Diversity in the globally intertwined giant barrel sponge species complex

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Impacts of host identity and geography on the prokaryotic community of giant barrel sponges at multiple spatial scales

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Submitted

ABSTRACT

Sponge holobionts are model systems for marine host–microorganism interactions. The understanding of the drivers of the sponge microbiome, therefore, shapes the general views in marine microbial ecology. A broader understanding of these interactions, furthermore, enhances the potential to utilize these microbes for biotechnological purposes. Most studies aiming to identify the drivers of the sponge microbiome focus on a single driver or on one spatial scale, leaving a hiatus in our understanding of the interplay of the drivers. In the present study, we assessed the importance of host identity and geography on prokaryotic communities at multiple spatial scales by comparing 73 giant barrel sponges (*Xestospongia* spp.) from Curaçao, Martinique, and Thailand. Geographic distance was the main driver of prokaryote communities at the global and regional scales, whereas host identity was a minor, albeit significant, driver. At a local scale in Curaçao, Martinique, and Thailand, the phylogenetic variation in the prokaryotic community was not related to inter-site distance, but rather to unidentified local site conditions while depth was an important driver in Martinique. Phylogeny was a more influential driver at the three localities than at the larger spatial scales. Together, this study shows that the relative importance of drivers of the sponge microbiome shifts across different spatial scales. Our results are in contrast with the assumption that host identity is the principal driver of the sponge microbial community. Instead, biogeographical differences should be comprehensively considered. Dispersal limitations seem crucial at large scale, while the importance of depth and local site differences shows that the prokaryote community of giant barrel sponges is flexible. This raises the question of whether this translates into adaptability to environmental change, potentially making them resilient to such changes.

INTRODUCTION

Sponges are an ancient animal lineage (Simion et al. 2017), and their evolutionary success has been suggested to be, at least in part, due to their intimate relationship with microbial symbionts (Taylor et al. 2007b). Their long evolutionary history has made sponge holobionts an important model system for marine host–microorganism interactions. As hosts, sponges are an important contributor to the diversity of the coral reef prokaryotic metacommunity (Chapter 6 of this thesis). Sponge-associated microorganisms perform a wide range of functional roles within sponge hosts (Webster and Thomas 2016). Among other things, they produce various secondary metabolites that help the sponge defend itself against pathogens and other harmful organisms (Pawlik 1993; Hentschel et al. 2012). These secondary metabolites have great potential for the pharmaceutical industry and have gained much attention over the past decades (Munro et al. 1999; Sipkema et al. 2005; Thomas et al. 2010). Some microbes living in sponges, e.g. Cyanobacteria, are capable of photosynthesis and provide their hosts with an extra source of energy (Usher 2008; Thacker and Freeman 2012). Other microbes play important roles in nutrient cycling within the wider ecosystem (Hoffman et al. 2009; Mohamed et al. 2010; de Goeij et al. 2013; Zhang et al. 2015).

The scientific community has placed substantial effort into studying the drivers of compositional variation in sponge-associated microbial communities, such as host identity and the environment. Host identity has generally been found to be a much more important driver of sponge microbial communities than the environment, particularly at higher taxonomic ranks (Thomas et al. 2016; Souza et al. 2017; Steinert et al. 2017). This host specificity has regularly been hypothesized to originate in the vertical transfer of microorganisms from parent to offspring which instigated co-evolution or co-diversification (Schmitt et al. 2008). However, it is also possible that these OTUs are acquired from the rare biosphere, after which they thrive in their respective hosts (Taylor et al. 2013; Reveillaud et al. 2014). Because of its link to host identity, the sponge microbiome is often considered to be stable across space and time (Erwin et al. 2012; Pita et al. 2013a, 2013b, 2018; Cárdenas et al. 2014; Hardoim and Costa 2014; Thomas et al. 2016; Gantt et al. 2017; Glasl et al. 2018). However, seasonal and spatial variation have been observed in certain species (Wichels et al. 2006; Cao et al. 2012; Turque et al. 2012; White et al. 2012; Luter et al. 2015; Weigel and Erwin 2016; Pita et al. 2018; Chapter 4 of this thesis). Hence, host-identity and environmental drivers may both contribute to prokaryote composition.

Most studies of the sponge microbiome have focused on only one driver, and no studies have hitherto compared the importance of multiple drivers at multiple spatial scales. Our knowledge of how drivers act together on the sponge prokaryote community thus represents an important hiatus. This fundamental knowledge, however, is essential to work on the main future directions in sponge holobiont research, recently identified by Pita et al. (2018). They highlighted the importance of determining how environmental factors alter

microbiome-mediated processes and the need to develop management solutions, which will ensure the maintenance of sponge holobiont functions at the ecosystem level (Pita et al. 2018). Furthermore, understanding the interplay between the various drivers is not only relevant for sponges, but also for other reef organisms whose microbial communities may be directly affected by those of sponges (Chapter 6 of this thesis).

In this study, we hypothesize that the relative contribution of drivers such as host identity and environmental conditions to the sponge prokaryotic community can shift at different spatial scales. This hypothesis was tested with a sampling design that includes sampling closely related and sympatric sponge species across multiple spatial scales. The widespread occurrence and recently unraveled phylogeny of giant barrel sponges (*Xestospongia* spp.) make them an ideal group for this purpose (Chapter 2 of this thesis). The most dominant prokaryotic phyla in giant barrel sponges include Proteobacteria, Actinobacteria, Chloroflexi, Nitrospirae, and Cyanobacteria, which were also among the most dominant groups in other high microbial abundance (HMA) sponge species (Thomas et al. 2016, Moitinho-Silva et al. 2017a, Chapter 4 of this thesis). Various drivers have been suggested to impact the giant barrel sponge prokaryote community, including host identity, geography (the location at which a sponge is collected) and depth (Montalvo and Hill 2011; Fiore et al. 2013a; Morrow et al. 2016; Chapter 4 of this thesis). Hence, giant barrel sponges can serve as a model to study the interplay between spatial and phylogenetic variation as drivers of sponge prokaryote communities.

METHODS

Study object

Giant barrel sponges are found across the entire Caribbean and are known as *Xestospongia muta*. However, this species consists of three reproductively isolated groups that have different morphologies and sterol compositions, and are believed to act as separate species (Fromont et al. 1994; López-Legentil and Pawlik 2009, Deignan et al. 2018; Chapter 2 of this thesis). In the Indo-Pacific, giant barrel sponges occur from the east coast of Africa and the Red Sea to Taiwan and New Caledonia and are referred to as *Xestospongia testudinaria*. This species complex consists of at least six genetically isolated groups, in some places with distinct morphological differences (Swierts et al. 2013; Bell et al. 2014; Chapter 2 this thesis). Although the different species have not yet been named, we use the variation in this species complex for our host-phylogenetic analyses.

A third described species, *Xestospongia bergquistia*, is confined to the Australian coast and, in contrast to other giant barrel sponges, lacks spongin fiber between the skeletal spicules (Fromont 1991). All of our samples contained spongin fiber; hence *X. bergquistia* was not included in the specimens used in the present study. Giant barrel sponges occur along a large depth range, from just below the surface, to depths exceeding 120 m. They are found in both clear and turbid waters and seem resilient to external stressors (McMurray et al. 2015).

Sample collection

We assessed the importance of host identity and geography on the prokaryotic community of giant barrel sponges by comparing their prokaryotic communities at different spatial scales. On a global scale, we tested samples from three Caribbean giant barrel sponge species against two Indo-Pacific species. At a regional scale, we compared the prokaryotic communities of three giant barrel sponge species from the Caribbean islands Curaçao and Martinique. And at a local scale, we compared giant barrel sponges from multiple reef sites within Curaçao, Martinique, and Thailand, respectively. Samples were collected in Curaçao, Martinique, and Thailand between September 2016 and March 2017 (Fig. 5.1a). In Curaçao, we collected 32 samples from six sites, in Martinique 25 samples from five sites and in Thailand sixteen samples from four sites (Fig. 5.1b-d). We chose different experimental setups in each of these localities in order to additionally explore multiple potential nominally defined environmental drivers. This was given preference over a uniform setup to compare one single driver as a proxy for environmental variation across these three localities. In Thailand, our setup focused on distance to shore, in Curaçao on coastal development and in Martinique on depth and light availability. The sponges in shallow water (<40 m) were sampled with an apple corer by SCUBA diving and the sponges from deeper water in Martinique (>80 m) were collected by dredging. The collected tissue was immediately stored in ethanol (98%) and kept in a cooler until they were finally stored at -20°C in the laboratory. A detailed description of the sites in Curaçao, Martinique, and Thailand is available in Appendix 5.1.

DNA analysis

Each sponge was barcoded for the I3-M11 partition of the mitochondrial CO1-gene and assigned to a species following the protocol and classification described in Chapter 2 of this thesis. Prokaryotic DNA extraction was performed as described in Chapter 4 of this thesis.

The 16S rRNA gene V3V4 variable region PCR primers 341F 5'-CCTACGGGNGGCWGCAG-3' and 785R 3'-GACTACHVGGGTATCTAATCC-5' with barcode on the forward primer were used in a 28 cycle PCR assay (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Pooled and purified PCR product was used to prepare the DNA library following the Illumina TruSeq DNA library preparation protocol. Next-generation, paired-end sequencing was performed at mrDNA Molecular Research LP (http://www.mrdnalab.com/; last checked 2016 11 18) on an Illumina MiSeq device (Illumina Inc, San Diego, CA, USA) following the manufacturer's guidelines. Sequences from each end were joined following Q25 quality trimming of the ends followed by reorienting any 3'-5' reads

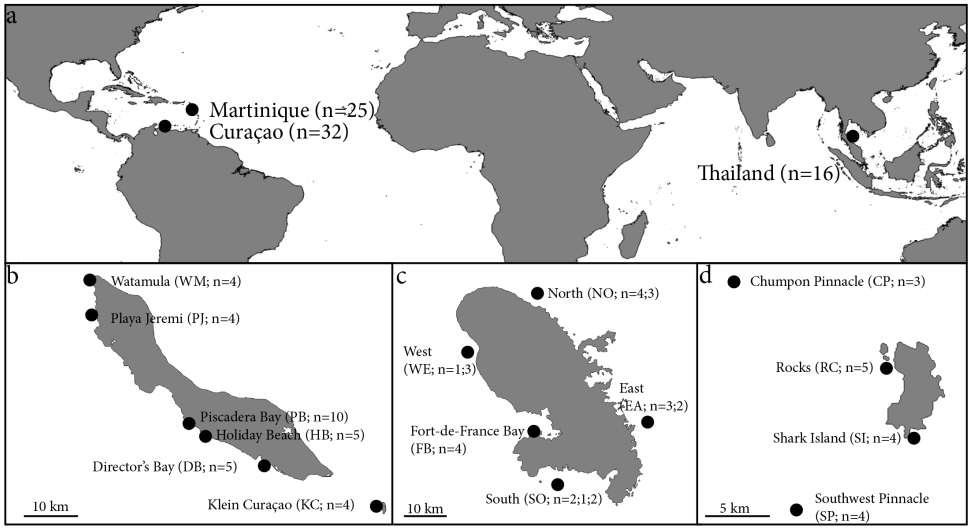


Figure 5.1. Maps indicating the sampled localities in the global oceans (a) and the sampling sites at the localities Curaçao (b), Martinique (c) and Thailand (d).

back into 5'-3', and removal of short reads (< 150 bp). The resultant files were analyzed using the QIIME (Quantitative Insights Into Microbial Ecology; (Caporaso et al. 2010) software package (http://www.qiime.org/; last checked 2017-01-20).

In QIIME, fasta and qual files were used as input for the split_libraries.py script. Default arguments were used except for the minimum sequence length, which was set at 250 bps after removal of forward primers and barcodes. In addition to user-defined cut-offs, the split_libraries.py script performs several quality filtering steps (http://qiime.org/scripts/split_libraries.html). OTUs were selected using the UPARSE pipeline (https://www.drive5.com/usearch/manual7/uparse_pipeline.html; last checked 2018 07 05) with usearch10 (Edgar 2013). The UPARSE pipeline (Edgar 2013) includes clustering, chimera checking and quality filtering on de-multiplexed sequences. Chimera checking was performed using the UCHIME algorithm (Edgar et al. 2011). Quality filtering in usearch10 filters noisy reads and results suggest its output is comparable to other denoisers such as AmpliconNoise, but is much less computationally expensive (Edgar and Flyvbjerg 2015). First, reads were filtered with the -fastq_filter command and the following arguments -fastq_trunclen 250 -fastq_maxee 0.5 -fastq_truncqual 15. Sequences were then dereplicated and sorted using the -derep_fulllength and -sortbysize commands. OTU (Operational Taxonomic Unit) clustering was performed using the -cluster_otus command followed by the -usearch_global command (using global alignment) with id set to 97% to map reads back to OTUs. AWK scripts were then used to convert the OTU files to QIIME format. In QIIME, representative sequences were selected using the pick_rep_set.py script in QIIME using the 'most_abundant' method.

Taxonomy was assigned to reference sequences of OTUs using default arguments in the assign_taxonomy.py script in QIIME with the rdp method (Wang et al. 2007). In the assign_taxonomy.py function, we used a fasta file containing reference sequences from the SILVA 128 QIIME release and the uclust classifier method to map sequences to the assigned taxonomy. The make_otu_table.py script in QIIME was used to generate a square matrix of OTUs x SAMPLES, followed by the single_rarefaction.py script to rarefy each sample to 25000 sequences. The rarefied table was used as input for further analyses. The DNA sequences generated in this study can be downloaded from the NCBI SRA: PRJNA554009.

Analyses

We performed our analyses on the prokaryotic community as a whole and on a subset that only included core Operational Taxonomic Units (OTUs) to assess what types of OTUs varied. In our dataset, an OTU was considered a core OTU when it occurred in each sample. Average relative abundances were calculated per bacterial phylum and class at global (Caribbean vs. Indo-Pacific; >15,000 km) and regional (Curaçao vs. Martinique; 800-1,000 km) scales. Mann-Whitney *U* tests were used to compare whether the abundances of these bacterial phyla and classes in the groups had similar distributions. The Mann-Whitney *U* tests were executed with the wilcox.test-function in the R package *stats*. The significance level was set at 0.05 and the p-values were corrected using the Benjamini-Hochberg procedure with an allowed false discovery rate of 0.1 (Benjamini and Hochberg 1995).

To compare beta diversity, we used principal coordinates analysis, an exploratory method, and mantel tests, an interpretative method (Paliy and Shankar 2016). Principal coordinates analysis is a preferred method in microbial ecology due to the capacity to use community composition measures and phylogenetic distances to calculate similarity or dissimilarity among microbial populations. The mantel test compares the microbial community distance matrix with an additional distance matrix of an independent set of variables. Both techniques complement each other in the analysis of microbial communities (Paliy and Shankar 2016). PCO ordinations were constructed for 'all samples' and for subsets of only 'Caribbean samples', 'Curaçao samples', 'Martinique samples' and 'Thailand samples'. PCO-values were calculated using the square root transformed data of 'all OTUs' and for a subset of 'core OTUs'. For this purpose, we used the ordinate-function in the R package *phyloseq* with the MDS method and Bray-Curtis distance (McMurdie and Holmes 2013). The PCO ordinations of the subset of core OTUs were plotted using different color codes to visualize differences regarding 'host-identity' and 'geography'. For each of the 'localities' (Curaçao, Martinique, Thailand), we also color-coded the samples for different local sites to explore which environmental factors are involved in driving compositional variation in the giant barrel sponge prokaryotic community. Using the adonis-function in the R package *phyloseq*, we performed PERMANOVA-tests comparing the PCO-values with location, host identity and, only for the 'local' subsets, different environmental factors. In Thailand, inshore

locations had a distance of <500 m to shore, and offshore locations of >5 km. In Curaçao, locations were considered city locations when they were located within 1 km of the urban area of Willemstad, the capital city of the island, and non-city locations when they were >20 km from the urban area of the city. In Martinique, samples from shallow reefs were sampled at a depth of <30 m, from deep reefs at a depth of >90 m and shallow caves at a depth of <30 m. We performed these PERMANOVA-tests on the PCO values of 'all OTUs' and of the subsets including only 'core OTUs' to compare which types of OTUs varied. The number of permutations was set at 9999 and the Benjamini-Hochberg Procedure was used as a post hoc test with an allowed false discovery rate of 0.1 (Benjamini and Hochberg 1995).

We tested for significant differences among local sites and host species in individual core OTUs in Curaçao, Martinique, and Thailand with an analysis of deviance using the glm-function in R-package *stats v3.6.0*. We first applied a generalized linear model (GLM) with the family argument set to 'quasibinomial'. In the 'quasibinomial' family, the dispersion parameter is not fixed at one so that it can model over-dispersion. Using the glm models, we tested for significant variation among local sites using the ANOVA-function in R with the F test, which is most appropriate when dispersion is estimated by moments, as is the case with quasibinomial fits.

In order to assess to what extent beta diversity or the variation in composition could be explained by geography, phylogeny or its combination, we constructed Bray-Curtis dissimilarity matrices of the prokaryotic communities using the vegdist-function in the R package *vegan,* geographic distance matrices using the distm-function in the R package *geosphere* with the Haversine-function and genetic distance matrices (phylogeny) using the dist.hamming-function in the R-package *phangorn* (Schliep 2011). The hamming-function is based on Hamming distance, which is a metric describing the minimum number of mutations required to convert one sequence into another sequence (Hamming 1950). For each set of samples, we used the multi.mantel-function from the R-package *phytools* (Revell 2012) to assess the amount of variation jointly explained by spatial and phylogenetic matrices. We then used the mantel.partial-function in the R-package *vegan* to obtain the amount of variation explained by distance only after partialing out the variation explained by phylogeny and the amount of variation explained by phylogeny only after partialing out the variation explained by distance. Through variance partitioning (Borcard et al. 1992), we subsequently obtained the amount of variation explained by distance only, phylogeny only, by distance and phylogeny combined (the spatially structured phylogenetic signal) and unexplained at all spatial scales from global to local.

Lastly, we used the simper-function in the R package *vegan* to identify significantly discriminating OTUs between sites, species, and environments within Curaçao, Martinique and, Thailand with the number of permutations set at 999 (Oksanen et al. 2007).

RESULTS

Dataset

In total, we retrieved 1,825,000 sequences, evenly distributed over 73 giant barrel sponge samples. These sequences were assigned to 8970 OTUs. The OTUs were assigned to 54 phyla, 135 classes and 194 orders (Appendix 5.2). The phylum Proteobacteria was the most abundant and diverse of all phyla with 921,510 sequences assigned to 4687 OTUs. Other diverse phyla included Bacteroidetes (760 OTUs), Planctomycetes (551), Acidobacteria (471), Chloroflexi (467), Actinobacteria (288) and Gemmatimonadetes (216) (Appendix 5.2). Although diverse, Planctomycetes and Bacteroidetes were not abundant with only 835 and 4039 sequence reads, respectively. In contrast, Nitrospirae (76), Cyanobacteria (137) and Tectomicrobia (46) were not diverse, but abundant with 274,497 (15.0% of all sequences), 76982 (4.2%) and 12288 (0.67%) sequence reads, respectively (Appendix 5.2). There was a clear positive relationship between OTU abundance and the number of samples in which the OTU was present (Appendix 5.3). In other words, abundant OTUs were also widespread.

Following our definition, there were 98 OTUs in the core (1.3% of the total number of OTUs), which together accounted for 1,447,732 sequences (79.3% of the total number of sequences; Appendix 5.2). The core consisted of OTUs assigned to the phyla Proteobacteria (63), Actinobacteria (11), Chloroflexi (10), Nitrospirae (7), Cyanobacteria (4), Acidobacteria (1), PAUC34f (1) and SBR1093 (1) (Appendix 5.2). The most abundant core members belonged to multiple bacterial phyla and classes (Appendix 5.4). In our dataset, between 49.8% and 85.5% of the giant barrel sponge prokaryotic community consisted of OTUs that were found in all of the sampled giant barrel sponges (Appendix 5.2).

Differences in total prokaryote community composition across scales

In the PCO analysis, samples from the Caribbean and the Indo-Pacific formed separate clusters (Fig. 5.2a). The first axis, which split the two oceans, explained 18.3% of the variation in the data set. The second axis, which explained 17.2% of the variation, was mostly related to the higher abundances of Chloroflexi (positive PCO values) or Cyanobacteria (negative PCO values) (Fig. 5.2e). The ocean of origin was a significant predictor of prokaryotic community composition and explained 19.6% of the variation in composition (Table 5.1). Dissimilarity between the prokaryotic communities of specimens was larger when they were spatially and genetically further apart (Fig. 5.3a,b). The relative abundances of Nitrospirae and SBR1093 were higher in the Indo-Pacific, whereas Gemmatimonadetes, PAUC34f, and Tectomicrobia were more abundant in the Caribbean (Table 5.2). Although 98 OTUs were present in all of our samples around the globe, 47 additional OTUs were present in all samples from the Caribbean but were not present in all samples from Thailand. An additional 65 OTUs were found in all samples from Thailand, but not in all Caribbean samples (Fig. 5.4). At this global scale, host identity explained more variance than geography (Fig. 5.2c; Table 5.1). This result, however, is inflated due to the fact that no species were shared between the two

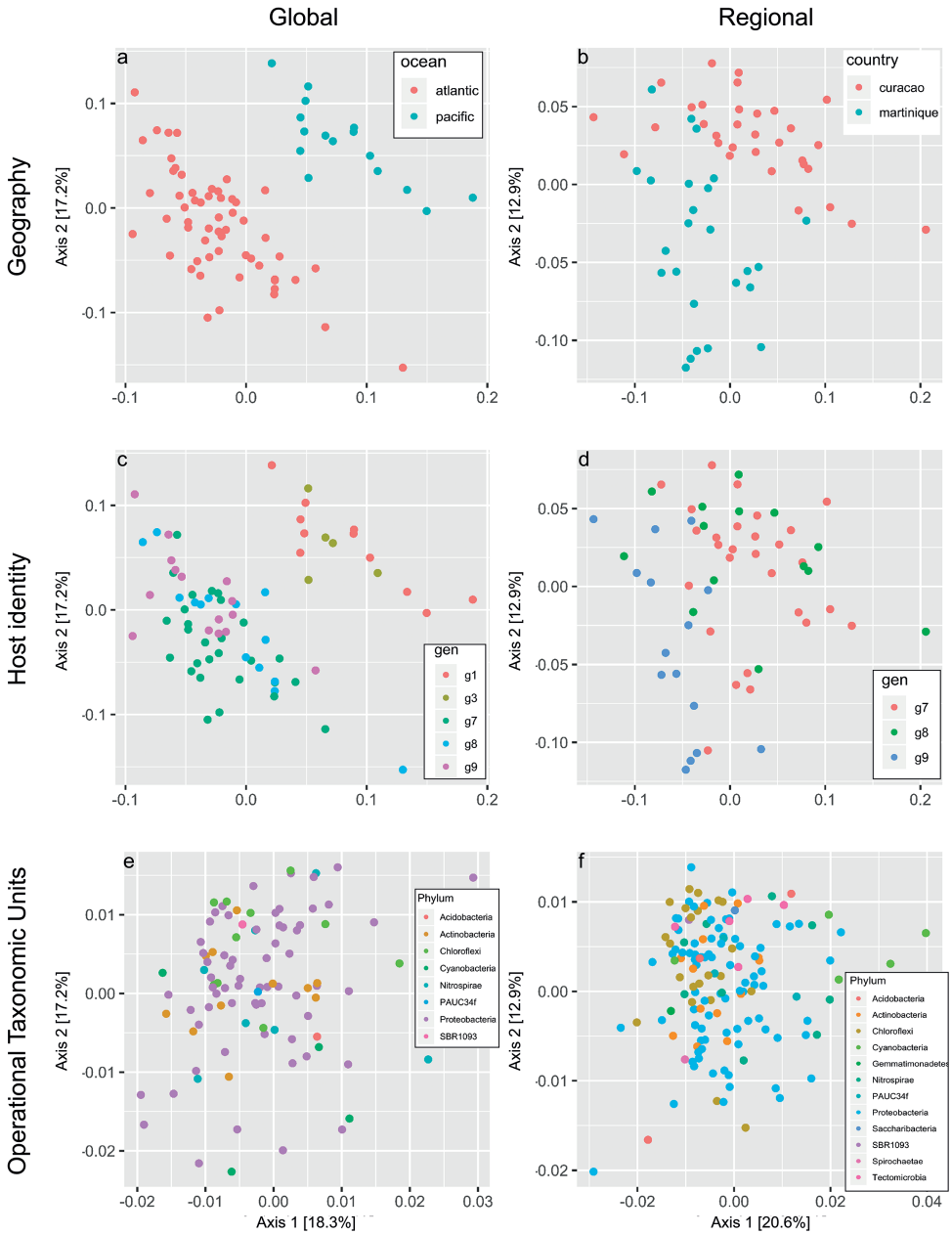


Figure 5.2. First and second axes of the Principal Coordinates Analysis based on our full dataset (a,c,e) and a subset including samples from the Caribbean (b,d,f). Each dot in the (a,b,c,d) graphs represents one sponge individual, and their positioning in the ordination is identical for (a) and (c) and for (b) and (d), the only difference being the color schemes. Colors in (a) indicate the ocean of origin and in (c) the species. Colors in (c) indicate the locality of origin and in (d) the species. In graphs (e) and (f) each dot indicates one core Operational Taxonomic Unit, color coded for phylum.

Table 5.1. Table including the statistical values from PERMANOVA test comparing the Principal Coordinates Analyses with geographic and phylogenetic variation. Asterisks indicate level of significance (* = <0.05; ** = <0.001; * = <0.001) after correcting for the number of tests using the Benjamini-Hochberg procedure with an allowed false discovery rate of 0.1.

	Geography				Phylogeny				Other			
Global	**F-value**	**R^2**	**p-value**		**F-Value**	**R^2**	**p-value**		**F-Value**	**R^2**	**p-value**	
Caribbean vs. Indo-Pacific	17.34	0.196	<0.001	***	6.81	0.286	<0.001	***				
Caribbean vs. Indo-Pacific core	10.51	0.129	<0.001	***	4.81	0.220	<0.001	***				
Regional												
Curaçao vs. Martinique	6.48	0.105	<0.001	***	3.27	0.108	<0.001	***				
Curaçao vs. Martinique core	6.50	0.106	<0.001	***	3.41	0.112	<0.001	***				
Local												
Thailand									**Inshore / Offshore**			
Thailand all	1.62	0.288	0.022	*	2.07	0.129	0.019	*	1.12	0.074	0.312	
Thailand all core	1.72	0.301	0.021	*	2.15	0.133	0.031	*	1.12	0.074	0.307	
Curaçao									**City / Non-city**			
Curaçao all	1.90	0.268	<0.001	***	2.02	0.123	0.017	*	1.37	0.044	0.149	
Curaçao all core	1.77	0.254	0.006	**	2.02	0.122	0.024	*	1.23	0.039	0.222	
Piscadera Bay all					2.13	0.379	0.033	*				
Piscadera Bay core					1.94	0.357	0.065					
Martinique									**Depth (deep / reef / cave)**			
Martinique all	1.21	0.194	0.112		1.34	0.109	0.077		1.76	0.138	0.006	**
Martinique all core	1.23	0.197	0.110		1.32	0.107	0.105		1.85	0.144	0.004	**
Martinique reef	1.63	0.419	0.015	*	1.12	0.170	0.289					
Martinique reef core	1.65	0.423	0.013	*	1.11	0.168	0.318					
Martinique deep all	0.95	0.363	0.555		0.96	0.243	0.543					
Martinique deep core	0.96	0.366	0.536		0.92	0.235	0.585					

oceans, which g1 and g3 occurring in the Pacific and g7, g8 and g9 occurring in the Atlantic. Within each ocean, no clustering by host identity seemed to occur (Fig. 5.2c).

At the regional scale, the giant barrel sponges from Curaçao and Martinique formed two separate clusters, primarily separated by the second axis which explained 12.9% of the variation (Fig. 5.2b). The island of origin was thus a significant predictor of the prokaryotic community composition and explained 10.5% of the variation in composition (Table 5.1). The first axis, which explained 20.6% of the variation was also related to the higher abundances of Chloroflexi (negative PCO values) or Cyanobacteria (positive PCO values) (Fig. 5.2f). Samples from Curaçao housed on average more than twice as many Cyanobacteria as samples from Martinique, while the latter harbored more Gammaproteobacteria and Nitrospinae (Table 5.2). In addition to the 145 OTUs that were present in each of the Caribbean samples, 36 other OTUs were also present in each sample from Martinique and five in each sample from Curaçao (Fig. 5.4). Host identity was also a significant predictor of the prokaryotic community and explained 10.8% of the variation in composition (Table 5.1). Species that were genetically and spatially closer had more similar prokaryotic community compositions (Fig. 5.3c,d). At this regional scale, host identity explained more variance than geography (Table 5.1), but also here this result is, at least partially, overestimated due to the unequal distribution of species among the localities.

In Curaçao, samples clustered by reef site along the first axis, which explained 32.0% of the variation (Fig. 5.5a). The second axis explained 8.7% of the variation and separated samples of the species 'g7' and 'g8' (Fid. 5.5d). The factors site and host identity were significant determinants of prokaryotic community composition (Table 5.1). Accordingly, sponges genetically (but not spatially) further apart had more dissimilar prokaryotic communities (Fig. 5.3e). The impacts of sampling site irrespective of geographic distance suggest that local environmental conditions play a role in structuring the prokaryote community (Fig. 5.3f). However, there was no apparent association between coastal development (i.e., whether a sponge was located near the city of Willemstad or in an area with low coastal development) and the variation in prokaryote composition (Fig. 5.5f; Table 5.1). Instead, the proportion of giant barrel sponge, coral, and algal covers varied among the sampled sites in Curaçao. A high abundance of giant barrel sponge individuals was found on reefs with high soft coral cover, high fleshy and calcareous algal cover, and a lower abundance of giant barrel sponges on reefs with low soft coral cover, low fleshy and calcareous algal cover, and high turf algal cover (Appendix 6.1). These factors, or other local environmental variables, which were not measured, may play a role in structuring the prokaryote community. Zooming in to one local community, at Piscadera Bay, showed that when removing most of the geographic variation, the sponges significantly clustered together based on host identity (Table 5.1; Appendix 5.5).

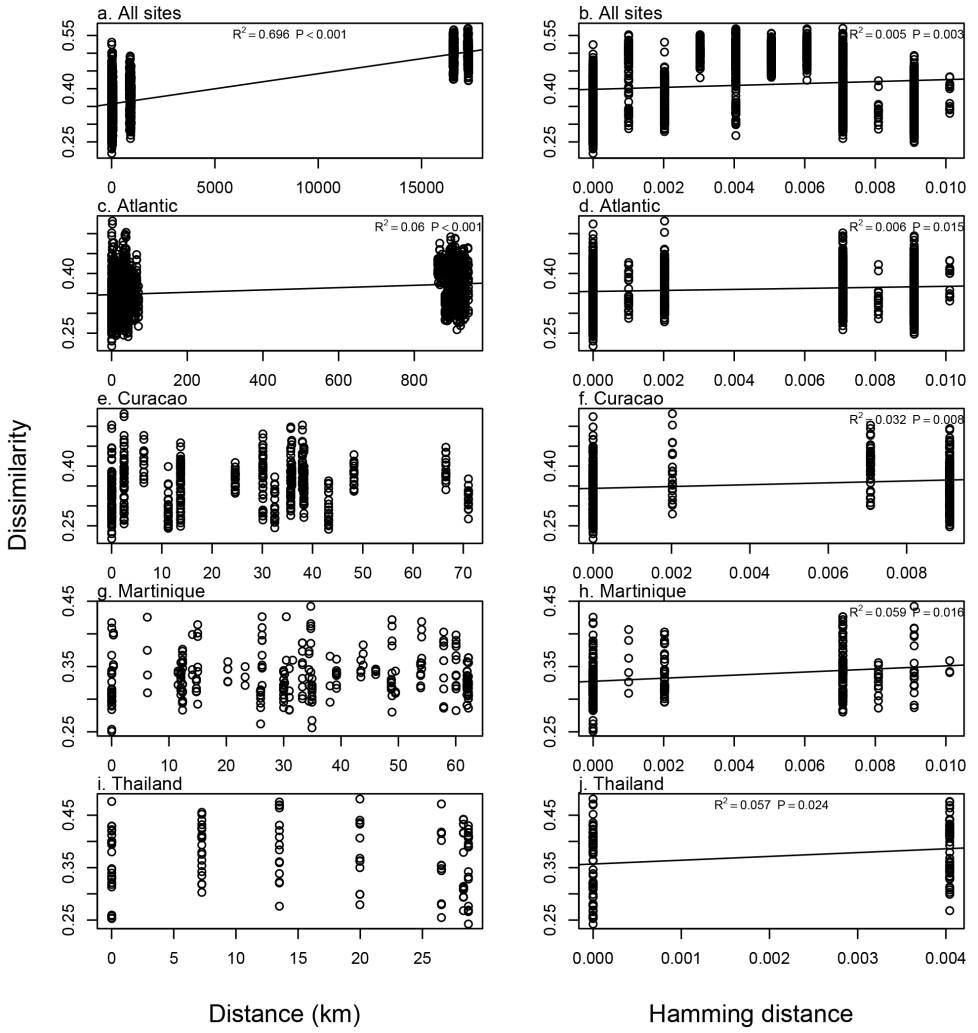


Figure 5.3. Scatterplots of dissimilarity x geographic distance and dissimilarity x genetic distance for 'all samples (global)' and for subsets of only 'Caribbean samples', 'Curaçao samples', 'Martinique samples' and 'Thailand samples'. We added regression lines that were calculated with the lm-function and the method set at "qr" to the plots if they had a significant fit, indicating a positive or negative relationship between geographic or genetic distance and dissimilarity to core prokaryotic community composition.

In Martinique, samples did not cluster according to sampling site or host identity (Fig. 5.5b,e; Table 5.1). Sponges genetically (but not spatially) further apart, however, had more dissimilar prokaryotic communities (Fig. 5.3g,h). Depth, however, proved a significant predictor of variation in prokaryote composition and explained 13.8% of the variation in composition (Fig. 5.5h; Table 5.1). Deeper waters were characterized by greater relative abundances of Nitrospirae, Tectomicrobia, and Spirochaetae, while sponges from shallower waters harbored

Table 5.2. Table including the average abundance of various bacterial phyla and classes in different regions and localities. Asterisks indicate significance level of the p–values after T-tests comparing the average abundance between the regions and localities and correcting for the number of tests using the Benjamini-Hochberg procedure with an allowed false discovery rate of 0.1 (* = <0.05; ** = <0.001; * = <0.001).

	Global			Regional		
Phylum/*Class*	**Caribbean**	**Pacific**		**Curaçao**	**Martinique**	
Proteobacteria	**50.28%**	**51.27%**		**49.05%**	**51.85%**	*
Gammaproteobacteria	34.48%	36.30%		32.71%	36.74%	***
Alphaproteobacteria	8.33%	9.13%		8.14%	8.58%	
Deltaproteobacteria	5.88%	4.48%	**	6.25%	5.41%	
JTB23	1.29%	1.04%		1.58%	0.91%	***
Betaproteobacteria	0.17%	0.09%		0.26%	0.05%	**
Epsilonproteobacteria	0.10%	0.14%	***	0.08%	0.13%	
Actinobacteria	**17.34%**	**17.66%**		**16.04%**	**18.99%**	**
Nitrospirae	**13.74%**	**19.68%**	***	**14.50%**	**12.76%**	
Chloroflexi	**6.94%**	**5.36%**	**	**7.28%**	**6.50%**	*
Caldilineae	4.01%	3.77%		4.15%	3.84%	
SAR202 clade	2.10%	1.09%	***	2.22%	1.93%	
Anaerolineae	0.61%	0.36%		0.67%	0.54%	
TK10	0.17%	0.12%		0.18%	0.15%	
Cyanobacteria	**4.84%**	**2.00%**	*	**6.34%**	**2.92%**	***
Gemmatimonadetes	**2.30%**	**0.58%**	***	**1.99%**	**2.69%**	*
Acidobacteria	**1.54%**	**1.36%**		**1.84%**	**1.16%**	
Holophagae	1.42%	1.22%		1.70%	1.06%	
Solibacteres	0.06%	0.07%		0.06%	0.06%	
Tectomicrobia	**0.86%**	**0.02%**	***	**0.78%**	**0.95%**	
Saccharibacteria	**0.36%**	**0.60%**		**0.39%**	**0.33%**	
PAUC34f	**0.46%**	**0.14%**	***	**0.50%**	**0.42%**	
Spirochaetae	**0.37%**	**0.33%**		**0.31%**	**0.45%**	**
SBR1093	**0.20%**	**0.31%**	**	**0.21%**	**0.20%**	
Bacteroidetes	**0.22%**	**0.22%**		**0.20%**	**0.25%**	
Bacteroidetes Incertae Sedis	0.10%	0.03%	***	0.07%	0.15%	
Flavobacteriia	0.07%	0.12%		0.05%	0.08%	
Poribacteria	**0.13%**	**0.05%**	*	**0.16%**	**0.11%**	
Nitrospinae	**0.09%**	**0.06%**		**0.06%**	**0.11%**	***
Parcubacteria	**0.08%**	**0.05%**	**	**0.10%**	**0.06%**	

more Cyanobacteria. When separating the dataset into shallow and deep locations, sampling site became a significant predictor of the prokaryotic community in shallow reef sponges, but not in deep reef sponges (Table 5.1). Host identity was not a significant driver at either depth range (Table 5.1).

In Thailand, samples from the four sites were separated by the first PCO axis (which explained 23.6% of the variation), although there was overlap among samples from different sites

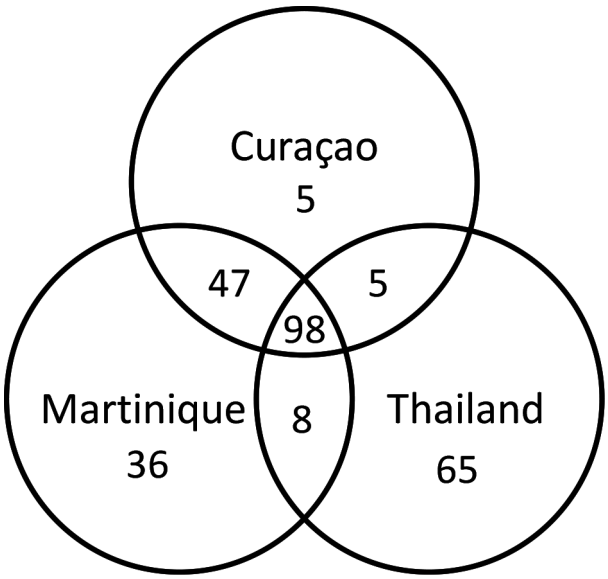


Figure 5.4. Venn-diagram indicating the number of core OTUs that are shared between Curaçao, Martinique and Thailand, or are unique to one of the sites.

(Fig. 5.5c). The second axis, which explained 18% of the variation, split the two species, although one sample from species g3 did not follow this pattern (Fig. 5.5f). Both sample site and host identity were significant predictors of the prokaryotic community and explained 28.8% and 12.9% of the variation in composition, respectively (Table 5.1). As in Curaçao, sponges spatially further apart did not have more dissimilar prokaryotic communities, suggesting that the sample site is a determinant of prokaryotic community composition due to local differences in environmental conditions that appear unrelated to geographic distance (Fig. 5.3i). Samples genetically further apart did have more dissimilar prokaryotic community compositions (Fig. 5.3j). Whether the sample originated in an inshore or offshore location did not appear to affect prokaryote composition (Fig. 5.5i; Table 5.1).

Patterns for the core community compared to the total community

Core and total prokaryote communities largely followed the same trends with respect to sample site, host identity or other drivers. The only exception was the subset of samples from Piscadera Bay in Curaçao, which showed a significant impact of host identity on the total prokaryote community, but not on the core community (Table 5.1). This subset, however, only consisted of ten samples, and this small number may have caused the lack of statistical support in the core community. Nonetheless, this general congruence of patterns between the core and total prokaryotic community indicates that the drivers not only affected the presence or absence of OTUs, but that they also affected the relative abundance of core OTUs.

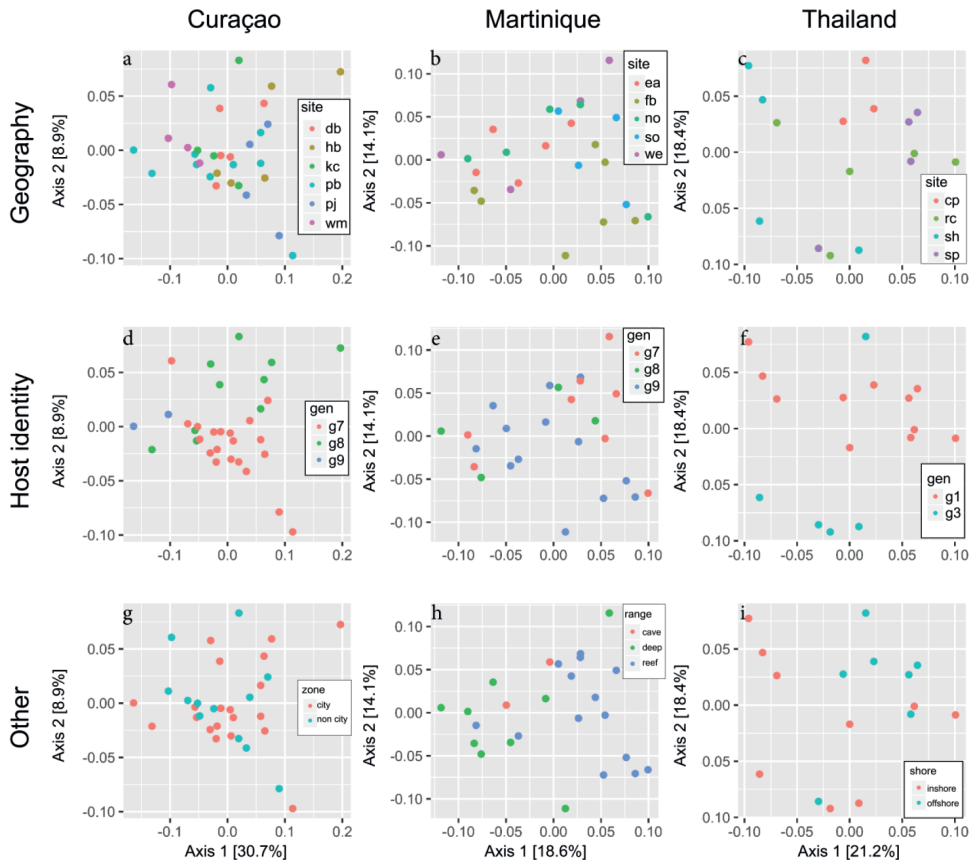


Figure 5.5. First and second axes of the Principal Coordinates Analyses based on the subsets subset including samples from Thailand (a,b,c), Curaçao (d,e,f) and Martinique (g,h,i). In each graph one dot represents one sponge individual, and their positioning in the ordination is identical for the graphs of Thailand (a,b,c), Curaçao (d,e,f) and Martinique (g,h,i), the only difference being the color schemes. Colors in (a), (d) and (g) indicate the site at which the specimens were sampled. Abbreviations of the sites are: cp = Chumpon Pinnacle, rc = Twin Rocks, sh = Shark Island, sp = Southwest Pinnacle, db = Director's Bay, hb = Holiday Beach, kc = Klein Curaçao, pb = Piscadera Bay, pj = Plaja Jeremy, wm = Watamula, ea = East, no = North, so = South, sw = Southwest, fb = Fort-De-France Bay. Colors in (b), (e) and (h) indicate the species identity of the host. Colors in (c) urban and rural environments. Colors in (f) indicate depth category or cave environment. Colors in (i) indicate whether the sponge sampled was from an inshore or offshore reef.

Core OTUs that differed with sample site and host identity

The abundance of some individual core OTUs in the localities Curaçao, Martinique and Thailand varied significantly with sample site and/or host identity based on the SIMPER analyses. In Curaçao, 2.6-19.0% of the core OTUs varied in abundance between two individual sites and 17.0-23.5% between two individual host species. In Martinique, these abundances varied between 4.4-9.9% and 7.2-7.7% respectively, and in Thailand, 4.9-9.8% and 17.2%.

Table 5.3. Table showing the amount of variation explained by 'distance only', 'phylogeny only', 'by distance and phylogeny combined (the spatially structured phylogenetic signal)' and 'unexplained' at all spatial scales from global to local according to our mantel tests. Asterisks indicate level of significance (* = <0.05; ** = <0.001; * = <0.001).

			F	R2	Pr(>|t|)	
All	Multi-mantel		2972.62	0.7	0.001	***
	Partial mantel	Distance only		0.696	0.001	***
		Phylogeny only		0.005	0.003	**
		Distance + Phylogeny		-0.002		
Atlantic	Multi-mantel		57.15	0.069	0.001	***
	Partial mantel	Distance only		0.06	0.001	***
		Phylogeny only		0.006	0.015	*
		Distance + Phylogeny		0.002		
Thailand	Multi-mantel		3.829	0.061	0.052	
	Partial mantel	Distance only		0	0.657	
		Phylogeny only		0.057	0.024	*
		Distance + Phylogeny		0.005		
Curaçao	Multi-mantel		9.46	0.037	0.025	*
	Partial mantel	Distance only		0	0.127	
		Phylogeny only		0.032	0.008	**
		Distance + Phylogeny		0.005		
Martinique	Multi-mantel		9.075	0.062	0.024	*
	Partial mantel	Distance only		0	0.176	
		Phylogeny only		0.059	0.016	*
		Distance + Phylogeny		0.004		

Eight OTUs varied significantly between at least two individual reef sites in each of the three localities. These OTUs were assigned to Proteobacteria (4), Nitrospira (2), SBR1093 (1) and Acidobacteria (1). OTU-108, a member of the order HOC36 within the Gammaproteobacteria, was the only core OTU that differed significantly with host identity in all three localities. This OTU was especially abundant in 'species g3' from Thailand and 'species g9' from Curaçao and Martinique, two genetically closely related species.

Twenty-six of the 181 core OTUs in Martinique significantly discriminated samples from different depths according to a SIMPER analysis. These OTUs were assigned to the Proteobacteria (16), Actinobacteria (3), Nitrospirae (2), Bacteroidetes (2), Chloroflexi (1), Spirochaetae (1) and Tectomicrobia (1).

Assessing the importance of distance and phylogeny using distance matrices

On a global scale there was a highly significant difference in composition between the total prokaryotic communities in different oceans. Geographic distance alone (the difference between sample sites after removing the variation attributable to phylogeny) was a significant predictor of compositional variation and explained 69.6% of this variation (Table 5.3; Fig. 5.3). Although phylogeny, when considered alone, was also a significant predictor, it only explained 0.5% of the variation in composition. The spatially structured phylogenetic component explained none of the variation in composition. At the regional scale, distance alone was also a significant predictor of compositional variation, but only explained 6% of the variation. Phylogeny alone was also a significant predictor at the regional scale, but only explained 0.6% of the variation with the spatially structured phylogenetic component explained 0.2% of the variation. In contrast to the global and regional scales, geographic distance was not a significant predictor of compositional variation between local sites in any of the three locations (Curaçao, Martinique, and Thailand). In contrast, the phylogeny alone component was a significant predictor at all three locations and explained between 3.2 and 5.9% of the variation in composition. The spatially structured phylogenetic component explained between 0.4 and 0.5% of the variation in composition.

DISCUSSION

The importance of geography vs phylogeny across scales

In the present study, we found that distance was a highly significant predictor of compositional variation in giant barrel sponge prokaryote communities at the global scale (distances >15,000 km) (Fig. 5.6). This finding is in line with a number of studies highlighting the importance of spatial processes in structuring communities of macrobes and microbes in sponges (e.g. Fiore et al. 2013a; Luter et al. 2015; Lesser et al. 2016; Morrow et al. 2016; Chapter 4 of this thesis). At the regional scale (distances 800-1,000 km), distance also proved a significant predictor of compositional variation albeit explaining much less variation (Fig. 5.6). At the local scale (distances 2-70 km), geographic distance did not contribute significantly to variation in the prokaryotic composition (Fig. 5.6). This pattern contrasts starkly with similar studies of terrestrial (Condit et al. 2002; Cleary et al. 2004; Cleary and Priadjati 2005; Keil et al. 2012) and marine (Becking et al. 2006; Watson et al. 2011) plants and animals, and marine microeukaryote communities (Zhang et al. 2018). These studies showed a rapid increase in dissimilarity at very small spatial scales followed by a very long tail where there was relatively little change in dissimilarity. One of the reasons for this relative lack of change is that similar environments are encountered at greater distances, for example, multiple mountain tops are encountered with similar communities. This relative lack of change is hypothesized to continue until, at very large scales, biogeographical barriers or climatic gradients further increase dissimilarity (Nekola and White 1999). The existence of a significant biogeographical barrier between Caribbean and Indo-Pacific populations of

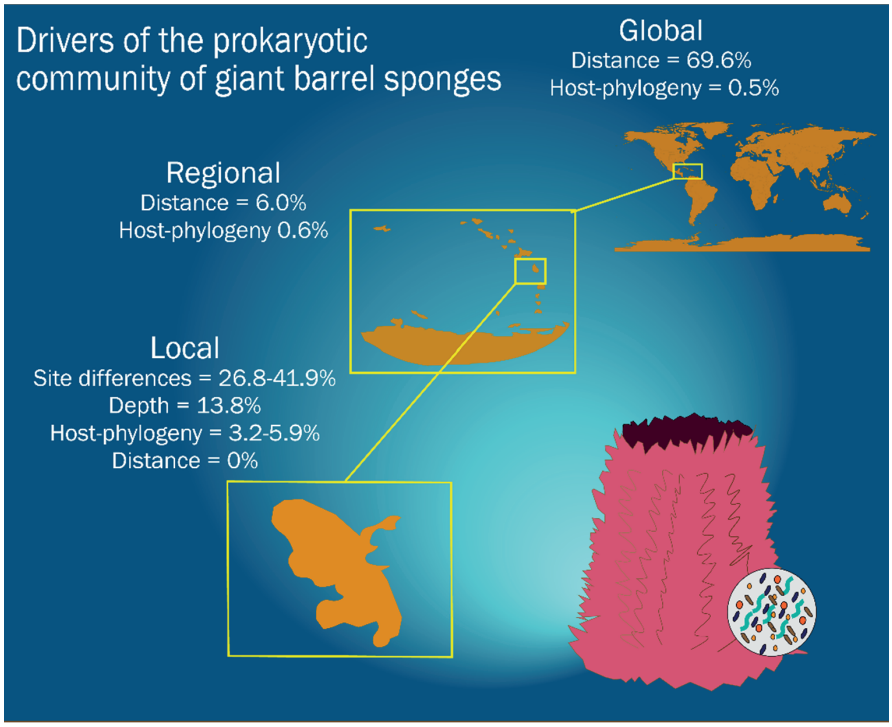


Figure 5.6. Overview of drivers of the prokaryotic community of giant barrel sponges (*Xestospongia* spp.) on different spatial scales.

giant barrel sponges is the most probable explanation for the pronounced distance-decay at a global scale. Although a biogeographical barrier is less obvious at a regional scale within the Caribbean basin, the significant geographic differences between Curaçao and Martinique might indicate that connectivity between Caribbean islands is somewhat more restricted than previously thought.

In contrast to the above, phylogenetic differences among host species explained only a very small amount of variation in composition at regional and global scales, after removing the spatial component. However, they were a more important determinant of compositional variation at local scales. These results indicate that the relative contribution of drivers such as distance and host phylogeny can shift at different spatial scales. Furthermore, other environmental parameters, for example, depth at local scales, can drive prokaryote community composition and obfuscate variation related to other spatial factors and host identity.

We used principal coordinates analyses and distance matrices to study the impact of geography and phylogeny on the prokaryote composition. Both techniques are

complementary and reveal different components of prokaryote community composition (Paliy and Shankar 2016). When geographic variation is inextricably entangled with variation in species identity, for example, it is difficult to infer to which driver the observed variation is truly related to (Marino et al. 2017). The co-variation in geography and host-phylogeny also affects the interpretation of drivers for giant barrel sponges, especially at the larger spatial scales. For example, giant barrel sponges from the Caribbean and the Indo-Pacific exist in different oceans and are assigned to different species. This can easily lead to the drawing of inaccurate conclusions. For example, Montalvo and Hill (2011) concluded that *X. muta* and *X. testudinaria* had different microbial communities due to being different species, but with the recent knowledge of their more complicated phylogeny, it has become clear that the differences in their microbial communities more likely reflect the different oceans they were sampled in (Chapter 4 of this thesis), although host identity is still expected to play a role. In the present study, we used distance matrices to show that distance alone, after removing the variation due to phylogeny, explained almost 70% of the variation in composition while phylogeny alone, after removing the impact of distance, explained less than 1%. Phylogeny seems to only affect specific prokaryotes such as OTU-108, a member of the order HOC36 that differed strongly with host identity in all three localities. This OTU was especially abundant in species g3 from Thailand and species g9 from Curaçao and Martinique. These genetic groups are both characterized by mitochondrial haplotype C5 and share a more common ancestor with one another than with any of the other genetic groups (Chapter 2 of this thesis).

At the smallest spatial scales, e.g., in Curaçao and Thailand, variation in the prokaryotic community was not related to distance, but rather due to differences among sampling sites. We were not able to identify which environmental variables were responsible for the differences. In Martinique, however, the differences between the prokaryotic communities were strongly correlated with depth. Depth-related shifts in the microbial community structure of giant barrel sponges have been previously detected at a depth range from 10 to 90 meters in sites surrounding the island Little Cayman in the Caribbean (Morrow et al. 2016). There, the researchers found a reduction in photosynthetic Cyanobacteria with depth, combined with an increase in Nitrospira. Although no cyanobacterial core OTUs and only two nitrospiral core OTUs differed with depth in Martinique, depth-related shifts for these taxa as a whole (i.e. including non-core OTUs) were also observed in this study. At first glance, this seems a perfect example of how different environmental conditions in deep *versus* shallow waters can structure prokaryotic communities. However, if only depth was important, we would expect shallow sponges from Martinique to be more similar to shallow sponges from other regions, like Curaçao, and this is not what we observed. Therefore, it is not merely the environmental differences related to depth that structure the distinct prokaryotic communities, but other factors too. Our results indicate that local site differences affect the abundance of bacterial phyla as a whole, rather than a change

in the relative abundance of various OTUs within each phylum that still add up to similar abundances at each location. Chlorofexi, Cyanobacteria, and Nitrospira are among the main phyla whose abundance varies strongly among locations. Chloroflexi are believed to play a key role in the degradation of dissolved organic matter from seawater (Bayer et al. 2018), Cyanobacteria are photosynthesizers (Burgsdorf et al. 2015) and members of the Nitrospira play a pivotal role in nitrification (Daims and Wagner 2018). The abundance of these phyla likely depends on the local availability of different resources.

The importance of drivers on the core community

Most of the observed patterns in the prokaryotic community of giant barrel sponges in relation to geographical variation, depth, and host identity were also present in the subset of core OTUs. This means that the variation is not necessarily only caused by the presence or absence of region-, depth range- or species-specific OTUs, but also by different relative abundances of core OTUs. So far, most evidence for environmental parameters that shape sponge microbiomes is derived from measured variation in the non-core microbial community (Schmitt et al. 2012; Pita et al. 2018). Differences in the presence and absence of certain non-core OTUs can be explained by the sponge hosts' ability to differentiate between alien and associated microbes, likely through the inherited immune system (Wilkinson et al. 1984; Wehrl et al. 2007). However, this concept fails to explain the differences in the relative abundances of core OTUs, which have to be regulated by different processes. The varying relative abundances of core OTUs between different giant barrel sponge species may be actively managed by the host sponge to fit specific environmental conditions or be a result of structural differences (such as pores, channels, choanocytes, etc.) between the giant barrel sponge species, which have yet to be identified.

In five different sponge species, Thomas et al. (2016) found a core community of seven to twenty OTUs per species, whereby they used a less stringent definition of a core OTU than we used (any OTU present in ≥ 85% of replicates). Despite our more stringent definition of a core OTU (any OTU present in 100% of replicates), by combining five closely related species distributed over multiple oceans, we found a core prokaryotic community that was five to fourteen times larger. This shows that the core prokaryotic community of giant barrel sponges is rich compared to other sponge species.

Implications for the functioning of coral reef ecosystems

This study illustrates the complex dynamics of the drivers that structure the composition of the giant barrel sponge prokaryotic community. It is important to translate how these complex dynamics cause differences in holobiont functioning and their cascading effects in the surrounding ecosystem (Pita et al. 2018). For example, the sponge holobiont can simultaneously perform nitrification and denitrification. The relative number of nitrifying microbes dictates whether the sponge acts as a source or sink of bioavailable nitrogen, often

one of the limiting resources in a marine ecosystem (Fiore et al. 2013b; 2015). With regards to photosynthesis, Cyanobacteria have a stronger association with giant barrel sponges in more oceanic environments or at elevated pCO_2 levels (Fiore et al. 2013b; Morrow et al. 2015; Lesser et al. 2016). Thus, the contribution of Cyanobacteria to the primary production of the ecosystem depends on the local environment of the sponge host (Wilkinson 1983). Besides changing the biochemistry, giant barrel sponges may also directly affect the prokaryotic community of sponge denizens or mobile organisms interacting with the sponges (Chapter 6 of this thesis).

Furthermore, the importance of depth and local site differences shows the flexibility of the prokaryote community of giant barrel sponges. Flexibility in host-symbiont interactions may translate into adaptability to environmental change, potentially making them more resilient or even acclimatized to such changes than other benthic coral reef organisms (Prazeres et al. 2017; Pita et al. 2018). The microbiome of corals, for example, is inflexible and adaptable (Pogoreutz et al. 2018). If the sponge holobiont is indeed better equipped to adapt to climate change, their increasing abundance on reefs worldwide may further accelerate (Bell et al. 2013; Deignan et al. 2018).

Pharmaceutical potential

The recent improvement of tools to study chemical compound profiles in sponges enables the identification of OTUs whose presence correlates with the production of such chemical compounds (Bayona et al. 2018). For example, Caribbean giant barrel sponges from the same location have been shown to harbor different sterol compositions (Fromont et al. 1994), and this chemical diversity may potentially be linked to OTUs that vary with host identity. As bacterial taxa in the sponge prokaryotic community can be influenced by different drivers, the production of chemical compounds can be similarly influenced by those drivers. It is especially appealing to try to identify (groups of) OTUs that are related to the production of secondary metabolites with pharmaceutical potential, such as members of the *Entotheonella,* which have been found in other marine sponges such as *Theonella swinhoei* and *Discodermia calyx* (Wilson et al. 2014; Wakimoto et al. 2014). This study shows that taking only host identity into account in such efforts may result in overlooking these OTUs and that spatial variation may also be important.

Conclusion

Considering the close and long-existing relationship between sponges and their microbial symbionts, we understand remarkably little about how this symbiosis is shaped by multiple drivers. The present study shows how the relative importance of drivers of microbial variation in sponges may shift across different spatial scales. We show that environmental drivers predominate at regional to global scales, while host identity is the dominant driver of prokaryote communities at local scales. With these findings, we expect that shortcomings in

the fundamental understanding of how these microbial communities develop can be solved and that the large pharmaceutical potential of sponges, in which their microbial symbionts are thought to play an essential role, can be better utilized.

ACKNOWLEDGEMENTS

We would like to thank Dr. Mark Vermeij of CARMABI and Roy Belderok for their support during field work in Curaçao. We thank Niels van der Windt for his advice about the field work in Thailand. We are grateful to the Muséum National d'Histoire Naturelle (MNHN) who invited us to participate in the MADIBENTHOS expedition, Martinique 2016 (Principal Investigator Philippe Bouchet). This work is part of the research programmes NWO-VIDI with project number 16.161.301 and ASPASIA (015.010.030), which are both (partly) financed by the Netherlands Organisation for Scientific Research (NWO). Furthermore, the fieldwork was supported by De Treubmaatschappij (Treub 2016b_De Voogd).

APPENDIX 5.1.

Detailed description of the sites in Curaçao, Martinique and Thailand.

Caribbean - Curacao

Curaçao is a Caribbean island that lies approximately 65 kilometers north of the Venezuelan coast. The Curaçaoan landmass is elongated in shape, with a long, sandy southern shoreline, and a rocky, long northern shoreline. The local bathymetry and oceanographic conditions mean that the southern side of the island hosts calm waters whose currents tend to run from East to West, whilst the northern coast has much rougher waters. The southern shoreline is more subject to human impact and is most populated in the capital city of Willemstad (population: 140 000), located to the Southeast of the island. The urbanization introduces large quantities of nutrients into the adjacent waters. It is expected that eutrophication diminishes as distance from the urban area decreases. The rest of the landmass is sparsely populated, with the eastern tip consecrated purely to nature conservation.

The Curacaoan section of the project involved sampling from six sites: Watamula (WM), Klein Curaçao (KC), Playa Jeremi (PJ), Piscadera Bay (PB), Holiday Beach (HB), and Director's Bay (DB). Klein Curaçao is a remote, uninhabited island with a leeward side with relatively high, yet patchy coral cover (Fig. 5.1b; Waitt Institute Report 2016). Director's Bay is a reef near a bay inlet with relatively high coral cover but relatively limpid water. Holiday Beach is a reef in a zone with high pressure from Willemstad, yet with relatively high coral cover, and low to medium turbidity. The reefs at Piscadera Bay receive considerable tidal sedimentation from the mangrove-lined bay. Playa Jeremi is a relatively secluded area, with intermediate to low coral cover and low turbidity. Watamula is located on the tip of the island, has a high abundance of giant barrel sponges, and intermediate to low coral cover. It is a site where the currents from the North and South sides mix. It is expected that Holiday Beach, Director's Bay, and Piscadera Bay were most affected by urbanization and associated eutrophication.

Caribbean - Martinique

Martinique is a volcanic island in The Lesser Antilles, stretching 70 km in length and 30 km in width (Fig. 5.1c). The five sampled sites around the island each have unique characteristics, derived from the surrounding environment. The northern part (NO) of the island is mountainous, heavily forested and catches most of the rainfall. The South (SO) is drier and more densely populated. Here 'Diamond Rock' is located, a small basalt island. Underwater is a ~30 m long tunnel, ending in a large overhang covered with sponges, 0 to 15 m deep, providing semi-dark conditions. The East (EA), or windward side, of Martinique, is exposed to the Atlantic Ocean and characterized by shallow coral reefs and cays. The West (WE), or leeward, side, is exposed to the Caribbean Sea and more sheltered. The reef slopes here descend steeply from the shore. The Fort-de-France Bay (WB) is a large inlet of the Caribbean Sea. The capital city of the island, Fort-de-France lies along the bay's northern coastline, but

most of the coastline is covered by extensive mangroves. The bay is shallow (<10 m) and very turbid. Deep samples (> 90 m) are collected in close proximity to the shallow samples, but usually further offshore.

Indo-Pacific - Thailand

Koh Tao is a small island in the Gulf of Thailand, approximately 60 kilometers from the mainland. It is a popular touristic destination, and sometimes called the diving capital of the world (Weterings 2011). Koh Tao has many regularly visited dive sites all around the island and a few sites that are more distant. In this study we compared two inshore sites, Shark Island (SH) and Twin Rocks (RC), with two offshore sites, Chumphon Pinnacle (CP) and Southwest Pinnacle (SP) (Fig. 5.1d). The sponges were collected from depths up to 25 meters.

Shark Island is a small uninhabited island situated in the Southeast of Koh Tao, approximately 300 m of the coast. Strong currents run along the fringes of the island, often resulting in heavy swell. The reef surrounding the island stretches from 5 until 25 meters deep, forming a mountainous underwater landscape. Twin Rocks lies northwest of Koh Tao, at a distance of approximately 0.5 km from the main island. Chumphon Pinnacle is a submerged pinnacle located around 8 km northwest of Koh Tao. The bottom of the pinnacle is located at 35 m depth and the pinnacle reaches its highest point at 14 m depth. Southwest Pinnacle includes of a series of seven submerged pinnacles approximately 8 km southwest of Koh Tao. The pinnacles start at a depth of 25-30 m and the shallowest point is reached at 5 m below the sea surface.

APPENDIX 5.2

Table 1. General information on phyla, classes and orders

Phylum	Seqs	OTUs	Class
Proteobacteria	921510	4687	Gammaproteobacteria
Actinobacteria	318055	288	Acidimicrobiia
Nitrospirae	274497	76	Acidimicrobiia
Chloroflexi	120321	467	Nitrospira
Cyanobacteria	76982	137	Alphaproteobacteria
Gemmatimonadetes	35052	216	Deltaproteobacteria
Acidobacteria	27426	471	Cyanobacteria
Tectomicrobia	12288	46	Caldilineae
Saccharibacteria	7511	63	BD2-11 terrestrial group
PAUC34f	7174	67	SAR202 clade
Spirochaetae	6614	128	Holophagae
SBR1093	4140	26	JTB23
Bacteroidetes	4039	760	uncultured bacterium
Poribacteria	2133	30	Anaerolineae
Nitrospinae	1459	24	uncultured Candidatus Saccharibacteria bacterium
Parcubacteria	1367	62	Spirochaetes
Planctomycetes	835	551	Ambiguous_taxa
Thaumarchaeota	691	19	TK10
Verrucomicrobia	585	160	uncultured delta proteobacterium
Firmicutes	529	148	Betaproteobacteria
Woesearchaeota (DHVEG-6)	431	30	Unassigned
Tenericutes	323	40	Epsilonproteobacteria
Marinimicrobia (SAR406 clade)	234	35	Bacteroidetes Incertae Sedis
Euryarchaeota	111	20	Flavobacteriia
Lentisphaerae	106	40	MD2898-B26
Deferribacteres	102	43	Solibacteres
Latescibacteria	89	64	Marine Group I
Chlamydiae	65	56	Subgroup 9
Peregrinibacteria	42	21	S085
Fusobacteria	35	12	ARKDMS-49
Ignavibacteriae	32	9	Cytophagia
Gracilibacteria	29	18	Clostridia
Omnitrophica	26	25	PAUC43f marine benthic group
Elusimicrobia	25	10	Mollicutes
Fibrobacteres	25	23	Sphingobacteriia
Chlorobi	15	11	Bacteroidia
Deinococcus-Thermus	12	9	Phycisphaerae
Unassigned	12	9	Planctomycetacia
Hydrogenedentes	11	9	Actinobacteria
BRC1	11	11	OPB35 soil group
TM6 (Dependentiae)	10	9	OM190

Seqs	OTUs	Order	Seqs	OTUs
636515	2170	uncultured	558023	303
317680	233	Acidimicrobiales	317680	233
317680	233	Nitrospirales	274497	76
274497	76	Desulfurellales	93123	109
155230	849	uncultured bacterium	93036	961
101746	1472	SubsectionI	75695	45
76923	102	Caldilineales	72299	84
72299	84	Rickettsiales	67365	122
34663	159	Rhodospirillales	51831	329
34208	188	KI89A clade	34342	185
25090	215	Rhodobacterales	32278	126
22508	36	Subgroup 10	25013	179
17240	285	Xanthomonadales	21402	184
10189	107	Unassigned	18336	522
7450	40	Ambiguous_taxa	11152	191
6614	128	Anaerolineales	10189	107
5632	52	uncultured Candidatus Saccharibacteria bacterium	7450	40
2881	36	HOC36	6771	53
2802	8	Oceanospirillales	6649	335
2736	62	Spirochaetales	6614	128
2086	138	Bdellovibrionales	3086	295
1970	29	Vibrionales	2847	88
1584	62	uncultured delta proteobacterium	2802	8
1396	254	Nitrosomonadales	2657	38
1266	18	Rhizobiales	2655	122
1106	44	uncultured Chloroflexi bacterium	2378	19
691	19	Cellvibrionales	2089	332
679	21	Campylobacterales	1970	29
646	8	Oligoflexales	1906	95
458	8	Order II	1552	53
426	151	Flavobacteriales	1396	254
390	122	Alteromonadales	1370	148
338	36	SubsectionIII	1201	42
323	40	Solibacterales	1106	44
304	209	NB1-j	892	276
286	55	Desulfobacterales	822	197
228	132	Unknown Order	791	68
228	136	SAR324 clade(Marine group B)	747	34
224	34	Gammaproteobacteria Incertae Sedis	563	114
215	18	SAR11 clade	462	6
210	153	Myxococcales	436	242

Table 1. (continued)

Phylum	Seqs	OTUs	Class
RBG-1 (Zixibacteria)	10	8	uncultured euryarchaeote
Aminicenantes	9	7	Subgroup 22
LCP-89	5	5	MD2896-B214
WS2	4	3	Blastocatellia
Armatimonadetes	3	3	SPOTSOCT00m83
WA-aaa01f12	3	3	Opitutae
Synergistetes	3	2	Thermoleophilia
Acetothermia	2	2	Bacilli
Candidatus Berkelbacteria	2	2	Verrucomicrobiae
AC1	1	1	Thermoplasmata
FCPU426	1	1	uncultured archaeon
Cloacimonetes	1	1	Subgroup 6
SR1 (Absconditabacteria)	1	1	Deferribacteres Incertae Sedis
Caldiserica	1	1	Chlamydiae
			Oligosphaeria
			vadinHA49
			Pla4 lineage
			Lentisphaeria
			Gemmatimonadetes
			R76-B128
			Pla3 lineage
			AEGEAN-245
			Tectomicrobia Incertae Sedis
			Proteobacteria Incertae Sedis
			Fusobacteriia
			Ignavibacteria
			ML635J-21
			Melainabacteria
			pltb-vmat-80
			Ardenticatenia
			WCHB1-41
			Elusimicrobia
			Dehalococcoidia
			Bacteroidetes BD2-2
			uncultured organism
			Subgroup 26
			KD4-96
			Fibrobacteria
			JG30-KF-CM66
			BD7-11
			Deferribacteres
			Subgroup 2
			Candidatus Campbellbacteria

Seqs	OTUs	Order	Seqs	OTUs
208	9	Cytophagales	426	151
192	122	Desulfovibrionales	420	48
186	2	Thiotrichales	419	64
172	7	E01-9C-26 marine group	400	43
167	16	Clostridiales	373	108
155	37	Chromatiales	306	50
140	13	Sphingobacteriales	304	209
128	18	Bacteroidales	278	50
122	52	Planctomycetales	227	135
111	20	uncultured euryarchaeote	208	9
108	6	Bradymonadales	193	90
99	29	BD7-8 marine group	182	10
85	40	Mycoplasmatales	180	13
65	56	Phycisphaerales	176	92
58	20	Blastocatellales	172	7
49	34	Arenicellales	155	34
49	42	PeM15	147	4
48	20	Gaiellales	137	11
48	18	Kordiimonadales	130	14
43	18	Puniceicoccales	127	27
42	28	uncultured archaeon	122	8
41	9	Verrucomicrobiales	122	52
37	6	Legionellales	121	63
36	6	Sphingomonadales	119	13
35	12	Thermoplasmatales	111	20
32	9	Bacillales	108	9
31	20	Entomoplasmatales	104	8
28	15	Parvularculales	103	16
27	7	Alphaproteobacteria Incertae Sedis	103	49
27	18	Caulobacterales	91	23
26	23	Chlamydiales	65	56
25	10	uncultured organism	63	49
24	11	Methylococcales	61	8
23	16	Subgroup 23	52	17
21	19	Salinisphaerales	49	11
20	7	Gemmatimonadales	48	18
20	2	Methylophilales	48	6
20	18	P.palmC41	46	11
18	5	Desulfarculales	40	27
18	18	Sva0071	37	11
17	3	Victivallales	36	13
16	3	Corynebacteriales	36	12
15	7	Fusobacteriales	35	12

Table 1. (continued)

Phylum	Seqs	OTUs	Class
			Chlorobia
			Subgroup 17
			Arctic97B-4 marine group
			Deinococci
			Subgroup 13
			Subgroup 21
			MACA-EFT26
			JdFBHP3
			Erysipelotrichia
			Skagenf62
			Subgroup 11
			Coriobacteriia
			Nitrospinia
			Spartobacteria
			Candidatus Falkowbacteria
			Chitinivibrionia
			Candidatus Adlerbacteria
			AT-s3-28
			Bacteroidetes vadinHA17
			OPB41
			Subgroup 18
			WCHB1-32
			Candidatus Moranbacteria
			Fimbriimonadia
			Synergistia
			Ktedonobacteria
			Chloroflexia
			Nitriliruptoria
			Belgica2005-10-ZG-3
			MD2902-B12
			uncultured crenarchaeote
			028H05-P-BN-P5
			ML602M-17
			Verrucomicrobia Incertae Sedis
			Candidatus Peribacteria
			Milano-WF1B-44
			Bacteroidetes VC2.1 Bac22
			Unknown Class
			SJA-15
			S0134 terrestrial group
			Negativicutes
			MD2896-B258
			Candidatus Azambacteria

Seqs	OTUs	Order	Seqs	OTUs
15	11	Sva0485	35	21
14	8	Order III	32	9
12	3	Ignavibacteriales	32	9
12	9	NB1-n	30	12
12	1	Aeromonadales	28	9
10	4	Sneathiellales	26	4
9	3	Acidithiobacillales	26	5
9	2	Propionibacteriales	25	9
9	6	Acanthopleuribacterales	24	18
7	2	CCM11a	24	21
7	4	uncultured Acidobacteria bacterium	21	17
6	4	Lactobacillales	20	9
5	2	Fibrobacterales	20	18
5	4	Deferribacterales	17	3
5	5	Syntrophobacterales	17	13
5	5	Hydrogenophilales	16	7
4	2	Halanaerobiales	16	13
4	3	Elusimicrobiales	15	1
3	1	Chlorobiales	15	11
3	2	SubsectionII	14	10
3	2	MB11C04 marine group	13	4
3	2	Lentisphaerales	12	7
3	2	SubsectionIV	11	3
3	3	Deinococcales	11	8
3	2	Micrococcales	11	7
2	1	CS-B046	10	5
2	2	HTA4	10	10
2	2	uncultured planctomycete	10	10
2	2	uncultured gamma proteobacterium	9	1
2	2	Pseudomonadales	9	1
2	2	Obscuribacterales	9	2
2	2	Gastranaerophilales	9	3
2	1	Erysipelotrichales	9	6
2	2	Vampirovibrionales	9	9
2	2	Bacteroidia Incertae Sedis	8	5
1	1	Desulfuromonadales	8	7
1	1	SS1-B-02-17	8	6
1	1	mle1-8	8	6
1	1	Emcibacterales	6	2
1	1	1013-28-CG33	6	3
1	1	Ardenticatenales	6	4
1	1	Coriobacteriales	6	4
1	1	Run-SP154	6	4

Table 1. (continued)

Phylum	Seqs	OTUs	Class
			MSBL8
			BJGMM-U56
			marine metagenome
			Candidatus Uhrbacteria
			Omnitrophica Incertae Sedis
			SB-5
			Latescibacteria Incertae Sedis
			uncultured Planctomycetales bacterium
			SGST604
			Caldisericia
			uncultured Microgenomates group bacterium
			SJA-68

Seqs	OTUs	Order	Seqs	OTUs
1	1	RS-B22	6	2
1	1	Lineage IIb	6	6
1	1	EC3	6	6
1	1	Nitrospinales	5	2
1	1	S-70	5	2
1	1	MSBL5	5	3
1	1	ss1-B-07-44	5	2
1	1	S26-47	5	3
1	1	Frankiales	5	2
1	1	vadinBA26	5	4
1	1	Rhodocyclales	5	4
1	1	Chthoniobacterales	5	4
		Enterobacteriales	5	5
		Acholeplasmatales	4	2
		Opitutales	4	2
		B103G10	4	2
		TRA3-20	4	2
		uncultured proteobacterium	4	3
		X35	4	3
		C86	4	3
		Oligosphaerales	4	3
		Chitinivibrionales	4	4
		A714019	3	1
		SS1-B-09-64	3	1
		F9P41300-M23	3	2
		Pasteurellales	3	1
		4-Org1-14	3	2
		uncultured Gemmatimonadetes bacterium	3	2
		MSBL9	3	2
		Fimbriimonadales	3	3
		uncultured Bacteroidetes bacterium	3	3
		Solirubrobacterales	3	2
		Synergistales	3	2
		Magnetococcales	3	3
		Mollicutes RF9	3	3
		marine metagenome	3	2
		43F-1404R	3	3
		Lineage IV	2	1
		SC-I-84	2	1
		MVP-21	2	1
		uncultured deep-sea bacterium	2	1
		Nitriliruptorales	2	2
		uncultured crenarchaeote	2	2

Table 1. (continued)

Phylum	Seqs	OTUs	Class

Seqs	OTUs	Order	Seqs	OTUs
		E6aD10	2	1
		OTU048-Xeno	1	1
		Neisseriales	1	1
		Kallotenuales	1	1
		Holophagales	1	1
		FW22	1	1
		DMI	1	1
		Caenarcaniphilales	1	1
		Selenomonadales	1	1
		uncultured Cytophagales bacterium	1	1
		possible order 07	1	1
		GB102	1	1
		Amsterdam-1B-07	1	1
		10bav-F6	1	1
		Thermales	1	1
		MSB-3A7 sediment group	1	1
		uncultured Planctomycetales bacterium	1	1
		GIF3	1	1
		Brocadiales	1	1
		MVP-88	1	1
		Mollicutes Incertae Sedis	1	1
		Caldisericales	1	1
		Tepidisphaerales	1	1
		Chloroflexales	1	1
		uncultured Microgenomates group bacterium	1	1
		Rs-M47	1	1
		Thermoanaerobacterales	1	1
		Sh765B-AG-111	1	1

APPENDIX 5.2

Table 2. Description of core OTUs

OTU	Sum	Number of samples	Domain	Phylum	Class	Order
6	125103	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
8	84801	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
2	71140	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
4	67225	73	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfurellales
1	61254	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
3	58869	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
22	47159	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
24	45009	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
35	42502	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
37	32052	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
18	30336	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
57	30213	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
27	28460	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
61	26094	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
46	24932	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
54	24022	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
32	22468	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
52	21982	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
36	21214	73	Bacteria	Acidobacteria	Holophagae	Subgroup 10
66	21186	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
44	20355	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
30	19280	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
33	19223	73	Bacteria	Proteobacteria	JTB23	uncultured bacterium
76	18561	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
55	18245	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
39	17919	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
58	16580	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
53	16191	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
56	16040	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
147	13996	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
79	12896	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
51	12413	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
65	12376	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
133	12371	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
114	12361	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
134	12035	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
73	11290	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
67	11092	73	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfurellales
112	11057	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
81	10212	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
82	9248	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales

Family	Genus	Species
Nitrospiraceae	Nitrospira	uncultured bacterium
Sva0996 marine group	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
Desulfurellaceae	G55	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
FamilyI	uncultured	Ambiguous_taxa
Unassigned	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
TK85	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
uncultured bacterium	uncultured bacterium	uncultured bacterium
Rhodospirillaceae	uncultured	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
SAR116 clade	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Rhodospirillaceae	uncultured	uncultured bacterium
Sva0996 marine group	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Desulfurellaceae	G55	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Rhodobacteraceae	uncultured	Unassigned

Table 2. (continued)

OTU	Sum	Number of samples	Domain	Phylum	Class	Order
84	8898	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
145	8679	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
129	8568	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
179	8417	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
68	8326	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
63	8282	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
74	7980	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
80	7966	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
70	7944	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
90	7551	73	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1711	7233	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
104	6678	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
87	6511	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
89	6488	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
105	6284	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
271	6096	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales
150	5991	73	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1377	5610	73	Bacteria	Nitrospirae	Nitrospira	Nitrospirales
146	5601	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
188	5443	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales
160	5256	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
194	5165	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
121	4960	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
144	4876	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
102	4828	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
182	4572	73	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales
156	4360	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
125	4290	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
154	4235	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
140	3923	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
204	3739	73	Bacteria	Chloroflexi	Caldilineae	Caldilineales
164	3706	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
995	3673	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
247	3566	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
240	3414	73	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfurellales
176	3145	73	Bacteria	Proteobacteria	Gammaproteobacteria	KI89A clade
108	3075	73	Bacteria	Proteobacteria	Gammaproteobacteria	HOC36
321	3031	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
11	2942	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
326	2907	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
187	2899	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
233	2779	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales

Family	Genus	Species
Nitrospiraceae	Nitrospira	uncultured bacterium
Unassigned	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
SAR116 clade	uncultured bacterium	uncultured bacterium
Unassigned	Unassigned	Unassigned
FamilyI	Synechococcus	uncultured bacterium
JTB255 marine benthic group	Unassigned	Unassigned
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
SAR116 clade	Unassigned	Unassigned
Rhodobacteraceae	Albidovulum	uncultured alpha proteobacterium
JTB255 marine benthic group	Unassigned	Unassigned
Nitrospiraceae	Nitrospira	uncultured bacterium
Unassigned	Unassigned	Unassigned
Rhodobacteraceae	uncultured	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Unassigned	Unassigned	Unassigned
Rhodospirillaceae	uncultured	Unassigned
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Unassigned	Unassigned	Unassigned
JTB255 marine benthic group	Unassigned	Unassigned
SAR116 clade	uncultured bacterium	uncultured bacterium
SAR116 clade	uncultured bacterium	uncultured bacterium
SAR116 clade	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Caldilineaceae	uncultured	uncultured Chloroflexi bacterium
Sva0996 marine group	uncultured bacterium	uncultured bacterium
Unassigned	Unassigned	Unassigned
Sva0996 marine group	Unassigned	Unassigned
Desulfurellaceae	G55	Unassigned
Unassigned	Unassigned	Unassigned
uncultured bacterium	uncultured bacterium	uncultured bacterium
Unassigned	Unassigned	Unassigned
FamilyI	Prochlorococcus	uncultured bacterium
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
SAR116 clade	Unassigned	Unassigned

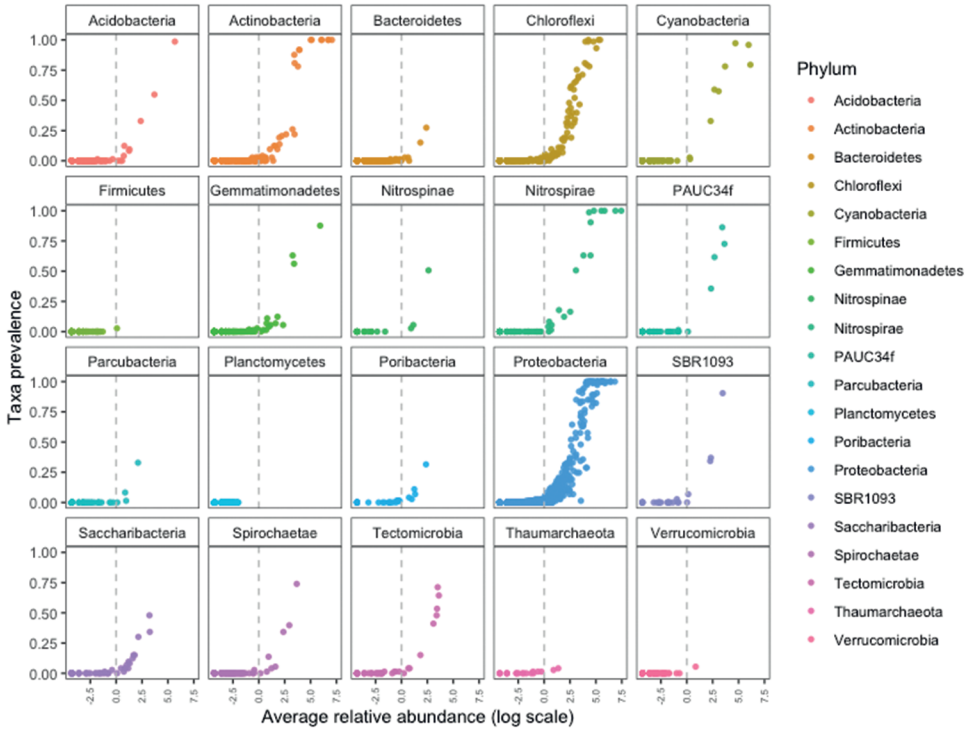
Table 2. (continued)

OTU	Sum	Number of samples	Domain	Phylum	Class	Order
3853	2535	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
130	2470	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
405	2377	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
221	2367	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
172	2328	73	Bacteria	SBR1093	Ambiguous_taxa	Ambiguous_taxa
207	2328	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
460	2249	73	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales
190	2123	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales
250	1709	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
260	1621	73	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales
236	1572	73	Bacteria	Cyanobacteria	Cyanobacteria	SubsectionI
257	1283	73	Bacteria	Chloroflexi	SAR202 clade	Unassigned
25	1087	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured
293	1057	73	Bacteria	PAUC34f	Unassigned	Unassigned
338	977	73	Bacteria	Proteobacteria	Gammaproteobacteria	uncultured

Family	Genus	Species
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Ambiguous_taxa	Ambiguous_taxa	Ambiguous_taxa
Sva0996 marine group	Unassigned	Unassigned
Sva0996 marine group	uncultured actinobacterium	uncultured actinobacterium
Rhodospirillaceae	OM75 clade	Unassigned
Unassigned	Unassigned	Unassigned
SAR116 clade	Unassigned	Unassigned
FamilyI	Synechococcus	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned
Unassigned	Unassigned	Unassigned

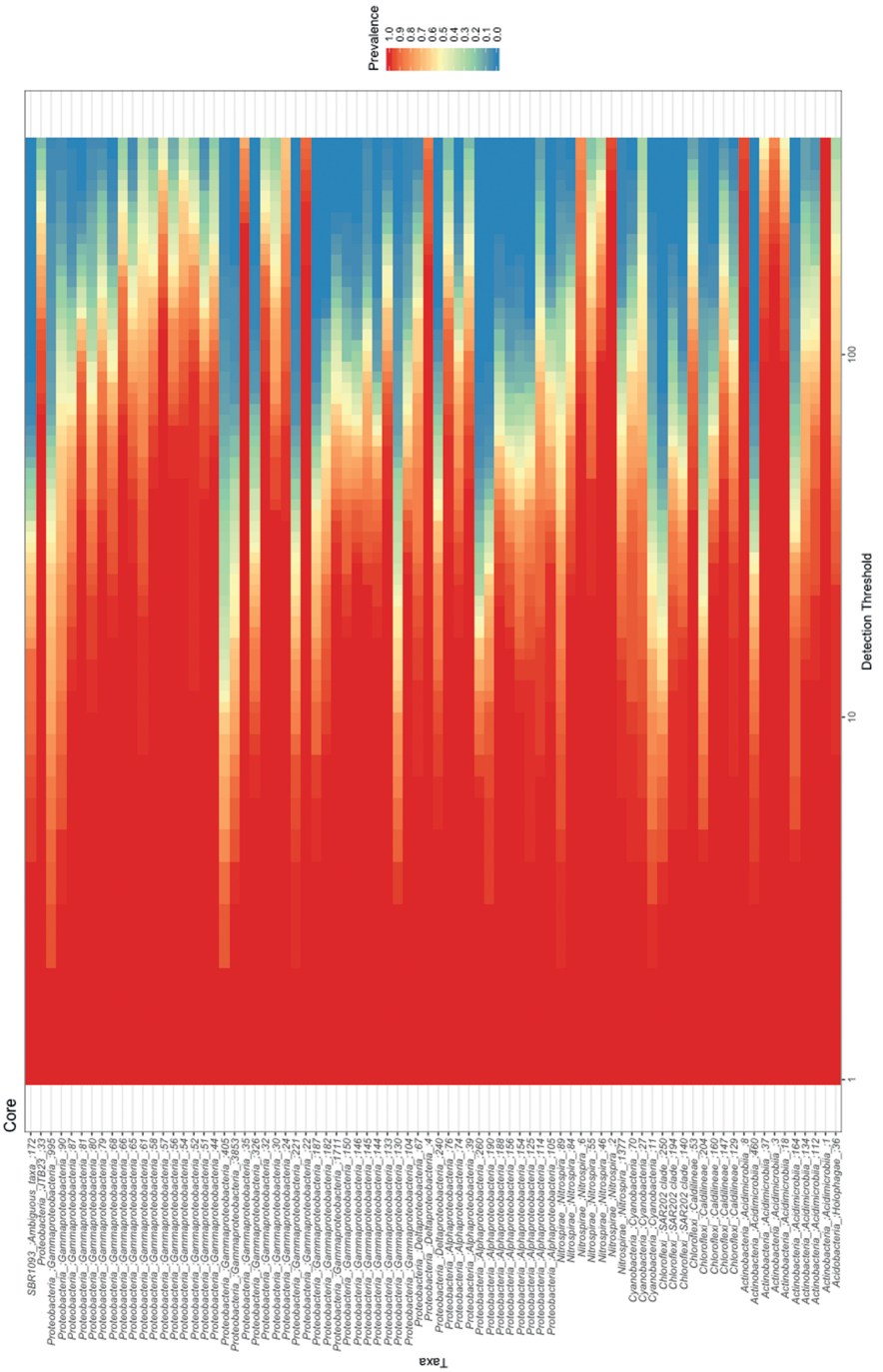
APPENDIX 5.3

Overview of OTU prevalences and their taxonomic affiliations.



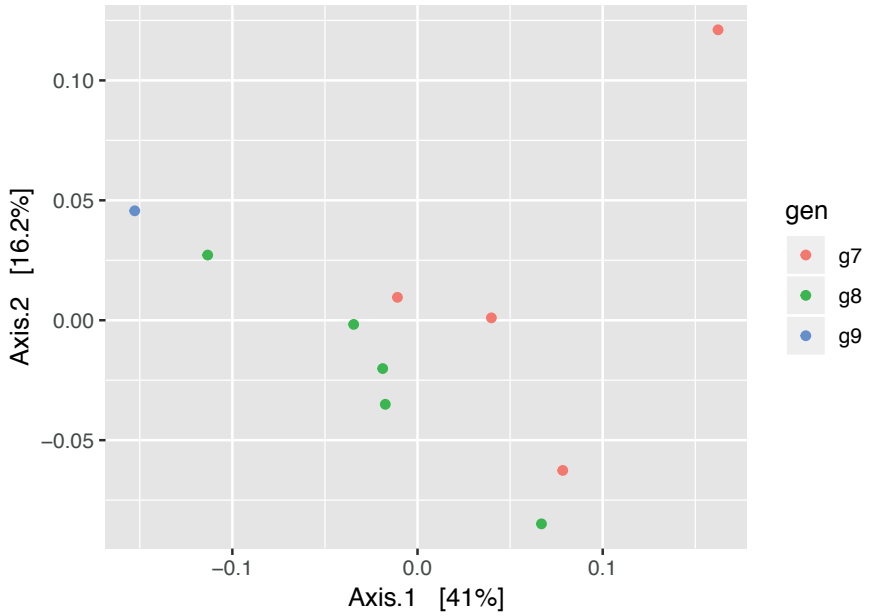
APPENDIX 5.4

Heat map of abundances of core OTUs.



APPENDIX 5.5

PCO of core community composition of 10 giant barrel sponge (Xestospongia spp.) samples from Piscadera Bay, Curaçao.



III

Section three: Reef Interactions