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Dactylella qiluensis, a new species of aquatic hyphomycete from China

Hongyan SU[a], Yu'e HAO[b], Shuoran LIU[a], Yunxia LI[a], Yonghong CAO[c], Minghui CHEN[c], Xijun SU[a] & Xiaoyan YANG[a]*

[a]College of Agriculture and biology, Dali University, Dali 671003, P.R. China. Email: yangxy.dlu@gmail.com*

[b]Department of Public Health of University of South China, Hengyang 421001, P. R. China

[c]Laboratory for Conservation and Utilization of Bioresources, Yunnan University, Kunming 650091, P.R. China

Abstract – A new *Dactylella* species lacking nematode-trapping devices was isolated from submerged wood during a survey of aquatic fungi in Qilu Lake, Yuxi, Yunnan province, China. Conidiophores are long and repetitive branched, forming 1-3 short conidia in sympodial arrangement at the apex. Conidia are commonly elongated fusoid, rostrate, slightly inflated at the middle, straight or curved, 5-9 septate, tapering evenly towards base, end cell elongate, tapering to a point at the distal, 88.9-143.1(120.8) × 8.2-11.2(9.9) µm, end cell long 22.4-40.2 µm. The new species is introduced, characterized and illustrated in this paper.

Orbiliales / nematophagous / *ITS* / Nematode-trapping fungi

INTRODUCTION

Since Ingold published his first paper concerning freshwater hyphomycetes isolated from England more than 60 years ago (Ingold, 1942), many freshwater hyphomycetes new to science have been discovered. Nematode-trapping fungi have also been investigated on a worldwide basis, and their distributions in soil, aquatic environments, and animal dung have been well studied (Juniper, 1953; Gray, 1987; Persmark & Jansson, 1997; Hay *et al.*, 1997; Ghahfarokhi *et al.*, 2004; Hao *et al.*, 2005). There have been several studies of freshwater fungi in China, including Yunnan Province, and also the nematode-trapping fungi from freshwater in Yunnan have been studied (Hao *et al.*, 2004; Hao *et al.*, 2005). However, the investigation of nematode-trapping fungi that do not trap nematodes in aquatic environments has been less studied.

Most nematode-trapping hyphomycetes are ascomycetes that form a monophyletic clade in Orbiliales (Hyde *et al.*, 2011). Recently, a new systematic classification was proposed by Li *et al.* (2005) based on sequence analyses of 28S rDNA, 5.8S rDNA, and β-tubulin genes. In terms of trapping devices, *Arthrobotrys* Corda 1839 is characterized by adhesive networks, *Dactylellina* Scholler, Hagedorn & Rubner 1999 by adhesive knobs, and *Drechslerella* Scholler, Hagedorn & Rubner 1999 by constricting-rings. Species with no trapping device were classified as *Dactylella* Grove 1884.

To investigate aquatic nematode-trapping hyphomycetes, waterlogged soil samples in Qilu Lake were collected in 2010. A previously unknown hyphomycete species was isolated from the waterlogged soil. Morphological and phylogenetic comparisons with its relative species show that it is a new species, which falls into the *Dactylella* genus based on the new systematic classification described above.

MATERIALS AND METHODS

While surveying predacious fungi in a plateau lake of Yunnan Province, China, soil samples from the bottom of Qilu lake in Yuxi County were obtained in September 2010. Two to five grams of soil was spread on Corn Meal Agar (CMA) plates as subsamples. After incubation at room temperature (about 23-25ºC) for 2-4 weeks, conidia of hyphomycetes were transferred into fresh CMA plates by sterile toothpicks to obtain pure cultures. For identification, the obtained pure cultures were inoculated on CMA plates, and incubated at 25ºC for 7-10 days, before taxonomic characters were measured and determined. Conidial sizes and the numbers of septa were calculated by measuring more than 100 conidia. Microscopic photographs were taken with an Olympus BX51 microscope. To induce trap formation, a 1×1 cm piece of agar at the centre of the plate was removed from a 7-day old culture to create an open area, where about 200 nematodes (*Panagrellus redivivus*) were placed after mycelia emerged from the cut margin.

Total DNAs were extracted from fresh mycelia as described by Turner (1997). A region containing the ITS region and the 5.8s rDNA gene was amplified by PCR using the primers described by White *et al.* (1990). PCR amplification conditions included an initial denaturation of 1 min at 94°C, followed by 30 cycles of 1 min at 94°C (denaturation), 1 min at 50°C (primer annealing), and 90 sec s at 74°C (extension), with a final extension step of 7 min at 74°C. PCR products were purified with a commercial Kit (TaKaRa Biotechnology Co., Ltd.), and sequenced by a LI-COR 4000L automatic sequencing system using a cycle sequencing Kit (ThermoSequenase) as described by Kindermann *et al.* (1998). The NCBI GenBank accession numbers for all the sequences included in the analysis were given in the phylogenetic tree.

ITS1, ITS2 and 5.8S rDNA complete sequences of the new fungal species were aligned with additional 19 sequences obtained from NCBI by MUSCLE (MUltiple Sequence Comparison by Log-Expectation) from the EBI (European Bioinformatics Institute) web server. Twenty taxa and 512 nucleotides were contained in the DNA matrix. The alignment was adjusted manually. Phylogenetic analysis was performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). Align-ment gaps were treated as missing data; sequence file was set as interleaved, and the GTR model was used as the substitution model. Markov chains were run for 1,000,000 generations, and trees were sampled every 10th generation, resulting in 100,000 trees, with an average standard deviation of split frequencies of about 0.0028. The first 25,000 burn-in phase trees were discarded, and the remaining 75,000 trees were used for calculating posterior probabilities in the consensus tree. The consensus tree was interpreted by the programme FigTree v1.3.1. (Rambaut and Drummond, 2010), and the new fungal species was presented at the bottom of the tree. The GenBank accession numbers of all the analyzed sequences were indicated after the taxa names on the phylogenetic tree.

RESULTS

Dactylella qiluensis H.Y.Su & M.H.Mo, **sp. nov.** Fig. 1

MycoBank: MB561219

Etymology: The species *Dactylella qiluensis* was named after Qilu Lake, Yuxi, Yunnan Province, China, where it was first collected.

Coloniis in CMA effusis, ad 24 mm diam, post 10 dies 21°C. Mycelio sparso, effuso, hyalino, septato, ramoso. Conidiophoris erectis simplicibus vel ramosis, septatis, 100~400 µm altis, basi 3.0~4.0 µm diam., ad apicem angustatis 2.5~3.0 µm crassis. Conidiis hyalinis, plerumque fusiformibus, 5~9 septatis (plerumque 7 septatis), 88.9-143.1(120.8) ×8.2-12.2(9.9) µm.

Colonies white on CMA at the beginning, and turning light pink as they expand. Growth quick, to 50 mm in 10 days at 25°C. Vegetative hyphae hyaline, Aerial mycelium sparse, spreading, hyphae hyaline, septate, branched, 2.5-4 µm wide. **Conidiophores** were septate and erect, mostly 100-400 µm high, 3-4 µm wide at the base, gradually tapering upwards to a width of 2.5-3.5 µm, after attaining a length of 200 to 300 µm, and producing a terminal conidium repeatedly growing out laterally at 100 to 200 µm below the apex to produce 1-2 new conidia on the apices of branches, thereby becoming repetitive and long branched. **Conidia** typically elongated fusoid and rostrate, slightly inflated in the middle, and straight or curved, with 5-9 septa, primarily 6-8 septa, tapering evenly towards the base. The endmost cells were elongate, tapering to a filament at the distal end, beak-like. The proportions of conidia with 5, 6, 7, 8 and 9 septa were 3%, 34%, 37%, 23%, 3% respectively. The size of the conidia is 88.9-143.1(120.8) × 8.2-12.2 (9.9) µm, and the length of the endmost cells was 22.4-40.2 µm. **Trapping organs** failed to form after nematodes were added to the culture on WA.

Holotype: YMF1.03532, collected from Qilu Lake, Yuxi, Yunnan Province, China on February 3th, 2010 by Xijun Su. The holotype and its culture (YMF1.03532) are deposited in the Key Laboratory of Microbial Fermentation, Yunnan University and Faculty of Agriculture and Biology, Dali University.

Phylogenetic analysis

A Bayesian tree was generated using ITS1, ITS2 and 5.8S rDNA complete sequences (Fig. 2). The new species was grouped with its relatives in one clade (species of *Dactylella* which produce no trapping devices). It was close to *D. intermedia* T.F. Li & X.Z. Liu 1998 (Li & Liu, 1998) with a posterior probability of 90%. Taxa of *Arthrobotrys*, *Drechslerella* and *Dactylellina* form three separate individual clades corresponding to the type of trapping devices (adhesive networks, constricting-rings and adhesive knobs, respectively) with high posterior probabilities. The tree also shows that the taxa without and with trapping devices separate at the root of the tree.

DISCUSSION

Although *Dactylella qiluensis* closely resembles *D. arnaudii* A.S. Yadav 1960 (Yadav, 1960) and *D. yunnanensis* K.Q. Zhang, X.Z. Liu & L. Cao 1994 (Zhang *et al.*, 1994) in conidial shape, especially in being rostrate, the conidia size

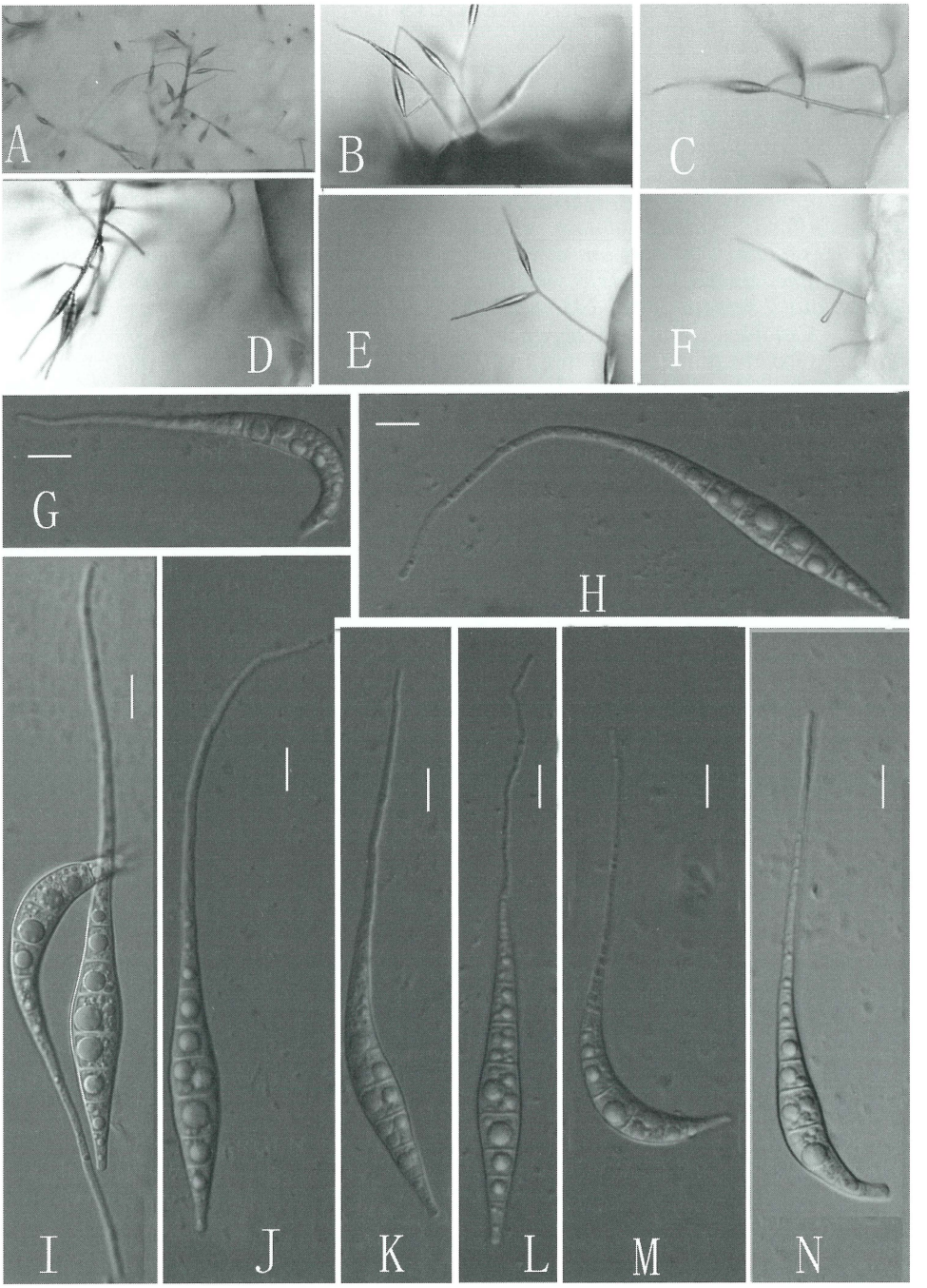

Fig. 1. *Dactylella qiluensis* (Holotype). **A-F.** Conidiophores and some conidia. Top views on conidiophore tips emerging from CMA culture. **G-N.** Conidia. Bars: A-N = 10 μm.

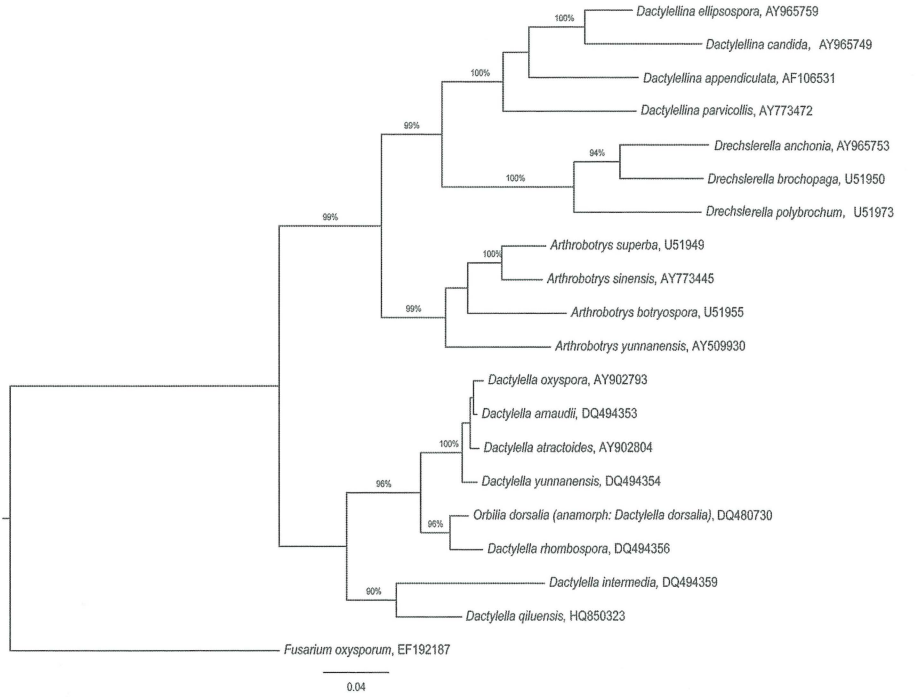

Fig. 2. Bayesian tree constructed based on ITS1, ITS2 and 5.8S rDNA complete sequences of hyphomycetes. Bayesian posterior probabilities over 90% were indicated on the branches. *Fusarium oxysporum* was set as outgroup. Scale bar at the bottom showed the expected changes per site.

and the branches of its conidiophores (Table 1) are different to those of these species. The conidia of *D. qiluensis* are obviously longer, and the conidiophores of *D. qiluensis* are long and repetitive branched, while those of *D. arnaudii* and *D. yunnanensis* are short, occasional, and single.

Phylogenetic analysis suggests that the new fungal species *D. qiluensis* is closely related to *D. intermedia* at the genetic level. However, the different branch lengths of these two taxa indicate different evolutionary changes: *D. intermedia*

Table 1. Morphological comparison of *Dactylella arnaudii*, *D. yunnanensis*, *D. intermedia*, *D. qiluensis*

	FEATURES			
	Conidia shape	*Size (μm)*	*Septation*	*Conidiophores*
Dactylella qiluensis	elongated fusoid, rostrate	88.9-143.1(120.8) × 8.7-11.2(9.9)	5-9	repetitive and long branched
D. arnaudii	fusiform, sometimes rostrate	29.5-92 × 3.3-9.9	3-12	shortly branched
D. yunnanensis	elongated fusoid, rostrate	66.5-106(84.0) × 8.5-13.5(10.0)	7-10	occasionally branched
D. intermedia	elongated fusoid	42.5-85.5 × 8-12	5-8	branched or not

seems to have changed more than *D. qiluensis* during the evolutionary process. Secondly, the tree shows that the group containing *D. qiluensis* and *D. intermedia* is separated from other species of *Dactylella* at the base of the clade, suggesting that this group has a different evolutionary pattern than the other groups of *Dactylella*. *D. qiluensis* and *D. intermedia* are different in their morphologies (Table 1) in that *D. intermedia* does not bear rostrate conidia, and its conidia are shorter than those of *D. qiluensis*.

Both phylogenetic and morphological analyses show that *D. qiluensis* is a distinct species in the genus *Dactylella*.

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