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Orbilia vermiformis sp. nov. and its anamorph

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Abstract—During our study of orbiliaceous fungi and their anamorphs, a specimen of *Orbilia* was collected and its anamorph culture was obtained. The morphological characteristics plus phylogenetic analyses based on nuclear rDNA sequences indicate that both the anamorph and teleomorph represent new novel distinct species. Both teleomorph and anamorph are described and illustrated. The teleomorph *Orbilia vermiformis* is similar to *O. crystallina*, but differing by its apothecia without solid, glassy processes at the margin, larger asci and ascospores, and the anamorph *Dactylella vermiformis* is characterized by branched conidiophores and 0-1-septate clavate conidia.

Key words—teleomorph-anamorph connection

Introduction

Xerointolerant species of the genus *Orbilia* Fr. are characterized by small, waxy, light-colored, semi-translucent apothecia, tiny asci and usually swollen paraphysis apices. This group of fungi is generally reported to occur on semi-moist decayed wood or bark and produces various anamorphs (Pfister 1997). The connection between *Orbiliaceae* and their anamorphs has been well established and many anamorph species have been reported recently (Mo et al. 2005a, b, Liu et al. 2005a, b, Yang & Liu 2005, Yu et al. 2006). However, anamorphs have been mainly isolated from species with subulate (Mo et al. 2005a, Webster et al. 1998, Pfister 1994, Pfister & Liftik 1995, Rubner 1996), subcylindrical (Liu et al. 2005a), globose and kidney-shaped (Pfister 1997) ascospores but not from species with helicoid ascospores. During our survey on the orbiliaceous fungi in China, an *Orbilia* species with helicoid ascospores was collected and its anamorph was obtained. The anamorph was placed within the genus *Dactylella* according to the system of Scholler et al. (1999). After detailed examination and phylogenetic analysis based on DNA sequences from

the internal transcribed spacer region (ITS) of the ribosomal RNA gene, we believe both teleomorph and anamorph were not described previously.

Materials and methods

The fresh holotype specimen of *Orbilia vermiformis* was collected from XiaoHeiJiang Forest Park, Pu'er County, Yunnan Province, China, in September, 2005, by Min Qiao. The anamorph was isolated and observed as the way described by Yu et al. (2006). The measurement of each character is derived from 50 repeats. To induce the formation of nematode-trapping organs, about 100 nematodes (*Panagrellus redivivus* Goodey) into a 1 × 1 square centimeter slot at the margins of the colony where the agar was removed.

The total DNA of the fungus was isolated from fresh mycelium as described by Turner et al. (1997). A region of rDNA, containing the ITS regions 4, 5 and the 5.8S rRNA, was amplified by PCR using the primers described by White et al. (1990). The parameters for PCR amplifications were as follows: 1 min at 94°C, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, 74°C for 90 s, and a final extension period of 7 min at 74°C. The PCR products were purified with a commercial Kit (TaKaRa Biotechnology Co., Ltd.), and sequenced (Kindermann et al. 1998).

DNA sequences were aligned using the ClustalX 1.83 and the BioEdit programs. Parsimony analysis was run using PAUP* 4.0b10 (Swofford 2002), with the following settings: gaps treated as missing, all characters weighted equally, used heuristic searches with TBR (tree-bisection-reconnection) as the branch-swapping algorithm and bootstrap values generated using 1000 replicates.

Taxonomic description

Teleomorph:

Orbilia vermiformis Baral, Z.F. Yu & K.Q. Zhang **sp. nov.** **FIGURE 1**

MycoBank # MB510463

Apothecia 1.2-2.0 mm in diam., solitaria vel gregaria, margine glabra vel minute crenulata, superficialia, sessilia, alba vel pallide lutea. Excipulum ectale textura angulare, cellullis 10-35 µm diam, marginem versus sine processibus solidis. Asci 39.3-62.0 × 3.8-5.0 µm, 8-spori, cylindraceo-clavati, apice truncati, basi angustati plerumque furcati, apice truncati vel rotundati. Ascosporae hyalinae, filiformes-subulatae, valde helicoideae, non-septatae, imbricate 2-4-seriatae, 8.8-11.5 × 1.0-1.5 µm, ad apicem cum vacuola refringente lacrimiforme, 1.3-1.8 × 0.8-1.0 µm. Paraphyses filiformes, apice non inflatae, usque 2.8-3.0 µm diam., hyalinae.

Etymology: *vermiformis*, referring to the worm-shaped ascospores.

Holotype: YMFT 1.01842, isolated from exposed rotten root of broad-leaved tree, XiaoHeiJiang Forest Park, Pu'er County, alt. 2100 m, Yunnan Province, PR China, Min Qiao. 3 Sep. 2005, a culture preserved in liquid nitrogen, permanent slide culture (YMF 1.01842), Isotype: H.B. 8313.

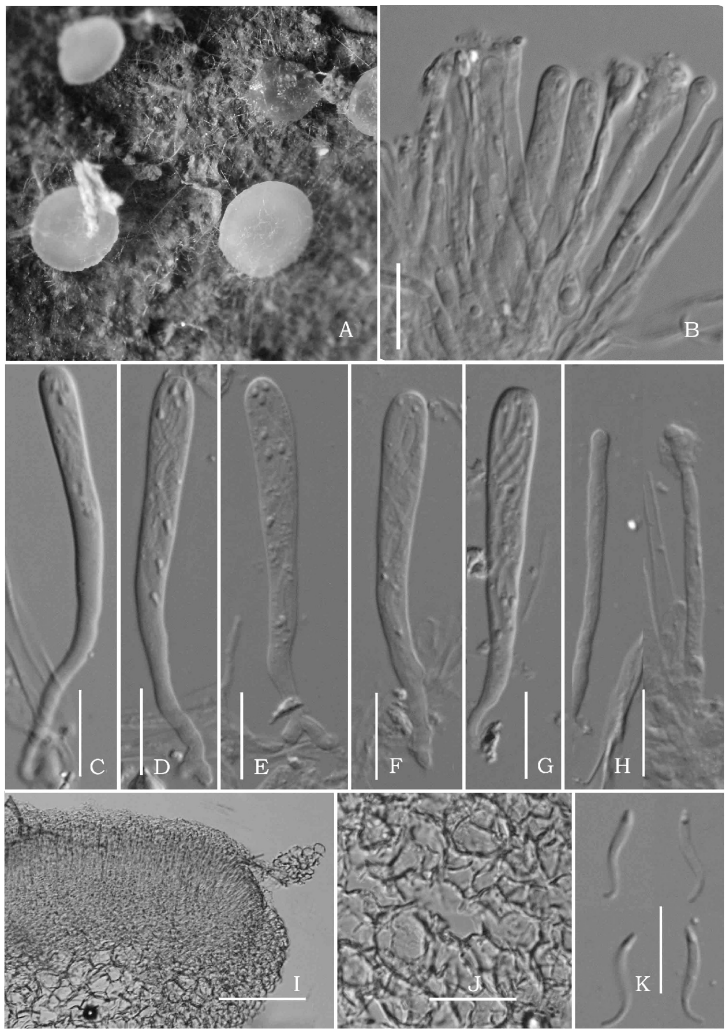

Fig 1. ***Orbilia vermiformis***. Holotype A. Fresh apothecia. B. Cluster of asci and paraphyses. C-G. Dead asci with living spores. H. Dead paraphyses. I. Median section of an apothecium. J. Cells of ectal excipulum. K. Living ascospores. Scale bars: B-H, J-K = 10 µm, I = 50 µm.

Apothecia scattered to gregarious, sessile, superficial on rotten wood. Disc 1.2-2.0 mm in diam., white or pale yellow throughout, waxy, not translucent when fresh, margin even or minutely crenulate. Ectal excipulum 250 µm thick in centre, composed of angular to prismatic cells from base to margin, hyaline, thin-walled, 25-45 × 10-35 µm, marginal cortical cells 13-17 × 8-13 µm, without glassy processes. Medullary excipulum 20 µm thick, textura intricata-

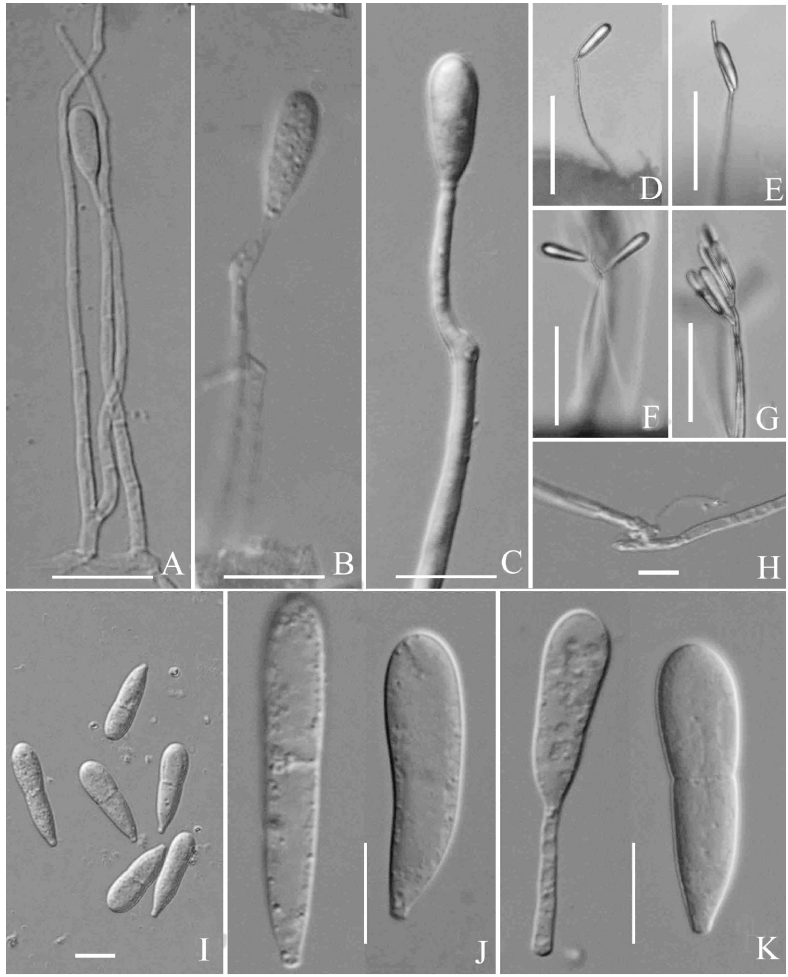

Fig. 2. ***Dactylella vermiformis***. Holotype. A-G. Living conidiophores with conidia. H. Joint of two conidiophore bases. I-K. Living conidia.

Scale bars: A-C, J-K = 10 µm, D-G = 50 µm, H-I = 10 µm.

angularis. Asci cylindric-clavate,rounded or strongly truncate with a distinct indentation (depending on direction of view) at the thin-walled apex, tapered and often forked at the base, 8-spored, 39.3-62.0 × 3.8-5.0 µm (living state), 45 × 3.3-3.7 µm (in KOH). Ascospores hyaline, non-septate, filiform, helicoid or sigmoid, 8.8-11.5 × 1.0-1.5 µm (living state) and 10-10.7 x 0.9-1.1 µm (in KOH), broadest above and slightly tapered to the rounded upper end, gradually tapered to a fine point at the base, lower spores in ascus inversely oriented, bearing a refractive tear-shaped spore body (SB) at the apex (broader end), 1.3-

1.8 × 0.8-1.0 µm, attachment of SBs to the apical wall of the ascospores partly visible. Paraphyses filiform, apex not or only very slightly enlarged to 2.8-3.0 µm, usually covered at the apex by a thin rough exudate, containing globose SCBs (cytoplasmic bodies).

Anamorph:

Dactylella vermiformis Z.F. Yu, Ying Zhang & K.Q. Zhang **sp. nov.** **FIGURE 2**
MycoBank # MB510462

Coloniae in agaro CMA , post 10 dies 25°C 40 mm diam. Mycelium sparsum, hyphis septatis, 3-4 µm latis. Conidiophora erecta, simplices vel ramose, 70-150 µm longa, 3 µm lata ad basim, 1.8-2 µm lata ad apicem. Conidia hyalina, clavata, 20-36.8 × 5.8-8.1 µm, 0-1 septata.

Etymology: species epithet refers to the teleomorph species.

Holotype: YMF 1.01842, permanent slide, XiaoHeiJiang Park, Pu'er County, Yunnan, P. R. China, alt. 2100 m, Min Qiao, Sep. 2005.

Colonies white, growing slowly on PDA, reaching 44 mm at 21°C after 10 days, 63 mm at 25°C, 61 mm at 28°C, no growth at 35°C, producing black pigment in the back of colonies. Colonies white, aerial mycelium sparse on CMA, 2.5-4 µm wide, reaching 30 mm at 22°C, 40 mm at 25°C, 45 mm at 28°C after 10 days, no growth at 35°C. Vegetative hyphae hyaline, branched and septate, 3-4 µm wide. Primary conidiophores septate, erect, simple or branched, 3 µm wide at the base and 1.8-2 µm wide above, 70-150 µm in length, single conidiophores with a single apical spore, most conidiophores growing in flexing knee with a spore at the flexing place, and another spore at the apex. Conidiophores often form loose bundles because of the joining of conidiophore bases. Conidia colorless, clavate, rounded at the distal end, constricted into bottleneck shape at the proximal end, straight, sometimes slightly curved, with (0-)1 septum (median or nearer to distal end), occasionally constricted at septum, 20.0-36.8 (25.3) × 5.8-8.1 (6.7) µm (living state). No trapping structures on WA were observed when nematodes were added.

Phylogenetic analysis

Maximum parsimony analysis of the ITS sequences (FIG. 3) yielded single tree based on 260 parsimony informative characters (constant characters are 221 and uninformative characters are 89). The MP tree had 1044 steps in length with a consistency index (CI) of 0.614 and a retention index (RI) of 0.6147. In our analysis, *Hyalorbilia brevistipitata* (a member of the family *Orbiliaceae*) was used as an outgroup. The ITS phylogenetic tree indicated that taxa forming adhesive knobs, forming constricting rings and forming networks clustered into different clades respectively, but four species of *Dactylella* located within different clade. *D. vermiformis* showed close affinities to species producing constricting rings and species producing adhesive knobs. Unfortunately, in our

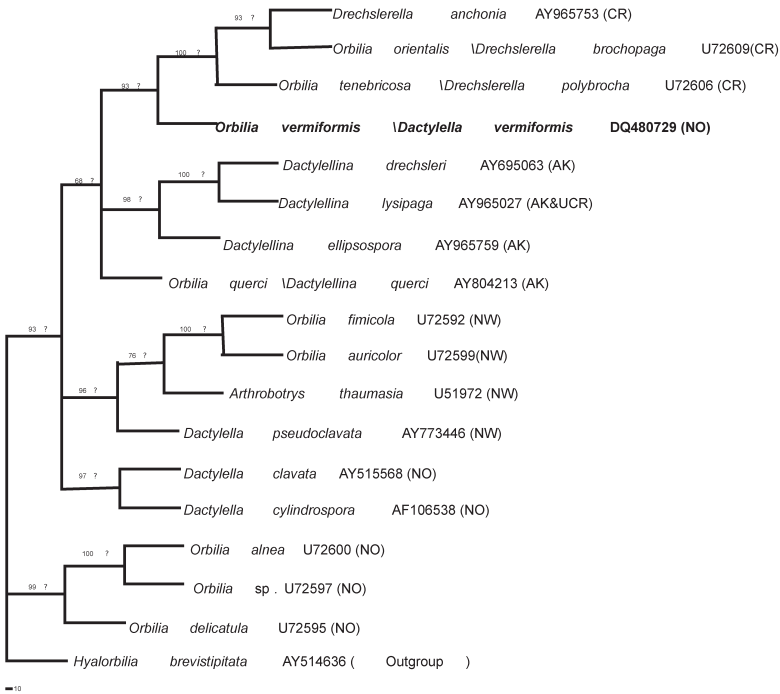

Fig. 3 Most parsimonious phylogenetic tree generated from a heuristic search based on the alignment of the ITS region sequences of some nematode-trapping fungi. Numbers above lines represent bootstrap values from 1000 replicates on all parsimony-informative characters, with only bootstrap values >50% shown. Except for the sequence of *O. vermiformis*, other sequences were obtained from the GenBank (accession numbers shown following each taxon name). The abbreviation in the parenthesis for each taxon refers to nematode-trapping device (or no device): AK, adhesive knobs; CR, constricting rings; NW, networks; UCR, non-constricting ring; NO, no-trapping advice.

molecular analyses no ITS sequence of species morphologically similar to *D. vermiformis* is available from GenBank, except *Dactylella pseudoclavata,* and these two species are phylogenetically distant.

Discussion

Morphologically, *D. vermiformis* is similar to *Dactylella arrhenopa* (Drechsler) K.Q. Zhang et al., *Dactylella polyctona* (Drechsler) K.Q. Zhang et al. and *Dactylella pseudoclavata* Z.Q. Miao & X.Z. Liu in producing clavate conidia with one septum (Zhang et al. 1995, Miao et al. 2003). However, conidia of *D. vermiformis* were larger with broader distal end than that of *D. arrhenopa*

and *D. polyctona*. Compared to *Dactylella pseudoclavata, D. vermiformis* has shorter conidia without nematode-trapping devices whereas *D. pseudoclavata* produces three-dimensional trapping networks (Table 1).

Table 1. Morphological comparison of *D. vermiformis* and similar species.

Dactylella species	Size of conidia (μm)	Trapping device	Number of conidial septa
D. pseudoclavata	30 – 45 × 8 – 11	three-dimensional networks	0-1
D. vermiformis	20.0 – 36 ×5.8 – 8.1	not observed	0-1
D. polyctona	12.7 – 21 ×2.3 – 2.8	not observed	1
D. arrhenopa	17 – 25 × 2.6 – 3.7	not observed	1

Orbilia vermiformis, the teleomorph of *Dactylella vermiformis*, is also distinctly different from other described species in the genus *Orbilia*. *O. vermiformis* is characterized by filiform, helicoid ascospores. Although similar ascospores exist in *Orbilia crystallina* (Quél.) Baral, those of *O. vermiformis* are broader and longer than that of *O. crystallina*. Other characteristics such as marginal cortical cells of *O. crystallina* being elongated and terminated by glassy and solid processes were not found in *O. vermiformis*, while the asci and paraphyses of *O. vermiformis* are distinctly larger than those of *O. crystallina*. In our present specimen, the apothecia of *O. vermiformis* were not translucent which is rather unusual, especially in xerointolerant species of *Orbilia*. However, in an American collection referable to *O. vermiformis* (see below) the apothecia were medium translucent.

A collection from Central America (Martinique, Le St. Esprit, Le Bois La Charles, bark of broad-leaved tree, C. Lechat, H.B. 8031) fits quite well with the type of *O. vermiformis*, except the narrower marginal cortical cells (9-15 × 5-7 μm). Also the presumed anamorph which was found on the natural substrate, resembles that of the type of *D. vermiformis* but the 1-septate conidia were found to be smaller (14.5-20.5 × 4.2-6.5 μm, in KOH). For the time being the specimen is considered to belong in the scope of *O. vermiformis*.

It was strange that *D. vermiformis* did not produce any nematode-trapping devices but was grouped with constricting ring forming species with 93% bootstrap support in phylogenetic tree. The possible reasons could be that the fungus lost its ability to form trapping devices or the fungus could capture other microscopic animals such as rotifers and rhizopods. The predatory ability of this fungus needs to be further investigated.

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