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Gongronella sichuanensis (Cunninghamellaceae, Mucorales), a new species isolated from soil in China

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Abstract

Gongronella is known as soil-borne fungi. During a survey of fungi in southwest China, four strains of the genus *Gongronella* were isolated from soil. One strain was identified as *G. butleri*, while the other three were proposed as a new species, *Gongronella sichuanensis* based on morphological observation as well as a phylogenetic analysis of ITS, LSU sequence data. The new taxon was described, illustrated and compared with known species. Meanwhile, the key of the genus *Gongronella* was updated and provided in this paper.

Key words: new species, molecular phylogeny, taxonomy, Mucorales

Introduction

The genus *Gongronella* Ribaldi (Cunninghamellaceae, Mucorales) was established to accommodate a single species, *G. urceolifera* Ribaldi (Ribaldi 1952). The main diagnostic criteria of *Gongronella* are the presence of a distinct globose apophysis and the reduced size of columellae (Upadhyay 1969). According to these features, Peyronel and Dal Vesco (1955) transferred *Absidia butleri* Lendn. 1926 to *Gongronella*, named as *G. butleri* (Lendn.) Peyronel & Dal Vesco. Thereafter, Hesseltine and Ellis (1961) introduced a new species, *G. lacrispora* Hesselt. & J.J. Ellis. Not until the last three years were several more species described. To date, the genus includes six species: *G. brasiliensi* C.A.F. de Souza, D.X. Lima & A.L. Santiago 2017, *G. butleri*, *G. guangdongensis* F. Liu, T.T. Liu & L. Cai 2015, *G. koreana* Hyang B. Lee & T.T.T. Nguyen 2015, *G. lacrispora* and *G. orasabula* Hyang B. Lee, K. Voigt, P.M. Kirk & T.T.T. Nguyen 2016 (Peyronel & Vesco 1955; Hesseltine & Ellis 1961; Adamčík *et al*. 2015; Ariyawansa *et al*. 2015; Li *et al*. 2016; Tibpromma *et al*. 2017).

Traditionally, the identification and classification of *Gongronella* was mainly based on their sporangial morphology. In recent years, molecular analysis has been used as an auxilliary tool to delimitate species, resulting in an increase of the number of new taxa (Adamčík *et al*. 2015; Ariyawansa *et al*. 2015; Li *et al*. 2016; Tibpromma *et al*. 2017). Despite the small number of described species, several studies have reported that members of the genus *Gongronella* have important biotechnological applications, such as the production of enzymes and antifungal proteins (Zhou *et al*. 2008; Wang *et al*. 2008; Wei *et al*. 2010).

During a survey of keratinolytic fungi in southwest China, four strains of *Gongronella* were isolated from soil. Based on rDNA phylogeny and morphology, one strain was identified as *G. butleri*, while the other three as a new species. This paper provides a phylogenetic tree, descriptions, and illustrations of the novel species.

Materials & Methods

Fungal isolation

Soil samples were collected from Chengdu City, Sichuan Province and Zunyi City, Guizhou Province, China. Isolation and purification of strains according to the methods described by Zhang *et al.* (2019). Four strains were obtained and

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deposited in the China General Microbiological Culture Collection Center (CGMCC) and in the Institute of Fungus Resources, Guizhou University (GZUIFR). Strains CGMCC 3.19651 (= GZUIFR-H25.4.1), CGMCC 3.19652 (= GZUIFR-H25.4.2), and CGMCC 3.19653 (= GZUIFR-H25.4.3) were isolated from Chengdu City. Strain GZUIFR-H20.4.1 (= CGMCC 3.19650) was isolated from Zunyi City. The culture of the type strain CGMCC 3.19651 was dried as holotype which was deposited in the Mycological Herbarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS 255616).

Morphology

Isolates were incubated on potato dextrose agar (PDA) and Czapek agar (CA) for examination of morphological characters. Colony was examined after 14 d at 25°C in darkness. Fungal microscopic features were observed and photographed with a OLYMPUS BX53 microscope (OLYMPUS, Japan) and processed with Ulead PhotoImpact 6.0.

DNA extraction, PCR amplification and nucleotide sequencing

Genomic DNA was extracted from fresh fungal mycelia using the BioTeke Fungus Genomic DNA Extraction Kit (DP2032, BioTeke, China), following the manufacturer's instructions. DNA samples were stored at -20 °C until used for polymerase chain reaction (PCR). Amplification and sequencing of the internal transcribed spacers (ITS1-5.8S-ITS2), the large subunit (LSU), the small subunit (SSU) of rRNA gene, translation elongation factor 1 alpha (EF1α) and part of the actin gene (*act*) were performed, respectively, with the primer pairs ITS1/ITS4 (White *et al*. 1990), LROR/LR7 (Vilgalys and Hester 1990), NS1/NS4 (White *et al*. 1990), EF1-983F/EF1-2218R (van den Brink *et al*. 2012) and ACT-F/ACT-R (Carbone and Kohn 1999). Sequencing was performed by TSINGKE Biological Technology (Kunming, China) using the corresponding primers.

Sequence alignment and phylogenetic analyses

The DNA sequences generated in this study were assembled using Lasergene software (version 6.0, DNASTAR). Sequence data, mainly from recent publications (Li *et al*. 2016; Tibpromma *et al*. 2017), were downloaded for analyses (Table 1). *Cunninghamella echinulata* (CBS 766.68) was chosen as an outgroup. Sequences alignments were performed by using MAFFT v.7.407 (Katoh and Standley 2013) and manually edited in MEGA 6.06 (Tamura *et al*. 2013). ITS and LSU sequences were concatenated by SequenceMatrix v.1.7.8 (Vaidya 2011). Molecular phylogenetic analyses of the combined ITS and LSU sequence data were obtained from maximum likelihood (ML) and Bayesian inference (BI) analyses. The best nucleotide substitution model was determined by Modeltest v3.7 (Posada and Crandall 1988), the best-fit model for BI is GTR+G under the Akaike Information Criterion (AIC).

In this study, due to the lack of reference sequences of other loci from *Gongronella* spp., only ITS+LSU phylogenetic analysis was finally performed. Maximum likelihood analyses was generated by using RAxML (Stamatakis and Alachiotis 2010) with the graphical user interface (GUI) (Silvestro and Michalak 2012) with 1,000 bootstrap replicates and search for best-scoring ML tree and the GTR model. Bayesian inference analyses were conducted with MrBayes v.3.2 (Ronquist *et al*. 2012) and a Bayesian posterior probability was determined by Markov Chain Monte Carlo sampling (MCMC). Two runs were executed simultaneously for 1,000,000 generations, saving trees every 500 generations. After the analyses was finished, each run was examined with the program Tracer v1.5 (Drummond and Rambaut 2007) to determine burn-in and confirm that both runs had converged. The final tree was submitted in TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S24328).

TABLE 1. Strains included in phylogenetic analyses.

Species	GenBank accession				
Strains	ITS	LSU	SSU	EF1α	*act*
Gongronella sichuanensis					
CGMCC 3.19651 T	**MK813373**	**MK813855**	**MK813849**	**MK820629**	**MK820625**
CGMCC 3.19652	**MK813374**	**MK813856**	**MK813850**	**MK820630**	**MK820626**
CGMCC 3.19653	**MK813375**	**MK813857**	**MK813851**	**MK820631**	**MK820627**
Gongronella lacrispora					
ATCC 24412	GU244498	---	---	---	---

.....continued on the next page

TABLE 1. (Continued)

Species	GenBank accession				
Strains	ITS	LSU	SSU	EF1α	*act*
Gongronella butleri					
CBS 216.58 **T**	MH857761	MH869292	---	---	---
CBS 415.67	MH859014	MH870714	---	---	---
CGMCC 3.19650	**MK813876**	**MK813888**	**MK813887**	**MK820632**	**MK820628**
Gongronella brasiliensis					
URM 7487 **T**	KY114930	KY114932	---	---	---
URM 7488	KY114931	KY114933	---	---	---
Gongronella koreana					
EML-TS2Bp **T**	KP636529	KP636530	KT321300	KP636528	KP636527
EML-TS2Bp-2	KP835545	KP835542	KT321301	KP835544	KP835543
Gongronella guangdongensis					
CGMCC 2.15212 **T**	KC462739	---	---	---	---
CGMCC 2.15213	KC462740	---	---	---	---
Gongronella orasabula					
EML-QF 12-1 **T**	KT936269	KT936263	KT936261	KT936267	KT936265
EML-QF 12-2	KT936270	KT936264	KT936262	KT936268	KT936266
Cunninghamella echinulata					
CBS 766.68	JN205894	MH877699	---	---	---

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

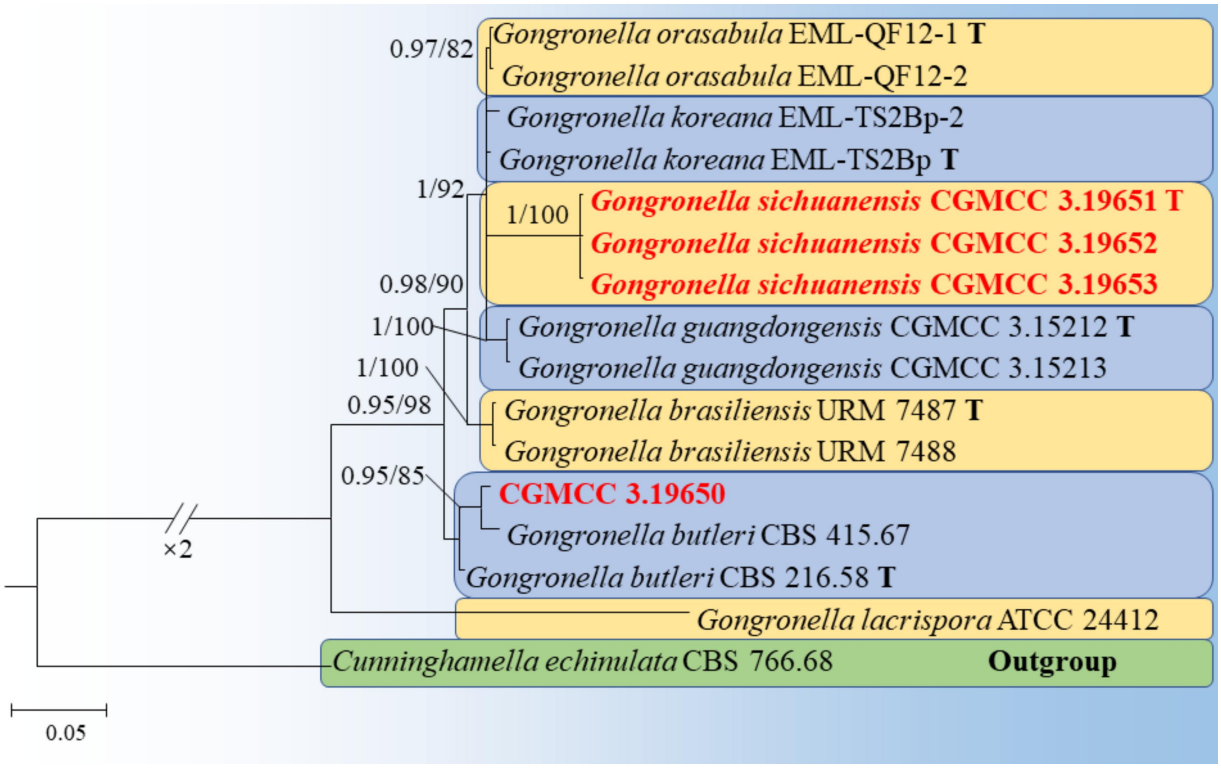

FIGURE 1. Phylogenetic tree of *Gongronella* based on the ITS+LSU dataset and *Cunninghamella echinulata* (CBS 766.68) as the outgroup taxon. Numbers at nodes are Bayesian posterior probabilities (left, BPP ≥ 0.95) and maximum likelihood bootstrap values (right, MLBS ≥ 80%). New isolates are in red and bold.

Results

Phylogenetic analyses

A concatenated aligned dataset of ITS rDNA and LSU rRNA sequences from 16 strains was 1020 bp (including gaps) long (Table 1) and was used for the phylogenetic analyses. The topologies obtained through Bayesian and maximum likelihood inferences were overall highly concordant. In the phylogenetic tree, the genus *Gongronella* was divided into two clades: *G. lacrispora* was a single clade, while the others clustered together with a well support value (BPP 0.95, MLBS 98%). Except *G. koreana*, each species monophyletically grouped. The strains CGMCC 3.19651, CGMCC 3.19652 and CGMCC 3.19653 isolated in this study were closely related to *G. guandongensis*, *G. koreana* and *G. orasabula*. Another isolate CGMCC 3.19650 closely clustered with *G. butleri*.

Taxonomy

Gongronella sichuanensis Zhi.Y. Zhang, Y.F. Han, W.H. Chen & Z.Q. Liang, *sp. nov.* (Fig. 2)

Mycobank No.: MB830671

Type:—CHINA. Sichuan Province: Chengdu City, Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital (N 30°39′55″, E 104°02′14″), soil, 10 September, 2016, Zhiyuan Zhang, holotype HMAS 255616, ex-holotype CGMCC 3.19651(= GZUIFR H25.4.1).

Colony on PDA white, 4–5 mm high, 67–68 mm in diam. in 14 days at 25°C, villiform, rounded, margin regular, reverse grey. Colony on CA grey, 1–2 mm high, 63 mm in diam. in 14 days at 25°C, villiform, rounded, margin regular, reverse grey. *Rhizoids* and *stolons* absent. Odourless. *Sporangiophores* erect, straight or slightly recumbent, solitary or a single branched, septate, smooth-walled, hyaline, 28.0–46.5 × 1.0–3.0 μm, always with one or two septa under the apophyses. *Sporangia* globose, subglobose, 10.5–26.5 μm, thin-walled, smooth, many-spored, always with an apophyses. *Apophyses* ellipsoidal to subglobose, hyaline, smooth, 4.5–8.5 × 4.5–6.0 μm in diam. *Columellae* hemispherical, 1.5–3.5 × 1.0–3.0 μm. *Sporangiospores* reniform, ovoid or ellipsoidal, smooth, 1.5–2.0 × 1.0–1.5 μm. *Chlamydospores* globose or obpyriform, 5.0–7.5 μm. *Zygospores* not observed.

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

Additional specimens examined:—The ex-isotype CGMCC 3.19652 and CGMCC 3.19653 were isolated from soil in Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, Chengdu City, Sichuan Province on 10 September 2016 by Zhiyuan Zhang.

Discussion

The genus *Gongronella* was established to accommodate *G. urceolifera* (currently *G. butleri*) for *Absidia*-like fungi having a globose apophysis with a constriction between the apophyses and the attachment of the sporangial wall (Adamčík *et al*. 2015). Members of the genus grow relatively slowly and inhabit strictly in the soil. The new species *G. sichuanensis* has morphological characters that fit the generic concepts very well. In the phylogenetic tree, *G. sichuanensis* is closely related to *G. orasabula*, *G. koreana* and *G. guangdongensis*. However, *G. sichuanensis* differs from *G. orasabula* by the presence of its chlamydospores (Li *et al*. 2016). *G. sichuanensis* is distinguished from *G. koreana* by the reniform, ovoid or ellipsoidal sporangiospores (Ariyawansa *et al*. 2015). Furthermore, *G. sichuanensis* differs from *G. guangdongensis* by the ellipsoidal to subglobose apophyses, and reniform, ovoid or ellipsoidal sporangiospores (Adamčík *et al*. 2015) (Table 2). The isolate CGMCC 3.19650 closely clustered with *G. butleri* in the phylogenetic tree, whose morphological characters were similar to the original description of *G. butleri* (Peyronel & Vesco 1955), so it was identified as the new isolate of *G. butleri*.

Gongronella taxonomy is primarily based on morphological characters and phylogenetic inferences of ITS sequences. In this paper, the phylogenetic analyses used ITS as well as LSU, the results are consistent with previous studies based on ITS (Li *et al*. 2016; Tibpromma *et al*. 2017). Therefore, the LSU data can be used together with ITS sequences as genetic data for resolving the interspecific relationship in the genus *Gongronella*.

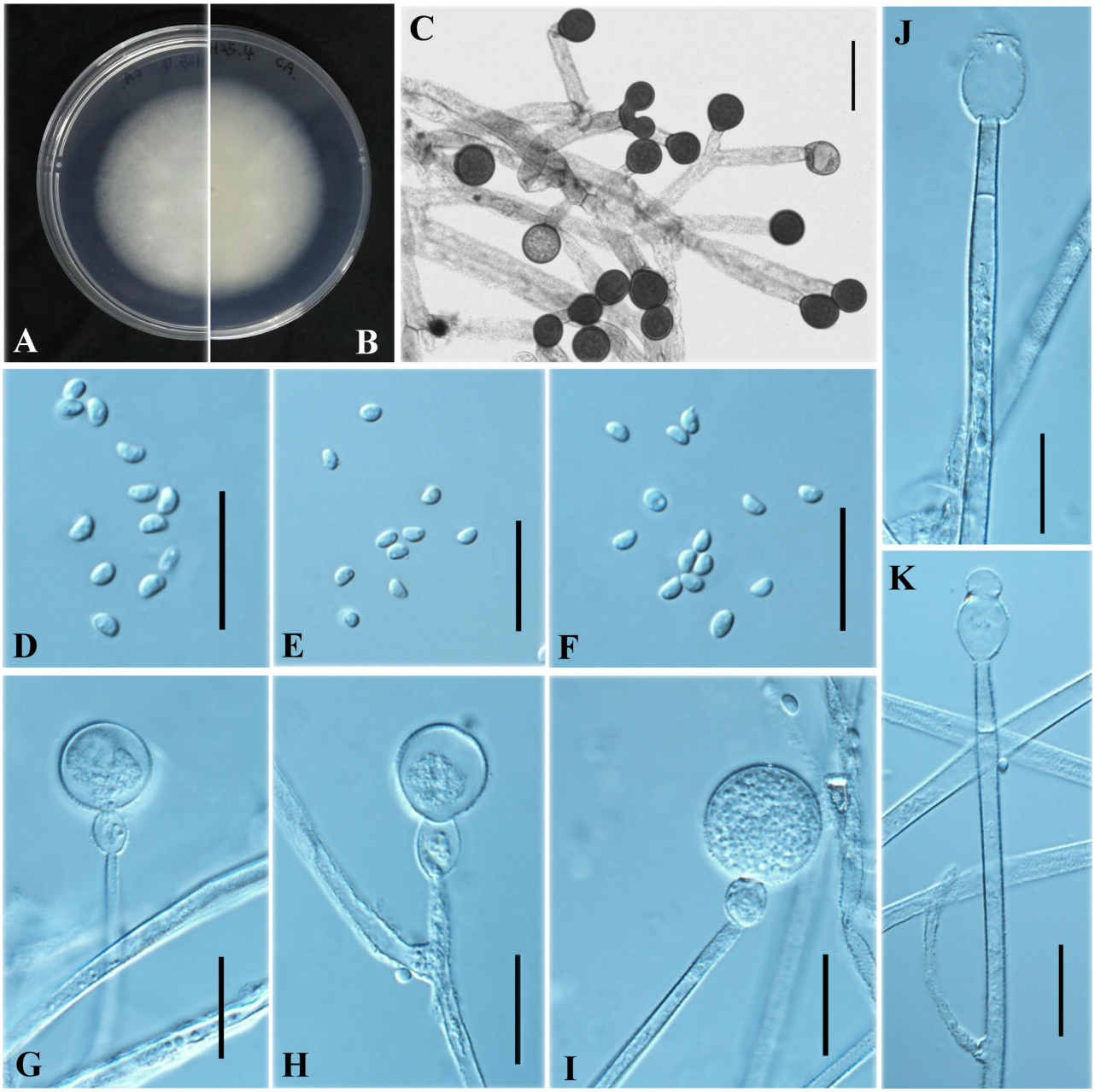

FIGURE 2. *Gongronella sichuanensis* (Holotype HMAS 255616). **A–B**. Colony on CA. **C**. Chlamydospore. **D–F**. Sporangiospores. **G–I**. Sporangiophores with variously shaped apophyses and sporangia. **J**. Apophyses. **K**. Columellae. Bars: C–K = 10 µm.

TABLE 2. Morphological comparison among *Gongronella sichuanensis* and the related species.

Species	Apophyses (µm)	Sporangiospores (µm)	Chlamydospores
G. guangdongensis	Hemispherical 5.5–9	globose 2–3	present
G. koreana	subglobose to pyriform 8.7–10×5.7–8.2	subglobose to ellipsoidal or bean-shaped 1.3–1.6×2.7–3.2	present
G. orasabula	globose, subglobose to pyriform 5–10×4.5–8.5	bean-shaped 2–3.5×2–2.5	absent
G. sichuanensis	ellipsoidal to subglobose 4.5–8.5×4.5–6.0	reniform, ovoid or ellipsoidal 1.5–2.0 × 1.0–1.5	present

Key to species of *Gongronella*

1 Zygospores absent **2**
– Zygospores present, globose ..... ***G. butleri***
2 Chlamydospores present **3**
– Chlamydospores absent ..... ***G. orasabula***
3 Rhizoids and stolons absent **4**
– Rhizoids and stolons present ..... ***G. brasiliensis***
4 Sporangiospores globose, subglobose to ellipsoidal or bean-shaped **5**
– Sporangiospores reniform, ovoid or ellipsoidal ..... ***G. sichuanensis***
5 Apophyses hemispherical **6**
– Apophyses subglobose to pyriform ..... ***G. koreana***
6 Columellae hemispherical ..... ***G. guangdongensis***
– Columellae dorsiventrally flattened to spherical, with a collar always present ..... ***G. lacrispora***

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