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Cryptic associated fungi and algae isolated from Antarctic epilithic lichens of the Victoria Land and the description of five new fungal species

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Abstract: Lichen thalli are niches for microorganisms, including microfungi, microalgae and non-photosynthetic bacteria, which form communities of variable composition, often shaped by the environmental conditions under which the lichen thalli develop. In Antarctica lichens represent an important group of organisms characterized by a high percentage of endemism, which have specialized to grow on rocks, as the predominant substrate for colonization. Here, Antarctic epilithic thalli of five endemic and three cosmopolitan lichen species have been investigated for their potential role as fungal species hotspots. The culturable fraction of the Antarctic lichen-associated fungi and algae was uncovered with the aim to isolate as many species as possible and find whether taxa are strictly connected to the Antarctic environment or are cosmopolitan species that particularly associate to lichens in harsh, extreme environments. Over 300 fungal and algal inocula grew in culture, among which we recognized species previously described, as well as five new fungal species in *Ascomycota* and *Basidiomycota*, namely: *Petrophila complexa sp. nov*., *Dactylospora endolichenica sp. nov*., *Knufia elegansiana sp. nov*., *Pseudeurotium lichenicum sp. nov*., and *Kurtzmanomyces lichenum sp. nov*. These species seem to select lichens as their preferred niche, both in Antarctica and worldwide. Indeed, the major representatives of the lichen mycobiota are fungi that do not show any specificity toward a particular lichen host species, rather toward the lichen thallus in general, as a structure in which spores, yeast cells and mycelia fragments thrive or rest. Particularly in Antarctica, where conditions on rock surfaces are far more selective than elsewhere, lichen thalli would be even more exploited as suitable niches by other fungi for their evolution and diversification.

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INTRODUCTION

Lichens are well-known self-sustaining and long-living symbioses in which a main fungal partner, i.e. the mycobiont, associates with a population of the main photosynthetic partners, i.e. the photobionts (green algae, cyanobacteria or both), to build as a stable structure the lichen thallus (Hawksworth & Honegger 1994). Within lichen thalli a multiplicity of microorganisms, including microfungi, microalgae and non-photosynthetic bacteria are housed (Hawksworth & Grube 2020), and form microbial communities of variable composition, often shaped by the environmental conditions under which the lichen thalli develop (e.g., U'Ren *et al.* 2010, 2012, Grube *et al.* 2015, Moya *et al.* 2017, Molins *et al.* 2021, Smith *et al.* 2020, Cometto *et al.* 2024). These lichen-associated microbial communities frequently occur cryptically, and in the past few years their species diversity has been better understood by DNA metabarcoding studies and culture isolations (Arnold *et al.* 2009, Fernández-Mendoza *et al.* 2017, Muggia & Grube 2018, Banchi *et al.* 2018, Yang *et al.* 2022, Beck et. al 2023, Cometto *et al.* 2023, 2024). For what concerns the eukaryotic fraction, the lichen-associated fungi represent a wide array of major lineages in *Ascomycota* and *Basidiomycota*, and include both filamentous and yeast species (Spribille *et al.*2018, Muggia & Grube 2018, Cometto *et al.* 2022b, 2023). The lichen-associated microalgae, instead, are majorly represented by the classes *Trebouxiophyceae* and *Ulvophyceae* (Moya *et al.* 2017, Molins *et al.* 2021).

In the last decade, metabarcoding studies have been applied to uncover in a comprehensive way this microbial taxonomic diversity cryptically occurring in lichens (U'Ren *et al.* 2010, Zhang *et al.* 2015, Spribille *et al.* 2016, Fernández-Mendoza *et al.* 2017, Banchi *et al.* 2018, Muggia & Grube

2018, Smith *et al.* 2020, Beck *et al.* 2023, Cometto *et al.* 2024). However, the isolation of culturable strains and their maintenance *in vitro* is still essential to integrate the knowledge on the morphological diversity of these cryptic communities. So far, an increasing number of studies have aimed at elucidating the diversity of the cryptically lichen-associated fungi and algae either with one or the other approach, but only a pair of studies have combined the metabarcoding and the culture-dependent approaches (Yang *et al.* 2022, Cometto *et al.* 2024). Recently, thanks to the successful isolation in culture of several strains coming from different geographic origins, new species have been formally described (Černajová & Škaloud 2019, Muggia *et al.* 2021, Cometto *et al.* 2023, Chang *et al.* 2023). In particular, within the subclass *Chaetothyriomycetidae* (*Eurotiomycetes*) the following taxa were described: the family *Pleostigmataceae* including three *Pleostigma* species (Muggia *et al.* 2021), the species *Melanina gundecimermanniae* (Muggia *et al.* 2021), ten new *Cladophialophora* (Chang *et al.* 2023, Cometto *et al.* 2023) and one new *Paracladophialophora* species (Cometto *et al.* 2023). Most of these species were isolated from lichens from very dry, cold and hot habitats and high altitude/alpine environments, hinting to the fact that lichens from extreme environments may serve as ecological niches and cradles of species diversification (Harutyunyan *et al.* 2008, Arnold *et al.* 2009, Quan *et al.* 2020, 2023).

Antarctica includes areas considered among the most extreme on Earth, reaching the limits for life sustainability. Together with bryophytes, only microbial communities composed of rock-inhabiting lichenized and not-lichenized fungi, green algae, bacteria, and cyanobacteria can outcompete and settle even throughout the most dry and cold interior areas of the continent (Øvstedal & Lewis Smith 2001, Selbmann *et al.* 2013a, b). In Antarctica, as well as at high altitudes, species must be able to cope with continuous fluctuations of temperatures, humidity/drought, salinity, oligotrophy, deposition of inorganic and organic nutrients, and high ultraviolet (UV) radiation (Gorbushina 2007, Onofri *et al.* 2007, De Los Ríos *et al.* 2014, Selbmann *et al.* 2015, Coleine *et al.* 2021, Greco *et al.* 2021). In Antarctica lichens represent an important group of organisms characterized by a high percentage of endemism (33–50 % in the continental Antarctica) with a total of 130 endemic species (Øvstedal & Lewis Smith 2001, Castello & Nimis 1997, 2000). Here, lichens have specialized to grow on rocks, as the predominant substrate for colonization (Nienow & Friedmann 1993), building either a crustose, thin, epilithic thallus, or strictly endolithic, with the thallus developing inside the rock substrate when conditions become incompatible with epilithic life (De los Ríos *et al.* 2005; species with foliose and fruticose growth forms are mostly found in coastal habitats).

In Antarctica, these so formed "cryptoendolithic lichen-dominated communities'' (Friedmann 1982, de la Torre *et al.* 2003) have been characterized for their fungal, bacterial and viral (Ettinger *et al.* 2023) composition and diversity (Selbmann *et al.* 2005, 2008, Muggia *et al.* 2021, Stoppiello *et al.* 2025), as they represent an environment — much more buffered from the environmental extremes (Casero *et al.* 2021, Perez-Fernandez *et al.* 2022) — where microorganisms can diversify. However, the Antarctic epilithic lichen thalli have not been investigated for their potential role as species hotspots since recently, when five endemic Antarctic (*Acarospora flavocordia*, *Buellia frigida*, *Lecanora fuscobrunnea*, *Lecanora physciella*, *Lecidea cancriformis*) and three cosmopolitan (*Pleopsidium chlorophanum*, *Rhizoplaca melanophthalma*, *Rusavskia elegans*) lichen species have been selected for metabarcoding studies tackling their associated fungal and bacterial diversity (Stoppiello *et al.* 2025). Here, we have integrated the molecular analyses of Stoppiello *et al.* (2025) by uncovering the culturable fraction of the lichen-associated fungi and algae (photobionts included) from Antarctica. We applied a culture approach aiming at isolating as many species as possible, to find whether taxa are strictly connected to the Antarctic environment or are cosmopolitan species that particularly associate to lichens in harsh, extreme conditions, as those recently described from thalli growing at high altitudes and in dry, highly irradiated habitats worldwide (Muggia *et al.* 2021, Cometto *et al.* 2022a, b, 2023).

MATERIAL AND METHODS

Sampling

The sampling campaign took place during the 37° Italian Antarctic expedition November 2021 – February 2022. A total of 14 localities were selected in the northern Victoria Land, distributed in the latitudinal range from 72° to 77° parallel (as reported in Stoppiello *et al.* 2025; Supplementary Table S1). If present, at least three samples of the eight selected species were collected at each site, from granitic and sandstone rocks which were colonized by both epilithic and endolithic lichens. The samples were aseptically removed using a geological hammer, collected in plastic sterile bags, transported and then stored at -20 °C in the laboratories at the University of Tuscia and at the University of Trieste, Italy, until downstream analyses. The species selected for this study were epilitic, ascomycetous chlorolichens (i.e., with green algae as photobionts), and were all fertile species producing apothecia. The species *Acarospora flavocordia*, *Buellia frigida*, *Lecanora fuscobrunnea*, *Lecanora physciella*, *Lecidea cancriformis* and *Pleopsidium chlorophanum* have a crustose, areolate and more or less placodioid thallus, firmly attached to the rock substrate, lacking a lower cortex. *Acarospora flavocordia*, *Buellia frigida*, *Lecanora fuscobrunnea*, *Lecanora physciella*, and *Lecidea cancriformis* are Antarctic endemic species, developing sometimes a inconspicuous thallus, often inside rock crevices, or even becoming endolithic in some case. *Pleopsidium chlorophanum* is an arctic-alpine species, bipolar in its distribution, which usually occur on vertical, rain-sheltered surfaces of siliceous, often metal-rich rocks and among rock crevices. The species *Rusavskia elegans* has a foliose thallus with strongly convex lobes tightly attached to the substrate, growing on well-lit natural rock outcrops as well as in strongly eutrophicated situation. It is a lichen with a worldwide distribution and very variable in colour, form, and substrate preference, as it grows on calcareous as well as slightly acidic (siliceous/granitic) rocks. *Rhizoplaca melanophthalma*, a cosmopolitan taxon as well, has a foliose-umbilicate to subsquamulose-umbilicate thallus, and is commonly found on bird's perching siliceous rocks.

Isolation of fungi and algae from lichen thalli

The isolation of microfungi and microalgae associated within the lichen thalli was performed for two thalli for each lichen species for each locality, following the protocol of Yamamoto *et al.* (2002). The lichen thalli were inspected to detect any symptom of infection by lichenicolous fungi and to avoid thalli showing any of them. The lichen material collected and examined was devoid of lichenicolous fungal infection and was processed further for the culture isolation experiments. Approximately 2 mm^2 fragments of lichen thalli were dissected with a sterile razor blade. The fragments included one or two thallus areolas and a few apothecia if present. The fragments were washed three times for 15 min with sterile water, followed by 30 min of washing with 500 µL of Tween20 diluted 1:10. A final washing step was performed rinsing the thallus fragments three times for 15 min with sterile water. The clean fragments were ground in sterile water under the hood and tiny thallus fragments were picked with a sterile bamboo stick and transferred into agar tubes. Different media were used to promote the growth of as many different fungal and algal species as possible: Trebouxia medium (TM; Ahmadjian 1987), Malt Yeast-extract (MY; Lilly & Barnett 1951), and Bold's Basal Medium (BBM; Nichols & Bold 1965). The media were supplemented with chloramphenicol [25 mg/mL] to reduce bacteria contamination. The tubes were incubated in a growth chamber under 17 °C, 20 µmol photons m^{-2} s^{-1}, with a light/dark cycle of 14/10 h, and grown for a period of 5–10 mo. Isolates that were grown up to 2–5 mm after a few months, were removed from the slant agar tubes, subcultured in Petri plates, and incubated for a further 2–4 mo to reach a sufficient biomass that could be used for their genetic and morphological characterization, as well as for their preservation as cryostock. The strains were firstly genetically characterized in the period September–November 2022, and afterwards were cryopreserved in a metabolically inactive state at -80 °C and -150 °C. The strains are deposited in the Culture Collection of Fungi from Extreme Environments, Mycological Section of the Italian Antarctic National Museum (MNA-CCFEE) at the University of Tuscia (Viterbo, Italy) and at the Mycotheca Universitatis Taurinensis (MUT) at the University of Turin (Turin, Italy).

DNA extraction, amplification and sequencing

The isolates were genetically identified, and DNA extraction followed the CTAB protocol of Cubero *et al.* (1999), with minor adjustments. The identity of all fungal strains was studied with sequences of at least two marker genes: i) the nuclear internal transcribed spacers (nucITS) and 5.8S rDNA ribosomal gene amplified with the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990), and ii) the D1/D2 domain of the 28S nuclear large ribosomal subunit (nucLSU) amplified with the primers LR0R and LR7 (Vilgalys & Hester 1990). To better delimit phylogenetic placement of those isolates (2 isolates) that were identified as belonging to the order *Capnodiales* in *Dothideomycetes*, we further amplified the markers RNA polymerase II second largest subunit (*RPB2*) and the nuclear small ribosomal subunit (nucSSU) using the primers fRPB2-5F (Liu *et al.* 1999) and fRPB2-414R (Quaedvlieg *et al.* 2012) and primers nuSSU-0072-5' and nuSSU-0852 (Gargas & Taylor 1992), respectively. Polymerase chain reactions (PCR) were prepared for a 25 µL final volume containing 5 µL DNA, 12.5 µL of AccuStart II PCR ToughMix, and 0.4 µL for each of the 10 µM primers.

The genetic identification and phylogenetic analyses of the photobionts were performed on algal sequences amplified directly from the lichen thalli and algal strains isolated in cultures. The analyses were based on two marker genes, i.e., the nuclear internal transcribed spacer of the ribosomal DNA (ITS rDNA) and the large subunit of the ribulose-1,5-biphosphate carboxylase (*rbcL*). The nucITS was amplified using the *Trebouxia*-specific primers ITS1T and ITS4T (Kroken & Taylor 2000), the *rbcL* was amplified with the primers rbcL803rev and rbcl320 (Nozaki *et al.* 1995), and the applied PCR conditions followed Muggia *et al.* (2008, 2010, 2014). The sequence identity of isolated algae was used to find correspondence between the sequence(s) obtained directly from the thallus DNA extraction amplified with the same photobiont specific primers.

All the amplicons were checked for their quality and size by 1 % agarose gel electrophoresis stained with SYBR® Green I nucleic acid gel stain (Sigma-Aldrich) and purified using Mag-Bind® Normalizer Kit (Omega bio-tek). Clean amplicons were sent for Sanger sequencing to Eurofins Genomics (Italy).

Phylogenetic analyses

The newly generated sequences were checked with sequences available in the GenBank database by BLAST similarity search (Altschul *et al.* 1997). Taxa that matched our sequences with an identity value higher than 97 % were selected to study the systematic placement of the isolates with phylogenetic analyses. The first blast comparison returned the similarity of the new sequences with three classes of *Ascomycota*, i.e. *Dothideomycetes*, *Eurotiomycetes* and *Leotiomycetes*, and with two classes of *Basidiomycota* represented by *Microbotryomycetes* and *Agaricostilbomycetes*, and the three orders *Cystobasidiales*, *Filobasidiales*, and *Tremellales*. For each group, a specific dataset was built (Supplementary Tables S2–S9). The complete datasets encompassed a broad range of taxonomic diversity, with the inclusion of genera and families that were closely related to the new sequences, as identified in previous research. In particular, the dataset of *Dothideomycetes* and *Eurotiomycetes* were based on Cometto *et al.* (2023). To better resolve the phylogeny of *Dactylospora* (*Eurotiomycetes*) we added also 47 sequences retrieved from GenBank (NCBI); the dataset of *Leotiomycetes* was based on Quijada *et al.* (2022). The datasets built for the classes and orders of the basidiomycetes were based on those presented by Cometto *et al.* (2022b) and references therein.

Sequence alignments for each locus (nucITS, nucLSU, nucSSU, *RPB2*, *rbcL*) and for each fungal taxon were prepared with MAFFT v. 7 (Katoh & Standley 2013) using the g-ins-I alignment strategy. The multilocus gene alignments were prepared in MEGA (Kumar *et al.* 2018). The nucITS nucITSphylogenetic reconstruction was made for all taxa recognized, while the multilocus datasets were prepared as follows: i) nucITS nucITSand nucLSU markers for *Agaricostilbomycetes*, *Chaetothyriales*, *Cystobasidiales* and *Leotiomycetes*; ii) nucITS, nucLSU, nucSSU and *RPB2* markers for *Capnodiales* (*Dothideomycetes*) (Supplementary Tables S2–S9).

To better understand the diversity inside the genus *Trebouxia* we compared the newly obtained sequences with datasets selected from previous studies by Muggia *et al.* (2020) and De Carolis *et al.* (2022) (Supplementary Tables S10, S11).

All the fungal taxa and photobiont datasets underwent Maximum Likelihood (ML) and Bayesian Inference (BI) analyses on the CIPRES web portal (Miller *et al.* 2011). For the ML analysis, the program RAxML v. 8.2.12 (Stamatakis 2014) with the GTRGAMMA substitution model and 1000 bootstrap replicates was used. In the case of BI, we employed MrBayes v. 3.2.7a (Huelsenbeck & Ronquist 2001) and ran two parallel runs with six chains each, extending over five million generations. The analysis began with a random tree and sampled every 100th step. We discarded the initial 25 % of the data as burn-in, and the corresponding posterior probabilities (PPs) were computed from the remaining trees. The multilocus datasets were analysed with both RAxML and BEAST (Huelsenbeck & Ronquist 2001, Stamatakis 2014) with the same settings of the single locus analyses.

Morphological and physiological analyses

The analyses of morphological and anatomical characters were performed on 11 fungal and three algal strains that were emerging as representatives of the new lineages and species identified, as explained below. The strains were analysed using standard microscopic techniques and documented with digital photographs. Analyses and photographs were performed on about 1-yr-old axenic subcultures. The following characters were studied for the fungal isolates: form of growth (filamentous vs yeast-like), melanisation of the hyphae, form and size of hyphal cells, branching of hyphae, development of conidiogenous cells and formation of conidia. Small fragments of mycelia were removed, and squashed sections were mounted in water and studied by light microscopy.

To describe the new *Kurtzmanomyces* species, a basidiomycetes yeast, physiological and morphological tests were performed for strain S91 and L3034 according to the protocols described by Kurtzman *et al.* (2011). The following solid media were used: ME agar (MEA; Oxoid), YPDA (yeast extract 1 %, peptone 1 %, dextrose 2 %, agar 2 %; Oxoid), potato dextrose agar (PDA; Oxoid), corn meal agar (CMA; Difco), and SGA (soytone 0.2 %, glucose 0.2 %, agar 2 %; Oxoid). Liquid growth tests were performed in 200 µL in 96 wells with final cell concentration of 5 × 106; microplates were sealed to prevent desiccation, incubated and measured with the optical density recorded at 580 nm. Physiological and morphological tests were performed at 20 °C for all the strains, in triplicate. Results were recorded every 7 d of incubation, for 8 wk. For determination of sexual compatibility, pairs of 7-d-old cultures were crossed on CMA, MEA and SGA, incubated at 20 °C for 1 wk and examined for production of mycelium and teliospores every week for 10 wk.

For microscopy, cultures were grown on YPD at 20 °C and studied with a Nikon Microphot-FX microscope with Nikon DS-Ri2 camera and Nikon Eclipse Ni microscope coupled with Nikon DS-Fi3 camera. Images of colony morphology were taken and described using an Optech dissecting microscope (GZ 808) couple with the Optech Camera IS 4K-8 B.

The following morphological traits were studied for the isolated algae: size of the cells, presence of autospores, and chloroplast type whether detectable.

All images were acquired with a ZeissAxioCam MRc5 digital camera fitted to the microscope, digitally processed, and slightly refined in sharpness and colour saturation with Adobe Photoshop v. 7.0. Photo plates were prepared with CorelDRAW X4.

RESULTS

Fungal diversity

A total of 328 fungal strains grew as axenic isolates and were further kept in culture. After fungal growth, we proceeded with species identification generating 220 nucITS, 37 nucLSU, one *RPB2*, and two nucSSU (these only for the isolates identified in *Capnodiales*) new sequences (Table 1). According to the blast search analyses the new nucITS sequences (total number reported in parenthesis) corresponded to the ascomycete classes *Dothideomycetes* (61), *Eurotiomycetes* (71), *Leotiomycetes* (19), and to the basidiomycetes *Agaricostilbomycetes* (9), *Cystobasidiales* (34), *Filobasidiales* (14), *Microbotryomycetes* (2) and *Tremellales* (10). The phylogenetic reconstructions either based on multilocus datasets (Figs 1–5) or on the nucITS nucITSlocus alone (Supplementary Figs S1–S5; Figs 6–8), were topological concordant with previous studies (Cometto *et al.* 2022b, 2023, and references therein; Quan *et al.* 2020, 2023). Morphological analyses of the taxa are presented in Figs 12–16.

Within *Dothideomycetes*, the new sequences in the order *Capnodiales* were 43 sequences belonging to *Elasticomyces elasticus* isolated from thalli of *A. flavocordia*, *B. frigida*, *L. cancriformis*, *L. physciella*, *R. elegans* and *R. melanophthalma* (Supplementary Fig. S1). A single isolate from the thallus of *R. elegans* L4530 corresponded to the genus *Catenulostroma*, which was nested in a clade together with other two sequences obtained by Antarctic *Catenulostroma* (by Santiago *et al.* 2015) and another seven sequences of endolichenic fungi isolated by Cometto *et al.* (2022b). Another single isolate from the thallus *R. melanophthalma* L4599 was identified as *Cladosporiales* sp. A supported clade of six new sequences was identified as *Meristemomyces* spp., isolated from thalli of *L. cancriformis* and *L. fuscobrunnea*. Seven strains belonging to three supported clades were found in the family *Extremaceae*: two corresponded to *Extremus antarcticus*, two to *Vermiconia flagrans* and three are still taxonomically unassigned. One strain (L4599) isolated from *R. melanophthalma* corresponded to *Cladosporiales* and two new strains (S71, S72), isolated from *L. cancriformis* (collected at Campo Icaro and Prior Island, respectively; Supplementary Table S1 and Supplementary Fig. S1), were identified as the new species *Petrophila complexa sp. nov.*, here described below (Fig. 1). The presented multi-locus phylogenetic reconstruction, based on the nuclear nucITS, nucSSU, nucLSU and *RPB2* loci, focused on the order *Capnodiales* (Fig. 1), supported the new *Petrophila* species by ML and Bayesian analyses. Morphological observations were performed on the strain S71 (Fig. 15).

The phylogenetic inference estimated for *Eurotiomycetes* and based on the nucITS locus (Supplementary Fig. S2) highlighted two new lineages. The first one was identified

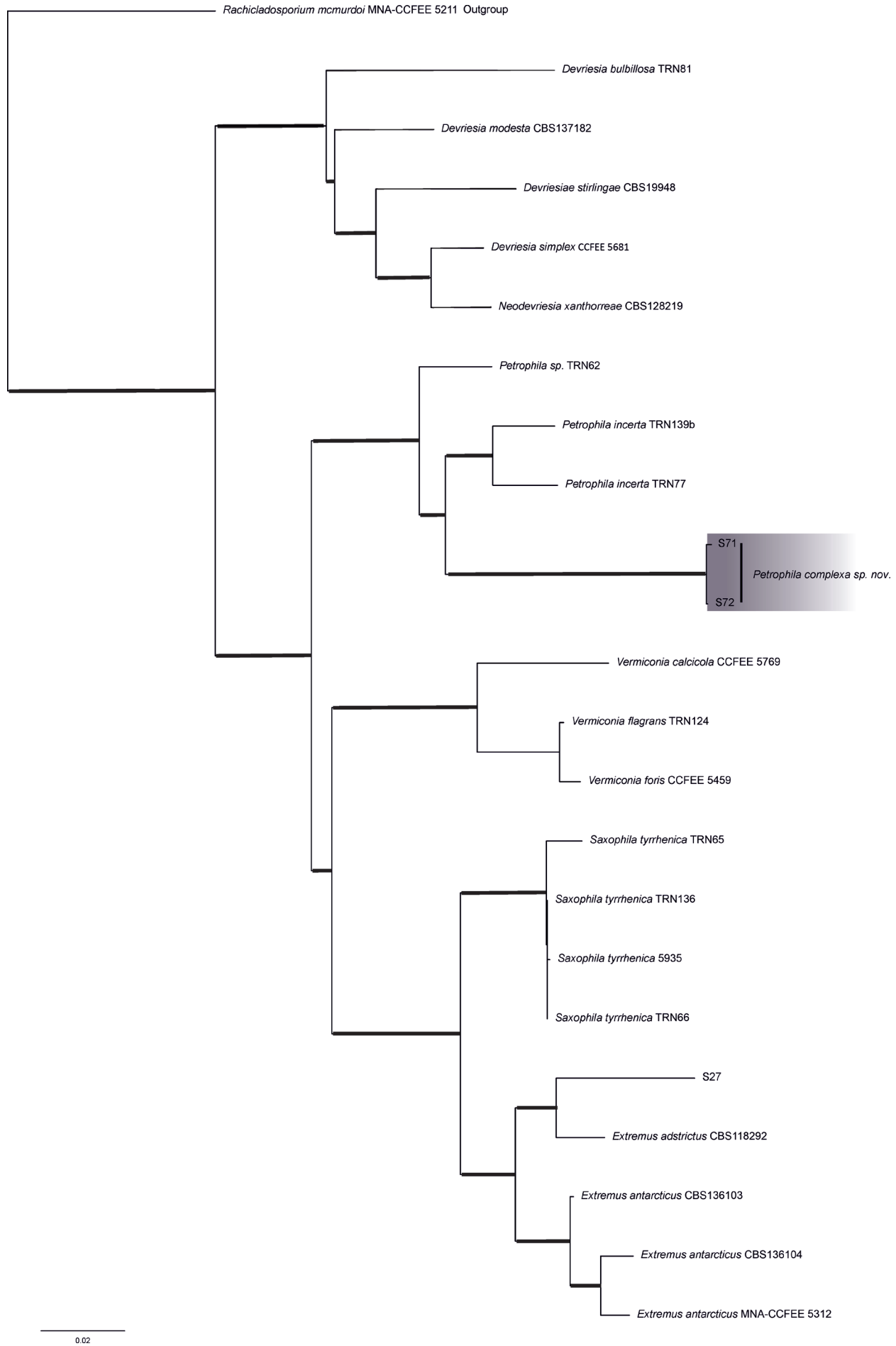

Fig. 1. Phylogenetic inference of *Capnodiales*, *Dothideomycetes* based on the concatenated nucITS-nucLSU-nucSSU-RPB2 dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

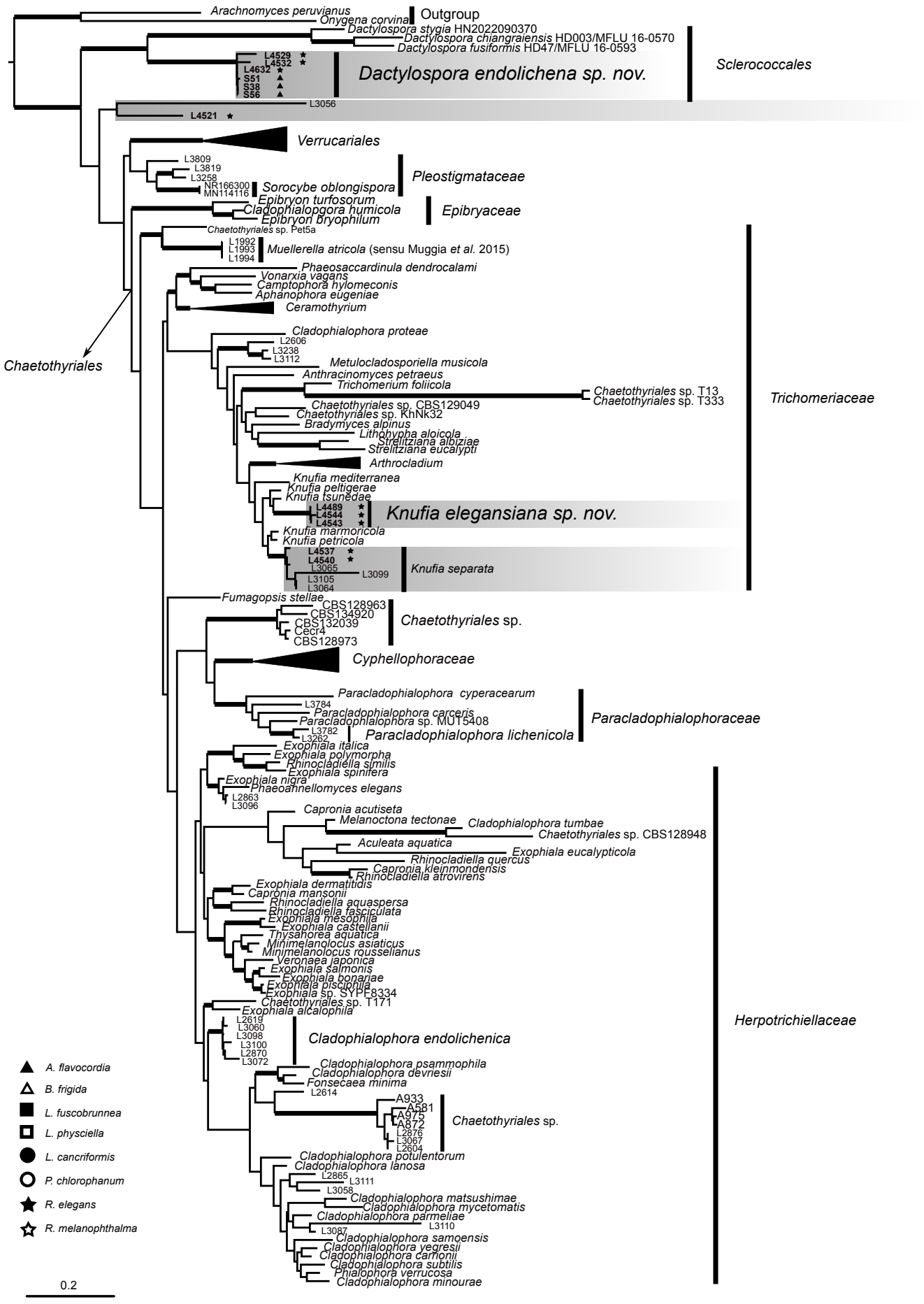

Fig. 2. Phylogenetic inference of *Chaetothyriales* based on the concatenated nucITS-nucLSU dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

Fig. 3. Phylogenetic inference of *Leotiomycetes* based on the concatenated nucITS-nucLSU dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

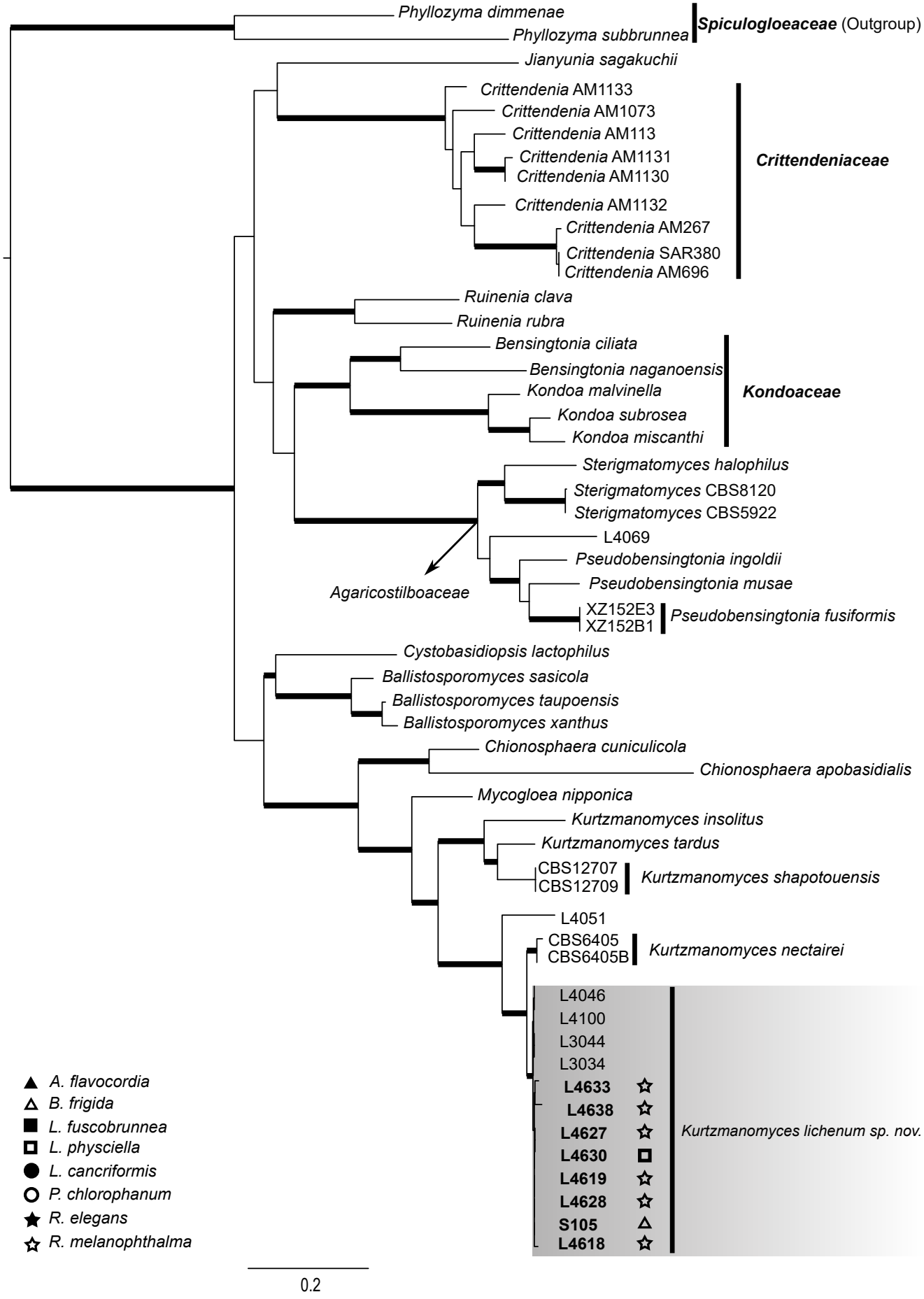

Fig. 4. Phylogenetic inference of *Agaricostilbomycetes* based on the concatenated nucITS-nucLSU dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend). *Agaricostilbomycetes* clades are named according to Millanes *et al.* (2021).

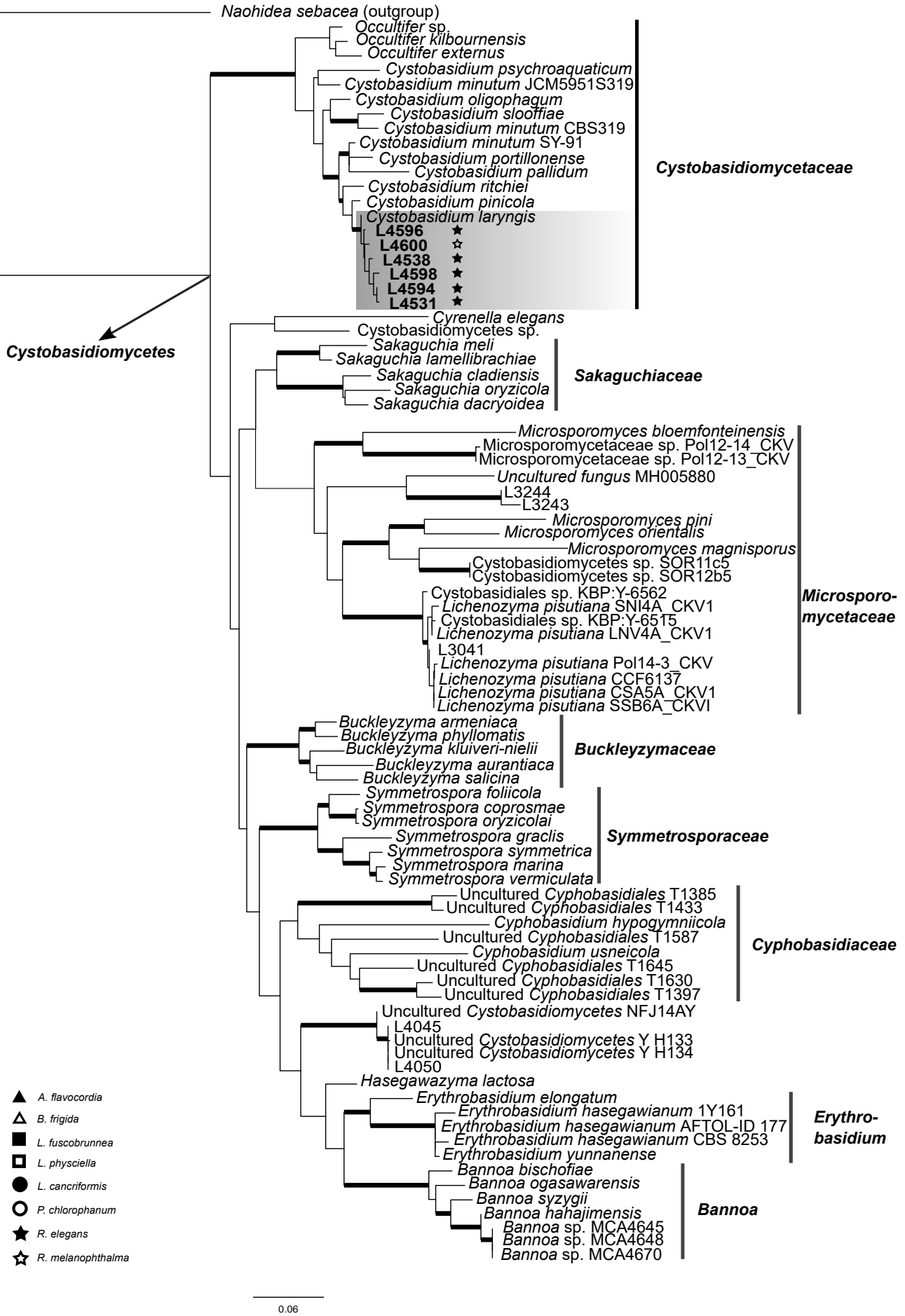

Fig. 5. Phylogenetic inference of *Cystobasidiomycetes* genera based on the concatenated nucITS-nucLSU dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. *Cystobasidiomycetes* clades are named according Černajová & Škaloud (2019), Millanes *et al.* (2016) and Cometto *et al.* (2022). Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

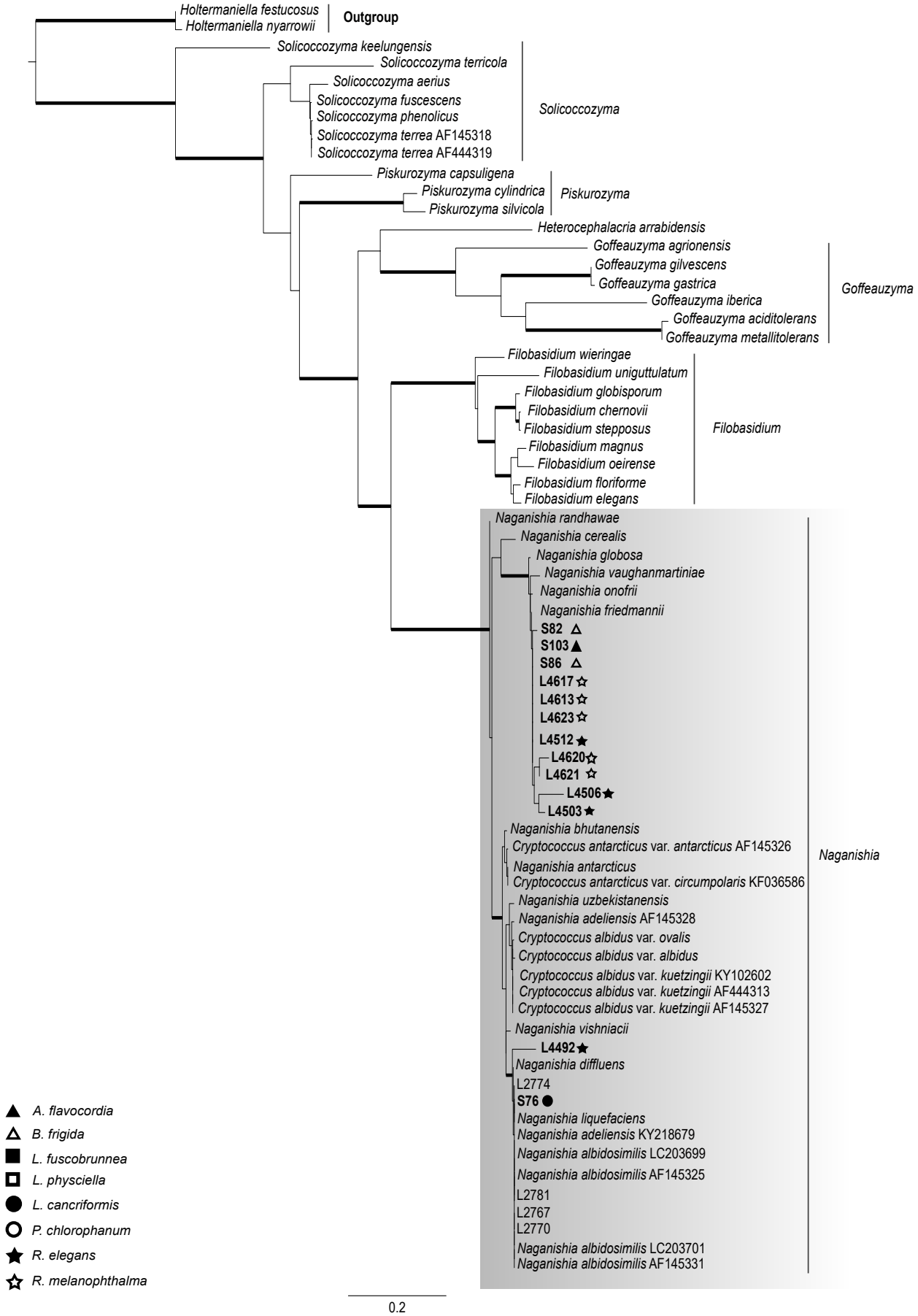

Fig. 6. Phylogenetic inference of *Filobasidiales* based on the nucITS dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. *Filobasidiales* clades are named according to Boekhout *et al.* (2011) and Liu *et al.* (2015a, b). Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

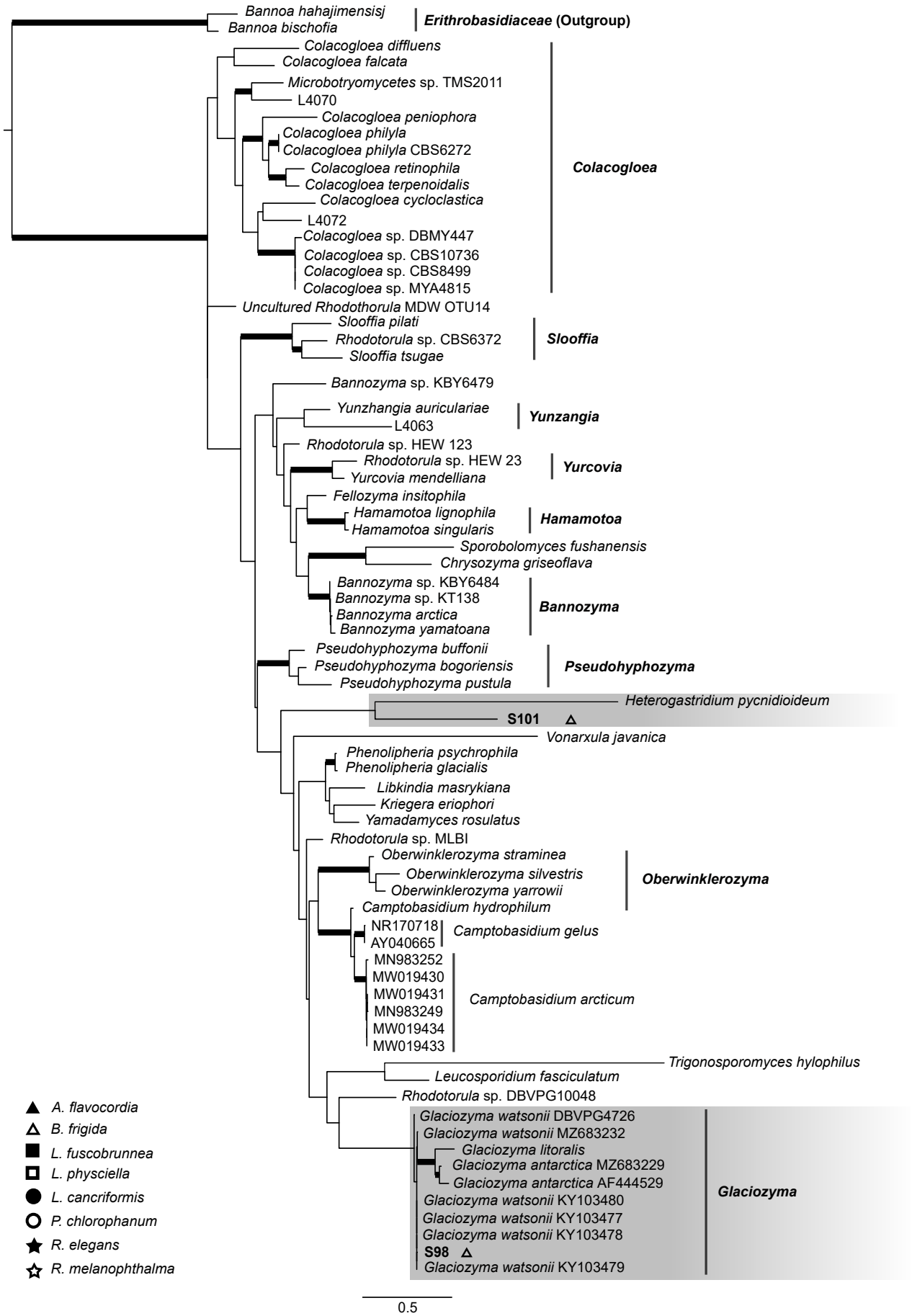

Fig. 7. Phylogenetic inference of *Microbotryomycetes* genus based on the nucITS dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. *Microbotryomycetes* clades are named according to Yurkov *et al.* (2016) and Kachalkin *et al.* (2019). Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

Fig. 8. Phylogenetic inference of *Tremellales* genera based on the nucITS dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. *Tremellales* clades are named according to Duarte *et al.* (2016), Millanes *et al.* (2011), Scorzetti *et al.* (2000), and Zamora *et al.* (2016). Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

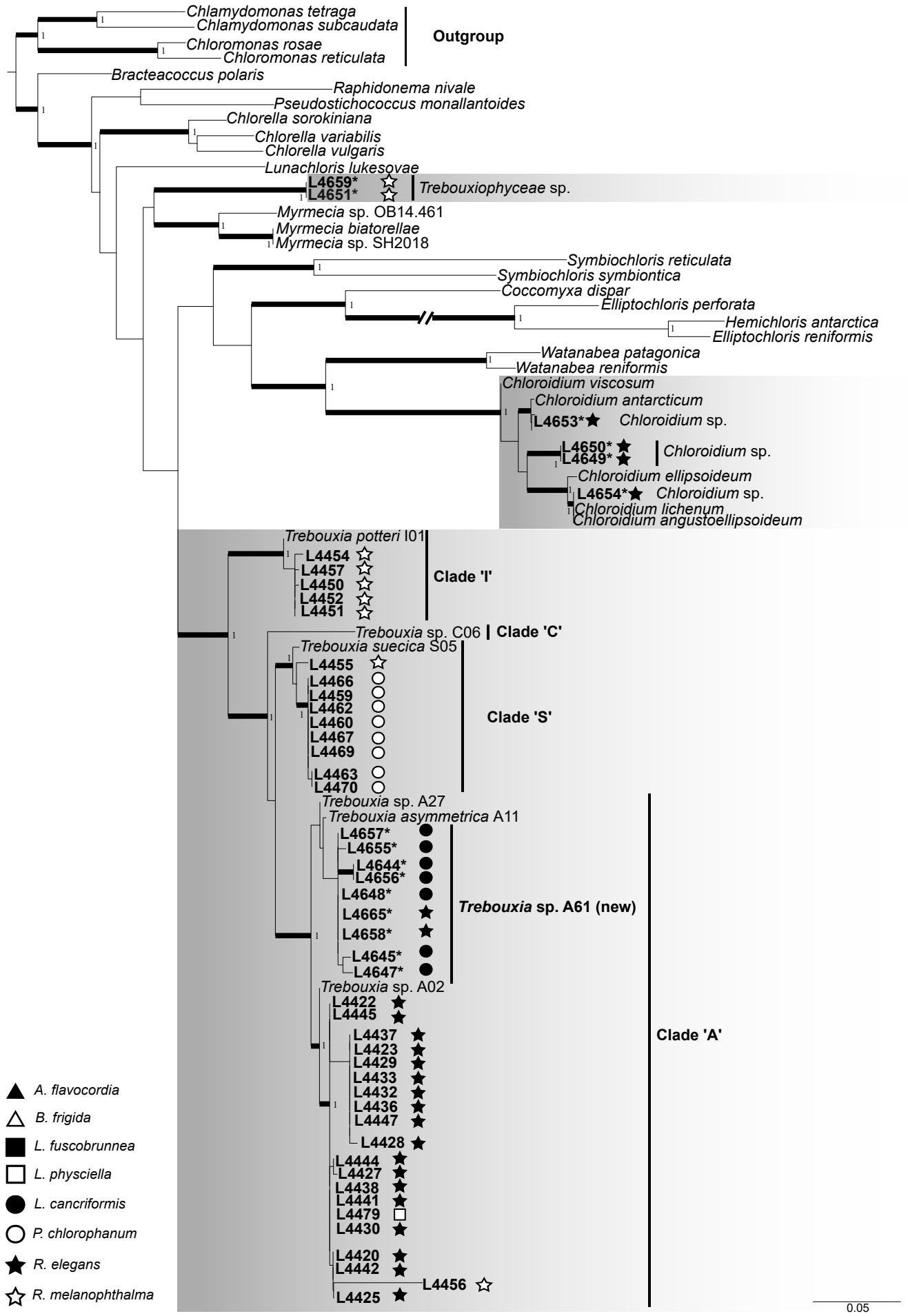

Fig. 9. Phylogenetic inference of *Trebouxiophyceae* based on the *rbcL* dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box.

Table 1. New sequences generated, the corresponding NCBI GenBank accession numbers and lichen species of origin of isolates collected from Victoria Land, Antarctica.

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
Catenulostroma sp.	L4530	PP781740	—	—	—	*Rusavskia elegans*	Random Hills
Chaetothyriales sp.	S23	PP781870	—	—	—	*Acarospora flavocordia*	Random Hills
	S24	PP781871	—	—	—	*Acarospora flavocordia*	Random Hills
Cladophialophora endolichena	S26	PP781873	—	—	—	*Acarospora flavocordia*	Random Hills
	S28	PP781875	—	—	—	*Acarospora flavocordia*	Random Hills
	S37	PP781884	—	—	—	*Acarospora flavocordia*	Random Hills
	S6	PP781904	—	—	—	*Acarospora flavocordia*	Random Hills
Cladophialophora humicola	L4541	PP781751	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4542	PP781752	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4557	PP781766	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4558	PP781767	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4559	PP781768	—	—	—	*Pleopsidium chlorophanum*	Mt Keinath
	L4560	PP781769	—	—	—	*Pleopsidium chlorophanum*	Mt Keinath
Cladosporiales sp.	L4599	PP781803	—	—	—	*Rhizoplaca melanophthalma*	Kay Island
Cystobasidiales sp.	L4494	PP781715	—	—	—	*Rusavskia elegans*	Random Hills
	L4584	PP781792	—	—	—	*Rusavskia elegans*	Random Hills
Cystobasidium sp.	L4531	PP781741	PP830974	—	—	*Rusavskia elegans*	Edmonson Point
	L4535	PP781745	—	—	—	*Rusavskia elegans*	Kay Island
	L4538	PP781748	PP830976	—	—	*Rusavskia elegans*	Kay Island
	L4594	PP781799	PP830980	—	—	*Rusavskia elegans*	Edmonson Point
	L4595	PP781800	—	—	—	*Rusavskia elegans*	Edmonson Point
	L4596	PP781801	PP830981	—	—	*Rusavskia elegans*	Kay Island
	L4598	PP781802	PP830982	—	—	*Rusavskia elegans*	Kay Island
	L4600	PP781804	PP830983	—	—	*Rhizoplaca melanophthalma*	Kay Island
	S102	PP781838	—	—	—	*Acarospora flavocordia*	Random Hills
	S104	PP781840	—	—	—	*Buellia frigida*	Star Nunatak
	S106	PP781842	—	—	—	*Acarospora flavocordia*	Kay Island
	S107	PP781843	—	—	—	*Acarospora flavocordia*	Kay Island
	S108	PP781844	—	—	—	*Acarospora flavocordia*	Random Hills
	S110	PP781846	—	—	—	*Acarospora flavocordia*	Kay Island
	S113	PP781849	—	—	—	*Lecidea cancriformis*	Random Hills

Table 1. (Continued)

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
	S116	PP781852	—	—	—	*Buellia frigida*	Random Hills
	S117	PP781853	—	—	—	*Buellia frigida*	Random Hills
	S118	PP781854	—	—	—	*Acarospora flavocordia*	Random Hills
	S75	PP781909	—	—	—	*Lecidea cancriformis*	Prior Island
	S77	PP781911	—	—	—	*Acarospora flavocordia*	Random Hills
	S78	PP781912	—	—	—	*Lecidea cancriformis*	Prior Island
	S79	PP781913	—	—	—	*Acarospora flavocordia*	Random Hills
	S83	PP781917	—	—	—	*Buellia frigida*	Kay Island
	S84	PP781918	—	—	—	*Buellia frigida*	Boulder Clay
	S85	PP781919	—	—	—	*Lecidea cancriformis*	Red Castle Ridge
	S87	PP781921	—	—	—	*Buellia frigida*	Kay Island
	S89	PP781922	—	—	—	*Lecidea cancriformis*	Red Castle Ridge
	S90	PP781923	—	—	—	*Acarospora flavocordia*	Random Hills
	S93	PP781926	—	—	—	*Acarospora flavocordia*	Kay Island
	S94	PP781927	—	—	—	*Buellia frigida*	Random Hills
	S95	PP781928	—	—	—	*Buellia frigida*	Random Hills
	S97	PP781929	—	—	—	*Lecidea cancriformis*	Inexpressible Island
Dactylospora endolichenica sp. nov.	L4529	PP781739	PP830973	—	—	*Rusavskia elegans*	Starnunatak
	L4532	PP781742	PP830975	—	—	*Rusavskia elegans*	Edmonson Point
	L4593	PP781798	—	—	—	*Rusavskia elegans*	Starnunatak
	L4632, ex-type	PP781832	PP830996	—	—	*Rusavskia elegans*	Random Hills
	S38	PP781885	PP831000	—	—	*Acarospora flavocordia*	Random Hills
	S42	PP781888	—	—	—	*Acarospora flavocordia*	Random Hills
	S51	PP781891	PP831001	—	—	*Acarospora flavocordia*	Random Hills
	S53	PP781892	—	—	—	*Acarospora flavocordia*	Random Hills
	S54	PP781893	—	—	—	*Acarospora flavocordia*	Random Hills
	S55	PP781894	—	—	—	*Acarospora flavocordia*	Random Hills
	S56	PP781895	PP831002	—	—	*Acarospora flavocordia*	Random Hills
	S57	PP781896	—	—	—	*Acarospora flavocordia*	Random Hills
Elasticomyces elasticus	L4500	PP781720	—	—	—	*Rusavskia elegans*	Random Hills
	L4508	PP781724	—	—	—	*Rusavskia elegans*	Botany Bay

Table 1. (Continued)

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
	L4509	PP781725	—	—	—	*Rusavskia elegans*	Botany Bay
	L4539	PP781749	—	—	—	*Rusavskia elegans*	Botany Bay
	L4561	PP781770	—	—	—	*Rhizoplaca melanophthalma*	Kay Island
	L4562	PP781771	—	—	—	*Lecanora physciella*	Random Hills
	L4563	PP781772	—	—	—	*Lecanora physciella*	Random Hills
	L4564	PP781773	—	—	—	*Lecanora physciella*	Random Hills
	L4565	PP781774	—	—	—	*Lecanora physciella*	Random Hills
	L4566	PP781775	—	—	—	*Lecanora physciella*	Random Hills
	L4567	PP781776	—	—	—	*Lecanora physciella*	Random Hills
	L4568	PP781777	—	—	—	*Lecanora physciella*	Random Hills
	L4569	PP781778	—	—	—	*Lecanora physciella*	Random Hills
	L4570	PP781779	—	—	—	*Lecanora physciella*	Random Hills
	L4571	PP781780	—	—	—	*Lecanora physciella*	Random Hills
	L4572	PP781781	—	—	—	*Lecanora physciella*	Random Hills
	L4573	PP781782	—	—	—	*Lecanora physciella*	Random Hills
	L4574	PP781783	—	—	—	*Lecanora physciella*	Random Hills
	L4575	PP781784	—	—	—	*Lecanora physciella*	Random Hills
	L4576	PP781785	—	—	—	*Lecanora physciella*	Random Hills
	L4577	PP781786	—	—	—	*Lecanora physciella*	Random Hills
	L4578	PP781787	—	—	—	*Lecanora physciella*	Random Hills
	L4579	PP781788	—	—	—	*Lecanora physciella*	Random Hills
	L4580	PP781789	—	—	—	*Lecanora physciella*	Random Hills
	L4581	PP781790	—	—	—	*Lecanora physciella*	Random Hills
	L4583	PP781791	—	—	—	*Rusavskia elegans*	Random Hills
	L4607	PP781810	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	S10	PP781845	—	—	—	*Acarospora flavocordia*	Random Hills
	S13	PP781859	—	—	—	*Buellia frigida*	Inexpressible Island
	S14	PP781860	—	—	—	*Buellia frigida*	Random Hills
	S15	PP781861	—	—	—	*Buellia frigida*	Inexpressible Island
	S16	PP781862	—	—	—	*Buellia frigida*	Random Hills
	S17	PP781863	—	—	—	*Buellia frigida*	Random Hills

Table 1. (Continued)

Organism	Isolate ID	nuclTS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
	S18	PP781864	—	—	—	*Buellia frigida*	Random Hills
	S19	PP781865	—	—	—	*Lecidea cancriformis*	Red Castle Ridge
	S1	PP781866	—	—	—	*Lecidea cancriformis*	Red Castle Ridge
	S20	PP781867	—	—	—	*Acarospora flavocordia*	Kay Island
	S21	PP781868	—	—	—	*Buellia frigida*	Random Hills
	S22	PP781869	—	—	—	*Buellia frigida*	Random Hills
	S25	PP781872	—	—	—	*Lecidea cancriformis*	Red Castle Ridge
	S31	PP781878	—	—	—	*Acarospora flavocordia*	Kay Island
	S34	PP781881	—	—	—	*Lecidea cancriformis*	Red Castle Ridge
	S4	PP781890	—	—	—	*Acarospora flavocordia*	Random Hills
	S5	PP781897	—	—	—	*Buellia frigida*	Random Hills
	S7	PP781914	—	—	—	*Acarospora flavocordia*	Random Hills
Extremaceae sp.	L4554	PP781763	—	—	—	*Rusavskia elegans*	Kay Island
	S27	PP781874	—	—	—	*Buellia frigida*	Boulder Clay
	S30	PP781877	—	—	—	*Buellia frigida*	Boulder Clay
	S64	PP781900	—	—	—	*Buellia frigida*	Random Hills
	S65	PP781901	—	—	—	*Buellia frigida*	Random Hills
Extremus sp.	S2	PP781876	—	—	—	*Acarospora flavocordia*	Random Hills
	S3	PP781887	—	—	—	*Acarospora flavocordia*	Random Hills
Glaciozyma watsonii	S98	PP781930	—	—	—	*Buellia frigida*	Random Hills
Knufia elegansiana sp. nov.	L4489, ex-type	PP781711	PP830970	—	—	*Rusavskia elegans*	Random Hills
	L4521	PP781733	PP830971	—	—	*Rusavskia elegans*	Starnunatak
	L4543	PP781753	PP830978	—	—	*Rusavskia elegans*	Starnunatak
	L4544	PP781754	PP830979	—	—	*Rusavskia elegans*	Starnunatak
	L4588	PP781793	—	—	—	*Rusavskia elegans*	Botany Bay
Knufia separata	L4490	PP781712	—	—	—	*Rusavskia elegans*	Random Hills
	L4493	PP781714	—	—	—	*Rusavskia elegans*	Random Hills
	L4495	PP781716	—	—	—	*Rusavskia elegans*	Random Hills
	L4496	PP781717	—	—	—	*Rusavskia elegans*	Random Hills
	L4497	PP781718	—	—	—	*Rusavskia elegans*	Random Hills
	L4498	PP781719	—	—	—	*Rusavskia elegans*	Random Hills

Table 1. (Continued)

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
	L4504	PP781722	—	—	—	*Rusavskia elegans*	Random Hills
	L4517	PP781730	—	—	—	*Rusavskia elegans*	Inexpressible Island
	L4527	PP781737	—	—	—	*Rusavskia elegans*	Starnunatak
	L4536	PP781746	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4537	PP781747	PP831006	—	—	*Rusavskia elegans*	Vegetation Island
	L4540	PP781750	PP830977	—	—	*Rusavskia elegans*	Vegetation Island
	L4546	PP781755	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4547	PP781756	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4548	PP781757	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4549	PP781758	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4550	PP781759	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4551	PP781760	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4552	PP781761	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4553	PP781762	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4555	PP781764	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4556	PP781765	—	—	—	*Rusavskia elegans*	Vegetation Island
	L4591	PP781796	—	—	—	*Rusavskia elegans*	Vegetation Island
	S11	PP781855	—	—	—	*Acarospora flavocordia*	Kay Island
	S12	PP781858	—	—	—	*Acarospora flavocordia*	Kay Island
	S35	PP781882	—	—	—	*Lecidea cancriformis*	Boulder Clay
	S36	PP781883	—	—	—	*Lecidea cancriformis*	Boulder Clay
Kurtzmanomyces lichenum sp. nov.	L4618	PP781820	PP830988	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4619	PP781821	PP830989	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4627	PP781829	PP830993	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4628	PP781830	PP830994	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4630	PP781831	PP830995	—	—	*Lecanora physciella*	Random Hills
	L4633	PP781833	PP830997	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4638	PP781835	PP830998	—	—	*Rhizoplaca melanophthalma*	Random Hills
	S105	PP781841	PP830999	—	—	*Buellia frigida*	Random Hills
	S91, ex-type	PP781924	PP831005	—	—	*Buellia frigida*	Star Nunatak
Meristemomyces sp.	S32	PP781879	—	—	—	*Lecidea cancriformis*	Random Hills

Table 1. (Continued)

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
	S33	PP781880	—	—	—	*Lecidea cancriformis*	Random Hills
	S60	PP781898	—	—	—	*Lecanora fuscobrunnea*	Pudding Butte
	S61	PP781899	—	—	—	*Lecanora fuscobrunnea*	Pudding Butte
	S66	PP781902	—	—	—	*Lecanora fuscobrunnea*	Boulder Clay
	S67	PP781903	—	—	—	*Lecanora fuscobrunnea*	Boulder Clay
Microbotryomycetes sp.	S101	PP781837	—	—	—	*Buellia frigida*	Campo Icaro
Naganishia friedmannii	L4492	PP781713	—	—	—	*Rusavskia elegans*	Random Hills
	L4503	PP781721	—	—	—	*Rusavskia elegans*	Random Hills
	L4506	PP781723	—	—	—	*Rusavskia elegans*	Random Hills
	L4512	PP781728	—	—	—	*Rusavskia elegans*	Botany Bay
	L4613	PP781816	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4617	PP781819	—	—	—	*Rhizoplaca melanophthalma*	Mt Keinath
	L4620	PP781822	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4621	PP781823	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4623	PP781825	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	S103	PP781839	—	—	—	*Acarospora flavocordia*	Random Hills
	S112	PP781848	—	—	—	*Acarospora flavocordia*	Kay Island
	S76	PP781910	—	—	—	*Acarospora flavocordia*	Random Hills
	S80	PP781915	—	—	—	*Acarospora flavocordia*	Random Hills
	S82	PP781916	—	—	—	*Acarospora flavocordia*	Random Hills
	S86	PP781920	—	—	—	*Buellia frigida*	Kay Island
Paracladophialophora lichenicola	S73	PP781907	—	—	—	*Buellia frigida*	Star Nunatak
	S74	PP781908	—	—	—	*Buellia frigida*	Star Nunatak
	L4528	PP781738	—	—	—	*Rusavskia elegans*	Starnunatak
Petrophila complexa sp. nov.	S71, ex-type	PP781905	PP831003	PP786395	—	*Lecidea cancriformis*	Campo Icaro
	S72	PP781906	PP831004	PP786396	PP971118	*Lecidea cancriformis*	Prior Island
Phaeoannellomyces sp.	S100	PP781836	—	—	—	*Acarospora flavocordia*	Random Hills
	S111	PP781847	—	—	—	*Acarospora flavocordia*	Kay Island
	S115	PP781851	—	—	—	*Acarospora flavocordia*	Random Hills
	S120	PP781856	—	—	—	*Acarospora flavocordia*	Random Hills
	S121	PP781857	—	—	—	*Acarospora flavocordia*	Random Hills

Table 1. (Continued)

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
	S39	PP781886	—	—	—	*Buellia frigida*	Inexpressible Island
	S45	PP781889	—	—	—	*Buellia frigida*	Inexpressible Island
	S92	PP781925	—	—	—	*Acarospora flavocordia*	Kay Island
Pseudeurotium lichenicum sp. nov.	L4523	PP781734	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4524	PP781735	PP830972	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4602	PP781805	PP830984	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4603	PP781806	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4604	PP781807	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4605	PP781808	PP830985	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4606	PP781809	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4608	PP781811	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4609	PP781812	PP830986	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4610	PP781813	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4611	PP781814	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4612	PP781815	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4614	PP781817	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4616	PP781818	PP830987	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4622	PP781824	PP830990	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4624	PP781826	PP830991	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4625, ex-type	PP781827	PP830992	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4626	PP781828	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
	L4636	PP781834	—	—	—	*Rhizoplaca melanophthalma*	Random Hills
Tremella macrobasidiata	L4510	PP781726	—	—	—	*Rusavskia elegans*	Random Hills
	L4511	PP781727	—	—	—	*Rusavskia elegans*	Botany Bay
	L4516	PP781729	—	—	—	*Rusavskia elegans*	Inexpressible Island
	L4519	PP781731	—	—	—	*Rusavskia elegans*	Random Hills
	L4520	PP781732	—	—	—	*Rusavskia elegans*	Inexpressible Island
	L4526	PP781736	—	—	—	*Rusavskia elegans*	Inexpressible Island
	L4589	PP781794	—	—	—	*Rusavskia elegans*	Inexpressible Island
	L4590	PP781795	—	—	—	*Rusavskia elegans*	Inexpressible Island
	L4592	PP781797	—	—	—	*Rusavskia elegans*	Starnunatak

Table 1. (Continued)

Organism	Isolate ID	nucITS	nucLSU	nucSSU	*RPB2*	Lichen species of origin	Sampling locality
Yeast lineage II (sensu Milannes *et al.* 2011)	L4533	PP781743	—	—	—	*Rusavskia elegans*	Edmonson Point
	L4534	PP781744	—	—	—	*Rusavskia elegans*	Edmonson Point
	S114	PP781850	—	—	—	*Lecidea cancriformis*	Random Hills

as a *Dactylospora* species within *Sclerococcales* and is represented by 12 isolates coming from three thalli of *A. flavocordia* collected at Random Hills and three thalli of *R. elegans* collected in Star Nunatak, Edmondson point, and Random Hills (Supplementary Table S1). The multi-locus phylogenetic reconstruction based on the combined nucITS and nucLSU loci (Fig. 2), confirmed the presence of this new *Dactylospora* clade supported by both ML and Bayesian inferences. The species is here described as *Dactylospora endolichenica sp. nov.* (see below), based on the morphological analyses performed on four strains (L4532, L4632, S38, S51; Fig. 12).

Six strains were assigned to the family *Epibryaceae* (Supplementary Fig. S2), isolated from two thalli of *R. elegans* and four thalli of *R. melanophthalma*. *Epibryaceae* here received full support by both the ML and Bayesian analyses.

The second new chaetothyrialean lineage (Fig. 2; Supplementary Fig. S2) is represented by five strains (L4488, L4489, L4521, L4543, and L4544), isolated from three thalli of *R. elegans* collected at Star Nunatak and Random Hills. It is fully supported and belong to the genus *Knufia* (*Trichomeriaceae*, *Chaetothyriales*), sister to a clade of three still undescribed *Knufia* strains. In the multilocus phylogeny (Fig. 2) this new *Knufia* lineage, described as the new species *Knufia elegansiana* (see below), was placed as sister taxon of *Knufia tsunedae*.

Most of the other strains in *Chaetothyriales*, a total of 25, corresponded to *Knufia separata* (Supplementary Fig. S2), together with other isolates obtained previously from lichens by Cometto *et al.* (2023). The new isolates of *Knufia separata* come from one thallus of *L. cancriformis* collected at Botanic Bay, one thallus from *A. flavocordia* collected at Kay Island, and nine thalli of *R. elegans* collected at Vegetation Island, Star Nunatak and Random Hills (Supplementary Fig. S2).

We also isolated three strains of the recently described species *Paracladophialophora lichenicola* (*Paracladophialophoraceae*), coming from thalli of *L. cancriformis* and *B. frigida*, and four strains of *Cladophialophora endolichena* (*Herpotrichiellaceae*), isolated from two thalli of *A. flavocordia*.

Eight strains formed a subclade within *Phaeoannellomyces elegans* and likely represent a new group of Antarctic haplotypes of this species. The last two strains S23 and S24 are so far identified as *Chaetothyriales* sp. within *Herpotrichiellaceae* and are placed unresolved in a clade with two *Capronia* and *Rhinocladiella* species and *Aculeata aquatica*.

The class *Leotiomycetes* is represented by 19 strains, and both the single locus (Supplementary Fig. S3) and the combined nucITS-nucLSU (Fig. 3) phylogenies placed them in a new clade close to the genera *Pseudeurotium*, *Thelebolus*, *Cleistothelebolus* and *Antarctomyces*. In particular, the combined dataset based on that of Quijada *et al.* (2022) placed this new clade as sister of *Pseudeurotium zonatum* (Fig. 3). All the strains were isolated from three thalli of *R. melanophthalma* collected at Random Hills and are here described as *Pseudeurotium lichenicum sp. nov.* (see below).

Within the *Basidiomycota*, the nucITS phylogenetic inference (Supplementary Fig. S4) of *Agaricostilbomycota* identified a new clade of *Kurtzmanomyces* represented by nine strains newly isolated form the Antarctic lichens and six strains previously isolate by other lichen thalli by

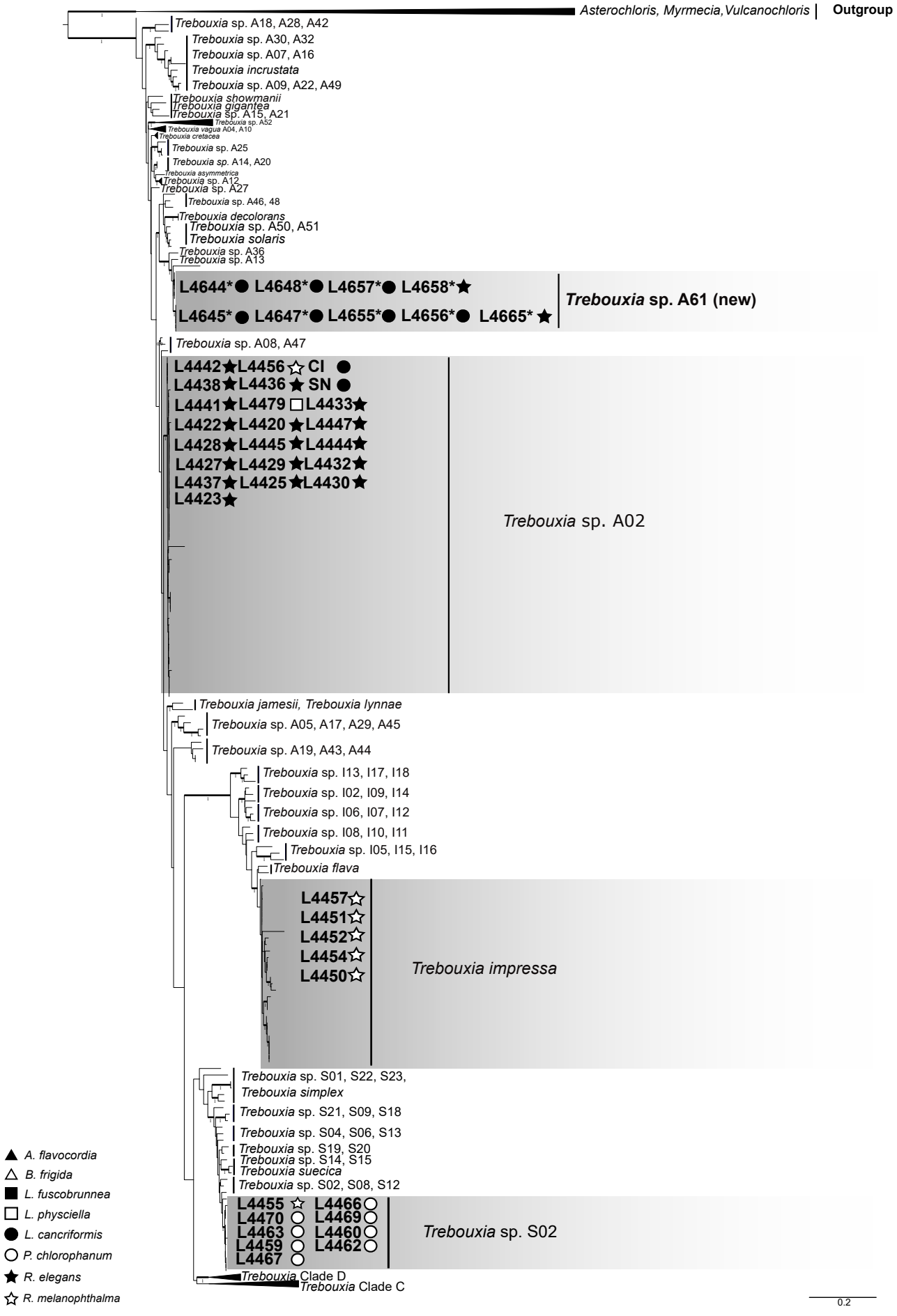

Fig. 10. Phylogenetic inference of the genus *Trebouxia* based on the nucITS dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. *Trebouxia* clades are named according to Muggia *et al.* (2020).

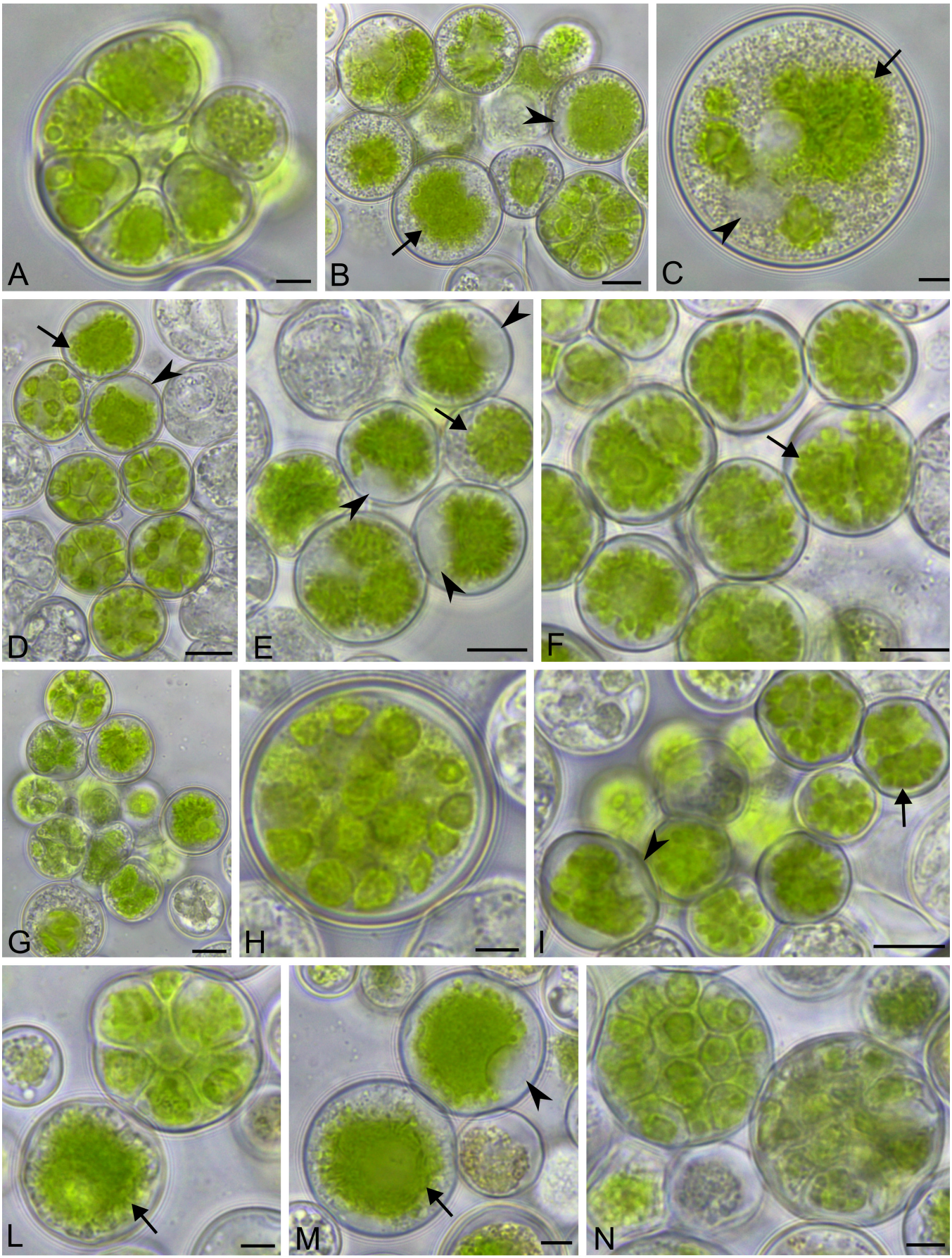

Fig. 11. Morphology of *Trebouxia* OTU A61. Cells observed by light microscopy: **A–C.** Strain L4644. **D–F.** Strain L4645. **G–I.** Strain L4647. **L–N.** Strain L4657. Black arrows indicate chloroplast lobes, and black arrowheads indicated the nucleus. Scale bars: A, C, H, L, M, N = 4 µm; B, D, E, F, G, I = 8 µm.

Cometto *et al.* (2022b), including the species *Tephromela atra* and *Rhizoplaca melanophthalma* of diverse geographic origins. This *Kurtzmanomyces* clade is monophyletic, fully supported, and sister to *Kurtzmanomyces nectairei.* The phylogenetic placement and the statistical support of the lineage was confirmed also by the multilocus analyses (ITS and nucLSU; Fig. 4). The strains belonging to this new clade, in this study, were isolated from one thallus of *B. frigida*, one of *L. physciella*, and two of *R. melanophthalma* (Fig. 4), all collected at Random Hills. The characterization of this new yeast species was done using strain S91 (Fig. 14; see below).

Within *Cystobasidiales* a total of 32 strains were identified as *Cystobasidium* (Fig. 5; Supplementary Fig. S5) and were isolated from *A. flavocordia* (10), *B. frigida* (8), *L. cancriformis* (6), *R. melanophthalma* (1), and *R. elegans* (7) (Supplementary Fig. S5). Of these, 27 strains corresponded to *C. laryngis*, while four strains are sister to *C. ritchiei* and one to *C. pallidum*. Two further strains (L4494, L4584) isolated from *R. elegans* were identified within *Cystobasidiales* at a basal position within the order (Supplementary Fig. S5).

Within *Filobasidiales* (Fig. 6), 11 strains isolated from *A. flavocordia* (1), *B. frigida* (2), *R. melanophthalma* (5), and *R. elegans* (3) were recognized as *Naganisha friedmannii* and are unresolved with *N. onofrii* and *N. vaughanmartiniae*. Another two strains isolated from thalli of *L. cancriformis* and *R. elegans* were placed in a clade and are partially unresolved together with *N. albidosimilis*, *N. diffluens*, *N. liquefaciens* and *N. adeliensis*, and with other four strains of *N. albidosimilis* previously isolated form lichens by Cometto *et al.* (2022b).

Microbotryomycetes was represented by only two strains isolated from *B. frigida* (Fig. 7), namely S101 and S98. Strain S101, found as sister taxon of *Heterogastridium pycnidioideum* and corresponded to this species (although branches subtending these two taxa are rather long). Strain S98 was recognized as *Glaciozyma watsonii*, as it is unresolved together with many other samples of *G. watsonii*. Ten new strains were identified within the order *Tremellales* (Fig. 8), eight of which were isolated from *R. elegans* and are identified as *Tremella macrobasidiata*, while the other two were isolated from *L. cancriformis* and *R. elegans* and were placed within the Yeast lineage II sensu Millanes *et al.* (2011), which still needs to be formally described. Both clades of *T. macrobasidiata* and Yeast Lineage II group several strains previously isolated from lichens (Cometto *et al.* 2023).

Algal diversity

Out of the eight lichen species studied, we obtained new isolates of microalgae from *L. physciella*, *L. cancriformis*, *P. chlorophanum*, *R. melanophthalma*, and *R. elegans*. In total, both from the isolated strains and the photobionts directly amplified from the thallus samples, we generated 45 nucITS and 48 *rbcL* new sequences, which were used to identify the taxa (Supplementary Table S10).

The phylogenetic analysis, based on the *rbcL* locus, revealed that all the photobiont sequences amplified from the lichen thalli belonged to the genus *Trebouxia* (Fig. 9). Specifically, we found that five sequences were associated to CLADE 'I', nine to CLADE 'S', and 29 to CLADE 'A' sensu Muggia *et al.* (2020). Another 15 new sequences were obtained from the axenic cultures: nine were identified as belonging to a new lineage of *Trebouxia* (better resolved in the nucITS phylogeny), two to the genus *Myrmecia* and four to the genus *Chloroidium*. The new lineage of *Trebouxia*, here identified as OUT A61, was isolated from *L. cancriformis* (strains L4648, L4644, L4657, L4647, L4655, L4645, L4656) and *R. elegans* (strains L4658, L4665).

The two strains of *Myrmecia* (L4651, L4659) formed a clade sister to the clade represented by *Myrmecia* sp. OB14.461, *Myrmecia* sp. SH2018 and *Myrmecia biatorellae*. The four strains recognized as *Chloroidium* (L4653, L4650, L4649, L4654), were either closely related to *Chloroidium antarcticum* (i.e., strain L4653), or sister to the clade formed by the three species *C. ellipsoideum*, *C. lichenum* and *C. angustoellipsoideum* (i.e., strains L4649 and L4650) in which the strain L4654 is nested.

The phylogenetic analysis based on the nucITS locus (Fig. 10; Supplementary Table S11) shows that 22 of the *Trebouxia* sequences amplified from the thalli of *L. physciella*, *L. cancriformis*, *R. elegans* and *R. melanophthalma* were identified as the lineage *Trebouxia* A02; nine sequences obtained from *P. chlorophanum* and *R. melanophthalma* were included in the lineage *Trebouxia* S02, and five sequences derived from *R. melanophthalma* were identified as *Trebouxia impressa*. The nine sequences amplified from axenically isolated strains from *L. cancriformis* and *R. elegans* formed a new, well-supported clade closely related to *Trebouxia* A13 and *Trebouxia* A36, here recognized as *Trebouxia* OTU A61.

Representative strains (L4644, L4645, L4647, L4657) of the new lineage *Trebouxia* OTU A61 were morphologically analysed (Fig. 11A–N) and revealed to be coccoid cells of 16–20 µm diameter, forming autospores (Fig. 11A, B, D, H, L, N). The chloroplast occupies most of the cytoplasm, confining the nucleus to a marginal part of the cell (Fig. 11C, E, M). Chloroplast lobes were not well evident, but crenulation could be appreciated in some cells, likely hinting to the 'crenulate' type of chloroplast (Fig. 11F, G, I; sensu Bordenave *et al.* 2022).

Taxonomy

Dactylospora endolichenica De Carolis & Muggia, ***sp. nov.*** MB 859817. Fig. 12.

Etymology: Residing cryptically within lichen thalli.

Typus: **Antarctica**, Victoria Land, Random Hills, isolated from the lichen thallus of *Rusavskia elegans* L4428 (TSB 44725), Jan. 2022, *L. Selbmann* (**holotype** MNA-CCFEE 6898, cryopreserved in a metabolically inactive state at -80 °C), ex-type culture L4632. *Dactylospora endolichenica* MNA-CCFEE 6898 is the unique identifier of the holotype sheet in the Antarctic National Museum - Culture Collection of Fungi from Extreme Environments (MNA-CCFEE) at the University of Tuscia, Viterbo.

Diagnosis: Endolichenic (i.e., cryptically present in lichen thalli) fungus derived likely from hyphae fragments or resting spores entrapped in the thalline matrix of the lichen hosts, growing in vitro rather fast; mycelium composed by a dense aggregate of hyaline hyphae that build a whitish-creamy colony with irregular margin, sometimes plaques are present. Most of the hyphae are composed by cylindrical

cells (2 × 15–20 µm) from which branches generate (Fig. 12). Pachybasium-like conidiophores (conidia-laden), ascocarps and ascospores not present in culture.

Distribution: reported for the first time as isolate from lichens growing on siliceous-granitic, and sandstone rocks in Northern Victoria Land, Antarctica (sites: Random Hills, Star Nunnatak, Edmonson Point). Isolated so far from the following lichen species: *A. flavocordia, R. elegans.*

Additional material examined: **Antarctica**, Northern Victoria Land, Random Hills on granitic rocks, endolichenic fungi isolated from thalli of *A. flavocordia* (strain numbers: S38, S42, S51, S53, S54, S55, S56 and S57); Northern Victoria

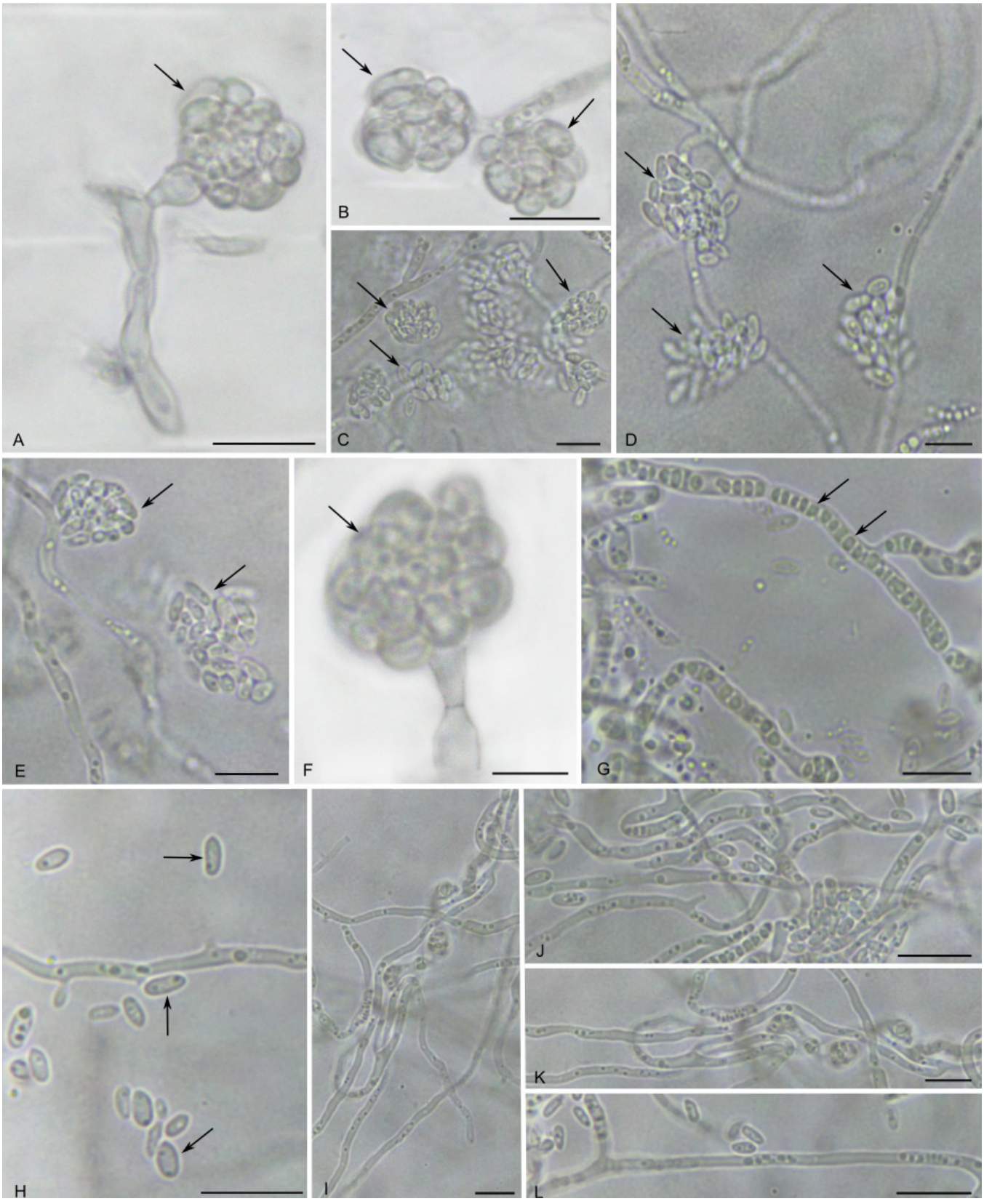

Fig. 12. Morphology of *Dactylospora endolichenica sp. nov.* **A–D.** Strain L4532. **E**, **F.** Strain L4632. **G**, **H.** Strain S38. **I–L.** Strain S51. Black arrows (in **A–F**, **H**) indicate conidiophores and conidiospores, and cellular inclusions (**G**). Scale bars: A–H, J–L = 5 µm; I = 10 µm.

Land, Star Nunatak, on granitic rocks, endolichenic fungi isolated from *R. elegans* L4440 thallus (TSB 44726), *L. Selbmann*, Jan. 2022, strain numbers L4529 and L4593; Northern Victoria Land, Edmonson Point, on granitic rocks, fungi isolated from *R. elegans* L4442 thallus (TSB 44727), *L. Selbmann*, Jan. 2022, strain number L4532.

Knufia elegansiana Stoppiello & Coleine, ***sp. nov.*** MB 859818. Fig. 13.

Etymology: From the lichen host species name, as originally isolated from thalli of *Rusavskia elegans*.

Typus: **Antarctica**, Victoria Land, Random Hills, isolated from the thallus of *Rusavskia elegans* (L4420, TSB 44728), Jan. 2022, *L. Selbmann* (**holotype** MNA-CCFEE 6899, cryopreserved in metabolically inactive state at -80 °C), ex-type culture L4489. *Knufia elegansiana* MNA-CCFEE 6899 is the unique identifier of the holotype sheet in the Antarctic National Museum - Culture Collection of Fungi from Extreme Environments (MNA-CCFEE) at the University of Tuscia, Viterbo.

Diagnosis: Endolichenic (i.e., cryptically present in lichen thalli) fungus derived likely from hyphae fragments or resting spores entrapped in the thalline matrix of the lichen hosts, growing in vitro slowly. The mycelium is composed by a dense aggregate of strongly melanized hyphae that builds a black granulose colony with irregular margin. Most of the hyphae are composed by cylindrical or rectangular cells of variable size (3–4 × 5–10 µm) from which branches generate (Fig. 13). Buddings and ramifications were observed.

Distribution: Reported for the first time as isolate from lichens growing on siliceous-granitic rocks in Northern Victoria Land, Antarctica (sites: Random Hills). Isolated so far from the lichen species *Rusavskia elegans.*

Additional material examined: **Antarctica**, Northern Victoria Land, Star Nunatak, on granitic rocks, endolichenic fungi isolated from *R. elegans* lichen thallus L4438 (TSB 44729), *L. Selbmann*, Jan. 2022, strain numbers L4521 and L4543; from *R. elegans* lichen thallus L4440 (TSB 44726), *L. Selbmann*, Jan. 2022, strain number L4544; from *R. elegans*

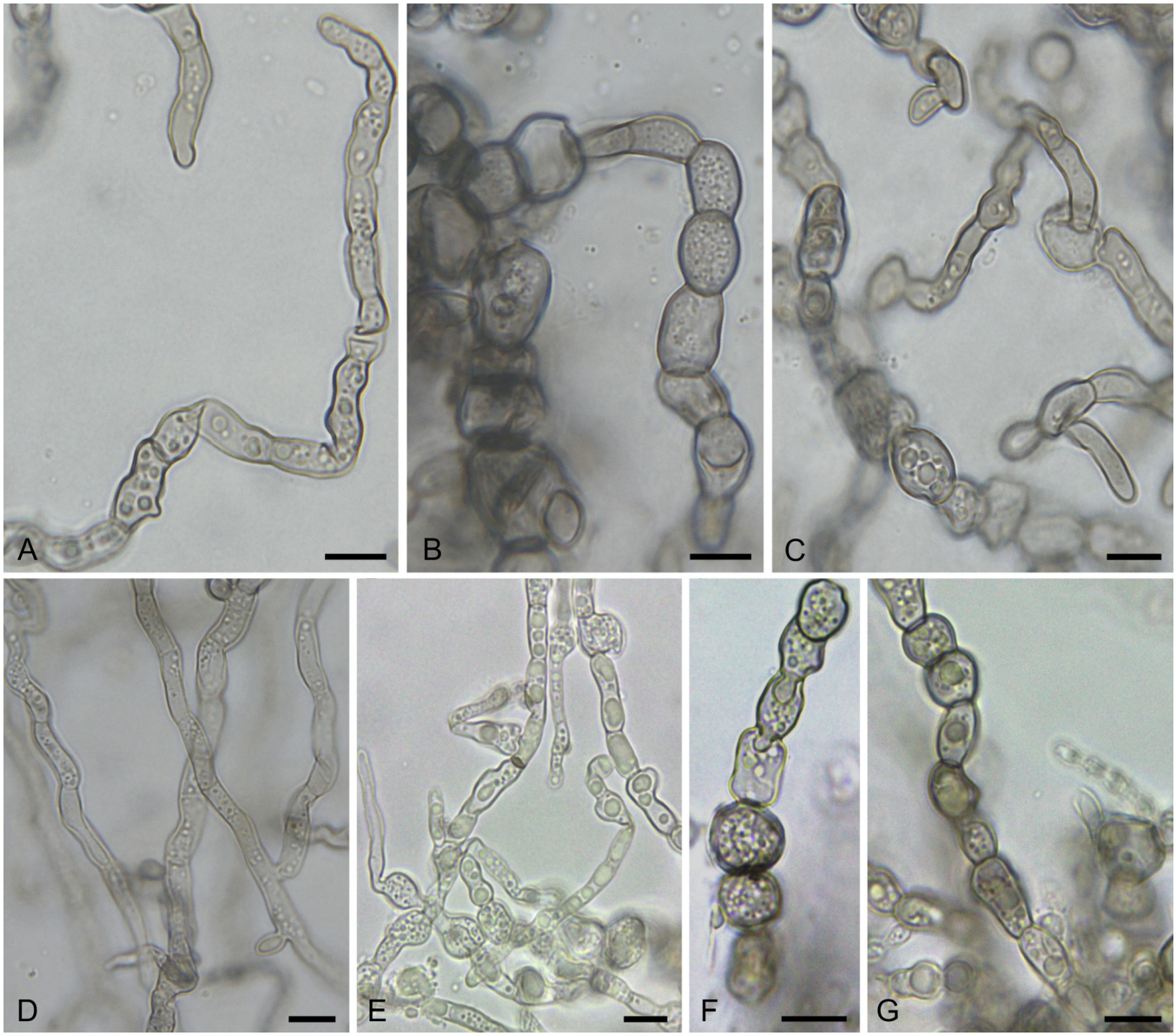

Fig. 13. Morphology of *Knufia elegansiana sp. nov.* **A–D.** Strain L4489. **E.** Strain L4543. **F**, **G.** Strain L4544. Filamentous, septate, and melanized hyphae (**A**, **D**), branching hyphae (**D, E**) and cytoplasmic inclusion (**E**) are visible. Scale bars = 10 ***µm.***

lichen thallus L4430 (TSB 44741), *L. Selbmann*, Jan. 2022, strain number L4588.

Kurtzmanomyces lichenum Turchetti & Muggia, ***sp. nov.*** MB 859815. Fig. 14.

Etymology: Associated to lichen thalli.

Typus: **Antarctica**, Victoria Land, Random Hills, isolated from a lichen thallus of *Buellia frigida*, Jan. 2022, *L. Selbmann* (**holotype** DBVPG 8077, cryopreserved in a metabolically inactive state at -80 °C), ex-type culture S91 = MNA-CCFEE 6772. *Kurtzmanomyces lichenum* DBVPG 8077 is the unique identifier in the Industrial Yeasts Collection DBVPG, Dep. of Agricultural, Food and Environmental Sciences, University of Perugia, Italy.

Diagnosis: Both studied strains (S91 and L3034) grew very slowly; after 10 d at 20 °C on PDA no colonies were visible. After 8 wk on PDA, the streak culture colonies are orange-coloured, raised, butyrous, with entire margins, smooth and dull surface, and 1.0–2.0 mm diam. (Fig. 14A). After 2 wk on YPD tubes (liquid medium) no growth was visible; only after 4 wk sediment was observed on the bottom of the tube and after eight weeks a ring on the surface of the liquid was noticed. After 2 wk on YPD, cells are round to ovoid, 2.3–7.0 × 1.5–4.6 µm in size, occur singly, and proliferating by multipolar budding (Fig. 14B, C). No pseudohyphae and true hyphae were observed. No mating was observed in the single strains and in the mixed cultures. Fermentation of glucose was negative. Growth on D-glucose, L-arabinose, sucrose, trehalose, cellobiose, salicin, and succinate was observed. Variable growth on D-galactose, D-arabinose, lactose, D-gluconate, and lactate was observed. No growth on L-sorbose, D-ribose, D-xylose, L-rhamnose, maltose, methyl-alpha-D-glucoside, arbutin, melibiose, raffinose, melezitose, glycerol, erythritol, ribitol, xylitol, D-glucitol, D-mannitol, galactitol, myo-inositol, glucono δ-lactone, D-glucoronate, D-galacturonate, citrate, methanol, ethanol, L-malate, hexadecane, N-acetyl d-glucosamine, and ethyl acetate was observed. Utilization of nitrogen sources: growth on nitrate, nitrite, ethylamine, cadaverine, and creatine was observed. Urea hydrolysis is negative, while Diazonium Blue B reaction was positive. Starch-like compounds were not produced. Maximum growth temperature: 20 °C. No growth was observed at 0.01 % cycloheximide.

Additional materials examined: **Antarctica**, Northern Victoria Land, Star Nunatak, on granitic rocks, endolichenic fungi isolated from *B. frigida* strain number S105, Jan. 2022, *L. Selbmann*; Northern Victoria Land, Random Hills, on granitic rocks, endolichenic fungi isolated from *R. melanophthalma* L4455 (TSB 44761), Jan. 2022, *L. Selbmann*, strain numbers L4618, L4619; Northern Victoria Land, Random Hills, on granitic rocks, endolichenic fungi isolated from *R. melanophthalma* L4457 (TSB 44762), Jan. 2022, *L. Selbmann*, strain numbers L4627, L4628, L4633, L4638; Northern Victoria Land, Random Hills, on granitic rocks, endolichenic fungi isolated from *L. physciella* L4479 (TSB 44784), Jan. 2022, *L. Selbmann*, strain number L4630. **USA**, Utah, Utah Co. Rock Canyon, on granitic rocks, endolichenic fungi isolated from *R. melanophthalma* L2637 (TSB 44792), Aug. 2019, *S.D. Leavitt*, strain number L3034; Idaho, Owyhee Co. Along Mud Flat Rd, 27.7 miles from Hishway 78, endolichenic fungi isolated from *R. melanophthalma* L2725, Aug. 2019, *S.D. Leavitt* 19-233, strain number L3044, L3045. **Italy**, Valle d'Aosta, Gressoney Valley, at Colle Pinter, 2800 m a.s.l., endolichenic fungi isolated from *R. melanophthalma* L3483 (TSB 42931), 22 Aug. 2020, *L. Muggia*, strain number L4089; Piemonte, prov. Turin, Val D' Ala, Ala di Stura, loc. Balme, from Balme path n. 228 towards Lago Ru, ca 1500 m a.s.l., on siliceous rocks, endolichenic fungi isolated from *Tephromela atra* L3556 (TSB 42728), 23 Aug. 2020, *L. Muggia*, strain number L4101; Piemonte, prov. Cuneo, Val Varaita-Val Maira, Colle di Sampeyre, on siliceous rocks W of the pass, 2250 m a.s.l., endolichenic fungi isolated from *Tephromela atra* L3643 (TSB 42738), 24 Aug. 2020, *L. Muggia*, strain number L4100.

Petrophila complexa Stoppiello & Selbmann, ***sp. nov.*** MB 859814. Fig. 15.

Etymology: Named after the unusual multiple structures that the fungus can exhibit, although it belongs to the group of black fungi, known for their very poor morphology.

Typus: **Antarctica**, Victoria Land, in Campo Icaro, isolated from lichen thallus of *Lecidea cancriformis*, Jan. 2022, *L. Selbmann* (**holotype** MNA-CCFEE 6751, cryopreserved in a metabolically inactive state at -80 °C), ex-type culture S71. *Petrophila complexa* MNA-CCFEE 6751 is the unique identifier of the holotype sheet in the Antarctic National Museum - Culture Collection of Fungi from Extreme Environments (MNA-CCFEE) at the University of Tuscia, Viterbo.

Diagnosis: Lichen-associated, asexual fungus. Strictly psychrophilic, optimal growth temperature 10 °C, unable to

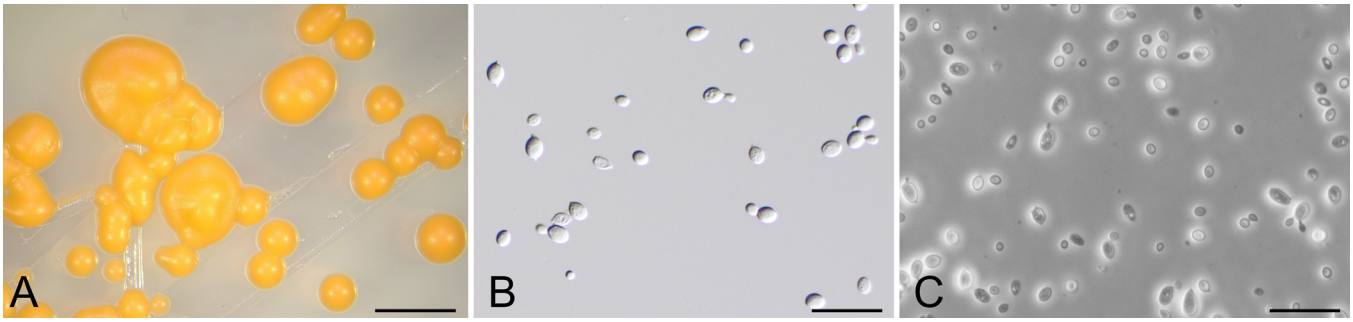

Fig. 14. Morphology of *Kurtzmanomyces lichenum sp. nov.*, strain S91. **A.** Cultures growing on YPD. B, D. Cells after 2 wk on YPD. Scale bars: A = 2 mm; B, C = 20 µm.

grow at 20 °C. Colonies black obverse and reverse, clumpy, slightly velvety, irregular, not flat margin. Very slowly growing in vitro, attaining 3–5 mm diam. in 1 month at optimal T. Hyphae septate, from brown to dark brown; cylindrical hyphae smooth (Fig. 15A), 3–3.5 µm wide, characterized by thick cell walls showing polar growth by enteroblastic proliferation and branching by lateral enteroblastic elongation (Fig. 15B). Torulose hyphae with cell wall 4.5 µm wide (Fig. 15C) seceding rhexolythically (Fig. 15D). Meristematic growth present (Fig. 15F). Conidiophores erect, micronematous, with intercalary or terminal conidiogenous cells producing 1-celled conidia (Fig. 15F). Intercalary or terminal chlamydospores 7.5 µm wide sporadically/sometimes present. Intercalary or terminal swollen cells 7.5 µm wide, eventually resulting in endoconidia formation, 3.2 µm wide (Fig. 15G–I), or evolving in resistant structures, globose or sub-globose (7–8 µm wide), with very thick cell wall.

Distribution: Continental Antarctica, isolated from the lichen *Lecidea cancriformis*, Campo Icaro and Prior Island, Northern Victoria Land.

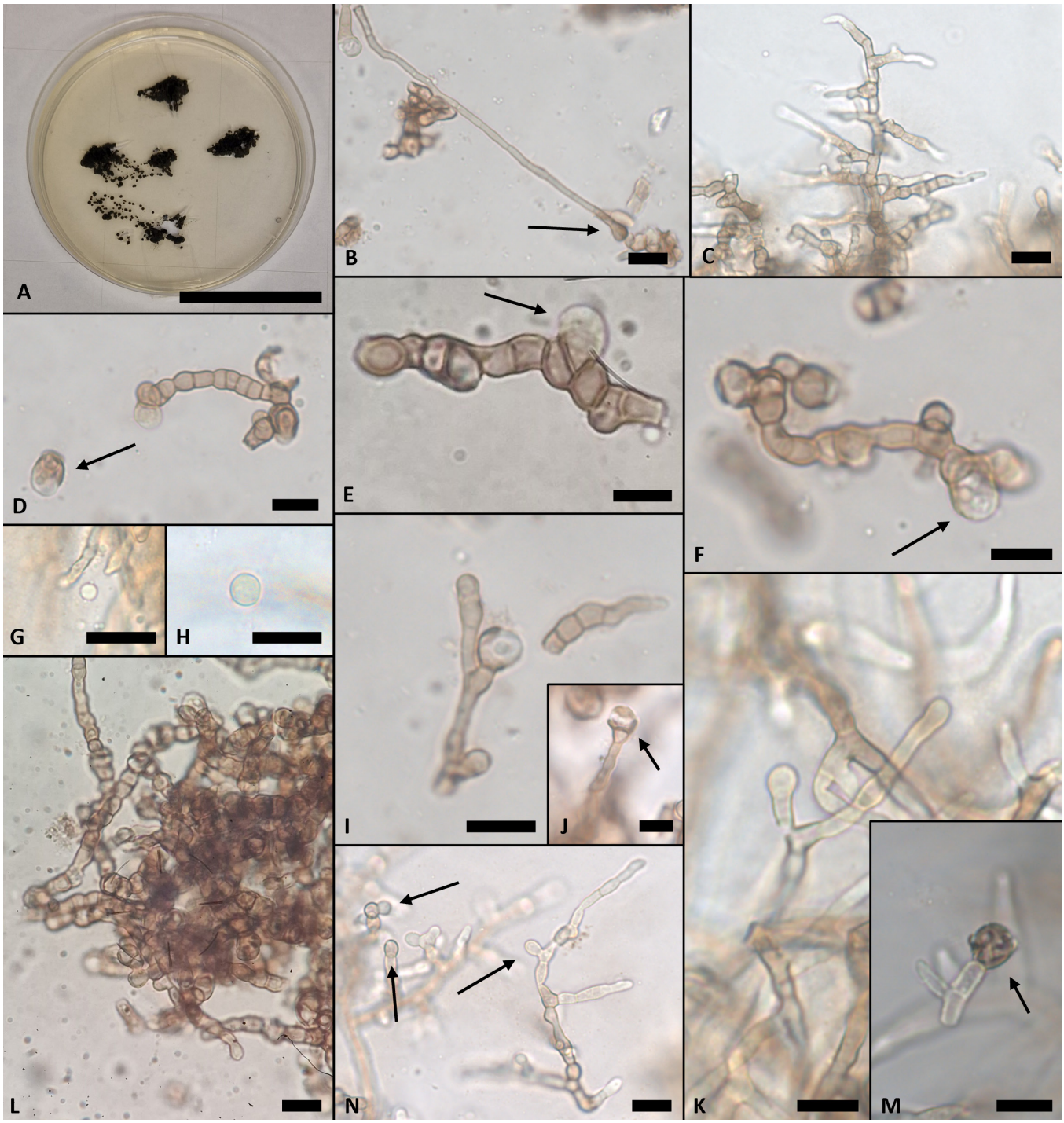

Fig. 15. A. Morphological characters of *Petrophila complexa sp. nov.*, strain MNA-CCFEE 6751. **B.** Torulose, septate hyphae, with enteroblastic proliferation (arrow). **C.** Branching by lateral enteroblastic elongation. **D, E.** Torulose hyphae with thicker cell wall 4.5 µm wide (**D**), seceding rhexolythically (**D**), and meristematic growth present are observed (**E**). **F.** Terminal conidiogenous cells producing 1-celled conidia (arrow). **G–J, M.** Intercalary or terminal swollen cells 7.5 µm wide, eventually resulting in endoconidia formation, 3.2 µm wide, or evolving in resistant structures, globose or sub-globose (7–8 µm wide), with very thick cell wall. **K, N.** Conidiophores erect, micronematous, with intercalary or terminal conidiogenous cells producing 1-celled conidia (arrows in N). **L.** Meristematic grown mycelium. Scale bars: A = 5 cm; B–H = 10 µm; I–M = 5 µm.

Additional material examined: **Antarctica**, Northern Victoria Land, Prior Island, on granitic rocks, endolichenic fungi isolated from *L. cancriformis*, Jan. 2022, *L. Selbmann*, strain number S72 (MNA-CCFEE 6752).

Notes: As reported for *P. incerta*, *P. complexa* displays intercalary conidiogenous cells producing 1-celled conidia. Unlike *P. incerta*, *P. complexa* displays endoconidiation and meristematic growth. *Petrophila complexa* is also peculiar for its ecology and distribution: differently from *P. incerta*, occurring on rocks in Spain, it occurs associated within lichen thalli in Antarctica.

Pseudeurotium lichenicum Selbmann & Muggia, ***sp. nov.*** MB 859819. Fig. 16.

Etymology: Associated to lichen thalli.

Typus: **Antarctica**, Victoria Land, Random Hills, isolated from a lichen thallus of *Rhizoplaca melanophthalma* L4456 (TSB 44730), Jan. 2022, *L. Selbmann* (**holotype** MNA-CCFEE 6900, cryopreserved in a metabolically inactive state at -80 °C), ex-type culture L4625. *Pseudeurotium lichenicum* MNA-CCFEE 6900 is the unique identifier of the holotype sheet in the Antarctic National Museum - Culture Collection of Fungi from Extreme Environments (MNA-CCFEE) at the University of Tuscia, Viterbo.

Diagnosis: Morphology studied on strains L4525 and L4625, endolichenic (i.e., cryptically present in lichen thalli) fungus derived likely from hyphae fragments entrapped in the thalline matrix of the lichen hosts, growing in vitro fast. The mycelium is composed by a dense aggregate of light melanized hyphae that builds a brownish colony with regular margin. Most of the hyphae are composed by cylindrical or rectangular cells of variable size (3–4 × 10–20 µm) from which branches generate; intracellular inclusions were often observed (Fig. 16).

Distribution: Reported for the first time as isolated from lichens growing on siliceous-granitic rocks in Victoria Land, Antarctica (sites: Random Hills). Isolated so far from the lichen species *R. melanophthalma*.

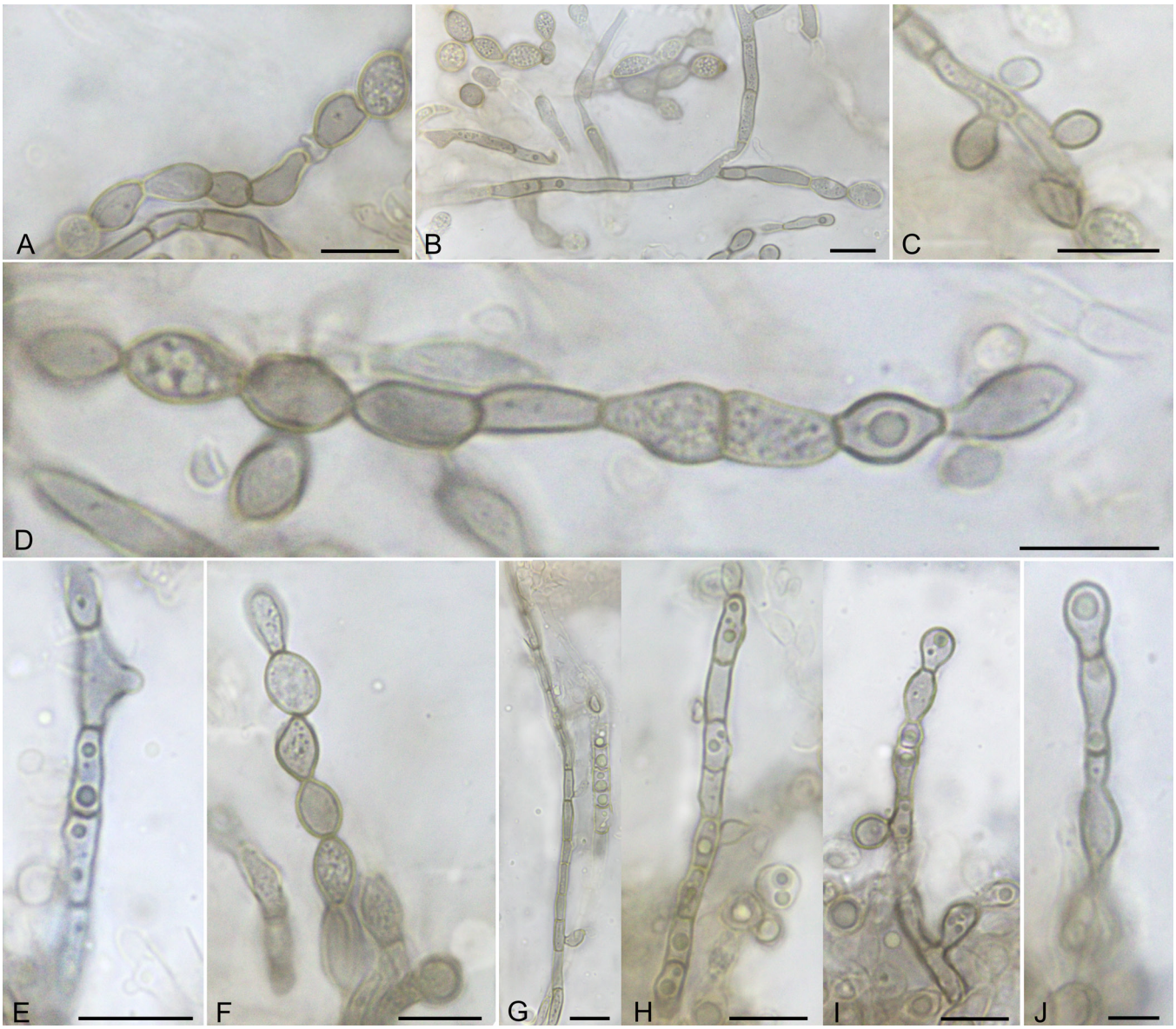

Fig. 16. Morphology of *Pseudeurotium lichenicum sp. nov*. **A–D.** Strain L4525. **E–J.** Strain L4625. Scale bars = 5 ***µm.***

Additional material examined: **Antarctica**, Northern Victoria Land, Random Hills on granitic rocks, endolichenic fungi isolated from *R. melanophthalma* L4456 (TSB 44730), Jan. 2022, *L. Selbmann*, strain numbers L4523, L4524, L4610, L4611, L4612, L4614, L4616; from *R. melanophthalma* L4451 (TSB 44731), Jan. 2022, *L. Selbmann*, strain numbers L4602, L4603, L4604, L4605, L4606, L4608, L4609; from *R. melanophthalma* L4452 (TSB 44732), Jan. 2022, *L. Selbmann*, strain numbers L4622, L4624, L4626, L4636.

DISCUSSION

High throughput sequencing has greatly improved the study of the lichen mycobiota by making available a large amount of data regarding the diversity of lichen-associated fungi. Still, these 'new generation' molecular techniques are affected by amplification biases and primer mismatches that may let some taxa go undetected but will likely be overcome by future metagenomic studies. Thus, an integrative taxonomic approach, in which molecular data are complemented by a culture-dependent methods still allows to reach a more comprehensive understanding of the hidden biodiversity and of the biology of the species. Concerning lichen-associated fungi, the isolation of environmental strains in axenic culture is already a well-established application, that has proven to be fundamental for a reliable description and characterization of new species cryptically inhabiting the lichen thalli (Girlanda *et al.* 1997, Arnold *et al.* 2009, Muggia *et al.* 2016, Smith *et al.* 2020, Muggia *et al.* 2021, Cometto *et al.* 2022b, 2023). However, many of these species are not always detected by the amplicon sequencing method. Here we were able to culture more than 300 fungal and algal strains from thalli of five Antarctic endemic (i.e., *A. flavocordia*, *B. frigida*, *L. fuscobrunnea*, *L. physciella*, *L. cancriformis*), and three cosmopolitan lichen species (i.e. *P. chlorophanum*, *R. melanophthalma* and *R. elegans*) collected in the Victoria Land, Antarctica. Among the isolated fungi we recognized species already known to science (Selbmann *et al.* 2008, 2013a, b, Egidi *et al.* 2014, Cometto *et al.* 2022a, b, 2023), as well as five taxa that are here newly described.

Most sequences belonged to the phylum *Ascomycota*, particularly to the big fungal classes *Dothideomycetes* and *Eurotiomycetes*, in which already most of the fungi isolated from epilithic lichens were recognized so far (Selbmann *et al.* 2013b, Muggia *et al.* 2016, Fernández-Mendoza *et al.* 2017, Cometto *et al.* 2023). The metabarcoding analyses performed on the same lichen samples (see Stoppiello *et al.* 2025) showed that *Basidiomycota* were present in lower numbers than *Ascomycota* (in line with other studies, e.g., Lendemer *et al.* 2019, Smith *et al.* 2020, Cometto *et al.* 2024), and indeed a corresponding lower number of fungal *Basidiomycota* isolates (yeasts and filamentous forms) was obtained. However, a few metabarcoding analyses performed from cold substrates (Debeljak & Baltar 2023) and from lichens (Cometto *et al.* 2024), showed the dominance of *Basidiomycota* within yeasts, which confirm the substantial amount of basidiomycetes yeasts grown in culture in the present research. This may be explained by the fact that there would be a lower inter-species competition of fewer fungi within the thalli due to the extreme and selective environmental conditions. Yeasts are known to adopt strategies to survive and thrive in extreme environments, such as production of cold-active enzymes, 'anti-freeze' compounds and extracellular polymers (Siddiqui & Cavicchioli 2006, Turk *et al.* 2011, Buzzini *et al.* 2012, Gunde-Cimerman *et al.* 2014), and this would facilitate their permanence within the lichen thalli in Antarctica. A key role could also be played by their production of exopolysaccharides that would make them more resistant toward the lichen secondary metabolites (Spribille *et al.* 2016).

Ascomycota *diversity*

The phylum *Ascomycota* is the most represented within the lichen mycobiota in general (U'Ren *et al.* 2010, 2012, Zhang *et al.* 2015, 2016, Fernàndez-Mendoza *et al.* 2017, Banchi *et al.* 2018). So far, *Eurotiomycetes*, *Dothideomycetes* and *Leotiomycetes* are the fungal classes most frequently associated to epilithic lichens with a great majority of taxa representatives of the polyextremotolerant black fungi (especially from the order *Capnodiales* and *Chaetothyriales*; Selbmann *et al.* 2013, Santiago *et al.* 2015, Muggia *et al.* 2022, Cometto *et al.* 2023). Black fungi have evolved adaptations that allow them to survive in environments such as Antarctica or high mountains (Onofri *et al.* 2007, De Los Ríos *et al.* 2014, Selbmann *et al.* 2015, Coleine *et al.* 2021, Cometto *et al.* 2022a).

In this study most of the black fungal strains belonged the order *Chaetothyriales*. This high abundance throughout the specimens examined could be related to the ancestral association of these guilds to lichen thalli which is apparently maintained across the evolution of *Chaetothyriomycetidae* (Gueidan *et al.* 2008, 2011, Quan *et al.* 2020, Muggia *et al.* 2021). Over 30 % of the black fungal isolates were *Elasticomyces elasticus*, a worldwide distributed species occurring exclusively in cold environments, specifically at high altitudes or polar locations and initially isolated from the thallus of *Usnea antarctica* collected in Kay Island, Antarctica (Selbmann *et al.* 2008). Despite having been also isolated from rocks, this high frequency may indicate a preference of the fungus for the endolichenic life-style.

Here we report four new species of ascomycetes inhabiting the lichen thalli, highlighting that there is a still unknown diversity to be uncovered. The species isolated from our samples belonged to the classes *Dothideomycetes*, *Eurotiomycetes* and *Leotiomycetes*. Within *Eurotiomycetes* in particular, the new taxa were recognized as *Dactylospora endolichenica sp. nov*. (*Dactylosporaceae*) and *Knufia elegansiana sp. nov*. (*Chaetothyriales*). *Dactylospora* is a genus of fungi comprising saprobic organisms that inhabit wood and liverworts but are also found in lichen thalli (Pang *et al.* 2014, Jaklitsch *et al.* 2016). The genus was reported in Antarctica from the South Shetland Islands as a lichenicolous fungus by Alstrup *et al.* (2018), who described the new species *Dactylospora antarctica* from fertile specimens parasitic on *Lecidea* thalli. In the present study we isolated *D. endolichenica* as a cryptic lichenicolous fungus from thalli of *A. flavocordia* and *R. elegans* collected in the continental region. The phylogeny of *Dactylospora* was resolved by Ekanayaka *et al.* (2019), with the recognition of the order *Dactylosporales* (*Eurotiomycetes*), but they did not include any sequences of *D. antarctica*, for which presently no sequences exist in the NCBI database. Our sequences formed a clade

fully supported within *Dactylospora* and closely related to *Sclerococcum*, which has been hypothesized to be the asexual morph of *Dactylospora* (Ekanayaka *et al.* 2019). The isolated strains of *D. endolichenica* present hyaline conidia, that substantially differ from those described previously for other *Dactylospora* species studied from environmental samples, i.e. uniseptate dark brown conidia (Olech *et al.* 1996, Pang *et al.* 2014, Alstrup *et al.* 2018, Ekanayaka *et al.* 2019). However, in Döbbeler & Buck (2017) atypical aseptate conidia were reported in *Dactylospora inopina*, and it might be that in the isolated strains we observed had immature conidia.

During the analysis of the *Chaetothyriales*, we also found *Paracladophialophora lichenicola* and *Cladophialophora endolichena*, which were recently isolated and described from several lichen species growing in extreme environments (Cometto *et al.* 2023). These fungi turned out to be strictly lichen-associated and were described only as isolates from lichen thalli coming from different sites in Europe and South America. Their presence among the isolates from Antarctica, and furthermore their isolation from lichen species endemic to Antarctica, i.e. *A. flavocordia* and *B. frigida*, further strengthens their association to lichen thalli. Our results also highlight that they have differentiated genetically from the already reported strains. Indeed, based on our phylogenetic analyses, the strains of both *P. lichenicola* and *C. endolichena* from Antarctica have unique nucleotide polymorphisms in the nucITS sequences that segregate them in their own clades.

The genus *Knufia* (*Chaetothyriales*), includes endolithic and saprotrophic species (Tsuneda *et al.* 2011, Mehrabi *et al.* 2018, Erdmann *et al.* 2022), and its presence within the lichen-dominated endolithic communities in Antarctica was already known and confirmed by culture isolations (Selbmann *et al.* 2013, Coleine *et al.* 2020). However, here *Knufia* was found to be one of the most abundant genera among the isolated endolichenic fungi. *Knufia* was abundantly found also using an amplicon sequencing approach by Stoppiello *et al.* (2025), and it is already reported multiple time as lichen isolates (Réblová *et al.* 2013, Selbmann *et al.* 2013, Cometto *et al.* 2023). Altogether these results confirm its ability to inhabit lichen thalli. Most of the newly isolated strains were recognized to be the endolithic species *Knufia separata*, recently described by Sun *et al.* (2020) as a rock inhabiting fungus from China. Interestingly, this species has been isolated from lichen thalli from Antarctica and from thalli of *R. melanophthalma* from South and North America collected at high elevations, above 3000 m (Cometto *et al.* 2022b). The ecology of *Knufia separata* confirms its nature as an extremophilic fungus (Sun *et al.* 2020), but the newly described species *Knufia elegansiana,* isolated from thalli of *R. elegans,* is a major result and hints to a further differentiation of this genus into new endolichenic taxa, likely exploring new niches for their evolution.

We recognized a new clade also within *Leotiomycetes*, representing the new species *Pseudeurotium lichenicum sp. nov*. (*Thelebolaceae*). The presence of this genus and its family *Thelebolaceae* in the Antarctic continent was reported for the mycobiota of permafrost and maritime Antarctic region especially in glaciers and aqueous environments, such as lake sediments, glacier layers or the peninsula region (Santiago *et al.* 2015, da Silva *et al.* 2020, Ogaki *et al.* 2020, Santos *et al.* 2020, Quijada *et al.* 2022). Our phylogenetic analyses reveal that the new clade of *Pseudeurotium* is closely related to *Antarctomyces* (de Menezes *et al.* 2017) and *Thelebolus*, thus evidence for the diversification of this lineage in Antarctica. The phylogenetic placement of this new lineage inside *Thelebolaceae* might be better solved in upcoming studies, as the phylogeny of the family is so far unresolved and needs further analyses (Quijada *et al.* 2022). The presence of this taxon inside the lichen thalli could suggest its parasitic lifestyle towards the lichen photobionts, as species of *Pseudeurotium* outside the Antarctic regions have been reported as parasites of algae and plants (Manzotti *et al.* 2020, Quijada *et al.* 2022). Whether *Pseudeurotium* develops a parasitic lifestyle toward the lichen photobiont(s) could be tested with co-culture experiments using the available strains in the future.

Our phylogenetic inference has highlighted a well-supported and separated clade in the genus *Petrophila*, order *Capnodiales*, here described as the new species *Petrophila complexa sp. nov.* The genus *Petrophila*, with the sole new species *P. incerta*, was introduced for the first time in 2014 (Egidi *et al.* 2014). The strains were isolated from rocks collected in the Mediterranean area, Mallorca, Spain. In this study, the genus *Petrophila* was found for the first time in Antarctica; the new species here described shows, in addition to a different geographic distribution, also a different ecology than *P. incerta*, being associated exclusively to lichen thalli of the endemic lichen species *L. cancriformis*.

Basidiomycota *diversity*

Basidiomycetous yeasts are an important component of the lichen mycobiota (Spribille *et al.* 2016, 2018, Cometto *et al.* 2022b). So far, some taxa could be isolated in cultures and described from boreal lichens and lichens from harsh, high altitude mountain environments (Černajová & Škaloud 2019, Cometto *et al.* 2022b). The culturable fraction of the basidiomycete mycobiota of Antarctic lichens could be studied here for the first time and interestingly we found again a few taxa previously isolated from lichens of different origins (Cometto *et al.* 2022b). Culturable basidiomycetous yeasts from Antarctic lichen thalli are majorly represented by *Cystobasidiomycetes*, *Tremellomycetes*, *Agaricostilbomycetes*, and *Filobasidiales*. Most of the newly isolated strains were identified as *Cystobasidium laryngis* (*Cystobasidiales*) and were closely related to other two species, i.e. *Cystobasidium ritchiei* and *C. pinicola*, that are known to be psychrophilic (Turchetti *et al.* 2018). The new strains of *C. laryngis* were isolated from three Antarctic endemic (*A. flavocordia, B. frigida, L. cancriformis)* and two cosmopolitan species (*R. elegans* and *R. melanophthalma*), suggesting that the occurrence of this yeast species is more dependent on the cold environmental conditions than on the ecology and distribution of the lichen hosts, as it was not isolated before from thalli of *R. melanophthalma* collected outside Antarctica (Cometto *et al.* 2022b).

Within *Agaricostilbomycetes* the new species of *Kurtzmanomyces lichenum sp. nov*. was represented by nine strains that formed a separate lineage with four other strains isolated previously by Cometto *et al.* (2022b) from the lichens *Tephromela atra* and *R. melanophthalma* from the Italian Alps and North America, respectively. This result strengthens the assumption that there are certain strains particularly

associated to the lichen thalli and distributed globally, and that deserve species description and taxonomical recognition as cryptic lichenicolous fungi, in the same way as already done for some cryptically occurring ascomycetes (Muggia *et al.* 2020, Cometto *et al.* 2023).

The genus *Naganisha* is also confirmed to occur in Antarctic lichens with the species *N. friedmannii* and *N. albidosimilis*. Both species are psychrophilic as their relatives were originally described from Antarctica and were already found in Antarctic soil and in lichen thalli of *Usnea antarctica*, *U. aurantiacoater* and *Ramalina terebrata* (Bab'eva & Golubev 1969, Vishniac 1985, Montes *et al.* 1999, Pavlova *et al.* 2002, Thomas-Hall *et al.* 2002, 2010, Duarte *et al.* 2013, 2016).

Species of the genus *Tremella* are known to be important lichen parasites distinguishable on the lichen thalli by their galls, in which the reproductive structures (basidia with basidiospores) are formed (Millanes *et al.* 2011). However, Cometto *et al.* (2022b) already reported the cryptic presence of *Tremella* in lichens and here we also confirm it, as *Tremella macrobasidiata* was isolated from thalli of *R. elegans* devoid of any symptom of infection. Interestingly, so far *T. macrobasidiata* was found strictly associated with *Lecanora* lichen species (Zamora *et al.* 2011, 2016, Tuovinen *et al.* 2021). Its cryptic presence in thalli of species other than *Lecanora*, could be explained by its dimorphic morphs, as it would be present as yeast cells among the mycobiont hyphae, instead of forming a filamentous mycelium and developing the typical galls. It may be hypothesized that the yeast growth is preferred by this fungus under harsh climatic/environmental conditions and thus it could occur cryptically and unspecific in different lichens.

Photobiont diversity

Lichens in Antarctica undergo several factors that limit the photosynthesis of their photobiont, such as low temperatures, lack of water, and high levels of UV radiation. Choosing a partner that can somehow mitigate these dangers is crucial for the survival of the lichen itself (Determeyer-Wiedmann *et al.* 2019). Studies on the diversity of photobionts in Antarctic lichens have previously shown that the genus *Trebouxia* is the most frequent lichen photobiont in these environments (Ruprecht *et al.* 2012, 2020, Wagner *et al.* 2020, Pérez-Ortega *et al.* 2012, 2023). A clear predominance of *Trebouxia* was found also in the analysed samples. This result is supported by the identification of the photobionts by both nucITS and *rbcL* markers. Most of the nucITS sequences obtained (48 %) belong to the *Trebouxia* A02 lineage (sensu Muggia *et al.* 2020), which has already been found as the main photobiont in Antarctic lichens (Pérez-Ortega *et al.* 2012, Ruprecht *et al.* 2012, 2020, Wagner *et al.* 2020, De Carolis *et al.* 2022). *Trebouxia* A02 associates as photobiont to many mycobiont genera and species worldwide, such as *Austrolecia*, *Buellia*, *Carbonea*, *Huea*, *Lecanora*, *Lecidea*, *Lecidella*, *Rhizoplaca* (Wagner *et al.* 2020); *Acarospora*, *Caloplaca*, *Polysporina*, *Sarcogyne*, and *Umbilicaria* (Pérez-Ortega *et al.* 2012); *R. melanophthalma* and *Tephromela atra* (De Carolis *et al.* 2022), and in this study we report it as the main photobiont of *R. elegans* and *L. physciella*. However, due to the lack of axenic cultured strains needed for the characterization of morphological and ultrastructural traits, this lineage still misses a formal species description.

The lineage *Trebouxia* S02 and *T. impressa* were the second most frequently recovered photobionts in the studied Antarctic thalli. *Trebouxia* S02 is already known for its occurrence in alpine environments, likely for its capacity to cope well with drought conditions, and thus able to thrive in the Antarctic (Garrido-Benavent *et al.* 2020, Ruprecht *et al.* 2012, 2020). This lineage has been previously reported for *Tephromela atra* and *R. melanophthalma* as well (Muggia *et al.* 2010, 2014, De Carolis *et al.* 2022), while here we report it for the first time for *Pleopsidium chlorophanum*. It is worth noting that the diversity of *Trebouxia* species found in the here selected lichens from Antarctica is very similar to that reported by De Carolis *et al.* (2022) for lichens from very high elevation. Indeed, the three lineages *Trebouxia* A02, *Trebouxia* S02 and *T. impressa*, seem to be the best adapted and the most present in harsh conditions, suggesting them as the best competitive lineages of *Trebouxia* in such extreme environments.

The new *Trebouxia* lineage OTU A61 was isolated from the thalli of *Lecidea cancriformis* and likely represents a new species. However, the strains grow relatively slowly in culture and only a future morphological and ultrastructural characterization could support and confirm its monophyly. Certainly, a description of a further *Trebouxia* species would highlight the extraordinary diversity and the evolutionary success of this genus as lichen photobiont.

Only six sequences revealed the presence of *Myrmecia* (L4651, L4659) and *Chloroidium* (L4653, L4654, L4650, L4649) among the algal isolates, while all the photobionts amplified from the thallus DNA extractions were confirmed to be *Trebouxia*. Indeed, the *Myrmecia* (L4651) strain was isolated from a thallus (*R. melanophthalma* L4452) of which the thallus sequence corresponded to *Trebouxia impressa*. However, the genus *Chloroidium* is known from Antarctic soils (Darienko *et al.* 2018, Veselá *et al.* 2024), and a new species was very recently even described as photobiont of the Antarctic lichen *Psoroma antarcticum* (Chae *et al.* 2025). Therefore, it is reasonable to assume that *Chloroidium* is present here as an epithalline or endolichenic secondary algae not specifically associated to the epilithic lichens here investigated.

CONCLUSIONS

The diversity of the mycobiota of Antarctic lichen species and the associated algae/photobionts is still far from being complete. The present study revealed an additional fraction of that hidden diversity of fungal and algal species residing in lichens, this time with a special focus on some crustose and sub-foliose/sub-fruticose species of Antarctic lichens. We presented further evidence that the lichen mycobiota is majorly composed by ascomycetous taxa and that basidiomycetes are represented by yeast species. Both asco- and basidiomycetes species seem to select lichens as their preferred niche in Antarctica and worldwide. Indeed, the major representatives of the lichen mycobiota are fungi that do not show a specificity toward a particular lichen host species, rather toward the lichen thallus in general as a structure in which spores, yeast cells and mycelia fragments thrive or rest. All the investigated species have *Trebouxia*

as principal photobiont, and this highlights the extraordinary diversity, plasticity, and the evolutionary success of this genus as symbiotic partner also under extreme environmental conditions.

In Antarctica, where living conditions on rock surfaces are far more selective than elsewhere, lichen thalli would be even more exploited as suitable niches by other fungi, microalgae concurring to their evolution. The role of lichens as arenas for microbial diversity and niche for diversification will become fundamental for the study of conservative strategies in Antarctica and other extreme environments.

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SUPPLEMENTARY MATERIAL

Fig. S1. Phylogenetic inference of *Dothideomycetes* based on the nuclear nucITS dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

Fig. S2. Phylogenetic inference of *Chaetothyriales* based on the nuclear nucITS dataset. Branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

Fig. S3. Phylogenetic inference of *Leotiomycetes* based on the concatenated nuclear nucITS dataset. branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

Fig. S4. Phylogenetic inference of *Agaricostilbomycetes* based on the nuclear nucITS dataset. branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend). *Agaricostilbomycetes* clades are named according to Millanes *et al.* (2021).

Fig. S5. Phylogenetic inference of *Cystobasidiomycetes* based on the nuclear nucITS dataset. branches in bold denote RAxML bootstrap support > 75 % and Bayesian posterior probabilities ≥ 0.9. Newly obtained sequences are in **bold** and enclosed in a grey box. *Cystobasidiomycetes* clades are named according to Černajová & Škaloud (2019) and Millanes *et al.* (2016). Symbols next to the strain number correspond to the lichen species from which they were isolated (see legend).

Table S1. Sampling information of localities and the collected lichen species.

Table S2A. Sequence dataset used in the phylogenetic analyses of *Dothideomycetes* presented in Fig. 1 and Supplementary Fig. S1; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S2B. Sequence dataset used in the phylogenetic analyses of *Dothideomycetes* presented in Fig. 1; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S3. Sequence dataset used in the phylogenetic analyses of *Chaetothyriomycetes* presented in Fig. 2 and Supplementary Fig. S2; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S4A. Sequence dataset used in the phylogenetic analyses of *Leotiomycetes* presented in Supplementary Fig. S3; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S4B. Sequence dataset used in the phylogenetic analyses of *Leotiomycetes* presented in Fig. 3; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI

accession numbers are reported.

Table S5. Sequence dataset used in the phylogenetic analyses of *Agaricostilbomycetes* presented in Fig. 4 and Supplementary Fig. S4; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S6. Sequence dataset used in the phylogenetic analyses of *Cystobasidiomycetes* presented in Fig. 5 and Supplementary Fig. S5; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S7. Sequence dataset used in the phylogenetic analyses of *Filobasidiales* presented in Fig. 6; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S8. Sequence dataset used in the phylogenetic analyses of *Microbotryomycetes* presented in Fig. 7; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S9. Sequence dataset used in the phylogenetic analyses of *Tremellales* presented in Fig. 8; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.

Table S10. New sequences generated for the algal isolates and from direct PCR from the thallus reported with their NCBI accessions, the name of the strain and the data of the original thallus from which the strains were isolated.

Table S11. Sequence dataset of the nuclear nucITS marker used in the phylogenetic analyses of the photobiont *Trebouxia* presented in Fig. 10; taxon name, voucher ID (if available), genetic markers, and the corresponding NCBI accession numbers are reported.