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Species diversity and major host–substrate associations of the genus Akanthomyces

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Abstract

Akanthomyces, a group of fungi with rich morphological and ecological diversity in Cordycipitaceae (Ascomycota, Hypocreales), has a wide distribution among diverse habitats. By surveying arthropod-pathogenic fungi in China and Southeast Asia over the last six years, nine *Akanthomyces* spp. were found and identified. Four of these were new species, and they were named *A. kunmingensis* and *A. subaraneicola* from China, *A. laosensis* from Laos, and *A. pseudonoctuidarum* from Thailand, and they were described and illustrated according to the morphological characteristics and molecular data. *Akanthomyces araneogenus*, which is isolated from spiders from different regions in China, Thailand, and Vietnam, is described as a newly recorded species from Thailand and Vietnam. The phylogenetic positions of the nine species were evaluated based on phylogenetic inferences according to five loci, namely, ITS, nr*LSU*, *TEF*, *RPB1*, and *RPB2*. In this study, we reviewed the research progress achieved for *Akanthomyces* regarding its taxonomy, species diversity, geographic distribution, and major host–substrate associations. The morphological characteristics of 35 species in *Akanthomyces*, including four novel species and 31 known taxa, were also compared.

Introduction

Akanthomyces is one of the oldest genera in the family Cordycipitaceae (Ascomycota, Hypocreales). This genus was established by Lebert in 1858 on the basis of the type species, *A. aculeatus*, which was found on a moth in France (Lebert 1858). Morphologically, *Akanthomyces* genera have been characterized asexually by white, cream, or flesh-colored cylindrical, attenuated synnematal growth covered by a hymenium-like layer of phialides producing one-celled catenulate conidia (Mains 1950; Samson and Evans 1974; Hsieh et al. 1997). These phialides are ellipsoidal, cylindrical, or narrowly cylindrical, and gradually or abruptly taper to a more or less distinct neck (Hsieh et al. 1997). Owing to extensive overlap in their morphological characteristics, *Akanthomyces* was once considered as a synonym of *Lecanicillium*, an anamorph within Cordycipitaceae with verticillium-like morphology (Gams and Zare 2001); however, many species originally described in *Lecanicillium* do not form a single monophyletic clade and are distributed throughout Cordycipitaceae (Wang et al. 2020). Kepler et al. (2017) phylogenetically established the genetic boundaries in Cordycipitaceae, and they proposed that *Lecanicillium* should be rejected and instead could be considered as a synonym of *Akanthomyces* (Kepler et al. 2017). Chiriví-Salomón et al. (2015) also showed that the type species of *Lecanicillium*, *L. lecanii* (as *Cordyceps confragosa*), as well as several other *Lecanicillium* species, namely, *L. attenuatum*, *L. muscarium*, and *L. sabanense*, fall within *Akanthomyces* (Chiriví-Salomón et al. 2015). The teleomorph of *Akanthomyces* was originally described as *Torrubiella*, and it was characterized by producing superficial perithecia on a loose mat of hyphae (subiculum) or a highly reduced non-stipitate stroma (Boudier 1885). According to the most complete taxonomic treatment of Cordycipitaceae to date by Kepler et al. (2017), this connection was verified by DNA sequencing. Since *Akanthomyces* was described earlier than *Torrubiella*, the recent taxonomic revision recommended *Akanthomyces* as the name of this genus (Kepler et al. 2017).

Over the past two decades, our efforts have been applied to the investigation of Cordycipitoid fungi, especially those located in China and Southeast Asia. To date, our study team has collected over 18,000 specimens and 7,500 strains of *Cordyceps* sensu lato, representing more than 450 species in total (Wang et al. 2020). These specimens and strains sufficiently revealed that Cordycipitaceae is the most complex group in Hypocreales with its varied morphological characteristics and wide-ranging hosts. Some of the genera with sexual and asexual morphs, such as *Akanthomyces* and *Hevansia*, share numerous similar morphological characteristics. The genus *Hevansia* was erected to accommodate asexual morphs on spiders that were previously described under *Akanthomyces*. The type species *Hevansia novoguineensis*, which was previously described as *Akanthomyces novoguineensis*, differs from *Akanthomyces* by the immersed perithecia of the teleomorph in a disk sitting at the top of a well-formed stipe (Aini et al. 2020); however, *H. novoguineensis* must now be an akanthomyces-like teleomorph (Kepler et al. 2017; Aini et al. 2020). *Akanthomyces*, *Samsoniella*, and *Cordyceps* species produce similar isaria-like asexual conidiogenous structures, such as flask-shaped phialides produced in whorls and conidia with divergent chains (Wang et al. 2020; Wang et al. 2022). Due to the extensive overlap in morphological characteristics and the lack of distinctive phenotypic variation, species in many genera, *Akanthomyces* in particular, are not easily classified and identified. Thus, more known species and new species in the genus *Akanthomyces* need to be introduced and supported by more detailed morphological and phylogenetic evidence in combination with a larger taxon sampling.

In surveys of arthropod-pathogenic fungi from different regions in Yunnan and Hunan Province, China; Chiang Rai Province, Thailand; Nghe An Province, Vietnam; and Oudomxay Province, Laos, over the last six years, approximately nine *Akanthomyces* spp. were located and identified (Table 1). In this study, we aimed to: 1) reveal the hidden species diversity of the genus *Akanthomyces* according to phylogenetic analyses and morphological observation, and 2) systematically review the geographical distribution and major host–substrate associations of *Akanthomyces* species by surveying the literature to the extent possible, and combining the results with those generated in our study.

Table 1
Specimen information and GenBank accession numbers for sequences used in this study

Species	Voucher information	Host/Substrate	GenBank accession numbers					Reference
			ITS	nr*LSU*	*TEF*	*RPB1*	*RPB2*	
Akanthomyces aculeatus	HUA 186145	–	–	MF416520	MF416465	–	–	Kepler et al. (2017)
Akanthomyces aculeatus	TS772	Lepidoptera; Sphingidae	KC519371	KC519370	KC519366	–	–	Sanjuan et al. (2014)
Akanthomyces araneicola	GY29011^{T}	Araneae; spider	MK942431	–	MK955950	MK955944	MK955947	Chen et al. (2019)
Akanthomyces araneogenus	GZUIF DX2^{T}	Araneae; spider	MH978179	–	MH978187	MH978182	MH978185	Chen et al. (2018)
Akanthomyces araneogenus	**YFCC 1811934**	**Araneae; spider**	**OQ509518**	**OQ509505**	**OQ506281**	**OQ511530**	**OQ511544**	**This study**
Akanthomyces araneogenus	**YFCC 2206935**	**Araneae; spider**	**OQ509519**	**OQ509506**	**OQ506282**	**OQ511531**	**OQ511545**	**This study**
Akanthomyces araneosus	KY11341^{T}	Araneae; spider	ON502826	ON502832	ON525443	–	ON525442	Chen et al. (2022)
Akanthomyces attenuatus	CBS 170.76^{T}	Lepidoptera; *Carpocapsa pomonella*	MH860970	OP752153	OP762607	OP762611	OP762615	Manfrino et al. (2022)
Akanthomyces coccidioperitheciatus	NHJ 6709	Araneae; spider	JN049865	EU369042	EU369025	EU369067	EU369086	Kepler et al. (2012)
Akanthomyces dipterigenus	CBS 126.27	Hemiptera; *Icerya purchasi*	AJ292385	KM283797	KM283820	KR064300	KM283862	Kepler et al. (2017)
Akanthomyces dipterigenus	**YFCC 2107933**	**Soil**	**OQ509520**	**OQ509507**	**OQ506283**	**OQ511532**	**OQ511546**	**This study**
Akanthomyces kanyawimiae	TBRC 7242	Araneae; spider	MF140751	MF140718	MF140838	MF140784	MF140808	Mongkolsamrit et al. (2018)
Akanthomyces kanyawimiae	TBRC 7243	Unidentified	MF140750	MF140717	MF140837	MF140783	MF140807	Mongkolsamrit et al. (2018)
Akanthomyces kunmingensis	**YFCC 1708939**	**Araneae; spider**	**OQ509521**	**OQ509508**	**OQ506284**	**OQ511533**	**OQ511547**	**This study**
Akanthomyces kunmingensis	**YFCC 1808940^{T}**	**Araneae; spider**	**OQ509522**	**OQ509509**	**OQ506285**	**OQ511534**	**OQ511548**	**This study**
Akanthomyces laosensis	**YFCC 1910941^{T}**	**Lepidoptera; Noctuidae**	**OQ509523**	**OQ509510**	**OQ506286**	**OQ511535**	**OQ511549**	**This study**
Akanthomyces laosensis	**YFCC 1910942**	**Lepidoptera; Noctuidae**	**OQ509524**	**OQ509511**	**OQ506287**	**OQ511536**	**OQ511550**	**This study**
Akanthomyces lecanii	CBS 101247	Hemiptera; *Coccus viridis*	JN049836	AF339555	DQ522359	DQ522407	DQ522466	Kepler et al. (2012)
Akanthomyces lepidopterorum	GZAC SD05151^{T}	Lepidoptera (pupa)	MT705973	–	–	–	MT727044	Chen et al. (2020)
Akanthomyces muscarius	CBS 455.70B	–	–	MH871560	–	–	–	Kepler et al. (2017)
Akanthomyces neoaraneogenus	GZU1031Lea^{T}	Araneae; spider	KX845703	–	KX845697	KX845699	KX845701	Chen et al. (2017)
Akanthomyces neocoleopterorum	GY11241^{T}	Coleoptera	MN093296	–	MN097813	MN097816	MN097812	Chen et al. (2020)
Akanthomyces neocoleopterorum	GY11242	Coleoptera	MN093298	–	MN097815	MN097817	MN097814	Chen et al. (2020)
Akanthomyces noctuidarum	BCC 36265^{T}	Lepidoptera; Noctuidae	MT356072	MT356084	MT477978	MT477994	MT477987	Aini et al. (2020)
Akanthomyces noctuidarum	BCC 47498	Lepidoptera; Noctuidae	MT356074	MT356086	MT477980	MT477996	MT477988	Aini et al. (2020)
Akanthomyces noctuidarum	BCC 28571	Lepidoptera; Noctuidae	MT356075	MT356087	MT477981	MT478009	MT478006	Aini et al. (2020)

Boldface: data generated in this study. Ex-type materials are marked with “T”.

Species	Voucher information	Host/Substrate	GenBank accession numbers					Reference
			ITS	nr*LSU*	*TEF*	*RPB1*	*RPB2*	
Akanthomyces pissodis	CBS 118231^{T}	Coleoptera; *Pissodes strobi*	–	KM283799	KM283822	KM283842	KM283864	Chen et al. (2020)
Akanthomyces pseudonoctuidarum	**YFCC 1808943^{T}**	**Lepidoptera; Noctuidae**	**OQ509525**	**OQ509512**	**OQ506288**	**OQ511537**	**OQ511551**	**This study**
Akanthomyces pseudonoctuidarum	**YFCC 1808944**	**Lepidoptera; Noctuidae**	**OQ509526**	**OQ509513**	**OQ506289**	**OQ511538**	**OQ511552**	**This study**
Akanthomyces pyralidarum	BCC 28816^{T}	Lepidoptera; Pyralidae	MT356080	MT356091	MT477982	MT478000	MT478007	Aini et al. (2020)
Akanthomyces pyralidarum	BCC 32191	Lepidoptera; Pyralidae	MT356081	MT356092	MT477983	MT478001	MT477989	Aini et al. (2020)
Akanthomyces sabanensis	ANDES-F 1023	Hemiptera; *Pulvinaria caballeroramosae*	KC633237	–	KC633267	KC875222	–	Kepler et al. (2017)
Akanthomyces sabanensis	ANDES-F 1024	Hemiptera; *Pulvinaria caballeroramosae*	KC633232	KC875225	KC633266	–	KC633249	Kepler et al. (2017)
Akanthomyces **sp.**	**YFCC 945**	**Soil**	**OQ509531**	–	**OQ506294**	**OQ511543**	**OQ511557**	**This study**
Akanthomyces subaraneicola	**YFCC 2107937^{T}**	**Araneae; spider**	**OQ509527**	**OQ509514**	**OQ506290**	**OQ511539**	**OQ511553**	**This study**
Akanthomyces subaraneicola	**YFCC 2107938**	**Araneae; spider**	**OQ509528**	**OQ509515**	**OQ506291**	**OQ511540**	**OQ511554**	**This study**
Akanthomyces sulphureus	TBRC 7248^{T}	Araneae; spider	MF140758	MF140722	MF140843	MF140787	MF140812	Mongkolsamrit et al. (2018)
Akanthomyces sulphureus	TBRC 7249	Araneae; spider	MF140757	MF140721	MF140842	MF140786	MF140734	Mongkolsamrit et al. (2018)
Akanthomyces sulphureus	**YFCC 1710936**	**Araneae; spider**	**OQ509529**	**OQ509516**	**OQ506292**	**OQ511541**	**OQ511555**	**This study**
Akanthomyces thailandicus	TBRC 7245^{T}	Araneae; spider	MF140754	–	MF140839	–	MF140809	Mongkolsamrit et al. (2018)
Akanthomyces tiankengensis	KY11571^{T}	Araneae; spider	ON502848	ON502825	ON525447	–	ON525446	Chen et al. (2022)
Akanthomyces tiankengensis	KY11572	Araneae; spider	ON502821	ON502827	ON525449	–	ON525448	Chen et al. (2022)
Akanthomyces tortricidarum	BCC 72638^{T}	Lepidoptera; Tortricidae	MT356076	MT356088	MT478004	MT477997	MT477992	Aini et al. (2020)
Akanthomyces tortricidarum	BCC 41868	Lepidoptera; Tortricidae	MT356077	MT356089	MT477985	MT477998	MT478008	Aini et al. (2020)
Akanthomyces tuberculatus	HUA 186131	Lepidoptera (adult moth)	–	MF416521	MF416466	–	–	Kepler et al. (2017)
Akanthomyces uredinophilus	KACC 44066	Rust	–	KM283784	KM283808	KM283830	KM283850	Park et al. (2016)
Akanthomyces uredinophilus	KACC 44082^{T}	Rust	–	KM283782	KM283806	KM283828	KM283848	Park et al. (2016)
Akanthomyces uredinophilus	KUN 101466	Insect	MG948305	MG948307	MG948315	MG948311	MG948313	Park et al. (2016)
Akanthomyces uredinophilus	KUN 101469	Insect	MG948306	MG948308	MG948316	MG948312	MG948314	Park et al. (2016)
Akanthomyces waltergamsii	TBRC 7251	Araneae; spider	MF140747	MF140713	MF140833	MF140781	MF140805	Mongkolsamrit et al. (2018)
Akanthomyces waltergamsii	TBRC 7252^{T}	Araneae; spider	MF140748	MF140714	MF140834	MF140782	MF140806	Mongkolsamrit et al. (2018)
Akanthomyces waltergamsii	**YFCC 883**	**Araneae; spider**	**OQ509530**	**OQ509517**	**OQ506293**	**OQ511542**	**OQ511556**	**This study**

Boldface: data generated in this study. Ex-type materials are marked with “T”.

Species	Voucher information	Host/Substrate	GenBank accession numbers					Reference
			ITS	nr*LSU*	*TEF*	*RPB1*	*RPB2*	
Akanthomyces zaquensis	HMAS 246915^T	Fungi; *Ophiocordyceps sinensis*	MT789699	MT789697	MT797812	MT797810	–	Wang et al. (2023)
Akanthomyces zaquensis	HMAS 246917	Fungi; *Ophiocordyceps sinensis*	MT789698	MT789696	MT797811	MT797809	–	Wang et al. (2023)
Samsoniella aurantia	TBRC 7271^T	Lepidoptera	MF140764	MF140728	MF140846	MF140791	MF140818	Mongkolsamrit et al. (2018)
Samsoniella inthanonensis	TBRC 7915^T	Lepidoptera (pupa)	MF140761	MF140725	MF140849	MF140790	MF140815	Mongkolsamrit et al. (2018)

Boldface: data generated in this study. Ex-type materials are marked with "T".

Materials And Methods

Soil and specimen collection

All of the soil samples were collected from Yunnan Province in China. Fungal specimens were obtained from six locations in 2017 and 2022, namely, two different locations in Yunnan Province, China, one location in Hunan Province, China, one location in Chiang Mai Province, Thailand, one location in Nghe An Province, Vietnam, and one location in Oudomxay Province, Laos. Soil samples and specimens were noted and photographed in the field, and then they were carefully put in plastic containers at a low temperature. After that, they were brought to the laboratory and stored at 4°C prior to examination and isolation.

Fungal isolation and culture

The *Akanthomyces* strains were isolated from the soil samples based on the methods described in our prior publications (Wang et al. 2015; Wang et al. 2023). Conidia developing on invertebrate cadavers were transplanted onto plates of potato dextrose agar (PDA; potato 200 g/L, dextrose 20 g/L, agar 20 g/L) and cultured at 25°C. Colonies of the isolated filamentous fungi appearing in the culture were transferred onto fresh PDA media. Each purified fungal strain was transferred to PDA slants and cultured at 25°C until its hyphae spread across the entire slope. The emerging fungal spores were washed with sterile physiological saline to form a suspension containing 1×10^3 cells/mL. To obtain monospore cultures, a sample of the spore suspension was placed on PDA on a Petri dish utilizing a sterile micropipette, and then the dish was incubated at 25°C. Voucher specimens and the corresponding isolated strains were deposited in the Yunnan Herbal Herbarium (YHH) and the Yunnan Fungal Culture Collection (YFCC), respectively, of Yunnan University, Kunming, China.

Morphological observations

The specimens were examined with an Olympus SZ61 stereomicroscope (Olympus Corporation, Tokyo, Japan). Fungal structures of the specimens, such as synnemata, phialides, and conidia, were mounted on glass slides with a drop of lactophenol cotton blue solution. Cultures on PDA slants were transferred to PDA plates, and then they were incubated at 25°C for 14 d. For morphological evaluation, microscope slides were prepared by placing mycelia from the cultures on PDA medium blocks (5 mm diameter) and then overlaid with a coverslip. Micro-morphological observations and measurements were performed with a light microscope (CX40, Olympus Corporation, Tokyo, Japan) and a scanning electron microscope (Quanta 200 FEG, FEI Company, Hillsboro, USA). The individual length and width measurements were recorded for 30–100 replicates, and included the absolute minima and maxima.

DNA extraction, PCR, and sequencing

The specimens and axenic living cultures were prepared for DNA extraction. Genomic DNA was extracted utilizing a Genomic DNA Purification kit (Qiagen GmbH, Hilden, Germany) based on the manufacturer's instructions. The primer pair ITS5/ITS4 was used to amplify a fraction of the internal transcribed spacer regions of the rDNA (ITS rDNA) (White et al. 1990). Primer pair LR5/LR0R (Vilgalys et al. 1990; Rehner et al. 1994) was used to amplify a fraction of the nuclear ribosomal large subunit (nr*LSU*), and EF1α-EF/EF1α-ER primers (Bischoff et al. 2006; Sung et al. 2007) were used to amplify translation elongation factor 1α (*TEF*). For amplification of the largest and second largest subunits of RNA polymerase II (*RPB1* and *RPB2*), PCR primer pairs RPB1-5'F/RPB1-5'R and RPB2-5'F/RPB2-5'R (Bischoff et al. 2006; Sung et al. 2007) were employed. All of the PCR reactions were performed in a final volume of 50 µL and contained 25 µL of 2 × Taq PCR Master Mix (Tiangen Biotech Co., Ltd., Beijing, China), 0.5 µL of each primer (10 µM), 1 µL of genomic DNA, and 23 µL of RNase-free water. Target gene amplification and sequencing were performed based on the methods detailed in our prior study (Wang et al. 2020).

Phylogenetic analyses

The phylogenetic analyses were based on five gene, namely, ITS, nr*LSU*, *TEF*, *RPB1*, and *RPB2*, sequences. The sequences were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/, accessed on March 1, 2023) and combined with those generated in our study. Taxon information and GenBank accession numbers are listed in Table 1. Sequences were aligned with MAFFT v.7 (http://mafft.cbrc.jp/alignment/server/, accessed on March 1, 2023). The aligned sequences were then manually corrected when necessary. After alignment, the sequences of the genes were concatenated. Conflicts among the five genes were resolved with PAUP* 4.0b10 (Swofford et al. 2002). The results showed that the phylogenetic signals for the five loci were congruent ($P = 0.02$). The data partitions were defined for the combined dataset with PartitionFinder V1.1.1 (Lanfear et al. 2012). Phylogenetic analyses were conducted utilizing BI and ML methods employing MrBayes v3.1.2 and RaxML v7.0.3, respectively (Ronquist et al. 2003; Stamatakis et al. 2008). The model selected for BI analysis was from jModelTest version 2.1.4 (Darriba et al. 2012). The following models were implemented in the analysis: GTR + I + G for partitions of ITS, nr*LSU*, and *TEF*, and GTR + I for partitions of *RPB1* and *RPB2*. The BI analysis was executed on MrBayes v3.1.2 for five million generations. GTR + FO + G was selected as the optimal model for ML analysis, and 1000 rapid bootstrap replicates were performed on the dataset.

Identification of host arthropods

The host arthropods of *Akanthomyces* spp. were identified on the basis of morphological characteristics, and they were further identified utilizing molecular analyses according to the mitochondrial cytochrome oxidase I gene (*cox1*) and mitochondrial cytochrome b gene (*cytb*). Genomic DNA was extracted from the head and leg areas of the cadavers of the hosts utilizing the CTAB method (Liu et al. 2001). The *cox1* and *cytb* loci were amplified with the primer pair Hep-cox1F/Hep-cox1R and Hep-cytbF/Hep-cytbR, respectively (Simon et al. 1994). Sequences were analyzed with MEGA v6.06 software (Tamura et al. 2013) and processed by Standard Nucleotide BLAST (GenBank, NCBI nucleotide database) to assess similarity with reported arthropod sequences.

Results

Sequencing and phylogenetic analyses

A combined five-gene dataset included sequences from 56 fungal taxa (Table 1). The final dataset consisted of 4,401 bp of sequence data (ITS 619 bp, nr*LSU* 896 bp, *TEF* 1,022 bp, *RPB1* 731 bp, and *RPB2* 1,133 bp). *Samsoniella aurantia* TBRC 7271 and *Samsoniella inthanonensis* TBRC 7915 within Cordycipitaceae were used as the outgroup sequences for this dataset. Both the Bayesian inference (BI) and maximum likelihood (ML) analyses produced trees with similar topologies that resolved the majority of the *Akanthomyces* lineages into separate terminal branches (Fig. 1). Among the hosts of *Akanthomyces*, Araneae (spider) and Lepidoptera (adult moth) are the two major orders. The majority of the spider pathogens were located at the top of the *Akanthomyces* clade, and adult moth entomopathogens were located at the bottom of the *Akanthomyces* clade (Fig. 1). The phylogenetic analyses also suggested the existence of distinct species in the spider pathogens and adult moth entomopathogens clade that we proposed as new species: *A. kunmingensis* and *A. subaraneicola*, which were found in the spider pathogens clade; and *A. laosensis* and *A. pseudonoctuidarum*, which were found in the adult moth entomopathogens clade (Fig. 1).

Morphological features

The morphological characteristics of the five species as well as photomicrographs of morphological structures are shown in Figs. 2, 3, 4, 5, and 6. The detailed fungal morphological descriptions are supplied in the Taxonomy section.

Taxonomy

In this study, *Akanthomyces* comprised at least 36 species with a cosmopolitan distribution (Table 2). A collection of 31 isolates of unknown identity were shown to represent four known species, four new species, and an undetermined species of *Akanthomyces*. The phylogenetic positions of the four known species were evaluated based on phylogenetic inferences according to five loci, namely, ITS, nr*LSU*, *TEF*, *RPB1*, and *RPB2*, including *A. araneogenus* from China, Thailand, and Vietnam, *A. dipterigenus* and *A. waltergamsii* from China, and *A. sulphureus* from Vietnam (see Table 2 and Fig. 1). The four new species, given the names *A. kunmingensis* and *A. subaraneicola* from China, *A. laosensis* from Laos, and *A. pseudonoctuidarum* from Thailand, were recognized according to morphological characteristics and molecular data. The isolate YFCC 945 from China represented unknown species in the genus *Akanthomyces*. Unfortunately, the isolate did not produce conidia or reproductive structures when grown on PDA and other media, and they were thus tentatively treated as an undetermined species of *Akanthomyces*, pending further investigation.

Table 2
Species diversity, host–substrate and geographic distribution of *Akanthomyces* species

Species	Host/Substrate	Know Distribution	References
Akanthomyces aculeatus	*Amphipyra pyramidoides* (Lepidoptera, Noctuidae); adult moth (Lepidoptera)	USA (Connecticut; Washington; Ontario); Brazil (Salvador)	Mains (1950)
Akanthomyces angustispora	Coleopterous larva	USA (Nashville)	Mains (1950)
Akanthomyces aranearum	Spider (Araneae)	USA (North Carolina; Maine); Ceylon; Netherlands; Ghana (Begoro); China	Mains (1950); Samson and Evans (1974); Hsieh et al. (1997); Zare and Gams (2001)
Akanthomyces araneicola	Spider (Araneae)	China (Guizhou)	Chen et al. (2019)
Akanthomyces araneogenus	**Spider (Araneae)**	China (Guizhou; **Yunnan**); **Thailand (Chiang Mai); Vietnam (Nghe An)**	Chen et al. (2018); **This study**
Akanthomyces araneosus	Spider (Araneae)	China (Guizhou)	Chen et al. (2022)
Akanthomyces attenuatus	*Cydia pomonella* (Lepidoptera, Tortricidae); leaf litter of *Acer saccharum*; *Symplocarpus foetidus* (plants); *Astrocaryum sciophilum* (plants)	Poland; USA; Canada; French	Zare and Gams (2001); Ellsworth et al. (2013); Barthélemy et al. (2019)
Akanthomyces clavata	*Hapithus agitator* (Orthoptera, Gryllidae)	USA (Florida)	Mains (1950)
Akanthomyces coccidioperitheciatus	Spider (Araneae)	Japan	Kepler et al. (2017); Johnson et al. (2009)
Akanthomyces dipterigenus	Hemiptera: *Icerya purchasi* (Coccidae); *Myzus persicae* (Aphididae); *Macrosiphoniella sanborni* (Aphididae); *Citrus aphid* (Aphididae); **soil**	UK; Sri Lanka; Peru; **China (Yunnan)**	Kepler et al. (2017); Zare and Gams (2001); **This study**
Akanthomyces fragilis	Orthopterous larva	Trinidad; Guiana; Brazil	Mains (1950); Petch (1937)
Akanthomyces gracilis	Hymenoptera, Formicidae (*Paltothyreus tarsatus*; *Platythyrea conradti*; *Polyrhachis militaris*; *Polyrhachis monista*; *Polyrhachis decemdentata*; *Camponotus brutus*; *Oecophylla longinoda*; *Crematogaster bequarti*; *Crematogaster clariventris*; *Macromischoides inermis*; *Macromischoides aculeatus*; *Dorylus* sp.); Coleoptera (beetle larvae, beetle imago); Lepidoptera larva; Hemiptera (Pyrrhocoridae; Cercopidae)	Ghana (Begoro); China (Guizhou)	Samson and Evans (1974); Liang et al. (2013)
Akanthomyces johnsonii	Leaf and stem (*Arctium* sp., *Begonia* sp., *Coffea* sp., *Dianthus* sp., *Ipomoea* sp., *Kalanchoe* sp., *Lycopersicon* sp., *Peperomia* sp., and *Sargassum* sp.); often associated with species of *Botryosporium*	Ghana; Indonesia; Australia (Great Barrier Reef); UK; USA; Canada	Vincent et al. (1988)
Akanthomyces kanyawimiae	Spider (Araneae)	Thailand (Phetchabun; Chanthaburi)	Mongkolsamrit et al. (2018)
Akanthomyces kunmingensis	**Spider (Araneae)**	**China (Yunnan)**	**This study**
Akanthomyces laosensis	**Adult moth (Lepidoptera, Noctuidae)**	**Laos (Oudomxay)**	**This study**

Boldface: data generated in this study.

Species	Host/Substrate	Know Distribution	References
Akanthomyces lecanii	Hemiptera, Coccidae: *Pulvinaria floccifera*; *Coccus viridis*; scale insect. *Tetranychus urticae* (Acari: Tetranychidae); *Pistacia vera* (plants); *Ammophila arenaria* (plants); *Dactylis glomerata* (plants); *Deschampsia flexuosa* (plants); *Elymus farctus* (plants); *Laretia acaulis* (plants); *Pinus sylvestris* (plants); *Shorea thumbuggaia* (plants); *Taxus baccata* (plants)	W. Indies; Dominican Republic; Peru; Jamaica; USA; Sri Lanka; Indonesia; Turkey; China; Iran; Spain; Finland; Chile; Italy; Poland; India	Kepler et al. (2017); Zare and Gams (2001); Dash et al. (2018); Dolatabad et al. (2017); Nicoletti and Becchimanzi (2020)
Akanthomyces lepidopterorum	Pupa of Lepidoptera	China (Guizhou)	Chen et al. (2020)
Akanthomyces muscarius	*Trialeurodes vaporariorum* (Hemiptera, Aleyrodidae); *Brachycaudus helichrysi* (Hemiptera, Aphididae); *Cecidophyopsis ribis* (Acari, Eriophyidae); *Cossus cossus* (Lepidoptera, Cossidae); *Zyginidia pullula* (Hemiptera, Cicadellidae); *Thrips tabaci* (Thysanoptera, Thripidae); peat; contaminated pesticide solution; *Pteridium aquilinum* (Pteridophyta); leaves of *Nypa fruticans* (Plants); *Hemileia vastatrix* (Fungi); water from domestic supply; laboratory glyphosate solution; *Acer campestre* (plants); *Laurus nobilis* (plants); *Myrtus communis* (plants); *Nypa fruticans* (plants); *Quercus robur* (plants); *Prunus cerasus* (plants); *cabbage plants*	UK; Italy; New Caledonia; Thailand; New Zealand	Kepler et al. (2017); Zare and Gams (2001); Nicoletti and Becchimanzi (2020); Vinit et al. (2018); Aghdam et al. (2017); Kuchár et al. (2019)
Akanthomyces neoaraneogenus	Spider (Araneae)	China (Guizhou)	Chen et al. (2017); Mains (1949)
Akanthomyces neocoleopterorum	Ladybug (Coleoptera)	China (Guizhou)	Chen et al. (2020)
Akanthomyces noctuidarum	Adult moth (Lepidoptera, Noctuidae)	Thailand (Narathiwat; Nakhon Ratchasima; Kamphaeng Phet)	Aini et al. (2020)
Akanthomyces pissodis	Adult of *Pissodes strobi* (Coleoptera, Curculionidae)	Canada	Chen et al. (2020); Cope and Leal (2005)
Akanthomyces pseudonoctuidarum	**Adult moth (Lepidoptera, Noctuidae)**	**Thailand (Chiang Mai)**	**This study**
Akanthomyces pyralidarum	Adult moth (Lepidoptera, Pyralidae)	Thailand (Kanchanaburi; Chiang Mai; Phetchabun)	Aini et al. (2020)
Akanthomyces ryukyuenis	Spider (Araneae)	Japan	Kobayasi and Shimizu (1982)
Akanthomyces sabanensis	*Pulvinaria caballeroramosae* (Hemiptera, Coccidae)	Colombia	Kepler et al. (2017); Chiriví-Salomón et al. (2015)
Akanthomyces subaraneicola	**Spider (Araneae)**	**China (Hunan; Yunnan)**	**This study**
Akanthomyces sulphureus	**Spider (Araneae)**	Thailand (Nakhon Ratchasima; Surat Thani); **Vietnam (Nghe An)**	Mongkolsamrit et al. (2018); **This study**
Akanthomyces thailandicus	Spider (Araneae)	Thailand (Chiang Mai)	Mongkolsamrit et al. (2018)
Akanthomyces tiankengensis	Spider (Araneae)	China (Guizhou)	Chen et al. (2022)
Akanthomyces tortricidarum	Adult moth (Lepidoptera, Tortricidae)	Thailand (Nakhon Ratchasima; Kamphaeng Phet)	Aini et al. (2020)

Boldface: data generated in this study.

Species	Host/Substrate	Know Distribution	References
Akanthomyces tuberculatus (= *A. pistillariaeformis*)	Adult moth (Lepidoptera); Hymenoptera, Formicidae; Hemiptera, Pyrrhocoridae	China (Zhejiang; Yunnan); Begoro; Trinidad	Mains (1950); Samson and Evans (1974); Liang et al. (2007)
Akanthomyces uredinophilus	Rust; decayed insect	Korea (Gangwon; North Chungcheong); China (Yunnan)	Park et al. (2016); Wei et al. (2018)
Akanthomyces waltergamsii	**Spider (Araneae)**	Thailand (Saraburi; Naknon Ratchasima); **China (Yunnan)**	Mongkolsamrit et al. (2018); **This study**
Akanthomyces zaquensis	The stroma and the sclerotium of *Ophiocordyceps sinensis* (Fungi)	China (Qinghai)	Wang et al. (2013)
Boldface: data generated in this study.			

Formal descriptions of the five *Akanthomyces* species

Akanthomyces araneogenus Z.Q. Liang, W.H. Chen and Y.F. Han, Phytotaxa 379(1): 69 (2018). (Fig. 2)

MycoBank: MB816114.

Description: **Sexual morph**: Undetermined. **Asexual morph**: See Chen et al. (2018). The descriptions that follow are based on other examined specimens that were obtained from Thailand and Vietnam. Mycosed hosts covered with white to pale yellow mycelia, occasionally several synnemata arise from all of the parts of the host. Colonies on PDA moderately fast-growing at 25°C, reaching a diameter of 25–36 mm in 14 days at 25°C, circular, middle bulge, white to yellowish, reverse yellowish. Hyphae smooth-walled, branched, septate, hyaline, 0.5–2.9 µm wide. Conidiophores smooth-walled, cylindrical, solitary, 10.6–22.4 × 1.3–2.6 µm (n = 30). Phialides consisting of a cylindrical, somewhat inflated base, verticillate on conidiophores, usually in whorls of 2–3, or solitary on hyphae, 8.1–17.8 × 1.1–3.6 µm (n = 30). Conidia smooth and hyaline, one-celled, globose, 1.6–2.4 µm in diameter, or ellipsoidal to fusiform, 2.2–4.1 × 1.1–2.3 µm (n = 50), often in chains. Size and shape of phialides and conidia similar in culture and on natural substratum.

Host

Spider (Araneae).

Habit

On the spiders on dead stems or emerging from leaf litter.

Distribution

Guizhou and Yunnan Province, China; Chiang Mai Province, Thailand; Nghe An Province, Vietnam.

Material examined: Thailand, Chiang Mai Province, Chiang Mai City, Queen Sirikit Botanic Garden (18.8990°N, 98.8604°E, 547 m above sea level), on a spider on a dead stem, November 20, 2018, Yao Wang (YHH 2301001; living culture: YFCC 1811934). Vietnam, Nghe An Province, Pu Mat National Park (18.9292°N, 104.5889°E, 621 m above sea level), on spiders emerging from leaf litter on the forest floor, April 28, 2017, Yao Wang (YHH 2301007, YHH 2301012; living culture: YFCC 1704946, YFCC 1704947). China, Yunnan Province, Dai Autonomous Prefecture of Xishuangbanna, Mengla County (21.1817°N, 101.7252°E, 875 m above sea level), on a spider on a dead stem, June 12, 2022, Zhi-Qin Wang (YHH 2301002; living culture: YFCC 2206935).

Commentary

In our phylogenetic analyses, *A. araneogenus* ex-type strain (GZUIF DX2) and *A. tiankengensis* ex-type isolate (KY11571) and our two samples isolated from the spiders formed a well-supported clade (Fig. 1). From a phylogenetic point of view, *A. tiankengensis* could not be distinguished from *A. araneogenus*, being inside the clade of the latter. Morphologically, previous morphological observations revealed several differences in the characteristics between *A. araneogenus* and *A. tiankengensis*, except they share the same hosts (Chen et al. 2018; Chen et al. 2022); however, our samples from different regions showed diversity of morphology in this study. The colony color and the shape and size of the phialides and conidia of *A. araneogenus* and *A. tiankengensis* among other morphological features have been noted in our samples. There is reason to believe that distinguishing the two species is difficult because of the extensive overlap in morphological characteristics. Thus, we propose that *A. tiankengensis* is a synonym of *A. araneogenus*.

Akanthomyces kunmingensis H. Yu bis, Y. Wang & Z.Q. Wang, sp. nov. (Fig. 3)

MycoBank: MB848307.

Etymology

Named after the location, Kunming City, where the species was collected.

Type: **China**, Yunnan Province, Kunming City, Wild Duck Lake Forest Park (25.2181°N, 102.8503°E, 2100 m above sea level), on a spider on a dead stem, August 14, 2018, collected by Yao Wang (holotype: YHH 16988; ex-type living culture: YFCC 1808940).

Description: **Sexual morph**: Undetermined. **Asexual morph**: Synnemata arising from spider body, cream to light yellow, erect, irregularly branched, producing a mass of conidia at the upper apex, powdery and floccose. Colonies on PDA reaching 15–20 mm in diameter after 14 days at 25°C, circular, white, and fluffy mycelium, middle bulge, reverse pale yellow to light brown. Hyphae smooth-walled, branched, septate, hyaline, 0.5–2.8 μm wide. Conidiophores smooth-walled, cylindrical, solitary, sometimes verticillate, 4.3–9.5 × 1.2–2.0 μm (n = 30). Phialides consisting of a cylindrical, somewhat inflated base, verticillate on conidiophores, usually in whorls of 4–5, or solitary on hyphae, 6.2–29.4 × 1.1–2.5 μm (n = 30). Conidia smooth and hyaline, ellipsoidal to long oval, one-celled, 1.9–3.5 × 1.1–1.8 μm (n = 50), often in chains. Size and shape of phialides and conidia similar in culture and on natural substratum.

Host

Spider (Araneae).

Habit

On spiders on dead stems.

Distribution

At present known only from Kunming City, Yunnan Province, China.

Other material examined: China, Yunnan Province, Kunming City, Songming County, Dashao Village (25.3924°N, 102.5589°E, 2700 m above sea level), on a spider on a dead stem, August 12, 2017, Yao Wang (YHH 2301006; living culture: YFCC 1708939).

Commentary

In regard to phylogenetic relationships, *A. kunmingensis* forms a distinct lineage in the genus *Akanthomyces*, and it is closely related to *A. sulphureus* and *A. waltergamsii* (Fig. 1). Morphologically, *A. kunmingensis* is so similar to *A. waltergamsii* that it was once referred to as *A. waltergamsii* by us (Wang et al. 2020); however, a morphological observation revealed a significant difference of conidia shapes between *A. kunmingensis* and *A. waltergamsii*. *Akanthomyces kunmingensis* usually produces a variety of shapes of conidia (viz., spherical, ellipsoidal to long oval, or fusiform), while *A. waltergamsii* produces only ellipsoidal and fusiform conidia. Moreover, *A. kunmingensis* can be distinguished from *A. sulphureus* and *A. waltergamsii* by its longer phialides (6.2–29.4 μm) and smaller conidia (1.9–3.5 × 1.1–1.8 μm) (Table 3).

Table 3
Morphological comparison of *Akanthomyces* species

Species	Perithecia (μm)	Asci (μm)	Part-spores (μm)	Synnemata (mm)	Conidiophores (μm)	Phialides (μm)	Conidia (μm)	Referer
Akanthomyces aculeata				Arising from various parts of the insect, terete, narrowing upward, 1–8 × 0.1–0.5, yellowish		Subcylindric or narrowly ellipsoid, 6–16 × 2.5–4, narrowing above to an acute apex terminated by a short sterigma up to 4 long	Broadly ellipsoid or obovoid often acute at the lower end, 3–6 × 2–3	Mains (
Akanthomyces aranearum				Arising from all parts of the host, cylindric to clavate, 0.8–10 × 0.1–0.2, simple or occasionally slightly branched, brown		Obovoid or ellipsoid 6–12 × 4–8, rounded above and abruptly narrowing into a short sterigma, asperulate	Narrowly obclavate often acute at the lower end, narrowing upward, rounded or obtuse at the upper end, 8–14 × 1.5–3	Mains (
Akanthomyces araneicola				Synnemata not observed	Mononematous, with single phialide or whorls of two to six phialides, or *Penicillium*-like from hyphae directly	Cylindrical, somewhat inflated base, 8.1–16.9 × 1.3–1.9, tapering to a thin neck	Mostly fusiform, 2.5–5.0 × 1.3–1.9	Chen et (2019)
Akanthomyces araneogenus				Occasionally several white synnemata arise from all parts of the host	Mononematous or synnematous, 21.6–48 × 1.2–2.2, *Penicillium*-like from hyphae directly	Cylindrical, somewhat inflated base, 4.3–17.3 × 0.9–3.1, tapering to a thin neck	Globose, 1.3–2.4 in diam, or ellipsoid, 2.1–3.3 × 1.1–1.6	Chen et (2018)
Akanthomyces araneosus				Synnemata not observed	Erect conidiophores usually arose from the aerial hyphae	Solitary or in groups of two, 16.9–18.1 × 1.3–1.9 with a cylindrical basal portion and tapered into a short, distinct neck	Fusiform, 3.1–5.0 × 1.0–1.8	Chen et (2022)
Akanthomyces angustispora				Arising from the body and head of the host, simple or branched, 8–13 × 0.2–0.6, flesh colored		Oblong or narrowly ellipsoid, 6–14 × 3–4, narrowing above into an acute apex terminated by a short sterigma	Narrowly clavate, 4.5–6 × 1.2–1.4	Mains (
Akanthomyces attenuatus						9–15.5 × 1–2	Cylindrical with attenuate base, occasionally 2-celled, 4.5–6.5 × 1.5–2.0	Kepler ((2017); and Ga (2001)
Akanthomyces clavata				Numerous, arising from various parts of the host, light brown, clavate, 0.5–2.0 × 0.06–0.25		Subcylindric, 17.1–21.4 × 2.8–4.3, narrowing above to acute apices, terminated by short sterigmata	Ellipsoid to oblong, 4.5–8.5 × 2.1–2.5	Mains (
Akanthomyces dipterigenus						20–40 × 1.2–2.7, tapering towards the apex	Ellipsoidal to oblong-oval, 5.0–10.5 × 1.5–2.5	Kepler ((2017); and Ga (2001)

Boldface: data generated in this study.

Species	Perithecia (μm)	Asci (μm)	Part-spores (μm)	Synnemata (mm)	Conidiophores (μm)	Phialides (μm)	Conidia (μm)	Referer
Akanthomyces fragilis				Numerous arising from all parts of the host, clavate, 0.7–1.5 × 0.03–0.09		Subcylindric to narrowly clavate, 7–10 × 2.5–3, verrucose in the upper portions	Subcylindric, somewhat narrowed and rounded at the ends, 6.5–9 × 1.5	Mains (
Akanthomyces gracilis				Arising from the natural body openings and intersegmental and appendage joints, usually white to yellow-brown, cylindrical, 0.7–3 × 0.1–0.5		Cylindrical basal part tapering to a slender neck, 7–10 × 1.5–2.5	Ellipsoidal to fusiform, 2.5–3 × 1–1.6	Samso Evans (
Akanthomyces johnsonii				Gregarious, white, 0.4–4 tall, with a stipe 0.025–0.1 wide, subulate to cylindrical	Unbranched or with metulae arising at right angles to the stipe hyphae, 4–6 × 2–3	10–20 long, ellipsoidal to cylindrical body 2.5–4 wide, tapering into a narrow neck 3–5 × 1–1.5	Broadly fusoid with more or less truncate poles with minute frills, 3–4 × (l–)1.5–2	Vincent (1988)
Akanthomyces kanyawimiae				Up to 1.5 long, up to 0.4 wide, covered by dense white to cream mycelia	Erect, verticillate with phialides in whorls of two to five	(8–)9–12(–15) × 2–3, with cylindrical basal portion,tapering into a long neck, (2–)3–5.5(–7) × 1–1.5	Cylindrical to ellipsoidal, (2–)2.5–3.5(–5) × (1.5–)2(–3)	Mongk et al. (2
Akanthomyces kunmingensis				**Cream to light yellow, erect, irregularly branched**	**Cylindrical, solitary, sometimes verticillate, with phialides in whorls of four to five 4.3–9.5 × 1.2–2.0**	**Cylindrical, somewhat inflated base, 6.2–29.4 × 1.1–2.5**	**Ellipsoidal to long oval, 1.9–3.5 × 1.1–1.8**	**This stu**
Akanthomyces laosensis				**Arising at the head and in the middle of the host body, white, up to 15.6 long, 0.6–1.3 wide, feathery to clavate with acute or blunt end**	**Monophialidic, produced along the synnemata, or solitary on hyphae in culture**	**Cylindrical, 11.5–30.0 × 2.0–4.2**	**Cylindrical or long oval, 4.1–9.8 × 2.3–4.2**	**This stu**
Akanthomyces lecanii	Ovoid, 350–650 × 200–375	200–350 × 3.5–4				Relatively short, 11–20(–30) × 1.3–1.8, aculeate and strongly tapering	Typically short-ellipsoidal, 2.5–3.5(–4.2) × 1–1.5	Kepler (2017); and Ga (2001); Shresth (2019)
Akanthomyces lepidopterorum				Synnemata not observed	Mononematous, with single phialide or two phialides	Cylindrical, somewhat inflated base, 12.7–25.8 × 1.4–1.7, tapering to a thin neck	Mostly cylindrical, 3.5–5.6 × 1.4–2.1, forming mostly globose heads	Chen et (2020)
Akanthomyces muscarius						(15–)20–35 × 1.0–1.7	Ellipsoidal to subcylindrical, (2–)2.5–5.5(–6) × 1–1.5(–1.8)	Kepler (2017); and Ga (2001)

Boldface: data generated in this study.

Species	Perithecia (μm)	Asci (μm)	Part-spores (μm)	Synnemata (mm)	Conidiophores (μm)	Phialides (μm)	Conidia (μm)	Referer
Akanthomyces neoaraneogenus				Synnemata not observed	Moderately branched, with (1−)2−6 (−8) phialides	30−64 × 1.1−3.2	Forming mostly globose heads, cylindric, 3.2−8.6 × 1.3−1.6	Chen et (2017); (1949)
Akanthomyces neocoleopterorum				Synnemata not observed	Mononematous, with single phialide or whorls of two to five phialides, or *Verticillium*-like from hyphae directly	Cylindrical, somewhat inflated base, 19.9−29.6 × 1.6−2.0, tapering to a thin neck	Mostly cylindrical, 3.3−6.6 × 1.5−1.8	Chen et (2020)
Akanthomyces noctuidarum	Ovoid, (530−)623−993(−1000) × (290−)308−413(−425)	(170−)196−423(−550) × (2−)2.7−3.8(−4)	(6−)7−10.7(−13) × 1	Arising from moth body and wing veins, white to cream, erect, cylindrical to clavate, (650−)668−1191(−1500) × (50−)53.4−102(−120) μm	Monophialidic or polyphialidic	Cylindrical with papillate end, hyaline, (5−)6.8−9(−10) × (1.8−)2−2.4(−3)	Cylindrical with round end, (3−)3.5−4.5(−6) × 1	Aini et (2020)
Akanthomyces pissodis				Synnemata not observed			Cylindrical to ovoid or oval, 4−9.2 × 1.6−2.4	Chen et (2020); and Le (2005)
Akanthomyces pseudonoctuidarum				**Arising from moth body, cream to light yellow, erect, cylindrical to clavate, 0.8−2 × 0.12−0.35**	**Cylindrical, solitary, 6.5−13.8 × 1.8−3.6**	**Cylindrical with papillate end, 6.8−26.0 × 2.1−3.6**	**Ellipsoidal to long oval, 2.6−6.4 × 1.5−2.2**	**This st**
Akanthomyces pyralidarum	Ovoid to obpyriform, (290−)342−580(−650) × (150−)186−291(−340)	(170−)222−329(−360) × (2−)2.5− 3.3(−4)	(5−)5.9−9.4(−12) × 1	Synnemata not observed	Not observed	Not observed	Not observed	Aini et (2020)
Akanthomyces ryukyuenis	Pyriformia, 570−630 × 170−250	5 wide, cap 3 wide	1 × 1−4					Kobaya (1982)
Akanthomyces sabanensis				Synnemata not observed	Generally arising from submerged hyphae, moderately branched	Solitary or in whorls of 2−4, 13−19 long, from 1.0−2.0 gradually tapering to 0.5−1.0	Ellipsoidal to ovoid, usually straight, 3.5−4.5 × 1.5−2.0	Kepler (2017); Salomo (2015)
Akanthomyces sulphureus	Narrowly ovoid, (650−)676(−680) × (240−)324.5(−330)	Up to 500 long, 2−3 wide	(300−)336(−450) × 1−1.5	Synnemata not observed	Erect, verticillate with phialides in whorls of two to three	(10−)16(−20) × 2−2.5, with a cylindrical basal portion, tapering into a thin neck, 1 × 0.5	Cylindrical to ellipsoidal, (4−)4.5−5.5(−6) × 2−3	Mongk et al. (2
Akanthomyces subaraneicola				**Synnemata not observed**	**Cylindrical, solitary or verticillate with phialides in whorls of two to five, 6.5−12.3 × 1.6−3.5**	**Cylindrical, somewhat inflated base, 12.1−38.2 × 1.3−3.2**	**Ellipsoidal to long oval, 3.0−5.4 × 1.8−3.4**	**This st**
Akanthomyces thailandicus	Narrowly ovoid, (700−)752−838(−850) × (300−)305−375(−400)	Up to 550 long, 5−7 wide	4−6 × 1−1.5	Synnemata not observed	Erect, forming verticillate branches with solitary phialides	(12−)13.5−21(−30) × 1−2, awl-shaped, *lecanicillium*-like	Cylindrical to ellipsoidal (3−)4−6(−7) × 1.5−2	Mongk et al. (2

Boldface: data generated in this study.

Species	Perithecia (μm)	Asci (μm)	Part-spores (μm)	Synnemata (mm)	Conidiophores (μm)	Phialides (μm)	Conidia (μm)	Referer
Akanthomyces tiankengensis				Synnemata not observed	Erect, usually arose from the aerial hyphae	Solitary or in groups of two, 13.9–17.1 × 1.1– 1.6 with a cylindrical basal portion and tapered into a short, distinct neck	Fusiform, 2.3–3.0 × 1.5–2.3	Chen et (2022)
Akanthomyces tortricidarum				Long synnemata arose at the head and in the middle of the host body, up to 5 long, 0.12–0.15 wide, cylindrical to clavate, short synnemata arose on moth body, wings, and legs, (197–)200–267(–300) × (15–)17.7–31.6(40–)μm, white to cream	Monophialidic or polyphialidic	Long synnemata: (5–)6–8(–10) × (1.8–)2–2.7(–3), short synnemata: (5–)6.2–8.3(–10) × (1.8–)2–2.5(–3), cylindrical to ellipsoidal with papillate end	Fusoid, long synnemata: (1–)2.5–3(–3.2) × (0.8–)1–1.4(–2), short synnemata: (1–)1.8–2.7(–3) × 1–2	Aini et a (2020)
Akanthomyces tuberculatus (= *A. pistillariaeformis*)	Narrowly ovoid or conoid, 420–900 × 180–370	300–600 × 4–5	2–6 × 0.5–1	Arising from all parts of the moths, clavate, 0.4–1.0 long, the stipe 0.025–0.05 thick		Subcylindric, 6–10 × 2–3, narrowing above into an acute apex terminated by a short sterigma 2–3 long	Fusoid to subcylindric narrowing at the ends, 2.5–5 × 1–1.5	Mains (
Akanthomyces uredinophilus				Synnemata not observed		Produced singly or in whorls of up to 3–4(–5) on prostrate hyphae, 20–60 × 1–2.5(–3)	Cylindric, oblong, or ellipsoid, 3–9 × 1.8–3	Park et (2016)
Akanthomyces waltergamsii				Arising on legs of spider, erect, up to 1.5 long, 0.1–0.12 wide	Usually forming verticillate branches with phialides in whorls of two to five	(10–)16(–22) × (1–)1.5(–2), with cylindrical to ellipsoidal basal portion, tapering into a thin neck, 1–3 × 1	Ellipsoidal or fusiform, (2–)3.5(–4) × 2–3	Mongk et al. (2
Akanthomyces zaquensis				Synnemata not observed		8.0–40.0 long, rarely over 100, 0.6–1.2 at the base, tapering to about 0.4 at the tips	Long-ellipsoidal to almost cylindrical, (1.5–)3.0–6.0(–7.0) × 0.5–1.2(–1.5)	Wang e (2013)

Boldface: data generated in this study.

Akanthomyces laosensis H. Yu bis & Y. Wang, sp. nov. (Fig. 4).

MycoBank: MB848308.

Etymology

Named after the location, Laos, where the species was collected.

Type: **Laos**, Oudomxay Province, Muang Xay County, Nagang Village (20.7143°N, 102.0957°E, 698 m above sea level), on the adult of Noctuidae on the underside of a dicotyledonous leaf, October 5, 2019, collected by Yao Wang (holotype: YHH 2301008; ex-holotype living culture: YFCC 1910941).

Description: Sexual morph: Undetermined. **Asexual morph**: Specimens examined in this study can be found on the underside of dicotyledonous leaves. Synnemata arose at the head and in the middle of the host body, white, up to 15.6 mm long and 0.6–1.3 mm wide, rarely branched, feathery to clavate with acute or blunt ends. Colonies on PDA moderately fast-growing at 25°C, reaching 23–26 mm in diameter in 14 days, circular, flat, white in the middle with a light yellow edge, reverse light yellow. Hyphae smooth-walled, branched, septate, hyaline, 0.8–3.5 μm wide. Conidiogenous cells monophialidic, produced along the synnemata, or solitary on hyphae in culture. Phialides smooth-walled, hyaline, cylindrical, 11.5–30.0 × 2.0–4.2 μm (n = 30). Conidia smooth and hyaline, cylindrical or long oval, one-celled, 4.1–9.8 × 2.3–4.2 μm (n = 30). Size and shape of phialides and conidia similar in culture and on natural substratum.

Host

Adult moth (Noctuidae, Lepidoptera).

Habit

On the adults of Noctuidae sp. on the underside of leaves of plants.

Distribution

Muang Xay County, Oudomxay Province, Laos.

Other material examined: Laos, Oudomxay Province, Muang Xay County, Nam Kit Park (20.6651°N, 102.0007°E, 695 m above sea level), on an adult moth on the underside of a leaf, October 1, 2019, Yao Wang (YHH 2301000; living culture: YFCC 1910942).

Commentary

Phylogenetically, A. laosensis forms a distinct lineage and is closely related to A. pyralidar*um* (Fig. 1). Morphologically, *A. laosensis* is distinctly different from *A. pyralidarum* because of its longer synnemata (up to 15.6 mm). Furthermore, *A. laosensis* was determined to occur on an adult of Noctuidae sp., while *A. pyralidarum* was located on an adult of Pyralidae sp. In fact, the species is easily distinguished from other known species in the genus of *Akanthomyces* by its longer phialides (11.5–30.0 μm) and larger conidia (4.1–9.8 × 2.3–4.2 μm) (Table 3).

Akanthomyces pseudonoctuidarum H. Yu bis & Y. Wang, sp. nov. (Fig. 5)

MycoBank: MB848309.

Etymology

Referring to macromorphological resemblance of *A. noctuidarum* and *A. pseudonoctuidarum* but phylogenetically distinct.

Type: **Thailand**, Chiang Mai Province, Chiang Mai City, Maejo Farm (18.9177°N, 99.0520°E, 317 m above sea level), on the adult of Noctuidae on the underside of a dicotyledonous leaf, August 22, 2018, collected by Hong Yu (holotype: YHH 2301010; ex-type living culture: YFCC 1808943).

Description: **Sexual morph**: Undetermined. **Asexual morph**: Synnemata arising from moth body, cream to light yellow, erect, simple, cylindrical to clavate, 800–2000 × 120–350 μm. Conidia and reproductive structures on natural substratum not observed. Colonies on PDA moderately fast-growing at 25°C, reaching a diameter of 25–28 mm within 14 days, circular, flat to raised, white and fluffy mycelium, reverse cream to pale yellow. Hypha smooth-walled, hyaline, septate, 1.0–2.9 μm wide. Conidiophores smooth-walled, cylindrical, solitary, 6.5–13.8 × 1.8–3.6 μm (n = 30). Conidiogenous cells monophialidic or polyphialidic. Phialides verticillate, usually in whorls of 2–3, or solitary on hyphae, cylindrical with papillate end, hyaline, 6.8–26.0 × 2.1–3.6 μm (n = 30). Conidia smooth and hyaline, ellipsoidal to long oval, one-celled, 2.6–6.4 × 1.5–2.2 μm (n = 30).

Host

Adult moth (Noctuidae, Lepidoptera).

Habit

On the adults of Noctuidae sp. on the underside of leaves of plants.

Distribution

Currently only known in Chiang Mai City, Chiang Mai Province, Thailand.

Other material examined: Thailand, Chiang Mai Province, Chiang Mai City, Queen Sirikit Botanic Garden (18.8990°N, 98.8605°E, 536 m above sea level), on an adult of Noctuidae, August 26, 2018, Yao Wang (YHH 2301011; living culture: YFCC 1808944).

Commentary: *Akanthomyces pseudonoctuidarum* is similar to its phylogenetically closely related species *A. noctuidarum* in macromorphology. They have the same hosts (the adults of Noctuidae sp.) and *Isaria*-like asexual conidiogenous structures, producing cream or light yellow synnemata; however, *A. pseudonoctuidarum* is easily recognized by its larger synnemata (800–2000 × 120–350 μm), longer phialides (6.8–26.0 μm), and larger conidia (2.6–6.4 × 1.5–2.2 μm) (Table 3). It was easily distinguished phylogenetically from *A. noctuidarum* (Fig. 1). Both the morphological study and phylogenetic analyses of combined ITS, nr*LSU*, *TEF*, *RPB1*, and *RPB2* sequence data supported that this fungus is a distinct species in the genus *Akanthomyces*.

Akanthomyces subaraneicola H. Yu bis, Y. Wang & Z.Q. Wang, sp. nov. (Fig. 6)

MycoBank: MB848310.

Etymology

“Subaraneicola” refers to morphologically resembling *A. araneicola* but phylogenetically distinct.

Type: **China**, Hunan Province, Huaihua City, Zhongpo National Forest Park (27.5724°N, 109.9664°E, 615 m above sea level), on a spider emerging from leaf litter on the forest floor, July 10, 2021, collected by Yao Wang (holotype: YHH 2301004; ex-type living culture: YFCC 2107937).

Description: Sexual morph: Undetermined. **Asexual morph**: Mycosed hosts covered by white to pale yellow mycelia, producing numerous powdery conidia, synnemata not observed. Colonies on PDA reaching 24–28 mm in diameter within 14 days at 25°C, circular, white, and fluffy mycelium in the center, cottony with a raised mycelial density at the outer ring, reverse white to pale yellow. Hyphae smooth-walled, branched, septate, hyaline, 1.6–3.2 μm wide. Conidiophores smooth-walled, cylindrical, solitary, sometimes verticillate, 6.5–12.3 × 1.6–3.5 μm (n = 30). Conidiogenous cells monophialidic or polyphialidic. Phialides consisting of a cylindrical, somewhat inflated base, verticillate on conidiophores, usually in whorls of 2–5, or solitary on hyphae, 12.1–38.2 × 1.3–3.2 μm (n = 30). Conidia smooth and hyaline, ellipsoidal to long oval, one-celled, 3.0–5.4 × 1.8–3.4 μm (n = 50), often in chains. Size and shape of phialides and conidia similar in culture and on natural substratum.

Host

Spider (Araneae).

Habit

On spiders on dead stems or emerging from leaf litter on the forest floor.

Distribution

Hunan and Yunnan Province, China.

Other material examined: China, Yunnan Province, Kunming City, Wild Duck Lake Forest Park (25.1244°N, 102.8716°E, 1900 m above sea level), on a spider on a dead stem, July 28, 2021, Yao Wang (YHH 2301005; living culture: YFCC 2107938).

Commentary

Morphologically, *A. subaraneicola* resembles the phylogenetic sister species *A. araneicola*. They were found to be parasitic on spiders (Araneae), and they are easily recognized by having white to pale yellow mycelia covering the hosts with a mass of conidia; however, our morphological observation revealed a significant difference in the shape and size of conidia between *A. subaraneicola* and *A. araneicola*. *Akanthomyces subaraneicola* usually produces large ellipsoidal to long oval conidia (3.0–5.4 × 1.8–3.4), while *A. araneicola* produces small fusiform conidia (2.5–5.0 × 1.3–1.9 μm) (Table 3). In addition, molecular phylogenetic analyses indicated that they are distinct species (Fig. 1).

Discussion

Akanthomyces is a common group of arthropod-pathogenic fungi found worldwide, with the highest species diversity occurring in subtropical and tropical regions, especially in China and Southeast Asia (see Table 2). Based on our update, there are at least 17 *Akanthomyces* species in China, and Yunnan Province has the most. With its unique geographical and ecological features, Yunnan Province is one of the regions with extremely rich biodiversity in Southwest China, and the resource of *Akanthomyces* is also extremely rich. There is also high species diversity of *Akanthomyces* in Southeast Asia, where more than 11 species have been recorded (Table 2). Thailand, Vietnam, and Laos are located in tropical regions with extremely rich biodiversity in Southeast Asia. The forests feature a rich biodiversity of both flora and fauna due to the tropical monsoon climate with high temperatures and rainfall (Lao et al. 2021). These have created a favorable environment for the development of arthropod-pathogenic fungi, including *Akanthomyces* spp.

Akanthomyces species inhabit diverse hosts/substrates that range from eight orders of Arthropoda, namely, Acari, Araneae, Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, and Thysanoptera, to plants, other fungi, peat, water, and rusts (see Table 2). Among the hosts of *Akanthomyces*, Araneae and Lepidoptera are the two major orders. Our study also found that the majority of *Akanthomyces* species are spider pathogens or adult moth entomopathogens, with the exception of a few other entomopathogens and generalists that have a remarkably broad host–substrate range (Table 2 and Fig. 1). In this study, we identified an extension of the host–substrate range to also include soil, as shown in Fig. 1.

Because of the difficulty of isolation and the limitation of cultivation conditions, studies on the development and application of *Akanthomyces* species are still currently limited. As generalists that have a remarkably broad host–substrate range, *A. gracilis* and *A. muscarius* have a high potential for interspecific transmission and biological control of pest insects (Samson and Evans 1974; Zare and Gams 2001; Nicoletti and Becchimanzi 2020; Kuchár et al. 2019). *Akanthomyces lecanii* is an effective mycoparasite of several rust fungi, green mold, and fungi causing root rot diseases (*Pythium ultimum*), as well as of several powdery mildew pathogens, and it is receiving increasing attention as a versatile biocontrol agent of a number of plant pathogens (Benhamou and Brodeur 2001). The members of the genus *Akanthomyces* contain species ranging from specialists with very narrow host ranges to generalists that attack a wide range of arthropods, and they might be used as an ideal model system for research on fungal arthropod pathology and fungal-pathogen speciation and host adaptation (Hu et al. 2014). Coleopterans, lepidopterans, and spiders are the major host groups of arthropod-pathogenic fungi within Hypocreales (Shrestha et al. 2019). The findings indicate that the majority of the hosts of *Akanthomyces* are distributed in lepidopterans and spiders, with a few in coleopterans (see Table 2). These arthropod-pathogenic fungi with special nutritional preferences are more likely to produce numerous distinctive bioactive compounds. It is hoped that this study will generate continued interest among mycologists, arachnologists, and related experts and researchers to use such fungal resources through in vitro growth and extraction of useful bio-active secondary metabolites (extrolites).

Fungal species diversity and their host–substrate associations are important aspects of fungal ecology. A strong taxonomic basis that is dependent on advances in nucleic acid sequence technology is one of the main fundamental needs in fungal ecology (Zhang et al. 2021), and is even crucial to studies on species diversity and their host–substrate associations. However, it is regrettable that a growing number of researchers have relied heavily on molecular biology techniques to the complete exclusion of fungal isolation and characterization utilizing classical methods (Zhang et al. 2021; Walker et al. 2019). Although the fungi research has entered the molecular era, phenotypic and culture-based studies are still an invaluable tool for fungal biology and ecology exploration (Walker et al. 2019). In addition to molecular data, morphological and ecological characteristics have a pivotal role in taxonomy and phylogenetic identification of fungi. In our work, we surveyed the literature to the greatest extent possible, combined that with the results of those obtained by morphological methods (optical microscope and electron microscop) in our study, to list and compare the morphological characteristics of 35 *Akanthomyces* species (Table 3). The morphological comparison revealed obvious differences in the size of ascospores and asci, morphology of the synnemata, conidiogenous structures, and conidial shape and size, although the morphological features generally overlapped. Our statistics showed that at least 20 *Akanthomyces* species are specialists with narrow host ranges, and they are either spider pathogens or adult moth entomopathogens (Table 2). They cause mortality of spiders and adult moths by nature. The cadavers are usually found attached to the underside of leaves or on tree trunks, barks, decaying logs, branches, grass, leaf litter, and forest floors (Shrestha et al. 2019). These ecological characteristics are phylogenetically informative for distinguishing species of *Akanthomyces*, and they contribute to the timely discovery of new *Akanthomyces* species in nature.

Conclusion

Akanthomyces is a common group of arthropod-pathogenic fungi found worldwide, with the highest species diversity occurring in subtropical and tropical regions, especially in China and Southeast Asia. *Akanthomyces* species inhabit diverse hosts/substrates that range from eight orders of Arthropoda, namely, Acari, Araneae, Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera, and Thysanoptera, to plants, other fungi, peat, water, rusts, and soil. Among the hosts of *Akanthomyces*, Araneae and Lepidoptera are the two major orders. In this study, a collection of 31 isolates of unknown identity were found to represent four known species, four new species, and an undetermined species of *Akanthomyces*. Phylogenetic and morphological analyses indicated that *A. tiankengensis* is a synonym of *A. araneogenus*. Our study significantly updated the taxonomy of *Akanthomyces* and enhanced our understanding of its species diversity, geographic distribution, host affiliations, and ecological diversity.

Declarations

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Not applicable.

Authors’ contributions

YW was major contributors in writing the manuscript. Z-QW analyzed the data. YW, HY, SS, CT and V-MD collected samples. HY and RL modified and improved the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets presented in this study can be found in GenBank. The accession numbers can be found in the article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

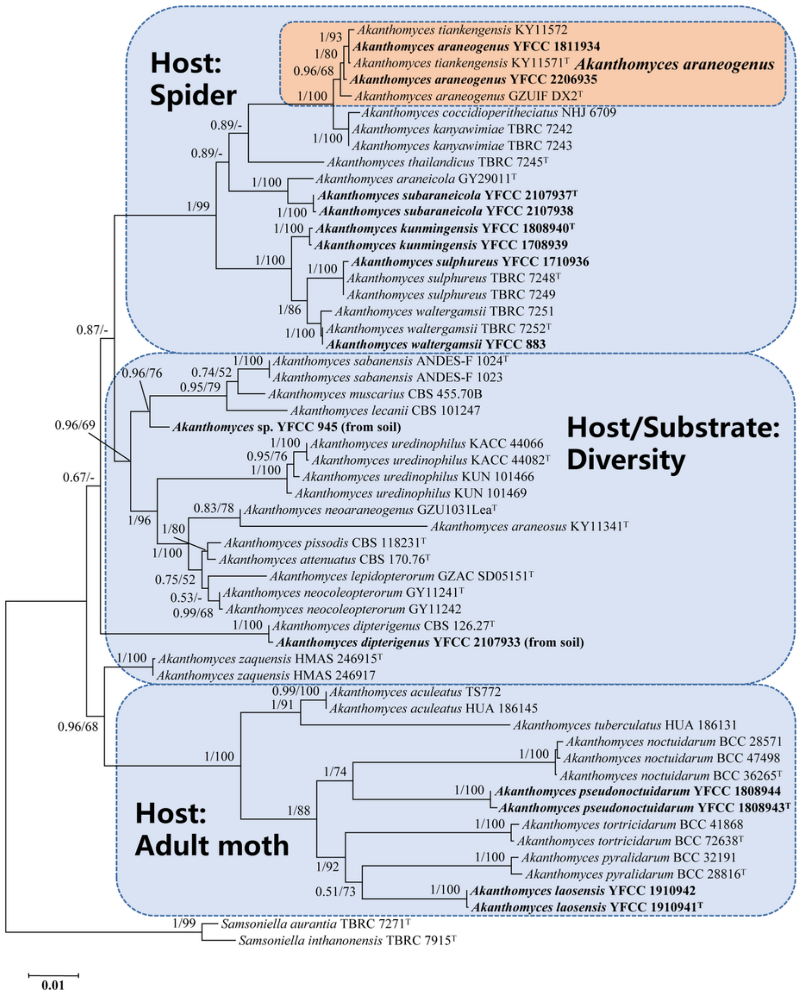

Figure 1

Phylogenetic tree of *Akanthomyces* species based on combined partial ITS + nr*LSU* + *TEF* + *RPB1* + *RPB2* sequences. Statistical support values (≥ 0.5/50%) are shown at the nodes for Bayesian inference (BI) posterior probabilities/maximum likelihood (ML) bootstrap support. Ex-type materials are marked with “T”. Isolates in bold type are those analysed in this study

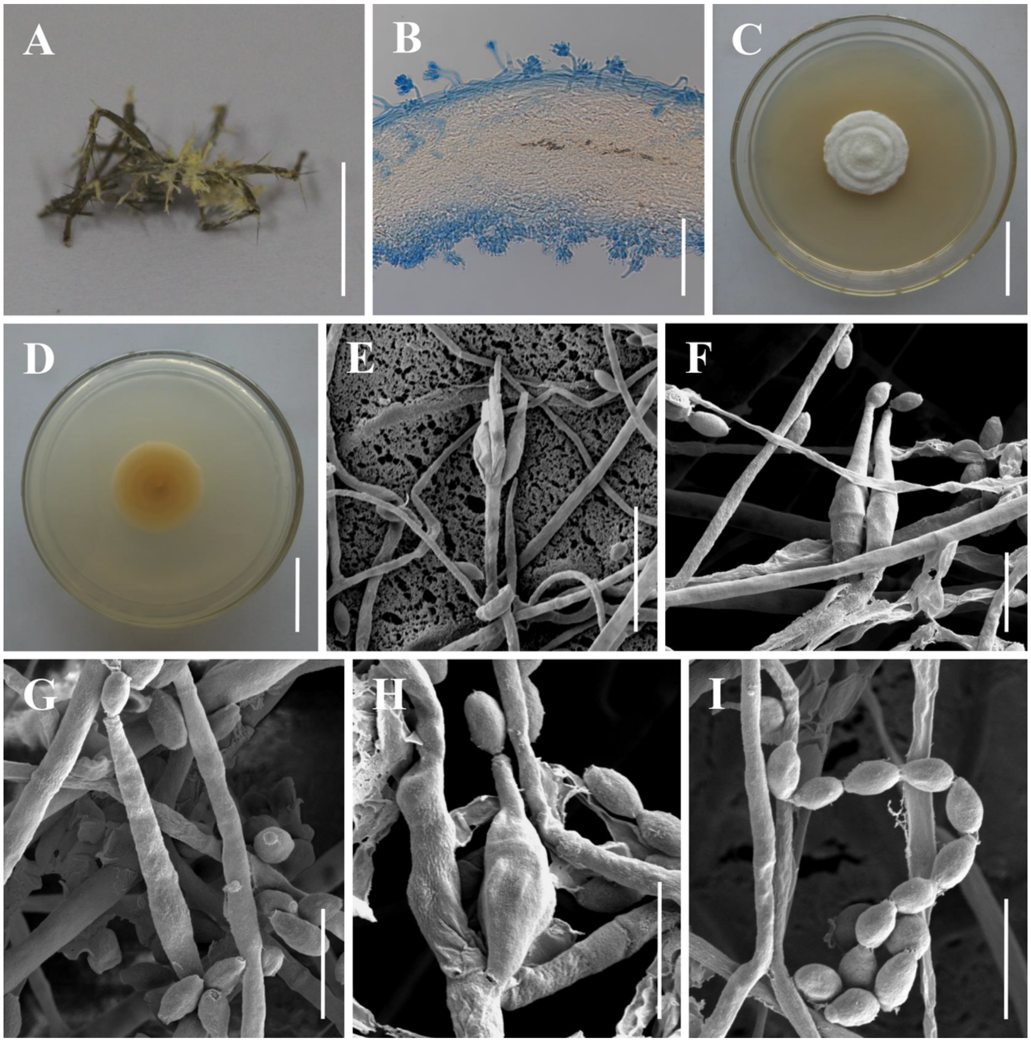

Figure 2

Morphology of *Akanthomyces araneogenus.* (A) Fungus on spider. (B) Conidiogenous structures on the host. (C–D) Culture character on PDA medium. (E–H)Conidiophores, conidiogenous cells and conidia. (I) Conidia. Scale bars: A = 5 mm; B = 30 μm; C–D = 30 mm; E = 10 μm; F–I = 5 μm

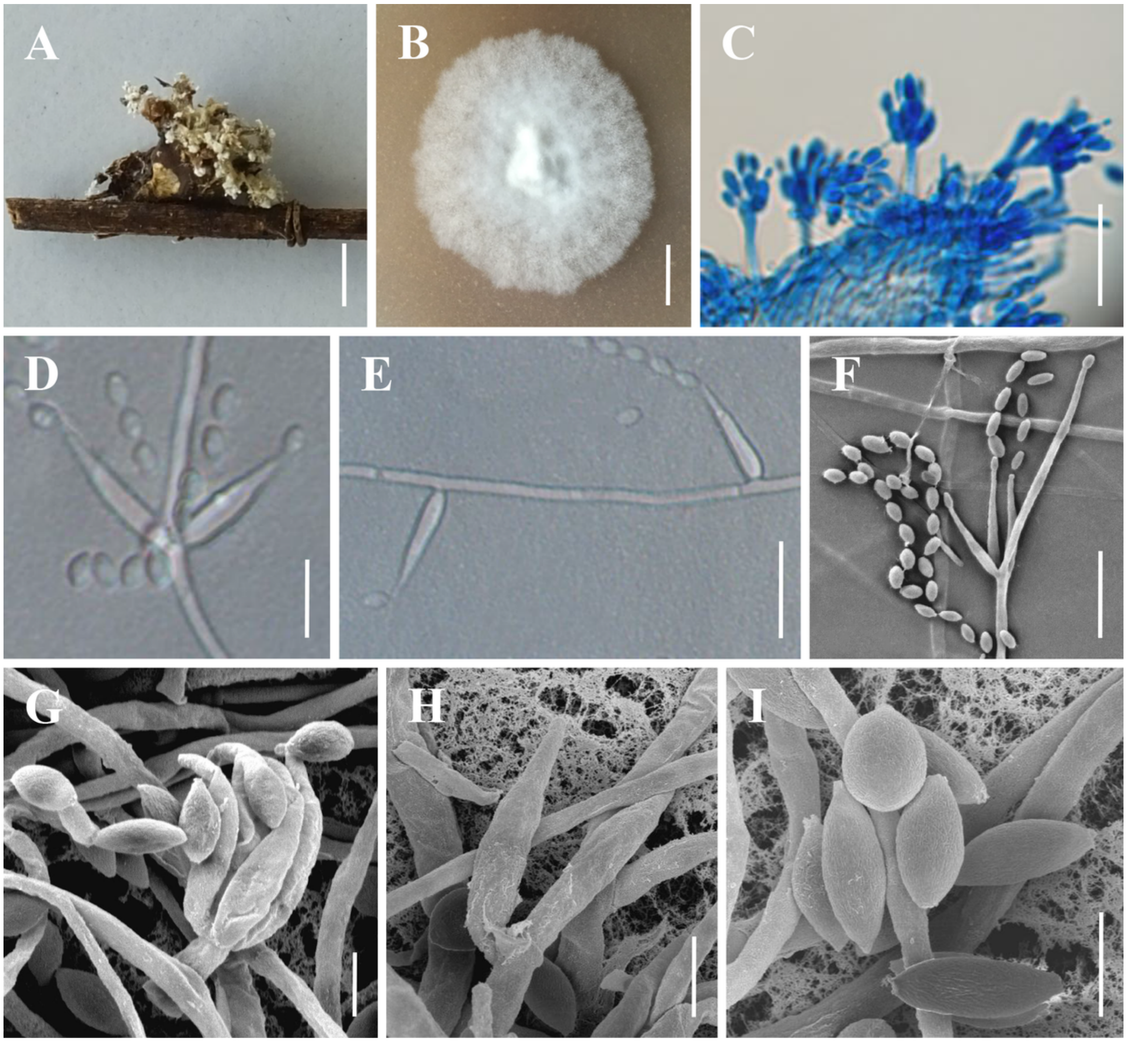

Figure 3

Morphology of *Akanthomyces kunmingensis*. (A) The type specimen (YHH 16988). (B) Culture character on PDA medium. (C) Conidiogenous structures on the host. (D–H) Conidiophores, conidiogenous cells and conidia. (I) Conidia. Scale bars: A = 3 mm; B = 10 mm; C, E–F = 10 μm; D = 5 μm; G–I = 2 μm

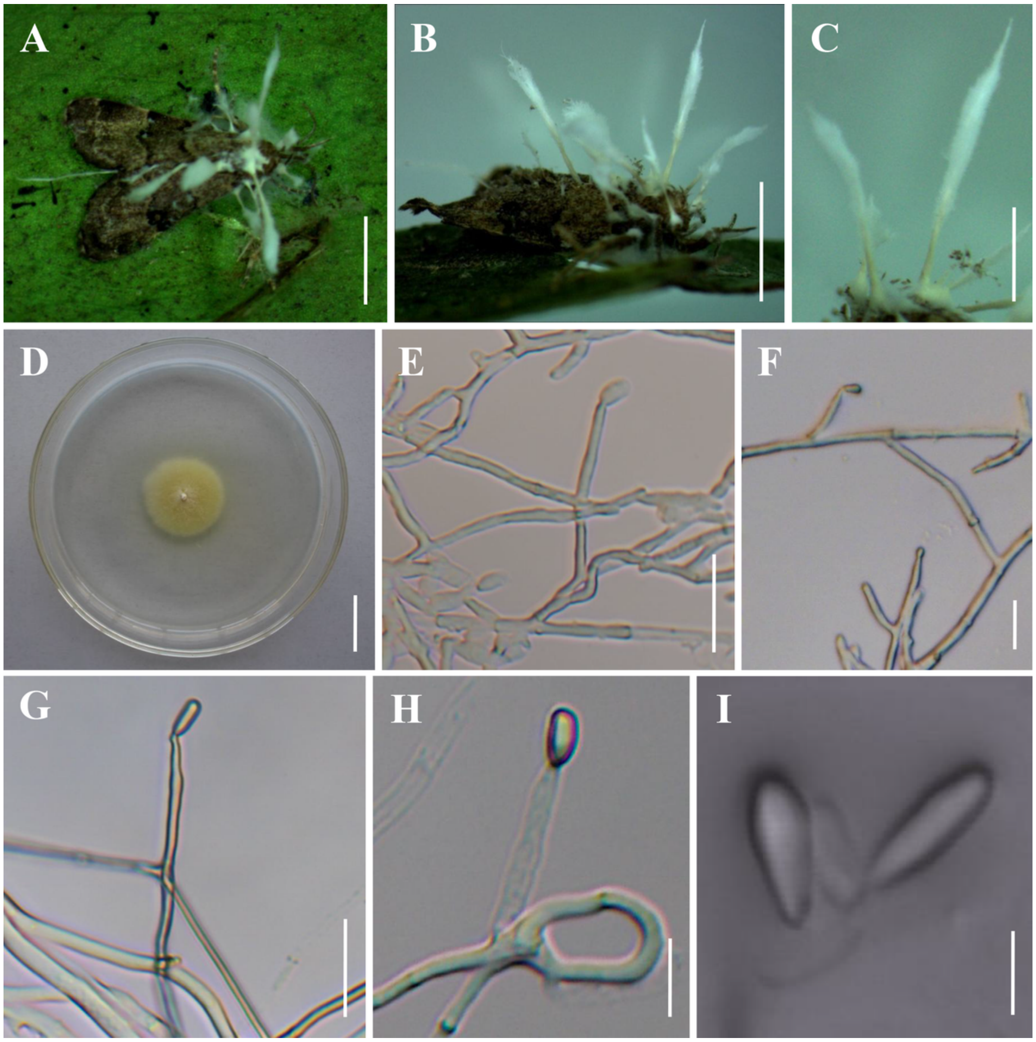

Figure 4

Morphology of *Akanthomyces laosensis*. (A–B) Fungus on adult moth. (C) Long synnemata. (D) Culture character on PDA medium. (E–H) Conidiophores, conidiogenous cells and conidia. (I) Conidia from long synnema. Scale bars: A–B = 10 mm; C = 5 mm; D = 20 mm; E–G = 20 μm; H = 10 μm; I = 5 μm

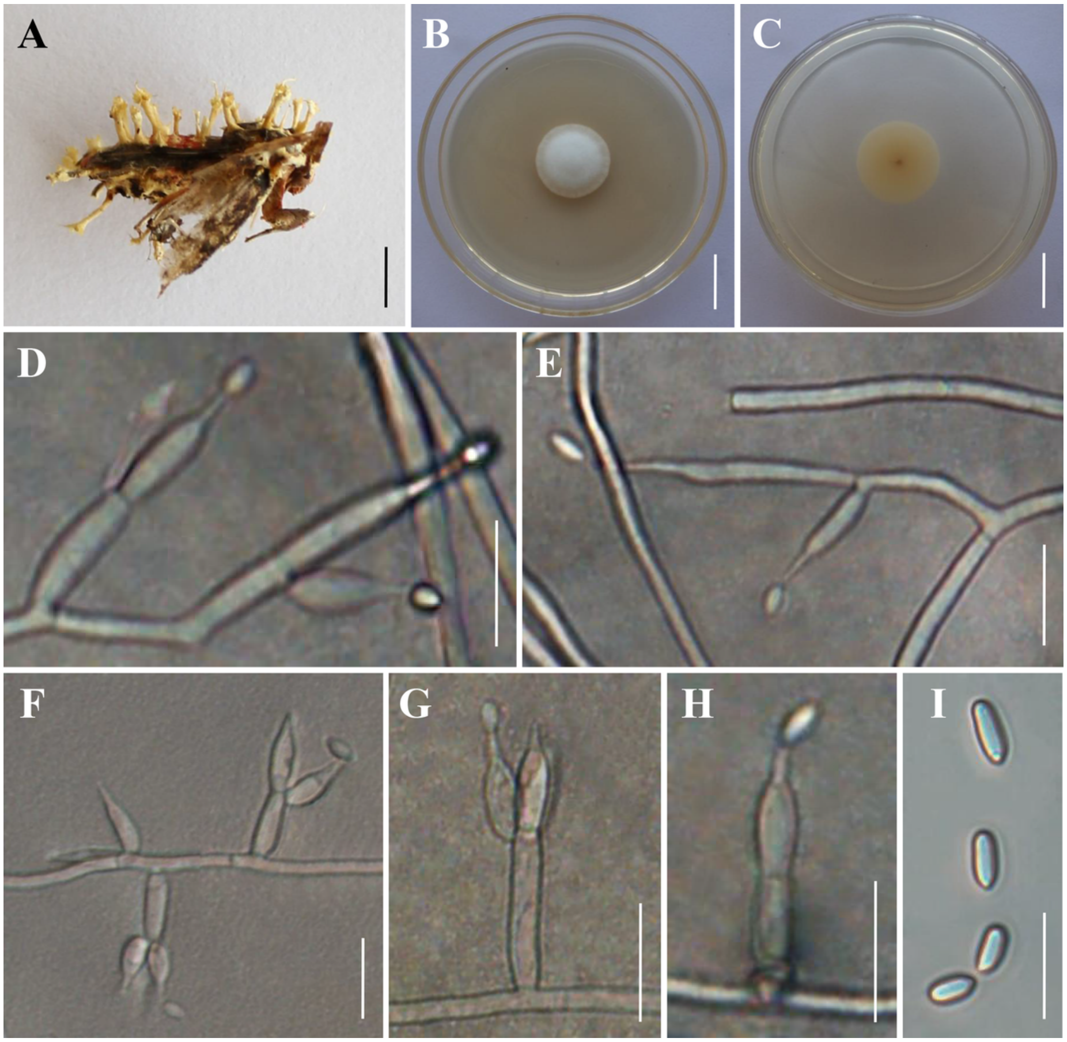

Figure 5

Morphology of *Akanthomyces pseudonoctuidarum*. (A) Adult moth infected by *A. pseudonoctuidarum*. (B–C) Culture character on PDA medium. (D–H) Conidiophores, conidiogenous cells and conidia. (I) Conidia. Scale bars: A = 2 mm; B–C = 20 mm; D–I = 10 μm

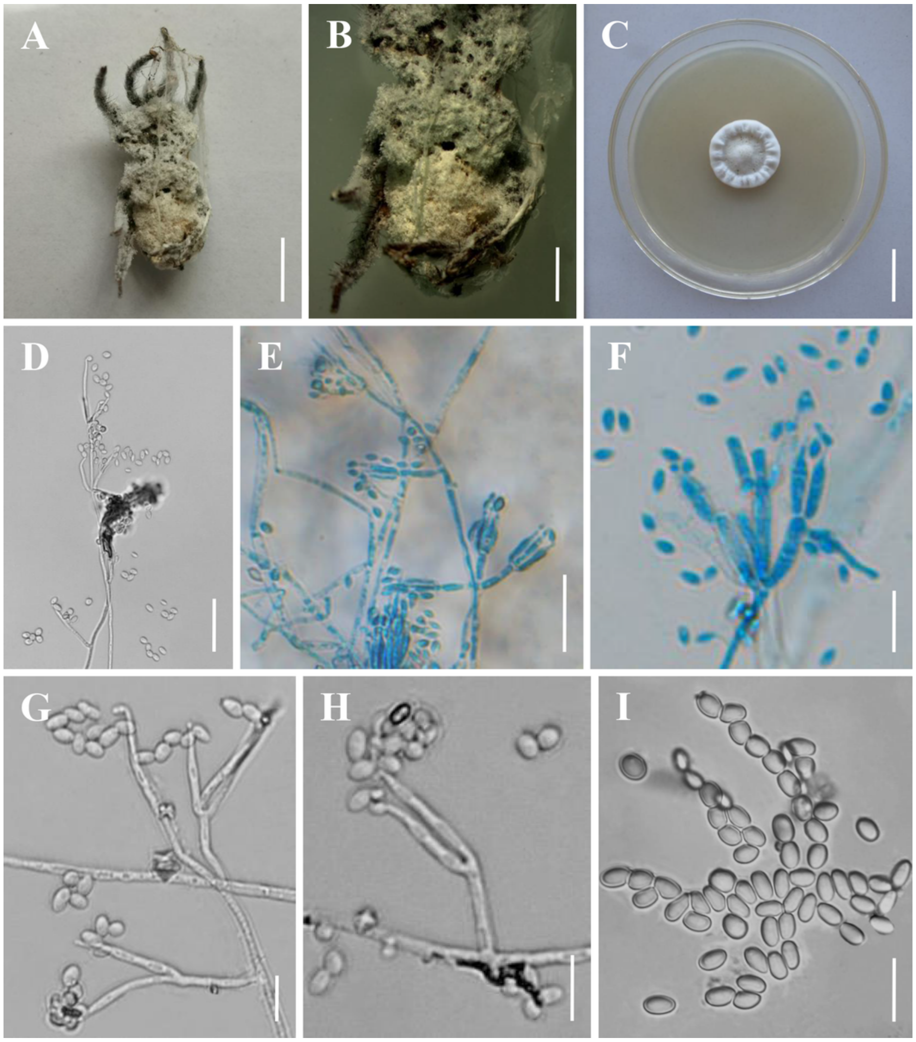

Figure 6

Morphology of *Akanthomyces subaraneicola*. (A–B) Fungus on spider. (C) Culture character on PDA medium. (D–H)Conidiophores, conidiogenous cells and conidia. (I) Conidia. Scale bars: A = 10 mm; B = 5 mm; C = 20 mm; D = 30 μm; E = 20 μm; F–I = 10 μm