Phytotaxa 432 (2): 119–124
https://www.mapress.com/j/pt/

Article

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

https://doi.org/10.11646/phytotaxa.432.2.2

Akanthomyces neocoleopterorum, a new verticillium-like species

WAN-HAO CHEN[1], YAN-FENG HAN[2*], JIAN-DONG LIANG[1] & ZONG-QI LIANG[2]
[1]*Department of Microbiology, Basic Medical School, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, China*
[2]*Institute of Fungus Resources, Guizhou University, Guiyang, Guizhou 550025, China*
**Corresponding author: swallow1128@126.com*

Abstract

A new species, *Akanthomyces neocoleopterorum*, which was isolated from an infected ladybug, is introduced. Morphological comparisons with extant species and DNA-based phylogenies from analysis of a multigene dataset support the establishment of the new species. It differs from other species by having mononematous and verticillium-like conidiophores, longer phialides, and mostly cylindrical conidia. Both the morphological identification and phylogenetic analysis of combined *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* sequence data support *A. neocoleopterorum* as a new species in the genus *Akanthomyces*.

Keywords: Coleoptera, mononematous, morphology, phylogeny, verticillium-like

Introduction

The genus *Akanthomyces* was established by Lebert in 1858 for a species, *A. aculeatus* Lebert, found on a moth in Europe. Mains (1950) emended and revised the genus *Akanthomyces* and characterized it by cylindrical synnemata covered by a hymenium-like layer of phialides producing one-celled catenulate conidia. He also described and illustrated three species, *A. aculeatus* Lebert, *A. ampullifer* (Petch) Mains and *A. aranearum* (Petch) Mains. Samson & Evans (1974) published a review of *Akanthomyces* and treated four species, *A. arachnophilus* (Petch) Samson & H.C. Evans, *A. aranearum*, *A. gracilis* Samson & H.C. Evans and *A. pistillariiformis* (Pat.) Samson & H.C. Evans. Since then, several species of *Akanthomyces* associated with insects or spiders have been described by Koval (1977), Vincent *et al*. (1988), Hywel-Jones (1996), Hsieh *et al*. (1997) and 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 combined *L. attenuatum* Zare & W. Gams, *L. lecanii* (Zimm.) Zare & W. Gams, *L. longisporum* (Petch) Zare & W. Gams, *L. muscarium* (Petch) Zare & W. Gams and *L. sabanense* Chir.-Salom., S. Restrepo & T.I. Sanjuan into *Akanthomyces*. They also transferred *A. arachnophilus*, *A. cinereus* Hywel-Jones, *A. koratensis* Hywel-Jones, *A. longisporus* B. Huang, S.B. Wang, M.Z. Fan & Z.Z. Li, *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) reported five spider-associated species, *Akanthomyces kanyawimiae* Mongkols. *et al*., *A. ryukyuenis* Mongkols. *et al*., *A. sulphureus* Mongkols. *et al*., *A. thailandicus* Mongkols., Spatafora & Luangsa-ard and *A. waltergamsii* Mongkols. *et al*. Chen *et al*. (2018, 2019) described two spider pathogenic species *A. araneogenum* Z.Q. Liang, W.H. Chen & Y.F. Han (now treated as *A. araneogenus* Z.Q. Liang, W.H. Chen & Y.F. Han) and *A. araneicola* W.H. Chen *et al.* Shrestha *et al.* (2019) transferred *Lecanicillium araneogenum* Wan H. Chen *et al.* to *Akanthomyces neoaraneogenum* (W.H. Chen *et al*.) W.H. Chen, Y.F. Han & Z.Q. Liang. Currently, *Akanthomyces* consists of 23 species that have been isolated from soil, insects and spiders (Chen *et al*. 2018, 2019, Shrestha *et al*. 2019).

An infected insect specimen was found during a survey of araneogenous fungi and its allies from southwestern China. It is described here as *Akanthomyces neocoleopterorum sp. nov*. and is supported by morphological characters and a phylogenic analysis.

Accepted by Eric McKenzie: 6 Dec. 2019; published: 10 Feb. 2020

Materials and methods

Specimen collection and identification

A fungus infected insect specimen was collected in Tongmuling, Guiyang city (N 26°23′25.92″, E 106°41′3.35″), on 9 November 2018. The fungus was isolated and cultured on an agar plate containing improved potato dextrose agar (PDA, 1 % w/v peptone) medium.

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 fresh hyphae were observed with an optical microscope (OM, DM4 B, Leica, Germany) following pretreatment with lactophenol cotton blue solution or normal saline. 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*. (2011). 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 using 983F/2218R and RPB2-5F/RPB2-7Cr primers 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 using ITS4/ITS5 primers 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*, *RPB1*, *RPB2* and *TEF* were selected based on Kepler *et al*. (2017), Mongkolsamrit *et al*. (2018), Chen *et al*. (2018, 2019) 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 24836.

Results

Sequencing and phylogenetic analysis

The *ITS*, *LSU*, *RPB1*, *RPB2* and *TEF* sequences from strain GY11241 were deposited in GenBank with accession numbers MN093295, MN09396, MN097816, MN097812 and MN097813, respectively. The concatenated alignment of *ITS+LSU+RPB1+RPB2+TEF* sequences was 2608 bp long. The three sets of sequences, from strains GY11241 and GY11242, formed a clade in both ML and Bayesian analyses (Fig. 1).

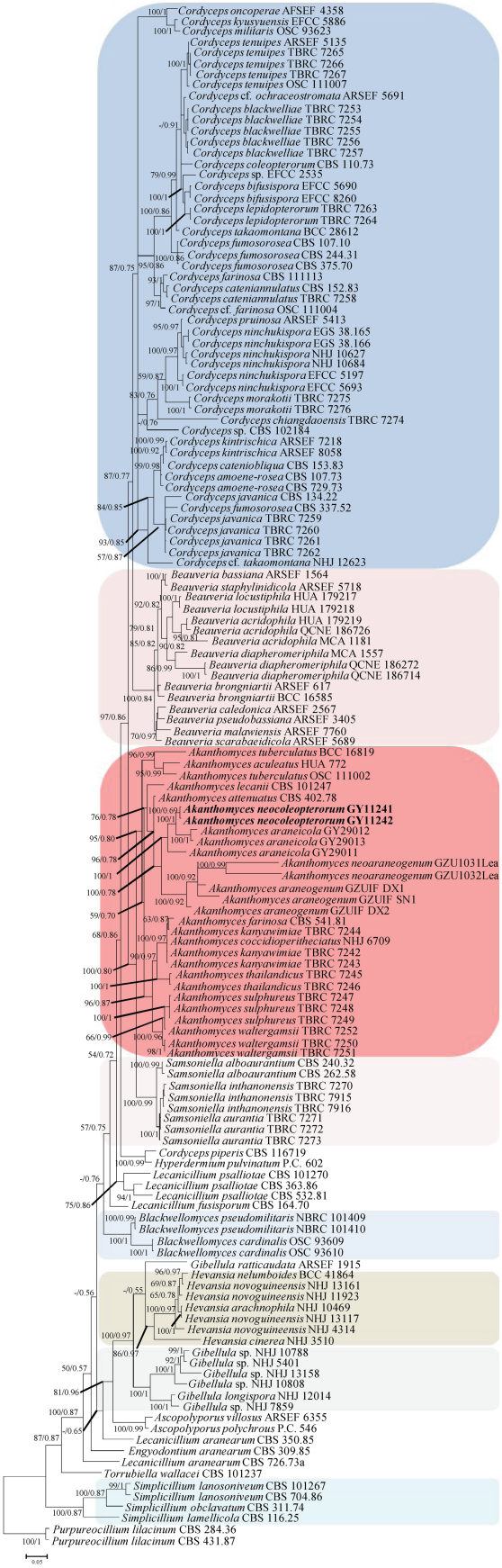

FIGURE 1. Phylogenetic analysis of *Akanthomyces neocoleopterorum* (strains GY11241, GY11242) and related species based on combined partial *ITS+LSU+RPB1+RPB2+TEF* sequences. Statistical support values (⩾50 %) are shown at nodes, and presented as bootstrap values/Bayesian posterior probabilities.

Taxonomy

Akanthomyces neocoleopterorum W.H. Chen, Y.F. Han, J.D. Liang & Z.Q. Liang, *sp. nov*. (Fig. 2) Mycobank No.: MB 832101

Type:—CHINA. Guizhou Province: Guiyang City, Tongmuling (N 26°23′25.92″, E 106°41′3.35″), on a ladybug, 9 November 2018, Wanhao Chen, holotype GZAC GY1124; ex-type culture GZAC GY11241.

Colonies on PDA, attaining a diameter of 51–53 mm after 14 days at 25 °C, white to yellowish, powdery, thin; reverse yellowish. *Hyphae* septate, hyaline, smooth-walled, 1.7–2.4 µm wide. *Conidiophores* mononematous, hyaline, smooth-walled, with single phialide or whorls of 2–5 phialides, or verticillium-like from hyphae directly. *Phialides* consisting of a cylindrical, somewhat inflated base, 19.9–29.6 × 1.6–2.0 µm, tapering to a thin neck. *Conidia* hyaline, smooth-walled, mostly cylindrical, 3.3–6.6 × 1.5–1.8 µm, forming divergent and basipetal chains. In culture both phialides and conidia are of similar general shape and size to those found on the ladybug.

Etymology:—referring to a new insect host in order Coleoptera.

Additional strains examined:—CHINA. Guizhou Province: Guiyang City, Tongmuling (N 26°23′25.92″, E 106°41′3.35″), on a ladybug, 9 November 2018, Wanhao Chen (GY11242). Sequences from this strain have been deposited in GenBank with accession numbers: *ITS*=MN09397, *LSU*=MN09398, *RPB1*=MN097817, *RPB2*= MN097814, *TEF*= MN097815.

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

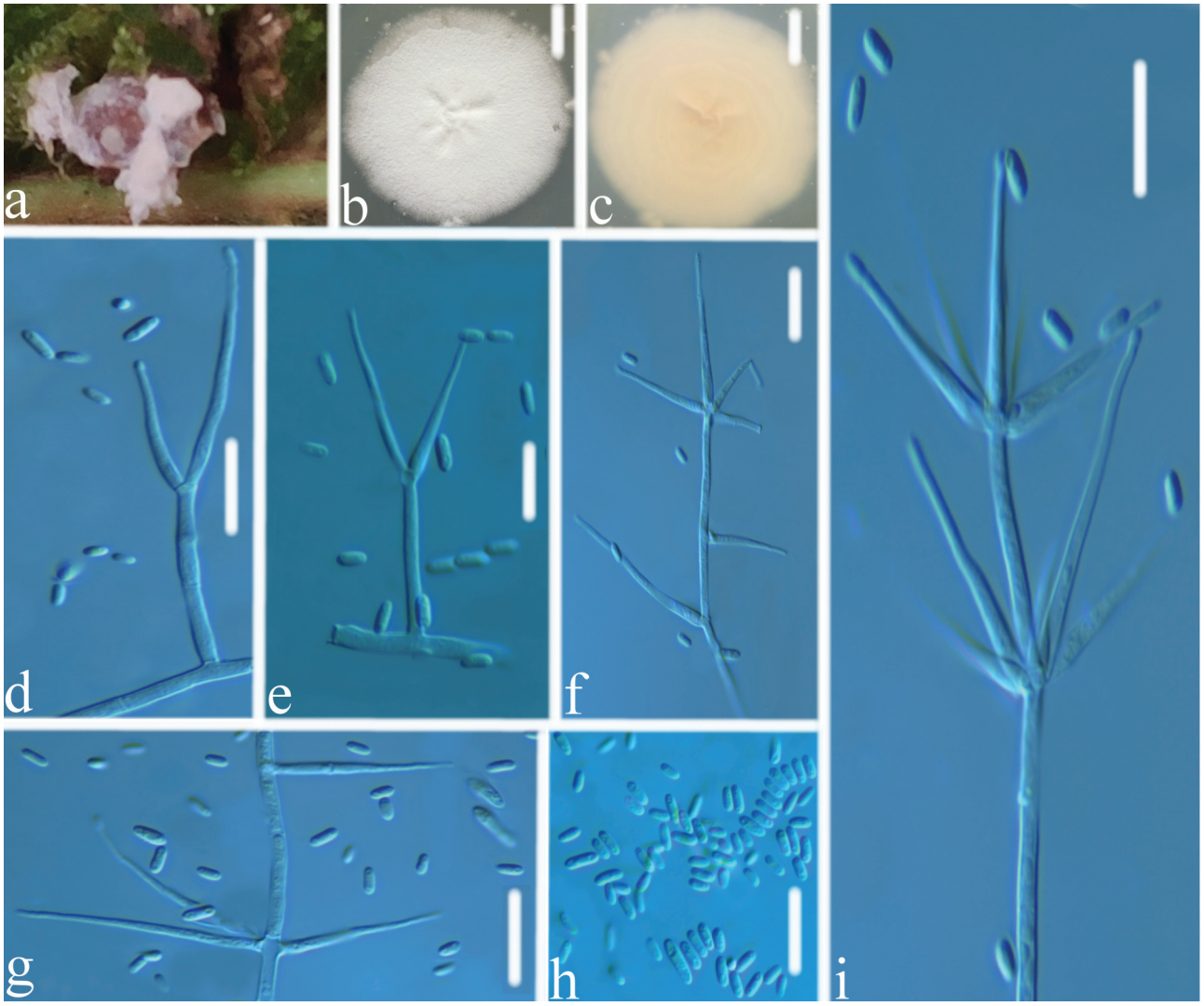

FIGURE 2. *Akanthomyces neocoleopterorum sp. nov.* **a.** Infected ladybug, **b, c.** Colony on PDA after 14 d at 25 °C, **d–g, i.** Conidiophores, conidiogenous cells and conidia, **h.** Conidia. Bars: b, c = 10 mm; d–i = 10 µm.

Discussion

As originally described, the characters of *Akanthomyces* were white, cream or flesh-colored cylindrical, attenuated synnemata covered with a hymenium of phialides. These phialides are either 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). Kepler *et al*. (2017) treated *Lecancillium* W. Gams & Zare as a junior synonym of *Akanthomyces*. The typical characters of strain GY11241 easily identified it as belonging to *Akanthomyces* and it is distinguished from other species by its mononematous and verticillium-like conidiophores, longer phialides (19.9–29.6 × 1.6–2.0 μm), and mostly cylindrical conidia (3.3–6.6 × 1.5–1.8 μm).

Concatenated analyses of *ITS*, *LSU*, *TEF*, *RPB1* and *RPB2* produced ML and Bayesian trees that were largely congruent. Most branches were strongly supported in both analyses. The two strains of *Akanthomyces neocoleopterorum* clustered together, distinct from other *Akanthomyces* species. Thus, molecular phylogenetic results supported the morphologically based conclusion that strain GY11241 is a new species in the genus *Akanthomyces*, described here as *A. neocoleopterorum*.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 31460010, 31860002), the Doctoral Fund of Guizhou University of Traditional 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 (1): 66–72. https://doi.org/10.11646/phytotaxa.379.1.6

Chen, W.H., Liu, C., Han, Y.F., Liang, J.D., Tian, W.Y. & Liang, Z.Q. (2019) *Akanthomyces araneicola*, a new araneogenous species from Southwest China. *Phytotaxa* 409 (4): 227–232. https://doi.org/10.11646/phytotaxa.409.4.5

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. (2011) 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
Shrestha, B., Kubátová, A., Tanaka, E., Oh, J., Yoon, D.H., Sung, J.M. & Sung, G.H. (2019) Spider-pathogenic fungi within Hypocreales (Ascomycota): their current nomenclature, diversity, and distribution. *Mycological Progress* 18 (8): 983–1003. https://doi.org/10.1007/s11557-019-01512-3
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