Mycosphere 9(3): 444–461 (2018) www.mycosphere.org ISSN 2077 7019

Article

Doi 10.5943/mycosphere/9/3/2

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Lignicolous freshwater fungi from China II: Novel *Distoseptispora* (Distoseptisporaceae) species from northwestern Yunnan Province and a suggested unified method for studying lignicolous freshwater fungi

Luo ZL[1,2], Hyde KD[2], Liu JK[3,4], Bhat DJ[5], Bao DF[1,2,4], Li WL[1] and Su HY[1*]

[1]*College of Agriculture & Biological Sciences, Dali University, Dali 671003, Yunnan, P.R. China*
[2]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Guizhou Key Laboratory of Agricultural Biotechnology, Guizhou Academy of Agricultural Sciences, Guiyang 550006, P.R. China*
[4]*Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand*
[5]*128/1-J, Azad Housing Society, Curca, Goa Velha-403108, India*

Luo ZL, Hyde KD, Liu JK, Bhat DJ, Bao DF, Li WL, Su HY 2018 – Lignicolous freshwater fungi from China II: Novel *Distoseptispora* (Distoseptisporaceae) species from northwestern Yunnan Province and a suggested unified method for studying lignicolous freshwater fungi. Mycosphere 9(3), 444–461, Doi 10.5943/mycosphere/9/3/2

Abstract

This is the second in a series of papers on lignicolous freshwater fungi from China. In this paper, eight fresh collections of asexual morphs of *Distoseptispora*, isolated from submerged wood in northwestern Yunnan Province, China, are characterized based on morphological characters and phylogenetic analyses of combined ITS, LSU, RPB2 and TEF1α sequence data. Four new *Distoseptispora* species (*D. cangshanensis*, *D. obpyriformis*, *D. rostrata* and *D. submersa*) are introduced, described and illustrated, with notes on their taxonomy and phylogeny. Newly generated molecular data of *Distoseptispora fluminicola* is also provided. We also provide a unified method for studying lignicolous freshwater fungi to standardize the findings of future Asian studies.

Key word – Asexual fungi – Methodology – Phylogeny – Sordariomycetes – Taxonomy

Introduction

Lignicolous freshwater fungi play an important role in nutrient and carbon cycling, biological diversity and ecosystem functioning in freshwater ecosystems (Palmer et al. 1997, Yuen et al. 1998, Bucher et al. 2004, Vijaykrishna et al. 2005, Hyde et al. 2016a). There have been some studies on lignicolous freshwater fungi in Yunnan Province, China (Cai et al. 2002, Luo et al. 2004, 2016, 2017, 2018, Liu et al. 2015, Su et al. 2015, 2016, Zhu et al. 2016) and in this paper, we deal with the genus *Distoseptispora* K.D. Hyde, McKenzie & Maharachch. which belongs to Distoseptisporaceae, Sordariomycetes.

The family Distoseptisporaceae K.D. Hyde & McKenzie was introduced by Su et al. (2016) with a single genus *Distoseptispora* to accommodate two Sporidesmium-like species. Yang et al. (2018) emended the description of the genus *Distoseptispora* which is characterized by

Submitted 26 February 2018, Accepted 21 April 2018, Published 21 May 2018
Corresponding Author: Hong-Yan Su – e-mail – suhongyan16@163.com

macronematous, septate, unbranched, olivaceous to brown conidiophores, monoblastic, holoblastic, determinate, terminal conidiogenous cells and acrogenous, olivaceous, brown or yellowish/reddish brown, euseptate or distoseptate conidia, with a basal cell with cross walls and a basal scar. Currently, there are nine species in *Distoseptispora* with six species known from freshwater habitats (Su et al. 2016, Hyde et al. 2016b, Yang et al. 2018).

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. 2016a) and this is the second in a series of papers on these fungi from China (Li et al. 2017). Eight isolates of *Distoseptispora* species were collected from submerged decaying wood in northwestern Yunnan Province, China. Four new species, viz. *Distoseptispora cangshanensis*, *D. obpyriformis*, *D. rostrata*, *D. submersa* are introduced based on morphological characters and phylogenetic analyses. Newly generated molecular data of *Distoseptispora fluminicola* is also provided. As several research groups are looking at freshwater fungi in Asia, we also update the methods for studying lignicolous freshwater fungi, in order to provide a standardized approach.

Methods to study lignicolous freshwater fungi

Field collection

Lignicolous freshwater fungi can be found on decaying wood submerged in creeks, dams, lakes, ponds, pools, rivers, streams or swamps (Goh & Hyde 1996, Hyde & Goh 1998a, Wong et al. 1998). The woody substrates collected from freshwater habitats are ideally less than 3 cm in diameter and ca 15 cm long, thus they can easily be examined under the microscope. These substrates include part of tree trunks, branches, twigs and litter and are variable in size and length. Substrates trapped between stones and rocks in riffles or those submerged at the bottom of freshwater are preferred, as these are more likely to have been in freshwater for long time and support freshwater fungi (Tsui et al. 2003). It is advisable not to collect floating woody substrates as these may support many terrestrial taxa. Species area curves and trend lines of sample size (number of woody substrates) with species richness in previous studies showed that the number of fungi increased quickly at first and then reached asymptote at around 50 samples (Tsui et al. 2000, Ho et al. 2001). The studies carried out by Hyde and co-workers were based on collections of 100 or more woody substrates from Australia, Britain, Seychelles and South Africa, however, the species diversity are similar to those found on 50 samples (Hyde & Goh 1997, 1998 a, b, Hyde et al. 1998). It is therefore suggested that 50 samples are collected from each selected collecting site (from downstream to upstream of rivers and streams or around lakes, ponds and dams) as 50 is an optimum number for each collection site discovering most diversity.

Submerged wood baits can also be used to investigate the fungal diversity of lignicolous freshwater fungi especially if standardization of the submergence time, type of wood, or the stage of decay is determined (Jones & Hyde 1988, Sivichai et al. 2000, Tsui et al. 2001). Usually, a native tree should be chosen as wood baits and should be of the same size for standardization. The woody baits must be sterilized by autoclaving at 1.5kgf/cm2 at 121℃ for 15 minutes or alternatively sun dried which avoids changes in the wood structures. Wood blocks are strung together as ladders and submerged at the sites.

In this study, specimens of submerged decaying wood were collected respectively from Nujiang River, Jinsha River and Cangshan Mountain, Yunnan, China.

Incubation

Specimens of submerged decaying wood should be returned to the laboratory in plastic bags to avoid moisture loss. The samples are further incubated at room temperature in plastic containers or plastic bags with moistened sterilized tissue paper (Tsui et al. 2000).

Morphological studies

Samples are examined, after incubation, regularly for up to three months using a dissecting

microscope (Tsui et al. 2003). For sexual morphs and coelomycetous fungi, hand sections of ascomatal structures or pycnidial structures are made using a razor blade (Chomnunti et al. 2014). Thin sections are mounted in distilled water for microscopic study and photomicrography. Ascomata, asci, ascospores, paraphyses or pseudoparaphyses, conidiomata, conidia and conidiogenous structures are examined under compound microscope (such as Nikon ECLIPSE 80i) can be photographed by digital camera (such as Canon 550D) fitted to a compound microscope (Nikon ECLIPSE 80i). Microscopic characters of hyphomycetes (conidiophores, conidia and conidiogenous cells) are captured with a digital camera fitted to a compound microscope. Measurements are made with the Tarosoft (R) Image Frame Work program and images used for figures processed with Adobe Photoshop CS6 Extended version 13.0 software (Adobe Systems, USA).

Single spore isolation

There are three main groups of lignicolous freshwater fungi, i.e. ascomycetes, coelomycetes and hyphomycetes, which have different types of fructifications. The methods for isolation may therefore be different.

Ascomata/conidiomata are removed from the substrate surface using fine forceps or cut by using razor blade for immersed ascomata/conidiomata. Spore masses are transferred with a sterilized needle or fine forceps to a drop of sterile water on a small glass container or a flamed microscope slide (Chomnunti et al. 2014). For hyphomycetes, using a needle to stick the conidia and avoid touching the substrate, should dislodge conidia that will stick to the needle and can be placed in a drop of water. If the conidia are not easy to stick to the needle, a single fruiting body including conidiophore and conidia can be picked and placed into a drop of water. The drop of water is then examined under a dissecting microscope to confirm that enough of and the correct spores have been transferred.

The agitated spore suspension is then sucked into a sterilized pipette or Pasteur syringe. Small drops are placed on 2 % water agar (WA), potato dextrose agar (PDA) or malt extract agar (MEA) in the centre of pre-marked squares in a grid on the bottom of a Petri dish and incubated at room temperature or in an incubator (25°C) overnight. After 12 hours, the plates are examined for single germinating spores under a dissecting microscope at high magnification. Germinating spores are transferred separately to at least two new MEA/PDA plates. Spores normally germinate within 12–24 hours and should be transferred immediately by picking up single spores with a small piece of agar using a fine needle (Chomnunti et al. 2014). From our experience, bacteria or moulds will overgrow the Petri dish within three days after single spore suspensions are placed on plates. In this case, the hypha of the germinated spore will be contaminated. Therefore, transfer to fresh plates must be carried out early on. Otherwise, it will be impossible to make sure that single spore cultures of the correct species have been obtained. Four to six spores can be placed at opposite sides of the Petri dish. Some spores should be examined under the compound microscope to confirm the correct spore types or species has been obtained. If identical spores have been picked for the initial Petri dishes, all colonies should be similar.

Preparation of herbarium material

Herbarium material is essential for describing new species and important to keep records so that published data are verifiable (Chomnunti et al. 2014). Herbarium material should be prepared as early as possible by cutting a small piece of wood containing the single species, rather than depositing samples with multiple species. Ideally, prepare the herbarium specimens from a portion of your sample at the beginning of the study, as with prolonged incubation the taxon may have disappeared. Dried cultures can also be used for herbarium material. All dried material should be placed in containers or herbarium packets, labeled and deposited in an international herbarium. In this study, original samples (dry wood with fungi) are deposited at the herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS) and Mae Fah Luang University (MFLU).

Storage of cultures

The main problem when working with fungal cultures is contamination by other fungi or mites. The risk increases with incubation time. The best way to solve this problem is therefore to deposit pure cultures in more than one culture collection as early as possible and not only when a culture collection number is needed for a publication (Chomnunti et al. 2014). Another method is to add Ivermectin to the agar just before pouring plates. In this study, pure cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Dali University Culture Collection (DLUCC).

DNA extraction, PCR amplification and sequencing

The eukaryotic rRNA cistron comprises the 18S, 5.8S, and 28S rRNA genes transcribed as a unit by RNA polymerase I (Schoch et al. 2012), and has been used for fungal diagnostics and phylogenetic analyses for more than 20 years (Begerow et al. 2010, Schoch et al. 2012). Additionally, protein-coding genes are widely used in mycology for phylogenetic analyses or species identification as they are generally superior to rRNA genes for resolving relationships at various taxonomic levels (Schoch et al. 2009, Maharachchikumbura et al. 2016). Tanaka et al. (2015), Liu et al. (2017) included the TEF1α locus in the multi-gene analyses of Massarineae and Dothideomycetes respectively, which resolved many ascomycete lineages well, and is a marker of good resolution at the generic level and below (Hyde et al. 2017). Maharachchikumbura et al. (2016) used LSU, SSU, RPB2 and TEF1α sequence data to do the multi-gene analyses to show families and order relationships within the class Sordariomycetes. We suggest that both ribosomal genes and protein genes should normally be sequenced and used in analyses.

In order to make sure the culture is isolated from the correct species and duplicated in the phylogenetic analysis, at least two cultures, ideally isolated from different specimens are needed for each species. In this study, we extracted genomic DNA from fresh fungal mycelium grown on PDA at 25–27 ℃ and used a EZ gene TM Fungal gDNA kit (GD2416) to extract DNA according to the manufacturer's instructions. The gene regions of the large subunit of the nuclear ribosomal DNA (LSU), the internal transcribed spacers (ITS), the translation elongation factor (TEF1α) and RNA polymerase II subunit 2 (RPB2) were amplified using the primer pairs LROR/LR7 (Vilgalys & Hester 1990), ITS5/ITS4 (White et al. 1990), 983F/2218R, fRPB2-5F/fRPB2-7cR (Liu et al. 1999) respectively. Primer sequences are available at the WASABI database at the AFTOL website (aftol.org). The PCR mixture contained 12.5 µl of 2×Power Taq PCR MasterMix (a premix and ready to use solution, including 0.1 Units/µl Taq DNA Polymerase, 500 µm dNTP Mixture each (dATP, dCTP, dGTP, dTTP), 20 mm Tris–HCl pH 8.3, 100MmKCl, 3 mMMgCl$_2$, stabilizer and enhancer), 1 µl of each primer including forward primer and reverse primer (10 µm), 1 µl template DNA extract and 9.5 µl deionised water. The PCR thermal cycle program for ITS and LSU amplification was as follows: initial denaturation at 94 ℃ for 3 mins, followed by 40 cycles of denaturation at 95 ℃ for 30 seconds, annealing at 55 ℃ for 50 seconds, elongation at 72 ℃ for 1 min. Regions of RPB2 and TEF1α were amplified with initial denaturation at 95 ℃ for 5 mins, followed by 40 cycles of denaturation at 95 ℃ for 1 min, annealing at 54 ℃ for 90 seconds, elongation at 72 ℃ for 90 seconds, and the final extension at 72 ℃ for 10 mins included for each condition of amplification. PCR products were then purified using mini-columns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27–9602–01). The sequences were carried out at Beijing Tsingke Biological Engineering Technology and Services Co., Ltd (Beijing, P.R. China).

Phylogenetic analysis and species recognition

Sequence data for relevant strains were downloaded from GenBank following recent publications (Hyde et al. 2016b, Su et al. 2016, Xia et al. 2017, Yang et al. 2018). Consensus sequences were assembled with Sequencher 4.9 for Windows (Gene Codes Corp., Ann Arbor, Michigan) and aligned using MAFFT v.7.110 online program (http://mafft.cbrc.jp/alignment/server/) (Katoh & Standley 2013) and manually adjusted via BioEdit

v7.2.3 (Hall 1999). Phylogenetic analyses were performed by using PAUP v.4.0b10 (Swofford 2002) for maximum parsimony (MP) and MrBayes v.3.2.2 (Ronquist et al. 2012) for Bayesian analyses.

Phylogeny website tools “ALTER” (Glez-Peña et al. 2010) were used to transform the alignment fasta to Phylip file 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 –22813.235538. RAxML bootstrap support values greater than 75 % are given above at the branches (Fig. 1).

Bayesian analyses were performed by using PAUP v.4.0b10 (Swofford 2002) and MrBayes v3.2.2 (Ronquist et al. 2012). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.0b4. Six simultaneous Markov Chains were run for 1 million generations and trees were sampled every 100th generation (resulting in 10000 trees). 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.

All new sequence data generated in this study are deposited in GenBank (Table 1) and alignments are submitted in TreeBASE (www.treebase.org, submission number 22352). Resulting trees were viewed in Treeview (Page 1996). The terminals of the phylogenetic tree (Fig. 1) are labeled with species and the isolates/culture collection codes as provided in GenBank.

Diversity analysis

To compare the number of species for each locality, the number of all species will be calculated and then final numbers of species will be compared. Species diversity should be calculated using Shannon’s diversity index *H’* (Shannon & Weaver 1963):

$$\mathrm{H} = -\sum_{i=1}^{s} Pi \ln pi, pi = Ni / \mathrm{N}$$

–N_i is individual number of i species
–N is individual number of all species
–P_i is the proportion of i species

Then the Evenness (E) is calculated using the formula: Evenness (E) = H′/lnS.

Simpson's Diversity Index (1-D) is used to compare with Shannon’s diversity index and the formula is as follow: D = Σ n(n-1)/N(N-1) where n is the total number of organisms of a particular species and N is the total number of organisms of all species.

Index of similarity was calculated using Sorensen’s formula to determine the similarity in species occurrences (Odum 1971). The similarity values range from 0 to 1 (1 meaning very similar, 0 indicating no similarity) by using the following formula:

(S’) = 2C / (A + B) where S’ is the degree of similarity, A and B are the number of species at site A and site B respectively, C is the number of species common to both collections.

Although this is not an ecological study, we provide a standardized approach to study the lignicolous freshwater fungi in Asia. In this study, however, we deal with the fungal taxonomy and phylogeny as is essential to give names to all taxa before we can discuss their ecology. Ecological studies will be carried out in the future once we obtain enough data from different rivers and streams.

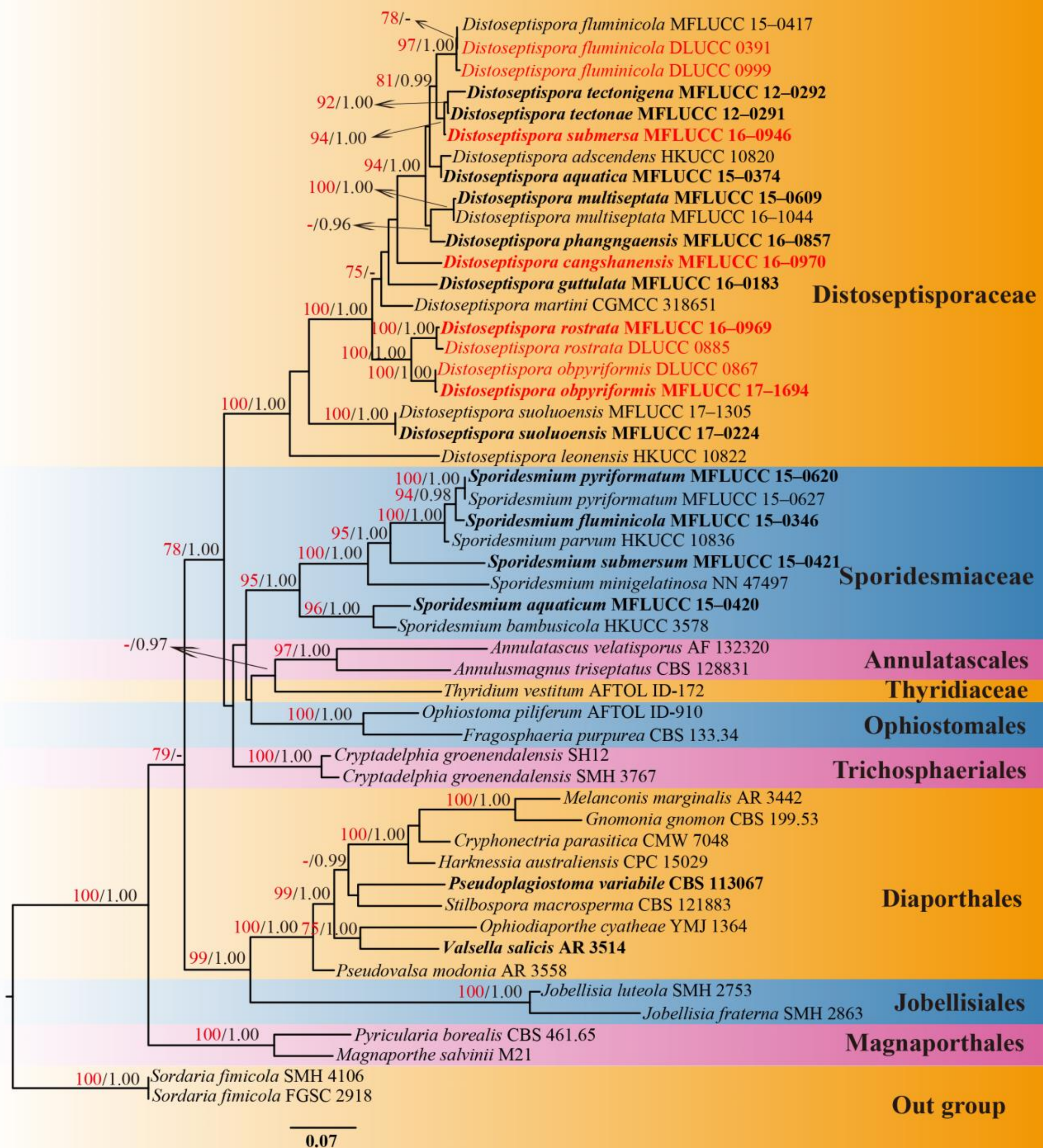

Figure 1 – Phylogram generated from maximum likelihood analysis (RAxML) based on combined ITS, LSU, RPB2 and TEF1α sequence data from selected taxa in Sordariomycetes. Bootstrap support values for maximum likelihood (ML) greater than 75% and Bayesian posterior probabilities (PP) greater than 0.95 are given above the nodes. The tree is rooted to *Sordaria fimicola* (SMH 4106, FGSC 2918). Newly generated sequences are indicated in red and ex-type strains are in bold.

Results

Phylogenetic analysis

Eight isolates of hyphomycetous taxa were obtained from submerged decaying wood, and they were assigned to the family *Distoseptisporaceae*. Phylogenetic analysis of combined ITS, LSU, RPB2 and TEF1α sequence data and morphological characters were used to assign the species and four novel species are introduced in this paper and compared with similar species (Table 2).

Table 1 Isolates and sequences used in this study (newly generated sequences are indicated in bold, ex-type strains are indicated in * after collection number).

Species	Collection/Isolate number	GenBank accession number			
		ITS	LSU	RPB2	TEF1α
Annulatascus velatisporus	HKUCC 3701	–	AF132320	–	–
Annulusmagnus triseptatus	CBS 128831	–	GQ996540	JQ429258	–
Cryphonectria parasitica	CMW 7084	JN942325	JN940858	–	–
Cryptadelphia groenendalensis	SH 12	–	EU528007	–	–
C. groenendalensis	SMH 3767	–	EU528001	–	–
Distoseptispora adscendens	HKUCC 10820	–	DQ408561	DQ435092	–
D. aquatica	MFLUCC 15-0374*	MF077552	KU376268	–	–
D. cangshanensis	**MFLUCC 16-0970***	**MG979754**	**MG979761**	**–**	**MG988419**
D. fluminicola	MFLUCC 15-0417*	MF077553	KU376270	–	–
D. fluminicola	**DLUCC 0391**	**MG979755**	**MG979762**	**–**	**MG988420**
D. fluminicola	**DLUCC 0999**	**MG979756**	**MG979763**	**–**	**MG988421**
D. guttulata	MFLUCC 16-0183*	MF077543	MF077554	–	MF135651
D. obpyriformis	**MFLUCC 17-1694***	–	**MG979764**	**MG988415**	**MG988422**
D. obpyriformis	**DLUCC 0867**	**MG979757**	**MG979765**	**MG988416**	**MG988423**
D. leonensisi	HKUCC 10822	–	DQ408566	DQ435089	–
D. martini	CGMCC 318651	KU999975	KX033566	–	–
D. multiseptata	MFLUCC 16-1044	MF077544	MF077555	MF135644	MF135652
D. multiseptata	MFLUCC 15-0609*	KX710145	KX710140	–	MF135659
D. phangngaensis	MFLUCC 16-0857*	MF077545	MF077556	–	MF135653
D. rostrata	**MFLUCC 16-0969***	**MG979758**	**MG979766**	**MG988417**	**MG988424**
D. rostrata	**DLUCC 0885**	**MG979759**	**MG979767**	**–**	**MG988425**
D. submersa	**MFLUCC 16-0946***	**MG979760**	**MG979768**	**MG988418**	**MG988426**
D. suoluoensis	MFLUCC 17-0224*	MF077546	MF077557	–	MF135654
D. suoluoensis	MFLUCC 17-1305	MF077547	MF077558	–	–
D. tectonae	MFLUCC 12-0291*	KX751711	KX751713	KX751708	KX751710
D. tectonigena	MFLUCC 12-0292*	KX751712	KX751714	KX751709	–
Fragosphaeria purpurea	CBS 133.34	AB278192	AB189154	–	–
Gnomonia gnomon	CBS 199.53	AY818956	AF408361	DQ470922	DQ471094
Harknessia australiensis	CPC 15029	JQ706085	JQ706211	–	–
Jobellisia fraternal	SMH 2863	–	AY346285	–	–
J. luteola	SMH 2753	–	AY346286	–	–
Magnaporthe salvinii	M 21	–	JF414887	–	JF710406
Melanconis marginalis	AR 3442	–	AF408373	EU219301	EU221991
Ophiodiaporthe cyatheae	YMJ 1364	JX570889	JX570891	JX570893	–
Pseudoplagiostoma variabile	CBS 113067*	GU973536	GU973611	–	–
Pseudovalsa modonia	AR 3558	–	EU683073	–	–

Table 1 Continued.

Species	Collection/Isolate number	GenBank accession number			
		ITS	LSU	RPB2	TEF1α
Pyricularia borealis	CBS 461.65	KM009162	KM009150	–	KM009198
Sordaria fimicola	SMH 4106	–	AY780079	AY780194	–
Sordaria fimicola	FGSC 2918	–	FR774289	–	FR774388
Sporidesmium aquaticum	MFLUCC 15-0420*	–	KU376273	–	–
S. bambusicola	HKUCC 3578	–	DQ408562	–	–
S. fluminicola	MFLUCC 15-0346*	–	KU376271	–	–
S. minigelatinosa	NN 47497	–	DQ408567	DQ435090	–
S. parvum	HKUCC 10836	–	DQ408558	–	–
S. pyriformatum	MFLUCC 15-0620*	KX710146	KX710141	MF135649	MF135662
S. pyriformatum	MFLUCC 15-0627	KX710148	KX710143	MF135650	MF135663
Stilbospora macrosperma	CBS 121883	JX517290	JX517299	–	–
Valsa ambiens	AR 3514	–	EU255210	EU219346	EU222018

Table 2 Habitat and morphological comparison among species of *Distoseptispora.*

Species	Habitats	Conidiophores	Conidia	References
Distoseptispora aquatica	Freshwater	29–41 × 7–9 µm, tapering distally, robust at base, dark brown, 1–3-septate	110–157 × 13.5–16.5 µm, obclavate, elongated, dark brown with bluish green to malachite green tinge, 15–28-distoseptate	Su et al. 2016
D. cangshanensis	Freshwater	44–68 × 4–8 µm, cylindrical, mid olivaceous to brown, 1–5-septate	58–166(–287) × 10–14 µm, obclavate or lanceolate, olivaceous or brown, multi-distoseptate	This study
D. fluminicola	Freshwater	21–33 × 5.5–6.5 µm, cylindrical, olive-green, 1–3-septate	125–250 × 13–15 µm, oblong, obclavate or cylindrical, brown with green tinge, 17–34-distoseptate	Su et al. 2016
D. guttulata	Freshwater	55–90 (–145) × 3.5–5.5 µm, cylindrical, mid or dark brown, 3–4(–10)-septate	75–130(–165) × 7–11 µm, obclavate or lanceolate, rostrate, mid to dark brown or olivaceous, 11–14(–20)-euseptate	Yang et al. 2018
D. obpyriformis	Freshwater	97–119 × 5–7 µm, cylindrical, pale to dark brown, 5–6(–10)-septate	53–71 × 12–16 µm, obpyriform, olivaceous to pale or dark brown, 9–11-distoseptate	This study
D. martinii	Terrestrial	50–110 × 3.5–4.5 µm, cylindrical, dark brown the most part, paler towards the apex, 4–9-septate	15–20 × 11–16 µm, transversal ellipsoid, oblate or subglobose, muriform, pale brown to brown	Xia et al. 2017
D. multiseptata	Freshwater	23–65 × 4.5–8.5 µm, slightly tapering distally, truncate at the apex, brown, 2–3-septate	95–290 × 11–20 µm, obclavate, rostrate, dark-olivaceous green, multi-distoseptate	Hyde et al. 2016b
D. phangngaensis	Freshwater	18–30 (–40) × 4.3–6.5 µm, tapering distally, brown, 2–3-septate	165–350 × 14–19 µm, elongate, obclavate, rostrate, dark olivaceous to mid or dark brown, multi-distoseptate	Yang et al. 2018

Table 2 Continued.

Species	Habitats	Conidiophores	Conidia	References
D. rostrata	Freshwater	82–126 × 5–7 µm, cylindrical, pale brown to brown, 4–7-septate	115–155 × 9–11 µm, obclavate or lanceolate, rostrate, olivaceous to pale brown, (15–)18–23-distoseptate	This study
D. submersa	Freshwater	55–73 × 7–9 µm, cylindrical, brown to dark brown, 4–5-septate	95–123 × 15–19 µm, obclavate, lanceolate or obpyriform, mid olivaceous to brown, 17–23(–28)-distoseptate	This study
D. suoluoensis	Freshwater	80–250 × 4.5–5.8 µm, cylindrical, dark brown, paler at the apical part, septate	(65–)80–125(–145) × 8–13 µm, narrowly obclavate or obspathulate, yellow brown or dark olivaceous, verrucose, 8–10-euseptate	Yang et al. 2018
D. tectonae	Terrestrial	Up to 40 × 4–6 µm, cylindrical, pale brown to dark brown, 2–4-septate	(90–)130–140(–170) × 13–14 µm, cylindric-obclavate, elongate, dark reddish brown, verrucose, 20–28-distoseptate	Hyde et al. 2016b
D. tectonigena	Terrestrial	Up to 110 × 5–11 µm, cylindrical, pale brown to dark brown, septate	148–225(–360) × 11–12 µm, cylindric-obclavate, elongate, dark reddish brown, 20–46-distoseptate	Hyde et al. 2016b

A combined dataset of 3069 characters (ITS, LSU, RPB2 and TEF1α) including gaps with 51 taxa analyzed using RAxML and Bayesian analyses resulted in trees which were topologically congruent with respect to the position of the new taxa investigated. Fig. 1 represents the phylogram generated using ML analysis (value of likelihood: –22813.235538). Twenty-one taxa of *Distoseptisporaceae* including four new species formed a monotypic clade among the selected families or orders of Sordariomycetes with strong support (100% ML and 1.00 PP). The newly collected *Distoseptispora fluminicola* isolates cluster with its ex-type strain with high support (97% ML and 1.00 PP). A strain of the new species *Distoseptispora submersa* clustered with *D. tectonigena* and *D. tectonae* in a well-supported monophyletic clade (94% ML and 1.00 PP). The isolate of *D. cangshanensis* forms a distinct clade among the species of *Distoseptispora*, but is weakly supported. *Distoseptispora rostrata* clusters with *D. obpyriformis* in a strongly-supported monophyletic clade (100% ML and 1.00 PP) between *D. suoluoensis* and *D. martini.*

Taxonomy

Distoseptispora cangshanensis Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 2

Index Fungorum number: IF554289; Facesoffungi number: FoF04193

Etymology – Referring to the collection site from Cangshan Mountain in China.

Holotype – MFLU 18–0474

Saprobic on decaying, submerged wood in freshwater habitats. Sexual morph: Undetermined. Asexual morph: *Colonies* effuse, olivaceous or brown, hairy or velvety. *Mycelium* mostly immersed, consisting of branched, septate, smooth, subhyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, mid-olivaceous to brown, solitary, 1–5-septate, erect, straight or flexuous, unbranched, smooth, cylindrical, 44–68 µm long ($\bar{x}$ = 56 µm, SD = 12, n =15), 4–8 µm wide ($\bar{x}$ = 6 µm, SD = 2, n = 15), truncate at the apex. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, subhyaline to pale brown, cylindrical. *Conidia* acrogenous, solitary, obclavate or lanceolate, rostrate, straight or slightly curved, multi-distoseptate, olivaceous or brown, tapering towards the rounded apex, truncate at the base, 58–166(–287) µm long ($\bar{x}$ = 112 µm, SD = 54, n = 30), 10–14 µm wide ($\bar{x}$ = 12 µm, SD =2, n = 30) at the broadest

part, 4–6 µm wide ($\bar{x}$ = 5 µm, SD =1, n = 30) at the apex, slightly constricted at septa, smooth-walled.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in a stream in Cangshan Mountain, May 2014, Q. Dai, S-220 (MFLU 18–0474, holotype), ex-type living culture MFLUCC 16–0970.

Notes – *Distoseptispora cangshanensis* is mostly similar to *D. rostrata* in having cylindrical, septate conidiophores, and the same shape, coloured, multi-distoseptate and similar sized conidia. However, they can be distinguished by DNA sequence data, that have 13bp (base pair), 46bp and 56bp nucleotide differences in LSU, ITS and TEF1α respectively, when compared to *D. cangshanensis* and *D. rostrata* by using single gene region sequence data (Jeewon & Hyde 2016). *Distoseptispora cangshanensis* also shares similar characters with *D. guttulata* in having cylindrical, septate conidiophores and obclavate or lanceolate, olivaceous or brown conidia. However, *D. cangshanensis* differs from *D. guttulata* by its distoseptate conidia, while *D. guttulata* has euseptate conidia and *D. cangshanensis* has shorter conidiophore (44–68 vs 55–145 µm) (Yang et al. 2018).

Distoseptispora obpyriformis Z.L. Luo & H.Y. Su, sp. nov. Fig. 3

Index Fungorum number: IF 554290; Facesoffungi number: FoF04194

Etymology – Referring to the obpyriform conidia of this fungus.

Holotype – MFLU 18–0476

Saprobic on decaying, submerged wood in freshwater habitats. Sexual morph: undetermined. Asexual morph: *Colonies* effuse, olivaceous or dark brown, hairy, velvety. *Mycelium* mostly immersed, consisting of branched, septate, smooth, subhyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, pale to dark brown, solitary, 5–6(–10)-septate, erect, straight or slightly flexuous, unbranched, smooth, cylindrical, 97–119 µm long ($\bar{x}$ = 108 µm, SD = 11, n = 20), 5–7 µm wide ($\bar{x}$ = 6 µm, SD = 1, n = 20), rounded at the apex. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, pale to dark brown, cylindrical. *Conidia* acrogenous, solitary, obpyriform, 9–11-distoseptate, thick-walled, olivaceous to pale or dark brown, tapering towards the rounded apex, slightly curved, truncate at the base, guttulate, 53–71 µm long ($\bar{x}$ = 62 µm, SD = 9, n = 25), 12–16 µm wide ($\bar{x}$ = 14 µm, SD = 2, n = 25), smooth-walled.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Nujiang River, May 2015, Z.L. Luo, S-769 (MFLU 18–0476, holotype), ex-type living culture MFLUCC 17–1694; October 2016, Z.L. Luo, S-867 (MFLU 18–0477, paratype) living culture DLUCC 0867.

Notes – Two specimens of *Distoseptispora obpyriformis* were collected from Nujiang River but in different collecting seasons and sites. *Distoseptispora obpyriformis* shares similar morphological characters with *D. rostrata* in the shape, colour and size of its conidiophores, however, *D. obpyriformis* differs from *D. rostrata* in having obpyriform, shorter conidia (53–71 vs 115–155 µm) and they are also phylogenetically distinct (Fig. 1). Additionally, with the exception of *D. martinii*, the short conidia of *D. obpyriformis* are also different from the longer conidia of other *Distoseptispora* species (Table 2).

Distoseptispora rostrata Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 4

Index Fungorum number: IF 554291; Facesoffungi number: FoF04195

Etymology – Referring to the rostrate conidia of this fungus.

Holotype – MFLU 18–0479

Saprobic on decaying, submerged wood in freshwater habitats. Sexual morph: undetermined. Asexual morph: *Colonies* effuse, olivaceous or brown, hairy or velvety. *Mycelium* mostly immersed, consisting of branched, septate, smooth, subhyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, pale brown to brown, solitary, 4–7-septate, erect, straight or slightly flexuous, unbranched, smooth, cylindrical, 82–126 µm long ($\bar{x}$ = 104 µm, SD = 22, n =15), 5–7 µm wide ($\bar{x}$ = 6 µm, SD = 1, n = 15), rounded at the apex. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, pale to dark brown, cylindrical, sometimes with

percurrent proliferation. *Conidia* acrogenous, solitary, obclavate or lanceolate, rostrate, straight or slightly curved, (15–)18–23-distoseptate, olivaceous to pale brown, slightly tapering towards the rounded apex, truncate at the base, 115–155 µm long ($\bar{x}$ = 135 µm, SD = 20, n = 30), 9–11 µm wide ($\bar{x}$ = 10 µm, SD =1, n = 30), smooth-walled.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Nujiang River, May 2015, X.J. Su, S-351 (MFLU 18–0479, holotype, HKAS 92781, isotype), ex-type living culture MFLUCC 16–0969; October 2016, Z.L. Luo, S-885 (MFLU 18–0475, paratype) living culture DLUCC 0885.

Notes – *Distoseptispora rostrata* resembles *D. guttulata* in having cylindrical, pale brown to brown, septate conidiophores and obclavate or lanceolate, rostrate, olivaceous conidia. However, *D. rostrata* can be distinguished from *D. guttulata* by its (15–)18–23-distoseptate conidia, while *D. guttulata* has 11–14(–20)-euseptate conidia. The multi-gene phylogenetic analyses also showed that they are different species (Fig. 1).

Distoseptispora submersa Z.L. Luo, K.D. Hyde & H.Y. Su, sp. nov. Fig. 5

Index Fungorum number: IF554292; Facesoffungi number: FoF04196

Etymology – Referring to the submerged habitats of the fungus

Holotype – MFLU 18–0478

Saprobic on decaying, submerged wood in freshwater habitats. Sexual morph: undetermined. Asexual morph: *Colonies* effuse, olivaceous or black, hairy or velvety. *Mycelium* mostly immersed, consisting of branched, septate, smooth, hyaline to pale brown hyphae. *Conidiophores* macronematous, mononematous, brown to dark brown, solitary, 4–5-septate, erect, straight or flexuous, unbranched, smooth, cylindrical, rarely percurrently proliferating, 55–73 µm long ($\bar{x}$ = 64 µm, SD = 9, n =15), 7–9 µm wide ($\bar{x}$ = 8 µm, SD = 1, n = 15), truncate at the apex. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, brown, cylindrical. *Conidia* acrogenous, solitary, obclavate, lanceolate, rostrate, straight or slightly curved, 17–23(–28)-distoseptate, mid olivaceous to brown, tapering towards the rounded apex, truncate at the base, 95–123 µm long ($\bar{x}$ = 109 µm, SD = 14, n = 20), 15–19 µm wide ($\bar{x}$ = 17 µm, SD =2, n = 20), smooth-walled.

Material examined – CHINA, Yunnan Province, saprobic on decaying wood submerged in Nujiang River, May 2015, Q. Dai, S-301 (MFLU 18–0478, holotype, HKAS 92806, isotype), ex-type living culture MFLUCC 16–0946.

Notes – *Distoseptispora submersa* agrees with the generic concept of *Distoseptispora* in having macronematous, olivaceous to brown, cylindrical conidiophores, monoblastic, integrated, determinate, terminal conidiogenous cells and obclavate, lanceolate, rostrate, distoseptate conidia (Yang et al. 2018). *Distoseptispora submersa* is phylogenetically close to *D. tectonae*, but *D. submersa* have larger conidiophores (55–73 × 7–9 µm vs up to 40 × 4–6 µm) and shorter conidia (95–123 vs 130–140 µm) (Table 2).

Discussion

Sporidesmium-like taxa are commonly collected from terrestrial habitats (Wu & Zhuang 2007), but they have frequently been recorded from submerged decaying wood in freshwater (Hyde & Goh 1998a, Ho et al. 2001, Cai et al. 2003, Hyde et al. 2016b, Su et al. 2016, Yang et al. 2018). *Sporidesmium* and its related genera are an interesting group as they share similar characters in having holoblastic, septate conidia and monoblastic, determinate or percurrent conidiogenous cells, and are difficult to classify based on morphology alone (Shenoy et al. 2006, Su et al. 2016, Yang et al. 2018). In this study, we collected five sporidesmium-like taxa from rivers and streams in northwestern Yunnan, China. Phylogenetic analyses show that eight hyphomycetous strains are positioned in Distoseptisporaceae in a robust clade. Four new *Distoseptispora* species are introduced in this paper based on morphology and molecular sequence data.

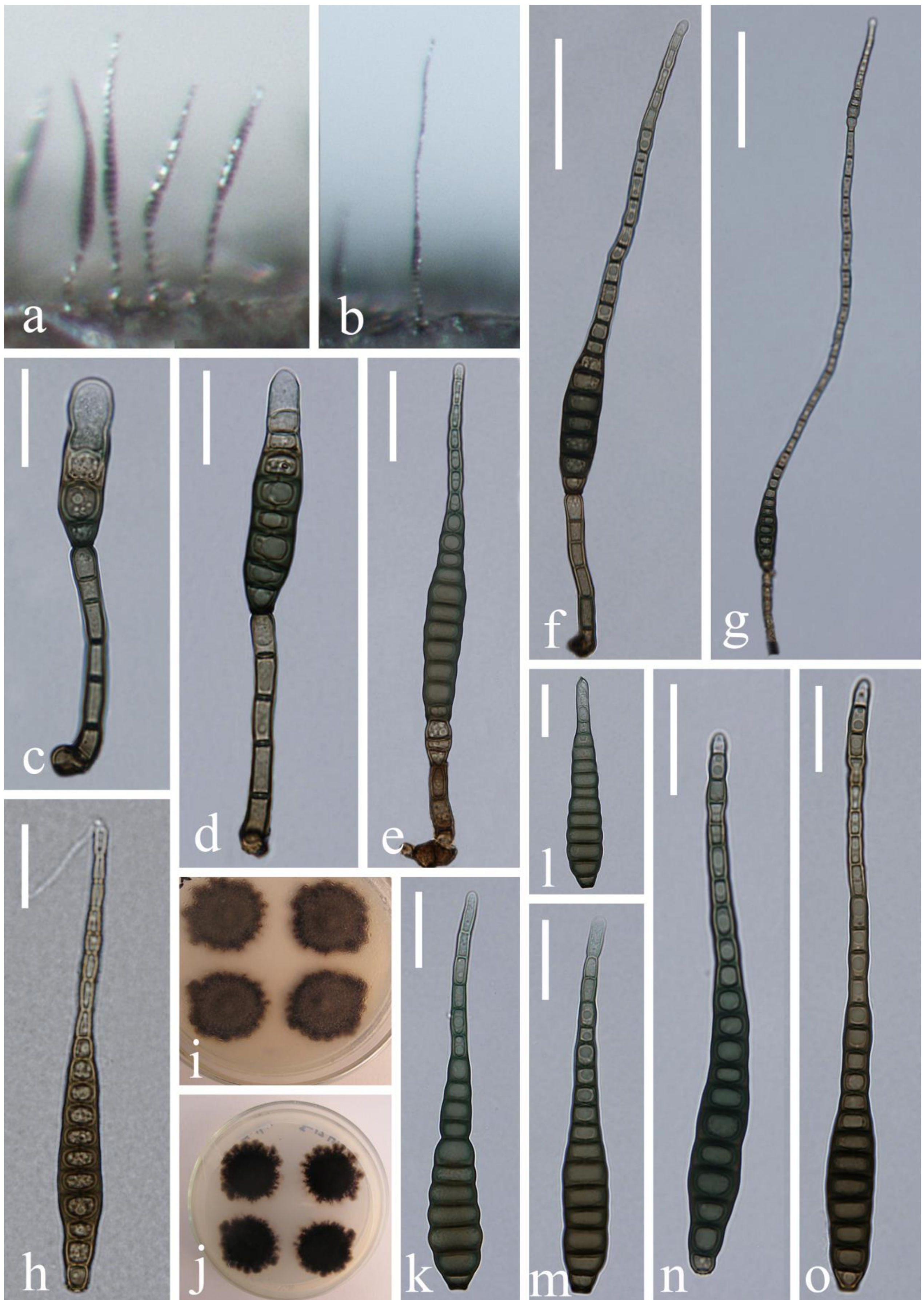

Figure 2 – *Distoseptispora cangshanensis* (MFLU 18–0474, holotype). a, b Colonies on substrate. c–g Conidiophores with conidia. k–o Conidia. h Germinating conidium. i, j Culture on PDA (j from below). Scale bars: f, g = 60 µm, c–e, h, k–o = 30 µm.

Figure 3 – *Distoseptispora obpyriformis* (MFLU 18–0476, holotype). a Colonies on substrate. b–d Conidiophores and conidia. e, f Conidiogenesis. g–i Conidia. j Germinating conidium. k, l Culture on PDA after 21 days (l from below). Scale bars: b–d = 50 µm, e–j = 30 µm.

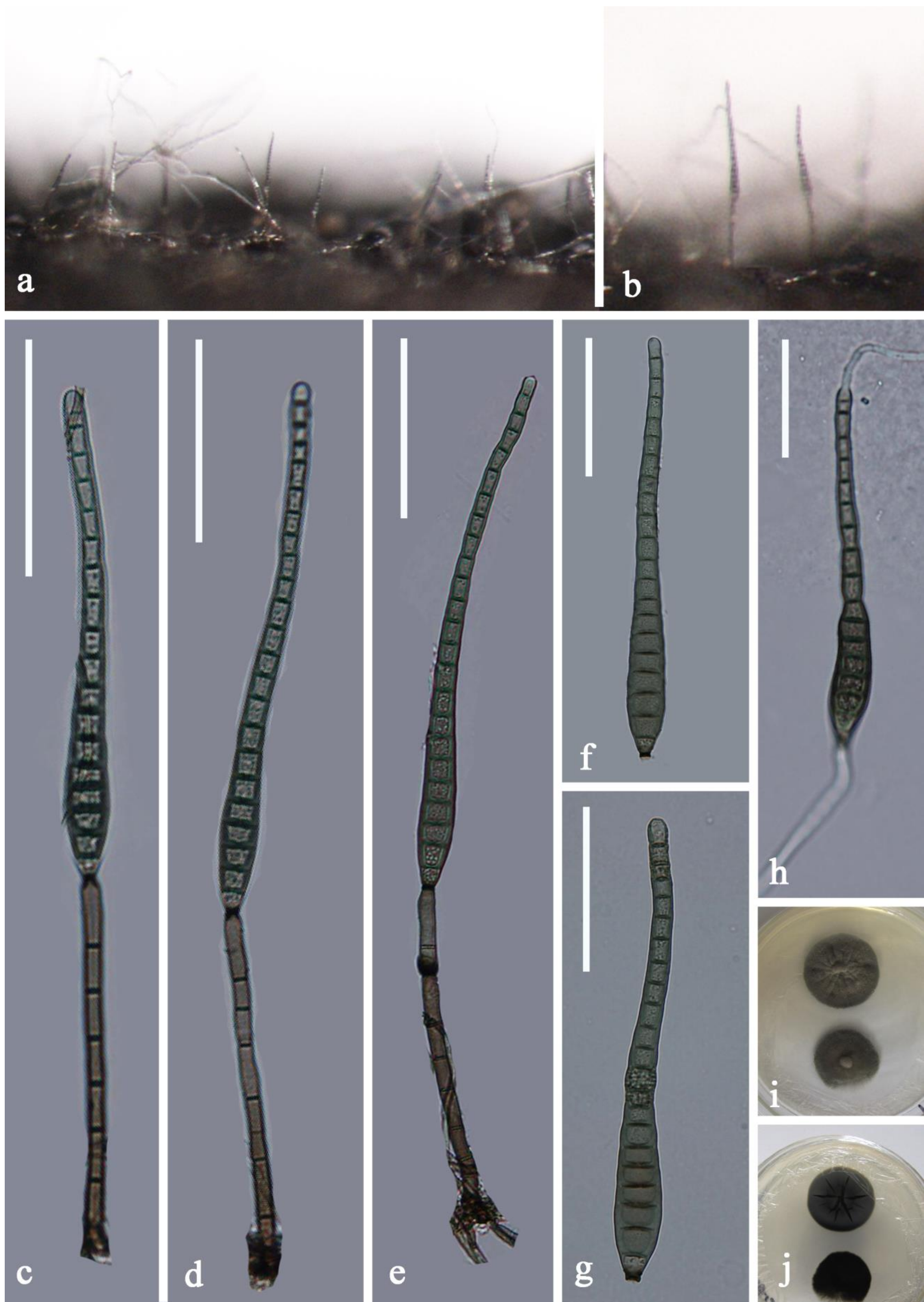

Figure 4 – *Distoseptispora rostrata* (MFLU 18–0479, holotype) a, b Colonies on substrate. c–e Conidiophores and conidia. f, g Conidia. h Germinating conidium. i, j Culture on PDA after 21 days (l from below). Scale bars: c–e = 70 µm, f–h = 50 µm.

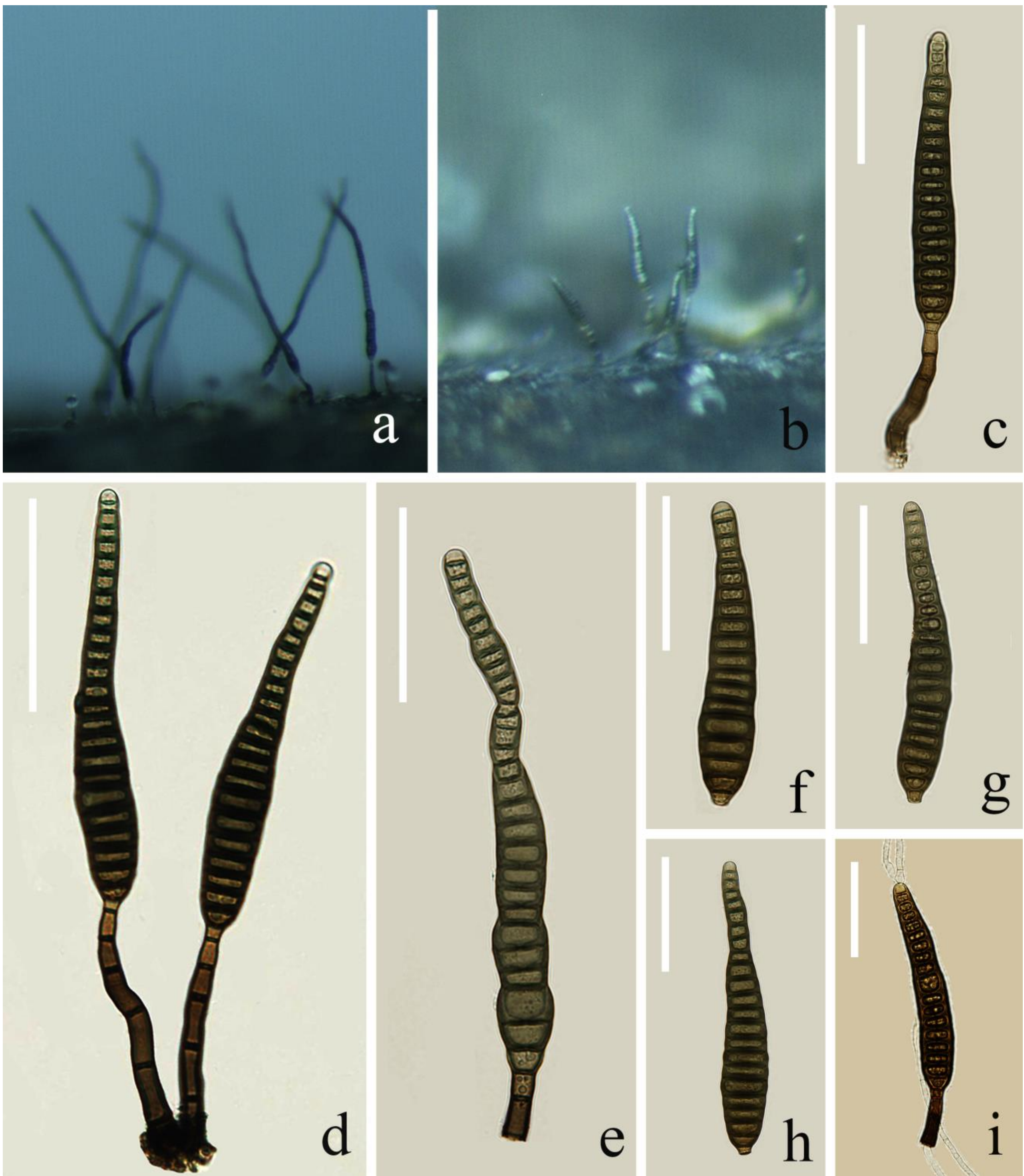

Figure 5 – *Distoseptispora submersa* (MFLU 18–0478, holotype) a, b Colonies on substrate. c, d Conidiophores and conidia. e Conidiogenesis with conidia. f–h Conidia. i Germinating conidium. Scale bars: c–i = 50 µm.

Distoseptispora is an asexual genus and there are presently no reports on the sexual morph of this genus. There are nine species presently accepted in *Distoseptispora*, with five species reported from Thailand (Hyde et al. 2016b, Yang et al. 2018) and four species from southwestern China (Su et al. 2016, Yang et al. 2018). All *Distoseptispora* species are saprobic and isolated from the decaying wood in terrestrial or aquatic habitats in tropical or subtropical regions. Xia et al. (2017) transferred *Acrodictys martinii* to the genus *Distoseptispora* as *D. martinii* based on their phylogenetic analysis, but this species is easily distinguished from other species in *Distoseptispora*

(Distoseptisporaceae) by its transversal ellipsoid, oblate or subglobose, muriform conidia. Most Acrodictys-like species belong to Acrodictyaceae, Junewangiaceae or Savoryellaceae (Xia et al. 2017).

Acknowledgments

We would like to thank the National Natural Science Foundation of China (NSFC 31660008, 31460015) and "Collaborative Innovation Center for Biodiversity and Conservation in the Three Parallel Rivers Region of China" for financial and laboratory support. Zong-Long Luo thanks Dr. Shaun Pennycook from Landcare Research, Auckland, New Zealand, for advising on the taxon name and Jing Yang, Yan-Mei Zhang and Hong-Wei Shen are acknowledged for their help on phylogenetic and morphological work. Jian-Kui Liu thanks Chiang Mai University (Chiang Mai, Thailand) for the offer of a Post-Doctoral Fellowship.

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