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Akanthomyces araneogenum, a new *Isaria*-like araneogenous species

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Abstract

During a survey of araneogenous fungi from Guizhou Province, China, a new species, *Akanthomyces araneogenum*, was isolated from a spider, *Araneus* sp. It differs from other *Akanthomyces* species by its spider host, *Isaria*-like conidiogenous structure, and mostly globose and smaller conidia (1.6–2.2 µm). Multi-locus (*ITS*, *LSU*, *RPB1*, *RPB2* and *TEF*) phylogenetic analysis confirmed that *A. araneogenum* is distinct from other species. The new species is formally described and illustrated, and compared with similar species.

Keywords: *Isaria*-like, morphology, phylogeny, spider

Introduction

Araneogenous or araneopathogenic fungi are spider-pathogenic fungi (Evans & Samson 1987). They have distinctive bioactive compounds due to their specific nutritional preference and unique hosts that show great potential for applications in medicine and health (Humber 2008, Molnár *et al*. 2010, Chen *et al*. 2017). These bioactive compounds include cyclopeptides (Lang *et al*. 2005), alkaloids (Isaka *et al*. 2010, 2013, Fukuda *et al*. 2014), carboxamide derivatives (Helaly *et al*. 2017), and especially exo-biopolymers, which have great potential for application development (Madla *et al*. 2005, Prathumpai *et al*. 2012).

Evans (2013) reported that araneogenous fungi include the sexual genera *Cordyceps* sensu lato and *Torrubiella* Boud., and the asexual genera *Akanthomyces* Lebert, *Clathroconium* Samson & H.C. Evans, *Gibellula* Cavara, *Granulomanus* de Hoog & Samson, *Hirsutella* Pat., *Hymenostilbe* Petch, *Isaria* Pers., *Lecanicillium* W. Gams & Zare, and *Nomuraea* Maubl. Quandt *et al*. (2014) transferred *Nomuraea atypicola* (Yasuda) Samson to the genus *Purpureocillium* Luangsa-ard, Hywel-Jones, Houbraken & Samson and named it *P. atypicola* (Yasuda) Spatafora, Hywel-Jones & Luangsa-ard. Chen *et al*. (2016, 2017) reported two genera, *Beauveria* Vuill. and *Clonostachys* Corda as araneogenous. Kepler *et al*. (2017) synonymsed *Granulomanus* with *Gibellula*, transferred *Torrubiella* and *Lecanicillium* to *Akanthomyces*, and *Isaria* to *Cordyceps*, and proposed a new genus, *Hevansia* Luangsa-ard, Hywel-Jones & Spatafora to accommodate *Akanthomyces novoguineensis* Samson & B.L. Brady. To date, known araneogenous fungi genera include *Cordyceps*, and its related anamorphic genera *Akanthomyces*, *Beauveria*, *Clathoconium*, *Clonostachys*, *Gibellula*, *Hevansia*, *Hirsutella*, *Hymenostibe*, *Nomuraea*, and *Purpureocillium*.

Akanthomyces was established by Lebert in 1858 to accommodate *A. aculeatus* Leb., which was found in France. The genus was emended and revised by Mains (1950). Several new species were reported later (Samson & Evans 1974, Koval 1977, Vincent *et al*. 1988, Hywel-Jones 1996, Hsieh *et al*. 1997, Huang *et al*. 2000, Kepler *et al*. 2017, Mongkolsamrit *et al*. 2018). Currently, the genus *Akanthomyces* consists of 19 species, and has been isolated from soil, insects and spiders. Twelve species have a spider host: *A. arachnophilus* (Petch) Samson & H.C. Evans, *A. aranearum* (Petch) Mains, *A. cinereus* Hywel-Jones, *A. kanyawimiae* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, *A. koratensis* Hywel-Jones, *A. lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha, *A. longisporus* B. Huang, S.B. Wang, M.Z. Fan & Z.Z. Li, *A. ovalongatus* L.S. Hsieh, Tzean & W.J. Wu, *A. sulphureus* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, *A. thailandicus* Mongkols., Spatafora & Luangsa-ard, *A. waltergamsii* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, and *A. websteri* Hywel-Jones.

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During a survey of araneogenous fungi from Guizhou Province, China, we isolated *Akanthomyces* with spider hosts. Morphological and molecular phylogenetic analysis suggested that these isolates were a new species, which is described here as *Akanthomyces araneogenum sp. nov*.

Materials & methods

Specimen collection and isolation

Fungus infected spiders were collected from Guizhou Province, China, in October 2015. The strain GZUIF DX2 was obtained from a naturally infected spider specimen (GZU201510311) by culturing on improved potato dextrose agar (PDA, 1% w/v peptone) medium.

Strain culture and identification

The isolated strain was incubated on Sabouraud's dextrose agar and PDA at 25 °C for 14 days. The morphological characteristics of the strain were examined using classical mycological techniques based on growth rate, and macroscopic and microscopic characteristics. The ex-type culture and a dried-culture of the holotype specimen are deposited in GZAC, Guizhou University, Guiyang, China.

DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out according to Liang *et al*. (2009). The extracted DNA was stored at −20 °C. Amplification of large subunit ribosomal RNA (*LSU*) genes was performed with NS1-1/AB28 primers (Curran *et al*. 1994). Translation elongation factor 1 alpha (*TEF*) and RNA polymerase II largest subunit 2 (*RPB2*) were amplified according to van den Brink *et al*. (2012). RNA polymerase II largest subunit 1 (*RPB1*) was amplified with the primer pair CRPB1 and RPB1-Cr (Castlebury *et al*. 2004). The internal transcribed spacer (*ITS*) region was amplified by PCR according to the procedures described by White *et al*. (1990). PCR products were purified using the UNIQ-10 column PCR products purification kit [no. SK1141; Sangon Biotech (Shanghai) Co., Shanghai, China] according to the manufacturer's protocol and sequenced at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

Sequence alignment and phylogenetic analyses

The DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of *ITS*, *LSU* rRNA, *RPB1*, *RPB2* and *TEF* were based on Kepler *et al*. (2017) and Mongkolsamrit *et al*. (2018). Multiple sequence alignments for *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* were carried out using MAFFT v7.037b (Katoh *et al*. 2013). Sequence editing was performed with MEGA6 (Tamura *et al*. 2013) and the resulting output was in Fasta file format. The concatenated *ITS+LSU+RPB1+RPB2+TEF* sequences were assembled by SequenceMatrix 1.7.8 (Vaidya 2011). Gene concordance was assessed with the 'hompart' command in PAUP4.0b10 (Swofford 2002).

The combined data set of five genes was analyzed phylogenetically using Bayesian MCMC and maximum likelihood (ML). For the Bayesian analysis, two runs were executed simultaneously for 10,000,000 generations, saving trees every 500 generations, with the GTR+G nucleotide substitution model across all partitions, in MrBayes 3.2 (Ronquist *et al*. 2012). After the analysis was finished, each run was examined with the program Tracer v1.5 (Drummond & Rambaut 2007) to determine burn-in and confirm that both runs had converged. For the ML analysis in RAxML (Stamatakis 2014), the GTRGAMMA model was used for all partitions, in accordance with recommendations in the RAxML manual against the use of invariant sites. The analyses were performed using the CIPRES web portal (Miller *et al*. 2010). The final alignment is available from TreeBASE under submission ID 23389.

Results

Sequencing and phylogenetic analysis

The *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* sequences from strain GZUIF DX2 were deposited in GenBank with accession numbers KU893153, MH978179, MH978182, MH978185 and MH978187, respectively. The concatenated alignment

of *ITS+LSU+RPB1+RPB2+TEF* sequences was 2080 bp long. The three sets of sequences, from strains GZUIF DX1, GZUIF DX2 and GZUIF SN1, formed a clade in both ML and Bayesian analyses (Fig. 1).

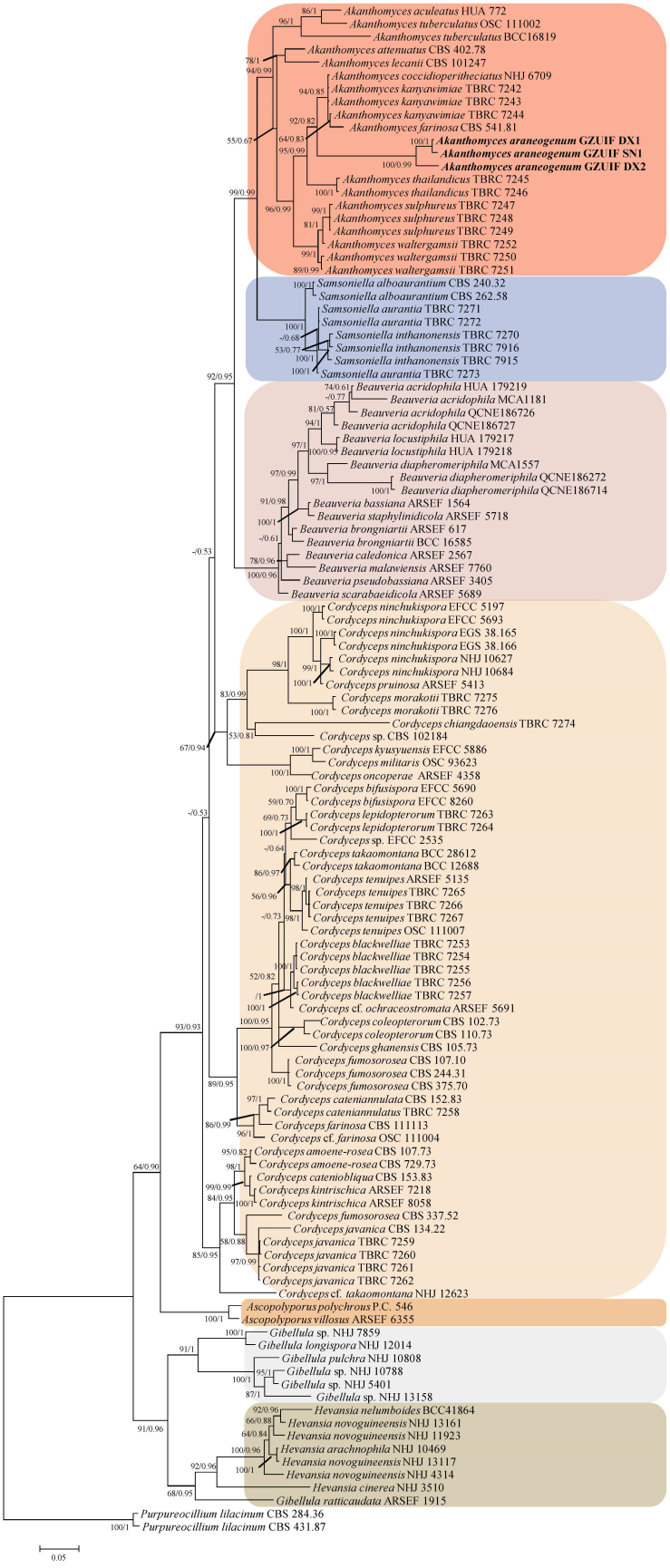

FIGURE 1. Phylogenetic analysis of *Akanthomyces araneogenum* (strains GZUIF DX1, GZUIF DX2, GZUIF SN1) and related species based on combined partial *ITS+LSU+RPB1+RPB2+TEF* sequences. Statistical support values (≥50 %) are shown at nodes, and presented as bootstrap values/Bayesian posterior probabilities.

Taxonomy

Akanthomyces araneogenum Z.Q. Liang, W.H. Chen & Y.F. Han, *sp. nov.* (Fig. 2)

Mycobank No.: MB816114

Type:—CHINA. Guizhou Province: Guiyang City, Tonguing (N 26°23′25.92″, E 106°41′3.35″), on the spider *Araneus* sp. in pinewood, 31 October 2015, Wanhao Chen, holotype GZU201510311, ex-type culture GZUIF DX2.

Spider host covered by white mycelial, numerous conidiophores arise in a dense layer, occasionally several white synnemata arise from all parts of the host. Hyphae septate, hyaline, smooth-walled, 1.4–2.2 µm wide. Conidiophores mononematous or synnematous, hyaline, smooth-walled, 21.6–48 × 1.2–2.2 µm, penicillium-like from hyphae directly. Phialides consisting of a cylindrical, somewhat inflated base, 4.3–17.3 × 0.9–3.1 µm, tapering to a thin neck. Conidia hyaline, smooth-walled, globose, 1.3–2.4 µm in diam, or ellipsoid, 2.1–3.3 × 1.1–1.6 µm, forming divergent basipetal chains.

Colony on Czapek agar, attaining a diameter of 31 mm after 14 days at 25 °C, white to yellowish, powdery, thin, cracked; reverse yellowish. Hyphae septate, hyaline, smooth-walled, 2.2–3.2 µm wide. Conidiophores mononematous, hyaline, smooth-walled, 78–121 × 2.2 µm, with single phialide or whorls of 2–3 phialides, or penicillium-like from hyphae directly. Phialides consisting of a cylindrical, somewhat inflated base, 12–17 × 1.1–3.2 µm, tapering to a thin neck. Conidia hyaline, smooth-walled, mostly globose, 1.6–2.2 µm in diam, or ellipsoid, 2.2–3.3 × 1.1 µm, forming divergent and basipetal chains.

Etymology:—referring to the fungus that is colonizing a host spider.

Additional specimens examined:—CHINA. Guizhou Province: Guiyang City, Tongmuling (N 26°23′25.92″, E 106°41′3.35″), on the spider *Araneus* sp. in pinewood, 31 October 2015, Wanhao Chen (GZUIF DX1). Sequences from this strain have been deposited in GenBank with accession numbers: MH978178=*LSU rRNA*, MH978181=*RPB1*, MH978184=*RPB2*, KU893152=*ITS rRNA*. CHINA. Guizhou Province: Guiyang City, Longdongbao (N 26°33′26.20″, E 106°45′12.00″), on the spider *Araneus* sp. in pinewood, 6 October 2015, Wanhao Chen (GZUIF SN1). Sequences from this strain have been deposited in GenBank with accession numbers: MH978188=*TEF*, MH978180=*LSU rRNA*, MH978183=*RPB1*, MH978186=*RPB2*, MH978177=*ITS rRNA*.

Known distribution:—Tongmuling and Longdongbao, Guiyang, Guizhou Province, China.

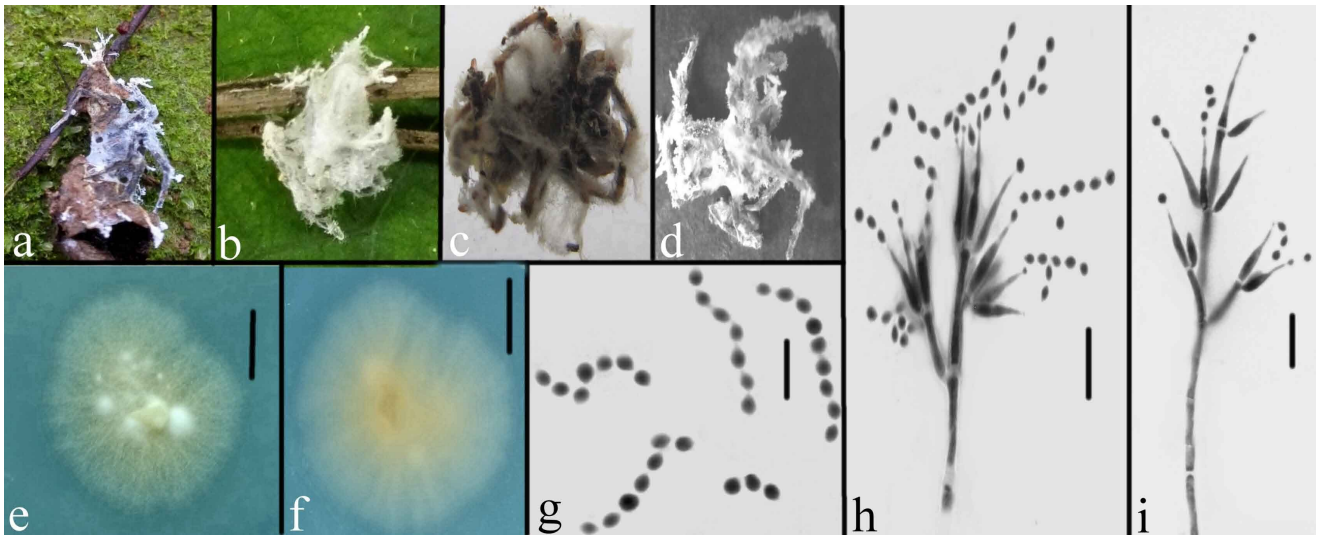

FIGURE 2. *Akanthomyces araneogenum* (holotype GZU201510311) a–d. Infected spider host. e, f. Colony (top and reverse view, respectively) on the Czapek agar medium after 14 d at 25 °C. g. Conidia. h, i. Conidiophores, conidiogenous cells and conidia on Czapek agar medium. Scale bars: e, f =10 mm, g, h, i=10 µm.

Discussion

As originally described, the typical characters of *Akanthomyces* are white, cream or flesh-coloured cylindrical, and/or attenuated synnemata covered with a hymenium of phialides. The conidiogenous cells are ellipsoidal, cylindrical,

or narrowly cylindrical and gradually or abruptly tapering to a more or less distinct neck. Conidia are unicellular, hyaline, in short or long chains (Lebert 1858, Mains 1950, Vincent *et al*. 1988, Hsieh *et al*. 1997). The morphological characters of strain GZUIF DX2 suggest it could be include in either *Akanthomyces* or *Isaria*. Mongkolsamrit *et al*. (2018) indicated that *Isaria* shares morphological characters with other genera, which has resulted in a turbulent taxonomic history, and reported some *Isaria*-like *Akanthomyces* species. Additionally, both genera have spider hosts. Strain GZUIF DX2 can be easily distinguished from *Akanthomyces* and *Isaria* species by its *Isaria*-like conidiogenous structure with mostly globose and smaller conidia (1.6–2.2 μm). Thus, morphological characters suggested that strain GZUIF DX2 is a new species of *Akanthomyces*.

Analyses of concatenated *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* sequences produced ML and Bayesian trees that were largely congruent. Most branches were strongly supported in both analyses. The three strains of *A. araneogenum* clustered with other *Akanthomyces* spp., and not with *Isaria* spp. (now treated as *Cordyceps* spp.), which supported the results of morphological analysis. The three strains of *A. araneogenum* clustered together, distinct from other *Akanthomyces* species. Thus, both molecular phylogenetic results and the morphology support description of the new species, *A. araneogenum*.

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