Hydrothermal activity and water mass composition shape microbial eukaryote diversity and biogeography in the Okinawa Trough

Margaret Mars Brisbin[a], Asa E. Conover[a], Satoshi Mitarai[a]

[a] Marine Biophysics Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Correspondance
M. Mars Brisbin, Marine Biophysics Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Okinawa, Japan
e-mail: margaret.marsbrisbin@oist.jp

Abstract

Microbial eukaryotes (protists) contribute substantially to ecological functioning in marine ecosystems, but factors shaping protist diversity, such as dispersal barriers and environmental selection, remain difficult to parse. Deep-sea water masses, which form geographic barriers, and hydrothermal vents, which represent isolated productivity hotspots, are ideal opportunities for studying the effects of dispersal barriers and environmental selection on protist communities. The Okinawa Trough, a deep, back-arc spreading basin, contains distinct water masses in the northern and southern regions and at least twenty-five active hydrothermal vents. In this study we used metabarcoding to characterize protistan communities from fourteen stations spanning the length of the Okinawa Trough, including three hydrothermal vent sites. Significant differences in community by region and water mass were present, and protist communities in hydrothermally-influenced bottom-water were significantly different from communities in other bottom waters. Amplicon sequence variants enriched at vent sites largely derived from cosmopolitan protists and many were identical to sequences recovered from other vents. However, a putatively symbiotic ciliate appeared endemic to Okinawa Trough vents. Results support the view that most protists are cosmopolitan opportunists, but moderate endemism exists. Furthermore, dispersal limitation in back-arc basins may contribute to allopatric differentiation, especially if protists rely on endemic hosts.

Introduction

Microbial unicellular eukaryotes (protists) are important contributors to all marine ecosystems, from the sunlit surface waters (1) to the deep, dark bathypelagic (2). The ongoing and fast-paced accumulation of environmental genetic data is revealing extensive phylogenetic and functional diversity among protists, especially in extreme environments like the deep sea and hydrothermal vents (3). Despite global sampling efforts (4,5), protistan biogeography is still a complicated issue and endemism versus

cosmopolitanism remain competing theories (6,7). Deep-sea water masses (8) and hydrothermal vents have proven useful testing grounds for the relative roles of environmental selection and limited dispersal in shaping prokaryotic marine biogeography (9). Comparable studies on marine protists, however, remain sparse (10).

The Okinawa Trough (OT), a deep (> 2000 m) back-arc spreading basin within the East China Sea (ECS), represents an ideal location to investigate how deep-sea water mass composition and hydrothermal activity influence protist diversity and biogeography. The OT is bounded by the Ryukyu Archipelago island chain to the east, which separates the ECS from the Philippine Sea. The Kuroshio Current, the warm western boundary current of the North Pacific subtropical gyre, enters the OT from the south near Taiwan and travels northward. In the south, Kuroshio Intermediate Water (KIW), a mixture of high-salinity South China Sea Intermediate Water (SCSIW) and low-salinity North Pacific Intermediate Water (NPIW), flows into and fills the southern OT (11). The Kerama Gap, the deepest channel connecting the OT and the Pacific Ocean, allows additional NPIW to enter the trough, with the majority flowing into and filling the northern trough as it mixes with KIW (11). The topography and current structure of the region, therefore, leads to distinct water mass compositions occupying the deep waters of the southern (45% NPIW and 55% SCSIW) and northern OT (75% NPIW and 25% SCSIW) (11), thus allowing for the study of protist community structure in differently composed deep water masses.

As a back-arc spreading basin, there is extensive hydrothermal activity throughout the Okinawa Trough (12). The InterRidge Database v3.4 includes 25 active hydrothermal sites in the Okinawa Trough (13), but this figure likely remains an underestimate as new vents continue to be discovered (14,15). The hydrothermal vents in the Okinawa Trough are distinct from those found in the Mid-Ocean Ridge and Eastern Pacific, primarily due to thick layers of terrigenous sediments overlaying vent sites. Compared to sediment-starved vent systems, Okinawa Trough vent fluids are typically low pH with high concentrations of CO2, NH4+, boron, iodine, potassium, and lithium (16) and have high methane concentrations (17). Unique physicochemical conditions associated with different vent systems—such as those associated with OT vents—give rise to heterogeneous biological communities (9), warranting careful study of diverse hydrothermal systems. Despite this, the Okinawa Trough vent fields have not been studied nearly as extensively as Eastern Pacific vents (12).

To investigate the influence of water mass composition on protist diversity and biogeography, we compared community compositions in samples collected from the surface, subsurface chlorophyll maximum, middle, and bottom water layers in the Southern OT, Kerama Gap, and Northern OT. To determine the influence of hydrothermal activity on protist communities we compared the community composition in bottom water samples collected near hydrothermal vents with bottom water samples collected at sites without hydrothermal activity. We purposefully analyzed amplicon

sequence variants (ASVs) in order to maximize the amount of diversity included in the study (18), and while community compositions at higher taxonomic levels appeared similar between sites, communities associated with different water masses were significantly different at the ASV level. In addition to being the first comprehensive survey of eukaryotic microbial diversity in the Okinawa Trough, this study demonstrates different microbial eukaryotic community compositions are associated with varying water mass composition and active hydrothermal vents in the Western Pacific. Lastly, ASVs enriched at vents largely derived from cosmopolitan protists, emphasizing the importance of the rare biosphere and supporting the “everything is everywhere, but the environment selects” hypothesis (19). However, ASVs enriched at vent sites also appear to derive from endemics, indicating that dispersal limitation in a back-arc basin (12) may contribute to allopatric differentiation.

Materials and Methods

Sampling locations

Water samples were collected from 14 stations along the Ryukyu Archipelago during the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) MR17-03C cruise from May 29 to June 13, 2017 (Figure 1). Stations 8, 9, 10, and 11 are located in the Southern Okinawa Trough (SOT); stations 3, 4, 2, 5 make up a transect of the Kerama Gap (KG) from east to west; stations 12, 13, 14, 15, 17, and 18 are considered Northern Okinawa Trough (NOT) stations. Station 10 is the Hatoma Knoll (16), and station 2 is the ANA site of the Daisan-Kume Knoll (14), both of which are confirmed, active hydrothermal vents. In addition, station 11 is near the Yonaguni Knoll (20), station 5 is near the Higa site (14), and station 12 is near the North Knoll of the Iheya Ridge (21).

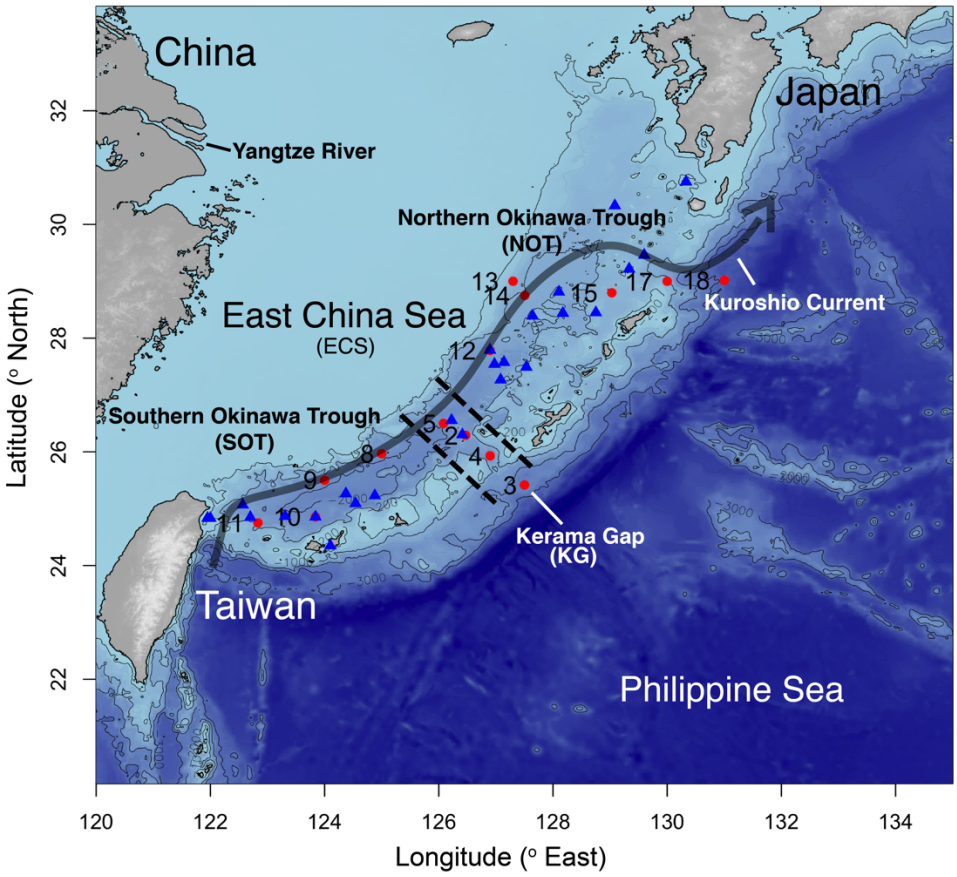

Figure 1. Sampling locations in the Okinawa Trough. Numbered red circles denote sampling stations where water was collected during the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) MR17-03C cruise in May and June 2017. Blue triangles indicate active hydrothermal sites according to the InterRidge Vents Database v3.4 (https://vents-data.interridge.org/ventfields-osm-map, accessed 05/08/2019). Bathymetry data was accessed and plotted through the function getNOAA.bathy in R package marmap. The 200, 1000, 2000, and 3000 m isobaths are plotted and labeled. Station 10 is the Hatoma Knoll in the Southern Okinawa Trough and station 2 is the ANA site at the Daisan-Kume Knoll in the Middle Okinawa Trough. Station 11 is near the Yonaguni Knoll IV, station 5 is near the Higa vent site, and station 12 is near the North Knoll of the Iheya Ridge.

Sample collection

A Niskin rosette with 30 10-L bottles and fitted with a conductivity-temperature-depth (CTD) probe (SBE 911plus, Sea-Bird Scientific, Bellevue, WA) was deployed at each cruise station to collect water from the subsurface chlorophyll maximum (SCM), the middle of the water column (mid), and 10 m above the seafloor (bottom). Surface seawater was collected by bucket alongside the research vessel. Two replicates of 4.5 liters (surface) from separate bucket casts or 5 liters from separate Niskin bottles (SCM, mid, bottom) were sequentially filtered under a gentle vacuum through 10.0-µm and 0.2-µm pore-size polytetrafluoroethylene (PTFE) filters (Millipore, Burlington, MA) to size-

fractionate the microbial community. Filters were flash-frozen in liquid nitrogen and stored at -80°C. Temperature, salinity, dissolved oxygen, fluorescence, and turbidity profiles were recorded by CTD probe at each station. Colored dissolved organic matter (CDOM), and nutrient (nitrate, nitrite, phosphate, and silicate) concentrations were analyzed by Marine Works Japan Ocean Chemistry Analysis Section onboard with water collected by Niskin bottle.

DNA extraction and sequencing library preparation

DNA was extracted from PTFE filters (n = 224, two replicates of two filter pore-sizes at four depths from 14 stations) following manufacturer protocols for the DNeasy PowerWater Kit (Qiagen, Hilden, Germany) including the optional heating step for 10 min at 65°C to fully lyse cells. Extracted DNA was quantified with the Qubit dsDNA High Sensitivity Assay (ThermoFisher, Waltham, MA). Sequencing libraries were prepared following the Illumina 16S Metagenomic Sequencing Library Preparation manual, but with universal eukaryotic primers for the V4 region of the eukaryotic 18S rRNA gene (F: CCAGCASCYGCGGTAATTCC (22), R: ACTTTCGTTCTTGATYR (23)) and 58°C annealing temperature in the initial PCR. Amplicon libraries were sequenced by the Okinawa Institute of Science and Technology (OIST) DNA Sequencing Section on the Illumina MiSeq platform with 2x300-bp v3 chemistry. Amplification and sequencing were successful for 211 samples and at least one replicate succeeded for each sample type.

Amplicon sequence analysis

Sequence data from each of four flow-cells were denoised separately using the Divisive Amplicon Denoising Algorithm (24) through the DADA2 plug-in for QIIME 2 (25). The resulting ASV tables were merged before taxonomy was assigned to ASVs using a naive Bayes classifier trained on the Protist Ribosomal Reference (PR2) database (26) with the QIIME 2 feature-classifier plug-in (27). We imported results into the R statistical environment (28) for further analysis with the bioconductor package phyloseq (29). Sequences were initially filtered to remove those that were not assigned taxonomy at the Kingdom level—likely data artifacts—and all metazoan sequences. We did not apply prevalence or minimum abundance filtering since samples were from varied locations and depths.

Statistical analyses

In an effort to minimize the compositional bias inherent in metabarcoding data we used the Aitchison distance between sample types when testing whether different sample types had distinct community compositions (30). To find the Aitchison distance a centered log-ratio (clr) transformation was performed on ASV count data with the R package CoDaSeq (30) before computing the Euclidean distance between samples with the R package vegan (31). The Aitchison distance was then used in Permutational

Multivariate Analyses of Variance (PERMANOVA, 999 permutations) with the vegan function adonis to test whether community composition was significantly different by water mass in the four depth layers or by vent presence/absence in the bottom samples. If the community composition varied significantly by the variable tested, we applied pairwise PERMANOVA. Lastly, we used the DESeq2 bioconductor package (32) to determine which ASVs were differentially abundant in bottom water from sites influenced by hydrothermal activity compared to bottom water at other sites. The data and code necessary to reproduce the statistical analyses are available on github (https://github.com/maggimars/OkinawaTroughProtists), including an interactive online document: https://maggimars.github.io/OkinawaTroughProtists/OTprotists.html.

Results

Protist diversity in the Okinawa Trough

Overall, 31.5 million sequencing reads were generated, with 34,631–421,992 sequencing reads per sample (mean = 144,604), and are available from the NCBI Sequencing Read Archive: accession PRJNA546472. Following denoising 16.8 million sequences remained with 1,724–215,842 sequences per sample (mean = 77,342). A total of 22,656 ASVs were identified in our dataset, with 49–1906 observed ASVs per sample (mean = 730). Subsurface chlorophyll maximum (SCM) samples had the highest richness estimates in both size-fractions (Figure 2) when estimated using the R package breakaway (33). SCM samples were significantly more diverse than samples from other depths when we applied breakaway's betta function (34). The community composition in all samples formed 3 distinct clusters—surface, SCM, and mid/bottom—in a Principal Coordinate Analysis (PCoA) plot based on Aitchison distance between samples; water depth was the strongest determinant of community structure in our samples (Figure 3). When samples from each depth were analyzed separately, samples clustered by filter pore-size for surface, SCM and mid water samples, but not bottom water samples (Supplemental Figure 1A–D), which could be due to detrital and/or extracellular DNA making up a larger portion of sequences in bottom samples.

Surface samples were dominated by Dinoflagellata, Haptophyta, and Radiolaria in the larger size-fraction and Dinoflagellata, Haptophyta, Ochrophyta and MAST groups in the smaller size-fraction (Figure 4). In the SCM, Dinoflagellata, Haptophyta, Ochrophyta and Radiolaria were most abundant in the larger fraction and Chlorophyta, Dinoflagellata, Haptophyta, Ochrophyta, Radiolaria, and MAST groups were most abundant in the smaller size-fraction. The mid-water and bottom-water samples were dominated by Dinoflagellata and Radiolaria in both size-fractions. Interestingly, bottom water samples from stations 2 and 10, the hydrothermal vent sites, as well as Station 11, included noticeably higher proportions of Ciliophora, Ochrophyta, and MAST groups.

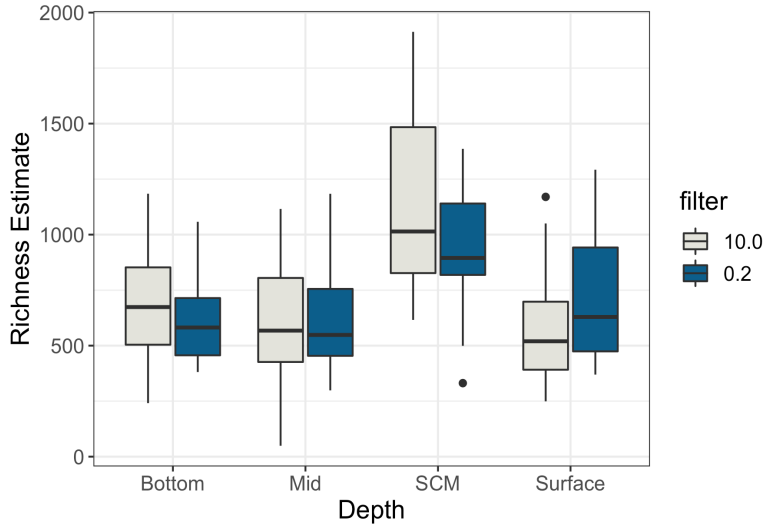

Figure 2. Richness estimates for protistan communities in small and large size-fractions from four depths in the Okinawa Trough. Blue-boxes depict upper and lower quartiles of modeled ASV richness in protist communities collected on 0.2 µm pore-size filters; whiskers depict range and points are outliers. Tan-boxes depict modeled ASV richness in protist communities collected on 10.0 µm pore-size filters. The Subsurface Chlorophyll Maximum (SCM) had significantly higher richness than the other depth layers when tested with the betta function in the R package breakaway ($p < 0.001$). Plot was made using the R package ggplot2.

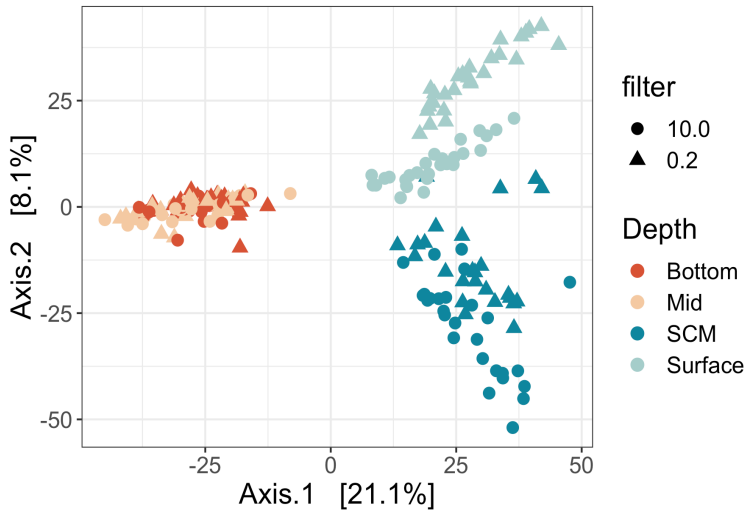

Figure 3. Principal coordinates analysis of Aitchison distances between size-fractionated protist communities in four depth layers. Point color indicates the depth layer from which samples were collected and point shape reflects the filter pore-size used to collect samples in µm. Samples form three main clusters by depth: surface, subsurface chlorophyll maximum (SCM), and mid/bottom. When PCoA is performed and plotted for depth layers individually, clusters by filter pore-size are visible in surface, SCM, and mid-water samples, but not bottom water samples (Supplemental Figure 1A–D). PCoA analysis on Aitchison distances was done with R packages CoDaSeq and Phyloseq, and the result was plotted with ggplot2.

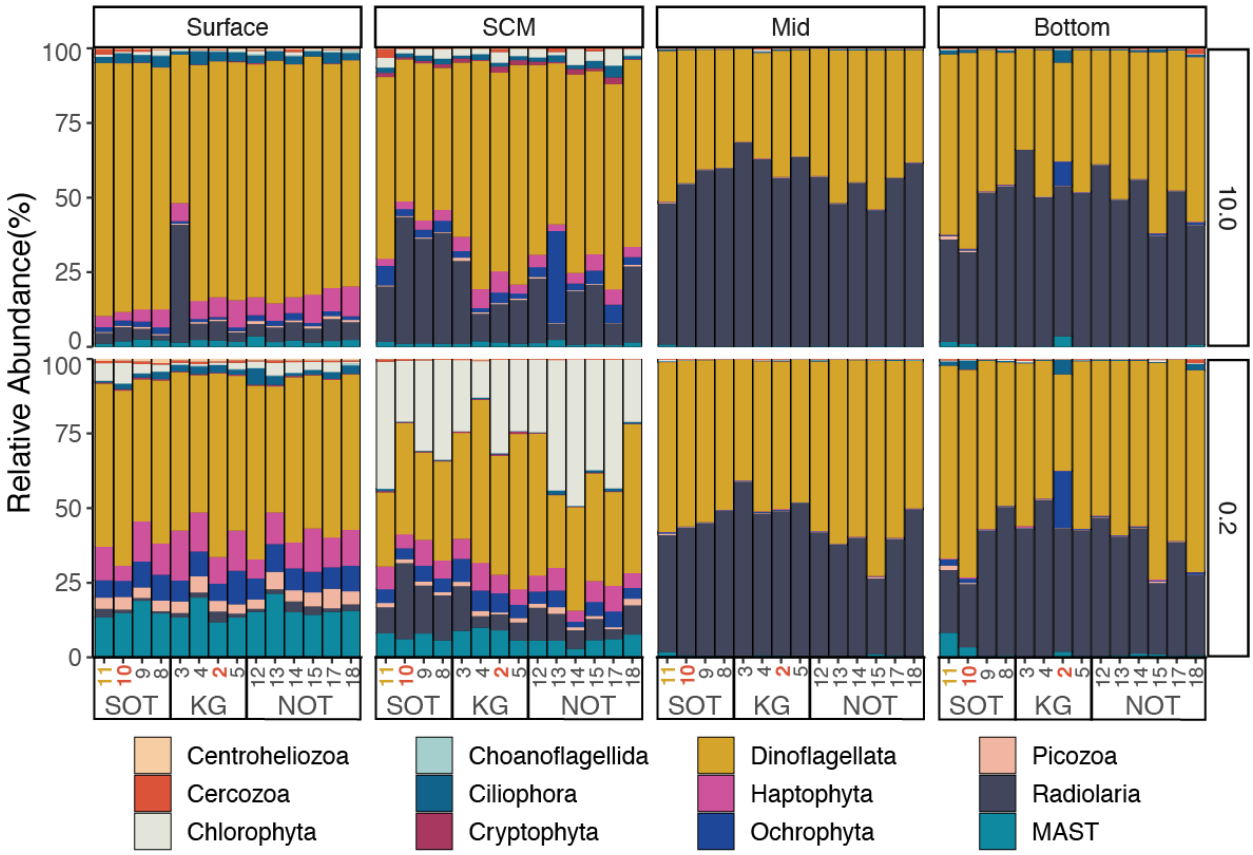

Figure 4. Relative abundance of major protist groups in size-fractionated samples from four depths in the Okinawa Trough (OT). Sampling stations are ordered on the x-axis from south to north: Southern Okinawa Trough (SOT) stations include 11, 10, 9, and 8; Kerama Gap (KG) stations include 3, 4, 2, and 5; Northern Okinawa Trough (NOT) stations include 12, 13, 14, 15, and 17. The plot is faceted by sampling depth (columns) and filter pore-size in µm (rows). When replicates were available for a particle station/depth/filter combinations, replicates were collapsed and represented with a single stacked bar. Regional community differences are not visible at the high taxonomic level represented in the plot, but there are visible differences by depth and filter pore-size. Station 10, the Hatoma Knoll vent field, and station 2, the ANA vent at the Daisan-Kume Knoll (highlighted in red), have anomalous bottom water communities compared to other other stations except station 11 (highlighted yellow). The relative abundance plot was created with R package ggplot2.

Protist biogeography in the Okinawa Trough

To investigate biogeography of protist communities in the OT, we subset sample replicates by depth-layer, filter pore-size, and region (SOT, KG, NOT). We then performed Permutational Multivariate Analyses of Variance (PERMANOVA, 999 permutations) by region on the Aitchison distances between samples in each of the depth/filter subsets with the adonis function in R package vegan (31). If the PERMANOVA p-value was less than or equal to 0.05, we performed pairwise PERMANOVA to determine which regions had statistically significantly different communities. The PERMANOVA results are summarized in Figure 5: in the surface,

SCM, and mid-water protist communities were significantly different between the NOT and the KG/SOT, whereas the pattern was reversed in the bottom waters.

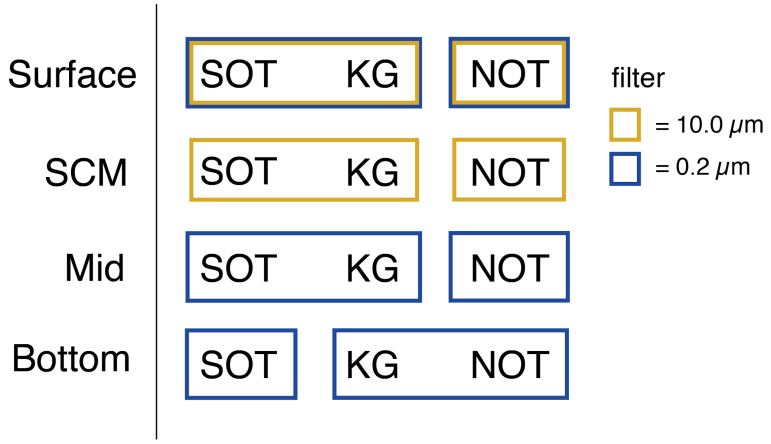

Figure 5. Schematic of PERMANOVA results comparing protist community composition between size-fractionated samples from four depth layers in the Southern Okinawa Trough (SOT), Kerama Gap (KG), and Northern Okinawa Trough (NOT). Breaks between boxes denote a significant difference ($p < 0.05$) between groups, as determined by pairwise PERMANOVA performed with the R function adonis on Aitchison distances between samples. Boxes are omitted in layers for which PERMANOVA found no significant effect of geographical grouping. Significant differences in community composition were present between NOT samples and KG/SOT samples in the surface, SCM, and mid-water column. The pattern was reversed in bottom water samples.

Protist communities at hydrothermal vent sites

Of the fourteen sampling stations, two were directly over confirmed, active hydrothermal vent sites: station 10 at the Hatoma Knoll in the Southern Okinawa Trough and station 2 at the ANA site in the Middle Trough/Kerama Gap. The relative abundance of major plankton groups (Figure 6) suggests that station 11 has a similar protist community to stations 2 and 10 and could therefore be being influenced by nearby hydrothermal activity at the Yonaguni Knoll (20). However, stations 5 and 12 are also geographically close to vent sites (13). Physicochemical parameters were, therefore, used to determine if the water sampled at stations 11, 5 or 12 was influenced by nearby hydrothermal activity. Stations 10 and 2, the active vent sites, exhibited a substantial turbidity and CDOM maximum above the seafloor, which is typical for OT vent plumes (16,35). Station 11 also exhibited a turbidity maximum above the seafloor, which none of the other sites shared, and exhibited increased CDOM compared to other sites (Figure 6, Supplemental Figure 2). Based on physicochemical parameters, it is likely that we sampled the plume from the Yonaguni Knoll when we collected bottom water at station 11. Hence, we considered station 11 as hydrothermally-influenced in the ensuing analyses.

To evaluate the effect of hydrothermal activity on protist diversity, we first checked whether alpha diversity was significantly different at hydrothermally-influenced sites; hydrothermal activity did not significantly increase or decrease richness in bottom water samples (Figure 7). To determine if hydrothermal activity altered community composition, we performed PERMANOVA by sample type (vent, plume, no hydrothermal activity) on the Aitchison distances between all bottom water samples. We did not subset the bottom water samples by filter type since bottom water samples did not cluster by filter type in PCoA (Supplemental Figure 1D). Community compositions at hydrothermally-influenced sites were significantly different from other bottom water samples ($p = 0.001$). Pairwise PERMANOVA revealed that the hydrothermally-influenced communities were both significantly different from the non-vent communities but were not significantly different from each other. These results demonstrate that deep waters affected by hydrothermal vents contain distinct protist communities from those found in surrounding waters. Additionally, since species richness did not vary significantly between bottom samples, the difference in community composition detected should primarily be due to differing relative contributions of plankton groups, rather than higher or lower total diversity.

Lastly, we investigated if some protist ASVs were specifically associated with hydrothermal sites by testing whether there were differentially abundant ASVs in the vent samples compared to the rest of the bottom water samples using DESeq2 (32). An ASV was considered significantly differentially abundant when the False Discovery Rate adjusted p-value was less than or equal to 0.01. Overall, 45 ASVs were significantly differentially abundant between the vent samples and the rest of the bottom water samples. Of the differentially abundant ASVs, 30 were more abundant at sites with hydrothermal influence than at the other sites, and included Dinophyceae, Syndiniales, Chrysophyceae, MAST, RAD-A and RAD-B Radiolarians, Oligohymenophorea and Spirotrichea ciliates, and Picozoa (Figure 8).

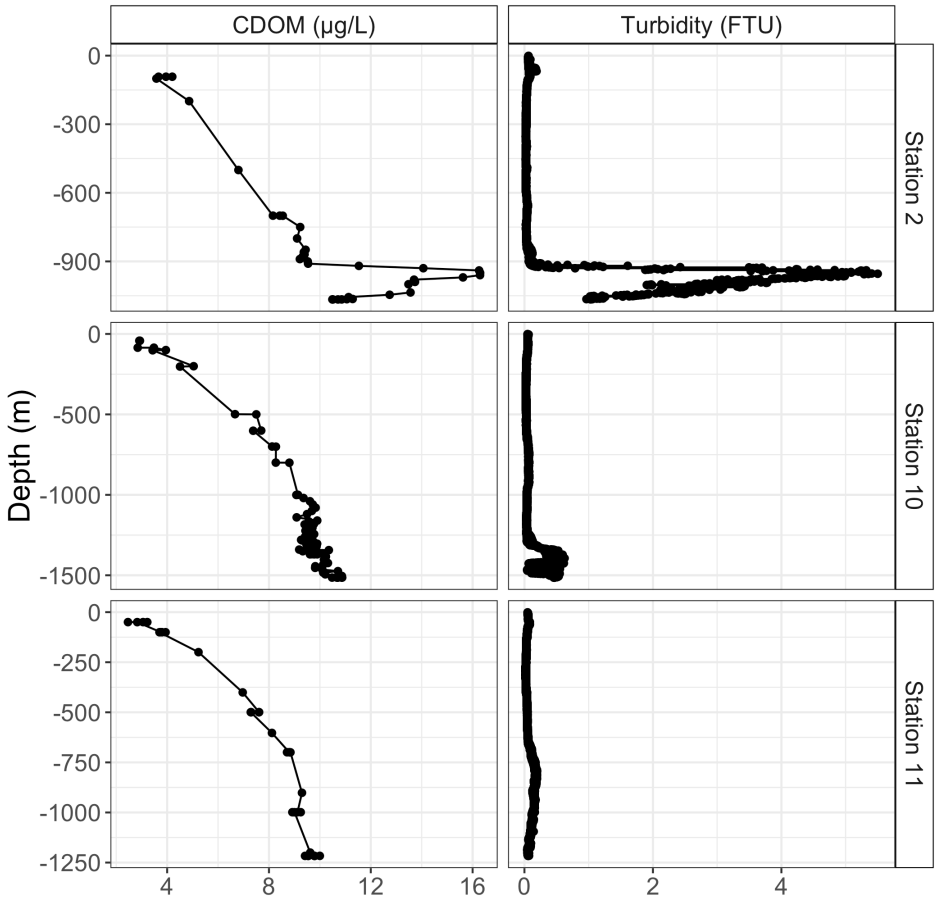

Figure 6. Depth profiles of colored dissolved organic matter (CDOM) (µg/L) and turbidity (FTU) at stations influenced by hydrothermal activity (stations 2, 10, 11). CDOM measurements were made from water collected in Niskin bottles during the Niskin Rosette and CTD cast at each station; turbidity was measured directly by CTD sensor during the same casts. Deep CDOM and turbidity maxima, which indicate hydrothermal activity in the Okinawa Trough (35), are visible in profiles from stations 2, 10, and 11, but no other stations (Supplemental Figure 2). Depth profiles were plotted with R package ggplot2.

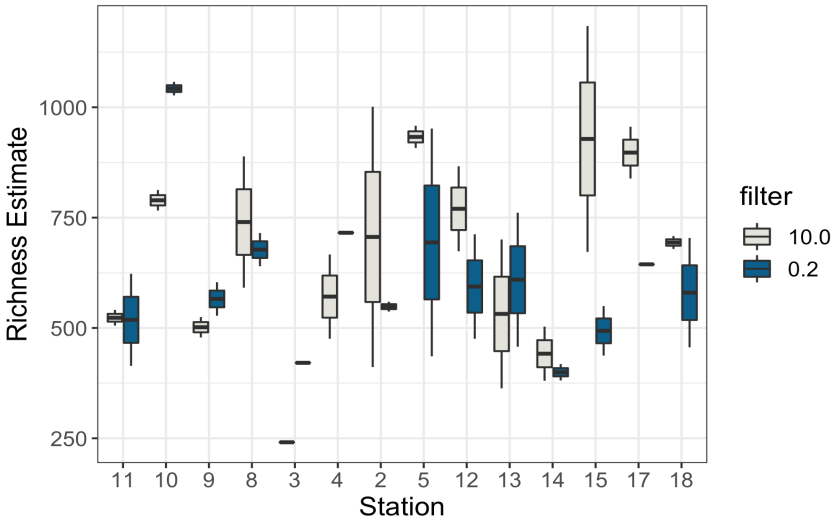

Figure 7. Richness estimates for protistan communities in small and large size-fractions from bottom water samples collected in the Okinawa Trough. Blue-boxes depict upper and lower quartiles of modeled ASV richness in protist communities collected on 0.2 µm pore-size filters; whiskers depict range. Tan-boxes depict modeled ASV richness in protist communities collected on 10.0 µm pore-size filters. Hydrothermally influenced sites (stations 2, 10, 11) did not have significantly different richness when compared with the rest of the bottom water samples using the betta function in the R package breakaway. Plot was made with R package ggplot2.

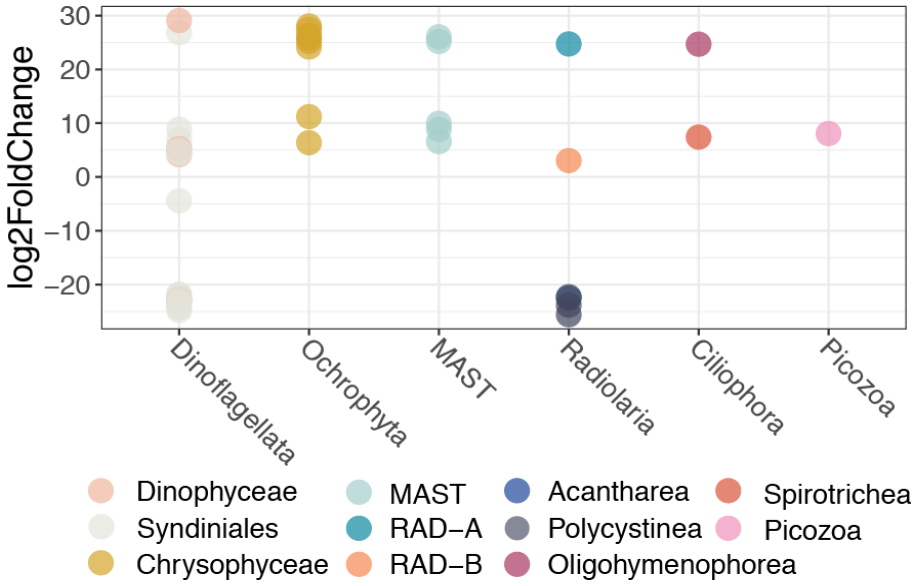

Figure 8. Log2 fold-change of significantly differentially abundant ASVs in bottom water influenced by hydrothermal activity. The DESeq function in the DESeq2 package was used to test whether ASVs were significantly more (positive log2 fold-change) or less (negative log2 fold-change) abundant in bottom water samples from Stations 10, 11, and 2 compared to bottom water samples from the other sites. Each point represents a single significantly differentially abundant ASV and taxonomic groupings are indicated on the x-axis and by point color. ASVs were considered significantly differentially abundant if False Discovery Rate adjusted p-values were < 0.01. Fold-change plot was made with R package ggplot2.

Discussion

Due to their small size, protists, like bacteria, face few dispersal barriers in the ocean (36). In addition, rapid asexual reproduction in many protists, as well as the ability to form resting cysts, increases their dispersal potential. As a result, many protists are cosmopolitan, suggesting that global protist diversity is low while local diversity in any given place is relatively high (37,38). Whether or not this is the case depends largely on how protist species and diversity are defined. For example, if a higher level of genetic diversity is included in a species definition, global diversity will be lower. Conversely, if less genetic differentiation is included in a species definition, endemism and global diversity will be higher (6). This has been demonstrated in bacteria through studies of biogeography at deep sea hydrothermal vents: only when microdiversity—diversity at the single nucleotide polymorphism level—is considered does geographic population structure emerge (17,39). By analyzing ASVs, which capture the highest amount of diversity available in metabarcoding datasets, we investigated whether biogeographic patterns exist among protists in the Okinawa Trough in regard to water mass composition and proximity to hydrothermal vents. Our results show significantly different protist communities in different water masses and near vent sites.

Biodiversity and biogeography

Consistent with previous studies on protist biogeography, our results demonstrate relatively weak biogeographic patterns in the Okinawa Trough, with the strongest influence on community composition being depth (40). Depth-dependent diversity patterns were similar to metabarcoding results from other regions, with higher diversity in the surface and SCM than the meso- and bathypelagic (40,41). In addition, overall community structure by depth was comparable to global patterns: Dinoflagellata, Haptophyta, Ochrophyta, Picozoa, Radiolaria and Marine Stramenopiles (MASTs) were abundant in the surface and SCM while Dinoflagellata and Radiolaria dominated in the meso- and bathypelagic (5). Despite weak biogeographic patterns overall, significant differences in community composition did exist by region and water mass composition when considered at the ASV level. Similarly, Agogué et al. (2011) found water mass composition was the best predictor of prokaryotic community composition in the Atlantic Ocean (8), and Pernice et al. (2016) found water mass composition was the best predictor of protistan community composition in the deep waters of the Atlantic, South and Equatorial Pacific, and Indian oceans (5).

The Okinawa Trough is divided into Southern (SOT) and Northern (NOT) regions based on the location of the Kerama Gap and bottom water characteristics. The bottom water in the SOT is 45% North Pacific Intermediate Water (NPIW) and 55% South China Sea Intermediate Water (SCSIW). Additional NPIW flows into the NOT through the Kerama Gap, increasing the contribution of NPIW in the NOT to 75% and decreasing salinity (11). In agreement with the prevailing oceanography, the protist

375 communities in the bottom waters of the NOT and Kerama Gap were significantly different from those in the SOT. Water masses in the ocean may either act as geographic boundaries to dispersal or select for microbes adapted to specific environmental conditions (8). Our results, therefore, could be interpreted as evidence that different communities are associated with NPIW and SCSIW and are mixed in
380 different ratios in the SOT and NOT, thus supporting water masses as dispersal barriers. Conversely, the results could also indicate that the same protists are present to some extent in both water masses and become more or less abundant in response to changing environmental conditions, such as salinity.

Although water can flow into the OT through the Kerama Gap and move
385 Northward with the Kuroshio Current, this transport pattern is inconsistent and responds to shifts in the Kuroshio Current axis and the strength of the Ryukyu Current—the weaker western boundary current to the east of the Ryukyu Islands (42). The communities in the surface, SCM, and mid-water, were not significantly different between the SOT and Kerama Gap, but communities in the NOT were different from the
390 SOT and Kerama Gap. These results suggest that water was moving from the ECS to the Pacific at the time of sampling. Our results could further be influenced by Changjiang (a.k.a. Yangtze) Diluted Water (CDW) and Kuroshio water mixing at the continental shelf near station 13. The Changjiang/Yangtze is the longest river in China—the 3rd largest in the world—and transports huge amounts of terrestrial
395 nutrients and anthropogenic pollutants into the northern ECS. The river discharge eutrophifies the continental shelf and causes blooms of diatoms, dinoflagellates, and haptophytes (43). The river output further creates a salinity gradient from 31 ppt at the coast to 34.5 ppt at the continental shelf (43). Surface and SCM water at station 13 had lower salinity, higher nutrient concentrations, and increased chlorophyll fluorescence
400 compared to other sampling sites in the NOT (Supplemental Figure 3); riverine input and CDW are thus likely affecting the protist community composition in the NOT. The regional differences in community composition we observed in the surface waters likely arise from a combination of environmental selection and oceanographic barriers.

405 ***Protist communities and hydrothermal vent sites***

The protist communities associated with deep-sea hydrothermal vents in the Okinawa Trough were significantly different from the communities in deep-sea samples without hydrothermal influence. Despite geographic separation and varying geochemical properties—vents in the middle Okinawa Trough (ANA) have more overlying terrigenous
410 sediment and, therefore, more organic carbon in the vent fluids than Southern Okinawa Trough vents (Hatoma, Yonaguni) (35)—the vent-associated communities were similar at all three vent sites. Like Sauvadet et al. (2010), we found Syndiniales, Ciliophora, and MAST (Marine Stramenopiles) enriched in bottom waters near hydrothermal sites compared to other deep-sea locations (3); Ochrophyta, environmental clades of

Radiolaria, and Picozoa were additionally enriched near the vent sites in this study (Figures 4, 8). Quantitative models of deep-water circulation suggest that back-arc basins, such as the Okinawa Trough, should be genetically well-connected, but that basin to basin dispersal is restricted (12). This conclusion rests on lateral transport within basins, which is supported by our findings; we detected a hydrothermal community signature 17 km from a vent site but not in overlying waters. Thus, a major remaining question is how similar vent-associated protists in the Okinawa Trough are to vent-associated protists in other hydrothermal regions and whether the protists enriched at vent sites have cosmopolitan distribution or are endemic to the region.

Dinoflagellate ASVs made up the majority of differentially abundant ASVs in vent samples (22 out of 45, Figure 8) and half of these were more abundant at vent sites. Among the enriched ASVs, 8 are in the parasitic order Syndiniales, including group I (clades 2 and 7) and group II (clades 1, 6, 12, 13, 14, and 16). The remaining enriched Dinoflagellata ASVs belong to the Order Dinophyceae but could not be further classified. Group II Syndiniales are common in sunlit, surface waters, whereas group I is common in suboxic and anoxic ecosystems. However, both groups I and II are found in surface and deep water and have individual clades that have only been recovered from suboxic or anoxic ecosystems (44). The majority of the enriched Syndiniales ASVs shared 100% identity with GenBank sequences (nr/nt, accessed 5/16/2019, (45,46)) from a wide variety of ecosystems throughout the global ocean, including surface waters near the poles, mesopelagic water near the California coast, 100 and 200 m oxygenated and micro-oxic North Atlantic waters, and at the sediment interface near the Juan de Fuca Ridge vent system (47,48); the Syndinales ASVs enriched in vent samples are clearly cosmopolitan organisms. Although they are not restricted to vent systems, parasitic Syndiniales do seem to thrive in vent systems and likely take advantage of increased host availability in such regions (49).

Ochrophyta accounted for the second-most enriched ASVs in vent samples (Figure 8). The Ochrophyta ASVs belong to several clades within the order Chrysophyceae. Two belong to undescribed clades of cosmopolitan uncultured environmental representatives (clades EC2H and EC2I), consisting of sequences from a diverse set of marine and freshwater environments (50). The remaining ASVs belong to the genus *Paraphysomonas*, which are colorless phagotrophs globally distributed in freshwater, marine, and soil ecosystems and have previously been recovered from vents (51). While several of the vent-associated ASVs share > 99%–100% identity vent-derived sequences (51), they are equally similar to sequences recovered from other marine environments. Therefore, while Ochrophyta ASVs belong to multiple Chrysophyte clades, one of which has been observed at a vent site before, all of these clades are very cosmopolitan, and all of the ASVs share high percent identities with sequences from diverse non-vent environments.

Five vent-associated ASVs belong to marine stramenopile lineages—MAST-1, -7, -8. Most of what is known about MAST lineages has been learned through environmental molecular surveys. Aggregate analyses of sequences collected through such studies have found that MAST-1, -7, and -8 are highly diverse, globally-distributed, and abundant in surface waters (52,53). Culture-independent techniques have demonstrated that MAST-1 (54), MAST-7 (55), and MAST-8 (52) are bactivorous flagellates. The vent-associated MAST ASVs therefore likely represent cosmopolitan bacterivores: MAST-1 and MAST-8 ASVs each share 99% (MAST-7) to 100% (MAST-1, -8) identity with sequences collected from surface waters globally, including from (anoxic) Saanich Inlet, Vancouver, Canada (56), the Scotian Shelf in the North Atlantic (57), and the Southern Ocean (58).

The RAD-A and RAD-B radiolarian groups, for which there are both one ASV enriched at vent sites, represent environmental clades that have no morphological description—other than being picoplankton—or known ecological roles (59). The RAD-A ASV shares > 99% identity with many GenBank sequences recovered from oxic and anoxic waters globally, including the Cariaco basin in the Caribbean Sea (48), the Gulf Stream (47), and the South East Pacific (60). The RAD-B ASV shares 100% identity with multiple sequences recovered from the East Pacific Rise (1500 m and 2500 m), the Arctic Ocean (500m), oxygenated water in the Cariaco Basin (48), the Juan de Fuca Ridge (61), and the Southern Ocean (62). While it is clear the radiolarian ASVs enriched at vents derive from cosmopolitan organisms, it is notable that identical and similar sequences have repeatedly been isolated from vent fields and anoxic regions; the RAD-A and -B ASVs may represent ubiquitous protists that are particularly suited to flourish in hydrothermal systems.

Previous studies indicated Ciliophora are abundant in sediments and bacterial mats near hydrothermal vents (63–65). It is unsurprising, then, that two Ciliophora ASVs were significantly more abundant in vent samples. The enriched Spirotrichea ASV (Figure 8) was classified to genus level by our classifier and belongs to *Leegaardiella*, a recently described genus collected in the North Atlantic (66). The *Leegardiella* ASV shared 100% coverage and identity with one sequence in GenBank—an uncultured eukaryote clone recovered from 2500 m at the East Pacific Rise—and had greater than 99% shared identity with another sequence recovered from the East Pacific Rise, also from 2500 m (47). However, the *Leegardiella* ASV also had 100% coverage and > 99% identity matches with several sequences recovered from Arctic surface water (67). This *Leegardiella* ASV may, therefore, derive from a widely distributed genus that opportunistically becomes more abundant at vent sites. The Oligohymenophorea ASV belongs to the environmental OLIGO 5 clade, but did not have > 96% identity matches with any sequences in GenBank; the closest match was to an uncultured eukaryote clone recovered from 500 m depth off the coast of California (47). The EukRef-Ciliophora curated Oligohymenophorea phylogeny groups the OLIGO 5 clade with the

subclass Scuticiliatia (68). While spirotrich ciliates are generally bacteriovores (63), Scuticiliatia ciliates are known to be symbionts of aquatic organisms (69), including giant hydrothermal bivalves (3). Corroborating our results, Zhao and Zu (2016) also found Spirotrichea and Scuticiliatia enriched among ciliates found near a vent site in the Northern Okinawa Trough (70). The higher degree of endemism seen in the Scuticiliatia ASV may be due to coevolution with an endemic host (71), since metazoa connectivity between vents in the Okinawa Trough and other vent fields is predicted to be low (12).

A single vent-associated ASV belonged to Picozoa. The first Picozoa species was described in 2013 (72) and Picozoa ecology remains poorly resolved. Information regarding their distribution derives from environmental molecular surveys, which have established five clades within Picozoa. While all Picozoa are marine, biogeographical patterns vary between clades: clade BP2 has only been found in surface waters, group 2 is mostly found in deep waters, and the other three groups are globally distributed throughout the water column (73). The vent-associated Picozoa ASV belongs to one of the cosmopolitan clades (group 1). The sequence shares 100% identity with three others in GenBank, from a variety of environments—surface water in the Arctic (74) and Southern (75) oceans, as well as mesopelagic water near southern California (47).

Conclusions and future directions

While the Okinawa Trough provides an ideal setting to investigate protist diversity and biogeography, this study represents the first comprehensive survey of protists in the region. We found protist communities varied by water mass composition predicted by regional oceanography, but the regional differences were only discernible if diversity was considered at the level of Amplicon Sequence Variants. We further found distinct protist communities associated with hydrothermal vent sites, with bacterivore, parasitic, and symbiotic protists enriched at vent sites. The enriched protists were mostly globally distributed and many were previously found at other vent sites. While these results support the view that protists are cosmopolitan opportunists, at least one ciliate ASV was not similar to any publicly available sequence, suggesting some protists may be endemic to certain hydrothermal regions and supporting moderate endemicity among protists (7). A major limitation of metabarcoding studies with DNA is that it is impossible to know whether sequences derive from actively metabolizing cells, dead or inactive cells, or even environmental DNA; future studies will benefit from incorporating methods that better delineate metabolic state. Additionally, metabarcoding studies suffer from focusing on a short sequence to assess diversity; metagenomic and metatranscriptomic approaches are becoming increasingly feasible for large-scale protist surveys, which will allow for finer resolution of diversity. In the future, comparisons between the Okinawa Trough and other hydrothermal regions will continue to advance our understanding of important protists in hydrothermal ecosystems and how hydrothermal activity influences protist diversity and biogeography in the global ocean. For now, a full understanding of

protist diversity and biogeography remains inaccessible, however, this study provides new evidence that different protists exhibit varying levels of endemism and ubiquity.

Acknowledgements

We thank the captain and crew of the JAMSTEC R/V Mirai for their assistance and support in sample collection. Hiromi Watanabe, Dhugal Lindsay, Mary M. Grossmann, and Yuko Hasagawa were instrumental in organizing and facilitating cruise sampling and Otis Brunner substantially contributed to seawater filtration. We are further grateful to Hiromi Watanbe for helping to access and interpret environmental data. We thank the OIST DNA sequencing section (Onna, Okinawa) for carrying out the sequencing. This work was funded by the Marine Biophysics Unit of the Okinawa Institute of Science and Technology Graduate University. MMB was supported by a Japan Society for the Promotion of Science DC1 graduate student fellowship.

References

1. Massana R. Eukaryotic picoplankton in surface oceans. Annu Rev Microbiol. 2011;65:91–110.

2. Edgcomb VP. Marine protist associations and environmental impacts across trophic levels in the twilight zone and below. Curr Opin Microbiol. 2016 Jun;31:169–75.

3. Sauvadet A-L, Gobet A, Guillou L. Comparative analysis between protist communities from the deep-sea pelagic ecosystem and specific deep hydrothermal habitats. Environ Microbiol. 2010;12(11):2946–64.

4. de Vargas C, Audic S, Henry N, Decelle J, Mahé F, Logares R, et al. Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. Science. 2015 May 22;348(6237):1261605.

5. Pernice MC, Giner CR, Logares R, Perera-Bel J, Acinas SG, Duarte CM, et al. Large variability of bathypelagic microbial eukaryotic communities across the world's oceans. ISME J. 2016 Apr;10(4):945–58.

6. Caron DA. Past President's address: protistan biogeography: why all the fuss? J Eukaryot Microbiol. 2009 Mar;56(2):105–12.

7. Foissner W. Protist diversity and distribution: some basic considerations. Biodivers Conserv. 2008 Feb 1;17(2):235–42.

8. Agogué H, Lamy D, Neal PR, Sogin ML. Water mass-specificity of bacterial communities in the North Atlantic revealed by massively parallel sequencing. Molecular [Internet]. 2011; Available from: https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-294X.2010.04932.x

9. Dick GJ. The microbiomes of deep-sea hydrothermal vents: distributed globally, shaped locally. Nat Rev Microbiol. 2019 May;17(5):271–83.

10. Takishita K. Diversity of Microbial Eukaryotes in Deep Sea Chemosynthetic Ecosystems Illuminated by Molecular Techniques. In: Ohtsuka S, Suzaki T, Horiguchi T, Suzuki N, Not F, editors. Marine Protists: Diversity and Dynamics. Tokyo: Springer Japan; 2015. p. 47–61.

11. Nakamura H, Nishina A, Liu Z, Tanaka F. Intermediate and deep water formation in the Okinawa Trough. Journal of [Internet]. 2013; Available from: https://onlinelibrary.wiley.com/doi/pdf/10.1002/2013JC009326

12. Mitarai S, Watanabe H, Nakajima Y, Shchepetkin AF, McWilliams JC. Quantifying dispersal from hydrothermal vent fields in the western Pacific Ocean. Proc Natl Acad Sci U S A. 2016 Mar 15;113(11):2976–81.

13. Beaulieu SE, Szafranski K. InterRidge Global Database of Active Submarine Hydrothermal Vent Fields, Version 3.4 [Internet]. 2018 [cited 2019 May 5]. Available from: http://vents-data.interridge.org

14. Makabe A., Tsutsumi S., Chen C., Torimoto J., Matsui Y., Shibuya T., Miyazaki J., Kitada K., Kawagucci S. Discovery of New Hydrothermal Vent Fields in the Mid- and southern-Okinawa Trough. In: Goldschmidt Abstracts. 2016. p. 1945.

15. Miyazaki J, Kawagucci S, Makabe A, Takahashi A, Kitada K, Torimoto J, et al. Deepest and hottest hydrothermal activity in the Okinawa Trough: the Yokosuka site at Yaeyama Knoll. R Soc Open Sci. 2017 Dec;4(12):171570.

16. Toki T, Itoh M, Iwata D, Ohshima S, Shinjo R, Ishibashi J-I, et al. Geochemical characteristics of hydrothermal fluids at Hatoma Knoll in the southern Okinawa Trough. Geochem J. 2016;50(6):493–525.

17. Mino S, Makita H, Toki T, Miyazaki J, Kato S, Watanabe H, et al. Biogeography of Persephonella in deep-sea hydrothermal vents of the Western Pacific. Front Microbiol. 2013 Apr 25;4:107.

18. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 2017 Dec;11(12):2639–43.

19. Baas Becking LGM. Geobiologie of inleiding tot de milieukunde. WP Van Stockum & Zoon; 1934.

20. Konno U, Tsunogai U, Nakagawa F, Nakaseama M, Ishibashi J-I, Nunoura T, et al. Liquid CO 2 venting on the seafloor: Yonaguni Knoll IV hydrothermal system, Okinawa Trough. Geophys Res Lett. 2006;33(16):725.

21. Nakajima R, Yamamoto H, Kawagucci S, Takaya Y, Nozaki T, Chen C, et al. Post-drilling changes in seabed landscape and megabenthos in a deep-sea hydrothermal system, the Iheya North field, Okinawa Trough. PLoS One. 2015 Apr 22;10(4):e0123095.

22. Stoeck T, Bass D, Nebel M, Christen R, Jones MDM, Breiner H-W, et al. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol. 2010 Mar;19 Suppl 1:21–31.

23. Mars Brisbin M, Mesrop LY, Grossmann MM, Mitarai S. Intra-host Symbiont Diversity and

Extended Symbiont Maintenance in Photosymbiotic Acantharea (Clade F). Front Microbiol. 2018 Aug 27;9:1998.

24. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016 Jul;13(7):581–3.

25. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol [Internet]. 2019 Jul 24; Available from: https://doi.org/10.1038/s41587-019-0209-9

26. Guillou L, Bachar D, Audic S, Bass D, Berney C, Bittner 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. 2013 Jan;41(Database issue):D597–604.

27. Bokulich NA, Kaehler BD, Rideout JR, Dillon M, Bolyen E, Knight R, et al. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome. 2018 May 17;6(1):90.

28. R Core Team. R: A language and environment for statistical computing [Internet]. 2018. Available from: https://www.R-project.org/

29. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013 Apr 22;8(4):e61217.

30. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. Front Microbiol [Internet]. 2017 [cited 2019 Mar 1];8. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2017.02224/full

31. Oksanen J, Guillaume Blanchet F, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan: Community Ecology Package. R package version 2.5-4 [Internet]. 2019. Available from: https://CRAN.R-project.org/package=vegan

32. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15(12):550.

33. Willis A, Bunge J. Estimating diversity via frequency ratios. Biometrics. 2015 Dec;71(4):1042–9.

34. Willis A, Bunge J, Whitman T. Improved detection of changes in species richness in high diversity microbial communities. J R Stat Soc C. 2017 Nov 22;66(5):963–77.

35. Zhang X, Sun Z, Fan D, Xu C, Wang L, Zhang X, et al. Compositional characteristics and sources of DIC and DOC in seawater of the Okinawa Trough, East China Sea. Cont Shelf Res. 2019 Feb 15;174:108–17.

36. Whittaker KA, Rynearson TA. Evidence for environmental and ecological selection in a microbe with no geographic limits to gene flow. Proc Natl Acad Sci U S A. 2017 Mar 7;114(10):2651–6.

37. Finlay BJ. Global dispersal of free-living microbial eukaryote species. Science. 2002 May

650 10;296(5570):1061–3.

38. Fenchel T, Finlay BJ. The Ubiquity of Small Species: Patterns of Local and Global Diversity. Bioscience. 2004 Aug 1;54(8):777–84.

39. Mino S, Nakagawa S, Makita H, Toki T, Miyazaki J, Sievert SM, et al. Endemicity of the cosmopolitan mesophilic chemolithoautotroph Sulfurimonas at deep-sea hydrothermal
655 vents. ISME J. 2017 Apr;11(4):909–19.

40. Countway PD, Gast RJ, Dennett MR, Savai P, Rose JM, Caron DA. Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western North Atlantic (Sargasso Sea and Gulf Stream). Environ Microbiol. 2007 May;9(5):1219–32.

41. Giner CR, Balagué V, Pernice MC, Duarte CM, Gasol JM, Logares R, et al. Marked
660 changes in diversity and relative activity of picoeukaryotes with depth in the global ocean. Available from: http://dx.doi.org/10.1101/552604

42. Jin B, Wang G, Liu Y, Zhang R. Interaction between the East China Sea Kuroshio and the Ryukyu Current as revealed by the self-organizing map. J Geophys Res. 2010 Dec 18;115(C12):937.

665 43. Lin YC, Chung CC, Gong GC, Chiang KP. Diversity and abundance of haptophytes in the East China Sea. Aquat Microb Ecol. 2014 Jun 12;72(3):227–40.

44. Guillou L, Viprey M, Chambouvet A, Welsh RM, Kirkham AR, Massana R, et al. Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). Environ Microbiol. 2008 Dec;10(12):3349–65.

670 45. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009 Dec 15;10:421.

46. Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2016 Jan 4;44(D1):D67–72.

47. Lie AAY, Liu Z, Hu SK, Jones AC, Kim DY, Countway PD, et al. Investigating microbial
675 eukaryotic diversity from a global census: insights from a comparison of pyrotag and full-length sequences of 18S rRNA genes. Appl Environ Microbiol. 2014 Jul;80(14):4363–73.

48. Edgcomb V, Orsi W, Bunge J, Jeon S, Christen R, Leslin C, et al. Protistan microbial observatory in the Cariaco Basin, Caribbean. I. Pyrosequencing vs Sanger insights into species richness. ISME J. 2011 Aug;5(8):1344–56.

680 49. Moreira D, López-García P. Are hydrothermal vents oases for parasitic protists? Trends Parasitol. 2003 Dec;19(12):556–8.

50. Scoble JM, Cavalier-Smith T. Scale evolution in Paraphysomonadida (Chrysophyceae): Sequence phylogeny and revised taxonomy of Paraphysomonas, new genus Clathromonas, and 25 new species. Eur J Protistol. 2014 Oct;50(5):551–92.

685 51. Atkins MS, Teske AP, Anderson OR. A Survey of Flagellate Diversity at Four Deep-Sea Hydrothermal Vents in the Eastern Pacific Ocean Using Structural and Molecular Approaches. J Eukaryot Microbiol. 2000;47(4):400–11.

52. Massana R, del Campo J, Sieracki ME, Audic S, Logares R. Exploring the uncultured microeukaryote majority in the oceans: reevaluation of ribogroups within stramenopiles. ISME J. 2014 Apr;8(4):854–66.

53. Massana R, Castresana J, Balagué V, Guillou L, Romari K, Groisillier A, et al. Phylogenetic and ecological analysis of novel marine stramenopiles. Appl Environ Microbiol. 2004 Jun;70(6):3528–34.

54. Massana R, Terrado R, Forn I, Lovejoy C, Pedrós-Alió C. Distribution and abundance of uncultured heterotrophic flagellates in the world oceans. Environ Microbiol. 2006 Sep;8(9):1515–22.

55. Frias-Lopez J, Thompson A, Waldbauer J, Chisholm SW. Use of stable isotope-labelled cells to identify active grazers of picocyanobacteria in ocean surface waters. Environ Microbiol. 2009 Feb;11(2):512–25.

56. Orsi W, Song YC, Hallam S, Edgcomb V. Effect of oxygen minimum zone formation on communities of marine protists. ISME J. 2012 Aug;6(8):1586–601.

57. Dasilva CR, Li WKW, Lovejoy C. Phylogenetic diversity of eukaryotic marine microbial plankton on the Scotian Shelf Northwestern Atlantic Ocean. J Plankton Res. 2014 Mar 1;36(2):344–63.

58. Massana R, Guillou L, Díez B, Pedrós-Alió C. Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. Appl Environ Microbiol. 2002 Sep;68(9):4554–8.

59. Not F, Gausling R, Azam F, Heidelberg JF, Worden AZ. Vertical distribution of picoeukaryotic diversity in the Sargasso Sea. Environ Microbiol. 2007 May;9(5):1233–52.

60. Shi XL, Marie D, Jardillier L, Scanlan DJ, Vaulot D. Groups without cultured representatives dominate eukaryotic picophytoplankton in the oligotrophic South East Pacific Ocean. PLoS One. 2009 Oct 29;4(10):e7657.

61. Jungbluth SP, Grote J, Lin H-T, Cowen JP, Rappé MS. Microbial diversity within basement fluids of the sediment-buried Juan de Fuca Ridge flank. ISME J. 2013 Jan;7(1):161–72.

62. Clarke LJ, Bestley S, Bissett A, Deagle BE. A globally distributed Syndiniales parasite dominates the Southern Ocean micro-eukaryote community near the sea-ice edge. ISME J. 2019 Mar;13(3):734–7.

63. Coyne KJ, Countway PD, Pilditch CA, Lee CK, Caron DA, Cary SC. Diversity and distributional patterns of ciliates in Guaymas Basin hydrothermal vent sediments. J Eukaryot Microbiol. 2013 Sep;60(5):433–47.

64. Pasulka A, Hu SK, Countway PD, Coyne KJ, Cary SC, Heidelberg KB, et al. SSU-rRNA Gene Sequencing Survey of Benthic Microbial Eukaryotes from Guaymas Basin Hydrothermal Vent. J Eukaryot Microbiol. 2019 Jul;66(4):637–53.

65. López-García P, Vereshchaka A, Moreira D. Eukaryotic diversity associated with carbonates and fluid-seawater interface in Lost City hydrothermal field. Environ Microbiol. 2007 Feb;9(2):546–54.

66. Santoferrara LF, Alder VV, McManus GB. Phylogeny, classification and diversity of Choreotrichia and Oligotrichia (Ciliophora, Spirotrichea). Mol Phylogenet Evol. 2017 Jul;112:12–22.

67. Terrado R, Scarcella K, Thaler M, Vincent WF, Lovejoy C. Small phytoplankton in Arctic seas: vulnerability to climate change. Biodiversity. 2013 Mar 1;14(1):2–18.

68. Boscaro V, Santoferrara LF, Zhang Q, Gentekaki E, Syberg-Olsen MJ, Del Campo J, et al. EukRef-Ciliophora: a manually curated, phylogeny-based database of small subunit rRNA gene sequences of ciliates. Environ Microbiol. 2018 Jun;20(6):2218–30.

69. Fan X, Hu X, Al-Farraj SA, Clamp JC, Song W. Morphological description of three marine ciliates (Ciliophora, Scuticociliatia), with establishment of a new genus and two new species. Eur J Protistol. 2011 Aug;47(3):186–96.

70. Zhao F, Xu K. Molecular diversity and distribution pattern of ciliates in sediments from deep-sea hydrothermal vents in the Okinawa Trough and adjacent sea areas. Deep Sea Res Part I. 2016 Oct 1;116:22–32.

71. Peek AS, Feldman RA, Lutz RA, Vrijenhoek RC. Cospeciation of chemoautotrophic bacteria and deep sea clams. Proc Natl Acad Sci U S A. 1998 Aug 18;95(17):9962–6.

72. Seenivasan R, Sausen N, Medlin LK, Melkonian M. Picomonas judraskeda gen. et sp. nov.: the first identified member of the Picozoa phylum nov., a widespread group of picoeukaryotes, formerly known as “picobiliphytes.” PLoS One. 2013 Mar 26;8(3):e59565.

73. Moreira D, López-García P. The rise and fall of Picobiliphytes: how assumed autotrophs turned out to be heterotrophs. Bioessays. 2014 May;36(5):468–74.

74. Terrado R, Medrinal E, Dasilva C, Thaler M, Vincent WF, Lovejoy C. Protist community composition during spring in an Arctic flaw lead polynya. Polar Biol. 2011 Dec 1;34(12):1901–14.

75. Díez B, Pedrós-Alió C, Massana R. Study of genetic diversity of eukaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. Appl Environ Microbiol. 2001 Jul;67(7):2932–41.