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A new *Myrmecridium* species from Guizhou, China

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Abstract — A strain of *Myrmecridium* was isolated from farmland soil in Guizhou Province, China. The strain produced distinctive obovoid or fusoid conidia that taper to a subtruncate darkened hilum at the base that distinguished it from other *Myrmecridium* species. Phylogenetic analysis of combined ITS1–5.8S–ITS2 and large subunit rDNA sequences supported this morphological separation. We describe *Myrmecridium obovoideum* as new and provide a key to *Myrmecridium* species.

Key words — asexual fungi, hyphomycetes, molecular phylogeny

Introduction

Arzanlou et al. (2007) established *Myrmecridium* Arzanlou et al. based on its hyaline mycelium with pale to unpigmented, pimple-like denticles that produce obovoid or fusiform conidia, tapering towards a narrowly truncate base with a slightly prominent unpigmented hilum and schizolytic conidial secession. The genus belongs in *Sordariomycetes* and comprises three species and two varieties (Arzanlou et al. 2007; Crous et al. 2011; Crous et al. 2012).

During a survey of anamorphic ascomycetes, we isolated a strain with characters of *Myrmecridium* (Arzanlou et al. 2007) from farmland soil in China.

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Combined ITS and LSU rDNA sequences were generated from the strain and analyzed to evaluate the morphological findings. Based on morphology and DNA sequence comparison, the strain is proposed as *Myrmecridium obovoideum*. In the present paper, we provide detailed illustrations and a description of this new species.

Materials & methods

Morphological and cultural studies

The *Myrmecridium* specimen — from a farmland soil sample collected from Huaxi District, Guizhou, China, in 2005 —is deposited in the herbarium of the Department of Plant Pathology, Shandong Agricultural University (HSAUP), and ex-type strains are conserved in HSAUP and the Herbarium of Plant Pathology, Guizhou University (HGUP). The specimen was examined in the laboratory and the taxon is described from cultures grown at 25°C on potato dextrose agar (PDA). Conidia and conidiophores were placed in a drop of 85% lactic acid and examined and photographed using a Nikon 80i microscope (Nikon Corporation, Japan) at 400× and 1000× magnification. The taxonomic determinations were made by comparing with descriptions in the literature.

DNA extraction, amplification and DNA sequencing

Genomic DNA was extracted from colonies grown on potato-dextrose agar (PDA), using the Fungal gDNA Kit GD2416 (Biomiga, CA, USA) following the manufacturer's instructions. The universal primers ITS1/ITS4 (White et al. 1990) were used for the ITS region (ITS1–5.8S–ITS2) amplification, and LR0R/LR5 were used for a segment of the large subunit rDNA (Vilgalys and Hester 1990). Amplification reactions were performed in a Bio-RAD PTC-200 thermocycler in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM $MgCl_2$, 0.25 mM of each dNTP, 0.5 U of Taq polymerase.

The PCR-amplified DNA fragments were fractionated in 1% agarose gels in 0.5× TBE buffer and visualized by ethidium bromide staining and UV illumination. Sequencing was performed with an ABI PRISM 3730 DNA autosequencer using either dRhodamine terminator or Big Dye Terminator chemistry (Life Technologies™, USA). Sequence data of the isolates used in the study were deposited in GenBank (Table 1). Alignments are available in TreeBASE (www.treebase.org/treebase-web/home.html) under the study ID 13733.

Phylogenetic analyses

Preliminary nucleotide sequence alignments were constructed using Clustal X 1.81 (Thompson et al. 1997). A partition homogeneity test (Farris et al. 1994) was applied to evaluate the feasibility of combining the datasets. Phylogenetic analyses of LSU sequences and combined ITS and LSU rDNA sequence were computed using MP analysis in PAUP* (Swofford 2002). In the MP analyses, trees were inferred using heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions, maxtrees were 5000, branches of zero length were collapsed and all parsimonious trees were saved. Measures calculated for parsimony

included tree length (TL), consistency index (CI), retention index (RI), and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were conducted with 1000 replications.

Results

Phylogenetic analysis

Partition homogeneity tests for combining the two (ITS and LSU) gene regions yielded a P-value of 0.104, and the two gene/regions were combined based on the tree topologies and P-value (Cunningham 1997, Dettman et

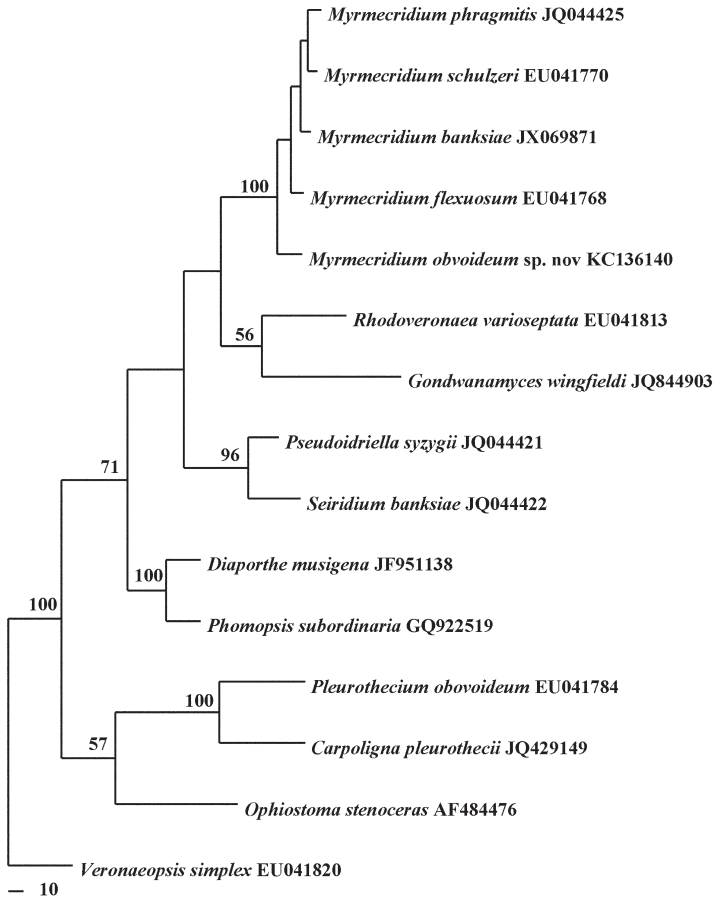

Fig. 1. One of the two equally most parsimonious trees of the analyzed ITS and LSU rDNA region/genes (284 of 1485 characters were parsimony informative). Bootstrap support values of less than 50% are not shown. The tree is rooted with *Pleurothecium obovoideum*.

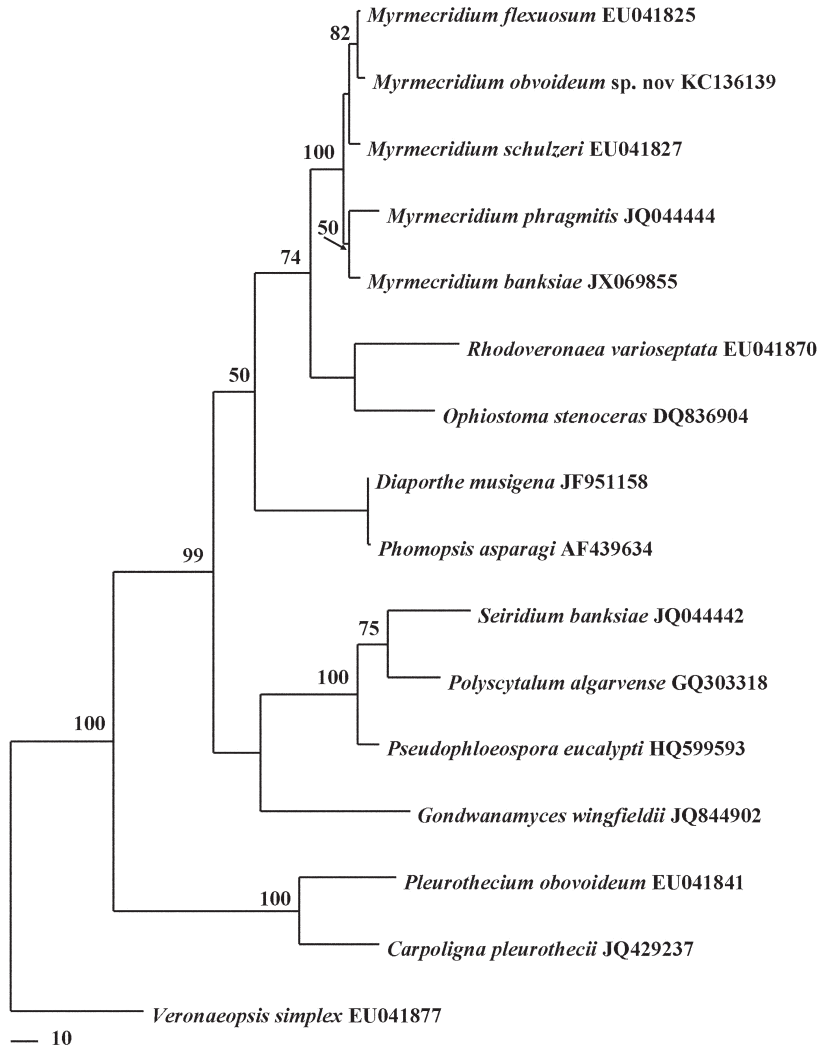

Fig. 2. The most parsimonious tree of the analyzed LSU rDNA gene (206 of 844 characters were parsimony informative). Bootstrap support values of less than 50% are not shown. The tree is rooted with *Veronaeopsis simplex*.

al. 2003). The aligned sequence data matrix contained five taxa (including the outgroup *Pleurothecium obovoideum*) and 1485 (546 ITS and 839 LSU) characters, of which 284 were parsimony informative. Two most parsimonious trees were obtained, with one chosen to represent the topology of the strict

consensus tree (Fig. 1; Tree Length (TL) = 380, CI = 0.956, RI = 0.467, HI = 0.044, and RC = 0.446). In this tree, five *Myrmecridium* species clustered together supported by a 100% bootstrap value. *Myrmecridium obovoideum* formed a single branch as the sister clade to the other four species with only 56% bootstrap support.

In order to evaluate the relationship of *Myrmecridium* with other ascomycetes, we also generated a parsimonious tree (Fig. 2) based on LSU rDNA gene region. The alignment with 16 taxa (*Veronaeopsis simplex* as outgroup) comprised 844 characters, of which 206 were parsimony-informative [Tree Length (TL) = 628, CI = 0.67, RI = 0.68, HI = 0.33, and RC = 0.45]. Only one most parsimonious tree was obtained to represent the topology of the strict consensus tree selected for presentation (Fig. 2). In this tree, the *Myrmecridium* group had a close relationship with *Rhodoveronaea* and *Ophiostoma* supported by a credible bootstrap value (74%), which was consistent with Arzanlou et al. (2007). However, the connection between *Rhodoveronaea* and *Ophiostoma* received weak support (<50%). Meanwhile, the placement of *M. obovoideum* was movable, because it showed a closer relationship with *M. flexuosum* (de Hoog) Arzanlou et al. supported by 82% bootstrap value, which differed from the analysis combined ITS and LSU rDNA gene regions.

Taxonomy

Myrmecridium obovoideum Jie, Y.L. Jiang, McKenzie & Yong Wang bis, **sp. nov.** Fig. 3

MycoBank MB 804082

Differs from other *Myrmecridium* species by its conidia with darkened hilum.

Type: China, Guizhou Province, Guiyang, Huaxi District, isolated from farmland soil, 5 November 2005, Y.L. Jiang (**Holotype**, HSAUP051001; **isotype**, HGUP0314; GenBank, KC136140, KC136139).

Etymology: in reference to the conidial shape of this new taxon.

Colonies on PDA at 25°C for 2 weeks reaching 30 mm diam. The surface effuse, grayish. Mycelium mostly superficial, partly immersed is contradictory with lacking aerial mycelium. Conidiophores straight to geniculate-sinuous, unbranched, subhyaline, 1–8-septate, ≤280 μm tall, 3–3.6 μm wide. Conidiogenous cells terminal, integrated, cylindrical, pale or reddish brown near the base, gradually becoming paler towards the apex, 50–210 μm, often with 1–6 thin septa, forming a rachis with scattered pimple-shaped denticles less than 1 μm long, sometimes fertile part slightly inflated. Conidia solitary, aseptate, subhyaline, pale brown, thin-walled, smooth to finely verrucose, surrounded by a wing-like gelatinous sheath, ≤1.5 μm thick, obovoid or fusoid, (7–)8–9(–10) × (3–)3.5(–4.2) μm, tapering to a subtruncate, slightly thickened hilum; darkened at rounded end.

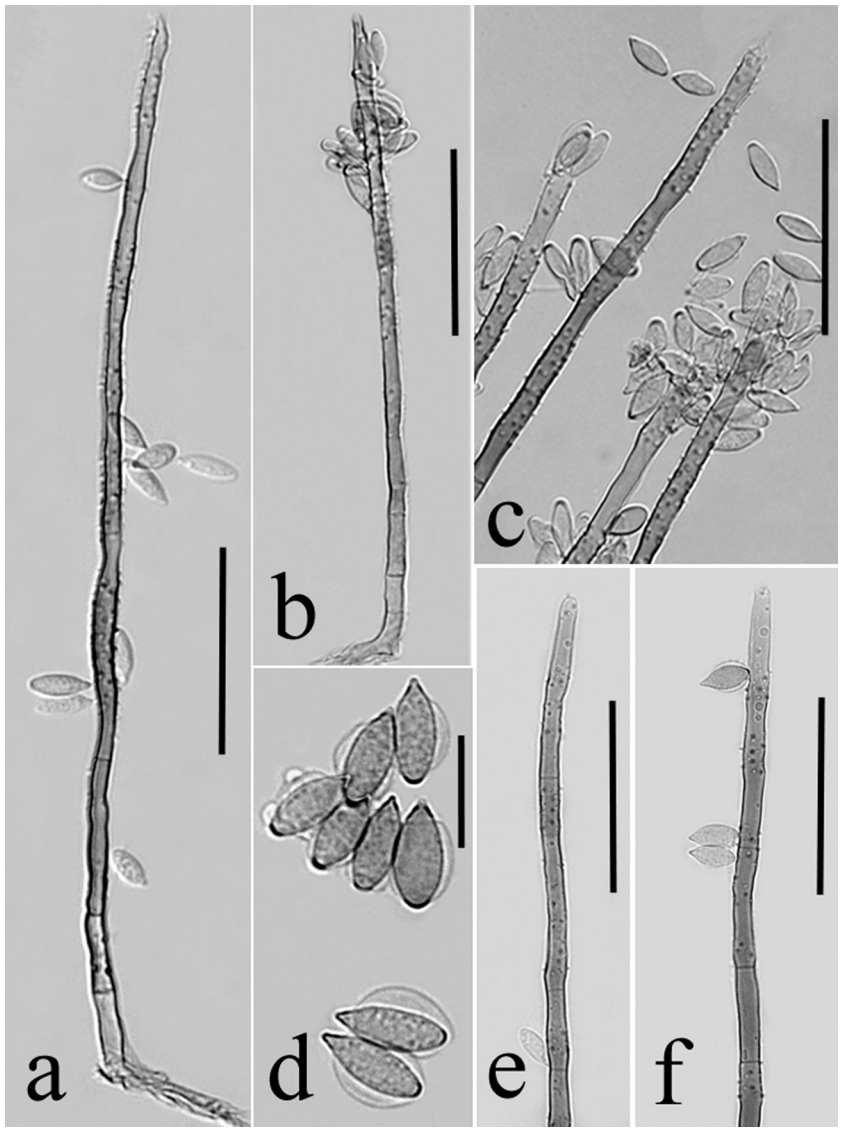

Fig. 3. *Myrmecridium obovoideum* (HSAUP051001, holotype) on PDA. a–b. Conidiophores. c. Rachis with pimple-shaped denticles and slightly inflated fertile part. d. Conidia surrounded by wing-like gelatinous sheaths. e–f. The thin septa of conidiogenous cells. Scale bars: a–c, e–f = 35 μm; d = 10 μm.

Discussion

Our LSU rDNA sequence analysis clustered *Myrmecridium* with *Rhodoveronaea* (sexual state = *Annulatascaceae*) and *Ophiostoma* (*Ophiostomataceae*) in the *Sordariomycetes*. Arzanlou et al. (2007) classified *Myrmecridium* as "*Incertae sedis* (*Sordariomycetes*)", and our results confirm this placement, without any indication of a family affinity.

Only *M. schulzeri* var. *tritici* (M.B. Ellis) Arzanlou et al. and *M. phragmitis* Crous produce septate conidia. *Myrmecridium banksiae* Crous differs from *M. obovoideum* by its larger conidia (9–14 × 2.5–3.5 µm); and *M. flexuosum* differs by its smaller conidia (5–9 × 3–4 µm). The conidia of *M. schulzeri* (Sacc.) Arzanlou et al. var. *schulzeri* (6–12 × 3–4 µm) are also larger than those of our species. In *Myrmecridium* only *M. obovoideum* has conidia with a darkened hilum, which enlarges the taxonomic criteria of *Myrmecridium* (Arzanlou et al. 2007). Our ITS and LSU rDNA sequence analyses also confirmed with high bootstrap value that our species differs from other *Myrmecridium* species. The combined morphological and phylogenetic analysis supports *M. obovoideum* as a new species.

Key to *Myrmecridium* species

1a. Hilum of conidia not darkened 2
1b. Hilum of conidia darkened . . . . . *M. obovoideum*
2a. Conidia without septate 3
2b. Septate conidia frequently observed. . . . . *M. schulzeri* var. *tritici*
3a. Conidia mostly less than 10 µm long 4
3b. Conidia up to 14 µm long . . . . . *M. banksiae*
4a. Conidia rounded at the base . . . . . *M. flexuosum*
4b. Conidia slightly acuminate at the base. . . . . *M. phragmitis*
4c. Conidia tapering to a subtruncate base . . . . . *M. schulzeri* var. *schulzeri*

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