Mortierellaceae from subalpine and alpine habitats: new species of *Entomortierella, Linnemannia, Mortierella, Podila* and *Tyroliella gen. nov*.

A. Telagathoti, M. Probst, E. Mandolini, U. Peintner*

Institute of Microbiology, University of Innsbruck, Technikerstrasse 25, 6020 Innsbruck, Austria

**Corresponding author*: Ursula Peintner, Ursula.Peintner@uibk.ac.at

Abstract: Fungi are incredibly diverse, but they are unexplored, especially in the subalpine and alpine zone. *Mortierellaceae* are certainly one of the most abundant, species-rich, and widely distributed cultivable soil fungal families in terrestrial habitats, including subalpine and alpine zones. The phylogeny of *Mortierellaceae* was recently resolved based on current state of the art molecular techniques, and the paraphyletic genus *Mortierella sensu lato* (*s.l.*) was divided into 13 monophyletic genera. Our extensive sampling campaigns in the Austrian Alps resulted in 139 different *Mortierellaceae* pure culture isolates representing 13 new species. For the definition of taxa, we applied both classical morphological criteria, as well as modern DNA-based methods. Phylogenetic relationships were resolved based on the ribosomal DNA internal transcribed spacer (rDNA ITS), the large subunit (LSU), and the DNA-directed RNA polymerase II largest subunit 1 (*RPB1*). In this study, we proposed a new genus and described 13 new species belonging to the genera *Entomortierella*, *Linnemannia*, *Mortierella* and *Podila*. In addition, we proposed eight new combinations, re-defined *E. jenkinii* at species level, defined a neotype for *M. alpina* and lecto- as well as epitypes for *M. fatshederae*, *M. jenkinii,* and *M. longigemmata.* The rDNA ITS region is generally applied as classical barcoding gene for fungi. However, the obtained phylogenetic resolution is often too low for an accurate identification of closely related species of *Mortierellaceae*, especially for small sampling sizes. In such cases, unambiguous identification can be obtained based on morphological characters of pure culture isolates. Therefore, we also provide dichotomous keys for species identification within phylogenetic lineages.

Key words: *Mucoromycota* systematics and taxonomy, multi-gene phylogeny, new taxa, systematics.
Taxonomic novelties: new genus: *Tyroliella* Telagathoti, Probst & Peintner; **New species:** *Entomortierella galaxiae* Telagathoti, M. Probst & Peintner, *Linnemannia bainierella* Telagathoti, M. Probst & Peintner, *Linnemannia stellaris* Telagathoti, M. Probst & Peintner, *Linnemannia nimbosa* Telagathoti, M. Probst & Peintner, *Linnemannia mannui* Telagathoti, M. Probst & Peintner, *Linnemannia friederikiana* Telagathoti, M. Probst & Peintner, *Linnemannia scordiella* Telagathoti, M. Probst & Peintner, *Linnemannia solitaria* Telagathoti, M. Probst & Peintner, *Mortierella triangularis* Telagathoti, M. Probst & Peintner, *Mortierella lapis* Telagathoti, M. Probst & Peintner, *Podila himami* Telagathoti, M. Probst & Peintner, *Podila occulta* Telagathoti, M. Probst & Peintner, *Tyroliella animus-liberi* Telagathoti, Probst & Peintner; **New combinations:** *Entomortierella basiparvispora* (W. Gams & Grinb.) Telagathoti, M. Probst & Peintner, *Entomortierella jenkinii* (A.L. Sm.) Telagathoti, M. Probst & Peintner; *Entomortierella sugadairana* (Y. Takash. *et al.*) Telagathoti, M. Probst & Peintner, *Linnemannia zonata* (Linnem. ex W. Gams) Telagathoti, M. Probst & Peintner, *Linnemannia fluviae* (Hyang B. Lee *et al.*) Telagathoti, M. Probst & Peintner, *Linnemannia biramosa* (Tiegh.) Telagathoti, M. Probst & Peintner, *Linnemannia cogitans* (Degawa) Telagathoti, M. Probst & Peintner, *Tyroliella pseudozygospora* (W. Gams & Carreiro) Telagathoti, M. Probst & Peintner; **Epitypifications (basionyms):** *Mortierella bainieri* var. *jenkinii* A.L. Sm., *Mortierella fatshederae* Linnem., *Mortierella longigemmata* Linnem. **Neotypification (basionym):** *Mortierella alpina* Peyronel.

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INTRODUCTION

The family *Mortierellaceae* belongs to the phylum *Mortierellomycota* (Tedersoo *et al.* 2018), which is phylogenetically distinct from the phylum *Mucoromycota*. The genus *Mortierella* was described in 1863 by Coemans based on the type *M. polycephala*. With almost 100 described species, *Mortierella* represents a comparatively species-rich genus in this group of zygomycetous fungi. *Mortierellaceae* species typically form white cottony aerial mycelium with a zonate/rosette pattern on different kinds of media, with garlic or freshly showered dog odour (Gams 1977, Petkovits *et al.* 2011, Wagner *et al.* 2013). The species of this fungal group are characterised by their coenocytic hyphae, branched/unbranched sporangiophores with basal swelling, or the production of rhizoids, ornamented/smooth sporangium, chlamydospores, and zygospores may be present. Both the homothallic and heterothallic species are reported in this family. Many *Mortierellaceae* species are known as important saprobic organisms living on organic matter and many other substrates (Linnemann 1941). Members of the family have a widespread distribution in the temperate zone, where they are almost cosmopolitan, and occur in a wide range of habitats: *Mortierella* spp. has been reported from bat dung (Degawa & Gams 2004), arthropods (Degawa & Tokumasu 1998, Werner *et al.* 2016), freshwater (Nguyen *et al.* 2019), and from the recently deglaciated barren ground in glacier forefields (Kuhnert *et al.* 2012, Dresch *et al.* 2019). Recent studies on the soil microbiota on a global scale reported *Mortierella* spp. to be important members of the soil core microbial community (Tedersoo *et al.* 2014, Zhang *et al.* 2019). Following their wide range of habitats, members of the genus *Mortierella* have a broad range of growth temperatures. *Mortierella* spp. belong to the winter active soil microbial community and form significant amounts of fungal biomass in the soil during both the snow-covered and vegetation period (Kuhnert *et al.* 2012). Members of *Mortierellaceae* are reported to produce polyunsaturated fatty acids and arachidonic acid, which are crucial for several biological functions in mammalians (Yadav *et al.* 2014), and are widely used for many commercial purposes (Singh

& Ward 1997) such as biofuel production (Du *et al.* 2018). Many *Mortierella* species have been reported to occur in agricultural soils, thereby promoting plant growth and litter decomposition (Ozimek & Hanaka 2021). They do not only enhance crop growth, but they can also reshape bacterial communities in the rhizosphere (Li *et al.* 2020). The ability of *Mortierellaceae* to grow at lower temperatures and under unfavourable environmental conditions could make them potential biocontrol agents. Recent studies reported that organic acids produced by these species have antifungal, nematocidal, and anti-bacterial properties (Shemshura *et al.* 2018). Few species even show significant nematocidal or insecticidal effects against wax moths, housefly larvae, and also *Meloidogyne* spp. (Edgington *et al.* 2014, DiLegge *et al.* 2019).

Despite our knowledge of *Mortierellaceae*, there are still many undescribed species, especially from alpine regions. These are of particular interest for understanding the diversity and evolutionary history of these globally distributed fungi. Alpine and subalpine habitats are usually characterised by poor nutrient availability, generally lower temperatures, and frequent freeze-thaw cycles. Our recent study on the distribution and diversity of *Mortierellaceae* species in the Austrian alpine range revealed a high diversity of 29 species occurring in subalpine and alpine habitats (Telagathoti *et al.* 2021), and we also isolated many unknown species belonging to this group.

We analysed the respective *Mortierellaceae* taxa by sequencing the rDNA-ITS region, the large subunit (28S ribosomal DNA sequences), and the RNA polymerase II largest subunit (*RPB1*) along with traditional micromorphological studies. Based on our combined approach, our isolates clearly belong to 13 novel species. In the framework of the *Mortierellaceae* phylogeny proposed by Vandepol *et al.* (2020), our 13 novel species belonging to five different genera, including a new genus, which we introduce here.

Besides this, we aimed to understand the evolutionary relationships and phylogenetic placement of our *Mortierellaceae* species based on the modern taxonomical concept as recently presented by Vandepol *et al.* (2020), and to propose easy and affordable protocols and keys for *Mortierella*, *Linnemannia*, *Podila*, *Entomortierella*, and the new genus' species based on identification from isolates in pure culture.

MATERIALS AND METHODS

Sampling sites and isolations

Soil samples up to a depth of 15 cm from the soil surface were collected from both snow cover (May 2019) and snow free period (mid-July 2019) from sub-alpine mixed *Pinus cembra* / *Picea abies* and pure *P. cembra* forests, alpine chalk dumps (Kalkschuttladen), alpine dwarf willow communities (*Salix herbacea*, *Salix reticulata*, *Dryas octopetala*) with an altitude ranging from 1 540–2 450 m (Table 1). For cultivation, a few grains of soil were directly plated on PDA media and incubated at 10 °C for 3 d. To obtain pure cultures of the *Mortierellaceae*, each colony was picked, replated, and incubated again at 10 °C for 5 d.

The strains used in this study are listed in Supplementary Table S1.

PCR and DNA sequencing

PCR was performed using the mycelium from 7-d-old cultures with a few modifications (Walch *et al.* 2016) as follows: a small amount of mycelium was suspended in 100 µL sterile water, homogenised, preheated at 85 °C for 15 min, and used as DNA template. The rDNA-ITS region was amplified by using standard primers ITS1 and ITS4 (White *et al.* 1990). In addition, the large subunit (LSU) was amplified using the primer pairs LR0R and LR5 (Hopple & Vilgalys 1994). The colony PCR was unsuccessful to amplify the *RPB1* gene; hence, the DNA was extracted using the CTAB method (Neuhauser *et al.* 2009). The primers used to amplify the *RPB1* gene are RPB1f and RPB1r (Vandepol *et al.* 2020). The PCR cycle conditions for the *RPB1* amplification was done using a touch up method (Visagie *et al.* 2014). Microsynth AG (Balgach, Switzerland) carried out unidirectional sequencing for ITS, LSU, and bidirectional sequencing for *RPB1*. The GenBank accession numbers of the sequences and further information on the environmental source of the isolates are available in Supplementary Table S1.

Phylogenetic analyses

A total of 139 ITS sequences were generated in this study. Two pure culture isolates per well-supported ITS lineage (> 90 % BPP) were sequenced for the LSU dataset, and one for the *RPB1* dataset. First, all sequences were manually checked for quality. Then, the preliminary identification was conducted based on the NCBI blastn searches. For the reference, the ex-type material sequences and the environmental sequences were retrieved from GenBank. The obtained sequences and the ex-type sequences were then aligned using MAFFT v. 7 (Katoh *et al.* 2017). The alignment was later checked with MEGA X (Kumar *et al.* 2018). The best Maximum likelihood (ML) model was calculated in MEGA X before the ML analysis. For the branch robustness evaluation, Maximum Parsimony based bootstrap was applied. Bootstrap analyses (1 000 replicates) were conducted by Subtree-Pruning Regrafting (SPR) algorithm level in which the initial trees were obtained. All positions with less than 95 % site coverage were eliminated for both the ML and Bootstrap search.

Branch robustness was checked with Bayesian Interference in MrBayes v. 3.2.6 (Ronquist *et al.* 2012). For the Markov Chain Monte Carlo (MCMC) analyses, four chains were run for 10 M generations, with trees being sampled every 5 000 generations. The analysis was stopped as the convergence diagnostic (average standard deviation of split frequencies) was below 0.05 after 10 M generations. From the 20 000 sampled trees (for each of the two runs), 25 % were discarded as burn-in before summary statistics were calculated (using sump and sumt commands). Diagnostic plots, as well as the convergence diagnostics EES (Estimated Sample Size; min ESS around 10 K) and PSRF (Potential Scale Reduction Factor; 1.000 for all parameters), indicated stationarity. The resulting trees were visualized with FigTree v. 1.4.4 (Rambaut 2010). The same approach was applied to both the large subunit and the *RPB1* analyses. Here, the evolutionary history was inferred by using the Maximum Likelihood method and General Reversible Chloroplast (cpREV) model.

Morphological studies

The *Mortierellaceae* isolates were grown for up to 2 mo on agar plates containing PDA (26.5 g/L of Potato dextrose agar), LCA (Takashima *et al.* 2019), Hempseed (50 g/L Hempseeds incubated overnight, filtered and autoclaved with 15 g/L agar), SNA (Nirenberg 1976), Water agar (WA; Samson *et al.* 1984), and Soil extraction (SE) (25 g autoclaved soil from the sampling sites and 15 g/L agar) at 16 °C and checked for reproductive structures. All isolates were examined via standard microscopic techniques using lactic acid, water, and

Table 1. Information of the sampling sites in Austria and the dominant plant communities.

Sampling sites	Altitude (m)	pH	Habitat
Kühtai (3 sites) Subalpine	1 842, 1 974, 2 011	3.4–3.6	*Pinus cembra*
Praxmar (3 sites) Subalpine	1 540–1 814	3.4–3.9	*Pinus cembra, Picea abies*
Patscherkofel Subalpine	2 260	3.5–3.7	*Pinus cembra*
Pfitscherjoch Alpine	2 261	4.0–4.3	*Salix reticulata*, *Salix herbacea*
Hafelekar Alpine	2 250	5.3–6.3	*Salix reticulata*, *Salix herbacea, Dryas octopetala,* chalk dumps
Haggen Subalpine	2 026	3.8–3.9	*Pinus cembra*
Obergurgl (2 sites) Glacier forefield	2 280, 2 450	7.0	Bare soil in the glacier forefield *Salix herbacea*

cotton blue. Microscopic documentation and measurements were made with a Nikon DS-Fi1 camera and the computer program NIS Elements v. 4.13.04. All measurements were made at 1 000-fold magnification. For a meaningful evaluation of spore size, at least 30 spores were measured. The reference type cultures were deposited at the Jena Microbial Resource Collection and the Westerdijk Fungal Biodiversity Institute, Utrecht (CBS) under the numbers listed in Supplementary Table S1. Additionally, cryo cultures in 10 % skim milk and 40 % glycerol are stored at -80 °C at the Institute of Microbiology in Innsbruck (Tyrol, Austria).

RESULTS

Taxonomy

The taxonomy of *Mortierellaceae* is presented per genus in alphabetical order. Dichotomous keys for a morphology-based identification of the treated species are provided in each group.

Entomortierella Vandepol & Bonito, Fungal Diversity 104: 281. 2020.

The genus *Entomortierella* represents the highest diversity in terms of morphology and ecology in the lineage of *Mortierellaceae*. *Entomortierella* species include both homothallic and heterothallic sexual morphs. Sporulation is usually abundant. Most of the species in this genus were reported to be associated with insects or arthropods/worms (Wagner *et al.* 2013). These *Entomortierella* species were recurrently isolated from soil, decaying plant matter, and roots. They are widely distributed in *Pinus cembra* and *Picea abies* forest soil, irrespective of the season (snow cover *vs.* snow free soil).

Type species: *Entomortierella lignicola* (G.W. Martin) Vandepol & Bonito

Entomortierella galaxiae Telagathoti, M. Probst & Peintner, ***sp. nov.*** Index Fungorum IF 559289. Figs 1, 2.

Etymology: *galaxiae* stands for "galaxy". Excessive sporulation was observed in these species, which looks like a milky way under the microscope, which is characteristic for this taxon. Hence, it is named as *Entomortierella galaxiae.*

Typus: **Austria**, Tirol, Haggen, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti (**holotype** IBF 20190163, culture ex-type CBS 149262 = JMRC SF015106, GenBank Acc. No. ITS MT380895, LSU MZ981751, *RPB1* ON774867).

Diagnosis: *Entomortierella galaxiae* differs from the closely related species *E. parvispora* and *E. basiparvispora* by the irregular, monopodial to sympodial branching pattern of the sporangiophores, by slightly smaller sporangiospores, and by the presence of a pronounced columella.

Colonies fast growing, white cottony mycelium not forming a typical rosette pattern on PDA, but a weak pattern on LCA. Sporulation abundant on PDA, LCA, WA, SE media, and Hempseed agar, most abundant on water and SE agar at 16 °C, musty odour. *Sporangiophores* arising from aerial mycelium and surface mycelium, no rhizoids, erect, mostly sympodially or monopodially branched, branching occurring at different levels all over the sporangiophore, shorter branches arising from the unseptated main branches, sometimes unbranched, 95–350(–660) µm tall (n = 30), tapering from 5–8 µm to 2.2–4.7 µm at the tip. *Sporangia* hyaline round, smooth-walled, 14–37(–49.5) µm (n = 30), multi-spored, after spore liberation with pronounced collarette and columella on all media. *Sporangiospores* hyaline, globose, smooth-walled, 2.5–3 µm diam (n = 30) (Mean ± SD = 2.7 ± 0.2). *Chlamydospores* absent. *Zygospores* unknown.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Additional strains examined: **Austria**, Tirol, Finkenberg, Pfitscherjoch, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 8 Aug. 2019, A. Telagathoti, isolate Pfs28; Praxmar, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti, isolate Pr1s18 (Supplementary Table S1).

Habitat: Subalpine to alpine areas, with *Pinus cembra* or *Salix reticulata* vegetation. Based on environmental sequences (> 99 %

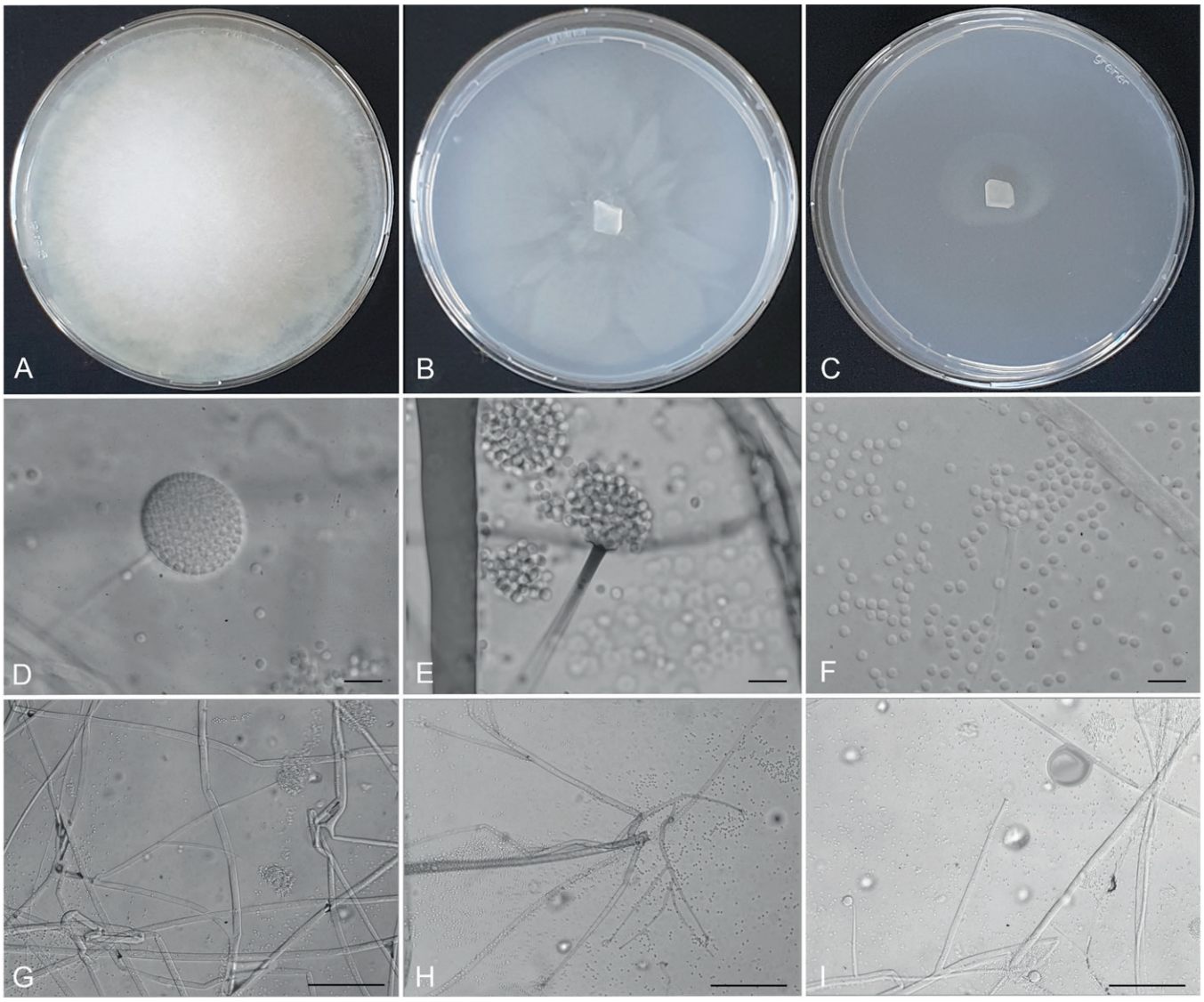

Fig. 1. Colony morphology of *Entomortierella galaxiae* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Entomortierella galaxiae*. **D.** Multi-spored sporangium attached to the sporangiophore. **E.** Apex of the sporangiophore after dehiscence of the peridium, with clear collarette and columella. **F.** Sporangiospores. **G.** Sporangiophores with sympodial branching pattern. **H.** Sporangiophores with irregular basitonous and mesotonous branching pattern. **I.** Sporangiophores with different size, a small with intact sporangium and a taller one where the sporangium already released the sporangiospores. All the microscopic structures of this type strain were observed on PDA. Scale bars: D–F = 10 µm; G–I = 20 µm.

sequence identity), this species was also detected in Poland (EU240039, KU727788) and Japan (MF423490).

Notes: *Entomortierella galaxiae* is morphologically closely related to *E. parvispora* and *E. basiparvispora*, which all fall in the *E. parvispora* species complex.

This complex represents one of two major phylogenetic lineages of the genus *Entomortierella* (Vandepol *et al.* 2020). Based on our phylogenetic analysis of rDNA ITS sequences (Fig. 3), the *E. parvispora* complex includes the following species: *E. basiparvispora, E. beljakovae, E. galaxiae, E. jenkinii, E. parvispora*, and *E. sugadairana. Entomortierella galaxiae* falls into the lineage of *M. sugadairana*, but it can be easily distinguished from this taxon based on morphological characters: *E. galaxiae* isolates are not producing zygospores; there are no rhizoids at the base of the much shorter sporangiophores. Moreover, the ecology is different: homothallic *M. sugadairana* NBRC 104553 (MF510830) was isolated from a decayed twig of *Fagus crenata,* while *E. galaxiae* was isolated from alpine soil.

Entomortierella galaxiae can be morphologically distinguished from the closely related *E. basiparvispora* based on the consistently basitonous ramification of sporangiophores, no trace of a columella and the larger spores. Spore size in the ex-type strain is 3–4 µm diam, but Gams & Grinb (1976) reported also isolates with spores up to 5 µm diam. The distinct columella clearly separates *E. galaxiae* from *E. parvispora* (Figs 1, 2).

Mortierella gracilis (Linnemann 1941) is considered as a synonym of *E. parvispora.* Strain CBS 304.52 (JX975859) represents a syntype. Based on our phylogenetic analysis, this is not a synonym of *E. parvispora*, but falls into a lineage with *E. basiparvispora*. Linnemann described *Mortierella gracilis* as follows: Sporangia somewhat bent, limp and delicate not common, arising without rhizoids from aerial hyphae, very different in height, branching scarcely present, usually only with 1–2 lateral branches peaking over the main branch, 160–600 µm, av. 200–300 µm, tapering from 5–8 to 2–3 µm, sporangiospores globose very small 2–3 µm. Additional material is needed to better circumscribe and define *M. gracilis*.

Mortierella debilis and *Mortierella debilis* var. *firmior* were reported to be morphologically related to *M. gracilis* (Zycha *et al.* 1969). The sporangiospores are 3–4 µm diam, chlamydospores are present. It is now considered a synonym of *Linnemannia elongata*.

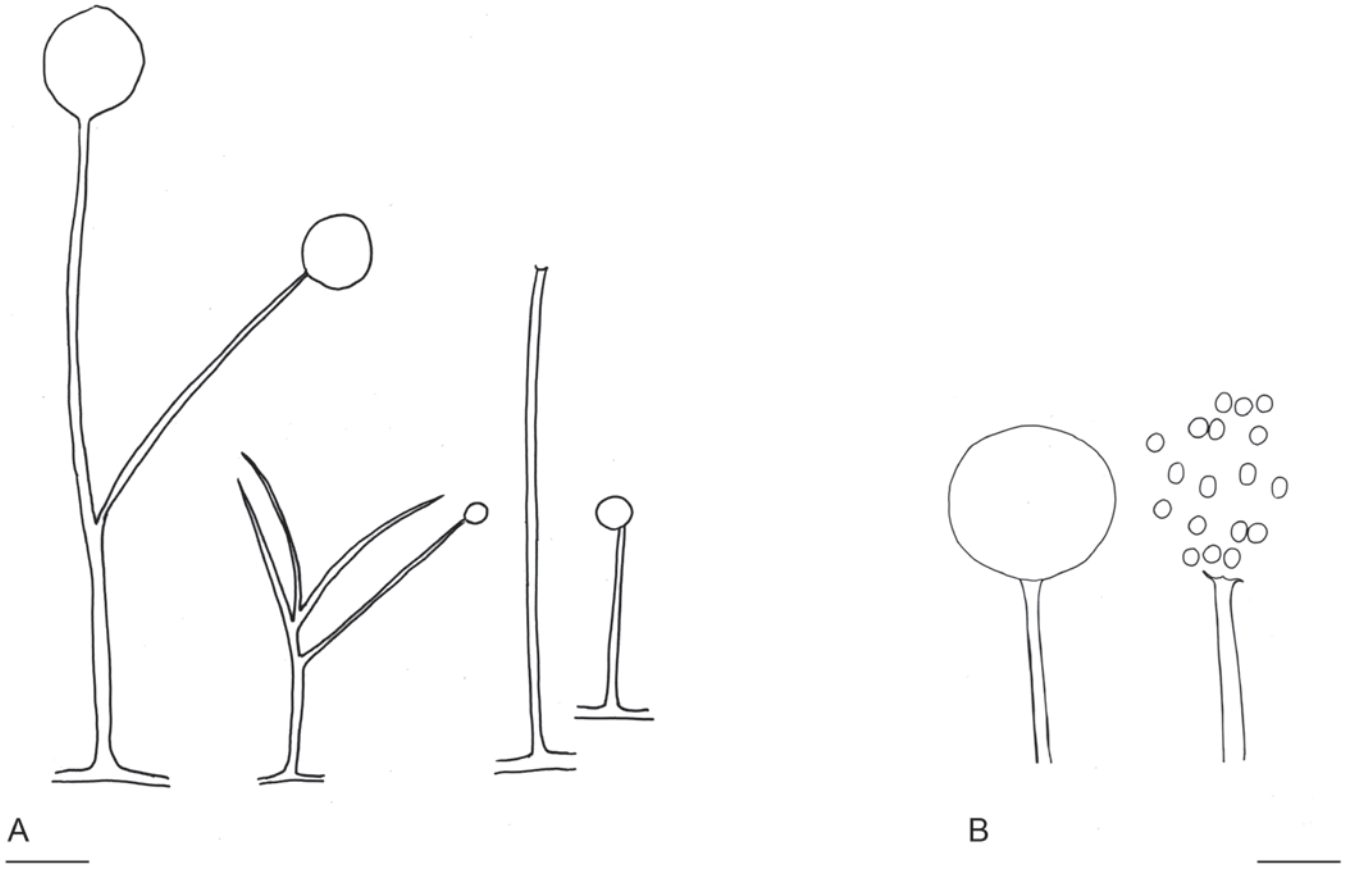

Fig. 2. *Entomortierella galaxiae.* **A.** Sporangiophores and sporangia. **B.** Sporangium, sporangiophore tip and sporangiospores. Scale bars: A = 20 µm; B = 10 µm.

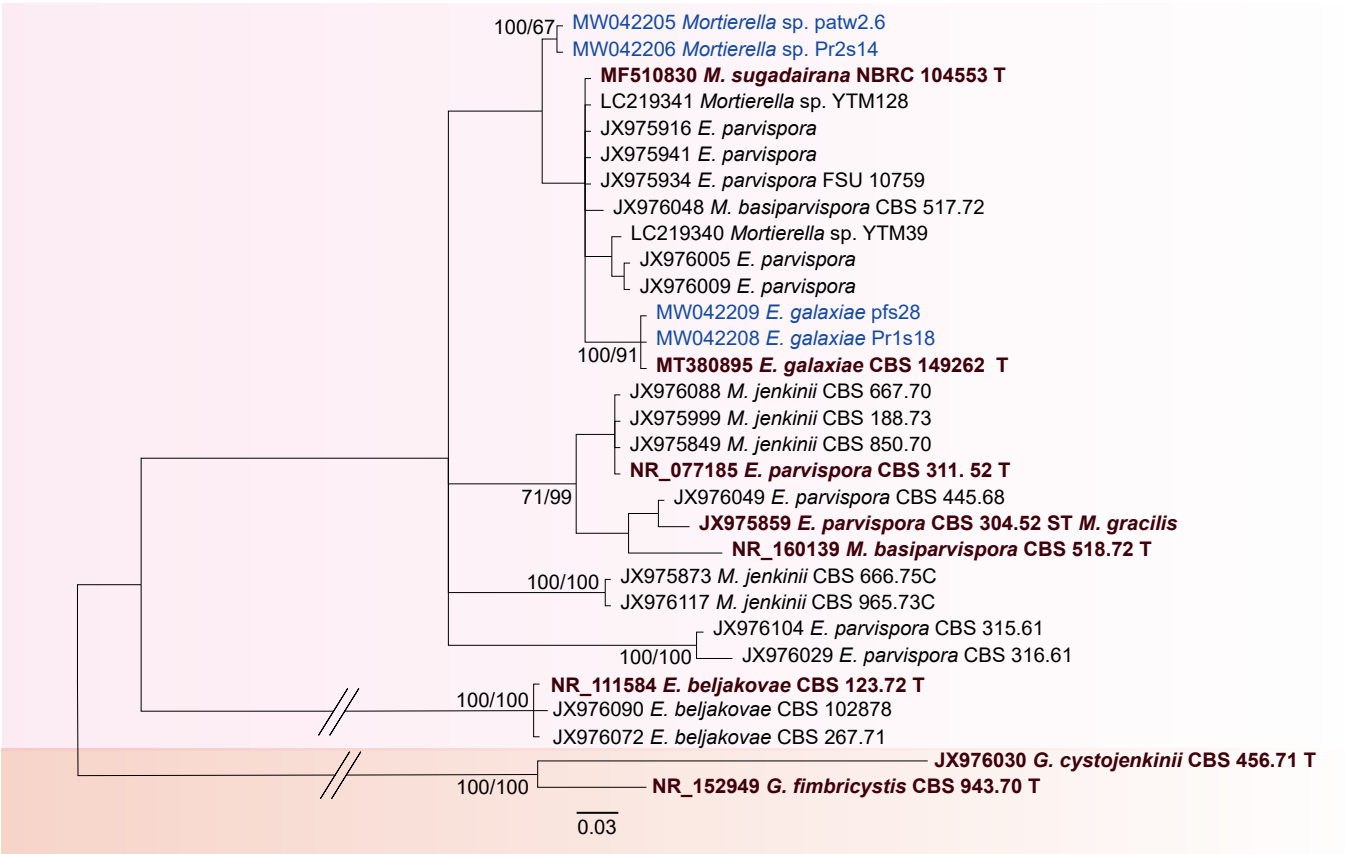

Fig. 3. Phylogenetic relationship of the *Entomortierella parvispora* complex based on rDNA-ITS sequences. The Maximum Likelihood Phylogram (log likelihood -2170.60) is shown, and the branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is provided above the respective branches. Newly generated sequences are highlighted in blue, sequences generated from typus are bold and red. *Gryganskiella cystojenkinii* and *G. fimbricystis* are used as outgroup (light brown).

Additional species in the *Entomortierella parvispora* lineage

Entomortierella basiparvispora (W. Gams & Grinb.) Telagathoti, M. Probst & Peintner, ***comb. nov.*** MycoBank MB 317882.
Basionym: *Mortierella basiparvispora* W. Gams & Grinb., Persoonia 9: 130. 1976.

Typus: **Chile**, Valdivia, Cordillera Pelada, soil, under *Fitzroya cupressoides,* unknown collection date and collector (**holotype** CBS 518.72 preserved as metabolically inactive culture, ex-type culture CBS 518.72.

Entomortierella jenkinii (A.L. Sm.) Telagathoti, M. Probst & Peintner, ***comb. et stat. nov.*** Index Fungorum: IF 559323.
Basionym: *Mortierella bainieri* var. *jenkinii* A.L. Sm., J. Bot. 36: 180. 1898.

Typus: **UK**, Newport, damp earth, Jan. 1897, Jenkin (A.L. Smith, 1898, New or rare British Fungi, J. Bot. 36: 180, figure in the middle of page on the right-hand side, **lectotype** designated here, MBT 10007673). **The Netherlands**, Wageningen, agricultural soil, 1970, Y.W. Veenbaas-Rijks (**epitype** CBS 667.70 designated here, MBT 10007674, preserved as metabolically inactive culture, ex-epitype culture CBS 667.70, GenBank Acc. No. MH871683).

Entomortierella sugadairana (Y. Takash. *et al.*) Telagathoti, M. Probst & Peintner, ***comb. nov.*** Index Fungorum IF 559304.
Basionym: *Mortierella sugadairana* Y. Takash. *et al.*, Mycoscience 59: 201. 2018.

Typus: **Japan**, Nagano, Sugadaira Research Station, Mountain Science Centre University of Tsukuba, isolated from decayed twig of *Fagus crenata*, collection date unknown, collected by S. Tokumasu (**holotype** KPM-NC0025509, ex-type culture NBRC 104335).

Key to the species of the *Entomortierella parvispora* complex

1a Sporangiophores with apophysis-like inflation; sporangiospores 8–12 µm ..... *E. beljakovae*
1b Sporangiophores without inflation underneath the sporangium 2

2a Sporangiophores large, up to 1 000 µm tall 3
2b Sporangiophores usually smaller than 500–600 µm 4

3a Homothallic species with zygospores produced, sporangiophores, mesotonously branched 150–1 100 µm, conspicuous rhizoids at base, sporangia multi-spored, globose sporangiospores 2–6 µm ..... *E. sugadairana*
3b No zygospores produced, sporangiophores with a long, unbranched basal part up to 600 µm long and 10–12 µm wide; sporangiospores ellipsoidal, 3.5–4.0 × 2.0–2.5 µm ..... *E. jenkinii*

4a Sporangiophores basitonously branched, 250–300 µm; globose sporangiospores 3–5 µm, chlamydospores absent . *E. basiparvispora*
4b Sporangiophores meso- to acrotonously branched; globose sporangiospores 2–3 µm diam 5

5a Without any trace of columella ..... *E. parvispora*
5b With distinct columella ..... *E. galaxiae*

Linnemannia Vandepol & Bonito, Fungal Diversity 104: 282. 2020.

The genus *Linnemannia* represents a comparatively species-rich lineage of *Mortierellaceae* including at least 26 species; six of them are newly described in this paper (Fig. 16). *Linnemannia* species are usually isolated from soil but have also been isolated from fungal sporocarps.

Linnemannia hyalina has abundantly, basitonously branched, tall sporangiophores (up to 2 000 µm), few spored sporangia, and abundant chlamydospores.

Type species: *Linnemannia hyalina* (Harz) Vandepol & Bonito

Linnemannia bainierella Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 4, 5. Index Fungorum IF 559290.

Etymology: *Linnemannia bainierella* is a diminutive form - using the Latin suffix -*ella*, and refers to the sister species *L. bainieri*. This species has smaller sporangiophores and sporangiospores than *L. bainieri*.

Typus: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti (**holotype** IBF 20190164, culture ex-type CBS 149293 = JMRC SF015105, GenBank Acc. No. ITS MT380864, LSU MZ981756, *RPB1* ON611699).

Diagnosis: *Linnemannia bainierella* has branched sporangiophores reaching 350–730 µm, multi-spored sporangia, and cylindrical or ovoid, smooth sporangiospores of 5.2–7.2 × 2.6–4.1 µm.

Colonies fast growing producing white cottony aerial mycelium at 16 °C on PDA, but extremely translucent with poor formation of aerial mycelium on LCA, SE, and WA. Sporulation on PDA, LCA, and WA, odour faintly of garlic. *Sporangiophores* arising from aerial and substrate mycelium, mesotonously branched, 350–600(–730) µm tall (n = 8), tapering from 6.6–10.1(–14) µm to 3.0–3.6 µm at the tip. *Sporangia* hyaline, round, smooth-walled, 19–33 µm diam (n = 5), multi-spored, after spore liberation with distinct collarette, no columella. *Sporangiospores* hyaline, cylindrical, smooth 5.0–7.0 × 2.5–4.0 µm (n = 30) (Mean ± SE = 4.9 ± 1.37 µm). *Chlamydospores* and *zygospores* not observed.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE, with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Additional strains examined: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti, cultures Pr1s12, Pr1s13, Pr1s20 (Supplementary Table S1).

Habitat: Subalpine forest soil in Austria.

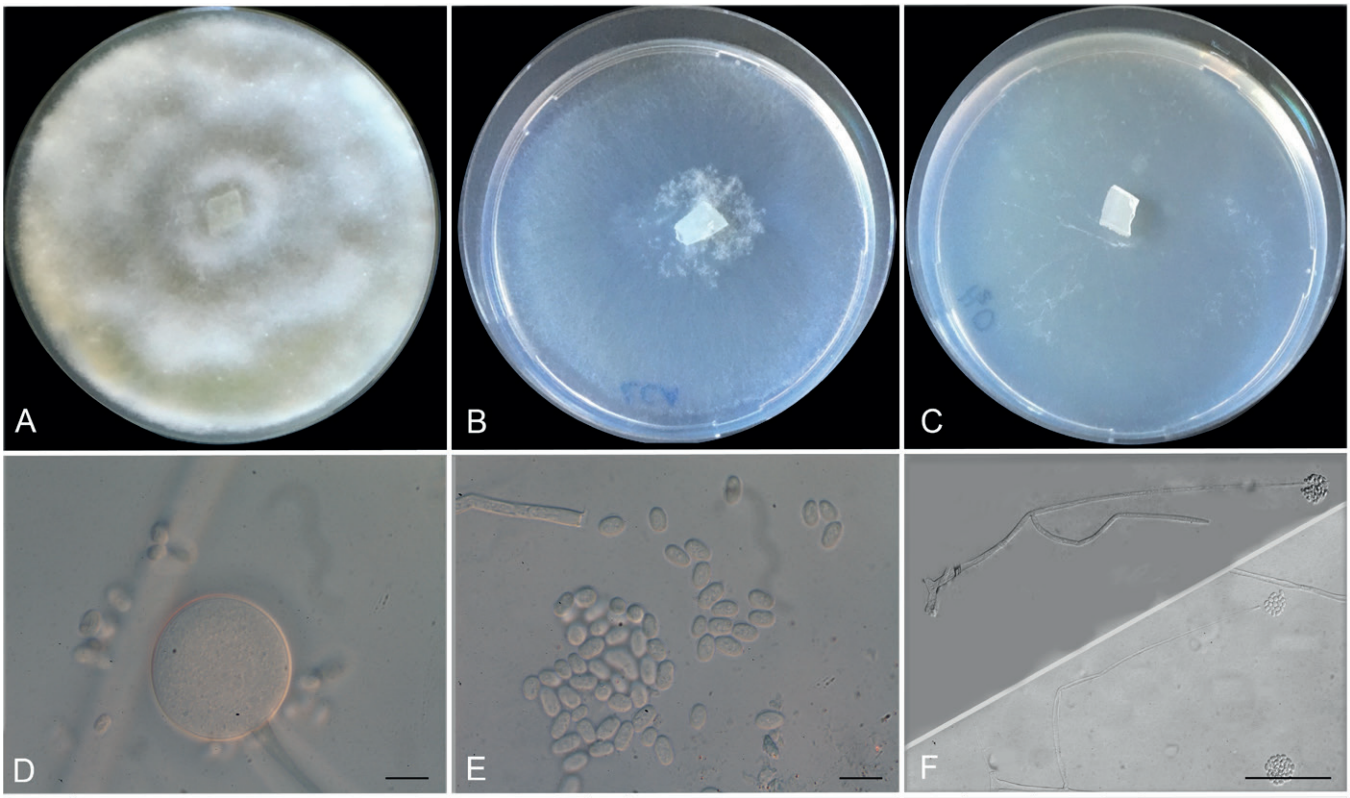

Fig. 4. Colony morphology of *Linnemannia bainierella* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Linnemannia bainierella*. **D.** Sporangium and sporangium filled with spores. **E.** Cylindrical sporangiospores and sporangiophore after dehiscence of peridium, with typical collarette at the tip. **F**. Unbranched and branched sporangiophore. All the microscopic structures of this ex-type strain were observed on LcA medium. Scale bars: D, E = 10 µm; F = 20 µm.

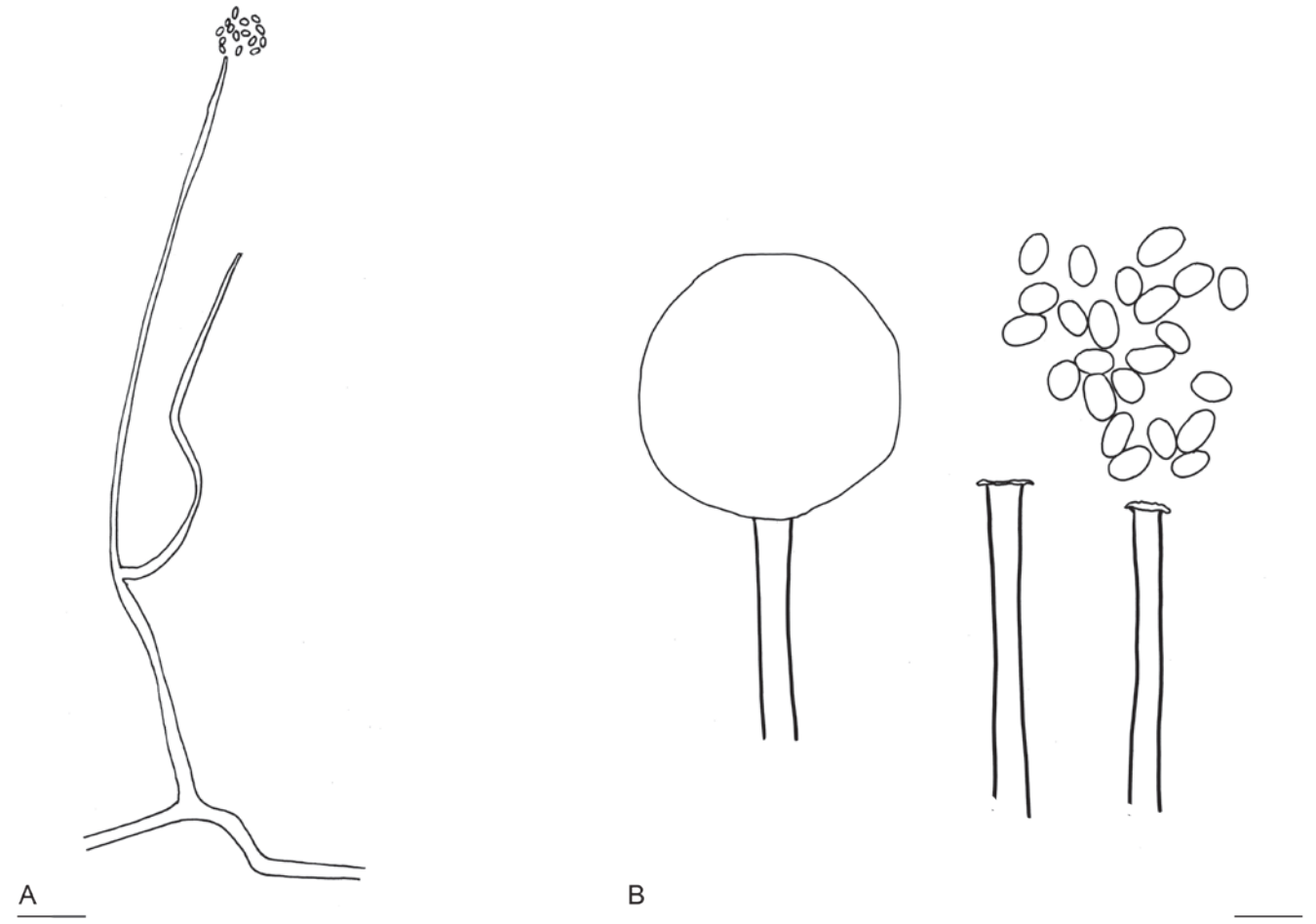

Fig. 5. *Linnemannia bainierella*. **A.** Sporangiophores. **B.** Sporangium, sporangiophore tip and sporangiospores. Scale bars: A = 40 µm; B = 10 µm.

Notes: *Linnemannia bainierella* is closely related to *L. bainieri*. The sister group relationship of the *L. bainieri* species complex could not be resolved based on rDNA or *RPB1* sequence analysis (Figs 29, 30), but based on tree topology this group seems to have an isolated position in the genus. The *L. bainieri* complex includes species with at least 500 µm tall, abundantly-branched sporangiophores, sporangia multi-spored, cylindrical sporangiospores > 4.5 µm long, no chlamydospores (Figs 4, 5).

Linnemannia friederikiana Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 6, 7. Index Fungorum IF 559293.

Etymology: This species is named in honour of Dr Friederike Göbl, an Austrian biologist. She is an early female pioneer in mycological research, especially concerning mycorrhiza of *Pinus* and *Picea* forests in alpine areas.

Typus: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti (**holotype** IBF 20190169, culture ex-type CBS 149263 = JMRC SF015090, GenBank Acc. No. ITS MW419933, LSU MZ981755, *RPB1* ON774872).

Diagnosis: *Linnemannia friederikiana* has up to 700 µm tall sporangiophores, sporangia producing irregularly shaped, roundish to ovoid, smooth or warty sporangiospores of 9.0–12.5 µm diam and chlamydospores produced in pairs or clusters.

Colonies fast growing, no pattern formed, with white cottony aerial mycelium on PDA, extremely translucent on LCA, SE, and WA, sporulation on PDA, LCA, and SE, odour unpleasant of wet dog. *Sporangiophores* arising from aerial and substrate mycelium, branched and unbranched sporangiophores present, unbranched sporangiophores 200–515 µm tall, pronounced branching on WA, mesotonously branched sporangiophores 292–700 µm tall (n = 30), tapering from 9.5 µm at the base to 2.8–5.0 µm at the tip. *Sporangia* hyaline, round, smooth-walled, 18–37 µm (n = 30) after spore liberation with collarette and columella. *Sporangiospores* hyaline, smooth-walled and spiny, irregular in shape, spores mainly produced on the substrate mycelium, roundish, 9.2–18.2 × 8.9–16.8 µm (Mean ± SE = 12.2 ± 2.6 µm) (n = 30). *Chlamydospores* thick-walled, appearing in pairs or cluster in the substrate mycelium on PDA and LCA. *Zygospores* not observed.

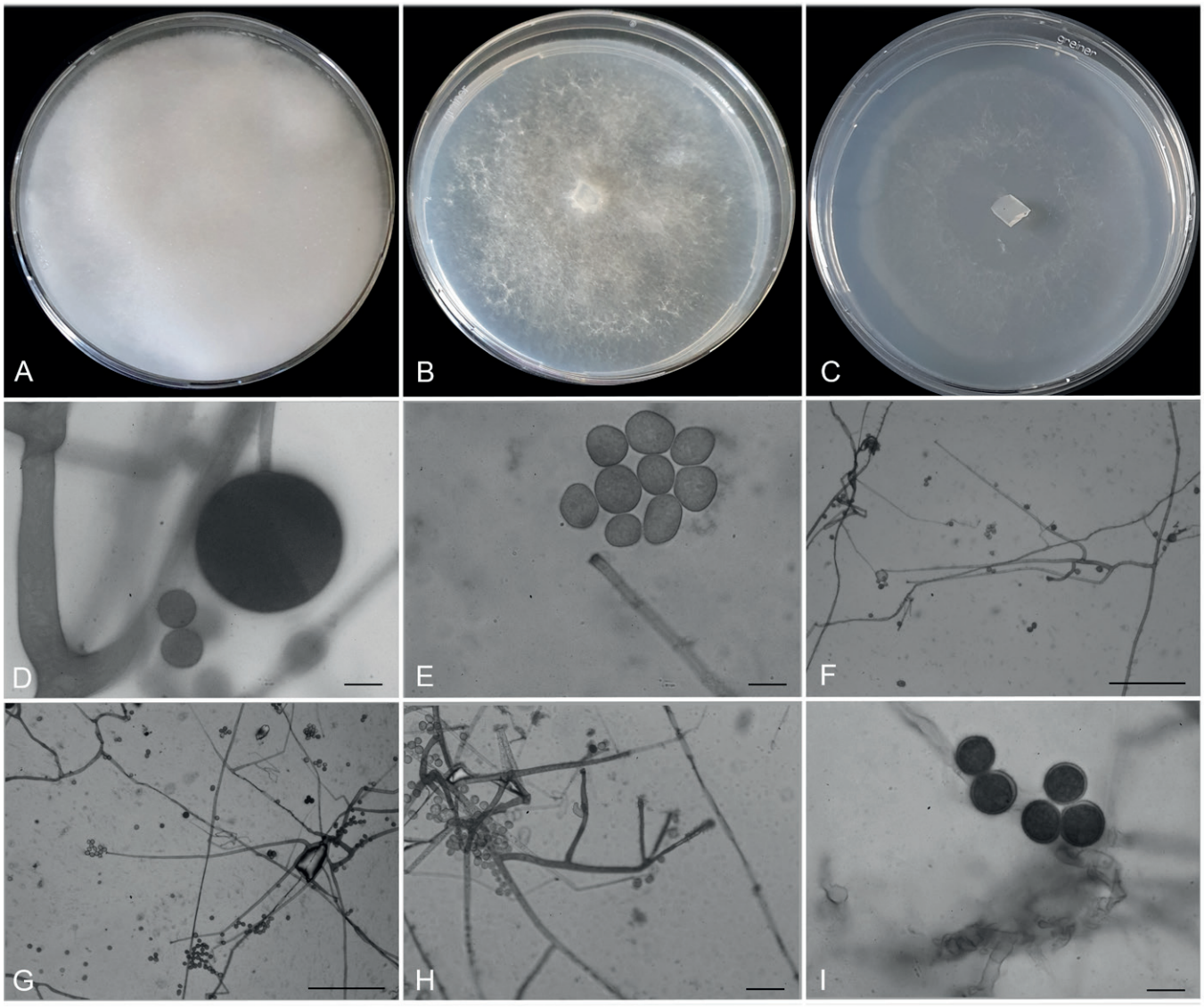

Fig. 6. Colony morphology of *Linnemannia friederikiana* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A**. PDA. **B**. LCA. **C**. WA. Asexual morphology of *Linnemannia friederikiana*. **D.** Sporangium. **E.** Sporangiospores and tip of sporangiophore without columella and collarette. **F–H.** Meso, unbranched, acrotonous branching of sporangiophore. **I.** Chlamydospores. (D–H) Sporangium, sporangiospores, tips, branching were observed on WA, whereas the chalmydospores (I) were observed on PDA medium. Scale bars: D, E, H, I = 10 µm; F, G = 20 µm.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C. At 25 °C with reduced aerial mycelium and sometimes colour of the mycelium changes to yellow due to the production of oil.

Additional strains examined: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest 5 Jun. 2019, A. Telagathoti, cultures Pr3s8, Pr3s12; Kühtai, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti,culture Ks1_7; Patscherkofel, soil from *Pinus cembra* forest, culture Patw2_7 (Supplementary Table S1).

Habitat: Coniferous forest soil; growing also during the snow-covered period.

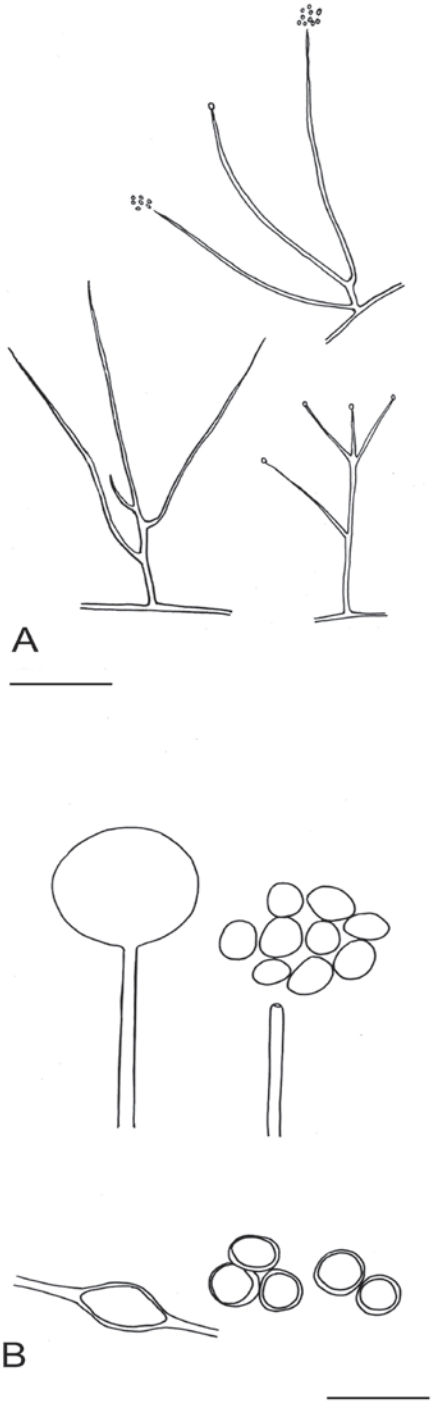

Fig. 7. *Linnemannia friederikiana.* **A.** Sporangiophores; **B.** Sporangium, sporangiophore tip, sporangiospores, chlamydospores. Scale bars: A = 20 µm; B = 10 µm.

Note: *Linnemannia friederikiana* differs from *L. zonata* by the azonate pattern of the mycelium and shorter sporangiophores.

Linnemannia mannui Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 8, 9. Index Fungorum IF 558847.

Etymology: Mannu = soil, in Telugu language. Telugu is a Dravidian language spoken in an area north of Madras, India, and running inland to Bellary. The literature, beginning in the 10th or 11th century, is mainly poetry and secular and religious epics.

Typus: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti (**holotype** IBF 20190167**,** culture ex-type CBS 149271 = JMRC SF015094, GenBank Acc. No. ITS MW042231, LSU MZ981765, *RPB1* ON774871).

Diagnosis: *Linnemannia mannui* has 150–450 µm tall, branched sporangiophores, and multi-spored sporangia producing cylindrical, smooth sporangiospores of 6.0–9.0 × 3.5–5.0 µm.

Colonies fast growing, white cottony mycelium producing abundant white exudate droplets on PDA at 16 °C, without pronounced pattern, sporulation poor on PDA, but good on WA and LCA, odour a mixture between garlic and fruity. *Sporangiophores* formed on aerial mycelium and substrate mycelium, then with rhizoids, unbranched or mesotonously or acrotonously branched 162–448 µm (n = 6) tall, tapering from a 4.8–7 µm wide base to 1.4–1.5 µm at the tip. If present, rhizoids base up to 14.2 µm in diam (n = 3); tips sometimes swollen up to 4 µm diam. *Sporangia* hyaline, round, smooth-walled, 21–27 µm in diam (n = 3); leaving an inconspicuous collarette after spore liberation; with columella. *Sporangiospores* hyaline, smooth-walled, cylindrical, 5.5–11.5 × 4.0–11.0 µm (n = 30) (Mean ± SE = 7.3 ± 1.8 µm). No *chlamydospores* or *zygospores* present.

Temperature requirements: Rapidly on PDA at 16 °C. Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Additional strain examined: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti, culture Pr2s23 (Supplementary Table S1).

Habitat: *Linnemannia mannui* was isolated from forest soil with *Pinus cembra* and *Picea abies* vegetation.

Note: *Linnemannia mannui* differs from *L. exigua* by the taller sporangiophores, cylindrical spore shape, and lacking chlamydospores.

Linnemannia nimbosa Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 10, 11. Index Fungorum IF 559292.

Etymology: *nimbus* = Latin cloud; referring to the cloudy and fluffy appearance of the colonies on PDA and LCA.

Typus: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 5 Jun. 2019, A. Telagathoti (**holotype** IBF 20190166, culture ex-type CBS 149264 = JMRC SF015092, GenBank Acc. No. ITS MW042228, LSU MZ981762, *RPB1* ON774865).

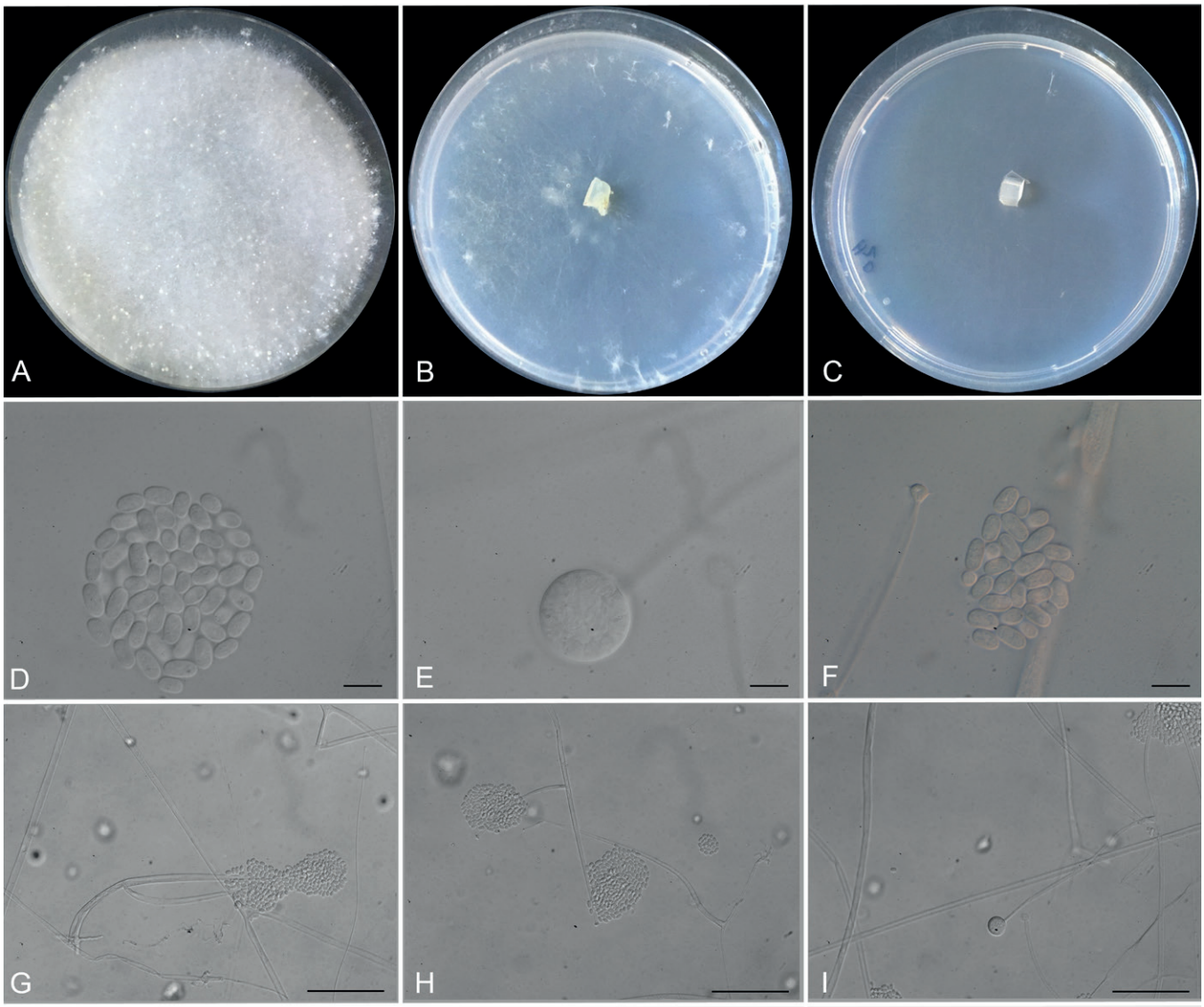

Fig. 8. Colony morphology of *Linnemannia mannui* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Linnemannia mannui.* **D.** Cylindrical spores. **E.** Globose sporangium. **F.** Typical collarette at the tip of the sporangiophore after dehiscence of peridium, sporangium with several sporangiospores. **G.** Mesotonously branched sporangiophore with rhizoids at the base. **H, I.** Acrotonously branched sporangiophore. All the microscopic features of this ex-type strain were observed on LCA. Scale bars: D–F =10 µm; G–I = 20 µm.

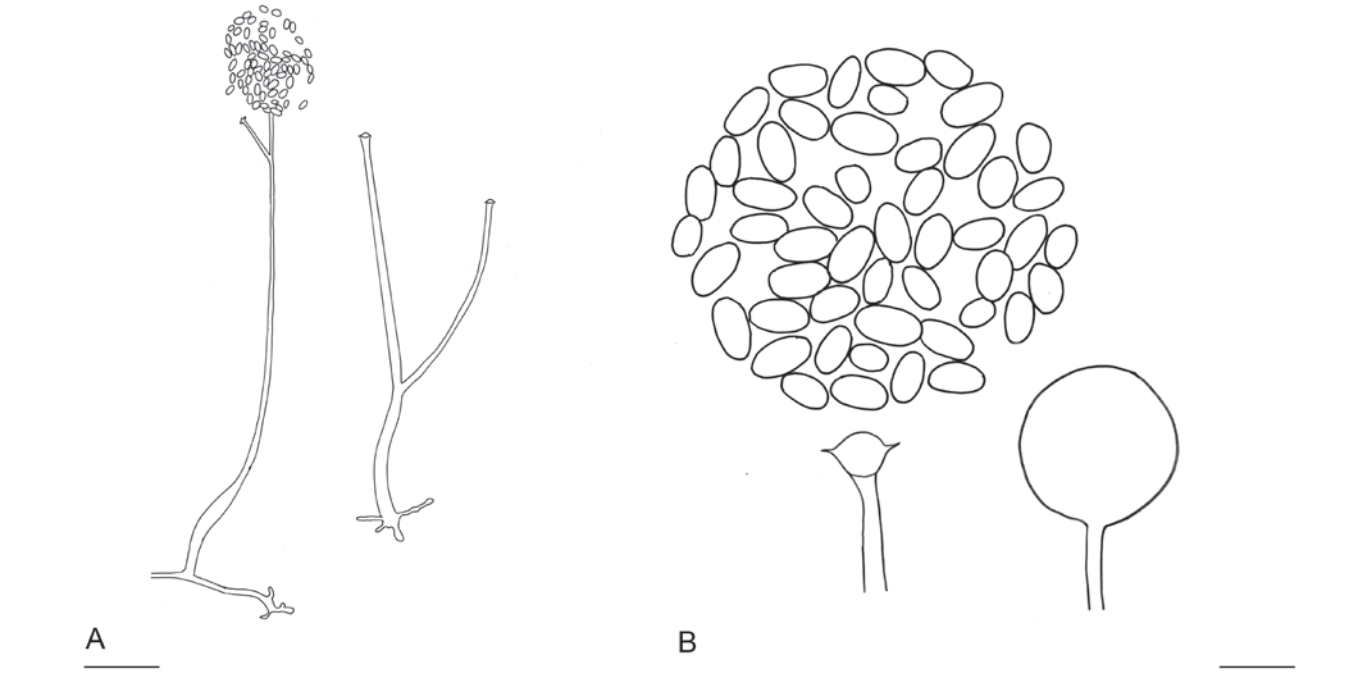

Fig. 9. *Linnemannia mannui.* **A.** Sporangiophores. **B.** Sporangium, sporangiophore tip with columella, sporangiospores. Scale bars: A = 40 µm; B = 10 µm.

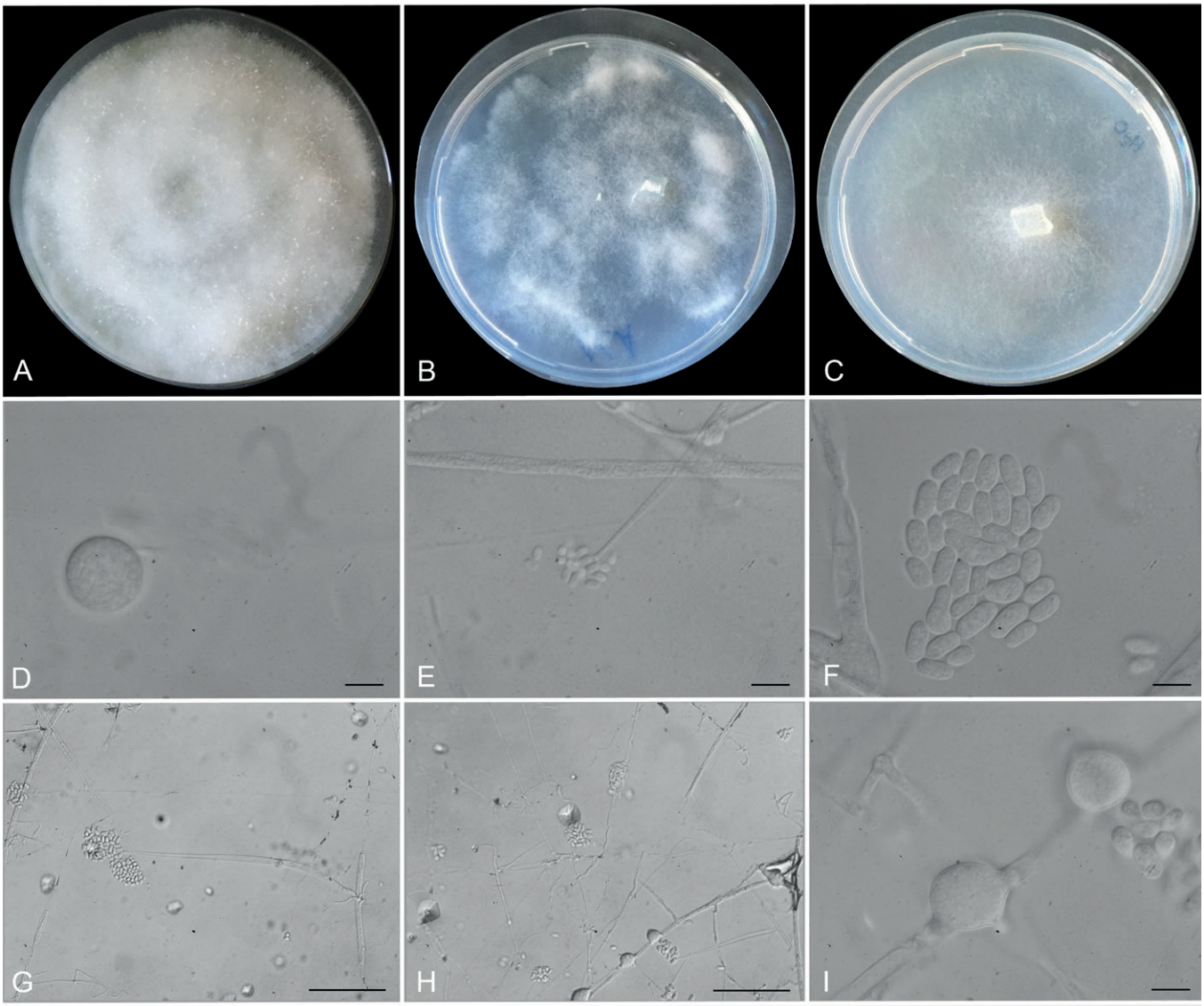

Fig. 10. Colony morphology of *Linnemannia nimbosa* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Linnemannia nimbosa.* **D.** Globose sporangium. **E.** Sporangium after dehiscence of peridium, with typical collarette at the tip of the sporangiophore sporangium with several sporangiospores. **F.** Cylindrical spores. **G, H.** Sporangiophore with and without the rhizoids at the base. **I.** Chlamydospores. All microscopic features of this ex-type strain were observed on SE agar. Scale bars: D–H, I = 10 µm; G, H = 20 µm.

Diagnosis: Sporangiophores unbranched < 200 µm tall, sporangiospores cylindrical, 7.0–11.5 × 3.5–6.5 µm. *Linnemannia nimbosa* differs from *L. exigua* by shorter sporangiophores and larger sporangiospores.

Colonies fast growing rapidly, mycelium white, cottony, without pronounced pattern on PDA. Sporulation observed on SE media, odour strong of garlic. *Sporangiophores* formed on the aerial mycelium, when on the substrate mycelium with occasional rhizoids, and unbranched or acrotonously branched, 100–391 µm (n = 25) tall, tapering from 4.2–6.9 µm to 1.6–2.6 µm at the tip (n = 3). *Sporangia* hyaline, round, smooth-walled, 12–31 µm (n = 30) in diam with few spores, peridium leaving an inconspicuous collarette and columella. *Sporangiospores* hyaline, smooth-walled, cylindrical, often also irregular, 7.0–11.5 × 3.5–6.5 µm (n = 30) (Mean ± SE = 6.95 ± 2.13 µm). Intercalary and terminal *chlamydospores* present in substrate mycelium.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10-12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Additional strains examined: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 5 Jun. 2019, A. Telagathoti, culture HFSF27; Obergurgl, Rotmoos glacier forefront, 16 Jun. 2019, A. Telagathoti, culture OBS9 (Supplementary Table S1).

Habitat: Subalpine forest or alpine snow-covered soil in Tyrol (Austria, Hafelekar and Obergurgl), with *Picea abies*, *Pinus cembra, Salix reticulata*, *S. herbacea, Dryas octopetala* vegetation or from chalk dumps. The soil pH ranged from 5 to 7.

Note: *Linnemannia nimbosa* differs from typical *L. exigua* by producing cylindrical sporangiospores, and unbranched sporangiophores with rhizoids at the base.

Linnemannia scordiella Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 12, 13. *Index Fungorum* IF 559294.

Etymology: Scordo = garlic, in Greek. Resembling the strong and pungent odour of garlic, without calling it “stinky”.

Typus: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 5 Jun. 2020, A. Telagathoti (**holotype** IBF

20200168, culture ex-type CBS 149265 = JMRC SF015087, GenBank Acc. No. ITS MW042238, LSU MZ981760, *RPB1* MZ779209).

Diagnosis: *Linnemannia scordiella* is characterised by unbranched sporangiophores producing multi-spored sporangia, a pronounced columella after spore liberation and smooth, ellipsoidal sporangiospores of 6.0–10 × 4.0–5.5 µm (n = 30). Chlamydospores are thick-walled, smooth, 15–30 µm diam. Colonies with strong odour of garlic.

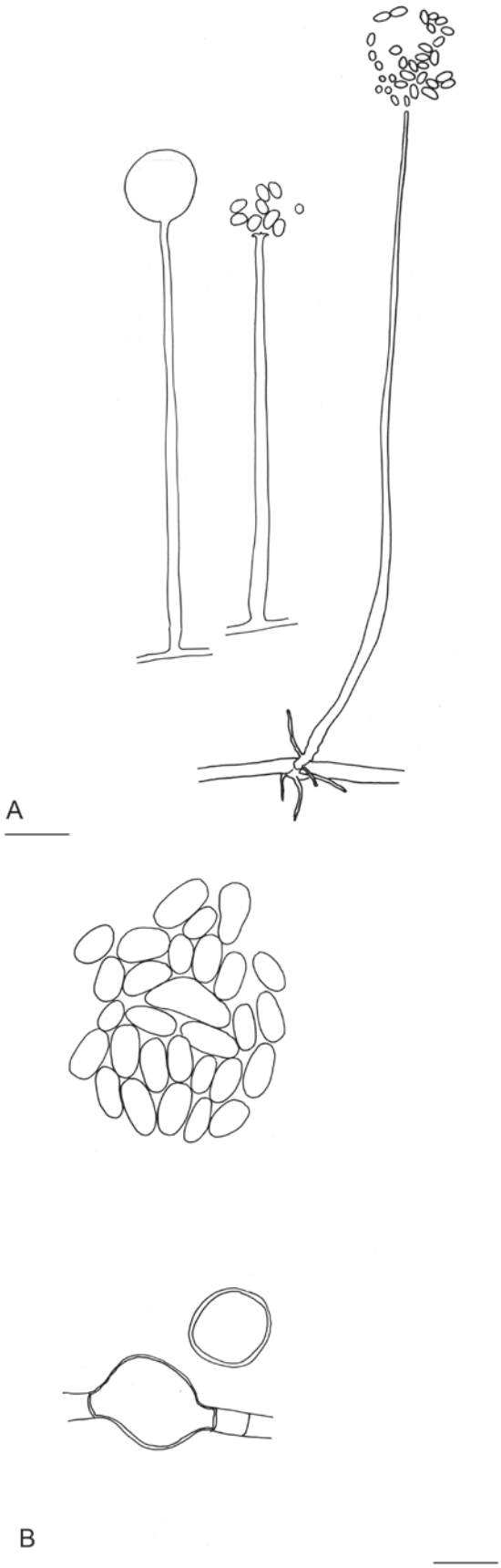

Fig. 11. *Linnemannia nimbosa.* **A.** Sporangiophores; **B.** Sporangiospores and chlamydospores. Scale bars: A = 20 µm, B = 10 µm.

Colonies fast growing with abundant white cottony aerial mycelium on PDA, colonies translucent and with poor aerial mycelium on LCA, SE, and WA, without pattern, sporulation on PDA, LCA and WA, odour strong of garlic. *Sporangiophores* often arising from the aerial mycelium, they are long and unbranched, 160–300 µm tall (n = 5), tapering from 6.4–7.7 µm at the base to 1.8–2.6 µm at the tip (n = 3), with distinct columella and collarette after spore liberation. *Sporangia* hyaline, smooth, round, multi-spored 16–33 µm (n = 3). *Sporangiospores* hyaline, smooth-walled, ellipsoidal, 6.0–10 × 4.0–5.5 µm (n = 30) (Mean ± SE = 6.07 ± 1.9 µm). *Chlamydospores* terminal and intercalary, thick-walled, smooth, 15–30 µm (n = 3). *Zygospores* not observed.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Additional strains examined: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 5 Jun. 2020, A. Telagathoti, cultures HFSF83, HFSF85 (Supplementary Table S1).

Habitat: Alpine dwarf willow habitats on calcareous soil.

Notes: *Linnemannia sclerotiella* differs from *L. scordiella* by its faint odour, branched sporangiophores few-spored sporangia, lack of columella, and larger chlamydospores covered by radiating hyphae. *Linnemannia sclerotiella* is clearly defined based on the ex-type culture CBS 529.68.

Linnemannia solitaria Telagathoti, M. Probst & Peintner, ***sp. nov.*** MycoBank MB 844592.

Typus: **Austria**, Tirol, Obergurgl, Rotmoos glacier forefield, bare soil, 10 Sep. 2016, A. Telagathoti, J. Falbesoner & P. Dresch (**holotype** IBF 20190175, culture ex-type CBS 149273 = JMRC SF013920, GenBank Acc. No. ITS MT279272, LSU MT279275).

Diagnosis: Sporangiophores unbranched, 50–200(–500) µm tall, tapering from 4–9 µm at the base to 3–4 µm near the tip, with columella. Sporangia many-spored, 15–22 µm diam with a conspicuous collarette after spore liberation. Sporangiospores subglobose to cylindrical-ovoid, smooth-walled, (6–)7–8(–10) × (5.5–)6–8 µm ($\overline{x}$ = 7.8 × 6.9 µm) (n = 30).

Description: Boonmee *et al*. (2021).

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Habitat: Alpine bare soil.

Notes: *Mortierella solitaria* Telagathoti *et al.*, Fungal Diversity 111: 301 (2021) is invalid (*nom. inval*. Art. 40.7.), and therefore it is validated here as a new *Linnemannia* species. *Linnemannia solitaria* is clearly distinct from all species in the *L. gamsii* complex: *L. gamsii* differs by branched sporangiophores, *L. fluviae* differs by branched sporangiophores with a bell-shaped apophysis.

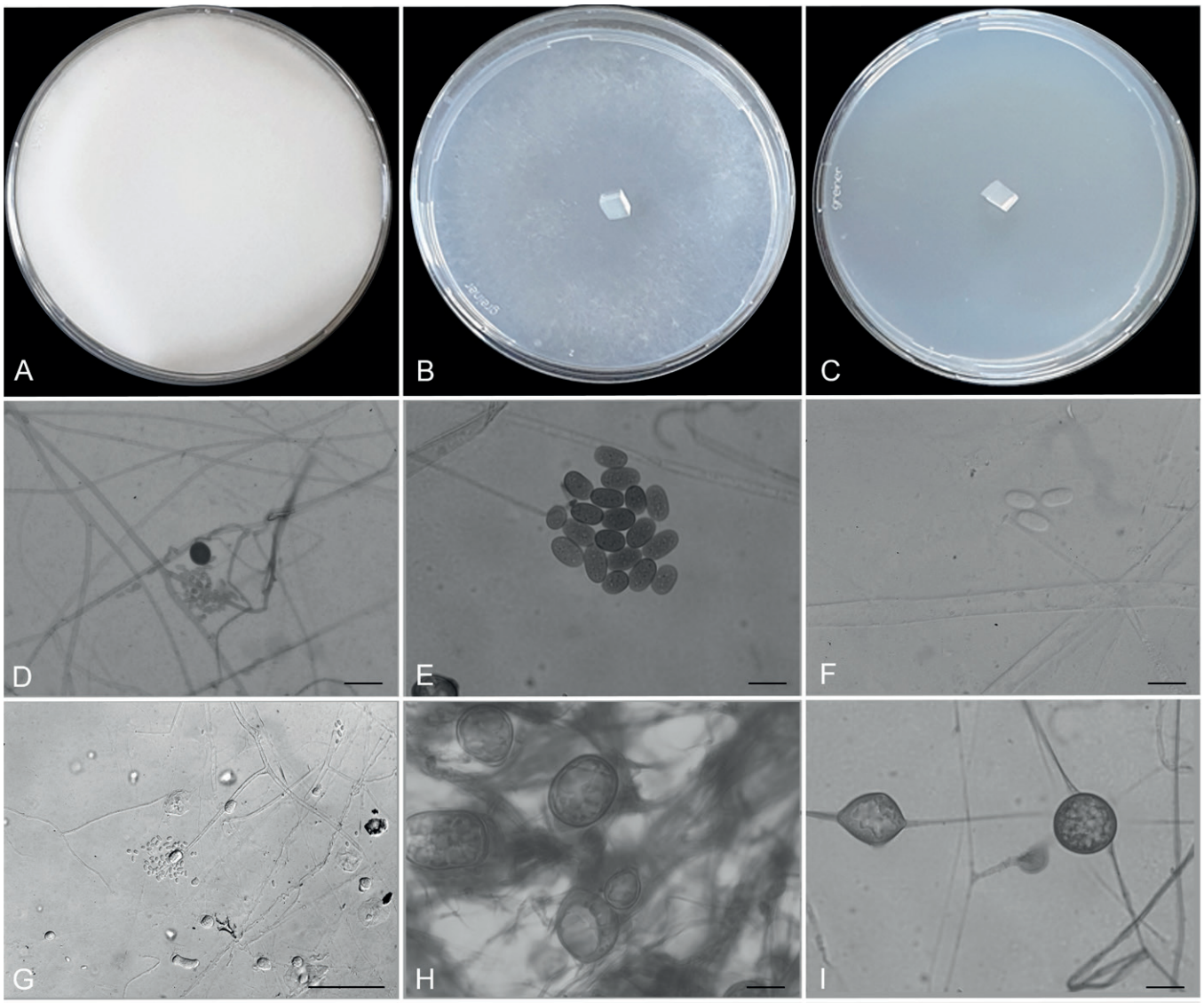

Fig. 12. Colony morphology of *Linnemannia scordiella* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Linnemannia scordiella*. **D.** Sporangia. **E.** Ellipsoidal sporangiospores. **F.** Dehiscence of peridium, with typical collarette and columella at the tip of the sporangiophore. **G.** Unbranched sporangiophore with several sporangiospores. **H, I.** Thick-walled, smooth chlamydospores. (D–G) Sporangia, sporangiospores, collarette and columella were observed on SE media whereas chlamydospores (H, I) were observed on PDA. Scale bars: D–F, H, I =10 µm; G = 20 µm.

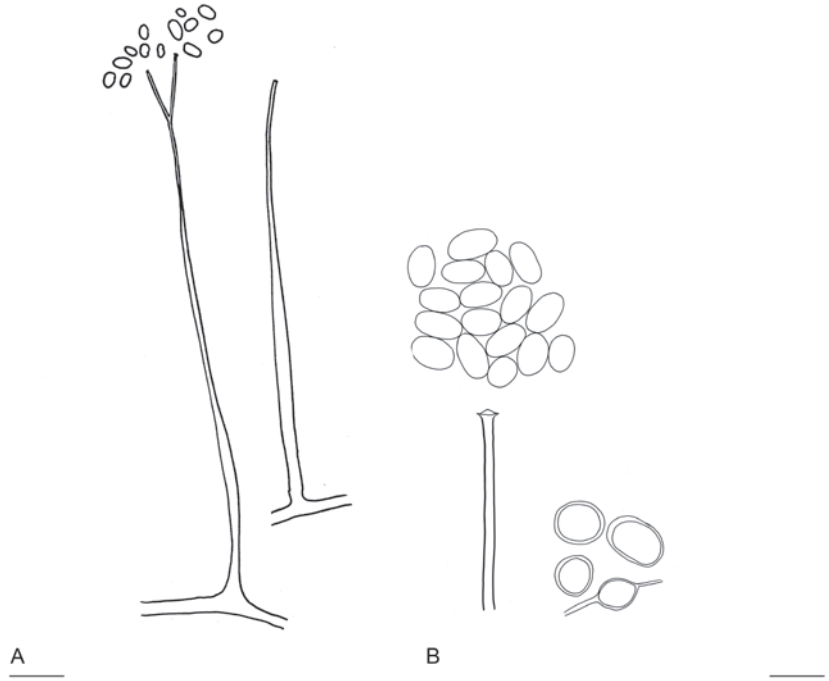

Fig. 13. *Linnemannia scordiella*. **A.** Sporangiophores. **B.** Sporangiophore tip with columella, sporangiospores and chlamydospores. Scale bars: A = 20 µm; B = 10 µm.

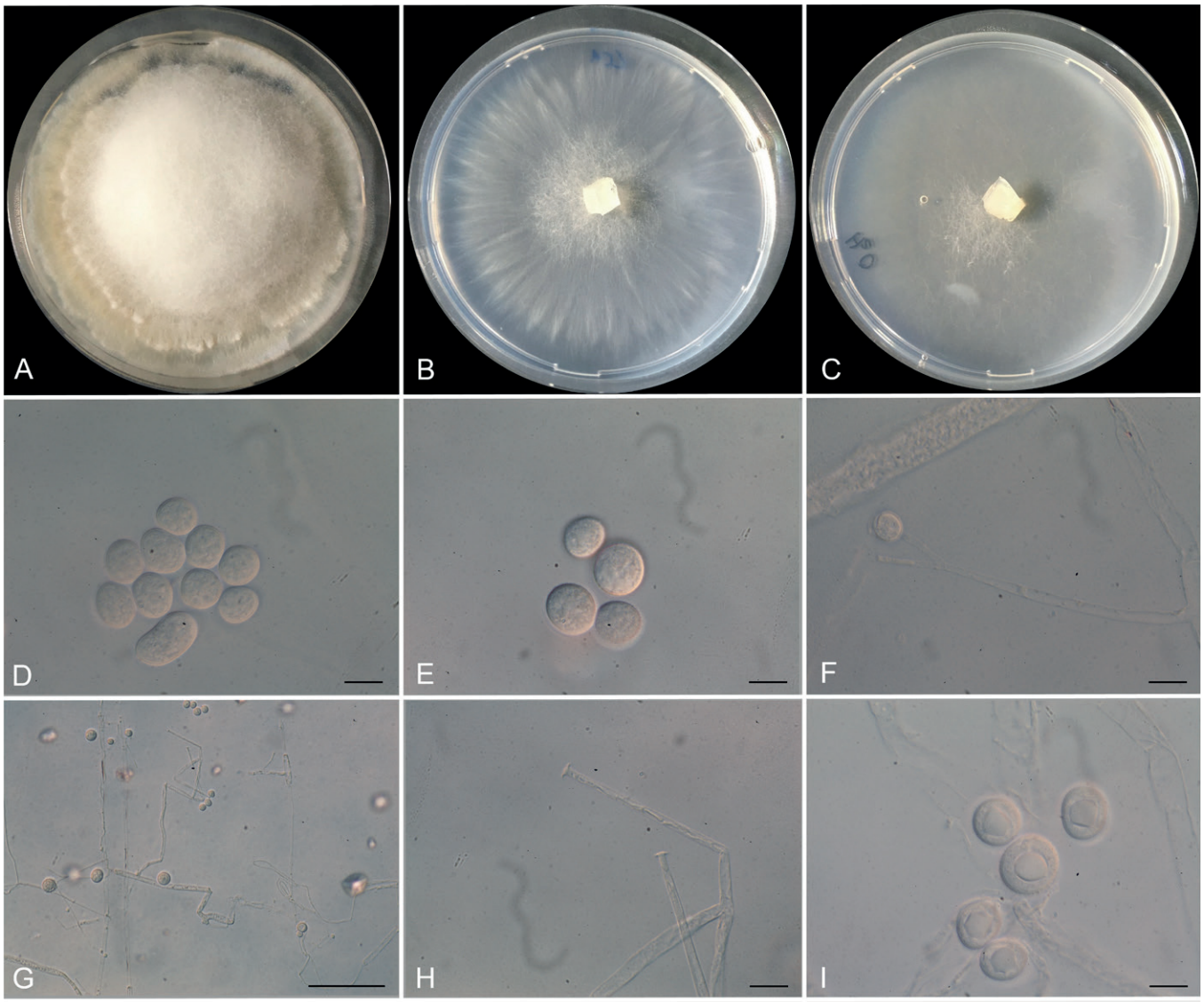

Fig. 14. Colony morphology of *Linnemannia stellaris* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Linnemannia stellaris.* **D, E.** Round, smooth and warted spores. **F, G.** Sporangiophores acrotonous branching. **H.** After the dehiscence of peridium, with typical collarette at the tip of the sporangiophore. **I.** Chlamydospores. All microscopic structures of this ex-type strain were observed on WA. Scale bars = 10 µm.

Linnemannia stellaris Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 14, 15. Index Fungorum IF 559291.

Etymology: *stellaris* = with the characteristic of stars. The culture appearance of this species on LCA resembles a star. The ornamented spores also conspicuously stand out between the smooth spores, and delight the passionate mycologist with their beautiful shape.

Typus: **Austria**, Tirol, Kühtai, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti (**holotype** IBF 20190165, culture ex-type CBS 149266 = JMRC SF015089, GenBank Acc. No. ITS MW042232, LSU MZ981764, *RPB1* ON774866).

Diagnosis: *Linnemannia stellaris* is characterised by colonies with a star-like growth pattern on LCA short, usually unbranched sporangiophores reaching 350 µm, the typical, few- to single-spored sporangioles with a collarette after spore liberation, and single-spored sporangioles producing warty spores.

Colonies fast growing on PDA at 16 °C, mycelium white, cottony, forming a rosette-like pattern with few rings on PDA, pattern star-like on LCA, sporulation poor on LCA, Hempseed, WA, as well as SE, no sporulation on PDA, odour musty, with a hint of garlic and crushed green leaves. *Sporangiophores* arising from aerial mycelium, often unbranched, sometimes acro- and mesotoneous branching, 123–264(–365) µm (n = 5) tall, tip tapering from 4–5 µm to 3 µm at the tip, and base not or only slightly enlarged (n = 3). *Sporangioles* hyaline, round, smooth, 11.5–28 µm diam (n = 30), with few spores or as single-spored sporangia, with collarette after spore liberation, with columella. *Sporangiospores* hyaline and of two types, ovoid to round, smooth or warty, 8.5–15.5 × 7.5–19.0 µm (n = 30) (Mean ± SE = 10.65 ± 2.1 µm). *Chlamydospores* terminal or intercalary, within the medium or on the surface, roundish, thick-walled, sometimes with oil droplets 11–15 µm diam. *Zygospores* unknown.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

Additional strains examined: **Austria**, Tirol, Kühtai, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti, culture Ks2_5; Patscherkofel, soil from *P. cembra* forest, 5 Jun. 2019, A. Telagathoti, culture Pats4_2; Praxmar,

soil from *P. cembra* forest, 5 Jun. 2019, A. Telagathoti, cultures Pr5, Pr10. Additional strains listed (Supplementary Table S1).

Habitat: Coniferous forest (*Pinus cembra* or *Picea abies*) soil in Austria.

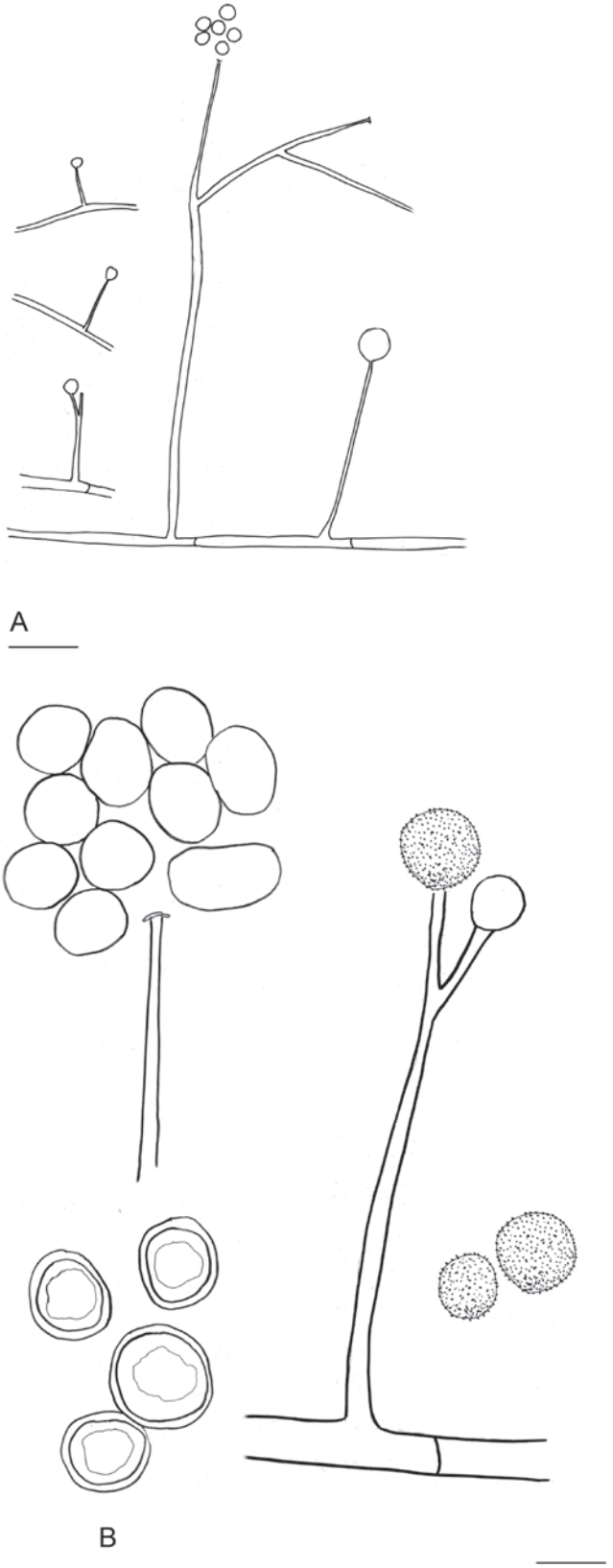

Fig. 15. *Linnemannia stellaris.* **A.** Sporangiophores. **B.** Sporangiophore tip with sporangiospores, sporangiole, spiny sporangiospores, chlamydospores with oil drops. Scale bars: A = 40 µm; B = 10 µm.

Notes: The genus *Linnemannia* is well-supported based on phylogenomics (Vandepol *et al.* 2020), as well as based on rDNA and *RBP1* phylogenies (Figs 29, 30). Within the genus there is support for the *Linnemannia bainieri* complex as well as the larger *L. gamsii* species complex including *L. camargensis, L. exigua, L. fluviae, L. gamsii, L. hyalina, L. mannui, L. nimbosa, L. schmuckeri, L. sclerotiella, L. solitaria, L. stellaris* and *L. zonata* (Fig. 16). The *L. bainieri* complex includes species with at least 500 µm tall, abundantly-branched sporangiophores, sporangia multi-spored, cylindrical sporangiospores > 4.5 µm long, no chlamydospores. The *L. gamsii* complex includes taxa with sporangiophores of 150–300(–500) µm length, at least sometimes-branched, with columella, sporangia multi-spored or few-spored, sporangiospores globose (> 6 µm diam) or cylindrical (length > around 10 µm), usually with chlamydospores. We isolated numerous strains (*e.g.* HSF41, HSF55, HSF78) (Fig. 16). However, we could not obtain sporulation irrespective of media or cultivation conditions.

Additional species of *Linnemannia*

Linnemannia fluviae (Hyang B. Lee *et al.*) Telagathoti, M. Probst & Peintner, ***comb. nov.*** Index Fungorum IF 559306.
Basionym: *Mortierella fluviae* Hyang B. Lee *et al*., Fungal Diversity 81: 254. 2016.

Typus: **Republic of Korea**, Jeonnam Province, Yeongsan River located in Gwangju (35°10'N 126°55'E), from a freshwater sample, 5 Feb. 2016, collector unknown, holotype EML-YR25716-1, preserved as metabolically inactive culture, ex-type culture EML-YR25716-1, GenBank Acc. No. ITS KX227755, LSU KX227753).

Linnemannia biramosa (Tiegh.) Telagathoti, M. Probst & Peintner, ***comb. nov.*** Index Fungorum IF 559307.
Basionym: *Mortierella biramosa* Tiegh., Ann. Sci. Nat., Bot. 1: 110. 1875.

Typus: Unknown.

Linnemannia cogitans (Degawa) Telagathoti, M. Probst & Peintner, ***comb. nov.*** Index Fungorum IF 559308.
Basionym: *Mortierella cogitans* Degawa, Mycologia 90: 1040. 1998.

Typus: **Japan**, Nagano, decaying tree bark, 26 Sep. 1996, Y. Degawa (**holotype** DM 303 (TNS), culture ex-type CBS 879.97, GenBank Acc. No. MH874286).

Linnemannia fatshederae (Linnem.) Telagathoti, M. Probst & Peintner, ***comb. nov.*** Index Fungorum IF 559309.
Basionym: *Mortierella fatshederae* Linnem., Mucorales, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe: 205. 1969.

Typus: **Germany**, in soil from a flowerpot containing *Fatshedera*, collection date and collector unknown (Zycha, Siepmann & Linnemann, 1969, Mucorales, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe: 205, fig. 98, **lectotype** designated here, MBT 10007675). **Spain**, soil, under *Pinus canarensis*, Feb. 1971, J.A. von Arx (**epitype** CBS 388.71 designated here, MBT 10007676, preserved as metabolically inactive culture, ex-epitype culture CBS 388.71, GenBank Acc. No. MH860177).

Linnemannia longigemmata (Linnem.) Telagathoti, M. Probst & Peintner, ***comb. nov.*** Index Fungorum IF 559310.
Basionym: *Mortierella longigemmata* Linnem., Mucorales, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe: 199. 1969.

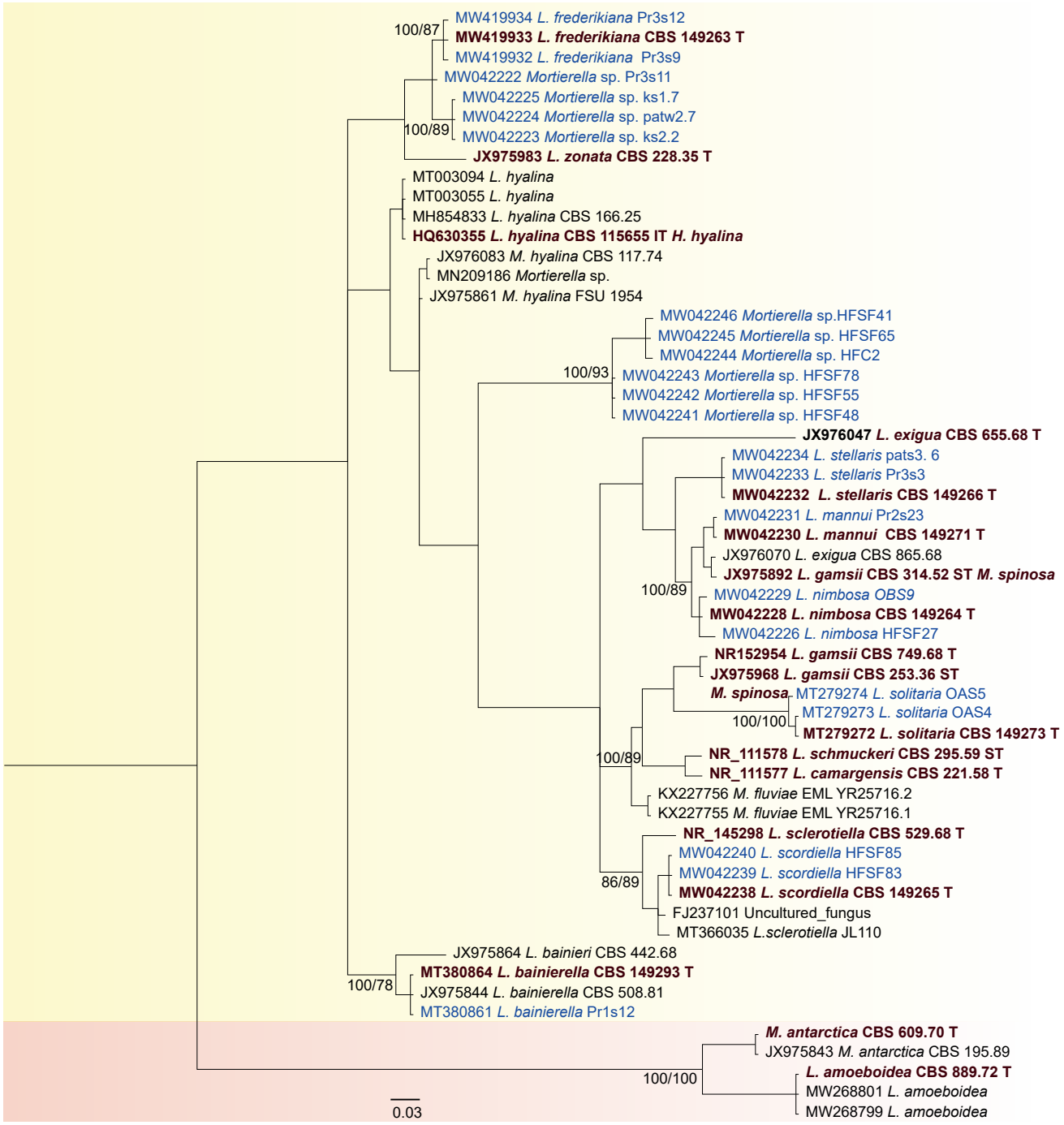

Fig. 16. Phylogenetic relationship of *Linnemannia* complex based on rDNA-ITS sequences. The Maximum Likelihood Phylogram (log likelihood -2703.19) is shown, and the branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is shown above the respective branches. Newly generated sequences are highlighted in blue, sequences generated from typus are bold and dark red. *Mortierella antarctica* and *Linnemannia amoeboidea* are used as an outgroup (light brown).

Typus: **Germany**, substrate, collection date and collector unknown (Zycha, Siepmann & Linnemann, 1969, Mucorales, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe: 199, fig. 93, **lectotype** designated here, MBT 10007677). **Germany**, Höglwald, soil, collection date and collector unknown, isolated in 1993 (**epitype** CBS 653.93 designated here, MBT 10007678, preserved as metabolically inactive culture, ex-epitype culture CBS 653.93, GenBank Acc. No. JX976055).

Linnemannia longigemmata has unbranched sporangiophores, 50–150 µm, one to few-spored sporangia, globose to slightly ovoid sporangiospores with 5–9 µm diam, and large, oil-filled chlamydospores up to 60 µm diam.

Mortierella claussenii has unbranched sporangiophores which are verticillately arranged on aerial hyphae, 7–17 µm long, sporangia (sporangiospores) globose, 5–6 µm diam. Sequences from the syntype material (CBS 294.59) and of other isolates (CBS 790.85) indicate a possible relationship to *Linnemannia camargensis* and *L. schmuckeri,* but with sequence identity of > 99 % only.

Mortierella tirolensis is morphologically similar to *M. schmuckeri,* but the original material was contaminated and lost (Zycha *et al.* 1969). Sporangiophores are 25–35 µm tall, sporangia (sporangiospores) are globose, 5–9 µm diam, anastomoses frequent. An epitype should be obtained before recombining this species into *Linnemannia*.

Key to the treated *Linnemannia* species

1a One-spored or few spored sporangiolas present, sometimes both 2
1b Sporangia multi-spored, sporangioles absent 7

2a One-spored sporangioles predominant, sporangiophores < 300 µm tall (for sporangiophores 50–150 µm: *L. longigemmata*) 3
2b With few-spored sporangioles only, chlamydospores abundant 6

3a Sporangia few- or single spored, sporangiophores 120–250 µm tall, unbranched or irregularly branched, sporangiospores of two types, warty stylospores and smooth sporangiospores present ..... *L. stellaris*
3b Sporangia always 1-spored 4

4a Sporangiophores < 50 µm long 5
4b Sporangiophores 80–200 µm tall, unbranched producing smooth-walled sporangia (sporangiospores) of 10–25 µm diam, chlamydospores small, ovoid ..... *L. zonata*

5a Sporangiophores 20–50 µm long, sporangia (sporangiospores) flattened 10–15 µm diam (for sporangia globose: *Mortierella tirolensis*) ..... *L. schmuckeri*
5b Sporangiophores 25–45 µm long, sporangia (sporangiospores) globose, 7–12 µm diam (for spores 5–6 µm diam: *Mortierella claussenii*) ..... *L. camargensis*

6a Large chlamydospores (50–100 µm diam) covered with short fimbriate hyphae, sporangia few-spored, sporangiospores globose, minutely striate, 6–10 µm ..... *L. sclerotiella*
6b Chlamydospores smooth, smaller, sporangiophores abundantly, basitonously branched, tall (200–2000 µm), sporangioles few-spored, sporangiospores more or less globose, mostly > 10 µm diam, abundant ..... *L. hyalina*

7a Sporangiospores cylindrical 8
7b Sporangiospores globose, rarely ellipsoid 14

8a Sporangiophores not branched 9
8b Branched sporangiophores present, sometimes mixed with unbranched ones 12

9a Chlamydospores absent, sporangiophores 150–450 µm tall, not branched but sometimes with mesotonous branching, sporangiospores 5.5–11.5 × 4.0–11.0 µm ..... *L. mannui*
9b Chlamydospores present 10

10a Chlamydospores with short hyphal outgrowths, sporangiophores 100–300 µm tall, usually not branched sporangiospores ovoid, 5–7 µm diam or 5 × 7 µm ..... *L. exigua*
10b Chlamydospores smooth 11

11a Sporangiophores 130–200 µm tall, unbranched, sometimes acrotonously branching, sporangia multi-spored, sporangiospores, 7.0–11.5 × 3.5–6 µm ..... *L. nimbosa*
11b Sporangiophores up to 300 µm tall (160–300 µm), sporangia multi-spored, sporangiospores cylindrical to ellipsoidal 6–10 × 4.0–5.5 µm, chlamydospores 15–30 µm diam ..... *L. scordiella*

12a At least 1 000 µm tall, abundantly branched sporangiophores present, sporangia multi-spored, cylindrical sporangiospores, 6–10 × 3–5µm ..... *L. bainieri*
12b Sporangiophores < 800 µm tall 13

13a Mesotonously branched sporangiophores up to 750 µm tall, multi-spored sporangia producing cylindrical sporangiospores, 4.5–7.0 × 3.0–4.5 µm ..... *L. bainierella*
13b Sporangiophores short, 150–450 µm tall, usually not branched, only sometimes mesotonously branching, sporangiospores, 5.5–11.5 × 4.0–11.0 µm, no chlamydospores ..... *L. mannui*

14a Sporangiophores unbranched 15
14b Sporangiophores branched 18

15a Sporangiophores short (50–200 µm); sporangiospores subglobose, 6–8 µm diam ..... *L. solitaria*
15b Sporangiophores taller 16

16a Sporangiospores cylindrical, only rarely ellipsoid, 6–10 × 4–5.5 µm, sporangiophores 160–300 µm tall, smooth chlamydospores 15–30 µm diam ..... *L. scordiella*

16b Sporangiospores clearly globose, subglobose or ovoid 17

17a Sporangiophores 100–300 µm tall, usually not branched sporangiospores ovoid, 5–7 µm diam or 5 × 7 µm, chlamydospores with short hyphal outgrowths ..... *L. exigua*
17b Unbranched sporangiophores 200–500 µm tall, multi-spored sporangia present, sporangiospores globose to ovoid, 9–12.5 µm diam, smooth chlamydospores present ..... *L. friederikiana*

18a If branching, then irregularly, tall branched and unbranched sporangiophores up to 700 µm, sporangia multi-spored, sporangiospores 9–12.5 µm diam ..... *L. friederikiana*
18b Sporangiophores more regularly branched 19

19a Sporangiophores 120–350 µm tall, acrotonously branched, some with bell-shaped apophysis, with columella, sporangiospores globose to ellipsoidal, 6.5–11.5 × 5.5–8.5 µm, chlamydospores absent ..... *L. fluviae*
19b Sporangiophores 200–400(–500) µm tall, racemously, mesotonously to acrotonously branched, sporangia multi-spored, without apophysis, sporangiospores are ellipsoid-globular < 10 µm diam (sporangiophores up to 500 µm tall, sporangiospores 6–12 µm diam: *L. gamsii* ST CBS 314.52) ..... *L. gamsii*

Mortierella Coem., Bull. Acad. Roy. Sci. Belgique, Cl. Sci. 15: 536. 1863.

The genus *Mortierella sensu stricto*, as recently amended, is now restricted to a phylogenetic lineage including the type of the genus *M. polycephala*. The genus *Mortierella* includes two major groups, the *M. polycephala* lineage and the *M. alpina* species complex (Vandepol *et al.* 2020). Here, we treat the *M. alpina* lineage in more detail. This complex includes *Mortierella* spp. with small, unbranched sporangiophores < 150 µm, often with distinctly widening base, multi-spored sporangia, sporangiophores usually with distinct collarette, and often with smell of garlic. Besides the shape of sporangiospores, also the shape of sporangia appears to be an important distinguishing character in the *M. alpina* complex. All our isolates are from alpine habitats. The rDNA ITS sequence-based phylogenetic analysis confirms that the *M. alpina* species complex includes four closely related species, which can also be separated by morphological characters: *M. alpina, M. antarctica, M. globalpina, M. lapis,* and *M. triangularis* (Fig. 20).

Type species: *Mortierella polycephala* Coem.

Mortierella alpina Peyronel I germi astmosferici dei fungi con micelio: 17. 1913. MycoBank MB 170280.

Typus: **Australia**, Victoria, sandy loam, collection date and collector unknown (**neotype** CBS 210.32 designated here, MBT 10007679, preserved as metabolically inactive culture, ex-neotype culture CBS 210.32, GenBank Acc. No. MH855290).

Culture characteristics: Domsch *et al*. (2007).

Notes: Misidentification is very common in the *M. alpina* species complex. Unfortunately, there is no authentic material available from Peyronel. In order to fix the name, CBS 210.32 was selected here as neotype: the culture characteristics and morphology are well-described (Domsch *et al.* 2007) and this morphological circumscription is widely accepted. CBS 210.32 is the ex-type culture of *M. renispora*, a later synonym of *M. alpina*. This culture was isolated from Australia, but Linnemann (Zycha *et al*. 1969) stated that it was frequently isolated from France and India as well. *Mortierella renispora* was originally distinguished from *M. alpina* based on the frequent and abundant occurrence of zygotes (about 500 µm). CBS 219.35 is the authentic culture of *M. acuminata*, which differs morphologically by forming only spiny sporangioles, but no sporangia.

Mortierella alpina produces deciduous sporangioles divided into small spores, in addition to the typical awl-shaped sporangiophores with a columella, and catenulate chlamydospores.

Mortierella lapis Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 17, 19. *Index Fungorum* IF 559297.

Etymology: *lapis* = stone, Latin. This species is named after the habitat it was isolated from, namely from gravel soil. Stone also refers to the alpine habitat.

Typus: **Austria**, Tirol, Obergurgl, Rotmoos glacier forefield, bare soil, 16 Jun. 2019, A. Telagathoti **(holotype** IBF 20190171, culture ex-type CBS 149267 = JMRC SF015098, GenBank Acc. No. ITS MT380877, LSU MZ981747, *RPB1* ON774869).

Diagnosis: *Mortierella lapis* is characterised by small, unbranched sporangiophores < 100 µm tall with swollen base, round sporangia without columella, and cylindrical sporangiospores 2.6–3.7 × 1.4–2.1 µm.

Colonies growing slowly at 10 °C with dense, white cottony aerial mycelium, with characteristic rosette pattern, sporulation poor on all media, odour of garlic. At 16 °C, growth was more rapid with less aerial mycelium on PDA. *Sporangiophores* simple, short and unbranched, arising from both the aerial and substrate mycelium; 50–80(–129) µm tall (n = 30), base irregularly swollen, often separated by a septum from the mycelium, sometimes with rhizoids, tapering from 4.7–8.2 µm at the base to 1.4–3.1 µm at the tip. The *sporangia* hyaline, smooth-walled, globose, 7–17 µm (n = 25) diam. Multi-spored, peridium leaving a distinct collarette on dehiscence, no columella. *Sporangiospores* hyaline, cylindrical, smooth, 2.6–3.7 **×** 1.4–2.1 µm (Mean ± SE = 2.5 ± 0.78 µm) (n = 31).

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 6–8 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C. At 25 °C with reduced aerial mycelium, and sometimes the mycelium becomes yellow due to oil production.

Additional strains examined: **Austria**, Tirol, Obergurgl, Rotmoos glacier forefield, bare soil, 16 Jun. 2019, A. Telagathoti, cultures OAS9, OAW4, OOW3 (Supplementary Table S1).

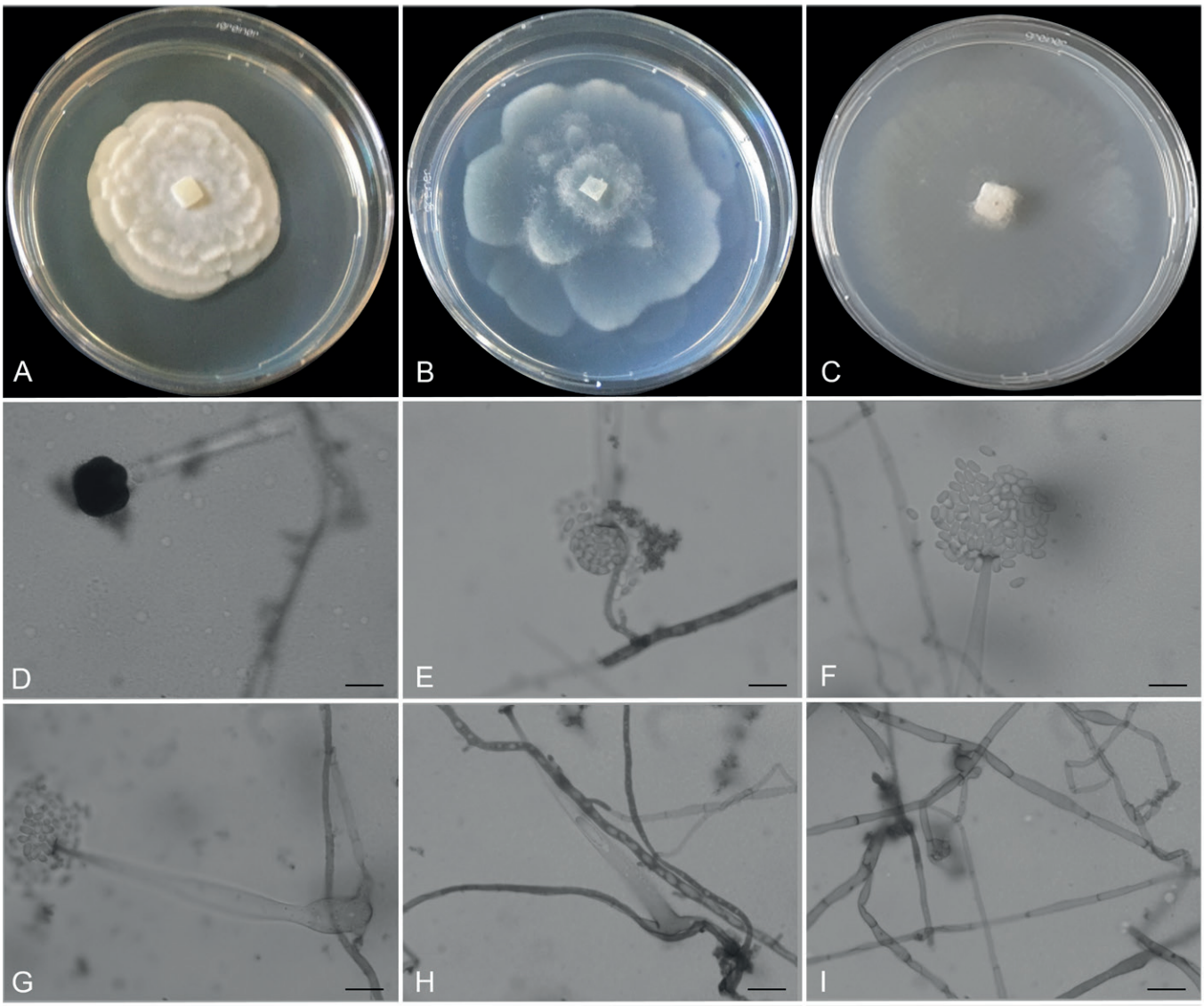

Fig. 17. Colony morphology of *Mortierella lapis* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Mortierella lapis*. **D, E.** Intact sporangia and the matured sporangia leaving sporangiospores. **F.** After the dehiscence of peridium, with typical collarette at the tip of the sporangiophore sporangium with several sporangiospores. **G, H.** Sporangiophores with swollen and wide base. **I.** Septated hyphae. All the microscopic structures for this ex-type strain were observed on SE media. Scale bars = 10 µm.

Habitat: Alpine habitats with low nutrient concentrations like glacier forefield bare terrain or sites with patchy alpine vegetation.

Notes: *Mortierella lapis* differs from *M. globalpina* by cylindrical spores, and by septa between sporangiophores and the rest of the mycelium. *Mortierella triangularis* differs by triangular sporangia and slightly larger sporangiospores (Fig. 19).

Mortierella triangularis Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 18, 19. Index Fungorum IF 559296.

Etymology: The name is based on the typical triangular shape of sporangia.

Typus: **Austria**, Tirol, Obergurgl, Rotmoos glacier forefield, bare soil, 16 Jun. 2019, A. Telagathoti, OAS8 (**holotype** IBF 20190170, culture ex-type CBS 149268 = JMRC SF015096, GenBank Acc. No. ITS MT380873, LSU MZ981741, *RPB1* ON774868).

Diagnosis: *Mortierella triangularis* is characterised by the presence of typical triangular multi-spored sporangia produced on short < 100 µm unbranched sporangiophores. The sporangiospores are ellipsoid, 2.6–4.1 × 1.7–2.6 µm. This species is differing from *M. globalpina* by the cylindrical sporangiospores, from *M. antarctica* by the much smaller sporangiospores produced, and from *M. alpina* by the lack of deciduous sporangia and chlamydospores.

Colonies growing very slowly at 0–16 °C on PDA, producing white cottony aerial mycelium on PDA, thin hyaline substrate mycelium with very reduced aerial mycelium on LCA and WA, with characteristic rosette pattern on PDA and LCA. Sporulation poor on Hempseed agar, SE, and WA. Odour faint. *Sporangiophores* arising from aerial and substrate mycelium, triangular-shaped (Fig. 19), sometimes with rhizoids 47–112 µm (n = 25) tall, tapering from the swollen base of 4 µm to 1.5–1.7 µm at the tip. *Sporangia* smooth, hyaline, often triangular when young, sometimes round, 11.5–19.5 × 9.5–19 µm (n = 30), upon dehiscence with collarette. *Sporangiospores* hyaline, smooth-walled, cylindrical, 2.6–4.1 µm × 1.7–2.6 µm (n = 30) (Mean ± SE = 2.9 ± 0.8 µm) subglobose in a few strains. *Chlamydospores* not observed. Some strains forming anastomoses, but no *zygospores*.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 6–8 mm

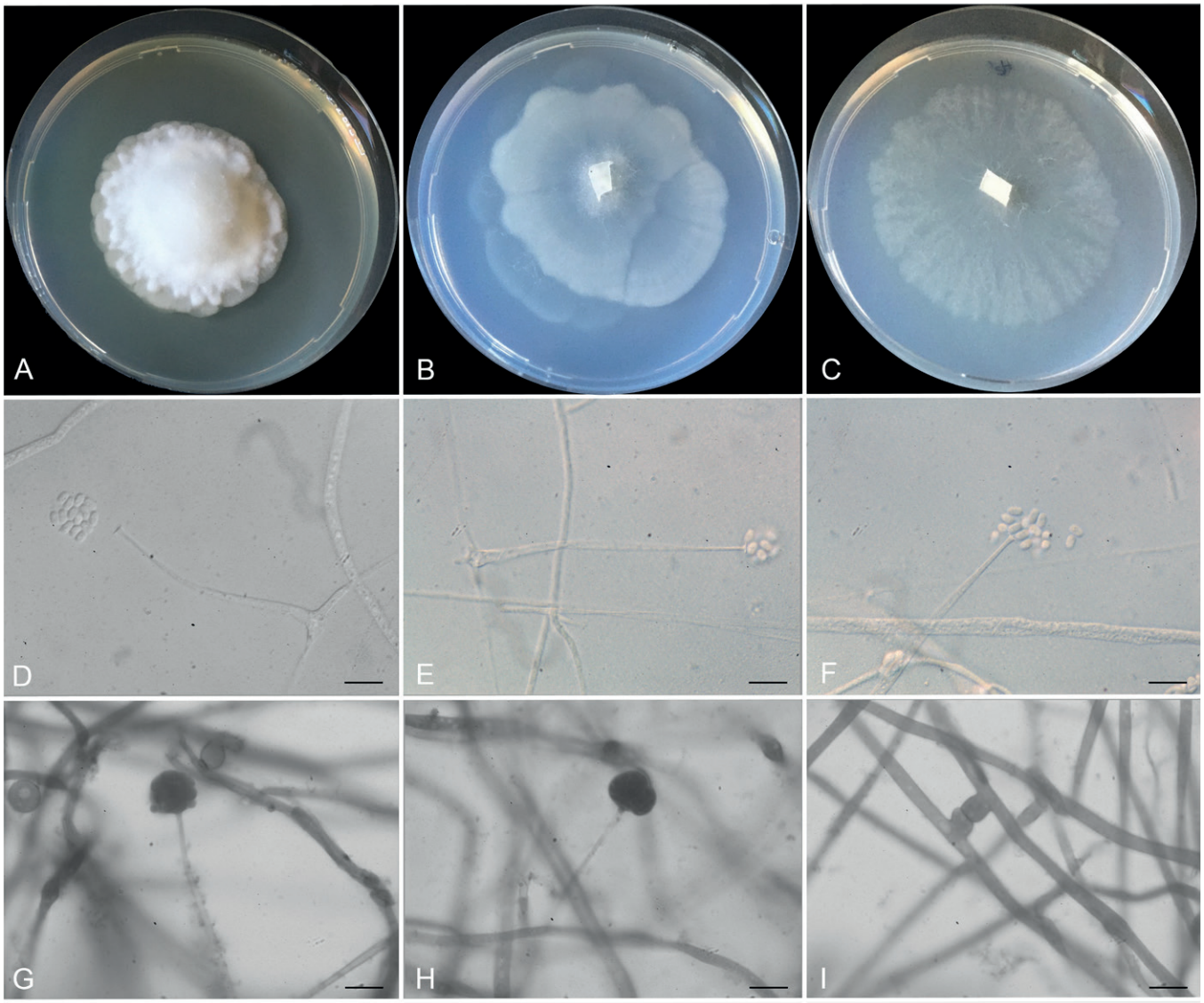

Fig. 18. Colony morphology of *Mortierella triangularis* grown on different media (9-cm-diam plates) at 16 °C after for 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Mortierella triangularis.* **D, E.** Sporangiophore with wide and sporangiophore with a swollen base. **F.** After dehiscence of peridium, with typical collarette at the tip of the sporangiophore, sporangium and several sporangiospores. **G, H.** Globose and triangular shaped sporangia. **I.** Hyphal side branches reminding of gametangia, forming anastomoses. All microscopic structures of this ex-type strain were observed on WA. Scale bars = 10 µm.

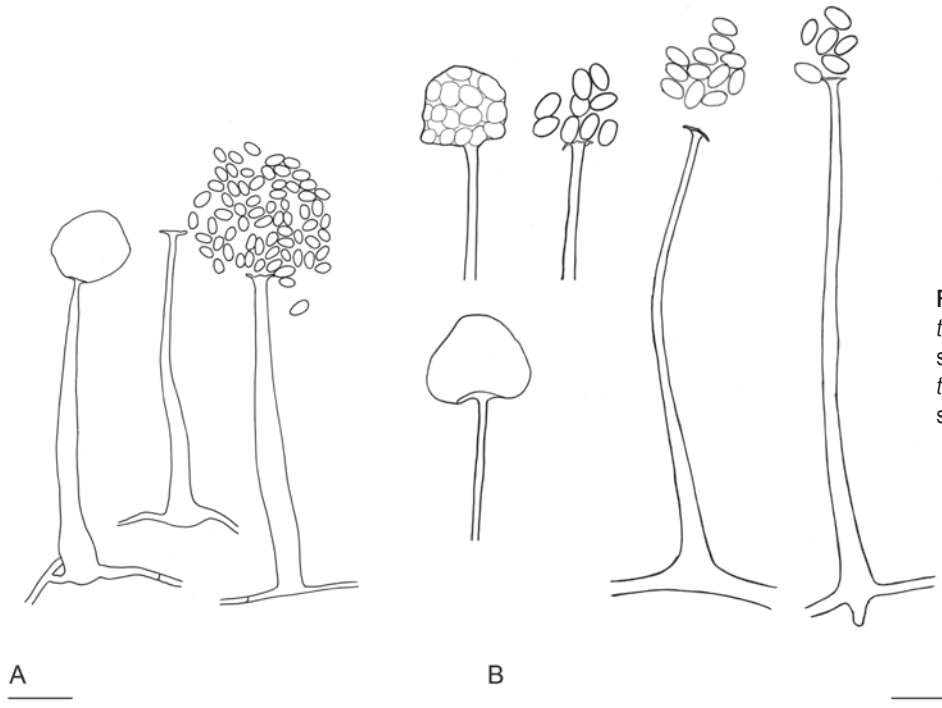

Fig. 19. *Mortierella lapis* and *Mortierella triangularis*. **A.** *Mortierella lapis* sporangiophore, sporangia and sporangiospores. **B.** *Mortierella triangularis* sporangiophore, sporangia and sporangiospores. Scale bars = 10 µm.

at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C. At 25 °C with reduced aerial mycelium and sometimes colour changes to yellow in the mycelium.

Additional strains examined: **Austria**, Tirol, Obergurgl, Rotmoos glacier forefield, bare soil, 16 Jun. 2019, A. Telagathoti, culture OBW1; Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 5 Jun. 2020, A. Telagathoti, cultures HFSF130, HFSF116, additional isolates (Supplementary Table S1).

Habitat: Soil with dwarf willow vegetation under snow fields or from the glacier forefields.

Key to species of the *Mortierella alpina* complex

1a Sporangiospores globose 2
1b Sporangiospores ellipsoid 3

2a Sporangiospores 3–10 µm, chlamydospores numerous ..... *M. antarctica*
2b Sporangiospores 3–5 µm, chlamydospores rare ..... *M. globalpina*

3a Deciduous sporangioles present in addition to multi-spored sporangia, smooth-walled chlamydospores occasionally present ..... *M. alpina*
3b Multi-spored sporangia present, deciduous sporangioles absent, cylindrical sporangiospores usually shorter than 4 µm 4

4a Sporangia globose ..... *M. lapis*
4b Sporangia triangular ..... *M. triangularis*

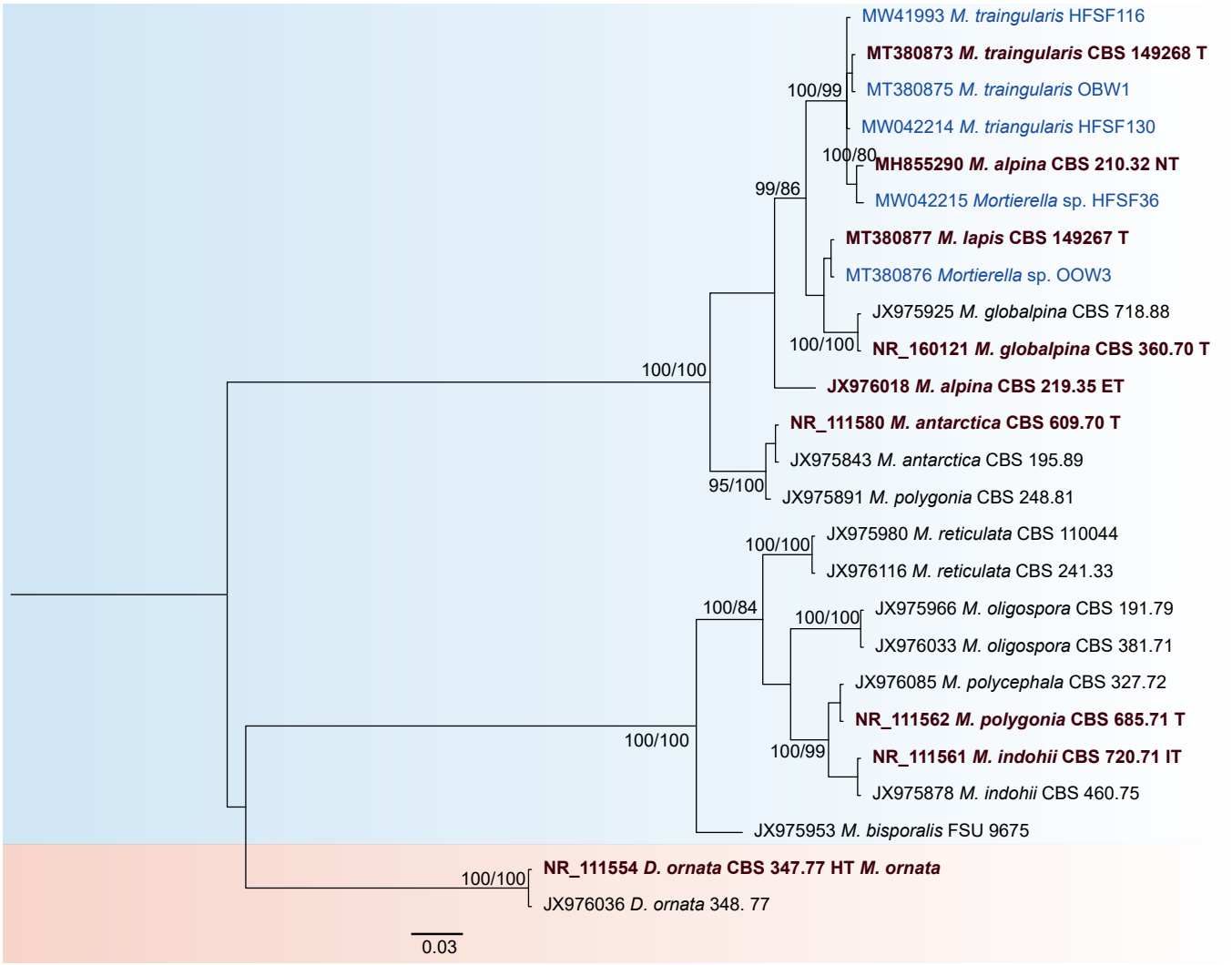

Fig. 20. Phylogenetic relationships of *Mortierella sensu stricto* based on rDNA-ITS sequences. The Maximum Likelihood Phylogram (log likelihood -1815.59) is shown, and the branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is shown above the respective branches. Sequences generated from the typus are highlighted in red. *Mortierella antarctica* and *Linnemannia amoeboidea* are used as an outgroup (light brown).

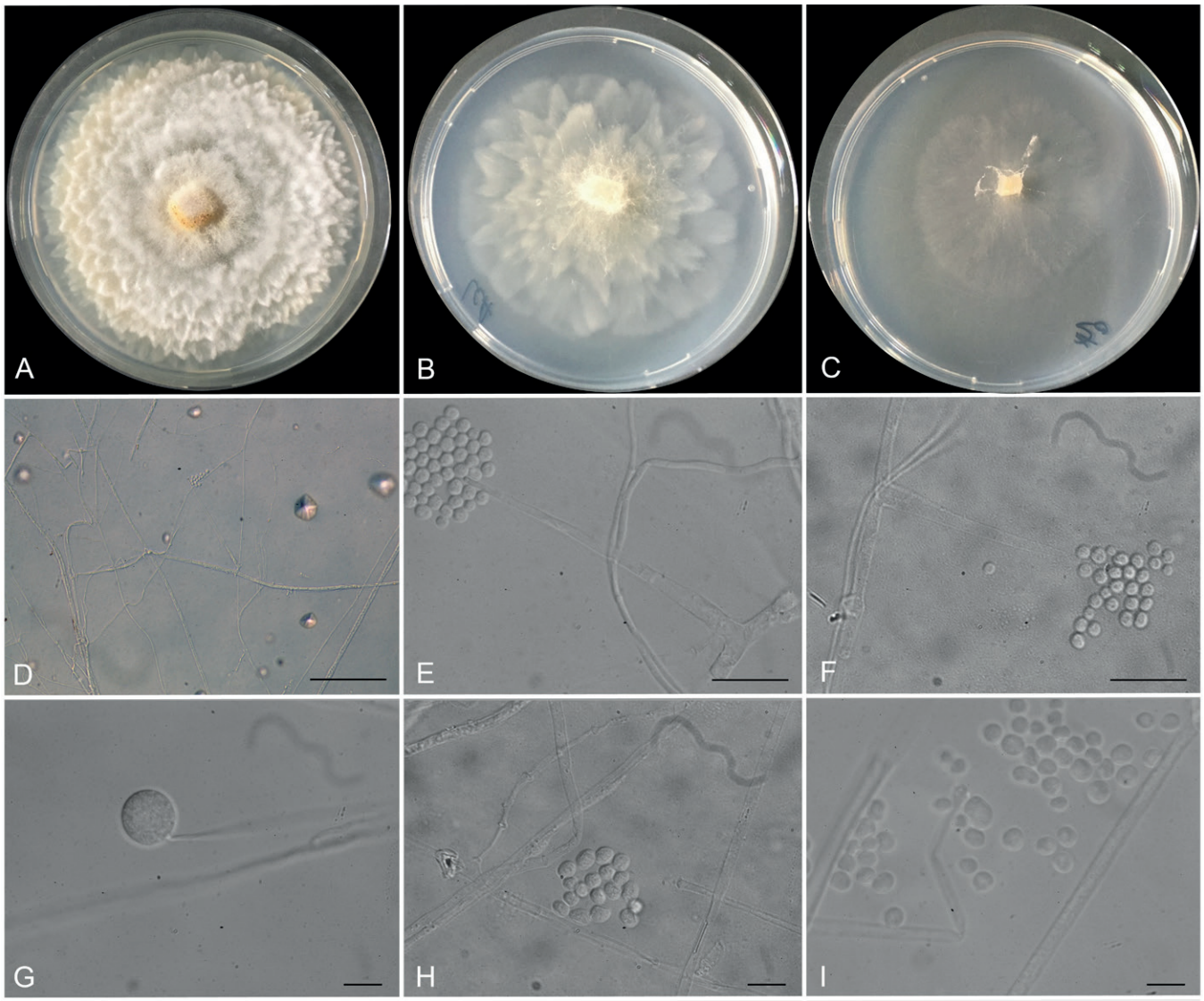

Fig. 21. Colony morphology of *Podila himami* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *Podila himami*. **D–F.** Single sporangiophore, with sporangiospores, columella, narrow tip, and a wider base. **G.** Sporangium. **H.** Sporangiophore tip with columella and sporangiospores. **I.** Sporangiospores with different size. (D–G) Single sporangiophore, sporangiospores, sporangia were observed on SE media whereas (H, I) the sporangiophore tip and sporangiospores were observed on LCA. Scale bars: D = 20 µm; E–I = 10 µm.

Podila Stajich *et al.*, Fungal Diversity 104: 284. 2020.

Podila species are generally isolated from forest soils, agricultural soils, compost or dung. Few *Podila* are reported as mycophilic fungi or mycoparasites (Rudakov 1978). Most of the species need taxonomic clarification. We here focussed on one of the several major phylogenetic lineages of the genus *Podila* (Vandepol *et al.* 2020), the *P. clonocystis* and *P. minutissima* lineage. All species in this group have short (< 120 µm tall) sporangiophores and globose sporangiospores.

Type species: *Podila minutissima* (Tiegh.) Vandepol & Bonito

Podila himami Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 21, 22. Index Fungorum IF 559298.

Etymology: *himam* = snow, in Telugu language. Telugu is a Dravidian language spoken in an area north of Madras, India, and running inland to Bellary. The literature, beginning in the 10th or 11th century, is mainly poetry and secular and religious epics. These species were isolated from the snow-covered soil only. Moreover, on LCA media the colonies resemble snowflakes.

Typus: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 16 Jun. 2020, A. Telagathoti (**holotype** IBF 20190172, culture ex-type CBS 149269 = JMRC SF015086, GenBank Acc. No. ITS MW042197, LSU MZ981749, *RPB1* ON645347).

Diagnosis: *Podila himami* has unbranched sporangiophores, globose sporangia producing globose spores of 2.5–4 µm, and is not forming chlamydospores. It differs by the unbranched sporangiophores from *P. verticillata* and *P. minutissima*, by the lack of chlamydospores from *P. clonocystis*, and by the smooth, few-spored sporangia from *P. humilis*, the latter producing 1-spored sporangia.

Colonies fast growing on PDA at 16 °C, white cottony mycelium differentiating in a narrow and dense flower-like pattern on PDA, and a broader flower pattern on LCA, producing yellow oily, droplets near to the inoculum after 10 d, good sporulation on WA and LCA, odour of garlic PDA, faintly fruity on LCA. *Sporangiophores* arising from the aerial hyphae, often single, unbranched or occasionally mesotonously branched, never verticillate, usually short, 50–150(–

172) µm (n = 25) tall, tapering from 2.3–5.5 µm at the base to 1.2–2.2 µm at the tip. *Sporangia* hyaline, round, smooth-walled, 6–14 µm diam (n = 30) with few spores, with columella. *Sporangiospores* hyaline, globose, smooth, 2.9–3.9 × 2.9–4.0 µm diam (n = 30) (Mean ± SE = 3.6 ± 0.3 µm) (Figs 21, 22). *Chlamydospores* and *zygospores* not observed.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C. At 25 °C with reduced aerial mycelium and sometimes the mycelium colour changes to yellow.

Additional strains examined: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 16 Jun. 2020, A. Telagathoti, cultures HFC24, HFSF60 (for additional strains see Supplementary Table S1).

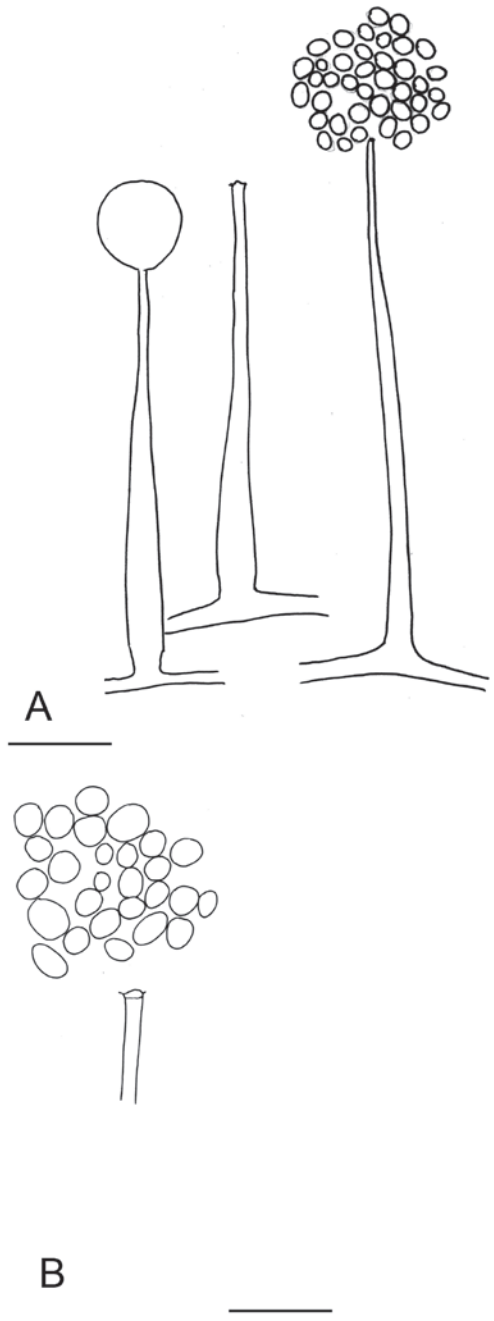

Fig. 22. *Podila himami.* **A.** Sporangiophores and sporangia. **B.** Sporangiophore tip with columella, sporangiospores. Scale bars: A = 20 µm; B = 10 µm.

Habitat: Isolated from alpine soil at an altitude of 2 250 m, with patchy *Salix reticulata*, *Salix herbacea, Dryas octopetala, chalk dumps* vegetation, and also in snow covered soil. Soil pH 5.3–6.3.

Notes: One additional strain (HFC24) deviated slightly from the holotype by sporangiophores with a narrow base of 2.7 µm diam and spore size: this strain produces a mixture of smaller and larger spores ranging from 2.5–4.3 µm diam and *Podila himami* can clearly be distinguished from both *P. minutissima* and *P. clonocystis* by mostly unbranched sporangiophores, and lack of pronounced columella and zygospores. *Podila minutissima* has often basitonously branched sporangiophores and forms zygospores. *Podila clonocystis* has long, slender, unbranched sporangiophores, which only occasionally form side branches, and typical chlamydospores of two types, round and consisting of broadened submerged hyphae. Our ITS sequence of *P. himami* is 100 % identical to CBS 226.35 (Fig. 25) (identified as *P. minutissima* by Linnemann, from Germany) and an isolate (LC515184) from Walker glacier, Canadian High Arctic, named *M. clonocystis.* Based on sequence similarity of > 99 % *P. himami* was also isolated in Japan (MF423505). Misidentification as *M. clonocystis P. minutissima* is likely. *Podila sossauensis* (*e.g.* CBS 176.74) was microscopically identified as *M. clonocystis* (Wagner *et al.* 2013), thus morphologically fitting well into this group.

Podila occulta Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 23, 24. Index Fungorum IF 559299.

Etymology: *occulta* = Latin buried or hidden. This species was isolated from snow-covered soil in an alpine habitat, and "*occultus*" refers to both, buried and hidden.

Typus: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 16 Jun. 2020, A. Telagathoti (**holotype** IBF 20190173, culture ex-type CBS 149270 = JMRC SF015103, GenBank Acc. No. ITS MW042216, LSU MZ981748, *RPB1* ON645348).

Diagnosis: *Podila occulta* has sympodially branched sporangiophores, and sporangia with several, not many, globose sporangiospores with a diam of 3.8–5.0 µm. It differs from *P. minutissima* by the smaller spores and presence of chlamydospores.

Colonies fast growing abundant white, cottony, aerial mycelium forms a rosette like pattern on PDA, no aerial mycelium on LCA, and slow growth with scarce aerial mycelium on WA, odour of garlic. Sporulation most abundant on SE at 16 °C, but also on PDA, WA, and LCA. *Sporangiophores* formed on aerial mycelium, unbranched or irregularly sympodially branched, 54–150(–170) µm (n = 30) tall, tapering from 4.5 µm at the base to 1.5 µm at the tip. *Sporangia* hyaline, round, smooth-walled, 14–22.5 µm (n = 30), few-spored, peridium leaving a conspicuous collarette, no columella. *Sporangiospores* hyaline, globose, smooth-walled, 3.6–5.7 µm (n = 30) (Mean ± SE = 4.5 ± 0.6 µm) (n = 30), clumping together in pattern similar to a bunch of flowers. *Chlamydospores* observed on LCA medium, round, 8.5–9.8 µm, hyphae of the substrate mycelium with scattered septate. *Zygospores* unknown.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

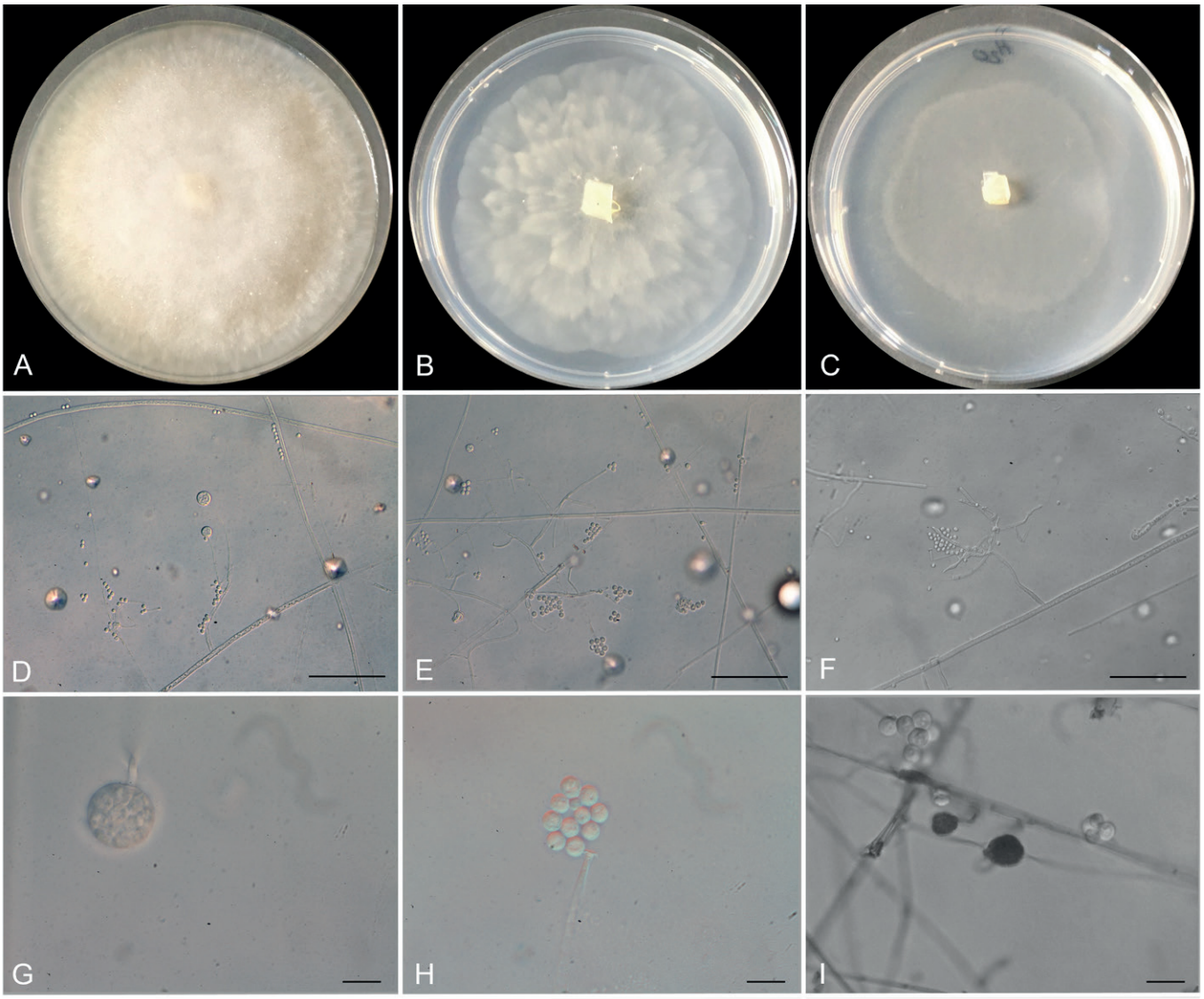

Fig. 23. Colony morphology of *Podila occulta* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA **C.** WA. Asexual morphology of *Podila occulta*. **D–F.** Branched sporangiophores. **G.** Sporangium with several sporangiospores. **H.** Sporangium after dehiscence of peridium, with typical collarette at the tip of the sporangiophore. **I.** Hyphal coils. All microscopic structures were observed on SE media except for the hyphal coils, which were observed on PDA media. Scale bars: D–F = 20 µm; G–I = 10 µm.

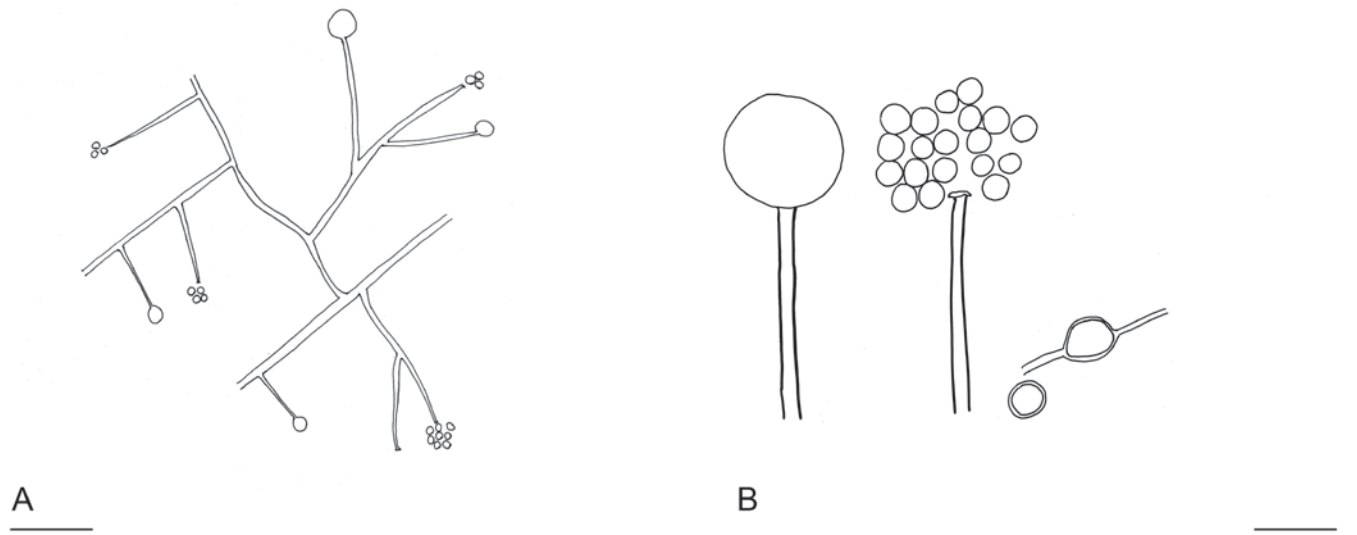

Fig. 24. *Podila occulta*. **A.** Sporangiophores on aerial hyphae. **B.** Sporangium, sporangiophore tip, sporangiospores and chlamydospores. Scale bars: A = 20 µm; B = 10 µm.

Additional strain examined: **Austria**, Tirol, Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 16 Jun. 2020, A. Telagathoti, culture HFSF52 (Supplementary Table S1).

Habitat: Calcareous soil covered by snowfields in the alpine region, covered with scattered alpine dwarf willow vegetation (*Dryas octopetala, Salix reticulata, S. retusa, Bistorta vivipara*). Soil temperature was -1.5 °C, soil pH 5.3–6.3.

Notes: *Podila occulta* differs clearly from *P. minutissima* var. *dubia* due to typically branched sporangiophores and smaller sporangiospores. This latter form was described for *Podila minutissima* isolates differing by fragile and tender, unbranched sporangiophores forming few-spored sporangia with spores 5–7 µm diam, without chlamydospores but with the tendency to form zygospores. It often forms hyphal coils (Linnemann 1941). *Podila epicladia* produces short, acrotonously branched sporangiophores < 200 µm with columella, globose sporangiospores 4–7 µm diam and scarce, lemon-shaped chlamydospores.

The species in the *Podila clonocystis* / *minutissima* lineage are difficult to resolve based on the phylogenetic resolution of ITS sequences, as they share 99 % sequence similarity. This resulted in a lot of confusion and misidentification around species in this group (Fig. 25). Phylogenetic clades of *Podila* with 99–100 % ITS sequence identity include morphologically different species, like already known for the *P. humils/verticillata* clade. However, identification based on ITS sequence analysis, only, is not reliable in such clades. The following species belong to this lineage: *P. clonocystis, P. epicladia, P. himami, P. horticola, P. minutissima, P. minutissima* var. *dubia, P. occulta* and *P. verticillata* (Fig. 28).

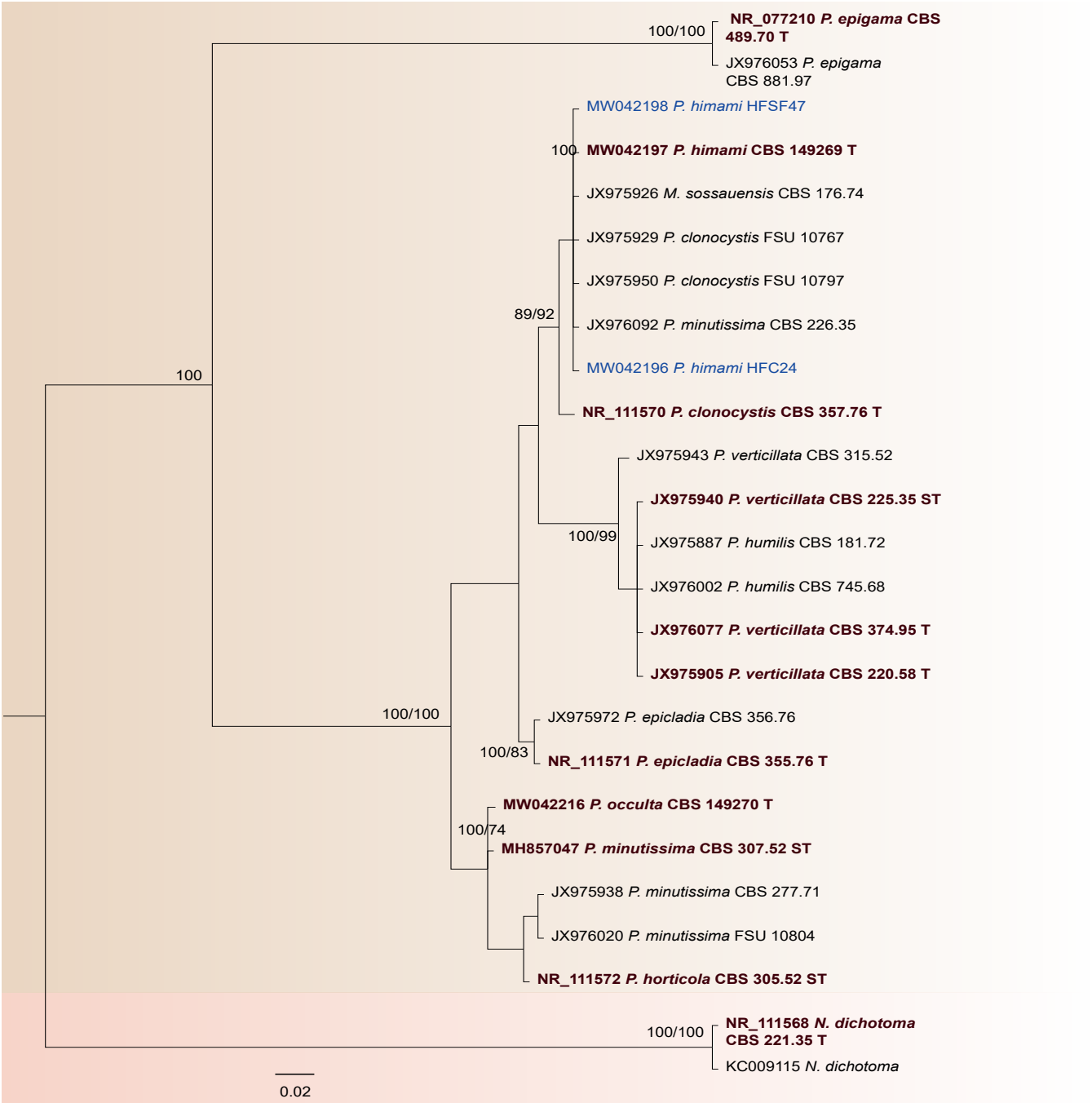

Fig. 25. Phylogenetic relationship of *Podila* species based on rDNA-ITS sequences. The Maximum Likelihood Phylogram (log likelihood -1604.45) is shown, and the branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is shown above the respective branches. Newly generated sequences are highlighted in blue, sequences generated from typus are bold and dark red. *Necromortierella dichotoma is* used as an outgroup (light brown).

Key to the *Podila clonocystis* / *minutissima* complex

1a Spinulose, 1-spored sporangioles present 3
1b One spored sporangioles absent, few- or multi-spored sporangia present 2

2a Few-spored sporangioles produced on single, unbranched, and occasionally mesotonously branched sporangiophores, with columella, sporangiospore 3–4 µm diam ..... *P. himami*
2b Sporangia multi-spored 4

3a One-spored sporangioles produced on always unbranched 40–100 µm tall sporangiophores, sporangiospores minutely spinulose ..... *P. horticola*
3b One-spored sporangioles produced on basitonously branched sporangiophores 50–200 µm tall, sporangiospores finely spinulose ..... *P. humilis*

4a Multi-spored sporangia produced on unbranched sporangiophores 5
4b Multi-spored sporangia produced on single, or sympodially and irregularly branched sporangiophores, sporangiospore 3.8–5.0 µm diam (for acrotonously branched sporangiophores see P. epicladia) ..... *P. occulta*

5a Chlamydospores present, consisting of widened hyphal branches of irregular shape, spore 2.5–4.0 µm diam ..... *P. clonocystis*
5b Chlamydospores absent 6

6a Multi-spored sporangia produced on unbranched sporangiophores sporangiospores almost globose, 5–7 µm diam ..... *P. minutissima* var. *dubia*
6b Multi-spored sporangia produced on basitonously branched sporangiophores, sporangiospores globose, 5–7 µm diam ..... *P. minutissima*

Tyroliella Telagathoti, M. Probst & Peintner*,* ***gen. nov.*** *Index Fungorum* IF 559302.

Etymology: This genus name refers to the Tyrolean origin of our fungal isolates. Tyrol is meant in a wider sense, including North-East- and South Tyrol. We also dedicate this genus to Tyrol to thank the Land Tirol for supporting our research. We keep the ending -ella as a reminder to *Mortierella*.

Diagnosis: Phylogenetic analyses of the rDNA region (Fig. 29) and the *RPB1* region (Fig. 30) clearly define the genus *Tyroliella,* as it represents a distinct lineage in the *Mortierellaceae.* The rDNA ITS sequences of *Tyroliella* spp. differ by > 14 %, and the *RPB1* region by > 16 % from the most similar sequences of *Necromortierella dichotoma* (CBS 221.35 ex-type culture). *Tyroliella* pure cultures produce abundant mycelium, multi-spored globose sporangia formed on simple or branched sporangiophores, sporangiospores are cylindrical, chlamydospores with typical hyphal appendages.

Generic description: *Tyroliella* species are characterised by branched or unbranched sporangiophores, round, smooth-walled sporangia without columella, cylindrical sporangiospores and chlamydospores with typical hyphal appendages. Based on the current stage of knowledge, it is impossible to morphologically define genera in *Mortierellaceae*, as currently recognised based on phylogenetic/phylogenomic lineages (Vandepol *et al.* 2020).

Type species: *Tyroliella animus-liberi* Telagathoti, M. Probst & Peintner

Tyroliella animus-liberi Telagathoti, M. Probst & Peintner, ***sp. nov.*** Figs 26, 27. Index Fungorum IF 559303.

Etymology: *animus liberi* = free spirit, Latin. The spirit feels free in high-altitude alpine areas, where this species was isolated. But we would also like to dedicate it to all persons with a free spirit.

Typus: **Austria**, Tirol, Finkenberg, Pfitscherjoch, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 8 Aug. 2019, A. Telagathoti (**holotype** IBF 20190174, culture ex-type CBS 149272 = JMRC SF015196, GenBank Acc. No. ITS MW042203, LSU MZ981752, *RPB1* ON774870).

Diagnosis: *Tyroliella animus-liberi* is characterised by short, unbranched sporangiophores up to 550 µm tall, hyaline, cylindrical sporangiospores, and single chlamydospores with typical hyphal outgrowths.

Colonies medium to fast growing, mycelium dense and cottony on PDA, up to 1 cm high, with numerous oil droplets which cause a pale yellowish surface colour on top, without clear pattern, sporulation on all the media but most abundant on SE, LCA and WA at 16 °C; odour of a wet dog. *Sporangiophores* arising from the aerial mycelium, simple, unbranched 78–190(–550) µm (n = 25) tall, tapering from 5.0 µm at the base to 1.5 µm at the tip. *Sporangia* hyaline, smooth-walled, round, 7–20(–27 µm) (n = 30), few-spored, with an inconspicuous minute collarette upon dehiscence, no columella. *Sporangiospores* regularly cylindrical to oblong, hyaline, smooth-walled, 3.0–5.7 × 2.2–3.3 µm (n = 30) (Mean ± SD = 3.4 ± 0.9 µm). *Chlamydospores* pale ochraceous, occurring individually with short, hyphal appendages, often dichotomously branched, up to 5 µm thick, up to 25 µm long. *Zygospores* not observed.

Temperature requirements: Optimum at 16 °C on PDA, LCA, Hempseed, WA, and SE with daily radial increments of 10–12 mm at 16 °C on PDA, temperature range from 4–25 °C, no growth at 30 °C.

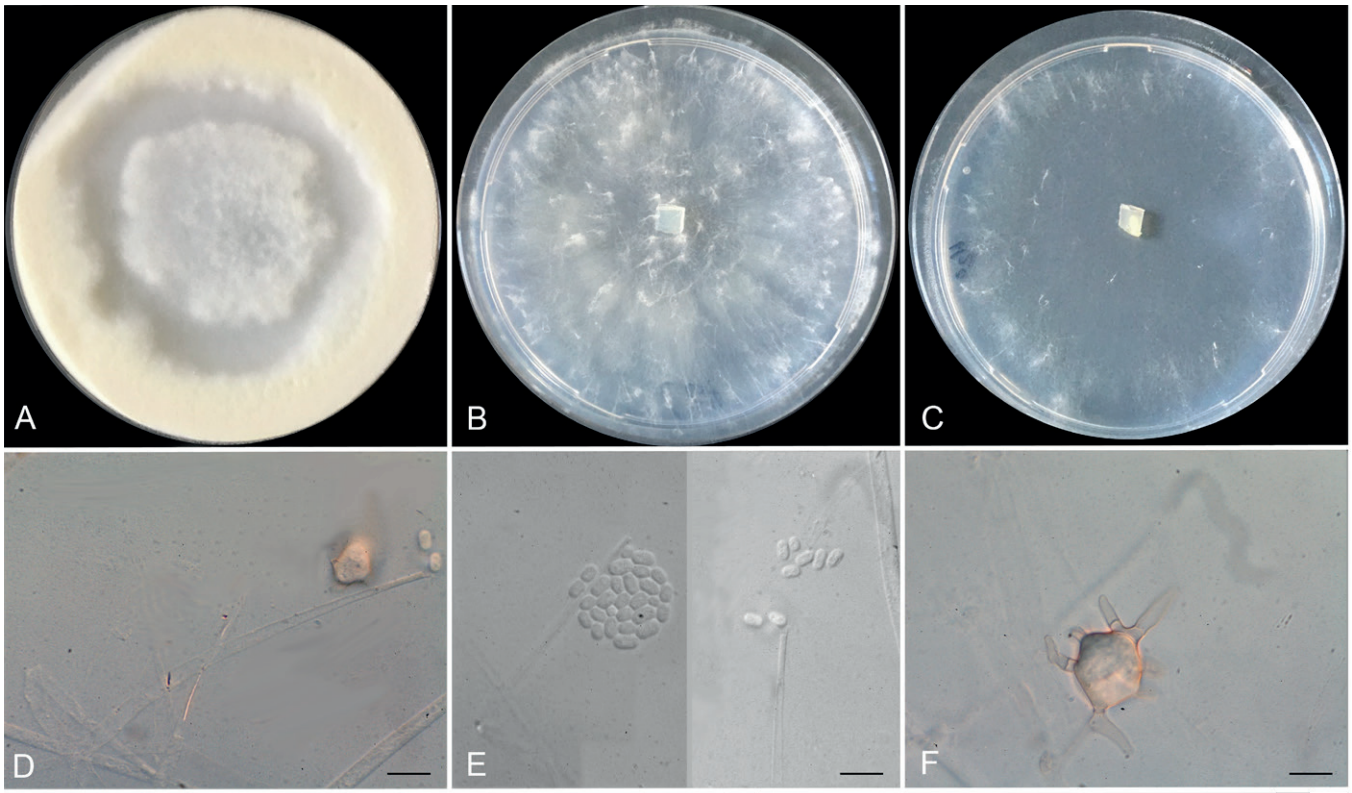

Fig. 26. Colony morphology of *Tyroliella animus-liberi* grown on different media (9-cm-diam plates) at 16 °C after 7 d incubation. **A.** PDA. **B.** LCA. **C.** WA. Asexual morphology of *T. animus-liberi.* **D.** Unbranched sporangiophore with slightly enlarged basal part. **E.** Sporangiospores after dehiscence or peridial wall. **F.** Typical, brownish pigmented chlamydospore with hyphal outgrowings. All the microscopic structures for this ex-type strain were observed on SE media. Scale bars = 20 µm.

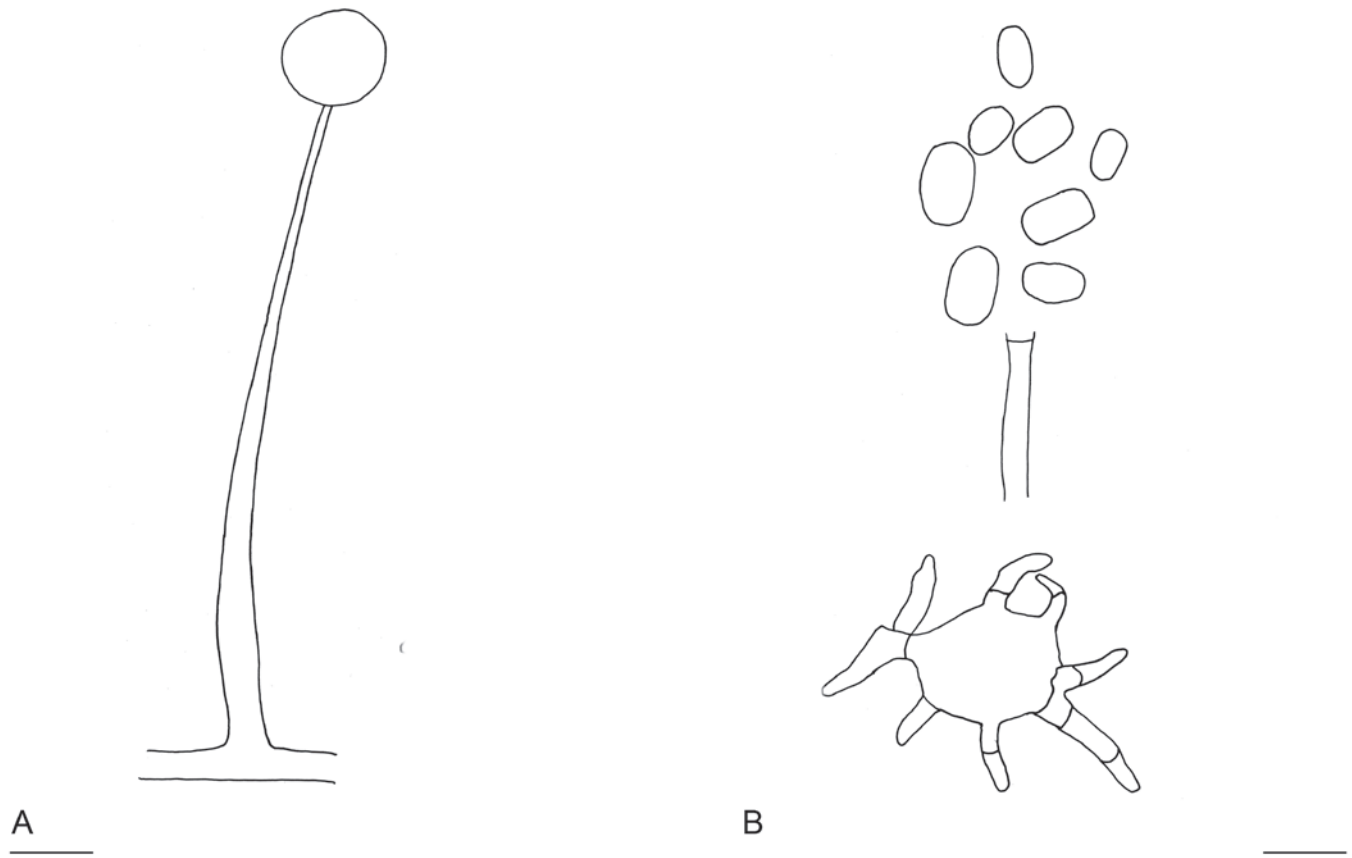

Fig. 27. *Tyroliella animus-liberi.* **A.** Sporangiophore with sporangium. **B.** Sporangiophore tip, sporangiospores and chlamydospore. Scale bars: A = 40 µm; B = 10 µm.

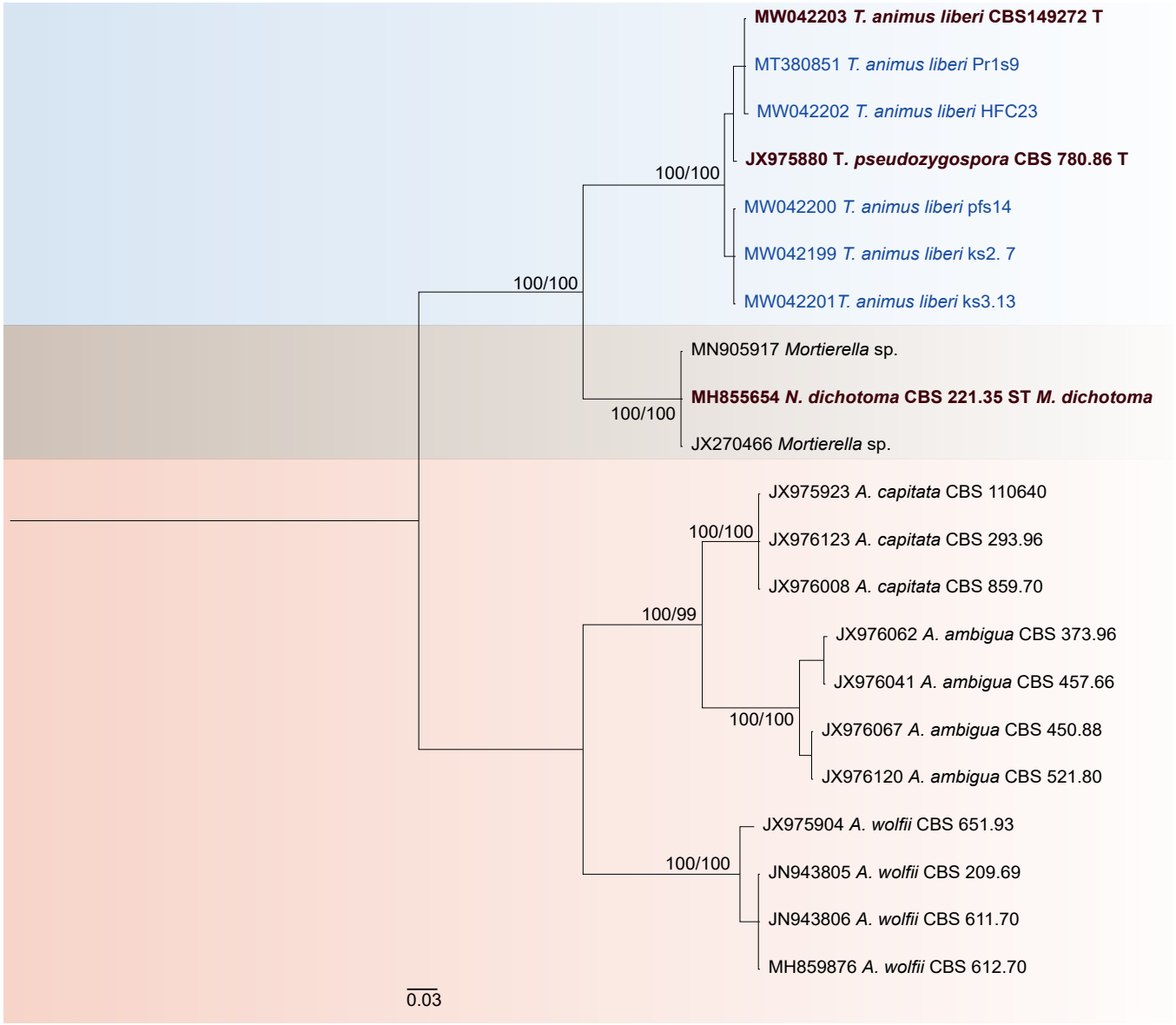

Fig. 28. Phylogenetic relationship of *Tyroliella animus-liberi* based on rDNA-ITS sequences. The Maximum Likelihood Phylogram (log likelihood -2339.17) is shown, and the branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is shown above the respective branches. Newly generated sequences are highlighted in blue, sequences generated from typus are bold and dark red. *Actinomortierella* is used as an outgroup (background light brown).

Additional strains examined: **Austria**, Tirol, Praxmar, soil from *Pinus cembra* forest 5 Jun. 2019, A. Telagathoti, culture Pr1s9; Innsbruck, Hafelekar, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 5 Jun. 2020, A. Telagathoti, culture HFC23; Kühtai, soil from *Pinus cembra* forest, 5 Jun. 2019, A. Telagathoti, cultures Ks3_13, Ks2_7; Finkenberg, Pfitscherjoch, soil covered by *Salix reticulata, S. herbacea, Dryas octopetala*, 8 Aug. 2019, A. Telagathoti, culture Pfs14 (for additional strains see Supplementary Table S1).

Habitat: *Tyroliella animus-liberi* was isolated in Austria from alpine and subalpine habitats (1 900–2 300 m) on calcareous or silicaceous soil with *Pinus cembra*, *Salix retusa, S. herbacea* and *Bistorta vivipara* vegetation.

Notes: *Tyroliella pseudozygospora* differs from *T. animus-liberi* by the much longer, basitonously branched sporangiophores (300–1 000 µm), the chlamydospores forming large clusters of up to 200 µm diam, and the typical garlic-like odour of the colonies.

Three additional strains (KS3_13, KS2_7, Pfs14) (Supplementary Table S1) could represent another *Tyroliella* species. They were isolated from subalpine *Pinus cembra* forest, and an alpine site with *Salix retusa* in Austria. However, no sporulation could be obtained for these strains, irrespective of different media, temperatures and treatments used. The colonies of strain KS3_13 differed from *T. animus-liberi* by a slightly different appearance on LCA and WA, and a more pleasant, fruity odour.

Phylogenetic analyses of the rDNA region (Fig. 29) and the *RPB1* region (Fig. 30) clearly confirm that the genus *Tyroliella* represents a distinct lineage in the *Mortierellaceae*. The sister group relationship to *Necromortierella* is clearly shown based on the rDNA ITS phylogeny (Fig. 28).

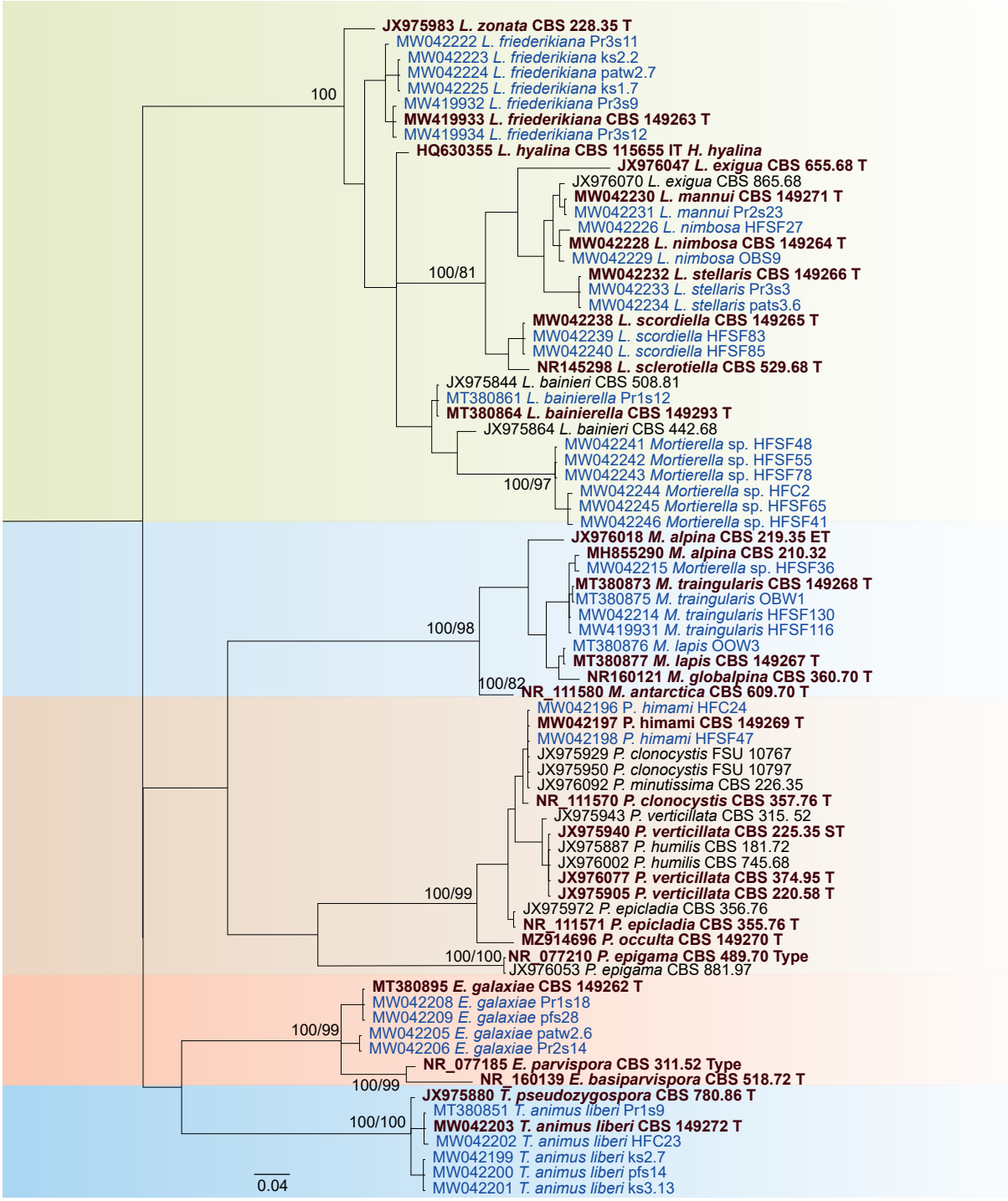

Fig. 29. Maximum Likelihood phylogram (log likelihood -4069.81) of isolated *Mortierellaceae* species based on rDNA-ITS region. For each clade, one or two isolate sequences were randomly picked. Our isolates were grouped into five genera (main colours) and nine well-supported lineages representing species groups (shaded colours). The branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is shown above the respective branches. Newly generated sequences are highlighted in blue, sequences generated from typus are bold and dark red.

Key to the species of *Tyroliella*

1a Sporangiophores 300–400(–1 000) µm long; sporangiospores cylindrical, longer than 5 µm (5–8 × 2.5–4.5 µm); chlamydospores formed within nests of short hyphal branches ..... *T. pseudozygospora*

1b Unbranched sporangiophores with a maximum length of 550 µm, hyaline; cylindrical sporangiospores usually < 6 µm long (3.0–5.7 × 2.2–3.3 µm), chlamydospores occurring individually with short, often dichotomously branched, hyphal appendages .... *T. animus-liberi*

Phylogenetic analysis based on rDNA sequences

A total of 139 isolates was used for phylogenetic delimitation and morphological characterisation (Supplementary Table S1). The blast analysis of the generated rDNA-ITS sequences showed > 95 % sequence similarity to sequences generated from type materials of related *Mortierellaceae* species, thus proving that they represent unique and new lineages. The evolutionary relationships

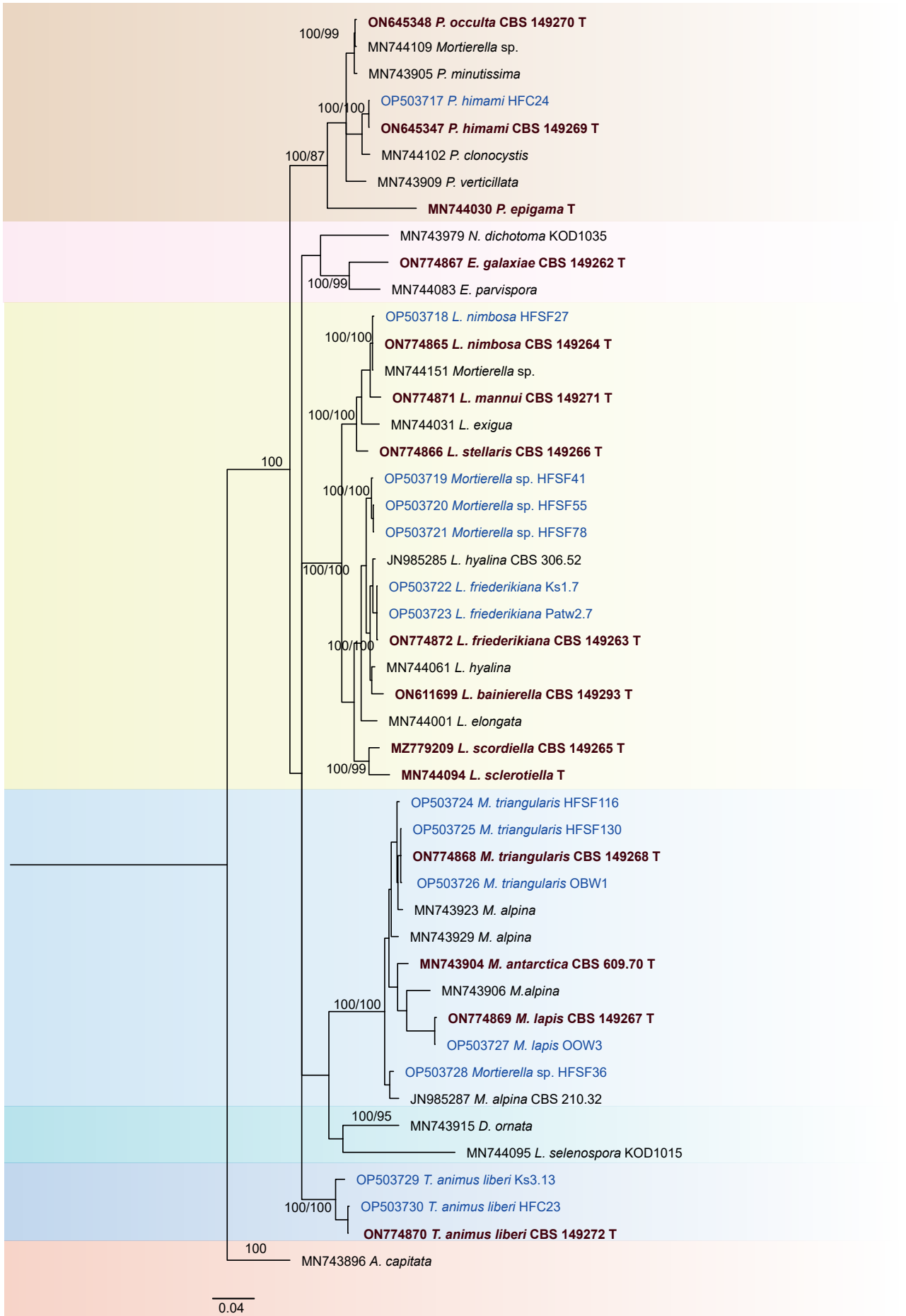

Fig. 30. Maximum Likelihood phylogram (loglikelihood -9496.62) of *Mortierellaceae* species based on *RPB1* sequences. Lineages with support of 100 % Bayesian Posterior Probability (BPP) are in bold in the phylogram. The branch support (Bayesian Posterior Probabilities / Parsimony Bootstrap support ≥ 70) is shown above the respective branches. Sequences generated from typus are highlighted in red. The colour-indicated lineages representing the genera *Podila, Entomortierella, Linnemannia, Mortierella,* and the new genus *Tyroliella* are well supported.

were well-resolved on species level in the ITS phylogeny (Fig. 29), but relationships among species or groups of species could not be resolved. The taxonomical resolution was still low when applying concatenated analysis of the ITS-LSU region (Supplementary Fig. S1). A multigene phylogeny could not be carried out due to insufficient *RPB1* sequences in the database.

Our isolates clustered into five different genera: *Entomortierella, Linnemannia, Mortierella, Podila,* and *Tyroliella.* Based on the ITS phylogeny (Fig. 29), 21 isolates belonged to two *Entomortierella* spp., 20 isolates belonged to two *Mortierella* spp., 59 strains belonged to six *Linnemannia* species, seven isolates belonged to two *Podila* species, and 32 isolates belonged to one *Tyroliella* species. Four singleton isolates were not further treated due to an insufficient number of replicates.

Phylogenetic analysis based on RNA polymerase II largest subunit *RPB1*

A total of 46 sequences of the *RPB1* gene, including the ex-type sequences available, were aligned to resolve the evolutionary relationships of *Mortierellaceae* (Fig. 30).

Within the *RPB1* phylogenetic tree, our isolates fell into five lineages representing the genera *Podila, Entomortierella, Linnemannia, Mortierella,* and the new genus *Tyroliella* (Fig. 30). The resolution on species level was very good compared to the rDNA phylogeny. However, unfortunately, there is still a huge lack of reference *RPB1* sequences, especially of sequences from type material, making it difficult to unambiguously assign our isolates to a species-based *RPB1* phylogeny only. For example, only the rDNA-based phylogeny showed that one of our clades is closely related to *Tyroliella pseudozygospora.* Also, the sister group relationship of *M. lapis* to *M. globalpina* could only be resolved based on rDNA phylogenies. However, the *RPB1* sequence of our isolates was basically for the circumscription of the *M. alpina* complex.

Distribution of *Mortierellaceae*

During our studies focussing on the fungal seasonal dynamics in glacier forefields, alpine habitats, and *Pinus cembra* forest soils in Austria, we isolated 139 pure culture isolates of *Mortierellaceae* spp. belonging to 13 new taxa. Based on the present work, we have increased the known diversity of *Mortierellaceae* in such habitats by 44 %. Our results further support that species of *Mortierellaceae* are diverse and widely distributed across all the habitats sampled (Table 1). However, several species appear to be specific, or at least more abundant, in certain habitats. For instance, *Entomortierella* species were generally widely distributed in *Pinus cembra* and *Picea abies* forest soil, irrespective of season (snow cover *vs*. snow free soil). Taxa belonging to *Linnemannia* were generally very abundant in all habitat types, with a few exceptions: *L. bainierella*, *L. stellaris, L. frederikiana,* and *L. mannui* appeared to be restricted to forest habitats, while *L. nimbosa* and *L. scordiella* were only isolated from alpine dwarf willow habitats. *Tyroliella* spp. were frequently and exclusively isolated from alpine and sub-alpine sites, most of them with quite acidic soil pH. Taxa belonging to the *Mortierella alpina* complex were also abundant, but only in glacier forefields. The species we detected were all psychrotolerant and appeared to be habitat specific. Species of the genus *Podila* were not often isolated in our habitats, and the few species we found, *P. himami* and *P. occulta,* might be specific to alpine dwarf willow habitats.

DISCUSSION

Identification of *Mortierellaceae* species

Mortierella in the classical sense (Gams 1977) is a polyphyletic genus including taxa with unique divergent ecological functions. Most of the *Mortierellaceae* species are easy to isolate and to sustain as pure cultures. It is also relatively easy to obtain their DNA even from old materials. Nevertheless, taxonomical studies on these ubiquitous fungi are quite laborious and require specific training. Therefore, they are low in number (Nagy *et al.* 2011a). The first extensive study based on a DNA sequencing approach revealed that the species of this family needed reclassification due to their high number of homoplastic traits; previous classifications were solely based on the phenotypic traits (Petkovits *et al.* 2011). The common sequence-based procedure of taxonomic classification based on the ribosomal marker region as barcoding genes is difficult to implement for *Mortierellaceae*. Across this family, the sequences of these marker genes are highly divergent. Also, the ribosomal large and small subunit are highly conserved and therefore often fail to support the higher-order phylogenetic relationships in this family (Wagner *et al.* 2013). Nevertheless, species or at least complexes of closely related taxa can usually be identified without problems based on their rDNA-ITS region. Most species in this family can be identified based on the rDNA-ITS, LSU region, as reported by a comprehensive study on *Mortierella s.l.* (Wagner *et al.* 2013). However, there are critical species, *e.g.*, *Mortierella alpina,* whose taxonomic identification remains difficult. Recently, Vandepol *et al.* (2020) resolved several pressing issues within the *Mortierellaceae* taxonomy using a multigene phylogenomic approach. Based on numerous, solid data, the polyphyletic *Mortierella s.l.* was purged and monophyletic lineages were recognised as well-defined genera. Thus, *Mortierellaceae* now includes 13 genera (Vandepol *et al.* 2020). Nevertheless, not all species were described yet, due to the species richness of this family. Moreover, not all described species could be recombined, due to the size of this taxonomic task. Identifying new fungal species is paramount, although the methodology applied for their placement into the taxonomy is subject to ongoing debate between taxonomists and bio-informaticians (Hibbett *et al.* 2016). With our study, we introduced one additional genus leading to 14 genera within *Mortierellaceae*.

Species identification based on a molecular phylogenetic marker gene approach is an easy and straight-forward approach, which can also be automatically applied by various pipelines due to the high amount of reference data present in molecular databases (Tedersoo *et al.* 2018). Some of the advantages of this approach are that it requires small amounts of the sample, and it is a relatively quick standard technique that can also be applied to environmental samples. Based on the huge amount of data we are currently acquiring as a scientific community, considerable information are available relating to the detected organisms (Savolainen *et al.* 2005). Moreover, this approach also has the potential to reveal undiscovered fungal groups in unexpected niches (Hibbett *et al.* 2016). Previously, the sequencing approach was routinely focussing on ribosomal markers; however, there is an ongoing trend of using multigene or even metagenome approaches for higher resolution. Doing so provides robust and powerful data, which helps to overcome significant misunderstandings (Tekpinar & Kalmer 2019) and is particularly helpful in classifying fungi in difficult groups or in so-called cryptic species.

As many benefits as molecular systematics offers, there are also disadvantages to this approach. The main drawback is that

it requires access to well-equipped laboratories and advanced computational infrastructure. Moreover, multigene approaches are especially expensive and time consuming (Thines *et al.* 2018). Additionally, a physical specimen and a pure culture isolate offer plenty of possibilities for further studies, and thus highly contribute not only to systematic and taxonomic revelations, but also to our understanding of the fungus' ecological relevance (Hofstetter *et al.* 2019). Last, but not least, public databases contain a vast number of incorrectly assigned sequences, thereby making the species classification more difficult.

A pure culture isolate offers the possibility for a morphological identification approach. Identifying a fungal species based on classical morphological techniques using simple staining methods and microscopy is highly economical, and provides rapid and reliable results, which often are sufficient for the identification of certain fungal taxa (Tekpinar & Kalmer 2019, Indunil Chinthani *et al.* 2020). The older classifications of *Mortierella s.l.* were based on such phenotypical identification schemes of pure culture isolates (Milko 1974, Gams 1977, Domsch *et al.* 2007). These morphological identification methods can still be used successfully, even though the nomenclature of the fungi in question has changed. This is especially valuable for taxa which have not been sequenced yet. However, morphological classification is often challenging, especially when working on fungal groups with a wide ecological amplitude and phenotypical plasticity. We observed this limitation in our study on *Mortierellaceae* taxa. Unfortunately, due to the high number of homoplastic characters, it is very difficult for an untrained person to identify *Mortierellaceae* genera. It is, therefore, highly recommendable to combine both the molecular sequencing and morphological approach for a reliable identification of species in *Mortierellaceae*. Henceforth, identification can be quickly and easily achieved based on rDNA ITS sequence analysis followed by a fast confirmation based on morphological characters, or *vice versa*. This also enables a quick identification of closely related species with nearly identical rDNA ITS sequences. Using this approach, we can identify critical species, and we can also shed light onto the diversity, evolving phenotypic traits and the ecology of these fungi.

Diversity and ecology of *Mortierellaceae*

Overall, we found a very high diversity of *Mortierellaceae* in alpine and subalpine habitats. We detected 29 species of *Entomortierella, Linnemannia*, *Mortierella,* and *Podila* based on our extensive sampling in Tyrolean (Austria) snow-covered and snow-free soil (Telagathoti *et al.* 2021). Here, we present 13 new species and the new genus *Tyroliella.* In comparison to the estimated total *Mortierellaceae* diversity of 126 species (Hibbett & Glotzer 2011, Nagy *et al.* 2011), this is a very high number of taxa present in this specific habitat. Consequently, we hypothesize that the true, global *Mortierellaceae* diversity might be higher than currently estimated. The diversity of *Mortierellaceae* taxa is comparatively well-known for temperate forests and agricultural soil (Takashima *et al.* 2018). However, until now, the sampling effort in alpine and arctic regions was quite limited, maybe due to the risks and difficulties involved in sampling in remote or inaccessible alpine regions. Moreover, there might be additional, unusual habitats (*e.g.* desert soil or steppe habitats), which are under-sampled, and which will probably contribute to a higher *Mortierellaceae* diversity.

The results of our study show that the genera *Linnemannia*, *Entomorterella,* and *Tyroliella* are widely distributed across alpine and subalpine habitats, as they were isolated from all soil types, irrespective of winter and summer conditions. These taxa are well adapted to the low temperatures (0–15 °C) (Telagathoti *et al.* 2021). In contrast, the *Podila* and the *Mortierella alpina* complex had a narrower ecological niche. The *M. alpina* complex appears to occur predominantly in arctic/alpine habitats, since most of the species were isolated from either glacier successional sites or snow-covered soil. *Podila* species were rather present in forest and agricultural sites. They have the ability to degrade cellulose and hemicellulose, which further explains their distribution and ecological role in decaying the dead plant material in those soils rich in organic matter (Varnaitė *et al.* 2008).

The genera of *Mortierellaceae* and their ecological importance

Members of the *Mortierellaceae* are common and ubiquitous fungi generally regarded as soil saprotrophs. *Mortierellaceae* taxa can also grow in acidic soils, and promote plant growth (Ozimek & Hanaka 2021). This could be an explanation for the globally very wide distribution of *Mortierellaceae* in soil (Tedersoo *et al.* 2014). The abilities to degrade cellulose and chitin and to grow at low temperatures are highly beneficial for these fungi, allowing them to thrive in otherwise unfavourable soil conditions. However, recent research highlighted additional ecological functions. *Mortierellaceae* were frequently detected in the plant soil rhizosphere, and *e.g. Linnemannia hyalina, Podila verticillata* are now widely recognised as important beneficial, plant growth-promoting fungi in agricultural soil (Büttner *et al.* 2021, Ozimek & Hanaka 2021). *Entomortierella* spp., in contrast, are known to be associated with insects or soilborne worms (Wagner *et al.* 2013). Besides their important function in the ecosystem, several species are also biotechnologically interesting. *Mortierella alpina* is one of the most promising arachidonic acid producing fungi (Shinmen *et al.* 1989, Singh & Ward 1997, Hao *et al.* 2015, Zhang *et al.* 2017).

Association of *Mortierellaceae* to soil bacteria

Recent studies on species diversity and distribution of *Mortierellaceae* across different habitats discovered associations and interactions of these fungi with bacteria (Takashima *et al.* 2018, Telagathoti *et al.* 2021). There is much attention towards the interactions between *Mortierellomycotina* and their endosymbionts such as *Mycoavidus cysteinexigens*, *Burkholderia*-related or *Mycoplasma*-related endobacteria (Fujimura *et al.* 2014, Ohshima *et al.* 2016, Bonfante & Desiro 2017, Uehling *et al.* 2017, Desirò *et al.* 2018, Takashima *et al.* 2018). So far, endobacteria were only reported for 22 species of *Mortierellaceae.* Moreover, *Mortierellaceae* are also frequently associated with free-living soil bacteria, especially *Pseudomonas* spp. (Telagathoti *et al.* 2021). Further investigations are needed to unravel the specificity and ecological role of these interactions across different *Mortierellaceae* genera.

Conclusions

Mortierellaceae represents a diverse and important group of fungi, and their distribution is highly dependent on environmental factors. Our extensive sampling on the alpine and sub-alpine habitats indicates that the diversity is still widely under-explored, in general and in particular in these habitats. Henceforth, the combination of the classical morphological identification techniques in combination with sequencing of the rDNA ITS barcoding gene allows for a straightforward and reliable identification of species, even in difficult species complexes.

DECLARATION ON CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Supplementary Material: https://studiesinmycology.org/

Fig. S1. Maximum Likelihood phylogram (log likelihood -7961.52) of our isolated *Mortierellaceae* species based on the concatenation of the rDNA-ITS and LSU region. Our isolates were grouped into five genera: the colour-indicated lineages representing the genera *Podila*, *Entomortierella*, the new genus *Tyroliella*, *Mortierella* and *Linnemannia*. Branch support values ≥ 70 (Bayesian Posterior Probabilities / Parsimony Bootstrap support) are shown above the respective branches. Sequences generated from typus are highlighted in red.

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Table S1. Information on the *Mortierellaceae* strains used in this study including GenBank accession numbers, CBS numbers, JMRC numbers, IBF herbarium numbers and data concerning the isolation of the respective strain. ITS sequences with 100 % sequence identity were submitted to GenBank only for 2–3 representative strains for each species, respectively (100 % identitiy).