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Article

Unveiling Species Diversity Within Early-Diverging Fungi from China VII: Seven New Species of *Cunninghamella* (Mucoromycota)

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Abstract: The fungal genus *Cunninghamella*, ubiquitously distributed as saprotrophic organisms with occasional endophytic and phytopathogenic manifestations, holds significant biomedical and biochemical importance. During systematic investigations of fungal biodiversity in China, seven novel taxa (*C. amphisporea* sp. nov., *C. cinerea* sp. nov., *C. flava* sp. nov., *C. hainanensis* sp. nov., *C. rhizoidea* sp. nov., *C. simplex* sp. nov., and *C. yunnanensis* sp. nov.) were delineated through integrated taxonomic approaches incorporating morphological characterization, multilocus phylogenetic analyses (ITS-LSU-TEF1 α), and physiological assessments. Phylogenetic reconstructions positioned these novel species within a well-supported clade along with *C. bainieri* and *C. verticillata*. New species and their diagnostic features are: *C. amphisporea* exhibiting dimorphic sporangiola production, *C. cinerea* distinguished by distinctive gray pigmentation in colony, *C. flava* characterized by yellow colony, *C. hainanensis* and *C. yunnanensis* bearing eponymous designations reflecting their geographic origins, and *C. simplex* displaying simplified sporangiophore branching. Comprehensive taxonomic descriptions accompanied by photomicrographic illustration are provided herein. This study constitutes the seventh installment in an ongoing series elucidating early-diverging fungal diversity in China, expanding the global *Cunninghamella* taxonomic inventory to 63 species and advancing our understanding of mucoralean phylogeny.

Keywords: Mucorales; basal fungi; fungal diversity; taxonomy; molecular phylogeny

1. Introduction

The genus *Cunninghamella* belongs to Mucoromycota, Mucoromycetes, Mucorales and Cunninghamellaceae [1]. It was established in 1930 by Matruchot and typified with *C. echinulata* (Thaxt.) Thaxt. ex Blakeslee [2,3]. Members of *Cunninghamella* are usually saprotrophs and sometimes endophytes, and they are ubiquitous globally in various environments, including soil, air, feces and humus [4–7]. Meantime, some species of *Cunninghamella* are opportunistic pathogens causing infections in immunocompromised populations [6,8–10]. Additionally, *Cunninghamella* represents a pivotal contribution to the field of biomedicine and biochemistry, producing a rich array of metabolites such as fatty acids, terpenes, nickel-iron carrier and sugars [11–13].

Cunninghamella species are characterised by the branching pattern of sporangiophores, the shape and size of terminal and lateral vesicles, and the shape, size and surface of sporangiola [2,4,14–18]. In a monographic study of 2001, Zheng and Chen [2] delimited *Cunninghamella* species on the basis of morphological characteristics and maximum growth temperatures. Nevertheless, identification of certain *Cunninghamella* remains challenging due to considerable phenotypic variabilities [2]. Later, Yu et al. [10], Liu et al. [19] and Walther et al. [3] reached a consensus among morphological

observations and molecular phylogenies of ITS rDNA (internal transcribed spacer of ribosomal DNA) and *TEF1 α* (translation elongation factor 1 alpha), overcoming the limitations of traditional classification methods and provided a more accurate and comprehensive way.

Currently, 56 *Cunninghamella* species are recorded in the Index Fungorum (<http://www.indexfungorum.org/>, accessed on 19 December 2024), and they are distributed in Antarctica, Australia, Belgium, Brazil, China, France, India, Japan, Korea, Papua New Guinea, Spain, Switzerland, Tanzania, Thailand and the United State [2,7,10,14,16,17,20–25].

Seven novel species of *Cunninghamella* were discovered based on molecular, morphological and physiological characteristics during the process of isolating fungi from soil samples collected from southern China. Phylogenetic trees, descriptions and illustrations of these novel species are provided herein. This is the seventh report of a serial work on diversity of Chinese early-diverging fungi [26–31].

2. Materials and Methods

2.1. Sample Collection and Strain Isolation

In 2024, soil samples were collected in Yunnan province, China. Soil sample collection was initiated by removal of surface contaminants (including leaf litter, debris, and particulates) using a sterilized stainless steel shovel. Following surface preparation, intact soil spanning the 5–10 cm subsurface horizon were extracted. Approximately 500 g of homogenized soil material was then transferred into sterile sample bags. All collected samples were immediately labeled with waterproof markers indicating collection date, GPS coordinates (± 3 m accuracy), and altitude, followed by a temporary storage at 4°C prior to laboratory processing [32,33]. Pure strains were isolated by a combination of plate dilution coating method and single spore separation method [34,35]. About 1 g of soil sample was added into a centrifugal tube containing 10 mL of sterile water. Then, 10^{-1} soil suspension was obtained by shaking. One milliliter of the 10^{-1} soil suspension was transferred into a centrifuge tube containing 9 mL of sterile water to prepare 10^{-2} soil suspension. In the same way, 10^{-3} and 10^{-4} soil suspensions were successfully obtained. A portion (200 μ L) of 10^{-3} and 10^{-4} soil suspensions were poured on the surface of Rose Bengal Chloramphenicol agar (RBC: peptone 5.00 g/L, glucose 10.00 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.50 g/L, KH_2PO_4 1.00 g/L rose bengal 0.05 g/L, chloramphenicol 0.10 g/L, agar 15.00 g/L) [36] containing 0.03% streptomycin sulfate, coated evenly with a glass coating rod, and then incubated in darkness at 25°C for 3–5 d. When colony grew, it was transferred onto Potato Dextrose Agar (PDA: 200 g potato, 20 g dextrose, 20 g agar, 1000 mL distilled water, pH 7.0) for further cultivation. After sporangium formation, sporangiospores were dispersed by suspension with sterile water. The single spores were picked up by a sterile inoculation ring and transferred to a PDA plate. The plate was placed in an incubator at 25°C for dark cultivation. All strains were stored at 4°C in 10% glycerol. The holotype materials were deposited in the China General Microbiological Culture Collection Center, Beijing, China (CGMCC) and Shandong Normal University, Jinan, China (XG). The holotype specimens were deposited in the Herbarium Mycologicum Academiae Sinicae, Beijing, China (Fungarium, HMAS). Taxonomic information of these new strains was uploaded to the Fungal Names repository (<https://nmdc.cn/fungalnames/>).

2.2. Morphology and Maximum Growth Temperature

After dark culture on PDA plate for 3–5 d, the phenotypic characteristics of fungi (obverse and reverse) were photographed using a high-definition color digital camera (DP80, Olympus, Tokyo, Japan). Micromorphological features of fungi were observed by a stereomicroscope (Olympus SZX10, OLYMPUS, Tokyo, Japan) and an optical microscope (BX53, Olympus, Tokyo, Japan). For the determination of the maximum growth temperature, all strains were initially cultured in the dark at 26°C for 3 d, and then the temperature was gradually increased by 1°C every day until the colony stopped growing [37–40].

2.3. DNA extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from mycelia by BeaverBeads Plant DNA Kit (Cat. No.: 70409–20; BEAVER Biomedical Engineering Co., Ltd.) [41,42]. Molecular markers ITS rDNA (internal

transcribed spacer of ribosomal RNA gene), LSU rDNA (large subunit of ribosomal RNA gene), and *TEF1 α* (translation elongation factor 1 alpha gene) were amplified by polymerase chain reaction (PCR), respectively, using the following primer pairs ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') / ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [43], LR0R (5'-GTA CCC GCT GAA CTT AAG C-3') / LR5 (5'-TCC TGA GGG AAA CTT CG-3') [44], and EF1-983F (5'-ATG ACA CCR ACR GCR ACR GTY TG-3') / EF1-2218R (5'-AACTTGCAGGCAATGTGG-3') [45]. Amplification procedure consisted of a predenaturation at 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing (ITS at 55°C for 30 s, LSU at 52°C for 30s; *TEF1 α* at 56°C for 1 min) and extension at 72°C for 1 min, and an extra extension at 72°C for 10 min. The amplification reaction was performed in a volume of 25 μ L, comprising 12 μ L 2 $\times$ Hieff Canace® Plus PCR Master Mix (Yeast Biotechnology, Cat No. 10154ES03), 10 μ L ddH₂O, 1 μ L forward and reverse primers (10 μ M) (TsingKe, Qingdao, China) and 1 μ L fungal genomic DNA (about 1 μ M). Completeness and concentration of extracted genomic DNA were evaluated by 1% agarose gel electrophoresis [46]. Gel recovery was performed using a Gel Extraction Kit (Cat# AE0101-C; Shandong Sparkiade Biotechnology Co., Ltd.). PCR products were sent to the Biosune Company Limited (Shanghai, China) for Sanger sequencing. Sequences were assembled and proofread using MEGA v.7.0 [47]. ITS, LSU and *TEF1 α* sequences were submitted to GenBank for BLAST similarity searches. All sequences newly acquired in this article were deposited at GenBank under the accession number in Table S1.

2.4. Phylogenetic Analyses

Phylogenetic analyses were performed based on the combined sequences of ITS, LSU and *TEF1 α* . Sequences both generated herein and retrieved from NCBI were presented in Table S1. Phylogenetic trees were reconstructed using maximum likelihood (ML) and Bayesian inference (BI) methods. ML analysis was performed using RAxML-HPC2 on XSEDE v.8.2.12 on the CIPRES Science Gateway website (<https://www.phylo.org/>) with 1,000 bootstrap replicates under the GTRGAMMA model [48]. For BI analysis, a quick start configured with an automatic stop option was executed on a Linux system server [49,50]. Random initial trees were used to run six simultaneously running Markov chains for 5,000,000 generations, with samples being taken every 1,000 generations. The first 25% of trees were discarded as burn-in, and the remaining trees were employed to measure the posterior probability (PP). The layout and adjustments of the phylogenetic tree were conducted on iTOL website (<https://itol.embl.de>), and then the final beautification was performed through Adobe Illustrator CC 2019.

3. Results

3.1. Phylogeny

The molecular dataset included 56 strains in *Cunninghamella*, with *Mucor janssenii* CBS 205.68 as an outgroup. The dataset consisted of 3,989 characters, covering ITS rDNA (1–1,692), LSU rDNA (1,693–2,912), and *TEF1 α* (2,913–3,989). There were 2,098 constant, 522 variable but parsimony-uninformative, and 1,369 parsimony-informative characters. MrModelTest indicated that Dirichlet fundamental frequency along with the GTR + I + G evolution pattern was suitable for both partitions in Bayesian Inference. The topology of the Maximum Likelihood (ML) evolutionary tree was highly congruent with that of the Bayesian Inference (BI) evolutionary tree. Therefore, the ML evolutionary tree was selected as a representative for detailed illustration (Figure 1). Fourteen strains of *Cunninghamella* isolated in this study were grouped into seven independent clades. The new species *C. simplex* was closely related to *C. bainieri* with full supports (MLBV = 100, BIPP = 1.00), and then next to another new species *C. cinerea* (MLBV = 100, BIPP = 0.98). The new species *C. flava* had a close relationship with *C. verticillata*. (MLBV = 80, BIPP = 0.95). The other four species each formed their own branches, that is *C. amphisporea* (MLBV = 100, BIPP = 1), *C. hainanensis* (MLBV = 100, BIPP = 1), *C. rhizoidea* (MLBV = 100, BIPP = 0.99), and *C. yunnanensis* (MLBV = 100, BIPP = 1).

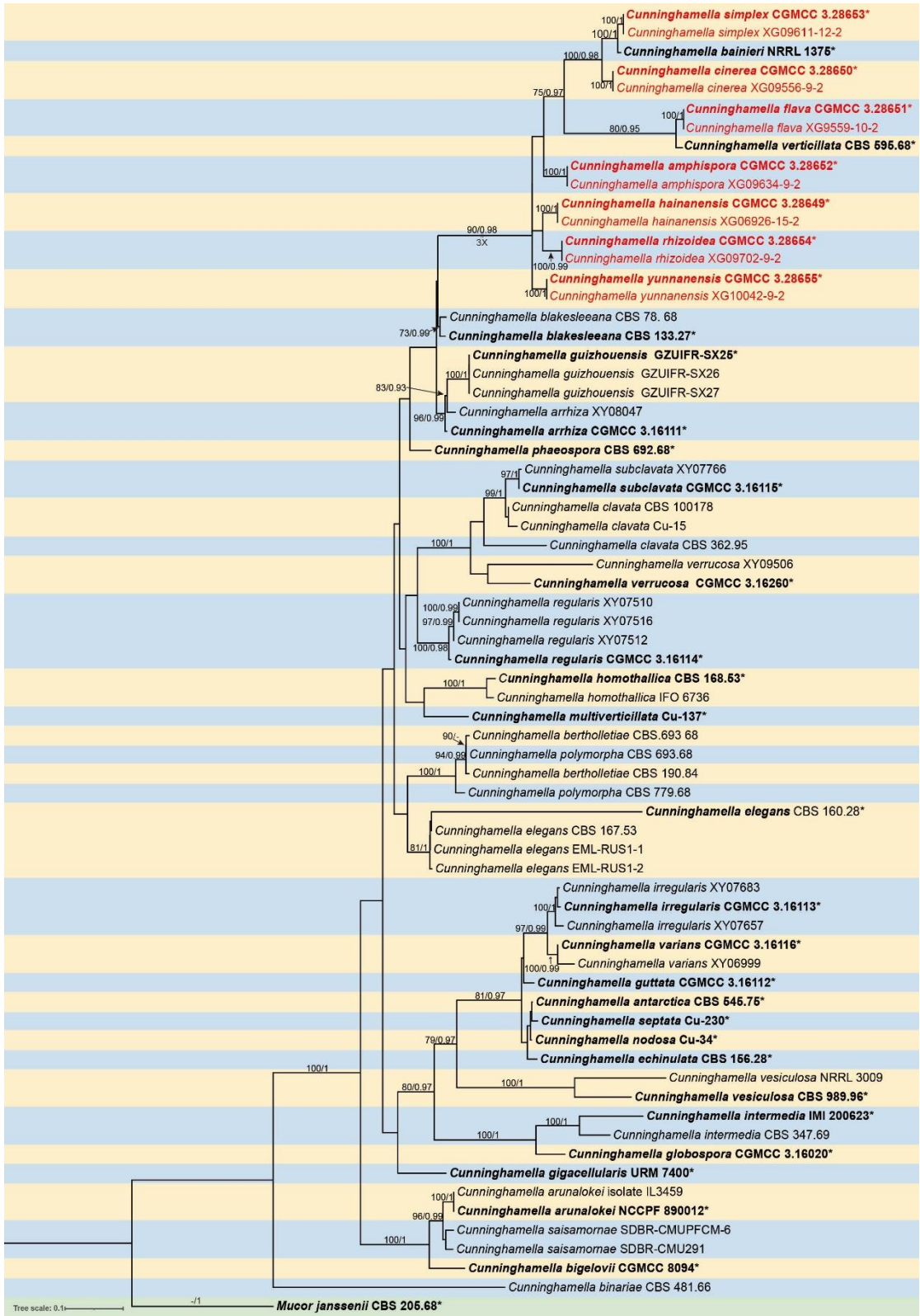
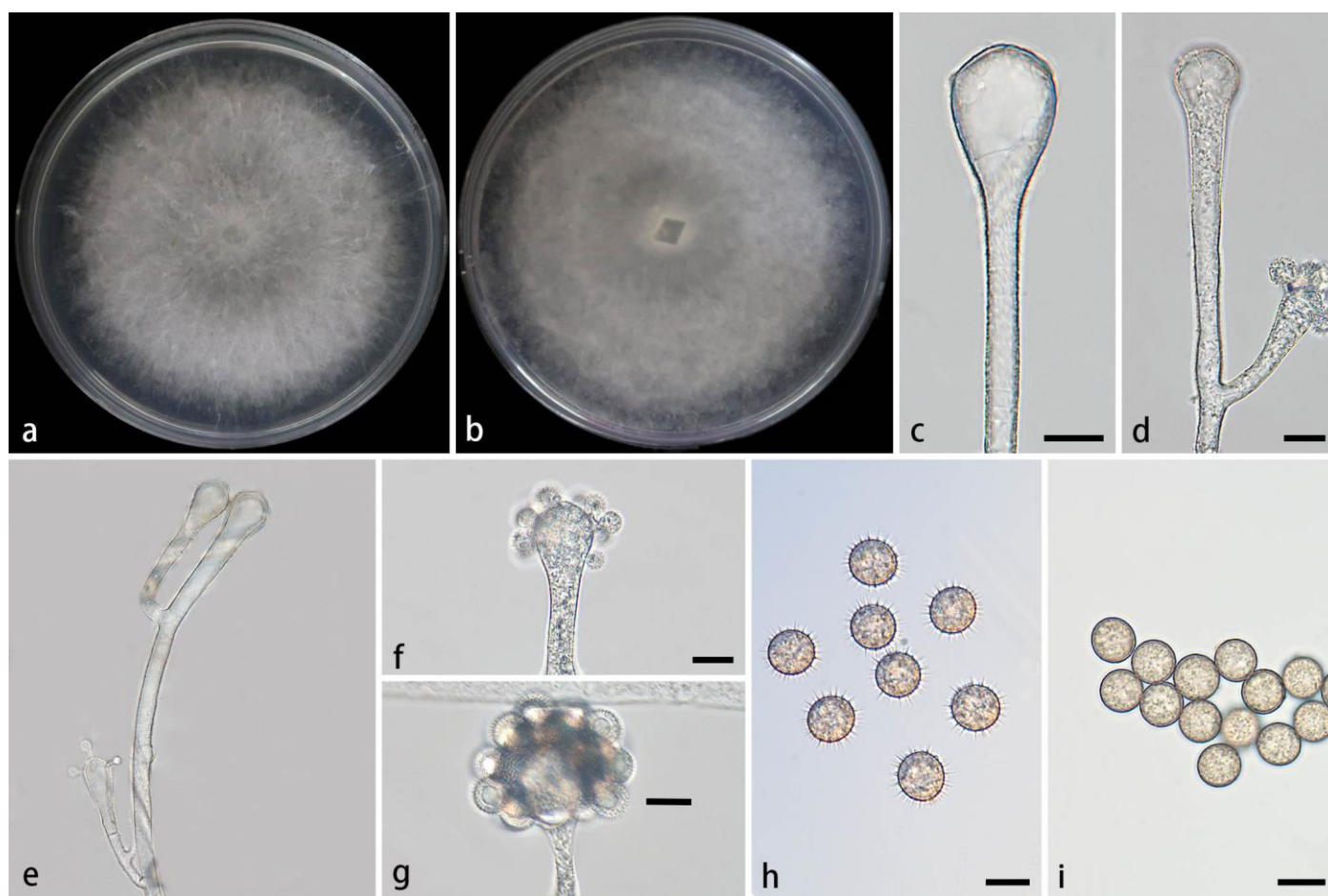


Figure 1. The Maximum Likelihood (ML) phylogenetic tree of *Cunninghamella* based on sequences of ITS, LSU rDNA and TEF1 α , with *Mucor janssenii* as an outgroup. Nodes are annotated with ML bootstrap values (MLBV $\geq$ 70%) and BI posterior probabilities (BIPP $\geq$ 0.9), separated by slashes "/". The bold black represents ex-type or ex-holotype strains with an asterisk "*". Strains isolated in this study are displayed in red. The scale at the bottom left indicates 0.1 substitutions per site.

3.2. Taxonomy

The descriptions and illustrations of seven novel *Cunninghamella* species are presented as follows.

3.2.1. *Cunninghamella amphisporea* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 2.

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

Figure 2. Morphologies of *Cunninghamella amphisporea* ex-holotype CGMCC 3.28652. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c-e) Sporangiphores with branching vesicles; (f-g) Vesicles bearing sporangia; (h) Spinose sporangia; (i) Smooth sporangia. Scale bars: (c-i) 10 µm.

Type—China, Yunnan Province, Puer City, Simao District, Yixiang Town, Dakai River section (22°60'26"N, 101°09'54"E, altitude 1582.73 m), from soil, 5 July 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353444, ex-holotype living culture CGMCC 3.28652 (= XG09634-9-1).

Etymology—The epithet *amphisporea* (Lat.) refers to the species producing two types of sporangia.

Description—Colonies on PDA at 26°C for 4 d, reaching 85 mm in diameter, fast growing with a growth rate of 21.25 mm/d, initially white, gradually becoming light gray, floccose. Hyphae branched, hyaline, smooth-walled, aseptate when juvenile, septate when old. Rhizoids not found. Stolons present. Sporangiphores arising from stolons or aerial hyphae, erect or few slightly bent, unbranched or simply branched, hyaline, single, never verticillate, always expanding upwards. 2.3–19.1 µm wide. Septa if present, single, usually just below the vesicles. Vesicles hemispherical, spherical, oval or pillar-shaped, hyaline, smooth-walled, terminally 6.4–56.6 µm long and 3.2–46.7 µm wide, laterally 8–18.9 µm long and 5.1–14.8 µm wide. Pedicels over the entire surface of vesicles, 1.4–3.4 µm long. Sporangia on pedicels, monosporous, globose, hyaline when young, dusky brown when old, thick-walled, 6.3–16.7 µm long, 6.3–15.8 µm wide, with or without spines. Spines 0.5–2.6 µm long. Chlamydospores absent. Zygospores not found.

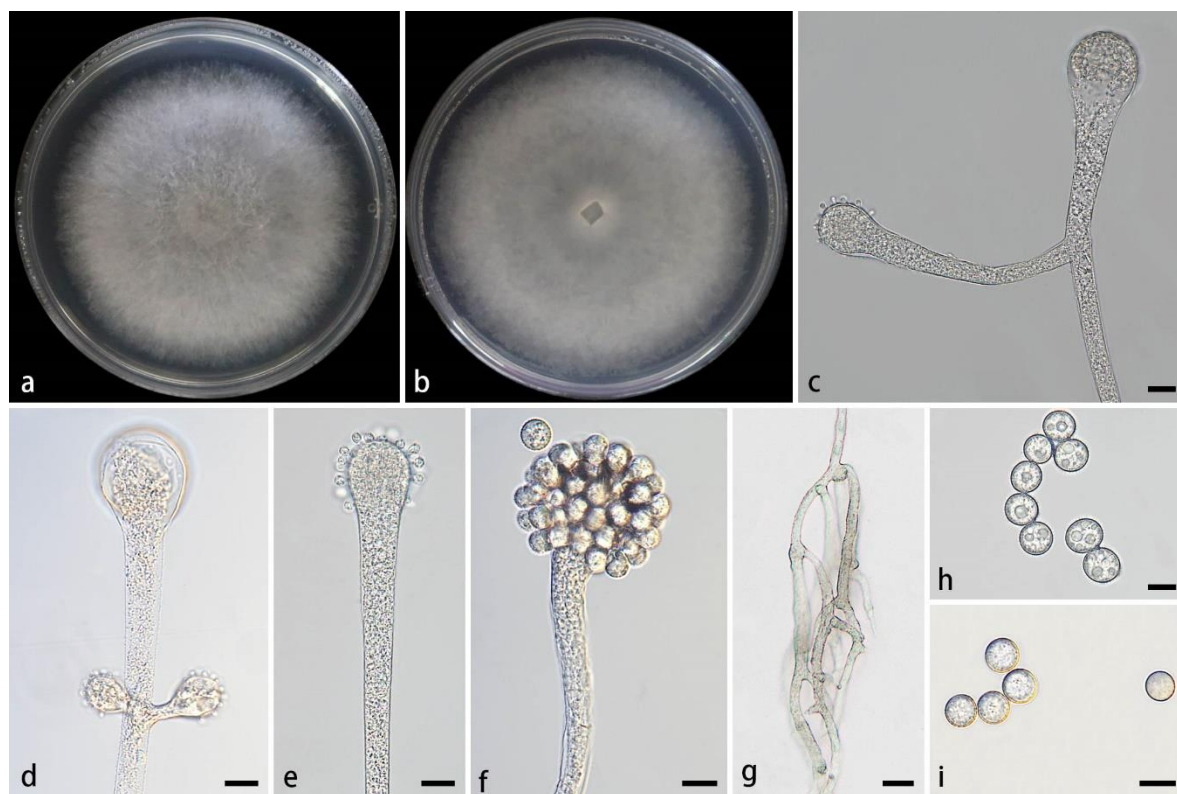
Maximum growth temperature—29°C..

Additional strains examined—China, Yunnan Province, Puer City, Simao District, Yixiang Town, Dakai River section (22°60'26"N, 101°09'54"E, altitude 1582.73 m), from a soil sample, 5 July 2024, M.F. Tao and X.Y. Ji, living culture XG09634-9-2.

GenBank accession numbers—CGMCC 3.28652 (ITS, PV089203; LSU, PV123104; *TEF1α*, PV200769), XG09634-9-2 (ITS, PV089204; LSU, PV123105; *TEF1α*, PV200770).

Notes—In the phylogenetic tree of ITS-LSU-*TEF1α*, two strains of the *Cunninghamella amphisporea* sp. nov. formed a fully supported independent clade (MLBV = 100, BIPP = 1.00; Fig. 1).

3.2.2. *Cunninghamella cinerea* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 3.



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

Figure 3. Morphologies of *Cunninghamella cinerea* ex-holotype CGMCC 3.28650. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c-d) Sporangiophores with branching vesicles; (e-f) Vesicles bearing sporangia; (g) Rhizoids; (h) Spinose sporangia; (i) Smooth sporangia. Scale bars: (c-f, h-i) 10 μ m; (g) 20 μ m.

Type—China, Yunnan Province, Puer City, Mojiang Hani Autonomous County, Tongguan Town (23°29'93"N, 101°39'17"E, altitude 1471.21 m), from soil, 4 July 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353442, ex-holotype living culture CGMCC 3.28650 (= XG09556-9-1).

Etymology—The epithet *cinerea* (Lat.) refers to gray colonies on PDA.

Description—Colonies on PDA at 26°C for 4 d, reaching 85 mm in diameter, fast growing with a growth rate of 21.25 mm/d, initially white, gradually turning to smoky gray with age, floccose. Hyphae branched, hyaline, smooth-walled, aseptate when juvenile, septate when mature. Rhizoids abundant, root-like, hyaline, often branched. Stolons present. Sporangiophores often arising from aerial hyphae, sometimes arising from stolons, erect or slightly bent, mainly unbranched or simply branched, hyaline, mainly single or recumbent, never verticillate, usually expanding upwards, 3.1–11.7 μ m wide. Septa rarely present, usually one or two below the vesicles in the main sporangiophores. Vesicles spherical to elliptic, usually hyaline, smooth, terminally 9.6–32.3 μ m long and 6.9–28.3 μ m wide, laterally 11.9–20.3 μ m long and 9.9–17.3 μ m wide. Pedicels covering the entire surface of vesicles, 1.2–2.2 μ m long. Sporangia on pedicels on vesicles, monosporous, globose to ovoid, hyaline when young, gradually dark brown when mature, thick-walled, 5.5–16.7 μ m long, 4.9–15.5 μ m wide, with or without spines. Spines 0.8–1.7 μ m long. Chlamydospores absent. Zygospores not found.

Maximum growth temperature—33°C.

Additional strains examined—China, Yunnan Province, Puer City, Mojiang Hani Autonomous County, Tongguan Town (23°29'93"N, 101°39'17"E, altitude 1471.21 m), from soil, 4 July 2024, M.F. Tao and X.Y. Ji, living culture XG09556-9-2.

GenBank accession numbers—CGMCC 3.28650 (ITS, PV089197; LSU, PV123098; *TEF1α*, PV172612), XG09556-9-2 (ITS, PV089198; LSU, PV123099; *TEF1α*, PV172613).

Notes—Based on the ITS-LSU-*TEF1α* phylogenetic tree, two strains of the *Cunninghamella cinerea* sp. nov. formed a well-supported clade (MLBV = 100, BIPP = 1.00; Fig. 1), exhibiting a close relationship to *C. simplex* and *C. bainieri* (MLBV = 100, BIPP = 0.98; Fig. 1). Morphologically, the new species was distinguished from *C. simplex* by larger terminal vesicles and lateral vesicles ($9.6\text{--}32.3 \times 6.9\text{--}28.3 \mu\text{m}$ vs $5.0\text{--}28.4 \times 4.1\text{--}25.8 \mu\text{m}$; $11.9\text{--}20.3 \times 9.9\text{--}17.3 \mu\text{m}$ vs $6.7\text{--}17 \times 3.8\text{--}10.8 \mu\text{m}$), shorter pedicles ($1.2\text{--}2.2 \mu\text{m}$ vs $1.1\text{--}2.9$), root-like rhizoids. The new species produced both spinose and smooth sporangia, while *C. simplex* produced only spinos sporangia. The new species differed from *C. bainieri* by non-verticillate sporangiophores, shorter pedicles ($1.2\text{--}2.2 \mu\text{m}$ vs $2.5\text{--}3.5$ (–6)). Besides, the new species had two shapes of sporangia (globose and ovoid), but *C. bainieri* had four shapes (ovoid, ellipsoid, globose and lacrymoid). Although both the new species and *C. bainieri* had rhizoids, the former was root-like and the latter was finger-like. Physiologically, the maximum growth temperature of the new species was 1°C lower than that of *C. bainieri* (33°C vs 34°C) [2].

3.2.3. *Cunninghamella flava* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 4.

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

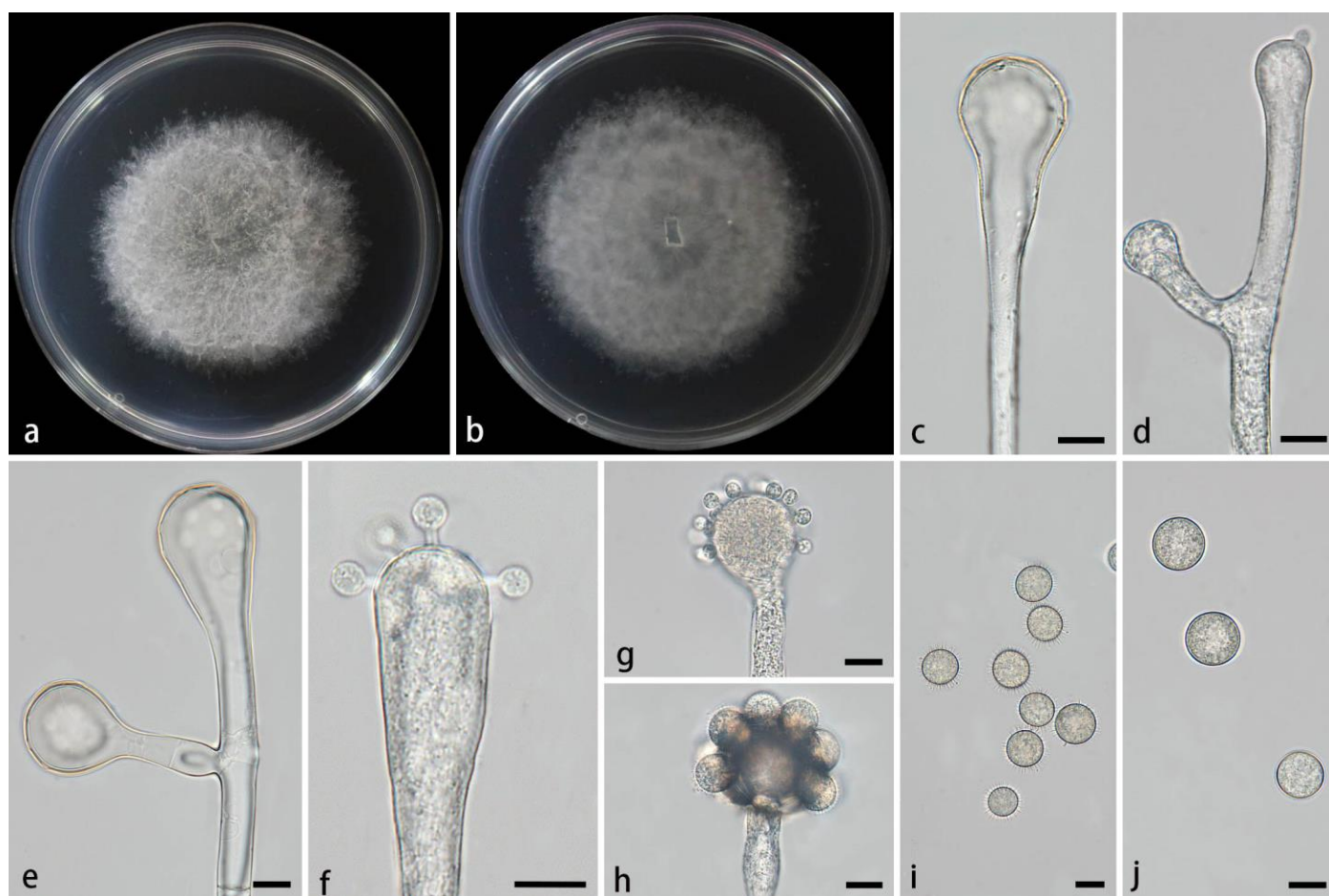


Figure 4. Morphologies of *Cunninghamella flava* ex-holotype CGMCC 3.28651. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c) Sporangiophores; (d–e) Sporangiophores with branching vesicles; (f–h) Vesicles bearing sporangia; (i) Spinose sporangia; (j) Smooth sporangia. Scale bars: (c–j) 10 μm .

Type—China, Yunnan Province, Puer City, Mojiang Hani Autonomous County, Tongguan Town (23°29'93"N, 101°39'17"E, altitude 1471.21 m), from soil, 4 July 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353443, ex-holotype living culture CGMCC 3.28651 (= XG9559-10-1).

Etymology—The epithet *flava* (Lat.) refers to the yellow colonies on PDA.

Description—Colonies on PDA at 26°C for 6 d, reaching 72 mm in diameter, fast growing with a growth rate of 12 mm/d, initially white, gradually turning to dry yellow with age, floccose. Hyphae branched, hyaline, smooth-walled, aseptate when juvenile, septate when mature. Rhizoids absent. Stolons present. Sporangioophores arising from aerial hyphae or stolons, erect or slightly bent, unbranched or 1–7 branched, hyaline, single, recumbent, opposite, in pairs, 1–3 verticillate, sometimes growing irregularly, swelling or bulging, then tapering upward, 3.8–25.6 µm wide. Septa if present, usually one or two below the vesicles in the main sporangioophores. Vesicles globose, pear-shaped, elliptic, usually hyaline, smooth, terminally 12.8–43.1 µm long and 11.3–44.5 µm wide, laterally 9.3–28.1 µm long and 9.4–25.3 µm wide. Pedicels on the entire surface of the vesicle, 1.6–2.2 µm long. Sporangiola on pedicles, monosporous, globose to ovoid, hyaline when young, gradually tawny when mature, thick-walled, 8.8–18.6 µm long, 8.6–18.7 µm wide, with or without spines. Spines 1.4–2.8 µm long. Chlamydospores absent. Zygosporangia not found.

Maximum growth temperature—33°C.

Additional strains examined—China, Yunnan Province, Puer City, Mojiang Hani Autonomous County, Tongguan Town (23°29'93"N, 101°39'17"E, altitude 1471.21 m), from soil, 4 July 2024, M.F. Tao and X.Y. Ji, living culture XG09559-10-2.

GenBank accession numbers—CGMCC 3.28651 (ITS, PV089199; LSU, PV123100; *TEF1α*, PV200765), XG09559-10-2 (ITS, PV089200; LSU, PV123101; *TEF1α*, PV200766).

Notes—Based on the ITS-LSU-*TEF1α* phylogenetic tree, two strains of the *Cunninghamella flava* sp. nov. formed a fully supported lineage (MLBV = 100, BIPP = 1.00; Fig. 1), closely related to *C. verticillata* (MLBV = 80, BIPP = 0.95; Fig. 1). However, they were different in terms of morphological features such as vesicles, pedicles, rhizoids, zygosporangia and zygosporangia. The vesicles of the new species were characterized by various shapes including globose, pear-shaped and elliptic, while vesicles were quite regular in *C. verticillata*, mainly depressed-globose, subglobose to globose. The pedicles of the new species were shorter than those of *C. verticillata* (1.6–2.2 µm vs 2.5–4 (–6.5) µm). Rhizoids were absent in the new species, whereas present in *C. verticillata*. Moreover, typical zygosporangia and zygosporangia were generated in *C. verticillata*, while absent in the new species. Physiologically, the maximum growth temperature of the new species was significantly lower than those of *C. verticillata* (33°C vs (37–) 39–42°C) [2].

3.2.4. *Cunninghamella hainanensis* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 5.

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

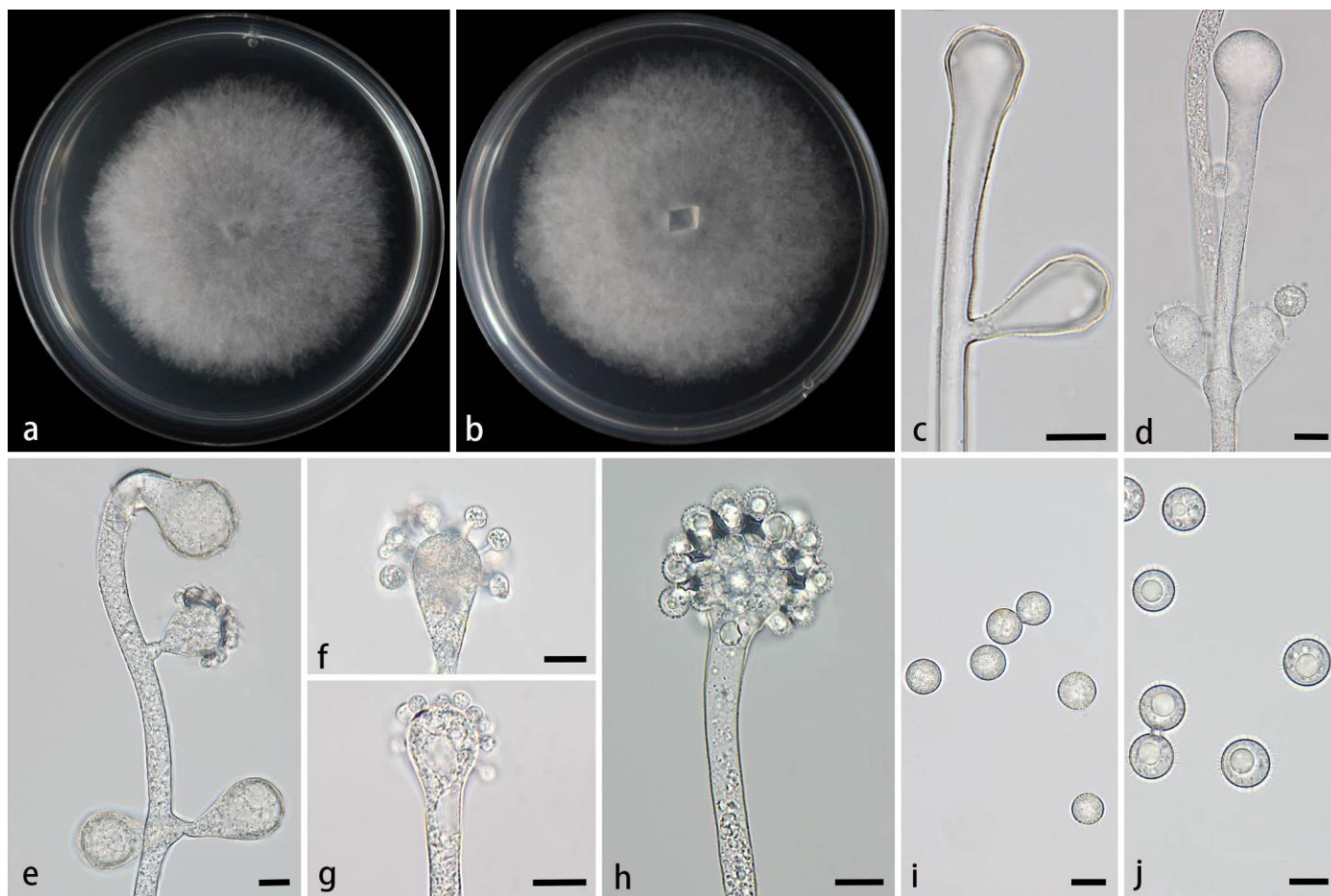


Figure 5. Morphologies of *Cunninghamella hainanensis* ex-holotype CGMCC 3.28649. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c-e) Sporangiphores with branching vesicles; (f-h) Vesicles bearing sporangiola; (i) Smooth sporangiola; (j) Spinose sporangiola. Scale bars: (c-j) 10 µm.

Type—China, Hainan Province, Danzhou City, near Hainan Tropical Botanical Garden (19°51'19"N, 109°50'08"E, altitude 108 m), from a soil sample, 14 February 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353441, ex-holotype living culture CGMCC 3.28649 (= XG06926-15-1).

Etymology—The epithet *hainanensis* (Lat.) refers to the location of the ex-holotype, Hainan Province, China.

Description—Colonies on PDA at 26°C for 4 d, reaching 85 mm in diameter, fast growing with a growth rate of 21.25 mm/d, initially white, gradually becoming light gray, floccose. Hyphae branched, hyaline, smooth-walled, aseptate when juvenile, septate with age. Rhizoids absent. Stolons present. Sporangiphores arising from stolons or aerial hyphae, mostly erect, a few slightly bent, occasionally verticillate, hyaline, single, unbranched or 1–3 branched, opposite, in pairs, 2.9–10.6 µm wide. Septa if present, usually one. Vesicles spherical, hemispherical, elliptic, smooth-walled, terminally 9.4–27 µm long and 7.9–26.5 µm wide, laterally 19.6–28.4 µm long and 12.2–22.9 µm wide. Pedicels on the entire surface of vesicles, 1.2–5.8 µm long. Sporangiola borne on pedicels, monosporous, mostly spherical, hyaline when young, dusky brown when old, thick-walled, 7.3–14 µm long, 7.8–13.4 µm wide, with or without spines. Spines 0.7–1.9 µm long. Chlamydospores absent. Zygosporangia not found.

Maximum growth temperature—29°C.

Additional strains examined—China, Hainan Province, Danzhou City, near Hainan Tropical Botanical Garden (19°51'19"N, 109°50'08"E, altitude 108 m), from a soil sample, 14 February 2024, M.F. Tao and X.Y. Ji, living culture XG06926-15-2.

GenBank accession numbers—CGMCC 3.28649 (ITS, PV089195; LSU, PV123096; *TEF1α*, PV172610), XG06926-15-2 (ITS, PV089196; LSU, PV123097; *TEF1α*, PV172611).

Notes—Based on the ITS-LSU-*TEF1α* sequence, two strains of the *Cunninghamella hainanensis* sp. nov. formed a distinct clade with full supports (MLBV = 100, BIPP = 1.00; Fig. 1), showing a close relatedness to *C. rhizoidea* (BIPP = 0.99; Fig. 1). Morphological comparisons revealed notable differences between these two new species in sporangiola, spines length and rhizoids. The sporangiola of the *C. hainanensis* were larger than those of *C. rhizoidea* ($7.3\text{--}14\ \mu\text{m} \times 7.79\text{--}13.4\ \mu\text{m}$ vs $6.2\text{--}13.4\ \mu\text{m} \times 5.6\text{--}12.9\ \mu\text{m}$) and exhibited two varieties (smooth and spinose vs only spinose). The spines of *C. hainanensis* were shorter than those of *C. rhizoidea* ($0.7\text{--}1.9\ \mu\text{m} \times 1.3\text{--}2.0\ \mu\text{m}$). Furthermore, *C. hainanensis* possessed numerous rhizoids, while the feature was absent in *C. rhizoidea*. Physiologically, *C. hainanensis* demonstrated a significantly lower maximum growth temperature than that of *C. rhizoidea* (29°C vs 33°C).

3.2.5. *Cunninghamella rhizoidea* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 6.

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

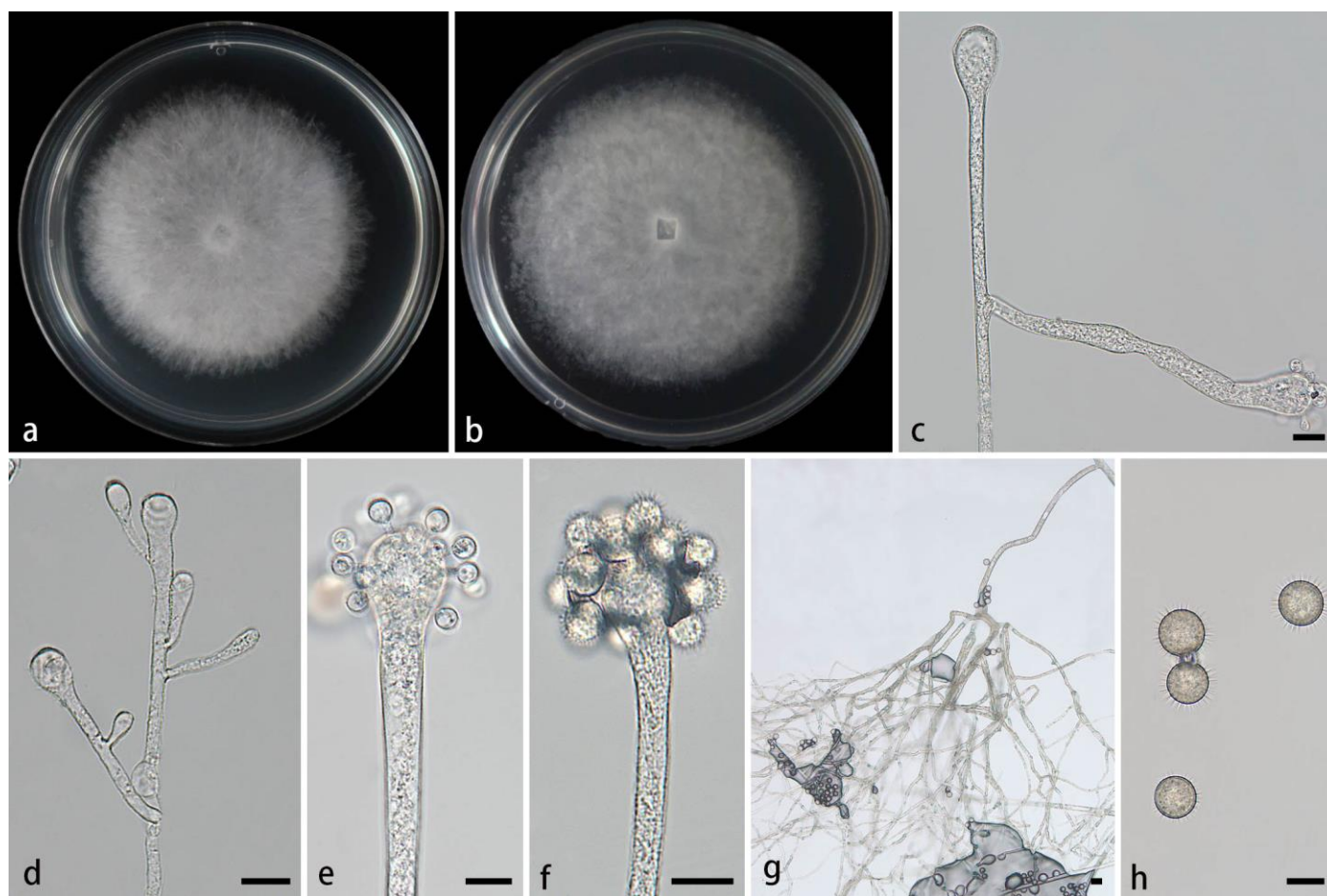


Figure 6. Morphologies of *Cunninghamella rhizoidea* ex-holotype CGMCC 3.28654. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c-d) Sporangiophores with branching vesicles; (e-f) Vesicles bearing sporangia; (g) Rhizoids; (h) Spinose sporangia. Scale bars: (c-f, h) 10 μm ; (g) 20 μm .

Type—China, Yunnan Province, Lincang City, Gengma Dai Autonomous County, He Pai Township ($23^\circ48'32''\text{N}$, $99^\circ41'66''\text{E}$, altitude 988.59 m), from soil, 7 July 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353446, ex-holotype living culture CGMCC 3.28654 (= XG09702-9-1).

Etymology—The epithet *rhizoidea* (Lat.) refers to the species producing abundant rhizoids.

Description—Colonies on PDA at 26°C for 4 days, reaching 85 mm in diameter, fast growing with a growth rate of 21.25 mm/d, initially white, gradually becoming gray with age, floccose. Hyphae branched, hyaline, smooth-walled, aseptate when adolescent, septate when mature. Rhizoids obviously present, root-like, complex branching, abundant. Stolons present. Sporangiophores forming from aerial hyphae or stolons, erect or slightly bent, mainly simple

branches or occasionally multiple branches, hyaline, single, recumbent, opposite, occasionally verticillate, sometimes having a swollen below the sporangiophores, 2.8–11.4 μm wide. Septa if observed, usually only one in sporangiophores. Vesicles globose, club-shaped, hyaline, smooth, terminally 7.9–35.3 μm long and 4.9–32.0 μm wide, laterally 2.7–26.6 μm long and 2.4–27.1 μm wide. Pedicels forming on the surface of the vesicle, 1.5–5.4 μm long. Sporangioles formed on pedicels on vesicles, monosporous, globose to ovoid, hyaline when young, gradually tea brown when mature, thick-walled, 6.2–13.4 μm long, 5.6–12.9 μm wide, with short spines. Spinse 1.3–2.0 μm long. Chlamydospores absent. Zygosporangia not found.

Maximum growth temperature—33°C.

Additional strains examined—China, Yunnan Province, Puer City, Mojiang Hani Autonomous County, Tongguan Town (23°29'93"N, 101°39'17"E, altitude 1471.21 m), from soil, 4 July 2024, M.F. Tao and X.Y. Ji, living culture XG09702-9-2.

GenBank accession numbers—CGMCC 3.28654 (ITS, PV089205; LSU, PV123106; *TEF1 α* , PV222157), XG09702-9-2 (ITS, PV089206; LSU, PV123107; *TEF1 α* , PV222158).

Notes—Based on the analysis of the ITS-LSU-*TEF1 α* sequence, two strains of the *Cunninghamella rhizoidea* sp. nov. formed a fully supported independent lineage (MLBV = 100, BIPP = 0.99; Fig. 1), exhibiting close genetic relatedness to *C. hainanensis* (BIPP = 0.99; Fig. 1). Morphological comparisons further highlighted significant differences between the two species in terms of sporangiola, spine length and rhizoids (see the note to *C. hainanensis* above).

3.2.6. *Cunninghamella simplex* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 7.

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

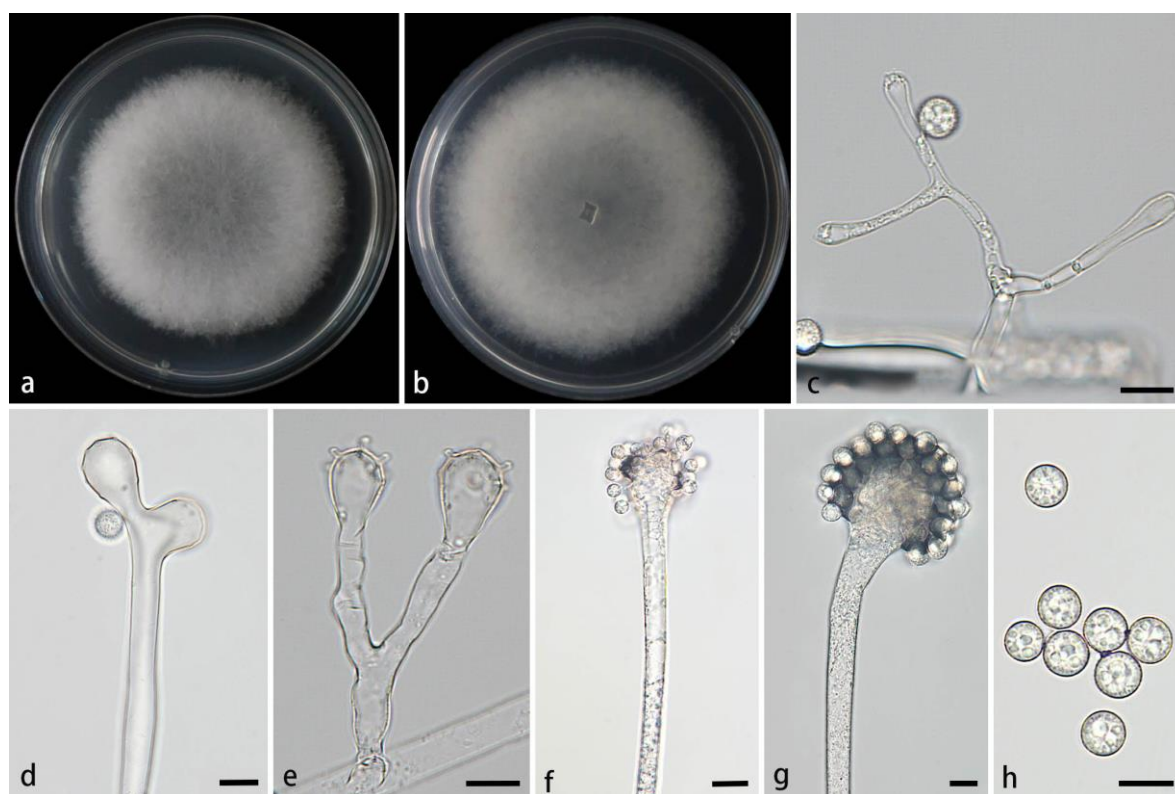


Figure 7. Morphologies of *Cunninghamella simplex* ex-holotype CGMCC 3.28653. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c-e) Sporangiophores with branching vesicles; (f-g) Vesicles bearing sporangia; (h) Smooth sporangia. Scale bars: (c-h) 10 μm .

Type—China, Yunnan Province, Puer City, Simao District, Yixiang Town, Yutang Section, Longlongba Jinyu Tea Estate (22°68'48"N, 101°07'32"E, altitude 1549.97 m), from soil, 5 July 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353445, ex-holotype living culture CGMCC 3.28653 (= XG09611-12-1).

Etymology—The epithet *simplex* (Lat.) refers to the simple branching pattern of sporangiophores.

Description—Colonies on PDA at 26°C for 4 d, reaching 85 mm in diameter, fast growing with a growth rate of 21.25 mm/d, initially white, gradually turning to dusky gray, floccose. Hyphae branched, hyaline, smooth-walled, aseptate when young, septate with age. Rhizoids absent. Stolons present. Sporangiophores arising from stolons or aerial hyphae, erect or slightly bent, hyaline, unbranched or simply branched, in pairs, never verticillate, 3.1–12.2 µm wide. Septa occasionally present below vesicles in the main sporangiophores, usually only one. Vesicles spherical, oval, cylindrical, usually hyaline, sometimes light brownish, smooth, terminally 5.0–28.4 µm long and 4.1–25.8 µm wide, laterally 6.7–17.0 µm long and 3.8–10.8 µm wide. Pedicels over the surface of vesicles, 1.1–2.9 µm long. Sporangiola borne on pedicels, monosporous, spherical to oval, hyaline when young, light brown with age, smooth, thick-walled, 5.8–10.2 µm long, 5.5–9.7 µm wide. Chlamydospores absent. Zygosporangia not found.

Maximum growth temperature—33°C.

Additional strains examined—China, Yunnan Province, Puer City, Simao District, Yixiang Town, Yutang Section, Longlongba Jinyu Tea Estate (22°68'48"N, 101°07'32"E, altitude 1549.97 m), from soil, 5 July 2024, M.F. Tao and X.Y. Ji, living culture XG09611-12-2.

GenBank accession numbers—CGMCC 3.28653 (ITS, PV089201; LSU, PV123102; *TEF1α*, PV200767), XG09611-12-2 (ITS, PV089202; LSU, PV123103; *TEF1α*, PV200768).

Notes—According to the ITS-LSU-*TEF1α* sequence, two strains of the *Cunninghamella simplex* sp. nov. constituted a distinct branch with full supports (MLBV = 100, BIPP = 1.00; Fig. 1), closely related to *C. bainieri* (MLBV = 100, BIPP = 1.00; Fig. 1). These two species were obviously different in the morphology of sporangiophores, sporangiola, vesicles, pedicels and rhizoids. The new species was lack of pseudoverticillate and verticillate sporangiophores, while *C. bainieri* produced. Additionally, the new species differed from *C. binariae* by the simply shaped sporangiola, spherical to oval, in contrast to ovoid to ellipsoid, globose and lacrymoid of various sporangiola in *C. bainieri*. The difference between the new species and *C. bainieri* on vesicles was that the new species generates spherical, oval and cylindrical forms, whereas *C. bainieri* produced spherical, hemispherical and oval shapes. The pedicels of the new species were shorter than those of *C. bainieri* (1.1–2.9 µm vs 2.5–3.5 (–6) µm). The new species lacked rhizoids, while *C. bainieri* gave rise to finger-like rhizoids. Physiologically, the maximum growth temperature of the new species was one degree lower than that of *C. bainieri* (33°C vs 34°C) [2].

3.2.7. *Cunninghamella yunnanensis* Z.Y. Ding & X.Y. Liu, sp. nov., Figure 8.

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

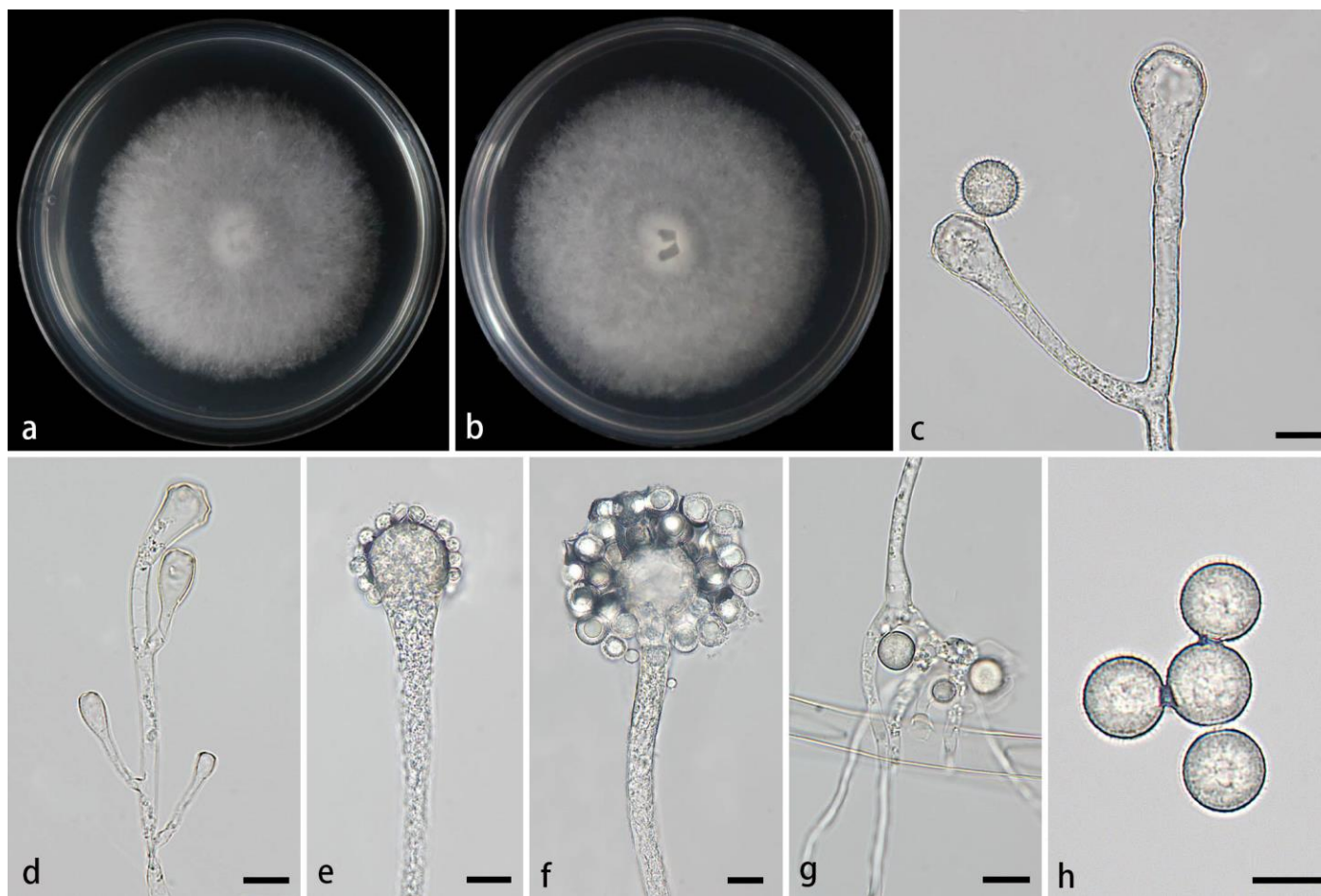


Figure 8. Morphologies of *Cunninghamella yunnanensis* ex-holotype CGMCC 3.28655. (a, b) Colonies on PDA, (a) obverse, (b) reverse; (c-d) Sporangiophores with branching vesicles; (e-f) Vesicles bearing sporangia; (g) Rhizoids; (h) Spinose sporangia. Scale bars: (c-h) 10 µm.

Type—China, Yunnan Province, Baoshan City, Longyang District, Lujiang Dam (24°92'66"N, 98°88'15"E, altitude 703.72 m), from soil, 10 July 2024, M.F. Tao and X.Y. Ji, holotype HMAS 353447, ex-holotype living culture CGMCC 3.28655 (= XG10042-9-1).

Etymology—The epithet *yunnanensis* (Lat.) refers to the location, Yunnan Province, China, where the ex-holotype was collected.

Description—Colonies on PDA at 26°C for 4 d, reaching 85 mm in diameter, fast growing with a growth rate of 21.25 mm/d, initially white, gradually becoming light gray with age, floccose. Hyphae branched, hyaline, smooth-walled, exuberant, aseptate when juvenile, septate when old. Rhizoids present, root-like, hyaline, rarely branched. Stolons present. Sporangiophores producing from stolons or aerial hyphae, erect or few slightly bent, mainly unbranched, or occasionally 1–7 branched, hyaline, straight or recumbent, few verticillate, usually expanding upwards, 1.8–14.6 µm wide. Septa sometimes one or two below the vesicles in the main sporangiophores. Vesicles hemispherical, spherical, oval, elliptic, hyaline, smooth, terminally 7.1–33.8 µm long and 4.4–33.5 µm wide, laterally 5.5–13.9 µm long and 3.2–12.4 µm wide. Pedicels present throughout the vesicles, 1.1–4.1 µm long. Sporangioles borne on pedicels, monosporous, mainly globose, sometimes oval, hyaline when young, grown when mature, thick-walled, 3.3–12.4 µm long, 3.3–12.0 µm wide, with short spines. Spines 0.7–2.0 µm long. Chlamydospores absent. Zygospores not found.

Maximum growth temperature—33°C.

Additional strains examined—China, Yunnan Province, Baoshan City, Longyang District, Lujiang Dam (24°92'66"N, 98°88'15"E, altitude 703.72 m), from soil, 10 July 2024, M.F. Tao and X.Y. Ji, living culture XG10042-9-2.

GenBank accession numbers—CGMCC 3.28655 (ITS, PV089207; LSU, PV123108; *TEF1α*, PV222159), XG10042-9-2 (ITS, PV089208; LSU, PV123109; *TEF1α*, PV222160).

Notes—Based on the ITS-LSU-TEF1 α phylogenetic tree, two strains of the *Cunninghamella yunnanensis* sp. nov. formed a fully supported independent lineage (MLBV = 100, BIPP = 1.00; Fig. 1), basal to all the other six new species as well as *C. verticillata* and *C. bainieri*.

4. Discussion

Currently, fungal taxonomists give paramount importance to molecular data when forming new taxa or evaluating species interconnections [51–53]. Specifically, sequences sourced from type materials are crucial for constructing a solid phylogenetic structure, serving as the foundation for a natural classification system [54]. Nonetheless, classical morphological characteristics and physiological characteristics continued to be widely acknowledged as a vital element in this process. The morphological, physiological characteristics and molecular data together provided a comprehensive picture of the species identity and evolutionary relationships [15,20]. In this study, seven novel species of *Cunninghamella* from southern China were identified based on morphological, physiological and molecular data from type strains. And the morphological characteristics of these seven novel species and their relatives were systematically compared herein (Table 1).

Table 1. morphological characteristics of *Cunninghamella* species.

Species	Colonies	Sporangiophores	Vesicles	Pedicels	Sporangiola	Reference
<i>C. hainanensis</i>	PDA: 26°C 4 d, 85 mm, 21.25 mm/d, initially white, gradually becoming light gray, floccose	2.9-10.6 μ m wide, mostly erect, a few slightly bent, occasionally verticillate, unbranched or 1–3 branched, opposite, in pairs	spherical, hemispherical, elliptic, terminal vesicles 9.4–27 $\times$ 7.9–26.5 μ m, lateral vesicles 19.6–28.4 $\times$ 12.2–22.9 μ m	1.2–5.8 μ m long	mostly spherical, 7.3–14 $\times$ 7.8–13.4 μ m, with or without spines, 0.7–1.9 μ m long	This study
<i>C. cinerea</i>	PDA: 26°C 4 d, 85 mm, 21.25 mm/d, initially white, gradually turning to smoky gray with age, floccose	3.1–11.7 μ m wide, erect or slightly bent, mainly unbranched or simply branched, mainly single or recumbent, never verticillate	spherical to elliptic, terminal vesicles 9.6–32.3 $\times$ 6.9–28.3 μ m, lateral vesicles 11.9–20.3 $\times$ 9.9–17.3 μ m	1.2–2.2 μ m long	globose to ovoid, 5.5–16.7 $\times$ 4.9–15.5 μ m, with or without spines, 0.8–1.7 μ m long	This study
<i>C. flava</i>	PDA: 26°C 6 d, 72 mm, 12 mm/d, initially white, gradually turning to dry yellow with age, floccose	3.8–25.6 μ m wide, erect or slightly bent, unbranched or 1–7 branched, recumbent, opposite, in pairs, 1–3 verticillate	globose, pear-shaped, elliptic, terminal vesicles 12.8–43.1 $\times$ 11.3–44.5 μ m, ateral vesicles 9.3–28.1 $\times$ 9.4–25.3 μ m	1.6–2.2 μ m long	globose to ovoid, 8.8–18.6 $\times$ 8.6–18.7 μ m wide, with or without spines, 1.4–2.8 μ m long	This study

Table 1. Cont.

Species	Colonies	Sporangiophores	Vesicles	Pedicels	Sporangiola	Reference
<i>C. amphispورا</i>	PDA: 26°C 4 d, 85 mm,	2.3–19.1 μ m wide, erect or few slightly	hemispherical, spherical, oval,	1.4–3.4 μ m long	Globose, 6.3–16.7 $\times$ 6.3–15.8	This study

	21.25 mm/d, initially white, gradually becoming light gray, floccose	bent, unbranched or simply branched, hyaline, single, no verticillate	pillar-shaped, terminal vesicles 6.4–56.6 × 3.2–46.7 μm, lateral vesicles 8–18.9 × 5.1–14.8 μm		μm, with or without spines, 0.5–2.6 μm long	
<i>C. simplex</i>	PDA: 26°C 4 d, 85 mm, 21.25 mm/d, initially white, gradually turning to dusky gray, floccose	3.1–12.2 μm wide, erect or slightly bent, hyaline, unbranched or simply branched, in pairs, never verticillate	spherical, oval, cylindrical, terminal vesicles 5.0–28.4 × 4.1–25.8 μm, lateral vesicles 6.7–17.0 × 3.8–10.8 μm	1.1–2.9 μm long	spherical to oval, 5.8–10.2 × 5.5–9.7 μm	This study
<i>C. rhizoidea</i>	PDA: 26°C 4 d, 85 mm, 21.25 mm/d, initially white, gradually becoming gray with age, floccose	.8–11.4 μm wide, erect branches or occasionally multiple branches, recumbent, opposite, occasionally verticillate	globose, club-shaped, terminal vesicles 7.9–35.3 × 4.9–32.0 μm; lateral vesicles 2.7–26.6 × 2.4–27.1 μm	1.5–5.4 μm long	globose to ovoid, 6.2–13.4 × 5.6–12.9 μm, with short spines, 1.3–2.0 μm long	This study
<i>C. yunnanensis</i>	PDA: 26°C 4 d, 85 mm, 21.25 mm/d, initially white, gradually becoming light gray with age, floccose	1.8–14.6 μm wide, rect or few slightly bent, mainly unbranched, or occasionally 1–7 branched, straight or recumbent, few verticillate	emispherical, spherical, oval, elliptic, terminal vesicles 7.1–33.8 × 4.4–33.5 μm; lateral vesicles 5.5–13.9 × 3.2–12.4 μm	1.1–4.1 μm long	mainly globose, sometimes oval, 3.3–12.4 × 3.3–12.0 μm wide, with short spines, 0.7–2.0 μm long	This study
<i>C. bainieri</i>	SMA: 27°C 4 d, 90mm, at first white, soon becoming light grey, to 'Light Mouse Grey', reverse cream, floccose	rect, bent, or recumbent, main axes of sporangiophores (8–) 11–21μm wide; primary branches (1–) 4–10 (–18) μm wide, monopodial, pseudoverticillate or verticillate in 1–2(–3) whorls of 3–8, typically in pairs	globose, subglobose to ovoid, sometimes irregular, axial vesicles 18.5–32(–40) μm; lateral ones (8–)13.5–30 μm	2.5–3.5 (–6) um long	ovoid to ellipsoid and 7–14.5 (–20) × 6.5–11 (–14.5) μm globose and 5.5–12.5μm, lacrymoid and 9–20 (–32.5) × 7–14.5 (–20) μm	[2]

Table 1. Cont.

Species	Colonies	Sporangiophores	Vesicles	Pedicels	Sporangiola	Reference
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		axial ones	
		slightly	Two kinds:
	erect, straight or	depressed-	globose,
	SMA: 28°C 5–6 recumbent, main axes	globose,	broadly
	d, 90mm, at first of sporangiophores	subglobose to	ellipsoid ovoid
	white, from the 7.5–17.5 (–25) μm; globose, 225–		and bluntly
C.	sixth day near branches (0–) 4–15 (– 50 (–70) μm; 2.5–4 (–		pointed at one
verticillata	‘Avellaneous’ to 25), verticillate to lateral ones 6.5) um		end, 6–17.5 (– [2]
	near ‘Colonial pseudoverticillate, usually long		20) × 5.5–13.5 (–
	Buff’, revcrse rarely singly or in globose to		17.5) μm; dark
	yellowish pairs, mostly simple, subglobose,		giant sp-
	cream, floccose very rarely re- sometimes		orangiola,
	branched broadly ovoid,		globose, 11.5-
	8 5–27.5 (–32.5)		17.5 (–25) μm
	μm		

The phylogenetic analysis of fourteen strains using combined ITS-LSU-*TEF1α* sequences revealed seven robust monophylies (Figure 1). *Cunninghamella hainanensis* and *C. rhizoidea* were sister to each other. Morphologically, the sporangiola of the former were larger than that of the latter. And the former had shorter spines and plentiful rhizoids. *C. cinerea* had close relationship with *C. simplex* and *C. bainieri*. Compared with *C. simplex*, *C. cinerea* had larger vesicles, shorter pedicels, rhizoids and spineless sporangiola. *C. cinerea* differed from *C. bainieri* by nonverticillate sporangiophores, shorter pedicels, as well as different shapes of rhizoids and sporangiola [2]. In contrast to *C. verticillata*, which had almost all globular vesicles, the vesicles were more varied in *C. flava* like globose, pear-shaped and elliptic. Additionally, *C. flava* had shorter pedicels and no rhizoids. More importantly, *C. flava* lacked the classical sexual stage, but *C. verticillata* was present [2]. In comparison to *C. cinerea*, *C. amphisporea* owned wider sporangiophores, larger lateral vesicles, smaller lateral vesicles, longer pedicels and spines. In comparison to *C. bainieri*, *C. simplex* had shorter pedicels, different shapes of vesicles and sporangiola. Sporangiophores were not pseudoverticillate and verticillate in *C. simplex*, but they were present in *C. bainieri* [2].

In addition, temperature also served as crucial factor in the categorization of fungi [14]. The maximum growth temperatures of the seven newly discovered species *C. amphisporea*, *C. hainanensis*, *C. cinerea*, *C. flava*, *C. rhizoidea*, *C. simplex* and *C. yunnanensis* were 29°C, 29°C, 33°C, 33°C, 33°C, 33°C, 33°C, respectively. Although the temperature tolerance of these new species was not very high, the maximum temperature of *C. verticillata*, a close relative of *C. flava*, was 42°C [2]. *C. amphisporea* and *C. hainanensis* are same in maximum growth temperature, but different in microscopic characteristics. In conclusion, both morphological and physiological traits are crucial for classifying fungi.

With the discovery of these seven new species, the genus *Cunninghamella* currently accommodates 63 species. This expansion not only broadens our taxonomic knowledge, but also paves the way for new research opportunities, promising to yield valuable insights into fungal diversity and function.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Supplementary Table S1: GenBank accession numbers of sequences used in this study; Supplementary File S1: the combined ITS-LSU- *TEF1α* sequence matrix used in this study.

Author Contributions: Z.Y. Ding was responsible for DNA sequencing, photo editing and paper drafting; Y. Jiang and W.X. Liu was responsible for data analyses; M.F. Tao, Y.X. Wang and X.Y. Ji collected soil samples; S. Wang was responsible for data analyses and manuscript revision; X.Y. Liu took charge of naming the new species, conceiving and revising the paper, and providing funding.

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Institutional Review Board Statement: Not applicable for studies involving humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The sequences from the present study were submitted to the NCBI database (<https://www.ncbi.nlm.nih.gov/>, accessed on 12 February 2025). The sequences were deposited in the GenBank database (Table S1).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Spatafora, J.W.; Chang, Y.; Benny, G.L.; Lazarus, K.; Smith, M.E.; Berbee, M.L.; Bonito, G.; Corradi, N.; Grigoriev, I.; Gryganskyi, A.; James, T.Y.; O'Donnell, K.; Roberson, R.W.; Taylor, T.N.; Uehling, J.; Vilgalys, R.; White, M.M.; Stajich, J.E. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*. **2016**, *108*, 1028–1046. <https://doi.org/10.3852/16-042>
2. Zheng, R.Y.; Chen, G.Q. A monograph of *Cunninghamella*. *Mycotaxon*. **2001**, *80*, 1–75.
3. Walther, G.; Pawłowska, J.; Alastruey-Izquierdo, A.; Wrzosek, M.; Rodriguez-Tudela, J.L.; Dolatabadi, S.; Chakrabarti, A.; de Hoog, G.S. DNA barcoding in *Mucorales*: an inventory of biodiversity. *Persoonia*. **2013**, *30*, 11–47. <https://doi.org/10.3767/003158513X665070>
4. Matruchot, L. Une Mucorinée purement conidienne, *Cunninghamella africana*. *Annales Mycologiques*. **1903**, *1*, 45–60.
5. Baijaj, U.; Mehrotra, B.S. The genus *Cunninghamella* - a reassessment. *Sydowia*. **1980**, *33*, 1–13.
6. Reed, M.D.A.; Body, P.B.; Austin, M.D.M.B.; Jr Frierson, M.D.H. *Cunninghamella bertholletiae* and *Pneumocystis carinii* pneumonia as a fatal complication of chronic lymphocytic leukemia. *Human Pathology*. **1988**, *19*, 1470–1472.
7. Nguyen, T.T.T.; Choi, Y.J.; Lee, H.B. Zygomycete fungi in Korea: *Cunninghamella bertholletiae*, *Cunninghamella echinulata*, and *Cunninghamella elegans*. *Mycobiology*. **2017**, *45*, 318–326. <https://doi.org/10.5941/MYCO.2017.45.4.318>
8. Gomes, M.Z.; Lewis, R.E.; Kontoyiannis, D.P. Mucormycosis caused by unusual mucormycetes, non-*Rhizopus*, -*Mucor*, and -*Lichtheimia*. *Clin. Microbiol. Rev.* **2011**, *24*, 411–45.
9. Kwon-Chung, K.J. Taxonomy of fungi causing mucormycosis and entomophthoromycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. *Clin. Infect. Dis.* **2012**, *54* Suppl 1, S8–S15. <https://doi.org/10.1093/cid/cir864>
10. Yu, J.; Walther, G.; Van Diepeningen, A.D.; Gerrits Van Den Ende, A.H.G.; Li, R.Y.; Moussa, T.A.A.; Almaghrabi, O.A.; De Hoog, G.S. DNA barcoding of clinically relevant *Cunninghamella* species. *Med. Mycol.* **2015**, *53*, 99–106. <https://doi.org/10.1093/mmy/myu079>
11. Fakas, S.; Papanikolaou, S.; Galiotou-Panayotou, M.; Komaitis, M.; Aggelis, G. Organic nitrogen of tomato waste hydrolysate enhances glucose uptake and lipid accumulation in *Cunninghamella echinulata*. *J. Appl. Microbiol.* **2008**, *105*, 1062–1070. <https://doi.org/10.1111/j.1365-2672.2008.03839.x>
12. Alakhras, R.; Bellou, S.; Fotaki, G.; Stephanou, G.; Demopoulos, N. A.; Papanikolaou, S.; Aggelis, G. Fatty acid lithium salts from *Cunninghamella echinulata* have cytotoxic and genotoxic effects on HL-60 human leukemia cells. *Eng. Life. Sci.* **2015**, *15*, 243–253. <https://doi.org/10.1002/elsc.201400208>
13. Zhao H, Lv ML, Liu Z, et al. High-yield oleaginous fungi and high-value microbial lipid resources from *Mucoromycota*. *Bioenerg. Res.* **2020**, *14*, 1196–1206. <https://doi.org/10.1007/s12155-020-10219-3>
14. Liu, C.W.; Liou, G.Y.; Chien, C.Y. New records of the genus *Cunninghamella* (*Mucorales*) in Taiwan. *Fungal Science*. **2005**, *20*, 1–9.
15. Guo, J.; Wang, H.; Liu, D.; Zhang, J.N.; Zhao, Y.H.; Liu, T.X.; Xin, Z.H. Isolation of *Cunninghamella bigelovii* sp. nov. CGMCC 8094 as a new endophytic oleaginous fungus from *Salicornia bigelovii*. *Mycol. Prog.* **2015**, *14*, 1–8. <https://doi.org/10.1007/s11557-015-1029-z>
16. Alves, A.L.; de Souza, C.A.; de Oliveira, R.J.; Cordeiro, T.R.; de Santiago, A.L. 2017 *Cunninghamella clavata* from Brazil: a new record for the western hemisphere. *Mycotaxon*. **2017**, *132*, 381–389.

17. Bragulat, M.R.; Castella, G.; Isidoro-Ayza, M.; Domingo, M.; Cabanes, F.J. Characterization and phylogenetic analysis of a *Cunninghamella bertholletiae* isolate from a bottlenose dolphin (*Tursiops truncatus*). *Revista Iberoamericana de Micología* **2017**, *34*, 215–219. <http://dx.doi.org/10.1016/j.riam.2017.03.002>
18. Weitzman, I.; Crist, M.Y. Studies with clinical isolates of *Cunninghamella* II. Physiological and morphological studies. *Mycologia* **1980**, *72*, 661–9.
19. Liu, X.Y.; Huang, H.; Zheng, R.Y. Relationships within *Cunninghamella* based on sequence analysis of ITS rDNA. *Mycotaxon* **2001**, *80*, 77–95.
20. Hyde, K.D.; Hongsanan, S.; Jeewon, R.; Bhat, D.J.; McKenzie, E.H.C.; Jones, E.B.G.; Phookamsak, R.; Ariyawansa, H.A.; Boonmee, S.; Zhao, Q.; Abdel-Aziz, F.A.; Abdel-Wahab, M.; Banmai, S.; Chomnunti, P.; Cui, B.K.; Daranagama, D.A.; Das, K.; Dayarathne, M.C.; De Silva, N.I.; Dissanayake, A.J.; Doilom, M.; Ekanayaka, A.H.; Gibertoni, T.B.; Góes-Neto, A.; Huang, S.K.; Jayasiri, S.C.; Jayawardena, R.S.; Konta, S.; Lee, H.B.; Li, W.J.; Lin, C.G.; Liu, J.K.; Lu, Y.Z.; Luo, Z.L.; Manawasinghe, I.S.; Manimohan, P.; Mapook, A.; Niskanen, T.; Norphanphoun, C.; Papizadeh, M.; Perera, R.H.; Phukhamsakda, C.; Richter, C.; De Santiago, A.L.C.M.; Drechsler-Santos, E.R.; Senanayake, I.C.; Tanaka, K. Fungal diversity notes 367–490: taxonomic and phylogenetic contributions to fungal taxa. *Fungal Diversity* **2016**, *80*, 1–270. <https://doi.org/10.1007/s13225-016-0373-x>
21. Suwannarach, N.; Kumla, J.; Supo, C.; Honda, Y.; Nakazawa, T.; Khanongnuch, C.; Wongputtisin, P. *Cunninghamella saisamornae* (*Cunninghamellaceae*, *Mucorales*), a new soil fungus from northern Thailand. *Phytotaxa* **2021**, *509*, 291–300. <https://doi.org/10.11646/phytotaxa.509.3.4>
22. Zhang, Z.Y.; Zhao, Y.X.; Shen, X.; Chen, W.H.; Han, Y.F.; Huang, J.Z.; Liang, Z.Q. Molecular phylogeny and morphology of *Cunninghamella guizhouensis* sp. nov. (*Cunninghamellaceae*, *Mucorales*), from soil in Guizhou, China. *Phytotaxa* **2020**, *455*, 31–39. <https://doi.org/10.11646/phytotaxa.455.1.4>
23. Wang, Y.J.; Zhao, T.; Wu, W.Y.; Wang, M.; Liu, X.Y. *Cunninghamella verrucosa* sp. nov. (*Mucorales*, *Mucoromycota*) from Guangdong province in China. *Phytotaxa* **2022**, *560*, 274–284.
24. Zhao, H.; Zhu, J.; Zong, T.K.; Liu, X.L.; Ren, L.Y.; Lin, Q.; Qiao, M.; Nie, Y.; Zhang, Z.D.; Liu, X.Y. Two new species in the family *Cunninghamellaceae* from China. *Mycobiology* **2021**, *49*, 142–150. <https://doi.org/10.1080/12298093.2021.1904555>
25. Zhao, H.; Nie, Y.; Zong, T.; Wang, K.; Lv, M.; Cui, Y.; Tohtirjap, A.; Chen, J.; Zhao, C.; Wu, F.; Cui, B.; Yuan, Y.; Dai, Y.; Liu, X.Y. Species diversity, updated classification and divergence times of the phylum *Mucoromycota*. *Fungal Diversity* **2023**, *123*, 49–157. <https://doi.org/10.1007/s13225-023-00525-4>
26. Zhao, H.; Nie, Y.; Huang, B.; Liu, X.Y. Unveiling species diversity within early-diverging fungi from China I: three new species of *Backusella* (*Backusellaceae*, *Mucoromycota*). *MycoKeys* **2024**, *109*, 285–304. <https://doi.org/10.3897/mycokeys.109.126029>
27. Tao, M.F.; Ding, Z.Y.; Wang, Y.X.; Zhang, Z.X.; Zhao, H.; Meng, Z.; Liu, X.Y. Unveiling species diversity within early-diverging fungi from China II: Three new species of *Absidia* (*Cunninghamellaceae*, *Mucoromycota*) from Hainan Province. *MycoKeys* **2024**, *110*, 255–272. <https://doi.org/10.3897/mycokeys.110.129120>
28. Wang, Y.X.; Zhao, H.; Jiang, Y.; Liu, X.Y.; Tao, M.F.; Liu, X.Y. Unveiling species diversity within early-diverging fungi from China III: Six new species and a new record of *Gongronella*. (*Cunninghamellaceae*, *Mucoromycota*). *MycoKeys* **2024**, *110*, 287–317. <https://doi.org/10.3897/mycokeys.110.130260>
29. Ding, Z.Y.; Ji, X.Y.; Tao, M.F.; Jiang, Y.; Liu, W.X.; Wang, Y.X.; Meng, Z.; Liu, X.Y. Unveiling species diversity within early-diverging fungi from China IV: Four new species of *Absidia* (*Cunninghamellaceae*, *Mucoromycota*). *MycoKeys* **2025**. (under review)
30. Ji, X.Y.; Ding, Z.Y.; Nie, Y.; Zhao, H.; Wang, S.; Huang, B.; Liu, X.Y. Unveiling species diversity: within early-diverging fungi. From China V: Five new species of *Absidia* (*Cunninghamellaceae*, *Mucoromycota*) *MycoKeys* **2025**. (under review)
31. Wang, Y.X.; Ding, Z.Y.; Ji, X.Y.; Meng, Z.; Liu, X.Y. Unveiling species diversity within early-diverging fungi from China VI: Four *Absidia* sp. nov. (*Mucorales*) in Guizhou and Hainan. *Microorganisms* **2025**. (under review)

32. Li, W. X.; Wei, Y. H.; Zou, Y.; Liu, P.; Li, Z.; Gontcharov, A. A.; Stephenson, S. L.; Wang, Q.; Zhang, S. H.; Li, Y. Dictyostelid cellular slime molds from the Russian Far East. *Protist* **2020**, *171*, 125756. <https://doi.org/10.1016/j.protis.2020.125756>
33. Zou, Y.; Hou, J. G.; Guo, S. N.; Li, C. T.; Li, Z.; Stephenson, S. L.; Pavlov, I. N.; Liu, P.; Li, Y. Diversity of dictyostelid cellular slime molds, including two species new to science, in forest soils of Changbai Mountain, China. *Microbiol. Spectr.* **2022**, *10*, e0240222. <https://doi.org/10.1128/spectrum.02402-22>
34. Zhao, H.; Nie, Y.; Zong, T.; Dai, Y.; Liu, X.Y. Three new species of *Absidia* (Mucoromycota) from China based on phylogeny, morphology and physiology. *Diversity* **2022**, *14*, 132. <https://doi.org/10.3390/d14020132>
35. Zhao, H.; Nie, Y.; Zong, T.; Wang, Y.; Wang, M.; Dai, Y.; Liu, X.Y. Species diversity and ecological habitat of *Absidia* (Cunninghamellaceae, Mucorales) with emphasis on five new species from forest and grassland soil in China. *Journal of Fungi* **2022**, *8*, 471. <https://doi.org/10.3390/d14020132>
36. Corry, J.E.L. Rose bengal chloramphenicol (RBC) agar. In: *Progress in Industrial Microbiology*; Elsevier: Amsterdam, The Netherlands, **1995**, pp. 431–433.
37. Zheng, R.Y.; Chen, G.Q.; Huang, H.; Li, X.Y. A monograph of *Rhizopus*. *Sydowia* **2007**, *59*, 273–372.
38. Zheng, R.Y.; Liu, X.Y.; Li, R.Y. More *Rhizomucor* causing human mucormycosis from China: *R. chlamydosporus* sp. nov. *Sydowia* **2009**, *61*, 135–147.
39. Zheng, R.Y.; Liu, X.Y. Taxa of *Pilaira* (Mucorales, Zygomycota) from China. *Nova Hedwigia* **2009**, *88*, 255–267. <https://doi.org/10.1127/0029-5035/2009/0088-0255>
40. Zong, T.K.; Zha, H.; Liu, X.L.; Ren, L.Y.; Zhao, C.L.; Liu, X.Y. Taxonomy and phylogeny of four new species in *Absidia* (Cunninghamellaceae, Mucorales) from China. *Frontiers in Microbiology* **2021**, *12*, 677836. <https://doi.org/10.3389/fmicb.2021.677836>
41. Doyle, J.J.; Doyle, J.L. Isolation of plant DNA from fresh tissue. *Focus* **1990**, *12*, 13–15. <https://doi.org/10.2307/2419362>
42. Guo, L.D.; Hyde, K.D.; Liew, E.C.Y. Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. *New Phytol.* **2000**, *147*, 617–630. <http://doi.org/10.1046/j.1469-8137.2000.00716.x>
43. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Academic Press: New York, NY, USA, **1990**; pp. 315–322.
44. Vilgalys, R.; Hester, M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J. Bacteriol.* **1990**, *172*, 4238–4246.
45. O'Donnell, K.; Lutzoni, F.M.; Ward, T.J.; Benny, G.L. Evolutionary relationships among mucoralean fungi *Zygomycota*: Evidence for family polyphyly on a large scale. *Mycologia* **2001**, *93*, 286–297. <https://doi.org/10.1080/00275514.2001.12063160>
46. Zhang, Z.; Liu, R.; Liu, S.; Mu, T.; Zhang, X.; Xia, J. Morphological and phylogenetic analyses reveal two new species of *Sporocadaceae* from Hainan, China. *MycoKeys* **2022**, *88*, 171–192. <https://doi.org/10.3897/mycokeys.88.82229>
47. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. <https://doi.org/10.1093/molbev/msw054>
48. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
49. Huelsenbeck, J.P.; Ronquist, F. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **2001**, *17*, 754–755. <https://doi.org/10.1093/bioinformatics/17.8.754>
50. Nylander, J. MrModeltest V2: Program distributed by the author. *Bioinformatics* **2004**, *24*, 581–583. <https://doi.org/10.1093/bioinformatics/btm388>
51. Bauer, R.; Garnica, S.; Oberwinkler, F.; Riess, K.; Weiß, M.; Begerow, D. *Entorrhizomycota*: a new fungal phylum reveals new perspectives on the evolution of fungi. *PLoS One* **2015**, *10*, e0128183. <https://doi.org/10.1371/journal.pone.0128183>
52. Jeewon, R.; Hyde, K.D. Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. *Mycosphere* **2016**, *7*, 1669–1677. <https://doi.org/10.5943/mycosphere/7/11/4>

53. Maharachchikumbura, S.S.N.; Hyde, K.D.; Jones, E.B.G.; McKenzie, E.H.C.; Bhat, J.D.; Dayarathne, M.C.; Huang, S.K.; Norphanphoun, C.; Senanayake, I.C.; Perera, R.H.; Shang, Q.J.; Xiao, Y.P.; D'souza, M.J.; Hongsanan, S.; Jayawardena, R.S.; Daranagama, D.A.; Konta, S.; Goonasekara, I.D.; Zhuang, W.Y.; Jeewon, R.; Phillips, A.J.L.; Abdel-Wahab, M.A.; Al-Sadi, A.M.; Bahkali, A.H.; Boonmee, S.; Boonyuen, N.; Cheewangkoon, R.; Dissanayake, A.J.; Kang, J.C.; Li, Q.R.; Liu, J.K.; Liu, X.Z.; Liu, Z.Y.; Luangsaard, J.J.; Pang, K.L.; Phookamsak, R.; Promputtha, I.; Suetrong, S.; Stadler, M.; Wen, T.C.; Wijayawardene, N.N. Families of *Sordariomycetes*. *Fungal Diversity* **2016**, *79*, 1–317. <https://doi.org/10.1007/s13225-016-0369-6>
54. Hu, D.M.; Wang, M.; Cai, L. Phylogenetic assessment and taxonomic revision of *Mariannaea*. *Mycological Progress* **2016**, *16*, 271–283. <https://doi.org/10.1007/s11557-016-1252-2>

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