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Gazing into the abyss: A glimpse into the diversity, distribution, and behaviour of heterotrophic protists from the deep-sea floor

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Abstract

The benthic biome of the deep-sea floor, one of the largest biomes on Earth, is dominated by diverse and highly productive heterotrophic protists, second only to prokaryotes in terms of biomass. Recent evidence suggests that these protists play a significant role in ocean biogeochemistry, representing an untapped source of knowledge. DNA metabarcoding and environmental sample sequencing have revealed that deep-sea abyssal protists exhibit high levels of specificity and diversity across local regions. This review aims to provide a comprehensive summary of the known heterotrophic protists from the deep-sea floor, their geographic distribution, and their interactions in terms of parasitism and predation. We offer an overview of the most abundant groups and discuss their potential ecological roles. We argue that the exploration of the biodiversity and species-specific features of these protists should be integrated into broader deep-sea research and assessments of how benthic biomes may respond to future environmental changes.

INTRODUCTION

Historically, the deep-sea floor was considered to be a monotonous habitat characterized by stable environmental conditions and (very) low species diversity (Dayton and Hessler, 1972). However, recent advances in molecular tools and approaches for studying microbial communities, as well as the increasing availability of a range of deep-sea vehicles and collection methods have revealed that deep-sea benthic biomes harbour an intriguingly vast diversity of unicellular eukaryotes, dominated by heterotrophic protists (e.g. Cordier et al., 2022; V. Edgcomb et al., 2009; Pawlowski, Christen, et al., 2011; Scheckenbach et al., 2010). These microeukaryotes are known to play important roles in driving primary and secondary

production, nutrient recycling, and carbon cycling, which are critical processes for the functioning of marine ecosystems (Worden et al., 2015). Barotolerant or barophilic omnivorous protists can feed on prokaryotes and smaller protists in porewaters of benthic sediments, as well as on a spectrum of organic particles within sediments (Cordier et al., 2019; V. P. Edgcomb, Orsi, Breiner, et al., 2011; V. P. Edgcomb, Pachiadaki, et al., 2016; Filker et al., 2013; Gooday, Schoenle, et al., 2020; Pawlowski, Fontaine, et al., 2011; Scheckenbach et al., 2010; Stoeck et al., 2003; Stock et al., 2012; Pachiadaki et al., 2014; Pachiadaki et al., 2016; Pasulka et al., 2019). While we focus here on the heterotrophic protists that based on available evidence dominate the active fraction of deep-sea sediments (Schoenle et al., 2021), we note that

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phytoplankton protists, particularly diatoms derived from sinking particles originating from overlying productive water columns, can be the dominant protists in some surficial deep-sea sediments and that a fraction (~18%) of those cells remain intact and presumably active for days or even weeks (Agusti et al., 2015). Although the deep-sea floor is arguably one of the largest biomes on Earth, save for the deep pelagic ocean (Webb et al., 2010), our knowledge of the biodiversity and functional roles of protists in this environment can at best be considered as limited, including studies of hydrothermal vents and cold seeps (V. P. Edgcomb et al., 2002; Pasulka et al., 2019), anoxic basins at bathyal depths (1000–3000 m water depth) (V. Edgcomb et al., 2009; V. P. Edgcomb, Orsi, Breiner, et al., 2011; V. P. Edgcomb, Pachiadaki, et al., 2016; Stoeck et al., 2003; Filker et al., 2013; Stoeck et al., 2014; Pachiadaki et al., 2014; Pachiadaki et al., 2016) and a few studies of abyssal and hadal trenches ranging from 6000 to 11,000 m depths (Cordier et al., 2019; Pawlowski, Fontaine, et al., 2011; Scheckenbach et al., 2010). Protists from surface waters and pelagic biomes are known to influence the global carbon cycle by forming the foundation of aquatic food webs and employing various nutritional strategies for survival in different conditions (Larsson et al., 2022; Mitra et al., 2014; Ward & Follows, 2016; Worden et al., 2015). In deep waters, where there is a complete absence of sunlight, protists are known to feed on a broad range of food particles, including detritus and other microeukaryotes in addition to prokaryotes and archaeal assemblages (Gage & Tyler, 1991; Hu et al., 2021; Pachiadaki et al., 2014). It is likely that deep-sea sedimental protists also play crucial roles in establishing benthic environments and have a significant influence on the cycling of carbon and other nutrients in marine ecosystems. Although many studies of deep-sea protists have focused on special localized settings such as hydrothermal vents, anoxic basins, and cold seeps, research on microeukaryote communities in abyssal sediments is extremely limited (Harris & Baker, 2012; Kostylev et al., 2001). Abyssal sediments are not only flat and smooth regions covered with muddy residues, but they also exhibit heterogeneous structures, such as submarine canyons, seamounts, and cold-water coral reefs, which may increase bioactivity and transportation of organic and inorganic particles (Devey et al., 2018; Ramirez-Llodra et al., 2010). Therefore, there is a need for more studies of abyssal sediment protist communities, as well as in situ experiments (e.g. grazing experiments and molecular analyses) to assess activities, including their roles as critical transmitters of bacterial carbon to metazoan communities, and as regulators of prokaryotic and archaeal activity (Azam et al., 1983; Bec et al., 2006; Gooday, Schoenle, et al., 2020).

We aim to provide an overview of the currently poorly known biodiversity of deep-sea floor protists, and their approximate geographic distributions, and we explore possible functions while taking into account their known behaviours and features. We limit our focus on the foraminiferans, due to the relatively large body of literature available on these protists and focus instead on the so-called ‘non-testate’ protists about which less is known. Not having a firm shell (i.e. tests), they are usually morphologically inconspicuous, not retained in geological records, and were until the present given much less attention. This is mostly caused by difficulties with their preservation and taxonomic determination (Gooday, Schoenle, et al., 2020). We argue that due to their overlooked abundance and the recently uncovered diversity of these deep-sea non-testate microeukaryotes, they likely play substantial roles in ecosystem functioning and carbon cycling. In addition, these sediment-dwelling protists exhibit high degrees of differences in terms of specific features, such as parasitic and/or predatorial behaviour, mobility, and morphological variability throughout different geographical locations. We choose to restrict ourselves to deep-sea sediments of 1000 m depth and greater, as there is no consensus on the minimum water depth required for sediments to be considered ‘deep’. This allows us to exclude sediments with shallow-water affinities on the uppermost continental slope that may be deposited during sea-level low stands or shed from carbonate platforms and reef slopes during high stands. Moreover, at these depths, there is a minimum risk of storms affecting bottom sediments (Hesse & Schacht, 2011; Tortorella et al., 2018).

THE FORAMINIFERA

Benthic foraminiferans have been extensively reviewed (Bernhard & Sen Gupta, 1999; Glock, 2023; Koho & Piña-Ochoa, 2012 and reference therein), showing that this diverse group includes many inhabitants of deep-sea sediments and low-oxygen habitats (Bernhard & Bowser, 1999; Glock, 2023). Benthic foraminiferans are used for palaeoceanographic reconstructions of past climate/ocean conditions due to the ability of testate forms to precipitate calcium carbonate tests that are preserved in the fossil record. Yet there are many soft-bodied monothalamous foraminiferans as well, that inhabit deep-sea sediments, such as Xenophyophores (Gooday et al., 2011). They encompass examples of agglutinated foraminiferans that take sediment grains and other substances to build their tests, using either an organic bioadhesive or, in some cases, calcite to bind the particles. Agglutinated foraminiferans are not often used for paleoceanography reconstructions due to their lower preservation potential. Interestingly, the tests of Xenophyophores can range in size from ~1 cm



to more than 20 cm (Gooday et al., 2011). In addition, there are soft-bodied species, often referred to as 'the-cate', which have an organic test. Some have adapted to oxygen-depleted/anoxic conditions including the acquisition of symbionts, alternatives to aerobic respiration (e.g. fermentation), use of high-energy phosphates, and peroxisome proliferation, as well as the ability to denitrify (Glock, 2023). These adaptations provide the ability to live in the sediments of silled basins and thus below the sea-floor surface where oxygen may not penetrate. In some benthic habitats, foraminiferans (and/or their symbionts) are important contributors to denitrification due to their high abundance (Choquel et al., 2021; Glud et al., 2009). Progress has been made in the use of live stains for visualizing active foraminiferans within the sediment fabric (Bernhard, 2003; Bernhard & Richardson, 2014), revealing that this important and diverse group remains active well below the sediment surface.

THE NON-TESTATE PROTISTS: FLAGELLATES, AMOEBAE, AND CILIATES

Unlike many foraminiferans, which are easily identified by their possession of prominent testate shells, morphological investigations of deep-sea non-testate protistan diversity have been challenging up until the turn of this century. This is due to the disruptions that can occur during the recovery of samples through the water column, which makes cultivation methods selective and significantly limit morphological analyses of flagellates, amoebae, and ciliates (Arndt et al., 2003; Schoenle et al., 2016, 2021). The development of culture-independent, high-throughput sequencing (HTS) and new methods for preserving samples in situ (V. Edgcomb, Orsi, Taylor, et al., 2011; V. P. Edgcomb & Pachiadaki, 2014) has dramatically enhanced our comprehension of microbial diversity in the deep-sea environment, followed by the establishment of vast public reference databases allowing comparative studies such as The *Tara* Oceans Consortium, The Marine Microbial Eukaryote Transcriptome Sequencing Project, and The Ocean Biogeographical Information System (Grassle, 2000; Keeling et al., 2014; Martín Míguez et al., 2019; Tara Oceans Consortium and Tara Oceans Expedition, 2017). While HTS and other environmental sequence sampling have enabled the detection of a broad range of non-foraminiferan diversity, its interpretation is limited by certain constraints, such as random sequencing errors, chimeric sequences, varying rDNA gene copy number from different eukaryotes, and biases of 'universal' primers (V. P. Edgcomb, Pachiadaki, et al., 2016; Flegontova et al., 2023; Gooday, Schoenle, et al., 2020; Mahé et al., 2015; Schoenle et al., 2016). Nevertheless, molecular and environmental studies have

confirmed that nearly all phylogenetic groups of non-testate protists are present in oceanic sediments below 1000 m depths (Cordier et al., 2022; Hohlfeld & Arndt, 2022; Rodríguez-Martínez et al., 2020; Schoenle et al., 2016). In most cases where deep-sea sediment cores have been studied for protists, data are presented only for total flagellates within targeted size ranges (e.g. reviewed in Bak & Nieuwland, 1997; Gooday, Durdin, & Smith, 2020). Where abundance estimations of heterotrophic flagellates have been made for deep-sea sediments, densities have been revealed of 100 to 10^5 cells/cm³ (Alongi, 1987; Arndt et al., 2003; Bak & Nieuwland, 1997; Burnett, 1981; Danovaro et al., 1998) and the proportion of unidentified benthic specimens increased with water depth, highlighting the potential that remains for discovery of new species in the deep sea (Hohlfeld & Arndt, 2022).

DEEP-SEA SEDIMENT-DWELLING PROTISTS

Although it is generally accepted that almost all phylogenetic groups of flagellated protists can be found in the deep-sea floor, there are only a few dozen confirmed observations of living specimens from these habitats, either from samples or isolated cultures (see Figure 1). Unlike many foraminiferans, which include numerous benthic and pelagic specialist taxa, many flagellates can be found in both seafloor and pelagic environments due to their small size, ranging from 2 to 8 μ m. While many can be free-living, small flocs of marine detritus are often a suitable habitat for them, and they are naturally mobile (Caron et al., 1982; Leadbeater & Green, 2000). As a result, aggregate-dwelling protists like kinetoplastids, diplomonids, ciliates, stramenopiles, and amoebae among others, can be found throughout the water column (Arndt et al., 2003; V. Edgcomb et al., 2009; Patterson et al., 1993; Pernice et al., 2015; Rocke et al., 2015; Schoenle et al., 2021). Many of these protists can withstand high pressure and remain active upon reaching the seabed through sedimentation (Schoenle et al., 2019; Živaljić et al., 2018, 2020) or through dispersal by currents. However, some barophilic flagellates may have been overlooked using traditional sampling methods during recovery from the deep sea. Cultures, direct counts of cells, or rDNA metabarcoding in deep-sea sediments (>1000 m depth) have recorded a wide range of taxa (Table 1).

PARASITIC AND PREDATORIAL DEEP-SEA PROTISTS: KEY PLAYERS IN ECOLOGICAL CYCLES?

Parasitism and predation are immensely successful modes of nutrition and parasitic organisms are highly

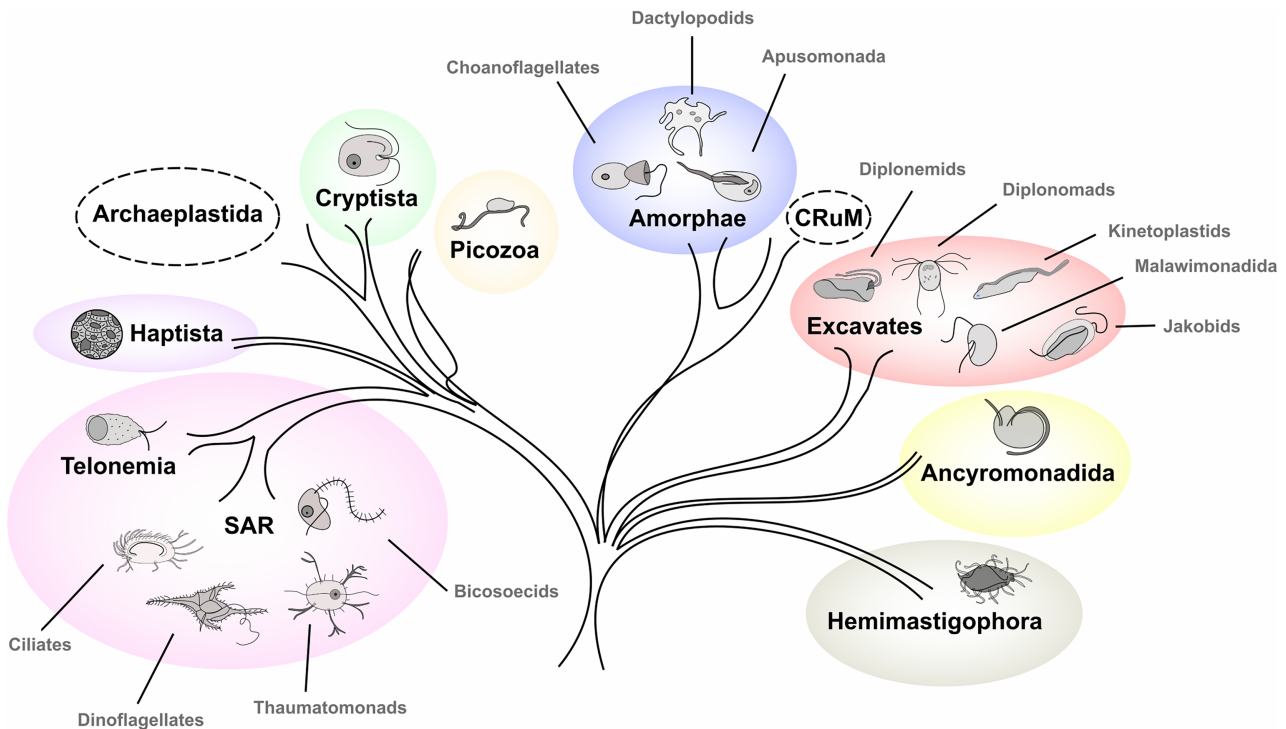


FIGURE 1 A phylogenetic tree of eukaryotes showcases representatives of protists discovered in deep-sea sediments through a combination of metabarcoding and direct observations. Each bubble within the tree signifies a distinct ‘supergroup’, with dotted bubbles indicating supergroups currently lacking recorded members known to inhabit the deep-sea floor. The graphical representation includes caricatures illustrating the most frequently observed protists, offering a visually engaging glimpse into the rich and diverse microcosm thriving in the abyssal depths.

abundant in most ecosystems. As such, they play key roles in gross marine ecological cycles, including but not limited to the carbon cycle and food webs (Field et al., 1998; Pascal et al., 2020; Verity & Smetacek, 1996; Worden et al., 2015). Numerous marine protists (a common example is Syndiniales) have been identified as parasitic agents of various hosts ranging from larger multicellular organisms such as lobsters, copepods, and other crustaceans down to other ubiquitous microeukaryotes found throughout the water column (i.e. dinoflagellates and ciliates) (Anderson & Harvey, 2020). Despite the importance of parasitism as an agent of plankton mortality and mobility, parasite–host dynamics remain poorly understood, especially within the context of the oceanic food webs and ecosystem models (Chambouvet et al., 2008; Fuhrman et al., 2015; Sehein et al., 2022; Skovgaard, 2005; Stentiford & Shields, 2005; Worden et al., 2015). Identifying and quantifying these interactions shall improve our understanding of how carbon and nutrients are cycled throughout the water column and down to the deep-sea sediment. In one of the first mass HTS-based studies, a remarkably high occurrence of operational taxonomic units (OTUs) belonging to the hypothesized parasitic ciliates annotated as DH148-5-EKD18 was documented in the deep-sea floor (approximately 3000 m) of the Southern Atlantic

Oceans, with the majority of hits belonging to known parasitic dinoflagellate taxa (López-García et al., 2001).

EXCAVATA

Perhaps the most well-known and studied parasitic taxa from within the excavates are the kinetoplastids. Although found in almost all terrestrial and aquatic environments, recent studies of deep-sea excavates revealed the presence of diverse kinetoplastids, abide at lower disproportional levels compared to other excavates (Hohlfeld & Arndt, 2022; Lara et al., 2009; Moreira & López-García, 2002; Salani et al., 2012; Schoenle et al., 2021). The ecological relevance of kinetoplastids, alongside their potential deep-sea sediment lineages reported thus far, make this group an interesting subject to investigate abyssal microbial eukaryotes. While some free-living kinetoplastids (*Bodo* and *Neobodo*) have been documented in the sediments of the Mediterranean Sea and South-Eastern Atlantic Ocean, many obligate parasitic species within the sub-order Prokinetoplastina (*Ichthyobodo*) have been found throughout the entirety of the Atlantic Ocean and specific locations in the Mediterranean Sea (e.g. Lerapetra Basin) (Salani et al., 2012; Schoenle et al., 2021). While environmental sequencing of these



TABLE 1 Taxa known to inhabit the deep-sea sediment.

Taxa	Source
Apusomonada	Patterson et al., 1993; Lee & Patterson, 2000; López-García et al., 2003; Zhang et al., 2018; Wu & Huang, 2019; Cordier et al., 2022; Hohlfeld & Arndt, 2022
Choanoflagellates	Patterson et al., 1993; Lee & Patterson, 2000; Arndt et al., 2017; Wu & Huang, 2019; Hohlfeld & Arndt, 2022
Dactylopodids	Cordier et al., 2019
Filastereans	Patterson et al., 1993; Arndt et al., 2017
Picozoa	Schön et al., 2021
Cryptista	Patterson et al., 1993; Lee & Patterson, 2000; Scheckenbach et al., 2010
Haptista	Wu & Huang, 2019; Selway et al., 2022
Telonemia	Patterson et al., 1993; Arndt et al., 2017; Wu & Huang, 2019; Hohlfeld & Arndt, 2022
Placidids	Arndt et al., 2017
Dinoflagellates	Scheckenbach et al., 2010; Bernhard et al., 2014; Wu & Huang, 2019; Cordier et al., 2022; Hohlfeld & Arndt, 2022
Thaumatomonads	Patterson et al., 1993; Cordier et al., 2019; Rodríguez-Martínez et al., 2020
Bicosoecids	Patterson et al., 1993; Lee & Patterson, 2000; Scheckenbach et al., 2010; Rodríguez-Martínez et al., 2020; Hohlfeld & Arndt, 2022
Chrysomonads	Cordier et al., 2022
Pedinellids	Patterson et al., 1993; Scheckenbach et al., 2010; Cleary & Durbin, 2016; Cordier et al., 2022
Ciliates	V. P. Edgcomb et al., 2002; Bernhard et al., 2003, 2014; Buck & Bernhard, 2004; Countway et al., 2007; Alexander et al., 2009; Sauvadet et al., 2010; Scheckenbach et al., 2010; Takishita et al., 2010; Coyne et al., 2013; Cleary & Durbin, 2016; Zhao & Xu, 2016; Schoenle et al., 2017; Zhao et al., 2017; Wu & Huang, 2019; Rodríguez-Martínez et al., 2020; Živaljić et al., 2020; Hohlfeld & Arndt, 2022
Cercomonads	Patterson et al., 1993; Lee & Patterson, 2000; Bernhard et al., 2014; Zhang et al., 2018; Wu & Huang, 2019; Cordier et al., 2022; Hohlfeld & Arndt, 2022
Diplonemids	Lee & Patterson, 2000; Lara et al., 2009; Scheckenbach et al., 2010; Flegontova et al., 2016; Cordier et al., 2022
Symbiontids	Buck et al., 2000; Yubuki et al., 2009
Kinetoplastids	Patterson et al., 1993; Lee & Patterson, 2000; Hausmann et al., 2002; Arndt et al., 2003; López-García et al., 2003; Scheckenbach et al., 2010; V. P. Edgcomb, Orsi, Breiner, et al., 2011; Salani et al., 2012; Cleary & Durbin, 2016; Hohlfeld & Arndt, 2022
Euglenids	Patterson et al., 1993; Arndt et al., 2003; Buck & Bernhard, 2004; Je Lee, 2006; Schoenle et al., 2019; Hohlfeld & Arndt, 2022
Malawimonadida	Arndt et al., 2017
Jakobids	Patterson et al., 1993
Diplonomads	Xu et al., 2016
Ancyromonadida	Patterson et al., 1993; Atkins et al., 1998; Arndt et al., 2003
Hemimastigophora	Arndt et al., 2003, 2017
Foraminifera	Bernhard & Bowser, 1999; Bernhard, 2003; Bernhard et al., 2003, 2014; Buck & Bernhard, 2004; Hohlfeld & Arndt, 2022

Note: Colours correspond to supergroups. For more detailed taxon and species assignments as well as approximate geographic locations, see Table S1.

kinetoplastids has revealed their presence in the sediment, it is still unknown which hosts they predominantly parasitize, let alone decipher specific host–parasite interactions. Thus, the exact role of these sediment-dwelling kinetoplastids remains enigmatic, with regards to both the parasitic and free-living species. However, the paramount ecological role of their terrestrial relatives (Stuart et al., 2008) leads us to speculate that marine kinetoplastids also play a key role in deep-sea ecological system dynamics.

While kinetoplastids were the dominant group of excavates in the fields of ecology and molecular biology of protists for the past half-century (Lukeš et al., 2023), recently another group has gained

attention given the rise of HTS-based studies and improved cultivation techniques (Tashyreva et al., 2022). Diplonemids primarily inhabit marine environments and have been considered rare and unimportant eukaryotes, although this notion has recently been rejected due to their extreme diversity and abundance, in addition to vastly outnumbering the previously discussed kinetoplastids (Schoenle et al., 2021; Tashyreva et al., 2022). Although found throughout the water column, diplomonads have been identified with high confidence to inhabit abyssal sediment communities, known as Deep-Sea Pelagic Diplonemids (DSPD), and have been proposed as crucial players in deep-sea ecosystems (Flegontova et al., 2020; Lara et al., 2009;



Schoenle et al., 2019; Tashyreva et al., 2022). While diplomid research is still considered to be in its infancy, with their exact lifestyles and feeding strategies remaining mostly enigmatic, there is growing confidence that their ubiquitous presence is crucial to marine ecosystems. Eukaryote-proximal isolation of various diplomids on the sea surface has suggested roles in predation and/or parasitism, mainly on crustaceans and other protists such as diatoms (Elbrächter et al., 1996; Tashyreva et al., 2022; Triemer & Ott, 1990). While it is currently assumed that closely related species of diplomids are found throughout the water column, it is thus likely that deep-sea sediment diplomids share similar feeding strategies that appear parasitic and predatorial as those described from their surface water relatives (Cordier et al., 2022; Heyden et al., 2004; Prokopchuk et al., 2022; Schnepf, 1994). In addition, diplomids have been shown to harbour numerous diverse bacterial symbionts (George et al., 2020; Tashyreva et al., 2018). While it is currently unknown whether DSPD species also host prokaryotic symbionts, it presents the question if symbionts may play a role in deep-sea acclimation (e.g. adaptation to anoxic conditions).

ALVEOLATA

Ciliates are an overwhelmingly diverse and widespread group of alveolate protists that includes many phagotrophic and parasitic taxa, and as such are vital components of deep-sea microbial food webs (Johansson, 2004). They are ubiquitous in marine environments and are commonly found in oxygen-depleted and anoxic habitats (Fenchel & Finlay, 1991; Lynn, 2010). Ciliates have been recorded from deep-sea sediments (>3500 m) at high abundancies ranging up to 700 cells/cm³ in the North Atlantic basins, supporting the notion that they are gross contributors to biogeochemical cycling on the sea floor (Cunningham & Ustach, 1992). Nearly all known representatives of phylogenetic groups within ciliates have been recorded through eDNA/HTS sampling at depths exceeding 1000 m throughout different oceanic regions (Table S1) (Alexander et al., 2009; Bernhard et al., 2003, 2014; Bernhard & Bowser, 1999; Cordier et al., 2022; Coyne et al., 2013; V. P. Edgcomb et al., 2002; Rodríguez-Martínez et al., 2020; Scheckenbach et al., 2010; Stock et al., 2013; Takishita et al., 2010; Wu & Huang, 2019; Zhao et al., 2017; Zhao & Xu, 2016). Although environmental screening has recovered vast amounts and diversity of ciliate OTUs through global marine waters, live observations of isolated deep ocean ciliates remain difficult due to dependence on conventional sampling approaches that rely on the collection of water samples using Niskin rosettes, and retrieval of sediment cores to the ocean

surface prior to preservation. These approaches expose fragile protists to hydrostatic stress (Schoenle et al., 2016; Živaljić et al., 2018, 2020). Studies of low oxygen/anoxic water columns have revealed novel protist lineages, including the first new ciliate class to be identified in over a decade (Orsi et al., 2012; Stoeck et al., 2003), illustrative of how little we know of protist diversity in some marine realms.

Common ciliate taxa detected along oxyclines and in anoxic water columns of the Cariaco Basin and the Baltic Sea include *Metopus* (Armophorida), *Frontonia* (Peniculida), *Strombidium* (Oligotrichida), *Euplotes* (Euplotida), *Oxytricha* (Sporadotrichida), *Cariacothrix* (Cariacotrichida), *Cyclidium* (Pleuronematida), *Epaxella* (Odontostomida), *Prorodon* (Prorodontida), and unaffiliated members of Karyorelictida, Colpodida, and Scuticociliata (Table S1) (Anderson et al., 2012; V. P. Edgcomb & Pachiadaki, 2014). Metopid ciliates are predators of bacteria known to inhabit anoxic marine sediments and often exhibit endosymbiont methanogens located in close proximity to their hydrogenosomes. The endosymbionts may convert hydrogenosomally produced hydrogen, carbon dioxide, and acetate into methane and water (Fenchel & Finlay, 1991). Species of *Frontonia*, *Euplotes*, and *Oxytricha* are bacterivorous and are commonly found in benthic marine habitats, however, they typically do not survive anoxia. Ciliates of the genus *Strombidium* can dominate water column oxyclines, such as in the Baltic Sea where they can reach up to 7 cells/ml (Anderson et al., 2012). Members of *Cyclidium*, *Epaxella*, scuticociliates, and karyorelictid ciliates are known to inhabit marine anoxic and sulfidic habitats (Dyer, 1989; Lynn, 2010). Studies of deep-sea water columns that incorporate microscopic observations are relatively few, but those indicating that ciliates can be quite abundant, and in anoxic waters can often host visible prokaryotic symbionts. For example, scanning electron microscopy analyses of samples from Cariaco Basin revealed $\sim 10^4/l$ ciliates in anoxic waters, including abundant scutocociliate types, and most exhibited visible epibiotic microbes, suggesting the symbionts play a role in the adaptation of those ciliates to anoxia both in water columns and in sediments (Orsi et al., 2012).

The broad spectrum of different ciliates described from the seafloor thus far indicates that they occupy diverse niches, preying on prokaryotes, detritus, and other protists. (Gooday, Schoenle, et al., 2020; Schoenle et al., 2017). Compared to flagellates, amoebae, and other protists, ciliates are relatively large. This difference in size facilitates smaller protists to establish parasitic relationships with ciliates, as is the case of some diplomids (Elbrächter et al., 1996). Studies of mesopelagic, bathypelagic, and hadal protist diversity and benthic protist communities below those waters have largely depended on DNA-based molecular data due to the challenges of retrieving intact/viable cells



from those depths and/or visualizing cells apart from marine sediments. While inferences about cell abundance based on abundance of ribosomal RNA marker genes must be made with caution due to known variations in gene copy number among protist taxa (Flegontova et al., 2023), early DNA-based studies of protist diversity close to the seafloor (>3000 m depth) along the haloclines of several deep hypersaline anoxic basins (DHABs) on the seafloors of the Mediterranean Sea revealed evidence that Alveolata dominated 18S rRNA signatures. A large fraction of these signatures represented ciliates (including unclassified taxa as well as *Trimyema*, *Strombidium*, *Metopus*, *Peritromus*, *Asomatida*, *Novistrombidium*, *Laboea*, *Salpingella*, and *Pseudotontonia*, depending on DHAB chemistry) (V. Edgcomb et al., 2009; Stock et al., 2012, 2013). An RNA-based study (Alexander et al., 2009) provided evidence for the activity of distinct ciliates at different positions along one DHAB halocline, and in some cases, ciliates (e.g. Oligohymenophorea, Spirotrichea, Plagiopylea, Phyllopharyngea, and Prostomatea) dominated recovered phylotypes. Scanning electron microscopy provided further evidence for intact and presumably living ciliates in several DHAB brine and halocline habitats (Orsi et al., 2012; Stock et al., 2013). Interestingly, as for Cariaco Basin anoxic water column scuticociliate morphotypes, the dominant ciliate imaged from the Urania DHAB halocline (fusiform ciliates present at $\sim 4 \times 10^5/L$) showed evidence of epibiotic prokaryotes, in this case affiliated with deltaproteobacteria (Orsi et al., 2012). Symbiotic relationships between prokaryotes and eukaryotes in deep-sea sediments are also observed, such as from Monterey Bay and Santa Barbara Basin (Buck & Bernhard, 2004). Light microscopy studies of sediments underlying three DHAB haloclines provided evidence of intact ciliates and foraminiferans, with increased diversity in those sediments (Bernhard et al., 2014).

Apicomplexans are a successful group of parasitic protists (Votýpka et al., 2017) known to infect a large variety of organisms, including other marine protists (Seeber & Steinfelder, 2016). Although found in marine ecosystems, eDNA sampling revealed a relatively low abundance of apicomplexans in deep-sea sediments compared to other alveolates (Cordier et al., 2022; Schoenle et al., 2021). Directly observed apicomplexans from deep-sea sediments are typically identified within their hosts, such as in the intestine of chaetognaths, Arctic krill, various copepods, molluscs, and amphipods (Takahashi et al., 2009; Théodoridès, 1989). While these apicomplexans are widespread on the seafloor, there is sparse knowledge as to their exact ecological impact other than the ability to infect diverse multicellular organisms.

Parasitic lifestyles are ubiquitous among different phylogenetic groups of dinoflagellates, with many members of Dinophyceae and Blastodiales being

parasites of various mesopelagic marine plankton (Coats, 1999). Several species of *Duboscquodinium*, *Tintinnophagus*, and *Duboscquella* are parasites of tintinnid ciliates lethal to their hosts and appear common and widespread throughout different marine locations (Coats et al., 2010). While the aforementioned dinoflagellate genera have not specifically been found in deep-sea sediment sampling, various OTUs have been recorded using universal eukaryote primers from a diverse set of global marine deep-sea beds (Alexander et al., 2009; Cordier et al., 2022; Countway et al., 2007; Coyne et al., 2013; V. P. Edgcomb et al., 2002; Rodríguez-Martínez et al., 2020; Sauvadet et al., 2010; Scheckenbach et al., 2010; Stock et al., 2013; Takahashi et al., 2009; Zhao et al., 2017; Zhao & Xu, 2016; Živaljić et al., 2020). Given the vast diversity of recorded dinoflagellate OTUs recovered from sediments, coupled with unambiguous parasitism and/or predatory behaviour identified in studied dinoflagellates from surface waters, it can be assumed with some degree of certainty that these yet unknown deep-sea sediment dinoflagellates have similar feeding patterns as their upper ocean relatives. In light of this assumption, one can only speculate on the vital role dinoflagellates play in ocean sediments. Due to the lack of direct observations of deep-sea floor dinoflagellates in situ, their ecological contributions are thus far speculative.

Another key feature hinting at widespread parasitic and/or predatory behaviour, abide non-specific, is the abundance and diversity of recovered enigmatic marine alveolate groups 1/2 (MALV I and MALV II) sequences from isolated radiolarians from different marine sediments. Radiolarians are a key reservoir for MALVs (of note: Syndiniales are within MALVs), implying a possible principal impact on predatory behaviour or symbiotic associations on the marine ecosystem (Bråte et al., 2012). However, despite the apparent importance of parasitic OTUs found throughout the eukaryote tree of life, the specific roles they fulfil in the deep-sea sediments remain unknown.

BIOGEOGRAPHIC DISTRIBUTION: DIVERSITY AND IDENTITY

While the use of environmental sequencing in uncovering deep-sea ecosystems has led to the discovery of distinct protists and the identification of previously unknown sequences, global comparisons of abyssal seafloor communities are to date still minimal (Figure 2 and Table S1) (Cordier et al., 2022; Countway et al., 2007; V. P. Edgcomb et al., 2002; Hohlfeld & Arndt, 2022; López-García et al., 2003). Specific kinetoplastid communities of *Rhynchomonas*, *Neobodo*, and *Perkinsella* show similar distribution patterns throughout the Eastern and Western Southern Atlantic basins and appear confined within sediment layers, with very

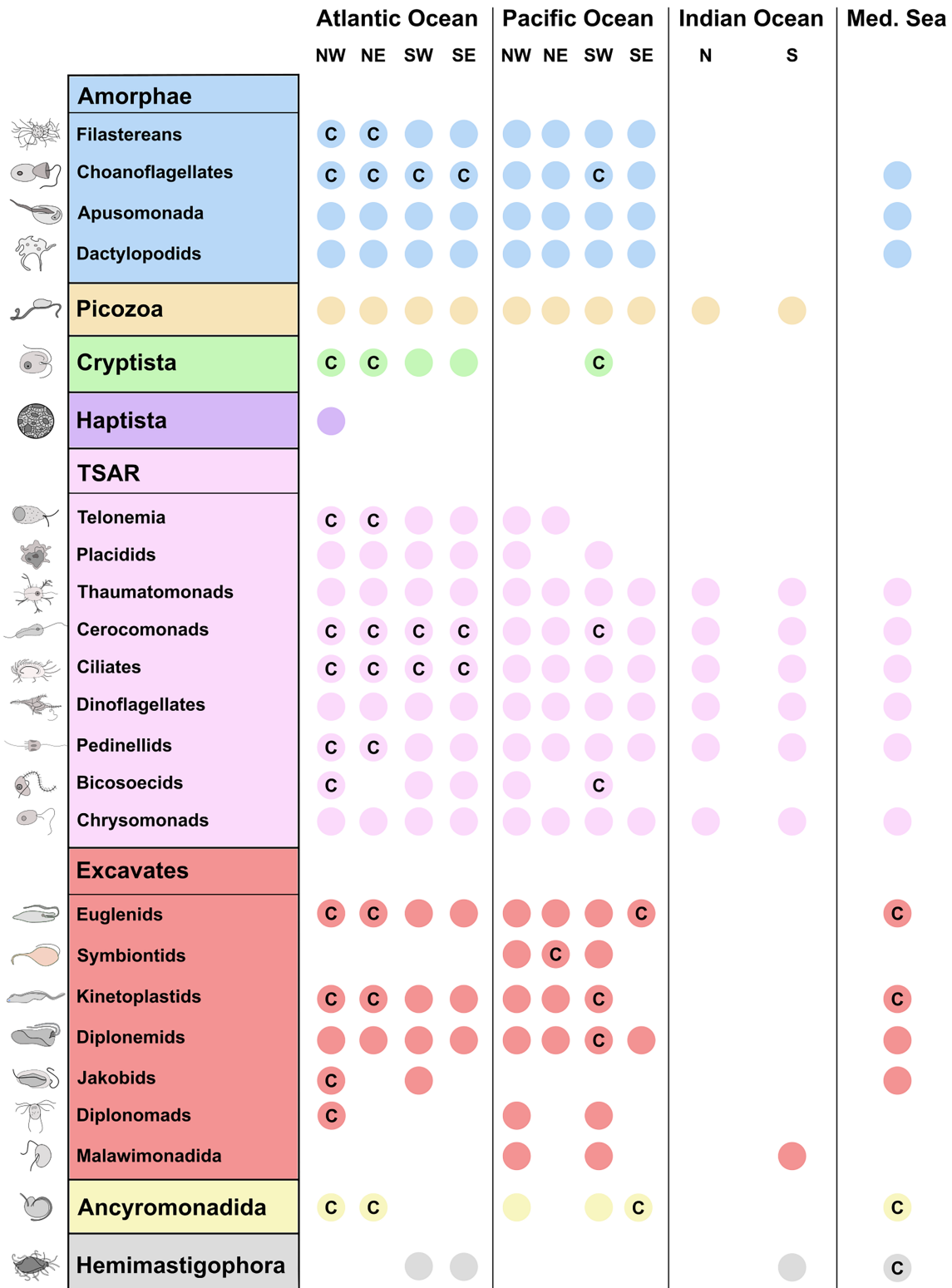


FIGURE 2 Geographical distribution of protist groups across four significant marine regions. Bubbles indicate the presence of distinct protist groups identified from environmental DNA and/or RNA sampling. Bubbles containing the letter 'C' denote instances where the identification of these protists has been verified through direct culture counts.

little abundance within pelagic communities (Cordier et al., 2022; Salani et al., 2012; Schoenle et al., 2021). *Bodo* has also been identified via culture studies near

the Australian coast at high abundancies on the deep-sea floor (Lee & Patterson, 2000), and fluorescent in situ hybridization suggests some novel kinetoplastids

to be abundant in haloclines near the seafloor of the Mediterranean Sea (V. Edgcomb, Orsi, Taylor, et al., 2011). Diverse species of kinetoplastids have also been recorded in the deep-sea beds of the Mediterranean exhibiting significantly different communities, indicating spatial structure at larger scales (Arndt et al., 2003; Salani et al., 2012). Diplonemid reads from throughout the Atlantic and Mediterranean have been recovered, including in two distinct regions in the Pacific (Cordier et al., 2022; Lara et al., 2009; Lee & Patterson, 2000; Scheckenbach et al., 2010). Of interest are the distinct OTUs annotated as DSPD diplomonids, although their representatives are not yet available in culture (Tashyreva et al., 2022). Among the excavates, isolated cultivation/eDNA studies have confirmed the presence of symbiontids (Pacific–California coastline), euglenids (Atlantic, Mediterranean, and Pacific), jakobids (North Atlantic), and diplomonads (Pacific) within the sediment of those geographic boundaries, but without context towards their abundance with respect to other OTUs (Arndt et al., 2003, 2017; Buck et al., 2000; Je Lee, 2006; Patterson et al., 1993; Yubuki et al., 2009).

Ciliates dominate the sediments of the Western Pacific with over 70% of recovered OTUs annotated as various ciliate genera from depths of 3800 m to shallower 810 m (Zhao et al., 2017; Zhao & Xu, 2016). While no discernible differences were seen at different seafloor depths, distinct ciliate OTUs were detected upon moving up the water column, excluding any horizontal distance-decay relationship and indicating that sedimental ciliates are specific. Ciliates appear not only to be overrepresented throughout the Pacific seafloor but are also highly abundant in Atlantic and Mediterranean samples (Table S1). As with ciliates, specific dinoflagellate OTUs are also widespread throughout the sediments and indicate a lack of horizontal distance-decay relationships (Cordier et al., 2022; Scheckenbach et al., 2010). However, the same studies revealed that environmental differences between the Cape/Namibian Abyssal and the Angola/Guinea Abyssal Plains display differences in the community structure of other protists, namely Telonema, Cryptophyceae, Chroanoflagellata, Heterokonta, and Haptophyta.

Deep-sea sediments appear to be much more diverse than oceanic waters and are comprised of communities that display clear biogeographic patterns at global scales and considerable patchiness at local sites (Cordier et al., 2022). It should be emphasized however, that these protist communities identified through HTS do not merely exhibit high diversity within defined biogeographic boundaries, but appear as a selected few specific genotypes/species within a set of OTUs dominating most reads (Table S1). Furthermore, these hits point to the fact that a large portion of the major species dominating deep-sea sediments are currently

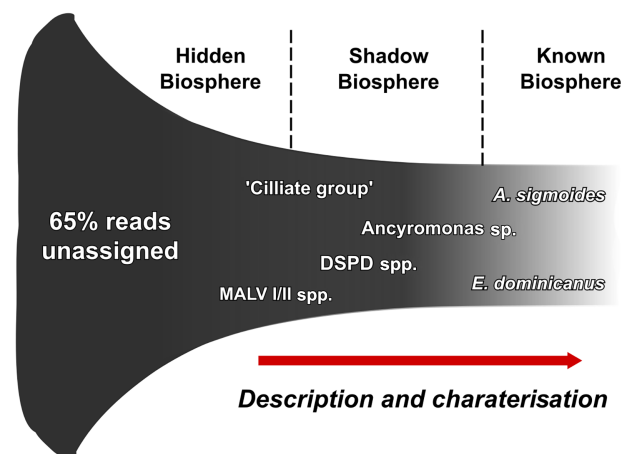


FIGURE 3 Research into protists from deep-sea sediments has barely scratched the surface. Only a small percentage of protists identified in deep sediments of the global seas have been properly described (Known Biosphere). eDNA can only characterize a fraction of reads from general protist operational taxonomic units (OTUs) (Shadow Biosphere) with a large portion currently annotated as ‘unassigned’ (Hidden Biosphere).

undescribed or uncharacterized, representing part of a ‘shadow biosphere’, in which we recognize their existence and approximate taxonomic grouping, yet know virtually nothing about them in terms of functionality and description (Figure 3). Hence, it is possible to only speculate about their role in deep-sea sediments using their closest previously characterized relatives as references (e.g. hypothesized feeding patterns of DSPD diplomonids using the described and cultivable species). Beyond this ‘shadow biosphere’ remains the ‘hidden biosphere’, encompassing eukaryotic eDNA hits but without assignment towards any discernible or known OTU (Figure 3). These eukaryotes represent anywhere from 15% to 65% of reads from the sediment layers of the Atlantic, Pacific, Indian Oceans, and Mediterranean Sea (Cordier et al., 2022).

CHALLENGES IN STUDYING DEEP-SEA FLOOR PROTISTS

Our limited understanding of bathyal and abyssal benthic protist communities relative to those in mesopelagic and, particularly, epipelagic sediments is due primarily to different logistical challenges of accessing these realms, which require dedicated ship time, coring devices, and the expertise to successfully apply them (e.g. box coring, multicoring rosettes, piston coring) or human-occupied or remotely operated deep-sea vehicles. In addition, artefacts can be introduced during sample recovery from deep sediments and water columns when those samples are not preserved in situ, which can hinder the recovery of intact cells and therefore bias our understanding of the taxa that inhabit this



realm. For instance, collection of water samples from the mesopelagic ocean using Niskin bottles was demonstrated to cause many fragile protists to lyse during transit to the ocean surface due to physico-chemical changes, for example, in pressure, temperature, and redox (V. P. Edgcomb, 2016; V. Edgcomb, Orsi, Taylor, et al., 2011), and researchers are faced with the same challenges for sediment samples from the deep sea. Studies of DNA recovered from deep-sea sediments (e.g. marker gene studies of diversity, metagenome studies of gene content) cannot inform on the fraction of active taxa, nor can they inform on active metabolisms because DNA recovered can derive from active, inactive but viable, and dead cells, or dissolved extracellular pool in marine sediments (Dell'Anno & Danovaro, 2005; Karl & Bailiff, 1989). For studies of labile molecules such as RNA and protein that can elucidate in situ protist activities, the physico-chemical changes associated with sample recovery influence the physiological poise of many surviving cells (V. P. Edgcomb, Taylor, et al., 2016; Feike et al., 2012). Therefore, they can bias our understanding of in situ activities and hence, the role that protists play in deep-sea microbial food webs. Development of new technologies that allow in situ preservation of sediment samples prior to recovery, or that allow incubation of sediment samples in situ with various tracers for selected metabolic activities will open new insights into the extent of diversity and metabolic activities of protists. For example, the remotely operated vehicle *Jason* deployed push cores into deep Mediterranean Sea sediments and injected and incubated those sediments in situ with CellTracker™ Green, a viability indicator that detects active esterases, allowing to differentiate the metabolically active fraction of the eukaryotic community (Bernhard et al., 2015). Until technologies that enable in situ preservation of samples (e.g. with protein or nucleic acid preservatives) or in situ, incubation studies become more widely available, protistologists and ecologists will not be able to provide a complete understanding of the contributions of protists from diverse taxonomic groups to deep-sea benthic food webs.

To summarize, utilizing HTS alone presents challenges, as it may generate artefacts and offer limited insights into the ecology and abundance of targeted deep-sea protists. To comprehend their biodiversity, a complementary approach involving direct observations and culture-dependent methods is essential. Analysing the functional aspects of their activity becomes particularly challenging due to the exposure of protists to significant pressure changes and alterations in temperature, oxygen, and redox conditions during sample handling. Reliable data on deep-sea protists' grazing behaviour and molecular characteristics are best obtained through in situ experiments at their targeted oceanic depths. In addition, it is important to

acknowledge potential biases in studying microbial communities based on rDNA metabarcoding, which include the accumulation and preservation of DNA from dead cells or the dissolved extracellular pool in marine sediments. Alternatives, such as rRNA-based methods may help mitigate these limitations.

CONCLUDING REMARKS

In closing, the benthic biome of the deep-sea floor is space-wise the largest ecosystem on Earth, yet we know so little about it. The available data indicates that after prokaryotes, protists are its most dynamic and productive component. To gain a comprehensive understanding of the global carbon cycle, it is crucial to investigate the role of protists in the deep-sea environment, including identifying the functional significance of various groups with regard to parasitic/predatory behaviour and their distribution patterns. Combined, results from various environmental studies have brought to light the dark abyss of the deep-sea sediments, revealing that it is indeed one of Earth's rich ecosystems (Arndt et al., 2017; Cordier et al., 2022; Hohlfeld & Arndt, 2022; Scheckenbach et al., 2010). This highlights the need for international efforts to study this fascinating and virtually untouched biome. By comprehending the role of protists, understanding their diversity, and unravelling key ecological features of deep benthic inhabitants, various missing elements of critical biogeochemical cycles can be elucidated, an issue more pressing with current global climate predictions and other outside factors such as deep-sea mining (DeVries, 2022; Gooday, Durden, & Smith, 2020). The effects of climate change, including warming, acidification, and deoxygenation, are likely to have profound impacts on deep-sea ecosystems, such as declining bottom-water oxygen concentration and food supply to the seafloor (Sweetman et al., 2017). Consequently, there may be global declines in sediment biomass, favouring small-sized organisms such as protists (Yool et al., 2017). While research into metazoan and prokaryote communities from the deep sea has taken centre stage, microeukaryotes have remained in the shadows, especially in terms of their greater function. Thus, we argue for a holistic approach where not only understanding the biodiversity of these sediment-dwelling protists is paramount, but comprehending how they interact with their environment in the context of life stages and feeding mechanisms (e.g. parasitism, predation) warrants further work. We hope that, with additional environmental studies targeting the last untouched frontiers of these critical biomes, alongside advances in molecular and microbial techniques, we will finally shed light on Earth's darkest corner.



AUTHOR CONTRIBUTIONS

Lawrence Rudy Cadena: Investigation; formal analysis; writing – original draft; data curation; methodology.

Virginia Edgcomb: Methodology; data curation; investigation; formal analysis; writing – original draft.

Julius Lukeš: Conceptualization; supervision; funding acquisition; visualization; project administration; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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SUPPORTING INFORMATION

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