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ORIGINAL ARTICLE

SSU-rRNA Gene Sequencing Survey of Benthic Microbial Eukaryotes from Guaymas Basin Hydrothermal Vent

Alexis Pasulka[a], Sarah K. Hu[b,1], Peter D. Countway[c], Kathryn J. Coyne[d], Stephen C. Cary[e], Karla B. Heidelberg[b] & David A. Caron[b]

a Biological Sciences Department, California Polytechnic State University, 1 Grand Avenue, San Luis Obispo, California, USA
b Department of Biological Sciences, University of Southern California, 3616 Trousdale Parkway, AHF 301 Los Angeles, Los Angeles, California, USA
c Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive, East Boothbay, Maine, USA
d College of Earth, Ocean, and Environment, University of Delaware, 700 Pilottown Road, Lewes, Delaware, USA
e Department of Biological Sciences, The University of Waikato, Private Bag 3105, Hamilton, New Zealand

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Correspondence
A. Pasulka, Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA, USA
e-mail: apasulka@calpoly.edu

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ABSTRACT

Microbial eukaryotes have important roles in marine food webs, but their diversity and activities in hydrothermal vent ecosystems are poorly characterized. In this study, we analyzed microbial eukaryotic communities associated with bacterial (*Beggiatoa*) mats in the 2,000 m deep-sea Guaymas Basin hydrothermal vent system using 18S rRNA gene high-throughput sequencing of the V4 region. We detected 6,954 distinct Operational Taxonomic Units (OTUs) across various mat systems. Of the sequences that aligned with known protistan phylotypes, most were affiliated with alveolates (especially dinoflagellates and ciliates) and cercozoans. OTU richness and community structure differed among sediment habitats (e.g. different mat types and cold sediments away from mats). Additionally, full-length 18S rRNA genes amplified and cloned from single cells revealed the identities of some of the most commonly encountered, active ciliates in this hydrothermal vent ecosystem. Observations and experiments were also conducted to demonstrate that ciliates were trophically active and ingesting fluorescent bacteria or *Beggiatoa* trichomes. Our work suggests that the active and diverse protistan community at the Guaymas Basin hydrothermal vent ecosystem likely consumes substantial amounts of bacterial biomass, and that the different habitats, often defined by distances of just a few 10s of cm, select for particular assemblages and levels of diversity.

HIGH-THROUGHPUT sequencing approaches have provided valuable insights into microbial eukaryote composition and diversity across a variety of ecosystems ranging from the sunlit surface of the oceans (de Vargas et al. 2015) to soils of tropical rainforests (Mahe et al. 2017). Improvements in sequence read lengths and numbers of reads are now providing unprecedented insight into the species composition and richness of natural microbial communities, with taxonomic resolution approaching that possible with whole gene sequences (Hu et al. 2015). Deeper sequencing efforts can effectively characterize the genetic diversity of complex microbial communities, characterizing both the highly abundant as well as the rare members of the community (Grattepanche et al. 2014; Santoferrara et al. 2014).

Cultivation-independent approaches, such as high-throughput sequencing, are particularly effective for characterizing protistan communities in marine environments that are hard to access and where microbes may be difficult to culture (e.g. the deep-sea; Arndt et al. 2003; Alexander et al. 2008). In fact, most of our knowledge of microbial eukaryotes living in extreme marine environments, such as deep-sea cold seeps and hydrothermal vents, is derived from such culture-independent approaches (Caron et al. 2012 and sources within). Sequencing surveys have enabled us to observe microbial eukaryote community patterns across environmental gradients within these types of extreme habitats (Coyne et al. 2013; Edgcomb et al. 2002; López-García et al. 2007;

Pasulka et al. 2016), such that we can begin to better understand the ecology, physiology, and trophic interactions of these organisms in chemosynthetic environments. However, to date, the majority of published work on hydrothermal vent-associated microbial eukaryotes has been derived from full-length 18S rRNA sequences, clone libraries or T-RFLP (Atkins et al. 2000; López-García et al. 2007; Moreira and López-García 2003; Xu et al. 2018). A greater understanding of ecologically important patterns in microbial eukaryotic composition and diversity can be gained from the application of modern high-throughput tag sequencing.

Guaymas Basin is a dynamic and hydrothermally active vent system, where the advection and production of a variety of different metabolic substrates support extensive benthic microbial diversity and activity (Meyer et al. 2013). These substrates sustain a unique microbial ecosystem that combines the characteristics of communities from hydrocarbon seeps and mid-ocean ridge hydrothermal vents resulting in a patchwork of habitats including high temperature fluids, porous carbonate rocks, and sediments covered with dense microbial mats (Teske et al. 2016). This patchwork of habitats, which exhibit a great variability in temperature, pH, and hydrogen sulfide concentrations are known to host a unique composition of microbial eukaryotes relative to the surrounding deep-sea (Edgcomb et al. 2002; López-García et al. 2003, 2007). Among the microbial eukaryotes, ciliates in particular have been shown to exhibit high diversity and numerical abundance in vent and methane seep ecosystems (Coyne et al. 2013; López-García et al. 2007; Small and Gross 1985). While direct evidence of ciliate trophic interactions (i.e. grazing) is still lacking, distribution patterns of ciliates in relation to stable isotope biomarkers and biogeochemical gradients (as proxies for regions of bacterial and archaeal productivity) suggest that these organisms play important trophic roles in chemosynthetic ecosystems, at least at methane seeps (Pasulka et al. 2016; Werne et al. 2002).

We used high-throughput sequencing of the V4 hypervariable region to characterize microbial eukaryote diversity and composition in the surface layers of the sediments inside and outside of microbial mats at the Guaymas Basin hydrothermal vent site. The presence of a colored mat covering the sediments (ranging from white to orange) is a visual indicator of a hotspot of high hydrocarbon flux and chemosynthetic production (Lichtschlag et al. 2010; Lloyd et al. 2010; Van Gaever et al. 2006). These mats are formed by dense assemblages of the giant sulfide oxidizing bacteria, *Beggiatoa* (Jannasch et al. 1989; McKay et al. 2012; Nelson et al. 1989) and have been shown to influence the composition and diversity of microbial eukaryotes (Coyne et al. 2013; Pasulka et al. 2016). This study builds on previous smaller scale sequencing efforts (e.g. full-length 18S rRNA sequences derived from clone libraries) conducted in this region (Coyne et al. 2013; Edgcomb et al. 2002) to (i) explore how patterns of microbial eukaryote composition and diversity vary across sediment features (e.g. microbial mats), (ii) determine if the same broad patterns of diversity hold true with a deeper depth of sequencing, and (iii) provide preliminary evidence of microbial eukaryotes actively grazing on bacteria to help describe their trophic relationships and activities in vent ecosystems.

METHODS

Study site and sample collection

Guaymas Basin is a hydrothermally active field in the Southern Guaymas Trench (Gulf of California, ~2,000 m water depth, 27°00.37 N to 27°00.49 N and 111°24.58 W to 111°24.44 W). All samples were collected using the DSV *Alvin* as part of R/V *Atlantis* expedition AT15-39 operated by Woods Hole Oceanographic Institution, Woods Hole, MA, in November 2008. Each mat system was defined by three habitats—(i) the center of the mat feature (dense mat-covered sediments), (ii) the edge of mat (sediments loosely covered with mat a short distance away from the center of the mat), and (iii) control sediments (non-mat-covered cold sediments 2–3 m away from mat edge). Sampling was conducted accordingly to procedures described in Coyne et al. (2013) and Campbell et al. (2013). Push cores were used to collect sediment from these three distinct habitats within one orange and two yellow mat systems (Fig. 1). In situ subsurface temperatures for most sites were measured before coring (< 50 cm away from the sample areas) by using either the external "High Temperature Probe" or the external "Heat-flow Probe" on the *Alvin* submersible (for probe details see McKay et al. 2012).

Immediately after collection, cores were tightly closed and stored upright in a fixed position for ascent to the surface. Upon arrival at the surface, cores were transferred to a 4 °C cold room for processing. Cores with intact layering of mats and sediments were subsampled for further analyses within 4–12 h after sampling on the seafloor. Replicate sediment cores from targeted microbial mats were collected separately for microbial eukaryotic community analyses and pore water geochemistry. Cores were placed on a piston and pushed up in centimeter increments for subsampling of different core horizons. The upper 1- and 2-cm horizons were sectioned and preserved for subsequent analyses. Samples for all molecular work were immediately frozen at −80 °C until extraction. Pore waters were analyzed for Fe, pH, and sulfide.

DNA extraction and PCR amplification

DNA was extracted from each sediment sample (in replicate) with the MoBio Power Soil Kit (MoBio Laboratories, Carlsbad, CA). Following extraction, DNA concentration was determined using the Qubit with HS DNA reagents (Thermo Fisher Scientific, Canoga Park, CA) and 0.1 ng/μl was used as the template DNA for first PCR reaction along with Q5 Hotstart (1× final concentration, New England Biolabs, NEB, Ipswich, MA), and 0.5 μM (final concentration) of each primer. The V4 region was amplified

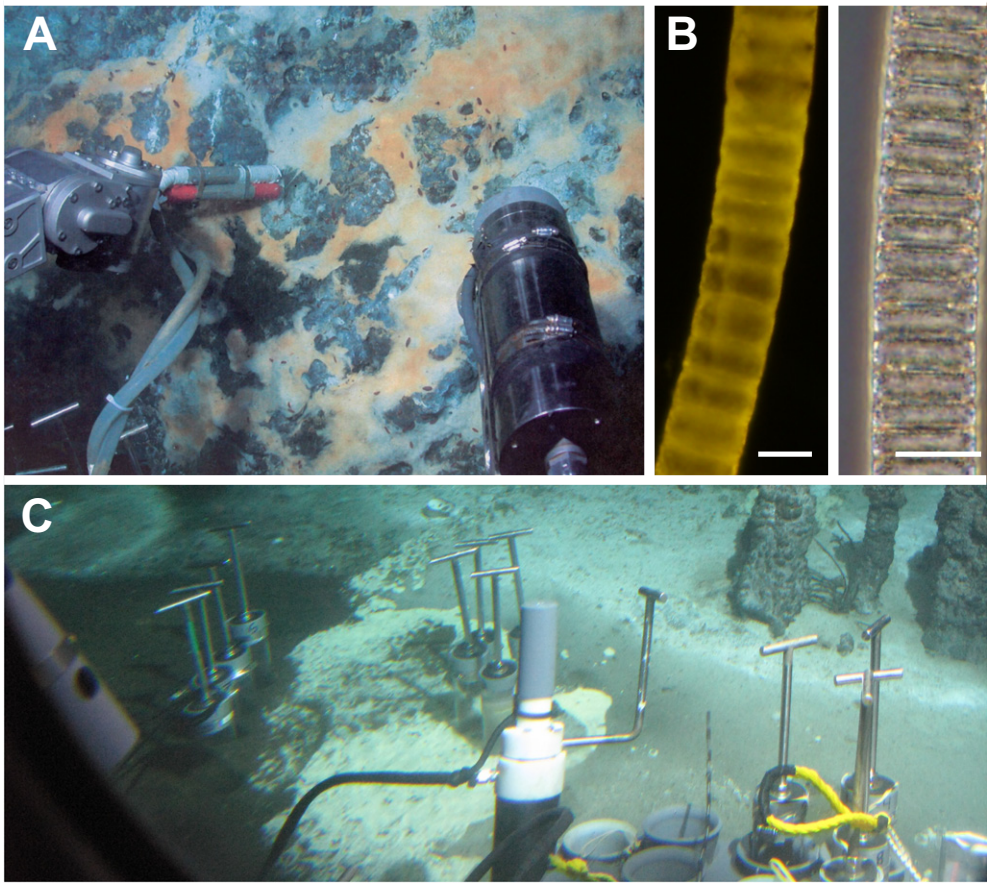

Figure 1 (**A**) Microbial mat at Guaymas Basin Hydrothermal vent. (**B**) Micrographs of *Beggiatoa* trichomes, the predominant bacteria that comprises the mats, by epifluorescence (left) and transmitted light (right) microscopy. Scale bar 10 μm. (**C**) Push cores deployed in the center and edge of the microbial mat.

using Stoeck et al. (2010) primers: EukF 5′-CCAGCA[G/C]C[C/T]GCGGTAATTCC-3′ and EukR 5′-ACTTTCGTTCTTGAT[C/T][G/A]A-3′ using the Illumina 2-step PCR method for amplification (www.illumina.com) as in Pasulka et al. (2016). Primers were modified with Illumina adaptors in the first PCR step, and primers with barcoded indices were used during the second PCR step in order to minimize PCR bias (Berry et al. 2011). The PCR protocol for V4 amplification employed an initial activation step at 98 °C for 2 min, followed by 12 cycles consisting of 98 °C for 10 s, 53 °C for 30 s and 72 °C for 30 s, followed by 18 cycles of 98 °C for 30 s, 48 °C for 30 s, and 72 °C for 30 s, with a final 10 min extension at 72 °C (adapted from Rodríguez-Martínez et al. 2012). For the second PCR step, 5 μl of the amplicon product from the first PCR was used as template with barcoded primers (0.5 μM final concentration), and the following thermal protocol: 98 °C for 2 min, followed by 10 cycles consisting of 98 °C for 10 s, 66 °C for 30 s, and 72 °C for 30 s, with a final extension of 2 min. For all PCR steps, amplification success and purity were checked by gel electrophoresis. Duplicate barcoded products were pooled and quantified with a Qubit fluorometer (Thermofisher Scientific, Canoga Park, CA). Barcoded amplicons were mixed together in equimolar amounts and purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA).

Illumina sequencing and sequence analysis

Paired-end 2 × 250 base pair sequencing was performed at Laragen, Inc. (Los Angeles, CA) on an Illumina MiSeq platform. The raw data were demultiplexed at Laragen, and sequences that had > 1 base pair mismatch on the 12-base pair barcode were removed. The sequences were processed using QIIME 1.8.0 (Caporaso et al. 2010). In short, paired ends were joined with a 25 bp minimum overlap, checked for the presence of at least one primer sequence, trimmed according to Q-scores (Q30 max acceptable), and sequences with ambiguous "N" base calls were removed. Sequences shorter than 150 bps were discarded. Chimeras were removed with vsearch (v1.11.1, Rognes et al. 2016) using default parameters. The remaining sequences were used to form de novo operational taxonomic units (OTUs) at a threshold of 97% similarity. Singleton OTUs were removed from the dataset and taxonomic assignments were made with representative sequences from each OTU.

Taxonomy was assigned using the Protist Ribosomal Database (PR2; Guillou et al. 2013). After sequence processing, five samples were removed from further analyses due to poor read recovery (Fig. S1). Metazoan OTUs were also removed prior to statistical analyses. Raw sequence data are available in the Sequence Read Archive (SRA) BioProject: PRJNA391741. Bioinformatic sequence analysis and downstream processing is available on GitHub (Table S1, https://github.com/apasulka/Guaymas-Basin-Benthic-Microbial-Eukaryotes).

Ciliate grazing observations and experiment

Orange and white *Beggiatoa* mats were collected with the Alvin slurp-samplers from the top of a flange vent at a depth of 1,990 m in the vicinity of "Rebecca's Roost", at coordinates $x = 2{,}617$, and $y = 1{,}173$ (27°0.63510′N, 111°24.41784′W), during Alvin dive 4,482. Mat material was pumped into sample chambers containing 0.2 μm filtered seawater from the vent ecosystem, transported to the surface on the Alvin science platform, and immediately placed at 4 °C upon recovery of the submersible. *Beggiatoa* mats and the affiliated microbial assemblage, including numerous ciliates of various sizes and shapes, were observed briefly by light microscopy (see below) prior to subsampling for preparation of a short-term grazing experiment at deep-sea pressure. Ciliates and *Beggiatoa* filaments exhibited active motility during these observations, suggesting that transport from the seafloor and transition to one atmosphere aboard the R/V Atlantis did not have a detrimental effect on these organisms. A subsample was removed immediately, and wet mounts were prepared for transmitted light and epifluorescence microscopy. The preparations were examined using low- and high-magnification compound microscopy for the presence of living protists, and for the presence of ingested prey.

A separate subsample of the *Beggiatoa* mat material was also decanted and gently mixed prior to distributing 160 ml into a sterile tissue culture flask, to which 1.5 ml of an equal mixture of two cultures of bacterial prey were added. The mixed bacterial prey included a green fluorescent protein (GFP) expressing *E. coli* (Gruber et al. 2009) and a GFP *Vibrio* sp. that were added to achieve a final concentration of GFP cells in the sample of $\sim 1 \times 10^6$ cells/ml. Both cultures of GFP-expressing cells were kindly provided to us by D.F. Gruber. The sample was gently mixed to distribute the GFP cells and then subsampled with sterile plastic transfer pipettes, such that several milliliters of water containing *Beggiatoa* filaments, ciliates, and GFP prey were drawn into the bulb portion of each pipette. The pipettes were inverted, and all experimental water was gently tapped down into the bulb and lower half of the pipette before the end of each pipette was sealed with a flat iron—care was taken to minimize the amount of air sealed in the pipette. Several control samples were preserved with formalin (1% final concentration) prior to the start of short-term incubations. Sealed pipettes containing the live microbial mat material were deposited into a stainless-steel pressure chamber filled with Milli-Q water, capped and pressurized to 200 atm using a hydraulic hand pump over a period of several minutes (Wirsen and Jannasch 1975). The pressure chamber was incubated for 90 min at room temperature (~20 °C) to approximate the temperature where the samples were collected atop the flange vent. Upon completion of the incubation period, the pressure chamber was vented, and sealed transfer pipettes were opened, and the grazing experiment stopped via cell fixation with formalin (1% final concentration). Wet mounts of the incubated microbial mat community were prepared for epifluorescence microscopy using an Olympus BX51TF-5 with image analysis system to examine the extent to which individual protists had ingested GFP-expressing bacterial cells and/or *Beggiatoa* filaments.

Microscopy, ciliate single-cell isolation, amplification, and sequencing

Microscopy of live and preserved material was opportunistically conducted during the study in order to obtain visual confirmation of the presence of live, active protists. For preserved material, samples were taken from the top 1 cm of the core, and preserved in 1% final concentration of buffered formalin, or immediately prepared as wet mounts on microscope slides at 4 °C for microscopy. Slides were examined immediately by transmitted light and epifluorescence microscopy and discarded after 10 min of observation. Individual cells obtained for DNA sequencing (large and abundant ciliates) were observed in live mat samples using an Olympus SZX10 stereoscope with image analysis system. Samples were placed in 1-ml glass chambers, and individual ciliates were removed by micropipetting, rinsed three times in 0.2 μm filtered seawater from the sampling site, and placed into sterile PCR tubes. In some cases, multiple individuals of the same morphotype were placed in the same tube in order to provide sufficient material for amplification, cloning, and sequencing. Isolated cells were stored frozen at −70 °C until lysis by three rounds of freeze/thaw. Frozen cells were transferred to a PCR thermal cycler and heated to 95 °C for 15 s before cooling to 4 °C. Heat-treated cells were transferred back to a −70 °C freezer until completely frozen. Two more rounds of heating and freezing were conducted to prepare the final cell lysates. These crude cell lysates were stored at −70 °C until whole genome amplification (WGA) using the Genomiphi v2 amplification kit (GE Life Sciences, Pittsburgh, PA). The general protocol was followed for Genomiphi reactions, using 1 μl of the crude cell lysate mixed with 9 μl of sample buffer. This mixture was heated to 95 °C for 3 min, cooled to 4 °C and transferred to ice. One μl of Genomiphi enzyme was gently mixed with 9 μl of reaction buffer and kept on ice until ready for use. The 10 μl aliquot of WGA reagents was added to the 10 μl sample aliquot and mixed by gentle pipetting. This 20 μl reaction mixture was incubated for 3 h at 30 °C to replicate the genomic DNA from the cell isolates using the random hexamer primers and phi29 DNA polymerase (included in the kit). Reactions were stopped by

heating to 65 °C for 10 min followed by chilling the reactions to 4 °C. WGA products were amplified with the 18S rRNA gene primers Euk-A and Euk-B (Medlin et al. 1988), cloned, and sequenced following protocols described previously by Countway et al. (2007, 2010). Clones with full-length amplicons were sequenced using a combination of T3 and T7 primers, matching sites on the pCR4-TOPO cloning vector (Thermofisher Scientific) and the internal 18S primer 570F, which binds at the start of the V4 region of the gene (Weekers et al. 1994). Individual reads were assembled into full-length 18S rRNA gene contigs in ChromasPro v2.1.8 (Technelysium). A total of 23 full-length 18S rRNA gene sequences resulted from picking live ciliates from Guaymas Basin hydrothermal vent samples.

RNA extraction, cDNA synthesis, cloning, and sequencing

A subsample of the same *Beggiatoa* mat material that was used for the grazing experiment described above was extracted with the MoBio PowerSoil RNA kit to obtain 18S rRNA sequences from the active members of the protist community. Extracted RNA was treated with DNAse I using the DNA-Free RNA Kit (Zymo Research) with 170 μl of RNA extract, 20 μl of DNAse buffer, and 10 μl of DNAse I. After digestion of the residual DNA, the RNA in the reaction mixture was purified and concentrated with the RNA spin-column provided in the kit and eluted with 16 μl RNAse-free water. First strand cDNA was synthesized using the Superscript III Reverse Transcriptase (RT) kit (Thermofisher Scientific) and the 18S rRNA gene-specific primer Euk-B. The reaction consisted of 1 μl of Euk-B (3 μM), 10 μl of the DNAse-treated RNA, 1 μl of dNTPs (10 mM each), and 1 μl of DNAse/RNAse-free water. This mixture was incubated at 65 °C for 5 min on a thermal cycler, transferred to ice for 1 minute, and centrifuged to spin down the tube contents. To this mixture, 4 μl of 5× First-Strand Buffer, 1 μl of DTT (0.1 M), and 1 μl of SuperScript III RT was added. The cDNA synthesis reaction was incubated at 55 °C for 60 min followed by heating to 70 °C for 15 min to stop the reaction. A parallel 'no-RT' reaction was carried out to test for residual DNA in the RNA sample and none was found, indicating complete digestion of DNA. Immediately following cDNA synthesis, residual RNA was removed from reaction mixtures by adding 1 μl of RNAse H (Thermofisher Scientific) to the cDNA and incubating at 37 °C for 20 min. PCR, cloning, and sequencing of 18S rRNA genes from cDNA followed the procedures described above. Seven full-length 18S rRNA gene sequences attributed to ciliates were assembled from the resulting cDNA clone library.

Phylogenetic reconstruction of full-length sequences

A phylogenetic tree containing 23 full-length ciliate sequences from Guaymas Basin plus closely related sequences from GenBank (Benson et al. 2013) and EukRef-Ciliophora (Boscaro et al. 2018) was constructed within the Geneious v9 software package (https://www.geneious.com), using the MUSCLE sequence aligner (Edgar 2004) and the Bayesian phylogenetic inference program, MrBayes v3.2 (Huelsenbeck and Ronquist 2001). The initial MUSCLE alignment was refined by manually trimming the nonoverlapping regions at the 5′ and 3′ end and subsequently realigned, resulting in an alignment spanning 1,600 nucleotide positions. Settings within MrBayes included selection of the "general time reversible" (GTR) nucleotide substitution model with rate variation set to "invgamma", indicating a gamma distribution of nucleotide substitution rates with a proportion of invariable nucleotide sites. The 18S rRNA gene from the dinoflagellate *Alexandrium* served as the outgroup for this phylogenetic tree.

Statistical analyses

All statistical analyses were done in R (R Development Core Team 2008). Nonmetric multidimensional scaling analysis (MDS) was used to visualize the microbial eukaryote composition data. Bray–Curtis similarity was used as the resemblance measure on fourth-root-transformed data for the MDS. Analysis of similarities (ANOSIM) was used to determine the significance of between-group differences in community structure [i.e. habitat (mat, edge, cold) and mat color]. Rarefaction curves were used to investigate the degree of sample saturation. Alpha diversity (OTU richness and Shannon diversity index—*H*′) was calculated using the "vegan" package.

RESULTS

Environmental characteristics

The benthic environments sampled in the study were dominated by two types of *Beggiatoa* microbial mat systems: orange ($n = 1$) and yellow ($n = 2$) (McKay et al. 2012). Within each mat system, the center and edge sediments had higher temperatures compared to the control (cold) sediments away from visible accumulations of *Beggiatoa* (Table 1). The center of the yellow mat had the highest recorded temperature (29 °C, Table 1) relative to all other sediment habitats sampled. Sulfide (mg/L) concentrations were higher in the centers and edges of the yellow mats (~3–29 mg/L) relative to the control sediments (< 1 mg/L); Table 1). Sulfide data were not available for much of the orange mat system. However, the orange mat edge and control sediments had the lowest sulfide concentrations (0.2 mg/L; Table 1). Iron (mg/L) concentrations were highly variable across each mat system (Table 1).

Sequencing statistics

Following sequence QC (see Methods) and removal of information for five samples with too few sequences

Table 1. Surface geochemistry

Habitat	MatColor	Sample ID	Dive	Temp	pH	S (mg/L)	Fe (mg/L)	Depth (m)	Lat	Lon
Mat	Yellow	1a/b	4,476	29.0	6.33	19.85	0.16	1987	27.031868	111.380328
Edge	Yellow	3	4,476	NA	6.63	28.63	NA	1987	27.031868	111.380328
Control	Yellow	5	4,476	NA	6.43	0.58	NA	1987	27.031868	111.380328
Mat	Yellow	7	4,477	2.9	6.94	3.29	0.34	2002	27.018312	111.382383
Control	Yellow	15*	4,477	NA	6.88	0.39	0.61	2002	27.01833	111.382363
Mat	Orange	9	4,481	19	7.09	ND	0.04	1988	27.022112	111.380157
Edge	Orange	11	4,481	9.7	7.15	0.2	0.04	1988	27.022112	111.380157
Control	Orange	14†	4,481	2.5	7.34	0.2	0.2	1988	27.022112	111.380157

Summary of geochemistry from a subset of samples that are representative of the mat surface at each site. NA denotes where data were unavailable. Sample IDs correspond to all other tables and figures.

*While there is no corresponding sediment core with a DNA extraction available for sample 15, this geochemical data pairs with sample 8 (yellow control sample from 2 to 3 cm depth, Table 2) due to proximity in location of the two samples.

†Sample 14 is not included in main figures, as there were too few sequences, but it portrays representative chemistry for a control site (Table 2 and Fig. S1).

(Table 2, Fig. S1), high-throughput sequencing of the V4 hypervariable region identified a range of 25,184–484,708 sequences per sample. Sequences clustered into 6,954 distinct OTUs (excluding global singletons). OTUs with only one sequence (singletons) or two sequences (doubletons) were on average < 1% of the sequence totals for individual samples (data shown for samples grouped by habitat in Table 3). Singletons and doubletons were on average 30% and 12% of the OTU total per sample, respectively (Fig. S1).

Overall microbial eukaryote community composition and diversity patterns

Community analysis revealed a complex and diverse microbial eukaryote assemblage thriving in the sediments associated with the Guaymas Basin hydrothermal vent ecosystem. While the majority of the community we examined via 18S rRNA was protistan in nature, we used the term microbial eukaryotes, because fungi were included in the full community analysis. The relative abundance of microbial eukaryotes

Table 2. Information about sampling locations including dive number and habitat type

Sample ID	Dive	Habitat	Sediment horizon	Total sequences	Total OTUs	Date
1a	4,476	Yellow mat	0–1 cm	237,176	521	23-11-2008
1b	4,476	Yellow mat	0–1 cm	484,570	582	23-11-2008
2	4,476	Yellow mat	1–2 cm	255,447	399	23-11-2008
2b*	4,476	Yellow mat	1–2 cm	107	38	23-11-2008
3b*	4,476	Edge of mat	0–1 cm	256	64	23-11-2008
3	4,476	Edge of mat	0–1 cm	79,927	290	23-11-2008
4a	4,476	Edge of mat	1–2 cm	68,170	249	23-11-2008
4b	4,476	Edge of mat	1–2 cm	57,612	176	23-11-2008
5a	4,476	Control	0–1 cm	385,676	1,357	23-11-2008
5b	4,476	Control	0–1 cm	363,065	1,518	23-11-2008
6a	4,476	Control	1–2 cm	224,654	942	23-11-2008
6b	4,476	Control	1–2 cm	120,328	673	23-11-2008
7	4,477	Yellow mat	0–1 cm	387,101	1,210	24-11-2008
8	4,477	Control	2–3 cm	381,712	1,312	24-11-2008
9	4,481	Orange mat	0–1 cm	446,922	878	28-11-2008
10a	4,481	Orange mat	1–2 cm	30,209	181	28-11-2008
10b	4,481	Orange mat	1–2 cm	25,174	175	28-11-2008
11	4,481	Edge of mat	0–1 cm	194,787	501	28-11-2008
13a*	4,481	Edge of mat	1–2 cm	814	97	28-11-2008
13b*	4,481	Edge of mat	1–2 cm	83	46	28-11-2008
14*	4,481	Control	0–1 cm	531	107	28-11-2008
12a	4,481	Control	1–2 cm	32,587	341	28-11-2008
12b	4,481	Control	1–2 cm	40,445	424	28-11-2008

All samples were collected from 2,000 m in depth. Samples with asterisks (*) were not included in the analysis, as these samples had too few sequences (and OTUs). Sample IDs correspond to all other tables and figures. Total sequences and OTUs reported for each sample do not include global singletons.

Table 3. Sequence analysis overview

	Yellow mat	Yellow edge	Yellow control	Orange mat	Orange edge	Orange control
Sequences (no singletons)	325,731 ± 137,862	68,570 ± 11,163	273,431 ± 124,436	167,435 ± 242,056	194,787	36,516 ± 5,556
Total OTUs	501 ± 93	238 ± 58	1,123 ± 386	411 ± 404	501	383 ± 59
Singleton OTUs	102 ± 12	53 ± 16	169 ± 34	63 ± 51	98	87 ± 14
Doubleton OTUs	50 ± 20	25 ± 12	183 ± 69	66 ± 80	79	46 ± 8
OTUs with > 2 sequences	349 ± 75	160 ± 32	771 ± 297	283 ± 274	324	250 ± 36

Table of OTU statistics averaged across sample types. Values as mean ± SD across each sample type. Five samples with too few sequences (see Table 1) were not included. Samples from edge of orange mat did not have any replicates.

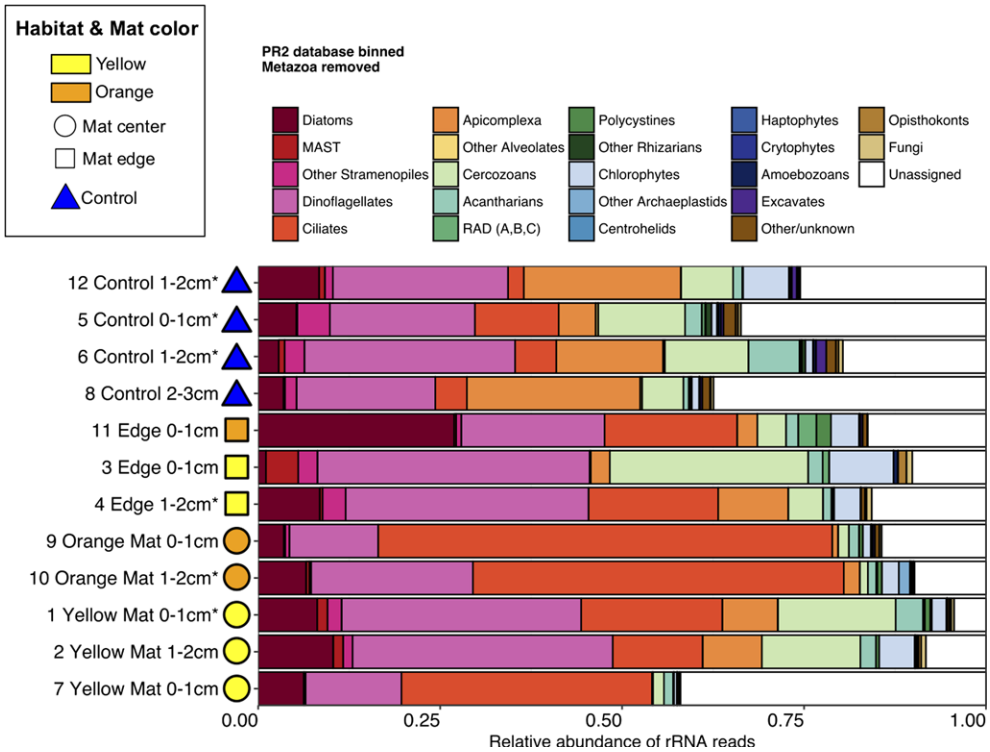

Figure 2 Overview of microbial eukaryote composition. Relative abundance of microbial eukaryotic rRNA reads across samples collected from Guaymas Basin hydrothermal vent sediments. OTUs were summed based on taxonomic group (bar plot colors). *Y*-axis legend color designates the color of mat sampled and shape indicates the type of habitat sampled (e.g. inside microbial mat, on the edge of a microbial mat or in adjacent cold/control mud). Asterisks (*) denote replicate samples pooled (see Table 2). The "Other/unknown" category includes a small percentage of OTUs which matched a reference in the PR2 database but are not fully annotated (i.e. "Eukaryota_X") and the "Unassigned" category includes OTUs that did not match any reference sequence in the PR2 database.

(grouped at the Phylum or major taxonomic group levels) varied within and across vent sediment habitats (e.g. in the center of microbial mat, on the edge of the microbial mat, or in adjacent control sediments; Fig. 2). Dinoflagellates, ciliates, apicomplexans, and cercozoans comprised the greatest proportion of microbial eukaryotic sequences (Fig. 2). Relative abundances of dinoflagellate OTUs did not vary dramatically between habitats, with the exception of a higher proportion of dinoflagellate sequences in the yellow mat systems relative to the orange mat systems (both edge and center, excluding yellow mat sample 7, Fig. 2). Ciliate OTUs exhibited higher relative abundances in microbial mat sediments relative to control sediments (Fig. 2). Ciliate OTUs also had higher relative abundances in the center of the orange mat compared to the yellow mat (Fig. 2). Apicomplexa were found to have higher relative abundances in the control sediments, while cercozoans had lower relative abundances in orange mat sediments relative to yellow mat sediments (except for sample 7, Fig. 2). Fungal OTUs exhibited low relative abundances across all samples (0.5–2.5% of the sequence total) with no visible pattern in relationship to sediment habitat types.

Multidimensional scaling (MDS) revealed that sediments from similar habitats (e.g. control, mat center, or

edge of mat; Fig. 3; ANOSIM $R = 0.47$, $P = 0.001$) harbored more similar microbial eukaryote communities, and that mat color was also correlated with the structure of the community (e.g. the orange mats had a different composition than the yellow mats; Fig. 3; ANOSIM $R = 0.23$, $P = 0.034$). However, there was also variability in the taxonomic composition of microbial eukaryote assemblages between mats of the same color. For example, a yellow mat center sample (Sample 7), fell as an outlier in multidimensional space relative to the other yellow mat sediments (Fig. 3) and had the highest OTU richness of all the mat samples (Table 2). Location within the mat also influenced community structure—the composition of microbial eukaryotes in the center and the edge of the microbial mats were significantly different from one another (Fig. 3; ANOSIM $R = 0.15$, $P = 0.18$). The main taxonomic groups driving differences in community composition across habitats were identified as ciliates, apicomplexans, and rhizarians (as determined via SIMPER and visualized in Fig. S4).

On average, control sediments had the highest OTU richness (Fig. 4A) and Shannon diversity (Fig. 4B), while sediments collected from the edge of mat samples had the lowest diversity overall (Fig. 4A, B). However, the sediments from the center of yellow mats had higher diversity on average than sediments from the center of the orange mats. None of the rarefaction curves reached an asymptote (Fig. 4A).

Comparison of OTUs between samples revealed that 295 OTUs were shared among all samples (Fig. 5A). Control sediments had the greatest number of unique OTUs (i.e. OTUs not shared with other habitats) relative to the other habitats (Fig. 5A). There were more OTUs shared between the center of the mat and the control sediments (474 shared OTUs) than between the center and edge of the mat (201 shared OTUs). Only 97 OTUs were shared between the edge of the mat and control sediments.

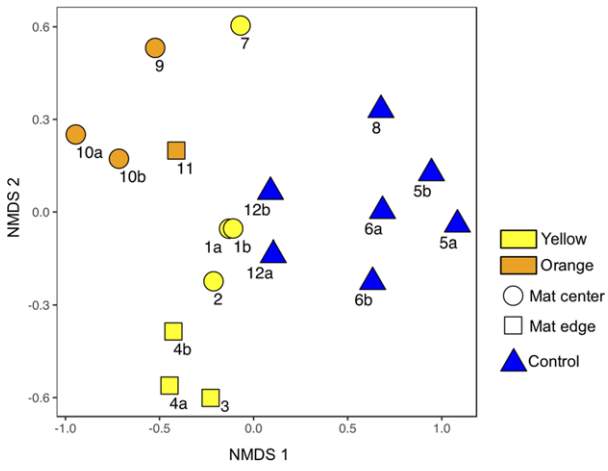

Figure 3 Two-dimensional MDS plot visualizing the relationship of microbial eukaryote composition (OTU level) across all sediment samples. Bray–Curtis similarity was used as the resemblance measure on fourth-root-transformed OTU abundance data. Shape indicates the type of habitat sampled (e.g. inside microbial mat, on the edge of a microbial mat or in adjacent cold/control mud). Each symbol is color coded by mat color (e.g. yellow or orange) or designation of a control sample.

Ciliate-specific composition and diversity patterns

We further examined ciliate community structure (at the class level) within and across vent sediment habitats due to the high relative abundances of ciliate OTUs in microbial mat sediments (Fig. 2, 6). Ciliate OTUs within the Spirotrichea were relatively abundant in control sediments and in the center of each mat (in some cases comprising > 50% of the ciliate sequences), but were less abundant at the edge of the mats (Fig. 6). Ciliate OTUs within the Oligohymenophorea and Litostomatea made up a greater proportion of the total ciliate community in control sediments relative to microbial mat sediments (Fig. 6). However, ciliate OTUs within the Heterotrichea made up a greater proportion of the total ciliate community in microbial mat samples compared to control sediments. Ciliate OTUs within the Ciliophora-10 made up a greater proportion of the total ciliate community in yellow mats (both in the center and on the edge of the mat) relative to all other samples, whereas ciliate OTUs within the Armophorea contributed to a greater proportion of the total ciliate community in the orange mats relative to all other samples (Fig. 6). Of the 808 ciliate OTUs (639,320 sequences), 52% of them shared < 95% similarity to sequences in the reference PR2 database. About 78% of ciliate OTUs shared < 97% similarity to sequences in the reference PR2 database.

Patterns of OTU richness among the ciliates followed the same broad patterns of total community OTUs-control sediments had the highest diversity, followed by the center of the mat, and then the edge of the mat (Fig. 5B). There were 28 ciliate OTUs shared among all samples, 55 ciliate OTUs shared between the center of the mat and control sediments, 26 ciliate OTUs shared between the center and edge of the mat, and only 8 ciliate OTUs shared between the edge of the mat and the control sediments. Sediments from the edge of the yellow mats had the lowest ciliate OTU richness relative to all other sediment habitats (Fig. 5B, 7 and Fig. S2).

OTU richness within ciliate classes varied across the different sediment habitats (Fig. 7). In general, ciliates within the Spirotrichea, Plagiopylea, Oligohymenophorea, Litostomatea, and Colpodea exhibited higher OTU richness relative to the other ciliate classes detected. The highest ciliate OTU richness within these classes was observed in the control sediments, with the exception of a few microbial mat samples (e.g. Samples 1 and 7). Most ciliate classes had a combination of OTUs that were unique within each habitat type as well as shared OTUs with the exception of ciliates within the Prostomatea and Phyllopharyngea. OTUs within these two classes were shared across all habitat types. Overall, control sediments had a higher proportion of unique OTUs relative to microbial mat samples (edge and mat center). However, ciliates within

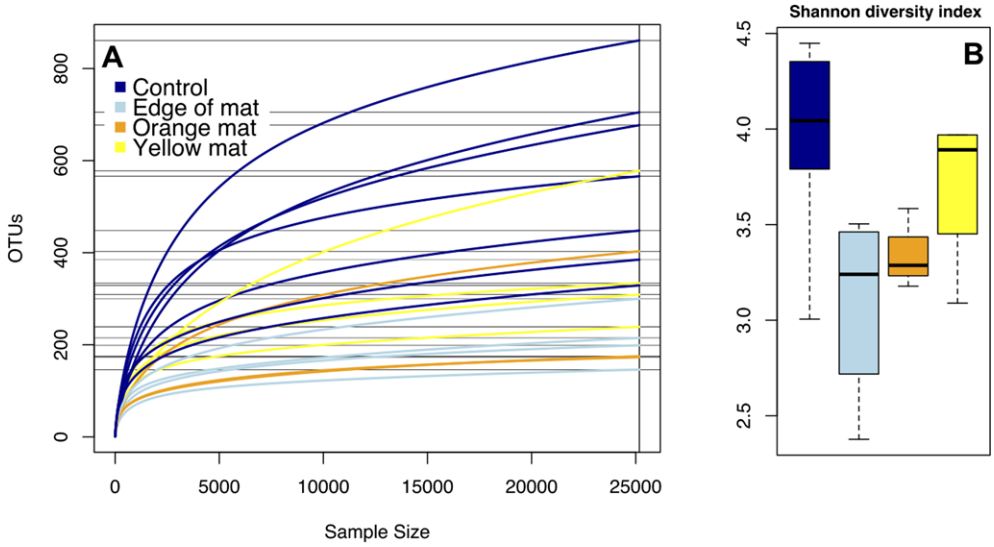

Figure 4 Species accumulation (rarefaction) curves (**A**) and Shannon Diversity Index (**B**) across all habitat types (indicated by color). All samples were subsampled to equal depth for alpha diversity metric calculations.

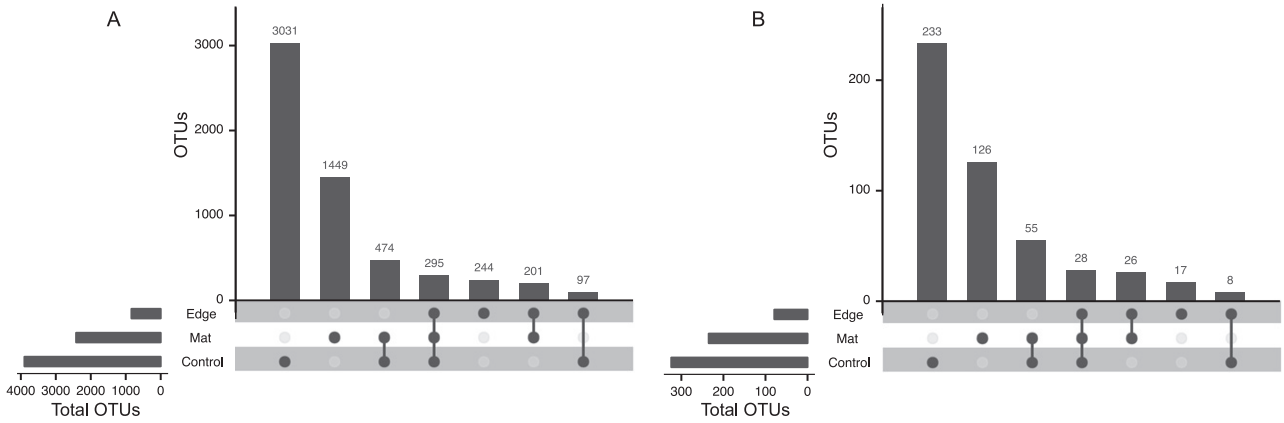

Figure 5 Distribution of unique and shared OTUs for (**A**) all OTUs and (**B**) ciliate only OTUs with respect to habitat designated by the filled-in circles in the matrix below each bar (all samples within edge, mat, or control were pooled regardless of mat color). Leftmost horizontal bars indicate the total number of OTUs in each sample type including shared OTUs.

the following ciliate classes had no unique OTUs in control sediments—Ciliophora-7, Ciliophora-5, Ciliophora-10, Cariacotrichea, Armophorea. Ciliates within the Ciliophora 7 and 5 were only found in control sediments, but ciliates within Ciliophora-10, Cariacotrichea, and Armophorea were also found in mat samples.

Microscopy of unpreserved sediment collected from the tops of cores confirmed high abundances of active microbial eukaryotes, predominantly ciliates, in the dense *Beggiatoa* mats. Live protists were rarely observed in control sediments, although there were living eukaryotes present in all samples. Microscopy, and ingestion experiments using fluorescent bacterial prey to demonstrate predation, also revealed that the ciliates were trophically active. The largest ciliate taxa showed evidence of the ingestion of *Beggiatoa* trichomes (trichome autofluorescence within food vacuoles) and other large particles within food vacuoles (Fig. 8A–D), while grazing experiments confirmed that many vent ciliates actively ingested GFP-expressing bacteria (Fig. 8E–H).

Full-length 18S rRNA gene sequences ($n = 23$) from individual micropipetted ciliates and a single cDNA-based clone library revealed the affinities of common taxa to five classes including Armophorea, Oligohymenophorea, Plagiopylea, Prostomatea, and Spirotrichea (as determined by placement on a phylogenetic tree Fig. S3). Three of the ciliate classes represented by sequences in the phylogenetic tree, including Armophorea, Prostomatea, and Spirotrichea, were only detected by sequencing full-length cDNA clones. Of the 808 ciliate OTUs (639,320 sequences)

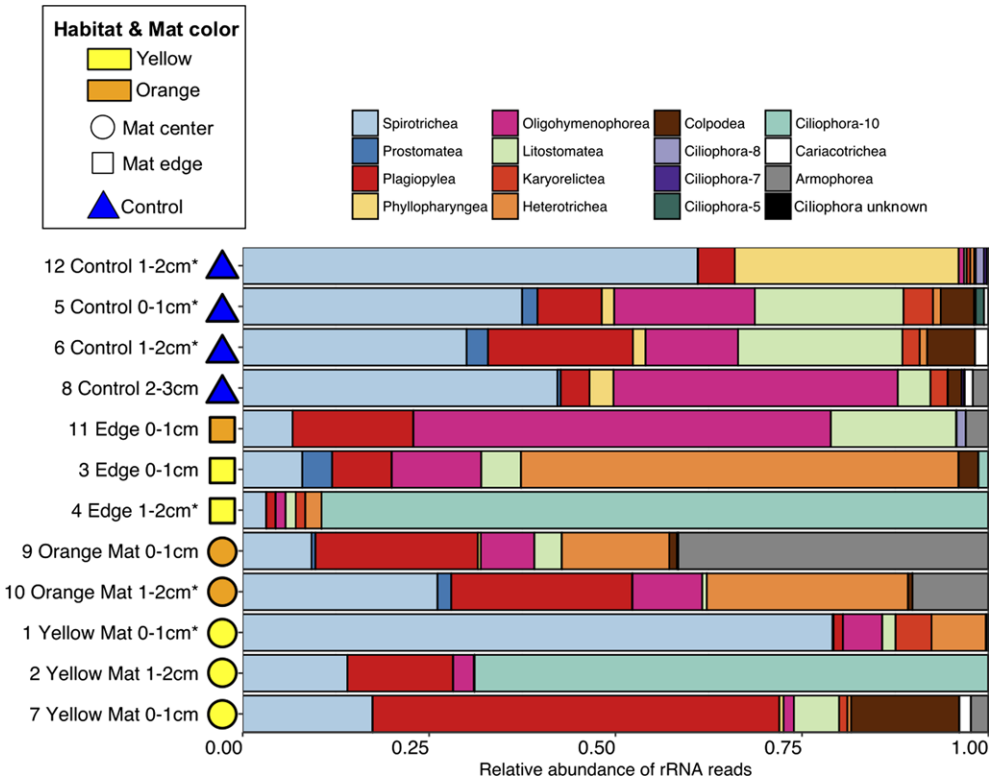

Figure 6 Overview of ciliate community composition. Relative abundance of microbial eukaryotic rRNA reads across samples collected from Guaymas Basin hydrothermal vent sediments. *Y*-axis legend color designates the color of mat sampled and shape indicates the type of habitat sampled (e.g. inside microbial mat, on the edge of a microbial mat or in adjacent cold/control mud). Asterisks (*) denote replicate samples pooled (see Table 2). The category "Ciliophora unknown" includes OTUs described no further than the ciliate taxonomic level. Further taxonomic classification of ciliates is summarized in Table S2.

determined from high-throughput tag sequencing, 32.3% of them (148 OTUs; 206,689 sequences) had a blast hit to one of the full length 18S rRNA gene sequences with a minimum sequence similarity of 97%.

DISCUSSION

Microbial eukaryotes, an assemblage typically dominated by protistan taxa, play key ecological roles in both benthic and pelagic marine ecosystems by mediating the transfer of organic matter and energy between different trophic levels (Caron et al. 2012; Edgcomb 2016; Pernice et al. 2015; Worden et al. 2015). Despite their presumed ecological importance, the taxonomic composition, species richness, and trophic activities of microbial eukaryotes in deep-sea chemosynthetic ecosystems remains poorly understood. We examined how local-scale processes may structure these communities by characterizing species richness and diversity patterns across sediment habitats at Guaymas Basin. These studies lend insight into the factors affecting community composition in these extreme habitats.

Results from this study confirmed several of the same broad patterns of microbial eukaryote diversity and composition that have been observed previously in this and other chemosynthetic ecosystems (Coyne et al. 2013; Edgcomb et al. 2002; Pasulka et al. 2016). These cross-study overarching patterns include: (i) a shift in community composition associated with sediment habitats, (ii) higher OTU richness in control (cold mud) sediments relative to warm sediments covered by microbial mats, and (iii) higher relative abundance of ciliate OTUs in microbial (*Beggiatoa*) mat sediments relative to control sediments. However, several major insights emerged from deeper sequencing of the 18S rRNA gene compared to smaller scale sequencing efforts that have been conducted in the past. We were able to obtain sequences for seven additional ciliate classes that had not previously been reported in Guaymas Basin sediments using shallower sequencing approaches. Furthermore, as might be expected, we detected higher absolute OTU richness in this study compared to previous studies in this region. Nonetheless, none of our samples reached an asymptote in our rarefaction curves (Fig. 4), suggesting deeper sequencing is still needed to fully characterize microbial eukaryote communities in these environments. Finally, as a result of deep sequencing we had the ability to characterize the patterns of OTU richness within microbial eukaryote classes, particularly within the ciliates (Fig. 7 and Fig. S2).

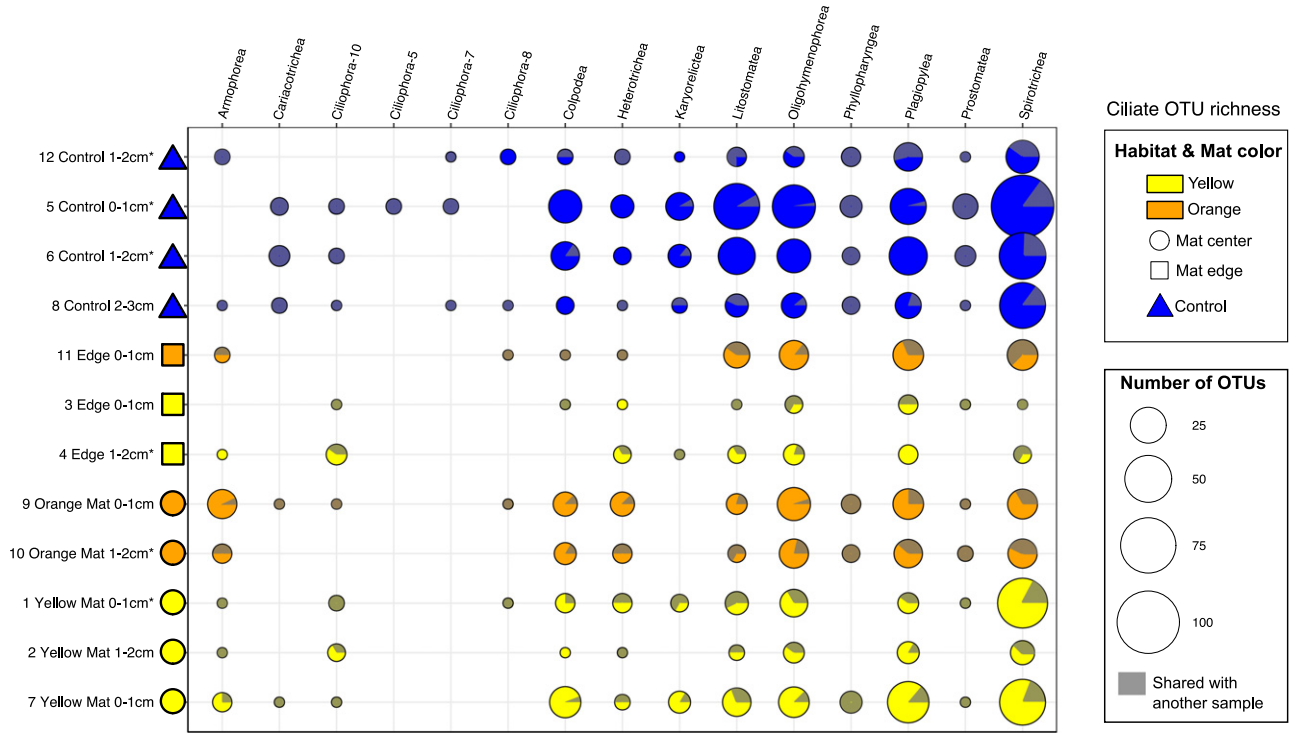

Figure 7 Ciliate OTU Richness. OTU richness (total number of OTUs) within ciliate classes across all samples; bubble size represents number of OTUs. *Y*-axis legend shape indicates the type of sample (e.g. inside microbial mat, on the edge of a microbial mat or in adjacent cold/control mud) and color designates the color of mat sampled. Shaded gray regions of each bubble (pie) denote the number of OTUs that were shared with another sample (not unique). Asterisks (*) denote replicate samples pooled (see Table 2).

Linking patterns in composition and diversity to environmental variability

The composition and diversity of microbial eukaryotes in Guaymas Basin vent sediments were correlated with local environmental conditions. Communities inhabiting mat-covered sediments were distinct from communities in control sediments, however, there was also considerable variability in the composition of microbial eukaryote communities between mats (Fig. 2, 3). Mat color has been linked to the trophic mode of *Beggiatoa* (Nikolaus et al. 2003) as well as the geochemical environment (McKay et al. 2012). While there was significant variability in the composition and diversity of microbial eukaryotes between orange and yellow mat systems, there was also variability between two mats of the same color (e.g. sample 7). Therefore, within the context of this dataset, we interpret these results to mean that the composition varies in relationship to a variety of environmental conditions (e.g. temperature and sulfide) that change on very small spatial scales across mats, not necessarily only linked to mat color. Temperature and porewater sulfide have been shown to play a key role in structuring faunal communities in chemosynthetic ecosystems (Bernardino et al. 2012; and sources within) as well as influence the distribution, composition and diversity of microbial eukaryotic communities in other types of reducing habitats (Edgcomb et al. 2002, 2011b; Orsi et al. 2011; Pasulka et al. 2016; Wylezich and Jurgens 2011). It is important to keep in mind, however, that variations in temperature and sulfide concentrations are correlated with a variety of other geochemical parameters and microbial processes within the sediments (Meyer et al. 2013 and sources within), all of which likely impact microbial eukaryote physiology and ecology. Therefore, characterizing the mechanisms responsible for the observed shifts in microbial eukaryote communities along these environmental gradients will be an important next step for understanding the ecology of microbial eukaryotes in vent ecosystems.

Examining variability in the composition and diversity of microbial eukaryotes along environmental gradients in vent sediments can also provide insight into how these communities shift over time in an ephemeral vent environment. In the last decade, with the use of molecular approaches, we have become more aware of the presence of low abundance background taxa that were likely below the detection limits for microscopy and culturing approaches in earlier studies (Caron 2009; Sogin et al. 2006). Whether or not these organisms play a role in ecosystem function is still unresolved, but it is possible that these less abundant taxa rapidly respond to changing environmental conditions and in turn perform important ecological activities (Caron and Countway 2009). Control sediments, which exhibited the highest diversity, also shared the greatest number of OTUs

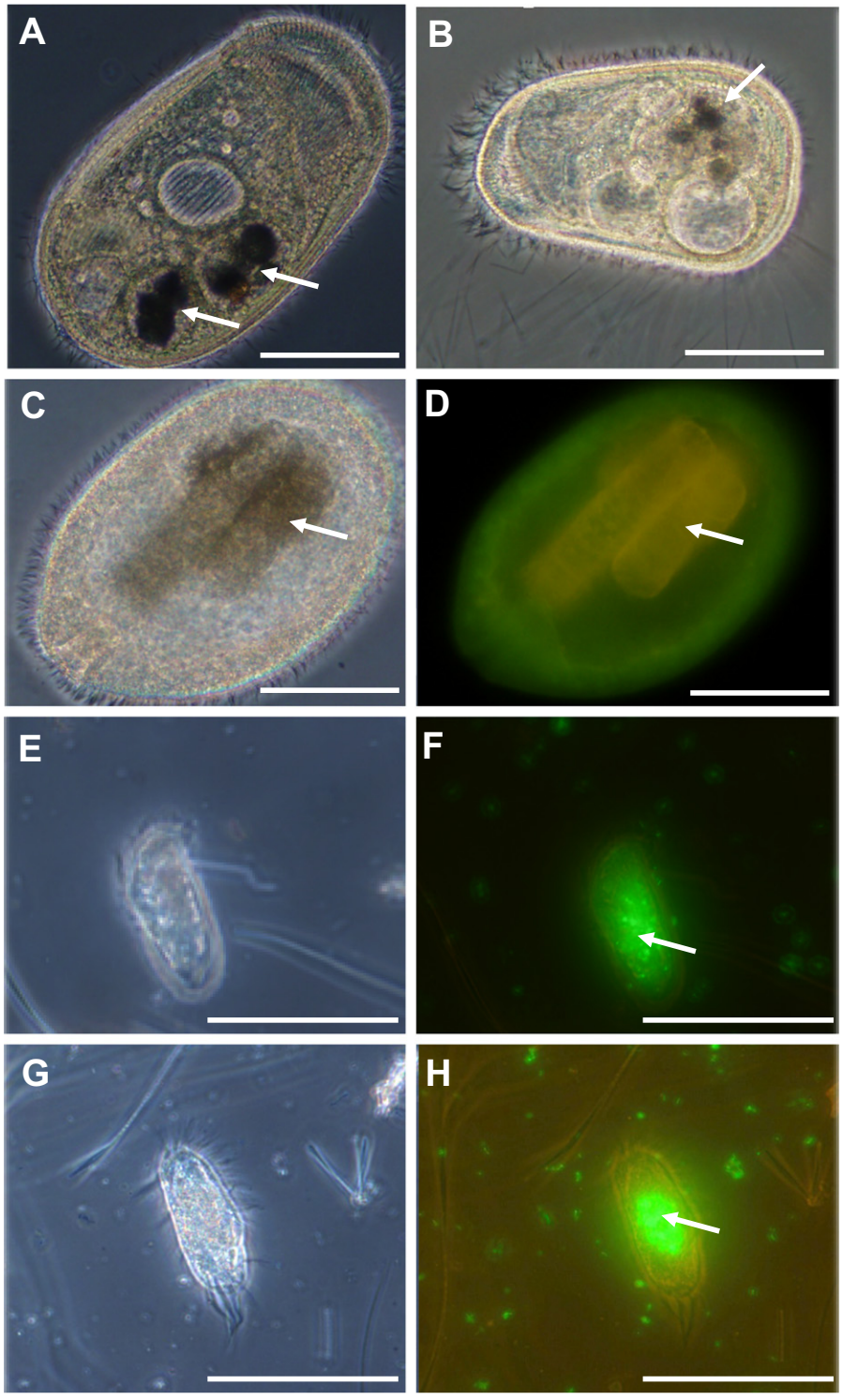

Figure 8 Transmitted light and epifluorescence micrographs of ciliates Guaymas Basin Beggiatoa mats containing ingested prey. Two large ciliates (**A, B**) containing unidentified prey in food vacuoles (arrows). A single large ciliate photographed using transmitted light (**C**) or epifluorescence microscopy (**D**) containing ingested trichomes of *Beggiatoa* (arrows). Paired transmitted light and epifluorescence microscopy (**E, F**) and (**G, H**) of two ciliates showing large numbers of ingested GFP-expressing bacterial cells (arrows). Marker bars = 100 μm (A–D) and 40 μm (E–H).

with sediments collected from the center of the mat (Fig. 5A). Furthermore, we found OTUs belonging to the Ciliophora-10, Cariacotrichea and Armphorea in the control sample which are all groups of ciliates known to be seep and/or anoxic habitat specific (Orsi et al. 2011, 2012; Lynn 2008; Takishita et al. 2010). These OTUs were present at a lower relative abundance in the control samples compared to the mat samples (e.g. rare taxa) and there were no unique OTUs within these classes in the control sediments (e.g. all OTUs were shared with mat habitats, Fig. 7). Taken together, these patterns may indicate that the microbial eukaryote community in the control sediments include community members capable of responding to variations in venting and seepage activity. Experiments in a deep-sea methane seep by Case et al. (2015) found that many bacterial and archaeal community members associated with carbonate rocks in active or inactive seepage areas persisted for up to 13 months after transplantation to a different activity level (i.e. inactive or active site, respectively). If microbial eukaryotes are also able to persist in sediments as levels of chemosynthetic activity shift due to variability in diffuse venting, this could explain the high OTU richness and co-existence of 'background' microbial eukaryotes and "chemosynthetic specialists" in the control sediments. Additionally, the overall reduced species richness (but high relative abundances of chemosynthetic specialists) within mat sediments may indicate that fewer "background" species can persist in the extreme environments of the mats (higher temperatures and concentrations of hydrogen sulfide). This pattern of low diversity, high abundance observed in the microbial mat sediments is consistent with the diversity and distribution patterns of metazoans within chemosynthetic habitats (Dando et al. 1994; Levin 2005; Sahling et al. 2002).

The edge of the mat-covered sediments exhibited the lowest diversity of all three habitats (Fig. 4). This pattern suggests that the edge region may provide the most challenging conditions for microbial eukaryotes because biogeochemical conditions fluctuate more rapidly over time (i.e. high frequency of disturbance), and therefore requires the organisms to quickly adapt to changing conditions. Disturbance is often thought to promote the cohabitation of ecologically different microorganisms (Fajemila et al. 2015; Galand et al. 2016). However, too great a frequency or intensity of disturbance is thought to result in a loss of diversity (Kim et al. 2013; Sommer 1995). In addition to physical and/or chemical disturbance, the edge of a mat may expose microbial eukaryote communities to different predators and/or food availability, both of which may impact their diversity and composition.

Fungi are recognized as important members of deep-sea ecosystems including but not limited to chemosynthetic habitats and the deep biosphere (Calvez et al. 2009; Edgcomb et al. 2011a; Pachiadaki et al. 2016). We found OTUs belonging to the Ascomycota, Chytridiomycota, Cryptomycota, Basidiomycota, as well as unassigned groups of fungi. Fungal sequences only made up a small fraction of the total community across all samples (0.5–2.5% of the sequence total) and there was no clear pattern in fungal diversity or relative abundance with habitat type. However, since the V4 region is not effective in capturing fungal diversity, especially compared to the ITS region (Nilsson et al. 2018), we can only be confident about the presence of the groups listed above, but not make inferences about their importance or biogeography in these ecosystems.

Diversity and trophic activity of ciliates

Ciliates are an important group of microbial eukaryotes known to consume bacteria and other protistan organisms. Characterizing their diversity and distributions is a first step toward understanding their ecological roles in difficult to access deep-sea benthic ecosystems. We recovered sequences from 11 of the 15 now described ciliate classes (Gao et al. 2016). Interestingly, four of the five most abundant and diverse classes observed in the Guaymas Basin vent sediments (Colpodea, Litostomatea, Oligohymenophorea, Spirotrichea, and Plagiopylea) are the same classes that have been described as abundant and diverse in the sunlit surface of the oceans (i.e. Tara Oceans Voyage; Gimmler et al. 2016). The only exception is the Plagiopylea. This discrepancy is consistent with the lifestyle of Plagiopyleans, which are thought to live in or above sulfide-containing sediment and feed predominantly on sulfur-oxidizing bacteria (Fenchel 1968; Lynn 2008; Schulz et al. 1990).

Oligohymenophorea and Spirotrichea, which have been described to be the most diverse assemblages within the ciliates (Lynn 2008), accounted for almost half (46% of the ciliate OTUs) of the total ciliate diversity in Guaymas vent sediments (Fig. 6). On average, about 25% of the OTUs within these classes were shared between habitats, suggesting that 75% were unique to each habitat (Fig. 5B). In contrast, OTUs within the classes Prostomatea and Phyllopharyngea were shared across all samples, with no habitat containing unique OTUs. The Prostomatea, along with the Plagiopylea, are thought to have the fewest number of species and genera among the ciliate classes (Lynn 2008). However, despite their lower diversity, prostomes have the ability to reach high abundances and become important grazers in the water column (Lynn 2008 and sources within). The Phyllopharyngea are a diverse class overall, however, sequences in the mats within this class were mainly affiliated with one subclass (Suctoria). The stalked body plan of this group is adapted to a sessile lifestyle, and they can either be attached to nonliving particulate material or ectosymbiotic (Lynn 2008). The distribution and OTU richness patterns of ciliates within the Prostomatea and Phyllopharyngea indicate that they are present in the sediments, but not necessarily strongly associated with seep environments.

More than half of the ciliate OTUs (52%) shared < 95% sequence similarity to sequences in the reference database. This value increased to 78% of the ciliate OTUs when we considered sequences that shared < 97% sequence similarity to the reference database. This finding suggests that deep-sea vent ecosystem sediments harbor

novel ciliate diversity that has yet to be described, or at least taxonomically resolved. Combining our short-read sequences with full-length 18S sequences from single ciliates enabled us to better resolve the taxonomic positions of a subset of our sequences (32% of the sequences, covering 5 classes of ciliates; Fig. S3). Furthermore, the ciliates for our full-length sequencing were selected from subsets of the same samples in which grazing experiments were performed, therefore, they likely represent active grazers in the sediments. Future work in these environments should pursue this combined approach so that more sequences can be interpreted with higher taxonomic resolution and related to common and active members of the community. This approach also addresses a major challenge with sequencing approaches in the deep-sea—the ability to differentiate between organisms that are trophically active and contribute to vent system food webs vs. DNA that may not be autochthonous to the environment (Pawlowski et al. 2011). Microscopy-based examination of sediment samples and isolation of live ciliates for single-cell sequencing (and grazing studies) conducted in the present study enabled us to make the link between a subset of sequences in our dataset and living organisms.

Our preliminary, but unique observations of active ciliate grazing on GFP-expressing bacteria and intact *Beggiatoa* trichomes, provided the first direct evidence of protists from deep-sea hydrothermal vents actively grazing on free-living bacteria and demonstrated a highly active cilate assemblage in the *Beggiatoa* mats (Fig. 8). Ciliates have been shown to play a role in the succession of shallow-water microbial mats through selective grazing, but the consumption of filamentous forms of bacteria has not been observed (Bernard and Fenchel 1995). The short-term grazing incubations were conducted at relevant deep-sea pressures (e.g. 200 atm.) using ciliates that were transported to the surface in an Alvin 'bio-box'. The depressurized ciliates were observed by microscopy at ambient lab temperature (~22 °C) prior to the start of the grazing experiment and were active. This active response by the vent ciliates in the mat community provided evidence that depressurization was not overly detrimental. Nevertheless, we re-pressurized our samples to ambient vent pressure to simulate the deep-sea hydrothermal vent ecosystem as closely as possible during the grazing experiment. The observation of ingested GFP-expressing bacterial prey within a high percentage of ciliates at the end of the experiment suggests that the pressure changes experienced by these grazers had minimal impact on their activities or physiology over the time-course of collection, transport, and experimentation. However, some ciliates appeared to have incorporated just a few GFP cells, others were clearly packed with GFP bacteria in food vacuoles (Fig. 8E–H).

CONCLUSIONS AND FUTURE DIRECTIONS

We are only beginning to understand the influence of microbial eukaryote diversity on ecosystem function in most marine ecosystems (Caron et al. 2012). Observational studies of microbial eukaryote assemblages in response to changing environmental conditions across a complex mosaic of habitats (mat, edge, and background sediments) are a key step toward understanding the ecological functions of microbial eukaryotes in deep-sea ecosystems. As revealed by our study, the Guaymas Basin hydrothermal vent systems are a reservoir of many as yet undescribed microbial eukaryotes. Local chemosynthetic activity influenced the relative abundances and diversity patterns of the entire community as well as the trophically active ciliate community. While our understanding of the role of microbial eukaryotes in deep-sea ecosystem function remains limited, it is becoming even more critical to integrate them into our understanding of chemosynthetic ecosystems, because these once untouched habitats are now being exploited for their resources (Thurber et al. 2014). One of the next challenges is to link patterns in composition and OTU richness to functional richness and ecosystem resilience. Does higher OTU richness provide functional redundancy or do new taxa provide new functions to an ecosystem? This type of information will eventually enable us to link community changes to function and prediction of how deep-sea ecosystem function may change in response to short-term environmental fluctuations, or long-term climate change (Levin and Bris 2015; Sweetman et al. 2017). Linking microbial eukaryotic biodiversity to ecology through culturing and additional paired molecular functional surveys will provide a deeper understanding of their influence on ecosystem function and causes for our observed community shifts.

LITERATURE CITED

Alexander, E., Stock, A., Breiner, H. W., Behnke, A., Bunge, J., Yakimov, M. M. & Stoeck, T. 2008. Microbial eukaryotes in the hypersaline anoxic L'Atalante deep-sea basin. *Environ. Microbiol.*, 11:360–381.

Arndt, H., Hausmann, K. & Wolf, M. 2003. Deep-sea heterotrophic nanoflagellates of the Eastern Mediterranean Sea: qualitative and quantitative aspects of their pelagic and benthic occurrence. *Mar. Ecol. Prog. Ser.*, 256:45–56.

Atkins, M. S., Teske, A. P. & Anderson, O. R. 2000. A survey of flagellate diversity at four deep-sea hydrothermal vents in the Eastern Pacific Ocean using structural and molecular approaches. *J. Eukaryot. Microbiol.*, 47:400–411.

Benson, D. A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J. & Sayers, E. W. 2013. GenBank. *Nucleic Acids Res.*, 41:D36–D42.

Bernard, C. & Fenchel, T. 1995. Mats of colourless sulphur bacteria. II. Structure, composition of biota and successional patterns. *Mar. Ecol. Prog. Ser.*, 128:171–179.

Bernardino, A. F., Levin, L. A., Thurber, A. R. & Smith, C. R. 2012. Comparative composition, diversity and trophic ecology of sediment macrofaunal at vents, seeps and organic falls. *PLoS ONE*, 4:e33515.

Berry, D., Mahfoudh, K. B., Wagner, M. & Loy, A. 2011. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl. Environ. Microbiol.*, 77:7846–7849.

Boscaro, V., Santoferrara, L. F., Zhang, Q., Gentekaki, E., Syberg-Olsen, M. J., del Campo, J. & Keeling, P. J. 2018. EukRef-Ciliophora: a manually curated, phylogeny-based database of small

subunit rRNA gene sequences of ciliates. *Environ. Microbiol.*, 20:2218–2230.

Calvez, T. L., Burgaud, G., Mahe, S., Barbier, G. & Vandenkoornhuyse, P. 2009. Fungal diversity in deep-sea hydrothermal ecosystems. *Appl. Environ. Microbiol.*, 75:6415–6421.

Campbell, B. J., Polson, S. W., Allen, L. Z., Williamson, S. J., Lee, C. K., Wommack, K. E. & Cary, S. C. 2013. Diffuse flow environments within basalt- and sediment based hydrothermal vent communities harbor specialized microbial communities. *Front. Microbiol.*, 4:182.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J. & Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, 7:335–336.

Caron, D. A. 2009. New accomplishments and approaches for assessing protistan diversity and ecology in natural ecosystems. *Bioscience*, 59:287–299.

Caron, D. A. & Countway, P. D. 2009. Hypotheses on the role of protistan rare biosphere in a changing world. *Aquat. Microb. Ecol.*, 57:227–238.

Caron, D. A., Countway, P. D., Jones, A. C., Kim, D. Y. & Schnetzer, A. 2012. Marine protistan diversity. *Annu. Rev. Mar. Sci.*, 4:467–493.

Case, D. H., Pasulka, A. L., Marlow, J. J., Grupe, B. M., Levin, L. A. & Orphan, V. J. 2015. Methane seep carbonates host distinct, diverse, and dynamic microbial assemblages. *MBio*, 6: e010348-15.

Countway, P. D., Gast, R. J., Dennett, M. R., Savai, P., Rose, J. M. & Caron, D. A. 2007. Distinct protistan assemblages characterize the euphotic zone and deep sea (2500 m) of the western North Atlantic (Sargasso Sea and Gulf Stream). *Environ. Microbiol.*, 9:1219–1232.

Countway, P. D., Vigil, P. D., Schnetzer, A., Moorthi, S. D. & Caron, D. A. 2010. Seasonal analysis of protistan community structure and diversity at the USC microbial observatory (San Pedro Channel, North Pacific Ocean). *Limnol. Oceanogr.*, 55:2381–2396.

Coyne, K. J., Countway, P. D., Pilditch, C. A., Lee, C. K., Caron, D. A. & Cary, S. C. 2013. Diversity and distributional patterns of ciliates in Guaymas Basin hydrothermal vent sediments. *J. Eukaryot. Microbiol.*, 60:433–447.

Dando, P. R., Bussmann, I., Niven, S. J., O'Hara, S. C. M., Schmaljohann, R. & Taylor, L. J. 1994. A methane seep area in the Skagerrak, the habitat of the pogonophore *Siboglinum poseidoni* and the bivalve mollusc *Thyasira sarsi*. *Mar. Ecol. Prog. Ser.*, 107:157–167.

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., Lara, E., Berney, C., Le Bescot, N., Probert, I., Carmichael, M., Poulain, J., Romac, S., Colin, S., Aury, J.-M., Bittner, L., Chaffron, S., Dunthorn, M., Engelen, S., Flegontova, O., Guidi, L., Horák, A., Jaillon, O., Lima-Mendez, G., Lukeš, J., Malviya, S., Morard, R., Mulot, M., Scalco, E., Siano, R., Vincent, F., Zingone, A., Dimier, C., Picheral, M., Searson, S., Kandels-Lewis, S., Acinas, S. G., Bork, P., Bowler, C., Gorsky, G., Grimsley, N., Hingamp, P., Iudicone, D., Not, F., Ogata, H., Pesant, S., Raes, J., Sieracki, M. E., Speich, S., Stemmann, L., Sunagawa, S., Weissenbach, J., Wincker, P. & Karsenti, E. 2015. Eukaryotic plankton diversity in the sunlit ocean. *Science*, 348:1261605.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.*, 32:1792–1797.

Edgcomb, V. P. 2016. Marine protist associations and environmental impacts across trophic levels in the twilight zone and below. *Curr. Opin. Microbiol.*, 31:169–175.

Edgcomb, V. P., Beaudoin, D., Gast, R., Biddle, J. F. & Teske, A. 2011a. Marine subsurface eukaryotes: the fungal majority. *Environ. Microbiol.*, 13:172–183.

Edgcomb, V. P., Kysela, D. T., Teske, A., de Vera Gomez, A. & Sogin, M. L. 2002. Benthic eukaryotic diversity in the Guaymas Basin hydrothermal vent environment. *Proc. Natl Acad. Sci. USA*, 99:7658–7662.

Edgcomb, V. P., Orsi, W., Bunge, J., Jeon, S., Christen, R., Leslin, C., Holder, M., Taylor, G. T., Suarez, P., Varela, R. & Epstein, S. 2011b. Protistan microbial observatory in the Cariaco Basin, Caribbean. I. Pyrosequencing vs Sanger insights into species richness. *ISME J.*, 5:1344–1356.

Fajemila, O. T., Langer, M. R. & Lipps, J. H. 2015. Spatial patterns in the distribution, diversity and abundance of benthic foraminifera around Moorea (Society Archipelago, French Polynesia. *PLoS ONE*, 10:e0145752.

Fenchel, T. 1968. The ecology of marine microbenthos II. The food of marine benthic ciliates. *Ophelia*, 5:73–121.

Galand, P. E., Lucas, S., Fagervold, S. K., Peru, E., Pruski, A. M., Vetion, G., Dupuy, C. & Guizien, K. 2016. Disturbance increases microbial community diversity and production in marine sediments. *Front. Microbiol.*, 7:1950.

Gao, F., Warren, A., Zhang, Q., Gong, J., Miao, M., Sun, P., Xu, D., Huang, J., Yi, Z. & Song, W. 2016. The all-data-based evolutionary hypothesis of ciliated protists with a revise classification of the phylum Ciliophora (Eukaryota, Alveolata). *Sci. Rep.*, 6:24874.

Gimmler, A., Korn, R., de Vargas, C., Audi, S. & Stoeck, T. 2016. The Tara Oceans voyage reveals global diversity and distribution patterns of marine planktonic ciliates. *Sci. Rep.*, 6:33555.

Grattepanche, J.-D., Santoferrara, L. F., McManus, G. B. & Katz, L. A. 2014. Diversity of diversity: conceptual and methodological differences in biodiversity estimates of eukaryotic microbes as compared to bacteria. *Trends Microbiol.*, 22:432–437.

Gruber, D. F., Tuorto, S. & Taghon, G. L. 2009. Growth phase and elemental stoichiometry of bacterial prey influences ciliate grazing selectivity. *J. Eukaryot. Microbiol.*, 56:466–471.

Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C., Burgaud, G., de Vargas, C., Decelle, J., del Campo, J., Dolan, J. R., Dunthorn, M., Edvardsen, B., Holzmann, M., Kooistra, W. H. C. F., Lara, E., Le Bescot, N., Logares, R., Mahe, F., Massana, R., Montresor, M., Morard, R., Not, F., Pawlowski, J., Probert, I., Sauvadet, A.-L., Siano, R., Stoeck, T., Vaulot, D., Zimmermann, P. & Christen, R. 2013. The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.*, 41:597–604.

Hu, S. K., Liu, Z., Lie, A. A. Y., Countway, P. D., Kim, D. Y., Jones, A. C., Gast, R. J., Cary, C., Sherr, E. B., Sherr, B. F. & Caron, D. A. 2015. Estimating protistan diversity using high-throughput sequencing. *J. Eukaryot. Microbiol.*, 62:688–693.

Huelsenbeck, J. P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17:754–755.

Jannasch, H. W., Nelson, D. C. & Wirsen, C. O. 1989. Massive natural occurrence of unusually large bacteria (*Beggiatoa* sp.) at a hydrothermal deep-sea vent site. *Nature*, 342:834–836.

Kim, M., Heo, E., Kang, H. & Adams, J. 2013. Changes in soil bacterial community structure with increasing disturbance frequency. *Microb. Ecol.*, 66:171–181.

Levin, L. A. 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. *Oceanogr. Mar. Biol. Annu. Rev.*, 43:1–46.

Levin, L. A. & Bris, N. L. 2015. The deep ocean under climate change. *Science*, 350:766–768.

Lichtschlag, A., Felden, J., Brüchert, V., Boetius, A. & De Beer, D. 2010. Geochemical processes and chemosynthetic primary production in different thiotrophic mats of the Håkon Mosby mud volcano (Barents Sea). *Limnol. Oceanogr.*, 55:931–949.

Lloyd, K. G., Albert, D. B., Biddle, J. F., Chanton, J. P., Pizarro, O. & Teske, A. 2010. Spatial structure and activity of sedimentary microbial communities underlying a Beggiatoa spp. mat in a Gulf of Mexico hydrocarbon seep. *PLoS ONE*, 5:e8738.

López-García, P., Philippe, H., Gaill, F. & Moreira, D. 2003. Autochthonous eukaryotic diversity in hydrothermal sediment and experimental micro-colonizers at the Mid-Atlantic Ridge. *Proc. Natl Acad. Sci. USA*, 100:697–702.

López-García, P., Vereshchaka, A. & Moreira, D. 2007. Eukaryotic diversity associated with carbonates and fluid- seawater interface in Lost City hydrothermal field. *Environ. Microbiol.*, 9:546–554.

Lynn, D. H. 2008. The ciliated protozoa: characterization, classification and guide to literature. Springer Science, Dordrecht.

Mahe, F., de Vargas, C., Bass, D., Czech, L., Stamatakis, A., Lara, E., Singer, D., Mayor, J., Bunge, J., Sernaker, S., Siemensmeyer, T., Trautmann, I., Romac, S., Berney, C., Kozlov, A., Mitchell, E. A. D., Seppey, C. V. W., Egge, E., Lentendu, G., Wirth, R., Trueba, G. & Dunthorn, M. 2017. Parasites dominate hyperdiverse soil protist communities in neotropical rainforests. *Nat. Ecol. Evol.*, 1:0091.

McKay, L. J., MacGregor, B. J., Biddle, J. F., Albert, D. B., Mendlovitz, H. P., Hoer, D. R., Lipp, J. S., Lloyd, K. G. & Teske, A. P. 2012. Spatial heterogeneity and underlying geochemistry of phylogenetically diverse orange and white Beggiatoa mats in Guaymas Basin hydrothermal sediments. *Deep Sea Res. Part I*, 67:21–31.

Medlin, L. K., Elwood, H. J., Stickel, S. & Sogin, M. L. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene*, 71:491–499.

Meyer, S., Wegener, G., Lloyd, K. G., Teske, A., Boetius, A. & Ramette, A. 2013. Microbial habitat connectivity across spatial scales and hydrothermal temperature gradients at Guaymas Basin. *Front. Microbiol.*, 4:207.

Moreira, D. & López-García, P. 2003. Are hydrothermal vents oases for parasitic protists? *Trends Parasitol.*, 19:556–558.

Nelson, D. C., Wirsen, C. O. & Jannasch, H. W. 1989. Characterization of large, autotrophic Beggiatoa spp. abundant at hydrothermal vents of the Guaymas Basin. *Appl. Environ. Microbiol.*, 55:2909–2917.

Nikolaus, R., Ammerman, J. W. & MacDonald, I. R. 2003. Distinct pigmentation and trophic modes in *Beggiatoa* from hydrocarbon seeps in the Gulf of Mexico. *Aquat. Microb. Ecol.*, 32:85–93.

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P. & Tedersoo, L. 2018. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nat. Rev. Microbiol.*, 1–15, https://doi.org/10.1038/s41579-018-0116-y

Orsi, W., Edgcomb, V., Faria, J., Foissner, W., Fowle, W. H., Hohmann, T., Suarez, P., Taylor, C., Taylor, G. T., Vdacny, P. & Epstein, S. S. 2012. Class Cariacotrichea, a novel ciliate taxon from the anoxic Cariaco Basin, Venezuela. *Int. J. Syst. Evol. Microbiol.*, 62:1425–1433.

Orsi, W., Edgcomb, V., Jeon, S., Leslin, C., Bunge, J., Taylor, G. T., Varela, R. & Epstein, S. 2011. Protistan microbial observatory in the Cariaco Basin, Caribbean. II. Habitat specialization. *ISME J.*, 5:1367–1373.

Pachiadaki, M. G., Redou, V., Beaudoin, D. J., Burgaud, G. & Edgcomb, V. P. 2016. Fungal and prokaryotic activities in the marine subsurface biosphere at Peru Margin and Canterbury Basin inferred from RNA-based analyses and microscopy. *Front. Microbiol.*, 7, https://doi.org/10.3389/fmicb.2016.00846

Pasulka, A. L., Levin, L. A., Steele, J. A., Case, D. H., Landry, M. R. & Orphan, V. J. 2016. Microbial eukaryotic distributions and diversity patterns in a deep-sea methane seep ecosystem. *Environ. Microbiol.*, 18:3022–3043.

Pawlowski, J., Christen, R., Lecroq, B., Bachar, D., Shahbazkia, H. R., Amaral-Zettler, L. & Guillou, L. 2011. Eukaryotic richness in the abyss: insights from pyrotag sequencing. *PLoS ONE*, 6:e18169.

Pernice, M. C., Giner, C. R., Logares, R., Perera-Bel, J., Acinas, S. G., Duarte, C. M., Gasol, J. M. & Massana, R. 2015. Large variability of bathypelagic microbial eukaryotic communities across the world's oceans. *ISME J.*, 10:945–958.

R Development Core Team 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Rodríguez-Martínez, R., Rocap, G., Logares, R., Romac, S. & Massana, R. 2012. Low evolutionary diversification in a widespread and abundant uncultured protist (MAST-4). *Mol. Biol. Evol.*, 29:1393–1406.

Rognes, T., Flouri, T., Nichols, B., Quince, C. & Mahé, F. 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ*, 4:e2584-22.

Sahling, H., Rickert, D., Lee, R. W., Linke, P. & Suess, E. 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Mar. Ecol. Prog. Ser.*, 231:121–138.

Santoferrara, L. F., Grattenpach, J.-D., Katz, L. A. & McManus, G. B. 2014. Pyrosequencing for assessing the diversity of eukaryotic microbes: analysis of data on marine planktonic ciliates and comparison with traditional methods. *Environ. Microbiol.*, 16:2752–2763.

Schulz, S., Wagener, S. & Pfennig, N. 1990. Utilization of various chemotrophic and phototrophic bacteria as food by the anaerobic ciliate Trimyema compressum. *Eur. J. Protistol.*, 26:122–131.

Small, E. G. & Gross, M. E. 1985. Preliminary observations of protistan organisms, especially ciliates, from the 21°N hydrothermal vent site. *Biol. Bull.*, 6:401–410.

Sogin, M. L., Morrison, H. G., Huber, J. A., Welch, D. M., Huse, S. M., Neal, P. R., Arrieta, J. M. & Herndl, G. J. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proc. Natl Acad. Sci. USA*, 103:12115–12120.

Sommer, U. 1995. An experimental test of the intermediate disturbance hypothesis using cultures of marine phytoplankton. *Limnol. Oceanogr.*, 40:1271–1277.

Stoeck, T., Bass, D., Neble, M., Christen, R., Jobes, M. D. M., Breiner, H.-W. & Richards, T. A. 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.*, 19:21–31.

Sweetman, A. K., Thurber, A. R., Smith, C. R., Levin, L. A., Mora, C., Wei, C. L., Gooday, A. J., Jones, D. O. B., Rex, M., Yasuhara, M., Ingels, J., Ruhl, H. A., Frieder, C. A., Danovaro, R.,

Würzberg, L., Baco, A., Grupe, B., Pasulka, A. L., Meyer, K. M., Dunlop, K. M., Henry, L. A. & Roberts, J. M. 2017. Major impacts of climate change on deep-sea benthic ecosystems. *Elem. Sci. Anth.*, 5:4.

Takishita, K., Kakizoe, N., Yoshida, T. & Maruyama, T. 2010. Molecular evidence that phylogenetically diverged ciliates are active in microbial mats of deep-sea cold-seep sediment. *J. Eukaryot. Microbiol.*, 57:76–86.

Teske, A., de Beer, D., McKay, L. J., Tivey, M. K., Biddle, J. F., Howe, D., Lloyd, K. G., Lever, M. A., Roy, H., Albert, D. B., Mendlovitz, H. P. & MacGregor, B. J. 2016. The Guaymas Basin hiking guide to hydrothermal mounds, chimneys, and microbial mats: complex seafloor expressions of subsurface hydrothermal circulation. *Front. Microbiol.*, 7:75.

Thurber, A. R., Sweetman, A. K., Narayanaswamy, B. E., Jones, D. O. B., Ingels, J. & Hansman, R. L. 2014. Ecosystem function and services provided by the deep sea. *Biogeosciences*, 11:3941–3963.

Van Gaever, S., Moodley, L., De Beer, D. & Vanreusel, A. 2006. Meiobenthos at the arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulfide-rich sediments. *Mar. Ecol. Prog. Ser.*, 321:143–155.

Weekers, P. H. H., Gast, R. J., Fuerst, P. A. & Byers, T. J. 1994. Sequence variations in small-subunit ribosomal-RNAs of *Hartmannella vermiformis* and their phylogenetic implications. *Mol. Biol. Evol.*, 11:684–690.

Werne, J. P., Bass, M. & Damste, J. S. S. 2002. Molecular isotopic tracing of carbon flow and trophic relationships in a methane-supported benthic microbial community. *Limnol. Oceanogr.*, 47:1694–1701.

Wirsen, C. O. & Jannasch, H. W. 1975. Activity of marine psychorophilic bacteria at elevated hydrostatic pressures and low-temperatures. *Mar. Biol.*, 31:201–208.

Worden, A. Z., Follows, M. J., Giovannoni, S. J., Wilken, S., Zimmerman, A. E. & Keeling, P. J. 2015. Rethinking the marine carbon cycle: factoring in the multifarious lifestyles of microbes. *Science*, 347:6223.

Wylezich, C. & Jurgens, K. 2011. Protist diversity in suboxic and sulfidic waters of the Black Sea. *Environ. Microbiol.*, 13:2939–2956.

Xu, W., Gong, L.-F., Pang, K. & Luo, Z. 2018. Fungal diversity in deep-sea sediments of a hydrothermal vent system in the Southwest Indian Ridge. *Deep Sea Res. Part I*, 131:16–26.

[1]Present address: Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, USA

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Visualization of OTU statistics across all samples.
Figure S2. Overview of microbial eukaryote OTU richness.
Figure S3. Bayesian phylogenetic tree of 18S rRNA gene sequences across 1,600 aligned nucleotide positions.
Figure S4. Relative abundance of ciliate, Apicomplexa, and Rhizaria sequences at the class level across all samples (number of sequences in each class were summed).
Table S1. Raw sequence OTU table.
Table S2. Ciliate sequence summary.