand (Yang et al. 2018)

***Dictyosporium triramosum*** Aramb., Cabello & Cazau, Mycotaxon 78: 185 (2001)

*Freshwater distribution*: Buenos Aires (Arambarri 2001)

\****Dictyosporium tubulatum*** J. Yang, K.D. Hyde & Z.Y. Liu, MycoKeys 36: 94 (2018)

*Freshwater distribution*: Thailand (Yang et al. 2018)

***Dictyosporium yunnanense*** L. Cai, K.D. Hyde & McKenzie [as ‘yunnanensis’], Cryptog. Mycol. 24(1): 7 (2003)

*Freshwater distribution*: Yunnan, China (Cai et al. 2003c)

\****Dictyosporium zhejiangense*** Wongs., H.K. Wang, K.D. Hyde & F.C. Lin, Cryptog. Mycol. 30(4): 358 (2009)

*Freshwater distribution*: Zhejiang, China (Wongsawas et al. 2009)

#### Key to freshwater *Dictyosporium* species (only species confirmed with molecular data)

1. Conidia without appendages.....2
1. Conidia with appendage.....3
2. Conidia (50–)95–140(–175)  $\mu\text{m} \times$  (27.5–)30–40(–52.5)  $\mu\text{m}$ .....***D. stellatum***
2. Conidia 23.5–40  $\mu\text{m} \times$  16–21.5  $\mu\text{m}$ .....***D. tetrasporum***
3. Conidia with sheath.....4
3. Conidia without sheath.....5
4. Conidia 60–85  $\mu\text{m} \times$  20–30  $\mu\text{m}$ .....***D. aquaticum***
4. Conidia (40–)43–54(–57)  $\mu\text{m} \times$  (20–)23–32(–36)  $\mu\text{m}$ .....***D. tratense***
5. Conidia with two types of appendages.....***D. palmae***
5. Conidia with one type of appendage.....6
6. Conidia > 45  $\mu\text{m}$  long.....***D. digitatum***
6. Conidia < 45  $\mu\text{m}$  long.....7
7. Conidia not complanate.....***D. olivaceosporum***
7. Conidia complanate.....8
8. Conidia consist of five rows of cells.....9
8. Conidia mostly consist of four rows of cells.....10
9. Appendages variable in shape.....***D. zhejiangense***
9. Appendages tubular.....***D. thailandicum***
10. Conidial appendage 22–34  $\times$  4–5  $\mu\text{m}$ ...***D. nigroapice***
10. Conidial appendage 19–24  $\times$  3.5–7  $\mu\text{m}$ .....***D. tubulatum***

***Digitodesmium*** P. M. Kirk, Trans. Br. mycol. Soc. 77: 284 (1981)

*Saprobic* on submerged bamboo, wood in freshwater or decaying wood, soil in terrestrial habitats. **Sexual morph**: Undetermined. **Asexual morph**: Hyphomycetous. *Conidiomata* sporodochial, punctiform, pulvinate, scattered, pale

brown to brown. *Mycelium* mostly immersed, composed of branched, septate, thin-walled, smooth, pale brown hyphae. *Conidiophores* semi-macronematous, mononematous, fasciculate, composed of moniliform, pale brown, septate, smooth hyphae. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal, determinate, globose to doliiform, minutely cicatrized. *Conidia* acrogenous, solitary, dry, digitate, cheir-oid, slightly divergent arms, euseptate, some with a hyaline gelatinous cap at the apex (Kirk 1981; Boonmee et al. 2016).

*Type species: Digitodesmium elegans* P.M. Kirk

*Notes: Digitodesmium* is unique by its digitate conidia and moniliform conidiophores (Kirk 1981; Boonmee et al. 2016). Tsui et al. (2006a) indicated that *Digitodesmium* might be a synonym of *Dictyosporium* because the phylogenetic position of *Digitodesmium* was within *Dictyosporium*, but with low bootstrap support. A similar result was shown in Cai et al. (2008). *Digitodesmium* was distinguished from *Dictyosporium* by its divergent conidial arms which were considered as an artificial feature by Tsui et al. (2006a) and Cai et al. (2008). Boonmee et al. (2016) introduced family Dictyosporiaceae to accommodate most cheirosporous hyphomycete genera and accepted *Digitodesmium* as a distinct genus based on multigene phylogenetic analyses. We think that the moniliform conidiophores of *Digitodesmium* are also important to distinguish this genus.

Three freshwater *Digitodesmium* species morphologically fit well with the generic concept of *Digitodesmium* in producing divergent conidial arms (Ho et al. 1999; Cai et al. 2002b, 2003b). The placement of *D. heptasporum* L. Cai & K. D. Hyde and *D. recurvum* W. H. Ho et al. needs to be confirmed with molecular data.

#### List of freshwater *Digitodesmium* species

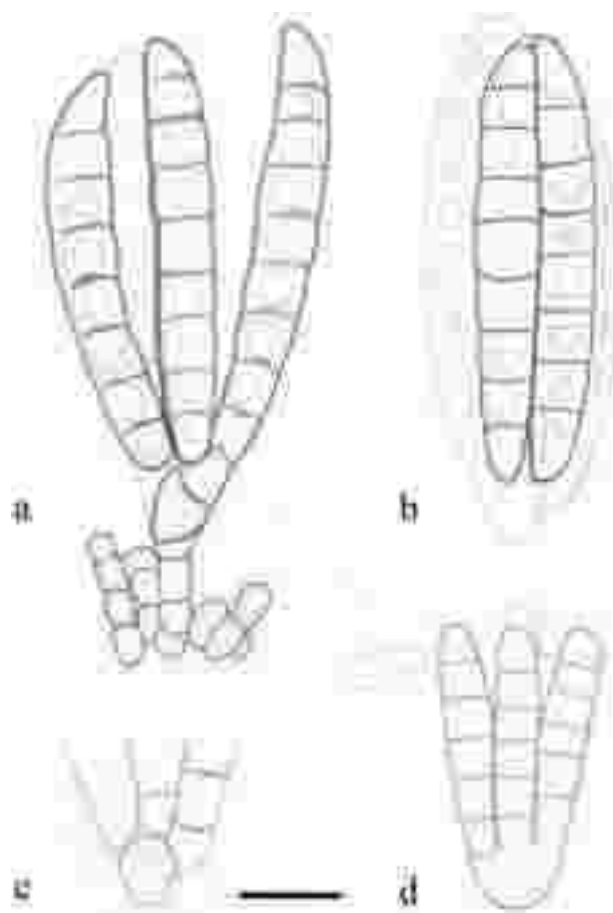
\**Digitodesmium bambusicola* L. Cai, K. Q. Zhang, McKenzie, W. H. Ho & K. D. Hyde, Nova Hedwigia 75: 528 (2002); Fig. 43d  
*Freshwater distribution:* Philippines (Cai et al. 2002b)

*Digitodesmium heptasporum* L. Cai & K. D. Hyde, Sydowia 55: 133 (2003)  
*Freshwater distribution:* Yunnan, China (Cai et al. 2003b)

*Digitodesmium recurvum* W. H. Ho, K. D. Hyde & Hodgkiss, Mycologia 91: 900 (1999); Fig. 43a–c  
*Freshwater distribution:* China (Ho et al. 1999)

#### Key to freshwater *Digitodesmium* species

1. Conidia with appendage.....*D. bambusicola*
1. Conidia without appendages.....2
2. Conidia 30–45 × 12–21 μm.....*D. recurvum*
2. Conidia 50–75 × 32.5–70 μm.....*D. heptasporum*



**Fig. 43** *Digitodesmium* spp. **a–c** *D. recurvum* (redrawn from Ho et al. (1999), HKU(M) 4552, **holotype**). **a** Conidiophore bearing conidium. **b** Conidium. **c** Conidium at the basal portion. **d** *D. bambusicola* (redrawn from Cai et al. (2002b), PDD 74494, **holotype**). **d** Conidium with apical or subapical appendages. Scale bars: **a–d** = 10 μm

*Pseudocoleophoma* Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 89 (2015)

*Saprobic* on decaying pod, leaves, stems and submerged wood. **Sexual morph:** *Ascomata* scattered or gregarious, immersed to erumpent, globose to subglobose, ostiolate. *Ostiolar neck* central, composed of subglobose, dark brown cells. *Peridium* composed of several layers of polygonal to rectangular cells. *Pseudoparaphyses* numerous, cellular, hypha-like, septate, branched, anastomosing. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, short pedicellate. *Ascospores* fusiform, septate, smooth, with a conspicuous, mucilaginous sheath (Tanaka et al. 2015). **Asexual morph:** Coelomycetous. *Conidiomata* immersed to erumpent, subglobose. *Ostiolar neck* well-developed, cylindrical, central. *Peridium* composed of several layers of slightly thick-walled, pale brown, polygonal to subglobose cells. *Conidiophores* absent. *Conidiogenous cells*

enteroblastic, phialidic, doliiform to lageniform. *Conidia* cylindrical, hyaline, aseptate or septate, smooth (Tanaka et al. 2015).

*Type species: Pseudocoleophoma calamagrostidis* Kaz. Tanaka & K. Hiray.

*Notes: Pseudocoleophoma* is characterized by fusi-form, septate ascospores with a conspicuous sheath and coleophoma-like conidia (Tanaka et al. 2015). *Pseudocoleophoma* was placed in Dictyosporiaceae based on molecular data (Tanaka et al. 2015; Boonmee et al. 2016; Yang et al. 2018). Four species are listed in Index Fungorum (2020). *Pseudocoleophoma typhicola* Kamolhan et al. (Hyde et al. 2016b) is known from only an asexual morph, while the other species have both sexual and asexual morphs. *Pseudocoleophoma typhicola* is the only species producing septate conidia (Tanaka et al. 2015; Hyde et al. 2016b; Jayasiri et al. 2019).

### List of freshwater *Pseudocoleophoma* species

\**Pseudocoleophoma typhicola* Kamolhan, Banmai, Boonmee, E.B.G. Jones & K.D. Hyde, Fungal Diversity 80: 34 (2016)

*Freshwater distribution:* UK (Hyde et al. 2016b)

*Jalapriya* M.J. D'souza, H.Y. Su, Z.L. Luo & K.D. Hyde, Fungal Diversity 80: 476 (2016)

*Saprobic* on decaying wood in freshwater or terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Colonies* effuse, dark brown to black. *Mycelium* immersed or partly superficial, consisting of branched, septate, smooth, subhyaline to pale brown hyphae. *Conidiophores* micronematous, unbranched, thin-walled, smooth, cylindrical. *Conidiogenous cells* holoblastic, integrated, determinate, terminal. *Conidia* acrogenous, solitary, cheiroid, complanate or non-complanate, smooth-walled, euseptate, with 5–7 rows of cells, rows converging or divergent at the apex, apical cells with or without appendages (Boonmee et al. 2016).

*Type species: Jalapriya pulchra* M.J. D'souza, H.Y. Su, Z.L. Luo & K.D. Hyde

*Notes: Jalapriya* was introduced to accommodate a new freshwater species *J. pulchra* and two new combinations of species from terrestrial habitats (Boonmee et al. 2016). The cheirosporous genera are morphologically very similar in the family and *Jalapriya* was established based on multi-gene phylogenetic analyses (Boonmee et al. 2016). *Jalapriya pulchra* is distinctive based on its complanate conidia with hyaline appendages on the apical cells.

### List of freshwater *Jalapriya* species

\**Jalapriya pulchra* M.J. D'souza, H.Y. Su, Z.L. Luo & K.D. Hyde, Fungal Diversity 80: 477 (2016); Fig. 44

*Freshwater distribution:* China (Boonmee et al. 2016)

*Pseudodictyosporium* Matsush., Bull. natn. Sci. Mus., Tokyo, N.S. 14: 473 (1971)

*Saprobic* on rotten leaves or submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiomata* on natural substratum sporodochial, superficial, punctiform to effuse, velvety, scattered to gregarious, pale brown to dark brown, olive. *Conidiophores* micronematous, aseptate, simple, hyaline to pale brown, smooth. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal, determinate, doliiform to cylindrical. *Conidia* acrogenous, solitary, dry, cheiroid, pale brown, smooth-walled, septate, composed of three rows of cells arising parallelly from a truncate basal cell, compactly, non-complanate, with or without appendages (Matsushima 1971; Li et al. 2017).

*Type species: Pseudodictyosporium wauense* Matsush.

*Notes: Pseudodictyosporium wauense* is a terrestrial species collected from New Guinea. It was re-collected from submerged wood in China and described as cheiroid conidia containing three parallel rows of cells arising from a basal cell (Li et al. 2017). The parallel rows of cells of *Pseudodictyosporium* are similar to *Vikalpa*, however, phylogeny separates them as distinct genera (Boonmee et al. 2016; Li et al. 2017; Yang et al. 2018).

### List of freshwater *Pseudodictyosporium* species

\**Pseudodictyosporium wauense* Matsush., Bull. natn. Sci. Mus., Tokyo 14(3): 473 (1971); Fig. 45

*Freshwater distribution:* China (Li et al. 2017)

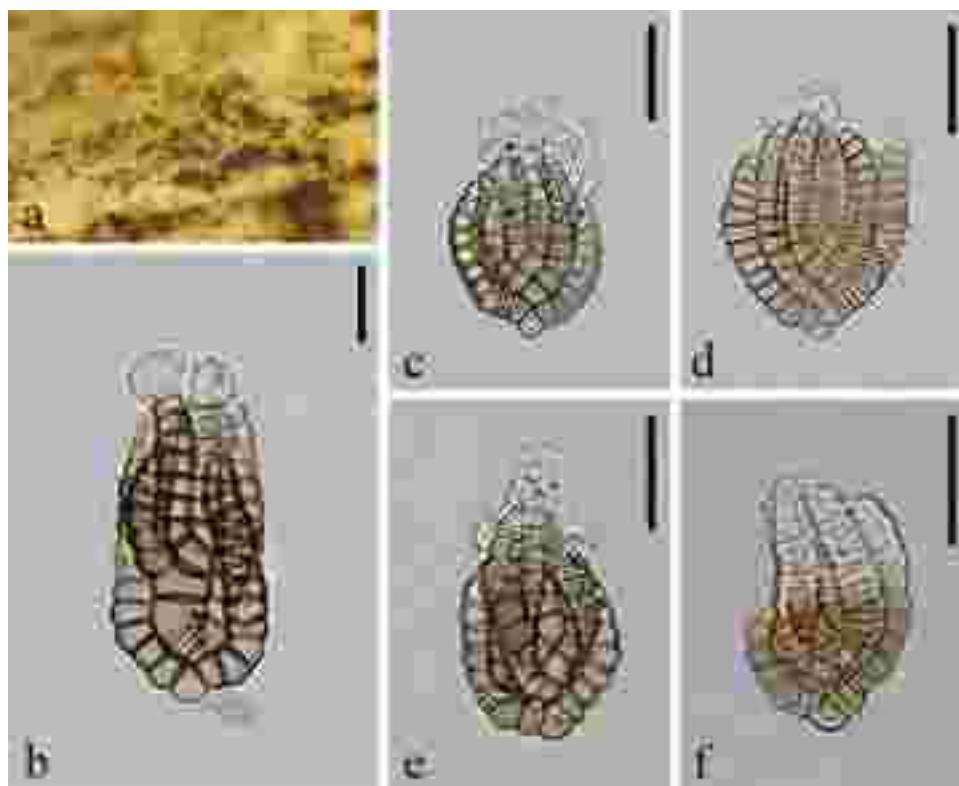
*Vikalpa* M.J. D'souza, Boonmee, Bhat & K.D. Hyde, Fungal Diversity 80: 479 (2016)

*Saprobic* on submerged wood in freshwater and decaying wood, leaves in terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. *Conidiomata* sporodochial, punctiform or effuse, scattered to gregarious, superficial, pale brown to dark brown, with or without a mucilage, inconspicuous covering. *Mycelium* immersed, composed of branched, septate, subhyaline to pale brown, smooth-walled hyphae. *Conidiophores* micronematous, aseptate, hyaline to pale brown. *Conidiogenous cells* holoblastic, integrated, terminal, determinate, cylindrical to doliiform. *Conidia* acrogenous, solitary, dry, cheiroid, pale brown, smooth-walled, euseptate or distoseptate, consisting of three rows of cells arised parallel on a truncate basal cell, closely compacted, non-complanate, with or without appendages (Boonmee et al. 2016).

*Type species: Vikalpa australiensis* (B. Sutton) M.J. D'souza, Boonmee & K.D. Hyde

*Notes: Vikalpa* was introduced for one new species and three new combinations transferred from *Dictyosporium*

**Fig. 44** *Jalapriya pulchra* (Material examined: CHINA, Yunnan Province, Dali, Cangshan Mountain, Lingquan stream, on submerged wood, March 2014, Z. Li, LQXM 47-1(S-013), HKAS 83979, **holotype**). **a** Colonies on submerged wood. **b–f** Conidia. Scale bars: **b** = 10  $\mu$ m, **c–f** = 20  $\mu$ m



(Boonmee et al. 2016). *Vikalpa* has non-complanate conidia consisting of three rows of cells arising parallel on a truncate basal cell, while *Dictyosporium* has complanate conidia with several unparallel rows of cells (Boonmee et al. 2016). The placement of *Vikalpa* was unstable, as it clustered basal to *Dictyosporium* in Boonmee et al. (2016), but distant from *Dictyosporium* (Yang et al. 2018). The freshwater species *V. lignicola* M.J. D'souza et al. was introduced based on its 3 rows and non-complanate conidia, but without molecular evidence (Boonmee et al. 2016).

#### List of freshwater *Vikalpa* species

*Vikalpa lignicola* M.J. D'souza, Bhat, H.Y. Su & K.D. Hyde, Fungal Diversity 80: 479 (2016); Fig. 46

*Freshwater distribution*: China (Boonmee et al. 2016)

**Didymosphaeriaceae** Munk, Dansk bot. Ark. 15(no. 2): 128 (1953)

#### Key to freshwater genera of Didymosphaeriaceae

1. Ascumata with a pore-like opening...*Didymosphaeria*
1. Ascumata without above character.....2
2. Asci cylindrical, ascospores broadly ellipsoidal, yellowish brown.....*Paraphaeosphaeria*

2. Asci elongate-clavate to sub-cylindrical, ascospores fusoid, hyaline or pale brown.....*Pseudotrichia*

**Didymosphaeria** Fuckel, Jb. nassau. Ver. Naturk. 23-24: 140 (1870) [1869-70]

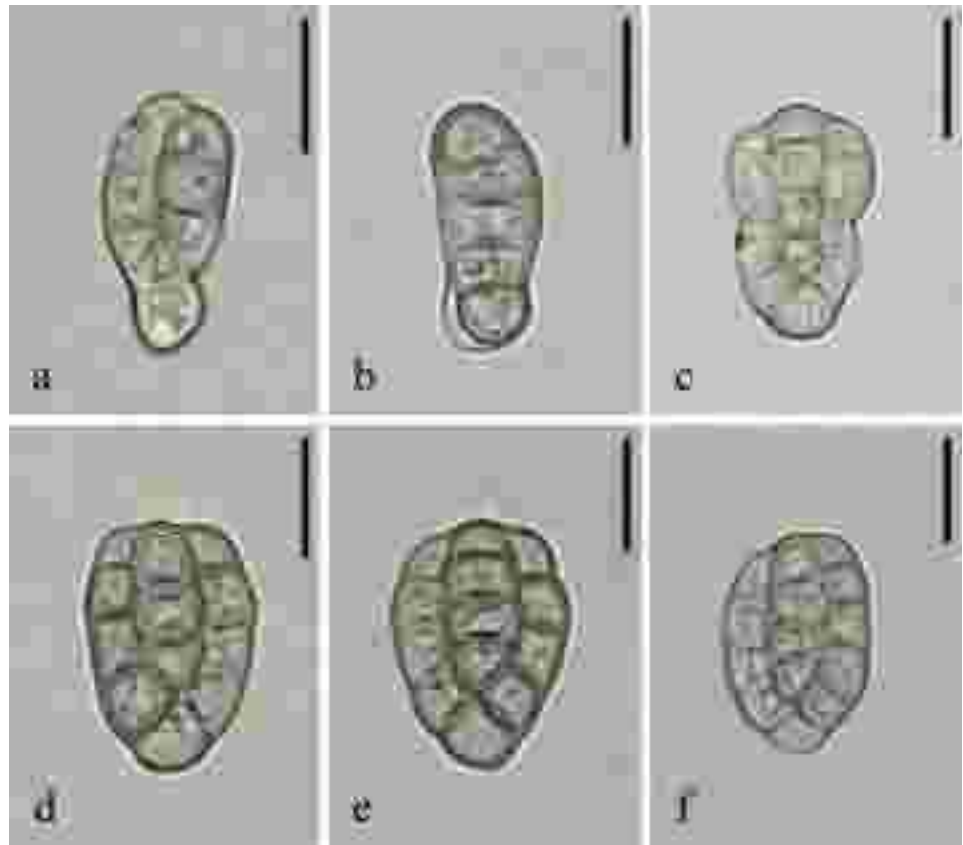
*Saprobic* on woody branches and herbaceous stems and leaves in freshwater or terrestrial habitats. **Sexual morph**: *Ascumata* solitary, scattered, or in small groups, immersed to erumpent, globose to ovoid, with ostiolate papilla. *Ostiole* with a pore-like opening, periphysate. *Peridium* one-layered, thin, composed of brown pseudoparenchymatous cells of *textura intricata*. *Pseudoparaphyses* dense, trabeculate, filiform, hyaline, indistinctly septate, anastomosing, mostly above the asci. *Asci* 8-spored, bitunicate, fissionate, cylindrical, with a furcated pedicel, apically rounded with an indistinct ocular chamber. *Ascospores* uniseriate, ellipsoidal, brown, 1-septate (Zhang et al. 2012b; Ariyawansa et al. 2014a). **Asexual morph**: Undetermined.

*Type species*: *Didymosphaeria futilis* (Berk. & Broome) Rehm

*Notes*: *Didymosphaeria* comprises over 520 epithets in Index Fungorum (2020). The type species *D. futilis* was examined by Ariyawansa et al. (2014a) who also introduced a new species *D. rubi-ulmifolii* Ariyaw. et al. based on immersed ascumata under clypeus with a single layered peridium and brown, 1-septate ascospores. *Didymosphaeria* represented by *D. rubi-ulmifolii* clustered with members of



**Fig. 45** *Pseudodictyosporium wauense* (Material examined: CHINA, Yunnan Province, Dali, saprobic on decaying wood submerged in a stream in Cangshan Mountain, June 2016, S.M. Tang, 3XP H 3–9–1, DLU 0801). **a–f** Conidia. Scale bars: **a–f** = 10  $\mu$ m



Didymosphaeriaceae (Liu et al. 2017a; this study, Fig. 2). The placement of *Didymosphaeria futilis* is questionable as two strains clustered in two different families (HKUCC 5834 in Cucurbitariaceae and CMW22186 in Didymellaceae) (Zhang et al. 2012b; Ariyawansa et al. 2014a). The freshwater species *D. pittospora* Udaiyan & Hosag. was proposed by Udaiyan and Hosagoudar (1991) but is an invalid name (Index Fungorum 2020) because the holotype was not indicated when introduced (Fig. 47).

#### List of freshwater *Didymosphaeria* species

*Didymosphaeria pittospora* Udaiyan & Hosag., J. Econ. Taxon. Bot. 15(3): 658 (1992) [1991]

*Freshwater distribution*: Tamil Nadu, India (Udaiyan and Hosagoudar 1991)

*Paraphaeosphaeria* O.E. Erikss., Ark. Bot., Ser. 2 6: 405 (1967)

*Saprobic* on various hosts in terrestrial and submerged wood in freshwater habitats. **Sexual morph**: *Ascomata* immersed to semi-immersed, depressed globose, with ostiolate papilla. *Ostiole* black, with a short neck, without periphyses. *Peridium* composed of several layers cells of *textura prismatica*. *Pseudoparaphyses* dense, trabeculate, filamentous, hyaline, septate. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical with a short,

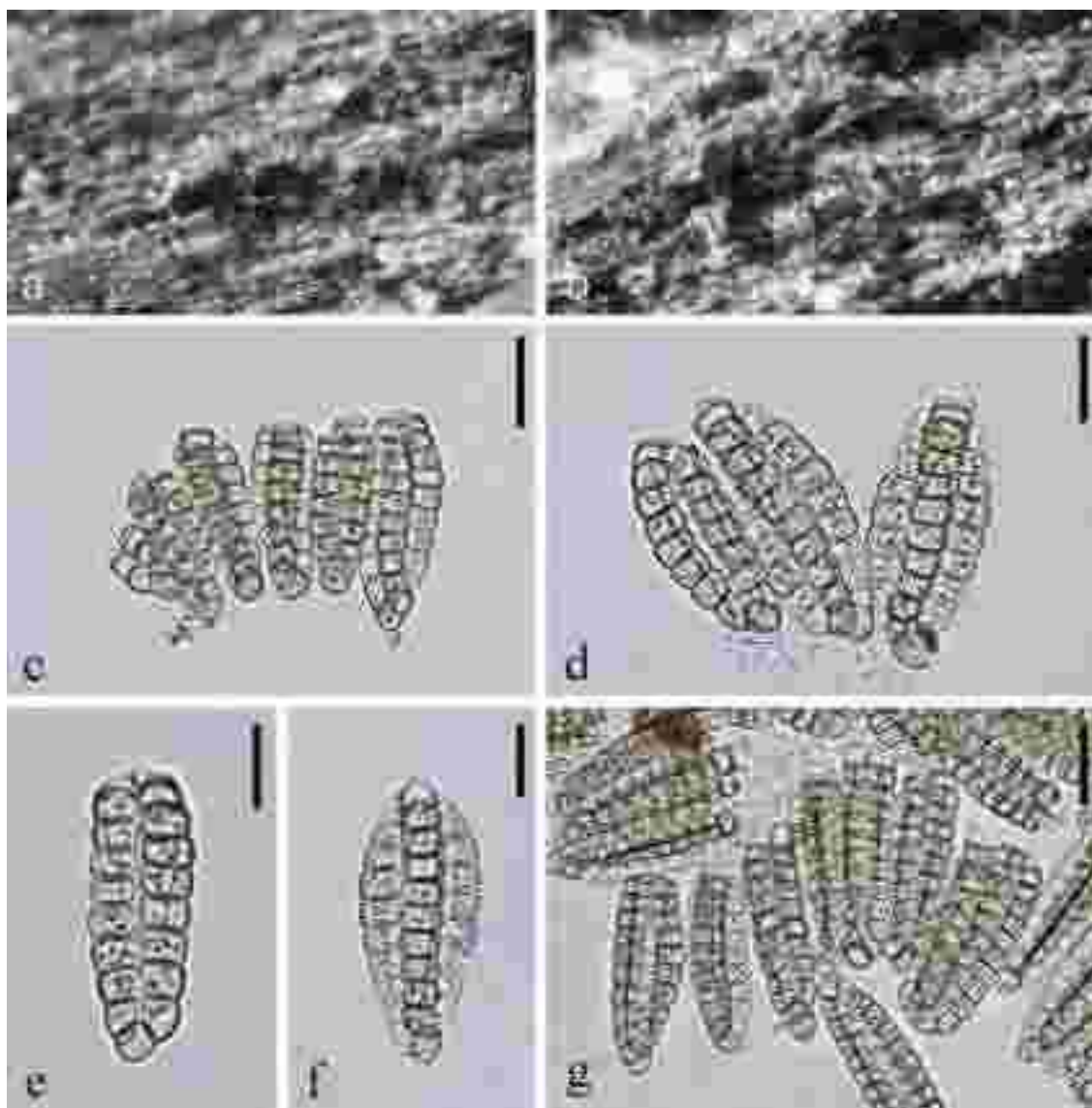
broad pedicel. *Ascospores* uniseriate or partially overlapping, broadly ellipsoidal, yellowish-brown, septate, smooth, with a thin sheath (Ariyawansa et al. 2014b). **Asexual morph**: Coelomycetous. *Conidiomata* eustromatic or pycnidial. *Conidiogenous cells* discrete or integrated, phialidic or annellidic, with percurrent proliferations. *Conidia* variable in shape, subglobose, ellipsoid to obovoid-pyriform, or more cylindrical, aseptate or 1-septate, smooth to verrucose (Verkley et al. 2014).

*Type species*: *Paraphaeosphaeria michotii* (Westend.) O.E. Erikss.

*Notes*: *Paraphaeosphaeria* was confirmed in Didymosphaeriaceae based on molecular data derived from epitype of *P. michotii* (Ariyawansa et al. 2014b). The asexual morph of *Paraphaeosphaeria* was reported as coelomycetous species, e.g. *P. angularis* Verkley & Aa, *P. arecacearum* Verkley et al. and *P. neglecta* Verkley et al. (Verkley et al. 2014). *Paraphaeosphaeria* is quite comparable to *Phaeosphaeria* I. Miyake, but phylogeny places them in Didymosphaeriaceae and Phaeosphaeriaceae, respectively (Liu et al. 2017a).

Two freshwater species, *Paraphaeosphaeria michotii* and *P. schoenoplecti* M.K.M. Wong et al., are similar in having 2-septate, yellowish brown ascospores, but can be distinguished by ascospores shape (cylindrical vs. long fusiform) and size (see key below) (Wong et al. 2000).

#### List of freshwater *Paraphaeosphaeria* species



**Fig. 46** *Vikalpa lignicola* (Material examined: CHINA, Yunnan Province, Dali, Cangshan Mountain, Xue Shan Stream, on decaying wood submerged in the stream, 11 August 2014, Z.L. Luo, XS-69-2,

MFLU 15-1506, **holotype**). **a, b** Colonies on submerged wood. **c–g** Conidia. Scale bars: **c, g** = 15  $\mu\text{m}$ , **d–f** = 10  $\mu\text{m}$

***Paraphaeosphaeria michotii*** (Westend.) O.E. Erikss., Arch. Botan. 6: 405 (1967)

*Basionym*: *Sphaeria michotii* Westend., Bull. Acad. R. Sci. Belg., Cl. Sci., sér. 2 7(5): 87 (1859)

*Synonymy*: see Index Fungorum (2020)

*Freshwater distribution*: England (Pugh and Mulder 1971)

***Paraphaeosphaeria schoenoplecti*** M.K.M. Wong, Goh & K.D. Hyde, Fungal Diversity 4: 172 (2000)

*Freshwater distribution*: Hong Kong, China (Wong et al. 2000)

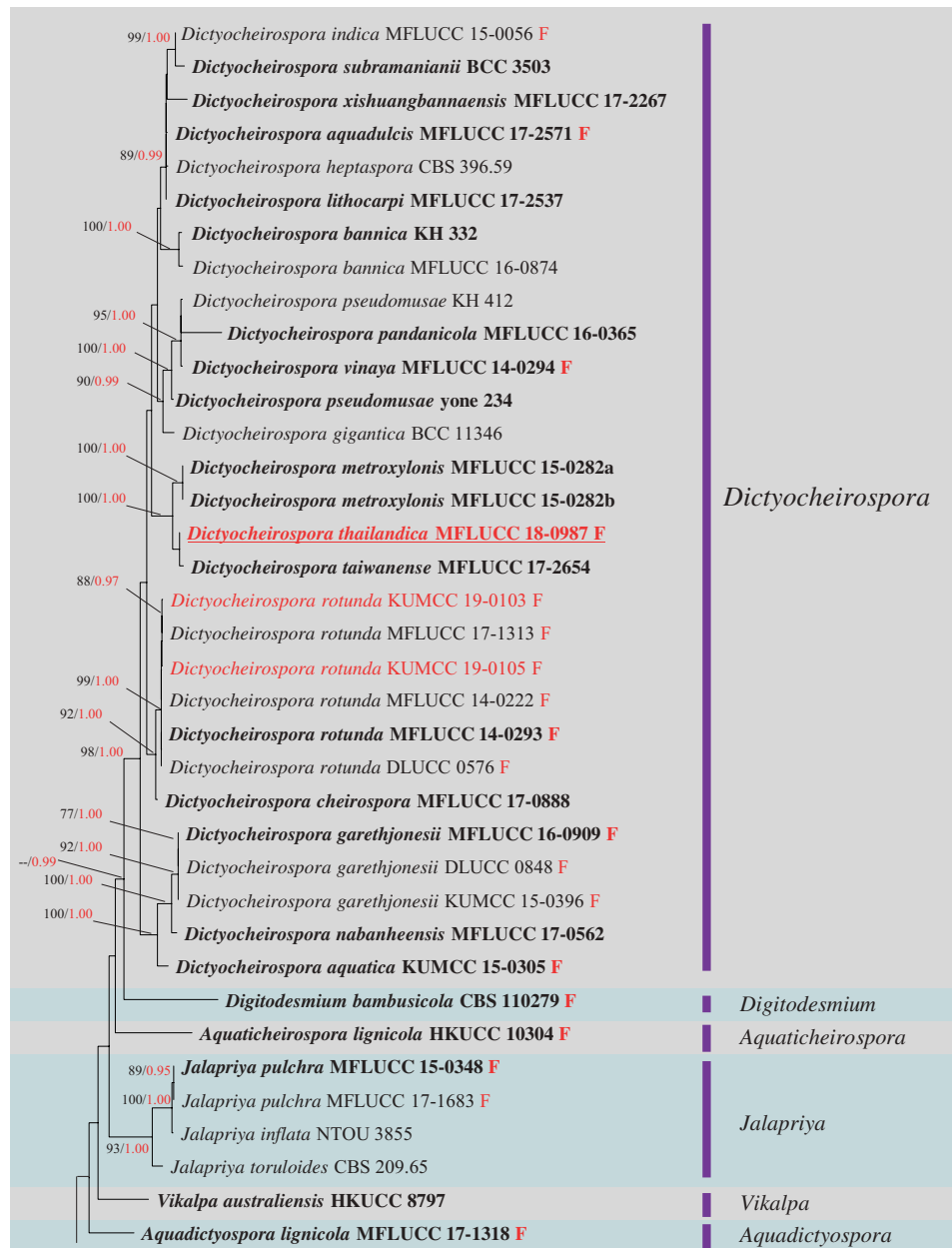
#### Key to freshwater *Paraphaeosphaeria* species

1. Ascospores (12–)16–24  $\times$  4–5(–6)  $\mu\text{m}$ .....*P. michotii*
1. Ascospores 27–31  $\times$  3.8–4  $\mu\text{m}$ .....*P. schoenoplecti*

***Pseudotrachia*** Kirschst., Annl. mycol. 37(1/2): 125 (1939)

*Saprobic* in terrestrial or rarely aquatic habitats. **Sexual morph**: *Ascomata* solitary or scattered, immersed to nearly superficial, globose to pyriform, carbonaceous or coriaceous, with ostiolate papilla. *Peridium* two-layered, comprising thick-walled cells of *textura angularis*, outer layer heavily pigmented, comprising small, dark brown to black cells, inner layer comprising lightly pigmented or hyaline

**Fig. 47** Phylogram generated from maximum likelihood analysis of combined ITS, LSU and TEF sequence data for species of Dictyosporiaceae. Bootstrap values for maximum likelihood equal to or greater than 75% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. The tree is rooted to *Lentithecium clonium* KT 1220 (Lentitheciaceae)



cells. *Pseudoparaphyses* numerous, cellular, narrow, hyaline, septate, unbranched. *Asci* 8-spored, bitunicate, fissitunicate, elongate clavate to sub-cylindrical, with narrow, long, furcate pedicellate, apically rounded with a minute ocular chamber. *Ascospores* uni- or bi-seriate, fusoid, straight to curved, hyaline or pale brown, 1- to multiseptate, smooth-walled, thick-walled (Thambugala et al. 2014; Tian et al. 2015). **Asexual morph:** Undetermined.

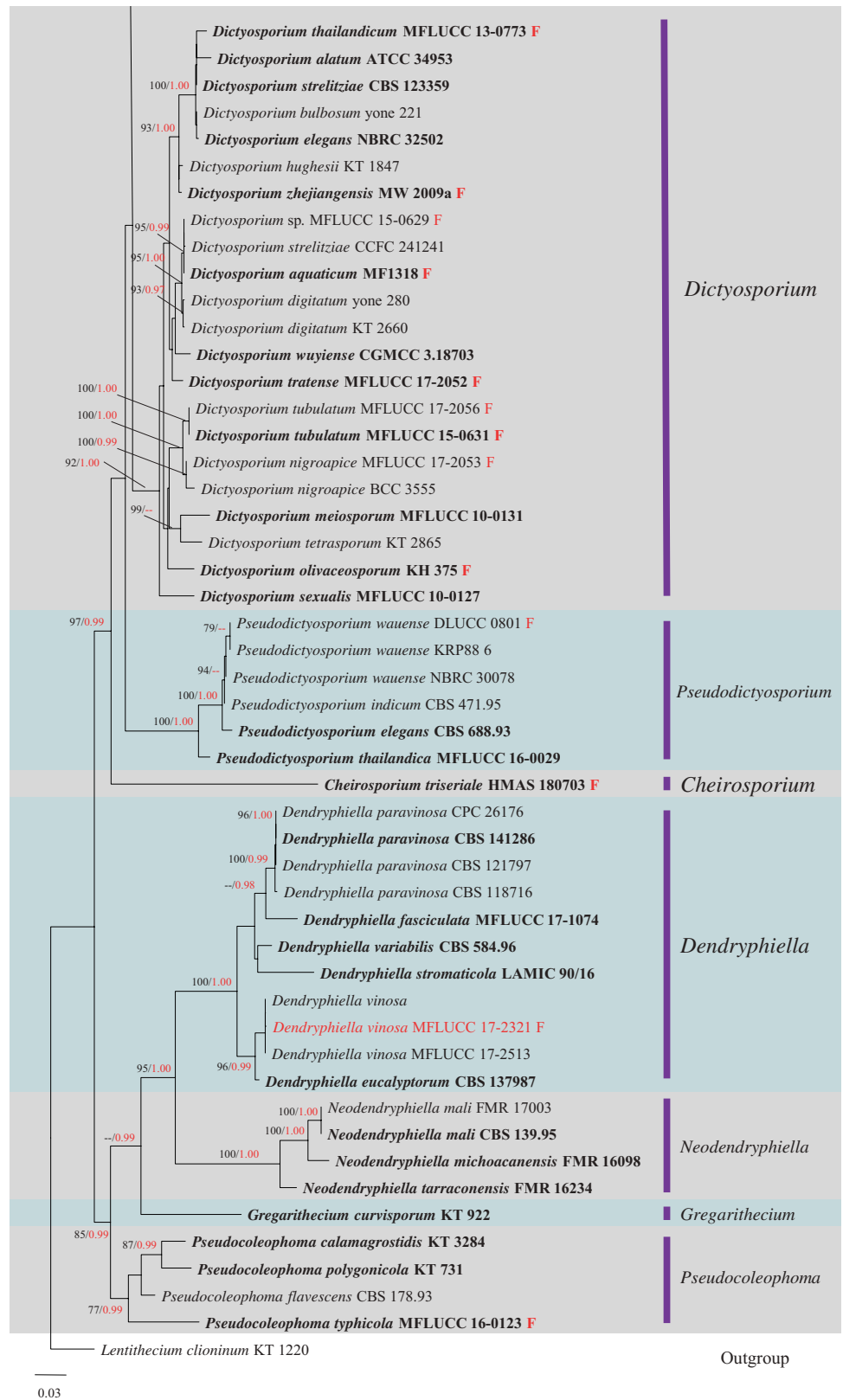
*Type species:* *Pseudotrichia stromatophila* Kirschst.

*Notes:* *Pseudotrichia* was placed in Didymosphaeriaceae by Thambugala et al. (2014) based on re-examination of the holotype of *P. stromatophila*. *Pseudotrichia* was polyphyletic as shown in Tian et al. (2015) as one strain of *P. guatopoensis*

Huhndorf formed an independent lineage in Pleosporales and two strains of *P. mutabilis* (Pers.) Wehm. clustered in Pleomasariaceae. The sequence data of the type species *P. stromatophila* and other *Pseudotrichia* species are needed to clarify the familial and generic status (Thambugala et al. 2014). *Pseudotrichia allequashensis* Fallah & Shearer differs from other species in the genus in having a narrow cylindrical neck with a clypeus and hyaline ascospores with three eusepta and two distosepta (Huhndorf 1994; Fallah and Shearer 2001). However, the placement of *P. allequashensis* in Didymosphaeriaceae needs confirmation with molecular data.

#### List of freshwater *Pseudotrichia* species

Fig. 47 (continued)



*Pseudotrichia allequashensis* Fallah & Shearer, Mycologia 93(3): 597 (2001)

*Freshwater distribution:* USA (Fallah and Shearer 2001)

### Key to freshwater Didymosphaeriaceae asexual genera

1. Conidiogenous cells phialidic or annellidic, with percurrent proliferations.....*Paraphaeosphaeria*
1. Conidiogenous cells phialidic, sometimes percurrent.....*Paraconiothyrium*

*Paraconiothyrium* Verkley, Stud. Mycol. 50(2): 327 (2004)

*Saprobic* on submerged wood in freshwater or plant materials in terrestrial habitats. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* eustromatic, simple or complex, rarely pycnidial. *Conidiogenous cells* discrete or integrated, phialidic, sometimes percurrent. *Conidia* narrowly ellipsoidal or short-cylindrical, aseptate or sometimes 1-septate, thin-walled, smooth-walled or minutely warted, hyaline when young, brown when mature (Verkley et al. 2004).

*Type species:* *Paraconiothyrium estuarinum* Verkley & Manuela Silva

*Notes:* *Paraconiothyrium* was introduced to accommodate the mycoparasite *P. minitans* (W.A. Campb.) Verkley and the other four species (Verkley et al. 2004). *Paraconiothyrium* species are coelomycetous and regarded as asexual morph of *Paraphaeosphaeria* because they clustered in a well-supported clade (Verkley et al. 2004). However, they clustered in two different clades in the later study of Wanasinghe et al. (2018b) who mentioned that the sexual morph of *Paraconiothyrium* is undetermined, which was in agreement with Wijayawardene et al. (2017). *Paraconiothyrium fuckelii* (Sacc.) Verkley & Gruyter was found from freshwater habitats in USA (Shearer 1972), but not confirmed by molecular data. The name *P. fuckelii* is illegitimate (Index Fungorum 2020) according to code, Rejection of names article 52. 1.

### List of freshwater *Paraconiothyrium* species

*Paraconiothyrium fuckelii* (Sacc.) Verkley & Gruyter, Stud. Mycol. 75: 25 (2012) [2013]

*Basionym:* *Coniothyrium fuckelii* Sacc., Fungi venet. nov. vel. Crit., Sér. 5: 200 (1878)

*Synonymy:* see Index Fungorum (2020)

*Freshwater distribution:* USA (Shearer 1972)

### Latoruaceae Crous, IMA Fungus 6(1): 176 (2015)

*Pseudoasteromassaria* M. Matsum. & Kaz. Tanaka, Fungal Diversity 75: 77 (2015)

*Saprobic* on submerged wood or parasitic on twigs. **Sexual morph:** *Ascomata* scattered, immersed, subglobose, brown to black, ostiolate. *Peridium* two-layered.

*Pseudoparaphyses* numerous, cellular, hyaline, septate, branched. *Asci* 8-spored, bitunicate, cylindrical to clavate, pedicellate. *Ascospores* bi- to tri-seriate, fusiform, brown, septate (Ariyawansa et al. 2015). **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, solitary or scattered, immersed or superficial, globose, ellipsoidal, conical, dark brown to black, ostiolate. *Peridium* two-layered. *Conidiophores* absent. *Conidiogenous cells* enteroblastic, phialidic, cylindrical or pyramidal, doliiform to ampulliform. *Conidia* variable in shape, truncate at the base, aseptate or septate, hyaline (Ariyawansa et al. 2015; Tibpromma et al. 2017).

*Type species:* *Pseudoasteromassaria fagi* M. Matsum. & Kaz. Tanaka

*Notes:* The type species *Pseudoasteromassaria fagi* was found on twigs with both sexual and asexual morphs, and it produces cylindrical, hyaline, septate conidia in culture (Ariyawansa et al. 2015). The second species *P. spadicea* W. Dong et al. was described from submerged wood in freshwater habitats. *Pseudoasteromassaria spadicea* differs from the asexual morph of *P. fagi* by its subglobose, aseptate conidia (Tibpromma et al. 2017). We report another species *P. aquatica* from Thailand.

### List of freshwater *Pseudoasteromassaria* species

\**Pseudoasteromassaria aquatica* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number:* IF557909; *Facesoffungi number:* FoF09252; Fig. 48

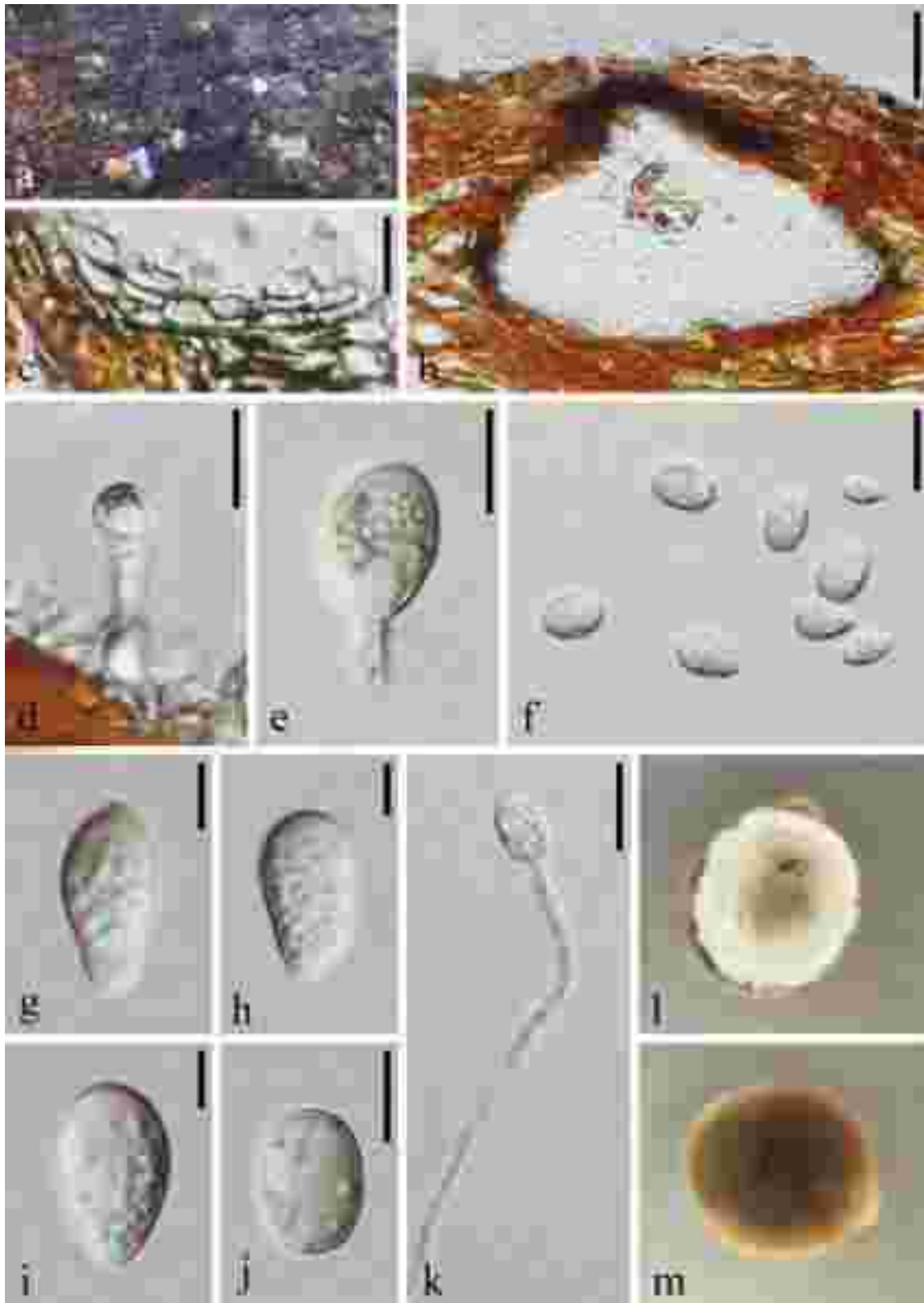
*Etymology:* in reference to aquatic habitat of the fungus  
*Holotype:* MFLU 18-1519

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph:** Undetermined. **Asexual morph:** *Pycnidia* 140–170 µm high, 210–250 µm diam., dark brown, scattered, immersed, conical, coriaceous, ostiolate. *Peridium* 15–20 µm thick, comprising 4–5 layers of brown to dark brown, irregular cells arranged in a *textura angularis*. *Conidiophores* reduced. *Conidiogenous cells* 5–8 × 2–4 µm ( $\bar{x}$  = 6.5 × 3 µm, n = 5), phialidic, determinate, discrete, cylindrical, hyaline, thin-walled. *Conidia* 12–18 × 8–13 µm ( $\bar{x}$  = 15.5 × 10.5 µm, n = 20), ellipsoidal, obovoid or irregular, aseptate, guttulate, hyaline, smooth, thin-walled, straight, lacking mucilaginous sheath.

*Culture characteristics:* On PDA, colony circular, reaching 15 mm in 50 days at 25 °C, white to grey from above, brown from below, surface rough, dry, raised, edge entire or undulate.

*Material examined:* THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat829-1 (MFLU 18-1519, **holotype**), ex-type living culture MFLUCC 18-1397; *ibid.*, hat829-2 (HKAS 105044, **isotype**), living culture KUMCC 19-0063.

*Notes:* *Pseudoasteromassaria aquatica* is morphologically similar to *P. spadicea* W. Dong et al. in having immersed pycnidia, phialidic conidiogenous cells, and hyaline, aseptate



**Fig. 48** *Pseudoasteromassaria aquatica* (MFLU 18-1519, holotype). **a** Conidiomata immersed in host. **b** Vertical section of pycnidium. **c** Structure of peridium. **d**, **e** Conidiogenous cells with conidia. **f–j**

Conidia. **k** Germinated conidium. **l**, **m** Colony on PDA (up-front, down-reverse). Scale bars: **b** = 50 μm, **c–e**, **j** = 10 μm, **f**, **k** = 20 μm, **g–i** = 5 μm

conidia (Tibpromma et al. 2017). However, *P. aquatica* has smaller pycnidia (140–170 × 210–250 µm vs. 290–320 × 460–480 µm) and slightly larger conidia (12–18 × 8–13 µm vs. 11–15 × 7–10 µm). Moreover, *P. aquatica* has ellipsoidal to obovoid conidia, while *P. spadicea* has subglobose conidia. Phylogenetic analysis supports them to be different species (Fig. 2).

\**Pseudoasteromassaria spadicea* W. Dong, H. Zhang & K.D. Hyde, Fungal Diversity 83: 59 (2017)

*Freshwater distribution:* Thailand (Tibpromma et al. 2017)

#### Key to freshwater *Pseudoasteromassaria* species

1. Pycnidia 140–170 µm high, 210–250 µm diam., conidia ellipsoidal to obovoid, 12–18 × 8–13 µm.....*P. aquatica*
1. Pycnidia 290–320 µm high, 460–480 µm diam., conidia mostly subglobose, 11–15 × 7–10 µm.....*P. spadicea*

**Lentitheciaceae** Y. Zhang et al., C.L. Schoch, J. Fourn., Crous & K.D. Hyde, Stud. Mycol. 64: 93 (2009)

#### Key to freshwater sexual genera of Lentitheciaceae

1. Ascromata with ostiolate papilla covered by short dark setae.....*Keissleriella*
1. Ascromata without setae.....2
2. Ascospores filiform.....*Poaceascoma*
2. Ascospores fusiform to cylindrical.....3
3. Ascospores surrounded by a large expanding sheath...  
.....*Tingoldiogo*
3. Ascospores without expanding sheath.....4
4. Ascospores fusiform .....*Lentithecium*
4. Ascospores cylindrical.....*Setoseptoria*

*Keissleriella* Höhn., Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 128: 582 (1919)

*Saprobic* on submerged wood in freshwater or dead wood in terrestrial habitats. **Sexual morph:** Ascromata immersed, solitary to gregarious, erumpent to nearly superficial, uniloculate, globose, dark brown to black, with ostiolate papilla, covered by dark setae or small blackened cells. *Peridium* thick, composed of pseudoparenchymatous cells and pale cells. *Pseudoparaphyses* dense, trabeculate, filiform, long, rarely septate, anastomosing and branching. *Asci* 4- or 8-spored, bitunicate, fission-tunicate, cylindro-clavate, with a furcate pedicel and a small ocular chamber. *Ascospores* medium-sized, uni- to bi-seriate, ellipsoidal to fusiform, or clavate, cylindrical, straight to slightly curved, hyaline to pale brown, or yellow, septate, sometimes with a vertical septum in central cells, constricted at the septa (Zhang et al. 2012b). **Asexual morph:** Coelomycetes, developed

in culture. *Pycnidia* erumpent becoming superficial, uniloculate, subglobose to hemispherical, glabrous, dark brown to black, with ostiolate papilla. *Mycelium* immersed. *Peridium* thin, composed of thin-walled, flattened cells. *Conidiophore* reduced. *Conidiogenous cells* holoblastic, determinate, lageniform, cylindrical to doliiform, hyaline, smooth, formed from the inner most layer of pycnidium wall. *Conidia* medium-sized, cylindrical to bone-shaped, straight to curved, hyaline to brown at aged, septate, smooth, thin-walled, without sheath (Tanaka et al. 2015).

*Type species:* *Keissleriella aesculi* (Höhn.) Höhn.

*Notes:* *Keissleriella* is an old genus comprising over 40 epithets in Index Fungorum (2020) with ca. 18 species confirmed with molecular data (Phookamsak et al. 2019). *Keissleriella* is characterized by globose ascromata, with a small black papilla and short black external setae (Zhang et al. 2012b). *Keissleriella* was shown to belong in Lentitheciaceae based on a few species (e.g. *K. cladophila* (Niessl) Corbaz and *K. linearis* E. Müll. ex Dennis) (Schoch et al. 2009; Zhang et al. 2012b), which has been accepted by other studies (Wijayawardene et al. 2017, 2018, 2020; Phookamsak et al. 2019). *Keissleriella* was regarded as congeneric with *Trichometasphaeria* because their species (*K. cladophila* (Niessl) Corbaz and *T. gloeospora* (Berk. & Curr.) L. Holm) clustered in one clade (Tanaka et al. 2015). However, Wijayawardene et al. (2017, 2020) treated them as distinct genera. Sequence data of their type species are needed to solve their phylogenetic relationships. All *Keissleriella* species clustered in a weakly supported clade, which probably indicated a polyphyletic nature.

*Keissleriella linearis* is the only species reported from freshwater habitats (Zhang et al. 2009d). It was isolated from dry culms of *Phragmites communis* in UK (Dennis 1964) and later collected from an artificial lake in France (Zhang et al. 2009d). *Keissleriella linearis* was transferred to *Lentithecium* based on phylogenetic analyses and examination of a fresh specimen (IFRD 2008) (Zhang et al. 2009d). With more sequences added, *K. linearis* was shown to be a member of *Keissleriella*, rather than *Lentithecium* (Tanaka et al. 2015; this study, Fig. 53). This result was consistent with Singtripop et al. (2015), who re-examined the specimen of *K. linearis* (IFRD 2008) and described the brown to dark brown setae covering ostiole which is the typical character of *Keissleriella*. In the phylogenetic analysis of Phookamsak et al. (2019), *K. linearis* grouped in *Lentithecium* but with low bootstrap support. We follow the results of Singtripop et al. (2015) and Tanaka et al. (2015) until more sequences are added to the family. *Keissleriella linearis* is characterized by immersed ascromata covered with a clypeus and numerous, dark brown, cylindrical bristles surrounding the ostiole, clavate asci and fusiform, hyaline, 3-septate ascospores with a thick, mucous sheath (Dennis 1964).

### List of freshwater *Keissleriella* species

\**Keissleriella linearis* E. Müll. ex Dennis, Kew Bull. 19(1): 120 (1964)

*Synonymy*: *Lentithecium lineare* (E. Müll. ex Dennis) K.D. Hyde, J. Fourn. & Ying Zhang, Fungal Diversity 38: 236 (2009)

*Freshwater distribution*: France (Zhang et al. 2009d)

*Lentithecium* K.D. Hyde, J. Fourn. & Ying Zhang, Fungal Diversity 38: 234 (2009)

*Saprobic* on submerged woody plant, grass in freshwater or dead leaf sheaths, stems, culms of grass in terrestrial habitats. **Sexual morph** *Ascomata* scattered to grouped, immersed to erumpent, uniloculate, globose to subglobose, glabrous, dark brown to black, with ostiolate papilla. *Peridium* composed of several layers of thin-walled cells. *Pseudoparaphyses* numerous, cellular, hyaline, septate, branched, persistent. *Asci* 8-spored, bitunicate, fissitunicate, clavate-cylindrical to oblong-cylindrical, broadly rounded at the apex, tapering to a short pedicel. *Ascospores* bi- or tri-seriate, fusiform, straight to slightly curved, hyaline to brown, septate, usually asymmetrical, smooth to roughed, with or without gelatinous sheath. **Asexual morph** *Conidiomata* pycnidial, solitary or aggregated, unilocular, immersed, erumpent to superficial, subglobose, ellipsoidal, dark brown to black, glabrous or surrounded by brown, septate, thick-walled hyphae, with ostiolate papilla. *Pycnidial wall* composed of several layers of dark cells of *textura angularis*, paler towards the inner layer. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, determinate, smooth-walled, hyaline, subglobose to pearshaped. *Conidia* variable in shape, hyaline, aseptate, smooth (Ryckegem 2001; Zhang et al. 2009d; Hyde et al. 2016b).

*Type species*: *Lentithecium fluviatile* (Aptroot & Van Ryck.) K.D. Hyde, J. Fourn. & Ying Zhang

*Notes*: *Lentithecium* was established to accommodate *L. aquaticum* Ying Zhang et al. and three new combinations *L. arundinaceum* (Sowerby) K.D. Hyde et al., *L. fluviatile* (type species) and *L. lineare* (E. Müll. ex Dennis) K.D. Hyde et al. (Zhang et al. 2009d). However, *L. arundinaceum* was later transferred to *Setoseptoria* based on phylogenetic analyses, although no morphological connection between *L. arundinaceum* (ascomycetes) and *S. phragmitis* Quaedv. et al. (type species, coelomycetes) was shown (Tanaka et al. 2015). *Lentithecium* was previously characterized by the lenticular *ascomata* (Zhang et al. 2009d) and later emended to globose after re-examination of the holotype of *L. fluviatile* (Hyde et al. 2013).

The freshwater species *Lentithecium clioninum* (Kaz. Tanaka et al.) Kaz. Tanaka & K. Hiray. and *L. pseudoclioninum* Kaz. Tanaka & K. Hiray. formed a well-supported clade with the type species, *L. fluviatile* (Fig. 53). *Lentithecium cangshanense* Z.L. Luo et al., *L. carbonneanum* J. Fourn.

et al., *L. unicellulare* Abdel-Aziz and a new species *L. kunmingense* clustered in a separated clade with low bootstrap support (Fig. 53), and close to the genus *Halobyssothecium* Dayarathne et al. *Lentithecium cangshanense*, collected from submerged wood, is probably conspecific with the marine species *L. voraginesporum* Abdel-Wahab et al., because of their nearly identical sequences (one nucleotide difference in LSU and SSU sequence data, respectively). Unfortunately, their ITS sequence data are lacking. Hyde et al. (2016b) distinguished them based on the size of *ascomata*, *asci* and *ascospores* which are considered as tiny differences in this study. More sequenced collections are needed in future.

*Lentithecium aquaticum* Ying Zhang et al., which was collected from submerged wood in France, clustered in *Lentithecium* (Zhang et al. 2009d). With more sequenced species added in the family, the placement of *L. aquaticum* appeared to be unstable and had a weak relationship with *Lentithecium* clade (Tanaka et al. 2015; Hyde et al. 2016b; Su et al. 2016a). The most recent publications showed it was phylogenetically distant from *Lentithecium* and clustered with the members of *Setoseptoria*, but with low bootstrap support (Wanasinghe et al. 2018b; Phookamsak et al. 2019). Our phylogenetic analysis show *L. aquaticum* forms a basal clade to *Darksidea*, *Halobyssothecium* and *Lentithecium* (Fig. 53). We exclude *L. aquaticum* from *Lentithecium* but more phylogenetic evidence is required.

*Lentithecium unicellulare* Abdel-Aziz, which was collected from submerged wood in the River Nile in Egypt, is the first asexual species in the genus (Hyde et al. 2016b). We report the second asexual species *L. kunmingense*, which was collected from China.

### List of freshwater *Lentithecium* species

\**Lentithecium cangshanense* Z.L. Luo, X.J. Su & K.D. Hyde, Phytotaxa 267: 65 (2016)

*Freshwater distribution*: China (Su et al. 2016a)

\**Lentithecium carbonneanum* J. Fourn., Raja & Oberlies, Persoonia 40: 295 (2018)

*Freshwater distribution*: France (Crous et al. 2018b)

\**Lentithecium clioninum* (Kaz. Tanaka et al.) Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 99 (2015); Fig. 49a–f

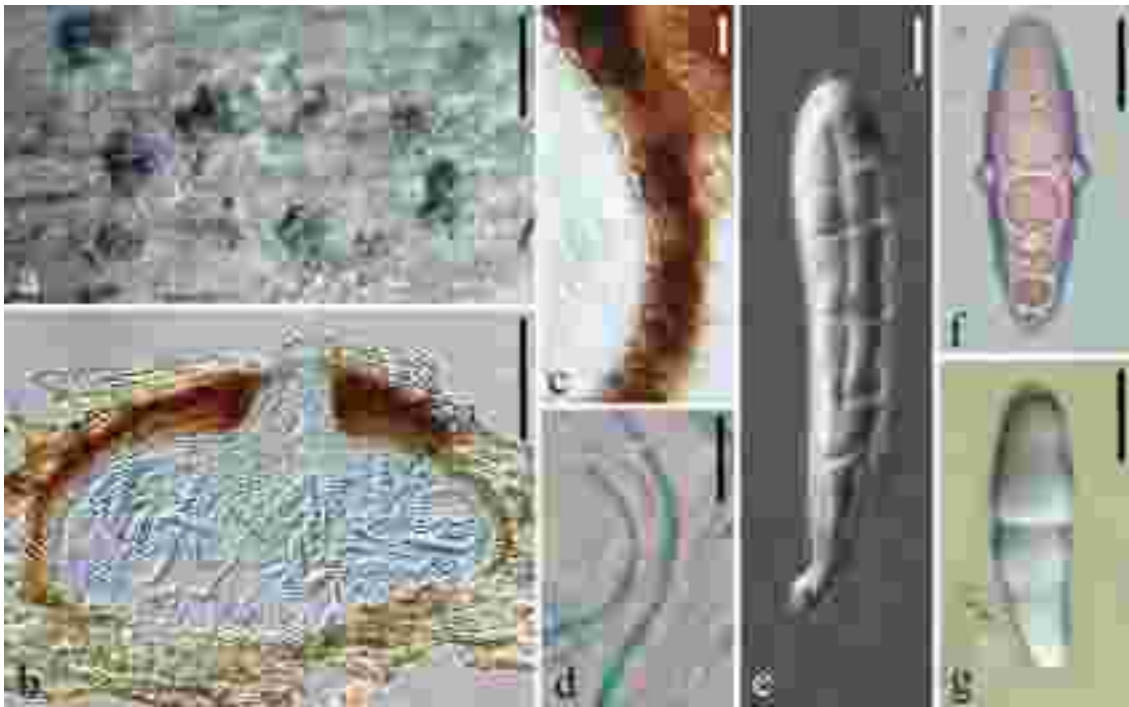
*Basionym*: *Massarina clionina* Kaz. Tanaka et al., Mycoscience 46(5): 288 (2005)

*Freshwater distribution*: Japan (Tanaka et al. 2005b)

\**Lentithecium kunmingense* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number*: IF557910; *Facesoffungi number*: FoF09253; Fig. 50





**Fig. 49** *Lentithecium* spp. (Material examined: JAPAN, Hokkaido, Akkeshi, Bekanbeushi-river, on submerged twigs of woody plant, 2 June 2003, K. Tanaka & S. Hatakeyama, KT 1149A, **holotype**; Hokkaido, Akkeshi, Toraihetsu-river, on submerged twigs of woody plant, 3 June 2003, K. Tanaka & S. Hatakeyama, KT 1220, **paratype**; Aomori, Hirosaki, Aoki, Mohei pond, on submerged twigs of woody

plant, 3 May 2003, K. Tanaka & N. Asama, KT 1113, **holotype**). **a–f** *L. clioninum* (**a–d** from KT 1149A, **holotype**; **e** from KT 1220, **paratype**; **f** from culture of KT 1149A). **a** Ascomata on the host surface. **b** Section of ascoma. **c** Peridium. **d** Pseudoparaphyses. **e** Ascus. **f** Ascospore. **g** Ascospore of *L. pseudoclioninum* (KT 1113, **holotype**). Scale bars: **a** = 500 μm, **b** = 100 μm, **c–g** = 10 μm

**Etymology:** referring to Kunming, where the holotype was collected

**Holotype:** HKAS 102150

**Saprobic** on decaying wood submerged in freshwater.

**Sexual morph:** Undetermined. **Asexual morph:** *Pycnidia* 210–250 μm high, 320–350 μm diam., scattered or solitary, unilocular, immersed to erumpent, ellipsoidal, dark brown to black, glabrous, with ostiolate papilla. *Peridium* 60–80 μm thick, composed of several layers of brown to black brown cells of *textura angularis*, hyaline to pale brown towards the inner layer which bearing conidiogenous cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5–19 × 2–5 μm ( $\bar{x}$  = 11.5 × 3.5 μm, n = 10), phialidic, determinate, smooth-walled, hyaline, subglobose to ellipsoidal. *Conidia* 8–14 × 5–8 μm ( $\bar{x}$  = 10.5 × 7 μm, n = 55), subglobose, ovate, ellipsoid, clavate, pyriform or irregular, with rounded or truncate base, hyaline, aseptate, several small to one big guttulate, smooth-walled.

**Culture characteristics:** On PDA, colony filamentous, reaching 45 mm in 25 days at 25 °C, black from above and below, surface dry, with sparse aerial mycelium, mostly immersed in culture, with a filiform edge.

**Material examined:** CHINA, Yunnan Province, Kunming University of Science and Technology, on submerged wood

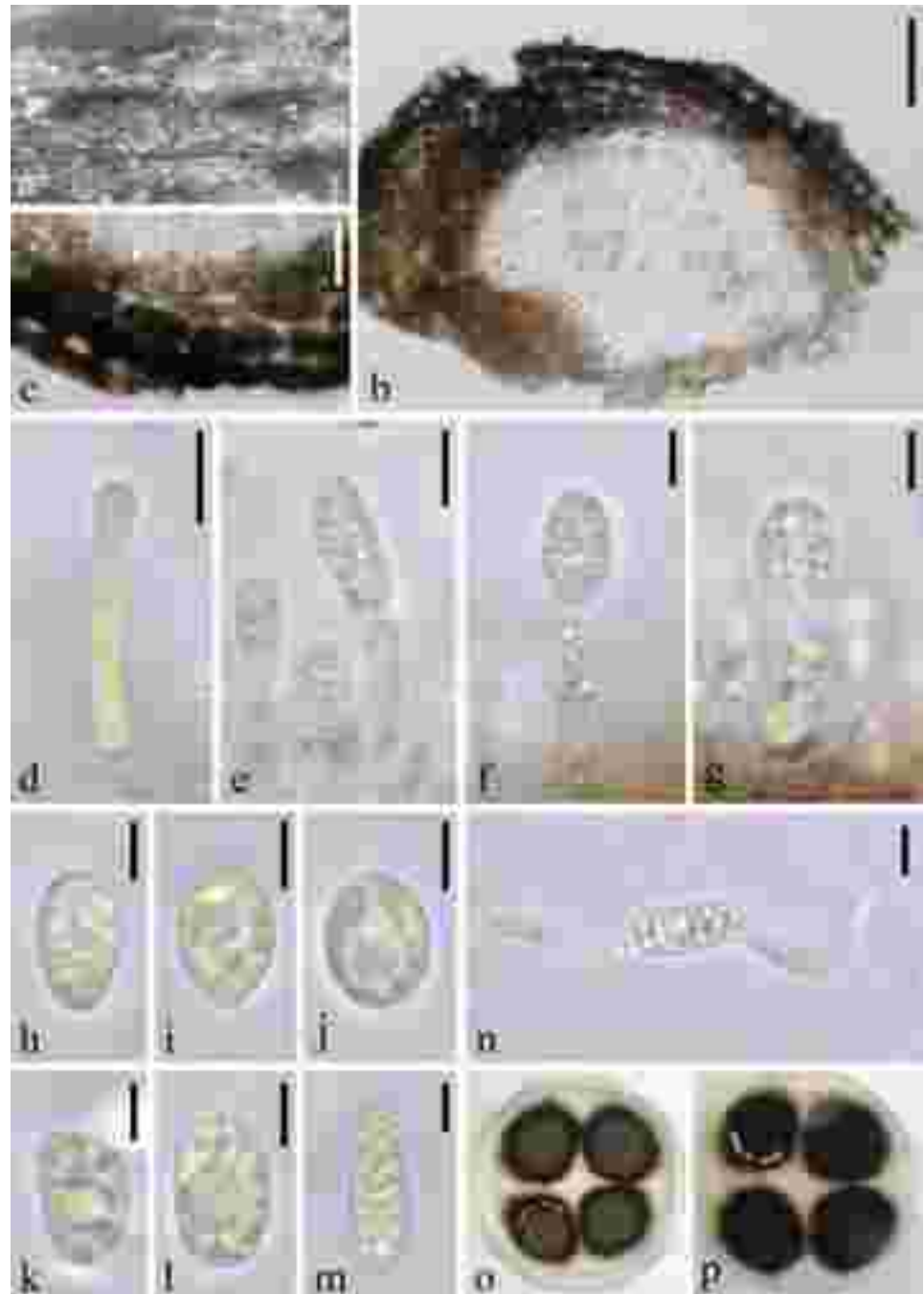
in a stream, 10 May 2017, C.X. Liu, L54 (HKAS 102150, **holotype**), ex-type living culture KUMCC 19-0101.

**Notes:** *Lentithecium kunmingense* clusters with *L. voragine-sporum* without bootstrap support (Fig. 53). *Lentithecium kunmingense* is a coelomycetous species, while *L. voragine-sporum* is an ascomycetous species (Hyde et al. 2016b). *Lentithecium voragine-sporum* only has LSU and SSU sequence data in GenBank, and there are thirteen and four nucleotide differences in LSU and SSU sequence data between *L. voragine-sporum* (CBS H-22560) and *L. kunmingense* (KUMCC 19-0101), respectively, which indicate them to be different species (Jeewon and Hyde 2016).

Only one asexual species *Lentithecium unicellulare* has been reported in *Lentithecium* (Hyde et al. 2016b). *Lentithecium kunmingense* is similar to *L. unicellulare* in having dark pycnidia, determinate, hyaline conidiogenous cells and variable, hyaline, aseptate conidia. However, *L. kunmingense* differs from *L. unicellulare* in having glabrous pycnidia and larger conidia (8–14 × 5–8 μm vs. 6–9 × 4–5 μm) (Hyde et al. 2016b). Phylogenetic analysis supports them to be different species (Fig. 53).

\**Lentithecium pseudoclioninum* Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 99 (2015); Fig. 49g

**Fig. 50** *Lentithecium kunmingense* (HKAS 102150, holotype). **a** Conidiomata immersed in host. **b** Vertical section of pycnidium. **c** Structure of peridium. **d–g** Conidiogenous cells with conidia. **h–m** Conidia. **n** Germinated conidium. **o, p** Colony on PDA (left-front, right-reverse). Scale bars: **b** = 50  $\mu$ m, **c** = 20  $\mu$ m, **d–n** = 5  $\mu$ m



*Freshwater distribution*: Japan (Tanaka et al. 2015)

\**Lentithecium unicellulare* Abdel-Aziz, Fungal Diversity 80: 53 (2016)

*Freshwater distribution*: Egypt (Hyde et al. 2016b)

#### Key to freshwater *Lentithecium* species

- |                       |   |  |                           |
|-----------------------|---|--|---------------------------|
| 1. Asexual morph..... | 2 | 2. Conidia 6–9 $\times$ 4–5 $\mu$ m.....                 | <i>L. unicellulare</i>    |
| 1. Sexual morph.....  | 3 | 2. Conidia 8–14 $\times$ 5–8 $\mu$ m.....                | <i>L. kunmingense</i>     |
|                       |   | 3. Ascospores hyaline.....                               | 4                         |
|                       |   | 3. Ascospores pigmented.....                             | 5                         |
|                       |   | 4. Ascospores surrounded by a wing-like sheath.....      |                           |
|                       |   | .....  | <i>L. clioninum</i>       |
|                       |   | 4. Ascospores lacking wing-like sheath.....              |                           |
|                       |   | .....  | <i>L. pseudoclioninum</i> |
|                       |   | 5. Asci > 100 $\mu$ m long, ascospores rough-walled..... |                           |
|                       |   | .....  | <i>L. carbonneanum</i>    |

5. Asci < 100 µm long, ascospores smooth-walled.....  
 .....*L. cangshanense*

*Poaceascoma* Phookamsak & K.D. Hyde, Cryptogamie, Mycologie 36: 231 (2015)

*Saprobic* on submerged bamboo, wood in freshwater or intertidal, dead stem in terrestrial habitats. **Sexual morph:** *Ascomata* solitary to gregarious, semi-immersed to erumpent, uniloculate, globose to subglobose, with or without setose, black, with a central ostiole, with short to long papilla. *Peridium* thick walled, of equal thickness, composed of several layers of dark brown to black, pseudoparenchymatous cells. *Pseudoparaphyses* dense, cellular, hyaline, septate, persistent, anastomosing above the asci, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, elongate-cylindrical, short pedicellate, apically rounded with an ocular chamber. *Ascospores* fasciculate, spirally arranged within the ascus, filiform, curved, hyaline, multi-septate, smooth, thin-walled, without sheath or appendage, ascospores often longer than asci (Phookamsak et al. 2015a). **Asexual morph:** Undetermined.

*Type species:* *Poaceascoma helicoides* Phook. & K.D. Hyde

*Notes:* *Poaceascoma* was introduced to accommodate ophiosphaerella-like species associated with Poaceae, which form semi-immersed to erumpent, setose ascomata with short to long necks and filiform ascospores spirally arranged in asci (Phookamsak et al. 2015a). *Poaceascoma* is a well-studied genus with sequence data available for all species in GenBank; they formed a monophyletic clade in the family with high bootstrap support (Phookamsak et al. 2015a; Luo et al. 2016a; Hyde et al. 2017, 2018a). Some genera also form filiform ascospores in Dothideomycetes, such as *Acanthophiobolus*, *Leptospora*, *Ophiobolus* and *Ophiosphaerella* (Shoemaker 1976; Boonmee et al. 2011; Zhang et al. 2012b; Boonmee et al. 2014). *Acanthophiobolus* differs from these genera in having rather smaller, superficial, apapillate ascomata (Boonmee et al. 2011, 2014). *Leptospora* is distinct in having possesses glabrous ascomata which often stain host tissues red or purple (Hyde et al. 2016b). *Ophiobolus* and *Ophiosphaerella* nested in Phaeosphaeriaceae (Phookamsak et al. 2014), which was phylogenetically distant from *Poaceascoma* (Lentitheciaceae).

*Poaceascoma aquaticum* Z.L. Luo & K.D. Hyde was collected from submerged bamboo in Thailand (Luo et al. 2016a). It was described as globose to subglobose ascomata without setae, cylindrical to cylindrical-clavate asci and pale brown to brown ascospores (Luo et al. 2016a). However, the ascospores were obviously hyaline in the illustration of Luo et al. (2016b).

#### List of freshwater *Poaceascoma* species

\**Poaceascoma aquaticum* Z.L. Luo & K.D. Hyde, Phytotaxa 253: 75 (2016)

*Freshwater distribution:* Thailand (Luo et al. 2016a)

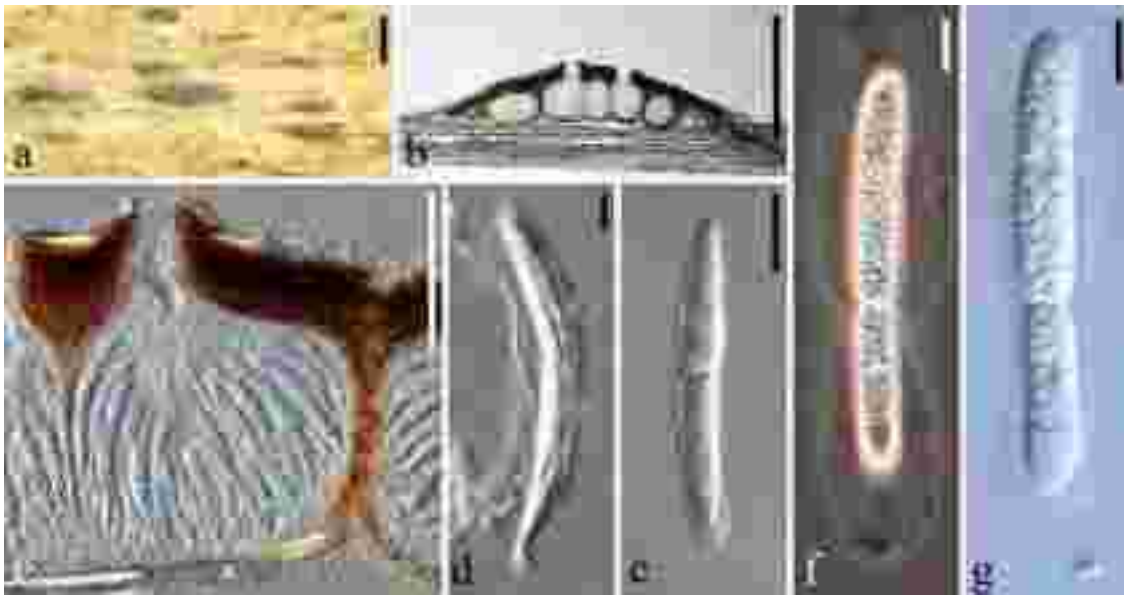
*Setoseptoria* Quaedvli., Verkley & Crous, Stud. Mycol. 75: 382 (2013)

*Saprobic* on decaying leaves or wood/branch in terrestrial or submerged wood in freshwater habitats. **Sexual morph:** *Ascomata* erumpent to superficial, scattered to gregarious, sometimes scattered beneath the host periderm or on decorticated wood, subglobose to hemispherical with flattened base, glabrous, sometimes with sparse brown hyphae at sides, dark brown to black, with a central ostiole, with or without short papilla. *Peridium* thin, composed of several layers of thin-walled cells. *Pseudoparaphyses* numerous, cellular, hyaline, septate, branched, persistent, anastomosing between and above the asci. *Asci* 8-spored, bitunicate, fissitunicate, clavate to cylindrical-clavate, or narrowly ovoid, pedicellate, rounded at the apex, with an indistinct or minute ocular chamber. *Ascospores* uni- to tri-seriate, cylindrical to narrowly fusiform, mostly hyaline, sometimes hyaline to pale olivaceous, septate, deeply constricted at the primary septum, mostly asymmetrical, smooth, thin-walled, surrounded by a gelatinous sheath (Tanaka et al. 2004; Hyde et al. 2017; Wanasinghe et al. 2018b). **Asexual morph:** Coelomycetous. *Pycnidia* immersed, uniloculate, globose, with brown, verruculose to warty setae, brown, ostiolate, somewhat papillate. *Peridium* composed of several layers of brown, angular cells which inwardly become hyaline. *Conidiophores* reduced to conidiogenous cells or with one supporting cell. *Conidiogenous cells* subcylindrical to doliiform, hyaline, smooth, apical region with several inconspicuous percurrent proliferations, or with periclinal thickening; collarette inconspicuous, or prominent, flared. *Conidia* subcylindrical, straight to somewhat curved, hyaline, smooth, transversely septate, thin-walled, without appendages or sheath (Quaedvlieg et al. 2013).

*Type species:* *Setoseptoria phragmitis* Quaedvli., Verkley & Crous

*Notes:* *Setoseptoria* was introduced for a coelomycetous species *S. phragmitis* which forms setose pycnidia and subcylindrical, (1–)3-septate, hyaline conidia that become olivaceous and verruculose in older cultures (Quaedvlieg et al. 2013). Another six ascomycetous species were added to the genus, but the asexual morphs were not produced in culture. These species forms a well-supported clade in our phylogenetic tree (Fig. 53), but lack strong bootstrap support between each other (Hyde et al. 2017; Wanasinghe et al. 2018b). Tanaka et al. (2015) mentioned that the species producing both sexual and asexual morphs were needed to confirm the validity of this generic treatment.

*Setoseptoria arundinacea* (Sowerby) Kaz. Tanaka & K. Hiray., a common species occurring on *Phragmites* culms,



**Fig. 51** *Setoseptoria* spp. (Material examined: JAPAN, Aomori, Hirosaki, Kadoke, Oowasawa-river, on dead culms of *Phragmites australis*, 29 July 2001, K. Tanaka, KT 600 = HHUF 27544; Hokkaido, Akkeshi, Ariake, small stream, on submerged stems of herbaceous plant, 3 June 2003, K. Tanaka & S. Hatakeyama, KT 1174

= HHUF 28293, **holotype**). **a–e** *S. arundinacea* (KT 600 = HHUF 27544). **a** Ascomata on the host surface. **b, c** Section of ascomata. **d** Ascus. **e** Ascospore. **f, g** Ascospores of *S. magniarundinacea* (culture of KT 1174 = CBS 139702). Scale bars: **a** = 1 mm, **b** = 500 μm, **c** = 100 μm, **d–g** = 10 μm

was also reported from freshwater habitats (Aptroot 1998; Tanaka and Harada 2003c; Luo et al. 2004; Zhang et al. 2009d). *Setoseptoria arundinacea* may consist of several cryptic species because two strains, KT 552 and KT 600 (Tanaka et al. 2015), did not form a clade with another two strains CBS 619.86 (Schoch et al. 2009) and CBS 123131 (Zhang et al. 2009c), which showed in Tanaka et al. (2015), Hyde et al. (2017), Wanasinghe et al. (2018b) and our study (Fig. 53). The epitype of this species is expected to solve this *S. arundinacea* complex. The second freshwater species *S. magniarundinacea* (Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray. has larger ascospores than *S. arundinacea* (Tanaka et al. 2004) (see key below).

#### List of freshwater *Setoseptoria* species

\**Setoseptoria arundinacea* (Sowerby) Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 101 (2015); Fig. 51a–e

*Basionym*: *Sphaeria arundinacea* Sowerby, Col. fig. Engl. Fung. Mushr. (London) 3: tab. 336 (1803)

*Synonymy*: Possible synonyms see Index Fungorum (2020)

*Freshwater distribution*: China (Luo et al. 2004), Denmark (Zhang et al. 2009d)

\**Setoseptoria magniarundinacea* (Kaz. Tanaka & Y. Harada) Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 102 (2015); Fig. 51f, g

*Basionym*: *Massarina magniarundinacea* Kaz. Tanaka & Y. Harada, Mycotaxon 90(2): 349 (2004)

*Freshwater distribution*: Japan (Tanaka and Harada 2004)

#### Key to freshwater *Setoseptoria* species

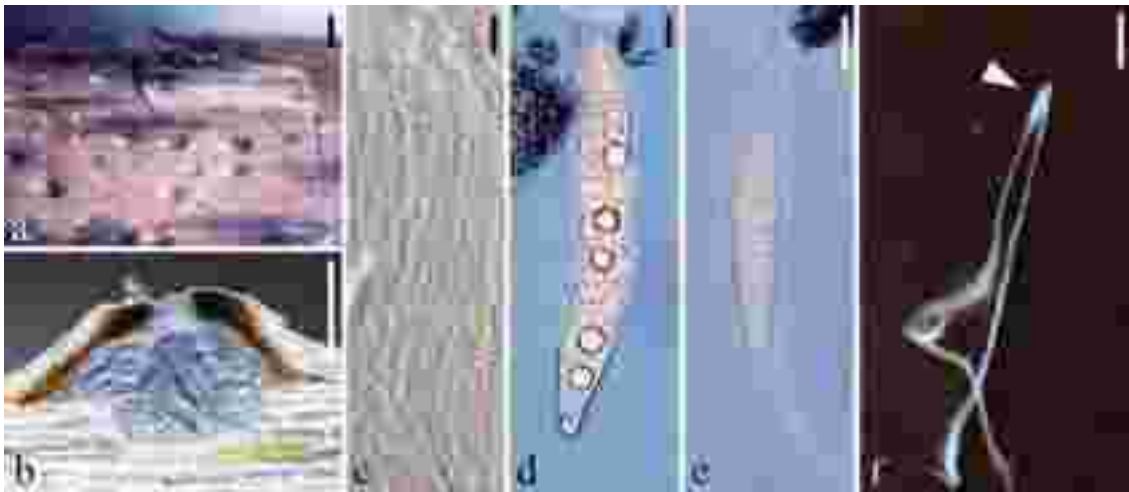
1. Ascospores  $23\text{--}40 \times 3.5\text{--}6 \mu\text{m}$ .....*S. arundinacea*
1. Ascospores  $67\text{--}82 \times 6.5\text{--}9 \mu\text{m}$ ...*S. magniarundinacea*

*Tingoldiagio* K. Hiray. & Kaz. Tanaka, Mycologia 102(3): 740 (2010)

*Saprobic* on submerged culms. **Sexual morph**: *Ascomata* scattered, immersed to erumpent, uniloculate, depressed globose to conical, with a flattened base, glabrous, black, with a central, rounded ostiole. *Peridium* composed of hyaline to brown, small cells. *Pseudoparaphyses* numerous, cellular, hyaline, septate, immersed in gel matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindro-clavate, rounded at the apex, with or without a shallow chamber. *Ascospores* bi- to triseriate, clavate, straight, asymmetrical, the upper cell often broader than the lower one, smooth, thin-walled, usually surrounded by a large, expanding gelatinous sheath (Hirayama et al. 2010; Xu et al. 2020). **Asexual morph**: Undetermined.

*Type species*: *Tingoldiagio graminicola* K. Hiray. & Kaz. Tanaka

*Notes*: *Tingoldiagio* resembles *Lindgomyces* in ascospore characters and aquatic habitats (Hirayama et al. 2010).



**Fig. 52** *Tingoldiagio graminicola* (Material examined: JAPAN, Aomori, Hirosaki, Kadoke, Oowasawa River, on submerged culms of *Phragmites japonica*, 28 September 2002, KT 891, **paratype**). **a, b, d, e, g** from KT 891, **paratype**. **c, f** from culture of KT 891. **a** Asc-

mata on the host surface. **b** Section of ascoma. **c** Pseudoparaphyses. **d** Ascus. **e** Ascospore. **f** Ascospore with an elongate gelatinous sheath in Indian Ink. Scale bars: **a** = 500  $\mu$ m, **b** = 100  $\mu$ m, **c–e** = 10  $\mu$ m, **f** = 50  $\mu$ m

However, *Tingoldiagio* differs by its depressed globose to mammiform ascomata with a wedge of palisade-like cells at the rim and graminicolous substrate. *Tingoldiagio* was treated as a synonym of *Lentithecium* due to the lenticular, immersed to erumpent ascomata, cylindro-clavate asci and hyaline, 1-septate ascospores with sheath (Zhang et al. 2012b). Later, its generic placement was supported by molecular studies (Tanaka et al. 2015; Hyde et al. 2016b; Wanasinghe et al. 2018b).

Freshwater species *Tingoldiagio graminicola* (type) has clavate, hyaline, 1-septate ascospores when young, becoming pale brown, 3-septate with age and with a fusiform gelatinous sheath which expands to form a long appendage in water (Hirayama et al. 2010). Other two freshwater species, *T. clavata* D.F. Bao et al. and *T. hydei* D.F. Bao et al., have several long or short, equatorial appendages, but lack the expanding sheath (Xu et al. 2020).

#### List of freshwater *Tingoldiagio* species

\**Tingoldiagio clavata* D.F. Bao, L. Xu & H.Y. Su, MycoKeys 65: 128 (2020)

*Freshwater distribution*: Thailand (Xu et al. 2020)

\**Tingoldiagio hydei* D.F. Bao, Z.L. Luo & H.Y. Su, MycoKeys 65: 126 (2020)

*Freshwater distribution*: Thailand (Xu et al. 2020)

\**Tingoldiagio graminicola* K. Hiray. & Kaz. Tanaka, Mycologia 102(3): 740 (2010); Fig. 52

*Freshwater distribution*: Japan (Hirayama et al. 2010)

#### Key to freshwater *Tingoldiagio* species

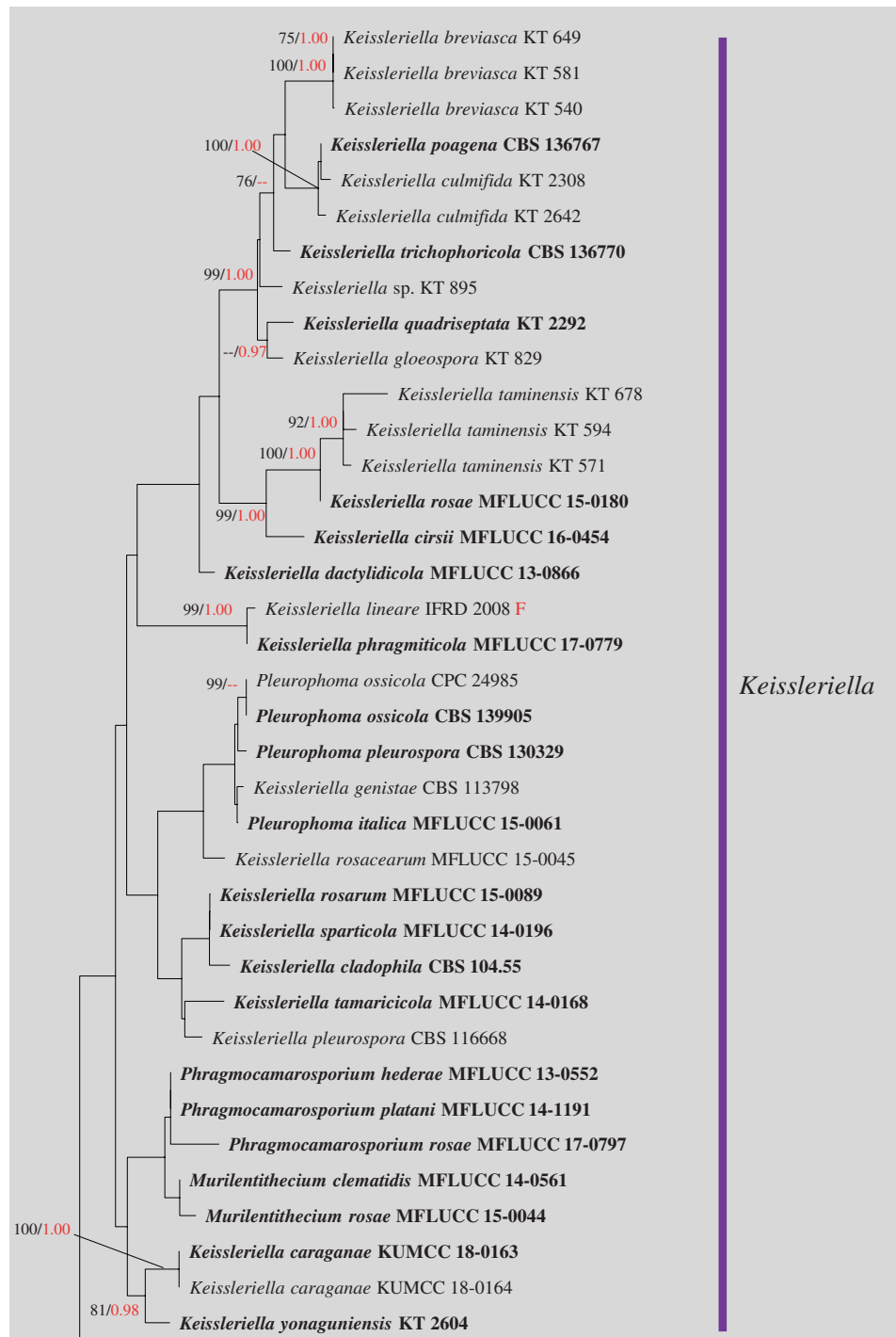
1. Ascospores without appendages at the septum.....*T. graminicola*
1. Ascospores with appendages at the septum.....2
2. Ascospores 48–51  $\times$  7.5–9  $\mu$ m.....*T. clavata*
2. Ascospores 37.5–42  $\times$  7.5–9  $\mu$ m.....*T. hydei*

**Lindgomycetaceae** K. Hiray., Kaz. Tanaka & Shearer, Mycologia 102(3): 733 (2010)

#### Key to freshwater sexual genera of Lindgomycetaceae

1. Ascromata with a clypeus.....*Neolindgomyces*
1. Ascromata without clypeus.....2
2. Ascospores < 3-septate.....3
2. Ascospores > 3-septate.....4
3. Ascospores hyaline to pale brown, 1(–2)-septate, or only brown and > 3-septate in old ascospores...*Lindgomyces*
3. Ascospores hyaline, 1-septate.....*Hongkongmyces*
4. Ascospores hyaline to golden-pale brown.....*Arundellina*
4. Ascospores brown to dark brown.....5
5. Ascospores fusiform to clavate, transversely septate, occasionally with one longitudinal or oblique septa, asci 4- to 8-spored.....*Lolia*
5. Ascospores fusiform or vermiform, transversely septate, without longitudinal or oblique septa, asci 8-spored.....*Aquimassariosphaeria*

**Fig. 53** Phylogram generated from maximum likelihood analysis of combined LSU, SSU, ITS and TEF sequence data for species of Lentitheciaceae. Bootstrap values for maximum likelihood equal to or greater than 75% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. The tree is rooted to *Massarina cisti* CBS 266.62, *M. eburnea* CBS 473.64 and *M. eburnea* H 3953 (Massariaceae)

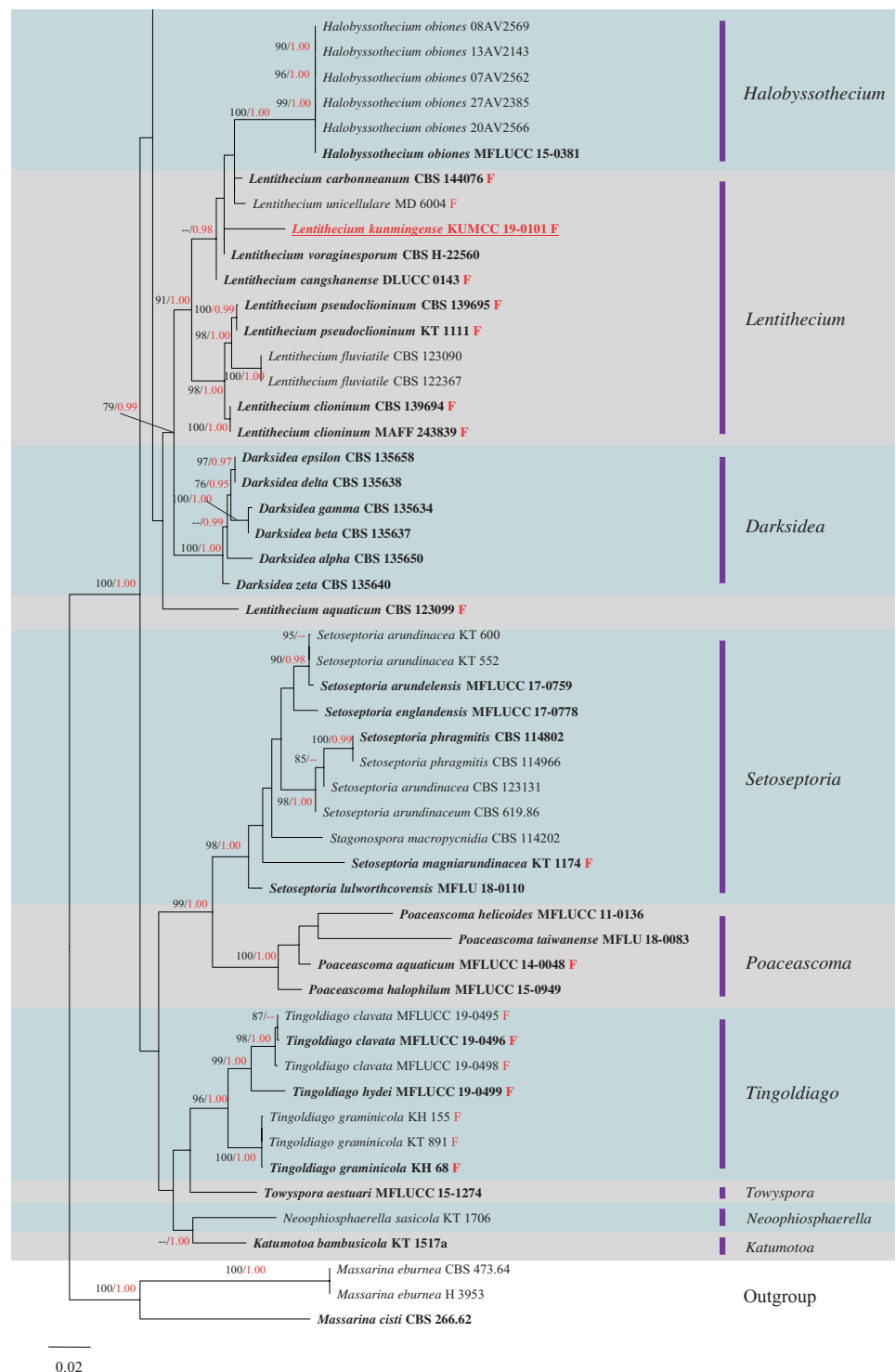


*Arundellina* Wanas., E.B.G. Jones & K.D. Hyde, Fungal Diversity 80: 59 (2016)

*Saprobic* on dead submerged stem. **Sexual morph:** *Ascomata* solitary to scattered, immersed, uniloculate, globose, glabrous, dark brown to black, coriaceous, with ostiolate papilla. *Peridium* 4–5 layered, outer layer heavily pigmented, comprising reddish to dark brown, thick-walled cells of *textura angularis*, inner layer comprising hyaline,

thin-walled cells of *textura angularis*. *Pseudoparaphyses* numerous, cellular, septate, branched. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindrical-clavate, pedicellate, with an ocular chamber. *Ascospores* overlapping uni- to bi-seriate, fusiform, hyaline to golden-pale brown, transversely septate, with pointed ends (Hyde et al. 2016b). **Asexual morph:** Undetermined.

Fig. 53 (continued)

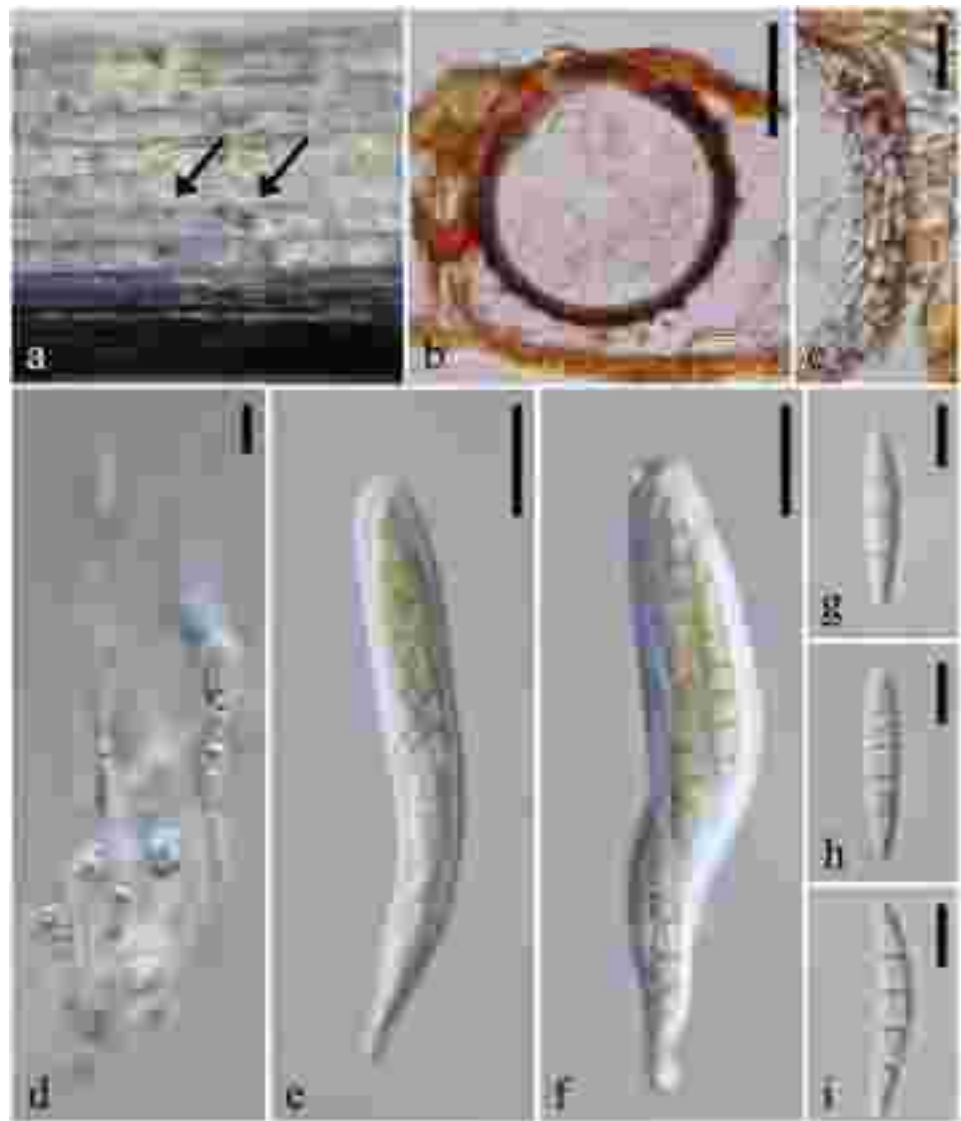


*Type species: Arundellina typhae* Wanas., E.B.G. Jones & K.D. Hyde

*Notes: Arundellina* is a monotypic genus introduced for *A. typhae*, which was isolated from submerged stems

of *Typha* sp. (Typhaceae) in Arun River in UK (Hyde et al. 2016b). The combined LSU and SSU sequence data placed it in Lindgomycetaceae (Hyde et al. 2016b), which is confirmed in this study (Fig. 2). The globose ascomata,

**Fig. 54** *Arundellina typhae* (Material examined: UK, England, Arun River, on dead submerged stem of *Typha* sp. (Typhaceae), 6 April 2015, E.B.G. Jones, GJ122, MFLU 16-1276, **holotype**). **a** Appearance of immersed ascomata on host substrate (arrows). **b** Section of ascoma. **c** Peridium. **d** Pseudoparaphyses. **e, f** Asci. **g–i** Ascospores. Scale bars: **b** = 100  $\mu$ m, **c, e, f** = 20  $\mu$ m, **d** = 5  $\mu$ m, **g–i** = 10  $\mu$ m



cylindric-clavate asci with thick-walled apex, fusiform, 3–4 transversely septate, golden-pale brown ascospores with pointed ends of *A. typhae* are unique in Lindgomycetaceae (Fig. 53).

#### List of freshwater *Arundellina* species

\**Arundellina typhae* Wanas., E.B.G. Jones & K.D. Hyde, Fungal Diversity 80: 61 (2016); Fig. 54

*Freshwater distribution*: UK (Hyde et al. 2016b)

*Hongkongmyces* C.C.C. Tsang et al., Medical Mycol. 52(7): 740 (2014)

*Parasitic* on biopsy tissues of an infected foot of a patient or saprobic on submerged wood. **Sexual morph**: *Ascomata* immersed to semi-immersed, scattered or gregarious, subglobose, coriaceous, dark brown to black, with papilla. *Peridium* composed of several layers of dark brown cells of *textura epidermoidea* or *textura angularis*.

*Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, septate, branched. *Asci* 8-spored, bitunicate, fisitunicate, cylindric-clavate, pedicellate. *Ascospores* overlapping biseriate, broad-fusiform, sometimes tapering towards the ends, hyaline, septate, surrounded with mucilaginous sheath (Hyde et al. 2017). **Asexual morph**: Coelomycetous. *Conidiomata* pycnidial, globose to ampulliform or ellipsoidal, dark brown to black, with a central ostiole to multiple ostioles. *Peridium* two-layered, or sometimes poorly developed and thinner at the base. *Conidiophores* reduced. *Conidiogenous cells* phialidic, sometimes with sympodial proliferations, discrete, subulate to ampulliform or subcylindrical, hyaline, smooth. *Conidia* white in mass, hyaline, solitary, ellipsoid to obovoid, globose, subglobose, lacking mucilaginous sheath (Crous et al. 2018b).

*Type species*: *Hongkongmyces pedis* C.C.C. Tsang et al.

*Notes*: *Hongkongmyces pedis* was isolated from biopsy tissues of an infected foot of a patient and it did not produce



fruiting bodies or conidia in culture (Tsang et al. 2014). Later, an asexual morph *H. snookiorum* Raudabaugh et al. was isolated from submerged wood (Crous et al. 2018b). In our phylogenetic analysis (Fig. 2), the sexual species *H. thailandicus* Phukhams. & K.D. Hyde and the new species *H. aquaticus* forms a weakly-supported clade, separated from the type species, *H. pedis*. In previous studies, *H. thailandicus* also had a weak relationship with *H. pedis* (Hyde et al. 2017; Crous et al. 2018b). This group needs further study with more collections.

#### List of freshwater *Hongkongmyces* species

\**Hongkongmyces aquaticus* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number*: IF557911; *Facesoffungi number*: FoF09254; Fig. 55

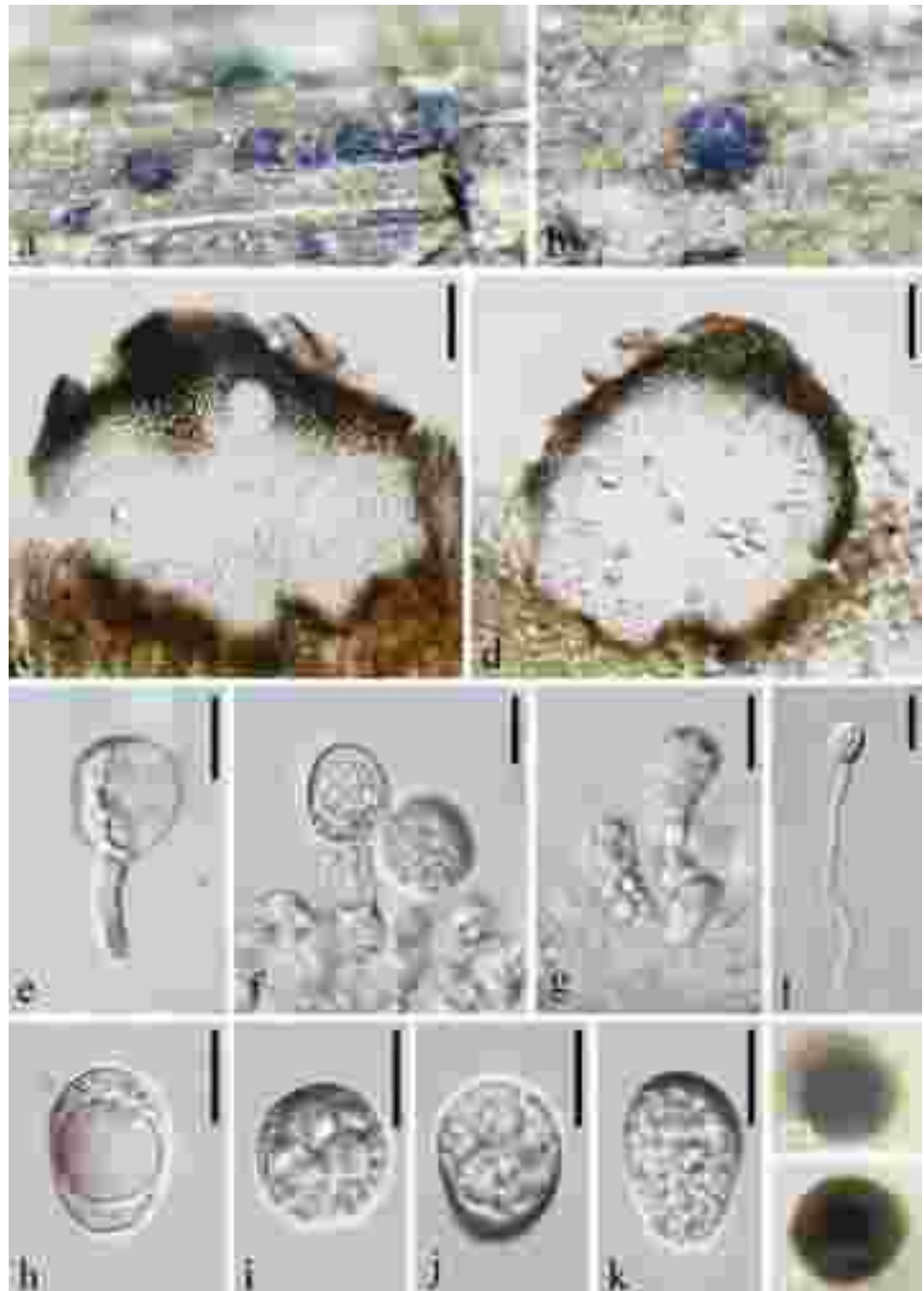
*Etymology*: in reference to aquatic habitat of this fungus

*Holotype*: MFLU 18-1516

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: Undetermined. **Asexual morph**: *Pycnidia* 270–320 µm high, 300–350 µm diam., dark brown to black,

**Fig. 55** *Hongkongmyces aquaticus* (MFLU 18-1516, **holotype**). **a, b** Appearance of black conidiomata on host. **c, d** Vertical section of pycnidia. **e–g** Conidiogenous cells with conidia. **h–k** Conidia. **l** Germinated conidium. **m, n** Colony on PDA (up-front, down-reverse). Scale bars: **c, d** = 50 µm, **e–k** = 10 µm, **l** = 20 µm



scattered, semi-immersed or erumpent to host surface, globose or ellipsoidal, coriaceous, ostiolate. *Peridium* 50–70 µm thick at the sides, poorly developed and thinner at the base, 30–40 µm thick, composed of dark brown, large, compressed cells of *textura angularis*. *Conidiophores* reduced. *Conidiogenous cells* 9–12 × 3–7 µm ( $\bar{x}$  = 10.5 × 5 µm, n = 5), phialidic, determinate, cylindrical to subcylindrical, hyaline, thin-walled. *Conidia* 14–19 × 11–15 µm ( $\bar{x}$  = 16.5 × 12.5 µm, n = 20), globose, subglobose or obovoid, aseptate, guttulate, hyaline, smooth, thin-walled, straight, lacking mucilaginous sheath.

**Culture characteristics:** On PDA, colony circular, reaching 15 mm in 10 days at 25 °C, grey to brown from above, dark brown from below, surface rough, dry, raised, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, 20180525-1 (MFLU 18-1516, **holotype**), ex-type living culture MFLUCC 18-1150; *ibid.*, 20180525-2 (HKAS 105003, **isotype**), ex-type living culture KUMCC 19-0019.

**Notes:** *Hongkongmyces aquaticus* resembles *H. snookiorum* in having phialidic conidiogenous cells and hyaline, variable shaped conidia, but it lacks sympodial proliferations (Crous et al. 2018b). Additionally, *H. aquaticus* has larger conidia (14–19 × 11–15 µm vs. 4.5–5.5 × 3.5–4 µm). Both species were collected from freshwater habitats, but from different substrates (submerged wood vs. submerged detritus). Phylogenetic analysis supports them to be different species (Fig. 2).

\**Hongkongmyces snookiorum* Raudabaugh, Iturr. & A.N. Mill., *Persoonia* 40: 289 (2018)

**Freshwater distribution:** USA (Crous et al. 2018b)

#### Key to freshwater *Hongkongmyces* species

1. Conidia 4.5–5.5 × 3.5–4 µm.....*H. snookiorum*
1. Conidia 14–19 × 11–15 µm.....*H. aquaticus*

#### *Aquimassariosphaeria* W. Dong & Doilom, **gen. nov.**

**Index Fungorum number:** IF557825; **Facesoffungi number:** FoF08733

**Etymology:** referring to type species was collected from aquatic habitat and its morphological similarity to *Massariosphaeria*

**Saprobic** on submerged wood or dry branches. **Sexual morph:** *Ascomata* scattered or solitary, immersed, erumpent to superficial, subglobose to ellipsoidal, black, coriaceous, with ostiolate papilla, sometimes stain substrate purple. *Peridium* two-layered, outer layer comprising several layers of dark brown to black, thin-walled, compressed cells, inner layer comprising several layers of pale brown to hyaline, thin-walled, large cells of *textura angularis*. *Pseudoparaphyses* numerous, cellular, hyaline, septate, embedded in a

matrix. *Asci* 8-spored, bitunicate, narrowly clavate, short pedicellate, apically rounded. *Ascospores* bi- to tri-seriate, narrowly fusiform or vermiform, straight or curved, brown, transversely septate, thin-walled, with or without a sheath.

**Asexual morph:** Undetermined.

**Type species:** *Aquimassariosphaeria kunmingensis* W. Dong, Doilom & K.D. Hyde

**Notes:** *Aquimassariosphaeria* is characterized by its immersed, erumpent to superficial ascomata, sometimes staining substrate purple, and narrowly fusiform or vermiform, brown, transversely septate ascospores. Morphologically, *Aquimassariosphaeria* is similar to *Lolia* in having fusiform to clavate, multiple transversely septate, brown ascospores, but the conidia of the latter equipped with apical, sub-apical and basal appendages, which are never formed in *Aquimassariosphaeria*. Phylogenetic analyses also support them to be different genera (Figs. 2, 61). Additionally, the type species of *Massariosphaeria*, *M. phaeospora* (E. Müll.) Crivelli, clusters in Cyclothyriellaceae (Jaklitsch and Voglmayr 2016), while *M. typhicola* (CBS 609.86) clusters in Lindgomycetaceae (Fig. 2). Although Ariyawansa et al. (2015) transferred *Neomassariosphaeria* Y. Zhang et al. from Amniculicolaceae to Lindgomycetaceae, to accommodate *Massariosphaeria typhicola*, we do not accept in this study (see notes under *Neomassariosphaeria*, Amniculicolaceae). A new genus *Aquimassariosphaeria* is, therefore, established to accommodate *Massariosphaeria typhicola* and the new species *A. kunmingensis*.

#### List of freshwater *Aquimassariosphaeria* species

\**Aquimassariosphaeria kunmingensis* W. Dong, Doilom & K.D. Hyde, **sp. nov.**

**Index Fungorum number:** IF557912; **Facesoffungi number:** FoF08734; Fig. 56

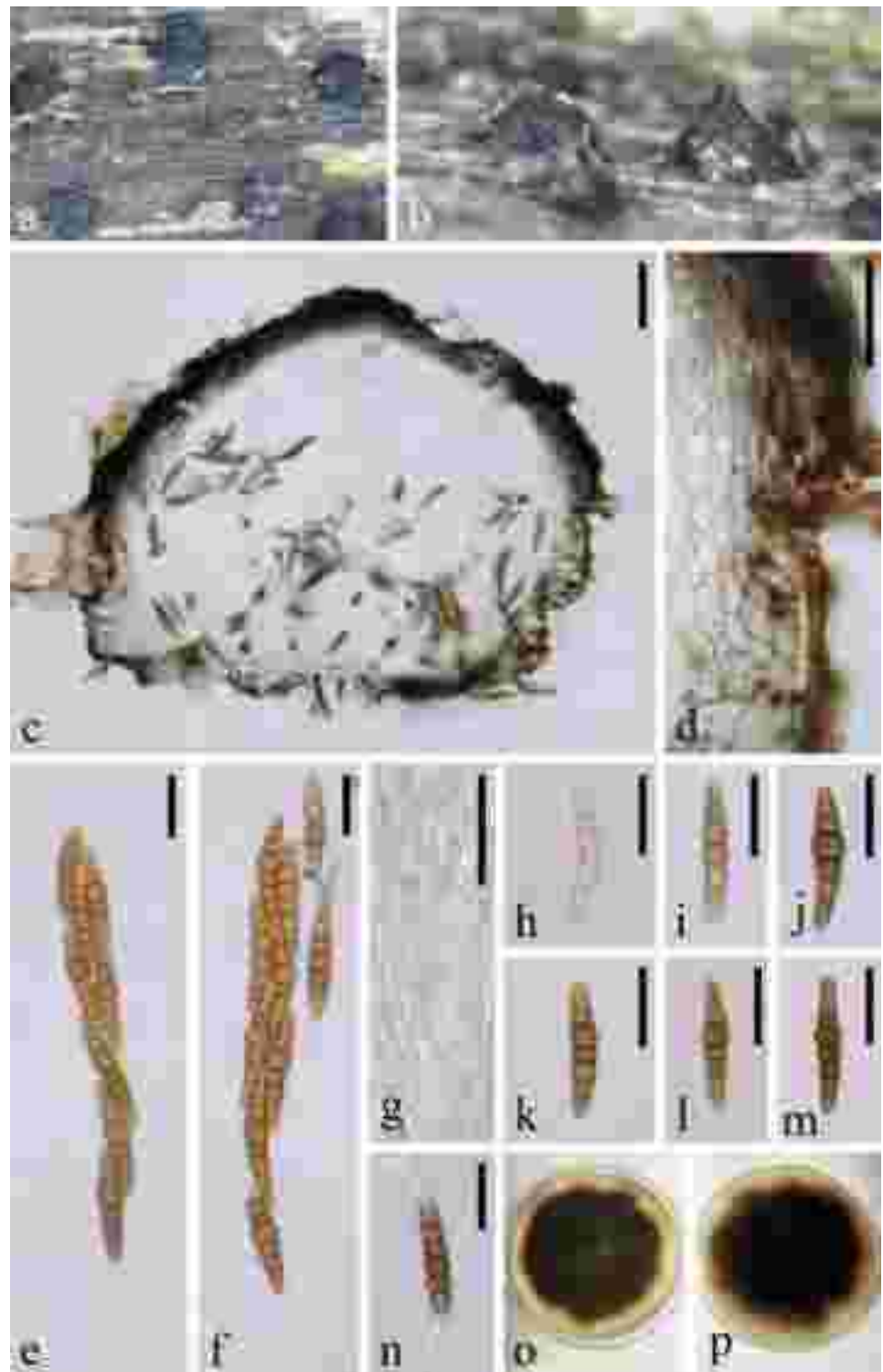
**Etymology:** referring to Kunming, where the holotype was collected

**Holotype:** HKAS 102148

**Saprobic** on decaying wood submerged in freshwater.

**Sexual morph:** *Ascomata* 300–350 × 370–400 µm, scattered or solitary, erumpent, mostly superficial, subglobose to ellipsoidal, black, coriaceous, with ostiolate papilla. *Peridium* 30–40 µm thick, two-layered, outer layer comprising 4–6 layers of dark brown or black, thin-walled, compressed cells of *textura angularis* or irregular cells, inner layer comprising 3–5 layers of pale brown to hyaline, thin-walled, large cells of *textura angularis*. *Pseudoparaphyses* 2 µm diam., numerous, cellular, hyaline, unbranched, septate, embedded in a matrix. *Asci* 160–200 × 15–18 µm ( $\bar{x}$  = 180 × 17 µm, n = 5), 8-spored, bitunicate, narrowly clavate, short pedicellate, apically rounded. *Ascospores* 35–46(–55) × 6.5–9.5 µm ( $\bar{x}$  = 40 × 7.8 µm, n = 25), bi- to tri-seriate in the upper part, uni-seriate in the lower part, narrowly fusiform or vermiform,

**Fig. 56** *Aquimassariosphaeria kunmingensis* (HKAS 102148). **a, b** Appearance of ascomata on host substrate. **c** Vertical section of ascoma. **d** Structure of peridium. **e, f** Bitunicate asci. **g** Pseudoparaphyses. **h–m** Ascospores. **n** Germinated ascospore. **o, p** Colony on PDA (left-front, right-reverse). Scale bars: **c** = 50  $\mu$ m, **d–n** = 20  $\mu$ m



occasionally narrowly clavate with elongate basal cells, with rounded ends, brown, 6–7-septate, occasionally 5- or 8-septate, constricted at the septa, asymmetric, obviously enlarged at the third or fourth cell from the apex, straight or curved, smooth, thin-walled, without a sheath. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 10 mm in 15 days at 25 °C, black from above, dark brown from below, surface rough, with sparse mycelium, mostly immersed in culture, dry, raised, edge entire.

**Material examined:** CHINA, Yunnan Province, Kunming University of Science & Technology, on submerged wood

in a stream, 10 May 2017, C.X. Liu, L47 (HKAS 102148), living culture KUMCC 18-1019.

*Notes:* *Aquimassariosphaeria kunmingensis* clusters with *A. typhicola* with moderate bootstrap support (Figs. 2, 61). *Aquimassariosphaeria kunmingensis* has superficial ascumata and thin-walled, smooth ascospores without a sheath, while the latter has completely immersed ascumata that staining substrate purple and thick-walled, heavily grained sculpted ascospores with a well-defined, mucilaginous sheath (Leuchtmann 1984). Comparison of single genes between *A. kunmingensis* (KUMCC 18-1019) and *A. typhicola* (CBS 609.86) shows that there are 4, 1 and 16 nucleotide differences in LSU, SSU and RPB2 sequence data, respectively, which supports them to be different species.

\**Aquimassariosphaeria typhicola* (P. Karst.) W. Dong & Doilom, *comb. nov.*

*Index Fungorum number:* IF557913; *Facesoffungi number:* FoF09255

*Basionym:* *Leptosphaeria typhicola* P. Karst., *Bidr. Känn. Finl. Nat. Folk* 23: 100 (1873)

*Synonymy:* *Massariosphaeria typhicola* (P. Karst.) Leuchtm., *Sydowia* 37: 168 (1984)

*Other synonyms* see *Index Fungorum* (2020), but *Neomassariosphaeria typhicola* (P. Karst.) Y. Zhang ter, J. Fourn. & K.D. Hyde is not accepted as a synonymy in this study

*Possible freshwater distribution:* USA (Fallah and Shearer 2001)

*Notes:* *Massariosphaeria typhicola* was transferred from *Leptosphaeria* based on some collections ZT 9428, ZT 9430, ZT 9431 and ZT 9435, and the description and drawing of Berlese (1894), which were designed from the original material by Karsten. The holotype of *Leptosphaeria typhicola* was not indicated when published and few information of *L. typhicola* can be obtained in Karsten (1873) and Berlese (1894). Leuchtmann (1984) mentioned that *M. typhicola* showed variability in the shape, size and septation of ascospores. Some authors named several strains CBS 123126, CBS 609.86, KT 667 and KT 797 as *M. typhicola* (Leuchtmann 1984; Tanaka and Harada 2004; Zhang et al. 2009c). However, phylogenetic analyses placed CBS 123126 in Amniculicolaceae (Zhang et al. 2009c, 2012b) and KT 667, KT 797, CBS 609.86 in Lindgomycetaceae (Hyde et al. 2016b; this study, Fig. 2). It is, therefore, considerably important to identify *M. typhicola* and its relatives with DNA sequence data. To solve the taxonomic confusion of *M. typhicola*, we select strain CBS 609.86 (specimen ZT 9428) designated by Leuchtmann (1984) as its representative materials. The ascospores from a drawing of *M. typhicola* based on four specimens ZT 9428, ZT 9430, ZT 9431 and ZT 9435, share similar morphology to the new species

*Aquimassariosphaeria kunmingensis* (Leuchtmann 1984). We, therefore, place two species in the same genus *Aquimassariosphaeria*. The examination and description for the specimen ZT 9428 is expected.

Our phylogenetic analyses show that KT 667 and KT 797 represent a different species with CBS 609.86 (Figs. 2, 61). Since the very less morphological information of KT 667 and KT 797 in Tanaka and Harada (2004), the real identity of these two strains are uncertain, only if their specimens are re-examined in the future. KT 667 and KT 797 probably represent another new genus, but the establishment needs evidence from their specimens or new collections with sufficient DNA sequence data. We name KT 667 and KT 797 as *Massariosphaeria* sp. in this study. The strain CBS 123126 has been treated as a different species *Neomassariosphaeria typhicola* based on morphology and phylogeny in this study (see notes under *Neomassariosphaeria*, Amniculicolaceae).

*Aquimassariosphaeria typhicola* was reported from freshwater habitats in USA with detailed description and illustration (Fallah and Shearer 2001). This USA specimen has shorter and thinner asci ((93–)113–133 × 11.5–18 µm vs. 100–160 × 15–25 µm) and shorter ascospores (32–36 × 6–8 µm vs. 26–52 × 6–11 µm) than collections of Leuchtmann (1984). Sequence data of the USA specimen are needed to confirm if *A. typhicola* occurred in freshwater environment in USA.

#### Key to freshwater *Aquimassariosphaeria* species

1. Ascospores smooth.....*A. kunmingensis*
1. Ascospores heavily grained sculpted.....*A. typhicola*

*Lindgomyces* K. Hiray., Kaz. Tanaka & Shearer, *Mycologia* 102(3): 733 (2010)

*Saprobic* on submerged woody plant. **Sexual morph:** *Ascomata* scattered to clustered, semi-immersed to nearly superficial, ellipsoidal or lenticular, subglobose to globose, uniloculate, glabrous, dark brown to black, with ostiolate papilla. *Peridium* composed of hyaline to brown, small, thin-walled cells, sometimes poorly developed at the base. *Pseudoparaphyses* numerous, cellular, hyaline, septate, branched, anastomosing. *Asci* 8-spored bitunicate, fissitunicate, cylindrical to clavate, sessile to minutely pedicellate, rounded at the apex, with an ocular chamber. *Ascospores* bi- to tri-seriate, fusiform to cylindrical, straight to slightly curved, hyaline to pale brown, with a median primary septum, smooth or finely verruculose, thin-walled, usually covered with a sheath or bearing bipolar mucilaginous appendages. Senescent ascospores brown, > 3-septate (Hirayama et al. 2010). **Asexual morph:** Undetermined.

*Type species:* *Lindgomyces ingoldianus* (Shearer & K.D. Hyde) K. Hiray., Kaz. Tanaka & Shearer

**Notes:** All *Lindgomyces* species have been reported from freshwater habitats (see list below). *Lindgomyces* was typified by *Massarina ingoldiana* Shearer & K.D. Hyde, which is a species complex with some strains assigned to *Lindgomyces*, and others to *Tingoldiagio* (Hirayama et al. 2010). The fusiform to cylindrical ascospores with a sheath or bearing bipolar mucilaginous appendages are main characters of *Lindgomyces*. *Lindgomyces aquaticus* sp. nov. is introduced in this study.

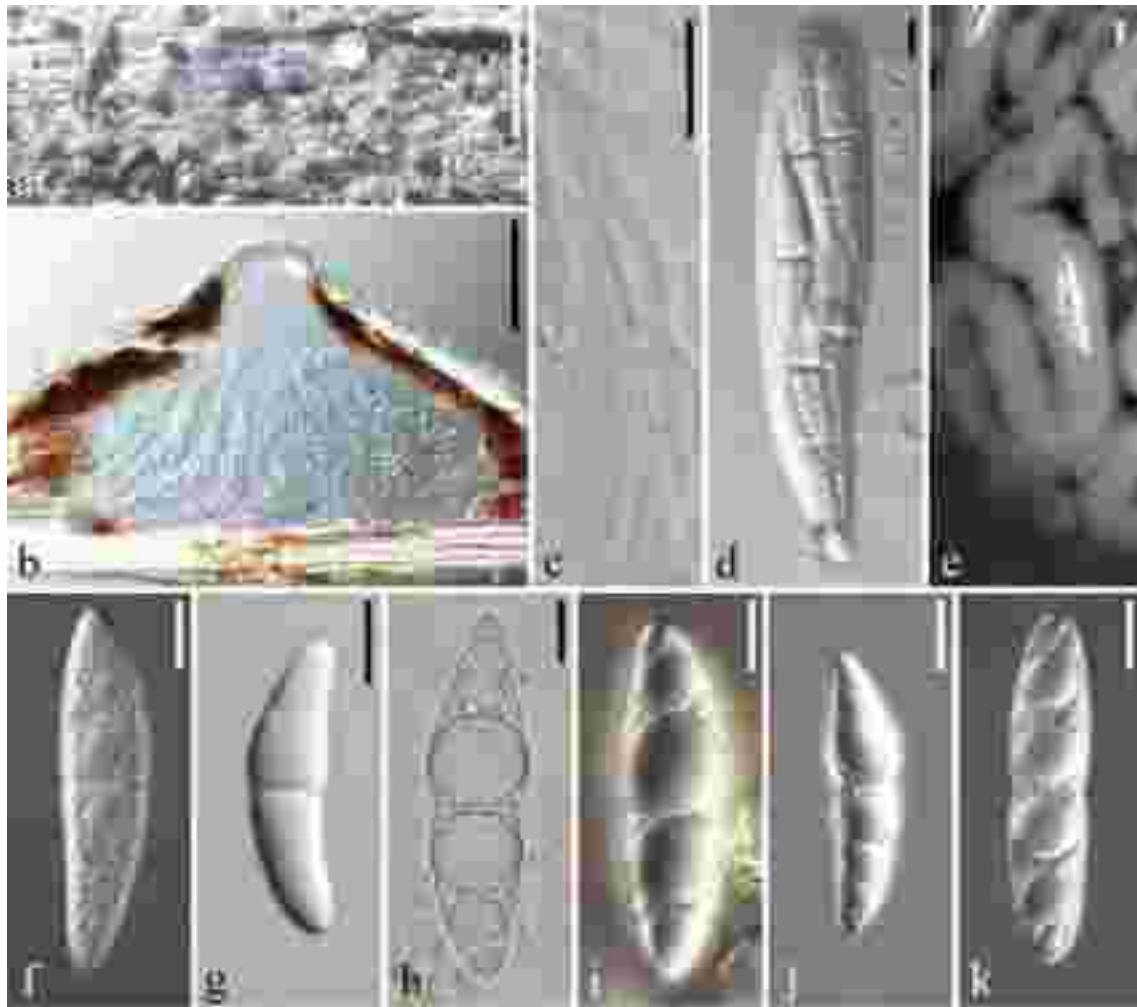
### List of freshwater *Lindgomyces* species

\**Lindgomyces angustiascus* Raja, A.N. Mill. & Shearer, Mycoscience 54(5): 357 (2013)

*Freshwater distribution:* USA (Raja et al. 2013a)

\**Lindgomyces apiculatus* K. Hiray. & Kaz. Tanaka, Mycologia 103(6): 1424 (2011); Fig. 57g

*Freshwater distribution:* Japan (Raja et al. 2011)



**Fig. 57** *Lindgomyces* spp. (Material examined: USA, Wisconsin, Adams County, Lemonweir River, on submerged decorticated woody debris, 31 July 1992, CAS and JLC, A-39-1, ILLS 52289, **holotype**; JAPAN, Okinawa, Iriomote, Oomijya River, on submerged decorticated woody debris, 28 September 2007, KH 100; *ibid.*, Aomori, Hirosaki, Aoki, Mohei-pond, on submerged twigs of woody plant, 3 May 2003, KT 1108, HHUF 28988, **holotype**; *ibid.*, Hokkaido, Akkeshi, Ootakita, Sattebetsu River, on submerged wood, 7 September 2003, KT 1399, HHUF 28194, **holotype**; *ibid.*, Okinawa, Kunigami, Aha, Tanagakumui, small river, on submerged dead twigs of woody plant, 19 May 2015, K. Tanaka et al., KT 3531, HHUF 30498, **holotype**; *ibid.*, Aomori, Nishimeya, Seisyu trail, Ooshirōsawa River, on submerged dead twigs of woody plant, 28 August

2010, K. Tanaka et al., KT 2742, HHUF 30513, **holotype**; *ibid.*, Hirosaki, Aoki, Mohei Pond, on submerged woody debris, 7 December 2002, KT 966, HHUF 27883, **holotype**). **a–f** *L. ingoldianus* (**a**, **c**, **d** from ILLS52289, **holotype**; **b** from KH 100; **e**, **f** from culture of ATCC 200398). **a** Ascomata on the host surface. **b** Section of ascus. **c** Pseudoparaphyses. **d** Ascus. **e** Ascospore with an elongate sheath. **f** Ascospore. **g** Ascospore of *L. apiculatus* (KT 1108, **holotype**). **h** Ascospore of *L. breviappendiculatus* (culture of KT 1399). **i** Ascospore of *L. okinawaensis* (culture of KT 3531). **j** Ascospore of *L. pseudomadisonensis* (culture of KT 2742). **k** Ascospore of *L. rotundatus* (culture of KT 966). Scale bars: **a** = 1000  $\mu$ m, **b** = 100  $\mu$ m, **c–k** = 10  $\mu$ m

**Fig. 58** *Lindgomyces aquaticus* (MFLU 18-1711, **holotype**). **a, b** Appearance of ascomata on host surface. **c** Vertical section of ascoma. **d** Structure of peridium. **e–g** Bitunicate asci. **h** Ascus embedded in pseudo-paraphyses. **i–k** Ascospores. **l** Ascospore in Indian Ink. **m** Germinated ascospore. **n, o** Colony on PDA (left-front, right-reverse). Scale bars: **c, m** = 50  $\mu$ m, **d–l** = 20  $\mu$ m



**\**Lindgomyces aquaticus*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum* number: IF557914; *Facesoffungi* number: FoF09256; Fig. 58

*Etymology*: referring to aquatic habitat of this fungus

*Holotype*: MFLU 18-1711

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Ascomata* 160–200  $\mu$ m high, 220–250  $\mu$ m wide, black, scattered, semi-immersed to nearly superficial, ellipsoidal or lenticular, coriaceous. *Peridium* 1.5–2.5  $\mu$ m, comprising several layers of brown, thin-walled, large

cells of *textura angularis* or subglobose cells, flattened and poorly developed at the base. *Pseudoparaphyses* 1.5–2.5  $\mu$ m diam., sparse, cellular, hypha-like, hyaline, septate, persistent, embedded in a gelatinous matrix. *Asci* 150–250  $\times$  33–43  $\mu$ m ( $\bar{x}$  = 210  $\times$  39  $\mu$ m,  $n$  = 10), 8-spored, bitunicate, mostly broadly clavate, rarely narrowly clavate, or cylindrical, apically rounded with well-developed ocular chamber. *Ascospores* (59–)68–71  $\times$  12–16  $\mu$ m ( $\bar{x}$  = 70  $\times$  14  $\mu$ m,  $n$  = 15), overlapping bi- to tri-seriate, straight or slightly curved, hyaline and 1-septate when young, slightly constricted at the septa, turn to pale brown and 5-septate when germinated,

fusiform with rounded or acute ends, with minute and some large guttulate, thin-walled, smooth, lacking a mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 15 mm in 20 days at 25 °C, grey to brown from above, black from below, surface rough, dry, raised, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat8115-1 (MFLU 18-1711, **holotype**), ex-type living culture MFLUCC 18-1416; *ibid.*, hat8115-2 (HKAS 105029, **isotype**), ex-type living culture KUMCC 19-0044.

**Notes:** *Lindgomyces aquaticus* clusters with an unnamed species *Lindgomyces* sp. KH241 with high bootstrap support (Figs. 2, 61). *Lindgomyces aquaticus* differs from KH241 by its larger asci (150–250 × 33–43 µm vs. 125–194 × 20.5–29 µm), ascospores ((59–)68–71 × 12–16 µm vs. 47–62 × 8–12 µm) and different habitats (freshwater vs. intertidal) (Hirayama et al. 2010). A large, sticky sheath is observed in KH241, but not in our collection MFLUCC 18-1416. Comparison of single gene between *L. aquaticus* (MFLUCC 18-1416) and *Lindgomyces* sp. (KH241) shows that there are one, zero and seven nucleotide differences in LSU, SSU and ITS sequence data, respectively. Morphological characters mentioned above can easily separate them to be different species. *Lindgomyces aquaticus* also differs from *L. ingoldianus* (type) in having wider asci (150–250 × 33–43 µm vs. (110–)120–167.5(–182.5) × 25–32.5 µm), larger ascospores ((59–)68–71 × 12–16 vs. (47–)50–59 × 9–11(–12)) and without sheath (Hirayama et al. 2010). We therefore introduce *L. aquaticus* sp. nov. and retain KH241 as *Lindgomyces* sp.

\**Lindgomyces breviappendiculatus* (Kaz. Tanaka, Sat. Hatak. & Y. Harada) K. Hiray. & Kaz. Tanaka, Mycologia 102(3): 738 (2010); Fig. 57h

**Basionym:** *Lophiostoma breviappendiculatum* Kaz. Tanaka, Sat. Hatak. & Y. Harada, Mycoscience 46(5): 288 (2005)

**Freshwater distribution:** Japan (Tanaka et al. 2005b; Hirayama et al. 2010)

\**Lindgomyces carolinensis* Raja, J. Fourn., Paguigan & Oberlies, Mycol. Progr. 16(5): 542 (2017)

**Freshwater distribution:** North Carolina (Raja et al. 2017)

\**Lindgomyces cigarosporus* Raja, J. Fourn., Paguigan & Oberlies [as ‘cigarospora’], Mycol. Progr. 16(5): 543 (2017)

**Freshwater distribution:** North Carolina (Raja et al. 2017)

\**Lindgomyces cinctosporus* Raja, A.N. Mill. & Shearer [as ‘cinctosporae’], Mycologia 86102(3): 738 (2010)

**Freshwater distribution:** USA (Hirayama et al. 2010)

\**Lindgomyces griseosporus* Y. Zhang ter, J. Fourn. & K.D. Hyde, Mycoscience 56(1): 43–48 (2014)

**Freshwater distribution:** France and Spain (Zhang et al. 2014c)

\**Lindgomyces ingoldianus* (Shearer & K.D. Hyde) K. Hiray., Kaz. Tanaka & Shearer, 88Mycologia 102(3): 733 (2010); Fig. 57a–f

**Basionym:** *Massarina ingoldiana* Shearer & K.D. Hyde, Mycologia 89(1): 114 (1997)

**Freshwater distribution:** Australia (Shearer and Hyde 1997), Brunei (Shearer and Hyde 1997), China (Tsui et al. 2000), Japan (Hirayama et al. 2010), Malaysia (Hyde and Aptroot 1998b), Thailand (Sivichai et al. 2002), USA (Shearer and Hyde 1997)

\**Lindgomyces lemonweirensis* Raja, A.N. Mill. & Shearer, Mycologia 103(6): 1428 (2011)

**Freshwater distribution:** USA (Raja et al. 2011)

\**Lindgomyces madisonensis* Raja & Oberlies, Persoonia, Mol. Phyl. Evol. Fungi 35: 307 90(2015)

**Freshwater distribution:** USA (Crous et al. 2015c)

\**Lindgomyces okinawaensis* Tak. Takah. & Kaz. Tanaka, Fungal Diversity 78: 35 (2016); Fig. 57i

**Freshwater distribution:** Japan (Li et al. 2016a)

\**Lindgomyces pseudomadisonensis* Tak. Takah. & Kaz. Tanaka, Fungal Diversity 81 (2016); Fig. 57j

**Freshwater distribution:** Japan (Hyde et al. 2016b)

\**Lindgomyces rotundatus* K. Hiray. & Kaz. Tanaka, Mycologia 102(3): 733 (2010); Fig. 57k

**Freshwater distribution:** Japan and USA (Hirayama et al. 2010)

#### Key to freshwater *Lindgomyces* species

1. Ascospores hyaline, smooth.....2
  1. Ascospores grey-brown, smooth to verruculose.....*L. griseosporus*
  2. Ascospores surrounded by a large, expanding gelatinous sheath.....3
    2. Ascospores without expanding sheath.....4
    3. Ascospores fusiform with acute ends.....*L. ingoldianus*
    3. Ascospores cylindrical with rounded ends.....*L. rotundatus*
    4. Ascospores with terminal gelatinous appendages.....5
    4. Ascospores without terminal gelatinous appendages.....10

5. Ascospores < 43 µm long.....6  
 5. Ascospores > 43 µm long.....7  
 6. Asci 100–157 × 14–16 µm.....*L. madisonensis*  
 6. Asci 85–125 × 17–25(–27) µm.....*L. apiculatus*  
 7. Sheath evanescent.....*L. carolinensis*  
 7. Sheath not evanescent.....8  
 8. Ascospores up to 70 µm long, with short, rounded, terminal ephemeral appendages.....*L. cigarosporus*  
 8. Ascospores < 60 µm long.....9  
 9. Ascospores 47–58 × 9–12 µm.....*L. angustiascus*  
 9. Ascospores (40–)44–60 × (9.5–)11–17.5 µm.....  
 .....*L. breviappendiculatus*  
 10. Ascospores < 10 µm wide.....*L. pseudomadisonensis*  
 10. Ascospores > 10 µm wide.....11  
 11. Ascospores without sheath.....12  
 11. Ascospores with a sheath.....13  
 12. Ascospores (59–)68–71 × 12–16 µm.....*L. aquaticus*  
 12. Ascospores (38–)40–48(–51) × (10–)12–19 µm.....  
 .....*L. okinawaensis*  
 13. Ascospores 40–58 × 10–18 µm, with an amorphous gelatinous sheath.....*L. cinctosporus*  
 13. Ascospores 30–44 × 10–15 µm, with an oval, ephemeral sheath.....*L. lemonweirensis*

*Neolindgomycetes* Jayasiri, E.B.G. Jones & K.D. Hyde, Mycosphere 10(1): 79 (2019)

*Saprobic* on *Pandanus* sp or submerged wood. **Sexual morph:** *Ascomata* scattered to gregarious, immersed, depressed globose or conical with flattened base, dark brown to black, coriaceous, with a small clypeus ostiole. *Ostiole* with a pore-like opening, central, with a reduced crest, with periphyses made up of lightly pigmented, pseudoparenchymatous cells. *Peridium* composed of dark brown to black, somewhat flattened cells of *textura angularis*, fusing and indistinguishable from the host tissues, with inner stratum comprising hyaline cell layers of *textura angularis*. *Pseudoparaphyses* numerous, trabeculate, filamentous, hyaline, septate, branched. *Asci* 8-spored, bitunicate, fissitunicate, cylindric-clavate, short pedicellate, apex rounded with a minute ocular chamber or inconspicuous apical thickening. *Ascospores* uni- to bi-seriate, overlapping, hyaline, cylindrical or fusiform with narrow, acute ends, septate, smooth-walled, with a mucilaginous sheath (Hyde and Goh 1999b; Jayasiri et al. 2019). **Asexual morph:** Undetermined.

*Type species:* *Neolindgomycetes pandani* Jayasiri, E.B.G. Jones & K.D. Hyde

*Notes:* *Neolindgomycetes* was introduced for *N. pandani* and a known species *Quintaria submersa* K.D. Hyde & Goh collected from freshwater habitats (Hyde and Goh 1999b; Jayasiri et al. 2019). *Neolindgomycetes* is distinctive in Lindgomycetaceae by its carbonaceous peridium and presence of clypeus (Jayasiri et al. 2019). It formed a basal clade in the family with high bootstrap support (Jayasiri et al. 2019),

but showed low support in Fig. 2 and moderate support in Fig. 61.

#### List of freshwater *Neolindgomycetes* species

\**Neolindgomycetes submersus* (K.D. Hyde & Goh) Jayasiri & K.D. Hyde [as ‘submersa’] Mycosphere 10(1): 81 (2019)

*Basionym:* *Quintaria submersa* K.D. Hyde & Goh, Nova Hedwigia 68(1-2): 262 (1999)

*Freshwater distribution:* Australia (Hyde and Goh 1999b)

#### Key to freshwater asexual genera of Lindgomycetaceae

1. Conidia with apical, subapical and basal appendages...  
.....*Lolia*
1. Conidia without appendages.....2
2. Conidiogenous cells holoblastic.....*Clohesyomyces*
2. Conidiogenous cells enteroblastic.....*Hongkongmyces*

*Clohesyomyces* K.D. Hyde, Aust. Syst. Bot. 6(2): 170 (1993)

*Saprobic* on submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata* pycnidial, scattered, subglobose to ellipsoidal, compressed lenticular, dark brown to black, semi-immersed to erumpent, unilocular, ostiolate. *Pycnidial wall* two-layered. *Ostiole* dark-brown, circular. *Conidiophores* reduced. *Conidiogenous cells* holoblastic, determinate, discrete, hyaline, smooth. *Conidia* variable in shape, usually ellipsoidal, mostly 1 median-septate, guttulate, hyaline, smooth, thin-walled, straight or slightly curved, surrounded by a mucilaginous sheath (Hyde 1993b).

*Type species:* *Clohesyomyces aquaticus* K.D. Hyde

*Notes:* *Clohesyomyces* was introduced to accommodate a single species *C. aquaticus* collected from a freshwater habitat in Australia (Hyde 1993b). Other collections were reported from freshwater habitats in China (Cai et al. 2006b; this study) and Thailand (Zhang et al. 2012a; this study). The phylogenetic position of *Clohesyomyces* was confirmed in Lindgomycetaceae (Zhang et al. 2012a; Raja et al. 2017) and this study (Fig. 2). The freshwater materials of *C. aquaticus* collected by Zhang et al. (2012a) was re-examined and described by Li et al. (2020) and this study (Fig. 59). We also report two additional freshwater collections obtained from China and Thailand (Fig. 59).

#### List of freshwater *Clohesyomyces* species

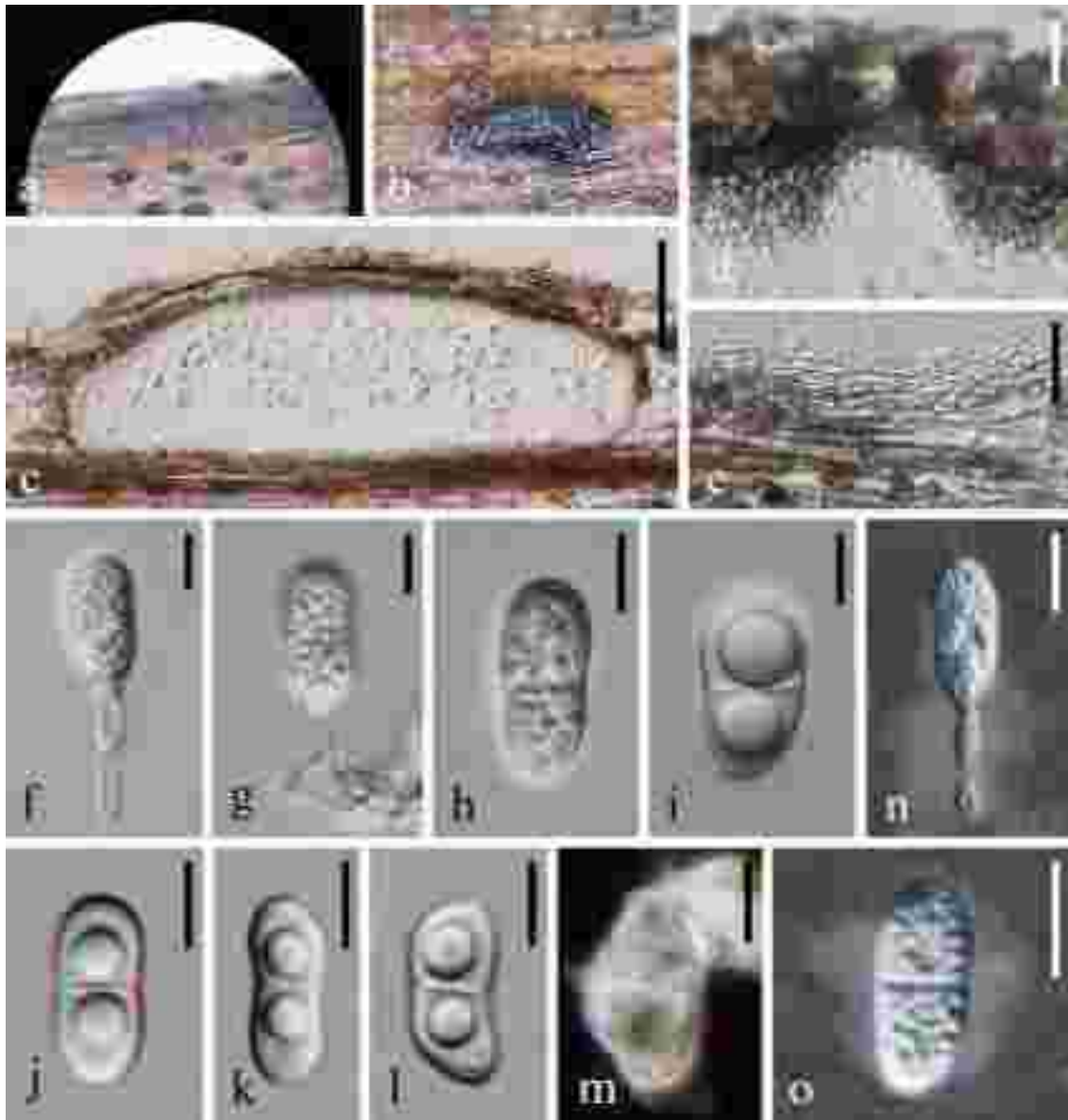
\**Clohesyomyces aquaticus* K.D. Hyde, Aust. Syst. Bot. 6(2): 170 (1993)

*Facesoffungi number:* FoF07165; Fig. 59

*Freshwater distribution:* Australia (Hyde 1993b), China (Cai et al. 2006b; this study), Thailand (Zhang et al. 2012a; this study)

*Saprobic* on submerged wood in freshwater habitats. **Sexual morph:** Undetermined. **Asexual morph:** *Conidiomata*





**Fig. 59** *Clohesyomyces aquaticus* (a–i from MFLU 18-1159, j–m from MFLU 15-2699, n–o from MFLU 11-1112). a, b Appearance of pycnidia on host surface. c Vertical section of pycnidium. d Ostiole.

e Peridium. f, g Conidiogenous cells with conidia. h, i Conidia. j–m, o Conidia. (m, o in Indian Ink). n Conidiogenous cell with conidium. Scale bars: c = 100  $\mu$ m, d, e = 20  $\mu$ m, f–o = 10  $\mu$ m

pycnidial, scattered, 160–300  $\mu$ m high, 180–850  $\mu$ m diam., subglobose to ellipsoidal, compressed lenticular, dark brown to black, semi-immersed to erumpent, unilocular, ostiolate. *Ostiole* dark brown, central, circular. *Peridium* 25–60  $\mu$ m thick, composed of two layers, with outer wall comprising several layers of dark brown cells, inner wall comprising 3–5 layers of brown-walled rectangle cells of *textura porrecta*. *Conidiophores* reduced. *Conidiogenous cells* holoblastic, up to 20  $\mu$ m long, determinate, discrete, cylindrical to subcylindrical, hyaline, smooth, forming from the inner layer cells of the pycnidial wall. *Conidia* 16–29  $\times$  7–16  $\mu$ m ( $\bar{x}$  = 21.1  $\times$  8.7  $\mu$ m, n = 10), cylindrical to subcylindrical or ellipsoid,

irregular, mostly 1-septate, slightly constricted at the septum, one prominent guttulate in each cell, some with additional small guttules, hyaline, smooth, thin-walled, truncate at the base, obtuse at the apex, straight or slightly curved, surrounded by an irregular, thin mucilaginous sheath.

*Culture characteristics*: On PDA, colony circular, slow growing, 40 mm in 30 days at 25  $^{\circ}$ C, brown to grey from above, brown to yellow from reverse, raised, felty wooly, fairly dense, initially aerial with a smooth edge, becoming rough after 20 days, staining agar yellow.

*Material examined*: THAILAND, Prachuap Khiri Khan Province, on submerged wood in a small river, 30 July 2015,

K.D. Hyde, 13A (MFLU 15-2699), living culture MFLUCC 15-0979; CHINA, Yunnan Province, on submerged wood in a small river, 25 November 2017, G.N. Wang, H58A-1 (MFLU 18-1159), living culture MFLUCC 18-1037; *ibid.*, H58A-2 (HKAS 101735), living culture KUMCC 18-0087.

**Additional material examined:** THAILAND, Chiang Mai Province, Doi Inthanon, on submerged wood, 16 November 2010, H. Zhang, d66 (MFLU 11-1112).

**Notes:** Our new collections MFLUCC 15-0979 and MFLUCC 18-1037 are identified as *Clohesyomyces aquaticus* based on identical LSU and ITS sequence data with MFLUCC 11-0092, and phylogenetic analysis (Figs. 2, 61). The conidial size of *C. aquaticus* varies in different collections, ranging from 15–31 × 7–16 µm. Moreover, MFLUCC 18-1037 has larger diameter of conidiomata than the holotype BRIP 20092 (260–300 µm high, 800–850 µm diam. vs. 247–390 µm high, 156–260 µm diam.) (Hyde 1993b; Zhang et al. 2012a).

**Hongkongmyces** C.C.C. Tsang et al., *Medical Mycol.* 52(7): 740 (2014)

(see entry below freshwater sexual genera of Lindgomycetaceae)

**Lolia** Abdel-Aziz & Abdel-Wahab, *Mycotaxon* 114: 36 (2011)

**Saprobic** on submerged decayed stem. **Sexual morph:** *Ascomata* solitary or aggregated in stroma, immersed to erumpent, uniloculate, globose to subglobose, sometimes with flattened base, glabrous, dark brown to black, coriaceous to sub-carbonaceous, with cylindrical ostiolar canals, with ostiolate papilla, usually stain the substrate purple. *Peridium* two-layered, comprising hyaline to yellow-brown, to dark brown, thin-walled cells of *textura angularis*. *Pseudoparaphyses* numerous, cellular, thin, hypha-like, septate, branched, persistent, emerged into the ostiolar canal. *Asci* 4- to 8-spored, bitunicate, fissitunicate, cylindrical-clavate, pedicellate, with ocular chamber. *Ascospores* uni- to bi-seriate, fusiform to clavate, with rounded ends, straight or slightly curved, yellow- to reddish- to dark-brown, transversely septate, occasionally with one longitudinal or oblique septum, smooth- or rough-walled, surrounded by prominent gelatinous sheath (Abdel-Aziz 2016a). **Asexual morph:** Coelomycetous. *Conidiomata* solitary or aggregated, superficial, acervular, pearl white to dull yellow. *Peridium* composed of hyaline cells of *textura intricata*, embedded in gel. *Conidiophores* lining the acervuli wall, branched and septate, hyaline, smooth, embedded in gel. *Conidiogenous cells* holoblastic, terminal, cylindrical to sub-cylindrical, hyaline, smooth, bearing a single conidium. *Conidia* solitary, ellipsoidal, straight, hyaline, aseptate, smooth, thin-walled, with apical, sub-apical and basal appendages (Abdel-Aziz and Abdel-Wahab 2010).

**Type species:** *Lolia aquatica* Abdel-Aziz & Abdel-Wahab

**Notes:** *Lolia* was introduced by Abdel-Aziz and Abdel-Wahab (2010) to accommodate the asexual species *L. aquatica* collected on decayed stem of *Phragmites australis* from Egypt. *Lolia* is characterized by acervular conidiomata, clavate, ellipsoidal, cylindrical conidia with apical, sub-apical and basal appendages (Abdel-Aziz and Abdel-Wahab 2010). Its sexual morph was later collected on submerged decayed stem of *Phragmites australis* from River Nile (Abdel-Aziz 2016a). The authors assigned two holotypes, IMI 398675 and CBS H-22130, based on both asexual and sexual morphs, respectively, which does not conform to “One fungus, one name” (Abdel-Aziz and Abdel-Wahab 2010; Abdel-Aziz 2016a). We select the first holotype (IMI 398675, ex-type living culture MF644) as the exclusive holotype.

The second species *L. dictyospora* Abdel-Aziz was also collected from River Nile, but on submerged decayed rachis of *Phoenix dactylifera* (Abdel-Aziz 2016a). The special characters of *L. dictyospora*, such as 4- to 8-spored asci and clavate ascospores occasionally with one longitudinal or oblique septa, distinguish it from *L. aquatica* (Abdel-Aziz 2016a). *Lolia aquatica* has cellular, hypha-like pseudoparaphyses, while *L. dictyospora* has trabeculate, filiform pseudoparaphyses. Two species cluster together, but without bootstrap support (Abdel-Aziz 2016a; this study, Figs. 2, 61).

#### List of freshwater *Lolia* species

\****Lolia aquatica*** Abdel-Aziz & Abdel-Wahab, *Mycotaxon* 114: 36 (2011); Fig. 60a–j

**Freshwater distribution:** Egypt (Abdel-Aziz and Abdel-Wahab 2010; Abdel-Aziz 2016a)

\****Lolia dictyospora*** Abdel-Aziz, *Phytotaxa* 267(4): 283 (2016); Fig. 60k–n

**Freshwater distribution:** Egypt (Abdel-Aziz 2016a)

#### Key to freshwater *Lolia* species

1. Asci 8-spored, ascospores 26–37 × 6–9 µm..... *L. aquatica*
1. Asci 4–8-spored, ascospores 14–19 × 4–7 µm..... *L. dictyospora*

**Longipedicellataceae** Phukhams., Bhat & K.D. Hyde, *Mycosphere* 7(11): 1722 (2016)

#### Key to freshwater genera of Longipedicellataceae

1. Sexual morph..... *Longipedicellata*
1. Asexual morph..... 2



**Fig. 60** *Lolia* spp. (Material examined: EGYPT, Sohag, El Balyana city, on decayed stem of *Phragmites australis* (Cav.) Steud. at irrigation canal, March 2005, F.A. Abdel-Aziz, IMI 398675, **holotype**; Sohag, River Nile, on submerged decayed stem of *Phragmites australis* (Poaceae), 14 August 2012, F.A. Abdel-Aziz, CBS H-22130; Sohag, River Nile, on submerged decayed rachis of *Phoenix dactylifera* (Arecaceae), 14 August 2012, F.A. Abdel-Aziz, CBS H-22131). **a–e**

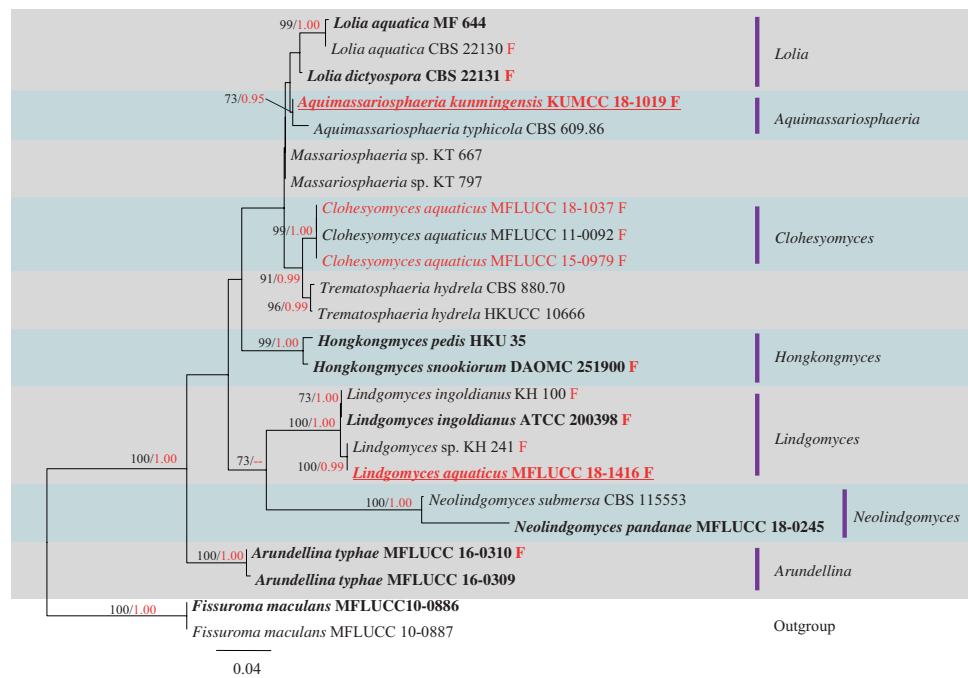
Sexual morph of *Lolia aquatica* (CBS H-22130). **a** Vertical section of ascoma. **b, c** Bitunicate asci. **d** Ascospore with a large gelatinous sheath. **e** Ascospore in Indian Ink. **f–j** Asexual morph of *L. aquatica* (IMI 398675, **holotype**). **f–g** Vertical section through the gelatinous acervular (**f** in phase contrast). **h–j** Conidia. **k–n** *L. dictyospora* (CBS H-22131, **holotype**). **k–m** Asci. **n** Ascospores surrounded by a large sheath. Scale bars: **a, f, g** = 100  $\mu$ m, **b, c** = 30  $\mu$ m, **d, e, h–n** = 10  $\mu$ m

1. Conidiophores semi-macronematous, slender, conidiogenous cells cuneiform, conidia variable in shape.....  
.....*Submersispora*
1. Conidiophores semi-macronematous or reduced, conidiogenous cells reduced or absent, conidia broadly fusiform.....*Pseudoxylomyces*

*Longipedicellata* H. Zhang, K.D. Hyde & J.K. Liu, *Phyto-taxa* 247(2): 102 (2016)

*Saprobic* on dead and submerged woody materials. **Sexual morph:** *Ascomata* scattered or clustered, immersed, beneath a blackened pseudoclypeus, lenticular, uniloculate, glabrous, brown to black, coriaceous, sometimes ostio-late. *Ostiole* relatively large, central, short, dark. *Peridium* thin, comprising several layers of brown, thin-walled, flattened cells of *textura angularis*, outer layer somewhat

**Fig. 61** Phylogram generated from maximum likelihood analysis of combined LSU, SSU, ITS, TEF and RPB2 sequence data for species of Lindgomycetaceae. Bootstrap values for maximum likelihood equal to or greater than 70% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. The tree is rooted to *Fissuroma maculans* (MFLUCC10-0886 and MFLUCC10-0887) (Aigialaceae)



carbonaceous, easily cracked. *Pseudoparaphyses* absent, few or numerous, cellular, hyphae-like, hyaline, sparsely septate. *Asci* 8-spored, bitunicate, clavate, long pedicellate, with an ocular chamber. *Ascospores* bi- to tri-seriate, broadly fusiform to ellipsoidal, hyaline, 1-septate, slightly asymmetrical, the upper cell often shorter and broader than the lower one, smooth, thin-walled, surrounded by mucilaginous sheath (Zhang et al. 2016). **Asexual morph:** Hyphomycetous. *Mycelium* composed of hyaline to dark brown, septate, branched, smooth hyphae. *Conidiophores* and *conidiogenous cells* unknown. *Conidia* chlamydosporous moniloid, in long acropetal, branched chains, catenate, doliiform, subglubose to oval, brown to reddish-brown, rough-walled (Phukhamsakda et al. 2016) (Fig. 61).

**Type species:** *Longipedicellata aptrootii* (K.D. Hyde & S.W. Wong) H. Zhang, K.D. Hyde & J.K. Liu

**Notes:** *Longipedicellata* was introduced in Bambusicolaceae to accommodate *Didymella aptrootii* K.D. Hyde & S.W. Wong based on combined LSU, SSU, RPB2 and TEF sequence data (Zhang et al. 2016). However, it clustered in Bambusicolaceae with low bootstrap support (Zhang et al. 2016). Phukhamsakda et al. (2016) re-collected this species from Thailand and showed a close relationship between *Pseudoxylomyces elegans* (Goh et al.) Kaz. Tanaka & K. Hiray. and *Longipedicellata aptrootii* which represented a new family Longipedicellataceae. They also reported the asexual morph of *L. aptrootii* which formed in the culture. We report *L. aquatica* sp. nov. in this study.

#### List of freshwater *Longipedicellata* species

**\**Longipedicellata aptrootii*** (K.D. Hyde & S.W. Wong) H. Zhang, K.D. Hyde & J.K. Liu, *Phytotaxa* 247(2): 104 (2016); Fig. 62

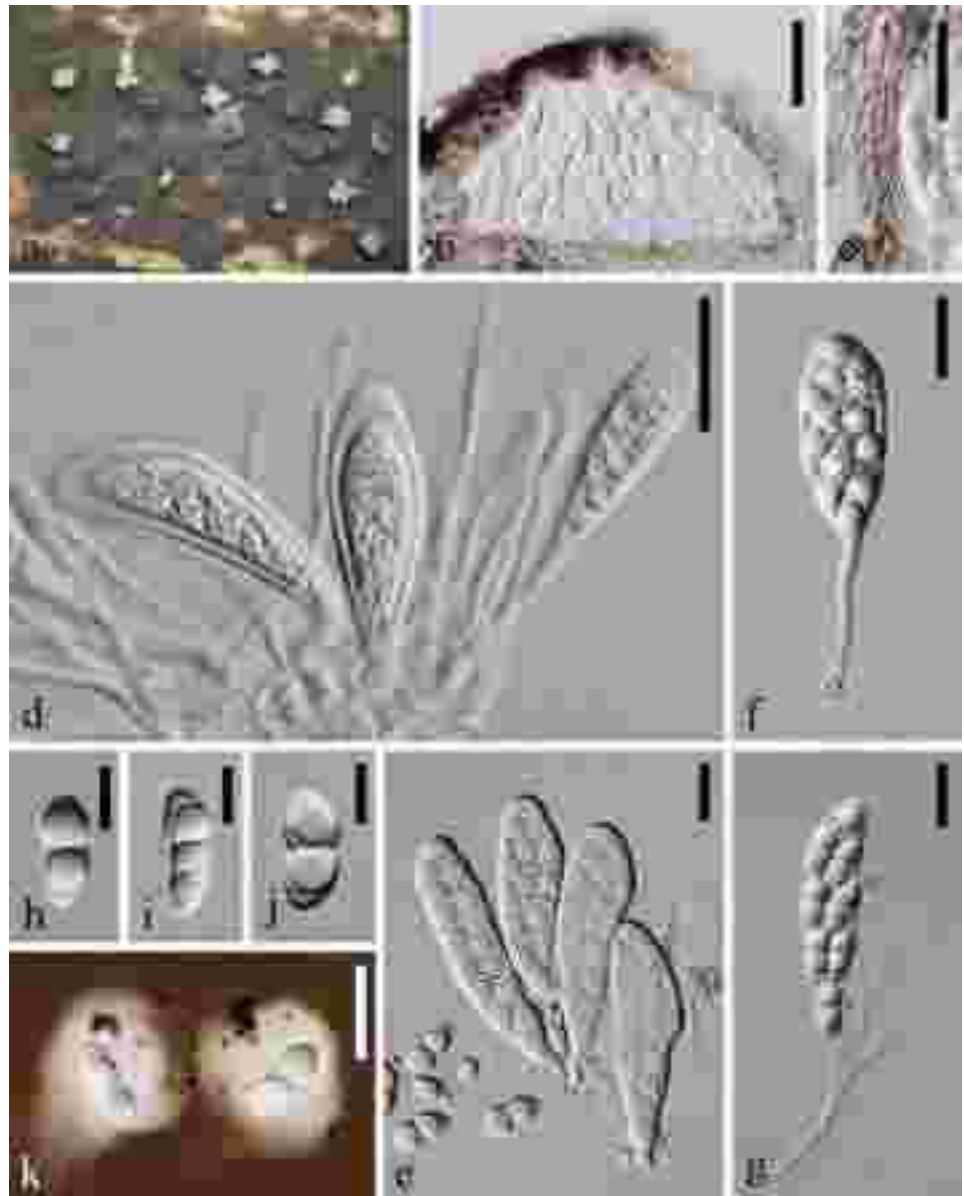
**Basionym:** *Didymella aptrootii* K.D. Hyde & S.W. Wong, *Australas. Mycol.* 18 (3): 54 (1999)

**Freshwater distribution:** China (Hyde and Wong 1998), Malaysia (Hyde and Wong 1998), Philippines (Hyde and Wong 1998; Cai et al. 2003a), Thailand (Kurniawati et al. 2010; Zhang et al. 2016; this study)

**Saprobic** on submerged wood in freshwater. **Sexual morph:** *Ascomata* 150–170 µm high, 190–220 µm diam., immersed or semi-immersed, scattered or clustered, lenticular, covered with a blackened pseudoclypeus, black, coriaceous, ostiolate. *Peridium* 10–15 µm thick, comprising several layers of brown, thin-walled, flattened cells of *textura angularis*. *Pseudoparaphyses* 2–2.5 µm wide, numerous, cellular, hyphae-like, hyaline, sparsely septate, unbranched. *Asci* 80–115 × 19–26 µm ( $\bar{x}$  = 95 × 22 µm, n = 10), 8-spored, bitunicate, fissitunicate, broadly clavate, with a long, straight or twisted pedicel and an ocular chamber. *Ascospores* 17.5–22 × 8–8.5 µm ( $\bar{x}$  = 19 × 8 µm, n = 15), bi- to tri-seriate, ellipsoidal to broadly fusiform, rounded at both ends, hyaline, 1-septate, constricted at the septum, the upper cell slightly broader and shorter than the lower cell, guttulate, thin-walled, surrounded by a large, irregular, mucilaginous sheath, 10–15 µm wide. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 15 mm in 30 days at 25 °C, black in inner layer and white

**Fig. 62** *Longipedicellata aptrootii* (a–c from MFLU 10-0162, d–k from MFLU 18-1000). **a** Ascomata on the host surface. **b** Section of ascoma. **c** Peridium. **d–g** Asci. **h–k** Ascospores. **k** Ascospores in Indian Ink. Scale bars: **b** = 50  $\mu$ m, **c, h–j** = 10  $\mu$ m, **d–g, k** = 20  $\mu$ m



in outer layer from above, black from below, surface fluffy, with fairly dense mycelium, raised, with a smooth edge.

**Material examined:** THAILAND, Phayao Province, on submerged wood in a stream, 23 February 2018, X.D. Yu, Y22 (MFLU 18-1000), living culture MFLUCC 18-0988.

**Additional material examined:** THAILAND, Chiang Rai Province, on submerged bamboo, 18 January 2010, H. Zhang (MFLU 10-0162).

**Notes:** Our new collection MFLUCC 18-0988 is identified as *Longipedicellata aptrootii* based on their identical LSU and ITS sequence data between MFLUCC 18-0988 and MFLUCC 10-0297, and phylogenetic analysis (Figs. 2, 68). Our collection has wider asci (80–115  $\times$  19–26  $\mu$ m vs. 70–100  $\times$  17–20  $\mu$ m) than the reference specimen in Zhang

et al. (2016). We found numerous pseudoparaphyses in our collection, which were not mentioned in Zhang et al. (2016).

**\**Longipedicellata aquatica*** W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

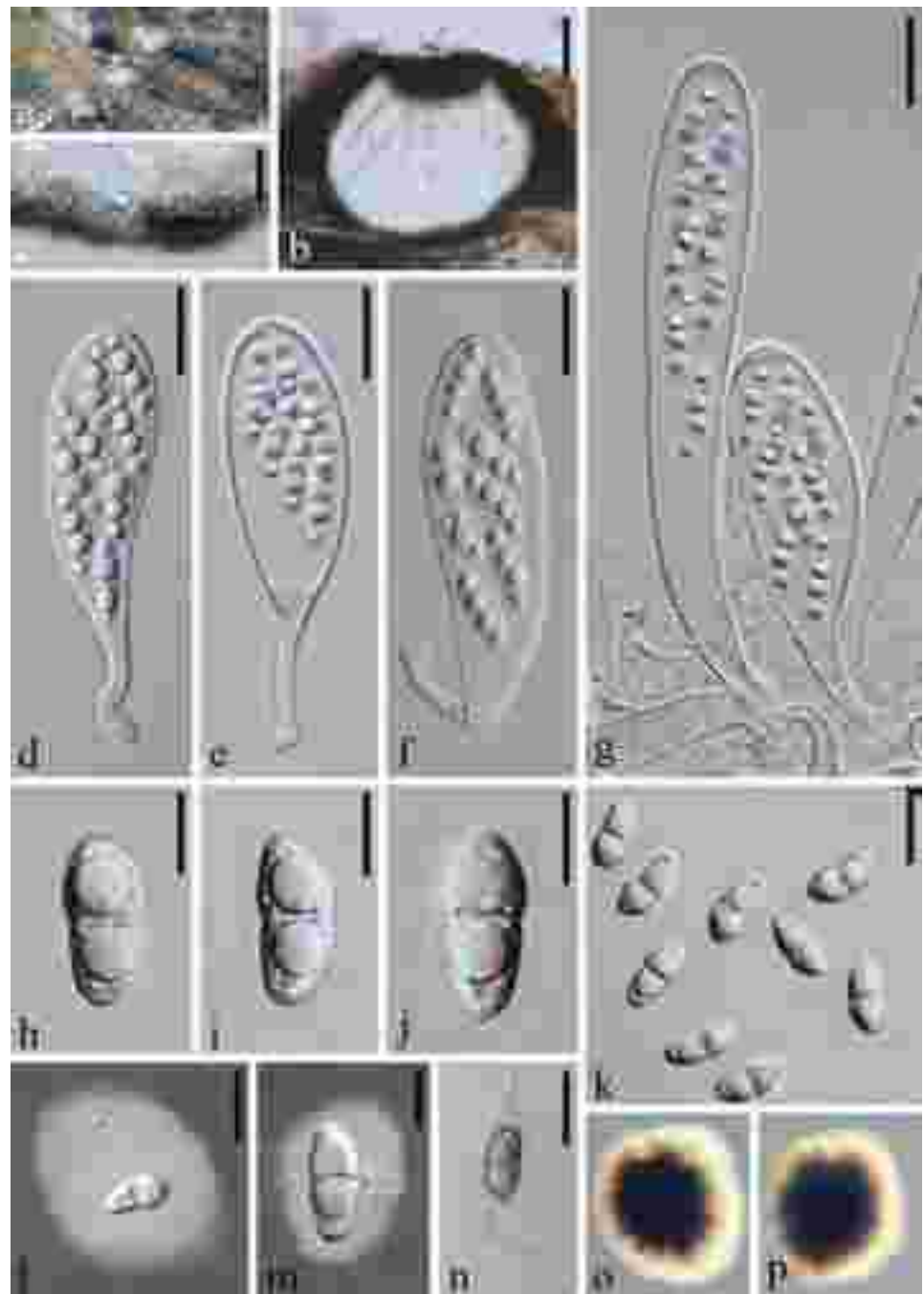
**Index Fungorum number:** IF557915; **Facesoffungi number:** FoF09257; **Fig. 63**

**Etymology:** referring to aquatic habitat of this fungus

**Holotype:** MFLU 17-1686

**Saprobic** on submerged wood in freshwater. **Sexual morph:** *Ascomata* 150–200  $\mu$ m high, 200–250  $\mu$ m diam., lenticular or subglobose, immersed or semi-immersed, covered with a blackened pseudoclypeus, black, with mass of grey-white ascospores pouring out when mature, scattered or clustered, coriaceous, ostiolate. *Peridium* 5–10  $\mu$ m thick,

**Fig. 63** *Longipedicellata aquatica* (MFLU 17-1686, holotype). **a** Appearance of black ascomata on host. **b** Vertical section of ascoma. **c** Structure of peridium. **d–g** Bitunicate asci. (**g** Elongate asci with pseudoparaphyses). **h–k** Ascospores. **l, m** Ascospores in Indian Ink. **n** Geminated ascospore. **o, p** Colony on PDA (left-front, right-reverse). Scale bars: **b** = 50  $\mu$ m, **c–g, k, l, n** = 20  $\mu$ m, **h–j, m** = 10  $\mu$ m



comprising several layers of brown to black, thin-walled cells of *textura angularis*. *Pseudoparaphyses* sparse, cellular, hyphae-like, hyaline, sparsely septate. *Asci* 85–120  $\times$  22–27  $\mu$ m ( $\bar{x}$  = 100  $\times$  24  $\mu$ m,  $n$  = 20), 8-spored, bitunicate, fissitunicate, narrowly to broadly clavate, elongate soon when released in water, up to 180  $\mu$ m long, with a long, straight or twisted pedicel and an ocular chamber. *Ascospores* 19–22  $\times$  8.5–10.5  $\mu$ m ( $\bar{x}$  = 2  $\times$  9.5  $\mu$ m,  $n$  = 20), bi- to tri-seriate, ellipsoidal to broadly fusiform, rounded at both ends, obtuse, hyaline, 1-septate, constricted at the

septum, the upper cell slightly broader and shorter than the lower cell, guttulate, with two prominent guttulate in each cell, becoming one big guttulate when mature, thin-walled, surrounded by a large, obvious, ellipsoidal, mucilaginous sheath, 7–27  $\mu$ m wide, deeply constricted near the ascospores septa. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony filamentous, reaching 20 mm in 15 days at 25  $^{\circ}$ C, black with brown margin from above, black from below, surface fluffy, with aerial mycelium, raised, with a smooth edge.

**Material examined:** THAILAND, Chiang Mai Province, Phrao District, on submerged wood in a stream, 1 September 2017, G.N. Wang, g7 (MFLU 17-1686, **holotype**), ex-type living culture MFLUCC 17-2334; Nakhon Phanom Province, on submerged decaying wood of unidentified plants, 12 December 2018, H. Yang, T12 (MFLU 19-0530), living culture MFLUCC 19-0324; Mukdahan Province, on submerged decaying wood of unidentified plants, 13 December 2018, H. Yang, T45 (MFLU 19-0551), living culture MFLUCC 19-0340.

**Notes:** *Longipedicellata aquatica* clusters with *L. aptrootii* with high bootstrap support (Figs. 2, 68). The asci of *L. aquatica* quickly become elongate when released in water, up to 180 µm long, but not in *L. aptrootii* (Zhang et al. 2016). Additionally, their ascospore sheaths are different (large, obvious, ellipsoidal, mucilaginous sheath, deeply constricted near the ascospore septa in *L. aquatica* vs. large, irregular, mucilaginous sheath in *L. aptrootii*). *Longipedicellata aquatica* is more similar to one collection of *L. aptrootii* (MFLU 16-0032) in having similar ascus size, but differs in having shorter ascospores (19–22 × 8.5–10.5 µm vs. 19–26 µm × 7–11 µm) (Phukhamsakda et al. 2016). After comparison of their single genes, there are three and 21 nucleotide differences in LSU and ITS sequence data, between *L. aquatica* MFLUCC 17-2334 and *L. aptrootii* MFLUCC 10-0297, respectively, which indicates that they are distinct species (Jeewon and Hyde 2016). *Longipedicellata aquatica* sp. nov. is therefore introduced here.

#### Key to freshwater *Longipedicellata* species

1. Asci 70–100 × 17–20 µm, ascospores surrounded by irregular sheath.....*L. aptrootii*
1. Asci 85–120 × 22–27 µm, ascospores surrounded by ellipsoidal sheath, which is deeply constricted near the ascospores septa.....*L. aquatica*

*Pseudoxylomyces* Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 126 (2015)

**Saprobic** on submerged wood. **Sexual morph:** Undetermined. **Asexual morph:** Colonies on natural substratum, scattered to gregarious, dark brown to black, glistening. *Mycelium* mostly immersed, pale brown to reddish brown. *Stromata* lacking. *Conidiophores* septate, brown, branched or not developed. *Conidiogenous cells* holoblastic. *Conidia* single, broadly fusiform or ellipsoidal, straight, with several transverse septa of thick-walled, yellowish brown to dark brown, with paler end cells, without sheath or appendages (Tanaka et al. 2015).

**Type species:** *Pseudoxylomyces elegans* (Goh, W.H. Ho, K.D. Hyde & C.K.M. Tsui) Kaz. Tanaka & K. Hiray.

**Notes:** *Pseudoxylomyces* was proposed to accommodate *Xylomyces elegans* Goh et al., which was isolated from submerged wood in Australia (Goh et al. 1997; Tanaka et al. 2015). *Xylomyces* is characterized as lacking conidiophores and conidiogenous cells, but has large chlamydo-spores (Goos et al. 1977; Goh et al. 1997). In contrast, *Pseudoxylomyces* is quite distinctive in producing broadly fusiform conidia at the tip of the conidiophores (Tanaka et al. 2015). One new species *P. aquaticus* is introduced in *Pseudoxylomyces* in this study.

#### List of freshwater *Pseudoxylomyces* species

\**Pseudoxylomyces aquaticus* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

**Index Fungorum number:** IF557916; **Facesoffungi number:** FoF09258; Fig. 64

**Etymology:** referring to aquatic habitat of this fungus

**Holotype:** HKAS 100937

**Saprobic** on decaying, submerged wood in freshwater.

**Sexual morph:** Undetermined. **Asexual morph:** Colonies scattered or gregarious, effuse, black. *Mycelium* mostly immersed in natural substratum, hyaline to pale brown. *Conidiophores* reduced or absent. *Conidiogenesis* holoblastic. *Conidia* 37–52 × 13–17 µm ( $\bar{x}$  = 43 × 15 µm, n = 20), solitary, dry, broadly fusiform, with 5 thick septa, distinctly constricted at the septa, brown, sometimes the two central cells swollen, guttulate when young, sometimes pale brown at bipolar cells which are easily wizened when old, rough-walled, verruculose, straight, thick-walled.

**Culture characteristics:** On PDA, colony circular, reaching 20 mm in 20 days at 25 °C, pale brown to white from above and below, surface rough, with sparse mycelium, dry, edge entire.

**Material examined:** THAILAND, Chiang Mai Province, Phrao District, on submerged wood in a stream, 1 September 2017, G.N. Wang, 4.14 (HKAS 100937, **holotype**), ex-type living culture KUMCC 17-0312.

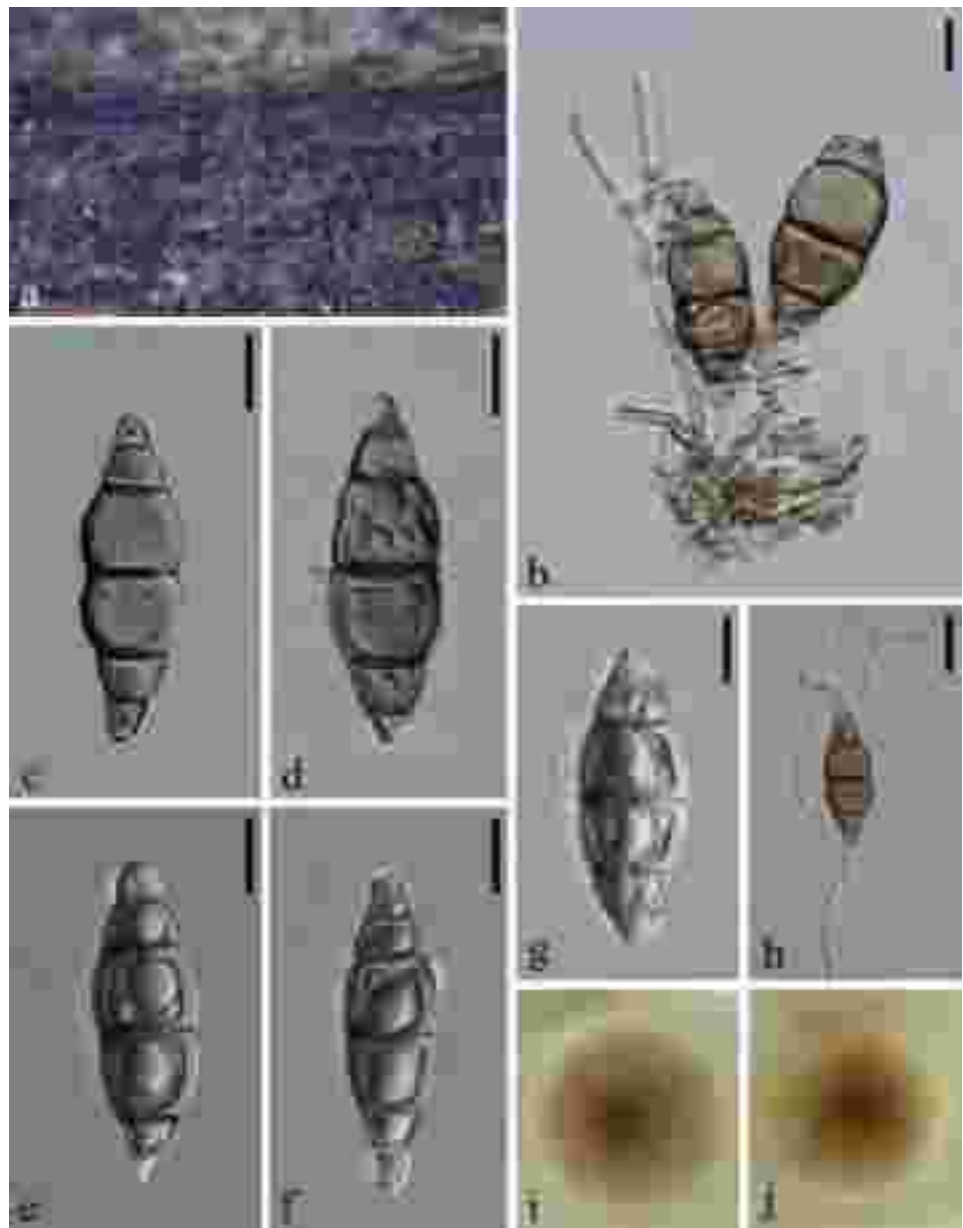
**Notes:** *Pseudoxylomyces aquaticus* shares similar characters with the type species *P. elegans* in having broadly fusiform conidia with thick septa (Goh et al. 1997; Tanaka et al. 2015). However, *P. aquaticus* has smaller conidia (37–52 × 13–17 µm vs. 66–104 × (19–)26–40 µm), fewer and stable number of septa (5-septate vs. 4–7-septate) and different pigmentation (brown vs. yellowish brown to orange brown) (Goh et al. 1997; Tanaka et al. 2015). Phylogenetic analysis supports them to be different species (Figs. 2, 68).

\**Pseudoxylomyces elegans* (Goh et al.) Kaz. Tanaka & K. Hiray., Stud. Mycol. 82: 126 (2015)

**Facesoffungi number:** FoF09259; Figs. 65, 66

**Basionym:** *Xylomyces elegans* Goh, W.H. Ho, K.D. Hyde & K.M. Tsui, Mycol. Res. 101(11): 1324 (1997)

**Fig. 64** *Pseudoxylomyces aquaticus* (HKAS 100937, holotype). **a** Colonies on submerged wood. **b–g** Conidia. **h** Germinated conidium. **i, j** Colony on PDA (left-front, right-reverse). Scale bars: **b–g** = 10  $\mu\text{m}$ , **h** = 20  $\mu\text{m}$



**Freshwater distribution:** Australia (Goh et al. 1997), Brazil (Barbosa and Gusmão 2011), China (Tsui and Hyde 2004), India (Patil and Borse 2015), Japan (Tanaka et al. 2015), Seychelles (Goh et al. 1997), Thailand (Sivichai et al. 2000; this study), USA (Raja et al. 2007)

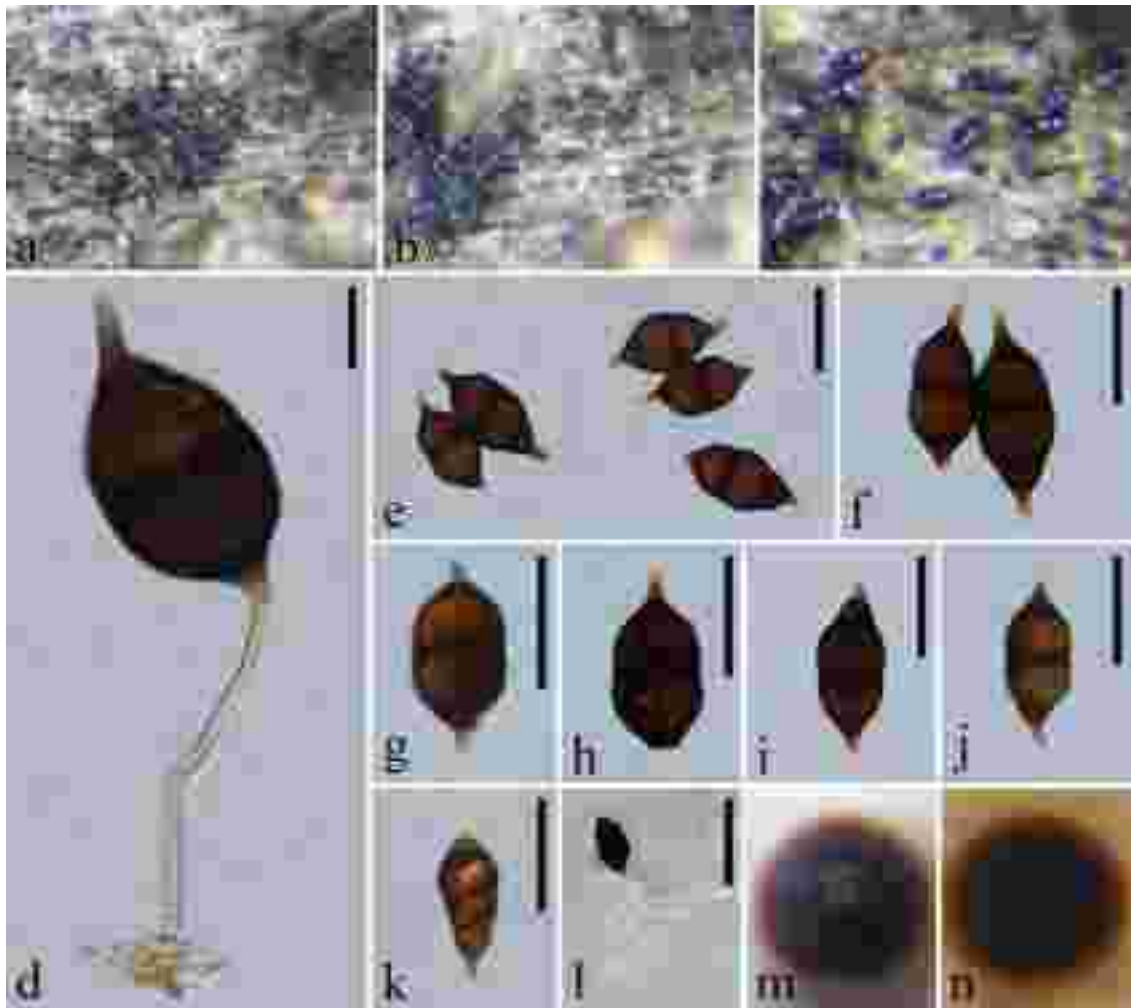
**Saprobic** on decaying, submerged wood in freshwater.

**Sexual morph:** Undetermined. **Asexual morph:** Colonies gregarious or scattered, effuse, black, glistening. Mycelium partly immersed in natural substratum, composed of hyaline to pale brown, septate hyphae. Conidiophores up to 70  $\mu\text{m}$  long, semi-macronematous, mononematous, unbranched, septate, not constricted at the septa, smooth, hyaline to pale brown to brown, thin-walled. Conidiogenous cells 16–20  $\times$  3.5–4.5  $\mu\text{m}$ , holoblastic, monoblastic, integrated,

determinate, terminal, cylindrical, hyaline to pale brown, smooth-walled. Conidia (70–)80–90(–100)  $\times$  29–37(–40.5)  $\mu\text{m}$  ( $\bar{x}$  = 87.5  $\times$  35  $\mu\text{m}$ ,  $n$  = 15), solitary, dry, mostly broadly ellipsoidal with suddenly tapering ending cells, 5–6(–7) thick septate, distinctly constricted and darker at the septa, two central cells mostly swollen and greatly enlarged, which almost occupy 1/2–3/5 of the whole conidia, reddish brown to dark brown, guttulate when young, straight or slightly curved, minutely rough-surfaced, thick-walled; ending cells long or short, subcylindrical, paler, rounded and easily wizened when old.

**Culture characteristics:** On PDA, colony circular, reaching 30 mm in 35 days at 25  $^{\circ}\text{C}$ , reddish brown to pale grey from above, reddish brown from below, surface rough, with





**Fig. 65** *Pseudoxylomyces elegans* (MFLU 17-1712). **a–c** Colonies on submerged wood. **d** Conidiophore bearing conidium. **e–k** Conidia. **l** Germinated conidium. **m, n** Colony on PDA (left-front, right-reverse). Scale bars: **d** = 20  $\mu$ m, **e–k** = 50  $\mu$ m, **l** = 100  $\mu$ m

sparse mycelium, mostly immersed in culture, dry, edge entire.

**Material examined:** THAILAND, Nan Province, on submerged wood in a stream, 4 August 2017, Saranyaphat Boonmee, DP8-1 (MFLU 17-1712), living culture MFLUCC 17-2350; *ibid.*, DP8-2, living culture MFLUCC 17-2351.

**Additional material examined:** JAPAN, Okinawa, Isl. Iriomote, Oomijya-river, on submerged twigs of woody plant, 12 July 2011, K. Tanaka & K. Hirayama, KT 2887 = HHUF 30139, culture MAFF 243852.

**Notes:** Our collection MFLUCC 17-2350 clusters with *Pseudoxylomyces elegans* (KT 2887) with strong bootstrap support (Figs. 2, 68). They share similar morphology in conidial size, pigmentation and septa (Goh et al. 1997; Tanaka et al. 2015). Comparing individual nucleotides between MFLUCC 17-2350 and KT 2887, we noticed 100% similarities in LSU sequence data, nine and eight nucleotide differences in ITS and TEF sequence data, respectively.

Based on their very similar morphology and no resolution in the phylogenetic tree (Figs. 2, 68), MFLUCC 17-2350 is, therefore, identified as *P. elegans*. The conidia of *P. elegans* are smooth-walled in HKU (M) 3242 (Goh et al. 1997), we observed the rough-walled conidia in our collection MFLUCC 17-2350. The conidia in our collection mostly have two swollen and greatly enlarged, central cells, which are not much obvious in HKU (M) 3242 and KT 2887. The strain SS1077 clusters basal to *P. elegans* with high bootstrap support (Fig. 68). SS1077 is named as *P. elegans* in Prihatini et al. (2008), the real identity of SS1077 is, however, better confirmed until more evidences are obtained. We name SS1077 as *Pseudoxylomyces* sp. as its morphology is unavailable and only ITS sequence data is deposited in GenBank.

#### Key to freshwater *Pseudoxylomyces* species



**Fig. 66** *Pseudoxylomyces elegans* (MAFF 243852 = KT 2887). **a, b** Asexual morph produced on agar medium. **c** Conidiophores and conidia. **d, e** Conidia. Scale bars: **a** = 1 mm, **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d, e** = 20  $\mu$ m

1. Conidia 37–52  $\times$  13–17  $\mu$ m.....*P. aquaticus*  
 1. Conidia 66–104  $\times$  (19–)26–40  $\mu$ m.....*P. elegans*

***Submersispora*** W. Dong, H. Zhang & K.D. Hyde, *gen. nov.*

*Index Fungorum number*: IF557805; *Facesoffungi number*: FoF08719

*Etymology*: referring to submerged habitat of this fungus

*Saprobic* on decaying, submerged wood in freshwater. **Sexual morph**: Undetermined. **Asexual morph**: Colonies broadly punctiform, gregarious or scattered or solitary, raised, black. *Mycelium* mostly immersed in natural substratum, consisting of branched, septate, thin-walled, smooth, hyaline to pale brown hyphae. *Conidiophores* semi-macronematous, mononematous, cylindrical, slender, unbranched, septate, smooth, pale brown, thin-walled. *Conidiogenous cells* monoblastic, holoblastic, integrated, determinate, cuneiform, pale brown, smooth. *Conidia* acrogenous, solitary, dry, variable in shape, septate, brown to dark black, smooth, thin-walled.

*Type species*: *Submersispora variabilis* W. Dong, H. Zhang & K.D. Hyde

*Notes*: Two freshwater genera *Longipedicellata* and *Pseudoxylomyces* are accommodated in Longipedicellataceae (Goh et al. 1997; Tanaka et al. 2015; Phukhamsakda et al. 2016; Zhang et al. 2016). Phylogenetic analyses show that our collection MFLUCC 17-2360 nests between *Longipedicellata* and *Pseudoxylomyces* with strong bootstrap support (Figs. 2, 68). Both *Longipedicellata* and *Pseudoxylomyces* have chlamydospore-like structures or chlamydospores (Goh et al. 1997; Phukhamsakda et al. 2016). Our collection

resembles *Pseudoxylomyces* in having holoblastic conidiogenous cells, but differs in having slender, semi-macronematous conidiophores and variable shaped conidia. Thus, the third freshwater genus *Submersispora* is introduced in this study.

**List of freshwater *Submersispora* species**

\**Submersispora variabilis* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

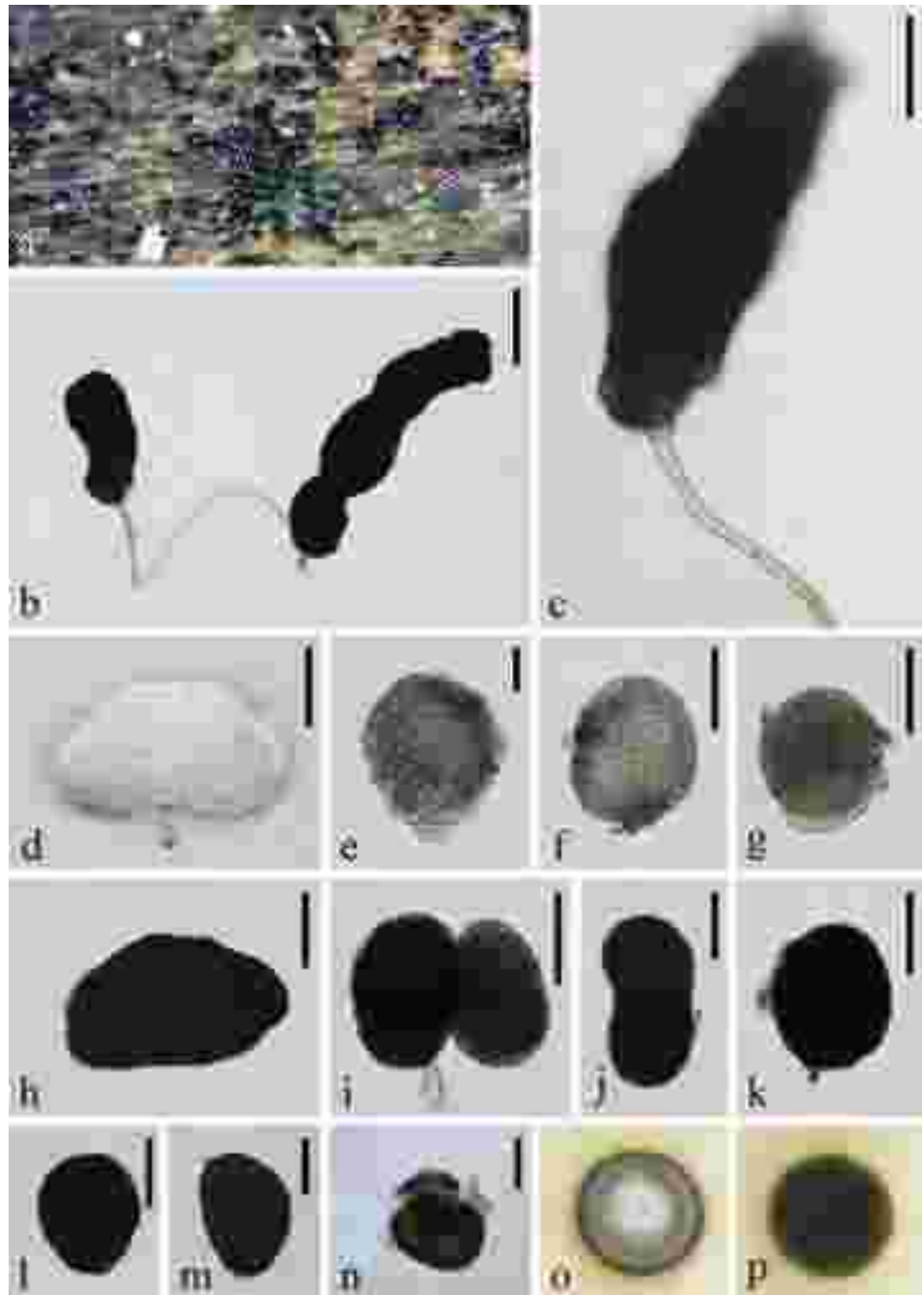
*Index Fungorum number*: IF557917; *Facesoffungi number*: FoF08720; Fig. 67

*Etymology*: referring to variable shape of conidia

*Holotype*: MFLU 17-1719

*Saprobic* on decaying, submerged wood in freshwater. **Sexual morph**: Undetermined. **Asexual morph**: Colonies broadly punctiform, gregarious or scattered or solitary, raised, black. *Mycelium* mostly immersed in natural substratum, consisting of branched, septate, thin-walled, smooth, hyaline to pale brown hyphae. *Conidiophores* up to 230  $\mu$ m long, 3  $\mu$ m wide, semi-macronematous, mononematous, cylindrical, slender, unbranched, septate, slightly constricted at the septa, smooth, pale brown, thin-walled. *Conidiogenous cells* 5.2–6.4  $\times$  5.5–6  $\mu$ m ( $\bar{x}$  = 5.8  $\times$  5.7  $\mu$ m,  $n$  = 5), monoblastic, holoblastic, integrated, determinate, cuneiform, pale brown, smooth. *Conidia* 26.5–185.5  $\times$  35–90  $\mu$ m ( $\bar{x}$  = 96.5  $\times$  65.7  $\mu$ m,  $n$  = 30), acrogenous, solitary, dry, variable in shape, mostly subglobose, ellipsoidal, oblong, irregular, septate, muriform, slightly constricted at the septa, comprising large number of angular cells, hyaline to brown when young, dark black and invisible for septa when mature, smooth, thin-walled.

**Fig. 67** *Submersispora variabilis* (MFLU 17-1719, **holotype**). **a** Colonies on submerged wood. **b** Conidiophores with conidia. **c** Conidium, conidiogenous cell and conidiophore. **d–m** Conidia. **n** Germinated conidium. **o, p** Colony on PDA (left-front, right-reverse). Scale bars: **b, f–n** = 50  $\mu$ m, **c–e** = 20  $\mu$ m



*Culture characteristics:* On PDA, colony circular, reaching 30 mm in 15 days at 25 °C, grey in the middle with dark brown margin from above, dark brown from below, surface rough, fluffy, with dense mycelium, dry, raised, edge entire.

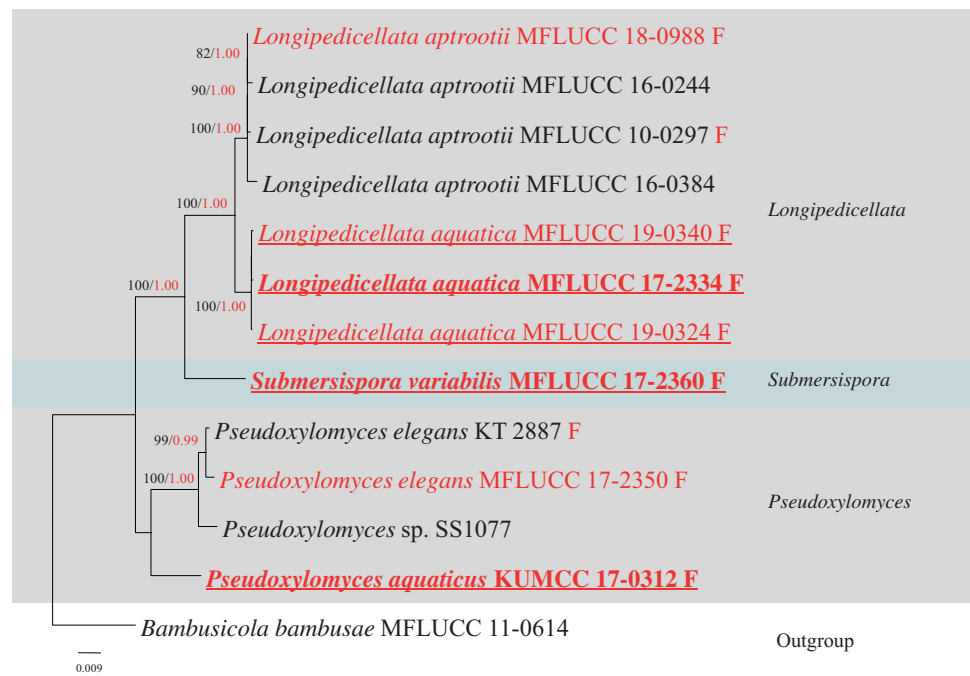
*Material examined:* THAILAND, Chiang Mai Province, MRC, on submerged wood in a stream, 1 September 2017, X.D. Yu, 2A (MFLU 17-1719, **holotype**), ex-type living culture MFLUCC 17-2360.

**Lophiostomataceae** Sacc. [as ‘Lophiostomaceae’], Syll. fung. (Abellini) 2: 672 (1883)

**Key to freshwater genera of Lophiostomataceae**

1. Ascospores pigmented.....2
1. Ascospores hyaline.....4
2. Ascospores evenly coloured, yellowish brown to dark brown.....3

**Fig. 68** Phylogram generated from maximum likelihood analysis of combined LSU, SSU, ITS and TEF sequence data for species of Longipedicellataceae. Bootstrap values for maximum likelihood equal to or greater than 75% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. The tree is rooted to *Bambusicola bambusae* MFLUCC11-0614 (Bambusicolaceae)



2. Ascospores pale yellowish to olivaceous brown, but pale at the terminal cells.....*Neotrematosphaeria*
3. Ascospores fusiform, often with hyaline appendages...  
.....*Biappendiculispora*
3. Ascospores cigar-shaped or ellipsoidal-fusiform, sometimes with terminal appendages.....*Sigarispora*
4. Ascospores with longest sheath drawn out to form polar appendages with tips of the sheath capped.....*Pseudocapulatispora*
4. Ascospores appendages not as above.....5
5. Ascospores with a mucilaginous collar around its equator, surrounded by a spreading papilionaceous sheath...  
.....*Vaginatispora*
5. Ascospores without a spreading papilionaceous sheath.....6
6. Ascospores with globose appendages at both ends.....  
.....*Neovaginatispora*
6. Ascospores without globose appendages.....7
7. Ascospores with an internal chamber and a narrow sheath drawn out at both ends.....*Crassiclypeus*
7. Ascospores without an internal chamber or a sheath...8
8. Ascomata sometimes with short papilla.....*Lentistoma*
8. Ascomata often with crest-like apex.....9
9. Ascospores with a narrow, bipolar sheath and coelomycetous asexual morph.....*Flabellascoma*
9. Ascospores not only as above.....*Lophiostoma*

*Biappendiculispora* Thambug., Kaz. Tanaka & K.D. Hyde, Fungal Diversity 74: 214 (2015)

*Saprobic* on dead herbaceous stems or culms of Poaceae or submerged wood. **Sexual morph:** *Ascomata* solitary to gregarious, immersed, with papilla erumpent through host surface, reddish brown to black, subglobose, coriaceous, ostiolate. *Ostiole* variable in shape, central, with a pore-like opening, plugged by gelatinous tissue, made of hyaline, pseudoparenchymatous cells. *Peridium* composed of a single stratum, with several layers of cells of *textura angularis*, fusing with the host tissues in outer layer. *Pseudoparaphyses* numerous, cellular, hypha-like, hyaline, septate, branched. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with short, bulbous pedicel, with an ocular chamber. *Ascospores* uni- to bi-seriate, fusiform, with acute ends, septate, constricted at the septa, pale yellowish to brown, with hyaline appendages (Thambugala et al. 2015; Bao et al. 2019c). **Asexual morph:** Undetermined.

*Type species:* *Biappendiculispora japonica* Thambug., Wanas., Kaz. Tanaka & K.D. Hyde

*Notes:* The monotypic genus *Biappendiculispora* was introduced for *B. japonica* (Thambugala et al. 2015) which was initially described as *Lophiostoma caulium* “var. f” (Tanaka and Harada 2003b). Three strains of *B. japonica* collected in Japan clustered in a separated clade with *Lophiostoma* (Thambugala et al. 2015; Bao et al. 2019c; this study, Fig. 74). Bao et al. (2019c) found this species from freshwater in China.

#### List of freshwater *Biappendiculispora* species

\**Biappendiculispora japonica* Thambug., Wanas., Kaz. Tanaka & K.D. Hyde, Fungal Diversity 74: 214 (2015)

*Freshwater distribution:* China (Bao et al. 2019c)

**Crassiclypeus** A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 167 (2018)

*Saprobic* on submerged dead twigs of woody plant. **Sexual morph:** *Ascomata* scattered to gregarious, immersed, subglobose, dark brown to black, ostiolate. *Ostiolar neck* crest-like, elongate, laterally compressed, surrounded by a well-developed clypeus. *Peridium* uniform, composed of elongate, brown cells, surrounded by brown hyphae. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, septate, branching and anastomosing. *Asci* 8-spored, bitunicate, fissitunicate, clavate, pedicellate, apically rounded with an ocular chamber. *Ascospores* fusiform, hyaline, septate, smooth, with an internal chamber and a narrow sheath which drawn out at both ends (Hashimoto et al. 2018). **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, superficial to immersed, scattered or clustered in small groups, globose to subglobose. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* phialidic, ampliform, hyaline, smooth. *Conidia* subglobose with rounded ends, hyaline, aseptate, smooth (Hashimoto et al. 2018).

*Type species:* *Crassiclypeus aquaticus* A. Hashim., K. Hiray. & Kaz. Tanaka

*Notes:* *Crassiclypeus* was introduced to accommodate the freshwater species *C. aquaticus* collected in Japan (Hashimoto et al. 2018). The asexual morph has subglobose conidia formed in culture (Hashimoto et al. 2018). *Crassiclypeus aquaticus* clusters basal to *Flabellascoma* with moderate bootstrap support (Fig. 74). *Crassiclypeus aquaticus* shares similar morphological characters with *F. fusiforme* in having fusiform, hyaline ascospores with a narrow, bipolar sheath. However, an internal chamber was not observed in the ascospores of *F. fusiforme* (see description of *F. fusiforme* below).

#### List of freshwater *Crassiclypeus* species

\**Crassiclypeus aquaticus* A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 167 (2018)

*Freshwater distribution:* Japan (Hashimoto et al. 2018).

**Flabellascoma** A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 167 (2018)

*Saprobic* on plant materials and submerged wood. **Sexual morph:** *Ascomata* scattered, immersed with neck erumpent through host surface, subglobose to ellipsoidal. *Ostiolar neck* elongate, laterally compressed. *Peridium* composed of elongate, brown to dark brown cells. *Pseudoparaphyses* numerous, cellular, hypha-like, hyaline, septate, branching and anastomosing. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate. *Ascospores* fusiform, hyaline, septate, with a narrow, bipolar sheath

(Hashimoto et al. 2018). **Asexual morph:** Coelomycetous. *Conidiomata* pycnidial, globose to subglobose. *Peridium* composed of subglobose to rectangular, brown cells. *Conidiophores* absent. *Conidiogenous cells* holoblastic, cylindrical or ampliform, hyaline, smooth. *Conidia* subglobose with rounded ends, hyaline, smooth, aseptate (Hashimoto et al. 2018) (Fig. 68).

*Type species:* *Flabellascoma minimum* A. Hashim., K. Hiray. & Kaz. Tanaka

*Notes:* *Flabellascoma* was introduced to accommodate *F. cycadicola* A. Hashim. et al. and *F. minimum*. *Flabellascoma* is morphologically similar to *Pseudolophiostoma* Thambug. et al. in *ascomata* with a well-developed, crest-like ostiolar neck (Thambugala et al. 2015; Hashimoto et al. 2018), but phylogeny separates them as distinct genera (Hashimoto et al. 2018; this study, Fig. 74). Two freshwater species *F. aquaticum* D.F. Bao et al. and *F. fusiforme* D.F. Bao et al. are morphologically very similar, the former has a long, cylindrical neck, but the latter has a short, crest-like neck (Bao et al. 2019c). They also can be distinguished by the size of *asci* (see key below).

#### List of freshwater *Flabellascoma* species

\**Flabellascoma aquaticum* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Mycosphere 10, 1085 (2019)

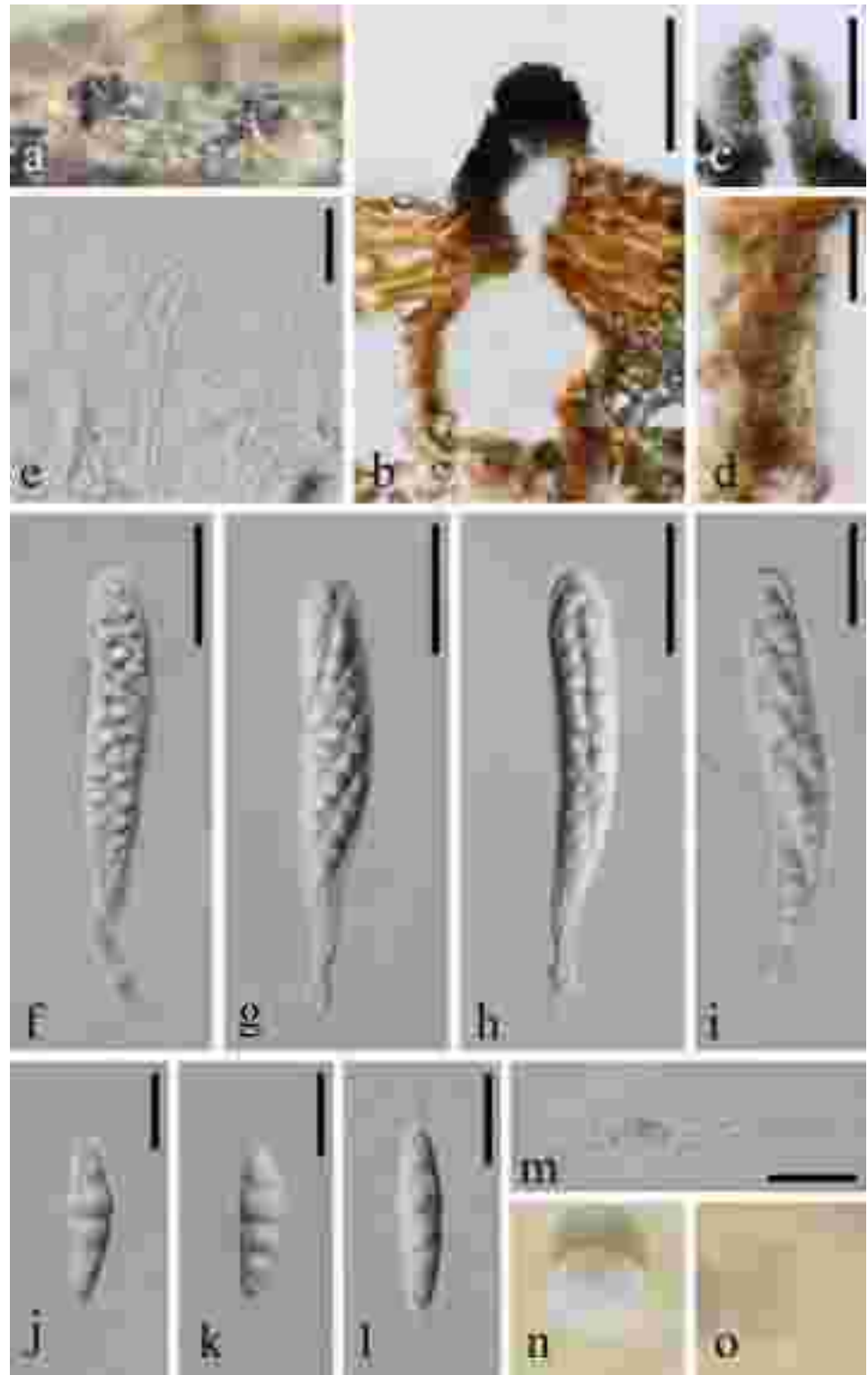
*Freshwater distribution:* China (Bao et al. 2019c)

\**Flabellascoma fusiforme* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Mycosphere 10, 1089 (2019); Fig. 69

*Freshwater distribution:* China (Bao et al. 2019c; this study)

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* 160–180 µm high, 140–150 µm diam., black, scattered, immersed with neck erumpent through host surface, subglobose. *Ostiolar neck* crest-like, elongate, compressed, 130–150 µm high, 75–85 µm wide, composed of several layers of brown to black, thick-walled cells of *textura angularis* or globose cells. *Peridium* 15–25 µm thick, composed of brown to dark brown, thin-walled cells of *textura angularis* or rectangular cells. *Pseudoparaphyses* 2 µm wide, numerous, trabeculate, hyaline, sparsely septate, indistinctly branched. *Asci* 8-spored, bitunicate, clavate, 66–87 × 8–11.5 µm ( $\bar{x}$  = 74.8 × 10 µm, n = 10), apically rounded, with an ocular chamber and a short pedicel. *Ascospores* 15–22 × 4–6 µm ( $\bar{x}$  = 18 × 5 µm, n = 10), fusiform, biserial, straight or slightly curved, hyaline, 1-septate, deeply constricted at the septum, slightly constricted at a quarter, the upper cell slightly broader than the lower one, sharply or gradually narrowed towards two ends, two prominent guttulate in each cell, thin-walled, smooth, with a narrow, hyaline, lateral narrowly pad-like sheath, 3.5–7 µm long at both ends. **Asexual morph:** Undetermined.

**Fig. 69** *Flabellascoma fusi-forme* (MFLU 18-1167). **a** Appearance of necks erumpent through host substrate. **b** Vertical section of ascoma. **c** Vertical section of neck. **d** Structure of peridium. **e** Pseudoparaphyses. **f–i** Bitunicate asci. **j–l** Ascospores. **m** Germinated ascospore. **n, o** Colony on PDA (left-front, right-reverse). Scale bars: **b** = 100  $\mu$ m, **c** = 50  $\mu$ m, **d–i** = 20  $\mu$ m, **j–l** = 10  $\mu$ m, **m** = 40  $\mu$ m



**Culture characteristics:** On PDA, colony circular, reaching 5 mm in 15 days at 25 °C, white from above, pale brown from below, surface rough, with dense mycelium, dry, raised, edge entire.

**Material examined:** CHINA, Yunnan Province, Pingbian, on submerged wood in a stream, 20 September 2017, W. Dong, PB30A (MFLU 18-1167), living culture MFLUCC 18-1019.

**Notes:** Our collection MFLUCC 18-1019 has identical LSU and ITS sequence data to *F. fusiforme*, which indicates that they are conspecific. Our collection has smaller ascospores (160–180 × 140–150 μm vs. 310–420 × 320–380 μm) and a thinner peridium (15–25 μm vs. 25–50 μm) than the holotype (Bao et al. 2019c). Phylogenetic analysis also supports that they are the same species (Fig. 74).

#### Key to freshwater *Flabellascoma* species

1. Asci 66–80 × 10–12 μm.....*F. fusiforme*
1. Asci 48–72 × 8–9 μm.....*F. aquaticum*

***Lentistoma*** A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 169 (2018)

**Saprobic** on woody plant or submerged wood. **Sexual morph:** *Ascomata* scattered, immersed to erumpent, subglobose or conical, with ostiolar, elongate, laterally compressed neck, surrounded by a well-developed clypeus or with ostiolate, short papilla. *Peridium* composed of globose, brown or black cells. *Pseudoparaphyses* numerous, cellular, hypha-like, narrow, indistinctly septate, branching and anastomosing. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate. *Ascospores* fusiform, hyaline, septate, with or without a bipolar sheath (Hashimoto et al. 2018). **Asexual morph:** Undetermined.

**Type species:** *Lentistoma bipolare* (K.D. Hyde) A. Hashim., K. Hiray. & Kaz. Tanaka

**Notes:** *Lentistoma* was introduced based on *Massarina bipolaris* K.D. Hyde (Hashimoto et al. 2018), which was collected from woody plant or submerged wood. We introduce the second *Lentistoma* species which was collected from submerged wood in Thailand.

#### List of freshwater *Lentistoma* species

\**Lentistoma aquaticum* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

**Index Fungorum number:** IF557918; **Facesoffungi number:** FoF09260; Fig. 70

**Etymology:** referring to aquatic habitat of this fungus

**Holotype:** MFLU 18-1718

**Saprobic** on decaying wood submerged in freshwater. **Sexual morph:** *Ascomata* 270–320 μm high, 580–620 μm wide, black, scattered, immersed to erumpent, conical, carbonaceous, with ostiolar, short papilla. *Peridium* 50–70 μm at the sides, 20–30 μm at the base, comprising carbonaceous, occluded dark cells, easily cracked, flattened at the base. *Pseudoparaphyses* 3.5 μm wide, numerous, cellular, hypha-like, hyaline, distantly septate. *Asci* (90–)130–160 × (12–)15–19(–21) μm ( $\bar{x}$  = 145 × 17 μm, n = 10), 8-spored, bitunicate, narrowly to broadly clavate, short pedicellate, apically rounded with well-developed ocular chamber.

*Ascospores* 38–43 × 6.5–8.5 μm ( $\bar{x}$  = 40 × 7.5 μm, n = 20), biseriate or overlapping triseriate, slightly curved, hyaline, 1-septate, constricted at the septa, fusiform, guttulate, thin-walled, smooth, lacking a mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony irregular, reaching 15 mm in 15 days at 25 °C, white from above, white to pale yellow from below, surface rough, dry, raised, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat3106-1 (MFLU 18-1718, **holotype**), ex-type living culture MFLUCC 18-1275; *ibid.*, hat3106-2 (HKAS 105032, **isotype**), ex-type living culture KUMCC 19-0047.

**Notes:** *Lentistoma aquaticum* clusters with *L. bipolare* with strong bootstrap support (Fig. 74). *Lentistoma bipolare* has crest-like, elongate, laterally compressed neck, while *L. aquaticum* has ostiolate and short papilla. *Lentistoma aquaticum* has longer and wider asci ((90–)130–160 × (12–)15–19(–21) μm vs. (82–) 105–140 × 8–15 μm) and longer ascospores (38–43 × 6.5–8.5 μm vs. 20–33 × 5.5–9(–11) μm) than *L. bipolare* (Hashimoto et al. 2018). Additionally, an internal chamber and a narrow sheath drawn out at both ends are described for the ascospores of *L. bipolare*, but not *L. aquaticum* (Hashimoto et al. 2018). There are 3, 31 and 13 nucleotide differences in LSU, ITS and TEF sequence data, respectively, between *L. aquaticum* MFLUCC 18-1275 and *L. bipolare* CBS 115375 (ex-type). Thus, *L. aquaticum* sp. nov. is introduced according to the guideline of Jeewon and Hyde (2016).

\**Lentistoma bipolare* (K.D. Hyde) A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 169 (2018)

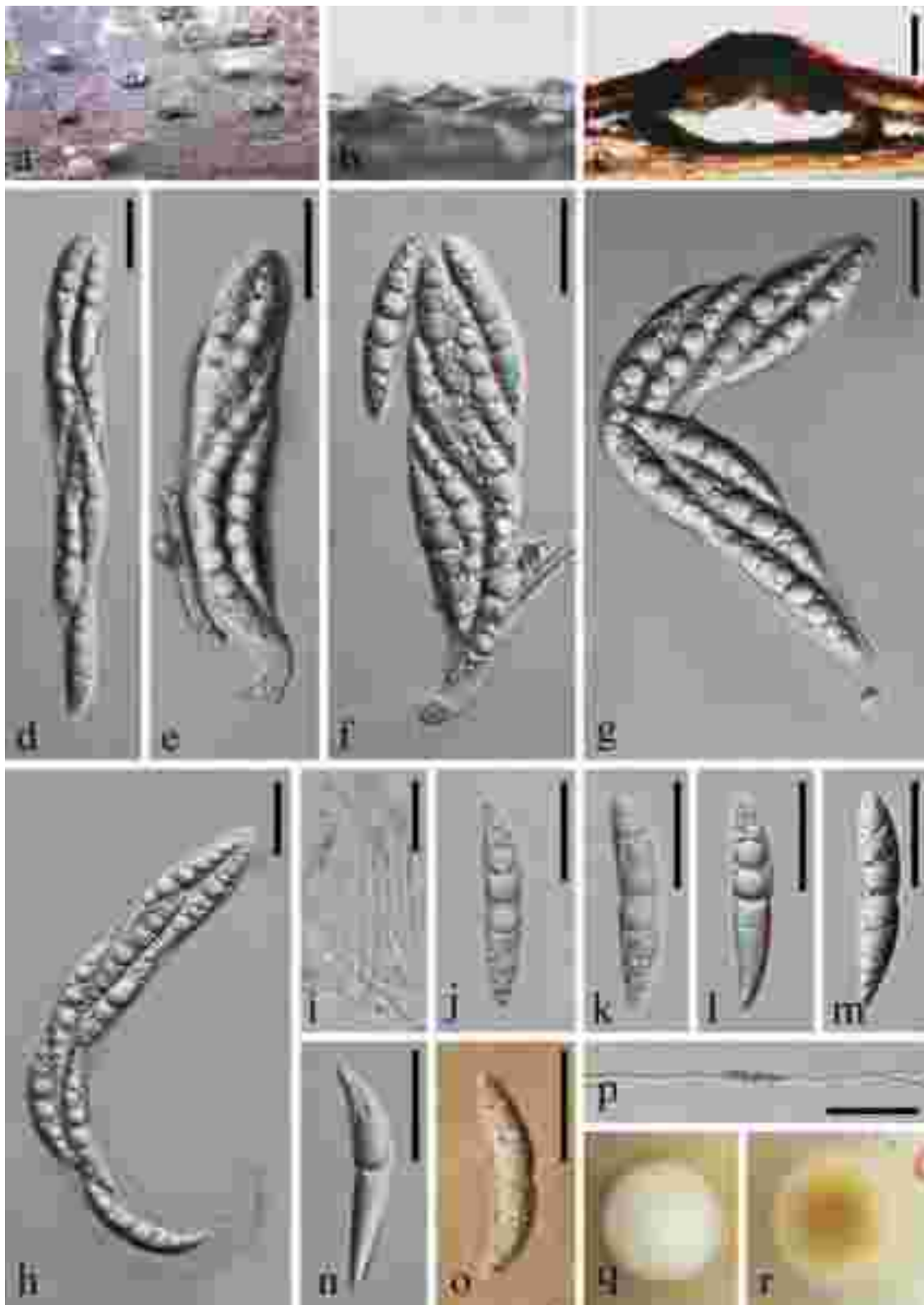
**Basionym:** *Massarina bipolaris* K.D. Hyde, Nova Hedwigia 61(1-2): 131 (1995)

**Synonymy:** *Lophiostoma bipolare* (K.D. Hyde) E.C.Y. Liew, Aptroot & K.D. Hyde, Mycologia 94(5): 812 (2002)

**Freshwater distribution:** Australia (Hyde 1995c; Hyde and Aptroot 1998b), Brunei (Ho et al. 2001), China (Hyde 1995a; Tsui et al. 2000; Ho et al. 2001, 2002; Luo et al. 2004), Japan (Hashimoto et al. 2018), Malaysia (Hyde and Aptroot 1998b), Seychelles (Hyde and Goh 1998b), South Africa (Hyde and Goh 1998b), Thailand (Sivichai et al. 2000, 2002), USA (Raja et al. 2009b)

#### Key to freshwater *Lentistoma* species

1. *Ascomata* with ostiolar, elongate, laterally compressed neck, surrounded by a well-developed clypeus, ascospores 20–33 × 5.5–9(–11) μm, with a narrow sheath.....*L. bipolare*
1. *Ascomata* with ostiolar, short papilla, ascospores 38–43 × 6.5–8.5 μm, without sheath.....*L. aquaticum*



**Fig. 70** *Lentistoma aquaticum* (MFLU 18-1718, **holotype**). **a**, **b** Appearance of ascomata on host surface. **c** Vertical section of ascoma. **d–h** Bitunicate asci. **i** Pseudoparaphyses. **j–n** Ascospores.

**o** Ascospore in Indian Ink. **p** Germinated ascospore. **q**, **r** Colony on PDA (left-front, right-reverse). Scale bars: **c** = 100  $\mu$ m, **d–o** = 20  $\mu$ m, **p** = 50  $\mu$ m



**Lophiostoma** Ces. & De Not., Comm. Soc. crittog. Ital. 1(4): 219 (1863)

*Saprobic* on herbaceous and woody substrates in terrestrial and aquatic habitats. **Sexual morph:** *Ascomata* scattered to gregarious, immersed to semi-immersed, papilla erumpent through host surface, uniloculate, globose to subglobose, glabrous, dark brown to black, coriaceous to carbonaceous, ostiolate. *Ostiole* slit-like, with crest-like apex, usually with a pore opening, plugged by gelatinous tissue. *Peridium* wider at the apex and thinner at the base, composed of a single stratum, comprising several layers of lightly pigmented to dark brown, thin-walled cells, outer layer fusing and indistinguishable from the host tissues. *Pseudoparaphyses* numerous, cellular, hypha-like, septate, branched, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindro-clavate, short to long pedicellate, rounded at the apex with an ocular chamber. *Ascospores* uni- or partially bi-seriate, fusiform, straight to slightly curved, hyaline to yellowish brown, 1- to multi-septate, sometimes with 3–5-eusepta, constricted at the central septum (Thambugala et al. 2015). **Asexual morph:** Coelomycetous. *Conidiomata* scattered or clustered, subglobose with a flattened base, covered with brownish, septate hyphae. *Neck* central, cylindrical, composed of dark brown, polygonal to subglobose cells. *Pycnidial wall* slightly thinner at the base, composed of subglobose to polygonal cells. *Conidiophores* septate, branched, hyaline. *Conidiogenous cells* phialidic. *Conidia* ellipsoidal to obovoid, aseptate, hyaline, smooth (Tanaka and Harada 2003b).

*Type species:* *Lophiostoma macrostomum* (Tode) Ces. & De Not.

*Notes:* *Lophiostoma* is an old genus with over 400 epithets recorded (Index Fungorum 2020). *Lophiostoma* was confirmed in Lophiostomataceae based on multigene phylogenetic analyses (Thambugala et al. 2015). Hashimoto et al. (2018) revisited 11 species that were classified as *Lophiostoma bipolare* complex, and proposed five new genera. The asexual morph was found in the culture of *L. semiliberum* (Desm.) Ces. & De Not. and characterized by subglobose conidiomata covered by hyphae, phialidic conidiogenous cells and ellipsoid to obovoid conidia (Tanaka and Harada 2003b). Six species have been found from freshwater habitats, but none of them is confirmed by molecular evidence. Of the six freshwater species, *L. purpurascens* is the only species staining the substrate purple (Hyde and Aptroot 1998b). Staining the substrate purple was also observed in other freshwater species, e.g. *Ammiculicola parva*, *Jahnula purpurea*, *Purpureofaciens aquatica* (Zhang et al. 2009b; Fournier et al. 2015; this study). Fresh collections are needed to verify the taxonomic placement of these freshwater species.

#### List of freshwater *Lophiostoma* species

***Lophiostoma aquaticum*** (J. Webster) Aptroot & K.D. Hyde, Fungal Diversity Res. Ser. 7: 106 (2002)

*Basionym:* *Massarina aquatica* J. Webster, Trans. Br. mycol. Soc. 48(3): 451 (1965)

*Freshwater distribution:* Brunei (Ho et al. 2001), England (Webster 1965), Malaysia (Ho et al. 2001), South Africa (Hyde and Aptroot 1998b; Hyde et al. 1998)

***Lophiostoma frondisubmersum*** (K.D. Hyde) E.C.Y. Liew, Aptroot & K.D. Hyde, Mycologia 94(5): 812 (2002)

*Basionym:* *Massarina fronsisubmersa* K.D. Hyde, Mycol. Res. 98(7): 724 (1994)

*Freshwater distribution:* New Guinea (Hyde 1994), USA (Raja et al. 2009b)

***Lophiostoma lunisporum*** (K.D. Hyde & Goh) Aptroot & K.D. Hyde, Fungal Diversity Res. Ser. 7: 107 (2002)

*Basionym:* *Massarina lunispora* K.D. Hyde & Goh, Mycol. Res. 102(6): 741 (1998)

*Freshwater distribution:* Australia (Hyde and Goh 1998a)

***Lophiostoma maquilangense*** K.D. Hyde and Aptroot, Nova Hedwigia 66(3–4): 499 (1998)

*Freshwater distribution:* Philippines (Hyde and Aptroot 1998b)

***Lophiostoma proprietunicatum*** K.M. Tsui, K.D. Hyde & Hodgkiss, Fungal Diversity Res. Ser. 7: 107 (2002)

*Basionym:* *Massarina proprietunicata* K.M. Tsui, K.D. Hyde & Hodgkiss, Mycol. Res. 103(12): 1575 (1999)

*Freshwater distribution:* China (Tsui et al. 1999)

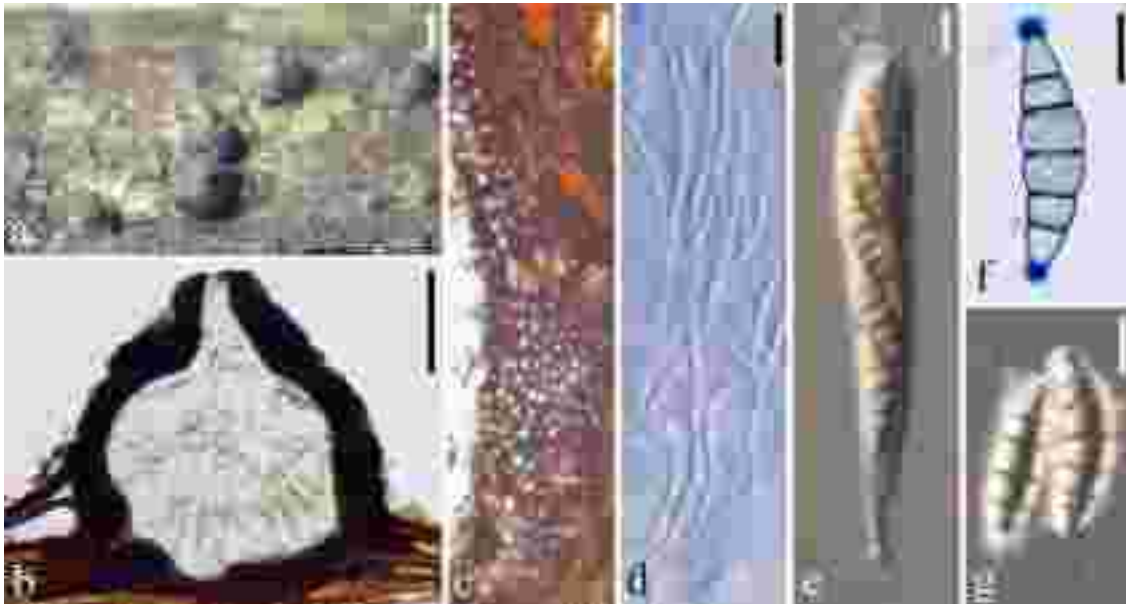
***Lophiostoma purpurascens*** (K.D. Hyde & Aptroot) Aptroot & K.D. Hyde, Fungal Diversity Res. Ser. 7: 108 (2002)

*Basionym:* *Massarina purpurascens* K.D. Hyde & Aptroot, Nova Hedwigia 66(3–4): 496 (1998)

*Freshwater distribution:* Australia (Hyde and Aptroot 1998b), China (Tsui et al. 2000), New Guinea (Hyde and Aptroot 1998b)

#### Key to freshwater *Lophiostoma* species

1. Often stain the substrate purple.....*L. purpurascens*
1. Do not stain substrate purple.....2
2. Ascospores hyaline, 1-septate.....3
2. Ascospores initially hyaline, 1-septate, becoming pale brown, 3-septate with age.....*L. aquaticum*
3. Ascospores with appendages.....4
3. Ascospores without appendages.....*L. lunisporum*
4. Ascromata immersed beneath a darkened, slightly raised pseudostromata.....*L. maquilangense*
4. Ascromata not as above.....5
5. Ascospore appendages > 18 µm...*L. proprietunicatum*



**Fig. 71** *Neotrematosphaeria biappendiculata* (Material examined: JAPAN, Serisawa-park (pond), Matsukitai, Hirosaki, Aomori, on submerged twigs, 7 December 2002, KT & NA 1124, HHUF 28026, **holotype**; Mohei-pond, Aoki, Hirosaki, Aomori, on submerged twigs, 27 September 2003, KT & NA 1489a, HHUF 28306, **paratype**). **a–c**

from KT 1489a, **paratype**. **a** Ascomata on the host surface. **b** Section of ascoma. **c** Structure of peridium. **d–g** from KT 1124, **holotype**. **d** Pseudoparaphyses. **e** Ascus. **f, g** Ascospores (f in black-blue ink. Note the pad-like appendages at both ends). Scale bars: **a** = 500  $\mu$ m, **b** = 250  $\mu$ m, **c–g** = 10  $\mu$ m

##### 5. Ascospore appendages < 18 $\mu$ m...*L. frondisubmersum*

*Neotrematosphaeria* Thambugala, Kaz. Tanaka & K.D. Hyde, Fungal Diversity 74: 223 (2015)

*Saprobic* on submerged, dead twigs. **Sexual morph:** *Ascomata* solitary, semi-immersed to erumpent, uniloculate, subglobose, glabrous, black, coriaceous to carbonaceous, with ostiolate papilla, with a crest-like apex and a pore-like opening. *Peridium* broad at sides, poorly developed at the base, composed of a single stratum, with several layers of dark brown to black cells of *textura prismatica*. *Pseudoparaphyses* numerous, cellular, filiform, indistinctly septate, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with a long pedicel, rounded at the apex, with an ocular chamber. *Ascospores* biseriolate to uniseriate near the base, fusiform, mostly curved, pale yellowish to olivaceous brown, but pale at the terminal cells, septate, with bipolar appendages (Tanaka et al. 2005a; Thambugala et al. 2015). **Asexual morph:** Coelomycetes, pleurophomopsis-like. *Conidiomata* erumpent to superficial, uniloculate, globose, with thick, brown, sparse hyphae at sides, with ostiolate papilla. *Peridium* composed of polygonal to globose cells. *Conidiophores* cylindrical, branched, 0–1-septate. *Conidiogenous cells* phialidic. *Conidia* extremely small-sized, subglobose to ellipsoid, straight, hyaline, aseptate, smooth (Tanaka et al. 2005a).

*Type species: Neotrematosphaeria biappendiculata* (Kaz. Tanaka, Y. Harada & M.E. Barr) Thambug., Kaz. Tanaka & K.D. Hyde

*Notes:* The monotypic genus *Neotrematosphaeria* was introduced to accommodate *Trematosphaeria biappendiculata* Kaz. Tanaka et al. which was isolated from submerged wood in freshwater in Northern Japan (Tanaka et al. 2005a; Thambugala et al. 2015). The pale yellowish to olivaceous brown, 5(–7)-septate ascospores with globose to conical terminal appendages can distinguish *Neotrematosphaeria* from other genera in Lophiostomataceae.

##### List of freshwater *Neotrematosphaeria* species

\**Neotrematosphaeria biappendiculata* (Kaz. Tanaka, Y. Harada & M.E. Barr) Thambugala, Kaz. Tanaka & K.D. Hyde; Fig. 71

*Basionym: Trematosphaeria biappendiculata* Kaz. Tanaka, Y. Harada & M.E. Barr, Fungal Diversity 19: 149 (2005)

*Freshwater distribution:* Japan (Tanaka et al. 2005a)

*Neovaginatispora* A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 188 (2018)

*Saprobic* on dead herbaceous twigs and submerged wood. **Sexual morph:** *Ascomata* solitary, semi-immersed to erumpent, black, subglobose, coriaceous, ostiolate. *Ostiole* rounded or slit-like, central, with a pore-like opening. *Peridium* uneven in width, thinner at the base, two-layered,

outer layer fusing with the host cells, inner layer comprising hyaline cells of *textura angularis*. *Pseudoparaphyses* numerous, cellular, hypha-like, septate. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, with a short, bulbous pedicel, with an indistinct ocular chamber. *Ascospores* uni- to biserial, fusiform with acute ends, septate, constricted at the septum, hyaline, smooth-walled, with globose appendages at both ends (Hashimoto et al. 2018; Bao et al. 2019c). **Asexual morph:** Undetermined.

*Type species: Neovaginatispora fuckelii* (Sacc.) A. Hashim., K. Hiray. & Kaz. Tanaka

*Notes: Neovaginatispora* was proposed for *Vaginatispora fuckelii* (Sacc.) Thambug. et al. due to its thinner (up to 25 µm thick) and uniformly thick peridium (Hashimoto et al. 2018). However, the freshwater collection of *Neovaginatispora fuckelii* found by Bao et al. (2019c) has thicker peridium (up to 40 µm thick) which is thinner at the base. The specimen HHUF 27325 examined by Thambugala et al. (2015) also showed the uneven peridium width. Phylogenetic analyses based on four strains of *N. fuckelii* guaranteed *Neovaginatispora* as a distinct genus (Hashimoto et al. 2018; Bao et al. 2019c; this study, Fig. 74).

#### List of freshwater *Neovaginatispora* species

\**Neovaginatispora fuckelii* (Sacc.) A. Hashim., K. Hiray. & Kaz. Tanaka, Stud. Mycol. 90: 188 (2018)

*Basionym: Lophiostoma fuckelii* Sacc., Michelia 1(no. 3): 336 (1878)

*Synonymy:* see Index Fungorum (2020)

*Freshwater distribution:* China (Bao et al. 2019c)

*Pseudocapulatispora* Mapook & K.D. Hyde, Fungal Diversity 101: 47 (2020)

*Saprobic* on submerged wood or dead stems. **Sexual morph:** *Ascomata* brown to black, solitary or scattered, immersed with papilla erumpent through host surface, ellipsoidal or ovoid, coriaceous, ostiolate. *Ostiolar* papilla short, blackened, compressed, crest-like. *Peridium* composed of several layers of brown to dark brown, thin-walled cells. *Pseudoparaphyses* numerous, trabeculate, filiform, hyaline, sparsely septate, branched. *Asci* 8-spored, bitunicate, mostly broadly clavate, rarely cylindrical-clavate, apically narrowly rounded, with an ocular chamber and a short pedicel. *Ascospores* biserial, broadly fusiform, hyaline, septate, mostly rounded or rarely sharply narrowed at two ends, guttulate, thin-walled, smooth, with a narrow, hyaline, elongate sheath (Mapook et al. 2020). **Asexual morph:** Undetermined.

*Type species: Pseudocapulatispora longiappendiculata* Mapook & K.D. Hyde

*Notes: Pseudocapulatispora longiappendiculata* was isolated from a dead stem of *Chromolaena odorata* in Thailand (Mapook et al. 2020). *Pseudocapulatispora* is distinct

in Lophiostomataceae by its sheath drawn out to form polar appendages with tips of the sheath capped (Mapook et al. 2020). *Capulatispora* Thambug. & K.D. Hyde has a similar sheath but it mostly has cylindrical-clavate asci and fusiform ascospores with narrowed and acute ends (Thambugala et al. 2015). Phylogenetic analysis supported them as distinct genera (Thambugala et al. 2015; Mapook et al. 2020). We collected a new specimen of *P. longiappendiculata* from freshwater habitats.

#### List of freshwater *Pseudocapulatispora* species

\**Pseudocapulatispora longiappendiculata* Mapook & K.D. Hyde, Fungal Diversity 101: 48 (2020); Fig. 72

*Freshwater distribution:* China (this study)

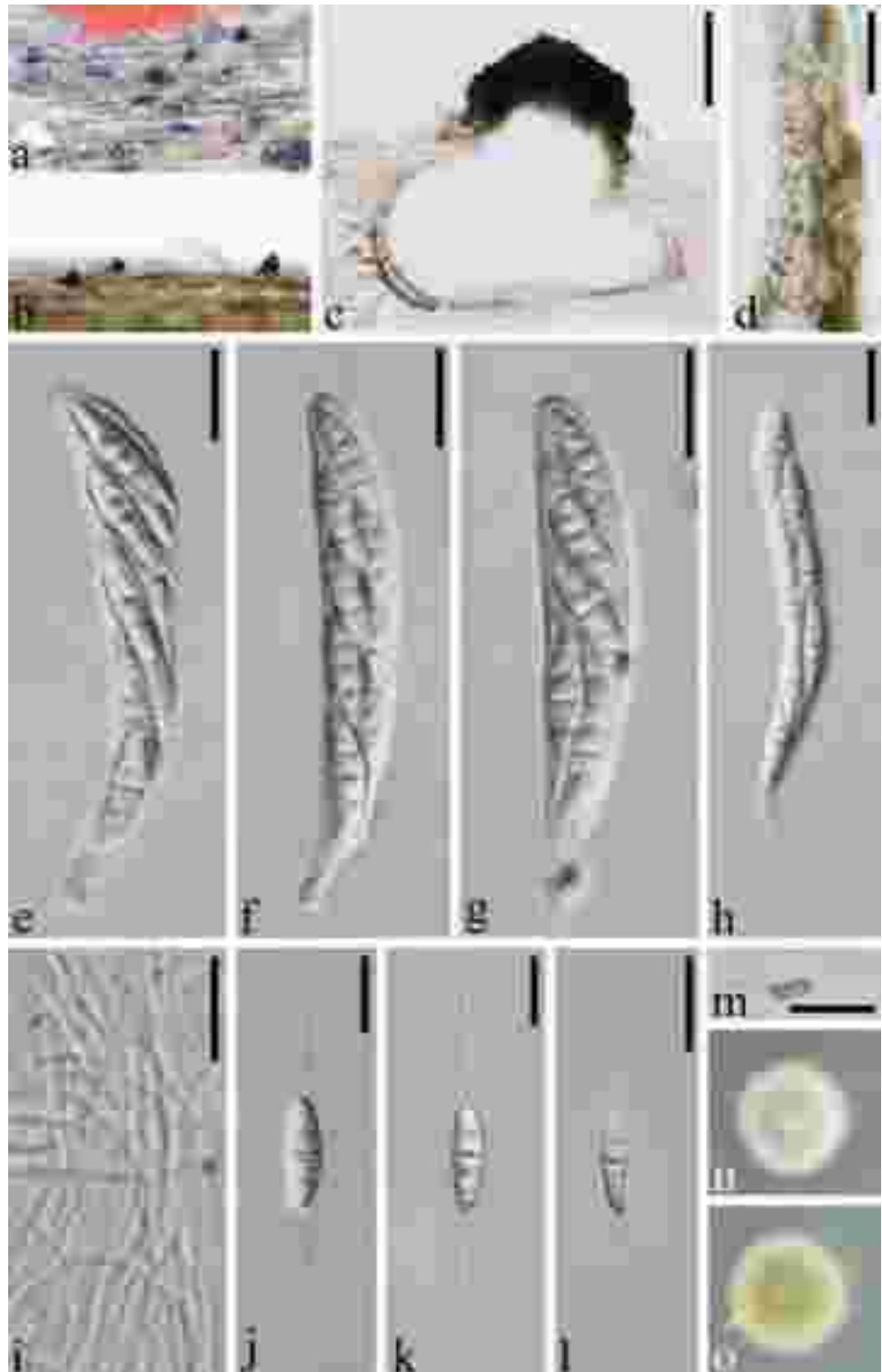
*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* 270–310 µm high, 310–330 µm diam., black, scattered, immersed with papilla erumpent through host surface, ellipsoidal, ostiolate. *Ostiolar* papilla short, blackened, compressed, 110–130 µm high, 160–180 µm wide, composed of dark brown to black, thick-walled cells of *textura angularis* or subglobose cells, 20–25 µm thick at the base, 70–80 µm thick at the top. *Peridium* 15–30 µm thick, composed of several layers of brown to dark brown, thin-walled cells of *textura angularis* or subglobose cells. *Pseudoparaphyses* 2 µm wide, numerous, trabeculate, filiform, hyaline, sparsely septate, branched. *Asci* 8-spored, bitunicate, mostly broadly clavate, rarely cylindrical-clavate, slightly to obviously curved, (90–)105–130 × 12–17 µm ( $\bar{x}$  = 115 × 14.5 µm, n = 10), apically narrowly rounded, with an ocular chamber and a short pedicel. *Ascospores* (20.5–)25.5–30 × 6–9 µm ( $\bar{x}$  = 27 × 7 µm, n = 10), biserial, ellipsoidal, mostly with rounded or rarely acute ends, straight or slightly curved, hyaline, 1-septate, slightly constricted at the to septum, slightly constricted at a quarter, guttulate, thin-walled, smooth, with a narrow, hyaline, elongate sheath with globose ending, 30 µm long. **Asexual morph:** Undetermined.

*Culture characteristics:* On PDA, colony circular, reaching 5 mm in 15 days at 25 °C, white to grey from above, pale yellow to pale brown from below, surface rough, with dense mycelium, dry, raised, edge entire.

*Material examined:* CHINA, Yunnan Province, Dehong, on submerged wood in a stream, 25 November 2017, G.N. Wang, H39A-1 (MFLU 18-1188), living culture MFLUCC 18-1027; *ibid.*, H39A-2 (HKAS 101722), living culture KUMCC 18-0072.

*Notes:* Phylogenetic analysis shows our collection MFLUCC 18-1027 is *P. longiappendiculata* (Fig. 74). MFLUCC 18-1027 has shorter appendages (30 µm vs. 40 µm) than the holotype (Mapook et al. 2020). This is a new habitat and new geographical record for *P. longiappendiculata* from freshwater in China.

**Fig. 72** *Pseudocapulatispora longiappendiculata* (MFLU 18-1188, new habitat and geographical record). **a**, **b** Appearance of ostiolar papilla erumpent through host substrate. **c** Vertical section of ascoma. **d** Structure of peridium. **e–h** Bitunicate asci. **i** Pseudoparaphyses. **j–l** Ascospores. **m** Germinated ascospore. **n**, **o** Colony on PDA (up-front, down-reverse). Scale bars: **c** = 100  $\mu$ m, **d–l** = 20  $\mu$ m, **m** = 50  $\mu$ m



*Sigarispora* Thambug. & K.D. Hyde, Fungal Diversity 74: 238 (2015)

*Saprobic* on decaying culms of grasses (Poaceae), dead herbaceous stems or submerged wood. **Sexual morph:** *Ascomata* solitary, scattered to gregarious, semi-immersed to immersed, with papilla erumpent through host surface,

globose to subglobose, black, ostiolate. *Ostirole* crest- or slit-like, central, rounded, with a pore-like opening and plugged by gelatinous tissue, comprising pseudoparenchymatous cells. *Peridium* composed of light to dark brown, small, thin-walled cells of *textura angularis*, fusing with the host tissues in outer layer. *Pseudoparaphyses* numerous, cellular,

long, hyaline, septate, branched. *Asci* 8-spored, bitunicate, fissionic, cylindrical-clavate, pedicellate, with an ocular chamber. *Ascospores* uni- to bi-seriate, yellowish brown to dark brown, cigar-shaped or ellipsoidal-fusiform, transversely septate or muriform, smooth-walled, with or without a mucilaginous sheath, sometimes with terminal appendages (Thambugala et al. 2015). **Asexual morph:** Undetermined.

*Type species:* *Sigarispora ravennica* (Tibpromma, Camporesi & K.D. Hyde) Thambugala & K.D. Hyde

*Notes:* *Sigarispora* was introduced for some lophiostoma-like species (Thambugala et al. 2015). *Sigarispora* is phylogenetically separated from *Lophiostoma* (Thambugala et al. 2015); this study, Fig. 74) and characterized by yellowish brown to dark brown, cigar-shaped or ellipsoidal-fusiform ascospores which are unlike the hyaline to yellowish brown, fusiform ascospores of *Lophiostoma*. Bao et al. (2019c) reported a freshwater species *S. clavata* D.F. Bao et al. from China and characterized by dark brown to yellowish brown, ellipsoidal to clavate ascospores.

#### List of freshwater *Sigarispora* species

\**Sigarispora clavata* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, *Mycosphere* 10: 1090 (2019)

*Freshwater distribution:* China (Bao et al. 2019c)

*Vaginatispora* K.D. Hyde, *Nova Hedwigia* 61(1–2): 234 (1995)

*Saprobic* on submerged wood, intertidal wood, dead twigs, endocarp or fallen fruit pericarp. **Sexual morph:** *Ascomata* solitary or scattered, immersed to erumpent, uniloculate, subglobose, glabrous, dark brown to black. *Ostiolar neck* crest-like, elongate, laterally compressed, composed of globose to elongate, brown to black cells, with a pore-like opening and hyaline periphyses. *Peridium* unequal in thickness, two-layered, outer layer comprising somewhat flattened cells, fusing and indistinguishable from the host tissues, inner layer comprising lightly pigmented to hyaline cells. *Pseudoparaphyses* numerous, cellular, hypha-like, hyaline, septate, anastomosing above the *asci*. *Asci* 8-spored, bitunicate, fissionic, cylindrical to clavate, with a short or long pedicel, apically round with an ocular chamber. *Ascospores* uni- to bi-seriate, narrowly ellipsoidal or fusiform, straight to slightly curved, hyaline, 1-septate, occasionally producing pseudosepta, septum mostly median, upper cell slightly broader than lower cell, smooth, thin-walled, with bipolar appendages or entire sheath (Hyde 1995b). **Asexual morph:** Undetermined.

*Type species:* *Vaginatispora aquatica* K.D. Hyde

*Notes:* *Vaginatispora aquatica* was collected from North Queensland, Australia (Hyde 1995b). Zhang et al. (2014a) transferred *V. aquatica* to *Lophiostoma* because it formed a basal clade to *Lophiostoma* species with high bootstrap support and morphologically resembles *L. macrostomum* (type

species), in having immersed to erumpent *ascmata* with a slit-like ostiole, an unequally thick *peridium*, clavate *asci*, and hyaline *ascospores* (Hyde 1995b; Zhang et al. 2014a). With more sequences added in Lophiostomataceae, *Vaginatispora* (typified by *V. aquatica*) was accepted as a distinct genus in Lophiostomataceae (Thambugala et al. 2015). The freshwater collections of *V. armatispora* (K.D. Hyde et al.) Wanas. et al. were found from Thailand (Bao et al. 2019c; Hyde et al. 2019).

#### List of freshwater *Vaginatispora* species

\**Vaginatispora aquatica* K.D. Hyde, *Nova Hedwigia* 61(1–2): 235 (1995); Fig. 73

*Synonymy:* *Lophiostoma vaginatispora* H. Zhang & K.D. Hyde, *Phytotaxa* 176(1): 177 (2014)

*Freshwater distribution:* Australia (Hyde 1995b), China (Tsui et al. 2000), Thailand (Zhang et al. 2014a)

\**Vaginatispora armatispora* (K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones) Wanas., E.B.G. Jones & K.D. Hyde, *Stud. Fung.* 1(1): 62 (2016)

*Basionym:* *Massarina armatispora* K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones, *Bot. Mar.* 35(4): 325 (1992)

*Synonymy:* *Lophiostoma armatisporum* (K.D. Hyde, Vrijmoed, Chinnaraj & E.B.G. Jones) E.C.Y. Liew, Aptroot & K.D. Hyde, *Mycologia* 94(5): 812 (2002)

*Freshwater distribution:* Thailand (Hu et al. 2010b; Bao et al. 2019c; Hyde et al. 2019)

#### Key to freshwater *Vaginatispora* species

1. Ascospores 33–45 × 11–12.5 μm, with a mucilaginous collar around its equator, surrounded by a spreading papilionaceous sheath..... *V. aquatica*
1. Ascospores 22–34 × 5.5–9 μm, surrounded by a narrow mucilaginous sheath, with distinct hyaline appendages at both ends..... *V. armatispora*

**Lophiotremataceae** K. Hiray. & Kaz. Tanaka, *Mycoscience* 52(6): 405 (2011)

*Lophiotrema* Sacc., *Michelia* 1(no. 3): 338 (1878)

*Saprobic* on woody and herbaceous substrates or submerged wood. **Sexual morph:** *Ascomata* scattered to crowded, mostly immersed, rarely erumpent, uniloculate, globose, subglobose to ovoid, glabrous, dark brown to black, opening compressed, with a slit-like ostiole. *Peridium* composed of pale brown, small, thin-walled cells of *textura angularis* to *globosa*. *Pseudoparaphyses* numerous, cellular, hyaline, septate, occasionally anastomosing or branching. *Asci* 8-spored, bitunicate, fissionic, cylindrical, sessile to minutely pedicellate, rounded at the apex, with



**Fig. 73** *Vaginatisspora aquatica* (Material examined: THAILAND, Chiang Mai Province, Doi Inthanon, 16 November 2010, H. Zhang, MFLU 11-1115). **a, b** Appearance of ascomata on wood. **c** Section

of ascoma. **d** Papilla of ascoma. **e** Ascus. **f, g** Ascospores with wide papilionaceous sheath. (**g** in Indian Ink). Scale bars: **c, d** = 100  $\mu$ m, **e** = 30  $\mu$ m, **f** = 20  $\mu$ m, **g** = 40  $\mu$ m

an ocular chamber. *Ascospores* uni- to bi-seriate, fusiform to cylindrical, straight to slightly curved, hyaline to brown, septate, symmetrical, smooth, thin-walled (Hirayama and Tanaka 2011; Hyde et al. 2013). **Asexual morph:** probably pleurophomopsis-like (Leuchtmann 1985).

*Type species:* *Lophiotrema nucula* (Fr.) Sacc.

*Notes:* *Lophiotrema* is distinguished from *Lophiostoma* by subglobose to globose ascomata, peridium composed thin-walled cells of *textura angularis* to *globosa*, cylindrical asci with a short pedicel, and hyaline to brown, septate ascospores (Hirayama and Tanaka 2011; Hyde et al. 2013). Hirayama and Tanaka (2011) mentioned that the ascus shape, including length of the ascus pedicel, was a reliable taxonomic indicator to separate these two genera (cylindrical, up to 15  $\mu$ m long in *Lophiotrema* vs. clavate, mostly (10–)15–30  $\mu$ m long in *Lophiostoma*). Based on multigene phylogenetic analysis, *Lophiotrema* was placed in Lophiotremataceae, which was distant from Lophiostomataceae (Hirayama and Tanaka 2011). *Pleurophomopsis* Petr. was linked to the asexual morph of *Lophiotrema*, which formed in culture of *L. nucula* (Leuchtmann 1985), but Hyde et al. (2013) considered that it was probably a spermatial state.

The freshwater species *Lophiotrema rubi* (Fuckel) Y. Zhang et al. is characterized by hemispherical to pyriform ascomata with black clypeus formed around the neck, broadly cylindrical asci with a hoof-shaped pedicel, and broadly fusiform, hyaline ascospores with bipolar appendages (Fallah and Shearer 2001). *Lophiotrema rubi* was transferred from *Massarina rubi* (CBS 691.95) based on multigene phylogenetic

analysis (Zhang et al. 2009c). *Lophiotrema rubi* is likely an immigrant to freshwater because most reports are from terrestrial habitats (Fallah and Shearer 2001).

#### List of freshwater *Lophiotrema* species

*Lophiotrema rubi* (Fuckel) Y. Zhang et al., C.L. Schoch & K.D. Hyde, Stud. Mycol. 64: 97 (2009)

*Basionym:* *Massaria rubi* Fuckel, Jb. nassau. Ver. Naturk. 25-26: 303 (1871)

*Synonymy:* *Didymellina raphithamni* Keissl., Nat. Hist. Juan Fernandez Easter Isl. 2: 480 (1927)

*Lophiostoma rubi* (Fuckel) E.C.Y. Liew, Aptroot & K.D. Hyde, Mycologia 94(5): 812 (2002)

*Lophiotrema emergens* P. Karst., Hedwigia 22: 42 (1883)

*Massarina emergens* (P. Karst.) L. Holm, Les Pleosporaceae: 149 (1957)

*Massarina rubi* (Fuckel) Sacc., Syll. fung. (Abellini) 2: 155 (1883)

*Mycosphaerella raphithamni* (Keissl.) Petr., Annls mycol. 38(2/4): 221 (1940)

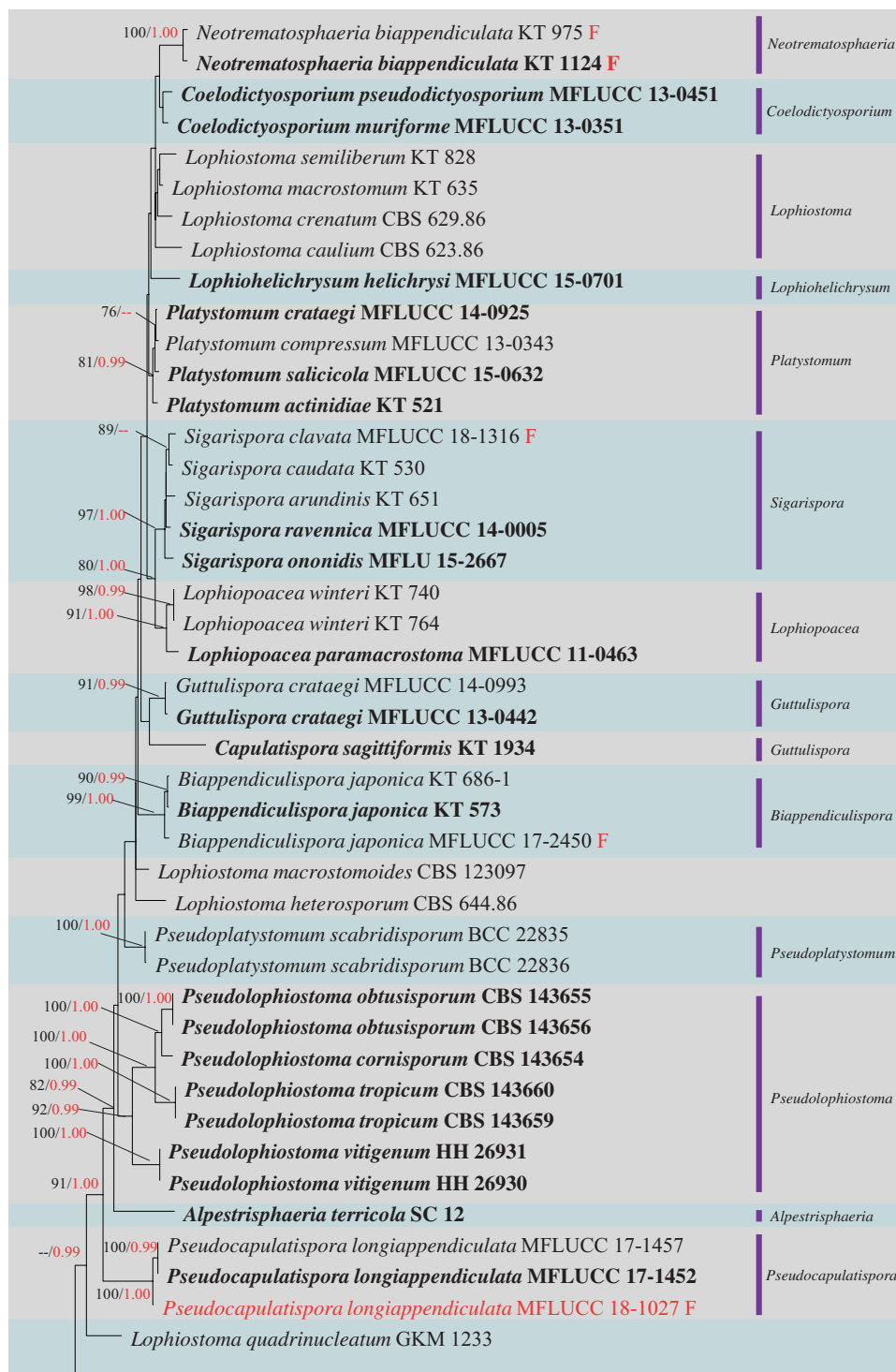
*Freshwater distribution:* USA (Fallah and Shearer 2001)

**Massarinaceae** Munk, Friesia 5(3-5): 305 (1956)

#### Key to freshwater genera of Massarinaceae

1. Ascospores basically symmetric, unevenly coloured, brown in central two cells and paler in end cells at maturity.....*Bysothecium*

**Fig. 74** Phylogram generated from maximum likelihood analysis of combined LSU, SSU, ITS, TEF and RPB2 sequence data for species of Lophiostomataceae. Bootstrap values for maximum likelihood equal to or greater than 75% and Bayesian posterior probabilities equal to or greater than 0.95 are placed near the branches as ML/BYPP. Newly generated sequences are in red and ex-type strains are in bold. The new species introduced in this study are indicated with underline. Freshwater strains are indicated with a red letter “F”. The tree is rooted to *Teichospora rubriostiolata* TR 7 and *T. trabicola* C134 (Teichosporaceae)



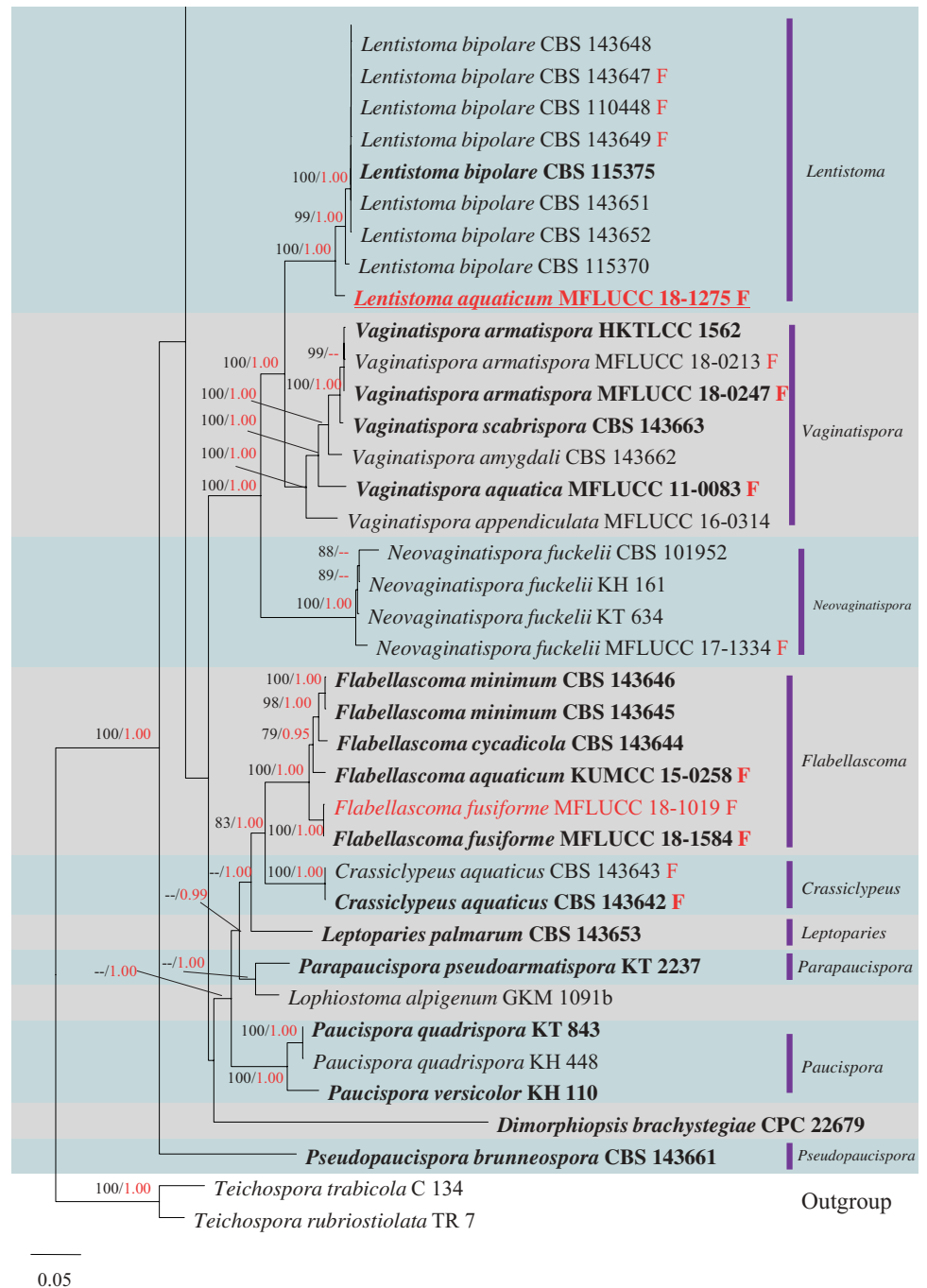
1. Ascospores asymmetric, upper cell longer and larger than lower cell, evenly coloured, medium to dark brown at maturity.....*Helminthosporium*

*Byssothecium* Fuckel, Bot. Ztg. 19: 251 (1861)

*Saprobic* on submerged wood in freshwater or decaying wood in terrestrial habitats. **Sexual morph:** *Ascomata*

gregarious, erumpent to superficial, uniloculate, subglobose, ovoid to obpyriform, black, coriaceous, with a central ostiole papilla. *Peridium* composed of brown, rectangle, pseudoparenchymatous cells, somewhat thinner at base. *Pseudoparaphyses* dense, cellular, hypha-like, hyaline, septate, embedded in a gelatinous matrix, anastomosing between and above the asci. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to clavate, pedicellate. *Ascospores* biseriate at

Fig. 74 (continued)



the apex, becoming uniseriate near the base, ellipsoidal to fusiform, straight to slightly curved, hyaline to brown, usually paler at end cells, septate, basically symmetric, smooth to minutely verrucose, thin-walled (Crane et al. 1992; Pem et al. 2019b). **Asexual morph:** chaetophoma-like (Boise 1983).

*Type species:* *Byssothecium circinans* Fuckel

*Notes:* *Byssothecium* is characterized by ellipsoidal to fusiform, hyaline to brown ascospores usually with bipolar paler cells (Crane et al. 1992; Pem et al. 2019b). The

asexual morph of *B. circinans* was produced in culture and characterized by minute conidiomata, phialidic conidiogenous cells and hyaline, aseptate conidia, which are similar to *Chaetophoma* (Boise 1983).

*Savoryella* E.B.G. Jones & R.A. Eaton shares similar morphological characters with *Byssothecium* in having cylindrical asci, ellipsoidal, 3-septate ascospores with brown central cells and paler end cells (Jones and Eaton 1969; Dayarathne et al. 2019). The ascomata have a long neck in *Savoryella*, but it is lacking in *Byssothecium*. Phylogeny



separates them in different classes: *Byssothecium* in Dothideomycetes (Lumbsch and Lindemuth 2001), and *Savoryella* in Sordariomycetes (Dayarathne et al. 2019) (Fig. 74).

One putative strain of *Byssothecium circinans* (CBS 675.92) clustered in Massarinaceae in Schoch et al. (2009). Zhang et al. (2009d) found that *B. circinans* clustered basal to Melanommataceae based on combined LSU and SSU dataset, while clustered in Massarinaceae based on RPB2 dataset. Because *B. circinans* considerably differs from *Massarina* in morphology, such as black, erumpent ascomata, ellipsoidal to fusiform ascospores with pigmented central cells and hyaline end cells (Crane et al. 1992), and had an unstable relationships in the phylogenetic tree (Zhang et al. 2009d). *Byssothecium circinans* was not assigned to Melanommataceae and treated as an unresolved species (Zhang et al. 2009c, d). Based on combined LSU, SSU and RPB2 dataset, *B. circinans* formed a stable clade in Melanommataceae (Zhang et al. 2012b; Hyde et al. 2013; Tanaka et al. 2015; Voglmayr and Jaklitsch 2017; Pem et al. 2019b; this study, Fig. 2).

The morphology of strain *Byssothecium circinans* (CBS 675.92) is unavailable, a specimen G-K 18367 of *B. circinans* was examined by Pem et al. (2019b) and also examined in this study (Fig. 75). Two *Byssothecium* species were reported from freshwater habitats (see list below), but not confirmed by molecular data.

#### List of freshwater *Byssothecium* species

\**Byssothecium circinans* Fuckel, Bot. Ztg. 19: 251 (1861); Fig. 75

*Synonymy*: Possible synonymy see Index Fungorum (2020)

*Freshwater distribution*: Hungary (Révay and Gönczöl 1990; Gönczöl and Révay 1993)

*Byssothecium flumineum* J.L. Crane, Shearer & Huhndorf, Mycologia 84(2): 236 (1992)

*Freshwater distribution*: USA (Crane et al. 1992)

#### Key to freshwater *Byssothecium* species

1. Ascospores 7.3–9.2 µm wide.....*B. flumineum*
1. Ascospores 10–12 µm wide.....*B. circinans*

*Helminthosporium* Link, Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 10 (1809)

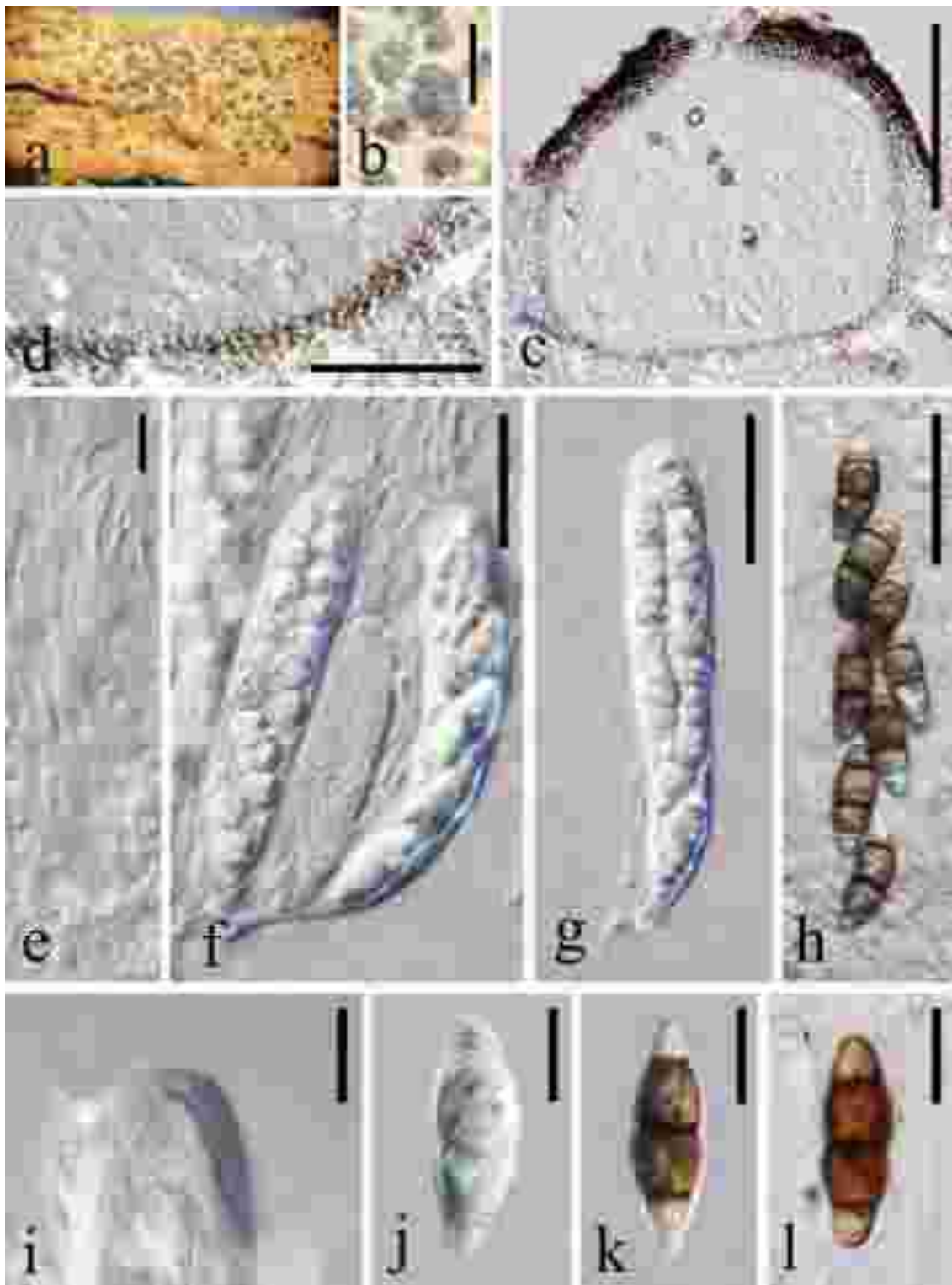
*Synonym*: *Exosporium* Link, Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 9 (1809)

*Saprobic* on submerged wood, various dead plant material or rarely parasitic on plants or fungicolous. **Sexual morph**: *Pseudostromata* dark brown or reddish brown, pseudoparenchymatous, of thick-walled dark brown cells; margin mostly

composed of dark brown, verrucose hyphae. *Ascomata* immersed in pseudostromata, solitary or in small groups, large, often strongly depressed, or globose, dark brown to black, with a central, inconspicuous ostiole. *Peridium* pseudoparenchymatous. *Pseudoparaphyses* numerous, cellular, septate, branched, anastomosing, narrow, usually embedded in a gel matrix. *Asci* 8-spored, bitunicate, fissitunicate, clavate or fusoid, short pedicellate. *Ascospores* irregularly biseriate, fusoid, ellipsoidal, obovoid, occasionally oblong, asymmetric, with one primary, eccentric, deeply constricted euseptum, upper cell longer and larger than lower cell, often with several transverse or oblique distosepta, rarely with a longitudinal distoseptum in the larger cell, slightly or not constricted at the secondary distosepta, hyaline when young, becoming medium to dark brown when mature, with subacute to rounded end cells, smooth or verruculose, sometimes with longitudinal striae, surrounded by a thick gelatinous sheath (Voglmayr and Jaklitsch 2017). **Asexual morph**: *Colonies* on natural substrate conspicuous, effuse to punctiform, hairy, brown to black. *Mycelium* mostly immersed, composed of branched, septate hyphae. *Conidiophores* macronematous, mononematous, arising solitarily or in fascicles, erect, mostly unbranched, straight or flexuous, cylindrical or subulate, with a well-defined small pore at the apex, and often with several lateral pores beneath the upper septa, ceasing growth with the formation of a terminal conidium, usually not proliferating, pale to dark brown, smooth or occasionally verruculose. *Conidiogenous cells* enteroblastic, polytretic, integrated, determinate, terminal and intercalary, subcylindrical. *Conidia* mostly solitary, acropleurogenous, obclavate, obpyriform to lageniform, mostly rostrate, straight or curved, pale brown to brown, distoseptate, smooth, with a paler apical cell and truncate base, often with a prominent, dark brown or black scar at the base (Ellis 1971; Zhu et al. 2016; Voglmayr and Jaklitsch 2017).

*Type species*: *Helminthosporium velutinum* Link [as ‘Helmisporium’], Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 10 (1809)

*Notes*: *Helminthosporium* is an old genus which was introduced in 1809 and currently comprises over 750 epithets in Index Fungorum (2020). *Helminthosporium* is morphologically similar to *Corynespora* Güssow and *Exosporium* Link as all of them have distoseptate conidia with tapering apex and truncate base, growing through a wide pore at the apex of the conidiophores (Ellis 1971; Voglmayr and Jaklitsch 2017). *Exosporium* has been synonymized with *Helminthosporium* based on their very similar morphological characters (Fries 1832) and this was accepted by Voglmayr and Jaklitsch (2017) with molecular data supported. *Corynespora* differs from *Helminthosporium* as conidiophores have successive proliferations (up to four), while they are not described in *Helminthosporium* (Voglmayr and Jaklitsch 2017). Phylogenetically, *Corynespora* has been



**Fig. 75** *Byssothecium circinans* (Material examined: GERMANY, Sabine, M. Huhndorf, 10 December 1993, G-K 18367). **a, b** Ascomata on the host surface. **c** Section of ascoma. **d** Peridium. **e** Pseudo-

paraphyses. **f–i** Bitunicate asci. **j–l** Ascospores. Scale bars: **b** = 200  $\mu$ m, **c** = 100  $\mu$ m, **d** = 30  $\mu$ m, **e** = 5  $\mu$ m, **f–h** = 40  $\mu$ m, **i–l** = 15  $\mu$ m

placed in Corynesporascaceae, while *Helminthosporium* clustered in Massarinaceae (Voglmayr and Jaklitsch 2017; this study, Fig. 2).

The massaria- and splachnonema-like sexual morphs have been linked to *Helminthosporium* based on cultural studies and sequence data (Tanaka et al. 2015; Voglmayr and Jaklitsch 2017). Both sexual and asexual morphs can be observed in *H. massarinum* Kaz. Tanaka et al., *H. microsorum* D. Sacc., *H. oligosporum* (Corda) S. Hughes, *H. quercicola* (M.E. Barr) Voglmayr & Jaklitsch, *H. quercinum* Voglmayr & Jaklitsch. and *H. tiliae* (Link) Fr. (Tanaka et al. 2015; Voglmayr and Jaklitsch 2017). The sexual morph of the type species *H. velutinum* Link is still unknown. The sexual genus *Byssosphaeria* has a relationship with *Helminthosporium* in Massarinaceae (Fig. 2), however, they can be distinguished by ascospore characters (see key to freshwater genera of Massarinaceae).

*Helminthosporium* species are commonly collected from terrestrial habitats worldwide as mentioned in Voglmayr and Jaklitsch (2017). Zhu et al. (2016) reported two species *H. aquaticum* H.Y. Su et al. and *H. velutinum* (type) from freshwater habitats in China (see list below) and no sexual morphs were observed. We do not make a key for these two species as they are morphologically very similar and they have overlapping size in all structures. Even though, multi-gene phylogenetic analyses supported them to be different species (Zhu et al. 2016; Voglmayr and Jaklitsch 2017). We believe that sexual morphs of *H. aquaticum* and *H. velutinum* will separate them clearly in future.

#### List of freshwater *Helminthosporium* species

\**Helminthosporium aquaticum* H.Y. Su, Z.L. Luo & K.D. Hyde, Phytotaxa 253(3): 184 (2016)

*Freshwater distribution*: Yunnan, China (Zhu et al. 2016)

\**Helminthosporium velutinum* Link [as ‘Helmisporium’], Mag. Gesell. naturf. Freunde, Berlin 3(1-2): 10 (1809)

*Freshwater distribution*: Yunnan, China (Zhu et al. 2016)

**Melanommataceae** G. Winter [as ‘Melanommeae’], Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 1.2: 220 (1885)

#### Key to freshwater genera of Melanommataceae

1. Asexual morph.....*Camposporium*
1. Sexual morph.....2
2. Ascospores dimorphic.....*Mamillisphaeria*
2. Ascospores monomorphic.....3
3. Ascomata glabrous.....*Melanomma*
3. Ascomata spread on substrate as a subiculum or covered by hyphae.....4
4. Ascomata immersed or erumpent.....*Herpotrichia*

4. Ascomata superficial, with red or orange at pore.....*Byssosphaeria*

***Byssosphaeria*** Cooke, Grevillea 7(no. 43): 84 (1879)

*Saprobic* on woody angiosperms or submerged wood.

**Sexual morph**: Ascomata solitary, scattered to gregarious, superficial, uniloculate, globose, subglobose or ovoid, with basal subiculum and covered by thick, branched hyphae, dark brown to black, coriaceous, central ostiolate, red or orange at pore, without papilla. *Peridium* two-layered, outer layer composed of irregular, thick-walled, brown to dark brown cells of *textura epidermoidea*, inner layer composed of small thin-walled, pale brown cells. *Pseudoparaphyses* dense, trabeculate, long, embedded in mucilage, anastomosing between and above asci. *Asci* 8-spored, bitunicate, fisitunicate, cylindro-clavate to clavate, with short pedicel, apically rounded with an ocular chamber. *Ascospores* uni- to bi-seriate, fusiform, tapering at both ends, mostly straight, sometimes slightly curved, hyaline to pale brown, 1-septate, verrucose (Tian et al. 2015). **Asexual morph**: Coelomycetous. *Pycnidia* formed in culture. *Conidiogenous cells* phialidic, lining cavity. *Conidia* hyaline, ellipsoid or subglobose (Barr 1984).

*Type species*: *Byssosphaeria keithii* (Berk. & Broome) Cooke [as ‘keitii’]

*Notes*: *Byssosphaeria* is characterized by superficial ascomata with bright yellow, orange or red, flat apices around the ostiole, with thick, branched hyphae, and hyaline to pale brown ascospores (Tian et al. 2015). *Byssosphaeria* differs from *Herpotrichia* Fuckel in having superficial ascomata with red or orange at pore (Zhang et al. 2012b; Tian et al. 2015). *Byssosphaeria* was shown to belong to Melanommataceae based on molecular data of some species (Zhang et al. 2012b; Tian et al. 2015). Sequence data of the type species *B. keithii* are unavailable. The freshwater species *B. schiedermayeriana* (Fuckel) M.E. Barr was found from Seychelles, but not supported by molecular data. Three terrestrial strains of *B. schiedermayeriana* clustered in Melanommataceae with good bootstrap support (Liu et al. 2015; Tian et al. 2015).

#### List of freshwater *Byssosphaeria* species

\**Byssosphaeria schiedermayeriana* (Fuckel) M.E. Barr, Mycotaxon 20(1): 34 (1984)

*Basionym*: *Herpotrichia schiedermayeriana* Fuckel, Jb. Nassau. Ver. Naturk. 27-28: 27 (1874)

*Synonymy*: see Index Fungorum (2020)

*Freshwater distribution*: Seychelles (Hyde and Goh 1998b)

*Camposporium* Harkn., Bull. Calif. Acad. Sci. 1(no. 1): 37 (1884)

*Saprobic* on decaying wood, bark and fruit of various trees and shrubs in terrestrial or freshwater habitats. **Sexual morph:** Undetermined. **Asexual morph:** Colonies effuse, grey, brown to black. *Mycelium* immersed or partly superficial on host substrate. *Conidiophores* macro- or micronematous, mononematous, often short, straight or irregularly bent, unbranched, pale brown to dark brown. *Conidiogenous cells* mono- or polyblastic, integrated, terminal, sympodial, cylindrical or subulate, denticulate. *Conidia* solitary, dry, multi-septate, pale brown, often unevenly coloured, the end cells subhyaline, smooth, mostly cylindrical, rounded or tapering at the apex, sometimes rostrate, with or without appendages (Ellis 1971; Hyde et al. 2020b).

*Type species:* *Camposporium antennatum* Harkn.

*Notes:* *Camposporium* is an old genus and characterized by short conidiophores, denticulate conidiogenous cells, and cylindrical, multi-septate, uneven coloured conidia often with single or several cylindrical appendages (Ellis 1971; Hyde et al. 2020b). *Camposporium* had a relationship with *Fusicnidium* Jun F. Li et al., but they can be distinguished by conidiogenous cells and conidial shape (Li et al. 2017; Hyde et al. 2020b). They are treated as distinct genera until their relationships are well-resolved (Hyde et al. 2020b). The type species, *C. antennatum*, represented by a strain CBS 113441 was placed in Melanommataceae (Hyde et al. 2020b). Other *Camposporium* species clustered with *C. antennatum*, but with low bootstrap support (Hyde et al. 2020b). A freshwater species *C. septatum* N.G. Liu et al. formed a well-supported clade with *Fusicnidium* species (Hyde et al. 2020b). However, *C. septatum* was placed in *Camposporium*, rather than *Fusicnidium*, based on its monoblastic conidiogenous cells and cylindrical conidia with several appendages at the apex (Hyde et al. 2020b). Both *C. appendiculatum* D.F. Bao et al. and *C. multiseptatum* D.F. Bao et al. have tapering apical cell with truncate ends, while *C. antennatum* has rounded apex (Ellis 1971; Hyde et al. 2020b). *Camposporium hyalinum* Abdullah and *C. marylandicum* Shearer have the typical apical appendages of *Camposporium*, but they have hyaline conidia which are different from the brown, unevenly coloured conidia of the other freshwater species (Shearer 1974; Abdullah 1980; Hyde et al. 2020b). The molecular data of these two species are needed to confirm their phylogenetic placement. *Camposporium* species need further study with more collections.

#### List of freshwater *Camposporium* species

\**Camposporium appendiculatum* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Fungal Diversity 100: 77 (2020)

*Freshwater distribution:* China (Hyde et al. 2020b)

*Camposporium hyalinum* Abdullah, Trans. Br. mycol. Soc. 75(3): 514 (1981) [1980]

*Freshwater distribution:* UK (Abdullah 1980)

*Camposporium marylandicum* Shearer, Mycologia 66(1): 16 (1974)

*Freshwater distribution:* USA (Shearer 1974)

\**Camposporium multiseptatum* D.F. Bao, Z.L. Luo, K.D. Hyde & H.Y. Su, Fungal Diversity 100: 82 (2020)

*Freshwater distribution:* China (Hyde et al. 2020b)

\**Camposporium pellucidum* (Grove) S. Hughes, Mycol. Pap. 36: 9 (1951)

*Freshwater distribution:* China (Hyde et al. 2020b)

\**Camposporium septatum* N.G. Liu, J.K. Liu & K.D. Hyde, Fungal Diversity 100: 85 (2020)

*Freshwater distribution:* Thailand (Hyde et al. 2020b)

#### Key to freshwater *Camposporium* species

1. Conidial apex without appendages.....*C. pellucidum*
1. Conidial apex with appendages.....2
2. Conidia with 2–3 appendages.....*C. septatum*
2. Conidia with a single appendage.....3
3. Conidia 2–4(–6)-septate .....*C. hyalinum*
3. Conidia > 5-septate.....4
4. Conidia 5–10-septate.....*C. marylandicum*
4. Conidia 10–13-septate.....5
5. Conidial appendages 72–114 µm long.....  
.....*C. appendiculatum*
5. Conidial appendages 11–17 µm long.....  
.....*C. multiseptatum*

*Herpotrichia* Fuckel, Fungi rhenani exsicc., suppl., fasc. 7(nos 2101–2200): no. 2171 (1868)

*Saprobic*, parasitic or hyperparasitic in terrestrial or freshwater habitats. **Sexual morph:** *Ascomata* scattered to gregarious, immersed, erumpent to nearly superficial, subglobose to pyriform, dark brown to black, spreading on substrate as a subiculum, coriaceous, roughened, with ostiolate papilla. *Peridium* two-layered, outer layer composed of pigmented, thick-walled cells of *textura angularis*, inner layer composed of light pigmented, thin-walled cells of *textura prismatica*. *Pseudoparaphyses* dense, cellular, hypha-like, hyaline, septate, branched and anastomosing above asci, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical to cylindro-clavate, with a furcate pedicel, apically rounded with an ocular chamber. *Ascospores* uni- to bi-seriate, fusoid, ellipsoidal or oblong, straight, hyaline to pale brown, 1-septate, thin-walled, smooth or verruculose (Tian et al. 2015). **Asexual morph:** Coelomycetous. pyrenochaeta- or pyrenochaeta-like. *Pycnidia* solitary or gregarious, superficial, globose to pyriform or hemispherical, with or without hairs, with ostiolate

papilla, with a long or short neck. *Peridium* composed of cells of *textura angularis*. *Conidiophores* absent. *Conidiogenous cells* phialidic, simple, cylindrical, branched. *Conidia* globose to ovoid or cylindrical, straight or curved, hyaline, aseptate (Bose 1961; Sivanesan 1984; Tian et al. 2015).

*Type species: Herpotrichia herpotrichoides* (Fuckel) P.F. Cannon

*Notes:* The type species of *Herpotrichia* was not indicated when it was introduced (Fuckel 1868). *Herpotrichia herpotrichoides* was accepted as the type species (Cannon 1982; Wijayawardene et al. (2017). The holotype of *H. rubi* Fuckel (an earlier name of *H. herpotrichoides*) was examined by Tian et al. (2015) and characterized by ascomata spreading on substrate as a subiculum and with a black, roughened wall. *Herpotrichia* is polyphyletic (Tian et al. 2015). *Herpotrichia dalisayi* K.D. Hyde & Aptroot is the only freshwater species in the genus (Hyde and Aptroot 1998a). *Herpotrichia dalisayi* is characterized by superficial or partially immersed ascomata with a tangle of dark brown subiculum and 1–5-septate ascospores, which are characteristics of *Herpotrichia* (Hyde and Aptroot 1998a). However, the phylogenetic placement of *H. dalisayi* in *Herpotrichia* is pending confirmation with molecular data. *Herpotrichia dalisayi* has larger ascospores (53–60 × 16–18 µm) than other *Herpotrichia* species (Barr 1984; Hyde and Aptroot 1998a).

#### List of freshwater *Herpotrichia* species

*Herpotrichia dalisayi* K.D. Hyde & Aptroot, Nova Hedwigia 66(1-2): 249 (1998)

*Freshwater distribution:* Philippines (Hyde and Aptroot 1998a)

*Mamillisphaeria* K.D. Hyde, S.W. Wong & E.B.G. Jones, Nova Hedwigia 62(3–4): 514 (1996)

*Saprobic* on submerged wood. **Sexual morph:** *Ascomata* scattered to gregarious, superficial, conical, dark brown to black, carbonaceous, with ostiolate papilla, under a pseudostroma that forms a thin layer on the host surface. *Peridium* thick, composed of several layers of compressed, hyaline cells, with palisade-like cells at the sides. *Pseudoparaphyses* dense, trabeculate, long, hyaline, septate, branching and anastomosing above the asci, embedded in a gelatinous matrix. Two types of asci and ascospores: TYPE 1: *Asci* 8-spored, bitunicate, fission-tunicate, cylindro-clavate, short pedicellate, apically rounded with an ocular chamber. *Ascospores* 2–4-seriate, ellipsoidal, hyaline, 1-septate, symmetric, with pad-like, mucilaginous appendage at each end and with some mucilage associated around the spore. TYPE 2: *Asci* 8-spored, bitunicate, fission-tunicate, cylindrical, pedicellate, with an ocular chamber and faint apical ring. *Ascospores* uni- to bi-seriate, ellipsoidal-fusoid, brown,

1-septate, asymmetric, with a thin mucilaginous sheath (Tian et al. 2015). **Asexual morph:** Undetermined.

*Type species: Mamillisphaeria dimorphospora* K.D. Hyde, S.W. Wong & E.B.G. Jones

*Notes:* The monotypic genus *Mamillisphaeria* is typified by a freshwater species *M. dimorphospora* which is strikingly characterized by dimorphic ascospores (Hyde et al. 1996b), reminiscent of *Aliquandostipite siamensis* (Pang et al. 2002). However, they differ in ascomata (globose to subglobose, with stalk in *A. siamensis* vs. conical, without stalk in *M. dimorphospora*) and sheath (ascospores without sheath in *A. siamensis* vs. both types of ascospores with mucilaginous sheaths in *M. dimorphospora*) (Hyde et al. 1996b; Pang et al. 2002). *Aliquandostipite siamensis* clustered in Aliquandostipitaceae (Fig. 14), while Tian et al. (2015) placed *M. dimorphospora* in Melanommataceae, but based on morphology. Sequence data of *M. dimorphospora* is needed to clarify its phylogenetic placement.

#### List of freshwater *Mamillisphaeria* species

*Mamillisphaeria dimorphospora* K.D. Hyde, S.W. Wong & E.B.G. Jones, Nova Hedwigia 62(3–4): 515 (1996); Fig. 76

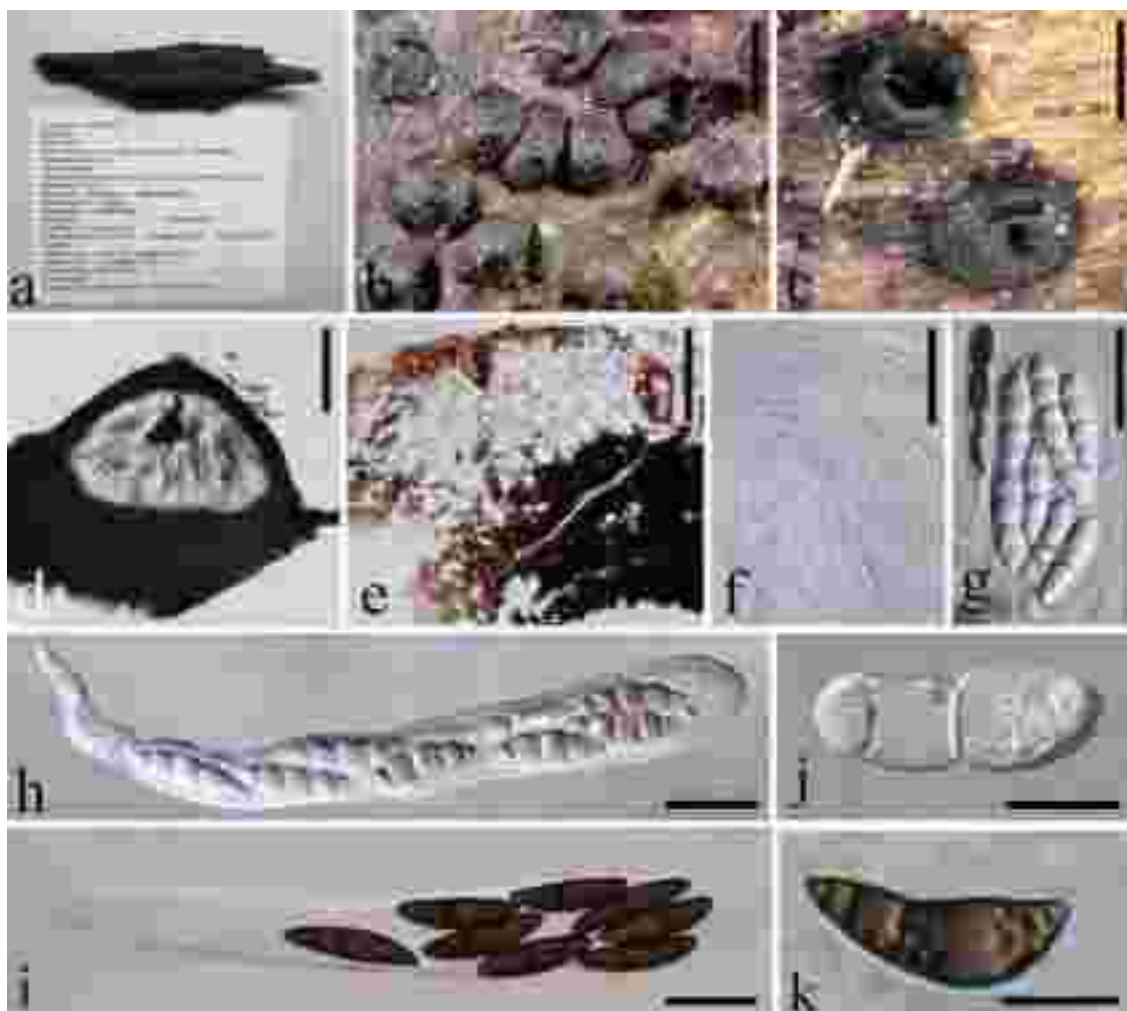
*Freshwater distribution:* Australia (Bareen and Iqbal 1994; Hyde et al. 1996b; Vijaykrishna and Hyde 2006), Brunei (Ho et al. 2001), Malaysia (Ho et al. 2001)

*Melanomma* Nitschke ex Fuckel, Jb. nassau. Ver. Naturk. 23–24: 159 (1870) [1869–70]

*Saprobic* or parasitic on wood and bark in terrestrial and freshwater habitats. **Sexual morph:** *Ascomata* gregarious, immersed, erumpent to nearly superficial, uniloculate, globose to subglobose, conical, often laterally flattened, black, coriaceous, often puckered or sulcate, with ostiolate papilla. *Peridium* two-layered, arranged in *textura angularis*, outer layer comprising small, heavily pigmented, thick-walled cells, inner layer comprising of hyaline to lightly pigmented, thin-walled cells. *Pseudoparaphyses* dense, trabeculate, filamentous, hyaline, indistinctly septate, branching and rarely anastomosing above the asci. *Asci* 8-spored, bitunicate, cylindrical to fusoid, with a short pedicel, apically rounded with an ocular chamber. *Ascospores* uniseriate, fusoid, pale brown to brown, septate, the second cell from the apex slightly wider than the others, smooth-walled (Tian et al. 2015). **Asexual morph:** Coelomycetous. *Conidiomata* superficial, globose, black, ostiolate. *Mycelium* immersed. *Peridium* two-layered. *Conidiophores* cylindrical, septate, hyaline. *Conidiogenous cells* phialidic, hyaline, smooth. *Conidia* cylindrical or ellipsoidal, hyaline, aseptate (Sutton 1980).

*Type species: Melanomma pulvis-pyrius* (Pers.) Fuckel

*Notes:* As the holotype of *Melanomma pulvis-pyrius* was not validly designated, a neotype and epitype were designated (Holm 1957; Zhang et al. 2008b). *Melanomma*



**Fig. 76** *Mamillisphaeria dimorphospora* (BRIP 22967a). (g, j Type 1, h, i, k Type 2) a Specimen and herbarium label. b, c Ascomata on host surface with a small papilla. d Vertical section of ascoma. e

Structure of peridium. f Trabeculate pseudoparaphyses. g–i Asci. j, k Ascospores. Scale bars: b = 1 mm, c = 500  $\mu$ m, d = 100  $\mu$ m, e = 50  $\mu$ m, f–i = 20  $\mu$ m, j, k = 10  $\mu$ m

*pulvis-pyrius* clustered with an asexual morphic species *Aposphaeria populina* Died., but they are not recognized as congeneric until the type species of *Aposphaeria* is obtained (Tian et al. 2015). The freshwater species *M. australiense* K.D. Hyde & Goh normally has larger ascospores than other *Melanomma* species (Hyde and Goh 1999b). The phylogenetic placement of *M. australiense* needs confirmation by molecular data.

#### List of freshwater *Melanomma* species

*Melanomma australiense* K.D. Hyde & Goh, Nova Hedwigia 68(1–2): 256 (1999); Fig. 77

*Freshwater distribution:* Australia (Hyde and Goh 1999b; Vijaykrishna and Hyde 2006)

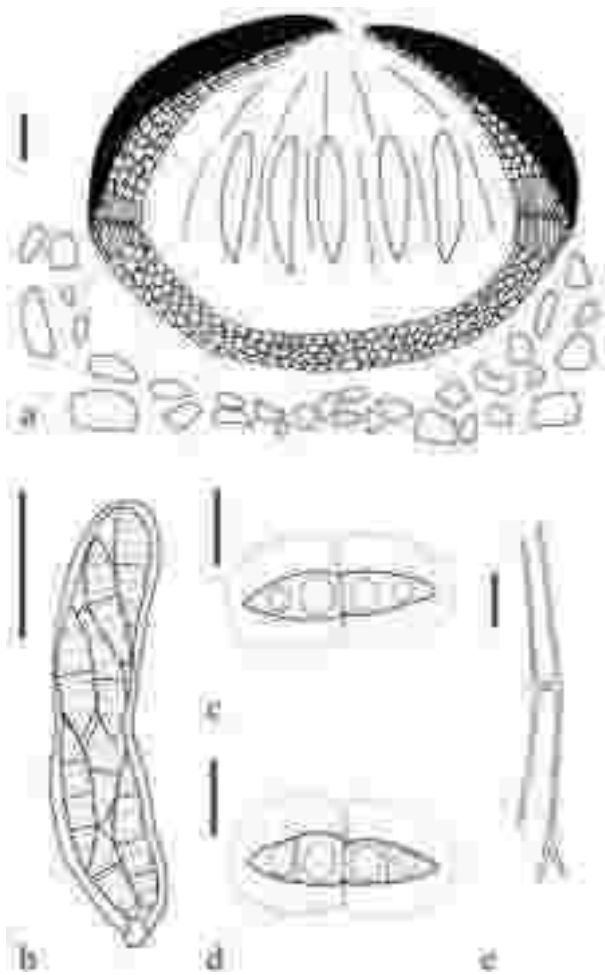
**Morosphaeriaceae** Suetrong, Sakay., E.B.G. Jones & C.L. Schoch, Stud. Mycol. 64: 161 (2009)

#### Key to freshwater genera of Morosphaeriaceae

1. Asci with uncoiled endoascus and hyaline ascospores...  
.....*Aquihelicascus*
1. Asci with coiled endoascus and brown ascospores.....  
.....*Neohelicascus*

*Helicascus* Kohlm., Can. J. Bot. 47: 1471 (1969)

*Saprobic* on rotten roots in sea water, intertidal wood of mangrove. **Sexual morph:** *Pseudostromata* immersed to semi-immersed, lenticular, solitary to clustered, dark brown to black, carbonaceous, enclosing single or multilocules, which are horizontally arranged under a black pseudoclypeus, ostiolate. *Pseudoclypeus* composed of host cells enclosed in black fungal hyphae, isodiametric or elongate cells formed between locules. *Pseudoparaphyses* numerous,



**Fig. 77** *Melanomma australiense* (redrawn from (Hyde and Goh 1999b), HKU(M) 403, **holotype**). **a** Section of ascoma. **b** Bitunicate ascus. **c, d** Ascospores. **e** Pseudoparaphyses. Scale bars: **a, b** = 50  $\mu$ m, **c, d** = 20  $\mu$ m, **e** = 10  $\mu$ m

trabeculate, filiform, persistent, hyaline, septate, anastomosing, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, subcylindrical, pedicellate, with an ocular chamber, endoascus coiled at first and finally stretching in water. *Ascospores* uniseriate, obovoid, straight to slightly curved, brown, asymmetrical, unequally 2-celled, with larger apical cells and smaller basal cells, sometimes at one or both ends apiculate, septate, smooth or verrucose, with or without a mucilaginous sheath. **Asexual morph:** Undetermined.

*Type species:* *Helicascus kanaloanus* Kohlm.

*Notes:* *Helicascus kanaloanus* was isolated from rotten roots in sea water (Kohlmeyer 1969). Sequences are available in GenBank for all *Helicascus* species. *Helicascus* comprises three species, viz. *H. kanaloanus*, *H. mangrovei* Preedanon et al. and *H. nypae* K.D. Hyde, representing a distinct marine genus which is characterized by subcylindrical asci, uniseriate, obovoid, brown, asymmetrical ascospores. We exclude other species to *Helicascus* and place them in

two new genera, *Aquihelicascus* and *Neohelicascus*, based on strong morphological and molecular evidence.

*Aquihelicascus* W. Dong, H. Zhang & Doilom, **gen. nov.**

*Index Fungorum* number: IF557806; *Facesoffungi* number: FoF08721

*Etymology:* referring to the aquatic habitat and its similarity to *Helicascus*

*Saprobic* on submerged wood. **Sexual morph:** *Pseudostromata* scattered, comprising brown to black fungal material growing in cortex of host cells, to uni- or multi-loculate, flattened at the basal region, horizontally arranged under the pseudostroma, carbonaceous or coriaceous, visible on the host surface as blackened ostiolar dots. *Locules* immersed, compressed subglobose to lenticular or sometimes triangular, with ostiolate papilla. *Ostiole* central, rounded, periphysate. *Peridium* comprising several layers of thin-walled angular cells, hyaline inwardly and dark at the outside, fusing with the host cells. *Pseudoparaphyses* numerous, cellular, hyaline, indistinctly septate, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, clavate, long pedicellate, endoascus uncoiled, apically rounded, with an indistinct or distinct, trapezoidal ocular chamber. *Ascospores* mostly biseriolate, sometimes overlapping uniseriate, straight or slightly curved, ellipsoidal to fusiform, with rounded ends, 1-septate, almost symmetrical, constricted at the septum, hyaline, smooth, thin-walled, lacking a mucilaginous sheath or appendage. **Asexual morph:** Undetermined.

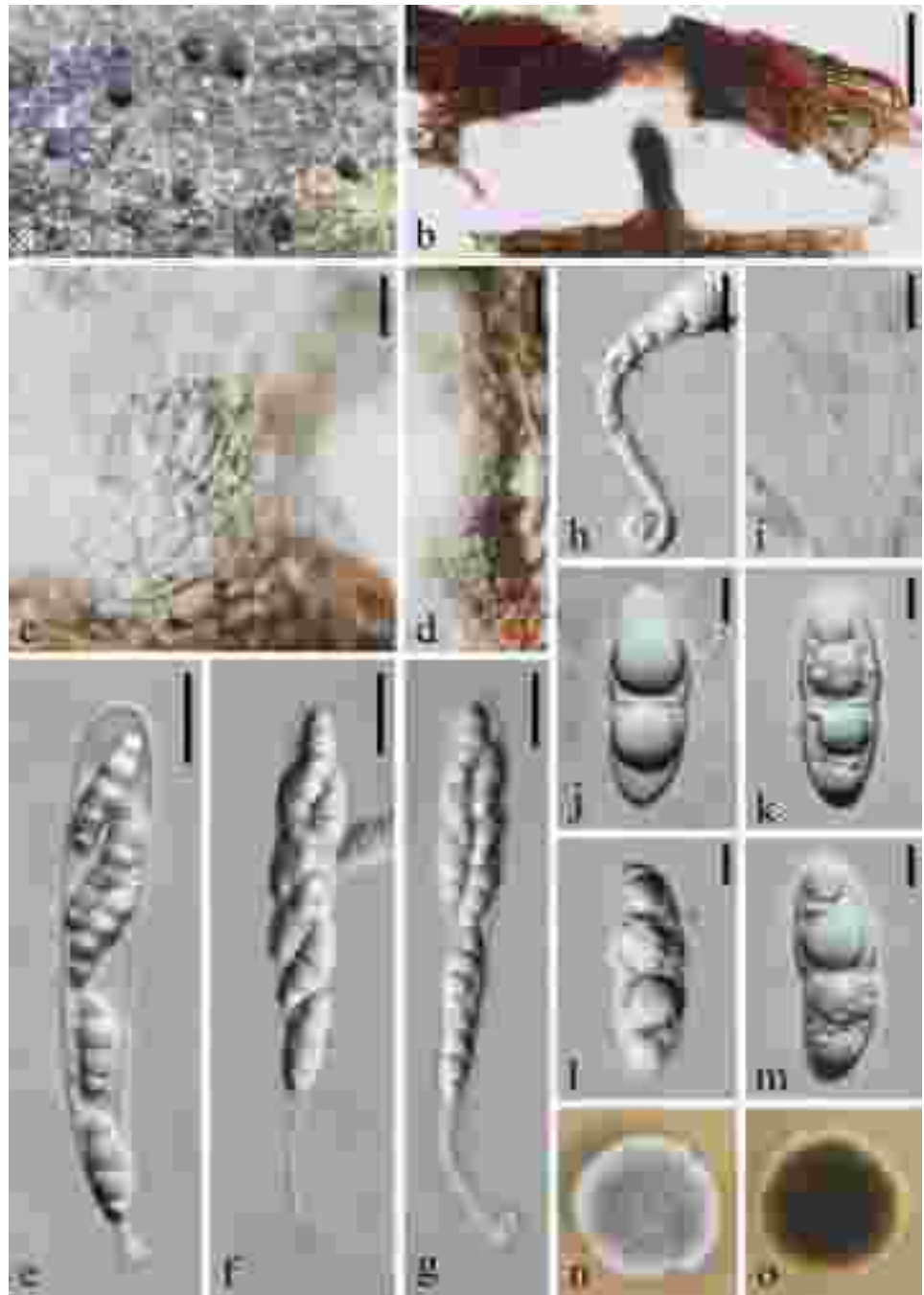
*Type species:* *Aquihelicascus thalassioideus* (K.D. Hyde & Aptroot) W. Dong & H. Zhang

*Notes:* *Aquihelicascus* is introduced to accommodate one new combination *A. thalassioideus* and two new species *A. songkhlaensis* and *A. yunnanensis*. *Aquihelicascus* differs from *Helicascus* in having clavate asci with uncoiled endoascus, and biseriolate, ellipsoidal, symmetrical, hyaline ascospores with rounded ends. In contrast, *Helicascus* has subcylindrical asci with coiled endoascus, and uniseriate, obovoid, asymmetrical, brown ascospores with apiculate ends (Kohlmeyer 1969; Hyde 1991; Preedanon et al. 2017). All *Aquihelicascus* species were collected from freshwater habitats, whereas *Helicascus* species are from marine habitats. *Aquihelicascus* and *Helicascus* clustered separately in phylogenetic analyses (Zhang et al. 2013a; Luo et al. 2016b; Preedanon et al. 2017; this study, Fig. 84) which supports the placement of two different genera.

*Aquihelicascus* species have hyaline ascospores and are morphologically very similar. Among them, *A. songkhlaensis* possesses two-loculate pseudostromata and *A. thalassioideus* has the widest peridium. The phylogenetic analysis and single gene comparison can easily separate them.

#### List of freshwater *Aquihelicascus* species

**Fig. 78** *Aquihelicascus songkhlaensis* (MFLU 18-1511, holotype). **a** Immersed ascomata with papilla erumpent through host surface. **b** Vertical section of pseudostroma. **c** Structure of medium peridium. **d** Structure of lateral peridium. **e–g** Bitunicate asci. **h** Pedicel. **i** Pseudoparaphyses. **j** Ascospore in Indian Ink. **k–m** Ascospores. **n, o** Colony on PDA (left-front, right-reverse). Scale bars: **b** = 200  $\mu$ m, **c–g, i** = 20  $\mu$ m, **h** = 10  $\mu$ m, **j–m** = 5  $\mu$ m



\**Aquihelicascus songkhlaensis* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum* number: IF557919; *Facesoffungi* number: FoF09261; Fig. 78

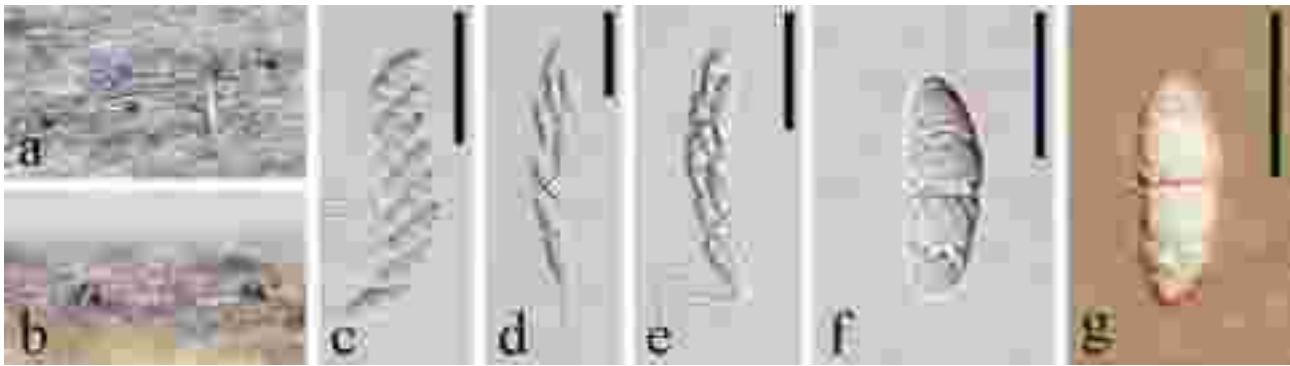
*Etymology*: referring to songkhla, where the holotype was collected

*Holotype*: MFLU 18-1511

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph**: *Pseudostromata* 380–430  $\mu$ m high, 820–870  $\mu$ m wide, scattered, comprising brown to black

fungal tissues growing in cortex of host, with two locules, flattened at the basal region, horizontally arranged under the pseudostroma, visible on the host surface as blackened ostiolar dots. *Locules* 170–270  $\times$  350–430  $\mu$ m, immersed, lenticular, coriaceous, with ostiolate papilla. *Ostiole* converging at the centre, subcylindrical, black. *Peridium* of locules, 25–35  $\mu$ m thick, comprising 8–11 layers of brown to dark brown, thin-walled, compressed cells of *textura angularis*, fusing with the host cells. *Pseudoparaphyses* 3  $\mu$ m diam., numerous, cellular, hypha-like, hyaline, distantly septate.





**Fig. 79** *Aquihelicascus thalassioideus* (MFLU 18-1705). **a, b** Immersed pseudostromata with blackened ostiolar dots. **c** Ascus embedded in pseudoparaphyses. **d, e** Bitunicate asci. **f, g** Ascospores. Scale bars: **c–e** = 50  $\mu\text{m}$ , **f, g** = 20  $\mu\text{m}$

*Asci* 110–145(–160)  $\times$  16–18  $\mu\text{m}$  ( $\bar{x}$  = 130  $\times$  17  $\mu\text{m}$ ,  $n$  = 10), 8-spored, bitunicate, clavate, long pedicellate, up to 50  $\mu\text{m}$  long, apically rounded with an indistinct ocular chamber. *Ascospores* 24–28  $\times$  8–10  $\mu\text{m}$  ( $\bar{x}$  = 25.7  $\times$  9.4  $\mu\text{m}$ ,  $n$  = 20), overlapping biseriate, straight or slightly curved, hyaline, 1-septate, constricted at the septum, symmetrical, ellipsoidal with rounded ends, with 2–4 prominent big guttules when immature, smooth, thin-walled, lacking a mucilaginous sheath. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 10 mm in 13 days at 25  $^{\circ}\text{C}$ , white to pale grey from above, dark brown from below, surface rough, dry, raised, edge entire.

**Material examined:** THAILAND, Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, 20180508-1 (MFLU 18-1511, **holotype**), ex-type living culture MFLUCC 18-1154; *ibid.*, 20180508-2 (HKAS 105006, **isotype**), ex-type living culture KUMCC 19-0022; Nakhon Si Thammarat Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat6107-1 (MFLU 18-1716), living culture MFLUCC 18-1273; *ibid.*, hat6107-2 (HKAS 105028), living culture KUMCC 19-0043; Songkhla Province, on submerged wood in a stream, 10 May 2018, W. Dong, hat8114-1 (MFLU 18-1702), living culture MFLUCC 18-1278; *ibid.*, hat8114-2 (HKAS 105036), living culture KUMCC 19-0051.

**Notes:** *Aquihelicascus songkhlaensis* clusters with *A. yunnanensis* in our phylogenetic tree (Fig. 84). They share similar morphological characters of clavate asci, ellipsoidal, hyaline, 1-septate, symmetrical ascospores and are from freshwater habitats and they have overlapping ascospore size. However, *A. songkhlaensis* can be distinguished by its two-loculate pseudostromata contrasting with one-loculate in *A. yunnanensis*. There are 12, 16 and 14 nucleotide differences between ex-type strains of the two species in LSU, ITS and TEF sequence data, respectively. Following the

guideline of Jeewon and Hyde (2016), *A. songkhlaensis* sp. nov. is introduced here.

\**Aquihelicascus thalassioideus* (K.D. Hyde & Aptroot) W. Dong & H. Zhang, **comb. nov.**

*Index Fungorum number:* IF557920; *Facesoffungi number:* FoF09262; Fig. 79

*Basionym:* *Massarina thalassioidea* K.D. Hyde & Aptroot, Nova Hedwigia 66(3–4): 498 (1998)

*Synonymy:* *Helicascus thalassioideus* (K.D. Hyde & Aptroot) H. Zhang & K.D. Hyde, Sydowia 65(1): 159 (2013)

*Freshwater distribution:* Australia (Hyde and Aptroot 1998b), Brunei (Hyde and Aptroot 1998b), China (Tsui et al. 2000; Ho et al. 2001; Luo et al. 2004), French West Indies, Martinique (Zhang et al. 2015), Peru (Shearer et al. 2015), Japan (Tanaka et al. 2015), Philippines (Hyde and Aptroot 1998b), Thailand (Kurniawati et al. 2010; Zhang et al. 2013a; this study)

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph:** *Pseudostromata* clustered, immersed, visible on the host surface as blackened ostiolar dots. *Pseudoparaphyses* 1–3  $\mu\text{m}$  diam., abundant, cellular, hypha-like, hyaline, septate, embedded in a gelatinous matrix. *Asci* 150–190  $\times$  14.5–23  $\mu\text{m}$  ( $\bar{x}$  = 175  $\times$  17.5  $\mu\text{m}$ ,  $n$  = 5), 8-spored, bitunicate, fissitunicate, clavate, long pedicellate, up to 90  $\mu\text{m}$  long. *Ascospores* 24–28  $\times$  7–10  $\mu\text{m}$  ( $\bar{x}$  = 25.5  $\times$  8.5  $\mu\text{m}$ ,  $n$  = 25), overlapping uni- to bi-seriate, sometimes overlapping tri-seriate, ellipsoidal, hyaline, 1-septate, constricted at septum, curved, thin-walled, smooth. **Asexual morph:** Undetermined (detailed description see Hyde and Aptroot (1998b) and Zhang et al. (2013a)).

**Material examined:** THAILAND, Chiang Rai Province, on submerged wood in a stream, 1 July 2018, W. Dong, CR134-1 (MFLU 18-1705), living culture KUMCC 19-0094; *ibid.*, CR134-2 (HKAS 105076).

**Notes:** Our new collection KUMCC 19-0094 is identified as *Aquihelicascus thalassioideus* based on multigene

phylogenetic analysis (Fig. 84). The morphological features of our collection also fits well with *A. thalassioideus* except for the long pedicel, which is probably because of the pedicel spreading in water (Fig. 79). Most publications do not report an ascospore sheath for *A. thalassioideus* except Zhang et al. (2015) who noted some fugacious mucilaginous remnants visible in Indian Ink when ascospores were just released from the asci. This character was not observed in our collection.

\**Aquihelicascus yunnanensis* W. Dong, H. Zhang & K.D. Hyde, *sp. nov.*

*Index Fungorum number*: IF557921; *Facesoffungi number*: FoF09263; Fig. 80

*Etymology*: referring to Yunnan, where the holotype was collected

*Holotype*: MFLU 18-1170

*Saprobic* on decaying wood submerged in freshwater.

**Sexual morph**: *Pseudostromata* 150–180 µm high, 420–480 µm diam., scattered, comprising brown to black fungal tissues growing in cortex of host, uniloculate, lenticular, immersed with blackened ostiolar dots on the host surface. *Ostiole* central, opening rounded, periphysate. *Peridium* 15–25 µm thick, comprising several layers of brown to dark brown, thin-walled, compressed, elongate cells of *textura angularis*, pale brown to hyaline inwardly. *Pseudoparaphyses* 1.5–2.5 µm diam., numerous, cellular, hypha-like, short, hyaline, sparsely septate, unbranched. *Asci* 120–140 × 13.5–19 µm ( $\bar{x}$  = 132 × 17 µm, n = 10), 8-spored, bitunicate, clavate, apically narrowly rounded, with a distinct, trapezoidal chamber, long pedicellate, up to 40 µm. *Ascospores* 25–28 × 9–10.5 µm ( $\bar{x}$  = 27 × 10 µm, n = 10), biseriate, ellipsoidal, rounded at both ends, straight or slightly curved, hyaline, 1-septate, constricted at the septum, symmetrical, guttulate, two prominent guttules near the septa and with additionally smaller ones beside, smooth, thin-walled, lacking a mucilaginous sheath. **Asexual morph**: Undetermined.

*Culture characteristics*: On PDA, colony circular, reaching 5 mm in 15 days at 25 °C, white to grey from above, brown from below, surface rough, fluffy, with dense mycelium, dry, raised, edge entire.

*Material examined*: CHINA, Yunnan Province, Dehong, on submerged wood in a stream, 25 November 2017, G.N. Wang, H35A-1 (MFLU 18-1170, **holotype**), ex-type living culture MFLUCC 18-1025; *ibid.*, H35A-2 (HKAS 101719, **isotype**), ex-type living culture KUMCC 18-0069.

*Notes*: *Aquihelicascus yunnanensis* clusters with *A. songkhlaensis* in our multigene phylogenetic analysis (Fig. 84). However, the number of locules in pseudostromata, single gene comparison (see notes under *A. songkhlaensis*) and phylogenetic analysis (Fig. 84) separate them to be different species.

*Aquihelicascus yunnanensis* is morphologically similar to *A. thalassioideus* in having clavate asci, and ellipsoidal, hyaline, 1-septate, guttulate ascospores (Zhang et al. 2013a). However, *A. yunnanensis* has a thinner peridium (15–25 µm vs. 70 µm) than *A. thalassioideus*. There are 10 and 32 nucleotide differences in LSU and ITS sequence data between *A. yunnanensis* (MFLUCC 18-1025) and *A. thalassioideus* (MFLUCC 10-0911), respectively, which indicates them to be different species.

#### Key to freshwater *Aquihelicascus* species

1. Pseudostromata multi-loculate.....*A. songkhlaensis*
1. Pseudostromata uni-loculate.....2
2. Peridium 15–25 µm thick.....*A. yunnanensis*
2. Peridium up to 70 µm thick.....*A. thalassioideus*

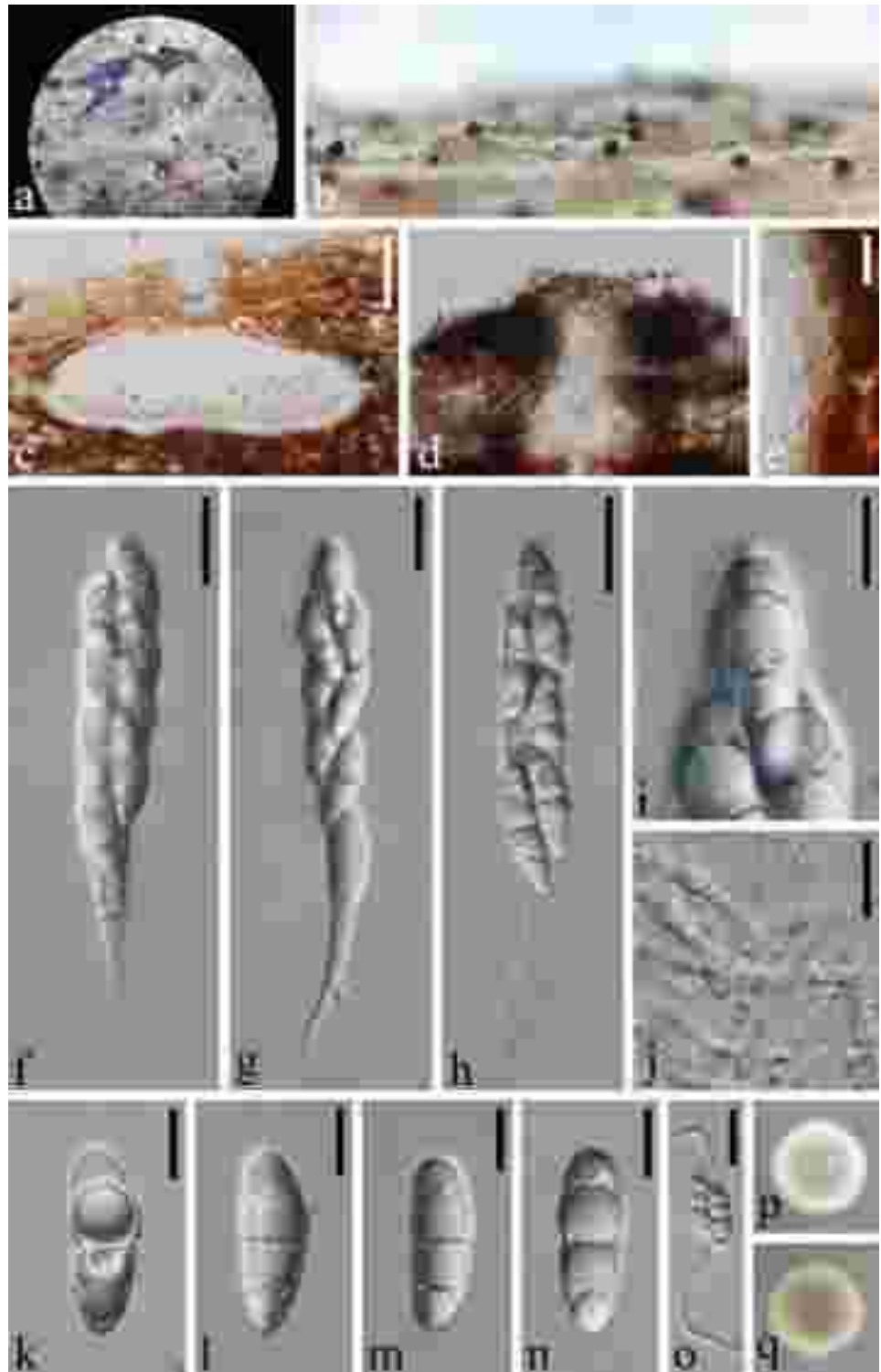
*Neohelicascus* W. Dong, H. Zhang, K.D. Hyde & Doilom, *gen. nov.*

*Index Fungorum number*: IF557807; *Facesoffungi number*: FoF08722

*Etymology*: referring to its morphological similarity to *Helicascus*

*Saprobic* on submerged wood. **Sexual morph**: *Pseudostromata* scattered, comprising brown to black fungal material growing in cortex of host, unil- or multi-loculate, flattened at the basal region, horizontally arranged under the pseudostroma, visible on the host surface as blackened ostiolar dots. *Locules* immersed, lenticular to ampulliform, ostiolate. *Ostiole* converging at the centre, uniting into one common, central pore, periphysate. *Peridium* of locules comprising several layers of brown, thin-walled angular cells, paler inwardly, fusing with the host cells. *Pseudoparaphyses* numerous, cellular, hypha-like, hyaline, septate, embedded in a gelatinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, clavate, long pedicellate, base of endoascus long, narrow and coiled within ectoascus, ectoascus uncoiling to form a long tail-like extension, apically rounded with a cylindrical ocular chamber. *Ascospores* mostly biseriate, straight or slightly curved, ellipsoidal to broadly fusiform, with rounded ends, 1–(2–3)-septate, apical cell slightly larger than basal cell, brown, thin-walled, smooth- or rough-walled, with or without a deliquescing sheath (Zhang et al. 2013a). **Asexual morph**: Coelomycetous. *Pycnidia* solitary, superficial, with the base immersed, uniloculate, globose to subglobose, brown, coriaceous, central ostiolate, with minute papilla. *Peridium* composed of brown, thin-walled cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* holoblastic, determinate, cylindrical to subcylindrical, hyaline, smooth. *Conidia* ellipsoid to obovoid, hyaline, aseptate, occasionally two-celled,

**Fig. 80** *Aquihelicascus yunnanensis* (MFLU 18-1170, holotype). **a, b** Immersed ascomata with blackened ostiolar dots on the host surface. **c** Vertical section of ascoma. **d** Structure of ostiole. **e** Structure of peridium. **f–i** Bitunicate asci. **j** Pseudoparaphyses. **k–n** Ascospores. **o** Germinated ascospores. **p** Colony on PDA (up-front, down-reverse). Scale bars: **c** = 100  $\mu$ m, **d** = 50  $\mu$ m, **e, i–n** = 10  $\mu$ m, **f–h, o** = 20  $\mu$ m



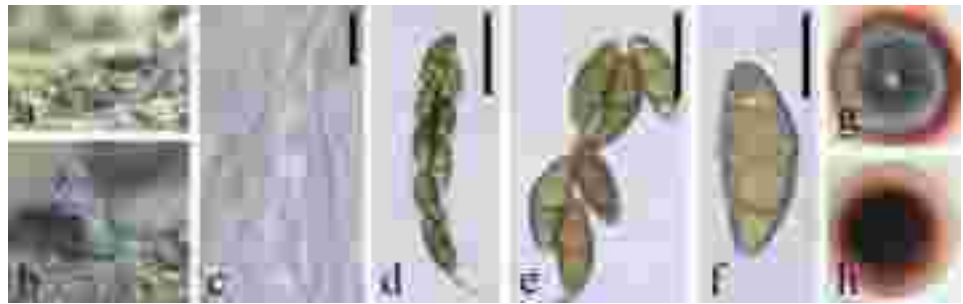
smooth, thin-walled, without sheath or appendage (Zhang et al. 2013a).

*Type species: Neohelicascus aquaticus* (H. Zhang & K. D. Hyde) W. Dong & H. Zhang

*Notes: Neohelicascus* is introduced to accommodate the new species *N. submersus* H. Yang et al. and another seven

new combinations as listed below. *Neohelicascus* is distinguished from *Aquihelicascus* in producing brown ascospores and the base of endoascus is long, narrow and coiled within ectoascus which uncoils to form a long tail-like extension. In contrast, *Aquihelicascus* possesses hyaline ascospores and

**Fig. 81** *Neohelicasus aquaticus* (HKAS 102145). **a**, **b** Immersed pseudostromata with blackened ostiolar dots. **c** Pseudoparaphyses. **d** Bitunicate ascus. **e**, **f** Ascospores. **g**, **h** Colony on PDA (up-front, down-reverse). Scale bars: **c**, **f** = 10  $\mu$ m, **d** = 30  $\mu$ m, **e** = 20  $\mu$ m



an uncoiled endoascus. Phylogenetic analysis supports two genera, *Aquihelicasus* and *Neohelicasus* (Fig. 84).

The ascospores of *Neohelicasus egyptiacus* and *N. elaterascus* are surrounded by a defined gelatinous sheath, which is absent in *N. unilocularis* (Zhang et al. 2013a, 2015). *Neohelicasus gallicus* is surrounded by some fugacious mucilaginous remnants, but only seen in ascospores that are just released from the asci (Zhang et al. 2014b). *Neohelicasus aquaticus* was characterized by 1–(2– or 3–) -septate ascospores with a deliquescent sheath (Zhang et al. 2013a), although Tanaka et al. (2015) observed only 1-septate ascospores without a sheath from a Japanese collection. *Neohelicasus aquaticus* is the only species producing asexual morph in culture (Zhang et al. 2013a).

#### List of freshwater *Neohelicasus* species

\**Neohelicasus aquaticus* (H. Zhang & K. D. Hyde) W. Dong, K.D. Hyde & H. Zhang, **comb. nov.**

*Index Fungorum number*: IF557922; *Facesoffungi number*: FoF09264; Fig. 81

*Basionym*: *Helicascus aquaticus* H. Zhang & K.D. Hyde, *Sydowia* 65(1): 155 (2013)

*Synonymy*: *Helicascus alatus* M. Zeng, S.K. Huang, Q. Zhao & K.D. Hyde, *Phytotaxa* 351(3): 215 (2018)

*Freshwater distribution*: China (Zeng et al. 2018; this study), Japan (Tanaka et al. 2015), Thailand (Zhang et al. 2013a)

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph**: *Pseudostromata* clustered, immersed, ampulliform, coriaceous, with ostiolate papilla, visible on the host surface as blackened ostiolar dots. *Pseudoparaphyses* 1–3  $\mu$ m diam., abundant, cellular, hypha-like, hyaline, septate, embedded in a gelatinous matrix. *Asci* 90–120  $\times$  14–21  $\mu$ m ( $\bar{x}$  = 110  $\times$  19  $\mu$ m,  $n$  = 5), 8-spored, bitunicate, fissitunicate, clavate, pedicellate. *Ascospores* 20–23  $\times$  8–10  $\mu$ m ( $\bar{x}$  = 22  $\times$  8.5  $\mu$ m,  $n$  = 15), mostly biserial, ellipsoidal, brown, 1-septate, slightly constricted at septum, asymmetrical, apical cell usually longer than basal cell, curved, thin-walled, smooth, with a deliquescent sheath. **Asexual morph**: Undetermined.

*Culture characteristics*: On PDA, colony circular, reaching 40 mm in 50 days at 25 °C, reddish brown to black

from above, reddish brown from below, surface rough, dry, umbonate, edge entire.

*Material examined*: CHINA, Yunnan Province, Qujing, on submerged wood in a stream, 10 May 2017, C.X. Liu, L25 (HKAS 102145), living culture KUMCC 19-0107.

*Notes*: The LSU and ITS sequence data from our collection HKAS 102145 shows 100% identity with the sequences of *Helicascus aquaticus* (MFLUCC 10-0918). HKAS 102145 fits well with the morphological features of *H. aquaticus* (Zhang et al. 2013a). However, we did not observe the 3-septate ascospores and asexual morph in culture.

*Helicascus alatus* was introduced by Zeng et al. (2018) based on multigene phylogenetic analysis and morphological characters. Zeng et al. (2018) considered that *H. alatus* differs from *H. aquaticus* in smaller ascomata, short pedicels and presence of a sheath. However, a comparison of nucleotides shows that *H. alatus* (MFLUCC 17-2300) and *H. aquaticus* (MFLUCC 10-0918) have identical sequences in LSU and ITS sequence data. Additionally, there are only two nucleotide differences in TEF sequence data between *H. alatus* (MFLUCC 17-2300) and *H. aquaticus* (MAFF 243866), thus *H. alatus* is a synonymy of *H. aquaticus*. Phylogenetic analysis supports these strains to be the same species (Fig. 84).

\**Neohelicasus chiangraiensis* (Z.L. Luo, J.K. Liu, H.Y. Su & K.D. Hyde) W. Dong, K.D. Hyde & H. Zhang, **comb. nov.**

*Index Fungorum number*: IF557923; *Facesoffungi number*: FoF09265

*Basionym*: *Helicascus chiangraiensis* Z.L. Luo, H.Y. Su & K.D. Hyde, *Phytotaxa* 270(3): 185 (2016)

*Freshwater distribution*: Thailand (Luo et al. 2016b)

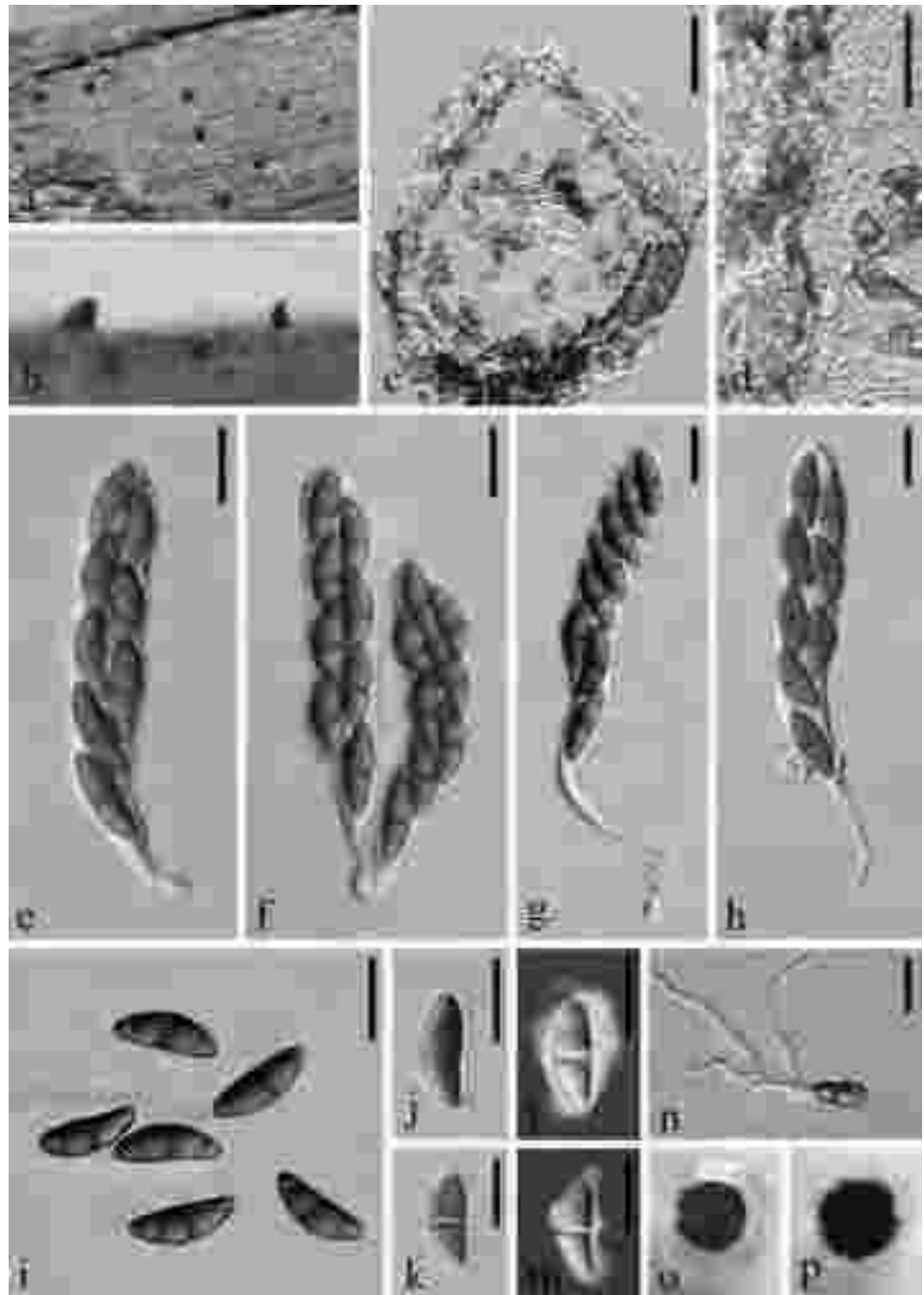
\**Neohelicasus egyptiacus* (Abdel-Wahab & Abdel-Aziz) W. Dong, K.D. Hyde & H. Zhang, **comb. nov.**

*Index Fungorum number*: IF557924; *Facesoffungi number*: FoF09266

*Basionym*: *Helicascus egyptiacus* Abdel-Wahab & Abdel-Aziz [as ‘aegyptiacus’], *Sydowia* 65(1): 153 (2013)

*Freshwater distribution*: Egypt (Zhang et al. 2013a)

**Fig. 82** *Neohelicascus elaterascus* (c, d, h, j–m from MFLU 18-1007, e–g, i, n–p from MFLU 18-0997). a, b Immersed pseudostromata with blackened ostiolar dots. c Vertical section of pseudostroma. d Structure of peridium. e–h Bitunicate asci. i–m Ascospores. (l, m in Indian Ink). n Germinated ascospore. o, p Colony on PDA (left-front, right-reverse). Scale bars: c = 50  $\mu$ m, d–n = 20  $\mu$ m



\**Neohelicascus elaterascus* (Shearer) W. Dong, K.D. Hyde & H. Zhang, *comb. nov.*

*Index Fungorum number*: IF557925; *Facesoffungi number*: FoF09267; Fig. 82

*Basionym*: *Kirschsteiniothelia elaterascus* Shearer, *Mycologia* 85:963 (1993).

*Synonymy*: *Morosphaeria elaterascus* (Shearer) S. Boonmee & K.D. Hyde, *Mycologia* 103: 705 (2012)

*Helicascus elaterascus* (Shearer) H. Zhang & K.D. Hyde, *Sydowia* 65: 158 (2013)

*Freshwater distribution*: Brunei (Ho et al. 2001), Chile (Shearer 1993b), China (Tsui et al. 2000; Luo et al. 2004), Japan (Tanaka et al. 2015), Peru (Shearer et al. 2015), South Africa (Hyde et al. 1998), Thailand (Hyde et al. 2020a; this study), USA (Shearer 1993b; Raja et al. 2009b)

*Saprobic* on decaying wood submerged in freshwater. **Sexual morph**: *Pseudostromata* 205–250  $\mu$ m high, 170–200  $\mu$ m diam., clustered, immersed, unilocular, mammiform, coriaceous, with ostiolate papilla, visible on the host surface as blackened ostiolar dots. *Peridium* 20–30  $\mu$ m thick,

comprising several layers of brown, thin-walled, pseudo-parenchymatous cells, outer layer partially occluded with the host cells, deeply pigmented, inwardly hyaline, forming a *textura angularis* in the upper regions and a *textura prismatica* at sides and angles of base. *Pseudoparaphyses* 1–3  $\mu\text{m}$  diam., abundant, cellular, cylindrical, hyaline, sparsely septate. *Asci* 95–130  $\times$  17–24  $\mu\text{m}$  ( $\bar{x}$  = 112  $\times$  21  $\mu\text{m}$ ,  $n$  = 15), 8-spored, bitunicate, fissitunicate, clavate, endoascus separating from ectoascus at time of ectoascus dehiscence, base of endoascus long, narrow and coiled within ectoascus, up to 210  $\mu\text{m}$  long. *Ascospores* 23–28  $\times$  8–11  $\mu\text{m}$  ( $\bar{x}$  = 25  $\times$  9  $\mu\text{m}$ ,  $n$  = 25), overlapping uni- or bi-seriate, sometimes overlapping tri-seriate, ellipsoidal, brown, 1-septate, constricted at septum, asymmetrical, apical cell slightly longer than basal cell, curved, thin-walled, smooth in optical microscope, surrounded by a gelatinous sheath. **Asexual morph:** Undetermined.

**Culture characteristics:** On PDA, colony circular, reaching 40 mm in 45 days at 25 °C, dark brown to black from above, black from below, surface rough, dry, raised, edge undulate.

**Material examined:** THAILAND, Phayao Province, on submerged wood in a stream, 23 February 2018, X.D. Yu, Y19 (MFLU 18-0997), living culture MFLUCC 18-0985; Chiang Mai Province, Mushroom Research Center, on submerged wood in a stream, 2 April 2018, X.D. Yu, Y29 (MFLU 18-1007), living culture MFLUCC 18-0993.

**Notes:** Our new collections MFLUCC 18-0985 and MFLUCC 18-0993 are identified as *Neohelicasus elaterascus* based on morphological and molecular evidence (Fig. 84). Shearer (1993b) described verrucose ascospores from the holotype (C-76-1), while our collections have smooth ascospores which are also described by Hyde et al. (2020a). *Helicasus elaterascus* is commonly reported on submerged wood from many countries as listed above.

\**Neohelicasus gallicus* (Y. Zhang ter & J. Fourn) W. Dong, K.D. Hyde & H. Zhang, **comb. nov.**

**Index Fungorum number:** IF557926; **Facesoffungi number:** FoF09268

**Basionym:** *Helicasus gallicus* Y. Zhang ter & J. Fourn., Phytotaxa 183: 185 (2014)

**Freshwater distribution:** France (Zhang et al. 2014b)

\**Neohelicasus submersus* H. Yang, W. Dong, K.D. Hyde & H. Zhang, **sp. nov.**

**Index Fungorum number:** IF557927; **Facesoffungi number:** FoF09269; Fig. 83

**Etymology:** referring to submerged habitat of this fungus

**Holotype:** MFLU 20-0436

**Saprobic** on submerged wood. **Sexual morph:** *Pseudostromata* 485–540  $\mu\text{m}$  high, 315–510  $\mu\text{m}$  diam., scattered, comprising dark brown to black fungal tissues growing in

cortex of host, immersed, unilocular, lenticular, flattened at the base, black, with wide ostiole, visible as elongate, prominent, black neck erumpent on the host surface. *Neck* 310–320  $\times$  125–165  $\mu\text{m}$ , central, cylindrical, with periphyses. *Peridium* 20–45  $\mu\text{m}$  thick, comprising several layers of dark brown cells of *textura epidermoidea*. *Pseudoparaphyses* 1.5–2.5  $\mu\text{m}$  wide, numerous, cellular, cylindrical, hyaline, indistinctly septate. *Asci* 120–165  $\times$  17.5–21  $\mu\text{m}$  ( $\bar{x}$  = 143  $\times$  19  $\mu\text{m}$ ,  $n$  = 10), 8-spored, bitunicate, fissitunicate, cylindrical to narrowly clavate, apically rounded, base of endoascus long, narrow and coiled within ectoascus, up to 150  $\mu\text{m}$  long. *Ascospores* 23.5–31  $\times$  8.5–11.5  $\mu\text{m}$ , ( $\bar{x}$  = 27  $\times$  10  $\mu\text{m}$ ,  $n$  = 30), obliquely arranged, overlapping uniseriate or occasionally biseriate, ellipsoidal to fusiform, slightly curved, 1-septate, constricted at the septum, upper cell slightly wider than lower cell, hyaline when young, become brown when mature, guttulate, thin-walled, smooth, covered by a distinct gelatinous sheath, 1.5–5.5  $\mu\text{m}$  wide, showing 15–27  $\mu\text{m}$  wide in Indian Ink. **Asexual morph:** Undetermined.

**Cultural characteristics:** Conidia germinated on PDA within 24 hours. Germ tubes arising from end of the ascospore. Colonies on PDA reaching 30 mm diameter in 30 days at 20–25 °C, initially white, turning reddish brown to dark brown after 15 days, with dense, hairy mycelium on the surface, with undulate, red margin, reverse reddish brown.

**Material examined:** CHINA, Yunnan Province, a small river in Puzhehei wetland, on submerged wood, 23 June 2019, H. Yang, P36 (MFLU 20-0436, **holotype**), ex-type living culture KUMCC 20–0153.

**Notes:** *Neohelicasus submersus* forms a well-supported clade with four species *N. elaterascus*, *N. egyptiacus*, *N. unilocularis* and *N. uniseptatus* (Fig. 84). *Neohelicasus submersus* resembles *N. elaterascus* in having unilocular pseudostromata, and ellipsoidal to fusiform, brown ascospores with a distinct gelatinous sheath (Shearer 1993b). However, *N. elaterascus* has shorter and wider asci (95–149  $\times$  (14–)18–25(–38)  $\mu\text{m}$  vs. 120–165  $\times$  17.5–21  $\mu\text{m}$ ) and ascospores are mostly biseriate and verrucose in the holotype C-76-1 (Shearer 1993b). Our two collections of *N. elaterascus* (MFLUCC 18-0993 and MFLUCC 18-0985) have smooth ascospores, which are similar to *N. submersus*, but phylogenetic analysis separate them as distinct species (Fig. 84).

*Neohelicasus submersus* is phylogenetically closest to *N. unilocularis* (Fig. 84). They have overlapping ascospore size, however, *N. submersus* has longer and thinner asci (120–165  $\times$  17.5–21  $\mu\text{m}$  vs. 70–75  $\times$  22–27  $\mu\text{m}$ ) than *N. unilocularis* (Zhang et al. 2015). *Neohelicasus submersus* has a distinct gelatinous ascospore sheath, 1.5–5.5  $\mu\text{m}$  wide, showing 15–27  $\mu\text{m}$  wide in Indian Ink, but it is lacking in *N. unilocularis*. There are two and 39 nucleotide differences in LSU and ITS sequence data between *N. submersus*