

Monsoonal Variation in Singapore Labyrinthulomycetes Community

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Abstract - *Labyrinthulomycetes* is a group of ubiquitous saprophytic protists found in marine and estuarine environments. *Labyrinthulomycetes* are capable of parasitism and commensalism and known as consumers of phytoplankton litter. While *Labyrinthulomycetes* have been mostly studied and documented in temperate regions, understanding their interactions and variability in the tropics are lacking. They have been found to be strongly associated with particulate organic matter (POM), potentially contributing to vertical export from the surface into the dark ocean. We have conducted a pilot study to assess the diversity and community structure of *Labyrinthulomycetes* associated to suspended sediment particles in Singapore waters. Suspended sediment particles were collected monthly during a period of one year with the deployment of SedPods (flat cylindrical concrete unit with 15 cm height and 15 cm diameter) on the reef slope off Kusu Island. *Labyrinthulomycetes* community composition was analysed using metabarcoding of the 18S rRNA gene. Environmental parameters such as nitrates and silicates were obtained from water column samples between April 2018 and April 2019. Results indicate that diversity follows changes in nutrient availability linked to monsoon. The relative abundance of genus *Aplanochytrium* increased with concentrations of nitrates and silicates. The abundance of metabarcodes assigned to the *Thraustochytriceae* family increased during the South-West monsoon, possibly influenced by the increased availability of detritus and organic matter from riverine inputs from the Malacca Straits and Sumatran peatlands

Keywords - *Labyrinthulomycetes*, Monsoon, Sediment, Seawater, 18s rRNA, Metabarcoding

1 INTRODUCTION

Labyrinthulomycetes are a group of ubiquitous marine heterotrophs which contribute largely to our ocean's carbon fixation capabilities. Our oceans currently uptake about 25% of carbon dioxide released into our atmosphere (Heinze et al, 2015). This carbon is broken down via the biological carbon pump (BCP) and the microbial carbon pump (MCP). The BCP fixes carbon from inorganic sources such as CO₂ into particulate organic

carbon (POC) and causes them to sink after aggregating, while the MCP degrades organic carbon sources from a highly degradable state to a recalcitrant state, both of which are facilitated by *Labyrinthulomycetes* (Polimene et al, 2017). These microbes are from the supergroup Stramenopila and get their name from the ectoplasmic net they secrete from their bothrosome which spread out into labyrinth-like structures. *Labyrinthulomycetes* contribute to the BCP by aggregation of POC via their ectoplasmic nets, causing POC to sink faster due to gravitational action (Bai et al, 2021). *Labyrinthulomycetes* also contribute to the MCP by degrading plant-based and terrestrial detritus by secretion of extracellular enzymes (Taoka et al, 2008).

Labyrinthulomycetes have shown variability with availability of plant detritus and some living plant matter. *Thraustochytrids* have been isolated from decaying plant matter, and are capable of secreting enzymes into the detritus via the ectoplasmic net which break down plant matter and provide nutrients to the cell (Raghukumar, 2002). *Thraustochytrids* are known to secrete a wide variety of enzymes such as various lipases and proteases which aid them in the degradation of organic matter (Bongiomi et al, 2005). The variety of enzymes produced and the high enzymatic activities of *Thraustochytrids* suggest that while they are usually not prominent in the microbial community, they contribute greatly to the degradation of recalcitrant organic matter.

Labyrinthulomycetes depend heavily on nutrients in the water column for growth and proliferation. In a coastal environments like an equatorial shelf sea, nutrient concentrations vary largely during different monsoon periods – the North East (NE) Monsoon, which takes place from mid-November to March, the South West (SW) Monsoon, which takes place from mid-May to mid-September, and the Intermonsoon periods (Zhou et al., 2021). These monsoons are driven by differences in temperature between the land and ocean, forming pressure cells which change prevailing wind and current directions. Changes in current direction also change sources of nutrient input. The SW monsoon brings about large concentrations of nitrates and silicates from the peatlands of Sumatra, while the NE monsoon

brings water with high salinity from the South China Sea into the Singapore Strait.

While studies of nutrient concentration and sedimentation in the region are well established, the response of the microbial community to changes in sedimentation or nutrient concentration has yet to be documented. Hence, this paper aims to reveal the variation of Labyrinthulomycetes in response to monsoonal changes in nutrients and sedimentation, and find out the global distribution of key taxa found in this study using the metaPR² application.

2 METHODOLOGY

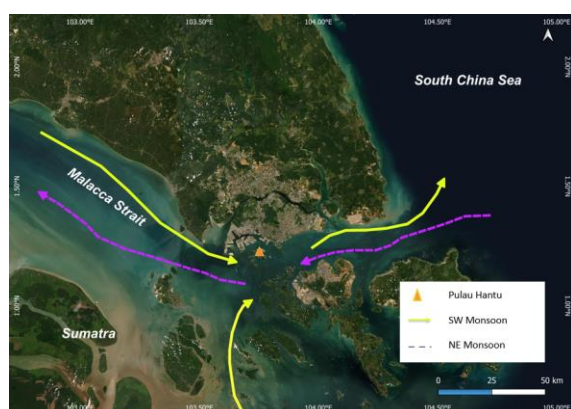


Figure 1: Current Reversals during the different monsoon periods, adapted from Zhou et al., 2021

2.1 STUDY SITE

The study was conducted off the coast of Pulau Hantu (1.226247°N, 103.747049°E). Being situated in the Singapore Strait, changes in nutrient concentrations are driven by current reversals during the NE and SW monsoon periods, while sedimentation is primarily driven by tidal flow and the presence of suspended sediment in the water column (Morgan et al., 2020).

2.2 ENVIRONMENTAL SAMPLE COLLECTION

Sediments and seawater samples were collected at Pulau Hantu over a period of 12 months, from April 2018 to March 2019. Sediments were accumulated on sediment pods and sedimentation rates were calculated as part of a study on vertical reef compression (Morgan et al., 2020). Accumulated sediment was gently scrubbed and flushed from the sediment pods surface with deionised water in the lab. The collected material was dried at 60°C overnight, or until all moisture was removed, and then weighed. It was stored at -80°C until DNA extraction. Seawater samples were collected using a Niskin bottle at a depth of 5 metres as part of a study on dissolved organic carbon and ocean acidification (Zhou et al., 2021).

2.3 ENVIRONMENTAL SAMPLE ANALYSIS

Sediment composition was categorised into total organic carbon (TOC), total inorganic carbon (TIC), and clastic sediment – non-organic sediment, using the loss-on-ignition (LOI) method (Heiri et al., 2001). Sediment was weighed to about 3g per sample and combusted at 550°C for 24 hours before being let to cool and weighed, with the difference in mass being the amount of organic carbon. Once cooled, the samples are put into a furnace at 950°C to combust the inorganic carbon, before being reweighed. The amount of inorganic carbon would be the difference in mass. Nutrient analysis of seawater samples was conducted using a SEAL AA3 segmented-flow analyzer following standard SEAL methods for seawater analysis, and chlorophyll-a was extracted and measured on a HORIBA Fluoro-max-4 fluorometer (Zhou et al., 2021).

2.4 DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

DNA was extracted from the sediment using the Qiagen DNeasy PowerBiofilm kit, following protocol from Moynihan modified from Sunagawa et al., 2010. DNA extracted was amplified using Labyrinthulomycetes-specific primers LABY-A (forward, 5'-GGGATCGAAGATGATTAG -3') and LABY-Y (reverse, 5'-CWCRAACTTCCTTCCGGT -3') (Stokes et al., 2002). PCR reactions were prepared as follows: for every 30ul reaction, 15ul KAPA HiFi HotStart ReadyMix (KAPABIOSYSTEMS, Cape Town, South Africa), 0.1uM of 0.75ul Forward Primer and Reverse Primer, 1.2ul BSA, and 5-10ng DNA template were used, and the remaining volume was topped up with nuclease free water. The reaction mixtures were cycled 35 times at 94°C for 30 seconds (denaturing), at 50°C for 30 seconds (annealing) and at 72°C for 1.5 minutes (extension). After which a final extension at 72°C for 5 minutes completed the amplification of the Labyrinthulomycetes-specific region of the 18s rRNA (Stokes et al., 2002). Final DNA concentration was measured using PicoGreen (Thermo Fisher Scientific, Waltham, MA, USA). DNA samples were purified, barcoded, and sequenced by the GeT-PlaGe platform of GenoToul (INRA Auzerville, France) using an Illumina MiSeq platform (2 x 300 bp).

2.5 DATA PROCESSING

Returned sequences were processed with R software using the *dada2* package (Callahan et al., 2016; R Development Core Team 2013). Reads were first visualised using the *plotQualityProfile*

function, before being filtered and trimmed to remove low quality reads using the *filterAndTrim* function using parameters *truncLen* = 230,220, *minLen* = 200, 180, *truncQ* = 5 and *maxEE* = 6. Errors in sequencing were identified using the function *learnErrors* and those errors were accounted for during the merging of forward and reverse reads using the function *mergePairs*. Chimeras were removed with the function *removeBimeraDenovo* using the “pooled” method. The *assignTaxonomy* function from the *dada2* package was used for taxonomic assignation. A Phyloseq object was created using the *merge_phyloseq* function from the *phyloseq* package by combining an OTU (organisational taxonomic unit) table, a sample data table and a table of taxonomic description. For visualisation purposes, metazoans and fungi were removed using *dplyr* and *tidyr*, and reads were normalized using the mean of the dataset for visualisation of relative abundances in the form of bar plots using *ggplot2* (Wickham, 2016; Wickham et al., 2022, Wickham and Girlich, 2022). The relative abundances of the top families of Labyrinthulomycetes and the top clades of those families were visualized on treemaps using the *treemapify* function (Wilcox, 2022). Scripts for data-processing are available at https://github.com/bteooo/dada2_LABY.

3 RESULTS

3.1 MONSOONAL VARIATION OF NUTRIENT CONCENTRATIONS

Hantu Island experiences varying nutrient concentrations during the different monsoon seasons (Figure 2). During the SW monsoon, nitrate and silicate concentrations are high, while salinity is low. During the NE monsoon, the opposite is true, with lower nitrate and silicate concentrations, but higher salinity.

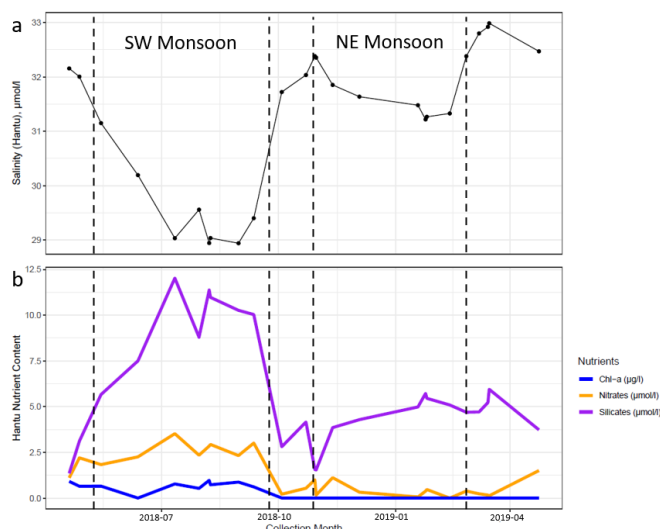


Figure 2: Difference in Salinity (a) and Nutrient Concentration (b) during the NE and SW monsoon

3.2 MONSOONAL VARIATION OF LABYRINTHULOMYCETES COMMUNITY

A total of 770 amplicon sequence variants (ASVs) were detected via sequencing, of which the top 4 families (Labyrinthulaceae, Labyrinthulomycetes_X_LAB1/6/8, Oblongichytridiaceae and Thraustochytriaceae) and top 10 clades of those families were chosen for analysis (Fig 3).

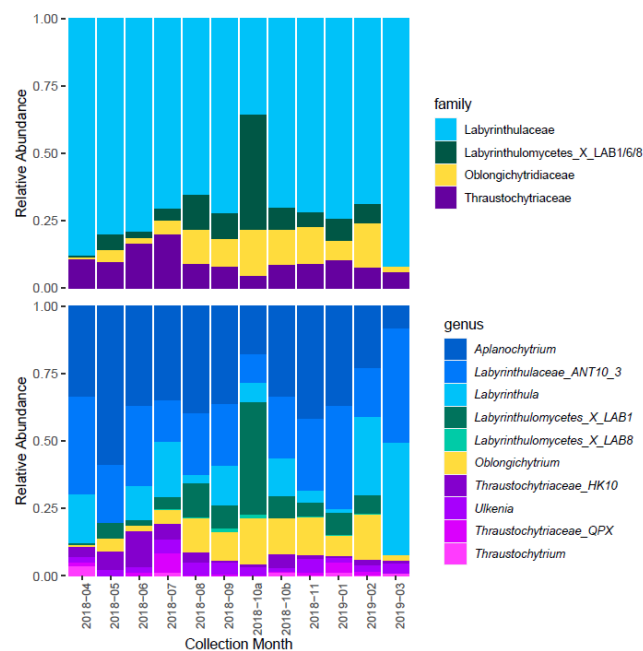


Figure 3: Labyrinthulomycete community composition throughout the study period

3.2.1 Labyrinthulaceae

Of the 4 families, Labyrinthulaceae had the highest average relative abundance of 72.4% across the study period (Fig 4). The top 3 clades include *Labyrinthula*, *Labyrinthulaceae_ANT10_3*, and *Aplanochytrium*. Labyrinthulaceae are most abundant in April 2018 and March 2019, at close to the end of the NE monsoon, having a relative abundance of 87.9% and 92.1% respectively, while being the least present in the middle of the late Intermonsoon, in October 2018, with a relative abundance of only 35.7%. *Aplanochytrium*, one of its most representative clade, tend to follow trends in silicate and nitrate concentrations. As nitrate and silicate concentrations spike in the middle of the SW monsoon, the relative abundances of *Aplanochytrium* increase. Similarly, when nitrate concentrations dip to its lowest in October 2018, the relative abundance of *Aplanochytrium* were at their lowest.

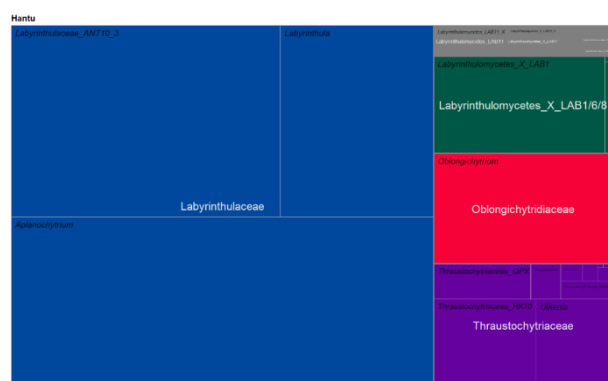


Figure 4: Labyrinthulomycetes community treemap

3.2.2 Thraustochytriceae

The family Thraustochytriceae was the least abundant amongst the top 4 families, and comprises of the clades Thraustochytriceae_HK10, Ulkenia, Thraustochytriceae_QPX and Thraustochytrium. Similar to Labyrinthulaceae, the family Thraustochytriceae have the highest lowest abundance in October 2018, in the middle of the late Intermonsoon, with a relative abundance of 4.21%. However, its relative abundance peaks in the middle of the SW monsoon and declines steadily afterward.

3.4 GLOBAL DISTRIBUTION OF KEY TAXA PRESENT IN THE STUDY



Figure 5: Global Distribution of Labyrinthulomycetes, visualised using the metaPR² application



Figure 6: Focus of Labyrinthulomycetes-related studies in the Atlantic Ocean and Mediterranean Sea

4 DISCUSSION

4.1 THRAUSTOCHYTRID GROWTH DRIVEN BY INCREASES IN ORGANIC DETRITUS

Thraustochytrids play a key part in organic matter decomposition (Bongiomi, 2012). During the SW monsoon, current reversals carry organic material from the peatlands of Sumatra and riverine input from the Malacca Strait into the study site. Thraustochytrid populations increase in the middle of the SW monsoon, but decrease from May onward, while particulate organic carbon (POC) increases at the beginning of the SW monsoon and generally decrease afterward. This suggests possible delayed dependence of thraustochytrid populations on the amount of organic material available. The significant delay of thraustochytrid population increase with respect to POC concentration could be due to the presence of phenolic compounds. The presence of phenolic compounds on live algae was proposed to inhibit thraustochytrid growth, and deter zoospore accumulation (Raghukumar, 2002; Fan et al, 2002). Phenolic compounds are known to leach intensely from detritus at early stages of decomposition, supporting the idea of delayed dependence as thraustochytrids can only break down the organic matter once the phenolic compounds have been decomposed.

The trends in thraustochytrid population and levels of POC elucidates at a positive relationship between the two. Studies in the Mediterranean have revealed positive correlations between thraustochytrid populations and particulate organic matter (POC), supporting their role as marine decomposers. (Bongiomi, 2012). To facilitate the decomposition of organic material in the form of plant detritus, thraustochytrids produce enzymes which can break down a wide range of polysaccharides such as starch and laminarin (Raghukumar, 2002). Thraustochytrids are even

capable of using pine pollen, which is known for its recalcitrant sporopollenin outer layer, as a form of nutrition. They extend their ectoplasmic net through the sporopollenin and extract nutrients within the pine pollen to nourish the thallus which is outside.

4.2 THRAUSTOCHYTRID MUTUALISMS IN MARINE ENVIRONMENTS

Besides playing key roles in marine decomposition, thraustochytrids have been proposed to exhibit mutualism with scleractinian corals, helping them recover from thermal stress events (Harel et al, 2008). High thermal stress results in a decrease in PUFA in corals, decreasing their resilience to thermal stress events as decreases in PUFA are associated with decreased symbiodiniaceae density, chlorophyll content and fatty acid content (Lim et al, 2017). Studies have shown that corals with thraustochytrids found on their surface and mucus survive bleaching event better than their counterparts without thraustochytrids, thus suggesting that thraustochytrids could be providing their coral hosts with PUFA, in exchange for a carbon source within the coral mucus (Harel et al, 2008).

4.3 APLANOCHYTRIUM GROWTH FOLLOWING TRENDS IN NITRATES AND SILICATES

Aplanochytrium are known to be grazers of diatoms which require nutrients such as silicates to build their walls and nitrates as electron acceptors in the absence of light and oxygen (Hamamoto and Honda, 2019; Merz et al, 2020; Turner et al, 2017). As the SW monsoon brings in large concentrations of silicates and nitrates, Chl- α concentrations increase as well. This increase could be associated with an increase in diatom populations, which in turn provides more prey for the *Aplanochytrium*.

Aplanochytrium aid in silica cycling by accelerating the rate of remineralisation of biogenic silica. *Aplanochytrium* use their ectoplasmic net to penetrate the silica shells of diatoms, accessing feeding on the nutrients within the diatom (Hamamoto and Honda, 2019). When diatoms die, their silica frustules sink to the bottom of the ocean via gravity and either dissolve or sink to the bottom of the ocean and form part of deep-sea sediment, depending on factors such as pH and ocean thermodynamics (Loucaides et al, 2008).

4.4 GLOBAL DISTRIBUTION OF TAXA PRESENT IN THE STUDY

The global distribution of Labyrinthulomycetes was generated using the metaPR² application. The metaPR² application is a database which contains 18s rRNA metabarcodes of 41 datasets which have been referenced against the PR² sequence

database, comprising of over 4000 samples and 90,000 ASVs (Vaulot et al, 2022).

Majority of Labyrinthulomycetes are generally coastal taxa, with Labyrinthulomycetes_X being an exception as it is found both in the open ocean and near the coast. The presence of Labyrinthulomycetes along the coast is testament to its role in the degradation of organic matter as plant detritus and other forms of organic matter leach into the ocean from the coast.

While Labyrinthulomycetes communities have been well studied around the Atlantic Ocean and the Mediterranean Sea, little or no studies have been conducted in the tropics or in Asia. The monsoonal variation in the Labyrinthulomycetes community in Singapore suggests that the interactions between Labyrinthulomycetes and other members of the microbial community might differ from literature, and more studies need to be conducted to understand the regional Labyrinthulomycetes community better.

5 CONCLUSION

The monsoonal variation in nutrient concentration has largely influenced the Labyrinthulomycetes community structure and we have elucidated certain relationships in this study. While the input of organic matter from the rivers along the Malacca Strait and peatlands of Sumatra during the SW monsoon provides Thraustochytrids with a source of nutrition, there seems to be a delayed dependence possibly due to the presence of phenolic compounds at the beginning of organic matter degradation, known to deter Thraustochytrid growth. Additionally, the influx of silicates and nitrates at the beginning of the SW monsoon improves growth conditions for diatoms, which serve as prey for *Aplanochytrium*. This could explain why the relative abundance of *Aplanochytrium* follows trends in nitrate and silicate concentrations. Lastly, the use of the metaPR² application highlights the lack of Labyrinthulomycetes-focused studies in Singapore and in the region, and combined with the observed monsoonal variation in Labyrinthulomycetes community structure, provide a basis for more Labyrinthulomycetes-focused studies in the region.

ACKNOWLEDGMENT

We thank Assistant Professor Adriana Lopes dos Santos, Professor David Vaulot for their comments and suggestions that improved the flow and clarity of the paper. The authors would also like to thank Denise Ong Rui Ying (Asian School of the Environment) for her guidance and input regarding data processing. I would like to acknowledge the funding support from Nanyang Technological

University – URECA Undergraduate Research Programme for this research project.

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APPENDIX



Figure 1: Current Reversals during the different monsoon periods, adapted from Zhou et al., 2021

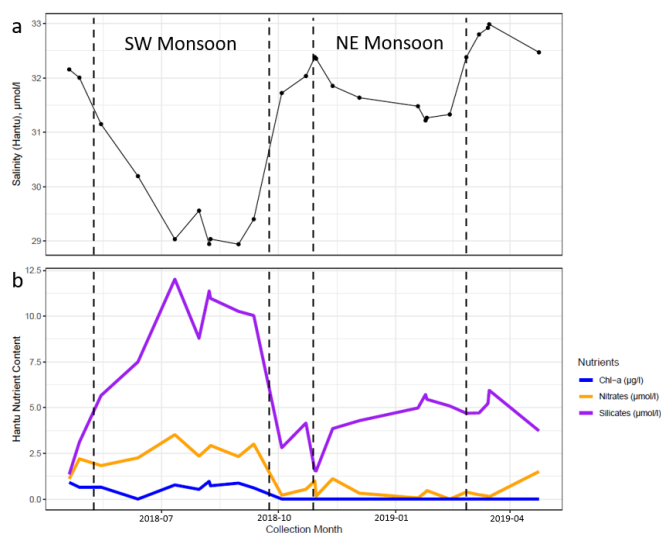


Figure 2: Difference in Salinity (a) and Nutrient Concentration (b) during the NE and SW monsoon

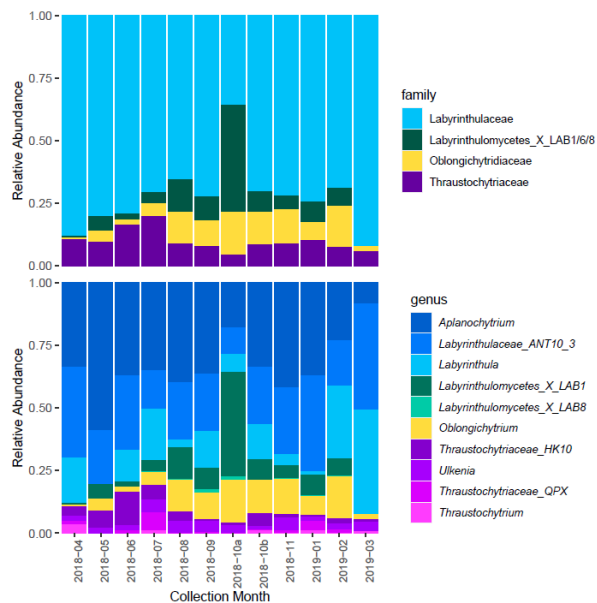


Figure 3: Labyrinthulomycete community composition throughout the study period

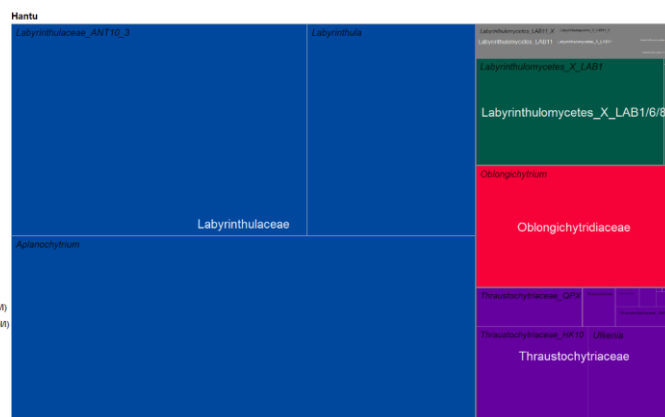


Figure 4: Labyrinthulomycetes community treemap



Figure 5: Global Distribution of Labyrinthulomycetes, visualised using the metaPR² application

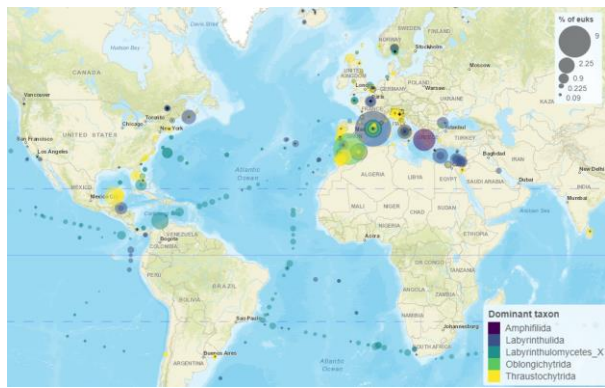


Figure 6: Focus of Labyrinthulomycetes-related studies in the Atlantic Ocean and Mediterranean