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Orbilia dorsalia sp. nov., the teleomorph of *Dactylella dorsalia* sp. nov.

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Abstract – In this study, *Orbilia dorsalia* and its anamorph are described. *O. dorsalia* is characterized by having an ellipsoid-shaped spore body in the subulate ascospores. Its anamorph produces fusoid conidia with 5-9 septa without trapping structure formation with the challenge of nematodes. Therefore it assigns in *Dactylella* and is named *D. dorsalia*. The analysis of ribosomal DNA ITS sequences of morphologically similar species (including teleomorphs and anamorphs) and representative species of *Dactylella* genus also support the establishment of the new species. Fortunately, the apothecia were formed on the cultures of *D. dorsalia* with nematode addition.

***Dactylella dorsalia* / ITS sequences / *Orbilia dorsalia* / Teleomorph-anamorph connection**

INTRODUCTION

Orbiliaceae is a kind of widely distributed fungi. The classification history of *Orbiliaceae* has been reviewed in recent papers (Liu *et al.*, 2005; Mo *et al.*, 2005). Liu (2005) and Mo (2005), as well as others obtained nematode-trapping anamorphs from ascospores of *Orbilia* spp. (Pfister, 1994, 1995; Webster *et al.*, 1998). It is difficult to induce the teleomorphs on the pure cultures. Although Drechsler (1937) observed small immature apothecia on a culture of *Arthrobotrys superba* Corda, and Zachariah (1983) found fruitbodies on the culture of a natural auxotroph of *Arthrobotrys dactyloides* Drechsler, the apothecia are immature. Apothecia were formed on the culture of non-predacious *Dactylella rhopalota* Drechsler with bacteria challenge and identified as *Orbilia* sp. (Zachariah, 1989). Rubner *et al.* (1996) observed apothecia of *Orbilia auricolor* (Bloxam *ex* Berk. & Broome) Saccardo on pure culture of *Monacrosporium psychrophilum* (Drechsler) Cooke & Dickinson. However, there were no detail examination and description of mature apothecia on culture. During studying the connection of *Orbilia* spp. and their anamorphs, an anamorph was resulted from ascospores *Orbilia* sp. The mature apothecia were formed where agar was removed and nematodes were added to induce trapping structures (Gao *et al.*, 1996). After detailed study, both teleomorph and anamorph represent new taxon and we describe them in this paper.

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MATERIALS AND METHODS

The fresh specimens of *Orbilia* were collected from the reverse surface of decayed bark (Euphordiaceae) from Xishuangbanna tropical botanical garden, Yunnan province, China in August, 2005 by Zhang Ying. To isolate anamorph, three apothecia were stuck on the lid of a Petri-dish with its hymenium upside down to shoot ascospores on the surface of CMA (20g corn, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water). Petri-dishes with apothecia were placed for 4-6 days at room temperature until ascospores deposit viewable on the CMA. The ascospores were transferred to another CMA plates. After incubating 7-10 days at 25•, cultures were observed and measured with an Olympus B51 microscope with differential interference contrast. All microscopic characteristics were measured from 50 individuals in water mounts. Trapping devices were induced by adding about 100 nematodes (*Panagrellus redivivus* Goodey) into a 1 cm × 1 cm square slot at the margins of the colony where the agar was removed.

Total DNA was extracted from fresh mycelium as described by Turner (1997). A region of nuclear rDNA, containing the ITS regions 4, 5 and the 5.8s rRNA gene was amplified by PCR using the primers described by White *et al.* (1990). The parameters of PCR amplifications are as follows: 1 min initial denaturation at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min primer annealing at 50°C, 90 s extension at 74°C, 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 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). The NCBI GenBank accession numbers for all sequences included in the analysis are given in the phylogenetic tree.

DNA sequences were aligned using ClustalX 1.83 and corrected by visual inspection using BioEdit sequence alignment editor. Parsimony analysis was run in PAUP* 4.0b10 (Swofford, 2002), with the following settings: gaps treated as missing, all characters equally weighted, using heuristic searches with TBR (tree-bisection-reconnection) as branch-swapping algorithm, initial "MaxTrees" setting at 100; bootstrap values were generated using the settings 1000 replications.

RESULTS

Morphological descriptions

Teleomorph:

Orbilia dorsalia Y. Zhang, Z. F. Yu & K. Q. Zhang, **sp.nov.** (Fig. 1).

Etymology: referring to the site of substrate where this fungus growing.

Apothecia 0.6-1.2 mm in diam., solitaria vel gregaria, superficialia, translucentia, sessilia, concavo. Excipulum ectale texturae angulare, 6.0~10.0 μm diam. Asci 25~28.8 × 3~3.8 μm, 8-spori, cylindraceo-clavati, ad basin angustati, ad apicem truncati. Ascospori 7~9.8 × 1.0~1.5 μm, non septati, curvati, subulati, ad partem ditalem angustati atque acuti, ad partem proximalem obtuse, inclusionem ellipticus continentes. Paraphyses filiformes, apice usque 2.5~3.0 μm.

Apothecia superficial, sessile, gregarious on decayed bark, yellow and translucent throughout. *Disc* 0.6~1.2 mm in diam., margin even, with small dentical,

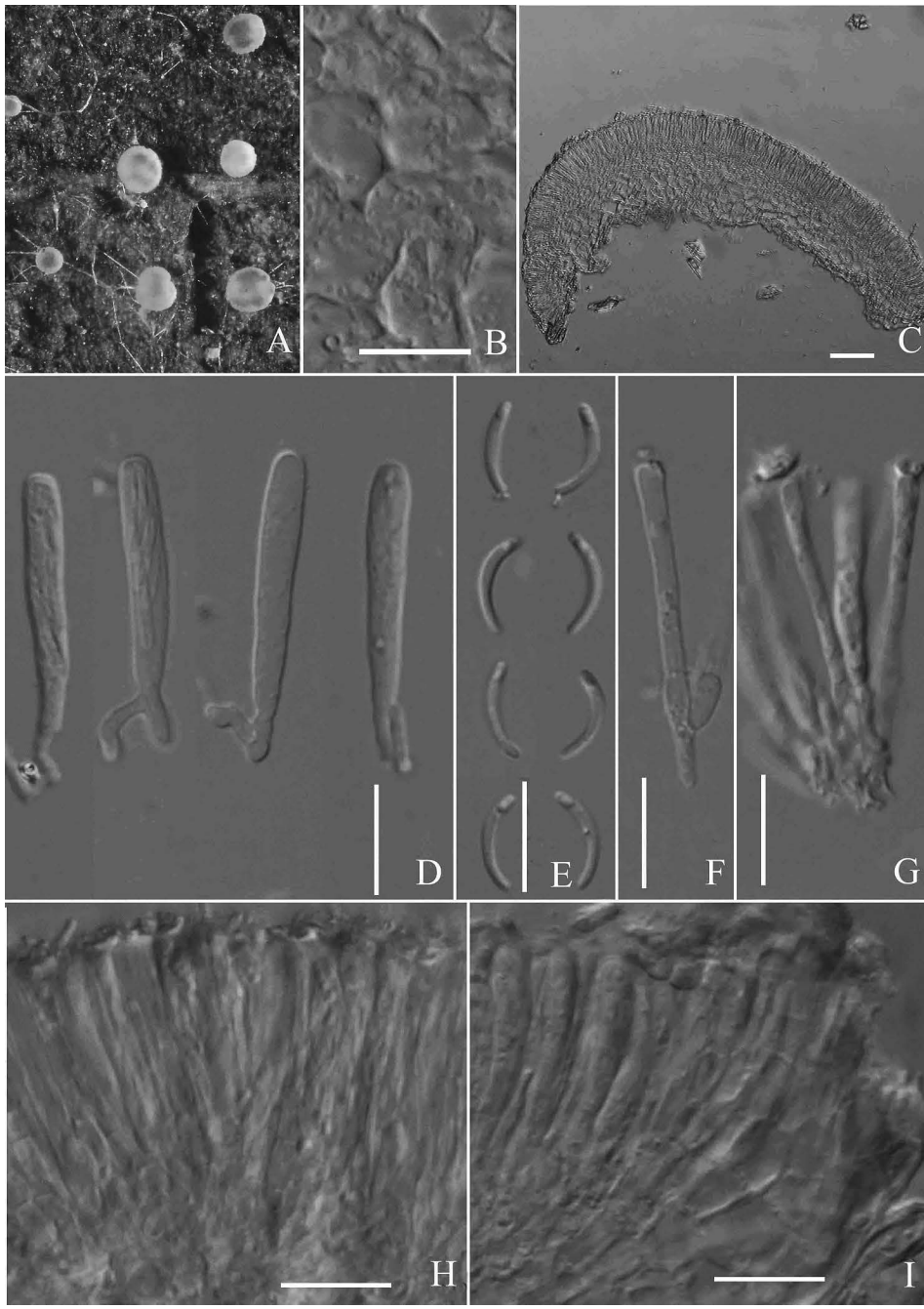

Fig. 1. *Orbilia dorsalia* (YMFT 1.01835). **A.** Fresh apothecia. **B.** Cells of medullary excipulum. **C.** Vertical section of an apothecium. **D.** Asci. **E.** Living ascospores. **F-G.** Paraphyses. **H.** Hymenium. **I.** Margin of ectal excipulum cells. Bars: C = 20 µm; B, D-I = 10 µm.

centrally attached. Ectal excipulum a textura angulare, 6.0~10.0 _m in diam., with thin or only slightly thickened walls. Subhymenium poor-developed. *Asci* non-septate, 8-spored, cylindric, rounded or truncate-rounded at the apex, tapered and often forked at the base, 25~28.8 × 3~3.8 µm. Ascospores hyaline, subulate, curved, distal end sometimes slightly tapered, proximal end obtuse, 7~9.8 × 1.0~1.5 µm, a refractive ellipse SB (spore body) at proximal end in living mature ascospores, not attached the ascospores, 1.2~1.3 × 0.8~1.0 µm. Paraphyses 1-3 septate, filiform, not or only slightly enlarged to 2.5~3.0 µm in diam. at the apex, apices encrusted.

Apothecia induced in culture 0.3~1.4 mm diam., as well as existence of immature apothecia. Other microscopic characteristics are consistent with that of apothecia growing on decayed bark. (Fig. 3, Fig. 4).

Anamorph:

Dactylella dorsalia Y. Zhang, Z. F. Yu & K. Q. Zhang, **sp. nov.** (Fig. 2)

Etymology: species epithet refers to similar conidia to *D. oxyspora.*

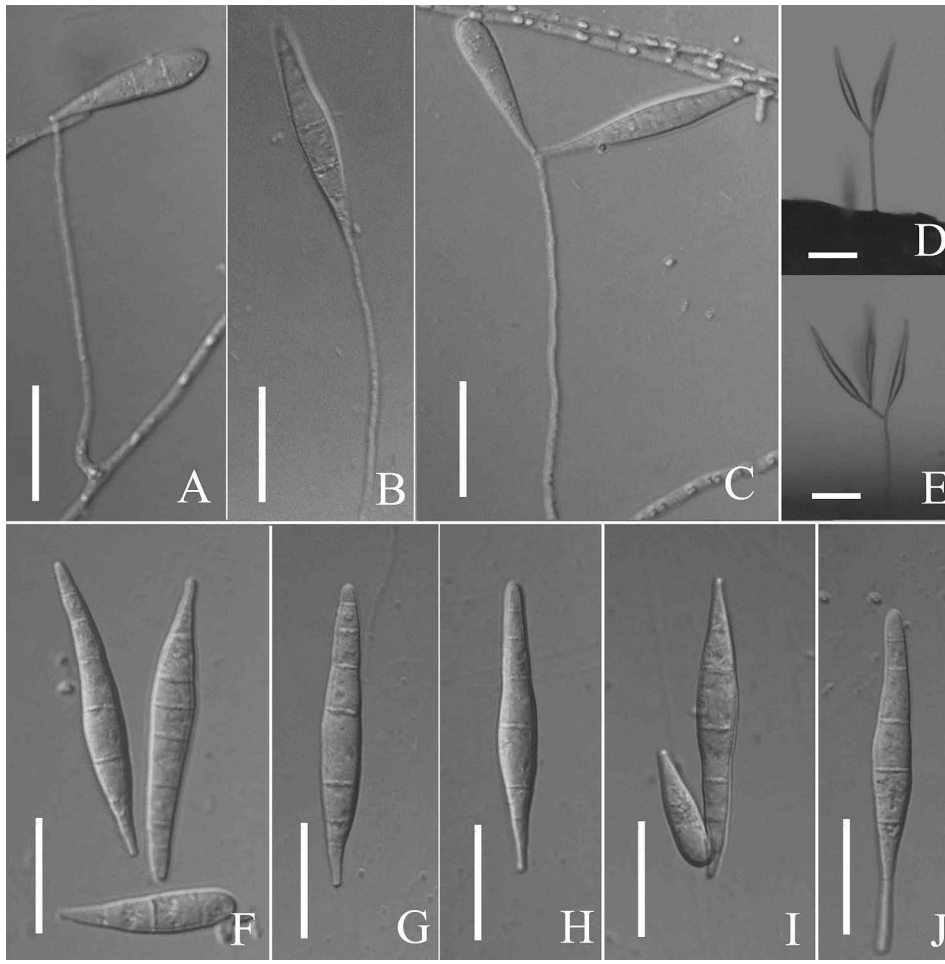

Fig. 2. *Dactylella dorsalia*. (YMF1.01835) **A-E.** Conidiophores with conidia. **F-J.** Conidia. Bars: A-C, F-J = 10 µm, D, E = 50 µm.

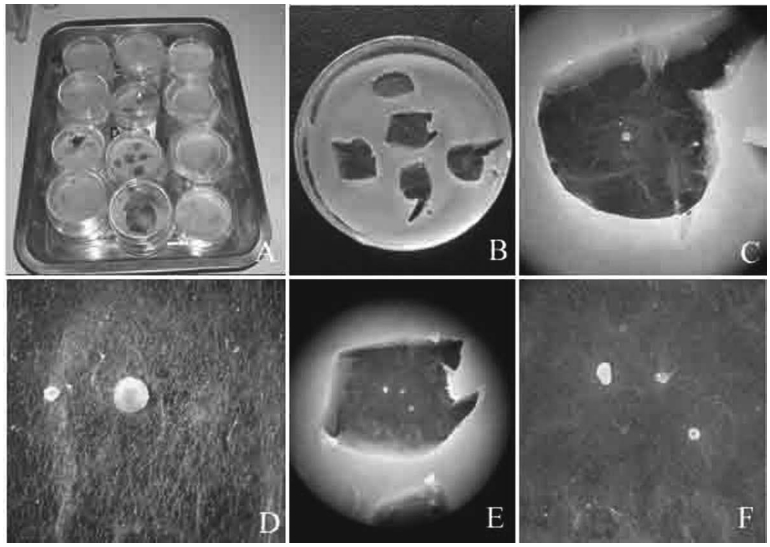

Fig. 3. **A-B.** Showing environment apothecia were growing. **C-F.** Apothecia growing from cultures.

Coloniae in CMA effusae, ad 24 mm diam, post 10 dies 21°C. Mycelium sparsum, effusum, hyalinum, septatum, romosum. Conidiophora erecta simples vel ramose, septata, 50~300 μm alt, basi 3.5~4.0 μm, ad apicem angustata 2.5~3.0 μm crassa. Conidia hyalinis, hyaline, plerumque fusiformbus, 5~9 septatia (plerumque 7 septatis), 22.0~30.3(32.5) ×3.3~4 μm.

Colonies white on PDA, growing slowly, 24 mm at 21°C after 10 days, 38 mm at 25°C, 30 mm at 28°C, no growth at 35°C. Colorless, appressed agar on CMA, reached 50 mm after 10 days at 21°C, 56 mm at 25°C, 70 mm at 28°C, no growth at 35°C. Aerial mycelium sparse, hyphae hyaline, septate, branched, 2.5~4 μm wide. Conidiophores simply branched, mostly 50~300 μm high, 3.5~4.0 μm at the base, gradually tapering upward to a width of 2.5~3 μm at the tip where bearing 1~2 apical spore. Conidia were commonly elongate fusoid, slightly inflated at the medium, tapering evenly towards each ends, straight, with 5~9 septate, the proportion of conidia with 5, 6, 7, 8 and 9 septa was 4%, 20%, 40%, 23%, 13% respectively, 22.0~30.3(32.5) × 3.3~4 μm. Trapping organs failed to produce after nematodes were added in culture on WA.

Holotype: PR China, Yunnan, Xishuangbanna County, tropical botanical garden, alt. 550 m, 5 August 2005. A dried voucher specimen (YMFT 1.01835), permanent slide (YMFD 1.01835) and culture (YMF1.01835) were deposited in the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan Province, PR China.

DNA sequencing and phylogenetic analysis

To get molecular evidence that *O. dorsalia* is a new species, its ITS sequence was compared with those of other related species of *Orbilia* and predacious hyphomycetes from GenBank. Of 557 total characters, 197 characters were constant, 116 variable characters were parsimony-uninformative. The

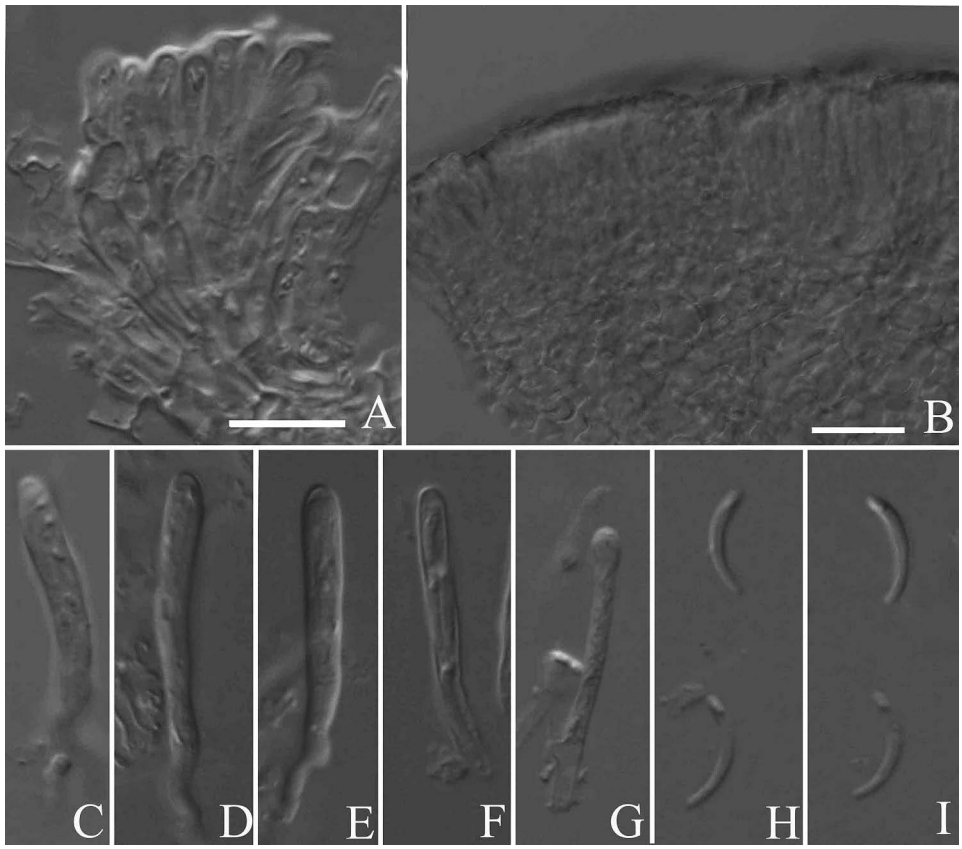

Fig. 4. Microscopic characteristics of *Orbilia dorsalia* growed from cultures. **A.** Margin of ectal excipulum cells. **B.** Part vertical section of an apothecium. **C-F.** Asci. **G.** Paraphyses. **H-I.** Ascospores. Bars: B = 20 μm; A, C-I = 10 μm.

number of parsimony-informative characters is 244. With *Trichothecium roseum* as outgroup, a single most parsimonious phylogenetic tree was generated. This tree shows that, according to different trapping devices, predacious fungi form three clades, with high bootstrap values respectively. The result was consistent with Li *et al.*'s (2005). *O. dorsalia*, *D. oxyspora* (Sacc. & Marchal) Matsush. *D. atractoides* Drechsler, and *D. asthenopaga* (Drechsler) M. Scholler, Hagedorn & A. Rubner, formed a single clade, with 100% bootstrap support. Except for *D. asthenopaga*, other three taxa can not produce trapping device.

DISCUSSION

Morphologically, *O. dorsalia* most resembles *Orbilia fimicoloides* J. Webster & Spooner (Webster *et al.*, 1998) in having subulate ascospores, no-enlarged apex of paraphyses, crust-like secretion on the apices of paraphyses, and

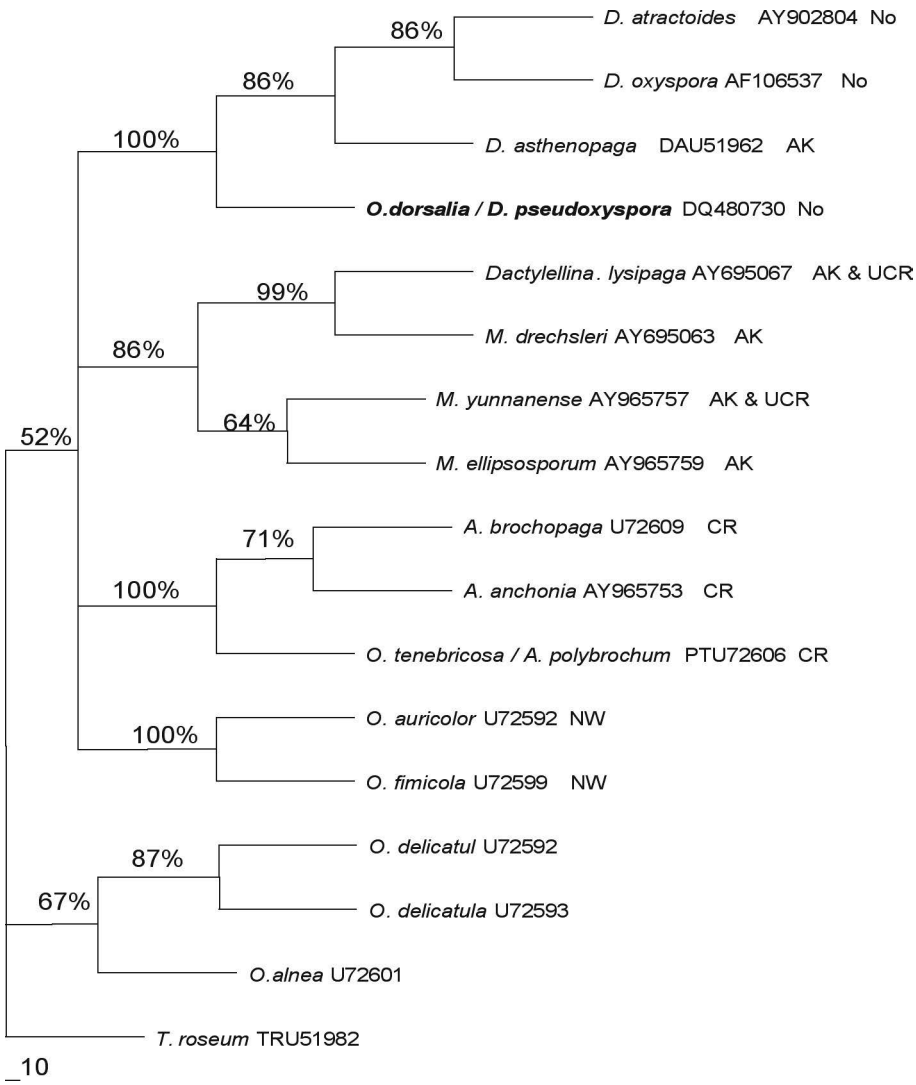

Fig. 5. Most parsimonious phylogenetic tree generated from a heuristic search based on the alignment of the ITS region sequences of predacious fungi and *Orbilia* spp. Numbers above lines represent bootstrap values from 1000 replicates on all parsimony-informative characters, with only bootstrap > 50% shown. Tree length = 961, consistency index (CI) = 0.6556, homoplasy index (HI) = 0.3444, retention index (RI) = 0.6548. AK = adhesive knobs; CR = constricting rings; NW = networks; UCR = non-constricting ring, No = no-trapping advice.

producing *Dactylella* sp. anamorph. However, *O. dorsalia* is different from *O. fimicoloides* by shorter asci (*O. fimicoloides* 30~35 × 3~3.5 μm, *O. dorsalia* 25~28.8 × 3~3.8 μm), shorter and wider ascospores (*O. fimicoloides* 8~10.5 × 0.9~1.0 μm, *O. dorsalia* 7~9.8 × 1.0~1.5 μm). SB is the key characteristic to separate species (Baral, pers. comm.), but SBs of *O. fimicoloides* was not described. In addition, their anamorphs (*D. oxyspora* and *D. dorsalia*) are also

different. Although the shape of conidia is similar, the size differs greatly (*D. dorsalia* 22.0~30.3(32.5) × 3.3~4 μm, *D. oxyspora* 60~100 × 9~13 μm), as well as the septa (*D. dorsalia* 5~9 septa, *D. oxyspora* 6~12 septa). The similarity of ITS sequences between those two species was 87.58%. Compared with similarities of other two species in the phylogenetic tree, for example, the similarity between *O. oxyspora* and *D. asthenopagum* is 92.62%, *O. fimicola* and *O. auricolor* is 98.8%, the similarity 87.58% supports *O. dorsalia* as a distinct species from *D. oxyspora*. *O. dorsalia* also resembles *Orbilia fimicola* Jeng & Krug (Jeng & Krug, 1977) in having subulate ascospores. However, *O. fimicola* is different from *O. dorsalia* by having inflating paraphyses apice covered with brown granules, and its anamorph is *Arthrobotrys superba*. SB of *O. fimicola* was also not occurred in the original description. *O. dorsalia* also resembles *O. auricolor* in having subulate ascospores, but *O. auricolor* distinguishes from *O. dorsalia* in having inflating paraphyses apice which are not encrusted. *O. auricolor* is a species complex which produced different *Arthrobotry* anamorphs, including *A. oligospora* Fresen., *A. cladodes* Drechsler var. *macroides* Drechsler (Pfister & Liftik, 1995), A. *psychrophilum* (Drechsler) M. Scholler, Hagedorn & A. Rubner (Rubner, 1996), *A. yunnanensis* M. H. Mo et K. Q. Zhang (Mo *et al.*, 2005). Except for tear-shaped SB of teleomorph of *A. yunnanensis*, there were no SBs described in other specimens. Fortunately, in our survey of these fungi, *A. oligospora*, *A. cladodes* var. *macroides* were isolated from *O. auricolor* complex and SBs were observed. Comparing to *O. dorsalia*, SB of teleomorph of *A. oligospora* is tear-shaped, distinctly attached to apex of spore, teleomorph of *A. cladodes* var. *macroides* is tear-shaped to ellipsoid, sometimes attached to apex of spore. However, *O. dorsalia* is ellipsoid, and does not attach to apex of spore.

The anamorph of *O. dorsalia* resembles *D. oxyspora* and *D. atractoides* Drechsler in the shape of conidia. Differences between *D. dorsalia* and *D. oxyspora* have been discussed above. *D. atractoides* differs from *D. dorsalia* both in the size and the septa of conidia (*D. atractoides* 32.5~90 (56) × 7.5~12.5 (9) μm, *D. oxyspora* 22.0~30.3(32.5) × 3.3~4 μm, the former is mainly 4~6-septa, the latter is mainly 6~8 septa). In addition, their branching mode of conidiophores differs greatly.

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