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Two *Arthrobotrys* anamorphs from *Orbilia auricolor*

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Abstract: Cultures derived from ascospores of two collections both referable to *Orbilia auricolor* produced anamorphs which were assigned to *Arthrobotrys cladodes* var. *macroides* and *A. oligospora* var. *oligospora*. These morphologically distinct isolates formed nematode-capturing hyphal networks when nematodes were present. Descriptions of the *Arthrobotrys* isolates are given. At least one other nematophagous hyphomycete is connected with a teleomorph that can be referred to *O. auricolor* suggesting that *O. auricolor* is not a single entity but a species complex.

Key Words: *Arthrobotrys*, Helotiales, nematophagous, *Orbilia*

INTRODUCTION

Pfister (1994) reported an *Arthrobotrys* Corda anamorph of *Orbilia fimicola* Jeng & J. C. Krug and reviewed the earlier literature in which *Orbilia* Fr. species had been indirectly implicated in the life history of *Arthrobotrys* species. More recently, Rubner and Baral (1994 pers. comm.) reported that a culture of the nematophagous *Monacrosporium psychrophilum* (Drechsler) R. C. Cooke & C. H. Dickinson produced a discomycete in culture which they identified as *Orbilia auricolor* (A. Bloxam ex Berk. & Broome) Sacc. To date, six genera of hyphomycetes have been reported to be connected to *Orbilia* species: *Dicranidion* Harkn. (Berthet, 1964; Korf, 1992), *Arthrobotrys* (Pfister, 1994), *Dactylella* Grove (Thakur and Zachariah, 1989), *Monacrosporium* Oudem. (Rubner and Baral, pers. comm.), *Anguillospora* Ingold (Webster and Descals, 1979) and an unnamed genus (Haines and Egger, 1982). In this paper we report that two *Arthrobotrys* species were isolated from ascospores of two specimens of members of the genus *Orbilia*. Both specimens used in this study are referable to *O. auricolor sensu* Spooner (1987), a taxon which has frequently been known under the name *O. curvatispora* Boud. Our report highlights not only the connection of *Orbilia* species with *Arthrobotrys* but also the taxonomic confusion in the genus *Orbilia*.

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MATERIALS AND METHODS

Ascospore deposits were obtained from the following ascomatal specimens: on decorticated wood from a swampy area, Purgatory Tract, Westwood, Massachusetts, July 1994, Pfister and Liftik (culture no. 90), and on a crustose lichen on a rock that had been incubated in a moist chamber (culture nos. 45 and 55). Voucher specimens are deposited in FH.

Whole fresh ascomata were attached to petri plate tops using petroleum jelly and placed over malt extract-yeast extract agar (MEYE) (Lilly and Barnett, 1951). Ascospores were discharged within 24 h, and short germ tubes developed after 2–7 days. Stock cultures were maintained on potato-dextrose agar (BBL 11550), cornmeal agar (BBL 11132) and MEYE. Cultures observed in the study were stored at room temperature in ambient light.

To determine trap formation, petri plates containing cornmeal agar were inoculated and the fungus was allowed to grow to the margins of the plate. Living nematodes (*Cephalobus* sp., Carolina Biological Supply L 278), maintained on potato plugs in distilled water, were looped onto a microscope slide, mixed with one or two drops of water and then added to the plates. Plates were examined directly under a compound microscope at 100–200×.

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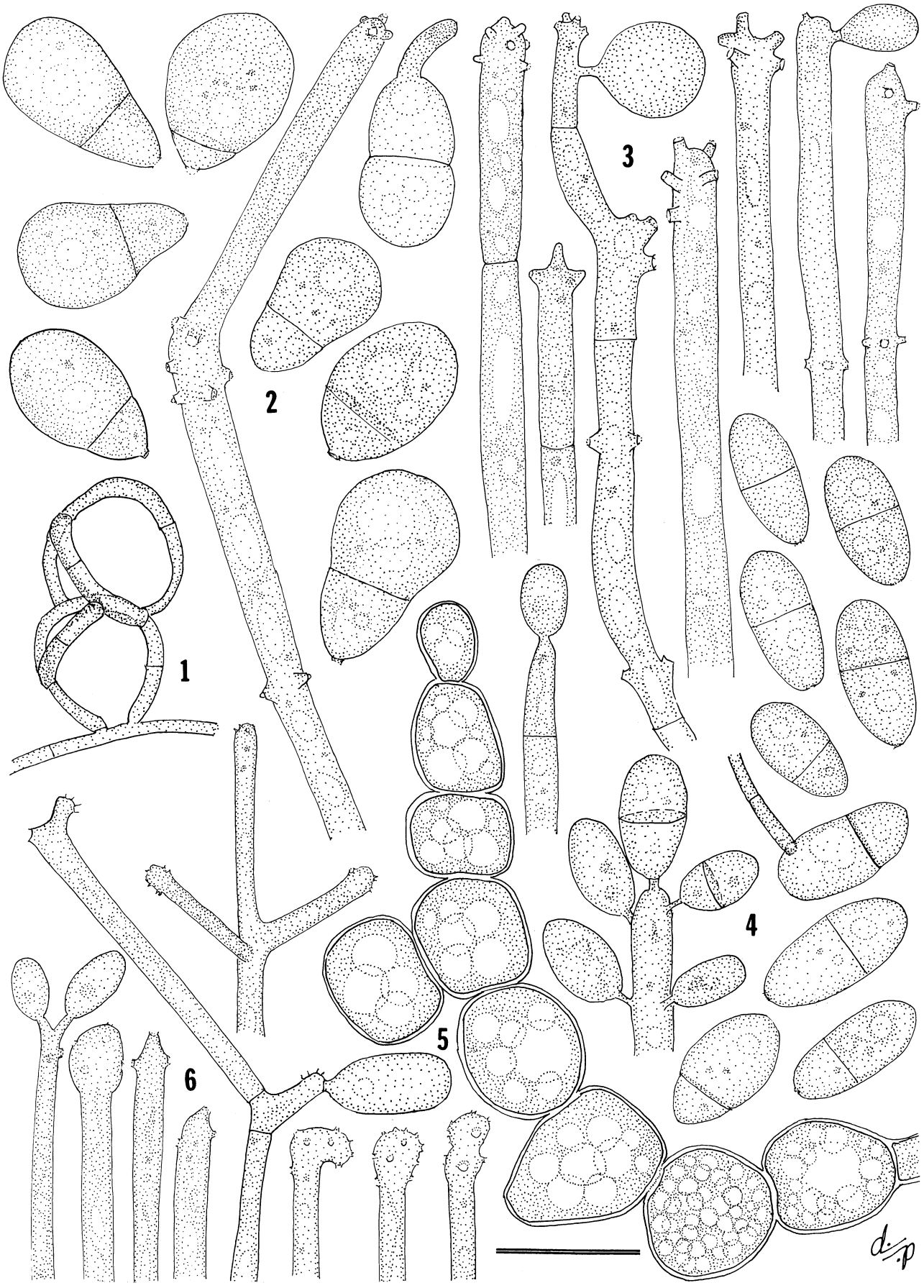
FIGS. 1–6. Microscopic features illustrated from cultures on MEYE of *Arthrobotrys oligospora* var. *oligospora* (culture no. 45) and *A. cladodes* var. *macroides* (culture no. 90). 1–3. *A. oligospora* var. *oligospora*. 1. Trap formed in nematode infested culture. 2. Conidia and conidiogenous cell. 3. Various conidiogenous cells and developing conidia. 4–6. *A. cladodes* var. *macroides*. 4. Conidia and conidiogenous cells. 5. Chlamydospore. 6. Conidiogenous cells and developing conidia. Scale = 16 μm.



For slide culture, a microscope slide, cover slip, glass support rod, and Whatman no. 1 (7-cm) filter paper were placed in a glass petri plate and autoclaved. A sterile 2-cm square of CMA was placed on the microscope slide. Two small amounts of each *Arthrobotrys* culture were transferred to the edges of the CMA blocks. The blocks were then topped with the cover slip. The filter paper was moistened with 2 ml of sterile water. Observations were made after 3–6 days of growth after which they were refrigerated and periodically examined.

Photographs were taken using an Olympus BH-2 microscope with bright field and Nomarski optics.

RESULTS

Both collections of ascomata used in this study could be referred to *Orbilia auricolor* using currently available keys (Spooner, 1987; Korf, 1992) and an unpublished study guide prepared by O. H. Baral. *Orbilia auricolor* is characterized as follows: Ascomata up to 1 mm diam, yellow to orange, pulvinate to turbinate; asci eight-spored, 30–40 × 3–4 μm, cylindric, J-, tapered toward the base and often forked, at the apex truncate without an obvious pore; ascospores curved, narrowly clavate, broad and rounded at one end, narrowing to an acute point at the other end, nonseptate containing a single inclusion which stains intensely in cresyl blue, 8–12.5 × 0.9–1.5 μm; paraphyses branching below, abruptly swelling to become capitate above reaching a diameter of 3–3.5 μm; excipulum of angular cells.

The anamorphs derived from the collections used here belong to different species of *Arthrobotrys* based on the published accounts of the genus by Cooke and Godfrey (1964), Haard (1968) and van Oorschot (1985). Culture nos. 45 and 55, derived from the material on the crustose lichen, were referred to *A. oligospora* Fresen. var. *oligospora*. Culture no. 90, derived from an *Orbilia* collected on decayed wood, produced an anamorph referable to *A. cladodes* Drechsler var. *macroides* Drechsler. Descriptions of the anamorphs follow:

Arthrobotrys oligospora Fresen. var. *oligospora* (culture nos. 45 and 55). FIGS. 1–3,7–9

Ascospores germinated after 2–3 days. Cultures on MEYE at first white becoming rose within 1 wk, growth cottony in places with hyphal strands forming across the surface of the medium. On CMA growth is uniform but sparse, remaining white. Conidiophores erect, with whorls of conidia. Conidia blastic, hologenous, 14–28 × 10–14 μm, obovoid, one septate, often constricted at the septum, the proximal cell smaller than the distal cell. Conidia basipetally produced, at several closely spaced loci, on somewhat inflated areas of the conidiogenous cells; secession is schizolytic, leaving protuberant, open, unthickened denticles with frills. Conidiogenous cells are indeterminant, persistent, with hologenous sympodial, resumptive proliferation.

Cultures with nematodes producing three-celled rings singly or aggregated into multidimensional networks (FIGS. 1, 7–9). Many traps formed within 12 h; nearly all the nematodes were trapped within 3 h.

Arthrobotrys cladodes Drechsler var. *macroides* Drechsler (culture no. 90). FIGS. 4–6, 10–12

Ascospores germinated within 4 days. Cultures on MEYE white becoming pale tan, forming hyphal strands on surface and cottony patches, remaining white. On CMA growth is sparse, remaining white. Conidiophores erect, branching, not proliferating; each branch terminating in a loose whorl of conidia. Conidia blastic, hologenous, 18–20 × 6–8 μm, elongate ellipsoid, with a single septum, the cells nearly equal, without constrictions at the septa. Conidia basipetally produced at several closely spaced loci on a sometimes inflated, often distorted, terminal area of the conidiogenous cell; secession schizolytic, leaving a slight protuberant, unthickened, open denticle with a slight frill. Conidiogenous cell determinant, persistent.

Cultures with nematodes normally produced three-celled rings singly or proliferating to produce multidimensional networks (FIGS. 10–12). Traps produced within 12 h, somewhat sparsely produced.

DISCUSSION

That collections of seemingly the same teleomorph produce morphologically distinct anamorphs strongly suggests that current concepts of *Orbilia auricolor* need critical reevaluation and that a complex of species is represented. The two collections from which cultures were derived do differ slightly in ascomatal color and shape. Ascomata that gave rise to *A. oligospora* var. *oligospora* were white or pale tan, were broadly attached to the substrate and broadly turbinate. Asco-

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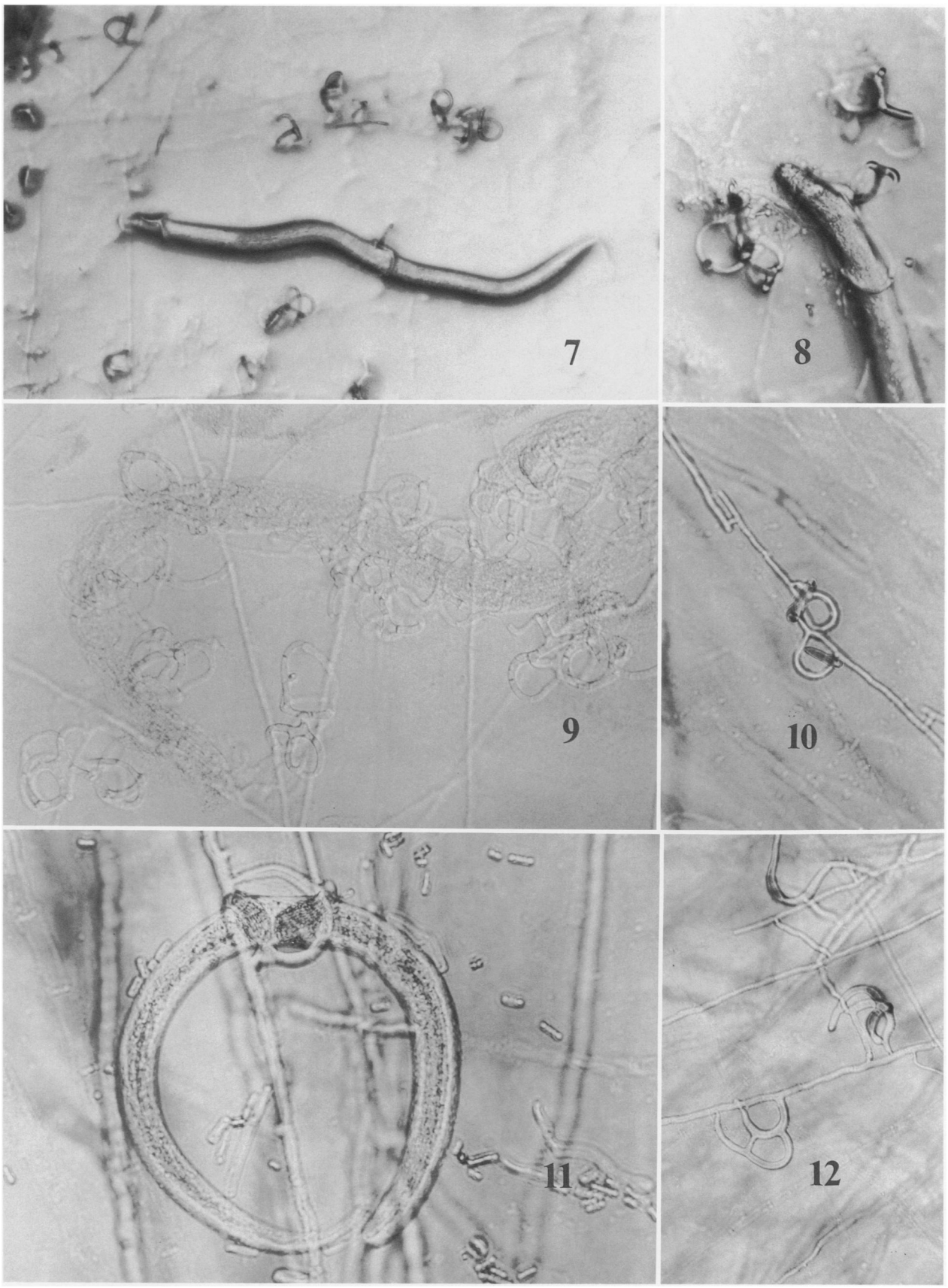
FIGS. 7–12. Trapped nematodes and trapping structures produced in nematode infested cultures of *A. oligospora* var. *oligospora* (culture no. 45) and *A. cladodes* var. *macroides* (culture no. 90). 7–9. *A. oligospora* var. *oligospora* showing traps and trapped nematodes at various stages. FIG. 7, ×100; FIG. 8, ×200; FIG. 9. ×400. 10–12. *A. cladodes* var. *macroides* showing traps and a trapped nematode FIGS. 10 and 12, ×200; FIG. 11, ×400.



mata that gave rise to *A. cladodes* var. *macroides* were yellow to orange and were centrally attached and lenticular. No type or authentic material of *O. auricolor* or of the several synonyms listed by Spooner (1987) was examined and, since fresh material is essential for accurate identification of species of *Orbilia,* such studies would be inconclusive. In addition to the present report, Rubner and Baral (pers. comm.) found that *Monacrosporium psychrophilum* produced in culture a discomycete also referable to *O. auricolor.* Further evaluation of the morphology of these teleomorphic taxa will be necessary to fully resolve the entities in this complex.

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