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## Drechslerella brochopaga, the anamorph of Orbilia (Hyalinia) orientalis

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*Abstract*—Cultures derived from three collections of *Orbilia* (formerly *Hyalinia*) *orientalis* produced an anamorph, which could be identified as *Drechslerella brochopaga*. The cultures formed constricting rings when nematodes *Panagrellus redivivus* were added. Both teleomorph and anamorph are illustrated and described. In addition, the new combination *Orbilia tenebricosa* is proposed.

Keywords-Orbilia orientalis, anamorph-teleomorph connection

#### Introduction

The genus *Orbilia* Fr. is a globally distributed genus comprising more than 200 species (Baral et al., in prep.). Its history was simply reviewed by Liu et al. (2005a). *Orbilia* has not been a major focus of mycologists because of the perceived low economic importance of the genus and minute size of the apothecia (Alexopoulos et al. 1996). However, Pfister (1994) reported predacious capabilities for some species of *Orbilia* and their *Arthrobotrys* anamorphs, and further data on nematode trapping in *Orbiliaceae* were subsequently supplied by Liu et al. (2005a, b) and Mo et al. (2005a, b). These authors also summarized known anamorphs of *Orbilia* (Liu et al. 2005a, Mo et al. 2005a). However, *Helicoon* Morgan (Pfister 1997) was excluded because its teleomorph was separated from other *Orbilia* spp. in the analysis of ITS regions (Hagedorn & Scholler 1999), and an *Idriella*-like anamorph (Haines & Egger 1982) was excluded because its teleomorph was not referable to *Orbilia* (Baral et al., in prep.).

The genus concept of predacious hyphomycetes was emended by Scholler et al. (1999) on the basis of ITS and 18S rDNA sequence data (Hagedorn & Scholler 1999) and, according to the phylogenies inferred from sequence analyses of 28S rDNA, 5.8S rDNA and ß-tubulin genes, further emended by Li et al. (2006).

Pfister (1997) and Scholler et al. (1999) included the sequence data of one and two strains of *Drechslerella brochopaga* (at that time without known teleomorph connection) respectively in their phylogenetic study of *Orbiliaceae*. During study of *Orbilia* and their

anamorphs, we found that *Orbilia orientalis* produced a nematode-trapping anamorph. After a detailed examination, the fungus was identified as *Drechslerella brochopaga*.

#### Material and methods

Fresh specimens of *Orbilia orientalis* were collected in China (on decaying bark of broadleaved tree in a forest at 848 m altitude, located in Jinggangshan Park of Jian City, Jiangxi province, in October 2005, Y. Zhang. A dried voucher specimen was deposited in the Laboratory for Conservation and Utilization of Bio-resource, Yunnan Province, China. YMFT 1.01892) and in France (on rotten bark on lower face of *Corylus avellana* branch lying on ground, c. 20 m altitude, N of St. Gemme la Plaine, Dept. Vendée, 6.VI.2003, J. L. Surault, H.B. 7379; on rottten wood of *Carpinus betulus* branch lying on moist ground, c. 110 m altitude, Forêt d'Aulnay, dept. Deux-Sèvres, 28.VI.2006, P. Tanchaud, H.B. 8146a). To isolate its anamorph, several apothecia were fixed to the lid of a Petri dish with their hymenia upside down to shoot ascospores on the surface of CMA (20 g corn meal, 18g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 ml distilled water).

Spore deposits from all apothecia produced the same anamorph. The Petri-dishes with apothecia were placed 4-6 days at room temperature until ascospore germination was seen on the CMA. The ascospores were transferred onto PDA plates and CMA plates respectively. After incubating 7-10 days at 25°, conidiophores and conidia were observed and measured with an Olympus B51 microscope with differential interference contrast and a Zeiss Standard 20 microscope. Trapping organs were induced by adding about 100 nematodes (*Panagrellus redivivus* Goodey) into a 1cm × 1cm square slot at the margins of the colony where the agar was removed.

In one of the two French collections (H.B. 7379), the anamorph (conidiophores and conidia) was only obtained around the apothecium on the lid of the Petri dish; a sterile transfer of mycelium and conidia in order to obtain a pure culture failed.

#### **Taxonomic Description**

### Teleomorph:

#### Orbilia orientalis (Raitv.) Baral,

- in Krieglsteiner, Regensburger Mykologische Schriften 9(1): 271. 1999
- = Hyalinia orientalis Raitv., in Azbukina, Plantae non vasculares, Fungi et Bryopsida Orientis extremis Sovietici, Fungi, Tomus 2, Ascomycetes, p. 362, 1991

Apothecia 0.8-1.2 mm in diam. (0.3-3 mm, France), superficial, with a distinct stalk up to 0.2-0.3 mm high, scattered to gregarious on decayed bark, white and translucent throughout when moist, pale brown when dry. Disc slightly concave to flat, margin even and smooth (distinctly crenulate-toothed in French specimens). Ectal excipulum of angular or globose cells, 8.0-13.8  $\mu$ m in diam. (near base 18-42 × 12-38  $\mu$ m, on flanks 15-30 × 12-20  $\mu$ m, France), with thin or only slightly thickened walls, towards margin of t. prismatica-angularis oriented at a high angle, marginal cells tipped by glassy processes (only observed in French specimens) up to 30-40 × 3.5-4.5  $\mu$ m, curved outwards, agglutinated to form rounded teeth. Medullary excipulum rather thick, of t. globulosa (-prismatica) with intermingled hyphae, subhymenium poorly developed.

FIGURE 1

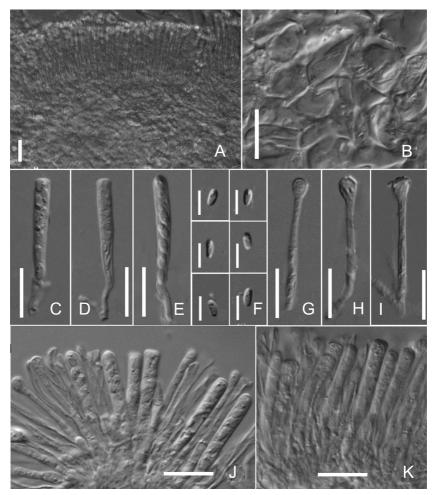


Fig. 1. Orbilia orientalis (YMFT 1. 01829) A. Hymenium B. Cells of ectal excipulum. C-E. Dead asci. F. Living ascospores with globose SBs. G-I. Dead paraphyses. J-K. Cluster of dead asci and paraphyses with living spores. Scale bar: A-E, G-K =  $10 \mu m$ , F =  $5 \mu m$ .

Asci 29.5-38 × 3.2-4(-4.5) µm (living state, France), 8-spored, lower (2-) 3-5(-6) spores inversely oriented (with spore body towards ascus base), cylindric, rounded or truncate at the apex (depending on side of view), tapered and often forked at the base (L, h-or H-shaped). Ascospores hyaline, non-septate, cylindric-ellipsoid, sometimes slightly tapered at lower end, 3.1-3.9×1.6-1.8 µm (living state, France: 2.7-3.7 × 1.3-1.6(-1.8) µm), with a refractive rod-shaped spore body (SB) at upper end in living mature ascospores, 1.2-1.4 × 0.3-0.5 µm in diam. (France: SB rod-shaped, attached to apex by a very fine

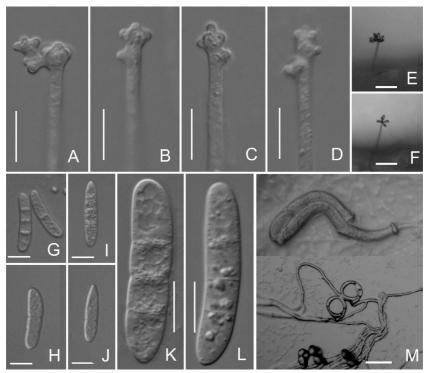


Fig. 2. Drechslerella brochopaga (YMF 1. 01829) A-F. Conidiophores. G-L. Conidia. M. Constricting rings. Scale bars: A-D, G-L=10 µm; E-F, M=50 µm.

filum, total size  $1.2-1.7 \times 0.15-0.2(-0.3) \mu m$ , SB often situated at lateral wall of spore). Paraphyses  $1.3-2.0 \mu m$  wide, enlarged to  $(2.3-)2.8-3.7(-4) \mu m$  in diam. at the medium to rather strongly clavate or capitate apex, terminal cell much longer than lower cells, apex covered by a rough thin layer of exudate.

#### Anamorph:

## Drechslerella brochopaga (Drechsler) M. Scholler, Hagedorn & A. Rubner,

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Sydowia 51: 99, 1999
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FIGURE 2

- Basionym: Dactylella brochopaga Drechsler, Mycologia 29: 517, 1937
  - = Dactylariopsis brochopaga (Drechsler) Mekht., Mikol. Fitopatol. 1: 278, 1967
  - = Arthrobotrys brochopaga (Drechsler) S. Schenck, W.B. Kendr. & Pramer, Can. J. Bot. 55: 982, 1977
  - = Candelabrella brochopaga (Drechsler) Subram., Kavaka 5: 95, 1978 ("1977")

Colonies colorless, spreading on CMA, reaching 48 mm after 14 days at 25°. Vegetative hyphae hyaline, septate, 3.5-4  $\mu$ m wide. Aerial mycelium sparse, hyaline, septate, branched, 2.5-4  $\mu$ m wide. Conidiophores hyaline, erect, septate, mostly 380-430  $\mu$ m high, 3.5-4.0  $\mu$ m at the base, tapering upward to a width of 2-2.5  $\mu$ m at the apex, there bearing

short blunt denticles 2-10  $\mu$ m in length, whereon bearing 3-10 conidia in radiating capitate arrangement, occasionally producing up to 13 conidia in more scattered, irregularly racemose arrangement. Conidia commonly cylindric-oblong, rounded at the distal end, shortly tapered at the somewhat truncate base, 20-36 × 5-7.5  $\mu$ m (living state, France: (27.5-)31-39(-45) × 6.5-7(-9)  $\mu$ m), slightly to medium curved especially near distal end (straight in front view), with (1-)3 septa, predominantly 3-septate, then terminal cells often exceeding the length of the middle cells by a third or even a half (not so distinctly longer in French material). Many constricting rings with an outer diam. of 30-40  $\mu$ m were observed on the CMA plates challenged with nematodes.

#### Discussion

*Orbilia orientalis* was described by Raitviir (1991) in the genus *Hyalinia* Boud., based on the presence of long glassy processes at the apothecial margin (described as "solid glassy hairs  $40-60 \times 3-5 \mu m$ "). Baral (1994) has shown that such hair characters frequently vary among closely related taxa or even within a species, and therefore have little taxonomic value at generic level. Hence, *Hyalinia* is here treated as a synonym of *Orbilia*.

*O. orientalis* is known to us from a few records from Europe and Asia. In our collections, the ascospores are more obtuse and the paraphyses more capitate than described in the protologue (spores pointed and paraphyses only slightly clavate). However, in a restudy of the holotype specimen (TAA) by one of us (H.B.) it was found that the ascospores were actually obtuse and the paraphyses distinctly capitate. We are therefore convinced that our collections from China and Europe are conspecific with the type collection from the Far East of Russia (region of Ussur, Primorsk, north of Vladivostok).

In the collection from China, the glassy processes were very short or perhaps even absent, the apothecial margin therefore smooth. Since the other characters including the anamorph were found to be very similar among our specimens, we conclude that the presence versus absence of such processes has little taxonomic value at species level in *O. orientalis*.

*Orbilia orientalis* is very close to *Orbilia tenebricosa* (Svrček) Baral **comb. nov**. (Basionym: *Patinella tenebricosa* Svrček, Česká Mykol. 31: 135, 1977), from which it differs in distinctly shorter and partly thicker ascospores, also in the absence of apothecial pigmentation. For *O. tenebricosa* (sub *Patinella*) Pfister (1997) reported *Drechslerella polybrocha* (Drechsler) M. Scholler et al. as anamorph. This was the first report of an anamorph with constricting rings in the *Orbiliaceae*. The present paper is the second report of such an anamorph-teleomorph connection.

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#### Literature Cited

- Alexopoulos CJ, Mims CW, Blackwell M. 1996. Introductory Mycology. Fourth Edition. New York: John Wiley and Sons, Inc: 355.
- Baral HO. 1994. Comments on "Outline of the ascomycetes 1993". Systema Ascomycetum 13:113-128.
- Baral HO, Marson G, Weber E. (in prep.). Monograph of Orbiliomycetes.
- Haines JH, Egger, KN 1982. A new species of Orbilia from Canada. Mycotaxon 16: 107-113.
- Hagedorn G, Scholler M. 1999. A reevaluation of predatory orbiliaceous fungi. I. Phylogenetic analysis using rDNA sequence data. Sydowia 51: 27-48.
- Li Y, Hyde KD, Jeewon R, Cai L, Vijaykrishna D, Zhang KQ. 2006. Phylogenetics and evolution of nematode-trapping fungi estimated from nuclear & protein coding genes. Mycologia 97: 1034-1046.
- Lin B, Liu XZ, Zhang WY. 2005a. Orbilia querci sp. nov. and its knob-forming nematophagous anamorph. FEMS Microbiol. Lett. 245: 99-105.
- Liu B, Liu XZ, Zhang WY. 2005b. A new species of *Hyalorbilia* and its anamorph from China. Nova Hedwigia 81: 145-155.
- Mo MH, Zhou W, Huang Y, Yu ZF, Zhang KQ. 2005a. A new species of *Dactylella* and its teleomorph. Mycotaxon 91: 185-192.
- Mo MH, Huang XW, Zhou W, Huang Y, Hao YE, Zhang KQ. 2005b. Arthrobotrys yunnanensis sp. nov., the fourth ananmorph of Orbilia auricolor. Fungal Diversity 18: 107-115
- Pfister DH. 1994. Orbilia fimicola, a nematophagous discomycetes and its Arthrobotrys anamorph. Mycologia 86: 451-453.
- Pfister DH. 1997. Castor, Pollux and life histories of fungi. Mycologia 89: 1-23.
- Raitviir A. 1991. Order *Helotiales* Nannf. (in Russian), In: Z. M. Azbukina, Plantae non vasculares, Fungi et Bryopsida Orientis extremis Sovietici, Fungi, Tomus 2, Ascomycetes, *Erysiphales*, *Clavicipitales*, *Helotiales*, p. 254-363.
- Scholler M, Hagedorn G, Rubner A. 1999. A reevaluation of predatory orbiliaceous fungi. II. A new generic concept. Sydowia 51: 89-113.