High and specific diversity of protists in the deep-sea basins dominated by diplonemids, kinetoplastids, ciliates and foraminiferans

Alexandra Schoenle, Manon Hohlfeld, Karoline Hermanns, Frédéric Mahé, Colomban de Vargas, Frank Nitsche, Hartmut Arndt

To cite this version:
Alexandra Schoenle, Manon Hohlfeld, Karoline Hermanns, Frédéric Mahé, Colomban de Vargas, et al.. High and specific diversity of protists in the deep-sea basins dominated by diplonemids, kinetoplastids, ciliates and foraminiferans. Communications Biology, Nature Publishing Group, 2021, 4 (1), pp.501. 10.1038/s42003-021-02012-5. hal-03208049

HAL Id: hal-03208049
https://hal.sorbonne-universite.fr/hal-03208049
Submitted on 26 Apr 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
High and specific diversity of protists in the deep-sea basins dominated by diplonemids, kinetoplastids, ciliates and foraminiferans

Alexandra Schoenle, Manon Hohlfeld, Karoline Hermanns, Frédéric Mahé, Colomban de Vargas, Frank Nitsche & Hartmut Arndt

Heterotrophic protists (unicellular eukaryotes) form a major link from bacteria and algae to higher trophic levels in the sunlit ocean. Their role on the deep seafloor, however, is only fragmentarily understood, despite their potential key function for global carbon cycling. Using the approach of combined DNA metabarcoding and cultivation-based surveys of 11 deep-sea regions, we show that protist communities, mostly overlooked in current deep-sea foodweb models, are highly specific, locally diverse and have little overlap to pelagic communities. Besides traditionally considered foraminiferans, tiny protists including diplonemids, kinetoplastids and ciliates were genetically highly diverse considerably exceeding the diversity of metazoans. Deep-sea protists, including many parasitic species, represent thus one of the most diverse biodiversity compartments of the Earth system, forming an essential link to metazoans.
Although deep-sea sediment life and its extraordinary representatives have been studied for more than two centuries, we still lack a firm understanding of diversity and ecological functions in the largest ecosystem of the biosphere due to the difficulty to access it. In the last two decades, the establishment of new tools for studying the molecular identity of microbial communities has revolutionized our understanding of the microbial world, and revealed a large and unique diversity of prokaryotes and previously unknown protistan lineages in surface waters and the deep sea. In parallel, morphological and molecular studies of cultured species have widening our perception of poorly represented branches of the tree of eukaryotic life. Despite the fundamental roles of protists in the food web of marine surface waters, we also investigated protist communities on a small scale to compare different deep-sea sites) in 11 regions in the Pacific and Atlantic Ocean (Fig. 1a–c, Supplementary Data 1, map created with Ocean Data View). Besides sampling on a large scale to compare different deep-sea regions, we also investigated protist communities on a small spatial scale (see Supplementary Data 1). We used the approach combining DNA metabarcoding of the hypervariable V9 region of the 18S rDNA and direct microscopic live observations (Fig. 1d) with cultivation of protists. Morphological and molecular characteristics of the cultures were obtained to verify results from DNA metabarcoding, and their potential of barotolerance was also investigated. Strict bioinformatic quality control led to a final eukaryotic dataset of ~47,000 operational taxonomic units (OTUs) (~70 million reads), of which the majority (87%) could be taxonomically assigned to groups of heterotrophic protists (Supplementary Tables 1 and 2). Keeping in mind that the number of sampled stations was more than twice as high, the eukaryotic richness in the euphotic zone of marine waters was also more than twice as high (~110,000 OTUs, the majority belonged to heterotrophic protistan groups) when compared to our deep-sea eukaryotic OTUs. Within the Malapina expedition, targeting the eukaryotic life in the deep water column, ~42,000 OTUs associated with picoeukaryotes could be recovered. Prostist richness in other benthic environments was lower when compared with our benthic deep-sea dataset. In the neotropical rainforests protist richness was much lower (~26,000 protist OTUs). Within marine coastal sediments, the protist diversity was found to be ~6000 OTUs. Comparing the number of eukaryotic deep-sea deep-sea OTUs with other environments shows that the diversity of deep-sea assemblages is higher than that of coastal sediment communities and has a comparable size as the marine pelagic communities. One should keep in mind that comparing our observed protist richness with studies from other environmental biomes is difficult due to the fact that some of them used different target regions and filtering/clustering methods. Therefore, we compared the eukaryotic community of the deep seafloor with that of de Vargas et al. from the sunlit ocean where similar filtering and clustering methods were used.

**Results and discussion**

Deep-sea metabarcoding approach. To explore protistan diversity in different deep-sea basins, we collected sediment samples from 20 sampling sites (3 bathyal sites, 15 abyssal sites, 2 hadal sites) in 11 regions in the Pacific and Atlantic Ocean (Fig. 1a–c, Supplementary Data 1, map created with Ocean Data View). Besides sampling on a large scale to compare different deep-sea regions, we also investigated protist communities on a small spatial scale (see Supplementary Data 1). We used the approach combining DNA metabarcoding of the hypervariable V9 region of the 18S rDNA and direct microscopic live observations (Fig. 1d) with cultivation of protists. Morphological and molecular characteristics of the cultures were obtained to verify results from DNA metabarcoding, and their potential of barotolerance was also investigated. Strict bioinformatic quality control led to a final eukaryotic dataset of ~47,000 operational taxonomic units (OTUs) (~70 million reads), of which the majority (87%) could be taxonomically assigned to groups of heterotrophic protists (Supplementary Tables 1 and 2). Keeping in mind that the number of sampled stations was more than twice as high, the eukaryotic richness in the euphotic zone of marine waters was also more than twice as high (~110,000 OTUs, the majority belonged to heterotrophic protistan groups) when compared to our deep-sea eukaryotic OTUs. Within the Malapina expedition, targeting the eukaryotic life in the deep water column, ~42,000 OTUs associated with picoeukaryotes could be recovered. Prostist richness in other benthic environments was lower when compared with our benthic deep-sea dataset. In the neotropical rainforests protist richness was much lower (~26,000 protist OTUs). Within marine coastal sediments, the protist diversity was found to be ~6000 OTUs. Comparing the number of eukaryotic deep-sea deep-sea OTUs with other environments shows that the diversity of deep-sea assemblages is higher than that of coastal sediment communities and has a comparable size as the marine pelagic communities. One should keep in mind that comparing our observed protist richness with studies from other environmental biomes is difficult due to the fact that some of them used different target regions and filtering/clustering methods. Therefore, we compared the eukaryotic community of the deep seafloor with that of de Vargas et al. from the sunlit ocean where similar filtering and clustering methods were used.

**Taxonomic assignment and link to deep-sea cultivable protists.** For the taxonomic assignment of sequences, we used a reference database called V9_DeepSea (Zenodo, Supplementary Fig. 1 and Data 2). Besides sequences from the Protist Ribosomal Reference database PR2 v4.11.1 (ref. 34), we included 102 in-house Sanger-sequenced strains (see Supplementary Data 2) of which the majority was isolated from deep-sea (57 strains) and marine surface waters (33 strains). We could recover 31 strains of these 102 cultivated marine protists (i.e. 21 deep-sea strains, 8 surface water strains) belonging to 20 species (19 OTUs, ~170,000 reads) with a V9 sequence similarity of 100% including Stramenopiles (bicosoecids, placidids), Discoba (kinetoplastids), Alveolata (ciliates), Obazoa (choanoflagellates), Rhizaria (cerozoans), and Cryptista (cryptophyceans). This highlights the importance of cultivation-based approaches for detailed molecular and morphological description of marine protists and the proper assignment of reads produced by NGS methods. Adding sequences from our strains increased the number of taxonomically assignable OTUs by 6% (273 OTUs, ~300,000 reads) with sequence similarities ranging from 80 to 100%. Overall, only 2.4% of our total protist OTUs were 100% identical to reference sequences (on average 90.4% similarity). This points to a specific and genetically distinct protist fauna in deep-sea sediments (Fig. 1d, e), which has previously been reported from studies targeting specific groups or using a smaller sampling size.

**High reference sequence similarity of diplonemids.** The Discoba had a higher proportion of OTUs with an overall higher similarity to reference sequences as compared to the other deep-sea protistan groups within our dataset. From the 7111 Discoba OTUs (sequence similarity ≥94%) ~89% (6300 OTUs) were associated with diplonemids. Pelagic diplonemids are depth stratified and more abundant and diverse in the deep ocean. The majority of the diplonemids are thought to have a parasitic lifestyle and one possibility is that they might be not as host specific as it is known for other protists (e.g. gregarines in
Another possibility could be that their recovery in molecular surveys might be better than for other protist lineages resulting in an over-representation in public databases. But these are only thoughts and further detailed studies of this interesting and important taxon are necessary.

When assessing protist diversity and saturation in our sampling effort, we could recover 71% of the total estimated sampling saturation of deep-sea heterotrophic protist OTUs by using incidence-based estimators (Fig. 1f). When considering the read abundance, saturation was nearly reached (Supplementary Fig. 2). We found great differences in OTU richness between the bathyal, the abyssal, and the hadal regions with only a small proportion of shared OTUs (Fig. 1f, g). Over half of them could only be detected in abyssal sediments, a result that might be biased by the higher sampling number of abyssal sites (Fig. 1g).

Deep-sea eukaryotic life compared with diversity in the sunlit ocean. A comparison with the Tara Oceans metabarcoding survey of eukaryotic diversity in the world sunlit ocean revealed a fundamental difference with only a small proportion of shared OTUs with our benthic deep-sea dataset (Fig. 2 and Supplementary Fig. 3B). We found 11 hyperdiverse deep-sea protist lineages (containing ≥1000 OTUs, Fig. 2c), particularly within the Discoba (diplonemids, kinetoplastids), Rhizaria (foraminiferans), Alveolata (dinoflagellates, MALV II, MALV I, ciliates), and cryptophyceans, which accounted together for more than half of all OTUs (~56%), but only 19% of the reads. A much higher richness characterized the deep-sea diplonemid (~27.7% of the total OTUs, ~4.6% of the total reads) and kinetoplastid flagellates (~3.8% of the total OTUs, ~1.4% of the total reads), foraminiferans (~8.2% of the total OTUs, ~2.5% of the total reads), ciliates (~6.7% of the total OTUs, ~2% of the total reads), and cryptophyceans (~2.4% of the total OTUs, ~1.8% of the total reads).

Sampling saturation and differences between depth zones. When assessing protist diversity and saturation in our sampling effort, we could recover 71% of the total estimated sampling saturation of deep-sea heterotrophic protist OTUs by using incidence-based estimators (Fig. 1f). When considering the read abundance, saturation was nearly reached (Supplementary Fig. 2). We found great differences in OTU richness between the bathyal, the abyssal, and the hadal regions with only a small proportion of shared OTUs (Fig. 1f, g). Over half of them could only be detected in abyssal sediments, a result that might be biased by the higher sampling number of abyssal sites (Fig. 1g).

insects). Another possibility could be that their recovery in molecular surveys might be better than for other protist lineages resulting in an over-representation in public databases. But these are only thoughts and further detailed studies of this interesting and important taxon are necessary.

Deep-sea eukaryotic life compared with diversity in the sunlit ocean. A comparison with the Tara Oceans metabarcoding survey of eukaryotic diversity in the world sunlit ocean revealed a fundamental difference with only a small proportion of shared OTUs with our benthic deep-sea dataset (Fig. 2 and Supplementary Fig. 3B). We found 11 hyperdiverse deep-sea protist lineages (containing ≥1000 OTUs, Fig. 2c), particularly within the Discoba (diplonemids, kinetoplastids), Rhizaria (foraminiferans), Alveolata (dinoflagellates, MALV II, MALV I, ciliates), and cryptophyceans, which accounted together for more than half of all OTUs (~56%), but only 19% of the reads. A much higher richness characterized the deep-sea diplonemid (~27.7% of the total OTUs, ~4.6% of the total reads) and kinetoplastid flagellates (~3.8% of the total OTUs, ~1.4% of the total reads), foraminiferans (~8.2% of the total OTUs, ~2.5% of the total reads), ciliates (~6.7% of the total OTUs, ~2% of the total reads), and cryptophyceans (~2.4% of the total OTUs, ~1.8% of the total reads).
Deep-branching eukaryotic taxonomic groups observed in the deep sea. Taxonomic groups include supergroups (see also Fig. 1), division (see also Fig. 3), and class/order (this figure) level as given in the PR2 database classification. Taxonomic groups, which are used in this figure (supergroups) and Fig. 3 (divisions), are colored. Asterisks indicate that >90% of reads within this lineage had a 80–85% sequence similarity to reference sequences. Deep-sea eukaryotes abundance expressed as numbers of rDNA reads. Scale of axis ranges from 0 to 1 million reads. Taxonomic groups with more than 1 million reads exceed the axis and are indicated with dark-purple bars and the number of reads is written within the bars (nine most abundant lineages with >1 million reads). Deep-sea eukaryotes’ richness expressed as numbers of OTUs. Scale of axis ranges from 0 to 1000 OTUs. Taxonomic groups containing >1000 OTUs exceed the axis and are indicated with dark-blue bars and the number of OTUs is written within the bars (11 hyperdiverse lineages containing >1000 OTUs). Percentage of rDNA reads and OTUs (calculated within each taxonomic group itself) with various ranges of sequence similarity (80–85%, 85–90%, 90–95%, 95–<100%, and 100%) to reference sequences. Sunlit ocean eukaryotic richness expressed as number of OTUs from the Tara Oceans global metabarcoding dataset. Taxonomic groups containing >1000 OTUs exceed the axis and are indicated with red bars and the number of OTUs is written within the bars.

reads), as compared to their surface water relatives (Fig. 2c, c). Richness was by far the highest in diplonemids, a feature that has also been observed in deep layers of the pelagic realm35 indicating their potential importance for deep ocean ecosystems not only in the pelagial (2.1% of the total read abundance), but also in deep-sea sediments (4.6% of the total read abundance). Local sedimentation of debris/marine snow as well as dark inorganic carbon fixation19,30 have challenged our understanding of organic carbon available for deep-sea microbial communities40–42. The high number of reads associated with phototrophic species within our deep-sea dataset, e.g., within the Archaeaplastida (mainly green microalgae from the family of Chlorococcales) and the Cryptophyta (mainly Cryptomonadales) might be due to sinking cells from surface waters down to the deep sea. On the other hand, the majority of them only had a low sequence similarity of 80–85% to Archaeaplastida and Cryptophyta in the reference database and might be associated to unknown taxonomic groups especially adapted to deep-sea conditions. Several studies have reported the presence of phototrophic protists in deep waters, suggesting that mixotrophy could help them to thrive in the aphotic zone43. There is also the possibility for those species to enter an encysted state upon sinking44.

*Cafeteria burkhardae* as potential global player in the marine realm. Particularly striking was the extremely high read abundance of bicosoecids, including one OTU (~2.6 million reads) 100% identical to the species *C. burkhardae* (Fig. 2b). *C. burkhardae* was detected at all investigated deep-sea sites, matching our observation of the dominance of this species during cultivation-based approaches of deep-sea protists from several deep-sea expeditions45. One could argue that the occurrence of one OTU in all samples might be due to cross-sample contamination. However, sediment samples were sampled during different expeditions and the sediment was processed and analyzed separately in the laboratory. Thus, a cross-sample contamination seems to be unlikely. Interestingly, *C. burkhardae* made also a majority of the bicosoecid reads from the Tara Oceans surface plankton metabarcoding dataset14,45 as well as within Malaspina metabarcoding targeting the water column from surface to bathypelagic waters. These occurrences in both pelagic and deep benthic ecosystems, together with recent experiments demonstrating survival at high hydrostatic pressures47, underline the cosmopolitan distribution of selected protist species in the world’s oceans across extreme environmental conditions.

Distributional patterns of deep-sea protist richness on small and large spatial scales. Each of the 27 sediment samples from the 11 investigated regions showed a highly distinct heterotrophic protist community (Fig. 3a) with the highest heterotrophic protist richness within the Alveolata, Discoba, and Rhizaria in each sediment sample (Fig. 3b), a pattern that has also been reported from previous bathyal and abyssal deep-sea floor studies20,24,25. However, diplonemids and dinoflagellates (mainly representatives of the marine alveolate (MALV) clusters) dominated the diversity at the deep seafloor (Fig. 3b). Stramenopiles (mainly bicosoecids) clearly dominated in regards of read abundances followed by high read abundances within the Alveolata, Discoba, and Rhizaria (Supplementary Fig. 4). The relative proportion of reads per sampling site and division level showed subtle differences (Supplementary Figs. 4 and 5). While the three bathyal stations from the Pacific Ocean formed a highly supported cluster, the two hadal regions from the North Atlantic Ocean clustered together with abyssal stations from the Atlantic (winter expedition) and Pacific Ocean (Fig. 3a). Furthermore, we observed distinct protist communities on much smaller spatial scale (stations NA4, NA8, NA9) from sediment samples extracted just a few meters apart from each other (Fig. 3a and Supplementary Fig. 6). This could be explained by the sediment patchiness at the abyssal seafloor, which can be very high as indicated by metazoan grazing tracks, or falls of larger organic particles (e.g. debris of macrophytes, wood or dead organisms from the pelagial; Fig. 1c). The high number (~60% OTUs) of heterotrophic protists being unique to one sediment sample and the low percentage (0.6% OTUs) of heterotrophic protists shared between all samples point to the potential of highly endemic protist communities in deep-sea sediments (Fig. 3c). Such a pattern has also been found for benthic deep-sea prokaryotes in different deep-sea basins4 and deep-sea Foraminifera46. The majority of “unique” heterotrophic protist OTUs had only a few reads, and several with 10–200 reads (Supplementary Fig. 7). The majority of the heterotrophic protist OTUs was represented by 16–64 reads (Supplementary Fig. 8). There was a high variation of unique protist OTUs and their taxonomic assignment per sampling site and depths (Supplementary Fig. 9). One could argue that this high dissimilarity and clustering could be the result of the high number of unique OTUs with low read abundances (Supplementary Fig. 7). However, even very conservative filtering steps (OTU abundances ≥50 or ≥100 reads) revealed a similar clustering of stations and still resulted in a great dissimilarity between protist communities on both small and large spatial scale (Supplementary Fig. 10).

Feeding modes of deep-sea protists. Abyssal plains are not flat or featureless, but rather strongly influenced, both by the underlying plate geology and subsequent sedimentary processes49, which could explain that we did not observe a homogeneous deep-sea diversity pattern. The majority of taxa recorded from the different deep-sea regions belonged either to bacterivorous groups (e.g. discicristates, stramenopiles, most cercomonads, several ciliates, foraminifers, lobose amoebae9, or forms parasitizing other eukaryotes (e.g. perkinseans, apicomplexans, and most MALV
Role of protists in the deep-sea food web. Our results provide a unique view on the genetic diversity and specificity of deep-sea protist communities and point to their very important though still underestimated role in shaping seafloor communities. The estimate of heterotrophic protist species richness (Fig. 1f) for the samples from the deep-sea floor was one order of magnitude higher than that of metazoans, a tendency also obtained from the pelagic (Fig. 2 and ref. 11). According to our data, protist communities comprise representatives of different trophic levels consisting of feeders on bacteria and archaeans, on detritus, dissolved organic carbon, small eukaryotes as well as parasites of protists and metazoans (illustrated in Fig. 3d). Thus, a major part of organic carbon in deep-sea sediments is channeled not only via long known deep-sea inhabiting foraminiferans but also through an unsuspected and extensive variety of small naked heterotrophic protists with different functions. These deep-sea protists form an essential link to metazoans via several trophic interactions between microbial and macrobial components derived from their molecular diversity. Highly diverse and abundant protists are embedded in deep-sea food webs on different trophic levels as feeders on prokaryotes and particulate and dissolved organic matter, as predators, as well as parasites of metazoans and protists.

Fig. 3 Distributional patterns and community composition of deep-sea heterotrophic protists. a Dendrogram cluster showing the similarity (Jaccard index) of heterotrophic protist communities of the 27 sediment samples in regard to species richness based on incidence-based data (presence/absence) using UPGMA clustering. The five clusters are supported by moderate to high bootstrap values. Multiple sediment samples were analyzed separately at stations marked with an asterisk for the investigation of small-scale distribution patterns. b Relative proportion of OTUs within the 27 deep-sea sediment samples related to the major taxonomic groups. Taxonomic groups (corresponding to division level in the PR2 database classification) are only separately shown, when the number of OTUs reached more than 1% within each sample. Otherwise, OTUs were clustered together into “Others”. “Unknown/Uncertain” OTUs have been either assigned to several taxonomic division levels or to sequences taxonomically assigned only to Eukaryota. c Relative proportion of shared (0.6%) and unique (57.6%) OTUs (heterotrophic protist richness) within all 27 sediment samples (obtained from 20 deep-sea stations). d Hypothetical deep-sea food web illustrating the generally ignored complex trophic interactions between microbial and macrobial components derived from their molecular diversity. Highly diverse and abundant protists are embedded in deep-sea food webs on different trophic levels as feeders on prokaryotes and particulate and dissolved organic matter, as predators, as well as parasites of metazoans and protists.

taxa among dinoflagellates). Deep-sea studies have observed protist grazing, indicating the potential of substantial reductions of the prokaryote standing stock due to protist grazing. However, the quantification of protist grazing in the deep sea still needs to be investigated. Members of several groups are known to feed also on other protists (e.g. several ciliates). Global and local differences in prokaryote diversity and abundance as a main food source, endemcity of macrofauna as important host for putative parasites might, amongst many other environmental factors varying across deep-sea habitats, shape deep-sea protist communities on small and large spatial scale. The impact of multiple processes and possible interactions, which might operate at the same time resulting in unique protist communities on the abyssal and hadal seafloor, still needs to be resolved.
addition, due to the parasitic lifestyle of many deep-sea protists (e.g. diplomonds, MALV II [35]), they might also act as important remineralizers of other protists and metazoans channeling carbon back to prokaryotes [34, 35]. Ammonia-oxidizing Archaea have shown to dominate microbial communities in abyssal clay in the North Atlantic Ocean [36]. Due to their high abundances, Archaea should also be considered as a potential food source for deep-sea protists. In a recent study, it was shown that the probably most common-metaheterotrophic flagellate taxon *Cafeteria* feeds on Archaea [37]. In addition, several protists from freshwater systems have been found to positively select Archaea as food source over Eubacteria [38]. New techniques and large-scale studies, as well as long-term surveys/time series, may further elucidate the diverse composition of seafloor communities over both space and time, which is critical to our understanding of global biogeochemical cycles in the Earth’s largest habitat.

**Methods**

**Sampling.** The highly diverse species composition of heterotrophic protists in the deep sea demanded a combination of culture-independent (metabarcoding) and culture-dependent methods [39]. Isolation and cultivation of deep-sea protists were carried out for 102 strains to create an extended reference database (see below). In addition, several studies were conducted most of the study area to test their survival at deep-sea pressure to check for their potential to belong to an active deep-sea community [28, 45, 47]. During four different expeditions in the Pacific and Atlantic Ocean on board of the research vessels *R/V Sonne* (SO237, SO223T and *R/V Meteor*), M79/1, M79/1) sediment samples from 20 different stations (3 abyssal, 2 hadal, 11 deep-sea basins/regions were collected using a Multi-Corer (MUC) (Supplementary Data 1). Temperature at the deep sea ranged between 2 and 4°C; salinity was about 36 PSU. Detailed data on the conditions are available from published cruise reports of M139 (https://doi.org/10.2312/cm_m139), M79/1 (https://doi.org/10.2312/cm_m79_1), SO237 (urn:nbn:de:gbv:46-00102735-15), and SO237 (https://doi.org/10.3829/EGOMAR_REP_NS_23_2013). Subsamples of the MUC were system taken from the upper 2 mm sediment layer by means of a sterile syringe. Only tubes with undisturbed sediment and overlying water were used for further analyses. For 17 stations (SA1–SA3, P1–P5, NA1–NA3, NA5–NA7, NA10–NA12) taken during expeditions SO237, SO223T, and M79/1, three replicate sediment samples from three MUCs (corresponds to one to two cores per MUC) were taken in total per station (Supplementary Data 1). For the three stations (NA4*, NA8*, NA9*) from the expedition M139, two to four replicates from three MUCs (corresponds to one to two cores per MUC) per station were taken (Supplementary Data 1). Samples were either fixated with 70% molecular biology graded ethanol and stored at −80°C or directly deep frozen at −80°C.

**DNA extraction, PCR amplification, and sequencing of 18S V9 rDNA metabarcodes.** Ethanol preserved sediments were treated in a speed vac for 45 min at 45°C to evaporate the ethanol. For 17 stations (see above) taken during expeditions SO237, SO223T and the environmental DNA was extracted from 1.5 g sediment of each replicate sample (a total of 1.5 g per station) using the DNeasy Power Lyzer Power Soil DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol (Supplementary Data 1). For the three stations from the expedition M139 (see above) the environmental DNA was extracted from an adapted sample volume using the same kit (Supplementary Data 1). Prior to the kit, sediment samples were pre-washed with three washing solutions to improve the success of DNA amplification by PCR in marine sediments [37]. Total DNA was quantified using a Nanodrop Spectrophotometer. For sediment samples taken during the expeditions SO237, SO223T, and M79/1, DNA of the three replicates per station were extracted in one concentration per PCR. Sediment samples from the expedition M139 were separately PCR amplified without prior pooling of DNA per station to investigate small-scale patterns of deep-sea protist diversity, PCR amplifications of the hypervariable V9 region of the 18S rDNA gene was performed with the Phusion High-Fidelity DNA Polymerase (Thermofisher) and the forward/reverse primer pair 1389F (5′-TGG TAC ACA CCG CCC-3′) and 1510R (5′-CCT TCY GTA GGT TCA CCT AC-3′) [38]. The PCR mixtures (25 µl final volume) contained 5 ng of total DNA template with 0.35 µM final concentration of each primer, 3% of DMSO, and 2× of GC buffer Phusion Master Mix (Finnzymes). PCR amplifications (98°C for 30 s; 25 cycles of 10 s at 98°C, 30 s at 57°C, 30 s at 72°C; and 72°C for 10 min) of all samples were carried out with a reduced number of cycles to avoid the formation of chimeras during the plateau phase of the reaction, and in triplicates (M139) or six replicates (SO237, SO223T, and M79/1) in order to smooth the intra-sample variance while obtaining sufficient amounts of amplicons for illumina sequencing. PCR products were checked by gel for amplification length. PCR products were pooled and purified using the PCR Purification Kit (Jena Bioscience, Jena, Germany). Bridge amplification and paired-end (2 × 150 bp) sequencing of the amplified fragments was performed using an Illumina Genome Analyzers IIX system at the Cologne Center of Genomics (CCG).

**Reference database.** Due to the lack of reference sequences for the V9 region in common public databases (e.g. NCBI, PR2), we generated a composite of the V9 region of 102 marine protist strains of the Heterotrophic Flagellate Collection Cologne (HFCC), of which several have not been published yet (Supplementary Data 2). Subsamples of a few milliliters of the sediment of the MUC samples (see above) suspension were cultivated in 50 ml tissue culture flasks (Sarstedt, Nümbrecht, Germany). Isolation was carried out using a micro-manipulator robot or microtiter plates (liquid aliquot method) [39]. All cultures were supplied with sterilized seawood or wheat grains as an organic food source for autochthonous bacteria. After isolation, the strains were cultivated in 50 ml tissue culture flasks (Sarstedt, Nümbrecht, Germany) filled with 30 ml Schmaltz-Pratt medium [34] (35 PSU; per liter 28.15 g NaCl, 0.67 g KCl, 5.51 g MgCl2 • 6 H2O, 6.92 g of KNO3, 1.45 g CaCl2 • 2 H2O, 0.10 g KH2PO4 • 3 H2O). The cultures were stored at 1°C in the dark. Isolates were characterized morphologically using AVEC high-resolution video microscopy and electron microscopy. For molecular studies, protistan cultures were concentrated by centrifugation (4000 × g, 20 min at 4°C, Megafuge 20K, Heraeus Instruments). Genomic DNA of each isolated protist strain was extracted using the Quick-gDNA™ Mini Prep Kit (Zymo Research, USA). We amplified a long sequence from the 18S rDNA to the 28S rDNA with the primers 18S For (5′-AAC CTG GTT GAT CCT GCC AGT-3′, ref. 61) binding at the beginning of the 18S rDNA and either NLR1126/22 (5′-GCT ATC TG GTG CAA GTG TCC G-3′, ref. 62) or NLR0989/24 (5′-AGG CAA TCC TTT GCC GGA AGT TAC-3′, ref. 63) binding in the 28S rDNA. PCR reactions were performed in 25 µl PCR reaction mixtures containing 5.5 µl ddH2O, 1.5 units TQA (Mastermix, VWR Germany), 2 µl DNA and 2.5 µl of each primer (forward and reverse) at a final concentration of 1.6 mM. The PCR conditions for amplifying the 18S-28S rDNA region were therefore generated: 72°C for 10 min. For bodonid strains, a different primer combination was used: 18S For Bodo (5′-CTG GTT GAT TCT GCC AGT-3′, ref. 65) + NLR1126/22 (5′-GCT ATC TG GTG CAA GTG TCC G-3′, ref. 62). Internal primers were used for sequencing (Supplementary Table 2). We established a new reference database for *Bodo* by combining the Illumina reads from our database with NCBI’s [41.111 (ref. 41) with the 102 sequences of marine protist strains of the Heterotrophic Flagellate Collection Cologne. Using Cutadapt [44], the final in-house reference database, called V9.DeepSea [47], was trimmed to the V9 region.

**Downstream analyses and taxonomic assignment.** Our bioinformatic pipeline (adapted from Frederic Mahe, https://github.com/frederic-mahe/swarm/wiki/Pred-v-metabarcoding-pipeline) allowed filtering of high-quality V9 rDNA reads/ amplicons and their clustering into OTUs (Supplementary Fig. 1). HiSeq sequencing resulted in ~223 million raw reads. Overlapping reads were assembled via VSEARCH v2.13.4 (ref. 64) using fastq_mergepairs with default parameters and –fastq_allowmergesterage resulting in ~209 million assembled reads for all stations. Paired reads were retained for downstream analyses if they contained both forward and reverse primers and were at least 70 bp long. Reads were quality filtered using cutadap and VSEARCH. Reads from all stations were combined in one file and de-replicated into strictly identical amplicons (metabarcodes) with VSEARCH while the information on their abundance was retained. Low abundance metabarcodes with a read abundance of one and two reads were removed from the dataset prior to clustering using the Python package scikit-metaclasses [45]. OTUs were discarded, when a phylogenetic placement within the kingdom level was not possible. Furthermore, OTUs assigned to Metazoa, Fungi, Archaeplastida, and exclusively phototrophic organisms, including several classes of Ochrophyta (Eustigmatophyceae, Pelagophyceae, Phaeophyceae, Phaeoathamniophyceae, Pingoophyceae, Raphidophyceae, Synurophyceae, Xanthophyceae, Bacillariophyceae, Chrysophyceae), Bacillariophyta, Filosa-Chlorarachnea within the cercozoans as well as the Cryptomonads within the Cryptophyta, were removed (Supplementary Table 1), resulting in a phylogenetic dataset of 40,623 heterotrophic protist OTUs (S2, S3). Except for Fig. 2, which compares the eukaryotic life of the deep sea with that of the eutrophic zone, we used the final heterotrophic protist dataset for all graphs.
our deep-sea NGS dataset, we downloaded the available “Database W4” containing the total V9 rDNA information organized at the metadata (unique sequences) level from the Tara Ocean project website (http://taraoceans.sb-roscoff.fr/EukDiv/extraction). This table contained all the 1,521,174 metacodes from the 47 sampled stations and the abundance information per metacode (in total 568,976,385 reads). We extracted this information together with the V9 sequence metacode and pooled these Tara Ocean metacodes with our deep-sea metacodes of 20 stations together in one file. Dereplication, clustering of metacodes in OTUs using Swarm, assigning the taxonomy of the representative OTU sequence to the V9-DeepSea reference database, and filtering (see steps in downstream analyses and taxonomic assignment) led to a final dataset of 123,120 eukaryotic OTUs and 589,807,407 reads. Taxonomic groups with more than 1,000 OTUs were here defined as hypervariable (see Fig. 2), as conducted within the framework of Tara Ocean41.

Statistics and reproducibility. Stampa plots were applied to visualize our taxonomic coverage assessment to the reference database sequences. A high proportion of environmental reads assigned with a high similarity to references indicates a good coverage, while low similarity values indicate a lack of coverage42. Statistical analyses were conducted with R v.3.5.2 and graphs were created with the R package “ggplot2”43. The alpha diversity of each of the stations was assessed based on several different indices with regard to species (OTU) richness and their evenness of distribution (read abundance) including the Shannon Wiener Index, effective number of species, Simpson’s Index, Pielou evenness, and Chao1 index (see Supplementary Table 2) implemented in the vegan package44. The total species richness and the species richness per depth region (bathyal, abyssal, hadal) were estimated with the incidence-based coverage estimator (ICE) using the “fossil” package. As we expected many rare species in deep-sea protist communities, we used ICE to appropriately estimate asymptotic species richness from datasets with many rare species45,46. Rarefaction curves were additionally used in order to investigate the degree of sample saturation by calling the function “trafezy” implemented in the “vegan” package71. We fit the Preston’s log-normal model to abundance (read) data by calling the function “prestonfit” within the “vegan” package, which groups species frequencies into doubling octave classes and estimates species richness with special emphasis on foraminifers and naked protists. Eur. J. Protistol. 75, 125721 (2020).

Caron, D. A. et al. Probing the evolution, ecology and physiology of marine protists using transcriptomics. Nat. Rev. Microbiol. 15, 6–20 (2017).

Jürgens, K. & Massana, R. In Microbial Ecology of the Oceans (ed. Kirchman, D. L.) 383–441 (Wiley, 2008).

Morán, M. A. The global ocean microbiome. Science 350,aa4855 (2015).

de Vargas, C. et al. Eukaryotic plankton diversity in the sunlit ocean. Science 348, 1261605 (2015).

Azam, F. et al. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257–263 (1983).

Patterson, D. J., Nygaard, C., Steenbeek, G. & Turley, C. M. Heterotrophic flagellates and other protists associated with oceanic detritus throughout the water column in the mid North Atlantic. J. Mar. Biol. Assoc. UK 73, 67 (1993).

Worden, A. Z. et al. Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. Science 347, 1237594 (2015).

Arndt, H. et al. In The Flagellates—Unity, Diversity and Evolution (eds Leadbeater, B. S. & Green, J. C.) 240–268 (Taylor & Francis Ltd, 2000).

Boenigk, J. & Arndt, H. Bacteriovory by heterotrophic flagellates: community structure and feeding strategies. Antonie van. Leeuwenhoek 81, 465–480 (2002).

Caron, D. A., Davis, P. G., Madin, L. P. & Sieburth, J. M. Heterotrophic bacteria and bacterivorous protozoa in oceanic macroaggregates. Science 218, 795–797 (1982).

Gooday, A. J. Biological responses to seasonally varying fluxes of organic matter to the ocean floor: a review. J. Oceanogr. 58, 303–332 (2002).

Molari, M., Manini, E. & Dell’Anno, A. Dark inorganic carbon fixation sustains the functioning of benthic deep-sea ecosystems. Glob. Biogeochem. Cycles 27, 212–221 (2013).

Pasulka, A. et al. SSU-rRNA gene sequencing survey of benthic microbial eukaryotes from Guaymas Basin hydrothermal vent. J. Eukaryot. Microbiol. 66, 637–653 (2019).

Stoeck, T., Taylor, G. T. & Epstein, S. S. Novel eukaryotes from the permanently anoxic Cariaco Basin (Caribbean Sea). Appl. Environ. Microbiol. 69, 5656–5663 (2003).

Pachadaki, M. G. et al. In situ grazing experiments apply new technology to gain insights into deep-sea microbial food webs. Deep Sea Res. Part II Top. Stud. Oceanogr. 129, 223–231 (2016).

Corrodi, T., Barrenechea, I., Lejzerowicz, F., Reo, E. & Pawlowski, J. Benthic foraminiferal DNA metacodes significantly vary along a gradient from abyssal to hadal depths and between each side of the Kuril-Kamchatka trench. Proc. Oceanogr. 178, 102175 (2019).

Pawlowski, J. et al. Eukaryotic richness in the abyss: insights from pyrotag sequencing. PLoS ONE 6, e18169 (2011).

Scheckenbach, F., Hausmann, K., Wylezich, C., Weitere, M. & Arndt, H. Large-scale patterns in biodiversity of microbial eukaryotes from the abyssal sea floor. Proc. Natl Acad. Sci. USA 107, 115–120 (2010).

Pernice, M. C. et al. Parasites dominate hyperdiverse soil protist communities in Neotropical rainforests. FEMS Microbiol. Ecol. 92, evo2016 (2016).

Schoenle, A., Hohlfeld, M., Hermanns, K. & Arndt, H. V9_DeepSea (Deep Sea Reference Database) [Data set]. Commun. Biol. 3(1), 10.5281/zenodo.3405675 (2021).
34. Guillou, L. et al. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41, D597–D604 (2013).

35. Fleglonova, O. et al. Extreme diversity of diplomed eukaryotes in the ocean. Curr. Biol. 26, 3060–3065 (2016).

36. Clifton, R. E., Janovy, J. & Percival, T. J. Host stadium specificity in the gregarine parasitizing Tenebrio molitor. J. Parasitol. 78, 334–337 (1992).

37. Leander, B. S. Marine gregarines: evolutionary prelude to the apicomplexan radiation? Trends Parasitol. 24, 60–67 (2008).

38. del Campo, J. et al. Assessing the diversity and distribution of apicomplexans in host and free-living environments using high-throughput amplicon data and a phylogenetically informed reference framework. Front. Microbiol. 10, 2373 (2019).

39. Herndl, G. J. & Reinthaler, T. Microbial control of the dark end of the biological pump. Nat. Geosci. 6, 718–724 (2013).

40. Baker, P. et al. Potential contribution of surface-dwelling Sargassum algae to deep-sea ecosystems in the southern North Atlantic. Deep-Sea Res. Part II Top. Stud. Oceanogr. 148, 21–34 (2018).

41. Boenf, D. et al. Biological composition and microbial dynamics of sinking particulate organic matter at abyssal depths in the oligotrophic open ocean. Proc. Natl Acad. Sci. USA 116, 11824–11832 (2019).

42. Krause-Jensen, D. & Duarte, C. M. Substantial role of macroalgae in marine primary productivity. Nat. Geosci. 10, 102–107 (2017).

43. Xu, D. et al. Pigmented microbial eukaryotes fuel the deep sea carbon pool in the tropical Western Pacific Ocean. Environ. Microbiol. 20, 3811–3824 (2018).

44. Agusti, S. et al. Ubiquitous healthy diatoms in the deep sea. Environ. Microbiol. 17, 906–920 (2015).

45. Schoenle, A. et al. Global comparison of bicosoecid flagellates isolated from the meromictic Lake Siusglets. Protist 166, 409–421 (2021).

46. Massana, R. et al. Gene expression during bacterivorous growth of a widespread marine heterotrophic flagellate. ISME J. 15, 154–167 (2021).

47. Žáviláč, S. et al. Survival of marine heterotrophic flagellates isolated from the surface and the deep sea at high hydrostatic pressure: literature review and own experiments. Deep Sea Res Part II Top. Stud. Oceanogr. 148, 251–259 (2018).

48. Lecroq, B. et al. Ultra-deep sequencing of foraminiferal microbarcodes unveils hidden richness of early monothalamous lineages in deep-sea sediments. Proc. Natl Acad. Sci. USA 108, 13177–13182 (2011).

49. Devey, C. W. et al. Habitat characterization of the Vema Fracture Zone and Puerto Rico Trench. Deep-Sea Res. Part II Top. Stud. Oceanogr. 148, 7–20 (2018).

50. Levin, L. A. & Sibuet, M. Understanding continental margin biodiversity: a new imperative. Annu. Rev. Mar. Sci. 4, 79–122 (2012).

51. Gooday, A. J. In Encyclopedia of Ocean Science (eds Cochran, J. et al.) 684–705 (Elsevier, 2019).

52. Stoll, H. & Wirth, O. The influence of hydrostatic pressure on the behaviour of three species of heterotrophic flagellates isolated from surface and deep-sea sediments of the South Atlantic based on SSU rDNA. Aquat. Microb. Ecol. 38, 239–247 (2005).

53. Park, J. S. & Simpson, A. G. B. Characterization of halotolerant Bacteroidales and Planctomycetes (Stramenopiles) that are distinct from marine forms, and the phylogenetic pattern of salinity preference in heterotrophic stramenopiles: novel halotolerant heterotrophic stramenopiles. Environ. Microbiol. 12, 1173–1184 (2010).

54. Moriya, M., Nakayama, T. & Inouye, I. Ularrrhete and rRNA sequence analysis of Wobblia lunata gen. et sp. nov., a new heterotrophic flagellate isolated from the seal sediment. Protist 151, 41–55 (2000).

55. Žáviláč, S. et al. Influence of hydrostatic pressure on the behaviour of three ciliate species isolated from the deep sea. Mar. Biol. 167, 63 (2020).

Acknowledgements
We are very grateful to the Capt. Oliver Meyer, Uwe Pahl, Rainer Hammacher, and the scientific and technical crews for valuable help during sampling and the excellent support during the expeditions S023T, S0237, M79/1, and M139. We thank Rosita Bieg, Bri-gitte Graf, and Barbıl Jendral (University of Cologne, Germany) for very valuable technical support. This work was supported by grants from the Federal Ministry of Education and Research (BMBF; ProtAbrys 03G0237B and 02WRM1364) and by the German Research Foundation (DFG; AR 288/5, 10, 15, 23; MerFit 17-97; MerFit 17-11; CRC 1211 B02/ 03 26823662) to H.A.; C.d.V. was supported by the French Government “Investissements d’Avenir” program OCEANOMICS (ANR-11-BTBR- 0008); F.N. was supported by German Research Foundation (FN 10973).

Author contributions
A.S., M.H., F.N., and H.A. were involved in the sampling of deep-sea sediment. M.H., K.H., H.A., F.N., and A.S. were involved in the cultivation and sequencing of marine protists for the reference database. M.H. and A.S. conducted the DNA extraction of sediments. A.S. conducted the bioinformatic analyses. F.M. and C.d.V. contributed data on from Tara Oceans and bioinformatics expertise. A.S. and H.A. wrote the manuscript. All authors reviewed the manuscript.

Funding
Open Access funding enabled and organized by Project DEAL.
Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s42003-021-02012-5.

Correspondence and requests for materials should be addressed to A.S. or H.A.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021