IMA Fungus

IMA Fungus 17: e177912 (2026)
DOI: 10.3897/imafungus.17.177912

Research Article

Cryptocalciella – a new *Mortierellaceae* genus from Alpine glacier forefields

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Abstract

Mortierellaceae are cosmopolitan, soil-inhabiting fungi that can be found in nearly all terrestrial habitat types and are therefore considered an essential part of the core soil microbiome. Many species of this family are known to endure harsh environments, including highly exposed and nutrient-depleted terrains such as glacier forefields. In these environments, microbial communities are taxonomically and functionally diverse, greatly contributing to nutrient cycling, soil organic matter formation, and plant establishment. However, there is growing understanding that *Mortierellaceae* diversity in these habitats remains largely undescribed.

In this study, we isolated multiple fungal strains belonging to a previously unknown *Mortierellaceae* taxon from early stages of soil development in calcareous glacier forefields of the Alps and comprehensively characterized them using different tools: physiological tests, detection of associated bacteria, and microscopic observations (e.g., light, fluorescence, and scanning electron microscopy) to visualize their morphology and surface structure. Additionally, whole-genome sequencing and phylogenomics were used to determine their placement within *Mortierellaceae*.

Our results show that the isolated strains represent a new species within a previously undescribed fungal genus. Due to the strains' origin in hidden, calcareous sediments of the earliest soil developmental stages at glacier forefields, we propose the name *Cryptocalciella humilis* Mandolini, Szedlacsek & Peintner for this fungus.

Key words: Alpine environment, bacterial associations, calcareous glacier forefields, early stages of soil development, fungal diversity, phylogenomics, whole-genome sequencing

Academic editor: César Marín
Received: 14 November 2025
Accepted: 14 January 2026
Published: 5 March 2026

Citation: Mandolini E, Szedlacsek S, Abramczyk B, Szucs A, Staykova A, Seybold A, Knapp M, Pawłowska J, Ladurner P, Peintner U (2026) *Cryptocalciella* – a new *Mortierellaceae* genus from Alpine glacier forefields. IMA Fungus 17: e177912. https://doi.org/10.3897/imafungus.17.177912

Introduction

Mortierellaceae are easily culturable fungi forming white, cottony mycelial colonies, with a typical zonate or rosette-like pattern. Many *Mortierellaceae* species are known to produce distinct smells (Wagner et al. 2013) and reproduce asexually by producing sporangiophores and sporangia. Some members have

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also been observed to reproduce sexually in form of zygospores. The first *Mortierella* species (*Mortierella polycephala* Coem.) was isolated from a mushroom and described by Coemans (1863) within *Mucoraceae*. The family *Mortierellaceae* was introduced within *Mucorales* by Luerssen (1879). In the subsequent decades, the taxonomy of this group was repeatedly modified; new genera and species were described, redefining the understanding of its diversity (e.g., Linnemann 1941). The first systematic revision of the family was prepared by Gams (1977), who proposed nine sections based on morphology: *Actinomortierella*, *Alpina*, *Haplosporangium*, *Hygrophila*, *Mortierella*, *Schmuckeri*, *Simplex*, *Spinosa*, and *Stylospora*. The first molecular phylogenetic studies of *Mortierellaceae* were carried out by Nagy et al. (2011) and Wagner et al. (2013). The phylogeny was reconstructed based on rDNA ITS and LSU sequences obtained from type materials, and the family was subdivided into seven clades, partially overlapping with the system proposed by Gams (1977). However, the authors refrained from introducing significant taxonomic changes. More recently, Vandepol et al. (2020) revised the family using multi-gene phylogenetics based on low-coverage genome sequencing and proposed several new genera for *Mortierella* s.l., e.g., *Podila* Stajich, Vandepol & Bonito, *Linnemannia* Vandepol & Bonito, and *Entomortierella* Vandepol & Bonito. Due to the vast *Mortierellaceae* diversity, not all species known at the time were included in this study; thus, not all valid *Mortierella* species epithets were recombined into the respective, updated genera. Moreover, since the 2020 revision, several new taxa have been described (e.g., Telagathoti et al. 2022; Crous et al. 2023; Mou and Bau 2024). Currently, the family *Mortierellaceae* includes 22 genera and 281 species names registered in MycoBank (accessed 04 July 2025), but due to inconsistencies in the approaches used to introduce taxonomic changes, many of the currently recognized genera within the family remain polyphyletic.

Mortierellaceae fungi are well-known soil saprotrophic generalists colonizing a wide range of habitats. The rapid development of eDNA sequencing methods has recently revealed high *Mortierellaceae* diversity in various habitats (Tedersoo et al. 2014). Although preliminary studies suggested that nearly all *Mortierellaceae* species were most likely already described (Nagy et al. 2011), several new species and even a new genus were recently described from alpine habitats (Telagathoti et al. 2022), suggesting that these habitats can still harbor unknown fungal diversity. *Mortierellaceae* were recently reported from snow mats (Schmidt et al. 2008) and from recently deglaciated barren ground in glacier forefields (Cazzolla Gatti et al. 2018; Dresch et al. 2019). Due to their high abundance and diversity in alpine habitats (Dresch et al. 2019; Telagathoti et al. 2021), *Mortierellaceae* are considered pioneer organisms in soil microbial communities, and they appear to be crucial for soil organic matter build-up. This role is particularly pronounced at sites where the activity of other organisms is often limited due to low temperatures and nutrient limitation. As alpine habitats are highly endangered due to their rapid disappearance resulting from global warming (Schwager and Berg 2019), the isolation, characterization, and preservation of undescribed *Mortierellaceae* diversity from these locations is needed.

This group of fungi has also been shown to commonly harbor endohyphal (Bonfante and Desirò 2017; Uehling et al. 2017; Desirò et al. 2018; Takashima et al. 2018) or epihyphal-associated bacteria, which appear to be very important in alpine habitats (Telagathoti et al. 2021). In natural habitats,

the interactions between fungi and bacteria are diverse, affecting both organisms' growth, movement, and physiology, including stress resistance (Deveau et al. 2018). While endohyphal interactions occur with putatively obligate endohyphal bacteria, such as *Mycoavidus* (Ohshima et al. 2016), epihyphal associations occur with different bacterial lineages (Telagathoti et al. 2021). Bacterial endosymbionts have been shown to protect hosts from mycophagous nematodes (Büttner et al. 2021) and to provide protection to associated plant roots, as reported for *M. globalpina* W. Gams & Veenbaas-Rijks (DiLegge et al. 2019). Furthermore, epihyphal bacterial associations were shown to increase with higher altitudes, suggesting that these interactions may confer a competitive advantage in these environments (Telagathoti et al. 2021). Moreover, the influence of bacteria on the growth and volatilome of the fungal host has been demonstrated (Probst et al. 2023). Due to the high prevalence of interactions with bacteria in the *Mortierellaceae* family, and the impact these interactions can have on host physiology, the detection and description of these bacteria should be considered in physiological studies.

The main goal of this paper is to characterize and formally describe a new *Mortierellaceae* fungus isolated from early stages of soil development in calcareous glacier forefields of the Alps. A combination of culture-based techniques, physiological tests, different types of microscopic observations, molecular detection of interacting bacteria, whole-genome sequencing, and phylogenomic analysis are combined to provide the most comprehensive characterization of the isolates. Moreover, four additional *Mortierellaceae* genomes were sequenced using Oxford Nanopore technology to provide a better representation of the genetic diversity of the family. Our species concept is based on a polyphasic approach, which is applied to delimit the newly proposed genus and the new species from other *Mortierellaceae* taxa. Differences in both phylogenetic or phylogenomic distance and morphological characters of fungal isolates are considered the minimum and essential criteria for taxon delimitation within this fungal lineage.

Materials and methods

Isolation and cultivation

Soil samples from two glacier forefields of the European Alps were collected: Dachstein (AT, 2250–2700 m a.s.l.) and Marmolada (IT, 2760 m a.s.l.). These glacier forefields are characterized by calcite [$CaCO_3$] or dolomite [$CaMg(CO_3)_2$] bedrock, with minimal to no vegetation and low amounts of soil organic material. Soils mainly had a coarse and sandy texture. A detailed description of the investigated sites, the sampling method, and soil properties is provided by Mandolini et al. (2025).

A total of 10 soil samples, five from each glacier forefield, were considered. For isolation, a few soil grains were directly plated on PDA (Suppl. material 2: table S1) and LCA medium (Suppl. material 2: table S2) (Takashima et al. 2019) and incubated for 6–8 days at 10 °C. To obtain pure cultures, colonies morphologically resembling typical *Mortierellaceae* phenotypes (white, cottony mycelial colonies with a typical zonate or rosette-like pattern) were picked, replated, and incubated again for 7–10 days at 10 °C.

Isolate M1D2 was chosen as the type strain (IBF20210222) and is referred to as *Cryptocalciella humilis* throughout the manuscript. This strain was used for all examinations described below (phylogenomic analysis, physiological testing, microscopy, and stress assays) either on PDA or in potato dextrose broth (PDB). To further evaluate the morphological variability within *C. humilis*, an additional strain (IBF20210223) was microscopically examined. For cultivation in PDB, a layer of sterile glass beads was provided as a structural surface for mycelial growth. In cases where the fungus needed to be cured of its associated bacteria, media were supplemented with streptomycin sulfate (0.1 g/L; Carl Roth, Germany) and tetracycline (0.05 g/L; Carl Roth, Germany).

Two reference cultures (M1D2 and D4A4) were deposited at the Westerdijk Fungal Biodiversity Institute, Utrecht (CBS). Additionally, cryocultures in 20% skim milk and 50% glycerol are stored at −80 °C at the Department of Microbiology of the Universität Innsbruck (Innsbruck, Austria).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from pure fungal isolates to amplify the rDNA ITS region and to detect co-occurring bacteria (see below). The rDNA ITS sequence was selected for initial species identification, since most species within the *Mortierellaceae* family can be sufficiently distinguished using this marker region (Wagner et al. 2013; Telagathoti et al. 2022). A small fragment of mycelium from 5-day-old cultures was suspended in 100 µL sterile PCR-grade water, homogenized with a synthetic pestle, and heated at 85 °C for 15 minutes. The suspension was then frozen at −20 °C for later use or directly used for amplification (Walch et al. 2016). The rDNA ITS region was amplified using standard primers ITS1 and ITS4 (White et al. 1990) (Suppl. material 2: table S8). Unidirectional Sanger sequencing of the rDNA ITS amplicons was performed by MicroSynth AG (Balgach, Switzerland).

In addition, LSU and RPB1 genes of four selected strains (M1D2, M7B40, M7B35, D4A5) were amplified and sequenced to further assess genetic variability within *C. humilis*. The four isolates were selected based on single-nucleotide variations in their sequences and differences in their locations of origin. The LSU was amplified using the primer pair LR0R and LR5 (Hopple Jr. and Vilgalys 1994) (Suppl. material 2: table S9). RPB1 sequences were obtained using a touch-up method (Visagie et al. 2014) with primers RPB1f and RPB1r (Vandepol et al. 2020) (Suppl. material 2: table S10). Bidirectional Sanger sequencing of LSU and RPB1 amplicons was performed by MicroSynth AG (Balgach, Switzerland). Distance matrices of the rDNA ITS and RPB1 regions were generated to compare genetic variability among *C. humilis* strains.

Sequence quality was checked using Geneious Prime 2023.2.1. Sequence ends were trimmed to remove low base-call-quality nucleotides. Sequences of low quality (e.g., inconsistent or unclear signal peaks throughout a significant portion of the sequence) were discarded. Final consensus sequences were submitted to GenBank (Suppl. material 2: table S12). A detailed description of the phylogenetic analysis based on rDNA ITS sequences of all obtained isolates is provided by Szedlacsek et al. (2025).

Whole genome sequencing and assembly

The genome of *Cryptocalciella humilis* M1D2 was extracted, sequenced, and assembled to investigate gene content and to perform phylogenomic placement of the new taxon. In addition, ex-type cultures of four further species were selected for whole-genome sequencing to better represent genetic diversity within the family: *Entomortierella ferrotolerans* Abramczyk & Nowak (CBS 151316), *Bonitomyces buffelskloofinus* Crous (CBS 150057), *Linnemannia biramosa* (Tieghem) Telagathoti, Probst & Peintner (CBS 370.95), and *Gamsiella stylospora* (Dixon-Stewart) Vandepol & Bonito (CBS 211.32).

For *C. humilis*, DNA extraction was performed from cultures grown in PDB supplemented with antibiotics (streptomycin sulfate 0.1 g/L and tetracycline 0.05 g/L) for 7 days at 16 °C. Mycelium was washed with PCR-grade distilled water and disrupted by one cycle of 30 seconds using a Precellys Evolution homogenizer with micro glass beads (Precellys Lysing Kit CK28). High-molecular-weight DNA was obtained using a CTAB extraction protocol. All pipetting steps were performed as gently as possible, and vortexing was avoided and replaced with gentle flicking to minimize DNA fragmentation. Genomic DNA libraries were prepared using the Ligation Sequencing Kit (SQK-LSK109) and sequenced on a PromethION R9 flow cell. Data were base-called with Dorado 0.9.1 (https://github.com/nanoporetech/dorado) using the high-accuracy model. Reads were preprocessed with Chopper v0.9.1 (De Coster and Rademakers 2023), trimming 50 bp from each end. Only reads longer than 15,000 bp with a minimum average quality score of Q14 were retained for downstream analyses. Genome assembly was performed using Flye v2.9.5 (Kolmogorov et al. 2019), and quality assessment of the final contigs was conducted using BUSCO v5.7.1 (Seppey et al. 2019). Short contigs (<5000 bp) were removed prior to genome annotation. Annotation was performed using funannotate v1.8.17 (Palmer and Stajich 2020). Signal peptide prediction was conducted using SignalP v6.0 (Teufel et al. 2022), and secondary metabolite biosynthetic gene clusters were identified using antiSMASH v8.0 (Blin et al. 2025). Raw reads and annotated genome sequences were deposited in NCBI under BioProject PRJNA1272301.

For *E. ferrotolerans*, *B. buffelskloofinus*, *L. biramosa*, and *G. stylospora*, cultures were grown in PDB supplemented with ciprofloxacin (0.1 g/L). Cultures were centrifuged, rinsed with PCR-grade distilled water, frozen at −80 °C, and ground in liquid nitrogen. Genomic DNA was extracted using the Bacterial & Yeast Genomic DNA Purification Kit (EURx). Libraries were prepared using the Ligation Sequencing Kit v14 (Oxford Nanopore Technologies) and sequenced on a Nanopore MinION device using an R10.4.1 flow cell; an R9.4.1 flow cell was used for *E. ferrotolerans*. Basecalling was performed using Dorado 0.9.1 (https://github.com/nanoporetech/dorado) with the high-accuracy model. Raw-read quality was assessed using FASTQC v0.12.1 (https://github.com/s-andrews/FastQC). Reads were trimmed and filtered based on quality assessment using Filtlong v0.2.1 (https://github.com/rrwick/Filtlong) and FastP v0.23.2 (Chen 2023). Genome assemblies were generated using Flye v2.9.5 (Kolmogorov et al. 2019), and BUSCO v5.7.1 assessments were performed for all assemblies (Seppey et al. 2019). Assembled genomes and raw reads for *E. ferrotolerans*, *B. buffelskloofinus*, *L. biramosa*, and *G. stylospora* were deposited in NCBI under BioProject PRJNA1285402. Assembly accession numbers for all genomes are provided in Suppl. material 1.

Phylogenomic analysis

All *Mortierellaceae* genomes available in the GenBank NCBI database were downloaded. The taxonomic placement of isolates was consulted using materials provided by Vandepol et al. (2020). A preliminary maximum likelihood phylogenomic tree was inferred using the Universal Fungal Core Genes (UFCG) pipeline (Kim et al. 2023). The final tree was calculated using the GTR+G4 substitution model and based on a concatenated alignment of 62 molecular markers (147,499 characters; 96,870 parsimony-informative sites) extracted from 69 genomic sequences. Based on these results, a representative subset of genomes was selected. Genomes of isolates not identified to species level were discarded. Metadata for all genomes used in the final phylogenomic analysis are available in Suppl. material 1.

As publicly available *Mortierellaceae* genomes were shown to contain *Mycoplasma*-related endohyphal bacterial sequences (Longley et al. 2024), all genomes were cleaned of bacterial contamination using Tiara v1.0.3 (Karlicki et al. 2022). The minimum sequence length was set to 1000 bp. All contigs classified as mitochondrial, eukaryotic, or unknown were selected for further analysis. The phylogenomic tree was inferred using the UFCG pipeline (Lee et al. 2022). Nucleotide sequences were aligned with MAFFT (Katoh and Standley 2013). Multiple-copy orthologs were included in the analysis together with the rDNA ITS region. A maximum likelihood phylogenetic tree was inferred using RAxML (Stamatakis 2014). The concatenated alignment was then extracted and used for Bayesian inference analysis in MrBayes v3.2.7 (Ronquist et al. 2012), installed on a server with an AMD EPYC 7713 64-core processor. For both analyses, the GTR+G4 substitution model was applied. Files containing the concatenated alignments used to calculate the trees are available in a GitHub repository (https://github.com/rainbowmycelium/UFCG_alignments).

Morphological description and microscopy

For macro- and microscopic characterization, *Cryptocalciella humilis* was grown on PDA (Suppl. material 2: table S1), LCA (Suppl. material 2: table S2), and soil extract agar (SEA; Suppl. material 2: table S3) for a minimum of 7 days at 4 °C, 10 °C, and 16 °C.

All images for macroscopic characterization were taken using a Canon EOS R10 (model DS126842), custom white-balanced, and adjusted for brightness. Image editing was performed using GIMP v3.0.4 and Inkscape v1.4.

Pure cultures were further examined using light microscopy to characterize sterile elements (i.e., hyphal morphologies) and reproductive structures (i.e., sporangia and spores). A small portion of the mycelium was picked with a sterile needle and mounted on a microscope slide using water, cotton blue, or Melzer's reagent. Microscopic images for measurements and characterization were obtained using a Nikon Eclipse E600 microscope with an Imaging Source camera lens (DFK38UX267) and a Nikon Optiphot-2 microscope with a Nikon DS-Fi3 camera. A minimum of 20 measurements were carried out for characterization of sporangiophore and sporangium morphologies. A stereo magnifier Nikon SMZ800 equipped with a Nikon DS-Fi3 camera was used to visualize entire bundles of sporangiophores.

To determine whether the observed bulbous gemmae within the mycelium contained genomic material (e.g., DNA), DAPI staining was performed, and stained material was visualized using fluorescence microscopy. Briefly, a small fragment of the mycelium was picked with a sterile needle and mounted on a microscope slide. Then, 300 µl of DAPI solution (30 µl of 1 mg/µL stock concentration and 270 µl PBS) was added, and the material was incubated in the dark at room temperature for 10 minutes. The material was rinsed three times with PBS and visualized using an inverted fluorescence microscope (Nikon Eclipse Ti-2). All microscopic images were visualized and edited using NIS-Elements D and GIMP v3.0.4 and processed with Inkscape v1.4.

To further investigate the characteristics of the bulbous gemmae and their complex placement within the hyphal network, scanning electron microscopy (SEM) was performed. Ten-day-old mycelial material from cultures grown on PDA was transferred onto pieces of 0.2 µm membrane filter paper (Merck, Darmstadt, Germany). Filter paper pieces were mounted on 12.7 mm SEM stubs (Ted Pella, Redding, CA, USA) using 12 mm carbon tabs (Science Services, München, Germany) and sputter-coated with 20 nm gold (Safematic, Zizers, Switzerland). Prepared samples were loaded and imaged using a Tescan Clara SEM (Tescan, Brno, Czechia).

Definition of terms in *Mortierellaceae*: gemmae and chlamydospores

Gemmae are small hyphal outgrowths (often globose and hyaline) produced on substrate or aerial hyphae, which may detach or remain clustered and may serve as clonal propagules; they occur in dense or loose clusters or chains. They are usually thin-walled and often not separated by septa from the coenocytic mycelium.

Chlamydospores are thick-walled, asexual propagules formed by modification of vegetative hyphae. They are often separated by septa from the rest of the thallus. They function primarily as survival structures, allowing persistence under unfavorable environmental conditions, and may also contribute to dispersal (Gams 1977; Wagner et al. 2013; Benny et al. 2016).

Physiological assays

Growth of *Cryptocalciella humilis* was tested under different temperature, pH, and salinity conditions. Initially, an agar plug of approximately 2 × 2 mm was cut from pure cultures and transferred to the center of assay PDA plates. For the temperature assay, growth was tested by incubating cultures for at least 7 days at 4 °C, 10 °C, 16 °C, 20 °C, 25 °C, and 30 °C. For the pH assay, growth was tested on PDA medium adjusted to different pH levels (pH 2, pH 4, pH 6, pH 8, pH 10, and pH 12). The pH was adjusted using HCl (1 M) or NaOH (3 M), respectively. Plates were incubated for 7 days at 16 °C. The salinity assay was performed by adding NaCl at concentrations of 0%, 1%, 2%, and 3%. Plates were incubated at 16 °C for 7 days. Growth was documented by photographing the plates from day 3 through day 7.

Different nutrient assays were conducted to assess the metabolic potential of *C. humilis*, namely chitin-solubilizing enzyme activity, protease activity, phosphate-solubilizing activity, and degradation of different carbon sources. Pre-cultures were grown in PDB for 7 days at 16 °C. Small mycelial pieces were then

collected, rinsed with sterile water to avoid transfer of dextrose, and inoculated onto assay plates. All plates were incubated for 14 days at 16 °C. To test for chitinolytic activity, chitin agar plates were prepared with bromocresol purple as a pH indicator (Agrawal and Kotasthane 2012) (Suppl. material 2: table S4). Plates were adjusted to pH 5.1. If the organism possessed chitin-solubilizing enzymes, the agar turned purple as a result of bromocresol purple responding to pH changes. A fungal strain belonging to the genus *Beauveria* was used as a positive control (Segura-Vega et al. 2024). Protease activity was assessed using casein agar plates (Suppl. material 2: table S5) (Aneja 2007). To test the enzymatic ability to utilize alternative carbon sources, C-source agar plates were prepared by adding one of three carbon sources: carboxymethyl cellulose, pectin, or starch (Suppl. material 2: table S6) (Aneja 2007). After sufficient growth was observed, the agar surface was treated with Lugol's iodine solution (6.7 g/L potassium iodide, 3.3 g iodine, 1 L distilled water) to visualize halos resulting from carbon source degradation. Phosphate-solubilizing activity was assessed using modified Pikovskaya agar plates (Suppl. material 2: table S7) (Rao and Sinha 1963; Subba-Rao 1977).

Growth was documented by photographing plates with a Canon EOS R10. Measurements of radial mycelial growth were performed on images by marking colony edges using the polygon tool in ImageJ v1.54 and calculating Feret's diameter. All assays were conducted in triplicates.

Screening for associated bacteria

Screening for fungus-associated bacteria was based on DNA extracts obtained from pure fungal cultures. For bacterial detection, primers 8f and 1492r (Uehling et al. 2017) were used to amplify the 16S rRNA gene (Suppl. material 2: table S11). All amplicons were sequenced using unidirectional Sanger sequencing (Microsynth AG, Balgach, Switzerland).

Results

A total of 44 *Cryptocalciella humilis* pure cultures were obtained (Suppl. material 2: table S12), with 19 and 25 isolates derived from Marmolada and Dachstein, respectively. Genetic variability among different *C. humilis* strains was detected within and across the two glacier forefields (Suppl. material 2: table S13).

Phylogenomic analysis

Quality assessment of the sequenced *Mortierellaceae* genomes showed BUSCO scores above 98%. Detailed BUSCO results, together with sequencing coverage, genome sizes, and numbers of contigs obtained after assembly, are provided in Suppl. material 2: table S14.

Phylogenomic analyses using RAxML and MrBayes produced trees with identical topologies. Posterior probabilities for all clades in Bayesian inference equaled one. Maximum likelihood analysis showed some uncertainty in phylogenetic placement within the *Mortierella alpina* clade. Furthermore, the overall results revealed that several genera within *Mortierellaceae* remain polyphyletic, including *Linnemannia* Vandepol & Bonito and *Entomortierella* Vandepol & Bonito. The phylogenomic position of *Cryptocalciella humilis* was congruent and

highly supported in both RAxML and MrBayes analyses. The analysis clearly showed that *C. humilis* belongs to *Mortierellaceae* (Fig. 1). The phylogenomic tree is available as a nexus file in Suppl. material 3. Sequences derived from *C. humilis* formed a lineage with *Modicella* Kanouse, *Benniella* Vandepol & Bonito, and *Gamsiella* (R.K. Benj.) Benny & M. Blackw., with a well-supported sister-group relationship to *Gamsiella*. *Dissophora* Thaxt. and *Bonitomyces* Crous represented the closest related lineage to this clade.

Taxonomy

Cryptocalciella Mandolini, Szedlacsek & Peintner, gen. nov.

MycoBank No: 859718

Etymology. The name *Cryptocalciella* derives from Greek *κρῠπτός* (hidden, concealed), and *χάλιξ* (pebble), later Latin *calx* (limestone). It refers to the concealed physiological abilities of the type species of this genus and to its ability to live hidden in calcareous sediments of the earliest soil developmental stages at glacier forefields. The ending "-ella" should provide a link to the family *Mortierellaceae*.

Diagnosis. Phylogenomic (high coverage genome) and phylogenetic (rDNA) analyses clearly identify *Cryptocalciella* as a distinct lineage in the *Mortierellaceae*, with a sister-group relationship to *Gamsiella* (Fig. 1). *Cryptocalciella* pure cultures produce abundant mycelium on PDA and a large amount of intercalar and terminal swollen, bulbous gemmae. Sporulation occurs on minimal media (LCA or SEA) with large, unbranched sporangiophores resembling a crystal chandelier producing spherical sporangia with a columella. Smooth, ovoid to phaseoliform sporangiospores have a hyaline episporal layer.

Type species. *Cryptocalciella humilis* Mandolini, Szedlacsek & Peintner.

Generic description. *Cryptocalciella* species are characterized by fast-growing colonies and the formation of typical, bulbous gemmae formed in clusters in the mycelium (Fig. 3). They produce large, unbranched sporangiophores originating in bundles, thus resembling crystal chandeliers; at the sporangiophore tip, spherical sporangia with a columella are produced, which contain smooth, ovoid to phaseoliform sporangiospores surrounded by a hyaline, episporal layer (Fig. 4). The nutrient-poor habitat and the psychrotolerance discriminate it clearly from similar genera producing large amounts of gemmae, e.g., *Benniella*. Species belonging to the sister-group *Gamsiella* are easily distinguished based on sporangiophore morphology.

Cryptocalciella humilis Mandolini, Szedlacsek & Peintner, sp. nov.

MycoBank No: 859719

Etymology. From Latin *humilis* (low, humble)—referring to the nutrient-poor, cold habitat where this species originated from and the low nutrient requirements this species has. Furthermore, the name indicates this species' courtesy, which allows it to carry selected bacteria on its hyphae. This enables it to potentially achieve something greater together: colonizing these difficult habitats and making them habitable for others.

Figure 1. Maximum likelihood phylogenomic tree of the *Mortierellaceae* family inferred using the UFCG pipeline. All bootstrap support values are shown on the branches. The new genus is shown in bold and highlighted in red. Sister-clade genera are highlighted in two different colors (green and gray). Sequences derived from ex-type strains are marked with "T". Genus names shown on the right follow Vandepol et al. (2020). Genomic sequences originating from Vandepol et al. (2020) are marked with asterisks. The tree was artificially rooted using *Umbelopsis isabellina* (Oudem.) W. Gams and *Umbelopsis* sp.

Diagnosis. *Cryptocalciella humilis* colonies are fast-growing (PDA, 20 °C), mycelia develop abundant clusters of terminal and intercalar bulbous gemmae. Large, unbranched sporangiophores are formed in bundles, resembling a crystal chandelier, sporangiophores (262) 373–758 (1107) × (28) 37–57 (76) µm, tapered below the tip with a slightly pronounced apophysis and collarette after spore liberation. Each sporangiophore produces an apical, spherical multispored sporangium; sporangiospores smooth, ovoid to phaseoliform, (4.8) 7.6–10.6 (16.7) × (3.2) 4.2–5.9 (10.7) µm, with a hyaline episporal layer. pH-optimum 8–10, no growth at 25 °C and above.

Typus. Italy, Trentino, Marmolada glacier forefield, Mesozoic latemar limestone and dolomite sandy-rocky sediments with no or scattered vegetation cover (*Arabis alpina* L., *Arabis caerulea* Haenke, *Cerastium uniflorum* Thom. Ex. Rchb., *Poa minor* Gaudin) 46.441896, 11.859656, 2760 m a.s.l., 18. Aug. 2021, soil collected by E. Mandolini (Mandolini et al. 2025) (holotype IBF20210222, culture ex-type CBS 154075, GenBank Acc. No. ITS PV434959).

Description. Colonies fast growing, with an increase of 6.4–7.3 mm per day on PDA (20 °C) (Fig. 6), mycelium dense, white, with varying growth patterns ranging from foamy concentric rings to extended wave-like lobes to flowery shapes with varying rosette sizes (Fig. 2). Aerial mycelium usually not very pronounced. Cultures on poor media (LCA and SEA) with fast radial growth, but mainly in the substrate, with only fine and plain aerial mycelium and without elaborate growth patterns with the only exception on LCA where colonies develop a thin white ring of accumulated bulbous gemmae on the external colony margin, occasionally also the centre (Figs 2, 3). Colonies with a foul acidic odor over time. Hyphae coenocytic, usually without septa, hyphal diameter variable, ranging from 2.9–4.7 mm (n = 100), sometimes thinning out into very fine hyphae of 1–2 µm diam., hyphae single or as hyphal bundles, grow straight or in spiral form, and then resulting in dense, intermingled hyphal areas. Branching irregularly, formation of septa only in cases when the cytoplasm is resorbed and hyphae or branches persist as empty tubes, in this case often also with several consecutive septa, indicating a stepwise resorption of the content. Bulbous gemmae develop terminal on unseptated hyphae, very abundant, frequently branching, forming clusters of bulbs, predominantly formed on the surface of the colony, (11.9) 14.3–18.5 (22.5) × (11.5) 13.8–17.6 (21.2) µm (n = 100) (Fig. 3). Gemmae also formed intercalary (5.2) 10.6–16.0 (18.8) × (4.6) 10.7–15.7 (18.3) µm (n = 50). Both gemmae (intercalar and terminal) also occurring submerged in the agar, appearing after about one week, but latest after 14 days on PDA at 16 °C. Large areas of older cultures consisting of nest of bulbous structures only. Nuclei located irregularly in the mycelium and present also in the bulbous gemmae (Fig. 3). When hyphae with bulbous structures are abandoned, the bulb is left behind empty as well (Fig. 3). No detachment of bulbs detected. Oil-like vesicles in the cellular content observable in both vegetative hyphae and gemmae (intercalar and terminal), especially when grown on PDA. On LCA condensed and large nests of those bulbous structures can be located only in the white ring area of the colony. Sporulation observed after several months, once the cultivation temperature was lowered from 16 °C to 4 °C during the incubation period. Simple, erect, unbranched sporangiophores with (262) 373–758 (1107) µm (n = 39) in length, protruding from the substrate,

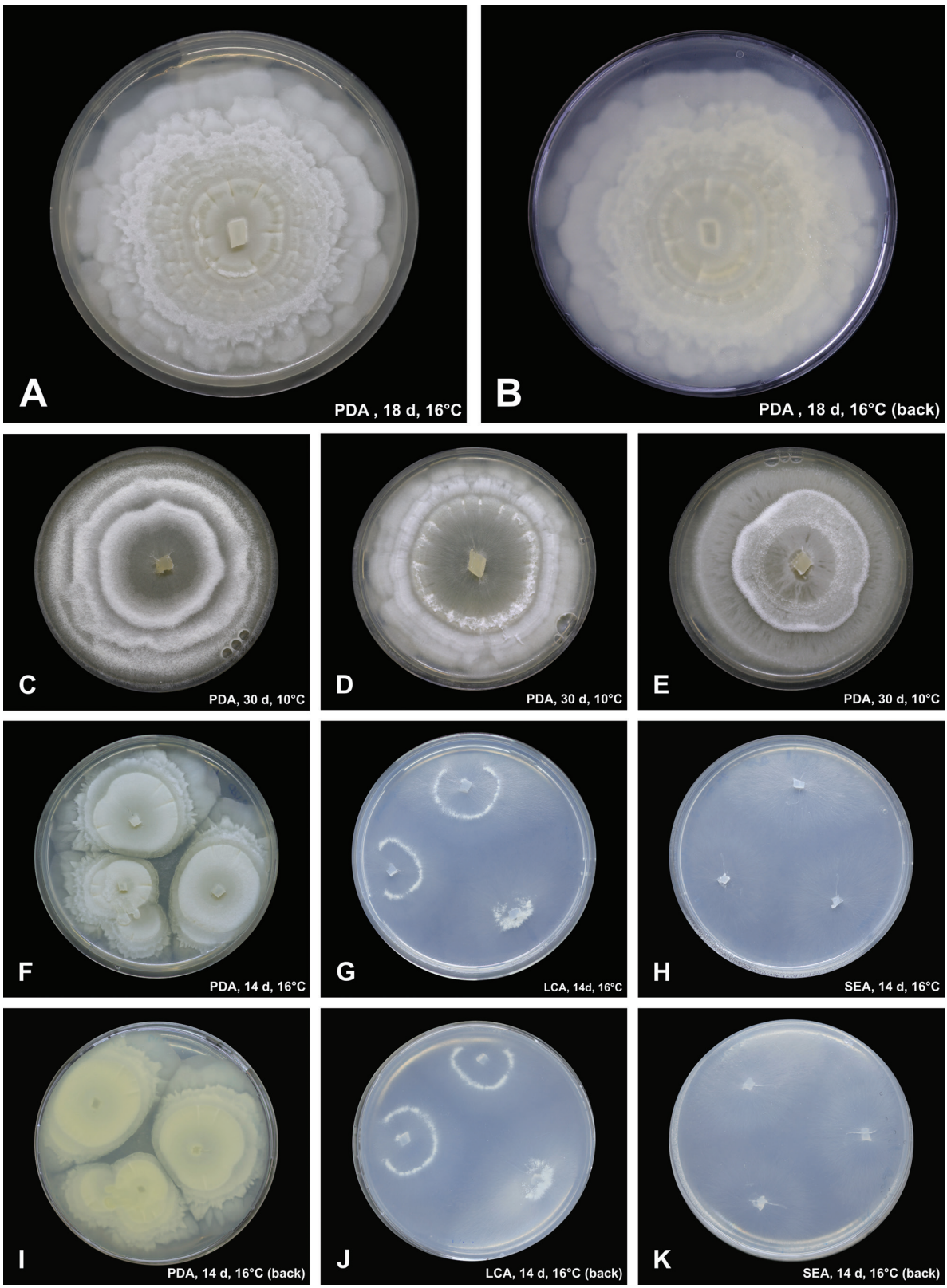

Figure 2. *Cryptocalciella humilis* in culture. **A, B** Ex-type strain (IBF20210222) of *C. humilis* on PDA. **C–E** Additional strains showing morphological variability of *C. humilis* on PDA; GenBank accession numbers of the rDNA ITS region of the depicted strains: **C** PV434964, **D** PV434965, and **E** PV434956. **F–K** Growth pattern of *C. humilis* (IBF20210222) on different media (PDA, LCA, and SEA); dense mycelial formation in various macromorphological forms occurs on PDA; no elaborate growth patterns were observed on nutrient-poor media, with the exception of a white ring area on LCA where concentrated nests of gemmae are produced. Diameter of all agar plates = 8 cm.

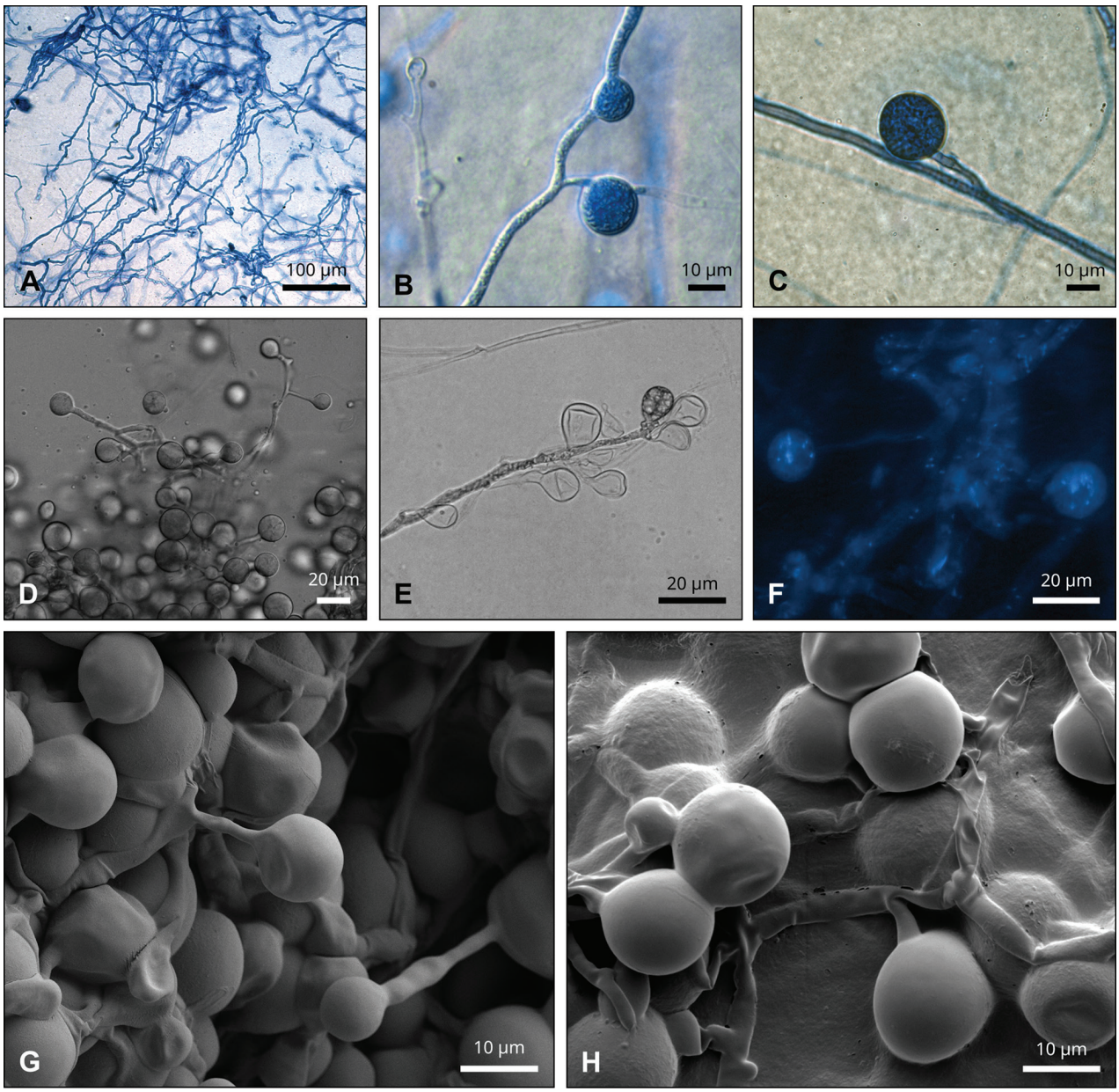

Figure 3. Microscopy images of *Cryptocalciella humilis*. **A** Mycelium at early stages of development; hyphae grow in coiled and spiral forms or aggregate (13 days; 4 °C, PDA). **B–H** Overview of gemmae in the mycelium; once bulb formation starts, these structures become very abundant. **B** Intercalary gemmae structures with adjacent empty hyphae and septa. **C** Terminal gemma structure. **D** A nest of bulbous structures from the white ring area of a colony on LCA. **E** Vital hypha filled with cytoplasm and empty terminal gemmae. **F** Mycelium and bulbs showing bright nuclei after DAPI staining. **G–H** SEM images showing nests of bulbous gemmae.

usually originating from nests of gemmae; formed in bundles with an appearance resembling a crystal chandelier or a flower bouquet (Fig. 5). Sporangiophore base reaching a diameter of (28) 37–57 (76) µm (n = 48), tapering to (17.0) 21.9–33.5 (42.7) µm (n = 53) below the tip, and ending in a slightly pronounced apical inflation (apophysis). Sporangia single, mostly spherical, with (78) 88–138 (167) µm (n = 21) diameter, ochraceous before maturation with a discreet columella (5.6) 9.2–19.7 (26.5) µm (n = 53) in height after spore liberation. Peridia deliquescing, leaving a conspicuous collarette after aperture, with (21.5) 31.5–52.0 (62.1) µm (n = 53) in diameter. Upon spore release,

masses of sporangiospores conflux into large drops (Fig. 4). Sporangiospores (4.8) 7.6–10.6 (16.7) × (3.2) 4.2–5.9 (10.7) µm (n = 211) in size, irregular, ovoid to phaseoliform, smooth, and surrounded by a hyaline episporal layer, often remaining in conjunction with other spores, giving the impression of having a slightly sticky surface, at least after the spore liberation (Fig. 4). Sporangiophores and sporangia sometimes encircled by solitary hyphae, leaving a net of hyphae as a cap after spore liberation. Both sporangia and spores are strongly cyanophil, sporangiophores appear dextrinoid with Melzer's reagent (iodide). Detailed drawings of *Cryptocalciella humilis* are presented in Fig. 5.

Growth requirements. Optimum temperature 16 °C–20 °C, with a daily radial increment of 6.4–7.3 mm. Growth was observed in a temperature range from 4 °C–20 °C. No growth at and above 25 °C. Optimum pH 8–10; daily radial increment of up to 8.6 (±0.1) mm (16 °C).

Additional strains examined. Austria, Upper Austria, Ramsau am Dachstein and Obertraun, sandy-rocky mesozoic limestones and dolomites sediments with rare patchy vegetation (*Arabis alpina*, *Arabis pumila*, *Cerastium uniflorum*, *Poa minor*, *Heliosperma pusillum* (Waldst. & Kit) Rchb., *Saxifraga aphylla* Sternb.) from the forefield of Hallstätter glacier forefront 47.48765, 13.618747, 2250–2700 m a.s.l., 03.07.2021, soil collected by E. Mandolini, and U. Peintner (Mandolini et al. 2025) (IBF20210223, CBS 154062, GenBank Acc. No. ITS PV435009).

Habitat and distribution. All isolates originate from calcareous glacier sediment at the earliest stages of soil development in glacier forefields (<20 years of ice-free sediment). The type specimen was cultured from barren ground close to the glacier ice front of the Marmolada Glacier in the Dolomites, Italy. Additional strains originate from the Hallstätter Ferner glacier in the Dachstein area, Austria. Based on data mining, *Cryptocalciella humilis* appears to be rare in other habitats.

Notes. This is currently the only representative of this genus, but it appears to be frequent in the two investigated, geographically distant calcareous glacier forefield habitats (Dachstein and Griessen) based on isolation-driven screening (Szedlacsek et al. 2025). Phylogenomic analyses clearly show that it represents a distinct lineage with a sister-group relationship to *Gamsiella* (Fig. 1). The closest reported morphological similarity to the striking sporangiophores of *C. humilis* is found in *Dissophora globulifera* (O. Rostr.) Vandepol & Bonito. The latter produces sporangia in dense tufts. Sporangiophores are 500–1000 µm long and possess a columella; sporangia are globose and 40–48 µm in diameter. Sporangiospores are globose, delicately echinulate, and 6–7 µm in diameter. *D. globulifera* has been isolated from agricultural soils in Europe and Japan, and environmental sequences indicate that it is a globally widespread taxon associated with forest soils (SH1019593.10FU). Based on sequence similarity, *D. globulifera* is not closely related to *Cryptocalciella* but instead shows a sister-group relationship to *Bonitomyces*. *Cryptocalciella* also shows some resemblance to *Mortierella rostafinskii* Brefeld, as originally depicted by Brefeld (Kuhlman and Hodges Jr. 1972). However, *M. rostafinskii* differs by the absence of a columella and by the fact that only a few (2–4) sporangiophores arise from a hyphal mass consisting of short branches, rather than from bulbous cells. Spores are oval to cylindric, regular, and thin-walled. Additionally, mature sporangia and empty sporangiophores of *C. humilis* show a high morphological resemblance to *Aquamortierella elegans* Embree & Indoh (Embree and Indoh 1967).

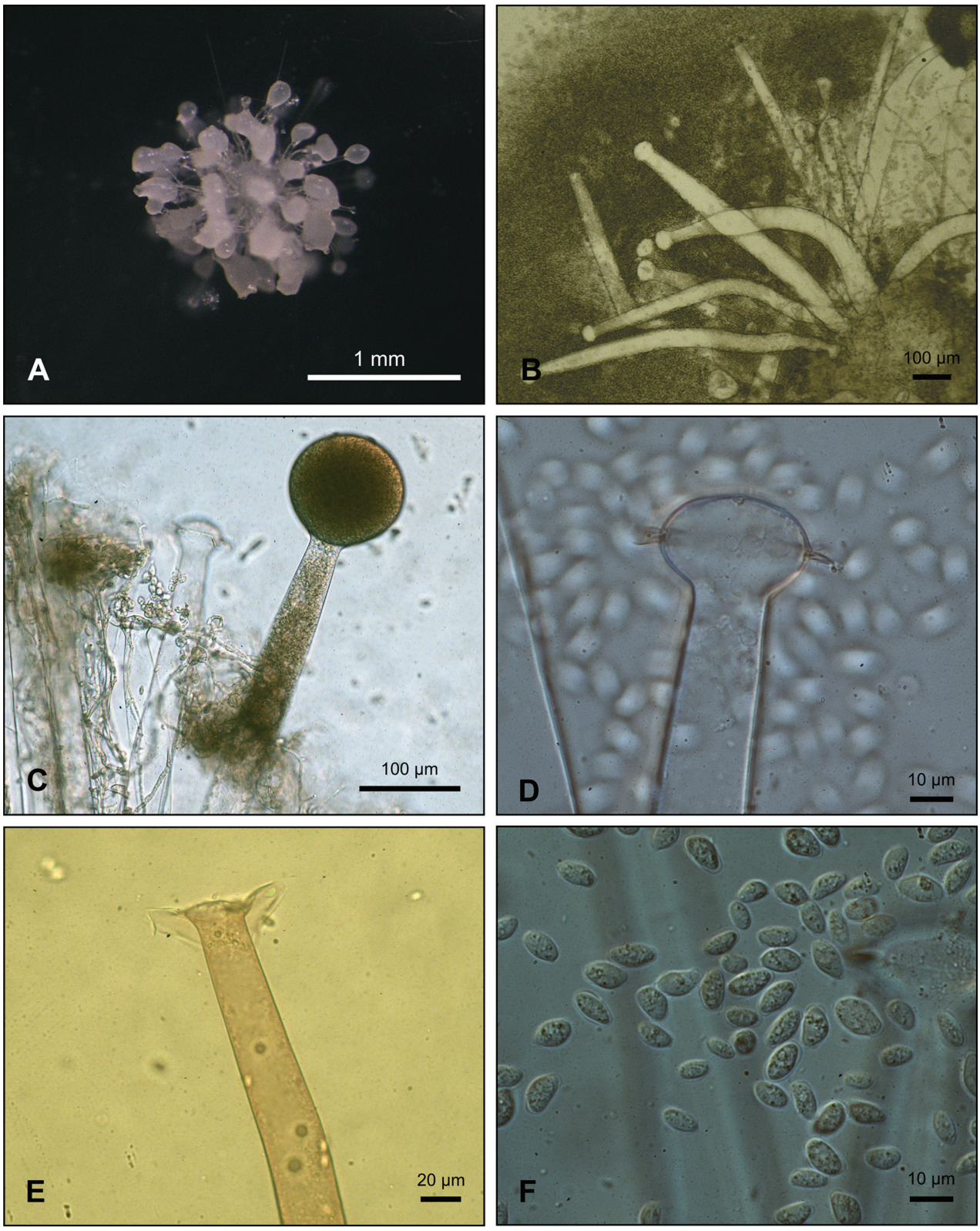

Figure 4. Reproductive structures of *Cryptocalciella humilis*. **A** Bundle of sporangiophores under the stereo magnifier with mostly intact sporangia; upon spore liberation, spore masses coalesce into larger drops. **B** Whole sporangiophores shrouded by masses of released sporangiospores. **C** Immature sporangia, spherical and ochraceously pigmented. **D** Empty sporangiophore with a pronounced collarette. **E** Dextrinoid appearance of sporangiophores in Melzer's reagent. **F** Sporangiospores with a hyaline episporal layer.

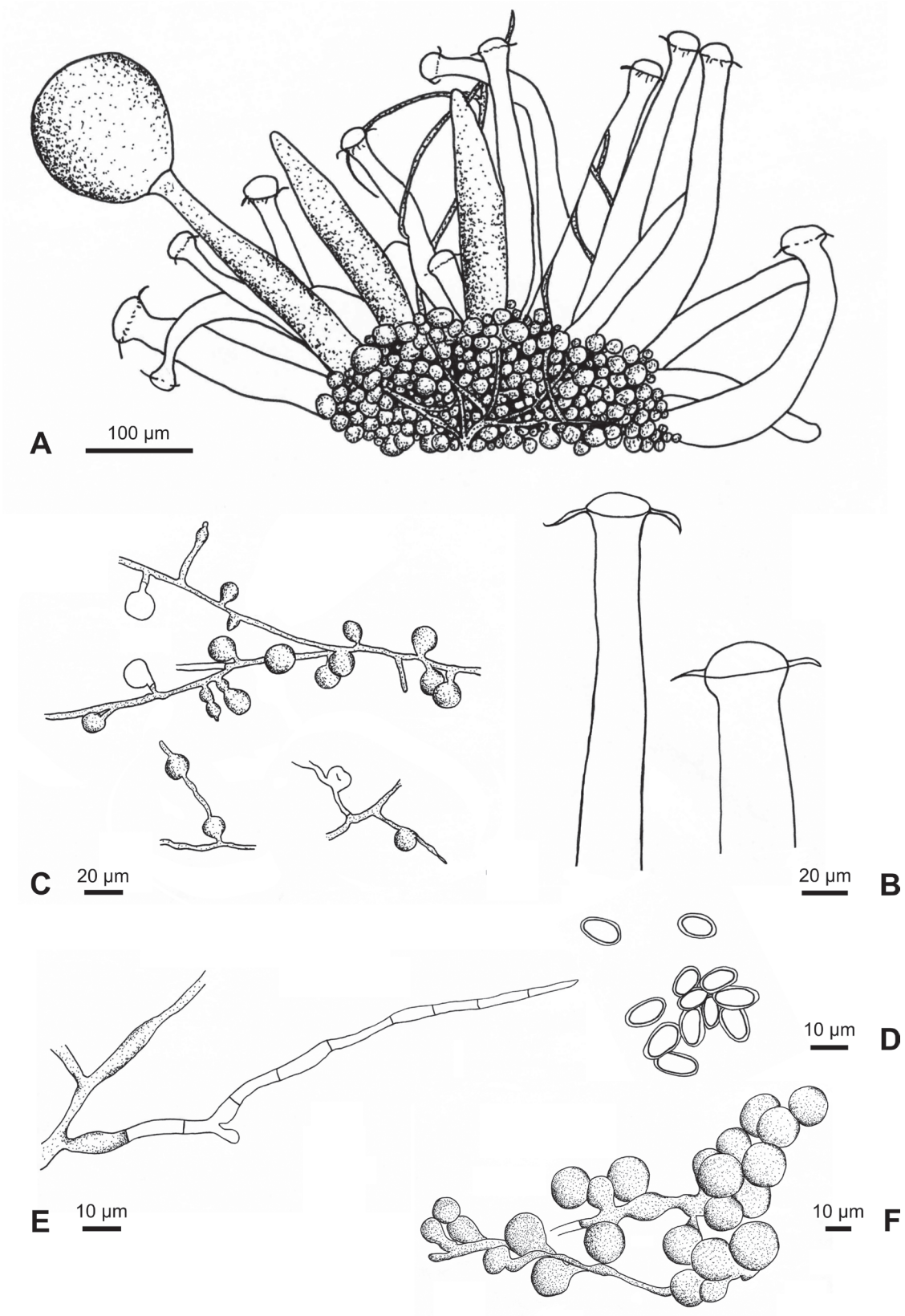

Figure 5. Drawings of *Cryptocalciella humilis*. **A** Bundle of sporangiophores with one immature sporangium and developing sporangiophores protruding from a nest of bulbous gemmae structures. **B** Empty sporangiophores with a pronounced collarette. **C** Hyphae with intercalary and terminal gemmae, cultivated on PDA. **D** Sporangiospores surrounded by a hyaline episporal layer. **E** Abandoned hyphal tip without cytoplasm and with several consecutive septa, cultivated on SEA. **F** Nest of bulbous gemmae originating from the white ring area of a culture grown on LCA. Drawings by Sophie Szedlacsek.

Both genera develop large, erect, non-septate sporangiophores that taper from base to tip, possess an apophysis, and form large spherical sporangia, leaving a distinct peridial collarette after spore liberation. However, sporangiospore morphology differs markedly. In *A. elegans*, sporangiospores are reniform to sinuate allantoid with vermiform appendages, whereas *C. humilis* produces ovoid to phaseoliform, smooth sporangiospores surrounded by a hyaline episporal layer. Double-walled sporangiospores are an unusual character in *Mortierellaceae* and have previously been reported only for *Lunasporangiospora chienii* (P.M. Kirk) Vandepol & Bonito and *Mortierella strangulata* van Tieghem (van Tieghem 1875).

Whole genome characteristics

The *Cryptocalciella humilis* genome assembly comprised 55 contigs, totaling 44.04 Mb in length, with a GC content of 50.47%. Assembly quality metrics included an N50 of 3.29 Mb and an N90 of 576.9 kb. A total of 11,609 protein-coding gene models were predicted, corresponding to 11,370 mRNA transcripts, with an average gene length of 2,205.17 bp. The annotation also identified 239 transfer RNA (tRNA) genes. Analysis of exon structure revealed 45,657 exons, including 9,681 multi-exon and 1,689 single-exon transcripts, with an average exon length of 419.16 bp. The predicted proteins had a mean length of 595.84 amino acids. Functional annotation, based on similarity searches and domain predictions, identified 7,354 transcripts with Gene Ontology (GO) terms, 8,664 with InterProScan domains, 8,989 with eggNOG orthologs, and 7,257 with Pfam domains. In addition, 229 carbohydrate-active enzymes (CAZymes), 387 MEROPS protease matches, and 648 putative secreted proteins were predicted (Suppl. material 2: table S15).

Physiological tests

Cryptocalciella humilis grew best at 20 °C, with a daily growth rate reaching 7.2 mm per day (Fig. 6). When grown at 16 °C, *C. humilis* showed a similar growth pattern to that at 20 °C but with higher standard deviations (Fig. 6). The fungus also grew at 4 °C, although at a reduced rate (Table 1). No growth was detected at temperatures of 25 °C or higher.

The optimal pH range for growth of *C. humilis* was between pH 8 and pH 10. Growth was observed at pH 4, but with a strongly reduced growth rate (Fig. 6, Table 1). Reduced aerial mycelium was observed at pH 10 and pH 12.

Growth of *C. humilis* decreased with increasing salinity. No growth was observed at NaCl concentrations of 3%, with a significant reduction in growth already evident at 2% salinity (Fig. 6, Table 1).

The chitin agar did not produce a color-changing reaction when inoculated with *Cryptocalciella humilis*, in contrast to the positive control (Suppl. material 2: fig. S1). Strongly reduced mycelial growth was observed on all chitin plates tested. The casein assay yielded positive results, as indicated by translucency in the inoculated plates compared with the sterile skim milk agar (Suppl. material 2: fig. S1). The phosphate agar showed no evidence of halo formation resulting from phosphorus solubilization. *C. humilis* was not able to solubilize complex carbon sources under the tested conditions, and none of the alternative carbon sources produced halos when treated with Lugol's iodine. Altogether, positive results were obtained only for the casein assay (Suppl. material 2: fig. S1).

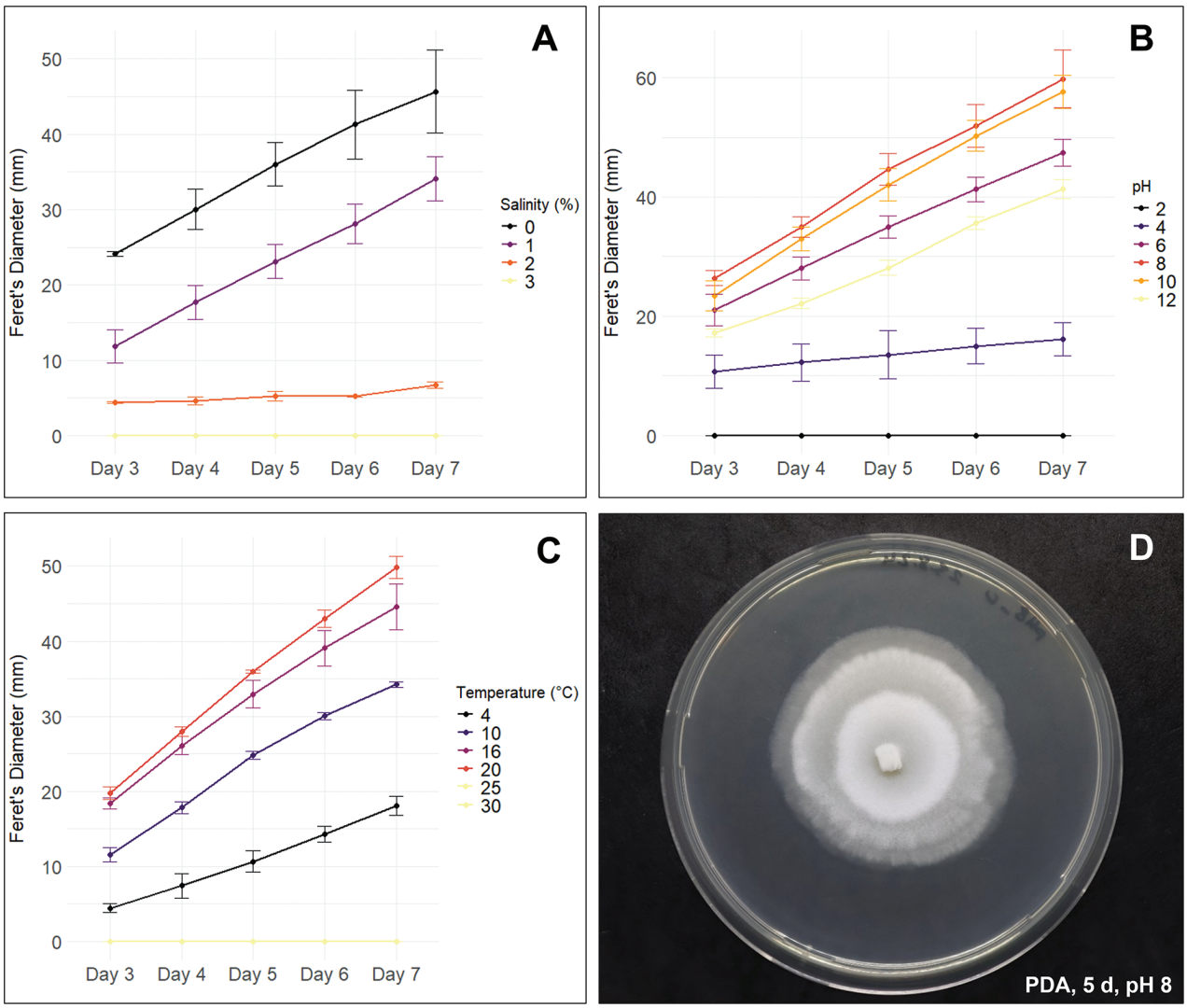

Figure 6. Growth rates of *Cryptocalciella humilis* for **A** salinity, **B** pH, and **C** temperature assays after 7 days of incubation. Data are shown as means (± SD) of Feret's diameters among biological replicates (n = 3). pH and salinity assays were carried out on PDA at 16 °C. **D** Example of a growth assay (pH assay) of *C. humilis* in which daily radial growth (rings of mycelium) is distinguishable from the central point of inoculation. Rings of mycelium correspond to time points days 3, 4, and 5 at pH 8 in **B**.

Associated bacteria

More than one third (36%, 16 out of 44) of the tested *C. humilis* isolates were associated with bacteria (Suppl. material 2: table S12). The obtained bacterial sequences clustered into a single OTU with 99% similarity over more than 500 bp. BLAST searches resulted in a 100% match with the type strain of *Pseudomonas svalbardensis* (PMCC 200367). Other potential matches included *P. mandelii* (e.g., QT-7), *P. caricapapayae* (NJ-DLW-5-A), *P. frigoris* (ZB1P45), *P. frederiksbergensis* (PgBE253), and *P. silesiensis* (NJ-XFW-4-A), which cannot be distinguished based on 16S rRNA sequences. One sequence with low quality was excluded from the analysis. Accession numbers for all obtained bacterial sequences are provided in Suppl. material 2: table S12.

Table 1. Mycelial growth rates of *Cryptocalciella humilis* under various assay conditions, measured as daily increases in radial growth from the central point of inoculation between days of the experiment (days 3–7). Values represent differences in mean growth between consecutive days.

Growth assay	Condition	Mycelium growth (mm/day)
Temperature	4 °C	+ 2.6 ± 0.15
	10 °C	+ 4.9 ± 0.04
	16 °C	+ 6.4 ± 0.35
	20 °C	+ 7.1 ± 0.17
	25 °C	0
	30 °C	0
pH	2	0
	4	+ 2.5 ± 0.25
	6	+ 6.8 ± 0.26
	8	+ 8.5 ± 0.56
	10	+ 8.2 ± 0.32
	12	+ 5.9 ± 0.19
Salinity	0%	+ 6.5 ± 0.46
	1%	+ 4.9 ± 0.30
	2%	+ 0.96 ± 0.04
	3%	0

Discussion

Phylogenomic placement and taxonomic distinction

The genus *Cryptocalciella* is a sister group to the genus *Gamsiella*. However, there are no shared morphological characters with representative species of the latter genus, and the habitats are also markedly different. Whereas *Gamsiella* strains were isolated from a decaying stump in Russia and sandy loam in Australia, *C. humilis* was isolated from alpine glacier forefields. The genus *Gamsiella* was defined based on the dichotomously branched sporangiophores of its type species, *Mortierella multidivaricata* R.K. Benj. In this species, the branches divide repeatedly until the terminal branches form two-spored sporangia on slender, elongate, attenuate pedicels, and colonies produce a garlic-like odor. Due to these characteristics, a subgenus *Gamsiella* was first established (Benjamin 1978) and was later elevated to genus rank (Benny and Blackwell 2004). *Mortierella multidivaricata* was originally isolated from debris collected beneath a rotting log in Sokolniki Park, Moscow, Russia (Benjamin 1978).

Mortierella stylospora Dixon-Stew. was only recently recombined into *Gamsiella* (Vandepol et al. 2020). However, the branches of this clade were already exceptionally long in earlier multi-gene phylogenies and are also comparatively long in our phylogenomic tree. *Mortierella stylospora* is characterized by well-developed stylospores borne on fine, erect aerial hyphae, and no sporangia have been observed (Dixon-Stewart 1932). The species was originally described from Australia.

Modicella and *Benniella* form a well-supported lineage that is sister to the *Cryptocalciella–Gamsiella* clade but exhibit markedly different morphologies. Species of *Modicella* are the only *Mortierellaceae* known to produce

macroscopic fruiting bodies in the form of small, whitish, round sporocarps. Their spores can germinate on artificial media and be grown axenically (Gerdemann and Trappe 1974). However, they form sporangia without a columella, and cultures produce a garlic-like odor, which is typical of many *Mortierellaceae*.

The genus *Benniella* shares some vegetative morphological traits with *Cryptocalciella*, but typical reproductive structures such as sporangia, stylospores, or zygospores have not been reported. Instead, *Benniella* produces chains or clusters of bulbous gemmae. Species of *Benniella* have been isolated from comparatively nutrient-rich soils in Australia from subhumid climate zones and from humid continental climates in Indiana, U.S.A. *Benniella* isolates were obtained using sterilized arthropod exoskeletons as bait, indicating an ability to degrade chitin (Vandepol et al. 2020). This contrasts with the psychrophilic *Cryptocalciella*, which is adapted to oligotrophic alpine habitats and was not able to degrade chitin. *Benniella* has also been reported as a plant endophytic fungus, and its ability to colonize plant hosts has been shown to depend on the presence of endohyphal bacteria, specifically *Mollicutes*-related endosymbionts (Longley et al. 2023). In contrast, no endohyphal bacteria were detected in *Cryptocalciella*, although regular associations with bacteria of the genus *Pseudomonas* were observed.

The next related lineage includes representative species of *Dissophora* and *Bonitomyces*. Due to their distinctive sporangiophores, species of this lineage are morphologically distinct from *Cryptocalciella*. *Dissophora* can be considered monophyletic only when excluding *D. globulifera* (O. Rostr.) Vandepol & Bonito (Wagner et al. 2013). *Bonitomyces* is sister to *Mortierella globulifera*, rendering *Dissophora* polyphyletic. *Bonitomyces* was defined based on *B. buffelskloofinus* Crous, which forms clusters of erect sporangiophores and is known only from dead twigs of an unidentified tree in South Africa (Crous et al. 2023).

Genome characteristics and comparative analysis

We generated a high-quality genome assembly for *Cryptocalciella humilis*, representing a previously undescribed genus closely related to *Gamsiella* and sharing a lineage with *Dissophora* within *Mortierellaceae*. The assembly exhibits strong continuity metrics, including an N50 of 3.29 Mb and an N90 of 576.9 kb, indicating a high level of completeness and low fragmentation. A high number of genes encoding carbohydrate-active enzymes (CAZymes), MEROPS-classified proteases, and 648 predicted secreted proteins were identified, which may reflect broad enzymatic capabilities. Interestingly, this was not detected in the physiological assays. We assume that saprotrophic degradation was not activated under the *in vitro* conditions applied in our experimental setup.

To contextualize this genome within *Mortierellaceae*, we compared it with available assemblies from representative genera, including *Dissophora decumbens* Thaxt., *Gamsiella multidivaricata*, *Benniella erionia* J. Liber & Bonito, and *Linnemannia elongata* (Linnem.) Vandepol & Bonito. While *Benniella* is often characterized by smaller gene sets and reduced enzymatic repertoires, the *Cryptocalciella* genome displays a more functionally complex profile, including an expanded secretome and elevated GC content. Compared with the closely related genera *Gamsiella* and *Dissophora*, the novel genome shares a similar size and gene count but exhibits a broader enzymatic repertoire and more extensive

secretion potential, suggesting greater metabolic and ecological plasticity. In contrast, *Benniella* species appear to be undergoing genomic streamlining, possibly due to niche specialization, which is not observed in the newly sequenced lineage. This metabolic and ecological plasticity is consistent with the habitat and ecological conditions of *Cryptocalciella humilis*. In glacier forefields, nutrient input is highly variable and unpredictable in timing. In addition, temperatures fluctuate, and the period favorable for growth is short (Bradley et al. 2014). This favors organisms that can utilize variable resources, store large amounts of nutrients, and use them during prolonged periods of scarcity to ensure survival. We observed this strategy in *Cryptocalciella*. Its mycelium stores large quantities of nutrients in the hyphae and gemmae, which are often reabsorbed together with the cytoplasm, leaving only empty hyphal walls behind.

Associated plants and bacteria

The high number of secreted proteins detected in the *Cryptocalciella* genome reflects its potential for environmental interactions. The isolates originate from the earliest stages of soil development, with only scattered and very few plants present. At the sampling sites, the occurring pioneer plants (*Arabis alpina*, *Arabis pumila*, *Cerastium uniflorum*, *Poa minor*, *Heliosperma pusillum*, *Saxifraga aphylla*) (Mandolini et al. 2025) are usually not associated with mycorrhizal fungi. However, it is possible that *Cryptocalciella* might have plant-beneficial properties, as reported for several other taxa of *Mortierellaceae* (Vandepol et al. 2020). This will be investigated in more detail in future studies.

Moreover, we often detected *Pseudomonas* taxa (Bacteria) in association with *Cryptocalciella humilis* mycelia. Similarly, representative species of *Pseudomonas* have been reported to be abundant in alpine and subalpine environments, in association with various *Mortierellaceae* species (Telagathoti et al. 2021). This indicates that these associations are not random and very likely have an important ecological function. *Pseudomonas* spp. are Gram-stain-negative, aerobic, rod-shaped, non-endospore-forming, and motile bacteria. We detected *Pseudomonas svalbardensis*, which is similar to strains isolated from a soil sample under red snow in the Ny-Ålesund region (Svalbard, High Arctic). Growth of this bacterium occurs at 4–30 °C (Ge et al. 2024). Many members of the genus *Pseudomonas* have been discovered in the Arctic and Antarctic regions and have important ecological functions (e.g., denitrification, dimethylsulfoniopropionate degradation, and plant growth promotion) in cold environments (See-Too et al. 2017; Hu et al. 2023). We therefore speculate that there might be a synergistic interaction between *Cryptocalciella humilis* and the associated *Pseudomonas* bacteria, enabling them to jointly colonize bare soil in glacier forefields.

The genomes generated in this study provide a valuable resource for future comparative genomics and evolutionary studies within *Mortierellaceae* and offer new insights into the diversification of ecological strategies in early-diverging fungi.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Adherence to national and international regulations

All the fungal strains used in this study have been legally obtained, respecting the Convention on Biological Diversity (Rio Convention).

Funding

This research was partially funded by Land Tirol (Austria) through the grant Tiroler Nachwuchsforscher*innenförderrung entitled Investigation on *Mortierellaceae* diversity and their distribution in alpine ecosystems (grant number: F.47849/5-2023). This research was funded in part by the Austrian Science Fund (FWF) (grant number: 10.55776/PIN4936824) and in part by the National Science Centre (Poland) in the context of the NCN Weave-UNISONO project entitled *Mortierellaceae* Functional Diversity in Alpine Ecosystems (grant number: 2024/06/Y/NZ8/00013).

Author contributions

Conceptualization: SS, UP, EM. Data curation: BMA, AS, SS, EM. Formal analysis: BMA, SS, PL, EM, AS. Funding acquisition: JP, UP. Investigation: MK, PL, EM, SS, AS, AS. Methodology: SS, EM, BMA. Project administration: UP, JP, EM. Resources: SS. Supervision: UP, JP. Validation: EM, SS, UP. Visualization: SS, EM. Writing - original draft: EM, SS, JP, BMA. Writing - review and editing: SS, BMA, JP, EM, UP.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information and in relevant public repositories under the number PRJNA1285402.

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Supplementary material 1

Metadata of phylogenomic analysis

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Data type: xlsx

Explanation note: Metadata of all taxa used in phylogenomic analysis.

Link: https://doi.org/10.3897/imafungus.17.177912.suppl1

Supplementary material 2

Additional information

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Data type: docx

Explanation note: Media recipes, PCR protocols, NCBI accession numbers of all isolates, genomic properties, distance matrices.

Link: https://doi.org/10.3897/imafungus.17.177912.suppl2

Supplementary material 3

Phylogenomic tree

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Data type: nex

Link: https://doi.org/10.3897/imafungus.17.177912.suppl3