Arthrobotrys yunnanensis sp. nov., the fourth anamorph of *Orbilia auricolor*

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A new species of predacious fungi, *Arthrobotrys yunnanensis*, is described and illustrated as the fourth anamorph of *Orbilia auricolor*. The fungus produces simple, erect conidiophores with several short apical denticles. The conidia are nonseptate or occasionally uniseptate, elongate ellipsoid-cylindrical or slightly clavate. In aged cultures it forms spherical to ellipsoidal chlamydospores. In the presence of nematodes, the fungus forms three-dimensional adhesive networks. In this paper the known anamorphs connected to the genus *Orbilia* also are summarized.

Key words: anamorph-teleomorph connection, *Arthrobotrys*, *Orbilia*, predacious fungi.

Introduction

Nematophagous fungi have been the subject of research over several decades in fundamental studies of their ecology, distribution and systematics, and as potential biological control agents of nematode pathogens of plants and animals (Li *et al.*, 2002; Liu and Zhang, 2003; Dong *et al.*, 2004). The predacious hyphomycetes in *Arthrobotrys* Corda and related genera, some with teleomorphs in *Orbilia* Fr. (*Ascomycota*, *Orbiliaceae*), destroy nematodes using several kinds of trapping devices: stalker and sessile adhesive knobs, two- or three-dimensional adhesive nets, and constricting and non-constricting hyphal rings (Scholler *et al.*, 1999). The known anamorphs of *Orbilia* include both predacious and non-predacious fungi (Table 1). The predacious forms fall into the genera *Arthrobotrys* Corda (Pfister, 1994; Pfister and Liftik, 1995), *Monacrosporium* Oudem. (Rubner, 1996; Liu *et al.*, 2002). Apparently non-predacious anamorphs of *Orbilia* include *Anguillospora* Ingold (Webster and

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Table 1. Anamorphs-teleomorph connection the genus *Orbilia*.

Teleomohps	Anamorphs	References
O. auricolor 1	**A. cladodes* Drechsler var. *macroides* Drechsler	Pfister and Liftik, 1995
O. auricolor 2	**A. oligospora* Fresen.	Pfister and Liftik, 1995
O. auricolor 3	**M. psychrophilum* (Drechsler) Cooke	Rubner, 1996
O. auricolor 4	**A. yunnanensis* M.H. Mo & K.Q. Zhang	This paper
O. fimicola	**A. superba* Corda	Pfister, 1994
O. sp.	**A. dactyloides* Drechsler	Zachariah, 1983
O. sp.	**M. doedycoides* (Drechsler) Cooke & Dickinson	Pfister, 1997
O. cunninghamii	**M. parvicolle* (Drechsler) Cooke & Dickinson	Liu *et al*., 2002
O. fimicoloides	*D.* cf. *oxyspora* (Sacc. & Marchal) Matsush.	Webster *et al*., 1998
O. alnea	*D.* sp.	Pfister, 1997
O. sp.	*D. rhopalota* Drechsler	Thakur and Zachariah, 1989
O. delicatula	*Dicranidion* sp.	Pfister, 1997
O. junci	*Dwayaangam junci* Kohlm.	Kohlmeyer *et al*., 1998
O. luteorubella	*Helicoön sessile* Margon	Pfister, 1997
O. luteorubella	*Anguillospora* sp.	Pfister, 1997
O. sp.	*Anguillospora rosea* J. Webster & Descals	Webster and Descals, 1979; Wesber, 1992; Pfister, 1997
O. piloboloides	*Idriella* sp.	Haines and Egger, 1982
O. trinacriifera	*Trinacrium* sp.	Matsushima, 1995
O. xanthostigma	*Dicranidion* sp.	Berthet, 1964; Korf, 1992; Pfister, 1997

Note: The predacious anamorphs were indicated by an asterisk (*) and the others were non-predacious species, for which no predacious organs or other evidence of nematode predation is known.

Descals, 1979; Pfister, 1997), *Dactylella* Grove (Thakur and Zachariah, 1989; Webster *et al.*, 1998), *Dicranidion* Harkn. (Berthet, 1964; Korf, 1992), *Dwayaangam* Subram. (Kohlmeyer *et al.*, 1998), *Helicoö n* Morgan (Pfister, 1997), *Idriella* P.E. Nelson & S. Wilh. (Haines and Egger, 1982) and *Trinacrium* Riess. (Matsushima, 1995).

While surveying the predacious fungi, we collected wet soil samples from Mt Xiaobailong, Yiliang, Yunnan, China on 15 August 2003. Subsamples of 2-5 g were spread on Corn Meal Agar (CMA) plates and stored at room temperature (about 20-28ºC). After incubation for 20 days, apothecia of an *Orbilia* were observed on the soil granules, and later also on other areas of the plates. For culture isolation, several fresh apothecia were attached to the lids of Petri dishes of CMA with medicated petroleum jelly. Ascospores were projected on the agar after 2-4 days and blocks with germinating ascospores were transferred into other CMA plates after germ tubes developed. Four

isolates were obtained from the deposited ascospores of four separate apothecia. For anamorph identification, the cultures were inoculated on CMA and incubated at 28ºC for 14-30 days and the taxonomic characters were measured and determined. To induce trap formation, a 2 cm^2 piece of agar in the center of the plate was removed from a 7-day old culture to create an open space. About 200 nematodes (*Panagrellus redivivus*) were added the free space after the mycelia emerged from the cut margin. Microscopic photographs of anamorph and teleomorph were taken from fresh living material mounted in water using an Olympus BX51 microscope.

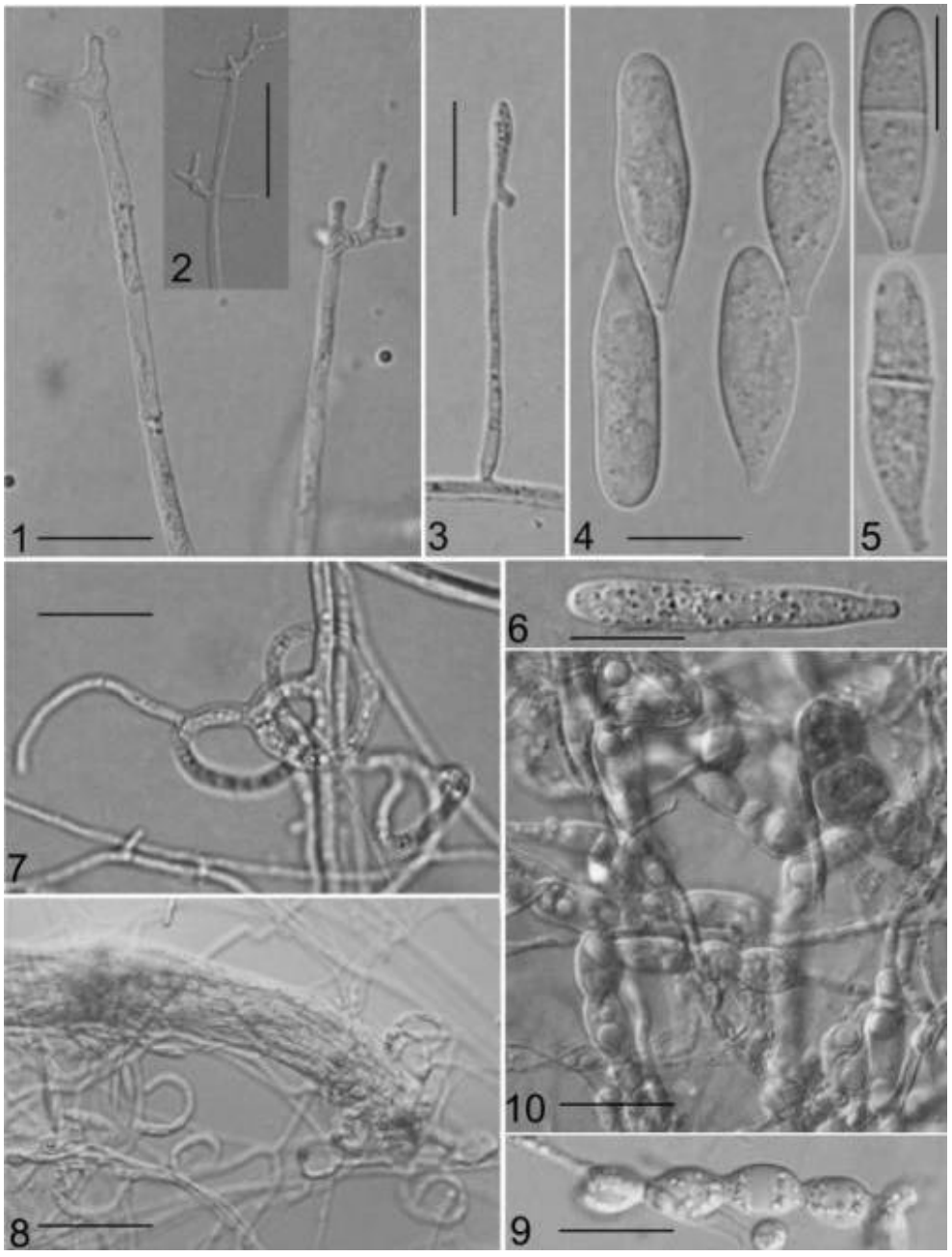
On CMA medium, the discharged ascospores germinated quickly and grew well. Four anamorph isolates were morphologically identical and can be referred to the genus *Arthrobotrys* Corda. Three morphologically distinct species of predacious anamorphs from the genera *Arthrobotrys* and *Monacrosporium* have been reported for a single species of *Orbilia*, *O. auricolor* (Berk. & Br.) Sacc., namely *A. oligospora* Fresen. (Pfister and Liftik, 1995), *A. cladodes* Drechsler var. *macroides* Drechsler (Pfister and Liftik, 1995) and *M. psychrophilum* Drechsler (Rubner, 1996). Morphologically, *O. auricolor* is a difficult species complex (Webster *et al.*, 1998). Seven predacious fungi have been reported as anamorphs of *Orbilia* (Table 1); the new species *A. yunnanensis* reported here represents an eighth, and the fourth known anamorph of *O. auricolor*.

Arthrobotrys yunnanensis M.H. Mo & K.Q. Zhang, **sp. nov.** (Figs. 1-10)

Etymology: The species epithet refers to the collection site of the species.

Coloniae in CMA effusae, ad 6 cm diam. post 5 dies 28 ºC. *Mycelium* sparsum, effusum, hyalinum, septatum, romosum, 2-4 µm laum. *Conidiophora* hyalina, simplicia, erecta, septata, non ramosa, plerumque 60-200 µm alta, basi 2-5 µm crassa, apice 1.5-2.4 µm crasso, efferenti 1-5 conidia sola de conidiogenis loci in perspicuis dendriculis in apicie aut prope apicem. *Conidia* hyalina, elongato ellipsoideo-cylindrica vel clavata, saepe non-septata, aliquanto uniseptata, 17.5-32.5 × 2.75-7.5 µm ($\overline{x}$ = 22.57 × 5.5 µm). *Reticula* tenacia quae vermiculos nematodeos capiunt evolventibus. *Chlamydosporae* globosae vel ellipsoideae, catenulatae.

Colonies growing rapidly on CMA medium, attaining 6 cm diam. in 5 days at 28ºC and mycelia spreading at the rate of 0.5 cm per 24 hours, conidiophores and conidia are produced after 4 days. *Mycelium* scanty, spreading, vegetative hyphae hyaline, septate and branched, mostly 2-4 µm wide. *Conidiophores* colorless, erect, simple, septate, frequently 60 to 200 µm high, 2 to 5 µm wide at the base and 1.5 to 2.4 µm at the tip, producing 1-5 conidia singly from conidiogenous loci on conspicuous denticles at and near the apex (Figs. 1-3). *Conidia* colorless, elongate ellipsoid-cylindrical (Figs. 4-5) or slightly clavate (Fig. 6), broadly rounded at the tip, rounded truncate at the narrowed base, sometimes constricted gradually at the distal part of conidia



Figs. 1-10. *Arthrobotrys yunnanensis* sp. nov. (from holotype: HT1.00593). **1, 2.** Conidiophores with short denticles. **3.** An unmature conidium attached to a conidiophore. **4-6.** Elongated ellipsoid-cylindrical or slightly clavate conidia. **7.** Three-dimensional adhesive networks. **8.** Trapped nematode in a three-dimensional adhesive networks. **9, 10.** Spherical to ellipsoidal chlamydospores. Bars: 1, 4-7, 9, 10 = 10 µm; 2, 3, 8 = 20 µm.

(Fig. 4), usually nonseptate (Figs. 4, 6), occasional uniseptate (<5%) at the center (Fig. 5), 17.5-32.5 × 2.75-7.5 µm ($\bar{x}$ = 22.57 × 5.5 µm).

Chlamydospores spherical to ellipsoidal, intercalary (Figs. 9-10). Trapping nematodes by three-dimensional adhesive networks (Figs. 7-8).

Habitat: Soil.

Known distribution: Yunnan, China.

Material examined: CHINA, Yunnan Province, Yiliang County, Mount Xiaobailong, 15 August 2003 (**holotype designated here**, HT1.00593, and a living culture, YMF1.00593, are deposited in the Herbarium of Laboratory for Conservation and Utilization of Bio-resources, Yunnan University).

Teleomorph: *Orbilia auricolor*.

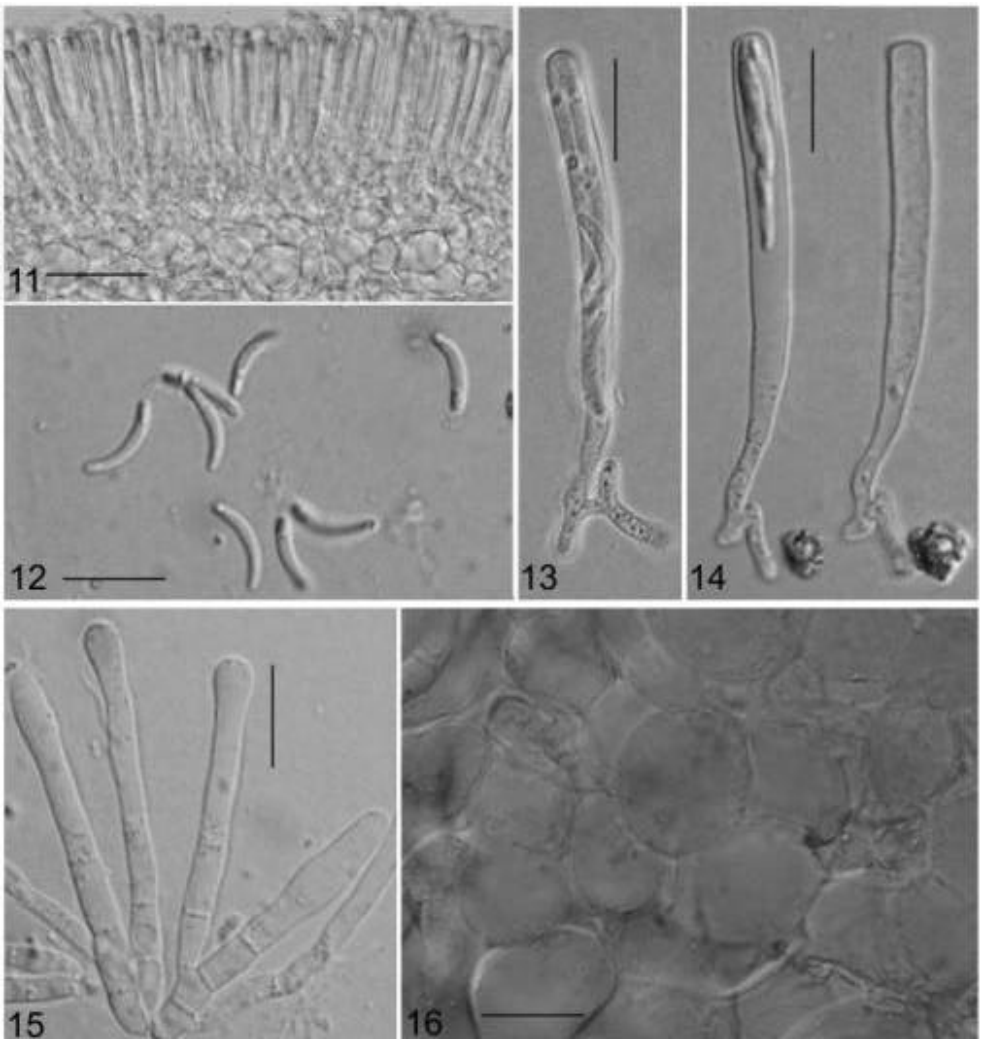
Orbilia auricolor (Figs. 11-16)

Apothecia superficial, sessile, pale cream. *Disc* 0.3-0.8 mm diam., smooth, plane, margin even. *Ectal excipulum* composed from base to margin of globose or subglobose cells, 6-12 µm diam., with thin or slightly thickened walls (Figs. 11, 16). *Asci* cylindric-clavate, tapered and often forked at the base, apex rounded or truncate-rounded, 8-spored, 30-45 × 3.5-5 µm in living state (Figs. 13-14). *Ascospores* banana-shaped or narrowly clavate, medium curved, one end narrower, broadest above and slightly trapped to the rounded proximal end, non-septate, containing an elongate tear-shaped spore body at the broader end, 9 × *ca.* 1.4 µm when shooting from asci and still in living state (Fig. 12). *Paraphyses* hyaline, cylindric-clavate, often branched below, septate in the lower part, 1.5-2 µm diam., slightly expanded to 2.5-3.5 µm at the apex, here not encrusted (Fig. 15).

Material examined: CHINA, Yunnan Province, Yiliang County, Mount Xiaobailong, 10 September 2003, Herbarium of Laboratory for Conservation and Utilization of Bio-resources, Yunnan University (Mo MH O 002).

The teleomorph clearly belongs in the *O. auricolor* complex and matches well with *O. auricolor* as described by Spooner (1987), but differs from his specimens in somewhat shorter ascospores with less tapering bases, and in the absence of encrustation on the paraphyses. The teleomorphic characters do not differ from Pfister's three specimens: *O. fimicola* (HB 5441, the teleomorph of *A. superba*), *O. auricolor* (HB 5442, the teleomorph of *A. oligospora*) and *O. auricolor* (HB 5443, the teleomorph of *A. cladodes* var. *macroides*) (Baral, pers. comm.). The teleomorphic characters also do not differ from Rubner's specimen (the teleomorph of *M. psychrophilum*) though the ascospores are somewhat shorter than that of Rubner's.

The genus *Arthrobotrys* was described by Corda (1839) based on the type *A. superba* Corda with the distinguishing characteristics of formation of conidia on sterigmata in a whorled pattern at the tip and nodes of the simple, erect and septate conidiophore, with two-celled conidia. Later, species with



Figs. 11-16. *Orbilia auricolor* (from the examined material: Mo MH O 002). **11.** Cluster of asci and paraphyses. **12.** Eight living ascospores projected from an ascus. **13.** Ascospores within the dead ascus. **14.** A turgescent living ascus (left) and an empty ascus (right) after projection of spores. **15.** Paraphyses. **16.** Globose to subglobose ectal excipulum cells. Bars: 11 = 40 µm; 12-14 = 10 µm; 15, 16 = 7 µm.

aseptate and multicelled conidia have been included (Schenck *et al.*, 1977). All species produce trapping mechanisms (Oorschot, 1985). Traditionally, for the taxonomy of predatory orbiliaceous fungi, the morphology of the conidia (shape, number and size of cells) and conidiophores (branching, modification of the apex) were preferentially used in delimiting genera. Most of the predatory hyphomycetes that do no form clamps are assigned to three genera,

Table 2. Morphological comparison of *Arthrobotrys yunnanensis* and its similar species.

Characters	***A. anomala***	***A. amerospora***	***A. botryospora***	***A. yunnanensis***
Conidia shape	Cylindric to long ellipsoidal	Ellipsoidal	Ellipsoidal	elongate ellipsoid-cylindrical or slightly clavate
Conidia size (μm)	13-22 × 3-7	13-31 × 10-20 ($\bar{x}$ = 23.6 × 15.9)	12-20 × 11-15	17.5-32.5 × 2.75-7.5 ($\bar{x}$ = 22.57 × 5.5)
Septation	Commonly 0, 1 septum only for germinated conidia	0	Commonly 0, occasionally 1	Commonly 0, occasionally 1
Modification of conidiogenous cell	Short denticle	Short denticle	Short denticle	Long denticle
Chlamydospore shape	Unknown	Spherical to elongate-ellipsoidal	Spherical to ovoid to elongate	Spherical to ellipsoidal
Predacious organ	Adhesive branches and networks	Adhesive networks	Adhesive hyphae and networks	Adhesive networks

Arthrobotrys, *Dactylella* and *Monacrosporium*. In this system, the trapping devices are only taken as a main characteristic for species but not genus identification. Recently, several molecular studies using ITS regions (Liou and Tzean, 1997; Pfister, 1997) and 18S rDNA (Ahré n *et al.*, 1998) found that the trapping devices are more important taxonomic characters than other morphological structures in delimiting genera. Ultimately, basing on the phylogenetic analysis using a 1.2 kb long fragment of 18 s rDNA, a new genus concept was proposed for predatory anamorphic *Orbiliaceae* by Scholler *et al.* (1999) in which the trapping device is the main morphological criterion for the delimitation of the genera. Four genera were defined in their taxonomic system and the species forming adhesive networks were assigned to the genus *Arthrobotrys* Corda emend. M. Scholler, Hagedorn and A. Rubner. Though their studies promoted the understanding systematics of predacious hyphomycetes, this new generic concept also needs improving. In following their system, it's difficult to class those species with two or more trapping devices. For example, *D. arcuata* Scheuer & J. Webster, which forms adhesive networks and adhesive knobs, was combined in the genus *Gamsylella* M. Scholler, Hagedorn & A. Rubner where species form stalked adhesive knobs (Scholler *et al.*, 1999). Here, we tentatively place our new species in *Arthrobotrys* Corda following the traditional generic concept.

Arthrobotrys yunnanensis differs from the three species of *Arthrobotrys* which form nonseptate, or occasionally uniseptate conidia, *A. anomala* (Barron

and Davidson, 1972), *A. amerospora* (Schenck *et al.*, 1977) and *A. botryospora* (Barron, 1979). *Arthrobotrys yunnanensis* is characterized by predominantly elongate ellipsoid-cylindrical or slightly clavate, non-septate conidia which are borne on distinct and long denticles. *Arthrobotrys yunnanensis* and *A. anomala* show similar conidial shape but differ in the size of conidia (Table 2). The conidia of *A. anomala* form one septum only when detached while the former are still nonseptate when detached. The new species is easy to distinguish from *A. botryospora* and *A. amerospora* by their different morphological characters of conidia (shape and size, see Table 2) and conidiophore (modification at apex). Furthermore, unlike *A. botryospora*, *A. yunnanensis* is not found to capture nematodes by adhesive hyphae, and unlike *A. amerospora*, which only produces nonseptate conidia, *A. yunnanensis* occasionally produces uniseptate conidia.

It is clear, as Pfister (1995) and Webster *et al.* (1998) mentioned, that *O. auricolor* is a species complex. It is difficult to distinguish members of this complex only on the basis of morphological characters of teleomorphs. So isolation and identification of more anamorhs would facilitate the taxonomy of the genus *Orbilia*.

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