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Orbilia querci sp. nov. and its knob-forming nematophagous anamorph

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Abstract

Orbilia querci, a new nematode-trapping fungus, was found on rotten wood of *Quercus* sp. in Huai-rou County, Beijing, China. It is characterized by having a tear-shaped spore body in the cylindrical ascospore. Pure culture was obtained from the ascospores. Conidiophores were simple or occasionally branched, bearing a single conidium on the tip. Conidia were spindle-shaped, mostly with 3-septa. Nematodes were captured by means of adhesive stalked knobs. The adhesive knobs were produced frequently on nutritional agar plates even in the absence of challenging nematodes. Its anamorph is placed in *Dactylellina* and named as *D. querci*. The sequence divergence of the ITS1 region between the fungus and the other knob-forming species tested was 23.8–33.4%, supporting *O. querci* as a distinct species. This is the first report of the connection between a knob-forming nematophagous hyphomycete and an *Orbilia* teleomorph.

Keywords: Orbiliaceous fungi; Nematophagous fungi; Teleomorph-anamorph connection; *Orbilia querci*; *Dactylellina querci*

1. Introduction

Orbiliaceous fungi include those which produce small, waxy, light-colored and semitranslucent apothecia with tiny asci and ascospores and a swollen paraphysis apex. Orbiliaceae, a family traditionally placed in the Helotiales, was excluded from this order according to molecular evidence. Recently, a new order Orbiliales and a new class Orbiliomycetes were proposed [1]. *Orbilia* Fr. and *Hyalorbilia* Baral and Marson are the only genera currently accepted in the family [2]. More than 200 species of the Orbiliaceae have been reported in the world, but most of them have not been re-evaluated, and very few of them were studied in culture. Recently, more attention has been paid to the orbiliaceous fungi because of their nematophagous anamorphs [3–6].

Nematode-trapping hyphomycetes have been classified by the morphology of conidiophores and conidia [7–11]. Rubner [12] revised the generic concepts of the predatory hyphomycetes based on trapping organs in her monographic treatment of the *Dactylella-Monacrosporium* complex. Later, Hagedorn and Scholler [13] and Scholler et al. [14] emended the predatory orbiliaceous fungi according to rDNA sequence data and the morphology of trapping organs assigned the adhesive knob

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forming species into *Dactylellina* M. Morelet and *Gamsylella* Scholler, Hagedorn and Rubner, respectively.

Net-work and constricting ring forming fungi have been demonstrated to link with *Orbilia* [3–5]. However, there were no connections established between adhesive knob-forming fungi and *Orbilia* teleomorphs [13]. During our recent study on the orbiliaceous fungi and their anamorphs, an interesting *Orbilia* specimen was collected from Huai-rou County, Beijing, China, and adhesive knob-forming anamorph produced from the ascospores. A new species of *Orbilia* and of *Dactylellina* are proposed and described.

2. Materials and methods

The fresh specimen of *Orbilia* was collected from Labagoumen Forest Park of Huai-rou County, Beijing, China in July, 2002. A dried voucher specimen was deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS 88781). The specimen was rehydrated and sectioned longitudinally at the thickness of 10–15 μm by freezing microtome; the living ascospores were observed directly from the tap water mount of living specimen.

An apothecium was stuck on the lid of a Petri dish with its hymenium upside down to shoot ascospores on the surface of water agar (15 g Bacto-Agar (Difco), 50 mg streptomycin, 30 mg chlortetracycline, 1000 ml distilled water). After germination, the ascospores were transferred onto PDA plates (Oxoid, UK). The morphological characteristics of the anamorph were observed and measured from cultures on PDA and CMA (Oxoid, UK) after incubation for 7–10 days at 25 °C. Trapping organs were induced by adding *Panagrellus redivivus* (L.) Goodey into a 10×10 mm square slot at the margins of the colony where the agar was removed and observed directly under a compound microscope after 1–3 days [15]. All microscopic characteristics were measured from 50 individuals in water mounts. Observations, measurements, and photographs were taken with an Olympus B51 microscope with differential interference contrast (DIC).

DNA was extracted from the pure cultures using a modified method of Doyle and Doyle [16]. Primers for PCR amplification of the internal transcribed spacer region of nr DNA were ITS5 and ITS4 described by White et al. [17]. PCR products were purified using the Go3S PCR Product Purification Kit and sequenced on an automated ABI 377 sequencer (PE). Related sequences were obtained from GenBank. All sequences were aligned with the Clustal X program [18] and adjusted visually where necessary in the program BioEdit sequence alignment editor [19]. Cladistic analyses using the neighbor-joining method [20] and maximum-parsimony method were performed with MEGA version 2.1 [21]. The neighbor-joining tree was constructed with Kimura 2-parameter model, including transitions and transversions and with pairwise deletion of gaps. The maximum-parsimony trees were searched using the Min-mini heuristic algorithm with a search factor of 3. The robustness of branches was assessed by bootstrap analysis with 1000 replicates. Details of strains used and GenBank accession numbers are given in Table 1.

3. Results

3.1. Morphological descriptions

Etymology: species epithet refers to the host genus (see Fig. 1).

Table 1
Species and sequence database accession numbers

Species	Strain no.	Geographic origin	Accession no.
Dactylellina drechsleri	AS 3.6773	Mainland China	AY773448
Dactylellina ellipsospora	CBS224.54	UK	U51971
Dactylellina ellipsospora	AS 3.6758	Mainland China	AY804214
Dactylellina haptotyla	CBS325.94	Canary Islands	AF106523
Dactylellina haptotyla	AS 3.6779	Mainland China	AY773470
Dactylellina leptospora	CBS560.92	USA	AF106529
Dactylellina leptospora	AS 3.6775	Mainland China	AY773466
Gamsylella arcuata	CBS174.89	UK	AF106527
Gamsylella gephyropaga	CBS178.37	UK	U51974
Gamsylella lobata	CBS329.94	Germany	AF106524
Gamsylella parvicollis	516	Mainland China	AY804215
Gamsylella phymatopaga	CCRC32875	Taiwan	U51970
Orbilia epipora	DHP107	USA	U72601[a]
Orbilia querci	AS 3.6762	Mainland China	AY804213
Patinella tenebricosa	DHP133	USA	U72606
Hyalorbilia brevistipitata	CBS 113946	Mainland China	AY514636

[a] U72601 under a tentative name *O. alnea* in GenBank and [5], Baral (unpublished) identified the specimen as *O. epipora* (Nyl.) Karsten.

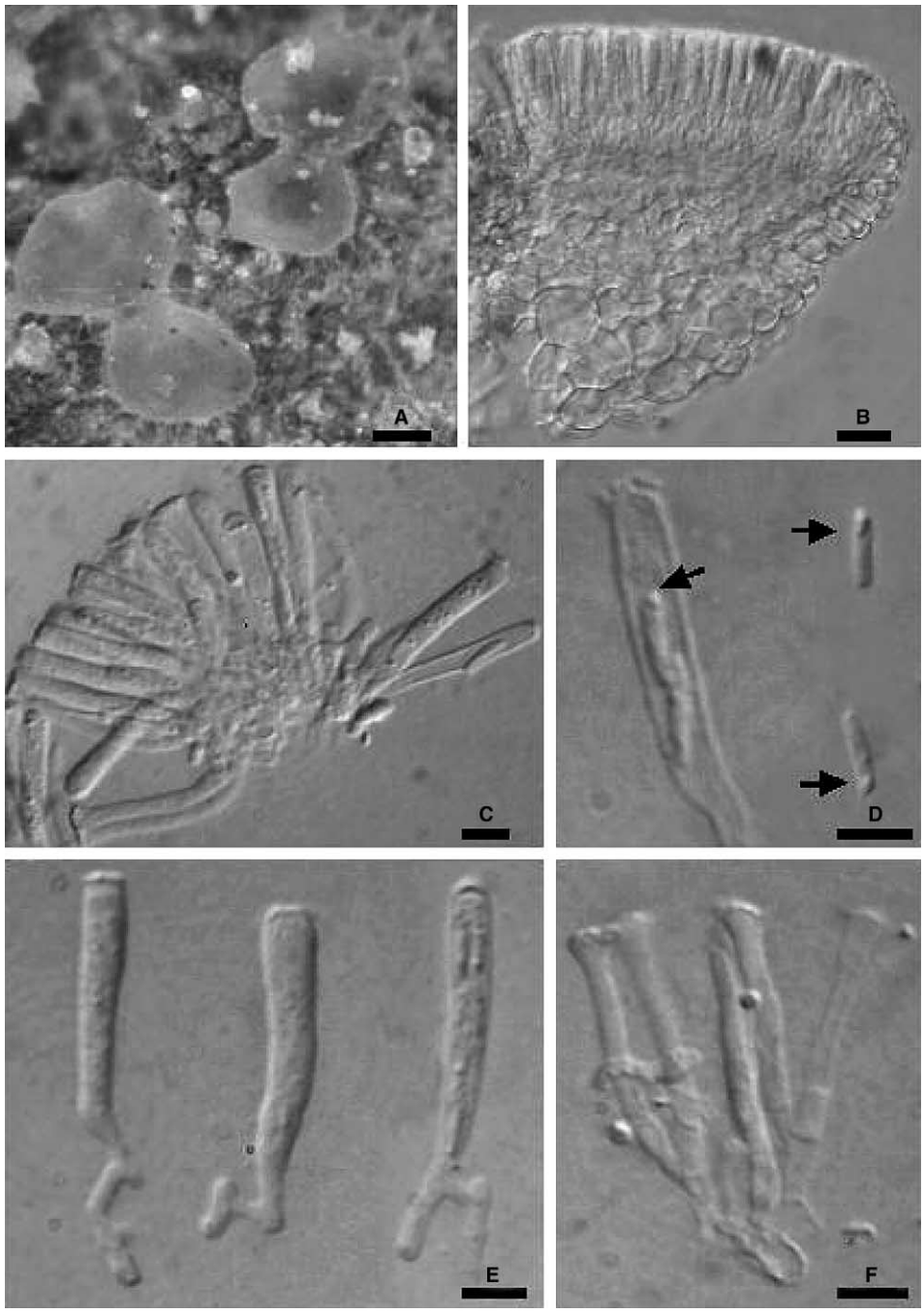

Fig. 1. *Orbilia querci*. (A) Dried apothecia. (B) Vertical section of an apothecium. (C)–(D) Asci and ascospores, arrow indicates the SB in living ascospore. E. Asci. F. Paraphyses. Bars A = 100 μm; B = 10 μm; (C)–(F) = 5 μm.

Apothecia 0.2–0.5 mm in diam., solitaria vel gregaria, superficialia, translucentia, sessilia, concavo. Excipulum ectale texturae angulare, cellulae isodiametrae, 6–10(−14) μm diam. Asci 18–30 × 2.5–3.0 μm, 8-spori, cylindraceo-clavati, basi angustati plerumque furcati, apice truncati vel rotundati. Ascosporae hyalinae, cylindri-coclavatae, non-septatae, imbricate biseriatae, 5.0–6.0 × 0.8–1.2 μm. Cellula apicalis cum vacuola refringens lacrimiformis vel ovata, 1.1–1.8 × 0.7–1.0 μm. Paraphyses filiformes, 1.5–2.0 μm diam., apice usque 2.0–2.5 diam., hyalinae.

Mycelium sparsum, hyphis hyalinis septatis laevibus compositum. Conidiophora hyalina, recta, simplicia, solitaria, septata, 130–180 μm alta, basi 5–6.5 μm crassa, ad apicem angustata 1.5–2.5 μm crassa. Conidia singularia, apicalia, hyalina, laevia, fusiformia, apicea obtuse,

25–32.5–40(−50) μm longa, 8–9.5–12 μm mm lata, 3- ad 5-septata, saepe 3-septata. Chlamydosporae desunt (see Fig. 2).

Apothecia superficial on rotten wood, gregarious, sessile, 0.2–0.5 mm in diam. Disc concave, smooth, translucent, whitish to pale yellow when fresh, brownish yellow when dried. Ectal excipulum of textura angularis, 78–96-μm thick, cells isodiametric, 6–10(−14) μm in diam. Medullary excipulum of textura intricate, 27–35-μm thick. Subhymenium poor-developed. Asci cylindrical-clavate, narrower and tapered towards the base, sometime forked at the base, apex truncate to rounded, 18–30 × 2.5–3.0 μm. Ascospores hyaline, subcylindrical to cylindrical-clavate, straight or sometimes slightly curved, non-septate, usually overlapping tightly and biseriate within ascus, 5.0–6.0 × 0.8–1.2 μm in water, spore body (SB) tear-shaped to short rod-shaped, 1.1–1.8 × 0.7–1.0 μm. Paraphyses filiform with a clavate to

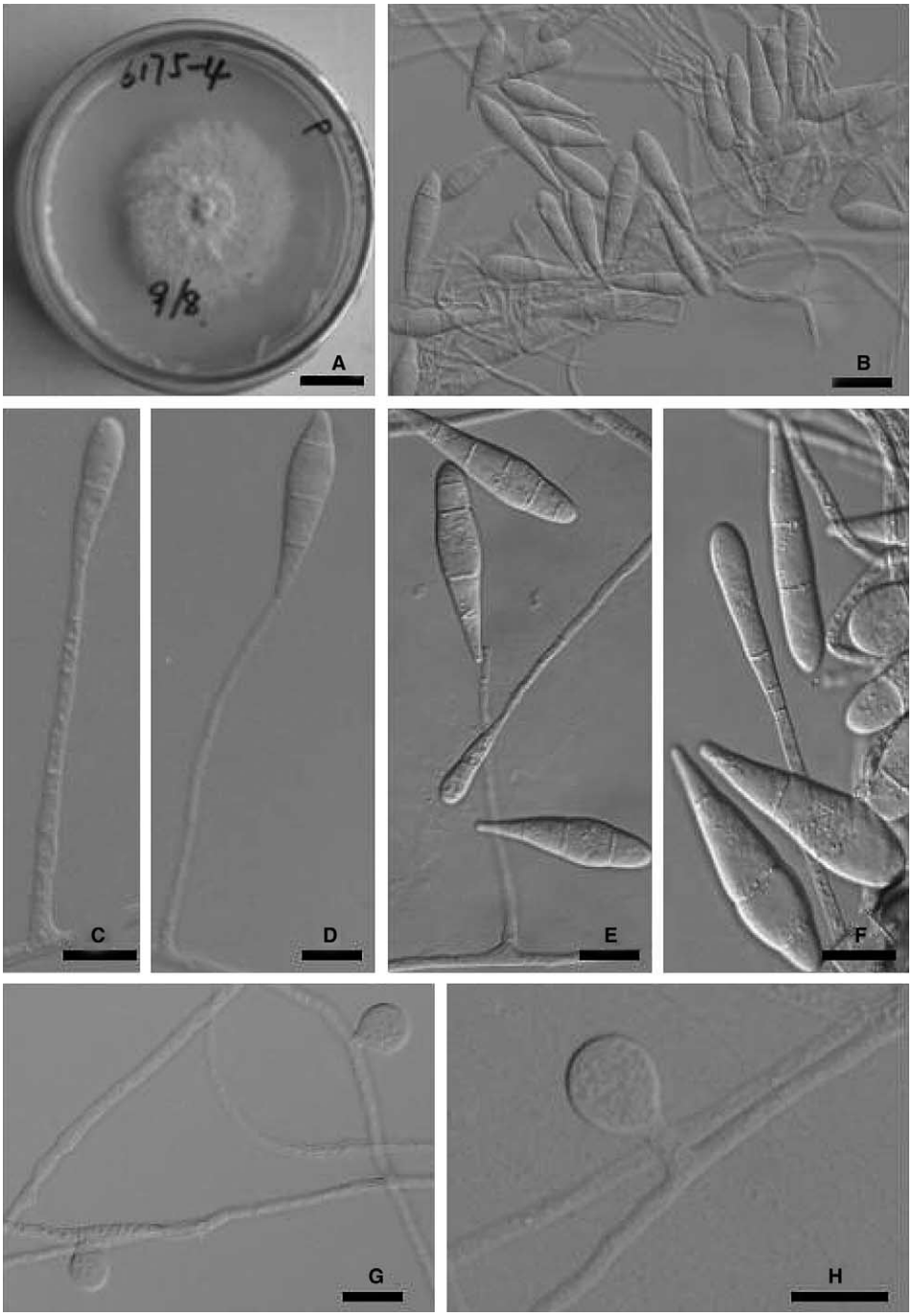

Fig. 2. *Dactylellina querci* (A) Colony on PDA. (B)–(F). Conidiophores and conidia, note the single conidium produced on each tip of the conidiophores. (G)–(H) Stalked knobs. Bars A = 2 cm; B = 20 μm; (C)–(H) = 10 μm.

capitate apex, hyaline, 18–22-μm long, 2.0–2.5-μm wide at apex and 1.5–2.0-μm wide below.

Colonies colorless on PDA or CMA, reached to 60–65 mm in diam. on PDA and 45 mm on CMA after 20 days culture at 23–25 °C. Aerial mycelium sparse, hyphae hyaline, septate, branched, 2.5–3.5-μm wide. Conidiophores mostly 130–180 μm high, 5–6.5-μm wide at the base, 1.5–2.5 μm at the tip, sometime branched near the apex, bearing a single conidium. Conidia were commonly spindle-shaped, slightly rounded at the distal end,

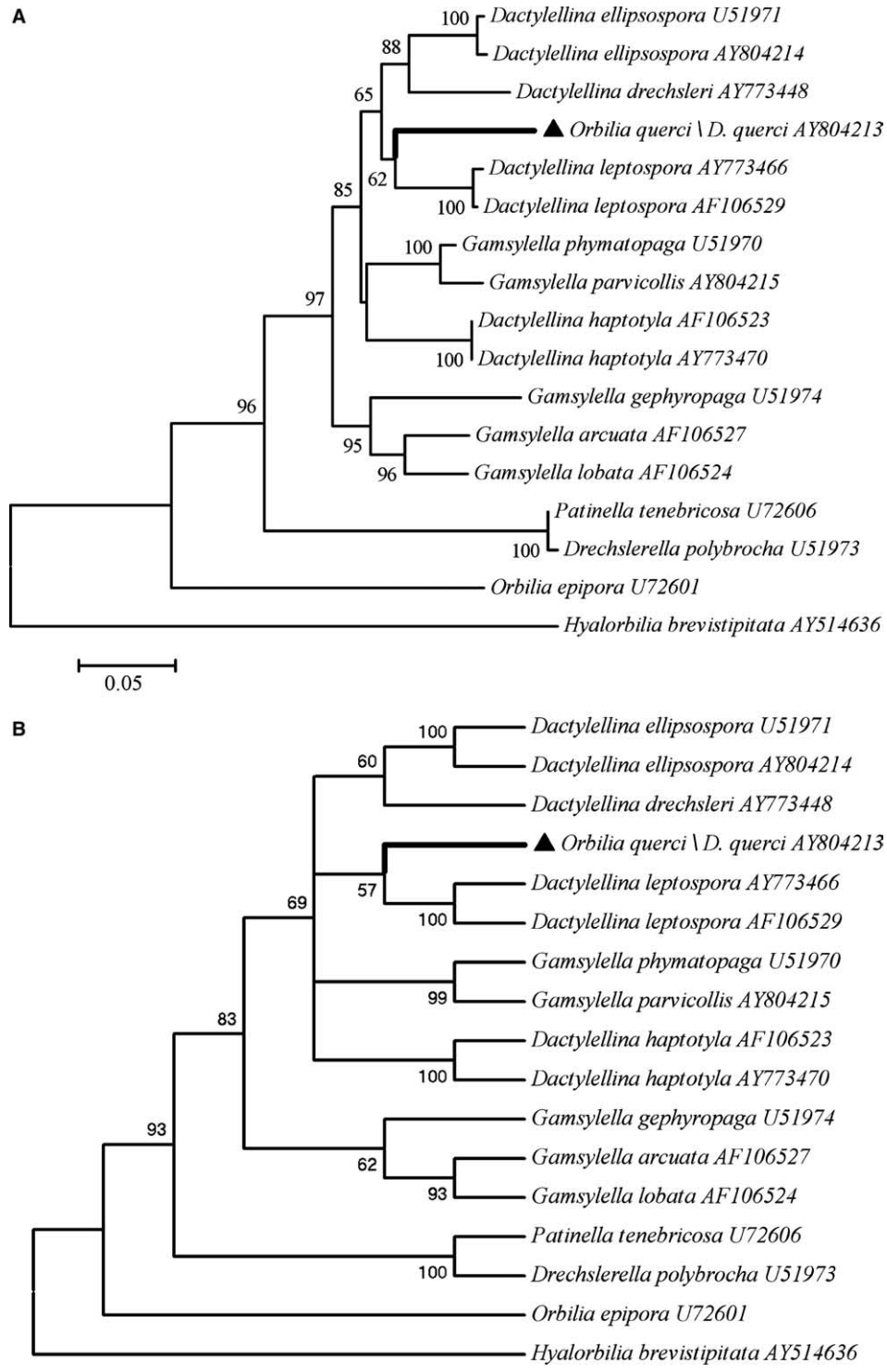

Fig. 3. Phylogenetic trees of *O. querci* and the related species based on ITS region sequence data, with trees rooted with *Hyalorbilia brevistipitata*. (A) A neighbor-joining tree obtained using Kimura 2-parameter distances. (B) The strict consensus of two maximum parsimony trees. Percentages at nodes represent levels of bootstrap support from 1000 replicates. Bootstrap values less than 50% are not shown.

narrowly truncate at the base, 25–32.5–40(−50) × 8–9.5–12 μm, with 3–5 and mainly 3-septa, but the middle cell is not much larger than the others. Chlamydospores not observed. Trapping nematodes by means of stalked knobs (0–5.5 μm), knobs spherical to subspherical, 8.0–12.0 × 7.5–10.0 μm. Knobs produced frequently on the nutritional agar plates even without challenging with nematode.

Holotype: PR China, Beijing, Huai-rou County, Labagoumen Forest Park, alt. 850 m, 10 July 2002, B. Liu 6175 (HMAS 88781), permanent slide culture (HMAS 88783).

Habitat: on rotten wood of *Quercus* sp.

3.2. DNA sequencing and phylogenetic analysis

The sequences of *O. querci* and the related species were compared. The total lengths of ITS1–5.8S–ITS2 rDNA regions of these species are 505–536 bases. The sequences obtained were compared with the ITS1 sequences of related species of *Dactylellina*. There was 77.2% similarity between *O. querci* and *D. leptospora* (Drechsler) Scholler et al., 73.5% with *Dactylellina ellipsospora* (Preuss) Scholler et al., 71.7% with *Dactylellina drechsleri* (Tarjan) Scholler et al., 67.6% with *Dactylellina haptotyla* (Drechsler) Scholler et al., 55.4% with *Orbilia epipora* (Nyl.) Karsten, and 52.4% with *Patinella tenebricosa* Svrček.

A neighbor-joining tree (Fig. 3(A)) was constructed based on sequences of the ITS region of *O. querci* and the related species, with *Hyalorbilia brevistipitata* Liu et al. [22] as the outgroup. The tree showed that the nematode-trapping species formed a clade with 96% bootstrap support, while *O. epipora* showed little homology with them. In the nematode-trapping clade, *P. tenebricosa* and its constricting ring forming anamorph *Drechslerella polybrocha* (Drechsler) Scholler et al. formed a subclade, while *Dactylellina* and *Gamsylella* species trapping nematodes with adhesive knobs or branches formed the other one with 97% bootstrap support. Two groups were recognized in the second subclades, one comprised 3 *Gamsylella* species and the other was of 5 *Dactylellina* species and 2 *Gamsylella* species, with which *O. querci* was grouped. *O. querci* was related to *D. leptospora* with a 62% bootstrap value. The tree indicated that *O. querci* is distinguishable from other knob-forming species tested although the relationships between *Dactylellina* and *Gamsylella* are poorly resolved. *O. querci* is also distinguishable from *O. epipora* and *P. tenebricosa*. Similar results were obtained with Maximum parsimony methods (Fig. 3(B)).

4. Discussion

Morphologically, the present species appears to be similar to *O. epipora* and *Orbilia rectispora* (Boud.) Baral in asci and paraphyses, but differs mainly in the shape of the spore body and in the size of the ascospores (Table 2). According to Baral's unpublished world monograph of the Orbiliomycetes, spore body (SB) is the key characteristic to separate species. The SB of our fungus is tear- to rod-shaped, 1.1–1.8 × 0.7–1.0 μm in size, while those of *O. epipora* and *O. rectispora* are globose (Baral, pers. comm.). The ascospores of *O. querci* are longer than those in *O. epipora* and shorter than that in *O. rectispora*, and its asci are slightly smaller than that in the two species. The spore arrangement in ascus of *O. querci* is usually tightly overlapping and biseriate, which is also different from the other two species. Furthermore, the anamorphs of both *O. epipora* and *O. rectispora* are *Dactylella* and not nematode-trapping (Baral, pers. comm.). *O. querci* also differs from *O. epipora* according to the sequence data; the similarity of the two species in the sequence of ITS1 region was only 54.4%. Regrettably, no sequence of *O. rectispora* is available to compare with *O. querci*.

The new species is also similar to *P. tenebricosa* in the size of ascospores, but the latter differs in dull gray-brown apothecia with a 46–60-μm long and 4–5-μm wide glass processes at margin [23]. The anamorph of *P. tenebricosa* is *Monacrosporium polybrochum* [5] which was later transferred to *Drechslerella* by Scholler et al. [14]. In addition to the different anamorph, the SBs of *P. tenebricosa* are filiform (1.5–2 × 0.3–0.6 μm) (Baral, pers. comm.) instead of tear-shaped and their ITS sequences are divergent.

The anamorph of *O. querci* resembles *D. drechsleri*, *D. ellipsospora* and *D. haptotyla* in the shape of conidia and adhesive knobs, but differs from them in that it lacks the largest cell in conidia and in having shorter stalks of adhesive knobs. The sequence divergence of

Table 2
Comparison of the morphological characteristics of *Orbilia querci* with other similar species

Species	Asci (μm)	Ascospores (μm)	Spore body (μm)
Orbilia epipora	25–42 × 3–4	2.7–4.5(−5) × 0.8–1.3	–
Orbilia rectispora	36–41 × 3–4	5.2–10(−13) × 1–1.7	1.2–2.0
Patinella tenebricosa	25–35 × 3–3.5	5–5.6 × 0.8–1	1.5–2.0 × 0.3–0.6
Orbilia querci	18–30 × 2.5–3.0	5.0–6.0 × 0.8–1.2	1.1–1.8 × 0.7–1.0

Data of *O. epipora*, *O. rectispora* and *P. tenebricosa* cited from Baral's unpublished world monograph of the Orbiliomycetes.

the ITS1 region between *O. querci* and the other knob-forming species tested is 23.8–33.4%, which supports the separation of *O. querci* from its related species.

The connection between orbiliacous fungi and nematode-trapping hyphomycetes has been well-established. Many nematode-trapping hyphomycetes derived from *Orbilia* have been described [1,5]. Among them, only adhesive network-forming *Arthrobotrys* and constricting ring-forming *Drechslerella* were known. This is the first evidence that a knob-forming nematode-trapping hyphomycete is connected with an *Orbilia* teleomorph.

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