



New species and records of *Dictyocheiropsora* from submerged wood in north-western Yunnan, China

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Abstract

Two new species of *Dictyocheiropsora*, *D. aquatica* and *D. garethjonesii*, isolated from submerged wood in aquatic habitats in north-western Yunnan, China, are introduced in this paper. The introductions are based on morphology and molecular analysis of DNA sequence data. We also recovered two fresh isolates of *Dictyocheiropsora rotunda* D' Souza, Bhat & K.D. Hyde, which is a first record for China. Descriptions and illustrations of *D. aquatica*, *D. garethjonesii* and *D. rotunda* are provided. The phylogenetic analysis of combined ITS and LSU sequence data place the isolates within the family *Dictyosporiaceae* (Pleosporales). A synopsis of characters of the accepted species of *Dictyocheiropsora* is provided.

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

Introduction

The order Pleosporales has been of great research interest in recent years and has undergone considerable revision (Zhang et al. 2009, 2012, Hyde et al. 2013, Ariyawansa et al. 2015). Boonmee et al. (2016) introduced the family *Dictyosporiaceae* (Pleosporales) to accommodate most cheiropsorous hyphomycete genera that are saprobes on decaying wood and plant debris in terrestrial and freshwater habitats. One of the diagnostic characteristics of *Dictyosporiaceae* is their multicellular cheiroid conidia, and these morphological features separate it from other families in the suborder Massarineae (Hyde et al. 2016a).

The genus *Dictyocheiropsora* D' Souza, Bhat & K.D. Hyde, introduced by Boonmee et al. (2016) with *Dictyocheiropsora rotunda* D' Souza, Bhat & K.D. Hyde as the type species, is characterized by dark sporodochial colonies that produce aero-aquatic, cheiroid, dictyosporous conidia (Boonmee et al. 2016). Presently, there are seven species accepted in the genus.

We are studying the freshwater lignicolous fungi in three rivers, viz. Jinsha, Lancang and Dulong, in north-western Yunnan Province, China (Hyde et al. 2016b, Luo et al. 2016). In this

study, five fresh collections of cheirosporous hyphomycetous taxa were made from submerged wood. Descriptions and illustrations are provided for two new species, *D. aquatica* and *D. garethjonesii* and for *D. rotunda*, which is a new record for China.

Material & methods

Isolation and morphology

Specimens of submerged decaying wood were collected from Jinsha, Lancang and Dulong rivers, in north-western Yunnan, China and brought to the laboratory in plastic bags. The samples were incubated in plastic boxes lined with moistened tissue paper at room temperature for one week and processed and examined following the methods described in Taylor & Hyde (2003). Morphological observations were made using a Motic SMZ 168 Series stereomicroscope and photographed by an OLYMPUS BX51 microscope imaging system. The fungal structures were measured using Image-Pro-Express software.

Single spore isolations were made to obtain the pure cultures as described in Chomnunti et al. (2014). The cultures are deposited in Mae Fah Luang University Culture Collection (MFLUCC), Culture collection of Kunming Institute of Botany (KUMCC) and Dali University (DLUCC). Herbarium specimens are deposited at the herbarium of Mae Fah Luang University (MFLU), the Herbarium of Cryptogams Kunming Institute of Botany Academia Sinica (HKAS) and Dali University (DLU). Facesoffungi and Index Fungorum numbers were obtained as in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from fresh fungal mycelium grown on PDA at room temperature. The EZ gene™ Fungal gDNA kit (GD2416) was used to extract DNA according to the manufacturer's instructions. ITS and LSU gene regions were amplified using the primer pairs ITS5/ITS4 and LROR/LR5. The final volume of the PCR reaction was 25 µL and 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, 100 mM KCl, 3 mM MgCl₂, stabilizer and enhancer), 1 µL of each primer (10 µM), 1 µL genomic DNA extract and 9.5 µL deionised water. The PCR thermal cycle program for ITS amplification was as follows: initially 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 1 minutes, annealing at 53 °C for 30 seconds, elongation at 72 °C for 1 min. The PCR thermal cycle program for LSU was followed as initially 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 52 °C for 40 seconds, elongation at 72 °C for 90 seconds. PCR products were purified using minicolumns, purification resin and buffer according to the manufacturer's protocols (Amersham product code: 27–9602–01). The sequences were carried by Beijing Tsingke Biological Engineering Technology and Services Co., Ltd (Beijing, P.R. China).

Phylogenetic analysis

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

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. Maximum parsimony analyses were performed using the heuristic search option with 1000 random taxa additions and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight and gaps

were treated as missing data. Maxtrees were unlimited, branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. Clade stability was assessed using a bootstrap (BT) analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Hillis & Bull 1993).

Bayesian analyses were performed by using PAUP v.4.0b10 (Swofford 2002) and MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003). The model of evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (PP) (Rannala & Yang 1996) were performed by Markov Chain Monte Carlo Sampling (BMCMC) in MrBayes v. 3.0b4 (Liu et al. 2012). Six simultaneous Markov Chains were run for 1 million generations and trees were sampled every 100th generation (Resulting 10000 total trees) (Cai et al. 2006). The first 2000 trees representing the burn-in phase of the analyses were discarded and the remaining 8000 (post burning) trees used for calculating posterior probabilities (PP) in the majority rule consensus tree (Cai et al. 2006, Liu et al. 2012).

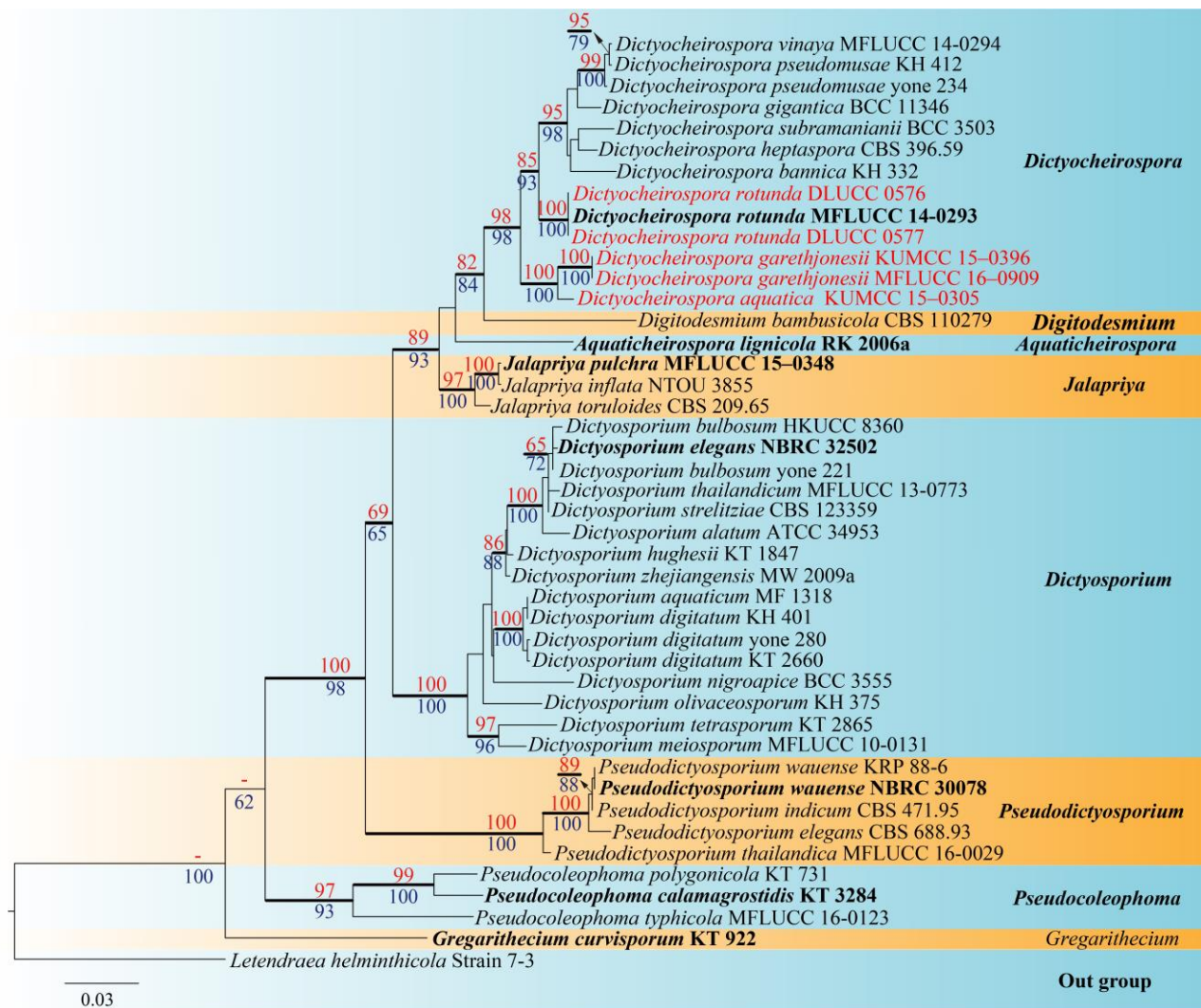


Fig. 1 Phylogram generated from Maximum likelihood analysis (RAxML) based on combined ITS and LSU sequence data for the family Dictyosporiaceae. Bootstrap support values for maximum likelihood (red) equal to or greater than 60% are given above the nodes. Bootstrap support values for maximum parsimony (blue) equal to or greater than 60% are given below the nodes. Branches with Bayesian posterior probabilities greater than 0.95 are in bold. The tree is rooted to *Letendreaa helminthicola* (strain 7–3). Newly generated sequences are indicated in red and generic types are highlighted in bold.

The phylogenetic analyses were carried out with combined ITS and LSU sequence data alignment to illustrate the placement of the isolates in Tubeufiaceae. All new sequence data generated in this study are deposited in GenBank (Table 1). All alignments are deposited in TreeBASE (www.treebase.org, submission number 20337). Phylogenetic trees were viewed in Treeview (Page 1996). The terminals of the tree (Fig. 1) are labelled 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 No.	GenBank Accession No.	
		ITS	LSU
<i>Aquaticheirosora lignicola</i>	RK 2006a*	AY864770	AY736378
<i>Dictyocheirosora aquatica</i>	KUMCC 15-0305*	KY320508	KY320513
<i>Dictyocheirosora bannica</i>	KH 332	LC014543	AB807513
<i>Dictyocheirosora Garethjonesii</i>	MFLUCC 16-0909*	KY320509	KY320514
<i>Dictyocheirosora Garethjonesii</i>	KUMCC 15-0396	KY320510	KY320515
<i>Dictyocheirosora gigantea</i>	BCC 11346	DQ018095	–
<i>Dictyocheirosora heptaspora</i>	CBS 396.59	DQ018090	–
<i>Dictyocheirosora rotunda</i>	MFLUCC 14-0293*	KU179099	KU179100
<i>Dictyocheirosora rotunda</i>	DLUCC 0576	KY320511	KY320516
<i>Dictyocheirosora rotunda</i>	DLUCC 0577	KY320512	KY320517
<i>Dictyocheirosora vinaya</i>	MFLUCC 14-0294*	KU179102	KU179103
<i>Dictyocheirosora pseudomusae</i>	KH 412	LC014549	AB807516
<i>Dictyocheirosora pseudomusae</i>	yone 234*	LC014550	AB807520
<i>Dictyocheirosora subramanianii</i>	BCC 3503	DQ018094	–
<i>Dictyosporium alatum</i>	ATCC34953*	NR077171	DQ018101
<i>Dictyosporium aquaticum</i>	MF1318*	KM610236	–
<i>Dictyosporium bulbosum</i>	HKUCC 8360	DQ018086	–
<i>Dictyosporium bulbosum</i>	yone 221	LC014544	AB807511
<i>Dictyosporium digitatum</i>	KH 401	LC014545	AB807515
<i>Dictyosporium digitatum</i>	KT 2660	LC014546	AB807518
<i>Dictyosporium digitatum</i>	yone 280	LC014547	AB807512
<i>Dictyosporium elegans</i>	NBRC 32502*	DQ018087	DQ018100
<i>Dictyosporium hughesii</i>	KT 1847	LC014548	AB807517
<i>Dictyosporium meiosporum</i>	MFLUCC 10-0131*	KP710944	KP710945
<i>Dictyosporium nigroapice</i>	BCC 3555	DQ018085	–
<i>Dictyosporium olivaceosporum</i>	KH 375	LC014542	AB807514
<i>Dictyosporium strelitziae</i>	CBS 123359*	FJ839618	FJ839653
<i>Dictyosporium tetrasporum</i>	KT 2865	LC014551	AB807519
<i>Dictyosporium thailandicum</i>	MFLUCC 13-0773*	KP716706	KP716707
<i>Dictyosporium zhejiangensis</i>	MW-2009a	FJ456893	–
<i>Digitodesmium bambusicola</i>	CBS 110279*	DQ018091	DQ018103
<i>Gregarithecium curvisporum</i>	KT 922*	AB809644	AB807547
<i>Jalapriya inflata</i>	NTOU 3855	JQ267362	JQ267363
<i>Jalapriya pulchra</i>	MFLUCC 15-0348	KU179108	KU179109
<i>Jalapriya toruloides</i>	CBS 209.65	DQ018093	DQ018104
<i>Letendraea helminthicola</i>	Strain 7-3	HM486428	AY016362
<i>Pseudocoleophoma calamagrostidis</i>	KT 3284*	LC014592	LC014609
<i>Pseudocoleophoma polygonicola</i>	KT 731*	AB809634	AB807546
<i>Pseudocoleophoma typhicola</i>	MFLUCC 16-0123*	KX576655	KX576656
<i>Pseudodictyosporium elegans</i>	CBS 688.93*	DQ018099	DQ018106
<i>Pseudodictyosporium indicum</i>	CBS 471.95	DQ018097	–
<i>Pseudodictyosporium thailandica</i>	MFLUCC 16-0029*	KX259520	KX259522
<i>Pseudodictyosporium wauense</i>	NBRC 30078	DQ018098	DQ018105
<i>Pseudodictyosporium wauense</i>	KRP88-6	HM036613	–

Results

Phylogenetic analyses

ITS and LSU sequence data were used to resolve the generic placement of the collections of *Dictyosporiaceae*. The alignment datasets included 44 taxa of which *Letendraea helminthicola* (strain 7–3) was used as the outgroup taxon. The combined datasets comprised 1265 characters including gaps. The best scoring RAxML tree was chosen as the backbone tree and is shown in Fig. 1.

The phylogenetic analyses show that all the new strains cluster in the genus *Dictyocheirospora* with high support (98% ML, 98% MP and 1.00 PP). Two isolates clustered with *Dictyocheirospora rotunda* with 100% ML, 100% MP and 1.00 PP. Three new isolates formed a distinct clade at the basal position of the genus *Dictyocheirospora* with strong support (100% ML, 100% MP and 1.00 PP), but *Dictyocheirospora garethjonesii* (MFLUCC 16–0909 and KUMCC 15–0396) clustered with *D. aquatica* (KUMCC 15–0305) in a sister group.

Taxonomy

Dictyocheirospora aquatica Z.L. Luo, D.J. Bhat & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF 552683; Facesoffungi number: FoF 02733; Fig. 2

Etymology – Referring to aquatic habitat of this fungus.

Holotype – HKAS 92714

Saprobic on decaying, submerged wood in freshwater. **Sexual morph:** Undetermined.

Asexual morph: Hyphomycetous. Colonies on natural substrate superficial, scattered. *Mycelium* immersed, composed of pale brown, smooth, septate, branched hyphae. *Conidiomata* sporodochial, 150–250 µm wide, dark brown to black. *Conidiophores* micronematous, undifferentiated from vegetative hyphae, short. *Conidiogenous cells* holoblastic, integrated, terminal, pale brown, smooth-walled. *Conidia* 34–42 × 12.5–19.5 µm (\bar{x} = 38 × 16 µm, n = 35), solitary, acrogenous, cheiroid, pale brown to brown, consisting of 5–6 rows of cells, with rows cylindrical, palmately divergent, inwardly curved at the tip, arising from a basal cell, without appendages, with each row composed of 8–10 cells, euseptate, slightly constricted at septa, smooth-walled.

Material examined – CHINA, Yunnan Province, Dulong River, on decaying submerged wood, 6 May 2015, Z.L. Luo, S-395 (HKAS 92714, **holotype**); ex-type living culture, KUMCC 15–0305.

Notes – *Dictyocheirospora aquatica* morphologically resembles *D. subramanianii* in having a similar conidial size and cylindrical, palmately divergent rows of cells, without appendages. However, *D. aquatica* differs from *D. subramanianii* in conidial shape with 5–6 separable rows, having 6–8 cells in each row, whereas the conidia in the latter has seven tightly appressed rows with each having 9–13 cells (Sutton 1985). Phylogenetic analysis also showed that *D. aquatica* is distinct from *D. subramanianii* (Fig. 1).

Dictyocheirospora garethjonesii Z.L. Luo, H.Y. Su & K.D. Hyde, *sp. nov.*

Index Fungorum number: IF 552684; Facesoffungi number: FoF 02734; Fig. 3

Etymology – In honour of Professor E.B.G. Jones for his major contributions to mycology.

Holotype – HKAS 92836

Saprobic on decaying, submerged wood in freshwater. **Sexual morph:** Undetermined.

Asexual morph: Hyphomycetous. Colonies on natural substrate superficial, scattered. *Mycelium* immersed, composed of brown, smooth, septate hyphae. *Conidiomata* sporodochial, dark brown, 200–300 µm wide. *Conidiophores* micronematous, undifferentiated from vegetative hyphae, short. *Conidiogenous cells* holoblastic, integrated, terminal, pale brown, cylindrical, smooth-walled. *Conidia* 45.5–54.5 × 15.5–24.5 µm (\bar{x} = 50 × 20 µm, n = 25), solitary, acrogenous, cheiroid, brown, consisting of 6–7 rows of cells, with rows digitate, cylindrical, inwardly curved at apex, arising from a basal cell, without appendages, with each row composed of 7–10 cells, euseptate, slightly constricted at septa.

Material examined – CHINA, Yunnan Province, Lancang River, on submerged wood, 25 April 2015, Z.L. Luo, S-310 (HKAS 92836, **holotype**), ex-type living culture MFLUCC 16–0909;

ibid. Jinsha River, on submerged wood, 18 April 2015, S.M. Tang, S-422 (DLU 0422); living culture, KUMCC 15-0396.

Notes – *Dictyocheirospora Garethjonesii* is morphologically similar to *D. rotunda* and *D. aquatica*. However, *D. Garethjonesii* differs from *D. rotunda* in having conidia with 6–7 rows with each row composed of 7–10 cells, whereas the conidia of *D. rotunda* have 5–7 rows with each row composed of 8–12 cells (Boonmee et al. 2016). *Dictyocheirospora Garethjonesii* differs from *D. aquatica* in having larger conidia ($45.5\text{--}54.5 \times 15.5\text{--}24.5 \mu\text{m}$ vs $34\text{--}42 \times 12.5\text{--}19.5 \mu\text{m}$) and conidia with 6–7 rows and each row composed of 7–10 cells, whereas the conidia of *D. aquatica* have 5–6 rows and each row composed of 6–8 cells (Table 2). Phylogenetic analysis also showed that *D. Garethjonesii* is distinct from *D. subramanianii* (Fig. 1).

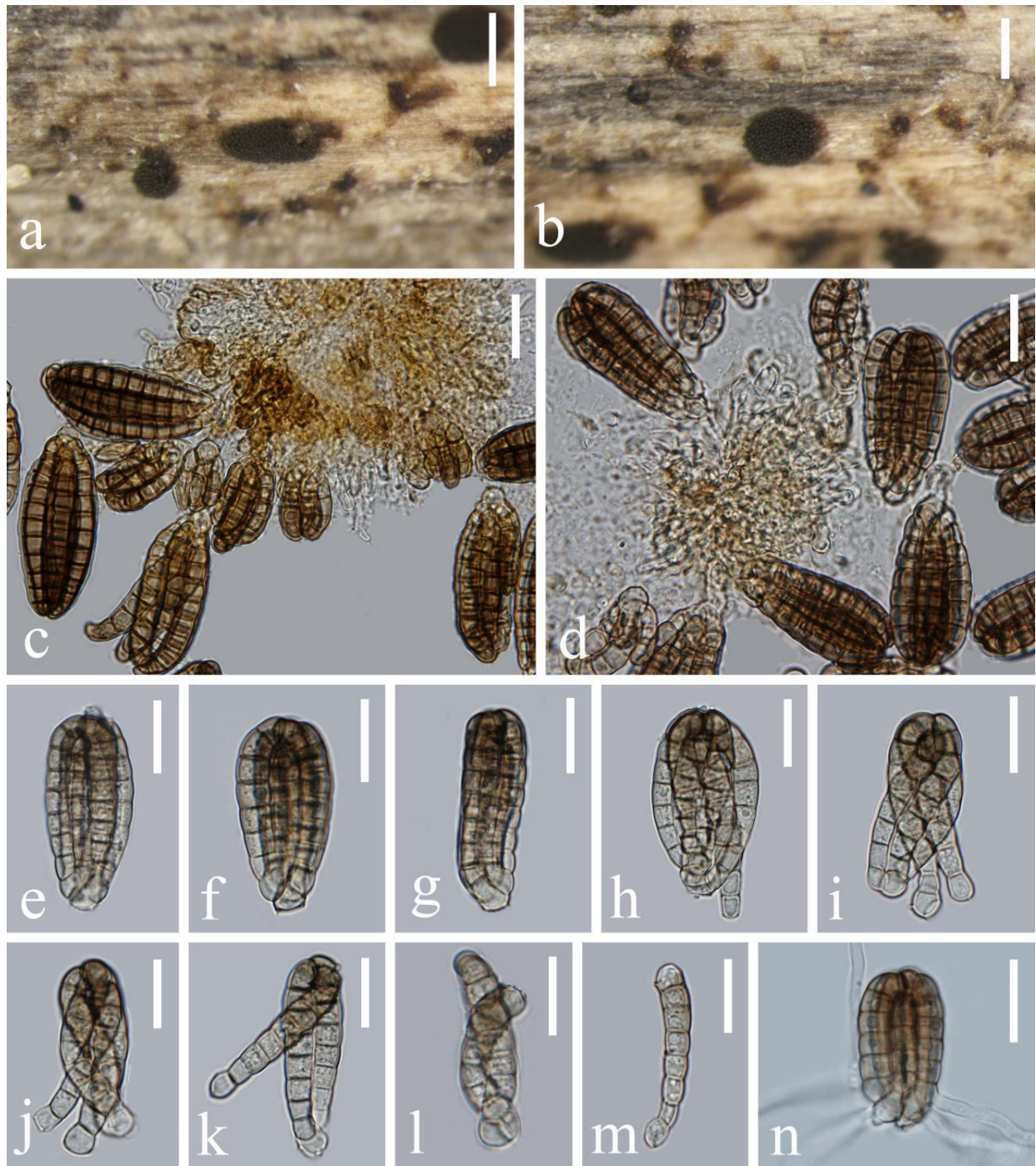


Fig. 2 – *Dictyocheirospora aquatica* (HKAS 92714, holotype) **a–b** Conidiomata on the substrate. **c–d** Squash mount of conidioma with conidiogenous cells. **e–g** Conidia. **h–m** Separable rows. **n** Germinating conidium. – Scale bars: **a–b** = 200 μm , **c–d** = 20 μm , **e–n** = 10 μm .

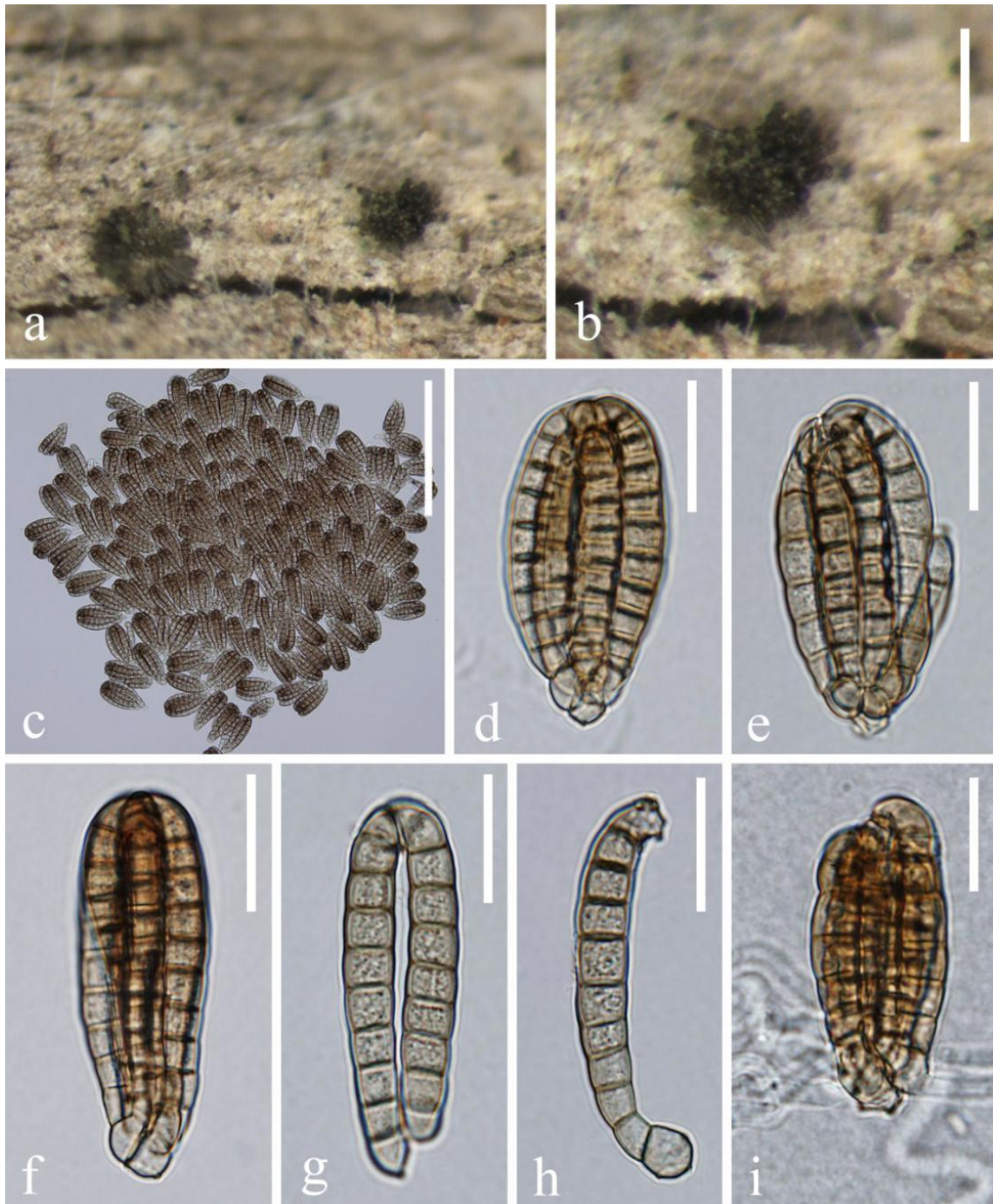


Fig. 3 – *Dictyocheirospora garethjonesii* (HKAS 92836, holotype) **a–b** Colonies on the substrate. **c** Squash mount of conidia. **d–f** Conidia. **g–h** Crushed conidia. **i** Germinating conidium. – **Scale bars:** **b** = 250 μ m, **c** = 150 μ m, **d–i** = 20 μ m.

Dictyocheirospora rotunda D'Souza, D.J. Bhat & K.D. Hyde, Fungal Diversity 80: 465

Facesoffungi number: FoF 01262; Fig. 4

Saprobic on decaying, submerged wood in freshwater. **Sexual morph:** Undetermined. **Asexual morph:** Hyphomycetous. Colonies on natural substrate scattered. *Mycelium* immersed, composed of brown, smooth, septate hyphae. *Conidiomata* sporodochial, dark brown to black, 150–250 μ m. *Conidiophores* micronematous, pale brown, smooth. *Conidiogenous cells* holoblastic, integrated, terminal, pale brown, cylindrical, smooth-walled. *Conidia* 49–55 \times 19.5–22.5 μ m (\bar{x} = 52 \times 21 μ m, n = 35), solitary, acrogenous, cheiroid, pale brown to brown, consisting of 5–7 rows of cells, rows digitate, cylindrical, inwardly curved at the tip, arising from a basal cell, without

appendages, with each row composed of 8–12 cells, euseptate, guttulate, slightly constricted at septa.

Material examined – CHINA, Yunnan Province, Jinsha River, on submerged wood, 19 April 2015, Z.L. Luo, JSJ H 11–7–1 (HKAS 92883), living culture, DLUCC 0576; *ibid.* 21 April 2015, Q Dai, JSJ H 35–1–1 (HKAS 92994), living culture, DLUCC 0577.

Notes – *Dictyocheirospora rotunda*, the type species of *Dictyocheirospora*, was introduced by Boonmee et al. (2016). Two new collections were from submerged wood in Jinsha River, during our study of lignicolous freshwater fungi of three parallel rivers in northwestern Yunnan Province, China. Morphologically our fresh collections fit perfectly well with the description of *Dictyocheirospora rotunda* (Boonmee et al. 2016). The phylogenetic analyses of our strains (DLUCC 0576 and DLUCC 0577) clustered with *D. rotunda* with high bootstrap support (100% ML, 100% MP and 1.00 PP).

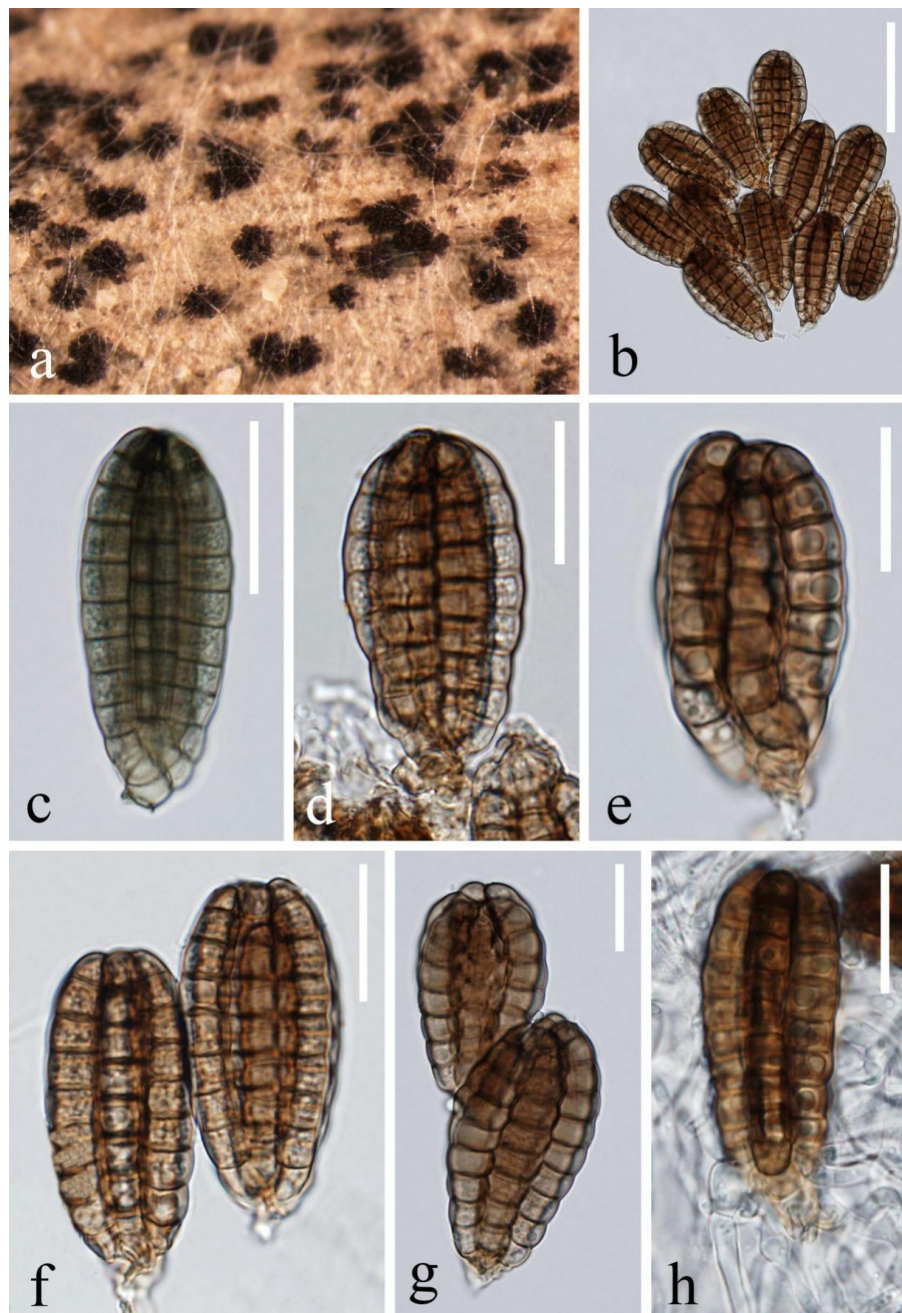


Fig. 4 – *Dictyocheirospora rotunda* (HKAS 92883) **a** Conidiomata on the substrate. **b** Squash mount of conidioma. **c–g** Conidia. **h** Germinating conidium. – **Scale bars:** **b** = 50 μ m; **c–h** = 20 μ m.

Table 2. A synopsis of characters of *Dictyocheirospora* species

Species	Colonies	Conidia				Substrate	Reference
		Size (µm)	Shape	No. of rows	No. of cells with each row		
<i>Dictyocheirospora aquatica</i>	Sporodochial	34–42 × 12.5–19.5	Cylindrical	5–6	6–8	Submerged decaying wood	This study
<i>D. bannica</i>	Punctiform	73–86 × 21–26	Ellipsoid to cylindrical	(5–) 7	17–19	Submerged twigs of woody plant	Boonmee et al. 2016
<i>D. garethjonesii</i>	Sporodochial	45.5–54.5 × 15.5–24.5	Cylindrical	6–7	7–10	Submerged decaying wood	This study
<i>D. gigantea</i>	Sporodochial	105–121 × 25–32	Cylindrical	7	19–22	Submerged decaying wood	Goh et al. 1999
<i>D. heptaspora</i>	Sporodochial	50–80 × 20–30	Cylindrical	7	No available	Palm and submerged wood	Goh et al. 1999
<i>D. rotunda</i>	Sporodochial	42–58 × 19–38	Cylindrical	5–7	8–12	Submerged decaying wood	Tanaka et al. 2015
<i>D. rotunda</i>	Sporodochial	49–55 × 19.5–22.5	Cylindrical	5–7	8–12	Submerged decaying wood	This study
<i>D. pseudomusae</i>	Sporodochial	61–78 × 19–29	Ellipsoid to cylindrical	(6–) 7	13–15	Submerged dead twigs of Bamboo	Tanaka et al. 2015
<i>D. subramanianii</i>	Sporodochial	33–42 × 16–20	Cylindrical	7	9–13	Palm	Sutton 1985
<i>D. vinaya</i>	Sporodochial	58–67 × 15.5–26.5	Cheiroid	6–7	9–13	Submerged decaying wood	Boonmee et al. 2016

Discussion

Freshwater taxa forming on the surface of wood, with multicellular, cheiroidconidia produced in sporodochia, were previously placed in the genus *Dictyosporium*. This fungal form is common in freshwater streams and swamps worldwide (Pinruan et al. 2007, Tsui et al. 2000, Ho et al. 2001, 2002). Boonmee et al. (2016), however revised the genus based on morphology and molecular analysis and accepted ten genera in Dictyosporiaceae. *Dictyocheiropora* is cosmopolitan in distribution and largely reported from freshwater habitats in Asia, Europe and Africa (Ellis 1971, Morris 1978, Mercado-Sierra 1981, Rao & de Hoog 1986, Matsushima 1993, Goh et al. 1999, Boonmee et al. 2016). The fresh collections used in this study are from submerged wood in freshwater lotic habitats in northwestern Yunnan Province, China.

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