Phytotaxa 333 (2): 243–250
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

https://doi.org/10.11646/phytotaxa.333.2.8

Beauveria majiangensis, a new entomopathogenic fungus from Guizhou, China

WAN-HAO CHEN[1,3], MAN LIU[2*], ZHEN-XING HUANG[2], GUANG-MING YANG[2], YAN-FENG HAN[3], JIAN-DONG LIANG[1] & ZONG-QI LIANG[3]

[1] *Department of Microbiology, Basic Medical School, Guiyang College of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China*

[2] *Guizhou Institute of Biology, Guiyang, Guizhou 550025, China*

[3] *Institute of Fungus Resources, Guizhou University, Guiyang, Guizhou 550025, China*

** Corresponding author email: freemyself516@hotmail.com*

Abstract

Beauveria majiangensis sp. nov., a fungal grub parasite, isolated from a blueberry farm in Guizhou Province, China, is herein described based on morphological and phylogenetic evidence. *Beauveria majiangensis* differs from other *Beauveria* species based on its indeterminate, denticulate rachis, cylindrical or sometimes subspherical conidiogenous cells, and ellipsoidal conidia. Phylogenetic analyses based on four loci (*TEF*, *RPB1*, *Bloc*, and *ITS*) strongly support that this strain is distinct within *Beauveria*.

Key words: *Beauveria*, grub, morphology, phylogeny, Coleoptera

Introduction

Beauveria is one of the most ubiquitous anamorphic genera of entomopathogenic fungi, and includes ecologically and economically important species (Posada & Vega 2005, Ownley *et al.* 2008, Roy *et al*. 2010); however, some *Beauveria* are endophytes or saprobes (Vega *et al*. 2008, Moonjely *et al*. 2016). 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 (Chen *et al*. 2013, Rehner *et al*. 2011).

The host taxa in which pathogenic *Beauveria* have been found include Araneae, Blattariae, Coleoptera, Diptera, Embioptera, Heteroptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mantodea, Neuroptera, Orthoptera, Siphonaptera, and Thysanoptera (Zimmermann 2007, Chen *et al*. 2017). There are 11 species with hosts in the order Coleoptera: *B. bassiana* (Bals.-Criv.) Vuill.; *B. brongniartii* (Sacc.) Petch; *B. amorpha* (Höhn.) Minnis, S.A. Rehner & Humber; *B. asiatica* S.A. Rehner & Humber; *B. caledonica* Bissett & Widden; *B. malawiensis* S.A. Rehner & Aquino de Muro; *B. pseudobassiana* S.A. Rehner & Humber; *B. sungii* S.A. Rehner & Humber; *B. varroae* S.A. Rehner & Humber; *B. lii* Sheng L. Zhang & B. Huang; and *B. hoplocheli* I. Robène-Soustrade & S. Nibouche.

Recently, entomopathogenic fungi were screened in Guizhou, China, and we isolated a grub-infecting *Beauveria* strain. Based on morphological characteristics and phylogenetic analysis, we concluded that this strain represents a new species and is described herein as *B. majiangensis*.

Materials and methods

Specimen collection and isolation

In December 2015, a fungus-infected grub specimen (GZU1214) was collected from a blueberry farm in Majiang, Qiandongnan Prefecture, Guizhou Province, China, by Man Liu of the Guizhou Institute of Biology. Strain GZU12141 was isolated from this infected grub specimen on improved potato dextrose agar (PDA, with 1% w/v peptone).

Accepted by Eric McKenzie: 30 Nov. 2017; published: 9 Jan. 2018

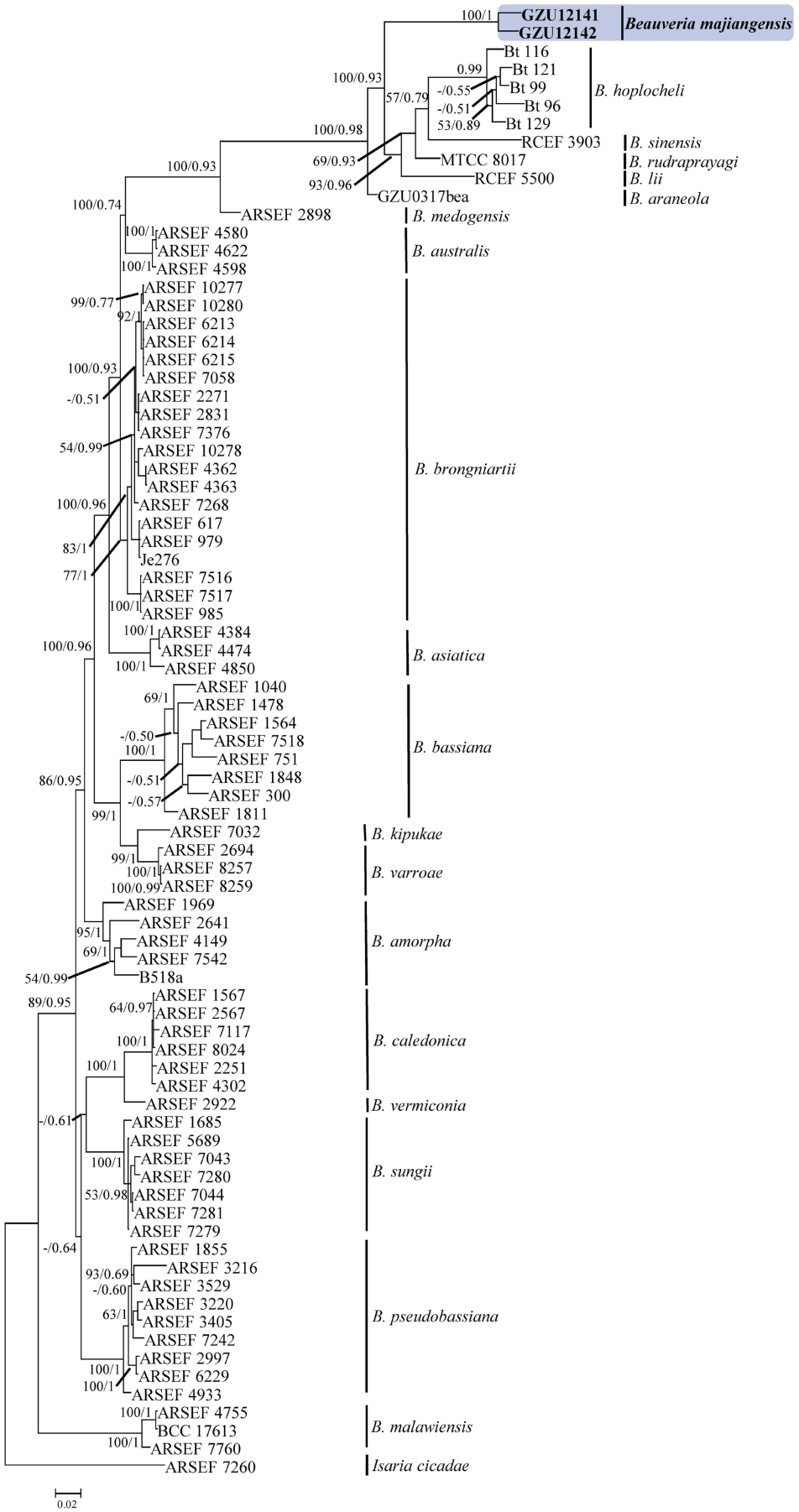

FIGURE 1. Phylogenetic analysis of GZU12141, GZU12142, and related *Beauveria* species based on combined partial *TEF+RPB1+Bloc* sequences. Statistical support values (≥50 %) are shown at nodes, and present bootstrap values/Bayesian posterior probabilities.

Strain culture and identification

Strain GZU12141 was incubated on Sabouraud's dextrose and potato dextrose agars at 25°C for 14 d. Morphological characteristics of the fungus were examined using classical mycological techniques based on growth rate, and macroscopic and microscopic characteristics. The ex-type culture and a dried-culture holotype specimen are deposited in GZAC, Guizhou University, Guiyang, China.

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. Taq enzyme and dNTP were from Shanghai Tiangen. Internal transcribed spacer (*ITS*), RNA polymerase II largest subunit (*RPB1*), B locus intergenic region (*Bloc*), and translation elongation factor 1 alpha (*TEF*), were amplified by polymerase chain reaction (PCR) according to the procedures described by White *et al*. (1990), Castlebury *et al*. (2004), Rehner *et al*. (2006), and van den Brink *et al*. (2012), respectively. 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 with the above PCR products at Sangon Biotech (Shanghai) Co. The resulting sequences were submitted to GenBank.

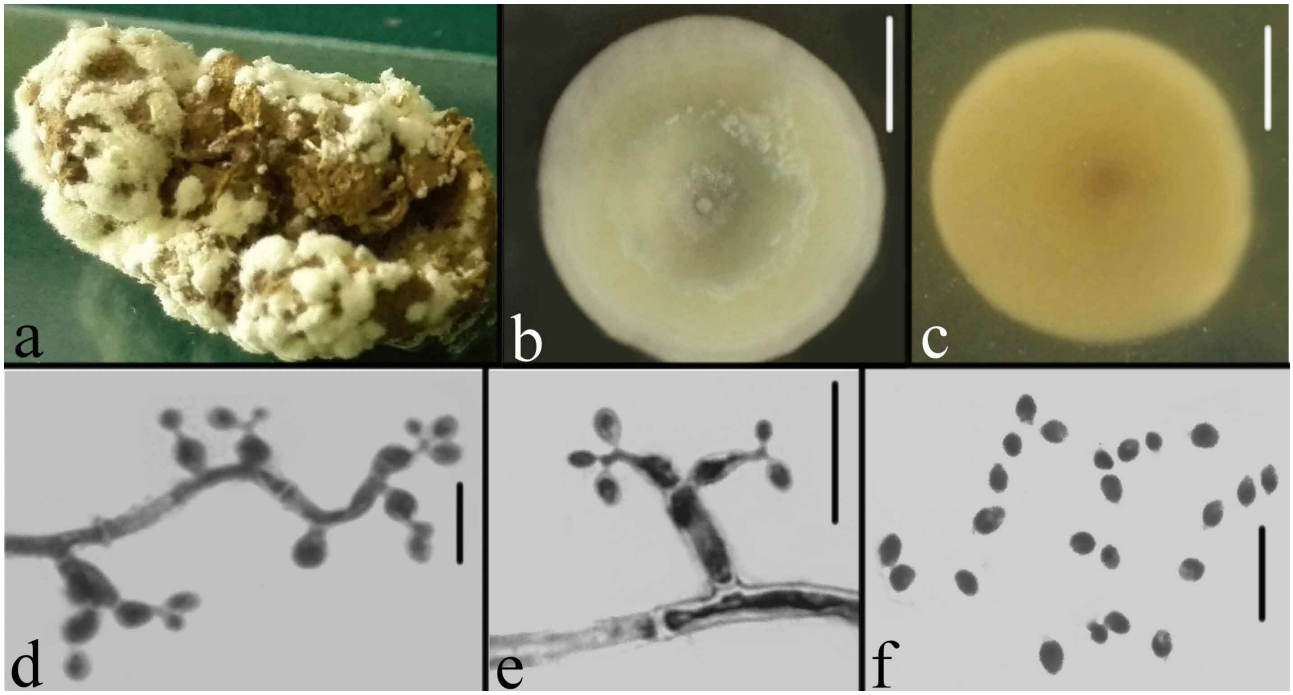

FIGURE 2. *Beauveria majiangensis* (holotype GZAC GZU1214) a. Infected grub. b, c. Colony (top and reverse view, respectively) on PDA after 14 d at 25 °C. d, e. Conidiogenous cells solitary and usually in dense lateral clusters. f. Conidia. Scale bars: b, c = 10 mm, d, e, f = 10 μm.

Sequence alignment and phylogenetic analyses

DNA sequences generated in this study were assembled and edited using Lasergene 6.0 (DNASTAR, Madison, WI, USA). *TEF*, *RPB1*, and *Bloc* sequences from 19 taxa (18 *Beauveria* isolates and one *Isaria cicadae* strain as outgroup), and *ITS* sequences from 20 taxa (19 *Beauveria* isolates and one *I. cicadae* strain as outgroup) were downloaded from GenBank, based on Agrawal *et al*. (2014), Ariyawansa *et al*. (2015), Chen *et al*. (2013), Chen *et al*. (2017), Imoulan *et al*. (2016), Robène-Soustrade *et al*. (2016), Rehner & Buckley (2005), Rehner *et al*. (2011), and Zhang *et al*. (2012). Multiple sequence alignments for *TEF*, *RPB1* and *Bloc* 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 *TEF+RPB1+Bloc* sequences were assembled by SequenceMatrix1.7.8 (Vaidya 2011). Gene concordance was assessed with the 'hompart' command in PAUP4.0b10 (Swofford 2002).

The combined three-gene (*TEF+RPB1+Bloc*) and *ITS* dataset was phylogenetically analyzed using MrBayes 3.2 (Ronquist *et al*. 2012). Two runs were simultaneously executed for 10,000,000 generations, saving a tree every 500 generations. The GTR+I+G and HKY+I+G nucleotide substitution models were used for *TEF+RPB1+Bloc* and *ITS* sequences, respectively, which were the best-fit substitution models for maximum likelihood analysis. The

GTRGAMMA model was used for all partitions in accordance with RAxML manual recommendations against invariant site use. All phylogenetic reconstructions were performed on the CIPRES web portal (Miller *et al*. 2010). The final alignment is available from TreeBASE (ID 21673).

Results

Phylogenetic analyses

TEF, *RPB1*, *Bloc* and *ITS* sequencing from GZU12141 was successful (GenBank accession no.: MG052640, MG052644, MG052639, and MG052642, respectively). The alignment lengths and number of taxa sampled for *TEF+RPB1 +Bloc* and *ITS* from GenBank were 2710 bp, from 19 taxa and 425 bp from 20 taxa, respectively. Strains GZU12141 (and a second isolate, GZU12142), formed a single clade in both combined data (*TEF+RPB1 +Bloc*) and *ITS* analyses (Figs 1, 3).

Taxonomy

Beauveria majiangensis W.H. Chen, M. Liu, Z.X. Huang, G.M. Yang, Y.F. Han, J.D. Liang & Z.Q. Liang *sp. nov.* (Fig. 2) MycoBank No.: MB823150

Type:—CHINA. Guizhou Province: Qiandongnan Prefecture, Majiang (N 26º42'47", E 107º43'37"), on a grub (Coleoptera: Scarabaeoidea) in blueberry farm, 14 December 2015, Man Liu, holotype GZAC GZU1214, ex-type culture GZAC GZU12141.

Colony growth and appearance similar on full-strength Sabouraud's dextrose and potato dextrose agars, 30.5 mm in diam., after 14 d at 25 °C, non-odorous, aerial mycelium white, dense, velutinous, powdery while sporulating; white to yellowish white. Reverse light aurantium in older potions. Vegetative hyphae septate, branched, hyaline, smooth-walled, 1.6–2.2 µm wide. Conidiogenous cells solitary or occurring in lateral clusters, base cylindrical or sometimes subspherical, 3.8–12.9 (–16.2) × 1.2–1.5 (–2.3) µm, apex with an indeterminate, denticulate rachis less than 1µm wide. Conidia 2.4–3.8 × 1.6–2.3 µm, Q = 1.6–2.6 (L^m = 2.1, W^m =1.4, Q^m =1.6), ellipsoidal, hyaline, aseptate, walls smooth and thin.

Etymology:—*majiangensis* named after the place, Majiang from which the fungus was collected.

Additional specimens examined:—CHINA. Guizhou Province: Qiandongnan Prefecture, Majiang (N 26º42′47″, E 107º43′37″), on a grub (Coleoptera: Scarabaeoidea) from a blueberry farm, 14 December 2015, Man Liu (GZAC GZU12142). Sequences from this strain were deposited in GenBank under accession numbers: MG052641= *TEF*, MG052645= *RPB1*, MG052638= *Bloc*, and MG052643= *ITS*.

Known distribution:—Guizhou Province, China.

Discussion

As originally described by de Hoog (1978), the main characters for *Beauveria* are basally inflated conidiogenous cells that sympodially produce conidia on divergent denticles. Based on these characteristics, strain GZU12141 clearly belongs to *Beauveria*. Six other *Beauveria* species produce an indeterminate, denticulate rachis similar to *B. majiangensis*: *B. caledonia*, *B. gryllotalpidicola*, *B. lii*, *B. loeiensis*, *B. medogensis*, and *B. rudraprayagi*. A comparative summary of the main characters of *B. majiangensis* and these other six species is provided (Table 1). *Beauveria majiangensis* is distinguished from *B. gryllotalpidicola*, *B. lii*, *B. loeiensis*, *B. medogensis*, and *B. rudraprayagi* based on its ellipsoidal conidia and their size. *B. majiangensis* is easily distinguished from *B. caledonia*, the latter possessing ellipsoidal to more or less cylindrical to conoidal conidiogenous cells. Thus, morphological characters confirm that *B. majiangensis* is a new *Beauveria* species.

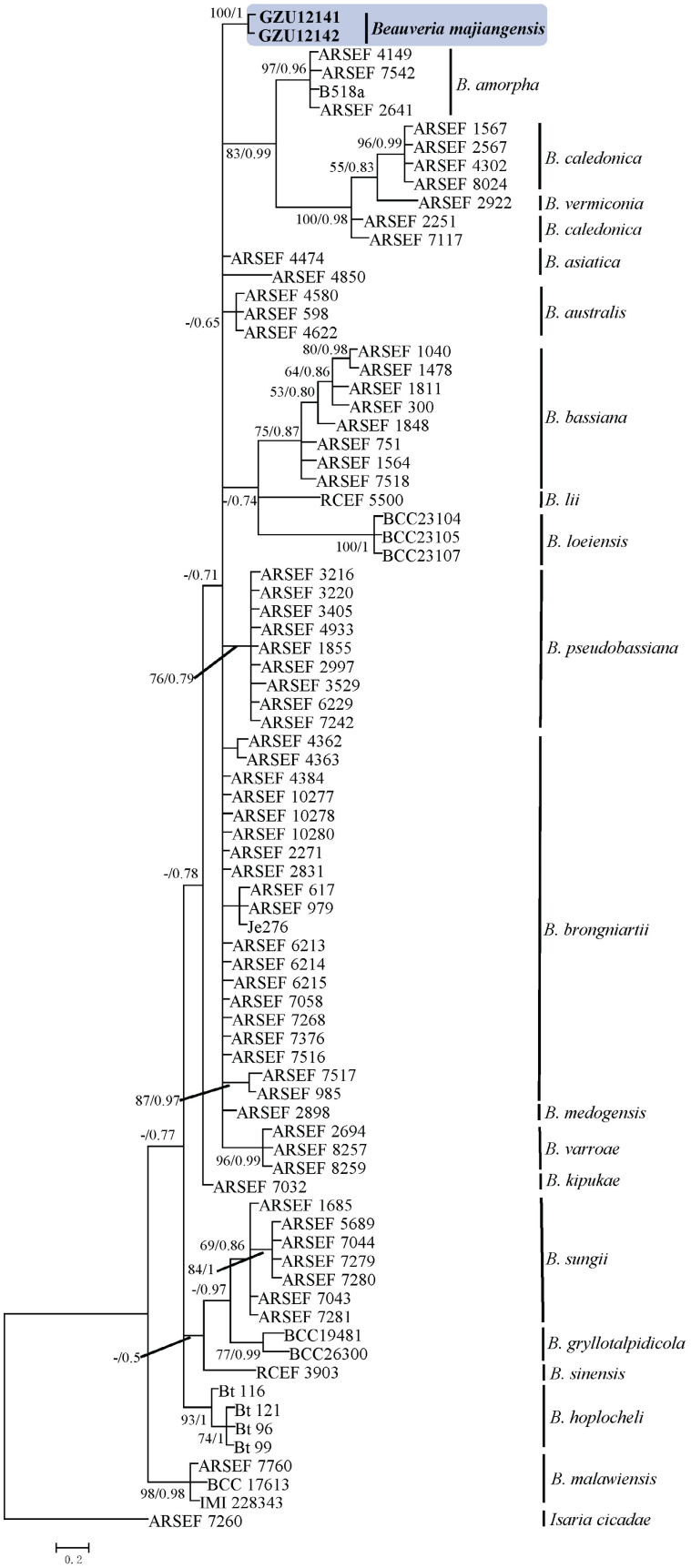

FIGURE 3. Phylogenetic analysis of GZU12141, GZU12142, and related *Beauveria* species based on *ITS* sequences. Statistical support values (≥50 %) are shown at nodes, and represent maximum likelihood bootstrap values/Bayesian posterior probabilities.

TABLE 1. Comparison of morphological characters between *Beauveria majiangensis* and its allies.

Species	Conidiogenous cells	Conidia (μm)
B. caledonia	ellipsoidal to conodial	ellipsoidal to more or less cylindrical, (2.4−)3.0−5.0(−6.5) × 1.0−1.8(−2.0)
B. gryllotalpidicola	flask-shaped	globose, 2 × 2
B. lii	ellipsoidal to cylindrical	ellipsoidal to cylindrical, (3.1–)4.3–6.5(–10.1) × (1.4–)2.1–2.6(–3.6)
B. loeiensis	cylindrical or narrowing at the tip	ellipsoidal to cylindrical, 3.5−6 × 1.5−2
B. medogensis	sub-spherical to flask-shaped	globose to subglobose, 2.0−3.0 × 2.0−3.5
B. rudraprayagi	sub-spherical to ampulliform	globose, subglobose, 2.5–4.0 × 2.5–4.0
B. majiangensis	cylindrical or sometimes subspherical	ellipsoidal, 2.4−3.8 × 1.6−2.3

The nuclear ribosomal *ITS* and *TEF* were first used to identify cryptic diversification among *Beauveria* spp. by Rehner & Buckley (2005). Rehner *et al*. (2011) proposed a multilocus phylogeny of *Beauveria* species based on partial *RPB1*, *RPB2*, *TEF*, and *Bloc* sequences, and noted that each of the four loci used to reconstruct the *Beauveria* phylogeny could be individually used for accurate placement of all species, based on multiple species-specific phylogenetically informative nucleotide characters. In this study, the concatenated *TEF+RPB1+Bloc* and *ITS* analyses produced maximum likelihood and Bayesian trees that were largely congruent. Most branches were strongly supported in both analyses. The two *B. majiangensis* strains clustered together and were distinct from other *Beauveria* species. Thus, molecular phylogenetic results supported the morphologically based conclusion that strain GZU12141 is a new *Beauveria* species.

Acknowledgements

We thank Mallory Eckstut, PhD, from Liwen Bianji, Edanz Editing China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript. This work was supported by Project of Major Special Sub-projects in Guizhou Province (Qiankehe Major Project [2015]6013-2) and Guizhou Province and Guizhou Academy of Sciences Cooperation Projects (Qiankeyuan SY [2017] 09).

References

Agrawal, Y., Mual, P. & Shenoy, B.D. (2014) Multi-gene genealogies reveal cryptic species *Beauveria rudraprayagi* sp nov from India. *Mycosphere* 5: 719–736.

Ariyawansa, H.A., Hyde, K.D., Jayasiri, S.C., Buyck, B., Kandawatte, W.T.C., Cui, Y.Y., Dai, D.Q., Dai, Y.C., Daranagama, D.A., Jayawardena, R.S., Lücking, R., Ghobad-Nejhad, M., Niskanen, T., Thambugala, K.M., Voigt, K., Zhao, R.L., Boonmee, S., Bahkali, A.H., Chen, J., Cui, B.K., Dayarathne, M.C., Dissanayake, A.J., Ekanayaka, A.H., Hashimoto, A., Hongsanan, S., Jones, E.B.G., Larsson, E., Lewis, D., Li, W.J., Li, Q.R., Liu, J.K., Luo, Z.L., Maharachchikumbura, S.S.N., Mapook, A., McKenzie, E.H.C., Norphanphoun, C., Pang, K.L., Perera, R.H., Phookamsak, R., Phukhamsakda, C., Randrianjohany, E., Senanayake, I.C., Singtripop, C., Shang, Q., Tanaka, K., Tian, Q., Tian, C.M., Tibpromma, S., Verbeken, A., Abdel-Wahab, M.A., Wanasinghe, D.N., Wijayawardene, N.N., Zhang, J.F., Zhang, H., Abdel-Aziz, F.A., Adamčík, S., Ammirati, J.F., Bulgakov, T., Cabral, A.L., Callaghan, T.M., Callac, P., Chang, C.H., Coca, L.F., Dal-Forno, M., Dollhofer, V., Fliegerová, K., Greiner, K., Griffith, G.W., Ho, H.M., Hofstetter, V., Jeewon, R., Kang, J.C., Kirk, P.M., Kytövuori, I., Lawrey, J.D., Li, J.X.H., Liu, Z.Y., Zhong, X.L., Liimatainen, K., Lumbsch, H.T., Matumura, M., Moncada, B., Nuankaew, S., Parnmen, S., Santiago, M.D.A., Sato, G., Sommai, S., Song, Y., De Souza, C.A.F., De Souza-Motta, C.M., Su, H.Y., Suetrong, S., Wang, Y., Wei, S.F., Wen, T.C., Shen, H., Yuan, H.S., Zhou, L.W., Reblova, M., Fournier, J. & Camporesi, E. (2015) Fungal diversity notes 111–252–Taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* 75: 1–248.
https://doi.org/10.1007/s13225-015-0346-5

Castlebury, L.A., Rossman, A.Y., Sung, G.H., Hyten, A.S. & Spatafora, J.W. (2004) Multigene phylogeny reveals new lineage for

Stachybotrys chartarum, the indoor air fungus. *Mycological Research* 108: 864–872.
https://doi.org/10.1017/S0953756204000607

Chandler, D., Goettel, M.S., Pell, J., Vega, F.E. & Wajnberg, E. (Eds.) (2010) *The ecology of fungal entomopathogens.* Dordrecht: Springer.
https://doi.org/10.1007/978-90-481-3966-8

Chen, M.J., Huang, B., Li, Z.Z. & Spatafora, J.W. (2013) Morphological and genetic characterization of *Beauveria sinensis* sp. nov. from China. *Mycotaxon* 124: 301–308.
https://doi.org/10.5248/124.301

Chen, W.H., Han, Y.F., Liang, Z.Q. & Jin, D.C. (2017) A new araneogenous fungus in the genus *Beauveria* from Guizhou, China. *Phytotaxa* 302: 57–64.
https://doi.org/10.11646/phytotaxa.302.1.5

de Hoog, G.S. (1978) Notes on some fungicolous Hyphomycetes and their relatives. *Persoonia* 10: 33–81.

Imoulan, A., Wu, H.J., Lu, W.L., Li, Y., Li, B.B., Yang, R.H., Wang, J.W., Wang, X.L., Kirk, P.M. & Yao, Y.J. (2016) *Beauveria medogensis* sp. nov., a new fungus of the entomopathogenic genus from China. *Journal of Invertebrate Pathology* 139: 74–81.
https://doi.org/10.1016/j.jip.2016.07.006

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
https://doi.org/10.1093/molbev/mst010

Liang, Z.Q., Han, Y.F., Chu, H.L. & Fox, R.T. (2009) Studies on the genus *Paecilomyces* in China V. *Taifanglania* gen. nov. for some monophialidic species. *Fungal Diversity* 34: 69–77.

Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES science gateway for inference of large phylogenetic trees. *In: Gateway computing environments workshop.* pp. 1–8.
https://doi.org/10.1109/GCE.2010.5676129

Moonjely, S., Barelli, L. & Bidochka, M.J. (2016) Insect pathogenic fungi as endophytes. *Advances in Genetics* 94: 107–135.
https://doi.org/10.1016/bs.adgen.2015.12.004

Ownley, B.H., Griffin, M.R., Klingeman, W.E., Gwinn, K.D., Moulton, J.K. & Pereira, R.M. (2008) *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of Invertebrate Pathology* 98: 267–270.
https://doi.org/10.1016/j.jip.2008.01.010

Posada, F.J. & Vega, F.E. (2005) A new method to evaluate the biocontrol potential of single spore isolates of fungal entomopathogens. *Journal of Insect Science* 5: 37.
https://doi.org/10.1093/jis/5.1.37

Rehner, S.A. & Buckley, E. (2005) A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia* 97: 84–98.
https://doi.org/10.1080/15572536.2006.11832842

Rehner, S.A., Posada, F., Buckley, E.P., Infante, F., Castillo, A. & Vega, F.E. (2006) Phylogenetic origins of African and Neotropical *Beauveria bassiana* sl pathogens of the coffee berry borer, *Hypothenemus hampei*. *Journal of Invertebrate Pathology* 93: 11–21.
https://doi.org/10.1016/j.jip.2006.04.005

Rehner, S.A., Minnis, A.M., Sung, G.H., Luangsa-ard, J.J., Devotto, L. & Humber, R.A. (2011) Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. *Mycologia* 103: 1055–1073.
https://doi.org/10.3852/10-302

Robène-Soustrade, I., Jouen, E., Pastou, D., Payet-Hoarau, M., Goble, T., Linderme, D., Lefeuvre, P., Calmès, C., Reynaud, B., Nibouche, S. & Costet, L. (2015) Description and phylogenetic placement of *Beauveria hoplocheli* sp. nov. used in the biological control of the sugarcane white grub, *Hoplochelus marginalis*, on Reunion Island. *Mycologia* 107: 1221–1232.
https://doi.org/10.3852/14-344

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
https://doi.org/10.1093/sysbio/sys029

Swofford, D.L. (2002) *PAUP* 4.0b10: phylogenetic analysis using parsimony (*and other methods).* Sunderland, MA: Sinauer, 142 pp.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30: 2725–2729.
https://doi.org/10.1093/molbev/mst197

Vaidya, G., Lohman, D.J. & Meier, R. (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27: 171–180.

https://doi.org/10.1111/j.1096-0031.2010.00329.x

van den Brink, J., Samson, R.A., Hagen, F., Boekhout, T. & de Vries, R.P. (2012) Phylogeny of the industrial relevant, thermophilic genera *Myceliophthora* and *Corynascus*. *Fungal Diversity* 52: 197–207.
https://doi.org/10.1007/s13225-011-0107-z

Vega, F.E., Posada, F., Aime, M.C., Pava-Ripoll, M., Infante, F. & Rehner, S.A. (2008) Entomopathogenic fungal endophytes. *Biological Control* 46: 72–82.
https://doi.org/10.1016/j.biocontrol.2008.01.008

White, T.J., Bruns, T., Lee, S.J.W.T. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18: 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, S.L., He, L.M., Chen, X. & Huang, B. (2013) *Beauveria lii* sp. nov. isolated from *Henosepilachna vigintioctopunctata*. *Mycotaxon* 121: 199–206.
https://doi.org/10.5248/121.199

Zimmermann, G. (2007) Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology* 17: 553–596.
https://doi.org/10.1080/09583150701309006