

# *Modicella guangxiensis* (Mortierellomycota, Mortierellaceae), a new species from southwestern karst areas of China

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## Abstract

## Background

*Modicella* Kanouse (1936) is the only genus of Mortierellaceae known to produce macroscopic fruiting bodies in the form of small, whitish, round sporocarps. Specimens which belong to *modicella* were collected during our field investigations in tropical karst areas of China. Based on morphological characteristics and phylogenetic analysis, a new species is introduced.

## New information

*Modicella guangxiensis* is described as a new species from tropical karst areas of China. The main distinguishing characteristics of *M. guangxiensis* are the number of sporangiospores per sporangium (11–18), the size of sporangiospores (14–34 × 12–27.5 μm) and the surface of some hyphae with hemispherical tuber. The phylogenetic analyses, based on the internal transcribed spacer (ITS) and the large subunit (LSU) regions of rDNA sequences using Bayesian (BA) and Maximum Likelihood (ML) methods showed that the new taxon is closely related to *M. reniformis*.

## Keywords

new taxon, taxonomy, phylogeny, sporocarp-forming fungi

## Introduction

*Modicella* Kanouse is a small genus of Mortierellaceae, typified by the species *Modicella malleola* (Harkn.) Gerd. & Trappe. This genus is the only genus of Mortierellaceae known to produce macroscopic fruiting bodies in the form of small, whitish, round sporocarps. They are morphologically Mortierellaceae-like in their acolumellate sporangium and garlic-like odour that is similar to that of other Mortierellaceae species (Vandepol et al. 2020).

At present, only three taxa have been recognised in *Modicella* (<http://www.indexfungorum.org>), namely *M. albostipitata* J.A. Cooper, *M. malleola* (Harkn.) Gerd. & Trappe and *M. reniformis* (Bres.) Gerd. & Trappe. The species of *Modicella* have usually been found on soil or within decomposing plant materials. *M. albostipitata* was found in New Zealand (Cooper and Park 2020); *M. malleola* has been collected in Europe, North America and China; *M. reniformis* has been collected in Brazil, Chile and Argentina (Smith et al. 2013).

During our field investigations in tropical karst areas of China, a sporocarp-forming fungus was discovered. Morphological study and phylogenetic analysis, based on ITS and LSU rDNA sequences, proved that it is new to science.

## Materials and methods

### Sampling, morphological observations and descriptions

One specimen was collected from the Guangxi Zhuang Autonomous Region, China and was dried in silica gel. Dried specimen preserved in the Herbarium of Guangxi Institute of Botany (IBK). Macroscopic characteristics are based on fresh specimens. Microscopic characteristics were obtained, based on dried specimens and examined with a light microscope (Olympus BX43F, Japan). Colour microscopic photos were taken by Mshot camera (Mshot MDX6-T, China). Measurements were made on the tissues mounted in purified water. Tissues were stained with 1% Congo Red solution or Lactate Carbolic Cotton Blue. The size of sporangia and sporangiospores was calculated, based on measurements of randomly sampled 42 sporangia and 44 sporangiospores.

### DNA extraction, PCR amplification and sequencing

Total DNA was extracted from dried specimens using a NuClean Plant Genomic DNA kit (CWBio). ITS4/ITS5 and LR0R/LR7 were taken as primer sequences to amplify the ITS and LSU regions (White et al. 1990). The PCR procedure for ITS and LSU was as follows: initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 40 s, 52°C for 40 s, 72°C for 1 min and final extension of 72°C for 10 min. The DNA sequencing was

completed by Shenggo Biological Technology Co. Ltd. Sequences derived in this study were deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank>).

## Data analysis

A total of 11 *Modicella* and allied species' sequences of ITS and LSU (including the new taxon) were used for molecular phylogenetic analyses, while sequences retrieved from GenBank (Table 1) mainly refer to Smith et al. (2013), Wagner et al. (2013) and Cooper and Park (2020). *Actinomortierella capitata* (CBS 859.70), *A. capitata* (CBS 110640), *A. wolfii* (CBS 209.69) and *A. wolfii* (CBS 612.70) were used as the outgroup, based on the earlier studies of Vandepol et al. (2020) and a recent bioRxiv preprint studies of Zhao et al. (2022).

Sequences of ITS and LSU were aligned separately with online MAFFT (Katoh et al. 2017) using the default settings. Prior to phylogenetic analysis, ambiguous sequences at the start and the end were deleted and gaps were manually adjusted to optimise the alignment using the default parameters in BioEdit v.7.2.5. (Hall 1999). The ITS and LSU sequences were concatenated as a combined file using SequenceMatrix (Vaidya et al. 2011). MrModelTest v.2.3 was used to estimate the optimal model (Nylander 2004). Bayesian Inference (BI) analysis was performed with MrBayes v.3.2.6 and four Markov Chains (MCMC) were run for one million generations, sampling every 100<sup>th</sup> generation (Ronquist and Huelsenbeck 2003). Maximum Likelihood (ML) bootstrap analysis was performed with rapid bootstrapping algorithm and 1000 replicates, followed by a ML tree search in raxmlGUI 2.0 (Edler et al. 2020). The tree was visualised by FigTree v.1.4.3 (Rambaut 2012). The final concatenated sequence alignments were deposited in TreeBase (<https://www.treebase.org/treebase-web/home.html>) with the submission ID 30895.

## Taxon treatment

### *Modicella guangxiensis* T. Bau & G. F. Mou, sp. nov.

- Fungal names number: [FN 571583](#)

#### Material

#### *Holotype*:

- a. kingdom: Fungi; phylum: Mortierellomycota; class: Mortierellomycetes; order: Mortierellales; family: Mortierellaceae; taxonRank: species; genus: *Modicella*; country: China; stateProvince: Guangxi; county: Longzhou; verbatimElevation: 191 m; verbatimLatitude: 22°27'53.59" N; verbatimLongitude: 106°55'38.42" E; identifiedBy: Guang-Fu Mou; type: Nonggang National Nature Reserve, on soil or decomposing plant materials, 15 August 2021, Guang-fu Mou, M2021081545 (holotype IBK!); institutionID: IBK; collectionID: M2021081545; institutionCode: Herbarium of Guangxi Institute of Botany (IBK); occurrenceID: 4499BC04-583D-535F-A55A-331F2F84111A

## Description

Sporocarps hemispherical to nearly spherical, base flattened or flat with a cavity; 1.5–4.5 mm in diameter, 1.5–3.0 mm in height; white when fresh and becoming pale yellow when dried; fragile; surface appearing granular when fresh and containing sporangia (Fig. 1A and B). Peridium absent. Columella absent. Basal hyphae white. Odour not recorded.

Sporangia subglobose, ellipsoid, (41)45–62(68) × (35)40–54(61) μm, hyaline, thin-walled, containing 11–15(18) sporangiospores (Fig. 1C–F); each sporangium is attached to a single subtending hypha (Fig. 1E). Sporangiospores subglobose, ellipsoid, ovoid or irregular, colourless, cyanophilous, with a significant oil-like content, (14)16–30(34) × (12)14–23.5(27.5) μm, 21 × 17 μm on average (Fig. 1G and H); some sporangiospores will elongate and constrict into two sporangiospores (Fig. 1H). There are three kinds of hyphae: hyphae of surface with hemispherical tuber (Fig. 1I), 3 μm in diameter, not easily found; hyphae of branched (Fig. 1J), 3–4.5 μm in diameter; hyphae linking with sporangium (Fig. 1K), wider in width, 5.5–6.8 μm, sometimes branched. All hyphae hyaline, thin-walled or slightly thick-walled, septa rarely seen. Zygospores not observed.

## Diagnosis

Differing from the known species by the number of sporangiospores per sporangia (11–18), the size of sporangiospores (14–34 × 12–27.5 μm) and the surface of some hyphae with hemispherical tuber.

## Etymology

*guangxiensis* (Lat.): referring to the locality of the type specimen, Guangxi Zhuang Autonomous Region of China.

## Distribution

So far, only known from Guangxi (CHINA).

## Ecology

It grows on broad-leaved forest soil or decomposing plant materials of karst areas.

## Analysis

### Phylogenetic analyses

The phylogenetic analyses were inferred from ITS+LSU ribosomal DNA datasets, which had 1635 (including gaps) base pairs. The ITS dataset had an alignment length of 694

(including gaps) base pairs representing 11 sequences. The LSU dataset had an alignment length of 941 (including gaps) base pairs representing eight sequences. The best-fit model used for Bayesian Inference (BI) analysis for the combined two-marker data subset (the two-marker dataset was treated individually) is the same, being the GTR+G model. For the Maximum Likelihood (ML) bootstrap analysis, the ITS and LSU datasets were treated as a whole, using the GTR+G model. The Bayesian Inference (BI) analysis and Maximum Likelihood (ML) bootstrap analysis obtained the same topology, this present manuscript showing the topology of Bayesian Inference (BI) analysis (Fig. 2). Maximum Likelihood bootstrap values (MLBS  $\geq 50\%$ ) and posterior probabilities values for BI (BPP  $\geq 0.85$ ) are given above each branch (BPP/BS), with the new taxon being in red font.

In the ITS+LSU analyses (Fig. 2), our specimen (red font) is grouped together with the species of *Modicella* with full support values (1.00 BPP and 100% BS). Within the *Modicella* clade, our specimen (red font) is in a independent clade differing from all known species of *Modicella*, with full support values of BI analyses (1.00 BPP) and strong statistical support values of ML analyses (76% BS).

## Discussion

*Modicella* is a small genus and also the only genus that produces fruiting bodies in Mortierellaceae. Phylogenetically, our specimen separated from the known species and formed a single clade receiving strong statistical support (1.00/76) (Fig. 2), confirming it as a distinct taxon.

Morphologically, *M. guangxiensis* differs from *M. allostipitata* by the sporocarps being smaller and without a distinct pseudostipe, the fewer number of sporangiospores per sporangia (11–18 vs.  $> 100$ ), the larger sporangiospores ( $14\text{--}34 \times 12\text{--}27.5 \mu\text{m}$  vs.  $10\text{--}16 \mu\text{m}$ ) (Cooper and Park 2020). The new species differs from *M. malleola* by the larger sporangiospores [ $14\text{--}34 \times 12\text{--}27.5 \mu\text{m}$  vs.  $6.5\text{--}23 \times 4\text{--}22 \mu\text{m}$  (Smith et al. 2013) or average  $21 \times 17 \mu\text{m}$  vs.  $8\text{--}12 \mu\text{m}$  (Thaxter 1922)]; *M. guangxiensis* differs from *M. reniformis* by the greater number of sporangiospores per sporangia (11–18 vs. 3–8) (Smith et al. 2013). In addition, the surface of hyphae with hemispherical tuber also differs from the known species.

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## References

- Cooper JA, Park D (2020) *Modicella albstipitata*, a new species of sporocarp-forming fungus from New Zealand (Mortierellaceae: Mortierellomycota). *Phytotaxa* 453 (3): 293-296. <https://doi.org/10.11646/phytotaxa.453.3.11>
- Edler D, Klein J, Antonelli A, Silvestro D (2020) raxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. *Methods in Ecology and Evolution* 12 (2): 1-5. <https://doi.org/10.1111/2041-210X.13512>
- Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41 (41): 95-98. <https://doi.org/10.1021/bk-1999-0734.ch008>
- Kato K, Rozewicki J, Yamada KD (2017) MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Briefings in Bioinformatics* 20 (4): 1160-1166. <https://doi.org/10.1093/bib/bbx108>
- Nylander J (2004) MrModeltest 2.3. Computer program and documentation distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.
- Rambaut A (2012) FigTree v1. 4. Molecular evolution, phylogenetics and epidemiology. University of Edinburgh, Institute of Evolutionary Biology.
- Ronquist F, Huelsenbeck J (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19 (12): 1572-1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Smith ME, Gryganskyi A, Bonito GM, Nouhra ER, Moreno-Arroyo B, Benny G (2013) Phylogenetic analysis of the genus *Modicella* reveals an independent evolutionary origin of sporocarp-forming fungi in the Mortierellales. *Fungal Genetics and Biology* 61: 61-68. <https://doi.org/10.1016/j.fgb.2013.10.001>
- Thaxter R (1922) A revision of the Endogoneae. *Proceedings of the American Academy of Arts and Sciences* 57 (12): 291-351. <https://doi.org/10.2307/20025921>
- Vaidya G, Lohman DJ, Meier R (2011) Sequencematrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27 (2): 171-180. <https://doi.org/10.1111/j.1096-0031.2010.00329.x>
- Vandepol N, Liber J, Desirò A, Na H, Kennedy M, Barry K, Grigoriev IV, Miller AN, O'Donnell K, Stajich J, Bonito GM (2020) Resolving the Mortierellaceae phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. *Fungal Diversity* 104 (1): 267-289. <https://doi.org/10.1007/s13225-020-00455-5>
- Wagner L, Stielow B, Hoffmann K, Petkovits T, Papp T, Vágvölgyi C, Hoog Sd, Verkleij G, Voigt K (2013) A comprehensive molecular phylogeny of the Mortierellales (Mortierellomycotina) based on nuclear ribosomal DNA. *Persoonia* 30 (1): 77-93. <https://doi.org/10.3767/003158513X666268>
- White TJ, Bruns TD, Lee SB, Taylor JW, Innis MA, Gelfand DH, Sninsky J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky J, White TJ (Eds) *PCR Protocols: A guide to methods and applications*. Academic Press, New York, 322 pp. <https://doi.org/10.1016/B978-0-12-372180-8.50042-1>

- Zhao H, Dai YC, Liu XY (2022) Outline and divergence time of subkingdom Mucoromyceta: two new phyla, five new orders, six new families and seventy-three new species. bioRxiv Preprint. <https://doi.org/10.1101/2022.07.05.498902>

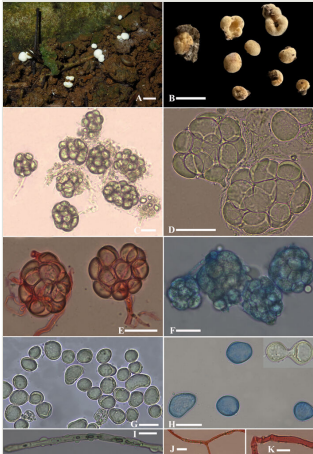


Figure 1.

Morphology of *Modicella guangxiensis*. **A** Sporocarps and its habitat; **B** Dried yellowish sporocarps; **C–F** Intact sporangia; **G, H** Sporangiospores; **I, J, K** Different kinds of hyphae. Scale bar A–B = 5 mm; C–F = 25  $\mu\text{m}$ ; G–H = 20  $\mu\text{m}$ ; I–K = 10  $\mu\text{m}$ . C, D, G, I. in purified water; E, J, K. in 1% Congo Red; F, H. in Cotton Blue; Photos by Guang-fu Mou, from M2021081545 (holotype IBK!).



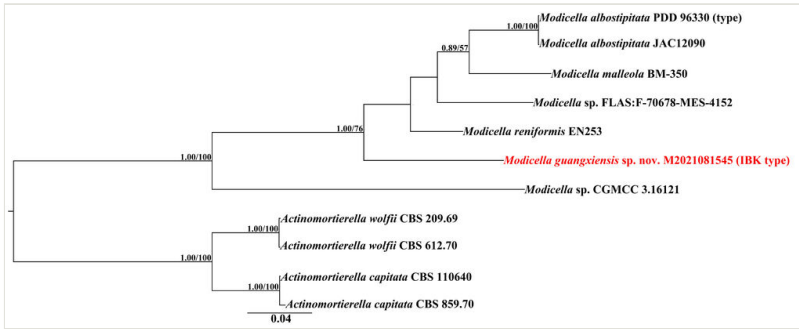


Figure 2.

Phylogenetic tree inferred from partial ITS+LSU sequences showing phylogenetic relationships of *Modicella guangxiensis*. Bayesian Inference (BPP  $\geq$  0.85) and Maximum Likelihood support values (BS  $\geq$  50) are shown (BPP/BS). New species is in red font.

Table 1.

Specimens used in phylogenetic analysis and GenBank accession numbers (the newly-generated sequences are in bold).

Species	Voucher/Strain number	GenBank Accession numbers	
		ITS	LSU
<i>Actinomortierella capitata</i>	CBS 859.70	MH859983	MH871779
<i>Actinomortierella capitata</i>	CBS 110640	JX975923	JX976163
<i>Actinomortierella wolfii</i>	CBS 209.69	MW577262	—
<i>Actinomortierella wolfii</i>	CBS 612.70	MH859876	MH871661
<i>Modicella allostipitata</i>	PDD 96330	NR_171266	NG_074467
<i>Modicella allostipitata</i>	JAC12090	MT649493	MT649494
<b><i>Modicella guangxiensis</i></b>	<b>M2021081545</b>	<b>OR711265</b>	<b>OR710909</b>
<i>Modicella malleola</i>	BM-350	KF053135	KF053131
<i>Modicella reniformis</i>	EN253	KF053136	KF053132
<i>Modicella</i> sp.	FLAS:F-70678-MES-4152	OP339685	—
<i>Modicella</i> sp.	CGMCC 3.16121	OL678160	—