Mycosphere 8(10): 1587–1597 (2017) www.mycosphere.org ISSN 2077 7019
Article
Doi 10.5943/mycosphere/8/10/1

Lignicolous freshwater fungi from China I : *Aquadictyospora lignicola* gen. et sp. nov. and new record of *Pseudodictyosporium wauense* from northwestern Yunnan Province

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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

Abstract

This is the first in a series of papers on lignicolous freshwater fungi from China. In this paper, eight fresh collections of taxa of *Dictyosporiaceae* from submerged wood in freshwater are characterized based on morphological characters and phylogenetic analyses of combined ITS, LSU and TEF1α sequence data. A new monotypic genus *Aquadictyospora,* with *A. lignicola* as the type species is introduced based on its distinct morphology and evidences from molecular phylogeny. In addition, detailed description and illustration of *Pseudodictyosporium wauense* from the fresh collection are provided and it is a new record for China.

Key words – Asexual fungi – *Dictyosporiaceae* – Phylogeny – Taxonomy

Introduction

Lignicolous freshwater fungi grow on submerged woody debris in freshwater streams, ponds, lakes and tree hollows (Goh & Hyde 1996, Wong et al. 1998). These taxa play an important role in the decomposition of submerged wood in aquatic habitats by breaking down lignocelluloses and releasing nutrients and are important in ecosystem functioning (Yuen et al. 1998, Bucher et al. 2004, Hyde et al. 2016). The lignicolous freshwater fungi are a taxonomically highly diverse group and mostly encountered in two major classes, Dothideomycetes and Sordariomycetes of the Ascomycota (Hyde et al. 2013, Wijayawardene et al. 2014, Maharachchikumbura et al. 2015, 2016, Liu et al. 2017).

Studies of freshwater fungi in China were first documented in 1988. He (1988) reported *Vibrissea truncorum* (Alb. & Schwein.) Fr. on submerged wood in Guizhou Province. This was probably the first record of lignicolous freshwater fungi in China. About ten years later,

Submitted 18 September 2017, Accepted 6 October 2017, Published 8 November 2017
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mycologists started to investigate the lignicolous freshwater fungi in Hong Kong and reported many new taxa (Tsui et al. 1997, 2000, 2001a, b, 2003, Goh et al. 1998, Hyde & Goh 1998, Ranghoo & Hyde 1998, Goh & Hyde 1999, Ho et al. 1999). In mainland China, investigations of lignicolous freshwater fungi mostly focused in the south to southwestern regions (Inderbitzin 2000, Jeewon et al. 2003, Cai et al. 2005, Hu et al. 2013). Cai et al. (2002) investigated the fungal diversity in Fuxian Lake in Yunnan Province and reported 64 higher fungi, with a new ascomycete *Pseudohalonectria fuxianii* L. Cai, K.M. Tsui, K.Q. Zhang & K.D. Hyde, while Luo et al. (2004) reported fungi from Dianchi Lake. Many new taxa have since been described including several new genera (Inderbitzin 2000, Jeewon et al. 2003, Cai et al. 2005, Cai & Hyde 2007, Wongsawas et al. 2009a, b , Hu et al. 2012, Liu et al. 2015, Su et al. 2015, 2016a, b, Luo et al. 2016, 2017, Wang et al. 2016, Zhu et al. 2016). Hu et al. (2013) also published a review paper of aquatic fungi in China.

Pleosporales is the largest order in the Dothideomycetes, and in recent years, various families and genera in the Pleosporales have undergone considerable revisions and their taxonomy and phylogeny have been reassessed (Goh et al. 1999, Tanaka et al. 2009, 2015, Zhang et al. 2012, Hyde et al. 2013). *Dictyosporiaceae* was introduced to accommodate a holomorphic group of Dothideomycetes that are saprobes on decaying wood and plants debris in terrestrial and freshwater habitats (Liu et al. 2015, Boonmee et al. 2016). The asexual morphs of *Dictyosporiaceae* are characterized by brown, multi-septate, cheirosporous conidia, produced from holoblastic conidiogenous cells, on micronematous conidiophores (Boonmee et al. 2016).

We are carrying out a survey of the diversity of lignicolous freshwater fungi along a north-south gradient in the Asian region (Hyde et al. 2016). To investigate the diversity of the freshwater fungi in China, we selected southwestern China (Yunnan Province), where there are several important rivers selected to study. This area is believed to have unique and diverse ecology and diversity, which have been shown by studies on plants and animals, as well as fungi (Yang et al. 2004). This is the first in a series of papers on these fungi in southwestern China, and a new genus *Aquadictyospora* which assign in *Dictyosporiaceae* (Pleosporales, Dothideomycetes) was established based on the morphological and phylogenetic analyses. In addition, a fresh collection was identified as *Pseudodictyosporium wauense* Matsush., the detailed description and illustration are provided, and it is the new record for China.

Materials & Methods

Submerged woody substrates were collected from a stream in Cangshan Mountain, Yunnan Province, China, and taken back to the laboratory in Zip-lock plastic bags. The samples were incubated in sterile humid plastic boxes for 1–3 weeks at 25–32℃, and processed following the methods described in Taylor & Hyde (2003). Specimens were studied under a Motic SMZ 168 Series stereoscope and photographed by OLYMPUS BX51 microscope imaging system. Measurements of the fungal structures were taken with Image-Pro-Express software.

The pure cultures were attempted by single spore isolation followed Chomnunti et al. (2014). The cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC) and Dali University Culture Collection (DLUCC). Herbarium specimens are deposited at the herbarium of Mae Fah Luang University (MFLU) and Dali University (DLU). Facesoffungi and Index Fungorum numbers were obtained as in Jayasiri et al. (2015) and Index Fungorum (2017).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh mycelia grown on PDA at 25–32℃ for 30 to 45 d. The EZ geneTM Fungal gDNA kit (GD2416) was used to extract DNA according to the manufacturer's instructions. The primer pairs ITS5/ITS4 (White et al 1990), LROR/LR7 (Vilgalys & Hester 1990) and EF1-983F/EF1-2218R (Carbone & Kohn 1999) were used to amplify the gene regions of ITS, LSU and TEF1α respectively. The PCR thermal cycle program for ITS and LSU amplification was as follows: initial denaturation of 94 °C for 3 mins, followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 50 seconds, elongation at 72 °C for 1 min. Regions of TEF1α was amplified with initial denaturation of 95 °C for 5 min, followed by 40

cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 90 seconds, elongation at 72 °C for 90 seconds, and the final extension at 72 °C for 10 mins included for each condition of amplification. PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amershamproduct code: 27–9602–01). The sequencing works were carried by Beijing Tsingke Biological Engineering Technology and Services Co., Ltd (Beijing, P.R. China).

Phylogenetic analysis

Sequence data for relevant strains were downloaded from GenBank following recent publications (Boonmee et al. 2016, Wang et al. 2016). 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). A maximum likelihood (ML) analysis was performed using RAxMLGUI v. 1.3 (Silvestro & Michalak 2011). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR+GAMMA substitution model.

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 1,000,000 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 to TreeBASE (www.treebase.org, submission number 21532). Resulted trees were viewed in Treeview (Page 1996). The terminals of the tree (Fig. 1) are labeled with species and the isolates/culture collection codes as provided in GenBank.

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	TEF1α
Aquaticheirospora lignicola	RK 2006a*	AY864770	AY736378	–
Aquadictyospora lignicola	**MFLUCC 17–1318***	**MF948621**	**MF948629**	**MF953164**
Cheirosporium triseriale	HMAS 180703*	EU413953	EU413954	–
Dendryphiella fasciculata	MFLUCC 17–1074*	MF399213	MF399214	–
Dendryphiella paravinosa	CBS 141286*	KX228258	KX228309	–
Dendryphiella eucalyptorum	CBS 137987	KJ869139	KJ869196	–
Dictyocheirospora aquatica	KUMCC 15–0305*	KY320508	KY320513	–
Dictyocheirospora bannica	KH 332	LC014543	AB807513	AB808489
Dictyocheirospora garethjonesii	MFLUCC 16–0909*	KY320509	KY320514	–
Dictyocheirospora garethjonesii	KUMCC 15–0396	KY320510	KY320515	–
Dictyocheirospora garethjonesii	**DLUCC 0848**	**MF948623**	**MF948631**	**MF953166**
Dictyocheirospora rotunda	MFLUCC 14–0293*	KU179099	KU179100	–
Dictyocheirospora rotunda	**DLUCC 0856**	**MF948624**	**MF948632**	**MF953167**
Dictyocheirospora rotunda	**MFLUCC 17–1687**	**MF948625**	**MF948633**	**MF953168**
Dictyocheirospora rotunda	**DLUCC 0747**	**MF948626**	**MF948634**	**MF953169**
Dictyocheirospora rotunda	**DLUCC 0804**	**MF948627**	**MF948635**	**MF953170**
Dictyocheirospora pseudomusae	KH 412	LC014549	AB807516	AB808492
Dictyocheirospora pseudomusae	yone 234*	LC014550	AB807520	AB808496
Dictyocheirospora vinaya	MFLUCC 14–0294*	KU179102	KU179103	–
Dictyosporium aquaticum	MF1318*	KM610236	–	–

Table 1 Continued.

Species	Collection/Isolate number	GenBank accession number		
		ITS	LSU	TEF1α
Dictyosporium elegans	NBRC 32502*	DQ018087	DQ018100	–
Dictyosporium hughesii	KT 1847	LC014548	AB807517	AB808493
Dictyosporium meiosporum	MFLUCC 10–0131*	KP710944	KP710945	–
Dictyosporium tetrasporum	KT 2865	LC014551	AB807519	AB808495
Dictyosporium thailandicum	MFLUCC 13–0773*	KP716706	KP716707	–
Digitodesmium bambusicola	CBS 110279*	DQ018091	DQ018103	–
Gregarithecium curvisporum	KT 922*	AB809644	AB807547	–
Jalapriya inflata	NTOU 3855	JQ267362	JQ267363	–
Jalapriya pulchra	MFLUCC 15–0348	KU179108	KU179109	–
Jalapriya pulchra	**MFLUCC 17–1683**	**MF948628**	**MF948636**	**MF953171**
Jalapriya toruloides	CBS 209.65	DQ018093	DQ018104	–
Periconia igniaria	CBS 379.86	LC014585	AB807566	AB808542
Periconia igniaria	CBS 845.96	LC014586	AB807567	AB808543
Pseudocoleophoma calamagrostidis	KT 3284*	LC014592	LC014609	LC014614
Pseudocoleophoma polygonicola	KT 731*	AB809634	AB807546	AB808522
Pseudocoleophoma typhicola	MFLUCC 16–0123*	KX576655	KX576656	–
Pseudodictyosporium elegans	CBS 688.93*	DQ018099	DQ018106	–
Pseudodictyosporium indicum	CBS 471.95	DQ018097	–	–
Pseudodictyosporium thailandica	MFLUCC 16–0029*	KX259520	KX259522	KX259526
Pseudodictyosporium wauense	NBRC 30078	DQ018098	DQ018105	–
Pseudodictyosporium wauense	KRP88–6	HM036613	–	–
Pseudodictyosporium wauense	**DLUCC 0801**	**MF948622**	**MF948630**	**MF953165**
Vikalpa australiensis	HKUCC 8797*	DQ018092	–	–

Results

Phylogenetic analyses

The combined ITS, LSU and TEF1α dataset consisted 43 sequences representing all genera of the *Dictyosporiaceae* and *Periconia igniaria* (CBS 379.86, CBS 845.96) was selected as out group. The alignment comprised 2674 characters, of which 2000 were constant, 485 parsimony-informative and 189 parsimony-uninformative. The best scoring RaxML tree is shown here (Figure 1) (value of likelihood: –12295.132955). The robust clade containing 12 genera (100 ML/1.00 Bayesian) has identical topologies in the multi-gene analyses (Fig. 1). In particular, the newly collected *Dictyocheirospora rotunda*, *D. garethjonesii* and *Jalapriya pulchra* isolates cluster with their type strain respectively with highly supported value (100 ML/1.00 Bayesian). The new isolate of *Pseudodictyosporium wauense* also cluster with its identified strains from previous studies. The isolate of *Aquadictyospora* formed a distinct clade among the genera of *Dictyosporiaceae.*

Taxonomy

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

Index Fungorum number: IF553861; Facesoffungi number: FoF03767

Etymology – referring to the aquatic habitats and shape of the conidia of the fungus.

Saprobic on submerged decaying wood. Sexual morph: Undetermined. Asexual morph: *Conidiomata* on natural substratum sporodochia, superficial, compact, scattered, circular or subglobose, dark brown to black, velvety. Mycelium immersed, consisted of septate, branched, smooth, thin-walled, hyaline hyphae. Conidiophores micronematous, reduced to conidiogenous cells, pale brown, smooth. Conidiogenous cells monoblastic. Conidia appearing broadly rounded, composed of 4–6 compactly arranged rows of uniformly medium brown cells in upper half, with a basal, subglobose, hyaline cell, not complanate. Conidial appendages absent.

Type species – *Aquadictyospora lignicola* Z.L. Luo, W.L. Li, K.D. Hyde & H.Y. Su

Aquadictyospora lignicola Z.L. Luo, W.L. Li, K.D. Hyde & H.Y. Su, sp. nov.

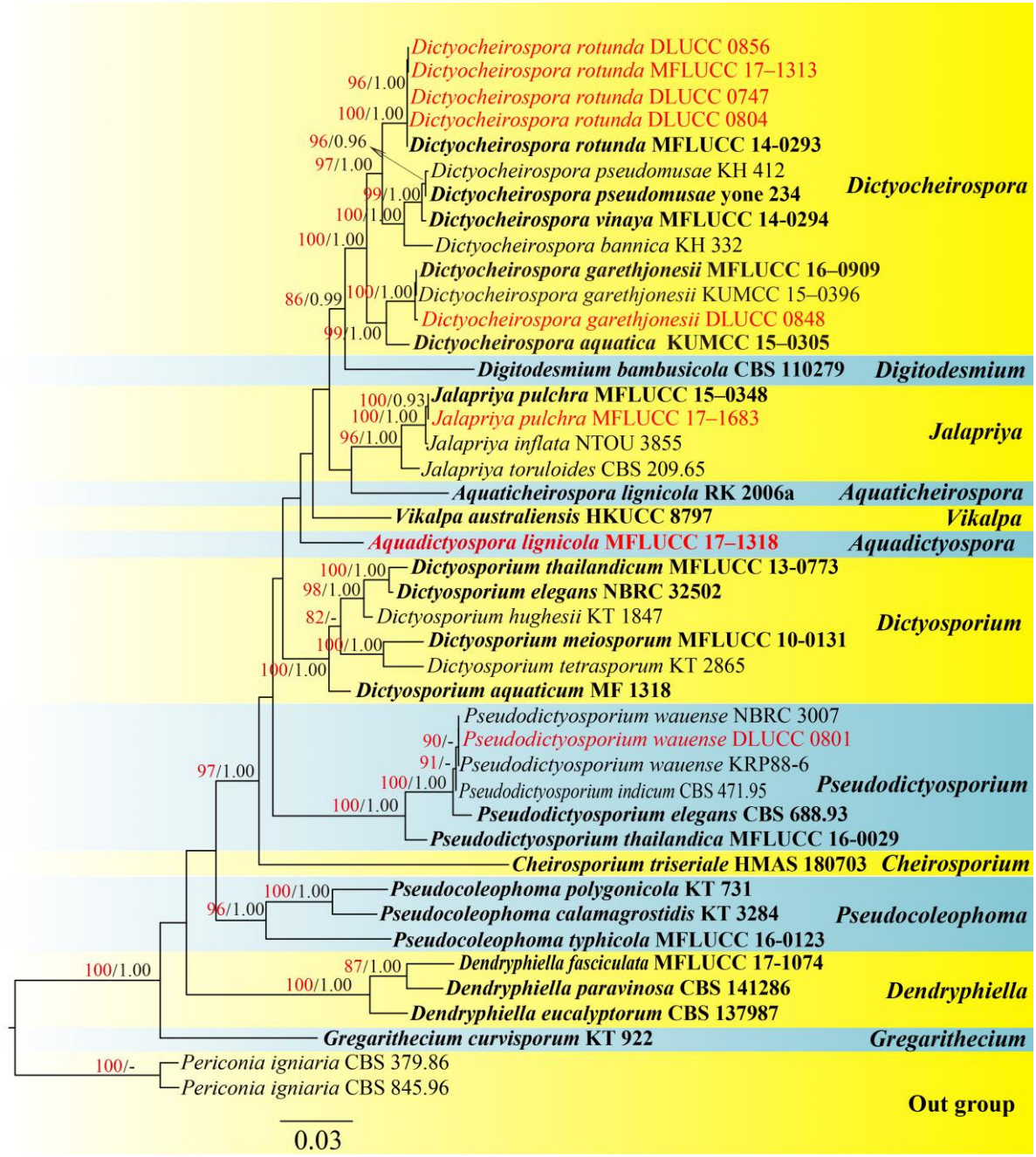

Figure 1 Phylogram generated from maximum likelihood analysis (RAxML) based on combined ITS, LSU and TEF1α sequence data of *Dictyosporiaceae*. Bootstrap support values for maximum likelihood (red) and Bayesian posterior probabilities (black) greater than 75% and 0.95 are given above the nodes. The tree is rooted to *Periconia igniaria* (CBS 379.86, CBS 845.96). Newly generated sequences are indicated in red and ex-type strains are in bold.

Index Fungorum number: IF553862; Facesoffungi number: FoF03768, Fig. 2

Etymology – The name lignicola is derived from the words 'lignum', meaning wood and cola, meaning habitat, referring to the habitat in which this fungus was found.

Holotype – MFLU 17–1422

Saprobic on submerged decayed wood in aquatic habitats. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Conidiomata on natural substratum sporodochia, 200 µm to 1 mm diam., superficial, compact, scattered, circular or subglobose, dark brown to black, velvety. Mycelium immersed, consisted of septate, branched, smooth, thin-walled, hyaline, 2–4 µm wide hyphae. Conidiophores micronematous, reduced, pale brown, smooth. Conidiogenous cells

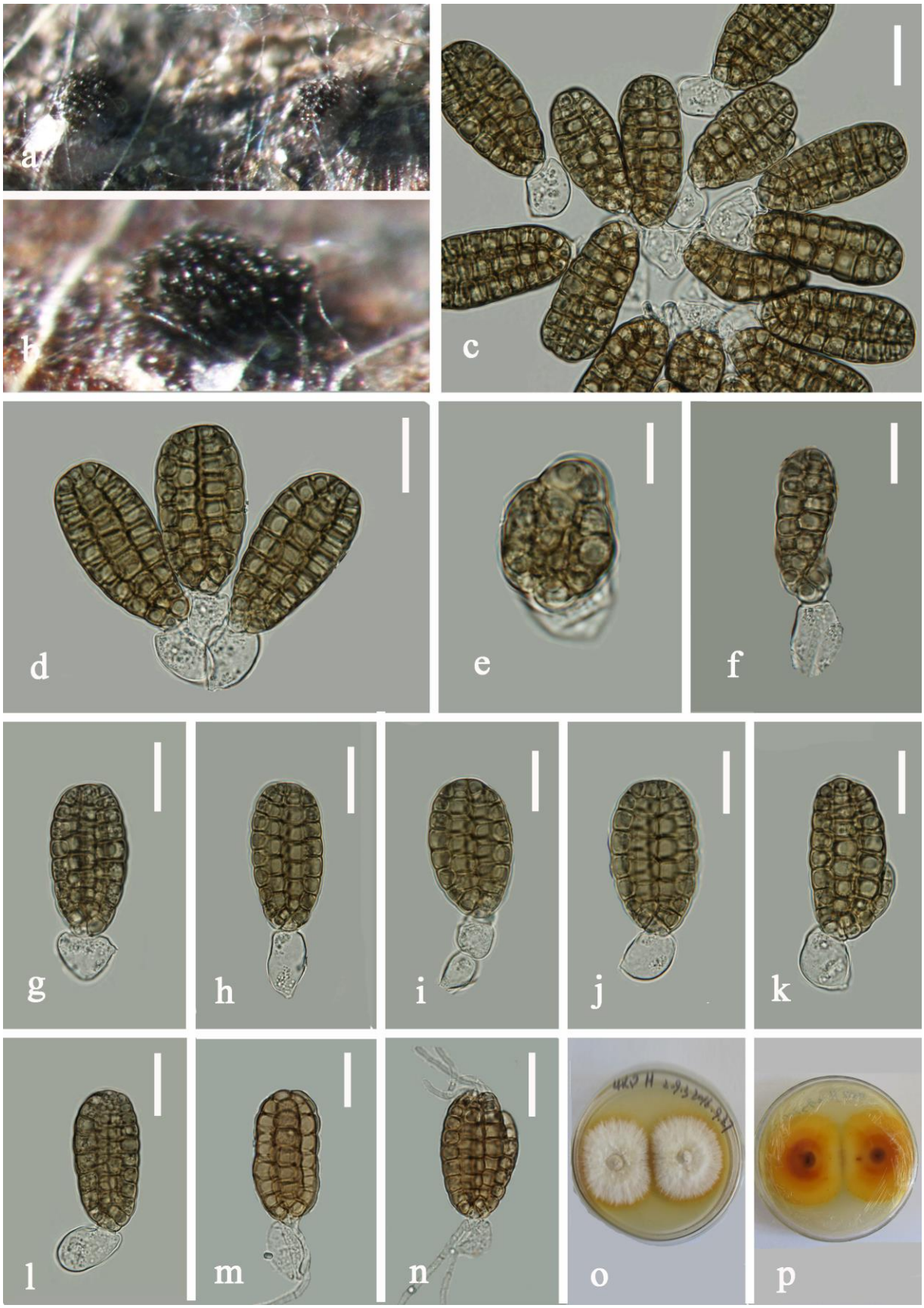

Figure 2 – *Aquadictyospora lignicola*. (MFLU 17–1422, holotype) a–b Colonies on wood. c–d Squash mount of conidia. e–i Conidia. m–n Germinating conidium. o–p Colonies on PDA from surface and reverse. – Scale bars: c–n = 25 µm.

holoblastic. Conidia appearing broadly rounded in upper half, composed of uniformly medium brown, compactly adpressed 4–6 apically curved rows of cells appearing muriform, cheiroid, 46–54 µm long ($\bar{x}$ = 50 µm, SD = 4, n = 40), 21–27 µm wide, ($\bar{x}$ = 24 µm, SD = 3, n = 40); in lower half with a broadly ovate to subglobose, hyaline, smooth basal cell, 16–22 µm long ($\bar{x}$ = 19 µm, SD = 3, n = 40), 12–15 µm wide, ($\bar{x}$ = 13.5 µm, SD = 1.5, n = 40), not complanate, secession schizolytic. Conidial appendages absent.

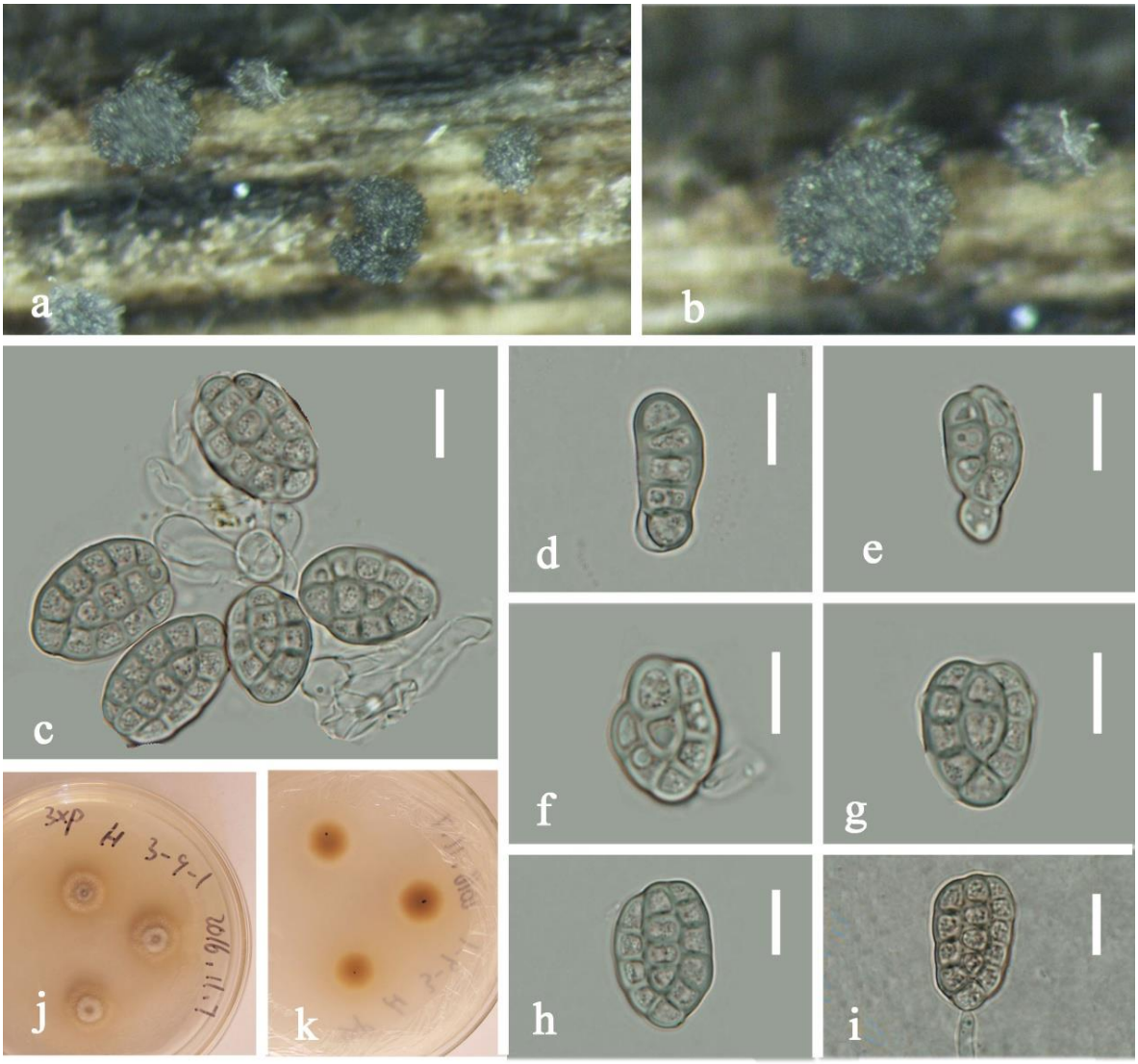

Figure 3 – *Pseudodictyosporium wauense* a–b Colonies on wood. c *Conidiogenous cells.* d–h Conidia. i Germinating conidium. j–k Colonies on PDA from surface and reverse. – Scale bars: c–i =10 µm.

Pseudodictyosporium wauense Matsush. Bull. natn. Sci. Mus., Tokyo 14(3): 473 (1971)

Facesoffungi number: FoF03769, Fig. 3

Saprobic on submerged decayed wood in aquatic habitats. Sexual morph: Undetermined. Asexual morph: Hyphomycetous. Conidiomata on natural substratum sporodochia, superficial, punctiform to effuse, scattered, sometimes coalescing, pale brown to dark brown, with or without a mucilage covering, rarely inconspicuous. Mycelium immersed, composed of septate, branched, subhyaline to pale brown, smooth-walled hyphae. Conidiophores micronematous, aseptate, simple, hyaline to pale brown, smooth. Conidiogenous cells integrated, holoblastic, terminal, determinate, doliiform to cylindrical. Conidia 19.5–22.5 µm long ($\bar{x}$ = 21 µm, SD = 1.5, n = 50), 12.5–15.5 µm wide, ($\bar{x}$ = 14 µm, SD = 1.5, n = 50), acrogenous, solitary, dry, cheiroid, very pale brown, smooth-walled,

euseptate or distoseptate, consisting of a truncate basal cell on which three rows of cells arise parallelly and compactly with all 3 rows in different planes, with or without appendages.

Material examined – CHINA, Yunnan Province, Dali, saprobic on decaying wood submerged in a stream in Cangshan Mountain, June 2016, S.M. Tang, 3XP H 3–9–1, (DLU 0801), living culture DLUCC 0801.

Notes – *Pseudodictyosporium wauense*, the type species of *Pseudodictyosporium*, was introduced by Matsushima et al. (1971). In this study, an isolate was obtained from submerged decaying wood collected in Yunnan Province, China. Its morphological characters identify the taxon as *Pseudodictyosporium wauense.* Based on the phylogenetic study (Fig. 1), our strain clusters with two other isolates of *P. wauense* (NBRC 3007, KRP88–6) with good branch support (90% ML). Therefore, the identification of this isolate is confirmed based on both morphology and phylogeny. It is a new record for China.

Acknowledgements

HongYan Su would like to thank the National Natural Science Foundation of China (NSFC 31460015, 31660008) and "Collaborative Innovation Center for Biodiversity and Conservation in the Three Parallel Rivers Region of China" for financial and laboratory support. ZongLong Luo thank Dr. Shaun Pennycook from Landcare Research, Auckland, New Zealand, for advising on the taxon name and thank RuXiao Wang, QiShan Zhou, ZhengPeng Li, QingXiong Ruan and HongWei Shen for their help on phylogeny and morphology work. JianKui Liu would like to thank the National Natural Science Foundation of China (NSFC 31600032) and Science and Technology Foundation of Guizhou Province (LH [2015]7061).

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