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A New *Dactylella* Species from *Orbilia alba*

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A new *Dactylella* species, *Dactylella alba* was isolated from the ascospores of *Orbilia alba* collected in Wenshan County, Yunnan Province, China. Conidiophores were either not branched or occasionally branched, bearing divergent sterigmata on the tip with single conidium on each. Conidia were elongated ellipsoids, 1-2 septate, mostly 1 septate. By combining the ITS sequence with morphological characteristics, a new anamorphic species is described and illustrated together with its teleomorph.

***Keywords*:** *Dactylella alba*, *Orbilia alba*, teleomorph-anamorph, ITS sequence

The fact that fungi can reproduce sexually and asexually (Alexopoulos *et al*., 1996) presents a major challenge for taxonomists in naming and classifying them, as a single entity has more than one type of reproductive morphology. At present, a whole fungus has been named both after its teleomorph and anamorph, as if it is two different species. Such a dual naming scheme goes against the principle of natural classification (Shenoy *et al*., 2007). Therefore, anamorph-teleomorph connections are important for the amalgamation of the two classification systems.

Orbilia Fries is known for its diverse anamorphs (Gams, 1995; Pfister, 1997; Chen *et al*., 2007; Shenoy *et al*., 2007) as it has produced about 10 anamorph genera, including nematode-trapping and non-trapping hyphomycetes (Pfister, 1997; Chen *et al*., 2007). Recently, new taxa were continuously reported from *Orbilia* (Liu *et al*., 2005; Mo *et al*., 2005; Yu *et al*., 2007a, 2007c), which has made *Orbilia* an important part of establishing anamorph-teleomorph of fungi.

Dactylella is a non-trapping anamorph. Six species thus far are known to be connected with *Orbilia* (Zachariah, 1983; Pfister, 1997; Webster *et al*., 1998; Yang and Liu, 2005; Yu *et al*., 2007a, 2007c). During our study of the *Orbilia* fungi and their anamorphs, a *Dactylella* species was isolated from *O. alba*. Conidiophores with branched or occasionally branched sterigmata on the tip have a candle-like structure, distinguishing *D. alba* from other *Dactylella* species. Therefore, a new *Dactylella* species is described in this paper by combining its morphological and ITS sequence characteristics.

Materials and Methods

Collection of teleomorph, isolation, and characterization of the anamorph

Fresh specimens of *O. alba* were collected from the decayed bark of a broad-leaved tree located in WenShan County, Yunnan Province, China (alt. 1272 m, 30 June 2006, Z.F. Yu. A dried voucher specimen was deposited to the Laboratory for Conservation and Utilization of Bio-resource, YMFT 1.01858). To isolate their anamorphs, several apothecia were fixed to the lid of a Petri-dish with their hymenia upside down so that ascospores would be directed to shoot towards the corn meal agar surface (CMA). The procedure was described in detail in previous paper (Yu *et al.*, 2006). All characteristics were observed and measured with an Olympus B51 microscope using differential interference contrast and a Zeiss Standard 20 microscope.

DNA extraction, PCR, and sequencing

Total DNA was isolated from fresh mycelium as described by Yu *et al*. (2007b). The primer pairs ITS4 and ITS5 (White *et al*., 1990) were used to amplify the complete ITS (including 5.8S) region. The parameters for PCR amplification were the same as described by Yu *et al*. (2007a). PCR products were purified with a commercial kit (TaKaRa, Japan). Both strands were sequenced with the same primers used in the amplification by a LICOR 4000L automatic sequencing system, using cycle sequencing with a Thermo-Sequenase-kit as described by Kindermann *et al*. (1998).

Phylogenetic analysis

We performed a parsimony analysis using ITS sequences of *Dactylella* species and representative *Orbilia* spp. with or without *Dactylella* anamorphs. The *Dactylella* species included in the phylogenetic analysis are found worldwide, incorporating species newly isolated from teleomorphs. *Dactylaria purpurella* was designated as an outgroup. GenBank accession was shown in the phylogenetic tree.

DNA sequences were aligned using CLUSTAL X 1.83 and the BioEdit programs. Parsimony analysis was performed using PAUP* 4.0b10 (Swofford, 2002) with the following settings: gaps treated as missing, all characteristics weighted equally, used heuristic searches with TBR (tree-bisection-

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reconnection) as the branch-swapping algorithm and bootstrap values generated using 1,000 replicates.

Results

Taxonomic description

***Dactylella alba* Z. F. Yu & K. Q. Zhang sp. nov.** (Fig. 1)

Coloniae in agaro albiae , post 4 dies 25°C ad 50 mm diam. Mycelium sparsum, hyphis septatis, 3.5~4 μm latis. Conidiophora hyalinae, septatae, erectae, simplices, 50~80 *μm altae, basi 3~3.5 μm crassae, sursum leviter fastigatae, subter apicem 2.5 μm crassae. ibi ex sterigmatibus brevibus obtusis 3~10 saepius 3~10 conidia in capitulum pulchrum radians aggregate ferentes vel subinde usque 11 conidia in parte superiore rarius digesta gerentes. Conidia hyalines, recta, elongate ellipsoidea, apice rotundata, basin versus paulo attenuate, 19~24.6 longa, 5.8~8.4 μm lata,1~2 septata, praecipue 1 septata.*

Etymology: species epithet refers to its teleomorph.

Holotype: P. R. China, Yunnan Province, Wenshan County, alt. 1272 m, substrate: *Orbilia alba* collected on decayed bark of a broad-leaved branch, June 2006, YMFT 1.01858, permanent slide culture (YMF 1.01858).

Teleomorph: *Orbilia alba* Dennis Kew Bull. 9: 295, 1954.

Colonies white, quick-growing on CMA medium, attaining a diameter of 50 mm in 4 days at 25°C. Vegetative hyphae hyaline, septate, 3.5~4 μm wide. Aerial mycelium sparse, hyaline, septate, branched, 2.5~4 μm wide. Conidiophores hyaline, septate, erect, simple or branched, 50 to 80 μm high, 3 to 3.5 μm wide at the base, tapering gradually upward to a width of 2.5 μm near the tip where it diverges.

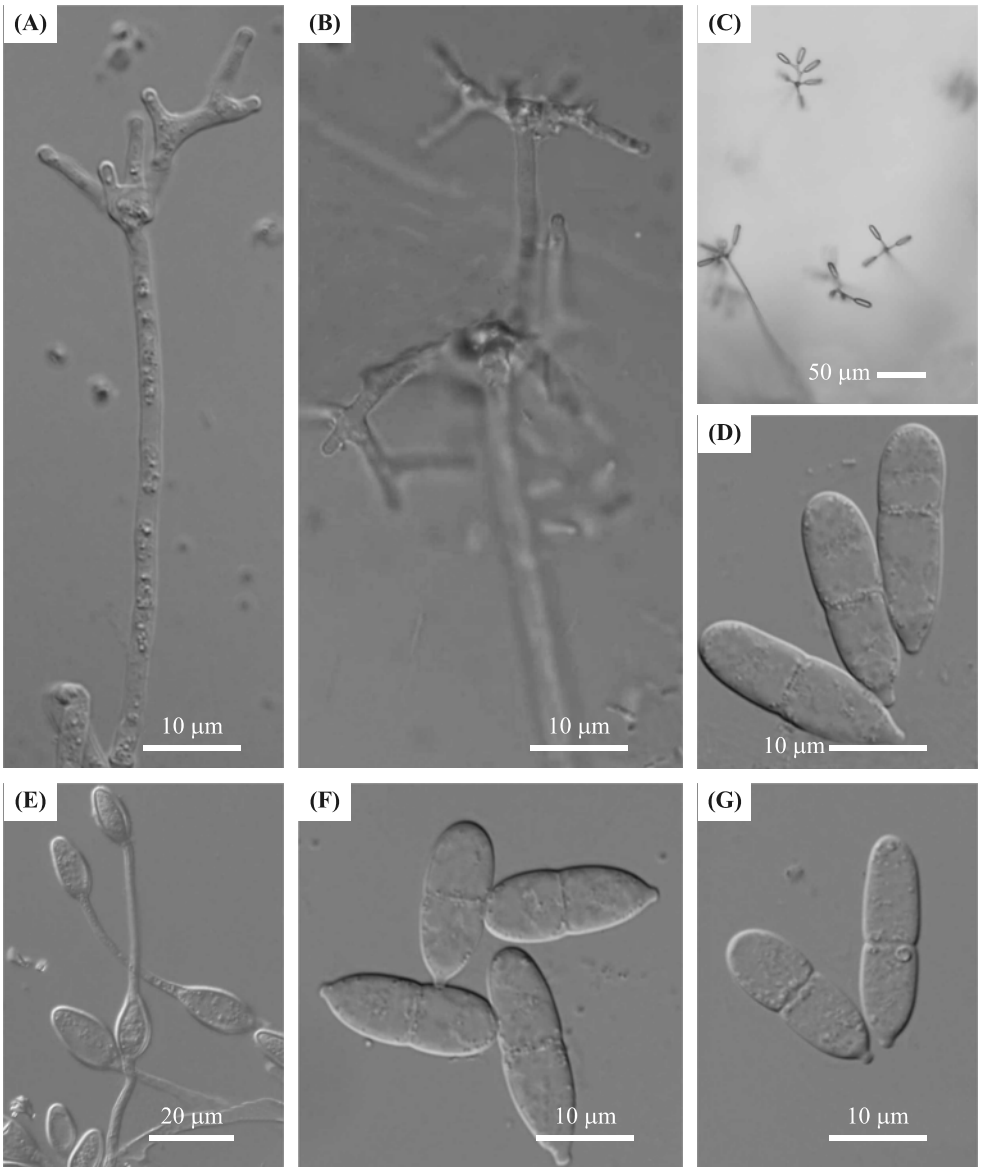

Fig. 1. Appearance of *D. alba* (YMF1.01858). (A, B) Conidiophores, (C) Conidiophores bearing conidia in clusters, (D, F, G) Conidia, (E) New type of spores similar to chlamydospore.

Simple or branched sterigmata, mostly 1.5 to 2.5 μm wide and 3.5 to 10 μm long, single conidia in each. Conidia hyaline, straight, elongated ellipsoid, broadly rounded at the wider distal end, constricted with a small, truncate protuberance at the base, 19~24.6×5.8~8.4 μm, 1~2 septate, proportion of conidia with 1, 2 septa 30% and 70%, respectively. A new type spore similar to chlamydospores sometimes produced from aerial mycelium developed from wider submerged mycelium, terminal to intercalary, solitary, hyaline, nonseptate, ellipsoidal or pyriform, 14~27×8.7~9.9 μm.

Orbilia alba **Dennis Kew Bull. 9: 295, 1954.** (Fig. 2)
Apothecia, gregarious, broadly sessile, whitish, translucent, round to somewhat undulating, flat, very thin, even margins, 2-6 mm in diameter. Ectal excipulum on the basal and flanking sides composed of angular or subglobose cells, (7-)10~20(-24)×(5-)10~15(-18) μm. Medullary excipulum strongly gelatinized. Asci 8-spored, (20.6-)26.2~33.8×3.4~3.8(-4.4) μm in living state, 22~31×2.7~3.2 μm in dead state, 4 of the spores inversely oriented (especially the lower ones), cylindric-clavate, tapered, often forked at the base. Apex medium truncate to rounded, thin-walled. Ascospores hyaline, non-septate, rod-shaped with one end obtuse and the other slightly tapered (especially in dead state), 3.6~5(-6.5)×0.7~1 μm in living state, 3.5~4.5×0.7~1 μm in dead state; spore bodies globose, approximately 0.5 μm diam., filled the obtuse ascospore apex at the upper end, attached to the inner apical wall. Paraphyses hyaline, subcylindrical to sublanceolate or spathulate with obtuse tips, 1.5~2.7 μm wide in dead state, slightly protruding, containing some paraphyses with capitate-clavate, tip 2.5~6 μm wide in dead state, sometimes covered with a layer of exudate about 1~1.3 μm thick.

Material examined: Decayed bark was taken from a branch of an unidentified broad-leaved tree lying on the ground of a subtropical forest in the karst region of P. R. China, Yunnan Province, Wenshan County, alt. 1272 m, on 30 June 2006, collected by Zefen Yu, YMFT1.01858.

Phylogenetic analysis

Parsimony analysis of the ITS sequences yielded a single, parsimonious tree based on 286 parsimony-informative characters (203 constant characters, 82 uninformative characters) (Fig. 3). The MP tree had 1410 steps in length with a consistency index (CI) of 0.522 and a retention index (RI) of 0.6897. All taxa were divided into four clades. Clade A is composed of worldwide *Dactylella* species isolated from soil, with the exception of *D. dorsalia* and *O. dorsalia* teleomorph (Yu *et al*., 2007c). Clade B is composed

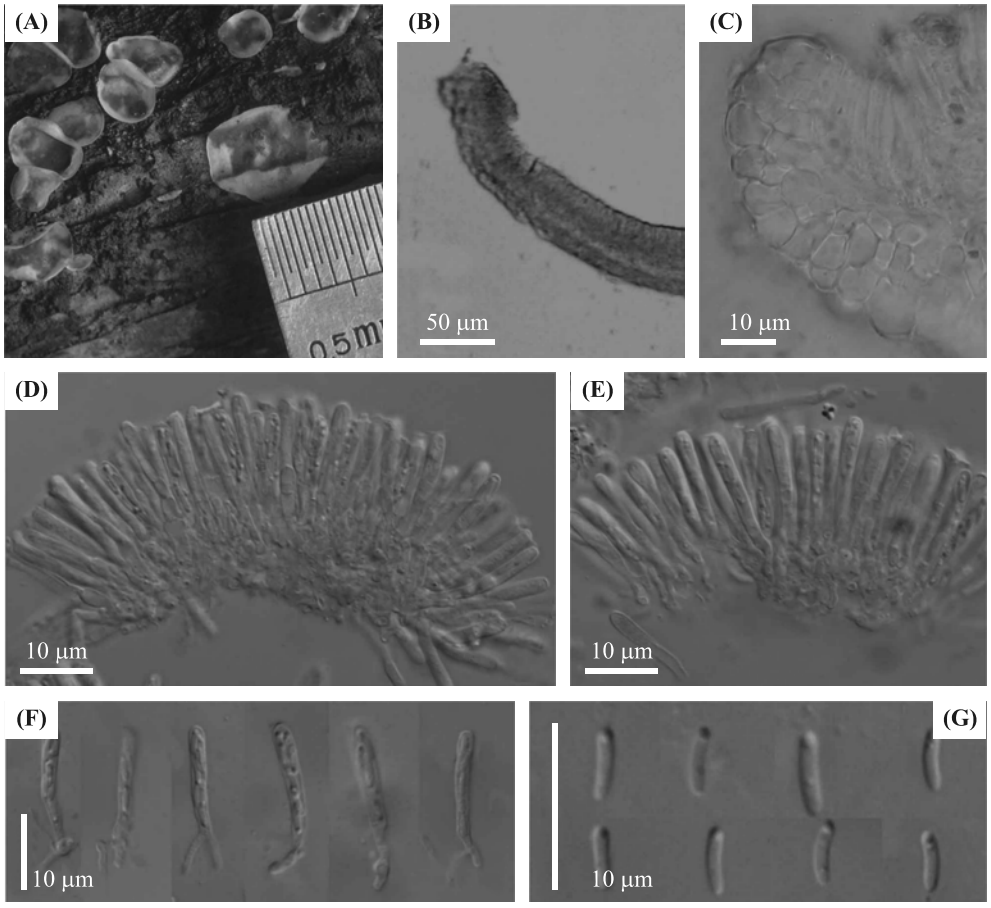

Fig. 2. *Orbilia alba* (YMFT 1.01858) (A) Rehydrated apothecia, (B, C) Vertical section of an apothecium, (D, E) Cluster of asci and paraphyses, (F) Dead asci, (G) Living ascospores.

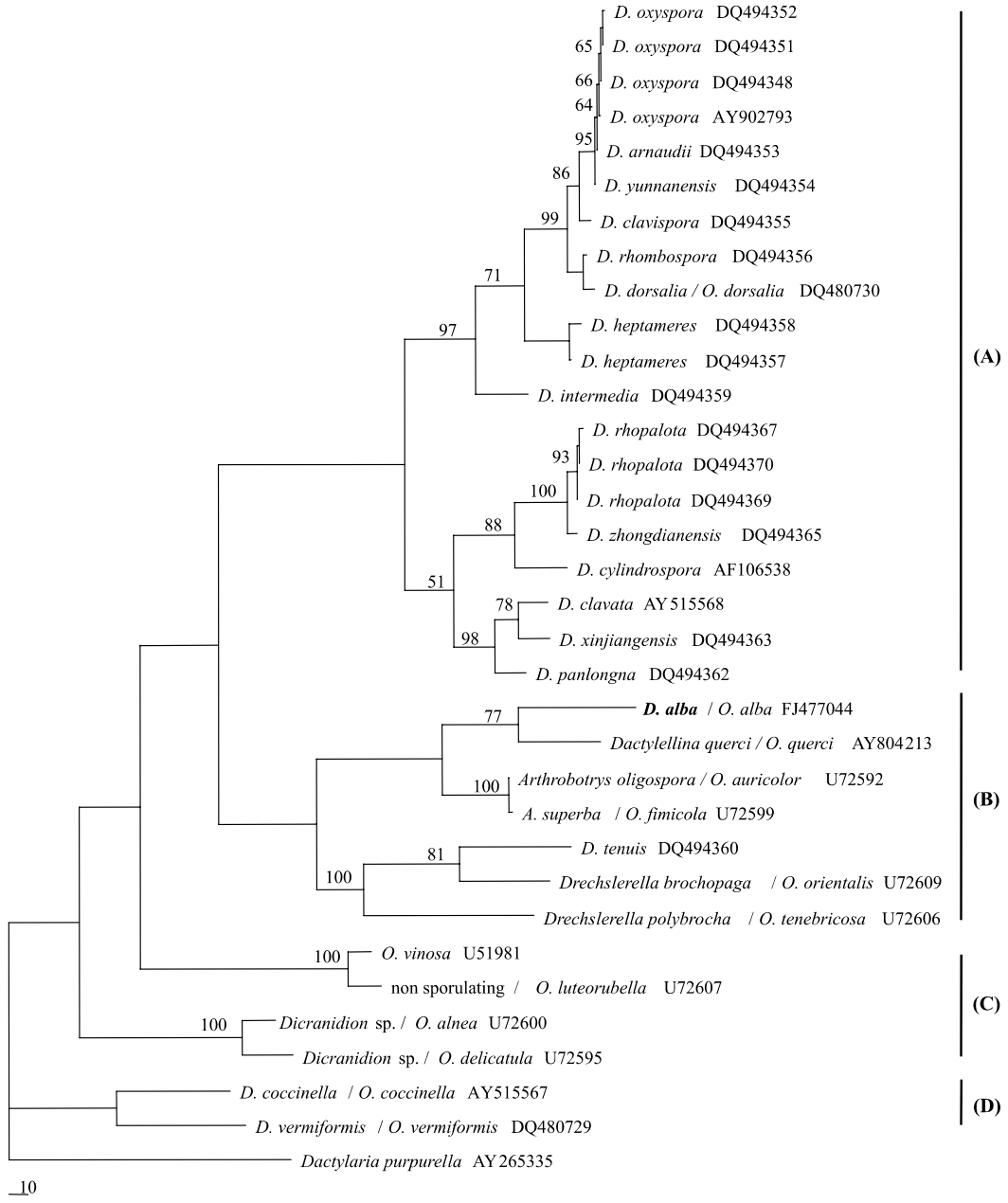

Fig. 3. Parsimonious phylogenetic tree generated from a heuristic search based on ITS region sequence alignment of common *Dactylella* species and representative *Orbilia* spp. Numbers above lines represent bootstrap values from 1,000 replicates on all parsimony-informative characters; only values >50% shown. Names above slant are anamorphs and those under slant are teleomorphs.

of 6 trapping nematode fungi and 2 *Dactylella* species, i.e. *D. alba* and *D. tenuis* Drechsler. *D. alba* is closely related to *Dactylellina querci*, which was isolated from *O. querci*. The reason these two taxa are closely related may be due to their teleomorphs possessing similar clavatae ascospores. Four *Orbilia* spp. constituted Clade C, their anamorphs being *Dicranidion* Harkness or non-sporulating. Clade D comprised of two *Dactylella* species isolated from teleomorphs. It is obvious that all *Dactylella* species did not fall into same clade. Chen *et al*. (2007) revised *Dactylella* based upon ITS sequence analysis and morphological characters. However, the heterogeneity of *Dactylella* still exists, especially when more and more *Dactylella* anamorphs of *Orbilia* are added.

Discussion

Dactylella was established by Grove with *D. minuta* Grove, which is incapable of preying on nematodes, as the type species. Main characters of the genus are saprophytic with erect conidiophores and simple. In addition, single conidia are located at the apex of conidiophores. The shape is ellipsoidal or fusoid or cylindrical and begins one-celled at first,

but later on they become 2- to many-septate as well as hyaline (Grove, 1884). Thereafter, additional descriptions of the genus were emended by other authors (Subramanian, 1963, 1977; Schenck *et al*., 1977; Rubner, 1996). Miao *et al*. (2003) reviewed its taxonomy variance and some predacious fungi were included in the *Dactylella* by some other authors. Recently, Rubner (1996) revised the generic concept and excluded the nematode-trapping species from this genus, which is currently widely accepted (Scholler *et al*., 1999; Li *et al*., 2005; Chen *et al*., 2007; Yu *et al*., 2007a, 2007c). Based on ITS sequence analysis and morphological characters, Chen *et al*. (2007) excluded five species from *Dactylella* for their inconsistent characters, resulting in only 28 *Dactylella* species. Many of these 28 species have more than 3 septa, however species with less than 3 include *D. clavispora* J. Chen, L.L. Xu, B. Liu & Xing Z. Liu, *D. teuis* Drechsler, *D. stenocrepis* Drechsler, *D. polyctona* (Drechsler) Zhang, Liu & Cao, *D. arrhenopa* (Drechsler) Zhang, Liu & Cao. Conidia of *D. polyctona* and *D. arrhenopa* have one septum, but are narrower than that of *D. alba* (*D. polyctona*, 2.3∼1.8 μm; *D. arrhenopa*, 2.6∼3.7 μm; *D. alba,* 5.8∼8.4 μm). Conidia of the other three species contain usually 3 septa, but occasionally only one.

D. alba is easily recognized by its conidiophores as it possesses obvious sterigmata at the tip and elongated ellipsoidal conidia with 1∼2 septa. Among the present *Dactylella* spp., only the conidiophore apex of *D. pulchra* (Linder) de Hoog & van Oorschot have similar sterigmata even though its conidia have more septa. Conidiophores apex of *D. alba* and *D. pulchra* are similar to that of *Arthrobotrys* Corda, however, their non-predacious characters and phylogenetic placement were consistent with *Dactylella*.

Acknowledgements

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