



## Lignicolous freshwater fungi in China III: Three new species and a new record of *Kirschsteiniothelia* from northwestern Yunnan Province

Bao DF<sup>1,2,3</sup>, Luo ZL<sup>1,2</sup>, Liu JK<sup>4</sup>, Bhat DJ<sup>5</sup>, Sarunya N<sup>3</sup>, Li WL<sup>1,6</sup>, Su HY<sup>1\*</sup> and Hyde KD<sup>2</sup>

<sup>1</sup>College of Agricultural & Biological Sciences, Dali University, Dali 671003, Yunnan, China

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

<sup>3</sup>Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>4</sup>Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang 550006, Guizhou, China

<sup>5</sup>128/1-J, Azad Housing Society, Curca, Goa Velha-403108, India

<sup>6</sup>College of Basic Medicine, Dali University, Dali 671003, Yunnan, China

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### Abstract

The diversity of lignicolous freshwater fungi of China is currently being studied. In this paper, fresh collections of *Kirschsteiniothelia* species from submerged wood in northwestern Yunnan Province, China, were reported. The phylogenetic analyses of combined ITS, LSU and SSU sequence data placed the isolates of the taxa within the family Kirschsteiniotheliaceae. Three new species, *Kirschsteiniothelia aquatica*, *K. cangshanensis* and *K. fluminicola* are introduced, based on their distinct morphology and evidences from molecular phylogeny. A detailed description of *Kirschsteiniothelia rostrata*, a new record for China is provided.

**Key words** – 3 new species – Asexual morphs – Kirschsteiniotheliaceae – Phylogeny – Taxonomy

### Introduction

The genus *Kirschsteiniothelia* was introduced by Hawksworth (1985a) with *K. aethiops* (Berk. & M.A. Curtis) D. Hawksw. as the type. *Kirschsteiniothelia* species are characterized by superficial, globose or subglobose, dark brown to black ascomata, fissitunicate, cylindrical-clavate asci, and dark brown, septate ascospores, with or without a mucilagenous sheath (Hawksworth 1985a, Boonmee et al. 2012, Hyde et al. 2013, Mehrabi et al. 2017).

*Kirschsteiniothelia aethiops* has been linked with the asexual genus *Dendryphiopsis* (Hughes 1953, Hawksworth 1985a, Boonmee et al. 2012, Hyde et al. 2013, Wijayawardene et al. 2017a, b). The genus *Dendryphiopsis* is characterized by macronematous, branched or unbranched, erect, septate, brown to dark brown conidiophores; terminal, determinate, monophialidic, conidiogenous cells; and cylindric-obclavate, septate, pale brown to dark brown conidia (Hyde et al. 2013, Su et al. 2016). Based on morphology and molecular data, Schoch et al. (2009) established the sexual and asexual connection of *Kirschsteiniothelia* (Schoch et al. 2009, Boonmee et al. 2012, Hyde et al. 2013, Wijayawardene et al. 2014). Furthermore, Boonmee et al. (2012) showed that *K. aethiops*

grouped with *D. atra* (Corda) S. Hughes, the type species of *Dendryphiopsis* based on phylogenetic analysis. Wijayawardene et al. (2014) therefore, proposed the synonymy of *Dendryphiopsis atra* under *Kirschsteiniothelia atra* (Corda) D. Hawksw.

*Kirschsteiniothelia* was previously placed in the family Pleosporaceae (Hawksworth 1985a, Barr 1987). Barr (1993) thought that *Kirschsteiniothelia* should be placed in Pleomassariaceae, based on the asexual morph connection and morphology. Subsequently, Schoch et al. (2006) showed that *K. aethiops* does not cluster with Pleosporaceae in their phylogenetic tree, thus they considered that the genus *Kirschsteiniothelia* should be transferred to a new family. Boonmee et al. (2012) established a new family Kirschsteiniotheliaceae to accommodate *Kirschsteiniothelia* in the class Dothideomycetes, based on morphological characters and phylogenetic analysis. In addition, *K. maritima* (Linder) D. Hawksw. was found to group in the Mytiliniaceae clade, as a sister group to *Mytilinidion* species. However, *Kirschsteiniothelia maritima* differed from *Mytilinidion* in morphology and in its marine habitat (Hawksworth 1985b, Kohlmeyer & Kohlmeyer 1979, Figueira & Barata 2007, Suetrong et al. 2009). Therefore, Boonmee et al. (2012) introduced a new genus, *Halokirschsteiniothelia*, to accommodate *K. maritima*. *Kirschsteiniothelia elaterascus* clustered in the same clade as *Morosphaeria*, and this species was transferred to *Morosphaeria* based on the phylogenetic analysis of Boonmee et al. (2012).

We are carrying out a survey on the diversity of lignicolous freshwater fungi along a north-south gradient in the Asian region (Hyde et al. 2016) and this is the third in a series of papers on these fungi from China (Li et al. 2017, Luo et al. 2018). In this study, we collected four sporidesmium-like hyphomycete taxa from submerged wood in streams and rivers in the west to northwest of Yunnan Province, China. In placing hyphomycetes in a natural classification, it is essential to obtain sequence data, as many morphologically similar taxa are shown to be unrelated (Shenoy et al. 2007, Su et al. 2016). We therefore used phylogenetic analyses to establish our new taxa with other species of *Kirschsteiniothelia*. In this paper, three new species, namely *Kirschsteiniothelia aquatica*, *K. cangshanensis* and *K. fluminicola* are introduced based on morphological characters and phylogenetic analyses. Newly generated molecular data, descriptions and illustrations of *Kirschsteiniothelia rostrata* are also provided.

## Materials & methods

### Isolation and morphology

Specimens of submerged wood were collected from Cangshan Mountain, Gaoligongshan Mountain, Jingsha River and Du long River, in the west of northwest of Yunnan, China. The process of morphological studies is following Luo et al. (2018). The morphological observations were made using a Motic SMZ 168 Series stereomicroscope and photographed by a Nikon E80i microscope-camera system. Measurements were made with the Tarosoft (R) Image Frame Work (Liu et al. 2010).

Single spore isolations were made to obtain pure cultures following the description by Chomnunti et al. (2014). Germinating spores were transferred to fresh PDA or MEA plates and incubated at room temperature 2–4 weeks. The cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC), Specimens (dry wood material with fungal material) are deposited in the herbarium of Mae Fah Luang University (MFLU).

### DNA extraction, PCR amplification, and sequencing

Fungal mycelium (500 mg) was scraped from surface of colonies grown on PDA plate or MEA plate, transferred into a 1.5 mL centrifuge tube and ground using liquid nitrogen. The EZ gene™ fungal gDNA kit (GD2416) was used to extract DNA according to the manufacturer's instructions. The primers ITS5/ITS4, LROR/LR5, NS1/NS4 were used for ITS, LSU and SSU gene regions respectively. The PCR mixture was including 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 MgCl<sub>2</sub>,

stabilizer and enhancer), 1 µL of each primer (10 µM), 1 µL genomic DNA extract and 9.5 µL deionized water. PCR thermal cycles for the amplification of the gene regions were as described in Su et al. (2015). PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer protocols (Amersham product code: 27–9602–01). The sequencing reactions were carried out by Shanghai Sangon Biological Engineering Technology and Services Co., Shanghai, P.R. China.

### Phylogenetic analysis

Raw sequences were assembled with Sequencher 4.9 for Windows (Gene Codes Corp., Ann Arbor, MI, USA). The consensus sequences were initially aligned using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/>) (Katoh & Standley 2013) and optimised manually when needed.

The phylogeny website tool “ALTER” (Glez-Peña et al. 2010) was used to transfer the alignment fasta file to Phylip format for RAxML analysis. Maximum likelihood (ML) analysis was performed at the CIPRES Science Gateway v.3.3 (<http://www.phylo.org/portal2/>, Miller et al. 2010) using RAxML v. 8.2.8 as part of the “RAxML-HPC BlackBox” tool (Stamatakis 2006, Stamatakis et al. 2008). All free model parameters were estimated by RAxML with ML estimates of 25 per site rate categories. The final ML search was conducted using the GTRGAMMA + I model. The best scoring tree was selected with a final likelihood value of –16566.464053. RAxML bootstrap support values greater than 75 % are given above at the branches (Fig. 1).

Bayesian analyses were performed by using MrBayes v3.0b4 (Ronquist & Huelsenbeck 2003). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.0b4 (Liu et al. 2012). Six simultaneous Markov Chains were run for one million generations, and trees were sampled every 100th generation (Resulting 10,000 total trees) (Cai et al. 2005). 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. 2012).

**Table 1** Isolates and sequences used in this study and newly generated sequences are indicated in bold

Taxon	Strain number	GenBank accession number		
		ITS	LSU	SSU
<i>Acrospermum adeanum</i>	M133	EU940180	EU940104	EU940031
<i>Acrospermum compressum</i>	M151	EU940161	EU940084	EU940012
<i>Acrospermum gramineum</i>	M152	EU940162	EU940085	EU940013
<i>Aliquandostipite crystallinus</i>	R 76–1	–	EF175651	EF175630
<i>Aliquandostipite khaoyaiensis</i>	CBS 118232	–	GU301796	–
<i>Dendryphiopsis atra</i>	AFTOL–ID 273	–	DQ678046	DQ677996
<i>Dyfrolomyces rhizophorae</i>	JK5456A	–	GU479799	GU479766
<i>Dyfrolomyces tiomanensis</i>	NTOU3636	–	KC692156	KC692155
<i>Flavobathelium epiphyllum</i>	MPN67	–	GU327717	JN887382
<i>Helicomycetes roseus</i>	CBS 283.51	AY916464	AY856881	AY856928
<i>Helicomycetes roseus</i>	MFLUCC 15–0343	KY320523	KY320540	–
<i>Jahnula aquatica</i>	R68–1	JN942354	EF175655	EF175632
<i>Jahnula bipileata</i>	F49–1	JN942353	EF175657	EF175635
<i>Jahnula sangamonensis</i>	A402–1B	JN942349	EF175661	EF175639
<i>Jahnula seychellensis</i>	SS 2113.2	–	EF175664	EF175643
<i>Kirschsteiniothelia aethiops</i>	CBS 109.53	–	AY016361	AY016344

**Table 1** Continued.

Taxon	Strain number	GenBank accession number		
		ITS	LSU	SSU
<i>K. aethiops</i>	<b>MFLUCC 16–1104</b>	<b>MH182583</b>	<b>MH182589</b>	<b>MH182615</b>
<i>K. aethiops</i>	<b>S–783</b>	<b>MH182586</b>	<b>MH182595</b>	<b>MH182617</b>
<i>K. aethiops</i>	MFLUCC 15–0424	KU500571	KU500578	KU500585
<i>K. aquatica</i>	<b>MFLUCC 17–1685</b>	<b>MH182587</b>	<b>MH182594</b>	<b>MH182618</b>
<i>K. arasbaranica</i>	IRAN 2509C	KX621986	KX621987	KS621988
<i>K. arasbaranica</i>	IRAN 2508C	KX621983	KX621984	KX621985
<i>K. cangshanensis</i>	<b>MFLUCC 16–1350</b>	<b>MH182584</b>	<b>MH182592</b>	–
<i>K. fluminicola</i>	<b>MFLUCC 16–1263</b>	<b>MH182582</b>	<b>MH182588</b>	–
<i>K. lignicola</i>	MFLUCC 10–0036	HQ441567	HQ441568	HQ441569
<i>K. phoenicis</i>	MFLUCC 18–0216	MG859978	MG860484	MG859979
<i>K. rostrata</i>	MFLUCC15–0619	KY697280	KY697276	KY697278
<i>K. rostrata</i>	<b>MFLUCC 16–1124</b>	–	<b>MH182590</b>	–
<i>K. submersa</i>	HA–2016	KU500570	KU500577	KU500584
<i>K. submersa</i>	<b>S-481</b>	–	<b>MH182591</b>	<b>MH182616</b>
<i>K. submersa</i>	<b>S-601</b>	<b>MH182585</b>	<b>MH182593</b>	–
<i>K. tectonae</i>	MFLUCC 12-0050	KU144916	KU764707	–
<i>K. thujina</i>	JF13210	KM982716	KM982718	KM982717
<i>Phyllobathelium anomalum</i>	MPN 242	–	GU327722	JN887386
<i>Pleospora herbarum</i>	CBS191.86	NR111243	GU238160	GU238232
<i>Pleospora herbarum</i>	MFLUCC 14–0920	KY659560	KY659563	KY659567
<i>Tubeufia helicomyces</i>	CBS 271.52	AY916461	AY856887	AY856933
<i>Tubeufia javanica</i>	MFLUCC 12–0545	KJ880034	KJ880036	KJ880035
<i>Tubeufia paludosa</i>	CBS 120503	–	GU301877	GU296203

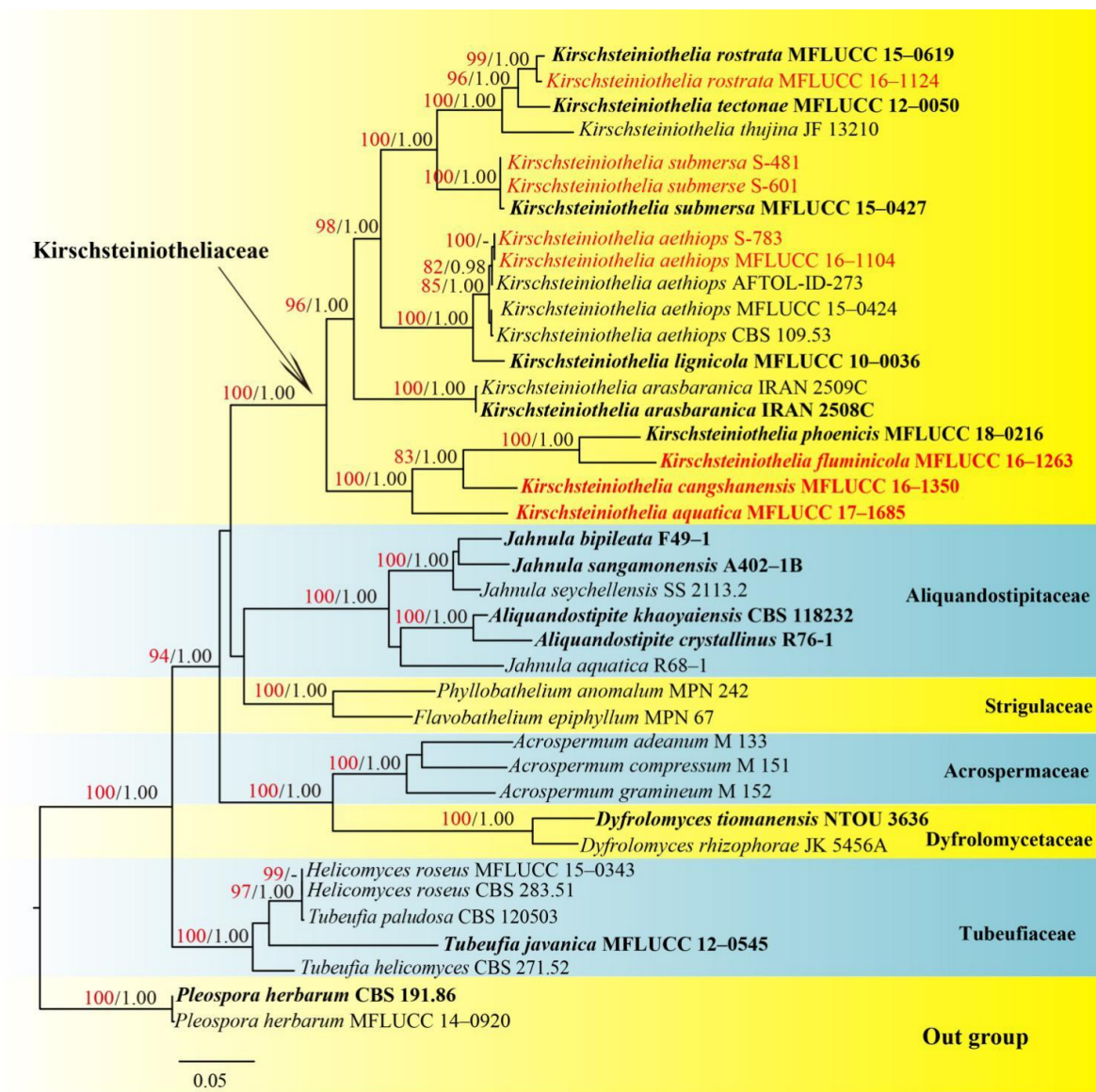
## Results

### Phylogenetic analyses

The combined sequence alignment comprised 39 taxa (Table 1), with *Pleospora herbarum* (CBS 19186, MFLUCC 14–0920) as the outgroup taxa, with 2248 characters including gaps. The result of maximum likelihood (ML) analysis based on combined ITS, LSU and SSU sequence data consisted of six families (Acrospermaceae, Aliquandostipitaceae, Dyfrolomycetaceae, Strigulaceae, Kirschsteiniotheliaceae and Tubeufiaceae) within the Dothideomycetes (Fig. 1). The phylogenetic analyses showed that all the new strains cluster in the family Kirschsteiniotheliaceae with high support (100% ML and 1.00 PP). Three newly generated isolates of *Kirschsteiniothelia* species and *Kirschsteiniothelia phoenicis* formed a monotypic lineage at the basal position of the family Kirschsteiniotheliaceae with strong support (100% ML and 1.00 PP). Four isolates in this lineage formed distinct clades which can be recognized as four different phylogenetic species and of which three were introduced as new species, namely *Kirschsteiniothelia aquatica*, *K. cangshanensis* and *K. fluminicola*.

### Taxonomy

In this section, we introduce three new species, *Kirschsteiniothelia aquatica*, *K. cangshanensis* and *K. fluminicola* with descriptions and illustrations, and also provide descriptions and illustrations for *K. rostrata* which is a new record for China.



**Figure 1** – Phylogenetic tree generated from maximum likelihood (ML) analysis based on combined ITS, LSU and SSU sequence data for some selected families within the Dothideomycetes. Bootstrap support values for maximum likelihood (ML) greater than 75% and Bayesian posterior probabilities (PP) greater than 0.95 are given above the nodes. Newly generated sequences are indicated in red and ex-type strains are in bold.

*Kirschsteinothelia aquatica* Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 2

Index Fungorum number: IF554783; Facesoffungi number: FoF04690

Holotype – MFLU 18–1077

Etymology – Referring to the aquatic habitat of this fungus.

*Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on natural substrate, hairy, dark brown. *Mycelium* partly superficial, partly immersed in the substrate, composed of septate, branched, smooth-walled hyphae. *Conidiophores* macronematous, mononematous, erect, straight or flexuous, unbranched, thick-walled, dark brown, septate, cylindrical, 114–151  $\mu\text{m}$  ( $\bar{x}$ =132.5  $\mu\text{m}$ , SD=18.5, n=10) long, 7–8  $\mu\text{m}$  ( $\bar{x}$ =7.5  $\mu\text{m}$ , SD=0.5, n=10) wide. *Conidiogenous cells* monoblastic, holoblastic, integrated, determinate or percurrently proliferating, terminal, dark brown, cylindrical. *Conidia* acrogenous, solitary, dry, straight or slightly curved, subhyaline and rounded at apex, truncate, thick-walled and dark brown at base, smooth, 35–46  $\mu\text{m}$  ( $\bar{x}$ =40.5  $\mu\text{m}$ , SD=5.5, n=20) long, 7.5–8.5  $\mu\text{m}$  ( $\bar{x}$ =8  $\mu\text{m}$ , SD=0.5, n=20) wide, sometimes percurrently proliferate at broken ends.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream in Cangshan Mountain, July 2015, H.W. Shen, XP H 1–12–1, S–708 (MFLU 18–1077, holotype), ex-type culture, MFLUCC 17–1685.

Notes – This fungus was collected from Cangshan Mountain, Yunnan Province, China. *Kirschsteiniothelia aquatica* resembles *K. cangshanensis* and *K. fluminicola* in having macronematous, straight or flexuous, unbranched, thick-walled, septate conidiophores, and solitary, septate, conidia. However, *K. aquatica* differs from *K. cangshanensis* in having longer conidiophores (114–151  $\mu\text{m}$  vs 105.5–135.5  $\mu\text{m}$ ), and subhyaline, truncate, thick-walled conidia, rounded at apex, and dark brown at base. *Kirschsteiniothelia aquatica* differs from *K. fluminicola* in having shorter and thinner conidiophores (114–151  $\times$  7–8  $\mu\text{m}$  vs 209–286  $\times$  7–9  $\mu\text{m}$ ). They are also phylogenetically distinct (Fig. 1).

***Kirschsteiniothelia cangshanensis*** Z.L. Luo, D.F. Bao, K.D. Hyde & H.Y. Su, sp. nov. Fig. 3

Index Fungorum number: IF554784; Facesoffungi number: FoF04691

Holotype – MFLU 17–1426

Etymology – Referring the fungus collected from Cangshan Mountain.

*Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, scattered, hairy, pale brown to brown. *Mycelium* mostly immersed, composed of pale brown to brown, septate, branched hyphae. *Conidiophores* macronematous, mononematous, erect, pale brown, septate, unbranched, cylindrical, percurrent, straight or flexuous, 105.5–135.5  $\mu\text{m}$  ( $\bar{x}$ =120.5  $\mu\text{m}$ , SD=15, n=20) long, 6–8  $\mu\text{m}$  ( $\bar{x}$ =7  $\mu\text{m}$ , SD=1, n=20) wide. *Conidiogenous* cells monoblastic, integrated, terminal, determinate, pale brown, cylindrical, percurrently proliferating. *Conidia* solitary, dry, obclavate, septate, straight or slightly curved, pale brown to brown, with a gelatinous sheath at apex, 33–43  $\mu\text{m}$  ( $\bar{x}$ =38  $\mu\text{m}$ , SD=5, n=20) long, 7.5–8.5  $\mu\text{m}$  ( $\bar{x}$ =8  $\mu\text{m}$ , SD=0.5, n=20) wide.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Jinsha River, April 2015, Z.L. Luo, S–561, (MFLU 17–1426, holotype), ex-type culture, MFLUCC 16–1350.

Notes – *Kirschsteiniothelia cangshanensis* shares similar characters with *K. fluminicola* in having macronematous, unbranched, cylindrical, septate, conidiophores and solitary, obclavate, septate, conidia. However, *K. cangshanensis* differs from *K. fluminicola* in having a gelatinous rounded sheath at the apex of shorter and thinner conidia (33–43  $\times$  7.5–8.5  $\mu\text{m}$  vs 47.5–86.5  $\times$  8–10  $\mu\text{m}$ ).

***Kirschsteiniothelia fluminicola*** Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 4

Index Fungorum number: IF555283; Facesoffungi number: FoF04692

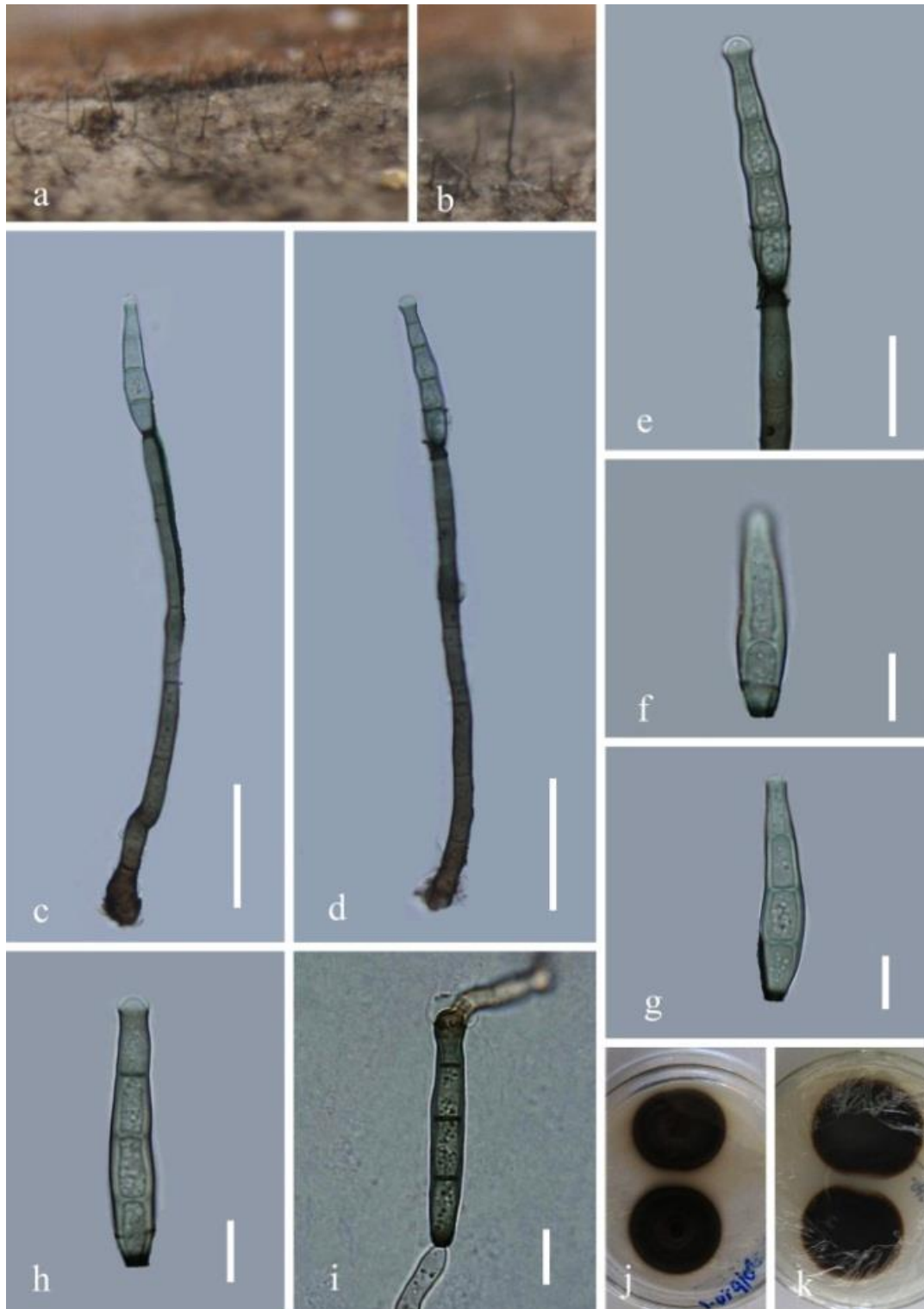
Holotype – MFLU 17–1427

Etymology – Referring to the fungus living in a stream.

*Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on natural substrate, hairy, dark brown. *Mycelium* immersed, dark brown to black, composed of unbranched, septate hyphae. *Conidiophores* erect, straight or flexuous, septate, smooth, cylindrical, dark brown to black, unbranched, percurrent, 209–286  $\mu\text{m}$  ( $\bar{x}$ =247.5  $\mu\text{m}$ , SD=38.5, n=20) long, 7–9  $\mu\text{m}$  ( $\bar{x}$ =8  $\mu\text{m}$ , SD=1, n=20) wide. *Conidiogenous cells* monoblastic, terminal, indeterminate, percurrently proliferating, cylindrical, dark brown. *Conidia* solitary to short-catenate, obclavate, rostrate, truncate at base, slender and rounded at apex, aseptate when immature, multi-septate at maturity, subhyaline to dark brown, with conspicuous, spherical guttules in almost all cells, 47.5–86.5  $\mu\text{m}$  ( $\bar{x}$ =67  $\mu\text{m}$ , SD=19.5, n=20) long, 8–10  $\mu\text{m}$  ( $\bar{x}$ =9  $\mu\text{m}$ , SD=1, n=20) wide.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Dulong River, May 2015, Z.L. Luo, S–315, (MFLU 17–1427, holotype), ex-type culture, MFLUCC 16–1263.

Notes – *Kirschsteiniothelia fluminicola* is introduced here based on distinct morphology and molecular data. *Kirschsteiniothelia fluminicola* is grouped in the family Kirschsteiniotheliaceae and it is sister to *K. phoenicis* (MFLUCC18–0216) as in Fig 1. *K. fluminicola* can be distinguished from other species in having short-catenate, slender conidia rounded at apex and multi-septate at maturity.



**Figure 2** – *Kirschsteiniothelia aquatica* (MFLU 18–1077, holotype) a, b Colonies on wood. c, d Conidiophore with conidia. e Conidiogenous cells and conidia. f–h conidia. i Germinating conidium. j, k Culture on PDA from surface and reverse. Scale bars: c, d = 40  $\mu\text{m}$ , e = 30  $\mu\text{m}$ , f–i = 10  $\mu\text{m}$ .



**Figure 3** – *Kirschsteiniothelia cangshanensis* (MFLU 17–1426, holotype) a Colonies on wood. b, c Conidiophore with conidia. d Conidiophore. e–f Conidiogenous cells and conidia. g–j Conidia. k Germinating conidium. l, m Culture on MEA from surface and reverse. Scale bars: b, c = 40  $\mu\text{m}$ , d, e = 30  $\mu\text{m}$ , f = 15  $\mu\text{m}$ , g–k = 10  $\mu\text{m}$ .





**Figure 4** – *Kirschsteiniothelia fluminicola* (MFLU 17–1427, holotype) a Colonies on wood. b–d Conidiophore with conidia. e, f Conidiogenous cells with conidia. g–j Conidia. k Germinating conidium. l, m Culture on PDA from surface and reverse. Scale bars: b = 40  $\mu$ m, c, d = 50  $\mu$ m, e = 30  $\mu$ m, f–k = 20  $\mu$ m.

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

Fig. 5

Index Fungorum number: IF552909

*Saprobic* on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: Colonies effuse on natural substrate, scattered, hairy, dark brown to black. Mycelium partly immersed, partly superficial on the substrate, composed of pale brown, septate, branched hyphae.

*Conidiophores* macronematous, mononematous, solitary, wide and slightly swollen at base, tapering towards apex, straight or slightly flexuous, smooth, brown to dark brown, unbranched, septate, 90–120  $\mu\text{m}$  ( $\bar{x}$ =105  $\mu\text{m}$ , SD=15, n=10) long, 7.5–8.5  $\mu\text{m}$  ( $\bar{x}$ =8  $\mu\text{m}$ , SD=0.5, n=10) wide. *Conidiogenous cells* holoblastic, monoblastic, integrated, terminal, determinate, cylindrical or lageniform, smooth, mid to dark brown. *Conidia* acrogenous, solitary, dry, olivaceous brown to brown, pale at apex, obclavate, rostrate, smooth, straight or curved, truncate at base, 6–17–euseptate, 77.5–108.5  $\mu\text{m}$  ( $\bar{x}$ =93  $\mu\text{m}$ , SD=15.5, n=20) long, 17.5–20.5  $\mu\text{m}$  ( $\bar{x}$ =19  $\mu\text{m}$ , SD=1.5, n=20) wide.

Material examined – CHINA, Yunnan Province, Baoshan City, saprobic on decaying wood submerged in a stream in Gaoligong Mountain, July 2016, S.M. Tang, GS H 37–1, S–441, living culture, MFLUCC 16–1124.

Notes – *Kirschsteiniothelia rostratas* introduced by Hyde et al. (2017), was found on decaying wood submerged in a freshwater stream in Thailand. Morphologically, our fresh collection fits well with *K. rostrata*, and the phylogenetic analysis showed that our isolate (MFLUCC 16–1124) clustered together with the ex-type culture (MFLUCC 15–0619) of *K. rostrata* (Fig. 1). Therefore we identified the fresh collection as *K. rostrata* and it is a new record for China.

## Discussion

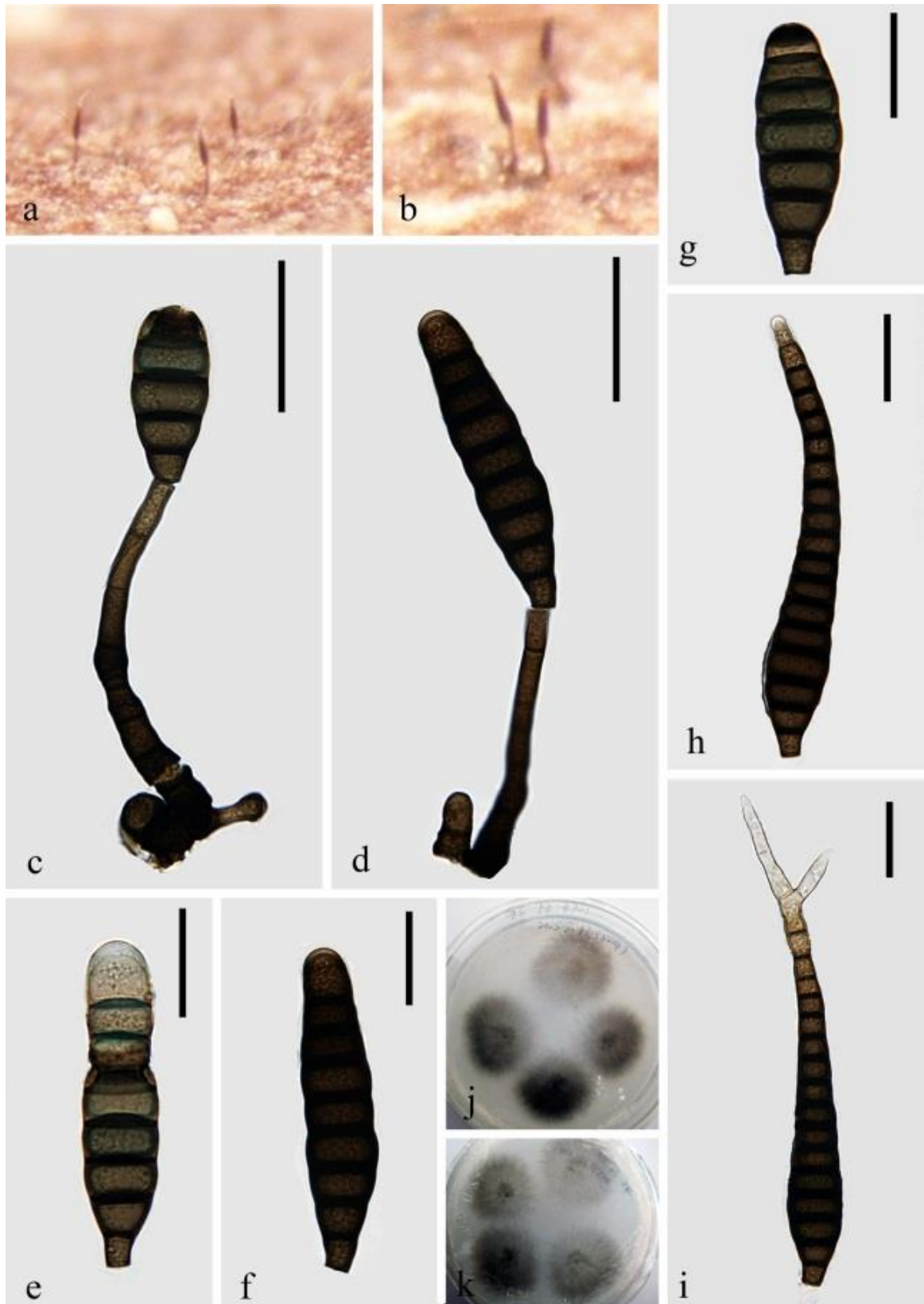
The genus *Kirschsteiniothelia* was introduced by Hawksworth (1985a) with *K. aethiops* (Berk. & M.A. Curtis) D. Hawksw as the type species. Previous studies on *Kirschsteiniothelia* mostly reported on the sexual states (Hawksworth 1985a, Shearer 1993, Hyde et al. 1997, Chen et al. 2006, Mehrabi et al. 2017, Boonmee et al. 2012). Several asexual species of *Kirschsteiniothelia* are now known, viz. *K. lignicola*, *K. emarceis*, *K. submersa*, *K. aethiops*, *K. tectonae* and *K. rostrata* (Boonmee et al. 2012, Su et al. 2015, Li et al. 2016, Hyde et al. 2017). In this study, we provide descriptions and illustrations for four species of *Kirschsteiniothelia* and out of which three are new.

Most of the species of *Kirschsteiniothelia* are widespread in the tropics and usually found on dead wood in terrestrial habitats (Mehrabi et al. 2017). In this study, the fresh collections are from submerged wood in freshwater habitats of Yunnan Province, China. *Kirschsteiniothelia rostrata* was collected from Gaoligongshan Mountain, whereas *K. aquatica* was collected from Canshan Mountain, *K. cangshanensis* from the Jinsha River and *K. fluminicola* from the Dulong River in Yunnan, China. The results showed that *Kirschsteiniothelia* species are widely distributed in the western Yunnan Province of China.

Boonmee et al. (2012) established a new family Kirschsteiniotheliaceae to accommodate *Kirschsteiniothelia sensu stricto* in the class Dothideomycetes. However, on phylogenetic analysis, species of *Kirschsteiniothelia* are found polyphyletic and classified in Capnodiales, Jahnuiales, Mytilinidiales and Pleosporales (Boonmee et al. 2012). Therefore, the placement of several *Kirschsteiniothelia* species remains uncertain based on morphology, until they are studied phylogenetically (Hawksworth 1985 a, b, Barr 1987, Lumbsch & Lindemuth 2001, Wang et al. 2004, Vijaykrishna et al. 2006, Nelsen et al. 2009, Ruibal et al. 2009, Shearer et al. 2009). Hernandez-Restrepo et al. (2017) found that Kirschsteiniotheliaceae is distantly related to other lineages representing different orders in Dothideomycetes in their phylogenetic analysis. Therefore a new order Kirschsteiniotheliales was introduced to accommodate the family Kirschsteiniotheliaceae, with *Kirschsteiniothelia* as the type genus and this is followed in Wijayawardene et al. (2018).

Supplementary note – In the paper “Li WL, Luo ZL, Liu JK, Bhat DJ, Bao DF, Su HY, Hyde KD (2017) Lignicolous freshwater fungi from China I : *Aquadictyospora lignicola* gen. et sp. nov. and new record of *Pseudodictyosporium wauense* from northwestern Yunnan Province. Mycosphere 8(10), 1587–1597, Doi 10.5943/mycosphere/8/10/1”, the “Material examined” and “Notes” for new species *Aquadictyospora lignicola* Z.L. Luo, W.L. Li, K.D. Hyde & H.Y. Su are missed due to typographical errors. We therefore would add the “Material examined” and “Notes” for species *Aquadictyospora lignicola* here as follows:

Material examined – CHINA, Yunnan Province, Dali, saprobic on decaying wood submerged in a stream in Cangshan Mountain, July 2016, H.Y. Su, 4XP H 2–9–3, (MFLU 17–1422, holotype), ex-type living culture MFLUCC 17–1318.



**Figure 5** – *Kirschsteiniothelia rostrata* (GS H 37–1) a, b Colonies on wood. c, d Conidiophore with conidia. e–h Conidia. i Germinating conidium. j, k Culture on PDA from surface and reverse. Scale bars: c = 30  $\mu$ m, d = 40  $\mu$ m, e–i = 25  $\mu$ m.

Notes – *Aquadictyospora* is morphologically similar to *Dictyocheirospora* in having solitary, terminal, cheiroid and pale brown conidia. However, *Aquadictyospora* differs from the latter by its hyaline basal conidial cells. *Aquadictyospora* is comparable to the monotypic genus *Bahugada* K.A. Reddy & V. Rao, typified by *B. sundara* K.A. Reddy & V. Rao (Reddy & Rao 1984), with its large hyaline basal cells in the conidia. *Bahugada* is distinguishable by its sparingly branched conidiophores and sympodial conidiogenous cells with broad denticles. The basal hyaline cell in *Aquadictyospora* is deeply constricted from above centre. The somewhat similar genera, *Dictyosporium*, *Digitodesmium* and *Jalapriya*, differ from *Aquadictyospora* in having conidia with appendages. The molecular phylogenetic study indicates its placement in *Dictyosporiaceae* as a genus which is phylogenetically related to the genera *Aquatichirospora*, *Dictyocheirospora*, *Digitodesmium*, *Jalapriya* and *Vikalpa*.

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