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Vertical community patterns of Labyrinthulomycetes protists reveal their potential importance in the oceanic biological pump

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Summary

The biological pump plays a vital role in exporting organic particles into the deep ocean for long-term carbon sequestration. However, much remains unknown about some of its key microbial players. In this study, Labyrinthulomycetes protists (LP) were used to understand the significance of heterotrophic microeukaryotes in the transport of particulate organic matter from the surface to the dark ocean. Unlike the sharp vertical decrease of prokaryotic biomass, the LP biomass only slightly decreased with depth and eventually exceeded prokaryotic biomass in the bathypelagic layer. Sequencing identified high diversity of the LP communities with a dominance of *Aplanochytrium* at all depths. Notably, ASVs that were observed in the surface layer comprised ~20% of ASVs and ~60% of sequences in each of the deeper (including bathypelagic) layers, suggesting potential vertical export of the LP populations to the deep ocean. Further analyses of the vertical patterns of the 50 most abundant ASVs revealed niche partitioning of LP phylotypes in the pelagic ocean, including those that could decompose organic detritus and/or facilitate the formation of fast-sinking particles. Overall, this study presents several lines of evidence that the LP can be an important component of the biological pump through their multiple ecotypes in the pelagic ocean.

Introduction

The oceans are responsible for about half of global primary production and regulate climate through the exchange of energy and matter with the atmosphere (Arrigo, 2007; Jiao *et al*., 2010; Sanders *et al*., 2014). Heterotrophic microbes play key roles in carbon biogeochemical processes through secondary production, remineralization and facilitation of particle aggregation/disaggregation (Cho and Azam, 1988; Smith *et al*., 1992; Kiørboe and Jackson, 2001; Steinberg *et al*., 2008; Collins *et al*., 2015; Boeuf *et al*., 2019; Duret *et al*., 2020). Therefore, microbes impact long-term carbon sequestration through both the biological and microbial pumps, which correspond to gravitational sinking of large particulate organic matter (POM) to the deep ocean and microbial transformation of biodegradable carbon to recalcitrant forms respectively (Jiao *et al*., 2010; Henson *et al*., 2012; Passow and Carlson, 2012). Until recently, most studies of microbe-mediated carbon sequestration have focused on prokaryotes (DeLong *et al*., 1993; López-Pérez *et al*., 2012; Crespo *et al*., 2013; Jiao *et al*., 2014; Fang *et al*., 2015; Mestre *et al*., 2017) and eukaryotic algae (Jardillier *et al*., 2010; Legendre *et al*., 2015). Among the heterotrophic microeukaryotes, a few phagotrophs, such as heterotrophic flagellates and ciliates, have been shown to form sinking particles or facilitate movement of organic matter into the microbial loop (Legendre and Le Fèvre, 1995; Legendre and

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Rassoulzadegan, 1996; Simon *et al*., 2002). However, much less is known about the roles of other heterotrophic microeukaryotes, especially those with mainly non-phagotrophic nutritional modes (Duret *et al*., 2020).

Nevertheless, increasing evidence has shown the direct involvement of diverse osmoheterotrophic microeukaryotes in marine carbon cycling (Worden *et al*., 2015; Boeuf *et al*., 2019; Duret *et al*., 2020). Particularly, nutrient-mineralizing eukaryotic decomposers, mainly fungi and Labyrinthulomycetes protists (LP), have been reported to dominate biomass on bathypelagic marine snow (Henson *et al*., 2012), suggesting an association with large, fast-sinking particles. Recently, diverse, ribosome-active LP have been identified as the major contributor to heterotrophic eukaryotic communities in oceanic sediments (Rodríguez-Martínez *et al*., 2020), further highlighting their importance in deep-sea ecosystems and potential dual roles in regulation of long-term carbon sequestration by facilitating POM sinking and/or remineralization. Therefore, the LP are a good target to examine the significance and effects of nutrient-mineralizing eukaryotic microbes in transport of POM from the surface to the dark ocean.

The LP are a group of heterotrophic unicellular protists ubiquitously present in the ocean and have a much larger cell size (3–20 μm) and higher C/N ratio (10.5–36) than those of bacterioplankton (<1 μm, 5.2–7.7 respectively) (Naganuma and Miura, 1997; Fukuda *et al*., 1998; Kimura *et al*., 1999). In fact, their cellular biomass can be $\sim 10^3$ times that of bacterioplankton (Fukuda *et al*., 1998; Naganuma *et al*., 1998; Kimura *et al*., 1999). They are well-documented as a major component of heterotrophic microbial communities that interact with planktonic prokaryotes and fungi in the coastal oceans (Bongiorni and Dini, 2002; Ueda *et al*., 2015; Liu *et al*., 2017; Xie *et al*., 2018; Bai *et al*., 2019; Xie *et al*., 2021). The LP have long been considered to be 'fungus-like' protists because most strains are osmotrophic saprophytes, using their ectoplasmic nets to attach to particulate detritus and then secrete hydrolases to break down the substrate (Raghukumar, 1986; Raghukumar, 2002), or living as parasites of algae, seagrasses and invertebrates (Ragan *et al*., 2000; Schärer *et al*., 2007; Sullivan *et al*., 2013; Hughes *et al*., 2018). Some strains are also reported to be capable of bacterivory in their amoeboid stages (Raghukumar, 1992) or to directly capture and decompose living diatoms using their specialized ectoplasmic nets (Hamamoto and Honda, 2019). The LP potentially facilitate the formation of fast-sinking aggregates due to both the ectoplasmic nets and extracellular polysaccharides (EPSs) (Li *et al*., 2013; Singh *et al*., 2014). Furthermore, several lines of evidence suggest that the LP in coastal oceans comprise multiple ecotypes with distinct ecological functions (Song *et al*., 2018; Xie *et al*., 2021). Therefore, we hypothesize that the LP in the pelagic ocean are composed of different ecotypes and together a key player influencing long-term carbon storage.

Our previous efforts identified 25 OTUs of the LP from Hawaiian oceanic waters using a clone library-based approach (Li *et al*., 2013). Since then, to the best of our knowledge, no further effort has been made to explore the diversity and community structure of the LP in pelagic waters. Importantly, the absence of vertical characterization of the LP communities hinders understanding of involvement in open ocean carbon export and storage processes. The South China Sea (SCS) is one of the largest marginal seas and because it contains multiple ecoregions, it represents a good location to investigate the LP community. To gain a greater insight into the hypothesis on the ecological role of LP in oceanic carbon cycle and storage, water samples were collected from more than 4000 km^2 of the pelagic SCS during three summer cruises (2016–2018). Here we report the abundance and biomass of LP through vertical profiles and provide the first line of molecular evidence that this group of protistan microbes includes different ecotypes that not only could facilitate carbon export from the surface to the deep ocean but also potentially decompose detritus in pelagic waters.

Results

Vertical abundance and biomass profiles of the Labyrinthulomycetes

The LP were detected in all the samples collected from the three cruises in the range of 10^4–10^5 cells L^{-1} (Fig. 1A, C, E). Despite high variance among the samples, their abundance generally displayed a slight decline with depth, with average abundance in the epipelagic (5–75 m), mesopelagic (200–500 m), and bathypelagic (1000–2000 m) layers of 9.2×10^4 cells L^{-1}, 6.2×10^4 cells L^{-1}, and 4.9×10^4 cells L^{-1} respectively. The LP abundance in the epipelagic layer was significantly higher than that in the mesopelagic and bathypelagic layers (Kruskal–Wallis test, $p < 0.05$), but the difference was not significant between the mesopelagic and bathypelagic layers (Kruskal–Wallis test, $p > 0.05$).

Compared to the less than twofold decline in the average LP abundance with depth, the average abundance of the prokaryotic plankton decreased by more than an order of magnitude from the epipelagic layer (7.8×10^8 cells L^{-1}) through the mesopelagic (1.5×10^8 cells L^{-1}) and bathypelagic (5.2×10^7 cells L^{-1}). Therefore, in the bathypelagic layer (and sometimes in the mesopelagic) the average free-living LP biomass was generally higher than that of the prokaryotic plankton at the same depth (Fig. 1B, D, F). The average biomass of the LP was

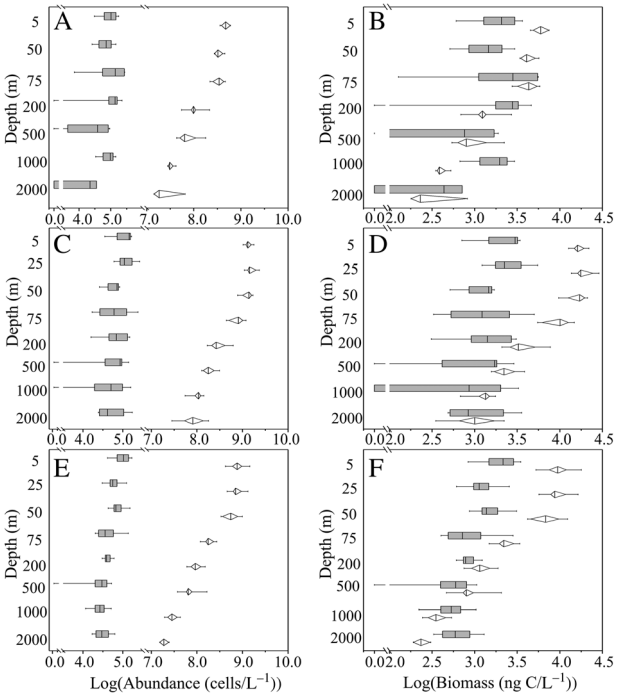

Fig. 1. Abundance and projected biomass of the Labyrinthulomycetes (Rectangles) and prokaryotic plankton (Diamonds) in the South China Sea for Cruise 2016 (A and B), Cruise 2017 (C and D) and Cruise 2018 (E and F). The boxes represent the ranges of the first and third quartiles, the line inside each box represent the median value, and the ends of the whiskers represent the lowest and highest datums.

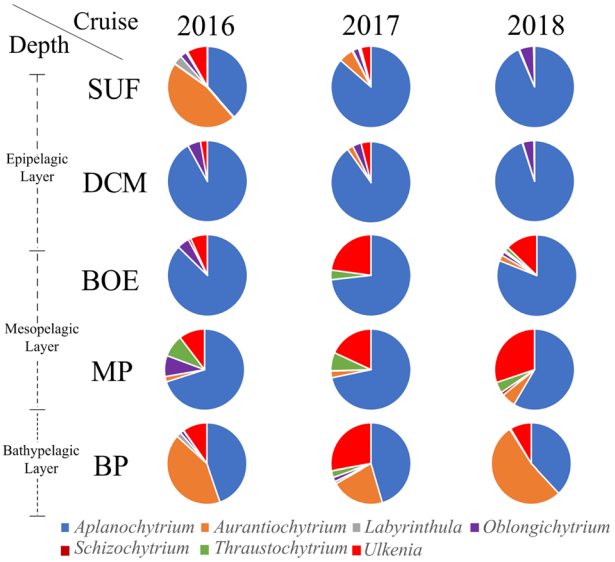

Fig. 2. Average relative abundance of different Labyrinthulomycetes genera (ASVs unclassified at the genus level not included) across depths and cruises. SUF: surface layer (5 m, eight samples); DCM: deep chlorophyll maximum layer (75 m, six samples); BOE: bottom of the euphotic layer (200 m, six samples); MP: mesopelagic layer (500 m, five samples); BP: bathypelagic layer (1000 or 2000 m, six samples). [Color figure can be viewed at wileyonlinelibrary.com]

1.01 μg C L^{-1} in the bathypelagic layer of SCS, which was 150.70% of the prokaryotic biomass. Clearly, the LP can comprise a larger fraction of living POM than prokaryotic plankton in the deep ocean.

Vertical variation of the Labyrinthulomycetes community composition

To understand the vertical profiles of the LP in the SCS, a total of 31 water samples collected from three cruises were sequenced. Quality-filtered LP sequence tags (972 554) were grouped into 1355 ASVs and annotated as members of *Ulkenia*, *Thraustochytrium*, *Schizochytrium*, *Oblongichytrium*, *Labyrinthula*, *Aurantiochytrium*, *Aplanochytrium* and unclassified Labyrinthulomycetes. While the relative composition of the annotated taxa varied among different layers (Fig. 2), *Aplanochytrium* was the most abundant genus across all depths, and was particularly dominant in the epipelagic except in the upper epipelagic of the 2016 cruise. *Aplanochytrium* reached its lowest relative abundance in the bathypelagic layer, suggesting it may not be well-adapted to the deep-sea environment. Conversely, the genera *Ulkenia* and *Thraustochytrium* reached their greatest relative abundances in the mesopelagic and bathypelagic layers (Fig. 2). Moreover, the genus *Aurantiochytrium* was unexpectedly found at high relative abundance in the bathypelagic layer. These patterns suggest that the LP genera have distinct adaptations to the depth-related environmental changes and may play different ecological roles at different depths. It is also notable that a large portion of the ASVs was classified as Labyrinthulomycetes, but not annotated to any known genera (Table S1), suggesting potential novel subgroups.

Although the alpha-diversity indexes (e.g. ASV richness and Shannon's diversity) of LP showed no significant change ($p > 0.05$, Kruskal–Wallis test) with depth (Table S2), Non-metric Multidimensional Scaling (NMDS) analysis revealed significant differences in ASV composition of the LP communities among different depths (PERMANOVA, $p < 0.05$) (Fig. 3; Table S3). In general, the LP community composition was most similar to that of neighbouring depths, suggesting persistent presence of some ASVs across multiple adjacent water layers. Surprisingly, Bray–Curtis dissimilarity between bathypelagic and surface communities was similar to communities from adjacent layers (Fig. 3). Across all water depths, ASVs that were observed in the surface layer comprised ~20% of ASVs and ~60% of sequences in other layers (Fig. 4), suggesting potential export of the surface LP populations deeper in the water column.

Vertical distribution patterns of the dominant Labyrinthulomycetes ASVs

To understand the ecological partitioning within the LP communities, the depth distribution of the 50 most abundant ASVs (with the highest average relative abundance across all samples) were examined in greater detail (Fig. 5). Based on the hierarchical clustering and LEfSe analysis, we identified several distinct vertical patterns.

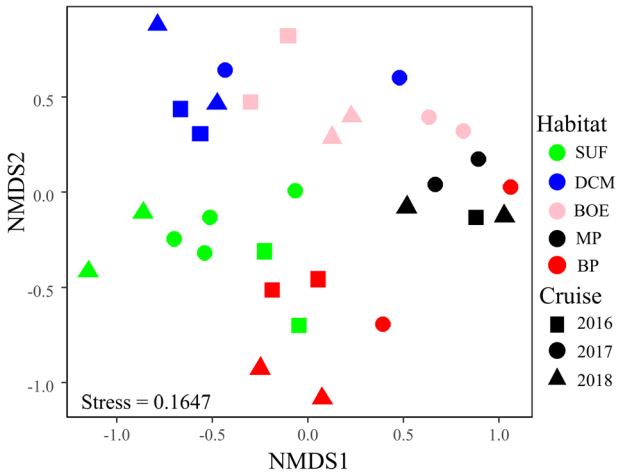

Fig. 3. Variation in Labyrinthulomycetes composition across different depths revealed by NMDS analysis. SUF: surface layer (5 m); DCM: deep chlorophyll maximum layer (75 m); BOE: bottom of the euphotic layer (200 m); MP: mesopelagic layer (500 m); BP: bathypelagic layer (1000 or 2000 m). [Color figure can be viewed at wileyonlinelibrary.com]

The ASVs in clusters 1 and 2 were found across all depths (Fig. 5); yet these two clusters dominated the LP communities in surface (24.36% of SUF sequences) and deep waters (51.26% of MP sequences and 31.20% of BP sequences) respectively, as revealed by their average relative abundance (Fig. S1) and the LEfSe identification of several members to be significant biomarkers for the surface (cluster 1) and mesopelagic/ bathypelagic (cluster 2) layers (Fig. 5), suggesting differential environmental preferences. The ASVs in cluster 3 were mostly annotated as members of the genus *Aurantiochytrium*. Statistically, this genus (Fig. 2) and these ASVs were rarely observed in the epipelagic and mesopelagic layers but frequently found in the bathypelagic (Fig. 5, Fig. S1), with half of the members showing a significantly higher abundance in the bathypelagic ocean compared to other depths (LDA > 2, $p < 0.05$), suggesting that they represent potential deep-sea specialists. Conversely, the ASVs in clusters 4 and 5 had the highest relative abundance in surface water samples (Figs 5 and S1), with nearly all ASVs within cluster 4 identified as surface water biomarkers (Fig. 5). Moreover, most of the ASVs in clusters 4 and 5 were also found in the bathypelagic layer, but rarely in the intervening mid-ocean depths. These ASVs could be adapted to the distinct surface and bathypelagic environments or could represent sub-ASV level niche differentiation. Alternatively, the ASVs in cluster 4 and cluster 5 might sink rapidly from the surface to the bathypelagic layer such that they were not captured in water samples from the middle layers, in which case,

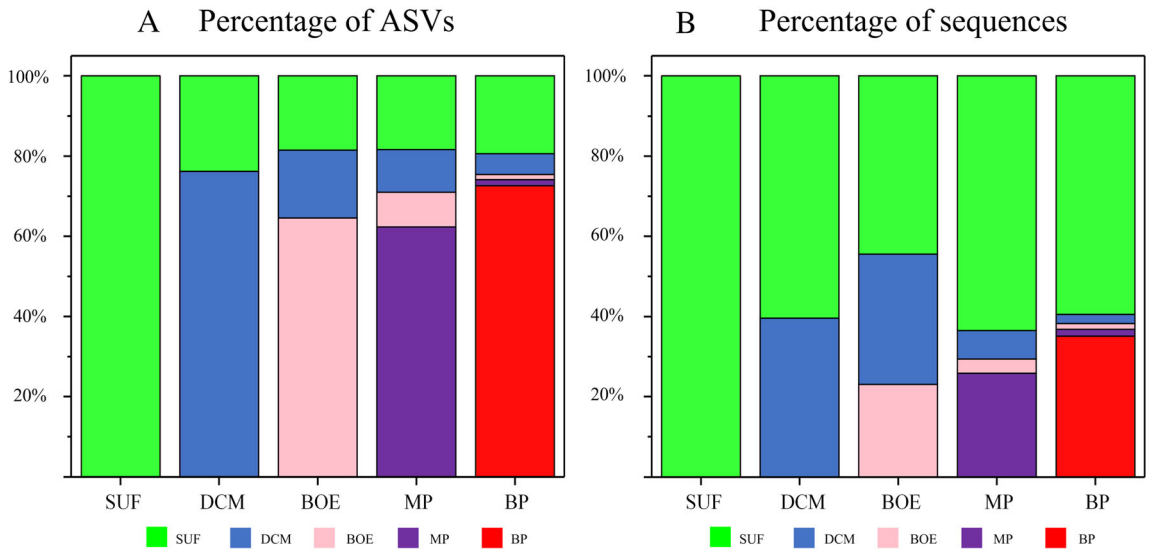

Fig. 4. Contribution of the Labyrinthulomycetes ASVs that were first detected at an upper layer (colour bar) to the communities at certain deeper layers (*y*-axis), based on the assumption of strictly downward mobility for the Labyrinthulomycetes protists. The contribution is expressed in percentages of shared ASV number (A) and shared sequence abundance (B). SUF: surface layer (5 m); DCM: deep chlorophyll maximum layer (75 m); BOE: bottom of the euphotic layer (200 m); MP: mesopelagic layer (500 m); BP: bathypelagic layer (1000 or 2000 m). [Color figure can be viewed at wileyonlinelibrary.com]

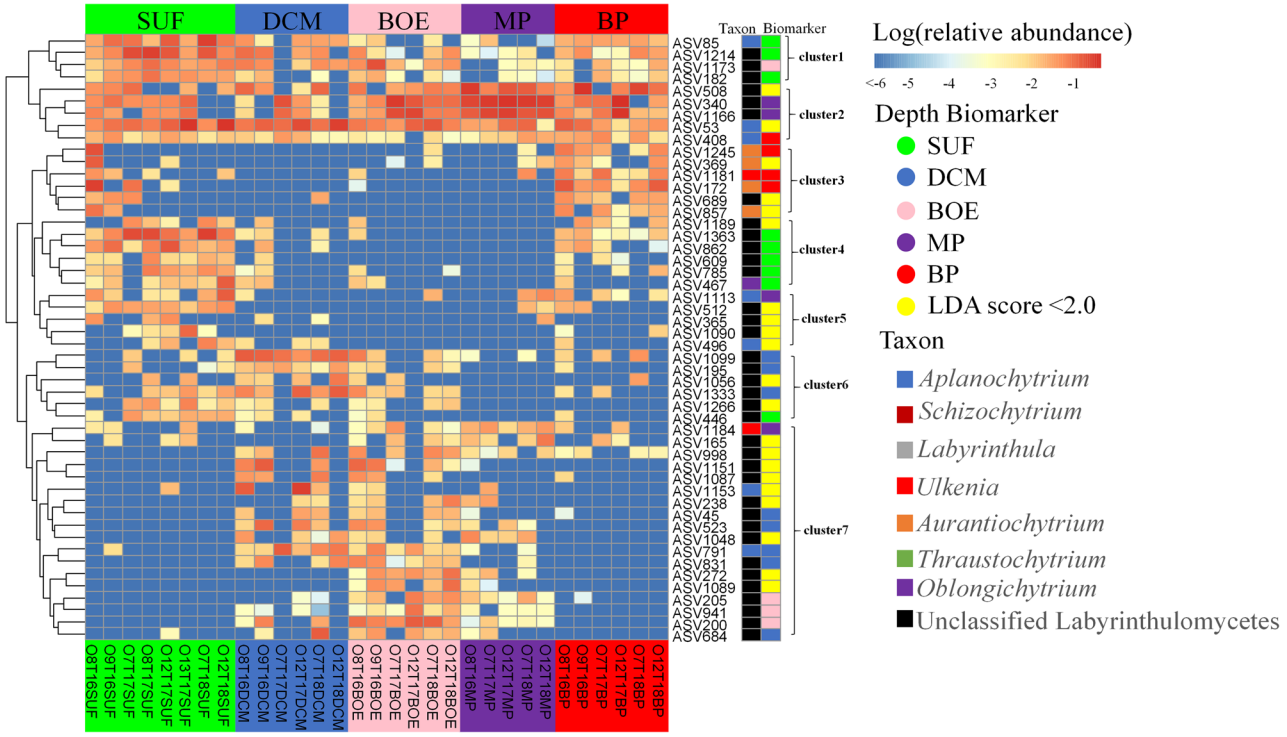

Fig. 5. Heatmap showing the distribution of the 50 most abundant Labyrinthulomycetes ASVs across different depths and stations. The ASVs (*y*-axis) were clustered by similarity of distribution patterns and annotated with taxonomic classification at the genus level and LEfSe identification results (depth biomarkers). The samples (*x*-axis) were arranged by sampling depths, years and stations and labelled with 'station + year + depth' below the heatmap columns. Different colours were used to distinguish the sampling depths and corresponding biomarkers. SUF (labelled green): surface layer (5 m); DCM (blue): deep chlorophyll maximum layer (75 m); BOE (pink): bottom of the euphotic layer (200 m); MP (purple): mesopelagic layer (500 m); BP (red): bathypelagic layer (1000 or 2000 m). [Color figure can be viewed at wileyonlinelibrary.com]

these ASVs could contribute to the biological pump. ASVs in clusters 6 and 7 were generally most abundant at the deep chlorophyll maximum and at the bottom of the euphotic zone but also were found in the mesopelagic zone (Figs 5 and S1). Overall, the vertical partitioning of the most abundant 50 ASVs suggested the potential involvement of the LP in export of surface POM into the deep ocean and also revealed distinct ecological niches of the LP in the pelagic ocean.

Discussion

Biomass importance and niche partitioning of the pelagic Labyrinthulomycetes

Over the past few decades, the LP have been well documented as the dominant nutrient-mineralizing eukaryotic decomposers whose biomass can often exceed that of bacterioplankton in coastal marine ecosystems (Kimura *et al*., 1999; Kimura and Naganuma, 2001; Ueda *et al*., 2015; Liu *et al*., 2017; Xie *et al*., 2018; Liu *et al*., 2019), but relatively few efforts have been made to examine their distribution and ecological importance in the open ocean, especially in bathypelagic waters (Raghukumar *et al*., 2001; Damare and Raghukumar, 2008; Li *et al*., 2013). This study reveals their persistence in the pelagic waters of the SCS, with abundance consistent with previous reports for other oceanic regions. In the pelagic waters of Mid Pacific Ocean, Arabian Sea, Equatorial Indian Ocean, and Greenland and Norwegian Seas, LP abundance was reported to reach up to $>10^5$ cells L^{-1} with the estimated biomass rivalling and occasionally exceeding that of the bacterioplankton (Raghukumar *et al*., 2001; Damare and Raghukumar, 2008; Li *et al*., 2013). Recently, the LP (along with fungi) were reported to dominate biomass on bathypelagic marine snow (Bochdansky *et al*., 2017) and were identified as the principal contributor to the heterotrophic eukaryotic rRNA sequences in the surface sediment of the East Pacific Ocean (Rodríguez-Martínez *et al*., 2020). These results align with our findings of free-living LP biomass rivalling the prokaryotic biomass in deep waters of the SCS (Fig. 1), and thus the existing evidence seems to support that the LP are a major component of microbial organic carbon of pelagic oceans at depth.

Furthermore, we observed relatively consistent abundance of LP with depth throughout the pelagic water

column (Fig. 1): unlike the over 10-fold decrease of bacterioplankton abundance with depth and depth-related decreases in mostly smaller and phagotrophic protists (Arístegui *et al*., 2009; Pernice *et al*., 2015), the abundance of the LP only displayed minimal decrease through epi-, meso- and bathy-pelagic layers (Fig. 1). The increased relative importance of the LP in the deep ocean suggests an ability to process recalcitrant organic matter at cold temperatures. While previous research has identified LP phylotypes' preferences for specific seasons and nearshore/offshore habitats (Ueda *et al*., 2015; Xie *et al*., 2021), here vertical partitioning patterns of the LP seem to divide LP ASVs into multiple depth-associated ecotypes (Fig. 5) such as the 'ubiquitous' (clusters 1 and 2), 'bathypelagic-layer favoured' (cluster 3), 'mid-depths disfavoured' (clusters 4 and 5) and 'mid-depths favoured' (clusters 6 and 7). The depth distributions of potential ecotypes may shed light on the ecological roles of LP in pelagic waters, but additional research is required to understand their specific niches and functions. Future work should focus on comparing the genomes and functional genes of the different ecotypes in pelagic waters.

Overall, our observations in the pelagic waters of the SCS complement previous work characterizing LP communities in the coastal oceans and reveal potentially important roles of this nutrient-mineralizing heterotrophic protistan taxon in the pelagic ecosystems that are moderated by niche partitioning through different ASV ecotypes.

Potential dual roles of Labyrinthulomycetes in oceanic carbon sequestration

The pelagic ocean is the largest habitat and a key site for long-term carbon storage in the biosphere (Arístegui *et al*., 2009). Our findings suggest a significant contribution of the LP to biological carbon stocks in the dark ocean, potentially through facilitating sinking organic particles formation in the surface oceans and/or *in situ* production. The similarities between surface and the bathypelagic populations support this hypothesis. This hypothesis also concurs with recent reports that the LP are a major component in the communities of bathypelagic marine snow and deep-sea sediments (Bochdansky *et al*., 2017; Rodríguez-Martínez *et al*., 2020). Most of the LP have been reported to be able to secrete EPSs and use their ectoplasmic nets to attach to detritus and living microbes, and thus can potentially facilitate aggregation of small organic particles to enhance the formation of large fast-sinking particles (Li *et al*., 2013; Hamamoto and Honda, 2019). Although the single vegetative cell of *Aplanochytrium* is 5–10 μm in diameter, data of TARA Ocean (https://www.embl.de/tara/) revealed high occurrence of this genus in particles larger than 20 μm, which also aligns with LP facilitation of particle formation (Hamamoto and Honda, 2019). In the pelagic ocean, these larger particles can reach the deep sea within days or weeks because of sinking velocities one order of magnitude higher than those of individual plankton (Christina and Passow, 2007). Thus, several lines of evidence identify LP as an integral component of the biological pump and a likely contributor to vertical carbon export.

However, as an important decomposer of marine detritus, the saprophytic LP could influence long-term carbon storage in more complex ways. LP blooms have been observed in microcosms and in the coastal waters of the Bohai Sea following a peak in bacterioplankton abundance (Xie *et al*., 2018). Likewise, their co-occurrence with bacteria-derived/colonized TEPs has been detected in the Equatorial Indian Ocean after the abundance of bacterioplankton surges (Damare and Raghukumar, 2008). Thus, the LP have been hypothesized to utilize organic resources left over by the heterotrophic bacteria (Damare and Raghukumar, 2008; Raghukumar and Damare, 2011; Xie *et al*., 2018). However, both recent field and laboratory results along with genomic evidence seem to support that some strains of the LP can function as initial decomposers for detritus including refractory lipids and cellulosic carbohydrates (Raghukumar *et al*., 1995; Bongiorni *et al*., 2005; Damare and Raghukumar, 2006; Taoka *et al*., 2009; Nagano *et al*., 2011; Song *et al*., 2018; Xie *et al*., 2021). In coastal waters, some LP phylotypes tend to bloom with fungi or eukaryotic algae, but a few dominant phylotypes are persistently abundant and correlated with bacterioplankton (Xie *et al*., 2021). In particular, the high abundance of ribosomally active LP in deep-sea sediments is consistent with their potential role as nutrient-mineralizing degraders of sinking and buried POM, which might enhance the remineralization of organic matter and reduce carbon sequestration in the deep ocean (Rodríguez-Martínez *et al*., 2020). Therefore, the LP are likely to play dual roles in the ocean carbon cycle and their net ecological effect on carbon sequestration in the pelagic ocean is still in need of further investigation.

While their potentially unique functionality is unclear, LP ecotypes partitioned with distinct vertical patterns suggesting their differential roles in carbon biogeochemical processes with potential influence on the biological pump. For example, the 'bathypelagic-layer favoured' and 'mid-depths favoured' LP ASV groups could grow *in situ* and may mainly contribute to the remineralization of organic matter in the dark ocean. However, the 'mid-depths disfavored' group, which were abundant in both the surface and bathypelagic layers but generally absent in the middle, suggests potentially rapid sinking through the water column and accumulation in the deep waters, and thus may significantly contribute to the biological

pump. Compared to the suspended and slowly sinking particles, the fast-sinking particles are usually more difficult to capture during seawater sampling, but the LP sinking speed and its effect on LP detection frequency at different depths await future verification using modelling approaches. An alternative possible explanation for the rare detection of this ecotype in the middle layers is excessive predation by deep-euphotic and mesopelagic zooplankton, in which case these LP populations could play an important role in supporting the nutrient and carbon flows through the food chains. Apparent micro/mesozooplankton predation on the LP cells has been observed in the pelagic ocean (Damare and Raghukumar, 2010; Damare and Raghukumar, 2015); many of the potential predators (e.g. ciliates, copepods) can peak in abundance around the deep chlorophyll maximum layer and remain non-negligible through the mesopelagic (Koppelmann and Frost, 2008; Wang *et al*., 2019), which may result in strong top-down control on the specific LP populations. Additionally, high abundance of this LP ecotype in the surface and bathypelagic waters could be attributed to potential survival strategies as K-selected specialists in the environments with limited organic resources that can be created by intense competition at the surface or by the 'picked-over' conditions of carbon in the bathypelagic layer. Finally, the 'ubiquitous' group could play more complex roles in carbon cycling and sequestration, potentially as free-living, slowly sinking generalists. Overall, along with the results of previous studies on ecological and metabolic traits of the LP, our results suggest that this nutrient-mineralizing protistan taxon can play dual roles in the long-term carbon storage of the pelagic ocean, i.e. facilitating POM sinking from the upper layers and promoting the remineralization of organic matter in the dark ocean.

Dominance of the genus Aplanochytrium

It is worth noting that this study for the first time confirmed the dominance of the genus *Aplanochytrium* and its universal presence in pelagic waters (Figs 2 and 5). Members of this genus have long been noted to be the dominant LP populations in coastal waters (Collado-Mercado *et al*., 2010; Liu *et al*., 2017; Xie *et al*., 2018; Bai *et al*., 2019). Of the 50 most abundant ASVs, seven *Aplanochytrium* ASVs accounted for 50% of the sequence tags that were annotated at the genus level and showed distinct vertical distributions (Fig. 5, clusters 1, 2, 5 and 7). Their multiple nutritional models further suggest the potential significance of this genus in the pelagic ocean carbon sequestration. Some members of *Aplanochytrium* have been isolated from decaying plant materials and can secrete cellulase (Bower, 1986; Nagano *et al*., 2011), while other strains are reported to live as parasites by consuming invertebrates (Burge *et al*., 2013). Some strains can directly live on algae (Sathe-Pathak *et al*., 1993; Hamamoto and Honda, 2019), which may explain its relatively higher abundance in the epipelagic compared to the meso- and bathypelagic zones (Fig. 2).

Conclusions

The biological pump contributes significantly to exporting organic carbon from the surface to depth for long-term storage in the pelagic ocean. However, the significance of nutrient-mineralizing microbial eukaryotes in this critical oceanic process remains poorly understood. Results of this study indicated that the LP, a group of marine unicellular osmoheterotrophic protistan microbes, displayed a relatively constant abundance profile through the surface to the bathypelagic layers in the SCS (Fig. 1). The biomass of the LP was less than 20% of the prokaryotic biomass in the upper epipelagic layers, but close to and/or higher than that of prokaryotes in the mesopelagic and bathypelagic layers respectively. Surface and bathypelagic layers shared a large number of LP ASVs, further supporting the potential role of LP in the carbon exporting processes. The genus *Aplanochytrium* was reported for the first time to be the predominant group of LP in pelagic waters. Together with previous reports, our results provide a critical piece of evidence to support the potentially important roles of the LP in carbon export from the surface to the dark ocean, beyond their well-known functions to decompose organic matter. Further investigation on their abundance and community structure in different size fractions from small to large particles would help to unravel the ecological and biogeochemical functions of LP in this important oceanic carbon storage process. Complementary analyses (e.g. sediment traps, metatranscriptomic sequencing, stable isotope probing and mesocosm experiments) of the different LP ecotypes would help to unravel their carbon metabolism dynamics across vertical profiles of the pelagic ocean.

Experimental procedures

Seawater sampling

Seawater samples were retrieved by the Sea Bird CTD rosette sampler during three summer cruises on the SCS in May–July 2016, June–August 2017 and June–August 2018 (Fig. S2; Table S4). Water samples were collected from epipelagic layer (5, 25, 50, 75 m), mesopelagic layer (200, 500 m) and bathypelagic layer (1000, 2000 m). For the flow cytometric (FCM) analysis of the LP, 4 ml of seawater was transferred into triplicate 5-ml cryovials, fixed with 0.22 μm filtered formaldehyde (2% final

concentration) and incubated for 3 h at 4°C (Duan *et al*., 2018; Xie *et al*., 2018). Another 1.5 ml of seawater was transferred into a 2-ml cryovial in triplicate, fixed with 0.22 μm filtered glutaraldehyde (0.5% final concentration) and incubated for 15 min at 4°C for the FCM analysis of prokaryotic plankton (Gasol and Del Giorgio, 2000). For investigation of the molecular diversity and community structure of the LP, 2 L of water samples were filtered through 0.22 μm polycarbonate Isopore Membrane Filters (Millipore, USA) and the resulting filters were stored at −80°C until the DNA extraction.

Abundance and biomass determination of Labyrinthulomycetes and prokaryotes

To determine their abundance, the LP and prokaryotic plankton were stained with acriflavine solution (0.5 g L^{-1} final concentration) and SYBR-I Green solution (1:500 dilution; Molecular Probes, USA) respectively, and counted with a FACS Calibur flow cytometer (BD-Biosciences, USA) following the procedures described in previous studies (Duan *et al*., 2018; Xie *et al*., 2018). Yellow-green fluorescent polystyrene latex beads (Molecular Probes) were added to individual FCM sample as an internal standard. A cell biomass value of 2.06×10^{-11} g carbon was used for estimating the LP biomass (Kimura *et al*., 1999). Oceanic prokaryotic plankton biomass was estimated on a value of 1.24×10^{-14} g of carbon per cell (Fukuda *et al*., 1998).

DNA extraction and high-throughput sequencing

To understand the community composition of the LP in different pelagic layers, eight samples from the surface layer (5 m) (SUF group), six samples from the deep chlorophyll maximum layer (75 m) (DCM group), six samples from the bottom of the euphotic layer (200 m) (BOE group), five samples from the mesopelagic layer (500 m) (MP group) and six samples from the bathypelagic layer (1000 m depth of Cruise 2016, 2000 m depth of Cruise 2017 and Cruise 2018) (BP group) were selected and subjected to high-throughput sequencing analysis (Table S5). The total DNA of these samples was extracted using E.Z.N.ATM Water DNA kit (OMEGA, USA), and the resulting DNA was suspended in 100 μl of sterile Milli-Q water. The quality of the total genomic DNA was monitored using a NanoDrop (Thermo Scientific, USA). The barcode specific primer set for the LP 18S rRNA gene, LABY-A (5′-GGGATCGAAGATGATTAG-3′) and LABY-Y (5′-CWCRAACTTCCTTCCGGT-3′), was used for the high-throughput sequencing analysis (Stokes *et al*., 2002). PCR reactions contained 20 ng of template DNA and 0.5 U DNA polymerase (Takara Bio, Japan) as well as a final concentration of 200 μM dNTPs, 2 mM $MgCl_2$ and 10 μM of each barcoding primer. The PCR reactions were thermo-cycled using the following protocol: 95°C for 5 min; 35 cycles of 60 s at 95°C, 60 s at 52°C and 60 s at 72°C; with a final extension at 72°C for 20 min (Liu *et al*., 2017). Triplicate PCR products for each sample were combined, purified with TIANquick Midi Purification Kit (Tiangen, China), then quantified with NanoDrop and sequenced on the Illumina Miseq platform at Allwegene Tech Company (Beijing, China). The raw sequences were deposited in NCBI under the BioProject PRJNA723995.

Sequence processing and bioinformatic analysis

The raw sequence reads were initially checked with FastQC software (version 0.11.5) in QIIME 1.8 (Caporaso *et al*., 2010). Barcodes, primers and low-quality ($Q < 20$) ends were trimmed off from the sequences. Then the DADA2 pipeline in QIIME 2 platform was used to remove potential chimaeras, denoise sequencing errors and resolve the remaining sequences into amplicon sequence variants (ASVs) (Callahan *et al*., 2016; Bolyen *et al*., 2019). Singleton ASVs were removed because they might be produced by sequencing errors. Based on the SILVA SSU rRNA database, ASVs were annotated using SilVAngs (version 1.9.3) (Quast *et al*., 2012). Non-Labyrinthulomycetes ASVs were then excluded from the feature table. Finally, a total of 972 554 sequences (73.3% of the non-singleton, quality-controlled sequences) were assigned to LP and retained for downstream analyses.

Statistical analysis

Alpha diversity indexes for each sample were calculated in QIIME 2, with an even rarefaction depth at 7900 sequences per sample (the least sequence number across all samples). NMDS analysis based on the Bray–Curtis distance was performed with the vegan package using R 3.4.2. Because of the non-normal distribution, the Kruskal–Wallis test was used to calculate significant differences of abundance, biomass and diversity indexes among different depths. Adonis analysis and pairwise permutation MANOVAs were applied to test for community differences across different depths after equal beta-dispersal was confirmed. The LEfSe analysis was conducted to identify biomarkers among the most abundant 50 ASV for different water layers, using the 'all-against-all' strategy, with alpha values for the factorial Kruskal–Wallis test among classes <0.05 and logarithmic LDA scores >2 considered as significant (Segata *et al*., 2011; Jalili *et al*., 2020).

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References

Arístegui, J., Gasol, J.M., Duarte, C.M., and Herndld, G.J. (2009) Microbial oceanography of the dark ocean's pelagic realm. *Limnol Oceanogr* **54**: 1501–1529.

Arrigo, K.R. (2007) Marine manipulations. *Nature* **450**: 491–492.

Bai, M., Sen, B., Wang, Q., Xie, Y., He, Y., and Wang, G. (2019) Molecular detection and spatiotemporal characterization of Labyrinthulomycete protist diversity in the coastal waters along the Pearl River Delta. *Microb Ecol* **77**: 394–405.

Bochdansky, A.B., Clouse, M.A., and Herndl, G.J. (2017) Eukaryotic microbes, principally fungi and labyrinthulomycetes, dominate biomass on bathypelagic marine snow. *ISME J* **11**: 362–373.

Boeuf, D., Edwards, B.R., Eppley, J.M., Hu, S.K., Poff, K.E., Romano, A.E., *et al*. (2019) Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean. *Proc Natl Acad Sci U S A* **116**: 11824–11832.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., *et al*. (2019) Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**: 852–857.

Bongiorni, L., and Dini, F. (2002) Distribution and abundance of thraustochytrids in different Mediterranean coastal habitats. *Aquat Microb Ecol* **30**: 49–56.

Bongiorni, L., Pusceddu, A., and Danovaro, R. (2005) Enzymatic activities of epiphytic and benthic thraustochytrids involved in organic matter degradation. *Aquat Microb Ecol* **41**: 299–305.

Bower, S.M. (1986) *Labyrinthuloides haliotidis* n.sp. (protozoa: Labyrinthomorpha), a pathogenic parasite of small juvenile abalone in a British Columbia mariculture facility. *Can J Zool* **65**: 1996–2007.

Burge, C.A., Mouchka, M.E., Harvell, C.D., and Roberts, S. (2013) Immune response of the Caribbean sea fan, *Gorgonia ventalina*, exposed to an *Aplanochytrium* parasite as revealed by transcriptome sequencing. *Front Physiol* **4**: 180.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**: 581–583.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al*. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–336.

Cho, B.C., and Azam, F. (1988) Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* **332**: 441–443.

Christina, L., and Passow, U. (2007) Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep Sea Res (II Top Stud Oceanogr)* **54**: 639–658.

Collado-Mercado, E., Radway, J.C., and Collier, J.L. (2010) Novel uncultivated labyrinthulomycetes revealed by 18S rDNA sequences from seawater and sediment samples. *Aquat Microb Ecol* **58**: 215–228.

Collins, J.R., Edwards, B.R., Thamatrakoln, K., Ossolinski, J. E., DiTullio, G.R., Bidle, K.D., *et al*. (2015) The multiple fates of sinking particles in the North Atlantic Ocean. *Global Biogeochem Cycles* **29**: 1471–1494.

Crespo, B.G., Pommier, T., Fernández-Gómez, B., and Pedrós-Alió, C. (2013) Taxonomic composition of the particle-attached and free-living bacterial assemblages in the Northwest Mediterranean Sea analyzed by pyrosequencing of the 16S rRNA. *MicrobiologyOpen* **2**: 541–552.

Damare, V., and Raghukumar, S. (2006) Morphology and physiology of the marine straminipilan fungi, the aplanochytrids isolated from the equatorial Indian Ocean. *Indian J Genet Plant Breed* **35**: 326–340.

Damare, V., and Raghukumar, S. (2008) Abundance of thraustochytrids and bacteria in the equatorial Indian Ocean, in relation to transparent exopolymeric particles (TEPs). *FEMS Microbiol Ecol* **65**: 40–49.

Damare, V., and Raghukumar, S. (2010) Association of the stramenopilan protists, the aplanochytrids, with zooplankton of the equatorial Indian Ocean. *Mar Ecol Prog Ser* **399**: 53–68.

Damare, V.S., and Raghukumar, S. (2015) Apparent grazing losses of Labyrinthulomycetes protists in oceanic and coastal waters: an experimental elucidation. *Ecol Res* **30**: 403–414.

DeLong, E.F., Franks, D.G., and Alldredge, A.L. (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnol Oceanogr* **38**: 924–934.

Duan, Y., Sen, B., Xie, N., Paterson, J.S., Chen, Z., and Wang, G. (2018) Flow cytometry for rapid enumeration and biomass quantification of thraustochytrids in coastal seawaters. *Microbes Environ* **33**: 195–204.

Duret, M.T., Lampitt, R.S., and Lam, P. (2020) Eukaryotic influence on the oceanic biological carbon pump in the Scotia Sea as revealed by 18S rRNA gene sequencing of suspended and sinking particles. *Limnol Oceanogr* **65**: S49–S70.

Fang, J., Zhang, L., Li, J., Kato, C., Tamburini, C., Zhang, Y., *et al*. (2015) The POM-DOM piezophilic microorganism continuum (PDPMC)-the role of piezophilic microorganisms in the global ocean carbon cycle. *Sci China Earth Sci* **58**: 106–115.

Fukuda, R., Ogawa, H., Nagata, T., and Koike, I. (1998) Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Appl Environ Microbiol* **64**: 3352–3358.

Gasol, J.M., and Del Giorgio, P.A. (2000) Using flow cytometry for counting natural planktonic bacteria and

understanding the structure of planktonic bacterial communities. *Sci Mar* **64**: 197–224.

Hamamoto, Y., and Honda, D. (2019) Nutritional intake of *Aplanochytrium* (Labyrinthulea, Stramenopiles) from living diatoms revealed by culture experiments suggesting the new prey–predator interactions in the grazing food web of the marine ecosystem. *PLoS One* **14**: e0208941.

Henson, S.A., Sanders, R., and Madsen, E. (2012) Global patterns in efficiency of particulate organic carbon export and transfer to the deep ocean. *Global Biogeochem Cycles* **26**: GB1028.

Hughes, R., Potouroglou, M., Ziauddin, Z., and Nicholls, J. (2018) Seagrass wasting disease: nitrate enrichment and exposure to a herbicide (Diuron) increases susceptibility of *Zostera marina* to infection. *Mar Pollut Bull* **134**: 94–98.

Jalili, V., Afgan, E., Gu, Q., Clements, D., Blankenberg, D., Goecks, J., *et al*. (2020) The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2020 update. *Nucleic Acids Res* **48**: 8205–8207.

Jardillier, L., Zubkov, M.V., Pearman, J., and Scanlan, D.J. (2010) Significant CO_2 fixation by small prymnesiophytes in the subtropical and tropical northeast Atlantic Ocean. *ISME J* **4**: 1180–1192.

Jiao, N., Herndl, G.J., Hansell, D.A., Benner, R., Kattner, G., Wilhelm, S.W., *et al*. (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. *Nat Rev Microbiol* **8**: 593–599.

Jiao, N., Robinson, C., Azam, F., Thomas, H., Baltar, F., Dang, H., *et al*. (2014) Mechanisms of microbial carbon sequestration in the ocean–future research directions. *Biogeosciences* **11**: 5285–5306.

Kimura, H., Fukuba, T., and Naganuma, T. (1999) Biomass of thraustochytrid protoctists in coastal water. *Mar Ecol Prog Ser* **189**: 27–33.

Kimura, H., and Naganuma, T. (2001) Thraustochytrids: a neglected agent of the marine microbial food chain. *Aquat Ecosyst Health Manag* **4**: 13–18.

Kiørboe, T., and Jackson, G.A. (2001) Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. *Limnol Oceanogr* **46**: 1309–1318.

Koppelmann, R., and Frost, J. (2008) The ecological role of zooplankton in the twilight and dark zones of the ocean. In *Biological Oceanography Research Trends*, Mertens, L.P. (ed). New York: Nova Science Publishers, pp. 67–130.

Legendre, L., and Le Fèvre, J. (1995) Microbial food webs and the export of biogenic carbon in oceans. *Aquat Microb Ecol* **9**: 69–77.

Legendre, L., and Rassoulzadegan, F. (1996) Food-web mediated export of biogenic carbon in oceans: hydrodynamic control. *Mar Ecol Prog Ser* **145**: 179–193.

Legendre, L., Rivkin, R.B., Weinbauer, M.G., Guidi, L., and Uitz, J. (2015) The microbial carbon pump concept: potential biogeochemical significance in the globally changing ocean. *Prog Oceanogr* **134**: 432–450.

Li, Q., Wang, X., Liu, X., Jiao, N., and Wang, G. (2013) Abundance and novel lineages of thraustochytrids in Hawaiian waters. *Microb Ecol* **66**: 823–830.

Liu, X., Sen, B., Zhao, Y., Bai, M., He, Y., Xie, Y., *et al*. (2019) Gradients of three coastal environments off the South China Sea and their impacts on the dynamics of heterotrophic microbial communities. *Sci Total Environ* **659**: 499–506.

Liu, Y., Singh, P., Liang, Y., Li, J., Xie, N., Song, Z., *et al*. (2017) Abundance and molecular diversity of thraustochytrids in coastal waters of southern China. *FEMS Microbiol Ecol* **93**: fix070.

López-Pérez, M., Gonzaga, A., Martin-Cuadrado, A.-B., Onyshchenko, O., Ghavidel, A., Ghai, R., and Rodriguez-Valera, F. (2012) Genomes of surface isolates of *Alteromonas macleodii*: the life of a widespread marine opportunistic copiotroph. *Sci Rep* **2**: 696.

Mestre, M., Borrull, E., Sala, M.M., and Gasol, J.M. (2017) Patterns of bacterial diversity in the marine planktonic particulate matter continuum. *ISME J* **11**: 999–1010.

Nagano, N., Matsui, S., Kuramura, T., Taoka, Y., Honda, D., and Hayashi, M. (2011) The distribution of extracellular cellulase activity in marine eukaryotes, thraustochytrids. *Mar Biotechnol* **13**: 133–136.

Naganuma, T., and Miura, S. (1997) Abundance, production and viability of bacterioplankton in the Seto Inland Sea, Japan. *J Oceanogr* **53**: 435–442.

Naganuma, T., Takasugi, H., and Kimura, H. (1998) Abundance of thraustochytrids in coastal plankton. *Mar Ecol Prog Ser* **162**: 105–110.

Passow, U., and Carlson, C.A. (2012) The biological pump in a high CO_2 world. *Mar Ecol Prog Ser* **470**: 249–271.

Pernice, M.C., Forn, I., Gomes, A., Lara, E., Alonso-Sáez, L., Arrieta, J.M., *et al*. (2015) Global abundance of planktonic heterotrophic protists in the deep ocean. *ISME J* **9**: 782–792.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., *et al*. (2012) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**: D590–D596.

Ragan, M.A., MacCallum, G.S., Murphy, C.A., Cannone, J.J., Gutell, R.R., and McGladdery, S.E. (2000) Protistan parasite QPX of hard-shell clam *Mercenaria mercenaria* is a member of Labyrinthulomycota. *Dis Aquat Organ* **42**: 185–190.

Raghukumar, C. (1986) Fungal parasites of the marine green algae, *Cladophora* and *Rhizoclonium*. *Bot Mar* **29**: 289–297.

Raghukumar, S. (1992) Bacterivory: a novel dual role for thraustochytrids in the sea. *Mar Biol* **113**: 165–169.

Raghukumar, S. (2002) Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). *Eur J Protistol* **38**: 127–145.

Raghukumar, S., and Damare, V.S. (2011) Increasing evidence for the important role of Labyrinthulomycetes in marine ecosystems. *Bot Mar* **54**: 3–11.

Raghukumar, S., Ramaiah, N., and Raghukumar, C. (2001) Dynamics of thraustochytrid protists in the water column of the Arabian Sea. *Aquat Microb Ecol* **24**: 175–186.

Raghukumar, S., Sathe-Pathak, V., Sharma, S., and Raghukumar, C. (1995) Thraustochytrid and fungal component of marine detritus. III. Field studies on decomposition of leaves of the mangrove *Rhizophora apiculata*. *Aquat Microb Ecol* **9**: 117–125.

Rodríguez-Martínez, R., Leonard, G., Milner, D.S., Sudek, S., Conway, M., Moore, K., *et al*. (2020) Controlled sampling of ribosomally active protistan diversity in sediment-surface layers identifies putative players in the marine carbon sink. *ISME J* **14**: 984–998.

Sanders, R., Henson, S.A., Koski, M., De La Rocha, C.L., Painter, S.C., Poulton, A.J., *et al*. (2014) The biological carbon pump in the North Atlantic. *Prog Oceanogr* **129**: 200–218.

Sathe-Pathak, V., Raghukumar, S., Raghukumar, C., and Sharma, S. (1993) Thraustochytrid and fungal component of marine detritus. 1. Field studies on decomposition of the brown alga *Sargassum cinereum* J. Ag. *Indian J Genet Plant Breed* **22**: 159–167.

Schärer, L., Knoflach, D., Vizoso, D.B., Rieger, G., and Peintner, U. (2007) Thraustochytrids as novel parasitic protists of marine free-living flatworms: *Thraustochytrium caudivorum* sp. nov. parasitizes *Macrostomum lignano*. *Mar Biol* **152**: 1095–1104.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C. (2011) Metagenomic biomarker discovery and explanation. *Genome Biol* **12**: R60.

Simon, M., Grossart, H.-P., Schweitzer, B., and Ploug, H. (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat Microb Ecol* **28**: 175–211.

Singh, P., Liu, Y., Li, L., and Wang, G. (2014) Ecological dynamics and biotechnological implications of thraustochytrids from marine habitats. *Appl Microbiol Biotechnol* **98**: 5789–5805.

Smith, D.C., Simon, M., Alldredge, A.L., and Azam, F. (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature* **359**: 139–142.

Song, Z., Stajich, J.E., Xie, Y., Liu, X., He, Y., Chen, J., *et al*. (2018) Comparative analysis reveals unexpected genome features of newly isolated Thraustochytrids strains: on ecological function and PUFAs biosynthesis. *BMC Genomics* **19**: 541.

Steinberg, D.K., Van Mooy, B.A., Buesseler, K.O., Boyd, P. W., Kobari, T., and Karl, D.M. (2008) Bacterial vs. zooplankton control of sinking particle flux in the ocean's twilight zone. *Limnol Oceanogr* **53**: 1327–1338.

Stokes, N., Calvo, L.R., Reece, K.S., and Burreson, E. (2002) Molecular diagnostics, field validation, and phylogenetic analysis of quahog parasite unknown (QPX), a pathogen of the hard clam *Mercenaria mercenaria*. *Dis Aquat Organ* **52**: 233–247.

Sullivan, B.K., Sherman, T.D., Damare, V.S., Lilje, O., and Gleason, F.H. (2013) Potential roles of *Labyrinthula* spp. in global seagrass population declines. *Fungal Ecol* **6**: 328–338.

Taoka, Y., Nagano, N., Okita, Y., Izumida, H., Sugimoto, S., and Hayashi, M. (2009) Extracellular enzymes produced by marine eukaryotes, thraustochytrids. *Biosci Biotechnol Biochem* **73**: 180–182.

Ueda, M., Nomura, Y., Doi, K., Nakajima, M., and Honda, D. (2015) Seasonal dynamics of culturable thraustochytrids (Labyrinthulomycetes, Stramenopiles) in estuarine and coastal waters. *Aquat Microb Ecol* **74**: 187–204.

Wang, C., Li, H., Zhao, L., Zhao, Y., Dong, Y., Zhang, W., and Xiao, T. (2019) Vertical distribution of planktonic ciliates in the oceanic and slope areas of the western Pacific Ocean. *Deep Sea Res (II Top Stud Oceanogr)* **167**: 70–78.

Worden, A.Z., Follows, M.J., Giovannoni, S.J., Wilken, S., Zimmerman, A.E., and Keeling, P.J. (2015) Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science* **347**: 1257594.

Xie, N., Hunt, D.E., Johnson, Z.I., He, Y., and Wang, G. (2021) Annual partitioning patterns of Labyrinthulomycetes protists reveal their multifaceted role in marine microbial food webs. *Appl Environ Microbiol* **87**: e01652-01620.

Xie, N., Sen, B., Song, Z., Zhao, Y., Chen, Z., Shi, W., *et al*. (2018) High phylogenetic diversity and abundance pattern of Labyrinthulomycete protists in the coastal waters of the Bohai Sea. *Environ Microbiol* **20**: 3042–3056.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supporting Information.