A new araneogenous fungus in the genus *Beauveria* from Guizhou, China

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**ABSTRACT**

*Beauveria araneola* sp. nov., a fungus parasitic on spiders, was isolated from a spider at the Experimental Farm of the Institute of Entomology, Guizhou University, China; and described with morphological and phylogenetic evidences. This species differs morphologically from other species in the genus by its long slender denticulate rachis, cylindrical to ellipsoid conidiogenous cells, and ellipsoidal to globose conidia. Phylogenetic analyses based on three-locus (*TEF*, *RPB1* and *Boc*) data strongly support the distinction of this fungus within the genus. Based on the phylogenetic results, *B. araneola* shares some pleiomorphic traits with soil-borne or entomogenous members of the genus, and is likely to have jumped from soil or insect hosts to spider.

**Key words:** *Beauveria*, host shift, morphology, phylogeny, spider

**Introduction**

Araneogenous or araneopathogenic fungi are fungi that attack spiders (Evans & Samson 1987). Their distinctive nutritional requirements for particular metabolites lead to specific host preferences. Recent studies of these fungi have been focused on bioactive compounds with fungistatic and bacteriostatic activities (Wipapat *et al.* 1998; Lee *et al.* 2005; Kuephadungphan *et al.* 2013; Bunbamrung *et al.* 2015), as well as new compounds including cyclic tetrapeptides (Lang *et al.* 2005), ergosterols and isariotin analogs (Asia *et al.* 2012), and alkaloids (Isaka *et al.* 2010, 2013). Biocompatible polymers have received particular attention (Madla *et al.* 2005).


*Beauveria* is one of the most ubiquitous anamorphic genera of entomopathogenic fungi. Members of *Beauveria* have branched, penicillate or trichodermoid conidiophores. Dense clusters of sympodial and globose or flask-shaped short conidiogenous cells, with an apical denticulate rachis, form on conidiophores and give rise to single-celled, hyaline conidia. Conidial shape in *Beauveria* can be globose, ellipsoidal, cylindrical, or comma-shaped (Chen *et al.* 2013; Rehner *et al.* 2011). The hosts of *Beauveria* species cover Heteroptera, Homoptera, Lepidoptera, Coleoptera, Hymenoptera, Diptera, Orthoptera, Siphonaptera, Mantodea, Thysanoptera, Neuroptera, Blattariae or Embioptera; only 13 species have acarina hosts, which belong to seven genera across six families (Zimmermann 2007; Li 1988).

Recently, while screening for acaropathogenic fungi in Guiyang, Guizhou, China, we isolated a *Beauveria* strain from a spider. Based on a combination of morphological characteristics and phylogenetic analysis, we conclude that it represents a new species, and describe it as *Beauveria araneola*.
Materials & methods

Collection of specimens and isolation
The fungus-infected spider specimen (GZU20150317) was collected in March 2015, from a vegetable field at the Experimental Farm of Guizhou University, Guiyang, Guizhou Province, China. A fungal strain GZU0317bea was isolated from the infected spider, on improved PDA with 1 % w/v peptone.

Strain culture and identification
The strain GZU0317bea was incubated on Sabouraud’s dextrose and PDA at 25 °C for 14 days. Morphological characteristics of the fungus were recorded using classical mycological techniques for growth rate as well as macroscopic and microscopic characteristics. The type culture and a dried ex-holotype culture (specimen) are deposited in GZAC, Guizhou University, Guiyang.

DNA extraction, PCR amplification and nucleotide sequencing
DNA extraction was performed according to Liang et al. (2009). The extracted DNA was stored at -20 °C. RNA polymerase II the largest subunit (RPB1) (Castlebury et al. 2004), Bloc loci (Rehner et al. 2006), and Translation elongation factor 1 alpha (TEF) (Houbraken et al. 2007) loci were amplified by polymerase chain reaction (PCR) according to the respective methods in the above references cited for each locus. Taq polymerase and dNTPs were obtained from Shanghai Tiangen. PCR products were purified using the UNIQ-10 column PCR Products Purification kit (no. SK1141; Sangon Biotech (Shanghai) Co. Ltd., Shanghai, China) according to the manufacturer’s protocol; these were sequenced with the respective PCR primers at Sangon Biotech (Shanghai) Co. Ltd. The resulting sequences from GZU0317bea 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, RPB1, and Bloc from 69 taxa (68 Beauveria isolates with Isaria tenuipes as outgroup). Those recorded by Ariyawansa (2015), Agrawal et al. (2014), Chen et al. (2013), Zhang et al. (2013), Rehner & Buckley (2005) and Rehner et al. (2011) were downloaded from GenBank. Multiple sequence alignments for TEF, RPB1, and Bloc were carried out using MAFFT (Katoh & Standley 2013) with the default settings. Manual editing of sequences was performed using MEGA6 (Tamura et al. 2013). Concordance among genes was assessed using the ‘hompart’ command of PAUP v4.0b10 (Swofford 2002). Sequences (TEF+RPB1+Bloc) were assembled into a single matrix using SequenceMatrix v1.7.8 (Vaidya et al. 2011).

Phylogenetic analysis of the combined dataset was performed using MrBayes 3.2 (Ronquist et al. 2012). Two MCMC chains were executed simultaneously for 10×10⁶ generations, recording trees every 500 generations, using the SYM+G nucleotide substitution model, chosen based on AIC in MrModeltest v2.2 (Nylander 2004). The GTRGAMMA model was used for all partitions in accordance with recommendations in the RAxML manual against the use of invariant sites. All phylogenetic analyses were performed on the CIPRES web portal (Miller et al. 2010). The final alignment is available from TreeBASE under submission ID 18576.

Results

Phylogenetic analysis
Sequencing of TEF, RPB1 and Bloc from the fungal strain GZU0317bea were successful (GenBank accession numbers KT961699, KT961701, and KT961698, respectively). The TEF+RPB1+Bloc alignment contained 3950 bp and 14 taxa. Phylogenies from likelihood and Bayesian analyses were largely congruent, with the majority of branches strongly supported in both analyses. As shown in Figure 1, the strain GZU0317bea formed a terminal branch and associated with Beauveria australis S.A. Rehner & Humber.
**FIGURE 1.** Phylogenetic tree inferred from the analysis of a concatenated dataset for the genes TEF, RPB1 and Bloc. Statistical support values (≥50%) are shown at nodes, for maximum likelihood/Bayesian method.

**Taxonomy**

*Beauveria araneola* W.H. Chen, Y.F. Han, Z.Q. Liang & D.C. Jin, *sp. nov.* (Figure 2)

*MycoBank No.*: MB815354

Type:—CHINA. Guizhou Province: Huaxi, N 26°42′, E 106°67′, 17 March 2015, Shuai Li (holotype GZAC150317, ex-type culture GZU0317bea and dried ex-type culture GZU0317bea.1).

Colony growth and appearance similar on full strength Sabouraud’s dextrose and potato dextrose agars, 40–46 mm in diam. After 14 days at 25 °C, colony non-odorous, white to yellowish white, with aerial mycelium white, dense,
velutinous, powdery while sporulating. Reverse light aurantium. Vegetative hyphae septate, branched, hyaline, smooth walled, 1.1–3.2 μm wide. Conidiogenous cells solitary or occurring in lateral clusters, with base subcylindrical or occasionally subspherical, 3.2–5.9 (–10.8) × 0.9–1.1(–1.3) μm, and sympodially branched neck tapering into a long slender denticulate rachis, geniculate or irregularly bent, 6.4–16.2 × 0.5–1.1 μm. Conidia 1.3–4.5 × 0.9–2.5 μm, Q = 1.9–3.2 (Lm = 2.7, Wm =1.4, Qm =1.6), ellipsoidal to globose, hyaline, aseptate, walls smooth and thin.

**Etymology:** — *araneola*, referring to its host spider.

**Distribution:** — Guizhou Province, China.

**Material examined:** — Dried specimen GZAC150317 (holotype) and its isolate GZU0317bea have been deposited at Guizhou University (GZAC).

Notes: The new species is similar to four other species in the genus *Beauveria* (Table 1), *Beauveria caledoninca* Bissett & Widden, *Beauveria lii* Sheng L. Zhang & B. Huang, *Beauveria sinensis* Ming J. Chen, Z.Z. Li & B. Huang and *Beauveria vermiconia* de Hoog & V. Rao. However, *Beauveria araneola* can be easily distinguished from these species by its long slender denticulate rachis, subcylindrical conidiogenous cells, and ellipsoidal to globose conidia.

**TABLE 1.** A comparison of morphological characters among *Beauveria araneola* and its allies

<table>
<thead>
<tr>
<th>Species name</th>
<th>Conidiogenous cells</th>
<th>Denticulate rachis (μm)</th>
<th>Conidia (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Beauveria caledoninca</em></td>
<td>ellipsoidal to conoidal</td>
<td>Indeterminate</td>
<td>ellipsoidal or cylindrical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3–5 × 1–1.8</td>
</tr>
<tr>
<td><em>Beauveria lii</em></td>
<td>ellipsoidal to cylindrical</td>
<td>Indeterminate</td>
<td>ellipsoidal to cylindrical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.3–6.5 × 2.1–2.6</td>
</tr>
<tr>
<td><em>Beauveria sinensis</em></td>
<td>flask-shaped to cylindrical</td>
<td>Geniculate or irregularly bent</td>
<td>Elongate ellipsoidal to cylindrical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3–5 × 1.5–2</td>
</tr>
<tr>
<td><em>Beauveria vermiconia</em></td>
<td>ellipsoidal to flask-shaped</td>
<td>Flexuose</td>
<td>Comma- or sickle-shaped</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 × 0.7–1</td>
<td>1–1.5 in face view</td>
</tr>
<tr>
<td><em>Beauveria araneola</em></td>
<td>subcylindrical, occasionally subspherical</td>
<td>Irregularly bent</td>
<td>Ellipsoidal to globose</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.4–16.2 × 0.5–1.1</td>
<td>2.4–4.3 × 1.1–2.2</td>
</tr>
</tbody>
</table>

**FIGURE 2.** *Beauveria araneola* sp. nov. a. Infected spider; b. The colony on PDA after 14d at 25°C; c. Conidia; d. e. Conidiogenous cells solitary and usually in dense lateral clusters. Bar: b = 10 mm; c, d, e = 10 μm.
Discussion

The evolutionary dynamics of fungi and their hosts are usually described either by coevolution, or by host shifts. Shifts often occur to new hosts that are evolutionarily distant but which occupy a common ecological niche (Vega et al. 2009). Nutrient requirements often determine whether host shifts occur. Relationships between insects and fungi have been described as biotrophy, necrotrophy and hemibiotrophy, *inter alia*. Nutritional requirements of members of Hypocreales are characterized by a shift in nutritional mode from plant-based to animal- and fungal-based nutrition. Prior to the common ancestor of Hypocreaceae/Clavicipitaceae, the dominant fungal ecologies include pathogens of plants and decomposers of plant debris, *i.e.* lifestyles reliant on plants or plant material as an immediate food source. The common ancestor of Hypocreaceae and Clavicipitaceae corresponds to a departure from plant-based nutrition to one that specializes on animals and fungi (Spatafora et al. 2007). Known fungal hosts are phylogenetically diverse, and include members of Urediniomycetes, Hymenomycetes (mushrooms and other fleshy fungi), and *Elaphomyces*, but significantly fewer species of Clavicipitaceae are known to be parasites of other fungi, than the number of known pathogens of animals (Mains 1957; Gams & van Zaayen 1982). Prediction of the characteristics and evolutionary placement of any given member should be based on the correlation between molecular-phylogenetic genealogy and nutritional preferences (Spatafora et al. 2007; Vega et al. 2009).

The phylogenetic relationships among members of *Beauveria* inferred from three nuclear loci were mainly consistent with those inferred from four nuclear loci (Rehner et al. 2011). Rehner et al. (2011) emphasized that each of the four loci used to reconstruct the phylogeny of *Beauveria* could be used individually for accurate placement of all species, based on multiple species-specific phylogenetically informative nucleotide characters.

In the Bayesian and maximum likelihood analyses of *Bloc, RPB1*, and *TEF* sequence data, *B. araneola* was the sister species of *Beauveria australis*, and they further clustered with *Beauveria brongniartii*. Both *B. australis* and *B. brongniartii* occur in soil and agricultural habitats and as pathogens of insects. Based on our molecular phylogeny, *B. araneola* may have plesiomorphically shared similar habitats with the soil-borne or entomogenous species of the genus, and have jumped from soil or insect to its spider host. Host is regarded as an important identification criterion in the taxonomy of entomopathogenic fungi (Nikoh & Fukatsu 2000), which also support the recognition of *B. araneola* as a new species.

The species in *Beauveria* show a wide range of nutritional preferences, which may provide an appropriate model taxon for study of entomopathogenic fungi as endophytes, plant disease antagonists, rhizosphere colonizers and plant growth-promoting fungi; as well as for studying the bodyguard hypothesis; and for understanding production strategies for fungal biocontrol agents and formulation of fungal propagules (Vega et al. 2009). High-throughput RNA-seq transcriptomic analysis reveals that *B. bassiana* can adapt to different environmental niches by activating well-defined gene sets (Xiao et al. 2012). As an araneogenous fungus co-evolved with its host spider, *B. araneola* may possess some special characteristics, such as the fibrinolytic enzyme seen in *Cordyceps militaris* (L.) Fr. on the pupae of silkworms (Kim et al. 2006; Cui et al. 2008; Choi et al. 2011), which may make it suitable for control of spider mites (Wekesa et al. 2015).

Morphologic characteristics, phylogenetic analysis and spider inhabitation support the recognition of *Beauveria araneola* as a distinct species.

Acknowledgements

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Reference

Agrawal, Y., Mual, P. & Shenoy, B.D. (2014) Multi-gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp nov. from India.


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