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Abstract

The Straminipila are characterized by their anterior flagellum with tripartite hairs and form a well-supported monophyletic branch of the larger Straminipila/Alveolata/Rhizaria (SAR) superkingdom. This is an account of the molecular systematics and phylogeny of osmotrophic and phagotrophic lineages of the Straminipila, comprising the slime nets and their thraustochytrid allies, as well as some lesser known lineages. The phylum Labyrinthulomycota *s. lat.* contains two main clades, one of which approximates to holocarpic thraustochytrids and the other to the labyrinthulids and aplanochytrids. Together with the flagellate bicosoecids and the proteromonads and opalinids, they form a monophyletic clade

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that is sister to the golden-brown algae and Oomycota. The systematics of the Labyrinthulomycota *s. lat.* is still in flux as recent studies employing environmental barcoding have revealed the presence of diverse lineages not branching within genera characterized in terms of their morphology. The current review deals primarily with the two major lineages of the Labyrinthulomycota *s. lat.* and discusses other lineages only briefly, due to the scarce knowledge about these organisms. Characteristics associated with zoosporegenesis and sexual reproduction are discussed in relation to other members of the Straminipila.

Keywords

Amoebae • Amphitremida • Aplanochytrids • Bothrosome • DHA (docosahexaenoic acids) • *Diplophrys* • Eelgrass wasting disease • Ecology • Ectoplasmic net • Labyrinthulida • Marine decomposers • Seagrass wasting disease • Scale coats • *Schizochytrium* • Slime nets • Thraustochytrida • Stramenopiles • Straminipila • Zoospore ultrastructure

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● Labyrinthulomycota/Labyrinthomorpha

●● Labyrinthulomycetes/Labyrinthulea

●●● Labyrinthulales/Labyrinthulida

●●●● Aplanochytriaceae/Aplanochytriidae (*Aplanochytrium* (including *Labyrinthuloides*))

●●●● “Stellarchytriaceae/Stellarchytriidae” (*Stellarchytrium*)

●●●● Labyrinthulaceae/Labyrinthulidae (*Labyrinthula*)

●●● Oblongichytriales/Oblongichytriida

●●●● Oblongichytriaceae/Oblongichytriidae (*Oblongichytrium*)

●●● Thraustochytriales/Thraustochytrida

●●●● Althornidiaceae/Althorniidae (*Althornia*)

●●●● Thraustochytriaceae/Thraustochytriidae (*Aurantiochytrium*, *Botryochytrium*, *Japanochytrium*, *Monorhizochytrium*, *Parietichytrium*, *Schizochytrium*, *Sicyoidochytrium*, *Thraustochytrium*, *Ulkenia*)

●●● Amphitremida

●●●● Amphitremidae (*Amphitrema*, *Archerella*, *Paramphitrema*)

●●●●Diplophrydaceae/Diplophryidae (*Diplophrys*)

●●●Amphifilida

●●●●Amphifilaceae/Amphifilidae (*Amphifila*)

●●●Sorodiplophryidae (*Fibrophrys*, *Sorodiplophrys*)

Summary classification of major lineages adapted from Tice et al. (2016). It should be noted that the higher level classification needs to be considered provisionally, as the deeper splits within Labyrinthulomycota are largely unresolved (Pan et al. 2017).

Introduction

General Characteristics

The osmotrophic fungus-like members of the kingdom Straminipila are characterized by absorptive nutrition and heterokont biflagellate zoospores. The term stramenopile was first introduced by Patterson (1989) in reference to the “straw hairs” (mastigonemes) that decorate the anterior flagella of this group of organisms (Fig. 1b, e). Dick (2001) pointed out this was an incorrect derivation of the Latin for “straw hair” and that the correct form should be straminipilous. However, Adl et al. (2005) favored the continued use of “stramenopile”, the form of the name that is most widely used (Lévesque 2011). It is now apparent that the Straminipila have their evolutionary origins in the sea and that many of the fungal-like organisms seem to be ecologically important and widespread pathogens of algae, animals, and plants (Beakes et al. 2012, 2014; Thines 2014).

Apart from the posteriorly uniflagellate chytrids all of the zoosporic organisms traditionally studied by mycologists can now be placed in the still contentious Straminipila/Alveolate/Rhizaria (SAR) superclade (Burki et al. 2008; Hackett et al. 2007; Reeb et al. 2009).

This account reviews one of the smaller groups within this lineage, the Labyrinthulomycota (predominantly labyrinthulids and thraustochytrids), and updates the pre-molecular account of the group published in the first edition of the Handbook of Protoctista by Porter (1990). Most members of the Labyrinthulomycota are heterotrophic colorless or yellowish protists that absorb nutrients in an absorptive (osmotrophic) or phagotrophic manner. They typically feed saprotrophically (but parasites are known, e.g., Schäfer et al. 2007) and are key players in the detrital food web, helping to break down often intractable plant and animal remains and making these substrates more accessible to grazing amoebae and ciliates (Raghukumar 2002; Bongiorno 2012). Many thraustochytrids can also feed phagotrophically (Raghukumar 1992), and some genera such as *Aurantiochytrium* (Fig. 2d) and *Ulkenia* (Fig. 4) have a free-living amoeboid stage. The Labyrinthulomycota are often referred to as “slime nets,” which relates to the feature shown by many of the crown genera, the formation of a network of fine, often branching and anastomosing, cytoplasmic threads (Figs. 1c, 2c, and 3a, b, d) that extend into the

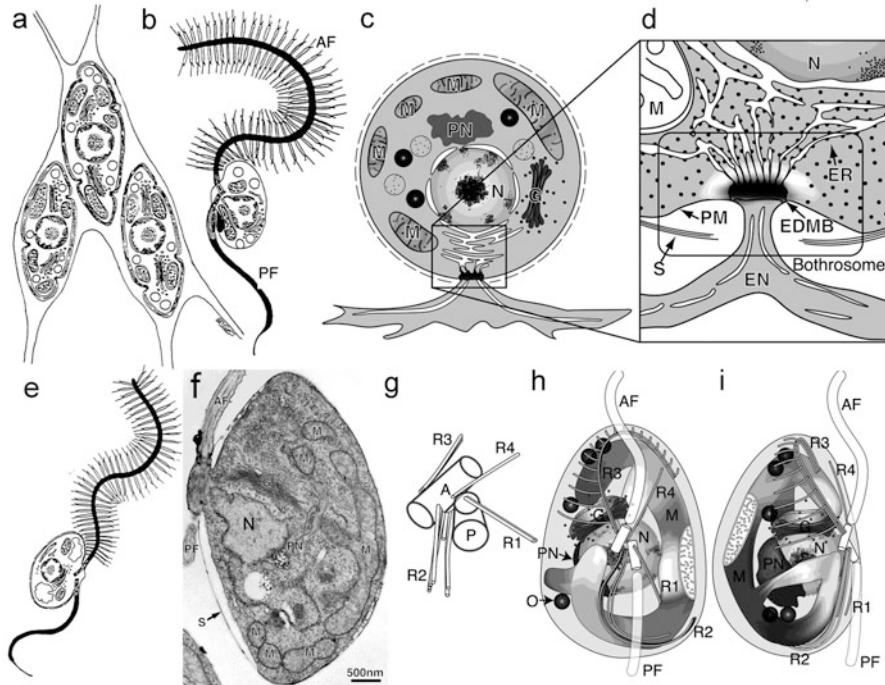


Fig. 1 Morphological features of labyrinthulids and thraustochytrids, part 1. Schematic drawing of *Labyrinthula* showing (a) uninucleate spindle-shaped cell bodies, which are coated in scales, containing mitochondria and Golgi dictyosomes and enveloping ectoplasmic net membrane and (b) biflagellate zoospore, with mastigonate anterior flagellum (AF) and shorter posterior flagellum (PF) with tapering terminal acroneme. Both adapted from Porter (1990). (c) Schematic drawings of the thraustochytrid *Schizochytrium aggregatum* thallus showing uninucleate (N) vegetative thallus and associated Golgi dictyosome (G) and surrounding mitochondria (M) and other organelles. (d) Schematic illustration of the bothrosome showing electron-dense plug material (EDMB), ectoplasmic net (EN), feeding endoplasmic reticulum (ER), plasma membrane (PM), and thallus scales (S). (e) Biflagellate zoospore, *Thraustochytrium* zoospore showing cell body covered in scales and anterior (AF) and posterior flagella (PF). From Porter (1990). (f) Transmission electron micrograph (TEM) showing a longitudinal profile of *S. aggregatum* zoospore, showing central nucleus (N) associated Golgi body (G) and paranuclear body (PN). (g–i) Diagrams of an *S. aggregatum* zoospore. (g) Ventral view of the flagellar roots showing the orientation anterior (A) and posterior (P) kinetosomes and their associated roots R1–R4. (h) Ventral and (i) right views of zoospore body showing the orientation of organelles and the flagellar apparatus: anterior basal body (A); anterior flagellum (AF); Golgi body; M, mitochondria; N, nucleus; P, posterior basal body; PN, paranuclear body (G); posterior flagellum (PF); flagellar roots (R1–A) (c, d, f, g–i) From Iwata et al. published in Protist <http://dx.doi.org/10.1016/j.protis.2016.12.002> Figs. 1, 3, and 5 with permission. All other photographs courtesy of Professor Daisuke Honda, Konan University http://syst.bio.konan-u.ac.jp/labybase/index_en.html

environment from the cell bodies and originating from a unique structure, now generally called the bothrosome (Figs. 1d and 2g, j; Porter 1990; Beakes et al. 2014). These threads provide adhesion to the substrate and absorb nutrients (as in

thraustochytrids) or form trackways along which the cell bodies glide (as in the labyrinthulids; Figs. 1a and 2h, i).

The group also includes a number of unicellular colorless protist genera such as *Amphifila*, *Amphitrema* (Fig. 3e), *Archerella* (Fig. 3f), *Diplophrys* (Fig. 3a–c), and *Sorodiplophrys* (Fig. 3d) that produce fine rhizopodia-like structures (Anderson and Cavalier-Smith 2012; Gomaa et al. 2013; Takahashi et al. 2014). Some such as *Ulkenia* and related genera also have an amoeboid phase (Fig. 2d) in their life cycle (Beakes et al. 2014; Karling 1981; Porter 1990; Yokoyama et al. 2007).

The unusual set of characteristics associated with the Labyrinthulomycota has hampered their taxonomic assignment. As summarized recently (Beakes et al. 2014), labyrinthulids have been assigned to various unrelated groups, such as the Rhizopoda, Mycota, Amoebozoa, and different phyla of the Straminipila, such as Chrysophyta and Oomycota. Based on phylogenetic evidence, as summarized by Gomaa et al. (2013; Fig. 5), the labyrinthulids do not belong to any of these groups and are probably best treated as an independent phylum in the Straminipila, the Labyrinthulomycota, as proposed by Porter (1990).

The recent application of molecular phylogenetic techniques including extensive environmental sampling and sequencing of DNA has, similar to the fungi of the kingdom Mycota (Jones et al. 2011), revealed many, as yet mostly undescribed, and often probably uncultivable, representatives of this group in diverse marine, freshwater, and terrestrial environments (Collado-Mercado et al. 2010; Diéz et al. 2001; Massana et al. 2002, 2006; Massana and Pedró-Alió 2008; Pan et al. 2017; Richards et al. 2012; Stoeck et al. 2003, 2006, 2007). This methodology has also revealed that several protist genera of previously unclear taxonomic affinity, such as *Amphitrema* and *Archerella*, which were formerly placed together with the filose testate amoebae, also belong in the Labyrinthulomycota (Gomaa et al. 2013; Pan et al. 2017; Tice et al. 2016).

Because of this phylogenetic uncertainty, as with many other protist groups, names in the Labyrinthulomycota have been published both according to zoological (ICZN) and botanical (ICBN/ICNfap) nomenclature. The majority of species within the traditional Labyrinthulomycota have been described by mycologists under the botanical code for nomenclature, whilst many recent changes were suggested under the code for zoological nomenclature and where possible both sets of nomenclature are given in this chapter.

Occurrence

The Labyrinthulomycota appear to be cosmopolitan and were considered to be saprotrophic or only weakly parasitic organisms, ubiquitous in marine and estuarine environments. The morphologically described part of the Labyrinthulomycota consists of a relatively small group of almost exclusively marine genera (Figs. 1b and 3) that typically feed saprotrophically and are an important part of the marine detrital food web (Raghukumar 2002; Bongiorni 2012). However, many thraustochytrids feed bacteriotropically (Raghukumar 2002), and some genera such as

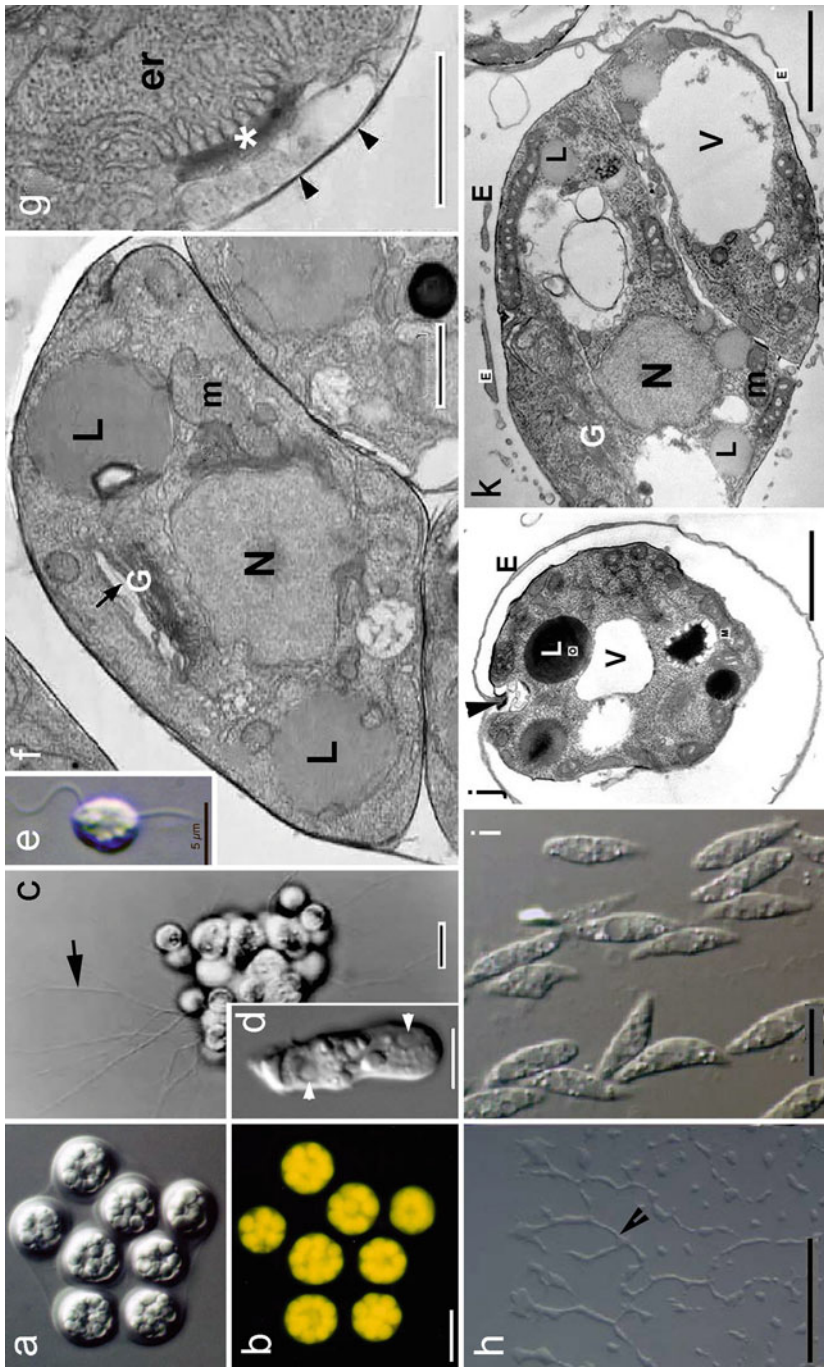


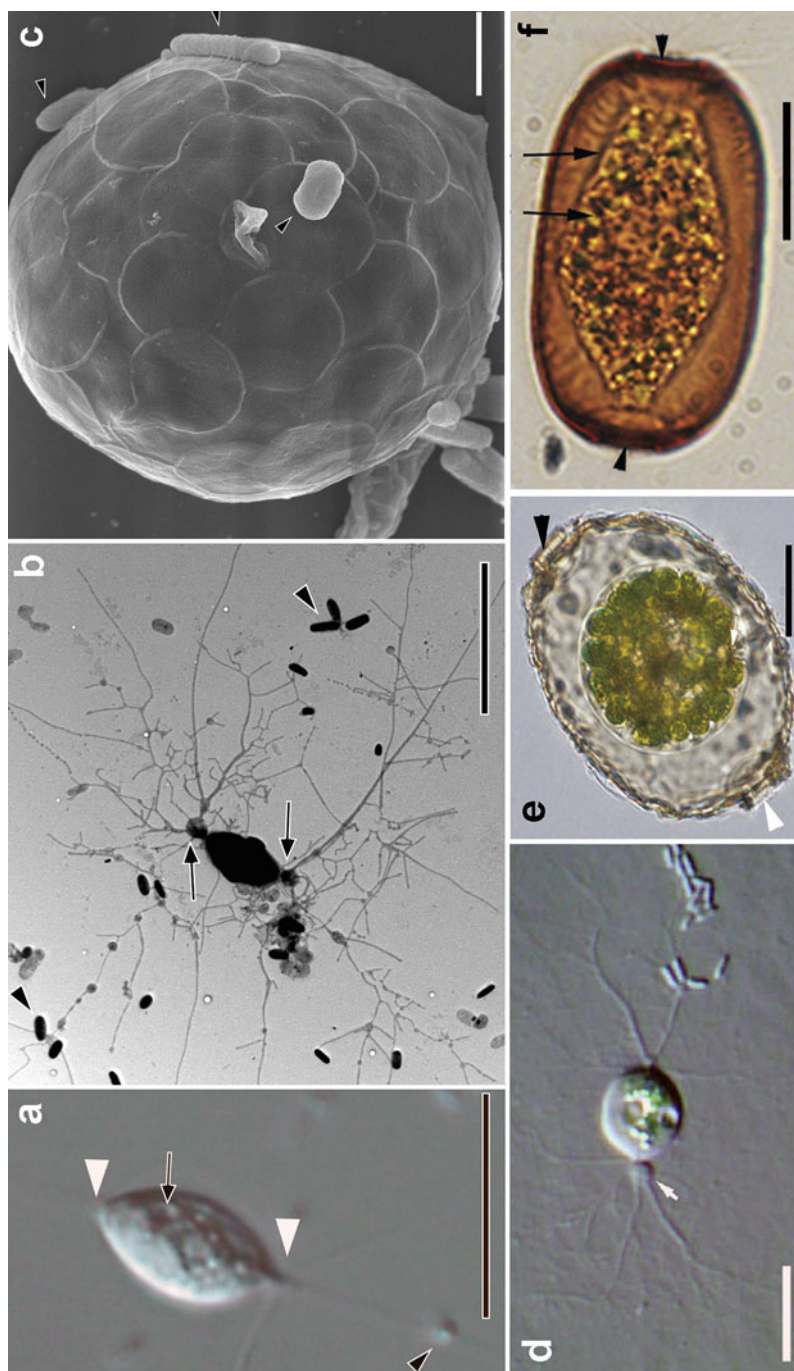
Fig. 2 (continued)

Ulkenia (Figs. 2 and 3h–j) have amoeboid stages engulfing their food. Thraustochytrids can be recovered in large numbers from marine sediments (Bongiorni 2012), including the deep sea (Raghukumar et al. 2001). Labyrinthulids are prevalent living on or within seaweeds and sea grasses, and there is an increasing evidence that they can live as parasites, commensals, or mutualists in plants (Bigelow et al. 2005; Bockelmann et al. 2012) and in other organisms, such as amoebae (Dykova et al. 2008) and mollusk tissues (e.g., Azevedo and Corral 1997).

However, most frequently they have been found associated with the surfaces of benthic algae, marine vascular plants, and detrital sediments (Porter 1990; Raghukumar 2002). Some, such as genus *Althornia*, are part of the free-floating eukaryotic plankton, and many others have been isolated from the marine water column (e.g., Collado-Mercado et al. 2010; Porter 1990), often in association with particulate “marine snow” (Naganuma et al. 2006; Raghukumar et al. 2001; Damare and Raghukumar 2008). Until the turn of the last millennium, the Labyrinthulomycota were considered to be exclusively marine organisms (Porter 1990), but about a decade ago, *Labyrinthula terrestris* has been described as a pathogen associated with turfgrass decline (Bigelow et al. 2005), and molecular studies have revealed an increasingly large number of freshwater members of this phylum (Anderson and Cavalier-Smith 2012; Gomaa et al. 2013; Richards et al. 2012). For instance, the testate protist genera *Archerella* and *Amphitrema* are common components of *Sphagnum* peatland, where they are often made visible by the endosymbiotic Trebouxiophyte algae (Fig. 3e, f) they contain (Gomaa et al. 2013). Many environmental sequences belonging to this clade have been isolated from anoxic sediments, which again suggests the habitats and roles occupied by these organisms is far more diverse than originally thought (Gomaa et al. 2013). Some members of the Labyrinthulomycota are genuine parasites which can have detrimental



Fig. 2 Morphological features of labyrinthulids and thraustochytrids, part 2. (a–f) Micrographs of the thraustochytrid *Aurantiochytrium limacinum*. DIC phase (a) and fluorescent (b) images of a colony of showing cells packed with oil globules which fluoresce orange when stained with Nile red. (c) Colony of vegetative cells growing on agar showing fine branching ectoplasmic net (arrowed) emanating from the body cells (scale bar = 10 μ m). (d) Amoeboid cell showing granular inclusions (arrowed) (scale bar = 5 μ m) (e) Biflagellate zoospore, showing typical ovoid morphology of the Thraustochytridiales (scale bar = 5 μ m). (f) TEM of vegetative thallus, showing central nucleus (N), associated Golgi dictyosome (G) and surrounding lipid (L) globules and mitochondria (m). (Scale bar = 1 μ m). (g) TEM of bothrosome at the surface of *Aplanochytrium* sp. SEK349 cell. Note the cisternae of endoplasmic reticulum feeding into the plaque of electron-dense plug material (asterisk). (h–k) Micrographs of *Labyrinthula* sp. AN-1565. (h) Branching “slime net” colony growing on surface of agar (scale bar = 0.5 mm). (i) DIC micrograph showing spindle-shaped colony cells which migrate along the enveloping ectoplasmic net (not visible) (Scale bar = 10 μ m). (j–k) Transmission electron micrographs showing transverse (j) and longitudinal (k) sections of thallus cells. Nuclei (N) are associated with a single Golgi dictyosome (G), and cytoplasm contains lipid globules (L), mitochondria (m), and vacuoles (V). The enveloping ectoplasmic net (E) originates from the bothrosome (arrow). (Scale bars = 5 μ m)

**Fig. 3** (continued)

environmental impacts (such as on seagrass beds) and cause diseases of economical and ecological importance (Hatai 2012).

Literature and History of Knowledge

Because they are a small group, there are no dedicated taxonomic monographs on the Labyrinthulomycota, although illustrations of the main thraustochytrid taxa were included in the monograph of simple holocarpic biflagellate fungal-like organisms by Karling (1981). They are also included in the major systematic review of straminipilous fungi by Dick (2001). *Labyrinthula* was first observed by Cienkowski (1867) associated with intertidal algae in the Black Sea. The genus *Thraustochytrium* was first observed by Sparrow (1936), who described *T. proliferum* associated with benthic algae from Woods Hole, Massachusetts, and later monographed zoosporic fungi from various habitats (Sparrow 1960, 1973, 1976). Thraustochytrids were initially included in the oomycetes until the mid-1970s, when ultrastructural investigations revealed significant differences with between them and other biflagellate “zoosporic fungi” of the oomycetes (reviewed by Beakes et al. 2014; Perkins 1976; Moss 1985, 1986; Porter 1990). Physiological aspects of the thraustochytrids were reviewed by Goldstein (1973) and again showed important differences with other biflagellate “fungi.” The next significant advances in knowledge came with the advent of molecular systematics. Molecular phylogeny confirmed that labyrinthulids and thraustochytrids were part of the straminipilous lineage as suggested by their ultrastructure (Patterson 1989), although the precise branching order of the various straminipilous clades remained poorly resolved (Leipe et al. 1994). It was not until the advent of multigene analyses based on conserved protein genes that there was a clearer understanding of how the main lineages were related (Tsui et al. 2009; Tsui and Vrijmoed 2012 – see later sections). Only recently have environmental sequencing projects greatly expanded the knowledge on the diversity, habitats, and distribution of Labyrinthulomycota s.lat., as outlined by Pan et al. (2017) (Fig. 5).



Fig. 3 Morphology of the Amphitremida. (a–c) Micrographs of *Diplophrys mutabilis* from freshwater. (a) Elongated fusiform cell, showing terminal origin of ectoplasmic elements (white arrows) and contractile vacuole (black arrow). (Scale bar = 10 μm). (b) Whole mount transmission electron micrograph of cell body showing radiating branched ectoplasmic elements from cell poles. Bacteria are also shown (arrowheads) (Scale bar = 10 μm). (c) SEM image of a lyophilized cell showing circular overlapping cells and attached bacteria. (Scale bar = 1 μm) (From Takahashi et al. 2014 Protist 165: 50–65 Figs. 1b, 2a, and 3a <http://dx.doi.org/10.1016/j.protis.2013.10.001> with permission). (d) DIC micrograph of an amoeba of coprophilic *Sorodiplophrys stercorea* showing anastomosing pseudopodia, with swellings (arrowed) (Scale bar = 10 μm) (From Tice et al. 2016, Fig. 1c *Journal of Eukaryote Microbiology* doi:10.1111/jeu.12311 with permission). (e) Brightfield micrograph of *Amphitrema wrightianum*, showing apical shell apertures (pseudostome), and green Trebouxiophyte endosymbionts. (Scale bar = 20 μm) (f) Brightfield micrograph of *Archerella flavum*, showing pigmented shell (test) with terminal pores. The protist cell is arrowed. (Scale bar = 20 μm) (From Gomaa et al. 2013 Fig. 1. PLoS ONE 8(1) <http://dx.doi.org/10.1371/journal.pone.0053046> with permission)

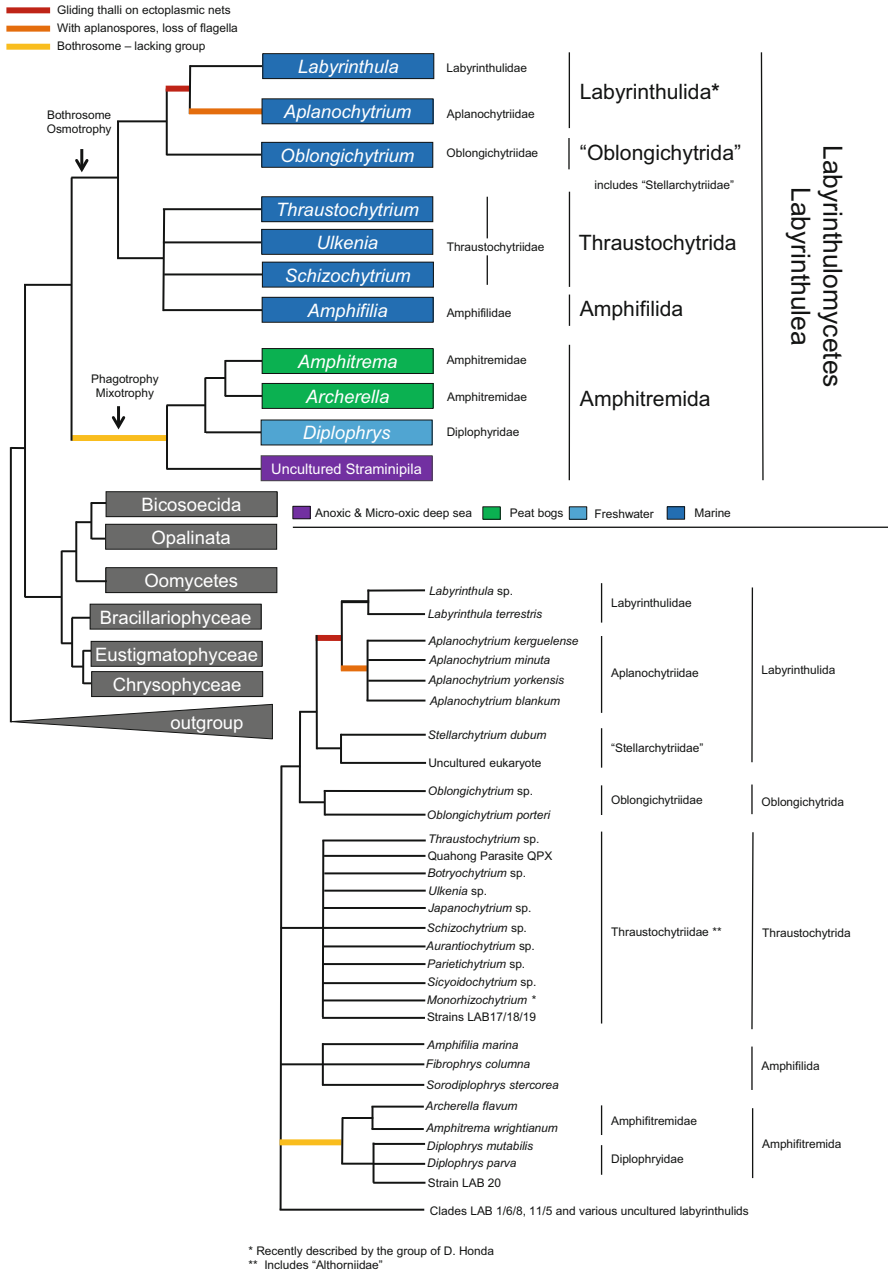


Fig. 5 Phylogeny. Trees summarizing phylogenetic relationships within the Labyrinthulomycota and with other Straminipila. Molecular phylogenetic scheme based on small subunit (SSU) rRNA gene sequences showing phylogenetic position *Archerella* and *Amphitrema* within the Amphitremida. This tree also shows relationship of Labyrinthulomycota and other heterokont

Thraustochytrid infections can cause serious economic losses to commercially reared shellfish. The most well-studied examples include the so-called “QPX thraustochytrid parasite” of the northern Quahog clam (Azevedo and Corral 1997; Lyons et al. 2005, 2007; Garcia-Vedrenne et al. 2013) and *Aplanochytrium haliotidis* infecting abalone (Bower 1987a, b; Bower et al. 1989). Other thraustochytrid species have also been shown to cause mass mortality amongst marine animals such as the nudibranch, *Tritonia diomedea* (McLean and Porter 1987), and cephalopods such as the lesser octopus, *Eledone cirrhosa* (Polglase 1980), and a squid, *Illex illecebrosus* (Jones and O’Dor 1983).

There has been much interest in exploiting marine thraustochytrids for a wide range of products they synthesize (particularly lipids; Fig. 2b), including the production of biodiesel, long-chain omega-3 fatty acids, and exopolysaccharides (Chang et al. 2012). In particular it is hoped to culture them as an alternative to fish as a source of polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), which are important dietary supplements for both animals (Miller et al. 2007) and humans (Kabayashi et al. 2011; Ragukumar 2008). Recently genetic manipulation has been used to improve fatty acid production in thraustochytrids (Kabayashi et al. 2011), and this work has been the main driving force behind sequencing the genome of *Aurantiochytrium* (Liu et al. 2016). Currently, *Schizochytrium* species are used for the commercial production of DHA (Winwood 2013). Squalene, a compound reported to reduce the incidence of coronary heart disease and cancer, accumulates in the thraustochytrid *Aurantiochytrium mangrovei* grown in the presence of the terbinafine (Fan et al. 2010).

Habitats and Ecology

Methodology for Detection and Enumeration Labyrinthulomycetes in the natural marine environment, including the water column, have been documented using various methods. Culture-based methods for determining and quantifying the prevalence of thraustochytrids in nature, using serial dilution and pine-pollen baiting, were pioneered by Gaertner (1968). The direct observation epifluorescence technique described by Raghukumar and Schaumann (1993) is another quantitative method that was considered to be a more sensitive and direct method for detecting and enumerating labyrinthulomycetes. More recently the introduction of molecular techniques involving sequencing of extracted environmental DNA and developing labyrinthulomycete-specific molecular probes has further extended the places where these organisms have been recorded from (Pan et al. 2017). Until now, they have been found in such extreme environments as arctic, subarctic, and antarctic habitats



Fig. 5 (continued) members of the Straminipila. The tree was adapted from Gomaa et al. 2013. PLoS One 8(1) <http://dx.doi.org/10.1371/journal.pone.0053046> with permission. Phylogenetic scheme for Labyrinthulomycota based on partial 18S rRNA sequences, including also environmental sequences. Adapted from Pan et al. 2017.

(Bahnweg and Sparrow 1974; Moro et al. 2003; Naganuma et al. 2006; Riemann and Schrage 1983; Stoeck et al. 2007), oceanic environments of the Indian Ocean (Damare and Raghukumar 2008, 2010), marine sediments (e.g., Bongiorno 2012), saline soils (Aschner 1958; Booth 1971; Bigelow et al. 2005), the deep sea (e.g., Amon 1978; Raghukumar et al. 2001), and shallow water hydrothermal vents (Colaco et al. 2006).

Environmental Tolerances of the Labyrinthulomycota Apparently, labyrinthulomycetes seem to have a wide range of tolerance to different salinity conditions. Some *Labyrinthula* isolates have been found associated with the roots and root hairs of trees in sandy soils irrigated with low salinity (4.3‰) water (Aschner 1958). Labyrinthulids have also been isolated from inland saline soils (Amon 1978). Many thraustochytrids (including isolates of the genera *Thraustochytrium*, *Schizochytrium*, and *Ulkenia*) have been isolated from habitats reflecting a wide range of salinities from weakly brackish waters (3‰) to briny salt evaporation ponds (150‰) indicating they may be thought of as euryhaline organisms (Jones and Harrison 1976). However, none of the species of thraustochytrids that Bahnweg (1979a, b) studied would grow in pure culture above a salinity of 40‰, and *Thraustochytrium pachydermum* appears to be one of the few species so far described that shows growth and zoospore formation at salinities up to 60‰ (Schneider 1981). There are also species and isolates found in habitats of more or less constant salinity and thus might actually be stenohaline.

The ability to withstand other extreme or fluctuating environmental or culture conditions has been reported amongst thraustochytrids (e.g., Bahnweg 1979a, b; Kuznetsov 1981) – some can apparently resist repeated cycles of drying and freezing (anabiosis). There are reports that thraustochytrids from both frozen arctic coastal soil samples and dried 50-year-old herbarium sheets of marine algae were successfully isolated and grown in culture (Kuznetsov 1981). Isolates of thraustochytrids also survived drying for several days (Jain et al. 2005) or even years (Kuznetsov 1981).

Habitats of Labyrinthulales Species of *Labyrinthula* are found in estuarine and near-shore marine habitats throughout the world associated with (or isolated from) organic detritus, macroalgae, diatoms, and particularly estuarine plants, such as mangroves and other marine vascular plants (Porter 1990). In hanging-drop or other laboratory cultures, the cells of *Labyrinthula* readily colonize a variety of vascular plant and algal tissues. They penetrate the cell walls and appear to decompose the cellular contents. In laboratory culture, labyrinthulids are capable of decomposing many different microorganisms as a substrate, including bacteria, yeast, hyphal fungi, diatoms, filamentous algae, and other thraustochytrids (Perkins 1976; Porter 1990). Species of *Labyrinthula* are reliably isolated from submerged moribund or adrift leaves of marine vascular plants and pieces of filamentous or thalloid macroalgae. It has long been believed that healthy algae and marine grasses do not contain *Labyrinthula* cells within their tissues (Porter 1990), although these organisms can be regularly isolated from their tissues and Bockelmann et al. (2012)

reported their endophytic presence. However, *Labyrinthula* is usually not necrotrophic but rather feeds on epibiotic microorganisms and decomposing plant and algal material (Porter 1990). Based on current knowledge, the genus *Labyrinthula* is primarily associated with coastal environments. The genus *Stellarchytrium* associated with starfish has recently been described (FioRito et al. 2016) and represents a case in which an actual organism was found for a group otherwise only known from environmental sequencing, in this case, the LAB1/6/8 clade (Pan et al. 2017).

Habitats of Thraustochytriales Species of thraustochytrids (which includes species now classified in both the Thraustochytriaceae and Aplanochytriaceae) have also been isolated from estuarine and marine habitats throughout the world. Members of the thraustochytrids are able to grow in culture on a variety of plant- and animal-derived substrates (Perkins 1973). For instance, they have been observed growing on the spore cases of vesicular-arbuscular mycorrhizal fungi from barrier sand dunes (Koske 1981). They are generally isolated from decomposing algal and plant material, as well as from sediments, although they may also be found in plankton collected in offshore trails (Damare and Raghukumar 2010). In general, thraustochytrids seem to be mostly surface inhabitants of particulate organic material, primarily saprotrophic in their nutrition. In tropical and sub-tropical areas, mangrove plants (e.g., *Avicennia*, *Bruguiera*, *Kandelia*, and *Rhizophora*) are probably the most well-studied habitat in which to find labyrinthulomycetes (e.g., Fan and Chen 2006; Leñaño 2001), where they appear to be primarily as saprobes colonizing the surface of organic detritus.

Thraustochytrids appear to be amongst the initial colonizers of fallen senescent mangrove leaves, alongside oomycetes (Thines 2014; Marano et al. 2016), and thus play an important role in nutrient cycling through exogenous production of their cellulase and xylanase degradation enzymes (Fan et al. 2002; Leñaño 2001; Raghukumar 2002; Raghukumar et al. 1994). Thraustochytrids can be recovered in large numbers from marine sediments including from the deep sea (Bongiorni 2012). This group thereby contributes significantly to the biomass in the estuarine or marine environment.

In contrast to labyrinthulids, thraustochytrids appear to grow poorly on living algae and vascular plants. This has been primarily attributed to the presence of secondary metabolites with antimicrobial properties which limit the growth and propagation of these organisms (Raghukumar 2002). However, 7 days after leaf fall, thraustochytrids were found colonizing fallen leaves of *Rhizophora apiculata* (Raghukumar et al. 1995). Labyrinthulomycetes isolated from mangrove areas include *Schizochytrium* sp., *Thraustochytrium* sp., *Ulkenia* sp., and several unidentified strains of *Labyrinthula* sp. and *Aplanochytrium* sp. (Leñaño 2001; Leander et al. 2004; Yokochi et al. 2001). The extensive colonization by thraustochytrid thalli on the surfaces of decomposing seaweeds has been noted (Miller and Jones 1983). Thraustochytrids, as epibionts, are probably feeding on other epibiotic microorganisms and decomposing plant and algal material. It is reported that the extent of colonization increased with the rate of decomposition; thus, they are probably saprotrophic followers of labyrinthulids, oomycetes, and

zoosporic fungi. Yokochi et al. (2001) reported not only *Labyrinthula* sp. as a saprobe on *Padina arborescens* and *Sargassum* sp. but also *Aplanochytrium* sp. on *Dictyota cervicornis*, *Chaetomorpha* sp., and *Cladophora* sp. (Leander et al. 2004). *Aplanochytrium minutum* and *Ulkenia visurgensis* were found associated with decaying *Sargassum cinereum* (Sathe-Pathak et al. 1993).

Some thraustochytrids may also be capable of necrotrophic/parasitoid growth on marine invertebrates, particularly mollusks such as nudibranchs (McLean and Porter 1987), squid (Jones and O'Dor 1983), and octopus (Polglase 1980). Thraustochytrids are also regular components of the gut microbiota of certain echinoids (Wagner-Merner et al. 1980) and have been found in a variety of Mediterranean sponges (Höhnk and Ulken 1979) and on the surface mucus of hermatypic corals (Harel et al. 2008). Thraustochytrids themselves may host viruses (Perkins 1976), e.g., herpes-type DNA virus particles (Kazama and Schornstein 1973). As herpes-type viruses are present in some vertebrates and invertebrates, (Segarra et al. 2010; Evans et al. 2017), this raises the possibility that thraustochytrids may be virus vectors for other organisms.

In spite of the many reports of thraustochytrids isolated from numerous substrates and locations, there have been surprisingly few direct observations of thraustochytrids in nature. *Schizochytrium*-like thalli were observed parasitizing colonies of the diatom *Thalassionema* collected from the North Sea (Gaertner 1979). Thraustochytrid-like thalli in Antarctic sediments fixed immediately after collection have been described (Riemann and Schrage 1983). Although diagnostic features were not presented, the thraustochytrids in these samples most closely resemble the genus *Aplanochytrium*.

Parasitic and Symbiotic Relationships Parasitism is another ecological strategy found in a few species of *Labyrinthula*. *Labyrinthula* spp. have been isolated from the marine algae *Chaetomorpha*, *Lyngbya*, *Cladophora*, *Rhizoclonium* (Raghukumar 1987a, b), and several marine vascular plants, such as *Cymodocea*, *Posidonia*, *Spartina*, *Thalassia*, and *Zostera* (Bockelmann et al. 2012; Garcias-Bonet et al. 2011; Stowell et al. 2005), although it is unlikely whether all these are parasitic associations. However, as mentioned previously, *Labyrinthula zosterae* has been identified as the cause of the wasting disease of eelgrasses (*Zostera capricorni*, and *Zostera marina*) resulting in the decline of eelgrass population (Armiger 1964; Muehlstein et al. 1988); and *Labyrinthula terrestris* has been identified as the cause of rapid blight on turfgrasses (Bigelow et al. 2005; Stowell et al. 2005; Craven et al. 2005; Olsen 2007). Garcias-Bonet et al. (2011) studied the occurrence and pathogenicity of *Labyrinthula* sp. in Mediterranean seagrass meadows. They found that their isolates could infect a number of different seagrass genera (*Posidonia*, *Cymodocea*, and *Zostera*) and indicates their isolate had a broader host range than found in most North American studies which indicated that pathogenicity was host genus-specific (Muehlstein et al. 1988; Short et al. 1993; Vergeer and den Hartog 1991, 1994). *Labyrinthuloides* (now classified as *Aplanochytrium*) *schizochytops* was commonly isolated from living plants of the seagrass *Halodule wrightii* and was thought to be living as an endophyte, although may also have been responsible for a

brownish discoloration on the host leaves (Quick 1974). The *Schizochytrium*-like thalli parasitizing the diatom *Thalassionema* (Gaertner 1979) were later followed by additional reports of thraustochytrids as diatom pathogens. For example, *Ulkenia amoeboidea* was found capable of infecting a number of diatoms, including *Coscinodiscus* sp., *Grammatophora* sp., *Melosira* sp., *Navicula* sp., and *Nitzschia* sp. (Raghukumar 2006). However, it remains unclear if diatom parasitism constitutes a major ecological niche of Labyrinthulomycota and if these infections have a significant ecological impact.

Many marine invertebrates (e.g., corals, clams, flatworms, sea stars, and sea urchin) have been reported to harbor labyrinthulomycetes, and in some, this relationship may be parasitic, as with *Aplanochytrium haliotidis* on abalone (Bower 1987b) and the QPX thraustochytrid parasite on Quahog clam (Azevedo and Corral 1997; Lyons et al. 2005, 2007). The latter has been most extensively studied as an animal pathogen, and its genome has recently been sequenced in order to try and understand the basis of virulence (Garcia-Verdrenne et al. 2013). Recently a newly recognized species, *Thraustochytrium caudivorum*, was shown to parasitize the marine free-living flatworm *Macrostomum lignano* (Schärer et al. 2007), causing lesions that can lead to the dissolution of the posterior part or even complete animal. Three newly described Labyrinthulales species, *Stellarchytrium dubum*, *Oblongichytrium porteri*, and *Aplanochytrium blankum*, were isolated from dermal tissues of ochre sea stars (*Pisaster ochraceus*) that were exhibiting symptoms of starfish wasting disease (Fiorito et al. 2016), although a direct causal relationship with the disease has yet to be established. Thraustochytrids are also capable of necrotrophic growth (perhaps parasitic) on marine invertebrates, particularly mollusks such as nudibranchs (McLean and Porter 1987), octopus (Polglase 1980), and squid (Jones and O'Dor 1983).

Other Labyrinthulomycota appear to have commensal relationships with their hosts such as *Labyrinthula* and *Oblongichytrium multirudimentale* on the coral *Fungia granulosa* (Kramarsky-Winter et al. 2006; Harel et al. 2008) or are saprobic such as *Aplanochytrium minuta* on scleractinian coral mucus (Raghukumar and Balasubramanian 1991). Thraustochytrids are regular components of the gut microbiota of certain echinoids (Wagner-Merner et al. 1980) and have been found in a variety of Mediterranean sponges (Höhnk and Ulken 1979), although details of these relationships are still unknown. Interestingly, thraustochytrids may host viruses (Perkins 1976), and herpes-type DNA virus particles have been described in a *Thraustochytrium* sp. (Kazama and Schornstein 1973). This is the only herpes-type virus to have been found in a host that is not a vertebrate and raises the possibility that thraustochytrids may be virus vectors for other organisms (Porter 1990). However, Labyrinthulomycota also carry RNA viruses of unknown host spectrum (Takao et al. 2005).

Freshwater and Terrestrial Labyrinthulomycota Until the advent of molecular systematics, it had been generally assumed that there were no genuinely freshwater or terrestrial members of the Labyrinthulomycota, although there were historic reports of *Labyrinthula* species infecting the freshwater alga *Vaucheria* (Zopf

1892). Recently it was shown that a number of phagotrophic freshwater protists (Fig. 3) such as the unicellular *Diplophrys parva* and *D. mutabilis* in the Thraustochytriales (Anderson and Cavalier-Smith 2012; Takahashi et al. 2014) and unicellular *Archella flavum* and *Amphitrema wrightianum* (Gomaa et al. 2013) and the sorocarpic *Sorodiplophrys stercorea* (Tice et al. 2016) in the Amphitremida all cluster in the Labyrinthulomycota clade. All of these heterotrophs are characterized by having fine filose pseudopodia. *Diplophrys parva* was isolated from the intestinal tract of a goldfish (Anderson and Cavalier-Smith 2012) and *D. mutabilis* from a freshwater lake (Takahashi et al. 2014), whereas *Archerella* and *Amphitrema* were both free-living protists isolated from wet Sphagnum moss (Gomaa et al. 2013). The coprophilic genus *Sorodiplophrys* was isolated from horse and cow dung (Tice et al. 2016). Environmental sampling has also revealed many more isolates belonging to the Amphitremida and Amphifilidae clades (Fig. 5), including isolates from various terrestrial soils, freshwater ecosystems, and anoxic sediments (Anderson and Cavalier-Smith 2012; Gomaa et al. 2013; Takahashi et al. 2014; Tice et al. 2016).

Characterization and Classification

Thallus (Cell) Morphology and Ultrastructure Members of the Labyrinthulaceae are characterized by forming colonies of spindle-shaped thalli (cells) that are ensheathed in a membranous ectoplasmic network which form a branched track system along which the cells freely migrate (Figs. 1a and 2h, i). Members of the Thraustochytriaceae on the other hand form ovoid or spherical thalli, which are associated with a fine ectoplasmic network of rhizoid (rhizopodia)-like threads (Fig. 3c–i) which act as anchoring and feeding structures (Perkins 1976; Bremer 1976; Moss 1985, 1986; Porter 1990). In terms of size and general appearance, this gives thraustochytrid thalli a superficial similarity to those of hyphochytrids and chytrid fungi (Karling 1981). The planktonic genus *Althornia* lacks rhizoids and absorbs nutrients directly from the environment (Karling 1981; Moss 1986; Porter 1990). The thalli of the Aplanochytridiaceae (now placed in the Labyrinthulales) superficially resemble thraustochytrids (Figs. 3g and 4) but are able to glide slowly along the surface of their ectoplasmic threads (Leander and Porter 2001). A number of previously enigmatic unicellular, sometimes colonial protists with fine-branching rhizopodia often arising bipolarly from the cells (Fig. 3a, b, d, f) have now been included in the Labyrinthulomycota, in a number of newly created families such as the Amphifilidae, Diplophryidae, and Sorodiplophryidae (Anderson and Cavalier-Smith 2012; Takahashi et al. 2014; Tice et al. 2016). In addition, the mixotrophic testate amoeba-like genera in the Amphitremidae, *Amphitrema*, *Archerella*, and *Paramphitrema* have cells protected in flask-shaped punctate shells (Fig. 3e, f) from which the rhizopodia emanate (Gomaa et al. 2013).

The Labyrinthulomycota have a typical straminipilous cytoplasmic ultrastructure with mitochondria with tubular-vesiculate cristae and prominent Golgi dictyosomes (Figs. 1f and 2f, g, j, k; Perkins 1976; Moss 1985, 1986; Porter 1990; Anderson and Cavalier-Smith 2012; Iwata et al. 2016). The cells usually contain cytoplasmic

vacuoles and oil globules (Figs. 1c and 2a, b, f, j, k). However, an ultrastructural feature that defines the Labyrinthulales and Thraustochytriales is that their ectoplasmic nets originate from the thallus body from a unique endomembrane complex associated with an electron-dense plaque on the plasma membrane (now known as the bothrosome – Figs. 1d and 2g) from which cisternae of endoplasmic reticulum radiate (Perkins 1976; Moss 1985, 1986; Porter 1990; Iwata et al. 2016). Previously this body has also been variously referred to as the sagenogenetosome (Perkins 1976) or sagenogen (Dykstra and Porter 1984). The early stages of the bothrosome complex development and net formation following zoospore settlement have recently been described in *Schizochytrium* by Iwata et al. (2016). This study has shown that the bothrosome forms within minutes of zoospore settlement at the anterior-ventral pole of the cell close to the Golgi body. Immunofluorescence labelling revealed that actin co-localized with newly formed bothrosome co-localized, and that within 18 min of settlement, the ectoplasmic net system had formed, with net filaments rich in actin (Iwata et al. 2016). The ectoplasmic net, unlike the rhizoid system of hyphochytrids and chytrids, is not walled (Figs. 1c, d, and 2j, k) and as well as containing actin, only contains cisternae of endomembrane (Moss 1985, 1986; Porter 1990; Takahashi et al. 2014). However, a classical bothrosome structure does not appear to be associated with slender rhizoids/filopodia of the unicellular protist-like members of the phylum in the Ampifilaceae (e.g., *Amphifila marina* – Dykstra and Porter 1984).

Another major difference between the thalli of the Labyrinthulomycota compared to other straminipilous fungi is that the thallus is surrounded by Golgi-derived ovoid, round or hexagonal scales (Fig. 3c; Perkins 1976; Moss 1985, 1986; Porter 1990) which are not cellulosic but composed of sulfated polysaccharides containing fucose or galactose (Bahnweg and Jäckle 1986; Honda et al. 1999; Moss 1985, 1986). In older thalli layers, scales can form a consolidated wall (Fig. 2f) around the thallus but do not coat the tracks or rhizoids (Perkins 1976; Dykstra and Porter 1984; Porter 1990). Surface scales are also a feature of the planktonic unicellular genus *Amphifila marina* (formerly *Diplophrys* – see Anderson and Cavalier-Smith 2012), which led Dykstra and Porter (1984) to suggest this enigmatic protist had Labyrinthulomycete affiliations. Cells of the freshwater heterotrophs *Diplophrys parva* (Anderson and Cavalier-Smith 2012) and *D. mutabilis* (Takahashi et al. 2014) are also coated in small Golgi-derived capsule-shaped or ovoid scales (Fig. 3c). In contrast, the cells of species in the Amphitremidae are contained in a thick rigid lightly pigmented shell (Fig. 3e, f; Gomaa et al. 2013).

Zoospore Formation and Fine Structure The ways in which these thalli differentiate into motile zoospores and proliferate have been the main defining characteristics (see Karling 1981) of the thraustochytrid genera (Fig. 4), although it now appears that this morphology is poorly correlated with underlying genetic relatedness (Yokohama and Honda 2007; Yokohama et al. 2007; Beakes et al. 2014). In some *Thraustochytrium* species, the whole thallus cytoplasm differentiates into biflagellate zoospores, which are released by the general splitting and disintegration of the thallus wall (Fig. 4; Karling 1981). In other *Thraustochytrium* spp., internal proliferation of

new thalli occurs from cytoplasm cleaved from the basal portion of thallus, concomitantly with the main compartment cleaving into zoospores (Karling 1981; Beakes et al. 2014). Development of the thallus in the genus *Aplanochytrium* is similar except that only non-motile aplanospores are formed (Fig. 4). The genus *Schizochytrium* has thalli which divide by successive bipartitions to form progressively smaller units (Figs. 2c and 4), in which the zoospores ultimately differentiate (Karling 1981). Several genera (*Botryochytrium*, *Parietichytrium*, *Sicyoidochytrium*, and *Ulkenia* – Fig. 4) in the Thraustochytriales have a more complex life cycle in which a free-living amoeboid cell (Figs. 2d and 4) is released from the original parental thallus and which then settles and eventually differentiates into zoospores (Beakes et al. 2014).

Straminipilous zoospores range in size between 3 and 15 μm (Dick 2001) and, as in the Labyrinthulomycota, many are reniform with laterally inserted flagella (Figs. 1b, e, f, and 2e). The straminipilous zoospore has a remarkably conserved overall organization and structure supporting the origin of this clade from a common flagellate ancestor (Tsui et al. 2009; Beakes et al. 2014). The anterior flagellum in all members of the Straminipila is decorated with two parallel rows of tripartite tubular hairs (TTH) and usually four to five times the overall zoospore body length (Fig. 1b, e; Perkins 1976; Porter 1990). The TTH are made of proteins and serve to reverse the flagellum thrust, in effect pulling straminipilous zoospores through the water (Dick 2001). Thraustochytrid, but not labyrinthulid, zoospores are unusual in that the zoospore body is also coated in small scales (Fig. 1e, f; Perkins 1976; Kazama 1980; Porter 1990).

All straminipilous flagellate cells share the same underlying flagellar rootlet system (Fig. 1g–i) which shows a remarkable degree of conservation throughout the lineage (Barr and Désaulniers 1989; Andersen et al. 1991; Dick 2001; Iwata et al. 2016). Zoospores of biflagellate members of the Straminipila have four rootlets, two associated with each flagellum (Andersen et al. 1991; Barr and Allan 1985; Barr and Désaulniers 1987; Iwata et al. 2016). The R3 anterior rootlet is composed of three microtubules, and curves around the anterior end of the zoospore and from which on one side emanate a series of microtubular ribs (Fig. 1h, i; Beakes et al. 2014; Iwata et al. 2016). Labyrinthulomycete zoospores appear to lack the striated fan between the kinetosomes that are a feature of oomycete zoospores (Fig. 1g; Barr 1981; Barr and Allan 1985; Porter 1990; Iwata et al. 2016). Unusually for members of the Straminipila, the Labyrinthulomycota do not have a typical transitional helix (TH) structure associated above the flagellar plate but do have a similarly placed cone-like structure and electron-dense plug (Barr and Allan 1985; Beakes et al. 2014; Cavalier-Smith and Chao 2006). Nuclear division has been investigated by Perkins (1970) and Kazama (1974) and shows similarity to other members of the Straminipila (Beakes et al. 2014).

Sexual Cycle Most members of the Straminipila appear to be diploid organisms that undergo gametic meiosis (Dick 2001; Sims et al. 2006). However, knowledge of the precise timing of meiosis and plasmogamy in the Labyrinthulomycota is still very uncertain (Porter 1990; Beakes et al. 2014). In labyrinthulids, evidence of meiosis

has been found in thalli dividing up to produce flagellate zoospores (Perkins and Amon 1969; Porter 1990), but precisely where syngamy takes place has still not been established. However, epibiotic resting spores are produced by some species of thraustochytrids, although it has not been established if these are the result of sexual reproduction (Karling 1981; Porter 1990).

Classification and Systematics

Even though investigations of the past three decades have revealed new species, cytological details, and development cycles, the complete life cycle of any species of *Labyrinthula* remains to be worked out (Porter 1990; Beakes et al. 2014). However, it has now been established that Labyrinthulomycetes together with the human pathogen *Blastocystis* and the ciliate-like opalinids formed one major, early diverging branch of the Straminipila and that the Hyphochytriomycota, Oomycota, and golden-brown photosynthetic Ochrophyta formed another separate lineage, although both share a common ancestor (Fig. 5).

Prior the era of molecular systematics, the Labyrinthulomycota were divided into two families, the Labyrinthulaceae and the Thraustochytriaceae (Karling 1981; Porter 1990; Dick 2001) within a single order, the Labyrinthulales (or Labyrinthulida). The Labyrinthulaceae contained a single genus, *Labyrinthula* whereas the Thraustochytriaceae had around a dozen genera, mainly defined by thallus morphology and differentiation (Perkins 1976; Karling 1981; Moss 1985; Porter 1990; Dick 2001). The first in-depth molecular systematic study of the group was carried out by Honda et al. (1999) based on SSU rRNA gene sequence comparisons. Their isolates fell into two major clades, which they named the “labyrinthulid phylogenetic group” (LPG) and the “thraustochytrid phylogenetic group” (TPG) (Honda et al. 1999). The LPG clade included *Labyrinthula* and *Aplanochytrium* (syn. *Labyrinthuloides*) in one subclade and *Schizochytrium minutum* and *Thraustochytrium multirudimentale* in another. The TPG clade contained genera such as *Schizochytrium*, *Ulkenia*, as well as many *Thraustochytrium* spp. (Honda et al. 1999). Rather than the straightforward separation of the labyrinthulids and thraustochytrids, these studies revealed for the first time that the labyrinthulids in particular were part of a more diverse monophyletic assemblage that included a number of species that had traditionally been considered to be thraustochytrids. However, the LPG and TPG clades correlated well with the sugar composition of their thallus walls (Honda et al. 1999), with genera in the LPG clade predominantly having fucose and those in the TPG clade having galactose as their major cell wall constituents (Honda et al. 1999). A concurrent study by Leander and Porter (2001) however, suggested there were three major clades within the Labyrinthulomycota. There was an additional clade that included two *Labyrinthuloides* species, *L. yorkensis*, and *L. minuta*. These were subsequently transferred to the genus *Aplanochytrium* in a new family, the Aplanochytriaceae/Aplanochytriidae (Anderson and Cavalier-Smith 2012; Leander et al. 2004), which was sister to the Labyrinthulaceae.

The third clade represented the residual Thraustochytriaceae, containing many of the traditional thraustochytrid genera together with the enigmatic, bothrosome-lacking, planktonic protist *Diplophrys* (now *Amphifila*) *marina* and two isolates of the Quahog clam pathogen (so-called QPX isolates). What these molecular studies also highlighted was that many of the traditional thraustochytrid genera, such as *Schizochytrium*, *Thraustochytrium*, and *Ulkenia*, which were based on patterns of thallus development, were paraphyletic or polyphyletic (Honda et al. 1999; Leander and Porter 2001; Leander et al. 2004) showing that traditional morphological characters were not good indicators of genetic relatedness. Subsequent studies have led to a radical revision in thraustochytrid nomenclature, with the introduction of many new genera (*Aurantiochytrium*, *Japanochytrium*, *Oblongichytrium*, *Parietichytrium*, *Sicyoidochytrium*, and *Stellarchytrium*) based on combined molecular and biochemical characteristics (Yokoyama and Honda 2007; Yokoyama et al. 2007; FioRito et al. 2016). A recent taxonomic analysis of labyrinthulomycetes phylogenies is shown in Fig. 5 (adapted Gomaa et al. 2013; Pan et al. 2017). The order Labyrinthulales *s. lat.* includes a number of genera (*Aplanochytrium*, *Stellarchytrium*, and some *Thraustochytrium* spp.) that would have previously been placed in the Thraustochytriaceae. Some recent analyses have also separated another, morphologically unremarkable, thraustochytrid-like clade encompassing the genus *Oblongichytrium*, into their own separate family (Oblongichytriidae; Fig. 5; Pan et al. 2017).

The taxonomic subdivision of the Labyrinthulomycota is still in flux and has changed significantly in the last decade as a result of molecular phylogenetic investigations of both the core labyrinthulids and thraustochytrids, but also other groups of heterotrophic protists that are now known to be related. As a consequence of the above taxonomic studies and a series of more recent phylogenetic investigations (Colladao-Mercado et al. 2010; Anderson and Cavalier-Smith 2012; FioRito et al. 2016; Gomaa et al. 2013; Pan et al. 2017; Takahashi et al. 2014; Tice et al. 2016), there seem to be four or five higher-level clades within the phylum (excluding clades only known from environmental sequencing), namely, Labyrinthulales/Labyrinthulida, Thraustochytriales/Thraustochytrida, “Amphifilales/Amphifilida,” Amphitremidales/Amphitremida, and “Oblongichytriales/Oblongichytrida.” However, what is becoming increasingly clear from environmental sequencing is that they are a diverse group of which the vast majority of species still awaits discovery (Worden and Not 2008; Collado-Mercado et al. 2010; Richards et al. 2012; Gomaa et al. 2013; Ueda et al. 2015; Pan et al. 2017). As for most environmental lineages, only partial SSU sequences are available which has been proven to have an insufficient resolution for the deeper splits of the Labyrinthulomycota; it remains unclear, how many of the lineages known only from environmental sequencing can be assigned to the orders given above. In the most comprehensive analysis of environmental sequences currently available, Pan et al. (2017) recognized several additional lineages of the Labyrinthulomycota basal to the known orders or in unresolved positions, which group in four clades, mostly with low to moderate support. These clades, such as the LAB1/6/8 clade containing *Stellarchytrium dubum*, might deserve family- or order-level status once their members have been studied in more detail, e.g., in multigenic phylogenies.

Class Labyrinthulomycetes/Labyrinthulomorpha Labyrinthulea? (Lister 1891) Olive ex Cavalier-Smith 1986

Order Labyrinthulales/Labyrinthulida E A Bessey 1950/Doffein 1901

Family Aplanochytriaceae/Aplanochytriidae Leander Ex Cavalier-Smith 2012

A monotypic family formerly included in thraustochytrids. They have typical ovoid to spherical thalli, attached to their substrate by a basal ectoplasmic net that only form non-motile aplanospores. However, unlike members of the Thraustochytriaceae, the thalli are able to slowly glide along the rhizoids. The genus, *Aplanochytrium* (which subsumes the genus *Labyrinthuloides*), contains around half a dozen described genera, but there are probably many undescribed species based on environmental sequencing.

Family Labyrinthulaceae/Labyrinthulidae Haeckel 1868/Cinekowska 1867

This family contains the classic “slime nets,” which form a colony of spindle-shaped thalli that are contained within a branching ectoplasmic network within which the cells migrate. The gliding motility of the cells, which at times is as fast as 100 $\mu\text{m}/\text{min}$, probably driven by a calcium-dependent contractile system of actin-like proteins in the ectoplasmic network (Nakatsuji and Bell 1980). Each cell has a single bothrosome connecting it to the ectoplasmic network. There are around a dozen or so species that have been recognized (Dick 2001). Most are saprotrophs associated with marine debris and decaying macroalgae and marine macrophytes. However, some species have been shown to be the causal agents responsible for the wasting disease of eelgrass (*Zostera*) beds (Muehlstein and Porter 1991) and the turfgrass dieback (Craven et al. 2005).

Family-Level Clade “Stellarchytriaceae/Stellarchytriidae” Undescribed, LAB 1/6/8

This clade, which possibly needs to be described as a new family or even order, is provisionally placed in the Labyrinthulales and contains various lineages known only from environmental sequencing (Pan et al. 2017) and the recently discovered species *Stellarchytrium dubum* (FioRito et al. 2016). *Stellarchytrium dubum* was isolated from diseased starfish, but its role in causing starfish wasting disease still needs to be investigated in detail.

Order Oblongichytriales/Oblongichytrida

Family Oblongichytriaceae/Oblongichytriidae Cavalier-Smith 2012

This monotypic family was first recognized as a result of molecular sequencing by Yokoyama and Honda (2007). It contains around a half-dozen species that were formerly included in the genus *Schizochytrium* based on thallus development. The family name is derived from their slender oblong zoospores they produce rather than the more ovoid zoospores typical of the Thraustochytriales. It appears to form an early diverging clade from the same root as the Labyrinthulales lineage (Fig. 5).

Order Thraustochytriales/Thraustochytrida Sparrow 1973

Even though it has now been split, this still is the largest and most diverse order in the Labyrinthulomycota with the most genera and species. They produce relatively small epibiontic thalli usually attached to substrate by a fine ectoplasmic network of fine-branched anastomosing filaments, which have role in both substrate attachment and feeding. Most are marine organisms that are saprotrophic epibiontic colonizers of a variety of marine detritus, but there are a number of pathogens, mostly of marine invertebrates.

Family Althornidiaceae/Althorniidae Jones and Alderman 1972

This monotypic and monospecies (*A. crouchii*) is the only truly planktonic thraustochytrid as it completely lacks the usual ectoplasmic network. It is also the only genus for which there is at present no sequence data, and therefore its taxonomic placement must be considered as provisional.

Family Thraustochytriaceae/Thraustochytriidae Sparrow ex Cejp 1959

Typically thraustochytrids are not colonial but grow by enlargement of cells which develop either into single ovoid or globular thalli or clusters of thalli depending if proliferation takes place before spore formation (traditionally referred to as sori). Within these, either zoospores are differentiated or an amoeboid stage is formed, which are both released by the breakdown of the thallus wall. There are at present six to eight genera within the family, a number of which have been recently created as a result of molecular studies (Yohoyama and Honda 2007; Yokoyama et al. 2007). Genera included in this family are *Aurantiochytrium*, *Botryochytrium*, *Japanochytrium*, *Monorhizochytrium*, *Parietichytrium*, *Schizochytrium*, *Thraustochytrium*, and *Ulkenia*. *Thraustochytrium* is the largest genus with around 20 described species.

Order "Amphitremidales"/Amphitremida Gomaa et al. 2013

Family "Amphitremidiaceae"/Amphitremidae Poch 1913

These organisms were formerly grouped with the testate amoebae. The cells are enclosed with ovoid, cup-shaped, or rectangular punctate shells (Gomaa et al. 2013). Many contain green algal trebouxiophyte endosymbionts and have a mixotrophic nutrition (Gomaa et al. 2013). There are currently three recognized genera, *Amphitrema*, *Archerella*, and *Paramphitrema*. Named species have been isolated from freshwater habitats, such as freshwater wetlands. However, environmental sequencing has revealed many uncultured sequences in a sister group from anoxic and micro-oxic deep-sea sediments.

Family "Diplophrydaceae"/Diplophryidae Cavalier-Smith 2012

This was one of the first of the colorless protist groups that was found associated with the Labyrinthulid clade, although the initial species studied *Diplophrys marina* (Dykstra and Porter 1984) has now been moved to the Amphifilida. The

Diplophryidae *s. str.* are small, largely freshwater heterotrophic protists with colorless spindle- to ovoid-shaped body cells from which a fine network of anastomosing filaments arises in bipolar fashion (Anderson and Cavalier-Smith 2012; Takahashi et al. 2014). The exact order placement of this family is not fully resolved, and it is placed with the Amphitremida on basis of recent phylogenetic investigations (Tice et al. 2016; Pan et al. 2017) although species also share morphological similarities with members of the next order.

Order “Amphifilales”/Amphifilida Cavalier Smith 2012

This is another order of colorless protists that have been phylogenetically elusive. They share many of the morphological characteristics of the genus *Diplophrys* described above, and the new genus was created by Anderson and Cavalier-Smith (2012) to contain the species *Diplophrys marina* which was in a separate clade from freshwater species of that genus. Another member of the family is the genus *Sorodiplophrys*, which had often been placed with dictyostelid amoebae (Tice et al. 2016).

Isolation Procedures

Thraustochytriales Isolation procedures have been summarized by Porter (1990) and are briefly reviewed in this account. Thraustochytrids can be isolated by plating tissue sections on seawater agar, peptone-yeast-glucose seawater agar (PYGSA, approximately 50% seawater), modified Vishniac’s medium (KMV), or vegetable juice seawater agar, amended with penicillin and streptomycin to prevent bacterial growth. Small pieces (1 cm² or less) of carefully rinsed (e.g., with sterile 50% seawater) tissue sections are placed on agar media and incubated at room temperature for 3 days or until thraustochytrid colonies are visible on the periphery of the tissue samples. Often the bottom surface of the tissue that is in direct contact with the agar is similarly colonized. Slide purification is the easiest method for obtaining an axenic culture of thraustochytrid. This is done by transferring a minute quantity of thraustochytrid cells, often with a fine glass needle, to a drop of sterile water on a slide then serially diluting until few cells or thalli are visible. Individual thalli can then be streaked to another agarised medium used in the isolation process. Baiting samples with pollen, especially from pines, is a method commonly used for isolating chytrids but similarly helpful when isolating thraustochytrids. For pollen baiting, the carefully rinsed substrate is placed in Petri dishes containing sterile-filtered seawater, onto which pollen grains (preferably sterilized) are dispersed. Colonization of pollen grains is usually evident within 2–10 days in the Petri dishes, but these may be held for several weeks if necessary. Thraustochytrid thalli can be observed on pollen grains with a dissecting microscope, ideally at high magnification (60–100×). Individual pollen grains can be transferred with a loop to agar plates or to small drops of sterile seawater from which zoospores, if released, can be picked up and streaked onto agar plates. Alternatively, colonized pollen grains can be transferred in mass to agar plates. Often, especially if a small initial inoculum is used, all of the

colonized pollen grains will have colonies of the same species of thraustochytrid. It has been noted that not all thraustochytrids readily colonize pollen grains; thus, if a synoptic collection is desired, a variety of isolation procedures should be used. Maintenance of thraustochytrids can be achieved by regular subculturing or cryopreservation in 10% glycerol.

Labyrinthulales Several methods for isolating members of the Labyrinthulales have been published (Amon 1978; FioRito et al. 2016; Garcias-Bonet et al. 2011; Yokochi et al. 2001). Moribund (discoloured) but not decomposed seagrass, marsh grass, mangrove litter, and algal fragments collected adrift or recently washed ashore are reliable sources for labyrinthulids. Organic sediments from marine and intertidal aerobic zones and tissues of invertebrate species may also yield labyrinthulids. Successful isolation has been reported with 1% serum seawater agar (SSA) but also with plain seawater agar. Half- to quarter-strength concentration of vegetable juice seawater agar and PYGSA amended with antibiotics (e.g., penicillin, streptomycin, or ampicillin) are similarly useful in isolating *Labyrinthula*. Often the thickness of agar media is minimized to ~2 mm (Yokochi et al. 2001) for ease in observing colonies with an inverted microscope. Similar to thraustochytrids, rinsed plant or algal materials are placed onto agar media and usually incubated at room temperature. Vividly swarming colonies radiating from tissue pieces are usually visible within 7 days of incubation. An agar block containing a swarm of labyrinthula can then be subcultured or cocultivated with marine yeast or bacteria (e.g., *Vibrio*, *Psychrobacter*). This method has been practiced often, since these microorganisms serve as host or food for labyrinthulids. However, maintaining a culture of labyrinthulids is challenging as isolate cessation after subculturing several times occurs, probably because the full life cycle is not concluded under these cultivation conditions.

Evolutionary History

In the absence of any fossil record for this group, all evolutionary speculation has to be based on the evidence of recent molecular phylogenetic studies. All of the osmotrophic fungal-like organisms studied by mycologists, except the plasmodiophorids, fall within the straminipilous branch of the chromalveolate assemblage (Fig. 1a adapted from Tsui et al. 2009; Fig. 1b from Moreira and López-García 2002). The kingdom Straminipila defined by Dick (2001) was often seen as synonymous with the kingdom Chromista (Cavalier-Smith and Chao 2006) which is the name often favored by online taxonomic databases, even though the chromista, as originally defined also contain organisms not belonging to the Straminipila or the SAR supergroup (Burki et al. 2009). However, Dick (2001) argued that because of the non-photosynthetic osmotrophic groups in this lineage that the etymologically correct name Straminipila would be a more appropriate kingdom name, as this would highlight the synapomorphy of a monophyletic group. Nonetheless, a widely adopted form of spelling for this kingdom is

Stramenopila (Adl et al. 2005; Lévesque 2011). Whether cryptophytes and haptophytes and their allies should also be considered as part of a wider supergroup is still debated (e.g., Reeb et al. 2009; Dorrell and Smith 2011).

Hyphochytrids and oomycetes are part of the lineage that shares a common ancestor with the photosynthetic ochrophytes (Tsui et al. 2009; Riisberg et al. 2009; Yubuki et al. 2010). It has recently been suggested that the stem origin of the Ochrophyta was around 571 millions of years ago, (a mean of estimates ranging from 735 to 434 million years ago: Brown and Sorhannus 2010). The Labyrinthulomycota are part of a sister clade, often collectively termed Bigyra which presumably evolved around the same time or only slightly earlier than the other osmotrophic Straminipila. The Labyrinthulomycota, Hyphochytriomycota, and Oomycota, as well as the ochrophyte straminipilous lineages share a common ancestor, which was most likely a photosynthetic mixotrophic marine flagellate (Tsui et al. 2009). The Labyrinthulomycota are part of one major straminipilous line and the Hyphochytriomycota and Oomycota of another (Fig. 5). This explains that whilst there are similarities between the Labyrinthulomycota and the other heterokont osmotrophs, they show much less in common than the other two groups.

The overall relationships between the major groups within the chromalveolate lineage, and the straminipilous groups in particular (see Beakes et al. 2014; Beakes and Thines, this volume), have been investigated using multiple protein-encoding genes (Tsui et al. 2009; Reeb et al. 2009; Riisberg et al. 2009). The statistically well-supported Alveolata kingdom, comprising Apicomplexa, Dinoflagellata, and Ciliata, forms the sister clade to the Straminipila (Keeling 2009). These can be divided into two main lineages: the first encompasses the bacteriotropic flagellate bicosoecids, the protistan gut-inhabiting opalinids (plus proteromonads and *Blastocystis* – equivalent to slopalinids defined by Patterson 1989) and the Labyrinthulomycota (Cavalier-Smith and Chao 2006; Tsui et al. 2009; Riisberg et al. 2009; Reeb et al. 2009), and the second straminipilous clade that includes the osmotrophic Hyphochytriomycota and Oomycota, the photosynthetic Ochrophyta, and a number of phagotrophic flagellates, such as *Developayella* and *Pirsonia* (Beakes et al. 2014). In this account, the Labyrinthulomycota is given phylum rank as in Porter (1990), and an emended Labyrinthulomycota *s. lat.* could be seen as containing other members of the phylum Bigyra (sensu Cavalier-Smith and Chao 2006), thereby replacing it.

The “chromalveolate hypothesis” proposes the red algal origin of the plastid in all chlorophyll *c*-containing algal groups (reviewed by Keeling 2009). However, recent comparative analyses of genomes of members of the Straminipila have led to the discovery of genes of green algal ancestry in both diatoms (Moustafa et al. 2011) and oomycetes (Richards et al. 2011; Jiang and Tyler 2012). It seems to cast doubt on such a simple “single-acquisition-multiple loss” interpretation (e.g., Maruyama et al. 2009; Stiller et al. 2009; Dorrell and Smith 2011). Theories involving multiple independent chloroplast acquisitions and horizontal gene transfer (HGT) have also been proposed as alternative explanations of the phylogenetic and genomic data (e.g., Martens et al. 2008; Stiller et al. 2009; Baurain et al. 2010). The eyespot in *Labyrinthula* zoospores (Perkins and Amon 1969) resembles those of photosynthetic

Straminipila and may be indicative of the remains of an ancestral chloroplast. However, the fact that such structures have only been observed in the most derived group does perhaps cast doubt on this suggested origin (Tsui et al. 2009). Thraustochytrids produce omega-3 PUFA using a desaturase enzyme that in algae is usually found in chloroplasts (Sargent et al. 1995). If, as suggested by Tsui et al. (2009), it is assumed the ancestor to the straminipilous lineage was a mixotrophic photosynthetic flagellate, then at least two independent plastid losses must have occurred in the straminipilous line, one prior to diversification of the Labyrinthulid clade and the other after the divergence of the ochrophytes leading to the heterotrophic Oomycota and related lineages (Beakes et al. 2014). Alternatively, if plastid loss was deeply seated within the straminipilous line, then an independent reacquisition of a chromistan type plastid must have occurred to give rise to the Ochrophyta as suggested by Leipe et al. (1994). Plastid genes have been widely found in sequenced oomycete genomes (see Lévesque et al. 2010; Jiang and Tyler 2012) but were not reported in the compact 18.8 Mb genome of the anaerobic human gut parasite *Blastocystis* (Doenoeud et al. 2011). The preliminary genome sequence for the thraustochytrid *Aurantiochytrium limacinum* has been recently released (Collier 2012), but preliminary analysis has not so far revealed evidence of genes of plastid origin (Collier personal communication).

The earliest-diverging Labyrinthulomycota clades appear to contain thraustochytrid clades that have still retained the ability of phagotrophic nutrition, which is considered to be the ancestral state (Tsui et al. 2009; Gomaa et al. 2013). The presence of endosymbiotic Trebouxiophyte algae in the cells of members of the Amphitremida indicates this group has retained the ability to feed phagotrophically (Gomaa et al. 2013).

According to the analysis of Tsui et al. (2009), the key evolutionary event in the evolution of the labyrinthulomycetes within the straminipilous lineage was the evolution of the naked ectoplasmic net. The most derived groups are the Labyrinthulids and Aplanochytrids which have lost the ability to feed phagotrophically and rely entirely on osmotrophic nutrition. But also they have evolved gliding movement on the ectoplasmic net. The Aplanochytrids became separated from the Labyrinthulids by the loss of flagella (although it would be interesting to know if flagella genes can be found in their genome) and the acquisition of polygonal scales (Tsui et al. 2009).

What has become apparent in the past decade is that the Labyrinthulomycota are a more diverse assemblage than previously thought (Fig. 5), both in terms of ecological niches they occupy and their morphology than was believed at the time of the last Handbook review (Porter 1990). It is likely, that many more species and hidden genera are yet to be discovered that will provide new insights into the origins and evolutionary development of this enigmatic group of protists.

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