Studies on the pollen-trapping fungal genus *Retiarius*: Taxonomy, phylogenetics, and metabolism with a special focus on western Canada.

by

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Abstract

Fungi can obtain nutrients from a variety of sources, including some which capture and degrade pollen grains. *Retiarius* Olivier (*Orbiliomycetes*, *Ascomycota*) is an anamorphic hyphomycete that traps airborne pollen grains with aerial hyphae. Only four species, described from South Africa and Hungary, are known within the genus. *Retiarius* has been widely reported in Europe, with several dispersed records world-wide including a single record from North America. In recent years, *Retiarius* has been detected in metagenome sequencing projects of lichens. In addition, microscopic observations of pollen grain deposits on the undersurfaces of lichens revealed hyphae infecting pollen grains. With these observations, I suspected that *Retiarius* was more prevalent in Western Canada than the previous sampling would suggest. In my thesis, I developed genus-specific PCR primers to screen for the presence of *Retiarius* on lichens and adjacent substrate from forests in Canada, the western US, and northern Mexico. I isolated strains in axenic culture of *Retiarius* from the lower surface of rock-dwelling lichens. Two of the strains were used in phenotype profiling experiments to characterize carbon and nitrogen utilization. The environmental screening suggested that *Retiarius* is ubiquitous in lichens and adjacent substrates of all forested ecosystems included in my sampling. In the Canadian Arctic, *Retiarius* was detected at low frequency rates. Phylogenetic analyses using a multilocus tree revealed two undescribed species: *Retiarius canadensis* nom. prov., and *Retiarius crescentus* nom. prov. Both were isolated from the lower surface of lichens, with morphological characteristics different from the known species of *Retiarius*. Using sequences derived from environmental sequencing, I identified an additional 17 putative species-level lineages of *Retiarius*. The phenotype profiling data were inconsistent in characterizing *Retiarius* carbon and nitrogen utilization. As a resource for future studies, the genome of *Retiarius crescentus* nom. prov. was sequenced and annotated, representing the first genome of a non-nematode trapping species in *Orbiliomycetes*. This research will serve as a benchmark for studying the genus *Retiarius*, expanding the current knowledge of its phylogeny and species richness. The evidence indicates a great diversity of species yet to be described, present in a wide variety of ecosystems across North America, and it might play a key role in nutrient cycling by tapping into the nutrient-rich pollen grains.

Preface

This thesis is an original work by Alejandro Huereca Delgado. Toby Spribille advised the approach and methodology. Sally Leys guided the boundaries of the study and training for sample preparations for electron microscopy. Carmen Allen contributed with genome analysis of *Retiarius crescentus* nom. prov., running data through the bioinformatics pipeline.

Dedication

To everyone I left behind in Mexico who supported me to chase the path of the lichenologist, my parents, Consuelo, and Alejandro. My siblings, Oscar, Sofia, Ricardo, and Cecilia. My dogs, Luna and Canon. My friends, Elda, Mendieta, Américo, Rogelio, Karla, Gisela, Gerardo, Héctor, Kevin, Meredith. Thank you, I will never forget the warmth you gave in the darkest moments. This achievement is also yours; I couldn't ever make it with you. Thank you for believing in me.

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Lichens have this mysterious power to allow you to achieve things you never imagined possible.

Alan Fryday.

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Glossary of Terms

Anamorphic fungi: fungal species that disseminate with asexual spores where mitosis has occurred.

Dendrotelmata: ephemeral water-filled tree holes.

Hyphomycete: anamorphic fungi producing spores directly from hyphae.

Ingoldian fungi: also known as aquatic hyphomycetes, are a fungal group that colonizes and decomposes organic matter like leaf litter and pollen in aquatic environments.

Phyllosphere: aerial part of the plant or the parts of a plant above the ground usually a surface of leaves, considered as a habitat for microorganisms.

Stemflow: the flow of intercepted water down the trunk or stem of a plant.

Teleomorphic fungi: fungal species that can sporulate with sexual spores as a result of meiosis.

Throughfall: portion of rainfall that reaches the soil or litter by falling through spaces in the canopy or by leaf drip.

1. Introduction

Fungi occur in and play an important role in every known biosphere on earth. Our understanding of fungi has focussed on conspicuous macrofungi or microfungi with economic or health impacts. Many inconspicuous fungal groups are understudied, such as the genus *Retiarius* (*Orbiliomycetes*), that specializes in capturing and degrading pollen grains. These anamorphic fungi have been widely reported in Europe, with isolated reports worldwide, but only once in North America. Aside from distribution and phylogenetic relationships, other aspects of *Retiarius* biology, such as metabolism in the genus, have not been explored. Considering the ability of *Retiarius* to consume pollen, mapping *Retiarius* occurrence in the environment could help unravel a possible role in incorporating nutrients derived from air-borne pollen into forested ecosystems.

Almost two centuries ago, researchers discovered that chytrid fungi use pollen as a nutrient source (Braun 1855, Goldstein 1960, Skvarla & Andregg 1972, Buaya & Thines 2023). Chytrids are zoosporic fungi with a single posterior flagellum (Powell 2016), that occur in aquatic and soil environments (Bills *et al.* 2004, Sparrow 1960). This group of fungi has been studied from various perspectives, including biodiversity inventories, taxonomic revisions (Hurdeal *et al.* 2023, Letcher *et al.* 2008), ecology, and seasonality (Letcher & Powel 2002, Powel 1993, Van Den Wyngaert *et al.* 2022). The role of chytrid fungi in nutrient transfer between trophic levels is well established (Kagami *et al.* 2007, 2014), specifically in making pollen nutrients available in aquatic systems (Kagami *et al.* 2017, Masclaux *et al.* 2013, Wurzbacher *et al.* 2014).

Other groups of fungi also consume pollen. The most comprehensive study exploring the consumption of pollen within *Dikarya*, the subkingdom of fungi encompassing the phyla *Ascomycota* and *Basidiomycota*, was carried out by Hutchison & Barron (1997). When testing 157 species of litter- and wood-decaying fungi (126 *Basidiomycota*, 28 *Ascomycota,* and two *Zygomycota*) in axenic culture experiments, 41 colonized pollen grains. Of these, thirty-nine were basidiomycetes, and two were ascomycetes. The two ascomycetes were *Amblyosporium botrytis* (incertae sedis, Wijayawardene *et al.* 2017) and *Chaetomium cochliodes* (*Sordariomycetes*). This study sparked more studies exploring the consumption of pollen by fungi, for example Czeczuga & Orlowska (2001) investigated the fungal community growing on floating pollen in freshwater habitats in Poland, reporting 82 species of Ingoldian and aero-aquatic fungi representing the class *Dothideomycetes*, *Leotiomycetes*, *Orbiliomycetes* and *Sordariomycetes* in 12 orders. Other studies have investigated the fungal infections affecting plants with economic or medical importance including *Botrytis cinerea* (Huang *et al.* 1999, Yanakawa 1984), *Colletotrichum acutatum* (Marques *et al.* 2013), *Coniothyrium minitans* and *Gliocladium catenulatum* (Huang *et al.* 2003), and *Cladosporium* spp., *Alternaria* sp., and *Aspergillus*/*Penicillium* (Magyar *et al.* 2022). Additionally, there have also been studies investigating ultrastructure of the fungal infection in pollen, such as in *Cercospora malvicola* (Classen *et al.* 2001), *Alternaria* sp. (Nair & Khan 1963), and in Olivier & Williams (1978).

Even though many fungal groups can break down pollen and obtain nutrients from it, few genera are considered to be “pollen-trapping” specialists. These include *Mycoceros*, *Retiarius* (*Orbiliomycetes*) and *Hexacladium* (*Ascomycota*, Incertae sedis), occurring on bark and in the phyllosphere of plants (Olivier 1978, 1983, Magyar *et al.* 2017b), and the recently described *Microstrobilinia* (*Leotiomycetes*) growing on *Picea* spp. cones, stromatizing tissue, and infecting pollen grains (Beenken *et al.* 2023). Given that pollen consumption in fungi is widespread, Barron (1992) hypothesized that low availability of nitrogen in oligotrophic environments has selected fungi to find alternative nitrogen sources.

Anamorphic fungi, previously called *Deuteromycetes*, or “Fungi Imperfecti”, are fungi reproducing vegetatively via mitospores, also called asexual conidiospores, or conidia (Boekhout *et al.* 2021, Kirk *et al.* 2008, Seifert *et al.* 2011, Sutton 1980). Based on their sporulating structure, they are classified into four major morphological categories formerly named as classes: agonomycetes (producing chlamydospores or sclerotia), blastomycetes (yeasts); coelomycetes (acervular or pycnidial fungi); and hyphomycetes (producing spores directly from hyphae). By the year 2021, an estimated 30,000 anamorphic species were known, but only by their asexual state (Wijayawardene et al 2022). At the time, less than 3% of anamorphic species (678) were linked to their sexual state (Shenoy *et al.* 2007, Wijayawardene *et al.* 2021).

Given the vast diversity and adaptability of fungi, it is not surprising that anamorphic species are ubiquitous in all ecosystems and found on every continent, inhabiting every microhabitat: littoral, deep sea and freshwater (Barone *et al.* 2022, Calabon *et al.* 2020, Jones *et al.* 2019), soil litter, ice/snow-covered soil, desert varnish, or rocks above 6500m (Allegrucci *et al.* 2015, Commeto *et al.* 2020, Parchet *et al.* 2012, Zhang *et al.* 2023), leaves, lichens, insects and mammals (Diederich *et al.* 2018, Marasinghe *et al.* 2023, Seyedmojtaba *et al.* 2018, Veg *et al.* 2013). Anamorphic fungi represent a source of untapped biodiversity, with thousands of species to be discovered and described (Hawksworth 1991, Hawksworth & Lücking 2016, Hawksworth & Rossman 1997, Wijayawardene *et al.* 2021, *et al.* 2022).

The class *Orbiliomycetes* Nannf. is an early divergent clade in *Pezizomycotina, Ascomycota* (Ericksson *et al.* 2003, Díaz-Escandón *et al.* 2023), containing an estimated 470 species, classified in one order (*Orbiliales*), one family (*Orbiliaceae*), and 10 genera (Fig. 1.; Baral *et al.* 2018, *et al.* 2020). Orbiliaceous species differ from other ascomycete classes by the combination of the absence of amyloidity in any tissue of the fungus; sterile hyphae often have globose organelles, soluble in KOH called cytoplasmic bodies (SCBs), and the presence of a membrane-bound vacuole derived from mitochondria inside meiotic spores (ascospores) called the spore body in species with sexual reproduction (Benny *et al.* 1978, Kumar *et al.* 2012). The function of the spore body is still unknown, but some evidence suggests it is filled with carbohydrates, putatively glucans, that disappear during ascospore germination (D. Pfister, unpublished data). According to Baral *et al.* (2020), all teleomorphic species are likely have an anamorphic state and vice versa. However, not all anamorphic species have sequence data, and species in culture rarely develop apothecia.

Anamorphic species in *Orbiliomycetes* are well documented. In particular, the genus *Arthrobotrys* (now a section of *Orbilia*), which create complex trapping organs and exhibit carnivorous behaviour by trapping micro-invertebrates like tardigrades, rotifers, amoeboid protists, and nematodes (Baral *et al.* 2020, Yang *et al.* 2012, Zopf 1888). In addition, many other anamorphic genera been described: *Anguillospora, Arthrobotrys, Brachyphoris, Curucispora, Dactylella, Dactylellina, Descalsia, Dicranidion, Drechslerella, Dwayaangam, Gamsylella, Helicoon, Lecophagus, Microdochiella, Pseudotripoconidium, Tridentaria, Trinacrium*, and *Vermispora*. However, apart from *Lecophagus*, most were synonymized under the genus *Orbilia* and *Hyalorbilia* (Baral *et al.* 2018, 2020). Today, after extensive taxonomic and phylogenetic revisions, only two anamorphic genera are recognized in *Orbiliomycetes*: *Mycoceros,* and *Retiarius* (Olivier 1978, Magyar *et al.* 2017b, Baral *et al.* 2018, 2020).

Retiarius Olivier (1978) was circumscribed to accommodate two species of anamorphic fungi from South Africa that capture air-borne pollen grains in the phyllosphere, *R. bovicornutus* and *R. superficiaris* (Olivier 1978). Magyar *et al.* (2017a) described two more species, *R. goenczoelii* and *R. revayae*, both occurring in bark fissures in Hungary. All species are characterized by the erect hyphae that they develop to capture pollen grains and produce conidia with Y or T shapes, referred to by their technical names dwayaangam and trinacrium-like, respectively (Fig. 2). Since D. Olivier described *Retiarius* (1978), the genus has been interpreted to be a “pollen-parasite”, due to its capture and degradation of pollen grains of *Pinaceae* (specially *Picea* and *Pinus*), *Salix* and *Tilia* (Baral *et al.* 2020, Magyar *et al.* 2017a, Olivier 1978). *Retiarius* species occur in microhabitats of tree bark and living leaves where air-borne pollen is deposited (Table 1), likely dispersed by splash-off during rain events (Olivier 1978). *Retiarius* has been reported from many countries across Europe, and there are isolated records from Asia, South Africa, and South America (Table 1). In North America, there is a single generic record in Canada, but no species name was assigned (Sokolski *et al.* 2006). Until now, published records of *Retiarius* have relied on detection of conidia, but no study has assessed occurrence using DNA data in a targeted environmental screening.

In a study to investigate how lichen-fungi interact with different algal partners in western Canada (Simon *et al.* in prep), an *Orbiliomycetes* fungus was detected in metagenomic data, and assigned tentatively to the genus *Retiarius*. In parallel, the presence of pollen deposits infected by fungal hyphae on the lower surface of lichens from forested areas of Alberta and British Columbia has been known for years (T. Spribille pers. obs.). However, the two could not be assumed with certainty to be linked, since the microscopic observations were not done in the same lichen sample sections used to generate metagenomes.

The widespread distribution of *Retiarius* and its detection in multiple metagenomic datasets raises the possibility that *Retiarius* is more widespread than previously thought. However, the species-level identity of the *Retiarius* detected in metagenomes remains unknown without characterized conidia or axenic cultures, and in absence of specific primers no survey of their presence in North American environments has been carried out. In my thesis, I investigated different aspects of the ecology, morphology and metabolism of the pollen-trapping fungal genus *Retiarius* in western Canada. At the outset of my project, my objectives were to: 1) test the *presence* of *Retiarius* on lichens and adjacent substrates in coniferous forests; 2) test the presence of *Retiarius* in treeless environments such as the Canadian Arctic, using lichens and adjacent substrates as proxies; 3) characterize the morphology and phylogenetic position of *Retiarius* speciesfound in Canada; and lastly, 4) characterize the utilization of carbon and nitrogen of two strains isolated in axenic culture using phenotype assays.

2. Materials and Methods

2.1 Field collection.

To assess the presence of *Retiarius* spp. in lichen samples and their adjacent substrate from coniferous forests, I carried out an opportunistic and exploratory sampling, collecting lichens occurring in boreal and montane forests in western Canada and northwestern United States. Macrolichens growing on rock, lignin, and diverse types of bark were collected in Alberta and British Columbia (Canada), and Montana (USA) (Fig. 3). Lichen specimens will be deposited in the Cryptogamic Herbarium of the University of Alberta (ALTA). The trees where lichens were collected belong to the genera *Abies*, *Alnus*, *Picea,* and *Pinus*. The targeted lichen fungi belonged to the orders: *Agaricales, Caliciales, Lecanorales, Lecidiales, Peltigerales, Teloschistales, Umbilicariales, Verrucariales*; and families: *Cladoniaceae, Collemataceae, Hygrophoraceae, Lopadiaceae, Parmeliaceae, Peltigeraceae, Physciaceae, Ramalinaceae, Sphaerophoraceae, Stereocaulaceae, Teloschistaceae, Umbilicariaceae,* and *Verrucariaceae* (Table 2).

To explore if *Retiarius* was present in ecoregions where its vegetation is not dominated by conifers, thus less abundant pollen rain, I screened lichen specimens and the adjacent substrate from the Canadian Arctic, loaned from lichen collections deposited in herbarium collections, facilitated by Troy McMullin (Canadian Nature Museum, CMN-CANL) and Erin Cox (University of Alberta-ALTA), in addition to lichens from Oregon, USA collected by Joseph Di Meglio, and lichens from temperate forests in northern Mexico collected by me in 2020, and Alejandra Peña Estrada in 2022 (Table 2, Fig. 3). Maps were produced using ArcMap 10.8.2 (ArcGIS) with the Albers equal-area conic and Robinson projections, using World Geodetic System 1984 (WGS84) coordinate reference system.

2.2 Isolation in axenic Culture.

To isolate *Retiarius* in pure cultures, I first inspected pollen grains deposited in the lower surface of *Umbilicaria phaea* and *U. torrefacta* using a dissecting microscope, Olympus SZX16, detecting hyaline hyphae growing on pollen grains, then I prepared microscope slides by scraping the pollen with a blade and visualize with a compound microscope, Zeiss AXIO A.1, detecting conidia potentially belonging potentially to *Retiarius*. After visual confirmation, I selected specimens with abundant amounts of pollen and wet the lichen by spraying sterile Milli-Q water.

Once lichen samples were wet, I scraped off pollen from the lower surface using a sterilized blade, collecting as much as possible in a 1.5ml Eppendorf with 1 ml of Milli-Q water, followed by a 10% dilution series. Then, I inoculated 200 ml of pollen suspension into Petri plates with culture media and antibiotic (see below), and incubate them for 5-10 days at 27°C. Once fungal growth was detected, I subcultured fungal colonies into pure axenic cultures, observing them every 4-5 days to detect early signs of contaminant fungal species. When culture of a single colony morphology was archived, and once the identity of the culture was confirmed to be *Retiarius* using Direct PCR (see 2.3 DNA extraction and sequencing section below), a collection number was assigned to each strain, using the code TSMC, standing for Toby Spribille Mycological Collection. The isolated strains of *Retiarius* will be deposited at the Westerdijk Fungal Biodiversity Institute, Netherlands (CBS), the Canadian Collection of Fungal Cultures, Canada (DAOMC) and the Centre for Global Microfungal Biodiversity, Canada (UAMH).

The recipes for the culture media used for strain isolation were potato-dextrose agar (PDA; Remel R4554312, ThermoFisher), 39 g/l with Milli-Q water and 2 ml/l of Chloramphenicol (C1919-5g, Sigma Aldrich) as antibiotic agent. Once the desired fungi were isolated in pure cultures, I subcultured them in oatmeal agar (OA), using commercial unflavored, unsweetened oatmeal (Quaker), 30 g/L and 20 g of agarose (B160-500, Fisher), which was cooked for 10 min and then filtered using a French Press, and finally autoclaved.

2.3 DNA extraction and sequencing.

To assess the presence of *Retiarius* using Sanger sequencing, I extracted and amplified DNA from 177 environmental samples, composed of lichen thalli and their adjacent substrate. To obtain samples from lichen thalli, I selected lichen specimens with and without conspicuous pollen deposits in the lower surface, using a dissecting microscope (Olympus SZX16). In the case of the substrate adjacent to the lichen, I selected pieces of bark and weathered lignum, twig fragments, and soil debris (Table 2). The selected samples, both lichen thalli and adjacent substrate, were freeze-dried at -80°C in an Ultra-Low Temperature freezer, and pulverized using a TissueLyser II (Qiagen, Germany). Then, I extracted DNA from the environmental samples using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Due to *Retiarius* morphological simplicity and the lack of conspicuous colony characteristics, its detection in situ is impossible, and is only achievable in a laboratory setting, using a compound microscope.

To test the presence of *Retiarius* without relying on the observation of the conidia, I developed *Retiarius-specific* primers for ITS (internal, transcribed spacer) using contig data derived from metagenomes produced by Antoine Simon in collaboration with the Symbiosis and Evolution Lab, at University of Alberta and available sequences of *Retiarius* deposited in GenBank-NCBI. I chose the ITS locus, because it is the most common region sequenced in fungi (Schoch *et al.* 2012), and because most available sequences are from this region. To ensure the effectiveness and specificity of the primers, i.e. to avoid amplification of other fungal DNA, I aligned the primers in a dataset of publicly available sequences of genera in *Orbiliaceae* and genera of lichen-fungi using AliView 1.28 (Larsson 2014) and Geneious 2023.1 (Kearse *et al.* 2012). To identify nucleotide regions specific to *Retiarius*, as well as discriminating non-target fungal sequences, I used the ThermoFisher Primer Designer Tool website (ThermoFisher Scientific, Waltham, Massachusetts, USA) to ensure primers were designed adequately: 1) primer G-C content ranges 40-60%; 2) primers do not bind together, avoiding primer dimers 3) avoid high melting temperature. To evaluate whether the primers amplified the target DNA, I processed the PCR product and sent it for sequencing (see next section) and compared the resulting sequences by the alignment of *Orbiliaceae* and lichen genera. After trying several combinations, I successfully produced two sets of primers (Table 3), which were used to screen lichen tissue and the adjacent substrate.

To extract DNA from axenic cultures, hyphal tissue was freeze-dried using liquid nitrogen and pulverized using a TissueLyser II (Qiagen, Germany). Pulverizing fungal tissue pre-frozen in Ultra-Low Temperature freezer at -80°C was not ideal because fungal tissue did not pulverize completely. Special care was taken in harvesting tissue, selecting areas of the culture where active growth is happening, meaning the tissue was alive, avoiding the central portion of the colony, in order to guarantee good DNA yield.

To identify the phylogenetic affinity of the fungal colonies isolated from pollen scraping without investing DNA extraction columns, I used a Direct-PCR technique following the “Yeast Colony PCR” protocol (Horecka & Chu 2017), which consists of taking a small piece of the colony, ideally the size of a sesame seed (not using weight as unit) into a 0.2 ml PCR tube with 50 µl sterilized water. Once transferred, I lysed the tissue by placing the PCR tubes in a Thermocycler with a custom program consisting with a single cycle at 99°C for 5 min to “burst” the cells used as DNA template in PCR reactions. Samples were stored at -20°C until sequences were obtained, and cultures were selected by way of the BLAST results related to *Orbiliomycetes* (see below for BLAST interpretation). Cultures with taxonomic affinity to *Orbiliomycetes* were subjected to DNA extraction (see above).

For PCR reactions, I used 2 µl of DNA template in a 22 µl reaction using the KAPA3G Plant Kit (Millipore, Sigma Aldrich, Munich, Germany) following the manufacturer’s protocol and specifications. Reactions were carried out using a 96 well plate Thermo-cycler Veriti 96-Well Fast, Applied Biosystems, Foster City, California, USA. For each primer region, I used the following programs: ITS initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension 72°C for 30 sec, with a final extension at 72°C for 7 min using the primers from Gardes & Bruns (1993); for custom *Retiarius* ITS, I used the same protocol; for nLSU, I used an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension 72°C for 1.5 min, with a final extension at 72°C for 7 min. For the amplification of environmental samples, I used the custom *Retiarius* ITS, and for DNA derived from the axenic using nSSU, ITS, nLSU, I used the following primer sets: for nSSU, NS1-N23; for ITS, ITS1F, and ITS4; for nLSU, LR0R LR7 (Table 3). The PCR products were checked by gel electrophoresis gel (1% agarose) stained with GelRed® Nucleic Acid Stain 10000X Water (Milipore, Sigma Aldrich, Munich, Germany). All successful PCR products that yielded positive bands were purified using Exonuclease I and Shrimp Alkaline Phosphatase enzymes (New England BioLabs, Inc., Ipswich, Massachusetts, USA). Sanger sequencing was performed on all PCR products and was processed in two sequencing facilities: Molecular Biology Service Unit (MBSU) at the Centennial Center of Interdisciplinary Sciences at the University of Alberta (Edmonton, Alberta, Canada), and Psomagen (Jamaica, New York, USA). Sequence quality was assessed by looking at electropherogram, trimming primer ends, and checking for ambiguities. Successful and usable sequences were then subject to BLAST query (Johnson *et al.* 2008) to determine the taxonomic proximity to *Orbiliomycetes* genera.

2.4 Taxon sampling.

For each phylogeny, ITS, LSU, and multilocus (SSU+ITS+LSU), newly generated sequences were incorporated into a curated dataset of the "*Lecophagus-Amphosoma*" clade in *Orbiliomycetes* selected from phylogenies produced in Baral *et al.* 2020. Each dataset contains representatives of the genera *Amphosoma*, *Bryorbilia*, *Lecophagus*, *Lilapila*, the three known sequenced species of *Retiarius* and *Orbilia* as the outgroup in all phylogenies. For the multilocus phylogeny, three *Orbilia* species were selected (Table 4): *O. crematomarginata* KM248772, *O. cucumispora* KT215231, and *O. scolecospora* KM248770. In the case of the ITS phylogeny, *Amphosoma atroolivaceum* KT380069*, A. resinicola* KT222389, and *Amphosoma sp.* MG386037*,* (Table 6), and *O. flavida* KT215228 for the LSU phylogeny (Table 5). For the ITS phylogeny, in addition to the sequences from the environmental screening, I used the newly produced sequences from cultured *Retiarius* spp. as BLAST query in NCBI to look for sequences with a high percentage of similarity to *Retiarius* deposited as "unculturable fungus", or "unknown ascomycota" in NCBI (Table 6). In LSU and multilocus phylogenies, only sequences associated with an identified taxon were included.

2.5 Phylogenetic analyzes.

To determine the phylogenetic relationship and diversity of the *Retiarius* species present in lichens and adjacent substrate, I calculated single gene trees (ITS and LSU), as well as a concatenated tree (ITS+LSU+SSU) using a maximum likelihood (ML) approach. To align, trim and concatenate multilocus datasets, I used custom Python scripts created by P. Resl, modified by T. Spribille & D. Díaz-Escandón, and implemented according to Resl *et al.* (2015). The script aligned the dataset with MAFFT v7 (Katoh & Standley, 2013), then trimmed using trimAl v1.2 (Capella-Gútierre *et al.* 2009) using the command "-

gappyout" to trim based on gap distribution. Maximum likelihood analyses were done in IQ-TREE v2.2.0 (Nguyen *et al.* 2015) with the partitioning command "-p" to evaluate evolutionary models for each gene. Trees were created using ultrafast bootstrapping of 1000 replicates to obtain branch support (Hoang *et al.* 2017), in addition to executing the Shimodaira-Hasegawa-like approximate likelihood-ratio test (SH-aLRT) with 1000 replicates (Guindon *et al.* 2010). Only branches with ≥95% ultrafast bootstrap values and ≥80% SH-aLRT were considered to be well supported (Nguyen *et al.* 2015). Phylogeny was inferred based on the best fitting mode by using IQ-Tree-based automated model finder (Kalyaanamoorthy *et al.* 2017) and bootstrap algorithm UFBoot (Minh *et al.* 2013). For single locus trees for ITS and LSU the same procedure was employed but using the command "-s" in IQ-TREE. Phylogenetic trees resulting from the analysis were visualized using FigTree v1.4.4 and edited with Adobe Illustrator 27.5.

2.6 Morphological characterization of fungal colonies in axenic cultures.

To characterize the morphology of the pollen-trapping fungi isolated in culture media, I followed traditional mycological descriptions and measured morphological features with taxonomic value to the genus according to Baral *et al.* (2020), and described the color of the colony, using the color nomenclature of Ridgway (1912). One culture of *Retiarius* species per locality was characterized. Twenty measurements of mycelial hyphae, conidium length, conidium width, main axis length, arm length, arm width, basal stipe height, basal stipe width, arm septation, chlamydospore length, and chlamydospore width were taken for each colony. The size of the colony was measured after 25 days. Measurements were taken from water mounts and are indicated as (minimum–) {X–SD}–{X+SD} (–maximum), where X is the arithmetic mean and SD the corresponding standard deviation, followed by the number of measurements. Colonies were inspected at 1000x on a Zeiss AXIO A.1 microscope in water and stained with lactophenol cotton blue. Measurements were made with Olympus cellSens software and micrographs were taken using an Olympus SC180 Color Camera.

In addition, the ex-type strain of *Retiarius bovicornutus* IMI 223460 was purchased from CABI, UK for morphological comparison with isolated colonies, as well to produce more sequences for phylogenetic analyses.

2.7 Identification of lichen samples.

To identify the lichen used as proxy for the environmental screening, every sample was identified to species, with some exceptions to genus, utilizing morphological characteristics and microchemical techniques, following Goward *et al.* (1994), Goward (1999), Brodo *et al.* (2016), and McCune & Geiser (2009). I used the nomenclature of lichen species names, following the checklist Lichens of North America, North of Mexico by T. L. Esslinger (Esslinger 2021). Morphological inspection of the thalli was made using a stereoscope (Olympus SZX16) and cross-sections by hand of the ascomata using a compound microscope (Zeiss AXIO A.1). The ascospores were measured in water or after being mounted in water with 10% KOH. Additionally, routine chemical tests (K: 10% potassium hydroxide; C: calcium hypochlorite; P: p-phenylenediamine crystals in 95% ethyl alcohol and UV: fluorescent reaction under UV light) were made on the lichen structures as needed depending on the taxonomic group (Orange *et al.* 2010).

2.8 Preparation of samples for scanning electron microscopy.

To visualize the hyphal networks associated with pollen deposits of *Umbilicaria* spp., I imaged the fungal-pollen interaction on the lower surface of the lichen, by Scanning Electron Microscopy using a Field Emission Scanning Electron Microscope, model Zeiss Sigma 300 VP (Zeiss Group, Oberkochen, Germany) at the Department of Earth & Atmospheric Sciences of the University of Alberta. Lichen samples were first inspected under the dissecting scope, and specimens with substantial pollen accumulation were selected. The samples were treated in a cocktail fixative composed of 5 ml of 8% glutaraldehyde, 10 ml of sodium acetate buffer pH 6.4, 2g of sucrose, and 5 ml of 4% osmium tetroxide (OsO_4) for a total volume of 20 ml (Harris & Shaw 1984). Samples were transferred to falcon tubes and fixed for 5 hours. After 5 hours, samples were rinsed three times with distilled water and then dehydrated in a series of washes through different concentrations of ethanol 30, 50, 70% for 10 min between washes. Once at 70% ethanol, dehydration continues with two washes of 95% and two washes at 100%, with 10 min between each wash. Specimens were then transferred to a sample holder to be critical-point dried under high pressure and high temperature In a Bal-Tec CPD 030 (Bal-Tec AG, Germany). Specimens were mounted on SEM aluminium stubs with carbon adhesive tape and finally coated with gold.

2.9 Phenotype Microarray assays.

To characterize utilization of carbon and nitrogen by *Retiarius* spp., I used three different sets of 96-well Phenotypic MicroArray^{TM} (PM) plates (Biolog Inc., Hayward CA, 94545, USA): PM1 and PM2A (carbon utilization) and PM3B (nitrogen utilization). Each well is pre-filled with a specific lyophilized nutrient source. An inoculum solution was prepared with fungal biomass and inoculation fluid Biolog FF-IF, the latter contained enough quantity of mineral nutrients and vitamins for cell viability (Blumenestein *et al.* 2015).

To prepare biomass for inoculum, *Retiarius* cultures were grown on liquid culture of Potato Dextrose Broth (BD Difco, New Jersey, USA) for 20-25 days at room temperature and visually inspected every 3-4 days to detect potential contamination. When enough biomass was produced, colonies were separated from the flask walls using sterilized wooden sticks and then transferred into sterilized filters to separate liquid medium from fungal colonies using vacuum. Fungal tissue was then transferred into a 50 ml Falcon tube and macerated with a sterilized pestle with 5 ml of Biolog FF-IF.

To inoculate the PM arrays, inoculation solution was homogenized to measure optical density (OD) using a spectrophotometer (Du® 730, Beckman Coulter), achieving 62% by adding small amounts of FF-IL to the suspension to obtain the desired OD according to the manufacturer’s protocol. In addition, solutions of glucose and potassium phosphate were mixed into the inoculum. Once the inoculation solution was ready, it was transferred to a sterilized extra deep Petri plate and 100 µl of inoculum was pipetted into each well of the PM on a series of triplicates per array. Plates were left at room temperature for incubation, with normal day-night light cycles, and stored in a plastic container to prevent external contamination.

Once PM was inoculated, data for the growth of fungal colony inferring cell density using measurements of OD at 750 nm using a microplate spectrophotometer reader (PowerWave XS, Biotek™) every 24 hours for 35 days. The first data point was measured one hour after inoculation of plates with mycelium mixed in IF-II. to detect any contamination, plates were checked daily before reading.

2.10 Genome Sequencing, Assemble, and Annotation.

To obtain DNA for genome sequencing, I selected the strain TSMC83 of *Retiarius crescentus* nom. prov., cultured in PDA media, following the same protocol described in section 2.3 DNA Extraction and Sequencing.

For genome sequencing, DNA extraction was sent by Carmen Allen, lab mate at the Evolution and Symbiosis Laboratory, to Genome Quebec sequencing facility (Quebec, Canada). DNA was sequenced using Illumina sequencing technology, in a NovaSeq600 sequencing instrument. DNA libraries were constructed by Genome Quebec technicians, using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina® and sequenced on an Illumina NovaSeq 6000 (S4 flow cell) using 150-bp paired-end reads. Carmen Allen processed the Illumina reads with metaWRAP 1.3.2 (Uritskiy *et al.* 2018) module to clean original reads. The genome was assembled using metaSPAdes 3.15.3 (Nurk *et al.* 2017). Genome annotation was performed using Funannotate pipeline (Palmer 2016). The assembled genome completeness was evaluated using BUSCO v5.3.2 (Manni *et al.* 2021).

3. Results

3.1 Phylogenetics confirm novel clades in *Retiarius*.

A total of 84 new sequences were generated in this study, comprising 25 of LSU and 59 of ITS, of which 33 are sequences derived from environmental screening isolates (Table 6) and 51 from axenic culture (ITS: 27 and LSU: 24, Table 4). PCR amplification of nSSU did not produce any usable data from axenic cultures. The single locus ITS alignment consisted of 66 isolates with 1056 characters, 709 were constant, 666 were variable sites and 252 were parsimony-informative. The best-fit model for ITS was IM3e+I+I+R3, calculated by ModelFinder in IQ-tree based on AIC. The single locus LSU alignment consists of 57 isolates with 839 characters, 639 were constant, 301 were variable sites and 157 were parsimony-informative. The best-fit model for LSU was TN+F+I+I+R2. The concatenated alignment consisted of three loci of 51 taxa with 2219 characters (nSSU: 475; ITS: 596, LSU: 1148) of which 1571 were constant sites, 875 were variable sites and 520 were parsimony-informative. The best-fit model per gene was: TNe+I+G4 for ITS, TN+F+R2 for LSU and K2P+I for nSSU. The difference in the model selection and site characteristics between the single-locus trees of ITS and LSU, and the concatenated alignment is caused because each tree has a different dataset, having more sequences in single-locus trees than the concatenated tree.

The resulting phylogenetic trees from Maximum Likelihood analyses using the concatenated nSSU+ITS+LSU and LSU datasets recover *Retiarius* as the sister genus of *Amphosoma*, belonging to the "*Lecophagus*-*Amphosoma*" clade within *Orbiliaceae*, concurring with earlier proposed phylogenies (Baral *et al.* 2020, Magyar *et al.* 2016a, 2017a, 2017b). Multilocus phylogenetic analysis resolves all genera form a monophyletic clade within the *Lecophagus-Amphosoma* clade, however, the LSU phylogeny recovers *Amphosoma* as polyphyletic, as in the phylogeny of Baral *et al.* (2020). The concatenated topology recovered the same topology as the one presented by Baral *et al.* (2020) (Fig. 4). However, LSU alone ended up producing a different topology than in the study previously mentioned (Fig. 5).

Thirty-one sequences derived from 17 cultures of *Retiarius*, isolated from pollen deposits in lower surface of *Umbilicaria* spp. (ITS 17; LSU 14), formed a well-supported clade in LSU (99.7/100) and multilocus phylogenies (99.2/100), with *R. revayae* KY352465 as sibling clade, here named as *Retiarius crescentus* nom. prov. (see section 4, Taxonomy). ITS phylogeny recovers *R. crescentus* nom. prov. in a single clade with high support (92.1/74), sibling to the "Ret 8" clade of environmental sequences. Given the strong support in all phylogenies and unique morphology within the genus, *R. crescentus* nom. prov. is considered a new species and described in this study (see section 4, Taxonomy).

Eighteen sequences produced from nine cultures of *Retiarius*, isolated from the same microhabitat (ITS 9; LSU 9), form a well-supported clade sibling to *R. bovicornutus* ex-type sequence KY352466 (strain IMI 223460) with 99.6/100 in LSU tree and 100/100 in the multilocus phylogeny, here named as *Retiarius canadensis* nom. prov. (see section 4, Taxonomy). ITS phylogeny recovers *R. canadensis* nom. prov. in a single clade, sibling to a clade formed by *R. bovicornutus* ex-type sequences, both related to *Retiarius* sp. AH-Ret12 produced from environmental screening.

The only available sequence of *R. bovicornutus* comes from the ex-type culture strain produced by Magyar *et al.* (2017a), and consists of the ITS and LSU gene regions. By purchasing the ex-type culture of *R. bovicornutus*, new sequences of the same available loci were produced to compare the nucleotide difference between the two species, due to nucleotide ambiguities in the available sequences. Comparing the ITS region, *R. canadensis* nom. prov. differs by 55-58 nucleotides compared to *R. bovicornutus* ex-type sequence KY352466, and 55-58 nucleotides compared to the produced sequence. In the case of the LSU gene, exists a discrepancy in the nucleotide sequences of *R. bovicornutus* between the sequence reported by Magyar *et al.* (2017a) and the newly generated sequence, differing from each other at nine nucleotide sites. Taking into account this difference, the LSU region of *R. canadensis* nom. prov. differs from *R. bovicornutus* by 11-15 nucleotides with KY352466 sequence and 20-24 nucleotides with the newly produced sequences. Given the phylogenetic support in all tree phylogenies and distinct morphometric data*, R. canadensis* nom. prov. is considered a new species and is described in this study (see section 4, Taxonomy).

3. 2 Environmental screening unveils the ubiquity of *Retiarius*.

A total of 177 samples were screened with specific primers for *Retiarius* (Table 2); 145 are lichens, of which 90 grow on rock or soil (75 saxicolous and 15 terricolous) and 55 grow on bark or lignin, and 32 samples were on adjacent substrate, 27 of which are bark, four soil debris, and one was from the phyllosphere. Of the 177, 89 samples produced no PCR product (classified as no band in Table 2), and 88 produced a band in gel electrophoresis. After sequencing, seven sequences amplified DNA of a different fungus than *Retiarius*, 33 sequences successfully amplified DNA of *Retiarius,* two produced band but no sequence, and 45 sequences were not usable. Though the latter group of sequences did amplify *Retiarius* DNA, they were not suitable for further analysis due to a high number of ambiguous nucleotides of non-specific sites and poor quality.

The 88 screened samples that yielded a band account for different microhabitats: bark 51 (36 corticolous lichens; 14 bark; one phyllosphere) and rock 37 (35 saxicolous lichens; one terricolous lichen; one from soil debris). Eighty-four of these samples come from forested ecosystems, from Humid Oak forest to Coastal Temperate rainforest to Boreal forest in North America, and only four samples from the Canadian Arctic and Subarctic tundra. From the previous samples, 33 produced *Retiarius* sequences: 10 corticolous lichens; 18 saxicolous lichens; and five bark (Table 2). By including these in the ITS phylogeny of the "*Lecophagus*-*Amphosoma*", the analyses recovered 17 clades within *Retiarius*, representing putative species-level lineages (Fig. 6). Based on current sampling, putative species are found both associated with lichen and their substrate, occurring in distinct geographic regions with different forest types (Table 2, Fig. 8).

While the genus-specific primers proved to be effective at amplifying *Retiarius* DNA in environmental samples, seven sequences amplified DNA of non-*Retiarius* fungi, five of them are *Orbiliaceae*. AH-Ret58, from *Parmotrema crinatum,* nests within *Hyalorbilia* (not shown in tree). In the case of AH-Ret68 from *Parmelia sulcata*, the BLAST search outcome suggests a high affinity to *Amphosoma* and *Lilapila* but does not align within the tree (data excluded from the tree). Three sequences nest in the genus *Lecophagus*, AH-Ret96 from *Polycauliona* aff. *polycarpa*, AH-Ret77 from the bark of *Crataegus* sp., and AH-Ret113 from *Lobaria pulmonaria*. AH-Ret96 forms a well-supported clade sister to *Lecophagus vermicola*. In the case of AH-Ret77 and AH-Ret113, both sequences group together and form a clade with high support, sister to *Lecophagus longisporus*. The two remaining sequences, AH-Ret64 from *Umbilicaria* sp. and AH-Ret112 from *Cetrelia cetrarioides* amplified DNA from non-orbiliaceous fungi AH-Ret64 sequence BLASTs to *Eurotiomycetes*, and AH-Ret112 amplified DNA of the lecanoromycete lichen host.

To explore the extent to which *Retiarius* may have been captured by environmental or amplicon sequencing and not recognized as such, I searched for matching sequences in GenBank using the newly produced sequences of *Retiarius canadensis* nom. prov. and *R. crescentu*s nom prov. as query search. The BLAST search resulted in 39 sequences deposited in NCBI as Uncultured fungus and/or Uncultured Ascomycota, only two deposited as *Retiarius* sp. and uncultured *Retiarius* (Table 6, Fig. 7). These sequences come from environmental DNA isolates, including 26 from leaves (phyllosphere), one from bark, six from soil/litter (leaf litter, roots, rhizosphere soil, soil), two from airborne deposition (house dust), one from mosses (bryophyte), and one from a water body (water pond). These sequences were produced in 13 studies, except for 5 sequences uploaded via direct submission, and detected *Retiarius* in different countries: Canada (Amend *et al.* 2010), China (Direct submission), Finland (Pitkäranta *et al.* 2008), France (Cordier *et al.* 2012), Germany (Dreyling *et al.* 2022), Italy (Victorino *et al.* 2021), Lithuania (Menkis *et al.* 2015, *et al.* 2019, Lynikienė *et al.* 2020, Marčiulynas *et al.* 2020, *et al.* 2022), New Zealand (Johnston *et al.* 2017), Norway (Kauserud *et al.* 2008), and USA (Alshuwaili *et al.* 2021) (Table 6, Fig. 9).

3.3 Carbon and nitrogen utilization of *Retiarius*.

I tested the nutrient consumption on a strain of the two novel species isolated in culture, *R. canadensis* nom. prov. (TSMC102) and *R. crescentus* nom. prov. (TSMC84) using Phenotype Microarray assays. Specifically, I evaluated the utilization of carbon with PM1 and PM2A, and with PM3B arrays, consisting of 190 carbon sources, and 95 nitrogen sources, respectability. The carbon sources in PM1 consisted of carbohydrates, amino acids, carboxylic acids, fatty acids and amides, while PM2A had alcohols, amines, esters, and polymers, as well as amino acids, carbohydrates and carboxylic acids. In the case of PM3B, the nitrogen sources consisted of amino acids, inorganic nitrogen sources, peptides, and other nitrogen sources. The experiment was carried out following the manufacturer's instructions, with an extended time due to the slow growth of the fungi. Every plate was done in triplicate to provide uniform data; however, the results were inconsistent throughout the replicates (Fig 9, 10, 11).

The results indicate that both species had different carbon and nitrogen uptakes. The strain TSMC102 had no growth on 145 of 190 carbon sources, slight growth on 21, and conspicuous growth on 25 (Fig. 10, 11). In the case of the nitrogen sources, there was no growth on 24 of 95 nitrogen sources, slight growth on 13 and conspicuous growth on 59 (Fig. 12). The strain TSMC84 grew profusely on only 9 carbon sources, moderately on 12, and did not grow on 165 of 190 (Fig. 10, 11). In the case of nitrogen sources, growth was detected on 39 nitrogen sources (Fig. 12), moderate growth on 16, and no growth was detected on 41 of 95 (Fig. 12). Notably, there was no color change due to pigment production in any of *R. crescentus* nom. prov. PM plates. The same was true of *R. canadensis* nom. prov., except for one amino acid, L-Tyrosine in PM3B, which turned from transparent to translucent dark brown during growth on PM plates. This color change also occurred in early and senescent growth stages on PDA media.

3.4 Draft Genome of *Retiarius crescentus* nom. prov.

The assembly of *R. crescentus* nom. prov. is composed of 624 scaffolds, with the N50 of 150,833 bp. The calculated genome size was 36.32 Mb, GC content of 38.04%, and 10331 genes predicted in BUSCO analysis. The draft genome assembly scored 95.9% completeness (0.7 % duplicated), 1.4% fragmentation, and it is missing 2.7% of the genes, in BUSCO analysis based on Ascomycota_DB10 (Table 9).

4. Taxonomy

4.1 *Retiarius canadensis* Huereca, Belosokhov & T. Sprib., nom. prov.

Fig. 17, 18.

Holotype: Canada, Alberta, Pincher Creek No. 9, Castle Provincial Park, 4.8 km in Ohagen Rd, 0.8km up hill. N 49.429500, W 14.379278, elev. 1540m a.s.l. isolated from the undersurface of *Umbilicaria phaea*, infecting *Pinaceae* pollen grains, June 06, 2021, A. Huereca, culture TSMC103, CBS 11875, DNA Isolate AH-Ret208. Holotype to be deposited in CBS (Westerdijk Fungal Biodiversity Institute, Netherlands); Isotype to be deposited in DAOMC (Canadian Collection of Fungal Cultures, Canada) and UAMH (Centre for Global Microfungal Biodiversity, Canada).

Diagnosis – Similar conidial shape to *Retiarius bovicornutus* but differing by having smaller conidia with shorter stipe (12-25 vs 8.9-13.8 µm) and shorter arms (25-50 vs 18.4-23.6 µm), as well different microhabitat (lower surface of *Umbilicaria* spp. vs phyllosphere).

Etymology: *canadensis*, Refers to its initial detection in Canada.

Description: *Somatic hyphae:* hyaline, 4.2-5.7 µm wide, septate, straight from slightly constricted to almost moniliform; aerial hyphae 6-7 µm wide, erect, moniliform; somatic cells with hyaline lipid bodies. Conidiophores absent; conidiogenous cells intercalary in somatic hyphae, smooth, hyaline**,** 2.2–4.5 µm**.** *Conidia* staurosporous, trinacrium-like, smooth, hyaline, SCBs not observed. (20-) 24.8-31.5 (-34.3) × (25.8-) 31.4-40.7 (-47.7) µm (n=58); stipe straight or slightly curved, tapering towards base, 1-2(3)-septate, (5.2) 8.9-13.8 (-17.1) × (3.5-) 4.4-5.4 (-6.3) µm (n=58). Conidium main axis curving upwards, 4-6 (-8) septate. Conidial arms curved upwards, 2-3 (-4) septate, tapering at the tips, (13.8-) 18.4-23.6 (-28) × (4.1-) 5-6 (-6.5) µm (n=116). Chlamydospores 6.3-9.3 × 6.1-7.7 µm. *Teleomorph* unknown.

Culture characteristics (25 days at 21 °C): Colonies on PDA 11.2-12.2 mm in diameter, color light shades ranging from cinereous to violet-gray, becoming darker from deep plumbeous to dark plumbeous, texture velvety to floccose, convex, wrinkled to sometimes complex; margin scalloped to undulate, sometimes irregular; exudate absent; soluble pigment dark brown; reverse color same shades but blurred by media. Colonies on OA 7.5-11.8 mm in diameter, color shades ranging from tilleul-buff to vinaceous-buff, sometimes with dark tinges of avellaneous; texture fine velvety to floccose; with radial furrowed to ridged wrinkles; not raised to pulvinate, margin from slightly undulate to almost scalloped, exudate absent; sometimes with slight dark soluble pigment; reverse color same shades but blurred by media.

Distribution and Ecology: *Retiarius canadensis* nom. prov. is known from the Thompson Plateau and the Rocky Mountains in southwestern Canada, at 1020-1540m above sea level (Fig. 13). It was isolated from the lower surface of lichens *Umbilicaria* (*U. phaea* and *U. torrefacta*), infecting pollen grains of *Pinaceae* (Fig. 18). The ecosystems where *R. canadensis* occurs are Douglas-fir forests in southern British Columbia, northwestern Montana, and southern Alberta.

Isolated strain localities:

Canada, British Columbia, Okanagan-Similkameen Regional District, East of Bankier town, N 49.694253, W 120.282587, elev. 1020m a.s.l., isolated from the undersurface of *Umbilicaria torrefacta* infecting *Pinaceae* pollen grains*,* August 08, 2021, A. Huereca, culture TSMC88, CBS 11876, DNA Isolate AH-Ret121; A. Huereca & C.C. Allen culture TSMC89, DNA Isolate AH-Ret122.

Canada, Alberta, Pincher Creek No. 9, Castle Provincial Park, 4.8 km in Ohagen Rd, 0.8km uphill. N 49.429500, W 114.379278, elev. 1540m a.s.l. isolated from undersurface of *Umbilicaria phaea*, infecting *Pinaceae* pollen grains, June 06, 2021, A. Huereca, culture TSMC102, DNA Isolate AH-Ret207; A. Huereca, culture TSMC103, DNA Isolate AH-Ret208; A. Huereca, culture TSMC104, DNA Isolate AH-Ret209; A. Huereca, culture TSMC106, DNA Isolate AH-Ret211; A. Huereca, culture TSMC107, DNA Isolate AH-Ret212; A. Huereca, culture TSMC108, DNA Isolate AH-Ret213.

USA, Montana, Stryker, Stillwater Ridge, East of Stryker, N 48.681362, W 114.759470, elev. 1047m a.s.l., isolated from the undersurface of *Umbilicaria torrefacta*, infecting *Pinaceae* pollen grains, April 30, 2023, A. Huereca, culture TSMC109, CBS 119626, DNA isolate AH-Ret216.

4.2 Taxonomic notes on *Retiarius bovicornutus* species complex.

Retiarius canadensis nom. prov. has similar conidial morphology to *R. bovicornutus* and *R. revayae*, but it differs in size and septation. The former has larger conidia in total size, 47-50 x 45-55 vs 24.8-31.5 × 31.5-40.7 µm, longer stipe, (12-)15-20(-25) vs 8.9-13.8 µm, longer and narrower conidial arms, (25-)30-45(-50) × 4-5 vs 18.4-23.6 × 5-6 µm, and more septa in arms, (2-) 4-5 vs 2-3 (-4). In the case of *R. revayae* has longer and narrower arms, 22-30 x 2-3.5 vs 18.4-23.6 x 5-6 µm; longer and narrower stipe, 14-22 × 2-3.5 vs 8.9-13.8 × 4.4-5.4 µm, more septa in stipe, 3-5 (-7) vs 1-2 (-3), and more septa in arms (3-) 4-7 vs 2-3 (-4).

Retiarius bovicornutus is the most documented species of the genus (Fig. 13). It was described from South Africa (Olivier 1978), later reported from Japan (Ando & Tabuki 1984), Peru (Matsushima 1993), India (Karamchand *et al.* 2021, Sridhar *et al.* 2006, Sridhar & Karamchand 2009), Italy (Magyar *et al.* 2005), Germany, Hungary, Romania, Sweden (Gönczöl & Révay 2004, 2006, Révay & Gönczöl 2010, 2011), France, Great Britain, Luxembourg (Baral *et al.* 2020), and Venezuela (Fernández-Da Silva & Smith-Briedis 2021). However, it appears the species concept has been applied in a broader sense, given the shape and morphometric data reported, which suggests the possibility that there are multiple species under the same name (Baral *et al.* 2020, Révay & Gönczöl 2010).

Most of the previously cited studies, illustrate the conidia of *R. bovicornutus,* but few provide measurements (Table 7). Baral *et al.* (2020) studied European samples from bark, reporting conidia with longer arms and more cylindrical, narrower stipe than the type protologue; samples from Luxembourg and France were similar in shape but slightly smaller than the type. One sample from Luxembourg (6.VI.2004) has a similar shape but is conspicuously smaller than the type. Only one was named as *R. bovicornutus* (CSVY/F/2698), other samples were considered as *R.* aff. *bovicornutus* or *R.* cf. *bovicornutus*. In contrast, two studies have described conidia developed from axenic culture: Ando & Tabuki (1984) from Japan and Matsushima (1993) from Peru, both with conidial arms distinctly shorter than the protologue type, both of which Baral *et al.* (2020) consider them as *R.* aff. *bovicornutus*.

Retiarius revayae was once thought to be a form of *R. bovicornutus*. Révay & Gönczöl (2011) inspected the community of canopy fungi occurring on twigs in angiosperms and conifers, reporting *R. bovicornutus* in all tree species and on 85% of all twig samples, especially in the upper surface of the twig. Gönczöl & Révay (2004) also detected *R. bovicornutus* in rainwater of stemflow samples. Révay & Gönczöl (2010) studied the fungal communities associated with rainwater deposited in the phyllosphere, and *R. bovicornutus* was detected. According to Magyar *et al.* (2017a), some illustrated conidia named *R. bovicornutus* in the previously mentioned studies actually belonged to *R. revayae.* This species was formally described from Hungary (Magyar *et al.* 2017a), and later reported from Luxembourg and South Africa by Baral *et al.* (2020), who noticed some morphological differences with the protologue, which led them to believe that there are more species with similar conidial form (Table 1, Fig. 14).

In addition to the morphological and phylogenetic distinction between *R. canadensis* nom. prov. and *R. bovicornutus*, they are found in different microhabitats. *R. canadensis* nom. prov. has been only found to be associated with pollen deposits in the undersurface of rock-dwelling lichens. In contrast, *R. bovicornutus* was described from the upper surface of angiosperms, and later reported from a wide variety in evergreen trees and shrubs, conifers, palm petioles, and epiphytic ferns. The known microhabitats are bark and twig surfaces (Baral *et al.* 2020, Révay & Gönczöl 2011), honeydew honey of conifers (Magyar *et al.* 2005), rainwater of stemflow (Gönczöl & Révay 2004, 2006), dendrotelma (Magyar *et al.* 2017c), rainwater of phyllosphere (Ando & Tabukio 1984, Gönczöl & Révay 2006, Karamchand *et al.* 2021, Révay & Gönczöl 2010), stream foam (Fernández-Da Silva & Smith-Briedis 2021) and phyllosphere (Baral *et al.* 2020, Matsushima 1993, Olivier 1978, Sridhar *et al.* 2006).

4.3 *Retiarius crescentus* Huereca, C.C. Allen & T. Sprib., nom. prov.

Fig. 19, 20.

Holotype: Canada, British Columbia, Okanagan-Similkameen Regional District, East of the town of Bankier, N 49.694253, W -120.282587, 1020m a.s.l., isolated from undersurface of *Umbilicaria torrefacta* infecting *Pinaceae* pollen grains*,* August 08, 2021, A Huereca, culture TSMC83, CBS 11877, DNA Isolate AH-Ret116. Holotype to be deposited in CBS (Westerdijk Fungal Biodiversity Institute, Netherlands); Isotype to be deposited in DAOMC (Canadian Collection of Fungal Cultures, Canada) and UAMH (Centre for Global Microfungal Biodiversity, Canada).

Diagnosis - *Retiarius crescentus* differs from all *Retiarius* species by canoe-shaped conidium with arms slightly to medium curved, one to two septa (rarely 3), and stipe absent to heavily reduced. Resembles *Lecophagus navicularis* by the shape of the conidium but differs in shorter arm length (17.3-21.7 µm vs 28-34 µm) with consistently three septa, different ecology (pollen-trapping vs rotifer trapping), and different microhabitat (lower surface of *Umbilicaria* spp. vs old manure).

Etymology: *crescentus*, in reference to its resemblance of a crescent moon,

Description: *Somatic hyphae*: hyaline, 4-6 µm wide, septate, straight, sometimes slightly constricted at septa; aerial hyphae 6-7 µm wide, erect, moniliform; cells with lipid bodies containing orange droplets, not carotenoids or glycogen (IKI reaction negative). Conidiophores absent; Conidiogenous cells intercalary in somatic hyphae, smooth, hyaline**,** 3-4 x 3-4 µm. *Conidia* staurosporous, canoe-shaped to allantoid with tapering tips, smooth, hyaline, SCBs not observed, (16.2-) 17.3-21.7 (-25.4) × (4.1-) 4.7-5.8 (-6.7) µm (n=39); stipe absent or heavily reduced, (0.3-) 0.34-0.74 (-1) × (1-) 1.2-2 (-2.4) µm (n=19). Conidium main axis straight, always with one septum in the middle. Occasionally, an additional one or two septa may be found in either or each of the arms. Conidial arms with conspicuously upward-curved tips, (7.4-) 8.5-10.7 (-12.3) × (3.8-) 4.3-5.1 (-5.7) µm (n=78). Chlamydospores 7.05-10.2 × 7.5-12.2 µm. *Teleomorph* unknown.

Culture characteristics (25 days at 21 °C): Colonies on PDA 10.7-12.46 mm in diameter, color light shades ranging from light salmon-orange to salmon-orange, becoming darker with shades ranging from hazel to mahogany red, sometimes darker with auburn tinges; texture velvety with abundant or sparse hair. Colonies convex, wrinkled to complex, with irregular to undulate margins; exudate absent, diffusible pigment rare, orange; reverse colour same shades but blurred by media. On OA 7.1-12.3 mm in diameter, color ranging between light salmon-orange to salmon-orange, rarely becoming darker with kaiser brown tinges; texture velvety in the center to almost glabrous towards the edge; colonies irregular, flat to slightly raised in the center, margin slightly to conspicuously undulate; exudate absent, diffusible pigment almost transparent to slightly salmon-orange or absent; reverse color same shades but blurred by media.

Distribution and Ecology: *Retiarius crescentus* nom. prov. is known from the Thompson Plateau, Columbia Mountains, and Rocky Mountains in southwestern Canada and at 745-1540m above sea level. It was isolated from the lower surface of the lichen genus *Umbilicaria* (*U. phaea* and *U. torrefacta*), infecting pollen grains of *Pinaceae* (Fig. 20). The ecosystems where *R. crescentus* occurs are Interior Cedar-Hemlock and Interior Douglas Fir Forest in southern British Columbia and southern Alberta (Fig. 15).

Isolated strain localities:

Canada, British Columbia, Central Kootenay, Nakusp community, Hot Springs Rd, west of Gardner Falls, N 50.278125, W -117.760820, elev. 745m a.s.l. isolated from undersurface of *Umbilicaria torrefacta* infecting *Pinaceae* pollen grains*,* August 08, 2021, A. Huereca, culture TSMC85, DNA Isolate AH-Ret118; A. Huereca & C.C. Allen culture TSMC87, CBS 11878, DNA Isolate AH-Ret120.

Canada, British Columbia, Okanagan-Similkameen Regional District, East of Bankier town, N 49.694253, W -120.282587, elev. 1020m a.s.l., isolated from the undersurface of *Umbilicaria torrefacta* infecting *Pinaceae* pollen grains*,* August 08, 2021, A. Huereca, culture TSMC84, DNA Isolate AH-Ret117; A. Huereca, culture TSMC86, DNA Isolate AH-Ret119.

Canada, Alberta, Pincher Creek No. 9, Castle Provincial Park, 4.8 km in Ohagen Rd, 0.8km up hill. N 49.429500, W -114.379278, elev. 1540m a.s.l. isolated from undersurface of *Umbilicaria phaea*, infecting *Pinaceae* pollen grains, June 06, 2021, A. Huereca, culture TSMC90, DNA Isolate AH-Ret192; A. Huereca, culture TSMC91, DNA Isolate AH-Ret193; A. Huereca, culture TSMC92, DNA Isolate AH-Ret194; A. Huereca, culture TSMC93, DNA Isolate AH-Ret195; A. Huereca, culture TSMC94, CBS 11879, DNA Isolate AH-Ret196; A. Huereca, culture TSMC95, DNA Isolate AH-Ret197; A. Huereca, culture TSMC96, DNA

Isolate AH-Ret198; A. Huereca, culture TSMC97, DNA Isolate AH-Ret199; A. Huereca, culture TSMC98, DNA Isolate AH-Ret200; A. Huereca, culture TSMC99, DNA Isolate AH-Ret201; A. Huereca, culture TSMC100, DNA Isolate AH-Ret202; A. Huereca, culture TSMC101, DNA Isolate AH-Ret206; A. Huereca, culture TSMC105, DNA Isolate AH-Ret210

4.4 Taxonomic remarks of *Retiarius crescentus* nom. prov. with other *Retiarius* species and similar Orbiliaceous anamorphs.

Retiarius crescentus nom. prov. is unique in the genus in its heavily reduced to absent main conidial axis, and producing canoe-shaped conidia, the shape and measurements of conidia being key features to distinguish *Retiarius* species (Table 8). D. T. Olivier (1978) described *R. superficiaris* and *R. bovicornutus*, the former designated as the type of the genus, characterized as having tripospermum-like three-armed conidia, sometimes 2- and 4-armed, and short main axis. *R. bovicornutus* develops Y-shaped conidia with two upwards curved arms, and 2-3 septa in the main axis. Then, Magyar *et al.* (2017a) described two species closely resembling R*. bovicornutus*, *R. revayae,* and *R. goenczoelii*, both with similar Y-shaped conidia. However, *R. goenczoelii* differs by having longer arms and more septa in them. In the case of *R. revayae,* it differs in having a longer main axis, with 3-5(-7) septa (Baral *et al.* 2020, Magyar *et al.* 2017a, Olivier 1978).

Among anamorphic *Orbiliomycetes*, two species of the genus *Lecophagus* have similar canoe-shaped conidia like *Retiarius crescentus* nom. prov.: *L. navicularis* (Tzean & G.L. Barron) M.W. Dick (syn. *Cephaliophora navicularis*), and *L. vermicola* D. Magyar, G. Marson, Z. Merényi & Baral. Both species are predatory fungi, developing adhesive knobs to capture rotifers and nematodes, respectably, while *R. crescentus* nom. prov. infects pollen grains. Aside from the different ecology, they differ in the number of conidiogenous cells producing two or more than 10, *L. vermicola* and *L. navicularis*, respectively (Magyar *et al.* 2016a, Tzean & Barron 1983), as well for developing adhesive knobs on the cells of conidia. In addition, *R. crescentus* nom. prov. differ from *L. navicularis* and *L. vermicola* in conidial size and arms septation, with 17.3-21.7 × 4.7-5.78 µm and 1-2 septa in the arms (rarely 3), whereas *L. navicularis* conidia are 28-34 × 6-9 µm and consistently 3 septa (Tzean & Barron 1983); and in the case of *L. vermicola*, conidia are distinctly larger and with more septa, 56-86 × 13-17 µm and (3-)7(-8) (Baral *et al.* 2020, Magyar *et al.* 2016a).

Mycoceros antennatissimus D. Magyar, is another orbiliaceous pollen-trapping fungus, that differs from all *Retiarius* species by having staurosporous conidia with repeated T and Y-shaped dichotomous branching in three-dimensional growth on its secondary and tertiary arms, with considerably larger size 55-90 x 70-190 µm (Magyar *et al.* 2017b). Remarkably, *M. antennatissimus* and *L. vermicola* occur in similar microhabitats to other *Retiarius* species, occurring on xeric bark fissures of trunks and branches of trees (Baral *et al.* 2020, Magyar *et al.* 2016a, 2017a, 2017b).

When D T. Olivier described *Retiarius* in phyllosphere samples from South Africa (1978), another pollen-trapping fungus was found growing intermixed with *R. superficiaris* colonies, *Hexacladium corynephorum* (Olivier 1983), and though it shares the same microhabitat and feeding ecology, it differs from *Retiarius* in various aspects. *Hexacladium* captures pollen grains with lateral clavate hyphae and conidia, while *Retiarius* does it with erect, aerial hyphae. *Retiarius* conidia also differs from *Hexacladium*, producing 2 to 4 arms and having a Y-T shape, while the conidia of the latter produce conidia with six radiating arms, connected to a principal axis. *Hexacladium* colonies are hyaline at the beginning, but the sclerotized after sporulation, becoming darker towards to drab-grey, when culture on PDA. *Hexacladium* has dark chlamydospores, in contrast to those of *Retiarius* which are hyaline. Lastly, *Hexacladium* may be potentially dependent to water to capture pollen (Olivier 1983, Seifert *et al.* 2011). The type culture strain (IMI 271061) is available at the CABI culture collection, but unfortunately, no sequences have been produced. In addition, its systematic position is unknown, with no clear affinity to any taxa, but its morphology resembles the genus *Varicosporium* (*Helotiales*, *Leotiomycetes*) (Seifert *et al.* 2011).

5. Discussion

5.1 High occurrence of *Retiarius* in lichens from forests of North America.

Traditionally, reports of *Retiarius* have come about due to its conspicuous conidia, facilitating its distinction from other fungal genera by morphology. This is the first study using *Retiarius*-specific primers to assess its presence in the environment without the need for culturing techniques or visualization by microscopy. The only record of the genus in North America is from Sokolski and colleagues (2006), who investigated the fungal communities associated with *Picea mariana* leaves and adjacent freshwater streams in eastern Canada, with no species name assigned. Apart from that one study, this is the first work to recognize that *Retiarius* is widespread in North America, occurring on lichens and adjacent substrates across Canada, northwestern US, and northern Mexico (Fig. 8).

The use of targeted environmental screening proved to be a reliable method to detect *Retiarius* in lichens and adjacent substrates, but this is not the only time DNA sequencing detects orbiliaceous species near lichens. Hofstetter *et al.* (2007) recovered five “contaminant” sequences with high similarity to *Lecophagus musicola* from DNA extractions of *Flavoparmelia caperata*, *Lecanora hybocarpa*, *Lobaria anomala*, *Lobarina scrobiculata* and *Usnea strigosa*. They hypothesized that the occurrence of these predatorial fungi in lichen thalli could provide protection against mycophagy from invertebrates. In the case of *Retiarius*, fourteen studies used large-scale environmental DNA sequencing approaches and generated *Orbiliomycetes* sequences to then catalog them as “uncultured fungus” or “uncultured *Ascomycota*” in NCBI (Table 6). However, after including them in a dataset with the newly generated sequences, these unclassified *Orbiliomycetes* are shown here to actually belong to *Retiarius* (Fig. 7).

The unclassified sequences were produced in surveys that analyzed fungal communities, isolating samples from various substrates. Eight studies detected the occurrence of *Retiarius* in samples of known microhabitats for the genus, bark, and stem (Dreyling *et al.* 2022, Liu *et al.* 2022), and phyllosphere (Cordier *et al.* 2012, Johnston *et al.* 2017, Lynikienė *et al.* 2020, Marčiulynas *et al.* 2022, Menkis *et al.* 2015, 2019). Two sequences were recovered from non-tree substrates: ground bryophyte from a boreal spruce forest (Kauserud *et al.* 2008), and leaf litter from a mixed temperate deciduous forest. *Retiarius* was also found occurring in human-disturbed environments, like the soil of agriculture plots (Victorino *et al.* 2021) and irrigation ponds of tree nurseries (Marčiulynas *et al.* 2020). The most striking occurrence was detected in indoor dust samples in an urban setting, a residential house, and a nursing home building (Amend *et al.* 2010, Pitkäranta *et al.* 2008).

My environmental screening results indicate a high frequency of occurrence on corticolous and saxicolous lichens, a previously unreported microhabitat for *Retiarius*. *Retiarius* was also detected from the adjacent substrate of lichens, bark, and soil debris. Although it was previously not examined, it is possible that *Retiarius* was present on corticolous lichens because of the ecology of *Retiarius*. This genus is part of a fungal community known as canopy fungi, also called terrestrial or arboreal aquatic hyphomycetes due to their similarity to Ingoldian fungi (Ando 1992, Carrol 1981, Révay & Gönczöl 2010, Sridhar 2009). These fungi appear not to be dispersed by wind due to their spore morphology, being more difficult to be lifted by air, such that they are not often recovered by aerial sampling (Magyar 2005, Sullivan *et al.* 2023). In fact, Magyar *et al.* (2021) suggested that the dispersal mechanism of canopy fungi happens thanks to water draining through the trees via stemflow, rainwater flowing through stem fissures from canopy to ground, or throughfall rainwater or condensation dripping from leaves to ground (Moffett 2000). This would explain why *Retiarius* is isolated from various microhabitats in trees like bark and twig surface, dendrotelmata (water-filled tree holes), honeydew, phyllosphere, and samples of throughfall and stemflow (Ando & Tabukio 1984, Baral *et al.* 2020, Ghate & Sridhar 2015, Gönczöl & Révay 2004, 2006, Karamchand *et al.* 2021, Magyar *et al.* 2005, 2017a, 2017c, Matsushima 1993, Olivier 1978, Révay & Gönczöl 2010, 2011, Sokolski *et al.* 2006, Sridhar & Karamchand 2009, Sridhar *et al.* 2006). Heavy rain events could also account for its presence in water bodies, like mountain streams and stream foam, explaining why they have been grouped in the Ingoldian fungi (Descals *et al.* 1995, Fernández-Da Silva & Smith-Briedis 2021, Kravetz 2020).

The presence of *Retiarius* in tree microhabitats could be explained by the passive deposition of pollen in the bark. Tree bark is considered a natural trap for airborne particles, such as dust, pollutants, and wind-dispersed spores like pollen grains or fungal spores (Fig. 16, Magyar 2008, Magyar *et al.* 2022). Pollen is commonly detected in bark, sometimes abundantly, so much so that bark is sometimes used to assess pollen deposition. It has been shown that pollen gets trapped in bark enclosures (Adam *et al.* 1967), in some cases retaining viable spores (Ranal 2004). Bark samples have been used to evaluate the composition of the flora, identifying the plant genus to which the pollen grain belongs and quantifying its abundance (Groenman-van Waateringe 1998, Song *et al.* 2013, Song *et al.* 2014). In the previously mentioned studies, differences were noted in the composition and quantity of pollen captured in bark, depending on the texture of the bark of the tree species, and in some cases, it stands out that *Pinaceae* pollen is found in considerable proportions, sometimes being the dominant group.

Lichens evidently colonize the outer zone of bark and are also considered natural traps in palynological studies (Carrol 1943, Faegri *et al.* 1989, Moore *et al.* 1991). For example, Song *et al.* (2013) evaluated the composition of pollen captured in different natural traps, including corticolous moss and lichen samples. Their results indicated that moss/lichen samples capture wind-dispersed pollen of *Pinus* and *Alnus*. Caramiello *et al.* (1991) tested the difference in pollen deposition among soil, mosses, and lichens, reporting less pollen in comparison to the other treatments. In the case of terricolous and saxicolous lichens, pollen deposition has been documented in polar environments. Nichols & Stolze (2017) documented pollen deposition in moss and lichen samplers across a transect from the boreal forest into arctic tundra in Canada, mainly detecting pollen from local flora, as well as *Picea* and *Pinus* pollen. Bera *et al.* (2012) found pollen and fungal spores deposited on lichens from Antarctica.

Considering the ecology of *Retiarius* involves trapping and infecting pollen grains in tree microhabitats, together with pollen deposition on bark and lichens, its presence in lichen thalli was always plausible. In a far-reaching scenario, the constant detection of *Retiarius* in environmental sampling, both in targeted and large-scale sequencing, suggests that its presence in forests is ubiquitous. If so, *Retiarius* might be one of the most common fungi in coniferous forests.

5.2 Low occurrence of *Retiarius* in lichens from the Canadian Arctic.

Lichens and mosses, both natural pollen traps and suitable *Retiarius* microhabitats, dominate the vegetation in polar environments. However, the environmental screening results recovered a low frequency of occurrence of *Retiarius*. Of the 177 screened samples, 65 come from ecosystems belonging to the Canadian subarctic and arctic, and *Retiarius* was detected in only four these (Fig. 6, 8; Table 2). Three samples, AH-Ret173, 174, and 175, come from localities located above latitude 67°, that is, above the Arctic Circle, but none of these produced a sequence usable for phylogenetic studies. However, AH-Ret161, from 61° latitude and considered subarctic tundra, did produce a usable sequence. The low availability of pollen at that latitude could explain the low rate of detection of *Retiarius* in the tundra. The abundance of vascular plants in these regions is dominated by perennial herbaceous and low-growing woody plants, with an evident absence of trees and annual plants (Peterson 2014). Although all plants produce pollen, the communities that produce pollen abundantly, and particularly conifer trees, are absent in this ecosystem. In lower latitudes adjacent to the Arctic, conifers are the dominant plant community in boreal-temperate forests, contributing annually to the pollen deposition phenomenon called pollen rain (Lee *et al.* 1996a). Due to their aerial pollination, these trees release millions of pollen grains into the air, with very few achieving their fertilization goal and thousands ending up deposited in the forest.

The amount of pollen per year varies depending on the forest type, the tree species, and the quantification method. Stark (1972) estimated 0.9-3 kg/ha deposited by *Pinus jeffreyi* in temperate forests in Nevada, and Fielding (1960) calculated 700 kg/ha in *P. radiata* forests. Studies in East Asia have estimated the deposition by *P. densiflora*, varies from 9.2-11.2 kg/ha to 17.7-27.5 kg/ha in Korea (Cho *et al.* 2003, Lee & Booth 2003). In contrast, estimates for the same species in Japan range from 55-96 kg/ha to 89-170 kg/ha (Saito *et al.* 1985, Sekiguchi *et al.* 1986). In the case of the boreal forest in North America, Lee *et al.* (1996a) calculated that *P. banksiana* produces an estimate of 19.6-24.6 kg/ha, while Doskey & Ugoagwu (1989) calculated 3.5-80 kg/ha in *P. resinosa* and *P. strobus* stands. One estimation stands out: according to Hesselman (1919), in the boreal forest of central and south Sweden, *Picea abies* produces around 75,000 tons of pollen every year. Regardless of the quantity, pollen is abundant in forests from adjacent latitudes to the Arctic. Some of these pollen grains have been found in palynological traps in the arctic tundra (Nichols & Stolze 2017).

The presence of exotic pollen grains in the Arctic is explained by events of complex, strong air masses, often associated with low-pressure systems, transporting pollen long distances from lower latitudes into the low and high Arctic (Rousseau *et al.* 2008). The composition of these pollen grains is diverse and varies in quantities, but conifers usually have larger concentrations than angiosperms (Campbell *et al.* 1999, Jędryczka *et al.* 2022). Different geographic sources have been identified and are, associated with different air current patterns. For example, Jędryczka *et al.* (2022) concluded that *Pinus* pollen detected in the Svalbard Archipelago comes from circumboreal regions 2000 km away from these islands, such as northern Canada, Scandinavia, and Siberia. Cyclones play an important role in dispersing pollen in Greenland, transporting pollen of tree species of eastern Canada and northeastern USA (Rousseau *et al.* 2008). In the Canadian Arctic, studies suggest that long-dispersal conifer pollen is transported from the adjacent boreal forest of the taiga to the tundra (Nichols & Stolze 2017). Levac & Barneoud (2018) documented pollen of *Larix laricina* in the High Arctic, likely from the prairie provinces in central Canada, while *Pinus* and *Picea* pollen deposited in the same region may come from eastern Canada (Campbell *et al.* 1999, Nichols *et al.* 1978).

Although the availability of pollen, both from annual perennials plants and conifers, would seem constant but not abundant, this does not explain the low detection of *Retiarius*. Another factor to consider is the dispersal of these fungi, usually thanks to rain events and rarely by air currents (Magyar *et al.* 2021). The extreme climatic conditions of the Arctic could also explain the low detection rate of *Retiarius*. Evidence suggests that filamentous fungi isolated from polar environments have evolved physiological mechanisms to withstand extreme low temperatures (Robinson 2001). However, at the moment it cannot be asserted that *Retiarius* has any physiological adaptations to survive these conditions, managing to persist annually, or if it is transported along with the pollen in these isolated dispersal events from the boreal forest into the Arctic.

5.3 Unexpected species diversity of *Retiarius* in North America.

Prior to this study, *Retiarius* was known from four species described in two studies: D. Olivier (1978) circumscribed the genus with *R. superficiaris* as the type species and *R. bovicornutus*, both isolated from leaf surfaces in South Africa. Almost 40 years after the description of the genus, D. Magyar described *R. goenczoelii* and *R. revayae* occurring on bark fissures in Hungary, sequencing the latter as well the ex-type strains of *R. bovicornutus* and *R. superficiaris*, placing the genus in the class *Orbiliomycetes* (Magyar *et al.* 2017a). The only species described with no sequences is *R. goenczoelii*; although its morphology is similar to that of other *Retiarius* species, its phylogenetic affinity is not certain (Baral *et al.* 2020). Here, I describe two new species, *R. canadensis* nom. prov. and *R. crescentus* nom. prov., occurring sympatrically in pollen grains located on the undersurface of *Umbilicaria* spp. in Canada and US (Fig. 18, 20). With these novelties, the genus *Retiarius* expands to six species, one of them with a unique conidial morphology previously not known to the genus (Table 8).

The use of *Retiarius*-specific primers for screening environmental samples revealed an astonishing level of phylogenetic diversity. After including the screening-derived sequences in an ITS phylogeny, the analyses revealed 17 clades that I interpret as putative species and two new species isolated in axenic culture and morphologically characterized (Fig 5). It is surprising that a fungal genus with four known species suddenly could contain more than 20 species when exploring a region of the planet without any species previously reported of *Retiarius*. Similarly, the recovery of 37 sequences deposited in NCBI classified as uncultured fungus, plus two as uncultured *Retiarius* and *Retiarius* sp., suggests that the species richness could be even higher. However, an estimate of how many putative species exist is not possible due to the length or completeness of these sequences.

Geographically, *Retiarius* distribution was previously thought to be centered in Europe, known from France, Germany, Great Britain, Hungary, Italy, Luxembourg, Romania, Spain, and Sweden, as well as from India, Japan, Peru, South Africa, and Venezuela (Table 1). This study confirms its occurrence in Canada, China, Finland, Lithuania, Mexico, New Zealand, Norway, and US, expanding its global distribution. Aside from *R. canadensis* nom. prov. and *R. crescentus* nom. prov., the newly reported occurrences are known solely from environmental sequences. Consequently, in the absence of morphological characteristics, particularly conidia, the key feature in *Retiarius* taxonomy, it is not possible to assign a species name. For example, while looking for *Retiarius* conidia on the lower surface of *Umbilicaria*, conidia with different morphology than those of the new species, probably belonging to one of the putative species detected in the environmental screening, were evident (Fig. 8, 15, 21).

5.4 Draft Genome Annotation

The diversity and taxonomy of *Orbiliomycetes* have been studied intensively in recent years (Baral *et al.* 2020, Quijada *et al.* 2020, Zhang *et al.* 2023), but genomic data has only been generated to study nematode-feeding species due to their unique carnivorous behavior. According to the BUSCO annotation, the genome of *Retiarius crescentus* nom. prov. has an estimated number of 10,331 genes and a genome size of 36.62 Mb. These values are similar to those reported in the available *Orbiliomycetes* genomes, some of which were available in a direct submission to GenBank, for example, the genome of *Dactylellina cylindrospora* with 10647 genes and 37.71 Mb in size (NCBI ID 88853); *D. entomopaga* with 10470 genes and 38.38 Mb (as *Arthrobotrys entomopaga* NCBI ID 88852), *Gamsylella cionopaga* 10716 genes and 43.12 Mb (as *Dactyllina cionopaga,* NCBI ID 8854), and *Orbilia brochopaga* 9919 genes and 35 Mb (as *Drechslerella brochopaga,* NCBI ID 81254). Five orbiliaceous genomes were produced in various studies, like the *A. flagrans* with 9927 genes and 36.64 Mb i (as *Duddingotnia flagrans*, Youssar *et al.* 2019); *D. haptotyla* with 10959 genes; and 40.4 Mb (as *Monacrosporium haptotylum*, Meerupati *et al.* 2013); *Dr. stenobrocha* with 7781 genes and 29.02 Mb (Liu *et al.* 2014); and *O. oligospora* with 11479 genes and 40.07 Mb (as *A. oligospora*, Yang *et al.* 2011). The genome of *D. dactyloides* was not annotated, but the genome size was reported with 38,63 Mb (Fan *et al.* 2021). The availability of the genome a non-nematode-feeding orbiliomycete will be useful for future phylogenomic studies in fungi, as well allow researchers to study the biology of *Retiarius* from a genomic perspective.

5.5 Metabolic assessment using phenotype microarrays.

Unfortunately, the experiment to characterize the carbon and nitrogen utilization of *Retiarius canadensis* nom. prov. and *R. crescentus* nom. prov. using Phenotype Microarrays failed due to inconsistent results in the three replicates. While there are substrates in which growth of the fungus was recorded in all three replicates, they are few. On most substrates where the fungus grew, it grew on only one of the three replicates, and on other substrates, only two of the three replicates showed growth. Hence, these results are unreliable and should be treated as preliminary data for the metabolic activity of these species. This discrepancy was potentially caused by inadequate maceration of the mycelium, leading to a lack of homogenized fungal mycelia in the inoculation solution. Consequently, during the pipetting process for plate inoculation, there was inconsistent sufficient in the amount of initial material available, resulting in instances where no growth was recorded.

In addition to the inoculation problem, there are other aspects to consider as to why Phenotype MicroArray technology may not be suitable for the genus *Retiarius*. Initially, Phenotype MicroArray was designed to test the metabolism of unicellular microorganisms, to identify bacteria based on their metabolism (Bochner & Savageau 1977, Bochner 1989). Subsequently it was used to study other unicellular microorganisms like yeast (Nielsen *et al.* 2005). In the following years, when this protocol was tried with filamentous fungi, it was initially without success. Tanzer *et al.* (2003) assessed three fungal species and found non-uniform growth, leading to highly variable optical density measurements. They concluded that methods for plate growth for the Phenotype MicroArray system have not been adjusted to filamentous fungi. This fostered research into optimizing spore concentration for each species, in order to have standardized results in the assays. However, other studies appeared not to encounter this problem, assessing the metabolism of various filamentous fungi species (C. Allen *et al.* in prep., Blumenstein *et al.* 2015, Nai *et al.* 2013).

Another reason that growth may have been hindered could be that conidia were nor used in the inoculation fluid, which could have impacted the homogeneity in the inoculation. Following the manufacturer's instructions, fungal biomass should be added to the inoculation fluid once sporulation is obtained in the culture, removing conidia and mycelial fragments with a sterile swab. This was not done with *Retiarius* because, at the time of the experiment, sporulation of the two *Retiarius* species had not been achieved because the correct medium that induces conidia sporulation (OA) had not been used.

Another difficulty was the growth rate of *Retiarius,* and the length of time used to read the optical density from plates. According to the manufacturers protocol, once the fungal biomass I inoculated and the incubation period is over, plates should be read every 24 hours, depending on how fast the fungi grow. This period ranges from 6 to 14 days since fungi usually exhibit fast growth rates (C. Allen *et al.* in prep., Blumenstein *et al.* 2015, Nai *et al.* 2013). In the case of *Retiarius*, growth rates in rich-nutrient media like PDA or Potato Dextrose broth took over 30 days or even more to produce enough biomass. Once *Retiarius* was inoculated into the 96-well plates, most of the substrates hindered the already slow growth rate even more, extending the time needed to collect data to 35 days. At the end of the experiment, it was noted that in some wells, the amount of the solution was relatively low. This can have two explanations: either the well lost the solution due to evaporation, or the colony used most of the inoculation fluid mixed with the substrate.

5.6 Potential Implications of *Retiarius* in Nutrient Cycling.

It is a fact that pollen is available in large quantities in ecosystems and that some fungi can feed on these grains, as in the case of *Retiarius*. It is used as an energy source because pollen has high concentrations of nitrogen, some not reliably available in oligotrophic ecosystems where fungi are selected to obtain this nutrient (Barron 1992). Pollen contributes substantial amounts of macronutrients such as nitrogen and phosphorus to ecosystems and high contents of polyunsaturated fatty acids (Banks & Highswander 2000, Greenfield 1999, Filipiak 2016, Masclaux *et al.* 2011). Studies have estimated the macronutrient input in pollen rain events, mainly in coniferous forests, like in Korea’s temperate forests, where pollen rain incorporates 0.19-0.55 kg/ha of nitrogen, 0.07-0.24 kg/ha of potassium and 0.01-0.07 kg/ha of phosphorus (Cho *et al.* 2003, Lee & Booth 2003). Lee *et al.* (1996b) estimated that pollen supplies the boreal forest with 0.34-0.49 kg/ha of nitrogen, 0.04-0.07 kg/ha of phosphorus, and 0.11-0.20 kg/ha of potassium. Thus, macronutrient deposition via pollen rain represents a temporary rich pulse of nutrients in oligotrophic environments (Filipiak 2016), releasing high concentrations of nutrients readily available to bacteria, fungi, and invertebrates (Greenfield 1999, Perez-Moreno & Read 2001, Shumilovskikh, *et al.* 2015, Stark 1972, Wurzbacher *et al.* 2014).

How fungi can break down pollen grains and access the nutrient content is poorly understood. The extracellular wall of pollen grains is composed of sporopollenin, an extremely stable and inert biopolymer (Brooks & Shaw 1978, Li *et al.* 2019). With this protection, pollen grains are difficult to degrade chemically; however, fungi can still break down pollen grains and obtain nutrients. According to microscopical observations, fungal hyphae penetration occurs in sites where pollen tube emergence takes place, leaving sporopollenin walls intact (Hutchinson & Barron 1997, Shumilovskikh *et al.* 2015, Stark 1972). Hutchison and Barron (1997) observed that hyphae are attracted to pollen grains, leading growth towards and around them, ultimately penetrating via the lysing of the pollen tube or physically through the germ pores. D. Olivier (1978) noted that *Retiarius* hyphae will grow around the circumference of the pollen grain, penetrating the dorsal region between the wings of pine pollen. In addition, according to Olivier's previous observations, no true appressorium formed, but a local thickening of the mucilaginous outer layer was noted, followed by a slight depression of the grain's wall, which suggests the hyphal penetration could be mechanical (Olivier & Williams 1975). Nevertheless, more research is needed to understand if the penetration happens thanks to enzymes or physical pressure in the pollen wall.

Nutrient cycling involving pollen-derived nutrients and fungi is known to some extent. Chytrid fungi serve as intermediate trophic level in water ecosystems; by transferring nutrients obtained from pollen grains to microinvertebrates, these fungi make pollen nutrients available to the ecosystem (Kagami *et al.* 2017, Masclaux *et al.* 2011, 2013; Rösel *et al.* 2011, Wurzbacher *et al.* 2014). Fungi occurring in forest litter are also known to access pollen nutrients (Hutchinson & Barron 1992, Stark 1972). Perez-Moreno and Read (2001) demonstrated that *Paxilus involutus*, an ectomycorrhizal fungus, is able to forage pollen grains, obtain nutrients, and then transfer them to *Betula pendula* seedlings. In this experiment, they found that mycorrhizae-inoculated seedlings grown with pollen had a significantly greater dry weight, nitrogen, and phosphorus content than those without access to pollen.

In the case of canopy fungi, like *Retiarius,* nutrient cycling has yet to be explored. Stone *et al.* (1996) hypothesized that canopy fungi were involved in nutrient cycling in the canopy by accessing airborne nutrients, then interacting with arthropods, or establishing symbiotic relationships, as well as obtaining nutrients from throughfall and stemflow water (Schroth *et al.* 2001). Unfortunately, there is still no study on a canopy fungi species involved in the nutrient transfer. Nevertheless, given how common *Retiarius* is in forested ecosystems and the availability of pollen every year, it seems reasonable to speculate it is yet another fungal genus with an essential role in ecosystems, with its ability to access a rich nutrient source inside pollen grains.

6. Conclusions

In this work, I confirm the presence of the pollen-trapping fungal genus *Retiarius* in North America, including the description of two species new to science, *Retiarius canadensis* nom. prov. and *Retiarius crescentus* nom. prov. In addition, using genus-specific primers proved to be a useful tool to detect *Retiarius* in the environment, revealing its ubiquity in forested ecosystems. In the Canadian Arctic, *Retiarius* is present but it was detected in low frequency, despite the availability of suitable microhabitats and pollen grains, though in low abundance. Also, the environmental screening revealed a high species richness awaiting to be isolated and formally described. By producing more *Retiarius* sequences and a comprehensive phylogeny, including unnamed environmental sequences at NCBI, this study will aid the identification of *Retiarius* sequences generated in studies of fungal communities or from axenic cultures. The sequencing of the genome of *Retiarius* crescentus nom. prov. opens the possibility of conducting comparative genomics studies with other fungal species aside from the nematode-trapping *Orbiliomycetes*. In addition, it will allow the study of its enzymatic machinery and processes involved in pollen degradation by non-chytrid fungi. Even though the metabolic assessment of *Retiarius* failed, understanding the metabolism of these fungi is crucial to deciphering the role *Retiarius* plays in nutrient cycling, as it may play a key factor in facilitating the transfer of nitrogen and phosphorus enclosed in pollen grains to forests, much as chytrid fungi do in aquatic environments.

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Bibliography

Adam, D.P., Ferguson, C.W. & LaMarch, V.C. Jr. 1967 Enclosed bark as a pollen trap. Science 157:1067–1068

Allegrucci, N., Bucsinszky, A. M., Arturi, M. F., & Cabello, M. N. 2015. Communities of anamorphic fungi on green leaves and leaf litter of native forests of *Scutia buxifolia* and *Celtis tala*: Composition, diversity, seasonality and substrate specificity. Revista Iberoamericana De Micología, 32(2), 71–78.

Alshuwaili, F., Anbagi, R. A., Nelsen, D., Semenova-Nelsen, T., & Stephenson, S. 2021. Mycobiome sequencing and analysis of the assemblages of fungi associated with leaf litter on the Fernow Experimental Forest in the Central Appalachian Mountains of West Virginia. Current Research in Environmental & Applied Mycology. Journal of Fungal Biology, 11(1), 315–332.

Amend, A.S., Seifert, K.A. & Bruns, T.D., 2010. Quantifying microbial communities with 454 pyrosequencing: does read abundance count? Mol. Ecol. 19, 5555–5565.

Ando, K. 1992. A study of terrestrial aquatic Hyphomycetes. Trans. mycol. Soc. Japan. 33, 415-425.

Ando, K. & Tubaki, K. 1984. Materials for the fungus flora of Japan (37). Trans. mycol. Soc. Japan 25, 395-398.

Banks, H.H. & Nighswander, J.E. 2000. Relative contribution of hemlock pollen to the phosphorus loading of the clear lake ecosystem near Minden, Ontario, p. 168–174. In McManus, K.A., Shields, K. S., & Souto, D.R. [eds.], Proceedings: Symposium on sustainable management of hemlock ecosystems in eastern North America. General Technical Report NE-267. U.S. Department of Agriculture, Forest Service, Northeastern Research Station.

Baral, H.O.; Weber, E.; Gams, W.; Hagedorn, G.; Liu, B.; Liu, X.Z.; Marson, G.; Marvanová, L.; Stadler, M.; Weiß, M. 2018. Generic names in the *Orbiliaceae* (*Orbiliomycetes*) and recommendations on which names should be protected or suppressed. – Mycol. Progr. 17: 5–31. –

Baral, H.O., Weber, E. & Marson G. 2020: Monograph of *Orbiliomycetes* (*Ascomycota*) based on vital taxonomy. Part I + II. National Museum of Natural History Luxembourg, 1752 pp.

Barone, G., Corinaldesi, C., Rastelli, E., Tangherlini, M., Varrella, S., Danovaro, R. & Dell'Anno, A. Local Environmental Conditions Promote High Turnover Diversity of Benthic Deep-Sea Fungi in the Ross Sea (Antarctica). 2022. Journal of Fungi. 8(1):65.

Beenken, L., Stroheker, S., Dubach, V. Schelegel, M., Queloz, V. & Gross, A. 2023. *Microstrobilinia castrans*, a new genus and species of the *Sclerotiniaceae* parasitizing pollen cones of *Picea* spp. *Mycological Progress* 22, 14

Benny, G. L., Samuelson, D. A., & Kimbrough, J. W. 1978. Ultrastructural studies on *Orbilia luteorubella* (*Discomycetes*). Canadian Journal of Botany, 56(17), 2006–2012.

Benny, G., Samuelson, D.A. & Kimbrough, J.W. 1978. Ultrastructural studies on *Orbilia luteorubella* (*Discomycetes*). Can J Bot 56:2006–2012.

Bera, S.K., Phartiyal, B. & Sharma, A. 2012. Evidence of Pollen-Spores retrieved from lichen patches distributed in Schirmacher Oasis and Ajacent Nunataks, East Antartica: A case study of pollen transport over Polar Region. International Journal of Earth Sciences and Engineering, 5 (4): 724-730.

Bills, G. F., Christensen, M., Powell, M., & Thorn, G. 2004. Saprobic soil fungi. In G. M. Mueller, G. F. Bills, & M. S. Foster (Eds.), Biodiversity of fungi: Inventory and monitoring methods (pp. 271–302). Oxford: Elsevier.

Blumenstein, K., Macaya-Sanz, D., Martín, J.A., Albrectsen, B.R. & Witzell, J. 2015. Phenotype MicroArrays as a complementary tool to next generation sequencing for characterization of tree endophytes. Front. Microbiol. 6:1033

Bochner, B. 1989. “Breathprints” at the Microbial Level. ASM News, 55, 536-539.

Bochner, B. R., & Savageau, M. A. 1977. Generalized indicator plate for genetic, metabolic, and taxonomic studies with microorganisms. Applied and environmental microbiology, 33(2), 434–444.

Boekhout, T., Amend, A. S., Baidouri, F. E., Gabaldón, T., Geml, J., Henning, T., Robert, V., Tan, C., Turchetti, B., Vu, D., Wang, Q., & Yurkov, A. 2021 Trends in yeast diversity discovery. Fungal Diversity, 114(1), 491–537.

Braun, A. 1855. Der Chytridium, eine Gattung einzelner Schmnarotzergewaichse auf Algen und Infusorien. Monatsher. Berlin Akad. 1855: 378-384.

Brodo, I. M., Sharnoff, S. D. & Sharnoff, S. 2001. Lichens of North America. Yale University Press, New Haven. 795 pp.

Brodo, I. M., Sharnoff, S. D. & Sharnoff, S. 2016. Keys to Lichens of North America, Revised and Expanded. Yale University Press, New Haven. 427 pp.

Brooks. J. 6; Shaw. B. 1978. Sporopollenin: a review of its chemistry, palaeochemistry and geochemistry. -Grana 17: 91-97. Uppsala 29 September 1978. ISSN 0017-3134.

Buaya, A.T. & Thines, M. 2023. *Ducellieriales* ord. nov. and evidence for a novel clade of endobiotic pollen-infecting “lagenidiaceous” *Peronosporomycetes*. Fungal Systematics and Evolution 12: 247–254.

Campbell, I. D., McDonald, K., Flannigan, M. D., & Kringayark, J. 1999. Long-distance transport of pollen into the Arctic. Nature 399, 29–30.

Capella-Gutiérrez, S., Silla-Martínez, J.M. & Gabaldón, T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics. 1;25(15):1972-1973

Caramiello. R., Siniscalco, C. & C Piervittori, R. 1991. The relationship between vegetation and pollen deposition in soil and in biological traps. - Grana 30: 291-300, 1991.

Carroll, G. 1943. The Use of Bryophytic Polsters and Mats in the Study of Recent Pollen Deposition. American Journal of Botany, 30(5), 361–366.

Carrol, G.C. 1981: Mycological inputs to ecosystem analysis. In: Wicklow, D.T. & G.C. Carroll (eds.): The Fungal Community. Its Organization and Role in the Ecosystem: 25–35. Marcel Dekker, New York

Cho, Y.-J., Kim, I. S., Kim, P.-G. & Lee E. J. 2003. Deposition of airborne pine pollen in a temperate pine forest. – Grana 42: 178–182.

Classen, B., Amelunxen, F., & Blaschek, W. 2000. Concentric Bodies in a Parasitic Fungus of *Malva sylvestris* (*Malvaceae*) Pollen. Journal of Phytopathology, 148(5), 313–317.

Cometto, A., Ametrano, C. G., & Muggia, L. 2022. Life on top: cryptoendolithic ascomycetes and microalgae isolated from over 6000 m altitude. Plant and Fungal Systematics, 67(1), pp.1-16.

Cordier, T., Robin, C., Capdevielle, X., Fabreguettes, O., Desprez-Loustau, M., & Vacher, C. 2012. The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. New Phytologist, 196(2), 510–519.

Crous, P. W., Wingfield, M. J., Burgess, T., Carnegie, A., Hardy, G., Smith, D. J., Summerell, B. A., Cano-Lira, J. F., Guarro, J., Houbraken, J., Lombard, L., Martín, M., Sandoval-Denis, M., Alexandrova, A. V., Barnes, C. W., Baseia, I. G., Bezerra, J. L., Guarnaccia, V., May, T., ... Rodríguez-Andrade, E. 2017. Fungal Planet description sheets: 625–715. Persoonia.

Descals, E., Pélaez F. & López-Llorca, L.V. 1995: Fungal spora of stream foam from central Spain II. Chorology, spore frequency and unknown forms. - Nova Hedwigia 60: 551–569.

Díaz-Escandón, D., Tagirdzhanova, G., Vanderpool, D., Allen, C.C.G., Aptroot, Češka, O., Hawksworth, D. L., Huereca, A., Knudsen, K., Kocourková, J., Lücking, R., Resl, P., & Spribille, T. 2022. Genome-level analyses resolve an ancient lineage of symbiotic ascomycetes. Current Biology: CB, 32 (23), 5209-5218

Diederich P, Lawrey J.D., Ertz, D. 2018. The 2018 classification and checklist of lichenicolous fungi, with 2000 non-lichenized, obligately lichenicolous taxa. Bryologist 121, 340–425.

Doskey, P. V. & Ugoagwu, B. J. 1989. Atmospheric deposition of macronutrients by pollen at a semi-remote site in northern Wisconsin. – Atmos. Environ. 3: 2761 – 2766

Dreyling, L., Schmitt, I., & Grande, F. D. 2022. Tree Size Drives Diversity and Community Structure of Microbial Communities on the Bark of Beech (*Fagus sylvatica*). Frontiers in Forests and Global Change, 5.

Eriksson, O.E., Baral, H.O., Currah, R.S., Hansen, K., Kurtzman, C.P., Læssøe, T. & Rambold, G. 2003. Notes on ascomycete systematics No. 3580– 3623. Myconet 9:91–103.

Esslinger, T. L. 2021. A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the Continental United States and Canada, version 24. Opuscula Philolichenum 20: 100-394.

Fernández-Da Silva, R. & Smith-Briedis, G. 2021. Hifomicetos acuáticos en los ríos Borburata y Patanemo (Puerto Cabello, Carabobo-Venezuela) como bioindicadores de calidad ambiental. Gestión y Ambiente 24(2), 98607.

Fielding, J.M. 1960. Branching and flowering characteristics of Monterey Pine. Forestry & Timber Bureau Bulletin No 37. Canberra.

Filipiak, M. .2016. Pollen Stoichiometry May Influence Detrital Terrestrial and Aquatic Food Webs. Front. Ecol. Evol. 4:138.

Gardes, M. & Bruns, T.D. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology, 2: 113-118.

Ghate, S.D. & Sridhar, K.R. 2015. Rain-borne fungi in stemflow and throughfall of six tropical palm species. – Czech Mycol. 67(1): 45–58.

Goldstein, S. 1960. Degradation of pollen by phycomycetes. Ecology 41: 543–545

Gönczöl, J. & Révay., A. 2004. Fungal spores in rainwater: stemflow, throughfall and gutter conidial assemblages. – Fungal Divers. 16: 67–86.

Gönczöl, J. & Révay., A. 2006. Species diversity of rainborne hyphomycete conidia from living trees. – Fungal Divers. 22: 37–54.

Goward, T. 1999. The lichens of British Columbia: illustrated keys. Part 2 – fruticose species. B C Ministry For Spec Rep. 9:1–319.

Goward, T., McCune, B. & Meidinger, D. 1994. The lichens of British Columbia: illustrated keys. Part 1 – foliose and squamulose species. B C Ministry For Spec Rep Ser. 8:1–181.

Greenfield, L. 1996. Plant pollen production in selected tree species. Canterbury Botanical Society Journal. 31, 10-13

Groenman-van Waateringe, W. 1998 Bark as a natural pollen trap. Rev Palaeobot Palynol 103:289–294

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59(3):307–321.

Harris P & Shaw G. 1984. Intermediate filaments, microtubules and microfilaments in epidermis of sea urchin tube foot. Cell Tissue Res. 236:27-33.

Hawksworth, D.L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research 95, 641–655.

Hawksworth, D.L., Lücking, R. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectrum 5, 79–95. Doi

Hawksworth, D.L. & Rossman, A.Y. 1997. Where are all the undescribed fungi? Phytopathology 87:888–891.

Hesselman, H. 1919. Lakttagelser over skogstradpollens spridnings-formaga. Meddelanden Fran Statens Skogforsoksanstalt 16: 27.

Hoang, D.T, Chernomor, O., von Haeseler, A., Minh B.Q. & Vinh, L.S. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. Mol. Biol. Evol., 35:518–522.

Horecka, J & Chu, M.A. 2017. Yeast Colony PCR: It doesn't get any easier than this!. protocols.io https://dx.doi.org/10.17504/protocols.io.gzwbx7e

Hopple, J. S., Jr. & R. Vilgalys. 1994. Phylogenetic relationship among coprinoid taxa and allies based on data from restriction site mapping of nuclear rDNA. Mycologia 86: 96-107.

Hofstetter, V., Miadlikowska, J., Kauff, F., & Lutzoni, F. 2007. Phylogenetic comparison of protein-coding versus ribosomal RNA-coding sequence data: A case study of the *Lecanoromycetes* (*Ascomycota*). Molecular Phylogenetics and Evolution, 44(1), 412–426.

Huang, H., Kokko, E. G., & Erickson, R. S. 1999. Infection of alfalfa pollen by *Botrytis cinerea*. Botanical Bulletin of Academia Sinica, 40(1), 101–106. https://ejournal.sinica.edu.tw/bbas/content/1999/1/bot41-12.html

Hung-Chang, H., Kokko, E.G., and Jenn-Wen, H. 2003. Infection of alfalfa (*Medicago sativa* L.) pollen by mycoparasitic fungi *Coniothyrium minitans* Campbell and *Gliocladium catenulatum* Gilmon and Abbott. Revista Mexicana de Fitopatología 21:117-122.

Hurdeal, V. G., Longcore, J. E., Jones, E. T., Simmons, D., Hyde, K. D., & Gentekaki, E. 2023. Integrative approach to species delimitation in *Rhizophydiales*: Novel species of *Angulomyces*, *Gorgonomyces*, and *Terramyces* from northern Thailand. Molecular Phylogenetics and Evolution, 180, 107706.

Hutchison, L. J. & Barron, G. L. 1997. Parasitism of pollen as a nutritional source for lignicolous Basidiomycota and other fungi. Mycol. Res. 101, 191–194.

James, T. Y., Letcher, P. M., Longcore, J. E., Mozley-Standridge, S. E., Porter, D. L., Powell, M. J., Griffith, G. W., & Vilgalys, R. 2006. A molecular phylogeny of the flagellated fungi (*Chytridiomycota*) and description of a new phylum (*Blastocladiomycota*). Mycologia, 98(6), 860–871.

Jędryczka M., Żuraw B., Zagórski P., Rodzik J., Mędrek K., Pidek I.A., Haratym W., Kaczmarek J. & Sadyś M. 2022. The origin of pine pollen grains captured from air at Calypsobyen, Svalbard. Polish Polar Research, 4 (3), 313-338.

Jones, E.B.G., Pang, K.L., Abdel-Wahab, M.A., Scholz, B., Hyde, K.D., Boekhout, T., Ebel, R., Rateb, M.E., Henderson, L., Sakayaroj, J., Suetrong, S., Dayarathne, M.C., Kumar, V., Raghukumar, S., Sridhar, K.R., Bahkali, A.H.A., Gleason, F.H., & Norphanphoun, C. 2019. An online resource for marine fungi. Fungal Divers 96:347–433.

Johnson, M. H., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S. M., & Madden, T. F. 2008. NCBI BLAST: a better web interface. Nucleic Acids Research, 36, W5–W9.

Johnston, P. R., Park, D., & Smissen, R. D. 2017. Comparing diversity of fungi from living leaves using culturing and high-throughput environmental sequencing. Mycologia, 109, (4), 643–654.

Kagami, M., De Bruin, A., Ibelings, B. W., & Van Donk, E. 2007. Parasitic chytrids: Their effects on phytoplankton communities and food-web-dynamics. Hydrobiologia, 578, 113–129

Kagami, M., Miki, T., & Takimoto, G. 2014. Mycoloop: Chytrids in aquatic food webs. Frontiers in Microbiology, 5, 166.

Kagami, M, Motoki, Y., Masclaux, H. & Bec, A. 2017 Carbon and nutrients of indigestible pollen are transferred to zooplankton by chytrid fungi. Freshwater Biol. 62: 954– 964.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T. K.F., von Haeseler, A. & Jermiin L.S. 2017 ModelFinder: Fast model selection for accurate phylogenetic estimates. Nature Methods, 14:587–589.

Karamchand, K. S., Sharathchandra, K. & Sridhar, K. R. 2021. Diversity of fungal spores in the canopy of two mangrove tree species of southwest India. International Journal of Agricultural Technology 17(6):2097-2110.

Katoh, K. & Standley, D.M. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution, 30(4), 772-780

Kauserud, H., Mathiesen, C., & Ohlson, M. 2008. High diversity of fungi associated with living parts of boreal forest bryophytes. Botany, 86(11), 1326–1333.

Kearse, M. D., Moir, R., Wilson, A. G., Stones-Havas, S., Cheung, M. C., Sturrock, S. S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. J. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649.

Kirk, P., Cannon, P., Minter, D. & Stalpers, J. 2008. Dictionary of the fungi, 10th ed. CABI Publishing, Wallingford

Kravetz, S. 2020. Efectos del impacto de actividades antrópicas en arroyos pampeanos sobre las comunidades acuáticas de hifomicetos. PhD Thesis. Universidad Nacional de Luján.

Kumar, T.K.A., Healy, R., Satafora, J.W., Blackwell, M., & McLaughlin, D.J. 2012. *Orbilia* ultrastructure, character evolution and phylogeny of *Pezizomycotina*. Mycologia 104:462–476

Larsson, A. 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics 30(22): 3276-3278.

Lee, E. J., & Booth, T. 2003. Macronutrient input from pollen in two regenerating pine stands in southeast Korea. Ecological Research, 18, 423–430

Lee, E. J., Kenkel, N. C., & Booth, T. 1996a. Pollen deposition in the boreal forest of west-central Canada. Canadian Journal of Botany, 74(8), 1265–1272.

Lee, J., Kenkel, T. & Booth, T. 1996b. Atmospheric deposition of macronutrients by pollen in the boreal forest. EcoScience 3, 304-309.

Letcher, P. M., & Powell, M. J. 2002. Frequency and distribution patterns of zoosporic fungi from moss-covered and exposed forest soils. Mycologia, 94, 761–771.

Letcher, P.M., Powell M.J. & Viusent M.C. 2008 Rediscovery of an unusual chytridiaceous fungus new to the order Rhizophydiales, Mycologia, 100:2, 325-334.

Levac, E., & Barneoud, P. 2018. Exotic pollen to the Canadian High Arctic: possible sources, transport mechanisms and implications of climate change. In AGU Fall Meeting Abstracts (Vol. 2018, pp. GH12A-04).

Li, F., Phyo, P., Jacobowitz, J., Hong, M., & Weng, J. (2018). The molecular structure of plant sporopollenin. Nature Plants, 5(1), 41–46.

Liu, L., Yang, C., Xu, X., Wang, X., Liu, M., Chen, R., Tan, F., Liu, Y., Lin, T. & Liu, Y. Unlocking the Changes of Phyllosphere Fungal Communities of Fishscale Bamboo (*Phyllostachys heteroclada*) under Rhombic-Spot Disease Stressed Conditions. Forests 2022, 13, 185

Lynikienė, J., Marčiulynienė, D., Marčiulynas, A., Gedminas, A., Vaičiukynė, M., & Menkis, A. 2020. Managed and Unmanaged Pinus sylvestris Forest Stands Harbour Similar Diversity and Composition of the Phyllosphere and Soil Fungi. Microorganisms, 8(2), 259.

Magyar D. 2005. Aerobiological studies on mycobiota. Ph.D. Thesis, Gödöllő: Szent István University

Magyar, D. 2008. The tree bark: a natural spore trap. Aspects of Applied Biology 89, 7-16.

Magyar, D., Göncöl, J., Révay, A., Grillenzoni, F. & Seijo-Coello, M.D.C. 2005: Stauro-and scolecoconidia in floral and honeydew honeys. – Fungal Diversity 20: 103–120.

Magyar, D., Merényi, Z., Bratek, Z., Baral, H., & Marson, G. 2016a. *Lecophagus vermicola* sp. nov., a nematophagous hyphomycete with an unusual hunting strategy. Mycological Progress, 15(10–11), 1137–1144.

Magyar, D., Mura-Mészáros, A. & Grillenzoni, F. 2016b: Fungal diversity in floral and honeydew honeys. – Acta Bot. Hung. 58: 145–166.

Magyar D., Merényi, Z., Körmöczi, P., Bratej. Z. & Kredics, L. 2017a Phylogenetic analysis and description of two new species of pollen parasitic *Retiarius* (anamorphic *Orbiliomycetes*). Nova Hedwigia 105:411–423.

Magyar, D., Merényi, Z., Udvardy, O., Kajtor-Apatini, D., Körmöczi, P., Fülöp, A., Bratek, Z. & Kredics, L. 2017b. *Mycoceros antennatissimus* gen. et sp. nov.: a mitosporic fungus capturing pollen grains. – Mycol. Progr. 17: 33–43.

Magyar, D., Vass, M., & Oros, G. 2017c. Dendrotelmata (water-filled tree holes) as fungal hotspots – along term study. Cryptogamie, Mycologie, 38 (1): 55-66.

Magyar, D., Van Stan, J.T. II & Sridhar K.R. 2021 Hypothesis and Theory: Fungal Spores in Stemflow and Potential Bark Sources. Front. For. Glob. Change 4:623758

Magyar, D., Van Stan, J.T. II. & Sridhar, K.R. 2022. Fungi of the “Bark Side”. Front. Young Minds 9:643131

Magyar, D., Krasznai, B., & Tóth, M. D. 2022. Microscopic fungi and other contaminants on airborne pollen grains of ragweed (*Ambrosia artemisiifolia* L.). Aerobiologia, 38(2), 217–231. https://doi.org/10.1007/s10453-022-09743-w

Manni, M., Berkeley, M.R., Seppey, M., Simão, F.A. & Zdobnov, E.M. 2021. BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. Mol. Biol. Evol.; 38: 4647-4654

Matsushima, T. 1993. Matsushima Mycological Memoirs 7. – Kobe, Japan.

Marasinghe, D. S., Hongsanan, S., Zeng, X., Jones, E. B. G., Chomnunti, P., Boonmee, S., & Hyde, K. D. 2023. Taxonomic monograph of epifoliar fungi. Fungal Diversity, 121(1), 139–334.

Marčiulynas, A., Marčiulynienė, D., Lynikienė, J., Gedminas, A., Vaičiukynė, M., & Menkis, A. 2020. Fungi and Oomycetes in the Irrigation Water of Forest Nurseries. Forests, 11(4), 459.

Marčiulynas, A., Marčiulynienė, D., Lynikienė, J., Bakys, R., & Menkis, A. 2022. Fungal Communities in Leaves and Roots of Healthy-Looking and Diseased Ulmus glabra. Microorganisms, 10(11), 2228.

Marques, J. P. R., Amorim, L., Spósito, M. B., Marin, D. R., & Appezzato-da-Glória, B. 2013. Infection of citrus pollen grains by *Colletotrichum acutatum*. European Journal of Plant Pathology, 136(1), 35–40.

Masclaux, H., Bec, A., Kagami, M., Perga,M.E., Simengando, T., Desvilettes, C., & Bourdier, G. 2011. Food quality of anemophilous plant pollen for zooplankton. Limnol.Oceanogr.56:939–946

Masclaux, H., Perga, M.E, Kagami, M., Desvilettes, C., Bourdier, G. & Bec, A. 2013. How pollen organic matter enters freshwater food webs, Limnology and Oceanography, 58 (4): 1185-1195pp.

McCune, B. & Geiser, L. 2009. Macrolichens of the Pacific Northwest, Second Ed. (2nd ed.). Corvallis: Oregon State University Press

Menkis, A., Marčiulynas, A., Gedminas, A., Lynikienė, J., & Povilaitienė, A. 2015. High-Throughput Sequencing Reveals Drastic Changes in Fungal Communities in the Phyllosphere of Norway Spruce (*Picea abies*) Following Invasion of the Spruce Bud Scale (*Physokermes piceae*). Microbial Ecology, 70(4), 904–911.

Menkis, A., Povilaitienė, A., Marčiulynas, A., Lynikienė, J., Gedminas, A., & Marčiulynienė, D. 2019. Occurrence of common phyllosphere fungi of horse-chestnut (*Aesculus hippocastanum*) is unrelated to degree of damage by leafminer (*Cameraria ohridella*). Scandinavian Journal of Forest Research, 34(1), 26–32.

Minh, B. Q., Nguyen, M. T., & Von Haeseler, A. 2013. Ultrafast Approximation for Phylogenetic Bootstrap. Molecular Biology and Evolution, 30(5), 1188–1195.

Moffett, M. W. 2000, What's “up”? A critical look at the basic terms of canopy biology. Biotropica 32: 569–596.

Nair, P. K. K., & Khan, H. A. 1963. Fungal Infection on the Pollen Grains of *Schizanthus pinnatus* Ruiz and Pav. Nature, 200(4913), 1335–1336.

Nai, C., Wong, H. Y., Pannenbecker, A., Broughton, W. J., Benoit, I., De Vries, R. P., Gueidan, C., & Gorbushina, A. A. 2013. Nutritional physiology of a rock-inhabiting, model microcolonial fungus from an ancestral lineage of the *Chaetothyriales* (*Ascomycetes*). Fungal Genetics and Biology, 56, 54–66.

Nguyen, L.T., Schmidt H.A., von Haeseler, A. & Minh, B.Q. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol Biol Evol 32:268–274

Nichols, H., Kelly, P. M., & Andrews, J. T. 1978 Holocene paleo-wind evidence from palynology in Baffin Island. Nature 273, 140–142.

Nichols, H. & Stolze, S. 2017. Modern pollen data from the Canadian Arctic 1972–1973. Sci. Data 4:170065 doi: 10.1038/sdata.2017.65

Nielsen, K., Cox, G. M., Litvintseva, A. P., Mylonakis, E., Malliaris, S. D., Benjamin, D. K., Giles, S. S., Mitchell, T. G., Casadevall, A., Perfect, J. R., & Heitman, J. 2005. *Cryptococcus neoformans* α Strains Preferentially Disseminate to the Central Nervous System during Coinfection. Infection and Immunity, 73(8), 4922–4933.

Nurk, S., Meleshko, D., Korobeynikov, A. & Pevzner, P.A. MetaSPAdes 2017. A new versatile metagenomic assembler. Genome Res. 27: 824-834

Olivier, D.L. 1978: *Retiarius* gen. nov.: phyllosphere fungi which capture wind-borne pollen grains. Trans. Br. mycol. Soc. 71: 193–201.

Olivier, D. L. 1983. Phyllosphere fungi which capture wind-borne pollen grains. II. *Hexacladium corynephorum* gen. et sp. nov. Transactions of the British Mycological Society 80, 237–245.

Olivier, D.L. & E.D.F. Williams. 1975. The penetration of *Pinus radiata* pollen by a parasitic fungus. Proc. Electr. Microsco. Soc. S. Africa. 5: 37-38.

Orange, A., James, P., White, F. C. & Orange, G. 2010. Microchemical Methods for the Identification of Lichens. British Lichen Society 102pp.

Palmer JM. 2016. Funannotate: a fungal genome annotation and comparative genomics pipeline. https://github.com/nextgenusfs/funannotate.

Parchert, K. J., Spilde, M., Porras-Alfaro, A., Nyberg, A., & Northup, D. E. 2012. Fungal Communities Associated with Rock Varnish in Black Canyon, New Mexico: Casual Inhabitants or Essential Partners? Geomicrobiology Journal, 29(8), 752–766.

Perez-Moreno, J. & Read, D. J. 2001. Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. New Phytol. 145, 30-309.

Peterson, K.M. 2014. Plants in Arctic Environments. In: Monson, R. (eds) Ecology and the Environment. The Plant Sciences, vol 8. 363–388. Springer, New York, NY.

Pitkäranta, M., Meklin, T., Hyvärinen, A., Paulin, L., Auvinen, P., Nevalainen, A., & Rintala, H. 2008. Analysis of Fungal Flora in Indoor Dust by Ribosomal DNA Sequence Analysis, Quantitative PCR, and Culture. Applied and Environmental Microbiology, 74(1), 233–244.

Powell, M. J. 1993. Looking at mycology with a Janus face. A glimpse at *Chytridiomycetes* active in the environment. Mycologia, 85, 1–20

Powell, M.J. 2016 Chytridiomycota. In: Archibald *et al.* (ed.), Handbook of the Protists. Springer International Publishing, Cham, Switzerland, pp 1–36

Ranal, M.A. 2004 Bark spore bank of ferns in a gallery forest of the ecological station of Panga, Uberlandia-MG, Brazil. Am Fern J 94: 57–69

Resl, P., Schneider, K., Westberg, M., Printzen, C., Palice, Z., Thor, G., Fryday, A. M., Mayrhofer, H., & Spribille, T. 2015. Diagnostics for a troubled backbone: testing topological hypotheses of trapelioid lichenized fungi in a large-scale phylogeny of *Ostropomycetidae* (*Lecanoromycetes*). Fungal Diversity, 73(1), 239–258.

Révay, Á. & J. Gönczöl. 2010. Rainborne hyphomycete conidia from evergreen trees. Nova Hedwigia 91: 151–163.

Révay, A. & J. Gönczöl. 2011. Canopy fungi ("terrestrial aquatic hyphomycetes") from twigs of living evergreen and deciduous trees in Hungary. – Nova Hedwigia 92: 303–31

Ridgway, R. 1912 Color standards and color nomenclature.Washington, D.C.

Robinson, C.H. 2001. Cold adaptation in Arctic and Antarctic fungi. New Phytologist, 151: 341-353

Rousseau, D.-D., Schevin, P., Ferrier, J., Jolly, D., Andreasen, T., Ascanius, S. E., Hendriksen, S.-E. & Poulsen, U. 2008. Long- distance pollen transport from North America to Greenland in spring, J. Geophys. Res., 113, G02013

Rösel, S., Rychła, A., Wurzbacher, C., & Grossart, H. 2011. Effects of pollen leaching and microbial degradation on organic carbon and nutrient availability in lake water. Aquatic Sciences, 74(1), 87–99.

Saito, H. & Takeoka, M. 1985. Pollen production rates in a young Japanese red pine forest. Japanese Journal of Ecology 35: 67–76.

Schoch, C. L., Seifert, K. A., Huhndorf, S. M., Robert, V., Spouge, J. L., Lévesque, C. A., Chen, W., Bolchacova, E., Voigt, K., Crous, P. W., Miller, A. N., Wingfield, M. J., Aime, M. C., An, K., Bai, F., Barreto, R. W., Begerow, D., Bergeron, M., Blackwell, *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America, 109(16), 6241–6246.

Schroth, G., Elias, M.E.A, Ugen, K., Seixas, R. & Zech, W. 2001. Nutrient fluxes in rainfall, throughfall and stemflow in tree-based land use systems and spontaneous tree vegetation of central Amazonia. Agriculture, Ecosystems and Environment 87, 37-49.

Seifert, K., Morgan-Jones, G., Gams, W. & Kendrick, B. 2011. The Genera of Hyphomycetes. CBS Biodiversity Series no. 9: 1–997. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherland

Sekiguchi, H., Nogawa, S., Saito, H. & Takeoka, M. 1986. Pollen production rates in middle-aged Japanese red pine stands. Journal of Japanese Forest Society 68: 143–149

Seyedmousavi, S., De Moraes Gimenes Bosco, S., De Hoog, S., Ebel, F., Elad, D., Gomes, R. R., Jacobsen, I. D., Jensen, H. E., Martel, A., Mignon, B., Pasmans, F., Piecková, E., Rodrigues, A. M., Singh, K., Vicente, V. A., Wibbelt, G., Wiederhold, N. P., & Guillot, J. 2018. Fungal infections in animals: a patchwork of different situations. Medical Mycology, 56(suppl_1), S165–S187.

Shenoy, B.D., Jeewon, R. & Hyde, K.D. 2007. Impact of DNA sequence-data on the taxonomy of anamorphic fungi. Fungal Diversity 26: 1-54.

Shumilovskikh, L. S., Schlütz, F., Achterberg, I., Kvitkina, A., Bauerochse, A., & Leuschner, H. H. 2015. Pollen as nutrient source in Holocene ombrotrophic bogs. Rev. Palaeobot. Palynol. 221, 171–178.

Skvarla, J. J. & Anderegg, D. E. 1972. Infestation of cedar pollen by *Rhizophidium* (*Chytridiomycetes*) Grana 12: 47- 51.

Song, X., Bera, S., Yao, Y., Blackmore, S., & Li, C. 2013. Natural traps of spores and pollen grains from the region surrounding Wenbi Reservoir, Yunnan, China. Kexue Tongbao, 58(S1), 162–168.

Song, X., Bera, S., Yao, Y., Ferguson, D. J. P., & Li, C. 2014. Tree barks as a natural trap for airborne spores and pollen grains from China. Chinese Science Bulletin, 59(19), 2331–2339.

Sparrow, F. K. 1960. The aquatic Phycomycetes (2nd ed. Rev.). Ann Arbor: University of Michigan Press

Sridhar, K.R. 2009: Fungi in the Tree Canopy: an Appraisal. – In: Rai, M. & Bridge, P.D (eds.): Applied Mycology – CAB International, UK, pp. 73–91

Sridhar, K.R., Karamchand, K. S. & Bhat, R. 2006. Arboreal water-borne hyphomycetes on oak-leaf basket fern *Drynaria quercifolia*. Sydowia 58: 309–320.

Sridhar, K. R., & Karamchand, K. S. 2009. Diversity of water-borne fungi in stemflow and throughfall of tree canopies in India. Sydowia 61, 327–344

Stark, N. 1972. Nutrient cycling pathways and litter fungi. – Bioscience 22: 355 – 360.

Stone, J.F., Sherwood, M.A., & Carrol, G.C. 1996. Canopy microfungi: function and diversity. Northwest Science 70, 37-45.

Środoń, A. 1960. Pollen spectra from Spitsbergen. Folia Quaternaria. 3, 1–17.

Stark, N. 1972 Nutrient cycling pathways and litter fungi. BioScience 22, 355-360.

Sutton, B.C. 1980. The Coelomycetes. Fungi Imperfecti with Pycnidia, Acervuli and Stromata. CMI, Kew, 696 p.

Sullivan, A. R., Karlsson, E., Svensson, D., Brindefalk, B., Villegas, J. A., Mikko, A., Bellieny, D., Siddique, A. B., Johansson, A.-M., Grahn, H., Sundell, D., Norman, A., Esseen, P.-A., Sjödin, A., Singh, N. J., Brodin, T., Forsman, M. & Stenberg, P. 2023. Airborne eDNA captures three decades of ecosystem biodiversity. bioRxiv. doi: https://doi.org/10.1101/2023.12.06.569882

Tanzer, M.M., Arst, H.N., Skalchunes, A.R., Coffin, M., Darveaux, B.A., Heiniger, R.W., Shuster, J.R., 2003. Global nutritional profiling for mutant and chemical mode- of-action analysis in filamentous fungi. Funct. Integr. Genom. 3, 160–170.

Tzean, S. S., & Barron, G. L. 1983. A new predatory hyphomycete capturing bdelloid rotifers in soil. Canadian Journal of Botany, 61(5), 1345–1348.

Uritskiy, G.V., DiRuggiero, J. & Taylor, J. 2018. MetaWRAP—a flexible pipeline for genome-resolved metagenomic data analysis. Microbiome 6, 158.

Van Den Wyngaert, S., Ganzert, L., Seto, K., Rojas-Jimenez, K., Agha, R., Berger, S. A., Woodhouse, J. N., Padisák, J., Wurzbacher, C., Kagami, M., & Grossart, H. 2022. Seasonality of parasitic and saprotrophic zoosporic fungi: linking sequence data to ecological traits. The ISME Journal, 16(9), 2242–2254.

Vega, F.E., Meyling, N.V., Luangsa-Ard, J.J. & Blackwell, M. 2012. Fungal entomopathogens In: Vega, F., Kaya, H.K., editors. Insect Pathology, 2nd ed San Diego: Academic Press; pp. 171–220.

Victorino, Í. M. M., Lumini, E., Caser, M., Orgiazzi, A., Demasi, S., Berruti, A., Scariot, V., & Bianciotto, V. 2021. Metabarcoding of Soil Fungal Communities Associated with Alpine Field-Grown Saffron (*Crocus sativus* L.) Inoculated with AM Fungi. Journal of Fungi, 7(1), 45.

Vilgalys, R. & Hester, M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus specie*s. Journal of Bacteriology, 172, 4239-4246.

Vilgalys, R. 1992. Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA https://sites.duke.edu/vilgalyslab/files/2017/08/rDNA-primers-for-fungi.pdf

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, Inc., New York.

Wijayawardene, N. N., Hyde, K. D., Rajeshkumar, K. C., Hawksworth, D. L., Madrid, H., Kirk, P. M., Crous, P. W., Singh, R., Crous, P. W., Kukwa, M., Lücking, R., Kurtzman, C. P., Yurkov, A., Haelewaters, D., Aptroot, A., Lumbsch, H. T., Timdal, E., Ertz, D., Etayo, J., et. al. 2017. Notes for genera: *Ascomycota*. Fungal Diversity, 86(1), 1–594.

Wijayawardene, N.N.; Phillips, A.J.L.; Tibpromma, S.; Dai, D.Q.; Selbmann, L.; Monteiro, J.S.; Aptroot, A.; Flakus, A.; Rajeshkumar, K.C.; Coleine, C.; *et al.* 2021. Looking for the undiscovered asexual taxa: Case studies from lesser studied life modes and habitats. Mycosphere.12, 1186–1229. Looking for the undiscovered asexual taxa: Case studies from lesser studied life modes and habitats. Mycosphere. 12, 1186–1229.

Wijayawardene, N. N., Phillips, A. J. L., Pereira, D., Dai, D., Aptroot, A., Monteiro, J. S., Druzhinina, I. S., Cai, F., Tian, C., Selbmann, L., Coleine, C., Castañeda-Ruiz, R. F., Kukwa, M., Flakus, A., Fiuza, P. O., Kirk, P. M., Rajeshkumar, K. C., Arachchi, I. S. L., Suwannarach, N., *et al.* 2022. Forecasting the number of species of asexually reproducing fungi (*Ascomycota* and *Basidiomycota*). Fungal Diversity, 114(1), 463–490.

Wurzbacher, C., Rösel, S., Rychła, A., & Grossart, H.P. 2014. Importance of Saprotrophic Freshwater Fungi for Pollen Degradation. PLOS ONE 9(4): e94643.

Yamakawa, T. 1984 The Effect of pollen on the infection of fruit vegetables with conidia of *Botrytis cinerea*. Proc. Kansai Pl. Prot. Soc. 26: 1-8.

Yang, E., Xu, L., Yang, Y., Zhang, X., Xiang, M., Wang, C., An, Z., & Liu, X. 2012. Origin and evolution of carnivorism in the *Ascomycota* (fungi). Proceedings of the National Academy of Sciences of the United States of America, 109(27), 10960–10965.

Zhang, Y., He, X., Baral, H., Qiao, M., Weiβ, M., Liu, B., Zhang, K., & Yu, Z. 2015. *Orbilia laevimarginata* sp. nov. and its asexual morph. Phytotaxa 203 (3) 25

Zhang, B., Li, X., Li, G. Wang, Q.M. & Wang, M. 2023. *Cadophora* species from marine glaciers in the Qinghai-Tibet Plateau: an example of unsuspected hidden biodiversity. IMA Fungus 13, 15

Zopf, W. 1888. Zur Kenntniss der Infections-Krankheiten niederer Tiere und Pflanzen. – Nova Acta Leop. Carol. Deut. Acad. Naturf. 52: 314–376

Appendix

Tables and Figures

Table 1. Compilation of *Retiarius* records from literature, including source of isolation, plant species where isolated from, and country reported from.

Species	Country	Detection	Isolation source	Substrate ID	Reference	Notes
Retiarius bovicornutus	Germany	Conidia	Twig surface	*Salix caprea*	Baral *et al.* 2020	-
Retiarius bovicornutus	Hungary	Conidia	Stemflow	*Chamaecyparis lawsoniana*	Gönczöl & Révay 2004	-
Retiarius bovicornutus	Hungary	Conidia	Stemflow	*Platanus hybrida*	Gönczöl & Révay 2004	-
Retiarius bovicornutus	Germany	Conidia	Stemflow	*Fagus sylvatica*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Germany	Conidia	Stemflow	*Prunus avium*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Germany	Conidia	Stemflow	*Carpinus betulus*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Hungary	Conidia	Stemflow	*Alnus glutinosa*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Hungary	Conidia	Stemflow	*Quercus cerris*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Hungary	Conidia	Throughfall	*Taxus baccata*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Romania	Conidia	Stemflow	*Picea abies*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Sweden	Conidia	Stemflow	*Picea abies*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	Sweden	Conidia	Stemflow	*Pinus sylvestris*	Gönczöl & Révay 2006	-
Retiarius bovicornutus	India	Conidia	Throughfall	*Avicennia officinalis*	Karamchand *et al.* 2021	-
Retiarius bovicornutus	Norway/Hungary	Conidia	Dendrotelma	*Acer platanoides*	Magyar *et al.* 2017c	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Chamaecyparis lawsoniana*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Bark	*Elaeagnus angustifolia*	Magyar 2008	
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Juniperus communis*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Taxus baccata*	Révay & Gönczöl 2011	-

Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Pinus sylvestris*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Pinus nigra*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Alnus glutinosa*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Carpinus betulus*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Fagus sylvatica*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Alnus glutinosa*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Carpinus betulus*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Hungary	Conidia	Twig upper surface	*Fagus sylvatica*	Révay & Gönczöl 2011	-
Retiarius bovicornutus	Venezuela	Conidia	Stream foam	-	Fernández-Da Silva & Smith-Briedis 2021	-
Retiarius bovicornutus	Italy	Conidia	Honeydew honey	*Abies alba*	Magyar *et al.* 2005	-
Retiarius bovicornutus	Italy	Conidia	Honeydew honey	*Picea excelsa*	Magyar *et al.* 2005	-
Retiarius bovicornutus	South Africa	Conidia	Phyllosphere	*Brabejum stellatifolium*	Olivier 1978	Type
Retiarius bovicornutus	Hungary	Conidia	Throughfall	*Chamaecyparis lawsoniana*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Hungary	Conidia	Throughfall	*Picea abies*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Hungary	Conidia	Throughfall	*Picea pungens*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Hungary	Conidia	Throughfall	*Thuja occidentalis*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Hungary	Conidia	Throughfall	*Cupressus arizonica*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Sweden	Conidia	Throughfall	*Picea abies*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Sweden	Conidia	Throughfall	*Pinus sylvestris*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	Sweden	Conidia	Throughfall	*Vaccinium myrtillus*	Révay & Gönczöl 2010	-
Retiarius bovicornutus	India	Conidia	Phyllosphere	*Drynaria quercifolia*	Sridhar *et al.* 2006	-
Retiarius bovicornutus	India	Conidia	Stemflow	*Ficus benghalensis*	Sridhar & Karamchand 2009	

Retiarius bovicornutus	India	Conidia	Stemflow	*Alstonia scholaris*	Sridhar & Karamchand 2009	
Retiarius aff. *bovicornutus*	Japan	Conidia	Throughfall	*Pinus densiflora*	Ando & Tabuki 1984	sensu Baral *et al.* 2020
Retiarius aff. *bovicornutus*	Peru	Conidia	Phyllosphere	Palm, decaying petioles	Matsushima 1993	sensu Baral *et al.* 2020
Retiarius aff. *bovicornutus*	Luxembourg	Conidia	Bark	*Robinia pseudoacacia*	Baral *et al.* 2020	-
Retiarius cf. *bovicornutus*	France	Conidia	Twig surface	*Pinus sylvestris*	Baral *et al.* 2020	-
Retiarius cf. *bovicornutus*	France	Conidia	Twig surface	*Pinus sylvestris*	Baral *et al.* 2020	-
Retiarius cf. *bovicornutus*	Great Britain	Conidia	Phyllosphere	*Rhodondendron* sp.	Baral *et al.* 2020	-
Retiarius cf. *bovicornutus*	Luxembourg	Conidia	Twig surface	*Clematis vitalba*	Baral *et al.* 2020	-
Retiarius cf. *bovicornutus*	Luxembourg	Conidia	Twig surface	*Salix caprea*	Baral *et al.* 2020	-
Retiarius aff. *revayae*	Luxembourg	Conidia	Twig surface	*Sambucus nigra*	Baral *et al.* 2020	-
Retiarius cf. *revayae*	South Africa	Conidia	Twig surface	*Pterocarpus* sp. (?)	Baral *et al.* 2020	-
Retiarius cf. *revayae*	South Africa	Conidia	Twig surface	*Vachelia* sp. (?)	Baral *et al.* 2020	-
Retiarius goenczoelii	Hungary	Conidia	Bark	*Platanus* x *acerifolia*	Magyar *et al.* 2017a	Type
Retiarius goenczoelii	Hungary	Conidia	Twig upper surface	No Data	Révay & Gönczöl 2011	sensu Magyar in Baral *et al.* 2020
Retiarius revayae	Hungary	Conidia	Stemflow	No Data	Gönczöl & Révay 2004	sensu Magyar in Gönczöl & Révay 2004
Retiarius revayae	Hungary	Conidia	Bark	*Taxus baccata*	Magyar *et al.* 2017a	Type
Retiarius revayae	ND	Conidia	Throughfall	No Data	Révay & Gönczöl 2010	sensu Magyar *et al.* 2017a
Retiarius revayae	Hungary	Conidia	Twig upper surface	No Data	Révay & Gönczöl 2011	sensu Magyar in Gönczöl & Révay 2011
Retiarius superficiaris	South Africa	Conidia	Phyllosphere	*Eriobotrya japonica*	Olivier 1978	Type
Retiarius sp.	Spain	Conidia	Stream foam	River	Descals *et al.* 1995	*R.* cf. *bovicornutus* ?
Retiarius sp.	India	Conidia	Throughfall	*Caryota urens*	Ghate & Sridhar 2015	-
Retiarius sp.	Romania	Conidia	Stemflow	*Picea abies*	Gönczöl & Révay 2006	-
Retiarius sp.	China	eDNA	Phyllosphere	*Phyllachora heterocladae*	Liu *et al.* 2022	-
Retiarius sp.	Canada	Conidia	Phyllosphere	*Picea mariana*	Sokolski *et al.* 2006	-
Retiarius sp. (?)	Argentina	Conidia	Freshwater stream	-	Kravetz 2020	Likely not *Retiarius*

Table 2. Table of samples of lichens and substrates screened for *Retiarius* using genus-specific primers and their Voucher ID, collector, and DNA extraction code. Specimens and substrates that produced usable sequence are in bold.

DNA Extraction Code	Specimen Voucher	Screened Substrate ID	Success	Growth Form	Substrate	Locality	Latitude	Longitude	Habitat
AH-Ret1	Alejandro Huereca *s.n.*	Pollen on *Umbilicaria hyperborea*	Usable	Umbilicate	Saxicolous	Pincher Creek, AB, CA.	49.427747	-114.38	Bedrock Meadow
AH-Ret2	Alejandro Huereca *s.n.*	Pollen on *Umbilicaria hyperborea*	Usable	*Umbilicate*	Saxicolous	Pincher Creek, AB, CA.	49.427747	-114.38	Bedrock Meadow
AH-Ret3	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea* with pollen	Usable	*Umbilicate*	Saxicolous	Pincher Creek, AB, CA.	49.427747	-114.38	Bedrock Meadow
AH-Ret4	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea*	Sequence - No usable	*Umbilicate*	Saxicolous	Pincher Creek, AB, CA.	49.427747	-114.38	Bedrock Meadow
AH-Ret5	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea*	Sequence - No usable	*Umbilicate*	Saxicolous	Pincher Creek, AB, CA.	49.427747	-114.38	Bedrock Meadow
AH-Ret6	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea* with pollen	Usable	*Umbilicate*	Saxicolous	Pincher Creek, AB, CA.	49.427747	-114.38	Bedrock Meadow
AH-Ret7	Alejandro Huereca *s.n.*	*Parmelia sulcata*	Sequence - No usable	*Foliose*	Corticolous	Pincher Creek, AB, CA.	49.425847	-114.3819	Montane coniferous forest
AH-Ret8	Alejandro Huereca *s.n.*	*Hypogymnia tubulosa*	Sequence - No usable	*Foliose*	Corticolous	Pincher Creek, AB, CA.	49.425847	-114.3819	Montane coniferous forest
AH-Ret9	Alejandro Huereca *s.n.*	*Hypogymnia physodes*	Usable	*Foliose*	Corticolous	Pincher Creek, AB, CA.	49.425847	-114.3819	Montane coniferous forest
AH-Ret10	Alejandro Huereca *s.n.*	*Hypogymnia physodes*	Sequence - No usable	*Foliose*	Corticolous	Pincher Creek, AB, CA.	49.425847	-114.3819	Montane coniferous forest
AH-Ret11	Alejandro Huereca *s.n.*	*Hypogymnia imshaugii*	Sequence - No usable	*Foliose*	Corticolous	Pincher Creek, AB, CA.	49.425847	-114.3819	Montane coniferous forest
AH-Ret12	Alejandro Huereca *s.n.*	*Umbilicaria phaea*	Usable	*Umbilicate*	Saxicolous	Okanagan-Similkameen, BC, CA.	49.16635	-120.5671	Douglas Fir forest

AH-Ret13	Alejandro Huereca *s.n.*	*Umbilicaria phaea*	Usable	*Umbilicate*	Saxicolous	Okanagan-Similkameen, BC, CA.	49.16635	-120.5671	Douglas Fir forest
AH-Ret14	Alejandro Huereca *s.n.*	*Umbilicaria polyphylla*	Sequence - No usable	*Umbilicate*	Saxicolous	Nakusp, BC, CA.	50.278125	-117.7608	Interior Cedar Hemlock Forest
AH-Ret15	Alejandro Huereca *s.n.*	*Umbilicaria polyphylla*	Sequence - No usable	*Umbilicate*	Saxicolous	Nakusp, BC, CA.	50.278125	-117.7608	Interior Cedar Hemlock Forest
AH-Ret16	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea*	Sequence - No usable	*Umbilicate*	Saxicolous	Nakusp, BC, CA.	50.278125	-117.7608	Interior Cedar Hemlock Forest
AH-Ret17	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea*	Usable	*Umbilicate*	Saxicolous	Nakusp, BC, CA.	50.278125	-117.7608	Interior Cedar Hemlock Forest
AH-Ret18	Alejandro Huereca *s.n.*	*Umbilicaria deusta*	Sequence - No usable	*Umbilicate*	Saxicolous	Okanagan-Similkameen, BC, CA.	49.694253	-120.2826	Douglas Fir forest
AH-Ret19	Alejandro Huereca *s.n.*	*Umbilicaria deusta*	Usable	*Umbilicate*	Saxicolous	Okanagan-Similkameen, BC, CA.	49.694253	-120.2826	Douglas Fir forest
AH-Ret20	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea*	Sequence - No usable	*Umbilicate*	Saxicolous	Okanagan-Similkameen, BC, CA.	49.694253	-120.2826	Douglas Fir forest
AH-Ret21	Alejandro Huereca *s.n.*	*Umbilicaria hyperborea*	Usable	*Umbilicate*	Saxicolous	Okanagan-Similkameen, BC, CA.	49.694253	-120.2826	Douglas Fir forest
AH-Ret22	Alejandro Huereca *s.n.*	*Abies balsamea* leaves	Sequence - No usable		Phyllosphere	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret23	Alejandro Huereca *s.n.*	Bark fragment of *Abies balsamea*	Sequence - No usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret24	Alejandro Huereca *s.n.*	*Parmelia* sulcata	Usable	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
Ah-Ret25	Alejandro Huereca *s.n.*	Bark fragment of AH-Ret24	Sequence - No usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest

AH-Ret26	Alejandro Huereca *s.n.*	*Alectoria sarmentosa*	Usable	*Fruticose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret27	Alejandro Huereca *s.n.*	Bark fragment of *Abies balsamea*	Sequence - No usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret28	Alejandro Huereca *s.n.*	*Tuckermanopsis orbata*	Usable	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret29	Alejandro Huereca *s.n.*	Bark fragment of AH-Ret28	No Band		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret30	Alejandro Huereca *s.n.*	*Imshaugia aleurites*	No Band	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret31	Alejandro Huereca *s.n.*	Bark fragment of AH-Ret30	Sequence - No usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret32	Alejandro Huereca *s.n.*	*Vulpicida prinastri*	Sequence - No usable	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret33	Alejandro Huereca *s.n.*	Twig fragment of AH-Ret32	Sequence - No usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret34	Alejandro Huereca *s.n.*	*Hypogymnia bitterii*	Usable	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret35	Alejandro Huereca *s.n.*	Bark fragment of AH-Ret34	No Band		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret36	Alejandro Huereca *s.n.*	*Melanohaela exasperatula*	Usable	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret37	Alejandro Huereca *s.n.*	Bark fragment (*Abies balsamea*) of R36	Usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret38	Alejandro Huereca *s.n.*	*Platismatia glauca*	Sequence - No usable	*Foliose*	Corticolous	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret39	Alejandro Huereca *s.n.*	Bark fragment of AH-Ret38	Sequence - No usable		Bark	Swan Hills, AB, CA.	54.796758	-115.8556	Boreal Forest
AH-Ret40	Toby Spribille *s.n.*	*Montanelia tominii*	Sequence - No usable	*Foliose*	Saxicolous	Libby, MT, US.	48.549217	-115.247987	Interior Douglas Fir Forest

AH-Ret41	Toby Spribille *s.n.*	*Dermatocarpon moulinsii*	Usable	*Umbilicate*	Saxicolous	Libby, MT, US.	48.549217	-115.247987	Interior Douglas Fir Forest
AH-Ret42	Toby Spribille *s.n.*	*Umbilicaria hyperborea*	Usable	*Umbilicate*	Saxicolous	Libby, MT, US.	48.549217	-115.247987	Interior Douglas Fir Forest
AH-Ret43	Toby Spribille *s.n.*	*Dermatocarpon moulinsii*	Sequence - No usable	*Umbilicate*	Saxicolous	Libby, MT, US.	48.549217	-115.247987	Interior Douglas Fir Forest
AH-Ret44	Toby Spribille *s.n.*	*Xanthoparmelia cumberlandia*	Usable	*Foliose*	Saxicolous	Libby, MT, US.	48.549217	-115.247987	Interior Douglas Fir Forest
AH-Ret45	Toby Spribille *s.n.*	*Parmelia saxatilis*	Usable	*Foliose*	Saxicolous	Libby, MT, US.	48.549217	-115.247987	Interior Douglas Fir Forest
AH-Ret49	Alejandro Huereca *s.n.*	*Stereocaulon grande*	Usable	*Fruticose*	Saxicolous	Clearwater, BC, CA.	51.868955	-120.0272	terior temperate rainforest
AH-Ret50	Alejandro Huereca *s.n.*	*Nephroma helveticum*	Sequence - No usable	*Foliose*	Corticolous	Clearwater, BC, CA.	51.868955	-120.0272	Interior temperate rainforest
AH-Ret51	Alejandro Huereca *s.n.*	Bark of *Picea glauca*	Usable		Bark	Clearwater, BC, CA.	51.868955	-120.0272	Interior temperate rainforest
AH-Ret52	Alejandro Huereca *s.n.*	*Platismatia herrei*	No Band	*Foliose*	Corticolous	Alert Bay, BC, CA.	50.446337	-126.9752	Coastal temperate rainforest
AH-Ret53	Alejandro Huereca *s.n.*	*Vulpicida prinastri*	Sequence - No usable	*Foliose*	Corticolous	Clearwater, BC, CA.	51.868955	-120.0272	Interior temperate rainforest
AH-Ret54	Alejandro Huereca *s.n.*	Bark of AH-Ret53	Usable		Bark	Clearwater, BC, CA.	51.868955	-120.0272	Interior temperate rainforest
AH-Ret55	Alejandro Huereca *s.n.*	*Xanthoparmelia* sp.	Sequence - No usable	*Foliose*	Saxicolous	Clearwater, BC, CA.	51.868955	-120.0272	Interior temperate rainforest
AH-Ret56	Alejandro Huereca *s.n.*	*Hypogymnia enteromorpha*	Sequence - No usable	*Foliose*	Corticolous	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest
AH-Ret57	Alejandro Huereca *s.n.*	Bark of AH-Ret56	No Band		Bark	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest
AH-Ret58	Alejandro Huereca *s.n.*	*Parmotrema crinatum*	Sequence - Non *Retiarius*	*Foliose*	Corticolous	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest

AH-Ret59	Alejandro Huereca *s.n.*	*Platismatia lacunosa*	No Band	*Foliose*	Corticolous	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest
AH-Ret60	Alejandro Huereca *s.n.*	Bark of *Alnus rubra*	Usable		Bark	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest
AH-Ret61	Alejandro Huereca *s.n.*	*Parmelia squarrosa*	Usable	*Foliose*	Saxicolous	Clearwater, BC, CA.	51.868955	-120.0272	Interior temperate rainforest
AH-Ret62	Alejandro Huereca *s.n.*	Bark of *Picea sitchensis*	No Band		Bark	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest
AH-Ret63	Alejandro Huereca *s.n.*	*Hypogymnia lophyrea*	No Band	*Foliose*	Corticolous	Mount Waddington, BC, CA.	50.387594	-127.968	Coastal temperate rainforest
AH-Ret64	Erin Cox 333	*Umbilicaria* sp.	Sequence - Non *Retiarius*	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.			Arctic Tundra
AH-Ret65	Erin Cox 378	*Umbilicaria decussata*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.			Arctic Tundra
AH-Ret66	Erin Cox 378	*Umbilicaria decussata*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.			Arctic Tundra
AH-Ret68	Joe DiMeglio *s.n.*	*Parmelia sulcata*	Sequence - Non *Retiarius*	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret69	Joe DiMeglio *s.n.*	*Sticta gretae*	No Band	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret70	Alejandro Huereca 772	*Lobariella* cf. *exonarta*	No Band	*Foliose*	Corticolous	Gomez Farías, TAM, MX.	23.048796	-99.24247	Montane humid mixed forest
AH-Ret71	Joe DiMeglio *s.n.*	*Nephroma helveticum*	No Band	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret72	Alejandro Huereca 660	*Sticta* sp.	Sequence - No usable	*Foliose*	Corticolous	Aramberri, NL, MX.	24.03668	-99.70818	Montane humid mixed forest
AH-Ret73	Alejandro Huereca *s.n.*	Bark of *Picea martinezii*	No Band		Bark	Aramberri, NL, MX.	24.03668	-99.70818	Montane humid mixed forest

AH-Ret74	Alejandro Huereca 772	*Sticta* sp.	No Band	*Foliose*	Corticolous	Ciudad Victoria, TAM, MX.	23.536803	-99.20016	Humid Oak forest
AH-Ret75	Alejandro Huereca *s.n.*	Bark of *Quercus* sp.	No Band		Bark	Ciudad Victoria, TAM, MX.	23.536803	-99.20016	Humid Oak forest
AH-Ret76	Joe DiMeglio *s.n.*	*Ramalina dilacerata*	No Band	*Fruticose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret77	Joe DiMeglio *s.n.*	Bark of *Crataegus* sp.	Sequence - Non *Retiarius*		Bark	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret78	Joe DiMeglio *s.n.*	*Hypogymnia tubulosa*	Sequence - No usable	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret79	Alejandro Huereca *s.n.*	Bark of *Malus* sp.	Sequence - No usable		Bark	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret80	Alejandro Huereca 767	*Emmanuelia* cf. *tenuis*	No Band	*Foliose*	Corticolous	Gomez Farías, TAM, MX.	23.048796	-99.24247	Montane humid mixed forest
AH-Ret81	Alejandro Huereca 673	*Emmanuelia* cf. *tenuis*	Sequence - No usable	*Foliose*	Corticolous	Aramberri, NL, MX.	24.03668	-99.70818	Montane humid mixed forest
AH-Ret82	Alejandro Huereca *s.n.*	Bark of *Picea martinezii*	Usable		Bark	Aramberri, NL, MX.	24.03668	-99.70818	Montane humid mixed forest
AH-Ret83	Alejandro Huereca 744	*Emmanuelia* cf. *tenuis*	No Band	*Foliose*	Corticolous	Ciudad Victoria, TAM, MX.	23.536803	-99.20016	Humid Oak forest
AH-R84	Alejandro Huereca *s.n.*	Bark of AH-Ret83	No Band		Bark	Ciudad Victoria, TAM, MX.	23.536803	-99.20016	Humid Oak forest
AH-Ret85	Alejandro Huereca 693	*Sticta* sp.	Sequence - No usable	*Foliose*	Corticolous	General Zaragoza, NL, MX.	23.88409	-99.79829	Montane humid mixed forest
AH-Ret86	Alejandro Huereca 743	*Emmanuelia* aff. *ravenelii*	No Band	*Foliose*	Corticolous	Ciudad Victoria, TAM, MX.	23.536803	-99.20016	Humid Oak forest

AH-Ret87	Alejandro Huereca *s.n.*	Bark fragment (Quercus) of AH-Ret86	No Band		Bark	Ciudad Victoria, TAM, MX.	23.536803	-99.20016	Humid Oak forest
AH-Ret88	Alejandro Huereca 695	*Sticta* sp.	Sequence - No usable	*Foliose*	Corticolous	Santiago, NL, MX.	25.3656	-100.1971	Montane humid mixed forest
AH-Ret89	Alejandro Huereca 688	*Sticta* sp.	Sequence - No usable	*Foliose*	Corticolous	Santiago, NL, MX.	25.3656	-100.1971	Montane humid mixed forest
AH-Ret90	Alejandro Huereca *s.n.*	Bark fragment (*Quercus*) of AH-Ret89	No Band		Bark	Santiago, NL, MX.	25.3656	-100.1971	Montane humid mixed forest
AH-Ret91	Alejandro Huereca 756	*Sticta* sp.	Sequence - No usable	*Foliose*	Corticolous	Gomez Farías, TAM, MX.	23.048796	-99.24247	Humid Oak forest
AH-Ret92	Joe DiMeglio *s.n.*	*Peltigera collina*	No Band	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret93	Joe DiMeglio *s.n.*	*Parmotrema perlatum*	Usable	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret94	Joe DiMeglio *s.n.*	Bark of AH-Ret93	Sequence - No usable		Bark	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret95	Joe DiMeglio *s.n.*	*Hypogymnia physodes*	No Band	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret96	Joe DiMeglio *s.n.*	*Polycaulonia aff. polycarpa*	Sequence - No *Retiarius*	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret97	Joe DiMeglio *s.n.*	Bark of AH-Ret96	No Band		Bark	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret98	Joe DiMeglio *s.n.*	*Physcia adscendens*	Sequence - No usable	*Foliose*	Corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret99	Joe DiMeglio *s.n.*	Bark of AH-Ret98	No Band		Bark	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret100	Joe DiMeglio *s.n.*	*Sphaerophorus venerabilis*	Sequence - No usable	*Fruticose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest

AH-Ret101	Joe DiMeglio *s.n.*	*Menegazzia subsimilis*	Usable	*Foliose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret102	Joe DiMeglio *s.n.*	Bark of AH-Ret101	No Band		Bark	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret103	Joe DiMeglio *s.n.*	*Nephroma helveticum*	Sequence - No usable	*Foliose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret104	Joe DiMeglio *s.n.*	*Pseudocyphellaria citrina*	Sequence - No usable	*Foliose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret105	Joe DiMeglio *s.n.*	*Hypotrachyna sinuosa*	Sequence - No usable	*Foliose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret106	Joe DiMeglio *s.n.*	*Platismatia norvegica*	Usable	*Foliose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret107	Alejandra Peña *s.n.*	*Lasallia papulosa*	Sequence - No usable	*Umbilicate*	Saxicolous	Valle de los Monjes, CHI, MX.	27.73251	-107.5595	Montane coniferous forest
AH-Ret108	Alejandra Peña *s.n.*	Substrate of AH-Ret109	Sequence - No usable		Debris	Valle de los Monjes, CHI, MX.	27.73251	-107.5595	Montane coniferous forest
AH-Ret109	Alejandra Peña *s.n.*	*Cora* sp.	No Band	*Foliose*	Saxicolous	Valle de los Monjes, CHI, MX.	27.73251	-107.5595	Montane coniferous forest
AH-Ret110	Alejandra Peña *s.n.*	*Cora* sp.	Band - No Sequence	*Foliose*	Saxicolous	- N/D	N/D	N/D	Montane coniferous forest
AH-Ret111	Alejandra Peña *s.n.*	Cora sp.	Band - No Sequence	*Foliose*	Saxicolous	N/D	N/D	N/D	Montane coniferous forest
AH-Ret112	Joe DiMeglio *s.n.*	*Cetrelia cetraroides*	Sequence - Non *Retiarius*	*Foliose*	corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret113	Joe DiMeglio *s.n.*	*Lobaria pulmonaria*	Sequence - Non *Retiarius*	*Foliose*	corticolous	Adair Village, OR, US.	44.701229	-123.2017	Douglas Fir forest
AH-Ret114	Joe DiMeglio *s.n.*	*Lobaria oregana*	No Band	*Foliose*	Corticolous	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest
AH-Ret115	Joe DiMeglio *s.n.*	Bark of AH-Ret114	No Band		Bark	Cascadia, OR, US.	44.399769	-122.4766	Douglas Fir forest

AH-Ret126	Erin Cox 1481	*Flavocetraria nivalis*	No Band	*Fruticose*	Terricolous	Kitikmeot Region, NU, CA.	69.132821	-105.0553	Arctic Tundra
AH-Ret127	Erin Cox *s.n.*	Substrate of AH-Ret128	No Band		Debris	Kitikmeot Region, NU, CA.	69.132821	-105.0553	Arctic Tundra
AH-Ret128	Erin Cox 1482	*Vulpicida tilesii*	No Band	*Foliose*	Terricolous	Kitikmeot Region, NU, CA.	69.132821	-105.0553	Arctic Tundra
AH-Ret129	Erin Cox 1483	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.132821	-105.0553	Arctic Tundra
AH-Ret130	Erin Cox 1484	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.132821	-105.0553	Arctic Tundra
AH-Ret131	Erin Cox 1274	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.128688	-105.0058	Arctic Tundra
AH-Ret132	Erin Cox 1276	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.128688	-105.0058	Arctic Tundra
AH-Ret133	Erin Cox 1277	*Collema* sp.	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	69.128688	-105.0058	Arctic Tundra
AH-Ret134	Erin Cox 1275	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.128688	-105.0058	Arctic Tundra
AH-Ret135	Erin Cox 1375	Substrate of *Cladina* sp	No Band		Debris	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret136	Erin Cox 1365.1	*Cetraria* sp.	No Band	*Fruticose*	Terricolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret137	Erin Cox 1366	*Cladonia* sp.	No Band	*Fruticose*	Terricolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret138	Erin Cox 1365	*Cetraria aculeata*	No Band	*Fruticose*	Terricolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret139	Erin Cox 1361	Substrate of *Cetraria*	No Band		Debris	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret140	Erin Cox 1363	*Flavocetria cucullata*	No Band	*Fruticose*	Terricolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret141	Erin Cox 1364	*Vulpicida tilesii*	No Band	*Foliose*	Terricolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret142	Erin Cox 1278	*Collema* sp.	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	69.128688	-105.0058	Arctic Tundra
AH-Ret143	Erin Cox 1370	*Collemataceae*	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret144	Erin Cox 1374	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.128687	-105.0046	Arctic Tundra
AH-Ret145	Erin Cox 1486	*Phaeophyscia* sp.	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	69.125627	-105.0022	Arctic Tundra
AH-Ret146	Erin Cox *s.n.*	*Umbilicaria* sp.	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	69.128688	-105.0058	Arctic Tundra
AH-Ret147	Troy McMullin 17675	*Parmelia saxatili*s	No Band	*Foliose*	Terricolous	Kivalliq Region, NU, CA.	61.111817	-94.0755	Arctic Tundra

AH-Ret148	Troy McMullin 17616	*Umbilicaria lyngei*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.105444	-94.16892	Arctic Tundra
AH-Ret149	Troy McMullin 17671	*Parmelia omphaloides*	No Band	*Foliose*	Terricolous	Kivalliq Region, NU, CA.	61.100556	-94.00256	Arctic Tundra
AH-Ret150	Troy McMullin 16175	*Umbilicaria muehlenbergii*	No Band	*Umbilicate*	Saxicolous	Avalon Peninsula, NL, CA.	47.23687	-53.23954	Arctic Tundra
AH-Ret151	Troy McMullin 16183	*Umbilicaria muehlenbergii*	No Band	*Umbilicate*	Saxicolous	Avalon Peninsula, NL, CA.	47.23789	-53.23946	Arctic Tundra
AH-Ret152	Troy McMullin 17612	*Umbilicaria polyphylla*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.103639	-94.15603	Arctic Tundra
AH-Ret153	Troy McMullin 20809	*Physconia muscigena*	No Band	*Foliose*	Terricolous	Qikiqtaaluk Region, NU, CA.	63.72363	-68.68901	Arctic Tundra
AH-Ret154	Troy McMullin 20776	*Umbilicaria deusta*	No Band	*Umbilicate*	Saxicolous	Qikiqtaaluk Region, NU, CA.	63.77204	-68.70029	Arctic Tundra
AH-Ret155	Troy McMullin 21935	*Pamelia sulcata*	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	67.93063	-116.9209	Arctic Tundra
AH-Ret156	Troy McMullin 20051	*Umbilicaria mammulata*	No Band	*Umbilicate*	Saxicolous	Thunder Bay District	48.34775	-88.89244	Arctic Tundra
AH-Ret157	Troy McMullin 20759	*Asahinea chrysantha*	No Band	*Foliose*	Terricolous	Qikiqtaaluk Region, NU, CA.	63.76102	-68.58034	Arctic Tundra
AH-Ret158	Troy McMullin 21933	*Parmelia sulcata*	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	67.93063	-116.9209	Arctic Tundra
AH-Ret159	Troy McMullin 37b	*Lopadium pezizoideum*	No Band	*Crustose*	Terricolous	Qikiqtaaluk, NU, CA.	70.7028	-69.6978	Arctic Tundra
AH-Ret160	Troy McMullin 17608	*Arctoparmelia incurva*	No Band	*Foliose*	Saxicolous	Kivalliq Region, NU, CA.	61.103639	-94.15603	Arctic Tundra
AH-Ret161	Troy McMullin 18056	*Umbilicaria cinereorufescens*	Usable	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.315139	-94.45708	Subarctic Tundra
AH-Ret162	Troy McMullin 17616	*Umbilicaria lyngei*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.105444	-94.16892	Arctic Tundra

AH-Ret163	Troy McMullin 1208	*Umbilicaria lyngei*	No Band	*Umbilicate*	Saxicolous	Inuvialuit Settlement Region, NWT, CA.	70.097	-124.671	Arctic Tundra
AH-Ret164	Troy McMullin 21041	*Umbilicaria americana*	Sequence - No usable	*Umbilicate*	Saxicolous	Thunder Bay District, ON, CA.	48.47506	-88.82361	Boreal Forest
AH-Ret165	Troy McMullin 20756	*Arctoparmelia separata*	No Band	*Foliose*	Saxicolous	Qikiqtaaluk Region, NU, CA.	63.74796	-68.55856	Arctic Tundra
AH-Ret166	Troy McMullin 15	*Parmelia sulcata*	Usable	*Foliose*	Saxicolous	Division No. 7 (Bonavista/Trinity), NL, CA.	48.5608	-53.8817	Boreal Forest
AH-Ret167	Troy McMullin 16754	*Parmelia squarrosa*	No Band	*Foliose*	Corticolous (recently fallen *Betula alleghaniensis*)	Division No. 1, NL, CA.	47.37271	-53.43902	Arctic Tundra
AH-Ret168	Troy McMullin 13918	*Nephroma arcticum*	No Band	*Foliose*	Terricolous	M.R.C. de la Haute-Gaspésie, QC, CA.	48.987934	-65.94831	Arctic Tundra
AH-Ret169	Troy McMullin 21238	*Umbilicaria virginis*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	67.74393	-115.3688	Arctic Tundra
AH-Ret170	Troy McMullin 13729	*Parmelia saxatilis*	No Band	*Foliose*	Conifer snag	M.R.C. de la Haute-Gaspésie, QC, CA.	48.886545	-66.62817	Subalpine Forest
AH-Ret171	Troy McMullin 16698	*Lobaria scrobiculata*	Usable	*Foliose*	Corticolous	Division No. 1, NL, CA.	47.37436	-53.44056	Boreal Forest
AH-Ret172	Troy McMullin 16662	*Parmelia sulcata*	No Band	*Foliose*	Corticolous	Division No. 1, NL, CA.	47.380278	-53.44472	Arctic Tundra
AH-Ret173	Troy McMullin 2319	*Cetrariella delisei*	Sequence - No usable	*Foliose*	Terricolous	Qikiqtaaluk Region, NU, CA.	79.42237	-90.69325	Arctic Tundra
AH-Ret174	Troy McMullin 37a	*Parmelia omphalodes*	Sequence - No usable	*Foliose*	Saxicolous	Qikiqtaaluk Region, NU, CA.	70.7028	-69.6978	Arctic Tundra
AH-Ret175	Troy McMullin 21097	*Lasallia pensylvanica*	Sequence - No usable	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	67.81063	-115.2041	Arctic Tundra
AH-Ret176	David Diaz Escandon *s.n.*	*Umbilicaria mammulata*	Usable	*Umbilicate*	Saxicolous	Holderness, NH, US.	43.727555	-71.5973	Temperate Deciduous Forest

AH-Ret177	Troy McMullin 21234	*Umbilicaria decussata*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	67.81063	-115.2041	Arctic Tundra
AH-Ret178	Troy McMullin 16741	*Peltigera collina*	No Band	*Foliose*	Corticolous	Division No. 1, NL, CA.	47.37397	-53.4404	Arctic Tundra
AH-Ret179	Troy McMullin 20788	*Arctoparmelia centrifuga*	No Band	*Foliose*	Saxicolous	Qikiqtaaluk Region, NU, CA.	63.74796	-68.55856	Arctic Tundra
AH-Ret180	Troy McMullin 21235	*Umbilicaria cinereofuscescens*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	67.81063	-115.2041	Arctic Tundra
AH-Ret181	Troy McMullin 21127	*Physconia muscigena*	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	67.93063	-116.9209	Arctic Tundra
AH-Ret182	Troy McMullin 21225	*Umbilicaria proboscidea*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	67.93063	-116.9209	Arctic Tundra
AH-Ret183	Troy McMullin 17838	*Umbilicaria arctica*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.094611	-94.0155	Arctic Tundra
AH-Ret184	Troy McMullin 17748	*Umbilicaria hyperborea var. hyperborea*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.103194	-94.01975	Arctic Tundra
AH-Ret185	Troy McMullin 17751	*Umbilicaria arctica*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.103194	-94.01975	Arctic Tundra
AH-Ret186	Troy McMullin 18477	*Umbilicaria deusta*	No Band	*Umbilicate*	Saxicolous	Municipality of Lac-Jacques-Cartier, QC, CA.	47.32642	-71.10176	Arctic Tundra
AH-Ret187	Troy McMullin 17621	*Umbilicaria deusta*	No Band	*Umbilicate*	Saxicolous	Kivalliq Region, NU, CA.	61.106	-94.18672	Arctic Tundra
AH-Ret188	Troy McMullin 17785	*Parmelia sulcata*	No Band	*Foliose*	Terricolous	Kivalliq Region, NU, CA.	61.097361	-94.00261	Arctic Tundra
AH-Ret189	Troy McMullin 23272	*Umbilicaria decussata*	No Band	*Umbilicate*	Saxicolous	Qikiqtaaluk Region, NU, CA.	79.4065	-90.7574	Arctic Tundra
AH-Ret190	Troy McMullin 21228	*Arctoparmelia separata*	No Band	*Foliose*	Saxicolous	Kitikmeot Region, NU, CA.	67.93063	-116.9209	Arctic Tundra
AH-Ret191	Troy McMullin 1135	*Umbilicaria decussata*	No Band	*Umbilicate*	Saxicolous	Kitikmeot Region, NU, CA.	68.9062	-114.3008	Arctic Tundra

Table 3. List of primers developed in this study to target *Retiarius* ribosomal DNA ITS1-5.8S-ITS2 to partial LSU, ITS1-5.8S-ITS2, nuclear Large subunit and nuclear Small subunit.

Gene Region	Abbreviation	5’→3’	Source
ITS (Forward)	ITS1	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns 1993
ITS (Reverse)	Ret4_ITS_R	CGTGCACTCGGCTTCTGACA	This study
ITS (Reverse)	Ret5_ITS_R	ACGGTTGATGGTCTCGAGTCAT	This study
LSU, 28S (Forward)	LROR	ACCCGCTGAACTTAAGC	Vilgalys unpublished (Lab Website)
LSU, 28S (Reverse)	LR7	TACTACCACCAAGATCT	Vilgalys & Hester 1990
nSSU, 18S (Forward)	NS1	GTAGTCATATGCTTGTCTC	Gargas & Taylor 1992
nSSU, 18S (Reverse)	NS23	GACTCAACACGGGGAAACTC	Gargas & Taylor 1992

Table 4. List of voucher of species and sequences used in nSSU, ITS and LSU phylogeny, Fig. 4. New sequences are highlighted in bold.

Species	Isolate ID	Country	Voucher	GenBank Accession			Source
				nSSU	ITS	LSU	
Amphosoma cf. *atroolivaceum*	MN151403	France	G.M. 2017-11-05.1	MN151403	MN151403	MN151403	Baral *et al.* 2020
Amphosoma aff. *resinicola*	MN151404	France	G.M. 2015-09-15.2	MN151404	MN151404	MN151404	Baral *et al.* 2020
Amphosoma atroolivaceum	MH221036	France	H.B. 9162b		MH221036	MH221036	Baral *et al.* 2020
Amphosoma atroolivaceum	KT380069	France	G.M. 2010-09-03		KT380069	KT380069	Baral *et al.* 2020
Amphosoma atroolivaceum	KT380058	France	G.M. 2009-09-27		KT380058		Direct Submission
Amphosoma atroolivaceum	KT222387	France	H.B. 9631b,		KT222387	KT222387	Baral *et al.* 2020
Amphosoma resinicola	KT222388	France	G.M. 2010-09-03 #2		KT222388	KT222388	Baral *et al.* 2020
Amphosoma resinicola	KT222389	France	G.M. 2011-09-19 #4		KT222389	KT222389	Baral *et al.* 2020
Bryorbilia arenicola	MK514443	Spain	E.R.D. 6987	MK514443	MK514443	MK514443	Baral *et al.* 2020
Lecophagus ellipsoideus	KT222385	Luxembourg	H.B. 9902a	KT222385	KT222385	KT222385	Baral *et al.* 2020
Lecophagus longisporus	KT215220	New Zealand	OAC 10849	KT215220	KT215220	KT215220	Baral *et al.* 2020
Lecophagus muscicola	ATCC 56071	Not indicated	ATCC 56071	AY635836	AY997058	DQ273799	James *et al.* 2006
Lecophagus vermicola	MD12	Hungary	K07/36		KU955283	KU955283	Magyar *et al.* 2016
Lilapila gallica	MT367524	France	G.M. 2019-07-30.5. #1914	MT367524	MT367524	MT367524	Baral *et al.* 2020
Lilapila jurana	MK473411	Switzerland	E.S. 17.06.18-48c	MK473411	MK473411	MK473411	Baral *et al.* 2020
Lilapila jurana	MT367523	France	G.M. 2019-07-30.5. #1917	MT367523	MT367523	MT367523	Baral *et al.* 2020
Lilapila oculispora	KT222413	France	H.B. 9162a	KT222413	KT222413	KT222413	Baral *et al.* 2020
Lilapila oculispora	MH221039	France	H.B. 10122	MH221039	MH221039	MH221039	Baral *et al.* 2020
Lilapila oculisporella	MH221044	France	G.M. 2016-08-14.1	MH221044	MH221044	MH221044	Baral *et al.* 2020
Lilapila oculisporella	MH221045	France	G.M. 2016-08-11.1	MH221045	MH221045	MH221045	Baral *et al.* 2020
Mycoceros antennatissimus	MD4/MD5	Hungary	BP 105172		KT186373	KT186370	Magyar *et al.* 2017b
Mycoceros antennatissimus	MD3/MD6	Hungary	BP 105172		KT186372	KT186371	Magyar *et al.* 2017b

Orbilia crenatomarginata	KM248772	Germany	H.B. 9265	KM248772	KM248772	KM248772	Zhang *et al.* 2015
Orbilia cucumispora	KT215231	USA	H.B. 6762a	KT215231	KT215231	KT215231	Baral *et al.* 2020
Orbilia scolecospora	KM248770	Spain	TFC Mic. 22307	KM248770	KM248770	KM248770	Zhang *et al.* 2015
Retiarius bovicornutus	KY352466	South Africa	IMI 223460		KY352466	KY352466	Magyar *et al.* 2017a
Retiarius bovicornutus	**AH-Ret215**	**South Africa**	**IMI 223460**		**PP414145**	**PP417912**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret121**	**Canada**	**TSMC88**		**PP414121**	**PP417895**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret122**	**Canada**	**TSMC89**		**PP414122**	**PP417896**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret208**	**Canada**	**TSMC103**		**PP414139**	**PP417907**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret209**	**Canada**	**TSMC104**		**PP414140**	**PP417908**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret211**	**Canada**	**TSMC106**		**PP414142**	**PP417909**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret212**	**Canada**	**TSMC107**		**PP414143**	**PP417910**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret213**	**Canada**	**TSMC108**		**PP414144**	**PP417911**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret216**	**USA**	**TSMC109**		**PP414146**	**PP417913**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret116**	**Canada**	**TSMC83**		**PP414116**	**PP417891**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret117**	**Canada**	**TSMC84**		**PP414117**	**PP417892**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret119**	**Canada**	**TSMC86**		**PP414119**	**PP417893**	**This study**

***Retiarius crescentus* nom. prov.**	**AH-Ret120**	**Canada**	**TSMC87**		**PP414120**	**PP417894**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret192**	**Canada**	**TSMC90**		**PP414127**	**PP417897**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret193**	**Canada**	**TSMC91**		**PP414128**	**PP417898**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret194**	**Canada**	**TSMC92**		**PP414129**	**PP417899**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret195**	**Canada**	**TSMC93**		**PP414130**	**PP417900**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret196**	**Canada**	**TSMC94**		**PP414131**	**PP417901**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret197**	**Canada**	**TSMC95**		**PP414132**	**PP417902**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret199**	**Canada**	**TSMC97**		**PP414134**	**PP417903**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret200**	**Canada**	**TSMC98**		**PP414135**	**PP417904**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret201**	**Canada**	**TSMC99**		**PP414136**	**PP417905**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret202**	**Canada**	**TSMC100**		**PP414137**	**PP417906**	**This study**
Retiarius revayae	KY352465	Hungary	BP 108633		KY352465	KY352465	Magyar *et al.* 2017a
Retiarius superficiaris	KY352467	South Africa	IMI 223459		KY352467	KY352467	Magyar *et al.* 2017a

Table 5. List of voucher of species and sequences used in LSU phylogeny, Fig. 5. New sequences are highlighted in bold.

Species	Isolate ID	Country	Voucher	GenBank Accession	Source
Amphosoma atroolivaceum	MH221036	France	H.B. 9162b	MH221036	Baral *et al.* 2020
Amphosoma atroolivaceum	KT380069	France	G.M. 2010-09-03	KT380069	Baral *et al.* 2020
Amphosoma atroolivaceum	KT222387	France	H.B. 9631b,	KT222387	Baral *et al.* 2020
Amphosoma resinicola	KT222388	France	G.M. 2010-09-03 #2	KT222388	Baral *et al.* 2020
Amphosoma sp. (*Amphosoma persooniae* nom. inval.)	MG386090	Australia	CBS 143171	MG386090	Crous *et al.* 2017
Bryorbilia arenicola	MK514443	Spain	E.R.D. 6987	MK514443	Baral *et al.* 2020
Lecophagus ellipsoideus	KT222385	Luxembourg	H.B. 9902a	KT222385	Baral *et al.* 2020
Lecophagus longisporus	KT215220	New Zealand	OAC 10849	KT215220	Baral *et al.* 2020
Lecophagus muscicola	ATCC 56071	Not indicated	ATCC 56071	DQ273799	James *et al.* 2006
Lecophagus vermicola	KU955283	Hungary	MD12	KU955283	Magyar *et al.* 2016a
Lecophagus vermicola	KU955284	Hungary	MD19	KU955284	Magyar *et al.* 2016a
Lilapila jurana	MK473411	Switzerland	E.S. 17.06.18-48c	MK473411	Baral *et al.* 2020
Lilapila jurana	MK473410	Switzerland	E.S. 18.01.31-48f	MK473410	Baral *et al.* 2020
Lilapila jurana	MK473419	Switzerland	E.S. 18.01.31-48g	MK473419	Baral *et al.* 2020
Lilapila jurana	MH221042	Switzerland	H.B. 10123	MH221042	Baral *et al.* 2020
Lilapila oculisporella	MH221044	France	G.M. 2016-08-14.1	MH221044	Baral *et al.* 2020
Lilapila oculisporella	MG372373	France	G.M. 2016-08-15.1	MG372373	Baral *et al.* 2020
Lilapila oculisporella	KY419170	France	G.M. 2015-08-30.4	KY419170	Baral *et al.* 2020
Lilapila oculisporella	KY419169	France	G.M. 2015-08-28.4	KY419169	Baral *et al.* 2020
Lilapila oculisporella	MH221045	France	G.M. 2016-08-11.1	MH221045	Baral *et al.* 2020
Lilapila oculisporella	MH221043	Switzerland	G.M. 2016-09-08.1	MH221043	Baral *et al.* 2020
Lilapila oculispora	MH221039	Switzerland	H.B. 10122	MH221039	Baral *et al.* 2020
Lilapila oculispora	MH221040	France	G.M. 2017-08-31.2	MH221040	Baral *et al.* 2020
Lilapila oculispora	KY419168	France	G.M. 2015-8-30.1	KY419168	Baral *et al.* 2020
Lilapila oculispora	KT222384	France	G.M. 2011-09-19.1	KT222384	Baral *et al.* 2020
Lilapila oculispora	KT222413	France	H.B. 1162a	KT222413	Baral *et al.* 2020
Lilapila oculispora	MH221041	France	G.M. 2017-08-31.1	MH221041	Baral *et al.* 2020
Mycoceros antennatissimus	KT186370	Hungary	MD5	KT186370	Magyar *et al.* 2017b
Mycoceros antennatissimus	KT186371	Hungary	MD6	KT186371	Magyar *et al.* 2017b
Orbilia flavida	KT215228	Croatia	H.B. 6716	KT215228	Baral *et al.* 2020
Retiarius bovicornutus	KY352466	South Africa	IMI 223460	KY352466	Magyar *et al.* 2017a
Retiarius bovicornutus	**AH-Ret215**	**South Africa**	**IMI 223460**	**PP417913**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret121**	**Canada**	**TSMC88**	**PP417895**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret122**	**Canada**	**TSMC89**	**PP417896**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret208**	**Canada**	**TSMC103**	**PP417895**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret209**	**Canada**	**TSMC104**	**PP417896**	**This study**

Retiarius canadensis nom. prov.	**AH-Ret211**	**Canada**	**TSMC106**	**PP417895**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret212**	**Canada**	**TSMC107**	**PP417896**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret213**	**Canada**	**TSMC108**	**PP417895**	**This study**
Retiarius canadensis nom. prov.	**AH-Ret216**	**USA**	**TSMC109**	**PP417913**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret116**	**Canada**	**TSMC83**	**PP417891**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret117**	**Canada**	**TSMC84**	**PP417892**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret119**	**Canada**	**TSMC86**	**PP417893**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret120**	**Canada**	**TSMC87**	**PP417894**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret192**	**Canada**	**TSMC90**	**PP417897**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret193**	**Canada**	**TSMC91**	**PP417898**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret194**	**Canada**	**TSMC92**	**PP417899**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret195**	**Canada**	**TSMC93**	**PP417900**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret196**	**Canada**	**TSMC94**	**PP417901**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret197**	**Canada**	**TSMC95**	**PP417902**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret199**	**Canada**	**TSMC97**	**PP417903**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret200**	**Canada**	**TSMC98**	**PP417904**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret201**	**Canada**	**TSMC99**	**PP417905**	**This study**
***Retiarius crescentus* nom. prov.**	**AH-Ret202**	**Canada**	**TSMC100**	**PP417906**	**This study**
Retiarius revayae	KY352465	Hungary	BP 108633	KY352465	Magyar *et al.* 2017a
Retiarius superficiaris	KY352467	South Africa	IMI 223459	KY352467	Magyar *et al.* 2017a

Table 6. List of voucher of species and sequences used in ITS phylogeny. Includes sequences derived from environmental screening of *Retiarius* using ITS, and unidentified sequences from NCBI, Fig 5,6. New sequences are highlighted in bold.

Name	Culture Strain	Isolate/Specimen Voucher	Isolation source	Isolation ID	Country	Latitude	Longitude	GenBank Accession	Refence
Amphosoma atroolivaceum	-	G.M. 2010-09-03.1	Resin	*Picea abies*	France	44.9981 N	6.1314 E	KT380069	Direct Submission
Amphosoma resinicola	-	G.M. 2011-09-19 #4	Resin	*Picea abies*	France	44.25 N	6.373 E	KT222389	Baral *et al.* 2020
Amphosoma sp.	-	CBS 143171	Bark	*Persoonia* sp.	Australia			MG386037	Crous *et al.* 2017
Lecophagus ellipsoideus	-	H.B. 9902a	Bark	*Clematis vitalba*	Luxembourg	49.48 N	6.1092 E	KT22238	Baral *et al.* 2020
Lecophagus longisporus	CBS 845.91	OAC 10849	Wood	-	New Zealand			KT215220	Baral *et al.* 2020
Lecophagus muscicola	ATCC 56071	AFTOL-ID183	Culture	-	-			AY997058	James *et al.* 2008
Lecophagus vermicola	-	MD12	Bark	*Platanus* x *acerifolia*	Hungary	47.476639 N	19.092444 E	KU955283	Magyar *et al.* 2016
Lecophagus vermicola	-	MD19	Bark	*Platanus* x *acerifolia*	Hungary	47.476639 N	19.092444 E	KU955284	Magyar *et al.* 2016
***Lecophagus* sp.**	**-**	**AH-Ret77**	**Bark**	***Crataegus* sp.**	**USA**	**44.701229 N**	**-123.2017 W**	**PP414110**	**This study**
***Lecophagus* sp.**	**-**	**AH-Ret96**	**Lichen**	***Polycauliona* aff. *polycarpa***	**USA**	**44.701229 N**	**-123.2017 W**	**PP414112**	**This study**
***Lecophagus* sp.**	**-**	**AH-Ret113**	**Lichen**	***Lobaria pulmonaria***	**USA**	**44.701229 N**	**-123.2017 W**	**PP414115**	**This study**
Retiarius bovicornutus	IMI 223460	IMI 223460	Phyllosphere	*Brabejum stellatifolium*	South Africa			KY352466	Magyar et. al. 2017a
Retiarius bovicornutus	IMI 223460	**AH-Ret215**	**Phyllosphere**	***Brabejum stellatifolium***	**South Africa**			**PP414145**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC88**	**AH-Ret121**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414121**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC89**	**AH-Ret122**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414122**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC102**	**AH-Ret207**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414138**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC103**	**AH-Ret208**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.425847 N**	**-114.3819 W**	**PP414139**	**This study**

***Retiarius canadensis* nom. prov.**	**TSMC104**	**AH-Ret209**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.425847 N**	**-114.3819 W**	**PP414140**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC106**	**AH-Ret211**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414142**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC107**	**AH-Ret212**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.425847 N**	**-114.3819 W**	**PP414143**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC108**	**AH-Ret213**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.425847 N**	**-114.3819 W**	**PP414144**	**This study**
***Retiarius canadensis* nom. prov.**	**TSMC109**	**AH-Ret216**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**USA**	**48.681362**	**-115.759470**	**PP414146**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC83**	**AH-Ret116**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414116**	**This study**
***Retiarius crescentus* nom prov.**	**-**	**AH-Ret1**	**Lichen**	**Pollen Scrape on *Umbilicaria***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414084**	**This study**
***Retiarius crescentus* nom prov.**	**-**	**AH-Ret2**	**Lichen**	**Pollen Scrape on *Umbilicaria***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414085**	**This study**
***Retiarius crescentus* nom prov.**	**-**	**AH-Ret3**	**Lichen**	**Pollen Scrape on *Umbilicaria***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414086**	**This study**
***Retiarius crescentus* nom prov.**	**-**	**AH-Ret6**	**Lichen**	**Pollen Scrape on *Umbilicaria***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414087**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC84**	**AH-Ret117**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414117**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC85**	**AH-Ret118**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**50.278125 N**	**-117.7608 W**	**PP414118**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC86**	**AH-Ret119**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414119**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC87**	**AH-Ret120**	**Lichen**	**Pollen from *Umbilicaria torrefacta***	**Canada**	**50.278125 N**	**-117.7608 W**	**PP414120**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC90**	**AH-Ret192**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414127**	**This study**

***Retiarius crescentus* nom prov.**	**TSMC91**	**AH-Ret193**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414128**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC92**	**AH-Ret194**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414129**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC93**	**AH-Ret195**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414130**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC94**	**AH-Ret196**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414131**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC95**	**AH-Ret197**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414132**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC96**	**AH-Ret198**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414133**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC97**	**AH-Ret199**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414134**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC98**	**AH-Ret200**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414135**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC99**	**AH-Ret201**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414136**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC100**	**AH-Ret202**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414137**	**This study**
***Retiarius crescentus* nom prov.**	**TSMC105**	**AH-Ret210**	**Lichen**	**Pollen from *Umbilicaria phaea***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414141**	**This study**
Retiarius revayae	-	BP 108633	Bark	*Taxus baccata*	Hungary	-	-	KY352465	Magyar et. al. 2017a
Retiarius superficiaris	-	IMI 223459	Phyllosphere	*Eriobotrya japonica*	South Africa			KY352467	Magyar et. al. 2017a
***Retiarius* sp.**	**-**	**AH-Ret9**	**Lichen**	***Hypogymnia physodes***	**Canada**	**49.429487 N**	**-114.3793 W**	**PP414088**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret12**	**Lichen**	***Umbilicaria phaea***	**Canada**	**49.16635 N**	**-120.5671 W**	**PP414089**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret13**	**Lichen**	***Umbilicaria phaea***	**Canada**	**49.16635 N**	**-120.5671 W**	**PP414090**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret17**	**Lichen**	***Umbilicaria torrefacta***	**Canada**	**50.278125 N**	**-117.7608 W**	**PP414091**	**This study**

***Retiarius* sp.**	**-**	**AH-Ret19**	**Lichen**	***Umbilicaria deusta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414092**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret21**	**Lichen**	***Umbilicaria torrefacta***	**Canada**	**49.694253 N**	**-120.2826 W**	**PP414093**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret24**	**Lichen**	***Parmelia sulcata***	**Canada**	**54.796758 N**	**-115.8556 W**	**PP414094**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret26**	**Lichen**	***Alectoria sarmentosa***	**Canada**	**54.796758 N**	**-115.8556 W**	**PP414095**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret28**	**Lichen**	***Tuckermanopsis orbata***	**Canada**	**54.796758 N**	**-115.8556 W**	**PP414096**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret34**	**Lichen**	***Hypogymnia bitteri***	**Canada**	**54.796758 N**	**-115.8556 W**	**PP414097**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret36**	**Lichen**	***Melanohalea exasperatula***	**Canada**	**54.796758 N**	**-115.8556 W**	**PP414098**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret37**	**Bark**	***Abies balsamea***	**Canada**	**54.796758 N**	**-115.8556 W**	**PP414099**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret41**	**Lichen**	***Dermatocarpon moulinsii***	**USA**	**48.549313 N**	**-115.2479 W**	**PP414100**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret42**	**Lichen**	***Umbilicaria torrefacta***	**USA**	**48.549313 N**	**-115.2479 W**	**PP414101**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret44**	**Lichen**	***Xanthoparmelia cumberlandia.***	**USA**	**48.549313 N**	**-115.2479 W**	**PP414102**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret45**	**Lichen**	***Parmelia saxatilis***	**USA**	**48.549313 N**	**-115.2479 W**	**PP414103**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret49**	**Lichen**	***Stereocaulon grande***	**Canada**	**51.868955 N**	**-120.0272 W**	**PP414104**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret51**	**Bark**	***Picea glauca***	**Canada**	**51.868955 N**	**-120.0272 W**	**PP414105**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret54**	**Bark**	**Unidentified (Angiosperm)**	**Canada**	**51.868955 N**	**-120.0272 W**	**PP414106**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret60**	**Bark**	***Alnus rubra***	**Canada**	**50.387594 N**	**-127.968 W**	**PP414107**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret61**	**Lichen**	***Parmelia squarrosa***	**Canada**	**51.868955 N**	**-120.0272 W**	**PP414108**	**This study**
***Retiarius* sp.**		**AH-Ret72**	**Lichen**	***Sticta* sp.**	**Mexico**	**24.03668 N**	**-99.70818 W**	**PP414109**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret82**	**Bark**	***Picea martinezii***	**Mexico**	**24.03668 N**	**-99.70818 W**	**PP414111**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret101**	**Lichen**	***Menegazzia subsimilis***	**USA**	**44.399769 N**	**-122.4766 W**	**PP414113**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret106**	**Lichen**	***Platismatia norvegica***	**USA**	**44.399769 N**	**-122.4766 W**	**PP414114**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret161**	**Lichen**	***Umbilicaria cinereorufescens***	**Canada**	**61.31513889 N**	**-94.45708 W**	**PP414123**	**This study**

***Retiarius* sp.**	**-**	**AH-Ret166**	**Lichen**	***Parmelia sulcata***	**Canada**	**48.5608 N**	**-53.8817 W**	**PP414124**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret171**	**Lichen**	***Lobarina scrobiculata***	**Canada**	**47.37436 N**	**-53.44056 W**	**PP414125**	**This study**
***Retiarius* sp.**	**-**	**AH-Ret176**	**Lichen**	***Umbilicaria mammulata***	**USA**	**43.727555 N**	**-71.5973 W**	**PP414126**	**This study**
Retiarius sp.	-	NWHC 46373-1399 3SD	-	-	Not indicated	-	-	MK794590	Direct Submission
Uncultured *Ascomycota*	-	BF-OTU267	House dust	House dust	Finland			AM901896	Pitkäranta *et al.* 2008
Uncultured *Ascomycota*	-	s C04 06	House dust	House dust	Canada	45.2556 N	-75.9204 W	GU931725	Amend *et al.* 2010
Uncultured fungus	-	E114D	Bryophyte	-	Norway	59.35 N	9.75 E	AM999605	Kauserud *et al.* 2008
Uncultured fungus	-	MOTU 2127 GYUGVSB 04I1PL0	Phyllosphere	*Fagus sylvatica*	France	42.892222 N	0.073333 E	JN904969	Cordier *et al.* 2012
Uncultured fungus	-	MOTU 3452 GOKCVWY 06G3JWR	Phyllosphere	*Fagus sylvatica*	France	43.096111 N	0.087222 E	JN905909	Cordier *et al.* 2012
Uncultured fungus	-	MOTU 375 GOKCVWY 06HGVJ2	Phyllosphere	*Fagus sylvatica*	France	42.976944 N	0.141389 E	JN906140	Cordier *et al.* 2012
Uncultured fungus	-	MOTU 4183 GYUGVSB 04IOD73	Phyllosphere	*Fagus sylvatica*	France	43.096111 N	0.087222 E	JN906429	Cordier *et al.* 2012
Uncultured fungus	-	2168 344	Phyllosphere	*Picea abies*	Lithuania	54.9 N	24.316667 E	KP897463	Menkis *et al.* 2015
Uncultured fungus	-	2168 388	Phyllosphere	*Picea abies*	Lithuania	54.9 N	24.316667 E	KP897503	Menkis *et al.* 2015
Uncultured fungus	-	2168 424	Phyllosphere	*Picea abies*	Lithuania	54.9 N	24.316667 E	KP897539	Menkis *et al.* 2015
Uncultured fungus	-	2168 471	Phyllosphere	*Picea abies*	Lithuania	54.9 N	24.316667 E	KP897586	Menkis *et al.* 2015
Uncultured fungus	-	FMOTU 2342	Soil	-	Not indicated			KU062216	Unpublished
Uncultured fungus	-	OTU F8 R22	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976000	Johnston *et al.* 2017
Uncultured fungus	-	OTU R214	Phyllosphere	*Nothofagus menziesii*	New Zealand	-39.363333 S	175.46833 E	MF976071	Johnston *et al.* 2017

Uncultured fungus	-	OTU R442	Phyllosphere	*Nothofagus menziesii*	New Zealand	-39.363333 S	175.46833 E	MF976258	Johnston *et al.* 2017
Uncultured fungus	-	OTU R393	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976260	Johnston *et al.* 2017
Uncultured fungus	-	OTU R568	Phyllosphere	*Nothofagus menziesii*	New Zealand	-39.363333 S	175.46833 E	MF976263	Johnston *et al.* 2017
Uncultured fungus	-	OTU F214 R697	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976553	Johnston *et al.* 2017
Uncultured fungus	-	OTU F242 R97	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976568	Johnston *et al.* 2017
Uncultured fungus	-	OTU F51 R71	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976575	Johnston *et al.* 2017
Uncultured fungus	-	OTU F398 R376	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976592	Johnston *et al.* 2017
Uncultured fungus	-	OTU F81 R245	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976602	Johnston *et al.* 2017
Uncultured fungus	-	OTU F237 R242	Phyllosphere	*Nothofagus menziesii*	New Zealand	-39.363333 S	175.46833 E	MF976653	Johnston *et al.* 2017
Uncultured fungus	-	OTU F265 R547	Phyllosphere	*Nothofagus menziesii*	New Zealand	-39.363333 S	175.46833 E	MF976658	Johnston *et al.* 2017
Uncultured fungus	-	OTU F35 R27	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976738	Johnston *et al.* 2017
Uncultured fungus	-	OTU F419 R213	Phyllosphere	*Nothofagus* sp.	New Zealand	-39.363333 S	175.46833 E	MF976764	Johnston *et al.* 2017
Uncultured fungus	-	2185 337	Phyllosphere	*Aesculus hippocastanum*	Lithuania	54.85 N	24.45 E	MG827709	Menkis *et al.* 2018
Uncultured fungus	-	2185 928	Phyllosphere	*Aesculus hippocastanum*	Lithuania	54.85 N	24.45 E	MG828112	Menkis *et al.* 2018
Uncultured fungus	-	2185 780	Phyllosphere	*Aesculus hippocastanum*	Lithuania	54.85 N	24.45 E	MG828147	Menkis *et al.* 2018
Uncultured fungus	-	3980 835	Phyllosphere	*Pinus sylvestris*	Lithuania	55.22 N	25.852222 E	MN903183	Lynikienė *et al.* 2020

Uncultured fungus	-	4248 606	Water Pond	-	Lithuania	54.50012 N	24.206 E	MT236683	Marčiulynas *et al.* 2020
Uncultured fungus	-	saf_F1008	Soil	-	Italy	45.751917 N	7.343611 E	MW163637	Victorino *et al.* 2021
Uncultured fungus	-	ASV_974_F	Bark	*Fagus sylvatica*	Germany	51.089243 N	10.432898 E	ON122975	Dreyling *et al.* 2022
Uncultured fungus	-	5238 294	Phyllosphere	*Ulmus glabra*	Lithuania	54.923794 N	23.762145 E	OP467540	Marčiulynas *et al.* 2022
Uncultured *Orbiliaceae*	-	-	Rhizosphere soil	-	China	ND	ND	LR603528	Direct Submission
Uncultured *Orbiliaceae*	-	-	Rhizosphere soil	-	China	ND	ND	LR820364	Direct Submission
Uncultured *Orbiliaceae*	-	-	Soil	-	China	ND	ND	OU498264	Direct Submission
Uncultured *Orbiliales*	-	OTU 1742	Roots	*Festuca brevipila*	Germany	52.46357713 N	14.503314 E	OX032132	Direct Submission
Uncultured *Retiarius*	-	OTU 9064	Leaf litter	-	USA	39.055833 N	-79.66833 W	MW337596	Alshuwaili *et al.* 2021

Table 7. Measurements of conidia in *Retiarius bovicornutus* agg., *R. canadensis* nom. prov. and *R. revayae*. Measurements in microns (µm). (^{T}) indicates the original description, * interpretation of type drawing of Oliver 1978 by H.O. Baral in Baral *et al.* 2020. Species identification following criteria in according Baral *et al.* 2020.

Species	Voucher	Country	Total size (height x width)	Stipe length	Stipe width	Stipe septa	Arms length	Arms width	Arms Septa	Reference
R. bovicornutus ^{T}	IMI 223460	South Africa	-	(12-) 15-20 (-25)	-	2-3	(25-) 30-45(-50)	-	4-5	Olivier 1978
*R. bovicornutus**	IMI 223460	South Africa	47-50 x 45-55	(12-) 15-20 (-25)	3.5-4.5 (-5.5)	(1-)2-3	(20-) 30-45 (-50)	4-5	(2-) 4-5	Baral *et al.* 2020
R. bovicornutus		Germany	38 x 43	-	-	-	30-32	5-5.5	-	Baral *et al.* 2020
R. aff. bovicornutus	TKB-C-1298	Japan	36-44 x 27-56	20-28	3.5-5.2	2-3	20-33.5	3.5-5.2	2-4	Ando & Tubaki 1984
R. aff. bovicornutus	MFC-9P086	Peru	19-35 x 25-50	14-24	-	1-3	11-30	4-5	2-3	Matsushima 1993
R. cf. *bovicornutus*	CSVY/F/2698	Great Britain	52-60 x 65-68	-	-	-	45-55	5-6	-	Baral *et al.* 2020
R. cf. *bovicornutus*	H.B. 6729c	France	25-45 x 56-65	-	-	-	-	-	-	Baral *et al.* 2020
R. cf. *bovicornutus*		Luxembourg	27-31 x 27-29	-	-	-	15-17	4-4.5	-	Baral *et al.* 2020
R. canadensis nom prov.	TSMC89	Canada	24.8-31.5 x 31.4-40.7	8.9-13.8	4.4-5.4	1-2 (-3)	18.4-23.6	5-6	2-3 (-4)	This study
R. revayae	BP 108633	Hungary	26-40 x 32-48	14-22	2-3.5	3-5 (-7)	22-30	2-3 (-3.5)	(3-) 4-7	Baral *et al.* 2020, Magyar *et al.* 2017a

Table 8. Comparative of conidial characteristics of the known *Retiarius* species, modified from Baral *et al.* 2020. Measurements stated in microns (µm), (^{T}) stands for collecting information of type locality.

Species	Total size of conidia (height x width)	Stipe (length x width)	Arms (length x width)	Conidial Shape	Ecology and Microhabitat ^{T}	Distribution
R. bovicornutus	47-50 x 45-55	(12-)15-20(-25) × 3.5-4.5(-5.5)	(25-)30-45(-50) × 4-5	Y-shaped	on leaves of *Brabejum stellatifolium*	South Africa
R. canadensis nom. prov.	24.8-31.5 x 31.5-40.7	8.9-13.8 × 4.4-5.4	18.4-23.6 × 5-6	Y-shaped	Undersurface of *Umbilicaria* spp.	Canada and USA
R. crescentus nom. prov.	17.3-21.7 x 4.7-5.8	(0.3-) 0.34-0.74 (-1) × (1-) 1.2-2 (-2.4), or absent	(7.4-) 8.5-10.7 (-12.3) x (3.8-) 4.3-5.1 (-5.7)	Canoe shaped	Undersurface of *Umbilicaria* spp.	Canada
R. goenczoelii	37-70 × 100-150	19-33 × 1.7-2.5(-3)	†42-90 × 2-2.5	Y/T-shaped	bark of *Platanus* × *acerifolia,* urban park	Hungary
R. revayae	26-40 × 32-48	14-22 × 2-3.5	22-30 × 2-3(-3.5)	Y-shaped	bark of *Taxus baccata,* urban park	Hungary
R. superficiaris	37-60 × 44-66	(8-)10-15 × 4-4.5(-5.5) 14-22	(20-)25-35(-45) × 4.5-6	dwayaangam-like, also Y or +-shaped	on leaves of *Eriobotrya japonica*	South Africa

Table 9. Summary statistics for the *Retiarius crescentus* nom. prov. genome assembly and annotation.

Characteristic	Value
Total assembly length (Mb)	36.32
No. of scaffolds	624
N_{50} (Mb)	0.15
L_{50}	76
No. of genes	10331
No. of coding-protein genes	10290
No. of complete single-copy orthologs	1636
No. of duplicate single-copy orthologs	12
No. of incomplete orthologs	24
No. of missing orthologs	46
% repeat	6.52
% GC	38.04
No. of tRNAS	41
No. of Phobius secretome genes	1092
No. of Phobius transmembrane proteins	2167
No. of antiSMASH biosynthetic gene clusters	17
No. of antiSMASH biosynthetic enzymes	31
No. of antiSMASH biosynthetic smCOGs	30
No. of CAZYmes	342
Total no. of annotations	10,290
ENA Project accession no.	PRJEB73517
Sequence Read Archive accession no.	XXX
Assembly accession no.	XXX

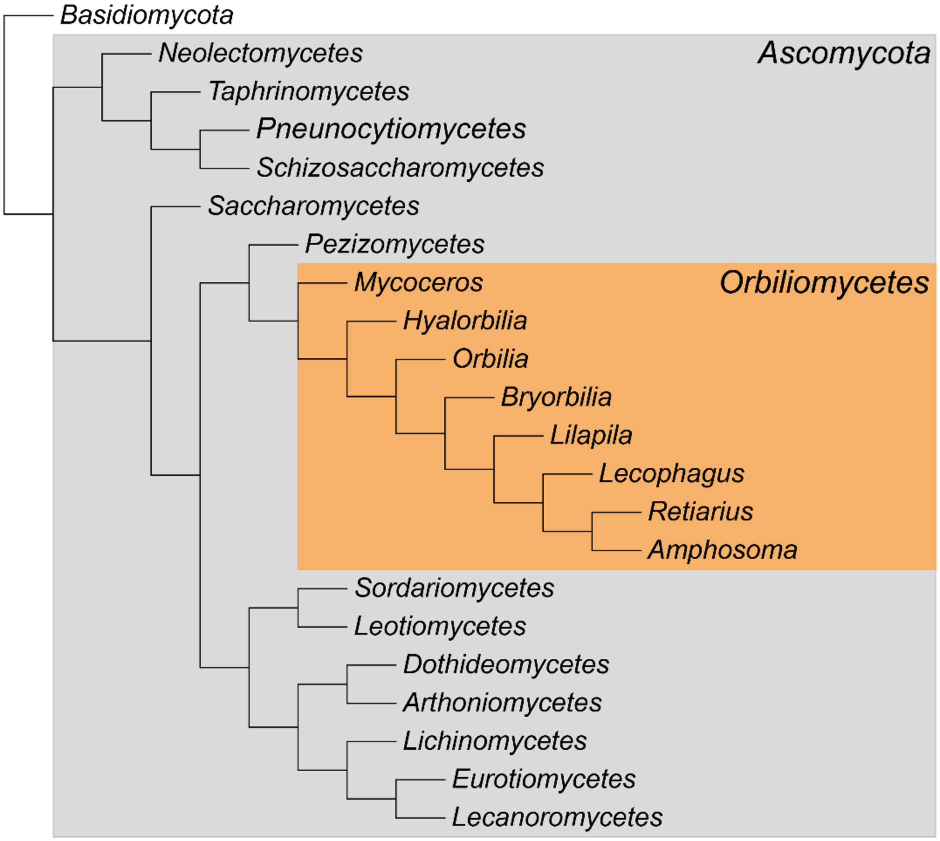

Fig. 1. Schematic representation of the classes in *Ascomycota*, with emphasis in genera of *Orbiliomycetes*. Illustration follows the topologies suggested in Díaz-Escandón et al. (2022) and Baral et al. (2020).

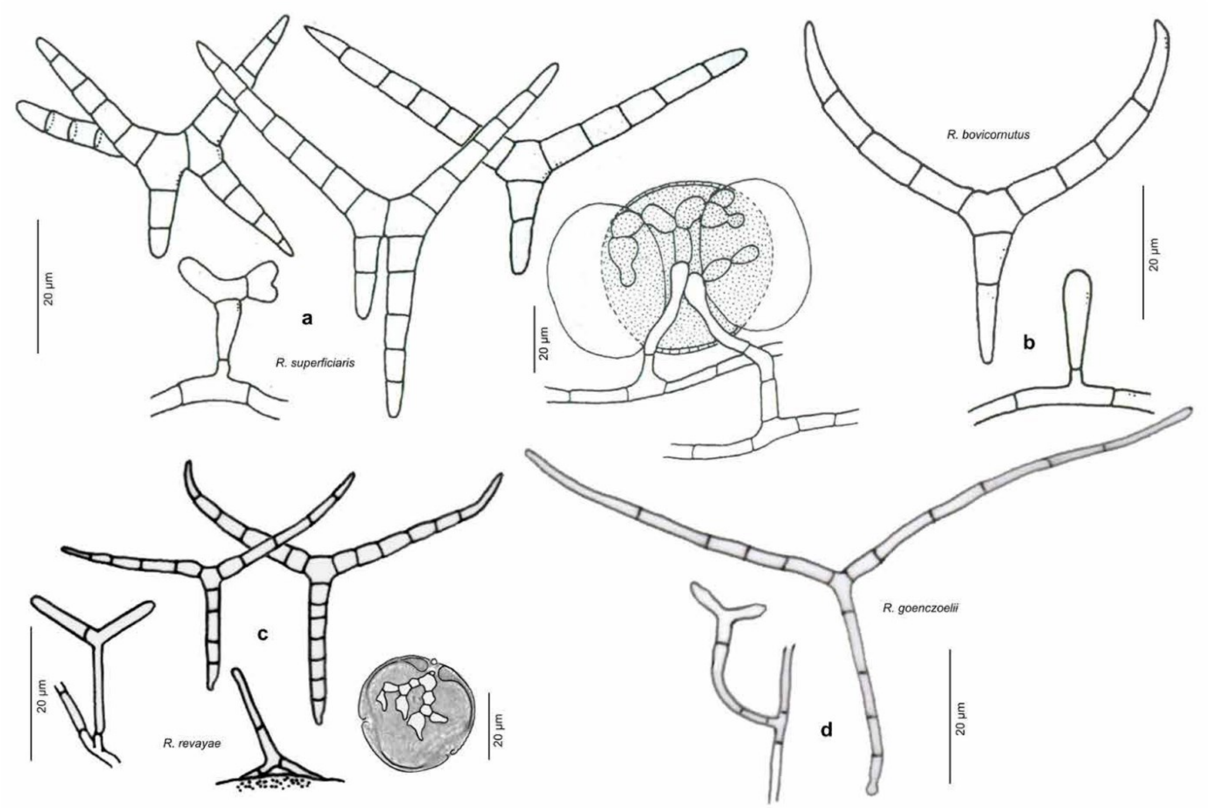

Fig. 2. Species of the genus *Retiarius* (conidia, conidiophores, and pollen infection), extracted from Baral *et al.* 2020. Original drawings from Olivier 1978 (a and b), and Magyar *et al.* 2017a (c and d).

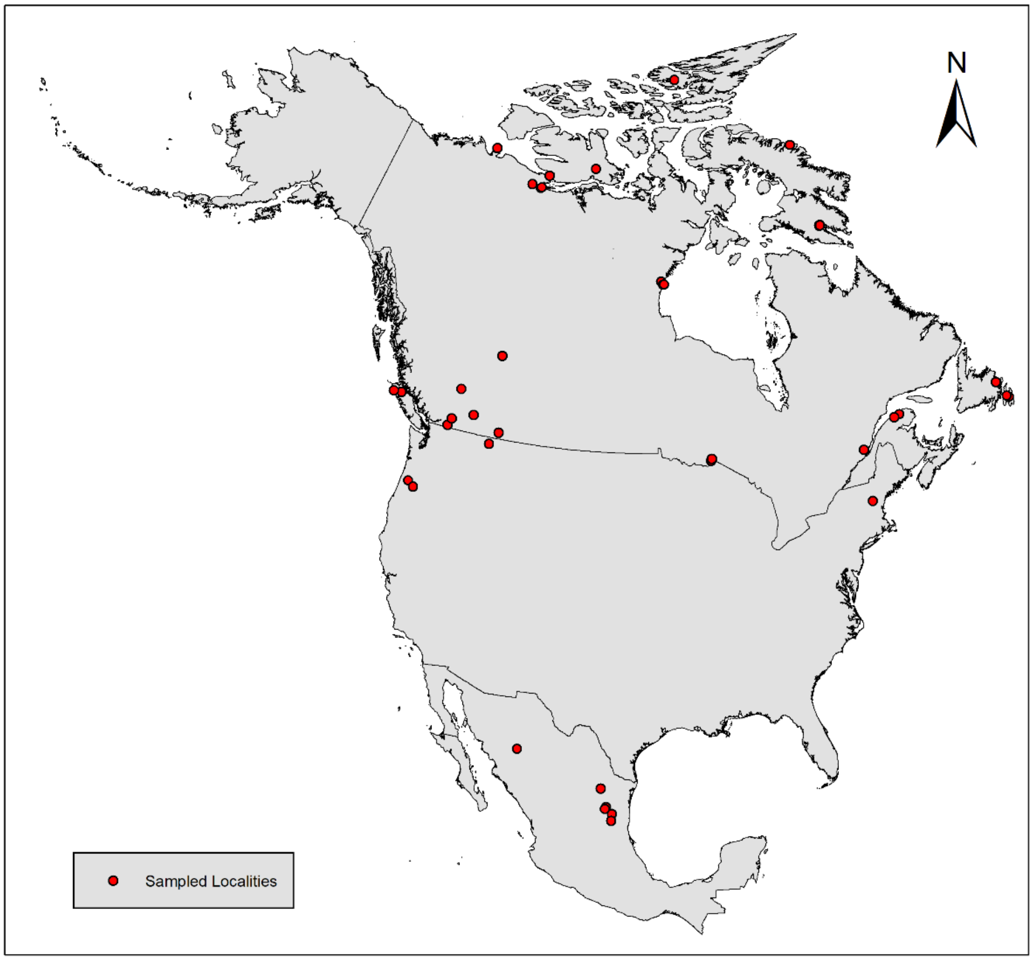

Fig. 3. Map of localities where samples were screened for *Retiarius*.

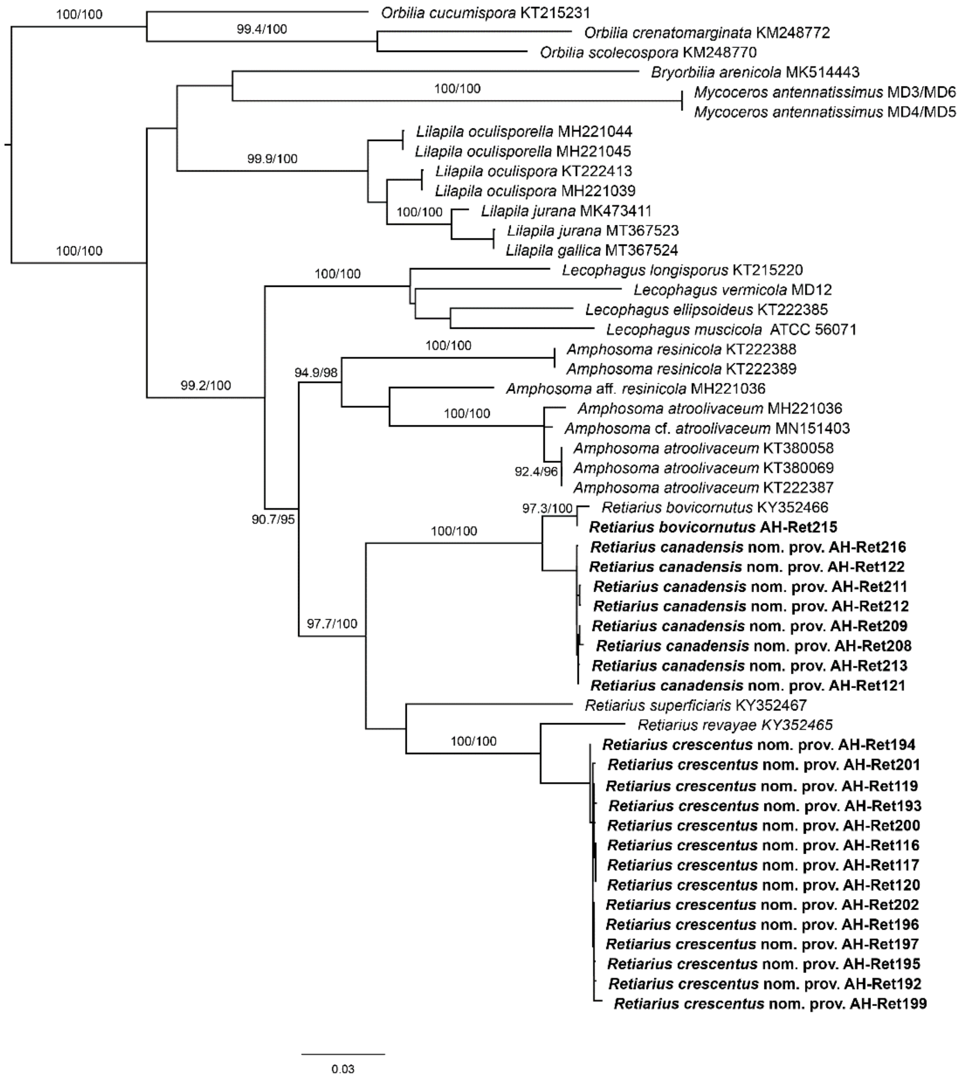

Fig. 4. Maximum likelihood phylogenetic tree of *Retiarius*, including genera in the *Lecophagus*-*Amphosoma* clade and *Mycoceros*, using *Orbilia crenatomarginata, O. cucumispora* and *O. scolespora* as outgroup based on nSSU+ITS+LSU. Only values in branches corresponding SH-alrt support ≥80% and ultrafast bootstrap support ≥95%. Bold sequences indicate those from this study; others are from GenBank.

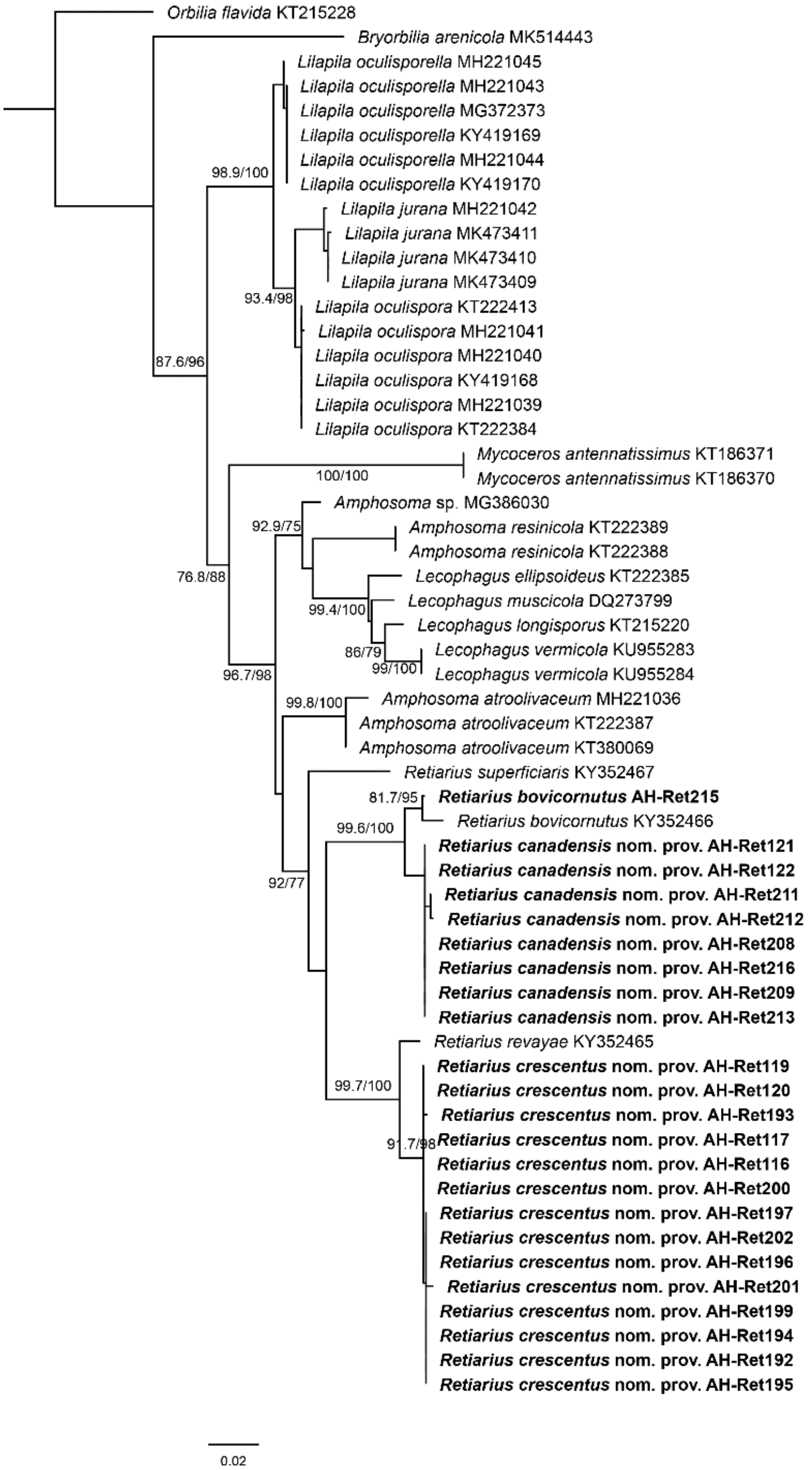

Fig. 5. Maximum likelihood phylogenetic tree of *Retiarius*, including genera in the *Lecophagus*-*Amphosoma* clade and *Mycoceros*, using *Orbilia flavida* as outgroup based on LSU. Only values in branches corresponding SH-alrt support ≥80% and ultrafast bootstrap support ≥95%. Bold sequences indicate those from this study; others are from GenBank.

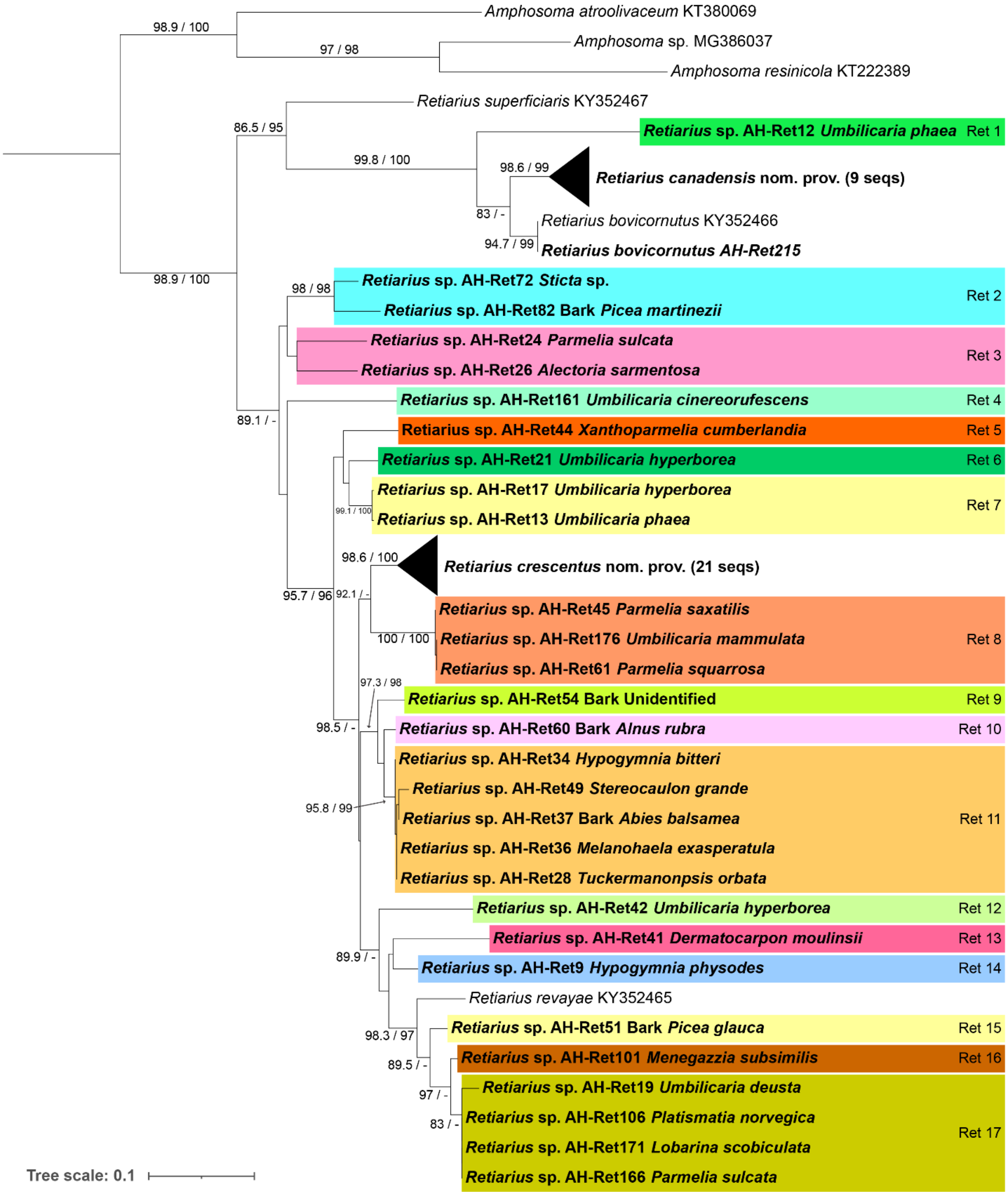

Fig. 6. Maximum likelihood phylogenetic tree ITS1-5.8s-ITS2 regions of environmental samples screened with custom primers for *Retiarius*, with *Amphosoma* spp. as outgroup. Only values in branches corresponding SH-alrt support ≥80% and ultrafast bootstrap support ≥95. Bold tips are sequences produced in this study. Color bars indicate the lineages that represent putative species.

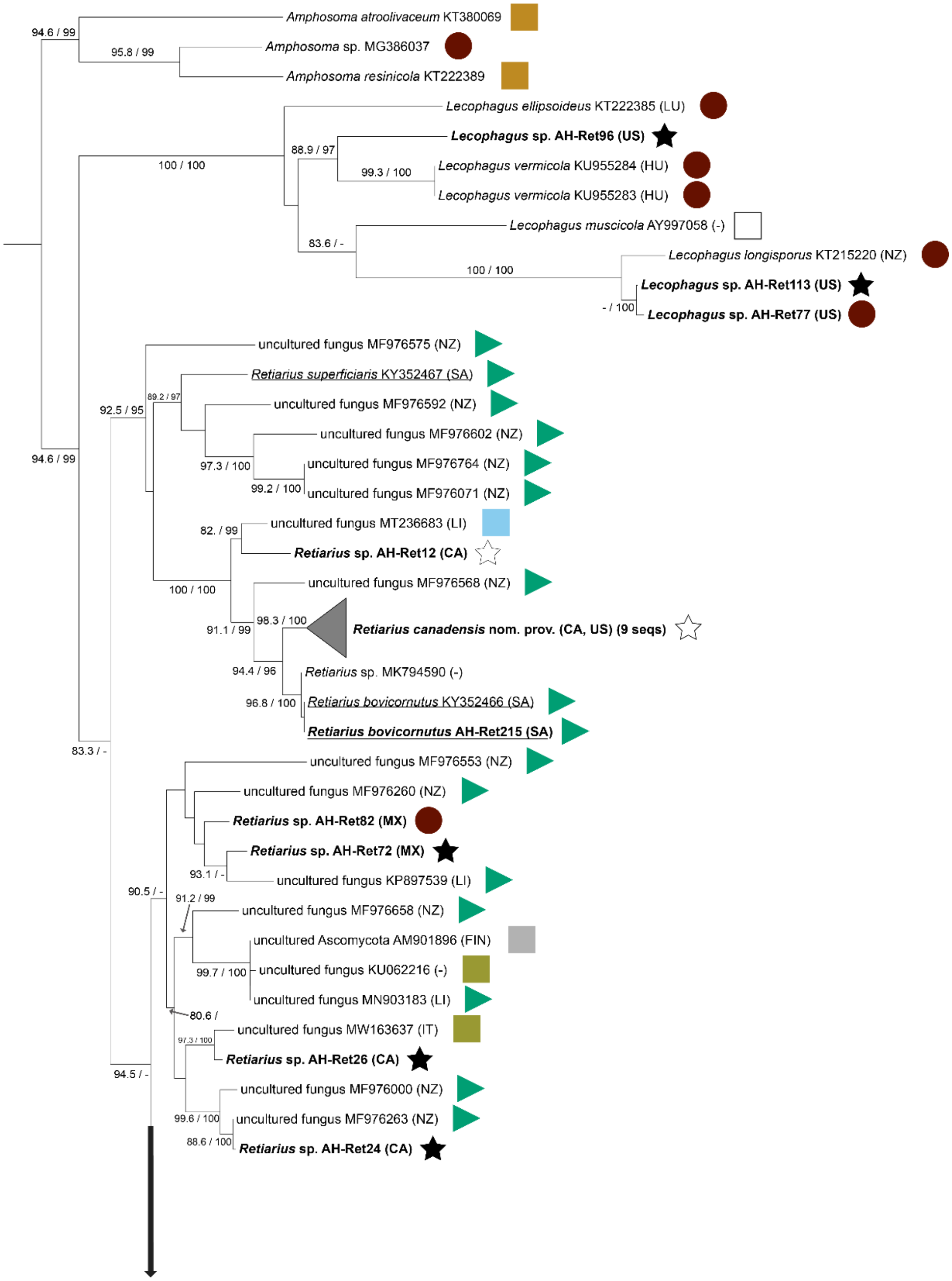

Fig. 7. Maximum likelihood phylogenetic tree ITS region of *Retiarius* and *Lecophagus* samples screened with custom primers for *Retiarius* and environmental sequences with high similarity to *Retiarius* deposited on GenBank, with *Amphosoma* spp. as outgroup. Only values in branches corresponding SH-alrt support ≥80% and ultrafast bootstrap support ≥95. Bold tips are sequences produced in this study, and underlined tips are sequences of known *Retiarius* species. Color legend indicate substrate were produced from. Abbreviations for countries: CA (Canada), CHI (China), FIN (Finland), FR (France), GER (Germany, HU (Hungary), IT (Italy), LT (Lithuania), MX (Mexico), NZ (New Zealand), NO (Norway), SA (South Africa), and US (United States of America).

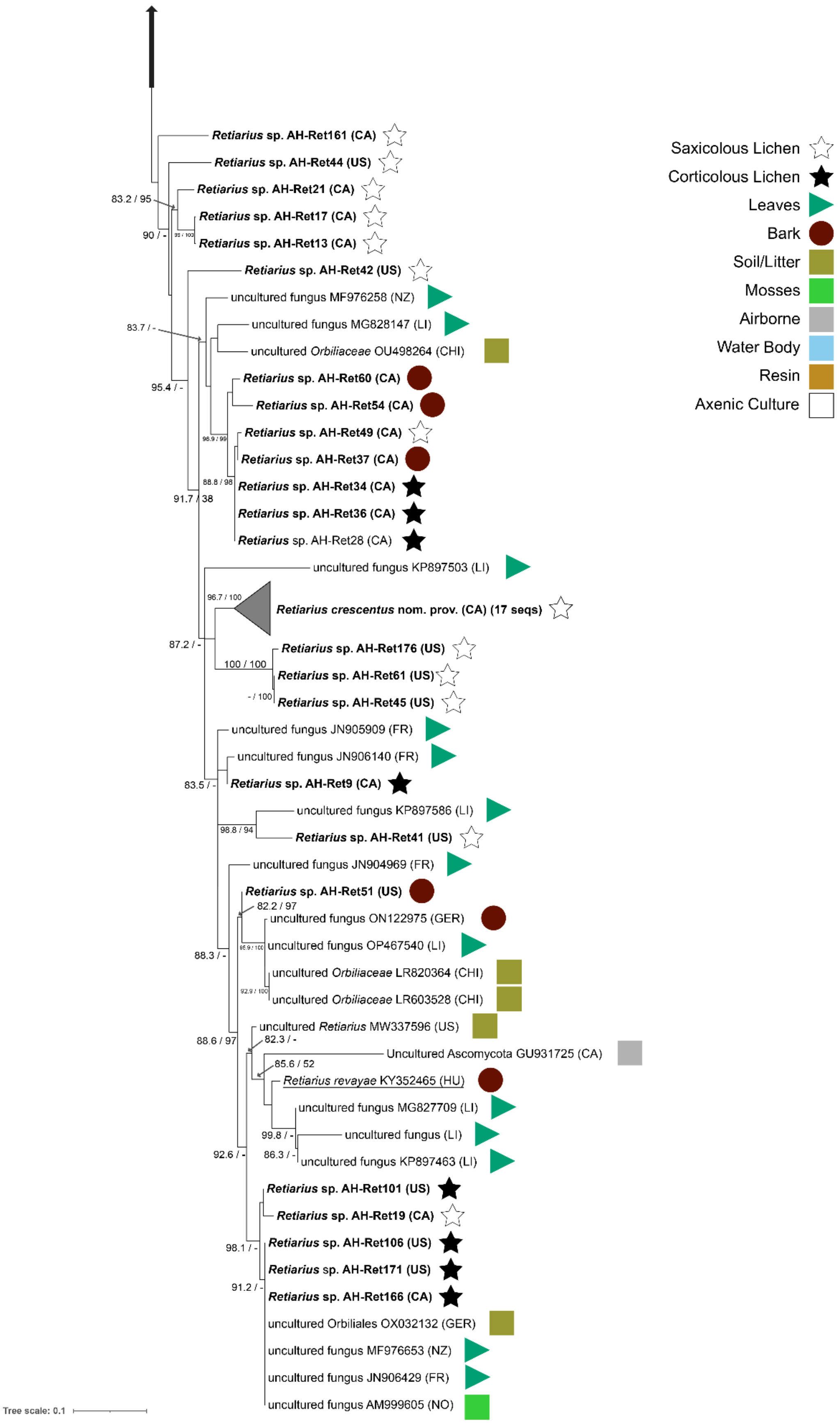

Fig. 7. Continued.

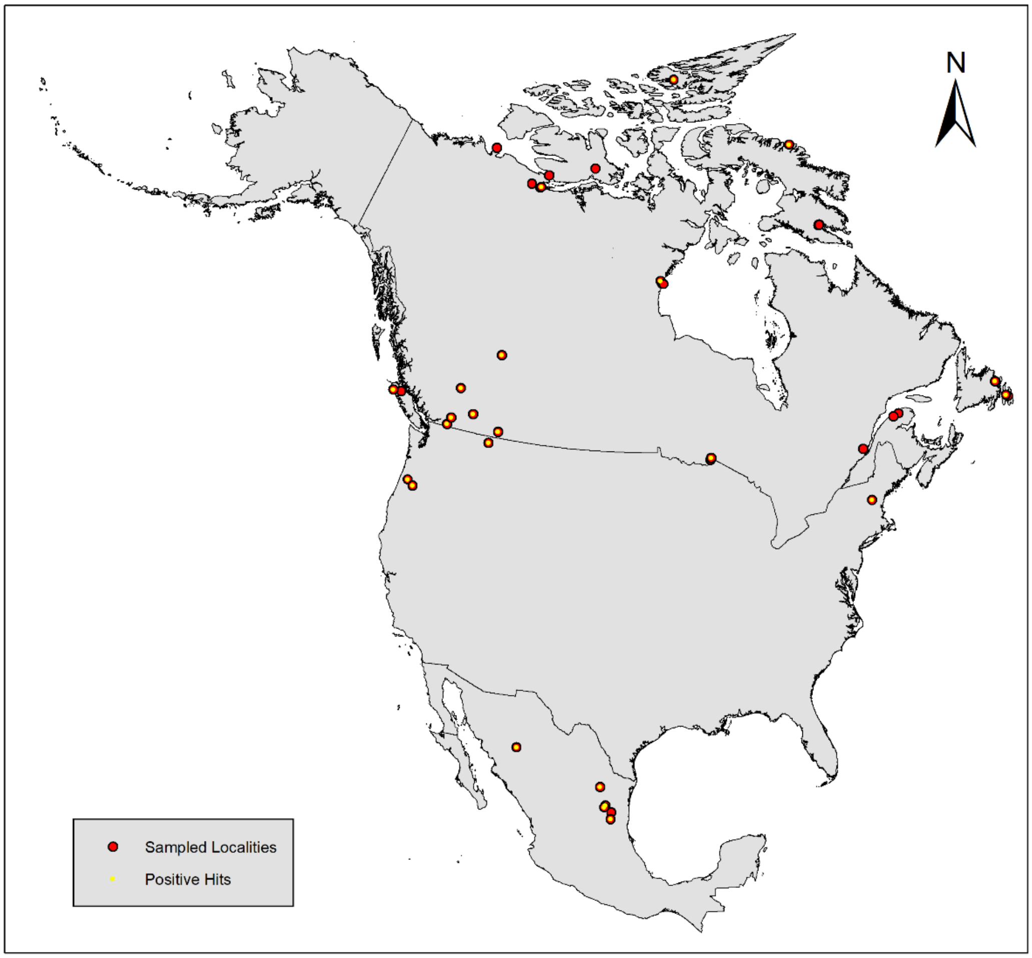

Fig. 8. Map of localities where screened samples of fungi from this study (red dots) showing those that were screened positive for *Retiarius* by PCR (yellow dots). Based on Table 2.

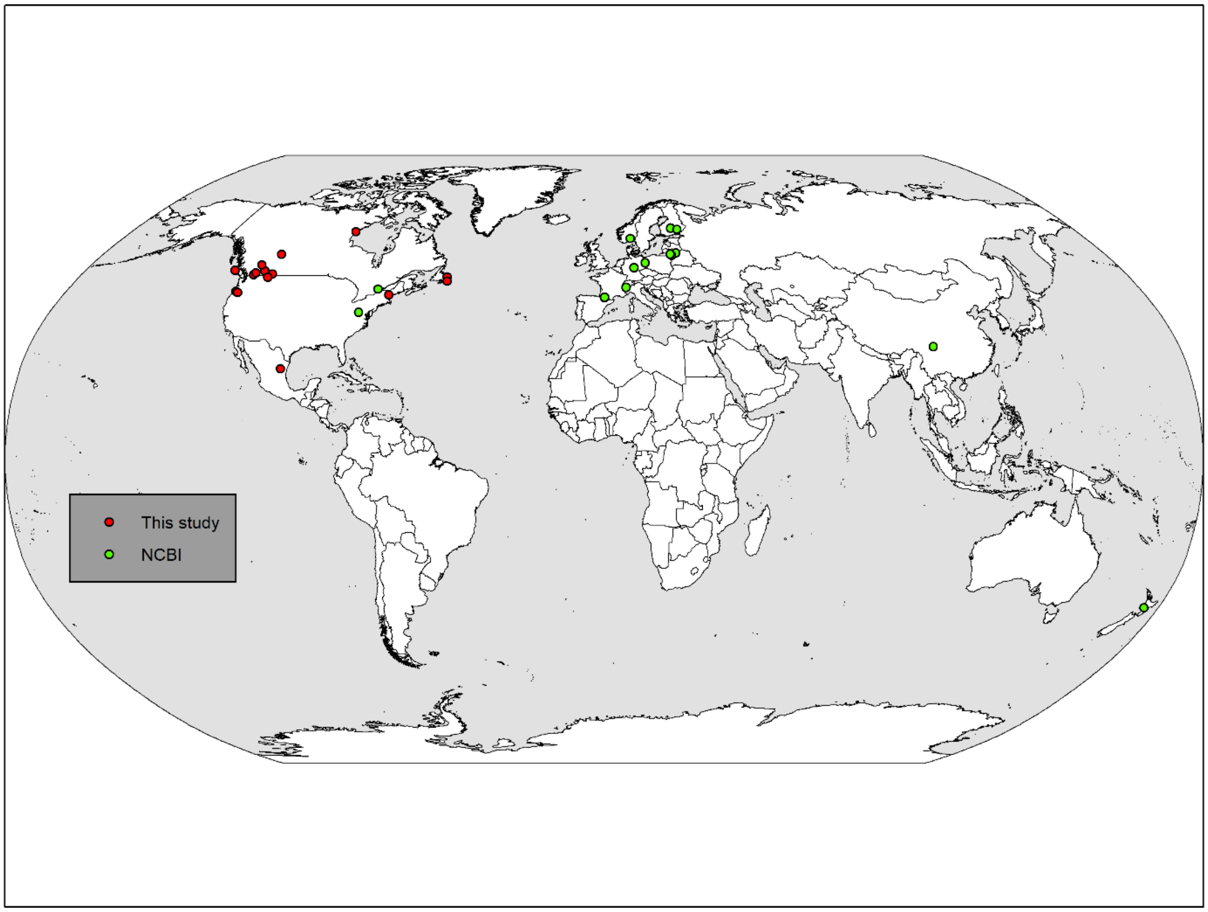

Fig. 9. Map of localities of environmental sequences of *Retiarius* deposited in NCBI (green dots), and produced in this study (red dots). Based on Table 6.

Nutrient source	Growth TSMC102	Rep. 1	Rep. 2	Rep. 3	Growth TSMC84	Rep. 1	Rep. 2	Rep. 3
Negative Control	-	0.05	0.054	0.071	-	0.045	0.056	0.053
L-Arabinose	+	0.118	0.711	0.066	+	0.17	0.142	0.863
N-Acetyl-D-Glucosamine	+	0.508	0.05	0.063	-	0.047	0.046	0.055
D-Saccharic Acid	-	0.047	0.052	0.052	-	0.043	0.046	0.051
Succinic Acid	-	0.055	0.048	0.05	-	0.045	0.055	0.049
D-Galactose	+	0.566	0.058	0.048	±	0.042	0.182	0.059
L-Aspartic Acid	-	0.054	0.053	0.068	-	0.044	0.045	0.049
L-Proline	-	0.056	0.051	0.05	-	0.041	0.045	0.055
D-Alanine	-	0.058	0.072	0.068	±	0.042	0.045	0.14
D-Trehalose	-	0.054	0.076	0.064	-	0.043	0.044	0.047
D-Mannose	±	0.055	0.057	0.142	-	0.05	0.05	0.049
Dulcitol	-	0.047	0.057	0.058	-	0.041	0.042	0.048
D-Serine	+	0.054	0.693	0.049	-	0.05	0.045	0.05
D-Sorbitol	-	0.049	0.048	0.069	+	0.046	0.315	0.052
Glycerol	-	0.051	0.061	0.053	-	0.046	0.051	0.049
L-Fucose	-	0.069	0.05	0.051	+	0.206	0.049	0.07
D-Glucuronic Acid	+	0.061	0.052	0.337	-	0.05	0.052	0.051
D-Gluconic Acid	-	0.048	0.053	0.049	-	0.046	0.046	0.05
D,L-alpha-Glycerolphosphate	-	0.07	0.061	0.081	-	0.046	0.046	0.051
D-Xylose	+	0.047	0.367	0.29	-	0.045	0.046	0.092
L-Lactic Acid	-	0.059	0.054	0.05	-	0.05	0.047	0.05
Formic Acid	-	0.049	0.053	0.056	-	0.047	0.048	0.05
D-Mannitol	+	0.049	0.055	0.314	-	0.048	0.045	0.05
L-Glutamic Acid	-	0.045	0.052	0.051	-	0.042	0.043	0.051
D-Glucose-6-Phosphate	-	0.053	0.059	0.055	-	0.045	0.061	0.047
D-Galactonic Acid-gamma-Lactone	-	0.048	0.051	0.062	-	0.047	0.05	0.052
D,L-Malic Acid	-	0.062	0.059	0.09	-	0.047	0.046	0.054
D-Ribose	±	0.074	0.122	0.053	-	0.067	0.049	0.056
Tween 20	±	0.048	0.114	0.058	-	0.047	0.047	0.052
L-Rhamnose	±	0.064	0.073	0.169	±	0.046	0.047	0.173
D-Fructose	+	0.049	0.135	0.502	-	0.05	0.047	0.05
Acetic Acid	-	0.054	0.056	0.06	-	0.055	0.059	0.062
alpha-D-Glucose	-	0.06	0.066	0.073	-	0.046	0.06	0.048
Maltose	-	0.062	0.052	0.068	+	0.048	0.047	0.295
D-Melibiose	-	0.058	0.049	0.076	-	0.047	0.046	0.064
Thymidine	-	0.056	0.065	0.055	-	0.049	0.044	0.048
L-Asparagine	-	0.047	0.049	0.048	-	0.045	0.052	0.053
D-Aspartic Acid	-	0.061	0.059	0.094	-	0.048	0.048	0.078
D-Glucosaminic Acid	±	0.063	0.05	0.153	-	0.048	0.054	0.055
1,2-Propanediol	-	0.047	0.075	0.091	-	0.057	0.048	0.052
Tween 40	±	0.057	0.062	0.125	+	0.048	0.051	0.717
alpha-Keto-Glutaric Acid	-	0.048	0.06	0.054	-	0.061	0.058	0.078
alpha-Keto-Butyric Acid	-	0.05	0.069	0.054	-	0.065	0.051	0.09
alpha-Methyl-D-Galactoside	-	0.094	0.062	0.08	-	0.049	0.047	0.063
alpha-D-Lactose	-	0.048	0.051	0.073	-	0.047	0.048	0.052
Lactulose	-	0.056	0.051	0.051	-	0.049	0.048	0.049
Sucrose	+	0.375	0.058	0.083	-	0.047	0.047	0.052
Uridine	-	0.051	0.067	0.063	-	0.043	0.046	0.048
L-Glutamine	±	0.053	0.049	0.176	-	0.05	0.043	0.048
m-Tartaric Acid	±	0.05	0.05	0.175	-	0.053	0.046	0.05
D-Glucose-1-Phosphate	-	0.051	0.064	0.078	-	0.05	0.046	0.051
D-Fructose-6-Phosphate	-	0.049	0.066	0.077	-	0.055	0.046	0.051
Tween 80	-	0.045	0.047	0.052	-	0.045	0.049	0.048
alpha-Hydroxy Glutaric Acid-gamma-Lactone	-	0.048	0.066	0.077	-	0.053	0.047	0.077
alpha-Hydroxy Butyric Acid	-	0.067	0.062	0.078	-	0.053	0.053	0.06
beta-Methyl-D-Glucoside	-	0.051	0.068	0.061	-	0.046	0.047	0.055

Adonitol	-	0.053	0.052	0.06	-	0.049	0.048	0.051
Maltotriose	+	0.107	0.067	0.395	-	0.047	0.046	0.05
2-Deoxy Adenosine	-	0.058	0.05	0.073	-	0.047	0.048	0.053
Adenosine	+	0.058	0.056	0.064	-	0.044	0.044	0.05
Glycyl-L-Aspartic Acid	-	0.049	0.048	0.063	-	0.047	0.046	0.052
Citric Acid	-	0.045	0.046	0.066	-	0.045	0.044	0.049
myo-Inositol	-	0.051	0.062	0.052	-	0.048	0.046	0.05
D-Threonine	+	0.048	0.05	0.372	-	0.048	0.047	0.053
Fumaric Acid	-	0.061	0.054	0.077	-	0.049	0.048	0.066
Bromo Succinic Acid	-	0.051	0.052	0.057	-	0.047	0.062	0.05
Propionic Acid	±	0.048	0.151	0.063	-	0.048	0.048	0.053
Mucic Acid	±	0.097	0.072	0.153	±	0.093	0.117	0.102
Glycolic Acid	+	0.311	0.082	0.086	-	0.047	0.047	0.049
Glyoxylic Acid	-	0.061	0.066	0.088	-	0.049	0.047	0.066
D-Cellobiose	±	0.056	0.049	0.107	-	0.045	0.047	0.054
Inosine	+	0.047	0.056	0.603	-	0.045	0.044	0.047
Glycyl-L-Glutamic Acid	-	0.056	0.054	0.083	-	0.048	0.048	0.05
Tricarballylic Acid	-	0.064	0.086	0.069	+	0.05	0.055	0.33
L-Serine	-	0.047	0.048	0.051	-	0.064	0.047	0.052
L-Threonine	+	0.065	0.051	0.62	-	0.048	0.049	0.054
L-Alanine	+	0.06	0.063	0.532	-	0.048	0.047	0.053
L-Alanyl-Glycine	-	0.061	0.053	0.051	±	0.101	0.047	0.051
Acetoacetic Acid	-	0.05	0.056	0.053	-	0.05	0.049	0.066
N-Acetyl-beta-D-Mannosamine	-	0.05	0.048	0.05	-	0.061	0.047	0.052
Mono Methyl Succinate	-	0.049	0.062	0.074	-	0.047	0.047	0.05
Methyl Pyruvate	+	0.114	0.209	0.068	+	0.514	0.092	0.047
D-Malic Acid	-	0.06	0.054	0.067	-	0.045	0.046	0.066
L-Malic Acid	-	0.054	0.061	0.08	-	0.048	0.043	0.046
Glycyl-L-Proline	+	0.042	0.045	0.881	+	0.438	0.052	0.048
p-Hydroxy Phenyl Acetic Acid	-	0.047	0.045	0.054	-	0.045	0.05	0.052
m-Hydroxy Phenyl Acetic Acid	-	0.046	0.047	0.056	-	0.045	0.044	0.049
Tyramine	-	0.046	0.046	0.048	-	0.046	0.046	0.049
D-Psicose	-	0.06	0.045	0.064	+	0.045	0.928	0.05
L-Lyxose	-	0.042	0.064	0.066	-	0.043	0.043	0.049
Glucuronamide	-	0.045	0.046	0.066	+	0.046	0.046	0.203
Pyruvic Acid	-	0.06	0.063	0.093	-	0.046	0.045	0.086
L-Galactonic Acid-gamma-Lactone	-	0.062	0.056	0.062	-	0.046	0.043	0.061
D-Galacturonic Acid	-	0.044	0.057	0.07	-	0.048	0.045	0.046
Phenylethylamine	-	0.044	0.049	0.054	-	0.044	0.045	0.049
2-Aminoethanol	-	0.046	0.061	0.061	-	0.052	0.045	0.052

Fig. 10. Utilization of carbon sources by two species of *Retiarius*: *R. canadensis* nom. prov. (TSMC102) and *R. crescentus* nom. prov. (TSMC84), using PM1 Biolog Phenotype MicroArray™ in triplicates. Optical density (OD_{750}) for each replicate represents mycelial growth on each well for 35 days. Growth is interpreted on at least one well: below 0.1 as negative growth (-), between 0.1-0.2 as dubious growth (±), and above 0.2 as positive growth (+). Respective growth is given by the following colors: gray below 0.1, green between 0.1 and 0.2, yellow 0.2 and 0.5, orange 0.5 and 1.0. and dark red above 1.0.

Nutrient source	Growth TSMC102	Rep. 1	Rep. 2	Rep. 3	Growth TSMC84	Rep. 1	Rep. 2	Rep. 3
Negative Control	-	0.048	0.048	0.056	-	0.044	0.055	0.048
Chondroitin Sulfate C	-	0.071	0.052	0.049	±	0.041	0.042	0.177
alpha-Cyclodextrin	+	0.442	0.656	0.313	+	0.461	0.319	0.288
beta-Cyclodextrin	±	0.071	0.069	0.108	-	0.067	0.063	0.057
gamma-Cyclodextrin	±	0.069	0.06	0.128	-	0.079	0.067	0.057
Dextrin	-	0.046	0.05	0.049	-	0.044	0.044	0.049
Gelatin	-	0.095	0.065	0.072	±	0.075	0.174	0.052
Glycogen	-	0.054	0.051	0.049	-	0.049	0.049	0.05
Inulin	-	0.064	0.072	0.077	±	0.058	0.098	0.101
Laminarin	-	0.05	0.065	0.049	-	0.048	0.066	0.05
Mannan	-	0.05	0.051	0.052	-	0.048	0.045	0.048
Pectin	-	0.052	0.049	0.054	+	0.242	0.051	0.05
N-Acetyl-D-Galactosamine	-	0.047	0.054	0.06	-	0.049	0.045	0.047
N-Acetyl-Neuraminic Acid	+	0.046	0.067	0.866	±	0.115	0.046	0.048
beta-D-Allose	-	0.047	0.06	0.073	-	0.045	0.057	0.048
Amygdalin	-	0.05	0.052	0.058	-	0.047	0.046	0.048
D-Arabinose	-	0.066	0.05	0.052	-	0.06	0.063	0.096
D-Arabitol	-	0.047	0.049	0.065	-	0.046	0.052	0.049
L-Arabitol	-	0.047	0.047	0.061	-	0.045	0.045	0.047
Arbutin	-	0.048	0.049	0.057	-	0.047	0.046	0.047
2-Deoxy-D-Ribose	-	0.049	0.049	0.079	-	0.047	0.049	0.051
i-Erythritol	-	0.051	0.049	0.051	±	0.156	0.11	0.049
D-Fucose	-	0.063	0.047	0.073	-	0.046	0.044	0.048
3-0-beta-D-Galactopyranosyl-D-Arabinose	-	0.046	0.047	0.046	-	0.046	0.046	0.046
Gentiobiose	-	0.078	0.056	0.058	-	0.044	0.043	0.048
L-Glucose	-	0.059	0.058	0.082	-	0.046	0.045	0.047
Lactitol	±	0.067	0.047	0.14	-	0.047	0.045	0.048
D-Melezitose	-	0.067	0.055	0.072	-	0.053	0.046	0.058
Maltitol	-	0.059	0.049	0.07	-	0.047	0.046	0.061
alpha-Methyl-D-Glucoside	-	0.048	0.056	0.074	-	0.047	0.046	0.051
beta-Methyl-D-Galactoside	-	0.05	0.048	0.05	-	0.046	0.046	0.055
3-Methyl Glucose	-	0.064	0.067	0.049	-	0.05	0.045	0.071
beta-Methyl-D-Glucuronic Acid	-	0.048	0.05	0.051	-	0.046	0.05	0.05
alpha-Methyl-D-Mannoside	-	0.053	0.058	0.076	-	0.048	0.047	0.055
beta-Methyl-D-Xyloside	-	0.05	0.048	0.048	-	0.046	0.046	0.047
Palatinose	±	0.059	0.055	0.141	-	0.049	0.044	0.047
D-Raffinose	-	0.048	0.045	0.052	-	0.044	0.046	0.05
Salicin	-	0.049	0.047	0.064	-	0.045	0.045	0.054
Sedoheptulosan	-	0.053	0.05	0.097	-	0.046	0.072	0.049
L-Sorbose	-	0.06	0.06	0.073	-	0.046	0.045	0.048
Stachyose	-	0.066	0.048	0.067	-	0.046	0.046	0.049
D-Tagatose	+	0.048	0.047	1.048	-	0.045	0.046	0.07
Turanose	+	0.058	0.053	0.451	-	0.046	0.047	0.049
Xylitol	-	0.049	0.048	0.067	-	0.047	0.045	0.05
N-Acetyl-D-Glucosaminitol	-	0.052	0.074	0.05	-	0.047	0.046	0.051
gamma-Amino Butyric Acid	-	0.053	0.053	0.08	-	0.046	0.048	0.077
delta-Amino Valeric Acid	±	0.124	0.047	0.051	-	0.069	0.045	0.086
Butyric Acid	-	0.048	0.048	0.051	-	0.049	0.046	0.047
Capric Acid	±	0.116	0.134	0.145	±	0.141	0.12	0.108
Caproic Acid	-	0.052	0.049	0.056	-	0.046	0.046	0.049
Citraconic Acid	-	0.049	0.064	0.075	-	0.047	0.045	0.05
Citramalic Acid	-	0.046	0.048	0.067	-	0.045	0.045	0.047
D-Glucosamine	-	0.048	0.047	0.05	-	0.047	0.046	0.048
2-Hydroxy Benzoic Acid	±	0.052	0.049	0.108	-	0.047	0.046	0.049

4-Hydroxy Benzoic Acid	-	0.059	0.055	0.062	-	0.047	0.046	0.053
beta-Hydroxy Butyric Acid	-	0.064	0.048	0.076	-	0.047	0.046	0.051
Glycolic Acid	+	0.06	0.049	0.641	-	0.061	0.048	0.064
alpha-Keto-Valeric Acid	-	0.05	0.049	0.052	-	0.066	0.084	0.074
Itaconic Acid	-	0.064	0.047	0.077	-	0.06	0.045	0.047
5-Keto-D-Gluconic Acid	-	0.046	0.055	0.047	-	0.045	0.047	0.046
D-Lactic Acid Methyl Ester	-	0.048	0.047	0.068	-	0.043	0.043	0.047
Malonic Acid	-	0.06	0.058	0.048	-	0.045	0.045	0.048
Melibionic Acid	-	0.065	0.054	0.058	+	0.051	0.07	0.318
Oxalic Acid	±	0.15	0.118	0.143	±	0.158	0.135	0.14
Oxalomalic Acid	-	0.046	0.046	0.047	-	0.046	0.044	0.047
Quinic Acid	-	0.05	0.059	0.048	-	0.046	0.045	0.047
D-Ribono-1,4-Lactone	±	0.139	0.046	0.079	-	0.07	0.067	0.048
Sebacic Acid	-	0.054	0.061	0.091	-	0.049	0.061	0.058
Sorbic Acid	-	0.05	0.052	0.053	-	0.047	0.049	0.049
Succinamic Acid	-	0.05	0.063	0.083	-	0.046	0.046	0.05
D-Tartaric Acid	-	0.05	0.05	0.051	±	0.045	0.044	0.146
L-Tartaric Acid	-	0.058	0.046	0.051	-	0.046	0.044	0.046
Acetamide	-	0.046	0.05	0.051	-	0.044	0.045	0.048
L-Alaninamide	-	0.051	0.051	0.07	-	0.046	0.046	0.068
N-Acetyl-L-Glutamic Acid	-	0.049	0.049	0.051	-	0.046	0.045	0.054
L-Arginine	-	0.051	0.062	0.074	-	0.048	0.045	0.055
Glycine	-	0.061	0.064	0.099	-	0.047	0.046	0.055
L-Histidine	-	0.051	0.047	0.072	-	0.046	0.046	0.056
L-Homoserine	-	0.051	0.047	0.074	-	0.046	0.045	0.051
Hydroxy-L-Proline	-	0.059	0.06	0.049	-	0.046	0.044	0.05
L-Isoleucine	-	0.06	0.048	0.072	-	0.046	0.047	0.048
L-Leucine	-	0.05	0.061	0.073	-	0.046	0.046	0.053
L-Lysine	-	0.058	0.059	0.082	-	0.045	0.046	0.049
L-Methionine	±	0.054	0.163	0.065	-	0.049	0.045	0.045
L-Ornithine	+	0.348	0.057	0.07	-	0.044	0.041	0.048
L-Phenylalanine	-	0.046	0.057	0.066	-	0.056	0.043	0.048
L-Pyroglutamic Acid	-	0.046	0.054	0.063	-	0.051	0.044	0.049
L-Valine	-	0.054	0.046	0.067	-	0.044	0.044	0.047
D,L-Carnitine	-	0.045	0.058	0.049	-	0.057	0.042	0.048
Sec-Butylamine	-	0.046	0.058	0.052	-	0.044	0.044	0.045
D,L-Octopamine	-	0.047	0.047	0.058	-	0.046	0.042	0.047
Putrescine	-	0.047	0.049	0.073	-	0.046	0.045	0.05
Dihydroxy Acetone	-	0.077	0.056	0.073	-	0.042	0.055	0.047
2,3-Butanediol	+	0.045	0.056	0.253	-	0.042	0.043	0.046
2,3-Butanedione		0.049	0.057	0.065	-	0.071	0.046	0.048
3-Hydroxy-2-Butanone	-	0.044	0.061	0.046	+	0.456	0.069	0.057

Fig. 11. Utilization of carbon sources by two species of *Retiarius*: *R. canadensis* nom. prov. (TSMC102) and *R. crescentus* nom. prov. (TSMC84), using PM2A Biolog Phenotype MicroArray^{TM} in triplicates. Optical density (OD_{750}) for each replicate represents mycelial growth on each well for 35 days. Growth is interpreted on at least one well: below 0.1 as negative growth (-), between 0.1-0.2 as dubious growth (±), and above 0.2 as positive growth (+). Respective growth is given by the following colors: gray below 0.1, green between 0.1 and 0.2, yellow 0.2 and 0.5, orange 0.5 and 1.0. and dark red above 1.0.

Nutrient source	**Growth** TSMC102	**Rep. 1**	**Rep. 2**	**Rep. 3**	**Growth** TSMC84	**Rep. 1**	**Rep. 2**	**Rep. 3**
Negative Control	+	0.045	0.208	0.198	-	0.051	0.051	0.048
Ammonia	+	0.044	0.071	1.015	±	0.058	0.044	0.141
Nitrite	-	0.046	0.047	0.061	-	0.045	0.046	0.049
Nitrate	+	0.438	0.102	0.222	+	0.237	0.048	0.192
Urea	+	1.355	0.822	0.057	-	0.042	0.045	0.048
Biuret	-	0.071	0.044	0.047	+	0.49	0.044	0.107
L-Alanine	+	1.319	0.995	0.047	-	0.043	0.047	0.045
L-Arginine	+	0.375	0.044	0.768	+	0.43	0.694	0.297
L-Asparagine	+	1.404	0.044	0.049	-	0.046	0.049	0.046
L-Aspartic Acid	+	1.318	0.056	0.061	+	0.046	0.075	1.165
L-Cysteine	±	0.05	0.147	0.158	±	0.075	0.184	0.092
L-Glutamic Acid	+	0.041	0.741	0.073	+	0.041	0.21	0.071
L-Glutamine	+	0.715	0.044	0.106	+	0.044	0.426	0.049
Glycine	+	0.046	0.862	0.092	+	0.146	0.299	0.166
L-Histidine	+	0.757	0.072	0.246	-	0.056	0.053	0.051
L-Isoleucine	+	1.297	0.182	0.183	-	0.073	0.06	0.047
L-Leucine	+	0.048	0.048	0.284	±	0.057	0.179	0.08
L-Lysine	+	0.97	0.048	0.377	-	0.046	0.059	0.047
L-Methionine	+	0.328	0.047	0.24	-	0.045	0.046	0.066
L-Phenylalanine	+	0.046	1.038	0.053	±	0.07	0.197	0.049
L-Proline	+	0.051	1.118	0.056	+	0.046	0.655	0.464
L-Serine	-	0.048	0.048	0.056	+	0.256	0.048	0.047
L-Threonine	+	0.695	0.641	0.059	±	0.174	0.051	0.046
L-Tryptophan	-	0.046	0.054	0.056	-	0.058	0.075	0.044
L-Tyrosine	+	0.442	0.658	0.607	-	0.088	0.09	0.07
L-Valine	+	0.046	0.354	0.052	±	0.08	0.129	0.056
D-Alanine	+	0.544	1.309	0.177	+	0.046	0.045	0.325
D-Asparagine	+	0.053	0.302	1.002	-	0.054	0.051	0.075
D-Aspartic Acid	+	0.594	0.048	0.121	-	0.052	0.051	0.048
D-Glutamic Acid	+	0.162	0.207	0.071	-	0.05	0.053	0.057
D-Lysine	±	0.181	0.049	0.058	-	0.049	0.046	0.047
D-Serine	±	0.171	0.057	0.062	-	0.051	0.05	0.057
D-Valine	-	0.05	0.094	0.057	-	0.057	0.058	0.049
L-Citrulline	+	1.163	0.046	0.61	±	0.137	0.048	0.048
L-Homoserine	+	0.076	0.842	0.087	+	0.427	0.749	0.046
L-Ornithine	+	0.046	1.048	0.491	+	0.744	0.054	1.557
N-Acetyl-L-Glutamic Acid	±	0.173	0.045	0.056	±	0.169	0.089	0.174
N-Phthaloyl-L-Glutamic Acid	+	0.271	0.239	0.512	+	0.048	0.389	0.656
L-Pyroglutamic Acid	±	0.054	0.048	0.172	-	0.047	0.046	0.049
Hydroxylamine	-	0.052	0.096	0.06	+	0.744	0.234	0.047
Methylamine	-	0.057	0.048	0.057	-	0.051	0.046	0.07
N-Amylamine	-	0.048	0.051	0.079	-	0.051	0.047	0.047
N-Butylamine	-	0.048	0.051	0.057	-	0.046	0.049	0.05
Ethylamine	-	0.053	0.048	0.088	±	0.047	0.144	0.05
Ethanolamine	+	0.051	0.752	0.052	+	0.114	0.267	0.318
Ethylenediamine	±	0.051	0.16	0.113	-	0.055	0.051	0.059
Putrescine	-	0.048	0.047	0.05	+	0.071	0.047	0.352
Agmatine	±	0.17	0.086	0.169	-	0.055	0.054	0.045
Histamine	±	0.046	0.182	0.052	±	0.058	0.044	0.133
beta-Phenylethyl-amine	-	0.058	0.081	0.066	±	0.05	0.045	0.145
Tyramine	+	0.048	0.432	0.197	+	0.09	0.108	0.575
Acetamide	+	0.14	0.047	0.379	±	0.082	0.192	0.125
Formamide	-	0.048	0.065	0.057	+	0.614	0.209	0.114
Glucuronamide	±	0.049	0.047	0.104	+	0.296	0.403	0.046
D,L-Lactamide	+	1.122	0.471	0.536	+	0.376	0.421	0.049
D-Glucosamine	-	0.092	0.046	0.068	±	0.114	0.047	0.048
D-Galactosamine	+	0.429	0.392	0.062	±	0.046	0.196	0.197
D-Mannosamine	+	0.294	0.05	0.156	+	0.134	0.049	0.217
N-Acetyl-D-glucosamine	+	0.158	0.511	0.087	-	0.045	0.046	0.047

N-Acetyl-D-galactosamine	-	0.064	0.042	0.053	±	0.176	0.043	0.052
N-Acetyl-D-mannosamine	±	0.131	0.052	0.051	+	0.17	0.043	0.235
Adenine	+	0.051	0.606	0.291	+	0.045	0.05	0.607
Adenosine	+	0.269	0.047	0.089	-	0.045	0.053	0.047
Cytidine	±	0.053	0.054	0.183	+	0.228	0.089	0.052
Cytosine	+	0.059	1.288	0.095	+	0.555	1.036	0.26
Guanine	+	0.256	0.103	0.313	-	0.054	0.052	0.05
Guanosine	±	0.073	0.049	0.197	-	0.048	0.051	0.048
Thymine	±	0.135	0.064	0.063	-	0.057	0.059	0.096
Thymidine	-	0.073	0.046	0.059	+	0.045	0.048	0.214
Uracil	-	0.051	0.05	0.063	-	0.067	0.088	0.049
Uridine	-	0.087	0.046	0.052	±	0.046	0.121	0.152
Inosine	+	0.445	0.054	0.095	+	0.045	0.942	0.105
Xanthine	-	0.053	0.053	0.056	-	0.052	0.052	0.056
Xanthosine	-	0.048	0.047	0.054	-	0.046	0.046	0.05
Uric Acid	+	0.059	0.244	0.437	+	0.053	0.06	0.374
Alloxan	-	0.05	0.049	0.059	-	0.05	0.074	0.048
Allantoin	+	0.048	0.365	0.086	+	0.046	0.047	0.204
Parabanic Acid	+	0.262	0.045	0.076	+	0.247	0.632	0.63
D,L-alpha-Amino-N-Butyric Acid	+	0.328	0.445	0.513	+	0.046	0.046	0.238
gamma-Amino-N-Butyric Acid	+	0.257	0.299	0.353	-	0.073	0.049	0.075
epsilon-Amino-N-Caproic Acid	+	0.051	0.216	0.059	+	0.046	0.54	0.048
D,L-alpha-Amino-Caprylic Acid	-	0.072	0.057	0.065	-	0.056	0.056	0.063
delta-Amino-N-Valeric Acid	-	0.047	0.054	0.05	+	0.193	0.405	0.187
alpha-Amino-N-Valeric Acid	+	0.657	0.055	0.333	-	0.045	0.044	0.045
Ala-Asp	+	0.945	0.195	0.056	+	0.043	0.051	1.404
Ala-Gln	+	0.264	0.927	1.335	+	0.045	0.29	0.471
Ala-Glu	+	0.081	0.045	0.262	-	0.048	0.043	0.048
Ala-Gly	+	0.879	0.652	0.149	+	0.043	0.043	0.793
Ala-His	-	0.056	0.047	0.057	-	0.045	0.046	0.046
Ala-Leu	+	0.048	1.11	0.239	+	0.074	0.068	0.5
Ala-Thr	+	1.433	0.044	0.243	+	0.083	0.201	0.494
Gly-Asn	+	0.641	0.849	0.071	-	0.044	0.043	0.043
Gly-Gln	+	0.179	1.251	0.334	+	0.073	0.042	0.303
Gly-Glu	+	0.046	0.734	0.077	-	0.066	0.043	0.044
Gly-Met	+	0.044	0.764	0.225	-	0.055	0.043	0.045
Met-Ala	+	0.317	0.047	0.091	-	0.052	0.048	0.048

Fig. 12. Utilization of nitrogen sources by two species of *Retiarius*: *R. canadensis* nom. prov. (TSMC102) and *R. crescentus* nom. prov. (TSMC84), using PM3B Biolog Phenotype MicroArray^{TM} in triplicates. Optical density (OD$_{750}$) for each replicate represents mycelial growth on each well for 35 days. Growth is interpreted on at least one well: below 0.1 as negative growth (-), between 0.1-0.2 as dubious growth (±), and above 0.2 as positive growth (+). Respective growth is given by the following colors: gray below 0.1, green between 0.1 and 0.2, yellow 0.2 and 0.5, orange 0.5 and 1.0. and dark red above 1.0.

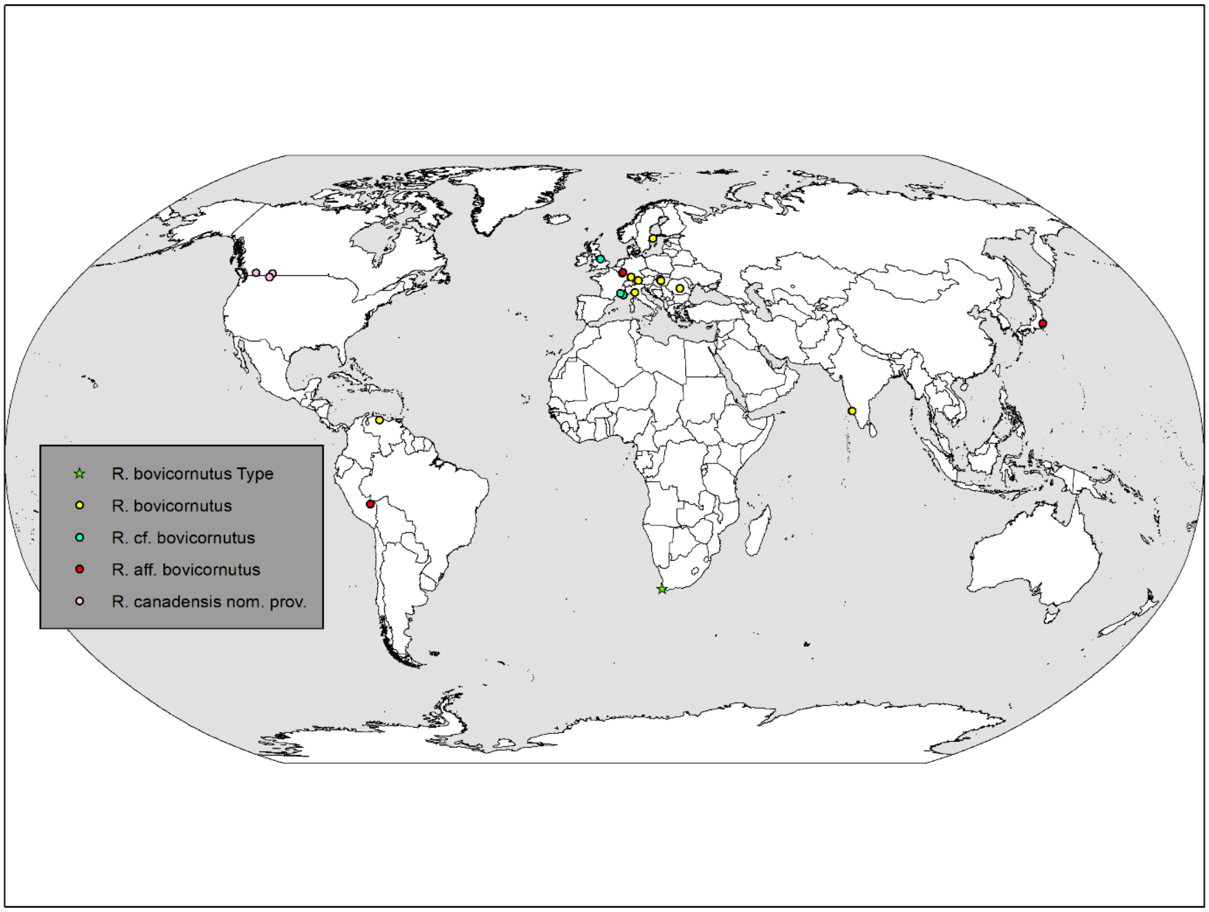

Fig. 13. Map showing the distribution of *Retiarius canadensis* nom. prov. and *R. bovicornutus* aggregate.

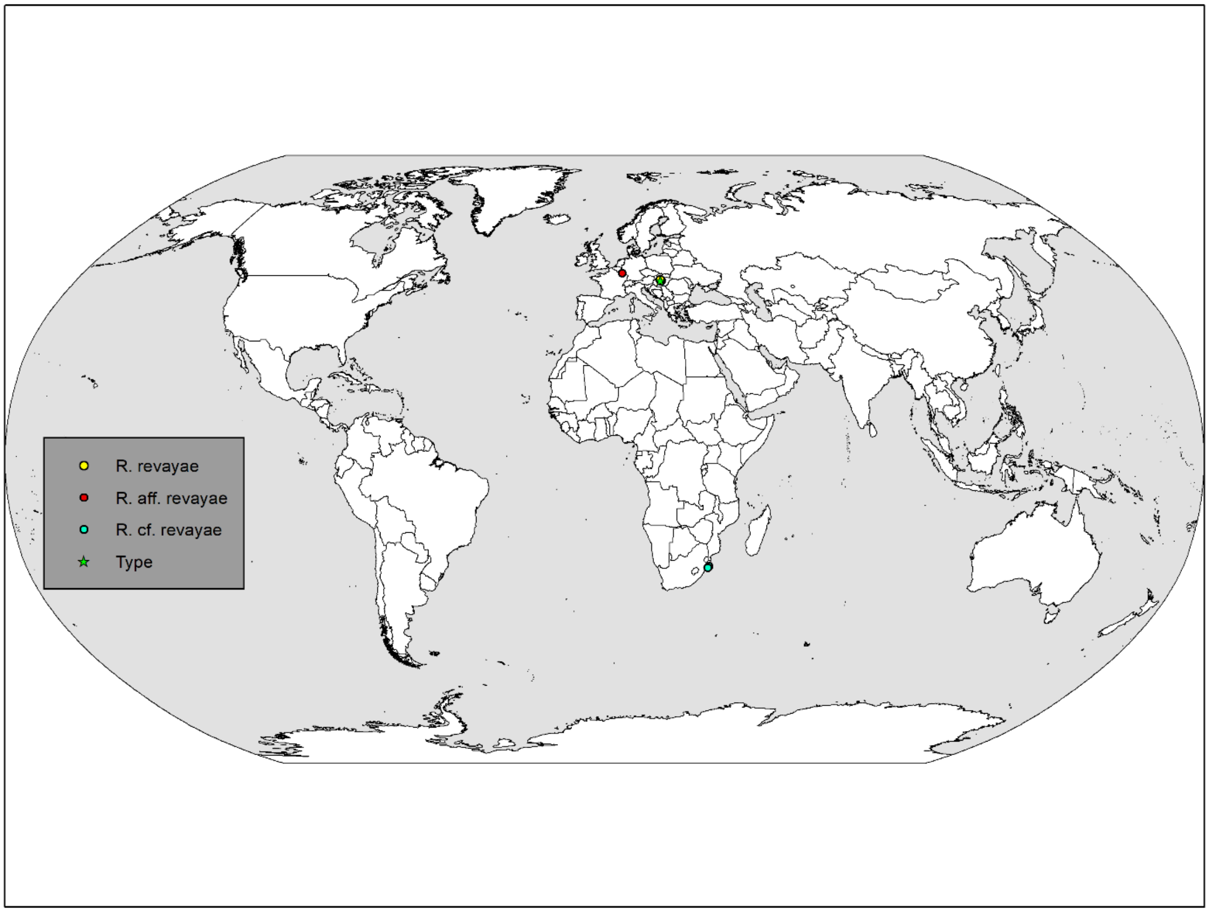

Fig. 14. Map of the distribution of *Retiarius revayae* aggregate.

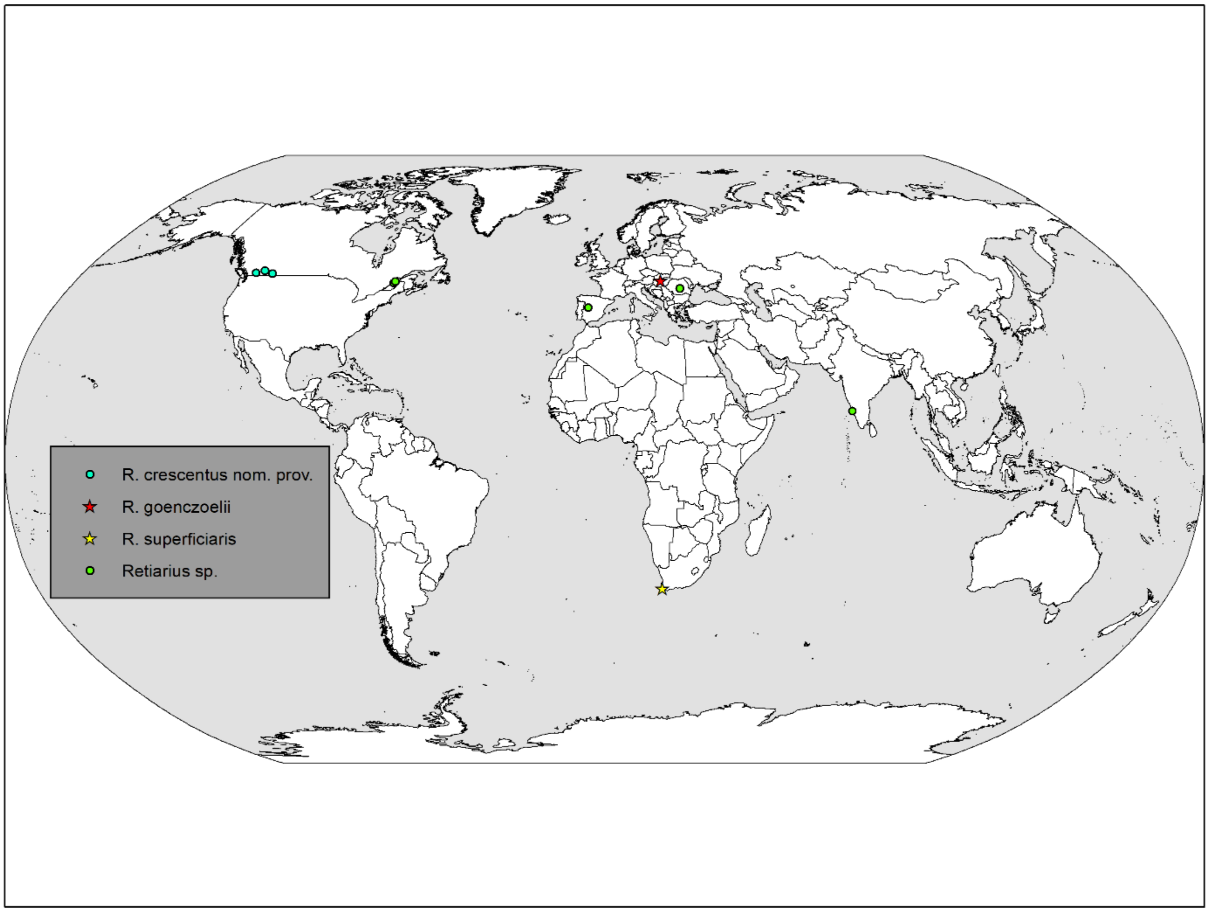

Fig. 15. Map of the distribution of *Retiarius crescentus* nom. prov., *R. goenczoelii, R. superficiaris* and records of undetermined *Retiarius* species.

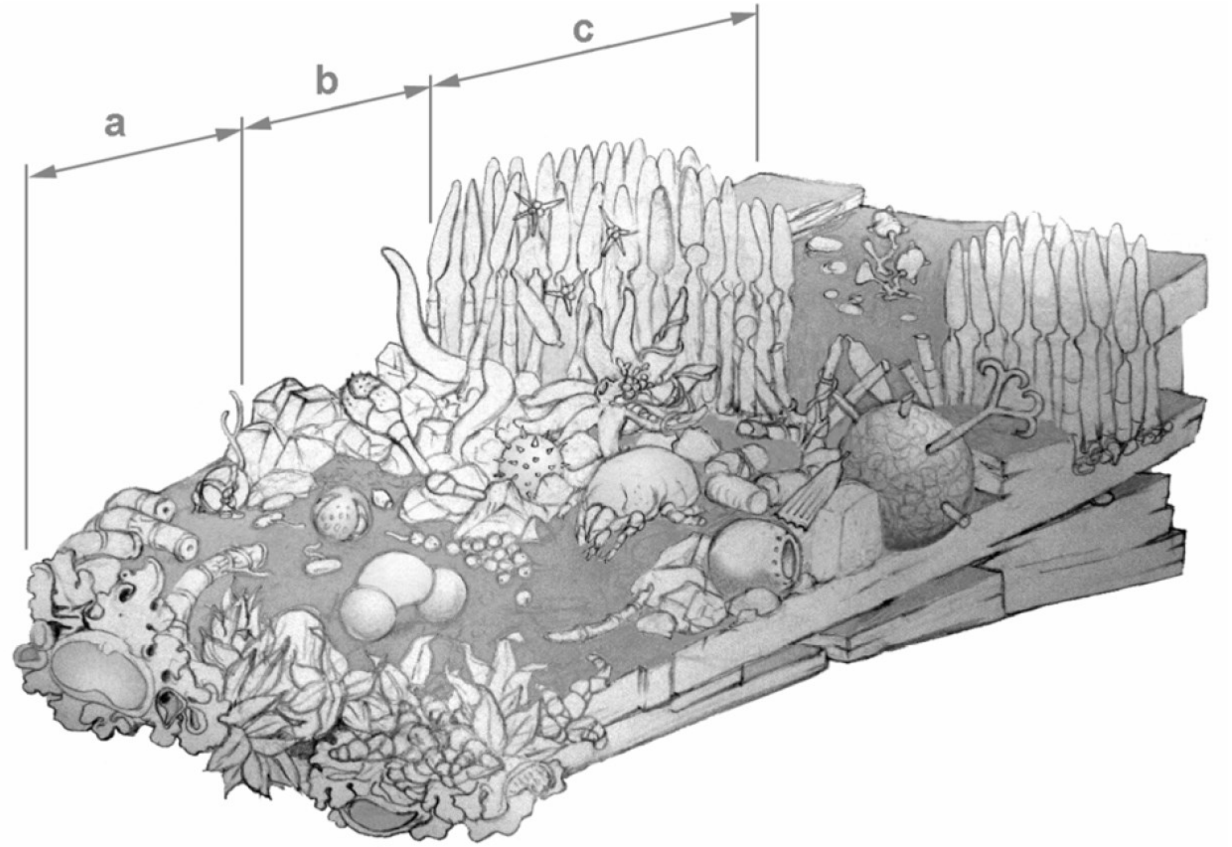

Fig. 16. Microhabitat zonation in bark fissures. a) Outer zone (lichens, melanized fungal mycelia, mosses); b) Deposition zone (inorganic dust, mites, nematodes, pollen grains, spores, testate amoebae); c) Inner zone (dematiaceous hyphomycetes). Reproduced from Fig. 1 in Magyar 2008

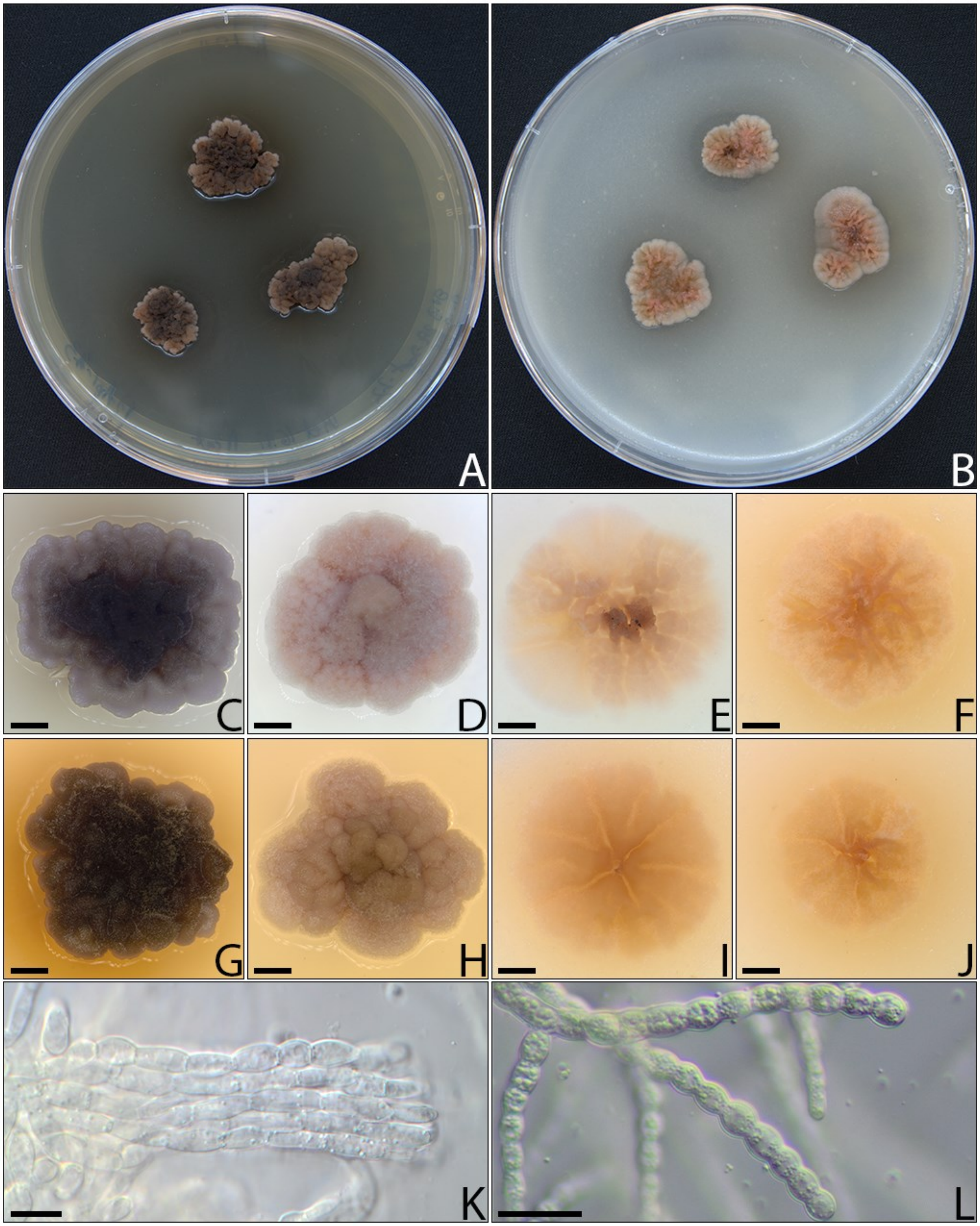

Fig. 17. Morphological features of *Retiarius canadensis* nom. prov. A, C, D, G, H: colonies on PDA; B, E., F. I, J: colonies on OA; K: somatic hyphae; L: chlamydospores. Scales: C, D, E, F, G, H, I, J: 2 mm, K: 10 µm, L: 20 µm.

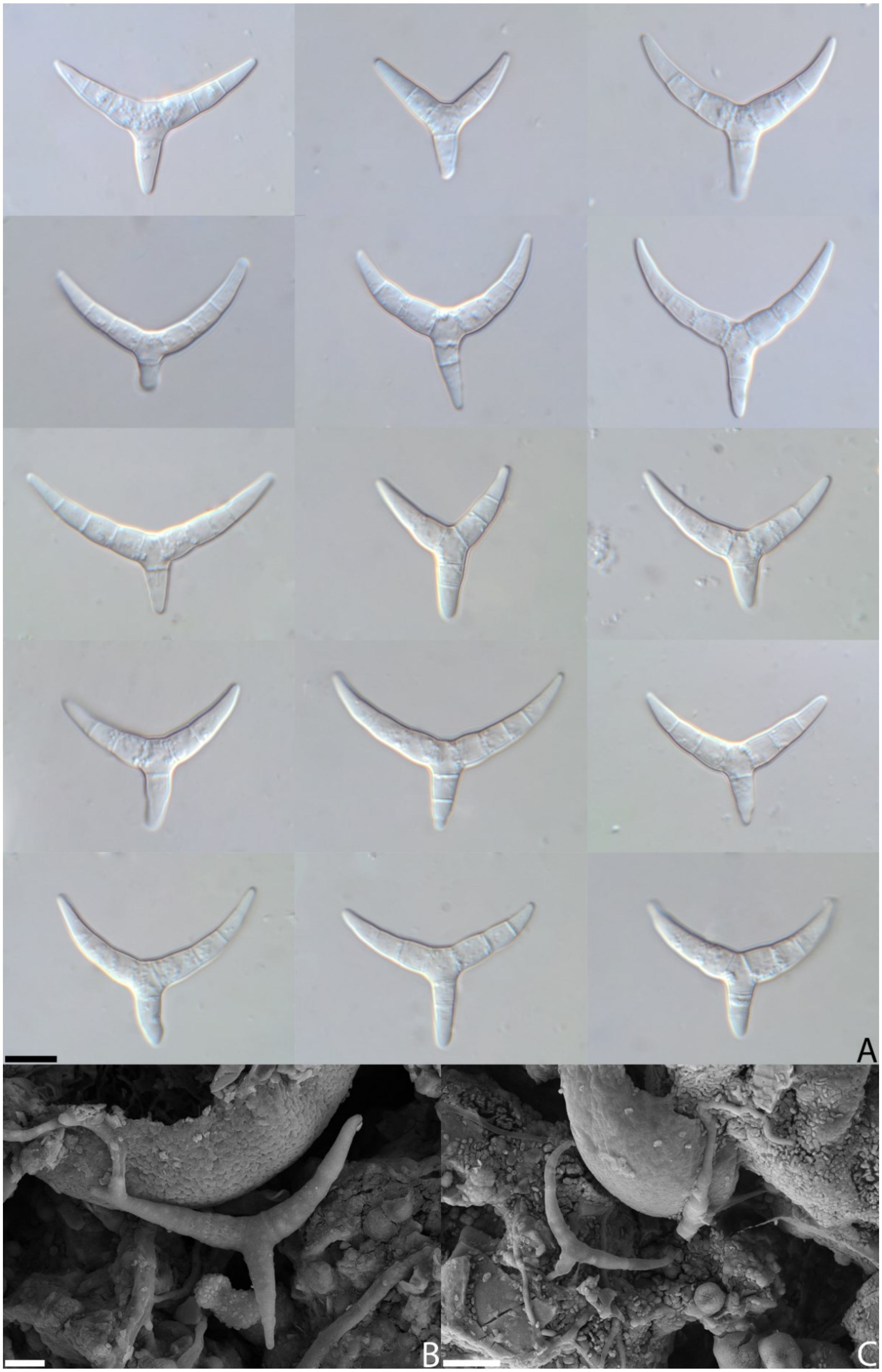

Fig. 18. Conidia of *Retiarius canadensis* nom. prov. A: Conidium in water. B, C: SEM micrograph conidium on pollen grain in undersurface of *Umbilicaria torrefacta*. Scales A: 10 µm; B: 4 µm; C: 10 µm.

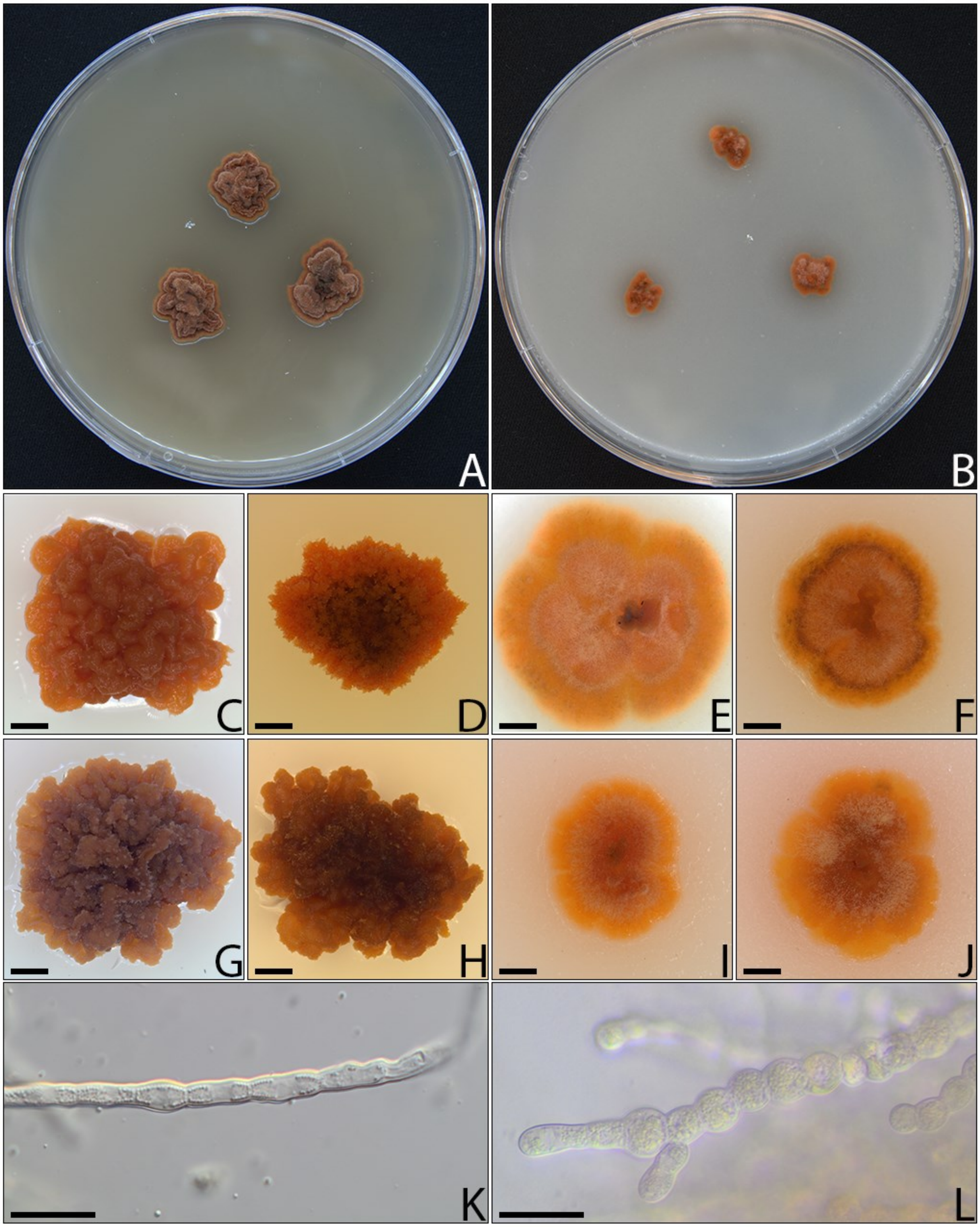

Fig. 19. Morphological features of *Retiarius crescentus* nom. prov. A, C, D, G, H: colonies on PDA; B, E., F. I, J: colonies on OA; K: somatic hyphae; L: chlamydospores. Scales: C, D, E, F, G, H, I, J: 2 mm, K: 10 µm, L: 20 µm.

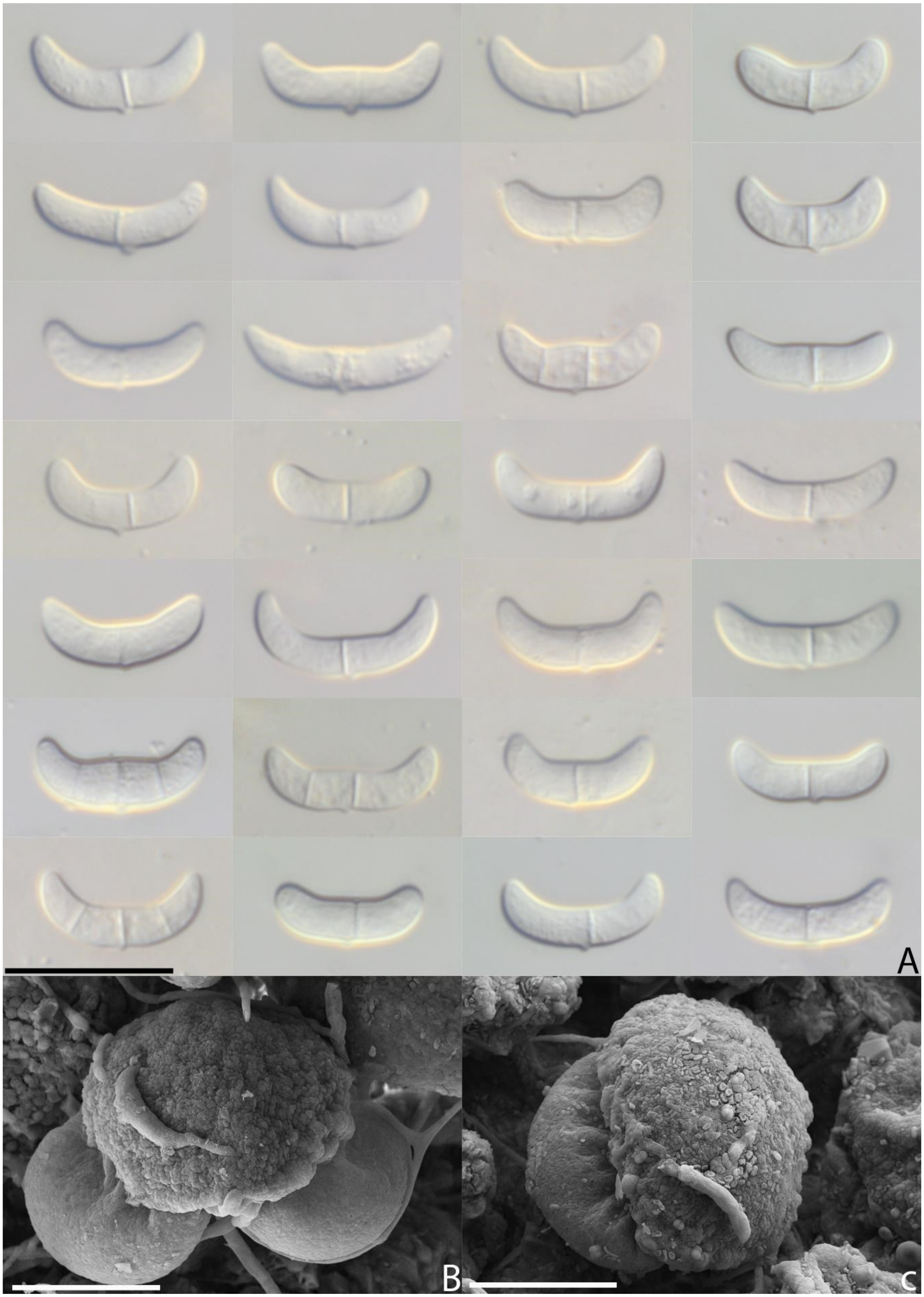

Fig. 20. Conidia of *Retiarius crescentus* nom. prov. A: Conidium in water. B, C: SEM micrograph conidium on pollen grain in undersurface of *Umbilicaria phaea*. Scales A: 10 μm; B, C: 10 μm.

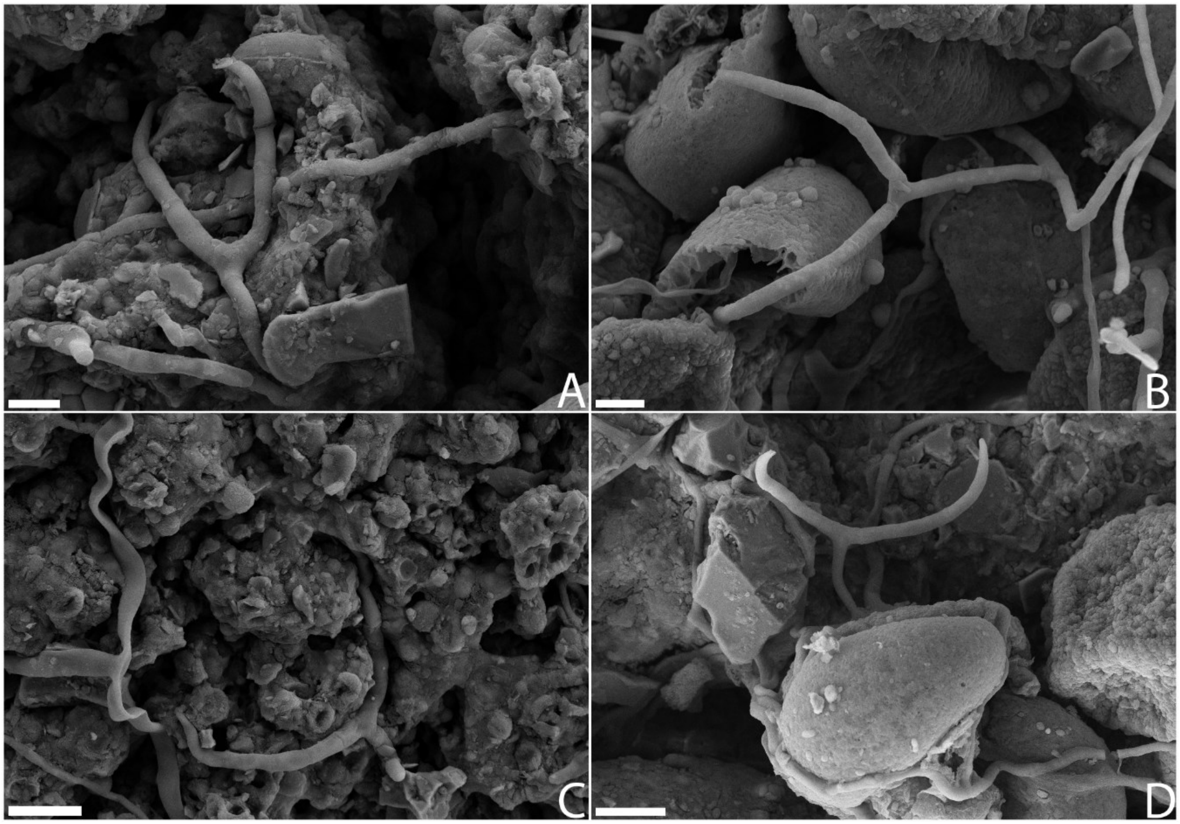

Fig. 21. Conidia of unidentified *Retiarius* species occurring among pollen grains in undersurface of *Umbilicaria phaea*. (SEM) Scales A, B: 5 μm; C, D: 10 μm.