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A new species of *Arthrographis* (Eremomycetaceae, Dothideomycetes), from the soil in Guizhou, China

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Abstract

During a survey of keratinolytic fungi in China, a new species, *Arthrographis multiformispora* was isolated from soil samples. Morphologically, *A. multiformispora* differs from other species in the genus by the presence of globose or subglobose chlamydospores and cylindrical arthroconidia. Phylogenetically, our four strains were clustered together with high support values and separated from other clades. We provided a description, illustrations, and phylogenetic tree for the new species.

Keywords: taxonomy, phylogeny, saprophytic fungi

Introduction

Cochet (1939) established the genus *Arthrographis* G. Cochet with *A. langeronii* as the type species, but it was invalid because it did not comply with the International Code of Botanical Nomenclature (ICBN). Subsequently, Sigler & Carmich. (1976) transferred *Oididendron kalrae* R.P. Tewari & Macph. to *Arthrographis* named *Arthrographis kalrae* (R.P. Tewari & Macph.) Sigler & J.W. Carmich and designated it as the type species. Giraldo *et al*. (2014) reprogrammed the phylogenetic circumscription of *Arthrographis,* demonstrating that *A. kalrae*, *A. arxii*, *A. chlamydospora*, *A. curvata*, *A. globosa* and *A. longispora* are valid species of the genus *Arthrographis*. Recently, Hernández-Restrepo *et al*. (2020) reported the new species *A. grakistii* Giraldo Lopez & Hern.-Restr. from in the Netherlands garden soil. Currently, the genus *Arthrographis* consists of 7 species.

Specie classification of the genus *Arthrographis* is generally recognized based on the morphological characteristics of their slow growth rate, the presence of 1-celled, cylindrical arthroconidia released schizolytically from dendritic conidiophores (Sigler & Carmichael 1976). Kang *et al*. (2010) found that the genus *Arthrographis was* polyphyly based on sequences analysis of SSU, ITS, and *RPB2*. Giraldo *et al*. (2014) carried out a detailed phenotypic, and phylogenetic analysis based on sequence data of the ITS region, actin (*ACT*), and chitin synthase genes (*CHS*), and the results were consistent with morphological findings.

The genus *Arthrographis* is widely distributed and acquired from the sea, soil and plants. During the investigation of soil keratinolytic fungi in China, four strains of *Arthrographis* were isolated. To determine their taxonomic location, multi-locus phylogenetic analysis was performed based on the internal transcribed spacer (ITS), large subunit ribosomal RNA gene (LSU), and a portion of the actin gene (*ACT*). We identify and describe a new species of *Arthrographis* from China.

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Materials & methods

Fungal isolation and Morphology

Soil samples were collected from Guiyang, Guizhou Province. We collected 3–10 cm below the soil surface and transported them to the laboratory in sterile Ziploc plastic bags. After being brought to the lab, they were treated and isolated according to the method of Zhang *et al*. (2020 a, b, 2021). The washed, sterile, and dried chicken feathers were mixed with soil samples and then wet with distilled water and cultured at darkroom temperature for 1 month. Then the target strains were isolated by dilution plate method. The purified strains were transferred to PDA and MEA plates for dark culture at 25 °C for 14 days. 25% lactic acid was used as the floating solution for film preparation, and the microstructures were observed and photographed under a microscope. The dried holotype was deposited in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), while ex-type living culture was deposited in the China General Microbiological Culture Collection Center (CGMCC), and all strains were deposited in the Institute of Fungus Resources, Guizhou University (GZAC), Guiyang City, Guizhou, China.

TABLE 1. Strains included in phylogenetic analyses.

Species	Strains	*ITS*	*LSU*	*ACT*
Arthrographis arxii	CBS 203.78 T	GQ272638	AB213426	HG316563
Arthrographis chlamydospora	CBS 135936 T	HG004554	HG004543	HG316560
Arthrographis curvata	CBS 135934 T	HG004556	HG004542	HG316558
Arthrographis globosa	UTHSC 11-757 T	HG004553	HG004541	HG316561
Arthrographis grakistii	CBS 145529	MN794359	MN794336	MN816497
	JW22015	MN794360	MN794337	MN816498
	JW22019	MN794361	MN794338	MN816499
	JW49011	MN794362	MN794339	MN816500
	JW49012	MN794363	MN794340	MN816501
	JW180011	MN794364	MN794341	MN816502
	CBS 145530	MN794365	MN794342	MN816503
	JW190018	MN794366	MN794343	MN816504
	JW209002	MN794367	MN794344	MN816505
	JW209003	MN794368	MN794345	MN816506
Arthrographis kalrae	CBS 693.77 T	AB116536	AB116544	HG316544
	JW 21004	MN794369	MN794346	MN816507
	CBS 145527	MN794370	MN794347	MN816508
	JW 21029	MN794371	MN794348	MN816509
Arthrographis longispora	CBS 135935 T	HG004555	HG004540	HG316559
	CBS 145528	MN794372	MN794349	MN816510
Arthrographis multiformispora	**CGMCC 3.20770 = GZUIFR 21.926 T**	**OL475525**	**OL475531**	**OL589245**
	GZUIFR 21.927	**OL475526**	**OL475532**	**OL589246**
	GZUIFR 21.928	**OL475527**	**OL475533**	**OL589247**
	GZUIFR 21.929	**OL475528**	**OL475534**	**OL589248**
Eremomyces bilateralis	CBS781.70 T	HG004552	HG004545	HG316562
Rhexothecium globosum	CBS955.73 T	MH860827	MH872561	——

Note: **T=**Ex-type; New isolates are in bold; The line "——" represents the absence of GenBank accession.

DNA extraction, PCR amplification, sequencing

According to Zhang *et al*. (2021), total genomic DNA was carried out. ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3' (White *et al*. 1990), LROR: ACCCGCTGAACTTAAGC and LR7: TACTACCACCAAGATCT (Vilgalys & Hester 1990), and ACT1: TGGGACGATATGGAIAAIATCTGGCA and ACT4: TCITCGTATICTIGCTIIGAIATCCACAT (Voigt & Wöstemeyer 2000) primers were used for amplification of internal transcribed spacers (ITS), the 28S nrRNA locus (LSU), and the actin gene (ACT), respectively. The PCR products were sent to Quintarabio (Wuhan, China) for purification and sequencing. The new sequences were submitted to the GenBank (Table 1).

Phylogenetic analyses

The ITS, LSU, and *ACT* sequences of *Arthrographis* and outgroup (*Eremomyces bilateralis* CBS 781.70 and *Rhexothecium globosum* CBS 955.73) were downloaded from GenBank (Table 1). The TBtools (Chen *et al*. 2020) were used for sequence processing (including name simplification, renaming, etc.). Each loci data set was aligned by MAFFT v7.037 (Katoh *et al*. 2013), with sequence editing and trimming implemented in trimAI (Capella-Gutierrez *et al*. 2009). The best-fit substitution model was selected using the Bayesian information criterion, in ModelFinder (Kalyaanamoorthy *et al.*, 2017). The "Concatenate Sequence" function in PhyloSuite v1.16 (Zhang *et al*. 2020) was used for the concatenation of loci.

The analysis was carried out using Bayesian inference (BI) and maximum likelihood (ML) methods. For Bayesian inference, MrBayes v3.2 (Ronquist *et al*. 2012) was used and Markov chain Monte Carlo (MCMC) simulations were run for 10^8 generations with a sampling frequency of every 10^3 generations and a burn-in of 25%. ML analysis was implemented in IQ-TREE v1.6.11 (Nguyen *et al*. 2015) with 10^4 bootstrap tests using the ultrafast algorithm (Minh *et al*. 2013). The above analyses were carried out in PhyloSuite v1.16 (Zhang *et al*. 2020).

Results

Phylogeny analysis

Based on a BLAST search using the ITS loci, our isolates were identified as belonging to the genus *Arthrographis*. To determine the phylogenetic position of these strains, we performed a multi-locus phylogenetic analysis. The dataset composed of ITS (1–511 bp), LSU (512–1030 bp), and ACT (1031–1801 bp) gene, comprising a total of 1,798 characters (including gaps), including 26 taxa with *Eremomyces bilateralis* (CBS 781.70) and *Rhexothecium globosum* (CBS 955.73) as the outgroup taxa (Fig. 1). The selected model for ML analysis and BI analysis were shown in Table 2.

TABLE 2. The best-fit substitution models are used in multi-locus phylogenetic construction.

	LSU	ITS	*ACT*
ML analysis	TIM2+F+I	GTR+F+R2	TIM+F+R2
BI analysis	GTR+F+I	GTR+F+I+G4	GTR+F+I+G4

The results showed that phylogenetic trees constructed by the BI and ML analyses had similar topological structures, and strains of each species were well clustered (Fig. 1). In the phylogenetic tree, our four strains (GZUIFR 21.926, GZUIFR 21.927, GZUIFR 21.928, and GZUIFR 21.929) were clustered together, with high support values (BI pp = posterior probability 1, ML BS 99) and separated from other clades (Fig. 1).

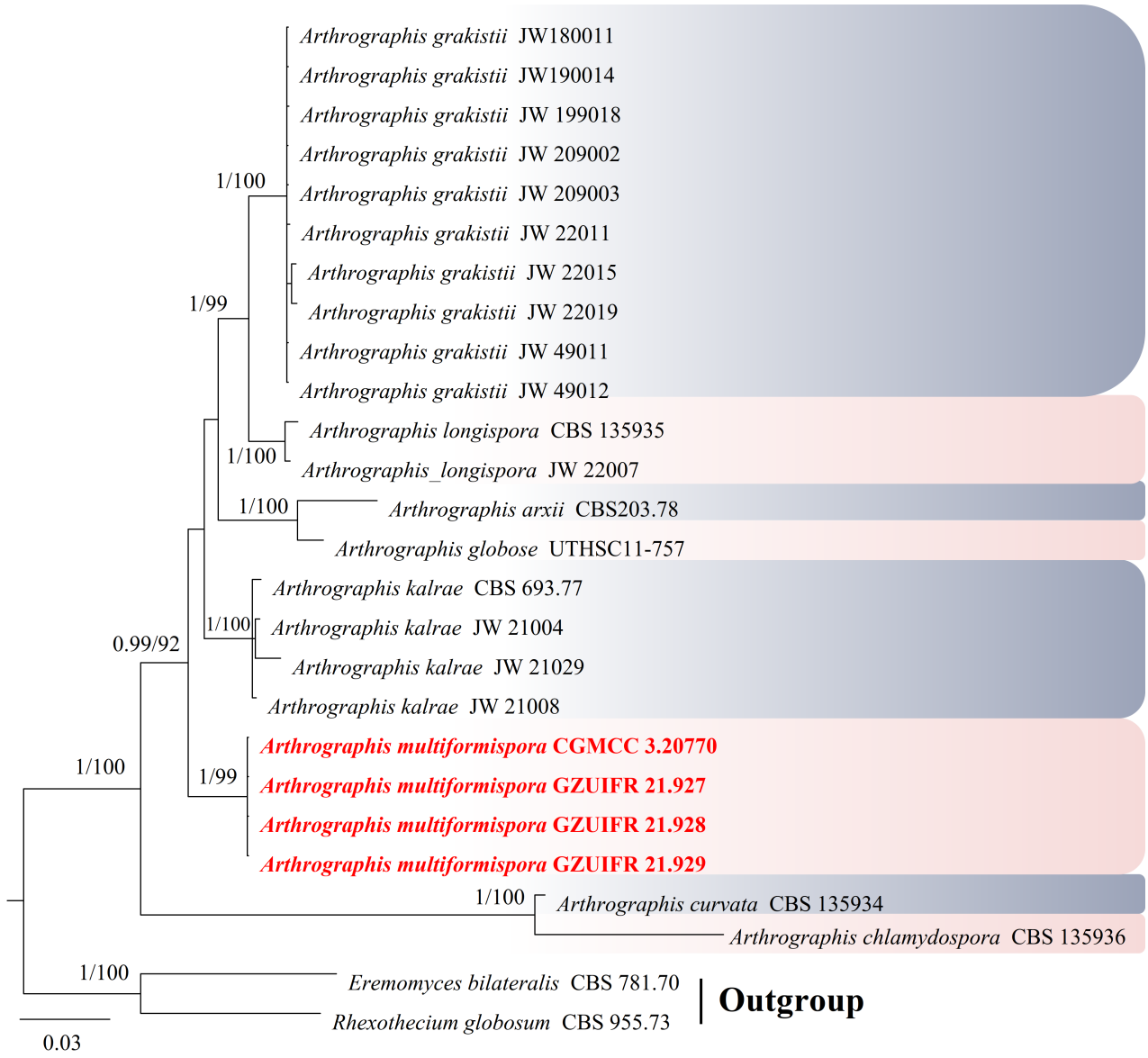

FIGURE 1. Phylogenetic tree of the genus *Arthrographis* constructed from ITS, LSU, and *ACT*. Notes: Statistical support values (BI/ML) were shown at nodes.

Taxonomy

Arthrographis multiformispora Xin Li, Y.F. Han & Z.Q. Liang, ***sp. nov.*** (Fig. 2)
Mycobank No.: MB841967

Type:—CHINA. Guizhou Province, Guiyang City, the green ground of Qianlingshan Park (N 26°60′, E 106°69′), soil, September 2016, Zhi-Yuan Zhang, dried holotype HMAS 351881, ex-holotype CGMCC 3.20770, *ibid*., GZUIFR 21.927.

Colonies slow-growing on PDA and MEA after 14 days of incubation at 25 °C; on PDA reaching up 23–25 mm in diameter; white, flat or raised, glabrous toward the periphery, reverse white to buff; On MEA, attaining a diameter of 22–24 mm; pale to white, umbonate at center and flat toward the periphery, powdery, reverse white. *Vegetative hyphae* septate, hyaline, smooth- and thin-walled, 0.5–2.0 µm diam. *Conidiophores* simple or branched, erect, up to 88.0 µm long, hyaline, smooth-walled. *Conidiogenous hyphae* simple or branched, erect, 0.5–3.0 µm wide, forming septa basipetally to form arthroconidia released by schizolythic secession. *Arthroconidia* unicellular, cylindrical with truncated or cuboid, straight or slightly curved, 0.5–3.0 × 1.5–5.5 µm, hyaline. *Chlamydospores* terminal, unicellular,

globose or subglobose, 2.5–4.0 × 3.5–4.5 µm. Non- trichosporiella-like synasexual morph in culture. Sexual morph not observed.

Etymology:—Referring to the presence of multiple types of spores.

Additional specimens examined:—CHINA, Guizhou Province, Guiyang City, the Affiliated Hospital of Guizhou Medical University, N 26°59′, E 106°71′, from soil beside a road, September 2016, Zhi-Yuan Zhang GZUIFR 21.928, *ibid*., GZUIFR 21.929.

Known distribution:—Guiyang City, Guizhou Province, China**.**

Notes:—Phylogenetically, our four strains (CGMCC 3.20770, GZUIFR 21.927, GZUIFR 21.928, and GZUIFR 21.929) clustered in a single clade with a high support value (BI pp = posterior probability 1, ML BS 99). Morphologically, member of the genus *Arthrographis* is generally recognized based on their slow growth rate, the presence of 1-celled, cylindrical arthroconidia released schizolytically from dendritic conidiophores (Sigler & Carmichael 1976). *A. multiformispora* differs from *A. curvata*, *A. grakistii*, *A. kalrae* and *A. arxii* in that it has no the trichosporiella-like synasexual morph (Giraldo *et al*. 2014; Hernández-Restrepo *et al*. 2020). *A. multiformispora* resembles *A. chlamydospora* in the presence of chlamydospores, but the conidiophores of the latter mostly repeatedly branched, while *A. multiformispora* is simple or branched (Giraldo *et al*. 2014). In conclusion, morphological and molecular phylogenetic results indicated that these new isolates are a new species in the genus *Arthrographis*, described here as *A. multiformispora*.

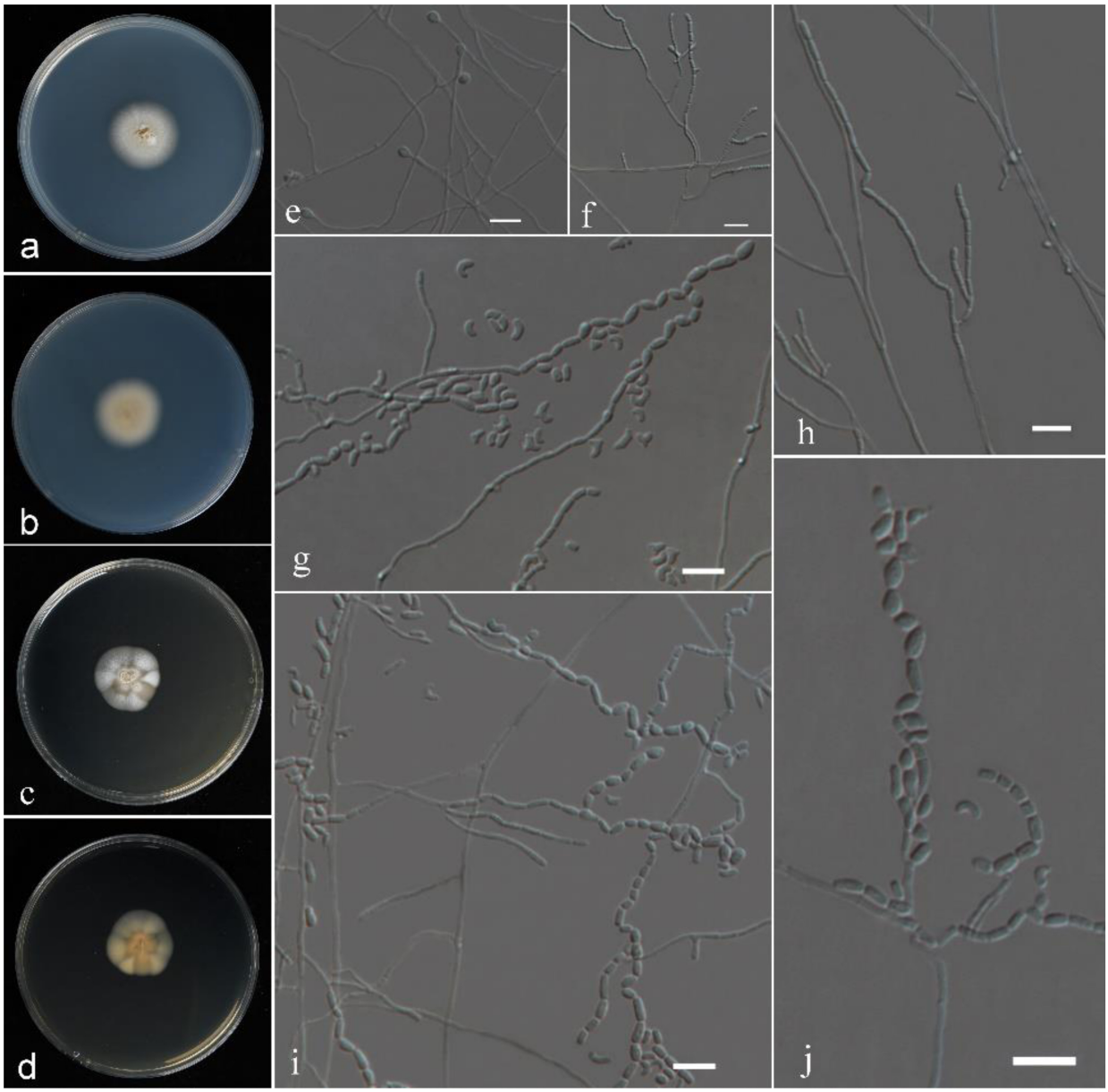

FIGURE 2. Morphology of *Arthrographis multiformispora sp. nov.* **a, b, c, d.** Colony on PDA and MEA after 14 d at 25 °C (upper surface and lower surface); **e.** chlamydospores**; g.** curved conidia**; f, h, i, j.** arthroconidia and branched conidiophores**.** Bars: e–h = 10 µm.

Discussion

In this study, four strains of *Arthrographis* were isolated from soil in Guizhou Province, China and identified by multi-loci phylogenetic analysis and morphological data. These isolates are a new species of *Arthrographis*, named *A. multiformispora*. *Arthrographis* spp. have diverse lifestyles and habitat sources – including saprophytes on soil, dung, and marine sediments (Giraldo *et al*. 2014, Hernández-Restrepo *et al*. 2020), and pathogens of animals (such as from human lesions) (Giraldo *et al.* 2014, Sigler & Carmichael 1976). Although *Arthrographis* spp. were initially isolated from soil, subsequent studies found that they also isolated from human lesions. In recent years, *Arthrographis* spp. is often considered as the source of opportunistic infections reported, such as infection of bronchus, toenail, cornea, etc., resulting in pulmonary infection (Tewari & Macpherson 1971), sinusitis (Chin-Hong *et al*. 2001), meningitis (Chin-Hong *et al*. 2001), keratitis (Biser *et al*. 2004), and onychomycosis (Yoshitsugu & Masaki 2010). Diagnosis and treatment of these cases are challenging due to the lack of global data on these uncommon invasive fungal infections (Boan *et al*. 2012). For example, *A. kalrae* can infect nails and cause onychomycosis due to their ability to utilize keratinous materials as a nutritional source (Yoshitsugu & Masaki 2010). Our new species of *A. multiformispora* were obtained from the soil beside a park road by the baiting technique (a method specifically designed for isolating keratinophilic fungi, Zhang *et al*. 2020). Therefore, it remains to be further studied whether *A. multiformispora* is an opportunistic infections pathogen to infect the skin and cause skin infection.

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