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Dictyosporium zhejiangense sp. nov., a new freshwater anamorphic fungus from China

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Abstract – During a study freshwater fungi on submerged wood in Zhejiang Province, China. New dictyosporous hyphomycete was collected. *Dictyosporium zhejiangense* sp. nov. is distinct in the genus in having complanate conidia with 5 rows of cells, with each apical cell of the outer rows provided with 1-3 appendages. Phylogenetic relationships of the new fungus with some *Dictyosporium* species are revealed based on ITS1-5.8s-ITS2 rDNA sequence data. *Dictyosporium zhejiangense* formed a clade together with *Dictyosporium* species which have flattened conidia bearing appendages. This new taxon is described, illustrated, compared with similar species and the phylogenetic relationships within the genus *Dictyosporium* are also discussed.

Freshwater fungi / hyphomycetes / taxonomy / systematics

INTRODUCTION

The genus *Dictyosporium* was established by Corda to accommodate sporodochial hyphomycetes with effuse or compact colonies, multiseptate, cheiroid conidia, micronematous and mononematous conidiophores (Ellis, 1971). Goh *et al.*, (1999) revised this genus and accepted 22 species. The ten additional species were later described including *D. triramosum* Arambarri, Cabello & Cazau (Arambarri *et al.*, 2001), *D. musae* Photita (Photita *et al.*, 2002), *D. taishanensis* G.Z. Zhao & T.Y. Zhang (Zhao & Zhang, 2003), *D. canisporum* L. Cai & K.D. Hyde, *D. tetraploides* L. Cai & K.D. Hyde (Cai *et al.* 2003a), *D. lakefuxianensis* L. Cai, K.D. Hyde et McKenzie, *D. yunnanensis* L. Cai, K.D. Hyde et McKenzie (Cai *et al.*, 2003b), *D. manglietiae* R. Kodsueb et McKenzie (Kodsueb *et al.*, 2006), *D. tetrasporum* L. Cai & K.D. Hyde (Cai & Hyde, 2007) and *D. freycinetiae* McKenzie (McKenzie, 2008).

The needs to link anamorphic fungi to their teleomorphs have been stressed by Shenoy *et al.* (2007a). *Dictyosporium* species and similar genera have been investigated using molecular techniques (Tsui *et al.*, 2006; Kodsueb *et al.*, 2007; Cai *et al.*, 2008). These studies showed that several cheirosporous genera have affinities and form a monophyletic grouping within the *Pleosporales*, species of which are also common in freshwater as inhabitants of decaying wood and

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cellulosic debris (Vijaykrishna & Hyde, 2006). However, the links between the anamorphs and teleomorphs have not been established in culture.

We are currently studying the biodiversity of freshwater fungi on submerged wood in China (e.g. Cai *et al.*, 2006; Cai & Hyde, 2007; Cai *et al.*, 2008; Jiang *et al.*, 2008; Wongsawas *et al.*, 2008). In the course of this research, we have collected one new species of *Dictyosporium* from a small stream in Songyang County, Lishui City, Zhejiang Province. Careful examination showed that it is new to science. This taxon is therefore, described, illustrated, and compared with similar species, and in addition DNA sequences from ITS1-5.8s-ITS2 rDNA region are analyzed to infer phylogenetics relationship within the genus *Dictyosporium*.

MATERIALS AND METHODS

Sample collection and specimen examination

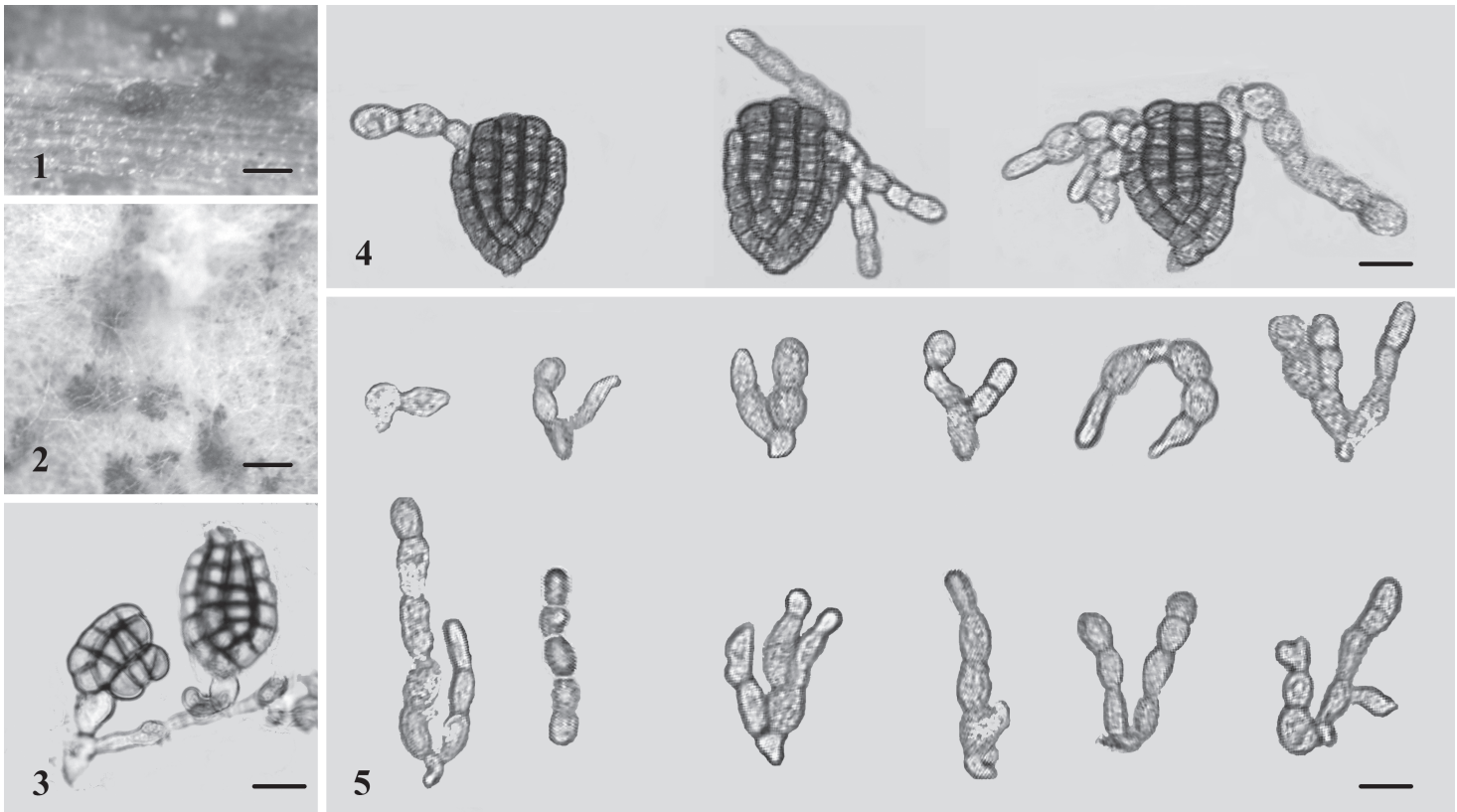
Wood samples which had been submerged for several months were randomly collected from a small stream at Songyang County, Lishui City, Zhejiang Province, P.R. China on October 2007 (28°34”15 ’N, 119°26”32 ’E, altitude 560 m). Samples were placed separately in snap lock plastic bags with sterile moist paper towels, incubated at room temperature and examined regularly within three months. Conidia of the fungus were measured at their widest point. The range between minimum and maximum values is provided. All observations were made with material mounted in water and examined under an Olympus BX-51 microscope equipped with an Olympus DP-50 digital camera system. Type specimen was deposited in Herbarium Mycologicum Academiae Sinicae (HMAS).

Fungal isolates and DNA extraction

Single spore isolation was obtained by methods as described by Choi *et al.* (1999). An isolate was grown on potato dextrose agar (PDA) for two months and total genomic DNA was extracted from fresh mycelia with a modified protocol of Doyle & Doyle (1987).

DNA amplification and sequencing

DNA amplification was performed by PCR. ITS1 and ITS4 primers were used to amplify the complete internal transcribed spacers 1, 2 and the 5.8S rDNA regions (White *et al.*, 1990). Amplification reactions were performed in a 50 µl reaction volume as follows: 1xPCR buffer, 0.2 mM of dNTP, 0.3 mM of each primer, 1.5 mM of $MgCl_2$, 1.5 units of Taq Polymerase and 5-10 ng of DNA (Hu *et al.*, 2007). The PCR thermal protocol consisted of an initial 2 min. at 94°C, 35 amplification cycles of 94°C 50 sec. denaturation, 55°C 1 min. annealing, 72°C 1 min. elongation, with a final extension step of 72°C for 5 min (modified from Shenoy *et al.*, 2007b). PCR products were examined on 1% agarose electrophoresis gel stained with ethidium bromide. PCR products were then purified using AxyPrep^{TM} (Axygen Scientific Inc, Union City, CA, USA) and sequenced using the above mentioned primers in an Applied Biosystems AB3730xl DNA analyzer.



Figs 1-5. Micrographs of *Dictyosporium zhejiangense* (from holotype). **1.** Sporodochia on natural substratum. **2.** Colonies and sporodochia on potato dextrose agar. **3.** Conidia, conidiogenous cells and conidiophores on potato dextrose agar. **4.** Conidia with appendages from woody substrata. **5.** Shapes of appendages. Bars: **1-2** = 200 µm, **3-5** = 10 µm.

Sequence alignment and phylogenetic analysis

Fifteen reference ITS1-5.8s-ITS2 rDNA sequences from GenBank including: eleven available sequences of *Dictyosporium* species (Tsui *et al.*, 2006), one sister taxon *Cheiorosporium triseriale* (Cai *et al.*, 2008), *Saccharicola bicolor* (AF455415) and *S. taiwanensis* (AF439464; as the outgroups), together with a novel sequence of *D. zhejiangense* (FJ456893) were aligned using BioEdit (Hall, 1999) and Clustal X (Thomson *et al.*, 1997). Ambiguously aligned regions in data set were excluded for analyses.

Phylogenetic analyses were performed using maximum parsimony in PAUP* 4.0b10 (Swofford, 2002). Characters were equally weighted and gaps were treated as missing data. Trees were derived using the heuristic search option with TBR (tree bisection-reconnection) branch swapping, 1K random addition sequences, branches of zero length were collapsed. Bootstrap analysis (BT) was used to determine clade stability with 1K replicates, each with 10 replicates of random stepwise addition of taxa. Tree was figured using Treeview (Page 1996).

The model of evolution was calculated by using Mrmodeltest 2.2 (Nylander, 2004). Posterior probabilities (PP) (Zhaxybayeva & Gogarten, 2002) were assessed by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001). Six simultaneous Markov chains were run for 1000K generations and trees were sampled every 100th generation. The first 100K trees were discarded as the burn-in phase of the analyses, and the remaining 900K trees were used for calculating posterior probabilities (PP) in the majority rule consensus tree.

RESULTS

Taxonomy

Dictyosporium zhejiangense Wongsawas, H.K. Wang, K.D. Hyde & F.C. Lin **sp. nov.**

Coloniae in substrato naturali sporodochialiae, atro-brunnea vel nigra. Mycelium plerumque in substrato immersum, ex hyphis ramosis, septatis, subhyalinis vel dilutum brunneae, laevibus compositum. Conidiophora pallide brunnea, micronemata, mononemata, laevia, septata, ramose, flexuosa, cylindrica. Cellulae conidiogenae in conidiophoris incorporatae, terminales, determinatae, cylindricae, doliiformes, subhyalinis vel dilutum brunneae. Conidiorum secessio rhexolytica. Conidia acrogena, solitaria, cheiroidea, laevibus, 25-35 × 17-24 µm, brunnea, complanata, ad septa modice constricti, in 23-37 cellulis, (4-)5-serielibus composita. Cellula apicalis exteriorum serietum cum 1-3 appendicibus tenuitunicatis, catenulata, eramosae, ramosae, subhyalina, 2-10 cellulis, laevia, 29-54 × 5.5-8 µm, cellula appendice forma valde variabili, constrictae ad septa.

Mycobank number: No. 515250.

Etymology: *zhejiangense*, in reference to the Province where the type was found.

Holotype: China, Zhejiang Province, Lishui City, Songyang County, on submerged wood, 24 October 2007, Wang H.K. & Wongsawas M., HMAS No. 196817.

Teleomorph: Unknown.

Habitat: Saprobic on submerged wood.
Distribution: P.R. China.

Colonies on natural substratum in the form of compact sporodochia, dark brown to black. **Mycelium** mostly immersed in substratum, composed of branched, septate, subhyaline to pale brown, smooth hyphae. **Conidiophores** pale brown, micronematous, mononematous, smooth, septate, branched, flexuous, cylindrical. **Conidiogenous cells** integrated, terminal, determinate, cylindrical, doliiform, subhyaline to pale brown. **Conidial secession** rhexolytic. **Conidia** acrogenous, solitary, cheiroid, smooth-walled, 25-35 × 17-24 μm (average = 30 × 20 μm, n = 30), brown, complanate, slightly constricted at the septa, consisting of 23-37 cells arranged mostly in 5 rows (rarely 4 rows). The two outer rows are usually shorter, the three central rows are darker than the outer rows, and the cells at the apical portion of the three central rows are swollen. The apical cells of the each outer row are provided with 1-3 appendages which are thin-walled, subhyaline, 2-10 celled, smooth, 29-54 × 5.5-8 μm, variable in shape, constrict at the septa. The appendages were frequently detached from conidia under slightly pressure and were absent in dried specimens.

Colonies on potato dextrose agar (PDA) growing slowly reaching 3.5 cm diameter at 25°C in 4 months. **Mycelium** cottony, white to pale orange, mostly superficial on the agar, composed of branched, septate, smooth, hyaline hyphae, 1.5-3 μm wide, producing an orangish-brown soluble pigment in the medium. **Sporodochia** slightly, irregularly, dark brown to black, scattered at the edge of

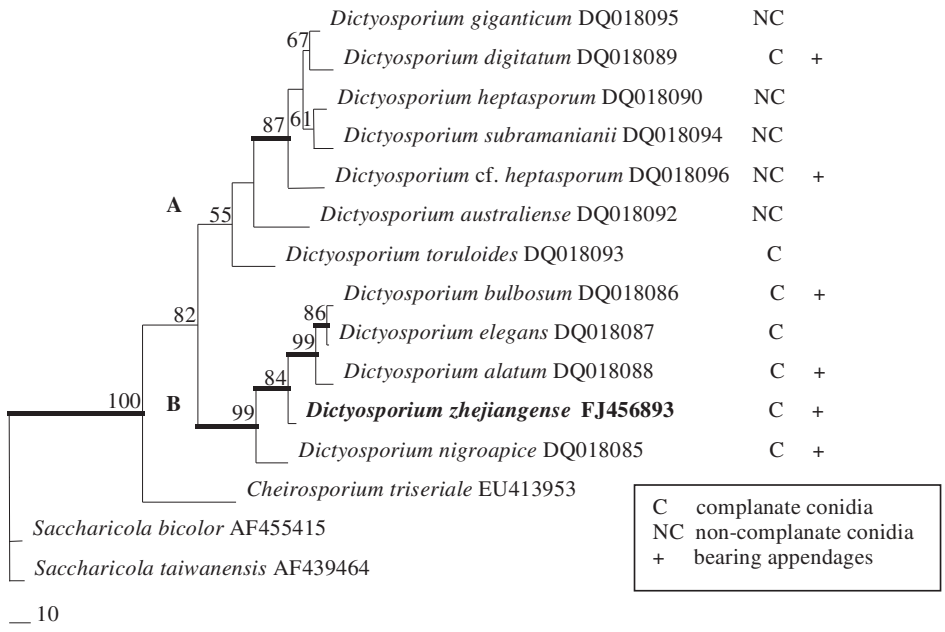


Fig 6. Phylogenetic tree generated from parsimony analysis based on ITS1-5.8s-ITS2 rDNA sequences. Bootstrap confidence values ≥ 50% are shown above branches. Thickened branches indicate Bayesian posterior probabilities ≥ 90%.

colony. **Conidiophores** light brown to brown, micronematous, mononematous, smooth, septate, branched and cylindrical. **Conidiogenous cells** integrated, terminal, determinate, cylindrical, doliiform, subhyaline to pale brown. **Conidial secession** rhexolytic. **Conidia** acrogenous, solitary, cheiroid, smooth-walled, 27-37 × 19-24 μm (average = 31 × 21 μm, n = 30), brown, complanate, slightly constricted at the septa, consisting of 22-33 cells arranged in 4-5 rows.

Phylogenetic analyses

The ITS1-5.8s-ITS2 rDNA data set consisted of 15 taxa. The final data set comprised 496 total characters; 292 characters were constant, 66 variable characters were parsimony-uninformative and 138 characters were parsimony informative. Parsimony analysis resulted in one tree (TL = 387, CI = 0.716, RI = 0.739, RC = 0.529, HI = 0.284). The genus *Dictyosporium* is monophyletic and well-separated from the sister taxon, *Cheirosporium triseriale*, with strong bootstrap value and posterior probabilities support (Cai *et al.*, 2008). Twelve taxa of *Dictyosporium* were divided into two clades (A and B) with high bootstrap confidentence (82%). *Dictyosporium toruloides* was basal to clade A with 55% bootstrap support (Fig. 6). The consensus tree from Bayesian analysis was slightly different to the parsimony tree. *Dictyosporium toruloides* was separate from Clade A and B and formed a sister relationship with 53% Bayesian probability (not shown).

DISCUSSION

Dictyosporium alatum Emden (Emden, 1975), *D. bulbosum* Tzean & J.L. Chen (Tzean & Chen, 1989), *D. canisporum* (Cai *et al.*, 2003a), *D. nigroapice* Goh, W.H. Ho & K.D. Hyde (Goh *et al.*, 1999), and *D. tetraseriale* Goh, Yanna & K.D. Hyde (Goh *et al.*, 1999) have complanate conidia with appendages that arise from each apical cells of the outer rows. Of these only *D. canisporum* has multicellular appendages (Table 1).

Dictyosporium zhejiangense differs from these species in having cheiroid conidia with 2-6 multicellular appendages provided from the two outer rows of conidia which have not been found in other species of *Dictyosporium*. *Dictyosporium zhejiangense* is most comparable to *D. canisporum*, which has similarly long and large appendages borne from the two outer rows of conidia. *Dictyosporium zhejiangense*, however, is distinguished in having conidia with mostly 5 rows of cells (vs. 4-5 rows), the conidia are smaller (25-35 × 17-24 μm vs. 32.5-47.5 × 20-25 μm), there are more appendages per conidia (2-6 vs. 2 appendages), the number of the appendages cells is greater (2-10 vs. 1-2 cells) and appendages are slightly narrower (29-54 × 5.5-8 vs. 24-51 × 6-10.5 μm).

Of 33 accepted species, there are eleven species of *Dictyosporium* that have conidia with variously developed appendages (Goh *et al.*, 1999; Photita *et al.*, 2002; Cai *et al.*, 2003a; Kodsueb *et al.*, 2006; McKenzie, 2008) and our study increases this group to twelve species. The function of the appendages is likely to be for attachment of spores through dispersal and is likely to be effected by environmental factors (Jones, 2006).

Table 1. Comparison of *Dictyosporium* species which complanate conidia bearing appendages at the apical cells of outer rows [based on Goh *et al.* (1999) and Cai *et al.* (2003a, 2003b)].

Species	*Conidia*		*Appendages*		
	No. of rows	*Size (μm)*	*No. per conidia*	*Size (μm)*	*No. of cell(s) and shape*
D. alatum	5	26-32 × 15-24	2	20-25 × 5	1 cell, allantoid or clavate
D. bulbosum	5(-6)	27-46 × 11-30	2	11-28 × 10-19	1 cell, sphaerical to obovoid
D. canisporum	4-5	32.5-47.5 × 20-25	2	24-51 × 6-10.5	1-2 cell (s), cylindrical
D. nigroapice	4	28-41 × 15-20	2	22-34 × 4-5	1 cell, cylindrical
D. tetraserile	4	24-40 × 14-20	2	24-35 × 3-8	1 cell, cylindrical
D. zhejiangense	(4-)5	25-35 × 17-24	2-6	29-54 × 5.5-8	2-10 cells, various shape

Dictyosporium zhejiangense clusters with species that have flattened conidia with outer rows of cells bearing appendages with high bootstrap support and posterior probabilities in the ITS1-5.8s-ITS2 rDNA phylogenetic tree (clade B). Nevertheless, the relation in this clade is not without exceptions; *D. elegans* has conidia flattened but without appendages (Goh *et al.*, 1999). *Dictyosporium canisporum* and *D. tetraserile*, species also which flattened conidia with appendages are not included in this analysis as they are not available in GenBank. The relationship in Clade A is more obscure since the species in this clade produce cylindrical or flattened conidia with or without appendages. The relationship between morphological and molecular characters is therefore not fully resolved. However, results from this study indicate that the appendages may have developed at some stages of the evolutionary convergence in one lineage.

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