



## *Lecanicillium araneogenum* sp. nov., a new araneogenous fungus

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### Abstract

During a survey of araneogenous fungi from Guiyang, Guizhou, China, a new species, *Lecanicillium araneogenum*, was isolated from a spider. It differs from other *Lecanicillium* species by its spider host, and cylindrical conidia. Multi-locus (*TEF*, *RPB1*, *RPB2*, *LSU* and *SSU rRNA*) phylogenetic analysis confirmed that *L. araneogenum* is distinct from other species. The new species is formally described and illustrated, and compared to similar species.

**Key words:** morphology, nutritional preferences, phylogeny, spider

### Introduction

Araneogenous or araneopathogenic fungi are fungi that live on, or are pathogenic to spiders (Evans & Samson 1987). These fungi receive a rich source of structurally diverse metabolites from their hosts, which contributes to the frequency of host infestation as well as the ability of these fungi to outcompete potential competitors (Molnár *et al.* 2010, Humber 2008).

The known araneogenous fungi belong to phylum Ascomycota, historically in the order Clavicipitales (now a junior synonym of Hypocreales), including genera such as *Cordyceps* sensu lato, and the asexual fungi (Evans 2013), *Akanthomyces* (Lebert 1858), *Clathroconium* (Samson & Evans 1982), *Granulomanus* (de Hoog 1978), *Hirsutella* (Patouillard 1892), *Hymenostilbe* (Petch 1931), *Isaria* (Persoon 1794), *Lecanicillium* (Gams & Zare 2001), *Nomuraea* (Maublanc 1903), and *Gibellula* (Cavara 1894).

The genus *Lecanicillium* has a wide range of hosts, including arthropods, nematodes, plants and fungi (Zare & Gams 2001). *Lecanicillium* spp. have potential for development as biological control agents effective against several plant diseases, pest insects and plant parasitic nematodes (Goettel *et al.* 2008). There are four species with spider hosts, *L. araneorum* (Petch) Zare & W. Gams, *L. araneicola* Sukarno & Kurihara, *L. lecanii* (Zimm.) Zare & W. Gams and *L. tenuipes* (Petch) Zare & W. Gams.

During a recent survey of araneogenous fungi from Guiyang, Guizhou, China, we isolated a *Lecanicillium* strain infecting a spider. The morphology and molecular phylogenetic analysis suggest that it is a new species. It is described here as *Lecanicillium araneogenum* sp. nov.

### Materials and methods

#### *Specimen collection and isolation*

A fungus infected spider specimen (GZU201510314) was collected from a pinewood in Tongmuling, Guiyang city (N 26°23'25.92", E 106°41'3.35"), in October 2015. Strain GZU1031Lea was isolated from this specimen, cultured on an agar plate containing improved potato dextrose agar (PDA, 1 % w/v peptone) medium.

#### *Strain culture and identification*

The isolated fungus was incubated on Sabouraud's dextrose agar and PDA at 25 °C for 14 d. Macroscopic and

microscopic morphological characteristics of the fungus were examined using classical mycological techniques and growth rate was determined. The ex-type culture and a dried culture holotype specimen are deposited in GZAC, Guizhou University, Guiyang.

#### **DNA extraction, PCR amplification and nucleotide sequencing**

DNA extraction was carried out according to Liang *et al.* (2009). The extracted DNA was stored at  $-20^{\circ}\text{C}$ . Amplification of small subunit ribosomal RNA (*SSU*) and large subunit ribosomal RNA (*LSU*) genes was performed with NS1/NS4 primers (White *et al.* 1990) and NS1-1/AB28 primers (Curran *et al.* 1994), respectively. Translation elongation factor 1 alpha (*TEF*) was amplified with forward primer 5'-GCCCCCGGCCATCGTACTTCAT-3' and reverse primer 5'-ATGACACCGACAGCGACGGTCTG-3' (van den Brink *et al.* 2012). Amplification of RNA polymerase II largest subunit 1 (*RPB1*) was with the primer pair CRPB1 and RPB1-Cr (Castlebury *et al.* 2004). For the amplification of RNA polymerase II largest subunit 2 (*RPB2*), the forward primer 5'-GACGACCGTG ATCACTTTGG-3' and the reverse primer 5'-CCCATGGCCTGTTTGCCCAT-3' were used (van den Brink *et al.* 2012). PCR products were purified using the UNIQ-10 column PCR Products Purification kit (no. SK1141; Sangon Biotech Co., Shanghai, China) according to the manufacturer's protocol, and sequenced with the above primers at Sangon Biotech. The resultant sequences of GZU1031Lea were submitted to GenBank.

#### **Sequence alignment and phylogenetic analyses**

DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of *TEF*, *LSU rRNA*, *RPB1*, *RPB2* and *SSU rRNA* from 22 taxa (21 *Lecanicillium* isolates and one *Simplicillium lanosoniveum* strain as outgroup), based on Zare & Gams (2001), Sukarno *et al.* (2009), Kaifuchi *et al.* (2013), Park *et al.* (2015), and Chirivi-Salomón *et al.* (2015) were downloaded from GenBank. Multiple sequence alignments for *TEF*, *LSU rRNA*, *RPB1*, *RPB2*, and *SSU rRNA* were constructed and carried out using MAFFT (Katoh & Standley 2013) with the default settings. Manual editing of sequences was performed in MEGA6 (Tamura *et al.* 2013). The concatenated sequences (*TEF+LSU+RPB1+RPB2+SSU*) were assembled using SequenceMatrix1.7.8 (Vaidya *et al.* 2011). Concordance between genes was assessed using the 'hompert' command of PAUP4.0b10 (Swofford 2002).

The combined data set of five genes was analyzed phylogenetically using Bayesian MCMC and Maximum Likelihood. For the Bayesian analysis, two runs were executed simultaneously for 10 000 000 generations, saving trees every 500 generations, with the GTR+I+G nucleotide substitution model across all partitions, in MrBayes 3.2 (Ronquist *et al.* 2012). After the analysis 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. Analyses were performed using the CIPRES web portal (Miller *et al.* 2010). The final alignment is available from TreeBASE under submission ID 19856.

## **Results**

#### **Sequencing and phylogenetic analysis**

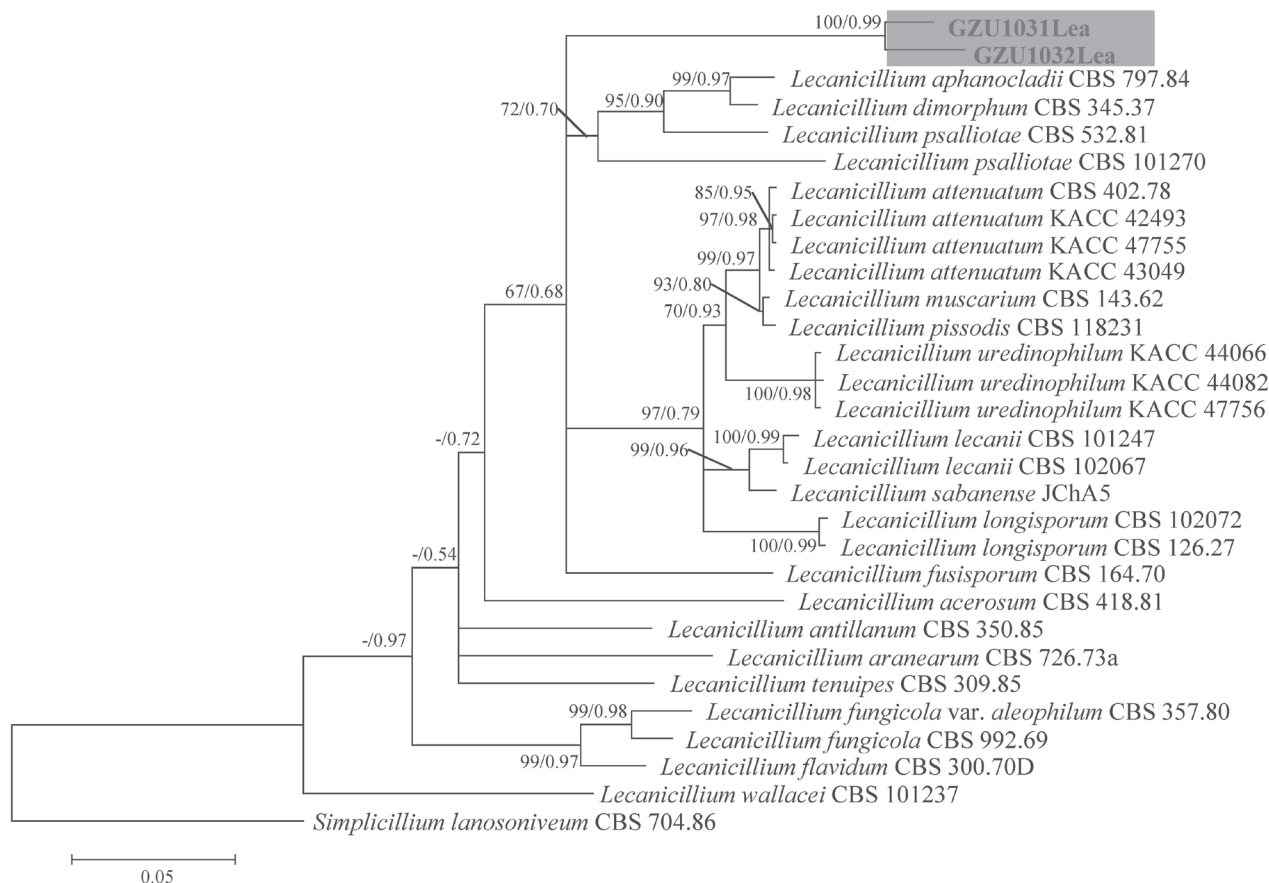
Sequences of *TEF*, *SSU rRNA*, *LSU rRNA*, *RPB1* and *RPB2* from strain GZU1031Lea were deposited in GenBank with accession numbers KX845697, KX845705, KX845703, KX845699 and KX845701, respectively. The concatenated alignment of *TEF+LSU+RPB1+RPB2+SSU* sequences was 2963 bp long. The two sets of sequences, from strains GZU1031Lea and GZU1032Lea, formed a clade in both ML and Bayesian analyses (Fig. 1).

## **Taxonomy**

*Lecanicillium araneogenum* W.H. Chen, Y.F. Han, J.D. Liang, Z.Q. Liang & D.C. Jin *sp. nov.* (Fig. 2)

Mycobank No.: MB818288

**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 GZU201510314, ex-type culture GZAC GZU1031Lea.



**FIGURE 1.** Phylogenetic analysis of strains GZU1031Lea, GZU1032Lea and related species based on combined partial *TEF*+*LSU*+*RPB1*+*RPB2*+*SSU* sequences. Statistical support values ( $\geq 50\%$ ) are shown at nodes, for maximum likelihood/Bayesian method.

Colonies on PDA 32 mm in diameter after 14 days at 25 °C, white to light grey, cottony, margin irregular; reverse light yellow. Odour indistinct. Vegetative hyphae 0.9–1.3  $\mu\text{m}$  wide, smooth-walled. Conidiophores usually arising from aerial hyphae, moderately branched. Phialides 30–64  $\times$  1.1–3.2  $\mu\text{m}$ , produced in whorls of (1–)2–6 (–8) on the conidiophores. Conidia forming mostly globose heads, 3.2–8.6  $\times$  1.3–1.6  $\mu\text{m}$ , cylindrical, aseptate, smooth-walled. In culture both phialides and conidia are of similar general shape and size to those found on spiders.

**Etymology:**—referring to a new fungus which has the ability to colonize the 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 (GZAC GZU1032Lea). Sequences from this strain have been deposited in GenBank with accession numbers: KX845698=*TEF*, KX845704=*LSU rRNA*, KX845670=*RPB1*, KX845702=*RPB2*, KX845706=*SSU rRNA*.

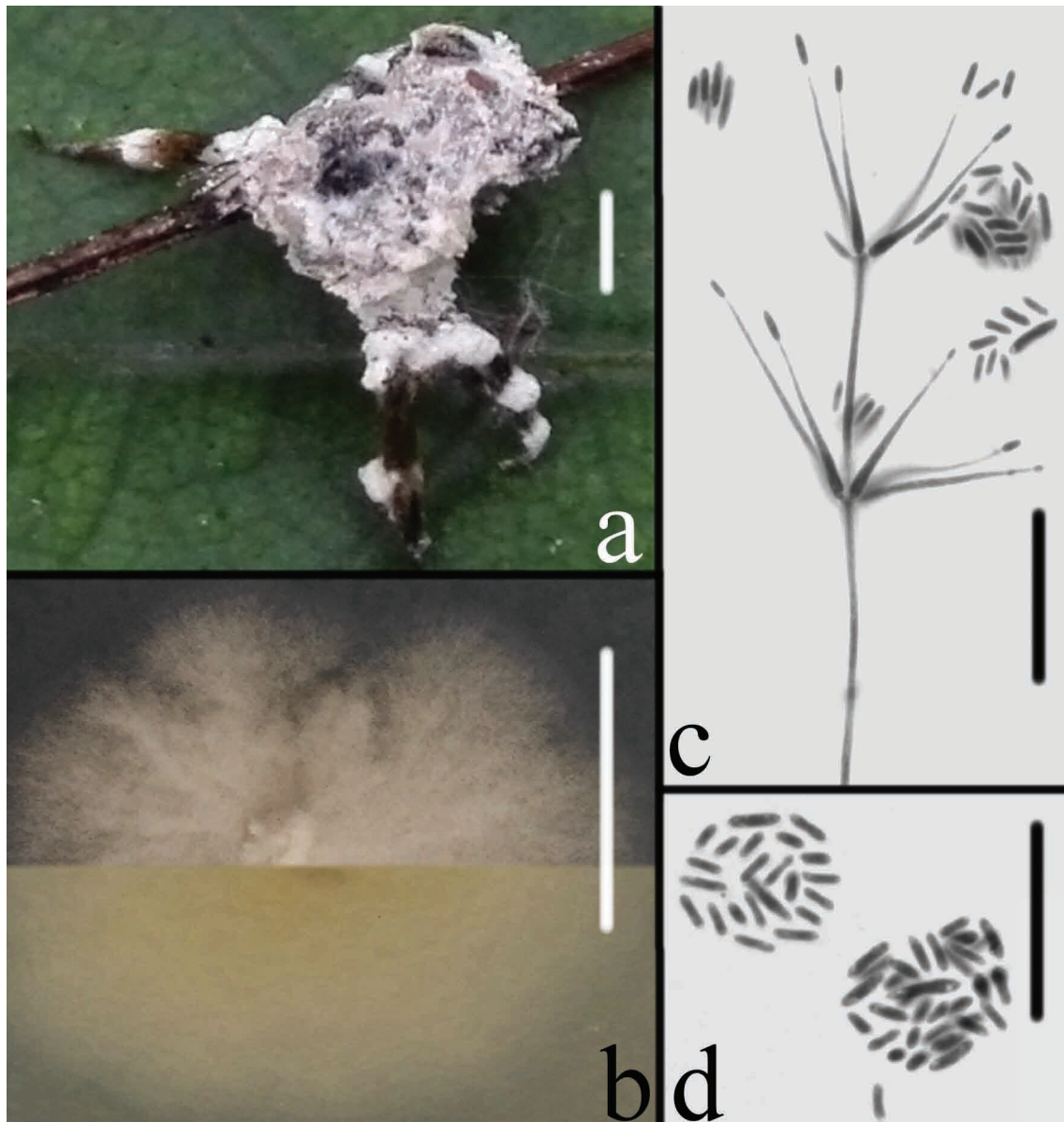
**Known distribution:**—Tongmuling, Guiyang, Guizhou Province, China.

## Discussion

As originally described by Zare & Gams (2001), the main taxonomic criteria for the genus *Lecanicillium* are: conidiophores that typically arise from aerial hyphae; erect short conidiophores bearing one or two whorls of phialides, in prostrate conidiophores numbers of phialides whorls or single phialides practically unlimited; phialides verticillate or solitary; conidia adhering in slimy heads or fascicles. On the basis of these characteristics, strains GZU1031Lea clearly belong to *Lecanicillium*. Four species in this genus have been reported from spider hosts (Evans 2013); strain GZU1031Lea can be distinguished from these other species by its distinctly cylindrical conidia (3.2–8.6  $\times$  1.3–2.2  $\mu\text{m}$ ). Strain GZU1031Lea is similar to *L. uredinophilum* in having cylindrical, oblong, or ellipsoid conidia (3–9  $\times$  1.8–3  $\mu\text{m}$ ), but the host of *L. uredinophilum* is a rust fungus, *Coleosporium* sp. (Spatafora *et al.* 2007). Thus, morphological

characters suggest that strain GZU1031Lea is a new species in the genus *Lecanicillium*, and it is described here as *L. araneogenum*.

Phylogenetic analyses of *Lecanicillium* have previously been based on the *ITS* region of ribosomal RNA (Zare & Gams 2001, Sukarno *et al.* 2009), *SSU* rRNA (Zare & Gams 2008, Kaifuchi *et al.* 2013), and combinations of *SSU* rRNA, *LSU* rRNA, *TEF*, *RPB1* and *RPB2* (Park *et al.* 2015, Chiriví-Salomón *et al.* 2015). In the present study, concatenated analyses of *SSU* rRNA, *LSU* rRNA, *TEF*, *RPB1* and *RPB2* produced ML and Bayesian trees that were largely congruent. The majority of branches were strongly supported in both analyses. The two strains of *Lecanicillium araneogenum* clustered together, distinct from other *Lecanicillium* species. Thus, molecular phylogenetic results supported the morphologically based conclusion that strain GZU1031Lea is a new species in the genus *Lecanicillium*, described here as *L. araneogenum*.



**FIGURE 2.** *Lecanicillium araneogenum* (Holotype GZU201510314) a. Infected spider. b. Colony (top and reverse view) on PDA after 14 d at 25°C. c. Verticillate conidiogenous cells on the conidiophores. d. Conidia forming mostly globose heads. Scale bars: a, b = 10 mm, c, d = 10  $\mu$ m.

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