

### RESEARCH ARTICLE

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# Diversity of culturable fungi in six Tibetan Plateau lakes, with descriptions of eight new taxa

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#### **ABSTRACT**

The combination of low temperatures, oligotrophic conditions, high altitude, and varying salinity in Tibetan Plateau lakes endows them with a wealth of distinctive fungal resources. This study aimed to investigate the diversity of culturable fungi in sediment and water samples collected from six lakes located on the Tibetan Plateau at elevations above 4,500 m. A total of 843 fungal strains were isolated by dilution plate method using four different media. Initial ITS analyses revealed that they belong to 156 species across 83 genera, spanning 50 families, 26 orders, 12 classes, and 6 phyla. Further morphological and multi-locus phylogenetic analyses were conducted, resulting in the formal descriptions of a new genus, *Xizangia*, and seven new species, including *Alternaria xizangensis*, *Emericellopsis ovoidea*, *Myceliophthora xizangica*, *Preussia cylindricalis*, *Preussia sedimenticola*, *Pseudeurotium sedimenticola*, and *Xizangia sedimenticola*. This research provides insights into the biodiversity of culturable fungi in the unique and extreme ecosystem, shedding light on the potential discovery of novel species and genera.

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#### **KEYWORDS**

Fungal diversity; multi-gene; phylogeny; salt lake; taxonomy

### 1. Introduction

The Qinghai-Tibetan Plateau (QTP) is the largest and highest (average 4,500 m above sea level) plateau on the Earth. It contains thousands of saline/hypersaline lakes, which possess a broad range of environmental gradients such as salinity (from 0.1 to 426.3 g/L) and pH (5.4–10.2) (Zheng 1997; Yang et al. 2016). Characterised by low temperature, low oxygen, high altitude, and intense UV radiation, lakes on the Tibetan Plateau serve as important repositories of biodiversity, providing insights into the evolutionary history and ecological dynamics of aquatic ecosystems. These extreme and unique ecosystems often harbour undiscovered microbial diversity with the potential to reveal novel species.

To date, investigations into the microbial diversity of Xizang's lakes have predominantly employed culture-independent methods (e.g. DNA sequencing) (Huang et al. 2014; Yang et al. 2016; Liu et al. 2021; Zhu et al. 2023) and focused on topics such as seasonal changes in microbial species composition (Liu

et al. 2013), the metabolic potential of communities in the carbon and nitrogen cycle (Wei et al. 2023), and mechanisms for coping with extremes (Li et al. 2020). However, few studies have been performed on the species composition and diversity of culturable fungi in Tibetan Plateau lakes. An examination of culturable filamentous fungi in Nam Co water revealed that the most frequently isolated genera were Lecanicillium, Mucor, and Penicillium, with Alternaria chlamydosporigena and Mucor hiemalis identified as the dominant species (Xue et al. 2022a). Notably, these fungi are commonly found in the natural environment. In addition, there has been a paucity of research on the diversity of culturable microbes in the sediments of Xizang's lakes, with particularly limited investigation into the diversity of fungal taxa.

Impacted by the development of high-throughput sequencing technologies, recent years have increasingly favoured the application of these molecular techniques instead of traditional culture methods for microbial resources and diversity studies (Amend

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et al. 2019). Nonetheless, the isolation of strains remains crucial for understanding microorganisms, serving as the foundational material for subsequent investigations into microbial physiology, genetic evolution, as well as exploitation (Richards et al. 2012). This manuscript delves into the fungal diversity of culturable isolates derived from Xizang's lakes.

### 2. Material and methods

### 2.1. Sampling collection and isolation

Samples of water and sediment were collected from six lakes located on the Tibetan Plateau, each with varying salinity levels: Angda'er Co (32.58 g/L), Dagze Co (15.93 g/L), Gomang Co (7.66 g/L), Gyaring Co (0.22 g/L), Nam Co (1.12 g/L), and Siling Co (8.12 g/L). Three sediment samples of different depths from each lake were collected using Grab's mud collector and then placed in sterile self-sealing bags. Two water samples from the surface and bottom of each lake were collected using the microporous membrane filtration method by filtering 500 mL of water samples through a 0.45-µm polyester fibre microporous membrane (47 mm, Millipore). All the sediment and water samples were transported to the laboratory at 4 °C for fungal isolation.

Fungi were isolated from sediments using either the dilution plate method (Zhang et al. 2015) or by directly coating sediment particles onto media plates. For the dilution plate method, one gram of each sediment sample was suspended in 9 mL of sterile water in a 15-mL sterile centrifuge tube. The tubes were shaken using a vortex oscillator. The suspension was then diluted to a series of concentrations, i.e.  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$ . One hundred microlitres of suspensions from each concentration were spread onto 1/100 PDA (Potato Dextrose Agar), 1/100 PmTG (Peptonised milk-Tryptone-Glucose, 1g peptonised milk, 1g tryptone, 5 g glucose, 10 g agar, 1 L H<sub>2</sub>O), 1/100 CMA (Corn Meal Agar) and 1/100 CDA (Czapek Dox Agar) containing ampicillin (50 µg/mL) and streptomycin (50 µg/mL) along with the corresponding salinity of sea salt with three replicates. For aqueous fungi, the 0.45-µm filter membranes of the aqueous samples were first vortexed with 2 mL of sterile water by oscillation, and then the suspension, as well as dilutions to  $10^{-1}$  and  $10^{-2}$ , were spread onto 1/100 PDA. All the plates were incubated at 15 °C for 3-4 weeks, from which the single colonies were picked up and inoculated onto new PDA plates every two days. All fungal strains were stored at 4 °C for subsequent studies.

# 2.2. Molecular analyses

Genomic DNA was extracted from fresh fungal mycelium grown for 7 d on PDA, following the Cetyltrimethylammonium bromide (CTAB) method (Guo et al. 2000). The internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS), the large subunit (LSU) rDNA, the small subunit (SSU) rDNA, the translation elongation factor 1-alpha (tef-1α), RNA polymerase II subunit (rpb2), and β-tubulin (tub2) regions were amplified using primer pairs ITS1/ ITS4 (White et al. 1990), LROR/LR5 (Vilgalys and Hester 1990), NS1/NS4 (White et al. 1990), 983F/2218 R (Rehner and Buckley 2005), RPB2-5F2/fRPB2-7cR (Liu et al. 1999; Sung et al. 2007), and Bt2a/Bt2b (Glass and Donaldson 1995), respectively. Amplification reactions were performed in a 25 µL reaction volume including 2.5 µL 10× PCR Buffer (Vazyme, Nanjing, China), 2 mmol/L MgCl<sub>2</sub>, 50 µmol/L dNTPs, 0.1 µmol/L of each forward and reverse primer, 0.5 U Tag DNA polymerase and 1 ng genomic DNA. PCR parameters were as follows: 94 °C for 10 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s, and a final elongation step at 72 °C for 10 min. The annealing temperature for each gene was 52 °C for LSU, SSU, ITS, and 57 °C for rpb2, tef-1a, and tub2. Sequencing reactions were performed by SinoGenoMax (Beijing, China). All ITS sequences of the obtained strains were subjected to BLASTn searches using UNITE and GenBank databases and assigned to possible genera and species. Furthermore, the ITS phylogenetic tree of all strains was constructed to show their isolation origin and taxonomic status as a whole.

To clarify the taxonomic status of potential new taxonomic units of interest to us in this study, phylogenetic analyses were performed based on ITS, LSU, and genetic markers recommended in recent publications, such as tef1-a, rpb2, and tub2. All the information on reference strains is shown in Tables S2-S7. All sequences of different loci were aligned using MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/) (Katoh and Toh 2010) and edited manually using MEGA v. 7.0

(Kumar et al. 2016) separately. Individual alignments were then concatenated and used for phylogenetic analysis. Maximum Likelihood (ML) and Bayesian inference (BI) methods were used to construct the phylogenetic trees. The ML analyses were implemented using RAxML-HPC Blackbox v. 8.2.12 (Stamatakis 2014) with 1,000 replicates under the GTR-GAMMA model. The robustness of branches was assessed by bootstrap analysis with 1,000 replicates. For Bayesian analysis, the best model of evolution was estimated using MrModeltest v. 2.4 (Guindon et al. 2003). Posterior probabilities (PP) (Rannala and Yang 1996; Zhaxybayeva and Gogarten 2002) were calculated by Markov Chain Monte Carlo sampling (MCMC) in MrBayes on XSEDE v. 3.2.7a (Huelsenbeck and Ronquist 2001), using the estimated evolutionary models. Six simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 1,000th generation. The first 2,000 trees, representing the burn-in phase of the analyses, were discarded and the remaining 8,000 trees were used to calculate posterior probabilities (PP) in the majority rule consensus tree. The final trees were visualised in FigTree v 1.4.0. Descriptions and illustrations of taxonomic novelties were deposited in Fungal Names (Wang et al. 2023).

# 2.3. Morphological observation

Strains of potentially new species were transferred to new plates of PDA, OA (Oatmeal Agar), and SNA (Synthetic Nutrient-poor Agar) and were incubated for at least 7 d at 15 °C. Cultures were examined periodically for the development of reproductive structures. Morphological characteristics were examined and photo-documented using a Nikon 80i microscope with differential interference contrast. At least 30 measurements for each structure were made according to methods described by Liu et al. (2012). The dry cultures were deposited in the Fungarium of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China (HMAS), while living cultures were deposited in the China General Microbiological Culture Collection Center (CGMCC) and LC Culture Collection (personal culture collection held in the Lei Cai's lab).

### 3. Results

In this study, a total of 843 fungal strains were isolated and assigned to 6 phyla, 12 classes, 26 orders, 50 families, 83 genera, and 156 species based on the primary analyses by employing a BLASTn search in UNITE or GenBank using ITS sequences (Figure 1a,b, Table S1). Furthermore, ITS sequence similarity comparisons identified 26 species with less than 98% similarity to sequences in NCBI, and 60 species with less than 99% similarity (see Table S1 and Figure 1d), indicating their potential novelty.

The top 10 most frequently segregated genera were, in order, Pseudogymnoascus, Pseudeurotium, Cadophora, Alternaria, Penicillium, Chaetomium, Fusarium, Candida, Rhizopus, and Aspergillus (Table S1, Figure 1c). The 10 genera with the highest number of species obtained, in descending order, were Penicillium (11), Fusarium (7), Cadophora (6), Alternaria (6), Aspergillus (6), Cladosporium (6), Chaetomium (5), Emericellopsis (5), Preussia (5), and Pseudogymnoascus (3) (Figure 1c). The top 10 most frequently isolated species were, in order, Pseudogymnoascus roseus (ITS Iden. 99.1%), Cadophora constrictospora (96.5%), Pseudeurotium hygrophilum (99.3%), Alternaria chlamydosporigena (99.4%), Chaetomium globosum (100%),Pseudeurotium desertorum (98.4%), Candida vartiovaarae (100%), Penicillium citrinum (100%), Pseudogymnoascus appendiculatus (98%), and Rhizopus arrhizus (99.7%) (Table S1).

Based on multi-locus phylogenetic analyses and morphological characterisation, a new genus and seven new species were officially described and illustrated in this study, which belonged to Alternaria, Emericellopsis, Myceliophthora, Preussia, Pseudeurotium, and Xizangia.

### **Taxonomy**

Alternaria xizangensis F. Liu, S. Song & L. Cai, sp. nov., Figure 2

Fungal name: FN571892.

Etymology: Named after the place where the fungus was collected, Xizang.

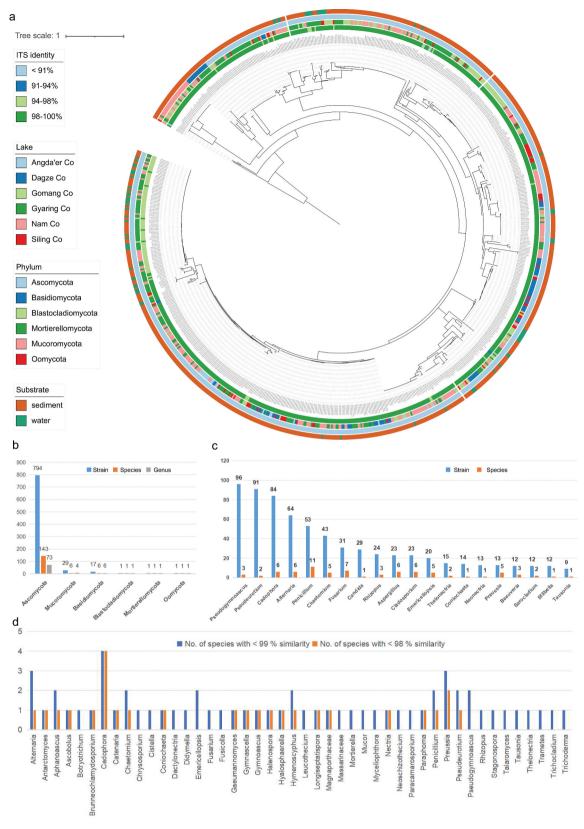


Figure 1. Culturable fungi in six Tibetan Plateau lakes. (a) Phylogenetic tree of all strains obtained in six lakes based on ITS sequences. (b) The number of fungal genera, species, and strains in different phyla obtained in this study. (c) Number of strains of the top 20 genera with the highest frequency of isolation and their corresponding number of species. (d) The number of species with less than 98% and 99% ITS sequence similarity to known species in the NCBI database.

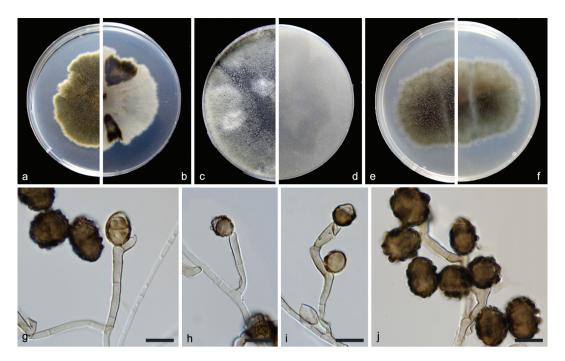


Figure 2. Alternaria xizangensis (from ex-holotype CGMCC 3.27272). (a–b) Surface and reverse of the colony on PDA. (c–d) Surface and reverse of the colony on OA. (e–f) Surface and reverse of the colony on MEA. (g–j) Conidiophores and conidia. Scale bars:  $g-j=10 \mu m$ .

Description: Conidiophores solitary or clustered, geniculate, frequently proliferating sympodially, pale brown to brown, 9–42 × 2–4  $\mu$ m. Conidia commonly solitary or several in a chain, varied from subsphaeroid, obovoid, obclavate to ellipsoid, brown to dark brown, verrucose, 9–18 × 7–17  $\mu$ m (av.  $\pm$  SD: 14.1  $\pm$  2.1 × 12.2  $\pm$  2.4  $\mu$ m), mature conidia with 0–1 transverse and 0–1 longitudinal septa.

*Material examined*: China, Xizang, Siling Co, N 31.7030, E 88.7354, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352877 (**holotype**), **ex-holotype living culture** CGMCC 3.27272 = LC19458 = S2.

Notes: A BLASTn search in NCBI using the ITS sequence of the type strain of Alternaria xizangensis indicated its close relationship with species in the Alternaria section Ulocladioides, with the species showing the highest similarity being A. terricola (96%). Multilocus phylogenetic analysis revealed that A. xizangensis is closely related to A. atra and A. multiformis (Figure 3), but differs from both by 21 bp in the ITS sequences. Morphologically, A. xizangensis differs from A. atra in the number of transepta (0–1 vs. 1–3) and longisepta (0–1 vs. 1–2) in conidia, and it differs from A. multiformis in producing smaller conidia (9–18 × 7–17 μm vs. 22–24 × 13–18 μm) (Simmons 1998).

**Emericellopsis ovoidea** F. Liu, S. Song & L. Cai, **sp. nov.**, Figure 4

Fungal name: FN571893.

Etymology: Referring to the obovate conidia.

Description: Mycelium consists of branched, septate, hyaline, smooth, and thin-walled hyphae up to 2 μm wide. Conidiophores are produced by immersed or superficial hyphae, erect or slightly curved, single or branched, 2–2.5 μm wide at base, hyaline, smooth wall. Conidiogenous cells erect, cylindrical,  $11-20\times1-2$  μm (av.  $\pm$  SD:  $16.3\pm2.9\times1.9\pm0.3$  μm). Conidia obovate, sometimes ellipsoidal, aseptate, rarely 1-spetum, hyaline, smooth wall,  $3.5-7.5\times2-3.5$  μm (av.  $\pm$  SD:  $5.0\pm0.9\times2.7\pm0.3$  μm).

Materials examined: China, Xizang, Siling Co, N 31.8011, E 88.7288, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352878 (holotype), ex-type living culture CGMCC 3.27273 = LC19461 = S58; ibid. LC19490 = SLC1018, LC19498 = SLC2007, LC19504 = SLC2016.

Notes: Based on the blastn searches using GenBank and UNITE databases, the species with the highest similarity to *Emericellopsis ovoidea* are *E. pallida*, *E. maritima* (99.41%), and *E. maritima* (98.52%), respectively. Through the multi-locus

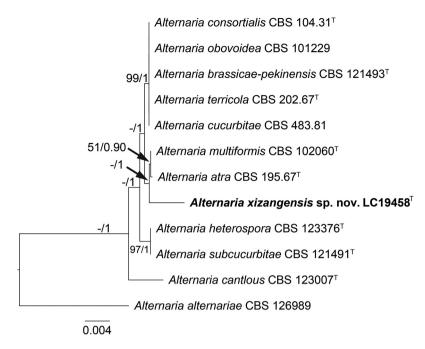


Figure 3. Maximum likelihood (ML) tree of Alternaria sect. Ulocladioides based on ITS, LSU, SSU, gapdh, rpb2, and tef1-a sequences. The tree is rooted with A. alternariae (CBS 126989). The tree topology of the ML analysis was similar to the Bl. ML bootstrap values (≥50%) and Bayesian posterior probability (≥0.9) are indicated along branches (ML/PP). Novel species are in bold font and "T" indicates type derived sequences.

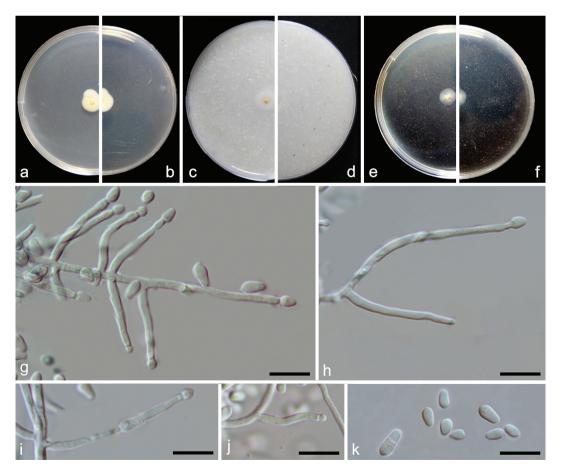
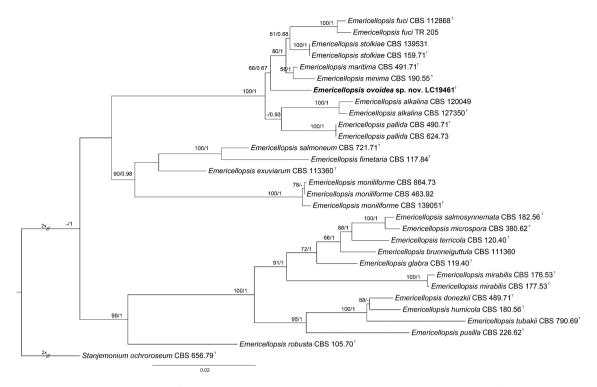


Figure 4. Emericellopsis ovoidea (from ex-holotype CGMCC 3.27273). (a-b) Surface and reverse of the colony on PDA. (c-d) Surface and reverse of the colony on OA. (e–f) Surface and reverse of the colony on MEA. (g–j) Conidiogenous cells and conidia. (k) Conidia. Scale bars:  $g-k = 10 \mu m$ .



**Figure 5.** Maximum likelihood (ML) tree of *Emericellopsis* and allied genera based on ITS, LSU, rpb2, and tef1-a sequences. The tree is rooted with *Stanjemonium ochroroseum* (CBS 656.79). Tree topology of the ML analysis was similar to the Bl. ML bootstrap values (≥50%) and Bayesian posterior probability (≥0.9) are indicated along branches (ML/PP). Novel species are in bold font and "T" indicates type derived sequences.

analysis, we noticed that *E. ovoidea* represents a distinct clade and has a close relationship with *E. alkalina, E. pallida, E. minima*, and *E. maritima* (Figure 5). Morphologically, *E. ovoidea* differs from *E. maritima* in producing relatively smaller conidia  $(3.5-7.5\times2-3.5\,\mu\text{m}\ vs.\ 6.5-9\times2-4\,\mu\text{m})$  (Belyakova 1970), and from *E. alkalina* and *E. minima* in the length of conidiogenous cells  $(11-20\,\mu\text{m}\ in\ E.\ ovoidea\ vs.\ 20-35\,\mu\text{m}\ in\ E.\ alkalina,\ 20-35\,\mu\text{m}\ in\ E.\ minima)$  (Stolk 1955; Grum-Grzhimaylo et al. 2013).

# *Myceliophthora xizangica* F. Liu, S. Song & L. Cai, **sp. nov**., Figure 6

Fungal name: FN571894.

*Etymology*: Named after the place where the fungus was collected, Xizang.

Description: Vegetative hyphae hyaline, smooth, branched, septate, about 1  $\mu$ m wide. Conidiophores hayline, branched, septate, 1–28.5 × 1–3  $\mu$ m, sometimes reduced to conidiogenous cells. Conidiogenous cells originating laterally or terminally from hyphae or conidiophores, subcylindrical, pyriform, subglobose or ampulliform, 1–8.4 × 1.5–5.5  $\mu$ m. Conidia

solitary, or in short chains, aseptate, smooth, hyaline, globose, subglobose, ovoid, or ellipsoidal, with a truncate base,  $3.5-5.5\times3-4~\mu m$  (av.  $\pm$  SD:  $4.6\pm0.6\times3.7\pm0.2~\mu m$ ).

*Materials examined*: China, Xizang, Gomang Co, N 31.1388, E 89.1987, 4,629 m, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352879 (**holotype**), **extype living culture** CGMCC 3.27275 = LC20320 = G117; Siling Co, N 31.703, E 88.7354, 4,541.58 m, from lake sediment, Aug. 2021, J.E. Huang, living cultures LC20379 = SLCS3, LC20380 = GMC63, LC20381 = GMC64.

Notes: Myceliophthora xizangica is closely related to M. lutea (Figure 7), but shares low sequence similarity with the latter at ITS (98%, 443/448), rpb2 (94.66%, 443/468), and tef1-a (93.27%, 679/728). Morphologically, it differs from M. lutea in producing branched conidiophores and subcylindrical conidiogenous cells (Wang et al. 2022).

# **Preussia cylindricalis** F. Liu, S. Song & L. Cai, **sp. nov**., Figure 8

Fungal name: FN571895.

Etymology: Referring to the cylindrical conidia.

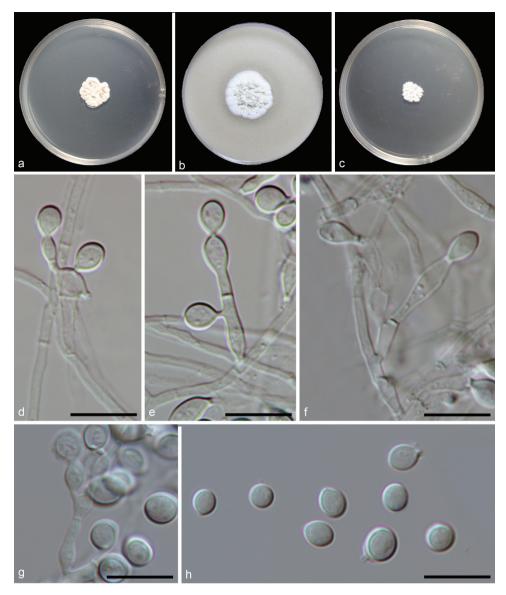


Figure 6. Myceliophthora xizangica (from ex-holotype CGMCC 3.27275). (a-c) Surface of colonies on PDA, OA, and MEA, respectively. (d-g) Conidiophores and conidia. (h) Conidia. Scale bars:  $d-h=10 \mu m$ .

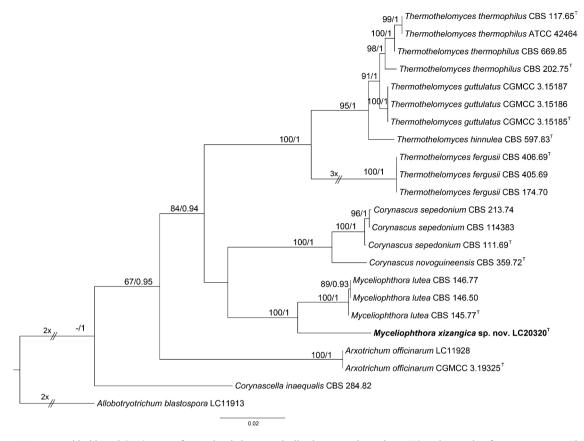
Description: Conidiomata pycnidial, black, spherical, clustered, 117–173 μm or Conidiophores reduced to conidiogenous cells lining the inner cavity, hyaline, smooth, phialidic, 2.5- $4 \times 1.5 - 3.5 \,\mu\text{m}$  (av.  $\pm$  SD:  $3.6 \pm 0.5 \times 2.79 \pm 0.7 \,\mu\text{m}$ ). Conidia ellipsoid with obtuse ends, hyaline, aseptate, smooth, 1-guttulate,  $1.5-2.5 \times 1-1.5 \,\mu m$  (av.  $\pm$  SD:  $2.2 \pm 0.2 \times 1.4 \pm 0.1 \,\mu m$ ).

Material examined: China, Xizang, Nam Co, N 30.8693, E 91.0386, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352880 (holotype), extype living culture CGMCC 3.27271 = LC19384 = NMC1013.

Notes: Although represented by a single strain, Preussia cylindricalis is phylogenetically distinct from all currently accepted species of this genus (Figure 9). It has a low ITS sequence similarity of 96.77% (510/527) with its closest relative P. typharum. Considering that the asexual morph of P. typharum has not been observed, we could not make a morphological comparison between the two species in this study.

Preussia sedimenticola F. Liu, S. Song & L. Cai, sp. nov., Figure 10

Fungal name: FN571896.



**Figure 7.** Maximum likelihood (ML) tree of *Myceliophthora* and allied genera based on ITS, *rpb2*, and *tef1-a* sequences. The tree is rooted with *Allobotryotrichum blastospora* (LC11913). Tree topology of the ML analysis was similar to the Bl. ML bootstrap values (≥50%) and Bayesian posterior probability (≥0.9) are indicated along branches (ML/PP). Novel species are in bold font and "T" indicates type derived sequences.

*Etymology*: Referring to the substrate where the fungus was isolated.

Description: Conidiophores hyaline, branched, or reduced to conidiogenous cells. Conidiogenous cells originating laterally or terminally from hyphae or conidiophores, cylindrical, hyaline, 2–14.5  $\times$  1–2 μm. Conidia hyaline, aseptate, ovoid with truncate base, globose, ellipsoid, solitary or rarely in chains, 2.5–4.5  $\times$  2–3 μm (av.  $\pm$  SD: 3.5  $\pm$  0.4  $\times$  2.8  $\pm$  0.3 μm).

Materials examined: China, Xizang, Dagze Co, N 31.8237, E 87.4713, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352881 (holotype), ex-type living culture CGMCC 3.27276 = LC20072 = DZC1014;ibid. LC20382 = DZC1035, LC20383 = DZC1043;Siling Co, Ν 31.8011, E 88.7288, from lake sediment, Aug. 2021, J.E. living cultures LC19477 = SLC1001, LC20384 = SLC1002, LC20385 = SLC2003.

Notes: Six strains of Preussia sedimenticola formed a distinct clade on the LSU-ITS

phylogenetic tree (Figure 9). Although *P. sedimenticola* is most closely related to *P. cylindricalis*, and both were isolated from lakes on the Tibetan Plateau, the two species exhibit distinct morphological differences. The conidiogenous cells of *P. sedimenticola* mostly form from hyphae and are cylindrical, whereas those of *P. cylindricalis* are formed in pycnidia and are phialidic in shape. Additionally, *P. sedimenticola* produces larger conidia than *P. cylindricalis* (2.5–4.5  $\times$  2–3  $\mu$ m *vs.* 1.5–2.5  $\times$  1–1.5  $\mu$ m).

**Pseudeurotium desertorum** Mouch., Revue Mycol., Paris 36(2): 123. 1971. Figure 11

Description: Hyphae hyaline, septate, branched, smooth, 1.5 μm wide. Asci spherical or nearly spherical, pyriform, 8-ascospore, thin-walled, smooth,  $8-14\times6-10$  μm. Ascospores globose, hyaline to pale brown, aseptate, smooth,  $3-4.5\times3-4$  μm (av.  $\pm$  SD:  $3.42\pm0.33\times3.35\pm0.23$  μm). Conidiophores reduced to conidiogenous cells, originating from

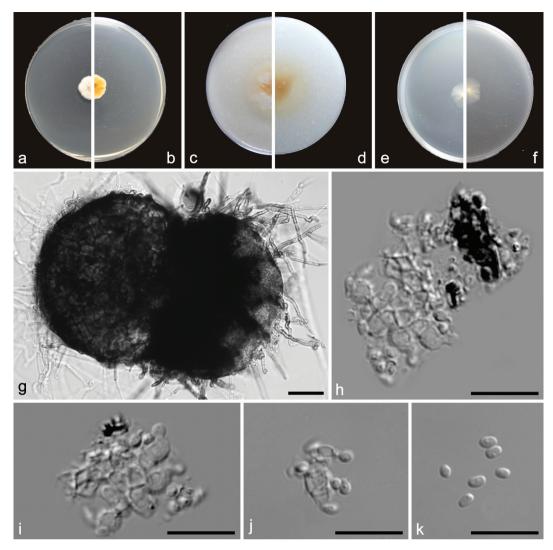


Figure 8. Preussia cylindricalis (from ex-holotype CGMCC 3.27271). (a-b) Surface and reverse of the colony on PDA. (c-d) Surface and reverse of the colony on OA. (e–f) Surface and reverse of the colony on MEA. (g) Pycnidium. (h–j) Conidiogenous cells and conidia. (k) Conidia. Scale bars:  $g = 25 \mu m$ ;  $h-k = 10 \mu m$ .

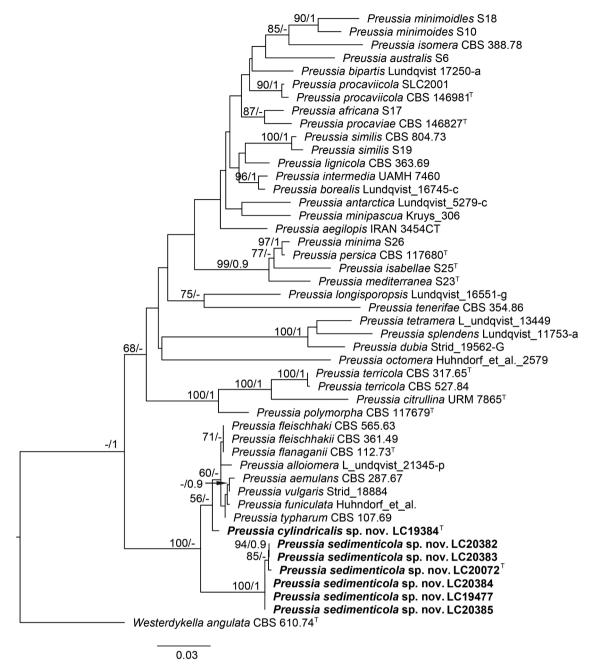
hyphae, hyaline, and aseptate. Conidiogenou cells cylindrical, clavate, obclavate, straight or curved, 1- $15 \times 1-2 \,\mu m$ . Conidia aseptate, globose, subglobose to broadly ellipsoidal,  $4-7 \times 3.5-4.5 \,\mu\text{m}$  (av.  $\pm$  SD:  $4.97 \pm 0.6 \times 4.08 \pm 0.27 \mu m$ ).

Materials examined: China, Xizang, Angda'er Co, 32.6684, E 89.5275, from lake sediment, Aug. 2021, J.E. Huang, living cultures LC19712 = ADECS193, LC19832 = ADECS495, LC19715 = ADECS198; Nam Co, N 30.8693, E 91.0386, from lake sediment, Aug. 2021, J.E. Huang, living culture LC19441 = NMC4005; Siling Co, N 31.703, E 88.7354, from lake sediment, Aug. 2021, J.E. Huang, living culture LC19502 = SLC2013.

Notes: Many Pseudeurotium desertorum strains were isolated from sediments of lakes on the Tibetan Plateau in this study. In terms of phylogenetic tree topology, intraspecific variation was observed in the species (Figure 12). However, as the sequences of these isolates obtained in this differed from the type strain P. desertorum by only 2-4 base pairs, we identified all these strains as P. desertorum and updated their morphological description herein.

Pseudeurotium sedimenticola F. Liu, S. Song & L. Cai, sp. nov., Figure 13

Fungal name: FN571897.



**Figure 9.** Maximum likelihood (ML) tree of *Preussia* and allied genera based on ITS and LSU sequences. The tree is rooted with *Westerdykella angulata* (CBS 610.74). Tree topology of the ML analysis was similar to the Bl. ML bootstrap values (≥50%) and Bayesian posterior probability (≥0.9) are indicated along branches (ML/PP). Novel species are in bold font and "T" indicates type derived sequences.

*Etymology*: Referring to the substrate where the fungus was isolated.

Description: Conidiophores reduced to conidiogenous cells, originating laterally or terminally from hyphae. Terminal conidiogenous cells lageniform, often bent or sinuous, 3–12 μm; intercalary conidiogenous cells similar to terminal cells or ampuliform, or reduced to small denticles on intercalary cells. Conidia aseptate,

hyaline, subglobose or obovoid,  $3-4\times2-3~\mu m$  (av.  $\pm$  SD:  $3.4\pm0.4\times2.6\pm0.2~\mu m$ ).

*Material examined*: China, Xizang, Angda'er Co, N 32.6801, E 89.5327, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352882 (**holotype**).

*Notes*: Although represented by single strain, *P. sedimenticola* forms a distinct clade on the ITS tree (Figure 12) and has 98.6% ITS sequence similarity to

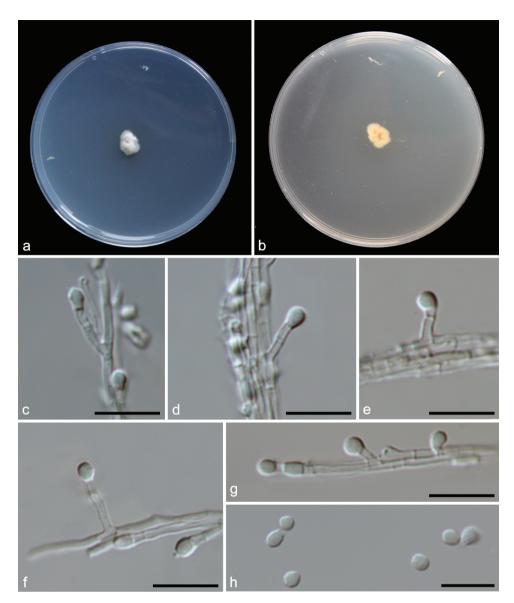


Figure 10. Preussia sedimenticola (from ex-holotype CGMCC 3.27276). (a-b) Surface and reverse colony on PDA. (c-g) Conidiogenous cells and conidia. (h) Conidia. Scale bars:  $c-h = 10 \mu m$ .

the closely related species P. desertorum. Morphologically, P. sedimenticola differs from P. desertorum in producing smaller conidia  $(3-4\times2 3 \mu m \ vs. \ 4-7 \times 3.5-4.5 \ \mu m).$ 

# Xizangia F. Liu & L. Cai, gen. nov.

Fungal name: FN571890.

Etymology: Named after the place where we collected the ex-type culture, Xizang.

Description: Conidiogenous cells arising laterally from aerial hyphae, hyaline, phialidic or cylindrical, aseptate or septate. Conidia hyaline, smooth, aseptate, globose or ovoid, guttulate.

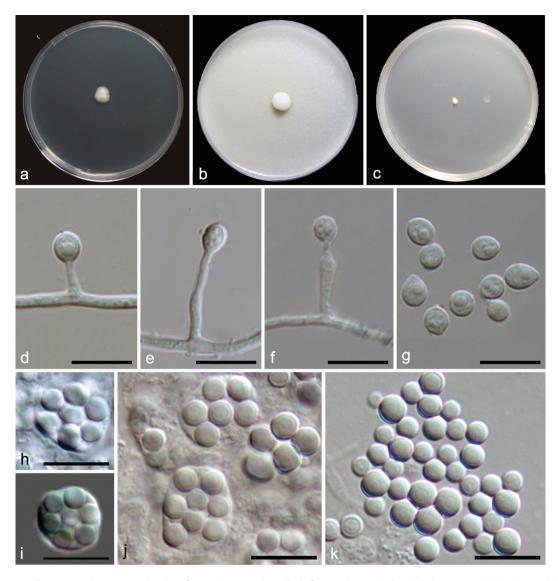
Type species: Xizangia sedimenticola F. Liu, S. Song & L. Cai, sp. nov.

Xizangia sedimenticola F. Liu, S. Song & L. Cai, sp. nov., Figure 14

Fungal name: FN571898.

Etymology: Referring to the substrate where the fungus was collected, sediment.

Description: Hyphae smooth, hyaline, septate, branched, 1–1.5 µm wide. Conidiophores arising



**Figure 11.** Pseudeurotium desertorum (a, d–g from LC19441; b–c, h–k from LC19712). (a–c) Colonies on PDA, OA, and MEA. (d–f) Conidiogenous cells and conidia. (g) Conidia. (h–j) Asci and ascospores. (k) Ascospores. Scale bars: d–k = 10 μm.

laterally from aerial hyphae, solitary, erect or curved, hyaline, reduced to conidiogenous cells, 0–2-septa, 8–  $42 \times 1-3~\mu m$ . *Conidia* hyaline, aseptate, smooth, solitary, globose or ovoid, 1–3 oil droplets, 2–2.5  $\mu m$  diam.

*Material examined*: China, Xizang, Siling Co, N 31.8011, E 88.7288, from lake sediment, Aug. 2021, J.E. Huang, HMAS 352883 (**holotype**), **ex-type living culture** CGMCC 3.27274 = LC19528 = SLCS12.

Notes: Based on the BLASTn searches of NCBI GenBank nucleotide database, the closest hits using the LSU+ITS, rpb2 and tub2 sequences of Xizangia sedimenticola are species in Chaetomiaceae such as Chaetomium spp., Stolonocarpus spp., and Botryoderma spp. (<90%); Stellatospora spp., Staphylotrichum spp., and

Madurella spp. (<78%); Acrophialophora spp., Myceliophthora spp., and Colleriella spp. (<80%), respectively. But it is phylogenetically and morphologically distinct from these genera. Based on our phylogenetic analysis, the monotypic species X. sedimenticola is located in the polyphyletic Lasiosphaeriaceae and is distinct from other genera (Figure 15).

# 4. Discussion

The lakes on the Tibetan Plateau are characterised by low temperatures, which provide a suitable environment for psychrophilic and psychrotrophic fungi. Both psychrophilic and psychrotrophic fungi

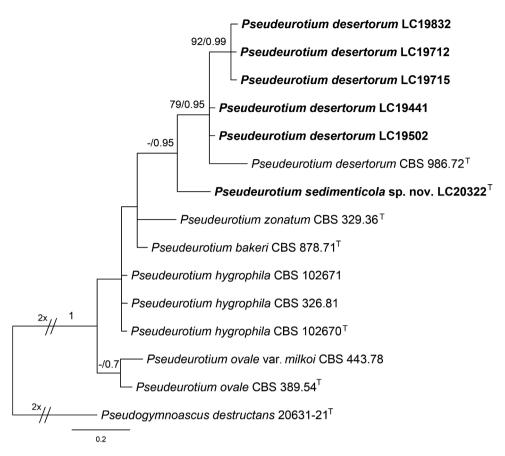
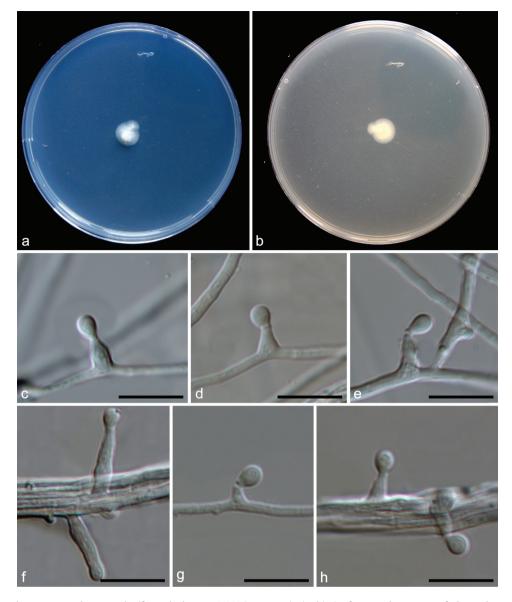


Figure 12. Maximum likelihood (ML) tree of *Pseudeurotium* based on ITS sequences. The tree topology of the ML analysis was similar to the BI. ML bootstrap values (≥50%) and Bayesian posterior probability (≥0.9) are indicated along branches (ML/PP). "T" indicates typederived sequences.

demonstrate the capability to thrive at 0 °C. Psychrophilic fungi exhibit an optimal growth temperature of approximately 15 °C or lower, with a maximum growth temperature of 20 °C or below. In contrast, psychrotrophic fungi have a maximum growth temperature that exceeds 20 °C (Morita 1975; Gounot 1986). To our knowledge, the diversity of cold-adapted fungi in lake sediments has been poorly studied. In an investigation of fungi obtained from soil and lake sediment in the Skarvsnes ice-free area, Antarctica, the coldadapted fungi were classified into five genera of Ascomycota (Embellisia, Phoma, Geomyces, Tetracladium, Thelebolus) and five genera of Basidiomycota (Mrakia, Cryptococcus, Dioszegia, Rhodotorula, Leucosporidium) (Tsuji et al. 2013). Interestingly, our study only obtained one species of Thelebolus from the above taxa, indicating a high degree of variability in fungal species found in different lake sediments.

Until now, research on culturable fungi in Xizang's lakes has been very limited, with most studies focusing on fungal diversity in Nam Co. The dominant fungal taxa isolated from water and sediments of Nam Co in our study differed from those previously reported by Xue et al. (2022a, 2022b). In Xue et al. (2022a, 2022b), the genera with the highest frequency of isolation from Nam Co water were Penicillium, Lecanicillium, Mucor, Alternaria, Fusarium, and Cladosporium. However, in our study, in terms of both the number of strains isolated and the frequency of taxa isolation, the prevailing fungi encompass not only ubiquitous environmental genera like Alternaria, Penicillium, and Aspergillus, but also taxa frequently isolated and documented in cold environments, such as glaciers, mangroves, oceans, or the exemplified by Cadophora Antarctic, Pseudeurotium (Tsuji et al. 2013; Zhang et al. 2022). This finding suggests that the culturable



**Figure 13.** *Pseudeurotium sedimenticola* (from holotype HMAS 352882). (a–b) Surface and reverse of the colony on PDA. (c–h) Conidiophore and conidia. Scale bars:  $c-h = 10 \mu m$ .

fungal diversity in Nam Co water is more diverse than previously thought.

It is well known that increased glacier ablation due to global warming and large inputs of glacier meltwater have altered the salinity and nutrients in the Tibetan Plateau lakes (Milner et al. 2009), simultaneously, microorganisms enter the lakes with the glacial meltwater. Thus, we suspect that some microorganisms in the lakes are of glacial origin. Combining the results of this study with the literature review, we find that *Cadophora* is one of the most common genera found in both marine glaciers (Zhang et al. 2022) and lakes (Table S1, Figure 1) in the Qinghai-Tibetan Plateau. To our

knowledge, this genus has been consistently isolated as plant pathogens or root colonisers from northern temperate regions or as decomposers from the cold Arctic and Antarctic locations (Blanchette et al. 2004, 2010, 2016; Gonçalves et al. 2012; Furbino et al. 2014; Nagano et al. 2017; Durán et al. 2019), and it possesses a notably elevated number of genes associated with stress tolerance and resource uptake (Egidi et al. 2019). In the present study, six *Cadophora* species were isolated and identified. Remarkably, four of these species exhibited less than 98% sequence similarity to known species in the NCBI database (Table S1), suggesting their potential as

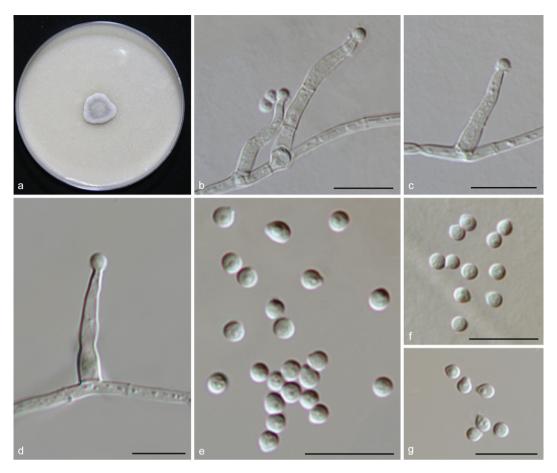


Figure 14. Xizangia sedimenticola (from ex-holotype CGMCC 3.27274). (a) Surface of the colony on OA. (b-d) Conidiophores and conidia. (e-q) Conidia. Scale bars:  $b-q = 10 \mu m$ .

new species. This discovery implies that cold environments might harbour a greater diversity of Cadophora species. Additional research is imperative to verify whether these species originated from glaciers or were transported into the lake through glacial meltwater.

Moreover, many fungi obtained in this study are plant-pathogenic. For instance, Fusarium verticillioides, a fungal species most frequently documented for infecting maize (Zea mays) and known for its production of mutagenic chemical compound fusarin C (Satterlee et al. 2022), has been isolated at a high frequency from sediment samples collected from Gyaring Co and Nam Co. Some other common plant and human pathogenic fungi isolated in this study include Fusarium fujikuroi (Yilmaz et al. 2021), F. oxysporum (Dean et al. 2012), and Truncatella angustata (Jagielski et al. 2015), etc. Moreover, this study isolated several common phytopathogenic fungal genera, including Didymella, Alternaria, Fusarium, Fusicolla,

Neonectria, Paraphoma, Plectosphaerella, Stagonospora (Table S1), with some species exhibiting less than 99% ITS similarity to the known species. Considering the pronounced diversity within rapidly differentiating phytopathogenic fungal taxa of these genera, even strains with 99% ITS similarity may represent different species (Hou et al. 2020; Han et al. 2023). In other words, they are likely to be emerging or newly discovered phytopathogenic fungi. For example, Myceliophthora tibetea sp. nov. shares 99.03% ITS sequence similarity with the known species Myceliophthora lutea, but based on multigene phylogenetic analyses and comparisons of morphological characters, we find that they are closely related but separate species (Figure 6). Hence, it is imperative to accurately distinguish these cryptic species and assess their pathogenicity in upcoming studies, especially concerning economically important local plant species in Xizang. This evaluation is vital for understanding the potential risks they pose and for implementing



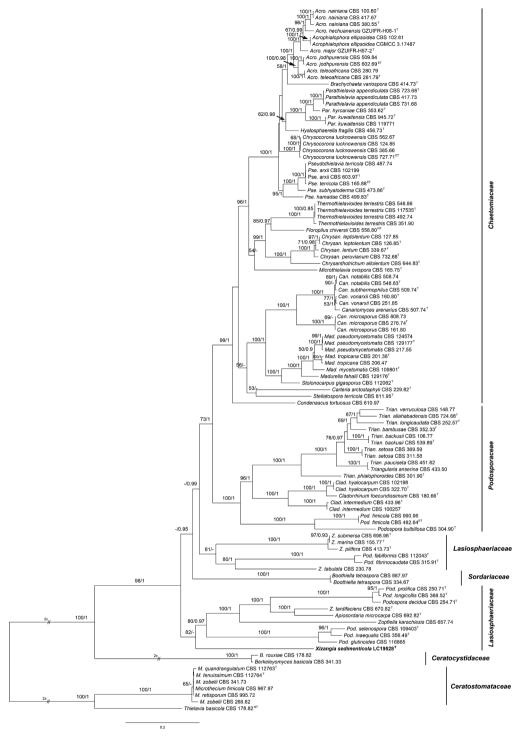


Figure 15. Phylogenetic tree resulting from maximum likelihood (ML) analysis of the concatenated partial rpb2, tub2, ITS, and LSU gene region alignment. Tree topology of the ML analysis was similar to the Bl. ML bootstrap values (≥50%) and Bayesian posterior probability (≥0.9) are indicated along branches (ML/PP). Type strains are marked with "T" (type), "ET" (epitype) or "NT" (neotype) after the culture number. The scale bar shows the expected number of changes per site.



monitoring and alert systems for these potentially pathogenic fungal species in the ecologically sensitive region of Xizang.

# 5. Conclusions

In conclusion, our examination of the culturable mycobiota in six lakes revealed a rich fungal diversity and a substantial level of species novelty, with the formal description of eight new taxa in this study. Additional potentially new taxonomic units will be documented in subsequent studies, accompanied by analyses of the evolutionary origins of taxa exhibiting distinctive distributions in this environment. Future research endeavours should integrate cultureindependent methods to more comprehensively elucidate the overall landscape of fungal diversity, community compositions, and potential ecological roles of fungi in the lakes of Xizang.

### Disclosure statement

No potential conflict of interest was reported by the author(s).

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### **Author contributions**

F.L. and D.P. conceived the project. D.P., J.H., and Z. N. contributed to the sample collection. S.S., J.H., F.L., and D.P. performed lab experiments and data analyses. J.H. and S.L. contributed to the deposition of sequences and type specimens. F.L. is mainly responsible for drafting and revising the manuscript, with assistance from D.P., J.H., L.C., and X.Z. in manuscript revision.

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