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Molecular phylogeny and morphological characterization of asexual fungi (Tubeufiaceae) from freshwater habitats in Yunnan, China

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Abstract – The diversity of lignicolous freshwater fungi along a north-south latitudinal gradient are currently being studied in Asia. In this paper, we report on 18 collections of asexual morphs of Tubeufiaceae from submerged wood in rivers, streams and a lake in Yunnan Province, China. Taxa are characterized based on morphological characters and analyses of ITS, LSU and TEF1α sequence data. The new genera, *Muripulchra* with a single species, *M. aquatica* and *Neohelicomyces* with three new taxa (*N. aquaticus*, *N. grandisporus*, *N. submersus*) are introduced. *Muripulchra* is characterized by micronematous conidiophores and obpyriform, septate to muriform conidia. *Neohelicomyces* is characterized macronematous conidiophores and multi-septate, helicoid conidia. *Tubeufia aquatica* is introduced as a new species and its phylogenetic relationships with other taxa is discussed. The phylogenetic analyses of a concatenated ITS, LSU and TEF1α dataset place all collections in the family Tubeufiaceae (Tubeufiales) and provide evidence to support the establishment of our new taxa. The asexual morph of *Tubeufia cylindrothecia,* the type species of *Helicomyces* (*H. roseus*) are described herein, phylogenetic relationships assessed and reference specimens are given for these two species. Descriptions and illustrations for the new genera and species are provided with notes on their taxonomy and phylogeny.

Asexual morph / Freshwater fungi / Phylogeny / Taxonomy / Tubeufiaceae

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INTRODUCTION

Aquatic hyphomycetes are a diverse group of asexual morphs involved in litter degradation in freshwater ecosystems (Goh & Hyde 1996; Tsui & Hyde 2004; Vijaykrishna *et al.* 2005; Raja *et al.* 2007; Kodsueb *et al.* 2007; Krauss *et al.* 2011; Su *et al.* 2015, 2016). Goh & Hyde (1996) recognized four biological groups of freshwater hyphomycetes classified as the ingoldian, aeroaquatic, terrestrial-aquatic and submerged-aquatic species. Most of the species are adapted to living in aquatic habitats and have specialized propagules for effective dispersal in water (Gulis *et al.* 2005). These taxa have a worldwide distribution, with more than 300 estimated species, most recorded from temperate and fewer from tropical and subtropical regions (Goh & Hyde 1996; Ho *et al.* 2002, Shearer *et al.* 2006; Zhang *et al.* 2008 a, b; Graca *et al.* 2016). However, several freshwater hyphomycetes have recently been recorded from tropical regions (Sivichai *et al.* 2000; Cai *et al.* 2006a, Hyde *et al.* 2016a; Yang *et al.* 2015, 2016 a, b).

Barr (1979) established the family Tubeufiaceae in Pleosporales based on the generic type *Tubeufia* and included other five genera (*Letendraeopsis*, *Melioliphila*, *Podonectria*, *Rebentischia*, *Thaxteriella*). Several genera have since been assigned to Tubeufiaceae with the numbers from six to ten (Barr 1979, 1980), 12 (Rossman 1987), 21 (Kirk *et al.* 2001) and even 32 (Kirk *et al.* 2008). Boonmee *et al.* (2014) revisited Tubeufiaceae, introduced a new order Tubeufiales to accommodate this monophyletic group, which had previously been referred to Pleosporales and accepted 19 genera (including five asexual genera). Subsequently, Wijayawardene *et al.* (2014) recognized 21 genera in Tubeufiaceae, while Doilom *et al.* (2017) accepted 20 genera.

Penzig & Saccardo (1897) introduced the genus *Tubeufia* with *T. javanica* as the type from *Bambusa emarcidis* from Java, Indonesia. Currently, the genus *Tubeufia* comprises 27 species, 14 species whose placement are confirmed with molecular data, and to date nine species are reported from freshwater habitats (Lu *et al.* 2017). Some *Tubeufia* species reproduce asexually in culture and have been connected to asexual genera, such as *Aquaphila*, *Helicoma*, *Helicomyces* or *Helicosporium*. Most of these established links have been confirmed by cultural methods and supported by DNA sequence data (Tsui *et al.* 2006, 2007; Promputtha & Miller 2010; Sánchez & Bianchinotti 2010; Sánchez *et al.* 2012; Boonmee *et al.* 2014; Lu *et al.* 2017). The genus *Helicomyces* established by Link (1809) with *H. roseus* as type species, is characterized by helicoid, tightly or loosely coiled, multi-septate, filamentous, hyaline, white, pinkish and brown conidia (Link 1809; Boonmee *et al.* 2014).

We are investigating lignicolous freshwater fungi along a north-south latitudinal gradient in Asian and Australasian region (Hyde *et al.* 2016b). There have been a few studies on freshwater fungi in Yunnan Province, China (Cai *et al.* 2002; Luo *et al.* 2004; Liu *et al.* 2015; Su *et al.* 2015, 2016; Zhu *et al.* 2016, but of the asexual Tubeufiaceae species, only *Helicomyces roseus* has been recorded from freshwater habitats in Yunnan Province (Cai *et al.* 2002; Luo *et al.* 2004; Hu *et al.* 2013). In this study, 18 fresh collections of hyphomycetes which belong to the family Tubeufiaceae were made from submerged wood in rivers, streams and a lake in Yunnan Province. New descriptions and illustrations are provided for two new genera, five new species, the asexual morph of *Tubeufia cylindrothecia* and the type species of *Helicomyces*, reference specimen are provided for these two species. A comprehensive molecular phylogenetic analysis is also performed to clarify species relationships, as well as to provide support for the establishment of novel taxa.

MATERIALS AND METHODS

Isolation and morphology

Specimens of submerged decaying wood were collected from streams in Cangshan Mountain, Erhai Lake, Nujiang River, Jinsha River and Lancang River in the west and northwest of Yunnan Province in China and returned to the laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week and examined following the methods described in Taylor & Hyde (2003). Morphological observations were made using a Motic SMZ 168 Series stereomicroscope and photographed by an OLYMPUS BX51 microscope imaging system. The fungal structures were measured using Image-Pro-Express software.

Single spore isolations were made to obtain pure cultures (Chomnunti *et al.* 2014). Germinating conidia were individually transferred to fresh PDA or MEA plates, followed by observation under a stereomicroscope. The cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC), Culture collection of Kunming Institute of Botany (KUMCC) and Dali University (DLUCC). Specimens (dry wood material with fungal material) are deposited in the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS), Mae Fah Luang University (MFLU) and Dali University (DLU). Facesoffungi number was registered as in Jayasiri *et al.* (2015). New species are introduced using data following the recommendations of Jeewon & Hyde (2016).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh fungal mycelium grown on PDA at room temperature. The EZ geneTM Fungal gDNA kit (GD2416) was used to extract DNA according to the manufacturer's instructions. ITS, LSU and TEF1α gene regions were amplified using the primer pairs ITS5/ITS4, LROR/LR5 and EF1-983F/ EF1-2218R. The final volume of the PCR reaction was 25 µl and contained 12.5 µl of 2×Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µl Taq DNA Polymerase, 500 µm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mM Tris-HCl pH 8.3, 100 Mm KCl, 3 mM MgCl2, stabilizer and enhancer), 1 µl of each primer (10 µM), 1 µl genomic DNA extract and 9.5 µl deionised water. The PCR thermal cycle program for ITS amplification was as follows: initial denaturation of 95°C for 3 mins, followed by 35 cycles of denaturation at 95°C for 1 mins, annealing at 53°C for 30 seconds, elongation at 72°C for 1 mins. The PCR thermal cycle program for LSU was as follows: 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 40 seconds, elongation at 72°C for 90 seconds. TEF1α region was amplified in the conditions with and initial denaturation of 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 40 seconds, annealing at 55°C for 50 seconds, elongation at 72°C for 90 seconds, and the final extension at 72°C for 10 mins included for each condition of amplification. PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27-9602-01). DNA sequencing were performed by same PCR primers described above by Beijing Tsingke Biological Engineering Technology and Services Co., Ltd (Beijing, P.R. China).

Phylogenetic analyses

Sequence data for relevant strains were downloaded from GenBank following data from recent publications (Kodsueb *et al* 2006; Schoch *et al.* 2006; Tsui *et al.* 2006, 2007; Promputtha & Miller 2010; Boonmee *et al.* 2011, 2014; Sánchez *et al.* 2012; Slippers *et al.* 2013; Suetrong *et al.* 2014; Doilom *et al.* 2016; Hyde *et al.* 2016a; Lu *et al.* 2017). Raw sequences were assembled with Sequencher 4.9 for Windows (Gene Codes Corp., Ann Arbor, Michigan). The consensus sequences were initially aligned using MAFFTv.7 (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and optimized manually when needed. A maximum likelihood analysis (ML) was performed using RAxMLGUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR + GAMMA substitution model.

Maximum parsimony analyses (MP) were performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm (Jeewon *et al.* 2002, 2003). All characters were unordered and of equal weight and gaps were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993, Jeewon *et al* 2013).

Bayesian analyses were performed by using PAUP v.4.0b10 (Swofford 2002) and MrBayes v3.2.2 (Ronquist *et al.* 2012). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.0b4 (Liu *et al.* 2011). Six simultaneous Markov Chains were run for 1 million generations and trees were sampled every 100th generation (resulting in 10000 trees) (Cai *et al.* 2006b). The first 2000 trees representing the burn-in phase of the analyses were discarded and the remaining 8000 (post burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai *et al.* 2006, Liu *et al.* 2011).

The phylogenetic analyses were carried out with combined ITS, LSU and TEF1α sequence data alignment to illustrate the placement of the isolates in Tubeufiaceae. All new sequence data generated in this study are deposited in GenBank (Table 1). All alignments are deposited in TreeBASE (www.treebase.org, submission number 20541). Phylogenetic trees were viewed in Treeview (Page 1996). The terminals of the tree (Fig. 1) are labeled with species and the isolates/culture collection codes as provided in GenBank.

RESULTS

Phylogenetic analyses

A combined dataset of 2307 characters (LSU, ITS and TEF1α sequence data) with 90 taxa analyzed using MP, ML and Bayesian analyses resulted in trees which were topologically congruent with respect to the position of the new taxa

Table 1. Isolates and sequences used in this study (newly generated sequences are indicated in bold)

Species	*Collection/Isolate number*	*GenBank accession number*			*References*
		LSU	*ITS*	*TEF1α*	
Acanthohelicospora aurea	NBRC 7098	AY856894	AY916478	–	Tsui *et al*. (2006)
Acanthohelicospora guianense	UAMH 1699	AY856891	AY916479	–	Tsui *et al*. (2006)
Acanthohelicospora pinicola	MFLUCC 10-0116	KF301534	KF301526	KF301555	Boonmee *et al*. (2014)
Acanthostigma chiangmaiensis	MFLUCC 10-0125	JN865197	JN865209	KF301560	Boonmee *et al*. (2011)
Acanthostigma patagonicum	BBB MVB573	JN127359	JN127358	–	Sánchez *et al*. (2012)
Acanthostigma perpusillum	UAMH 7237	AY856892	AY916492	–	Tsui *et al*. (2006)
Acanthostigmina multiseptatum	ANM 475	GQ850492	GQ856145	–	Promputtha & Miller (2010)
Acanthostigmina multiseptatum	ANM 665	GQ850493	GQ856144	–	Promputtha & Miller (2010)
Aquaphila albicans	BCC 3463	DQ341100	DQ341097	–	Tsui *et al*. (2007)
Aquaphila albicans	BCC 3543	DQ341101	DQ341096	–	Tsui *et al*. (2007)
Aquaphila albicans	BCC 3520	DQ341102	DQ341098	–	Tsui *et al*. (2007)
Aquaphila albicans	MFLUCC 16-0010	KX454166	KX454165	–	Hyde *et al*. (2016a)
Aquaphila albicans	MFLUCC 16-0020	KX454168	KX454167	–	Hyde *et al*. (2016a)
Boerlagiomyces macrospora	MFLUCC 12-0388	KU764712	KU144927	KU872750	Doilom *et al*. (2017)
Botryosphaeria dothidea	CBS 115476	DQ377852	DQ677998	DQ767637	Schoch *et al*. (2006); Slippers *et al*. (2013)
Chlamydotubeufia helicospora	MFLUCC 16-0213	KX454170	KX454169	–	Hyde *et al*. (2016a)
Chlamydotubeufia khunkornensis	MFLUCC 10-0117	JN865189	JN865201	KF301565	Boonmee *et al*. 2011
Chlamydotubeufia khunkornensis	MFLUCC 10-0118	JN865190	JN865202	KF301564	Boonmee *et al*. (2011)
Helicangiospora lignicola	MFLUCC 11-0378	KF301531	KF301523	KF301552	Boonmee *et al*. (2014)
Helicoma ambiens	UAMH 10533	AY856916	AY916451	–	Tsui *et al*. (2006)
Helicoma conicodentatum	UBC F14998	AY856869	AY916450	–	Tsui *et al*. (2006)
Helicoma dennisii	NBRC 30667	AY856897	AY916455	–	Tsui *et al*. (2006)
Helicoma inthanonense	MFLUCC 11-0003	JN865199	JN865211	–	Boonmee *et al*. (2011)
Helicoma khunkornense	MFLUCC 10-0119	JN865191	JN865203	–	Boonmee *et al*. (2011)
Helicoma linderi	NBRC 9207	AY856895	AY916454	–	Tsui *et al*. (2006)
Helicoma miscanthi	MFLUCC 11-0375	KF301533	KF301525	KF301554	Boonmee *et al*. (2014)
Helicoma muelleri	CBS 964.69	AY856877	AY916453	–	Tsui *et al*. (2006)
Helicoma rugosa	UBC F13877	AY856917	AY916452	–	Tsui *et al*. (2006)

Table 1. Isolates and sequences used in this study (newly generated sequences are indicated in bold) *(continued)*

Species	*Collection/Isolate number*	*GenBank accession number*			*References*
		LSU	*ITS*	*TEF1α*	
Helicoma siamense	MFLUCC 10-0120	JN865192	JN865204	KF301558	Boonmee *et al*. (2011)
Helicoma vaccinii	CBS 216.90	AY856879	AY916486	–	Tsui *et al*. (2006)
Helicomyces indicum	CBS 374.93	AY856885	AY916477	–	Tsui *et al*. (2006)
Helicomyces paludosa	CBS 120503	DQ341103	DQ341095	–	Tsui *et al*. (2007)
Helicomyces roseus	CBS 283.51	DQ678083	–	DQ677928	Tsui *et al*. (2006)
Helicomyces roseus	**MFLUCC 15-0343**	**KY320540**	**KY320523**	–	This study
Helicomyces roseus	**KUMCC 15-0430**	**KY320541**	**KY320524**	**KY320557**	This study
Helicomyces roseus	**KUMCC 15-0322**	**KY320542**	**KY320525**	**KY320558**	This study
Helicomyces roseus	**KUMCC 15-0281**	**KY320543**	**KY320526**	**KY320559**	This study
Helicomyces roseus	**KUMCC 15-0411**	**KY320544**	**KY320527**	**KY320560**	This study
Helicomyces talbotii	MUCL 33010	AY856874	AY916465	–	Tsui *et al*. (2006)
Helicosporium cereum	NBRC 9014	AY856903	AY916489	–	Tsui *et al*. (2006)
Helicosporium guianense	CBS 269.52	AY856893	AY916487	–	Tsui *et al*. (2006)
Helicosporium vegetum	CBS 941.72	AY856883	AY916488	–	Tsui *et al*. (2006)
Helicosporium vegetum	BCC 8125	AY856909	AY916491	–	Tsui *et al*. (2006)
Helicosporium vegetum	BCC 3332	AY856907	AY916490	–	Tsui *et al*. (2006)
Manoharachariella tectonae	MFLUCC 12-0170	KU764705	KU144935	KU872762	Doilom *et al*. (2016)
Muripulchra aquatica	**DLUCC 0571**	**KY320548**	**KY320531**	–	This study
Muripulchra aquatica	**MFLUCC 15-0249**	**KY320549**	**KY320532**	–	This study
Muripulchra aquatica	**KUMCC 15-0245**	**KY320550**	**KY320533**	**KY320563**	This study
Muripulchra aquatica	**KUMCC 15-0276**	**KY320551**	**KY320534**	**KY320564**	This study
Neoacanthostigma filiforme	ANM 101	GQ850495	–	–	Promputtha & Miller (2010)
Neoacanthostigma filiforme	ANM 514	GQ850494	GQ856146	–	Promputtha & Miller (2010)
Neoacanthostigma fusiforme	MFLUCC 11-0510	KF301537	KF301529	–	Boonmee *et al*. (2014)
Neoacanthostigma septoconstrictum	ANM 536.1	GQ850491	GQ856143	–	Promputtha & Miller (2010)
Neohelicomyces aquaticus	**MFLUCC 16-0993**	**KY320545**	**KY320528**	**KY320561**	This study
Neohelicomyces aquaticus	**KUMCC 15-0463**	**KY320546**	**KY320529**	**KY320562**	This study
Neohelicomyces grandisporus	**KUMCC 15-0470**	**KX454175**	**KX454165**	–	This study
Neohelicomyces submersus	**MFLUCC 16-1106 (KUMCC 15-0251)**	**KY320547**	**KY320530**	–	This study
Tamhinispora indica	NFCCI 2924	KC469283	KC469282	–	Rajeshkumar & Sharma (2013)

Table 1. Isolates and sequences used in this study (newly generated sequences are indicated in bold) *(continued)*

Species	Collection/Isolate number	GenBank accession number			References
		LSU	ITS	TEF1α	
Thaxteriellopsis lignicola	MFLUCC 10-0121	JN865193	JN865205	–	Boonmee *et al*. (2011)
Thaxteriellopsis lignicola	MFLUCC 10-0122	JN865194	JN865206	KF301563	Boonmee *et al*. (2011)
Thaxteriellopsis lignicola	MFLUCC 10-0123	JN865195	JN865207	KF301562	Boonmee *et al*. (2011)
Thaxteriellopsis lignicola	MFLUCC 10-0124	JN865196	JN865208	KF301561	Boonmee *et al*. (2011)
Thaxteriellopsis lignicola	MFLUCC 15-0898	KU764711	KU144926	KU872749	Doilom *et al*. (2017)
Tubeufia aquatica	**MFLUCC 16-1249 (DLUCC 0575)**	**KY320539**	**KY320522**	**KY320556**	This study
Tubeufia aquatica	**DLUCC 0574**	**KY320538**	**-**	**KY320555**	This study
Tubeufia chiangmaiensis	MFLUCC 11-0514	KF301538	KF301530	KF301557	Boonmee *et al*. (2014)
Tubeufia cylindrothecia	BCC 3559	AY849965	–	–	Kodsueb *et al* (2006)
Tubeufia cylindrothecia	**DLUCC 0572**	**KY320537**	**KY320520**	**KY320554**	This study
Tubeufia cylindrothecia	**MFLUCC 16-1253 (KUMCC 15-0335)**	**KY320536**	**KY320519**	**KY320553**	This study
Tubeufia cylindrothecia	**MFLUCC 16-1283 (DLUCC 0573)**	**KY320535**	**KY320518**	**KY320552**	This study
Tubeufia filiformis	MFLUCC 16-1128	KY092407	–	KY117028	Lu *et al*. (2017)
Tubeufia filiformis	MFLUCC 16-1135	KY092411	KY092416	KY117032	Lu *et al*. (2017)
Tubeufia helicomyces	CBS 245.49	DQ767654	–	DQ767638	Schoch *et al*. (2006)
Tubeufia helicomyces	CBS 271.52	AY856887	AY916461	–	Tsui *et al*. (2006)
Tubeufia hyalospora	MFLUCC 15-1250	KX454179	–	–	Hyde *et al*. (2016)
Tubeufia intermedium	ATCC 22621	AY856912	–	–	Tsui *et al*. (2006)
Tubeufia javanica	MFLUCC 12-0545	KJ880036	KJ880034	KJ880037	Boonmee *et al*. (2014)
Tubeufia latispora	MFLUCC 16-0027	KY092412	KY092417	KY117033	Lu *et al*. (2017)
Tubeufia laxispora	MFLUCC 16-0219	KY092409	KY092414	KY117030	Lu *et al*. (2017)
Tubeufia laxispora	MFLUCC 16-0232	KY092408	KY092413	KY117029	Lu *et al*. (2017)
Tubeufia lilliputeus	NBRC 32664	AY856899	AY916483	–	Tsui *et al*. (2006)
Tubeufia mackenziei	MFLUCC 16-0222	KY092410	KY092415	KY117031	Lu *et al*. (2017)
Tubeufia roseohelicospora	MFLUCC 15-1247	KX454178	KX454177	–	Hyde *et al*. (2016a)
Tubeufia roseus	BCC 8808 (SS1014)	AY856910	AY916481	–	Tsui *et al*. (2006)
Tubeufia roseus	BCC 3381	AY787932	–	–	Kodsueb *et al* (2006)
Tubeufia tectonae	MFLUCC 12-0392	KU764706	KU144923	–	Doilom *et al*. (2017)
Wiesneriomyces conjunctosporus	BCC 40615	KJ425454	–	–	Suetrong *et al*. (2014)
Wiesneriomyces conjunctosporus	BCC 40633	KJ425455	–	–	Suetrong *et al*. (2014)
Wiesneriomyces laurinus	BCC 40684	KJ425461	–	–	Suetrong *et al*. (2014)
Wiesneriomyces laurinus	BCC 9453	KJ425458	–	–	Suetrong *et al*. (2014)

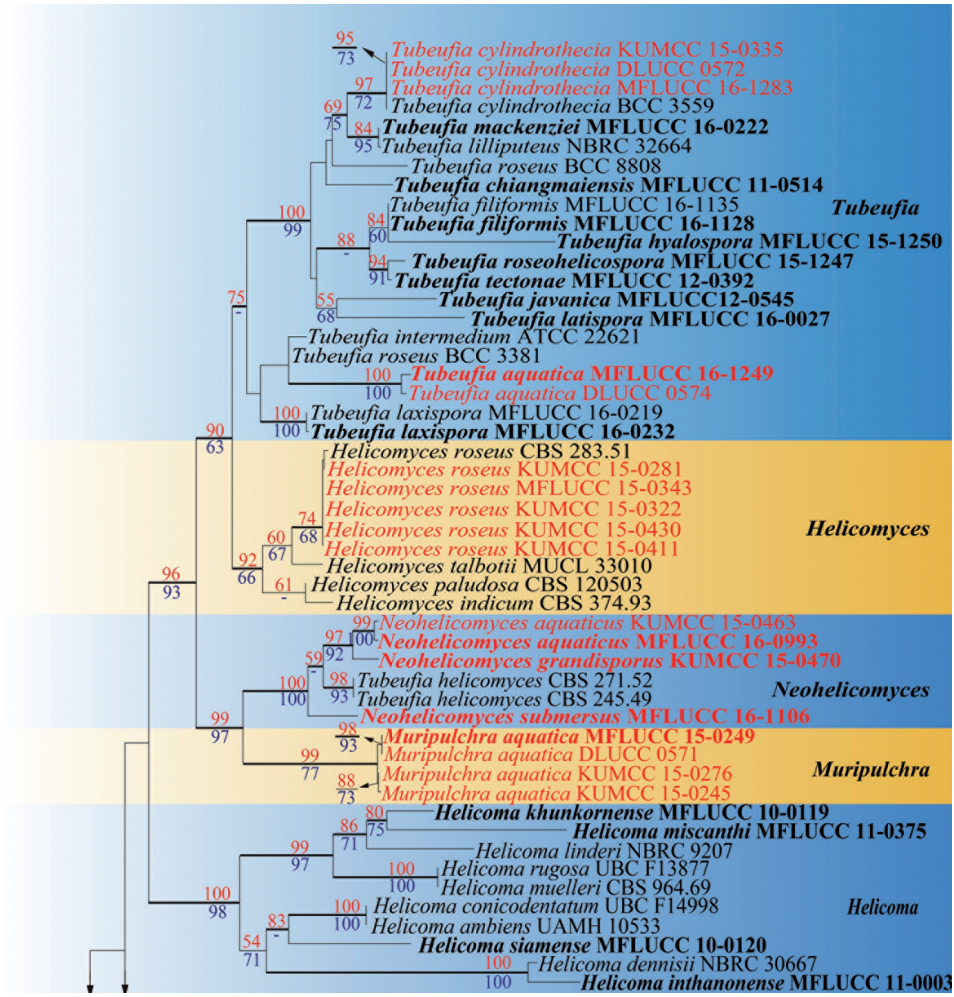

Fig. 1. Phylogram generated from maximum likelihood analysis (RAxML) based on combined ITS, LSU and TEF1α sequenced data from taxa of the family Tubeufiaceae. Bootstrap support values for maximum likelihood (red) and maximum parsimony (blue) equal to or greater than 50% are given above the nodes. Branches with Bayesian posterior probabilities greater than 0.95 are in bold. The tree is rooted to *Botryosphaeria dothidea* (CBS 115476). Newly generated sequences are indicated in red and ex-type strains are in bold.

investigated herein. Figure 1 represents the phylogram generated under the ML analysis (value of likelihood: –18133.722450). Bootstrap support values for maximum likelihood (red) equal to or greater than 50% are given above the nodes. Bootstrap support values for maximum parsimony (blue) equal to or greater than 50% are given below the nodes. Branches with Bayesian posterior probabilities greater than 0.95 are in bold. All species collected in this study are highly supported in the multigene sequence analyses as belonging to the family Tubeufiaceace. In particular, *Tubeufia javanica* clusters with *T. latispora* and our new *T. aquatica*

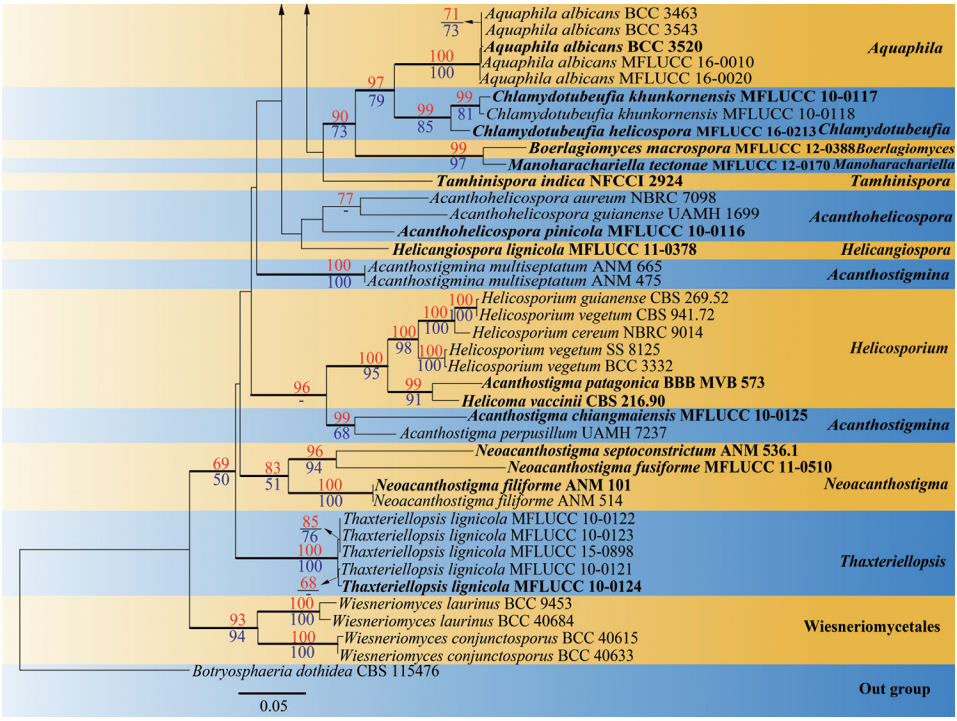

Fig. 1 *(continued)*. Phylogram generated from Maximum Likelihood analysis (RAxML) based on combined ITS, LSU and TEF1α sequenced data of the family Tubeufiaceae. Bootstrap support values for Maximum Likelihood (ML, red) and maximum parsimony (MP, blue) equal to or greater than 50% are given above the nodes. Branches with Bayesian posterior probabilities greater than 0.95 are in bold. The tree is rooted to *Botryosphaeria dothidea* (CBS 115476). Newly generated sequences are indicated in red and ex-type strains are in bold.

isolates constitute an independent lineage with high bootstrap support (100 ML, 100 MP, 1.00 BYPP). The newly collected *Helicomyces roseus* isolate clusters with other *Helicomyces* species in a strongly-supported monophyletic clade (92 ML, 66 MP, 1.00 Bayesian). Our analyses also place our new genus *Neohelicomyces* in another monophyletic clade with *Tubeufia helicomyces* nested in between. *Neohelicomyces aquaticus* clusters with *N. grandisporus* with high bootstrap support (97 ML, 92 MP, 1.00 Bayesian), while *N. submersus* is basal to other members of this clade. The monophyly of *Muripulchra* is also well-supported (99 ML, 77 MP, 1.00 Bayesian) in the combined analysis and phylogeny strongly supports all *M. aquatica* isolates as distinct to other genera.

Taxonomy

In this section, we introduced two new genera, viz. *Muripulchra* with one new species, *Neohelicomyces* with three new species, a new *Tubeufia* asexual morph species, and provide descriptions and illustrations for the asexual morphs of *Tubeufia cylindrothecia* and the type species of *Helicomyces* (*H. roseus*).

Helicomyces roseus Link, *Magazin Ges. Naturf. Freunde*, Berlin 3: 21, 1809.

Facesoffungi number: FoF 02651, Fig. 2

Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. ***Asexual morph***: *Colonies* on the substratum superficial, effuse, gregarious, white. *Mycelium* composed of partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of crowded conidia. *Conidiophores* pale brown, micronematous, mononematous, septate, branched, 26-53 μm ($\bar{x}$ = 39.5 μm, SD = 13.5, n = 10) long, 4-5 μm ($\bar{x}$ = 4.5 μm, SD = 0.5, n = 10) wide, smooth-walled. *Conidiogenous cells* holoblastic, monoblastic, integrated, smooth, each with single conidium. *Conidia* 126.5-237.5 μm ($\bar{x}$ = 182 μm, SD = 55.5, n = 20) long, 4-7 μm ($\bar{x}$ = 5.5 μm, SD = 1.5, n = 20) wide, helicoid, with conidial filament loosely coiled 1-2½ times, rounded at apical end, pale brown, smooth-walled.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream in Cangshan Mountain, March 2014, Z.L. Luo, S-051 (HKAS 83995, **reference specimen designated here**), living culture, MFLUCC 15-0343; Langcang River, April 2015, X.C. Tao, S-305 (HKAS 92825), living culture, KUMCC 15-0322; Jinsha River, April 2015, Z.L. Luo, JSJ H 5-1-1 (DLU 292), living culture, KUMCC 15-0430; Dulong River, May 2015, X.C. Tao, HD1-10-8 (DLU 509), living culture, KUMCC 15-0281; Jinsha River, April 2015, X.J. Su, JSJ H 25-18-1 (DLU 383), living culture, KUMCC 15-0411.

Notes: The type species of the genus *Helicomyces*, *H. roseus* is commonly encountered in freshwater habitats worldwide (Link, 1809, Barr, 1980, Sivichai *et al.* 2002, Zhao *et al.* 2007, Hu *et al.* 2013). In our study, five isolates were made from submerged decaying wood collected in Yunnan Province, China. Morphological characters such as micronematous, short, erect conidiophores, holoblastic, monoblastic, integrated conidiogenous cells, and conidia becoming loosely uncoiled in water, rounded at apex and the size of conidiophores and conidia fit well with *H. roseus*. Acording to our phylogenetic study (Fig. 1), our strain clusters with *Helicomyces roseus* (CBS 283.51). We therefore identify our isolate as *Helicomyces roseus* based on morphology and phylogeny.

Muripulchra Z.L. Luo, H.Y. Su & K.D. Hyde, ***gen. nov.***

MycoBank number: MB 818825; *Facesoffungi number*: FoF 02647

Etymology: 'Muri' referring to muriform conidia, 'pulchra' meaning beautiful in Latin.

Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. ***Asexual morph***: *Colonies* effuse, punctiform, superficial, sporodochial, dark brown. *Mycelium* composed of immersed or partly superficial, septate, branched, brown hyphae, forming sporodochia with a stromatic base. *Conidiophores* micronematous, arising from stroma. *Conidiogenous cells* holoblastic, erect, smooth, pale brown, narrow at the base, wide above, cylindrical, with narrow cell-lumen, arising directly on stroma. *Conidia* obpyriform, rounded at top, truncate at base, septate to muriform, longitudinally septate in above 2-4 cells, smooth, thick-walled, granulate, often carrying part of conidiogenous cells at the base as a broken frill.

Type species: ***Muripulchra aquatica*** Z.L. Luo, H.Y. Su & K.D. Hyde

Muripulchra aquatica Z.L. Luo, H.Y. Su & K.D. Hyde, ***sp. nov.***

MycoBank number: MB 818826; *Facesoffungi number*: FoF 02648, Fig. 3

Holotype: HKAS 83980

Etymology: referring to aquatic habitat of this taxon.

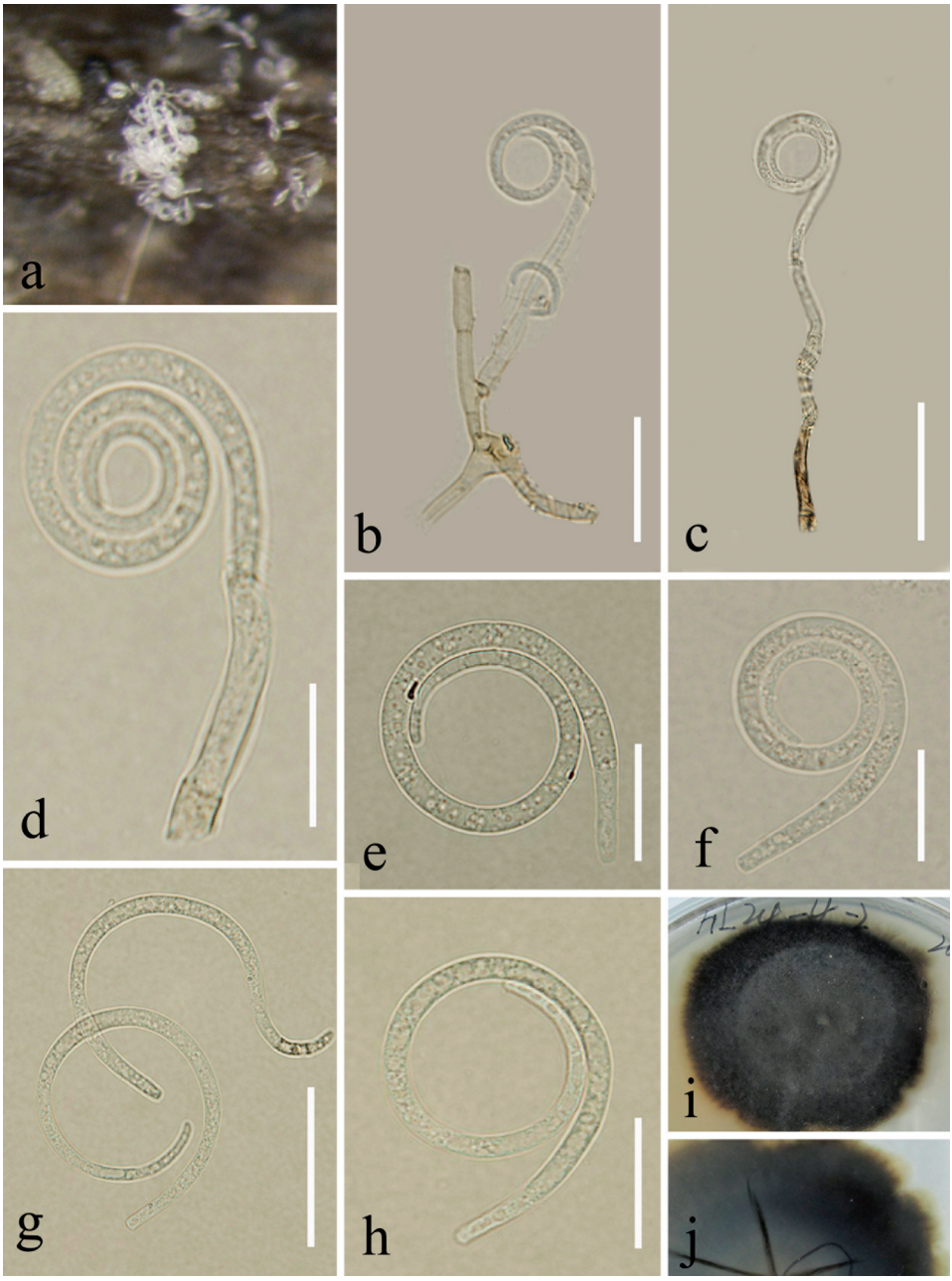

Fig. 2. ***Helicomyces roseus*** (HKAS 83995, **reference specimen**). **a.** Colonies on wood. **b, c.** Conidiophores with attached conidium. **d.** Conidiogenous cells with conidium. **e-h.** Conidia. **i**, **j.** Colonies on PDA from surface and reverse. Scale bars: **b, c** = 50 μm, **d-h** = 30 μm.

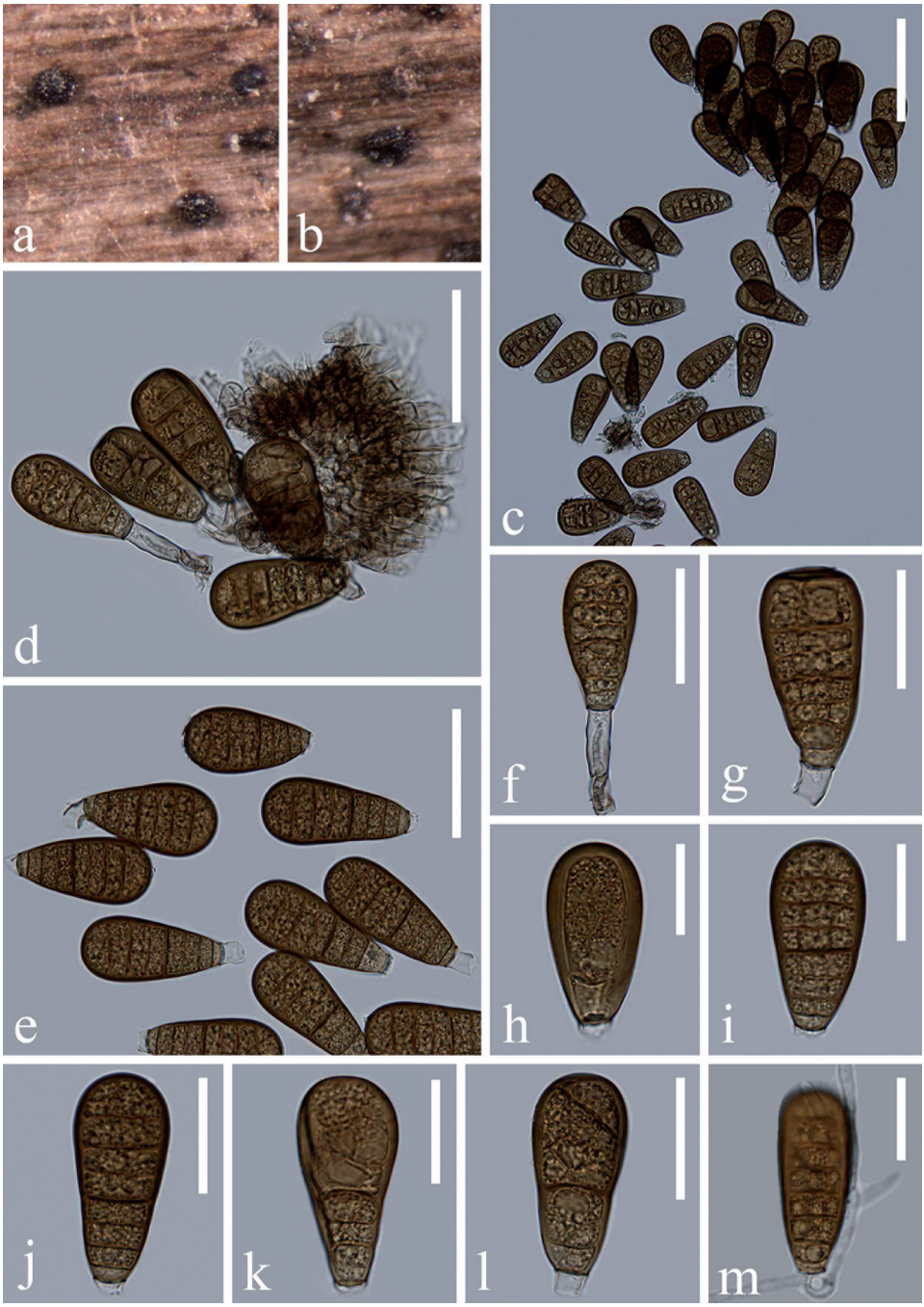

Fig. 3. ***Muripulchra aquatica*** (HKAS 83980, **holotype**). **a, b.** Colonies on wood. **d, f, g.** Conidia with conidiogenous cells. **c, e, h-l.** Conidia. **m.** Germinating conidia. Scale bars: **c** = 50 µm, **d, e** = 40 µm, **f, g, j, l** = 30 µm, **h, i, k, m** = 20 µm.

Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. ***Asexual morph***: *Colonies* effuse to punctiform, superficial, sporodochial. *Mycelium* composed of immersed or partly superficial, septate, branched, pale brown hyphae, forming sporodochial stroma. *Conidiophores* micronematous. *Conidiogenous cells* holoblastic, erect, smooth, pale brown, narrow at the base, wide above, cylindrical, with narrow cell-lumen, arising directly on stroma, 9.5-12.5 *μm* long ($\bar{x}$ = 11 *μm*, SD = 1.5, n = 10), 3.5-4.5 *μm* wide ($\bar{x}$ = 4 *μm*, SD = 0.5, n = 10). *Conidia* obpyriform, rounded to flat at top, truncate at base, 6-7-septate, muriform, longitudinally septate in above 2-4 cells, smooth, thick-walled, granulate, dark brown, 40.5-47.5 *μm* long ($\bar{x}$ = 44 *μm*, SD = 3.5, n = 25), 20-23 *μm* wide ($\bar{x}$ = 21.5 *μm*, SD = 1.5, n = 25), often carrying part of conidiogenous cell at the base as a broken frill.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in Linquan stream in the Cangshan Mountain, March 2014, H.Y. Su, S-014, (HKAS 83980, **holotype**), ex-type living culture, MFLUCC 15-0249; Dulong River, May 2015, Z.L. Luo, HD 4-1-3 (HKAS 92723), living culture, DLUCC 0571; Cangshan Mountain, October 2014, Z.L. Luo, S-281, (DLU 281), living culture, KUMCC 15-0245; Gaoligong Mountain, July 2015, X.J. Su, S-433, (DLU 433), living culture, KUMCC 15-0276.

Notes: *Muripulchra aquatica* resembles *Thyrostroma* eucalypti in having effuse, superficial, sporodochial colonies and septate to muriform conidia, but Muripulchra *aquatica* differs in having micronematous conidiophores, obpyriform conidia often carrying part of conidiogenous cells at the base as a broken frill (Yuan *et al.* 1990). In addition *Thyrostroma* species have been referred to the family Botryosphaeriaceae (Phillips *et al* 2008; Slippers *et al.* 2013) and therefore we reckon our new genus is distinct as it belongs to the Tubeufiaceae.

Muripulchra aquatica is also morphological similar to *Bactrodesmium gabretae* with superficial, sporodochial colonies, simple and inconspicuous conidiophores and transverse septa conidia. However, *Muripulchra aquatica* differs in having unbranched conidiophores, obpyriform, 6-7-septate, bigger conidia (40.5-47.5 × 20-23 *μm* vs 13-19(24) × 9-13.5 *μm*). Phylogenetic analysis showed that *Muripulchra aquatica* belong in Tubeufiales whereas *Bactrodesmium gabretae* is placed in Pleosporales (Tanaka *et al.* 2015).

Neohelicomyces Z.L. Luo, D.J. Bhat & K.D. Hyde, ***gen. nov.***

MycoBank number: MB 818820; *Facesoffungi number*: FoF 02643

Etymology: The generic epithet, *neo* (Lat., new), refers to the similarity to *Helicomyces*.

Saprobic on submerged decaying wood. *Colonies* on the substratum superficial, effuse, gregarious, white. *Mycelium* composed of partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of crowded, glistening conidia. *Conidiophores* macronematous, mononematous, erect, septate, sparsely branched, pale brown, arising directly on substrate, glistening, light-coloured, smooth-walled, fertile below half, remaining sterile above the half. *Conidiogenous cells* monoblastic, holoblastic, integrated, with lateral minute denticles each with single conidium. *Conidia* helicoid, with conidial filament, tightly to loosely coiled, rounded at apical end, pale brown, multi-septate, smooth-walled, guttulate.

Type species: ***Neohelicomyces aquaticus*** Z.L. Luo, Bhat & K.D. Hyde.

Neohelicomyces aquaticus Z.L. Luo, D.J. Bhat & K.D. Hyde, ***sp. nov.***

MycoBank number: MB 818822; *Facesoffungi number*: FoF 02644, Fig. 4
Holotype: MFLU 16-2543
Etymology: referring to aquatic habitats of this fungus.

Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. ***Asexual morph***: *Colonies* on the substratum superficial, effuse, gregarious, white. *Mycelium* composed of partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of crowded, glistening conidia. *Conidiophores* macronematous, mononematous, erect, septate, sparsely branched, pale brown, arising directly on substrate, light-coloured, 240.5-335.5 *μm* long ($\bar{x}$ = 288 *μm*, SD = 47.5, n = 10), 5-6 *μm* wide ($\bar{x}$ = 5.5 *μm*, SD = 0.5, n = 10), smooth-walled, fertile below half, remaining sterile at the tip. *Conidiogenous cells* monoblastic, holoblastic, integrated, with lateral minute denticles each with single conidium. *Conidia* 154.5-179.5 *μm* long ($\bar{x}$ = 167 *μm*, SD = 12.5, n = 20), 2.5-3.5 *μm* wide, ($\bar{x}$ = 3 *μm*, SD = 0.5, n = 20), helicoid, with conidial filament coiled, 2-2½ times, tightly to loosely coiled, rounded at both ends, pale brown, multi-septate, smooth-walled, guttulate.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in the Nujiang River, May 2015, Z.L. Luo, N1-7 (MFLU 16-2543, **holotype**), ex-type living culture, MFLUCC 16-0993, KUMCC 15-0466; Nujiang River, May 2015, Q. Dai, N1-2 (HKAS 92811), living culture, KUMCC 15-0463.

Notes: *Neohelicomyces aquaticus* was collected from the Nujiang River during our study of lignicolous freshwater fungi at the three parallel rivers region in north-western Yunnan Province. *Neohelicomyces aquaticus* resembles *N. submersus* and *N. grandisporus* in having macronematous, septate conidiophores and helicoid conidia with tightly to loosely coiled conidial filament. However, *N. aquaticus* differs from *N. submersus* in having longer conidiophores (240.5-335.5 *μm* vs 172-285 *μm*) and conidia coiled 2-2½ vs 3-3½ times. *Neohelicomyces aquaticus* differs from *N. grandisporus* in having longer and wider conidiophores (240.5-335.5 × 5-6 *μm* vs 107-161 × 5-6 *μm*) and conidia coiled 2-2½ vs 1-1½ times. The phylogenetic data also confirms them as distinct taxa.

Neohelicomyces submersus Z.L. Luo, H.Y. Su & K.D. Hyde, ***sp. nov.***

MycoBank number: MB 818823; *Facesoffungi number*: FoF 02645, Fig. 5
Holotype: HKAS 93065
Etymology: referring to submerged habitat of this taxon.

Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. ***Asexual morph***: *Colonies* on substratum superficial, effuse, gregarious, white. *Mycelium* composed of partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of crowded, glistening conidia. *Conidiophores* macronematous, mononematous, erect, septate, branched, pale brown to brown, arising directly on substrate, 172-285 *μm* long ($\bar{x}$ = 228.5 *μm*, SD = 56.5, n = 10), 3.5-4.5 *μm* wide ($\bar{x}$ = 4 *μm*, SD = 0.5, n = 10), smooth-walled. *Conidiogenous cells* monoblastic, holoblastic, integrated, intercalary, with lateral minute denticles each with single conidium. *Conidia* 142.5-207.5 *μm* long ($\bar{x}$ = 175 *μm*, SD = 32.5, n = 20), 2.5-3.5 *μm* wide ($\bar{x}$ = 3 *μm*, SD = 0.5, n = 20), helicoid, with conidial filament coiled 3-3½ times, tightly coiled, rounded at apical end, pale brown, multi-septate, smooth-walled, granulate.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in Heilong stream in the Cangshan Mountain, Jan 2015, X.Y. Liu,

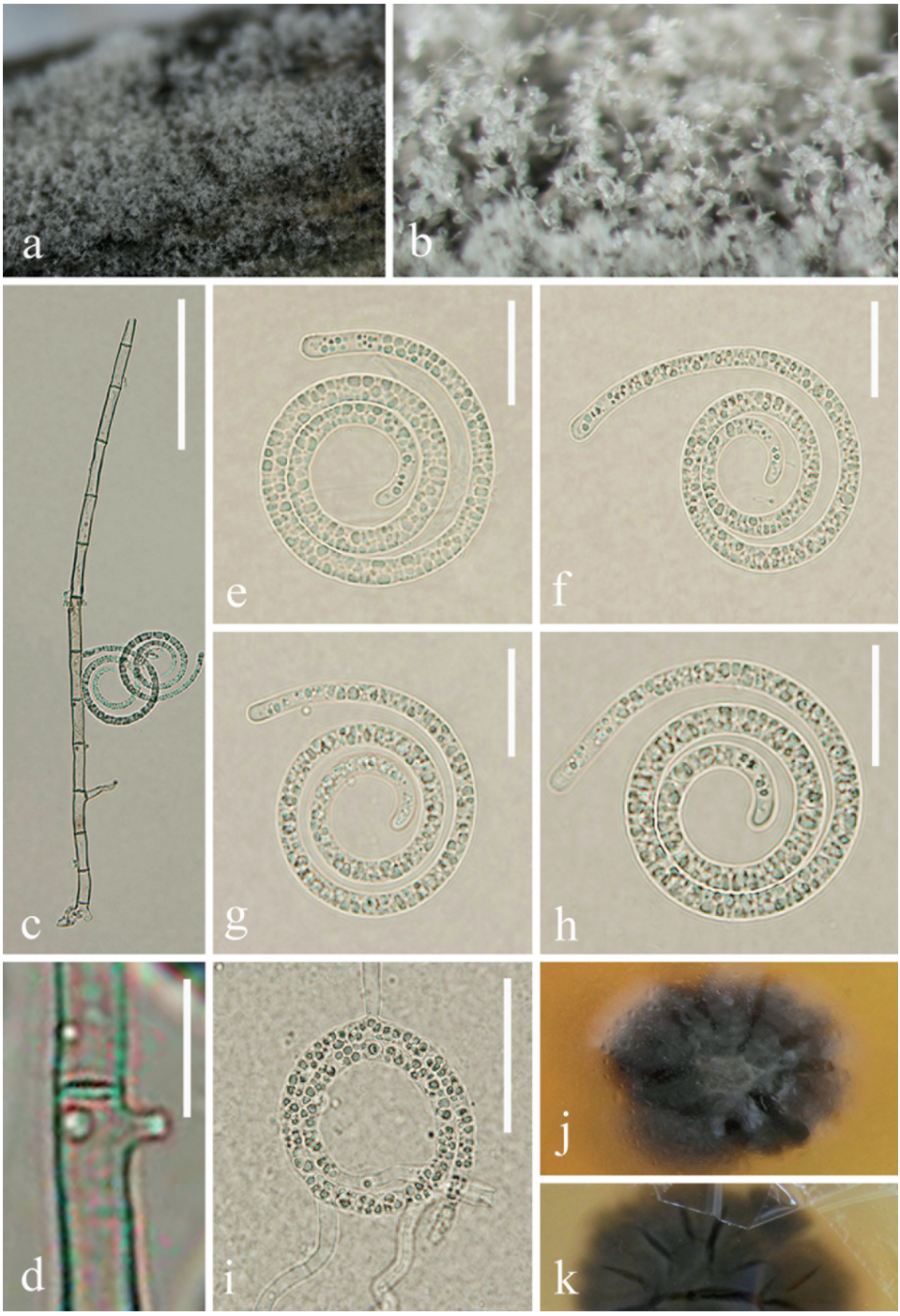

Fig. 4. ***Neohelicomyces aquaticus*** (MFLU 16-2543, **holotype**). **a, b.** Colonies on wood. **c.** Conidiophores with attached conidium. **d.** Conidiogenous cells. **e-h.** Conidia. **i.** Germinating conidia. **j, k.** Colonies on PDA from surface and reverse. Scale bars: **c** = 100 µm, **d-h** = 15 µm, **i**= 30 µm.

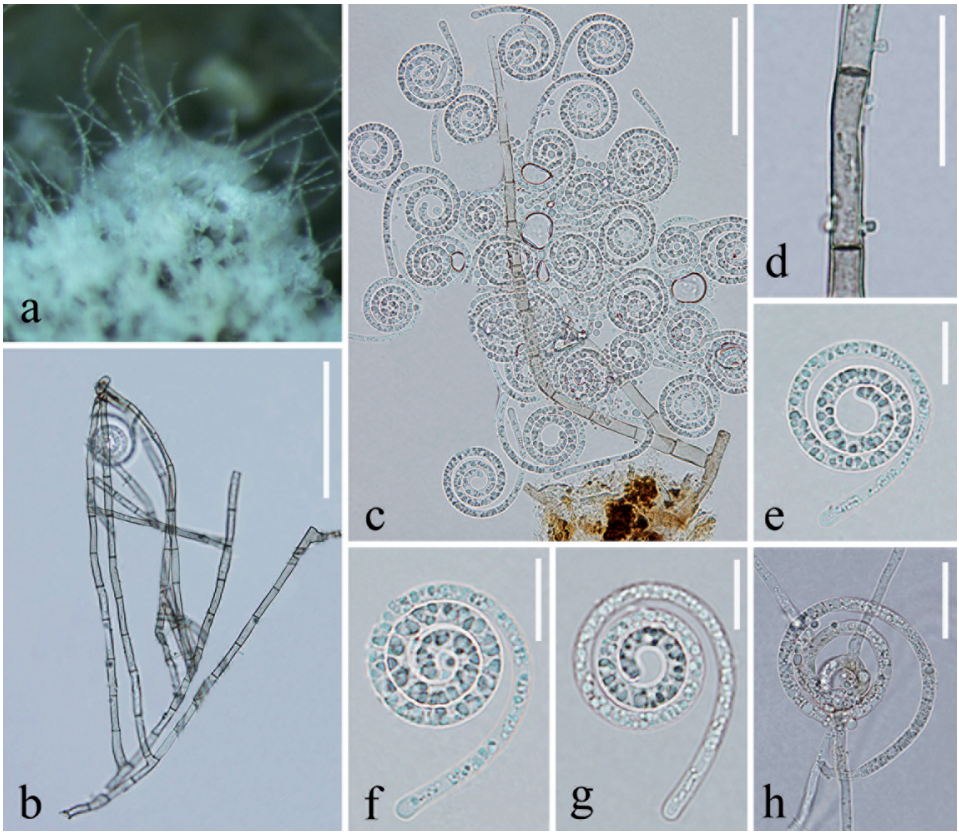

Fig. 5. ***Neohelicomyces submersus*** (HKAS 93065, **holotype**). **a.** Colonies on wood. **b, c.** Conidiophores with attached conidium. **d.** Conidiogenous cells. **e-g.** Conidia. **h.** Germinating conidia. Scale bars: **b, c** = 70 µm, **d-h** = 20 µm.

4HLXM H 2-1 (HKAS 93065, **holotype**), ex-type living culture, MFLUCC 16-1106, KUMCC 15-0251.

Notes: *Neohelicomyces submersus* share similar characters with *N. aquaticus* and *N. grandisporus* in having macronematous, septate, branched conidiophores, helicoid, coiled conidia. However, *Neohelicomyces submersus* differs from *N. aquaticus* in having shorter and thinner conidiophores, conidia coiled 3-3½ vs 2-2½ times and differs from *N. grandisporus* in having longer conidiophores (172-285 vs 107-161 *µm*) and conidia coiled 3-3½ vs 1-1½ times. Phylogenetic analyses position *N. submersus* as a distinct taxon basal to other *Neohelicomyces* as well as *Tubeufia helicomyces*.

Neohelicomyces grandisporus Z.L. Luo, Boonmee & K.D. Hyde, ***sp. nov.***

MycoBank number: MB 818824; *Facesoffungi number*: FoF 02646, Fig. 6
Holotype: HKAS 92812
Etymology: 'grandisporus' referring to large filamentous conidia.
Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. **Asexual morph**: *Colonies* on the substratum superficial, effuse, gregarious, hyaline

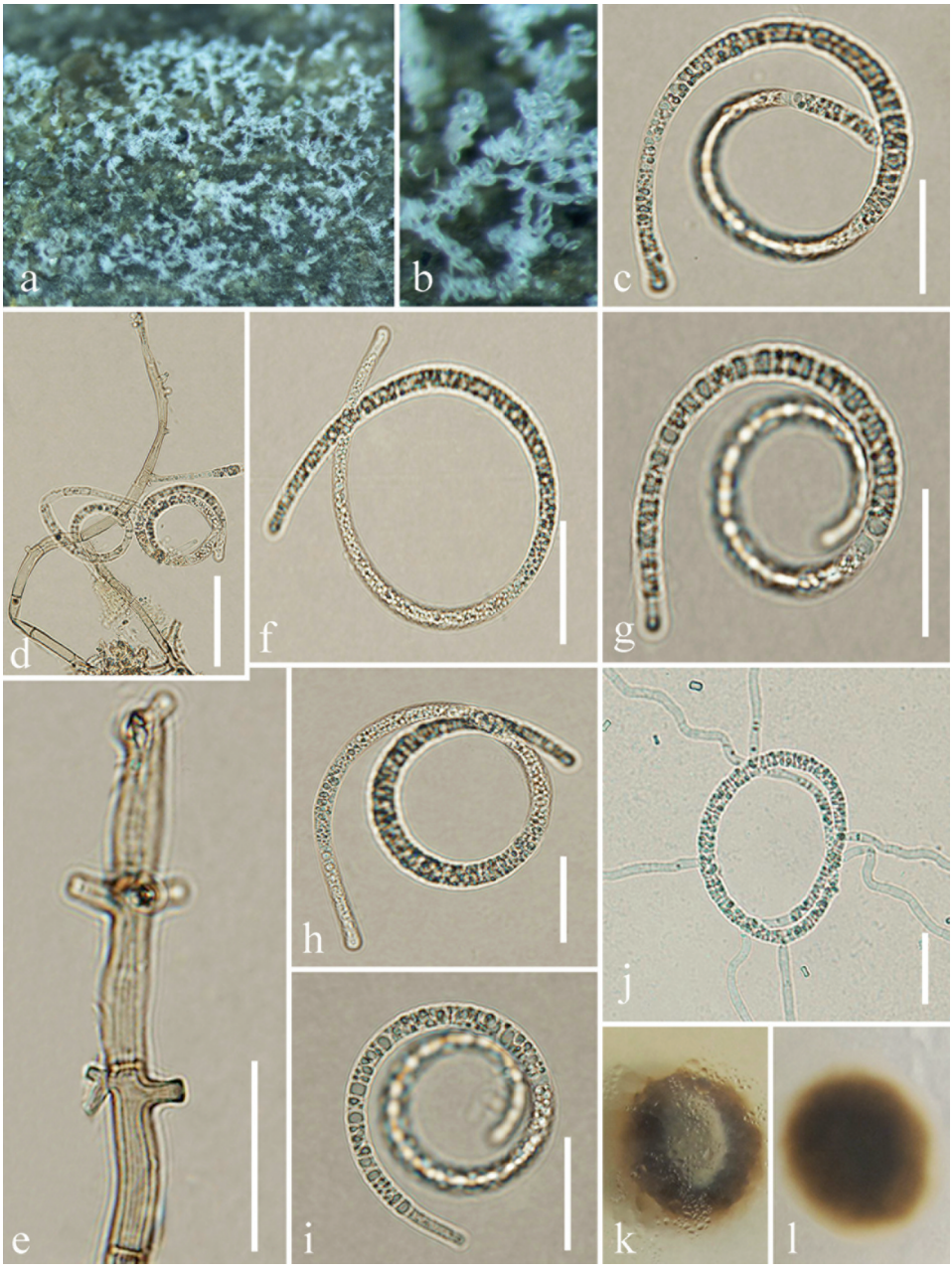

Fig. 6. ***Neohelicomyces grandisporus*** (HKAS 92812, **holotype**). **a, b.** Colonies on wood. **d.** Conidiophores with attached conidium. **e.** Conidiogenous cells with conidiophores. **c, f-i.** Conidia. **i.** Germinating conidium. **k, l.** Colonies on PDA from surface and reverse. Scale bars: **d** = 35 µm, **c, f, j** = 25 µm, **e, g-i** = 20 µm.

to white. *Mycelium* partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of crowded, glistening conidia. *Conidiophores* pale brown, macronematous, erect, septate, branched, 107-161 *μm* long ($\bar{x}$ = 134 *μm*, SD = 27, n = 10), 4-5 *μm* wide ($\bar{x}$ = 4.5 *μm*, SD = 0.5, n = 10), smooth-walled. *Conidiogenous cells* holoblastic, polyblastic, integrated, each with single conidium. *Conidia* 165-214 *μm* long ($\bar{x}$ = 189 *μm*, SD = 25, n = 30), conidial filament 4.5-5.5 *μm* wide ($\bar{x}$ = 5 *μm*, SD = 0.5, n = 30), loosely coiled 1-1½ times, rounded at apical end, pale brown to brown, smooth-walled.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in the Nujiang River, May 2015, Z.L. Luo, N2-4-1 (HKAS 92812, **holotype**), ex-type living culture, KUMCC 15-0470, MFLUCC.

Notes: *Neohelicomyces grandisporus* resembles *N. aquaticus* in having macronematous, septate conidiophores, helicoid, tightly to loosely coiled conidia. However, *N. grandisporus* differs from *N. aquaticus* in having shorter conidiophores (107-161 vs 240-335.5 *μm*), polyblastic conidiogenous cells, wider conidia (4.5-5.5 vs 2.5-3.5 *μm*) and conidial filaments coiled 1-1½ vs 2-2½ times. The polyblastic conidiogenous loci appear in a whorl below the upper septum in each cell (Fig. 6, e). The phylogenetic analysis (Fig. 1) showed that *N. grandisporus* separates from other species of *Neohelicomyces* with strong bootstrap support (97% ML/ 92 MP / 1.00 BYPP, Fig. 1).

Tubeufia aquatica Z.L. Luo, D.J. Bhat & K.D. Hyde, ***sp. nov.***

MycoBank number: MB 818827; *Facesoffungi number*: FoF 02649, Fig. 7

Holotype: MFLU 16-2544

Etymology: referring to aquatic habitats of this fungus.

Saprobic on submerged decaying wood. ***Sexual morph***: Undetermined. ***Asexual morph***: *Colonies* on the substratum superficial, effuse, gregarious, white, glistening. *Mycelium* composed of partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of crowded conidia. *Conidiophores* brown to dark brown, macronematous, septate, branched, indeterminate, erect, flexuous, 109.5-189.5 *μm* long ($\bar{x}$ = 149.5 *μm*, SD = 40, n = 10), 5.5-6.5 *μm* wide ($\bar{x}$ = 6 *μm*, SD = 0.5, n = 10), smooth. *Conidiogenous cells* holoblastic, polyblastic, integrated, terminal and later becoming intercalary, smooth. *Conidia* 96.5-122.5 *μm* long ($\bar{x}$ = 109.5 *μm*, SD = 13, n = 20), 4-5 *μm* wide ($\bar{x}$ = 4.5 *μm*, SD = 0.5, n = 20), helicoid, with conidial filament closely coiled 2-2½ times, rounded at apical end, narrowly truncate at base, hyaline to pale brown, smooth, multi-septate, guttulate, smooth-walled.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in Erhai Lake, June 2015, Z.L. Luo, 3EHL H 60-1 (MFLU 16-2544, **holotype**), ex-type living culture, MFLUCC 16-1249; Lancang River, April 2015, Z.L. Luo, HL 9-7-2, (MFLU 16-2545), living culture, DLUCC 0574.

Notes: *Tubeufia aquatica* was collected from the Lancang River and Erhai lake in Yunnan Province. *Tubeufia aquatica* resembles the asexual morph of T. cylindrothecia which we also introduce in this paper in having macronematous, septate conidiophores and helicoid, smooth, multi-septate, guttulate conidia. However, *T. aquatica* differs in having branched, longer conidiophores (109.5-189.5 vs 50-81 *μm*) and shorter conidia (96.5-122.5 vs 256-314 *μm*). Phylogenetic analysis also showed that the two isolates of *Tubeufia aquatica* formed a separate clade in the genus *Tubeufia* (Fig. 1).

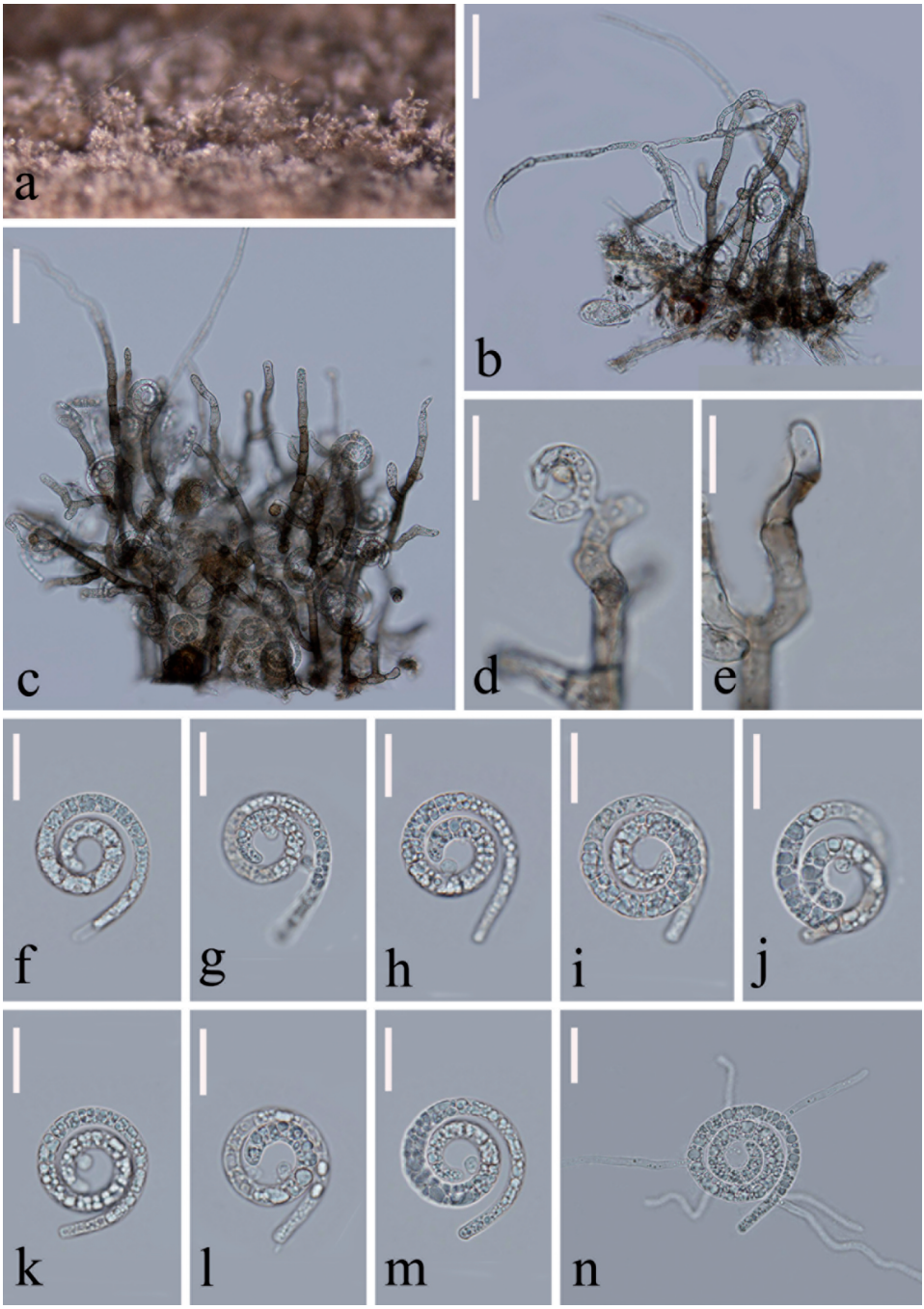

Fig. 7. ***Tubeufia aquatica*** (MFLU 16-2544, **holotype**). **a.** Colonies on wood. **b, c.** Conidiophores with attached conidium. **d.** Conidiogenous cells with conidium. e Conidiogenous cells. **f-m.** Conidia. **n.** Germinating conidia. Scale bars: **b**, **c** = 50 µm, **d-n** = 15 µm.

Tubeufia cylindrothecia (Seaver) Höhn Sber. Akad. Wiss. Wien, Math.-naturw. Kl., Abt. 1 128: 562 (1919)

Facesoffungi number: FoF 02650, Fig. 8

Saprobic on submerged decaying wood. ***Sexual morph***: See Seaver (1909). ***Asexual morph***: *Colonies* on the substratum superficial, effuse, gregarious, white. *Mycelium* composed of partly immersed, partly superficial, hyaline to pale brown, septate, sparsely branched hyphae, with masses of conidia. *Conidiophores* pale brown, mononematous, macronematous, septate, unbranched, erect, flexuous, 50-81 *μm* long ($\bar{x}$ = 65.5 *μm*, SD = 15.5, n = 10), 5-7 *μm* wide ($\bar{x}$ = 6 *μm*, SD = 1, n = 10), smooth-walled. *Conidiogenous cells* holoblastic, monoblastic, integrated, smooth, terminal, each with single conidium. *Conidia* 256-314 *μm* long ($\bar{x}$ = 285 *μm*, SD = 29, n = 20), 4.5-5.5 *μm* wide ($\bar{x}$ = 5 *μm*, SD = 0.5, n = 20), helicoid, with conidial filament loosely coiled 1½-3½ times, rounded at apical end, narrowly truncate at base, hyaline to pale brown, multi-septate, guttulate, smooth.

Material examined: CHINA, Yunnan Province, saprobic on decaying wood submerged in Jinsha River, April 2015, Z.L. Luo, JSJ H 8-17-1 (MFLU 16-2547, **reference specimen designated here**), living culture, MFLUCC 16-1283; Lancang River, April 2015, Z.L. Luo, HL 7-14-1 (HKAS 92868), living culture, MFLUCC 16-1253, KUMCC 15-0335; Lancang River, April 2015, Z.L. Luo, HL 10-9-1, (MFLU 16-2546), living culture, DLUCC 572.

Notes: *Tubeufia cylindrothecia* is frequently associated with the asexual morph referred to as *Helicomyces roseus* based on morphological studies (Seaver & Waterston, 1940, Barr, 1980, Zhao *et al.* 2007). However, the phylogenetic analyses did not support this observation (Kodsueb *et al.* 2006, Boonmee *et al.* 2011, 2014). In the present study, we collected three helicosporous taxa from freshwater habitats and phylogenetic analyses show them to cluster with *T. cylindrothecia* with strong support (97% ML/ 72 MP / 0.98 BYPP, Fig. 1). Based on morphological characters and molecular data, we therefore illustrate, describe and assign these three isolates (MFLUCC 16-1283, MFLUCC 16-1253 and DLUCC 0572) under sexual named *Tubeufia cylindrothecia*.

DISCUSSION

The family Tubeufiaceae, as currently circumscribed accommodates a number of helicosporous hyphomycetous genera with 20 recognized genera (Doilom *et al.* 2017). Species within this family exhibit great spore diversity, mostly occur on terrestrial woody substrates and are mainly characterized by hyphomycetous asexual morph with helicosporous conidia. Recently, a new order *Tubeufiales* has been established to accommodate these species (Boonmee *et al.* 2014). Helicosporous hyphomycetes species in the family Tubeufiaceae are characterized by conidia which are true helicospores, that curve through at least 180° in one plane as they extend in length (two-dimension) (Goos, 1985a, 1986, 1989, Tsui *et al.* 2006). *Helicomyces* Link (1809), *Helicosporium* Nees (1817) and *Helicoma* Corda (1837) are the three earliest described helicosporous hyphomycete genera. These genera were traditionally delineated by Goos (1985b, 1986, 1989) based on different aspects of conidia (including their filaments) and conidiophores. This viewpoint is still followed to identify these three genera to date and we follow in the same scheme to diagnose species collected in this study.

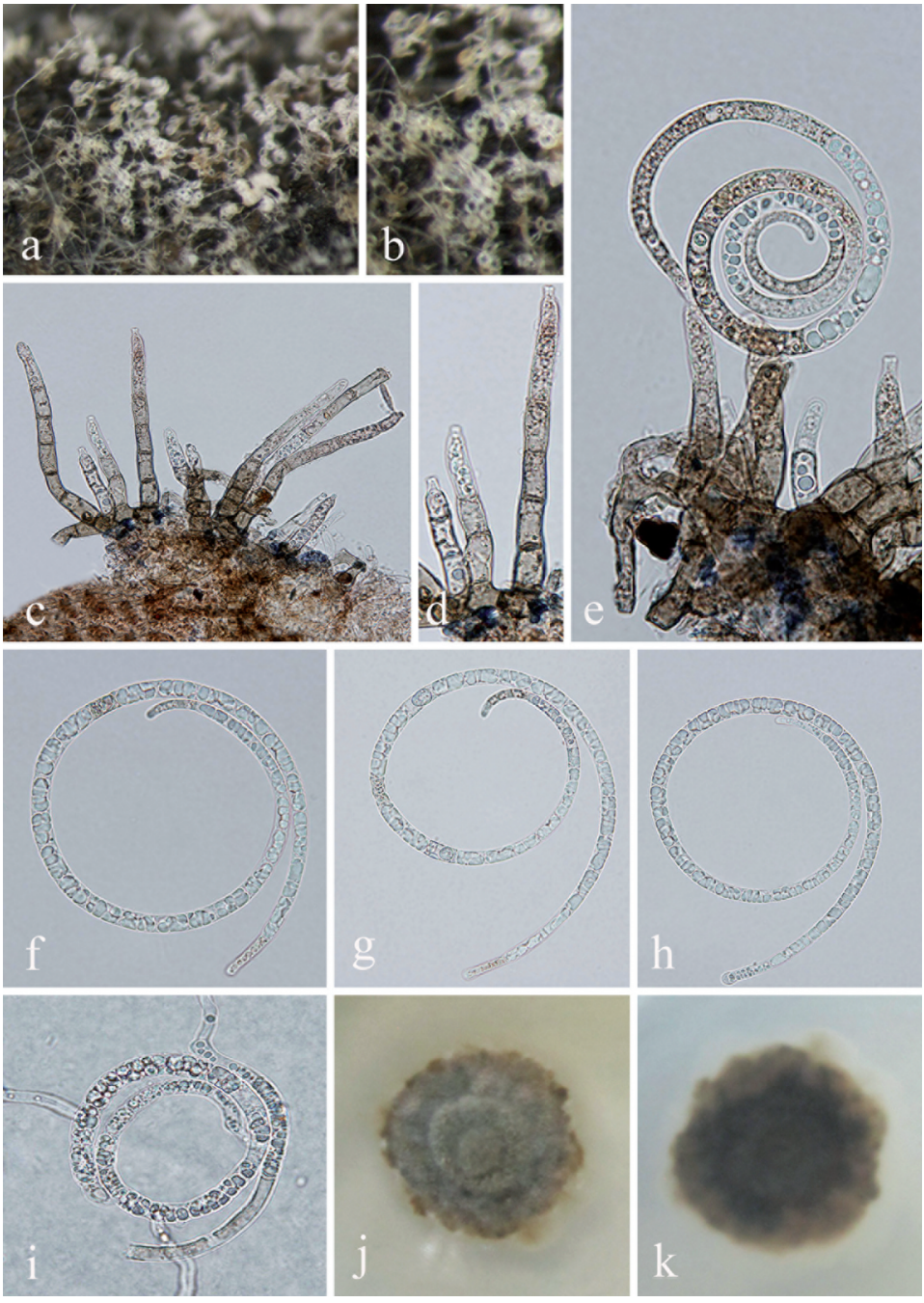

Fig. 8. ***Tubeufia cylindrothecia*** (MFLU 16-2547, **reference specimen**). **a, b.** Colonies on wood. **c.** Conidiophores. **d.** Conidiogenous cells. **e.** Conidiophores with attached conidium. **f-h.** Conidia. **i.** Germinating conidia. **j, k.** Colonies on PDA from surface and reverse. Scale bars: **c** = 50 µm, **d-i** = 30 µm.

This study provides an illustration of *Helicomyces roseus* from freshwater habitats. Based on morphological characters (such as the wide helicoid and loosely coiled conidia; Fig. 2) and DNA sequence based phylogeny, there is support to identify our new collection as *H. roseus*. Multigene phylogeny supports our new collection together with other representatives of *H. roseus* collected from freshwater habitats in a strongly monophyletic clade with *H. talboti, H. indicum* and *H. paludosa* as sister taxa (Fig. 1).

Our examination of decayed submerged woods from freshwater reveals the presence of an unusual group of species whose morphological characters are different from other helicosporous taxa. A new genus, *Neohelicomyces*, is therefore erected to accommodate three new species in this study. *Neohelicomyces* differs from its close allies, especially from *Helicomyces* in having elongate, erect, conspicuous conidiophores and differs from *Helicosporium* based on conidial coiling ratio, size (Tsui *et al.* 2006). DNA based multigene phylogeny also positions *Neohelicomyces* in a separate lineage which is strongly supported and phylogenetically distinct from *Helicomyces* (Fig. 1). Results therefore provide further evidence to support the establishment of the new genus. At the species level in this new genus, we also take note of some morphological differences and herein follow the recommendations outlined by Jeewon & Hyde (2016) based on morphology and phylogeny to justify our taxonomic viewpoint and describe three new species. We noted striking differences in conidiophore, conidial dimensions and the number of coils in the conidia which provides reliable morphological markers in species segregation among *Neohelicomyces*. These morphological differences provide sufficient grounds to demarcate our species herein and are also in concordance with previous studies whereby these morphs have been used to differentiate *Helicomyces* and allied species (Tsui *et al.* 2006; Zhao *et al.* 2007).

Another peculiar finding in this study is the phylogenetic placement of *Tubeufia helicomyces*, which is embedded in between *Neohelicomyces* species (Fig. 1) and distinct from other *Tubeufia* species sampled. This phylogenetic uniqueness strongly calls for a possible re-circumscription of this species in the future and to verify whether *Neohelicomyces* could be paraphyletic.

Our DNA sequence data strongly support placement of *Muripulchra* as new genus given its distinct lineage. Phylogeny clearly indicates that all *Muripulchra aquatica* isolates belong to a monotypic monophyletic clade supported by high bootstrap values and basal to *Neohelicomyces* (Fig. 1). The former is characterized by specific sporodochial colonies and cylindrical conidia with narrow cell-lumen which make them distinct from *Neohelicomyces* (Fig. 3). We also noted that two *Muripulchra aquatica* (DLUCC 0571 and MFLUCC 15-0249) cluster together with high support and phylogenetically apart from the other two morphologically similar isolates. Following Jeewon and Hyde's (2016) recommendations on species delimitation for new species, we delved into pairwise dissimilarities of DNA sequences and noted that there are indeed differences in the ribosomal ITS sequences that possibly explain a close phylogenetic relatedness between them and their slight phylogenetic divergence to the other isolates. There are three noticeable nucleotide differences among the 560 nucleotides analysed between *Muripulchra aquatica* isolates (DLUCC 0571 and MFLUCC 15-0249) and the other ones (KUMCC 15-0245 and KUMCC 15-0276). Major differences are as follows: Nucleotide **G** instead of **A** at position 108; Nucleotide **C** instead of **T** at position 386 and a **T** insertion at position 390. We reckon that further sampling among species of this new genus could reveal that they are potentially genetically highly divergent. However, at present, we could not identify any morphological apomorphy to the sister pair of

Muripulchra aquatica isolates DLUCC 0571 and MFLUCC 15-0249 despite a slight phylogenetic distinctiveness to other isolates.

Tubeufia aquatica is described here as a new taxon that fits into the generic concept of *Tubeufia* in having helicoid conidia (Boonmee *et al.* 2014; Lu *et al.* 2017). DNA sequence based data affiliate our new taxon to other *Tubeufia* species but clearly indicates that their isolates constitute an independent and strongly supported monophyletic lineage that supports new species status (Fig 1). There are also some noteworthy base pair differences in the ITS regions of *Tubeufia aquatica* as compared to *T. laxispora* to which they are related and major differences are as follows: Nucleotide **T** instead of **C** at position 21, 23, 90, 99, 100, 101, 149, 404, 422; Nucleotide **A** instead of **T** at position 63, 86, 146, 152; Nucleotide **C** instead of **A** at position 65, 395; Nucleotide **A** instead of **C** at position 91, 102; Nucleotide **A** instead of **G** at position 105, 106, 121, 122, 170, 398, 403; Nucleotide **C** instead of **G** at position 128, 129.

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