Mycologia, 103(1), 2011, pp. 164–173. DOI: 10.3852/10-102

Pseudotripoconidium, a new anamorph genus connected to *Orbilia*

Ze-Fen Yu*
Min Qiao*
Ying Zhang
Li Qin
Ke-Qin Zhang[1]

Laboratory for Conservation and Utilization of Bio-resources, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, Yunnan 650091, P. R. China

Abstract: A new anamorphic fungus is described based on four isolates from ascospores of *Orbilia* aff. *luteorubella*. This fungus differs from previously known *Orbilia* anamorphs in producing inversely pyramidal, unicellular conidia with several protuberances at their distal end. Conidia produce 1–7 prominent denticles that emerge from a node at the conidiophore apex. Conidiogenesis is holoblastic. Because phylogenetic analysis indicated greater than 90% ITS sequence similarities among the four isolates they are treated here as a single species. In the sequence analysis of the internal transcribed spacer region (ITS) these isolates and other sequences identified as *O.* aff. *luteorubella* were nested within *Orbilia* and formed a clade with 99% bootstrap support. This clade is separated from nematode-trapping species of *Orbilia*. Based on both morphological and molecular analyses, we propose a new genus, *Pseudotripoconidium*.

Key words: anamorph-teleomorph, conidiogenesis, *Orbilia* aff. *luteorubella*, systematics

INTRODUCTION

Family Orbiliaceae was introduced by Nannfeldt (1932), and today it includes three genera, *Orbilia*, *Hyalorbilia* and *Pseudorbilia* (Zhang et al. 2007). Species of *Orbilia* and *Hyalorbilia* occur on dung, decayed wood or dead twigs that are either moist on the ground or dry on standing plants. Little attention was given this group of fungi in part because of a perceived economic unimportance and because of the minute apothecia (Alexopoulos et al. 1996). But the connection of this group to diverse nematode-capturing anamorphs has attracted the attention of many mycologists.

The known anamorphs of *Orbilia* include both predacious and apparently non-predacious fungi. The form genera of predacious members are *Arthrobotrys* Corda (Pfister 1994, Pfister and Liftik 1995), *Dactylellina* M. Morelet (Liu et al. 2005) and *Drechslerella* Subram (Pfister 1997). Non-predacious anamorphs of *Orbilia* include *Dactylella* Grove (Thakur and Zachariah 1989, Webster et al. 1998), *Dicranidion* Harkn (Berthet 1964, Korf 1992, Pfister 1997), *Anguillospora* Ingold (Webster and Descals 1979, Pfister 1997, Baschien et al. 2006), *Trinacrium* Riess (Matsushima 1995, Pfister 1997) and *Dwayaangam* Subram. (Kohlmeyer et al. 1998). *Helicoon* Morgan (Pfister 1997) was reported to be an anamorph of *Orbilia*, but it was excluded because the relevant isolate was separated from other *Orbilia* spp. in an analysis of ITS rDNA sequences (Hagedorn and Scholler 1999). A morphologically *Idriella*-like fungus also was reported by Haines and Egger (1982) to be linked to *Orbilia piloboloides*. However species recognized in *Idriella* P.E. Nelson and S. Wilh. are morphologically different from this type of *Orbilia* anamorph and probably unrelated to Orbiliomycetes (H.O. Baral and E. Weber pers comm).

Among the known anamorphs of *Orbilia*, nematophagous fungi have attracted researchers by their wonderful trapping devices (Pfister 1997, Liu et al. 2005, Mo et al. 2005). Indeed their distribution, ecology and systematics have been the subject of research for several decades (Drechsler 1937, Liou and Tsean 1997, Li et al. 2000, Scholler et al. 1999, Li et al. 2005). The non-predacious anamorphs mainly include those that produce filamentous, multiseptate and hyaline conidia, with unclear economic importance.

Orbilia luteorubella (Nyl.) P. Karst. is a widely distributed species, which has been recorded in Finland, Macaronesia and North America (Korf 1992, Pfister 1997). Pfister (1997) reported two anamorphs under the name *O. luteorubella*; they were *Helicoon sessile* and *Anguillospora* sp. However Scholler (1999) expressed doubt that *Helicoon* was an anamorph of *Orbilia* according to ITS analysis. In the present paper we report the anamorph-teleomorph connection of conidial fungi cultured from a species similar to *O. luteorubella*. We describe these anamorphs in a new genus, *Pseudotripoconidium*.

MATERIALS AND METHODS

Collection of teleomorph.—Four fresh specimens of *Orbilia* aff. *luteorubella* were collected from decaying branches on

Submitted 13 Apr 2010; accepted for publication 26 May 2010.
* These authors contributed equally to this work.
[1] Corresponding author. E-mail: kqzhang111@yahoo.com.cn

forest floors in four regions. The morphological description and photographs were carried out with water mounts. An Olympus BX51 microscope with differential interference contrast was used to document microscopic features. Asci and living ascospores were observed and measured with fresh specimens. For observation of excipula structures apothecia were sectioned longitudinally (10–15 μm thick) with a Leica CM3050 S freezing microtome.

Isolation and characterization of the anamorph.—To isolate the fungus in culture three apothecia from each collection were attached to the lids of Petri dishes so that the hymenium was positioned downward to expel ascospores onto the surface of CMA plates (20 g cornmeal, 18–20 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 mL distilled water). The dishes were incubated 4–6 d at room temperature until ascospores germinated, then ascospores were excised and transferred onto CMA plates. After 15 d incubation at 25 C conidiophores and conidia of several repeated samples were observed and measured. Microscopic measurements were obtained from 40 individuals.

Colony morphology was observed on CMA and freshly prepared potato dextrose agar (PDA, broth from 200 g potato, 20 g dextrose, 18–20 g agar, 1000 mL distilled water). Growth rates were obtained by placing 0.5 × 0.5 cm square blocks cut from a colony on CMA plates. After 10 d at 21, 25, 28 and 35 C diameters were measured. To stimulate formation of trapping organs 100 nematodes (*Panagrellus redivivus* Goody) were added to a 1 cm × 1 cm square slot at the margin of the colony where the agar was removed.

Isolated anamorphs were maintained on CMA slants at Key Laboratory of Industrial Microbiology and Fermentation Technology of Yunnan (YMF), Kunming, Yunnan Province, P. R. China. Teleomorphic specimens are indicated by adding a T to YMF numbers. In addition cultures were deposited at the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands (CBS).

Scanning (SEM) and transmission electron microscopy (TEM).—For SEM studies strains were grown on CMA plates 10 d. Blocks (1 cm × 1 cm) Conidiophores and conidia were excised, fixed with a 2.5% glutaraldehyde solution at 4 C for 4 h in a 1.5 mL Eppendorf tube, and the glutaradehyde solution was carefully removed and discarded. Fixed conidia were rinsed three times in 1 mL 0.2 M phosphate buffer (pH 7.2), followed by brief centrifugation to remove the buffer with a pipette. Then 300 μL 1% OsO_4 was added to the tube and these conidia were postfixed in the same buffer 1 h at room temperature. A graded acetone series was used to dehydrate the specimens, and each step was followed by a brief centrifugation to remove liquid. Specimens were exchanged in an isoamyl acetate series, dried with an HCP-2 critical point dryer (Hitachi) 7 h, mounted on aluminum stubs, coated with a gold-palladium mixture by an IB-3 ion coater (Eiko) and viewed with a scanning electron microscope (Philips XL 30 ESEM) at 20–30 kV (Samson et al. 1979, Luo et al. 2007). TEM samples were embedded in epoxy resin (Epon 812) after fixation and dehydration and cut with glass knives. Ultrathin sections were mounted on formvar film on 100 mesh copper grids and stained with uranyl acetate and lead citrate. Observations were taken with a JEM-100 CX transmission electron microscope (JEOL) at 60 kV.

DNA extraction, PCR and sequencing.—Total DNA was isolated from fresh mycelium as described by Turner et al. (1997). Primer pairs ITS4 and ITS5 (White et al. 1990) were used to amplify the complete ITS (including 5.8 S). Parameters for PCR amplifications were 1 min initial denaturation at 94 C, followed by 30 cycles of 1 min denaturation at 94 C, 1 min primer annealing at 50 C, 90 s extension at 74 C and a final extension 7 min at 74 C. PCR products were purified with a commercial kit (TaKaRa Biotechnology Co. Ltd.) and sequenced on both strands with the same primers that were used for amplification, with a LI-COR 4000L automatic sequencing system, using cycle sequencing with the Thermo Sequenase kit as described by Kindermann et al. (1998).

Phylogenetic analysis.—We performed parsimony analysis comparing ITS sequences of our four isolates with sequences obtained from GenBank for *Orbilia* species having well characterized anamorphs (TABLE I). Except for *Trinacrium* and *Anguillospra*, all anamorphic genera of *Orbilia* were included. Another species of *Dwayaangam*, *D. colodena*, was used as representative species because ITS of *Dwayaangam junci*, the first connected with *Orbilia* (Kohlmeyer et al. 1998), cannot be obtained.

DNA sequences were aligned with Clustal X 1.83. Manual gap adjustments were made to improve the alignment with BIOEDIT (Hall 1999). Parsimony analysis was run in PAUP* 4.0b10 (Swofford 2002). Gaps were treated as missing data, all characters were equally weighted, initial MAXTREES setting was 100, and all trees were obtained by running the heuristic searches with tree bisection reconnection (TBR) as branch swapping algorithm and up to 100 random addition sequence replications. To assess the relative support for each clade bootstrap values were calculated from 1000 replicate analyses with the heuristic search strategy and random additional sequences of the taxa. Related data were submitted to TreeBASE. Submission ID is 10455, and study URL is http:// purl.org/ phylo/treease/ phylows/study/TB2:S10465.

TAXONOMY

Orbilia aff. *luteorubella* (Nyl.) P. Karst., Not. Sällsk. Fauna Fl. Fenn. 11:248. 1870

= *Peziza luteorubella* Nyl., Not. Sällsk. Fauna Fl. Fenn. 10:55. 1869. FIGS. 1–8

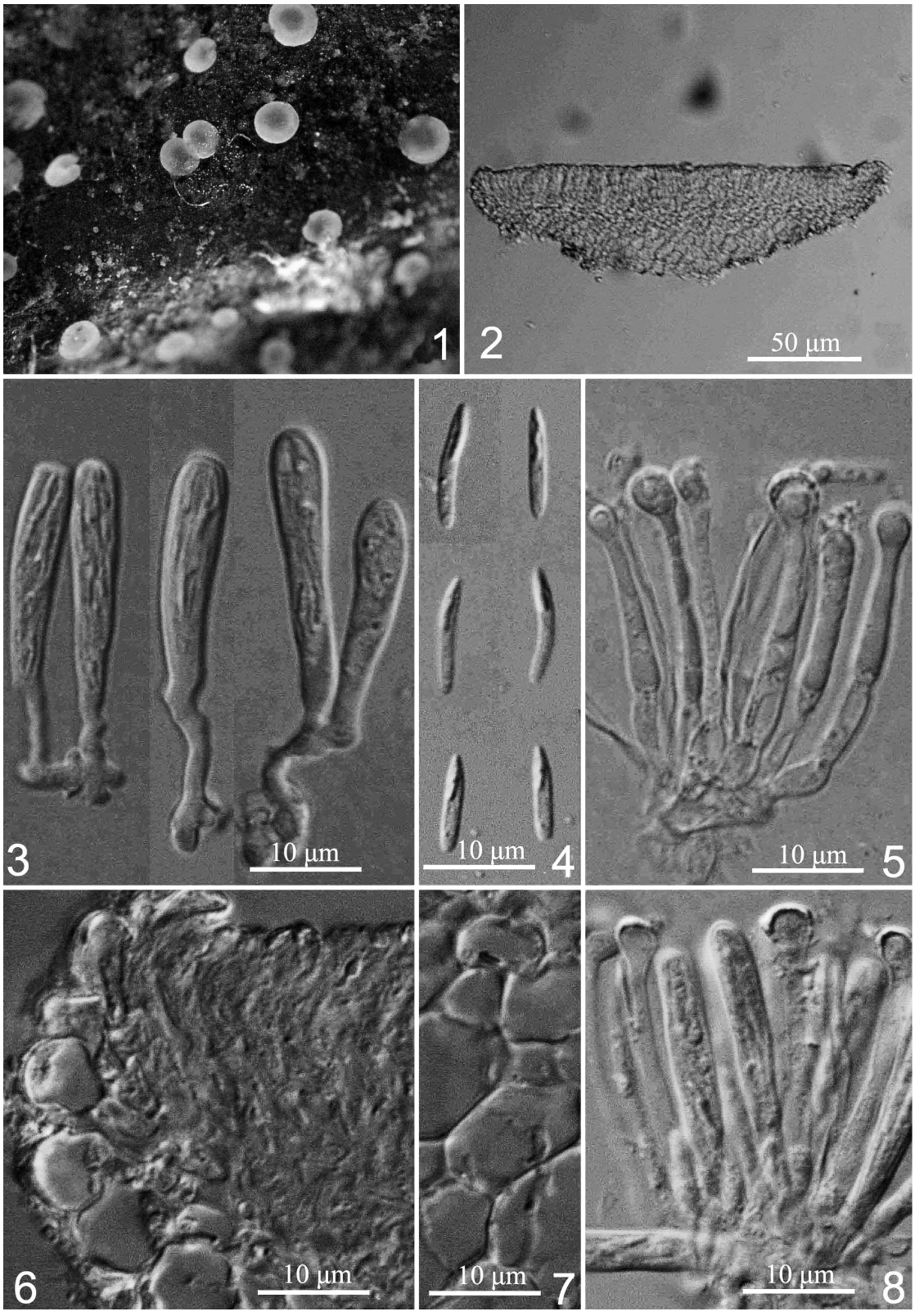
Apothecia 0.7–2.0 mm diam, superficial, sessile, scattered on decayed twig, pale yellowish red and translucent throughout when moist, and yellow when dry. Disk slightly concave to flat, margin even, smooth, centrally attached. Asci 29.8–35 × 4.3–5 μm (living state, YMFT 1.01848: 28–34 × 4.0–5 μm), eight-spored, lower 2–3 spores inversely oriented (with spore body toward ascus base), cylindrical, rounded or truncate at the apex (depending on view), tapered and often forked at the base (mostly L-shaped).

TABLE I. Detailed information of strains of *Orbilia* spp. in this study

Strain number	Teleomorph/anamorph	Conidia of anamorph	GenBank Acc. No.	Reference
YMF 1.01859	*O. alba/Dac. alba*	elongate ellipsoid, 1–2-septate	FJ477044	Yu et al. 2009a
D.H.P. 91	*O. alnea/Dicranidion* sp.	Y-shaped	U72600	Pfister 1997
YMF 1.00593	*O. auricolor* agg.1/ *A. yunnanensis*	elongate ellipsoid, cylindrical, slightly clavate, usually nonseptate	AY509930	Mo et al. 2005
CBS 319.94	*O. auricolor* agg.2/ *A. psychrophiulum*	ellipsoidal-fusoid, 1–5-septate	U51977	Rubner 1996
D.H.P. 90	*O. auricolor* agg.3/*A. oligospora*	pyriform or obovoid, one-septate	U72592	Pfister 1995
YMF 1.01839	*O. auricolor* agg.4/*A. cladodes*	ellipsoidal, or elongate obovoid, one-septate	FJ557236	Pfister 1995
unknown	*O. coccinella/Dac. coccinella*	cylindrical, 1–7-septate	AY515567	Yang et al. 2005
D.H.P. 108	*O. delicatula/Dicranidion* sp.	Y-shaped	U72595	Pfister 1997
YMF 1.01835	*O. dorsalia/Dac. dorsalia*	elongate fusoid, 4–6-septate	DQ480730	Yu et al. 2007a
D.H.P. 60	*O. fimicola/A. superba*	ellipsoidal, 1-septate	U72599	Pfister 1994
CBS 280.70	*O. fimicoloides/Dac. oxyspora*	elongate fusoid or clavate, 4-6-septate	AY902793	Webster et al. 1998
D.H.P. 212	*O. orientalis/Dre. brochopaga*	cylindrical-oblong, 1–3-septate	U72609	Yu et al. 2006
unknown	*O. querci/Dactylellina querci*	spindle-shaped, 3–5-septate	AY804213	Liu et al. 2005
D.H.P. 133	*O. tenebricosa/Dre. polybrocha*	broad-ovoid, one septum	U72606	Pfister 1997
YMF 1.01863	*Orbilia* sp./*Dre. yunnanensis*	elongate ellipsoidal, 0–1 septum	FJ185262	Yu et al. 2009b
T5.2	unknown	usually six arms	AY746342	Sokolski et al. 2006
4.37	unknown	usually six arms	AY746341	Sokolski et al. 2006
D.H.P. 146	*O.* aff. *luteorubella*	non-sporulating	U72607	Pfister 1997
D.H.P. 125	contamination in culture of *O. luteorubella*	non-sporulating	U72604	Pfister 1997
YMF 1.01843 CBS 121220	*O.* aff. *luteorubella/P. sinense*	inversely pyramidal, with 2–3 nipple-shaped lateral protuberances at distal end	DQ480727	This paper
YMF 1.01848 CBS 121221	*O.* aff. *luteorubella/P. sinense*	inversely pyramidal, with 4–5 nipple-shaped lateral protuberances at distal end (FIGS. 26–35)	EF026114	This paper
YMF 1.03007 CBS 125670	*O.* aff. *luteorubella/P. sinense*	inversely pyramidal, with 5–7 nipple-shaped lateral protuberances at distal end (FIGS. 24, 25)	GU188277	This paper
YMF 1.3475	*O.* aff. *luteorubella/P. sinense*	inversely pyramidal, with 2–3 nipple-shaped lateral protuberances at distal end	GU188276	This paper
CGMCC 3.13369	*O.* cf. *luteorubella*/unknown	unknown	FJ719770	Jiang, X et al. unpubl
629	*A. vermicola*	elongate-ellipsiodal to broadly fusiform, 2(–3)-septate	AY773454	Yang et al. 2007
YMF 1.01842	*O. vermiformis/Dac. vermiformis*	clavate, (0)–1 septum	DQ480729	Yu et al. 2007b

Ascospores hyaline, nonseptate, fusoid-bacilliform, straight or slightly curved, tapered at upper end, rounded at lower end, 7.8–10 × 1.3–1.6 μm (living state, YMFT 1.01848: 4.7–5.4(7.4) × 1.2–1.5 μm; YMFT 1.03007: 9.4–12.3 × 0.8–1.3 μm), with a refractive filiform spore body (SB, occasionally tear-shaped in YMFT 1.01848) at upper end in living mature ascospores, straight or slightly curved, attached to apex, 3.3–4.5 × 0.5–1.0 μm (YMFT 1.01848: 3.1–4.3 × 0.5–1.0 μm).

Specimen examined: YMFT 1.01843, CHINA, YUNNAN PROVINCE: Pu'er County, XiaoHeJiang Forest Park, 22°46′N, 100°58′E, 2500 m, 9 Jun 2005, M. Qiao; YMFT 1.01848, CHINA, YUNNAN PROVINCE: YingJiang County, TongBiGuan Nature Reserve, 24°39′N, 97°38′E, 2000 m, 25 Apr 2006, M. Qiao; YMFT 1.03007, CHINA, YUNNAN PROVINCE:



FIGS. 1–8. *Orbilia luteorubella* (YMFT 1.01843). 1. Apothecia. 2. Vertical section of an apothecium. 3. Asci. 4. Living ascospores with filiform SBs. 5. Cluster of living paraphyses. 6. Median section of apothecial margin. 7. Cells of inner part of ectal excipulum. 8. Cluster of dead asci and paraphyses.

Kunming, XiShan Forest Park, 25°03′N, 102°42′E, 2400 m, 20 Jun 2007, S.F. Li; YMFT 1.03475, CHINA, YUNNAN PROVINCE: TongHai County, XiuShan Forest Park, 24°06′N, 102°45′E, 2400 m, 15 Sep 2008, S.F. Li. All specimens were collected on fallen, unidentified decaying branches.

Pseudotripoconidium Z.F. Yu et K.Q. Zhang, gen. nov. MycoBank MB510511

Coloniae albidae vel hyalinae. Conidiophora hyalina, erecta, septata, simplicia vel parce ramosa. Denticuli divergentes in capitulo laxo dispositi. Conidia hyalina, aseptata, obpyramidalia, protuberantias mastoideas in parte superiore proferentia.

Colonies white on PDA. Colorless to white, appressed to the agar on CMA. Aerial mycelium sparse, hyphae hyaline, septate, branched. Conidiophores hyaline, septate, erect, simple or occasionally branched, bearing divergent, slightly tapering denticles at the tip. Conidia hyaline, non-septate, inversely pyramidal, somewhat flattened at the base, expanding gradually toward the distal end, with nipple-shaped lateral protuberances. No trapping devices were found.

Etymology. *Pseudotripoconidium* refers to the conidial shape resembling *Tripoconidium*.

Type species: Pseudotripoconidium sinense Z.F. Yu et K.Q. Zhang.

Pseudotripoconidium sinense Z.F. Yu et K.Q. Zhang, sp. nov. FIGS. 9–23

MycoBank MB510512

Coloniae post 10 dies 21 C 24 mm diam, albidae. Mycelium effusum, hyphis hyalinis, septatis, 2.5–4 µm latis. Conidiophora hyalina, erecta, septata, simplicia vel parce ramosa, 110–140 µm alta, ad basim cirea 3 µm lata, sursum leviter fastigata, apicem versus circa 1 µm lata, 1–7 denticulos in capitulo laxo ferentia. Conidia hyalina, aseptata, obpyramidalia, basi truncata, 5–9 µm × 3–4.3 µm, 2–3 mastoideas protuberatias in parte distali proferentia. Chlamydosporae globosae vel ellipsoideae, catenulatae, 7.5–14.5 µm diam.

Colonies white on PDA, growing slowly, 24 mm at 21 C after 10 d, 36 mm at 25 C, 34 mm at 28 C, no growth at 35 C. Colonies colorless; appressed to agar on CMA, reaching 24 mm after 10 d at 21 C, 30 mm at 25 C, 28 mm at 28 C, no growth at 35 C. Aerial mycelium sparse, hyphae hyaline, septate, branched, 2.5–4 µm wide. Conidiophores hyaline, septate, erect, simple or occasionally branched, 110–140 µm high, 2.5–3.0 µm wide at base, gradually tapering to 1 µm near tip, bearing divergent, slightly tapering denticles. Simple conidiophores forming a single apical conidium (FIG. 10), but most often 3–7 denticles present near the apex (FIG. 9). Denticles 1.5–2 µm long and 1–2 µm wide, the apical one 2.5–5 µm long. Denticles generally arising laterally from the conidiophore in perpendicular direction. Occasionally the main conidiophore produces several branches, singly or pairs. Conidia hyaline, non-septate, inversely pyramidal, somewhat truncate at the proximal end, where connected to the denticle (FIG. 12), in the upper part somewhat compressed, appearing lens-shaped when viewed from above (FIG. 16), with 2–3 nipple-shaped lateral protuberances at the distal end. Under the light microscope conidia triangular or unequally quadrangular, with 1–5 globose, and KOH-inert droplets of 1.3–2.5 µm diam. Conidia 5–9 µm long, 3–4.3 µm wide in the broadest part, including protuberances. Chlamydospores formed frequently in older cultures, subglobose to ellipsoidal, forming intercalary chains, 7.5–14.5 µm diam (FIG. 13).

Holotype. Yunnan Province, China. Specimen examined: Xiaoheijiang Park, Pu'er County, anamorph was isolated from *Orbilia* aff. *luteorubella* growing on decayed branches, collected 9 Jun 2005 by M. Qiao, isolated 12 Jun 2005 by Z.F. Yu (holotype YMF 1.01843, isotype CBS 121220).

Etymology: sinense refers to China, the country of its origin.

Known distribution: Southern China.

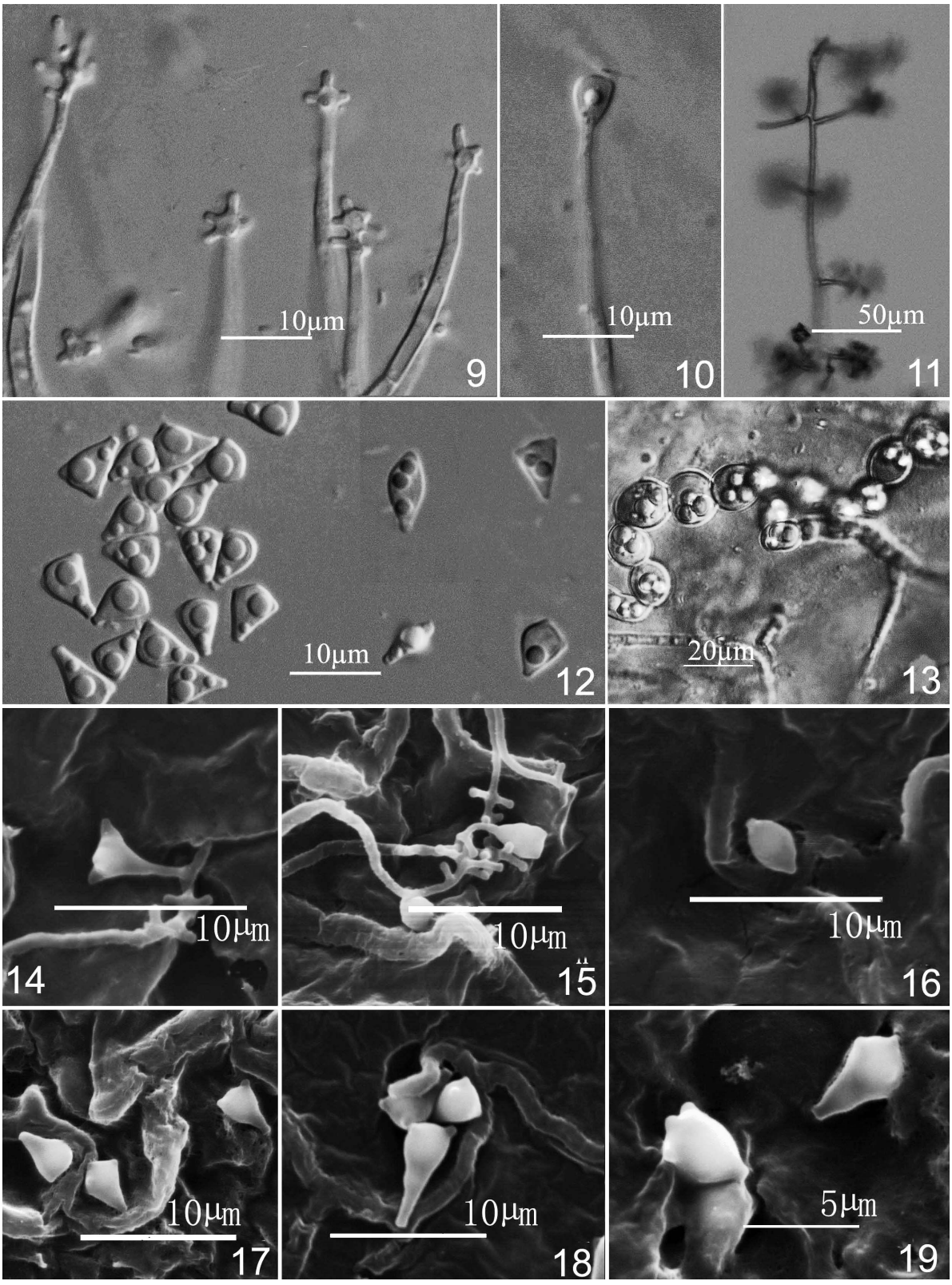
Habitat: On decayed branches.

Phylogenetic analysis.—Parsimony analysis of the ITS sequences (FIG. 36) yielded a single most parsimonious tree based on 291 parsimony informative characters (166 constant, 64 uninformative). The MP tree was 1298 steps long with a consistency index (CI) 0.5370 and a retention index (RI) 0.6589. In our analysis *Ascobolus crenulatus* (a member of family Pezizomycetes, GenBank accession member DQ491504) was used as outgroup. In the tree all predacious species clustered within a single clade, supported by 80% bootstrap. Except strain D.H.P. 125, all isolates of the *O.* aff. *luteorubella* formed a subclade with 99% bootstrap support. *Dwayaangam* did not fall into *Orbilia*; perhaps relevant sequences cannot be obtained.

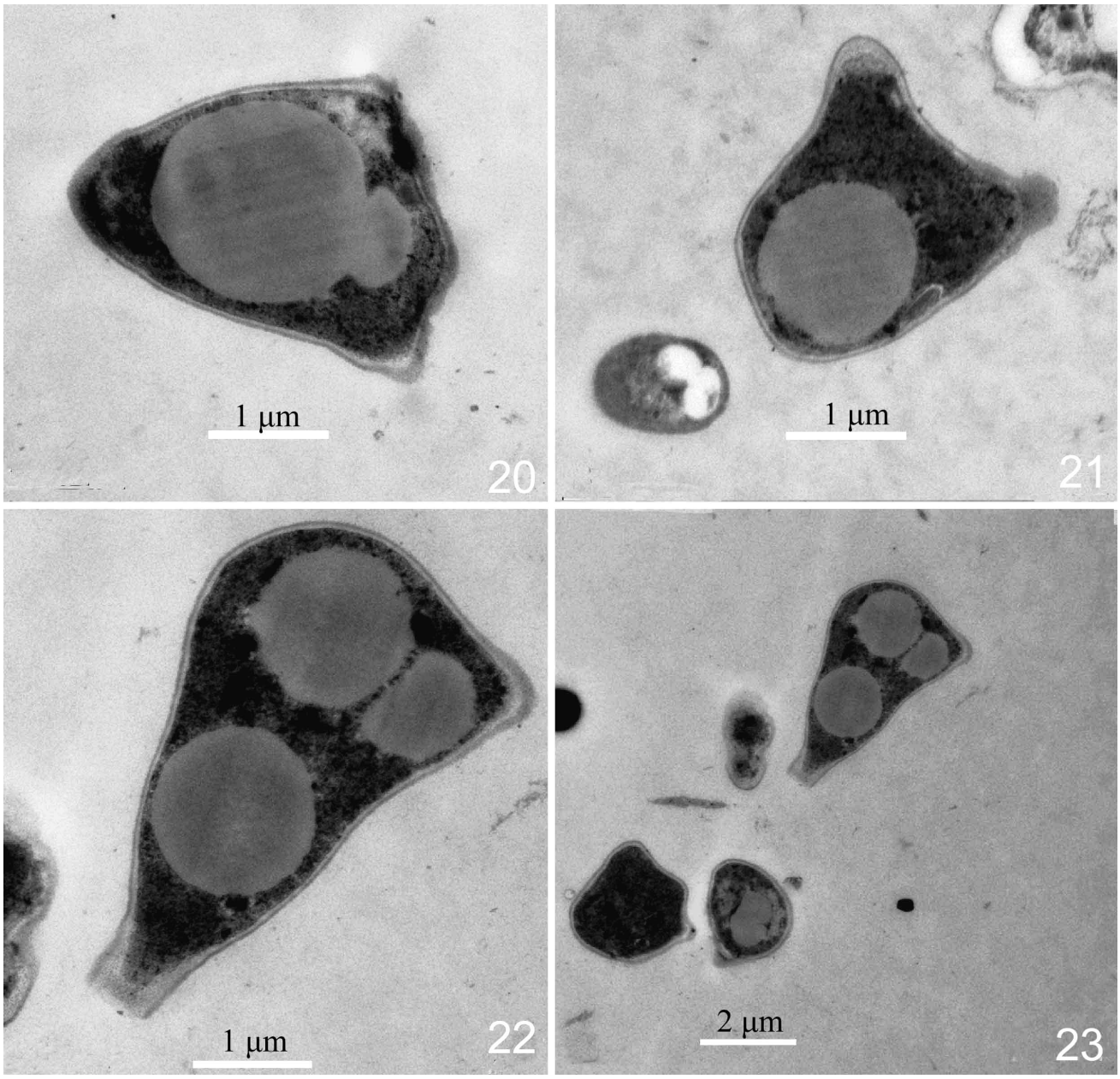
DISCUSSION

Several anamorphs of *O. luteorubella* have been mentioned. A non-sporulating strain isolated from *O. luteorubella* (D.H.P. 125) and an independently sequenced *Helicoon sessile* isolate formed a clade entirely unrelated to *Orbilia*, according to the analysis of ITS sequences of main nematophagous members of Orbiliaceae (Hagedorn and Scholler 1999). Another non-sporulating culture from *O. luteorubella* (D.H.P. 146) properly falls in *Orbilia*. The four isolates forming *Pseudotripoconidium* clustered with strain D.H.P. 146 and fell into *Orbilia* while D.H.P. 125 did not cluster within *Orbilia* in our present analysis.

Pfister (1997) also reported an *Anguillospora* anamorph obtained from *Orbilia* that he tentatively identified as *O. luteorubella*. The uncertainty was due to difficulties of *Orbilia* identification. Descals et al.



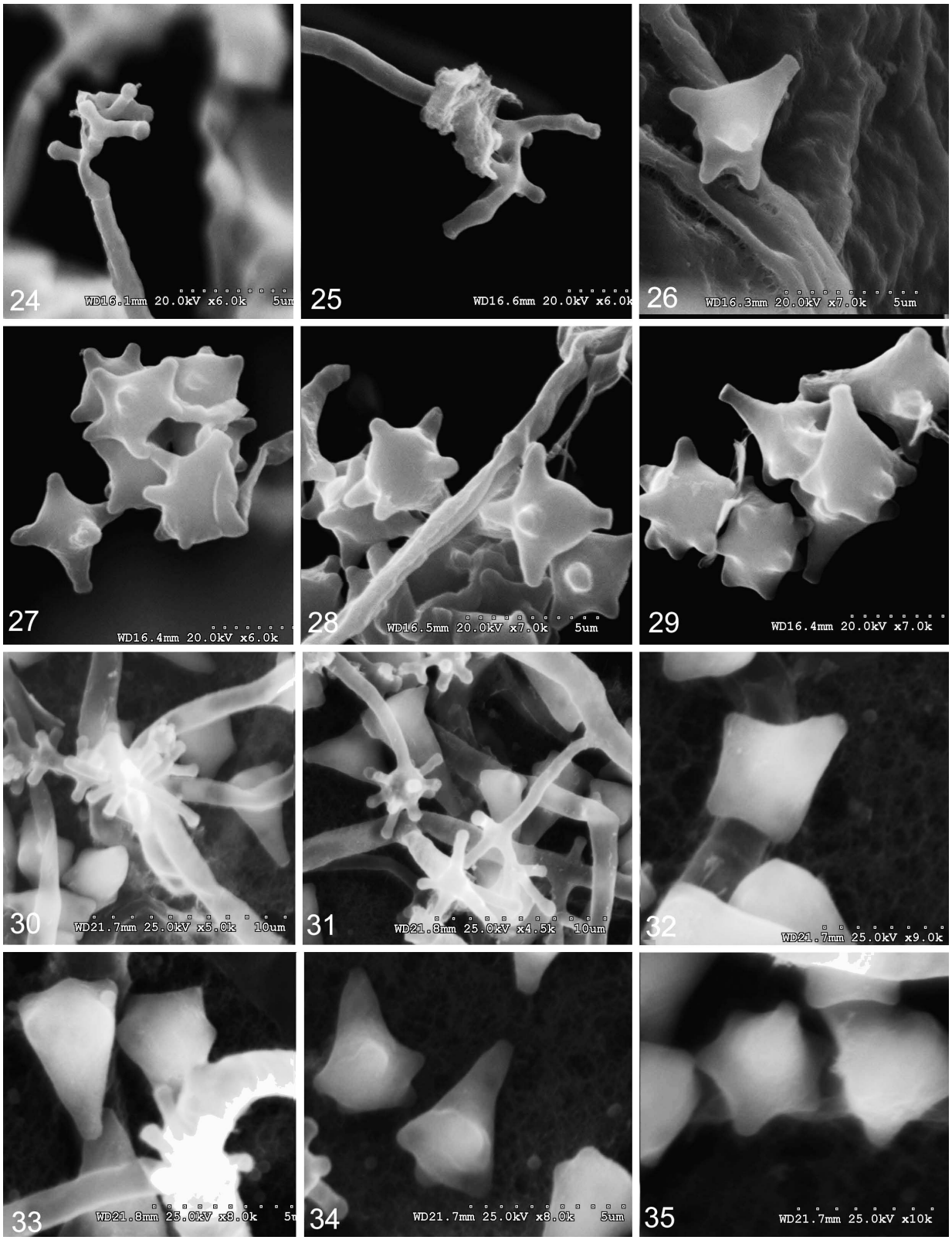
FIGS. 9–19. *Pseudotripoconidium sinense* (Holotype CBS 121220 or YMF 1.01843). 9. Conidiophores with denticles. 10. Conidiophore bearing a conidium. 11. Conidiophore with secondary branches. 12. Conidia. 13. Chlamydospores. 14. Conidium seen above, showing the lateral compression. 15–19. Conidia with 2–3 nipple-shaped protuberances.



FIGS. 20–23. Transmission electron micrograph of *Pseudotripoconidium sinense* conidia (from holotype). 20. Longitudinal section showing two guttules. 21. Longitudinal section showing one guttule and two protuberances. 22. Longitudinal section showing three guttules. 23. Section of three conidia.

(1998) suggested that the *Anguillospora* anamorph illustrated by Pfister was *A. rosea*, but because Pfister did not provide apothecial characters Descals et al. hesitated to formally link *O. luteorubella* and *A. rosea*. However *Anguillospora rosea* Webster and Descals was genetically connected to *Orbilia* by Belliveau and Bärlocher (2005). Based on the description provided by Descals et al. (1998) and the illustration of the teleomorph of [ex] A430–18-10, we think that the teleomorph they were discussing was either *O. luteorubella* or *O. sarraziniana* Boud. Molecular studies have shown that *Anguillospora* is a heterogeneous Ingoldian anamorph genus (Baschien 2006, E. Weber and H.O. Baral pers comm). It is affiliated with at least three different classes of ascomycetes; the type, *A. longissima* (Sacc. & Syd.) Ingold, belongs in Dothideomycetes, *A. rosea* in Orbiliomycetes and *A. fustiformis* Maranová and Descals belongs in Leotiomycetes.

Four teleomorphic specimens from different localities were identified as possibly conspecific with *O. luteorubella* based on various morphological characters in our present research (FIGS. 1–8. YMFT



FIGS. 24–35. *P. sinense* (from YMF 1.03007 and 1.01848). 24–25. Conidiophores with denticles of YMF 1.03007. 26–29. Conidia with 5–7 nipple-shaped protuberances of YMF 1.03007. 30–31. Conidiophores with denticles of YMF 1.01848. 32–35. Conidia with 4–5 nipple-shaped protuberances of YMF 1.01848.

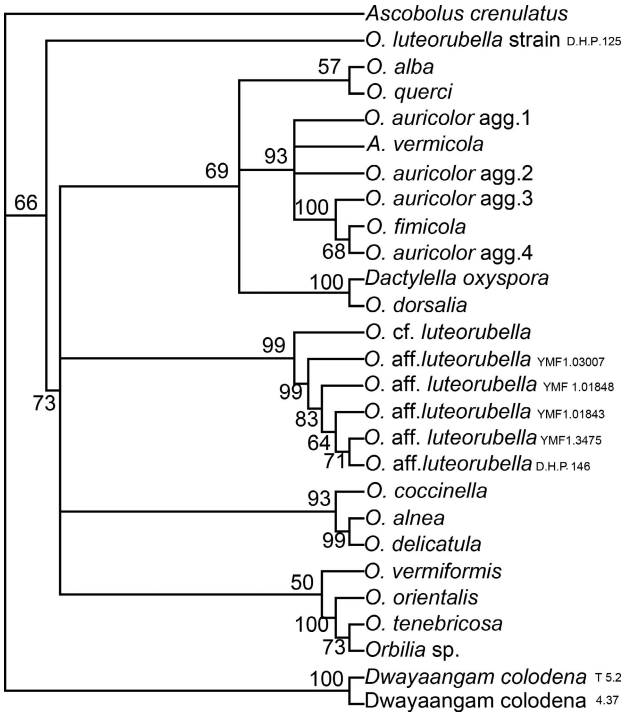


FIG. 36. Most parsimonious phylogenetic tree generated from a heuristic search based on the alignment of the ITS region sequences of *Orbilia* spp. with known anamorphs. Numbers above lines represent bootstrap values from 1000 replicates on all parsimony informative characters; only values >50% shown.

1.01843). The anamorphs produced by these isolates could not be distinguished with light microscopy, but the conidia of these isolates differed in the number of protuberances when viewed with SEM. YMF 1.03475 and YMF 1.01843 both had conidia with up to three protuberances, while YMF 1.01848 and YMF 1.03007 conidia respectively had up to five and seven protuberances. However the homology of ITS sequences was above 90%, therefore we treated these isolates as a single entity. Strains of the *O. luteorubella* aggregate formed a single clade separate from predacious fungi in our phylogenetic analysis. Only nematodes were added to test the trapping ability of *P. sinense* in our present study. Baral (pers comm) suggested that Orbiliaceae might trap other microscopic animals instead of nematodes. Trapping capabilities of these fungi against other invertebrates should be studied further to explore this possibility.

Morphologically *Pseudotripoconidium* resembles *Tuberculispora* Deighton and Piroz (1972) and *Coronospora* Matsush (1975) in having protuberances on their conidia. However conidia of *Tuberculispora* are subglobose and those of *Coronospora* are one-septate and the conidiophores are basally dark brown. *Pseudotripoconidium* also resembles *Tripoconidium aphanophagum* (Drechsler 1937, Subramanian 1977) and *Triposporina uredinicola* Höhn (type species of *Triposporina*) in having obpyramidal conidia with protuberances, but the basal part of the conidia of these two species are divided by two transverse septa and their protuberances are always bilobed, and septa are at the base of protuberances, which divide them from the other part of the conidia. In addition conspicuous annulations are near the tip of conidiophores of *T. uredinicola*, which distinguishes it from *Pseudotripoconidium* by the sporulating mode.

Nomen nudum was inadvertently created when an unpublished manuscript name was picked up in Index Fungorum. A citation is given for a paper that was never published. Later we used this name, still nomen nudum, in one of our papers (Guo et al. 2009). All these references refer to the same material, now here validly published.

ACKNOWLEDGMENTS

This work was jointly financed by National Basic Research Program of China (2007CB411600), National Natural Science Foundation Program of PR China (30860004, 31060008), Grants from the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI020) Major State Basic Research Development Program (2009CB125905) and Research Project (2009DA002). H.O. Baral and E. Weber from Tübingen, Germany, are thanked for their detailed comments on this paper and identification of teleomorph specimens. Prof J.P. Xu from McMaster University and all anonymous reviewers are thanked for their detailed comments on this paper.

LITERATURE CITED

Alexopoulos CJ, Mims CW, Blackwell M. 1996. In: Introductory Mycology. 4th ed. New York: John Wiley & Sons. 355 p.

Baschien C, Marvanov L, Szewzyk U. 2006. Phylogeny of selected aquatic hyphomycetes based on morphological and molecular data. Nova Hedwig 83:311–352, doi:10.1127/0029-5035/2006/0083-0311.

Belliveau Michel JR, Bärlocher F. 2005. Molecular evidence confirms multiple origins of aquatic hyphomycetes. Mycol Res 109:1407–1417, doi:10.1017/S0953756205004119.

Berthet P. 1964. Formes conidiennes de divers discomycètes. Bull Trim Soc Mycol Fr 80:125–149.

Deighton FC, Pirozynski KA. 1972. CMI papers 128:92–94.

Descals E, Marvanová L, Webster J. 1998. New taxa and combinations of aquatic hyphomycetes. Can J Bot 76: 1647–1569, doi:10.1139/cjb-76-9-1647.

Drechsler C. 1937. Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447–552, doi:10.2307/3754331.

Guo JW, Yu ZF, Li CY, Zhang KQ. 2009. The evaluation of sexual reproduction capacity of orbiliaceous anamorphs. Mycosystema 28:692–697.

Hagedorn G, Scholler M. 1999. A reevaluation of predatory orbiliaceous fungi I. Phylogenetic analysis using rDNA sequence data. Sydowia 51:27–48.

Haines JH, Egger KN. 1982. A new species of *Orbilia* from Canada. Mycotaxon 16:107–113.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98.

Kindermann J, EL-Ayouti Y, Samuels GJ, Kubicek CP. 1998. Phylogeny of the genus *Trichoderma* based on sequence analysis of the internal transcribed spacer region 1 of the rDNA clade. Fungal Genet Biol 24:298–309, doi:10.1006/fgbi.1998.1049.

Kohlmeyer J, Baral HO, Volkmann-Kohlmeyer B. 1998. Fungi on *Juncus roemerianus*. A new *Orbilia* with Ingoldian anamorph. Mycologia 90:303–309, doi:10.2307/3761307.

Korf RP. 1992. A preliminary discomycete flora of Macronesia 8. Orbiliaceae. Mycotaxon 45:503–510.

Li TF, Zhang KQ, Liu XZ. 2000. Taxonomy of nematophagous fungi. Beijing: Chinese Science & Technological Press. 313 p.

Li Y, Hyde KD, Jeewon R, Cai L, Vijaykrishna D, Zhang KQ. 2005. Phylogenetics and evolution of nematode-trapping fungi estimated from nuclear and protein coding genes. Mycologia 97:1034–1046, doi:10.3852/mycologia.97.5.1034.

Liou GY, Tsean SS. 1997. Phylogenic of the genus Arthrobotrys and allied nematode-trapping fungi based on rDNA sequences. Mycologia 89:876–884, doi:10.2307/3761108.

Liu B, Liu XZ, Zhang WY. 2005. *Orbilia quercus* sp. nov. and its knob-forming nematophagous anamorph. FEMS Microbiol Lett 245:99–105, doi:10.1016/j.femsle.2005.02.027.

Luo H, Liu YJ, Fang L, Li X, Tang NH, Zhang KQ. 2007. *Coprinus comatus* damages nematode cuticles mechanically with spiny balls and produces potent toxins to immobilize nematodes. Appl Environ Microb 73:3916–3923, doi:10.1128/AEM.02770-06.

Matsushima T. 1975. Icones microfungorum a Matsushima lectorum. Kobe, Japan: Published by the author. 209 p.

———. 1995. Matsushima Mycological Memoirs. Vol. 8. Kobe, Japan: Matsushima Fungus Collection. 44 p.

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.

Nannfeldt JA. 1932. Studien uber die Morphologie und Systematik der nicht-lichenisierten inoperculaten Discomyceten. Nova Acta Regiae Societatis Scientiarum Upsaliensis Ser. IV 8:5–337.

Pfister DH. 1994. *Orbilia fimicola*, a nematophagous discomycetes and its *Arthrobotrys* anamorph. Mycologia 86:451–453, doi:10.2307/3760578.

———. 1997. Castor, Pollux and life histories of fungi. Mycologia 89:1–23, doi:10.2307/3761168.

———, Liftik ME. 1995. Two *Arthrobotrys* anamorphs from *Orbilia auricolor*. Mycologia 87:684–688, doi:10.2307/3760812.

Rubner A. 1996. Revision of predacious hyphomycetes in the *Dactylella-Monacrosporium* complex. Stud Mycol 39:1–134.

Samson RA, Stalpers JA, Verkerke W. 1979. A simplified technique to prepare fungal specimens for scanning electron microscopy. Cytobios 24:7–11.

Scholler M, Hagedorn G, Rubner A. 1999. A reevaluation of predatory orbiliaceous fungi II. A new generic concept. Sydowia 51:89–113.

Sokolski S, Piché Y, Laitung B, Bérubé JA. 2006. Streams in Quebec boreal and mixed-wood forests reveal a new aquatic hyphomycete species, *Dwayaangam colodena* sp. nov. Mycologia 98:628–636, doi:10.3852/mycologia.98.4.628.

Subramanian CV. 1977. Revisions of Hyphomycetes I. Kavaka 5:93–98.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0. Sunderland, Massachusetts: Sinauer Associates.

Thakur S, Zachariah K. 1989. Response of the fungus *Dactylella rhopalota* to bacteria. Plant Soil 120:87–93, doi:10.1007/BF02370294.

Turner D, Kovacs W, Kuhls K, Lieckfeldt E, Peter B, Arisan-atac I, Strauss J, Samuels GJ, Börner T, Kubicek CP. 1997. Biogeography and phenotypic variation in *Trichoderma* sect. *Longibrachiatum* and associated *Hypocrea* species. Mycol Res 101:449–459, doi:10.1017/S0953756296002845.

Webster J, Descals E. 1979. The teleomorphs of waterborne hyphomycetes from freshwater. In: Kendrick B, ed. The whole fungus. Ottawa, Canada: National Museum of Natural Sciences. p 41–451.

———, Henrici A, Spooner B. 1998. *Orbilia fimicoloides* sp. nov., the teleomorph of *Dactylella* cf. *oxyspora*. Mycol Res 102:99–102, doi:10.1017/S0953756297004747.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. San Diego, California: Academic Press. p 315–322.

Yang Y, Liu XZ. 2005. *Dactylella coccinella* sp. nov., an anamorph species. Mycotaxon 91:127–132.

———, Yang E, An ZQ, Liu ZX. 2007. Evolution of nematode-trapping cells of predatory Fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. PNAS 104:8379–8384, doi:10.1073/pnas.0702770104.

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. 2007a. *Orbilia dorsalia* sp.nov., the teleomorph of *Dactylella pseudoxyspora* sp.nov. Cryptogamie Mycol 28:55–63.

———, ———, ———, ———. 2007b. *Orbilia vermiformis* sp. nov. and its anamorph. Mycotaxon 99:271–278.

———, Kong YJ, Qiao M, Guo JW, Zhang KQ. 2009a. A new *Dactylella* species from *Orbilia alba*. J Microbiol 47(3):265–269, doi:10.1007/s12275-008-0301-1.

———, Qin L, Zhang Y, Qiao M, Kong YJ, Zhang KQ. 2009b. A new *Drechslerella* species isolated from *Orbilia* cf. *orientalis*. Mycotaxon 110:253–259.

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