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Unveiling Species Diversity Within Early-Diverging Fungi from China VIII: Four New Species in *Mortierellaceae* (*Mortierellomycota*)

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Abstract: The fungal family *Mortierellaceae* represents ubiquitous and ecologically significant components of soil ecosystems across terrestrial habitats. Through an integrative taxonomic approach combining multi-locus phylogenetic analyses (ITS, LSU, SSU rDNA, *RPB1*, and *Act*) with detailed morphological examinations of rhizosphere soil isolates, four novel species within this family were proposed. This study describes and illustrates these taxa, elucidating their morphological distinctions from allied species and phylogenetic relationships within the family. *Linnemannia rotunda* sp. nov. (closely related to *L. longigemmata*) is distinguished by its globose sporangia and sporangiospores. *Mortierella acuta* sp. nov. is characterized by spiky collarettes. *Mortierella oedema* sp. nov. (a sister to *M. macrocystis*) exhibits distinctive ampulliform swellings. *Mortierella tibetensis* sp. nov. (clustering with *M. parvispora*), named for its geographic origin in Tibet. As the eighth installment in a systematic investigation of early-diverging fungal diversity in China, this work expands the global taxonomic inventory of *Mortierellaceae* to 148 species, underscoring the ongoing discovery of cryptic biodiversity within this ecologically pivotal group.

Keywords: *Linnemannia*; *Mortierella*; *Mortierellales*; multi-gene phylogeny; taxonomy

1. Introduction

Mortierellaceae, belonging to *Mortierellomycota*, *Mortierellomycotina*, *Mortierellomycetes* and *Mortierellales* (http://www.indexfungorum.org/, accessed on 14 February 2025) [1,2], typically form white, cottony zonate or rosette-like colonies, with a distinctive odor reminiscent of garlic or a freshly bathed dog [3–5] A swelling is usually produced at the base of sporangiophores. *Mortierellaceae* species are considered to be an important saprophyte [6], usually detected and isolated from soil, plant remains, insect guts, mosses, and living plant roots [3] This family is ubiquitous and widely distributed. GBIF database documents *Mortierellaceae* from Estonia (92,697 records), Australia (35,514), Czechia (27,899), Russian Federation (15,180), Colombia (13,733), United States of America (13,424), Italy (13,417), Lithuania (11,343), Sweden (11,074) and Norway (422), (https://www.gbif.org/, accessed on 24 February 2025). Recent studies on soil microbial communities across the globe have shown that species of *Mortierellaceae* are important members of the soil core microbiome. [7,8] Owing to their broad habitats, members of the genus *Mortierella* are able to grow at a wide range of temperatures. They live in the winter-active soil microbial community, forming substantial fungal biomass in the soil during both the snow-covered and the vegetative periods. [9] They produce polyunsaturated fatty acids, such as arachidonic acid, which are crucial for several biological functions in mammals. [10–12] These biological functions are widely used in commercial production, for example, in the production of biofuels. [13,14] Many *Mortierellaceae* species have the ability to promote plant growth, to decompose plant litter, and to remodel rhizosphere microbial communities. [15,16] Some species are also biological control agents, producing active antimicrobial metabolites. [17].

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Over the past few years, *Mortierellaceae* has experienced an influx of a large number of new species. [18] It currently accommodates 17 genera and 144 species. Among them, the *Mortierella* is the most species-rich genus, with 80 species. It is followed by *Linnemannia*, with 24 species recorded. (https://www.catalogueoflife.org/, accessed on 15 February 2025).

In this paper, four new species, *Linnemannia rotunda* sp. nov., *Mortierella acuta* sp. nov., *M. oedema* sp. nov. and *M. tibetensis* sp. nov., were described from soil samples in China (Yunnan, Shandong and Tibet) based on evidence of molecular phylogeny, morphological characteristic and growth temperature. This is the eighth report of a serial work on diversity of Chinese early-diverging fungi [19–25]

2. Materials and Methods

2.1. Isolation and Morphology

In 2024, soil samples were collected in Yunnan, Tibet, and Shandong, following the methods by Zou et al. [26] and Liu et al. [27] Each sample (approximately 100 g) was placed into a sterile bag and labeled with date, vegetation type, altitude, latitude, and longitude. All samples were stored at 4°C after being transported to the laboratory. Pure strains were isolated from the soil samples using a combination of soil dilution plating and moist-chamber cultivation methods. [28]. Approximately 1 g of soil sample was placed into a 10 mL centrifuge tube containing 10 mL of sterile water and agitated on a shaker for 25 min to prepare a soil suspension. One milliliter of the initial suspension was added to nine milliliters of sterile water to obtain a 10^{-2} soil suspension. The process was repeated to achieve 10^{-3} and 10^{-4} soil suspensions. Approximately 200 μL of the 10^{-3} and 10^{-4} soil suspensions were pipette to the rose bengal chloramphenicol agar (RBC: peptone 5.00 g/L, KH_2PO_4 1.00 g/L, $MgSO_4 \cdot 7H_2O$ 0.50 g/L, rose bengal 0.05 g/L, glucose 10.00 g/L, chloramphenicol 0.10 g/L, agar 15.00 g/L), and evenly dispersed using a sterile triangular glass spreader. [29] The plates were cultivated at 26 ℃ in the dark for 2–5 d. Subsequently, the agar containing mycelia at the edge of the colonies was transferred to fresh potato dextrose agar (PDA: glucose 20 g/L, potato 200 g/L, agar 20 g/L). Macroscopic images were captured using a digital camera (Canon PowerShot G7X, Canon, Tokyo, Japan). For the moist-chamber method, 1 g of soil was evenly spread on the surface of PDA plates, sealed with a parafilm and incubated invertedly at 26 ℃ in the dark. After 2–3 d, target strains were purified by streaking with an inoculation loop. Two days later, the agar containing mycelia at the colony edge was transferred to fresh PDA and cultured as described above. A drop of lactic acid phenol cotton blue staining solution was mounted on the glass slide. Then, a small piece of tape was touched to the surface of the mycelia, making part of the hyphae adhere to it. It was then soaked in the lactic acid phenol cotton blue staining solution. The microscopic morphological characteristics of the fungi were observed using a stereoscope (Olympus SZX10, OLYMPUS, Tokyo, Japan) and a light microscope (Olympus BX53, OLYMPUS, Tokyo, Japan), and images were captured with a high-definition color digital camera (Olympus DP80 OLYMPU, Tokyo, Japan). **[20–25,30]** Structural measurements were conducted using Digimizer software (v5.6.0), with at least 25 individuals measured for each trait. The minimum and maximum growth temperature was determined using a gradient method. The culture was initially incubated at 10°C for two days, and then the temperature was reduced by 1°C each day until no further growth. This temperature was defined as the minimum growth temperature. The culture was initially incubated at 25°C for two days, and then the temperature was increased by 1°C each day until no further growth. This temperature was defined as the maximum growth temperature. All strains were kept in 10% sterilized glycerin at -20℃. The living cultures were stored in the China Microbiological Culture Collection Center, Beijing, China (CGMCC). Equivalent strains were preserved in the Shandong Normal University Culture Collection (XG). Dry culture of types was submitted to the Herbarium Mycologicum Academiae Sinicae, Beijing, China (Fungarium; HMAS). The taxonomic information was deposited to the Fungal Names repository (https://nmdc.cn/fungalnames/).

2.2. DNA Extraction, PCR Amplification, and Sequencing

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The DNA extraction kit (Cat. No.: 70409-20; BEAVER Biomedical Engineering Co., Ltd.) was employed for genomic DNA extraction, following the manufacturer's instructions. The ITS, LSU, SSU, *RPB1* and *Act* regions were amplified using the primer pairs and programs specified in Table 1. The final volume of the PCR reaction mixture is 25 μL, comprising 12.5 uL of 2 × Hieff Canace Plus PCR Master Mix with dye (Yeasen Biotechnology, Cat No. 10154ES03), 9.5 μL of ddH_2O, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM) and 1 μL of template genomic DNA (1 ng/μL). PCR products were visualized at 254 nm on a 2% agarose electrophoresis gel. [31], and purified using a gel extraction kit (Cat# AE0101-C, Shandong Sparkjade Biotechnology Co., Ltd.). DNA sequencing was performed by Tsingke Biotechnology (Beijing, China). All sequences generated in this study were deposited in GenBank.

Table 1. PCR information used in this study.

Loci	PCR primers	Primer sequence (5′ – 3′)	PCR cycles	References
ITS	ITS5	GGA AGT AAA AGT CGT AAC AAG G	95 °C 5 min; (95 °C: 30 s, 55 °C: 30 s, 72 °C: 1 min) × 35 cycles; 72 °C 10 min	[32]
	ITS4	TCC TCC GCT TAT TGA TAT GC		
LSU	LR0R	GTA CCC GCT GAA CTT AAG C	95 °C 5 min; (94 °C: 30 s, 52 °C: 45 s, 72 °C: 1.5 min) × 30 cycles; 72 °C 10 min	[33]
	LR5	TCC TGA GGG AAA CTT CG		
RPB1	RPB1-Af	GAR TGY CCD GGD CAY TTY GG	95 °C 3 min; (94 °C: 40 s, 60 °C: 40 s, 72 °C: 2 min) × 9 (94 °C: 45 s, 55 °C: 1.5 min, 72 °C: 2 min) × 37 cycles; 72 °C 10 min	[34]
	RPB1-Cr	CCN GCD ATN TCR TTR TCC ATR TA		
Act	ACT-1	TGG GAC GAT ATG GAI AAI ATC TGG CA	95 °C 3 min; (95 °C: 60 s, 55 °C: 60 s, 72 °C: 1 min) × 30 cycles; 72 °C 10 min	[35]
	ACT-4R	TC ITC GTA TIC TIG CTI IGA IAT CCA CA T		
SSU	NS1	GTA GTC ATA TGC TTG TCT CC	95 °C 5 min; (94 °C: 60 s, 54 °C: 50 s, 72 °C: 1 min) × 37 cycles; 72 °C 10 min	[32]
	NS4	CTT CCG TCA ATT CCT TTA AG		

2.3. Phylogenetic Analyses

Newly acquired sequence data were processed using MEGA v.7.0 to ensure consistency. [36,37] Reference sequences were downloaded from GenBank according to a study on *Mortierellacea*e by Telagathoti et al. [18] Phylogenetic analyses were conducted for each marker, as well as a concatenation of ITS-LSU-SSU-*RPB1*-*Act*. The phylogeny of *Mortierellacea*e was inferred using both maximum likelihood (ML) and Bayesian inference (BI) algorithms. [38,39] These algorithms were integrated with the CIPRES Science Portal (https://www.phylo.org/, accessed February 15, 2025). ML analysis was carried out with 1,000 bootstrap replicates using RaxML 8.2.4 (https://www.phylo.org/) in CIPRES Science Gateway V. 3.3. [40,41] BI analysis was performed using the GTR + I + G model and sampling frequence of once per 1,000 generations. Eight cold Markov chains were run simultaneously for two million generations. [42,43] The phylogenetic trees resulted were optimized with the iTOL (https://itol.embl.de, accessed February 15, 2024), and refined using Adobe Illustrator CC 2019.[20]

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3. Results

3.1. Molecular Phylogeny

For *Linnemannia*, phylogenetic analyses were performed on a dataset containing 31 strains, representing 25 species, with *Mortierella cogitans* (CBS 879.97) as an outgroup. The sequence matrix comprises a total of 4,527 concatenated characters: 1–634 (ITS), 635–1,622 (LSU), 1,623–2,498 (SSU), 2,499–3,700 (*RPB1*), and 3,701–4,527 (*Act*). Among these characters, 829 are parsimony-informative, along with 3,530 constant and 168 parsimony-uninformative. Bayesian tree topology is congruent with that of the ML tree (**Figure 1**).

For *Mortierella*, phylogenetic analyses were performed on a dataset containing 87 strains, representing 74 species, with *Umbelopsis isabelline* (NRLL 1757) and *U. actotrophica* (CBS 31093) employed as outgroups. The sequence matrix comprises a total of 4,527 concatenated characters: 1–990 (ITS), 991–1,959 (LSU), 1,960–3,042 (SSU), 3,043–4,401 (*RPB1*), and 4,402–5,273 (*Act*). Among these, 1,828 are parsimony-informative along with 2,542 constant and 903 parsimony-uninformative. Bayesian tree topology is consistent with the ML tree (**Figure 2**).

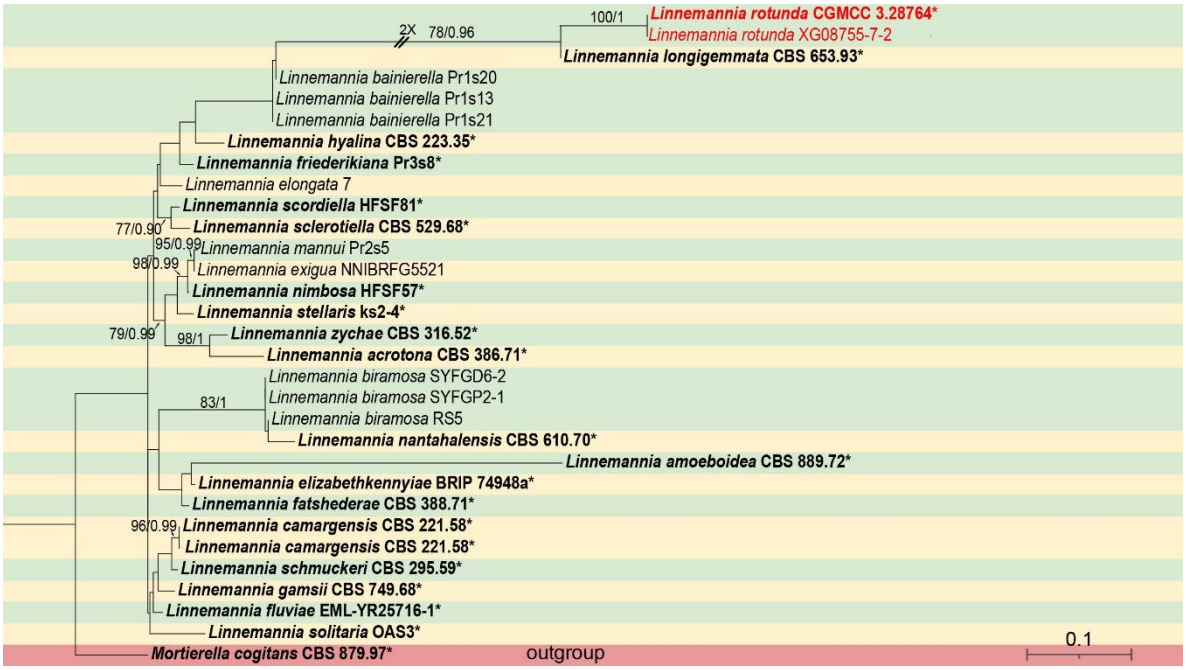

Figure 1. The ML phylogenetic tree of the genus *Linnemannia* based on the concatenated alignment of ITS, LSU, SSU, *RPB1* and *Act* sequences, with *Mortierella cogitans* serving as outgroup. Branches are labeled with Maximum Likelihood Bootstrap Value (left, MLBV≥70) and Bayesian Inference Posterior Probability (right, BIPP≥0.90), which are separated by a slash "/". New species are highlighted in red. Branches shortened due to space constrains are indicated by double slashes "//" and the number of folds. Strains marked with an asterisk "*" and in bold represent ex-type or ex-holotypes. The bottom-right scale bar indicates 0.1 substitutions per site.

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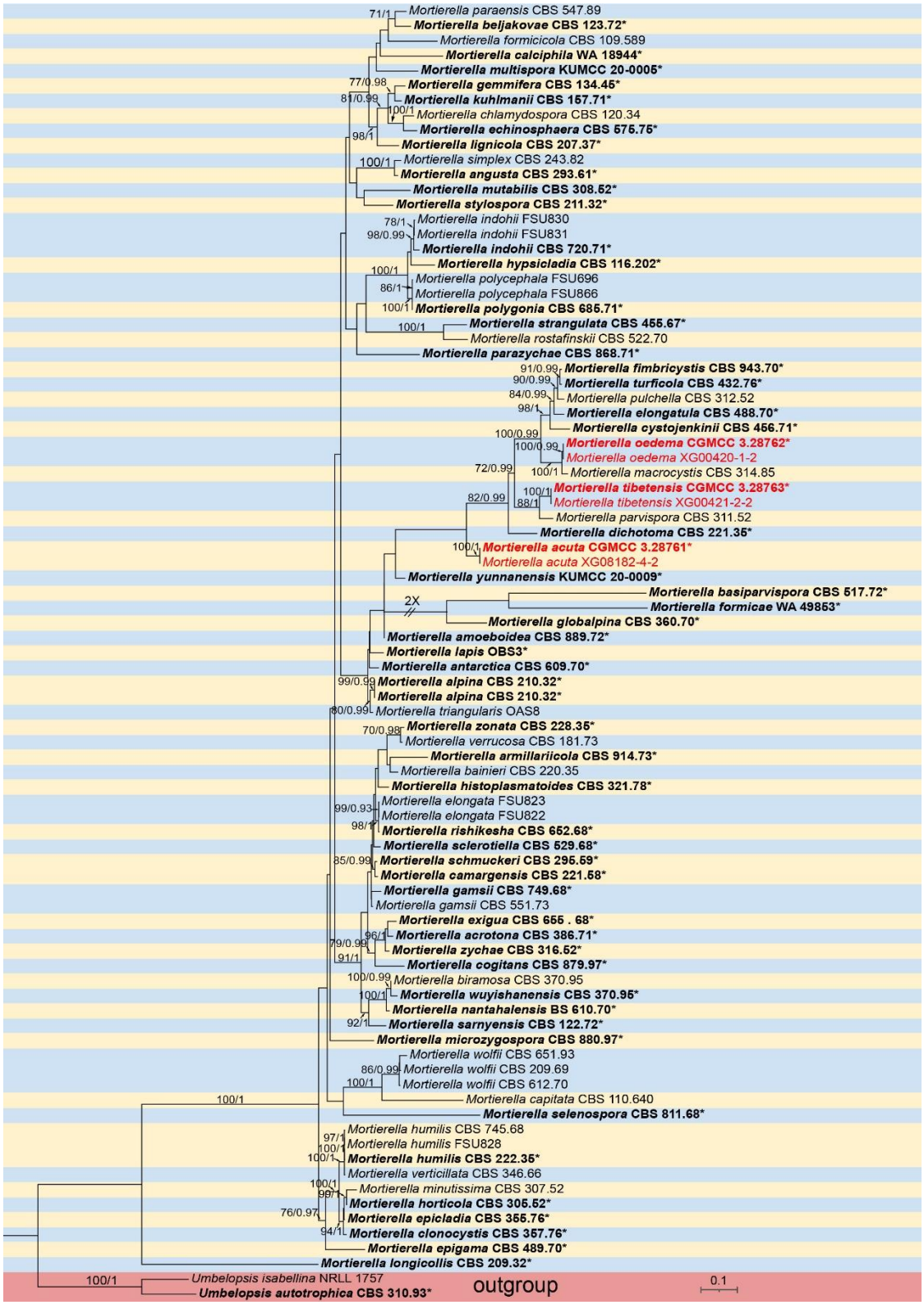

Figure 2. The ML phylogenetic tree of the genus Mortierella based on the concatenated alignment of ITS, LSU, SSU, RPB1 and Act sequences, with Umbelopsis isabellina and U. actotrophica serving as outgroups. Branches are labeled with Maximum Likelihood Bootstrap Value (left, MLBV≥70) and Bayesian Inference Posterior Probability (right, BIPP≥0.90), which are separated by a slash "/". New species are highlighted in red. Branches shortened due to space constrains are indicated by double slashes "//" and the number of folds. Strains marked with an asterisk"*" and in bold represent ex-types or ex-holotypes. The bottom-right scale bar indicates 0.1 substitutions per site.

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3.2. Taxonomy

3.2.1. *Linnemannia rotunda* X.Y. Ji, Y. Jiang & X.Y. Liu, sp. nov. Figure 3

Fungal Names—FN##### (to be applied after review)

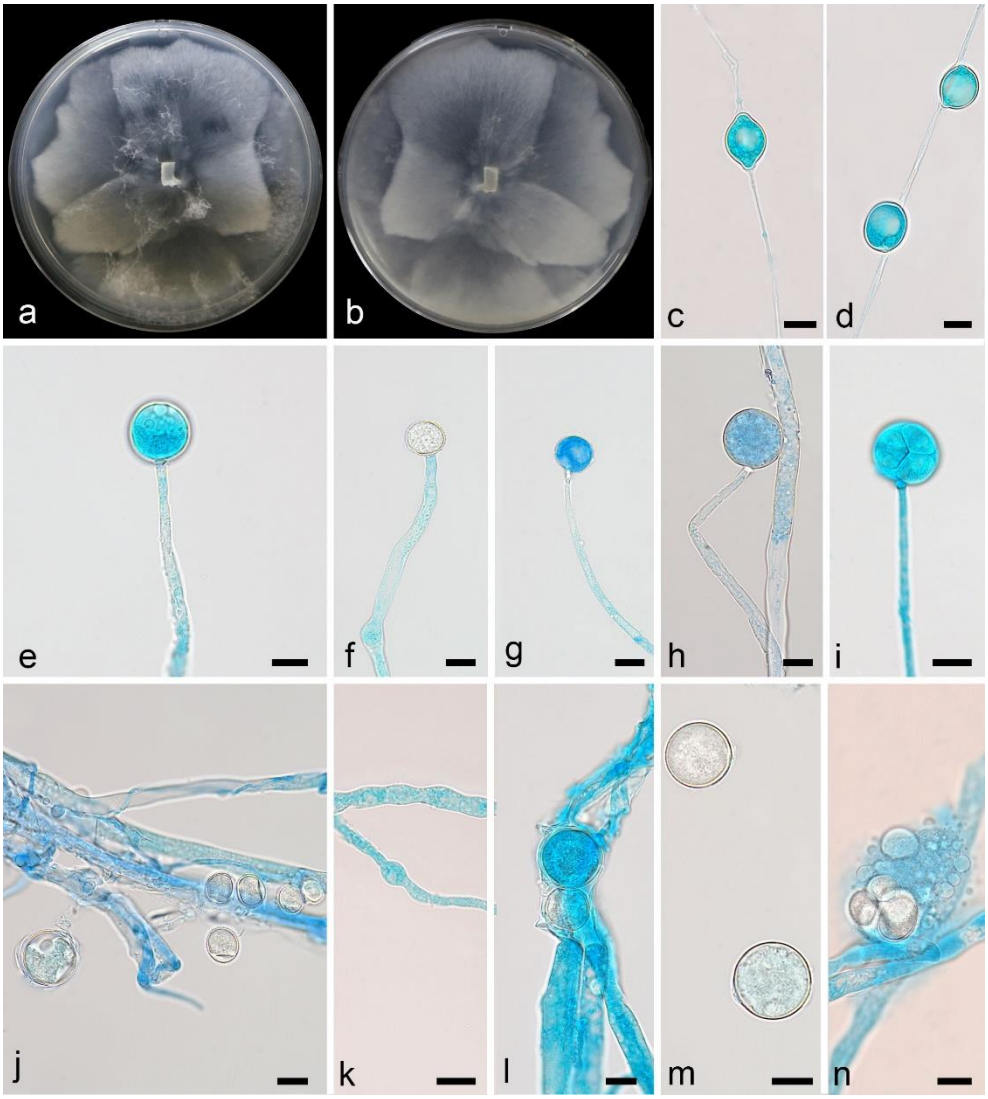

Figure 3. *Linnemannia rotunda* ex-holotype CGMCC 3.28764. (**a**, **b**) Colonies on PDA (**a** obverse, **b** reverse); (**c**, **d**) Chlamydospores; (**e**-**i**) Sporangia; (**k**) Typical swollen hyphae; (**j**, **l**-**n**) Sporangiospores; Scale bars: (**c**-**n**) 10 μm.

Type—China, Yunnan Province, Yuxi City, Xinping Dai Autonomous Country (23°56'39''N, 101°30'1''E, altitude 2397.53 m), from soil, 14 May 2024, X.Y. Ji, holotype HMAS 353518, ex-holotype living culture CGMCC 3.28764 (=XG08755-7-1).

Etymology—The ''*rotunda*'' (Lat.) refers to the round shape of sporangia and sporangiospores.

Description—Colonies on PDA at 16°C for 5 d, reaching 88 mm diameter, fast growing with a rate of 17.6 mm/d, garlic smell, with sparse aerial mycelia. Hyphae hyaline, 1.7–9.4 μm in diameter, sometimes swollen. Sporangiophores erect or slightly bent, unbranched, 25.5–146.0 μm long, 1.9–5.0 μm wide, sometimes with a swelling beneath sporangia. Sporangia oval to round, smooth, multi-spored, 10.4–22.3 μm long, 10.7–22.6 μm wide. Columellae present but usually tiny. Sporangiospores smooth, hyaline, mostly round, 9.6–19.0 μm in diameter. Chlamydospores present, mostly oval, 10.1–22.0 μm long, 6.6–15.6 μm wide. Zygospores not found.

Temperature requirements—Minimum growth temperature 4°C, and maximum growth temperature 28°C.

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Additional specimen examined—China, Yunnan Province, Yuxi City, Xinping Dai Autonomous Country (23°56'39''N, 101°30'1''E, altitude 2397.53m), from soil, 14 May 2024, X.Y. Ji and X.Y. Liu, living culture XG08755-7-2.

Notes—The ITS rDNA phylogenetic analysis showed that the new species *Linnemannia rotunda* was closely related to *L. longigemmata* (MLBV=78, BIPP=0.96, Figure 1) [18] The new species is distinguished from *L. longigemmata* by 61/634 characters. Morphologically, compared to *L. longigemmata*, the new species has a shorter sporangiophores (25.5–146.0 μm vs 50–150.0 μm).

3.2.2. *Mortierella acuta* X.Y. Ji, Y. Jiang & X.Y. Liu, sp. nov. Figure 4

Fungal Names—FN##### (to be applied after review)

Type—China, Shandong Province, Tai'an City, Mount Tai (36°11'49''N, 117°7'16''E, altitude 155.8m), from soil, 12 March 2024, X.Y. Ji, holotype HMAS 353516, ex-holotype living culture CGMCC 3.28761 (=XG08182-4-1).

Etymology—The epithet ''*acuta*'' (Lat.) refers to the spiky collarette.

Description—Colonies on PDA at 16°C for 6 d, attaining 64 mm diameter, moderately fast growing with a rate of 10.6 mm/d, garlic smell, white cottony, with a rose pattern, luxuriant and velvety after 20 d of cultivation. Hyphae hyaline, upright or bent. Sporangiophores arising from aerial mycelia, erect or slightly bent, unbranched, 22.4–72.4 μm in height, tapering from 2.8–4.3 μm to at the base to 1.0–1.4 μm at the apex. Sporangia almost spherical in shape, smooth, deliquescent, multi-spored, 9.5–12.6 μm in diameter. Columellae absent. Collarettes present. Sporangiospores transparent, mostly oval, 2.6–3.4 μm long, 1.4–1.7 μm wide. Chlamydospores present. Zygospores absent.

Temperature requirements—Minimum growth temperature 4°C, and maximum growth temperature 29°C.

Additional specimen examined—China, Shandong Province, Tai'an City, Mount Tai (36°11'49''N, 117°7'16''E, altitude 155.8m), from soil, 12 March 2024, X.Y. Ji and X.Y. Liu, living culture XG08182-4-2.

Notes—Phylogenetic analysis of the two combined genes of ITS and LSU showed that the new species *M. acuta* forms an independent and fully supported clade (MLBV = 100, BIPP = 1.00).

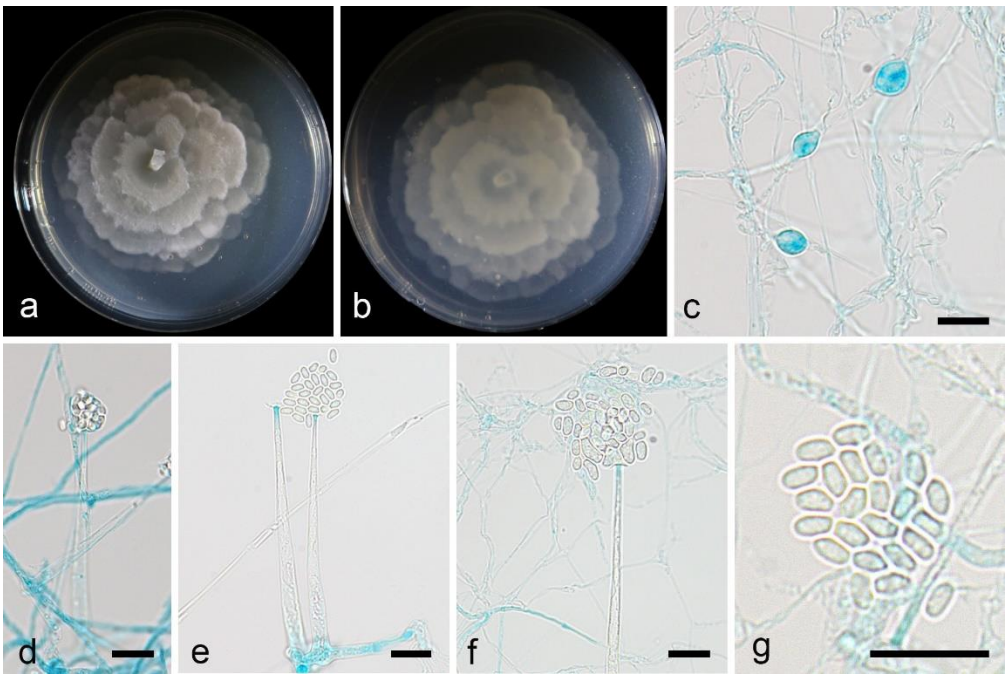

Figure 4. *Mortierella acuta* ex-holotype CGMCC 3.28761. (**a**, **b**) Colonies on PDA (**a** obverse, **b** reverse); (**c**) Chlamydospores; (**d**) Sporangia; (**e**, **f**) Deliquescent sporagnia releasing sporangiospores and leaving obvious collarettes; (**g**) Sporangiospores; Scale bars:(**c**-**i**) 10 μm.

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3.2.3. *Mortierella oedema* X.Y. Ji, Y. Jiang & X.Y. Liu, sp. nov. Figure 5

Fungal Names—FN##### (to be applied after review)

Type—China, Tibet, Shigatse City, Yadong County (27°24'37''N, 88°54'23''E, altitude 3535m), from soil, 25 June 2024, X.Y. Ji, holotype HMAS 353517, ex-holotype living culture CGMCC 3.28762 (=XG00420-1-1).

Etymology—The ‘’*oedema*’’ (Lat.) refers to the swelling of hyphae.

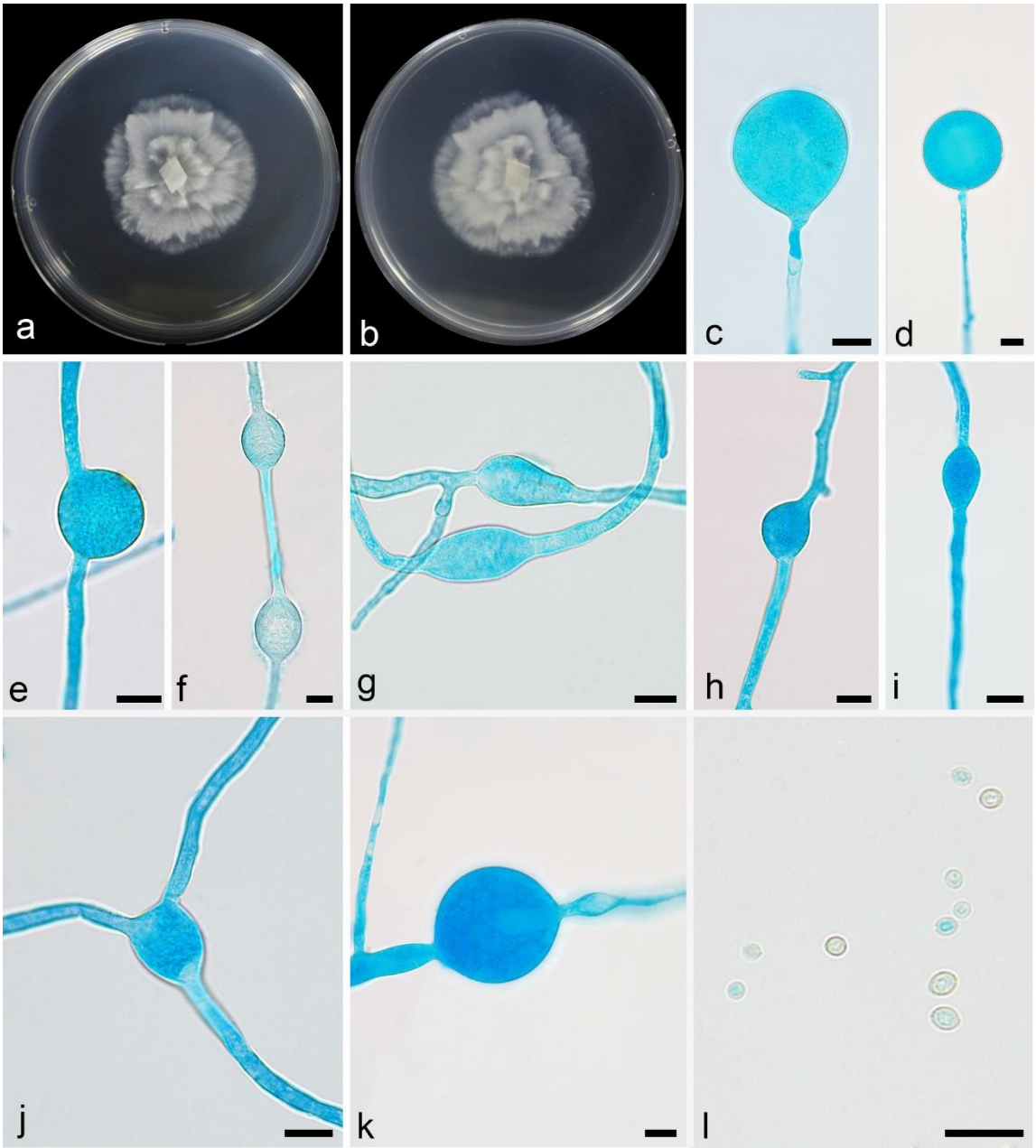

Figure 5. *Mortierella oedema* ex-holotype CGMCC 3.28762. (**a**, **b**) Colonies on PDA (**a** obverse, **b** reverse); (**c**, **d**) Sporangia; (**e**-**i**, **k**) Chlamydospores; (**j**) Typical swollen hyphae; (**l**) Sporangiospores; Scale bars:(**c**-**l**) 10 μm.

Description—Colonies on PDA at 16°C for 5 d, reaching 45 mm diameter, slow growing with a rate of 9 mm/d, garlic smell, sparse aerial mycelia, with characteristic rose pattern. Hyphae hyaline, light brown with age, 1.7–5.1 μm wide, sometimes swollen. Sporangia oval or spherical, smooth, deliquescent, hyaline, 30.7–42.5 μm in diameter. Columellae absent. Collarettes absent. Sporangiospores hyaline, smooth, oval or round, 2.2–3.1 μm long, 2.0–2.9 μm wide. Chlamydospores abundant, oval, round and irregular, 7.7–43.1 μm long and 6.9–37.7 μm wide. Zygospores not found.

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Temperature requirements—Minimum growth temperature 4°C, and maximum growth temperature 28°C.

Additional specimen examined—China, Tibet, Shigatse City, Yadong County (27°24'37''N, 88°54'23''E, altitude 3535m), from soil, 25 June 2024, X.Y. Ji, living culture XG00420-1-2.

Notes—The ITS rDNA phylogenetic analysis showed that the new species *M. oedema* is closely related to *M. macrocystis* (MLBV=100, BIPP=1, Figure 2) [44] It is distinguished from *M. macrocystis* by 48/631 characters in ITS sequences. In sporangiospore shape, the new species is oval or round while *M. macrocystis* almost spherical. In chlamydospore shape, the new species is various (oval, round and irregular) while *M. macrocystis* globose.

3.2.4. *Mortierella tibetensis* X.Y. Ji, Y. Jiang & X.Y. Liu, sp. nov. Figure 6

Fungal Names—FN##### (to be applied after review)

Type—China, Tibet, Shigatse City, Yadong County, (27°21'53''N, 88°58'26''E, altitude 3535m), 2827m, from soil, 26 June 2024, X.Y. Ji, holotype HMAS 353519, ex-holotype living culture CGMCC 3.28763 (=XG00421-2-1).

Etymology—The ''*tibetensis*'' (Lat.) refers to Tibet Autonomous Region of China where the type was collected.

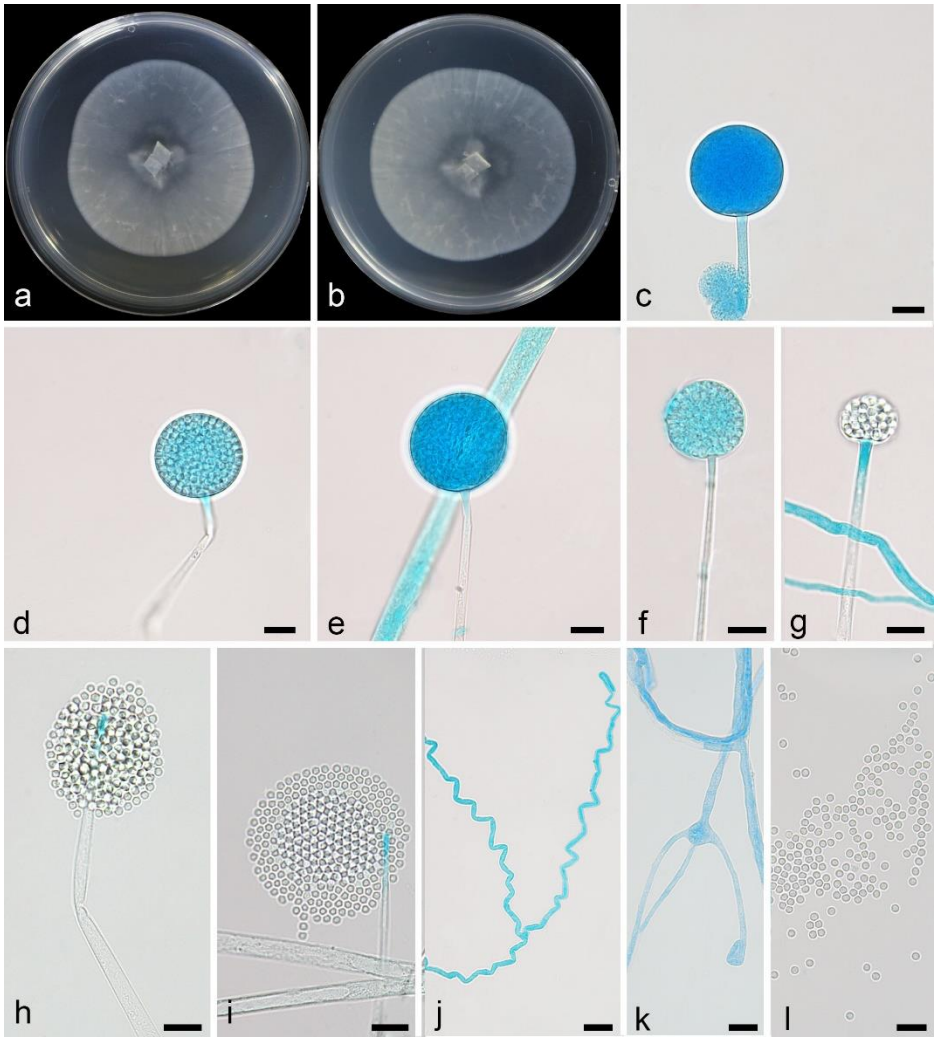

Figure 6. *Mortierella tibetensis* ex-holotype CGMCC 3.28763 (**a**, **b**) Colonies on PDA (**a** obverse, **b** reverse); (**c**, **g**) Sporangia; (**h**, **i**) Deliquescent sporangia releasing sporangiospores; (**j**) Curved hyphae; (**k**) Typical swollen hyphae; (**l**) Sporangiospores; Scale bars: (**c-l**) 10 μm.

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Description—Colonies on PDA at 16°C for 7 d, reaching 59 mm diameter, slow growing with a rate of 8.4 mm/d, garlic smell and a wet dog smell, with sparse aerial mycelia. Hyphae hyaline, upright or bent, sometimes swollen. Sporangiophores arising from aerial mycelia, erect or slightly bent, unbranched, 112–406 μm in height, tapering from 4.2–6.9 μm to at the base to 1.6–2.6 μm at the apex. Sporangia almost spherical in shape, smooth, deliquescent, multi-spored, 12.8–30.3 μm in diameter. Columellae absent. Collarettes absent. Sporangiospores hyaline, mostly pentagonal or hexagonal, smooth, 1.8–5.9 μm vertical heigh. Chlamydospores absent. Zygospores not observed.

Temperature requirements—Minimum growth temperature 4°C, and maximum growth temperature 29°C.

Additional specimen examined—China, Tibet, Shigatse City, Yadong County (27°21'53''N, 88°58'26''E, altitude 3535m), from soil, 26 June 2024, X.Y. Ji and X.Y. Liu, living culture XG00421-2-2.

Notes—Phylogenetic analysis of three loci (ITS, LSU, SSU) showed that the new species *M. tibetensis* was closely related to *M. parvispora* (MLBV=88, BIPP=1, Figure 2) [45]. The new species is distinguished from *M. parvispora* by 49/648, 18/969 and 25/1019 characters in ITS, LSU and SSU sequences, respectively. Due to lack of morphological description in protologue for *M. parvispora*, no comparisons are able to be made.

4. Discussion

Mortierellaceae is a fungal family with an extremely high ecological and physiological diversity that allows it to be widely distributed worldwide. [46] It covers a wide range of ecosystems from cold regions to temperate and tropical. For example, some species exhibit significant activity in alpine and polar environments, while others are widespread in temperate and tropical soils. Members of this family typically live in soil, but also have extensive associations with plant roots, insect guts, and other microorganisms. [47] For example, some bacterial species of *Pseudomonas* often live in symbiosis with *Mortierellaceae* fungi, and this symbiosis may have an impact on the fungi's volatile organic compounds (VOCs). Some soil samples in Yunnan Province, Tibet Autonomous Region and Shandong Province were investigated in this study. The city Yuxi in Yunnan Province have a subtropical monsoon climate with complex terrain, mild and humid terrain, and abundant precipitation. The climatic environment is conducive to the growth of various microorganisms. Tai'an City, Shandong Province belongs to the warm temperate continental semi-humid monsoon climate zone, with four distinct seasons, suitable cold and summer, synchronized light and temperature, and rain and heat in the same season. Yadong County of Tibet Autonomous Region, has a plateau and mountainous climate, with significant seasonal changes and extreme weather phenomena. [48,49] Four new species of the family *Mortierellaceae* were obtained from these regions.

In recent years, the phylogenetic analysis of the *Mortierellaceae* family has primarily relied on morphological characteristics and the ITS + LSU + SSU sequences. In this study, the phylogenetic tree was reconstructed with the additional inclusion of *Act* and *RPB1* protein-coding sequences. The results were largely consistent with those from previous studies based on ITS + LSU. Based on morphology and molecular phylogenetic analyses, four novel species were identified in the family *Mortierellaceae*, namely *L. rotunda* sp. nov., *M. acuta* sp. nov., *M. oedema* sp. nov. and *M. tibetensis* sp. nov. By analyzing these data, strong supports were obtained for the clades of these species (Fig 1: *L. rotunda* 100 MLBV and 1.00 BIPP; Figure 2: *M. acuta* 100 MLBV and 1.00 BIPP, *M. oedema* 100 MLBV and 0.99 BIPP, *M. tibetensis* 100 MLBV and 1.00 BIPP). At the same time, in terms of morphological structure and physiology, we also found some differences between these four newly discovered species and their relatives. They have great differences in sporangiospores size, stolon width and sporangium size; However, other features are not described in the protologues of those rlatives. [18,44,45,50,51]

Some species of the *Mortierellaceae* family play important roles in ecosystems, such as promoting plant growth, breaking down plant debris, and remodeling rhizosphere microbial communities. In addition, fungi in this family have important applications in the field of biotechnology, for example as industrial producers of polyunsaturated fatty acids such as arachidonic acid; Metabolites of some species act as antibacterial and insecticides. [52,53] In recent years, significant progress has been made

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in taxonomic research in the family *Mortierellaceae*. Through multi-locus phylogenetic analyses, the *Mortierella* s.l. was reclassified into 13 monophyletic genera. These taxonomic adjustments provide a clearer framework for future research and contribute to a better understanding of the diversity and evolution. However, there are still many unsampled areas, especially in the geographical and ecological distribution on a global scale, which needs to be further explored.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. The GenBank accession number of the sequence used in this study (Table S2, S3).

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Data availability: The sequences of this study have been submitted to the NCBI database (https://www.ncbi.nlm.nih.gov/, accessed February 15, 2025) with accession numbers were shown in Tables S2 and S3.

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