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Dactylella zhongdianensis sp. nov, a new predacious antagonist of nematodes

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Abstract—A new predacious fungus, *Dactylella zhongdianensis*, is described and illustrated from Zhongdian in western Yunnan, China. This fungus is characterized by its solitary clavate, multiseptate conidia borne on erect unbranched or branched conidiophores. It forms adhesive networks as a capture device in the presence of nematodes. Infection, host specificity, and pathogenicity of the new species to nematodes are also described.

Key words—nematode-trapping fungi

Introduction

Nematophagous fungi have been the subjects of research over several decades in fundamental studies of their ecology, distribution and systematics, and as potential biological control agents of nematode pathogens of plants and animals (Li et al. 2002). These fungi, which comprise a diverse range of fungi able to infect and digest nematodes, can be divided into four categories: endoparasitic fungi, nematode-trapping fungi, fungi that parasitize eggs and females, and toxin-producing fungi (Barron & Thorn 1987, Dackman et al. 1992). Nematode-trapping fungi are unique, however, in their morphological adaptation to the predacious habit and in their ability to capture and consume prey. These fungi have a more complex relationship with their nematode host, since they also have an ability to live saprophytically. They form different hyphal structures (adhesive nets, knobs, branches or hyphae, constricting rings or non-constricting rings) in order to capture nematodes (Barron 1977). Nematode-trapping fungi have been known for over a century. Ecological surveys on their occurrence indicate that this group is found throughout the world, in all types of climate and in all the habitats examined (Gray 1987). The biocontrol potential of nematode-trapping

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fungi has also been considered (Chandrawathani et al. 1998, Fernández et al. 1999, Manueli et al. 1999). There have been some recent exciting advances in the control of nematode parasites of livestock, by exploiting the nematophagous properties of nematode-trapping fungi. Among these predators, *Arthrobotrys flagrans* (Dudd. 1950) Sidorova, Gorlenko & Nalepina [=*Duddingtonia flagrans* (Dudd. 1950) RC Cooke (Cooke 1969)] has been selected for commercial development (Fernández et al. 1999).

Here, we report a new predacious fungus, *Dactylella zhongdianensis*, and outline its infection and pathogenicity to nematodes.

Materials and methods

Isolation of the fungus

While surveying the predacious fungi, we collected soil samples from Zhongdian, Yunnan, China on 7 September 2003. Subsamples of 1-2 g were spread on Corn Meal Agar (CMA) plates and stored at room temperature (about 20-28˚C). After incubation for 20 days, the entire surface of the petri dishes were observed under a dissecting microscope and the conidia of nematode-trapping fungi were isolated to CMA plates by sterile toothpick. For identification, the cultures were incubated on CMA for 14-30 days at 28˚C and the taxonomic characters were measured and determined. The trap formation was induced by nematode *Panagrellus redivivus* (Linne 1767) Goodey according to the description of Lei et al. (1998). Microscopic photographs of the fungus were taken from fresh living material mounted in water using an Olympus BX51 microscope.

Infection and pathogenicity of the fungus to nematodes

Two plant-parasitic nematodes, *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle, *Meloidogyne arenaria* (Neal 1889) Chitwood and one free-living nematode *P. redivivus* were used in the tests. *B. xylophilus* and *P. redivivus* were cultured on media and stored in our lab. The larvae of *M. arenaria* were isolated from root knots of tobacco from Yiliang, Kunming, China. Predation rates were used to evaluate the pathogenicity of the fungus against nematodes. For the pathogenicity test, the fungus was inoculated on Water Agar (WA) plates. After incubation at 28˚C for two weeks, about 200 individuals per nematode species were added to the plates. Each experiment was carried out in triplicate. The predation rate was calculated by recording the proportion of nematodes trapped by predacious structures and calculated using the following formula:

Predation rate = Number of nematodes trapped by networks/Total number of nematodes observed×100%.

Results and discussion

Taxonomic description

Dactylella zhongdianensis J. Zhang & K.Q. Zhang **sp. nov.** (Figs 1-7)

Coloniae in extracto granorae zeae maydae cum agaro albidae. Hyphae hyalinae, septatae, ramosae, 2-3 µm latae. Conidiophora hyalinae, erectae, sptatae, simplicae, parca ramosae, 20-107.5 µm longae, basi 2.5-4.5 µm crassae, apice 1-2 µm crassae,

ibi uni-conidium ferentes. Conidiae hyalinae, clavatae, plerumque levitr curvae, 2-6 septatae, praecipue 3-septae, 17.5-35(28.3)×5-10(7.2) μm. Reticulis tenacia quae vermiculos nematodeos capiunt evolventibus.

Etymology: in reference to Zhongdian, the place where the fungus was first isolated.

Holotype here designated: YMF1.00568D, Zhongdian, Province Yunnan, China, 7 September 2003, Jing Zhang. The holotype and its culture (YMF1.00568) are deposited in the Laboratory for Conservation and Utilization of Bio-resources, Yunnan, P. R. China.

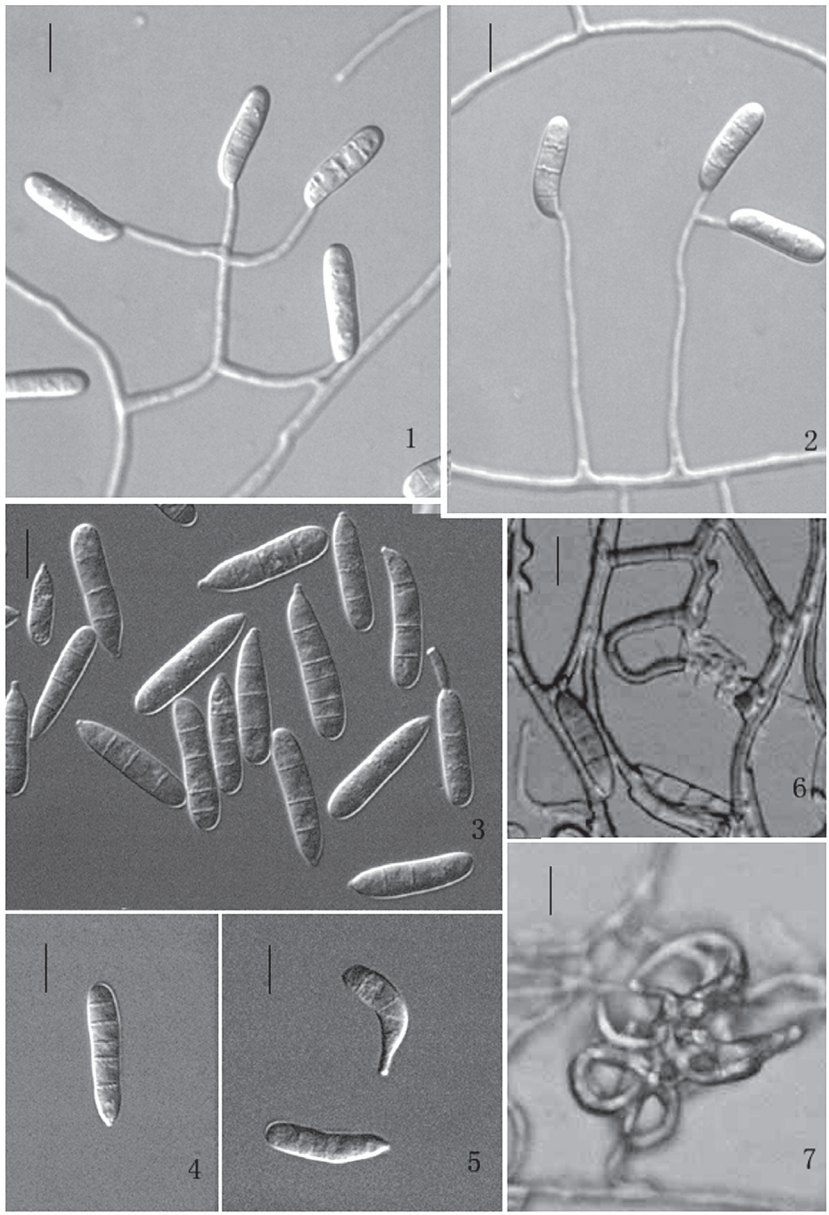
Colonies on CMA medium whitish at first and later granular for the mass spores, aerial hyphae hyaline, septate, branched, 2-3 μm wide. Conidiophores hyaline, erect, septate, unbranched or forming 2-5 branches, 20-117.5 μm high, 2.5-4.5 μm wide at the base, tapering upward gradually to 1-2 μm wide at the tip, and bearing there a single conidium. Conidia hyaline, mostly clavate, rarely curved or slightly curved at basal end, rounded distally, producing umbilicus at the base, 2-6-septate, mainly 3-septate, 17.5-35×28.3×5-10×7.2 μm. The proportion of conidia with 2, 3, 4, 5 and 6 septa is 8%, 82.4%, 7.5%, 1.6% and 0.5% respectively. The predacious device exhibits a three-dimensional adhesive network with small dense loops.

The genus *Dactylella* was established by Grove (1884) based on the type of *D. minuta* Grove 1884, and its circumscription was emended several times by different authors (Schenck et al. 1977, Van Oorschot 1985, Zhang et al. 1994) and recently was summarized by Miao et al. (2003) in detail. We prefer to place the new species in *Dactylella* following the generic concept of Subramanian (1963), who characterized *Dactylella* as having primarily cylindrical, ellipsoidal, clavate or fusoid conidia that are multi-septate (without a large inflated central cell) and solitarily borne on the conidiophore.

The new species resembles *D. pseudoclavata* Z.Q. Miao & X.Z. Liu 2003 (Miao et al. 2003) and *D. shizishanna* X.F. Liu & K.Q. Zhang 2003 (Liu & Zhang 2003) in conidial shape and the kind of predatory device, but can be distinguished as follows. *D. zhongdianensis* forms shorter conidia [17.5-35(28.3)×5-10(7.2) μm] than those of *D. shizishanna* [22.5-73.8(50.6)×5-10(6.6) μm]. The conidia of *D. zhongdianensis* usually have 2-6 (mainly 3) septa and show an umbilicus at the base, but *D. shizishanna* produces conidia with 2-9 (mainly 3-7) septa and without the umbilicus, while *D. pseudoclavata* produces 0-1-septate conidia with a bottleneck shaped base. *D. zhongdianensis* also resembles *D. clavata* R.H. Gao, M.H. Sun & X.Z. Liu 1995 (Gao et al. 1995), *D. cylindrospora* (RC Cooke 1969) A. Rubner (Rubner 1996), *D. rhopalota* Drechsler 1943 (Drechsler 1943), and *D. submersa* (Ingold 1944) Nisson (Ingold 1944) in conidial shape and size. However, the four similar speceis do not produce predacious devices when confronted by nematodes.

Infection and pathogenicity of *Dactylella zhongdianensis* to nematodes

On WA plates, the fungus extended in all directions from the inoculum into the surrounding substratum after stored at 28˚C for 3 days and obtained a 9 cm diam colony in 11 days. Two days after adding the nematodes, the radiating hyphae gave rise at varying intervals to three-dimensional networks. Nematodes that in their continuous



Figs.1-7. *Dactylella zhongdianensis* J.Zhang & K.Q.Zhang. 1-2. Conidiophores with conidia. 3-5. Conidia. 6. The radiating hyphae begin to give rise to form trap device. 7. Three-dimensional adhesive networks. Bar=10μm.

movement happened to run afoul of these structures were held fast despite their energetic struggles to escape. Following narrow perforation of the integument, the fungus infected the struggling nematode by intruding globose infective bodies from which assimilative hyphae then extended to invade the fleshy interior. At first the assimilative hyphae were badly obscured from view due to the globose degeneration of the animal's musculature and organs. The assimilative hyphae became more clearly visible after the degenerating material had been largely absorbed,. Eventually the assimilative hyphae were wholly emptied of contents by withdrawing their protoplasm backward into the external mycelium and, together with the collapsing pellicle, vanishing from sight. With ample nourishment being obtained through destruction of many nematodes, the fungus soon produced conidiophores and conidia from the dead animals. The fungus displayed no host prey specificity in infection pattern and capture capability when tested with three nematode species. The fungus was able to trap and consume the nematodes *B. xylophilus*, *M. arenaria*, and *P. redivivus* showing predation rates of 75.6%, 80.3% and 78.5% respectively, and the rates are not significantly different ($P > 0.01$). Our result showing that the predator lacked host specificity agrees with previous reports (for a review see: Gray 1987).

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