New ITS rDNA barcodes clarify phylogenetic relationships and biogeography of aquatic hyphomycetes

Ricardo Duarte (ricardofilipeduarte@bio.uminho.pt)
UMinho CBMA: Universidade do Minho Centro de Biologia Molecular e Ambiental
https://orcid.org/0000-0002-2333-6127
Isabel Fernandes
UMinho CBMA: Universidade do Minho Centro de Biologia Molecular e Ambiental
Vladislav Gulis
Coastal Carolina University
Fernanda Cássio
UMinho CBMA: Universidade do Minho Centro de Biologia Molecular e Ambiental
Cláudia Pascoal
UMinho CBMA: Universidade do Minho Centro de Biologia Molecular e Ambiental

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Abstract

Aquatic hyphomycetes are key microbial decomposers of allochthonous organic matter in freshwater ecosystems. Although their importance in carbon flow and food webs in streams is widely recognized, there are still gaps in our understanding of their phylogeny, molecular diversity and distribution patterns. Our study utilized the growing database of ITS rDNA barcodes of aquatic hyphomycetes (1252 sequences) and aimed to *(i)* produce new barcodes for some lesser-known taxa of aquatic hyphomycetes, *(ii)* clarify the phylogeny of aquatic hyphomycetes, including the placement of some taxa at the class or order level based on molecular data, and *(iii)* provide insights into biogeography of this group. This study increased the number of aquatic hyphomycetes with available ITS barcode from 120 species (out of ~ 300 species described) to 136 species. Phylogenetically, the 136 species of aquatic hyphomycetes were distributed between two phyla (Ascomycota and Basidiomycota), 6 classes and 10 orders of fungi. However, 36 species remain *incertae sedis* without attribution at the order level. The geographical distribution of species with available ITS sequences included 50 countries from 5 continents. North American and European countries showed the highest number of aquatic hyphomycete species with ITS barcodes, with 6 countries having more than 20 species: Portugal (34 species), United States of America (33 species), United Kingdom (29 species), Germany (28 species), France (22 species) and Czech Republic (21 species).

Introduction

Aquatic hyphomycetes are the major microbial decomposers of plant litter in streams (Pascoal and Cássio 2004; Gessner *et al.*2007). They play a pivotal role in these ecosystems by driving carbon and nutrient cycling and channeling energy to higher trophic levels (Graça and Canhoto 2006), thereby contributing to the functioning of freshwater ecosystems. Aquatic hyphomycetes comprise over 300 species of fungi (Descals 1997; Shearer *et al.*2007) with a worldwide distribution (Duarte *et al.*2016). However, the occurrence of individual species is likely to be limited to certain latitudes and/or altitudes (Chauvet 1991; Jabiol *et al.*2013; Seena *et al.*2019) and influenced by physical characteristics of streams and rivers as well as water chemistry (Ferreira *et al.*2014; Duarte *et al.* 2017; Gulis *et al.*2019). The majority of aquatic hyphomycetes belong to the phylum Ascomycota (Barlocher 1992; Shearer *et al.*2007). A large number of species are in the class Leotiomycetes, while others are distributed among Sordariomycetes, Dothideomycetes, Orbiliomycetes and Pezizomycetes (Belliveau and Bärlocher 2005; Baschien *et al.*2006; Campbell *et al.*2006, 2009). Yet, the taxonomic positioning of many species of aquatic hyphomycetes remains undefined due to the lack of either teleomorph observations or molecular data.

Conidial morphology still plays a large role in the taxonomy of aquatic hyphomycetes (Baschien *et al.*2013; Gulis *et al.* 2020), with many species producing stauroconidia (mostly tetraradiate spores), variously branched spores, or scolecoconidia (sigmoid, variously curved or substraight spores). Along with spore shapes, the details of conidiogenesis are also traditionally used in systematics (Alexopoulos *et al.*1996; Marvanová 1997). However, the conidial morphologies of aquatic hyphomycetes are believed to

have evolved convergently as independent adaptations to similar environmental pressures in different phylogenetic lineages of fungi, making conidial shape an unreliable indicator of phylogenetic relationships (Campbell *et al.*2006). For some years now, great efforts in fungal taxonomy and systematics focus on comparisons of nucleotide sequences of select genes instead of, or in addition to, phenotypic characters (Johnston *et al.*2019). DNA sequences are increasingly used to investigate anamorph/teleomorph connections and phylogenetic relationships among fungal taxa (Bruns *et al.*1991; Taylor 1993; Berbee and Taylor 2001; Sati and Pathak 2016). In addition, molecular barcodes, including ITS rDNA sequence data (Schoch *et al.*2012; see also below), are invaluable in studies dealing with analyses of fungal community structure from environmental samples (Duarte *et al.* 2014b; Fernandes *et al.*2015; Seena *et al.*2019). Compared to morphology, molecular data provide considerably more information for phylogenetic analyses and therefore have improved resolving power. For instance, molecular data showed that several genera of aquatic hyphomycetes are polyphyletic (Campbell *et al.*2009; Baschien *et al.*2013; Johnston and Baschien 2020), helped to connect anamorphs to teleomorphs (Sati and Pathak 2016) and suggested that many aquatic hyphomycetes have relatives of terrestrial origin (Baschien *et al.*2013).

In an attempt to better understand phylogenetic relationships among aquatic hyphomycetes, Duarte *et al.* (2014a) found that only 26% of all described species had an internal transcribed spacer (ITS) rDNA barcode available. Although there has been an effort to sequence different loci (including ITS) from isolates of aquatic hyphomycetes since then (Baschien *et al.*2013; Duarte *et al.*2015; Seena *et al.*2018; Johnston and Baschien 2020), a large scale comprehensive analysis of available ITS barcodes of aquatic hyphomycetes is still missing. Here, we analyzed sequence data from the ITS rDNA (most widely used DNA barcode for fungi (Schoch *et al.* 2012)) from all species of aquatic hyphomycetes available in GenBank (120 species), and also included new sequences generated in this study in our labs from 54 pure cultures (42 species, 16 of them sequenced for the first time). The objectives of our study were to *(i)* provide new barcodes of aquatic hyphomycetes, *(ii)* clarify the phylogeny of aquatic hyphomycetes, including the placement of some taxa at the class or order level based on molecular data, and *(iii)* provide insights into biogeography of the group.

Materials And Methods

Dataset compilation

We assembled a dataset by compiling available information on aquatic hyphomycetes from literature and public databases, using as query the terms “aquatic hyphomycetes”, “Ingoldian fungi” and “Ingoldian hyphomycetes”. Species’ accepted name, synonyms and the information on the teleomorph and anamorph connections from three databases – Mycobank (https://www.mycobank.org/), Index Fungorum (indexfungorum.org) and NCBI Taxonomy (https://www.ncbi.nlm.nih.gov/taxonomy), was compiled (Supplementary Data S1). In addition, ITS rDNA sequences were downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/; data collected in January 2021).

New ITS barcodes

Fungi from environmental samples (submerged decaying plant litter or stream foam) were isolated according to Pascoal *et al.* (2005) and Descals (2005). Pure cultures were grown at 15 °C on 1% malt extract agar for approximately 15-20 days before DNA extraction. DNA extractions were performed using the DNeasy PowerSoil Kit or DNeasy UltraClean Microbial Kit (Qiagen) according to the manufacturer's instructions. PCR amplifications were performed according to Duarte *et al.* (2012) or Baschien *et al.* (2013), targeting the entire fungal ITS1-5.8S-ITS2 region of the rDNA, by using primer pairs ITS1F/ITS4 (White *et al.* 1990; Gardes and Bruns 1993) or SR6R/LR1 (Vilgalys and Hester 1990). PCR products were checked on an agarose gel to confirm the presence of the desired band and then purified using the PureLink PCR purification Kit (Invitrogen) or ExoSAP-IT PCR product cleanup reagent (Applied Biosystems) according to the manufacturer's instructions. DNA concentrations were checked with a NanoDrop spectrophotometer (ND-1000, Thermo Fisher Scientific). The amplicons were sequenced in both directions either at StabVida (Oeiras, Portugal) or Eurofins Genomics (Louisville, KY, USA).

Isolates are maintained in culture collections, in particular at the Centre of Molecular and Environmental Biology (CBMA) of University of Minho, Portugal, at the Czech Collection of Microorganisms (CCM), Czech Republic, and at Coastal Carolina University, USA. The geographical origin and the substrate of all fungal isolates are given in Table 1.

Phylogenetic analysis

Consensus sequences of ITS region were obtained with BioEdit software, version 7.2.5 (Hall 1999), and were deposited in GenBank, under the accession numbers shown in Table 1. To analyze phylogenetic relationships of aquatic hyphomycetes, we used the assembled dataset of 1252 sequences and aligned them using the multiple sequence alignment algorithm FFT-NS2 implemented in MAFFT software version 7 (Katoh and Standley 2013; Katoh *et al.* 2018). Maximum likelihood (ML) phylogeny was inferred using IQ-TREE based on the best-fitting model (SYM+I+G4) according to Bayesian Information Criterion (BIC), after testing 88 DNA models (Nguyen *et al.* 2015; Trifinopoulos *et al.* 2016), with automated model finder (Kalyaanamoorthy *et al.* 2017) and the bootstrap algorithm UFBoot (Hoang *et al.* 2017). Branch support was assessed with bootstrap analysis (1000 replicates) (Felsenstein 1985). Dissimilarity between DNA sequences assessed within and between genera was calculated using MEGA-X software (Kumar *et al.* 2018). The ITS sequence of *Mucor hiemalis* (type strain CBS 201.65, GenBank accession number NR_152948.1) was used as the outgroup to root the trees. Phylograms were pruned, formatted and colored in iTOL (Letunic and Bork 2007, 2019).

Results And Discussion

We compiled a dataset summarizing data regarding described species of aquatic hyphomycetes (Supplementary Data S1). Our dataset combines taxonomic data for a total of 325 species, in particular, the accepted name, synonyms, basionym, teleomorph and anamorph, as well as the taxonomic placement (phylum, class, order and family). Results showed a high taxonomic diversity of aquatic hyphomycetes that were distributed among two phyla (Ascomycota and Basidiomycota), 8 classes, 16 orders, 22 families and 124 genera. We searched GenBank for the 325 species of aquatic hyphomycetes, of those, ITS rDNA sequences were found for 120 species, 1074 strains, with a total of 1198 sequences since some strains had more than one sequence deposited, as they were sequenced by different researchers (Supplementary Data S2). Only sequences of aquatic hyphomycetes identified to the species level were considered (data for specimens identified to the genus level were ignored, e.g., *Flagellospora* sp.). We expanded this database by including new ITS rDNA sequences generated in this study from 54 pure cultures of 42 species of aquatic hyphomycetes, 16 of them belonging to species for which no ITS data was yet available (Table 1). The 1252 barcodes considered in our study (136 species, 1127 strains) have an average size of 504 base pairs, with some isolates having longer barcodes (up to 975 base pairs), due to the existence of long inserts in the ITS region, especially among Dothideomycetes (data not shown).

Our database represents the largest number of taxa and barcodes considered so far, much greater than dealt with in other studies, e.g., 19 species, 94 isolates (Seena *et al*. 2010); 7 species, 21 strains (Letourneau *et al*. 2010); 6 species, 130 isolates (Duarte *et al*. 2012); 75 species (Baschien *et al*. 2013). In addition, we generated 54 new ITS barcodes (Table 1). Our results increased the percentage of species of aquatic hyphomycetes with available ITS barcode from 37% (120 species out of 325) to 42% (136 species out of 325). The latter number still appears to be low emphasizing the need to generate even more barcodes to better understand genetic diversity as well as to facilitate studies in molecular fungal ecology (Barlocher 2010; Nilsson *et al.* 2019). At the same time, it is highly likely that some of the 325 species names in our database will be eventually synonymized with others, in part due to new molecular evidence becoming available in the future. Thus, we believe that we likely covered about 50% or more of the known diversity of aquatic hyphomycetes.

As expected, a much higher number of aquatic hyphomycetes showed affinity to ascomycetes than to basidiomycetes (Figure 1). In Ascomycota, the highest number of species were found to belong to Leotiomycetes (83 species, Figure 1A), with ~700 strains with available ITS sequences (56% of the total sequences considered in our study). Very few species were found to belong to the Basidiomycota classes: Agaricomycetes and Classiculomycetes, with only two and one species attributed, respectively. In terms of orders (Figure 1B), Helotiales showed the highest number of species and strains – 78 and 693, respectively. Notably, a considerable number of species (36 species; 26% of all species considered) could not be attributed to any order (*incertae sedis*). Clearly, additional barcodes should be generated for less represented taxa in order to better understand genetic diversity and phylogenetic position of some species of aquatic hyphomycetes.

The phylogenetic relationships of aquatic hyphomycetes are shown in Fig. 2 (phylogram in traditional rectangular format is shown in Supplementary Data S3). The proposed phylogeny divides the 136 species between two phyla: Ascomycota (Fig. 2, green) and Basidiomycota (Fig. 2, blue), and displays the separation of 6 classes and 10 orders into well-defined clades.

While the general topology of the tree based on ITS rDNA sequences of aquatic hyphomycetes (Fig. 2) was reasonable, some species and genera were problematic or not properly resolved. For example, in case of *Tetracladium,* even though the genus represents a well-defined clade, no clear separation among the seven species was evident. Similar results were previously reported using sequences of 18S rDNA (Nikolcheva and Bärlocher 2002), 28S rDNA (Wang *et al*. 2015), and ITS+28S (Baschien *et al.* 2013) regions. The five species of the genus *Lemonniera* also clustered in a well-defined clade, but, as for *Tetracladium*, no clear separation among the species was evident. Interestingly, our results positioned all five species within Leotiomycetes, Helotiales. This contrasts with previous results based on 28S region, where *L. pseudofloscula* was positioned within Dothideomycetes, Pleosporales (Campbell *et al.* 2006). Also, the genus *Fontanospora* was split into 4 groups: one group with *F. eccentrica* only, a second with *F. fusiramosa* only, a third group with *F. alternibrachiata*, and a final one with a mix of *F. fusiramosa*, *F. eccentrica* and *Articulospora tetracladia* (the latter isolate most probably corresponds to a misidentification, since all the other isolates of *A. tetracladia* clustered together). *Fontanospora* was previously reported to be polyphyletic based on analysis of 28S rDNA (Campbell *et al.* 2009). Isolates of *Filosporella versimorpha* (2) and *F. fistucella* (5) are intermingled on a tree, and the same pattern was observed for isolates of *Alatospora flagellata* (2), *A. acuminata* (27) and *Flagellospora leucorhynchos* (1) suggesting that using just ITS rDNA sequences is not sufficient to resolve their phylogenetic relationships. *Anguillospora crassa* separated into two distinct groups with *Tricladium obesum* and *Anguillospora furtiva* being phylogenetically close; all these species belong to a recently described family Tricladiaceae (Johnston and Baschien 2020). In our analysis, isolates of *Tumularia aquatica* are separated into two groups within Dothideomycetes. One group clustered with *Colispora cavincola*, *C. elongata*, *Clavariopsis aquatica* and *Tumularia tuberculata*. The other group (2 isolates) formed a separate clade, distant from the previous one. This may suggest a misidentification of the strains and highlights the importance of using ex-type strains with available DNA barcodes to help in the identification of problematic isolates (Vu *et al*. 2019). Strains identified as *Speiropsis pedatospora* were also separated into two groups. One group (including ex-type culture) clustered with *Speiropsis scopiformis* within Dothidiomycetes and close to the order Tubeufiales. In a recent study based on ITS + 28S sequences, *S. pedatospora* was positioned in the family Weisneriomycetaceae, as a sister group to Tubeufiales (Pratibha *et al.* 2016). The second group contains likely misidentified isolates (SS2229 and SS2236) and are placed in Jahnulales, Dothideomycetes (Prihatini et al. 2008; Suetrong et al. 2011; Dong et al. 2020). ITS region does not seem suitable to resolve the phylogeny of *Wiesneriomyces laurinus*, since isolates of this species were split into three groups with other species in between (*Speiropsis* spp., *Phalangispora nawawii* and *P. constricta*). The analysis of both 18S and 28S regions seems to have better resolving power for *Wiesneriomyces laurinus* (Suetrong *et al*. 2014). We also found problems with a few isolates that did not group together with the remaining isolates of the same species, possibly due

to misidentification: *Amniculicola longissima* WPRHD03, *Neonectria lugdunensis* NRRL-20592, *Flagellospora curvula* 30-67, *Anguillospora furtiva* NBRC-103659, *Varicosporium elodeae* AU-CRYP05 and *Articulospora tetracladia* CCM F-12313,

The robust phylogeny of aquatic hyphomycetes was also used to propose the following taxonomic placement for some species with *incertae sedis* classification (black color code in Fig. 2). *(i) Campylospora* and *Lunulospora* species are clearly placed within the order Hypocreales in Sordariomycetes (Fig. 2 and Supplementary Data S3) with high robustness (bootstraps ≥ 99%). *(ii) Tumularia aquatica* (excluding the two ambiguous sequences) and *Tumularia tuberculata* both group within Dothidiomycetes, and close to species of the order Pleosporales. *(iii)* Three species (*Goniopila monticola*, *Culicidospora aquatica* and *C. gravida*) with no clear phylogenetic affinity or with contradictory classification among databases (Mycobank vs Index Fungorum vs NCBI) were clearly positioned within the order Helotiales in Leotiomycetes. *C. gravida* was attributed to Helotiales based on ITS barcode produced for the first time in our study. More sequences from *C. aquatica* and *C. gravida* are needed to increase robustness of these observations. *(iv) Speiropsis scopiformis*, *Phalangispora nawawii* and *Phalangispora constricta* are within Dothidiomycetes, but further analyses are needed to define their position in terms of order. Other species were attributed to classes, but because only 1 sequence of each species is available, these observations need to be confirmed in the future. *(v) Retiarius bovicornutus* and *Isthmotricladia gombakiensis* are clustered close to *Dactylellina appendiculata* within the Orbiliomycetes. *(vi) Heliscella stellata* are clustered close to *Stenocladiella neglecta* and *Isthmomyces lanceatus* in Dothidiomycetes. *(vii) Lateriramulosa uni-inflata, Colispora cavincola* and *C. elongata* are also positioned in Dothidiomycetes.

The highest average evolutionary divergence for all ITS sequences of aquatic hyphomycetes was found between genera *Stenocladiella* and *Classicula* (0.647; Supplementary Data S4). Actually, *S. neglecta* is an ascomycete (Dothidiomycetes) while *C. sinensis* is a basidiomycete (Classiculomycetes). Regarding the average evolutionary divergence within genus (between 2 or more species of same genus), the highest was observed for *Mycofalcella* (0.22) and the lowest for *Aquanectria* (0.0081) and *Variocladium* (0.0085). The genus *Mycofalcella* comprises 2 species: *M. calcarata* recently repositioned in the family *Tricladiaceae* (Helotiales, Leotiomycetes) (Johnston and Baschien 2020), and *M. iqbalii*, also connected to Tricladiaceae (Helotiales, Leotiomycetes) according to Mycobank and Index Fungorum. However, in our analysis, *M. iqbalii* is positioned within Dothideomycetes, which explains high evolutionary divergence within this genus. Future analysis is needed to likely transfer *M. iqbalii* to a new genus. *Aquanectria* (Hypocreales, Sordariomycetes) is a recent genus erected to accommodate two species (*A. penicillioides* and *A. submersa*) previously in the genera *Flagellospora* (as *F. penicillioides*) and *Heliscus* (as *H. submersus*), respectively (Lombard *et al.* 2015). The genus now includes five more species, described based on multilocus phylogenetic analyses (Huang *et al.* 2018; Gordillo and Decock 2019), but none of the new species is considered as aquatic hyphomycete. *Variocladium* contains two species, *V. giganteum* and *V. rangiferinum* (Helotiales, Leotiomycetes) (Descals *et al.* 1998; Campbell *et al.* 2009). Interestingly, the average evolutionary divergence within genera *Aquanectria* (0.0081) and *Variocladium* (0.0085) was lower than that of the species *Vibrissea flavovirens* (0.0086) (Supplementary Data S4). Thus, *Aquanectria*

and *Variocladium* illustrate a situation of quick morphological diversification and/or slow molecular evolution of ITS region.

In our study, 46 species have been represented by just a single sequence each. This may have affected our conclusions about the phylogeny of these particular species due to possible misidentification of isolates or inaccurate sequences deposited in public collections. We encourage continuous efforts to isolate and produce new DNA barcodes of aquatic fungi. In addition, having a larger number of DNA sequences from strains isolated from different geographical regions might provide an opportunity to address other questions like haplotype analysis of some aquatic hyphomycetes (Wirtz *et al.* 2012; Chiva *et al.* 2019).

The geographical origin of all species with available ITS sequences included a total of 50 countries from 5 continents (Fig. 3 and Supplementary Data S2). North American and European countries had the highest number of species of aquatic hyphomycetes with available ITS barcodes: Portugal (34 species), United States of America (33 species), United Kingdom (29 species), Germany (28 species), France (22 species) and Czech Republic (21 species). These findings suggest that a larger effort isolating and barcoding aquatic hyphomycetes is still needed, especially in some parts of the world without any representation, such as many countries in Africa and some in Asia. This trend was previously noted in a review that dealt with biogeography of aquatic hyphomycetes based on morphospecies (Duarte *et al.* 2016). Clearly, the number of species reported here for different countries does not necessarily indicate the level of biodiversity but rather reflects collecting efforts or the existing expertise in the individual countries.

Our study advanced the knowledge of phylogenetic relationships among aquatic hyphomycetes and their biogeography based on 1252 ITS rDNA barcodes. Phylogenetic analysis showed that 136 species of aquatic hyphomycetes were distributed between the fungal phyla Ascomycota and Basidiomycota, in 6 classes and 10 orders. We included new barcodes for 16 species and elucidated phylogenetic positions of some genera and species, which were previously classified as *incertae sedis,* to the level of class or order. Future studies should strive to increase the database of ITS sequences, especially focusing on species with still unclear phylogenetic relationships (*incertae sedis*) and sampling, isolating and sequencing aquatic hyphomycetes from geographically less explored regions, such as Africa and certain Asian countries. It would be also useful to explore extreme habitats (e.g., intermittent streams, polar regions and deserts). In addition to sequencing rDNA loci, a multilocus approach including structural gene analysis or comparison of entire genomes might help provide new insights into fungal classification (Johnston *et al.* 2019; Li *et al.* 2021). Whole genome sequencing and annotation will also facilitate studying phylogeography of aquatic hyphomycetes while environmental metagenomics will help to unravel patterns of their distribution in aquatic ecosystems, including those affected by anthropogenic change. These modern approaches that allow species detection in the absence of reproductive structures may open new avenues to fungal conservation (Barros and Seena 2022).

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Data collection and analysis were performed by Ricardo Franco-Duarte, Isabel Fernandes and Vlad Gulis. Funding acquisition was performed by Fernanda Cássio, Cláudia Pascoal and Vlad Gulis. The first draft of the manuscript was written by all authors. All authors read and approved the final manuscript.

Data Availability

Accession numbers for DNA sequences obtained for the first time in this study are listed in Table 1. Accession numbers for DNA sequences obtained from Genbank are listed in Supplementary Data S2.

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Table 1

Table 1: Sources and accession numbers of aquatic hyphomycete isolates sequenced in this study. Species with new ITS barcodes generated for the first time in this study are highlighted in bold.

Species name	Strain	Isolation country	Isolation substrate	GenBank accession number
Alatospora acuminata	UMB-223	Portugal	foam	OM273714
Alatospora acuminata	UMB-741	Portugal	leaves	MZ773535
Alatospora acuminata	UMB-902	Portugal	oak leaves	OM273715
Alatospora pulchella	UMB-1115	Portugal	oak leaves	MZ773536
Anguillospora crassa	UMB-217	Portugal	foam	OM273716
Anguillospora crassa	UMB-1150	Portugal	foam	MZ773539
Anguillospora crassa	VG33-1	USA	dead submerged tree roots	OM907724
Anguillospora curvula	VG69-4	USA	grass blades	OM907725
Anguillospora filiformis	UMB-016	Portugal	leaves	OM273717
Anguillospora filiformis	UMB-225	Portugal	leaves	MZ773533
Aquanectria penicillioides	VG205-1-2	USA	wood	OM907726
Arbusculina irregularis	**CCM F-23687**	Canada	unknown	OM273718
Arbusculina irregularis	**VG76-8**	USA	foam	OM906795
Articulospora atra	VG233-6	USA	wood	OM907727
Articulospora proliferata	VG229-6	USA	grasses	OM907728
Articulospora tetracladia	UMB-712	Portugal	foam	OK605572
Articulospora tetracladia	UMB-1144	Portugal	foam	OK605573
Casaresia sphagnorum	**VG7-1**	USA	*Quercus prinus* leaves	OM907729
Clavariana aquatica	**VG75-4**	USA	foam	OM907730
Clavatospora	VG80-6	USA	*Tilia* sp. leaves	OM907731

longibrachiata				
Culicidospora gravida	**VG39-4**	USA	foam	OM907732
Dendrosporomyces prolifer	**VG258-1**	USA	foam	OM906797
Dendrosporomyces prolifer	**VG98-3**	USA	foam	OM906796
Dimorphospora foliicola	UMB-215	Portugal	leaves	OM273719
Dimorphospora foliicola	UMB-1119	Portugal	oak leaves	MZ773538
Filosporella exilis	VG211-1	USA	grasses	OM907733
Filosporella fistucella	UMB-007	Portugal	water	OM273720
Fontanospora alternibrachiata	**VG8-4**	USA	*Rhododendron maximum* leaves	OM907734
Geniculospora inflata	**VG79-1**	USA	twigs	OM907735
Heliscella stellata	**VG254-5**	S. Korea	*Betula* sp. leaves	OM907736
Heliscina antennata	**VG50-2**	USA	artificial foam	OM907737
Hydrocina chaetocladia	UMB-1116	Portugal	oak leaves	MZ773531
Isthmotricladia gombakiensis	**VG113-5**	USA	foam	OM907738
Lateriramulosa uni-inflata	**VG80-7**	USA	unident. dicot leaves	OM907739
Lemonniera alabamensis	UMB-594	Portugal	leaves	MZ773530
Lemonniera aquatica	VG66-7	USA	sedges	OM907740
Lemonniera cornuta	VG77-4	USA	foam	OM907741
Lemonniera pseudofloscula	**VG30-2**	USA	*Acer rubrum* leaves	OM907742
Lemonniera terrestris	VG209-3	USA	leaves	OM907743
Mycofalcella calcarata	VG44-4	USA	decorticated branch	OM907744
Neonectria lugdunensis	UMB-161	Portugal	twigs	OK605576
Pleuropedium multiseptatum	**CCM F-46594**	Canada	unknown	OM273721

Pyramidospora constricta	**VG116-5**	USA	*Platanus* sp. leaves	OM907745
Pyramidospora ramificata	**VG54-1**	USA	unident. dicot leaves	OM907746
Tricladium curvisporum	**VG69-3**	USA	grasses	OM907747
Tricladium curvisporum	**VG242-1**	USA	grasses	OM907748
Tricladium splendens	UMB-414	Portugal	foam	OK605580
Tricladium splendens	UMB-1117	Portugal	oak leaves	MZ773537
Tumularia tuberculata	VG262-4	S. Korea	*Quercus* sp. leaves	OM907749
Tumularia tuberculata	VG264-4	S. Korea	*Quercus* sp. leaves	OM907750
Varicosporium elodeae	UMB-878	Portugal	foam	OK605582
Variocladium giganteum	VG43-4	USA	*Quercus* sp. leaves	OM907751
Variocladium rangiferinum	**VG71-1**	USA	sedges	OM907752

Figures

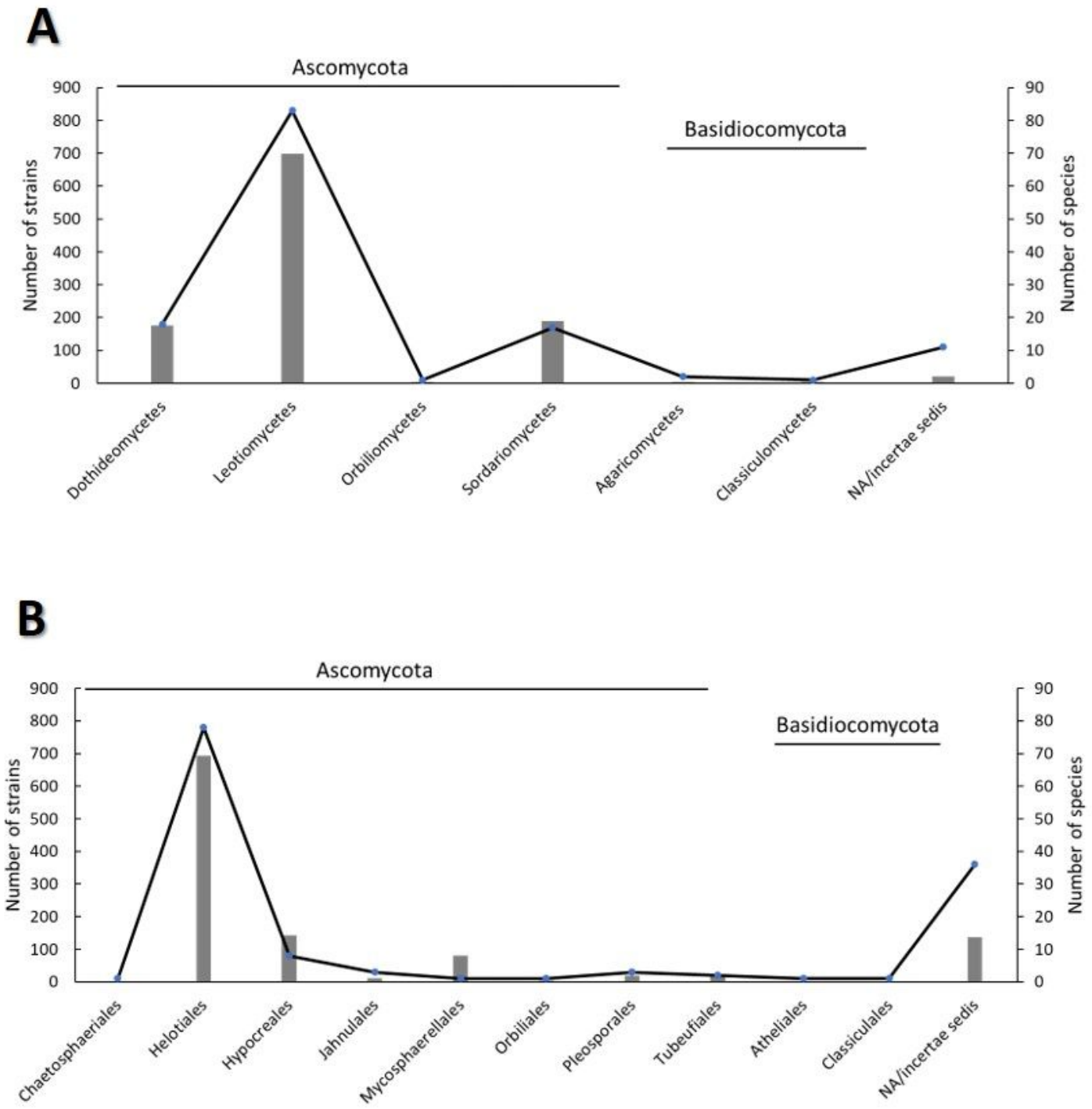

Figure 1

Distribution of aquatic hyphomycete strains (bars) and species (lines) among classes (A) and orders (B) of ascomycetes and basidiomycetes.

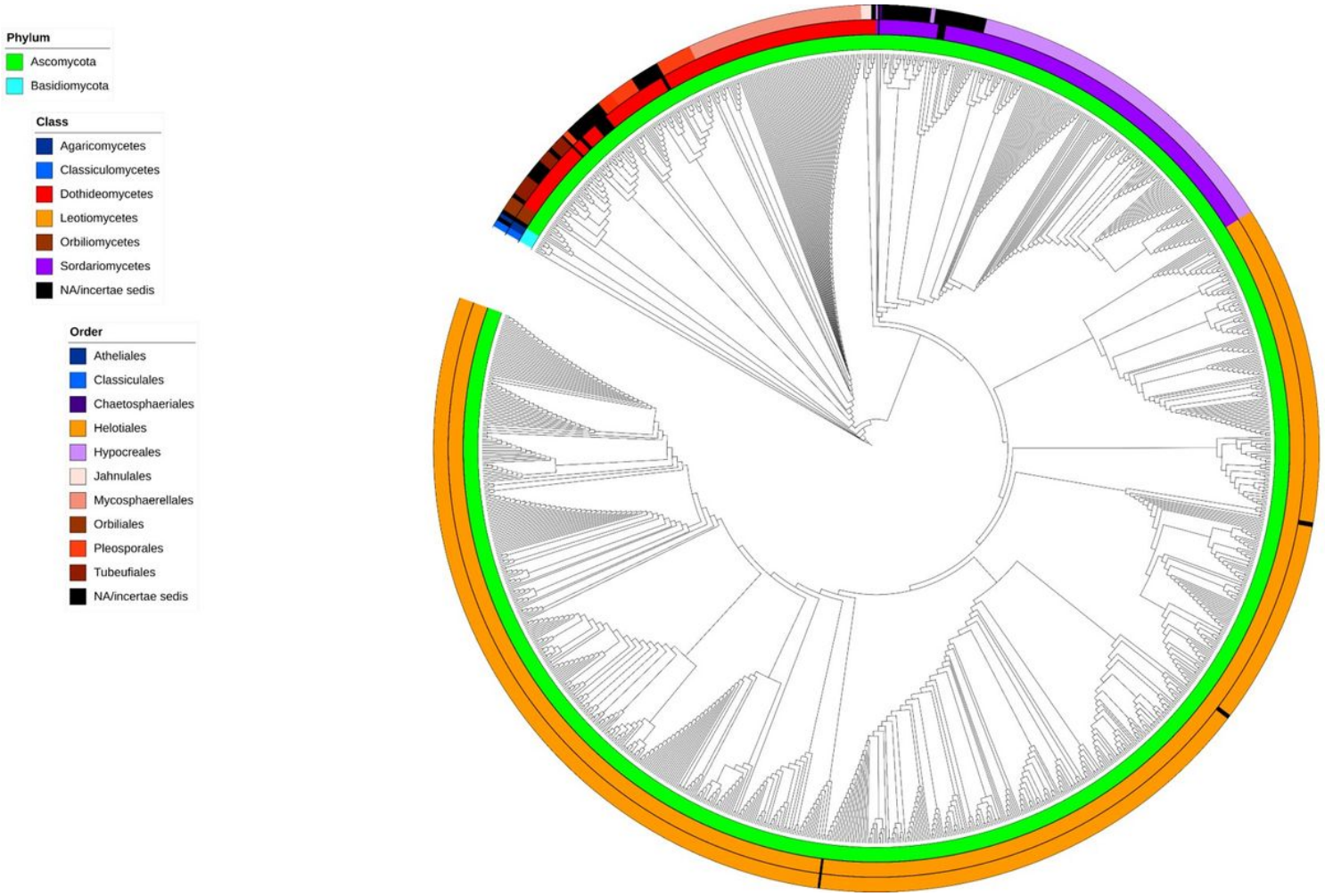

Figure 2

Phylogram of aquatic hyphomycetes based on 1248 ITS rDNA barcodes. Circle sections represent taxonomic divisions: inner circle – phyla, middle circle – classes, outer circle – orders.

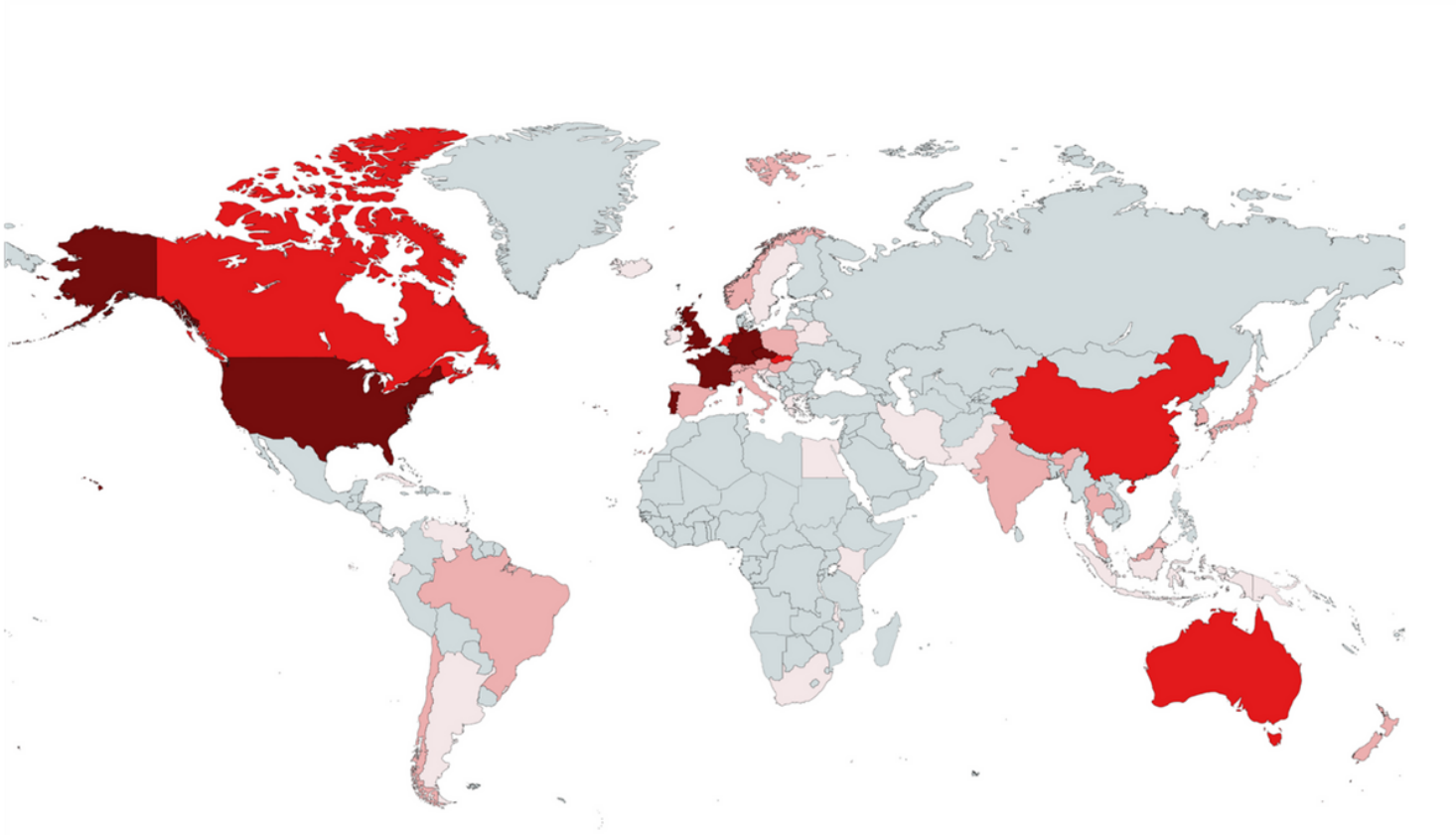

Figure 3

World map showing the countries of origin for strains/sequences of aquatic hyphomycetes used in this study. Colors indicate the number of different species obtained from each country: dark red – more than 30; red – from 11 to 29; light red – from 3 to 10; very light red – less than 3 species.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryDataS1.xlsx
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- SupplementaryDataS4.xlsx