Ann Microbiol (2011) 61:591–595
DOI 10.1007/s13213-010-0177-2

ORIGINAL ARTICLE

Dactylella pseudobrevistipitata, a new species from China

Li Qin · Min Qiao · Yue Yang · Guang-Zhu Yang · Kai-Ping Lu · Ke-Qin Zhang · Jian-Ping Xu · Ze-Fen Yu

Received: 9 June 2010 / Accepted: 26 November 2010 / Published online: 16 December 2010

Abstract An anamorphic fungus was isolated from fresh specimens of *Orbilia* species collected in Yunnan Province, China. The fungus was characterized by very short conidiophores and 1–5 septate cylindrical conidia, and most closely related phylogenetically to *Dactylella vermiformis* that produces branched conidiophores and 0–1 septate conidia, but the calculated similarity of ITS sequences was only 83% between the two fungus species. Considering the morphological characteristics and the calculated similarity value, a new species, *Dactylella pseudobrevistipitata*, was described with holotype YMF 1.03504.

Keywords *Dactylella pseudobrevistipitata* · Phylogenetic analysis · ITS sequences

Introduction

The genus *Dactylella* was set up by Grove in 1884 with the type species *D. minta* Grove. Main characters of the genus are saprophytic, conidiophores erect, simple, conidia borne singly at the apex of conidiophores, ellipsoidal or fusoid or cylindrical, one-celled at first, later 2– to many septate, hyaline (Grove 1884). In the type strain, the predacious character was not mentioned. Later, Drechsler (1937, 1950) described many new taxa of nematode-trapping species with similar conidia following this concept. Then, circumscription of the genus was emended several times by other authors (Subramanian 1963, 1977; Schenck et al. 1977; Rubner 1996), in which Ruber's genus concept is widely accepted (Scholler et al. 1999; Li et al. 2005; Yu et al. 2007a, b; Chen et al. 2007a, b, c). Chen et al. (2007a, b, c) further emended this genus and transferred those species with short conidiophores to *Vermispora* Deighton & Pirozynski and *Brachyphoris* J Chen, LL Xu, B Liu & XZ Liu. The latter is characterized by very short, simple conidiophores, and spindle-shaped, filiform or elongate fusoid conidia.

In our study of *Orbilia* and anamorphs from Yunnan Province, China, a strain was isolated from germinated ascospores of *Orbilia*. The *Orbilia* specimen was not identified in detail because there were two apothecia which were used to isolate the anamorph. The conidiophores of this anamorph are shorter than the conidia. Phylogenetic analysis inferred from ITS indicated that this species belongs to *Dactylella*, although it looks like *Brachyphoris* in having very short conidiophores; we described it as a new species of *Dactylella*, *D. pseudobrevistipitata*.

Materials and methods

Collection of teleomorph, isolation and characterization of the anamorph

Fresh specimens of an *Orbilia* species were collected on decaying bark of a broad-leaved tree from near Thermal Spring (500 m altitude), Yuanjiang County, Yunnan

Li Qin and Min Qiao contributed equally to this work.

L. Qin · M. Qiao · Y. Yang · G.-Z. Yang · K.-P. Lu · K.-Q. Zhang · Z.-F. Yu (✉)
Laboratory for Conservation and Utilization of Bio-Resources, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University,
Kunming, Yunnan 650091, People's Republic of China
e-mail: zfyuqm@hotmail.com

J.-P. Xu
Department of Biology, McMaster University,
1280 Main St. West,
Hamilton, Ontario L8S 4K1, Canada

Province, China, in September 2009 by Li Qin. To isolate its anamorph, two fresh apothecia were fixed to the lid of a Petri-dish with their hymenia upside down so that ascospores were deposited on the surface of CMA (20 g corn meal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1,000 ml distilled water). For other isolating details, see Yu et al. (2007a). Microscopic characters of anamorph were observed and measured with an Olympus B51 microscope with differential interference contrast. Conidial size, conidial septate and conidiophores size were calculated by measuring more than 50 samples. Trapping organs were induced by adding about 100 nematodes (*Panagrellus redivivus* Goodey) to a 1×1 cm slot at the margins of the colony created by removing the agar.

DNA extraction, PCR amplification and sequencing

Fresh mycelium was collected from the cultures growing on cellophane menbrane on the PDA plate surface. The total DNA was extracted from fresh mycelium as described by Yu et al. (2007b). The ITS region was amplified with the standard primers of ITS4 and ITS5 (White et al. 1990). The parameters for PCR amplification were as follows: 3 min at 95°C, followed by 30 cycles of 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min. A final extansion at 72°C for 10 min followed. The PCR products were purified with a commercial Kit (TaKaRa Biotechnology), and sequenced

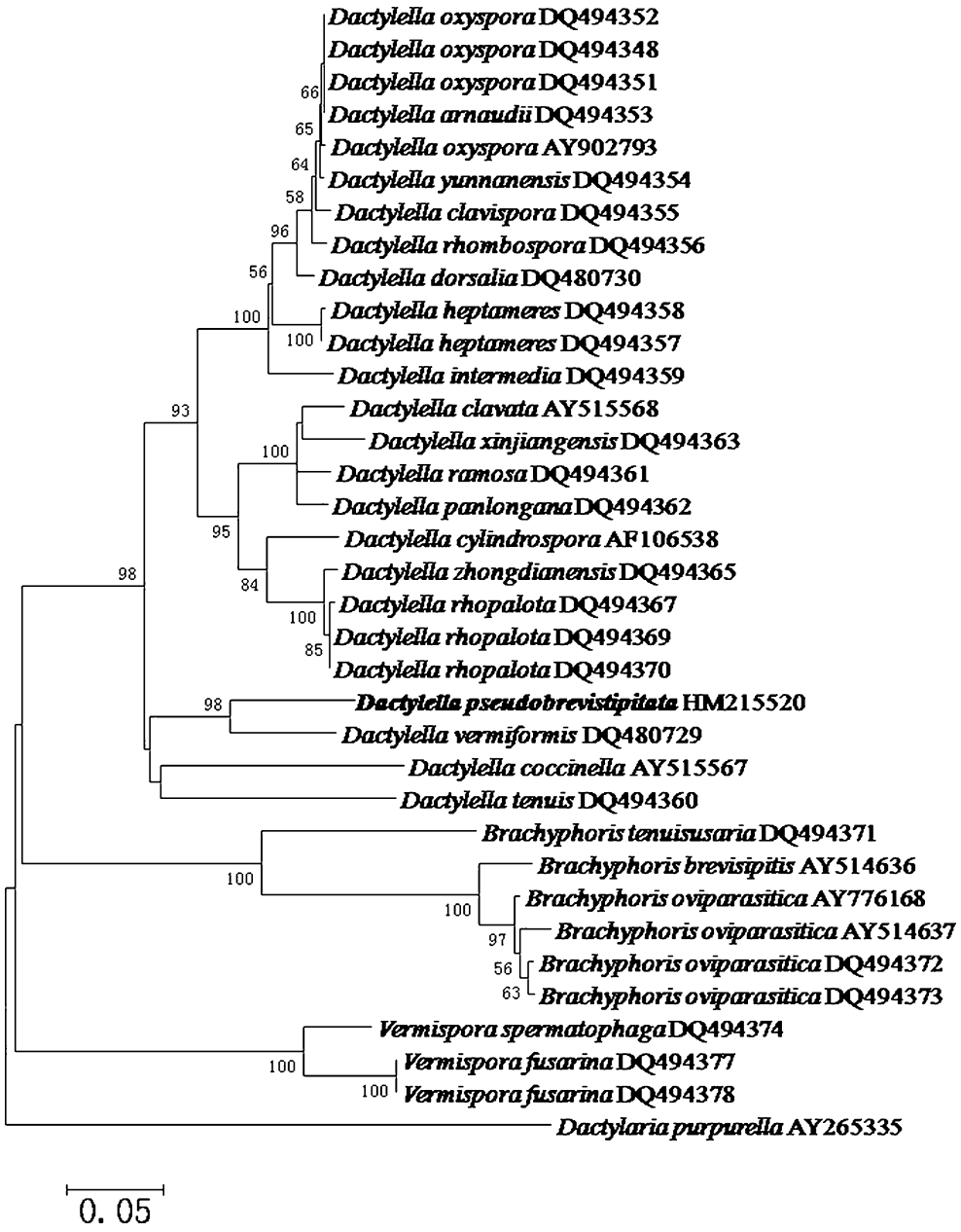

Fig. 1 A neighbor-joining tree inferred from the ITS DNA sequences. The *number* associated with each *branch* represents the percentage of 500 bootstraps supporting that branch. GenBank accession numbers are shown following each taxon name

on both strands using the same primers that were used for amplification, with an ABI 3730 DNA analyzer automatic sequencing system, using cycle sequencing with the Thermo Sequenase-kit.

Phylogenetic analysis

We performed a phylogenetic analysis using ITS sequences of *Dactylella*, *Vermispora* and *Brachyphoris* used in Chen et al. (2007a), with *Dactylaria purpurella* as the outgroup. In addition, two similar species morphologically similar to *D. pseudobrevistipitata*, *D. vermiformis* ZF Yu, Y Zhang & KQ Zhang and *D. coccinella* Y Yang & XZ Liu (Yang and Liu 2005) were included.

DNA sequences were aligned using Clustalx 1.83. Cladistic analyses using the neighbor-joining method were performed with MEGA version 3.1. The neighbor-joining tree was constructed with Kimura 2-parameter model, including transitions and transversions and with pairwise deletion of gaps; bootstrap repetition was 500.

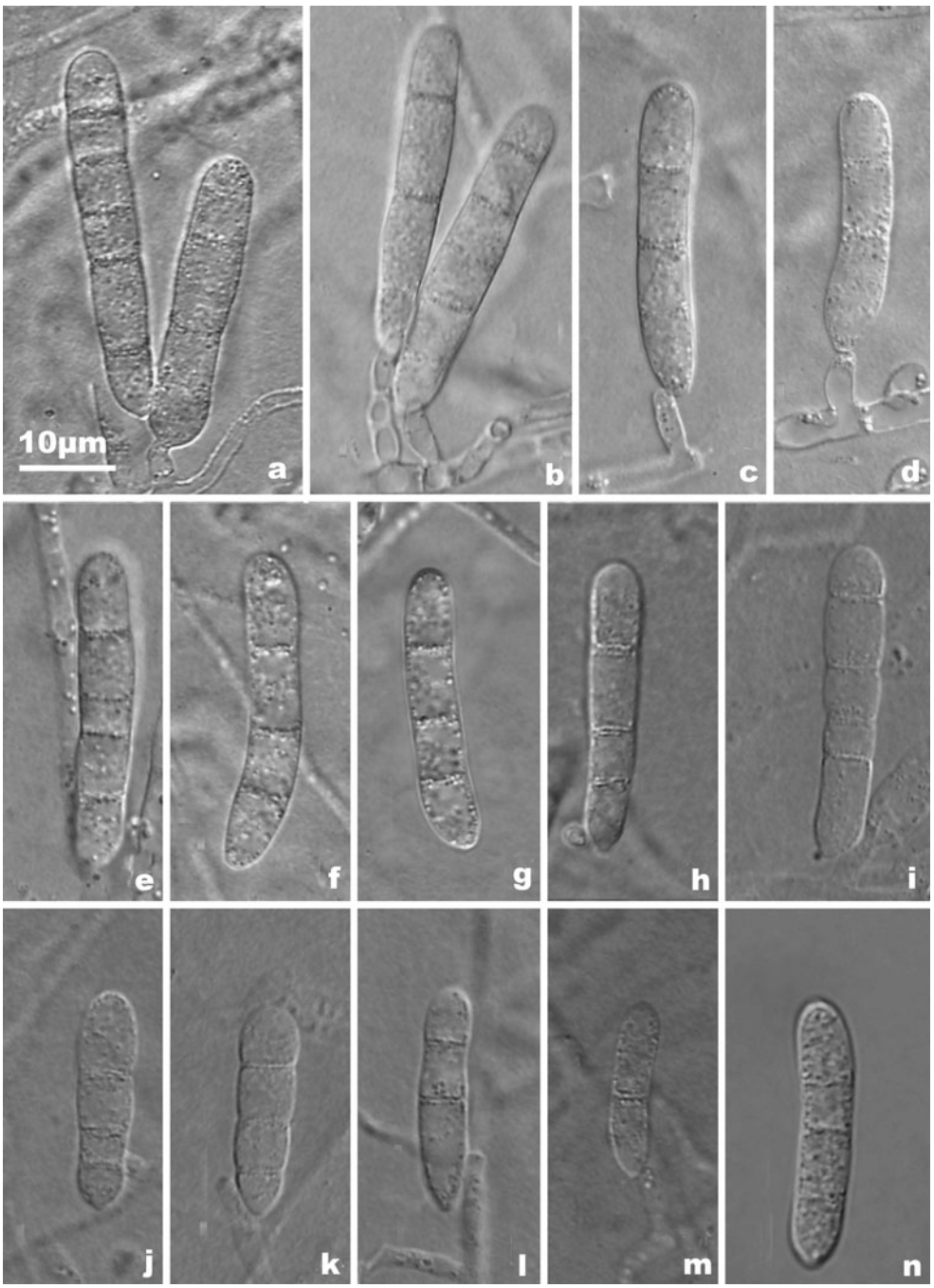

Fig. 2 *Dactylella pseudobrevistipitata*. Holotype YMF 1.03504. **a**–**d** Conidiophores with conidia. **e**–**n** Conidia

Results and discussion

A neighbor-joining tree was constructed based on 35 ITS sequences, which clustered into three different clades, *Dactylella*, *Vermispora* and *Brachyphoris* (Fig. 1). *D. pseudobrevistipitata* together with other three species, *D. tenuis* Drechsler, *D. vermiformis*, *D. coccinella*, formed a subclade within the *Dactylella* clade, which separated from most species of *Dactylella*. Among three species phylogenetically related to *D. pseudobrevistipitata*, *D. vermiformis* was most closely to *D. pseudobrevistipitata*, but the similarity of both ITS sequences was only 83%.

The conidiophores of *D. pseudobrevistipitata* were shorter than the conidia, which was in accordance with one of the basic characters of the *Brachyphoris* genus (Fig. 2). However, *D. pseudobrevistipitata* differed from all members of *Brachyphoris* in having cylindrical conidia. In the phylogenetic analysis based on ITS, it fell into the *Dactylella* clade with a high bootstrap number, so *D. pseudobrevistipitata* is the only species with shorter conidiophores than conidia in the present accepted species of *Dactylella* (Chen et al. 2007a). To discover whether the species with such short conidiophores should be separated from *Dactylella*, it will be necessary to collect other sibling species to carry out further investigations.

The phylogenetic analysis showed that *D. pseudobrevistipitata* clustaled with *D. vermiformis*, but their morphological characters were entirely different. The latter had branched conidiophores and 0–1 septate conidia. *D. pseudobrevistipitata* was most similar to *D. coccinella* in conidia shape, but the conidiophores of the latter were not longer than its conidia, but longer than those of *D. pseudobrevistipitata*. Furthermore, the conidia of *D. pseudobrevistipitata* were narrower than those of *D. coccinella*, and the latter had 1–7 septate conidia. Their ITS similarity was only 70%. The two species cannot trap nematodes.

Besides *D. coccinella*, *D. clavata* RH Gao, MH Sun and XZ Liu, *D. cylindrospora* (RC Cooke) A Rubner, *D. heptameres* Drechsler and *D. tenuis* were somewhat similar to *D. pseudobrevistipitata* in conidia shape. However, the first three were separated from *D. pseudobrevistipitata* phylogenetically, and the septate number of conidia of these species was different from *D. pseudobrevistipitata*: *D. clavata* was 1–7, *D. cylindrospora* was 3–7, *D. heptameres* was 3–6 and *D. tenuis* was 1–4.

Interestingly, in the subclade formed by *D. pseudobrevistipitata* and other three species, another two species, *D. vermiformis* and *D. coccinella*, were also isolated from teleomorphs; only *D. tenuis* has not been connected with teleomorph. Whether *Dactylella* spp. isolated from teleomorphs are separated from other *Dactylella* spp. phylogenetically, will need other isolations to confirm.

Dactylella pseudobrevistipitata L. Qin, M. Qiao & Z.F. Yu, sp. nov. Fig. 2

Coloniae in agaro CMA, post 15 dies 25°C 30 mm diam. Mycelium sparsum, hyphis septatis, 3–4 μm latis. Conidiophora erecta, simplices vel ramose, 2.0–11.3 μm longa, 2.5 μm lata ad basim, 1.9–3.2 μm lata ad apicem. Conidia hyalina, clavata, 13.3–39.0×3.5–5.6 μm, 1–5 septata.

Colonies white, growing slowly on PDA, reaching 50 mm at 25°C after 15 days. Colonies white, aerial mycelium sparse on CMA, reaching 30 mm at 25°C after 10 days. Vegetative hyphae hyaline, branched and septate, 3–4 μm wide. Producing a large number of conidia in natural light after 7 days at room temperature. Conidiophores septate, unbranched, initially erect, collapse on the agar surface in less than 2 days, 2.0–11.3 μm high, Conidia cylindrical, straight or slightly curved, smooth, 1–5 transverse septate, the proportion of conidia with 2, 3, or 4 septate is 13.3, 51.1 and 26.7% respectively, 13.3–39.0×3.5–5.6 μm. No traps and capture ability have been detected in pure cultures when induced by *P. redivivus*.

Etymology: The species was named after its short conidiophores, but the name of *Dactylella brevistipitata* was used in Liu et al. (2005), which was transferred to *Brachyphoris*, we adopted *Dactylella pseudobrevistipitata*.

Holotype: YMF1.03504, permanent slide, near Thermal Spring, Yuan-Jiang Country, Yunnan Province, P. R. China, alt. 500 m, collected by Li Qin on Sep. 18, 2009.

Acknowledgements This work was jointly financed by National Natural Science Foundation Program of PR China (30860004, 31060008), grants from the Young Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI020) and by Yunnan Province (2010CI106) and Research Project (2009DA002). Two anonymous reviewers are thanked for their detailed comments on this paper.

References

Chen J, Xu LL, Liu B, Liu XZ (2007a) Taxonomy of *Dactylella* complex and *Vermispora.* I. Generic concepts based on morphology and ITS sequences data. Fungal Divers 26:73–83

Chen J, Xu LL, Liu B, Liu XZ (2007b) Taxonomy of *Dactylella* complex and *Vermispora.* II. The genus *Dactylella*. Fungal Divers 26:85–126

Chen J, Xu LL, Liu B, Liu XZ (2007c) Taxonomy of *Dactylella* complex and *Vermispora.* III. A new genus *Brachyphoris* and revision of *Vermispora*. Fungal Divers 26:127–142

Drechsler C (1937) Some hyphomycetes that prey on free-living terricolous nematodes. Mycologia 29:447–552

Drechsler C (1950) Several species of *Dactylella* and *Dactylaria* that capture free-living nematodes. Mycologia 42:1–79

Grove WB (1884) New or noteworthy fungi. J Bot 22:195–201

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

Liu B, Liu XZ, Zhang WY (2005) A new species of *Hyalorbilia* and its anamorph from China. Nova Hedwig 81:145–155

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

Schenck S, Kendrick WB, Pramer D (1977) A new nematode-trapping species and a reevaluation of *Dactylaria* and *Arthrobotrys*. Can J Bot 55:977–985

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

Subramanian CV (1963) *Dactylella, Monacrosporium* and *Dactylina*. J Indian Bot Soc 42:291–300

Subramanian CV (1977) Revisions of hyphomycetes-I. Kavaka 5:93–98

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. Academic, San Diego, CA, pp 315–322

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

Yu ZF, Zhang Y, Qiao M, Baral HO, Weber E, Zhang KQ (2007a) *Drechslerella brochopaga*, the anamorph of *Orbilia (Hyalinia) orientalis*. Mycotaxon 96:163–168

Yu ZF, Qiao M, Zhang Y, Baral HO, Zhang KQ (2007b) *Orbilia vermiformis* sp. nov. and its anamorph. Mycotaxon 99:271–278