

# A Re-Visit to the Evolution and Ecophysiology of the Labyrinthulomycetes

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## 1. Introduction

The labyrinthulomycetes (also known as Labyrinthulomycota or Labyrinthulea) are marine heterotrophic fungus-like protists and belong to the eukaryotic Kingdom Stramenopiles (Honda et al., 1999, Tsui et al., 2009). Most labyrinthulomycete species are unicellular, and they are ubiquitous in the ocean, and their occurrence and distribution in water column and sediments have been well documented (Kimura et al., 1999, Naganuma et al., 1998, Raghukumar, 2002). Their main ecological role may be as saprotrophic decomposers, recycling nutrients in marine and coastal ecosystems, by chemical alteration of detritus through extra-cellular enzymes (Raghukumar, 2002, Taoka et al., 2009). Their role in facilitating the settlement of barnacle cyprids has also been demonstrated (Raghukumar et al., 2000).

Labyrinthulomycetes have been studied by mycologists, and two comprehensive reviews were published by Raghukumar and her co-workers on their ecology (Raghukumar, 2002, Raghukumar & Damare, 2011). In these reviews, the authors dealt mainly with the general ecological role of these organisms in the marine ecosystems; their associations/interactions with living or decaying plant materials, phytoplankton, animals and bacteria, either in sediments or in the oceanic water column. Their role in the marine food web either as “remineralizers” and possible “left-over” scavengers were also discussed.

Though labyrinthulomycetes belong in the Stramenopiles, they evolved a fungus-like, absorptive mode of osmotrophic nutrition by developing rhizoids on detritus. Convergently with true fungi and oomycetes (also in Stramenopiles), some labyrinthulomycetes are pathogenic, causing diseases such as turf grass and eelgrass wasting disease, and the hard clam disease ‘QPX’, a role discovered only over the last two decades (Bigelow et al., 2005, Craven et al., 2005, Muelstein et al. 1988, Stokes et al., 2002). Many representatives in labyrinthulomycetes accumulate high level of omega-3 long-chain polyunsaturated fatty acids (PUFAs), such as, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acids (DPA) within the cells, thus being an important component in the detrital food web (Findlay et al., 1986, Yongmanitchai & Ward 1989). As a result, a number

of species are currently serving as sources of valuable DHA used in dietary supplements and for DHA production in industry (Abril et al., 2000, Sijtsma & de Swaaf, 2004). Recent studies have also revealed their potential in carotenoid and squalene production (Carmona et al., 2003, Jiang et al. 2004), and as aquacultural feeds (Yamasaki et al., 2007).

The labyrinthulomycetes are important in nutrient recycling, and in the food and biotechnology industry. However their ecophysiology and evolution are not well understood. This chapter will bring together the latest information on their evolution, ecology and physiology. We also review some current approach to unravel their evolutionary origins and ecological role in the oceans and mangrove environment, particularly on the thraustochytrids.

## **2. Techniques for physiological, ecological and evolutionary investigation**

### **2.1 Isolation and cultivation**

Representatives of labyrinthulomycetes can be isolated from mangrove leaves, sediment, open water, and from the guts of marine invertebrates. Normally mangrove or marine samples collected are rinsed and directly placed on yeast extract-peptone (YEP) agar (Fan et al., 2002a, 2009). Alternatively samples are collected and placed in the centrifuge tubes/ test tubes containing 10 ml sterilized, full strength artificial/ natural seawater, together with small amount of sterilized pine pollen (approx. 50-100 pollens). The pine pollens are then aseptically placed on GYP agar [glucose 2 g, polypeptone 1 g, yeast extract 0.5 g, chloramphenicol 0.2 g, agar 15 g, seawater 500 ml, D water 500 ml] or YEP agar [yeast extract 1 g, mycological peptone 1 g, agar (technical grade) 15g and 1 L 15‰ artificial seawater] for microscopy and further isolation.

Similarly marine invertebrates are collected, and the diluted gut contents are plated onto various media (Porter, 1990, Tsui et al., 2009). Undiluted coelomic fluid samples can be directly plated onto corresponding media (Porter, 1990, Tsui et al., 2009). Plates were checked every 4 -5 days under a dissecting microscope. Transmission electron microscopy (TEM) can be carried out according to Honda et al. (1998). Colonies exhibiting thraustochytrid-like morphology can be sub-cultured several times until axenic. Thraustochytrid colonies can be maintained in sterile broth too [yeast extract 1g, mycological peptone 1g, glucose 10 g and 1L 15‰ artificial seawater prepared from artificial sea salts (Sigma)].

### **2.2 Fatty acids analysis**

Fatty acid profiles have become important biochemical characters in the delineation of genus, species, and isolates (Fan et al., 2009, Yokoyama et al., 2007a, b). Fatty acids composition are analysed using a modified method of Lepage & Roy (1984). The freeze-dried cells of labyrinthulomycetes are methylated with sulfuric acids in methanol with the addition of an internal standard (e.g. heptadecaenoic acid, C17:0). Then the fatty acid methyl esters (FAMES) are extracted by water and hexane (1:1). The FAMES (1µl) in the hexane layer were subjected to gas chromatography equipped with a flame ionization detector (Agilent 6890 GC-FID), and a DB-225 capillary column (30 mm 5 0.25 mm diam). Injector is held at 220°C with initial temperature at 90°C for 3 min then increases from 90°C to 210°C at 20°C/ min. The detector is held at 230°C and helium is used as carrier gas and the column

flow rate is 1ml/ min. The amount of DHA is identified and quantified by a comparison of retention time for laboratory standard and internal standard.

### 2.3 Carotenoid analysis

To characterize the carotenoid pigment composition of the taxa, cells are extracted with chloroform-methanol. The solvent is removed in vacuum to obtain a crude residue of the extract. The dried extraction is dissolved in a small amount of chloroform and applied to the column of silica gel packed by hexane. The fraction is reconstituted with methanol and loaded onto the HPLC instrument, which is capable of detecting UV-visible wavelength carotenoid spectra (Carmona et al., 2003).

### 2.4 DNA extraction, PCR and sequence analyses

For molecular phylogeny, cells of labyrinthulomycetes on agar or in liquid broth are harvested, and DNA is extracted by commercial kit. Primers of various genes are used to amplify corresponding fragments under the conditions in White et al. (1990) and Tsui et al. (2009). In case of having several fragments after PCR, products corresponding to the expected size are gel-purified and cloned into the vector pCR2.1 using the TOPO TA cloning kit (Invitrogen). Five to ten clones are sequenced using the vector primers and designed internal primers. Sequence data is then aligned with homologous sequences from a representative sampling of eukaryotes from GenBank databases with computer softwares, such as Clustal X (Thompson et al., 1997) or MacClade (Maddison & Maddison, 2000). Alignment data are subjected to various methods of phylogenetic analysis; Maximum Parsimony (MP), Neighbor Joining (NJ) and Maximum-likelihood (ML) using PAUP\*4.0 (Swofford, 2003) and Phylip 3.6 (Felsenstein et. al., 2002).

Culture independent methods are getting popular recently for environmental characterization. Clone libraries of SSU rRNA from water and environmental samples facilitate the investigation of natural communities and unknown lineages in various habitats (Massana et al., 2004a, b). Fluorescent *in situ* hybridisation probes (FISH) and quantitative PCR probes have also been developed for detection of thraustochytrids (Takao et al., 2007), and QPX from marine water simultaneously (Liu et al., 2009).

## 3. Position in the 'tree of life'

Labyrinthulomycetes have been traditionally classified under the Kingdom Fungi based on morphology, as well as their life histories and mode of nutrition. The labyrinthulomycetes presently belong to the Kingdom Stramenopiles, which also accommodate the photosynthetic ochrophytes (brown algae, golden brown algae and diatoms), along with the non-photosynthetic free-living bicoceans, and oomycetes which are well known as serious plant pathogens (Fig. 1) (Cavalier-Smith, 1998, Keeling et al., 2005, Leipe et al., 1994, Oudot-Le Secq et al., 2006, Tsui et al., 2009). Labyrinthulomycetes share Stramenopile characters in having cell walls of thin scales (Chamberlain & Moss, 1988), tubular mitochondria, and biflagellate zoospores with one smooth flagellum and one bearing tripartite tubular hairs (Patterson, 1989). Together with the alveolate relatives, which include the apicomplexa, ciliates and dinoflagellates, they form the super-kingdom "Chromalveolate" defined firstly in Baldalf et al. (2000).

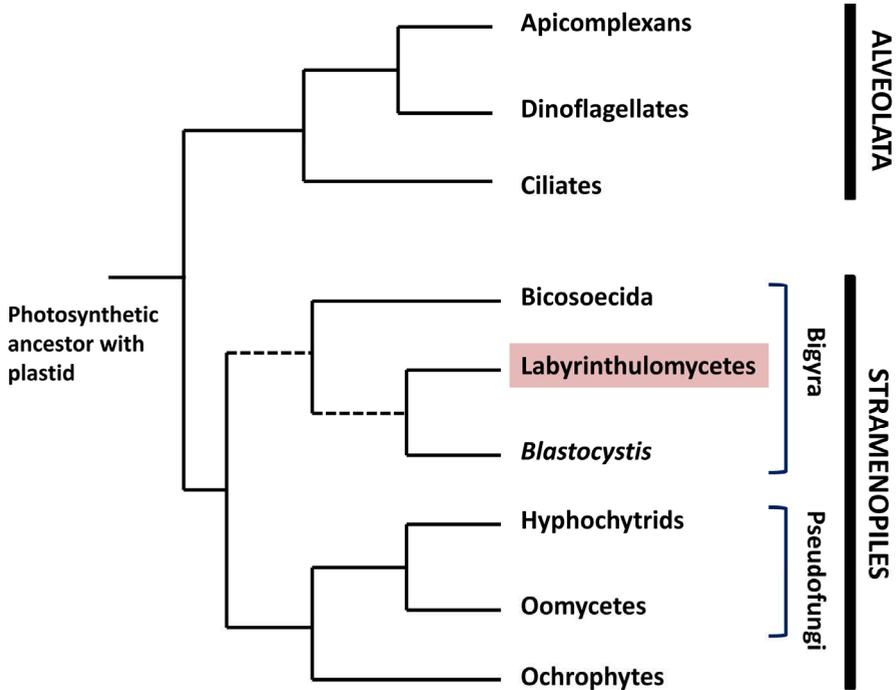


Fig. 1. A simplified phylogenetic tree showing the relationships among Labyrinthulomycetes and other members in Chromalveolata based on Riisberg et al. (2009) (dotted lines indicate unsolved relationship).

The Stramenopiles form a strong, monophyletic group, but the branching order among early-diverging lineages including the heterotrophic labyrinthulomycetes, bicoecida and oomycetes, and the photosynthetic ochrophytes has been difficult to resolve until recently (Cavalier-Smith, 1998, Keeling et al., 2005, Oudot-Le Secq et al., 2006, Tsui et al., 2009). Published phylogenies strongly support the oomycetes and photosynthetic ochrophytes as a monophyletic group (Tsui et al., 2009, Tyler et al., 2006). While the labyrinthulomycetes appeared as the closest relative to the Bicosoecida, and the phylum Bigyra diverged at the earliest bifurcation of ancestral stramenopiles based on three protein coding genes and SSU rRNA (Tsui et al., 2009). However the sister relationship between labyrinthulomycetes and Bicosoecida was not recovered with seven genes phylogenies when additional representatives of Bicosoecida and *Blastocystis* were included (Riisberg et al., 2009). The basal relationships among the labyrinthulomycetes, bicoecida and *Blastocystis* were unsolved and not supported (Riisberg et al., 2009), as previous SSU rDNA phylogenies (Cavalier-Smith et al., 1994, Van de Peer et al., 2000). Those studies either showed that the labyrinthulomycetes as the sister group of the bicoeceans or showed the labyrinthulomycetes, then bicoeceans emerging from successive divergences at the base of the stramenopiles (Cavalier-Smith & Chao 2006, Leipe et al., 1994). In contrast, Oudot-Le Secq et al. (2006)'s analysis of mitochondrial data showed the labyrinthulomycetes and oomycetes forming a monophyletic group.

No matter what is the branching order in the basal heterotrophic stramenopiles, evidence is accumulating that the ancestors of Stramenopiles and “Chromalveolate” were photosynthetic/ phagotrophic algae (mixotrophs) (Cavalier-Smith & Chao 2006, Harper et al., 2005). Therefore photosynthesis had been lost once in the oomycetes and at least once in the common ancestor to the bicoeceans and labyrinthulomycetes (Riisberg et al., 2009, Tsui et al., 2009). Phagotrophy is the main mode of nutrition in the bicoeceans, which feed on bacteria by the invagination of cell membrane (Boenigk & Arndt, 2002). This may be a shared primitive character for the bicoeceans and the labyrinthulomycetes too. In the labyrinthulomycetes lineage, phagotrophy may have preceded the development of an ectoplasm and cell wall. In addition to their dominant walled, osmotrophic vegetative stage, labyrinthulomycetes including *Thraustochytrium striatum*, *Aurantiochytrium mangrovei*, *Ullkenia* and *Labyrinthula* sp. can produce a transient phagotrophic amoeboid stage that ingests bacteria through the development of pseudopodia (Raghukumar, 1992). Oomycetes secrete enzymes and absorb dissolved nutrients across a continuous cell wall, while labyrinthulomycetes are believed to secrete enzymes and absorb dissolved nutrients across their wall-less ectoplasm (Moss, 1991), possibly reflecting the convergent origins of osmotrophy in these two groups.

It is well established that the plastids (cyanobacterial origin) of all photosynthetic stramenopiles originated from a common ancestor. So scientists are interested in the process of plastid loss or the lost of plastid function in those non-photosynthetic stramenopiles (Leipe et al., 1996). The identification of an apparently plastid-derived 6-phosphogluconate dehydrogenase gene and genes of algal origin in *Phytophthora infestans* (a non-photosynthetic stramenopile) supported it has a photosynthetic ancestor (Tyler et al., 2006). The labyrinthulomycetes also have characters that may have originated from ancestral chloroplasts. Many thraustochytrids produce omega-3 PUFA using desaturase and elongase which are usually located in chloroplasts (Sargent et al., 1995). A few members can be phototactic (e.g. *Labyrinthula* sp. (Perkins & Amon, 1969) and *Ullkenia* sp. (Amon & French, 2004)). The eyespot of *Labyrinthula* zoospores (Perkins & Amon, 1969) also resembles eyespots of other stramenopiles and it may mark the remains of an ancestral chloroplast. In the stramenopiles and in dinoflagellates, eyespots are either within the chloroplast (Motomura, 1994), or are believed to be derived from a chloroplast that underwent evolutionary reduction (Dodge, 1984). Eyespots are absent in the basal thraustochytrids and aplanochytrids (Chamberlain & Moss, 1988, Porter, 1990) and the phylogeny suggests that if these were the last remnants of chloroplasts/plastids, they must have undergone multiple, convergent losses in the labyrinthulomycetes.

#### 4. Phylogenetic relationships within the labyrinthulomycetes

The current taxonomic classification of labyrinthulomycetes is based on the framework of Porter (1990) and Dick (2001). They share a morphological synapomorphy in that their cells secrete an ‘ectoplasmic’ network, a radiating network of cytoplasm bound by a plasma membrane (Perkins, 1972). Cells extrude ectoplasm through an electron opaque organelle at the periphery of the cell body that is variously called a ‘bothrosome,’ (Porter, 1969) or a ‘sagenogenetosome’ (Perkins, 1972). The ectoplasmic network appears to help cells adhere to and penetrate substrates, and it secretes the digestive enzymes required to solubilize nutrients that can be absorbed by the cells (Raghukumar, 2002).

Morphologically they are divided into two major lineages - labyrinthulids and thraustochytrids, largely corresponding to the family Labyrinthaceae and Thraustochytriaceae. The labyrinthulids include the genera *Labyrinthula* and *Aplanochytrium* (Leander & Porter, 2001). In contrast to thraustochytrids, they are commonly recorded from living algae and seagrasses. The cell bodies of *Labyrinthula* are colonial and glide within the shared ectoplasmic net (containing spindle-shaped vegetative cells) that gives them their common name, 'net slime molds.' The vegetative cells multiply by mitotic division and reproduce by forming zoosporangia and biflagellate zoospores. The cell bodies of *Aplanochytrium* species also crawl via ectoplasmic filaments but unlike *Labyrinthula* species, cells are solitary, not colonial and they are not embedded in ectoplasm (Leander et al., 2004). In addition to the difference in the function of their ectoplasmic filaments, *Labyrinthula* species produce biflagellate zoospores with eyespots (Perkins & Amon, 1969) while *Aplanochytrium* species often reproduce by aplanospores rather than by zoospores. For *Aplanochytrium* species that do have zoospores, eyespots have not been reported (Leander et al., 2004, Porter, 1990).

The remaining labyrinthulomycete genera, commonly referred to as the 'thraustochytrids' produce unicellular, non-motile thalli and although they secrete an ectoplasmic network, they do not use the network for mobility as expressed in the labyrinthulids. Thraustochytrids are abundant heterotrophs in marine and mangroves habitats, and there are three major genera according to Porter (1990) - *Thraustochytrium*, *Schizochytrium*, and *Ulkenia*. The mode of zoospore production is the basis for genus differentiation. The cytoplasmic content of a vegetative cell develops into a zoosporangium, and then divides directly into zoospores in the genus *Thraustochytrium*. The cytoplasm escapes as an amoeboid mass, prior to the zoospore division in *Ulkenia*. *Schizochytrium* is characterised by the successive bipartition of a vegetative cell, resulting in the formation of the stages called the diad and the tetrad. Eventually the individual cells within a tetrad develop into zoosporangia and zoospores (Porter, 1990). However there is a high level of morphological variability and overlapping among the genera.

Molecular data consistently support the monophyly of the labyrinthulomycetes (Cavalier-Smith et al. 1994, Honda et al., 1999, Leipe et al., 1996). Multi-gene phylogenies divided them into two well-supported clades. Clade I includes only thraustochytrids, while Clade II includes the labyrinthulids, which include both gliding species and colonial species, as well as thraustochytrids (Fig. 2) (Honda et al., 1999, Tsui et al., 2009). So thraustochytrids that are nonmotile in their assimilative phase are paraphyletic. Also the nesting of labyrinthulids (representatives of *Aplanochytrium* and *Labyrinthula*) among thraustochytrids in Clade II suggested that the ectoplasmic trackways that allow gliding movement of *Aplanochytrium* and *Labyrinthula* had their origin in thraustochytrid's ectoplasmic networks used for anchorage and for nutrient absorption but not movement (Fig. 2) (Tsui et al., 2009).

Molecular data support the sister relationship between *Aplanochytrium* and *Labyrinthula* (Fig. 2) (Honda et al., 1999, Tsui et al. 2009, Yokoyama and Honda 2007a), but provide little resolution on the branching order of genera in thraustochytrids *sensu* Porter (1990) and earlier taxonomic treatment. None of the genera *Thraustochytrium*, *Schizochytrium* and *Ulkenia* were monophyletic, indicating that the morphological characters employed as taxonomic criteria are unreliable (Honda et al., 1999).

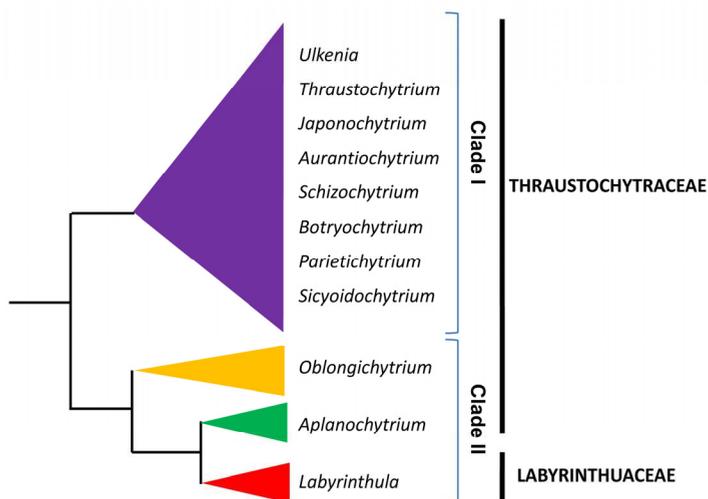


Fig. 2. A schematic summary of the phylogenetic relationships among the genera within the labyrinthulomycetes (modified from Tsui et al., 2009 and Yokoyama et al., 2007b).

Recent studies have delineated the thraustochytrids into multiple monophyletic genera with their morphology, biochemistry, and molecular data. Genera of *Oblongichytrium*, *Aurantiochytrium*, *Botryochytrium*, *Parietichytrium*, and *Sicyoidochytrium* have been erected during the taxonomic revisions of *Schizochytrium* and *Ulkenia* (Fig. 2) (Yokoyama et al., 2007a, b). For example, the genus *Aurantiochytrium* has been erected for a phylogenetic lineage of *Schizochytrium* species that could accumulate DHA for over 30% of the total fatty acids (Yokoyama et al., 2007a). Also the content of C18 and C20 precursor unsaturated fatty acids in *Aurantiochytrium* for DNA synthesis in the elongation/ desaturation pathway were much lower than those in the genera *Thraustochytrium* and *Schizochytrium* (Nagano et al., 2011).

## 5. Ecophysiology of thraustochytrids

Thraustochytrids, are ubiquitous in oceanic water column (Bahnweg & Sparrow, 1974, Raghukumar, 2002) and they are associated with the wide range of substrata and habitats; e.g. from both fresh and decaying algal surfaces (e.g. in UK waters - Miller & Jones, 1986; in Indian waters - Raghukumar 1986), from decaying leaves of sea grass (e.g. in US waters - Jensen et al., 1998), from decaying leaves of mangrove plants (e.g. in Hong Kong waters - Fan et al., 2002a) and from invertebrate tissues (e.g. in abalone tissues - Bower, 1987; in clam tissues - Azevedo & Corral 1997). Raghukumar & Damare (2011) gave a short concise chronological account of the development of the research of this group of organisms since their first discovery in US waters in the mid-30s (Sparrow, 1936).

In the past decade, there were two areas of research in thraustochytrids where efforts were concentrated; phylogeny studies based on molecular analysis as described earlier in this chapter and the physiology of production of compounds which have important nutritional values (e.g. fatty acids - Fan et al., 2001, 2007; squalene - Li et al., 2009). In the following

paragraphs, a review of some ecophysiological investigations of thraustochytrids isolated from decaying mangrove leaves in subtropical mangroves is presented (Fan et al., 2002a, b, Tsui et al. 2011, Wong et al., 2005).

Thraustochytrids are well adapted to the mangrove environment where salinity and temperature levels fluctuate daily, monthly and seasonally. A series of ecological and physiological investigations have been undertaken on various isolates of thraustochytrids isolated from the subtropical mangroves where salinity levels could vary between 5 and 34 ‰ in summer and winter in Hong Kong respectively (Fan et al., 2002a, b, Tsui et al., 2011, Wong et al. 2005). Some of these species were isolated from low saline waters (ca. 5‰). These isolates were, namely *Schizochytrium* sp. KF1, *Aurantiocytrium mangrovei* KF-2, KF-7 KF-12, *Thraustochytrium striatum* KF-9, and *Ulkenia* KF-13. Their growth response under different salinities (distilled water, 7.5 – 30 ‰), pH (4 – 9) and temperature (15 – 30 °C) levels in yeast extract –peptone-glucose seawater (YPGS) broth were reported (Fan et al. 2002a). In general, all cultures grew equally well in all tested pH levels, and the overall optimal temperature range was at 22 – 25°C between 7.5 and 30‰ salinity levels. *Aurantiocytrium* and *Schizochytrium* isolates produced overall higher dry weight biomass (ca. 150 – 300 mg/50mL) at all tested temperature and salinity levels compared to *Ulkenia* and *Thraustochytrium* isolates. Although each isolate had their own specific, optimal response to varying salinities and temperature levels, the interaction of salinity and temperature affected their growth significantly ( $P < 0.001$ ) (Fan et al., 2002a, b).

The zoospore production capacity and their motility profile are also highly influenced by salinity (Tsui et al., 2011). A summary of the zoospore features at various salinities of *Schizochytrium* sp.KF1, *Aurantiocytrium mangrovei* KF-6, *Thraustochytrium striatum* KF-9 and *Ulkenia* KF-13. is shown in Table 1. Zoospores of thraustochytrids were also strongly attracted to the mangrove leaf extracts when comparing to various amino acids and carbohydrates (Fan et al., 2002b). Zoospores of *A. mangrovei* KF-6 showed highest response followed by *Ulkenia* sp. KF-13 whereas those of *T. striatum* KF-9 were very weak, showing almost no differentiation amongst all the test compounds. The summary data shown in Table 1, and the results of the chemotactic response experiment (Table II in Fan et al., 2002b) indicate the overall competitiveness of these strains in the mangrove environment where fluctuating saline waters could be encountered within each tidal cycle throughout the year.

*Aurantiocytrium mangrovei* was the most abundant thraustochytrid species in the Hong Kong mangroves, followed by *Schizochytrium* spp. and *Ulkenia* spp., whereas *Thraustochytrium* spp. were seldom encountered (Vrijmoed unpublished). The very small number of zoospores being produced by *T. striatum* (Table 1) coupled with the weak chemotactic response to mangrove leaf extracts and nutrients and the overall low biomass produced in batch cultures may explain their low occurrence in spite of the fairly active zoospores after release from the zoosporangium. The average zoospore production capacity of *Ulkenia* sp. was nearly 10-fold of that of *Thraustochytrium* sp. However, its moderate motility and lowest VCL and VSL amongst the test strains lower the chance of the zoospores to locate a substrate for settlement and growth. *A. mangrovei* had the highest growth rate in batch cultures; its zoospores were also most attracted to mangrove leaf extracts and nutrients. Their VSL and VSL were high which compensate the moderate zoospore production amount and the average motility % within a 4h period. These are the probable reasons for their dominance in the subtropical mangroves in Hong Kong (Tsui et al., 2011).

	<i>Schizochytrium</i> sp.KF1	<i>Aurantiochytrium</i> <i>mangrovei</i> KF-6	<i>Thraustochytrium</i> <i>striatum</i> KF-9	<i>Ulkenia</i> KF-13	General Comments
Average Zoospore production <sup>a</sup> (x10 <sup>3</sup> mL <sup>-1</sup> )	19.34	29.04	4.88	40.30	zoospore production of all strains suppressed at levels > 15‰
Average motility <sup>b</sup> within a 4h-period (%)	86.6	78.1	91.7	84.3	Motility of all strains remained at 90 to 100% after 2h but were reduced 60 - 90% after 4h.
Average curvilinear velocity (VCL) <sup>b</sup> (µm sec <sup>-1</sup> ) within a 4h-period	89.3	99.2	103.1	71.0	Not applicable
Average straight line velocity (VSL) <sup>c</sup> (µm sec <sup>-1</sup> ) within a 4h-period	60.2	70.6	71.3	35.8	Not applicable

<sup>a</sup> The motility of zoospores was recorded using the image analysis system consisting of a phase contrast microscope with a lens at 20x10 magnification (Olympics BX50 Japan) equipped with a progressive scan charged-coupled device (CCD) camera (Basler Scout, SCA640-70FM, Ahrensburg, Germany).

<sup>b</sup> Zoospores were induced from 2-day old cultures in yeast extract peptone plates flooded separately with distilled water, and artificial seawater at 7.5, 15, 22.5 and 30‰.

<sup>c</sup> VCL - the time average velocity of the zoospore head along its actual trajectory.

<sup>d</sup> VSL- the time average velocity of the zoospore head along the straight line between its first detected position and its last position.

Table 1. A summary of zoospore profile of mangrove thraustochytrids (adapted from Tsui et al., 2011).

The temporal variation of abundance of thraustochytrids in decaying mangrove leaves (*Kandelia obovata*) and sediments were also investigated, and the results indicate that thraustochytrid abundance in decaying leaves were much higher (4.8x10<sup>3</sup> - 5.6x10<sup>5</sup> CFUg<sup>-1</sup> of oven-dried weight of leaves) compared with the levels in surface sediments (1.0x10<sup>2</sup> - 1.6x10<sup>3</sup> CFUg<sup>-1</sup> of oven-dried weight of sediment) (Wong et al., 2005). Thraustochytrids colonies were enumerated by spreading the leaf homogenate and sediment suspension on YEP agar plates incorporated with antibiotics and incubated at 25 °C for two days. This is

supported by a similar pattern of thraustochytrid occurrence in the samples, being an average of 85.5% vs. 57.5% in leaves and sediments respectively. However statistical analyses revealed no significant correlations in the occurrence between leaves and sediments, as well as between the samples and the air temperature and water salinities.

Data of several experiments indicate that thraustochytrids provide the necessary long-chain polyunsaturated fatty acids (LCPUFAs) to marine organisms which cannot synthesize them. Mangrove crabs (e.g. *Parasesarma affinis* and *Parasesarma bidens*) which mainly ingest decay leaves (Lee & Kwok, 2002) would be enriched with the LCPUFAs laden in the leaves. Partially digested thraustochytrid cells were also detected amongst diatom skeletons in the gut content of the mudskipper *Boleophthalmus pectinirostris* which are prevalent in the intertidal mangrove shores in Hong Kong (Vrijmoed, unpublished data). Mudskippers sieved sediment to obtain their food. So there is partial evidence on the importance of thraustochytrids in the food web in the mangrove ecosystem.

## 6. Future research and conclusion

Labyrinthulomycetes occupy an important position in the eukaryote tree of life and they play a critical role in the ecosystems by upgrading the 'nutritional value of detritus' due to their ability to produce LCPUFAs. Although labyrinthulomycetes, specifically the labyrinthulids, are important ecologically, there is no formal estimate to the number of species but many unknown representatives have been described only from sequences in metagenomics studies from marine ecosystems (Massana et al., 2004a, Not et al., 2007). Currently four labyrinthulomycete genomes are being sequenced at Joint Genome Institute. The data will offer genome-scale insight into the physiology of an ecological and biotechnological significant group of organisms. For example, the genome data will provide new information about the genetic basis for the ectoplasmic net development, and virulence to organisms and their evolutionary history. The genome data will also provide specific insight into genetic basis for differences between species that are of ecological and biotechnological relevance. Additionally, the information will make possible further investigations of degrading enzymes of biotechnological interest.

## 7. Acknowledgements

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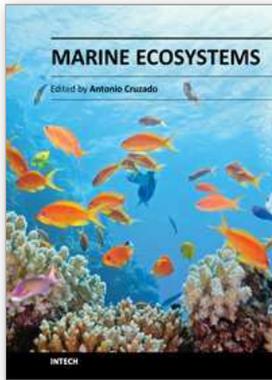
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Marine ecosystems, a very wide topic, includes many different processes, groups of organisms and geographical peculiarities. The objective of this book is to present various topics of great importance for understanding the marine ecosystems, what they are, how they work and how we can model them in order to forecast their behaviour under changing conditions. They have been thoroughly reviewed and accepted for publication. The chapters cover aspects such as: Threats to ultraoligotrophic marine ecosystems (Ch. 1); Modelling the pelagic ecosystem dynamics: the NW Mediterranean (Ch. 2); The marine ecosystem of the Sub-antarctic, Prince Edward Islands (Ch. 3); Meiofauna as a tool for marine ecosystem biomonitoring (Ch. 4); Chemical interactions in Antarctic marine benthic ecosystems (Ch. 5); An Interdisciplinary Approach on Erosion Mitigation for Coral Reef Protection- A Case Study from the Eastern Caribbean (Ch. 6); A revisit to the evolution and ecophysiology of the Labyrinthulomycetes (Ch. 7); Seabed mapping and marine spatial planning: a case-study from a Swedish marine protected area (Ch. 8); Management strategies to limit the impact of bottom trawling on VMEs in the High Seas of the SW Atlantic (Ch. 9); Hydrocarbon contamination and the swimming behavior of the estuarine copepod *Eurytemora affinis* (Ch. 10), and Interactions between marine ecosystems and tourism on the Adriatic and Mediterranean (Ch. 11).

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