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Gongronella zunyiensis sp. nov. (Cunninghamellaceae, Mucorales) isolated from rhizosphere soil in China

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Abstract

During a survey of rhizosphere soil fungi of *Eucommia ulmoides* in China, three *Gongronella* strains were isolated. Phylogenetic analysis showed that these strains grouped into a separate subclade, closely related to *Gongronella sichuanensis*. The new strains could be distinguished from *G. sichuanensis* by the smooth-walled, subglobose, or conical-cylindrical apophyses. Both phylogenetic analysis and morphological characteristics supported the three strains being a new species, named as *Gongronella zunyiensis*.

Keywords: 1 new species, key, morphology, mucorales, phylogeny, rhizosphere soil

Introduction

Gongronella was established to accommodate a single species, *G. urceolifera* Ribaldi (Ribaldi 1952). Peyronel & Vesco (1955) transferred *Absidia butleri* Lendn. to *Gongronella,* as *G. butleri* (Lendn.) Peyronel & Dal Vesco. Based on the presence of a characteristic apophysis, *G. urceolifera* was found to be synonymous with *Absidia butleri* (Peyronel & Vesco 1955, Pici 1955).

Six species, *Gongronella lacrispora* Hesseltine & Ellis, *G. guangdongensis* F. Liu, T.T. Liu & L. Cai, *G. koreana* Hyang B. Lee & T.T.T. Nguyen, *G. orasabula* Hyang B. Lee, K. Voigt, P.M. Kirk & T.T.T. Nguyen, *G. brasiliensi* C.A.F. de Souza, D.X Lima & A.L. Santiago, and *G. sichuanensis* Zhi.Y. Zhang, Y.F. Han, W.H. Chen & Z.Q. Liang were reported later by Hesseltine & Ellis (1961), Adamčík *et al*. (2015), Ariyawansa *et al*. (2015), Li *et al*. (2016), Tibpromma *et al.* (2017) and Zhang *et al.* (2019), respectively. Currently, the genus *Gongronella* consists of seven species.

Gongronella species have been discovered to produce many bioactive compounds, such as enzymes (Zhou *et al*. 2008, Wang *et al*. 2008, Wei *et al*. 2010, Santos *et al.* 2016). Dong *et al*. (2018) reported that *Gongronella* sp. may promote plant growth by secreting organic acid and facilitating phosphate acquisition. *Gongronella* species have been mostly isolated from soil (Adamčík *et al*. 2015, Ariyawansa *et al*. 2015, Li *et al*. 2016, Zhang *et al*. 2019). *G. butleri* was isolated from roots of *Cocos nucifera* from Malaysia (Lendner 1926).

Eucommia ulmoides Oliver (Eucommiaceae), an indigenously traditional medicine tree in China, is the sole species of the genus *Eucommia*. During a survey of the rhizosphere soil of *E. ulmoides* in Zunyi, Guizhou Province, China, three *Gongronella* strains were isolated. Both phylogenetic analysis and morphological characteristics supported that these three strains were a new species and they are named as *Gongronella zunyiensis sp. nov*.

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Materials & methods

Rhizosphere soil collection and fungal isolation

The rhizosphere samples were collected from Zunyi City, Guizhou Province, China (N: 29°41′47″, E: 111°16′08″). Soil samples were mixed with sterilized water in an Erlenmeyer flask, and 1 mL suspensions were evenly spread on Martin's medium and incubated at 25°C. Three fungal strains (GZU20180911.1, GZU20180911.2, GZU20180911.3) were isolated from the rhizosphere soil of *Eucommia ulmoides*. They were purified on potato dextrose agar (PDA) and maintained on PDA slants stored at −70°C at the Institute of Fungus Resources, Guizhou University (GZAC). Ex-type culture and ex-isotypes were deposited in China General Microbiological Culture Collection Center (as CGMCC 3.19899, CGMCC 3.19900 and CGMCC 3.19901), and dried culture specimens were deposited in Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS 255626, HMAS 255627 and HMAS 255628).

Strain culture and morphological identification

The isolated strains were incubated on PDA at 25 °C for 14 d. Macroscopic and microscopic morphological characteristics were examined and photographed with an Olympus BX53 microscope (OLYMPUS, Japan). Diagnosis features were illustrated on the basis of these observations. They were morphologically identified according to colony characteristics and conidiogenous structures.

DNA extraction, PCR amplification and nucleotide sequencing

DNA extraction was carried out using the BioTeke Fungus Genomic DNA Extraction Kit (DP2032, BioTeke, China). The extracted DNA was stored at −20 °C. Amplification of the nuclear ribosomal ITS and large subunit ribosomal RNA (LSU) genes were performed with NS1/NS4 (White *et al*. 1990) and LROR/LR7 (Vilgalys & Hester 1990). The PCR products were sequenced with the above primers at TSINGKE Biological Technology (Kunming, China). The sequences of three strains were submitted to GenBank (ITS: MN453853, MN453854, MN453855; LSU: MN453856, MN453857, MN453858).

Sequence alignment and phylogenetic analysis

DNA sequences generated in this study were assembled and edited using Lasergene software (version 6.0, DNASTAR). Sequences of ITS and LSU were selected based on Li *et al*. (2016), Tibpromma *et al.* (2017), and Zhang *et al.* (2019). *Cunninghamella echinulata* (CBS 766.68) was selected as the outgroup in the phylogenetic analysis. Sequence alignments were carried out using MAFFT v.7.407 (Katoh & Standley 2013) with the default settings. Manual editing of sequences was performed in MEGA6 (Tamura *et al*. 2013). The sequences dataset (ITS+LSU) were concatenated using SequenceMatrix1.7.8 (Vaidya *et al*. 2011). Concordance between genes was assessed using the 'hompart' command of PAUP4.0b10 (Swofford 2002).

The combined data set of ITS+LSU genes were analyzed phylogenetically using maximum likelihood (ML) and Bayesian MCMC (BI). The maximum likelihood analysis was generated using RAxML (Stamatakis & Alachiotis 2010) with the graphical user interface (GUI) (Silvestro & Michalak 2012) with 1,000 bootstrap replicates and searched for best-scoring ML tree and the GTR model. For the Bayesian analysis, two runs were executed simultaneously for 1,000,000 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. The final alignment is available from TreeBASE under submission 25269.

Results

Phylogenetic analysis

The concatenated alignment of ITS+LSU sequences was 1026 bp (ITS: 632, LSU: 494) long. In the phylogenetic tree (Fig. 1), *Gongronella* species were divided into two clades, *G. lacrispora* formed a single clade and the others clustered together with well-supported values (BPP 0.93, MLBS 97%). Except for *G. koreana*, each species was monophyletically grouped. The three new strains (CGMCC 3.19899, CGMCC 3.19900 and CGMCC 3.19901) clustered with *G. sichuanensis* in a subclade (0.98/99).

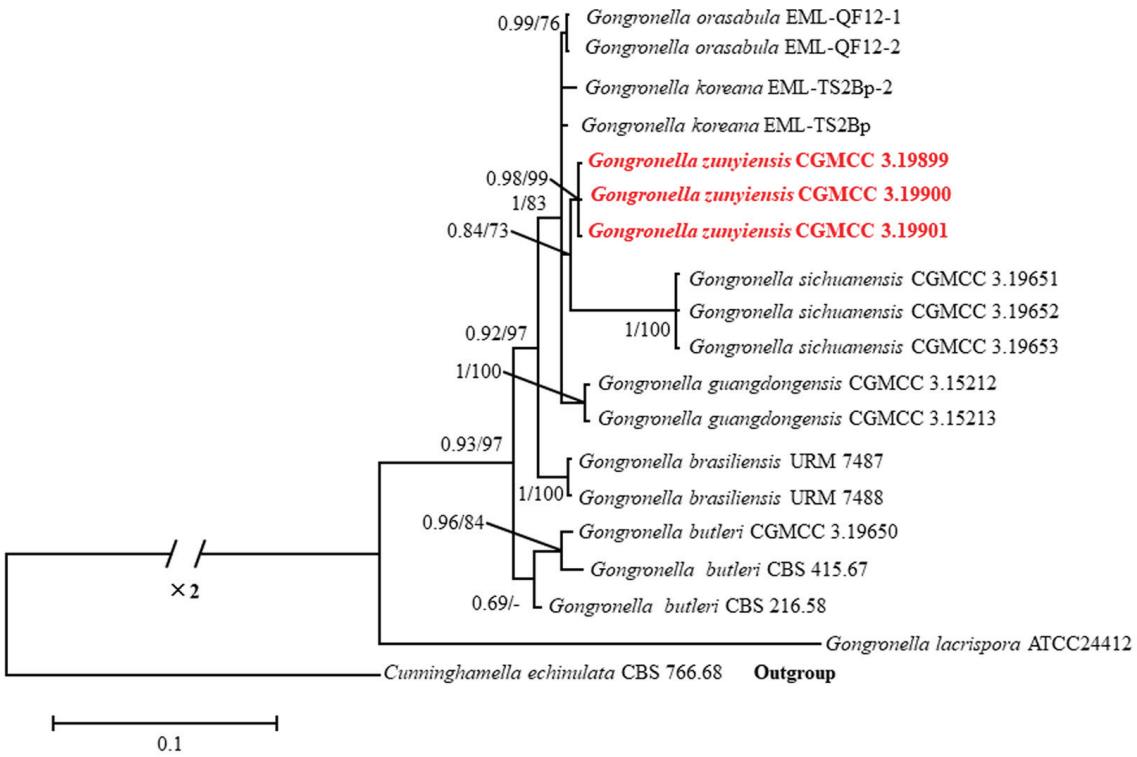

FIGURE 1. Phylogenetic tree of *Gongronella* strains CGMCC 3.19899, CGMCC 3.19900, CGMCC3.19901 and related species based on combined partial ITS+LSU sequences. Statistical support values (≥50 %) are shown at nodes, for Bayesian method / maximum likelihood. New isolates are in red.

Taxonomy

Gongronella zunyiensis C.B. Dong, Y.F. Han & Z.Q. Liang *sp. nov*. (Fig. 2) MycoBank No.: MB 833095

Type:—CHINA. Guizhou Province: Zunyi City, Fengxiang town (N: 29°41′47″, E: 111°16′08″), rhizosphere soil of *Eucommia ulmoides*, 11 August 2017, Dong Chunbo, holotype HMAS 255626, ex-holotype CGMCC 3.19899.

Colonies on PDA white, rhizoids frequent, slightly branched, long or short, 3–6 mm high, 70–75 mm in diam. after 14 d at 25 °C, villiform, rounded, margin irregular, reverse grey-white. Aerial mycelia smooth, old mycelia becoming rough. *Sporangiophores* rough or smooth, erect, growing directly from the aerial mycelia, 1.5–4.0 µm wide and variable in length, many-branched, and some displaying septa clearly below the apophysis, separating the sporangium from the sporangiophore. *Sporangia* subglobose to globose, many-spored, always with an apophysis, sporangial wall thin and smooth, 11.0–19.5 µm. *Apophyses* smooth-walled, subglobose, 3.5–9.5 µm or conical-cylindrical, 4.0–7.0 × 5.0–9.0 µm. *Sporangiospores* subglobose, reniform, 1.5–2.0 × 2.0–3.5 µm. *Columellae* hemispherical and globose, 2.0–3.0 × 3.5–7.0 µm, collarette usually present. *Chlamydospores* terminal or lateral, globose or subglobose, 7.0–10.5 µm. *Zygospores* not observed.

Etymology:—Refers to the region from which the fungus was isolated.

Additional specimens examined:—The dried cultures HMAS 255627 and HMAS 255628, and the ex-isotypes CGMCC 3.19900 and CGMCC 3.19901 were isolated from rhizosphere soil of *E. ulmoides* in Fengxiang town, Zunyi City, Guizhou Province on 10 September 2017 by Chunbo Dong.

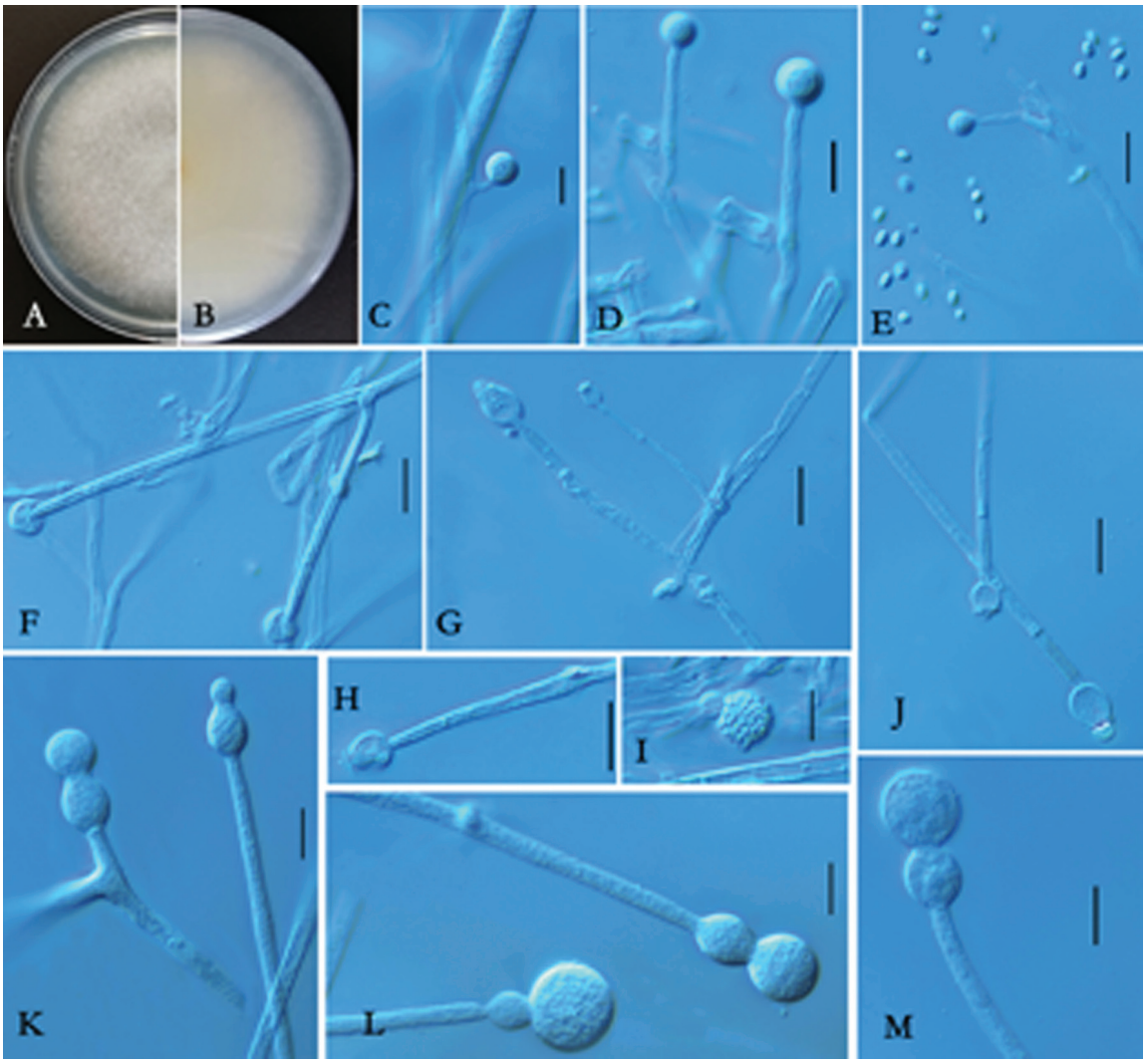

FIGURE 2. Morphology of *Gongronella zunyiensis*. **A**–**B**. Colony on potato dextrose agar (top and reverse). **C**–**E**. Chlamydospore and sporangiospores. **F**–**H, J**. Sporangiophores with short columella, apophysis, and collarette. **I, K**–**M**. Sporangiophores with variously shaped apophysis and sporangia. Bars C–M = 10 µm.

Discussion

The nuclear ribosomal ITS has been widely used for phylogenetic analysis in the genus *Gongronella* (Adamčík *et al*. 2015, Ariyawansa *et al*. 2015, Li *et al*. 2016, Tibpromma *et al*. 2017). The ITS and LSU sequences were first used to analyse the relationship among *Gongronella* species by Zhang *et al*. (2019). In the present study, ITS and LSU sequences were also used for identification of three strains. The new strains clustered into a separate subclade, and had a close relationship with *G. sichuanensis*. *Gongronella zunyiensis* was distinguished from *G. sichuanensis* by its subglobose, or conical-cylindrical apophyses (Table 1), which supported the results of molecular phylogenetic analysis. Therefore, both molecular phylogenetic results and morphology confirmed the new strains as a new species, *G. zunyiensis*.

Key to species of *Gongronella*

1. Zygospores present ..... *G. butleri*
1. Zygospores absent 2
2. Chlamydospores absent ..... *G. orasabula*
2. Chlamydospores present 3
3. Chlamydospores intercalary ..... *G. guangdongensis*
3. Chlamydospores terminal or lateral 4

4. Rhizoids and stolons present ..... *G. brasiliensis*
4. Rhizoids and stolons absent.....5
5. Sporangiospores reniform, ovoid or ellipsoidal ..... *G. sichuanensis*
5. Sporangiospores globose, subglobose to ellipsoidal or bean-shaped.....6
6. Apophyses conical-cylindrical ..... *G. zunyiensis*
6. Apophyses hemispherical, subglobose to pyriform.....7
7. Columellae hemispherical and globose.....*G. koreana*
7. Columellae dorsiventrally flattened to spherical, with a collar always present ..... *G. lacrispora*

TABLE 1. Morphological comparison of *Gongronella zunyiensis* and *G. sichuanensis.*

Species	Apophyses (μm)	Sporangiospores (μm)	Columellae (μm)	Sporangia (μm)	Chlamydospores (μm)
G. sichuanensis	Hemispherical, 2.5–4	Fusiform, reniform, 1.3–3.8×1.3–1.7	Hemispherical, 1.5–3.5×1.0–3.0	Globose, subglobose, 10.5–26.5	Present
G. zunyiensis	Subglobose, 3.5–9.5, or conical-cylindrical, 4.0–7.0×5.0–9.0	Subglobose or reniform, 1.5–2.0×2.0–3.5	Hemispherical and globose, 2.0–3.0×3.5–7.0	Globose or subglobose, 11.0–19.5	Present

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