ISSN (print) 0093-4666 ISSN (online) 2154-8889

MYCOTAXON

http://dx.doi.org/10.5248/125.183

Volume 125, pp. 183–188 *July–September 2013*

A new *Drechslerella* species from Hainan, China

Jian-Ying Li[#], Wen-Yun Qian[#], Min Qiao, Ya-Li Bai & Ze-Fen Yu[*]

Laboratory for Conservation and Utilization of Bio-resources,
Key Laboratory for Microbial Resources of the Ministry of Education,
Yunnan University, Kunming, Yunnan, 650091, P. R. China

[*]Correspondence to: *zefenyu@eyou.com*

Abstract — *Drechslerella hainanensis* sp. nov., isolated from soil sampled from Wuzhi Mountain, Hainan Province, China, is described. The new species, placed in *Drechslerella* because it forms constricting rings in the presence of nematodes, is characterized by unbranched conidiophores and 0–2-septate subcylindric–ellipsoidal macroconidia. The morphological and phylogenetic differences between *D. hainanensis* and similar species are discussed.

Key words — ITS, nematode trapping fungi, orbiliaceous, phylogenetic placement

Introduction

Scholler et al. provided evidence by detailed molecular analyses to propose new generic concepts for orbiliaceous nematode-trapping fungi based on the shape of trapping device (Hagedorn & Scholler 1999, Scholler et al. 1999). Li et al. (2005) also redefined the systematic classification of nematode-trapping fungi based on phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA, and β-tubulin genes and confirmed that it is credible to classify nematode-trapping fungi based on the shapes of their trapping devices. According to the present systematics of nematode-trapping fungi, *Drechslerella* Subram. is characterized by three-celled constricting ring traps.

In our survey of nematode-trapping fungi from Hainan Province, a new species was isolated from soil, which we referred to *Drechslerella* based on its formation of three-celled constricting ring traps. In this paper, we describe and illustrate the new species and discuss its phylogenetic placement determined by ITS sequence analyses.

[#] Jian-Ying Li & Wen-Yun Qian contributed equally to this work

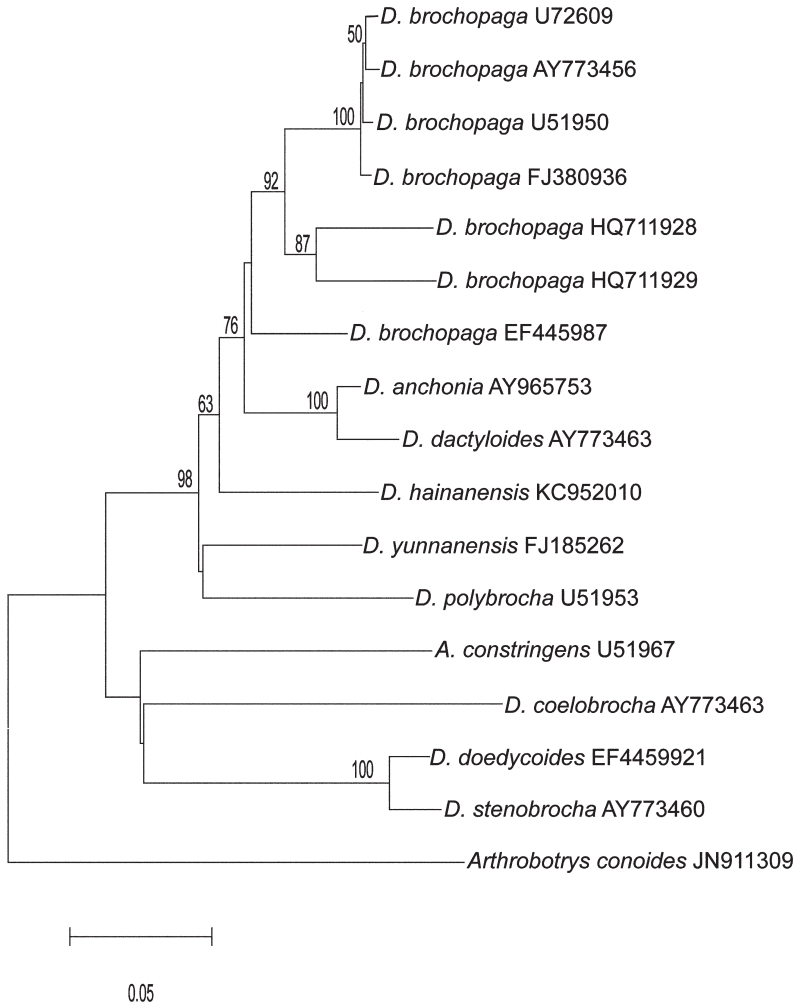

Plate 1. Phylogenetic tree of *Drechslerella hainanensis* and nine other *Drechslerella* species, including *Arthrobotrys constringens* (= *D. effusa*), using the neighbor joining method based on ITS region sequence data. *Arthrobotrys conoides* was used as outgroup. Bootstrap values <50% are not shown.

Materials & methods

Collection of samples, isolation and characterization

Soil samples (30 g) were collected from the forest in Wuzhi Mountain, Hainan Province, China. Each sample was placed in zip-locked plastic bags and labeled.

Samples of 0.5–1g soil were sprinkled onto CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water) inoculated with sterile *Paragrellus redivius* (free-living nematode) and incubated at 25°C, following methods described by Duddington (1955) and Wyborn et al. (1969). After one month, samples were examined using a dissecting microscope. Single spores were isolated with a sterilized toothpick and were cultivated on CMA at 25°C, maintained on the same medium as agar slants and stored at 4°C. Morphological observations were made from CMA after incubation at 28°C for one week.

Specimens and cultures were conserved in the Herbarium of the Laboratory for Conservation and Utilization of Bio-resources (YMF), Yunnan University, Kunming, Yunnan, P.R. China.

DNA extraction, PCR and sequencing

Total DNA was isolated from fresh mycelium as described by Turner et al. (1997). Primer pairs ITS4 and ITS5 (White et al. 1990) were used to amplify the complete ITS. PCR amplification parameters followed Yu et al. (2007). The PCR products were purified with a commercial Kit (Bioteke Biotechnology Co., Ltd., China) and sequenced on both strands with the same primers that were used for amplification with the aid of a LI-COR 4000L automatic sequencing system, using cycle sequencing with the ThermoSequenase-kit as described by Kindermann et al. (1998).

Phylogenetic analysis

Using the new ITS sequence from our culture and the 15 available GenBank ITS sequences from nine other *Drechslerella* species, we performed cladistic analyses with MEGA version 4.1 using the neighbor-joining method. The neighbor-joining tree was constructed with Kimura 2-parameter model, including transitions and transversions with pairwise gap deletion.

Results

A neighbor-joining tree (Plate 1) was generated from ITS sequences from *D. hainanensis* and nine other *Drechslerella* species. *Arthrobotrys conoides* Drechsler, a fungus trapping by means of adhesive three-dimensional networks, was selected as outgroup. The phylogenetic tree shows that *D. hainanensis* clusters as a species separate from *D. doedycoides* (Drechsler) M. Scholler et al., *D. effusa* (Jarow.) M. Scholler et al., and *D. polybrocha* (Drechsler) M. Scholler et al.

Taxonomy

Drechslerella hainanensis Jian Y. Li & Z.F. Yu, **sp. nov.** Plate 2

MycoBank MB 804093

Differs from *Drechslerella effusa* by its production of only a single macroconidium at the conidiophore apex.

Type: PR China, Hainan Province, Wuzhi Mountain, Atuoling Provincial Park, 18°48′N 109°34′E, elev. 514 m, isolated from forest soil, Dec. 2010, Y. L. Bai (**Holotype**, YMF 1.03963; ex-type culture, YMF 1.036931).

Etymology: *hainanensis* refers to the province in which the species was found.

Mycelium slow-growing, colonies hyaline to white, reaching ≤25 mm diam. after 10 days on CMA at 25°C. Vegetative hyphae hyaline, septate, 1.2–4.9 µm wide. Aerial mycelium sparse, hyaline, septate, branched. Macroconidiophores hyaline, septate, erect, unbranched, 98–109 × 3.5–5.0 µm, each tip bearing one conidium. Macroconidia hyaline, straight, subcylindric-ellipsoidal, 32.5–43 µm long, 17.0–25 µm at the broadest part, (0–)1–2 septate (0 in 18.3%; 1 in 40.2%; 2 in 41.5%; n = 80). Microconidia frequently observed. Microconidiophores hyaline, septate, erect, unbranched, 40–80 × 2.2–3.3 µm, sometimes narrower near apex, apices with 2–4 4.5–6.0 long denticles, each bearing one conidium. Microconidia hyaline, straight, elongate ellipsoid-clavate, rounded at the apex, 18.2–22.8 × 4.2–5.3 µm, (0–)1 septate. Trapping nematodes by means of spontaneously formed, stalked, three-celled constricting rings with triangular inner thickenings at the septa. At non-constricted points, outer diameter = 22–35.5 µm, inner diameter = 9.0–15.5 µm.

Discussion

Drechslerella heterospora (Drechsler) M. Scholler et al., *D. stenobrocha* (Drechsler) M. Scholler et al., and *D. effusa* resemble *D. hainanensis* in their cylindric-ellipsoid shaped macroconidia. *Drechslerella heterospora* differs from *D. hainensis* by its distinctly narrower microconidia and *D. stenobrocha* differs from *D. hainensis* by its distinctly narrower macroconidia. *Drechslerella effusa* fits quite well with *D. hainanensis* in macroconidial size and number of septa but differs in producing up to 12 or more macroconidia near the conidiophore apex, whereas the macroconidia of *D. hainanensis* always grow singly at the tip. Unlike *D. hainanensis*, *D. doedycoides* macroconidia are broadly ellipsoid-fusoid with narrowed ends and 2(–3)-septate, while those of *D. polybrocha* have only one sub-basal septum.

Our ITS rDNA phylogenetic tree supports *D. hainanensis* as separate from *D. doedycoides*, *Arthrobotrys constringens* (= *D. effusa*), *D. polybrocha*, and *D. stenobrocha*. We included a sequence from the type of *Arthrobotrys constringens* Dowsett et al. because no sequence was available from the *D. effusa* type; Oorschot (1985) treated these two species as synonymous, although they differ somewhat in microconidial size. Although *D. hainanensis* is very close to the clade formed by *D. brochopaga* (Drechsler) M. Scholler et al., *D. anchonia* (Drechsler) M. Scholler et al., and *D. dactyloides* (Drechsler) M. Scholler et al., in these three species the conidiophore apex bears more than one conidium, thus forming a radiating capitate arrangement, and their conidial shape and septation also differ from those in *D. hainanensis*.

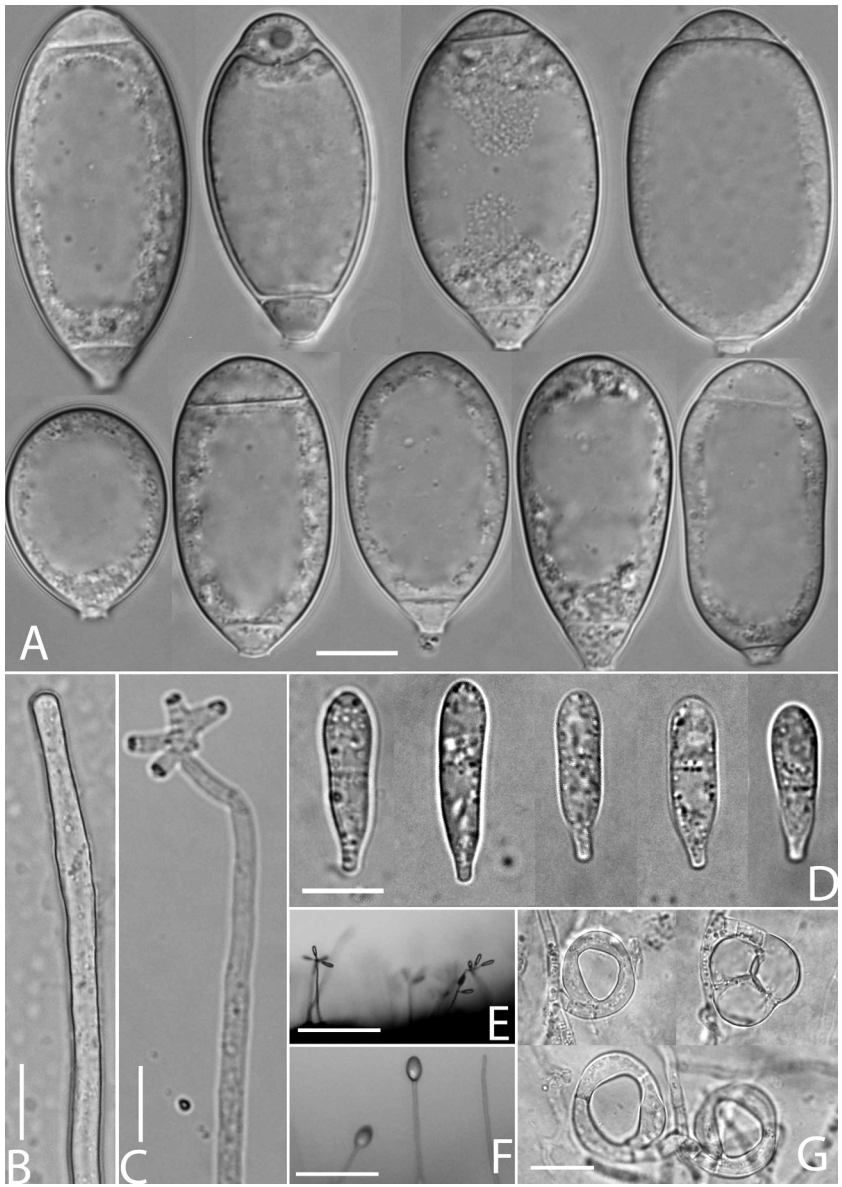

Plate 2. *Drechslerella hainanensis* (**Holotype**, YMF 1.03963): A. Macroconidia; B, C. Macroconidiophore; D. Microconidia; E. Microconidiophores and microconidia; F. Macroconidiophore and macroconidia; G. Constricting rings. Scale bars: A–D = 10 μm; E, F = 50 μm; G = 20 μm.

Acknowledgements

This work was jointly financed by National Basic Research Program of China ('973'Program: 2013CB127503) and National Natural Science Foundation Program of PR China (31060008, 31160008, 31260007, 31360130), Grants from the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI020). China National Tobacco Corporation (grant no. 110201002023) and Yunnan Branch of China Tobacco Industrial Corporation (grant no. 2010YN17). We are very grateful to Dr. D. M. Hu and Prof. H.-O. Baral for critically reviewing the manuscript and providing precious suggestions on this paper.

Literature cited

Duddington CL. 1955. Notes on the technique of handing predacious fungi. Transactions of the British Mycological Society 38: 97–103. http://dx.doi.org/10.1016/S0007-1536(55)80021-6

Hagedorn G, Scholler M. 1999. A reevaluation of predatory orbiliaceous fungi. I. Phylogenetic analysis using rDNA sequence data. Sydowia 51: 27–48.

Kindermann J, El-Ayouti Y, Samuels GJ, Kubicek CP. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genetic Biology 24: 298–309. http://dx.doi.org/10.1006/fgbi. 1998.1049

Li Y, Hyde KD, Jeewon R, Cai L, Vijaykrishna D, Zhang KQ. 2005. Phylogenetics and evolution of nematode-trapping fungi (*Orbiliales*) estimated from nuclear and protein coding genes. Mycologia, 97(5): 1034–1046. http://dx.doi.org/10.3852/mycologia. 97.5.1034

Oorschot CAN (1985) Taxonomy of the *Dactylaria* complex V. A review of *Arthrobotrys* and allied genera. Studies in Mycology 26: 61–96.

Scholler M, Hagedorn, Rubner A. 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 50(1): 89–93.

Turner D, Kovacs W, Kuhls K, Lieckfeldt E, Peter B, Arisan-Atac I, Strauss J, Samuels GJ, Börner T, Kubicek CP. 1997. Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycological Research 101: 449–459. http://dx.doi.org/10.1017/S0953756296002845

Wyborn CHE, Priest D, Duddington CL. 1969. Selective technique for the determination of nematophagous fungi in soil. Soil Biology & Biochemistry 1: 101–102. http:/dx.doi.org/10.1016/0038-0717(69)90039-X

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis et al. (eds). PCR Protocols: a guide to methods and applications. Academic Press, San Diego, CA.

Yu ZF, Zhang Y, Qiao M, Zhang KQ. 2007. *Orbilia dorsalia* sp. nov., the teleomorph of *Dactylella dorsalia* sp. nov. Cryptogamie, Mycologie 28: 289–294.