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ORIGINAL PAPER

High genetic diversity of *Labyrinthula* spp. inhabiting mangrove leaf detritus in Okinawa, Japan

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Abstract

The heterotrophic marine protist *Labyrinthula* plays various roles in the environment, from pathogen to symbiont to saprobic decomposer. Mangroves are notably rich in detritus that supports the saprotrophic lifestyle of these organisms, making this environment a hotspot for labyrinthulid biodiversity. In this study, *Labyrinthula* were isolated from decaying leaves found at 14 different mangrove sites in Okinawa, Japan (26°09'28.1"–26°44'34.8"N, 127°39'56.5"–128°18'55.6"E) in October 2024. A total of 61 isolates in 6 lineages were identified and characterized based on morphological and molecular data. Phylogenetic analyses based on the 18S rRNA gene and ITS region grouped all lineages with previously reported non-pathogenic labyrinthulid isolates. Two of the six lineages grouped with existing *Labyrinthula* species-letter groups "M" and "Q=V", one formed a hitherto unnamed clade with an existing sequence from the gill tissue of *Psetta maxima*, and three branched independently, indicating the discovery of three novel *Labyrinthula* spp. The four unnamed lineages have thus been designated as Lineages O1, O2, O3, and O4. Sequences in this study also grouped with other *Labyrinthula* spp. from mangrove leaves, seawater, seagrass, and turfgrass across different geographical locations, demonstrating the ecological plasticity of the group. The genetic records made available in this study provide the groundwork for further understanding the ecological role of *Labyrinthula* in mangroves and its cultivable diversity.

Keywords Biodiversity · Labyrinthulomycota · Phylogenetics · Saprotrophs · Systematics · Taxonomy

Introduction

The Labyrinthulomycetes (Labyrinthulomycota) are a group of heterotrophic protists classified within the Stramenopiles. This group consists of three major lineages, namely the thraustochytrids, labyrinthulids, and aplanochytrids (Leander and Porter 2001). The distinguishing characteristic of the Labyrinthulomycota is the ectoplasmic net: a network of fine, branching cytoplasmic threads extending from a unique structure called the bothrosome (Porter 1972). These threads form pathways on which the cell bodies glide and also play a role in substrate adhesion and nutrient absorption (Bennett et al. 2017). This unique feature has given rise to the name "slime nets," mainly used to describe the family Labyrinthulidae, of which there is only one genus, *Labyrinthula* (Beakes et al. 2014).

Members of *Labyrinthula* are characterized by colorless to yellowish spindle-shaped cells that migrate through an extensive branched system formed by a membranous ectoplasmic net. Each cell contains a visible central nucleus surrounded by lipid droplets (Jepps 1931) and is connected to

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the ectoplasmic net through a single bothrosome (Bennett et al. 2017). Their use of the ectoplasmic net distinguishes members of *Labyrinthula* from those of the genus *Aplanochytrium*, whose cells glide along the net rather than being embedded in it (Leander et al. 2004).

Labyrinthula are ubiquitous in marine, estuarine, and terrestrial environments. They have been isolated from various substrates, including algae (Pokorny 1967; Raghukumar 1987), vascular plants (Muehlstein 1992), and detrital materials (Sakata et al. 2000). They have also been found in association with amoeba (Dyková et al. 2008) and diatoms (Jepps 1931; Popova et al. 2020). Their ability to survive in a wide range of environments exemplifies their tolerance to a variety of environmental conditions.

Interest surrounding the genus has increased since *Labyrinthula zosterae* and *Labyrinthula terrestris* were identified to be the causative agents of the seagrass wasting disease and rapid blight, respectively (Muehlstein et al. 1991; Olsen 2007). Not all *Labyrinthula* are pathogens, however, with many of them functioning as saprobic decomposers associated with decaying macroalgae and plants (Bennett et al. 2017). More recently, *Labyrinthula* have started to attract attention for their ability to produce long-chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid or DHA, which is used in a variety of biotechnological applications from medicine to enriched animal feed (Kumon et al. 2006). The ability of saprotrophic *Labyrinthula* to produce LCPUFAs has been hypothesized to be ecologically relevant in aquatic food webs as a mechanism for upgrading detritus to nutritionally valuable compounds (Raghukumar 2002; Yoshioka et al. 2019).

Out of all the environments from which *Labyrinthula* have been isolated, mangroves are of interest due to their role as carbon sinks, making them an optimal environment for the saprotrophic activity of *Labyrinthula* (Raghukumar 2017). Mangroves produce large amounts of leaf litter which, when buried in waterlogged sediments, become highly accessible for decomposition (Inoue et al. 2024). The presence of *Labyrinthula* in mangroves has been confirmed by several studies across different regions of the world including Brazil, the Philippines, and China (Wahid et al. 2007; Boro et al. 2018; Wang et al. 2019). Among these studies, Wang et al. (2019) demonstrated a high culturable diversity of *Labyrinthula* in coastal mangroves in China. In Japan, *Labyrinthula* has also been isolated from mangrove leaves in Ishigaki, Okinawa (Honda et al. 1999) and other unspecified areas (Tsui et al. 2009). However, there is still a lack of isolates collected in the Nansei Islands at the southern tip of Japan (Suzuki et al. 2022). This area includes Okinawa Prefecture, which hosts 90% of Japan's mangrove population (Inoue et al. 2024) and thus likely holds biogeographical significance for *Labyrinthula* diversity. Mangroves in this area are isolated from the global mangrove distribution, which further emphasizes their importance for study and conservation (Thomas et al. 2022).

The goal of this study is to conduct an initial survey on the genetic diversity of saprotrophic *Labyrinthula* spp. found in mangroves in southern Japan (Okinawa) in order to establish a baseline for cataloging their cultivable diversity. It also aims to improve the current understanding of *Labyrinthula* systematics by including unique lineages from Okinawa. Results will also provide insights into the ecology of this group, laying the foundation for future research on the relationships of *Labyrinthula* with other organisms. Here, *Labyrinthula* were isolated from decaying leaves in various mangrove sites on Okinawa Island and cultured for further morphological and molecular analyses (18S rRNA gene and ITS region).

Materials and methods

Sample collection and establishment of cultures

Decomposing leaves were collected from mangrove forests across 14 different localities around Okinawa, Japan (26°09'28.1"–26°44'34.8"N, 127°39'56.5"–128°18'55.6"E) in October 2024 (Fig. 1a). Individual leaves were placed into plastic bags with a minimal amount of water from the site to maintain their moisture and kept cool during transportation from sampling sites to the laboratory (Fig. 1b).

Collected leaves were individually placed onto serum seawater agar (SSA) plates containing 0.8% agar (Nacalai Tesque, Inc., Kyoto, Japan) and 0.8% horse serum (Thermo Fisher Scientific, Auckland, New Zealand) based on a procedure modified from Muehlstein (1992). The media was prepared using filtered, autoclaved seawater. To limit bacterial growth, for every 500 mL of media, approximately 12 mg each of ampicillin sodium salt (Sigma-Aldrich, Co., St. Louis, MO, USA), streptomycin sulfate salt (Sigma-Aldrich, Co., St. Louis, MO, USA), and penicillin G potassium salt (MP Biomedicals, LLC, Irvine, CA, USA) were added following FioRito et al. (2016); the growth of diatoms was inhibited by adding 5 mg of germanium(IV) oxide, 4 N (Kanto Chemical Co. Inc., Tokyo, Japan) following Sullivan et al. 2017.

Plated leaves were either kept whole or cut into two to three smaller pieces. To isolate pure strains, *Labyrinthula* colonies branching out of the leaves were transferred from their original plates onto new SSA plates multiple times. For each transfer, two to four isolations were taken from each plate (Fig. 1c). The transfer was done by cutting 0.25–1 cm^2 agar blocks containing the *Labyrinthula* using

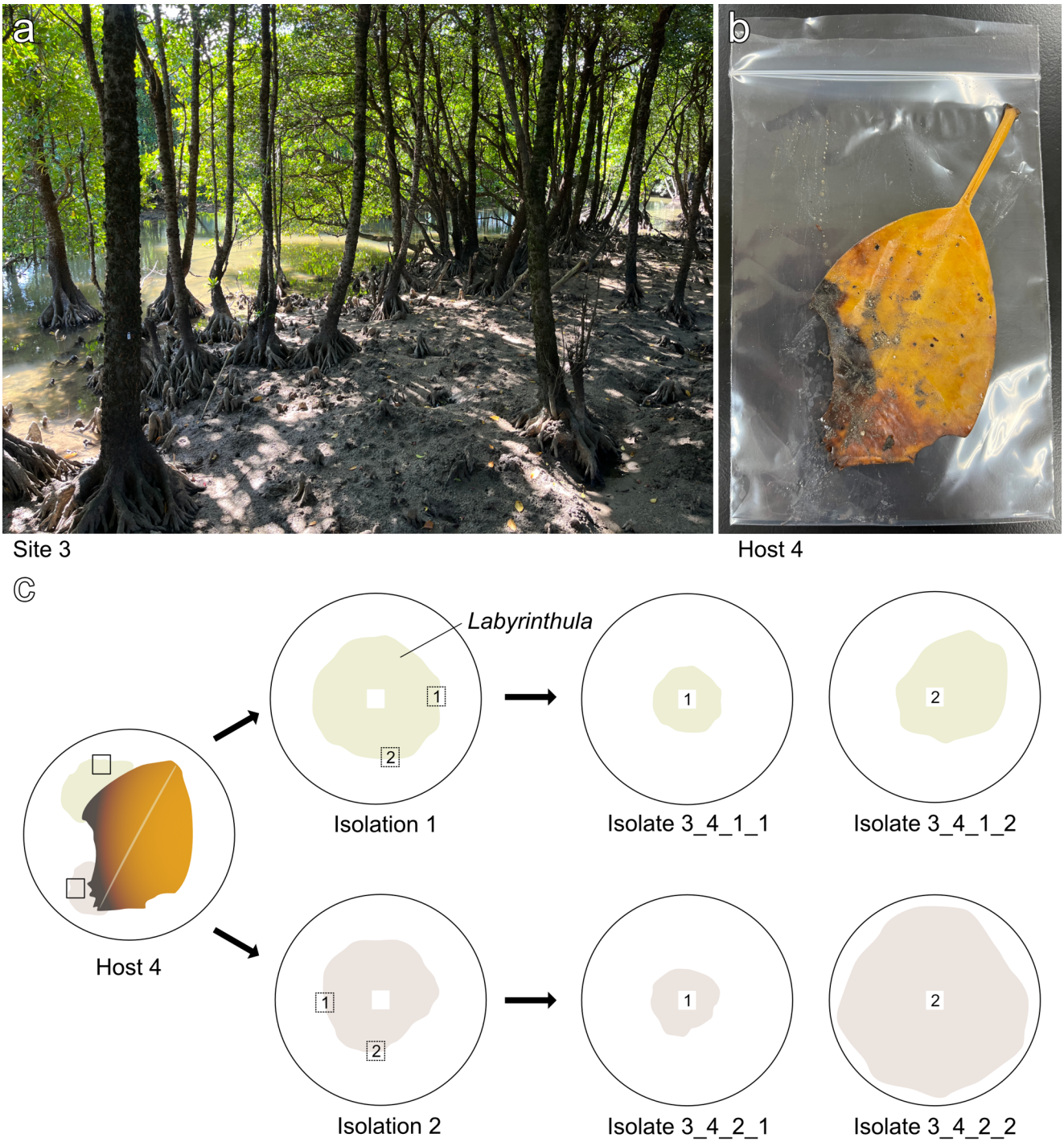

Fig. 1 Sample collection and establishment of *Labyrinthula* cultures. **(a)** Mangroves along Okukubi River (Site 3). **(b)** Decaying leaf (Host 4) taken from Site 3 used for establishment of *Labyrinthula* cultures. **(c)** Graphical summary of isolation and isolate naming scheme

a flame-sterilized razor and placing them onto a new SSA plate of the same composition, except without germanium oxide.

Plates were stored at 28 ± 2 °C and observed daily using an Olympus CKX53 inverted microscope (Olympus, Tokyo, Japan). Cultures were maintained by transfer to new plates every three to fifty days, depending on the growth of each isolate. Plates that exhibited weak growth and dry appearance were supplemented with drops of liquid media, which was prepared similarly to the SSA plates but without the addition of agar.

Light microscopy and cell measurement

Labyrinthula were imaged at 40X and 400X magnification using an Olympus CKX53 inverted microscope (Olympus, Tokyo, Japan) connected to a Canon EOS Kiss X9i digital camera (Canon, Tokyo, Japan). Cell measurements were taken using ImageJ (Schneider et al. 2012).

DNA extraction, PCR amplification and sequencing

For DNA extraction, the section of the agar that was imaged was cut out using a flame-sterilized razor and transferred into an Eppendorf tube. Genomic DNA was extracted using the MasterPure Complete DNA and RNA Purification Kit (Epicentre, Madison, WI, USA) following manufacturer protocols. Two DNA extractions were performed for each plate.

Direct PCR was performed on the extracted DNA using the SR1 (5'-TACCTGGTTGATCCTGCCAG-3') – 18SRF (5'-CCCGTGTTGAGTCAAATTAAG-3') and SR4 (5'-AGGGCAAGTCTGGTGCCAG-3') – SR12 (5'-CCTTCCGCAGGTTCACCTAC-3') primer pairs to amplify the 18S rRNA gene (Nakayama et al. 1996; Mo et al. 2002; Yamaguchi et al. 2006). Thermocycler conditions were as follows: initial denaturation at 94 °C for 10 min, 35 cycles at 98 °C for 10 s, 54 °C for 5 s, and 68 °C for 10 s, and a final extension at 68 °C for 5 min. For the ITS region, amplification was first performed using the SR1–852R-70 (5'-CGAACGATTTGCACGTCAG-3') primer pair (Nakayama et al. 1996; Yamada et al. 2013) under the following thermocycler conditions: initial denaturation at 94 °C for 10 min, 35 cycles at 98 °C for 10 s, 52 °C for 5 s, and 68 °C for 10 s, and a final extension at 68 °C for 5 min. After diluting the PCR products 1:100 times, the reaction was then nested using the Lp1F1 (5'-GTCCCTGCCCTTTGTACAC-3') – 25R1 (5'-CTTGGTCCGTGTTTCAAGAC-3') primer pair (Kogame et al. 1999; Howard et al. 2009) under the following thermocycler conditions: initial denaturation at 94 °C for 10 min, 27 cycles at 98 °C for 10 s, 58 °C for 5 s, and 68 °C for 10 s, and a final extension at 68 °C for 5 min. The PCR products were then purified using polyethylene glycol (PEG) and directly sequenced with BrilliantDye Terminator v1.1 (NimaGen, Nijmegen, Netherlands) according to manufacturer protocol on a 3130 genetic analyzer (Applied Biosystems, Waltham, MA, USA). Sequencing for the 18S rRNA gene was performed using the SR1, 18SRF, and SR12 primers. The Lp1F1 primer was used to sequence the ITS region.

Phylogenetic analysis of 18S rRNA gene and ITS region

Sanger sequencing reads for both the 18S rRNA gene and ITS region were processed using Geneious Prime 2025 (https://www.geneious.com). Contigs were assembled using the "De Novo Assemble" tool with low quality regions and ends manually trimmed. A BLAST search was run on the resulting consensus sequences for identification. A total of 69 closely-related sequences from GenBank and additional sequences based on published phylogenetic analyses conducted by Martin et al. (2016), Popova et al. (2020), and Pagenkopp Lohan et al. (2025) were compiled and aligned with the new sequences using Multiple Alignment using Fast Fourier Transform (MAFFT) (Katoh et al. 2002) (Table S1, Supplementary Material 1). The alignment was then trimmed using Gblocks (Talavera and Castresana 2007), resulting in a final alignment that was 1412 bps for 18S and 241 bps for ITS. The 18S and ITS alignments were then concatenated to make a 1653 bp alignment wherein 78 of the 131 sequences consisted of both genes. MEGA 11 (Stecher et al. 2020; Tamura et al. 2021) was used to calculate the p-distance matrix for percent identity (Agnew-Camiener et al. 2025).

Iqtree v1.6.12 (Nguyen et al. 2015) was used to select the best-fit model for both Maximum-Likelihood and Bayesian analyses. For the Maximum-Likelihood analysis of concatenated data (18S and ITS region sequences), the model GTF+F+R4 was selected under the Akaike Information Criterion with correction. The same model was selected for the 18S alignment, while the model TIM2+F+I+G4 was selected for the ITS alignment. Bootstrap analysis was conducted with 1,000 pseudo-replicates. MrBayes 3.2.7a (Huelsenbeck and Ronquist 2001) was used to calculate Bayesian Posterior Probabilities. For Bayesian analysis, the general time reversible (GTR) substitution model with gamma-distributed rate variation across sites and a proportion of invariable sites ("GTR+I+Γ") was selected. Four Markov Chain Monte Carlo (MCMC) chains were run with the general time reversible (GTR) substitution model for 10,000,000 generations and sampled every 100th generation. The default settings were used for all other parameters. After discarding the first 25% as burn-in, the posterior probability values were obtained from the resulting consensus tree.

Sequences generated from this study were deposited to NCBI. Accession numbers are listed in Table S2 (Supplementary Material 1). Each sequence was labelled as isolate X_Y_Z according to the site, host, and isolation they were taken from. Site number X ranges from 1 to 14 while host number Y ranges from 1 to 16. Isolate number varies depending on the number of re-isolations performed. An

example of the sequence naming scheme can be seen in Fig. 1c.

Results

Sample collection, culturing, and site distribution

A total of 198 leaves were collected across 14 sites, 8 of which were facing the East China Sea, while the other 6 were facing the Philippine Sea (Fig. 2). A minimum of 6 and a maximum of 20 leaves from each site were plated (Table 1). *Labyrinthula* were observed growing from the leaves in all sites within one to two days after culturing. From the 198 leaves plated, 47 leaves contained one isolate, while 14 leaves contained 2–3 isolates. A total of 61 isolates in 6 distinct lineages were successfully grown and identified. Two of these lineages have previously been defined by Martin et al. (2016) as "M" and "Q"/"V" (the former based on ITS data and the latter based on 18S data; it is henceforth referred to as "Q=V"), while the rest of the lineages have been designated as O1–O4.

Lineages Q=V, M, and O1 contained multiple isolates, while Lineages O2, O3, and O4 contained one isolate each. Lineage Q=V is composed of 22 isolates found at 10 different sites, Lineage M of 27 isolates at 11 different sites, and Lineage O1 of 8 isolates at 4 different sites.

As for their distribution around the island, Lineage O4 was found at a site facing the Philippine Sea, while Lineages O2 and O3 were found at sites facing the East China Sea. Other lineages were found at sites on both sides of the island. A detailed list of the isolates found at each site can be found in Table S2 (Supplementary Material 1).

Culture morphology

All cultures exhibited characteristic spindle-shaped cells with a singular central nucleus visibly connected by cytoplasmic threads. Streams of cells were shown to move through the ectoplasmic net. Differences in morphology, specifically in cell size (Table 2) and radiating pattern of the ectoplasmic net, were observed between each lineage.

In the lineages with the most isolates, Q=V (Fig. 3a) and M (Fig. 3b), the cells formed masses in some areas of the ectoplasmic net, especially towards the margins. However, this clumping was much more pronounced in Lineage Q=V, as the cells at the margins of the ectoplasmic net could no longer be distinguished from one another. The streams in Lineage Q=V also had multiple cells clumped together. On the other hand, isolates in Lineage M had slightly more spaced-out masses and streams wherein the individual cells were still visibly separated. Lineage Q=V also had smaller cells on average than Lineage M: the former had a cell length ranging from 5.6 to 13.2 µm (X±SD=10.2±1.6 µm, n=50) and a cell width ranging from 2.4 to 4.9 µm (X±SD=3.3±0.6 µm, n=50), while the latter had a cell length ranging from 7.2 to 16.4 µm (X±SD=11.4±2.2 µm, n=50) and a cell width ranging from 2.3 to 4.4 µm (X±SD=3.5±0.5 µm, n=50).

Lineage O1 (Fig. 3c) did not exhibit any clumping unlike Lineages Q=V and M. Isolates in this lineage had a cell length ranging from 7.6 to 12.1 µm (X±SD=9.7±1.0 µm, n=50) and a cell width ranging from 2.4 to 5.3 µm (X±SD=3.8±0.5 µm, n=50). They exhibited a radiating pattern that consisted of long, central streams of cells from which other smaller streams extended.

Singular isolates in Lineages O2–O4 also exhibited unique cell and colony characteristics. Lineage O2 (Fig. 3d) had the largest cells on average, with a cell length ranging from 10.6 to 15.5 µm (X±SD=13.2±1.8 µm, n=10) and a cell width ranging from 3.5 to 5.4 µm (X±SD=4.5±0.6 µm, n=10). Their unique radiating pattern consisted mainly of thick, circular streams of cells. On the other hand, Lineage O3 (Fig. 3e) had the smallest cells on average, with a cell length ranging from 7.2 to 10.6 µm (X±SD=9.4±1.1 µm, n=10) and a cell width ranging from 2.8 to 3.9 µm (X±SD=3.4±0.4 µm, n=10). The network formed here lacked any distinct central streams of cells. Lineage O4 (Fig. 3f) had a cell length ranging from 9.5 to 13.1 µm (X±SD=10.8±1.1 µm, n=10) and a cell width ranging from 2.8 to 4.3 µm (X±SD=3.4±0.5 µm, n=10). The colony here formed a large mass with cells accumulating at a smooth margin.

Phylogeny

Sequences of the 18S rRNA gene were obtained from 61 unique isolates. The same 61 isolates were sequenced for the ITS region; however, isolate 5_16_2_1 was not completely sequenced, likely due to mononucleotide slippage. Hence, only 60 ITS region sequences were obtained.

Maximum-Likelihood and Bayesian analyses were used to reconstruct both individual and concatenated phylogenies for the 18S rRNA gene and ITS region. The resulting tree of the genus *Labyrinthula* showed three distinct clades as described by Martin et al. (2016): "P" for pathogenic seagrass isolates including *Labyrinthula zosterae*, "T" for terrestrial isolates, and "N" for non-pathogenic isolates occurring on various aquatic vegetation. These three clades were present in the concatenated tree (Fig. 2) as well as the 18S (Fig. S1, Supplementary Material 3) and ITS (Fig. S2, Supplementary Material 3) trees, which all showed similar topology.

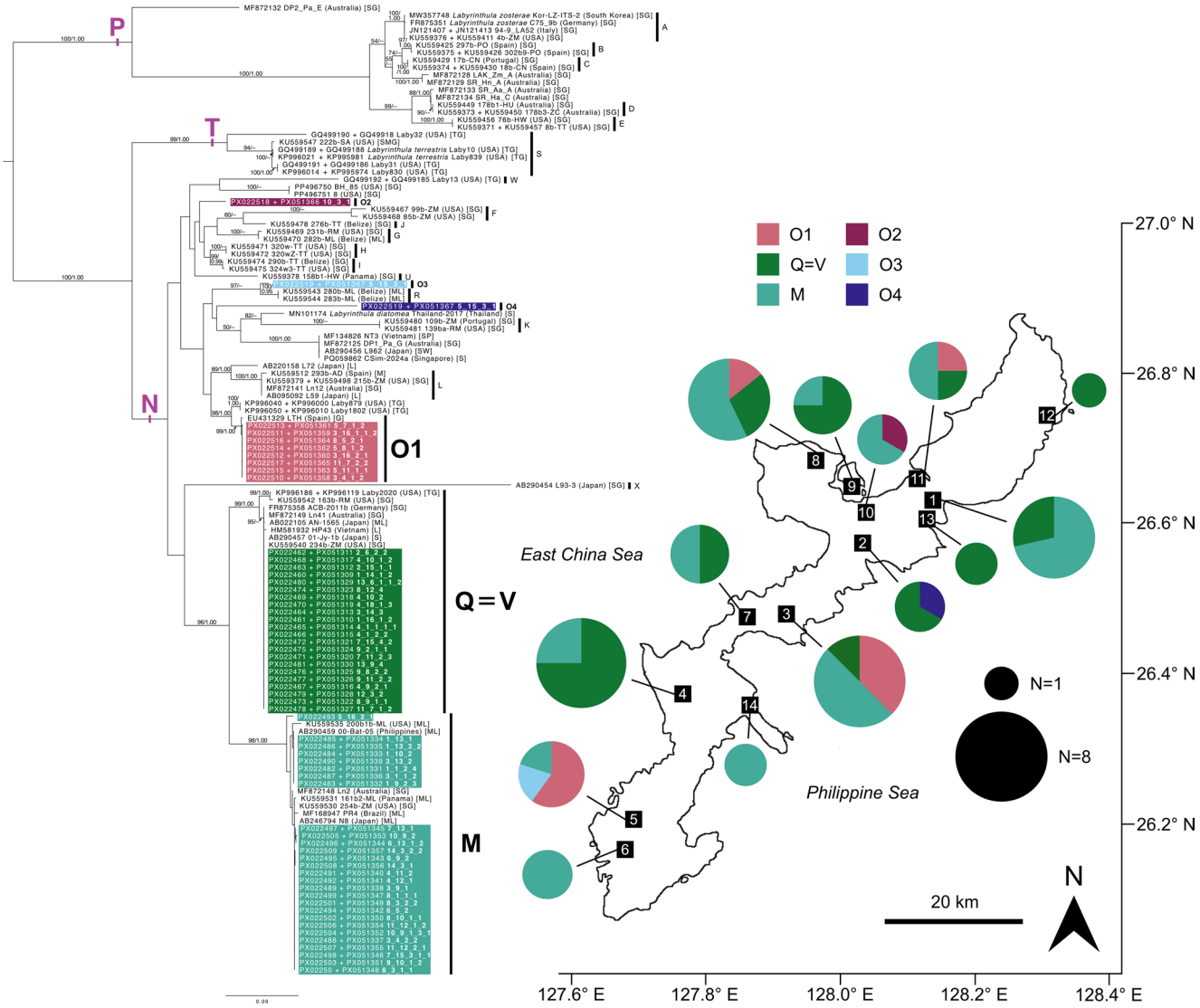

Fig. 2 Maximum-Likelihood (ML) tree inferred from concatenated 18S rRNA gene and ITS region sequences and map of sampling sites around Okinawa main island (see Table 1 for sampling site names corresponding to each number). A total of 61 isolates from this study as well as 69 additional *Labyrinthula* isolates from GenBank are contained in this tree. Maximum-Likelihood (ML) values over 50 and Bayesian Posterior Probabilities (BPP) over 0.95 are displayed at the nodes (ML/BPP). Capital letters in magenta indicate pathogenic clade P, non-pathogenic clade N, and terrestrial clade T. Capital letters in black are based on putative species-letter groupings by Martin et al. (2016), with the addition of new lineages O1–O4. The lineages containing the sequences in this study are in bold. Original hosts/substrates of the additional isolates are indicated in square brackets; these include seagrass [SG], turfgrass [TG], salt marsh grass [SMG], mangrove leaves [ML], seawater [S], shrimp pond [SP], seaweed [SW], leaves [L], macroalgae [M], and gill tissue [G]. Sequences generated in this study corresponding to six different lineages are highlighted. Pie charts indicate the proportion of sequences from each lineage found at each site

The 18S, ITS, and concatenated trees also all formed the putative species-letter groupings described by Martin et al. (2016). Groups F, G, H, I, J, K, and R were exclusive to the ITS tree, while groups U and X were exclusive to the 18S tree. All groups were present in the concatenated tree.

The sequences in this study were all grouped into the non-pathogenic clade N (100% ML/1.00 BPP), where they formed six distinct lineages. Sequences from the same host generally grouped into the same lineage except for the following pairs of isolates: 7_15_3_1_1 and 7_15_4_2, 11_7_1_2 and 11_7_2_2, and 3_4_1_2 and 3_4_2_2.

Two of the six identified lineages shared a high percent identity (Table 3) with existing putative species-letter groupings Q=V and M and were thus grouped therein. Lineage Q=V included other isolates obtained from seawater, turfgrass, leaf, and seagrass hosts, while Lineage M included other isolates obtained from mangrove leaf and seagrass hosts.

Table 1 Summary of sample collection data including sampling locations, number of leaves sampled, and number of isolates sequenced per site

Site Number	Site Name	Coordinates	No. of leaves sampled	No. of isolates sequenced
1	Kesaji River	26°36'17.8"N 128°08'39.0"E	18	7
2	Oura River	26°33'34.4"N 128°02'37.0"E	15	3
3	Okukubi River	26°27'34.8"N 127°56'12"E	18	8
4	Hija River	26°21'55.7"N 127°45'03.6"E	18	8
5	Lake Man	26°11'42.9"N 127°40'56.6"E	20	5(4)[a]
6	Onaga	26°09'28"N 127°39'56.5"E	14	3
7	Onna	26°28'51.0"N 127°50'49"E	17	4
8	Ooi River	26°41'09.5"N 127°58'30.0"E	12	7
9	Yohena	26°39'25.6"N 128°00'57.6"E	12	4
10	Makiya	26°38'01.7"N 128°02'05.0"E	12	3
11	Shirahama	26°39'34.0"N 128°07'14.2"E	12	4
12	Ada	26°44'34.8"N 128°18'55.6"E	12	1
13	Gesashi	26°36'12.4"N 128°08'31.6"E	12	2
14	Katsurenhae-baru	26°20'08.4"N 127°52'11.9"E	6	2

[a]Only 4 isolates were sequenced for ITS

Novel lineages O1–O4 all branched independently from existing putative-species letter groups. Lineage O1, which is composed of multiple isolates, grouped with exactly one other existing sequence obtained from an isolate of *Labyrinthula* sp. found in the gill tissue of *Psetta maxima.* The clade formed by these sequences is sister to a group containing isolates from turfgrass.

Lineages O2–O4 comprise only one isolate each, indicating the discovery of three new genetically distinct *Labyrinthula* spp.. The phylogenetic position of these lineages is variable between the 18S, ITS, and concatenated trees.

Within-lineage mean percent identity for Lineages Q=V, M, and O1 ranged from 99.7 to 99.9% for the 18S sequences and 98.7 to 99.9% for the ITS sequences. Among-lineage mean percent identity, which was calculated in comparison to the nearest sister clade of each lineage, ranged from 91.3 to 97.5% for 18S and 77.3 to 97.1% for ITS. The among-lineage percent identity values were calculated in comparison with the nearest neighbor (i.e. highest percent identity) to each lineage. There are no overlaps in the ranges of both 18S and ITS percent identity within and among lineages as shown in Table 3. The distance matrices used to calculate percent identity values may be viewed in Supplementary Material 2 for the 18S alignment and Supplementary Material 4 for the ITS alignment.

Discussion

Distribution of *Labyrinthula* in Okinawan mangroves

This study confirmed the presence of six distinct lineages of *Labyrinthula* spp. in Okinawan mangroves. Lineages Q=V, M, and O1, were ubiquitous on both sides of the island, and may have been transported either through human- or animal-mediated dispersal or through detritus movement in the water column (Trevathan-Tackett 2018). Lineages O2 and O3 were found on the side of the East China sea, while Lineage O4 was found on the side of the Philippine Sea. However, since only one isolate was successfully cultured and identified in each lineage, it cannot be determined whether these isolates are representative of their respective lineage's distribution.

Based on the number of isolates sampled at each site, Lineages Q=V and M appeared to be dominant in Okinawan mangroves. However, it is also possible that these numbers were boosted by the ability of the isolates from these lineages to survive in the given culture conditions. Similarly, the minimal presence of Lineages O2, O3, and O4 may be due to their inability to culture well or low abundance in nature.

Table 2 Cell size measurements. Comparison of cell length and width between each lineage

		Cell length (µm)				Cell width (µm)			
Lineage	***n***	Mean	Std	Min	Max	Mean	Std	Min	Max
Q=V	50	10.2	1.6	5.6	13.2	3.3	0.6	2.4	4.9
M	50	11.4	2.2	7.2	16.4	3.5	0.5	2.3	4.4
O1	50	9.7	1.0	7.6	12.1	3.8	0.5	2.4	5.3
O2	10	13.2	1.8	10.6	15.5	4.5	0.6	3.5	5.4
O3	10	9.4	1.1	7.2	10.6	3.4	0.4	2.8	3.9
O4	10	10.8	1.1	9.5	13.1	3.4	0.5	2.8	4.3

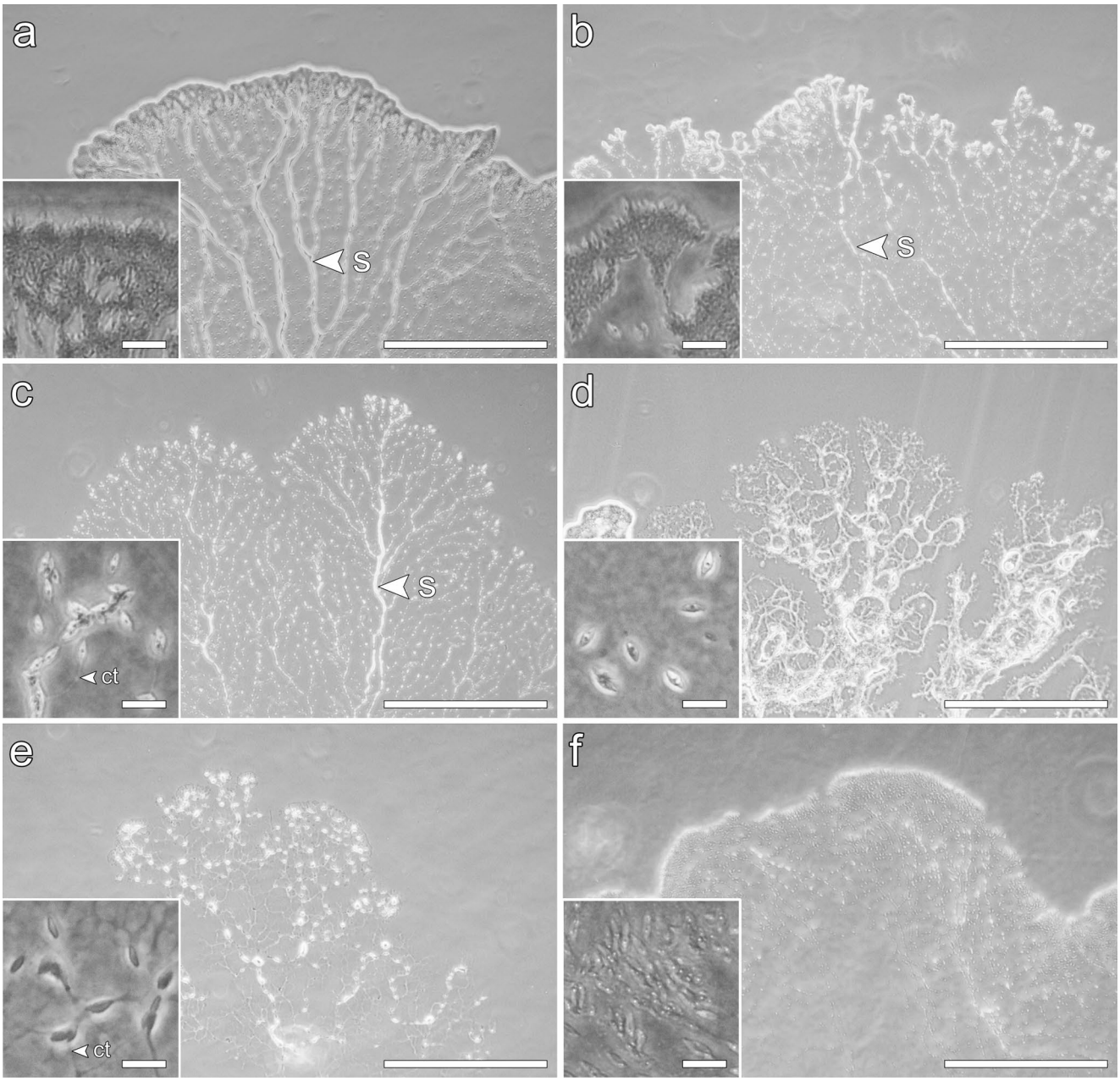

Fig. 3 Representative micrographs of *Labyrinthula* cultures on agar showing branching patterns of each lineage. Streams (s) and cytoplasmic threads (ct) are labeled. **(a)** Isolate 13_9_4 belonging to Lineage Q=V. **(b)** Isolate 11_12_1_2 belonging to Lineage M. **(c)** Isolate 3_16_2_1 belonging to Lineage O1. **(d)** Isolate 10_3_1 belonging to Lineage O2. **(e)** Isolate 5_15_3_1 belonging to Lineage O3. **(f)** Isolate 2_14_1_2_1 belonging to Lineage O4. Scale bars: 1 mm. Insets show spindle-shaped *Labyrinthula* cells from the same cultures. Scale bars: 20 µm

Labyrinthula culture morphology

Although new lineages have been discovered in this study, the lack of unifying morphological traits in each lineage makes it difficult to declare any new species. While the two dominant lineages demonstrated similarities in their morphology, such as the formation of masses towards the margins of the ectoplasmic net, this observation was not consistent throughout all isolates in both lineages. Each isolate had its own variations in radiating pattern and grew differently on the agar (Fig. S3, Supplementary Material 3). This observation agrees with previous studies that showed that the morphology of isolates of the same putative type can vary depending on age and culture conditions (Pokorny 1967; Muehlstein et al. 1991; Martin et al. 2016). Knight et al. (2025) observed that network morphology in

Table 3 Percent identities of 18S and ITS sequences. Comparisons were done within each lineage and with the nearest neighbor (i.e. highest percent identity) to each lineage. Calculations were based on the p-distance matrix (18S in Supplementary Material 2, ITS in Supplementary Material 4)

Lineage	18S mean % identity within lineage	18S mean % identity with (nearest neighbor)	ITS mean % identity within lineage	ITS mean % identity with (nearest neighbor)
Q=V	99.9	94.2 (M)	99.0	82.8 (M)
M	99.7	94.2 (Q=V)	98.7	82.8 (Q=V)
O1	99.9	97.5 (Laby1802, Laby879)	99.9	97.1 (Laby1802, Laby879)
O2	–	94.4 (O1)	–	89.9 (I)
O3	–	94.7 (L)	–	94.3 (R)
O4	–	91.3 (L)	–	77.3 (O3)

Labyrinthula varied depending on the environmental conditions above the agar; colonies with a liquid overlay formed sparse, filamentous networks while colonies exposed to air exhibited a dense, aggregated morphology. Hence, changes in colony morphology may have occurred after plate transfers in response to variations in moisture.

This environmental dependence of cell and colony morphology not only creates variations within putative types but also results in an overlap of traits between different groups. The high level of morphological variability within each lineage, as well as overlapping traits among different lineages, highlights the need for molecular phylogenetic analysis in reconsidering the taxonomic organization of *Labyrinthula* (Honda et al. 1999).

Phylogeny and ecological plasticity

Based on molecular phylogenetic analysis, the isolates in this study formed clades with existing sequences isolated from various hosts in different locations. This wide host-geographic range seems to be common for putative species-letter groups in the non-pathogenic clade as suggested by Martin et al. (2016). Though it is still unclear how *Labyrinthula* can disperse over such long distances, previous studies have confirmed the presence of labyrinthulids in ballast water, suggesting that maritime shipping is likely to have played a role in their transport (Pagenkopp Lohan et al. 2022).

The range of sites and substrates from which *Labyrinthula* were isolated confirms not only its cosmopolitan nature but also its ability to occupy several different ecological niches. For example, the strain LTH, which was grouped with mangrove samples from this study, was isolated along with the trophozoites of the amoeba *Thecamoeba hilla* in gill tissue (Dyková et al. 2008). The existence of both symbionts and saprotrophs in a single clade implies the broader ecological plasticity of *Labyrinthula.*

Despite this apparent plasticity, there is still a clear distinction between the pathogenic clade P and the non-pathogenic clade N containing the saprobic decomposers found in this study. Nonetheless, the existence of pathogenic strains within the isolates in this study cannot be ruled out as no pathogenicity tests were conducted. Trevathan-Tackett et al. (2018) also suggested the existence of within-clade and within-haplotype variability in pathogenicity for seagrass samples; the same may apply for the mangrove leaf samples found in this study.

Further study is needed to determine whether the same *Labyrinthula* spp. strains which act as saprobic decomposers to mangrove leaves are also capable of acting as pathogens to mangroves or even other hosts such as seagrass and macroalgae, especially since *Labyrinthula* are capable of moving between biological hosts through direct contact (Muehlstein 1992; Martin 2016). On the other hand, pathogenic lineages have also been shown to demonstrate the fatty acid production ability previously associated with saprotrophic *Labyrinthula* (Yoshioka et al. 2019), suggesting that some ecological roles may be shared between pathogenic and non-pathogenic groups. Additional data would be needed to ascertain the environmental conditions that give rise to these ecological changes, which will be especially helpful for studying pathogen evolution in the future.

Conclusion

This study is the first to demonstrate the genetic diversity of *Labyrinthula* spp. found in Okinawan mangroves. The separation of the isolates into six distinct lineages indicates that there exists a high level of genetic diversity within *Labyrinthula* sampled in mangroves. It is likely that their presence here is supported by the amount of detritus available in these carbon sinks that are utilized by these saprotrophic organisms, possibly to produce nutritionally valuable LCPUFAs (Yoshioka et al. 2019). Hence, given the existence of various lineages, there is potential for diversity in terms of chemical production. Further investigation into the chemical profiles of each lineage could help shed more light on the diversity and ecology of *Labyrinthula* in mangroves, as well as lay the groundwork for future biotechnological applications.

The ecological plasticity of *Labyrinthula* has also been highlighted through comparison with isolates obtained from a variety of substrates and locations. An interesting topic for further research would be investigating whether the saprotrophic *Labyrinthula* from mangroves can become symbiotic or even pathogenic under certain conditions. With the

wide range of *Labyrinthula* taking up different roles in the ecosystem, understanding and categorizing them based on their genetic diversity is the first essential step to both managing pathogenicity and harnessing the full potential of their chemical production.

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Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Natalia C. Araña, Siratee Riewluang, and Kevin C. Wakeman. The first draft of the manuscript was written by Natalia C. Araña, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The 18S and ITS sequence datasets generated during the current study are available at NCBI GenBank (https://www.ncbi.nlm.nih.gov/nuccore/) with the respective accession numbers: PX022460–PX022520; PX051309–PX051368.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to declare.

Ethics approval No permits were required for the collection of detrital mangrove leaves in Okinawa, Japan.

References

Agnew-Camiener MV, Eisenlord ME, Friedman CS, Schreier HJ, Burge CA (2025) Pathogenicity and phylogeny of *Labyrinthula* spp. isolated in Washington and Oregon, USA. J Eukaryot Microbiol 72:e13073. https://doi.org/10.1111/jeu.13073

Beakes GW, Honda D, Thines M (2014) 3 systematics of the stramenipila: Labyrinthulomycota, Hyphochytriomycota, and oomycota. In: McLaughlin DJ, Spatafora JW (eds) Systematics and evolution, 2nd edn. Springer, Berlin, Heidelberg, pp 39–97. https://doi.org/10.1007/978-3-642-55318-9_3

Bennett RM, Honda D, Beakes GW, Thines M (2017) Labyrinthulomycota. In: Archibald JM, Simpson AGB, Slamovits CH (eds) Handbook of the protists, 2nd edn. Springer, Cham, pp 507–542. https://doi.org/10.1007/978-3-319-28149-0_25

Boro MC, Harakava R, Pires-Zottarelli CLA (2018) Labyrinthulomycota from Brazilian mangrove swamps and coastal waters. Bot Mar 61:65–74. https://doi.org/10.1515/bot-2017-0052

Dyková I, Fiala I, Dvořáková H, Pecková H (2008) Living together: the marine amoeba *Thecamoeba Hilla* Schaeffer, 1926 and its endosymbiont *Labyrinthula* Sp. Eur J Protistol 44:308–316. https://doi.org/10.1016/j.ejop.2008.04.001

FioRito R, Leander C, Leander B (2016) Characterization of three novel species of labyrinthulomycota isolated from ochre sea stars (*Pisaster ochraceus*). Mar Biol 163:170. https://doi.org/10.1007/s00227-016-2944-5

Honda D, Yokochi T, Nakahara T, Raghukumar S, Nakagiri A, Schaumann K, Higashihara T (1999) Molecular phylogeny of labyrinthulids and thraustochytrids based on the sequencing of 18S ribosomal RNA gene. J Eukaryot Microbiol 46(6):637–647. https://doi.org/10.1111/j.1550-7408.1999.tb05141.x

Howard MDA, Smith GJ, Kudela RM (2009) Phylogenetic relationships of yessotoxin-producing dinoflagellates, based on the large subunit and internal transcribed spacer ribosomal DNA domains. Appl Environ Microbiol 75:54–63. https://doi.org/10.1128/AEM.00818-08

Huelsenbeck JP, Ronquist F (2001) MrBayes: bayesian inferences of phylogeny. Bioinformatics 17:754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Inoue T, Baba S, Akaji Y, Miura S, Oshiro N, Kezuka M, Suárez EL (2024) Mangroves of the South Kuroshio. Preprint at https://ecoevorxiv.org/repository/view/7161/

Jepps MW (1931) Note on a marine *Labyrinthula*. J Mar Biol Assoc UK 17:833–838. https://doi.org/10.1017/S0025315400052000

Katoh K, Misawa K, Kuma KI, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast fourier transform. Nucleic Acids Res 30:3059–3066. https://doi.org/10.1093/nar/gkf436

Knight JM, Gonzalez P, Fairhurst DJ, Melaugh G, Poon WCK (2025) Environmental dependence of colony morphologies in Labyrinthula species. Preprint at https://www.biorxiv.org/content/https://doi.org/10.1101/2025.04.01.646626v1

Kogame K, Horiguchi T, Masuda M (1999) Phylogeny of the order scytosiphonales (Phaeophyceae) based on DNA sequences of rbcL, partial rbcS, and partial LSU NrDNA. Phycologia 38:469–502. https://doi.org/10.2216/i0031-8884-38-6-496.1

Kumon Y, Yokoyama R, Haque Z, Yokochi T, Honda D, Nakahara T (2006) A new labyrinthulid isolate that produces only docosahexaenoic acid. Mar Biotechnol 8:170–177. https://doi.org/10.1007/s10126-005-5098-x

Leander CA, Porter D (2001) The labyrinthulomycota is comprised of three distinct lineages. Mycologia 93:459–464. https://doi.org/10.1080/00275514.2001.12063179

Leander CA, Porter D, Leander BS (2004) Comparative morphology and molecular phylogeny of aplanochytrids (Labyrinthulomycota). Eur J Protistol 40:317–328. https://doi.org/10.1016/j.ejop.2004.07.003

Martin DL, Chiari Y, Boone E, Sherman TD, Ross C, Wyllie-Echeverria S, Gaydos JK, Boettcher AA (2016) Functional, phylogenetic and host-geographic signatures of *Labyrinthula* spp. provide for putative species delimitation and a global-scale view of seagrass wasting disease. Estuaries Coasts 39:1403–1421. https://doi.org/10.1007/s12237-016-0087-z

Mo C, Douek J, Rinkevich B (2002) Development of a PCR strategy for thraustochytrid identification based on 18S rDNA sequence. Mar Biol 140:883–889. https://doi.org/10.1007/s00227-002-0778-9

Muehlstein LK (1992) The host-pathogen interaction in the wasting disease of eelgrass, *Zostera marina*. Can J Bot 70:2081–2088. https://doi.org/10.1139/b92-258

Muehlstein LK, Porter D, Short FT (1991) *Labyrinthula zosterae* sp. nov., the causative agent of wasting disease of eelgrass, *Zostera marina*. Mycologia 83:180–191. https://doi.org/10.2307/3759933

Nakayama T, Watanabe S, Mitsui K, Uchida H, Inouye I (1996) The phylogenetic relationship between the chlamydomonadales and chlorococcales inferred from 18SrDNA sequence data. Phycol Res 44:47–55. https://doi.org/10.1111/j.1440-1835.1996.tb00037.x

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) Iq-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300

Olsen MW (2007) *Labyrinthula terrestris*: a new pathogen of cool-season turfgrasses. Mol Plant Pathol 8:817–820. https://doi.org/10.1111/j.1364-3703.2007.00425.x

Pagenkopp Lohan KM, Darling JA, Ruiz GM (2022) International shipping as a potent vector for spreading marine parasites. Divers Distrib 28:1922–1933. https://doi.org/10.1111/ddi.13592

Pagenkopp Lohan KM, DiMaria R, Martin DL, Hughes AR, Peterson BJ, Boyer KE, Stachowicz JJ, Jorgensen P, Ruiz GM, Ross C (2025) Phylogeography of *Labyrinthula* species and strains shows high connectivity and low genetic variation across seagrass hosts and geographic locations in North America. Front Mar Sci. https://doi.org/10.3389/fmars.2024.1463968

Pokorny KS (1967) Labyrinthula. J Protozool 14:697–708. https://doi.org/10.1111/j.1550-7408.1967.tb02065.x

Popova OV, Belevich TA, Golyshev SA, Kireev II, Aleoshin VV (2020) *Labyrinthula Diatomea* n. sp.—a labyrinthulid associated with marine diatoms. J Eukaryot Microbiol 67:393–402. https://doi.org/10.1111/jeu.12789

Porter D (1972) Cell division in the marine slime mold, *Labyrinthula* sp., and the role of the bothrosome in extracellular membrane production. Protoplasma 74:427–448. https://doi.org/10.1007/BF01281960

Raghukumar C (1987) Fungal parasites of the green alga *Chaetomorpha media*. Dis Aquat Org 3:147–150. https://doi.org/10.3354/dao003147

Raghukumar S (2002) Ecology of the marine protists, the labyrinthulomycetes (thraustochytrids and labyrinthulids). Eur J Protistol 38:127–145. https://doi.org/10.1078/0932-4739-00832

Raghukumar S (2017) The Mangrove ecosystem. Fungi in coastal and oceanic marine ecosystems. Springer, Cham, pp 61–86. https://doi.org/10.1007/978-3-319-54304-8_5

Sakata T, Fujisawa T, Yoshikawa T (2000) Colony formation and fatty acid composition of marine labyrinthulid isolates grown on agar media. Fish Sci 66:84–90. https://doi.org/10.1046/j.1444-2906.2000.00012.x

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to imageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089

Stecher G, Tamura K, Kumar S (2020) Molecular evolutionary genetics analysis (MEGA) for MacOS. Mol Biol Evol 37:1237–1239. https://doi.org/10.1093/molbev/msz312

Sullivan BK, Robinson KL, Trevathan-Tackett SM, Lilje ES, Gleason FH, Lilje O (2017) The first isolation and characterisation of the protist *Labyrinthula* sp. in southeastern Australia. J Eukaryot Microbiol 64:504–513. https://doi.org/10.1111/jeu.12387

Suzuki T, Ueda M, Iwata I, Doi K, Nakazawa A, Yoshida M, Honda D, Ishida K (2022) Phylogenetic diversity and distribution of labyrinthulids in Japan. Agric Biotechnol 6:1142–1146

Talavera G, Castresana J (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst Biol 56(4):564–577. https://doi.org/10.1080/10635150701472164

Tamura K, Stecher G, Kumar S (2021) MEGA 11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Thomas M, Nakajima Y, Mitarai S (2022) Extremely stochastic connectivity of island mangroves. Front Mar Sci. https://doi.org/10.3389/fmars.2022.827590

Trevathan-Tackett SM, Sullivan BK, Robinson K, Lilje O, Macreadie PI, Gleason FH (2018) Pathogenic *Labyrinthula* associated with Australian seagrasses: considerations for seagrass wasting disease in the Southern hemisphere. Microbiol Res 206:74–81. https://doi.org/10.1016/j.micres.2017.10.003

Tsui CKM, Marshall W, Yokoyama R, Honda D, Lippmeier JC, Craven KD, Peterson PD, Berbee ML (2009) Labyrinthulomycetes phylogeny and its implications for the evolutionary loss of chloroplasts and gain of ectoplasmic gliding. Mol Phylogenet Evol 50:129–140. https://doi.org/10.1016/j.ympev.2008.09.027

Wahid MI, Yoshikawa T, Sakata T (2007) Bacteriolytic activity and growth of marine isolates of labyrinthulids on dead bacterial cells. Fish Sci 73:1286–1294. https://doi.org/10.1111/j.1444-2906.2007.01467.x

Wang Q, Ye H, Xie Y, He Y, Sen B, Wang G (2019) Culturable diversity and lipid production profile of labyrinthulomycete protists isolated from coastal mangrove habitats of China. Mar Drugs 17:268. https://doi.org/10.3390/md17050268

Yamada N, Terada R, Tanaka A, Horiguchi T (2013) *Bispinodinium angelaceum* gen. et sp. nov. (Dinophyceae), a new sand-dwelling dinoflagellate from the seafloor off Mageshima Island, Japan. J Phycol 49:555–569. https://doi.org/10.1111/jpy.12064

Yamaguchi A, Kawamura H, Horiguchi T (2006) A further phylogenetic study of the heterotrophic dinoflagellate genus, *protoperidinium* (Dinophyceae) based on small and large subunit ribosomal RNA gene sequences. Phycol Res 54:317–329. https://doi.org/10.1111/j.1440-1835.2006.00438.x

Yoshioka RM, Schram JB, Galloway AWE (2019) Eelgrass pathogen *Labyrinthula zosterae* synthesizes essential fatty acids. Dis Aquat Org 135:89–95. https://doi.org/10.3354/dao03382