Phoronids and their tubes harbor distinct microbiomes compared to surrounding sediment

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Keywords: phoronids, *Phoronopsis harmeri*, tube, sediment, microbiome, sulfur-cycling, Campylobacterales, Desulfobulbales, Desulfobacterales, metagenomics

Abstract

Phoronids are a phylum of animals with only ~12 described species, all of which are marine filter feeders that build external tubes for shelter and produce chemical deterrents against predators. Many tube-building invertebrates host distinct microbial communities and even have obligate symbionts for survival in sulfur-rich marine sediments. However, the microbiome of phoronids has yet to be comprehensively described. To address this, we surveyed the composition of the microbiome of the phoronid, *Phoronopsis harmeri*, using 16S rRNA gene amplicon and metagenomic sequencing. We found that the phoronid microbiome was dominated by members of the orders Campylobacterales, Desulfobulbales, and Desulfobacterales. We also found that the microbiomes of tubes and phoronids were less diverse than that of surrounding sediment, and that the microbiomes of phoronids, tubes and surrounding sediment were all distinctly structured. Based on analysis of metagenomic data, and even though we were only able to recover low quality MAGs of abundant taxa, we found preliminary evidence that taxa associated with phoronids and their tubes likely participate in sulfur cycling pathways. Future work should perform more robust metagenomic sequencing and chemical analysis to assess if there is a link between known phoronid chemical defenses and microorganisms. Overall, this study provides foundational insight into the microbial communities associated with phoronids and these initial findings suggest that these communities may play an important role in sulfur cycling in marine sediments.

Introduction

Phoronopsis harmeri [1], previously known as *P. viridis* [2], is a phoronid found in several small bays in central California, including Bodega Harbor. Phoronids are lophophorate organisms, aquatic invertebrates that have a specialized feeding structure made of tentacles, that live in marine sediment and build external tubes for shelter. Thus, phoronids are like other marine worm-like organisms that serve as ecosystem engineers through creation of dwellings such as tubes, mounds or other structures that alter sediment stability, enhance solute exchange and thus create distinct local environments [3–16]. *P. harmeri* is a digging phoronid and has an organic tube consisting of particles of the surrounding sediment [17]. These tubes consist of an inner organic cylinder made of thin films whose fibers form a thick net and an external layer [18]. Phoronid tubes can be 1 to 3 mm in diameter and up to 20 cm long, about twice the length of the phoronid [18,19]. While typically found inside their tubes, phoronids are not physically attached to their tubes, and in order to filter feed, they extend their green colored lophophores above the sediment [14,20,21]. Phoronid tubes can occur at very high densities and can lead to increased diversity of infaunal community members [19].

Studies of other marine ecosystem engineers, including polychaetes (e.g., *Escarpia* sp. [22], *Diopatra* sp. [23], *Neanthes* sp. [24]), tube worms (e.g., *Riftia* sp. [25], *Lamellibrachia* sp. [26]) and shrimp (e.g., *Upogebia* sp. [27]), have described important roles for the microbiome in host-environment interactions including metal resistance and sulfur cycling [28–30]. Often the microenvironment of the tube or burrow created by these marine organisms is found to have a distinct microbiome from surrounding sediments [22,23,28,31]. For example, blue mud shrimp burrows [27] introduce oxygen into local sediment which supports biogeochemical zonation around the burrow and may promote growth of sulfur cycling microbes. Free-living sulfur oxidizers and reducers have been reported to be enriched in association with marine ecosystem

engineers and their respective structures [27,31–34]. Bivalves in particular form tight symbiotic associations with sulfur oxidizing bacteria which provide the bivalve with primary production products, like sugars, in exchange for hydrogen sulfide or oxygen [35,36]. It has been suggested that the tube of phoronids may serve as a physical barrier against hydrogen sulfide and allow for a similar zone of oxygenation [37], and while bacteria have recently been observed to line the inside of phoronid tubes with microscopy [18], to date, no symbiotic or free-living sulfur cycling microbes have been confirmed to associate with phoronids.

In addition to tube-building, the phoronid, *P. harmeri,* is known to produce a chemical deterrent that is unpalatable to predators [20]. While all parts of the phoronid exhibit some predator deterrence, this feature is most concentrated in its green lophophore [20]. In *P. harmeri,* the chemical compound responsible has not yet been successfully isolated, likely due to high volatility or reactivity with air [20]. While many other structure-forming infaunal organisms also produce secondary metabolites to deter predators, historically few efforts have successfully isolated the responsible compounds [38–45] . Recent studies of other marine organisms (e.g., sea slugs, sponges) have attributed the origin of their chemical defense to symbiotic microbes [46,47]. Microscopic anatomy studies of phoronids have visualized bacteria inhabiting the lophophore organs of adults [48,49], leading us to speculate on whether there is a role for microbes in the production of the chemical defenses of phoronids and other structure-forming infaunal organisms.

In this foundational study, we use sequencing and analysis of 16S rRNA gene amplicons and metagenomes to achieve the following goals (i) survey the overall microbiome composition of the phoronid, *P. harmeri,* (ii) assess whether the microbiome differs between phoronids, tubes and surrounding sediment, and (iii) generate metagenome-assembled genomes (MAGs) in order to hypothesize functional roles for the dominant microbial associates of phoronids.

Methods

Sample Collection

Marine invertebrates were sampled as accidental bycatch during a previous study of the mycobiome of the seagrass, *Zostera marina* [50]. In that study, individual *Z. marina* and adjacent sediment, as well as unvegetated sediment, were cored using a modified 2.375-inch diameter PVC pipe. In total, three vegetated cores and one unvegetated core contained phoronids as bycatch. All four of these cores were collected from one site in Bodega Bay, CA, Westside Point (GPS: 38°19′10.67″N, 123° 3′13.71″W) in August 2016. A sediment sample was collected from each core using an 11 mm diameter straw. Cores were transported on dry ice to the lab and bycatch was removed using flame-sterilized tweezers. Individual phoronids were extracted from their associated tube and both phoronid and tube were placed into separate sterile 1.5 mL tubes. In total, four sediment samples, 13 phoronids (*Phoronopsis harmeri*), and 12 tubes were opportunistically collected. This included 11 paired sets of phoronids and their respective tubes. All samples were stored at -80 °C until processing.

Molecular methods

Samples were randomized prior to extraction using a random number generator. All marine invertebrate samples were surface cleaned and sterilized as in Rattray et al [51]. Briefly, this involved rinsing samples in autoclaved water, then immersing samples in 95% ethanol for 5 minutes, followed by a final rinse in autoclaved water. DNA was extracted from surface sterilized samples with the ZymoBIOMICS DNA Mini kit (Zymo Research, Irvine, CA) following the manufacturer’s protocol with samples bead beaten on the homogenize setting for 1 min using a BioSpec Products mini-bead beater. DNA was also extracted from three no-sample added (negative), three autoclaved water (negative), three 95% ethanol (negative), three 70% ethanol (negative) and three ZymoBIOMICS Microbial Community Standard (positive) controls (Zymo Research, Irvine, CA).

Sequence generation

For high-throughput amplicon library preparation and sequencing, samples were randomized and placed in 96-well plates to be sent to Zymo Research, Inc. via their ZymoBIOMICS Service. The 16S ribosomal RNA (rRNA) gene was amplified using the Earth Microbiome Project (EMP) 515F (Parada) and 806R (Apprill) primer set [52,53]. Libraries were produced using the Quick-16S™ NGS Library Preparation Kit (Zymo Research, Irvine, CA). PCR products were quantified with qPCR and pooled together based on equal molarity. The pooled library was cleaned with Select-a-Size DNA Clean & Concentrator™ (Zymo Research, Irvine, CA, United States), then quantified with TapeStation (Agilent, Santa Clara, CA, United States) and Qubit (Invitrogen, Carlsbad, CA, United States). The final library was sequenced on an Illumina MiSeq (Illumina, Inc., San Diego, CA, United States) with a v3 reagent kit (600 cycles) to generate 300 bp paired-end reads. The raw sequence reads generated for this 16S rRNA gene amplicon project were deposited at GenBank under BioProject ID PRJNA1016428.

Two paired samples (one phoronid [511], and one tube [512]) were chosen for metagenomic sequencing. DNA quality was checked on a 1.5% E-Gel (Thermo Fisher Scientific, Waltham, MA, United States). DNA was provided to the UC Davis Genome Center DNA Technologies Core for library preparation and sequencing as part of a shared multi-project sequencing run of nine samples. DNA libraries were sequenced on an Illumina MiSeq to generate 250 bp paired-end reads. The raw metagenomic sequence reads generated for this project were deposited at GenBank under BioProject ID PRJNA1016428.

16S rRNA gene amplicon processing

Primers were removed from reads using cutadapt v. 2.3 [54]. Reads were processed with DADA2 in R with the following parameters maxEE=c(2,2), truncLen=c(190, 190), and truncQ=2 [55–57]. Using DADA2, Reads were then denoised and merged to generate amplicon sequence

variant (ASV) count tables. Chimeric sequences were identified and removed using removeBimeraDenovo by “consensus” (1.22% of ASVs).

After removing chimeras, samples had a mean read count of 50,507 (range: 27,899 - 74,130). Taxonomy was inferred for ASVs using the RDP Naive Bayesian Classifier algorithm with the SILVA (v. 138) [58–60]. ASVs were aligned using DECIPHER [61,62]. A neighbor-joining tree was then constructed as a starting tree, and fit to a GTR+G+I (Generalized time-reversible with Gamma rate variation) maximum likelihood phylogeny using phangorn [63]. The resulting phylogeny was then rooted using a eukaryotic outgroup with APE [64].

To identify possible contaminants, we used decontam’s prevalence method with a threshold of 0.5, which will identify ASVs with a higher prevalence in negative controls than in true samples [65]. This threshold identified 113 possible contaminant ASVs which were removed from the final dataset. Negative and positive controls were subsequently removed at this point in the analysis. Further, all ASVs with taxonomic assignments in the Eukaryota or an unclassified kingdom, as well as to chloroplasts or mitochondria were removed. The resulting count table contained 4,295 ASVs.

Count tables were normalized for sequencing depth by subsetting without replacement to 10,000 sequences per sample. Rarefaction depths were chosen to balance rarefaction curve saturation with the minimum library size needed to maintain all samples.

16S rRNA gene amplicon data analysis

Relative abundance, alpha and beta diversity analyses were assessed using the tidyverse [66], ggplot2 [67], vegan [68] and phyloseq [69] packages in R. We visualized the relative abundance of the microbiome across sample types (phoronids, tubes, sediment) by transforming rarified

read counts to proportions and collapsing ASVs into taxonomic families. To better enable interpretation, we only visualized families or orders that represented a summed mean proportion of greater than 5% relative abundance across sample types.

To assess differences in alpha diversity across sample types, we calculated the Shannon index and then tested for significant differences using Kruskal-Wallis (KW) tests with 9,999 permutations. When the KW test resulted in a rejected null hypothesis ($P < 0.05$), we further applied *post hoc* Dunn tests and corrected the resulting *p*-values using the Benjamini-Hochberg method.

To assess differences in beta diversity across sample types, we subsampled to the desired rarefaction depth using rarefy_even_depth from phyloseq across 100 random iterations, calculated the Weighted Unifrac dissimilarity for each iterative dataset using the distance function from phyloseq, and then averaged across all iterations to generate an average dissimilarity matrix for the dataset. We visualized the average dissimilarity matrix as a principal coordinate analysis (PCoA) plot using the ordinate function in phyloseq. We tested for significant differences in beta diversity centroids (i.e., means of each group) across sample types using permutational multivariate analyses of variance (PERMANOVAs) with 9,999 permutations and by = “margin” using the adonis2 function. Vegetative status (i.e., with or without seagrass) was included as a covariate in the PERMANOVA model. *Post hoc* pairwise PERMANOVAs were performed to assess pairwise differences using the pairwise.adonis function from the pairwiseAdonis package [70] with *p*-values corrected using the Benjamini-Hochberg method. We also tested for significant differences in dispersion (i.e., variability) using the betadisper and permutest functions from the vegan package with 9,999 permutations.

Metagenomic read-based analysis

Adaptor removal and quality filtering of metagenomic reads was performed using BBDuk v. 38.90 [71] with the following parameters: ktrim=r k=23 mink=11 hdist=1 tpe tbo qtrim=rl trimq=10 maq=10. Taxonomic classification of quality-controlled reads was performed using Kraken2 v. 2.1.2 [72] against the NCBI non-redundant nucleotide database (downloaded May 3, 2023). Bracken v. 2.5 [73] was then run on the Kraken2 outputs to estimate the number of reads associated with each species in each individual sample. ETE3 [74] was used to obtain the full taxonomic lineage string of each identified species from NCBI.

Resulting Bracken count tables were imported into R for downstream analysis using the tidyverse [66], ggplot2 [67], vegan [68] and phyloseq [69] packages in R. Bracken counts were transformed into proportions. We visualized the relative abundance of the microbiome across metagenomic samples (one phoronid, one tube). To better enable interpretation, we only visualized families or orders that represented a summed mean proportion of greater than 5% relative abundance across samples.

Metagenome-assembled genome (MAG) analysis

Quality filtered reads were coassembled using MEGAHIT v1.2.9 [75]. The anvi'o v.7.1 pipeline was then used to identify and assess metagenome-assembled genomes (MAGs) [76]. Briefly, we first calculated read coverage against the coassembly using bowtie2 v. 2.4.2 and samtools v. 1.11 [77,78]. Then, we constructed a contig database for the coassembly using 'anvi-gen-contigs-database' and predicted genes using Prodigal v. 2.6.3 [79] and single-copy genes for bacteria [80] using HMMER v. 3.2.1 [81]. We then assigned taxonomy to genes using Kaiju v. 1.8.2 with the NCBI BLAST non-redundant protein database nr including fungi and microbial eukaryotes v. 2020-05-25 [82].

Anvi'o profiles were constructed for each individual sample for contigs > 1 Kb using 'anvi-profile' with the '--cluster-contigs' option. Individual sample profiles were subsequently merged using 'anvi-merge'. Contigs were automatically binned into MAGs using 'anvi-cluster-contigs' with four different algorithms, CONCOCT v. 1.1.0, BinSanity v.0.5.4, MaxBin v. 2.2.7 and METABAT v. 2.2.7 [83–86]. An optimized, non-redundant set of MAGs was then calculated using DASTOOL v. 1.1.2 [87]. Completeness and contamination of MAGs were assessed using 'anvi-summarize' and CheckM2 [88]. Inspection of MAGs was performed using 'anvi-refine' and manual refinement was performed as needed. Taxonomy was assigned to MAGs using GTDB-Tk v.2.2.6 [89], which uses both phylogenetic placement and average nucleotide identity (ANI) to make taxonomic assignments. METABOLIC v.4.0 was run using 'METABOLIC-G.pl' on both the coassembly and individual MAGs to identify genes related to sulfur cycling processes [90]. Only MAGs with ≥ 25% completion based on CheckM2 estimates are reported here.

Results

Phoronid microbiome is dominated by members of the order Campylobacterales

Analysis of the 16S rRNA gene amplicon data revealed that the taxonomic orders with the highest mean relative abundance associated with phoronids were the Campylobacterales, Verrucomicrobiales, Desulfobulbales, and Desulfobacterales (Figure 1A, Figure S1). For tubes, the orders with the highest average relative abundance were Campylobacterales, Desulfobulbales, Desulfobacterales, and Flavobacteriales. Finally, the most abundant orders associated with sediment were Campylobacterales, Desulfobulbales, Flavobacteriales, and Desulfobacterales. Overall, the most relatively abundant taxonomic orders associated with all three sample types were almost identical, possibly indicating that much of the phoronid and tube associated communities may be sourced from the surrounding sediment.

In comparison, a read-based analysis of the metagenomic data using Kraken2/Bracken found the taxonomic orders with the highest relative abundance associated with phoronids to be the Bacillales, Enterobacterales, Flavobacteriales, and Eubacteriales, very different from the 16S rRNA gene amplicon results (Figure 1B, Figure S2). The taxonomic orders with the highest relative abundance associated with tubes in the metagenomic read analysis (Burkholderiales, Desulfobacterales, Campylobacterales, and Hyphomicrobiales) were largely similar to the 16S rRNA gene amplicon results. There are several possible reasons for discrepancies between the metagenomic and the 16S rRNA gene amplicon data including primer bias [91,92], different naming conventions between databases [93,94], and the limitations of read-based taxonomic classifiers [95]. However, one major contributor here was likely the high-prevalence of host DNA in the phoronid metagenome, which may have limited our ability to accurately determine relative abundance of bacterial taxa. Metagenomic read analysis predicted that only 6.40% of reads were bacterial in the phoronid metagenome, while the remaining 93.09% were predicted to be eukaryotic. In comparison, analysis of the tube metagenome yielded a much higher proportion of bacterial reads (43.09%) compared to eukaryotic reads (56.11%).

Phoronids and tubes are distinct from surrounding sediment

We compared alpha diversity and community structure between phoronids, tubes and surrounding sediment using 16S rRNA gene amplicon data analysis. We found that phoronids, tubes and surrounding sediment had significantly different Shannon alpha diversity (Figure 2A; K-W test, $p < 0.05$). *Posthoc* tests revealed that phoronids and tubes had similar Shannon alpha diversity (Dunn test, $p > 0.05$) and both were less diverse than sediment ($p < 0.05$). Shannon alpha diversity was not significantly different across samples from different cores, vegetation status (whether a core was from within or outside of a seagrass bed), or between phoronid-tube pairs (K-W test, $p > 0.05$).

Phoronids, tubes and surrounding sediment also had significantly different community structure (Weighted UniFrac beta diversity) (Figure 2B; PERMANOVA; $p < 0.01$). *Posthoc* pairwise contrasts were all significantly different, indicating that phoronids, tubes and surrounding sediment all harbor distinctly structured communities ($p < 0.05$). Vegetation status also was associated with significantly different community structure (Figure S3; $p < 0.01$). Additionally, we found no significant differences in community variance, or dispersion, in terms of sample type or vegetation status (betadisper; $p > 0.05$).

Recovery of low-quality metagenome-assembled genomes and sulfur cycling genes

Despite limitations due to prevalence of host DNA in the phoronid sample (~93% represented eukaryotic reads), low sample size and sequencing depth, which precluded us from obtaining high (≥ 90% complete) or medium quality MAGs (≥ 50% complete) [96], we were still able to generate and report here on five low quality draft MAGs associated with phoronid tubes that were ≥ 25% complete (CheckM2; Table 1, Table S1). The taxonomic identities of the five low quality MAGs largely reflect the dominant taxonomic orders and families of phoronids and tubes found in the 16S rRNA gene amplicon data (i.e., Campylobacterales, Desulfobacterales, and Flavobacteriales). As a result of evolutionary novelty, incompleteness, or both, none of these MAGs was placed by GTDB-Tk into a species level taxonomic assignment with several of the MAGs only able to be placed at higher taxonomic levels (i.e., class, family).

Given the importance of sulfur cycling in marine ecosystems, we used METABOLIC to survey the sulfur cycling capacity of the metagenome co-assembly overall and the five low quality MAGs individually. Results from running METABOLIC on the co-assembly suggest that a majority of genes needed for the sulfur cycle are present in the microbial community associated with phoronids and tubes (Figure 3, Figure S4). Further, while we only have an incomplete picture of the genes present in the MAGs, METABOLIC found that some of the MAGs, in

particular PHOR-02, PHOR-04, PHOR-05, had key genes involved in a subset of these pathways. With PHOR-02 likely capable of sulfite oxidation, sulfate reduction and sulfite reduction, PHOR-04 capable of sulfur oxidation, and PHOR-05 capable of sulfite reduction. Genes involved in other key sulfur cycling pathways like sulfide oxidation, thiosulfate oxidation and thiosulfate disproportionation while not detected in the MAGs, were detected in the metagenomic co-assembly.

Discussion

Phoronid microbiome is similar to that of other marine organisms

The orders Campylobacterales, Desulfobulbales, and Desulfobacterales had the highest relative abundance in the 16S rRNA amplicon analysis of the microbiomes of phoronids, tubes, and their surrounding sediment. These orders have been reported as highly abundant members of the microbiomes of other marine organisms and their dwellings including shrimp [27,97], polychaetes [31,98,99], and tube worms [100]. Metagenomic read-based analysis similarly identified Campylobacterales and Desulfobacterales as relatively highly abundant in the microbiome of tubes. However, the metagenomic analysis of phoronids was less consistent with the 16S rRNA amplicon analysis likely due to several factors including 16S rRNA gene primer bias [91,92], different naming conventions between 16S rRNA gene and genomic databases [93,94], database age [101,102], and methodological limitations of short read-based taxonomic classifiers [95], which can be complicated by low coverage or host-dominated metagenomic sequencing.

Phoronid microbiome is dominated by known sulfur-cycling microbes

Sulfur cycling is a critical ecosystem process in the marine ecosystem and many marine organisms have known associations with microbes involved in the sulfur cycle [27,31–33,103]. It is thought that remineralization of a third of organic matter on the seafloor globally is facilitated

by sulfate-reducing microbes [104]. The orders Campylobacterales, Desulfobulbales, and Desulfobacterales all include taxa known to be involved in sulfur cycling [105,106]. Members of the Campylobacterales, such as *Sulfurovum* sp., include known sulfur-oxidizing chemolithoautotrophs and are often reported as chemosymbionts of marine animals such as tubeworms, shrimp, snails, polychaetes, and bivalves [30,107–114]. Members of the Desulfobulbales include filamentous cable bacteria which couple sulfur oxidation and oxygen or nitrate reduction via long distance electron transfer [115,116]. Finally, members of the Desulfobacterales include sulfate reducers and oxidizers, and symbionts of gutless marine oligochaetes [117,118]. Understanding the intricate relationships between phoronids and these sulfur-cycling microbes may shed light on the potential contributions of phoronids to sediment biogeochemical cycling and provide valuable insights into understanding broader marine ecosystem dynamics.

Tube habitat may help select for sulfur-cycling microbes from sediment

Phoronid tubes hosted a community that was distinct from both host phoronid and surrounding sediment, despite having a similar taxonomic composition to both. Distinct tube or burrow microbiomes have be reported for shrimp [27] and polychaetes [99,119], and it thought that these dwellings may provide conducive conditions for sulfur reduction, potentially explaining the prevalence of orders like Desulfobulbales and Desulfobacterales for which many of the known members are sulfate-reducers [7,31,119,120]. Desulfobulbales are thought to be able to tap into polychaete tubes which serve as oxygenated conduits facilitating sulfur cycling [121]. Similar findings in mud shrimp burrows suggest a role for these dwellings in providing a zone of oxygenation for sulfate-reducing microbes [27]. These results combined with observations of bacteria lining the inside of phoronid tubes [18], suggests that these tubes may be creating a microenvironment conducive to sulfur-cycling microbes, echoing the observations of other dwelling-building species.

The phoronids and their tubes also hosted less diverse microbiomes than that of surrounding sediment, which could indicate that there is some selection occurring. Host-associated drops in diversity have been reported in other studies of marine invertebrates in comparison to surrounding habitat [27,122]. However, a comprehensive analysis of marine invertebrate microbiomes found that while microbiomes were distinct from surrounding sediment, providing some evidence of host selection, there was no evidence of phylosymbiosis [122]. This suggests that local habitat is a defining driver of microbial community composition in marine species. Here we found a significant effect of vegetation status, which further supports the idea that local habit is important as seagrasses have previously reported to select for a distinct soil community compared to surrounding unvegetated sediment [123]. These findings underscore the intricate interplay between marine invertebrates and their microbial communities in shaping local ecosystems, as well as the role of local ecosystems in shaping these host-microbe interactions.

Functional analysis of MAGs support role in sulfur-cycling

We were able to generate five low quality draft MAGs including members of the dominant orders Campylobacteria and Desulfobacterales. These partial MAGs point towards involvement of abundant taxa and other microbial community members in sulfur cycling. We found that PHOR-02 (GCA-2746365 sp./Thiotrichaceae sp.) was likely capable of sulfite oxidation, sulfate reduction and sulfite reduction, PHOR-04 (Campylobacteria sp.) capable of sulfur oxidation, and PHOR-05 (Desulfosarcinaceae sp.) capable of sulfite reduction. However, we are likely under sampling the true functional potential of these MAGs due to their relatively incomplete status. For example, PHOR-01 (*Sulfurovum* sp.) is missing many of the genes required for different sulfur cycling processes, despite belonging to a genus that includes reported sulfate oxidizers [100,124,125]. Regardless, these partial MAGs provide initial insight into the possible function of microbes associated with phoronids and their tubes, depicting a microbial community likely

involved in marine sulfur cycling. Given that microscopic evidence suggests that there are symbiotic microbes associated with phoronids [48,49], future work should improve on these MAGs to enable investigation into other functional roles of these microbes in phoronid fitness, for example, whether microbes may play a role in host chemical defense or host nutrition.

Conclusion

While limited in scope, this work provides a foundation for understanding phoronid microbial communities. We found that phoronids and tubes were distinct in microbiome richness and community structure compared to nearby sediment. Further, we identified that phoronids and their tubes harbor distinct microbial communities. This suggests that phoronids and tubes are providing different niches for microbial community members. In summary, this work describes the microbiome of another marine tube-building organism that may have formed associations with microorganisms involved in sulfur metabolism to survive in marine ecosystems. These results also open the door to future exploration of whether there is a microbial contribution to phoronid biochemical defense against predators or host nutrition.

Author Contributions

CLE conceived and designed the experiments, performed sampling, analyzed the data, prepared figures and/or tables, wrote and reviewed drafts of the paper. JAE reviewed drafts of the paper.

Data availability

This 16S amplicon sequencing project and raw metagenomic sequencing reads have been deposited at GenBank under the accession no. PRJNA1016428. Individual MAG assemblies are available on Zenodo (https://zenodo.org/doi/10.5281/zenodo.11224923). All data and code

can be found in GitHub (https://github.com/casett/PhoronidMicrobiomes) and is archived on Zenodo (https://zenodo.org/doi/10.5281/zenodo.11225270).

Acknowledgements

We would like to thank Katherine Dynarski (ORCID: 0000-0001-5101-9666) and Sonia Ghose (ORCID: 0000-0001-5667-6876) for help with sample collection. We would like to thank John J. Stachowicz for use of his scientific sampling permit, California Department of Fish and Wildlife Scientific Collecting Permit # SC 4874.

Funding sources

This work was supported by a grant from the UC Davis Center for Population Biology to CLE. CLE is supported by the National Science Foundation (NSF) under a NSF Ocean Sciences Postdoctoral Fellowship (Award No. 2205744). Computations were performed using the computer clusters and data storage resources of the UC Riverside HPCC, which were funded by grants from NSF (MRI-2215705, MRI-1429826) and NIH (1S10OD016290-01A1). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Pixell HLM. Two New Species of the Phoronidea from Vancouver Island. J Cell Sci. 1912;s2-58: 257–284. doi:10.1242/jcs.s2-58.230.257

2. Hilton WA. Phoronidea from the coast of southern California. Journal of Entomology and Zoology. 2930;22: 33–35.

3. Kristensen E. Impact of polychaetes (Nereis spp. and Arenicola marina) on carbon biogeochemistry in coastal marine sediments†. Geochem Trans. 2001;2: 1–12. doi:10.1186/1467-4866-2-92

4. Laverock B, Tait K, Gilbert JA, Osborn AM, Widdicombe S. Impacts of bioturbation on temporal variation in bacterial and archaeal nitrogen-cycling gene abundance in coastal sediments. Environ Microbiol Rep. 2014;6: 113–121. doi:10.1111/1758-2229.12115

5. Fang J, Jiang W, Meng S, He W, Wang G, Guo E, et al. Polychaete Bioturbation Alters the Taxonomic Structure, Co-occurrence Network, and Functional Groups of Bacterial Communities in the Intertidal Flat. Microb Ecol. 2023;86: 112–126. doi:10.1007/s00248-022-02036-2

6. Taylor JD, Cunliffe M. Polychaete burrows harbour distinct microbial communities in oil-contaminated coastal sediments. Environ Microbiol Rep. 2015;7: 606–613. doi:10.1111/1758-2229.12292

7. Steward CC, Nold SC, Ringelberg DB, White DC, Lovell CR. Microbial biomass and community structures in the burrows of bromophenol producing and non-producing marine worms and surrounding sediments. Marine Ecology Progress Series. 1996;133: 149–165. Available: https://www.jstor.org/stable/24856113

8. Kristensen E, Jensen MH, Aller RC. Direct measurement of dissolved inorganic nitrogen exchange and denitrification in individual polychaete (Nereis virens) burrows. Journal of Marine Research. 1991;49. Available: https://elischolar.library.yale.edu/journal_of_marine_research/2005

9. Hüttel M. Influence of the lungworm Arenicola marina on porewater nutrient profiles of sand flat sediments. Mar Ecol Prog Ser. 1990;62: 241–248. Available: https://www.jstor.org/stable/24842152

10. Alongi DM. Microbes, meiofauna, and bacterial productivity on tubes constructed by the polychaete Capitella capitata. Mar Ecol Prog Ser. 1985;23: 207–208. Available: https://www.int-res.com/articles/meps/23/m023p207.pdf

11. Carey DA. Particle Resuspension in the Benthic Boundary Layer Induced by Flow around Polychaete Tubes. Can J Fish Aquat Sci. 1983 [cited 13 Sep 2023]. doi:10.1139/f83-291

12. Eckman JE. Hydrodynamic processes affecting benthic recruitment1. Limnol Oceanogr. 1983;28: 241–257. doi:10.4319/lo.1983.28.2.0241

13. Gallagher ED, Jumars PA, Trueblood DD. Facilitation of Soft-Bottom Benthic Succession By Tube Builders. Ecology. 1983;64: 1200–1216. doi:10.2307/1937829

14. Johnson AS. Hydrodynamic study of the functional morphology of the benthic suspension feeder Phoronopsis viridis (Phoronida). Mar Biol. 1988;100: 117–126. doi:10.1007/BF00392961

15. Friedrichs M, Graf G, Springer B. Skimming flow induced over a simulated polychaete tube lawn at low population densities. Mar Ecol Prog Ser. 2000;192: 219–228. doi:10.3354/meps192219

16. Hentschel BT, Larson AA. Hydrodynamic mediation of density-dependent growth and adult-juvenile interactions of a spionid polychaete. Limnol Oceanogr. 2006;51: 1031–1037. doi:10.4319/lo.2006.51.2.1031

17. Emig CC. The Biology of Phoronida. Advances in Marine Biology. Academic Press; 1982. pp. 1–89. doi:10.1016/S0065-2881(08)60086-3

18. Temereva E, Shcherbakova T, Tzetlin A. First data on the structure of tubes formed by phoronids. Zoology. 2020;143: 125849. doi:10.1016/j.zool.2020.125849

19. Larson AA, Stachowicz JJ, Hentschel BT. The effect of a tube-building phoronid on associated infaunal species diversity, composition and community structure. J Exp Mar Bio Ecol. 2009;381: 126–135. doi:10.1016/j.jembe.2009.09.015

20. Larson AA, Stachowicz JJ. Chemical defense of a soft-sediment dwelling phoronid against local epibenthic predators. Mar Ecol Prog Ser. 2009;374: 101–111. doi:10.3354/meps07767

21. Ronan TE. Structural and Paleoecological Aspects of a Modern Marine Soft-sediment Community: an Experimental Study. University of California, Davis . 1975. Available: https://www.jstor.org/stable/1541073

22. Medina-Silva R, Oliveira RR, Trindade FJ, Borges LGA, Lopes Simão TL, Augustin AH, et al. Microbiota associated with tubes of Escarpia sp. from cold seeps in the southwestern Atlantic Ocean constitutes a community distinct from that of surrounding marine sediment and water. Antonie Van Leeuwenhoek. 2017;111: 533–550. doi:10.1007/s10482-017-0975-7

23. Fuirst M, Ward CS, Schwaner C, Diana Z, Schultz TF, Rittschof D. Compositional and Functional Microbiome Variation Between Tubes of an Intertidal Polychaete and

Surrounding Marine Sediment. Front Mar Sci. 2021;8: 656506. doi:10.3389/fmars.2021.656506

24. Li M, Yang H, Gu J-D. Phylogenetic Diversity and Axial Distribution of Microbes in the Intestinal Tract of the Polychaete Neanthes glandicincta. Microb Ecol. 2009;58: 892–902. doi:10.1007/s00248-009-9550-8

25. López-García P, Gaill F, Moreira D. Wide bacterial diversity associated with tubes of the vent worm Riftia pachyptila. Environ Microbiol. 2002;4: 204–215. doi:10.1046/j.1462-2920.2002.00286.x

26. Duperron S, de Beer D, Zbinden M, Boetius A, Schipani V, Kahil N, et al. Molecular characterization of bacteria associated with the trophosome and the tube of Lamellibrachia sp., a siboglinid annelid from cold seeps in the eastern Mediterranean. FEMS Microbiol Ecol. 2009;69. doi:10.1111/j.1574-6941.2009.00724.x

27. Li C, Reimers CE, Chapman JW. Microbiome analyses and presence of cable bacteria in the burrow sediment of Upogebia pugettensis. Mar Ecol Prog Ser. 2020;648: 79–94. doi:10.3354/meps13421

28. Rincón-Tomás B, González FJ, Somoza L, Sauter K, Madureira P, Medialdea T, et al. Siboglinidae Tubes as an Additional Niche for Microbial Communities in the Gulf of Cádiz—A Microscopical Appraisal. Microorganisms. 2020;8. doi:10.3390/microorganisms8030367

29. Wilkins LGE, Leray M, O'Dea A, Yuen B, Peixoto RS, Pereira TJ, et al. Host-associated microbiomes drive structure and function of marine ecosystems. PLoS Biol. 2019;17: e3000533. doi:10.1371/journal.pbio.3000533

30. Dubilier N, Bergin C, Lott C. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat Rev Microbiol. 2008;6: 725–740. doi:10.1038/nrmicro1992

31. Matsui GY, Ringelberg DB, Lovell CR. Sulfate-Reducing Bacteria in Tubes Constructed by the Marine Infaunal Polychaete Diopatra cuprea. Appl Environ Microbiol. 2004;70: 7053. doi:10.1128/AEM.70.12.7053-7065.2004

32. King GM. Microbiomes of the Enteropneust, Saccoglossus bromophenolosus, and Associated Marine Intertidal Sediments of Cod Cove, Maine. Front Microbiol. 2018;9: 418872. doi:10.3389/fmicb.2018.03066

33. Hilário A, Capa M, Dahlgren TG, Halanych KM, Little CTS, Thornhill DJ, et al. New Perspectives on the Ecology and Evolution of Siboglinid Tubeworms. PLoS One. 2011;6: e16309. doi:10.1371/journal.pone.0016309

34. Martin BC, Middleton JA, Fraser MW, Marshall IPG, Scholz VV, Hausl B, et al. Cutting out the middle clam: lucinid endosymbiotic bacteria are also associated with seagrass roots worldwide. ISME J. 2020;14: 2901–2905. doi:10.1038/s41396-020-00771-3

35. Le Pennec M, Herry A, Beninger P. Feeding and digestive adaptations of bivalve molluscs to sulphide-rich habitats. Comp Biochem Physiol A Physiol. 1995;111: 183–189. doi:10.1016/0300-9629(94)00211-B

36. Cary SC, Vetter RD, Felbeck H. Characterization of Resource Utilization of Marine Invertebrates Harboring Chemoautotrophic Endosymbiotic Bacteria. Mar Ecol Prog Ser. 1989;55: 31–45.

37. Johnson RG. The Vertical Distribution of the Infauna of a Sand Flat. Ecology. 1967;48: 571–578. doi:10.2307/1936501

38. Woodin SA, Walla MD, Lincoln DE. Occurrence of brominated compounds in soft-bottom benthic organisms. J Exp Mar Bio Ecol. 1987;107: 209–217. doi:10.1016/0022-0981(87)90038-4

39. Kicklighter CE, Kubanek J, Barsby T, Hay ME. Palatability and defense of some tropical infaunal worms: alkylpyrrole sulfamates as deterrents to fish feeding. Mar Ecol Prog Ser. 2003;263: 299–306. doi:10.3354/meps263299

40. Kicklighter CE, Kubanek J, Hay ME. Do brominated natural products defend marine worms from consumers? Some do, most don't. Limnol Oceanogr. 2004;49: 430–441. doi:10.4319/lo.2004.49.2.0430

41. Kicklighter CE, Hay ME. Integrating prey defensive traits: contrasts of marine worms from temperate and tropical habitats. Ecol Monogr. 2006;76: 195–215. doi:10.1890/0012-9615(2006)076[0195:IPDTCO]2.0.CO;2

42. Gaston GR, Slattery M. Ecological function of chemical deterrents in a tropical polychaete, Eupolymnia crassicornis (Annelida, Terebellidae), in Belize. 2002 [cited 13 Sep 2023]. Available: https://www.ingentaconnect.com/content/umrsmas/bullmar/2002/00000070/00000003/art00007

43. Chemical Defense in the Egg Masses of Benthic Invertebrates: An Assessment of Antibacterial Activity in 39 Mollusks and 4 Polychaetes. J Invertebr Pathol. 2001;78: 109–118. doi:10.1006/jipa.2001.5047

44. Amsler CD, McClintock JB, Baker BJ. Secondary Metabolites as Mediators of Trophic Interactions Among Antarctic Marine Organisms1. Integr Comp Biol. 2015;41: 17–26. doi:10.1093/icb/41.1.17

45. Davis AR. Alkaloids and ascidian chemical defense: Evidence for the ecological role of natural products fromEudistoma olivaceum. Mar Biol. 1991;111: 375–379. doi:10.1007/BF01319409

46. Tianero MD, Balaich JN, Donia MS. Localized production of defence chemicals by intracellular symbionts of Haliclona sponges. Nature Microbiology. 2019;4: 1149–1159. doi:10.1038/s41564-019-0415-8

47. Zan J, Li Z, Tianero, Davis J, Hill RT, Donia MS. A microbial factory for defensive kahalalides in a tripartite marine symbiosis. Science. 2019;364. doi:10.1126/science.aaw6732

48. Temereva EN, Malakhov VV. Microscopic anatomy and ultrastructure of the lophophoral organs and adjacent epithelia of the lophophoral concavity and the anal papilla of Phoronopsis harmeri Pixell, 1912 (Lophophorata: Phoronida). Russ J Mar Biol. 2006;32: 340–352. doi:10.1134/S1063074006060034

49. Temereva EN, Malakhov VV, Yushin VV. Histology and Ultrastructure of the Body Wall in the Phoronid Phoronopsis harmeri. Russ J Mar Biol. 2001;27: 156–167. doi:10.1023/A:1016765418546

50. Ettinger CL, Eisen JA. Characterization of the Mycobiome of the Seagrass, , Reveals Putative Associations With Marine Chytrids. Front Microbiol. 2019;10: 2476. doi:10.3389/fmicb.2019.02476

51. Rattray RM, Perumbakkam S, Smith F, Craig AM. Microbiomic comparison of the intestine of the earthworm Eisenia fetida fed ergovaline. Curr Microbiol. 2010;60: 229–235. doi:10.1007/s00284-009-9530-8

52. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology. 2016;18: 1403–1414. doi:10.1111/1462-2920.13023

53. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R

gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology. 2015;75: 129–137. doi:10.3354/ame01753

54. Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal. 2011;17: 10. doi:10.14806/ej.17.1.200

55. Weinstein MM, Prem A, Jin M, Tang S, Bhasin JM. FIGARO: An efficient and objective tool for optimizing microbiome rRNA gene trimming parameters. Journal of Bimolecular Techniques. 2020. doi:10.1101/610394

56. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13: 581–583. doi:10.1038/nmeth.3869

57. The R Project for Statistical Computing. [cited 10 Feb 2023]. Available: https://www.r-project.org/

58. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73: 5261–5267. doi:10.1128/AEM.00062-07

59. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research. 2012;41: D590–D596. doi:10.1093/nar/gks1219

60. Yilmaz P, Parfrey LW, Yarza P, Gerken J, Pruesse E, Quast C, et al. The SILVA and “All-species Living Tree Project (LTP)” taxonomic frameworks. Nucleic Acids Research. 2014;42: D643–D648. doi:10.1093/nar/gkt1209

61. Wright ES. RNAconTest: comparing tools for noncoding RNA multiple sequence alignment

based on structural consistency. RNA. 2020;26: 531–540. doi:10.1261/rna.073015.119

62. Wright ES. Using DECIPHER v2.0 to Analyze Big Biological Sequence Data in R. The R Journal. 2016;8: 352–359. Available: https://journal.r-project.org/archive/2016-1/wright.pdf

63. Schliep KP. phangorn: phylogenetic analysis in R. Bioinformatics. 2010;27: 592–593. doi:10.1093/bioinformatics/btq706

64. Paradis E, Claude J, Strimmer K. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics. 2004;20: 289–290. doi:10.1093/bioinformatics/btg412

65. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome. 2018;6: 226. doi:10.1186/s40168-018-0605-2

66. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, et al. Welcome to the Tidyverse. Journal of Open Source Software. 2019;4: 1686. doi:10.21105/joss.01686

67. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer; 2016. Available: https://books.google.com/books/about/ggplot2.html?hl=&id=RTMFswEACAAJ

68. Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, et al. vegan: Community Ecology Package. 2022. Available: https://CRAN.R-project.org/package=vegan

69. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE. 2013;8: e61217. doi:10.1371/journal.pone.0061217

70. Martinez Arbizu P. pairwiseAdonis: Pairwise multilevel comparison using adonis. 2020. Available: https://github.com/pmartinezarbizu/pairwiseAdonis

71. Bushnell B. BBTools. 2022. Available: https://sourceforge.net/projects/bbmap/

72. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol. 2019;20: 1–13. doi:10.1186/s13059-019-1891-0

73. Lu J, Breitwieser FP, Thielen P, Salzberg SL. Bracken: estimating species abundance in metagenomics data. PeerJ Comput Sci. 2017;3: e104. doi:10.7717/peerj-cs.104

74. Huerta-Cepas J, Serra F, Bork P. ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. Mol Biol Evol. 2016;33: 1635. doi:10.1093/molbev/msw046

75. Li D, Liu C-M, Luo R, Sadakane K, Lam T-W. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics. 2015;31: 1674–1676. doi:10.1093/bioinformatics/btv033

76. Eren AM, Murat Eren A, Esen ÖC, Quince C, Vineis JH, Morrison HG, et al. Anvi'o: an advanced analysis and visualization platform for 'omics data. PeerJ. 2015. p. e1319. doi:10.7717/peerj.1319

77. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9: 357–359. doi:10.1038/nmeth.1923

78. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25: 2078–2079. doi:10.1093/bioinformatics/btp352

79. Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics. 2010;11: 119. doi:10.1186/1471-2105-11-119

80. Lee MD. GToTree: a user-friendly workflow for phylogenomics. Bioinformatics. 2019. pp.

4162–4164. doi:10.1093/bioinformatics/btz188

81. Eddy SR. Accelerated Profile HMM Searches. PLoS Comput Biol. 2011;7: e1002195. doi:10.1371/journal.pcbi.1002195

82. Menzel P, Ng KL, Krogh A. Fast and sensitive taxonomic classification for metagenomics with Kaiju. Nat Commun. 2016;7: 11257. doi:10.1038/ncomms11257

83. Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, et al. Binning metagenomic contigs by coverage and composition. Nat Methods. 2014;11: 1144–1146. doi:10.1038/nmeth.3103

84. Kang DD, Li F, Kirton E, Thomas A, Egan R, An H, et al. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. PeerJ. 2019;7: e7359. doi:10.7717/peerj.7359

85. Graham ED, Heidelberg JF, Tully BJ. BinSanity: unsupervised clustering of environmental microbial assemblies using coverage and affinity propagation. PeerJ. 2017;5: e3035. doi:10.7717/peerj.3035

86. Wu Y-W, Tang Y-H, Tringe SG, Simmons BA, Singer SW. MaxBin: an automated binning method to recover individual genomes from metagenomes using an expectation-maximization algorithm. Microbiome. 2014;2: 1–18. doi:10.1186/2049-2618-2-26

87. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, et al. Recovery of genomes from metagenomes via a dereplication, aggregation, and scoring strategy. doi:10.1101/107789

88. Chklovski A, Parks DH, Woodcroft BJ, Tyson GW. CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. Nat Methods.

2023;20: 1203–1212. doi:10.1038/s41592-023-01940-w

89. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics. 2019. doi:10.1093/bioinformatics/btz848

90. Zhou Z, Tran PQ, Breister AM, Liu Y, Kieft K, Cowley ES, et al. METABOLIC: high-throughput profiling of microbial genomes for functional traits, metabolism, biogeochemistry, and community-scale functional networks. Microbiome. 2022;10: 1–22. doi:10.1186/s40168-021-01213-8

91. Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, et al. Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics. Front Microbiol. 2016;7: 459. doi:10.3389/fmicb.2016.00459

92. Eloe-Fadrosh EA, Ivanova NN, Woyke T, Kyrpides NC. Metagenomics uncovers gaps in amplicon-based detection of microbial diversity. Nat Microbiol. 2016;1: 15032. doi:10.1038/nmicrobiol.2015.32

93. Balvočiūtė M, Huson DH. SILVA, RDP, Greengenes, NCBI and OTT — how do these taxonomies compare? BMC Genomics. 2017;18: 1–8. doi:10.1186/s12864-017-3501-4

94. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil P-A, et al. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol. 2018;36: 996–1004. doi:10.1038/nbt.4229

95. Portik DM, Brown CT, Pierce-Ward NT. Evaluation of taxonomic classification and profiling methods for long-read shotgun metagenomic sequencing datasets. BMC Bioinformatics. 2022;23: 1–39. doi:10.1186/s12859-022-05103-0

96. Bowers RM, Kyrpides NC, Stepanauskas R, Harmon-Smith M, Doud D, Reddy TBK, et al. Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol. 2017;35: 725–731. doi:10.1038/nbt.3893

97. Tokuda G, Yamada A, Nakano K, Arita NO, Yamasaki H. Colonization of Sulfurovum sp. on the gill surfaces of Alvinocaris longirostris, a deep-sea hydrothermal vent shrimp. Mar Ecol. 2008;29: 106–114. doi:10.1111/j.1439-0485.2007.00211.x

98. Lim SJ, Thompson LR, Young CM, Gaasterland T, Goodwin KD. Dominance of Sulfurospirillum in Metagenomes Associated with the Methane Ice Worm (Sirsoe methanicola). Appl Environ Microbiol. 2022 [cited 8 Sep 2023]. doi:10.1128/aem.00290-22

99. Pischedda L, Militon C, Gilbert F, Cuny P. Characterization of specificity of bacterial community structure within the burrow environment of the marine polychaete Hediste (Nereis) diversicolor. Res Microbiol. 2011;162: 1033–1042. doi:10.1016/j.resmic.2011.07.008

100. Giovannelli D, Chung M, Staley J, Starovoytov V, Le Bris N, Vetriani C. Sulfurovum riftiae sp. nov., a mesophilic, thiosulfate-oxidizing, nitrate-reducing chemolithoautotrophic epsilonproteobacterium isolated from the tube of the deep-sea hydrothermal vent polychaete Riftia pachyptila. Int J Syst Evol Microbiol. 2016;66: 2697–2701. doi:10.1099/ijsem.0.001106

101. Nasko DJ, Koren S, Phillippy AM, Treangen TJ. RefSeq database growth influences the accuracy of k-mer-based lowest common ancestor species identification. Genome Biol. 2018;19: 165. doi:10.1186/s13059-018-1554-6

102. Gray M, Zhao Z, Rosen GL. How Scalable Are Clade-Specific Marker K-Mer Based

Hash Methods for Metagenomic Taxonomic Classification? Front Signal Process. 2022;2: 842513. doi:10.3389/frsip.2022.842513

103. Wasmund K, Mußmann M, Loy A. The life sulfuric: microbial ecology of sulfur cycling in marine sediments. Environ Microbiol Rep. 2017;9: 323–344. doi:10.1111/1758-2229.12538

104. Bowles MW, Mogollón JM, Kasten S, Zabel M, Hinrichs KU. Global rates of marine sulfate reduction and implications for sub-sea-floor metabolic activities. Science. 2014;344. doi:10.1126/science.1249213

105. Robador A, Müller AL, Sawicka JE, Berry D, Hubert CR, Loy A, et al. Activity and community structures of sulfate-reducing microorganisms in polar, temperate and tropical marine sediments. ISME J. 2016;10. doi:10.1038/ismej.2015.157

106. Watanabe M, Higashioka Y, Kojima H, Fukui M. Desulfosarcina widdelii sp. nov. and Desulfosarcina alkanivorans sp. nov., hydrocarbon-degrading sulfate-reducing bacteria isolated from marine sediment and emended description of the genus Desulfosarcina. Int J Syst Evol Microbiol. 2017;67: 2994–2997. doi:10.1099/ijsem.0.002062

107. Wang J, Zheng Q, Wang S, Zeng J, Yuan Q, Zhong Y, et al. Characterization of two novel chemolithoautotrophic bacteria of Sulfurovum from marine coastal environments and further comparative genomic analyses revealed species differentiation among deep-sea hydrothermal vent and non-vent origins. Front Mar Sci. 2023;10: 1222526. doi:10.3389/fmars.2023.1222526

108. Sogin EM, Kleiner M, Borowski C, Gruber-Vodicka HR, Dubilier N. Life in the Dark: Phylogenetic and Physiological Diversity of Chemosynthetic Symbioses. Annu Rev Microbiol. 2021;75: 695–718. doi:10.1146/annurev-micro-051021-123130

109. Waite DW, Vanwonterghem I, Rinke C, Parks DH, Zhang Y, Takai K, et al. Comparative

Genomic Analysis of the Class Epsilonproteobacteria and Proposed Reclassification to Epsilonbacteraeota (phyl. nov.). Front Microbiol. 2017;8: 264454. doi:10.3389/fmicb.2017.00682

110. Campbell BJ, Engel AS, Porter ML, Takai K. The versatile ε-proteobacteria: key players in sulphidic habitats. Nat Rev Microbiol. 2006;4: 458–468. doi:10.1038/nrmicro1414

111. Assié A, Leisch N, Meier DV, Gruber-Vodicka H, Tegetmeyer HE, Meyerdierks A, et al. Horizontal acquisition of a patchwork Calvin cycle by symbiotic and free-living Campylobacterota (formerly Epsilonproteobacteria). ISME J. 2019;14: 104–122. doi:10.1038/s41396-019-0508-7

112. Beinart RA, Sanders JG, Faure B, Sylva SP, Lee RW, Becker EL, et al. Evidence for the role of endosymbionts in regional-scale habitat partitioning by hydrothermal vent symbioses. Proc Natl Acad Sci U S A. 2012;109. doi:10.1073/pnas.1202690109

113. Haddad A, Camacho F, Durand P, Cary SC. Phylogenetic characterization of the epibiotic bacteria associated with the hydrothermal vent polychaete Alvinella pompejana. Appl Environ Microbiol. 1995;61: 1679. doi:10.1128/aem.61.5.1679-1687.1995

114. Petersen JM, Ramette A, Lott C, Cambon-Bonavita MA, Zbinden M, Dubilier N. Dual symbiosis of the vent shrimp Rimicaris exoculata with filamentous gamma- and epsilonproteobacteria at four Mid-Atlantic Ridge hydrothermal vent fields. Environ Microbiol. 2010;12. doi:10.1111/j.1462-2920.2009.02129.x

115. Müller H, Marozava S, Probst AJ, Meckenstock RU. Groundwater cable bacteria conserve energy by sulfur disproportionation. ISME J. 2019;14: 623–634. doi:10.1038/s41396-019-0554-1

116. Müller H, Bosch J, Griebler C, Damgaard LR, Nielsen LP, Lueders T, et al. Long-

distance electron transfer by cable bacteria in aquifer sediments. ISME J. 2016;10: 2010–2019. doi:10.1038/ismej.2015.250

117. Dubilier N, Mülders C, Ferdelman T, de Beer D, Pernthaler A, Klein M, et al. Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature. 2001;411: 298–302. doi:10.1038/35077067

118. Galushko AS, Rozanova EP. Desulfobacterium cetonicum sp. nov., a sulfate-reducing bacterium oxidizing fatty acids and ketones. Microbiology (Mikrobiologiya). 1991;60: 742–746. Available: https://cir.nii.ac.jp/crid/1370013168861843586

119. Aller RC, Yingst JY. Biogeochemistry of tube-dwellings: A study of the sedentary polychaete Amphitrite ornata (Leidy). Journal of Marine Research. 1978;36. Available: https://elischolar.library.yale.edu/journal_of_marine_research/1429

120. Dobbs FC, Guckert JB. *Callianassa trilobata* (Crustacea: Thalassinidea) influences abundance of meiofauna and biomass, composition, and physiologic state of microbial communities within its burrow. Marine Ecology Progress Series,. 1988;45: 1–2. Available: https://www.jstor.org/stable/24827695

121. Aller RC, Aller JY, Zhu Q, Heilbrun C, Klingensmith I, Kaushik A. Worm tubes as conduits for the electrogenic microbial grid in marine sediments. Sci Adv. 2019;5: eaaw3651. doi:10.1126/sciadv.aaw3651

122. Boscaro V, Holt CC, Van Steenkiste NWL, Herranz M, Irwin NAT, Àlvarez-Campos P, et al. Microbiomes of microscopic marine invertebrates do not reveal signatures of phylosymbiosis. Nature Microbiology. 2022;7: 810–819. doi:10.1038/s41564-022-01125-9

123. Ettinger CL, Voerman SE, Lang JM, Stachowicz JJ, Eisen JA. Microbial communities in sediment from patches, but not the leaf or root microbiomes, vary in relation to distance

from patch edge. PeerJ. 2017;5: e3246. doi:10.7717/peerj.3246

124. Inagaki F, Takai K, Nealson KH, Horikoshi K. Sulfurovum lithotrophicum gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the ε-Proteobacteria isolated from Okinawa Trough hydrothermal sediments. Int J Syst Evol Microbiol. 2004;54: 1477–1482. doi:10.1099/ijs.0.03042-0

125. Mori K, Yamaguchi K, Hanada S. Sulfurovum denitrificans sp. nov., an obligately chemolithoautotrophic sulfur-oxidizing epsilonproteobacterium isolated from a hydrothermal field. Int J Syst Evol Microbiol. 2018;68: 2183–2187. doi:10.1099/ijsem.0.002803

Figures and Tables:

Figure 1. Mean relative abundance of orders associated with phoronids, tubes and surrounding sediment based on analysis of 16S rRNA gene amplicon and metagenomic data. Bar charts display the mean relative abundance of taxonomic orders for each sample type (phoronid, tube, sediment) colored by predicted order. Mean relative abundance based on 16S rRNA gene amplicon data is shown on top with bars representing standard error, with mean relative abundance of read-based Kraken2/Bracken2 analysis of metagenomic data shown on the bottom. Orders representing less than 5% mean relative abundance across datasets are not shown. Number of samples summarized as follows - Phoronid: n_{16S} = 13, $n_{Metagenomics}$ = 1; Tube: n_{16S} = 12, $n_{Metagenomics}$ = 1; Sediment: n_{16S} = 4.

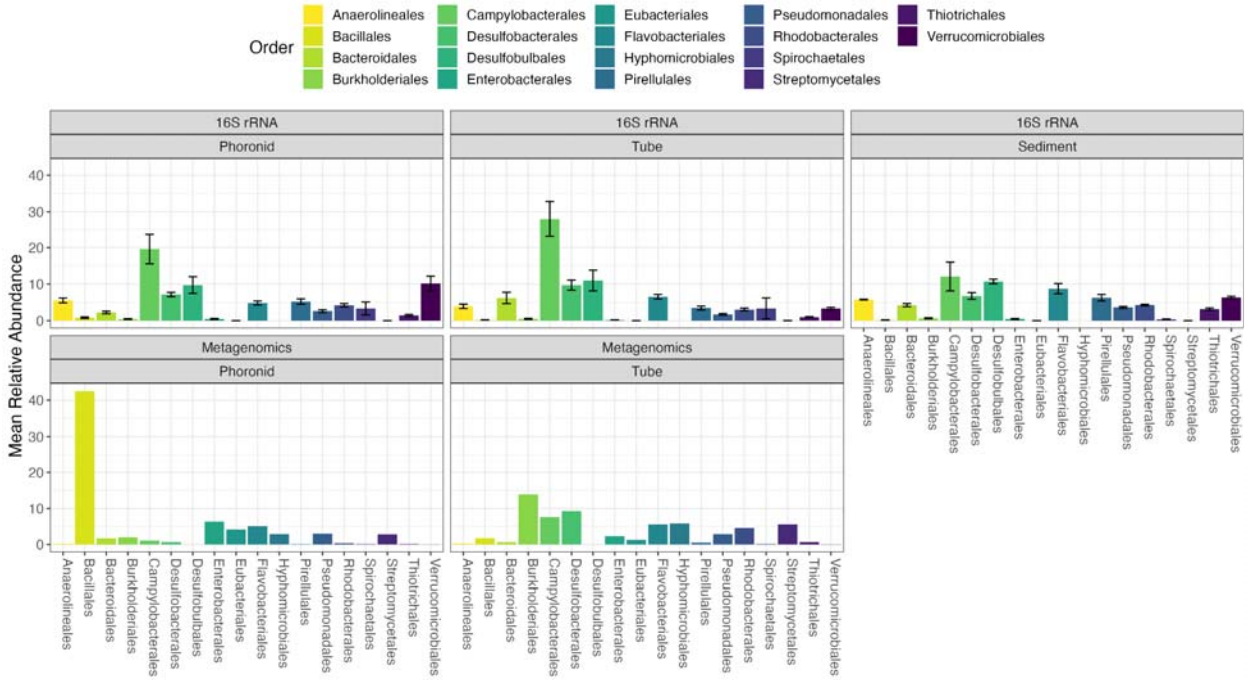


Figure 2. Phoronids and tubes have distinct microbial communities compared to surrounding sediment. (A) Shannon diversity was used to assess alpha diversity across sample types (phoronids, tubes, sediment). Comparisons that are significantly different from each other are notated by different letters (e.g., a vs. b). (B) Principal-coordinate analysis (PCoA) visualization of Weighted Unifrac distances shows individual samples designated as points with colors and shapes representing sample type: phoronids (green circle), tubes (yellow triangles), and sediment (blue squares). Ellipses represent the 95% confidence interval around the centroid of each group. Number of samples summarized in (A) and (B) as follows - Phoronid: *n* = 13; Tube: *n* = 12; Sediment: *n* = 4.

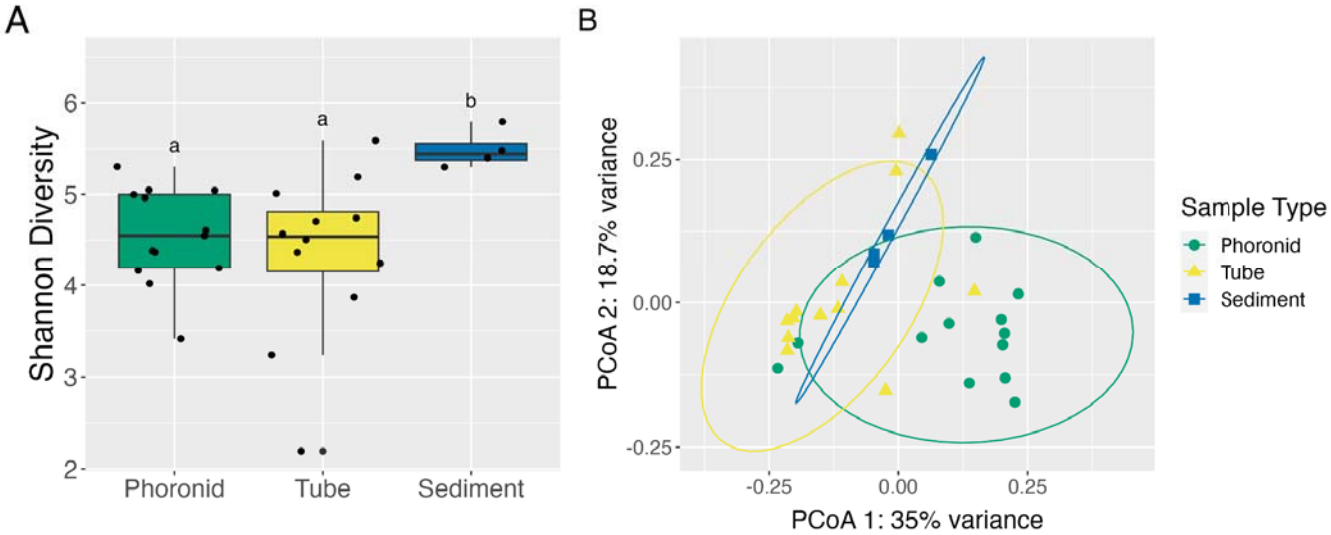


Figure 3. Predicted sulfur metabolism profiles. (A) Heatmap showing whether the metagenomic coassembly or individual MAGs had any genes detected by METABOLIC, which predicts metabolic and biogeochemical functional traits of input genomic data, related to each of the different sulfur cycling metabolisms and processes. A gene-level heatmap can be found in Figure S4. (B) Summary scheme of the sulfur cycle at the community scale. Each arrow represents a single step within the cycle. Red arrows indicate that the METABOLIC found the required genes for a step in the metagenomic coassembly, while black arrows indicate missing steps. Under each step is a box displaying the identifier(s) of any MAG(s) found by METABOLIC to have the genes needed for that step.

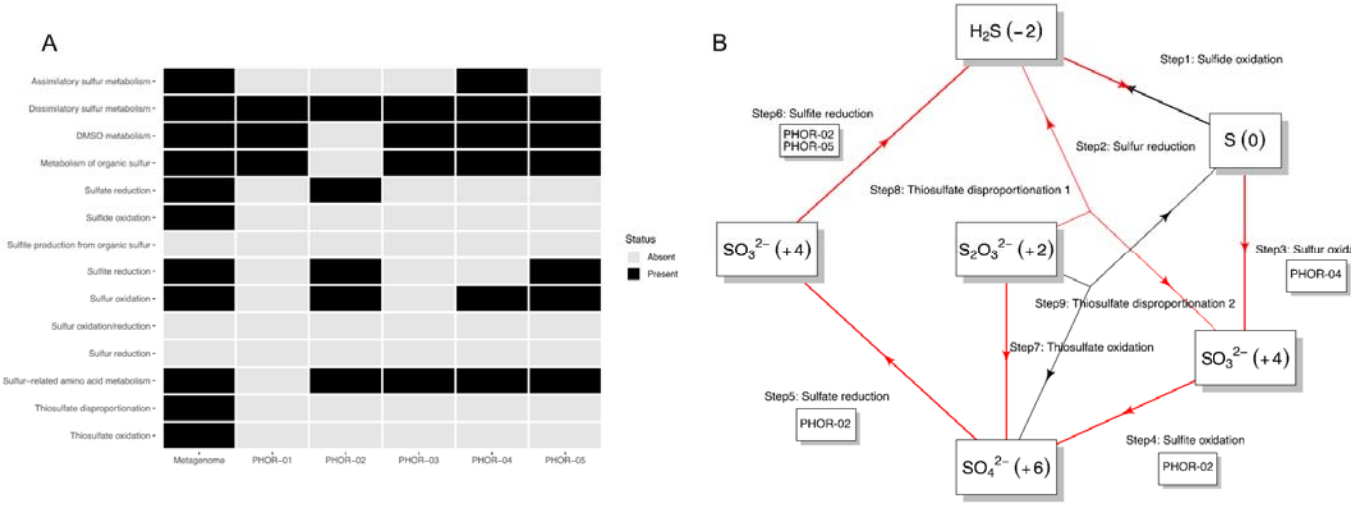
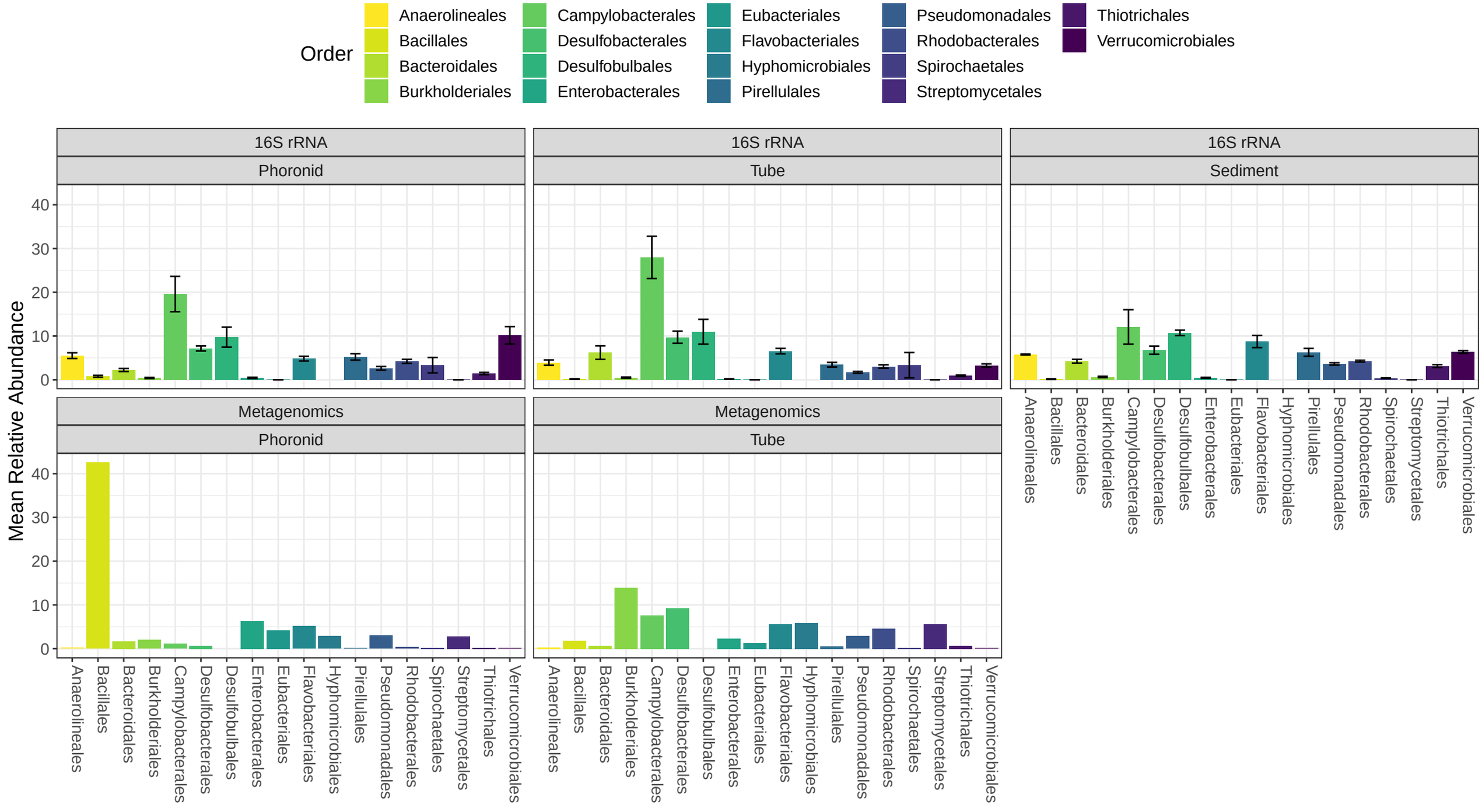
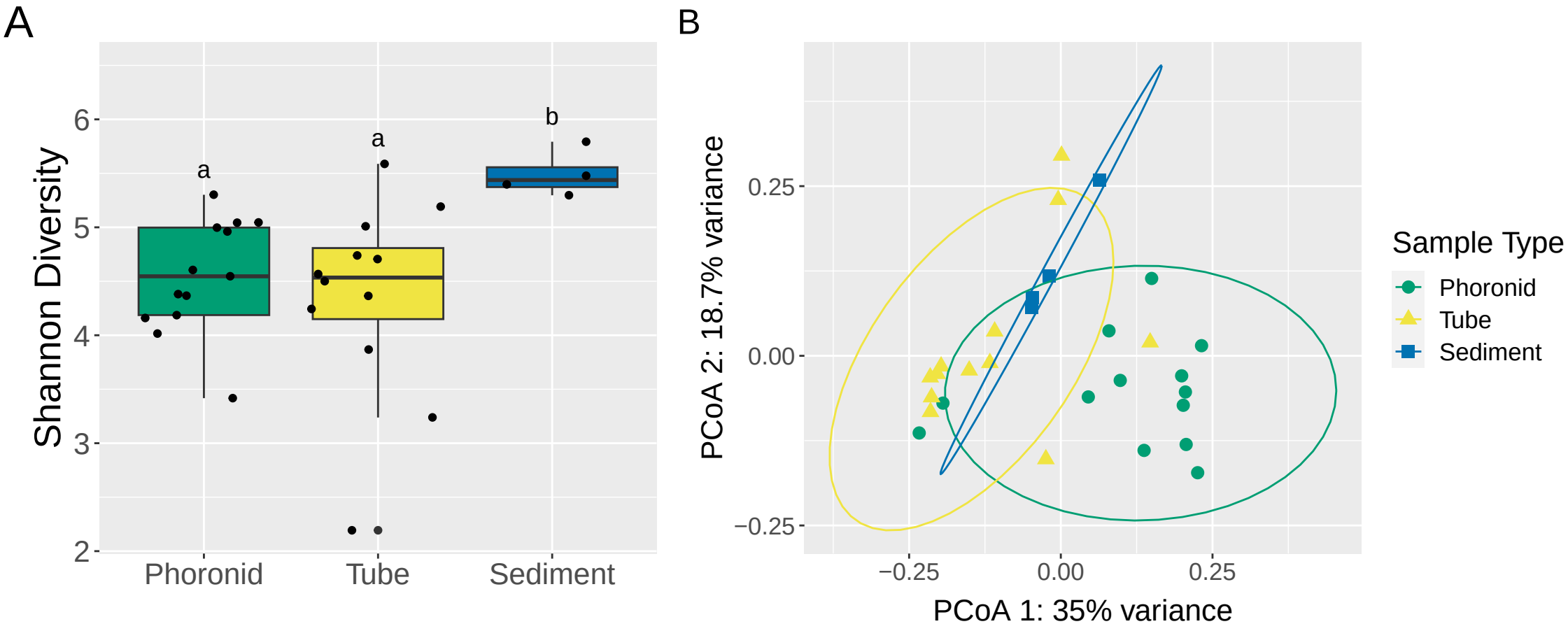
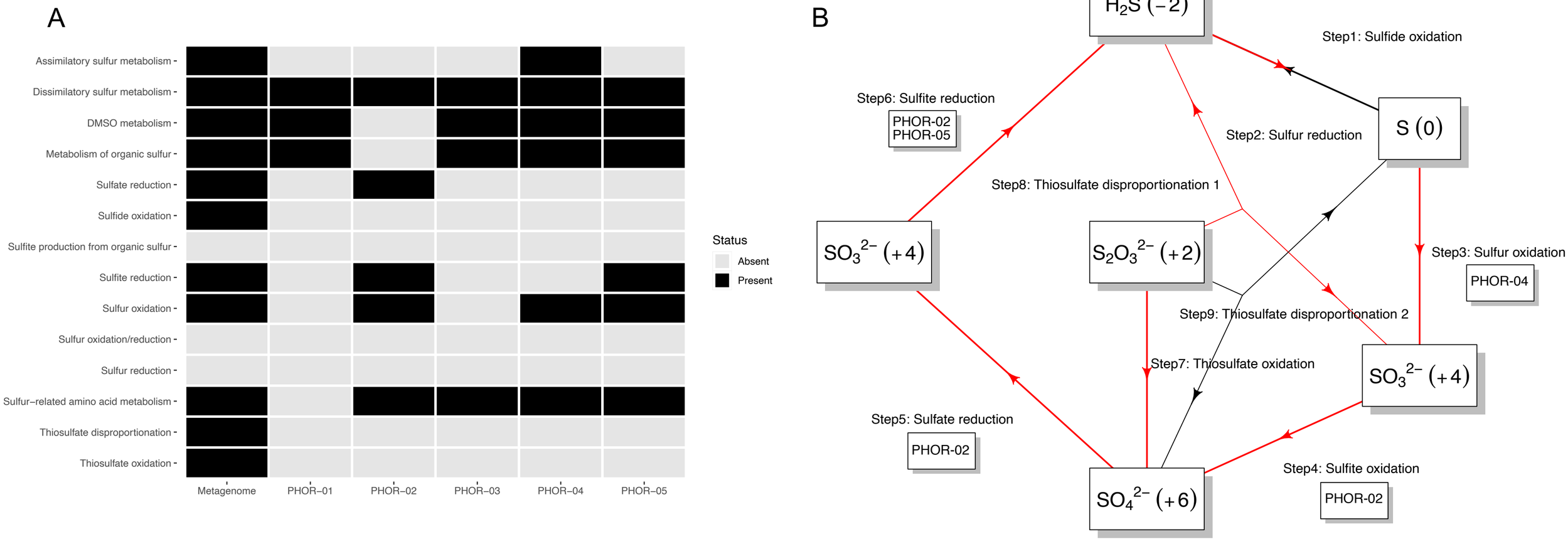


Table 1. Summary of genomic features of five draft MAGs. Genomic features are summarized for each MAG, including putative taxonomic identity (GTDB and NCBI), genome size (bp), number of contigs, N50, percent GC content, number of genes, completion and contamination estimates as generated by CheckM2 and anvi'o, and mean read coverage against each metagenome. MAGs are sorted by CheckM2 completion. See Table S1 for full GTDB taxonomy.

Bin identifier	**GTDB taxonomy**	**NCBI taxonomy**	**MAG size (bp)**	**No. of contigs**	**N_{50} (bp)**	**GC (%)**	**No. of genes**	**CheckM2 %**		**anvi'o %**		**Mean coverage (%)**	
								Completion	**Redundancy**	**Completion**	**Redundancy**	**Phoronid**	**Tube**
PHOR-01	*Sulfurovum* sp.	*Sulfurovum* sp.	1038522	362	3414	38	1362	48.78	2.14	40.85	4.23	0.01	8.25
PHOR-02	*GCA-2746365* sp.	Thiotrichaceae sp.	1523472	1074	1383	47	2316	42.83	7.9	47.89	2.82	0.00	2.90
PHOR-03	*Aureibaculum* sp.	Flavobacteriaceae sp.	2193434	1552	1364	34	3297	40.31	7.27	42.25	7.04	0.00	3.16
PHOR-04	Campylobacteria sp.	Campylobacteria sp.	1765533	1249	1392	50	2550	36.93	5.63	26.76	2.82	0.00	3.08
PHOR-05	Desulfosarcinaceae sp.	Desulfosarcinaceae sp.	2005203	1262	1608	56	2685	32.46	2.33	23.94	4.23	0.00	3.89


Order
Anaerolineales
Bacillales
Bacteroidales
Burkholderiales
Campylobacterales
Desulfobacterales
Desulfobulbales
Enterobacterales
Eubacteriales
Flavobacteriales
Hyphomicrobiales
Pirellulales
Pseudomonadales
Rhodobacterales
Spirochaetales
Streptomycetales
Thiotrichales
Verrucomicrobiales
16S rRNA
Phoronid
16S rRNA
Tube
16S rRNA
Sediment
Metagenomics
Phoronid
Metagenomics
Tube
Mean Relative Abundance
0
10
20
30
40
Anaerolineales
Bacillales
Bacteroidales
Burkholderiales
Campylobacterales
Desulfobacterales
Desulfobulbales
Enterobacterales
Eubacteriales
Flavobacteriales
Hyphomicrobiales
Pirellulales
Pseudomonadales
Rhodobacterales
Spirochaetales
Streptomycetales
Thiotrichales
Verrucomicrobiales


A
Shannon Diversity
6
5
4
3
2
a
a
b
Phoronid
Tube
Sediment
B
PCoA 2: 18.7% variance
0.25
0.00
−0.25
−0.25
0.00
0.25
PCoA 1: 35% variance
Sample Type
Phoronid
Tube
Sediment


A
Assimilatory sulfur metabolism
Dissimilatory sulfur metabolism
DMSO metabolism
Metabolism of organic sulfur
Sulfate reduction
Sulfide oxidation
Sulfite production from organic sulfur
Sulfite reduction
Sulfur oxidation
Sulfur oxidation/reduction
Sulfur reduction
Sulfur–related amino acid metabolism
Thiosulfate disproportionation
Thiosulfate oxidation
Metagenome
PHOR–01
PHOR–02
PHOR–03
PHOR–04
PHOR–05
Status
Absent
Present
B
H_2S (−2)
Step1: Sulfide oxidation
S (0)
Step2: Sulfur reduction
Step3: Sulfur oxidation
PHOR-04
SO_3^{2-} (+4)
Step4: Sulfite oxidation
PHOR-02
SO_4^{2-} (+6)
Step5: Sulfate reduction
PHOR-02
SO_3^{2-} (+4)
Step6: Sulfite reduction
PHOR-02
PHOR-05
$S_2O_3^{2-}$ (+2)
Step7: Thiosulfate oxidation
Step8: Thiosulfate disproportionation 1
Step9: Thiosulfate disproportionation 2