



Article

Doi 10.5943/mycosphere/8/9/9

Copyright © Guizhou Academy of Agricultural Sciences

***Neotubeufia* gen. nov. and *Tubeufia guangxiensis* sp. nov. (Tubeufiaceae) from freshwater habitats**

**Chaiwan N<sup>1</sup>, Lu YZ<sup>1,5</sup>, Tibpromma S<sup>1,2,3,4</sup>, Bhat DJ<sup>6</sup>, Hyde KD<sup>1,2,3,4</sup> and Boonmee S<sup>1\*</sup>**

<sup>1</sup> Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai, 57100, Thailand

<sup>2</sup> Mushroom Research Foundation, 128 M.3 Ban Pa Deng T. Pa Pae, A. Mae Taeng, Chiang Mai 50150, Thailand

<sup>3</sup> Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650201, Yunnan, People's Republic of China

<sup>4</sup> World Agroforestry Centre, East and Central Asia, Kunming 650201, Yunnan, P. R. China

<sup>5</sup> Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang, Guizhou Province 550025, P.R. China.

<sup>6</sup> Formerly at Department of Botany, Goa University, Goa, 403206, India

Chaiwan N, Lu YZ, Tibpromma S, Bhat DJ, Hyde KD, Boonmee S 2017 – *Neotubeufia* gen. nov. and *Tubeufia guangxiensis* sp. nov. (Tubeufiaceae) from freshwater habitats. Mycosphere 8(9), 1443–1456, Doi 10.5943/mycosphere/8/9/9

**Abstract**

During our fungal forays in freshwater streams of Thailand and China, two new taxa belonging to the family Tubeufiaceae were isolated from decaying submerged wood samples. A new genus *Neotubeufia* is introduced to accommodate a new species, *N. krabiensis*, which is comparable to *Tubeufia* in the features of ascomata, asci and ascospores, but found distinct with dark ascomata, cylindrical asci and cylindric-fusiform ascospores. A multigene phylogenetic analysis based on ITS, LSU, TEF1 $\alpha$  and RPB2 sequence data indicate that *Neotubeufia* formed a single clade distant from the *Tubeufia* clade. A new species, *Tubeufia guangxiensis* collected from decaying wood in a freshwater habitat in China is confirmed by comparing morphological characters with the type species *T. javanica* and other *Tubeufia* taxa together with phylogenetic analysis. Descriptions, illustrations and notes are provided for the new genus and new species.

**Key words** – decaying wood – freshwater fungi – new species – phylogeny – taxonomy

**Introduction**

The family Tubeufiaceae was introduced by Barr (1979) and Boonmee et al. (2014) accommodated the family in the order Tubeufiales. A comprehensive review of Tubeufiaceae by Boonmee et al. (2011) based on morphological re-examination and phylogenetic analysis included 20 genera in the family. Currently, the family comprises 24 genera (Wijayawardene et al. 2014, Doilom et al. 2017, Boonmee et al. 2014, Hyde et al. 2016a, Lu et al. 2017a, b, c, d, Brahmanage et al. 2017, Luo et al. 2017, Tanney & Miller 2017) with sexual and asexual

morphs. All sexual morph taxa are characterized by superficial, sphaerical, solitary, light to darkly pigmented ascomata, with or without setae, cylindrical clavate asci and cylindrical elongate to fusiform, multi-septate, hyaline to pigmented ascospores. The asexual morph taxa are commonly found as hyphomycetes, with helicosporous, phragmosporous and dictyosporous conidia (Barr 1980, Kodsueb et al. 2006, Boonmee et al. 2011, 2014, Lu et al. 2017a, b, Luo et al. 2017). Members of *Tubeufia* are saprobic, generally growing on dead woods (Boonmee et al. 2011, 2014, Hyde et al. 2013, 2016a). Species of *Tubeufia* colonizing woody substrates are common in terrestrial habitat (Goh & Hyde 1996, Ho et al. 2001, Cai et al. 2002, Luo et al. 2004, Zhao et al. 2007, Lu et al. 2017a). However, some species can be found in aquatic habitat e.g. *Tubeufia hyalospora* and *T. roseohelicospora* in their asexual form (Hyde et al. 2016a). Index Fungorum lists 55 records of *Tubeufia* (Index Fungorum 2017) while Wijayawardene et al. (2017) estimated 29 species.

In this study, a new genus and a new species of *Tubeufiaceae* are introduced from Thailand and Chinese collections. Detailed descriptions, illustrations, phylogenetic analyses and notes of the novel taxa are provided.

## Materials & Methods

### Isolates and morphology

Decaying wood was collected in freshwater stream in southern Thailand and from Guangxi Province in China and brought to the laboratory in Zip-lock bags. Samples were observed under a light microscope (Nikon ECLIPSE 80i compound microscope) and micro-morphological structures were photographed with a Canon EOS 600D digital camera fitted to the microscope. Measurements were made using Tarosoft (R) Image Frame Work program. Figures were processed with an Adobe Photoshop CS6 Extended version 10.0 software (Adobe Systems, USA).

Single spore isolation was made following the method described in Chomnunti et al. (2014). Germinating spores were aseptically transferred to fresh malt extract agar (MEA) plates and incubated at 28°C. Cultures were grown for 2–4 weeks and morphological characters, such as colour, colony and texture recorded. The specimens and living cultures were deposited in the Herbarium of Mae Fah Luang University (Herb. MFLU) and Culture Collection of Mae Fah Luang University (MFLUCC), Chiang Rai, Thailand and Thailand Bioresource Research Center (TBRC), Pathum Thani, Thailand. Facesoffungi and Index Fungorum numbers were submitted (Jayasiri et al. 2015, Index Fungorum 2017). New taxa were justified based on recommendations outlined by Jeewon & Hyde (2016).

### DNA extraction and PCR amplification

The extraction, amplification and sequencing of DNA followed the method of Lu et al. (2017a). Four genes were amplified with universal primers, namely the internal transcribed spacer region of ribosomal DNA (ITS: ITS5/ITS4) (White et al. 1990), large subunit nuclear ribosomal DNA (LSU: LROR/LR5) (Vilgalys & Hester 1990), the translation elongation factor 1-alpha gene (TEF1α: EF1-983F/EF1-2218R) (Rehner & Buckley 2005) and the RNA polymerase second largest subunit (RPB2: fRPB2-5f/fRPB2-7cR) gene (Liu et al. 1999). The PCR products were purified and sequenced with the same primers. The amplification reactions were carried out using the method described by Lu et al. (2017a). The quality of PCR products were checked on 1% agarose gel electrophoresis stained with ethidium bromide. The PCR products were sent for sequencing at Sangon Biotech, Shanghai, China.

**Table 1.** Taxa used in the phylogenetic analyses and their GenBank accession numbers. The new taxa are indicated with an asterisk.

Taxa	Culture accession No.	GenBank Accession No.				Reference
		ITS	LSU	TEF1 $\alpha$	RPB2	
<i>Acanthohelicospora aurea</i>	GZCC 16-0059	KY321322	KY321325	KY792599	MF589910	Lu et al. (2017c)
<i>Acanthohelicospora guianense</i>	UAMH 1699	AY916479	AY856891	–	–	Tsui et al. (2006)
<i>Acanthohelicospora pinicola</i>	<b>MFLUCC 10–0116</b>	KF301526	KF301534	KF301555	–	Boonmee et al. (2014)
<i>Acanthostigma perpusillum</i>	<b>UAMH 7237</b>	AY916492	AY856892	–	–	Tsui & Berbee (2006)
<i>Acanthostigmina multiseptatum</i>	ANM 665	GQ856144	GQ850493	–	–	Promptutha & Miller (2010)
<i>Acanthostigmina Chiangmaiensis</i>	MFLUCC 10–0125	JN865209	JN865197	KF301560	–	Boonmee et al. (2014)
<i>Acanthostigmina multiseptatum</i>	ANM 475	GQ856145	GQ850492	–	–	Promptutha & Miller (2010)
<i>Aquaphila albicans</i>	<b>BCC 3543</b>	DQ341096	DQ341101	–	–	Tsui et al. (2007)
<i>Aquaphila albicans</i>	MFLUCC 16–0010	KX454165	KX454166	KY117034	MF535255	Hyde et al. (2016a); Lu et al. (2017a)
<i>Berkleasmium concinnum</i>	DAOM 696499	KY611397	KY611399	–	–	Tanney & Miller (2017)
<i>Berkleasmium concinnum</i>	ILLS 80802	KY582484	–	–	–	Tanney & Miller (2017)
<i>Berkleasmium concinnum</i>	NB 789	KY611398	KY611400	–	–	Tanney & Miller (2017)
<i>Boerlagiomyces macrospora</i>	MFLUCC12–0388	KU144927	KU764712	KU872750	–	Doilom et al. (2017)
<i>Botryosphaeria dothidea</i>	CBS 115476	KF766151	KU764712	DQ767637	–	Schoch et al. (2006); Slippers et al. (2013)
<i>Chlamydotubeufia chlamydosporum</i>	CBS 160.69	AY916466	AY856875	–	–	Tsui et al. (2006)
<i>Chlamydotubeufia huaikangplaensis</i>	<b>MFLUCC10–0926</b>	JN865210	JN865198	–	–	Boonmee et al. (2011)
<i>Chlamydotubeufia krabiensis</i>	MFLUCC 16–1134	KY678767	KY678759	KY792598	MF535261	Hyde et al. (2017)
<i>Dictyospora thailandica</i>	MFLUCC 16–0001	KY873627	KY873622	KY873286	–	Brahmanage et al. (2017)
<i>Dictyospora thailandica</i>	MFLUCC 16–0215	KY873628	KY873623	KY873287	–	Brahmanage et al. (2017)
<i>Helicangiospora lignicola</i>	<b>MFLUCC 11–0378</b>	KF301523	KF301531	KF301552	–	Boonmee et al. (2011)
<i>Helicoma Chiangraiensis</i>	MLLUCC 10–0115	JN865200	JN865188	–	–	Boonmee et al. (2011)
<i>Helicoma khunkornense</i>	MFLUCC 10–0119	JN865203	JN865191	KF301559	–	Boonmee et al. (2011)
<i>Helicoma muelleri</i>	<b>CBS 964.69</b>	AY916453	AY856877	–	–	Tsui et al. (2006)
<i>Helicoma perelegans</i>	ATCC 22621	AY916463	–	–	–	Tsui et al. (2006)
<i>Helicomycetes indicum</i>	CBS 374.93	AY916477	AY856885	–	–	Tsui & Berbee (2006)
<i>Helicomycetes roseus</i>	KUMCC 15 0281	KY320526	KY320543	KY32055	–	Luo et al. (2017)
<i>Helicomycetes roseus</i>	BCC 8808	AY916481	AY856910	–	–	Tsui et al. (2007)
<i>Helicomycetes roseus</i>	CBS 283.51	AY916464	AY856881	–	–	Tsui et al. (2006)
<i>Helicomycetes talbotii</i>	MUCL 33010	AY916465	AY856874	–	–	Tsui & Berbee (2006)
<i>Helicosporium luteosporum</i>	MFLUCC 16–0226	KY321324	KY321327	KY792601	–	Lu et al. (2017c)
<i>Helicosporium flavum</i>	MFLUCC 16–1230	KY873626	KY873621	–	–	Brahmanage et al. (2017)
<i>Helicosporium luteosporum</i>	MFLUCC 16–1233	–	KY873624	–	–	Brahmanage et al. (2017)

**Table 1 continued.** Taxa used in the phylogenetic analyses and their GenBank accession numbers. The new taxa are indicated with an asterisk.

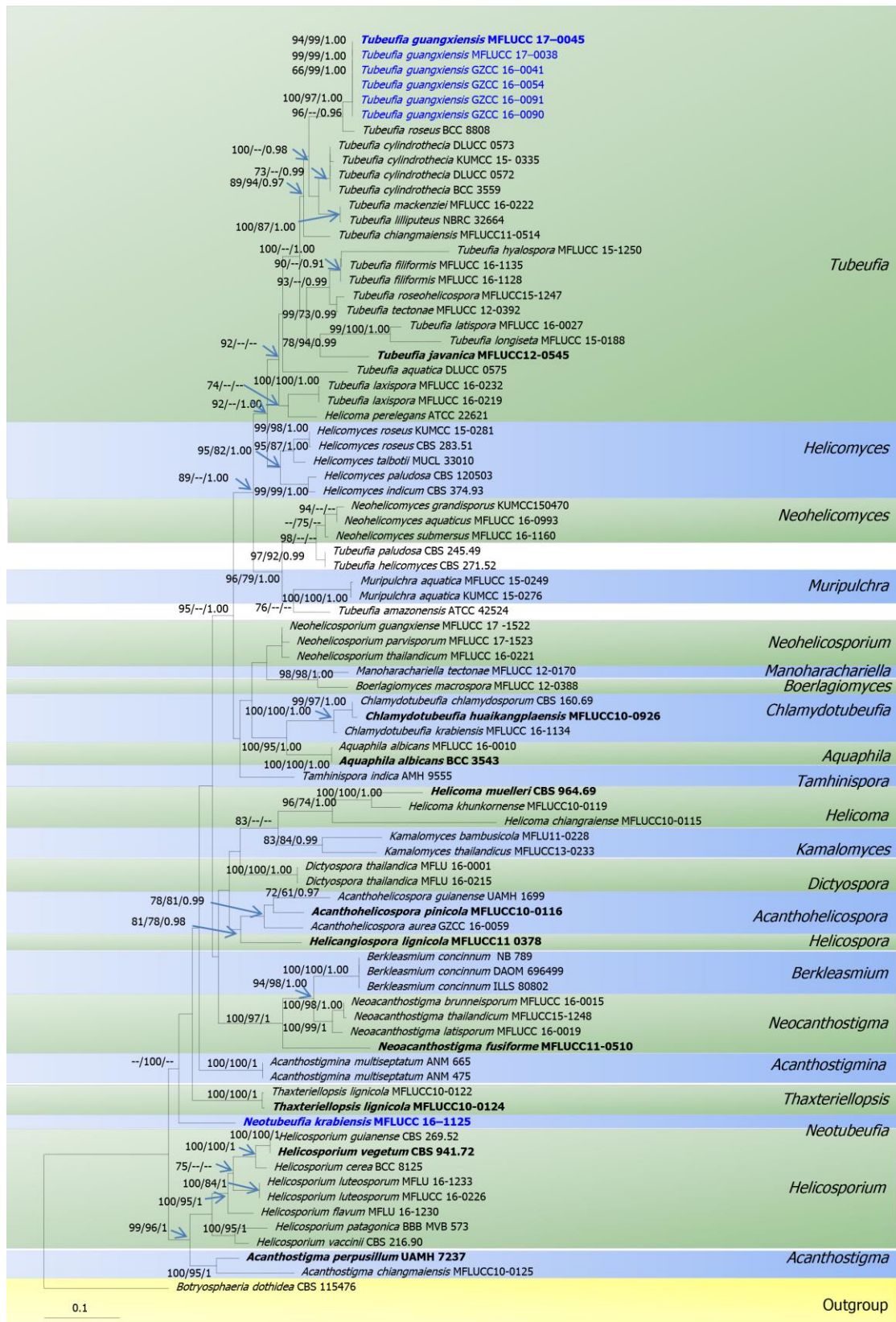
Taxa	Culture accession No.	GenBank Accession No.				Reference
		ITS	LSU	TEF1 $\alpha$	RPB2	
<i>Helicosporium guianense</i>	CBS 269.52	AY916487	AY856893	–	–	Tsui et al (2006)
<i>Helicosporium patagonica</i>	BBB MVB 573	JN127358	JN127359	–	–	Sanchez et al. (2011)
<i>Helicosporium vaccinii</i>	CBS 216.90	AY916486	AY856879	–	–	Sanchez et al. (2011)
<b><i>Helicosporium vegetum</i></b>	<b>CBS 941.72</b>	AY916488	AY856883	–	–	Tsui et al. (2007)
<i>Helicosporium cerea</i>	BCC 8125	AY916491	AY856909	–	–	Tsui et al. (2007)
<i>Kamalomyces bambusicola</i>	MFLU 11–0228	–	MF506880	–	–	Phookamsak et al. (2017)
<i>Kamalomyces thailandicus</i>	MFLUCC 13–0233	MF506884	MF506882	MF506886	–	Phookamsak et al. (2017)
<i>Manoharachariella tectonae</i>	MFLUCC 12–0170	KF301529	KF301537	KU872762	–	Boonmee et al. (2014)
<i>Muripulchra aquatica</i>	KUMCC 15–0276	KY320534	KY320551	KY320564	–	Luo et al. (2017)
<i>Muripulchra aquatica</i>	MFLUCC 15–0249	KY320532	KY320549	–	–	Luo et al. (2017)
<i>Neocanthostigma brunneisporum</i>	MFLUCC 16–0015	KY790446	KY790434	KY792610	–	Lu et al. (2017b)
<b><i>Neocanthostigma fusiforme</i></b>	<b>MFLUCC 11–0510</b>	KF301529	KF301537	KY792612	–	Boonmee et al. (2014)
<i>Neocanthostigma latisporum</i>	MFLUCC 16–0019	KY790449	KY790437	KY792613	MF535271	Lu et al. (2017b)
<i>Neocanthostigma thailandicum</i>	MFLUCC 15–1248	KX454176	–	–	–	Prompttha & Miller (2010)
<i>Neohelicomycetes aquaticus</i>	MFLUCC 16–0993	KY320528	KY320545	KY320561	–	Luo et al. (2017)
<i>Neohelicomycetes grandisporus</i>	KUMCC 150470	KX454173	KX454174	–	–	Boonmee et al. (2014)
<i>Neohelicomycetes submerses</i>	MFLUCC 16–1160	KY320530	KY320547	–	–	Luo et al. (2017a)
<i>Neohelicosporium guangxiense</i>	MFLUCC 17–1522	MF467922	MF467935	MF535248	MF535278	Lu et al. (2017d)
<i>Neohelicosporium parvisporum</i>	MFLUCC 17–1523	MF467926	MF467939	MF535252	MF535282	Lu et al. (2017d)
<i>Neohelicosporium thailandicum</i>	MFLUCC 16–0221	MF467928	MF467941	MF535253	MF535283	Lu et al. (2017d)
<b><i>Neotubeufia krabiensis</i>*</b>	<b>MFLUCC 16–1125</b>	MG012031	MG012024	MG012010	MG012017	This study
<i>Tamhinispora indica</i>	AMH 9555	KC469282	KC469283	–	–	Rajeshkumar & Sharma (2013)
<i>Thaxteriellopsis lignicola</i>	MFLUCC 10–0122	JN865206	JN865194	KF301563	–	Boonmee et al. (2011)
<b><i>Thaxteriellopsis lignicola</i></b>	<b>MFLUCC 10–0124</b>	JN865208	JN865196	KF301561	–	Boonmee et al. (2011)
<b><i>Tubeufia guangxiensis</i> *</b>	<b>MFLUCC 17–0045</b>	MG012025	MG012018	MG012004	MG012011	This study
<i>Tubeufia guangxiensis</i> *	MFLUCC 17–0038	MG012026	MG012019	MG012005	MG012012	This study
<i>Tubeufia guangxiensis</i> *	GZCC 16–0041	MG012027	MG012020	MG012006	MG012013	This study
<i>Tubeufia guangxiensis</i> *	GZCC 16–0054	MG012028	MG012021	MG012007	MG012014	This study
<i>Tubeufia guangxiensis</i> *	GZCC 16–0091	MG012029	MG012022	MG012008	MG012015	This study
<i>Tubeufia guangxiensis</i> *	GZCC 16–0090	MG012030	MG012023	MG012009	MG012016	This study
<i>Tubeufia amazonensis</i>	ATCC 42524	AY916458	AY856911	–	–	Tanney & Miller (2017)
<i>Tubeufia aquatica</i>	DLUCC 0575	KY320522	KY320539	KY320556	–	Luo et al. (2017)

**Table 1 continued.** Taxa used in the phylogenetic analyses and their GenBank accession numbers. The new taxa are indicated with an asterisk.

Taxa	Culture accession No.	GenBank Accession No.				Reference
		ITS	LSU	TEF1 $\alpha$	RPB2	
<i>Tubeufia Chiangmaiensis</i>	MFLUCC 11-0514	KF301530	KF301538	KF301557	–	Boonmee et al. (2014)
<i>Tubeufia cylindrothecia</i>	BCC 3559	–	AY849965	–	–	Kodsueb et al. (2006)
<i>Tubeufia cylindrothecia</i>	DLUCC 0572	KY320520	KY320537	KY320554	–	Luo et al. (2017)
<i>Tubeufia cylindrothecia</i>	DLUCC 0573	KY320518	KY320535	KY320552	–	Luo et al. (2017)
<i>Tubeufia cylindrothecia</i>	KUMCC 15-0335	KY320519	KY320536	KY320553	–	Luo et al. (2017)
<i>Tubeufia filiformis</i>	MFLUCC 16-1128	–	KY092407	KY117028	–	Lu et al. (2017a)
<i>Tubeufia filiformis</i>	MFLUCC 16-1135	KY092416	KY092411	–	–	Lu et al. (2017a)
<i>Tubeufia helicomyces</i>	CBS 271.52	AY916461	AY856887	–	–	Tsui et al. (2007)
<i>Tubeufia hyalospora</i>	MFLUCC 15-1250	–	KX454179	–	–	Hyde et al. (2016)
<b><i>Tubeufia javanica</i></b>	<b>MFLUCC 12-0545</b>	KJ880034	KJ880036	KJ880037	–	Boonmee et al. (2014)
<i>Tubeufia latispora</i>	MFLUCC 16-0027	KY092417	KY092412	KY117033	–	Lu et al. (2017a)
<i>Tubeufia laxispora</i>	MFLUCC 16-0219	KY092414	KY092409	KY117030	–	Lu et al. (2017a)
<i>Tubeufia laxispora</i>	MFLUCC 16-0232	KY092413	KY092408	KY117029	MF535287	Lu et al. (2017a)
<i>Tubeufia lilliputeus</i>	NBRC 32664	AY916483	AY856899	–	–	Tsui et al. (2007)
<i>Tubeufia mckenziei</i>	MFLUCC 16-0222	KY092415	KY092410	KY117031	MF535288	Lu et al. (2017a)
<i>Tubeufia longiseta</i>	MFLUCC 15-0188	KU 940133	–	–	–	Dai et al. (2016)
<i>Tubeufia paludosa</i>	CBS 120503	GU296203	GU301877	GU349024	–	Schoch et al (2009)
<i>Tubeufia paludosa</i>	CBS 245.49	GU566745	–	–	–	Ruibal et al. (2009)
<i>Tubeufia roseohelicospora</i>	MFLUCC 15-1247	KX454177	KX454178	–	–	Hyde et al. (2016a)
<i>Tubeufia tectonae</i>	MFLUCC 12-0392	KU144923	KU764705	KU872762	–	Doilom et al. (2017)

### Phylogenetic analysis

Sequence data of *Tubeufiaceae* and related taxa (Table 1) were downloaded from GenBank following recent publications (Brahmanage et al. 2017, Luo et al. 2017, Lu et al. 2017a, Tanney & Miller 2017). Gene sequences and genetic markers used for genera were based on the current publications and have commonly been used for the genera. The multiple sequence alignments were produced with MAFFT v. 7 (<http://mafft.cbrc.jp/alignment/server/index.html>) and BioEdit v. 7.0.5.2 (Hall 1999). The phylogenetic analyses were performed using maximum likelihood (ML), maximum parsimony (MP) and Bayesian posterior probabilities (BYPP) following Luo et al. (2017). The phylogenetic trees were figured in FigTree v. 1.4 (Rambaut 2012) and edited using Microsoft Office Power Point 2007 and Adobe illustrator CS3 (Adobe Systems Inc., USA). Sequences derived in this study were deposited in GenBank, while the alignments in TreeBASE ([www.treebase.org](http://www.treebase.org)).



**Figure 1** – Phylogram generated from RAXML based on combined ITS, LSU, TEF1 $\alpha$  and RPB2 sequence data. Bootstrap support values for maximum likelihood (ML, left) greater than 60%, maximum parsimony (MP, middle) greater than 60% and Bayesian posterior probabilities (PP, right) equal to or greater than 0.95 are indicated at the nodes. The ex-type strains are in bold and the new taxa in blue.

## Results

### Phylogenetic analyses

The combined sequence alignments comprised 84 taxa, with *Botryosphaeria dothidea* as the outgroup taxon. The dataset comprised 3678 characters (ITS, LSU, TEF1 $\alpha$  and RPB2 sequence data) after alignment. RAxML analysis yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value. The phylogenetic tree obtained in this study showed similar results to previous studies (Boonmee et al. 2014, Hyde et al. 2016a, Lu et al. 2017a, b, c, d, Brahmanage et al. 2017, Luo et al. 2017, Tanney & Miller 2017). Our two new taxa when included in the analysis formed a well-supported clade with *Tubeufia roseus*. Phylogenetic analysis shows that *Neotubeufia* is well-separated from all genera in the *Tubeufiaceae*, while the new species *Tubeufia guangxiensis* formed a sister clade with four species, *T. roseus*, *T. cylindrothecia*, *T. mackeniei* and *T. lilliputeus* with strong support (99% and 99% BS and 1.00 PP) but remained distinct.

***Neotubeufia* Chaiwan, Boonmee, Y.Z. Lu & Hyde, gen. nov.**

Fig. 2

Index Fungorum number: IF553871; Facesoffungi number: FoF03777

Etymology – neo – a new genus similar to *Tubeufia*.

*Saprobic* on submerged decaying wood. **Sexual morph:** *Ascomata* superficial, uniloculate, solitary, sometimes grouped, subglobose, dark-brown to black, lacking setae. *Peridium* comprising several layers of brown to dark brown cells of *textura angularis*. *Hamathecium* comprising numerous filiform, septate, hyaline pseudoparaphyses. *Asci* 8-spored, bitunicate, broad cylindrical, rounded at apex, pedicellate, sometimes sessile. *Ascospores* cylindric-fusiform, slightly curved, tapering towards rounded ends, 6–7-septate, guttulate when immature, hyaline, smooth-walled. **Asexual morph:** Undetermined.

*Type species* – *Neotubeufia krabiensis*

Notes – The dark, subglobose ascomata, cylindrical asci and cylindrical to cylindric-fusiform ascospores of *Neotubeufia krabiensis* are somewhat similar to characters of the taxa in *Tubeufia* (Boonmee et al. 2014, Luo et al. 2017a). *Neotubeufia* on the other hand, has blackened and black and rounded ascomata. Phylogenetically, *Neotubeufia krabiensis* does not group with any other taxa in *Tubeufiaceae* and formed a single, separate clade distant from *Tubeufia* clade with strong bootstrap support (100% MP). Therefore, the genus *Neotubeufia* is introduced to accommodate this unique species.

***Neotubeufia krabiensis* Chaiwan, Boonmee, Y.Z. Lu & Hyde, sp. nov.**

Index Fungorum number: IF553872; Facesoffungi number: FoF03778

Etymology – From the Latin –ensis, meaning associated with and Krabi in reference to the type locality of Krabi Province.

Holotype: MFLU17–1184

*Saprobic* on submerged decaying wood. **Sexual morph:** *Ascomata* 340–390  $\mu$ m high  $\times$  365–400  $\mu$ m diam, superficial, solitary, scattered, subglobose dark-brown to black, lacking setae. *Peridium* 58–68  $\mu$ m wide, comprising several layers brown to dark brown cells of *textura angularis*, outer layer darkened cells and inner layer pale brown to hyaline cells. *Hamathecium* comprising numerous filiform, septate, hyaline pseudoparaphyses. *Asci* 120–145  $\times$  12–15  $\mu$ m ( $\bar{x}$  = 132  $\times$  14  $\mu$ m, n = 20), 8-spored, bitunicate, cylindrical, apically rounded, long pedicellate or sessile. *Ascospores* 55–75  $\times$  5–6.5  $\mu$ m ( $\bar{x}$  = 66  $\times$  6  $\mu$ m, n = 20), fasciculate, cylindrical to cylindric-fusiform, elongated, slightly curved, tapering towards rounded ends, 6–7-septate, hyaline, guttulate, smooth-walled. **Asexual morph:** Undetermined.



Culture characteristics – Ascospores germinating on water agar and producing germ tubes within 12 hours. Colonies growing on MEA, circular, umbonate, rough and wrinkled at surface, edge entire, reaching 12 mm in 2 weeks at 28 °C, pale brown to brown, mycelium superficial and partially immersed, branched, septate, hyaline to pale brown, smooth.

Material examined – THAILAND, Krabi, Muang, on decaying wood in freshwater stream, 16 December 2015, Saranyaphat Boonmee, TP01–3 (MFLU 17–1184, **holotype**; GZAAS 17–0017 **paratype**), ex-type living culture, MFLUCC 16–1125 = TBRC \*\*\*.

Notes – *Neotubeufia krabiensis* found in a freshwater habitat and described as a new species, shares some similar morphological characters with *Tubeufia* species in that both have bitunicate, cylindrical asci and fasciculate, cylindrical to long cylindric-fusiform, elongate, slightly curved, phragmoseptate ascospores. However, phylogenetic analysis placed *N. krabiensis* in a separate clade from *Tubeufia* group with strong support. Therefore, *N. krabiensis* is clearly distinct taxon from *Tubeufia*. *Neotubeufia* differs from the type species *T. javanica* and other species in *Tubeufia* by its dark ascomata and cylindrical to cylindric-fusiform, multi-septate with distinctively guttulate ascospores.

***Tubeufia guangxiensis*** Chaiwan, Boonmee, Y.Z. Lu & Hyde, **sp. nov.**

Figs 3, 4

Index Fungorum number: IF553873; Faces of fungi number: FoF03779

Etymology – refers to the location where the fungus was collected, Guangxi, China. Holotype: GZAAS 16–0057

*Saprobic* on submerged decaying wood. **Sexual morph:** *Ascomata* 255–300 µm diam. × 140–160 µm high ( $\bar{x}$  = 275 × 150 µm, n = 5), superficial, subglobose, spherical to clavate-obovate oval to ellipsoid, narrow at apex, solitary, scattered, pale to yellowish. *Peridium* 25–29 µm wide, composed of several layers of white light brown cells of *textura angularis*. *Hamathecium* comprising numerous filiform, septate, hyaline, pseudoparaphyses. *Asci* 160–230 × 12–17 µm ( $\bar{x}$  = 197 × 14.5 µm, n = 20), 8-spored, bitunicate, cylindrical, rounded at apex, long pedicellate. *Ascospores* 135–155 × 3–5 µm ( $\bar{x}$  = 143 × 4 µm, n = 20), fasciculate, spiral, broadly elongate, cylindrical to sub-fusiform, slightly curved, tapering towards rounded ends, phragmosporous, 12–15-septate, guttulate, hyaline, smooth-walled. **Asexual morph:** hyphomycetous, helicosporous. *Conidiophores* 24–39 × 3.5–5 µm ( $\bar{x}$  = 32 × 4.5 µm, n = 10), mononematous, erect, short, septate, pale brown, smooth-walled. *Conidiogenous cells* 10–17 × 3.5–5 µm ( $\bar{x}$  = 13 × 4.5 µm, n = 10), monoblastic, holoblastic, integrated, terminal, cylindrical, each with single conidium. *Conidia* 45–70 × 6.5–9 µm ( $\bar{x}$  = 60 × 7.5 µm, n = 20) with 360–460 µm long, coiled 1½–2½ times when tight, becoming loosely coiled in water, rounded at apical end, up to 50-septate, slightly constricted at septa, hyaline, guttulate, smooth-walled.

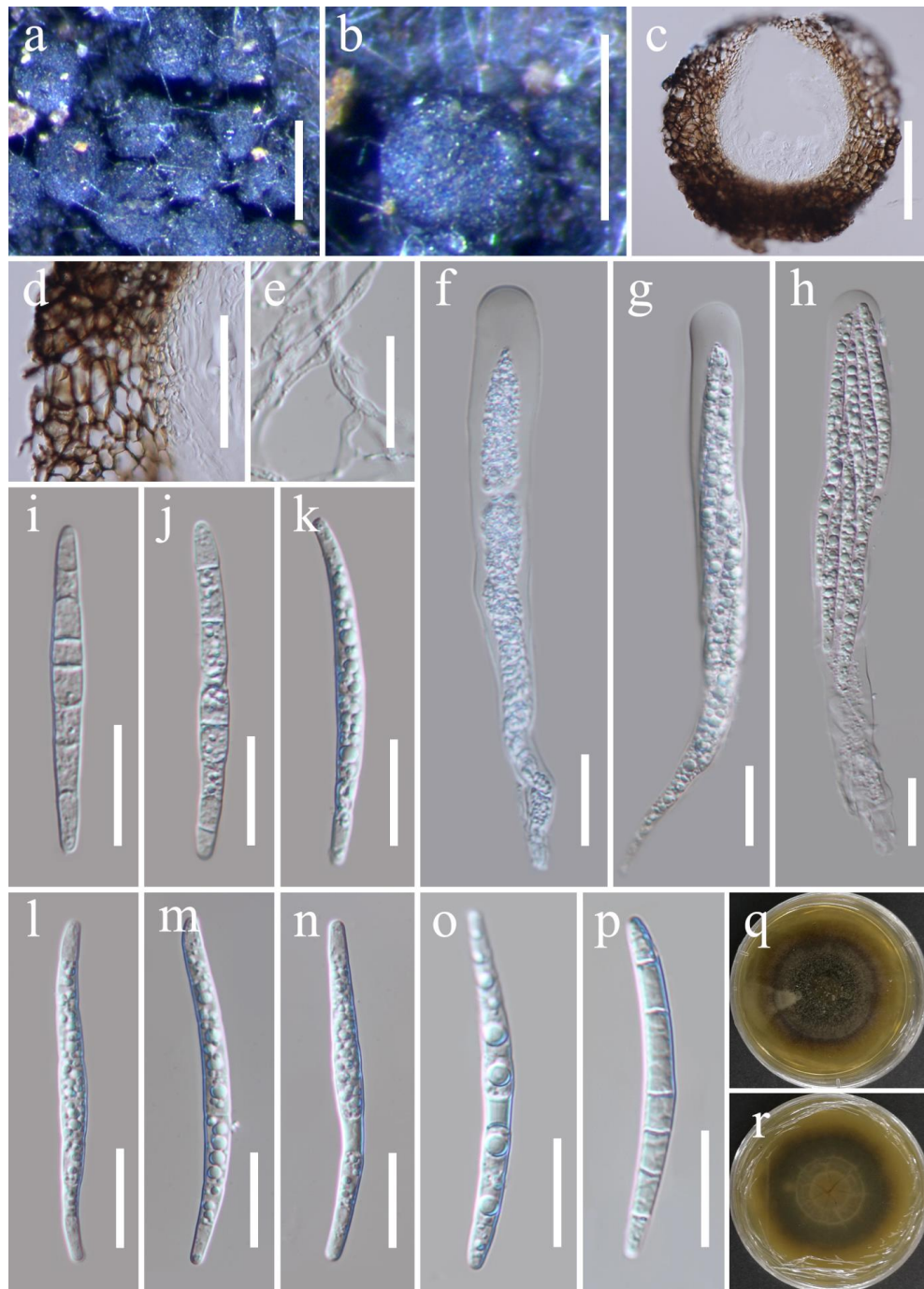
Culture characteristics – Ascospores germinating on water agar and producing germ tubes within 12 hours. Colonies growing on PDA, circular, with flat surface, edge entire, reaching 11 mm in 2 weeks at 28 °C, pale brown to brown, mycelium superficial and partially immersed, branched, septate, hyaline to pale brown, smooth.

Material examined – CHINA, Guangxi Province, Fang Cheng Gang City, on submerged decaying wood in a freshwater stream, 15 May 2016, Y.Z. Lu, JHC14–2 (GZAAS 16–0057, **holotype**), ex-type living culture, MFLUCC 17–0045 = GZCC 16–0045; *ibid.*, JHC01–3 (GZAAS 16–0042, **paratype**), ex-type living culture, MFLUCC 17–0038 = GZCC 16–0030; *ibid.*, JHC10 (GZAAS 16–0053, **paratype**), ex-type living culture, GZCC 16–0041; *Ibid.*, JHC21–5 (GZAAS 16–0066, **paratype**), ex-type living culture, GZCC 16–0054; *ibid.*, JHC25–1 (GZAAS 16–0103, **paratype**), ex-type living culture, GZCC 16–0091; *ibid.*, JHC26–2 (GZAAS 16–0102, **paratype**), ex-type living culture, GZCC 16–0090.

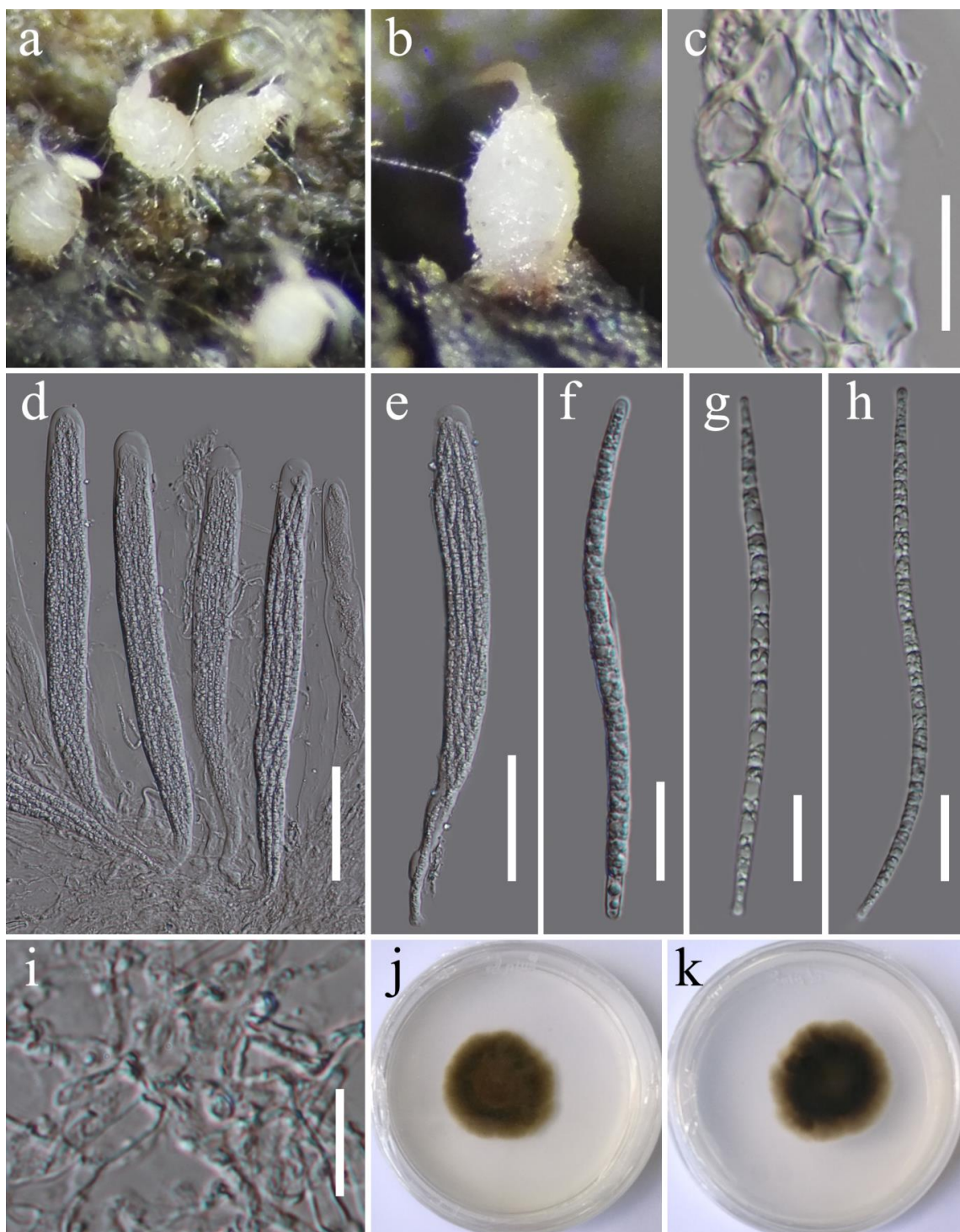
Notes – *Tubeufia guangxiensis* is a new species found in freshwater habitat in China. Morphologically it resembles the type species, *T. javanica* in having oval, pale to yellowish



ascomata with thin-walled, long cylindrical asci and ascospores. Phylogenetically, *T. guangxiensis* forms a sister clade to *T. roseus*, *T. cylindrothecia*, *T. mackeniei* and *T. lilliputeus* with strong-support (100% and 97% BS and 1.00 PP). *Tubeufia guangxiensis* differs from these species by having asexual morph found on submerged decaying wood in a freshwater stream (Boonmee et al. 2014, Luo et al. 2017a). The asexual morph of *T. guangxiensis* found on submerged decaying wood in freshwater habitat is helicosporous and appears similar to *T. hyalospora* and *T. roseohelicospora*, but differs by smaller mononematous conidiophores and coiled conidia. *T. javanica* has no asexual morph reported.

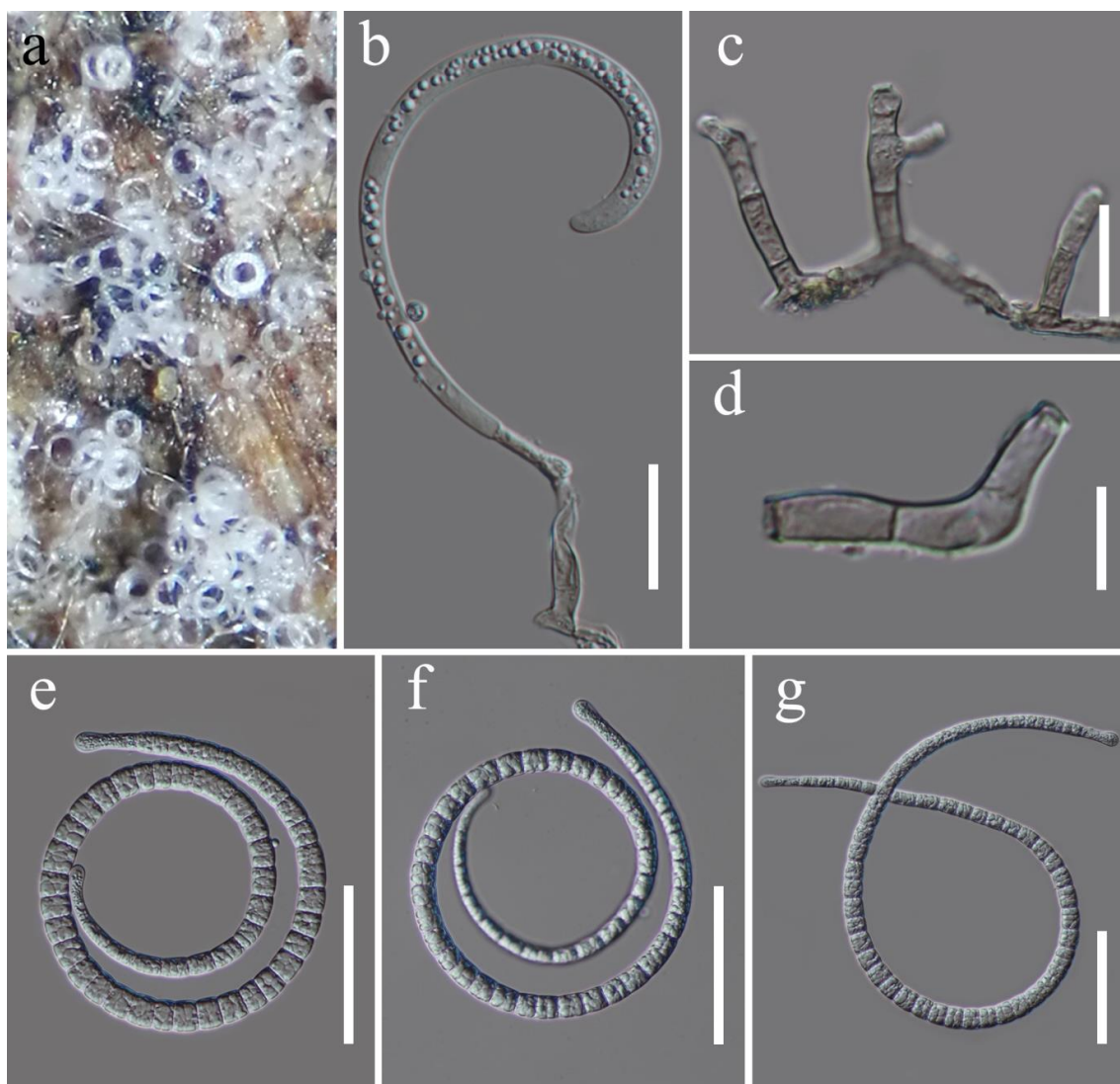


**Figure 2** – *Neotubeufia krabiensis* (MFLU 17–1184, **holotype**). **a, b** Superficial ascomata on substrate. **c** Cross section of ascoma. **d** Peridium. **e** Hamathecium. **f–h** Asci. **i–p** Ascospores. **q, r** Colonies on MEA from above and below at two months. Scale bars: a–b = 500  $\mu$ m, c = 100  $\mu$ m, d–p = 20  $\mu$ m.



**Figure 3** – Sexual morph of *Tubeufia guangxiensis* (GZAAS 16–0057, holotype). **a, b** Superficial ascomata on substrate. **c** Peridium. **d, e** Asci with hamathecium. **f–h** Ascospores. **i** Hamathecium. **j, k** Colonies on PDA from above and below at two months. Scale bars: **c, f–h** = 20  $\mu\text{m}$ , **d–e** = 50  $\mu\text{m}$ , **i** = 10  $\mu\text{m}$ .





**Figure 4** – Asexual morph of *Tubeufia guangxiensis* (GZAAS 16–0042, **paratype**). **a** Colonies on decaying wood. **b** Conidiophore with attached conidium. **c–d** Conidiophores and Conidiogenous cells. **e–g** Conidia. Scale bars: b–c = 20  $\mu$ m, d = 10  $\mu$ m, e–g = 50  $\mu$ m.

### Acknowledgements

S. Boonmee would like to thank the Thailand Research Fund (project No. TRG5880152) and the National Research Council of Thailand (no. 2560A30702021). K.D. Hyde would like to thank Molecular Biology Experimental Center for the help on molecular work, the Mushroom Research Foundation (MRF), Chiang Rai, Thailand. Napalai Chaiwan would like to thank Shaun Pennycook for nomenclatural advice and Dhanushka Nadeeshan for valuable suggestions and help.

### References

- Barr ME. 1979 – A classification of *Loculoascomycetes*. *Mycologia* 71, 935–957.
- Barr ME. 1980 – On the family *Tubeufiaceae* (*Pleosporales*). *Mycotaxon* 12, 137–167.
- Boonmee S, Zhang Y, Chomnunti P, Chukeatirote E. 2011 – Revision of lignicolous *Tubeufiaceae* based on morphological reexamination and phylogenetic analysis. *Fungal Diversity* 51, 63–102.

- Boonmee S, Rossmana AY, Liu JK, Li WJ. 2014 – *Tubeufiales*, ord. nov., integrating sexual and asexual generic names. Fungal Diversity 68, 239–298.
- Brahmanage RS, Lu YZ, Bhat DJ, Wanasinghe DN, et al. 2017 – Phylogenetic investigations on freshwater fungi in *Tubeufiaceae* (*Tubeufiales*) reveals the new genus *Dictyospora* and new species *Chlamydotubeufia aquatica* and *Helicosporium flavum*. Mycosphere 8, 917–933.
- Cai L, Tsui CKM, Zhang KQ, Hyde KD. 2002 – Aquatic fungi from Lake Fuxian, Yunnan, China. Fungal Diversity 9, 57–70.
- Chomnunti P, Hongsanan S, Hudson BA, Tian Q. 2014 – The sooty moulds. Fungal Diversity 66, 1–36.
- Dai DQ, Phookamsak R, Wijayawardene NN, Li WJ, 2016 – Bambusicolous fungi. Fungal Diversity 82, 1–105.
- Doilom M, Dissanayake AJ, Wanasinghe DN, Boonmee S, et al. 2017 – Microfungi on *Tectona grandis* (teak) in northern Thailand. Fungal Diversity 82, 107–182.
- Goh TK, Hyde KD. 1996 – Biodiversity of freshwater fungi. Journal of Industrial Microbiology & Biotechnology 17, 328–345
- Hall TA. 1999 – BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41, 95–98.
- Ho WH, Hyde KD, Hodgkiss IJ. 2001 – Fungal communities on submerged wood from streams in Brunei, Hong Kong, and Malaysia. Mycological Research 105, 1492–1501.
- Hyde KD, Jones GEB, Liu JK, Ariyawansa H. et al. 2013 – Families of Dothideomycetes. Fungal Diversity 63, 1–313.
- Hyde KD, Hongsanan S, Jeewon R, Bhat DJ, et al. 2016a – Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 80, 1–270.
- Hyde KD, Fryar S, Tian Q, Bahkali AH. et al. 2016b – Lignicolous freshwater fungi along a north south latitudinal gradient in the Asian/Australian region; can we predict the impact of global warming on biodiversity and function? Fungal ecology 19, 190–200.
- Hyde KD, Norphanphoun C, Abreu VP, Bazzicalupo A, et al. 2017 – Fungal diversity notes 603–708: taxonomic and phylogenetic notes on genera and species. Fungal Diversity (In press).
- Index Fungorum, 2017– <http://www.indexfungorum.org/Names/Names.asp>
- Jaysiri SC, Hyde KD, Ariyawansa HA, Bhat DJ, et al. 2015 – The Faces of Fungi database: fungal names linked with morphology, phylogeny and human impacts. Fungal Diversity 74, 3–18.
- Jeewon R, Hyde KD. 2016 – Establishing species boundaries and new taxa: recommendations to resolve taxonomic ambiguities. Mycosphere 7, 1669–1677.
- Katoh K and Standley DM 2017 – MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4), 772–780.
- Kodsueb R, Jeewon R, Vijaykrishna D, McKenzie EHC. 2006 – Systematic revision of *Tubeufiaceae* based on morphological and molecular data. Fungal Diversity 21, 105–130.
- Liu YJ, Whelen S, Hall BD. 1999 – Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution 16, 1799–1808.
- Lu YZ, Boonmee S, Dai DQ, Liu JK, et al. 2017a – Four new species of *Tubeufia* (*Tubeufiaceae*, *Tubeufiales*) from Thailand. Mycological Progress, 16, 403–417.
- Lu YZ, Boonmee S, Liu JK, Hyde KD, et al. 2017b – Novel *Neoacanthostigma* species from aquatic habitats. Cryptogamie, Mycologie 38(2), 169–190.

- Lu YZ, Boonmee S, Bhat DJ, Hyde KD, et al. 2017c – *Helicosporium luteosporum* sp. nov. and *Acanthohelicospora aurea* (Tubeufiaceae, Tubeufiales) from terrestrial habitats. Phytotaxa 319(3), 241–253.
- Lu YZ, Boonmee S, Liu JK, Hyde KD, et al. 2017d – Multi-gene phylogenetic analyses reveals *Neohelicosporium* gen. nov. and five new species of helicosporous hyphomycetes from aquatic habitats. Mycological Progress (In press).
- Luo ZL, Bhat DJ, Jeewon R, Boonmee S, et al. 2017 – Molecular phylogeny and morphological characterization of asexual fungi (Tubeufiaceae) from freshwater habitats in Yunnan, China. Cryptogamie Mycologie 38, 1–28.
- Phookamsak R, Lu YZ, Hyde KD, Jeewon R, 2017 – Taxonomic and phylogenetic appraisal of *Kamalomyces bambusicola* sp. nov. and *Kamalomyces thailandicus* sp. nov. (Tubeufiaceae, Tubeufiales) from Thailand. (In press).
- Promptutha I, Miller AN. 2010 – Three new species of *Acanthostigma* (Tubeufiaceae, Dothideomycetes) from Great Smoky Mountains National Park. Mycologia 102, 574–587.
- Rambaut A, 2012 – FigTree version 1.4.0. Available at <http://tree.bio.ed.ac.uk/software/figtree>.
- Rajeshkumar KC, Sharma R, 2013 – *Tamhinispora* a new genus belongs to family Tubeufiaceae from the Western Ghats, India based on morphology and phylogenetic analysis. Mycosphere 4(2), 165–175.
- Rehner SA, Buckley E. 2005 – A *Beauveria* phylogeny inferred from nuclear ITS and EF1- $\alpha$  sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97, 84–98.
- Ruibal C, Gueidan C, Selbmann L, Gorbushina, AA, 2009 – Phylogeny of rock-inhabiting fungi related to Dothideomycetes. Studies in Mycology 64, 123–133
- Sanchez RM, Miller AN, Bianchinotti MV. 2011 – A new species of *Acanthostigma* (Tubeufiaceae, Dothideomycetes) from the Southern Hemisphere. Mycologia (In press).
- Schoch CL, Shoemaker RA, Seifert KA, Hambleton S, et al. 2006 – A multigene phylogeny of the Dothideomycetes using four nuclear loci. Mycologia 98(6), 1041–1052.
- Slippers B, Boissin E, Phillips AJL, Groenewald JZ, et al. 2013 – Phylogenetic lineages in the *Botryosphaerales*: a systematic and evolutionary framework. Studies in Mycology 76, 31–49.
- Tanney J, Miller AN. 2017 – Asexual-sexual morph connection in the type species of *Berkleasmiium*. Biodiversity (Mycology and Botany), Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, 99–105.
- TreeBASE, 2017 – [www.treebase.org](http://www.treebase.org)
- Tsui CKM, Sivichai S, Berbee ML. 2006 – Molecular systematics of *Helicoma*, *Helicomycetes* and *Helicosporium* and their teleomorphs inferred from rDNA sequences. Mycologia 98, 4–104.
- Tsui CKM, Berbee ML. 2006 – Phylogenetic relationships and convergence of helicosporous fungi inferred from ribosomal DNA sequences. Molecular Phylogenetics and Evolution 39, 587–597.
- White TJ, Burns T, Lee S, Taylor JW. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, San Diego, California, USA, 315–322.
- Vilgalys R, Hester M. 1990 – Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172, 4238–4246.

- Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, et al. 2014 – Naming and outline of Dothideomycetes, including proposals for the protection or suppression of generic names. *Fungal Diversity* 69, 1–55.
- Wijayawardene NN, Hyde KD, Rajeshkumar KC, Hawksworth DL, et al. 2017 – Notes for genera: Ascomycota *Fungal Diversity* (In press).
- Zhao GZ, Liu X, Wu W. 2007 – *Helicosporous hyphomycetes* from China. *Fungal Diversity* 26, 313–524.