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Kirschsteiniothelia xishuangbannaensis sp. nov. from pará rubber (*Hevea brasiliensis*) in Yunnan, China

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Abstract

During surveys of saprobic fungi associated with pará rubber in Yunnan Province, China, a saprobic fungus morphologically resembles *Kirschsteiniothelia* was collected from a dead branch of pará rubber. *Kirschsteiniothelia xishuangbannaensis* is characterized by conidia having acrogenous, obclavate, rostrate, straight or curved, truncate at base, 3–8-septate, 1–2 hyaline globose/ampulliform mucilaginous sheaths around the apex. Multi-gene phylogenetic analyses of ITS, LSU and SSU showed our collection is closely affiliated to *K. thailandica* with 100% ML/1.00 BYPP statistical support. Both morphological characteristics and multi-gene phylogeny confirmed that our collection is a distinct species of *Kirschsteiniothelia*. Descriptions, micromorphological illustrations, and a phylogenetic tree showing the placement of the new taxon are provided.

Keywords – 1 new species – Hyphomycetes – *Kirschsteiniotheliaceae* – Multi-gene – Taxonomy

Introduction

Kirschsteiniotheliaceae was introduced in *Pleosporales* to accommodate a single genus *Kirschsteiniothelia* based on morphological and phylogenetic analyses (Boonmee et al. 2012). Hernandez-Restrepo et al. (2017) found that *Kirschsteiniotheliaceae* is phylogenetically distinct from other orders in *Dothideomycetes*. Thus, a new order *Kirschsteiniotheliales* was introduced to accommodate *Kirschsteiniotheliaceae*, with the type genus *Kirschsteiniothelia* (Hernandez-Restrepo et al. 2017, Hongsanan et al. 2020). The members of *Kirschsteiniotheliaceae* are commonly saprobic on dead or decaying wood (Bao et al. 2018, Rodríguez-Andrade et al. 2019, Sun et al. 2021, Verma et al. 2021).

Kirschsteiniothelia was introduced by Hawksworth (1985) and most species in this genus have been found in tropical or subtropical regions in terrestrial and freshwater habitats (Bao et al. 2018, Sun et al. 2021). The sexual morph of *Kirschsleiniothelia* has brown or black, superficial or semi-immersed, subglobose ascomata, cylindrical-clavate, bitunicate, 8-spored asci, brown to dark brown ascospores with smooth-walled and 1–2-septate (Hawksworth 1985, Boonmee et al. 2012, Hyde et al. 2013, Mehrabi et al. 2017). There are two types of asexual morphs in *Kirschsteiniothelia* as dendryphiopsis-like and sporidesmium-like (Sun et al. 2021).

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Dendryphiopsis-like asexual morph was reported linking with *Kirschsteiniothelia* (*K. lignicola, K. emarceis*), which was confirmed by Hawksworth (1985) and Boonmee et al. (2012). Su et al. (2016) reported a sporidesmium-like asexual morph (*K. submerse*) in *Kirschsteiniothelia*. Evidence that each of these is included in the LSU-SSU-ITS matrix (Boonmee et al. 2012, Su et al. 2016).

The pará rubber tree (*Hevea brasiliensis*) belongs to *Euphorbiaceae* and is native to the Amazon region. Rubber has great economic importance due to the latex, which is the source of natural rubber (Alvarenga & Carmo 2008). Knowledge of the fungi associated with rubber is important for pathologists, mycologists, and farmers. Senwanna et al. (2021) provided a checklist of fungi and fungus-like organisms associated with rubber. The taxa in the checklist belong to 67 orders, 168 families, and 513 genera from 59 countries. Very few taxa associated with rubber have been described based on both morphology and phylogeny, while most of the previous studies on fungi associated with rubber were based only on morphology (Senwanna et al. 2017, 2018, 2019, 2020, 2021, Xu et al. 2022a, b).

In this study, we introduce a new species isolated from a dead branch of *Hevea brasiliensis* in Xishuangbanna, Yunnan Province, China. The new species is confirmed based on morphological characteristics and phylogenetic analyses of combined LSU, ITS and SSU sequence data. In addition, we provide morphological characteristics, host, and location information of all known *Kirschsleiniothelia* species.

Material & methods

Sample collection and isolation

Specimens were collected from Xishuangbanna, Jinghong, Manlie, 28–30 °C, 330 m, 122°2'28"N, 100°44'23"E, Yunnan Province, China (September 2021), stored in sealable plastic bags and brought to the mycology laboratory. Morphological observations and single spore isolations were carried out following Senanayake et al. (2020). Morphological characteristics were observed using a stereomicroscope Leica S8AP0 and photographed with a Leica DM2500 camera connected to a Leica DMC4500 compound microscope. Measurements were made using Tarosoft (R) Image Frame Work software. Adobe Photoshop CS5 Extended Version 10.0 software was used to make photo-plates. Herbarium specimen of the new species was deposited at the Zhongkai University of Agriculture and Engineering (ZHKU). The living cultures were deposited at the culture collection of Zhongkai University of Agriculture and Engineering (ZHKUCC). Faces of fungi (FoF) numbers and Index Fungorum (IF) numbers were obtained as explained in Jayasiri et al. (2015) and Index Fungorum (2022).

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted directly from fresh mycelia that were scraped from a one-month-old culture using an E.Z.N.A. Forensic DNA Kit (BIO-TEK) in accordance with the manufacturer's protocol. Primer pairs ITS5 / ITS4 (White et al. 1990), LR0R / LR5 and NS1 / NS4 (Vilgalys & Hester 1990) were used to perform PCR amplifications for the large subunit ribosomal rRNA (LSU), the internal transcribed spacer (ITS) and the small subunit ribosomal rRNA (SSU), respectively. Polymerase chain reaction (PCR) amplifications were conducted using 25 µl PCR mixture containing 8.5 µl ddH$_2$O, 12.5 µl 2 × Master Mix (Bioteke Corporation, Beijing, China), 2 µl DNA template and 1 µl each reverse and forward primer (Tibpromma et al. 2018, Xu et al. 2022b). Purification and sequencing of PCR products were carried out in Kunming Tsingke, P.R. China.

Sequence alignment and phylogenetic analyses

Sequences generated in this study were subjected to BLAST searches in GenBank to check the closely related taxa to the new taxon. Sequences were also obtained from the recently published papers (Bao et al. 2018, Hongsanan et al. 2020) (Table 1). Multiple alignments were automatically made with MAFFT v. 7 available at the web server (http://mafft.cbrc.jp/alignment/server), using default settings (Katoh et al. 2019). The aligned sequences were trimmed using TrimAl V 1.2 with "gappyout" option (Capella-Gutiérrez et al. 2009). Data were converted from FASTA to PHYLIP and NEXUS formats using ALTER (Alignment Transformation Environment online, https://sing.ei.uvigo.es/ALTER) (Glez-Peña et al. 2010). Combined multi-gene phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian Inference (BYPP) analyses (Ronquist & Huelsenbeck 2003, Stamatakis 2006, Dissanayake et al. 2020). The ML analysis was done on the online platform CIPRES (https://www.phylo.org) using RAxML-HPC on XSEDE under the GTRGAMMA substitution model with 1,000 bootstrap replicates. Bayesian analysis was performed on the same platform CIPRES (https://www.phylo.org) using MrBayes. The GTR+I+G was used a substitution model of the combined ITS, LSU and SSU based on MrModeltest 2.3 (Nylander 2004). Six simultaneous Markov chains were run for 1,000,000 generations sampling every 1000th generation, halting the analyses when the average standard deviation of split frequencies was less than 0.01. Trees were visualized in FigTree v1.4.0, and the constructed tree was edited using Microsoft PowerPoint and saved as a PDF format.

Table 1 Names, strain/culture collection numbers and corresponding GenBank accession numbers of the taxa used in the phylogenetic analyses in this study. The newly generated sequences are shown in black bold while T indicates holotype or ex-type strains. Absence of GenBank accession numbers shown by "–".

Taxa	Strains	ITS	LSU	SSU
Acrospermum adeanum	M133	EU940180	EU940104	EU940031
Acrospermum compressum	M151	EU940161	EU940084	EU940012
Acrospermum gramineum	M152	EU940162	EU940085	EU940013
Aliquandostipite crystallinus	R 76–1	–	EF175651	EF175630
Aliquandostipite khaoyaiensis T	CBS 118232	–	GU301796	–
Dyfrolomyces rhizophorae	JK5456A	–	GU479799	GU479766
Dyfrolomyces tiomanensis	NTOU3636	–	KC692156	KC692155
Flavobathelium epiphyllum	MPN67	–	GU327717	JN887382
Halokirschsteiniothelia maritima	CBS 221.60	–	AY849943	AF053726
Helicomyces roseus	CBS 283.51	AY916464	AY856881	AY856928
Helicomyces roseus	MFLUCC 15–0343	KY320523	KY320540	–
Homortomyces combreti T	CPC 19808	JX517281	JX517291	–
Homortomyces tamaricis	MFLUCC 13–0280	KU752184	KU561874	KU870905
Homortomyces tamaricis T	MFLUCC 13–0441	NR_155161	NG_059495	–
Homortomyces tamaricis	MFLUCC 14–0167	KU934190	KU561875	–
Jahnula bipileata T	F49–1	JN942353	EF175657	EF175635
Jahnula sangamonensis	A402–1B	JN942349	EF175661	EF175639
Jahnula seychellensis	SS 2113.2	–	EF175664	EF175643
Kirschsteiniothelia aethiops	CBS 109.53	–	AY016361	AY016344
Kirschsteiniothelia aethiops	MFLUCC 16–1104	MH182583	MH182589	MH182615
Kirschsteiniothelia aethiops	S–783	MH182586	MH182595	MH182617
Kirschsteiniothelia aethiops	MFLUCC 15–0424	KU500571	KU500578	KU500585
Kirschsteiniothelia aquatica T	MFLUCC 16–1685	MH182587	MH182594	MH182618
Kirschsteiniothelia arasbaranica	IRAN 2509C	KX621986	KX621987	KX621988
Kirschsteiniothelia arasbaranica T	IRAN 2508C	KX621983	KX621984	KX621985
Kirschsteiniothelia atra T	DEN	MG602687	–	–
Kirschsteiniothelia cangshanensis T	MFLUCC 16–1350	MH182584	MH182592	–
Kirschsteiniothelia cangshanensis	GZCC19–0515	–	MW133829	MW134609
Kirschsteiniothelia ebriosa	CBS H–23379	–	LT985885	–
Kirschsteiniothelia emarceis T	MFLU 10–0037	NR_138375	NG_059454	–
Kirschsteiniothelia fluminicola T	MFLUCC 16–1263	MH182582	MH182588	–
Kirschsteiniothelia lignicola T	MFLUCC 10–0036	HQ441567	HQ441568	HQ441569
Kirschsteiniothelia phoenicis T	MFLUCC 18–0216	MG859978	MG860484	MG859979
Kirschsteiniothelia phoenicis	MFLU 18–0153	NR_158532	NG_064508	–
Kirschsteiniothelia rostrata	MFLUCC15–0619	KY697280	KY697276	NG_063633

Table 1 Continued.

Taxa	Strains	ITS	LSU	SSU
Kirschsteiniothelia rostrata	MFLUCC 16–1124	–	MH182590	–
Kirschsteiniothelia rostrata	MFLU 15–1154	NR_156318	NG_059790	KY697278
Kirschsteiniothelia submersa T	MFLUCC 15–0427	KU500570	KU500577	KU500584
Kirschsteiniothelia submersa	S–481	–	MH182591	MH182616
Kirschsteiniothelia submersa	S–601	MH182585	MH182593	–
Kirschsteiniothelia tectonae	MFLUCC 12–0050	KU144916	KU764707	–
Kirschsteiniothelia tectonae	MFLUCC 13–0470	KU144924	–	–
Kirschsteiniothelia thailandica T	MFLUCC 20–0116	MT985633	MT984443	MT984280
Kirschsteiniothelia thujina	JF13210	KM982716	KM982718	KM982717
Kirschsteiniothelia vinigena T	CBS H–23378	–	NG_075229	–
Kirschsteiniothelia xishuangbannaensis T	**ZHKUCC 22–0220**	**OP289566**	**OP289564**	**OP303181**
Kirschsteiniothelia xishuangbannaensis	**ZHKUCC 22–0221**	**OP289563**	**OP289565**	**OP303182**
Phyllobathelium anomalum	MPN 242	–	GU327722	JN887386
Stemphylium vesicarium	CBS 191.86	MH861935	GU238160	GU238232
Stemphylium vesicarium	MFLUCC 14–0920	KY659560	KY659563	KY659567
Tubeufia helicomyces	CBS 271.52	AY916461	AY856887	AY856933
Tubeufia javanica T	MFLUCC 12–0545	KJ880034	KJ880036	KJ880035

Result

Phylogenetic analyses

The combined sequence alignment comprised 52 taxa, with *Stemphylium vesicarium* (CBS 191.86, MFLUCC 14–0920) as the outgroup taxa. The matrix had 2442 characters including gaps, and after trimming it had 1559 distinct alignment patterns with 28.50% of undetermined characters or gaps (ITS:554; LSU:877; SSU:1011). The RAxML analysis of the combined dataset yielded a best scoring tree (Fig. 1) with a final ML optimization likelihood value of -23356.466779. The final RAxML tree is shown in Fig. 1. Phylogenetic analyses results showed that the new strains ZHKUCC 22–0220, and ZHKUCC 22–0221 forms a sister clade with *Kirschsteiniothelia thailandica* with high statistical support (100% ML/ 1.00 BYPP, Fig. 1).

Taxonomy

Kirschsteiniothelia xishuangbannaensis R.F. Xu & Tibpromma, sp. nov. Fig. 2

Index Fungorum Number: IF 559433; Facesoffungi Number: FoF12758

Etymology – The specific epithet “xishuangbannaensis” refers to Xishuangbanna, in Yunnan Province where the holotype was collected.

Saprobic on a dead branch of *Hevea brasiliensis*. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse on natural substrate, solitary or scattered, hairy, brown to dark brown. *Conidiophores* 35–150 × 5–15 µm ($\bar{x}$ = 104 × 10 µm, n = 10), macronematous, septate, curved, solitary, brown to dark brown, wide and slightly swollen at base, tapering towards apex. *Conidiogenous cells* 10–50 × 5–10 µm ($\bar{x}$ = 29 × 9 µm, n = 10), monoblastic, holoblastic, integrated, terminal, determinate, cylindrical or lageniform, smooth, brown to dark brown. *Conidia* 30–150 × 5–20 µm ($\bar{x}$ = 93 × 16 µm, n = 20), acrogenous, solitary, obclavate, rostrate, straight or curved, truncate at base, some have guttules, 3–8-septate, yellow-brown to brown, subhyaline or pale-brown at apex, 1–2 hyaline globose/ampulliform mucilaginous sheaths around the tip (mostly 1 mucilaginous sheath).

Culture characteristics – cultures on PDA, circular, flat, velvety, smooth, entire, reddish brown from above, dark brown in the middle and the edges are reddish brown, dark-brown from below.

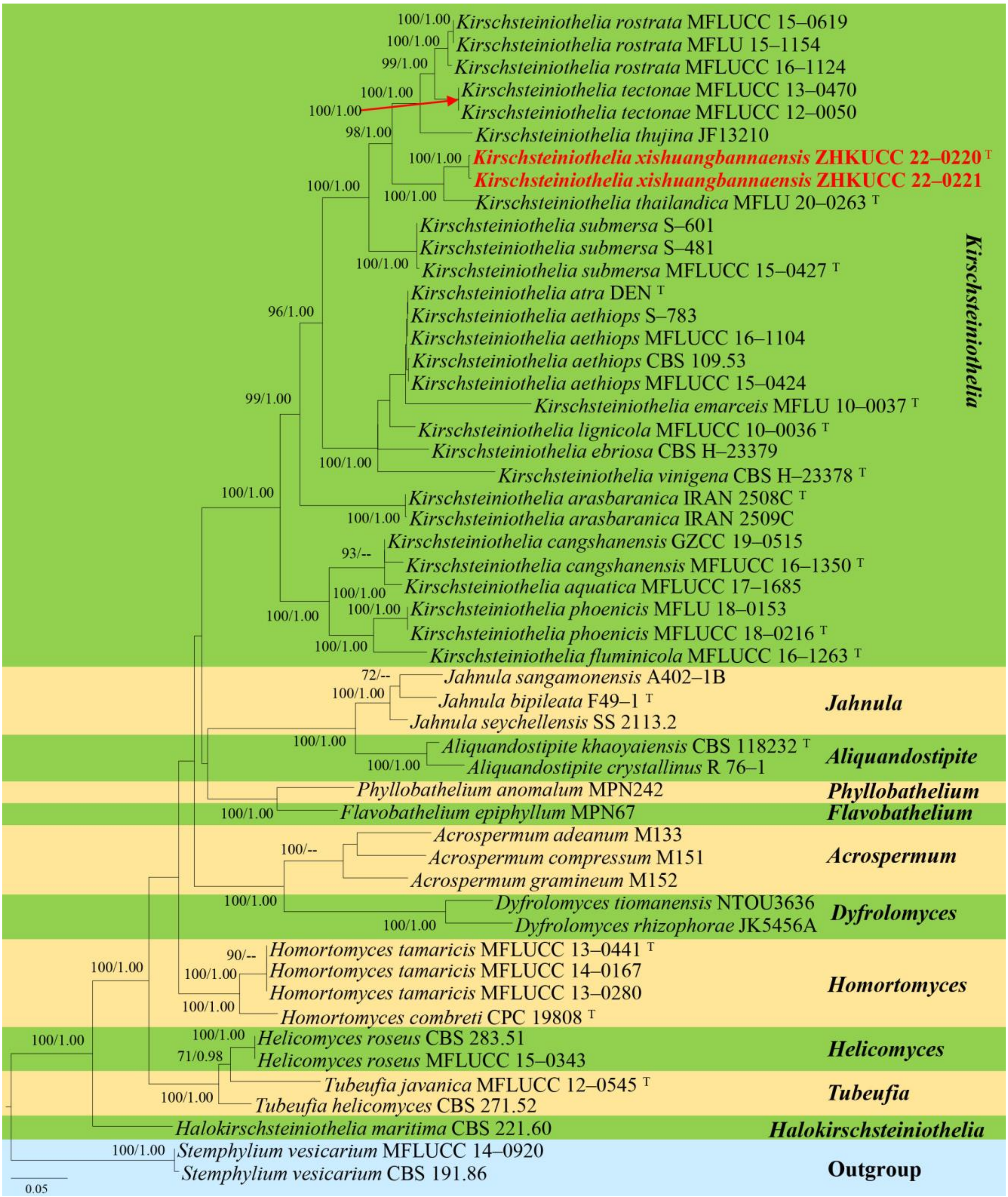

Fig. 1 – Maximum likelihood phylogenetic tree inferred from a combined dataset of ITS, LSU and SSU. The tree is rooted with *Stemphylium vesicarium* (CBS 191.86, MFLUCC 14–0920). Newly isolated strains are indicated in red bold font while T indicates holotype or ex-type strains. Bootstrap support values for maximum likelihood (ML) equal to or higher than 60% and Bayesian Probability (BYPP) equal to or higher than 0.95 are mentioned above/below the branches.

Material examined – CHINA, Yunnan Province, Xishuangbanna, Jinghong, Manlie, 28–30℃, 330 m, 122°2'28"N, 100°44'23"E, on a dead branch of *Hevea brasiliensis*, 15 September 2021, Ruifang Xu, XSBNR–44 (ZHKU 22–0126, holotype; ex-type culture ZHKUCC 22–0220 = ZHKUCC 22–0221).

Fig. 2 – *Kirschsteiniothelia xishuangbannaensis* (ZHKU 22–0126, holotype). A, B Colonies on the natural substrate. C–G Conidiophores with conidia (red arrows indicate mucilaginous sheaths). H–M Conidia (red arrows indicate mucilaginous sheaths). N Germinated conidium. O, P Colonies on PDA after one month. Scale bars: C–E, L = 50 µm, F, G = 100 µm, H–K, M, N = 30 µm.

Notes – The phylogenetic analyses showed *Kirschsteiniothelia xishuangbannaensis* forms a monophyletic clade with *K. thailandica* (100% ML/1.00 BYPP, Fig. 1). The base pair comparison of *K. xishuangbannaensis* and *K. thailandica* shows 55/487 bp differences in ITS (12.11%, gaps 10 bp), 13/803 bp differences in LSU (1.62%) and 2/1,011 bp differences in SSU (0.19%). Based on the recommendation of Jeewon & Hyde (2016), a minimum of >1.5% nucleotide differences in the ITS region is indicative of a new species. Morphology comparison of the new taxon (*K. xishuangbannaensis*) and *K. thailandica* revealed that *K. xishuangbannaensis* has longer conidiophores (35–150 vs. 55–93 µm), longer conidiogenous cells (10–50 vs. 9.5–21 µm), and longer conidia (30–150 vs. 74–110 µm). In addition, *K. thailandica* has olivaceous or brown conidia with a single hyaline mucilaginous sheath at the apex while *K. xishuangbannaensis* has yellow-brown to brown, 1–2 hyaline globose/ampulliform mucilaginous sheaths at the apex. Furthermore, the colour of culture colonies of *K. xishuangbannaensis* on PDA has dark-olivaceous whereas *K. thailandica* has reddish brown (Sun et al. 2021). Thus, *Kirschsteiniothelia xishuangbannaensis* is introduced as a new species, based on its distinct morphology, base pair differences and phylogenetic evidence.

Discussion

The survey of microfungi associated with pará rubber in Yunnan Province, China revealed a new species *Kirschsteiniothelia xishuangbannaensis*. There are 29 species in *Kirschsteiniothelia* (Wijayawardene et al. 2020), including 16 sexual species (Table 2), nine asexual species (Table 3) and four species known for both morphs (Table 4), and the members of this genus are distributed in 14 countries, and most species have been found in tropical or subtropical regions (Tables 2, 3, 4). In addition, most *Kirschsteiniothelia* species have been reported from terrestrial habitat, while *K. cangshanensis,* and *K. fluminicola* found from freshwater habitat (Bao et al. 2018).

Several species were reported from various freshwater and terrestrial habitats in China viz. *K. cangshanensis*, *K. fluminicola* and *K. rostrata* (Bao et al. 2018). This shows that *Kirschsteiniothelia* species are widely distributed in Yunnan Province, China. Based on morphological investigations at different stages of rubber leaf decay (new fallen, middle stage decaying leaves, old decaying leaves), *Kirschsteiniothelia* species were discovered in Thailand (Seephueak et al. 2010). The present study expands our knowledge of *Kirschsteiniothelia* and this is the first *Kirschsteiniothelia* species associated with rubber in China that was identified based on morphology and phylogeny.

Table 2 Sexual morph morphological, host, and location information of *Kirschsteiniothelia* species. Remarks: the symbol "–" denotes no information available.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
K. abietina	*Tsuga canadensis*	U.S.A.	scattered, minute depressed sub-hemispherical, thin, when young and immature elevating the surface of the bark into light brown or concolorous pustules, when mature becoming sub-erumpent, black at the apex, and readily removable from the surface	minute, papilliform	100–110 × 20 µm, oblong cylindrical	–	100–110 × 20 µm, oblong cylindrical, 7–8 spores	23–28 × 6–10 µm, irregularly biseriate, elliptical, rounded at the ends, slightly or not at all constricted at the septum, young hyaline, nucleosomes or granular, becoming brown	Wang et al. (2004)

Table 2 Continued.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
K. acerina	*Acer saccharum*	U.S.A.	185–240 × 145–200 µm, arising singly or in small groups, scattered, superficial, hemispherical, applanate at the base, black	–	20–40 µm thick, dark reddish-brown, *textura angularis*	cellular, persistent, regularly septate, branched and anastomosing, 1.5–2.0 µm thick	85–95 × 20–24 µm, subcylindrical, short-stalked, bitunicate, (4-)8-spores	22–26 × 8–11 µm, ellipsoid, rounded at the apices, 1-septate, the lower cell often somewhat smaller, slightly constricted at the septum, brown to dark brown	Hawksworth (1985)
K. arasbaranica	*Quercus petraea*	Iran	189–380 × 350–480 µm, slightly immersed in the substrate, subglobose to globose, scattered or loosely clustered in small groups, black, inside white	–	50–80 µm thick, comprising several layers of cells of *textura angularis*, inner layer cells hyaline, outer layer cells pale brown to dark	comprising numerous filiform 2–2.7 µm wide, hyaline, branched, embedded in a gelatinous matrix	120–180 × 30–40 µm, cylindrical to clavate, 8-spores, occasionally 4- or 6-spores, bitunicate, fissitunicate, with a short stalk up to 10 µm long, apically rounded, with an apical ocular chamber 5–8 µm wide	34–42 × 13–16µm, arranged 2- to 4-seriate in the lower 2/3 and 1-seriate in the upper 1/3 of asci, brown to dark brown at maturity, narrowly to broadly ellipsoidal with rounded apex, thick-walled, covered with a mucilaginous sheath, 1-septate, septum deeply constricted and submedian, the upper cell distinctly larger than the lower cell, each cell containing a	Mehrabi et al. (2017)
K. atkinsonii	*Freycinetia arnottii*	U.S.A.	500 µm diam, sub-cuticular, developing under raised, black, shiny	–	–	1–1.5 um diam, filamentous, simple or branched, septate, embedded	70–90 × 9–16 um, 8-spores, clavate, short	distinct large guttules 14–16 × 5–6 µm, 2-seriate, clavate, light	Hyde (1997)

Table 2 Continued.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
						in mucilage	pedicellate, thick-walled, bitunicate with an ocular chamber	brown, 1-septate	
K. atra	Dead wood	U.S.A.	300–600 µm diam, arising singly or in small groups, scattered, colonies not delimited, superficial except at the base which can remain immersed, hemispherical to subglobose, the base slightly to strongly applanate, black	short papilliform or plane	40–60 µm thick, dark brown thick-walled, *textura angularis*	2–3.5 µm diam, persistent, abundant, regularly septate, branched and anastomosing, the centrum sometimes turning blue in iodine.	70–90 × 12–15 µm, broadly, cylindrical to cylindric-clavate, short-stalked, fissitunicate, 4- or 8-spores	25–33 × 8.5–12 µm, ellipsoidal, rounded or somewhat constricted at the apices, 1-septate, the upper cell usually larger in size, somewhat constricted at the septum, brown, sometimes appearing almost smooth but at high magnification a delicate verruculose ornamentation is evident, not guttulate, lacking a distinct gelatinous sheath when mature	Hawksworth (1985)
K. dolioloides	*Pinus*	Switzerland	about 0.6 mm in diameter, singly or in small groups, superficially attached to the bare wood, conical to hemispherical, black, carbonaceous, very hard	roundish, on a short apical or lateral papilla	–	numerous, knotty, containing oil droplets	125–145× 25–34 µm, obclavate, with a short-curved peduncle, thick walled, at the rounded apex with pore, 8-spores	84–39 × 14–15 µm, 2-celled, with a slight constriction at the septum, young olivine, old dark brown	Wang et al. (2004)

Table 2 Continued.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
K. phileura	*Tilia American*	New York	semi-immersed	simplicity	–	–	elliptic, uniseptate	perithecia punctiform, small	Barr (1993)
K. phoenicis	*Phoenix paludosa*	Thailand	135–160 × 183–235µm, black, scattered, subglobose to globose or slightly conical in appearance, superficial with apical papilla or plane, base remaining immersed, usually flattened	–	laterally 24–34 µm wide, composed of 5–6 layers of *textura angularis*, with dark brown outer layer cells and inner layers pale brown	0.8–1.5 µm wide, branched, embedded in a gelatinous matrix	70–112 × 14–24 µm, bi-or tri-seriate in the middle of the ascus, cylindrical-clavate, straight or slightly curved, apically rounded, 8-spores, with a short pedicellate up to 5 µm long	18–27 × 5–7.5 µm, brown, ellipsoid, rounded or slightly pointed at the ends, 1-septate, septum sub-median and constricted, upper cell broader than the lower cell, each cell containing a distinct large guttulate, smooth-walled, with a mucilaginous sheath	Hyde et al. (2018)
K. proteae	*Protea cynaroides*	South Africa	a) up to 300 µm high, up to 390 µm wide, perithecium, intraepidermal, remain immersed with a tip of papilla reaching the surface or becoming superficial by weathering of the substrate	papillate	24–35 µm thick except for the ostiole region (43–52 µm thick), thickened and heavily pigmented towards the apex, consisting of two strata, outer stratum composed of a few layers of dark	hyaline, filamentous, septate, branched, 1–2 µm wide	54–72 × 6–8 µm, bitunicate, cylindrical to inflated cylindrical, ocular chamber	13–17 × 3–4 µm, pale brown to brown, fusoid, 1-septate, median or sub-median, smooth, at times cells become two large guttulate	Marincowitz et al. (2008)

Table 2 Continued.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
					brown, very thick-walled, compressed cells, individual cells indistinguishable, inner stratum composed of a few layers of hyaline, thin-walled, very compressed cells		0.8–1 × 0.5–2 µm		
K. populi	*Populus angustifolia*	Colorado	about 250–300 µm, scattered, small, globose, black, shining, not collapsing, obscurely perforate, base sunk in the wood	–	–	–	80–90 × 8 µm, cylindrical, short stipitate	12 × 6 µm, ovate, ends rounded, fuliginous, equally uniseptate, much constricted	Wang et al. (2004)
K. reticulata	Unknown twigs	Taiwan, China	320–450 × 180–220 µm, scattered, immersed at first, then erumpent through torn fissure of host, apex rounded, hyphae intercellular and intracellular in	–	20–40 µm thick at sides, *textura angularis*, at lateral where cells usually arranged in palisade-like rows, at base reduced, usually merged with underlying	branched, 1.5–2 µm wide	90–110 × 23–27 µm, obclavate, with a short stalk up to 15 µm long	ellipsoid, 17–23 × 7–10 µm, dark brown, 1-septate, septum constricted, median or occasionally slightly deviating from middle, with reticulate ornamentation on	Chen et al. (2006)

Table 2 Continued.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
			host, broad, 3–5 µm wide, deeply constricted where crossing host cell wall		host tissue			surface, covered with mucilaginous sheath	
K. smilacis	*Smilae*	Taiwan, China	450–600 ×200–300 µm, scattered, superficial in appearance, externally covered with a thin-layer of host epidermis, apex rounded	–	35–50 µm thick, composed of opaque and melanized cells, poorly developed at base where cells usually integrate with host tissue	1–2 µm wide, frequently branched	75–100 × 16–21 µm, obclavate, short-stalked, stalk up to 10 µm long	20–24 × 6–8 um, ellipsoid, pale brown, 1-septate, slightly constricted at septum, covered with mucilaginous sheath	Chen et al. (2006)
K. striatispora	*Juniperus nana*	Switzerland	150–250 × 120–150 µm, arising singly, scattered, erumpent, almost entirely superficial at maturity, hemispherical, base applanate, black, lacking a distinct basal fringe	almost plane to slightly papillate	15–32 µm thick, dark reddish-brown, *textura angularis*	cellular, persistent, septate, very irregularly branched, the cells irregular in shape, 2–3 µm thick.	65–75 × 9–11 µm, arising from the base of the ascomata cavity, vertically orientated, subcylindrical, short-stalked, bitunicate, with a distinct pimple-like internal apical beak, 4-spores	15–18 × 5.0–6.5 µm, over lapping uniseriately arranged in the asci, ellipsoid to somewhat soleiform, 1-septate, constricted at the septum or scarcely, the cells equal in size or the lower slightly smaller, apices rounded, reddish-brown, slightly granular at first but finally with up to five longitudinal or sinuate furrows in	Hawksworth (1985)

Table 2 Continued.

Species	Host	Country	Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	References
								face view at high magnifications using light microscopy	
K. thujina	*Abies balsamea/ Thuja occidentalis*	Canada/ U.S.A.	350–500 × 300–500 µm, arising singly or in small groups, scattered, originating in the outer layers of the host but soon erumpent and superficial at maturity, hemispherical, base applanate, black	short–papilliform to conical 75 µm wide	40–60 µm thick, deep reddish–brown, *textura angularis*	evidently cellular, persistent, abundant, rather sparsely septate, branched, 2.5–3.5 µm thick	100–140 × 17–22 µm, arising from the base of the ascomata cavity, vertically orientated, subcylindrical to elongate-clavate, short-stalked, bitunicate, 8-spores	36–50 × 15–17 µm, irregularly to distichously arranged in the asci, elongate–ellipsoid, slightly attenuated towards the apices, 1-septate, the cells mainly equal in size, the lower cell sometimes smaller, dark brown, apparently smooth walled, often guttulate	Hawksworth (1985)
K. umbrinoidea	Bark of *Aesculus hippocastanum*	Italy	superficial, subglobose	papillate	–	–	short-stalked, bitunicate	23–28 × 75–9 µm, oblong-fusiform, hyaline, two guttulate	Wang et al. (2004)
K. xera	bark of *Prunus*	New York	0.5 mm diam, black, carbonaceous, scattered or gregarious, covered at first, then emergent and superficial, embraced by the remains of the ruptured epidermis, smooth	inconspicuous	–	–	70–75 µm long, cylindrical, 8-spores	17–23 × 6–7 µm, uniseptate, constricted at the septum, cells somewhat unequal, guttules, with granulate contents, uniseriate or partly biseriate	Wang et al. (2004)

Table 3 Asexual morph morphological, host, and location information of *Kirschsteiniothelia* species. Remarks: the symbol "–" denotes no information available.

Species	Host	Country	Colony/Conidiomata	Conidiophore	Conidiogenous cells	Conidia	References
K. cangshanensis	Submerged wood	Yunnan, China	effuse, scattered, hairy, pale brown to brown	105.5–135.5 × 6–8 µm, macronematous, erect, pale brown, septate, unbranched, cylindrical, percurrent, straight or flexuous	monoblastic, integrated, terminal, determinate, pale brown, cylindrical	33–43 × 7.5–8.5 µm, solitary, dry, obclavate, septate, straight or slightly curved, pale brown to brown, with a gelatinous sheath at apex	Bao et al. (2018)
K. ebriosa	From sparkling wine	Spain	–	40–150 × 4 µm, macronematous, consisting of a straight or slightly sinuous, erect, dark brown, septate, thin- and smooth-walled to slightly verrucose stipe, bearing a few lateral branches and occasionally one branch at the top, branches are brown, thin- and smooth-walled, 1–5-septate, cylindrical, 30–50 × 4 µm, with rounded ends	branches, intercalary or terminal, determinate, cylindrical	8–14 × 4–5 µm, brown to dark brown, thin- and smooth-walled, 1–2(–5)-septate, sometimes solitary, mostly in branched acropetal chains of up to 5, cylindrical with rounded ends, sometimes slightly constricted at septum, produced laterally and terminally on the stipe, on the branches and on the conidia	Rodríguez-Andrade et al. (2019)
K. fluminicola	Submerged wood	China, Yunnan	effuse on natural substrate, hairy, dark brown	209–286 × 7–9 µm, erect, straight or flexuous, septate, smooth,	monoblastic, terminal, indeterminate,	47.5–86.5 × 8–10 µm, solitary to short-catenate,	Bao et al. (2018)

Table 3 Continued.

Species	Host	Country	Colony/Conidiomata	Conidiophore	Conidiogenous cells	Conidia	References
				cylindrical, dark brown to black, unbranched, percurrent	cylindrical, dark brown	obclavate, rostrate, truncate at base, slender and rounded at apex, aseptate when immature, multi-septate at maturity, subhyaline to dark brown, with conspicuous, spherical guttulate in almost all cells	
K. rostrata	Submerged wood	Thailand	effuse on natural substrate, scattered, hairy, dark brown to black	90–120 × 7.5–8.5 µm, macronematous, mononematous, solitary, wide and slightly swollen at base, tapering towards apex, straight or slightly flexuous, smooth, brown to dark brown, unbranched, septate	holoblastic, monoblastic, integrated, terminal, determinate, cylindrical or lageniform, smooth, mid to dark brown	77.5–108.5 × 17.5–20.5 µm, acrogenous, solitary, dry, olivaceous brown to brown, pale at apex, obclavate, rostrate, smooth, straight or curved, truncate at base, 6–17-septate	Bao et al. (2018)
K. shimlaensis	*Himachal Pradesh*	India	on natural substratum scattered, dark, brown to black	110–268 × 12–19 µm, macronematous, mononematous, single to loosely fasciculate, erect, straight to flexuous multi-septate, irregular or subscorpioid branched at the apex, dark brown,	18–24 × 8–10 µm, monoblastic, terminal or intercalary, cylindrical or doliiform, mostly discrete,	41–81 ×13–17.5 µm, solitary, obovoid, oblong, broad clavate or cylindrical, sometimes constricted near the middle or at	Verma et al. (2021)

Table 3 Continued.

Species	Host	Country	Colony/Conidiomata	Conidiophore	Conidiogenous cells	Conidia	References
				smooth	determinate, sometimes with enteroblastic, percurrent elongations, smooth, dark brown or brown	the supra basal cell, sub-truncate or rounded at the base, rounded at the apex, dark brown or black, smooth, guttulate, lumen aspect granulose, 2–5(–6)-septate	
K. submersa	decaying wood	Yunnan, China	effuse, brown or black, hairy	220–280 × 6–7 µm, macronematous, mononematous, blackish to brown, paler towards the apex, smooth, unbranched, thick-walled, multi-septate, straight to slightly flexuous, cylindrical, erect	holoblastic, integrated, terminal, determinate, percurrent, pale brown, cylindrical	37.5–51.5 × 8.5–9.5 µm, acrogenous, solitary, dry, smooth, obclavate, straight or slightly curved, truncate and darker at the base, rounded and paler at the apex, brown to pale brown, thick-walled, hyaline and thinner at the tip, 4–6-septate	Su et al. (2016)
K. tectonae	*Tectona grandis*	Thailand	natural substrate, superficial, hairy, dark brown, scattered, single or in groups	up to 200 µm long, 4–8 µm wide at the swollen base, superficial on host surface, macronematous, mononematous, simple, erect to slightly curved, unbranched or branched,	7.5–9.5 × 3.5–5 µm, monoblastic, integrated, terminal, cylindrical	135–150 × 16–17 µm, 9–25 or more transverse septa, cylindric-obclavate, elongate, straight or slightly	Li et al. (2016)

Table 3 Continued.

Species	Host	Country	Colony/Conidiomata	Conidiophore	Conidiogenous cells	Conidia	References
				septate, slightly constricted at septa, pale brown to dark brown, cylindrical		curved, rounded at the apex and slightly paler, with sheath at apex, truncate at the base, dark reddish brown, thick-walled, smooth	
K. thailandica	*Ficus microcarpa*	Thailand	scattered, effuse, brown to dark brown, hairy on natural substrate	55–93 × 7–10 µm, macronematous, solitary, erect, cylindrical, straight or slightly flexuous, unbranched, septate, brown to dark brown	9.5–21 × 7–10 µm, monoblastic, integrated, terminal, cylindrical, brown to dark brown	74–110 × 13–20 µm, acrogenous, straight, solitary, obclavate, smooth–walled, 6–8-distoseptate, shiny, olivaceous or brown, hyaline at apex, truncate at base, tapering towards apex, with a conspicuous, gelatinous, hyaline sheath around tip	Sun et al. (2021)
K. vinigena	cork stopper	Spain	–	macronematous, consisting in a straight or slightly sinuous, erect, dark brown, septate, thin- and smooth-walled to coarsely verrucose (specially at the base) stipe, 100–150 × 3 µm, bearing several lateral	branches, intercalary or terminal, determinate, and cylindrical	8–80 × 4–5 µm, holoblastic, dark brown, thin- and smooth-walled to coarsely verrucose, 1–2(–7)-septate, sometimes solitary, mostly	Rodríguez-Andrade et al. (2019)

Table 3 Continued.

Species	Host	Country	Colony/Conidiomata	Conidiophore	Conidiogenous cells	Conidia	References
				branches, which also branch irregularly, branches are abundant, brown, thin- and smooth-walled to coarsely verrucose (specially at the base), non-septate to 7-septate, barrel-shaped to cylindrical, 5–80 × 4 µm, with rounded ends		disposed in branched acropetal chains of up to 4, cylindrical, with rounded ends, sometimes slightly constricted at septa, produced laterally and terminally on the stipe, on the branches, and on other conidia	
K. xishuangbannaensis	*Hevea brasiliensis*	Yunnan, China	effuse on natural substrate, solitary or scattered, hairy, brown to dark brown	35–150 × 5–15 µm, macronematous, septate, curved, solitary, brown to dark brown, widely and slightly swollen at base, tapering towards apex	10–50 × 5–10 µm, monoblastic, holoblastic, integrated, terminal, determinate, cylindrical or lageniform, smooth, brown to dark brown	30–150 × 5–20 µm, acrogenous, solitary, obclavate, rostrate, straight or curved, truncate at base, some have guttules, 3–8-septate, yellow–brown to brown, subhyaline or pale-brown at apex, 1–2 hyaline globose / ampulliform mucilaginous sheaths around the tip (mostly 1 mucilaginous sheath)	This study

Table 4 Sexual and asexual morph morphological, host, and location information of *Kirschsteiniothelia* species. Remarks: the symbol "–" denotes no information available.

Species	Host	Country	Sexual morph						Asexual morph				References
			Ascomata	Ostiole	Peridium	Pseudoparaphyses	Asci	Ascospores	Colony / Conidiomata	Conidiophore	Conidiogenous cells	Conidia	
K. aethiops	*Querus*/ *Carpinus*/ dead wood/ submerged wood	Belgium/ Germany/ U.S.A./ China, Yunnan	300–600 µm diameter, arising singly or in small groups, scattered, colonies not delimited, hemispherical to subglobose	20–30 µm diameter, short papilliform	40–60 µm thick, dark brown, thick-walled, *textura angularis*	cellular, persistent, abundant, regularly septate, branched 2.5–3.5 µm thick	70–90 × 12–15µm, arising from the base of the ascomata cavity, vertically orientated, broadly subcylindrical, short-stalked, bitunicate, 4- or 8-spores	25–33 × 8.5–12.0 µm, ellipsoid, rounded or somewhat constricted at the apices, 1-septate, the upper cell usually larger in size, somewhat constricted at the septum, brown, sometimes appearing almost smooth	on the substratum superficial, effuse, gregarious, hairy, velvety, dark brown	245–355 × 8–10 µm, mononematous, macronematous, septate, usually with one or short branches near the apex, erect, straight or flexuous, smooth, dark brown and gradually paler towards the apex, cylindrical, 5–10-septate	integrated, sometimes percurrent, terminal, becoming intercalary, pale brown or subhyaline, lageniform, new cell developing from apical or subapical part of subtending cell	54–63 × 14–18 µm, acrogenous, solitary, dry, cylindrical, rounded at the apex, and narrowly truncate at the base, 3–4-septate, septa thick, smooth, brown	Hawksworth (1985), Su et al. (2016)
K. emarceis	Dead wood	Thailand	109–280 × 101–318 µm, dark brown to black, clustered, solitary or scattered, superficial, subglobose to globose, slightly immersed, base flattened, with central ostiole, but unclear, with a few sparse hair-like setae	–	12– 40 µm thick, composed of 3–4 layers, comprising cells of *textura angularis*, inner layer cells pale brown, but cells of outer layer black	1–2 µm wide, hyaline, embedded in a gelatinous matrix	88–140 × 18–24 µm, 8-spores, bitunicate, fissitunicate, cylindrical to clavate, apically rounded	25–28 × 8–9 µm, biseriate, ellipsoidal, slightly curved, septum median to supra-median, dull green, becoming brown to dark brown at maturity, one-septate, two-celled, smooth walled, lacking a mucilaginous sheath	–	162–271 × 7–14 µm, erect, branched apically, brown to dark brown, smooth	–	45–56 × 14–15 µm, oblong to clavate, grayish, brown to dark brown, 3–4 (–5) septate, constricted at septa, smooth-walled	Boonmee et al. (2012)

Table 4 Continued.

Species	Host	Country	Sexual morph						Asexual morph				References
			Ascomata	**Ostiole**	**Peridium**	**Pseudoparaphyses**	**Asci**	**Ascospores**	**Colony / Conidiomata**	**Conidiophore**	**Conidiogenous cells**	**Conidia**	
K. lignicola	Dead wood	Thailand	152–178 × 156–214 µm, superficial, subglobose to globose, dark brown to black, membranaceous, clustered, solitary, with central papilla 24 µm wide with 24 µm high	–	18–22 mm thick, comprising 3–4 layers of cells of *textura angularis*, inner layer cells pale brown, outer layer cells dark	filiform, 1–2 mm wide, hyaline, embedded in a gelatinous matrix	107–163 × 19–28.5 µm, bitunicate, fissitunicate, cylindrical to clavate, 8-spores with along pedicel 14.5–24 µm, apically rounded	27–30 × 10–12 µm, biseriate, ellipsoidal, slightly curved, 1(–2) septate, with median septum or in lower part, some ascospores with secondary septum, dull green, brown to dark brown at maturity, thick-walled	287–406 × 11–13 µm, erect, branched apically, dark brown, smooth, conidium forming at apex	–	–	39–48 × 21–25 µm, obovoid to broadly, 1–2 transverse septa, constricted at septa, smooth-walled, dark brown, rounded at ends	Boonmee et al. 2012
K. recessa	*Pyrus*/ dead wood	Italy/ U.S.A.	150–250 µm diameter, arising singly or more usually in small groups, scattered, superficial except at the base, hemispherical, base slightly rounded to applanate, black	short-papilliform	50–75 µm thick, dark reddish-brown, composed of thick-walled, *textura angularis*, cells mainly 4–7 µm diameter, the outer most often with the lumina becoming occluded, basal tissue similar in structure to	cellular, persistent, abundant, regularly septate, branched and anastomosing, 2–3 µm thick	90 × 10 µm, arising from the base of the ascomata cavity, vertically orientated, subcylindrical, short-stalked, bitunicate, 8–spores	15–17.5 × 5–6.5 µm, distichously arranged in the asci, elongate-ellipsoid, rounded at the apices, 1-septate, cells equal in size or the lower slightly smaller, slightly constricted at the septum, pale brown, almost smooth or with a very weak verruculose ornamentation	3.5–4.0 µm thick, erect, red–brown, septate, thick-walled	–	–	–	Hawksworth 1985

Table 4 Continued.

Species	Host	Country	Sexual morph						Asexual morph				References
			Ascomata	**Ostiole**	**Peridium**	**Pseudoparaphyses**	**Asci**	**Ascospores**	**Colony / Conidiomata**	**Conidiophore**	**Conidiogenous cells**	**Conidia**	
					the sides of the ascoma, well developed, mainly 8–20 µm thick, the cells tending to be vertically orientated and pallisade-like			, not guttulate, lacking a distinct gelatinous sheath					

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Accessibility of data

Provide identifiers of material and data deposited in (preferably publicly accessible) public herbaria, collections, databases and data repositories

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