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Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western North Atlantic (Sargasso Sea and Gulf Stream)

Peter D. Countway,[1]* Rebecca J. Gast,[2] Mark R. Dennett,[2] Pratik Savai,[1] Julie M. Rose[1] and David A. Caron[1]

[1]*Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.*
[2]*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA.*

Summary

Protistan diversity was characterized at three locations in the western North Atlantic (Sargasso Sea and Gulf Stream) by sequencing 18S rRNA genes in samples from euphotic (≤ 125 m) and bathypelagic depths (2500 m). A total of 923 partial-length protistan sequences were analysed, revealing 324 distinct operational taxonomic units (OTUs) determined by an automated OTU-calling program set to 95% sequence similarity. Most OTUs were comprised of only one or two sequences suggesting a large but rare pool of protistan diversity. Many OTUs from both depth strata were associated with recently described novel alveolate and stramenopile lineages while many OTUs from the bathypelagic were affiliated with Acantharea, Polycystinea and Euglenozoa and were not observed in euphotic zone libraries. Protistan assemblages from the euphotic zone and the deep sea were largely composed of distinct OTUs; only 28 of the 324 protistan OTUs were detected in both shallow and deep sea clone libraries. The diversity of protistan assemblages in the deep sea was distinctly lower than the diversity of euphotic zone assemblages. Protistan assemblages from the Gulf Stream were the most diverse for either depth strata. Overall, protistan assemblages from different stations but comparable depths were more similar than the assemblages from different depths at the same station. These data suggest that particular groups of protistan OTUs formed distinct 'shallow' and 'deep-sea' assemblages across widely spaced oceanic locales.

Received 22 August, 2006; accepted 15 December, 2006. *For correspondence. E-mail countway@usc.edu; Tel. (+1) 213 821 2123; Fax (+1) 213 740 8123.

Introduction

Microbial eukaryotes (protists) fulfil diverse functional roles and are essential members of marine microbial food webs (Pomeroy, 1974; Azam *et al*., 1983). Protists have been found at virtually all depths in the water column from the surface to the deepest reaches of the oceans. Phototrophic protists make significant contributions to global carbon fixation (Cullen, 2001) while phagotrophic forms consume large amounts of microbial biomass, serving as energetic links to higher trophic levels (Sanders *et al*., 2000; Sherr and Sherr, 2002). Substantial fractions of the biomass consumed by phagotrophic protists are returned to the environment as labile compounds, which are utilized by bacteria, archaea and phytoplankton to fuel further production (Caron *et al*., 1988; Barbeau *et al*., 1996). Most ecological studies of marine protistan assemblages have focused on the protists inhabiting the euphotic zone while deep-sea protistan assemblages remain largely uncharacterized. Although protistan primary production is limited to the euphotic zone, delivery of fixed carbon to the deep sea via sinking provides a link between surface-associated and deep-sea detritus-based, microbial food webs (Gooday, 2002).

Protistan assemblages in the deep sea are poorly characterized with respect to species diversity and abundance estimates. The earliest studies of these communities indicated very low abundances of small protists at great oceanic depths (Lighthart, 1969; Burnett, 1981; Alongi, 1987). More recent studies have confirmed these low abundances (generally < 100 cells ml^{-1}) in various deep-sea samples from meso- to abyssopelagic depths (Cho *et al*., 2000; Yamaguchi *et al*., 2002; Arndt *et al*., 2003). However, it is unclear whether the generally lower abundance of deep-sea protists equates to lower biodiversity.

Until recently, the composition of protistan assemblages in the deep sea was known largely from studies at hydrothermal vents (Small and Gross, 1985) or that focused on specific groups, primarily foraminifera (Gooday, 1986). Deep-sea foraminifera have received considerable attention because of their geological significance and morphological diversity. More recently, genetic approaches have begun to document the presence of previously unknown protistan lineages in the deep sea (López-García *et al*.,

2001; Edgcomb *et al*., 2002; López-García *et al*., 2003; Stoeck *et al*., 2003). Although interest in deep-sea protists is increasing, direct comparisons of deep-sea and surface-dwelling protistan assemblages have yet to be performed.

The diversity of natural protistan assemblages from the deep sea and the overlying euphotic zones at stations in the Western North Atlantic were compared by the analysis of 18S rRNA gene sequences obtained by environmental polymerase chain reaction (PCR), cloning and sequencing. Our results indicated that the protistan assemblages of the euphotic zone and deep sea were dominated by distinctly different taxa. Additionally, euphotic-zone assemblages from a particular site were more similar to euphotic-zone assemblages from the other sites than they were to the deep-sea assemblages from the same site. The overall protistan diversity in samples from surface waters was higher than diversity in deep-sea samples, presumably due in part to the presence of phototrophs in surface waters (largely absent from deep-sea samples) and the generally higher rates of energy transfer and biological activity in surface waters.

Table 1. Higher-level taxonomic distribution of protistan OTUs (defined by 95% sequence similarity) and the number of sequences comprising each taxonomic group.

Protistan taxonomy	OTUs	Sequences
Fungi	4	5
Choanoflagellida	1	1
Chlorophyta	4	7
Stramenopiles	24	42
Haptophyceae	4	6
Alveolata; Ciliophora	17	58
Alveolata; Dinophyceae	37	145
Alveolata; group I	38	102
Alveolata; group II	24	71
Alveolata; unclassified	70	138
Rhodophyta	2	3
Cryptophyta	3	4
Cercozoa	1	1
Acantharea	30	68
Polycystinea	24	173
Euglenozoa	34	72
Sticholonchidae	5	18
Telonema	2	9
Total	324	923

Taxonomic arrangement within the table follows the clockwise arrangement of groups depicted in the pie-charts (Fig. 1). Table does not include metazoan sequence data presented in the pie-charts.

Results

Taxonomic distribution of clones

The focus of this study was to investigate the depth and breadth of protistan diversity at the sampling sites by sequencing a relatively large number of clones for each sample. A total of 1098 partial-length rRNA gene sequences were obtained from six clone libraries (3 stations $\times$ 2 depths) after removing poor-quality and potentially chimeric sequences (Table S1). Three sequences were removed from further consideration because of their similarity to streptophytes. The remaining 1095 sequences were deposited in GenBank (accession numbers DQ917930–DQ919024). These 1095 sequences grouped into 344 operational taxonomic units (OTUs) representing approximately species-level distinctions (D.A. Caron *et al*., in preparation). The vast majority of sequences revealed in this study were protistan in origin but metazoans were also detected in all libraries (Fig. 1). Metazoan sequences comprised only ~6% (20 OTUs) of the total OTUs. Removal of metazoan OTUs from the data set resulted in a collection of 324 protistan OTUs (Table 1). The number of protistan sequences in each of the six libraries was fairly constant, ranging from 136 to 170 sequences, depending largely on the sequencing success rates for individual libraries (Table 2).

Protistan sequences from each station and depth were assigned to one of 18 higher-level taxonomic groups based on their BLAST (Altschul *et al*., 1997) identification (Fig. 1; Table 1). Each group comprised no more than 25% of the sequences in a particular library with the exception of polycystines in the deep-sea library from station 1 (Fig. 1A). The distribution of the 324 protistan OTUs in these taxonomic groups ranged from 1 to 70 OTUs per group (Table 1). Alveolate lineages including ciliates, dinoflagellates, group I, group II and unclassified alveolates made up large percentages of most of the shallow and deep libraries (Fig. 1), representing 57% of the detected diversity (186 of 324 OTUs). 'Unclassified Alveolata' contained 70 OTUs, the largest single collection of OTUs identified by BLAST analysis. Stramenopiles were a sizeable component of each shallow library, represented by 42 sequences and 24 OTUs, reflecting the large amount of diversity within this group. The sarcodine groups Acantharea (30 OTUs) and Polycystinea (24 OTUs) were detected at all stations and both depths, but were particularly well-represented in the deep libraries (Fig. 1, Table 1). Euglenozoa were also well-represented in deep libraries (34 OTUs). The remaining groups contained five or fewer OTUs (Table 1) comprising a diverse list of relatively rare phylotypes including members of the newly described protistan phylum, Telonemia (Shalchian-Tabrizi *et al*., 2006).

Rank abundance of OTUs

A rank abundance curve representing the frequency distribution of OTUs was constructed to examine the species richness and evenness of protistan assemblages for the entire set of shallow and deep sequences (Fig. 3). This

Table 2. Protistan diversity estimates for various combinations of the six clone libraries, ranging from the total database of 923 sequences comprising 324 operational taxonomic units (OTUs) to each of the six (station/depth) clone libraries.

Sample set	*N*	OTUs	H′	D_S^{-1}	Chao1 (95% CI)	ACE (95% CI)
Total	923	324	4.9	41.3	694 (574–873)	773 (648–946)
Stn. 1	294	137	4.1	20.1	366 (265–547)	392 (292–555)
Stn. 2	335	154	4.4	38.2	417 (302–620)	391 (299–542)
Stn. 3	294	162	4.6	54.5	629 (423–999)	719 (500–1080)
Shallow	465	217	4.8	51.1	490 (388–652)	572 (456–745)*
Deep	458	135	3.9	16.2	326 (237–494)	321 (247–445)*
Stn. 1 Shallow	142	94	4.2	45.7	247 (173–390)	301 (200–498)§
Stn. 1 Deep	152	50	2.9	6.4	119 (76–234)**	105 (75–171)§,†,‡
Stn. 2 Shallow	165	97	4.2	51.1	267 (182–439)	264 (185–415)†
Stn. 2 Deep	170	66	3.5	16.9	146 (101–251)	152 (107–250)††
Stn. 3 Shallow	158	105	4.3	56.1	376 (242–641)**	433 (280–719)‡,††
Stn. 3 Deep	136	65	3.6	20.1	171 (111–308)	236 (147–424)

N is the number of sequences, H′ is the Shannon diversity index, and D_S^{-1} is Simpson's diversity index. Chao1 and ACE are non-parametric species (OTU) richness estimators calculated from the OTU rank abundance data for each set of sequences. Paired symbols (*, **, §, †, ‡, or ††) represent significant differences between diversity estimates for specific, paired samples ($P < 0.05$) based on the non-overlap of the 95% confidence intervals (CI).

curve was a typical shape for a diverse assemblage (Lunn *et al*., 2004), with 311 of the 324 OTUs containing fewer than 10 sequences per OTU (Fig. 3). Thirty-eight per cent of the sequences belonged to 13 common OTUs (≥ 10 sequences per OTU) while singletons represented 62% of all OTUs but only 22% of the protistan sequences. The three most common OTUs included a polycystine from deep-sea libraries (101 sequences) and a dinoflagellate (77 sequences) and ciliate (36 sequences), both of which were found in euphotic-zone and deep-sea libraries. A total of 189 OTUs were only detected in shallow libraries while 107 OTUs were unique to deep-sea libraries. Only 28 OTUs were detected in both shallow and deep libraries and included seven of the 10 most abundant OTUs described above. See Table S1 for a complete listing of all OTUs.

Diversity indices and richness estimators

Univariate diversity indices (Shannon's H′ and Simpson's D_S^{-1}) were calculated for all clone libraries (Table 2). Both indices account for OTU evenness and richness but Simpson's index uses a wider numerical scale, making differences in observed diversity more apparent. The overall diversity for pooled libraries (923 sequences) was high (e.g. $D_S^{-1} = 41.3$, Table 2). Protistan assemblages, pooled for each station across depths, revealed increasing diversity from station 1 to station 3 (e.g. 20.1, 38.2 and 54.5 for D_S^{-1}). Diversity indices were higher for pooled shallow stations ($D_S^{-1} = 51.1$) compared with pooled deep stations (e.g. $D_S^{-1} = 16.2$). Comparisons of each of the individual libraries revealed remarkably similar levels of protistan diversity in euphotic-zone assemblages that were substantially higher than diversity estimates of the deep-sea assemblages. Highest diversity for both shallow and deep sea protistan assemblages occurred at station 3 (Fig. 2, Table 2).

Total protistan diversity was estimated by application of non-parametric richness estimators (Chao1 and ACE) to data depicted in OTU rank abundance curves (Fig. 3) following procedures outlined in the study by Hughes and colleagues (2001). Specifically, these estimators and their 95% confidence intervals (CI) were calculated from the abundance of each OTU in the 'rare' classes, defined as singletons and doubletons for Chao1 and all OTUs with 10 or fewer members for ACE (Chao and Shen, 2003–2005). Chao1 and ACE (with 95% CI) were calculated for the entire data set and indicated that the total protistan diversity from which the observed OTU distribution was drawn ranged from 694 (574–873) OTUs for Chao1 to 773 (648–946) OTUs for ACE (Table 2). Chao1, ACE and the number of observed OTUs (S_{OBS}) were calculated for all possible sample sizes using EstimateS 7.5 (Colwell, 2005), set for 100 randomizations and sampling without replacement. Plots of Chao1 and S_{OBS} deviated strongly from a 1:1 relationship but failed to reach saturation and increased continuously over the entire sample size (Fig. 4).

Estimates of total protistan diversity were not statistically different based on the overlap of their 95% CI (Hughes *et al*., 2001) when samples were pooled by station (e.g. all shallow and all deep sequences at a particular station) for both Chao1 and ACE estimators (Table 2). Chao1 and ACE richness estimates ranged from ~400 to 700 OTUs for each station, whereas pooling the samples by depth (across stations) resulted in estimates ranging from ~300 to 600 OTUs (Table 2). Chao1 estimates were not significantly different for pooled-shallow versus pooled-deep samples; however, ACE estimates indicated a significant difference between pooled-shallow

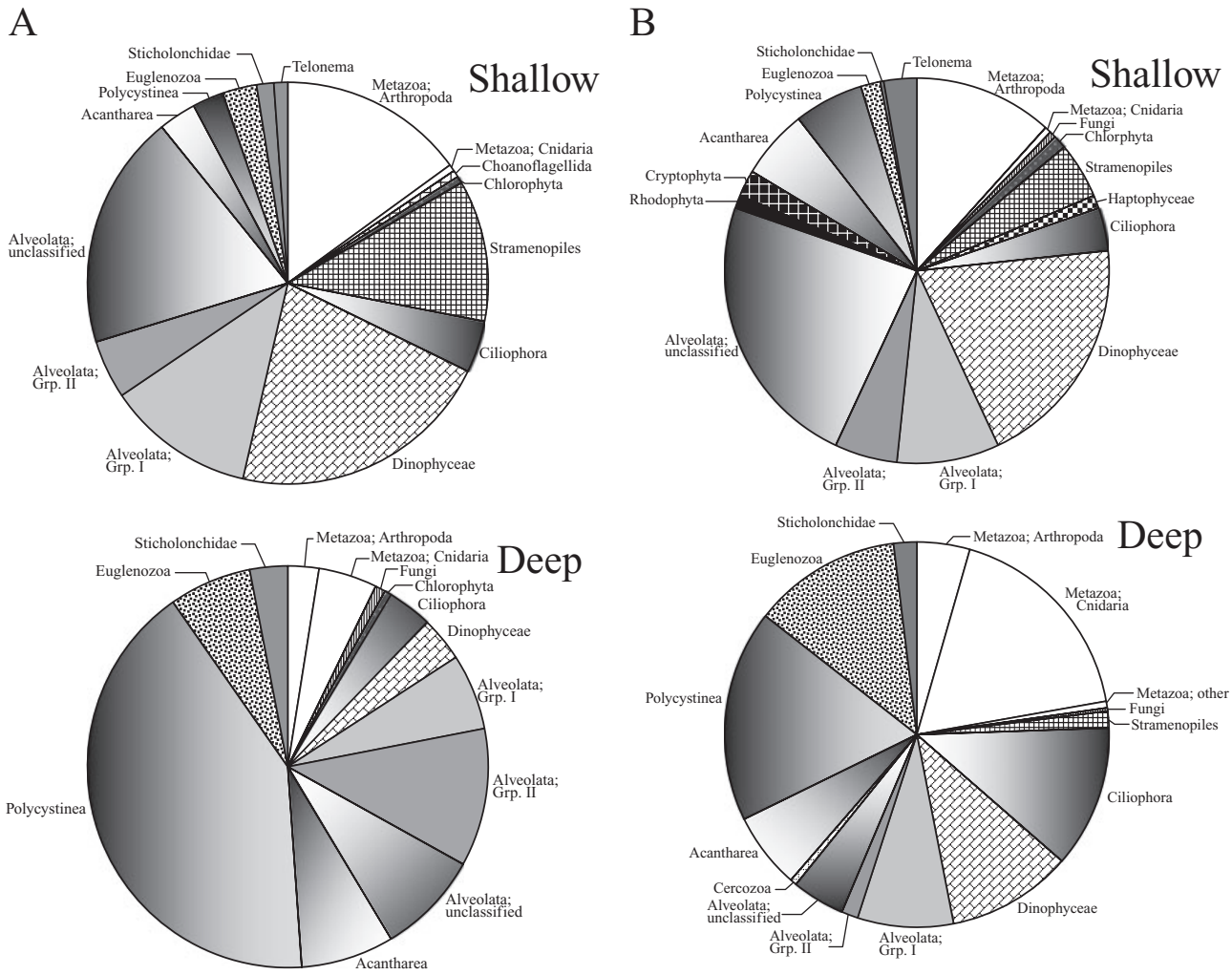

Fig. 1. Higher-level taxonomic distribution of microbial eukaryotes from shallow (integrated euphotic zone depths) and deep (2500 m) sampling depths based on putative identifications of partial-length 18S rRNA gene sequences resulting from BLAST searches of NCBI and ARB databases for station 1 (A), station 2 (B) and station 3 (C). Charts represent the entire data set of 1095 eukaryote sequences (both metazoan and protistan) that were submitted to GenBank.

and pooled-deep libraries (Table 2, *). Richness estimates for individual clone libraries indicated a total diversity in each sample ranging from ~100 to 400 OTUs. Highest diversity estimates occurred using shallow data sets; however, the only Chao1 estimates that were statistically different between depths were the estimates for shallow samples of station 3 and deep samples of station 1 (Table 2, **). ACE estimates were generally higher for all shallow assemblages, with significant differences occurring between the shallow and deep samples of station 1 (Table 2, §). Several additional significant differences between ACE diversity estimates of individual clone libraries were detected (Table 2, †, ‡ and ††). Overall, Chao1 and ACE estimates suggested two- to fourfold higher protistan diversity in the original seawater samples compared with the observed number of OTUs for each of the clone libraries.

Similarity of protistan assemblages

Protistan OTUs were normalized to relative abundances within a clone library and transformed by calculating the square-root of each value. Data transformation minimized the effect that OTUs with high relative abundance had on the comparisons of assemblage similarities while allowing the evenness of OTUs to factor into comparisons. Cluster analysis of Bray–Curtis coefficients for each pairwise comparison of libraries indicated significant ($P < 0.05$) differences in the composition of protistan assemblages from shallow (euphotic zone) and deep sea (2500 m) ecosystems (Fig. 5A). Overall, shallow and deep assemblages from different stations were ~30–40% similar within depth strata (Fig. 5A). The similarity value dropped to ~10% when shallow assemblages were compared with deep-sea assemblages (Fig. 5A). Analysis of the Bray–

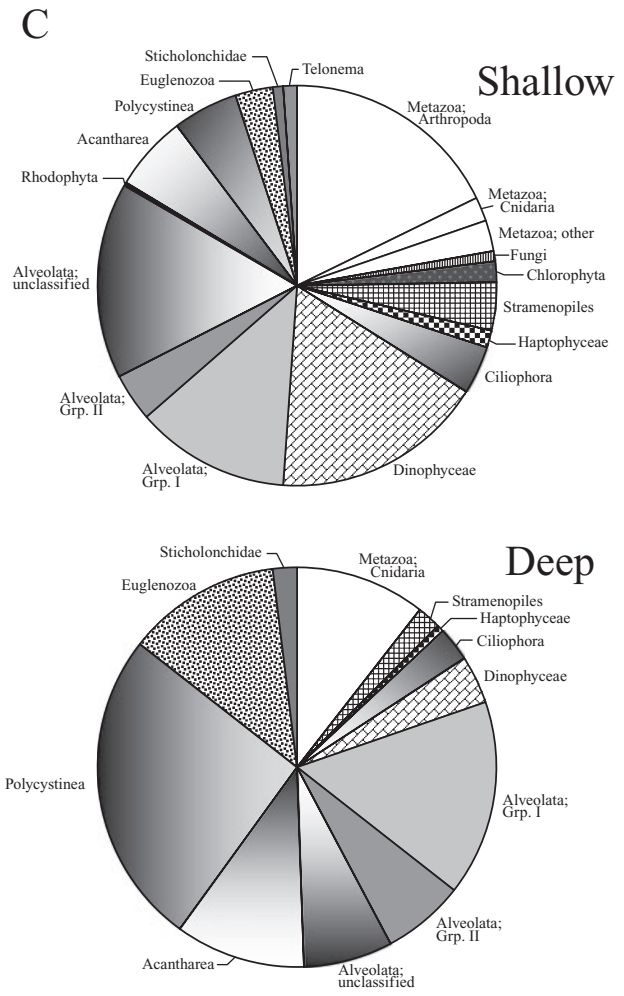

Fig. 1. *cont.*

Curtis resemblance matrix by non-metric multidimensional scaling (MDS) revealed trends similar to those resulting from cluster analysis, with differences among assemblages highlighted in three-dimensional space (Fig. 5B). Cluster analysis similarities were overlaid on the MDS plot (ovals in Fig. 5B) to accentuate the similarities and differences of the six clone libraries. The stress value for the three-dimensional MDS plot was zero, indicating a very strong correspondence between the distances on the plots and the original distances in the Bray–Curtis resemblance matrix (Clarke and Warwick, 2001).

Discussion

Taxonomic diversity and distribution of protistan assemblages

Molecular surveys of protistan assemblages have only recently begun to reveal the extent of protistan diversity and the distribution of similar phylotypes across the globe (Diez *et al*., 2001a; López-García *et al*., 2001; Moon-van der Staay *et al*., 2001; Edgcomb *et al*., 2002; López-García *et al*., 2003; Stoeck *et al*., 2003; Gast *et al*., 2004; Massana *et al*., 2004a; Romari and Vaulot, 2004; Countway *et al*., 2005; Behnke *et al*., 2006; Lovejoy *et al*., 2006; Worden, 2006; Zuendorf *et al*., 2006). Many 18S rRNA surveys have almost exclusively focused on identifying phylotypes via tree-building, without considering the quantitative diversity of protistan assemblages or their relative similarities. Two significant issues have impeded progress on making such comparisons. First, most molecular surveys have been conducted on relatively small scales because of the effort and cost of carrying out such work; and second, difficulties have arisen in

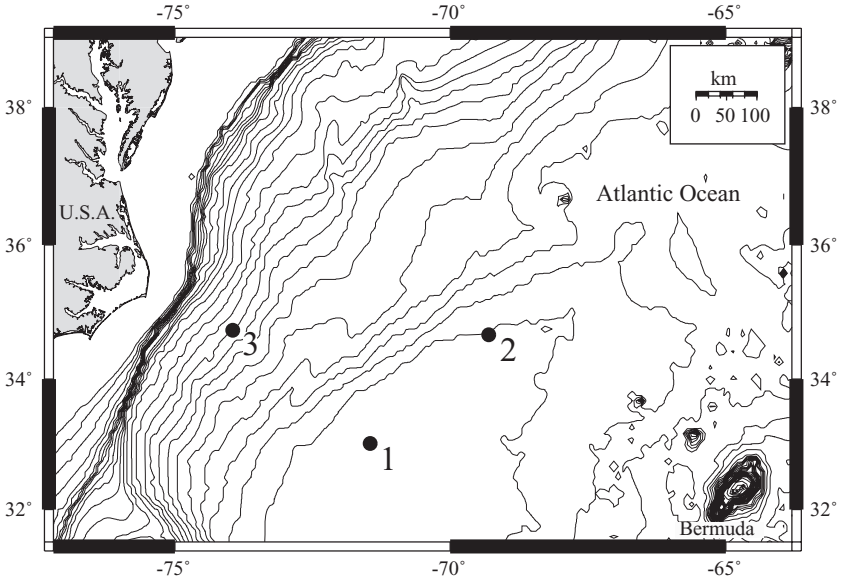

Fig. 2. Station locations and bottom depths in the western North Atlantic; station 1: 33.0168°N, 71.4552°W (~5300 m); station 2: 34.6667°N, 69.3000°W (~5200 m); station 3: 34.7325°N, 73.9468°W (~3600 m). Contours represent 200 m depth intervals with the deepest contour line (nearest stations 1 and 2) representing a depth of 5000 m. Map created with the 'Online Map Creation' tool designed by M. Weinelt (http://www.aquarius.geomar.de/omc/).

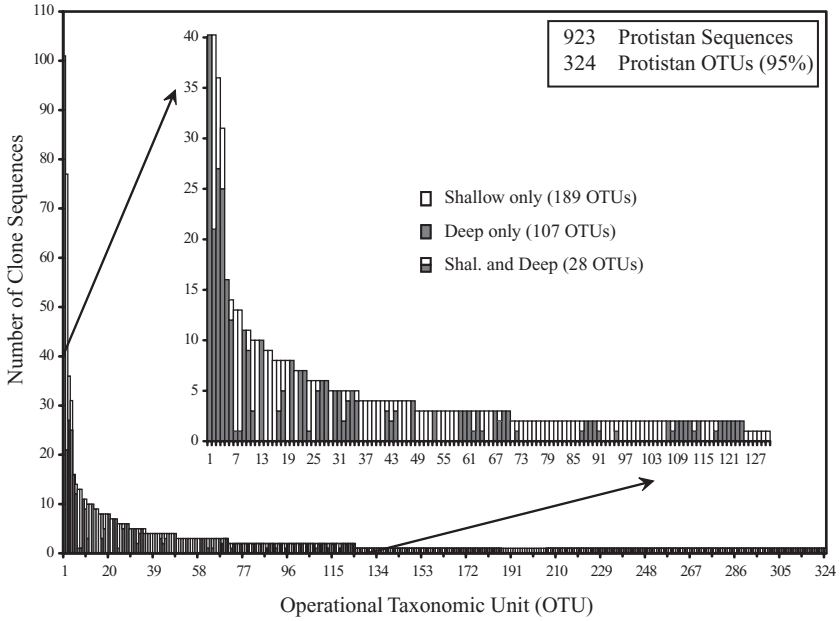

Fig. 3. Protistan rank abundance curve for the pooled data set of 923 sequences (metazoan sequences removed), depicting 324 unique operational taxonomic units (OTUs). Protistan OTUs were largely unique to either shallow (189 OTUs, white bars) or deep (107 OTUs, grey bars) sampling depths, with only 28 OTUs present in both shallow and deep libraries. Rank abundance curve highlighting OTUs with two or more members (inset).

establishing practical, widely adopted demarcations for defining protistan OTUs that would otherwise facilitate the kind of comparisons discussed above (D.A. Caron *et al.*, in preparation).

Despite these limitations, recent culture-independent protistan surveys have started to include estimates of diversity (Massana *et al.*, 2004a; Countway *et al.*, 2005; Behnke *et al.*, 2006; Zuendorf *et al.*, 2006) and biogeographic patterns; subjects that have received more attention in the bacterial and archaeal literature (Martiny *et al.*, 2006). Plankton are distributed heterogeneously over short scales of time and space (Scheffer *et al.*, 2003), which may lead to differences in local estimates of diversity. Differences in protistan diversity and assemblage composition over tens to hundreds of metres in the water column (Stoeck *et al.*, 2003; Lovejoy *et al.*, 2006) and spanning time scales of days (Massana and Jürgens, 2003; Countway *et al.*, 2005) are now being confirmed using high-resolution molecular approaches. The expansion and concerted application of these approaches have

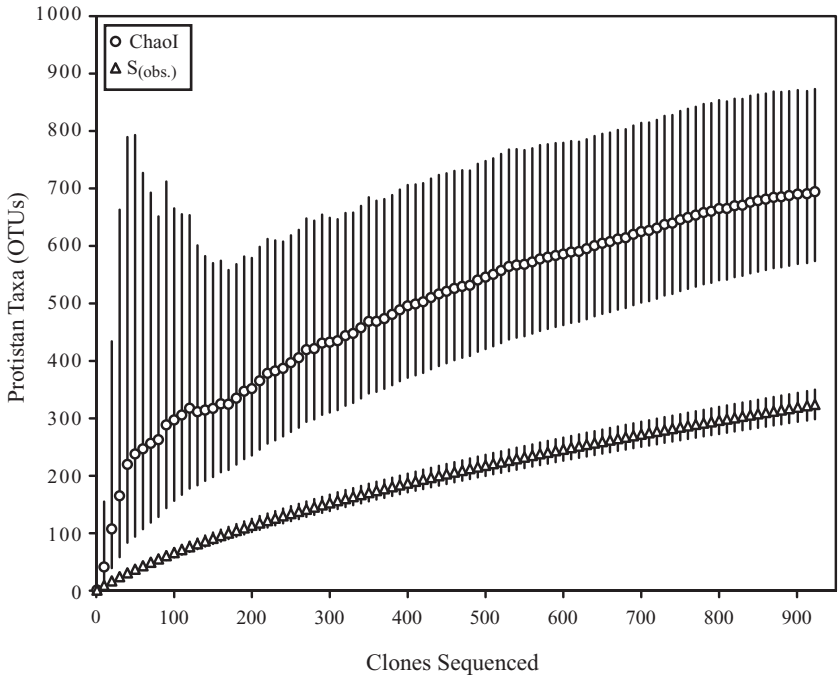

Fig. 4. Rarefaction curves for pooled protistan sequence data of the ChaoI diversity estimator (circles) and the number of observed species (triangles) for a given level of sequencing effort (±95% confidence interval). Curves were constructed from 100 randomizations, without replacement using EstimateS, version 7.5.

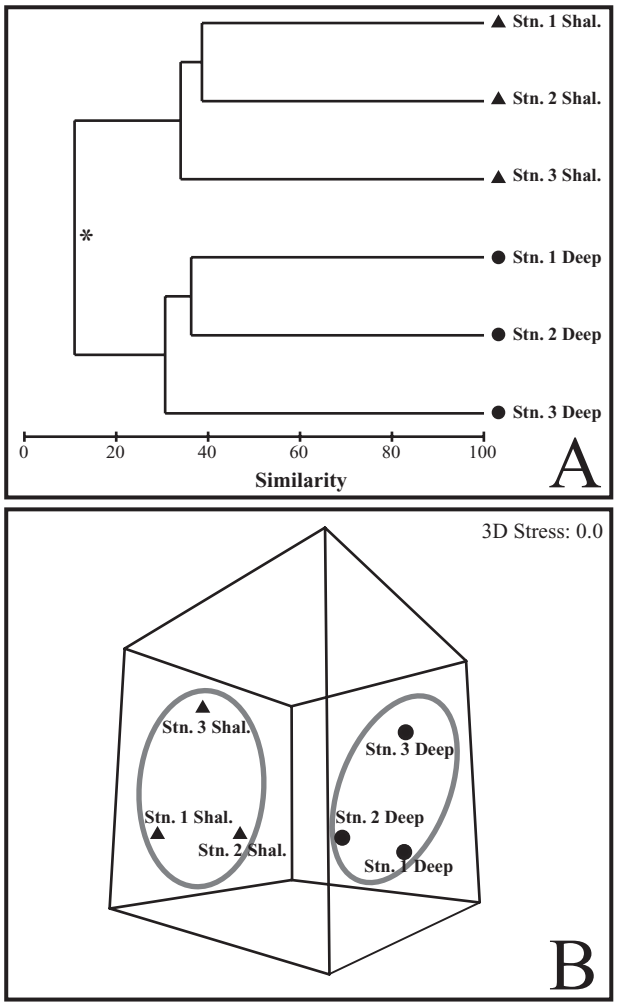

Fig. 5. A. UPGMA cluster diagram of Bray–Curtis similarities calculated from square-root transformed relative OTU abundances for each clone library. The asterisk at the node in the dendrogram separating shallow and deep libraries indicates significant compositional differences between shallow and deep protistan assemblages ($P < 0.05$) determined by the SIMPROF test.
B. Three-dimensional non-metric multidimensional scaling (MDS) plot constructed from a Bray–Curtis similarity matrix of square-root transformed relative OTU abundances. Ellipses around data points indicate similarities of protistan assemblages determined by cluster analysis.

begun to provide new insights into the global diversity and distributions of microbial eukaryotes.

The best BLAST matches for many of the sequences in the present study were sequences detected at diverse sites around the world; a result supporting the hypothesis that many protistan taxa are globally distributed (Fenchel and Finlay, 2004). Alveolates comprised major fractions of most clone libraries, with a high proportion putatively attributed to known ciliate and dinoflagellate lineages. A substantial number of representatives from newly discovered and largely uncultured protistan lineages were also observed. These phylotypes included novel group I and group II alveolates (Moreira and López-García, 2002), novel stramenopiles (Massana *et al*., 2002) and an array of novel phylotypes previously from a variety of diverse habitats (Dawson and Pace, 2002; Edgcomb *et al*., 2002; Stoeck and Epstein, 2003).

Novel alveolate groups I and II were routinely detected in both shallow and deep-sea libraries, with group I alveolates generally more abundant. *Amoebophrya* (a parasitic protist) has been reported as one of the only described members of the novel alveolate groups (Moreira and López-García, 2002). Groisillier and colleagues (2006) summarized 18S sequence data from a large number of environmental clone libraries and concluded that group II alveolates belong to the Syndiniales and that both novel alveolate groups form distinct lineages. Recent surveys have determined that the group II alveolate lineage is more diverse than the group I lineage (Groisillier *et al*., 2006; Lovejoy *et al*., 2006; Worden, 2006). We detected large numbers of these novel alveolate phylotypes, including 38 group I OTUs (102 clones) and 24 group II OTUs (71 clones). Group I and II alveolates have been reported from diverse habitats including the Antarctic Polar Front (López-García *et al*., 2001), Mediterranean Sea (Massana *et al*., 2004a), English Channel (Romari and Vaulot, 2004), North Pacific (Worden, 2006), North Atlantic (Countway *et al*., 2005) and Arctic oceans (Lovejoy *et al*., 2006).

'Unclassified alveolate' sequences had closest BLAST matches to unidentified taxa from previous culture-independent studies, but these clones clearly grouped within alveolate lineages when higher-level taxonomic affinities were considered. Many of the best BLAST matches to 'unclassified alveolates' sequences were sequences recovered from the English Channel (Romari and Vaulot, 2004), providing further evidence for the hypothesis of widespread distributions of undocumented protistan taxa.

Stramenopile sequences were present in all clone libraries except the deep-sea library from station 1. Although the total number of stramenopile sequences was relatively small (42 sequences) the observed diversity of this group was quite high (24 OTUs). Many stramenopile sequences in the present study were closely related to novel lineages that may include heterotrophic forms (Diez *et al*., 2001a; Massana *et al*., 2002; Massana *et al*., 2004b; 2006). Detection of these novel lineages in geographically distinct ecosystems including the North Atlantic (this study), Arctic Ocean (Lovejoy *et al*., 2006) and coastal North Pacific (Worden, 2006) supports speculation on the 'global distribution' and potential 'ecological significance' for many of these novel phylotypes.

Three major protistan groups (Polycystines, Acantharea and Euglenozoa) were well represented in the deep-sea libraries. The best BLAST matches for many of these deep-

water OTUs were sequences obtained from other deep-sea ecosystems including an anoxic basin in the Caribbean Sea (Stoeck *et al*., 2003), hydrothermal vents at Guaymas Basin (Edgcomb *et al*., 2002) and the deep sea near Antarctica (López-García *et al*., 2001). Polycystines represented nearly 20% of the total number of sequences but comprised only 7% of the total OTU count (Table 1). Despite high abundances of polycystine sequences in clone libraries it is not clear how effective our sampling approach was for this group because many polycystine species form large mineralized tests and gelatinous structures that might be removed by prefiltration (Caron and Swanberg, 1990). Acantharean OTUs were more abundant than polycystine OTUs even though fewer Acantharean sequences were obtained suggesting high Acantharean diversity. Many of the OTUs from these two major heterotrophic groups were not present in euphotic-zone clone libraries and it is presently unknown if these deep-sea phylotypes represent living, growing populations, or sinking populations from surface waters that were missed by our sampling.

Euglenozoan OTUs were more abundant in deep-sea than euphotic-zone clone libraries and represented a diverse protistan group. Euglenozoa have proven to be a more diverse group than previously believed (Von Der Heyden *et al*., 2004) with newly described members found in anoxic ecosystems (Stoeck and Epstein, 2003; Behnke *et al*., 2006; Stoeck *et al*., 2006; Zuendorf *et al*., 2006), the deep sea near Antarctica (López-García *et al*., 2001) and Monterey Bay (Buck *et al*., 2000). The ecological roles and precise phylogenetic affinities of these predominantly deep-sea euglenozoa remain to be characterized.

Observed and estimated protistan diversity

Cloning and sequencing revealed a total of 324 protistan OTUs in the present study. Operational taxonomic unit abundance ranged from 94 to 105 OTUs in shallow libraries and from 50 to 66 OTUs in deep libraries (Table 2). Most protistan OTUs were present as singletons (200 OTUs) or doubletons (54 OTUs) indicating a large number of rare taxa (Fig. 3), a characteristic of many protistan assemblages (Countway *et al*., 2005; Pedrós-Alió, 2006; Zuendorf *et al*., 2006). This result demonstrates the need for sequencing even larger clone libraries to reveal the many taxa present at very low abundance (Sogin *et al*., 2006). Discrete sampling at a single time point typically underestimates protistan diversity, with additional OTUs detected from the same sample subjected to repeated sampling (Countway *et al*., 2005). The previous study demonstrated the utility of environmental perturbations as a mechanism for revealing a greater proportion of rare OTUs in a parcel of water.

Rarefaction curves have indicated continuously increasing numbers of OTUs with increasing numbers of clones sequenced, implying that much of the microbial diversity is not captured by routine sampling approaches (Massana *et al*., 2004a; Romari and Vaulot, 2004; Countway *et al*., 2005; Behnke *et al*., 2006; Zuendorf *et al*., 2006). This trend was observed in the present study for individual clone libraries (data not shown) and for the entire data set of 923 sequences (Fig. 4) suggesting that larger libraries must be analysed or new approaches taken to reveal all of the diversity at a particular time and place.

The high diversity observed for natural microbial assemblages has resulted in the application of diversity estimations to bacterial studies (Hughes *et al*., 2001; Martin, 2002; Hill *et al*., 2003) and more recently to protistan studies (Massana *et al*., 2004a; Countway *et al*., 2005; Behnke *et al*., 2006; Zuendorf *et al*., 2006). To date, most studies of protistan diversity have sequenced representative clones from restriction fragment length polymorphism-based OTUs to investigate the breadth of diversity (Stoeck and Epstein, 2003; Massana *et al*., 2004a; Romari and Vaulot, 2004). The previous studies represent ground-breaking work and arrive at the same conclusion of 'high diversity' as the present study; however, the restriction fragment length polymorphism-based approach to OTU calling may have underestimated the overall protistan diversity.

Univariate diversity statistics (H′ and D_S^{-1}) have been applied previously to OTU data from bacterial clone libraries (Dunbar *et al*., 1999; Hill *et al*., 2003). These statistics do not permit comparisons of the specific types of OTUs in different samples, but are useful for summarizing the overall diversity in an ecosystem resulting from changes in environmental conditions or biological interactions. The scale of the Simpson index was better at highlighting the differences among samples in the present study and is generally thought to be more biologically meaningful than the Shannon index because the Simpson index is a probability of encounter rather than a measurement of 'information' (Brower *et al*., 1998).

Non-parametric richness estimators (Chao1 and ACE) were first applied to molecular surveys of bacterial assemblages (Hughes *et al*., 2001; Hill *et al*., 2003). More recently these diversity estimators have begun to appear in studies of protistan diversity (Massana *et al*., 2004a; Countway *et al*., 2005; Behnke *et al*., 2006; Zuendorf *et al*., 2006). Countway and colleagues (2005) reported an overall eukaryote Chao1 diversity estimate of 282 OTUs (95% CI = 229–381) while Chao1 estimates of picoeukaryotic diversity at an oligotrophic site in the Mediterranean Sea ranged from 77 to 171 OTUs (Massana *et al*., 2004a). The lower diversity estimates in the study by Massana and colleagues (2004a) were likely due to the

intentional exclusion of larger protists from their samples and possibly because of differences in how OTUs were defined.

The species richness estimates for the pooled clone libraries in the current study represent some of the highest values of protistan diversity yet reported (Table 2). Chao1 and ACE estimates for the individual libraries were lower but still notable, and ranged from ~100 to 400 OTUs. The highest Chao1 and ACE values from an individual clone library in the present study were 376 OTUs and 433 OTUs respectively. However, these estimates were undoubtedly conservative because they were based on data from clone libraries that had not reached saturation (e.g. Fig. 4). Nevertheless, these data imply that the total pool of protistan diversity from which our samples were drawn was substantially higher than the observed OTU diversity. In this sense, our observations are in general agreement with previous protistan studies (Massana *et al*., 2004a; Romari and Vaulot, 2004; Countway *et al*., 2005; Behnke *et al*., 2006; Lovejoy *et al*., 2006; Worden, 2006; Zuendorf *et al*., 2006). In general, estimates of the total diversity from the six individual clone libraries were high, and relatively similar within particular depth strata. The wide range of the Chao1 and ACE confidence intervals, a consequence of the large numbers of rare OTUs, made the detection of potential differences among our clone libraries difficult.

The relative similarities of Chao1 and ACE estimates between stations for similar depths in the present study (Table 2) may indicate that a finite number of ecological niches exist across wide regions of the ocean, especially within a particular depth horizon. We hypothesize that these niches may be occupied by different taxa at different locales and times, but that overall a similar number of dominant taxa may be supported at any given time and place. In support of the previous hypothesis, experimental evidence suggests that natural protistan assemblages often retain their overall level of diversity, even when the composition of the dominant taxa within these assemblages changes (Massana and Jürgens, 2003; Countway *et al*., 2005).

Protistan diversity revealed by recent culture-independent studies (López-García *et al*., 2001; Moon-van der Staay *et al*., 2001; Stoeck *et al*., 2003; Gast *et al*., 2004; Massana *et al*., 2004a; Romari and Vaulot, 2004; Countway *et al*., 2005; Behnke *et al*., 2006; Lovejoy *et al*., 2006; Worden, 2006; Zuendorf *et al*., 2006) indicates a picture of high diversity more typically associated with bacterial assemblages. Estimates of the total protistan diversity predicted from our six clone libraries ranged from ~100 to 400 OTUs per library whereas the overall estimate of diversity for the entire data set ranged from ~700 to 800 OTUs. Similarly, a metagenomic survey of bacteria in the Sargasso Sea yielded a Chao1 estimate of ~1000 OTUs (Venter *et al*., 2004) although the methodology employed was quite different than that used in the present study. Regardless, it is clear from these studies that oceanic ecosystems are characterized by protistan (and bacterial) assemblages of great taxonomic depth and breadth, which are still incompletely characterized because of large pools of rare phylotypes.

Diversity estimates based on the analysis of clone libraries are unavoidably affected by the predominance of rare phylotypes as well as biases associated with extraction, amplification, cloning and sequencing of DNA. Procedural biases associated with culture-independent analyses of complex microbial assemblages include: DNA extraction variability among organisms, PCR efficiency for different templates, chimera formation and the variable copy numbers of rRNA operons in different taxa. For a thorough review of these issues, see the study by von Wintzingerode and colleagues (1997). These biases have been discussed extensively in the literature and suggestions for their mitigation have been proposed (Polz and Cavanaugh, 1998; Thompson *et al*., 2002; Kurata *et al*., 2004; Acinas *et al*., 2005; Stoeck *et al*., 2006). Biological biases such as the effect of variable numbers of rRNA operons remain problematic for interpreting PCR-based assessments of microbial diversity and abundance (Zhu *et al*., 2005). Although diversity estimates from clone libraries may not accurately reflect the 'true' diversity in nature, PCR-based approaches provide a starting point for comparisons (Curtis and Sloan, 2004). New methods for assessing microbial diversity (with fewer inherent biases) are becoming more sophisticated and include: rRNA-based primer-independent clone libraries (Botero *et al*., 2005) and extremely high-throughput sequencing of short diagnostic sequence tags (Sogin *et al*., 2006).

Analysis of community similarity

Multivariate statistical approaches such as hierarchical cluster analysis, principal component analysis (PCA) and non-metric MDS have been used to compare microbial assemblages collected from different environments or experimental treatments (Diez *et al*., 2001b; Casamayor *et al*., 2002; Lawley *et al*., 2004). Recent studies have advocated the use of Bray–Curtis similarities for the comparison of microbial assemblages (Rees *et al*., 2004; Klaus *et al*., 2005) because joint OTU absences do not affect similarity calculations (Clarke and Warwick, 2001). Non-metric MDS has been favoured over PCA for several recent comparisons of microbial assemblages (Rees *et al*., 2004; Klaus *et al*., 2005) because PCA assumes that data are normally distributed (Clarke and Warwick, 2001), which is not always the case for OTUs in microbial

assemblages. Multidimensional scaling is powerful because it utilizes a similarity matrix that reflects differences in both the type and relative abundance of each OTU. Although MDS offers an approach for comparing microbial assemblages, it suffers from a lack of power when replicate number is low, which is typical for clone library-based studies of diversity.

The present study represents one of the first comparisons of sequence-based protistan OTUs collected from vastly different oceanic depths using cluster analysis and non-metric MDS. A previous study of marine protistan diversity utilized non-metric MDS plots to display the similarity of eukaryotic denaturing gradient gel electrophoresis patterns, indicating similar ordination for samples collected from similar depths over a period of 3 days (Diez *et al*., 2001b). Protistan assemblages from shallow stations in the present study were not significantly different based on a similarity profile (SIMPROF) test of the assemblages that were clustered using UPGMA; however, these shallow-water assemblages were significantly different from the deep-sea assemblages (Fig. 5A). The detection of differences between shallow and deep sea protistan assemblages might be expected given the presumed differences in the trophic structure of protistan assemblages from the euphotic zone and the deep sea. However, the observed similarity of protistan assemblages from comparable depths but different sites was relatively surprising because the sampling stations were separated by hundreds of kilometres and the assemblages were likely influenced by different physical and chemical processes.

Low stress value (0.0) indicated that the three-dimensional MDS plot (Fig. 5B) was an extremely good representation of the relationship between shallow and deep protistan assemblages. Comparisons of protistan assemblages in the present study were based upon clone libraries that had not reached saturation based on rarefaction analyses. However, it is unlikely that the detection of additional rare OTUs would have had much effect on the Bray–Curtis similarities calculated from relative clone abundances. Shallow samples were more similar to other shallow samples and deep samples were more similar to other deep samples even when OTU data were compared by presence/absence (e.g. equal weighting of abundant and rare types) instead of relative clone abundance (data not shown).

In summary, protistan assemblages from sites in the western North Atlantic were more genetically diverse than expected yet relatively similar (with respect to composition) over large distances at comparable depths. More than half of the protistan OTUs were present only as singletons, suggesting the presence of a large reservoir of rare phylotypes and assuming that PCR and cloning biases were minimally confounding factors. All major protistan groups were detected with particularly high occurrences of stramenopiles, ciliates, dinoflagellates, group I and II alveolates, acanthareans, polycystines and euglenozoans. Conservative estimates of the total protistan diversity ranged from ~700 to 800 OTUs. Multivariate analysis supported observations that shallow and deep-sea assemblages were composed of fundamentally different OTUs, and different levels of diversity. The observed trends support the idea that largely unique protistan assemblages inhabit the deep sea, with only minor contributions from surface-dwelling taxa.

Experimental procedures

Sample collection and processing

Samples for this study were collected at three sites in the western North Atlantic encompassing a broad oceanic region (~4.0×10^4 km^2). Two sites were oligotrophic stations in the Sargasso Sea (stations 1 and 2) while the third site (station 3) was in the Gulf Stream (Fig. 2). Coordinates and depths of the sites were: station 1: 33.0168°N, 71.4552°W (5300 m); station 2: 34.6667°N, 69.3000°W (5200 m); station 3: 34.7325°N, 73.9468°W (3600 m) and were occupied for several days beginning on 19, 21 and 24 August 2000 respectively. Seawater was collected from the euphotic zone in the middle of the surface mixed-layer and the deep chlorophyll *a* maximum (DCM) and from the bathypelagic zone at a depth of 2500 m. Sampling depths within the euphotic zone were 20 m and 125 m at station 1, 35 m and 100 m at station 2 and 15 m and 105 m at station 3 for mixed-layer and DCM samples respectively. Samples were transferred directly from Niskin bottles to acid-washed (5% HCl) polycarbonate carboys via gravity filtration through 200 μm Nitex mesh. Prefiltration through Nitex reduced the contribution of microbial metazoa to subsequent DNA extracts. Sample volumes varied with depth in proportion to protistan biomass and were 2 l in the euphotic zone and 20 l in the bathypelagic zone.

DNA collection and extraction

Samples were filtered through 47 mm GF/F filters (Whatman) and stored frozen in Cryo-vials until extraction. One millilitre of hot (~70°C) lysis buffer (100 mM Tris pH 8, 40 mM EDTA pH 8, 100 mM NaCl, 1% SDS) and 200 μl of 0.5 mm zircon beads were added to thawed samples. Sample lysis involved three rounds of bead-beating and heating that included 30 s of vortexing followed by heating at 70°C for 5 min. Lysates were transferred to 2 ml tubes, adjusted to a final concentration of 0.7 M NaCl and 1% CTAB (Sigma) and heated to 70°C for 10 min. Lysates were extracted with chloroform and DNA was precipitated from the aqueous layer with isopropanol (Gast *et al*., 2004). DNA was pelleted by centrifugation at 12 000 r.p.m. for 15 min; the supernatant was decanted and the pellet air-dried. DNA samples were re-suspended in sterile water and stored frozen at –20°C until use.

PCR for cloning and sequencing

18S rRNA genes were amplified from DNA extracts with universal eukaryote primers: Euk-A (5′-AACCTGGTTGATCC

TGCCAGT-3′) and Euk-B (5′-GATCCTTCTGCAGGTTCA CCTAC-3′) to generate products for TA cloning (Medlin *et al*., 1988). Polymerase chain reaction reagents were diluted to final concentrations of 0.5 μM of each primer, 1× Promega buffer B, 2.5 mM Promega $MgCl_2$, 250 μM Promega dNTPs, 300 ng μl^{-1} BSA (Sigma A-7030; Kirchman *et al*., 2001), plus 2.5 U of Promega *Taq* in buffer B and 1–2 μl of DNA extract in 50 μl reaction volumes. The thermal protocol for PCR included one cycle of 95°C for 2 min, 35 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 2.5 min, with a final extension at 72°C for 7 min. Three to four replicate PCRs were set up for each sample to ensure production of an adequate amount of product for cloning. Polymerase chain reaction products from the two euphotic-zone samples at each station were combined to form a single, depth-integrated 'shallow' sample.

Cloning and sequencing

Polymerase chain reaction products were separated on 1.2% SeaKem agarose (Cambrex) and products of the expected size were excised from the gels. DNA was extracted from gel slices using a gel recovery kit (Zymo). Purified DNA was eluted in sterile water and quantified by PicoGreen (Molecular Probes). Polymerase chain reaction products (30 ng) were ligated overnight at 4°C into pGEM-T Easy Vector (Promega). Ligation products (5 ng) were mixed with 40 μl of Electro10Blue cells (Stratagene) and electroporated in a 0.1 cm cuvette on a GenePulser Xcell (Bio-Rad) set to 1700 V, 600 Ω and 25 μF. Sterile SOC (960 μl) was added to the cells after shocking and the re-suspended cells were transferred to a sterile 15 ml BD Falcon tube for outgrowth at 37°C for 90 min, shaking at 250 r.p.m. Transformed cells (50–200 μl) were spread onto S-Gal (Sigma) plates containing ampicillin (100 μg ml^{-1}) for overnight growth at 37°C.

Insert positive colonies were picked with sterile toothpicks and grown for 18–24 h in deep-well culture blocks containing 1.25 ml of TB and ampicillin (100 μg ml^{-1}). Glycerol stocks of all clones were prepared and archived at −80°C prior to spin-down and collection of bacterial pellets. Plasmid DNA was extracted from pellets with the Wizard SV96 kit (Promega). Sequencing reactions were set up with plasmid DNA, DTCS reagents (Beckman–Coulter) and the sequencing primer Euk-570F (5′-GTAATTCCAGCTC CAATAGC-3′) and were followed by the analysis of products on a Beckman–Coulter CEQ8000 automated DNA sequencer.

Sequence processing, phylotype assignment and diversity estimates

DNA sequences were trimmed to remove low-quality regions from both the 5′ and 3′ ends using software provided with our CEQ8000 DNA sequencer. Sequence chromatograms were visually inspected using Chromas (Technelysium) to confirm trimming accuracy and resolve any base-calling problems. Putative identities were assigned for all sequences using BLAST (Altschul *et al*., 1997) based on comparisons with both NCBI (Benson *et al*., 2004) and ARB databases (Ludwig *et al*., 2004). High-level taxonomic identity was recorded for each sequence based on its nearest BLAST match containing taxonomic information (Fig. 1). Although most BLAST scores were much higher than 200, even relatively short sequences with scores near 200 were taxonomically informative because of their inclusion of a hypervariable region near their 5′ end. Sequences with scores < 200 were discarded because of reduced length or low quality. Potential sequence chimeras were flagged using Chimera Check (Cole *et al*., 2003). These procedures resulted in a total of 1098 sequences. Three streptophyte sequences were removed, leaving 1095 sequences in our study.

Pairwise sequence comparisons of the 1095 sequences (GenBank accessions DQ917930–DQ919024) were conducted with CLUSTALW (Thompson *et al*., 1994) as a first step in establishing OTUs using an automated OTU calling program (D.A. Caron *et al*., in preparation). Sequences were initially grouped into OTUs using a similarity of 95%. This similarity level was chosen based upon our analysis of the variability of more than 500 full-length 18S sequences derived from morphologically well-defined protists (D.A. Caron *et al*., in preparation). Intraspecies (strain level) and intragenus (species level) sequence variability was examined to define a similarity that would approximate species-level distinctions. Our automated process for calling OTUs from environmental sequence data sets involved a series of steps including: (i) initial establishment of OTUs (based on 95% sequence similarity); (ii) optimization of the placement of sequences within the OTUs through the comparison of all sequences in each OTU with all sequences in every other OTU; and (iii) a condensation process whereby OTUs not differing by more than 5% sequence dissimilarity (averaged over all sequences in any two OTUs) were merged into a single OTU. The level of sequence similarity (95%) for species-level discrimination used in this process is not high relative to values predicted from manually adjusted sequence alignments. However, our approach permits fully automated OTU calling, which has great desirability for ecological studies.

Rank abundance lists of OTUs served as input files for diversity estimation in SPADE (Chao and Shen, 2003–2005; Chao *et al*., 2005) and EstimateS (Colwell, 2005). Operational taxonomic unit distributions provided estimates of the clone library diversity as well as the total protistan diversity (e.g. the sampled plus non-sampled diversity). Shannon's (H′) and Simpson's (D_S^{-1}) diversity indices (Brower *et al*., 1998) were calculated from OTU abundance data along with the non-parametric richness estimators, Chao1 (Chao, 1987) and ACE (Chao and Lee, 1992). Protistan assemblages from different stations and depths were compared using the Bray–Curtis coefficient of similarity (*S*), which ranges over a scale of 0–100, where '100' indicates an identical assemblage (Bray and Curtis, 1957). Relative abundances of each of the 324 protistan OTUs were arranged in a matrix (six libraries by 324 OTUs) and transformed by square rooting each value. Transformation downweighted the effect of abundant OTUs on similarities. Bray–Curtis similarities were analysed by cluster analysis and non-metric MDS using PRIMER v.6 (Clarke and Warwick, 2001). A SIMPROF test was conducted in PRIMER to establish the significance of dendrogram nodes resulting from cluster analysis.

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Supplementary material

The following supplementary material is available for this article online:

Table S1. The following lists indicate the OTUs determined from 1098 rRNA gene sequences using an initial sequence similarity threshold of 95%. Operational taxonomic unit numbering reflects the order in which OTUs were generated by our automated OTU-calling program. The number in parentheses following the OTU number indicates the number of sequences comprising a particular OTU. Putative higher-level taxonomic identifications are provided for each OTU and were based on BLAST identifications (using NCBI and ARB databases) of sequences comprising each OTU. Specific clone names beginning with 'ENVP' appear below the taxonomic identifications and are searchable in the GenBank database. Three streptophyte sequences comprising OTUs 107 and 197 (see *** in Table) were removed from the data set leaving a total of 344 OTUs and 1095 sequences. Twenty of the remaining 344 OTUs are affiliated with microbial metazoa (see Arthropoda, Cnidaria, Ctenophora and Polychaeta below). These metazoan DNA sequences were submitted to GenBank but not considered in the diversity aspects of the current study. The remaining 923 sequences (324 OTUs) were identified as protistan in origin and formed the basis for our comparisons.

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Supplemental Table 1. The following lists indicate the OTUs determined from 1,098 rRNA gene sequences using an initial sequence similarity threshold of 95%. OTU numbering reflects the order in which OTUs were generated by our automated OTU-calling program. The number in parentheses following the OTU number indicates the number of sequences comprising a particular OTU. Putative higher-level taxonomic identifications are provided for each OTU and were based on BLAST identifications (using NCBI and ARB databases) of sequences comprising each OTU. Specific clone names beginning with 'ENVP' appear below the taxonomic identifications and are searchable in the GenBank database. Three streptophyte sequences comprising OTUs 107 and 197 (see *** in Table) were removed from the data set leaving a total of 344 OTUs and 1,095 sequences. Twenty of the remaining 344 OTUs were affiliated with microbial metazoa (see Arthropoda, Cnidaria, Ctenophora and Polychaeta below). These metazoan DNA sequences were submitted to GenBank but not considered in the diversity aspects of the current study. The remaining 923 sequences (324 OTUs) were identified as protistan in origin and formed the basis for our comparisons.

Similarity % = 95

OTU 1 (n = 3)
Stramenopile
==========
ENVP10203.00010
ENVP10203.00032
ENVP21819.00153

OTU 2 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00013
ENVP21819.00021

OTU 3 (n = 1)
Group II Alveolate
==========
ENVP10203.00033

OTU 4 (n = 1)
Polycystinea
==========
ENVP10203.00017

OTU 5 (n = 77)
Dinophyceae
==========
ENVP10203.00374
ENVP10203.00006
ENVP10203.00289
ENVP21819.00091
ENVP21819.00145
ENVP21819.00219
ENVP10203.00018
ENVP10203.00048
ENVP10203.00212
ENVP10203.00368
ENVP10203.00025
ENVP10203.00344
ENVP21819.00050
ENVP21819.00302
ENVP36162.00218
ENVP36162.00237
ENVP36162.00273
ENVP36162.00295
ENVP10203.00249
ENVP10203.00373
ENVP21819.00075
ENVP21819.00383
ENVP223.00212
ENVP36162.00232
ENVP10203.00050
ENVP10203.00007
ENVP10203.00193
ENVP107.00009
ENVP107.00193
ENVP21819.00045
ENVP21819.00060
ENVP21819.00077
ENVP21819.00148
ENVP21819.00299
ENVP223.00045
ENVP223.00051
ENVP223.00095
ENVP223.00116
ENVP223.00223
ENVP223.00263
ENVP36162.00216
ENVP36162.00300
ENVP36162.00309
ENVP366.00245
ENVP21819.00374
ENVP223.00102
ENVP36162.00071
ENVP36162.00219
ENVP10203.00079
ENVP21819.00365
ENVP223.00279
ENVP36162.00265
ENVP36162.00296
ENVP10203.00141
ENVP10203.00198
ENVP10203.00317
ENVP107.00039
ENVP107.00087
ENVP21819.00029
ENVP21819.00121
ENVP21819.00207
ENVP223.00235
ENVP36162.00069
ENVP366.00287
ENVP10203.00067
ENVP21819.00315
ENVP21819.00355
ENVP36162.00111
ENVP36162.00325
ENVP223.00073
ENVP223.00219
ENVP223.00234
ENVP36162.00176
ENVP36162.00211
ENVP36162.00272
ENVP107.00198
ENVP223.00069

OTU 6 (n = 1)
Stramenopile
==========
ENVP10203.00002

OTU 7 (n = 1)
Stramenopile
==========
ENVP10203.00020

OTU 8 (n = 1)
Stramenopile
==========
ENVP10203.00021

OTU 9 (n = 36)
Ciliophora
==========
ENVP10203.00022
ENVP10203.00349
ENVP36162.00223
ENVP21819.00193
ENVP21819.00014
ENVP223.00119
ENVP223.00137
ENVP223.00195
ENVP223.00262
ENVP223.00271
ENVP36162.00087
ENVP36162.00246
ENVP366.00039
ENVP107.00095
ENVP107.00244
ENVP107.00259
ENVP21819.00252
ENVP223.00011
ENVP223.00019
ENVP223.00003
ENVP223.00007
ENVP223.00080
ENVP223.00081
ENVP223.00111
ENVP223.00151
ENVP223.00161
ENVP223.00183
ENVP223.00194
ENVP223.00207
ENVP223.00226
ENVP223.00255
ENVP223.00272
ENVP223.00275
ENVP36162.00050
ENVP366.00008
ENVP366.00217

OTU 10 (n = 22)
Arthropoda
==========
ENVP10203.00023
ENVP10203.00026
ENVP10203.00055
ENVP10203.00058
ENVP10203.00082
ENVP10203.00094
ENVP10203.00104
ENVP10203.00231
ENVP10203.00297
ENVP21819.00348
ENVP36162.00029
ENVP36162.00039
ENVP36162.00053
ENVP36162.00130
ENVP36162.00173
ENVP36162.00184
ENVP36162.00200
ENVP36162.00329
ENVP36162.00341
ENVP36162.00346
ENVP36162.00358
ENVP36162.00376

OTU 11 (n = 10)
Unclassified
Alveolate
==========
ENVP10203.00024
ENVP10203.00046
ENVP107.00040
ENVP107.00156
ENVP21819.00041
ENVP21819.00151
ENVP21819.00272
ENVP36162.00163
ENVP36162.00257
ENVP366.00172

OTU 12 (n = 14)
Arthropoda
==========
ENVP10203.00027
ENVP10203.00239
ENVP36162.00127
ENVP21819.00027
ENVP21819.00249
ENVP21819.00338
ENVP21819.00352
ENVP21819.00380
ENVP36162.00360
ENVP21819.00019
ENVP21819.00068
ENVP21819.00373
ENVP36162.00326
ENVP36162.00332

OTU 13 (n = 1)
Stramenopile
==========
ENVP10203.00028

OTU 14 (n = 13)
Group I Alveolate
==========
ENVP10203.00029
ENVP10203.00008
ENVP10203.00226
ENVP10203.00311

ENVP21819.00093
ENVP21819.00204
ENVP21819.00232
ENVP21819.00237
ENVP21819.00375
ENVP36162.00098
ENVP36162.00334
ENVP10203.00301
ENVP366.00059

OTU 15 (n = 8)
Dinophyceae
==========
ENVP10203.00003
ENVP10203.00034
ENVP10203.00247
ENVP21819.00229
ENVP21819.00339
ENVP21819.00378
ENVP36162.00008
ENVP36162.00270

OTU 16 (n = 6)
Dinophyceae
==========
ENVP10203.00030
ENVP10203.00248
ENVP107.00206
ENVP21819.00013
ENVP21819.00079
ENVP21819.00384

OTU 17 (n = 6)
Acantharea
==========
ENVP10203.00031
ENVP36162.00015
ENVP36162.00121
ENVP36162.00189
ENVP36162.00231
ENVP36162.00323

OTU 18 (n = 2)
Ciliophora
==========
ENVP10203.00036
ENVP366.00286

OTU 19 (n = 1)
Group II Alveolate
==========
ENVP10203.00037

OTU 20 (n = 7)
Arthropoda
==========
ENVP10203.00040
ENVP21819.00012
ENVP36162.00328
ENVP10203.00361
ENVP36162.00213
ENVP36162.00344
ENVP36162.00349

OTU 21 (n = 7)
Telonema
==========
ENVP10203.00041
ENVP21819.00002
ENVP21819.00228
ENVP21819.00243
ENVP21819.00370
ENVP36162.00266
ENVP10203.00063

OTU 22 (n = 6)
Arthropoda
==========
ENVP10203.00042
ENVP10203.00175
ENVP21819.00198
ENVP21819.00231
ENVP21819.00301
ENVP36162.00150

OTU 23 (n = 9)
Dinophyceae
==========
ENVP21819.00038
ENVP21819.00004
ENVP21819.00202
ENVP36162.00052
ENVP36162.00229
ENVP10203.00044
ENVP10203.00162
ENVP10203.00306
ENVP21819.00313

OTU 24 (n = 3)
Stramenopile
==========
ENVP10203.00045
ENVP10203.00086
ENVP36162.00365

OTU 25 (n = 3)
Group II Alveolate
==========
ENVP10203.00047
ENVP21819.00254
ENVP36162.00140

OTU 26 (n = 8)
Dinophyceae
==========
ENVP10203.00005
ENVP21819.00250
ENVP223.00008
ENVP223.00184
ENVP107.00017
ENVP21819.00239
ENVP21819.00362
ENVP36162.00135

OTU 27 (n = 3)
Stramenopile
==========
ENVP10203.00051
ENVP10203.00227
ENVP36162.00255

OTU 28 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00052

OTU 29 (n = 4)
Arthropoda
==========
ENVP21819.00212
ENVP21819.00245
ENVP21819.00372
ENVP36162.00104

OTU 30 (n = 7)
Arthropoda
==========
ENVP10203.00056
ENVP10203.00163
ENVP21819.00023
ENVP21819.00048
ENVP21819.00297
ENVP21819.00325
ENVP21819.00328

OTU 31 (n = 1)
Group I Alveolate
==========
ENVP10203.00057

OTU 32 (n = 1)
Group I Alveolate
==========
ENVP10203.00059

OTU 33 (n = 4)
Unclassified
Alveolate
==========
ENVP10203.00060
ENVP21819.00323
ENVP21819.00369
ENVP36162.00227

OTU 34 (n = 4)
Unclassified
Alveolate
==========
ENVP10203.00061
ENVP21819.00046
ENVP21819.00268
ENVP36162.00160

OTU 35 (n = 8)
Unclassified
Alveolate
==========
ENVP10203.00076
ENVP21819.00356
ENVP107.00130
ENVP107.00199
ENVP107.00269
ENVP21819.00327
ENVP223.00021
ENVP223.00074

OTU 36 (n = 2)
Stramenopile
==========
ENVP10203.00069
ENVP21819.00197

OTU 37 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00070
ENVP21819.00109

OTU 38 (n = 10)
Group I Alveolate
==========
ENVP10203.00073
ENVP10203.00218
ENVP10203.00263
ENVP10203.00269
ENVP10203.00336
ENVP36162.00097
ENVP36162.00217
ENVP10203.00071
ENVP21819.00054
ENVP36162.00268

OTU 39 (n = 5)
Stramenopile
==========
ENVP10203.00072
ENVP10203.00255
ENVP21819.00066
ENVP21819.00164
ENVP21819.00330

OTU 40 (n = 1)
Dinophyceae
==========
ENVP10203.00074

OTU 41 (n = 1)
Dinophyceae
==========
ENVP10203.00075

OTU 42 (n = 3)
Group I Alveolate
==========
ENVP366.00196
ENVP366.00276
ENVP223.00232

OTU 43 (n = 4)
Euglenozoa
==========
ENVP10203.00080
ENVP10203.00350
ENVP21819.00294
ENVP36162.00314

OTU 44 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00081

OTU 45 (n = 1)
Stramenopile
==========
ENVP10203.00085

OTU 46 (n = 1)
Ciliophora
==========
ENVP10203.00092

OTU 47 (n = 13)
Arthropoda
==========
ENVP10203.00093
ENVP10203.00266
ENVP21819.00006
ENVP223.00015
ENVP223.00006
ENVP223.00118
ENVP223.00136
ENVP223.00190
ENVP223.00192
ENVP223.00196
ENVP223.00267
ENVP36162.00208
ENVP36162.00221

OTU 48 (n = 2)
Dinophyceae
==========
ENVP10203.00095
ENVP10203.00096

OTU 49 (n = 1)
Acantharea
==========
ENVP10203.00112

OTU 50 (n = 1)
Dinophyceae
==========
ENVP10203.00133

OTU 51 (n = 1)
Group I Alveolate
==========
ENVP10203.00137

OTU 52 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00152

OTU 53 (n = 6)
Cnidaria
==========
ENVP10203.00166
ENVP223.00022
ENVP223.00050

ENVP223.00075
ENVP223.00191
ENVP366.00146

OTU 54 (n = 1)
Dinophyceae
==========
ENVP10203.00169

OTU 55 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00177

OTU 56 (n = 4)
Chlorophyta
==========
ENVP10203.00196
ENVP21819.00071
ENVP36162.00079
ENVP36162.00222

OTU 57 (n = 1)
Stramenopile
==========
ENVP10203.00199

OTU 58 (n = 4)
Stramenopile
==========
ENVP10203.00200
ENVP21819.00089
ENVP21819.00213
ENVP36162.00367

OTU 59 (n = 3)
Unclassified
Alveolate
==========
ENVP10203.00202
ENVP21819.00163
ENVP21819.00225

OTU 60 (n = 2)
Group I Alveolate
==========
ENVP10203.00203
ENVP36162.00354

OTU 61 (n = 3)
Unclassified
Alveolate
==========
ENVP10203.00205
ENVP21819.00240
ENVP36162.00321

OTU 62 (n = 9)
Unclassified
Alveolate
==========
ENVP10203.00206
ENVP21819.00158
ENVP21819.00210
ENVP21819.00230
ENVP21819.00238
ENVP21819.00324
ENVP21819.00333
ENVP21819.00351
ENVP21819.00376

OTU 63 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00216
ENVP36162.00342

OTU 64 (n = 2)
Euglenozoa
==========
ENVP10203.00217
ENVP36162.00343

OTU 65 (n = 1)
Dinophyceae
==========
ENVP10203.00219

OTU 66 (n = 1)
Arthropoda
==========
ENVP10203.00222

OTU 67 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00224

OTU 68 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00225
ENVP10203.00313

OTU 69 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00228

OTU 70 (n = 1)
Euglenozoa
==========
ENVP10203.00230

OTU 71 (n = 1)
Group II Alveolate
==========
ENVP10203.00232

OTU 72 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00233
ENVP10203.00245

OTU 73 (n = 1)
Group I Alveolate
==========
ENVP10203.00234

OTU 74 (n = 3)
Group I Alveolate
==========
ENVP10203.00235
ENVP21819.00350
ENVP36162.00089

OTU 75 (n = 6)
Euglenozoa
==========
ENVP10203.00236
ENVP223.00156
ENVP223.00198
ENVP223.00211
ENVP223.00282
ENVP366.00200

OTU 76 (n = 1)
Polycystinea
==========
ENVP10203.00238

OTU 77 (n = 8)
Unclassified
Alveolate
==========
ENVP10203.00242
ENVP10203.00272
ENVP21819.00233
ENVP21819.00255
ENVP21819.00304
ENVP36162.00037
ENVP36162.00038
ENVP36162.00243

OTU 78 (n = 1)
Acantharea
==========
ENVP10203.00243

OTU 79 (n = 5)
Arthropoda
==========
ENVP10203.00246
ENVP223.00029
ENVP223.00084
ENVP36162.00028
ENVP36162.00251

OTU 80 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00251
ENVP36162.00080

OTU 81 (n = 1)
Ciliophora
==========
ENVP10203.00252

OTU 82 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00254
ENVP10203.00338

OTU 83 (n = 1)
Choanoflagellida
==========
ENVP10203.00258

OTU 84 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00259
ENVP36162.00340

OTU 85 (n = 3)
Group II Alveolate
==========
ENVP10203.00260
ENVP10203.00377
ENVP36162.00302

OTU 86 (n = 7)
Arthropoda
==========
ENVP10203.00264
ENVP10203.00298
ENVP10203.00329
ENVP36162.00106
ENVP36162.00194
ENVP36162.00363
ENVP36162.00368

OTU 87 (n = 1)
Sticholonchidae
==========
ENVP10203.00267

OTU 88 (n = 2)
Stramenopile
==========
ENVP10203.00270
ENVP21819.00086

OTU 89 (n = 1)
Group I Alveolate
==========
ENVP10203.00275

OTU 90 (n = 1)
Dinophyceae
==========
ENVP10203.00277

OTU 91 (n = 2)
Acantharea
==========
ENVP10203.00281
ENVP21819.00336

OTU 92 (n = 1)
Group II Alveolate
==========
ENVP10203.00300

OTU 93 (n = 13)
Group I Alveolate
==========
ENVP10203.00302
ENVP10203.00376
ENVP21819.00087
ENVP21819.00293
ENVP36162.00027
ENVP36162.00235
ENVP36162.00374
ENVP36162.00013
ENVP223.00082
ENVP21819.00076
ENVP36162.00136
ENVP36162.00148
ENVP36162.00059

OTU 94 (n = 3)
Group II Alveolate
==========
ENVP10203.00314
ENVP21819.00072
ENVP36162.00191

OTU 95 (n = 1)
Acantharea
==========
ENVP10203.00322

OTU 96 (n = 4)
Ciliophora
==========
ENVP10203.00324
ENVP21819.00182
ENVP36162.00271
ENVP36162.00350

OTU 97 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00335

OTU 98 (n = 1)
Unclassified
Alveolate
==========
ENVP10203.00337

OTU 99 (n = 1)
Stramenopile
==========
ENVP10203.00341

OTU 100 (n = 2)
Group I Alveolate
==========
ENVP10203.00343
ENVP21819.00316

OTU 101 (n = 1)
Dinophyceae
==========
ENVP10203.00345

OTU 102 (n = 2)
Acantharea
==========
ENVP10203.00346
ENVP223.00240

OTU 103 (n = 1)
Stramenopile
==========
ENVP10203.00356

OTU 104 (n = 8)
Polycystinea
==========
ENVP107.00001
ENVP107.00082
ENVP107.00122
ENVP107.00155
ENVP107.00226
ENVP107.00230
ENVP223.00094
ENVP366.00283

OTU 105 (n = 101)
Polycystinea
==========
ENVP107.00011
ENVP107.00012
ENVP107.00016
ENVP107.00025
ENVP107.00003
ENVP107.00041
ENVP107.00046
ENVP107.00004
ENVP107.00050
ENVP107.00057
ENVP107.00064
ENVP107.00008
ENVP107.00084
ENVP107.00086
ENVP107.00102
ENVP107.00107
ENVP107.00115
ENVP107.00121
ENVP107.00132
ENVP107.00142
ENVP107.00153
ENVP107.00165
ENVP107.00166
ENVP107.00167
ENVP107.00174
ENVP107.00179
ENVP107.00181
ENVP107.00196
ENVP107.00197
ENVP107.00220
ENVP107.00229
ENVP107.00232
ENVP107.00249
ENVP107.00250
ENVP107.00252
ENVP107.00255
ENVP107.00264
ENVP107.00268
ENVP107.00277
ENVP223.00046
ENVP223.00059
ENVP223.00076
ENVP223.00103
ENVP223.00141
ENVP223.00165
ENVP223.00166
ENVP223.00167
ENVP223.00177
ENVP223.00099
ENVP223.00221
ENVP223.00249
ENVP223.00287
ENVP366.00218
ENVP366.00244
ENVP366.00247
ENVP366.00273
ENVP107.00068
ENVP107.00173
ENVP107.00182
ENVP107.00195
ENVP107.00242
ENVP223.00146
ENVP223.00155
ENVP223.00174
ENVP223.00182
ENVP223.00189
ENVP107.00056
ENVP107.00061
ENVP107.00065
ENVP107.00074
ENVP107.00088
ENVP107.00162
ENVP107.00097
ENVP107.00237
ENVP223.00013
ENVP223.00024
ENVP223.00054
ENVP223.00200
ENVP223.00208
ENVP366.00043
ENVP366.00005
ENVP366.00120
ENVP366.00173
ENVP366.00269
ENVP107.00014
ENVP107.00047
ENVP107.00073
ENVP107.00145
ENVP107.00154
ENVP107.00168
ENVP107.00279
ENVP223.00117
ENVP223.00121
ENVP223.00139
ENVP223.00152
ENVP223.00154
ENVP223.00172
ENVP223.00253
ENVP223.00264
ENVP366.00038
ENVP366.00177

OTU 106 (n = 3)
Acantharea
==========
ENVP107.00015
ENVP107.00262
ENVP223.00133

OTU 107*** (n = 1)
Streptophyta
==========
ENVP366.00275

OTU 108 (n = 7)
Acantharea
==========
ENVP107.00002
ENVP107.00070
ENVP107.00278
ENVP223.00197
ENVP223.00236
ENVP366.00123
ENVP366.00246

OTU 109 (n = 61)
Cnidaria
==========
ENVP21819.00031
ENVP223.00037
ENVP223.00060
ENVP223.00086
ENVP223.00134
ENVP36162.00063
ENVP36162.00260
ENVP366.00137
ENVP366.00175
ENVP366.00278
ENVP107.00112
ENVP107.00267
ENVP107.00273
ENVP223.00004
ENVP223.00058
ENVP223.00066
ENVP223.00135
ENVP223.00153
ENVP223.00162
ENVP223.00098
ENVP223.00203
ENVP223.00205
ENVP223.00224
ENVP223.00243
ENVP223.00244
ENVP223.00246
ENVP223.00247
ENVP223.00270
ENVP223.00274
ENVP223.00276
ENVP223.00278
ENVP223.00283
ENVP36162.00180
ENVP366.00037
ENVP366.00209
ENVP366.00232
ENVP366.00265
ENVP107.00075
ENVP223.00056
ENVP36162.00085
ENVP107.00026
ENVP107.00099
ENVP107.00212
ENVP107.00231
ENVP107.00280
ENVP223.00012
ENVP223.00067
ENVP223.00078
ENVP223.00107
ENVP223.00149
ENVP223.00160
ENVP223.00214
ENVP223.00216
ENVP366.00142
ENVP366.00193
ENVP366.00219
ENVP366.00223
ENVP366.00248
ENVP366.00251
ENVP366.00259
ENVP366.00274

OTU 110 (n = 1)
Cnidaria
==========
ENVP107.00036

OTU 111 (n = 1)
Group II Alveolate
==========
ENVP107.00037

OTU 112 (n = 16)
Group II Alveolate
==========
ENVP107.00042
ENVP107.00062
ENVP107.00094
ENVP107.00210
ENVP107.00276
ENVP366.00060
ENVP366.00117
ENVP366.00124
ENVP366.00140
ENVP366.00160
ENVP366.00233
ENVP107.00161
ENVP366.00112
ENVP366.00113
ENVP366.00162
ENVP366.00189

OTU 113 (n = 6)
Acantharea
==========
ENVP107.00044
ENVP107.00148
ENVP223.00100
ENVP366.00025
ENVP366.00234
ENVP366.00238

OTU 114 (n = 11)
Euglenozoa
==========
ENVP107.00005
ENVP107.00120
ENVP107.00147
ENVP107.00223
ENVP223.00042
ENVP223.00053
ENVP366.00168
ENVP223.00113
ENVP223.00261
ENVP107.00007
ENVP223.00038

OTU 115 (n = 1)
Polycystinea
==========
ENVP107.00051

OTU 116 (n = 1)
Cnidaria
==========
ENVP107.00053

OTU 117 (n = 1)
Polycystinea
==========
ENVP107.00058

OTU 118 (n = 11)
Sticholonchidae
==========
ENVP223.00085
ENVP366.00044
ENVP366.00261
ENVP107.00006
ENVP107.00131
ENVP223.00217
ENVP223.00230
ENVP36162.00129
ENVP366.00249
ENVP107.00152
ENVP36162.00193

OTU 119 (n = 2)
Ciliophora
==========
ENVP107.00066
ENVP223.00130

OTU 120 (n = 3)
Group II Alveolate
==========
ENVP107.00067
ENVP223.00018
ENVP366.00170

OTU 121 (n = 6)
Group I Alveolate
==========
ENVP107.00071
ENVP107.00079
ENVP107.00104
ENVP107.00222
ENVP107.00261
ENVP107.00270

OTU 122 (n = 3)
Dinophyceae
==========
ENVP107.00077
ENVP223.00040
ENVP223.00088

OTU 123 (n = 5)
Polycystinea
==========
ENVP107.00078
ENVP107.00257
ENVP223.00122
ENVP223.00140
ENVP223.00242

OTU 124 (n = 1)
Chlorophyta
==========
ENVP107.00081

OTU 125 (n = 3)
Unclassified
Alveolate
==========
ENVP107.00083
ENVP21819.00335
ENVP21819.00344

OTU 126 (n = 1)
Group II Alveolate
==========
ENVP107.00085

OTU 127 (n = 1)
Unclassified
Alveolate
==========
ENVP107.00111

OTU 128 (n = 5)
Acantharea
==========
ENVP107.00119
ENVP223.00035
ENVP223.00170
ENVP223.00269
ENVP366.00213

OTU 129 (n = 1)
Unclassified
Alveolate
==========
ENVP107.00123

OTU 130 (n = 4)
Cnidaria
==========
ENVP107.00124
ENVP223.00079
ENVP223.00173
ENVP223.00229

OTU 131 (n = 1)
Ciliophora
==========
ENVP107.00125

OTU 132 (n = 2)
Unclassified
Alveolate
==========
ENVP107.00136
ENVP107.00258

OTU 133 (n = 1)
Unclassified
Alveolate
==========
ENVP107.00138

OTU 134 (n = 4)
Euglenozoa
==========
ENVP107.00159
ENVP107.00163
ENVP21819.00292
ENVP223.00077

OTU 135 (n = 1)
Group I Alveolate
==========
ENVP107.00172

OTU 136 (n = 1)
Euglenozoa
==========
ENVP107.00183

OTU 137 (n = 2)
Acantharea
==========
ENVP107.00201
ENVP223.00125

OTU 138 (n = 3)
Unclassified
Alveolate
==========
ENVP107.00204
ENVP107.00253
ENVP366.00144

OTU 139 (n = 4)
Sticholonchidae
==========
ENVP107.00208
ENVP21819.00122
ENVP223.00225
ENVP21819.00067

OTU 140 (n = 1)
Group I Alveolate
==========
ENVP107.00217

OTU 141 (n = 1)
Dinophyceae
==========
ENVP366.00271

OTU 142 (n = 1)
Euglenozoa
==========
ENVP107.00221

OTU 143 (n = 2)
Group I Alveolate
==========
ENVP107.00234
ENVP36162.00248

OTU 144 (n = 1)
Acantharea
==========
ENVP107.00235

OTU 145 (n = 1)
Acantharea
==========
ENVP107.00241

OTU 146 (n = 14)
Group II Alveolate
==========
ENVP10203.00015
ENVP107.00243
ENVP21819.00025
ENVP366.00190
ENVP366.00263
ENVP107.00194
ENVP366.00027
ENVP366.00049
ENVP107.00110
ENVP107.00256
ENVP366.00048
ENVP366.00174
ENVP366.00097
ENVP366.00272

OTU 147 (n = 1)
Euglenozoa
==========
ENVP107.00247

OTU 148 (n = 1)
Unclassified
Alveolate
==========
ENVP107.00254

OTU 149 (n = 1)
Fungi
==========
ENVP107.00265

OTU 150 (n = 1)
Acantharea
==========
ENVP366.00270

OTU 151 (n = 1)
Ciliophora
==========
ENVP107.00281

OTU 152 (n = 2)
Rhodophyta
==========
ENVP21819.00010
ENVP21819.00307

OTU 153 (n = 1)
Acantharea
==========
ENVP21819.00015

OTU 154 (n = 1)
Acantharea
==========
ENVP21819.00017

OTU 155 (n = 2)
Telonema
==========
ENVP21819.00018
ENVP36162.00331

OTU 156 (n = 1)
Cryptophyta
==========
ENVP21819.00024

OTU 157 (n = 1)
Acantharea
==========
ENVP21819.00026

OTU 158 (n = 1)
Group II Alveolate
==========
ENVP21819.00028

OTU 159 (n = 1)
Dinophyceae
==========
ENVP21819.00033

OTU 160 (n = 31)
Polycystinea
==========
ENVP21819.00039
ENVP366.00166
ENVP21819.00220
ENVP21819.00244
ENVP21819.00267
ENVP36162.00051
ENVP36162.00244
ENVP366.00129
ENVP366.00133
ENVP366.00161
ENVP366.00202
ENVP366.00260
ENVP366.00267
ENVP366.00051
ENVP366.00058
ENVP366.00128
ENVP366.00130
ENVP366.00167
ENVP366.00099
ENVP366.00204
ENVP366.00211
ENVP366.00220
ENVP366.00229
ENVP366.00231
ENVP366.00241
ENVP366.00243
ENVP366.00257
ENVP366.00111
ENVP366.00158
ENVP366.00268
ENVP366.00280

OTU 161 (n = 1)
Group I Alveolate
==========
ENVP21819.00040

OTU 162 (n = 2)
Cryptophyta
==========
ENVP21819.00043
ENVP21819.00147

OTU 163 (n = 1)
Group I Alveolate
==========
ENVP21819.00047

OTU 164 (n = 2)
Haptophyceae
==========
ENVP21819.00049
ENVP366.00164

OTU 165 (n = 1)
Dinophyceae
==========
ENVP21819.00005

OTU 166 (n = 2)
Group II Alveolate
==========
ENVP21819.00051
ENVP21819.00157

OTU 167 (n = 2)
Dinophyceae
==========
ENVP21819.00053
ENVP36162.00233

OTU 168 (n = 2)
Ciliophora
==========
ENVP21819.00055
ENVP36162.00225

OTU 169 (n = 2)
Polycystinea
==========
ENVP21819.00057
ENVP36162.00284

OTU 170 (n = 1)
Ciliophora
==========
ENVP21819.00059

OTU 171 (n = 1)
Acantharea
==========
ENVP21819.00062

OTU 172 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00064

OTU 173 (n = 1)
Fungi
==========
ENVP21819.00078

OTU 174 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00082

OTU 175 (n = 1)
Cryptophyta
==========
ENVP21819.00090

OTU 176 (n = 1)
Acantharea
==========
ENVP21819.00092

OTU 177 (n = 1)
Group I Alveolate
==========
ENVP21819.00096

OTU 178 (n = 5)
Unclassified
Alveolate
==========
ENVP21819.00110
ENVP366.00136
ENVP36162.00122
ENVP36162.00373
ENVP366.00042

OTU 179 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00136

OTU 180 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00141

OTU 181 (n = 3)
Unclassified
Alveolate
==========
ENVP21819.00146
ENVP21819.00364
ENVP366.00147

OTU 182 (n = 3)
Unclassified
Alveolate
==========
ENVP21819.00149
ENVP21819.00280
ENVP36162.00253

OTU 183 (n = 2)
Polycystinea
==========
ENVP21819.00186
ENVP21819.00309

OTU 184 (n = 2)
Polycystinea
==========
ENVP21819.00195
ENVP21819.00277

OTU 185 (n = 1)
Acantharea
==========
ENVP21819.00201

OTU 186 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00203

OTU 187 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00209

OTU 188 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00218

OTU 189 (n = 1)
Dinophyceae
==========
ENVP21819.00226

OTU 190 (n = 1)
Dinophyceae
==========
ENVP21819.00242

OTU 191 (n = 2)
Haptophyceae
==========
ENVP21819.00251
ENVP36162.00044

OTU 192 (n = 1)
Group II Alveolate
==========
ENVP21819.00253

OTU 193 (n = 1)
Chlorophyta
==========
ENVP21819.00256

OTU 194 (n = 3)
Unclassified
Alveolate
==========
ENVP21819.00261
ENVP36162.00234
ENVP36162.00256

OTU 195 (n = 1)
Euglenozoa
==========
ENVP21819.00265

OTU 196 (n = 2)
Group I Alveolate
==========
ENVP21819.00266
ENVP36162.00067

OTU 197*** (n = 2)
Streptophyta
==========
ENVP21819.00290
ENVP21819.00298

OTU 198 (n = 1)
Ciliophora
==========
ENVP21819.00308

OTU 199 (n = 1)
Group II Alveolate
==========
ENVP21819.00314

OTU 200 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00318

OTU 201 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00326

OTU 202
Unclassified
Alveolate (n = 2)
==========
ENVP21819.00341
ENVP36162.00230

OTU 203 (n = 1)
Group II Alveolate
==========
ENVP21819.00343

OTU 204 (n = 1)
Stramenopile
==========
ENVP21819.00347

OTU 205 (n = 1)
Unclassified
Alveolate
==========
ENVP21819.00353

OTU 206 (n = 1)
Acantharea
==========
ENVP21819.00358

OTU 207 (n = 2)
Polycystinea
==========
ENVP21819.00363
ENVP36162.00040

OTU 208 (n = 4)
Group I Alveolate
==========
ENVP21819.00371
ENVP223.00268
ENVP223.00163
ENVP366.00126

OTU 209 (n = 1)
Dinophyceae
==========
ENVP21819.00377

OTU 210 (n = 2)
Acantharea
==========
ENVP21819.00381
ENVP36162.00185

OTU 211 (n = 5)
Group I Alveolate
==========
ENVP223.00014
ENVP223.00148
ENVP223.00199
ENVP223.00286
ENVP36162.00348

OTU 212 (n = 2)
Group I Alveolate
==========
ENVP223.00017
ENVP223.00033

OTU 213 (n = 1)
Euglenozoa
==========
ENVP223.00002

OTU 214 (n = 1)
Euglenozoa
==========
ENVP223.00025

OTU 215 (n = 1)
Ciliophora
==========
ENVP223.00026

OTU 216 (n = 1)
Polycystinea
==========
ENVP223.00028

OTU 217 (n = 1)
Euglenozoa
==========
ENVP223.00030

OTU 218 (n = 3)
Group I Alveolate
==========
ENVP223.00031
ENVP223.00052
ENVP223.00070

OTU 219 (n = 1)
Euglenozoa
==========
ENVP223.00032

OTU 220 (n = 3)
Stramenopile
==========
ENVP223.00036
ENVP223.00047
ENVP36162.00198

OTU 221 (n = 10)
Euglenozoa
==========
ENVP223.00039
ENVP223.00043
ENVP223.00049
ENVP223.00065
ENVP223.00144
ENVP223.00228
ENVP223.00257
ENVP223.00259
ENVP366.00139
ENVP366.00206

OTU 222 (n = 1)
Cercozoa
==========
ENVP223.00041

OTU 223 (n = 1)
Dinophyceae
==========
ENVP223.00057

OTU 224 (n = 1)
Unclassified
Alveolate
==========
ENVP223.00061

OTU 225 (n = 1)
Unclassified
Alveolate
==========
ENVP223.00062

OTU 226 (n = 4)
Ctenophora
==========
ENVP223.00064
ENVP36162.00118
ENVP36162.00157
ENVP36162.00324

OTU 227 (n = 1)
Euglenozoa
==========
ENVP223.00068

OTU 228 (n = 2)
Unclassified
Alveolate
==========
ENVP10203.00066
ENVP223.00071

OTU 229 (n = 1)
Euglenozoa
==========
ENVP223.00087

OTU 230 (n = 1)
Unclassified
Alveolate
==========
ENVP223.00009

OTU 231 (n = 1)
Polycystinea
==========
ENVP223.00090

OTU 232 (n = 1)
Dinophyceae
==========
ENVP223.00091

OTU 233 (n = 1)
Unclassified
Alveolate
==========
ENVP223.00093

OTU 234 (n = 2)
Polycystinea
==========
ENVP223.00096
ENVP223.00254

OTU 235 (n = 3)
Unclassified
Alveolate
==========
ENVP223.00109
ENVP223.00115
ENVP223.00178

OTU 236 (n = 2)
Group II Alveolate
==========
ENVP223.00112
ENVP223.00132

OTU 237 (n = 1)
Euglenozoa
==========
ENVP223.00127

OTU 238 (n = 2)
Euglenozoa
==========
ENVP223.00142
ENVP366.00075

OTU 239 (n = 2)
Acantharea
==========
ENVP223.00150
ENVP366.00148

OTU 240 (n = 2)
Euglenozoa
==========
ENVP223.00158
ENVP36162.00375

OTU 241 (n = 1)
Stramenopile
==========
ENVP223.00168

OTU 242 (n = 1)
Dinophyceae
==========
ENVP223.00171

OTU 243 (n = 1)
Ciliophora
==========
ENVP223.00176

OTU 244 (n = 3)
Group I Alveolate
==========
ENVP223.00179
ENVP223.00245
ENVP366.00050

OTU 245 (n = 1)
Ciliophora
==========
ENVP223.00185

OTU 246 (n = 5)
Group I Alveolate
==========
ENVP223.00097
ENVP223.00213
ENVP366.00104
ENVP366.00159
ENVP366.00098

OTU 247 (n = 1)
Group I Alveolate
==========
ENVP223.00206

OTU 248 (n = 1)
Ciliophora
==========
ENVP223.00222

OTU 249 (n = 2)
Euglenozoa
==========
ENVP223.00238
ENVP366.00266

OTU 250 (n = 5)
Acantharea
==========
ENVP21819.00346
ENVP223.00231
ENVP223.00250
ENVP223.00260
ENVP223.00285

OTU 251 (n = 1)
Fungi
==========
ENVP223.00256

OTU 252 (n = 1)
Dinophyceae
==========
ENVP223.00266

OTU 253 (n = 1)
Group I Alveolate
==========
ENVP36162.00002

OTU 254 (n = 1)
Arthropoda
==========
ENVP36162.00003

OTU 255 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00026

OTU 256 (n = 4)
Group II Alveolate
==========
ENVP36162.00032
ENVP36162.00361
ENVP36162.00242
ENVP36162.00364

OTU 257 (n = 1)
Euglenozoa
==========
ENVP36162.00035

OTU 258 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00045

OTU 259 (n = 2)
Dinophyceae
==========
ENVP36162.00005
ENVP36162.00339

OTU 260 (n = 1)
Dinophyceae
==========
ENVP36162.00054

OTU 261 (n = 1)
Polycystinea
==========
ENVP36162.00055

OTU 262 (n = 1)
Dinophyceae
==========
ENVP36162.00057

OTU 263 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00062

OTU 264 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00064

OTU 265 (n = 1)
Ciliophora
==========
ENVP36162.00065

OTU 266 (n = 1)
Acantharea
==========
ENVP36162.00073

OTU 267 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00076

OTU 268 (n = 1)
Group II Alveolate
==========
ENVP36162.00082

OTU 269 (n = 1)
Stramenopile
==========
ENVP36162.00092

OTU 270 (n = 1)
Polycystinea
==========
ENVP36162.00093

OTU 271 (n = 4)
Acantharea
==========
ENVP36162.00105
ENVP36162.00131
ENVP36162.00153
ENVP36162.00207

OTU 272 (n = 1)
Group II Alveolate
==========
ENVP36162.00110

OTU 273 (n = 4)
Arthropoda
==========
ENVP36162.00020
ENVP36162.00115
ENVP36162.00303
ENVP36162.00371

OTU 274 (n = 2)
Fungi
==========
ENVP36162.00120
ENVP36162.00353

OTU 275 (n = 2)
Group I Alveolate
==========
ENVP36162.00123
ENVP36162.00169

OTU 276 (n = 1)
Group I Alveolate
==========
ENVP36162.00124

OTU 277 (n = 1)
Group I Alveolate
==========
ENVP36162.00128

OTU 278 (n = 1)
Euglenozoa
==========
ENVP36162.00132

OTU 279 (n = 2)
Polychaeta
==========
ENVP36162.00134
ENVP36162.00258

OTU 280 (n = 2)
Stramenopile
==========
ENVP36162.00146
ENVP366.00207

OTU 281 (n = 1)
Group I Alveolate
==========
ENVP36162.00152

OTU 282 (n = 1)
Stramenopile
==========
ENVP36162.00156

OTU 283 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00165

OTU 284 (n = 1)
Stramenopile
==========
ENVP36162.00187

OTU 285 (n = 1)
Polycystinea
==========
ENVP36162.00099

OTU 286 (n = 1)
Haptophyceae
==========
ENVP36162.00209

OTU 287 (n = 1)
Dinophyceae
==========
ENVP36162.00238

OTU 288 (n = 1)
Dinophyceae
==========
ENVP36162.00239

OTU 289 (n = 1)
Dinophyceae
==========
ENVP36162.00254

OTU 290 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00259

OTU 291 (n = 1)
Group I Alveolate
==========
ENVP36162.00267

OTU 292 (n = 1)
Dinophyceae
==========
ENVP36162.00279

OTU 293 (n = 1)
Rhodophyta
==========
ENVP36162.00289

OTU 294 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00290

OTU 295 (n = 1)
Dinophyceae
==========
ENVP36162.00294

OTU 296 (n = 1)
Polycystinea
==========
ENVP36162.00301

OTU 297 (n = 1)
Euglenozoa
==========
ENVP36162.00308

OTU 298 (n = 1)
Ciliophora
==========
ENVP36162.00317

OTU 299 (n = 4)
Polycystinea
==========
ENVP10203.00078
ENVP10203.00325
ENVP21819.00133
ENVP36162.00318

OTU 300 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00319

OTU 301 (n = 1)
Haptophyceae
==========
ENVP36162.00330

OTU 302 (n = 1)
Chlorophyta
==========
ENVP36162.00333

OTU 303 (n = 1)
Polycystinea
==========
ENVP36162.00335

OTU 304 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00336

OTU 305 (n = 1)
Group I Alveolate
==========
ENVP36162.00338

OTU 306 (n = 1)
Polycystinea
==========
ENVP36162.00347

OTU 307 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00351

OTU 308 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00359

OTU 309 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00362

OTU 310 (n = 2)
Arthropoda
==========
ENVP10203.00054
ENVP36162.00366

OTU 311 (n = 1)
Unclassified
Alveolate
==========
ENVP36162.00369

OTU 312 (n = 1)
Sticholonchidae
==========
ENVP36162.00370

OTU 313 (n = 1)
Dinophyceae
==========
ENVP36162.00377

OTU 314 (n = 1)
Group II Alveolate
==========
ENVP366.00015

OTU 315 (n = 7)
Group II Alveolate
==========
ENVP107.00018
ENVP107.00170
ENVP107.00207
ENVP107.00248
ENVP223.00005
ENVP366.00029
ENVP366.00003

OTU 316 (n = 1)
Unclassified
Alveolate
==========
ENVP366.00030

OTU 317 (n = 1)
Unclassified
Alveolate
==========
ENVP366.00031

OTU 318 (n = 2)
Euglenozoa
==========
ENVP366.00052
ENVP366.00064

OTU 319 (n = 1)
Group II Alveolate
==========
ENVP366.00053

OTU 320 (n = 1)
Stramenopile
==========
ENVP366.00006

OTU 321 (n = 1)
Group I Alveolate
==========
ENVP366.00061

OTU 322 (n = 4)
Acantharea
==========
ENVP366.00063
ENVP366.00153
ENVP366.00258
ENVP366.00281

OTU 323 (n = 1)
Euglenozoa
==========
ENVP366.00065

OTU 324 (n = 1)
Euglenozoa
==========
ENVP366.00007

OTU 325 (n = 1)
Unclassified
Alveolate
==========
ENVP366.00074

OTU 326 (n = 1)
Polycystinea
==========
ENVP366.00076

OTU 327 (n = 1)
Acantharea
==========
ENVP366.00086

OTU 328 (n = 2)
Euglenozoa
==========
ENVP366.00087
ENVP366.00154

OTU 329 (n = 1)
Stramenopile
==========
ENVP366.00101

OTU 330 (n = 1)
Unclassified
Alveolate
==========
ENVP366.00110

OTU 331 (n = 1)
Sticholonchidae
==========
ENVP366.00116

OTU 332 (n = 2)
Group I Alveolate
==========
ENVP366.00118
ENVP366.00151

OTU 333 (n = 2)
Euglenozoa
==========
ENVP107.00218
ENVP366.00122

OTU 334 (n = 1)
Group I Alveolate
==========
ENVP366.00127

OTU 335 (n = 1)
Euglenozoa
==========
ENVP366.00134

OTU 336 (n = 1)
Acantharea
==========
ENVP366.00135

OTU 337 (n = 2)
Euglenozoa
==========
ENVP366.00143
ENVP366.00165

OTU 338 (n = 1)
Euglenozoa
==========
ENVP366.00163

OTU 339 (n = 1)
Dinophyceae
==========
ENVP366.00230

OTU 340 (n = 1)
Polycystinea
==========
ENVP366.00235

OTU 341 (n = 1)
Euglenozoa
==========
ENVP366.00237

OTU 342 (n = 1)
Dinophyceae
==========
ENVP366.00240

OTU 343 (n = 1)
Dinophyceae
==========
ENVP366.00250

OTU 344 (n = 2)
Acantharea
==========
ENVP107.00266
ENVP366.00252

OTU 345 (n = 1)
Euglenozoa
==========
ENVP366.00255

OTU 346 (n = 1)
Group I Alveolate
==========
ENVP366.00264