Phytotaxa 409 (4): 227–232
https://www.mapress.com/j/pt/

Article

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

https://doi.org/10.11646/phytotaxa.409.4.5

Akanthomyces araneicola, a new araneogenous species from Southwest China

WAN-HAO CHEN[1], CHANG LIU[2], YAN-FENG HAN[3*], JIAN-DONG LIANG[1], WEI-YI TIAN[1] & ZONG-QI LIANG[3]

[1]*Department of Microbiology, Basic Medical School, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China*
[2]*School of Pharmacy, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China*
[3] *Institute of Fungus Resources, Guizhou University, Guiyang, Guizhou 550025, China*
**Corresponding author: swallow1128@126. com*

Abstract

During a survey of spider-pathogenic fungi from Southwest China, a new araneogenous species was found. The new species, *Akanthomyces araneicola*, differs from other species based on mostly smaller fusiform conidia (2.5–5.0 × 1.3–1.9 µm), mononematous conidiophores and, additionally, in parasitising a spider. Both the morphological identification and phylogenetic analysis of combined *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* sequence data support *A. araneicola* as a new species in the genus *Akanthomyces*.

Keywords: 1 new species, mononematous, morphology, phylogeny, spider

Introduction

Akanthomyces was established by Lebert to accommodate *A. aculeatus* Leb., which was found in France. Mains (1950) emended and revised the genus describing three species *A. aculeatus*, *A. ampullifer* (Petch) Mains and *A. aranearum* (Petch) Mains, and transferred three species to the new genus *Insecticola* Mains. Samson & Evans (1974) treated *Insecticola* as a synonym of *Akanthomyces*, and reported three species from Ghana, *A. arachnophilus* (Petch) Samson & H.C. Evans, *A. gracilis* Samson & H.C. Evans and *A. pistillariiformis* (Pat.) Samson & H.C. Evans. Several new species were reported later (Koval 1977, Vincent *et al*. 1988, Hywel-Jones 1996, Hsieh *et al*. 1997, Huang *et al*. 2000).

Kepler *et al*. (2017) re-evaluated the Cordycipitaceae by phylogenetic analysis and treated *Torrubiella* Boud. and *Lecancillium* W. Gams & Zare as junior synonyms of *Akanthomyces*, and transferred *Akanthomyces arachnophilus*, *A. cinereus* Hywel-Jones, *A. koratensis* Hywel-Jones, *A. longisporus* B. Huang, S.B. Wang, M.Z. Fan & Z.Z. Li, *Cordyceps nelumboides* Kobayasi & Shimizu, *A. novoguineensis* Samson & B.L. Brady, *A. ovalongatus* L.S. Hsieh, Tzean & W.J. Wu and *A. websteri* Hywel-Jones to the new genus *Hevansia* Luangsa-ard, Hywel-Jones & Spatafora. Mongkolsamrit *et al*. (2018) described some *Isaria*-like and spider host species: *Akanthomyces kanyawimiae* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, *A. sulphureus* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard, *A. thailandicus* Mongkols., Spatafora & Luangsa-ard and *A. waltergamsii* Mongkols., Noisrip., Thanakitp., Spatafora & Luangsa-ard. Currently, *Akanthomyces* consists of 20 species that have been isolated from soil, insects and spiders (Chen *et al*. 2018). Thirteen species have a spider host: *A. arachnophilus*, *A. aranearum*, *A. araneogenum* Z.Q. Liang, W.H. Chen & Y.F. Han, *A. cinereus*, *A. kanyawimiae*, *A. koratensis*, *A. lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha, *A. longisporus*, *A. ovalongatus*, *A. sulphureus*, *A. thailandicus*, *A. waltergamsii* and *A. websteri*.

During a survey of spider-pathogenic fungi from Southwest China, a new araneogenous species was found. It is described here as *Akanthomyces araneicola sp. nov.* and is supported by morphological characters and a phylogenic analysis.

Accepted by Eric McKenzie: 8 Jul. 2019; published: 12 Jul. 2019

Materials and methods

Specimen collection and isolation

A fungus infected spider specimen was collected from a pinewood in Tongmuling, Guiyang city (N 26°23′25.92″, E 106°41′3.35″), in July 2018. The fungus was isolated and cultured on agar plates containing improved potato dextrose agar (PDA, 1 % w/v peptone) medium.

Strain culture and identification

The isolated fungus was incubated on 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 °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 selected based on Kepler *et al*. (2017), Mongkolsamrit *et al*. (2018), Chen *et al*. (2018) and the result of a Blast search in GenBank. Multiple sequence alignments for *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* were carried out using MAFFT v7.037b (Katoh & Standley 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 v.1.7.8 (Vaidya *et al*. 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 24458.

Sequencing and phylogenetic analysis

The *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* sequences from strain GY29011 were deposited in GenBank with accession numbers MK942431, MK942430, MK955944, MK955947 and MK955950, respectively. The concatenated alignment of *ITS+LSU+RPB1+RPB2+TEF* sequences was 2639 bp long. The three sets of sequences, from strains GY29011, GY29012 and GY29013, formed a clade in both ML and Bayesian analyses (Fig. 1).

Results

Taxonomy

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

Mycobank: MB 831092

Type:—CHINA. Guizhou Province: Guiyang City, Tongmuling (N 26°23′25.92″, E 106°41′3.35″), on a spider, 31 July 2018, Wanhao Chen, holotype GZAC GY2901; ex-type culture GZAC GY29011.

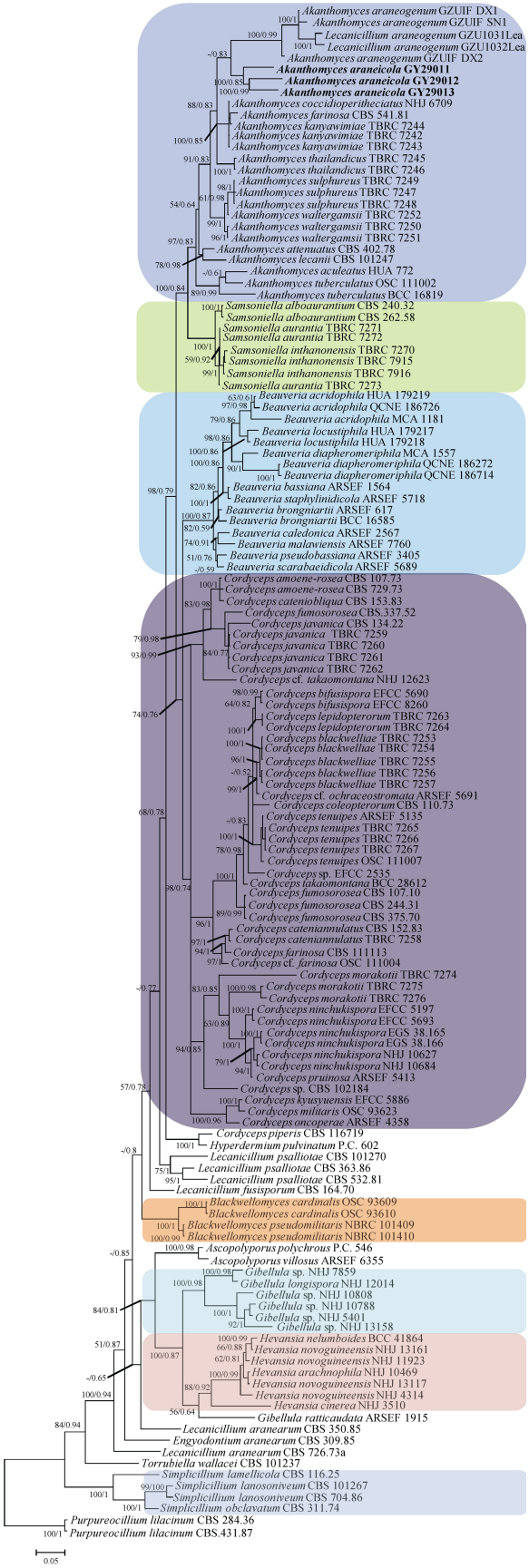

FIGURE 1. Phylogenetic analysis of *Akanthomyces araneicola* (strains GY29011, GY29012, GY29013) and related species based on combined partial *ITS+LSU+RPB1+RPB2+TEF* sequences. Statistical support values (≥50 %) are shown at nodes, and presented as bootstrap values/Bayesian posterior probabilities.

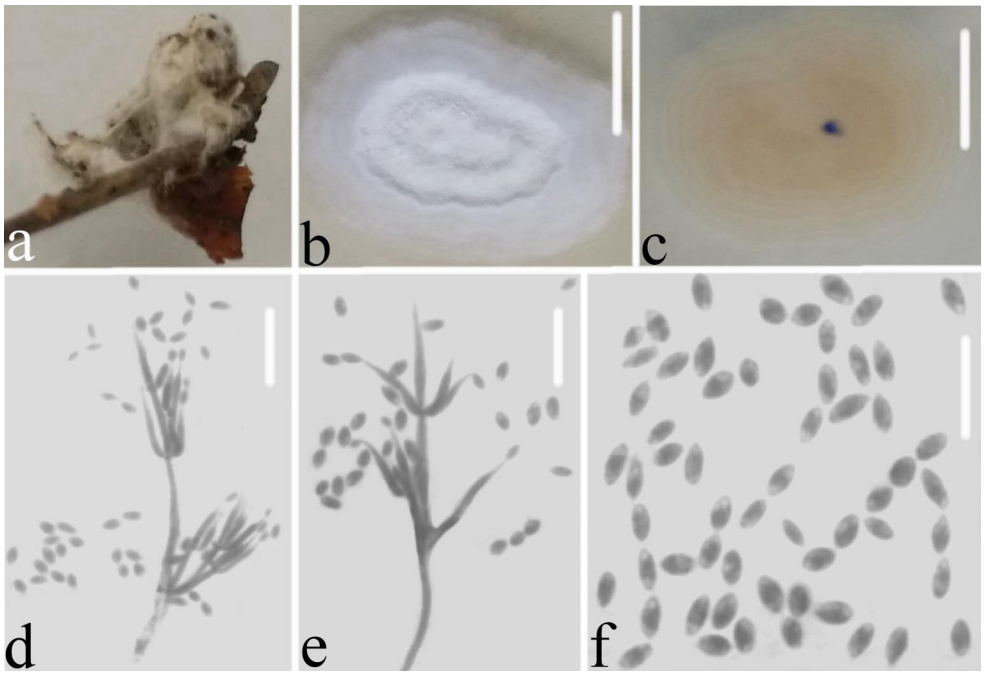

FIGURE 2. *Akanthomyces araneicola sp. nov.* **a.** Infected spider. **b, c.** Colony on PDA after 14 d at 25 °C. **d, e.** Conidiophores, conidiogenous cells and conidia. **f.** Conidia. Bars: **b, c** = 10 mm; **d–f** = 10 μm.

Colonies on PDA, attaining a diameter of 21–28 mm after 14 days at 25 °C, white to yellowish, powdery, thin; reverse yellowish. *Hyphae* septate, hyaline, smooth-walled, 1.3–2.5 μm wide. *Conidiophores* mononematous, hyaline, smooth-walled, with single phialide or whorls of 2–6 phialides, or penicillium-like from hyphae directly. *Phialides* consisting of a cylindrical, somewhat inflated base, 8.1–16.9 × 1.3–1.9 μm, tapering to a thin neck. *Conidia* hyaline, smooth-walled, mostly fusiform, 2.5–5.0 × 1.3–1.9 μm, forming divergent and basipetal chains. In culture both phialides and conidia are of similar general shape and size to those found on spiders.

Etymology:—referring to the ability to colonize spider.

Additional strains examined:—CHINA. Guizhou Province: Guiyang City, Tongmuling (N 26°23′25.92″, E 106°41′3.35″), on a spider, 31 July 2018, Wanhao Chen (GY29012). Sequences from this strain have been deposited in GenBank with accession numbers: *ITS*=MK942434, *LSU*=MK942435, *RPB1*=MK955945, *RPB2*=MK955948, *TEF*=MK955950; CHINA. Guizhou Province: Guiyang City, Tongmuling (N 26°23′25.92″, E 106°41′3.35″), on a spider, 31 July 2018, Wanhao Chen (GY29013). Sequences from this strain have been deposited in GenBank with accession numbers: *ITS*=MK942437, *LSU*=MK942436, *RPB1*=MK955946, *RPB2*=MK955949.

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

Discussion

As originally described, the typical characters of *Akanthomyces* are ellipsoidal, cylindrical, or narrowly cylindrical conidiogenous cells that gradually or abruptly taper to a more or less distinct neck (Lebert 1858, Mains 1950, Vincent *et al*. 1988, Hsieh *et al*. 1997). 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. The typical characters of the strain GY29011 easily identified it as belonging to *Akanthomyces* and it is distinguished from other species by mostly smaller fusiform conidia (2.5–5.0 × 1.3–1.9 μm), mononematous conidiophores and, additionally, in parasitising a spider. Thus, morphological characters suggested that strain GY29011 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. araneicola* clustered with other *Akanthomyces* spp., which supported the results of morphological analysis, but it is distinct from other *Akanthomyces* species. Thus, both molecular phylogenetic results and the morphology support description of the new species, *A. araneicola*.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 31460010, 31860002), the Doctoral Fund of Guiyang University of Chinese Medicine (3043-043170023), the National first-class construction discipline in Guizhou province (Chinese medical science) (GNYL[2017]008), and Engineering Research Center of General Higher Education in Guizhou Province (Qianjiaohe(2015)337).

References

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

Chen, W.H., Liu, C., Han, Y.F., Liang, J.D. & Liang, Z.Q. (2018) *Akanthomyces araneogenum*, a new *Isaria*-like araneogenous species. *Phytotaxa* 379: 66–72. https://doi.org/10.11646/phytotaxa.379.1.6

Curran, J., Driver, F., Ballard, J.W.O. & Milner, R.J. (1994) Phylogeny of *Metarhizium*: analysis of ribosomal DNA sequence data. *Mycological Research* 98: 547–552. https://doi.org/10.1016/S0953-7562(09)80478-4

Drummond, A. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* 7: 214. https://doi.org/10.1186/1471-2148-7-214

Hsieh, L.S., Tzean, S.S. & Wu, W.J. (1997) The genus *Akanthomyces* on spiders from Taiwan. *Mycologia* 89: 319–324. https://doi.org/10.1080/00275514.1997.12026788

Huang, B., Wang, S., Fan, M. & Li, Z. (2000) Two species of *Akanthomyces* from Dabieshan mountains. *Mycosystema* 19: 172–174.

Hywel-Jones, N. (1996) *Akanthomyces* on spiders in Thailand. *Mycological Research* 9: 1065–1070. https://doi.org/10.1016/S0953-7562(96)80214-0

Katoh, K. & Standley, D.M. (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30 (4): 772–780. https://doi.org/10.1093/molbev/mst010

Kepler, R.M., Luangsa-ard, J.J., Hywel-Jones, N.L., Quandt, C.A., Sung, G.H., Rehner, S.A., Aime, M.C., Henkel, T.W., Samjuan, T., Zare, R., Chen, M.J., Li, Z.Z., Rossman, A.Y., Spatafora, J.W. & Shrestha, B. (2017) A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). *IMA Fungus* 8: 335–353. https://doi.org/10.5598/imafungus.2017.08.02.08

Koval, E.Z. (1977) Species novae fungorum entomophilorum hyphomycetalum. *Novosti Sis tematihi vysskhikh i nizshikh Rastenii* 1976: 203–208.

Lebert, H. (1858) Ueber einige neue oder unvollkommen gekannte Krankherten der Insekten, welche durch Entwicklung niederer Pflanzen im lebenden Körper enstehen. *Zeitschrift für wissenschaftliche Zoologie* 9: 439–453.

Liang, J.D., Han, Y.F., Zhang, J.W., Du, W., Liang, Z.Q. & Li, Z.Z. (2009) Optimal culture conditions for keratinase production by a novel thermophilic *Myceliophthora thermophila* strain GZUIFR-H49-1. *Journal of Applied Microbiology* 110: 871–880. https://doi.org/10.1111/j.1365-2672.2011.04949.x

Mains, E.B. (1950) Entomogenous species of *Akanthomyces*, *Hymenostilbe* and *Insecticola* in North America. *Mycologia* 42: 566–589. https://doi.org/10.1080/00275514.1950.12017861

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

Mongkolsamrit, S., Noisripoom, W., Thanakitpipattana, D., Wutikhun, T., Spatafora, J.W. & Luangsa-ard, J. (2018) Disentangling cryptic species with *Isaria*-like morphs in Cordycipitaceae. *Mycologia* 110: 230–257. https://doi.org/10.1080/00275514.2018.1446651

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

Samson, R.A. & Evans, H.C. (1974) Notes on entomogenous fungi from Ghana: II. The genus *Akanthomyces*. *Acta Botanica Neerlandica*

23: 28–35.
https://doi.org/10.1111/j.1438-8677.1974.tb00913.x

Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
https://doi.org/10.1093/bioinformatics/btu033

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

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 (2): 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

Vincent, M.A., Seifert, K.A. & Samson, R.A. (1988) *Akanthomyces johnsonii*, a saprophytic synnematous hyphomycete. *Mycologia* 80: 685–688.
https://doi.org/10.1080/00275514.1988.12025601

White, T.J., Bruns, T., Lee, S. & Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (Eds.) *PCR protocols: a guide to methods and applications*. Academic Press, New York, pp. 315–322.
https://doi.org/10.1016/B978-0-12-372180-8.50042-1