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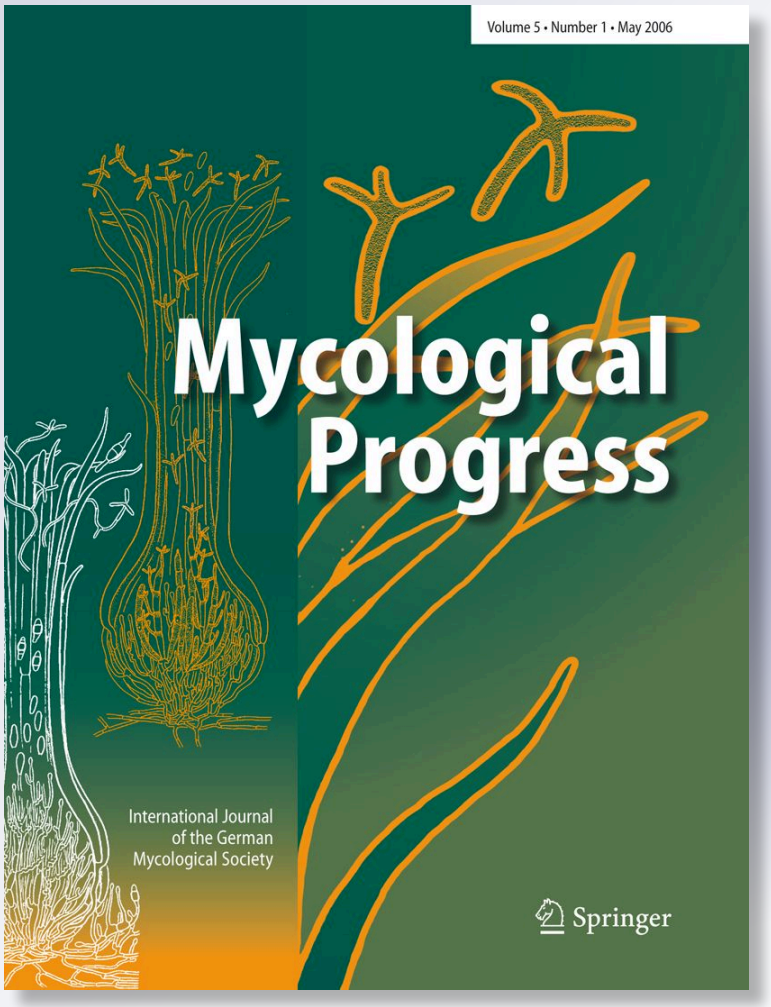

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ORIGINAL ARTICLE

Orbilia blumenaviensis and its *Arthrobotrys* anamorph

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Abstract A member of the nematophagous anamorph genus *Arthrobotrys* was isolated from its teleomorph *Orbilia blumenaviensis* comb. nov. (= *Orbilia fici*), a species closely related to *O. auricolor* but deviating in having lanceolate paraphyses. In the presence of nematodes, the anamorph forms three-dimensional adhesive networks. A trimorphism in its conidia was observed which vary in shape and number of septa. In the first isolate, two types of heteropolar conidia were obtained. These differ markedly from the type strain of *A. vermicola*, which forms mainly homopolar (fusoid) conidia. In a further ascospore isolate of *O. blumenaviensis*, however, mainly fusoid conidia referable to *A. vermicola* occurred. Combining morphological and phylogenetic analysis, we conclude that these isolates with differently shaped conidia and/or differences in the ITS rDNA (ca. 2–4%) belong to a single anamorph species, *A. vermicola*. Both teleomorph and anamorph are illustrated and described.

Min Qiao and Ying Zhang contributed equally to this work.

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Keywords Anamorph–teleomorph connection · *Arthrobotrys vermicola* · Nematophagous fungi · *Orbilia blumenaviensis*.

Abbreviations
* Living state
† Dead state

Introduction

Teleomorph–anamorph connections are helpful in solving taxonomic difficulties within the genus *Orbilia*, and in establishing the taxonomic position of anamorphic fungi. The family Orbiliaceae (Orbiliomycetes) presently includes three genera, *Orbilia* Fr., *Hyalorbilia* Baral & G. Marson, and *Pseudorbilia* Y. Zhang et al. (Zhang et al. 2007). Among these genera, *Orbilia* includes the highest number of species. In the *Dictionary of the Fungi* the genus comprises 58 accepted species (Kirk et al. 2008), while in fact many more species exist (Baral et al., unpublished). Distinction among these teleomorph species is often only possible if they are studied in the living state (Baral 1992), particularly by using the spore body in the living ascospores, which provides one of the most important characteristics. In this paper, a connection between *O. blumenaviensis* (Henn.) Baral & E. Weber and its *Arthrobotrys vermicola* (R.C. Cooke & Satchuth.) Rifai anamorph is reported.

Materials and methods

Collection of the specimens and isolation of the anamorph

While surveying orbiliaceous fungi, two specimens of *O. blumenaviensis* were collected. One was on decaying

angiosperm wood fallen on the ground of a broad-leaved subtropical evergreen forest located in Xiushan Forest Park (24°05′N, 102°45′E, 1,800–1,850 m asl) of Tonghai County, Yunnan Province, China, in July 2007, by S.F. Li and J.W. Guo. A dried voucher specimen was deposited in the Laboratory for Conservation and Utilization of Bio-resource, Yunnan Province, China (YMFT 1.03002). Another was on wood of a fallen trunk of an unidentified angiosperm near Wenleng town of Lianghe County (24°33′N, 98°07′E, 1,000 m asl), in August 2010, by Z.F. Yu and M. Qiao. A dried voucher specimen was deposited under the number YMFT 1.03606. Two further collections of *O. blumenaviensis* were made on the Lesser Antilles (Central America), on bark of unidentified angiosperm trees at L'Anse Noire, 5.5 km WSW of Les Trois-Îlets, Martinique, ~5 m asl, 10 Dec 2005, C. Lechat (H.B. 8029, C.L.L. 5645); Ravine Blondeau, Vieux Fort, Guadeloupe, 22 Nov 2006, C. Lechat (H.B. 8413a, C.L.L. 6016). Private herbaria H.B. = H.-O. Baral, C.L.L. = Christian Lechat. A soil isolate of *A. vermicola* was obtained from Dehong Canton, Yunnan Province, soil collected by H. Luo, July 2003, strain isolated by L. Cao, July 2003 (YMF 1.00534).

To isolate the anamorph, we followed the procedure described by Yu et al. (2006). Only apothecia of YMFT 1.03002 and YMFT 1.03606 were stuck on Petri dishes, whereas those from Central America were dry when received. The culture form soil was isolated following the detailed procedure given by Li et al. (2006). All characters including teleomorph were observed and measured in water mounts with an Olympus BX51 microscope with differential interference contrast, those from Central America with a Zeiss Standard 20. Trapping organs 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. In the specimens from Central America, the anamorph was only observed close to the apothecia on the natural substrate.

DNA extraction, PCR and sequencing

Genomic DNA was extracted from the mycelium collected from cultures growing on cellophane membrane on PDA according to the method described by Turner et al. (1997). Primer pairs ITS5 and ITS4 were used to amplify the complete internal transcribed spacer (White et al. 1990). The parameters for PCR amplifications are as follows: 1 min initial denaturation at 95 °C, followed by 30 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C, 1.5 min extension at 72 °C, and a final extension period of 10 min at 72 °C. The purified PCR products were directly sequenced on both strands with the same primers that were used for amplification.

Phylogenetic analysis

In order to confirm the morphological identity of the anamorph of *O. blumenaviensis*, we performed a phylogenetic analysis using ITS sequences of 7 strains, including the type strain of *A. vermicola*. Also included were the morphologically similar species *A. oligospora*, some representative species of the genus *Arthrobotrys*, and those strains from Genbank with high ITS sequence similarity. Genbank accession numbers are shown on the phylogenetic tree. Further information is shown in Table 1.

Table 1 Strains of *Arthrobotrys vermicola* used in our phylogenetic analysis

Genbank accession nos.	Strain no.	Origin	Substrate	Morphology of conidia	Type of conidia	Reference
GU178821		Uganda	Soil	Elongate ellipsiodal to broadly fusoid, 2(−3)-septate; also pyriform, 1-septate	c (b, a)	Cooke and Satchuthananthavale (1965), Oorschot (1985) type strain
U51944		Taiwan, China	Soil	Unknown	?c	Liou and Tzean (1997)
EU977508		Hong Kong, China	Soil	Ellipsoid-fusoid, 1–3-septate; also elongate pyriform, 1-septate	(a), b, c	Swe et al. (2008), isolate F1.103
AY773454		Beijing, China	Soil	Unknown	?c	Yang et al. (2007)
GQ121416	YMF 1.00534	Yunnan, China	Soil	Elongate ellipsiodal to broadly fusoid, 1(−3)-septate	c	This paper
FJ599809	YMFT 1.03002	Yunnan, China	Wood	Pear-shaped to obovoid, 1-septate; also elongate cylindric-clavate, 1–2-septate	a (b)	This paper
HQ595346	YMFT 1.03606	Yunnan, China	Wood	Elongate ellipsiodal to broadly fusoid, 1–3-septate	(a, b), c	This paper

DNA sequences were aligned using Clustalx 1.83. Cladistic analyses using the neighbor-joining method was performed with MEGA version 2.1. The neighbor-joining tree was constructed with the Kimura 2-parameter model, including transitions and transversions and with pairwise deletion of gaps; bootstrap repetition is 500. Similarity was evaluated by DNAman5.22.

Results

Description of the Chinese collections (YMFT 1.03002 and YMFT 1.03606)

Teleomorph

Orbilia blumenaviensis (Henn.) Baral & E. Weber, *comb. nov.* (Figs. 1 and 4h–j)

Basionym: *Helotium blumenaviense* Henn., Hedwigia 41: 24, 1902

= *Orbilia fici* Cash & Corner, Trans. Brit. Mycol. Soc. 41: 280, 1958

Apothecia 0.7–1.5 mm in diam. (YMFT 1.03606 1.5–6.0 mm), superficial, with a distinct stalk up to 0.2–0.3 mm high, scattered togregarious on decayed wood, smooth, margin even, yellow and translucent throughout when moist, pale brown when dry. **Ectal excipulum** composed of globose or subglobose cells, near base 20–43×15–38 μm, on flanks 15–30×12–25 μm. **Asci** cylindric-clavate, often forked at the base, apex medium truncate (rounded in side view), 8-spored, †28–39(−45)×2.6–3.7(−4.4) μm in dead state. **Ascospores** falcate, medium to strongly curved, lower end distinctly narrowed, upper end only slightly so, non-septate, containing a spore body at the broader end, with 1–2 minute lipid bodies in the middle, *(6.2–)8–11(−12)×1–1.2 μm when shot from asci and still in living state. **Spore body** rounded or elongate ellipsoid, 0.9–1.6×0.7–0.8 μm (1.5–2.5 μm including invisible connecting part). **Paraphyses** hyaline, cylindrical, with distinctly widened, lanceolate apex, septate below, †1.7–2.8 μm wide at widest point, not encrusted.

Anamorph

Arthrobotrys vermicola (R.C. Cooke & Satchuth.) Rifai, Reinwardtia 7: 371, 1968 (Figs. 2, 3 and 4a–g, k)

≡*Dactylaria vermicola* R.C. Cooke & Satchuth., Trans. Brit. Mycol. Soc. 49(1): 29, 1965

Colonies growing rapidly on CMA medium, attaining 40 mm diam. in 6 days at 28 °C. Mycelium spreading, vegetative hyphae hyaline, septate and branched, mostly 3–6 μm wide. **Conidiophores** colorless, produced on the mycelium growing at the fringe of the plate, appressed or erect, branched, septate; in isolate YMFT 1.03002 frequently 150–300 μm high when erect, often 700–1,500 μm when appressed, 4–8 μm wide at the base and 3.5–5.0 μm at the tip, often recommencing growth after the first group of conidia had been produced and a second head was then formed about 100 μm above the first. This process was repeated until 3–4 (−7) whorls of conidia were produced on a single conidiophore (Fig. 2c), each group with 2–8 conidia; in isolate YMFT 1.03606 conidiophores 113–129 μm high, with 1–5 conidia at tip, not proliferating (Fig. 4k). **Conidia:** in isolate YMFT 1.03002, two types of colorless conidia were simultaneously formed which mainly differ in their length-width ratio but also in the number of septa: type a with 1-septate, pear-shaped to obovoid, sometimes elongate ellipsoidal conidia being broadly rounded at the tip, rounded-truncate at the narrowed base, sometimes slightly gradually attenuated at the proximal end, *(16.4–)20.4–28.2×(9.8–)13.5–15.3 (−19) μm; type b with 1–2 septate, elongate cylindric-clavate, obconical conidia being broadly rounded at the tip, *(21.7–)30.0–34.5(−39.2)×12.7–14 μm. The proportion of conidial types a and b was 88 and 12% respectively. In isolate YMFT 1.03606 three types of conidia were formed: besides type a and b with similar size to that of YMFT 1.03002, predominantly ellipsoid-fusoid conidia occurred (here named type c), *(25–)33–35(−40)×(10.5–)13–16.3(−17.2) μm (com-

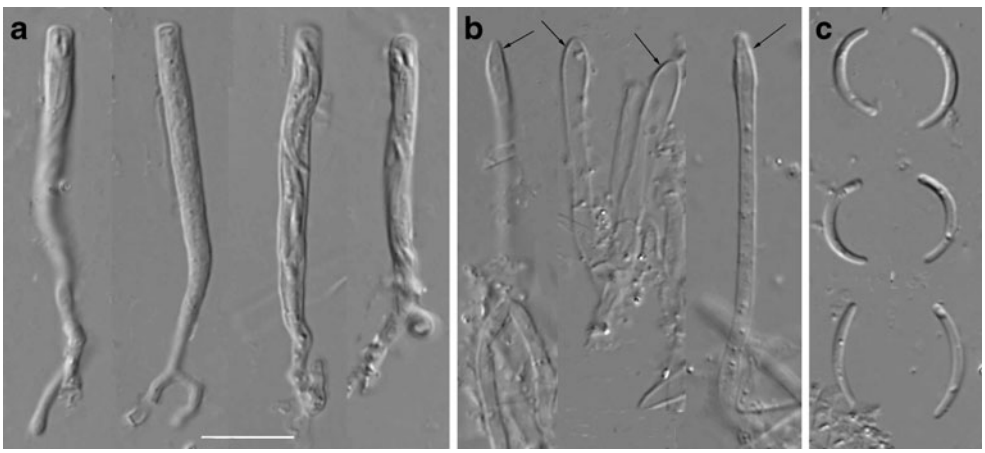

Fig. 1 *Orbilia blumenaviensis* (YMFT 1.03002). **a** Asci. **b** Paraphyses. **c** Ascospores. Ascospores in living state, asci and paraphyses in dead state. *Bar* 10 μm

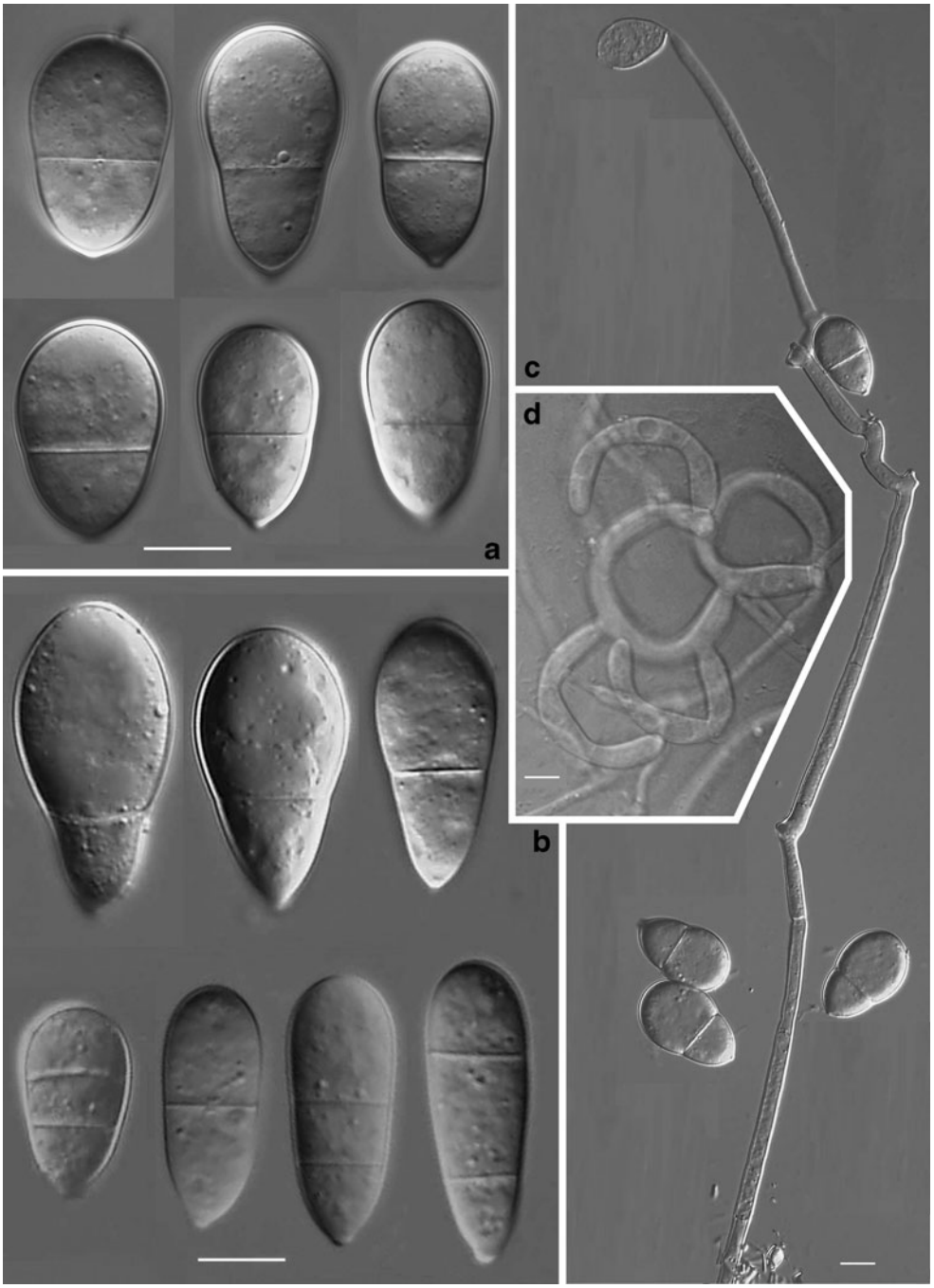

Fig. 2 *Arthrobotrys vermicola* (YMF 1.03002). **a** Conidia of type (a). **b** Conidia of type (b). **c** Conidiophore and conidia of type (a). **d** Adhesive network. Living state. *Bars* 10 μm

bined data of a, b, c), 1–3-septate (Fig. 4a–f). Nematodes are trapped by three-dimensional adhesive networks. In soil isolate YMF 1.00534, conidia ellipsoid-fusoid (type c), *26.5–31.8×12.4–16.3 μm (Fig. 3).

Phylogenetic analysis

A neighbor-joining tree (Fig. 5) was constructed based on sequences of the ITS region of *A. vermicola* and other *Arthrobotrys* species, with *Dactylaria purpurella* as outgroup. In the phylogenetic tree, 7 strains identified as *A. vermicola* formed a monophyletic clade with high bootstrap support. The ascospore isolates of *O. blumenaviensis* clustered within the *A. vermicola* clade.

Our molecular analysis of the ITS rDNA showed the following similarities between the type strain and our isolates: the ITS similarity is 97.83% with YMF 1.00534 (conidial type c), and 97.84% with YMFT 1.03606 (mainly type c). The strain of Swe et al. (2008) in which mainly type b and c occurred shows 98.4% similarity, and YMF 1.03002 with only types a and b 96.02%. A similarity

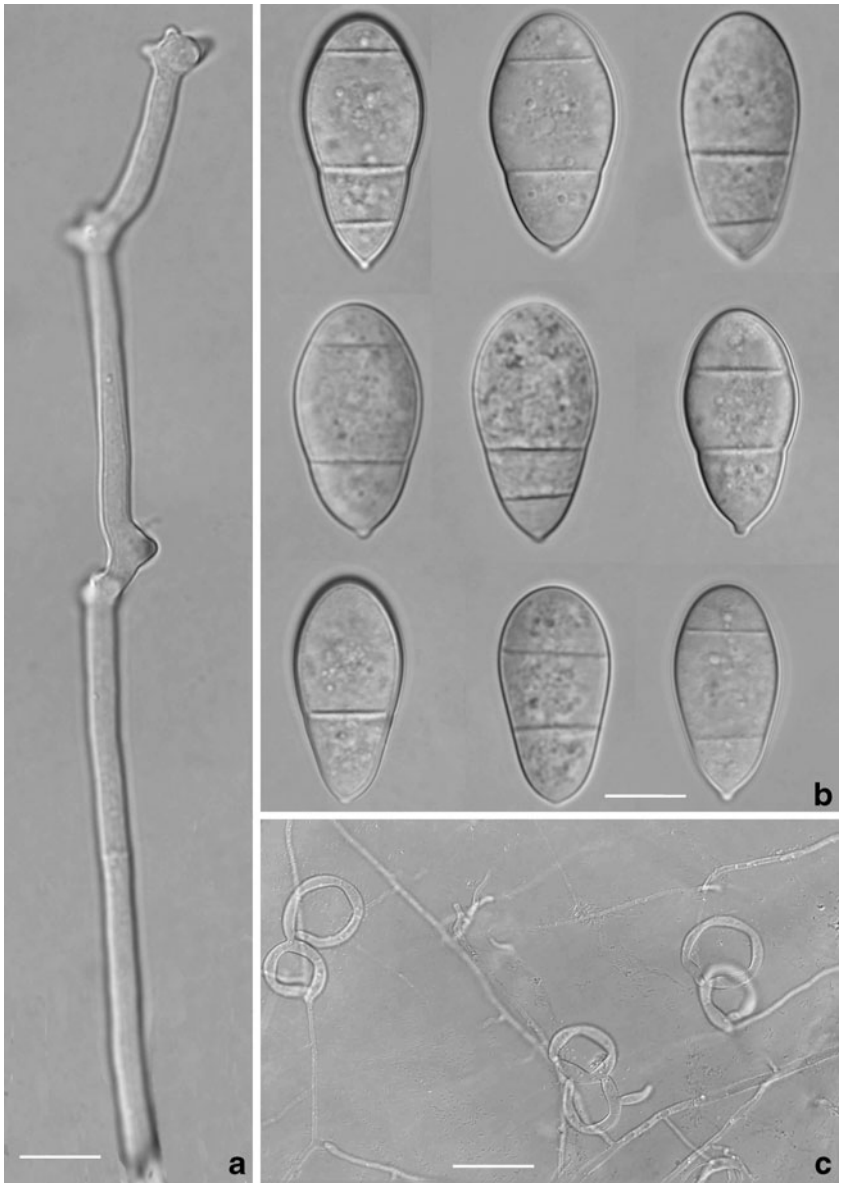

Fig. 3 *Arthrobotrys vermicola* (YMF 1.00534). **a** Conidiophore. **b** Conidia of type (c). **c** Adhesive network. Living state. *Bars* (**a**, **b**) 10 μm, (**c**) 50 μm

comparable to the latter (96.5%) is noted between YMF 1.03002 (types a and b) and YMF 1.00534 (type c), whereas a soil isolate from Beijing and YMF 1.03002 showed a similarity of 97.56%.

Discussion

As a result of reexamination of the type material (Baral, unpublished), *O. blumenaviensis* and *O. fici* are to be treated as synonyms, and are conspecific with several recent (sub)tropical collections, including the two Chinese ones treated here. The teleomorph is known from Central and South America and South East Asia. The type of *H. blumenaviense* is from southern Brasilia (St. Catarina, on a palm), that of *O. fici* from Singapore (on bark of *Ficus irregularis*).

Whereas the teleomorph morphology is quite homogenous in *O. blumenaviensis*, the available anamorphs showed a distinct variation which, at the first glance, seems to point to different species. The anamorph obtained in the ascospore isolate YMF 1.03002 of *O. blumenaviensis* differs from the type strain of *A. vermicola* in conidial shape. Strain YMF 1.03002 formed two types of heteropolar conidia (type a: pyriform to obovoid; type b: elongate clavate) at the same time, whereas Cooke and Satchuthananthavale (1965) described the conidia of the type strain as ellipsoid-fusoid (homopolar, here named type c). However, they also figured an elongate clavate, 4-septate conidium (type b) and a

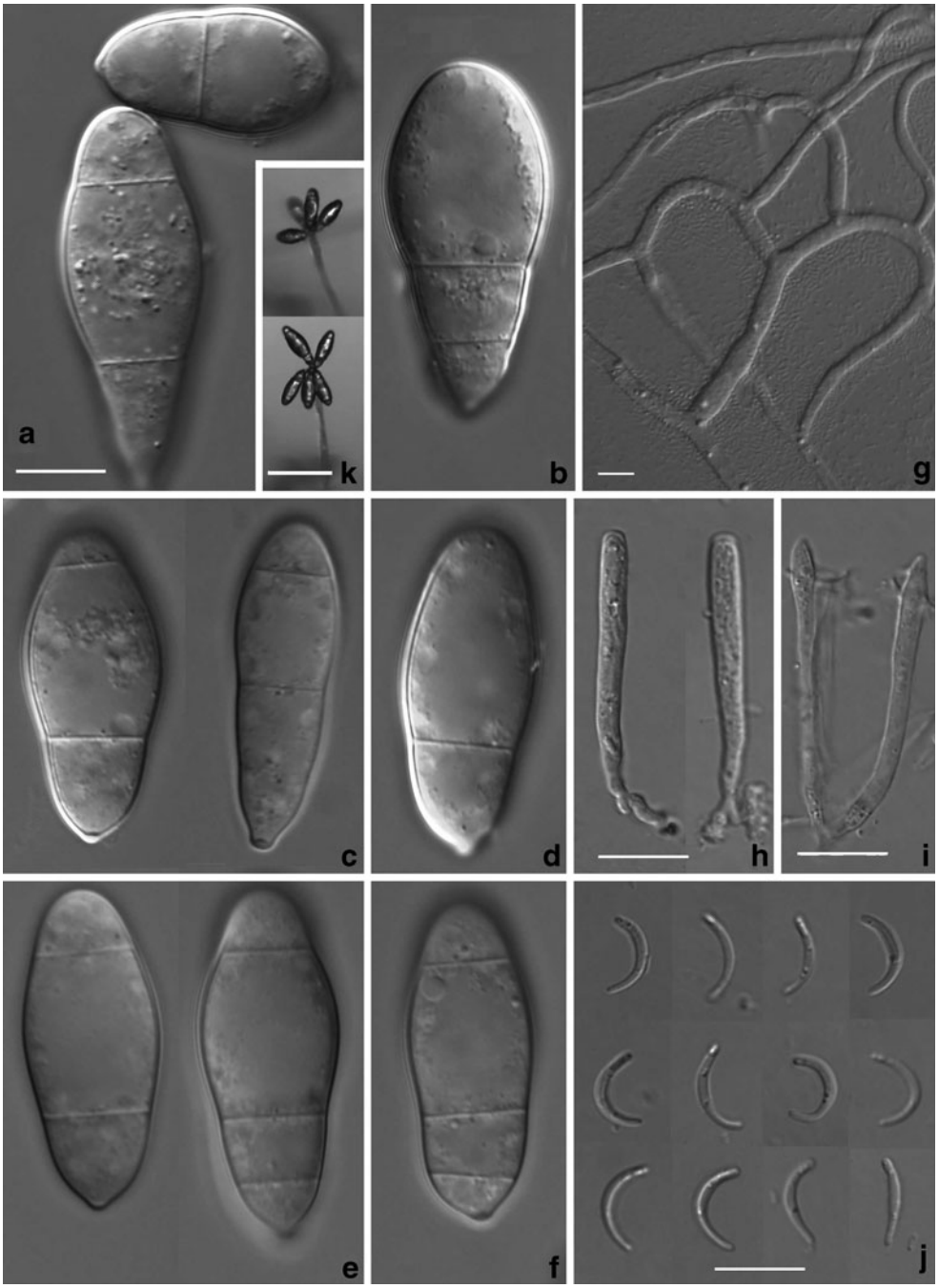

Fig. 4 *Orbilia blumenaviensis* and *Arthrobotrys vermicola* (YMFT 1.03606 and YMF 1.03606). **a** Conidia of types (a) and (c). **b** Conidium of type (b). **c**–**f** Conidia of type (c). **g** Adhesive network. **h** Asci. **i** Paraphyses. **j** Ascospores. **k** Conidiophores with conidia. Living state, except for asci and paraphyses. *Bars* 10 μm (**k** 50 μm)

1-septate conidium which comes close to type a. In her re-examination of the type culture, Oorschot (1985) reported besides ellipsoid to fusoid, (1–)2–3-septate conidia (type c) also obovoid (0–)1-septate (type a) and some elongate clavate, 2–3-septate conidia (type b). In the other ascospore isolate of *O. blumenaviensis* (YMFT 1.03606; Fig. 4) we found, besides of heteropolar conida, mainly fusoid conidia (type c). This isolate, therefore, better concurs with the type of *A. vermicola*. In the soil isolate (YMF 1.00534; Fig. 3), only conidia of type c were found which match well the type of *A. vermicola*. On the natural substrate of two specimens of *O. blumenaviensis* from the Caribbean region, only heteropolar conidia of type a were found.

Further literature reports show the variability in conidial shapes: in a non-sequenced soil isolate identified as *A. vermicola* (Zhang and Mo 2006) both homopolar (type c) and heteropolar conidia (type a) occurred. Also, Swe et al. (2008) illustrated under the name *A. vermicola* an isolate from mangrove and freshwater habitats in which the conidia are mainly of type b and c.

YMF 1.03002 formed long conidiophores with apical whorls of 2–8 conidia, the conidiophores showing recom-

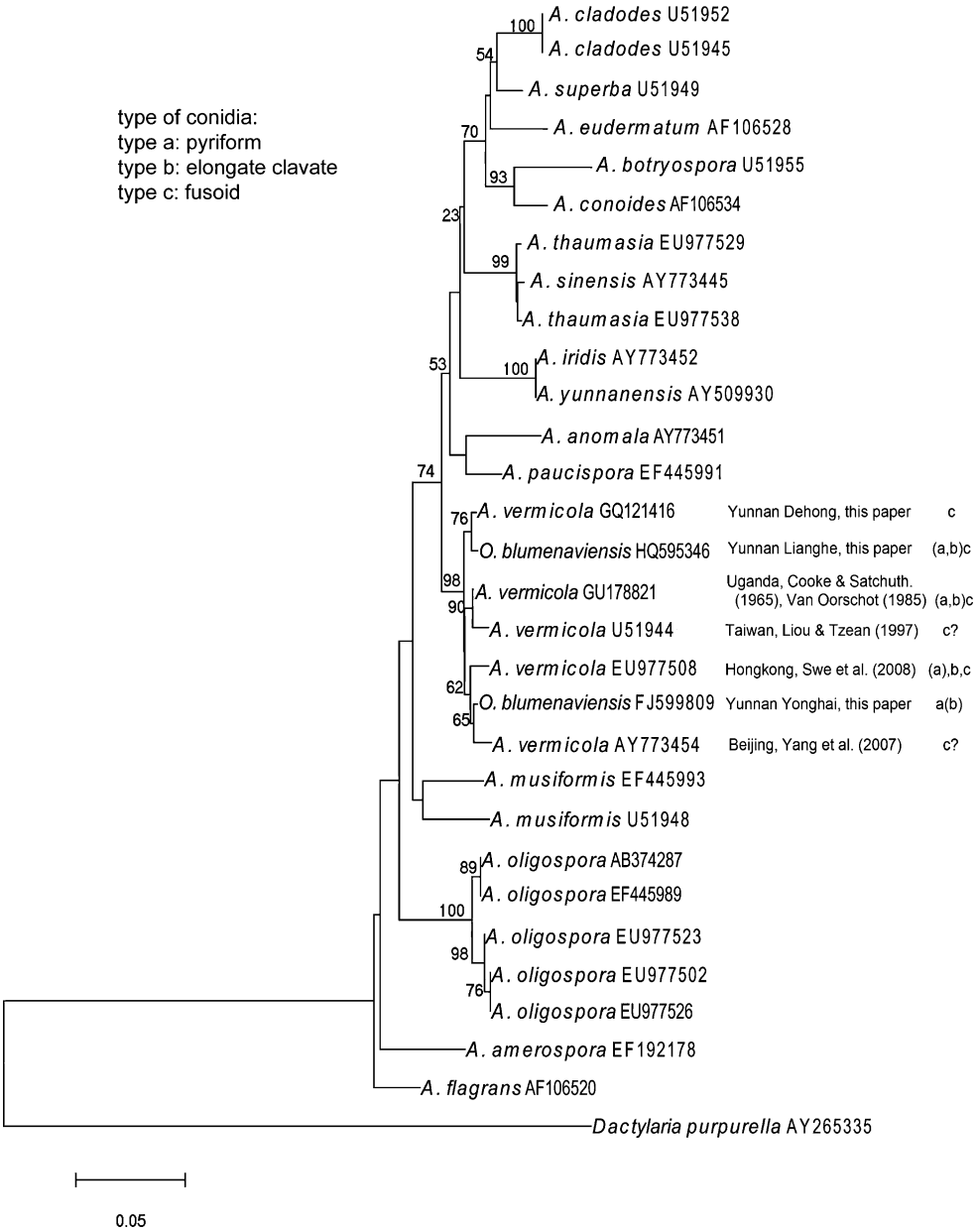

Fig. 5 Phylogenetic tree of *A. vermicola* and related species using the neighbor-joining method based on ITS region sequence data. *Dactylaria purpurella* was used as outgroup. Bootstrap values less than 50% are not shown

menced growth by forming 3–4(−7) whorls. In this respect, it closely resembles the original description of *Arthrobotrys vermicola*. YMFT 1.03606 closely resembles the type strain in conidial characters, but formed shorter conidiophores with only 1 whorl of 1–5 conidia.

The above evidences indicate that the conidial types a, b and c may simultaneously occur within a single strain of *A. vermicola*. Our results on the ITS similarity further support these morphological findings. At the present state of knowledge, a clear correlation between conidial shape and genetical data can hardly be recognized. For two of the strains included in the phylogenetic analysis no morphological data were available, therefore, the conidia are only assumed to be of type c (see Fig. 5). From the above we conlude that all these strains are conspecific, and that the anamorph of *O. blumenaviensis* is *A. vermicola*.

Orbilia blumenaviensis is a close relative of the species complex around *O. auricolor* (A. Bloxam ex Berk. & Broome) Sacc., for which hitherto four different anamorphs were recorded, depending on the isolate (Mo et al. 2005). Based on morphological characters of the teleomorph alone, species delimitation in this complex is usually very difficult. However, *O. blumenaviensis* is easily recognized by its lanceolate paraphyses in contrast to the ±capitate, never apically narrowed paraphyses characteristic of the *O. auricolor* complex. However, the anamorph of *O. blumenaviensis* also differs distinctly in its conidial characters from those reported for *O. auricolor*.

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References

Baral HO (1992) Vital versus herbarium taxonomy: morphological differences between living and dead cells of Ascomycetes, and their taxonomic implications. Mycotaxon 44:333–390

Cooke RC, Satchuthananthavale V (1965) Some nematode-trapping species of *Dactylaria*. Trans Br Mycol Soc 49:27–32

Kirk PM, Cannon PF, David JC et al (2008) Ainsworth & Bisby's dictionary of the fungi, 10th edn. CAB International, Wallingford

Li Y, Jeewon R, Hyde KD, Mo MH, Zhang KQ (2006) Two new species of nematode-trapping fungi: relationships inferred from morphology, rDNA and protein gene sequence analyses. Mycol Res 110:790–800

Liou GY, Tzean SS (1997) Phylogeny of the genus *Arthrobotrys* and allied nematode-trapping fungi based on rDNA sequences. Mycologia 89:876–884

Mo MH, Huang XW, Zhou W, Huang Y, Hao YE, Zhang KQ (2005) *Arthrobotrys yunnanensis* sp. nov., the fourth ananmorph of *Orbilia auricolor*. Fungal Divers 18:107–115

Oorschot CAN van (1985) Taxonomy of the *Dactylaria* complex. V. A review of *Arthrobotrys*, an allied genera. Stud Mycol 26:61–96

Swe A, Jeewoon R, Hyde KD (2008) Nematode-trapping fungi from mangrove habitats. Cryptogam Mycol 29:333–354

Turner D, Kovacs W, Kuhls K et al (1997) Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycol Res 101:449–459

White TJ, Bruns T, Lee S et al. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA et al (eds), PCR Protocols: a guide to methods and applications. Academic Press, San Diego, 315–322

Yang Y, Yang E, An Z, Liu XZ (2007) Evolution of nematode-trapping cells of predatory fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. Proc Natl Acad Sci USA 104:8379–8384

Yu ZF, Zhang Y, Qiao M, Baral HO, Weber E, Zhang KQ (2006) *Drechslerella brochopaga,* the anamorph of *Orbilia (Hyalinia) orientalis*. Mycotaxon 96:163–168

Zhang KQ, Mo MH (2006) Flora fungorum sinicorum, vol. 33. *Arthrobotrys* et genera cetera cognata. Science Press, Beijing (in Chinese)

Zhang Y, Yu ZF, Baral HO, Qiao M, Zhang KQ (2007) *Pseudorbilia* gen. nov. (Orbiliaceae) from Yunnan, China. Fungal Divers 26:305–312