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MICROBIOLOGY OF AQUATIC SYSTEMS

Composition of Archaea in Seawater, Sediment, and Sponges in the Kepulauan Seribu Reef System, Indonesia

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Abstract Coral reefs are among the most diverse and productive ecosystems in the world. Most research has, however, focused on eukaryotes such as corals and fishes. Recently, there has been increasing interest in the composition of prokaryotes, particularly those inhabiting corals and sponges, but these have mainly focused on bacteria. There have been very few studies of coral reef Archaea, despite the fact that Archaea have been shown to play crucial roles in nutrient dynamics, including nitrification and methanogenesis, of oligotrophic environments such as coral reefs. Here, we present the first study to assess Archaea in four different coral reef biotopes (seawater, sediment, and two sponge species, *Stylissa massa* and *Xestospongia testudinaria*). The archaeal community of both sponge species and sediment was dominated by *Crenarchaeota*, while the seawater community was dominated by *Euryarchaeota*. The biotope explained more than 72 % of the variation in archaeal composition. The number of operational taxonomic units (OTUs) was highest in sediment and seawater biotopes and substantially lower in both sponge hosts. No "sponge-specific" archaeal OTUs were found, i.e., OTUs found in both sponge species but absent from nonhost biotopes. Despite both sponge species hosting phylogenetically distinct microbial assemblages, there were only minor differences in Kyoto Encyclopedia of Genes and Genomes (KEGG) functional pathways. In contrast, most functional pathways differed significantly between microbiomes from sponges and nonhost biotopes including all energy metabolic pathways. With the exception of the methane and nitrogen metabolic pathway, all energy metabolic pathways were enriched in sponges when compared to nonhost biotopes.

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Introduction

Of the two known prokaryotic domains of life, Archaea are the least studied and understood. The archaeal domain was first described by Woese and Fox (1978). Since then, it has undergone several taxonomic amendments. Several phyla have been proposed, but a lack of consensus on some of the proposed phyla persists. At present, the domain Archaea consist of the following phyla: *Crenarchaeota*, *Thaumarchaeota*, *Euryarchaeota*, *Korarchaeota*, and *Nanoarchaeota*. The *Korarchaeota* phylum was based on 16S rRNA gene sequence amplification of data from environmental sequence studies [3, 25]. Recently, Brochier-Armanet et al. [8, 9] and Spang et al. [81] suggested that mesophilic *Crenarchaeota* differed from hyperthermophilic *Crenarchaeota* and proposed *Thaumarchaeota* (mesophilic *Crenarchaeota*) as a new phylum. Huber et al. [40] proposed the establishment of *Nanoarchaeota* as a new phylum based on the low similarity of *Nanoarchaeum equitans* sequences with known organisms; however, some other studies have suggested that *Nanoarchaeota* is a fast-evolving lineage of the *Euryarchaeota* phylum related to *Thermococcales* [7]. Other recently proposed taxa include the *Aigarchaeota* [57] and *Geoarchaeota* [45]. With the proposal of *Thaumarchaeota* as a new phylum, *Crenarchaeota* became restricted to a single class: *Thermoprotei*. This class is normally associated with extreme environments (e.g., hot and acidic environments) [58].

Thaumarchaeota, however, is still considered a *Crenarchaeota* class in some rRNA gene databases.

At the time of their discovery, Archaea were thought to exclusively inhabit extreme environments (high temperature, salinity, and/or pressure). Recently, culture independent methods have shown the domain to be much more widespread; it has been found in a variety of habitats (tropical and polar, terrestrial and aquatic, deep and shallow water) [77, 92, 96]. Members of Archaea have also been found inhabiting the tissues of various sponge species [62, 92], the mucous of scleractinian corals [77], sediment [11, 18], and the water column [20, 96].

Among the best known hosts of microbial communities [88, 91], marine sponges, are also abundant and conspicuous components of coral reef systems and play important functional roles both in the benthos and water column. Sponges have been shown to affect water column composition (filtration, secondary metabolite emanation, and nutrient cycling including nitrification) [5, 97]. Although sponges also feed on microbes [35, 49], they harbor a remarkable variety of microbial endosymbionts within their tissues. Microbes provide their sponge host with nutrients, aid in metabolic waste processing, and provide protection against ultraviolet light [34, 39, 91]. Sponges, in turn, offer a stable and nutrient-rich environment for microbes [36, 49].

Sponges have also been shown to host distinct microbial communities when compared to other nonsponge hosts or nonhost biotopes such as sediment or water [41]. Recent studies [85, 93], however, have shown that organisms previously thought to be found exclusively in sponges have also been found in nonsponge hosts or the nonhost environment. The extremely low concentrations of these organisms though in nonsponge samples had made them virtually impossible to detect using previous conventional molecular techniques, such as DGGE and clone libraries. It should be noted, however, that the presence of these organisms in nonsponge samples may also be the result of sponges releasing symbionts into the water column or sediment during spawning/injury events [85].

The majority of studies have showed bacteria to be more abundant than Archaea in marine sponges [48, 76, 86]. Twenty-six bacterial and two archaeal phyla were found in sponges from distinct locations around the world to date [84]. However, there are exceptions; the archaeon *Cenarchaeum symbiosum* dominates the *Axinella mexicana* microbial community representing more than 65 % of all prokaryotic cells [62]. This predominance suggests that Archaea play a major role in sponge metabolism. However, the exact roles of Archaea in sponges and in sediment and seawater remain largely unknown.

Despite this uncertainty, it is generally accepted that Archaea play an indispensable role in the transformation, degradation, and recycling of nutrients and organic matter [49, 92]. Several studies [11, 96] have suggested that Archaea and more specifically mesophilic *Crenarchaeota*, which use dissolved inorganic carbon as a carbon source [64], may be similar to or even surpass bacteria (β- and γ-proteobacteria) as mediators of oceanic nitrification [11, 96]. Francis et al. [31] reported a more widespread presence of ammonia-oxidizing Archaea (AOA) than ammonia-oxidizing bacteria in both the water column (Black Sea and Monterey Bay) and sediment (San Francisco Bay and Bahía del Tóbari). In sponges, a recent study [68] revealed the same pattern in four cold water sponges. Anaerobic Archaea belonging to the *Euryarchaeota* phylum also seem to be the only organisms capable of performing methanogenesis [90], which is the last step of carbon degradation and prevents the accumulation of organic compounds in the environment [43].

Archaeal community composition and symbiont–sponge relationships appear to be host dependent, for example, coral hosts do not seem to establish specific associations with Archaea since most of the archaeal sequences found in corals are also present in the water column [71]. Sponges, in contrast, have been shown to host distinct microbial communities when compared to other nonsponge hosts or nonhost biotopes [41]. Identifying the role and composition of Archaea and other microbes in different biotopes is thus essential in order to gain a better understanding of the coral reef ecosystem and the role of Archaea therein.

In the present study, we assessed the composition of Archaea in four biotopes, two nonhost (sediment and seawater) and two host (the sponge species *Stylissa massa* and *Xestospongia testudinaria*) in four reef sites in the Kepulauan Seribu reef system, Indonesia. Our goals were to (1) assess to what extent sponges contain unique archaeal communities when compared to communities of Archaea in the surrounding environment (seawater and sediment), (2) identify closely related organisms to abundant operational taxonomic units (OTUs) using Basic Local Alignment Search Tool (BLAST) search, (3) construct a phylogeny of abundant OTUs in order to assess to what extent biotopes host phylogenetically distinct lineages, and (4) assess to what extent metabolic pathways differ between Archaea in different biotopes.

Material and Methods

Study Site

The Jakarta Bay and Kepulauan Seribu coral reef system, also known as Thousand Islands (hereafter referred to as JBTI), is located to the northwest of Jakarta in the Java Sea (Fig. 1). This reef system consists of 105 islands or cay-crowned reefs [15] forming a coral island chain of about 80 km [67]. Thirteen rivers discharge into Jakarta Bay and represent important sources of organic and inorganic suspended matter (domestic sewage) as well as chemical pollutants and other

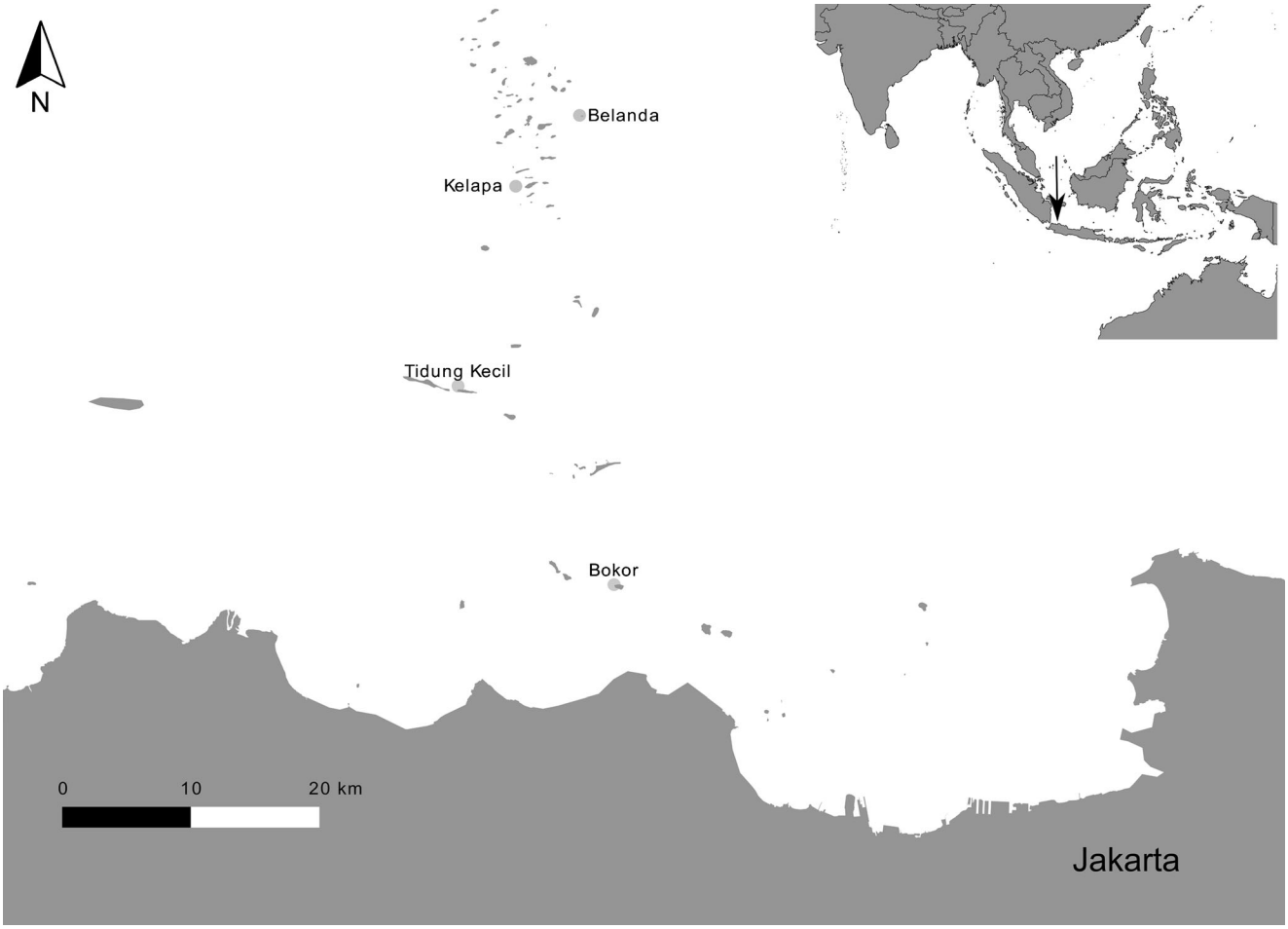

Fig. 1 Map of the study area (Jakarta Bay and Kepulauan Seribu coral reef system) showing the location of study sites sampled

substances [67]. Organic matter concentrations, however, decline strongly from in-to-offshore as do pollutant loads [14].

Sampling

Four sites (Belanda, Pulau Kelapa, Tidung Kecil, and Bokor) were surveyed using SCUBA between July 26th and the 10th of August 2011. At each site, samples were taken of sediment, seawater, and the sponges *S. massa* and *X. testudinaria.* The sediment samples were taken using the mini core method. Minicores, consisting of the top 5 cm of sediment, were collected using a plastic disposable syringe from which the end had been cut in order to facilitate sampling [12]. The two sponges studied are common reef sponges in the Indonesian archipelago although they inhabit different habitats. *Stylissa massa* (Carter, 1887) is a medium-sized orange colored sponge that mainly occurs in very shallow water (0.5–3 m) whereas the giant barrel sponge *X. testudinaria* (Lamarck, 1815) grows mostly in deeper waters (3–50 m). Specimens were identified to species by NJ de Voogd. Cores of both sponge species were sampled including segments of surface and interior in order to sample, as much as possible, the whole bacterial community. The seawater samples were collected by filtering 1 l [6, 80] of seawater through a Millipore® White Isopore Membrane Filter (GTTP04700, 47 mm diameter, 0.22 μm pore size). All samples were stored in 96 % EtOH [13, 63] and kept at temperatures lower than 4 °C immediately after collection. Once in the laboratory, samples were stored at −20 °C until DNA extraction.

DNA Extraction and Pyrosequencing

We isolated PCR-ready genomic DNA from seawater, sediment, and sponge samples using the FastDNA® SPIN Kit following the manufacturer's instructions. This is an extraction method frequently used for this purpose [13, 17, 89]. Briefly, we prepared sediment samples by centrifuging each one for 30 min at 4,400 rpm and 4 °C; the membrane filter (seawater sample) and sponge samples were each cut into small pieces. The whole membrane filter and 500 mg of sediment and sponge were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles. The microbial cell lysis was performed in the FastPrep® Instrument (Q Biogene) for 80 s at the recommended speed.

The extracted DNA was eluted into DNase/Pyrogen-Free Water to a final volume of 50 μl and stored at −20 °C until use. Pyrosequencing and sequence analysis were performed using previously described methods [13, 60] with the exception of the pick OTUs step where we used the recently developed UPARSE clustering method and chimera check [24] and the most recent Greengenes database (http://greengenes.secondgenome.com/downloads/database/13_5) for OTU picking and taxonomic assignment (see Online Resource 1 for a detailed description). In the most recent Greengenes release, the recently adopted phylum *Thaumarchaeota* is still considered a class of the *Crenarchaeota* phylum; in the present study, we follow the Greengenes taxonomy. The sequences generated in this study can be downloaded from the NCBI SRA: Accession number SRP023167.

Identification of Closely Related Organisms and Phylogeny of Abundant OTUs

Closely related organisms of numerically abundant OTUs (≥100 sequences) were identified using the NCBI BLAST command line "blastn" tool with the −db argument set to nt [98]. BLAST was also used to obtain sequences for cultured Archaea, which were included in a bootstrap consensus phylogenetic tree based on 1,000 replicate trees along with representative sequences belonging to all abundant OTUs; the tree was made using the Mega5 Program (http://www.megasoftware.net/; last checked 2012-11-20; [82] with the Nearest-Neighbor-Interchange and Generalized Time-Reversible model [83] with Gamma distributed and invariant sites).

Statistical Analysis

A square matrix containing the presence and abundances of all OTUs per sample was imported into R [87] using the read.table () function. Sequences not classified as Archaea (e.g., bacteria or unclassified at the level of domain, 7,865 sequences) and OTUs with <5 sequences were removed prior to statistical analysis. The OTU abundance matrix was $\log_{10}(x+1)$ transformed and a distance matrix constructed using the Bray–Curtis index with the vegdist() function in the vegan package [59] in R. The Bray–Curtis index is one of the most frequently applied (dis)similarity indices used in ecology [19, 50]. Variation in OTU composition among biotopes (*S. massa* and *X. testudinaria*, sediment, and seawater) was assessed with Principal Coordinates Analysis (PCO) using the cmdscale() function in R with the Bray–Curtis distance matrix as input. We tested for significant variation in composition among biotopes using the adonis() function in vegan. In the adonis analysis, the Bray–Curtis distance matrix of species composition was the response variable with biotope as independent variable. The number of permutations was set at 999; all other arguments used the default values set in the function. Weighted averages scores were computed for OTUs on the first two PCO axes using the wascores() function in the vegan package.

Metagenome Analysis

In the present study, we use PICRUSt [47] to predict the metagenome of each sample. PICRUSt is a bioinformatics tool that uses marker genes, in this case 16S rRNA, to predict metagenome gene functional content. These predictions are precalculated for genes in databases including Kyoto Encyclopedia of Genes and Genomes (KEGG) [42] and Clusters of Orthologous Groups of proteins (COG). In the present study, we used the KEGG database. Output of PICRUSt consists of a table of functional counts, i.e., KEGG Pathway counts by sample. Note that because of functional overlap, some orthologs can be represented in multiple pathways. Since KOs can belong to several pathways, we used the categorize_by_function.py script in PICRUSt to collapse the PICRUSt predictions at the level of the individual pathways. This table was in turn used as input for LEfSe [74]. Using LEfSe, we tested data for statistical significance, biological consistency, and effect size relevance among biotopes. Graphical representations of LEfSe results are presented hierarchically using cladograms with category, subcategory and individual pathway designations (Online Resource 2), and using barplots for selected functional individual pathways.

Results

The sequencing effort yielded 50,241 sequences, which were assigned to 4,669 OTUs after quality control, OTU picking, and removal of chimera. At the level of domain, 2,305 OTUs remained unidentified, and 1,428 OTUs were assigned to the bacteria domain; these were, however, not included in the statistical analysis. The final dataset included 42,313 sequences and 936 OTUs of which 146 OTUs remained unclassified at the phylum level.

All archaeal OTUs were assigned to two phyla, *Crenarchaeota* and *Euryarchaeota*. In addition to this, OTUs were assigned to 14 classes, 18 orders, 15 families, 12 genera, and 3 species. Of these, the classes *Thermoplasmata* and *Thaumarchaeota*, the orders *E2* and *Cenarchaeales*, the families Marine group II and *Cenarchaeaceae*, the genera Cenarchaeum and *Nitrosopumilus*, and the species *Cenarchaeum symbiosum* were the most abundant. Thirty-three OTUs from 12,356 sequence reads were identified from *X. testudinaria* hosts while 27 OTUs from 10,580 sequence reads were identified from *S. massa* hosts. With respect to the nonhost biotopes, 322 OTUs from 8,570 sequence reads were identified from seawater samples while 724 OTUs from 10,807 sequence reads were identified from sediment samples.

BLAST was used to find closely related organisms to all 37 abundant (≥100 sequences) OTUs (Table 1). The most abundant OTU overall was OTU-1, assigned to the species *Cenarchaeum symbiosum* and found predominantly in *S. massa* hosts and represented by 10,473 sequences. This OTU was also the only dominant symbiont found in *S. massa*; the remaining 26 OTUs found in this sponge had less than 40 sequences each. OTU-1 was closely related to an organism previously isolated from *Phakellia fusca* hosts in the South China Sea.

Table 1 List of most abundant OTUs (≥100 sequences) including OTU-numbers, number of sequences (reads), biotope where the OTUs were found (group), their taxonomic affiliation, GenBank GenInfo sequence identifiers (GI) of closely related organisms identified using BLAST and sequence identity (Sq ident) of these organisms with our representative OTU sequences

OTU	Reads	Group	Phylum	Class	Order	Family	Genus	Species	GI	Sq ident
1	10,473	Sm	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*Symbiosum*	*340764424*	100
2	6,250	Xt	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691426*	99.3
3	2,228	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*445066213*	96.5
4	1,601	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*220685393*	100
5	1,659	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*125381583*	100
6	1,592	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*321159150*	100
7	1,961	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*529279729*	100
8	801	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*383933392*	100
9	881	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Cenarchaeum*	*Symbiosum*	*321159184*	99.8
10	636	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*310871774*	98.4
11	729	Wt	[b]							
12	360	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*220685221*	100
13	375	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*394999368*	100
15	188	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*39546718*	100
16	222	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*44506621l*	96.5
17	267	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691432*	96.8
18	261	Xt[a]	[b]							
19	195	Sd	*Euryarchaeota*	*Thermoplasmata*	*E2*	*CCA47*			*364530814*	99.8
20	139	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*145651460*	100
22	105	Sd	*Euryarchaeota*	*Thermoplasmata*	*E2*	*DHVEG-1*			*364527263*	99.8
24	202	Sd	*Crenarchaeota*	*MCG*					*145651451*	99.5
28	174	Sd[a]	*Crenarchaeota*	*MBGB*					*389591832*	100
29	107	Sd	*Euryarchaeota*	*Thermoplasmata*	*E2*				*408718821*	99.8
39	100	Sd	[c]						*223031543*	97.9
229	2589	Xt	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691431*	99.5
236	202	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*125381472*	99.8
246	589	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*383933255*	100
317	203	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691434*	99.0
331	173	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*445066213*	96.5
399	374	Wt	*Euryarchaeota*	*Thermoplasmata*	*E2*	*Marine group II*			*83416103*	100
594	197	Xt[a]	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*305691432*	100
1,016	125	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*310871821*	100
1,519	273	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*548783414*	99.5
1,736	808	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*125381373*	100
2,921	377	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*			*529279806*	99.8
3,207	200	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*125381446*	100
4,233	641	Sd	*Crenarchaeota*	*Thaumarchaeota*	*Cenarchaeales*	*Cenarchaeaceae*	*Nitrosopumilus*		*529279817*	99.5

[a] Represent an OTU only present in a particular biotope

[b] Represent an OTU unclassified at the domain level (not included in the analysis)

[c] Represent an OTU unclassified at the phylum level

Only *X. testudinaria* and sediment hosted biotope-specific abundant OTUs. Of the nine abundant OTUs in *X. testudinaria*, seven were host-specific (3, 17, 18, 16, 317, 594, and 331); with the exception of OTU 18 (unclassified), all the remaining eight were assigned to the *Cenarchaeaceae* family. Of these, five were assigned to the genus *Nitrosopumilus*. The most abundant OTU was OTU-2, assigned to the genus *Nitrosopumilus* and closely related to an organism isolated from *Xestosposgia muta* hosts in Florida (Table 1).

In the sediment biotope, the recorded number of OTUs (724) was almost twice as much as the sum of all OTUs from the remaining three biotopes (382). Of the 724 OTUs, only 18 were considered abundant and only one of these was host-specific (OTU-28); this was assigned to the Marine Benthic Group B (MBGB) class. The most abundant OTU was OTU-7 assigned to the genus *Nitrosopumilus* and closely related to an organism isolated from sediment samples collected in Oujiang River, China.

In seawater samples, the most abundant OTU was OTU-4 assigned to Marine group II and closely related to an organism isolated from water samples collected in Arabian Sea. With the exception of OTU-11 (unclassified), all abundant OTUs in seawater were identified as belonging to the Marine group II family.

Higher Taxon Abundance

There were marked differences in the abundance of higher archaeal taxa among biotopes (Fig. 2). The *Euryarchaeota* were more abundant in nonhost biotopes than in sponge hosts. The abundance of *Euryarchaeota* in sponge hosts is largely due to the contribution of *S. massa* with four times more sequences than *X. testudinaria*; only 0.2 % of the *X. testudinaria* sequences were assigned to the *Euryarchaeota* phylum. In contrast, the *Crenarchaeota* were much more abundant in sponge hosts. The taxa Miscellaneous Crenarchaeotal Group (MCG; *Crenarchaeota*) and YLA114 (*Euryarchaeota*) were virtually all restricted to nonhost biotopes.

Of the 15 families found in this study, just two were detected (>0.1 %) in sponge hosts: *Cenarchaeaceae* (98 % *S. massa* and 99.8 % *X. testudinaria*) and Marine group II (1.95 %*S. massa* and 0.2 % *X. testudinaria*). The relative abundance of the most abundant OTU in each biotope was highest in *S. massa*, where more than 97 % of sequences on average belonged to a single OTU. For *X. testudinaria*, just over 69 % of sequences on average belonged to a single OTU. Dominance was lowest for the sediment biotope where just over 14 % of sequences belonged to a single OTU on average.

Importance of Biotope in Structuring Composition

There was a highly significant difference in archaeal composition among biotopes ($F_{3,12}=10.39$, $P<0.001$, $R^2=0.722$). Variation among biotopes thus explained 72 % of the variation in archaeal composition. A PCO ordination (Fig. 3) of the first two axes shows four distinct clusters representing samples from the four biotopes. Although forming distinct clusters, samples from sediment and seawater were closer to one another in the ordination than either to the sponge samples. Axis 1 of the PCO ordination separates samples from nonhost biotopes and both sponge hosts. Axis 2 separates samples from *S. massa* and *X. testudinaria* hosts.

Less than 1 % of OTUs were found in all four biotopes (6 of 936). Only 17.6 % of the sponge OTUs were shared between *S. massa* and *X. testudinaria* hosts (9 of 51); however, these OTUs were also shared with seawater and sediment. No OTUs were found in both sponge hosts that were not present in either sediment or seawater. Only 14.9 % of the OTUs (136 of 910) restricted to the nonhost biotopes were shared between seawater and sediment, and the majority (123 of 136) were not present in sponge hosts.

Phylogeny

In the phylogenetic tree (Fig. 4), there were two main clusters: (1) a cluster consisting of OTUs belonging to the *Crenarchaeota* phylum and (2) a cluster consisting of OTUs belonging to the *Euryarchaeota* phylum. Inside the main cluster of *Euryarchaeota*, the most abundant seawater OTUs belonging to Marine Group II formed a small distinct cluster. Inside the *Crenarchaeota* main cluster, *X. testudinaria*, *S. massa* and some sediment OTUs clustered together to form a cluster consisting of *Cenarchaeaceae* members. Some of the sediment and *X. testudinaria* OTUs present in the *Cenarchaeaceae* cluster grouped together to form a small cluster of members of the thaumarchaeon *Nitrosopumilus*, supported by high bootstrap value (82). OTUs found exclusively or predominantly in *X. testudinaria* formed a distinct and very well supported cluster (100).

Metagenome Analysis

Using LEfSe, we identified significant differences between the different biotopes (Online Resource 2). Differences in the top level functional categories between biotopes included enrichment of the cellular processes in *X. testudinaria*, environmental information processing in sediment, genetic information processing, and human diseases in *S. massa* and organismal systems in seawater. Differences in functional

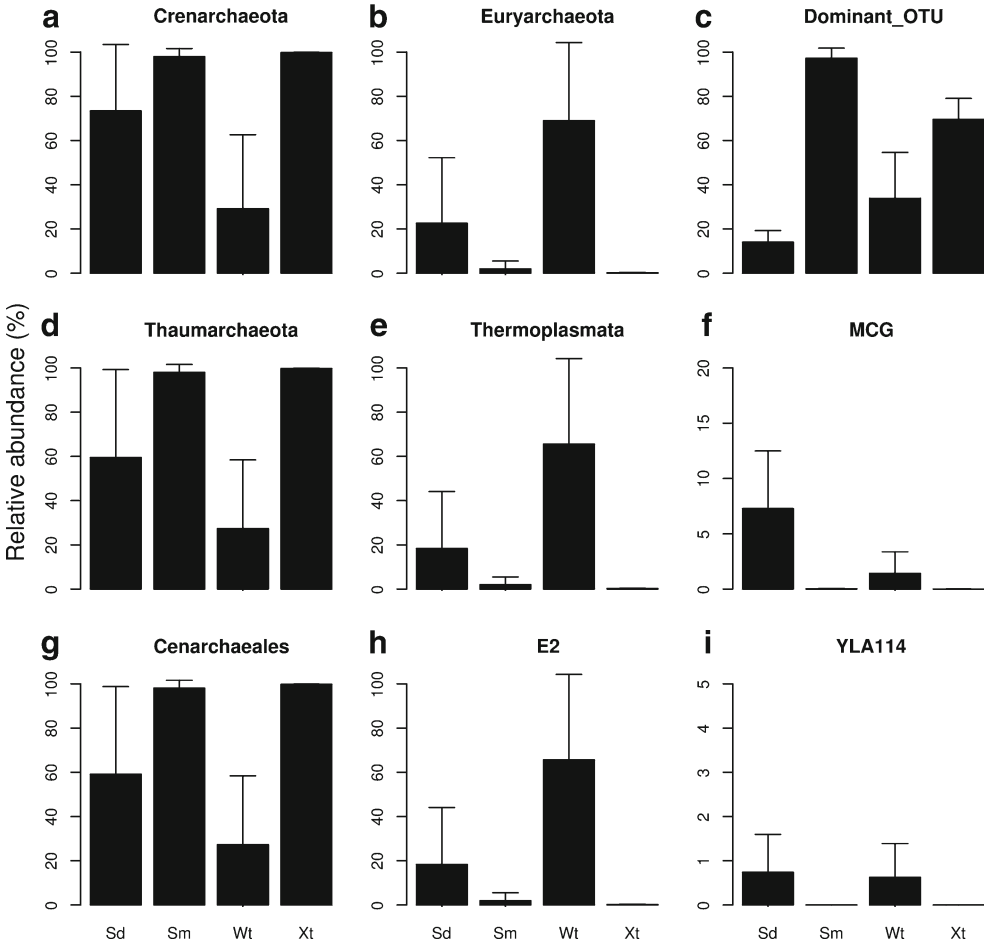

Fig. 2 Mean relative abundance of the most abundant archaeal phyla, classes, orders and the dominant OTU for samples from seawater (*Wt*), sediment (*Sd*), *S. massa* (*Sm*) and *X. testudinaria* (*Xt*). *Error bars* represent a single standard deviation. The dominant OTU represents the mean abundance for the single most abundant OTU in each sample, thus not necessarily the same OTU

subcategories between biotopes included enrichment of the amino acid metabolism and metabolism of cofactors and vitamins in *S. massa*; biosynthesis of other secondary metabolites, energy metabolism, and metabolism of terpenoids and polyketides in *X. testudinaria*; carbohydrate metabolism, enzyme families, lipid metabolism and xenobiotic biodegradation; and metabolism in seawater and glycan biosynthesis and metabolism in sediment. The relative abundance analysis of the functional individual pathways (Fig. 5) gives some insight into the differences observed among biotopes showing which individual pathways generated the significant differences in the top level functional categories and subcategories. Differences at this level included enrichment of the citrate cycle (TCA cycle); glycolysis/gluconeogenesis; ABC transporters; pentose and

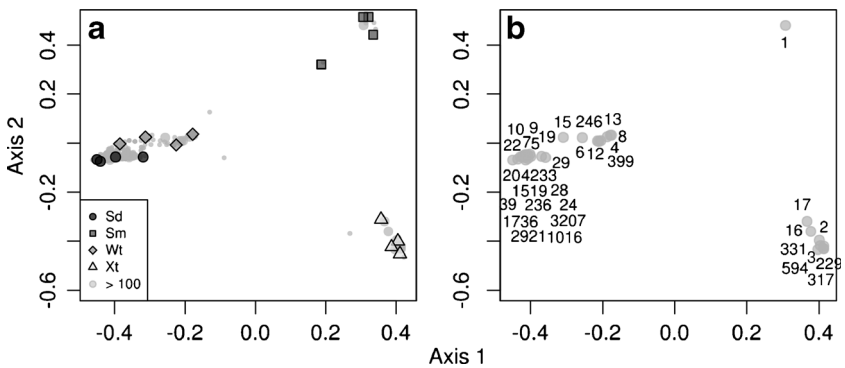

Fig. 3 Ordination showing the first two axes of the PCO analysis. **a** *Symbols* represent samples from seawater (Wt), sediment (Sd), *S. massa* (Sm) and *X. testudinaria* (Xt). Very small *circles* represent OTUs <100 sequence reads. **b** *Numbers* represent abundant (≥100 sequence reads) OTUs referred to in Table 1

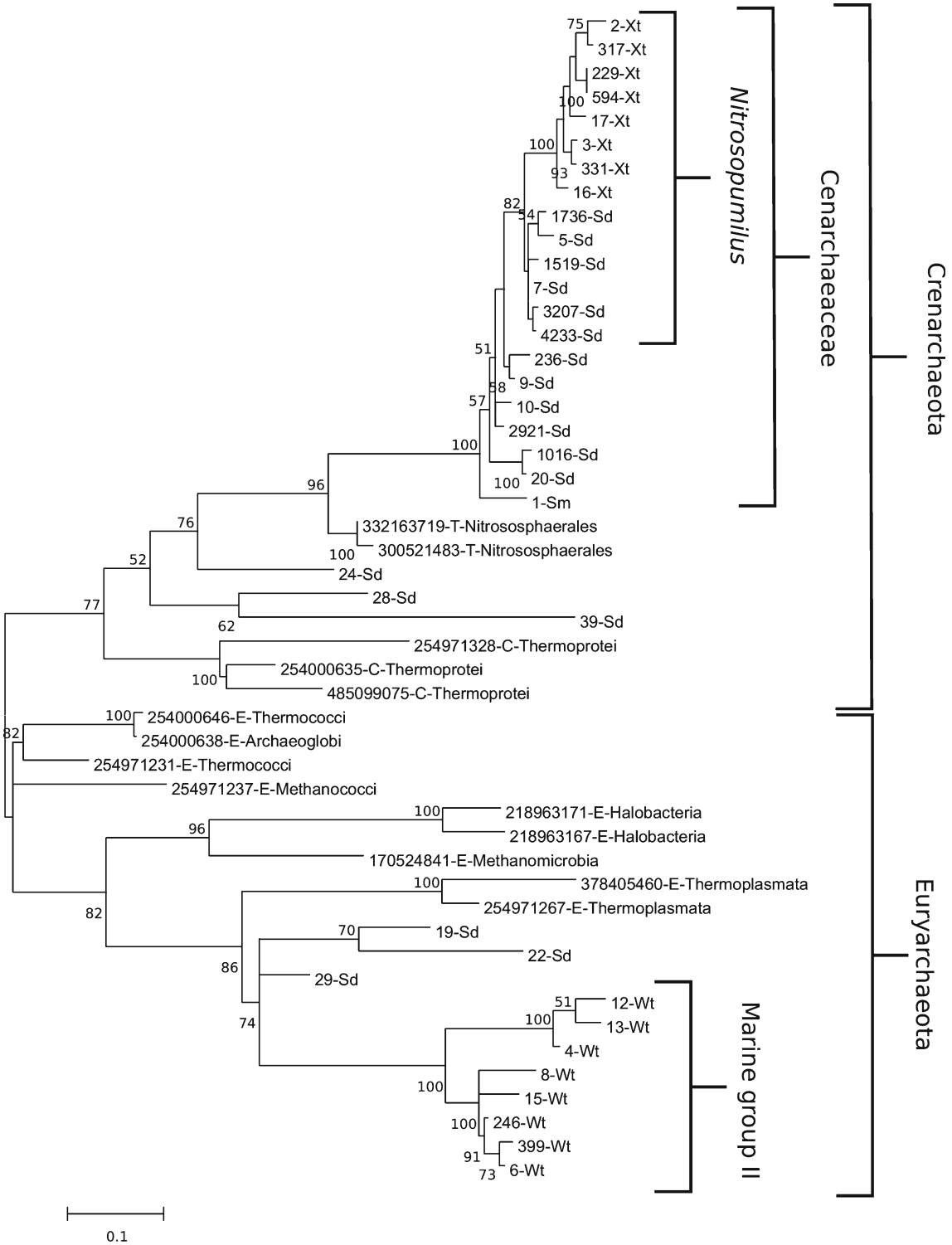

Fig. 4 Phylogenetic tree of the archaeal 16S rRNA gene sequences recovered from the studied biotopes (seawater, sediment, *S. massa* and *X. testudinaria*); bootstrap values lower than 50 % were omitted. The number of each OTU is indicated as are GenBank GenInfo sequence identifiers of cultured archeal sequences. OTUs are assigned to the following clusters: *Wt* mainly found in seawater biotope, *Sd* found in sediment biotope, *Sm* found in *S. massa* biotope and *Xt* found in *X. testudinaria* biotope

glucuronate interconversions (carbohydrate metabolism); isoquinoline alkaloid biosynthesis; methane metabolism (energy metabolism), beta-alanine metabolism (metabolism of other amino acids); aminobenzoate degradation; caprolactam degradation; chloroalkane and chloroalkene degradation; nitrotoluene degradation (xenobiotic biodegradation and

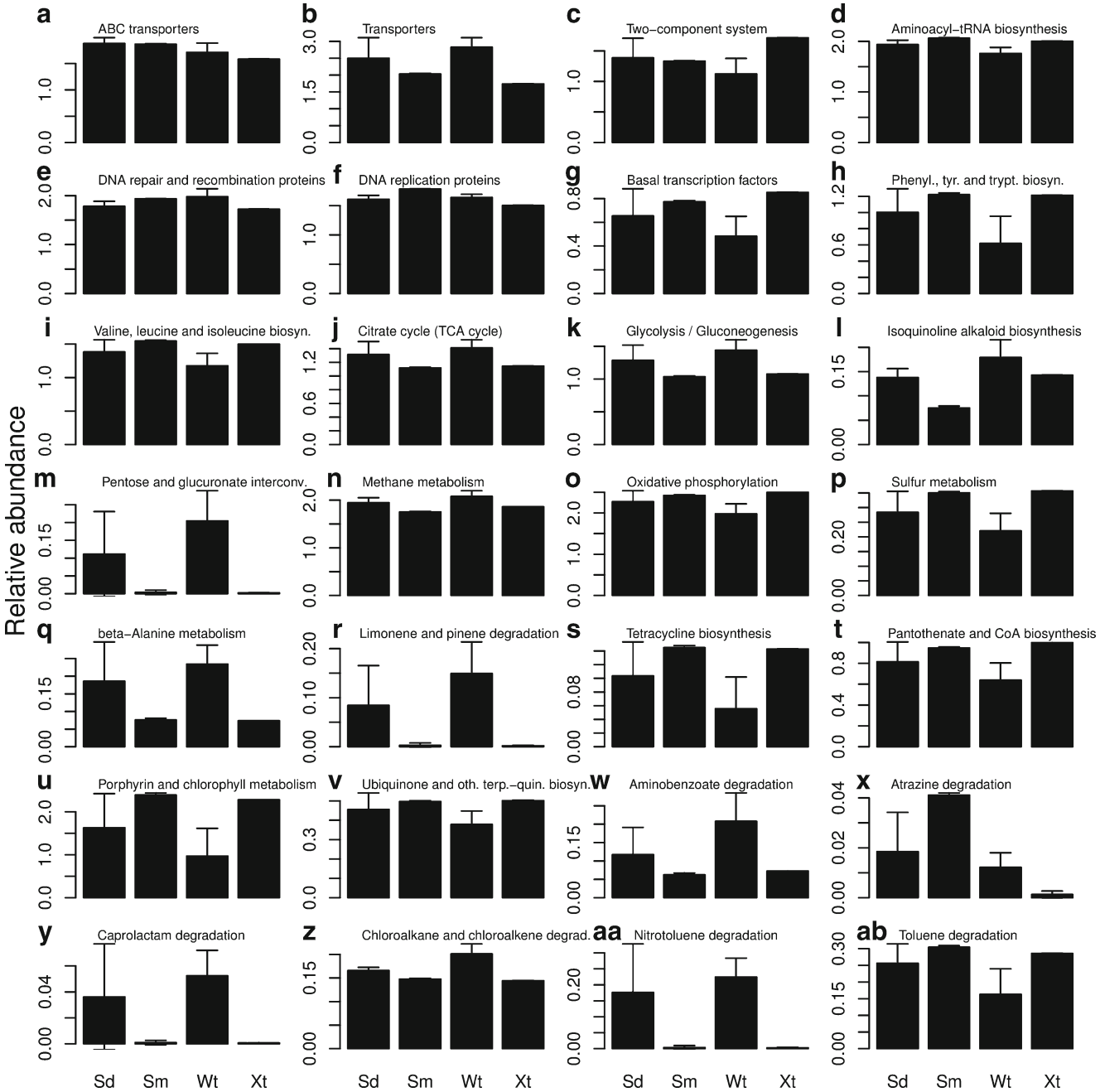

Fig. 5 Mean relative abundance of gene counts for selected functional individual pathways for samples from seawater (*Wt*), sediment (*Sd*), *S. massa* (*Sm*) and *X. testudinaria* (*Xt*). *Error bars* represent a single standard deviation. The individual pathways shown include the following KEGG categories: **a** ABC transporters, **b** transporters, **c** two-component system, **d** aminoacyl-tRNA biosynthesis, **e** DNA repair and recombination proteins, **f** DNA replication proteins, **g** basal transcription factors, **h** Phenylalanine, tyrosine, and tryptophan biosynthesis, **i** valine, leucine, and isoleucine biosynthesis, **j** citrate cycle (TCA cycle), **k** glycolysis/gluconeogenesis, **l** isoquinoline alkaloid biosynthesis, **m** pentose and glucuronate interconversions, **n** methane metabolism, **o** oxidative phosphorylation, **p** sulfur metabolism, **q** beta-alanine metabolism, **r** limonene and pinene degradation, **s** tetracycline biosynthesis, **t** pantothenate and CoA biosynthesis, **u** porphyrin and chlorophyll metabolism, **v** ubiquinone and other terpenoid-quinone biosynthesis, **w** aminobenzoate degradation, **x** atrazine degradation, **y** caprolactam degradation, **z** chloroalkane and chloroalkene degradation, **aa** nitrotoluene degradation and **ab** toluene degradation

metabolism) and limonene and pinene degradation (metabolism of terpenoids and polyketides) in nonhost biotopes and enrichment of the aminoacyl–tRNA biosynthesis (genetic information processing/translation); phenylalanine, tyrosine, and tryptophan biosynthesis; valine, leucine, and isoleucine biosynthesis (amino acid metabolism); basal transcription factors (genetic information processing/transcription); oxidative phosphorylation; sulfur metabolism (energy metabolism); tetracycline

biosynthesis (metabolism of terpenoids and polyketides); pantothenate and CoA biosynthesis; porphyrin and chlorophyll metabolism; ubiquinone and other terpenoid-quinone biosynthesis (metabolism of cofactors and vitamins); atrazine degradation; and toluene degradation (xenobiotic biodegradation and metabolism) in sponge biotopes.

Discussion

Higher Taxon Abundance

In line with previous studies [48, 60], only two archaeal phyla (*Euryarchaeota* and *Crenarchaeota*) were detected in our samples. *Crenarchaeota* were the most abundant phylum in all biotopes except seawater. Several studies have found sponges to be exclusively associated with *Crenarchaeota* [73, 88, 92]. Some other studies, however, observed *Euryarchaeota* in some sponge species albeit in low abundances [39, 48]. In sponge samples, most sequences belonged to the *Thaumarchaeota* class. Mesophilic *Crenarchaeota* (*Thaumarchaeota*) have been shown to be important players in geochemical cycles [8, 96]. Previous studies linked a high abundance of *Thaumarchaeota* to peaks in nitrification in the water column of the Dutch coastal North Sea and subsequent reduction of ammonia and accretion of nitrite concentrations [61, 96]; *Thaumarchaeota* may play a similar role in JBTI. The fact that the reefs of JBTI are subject to elevated levels of pollution [67] means that organisms capable of nitrifying toxic ammonia (NH_3) to nitrate (NO_3^-) may play a crucial role in maintaining a healthy coral reef environment [4, 72].

As expected, given the highly selective nature of sponges [35], the number of OTUs found in nonhost biotopes was substantially higher than in sponge hosts. In contrast, Lee et al. [48] found a less diverse archaeal community in Red Sea seawater samples than in *X. testudinaria*. This difference in archaeal composition between seawater and *X. testudinaria* in both regions may reflect geographic differences. However, methodological differences (mainly in sample preservation before extraction and in the hypervariable region and universal primers selected) may also be at least partially responsible for the differences in archaeal composition between both studies.

Stylissa massa shared a higher percentage of OTUs with nonhost biotopes than *X. testudinaria* (77.8 and 39.4 % respectively). Among other things, this may indicate a higher permeability of *S. massa* to incorporate environmental archaeal OTUs or more pronounced antimicrobial activity by *X. testudinaria*. *Xestospongia* species have previously been shown to produce compounds with antimicrobial activity [51] as has *S. massa* [70]. However, antimicrobial activity has been only demonstrated for bacteria and fungi [70, 99]; no antiarchaeal activity has been reported in sponges.

The majority of OTUs found in seawater samples were assigned to the phylum *Euryarchaeota* (68.7 %). However, in contrast to Lee et al. [48], where nearly all archaeal reads from seawater samples were classified as *Euryarchaeota*, in the present study, almost 30 % of the seawater sequences were assigned to *Crenarchaeota*, and of those, 94.6 % belongs to the class *Thaumarchaeota*. In line with our study, Qian et al. [65] reported a dominance of *Euryarchaeota* in the upper layers (2 and 50 m) of Red Sea waters. Other studies [22, 53], however, found *Crenarchaeota* to be the dominant phylotype in seawater samples. Almost all *Euryarchaeota* classes have methanogenic taxa [58]. In this study, besides the numerous *Thermoplasmata* class (196 OTUs 7,846 sequences), two other archaeal taxonomic classes known as methanogens were found: *Methanobacteria* (17 OTUs, 62 sequences) and *Methanomicrobia* (5 OTUs, 12 sequences). The predominance of the *Thermoplasmata* class in seawater indicates that methanogenesis and methane oxidation are important processes in this biotope. The results of our PICRUSt and LEfSe analysis support this hypothesis and showed significant enrichment of the methane metabolism pathway in seawater. The detection of methanogenic Archaea in oxic environments is not new. Despite being generally believed to be produced exclusively by strictly anaerobic Archaea, methane has been found to be supersaturated in oxygenated surface waters [33, 69]. This phenomenon has been called the "ocean methane paradox" [44], and several explanations have emerged in the literature [33, 44, 52, 54].

Marine group II (*Thermoplasmata/E2*) was the most abundant family (62.9 % of all sequences) in seawater. Only 1.95, 0.21, and 0.44 % of *S. massa*, *X. testudinaria*, and sediment sequences on average were assigned to this family. Wemheuer et al. [94] previously reported *Thermoplasmata* as the third most abundant archaeal class in seawater samples collected in the North Sea. DeLong and Pace [21] noted that E2 was predominantly found in marine plankton, and Holmes et al. [39] reported the presence of Marine Group II in three sponges from the Timor Sea, Australia (*Axechina raspailioides*, *Reniochalina stalagmitis*, and *Ptilocaulis sp.*). According to Baker et al. [2], Marine Group II may also be active players in the recycling of organic carbon and nitrogen. One hundred and forty-six OTUs remained unclassified at the phylum level. All of these potential novel taxa were found in sediment and/or seawater indicating that these are rich biotopes that require more intense research.

Importance of Biotope in Structuring Composition

The PCO ordination and phylogenetic tree revealed marked compositional differences among the four biotopes. The four biotopes studied here thus host compositionally and phylogenetically distinct communities of Archaea. In contrast to our study, Lee et al. [48] failed to distinguish differences in

archaeal composition among sponge species, although they did find compositional differences between sponges and seawater.

Metagenome

The contribution of sponge symbionts to the health, performance, and survival of their host is well known [32, 86]. According to Freeman et al. [32], around 50 % of sponge energy requirements are fulfilled by microbial processes. This is supported by our metagenomic results, which showed a significant enrichment of almost all functional individual pathways associated to the energy metabolism in *X. testudinaria*, namely, oxidative phosphorylation, carbon fixation, and sulfur metabolism. Although sponges acquire part of their nutritional requirements through filter feeding, some species rely mainly on their microbial community to fulfill their energy and carbon needs. For example, *Aplysina cauliformis* and *Neopetrosia subtriangularis* obtain about 77 % of their carbon needs from their microbial cells as opposed to only 27 % for *Niphates erecta* [32]. The higher abundance of enzymes encoding for carbon fixation in *X. testudinaria* indicates that this sponge species primarily relies on high microbe densities to acquire carbon. *Stylissa massa*, in contrast, may not rely so heavily on microbial symbiosis and obtains a higher part of its carbon from the filtered particulate organic matter, although this remains to be demonstrated. In addition to providing supplemental nutrition, *X. testudinaria* archaeal symbionts may also play an important role in detoxifying sponge tissues and metabolizing toxic by-products such as hydrogen sulfide through sulfur oxidation, as has been shown by Hoffmann et al. [37] and Radax et al. [68] for sulfur-oxidizing bacteria.

Nitrogen and methane metabolism were significantly enriched in seawater. Methane metabolism encompasses methanogenesis and methane oxidation; normally in the archaeal domain, both processes occur under strict anaerobic conditions [58]. Based on this, one would expect that these processes to be much more prevalent in sediment than in oxic seawater. In the present study, the significant enrichment of the methane metabolism pathway together with the predominance of OTUs assigned to methanogenic classes in seawater biotopes can be the result of physical transport from anoxic sediment [33, 69] or attachment of methanogens to microanoxic environments present in the water column such as fecal pellets, photoautotrophs, or marine snow particles [33, 52]. As happens in aerated soils [1], the methanogens present in JBTI seawater may also be able to survive under oxic conditions and become active only under favorable conditions. Moreover, *Thaumarchaeota* were recently associated with an aerobic process of methane production. *Nitrosopumilus maritimus* seems to be involved in methylphosphonic acid (MPn) biosynthesis which, in turn, is used by aerobic bacteria as a source of phosphorus to produce methane [54]. Although there were no *Nitrosopumilus* among the most abundant water OTUs, the number of *Nitrosopumilus* sequences in this biotope was not negligible (826 sequences).

The predominance of the archaeal ammonia monooxygenase subunit genes (AmoA) when compared to their bacterial counterparts in marine water and sediment biotopes is well known [31, 96] and suggests an important role of Archaea in global nitrogen cycling. In the present study, the nitrogen metabolism pathway was significantly enriched in seawater, which is surprising due to the low abundance of *Thaumarchaeota* in water when compared to sponge biotopes. Some studies have found nitrogen metabolism related functions enriched in different sponge species when compared to seawater samples [28]; however, these findings concern the entire prokaryotic community.

The sponge symbiotic relationship with photosynthetic microorganisms is well known [5, 26, 86, 95]. This consortium occurs more frequently in oligotrophic waters; sponges benefit from photosymbiotic derived nutrients while photosynthetic microbes benefit from metabolic end-products synthesized by sponges. Indeed, some sponge species obtain almost 50 % of their nutritional requirements from their photosynthetic symbionts [32, 95]. Cyanobacteria have been the photosymbiont more commonly reported in sponge hosts [26, 27]. Here, sponge archaeal communities were enriched in the function of porphyrin and chlorophyll metabolism associated with photosynthesis. Some Archaea are able to convert light into chemical energy via ATP synthesis [38]. These Archaea can be especially important to *S. massa* which, living in shallow water, may suffer from exposure to air during low tide and concomitant elevated UV exposure. The association with these symbionts may enable *S. massa* to deal with these stresses.

The higher expression of citrate cycle (TCA cycle) and glycolysis/gluconeogenesis pathways in archaeons living in nonhost biotopes appears to indicate that aerobic respiration is the primary carbon assimilation and energy generation process for these organisms.

Nutrient availability inside sponges is necessarily different when compared to nutrient availability in seawater or sediment; these differences can lead to the adoption of distinct nutritional strategies by the archaeal community, as shown by the higher relative abundance of transporters in nonhost biotopes. These transporters are involved in nutrient acquisition being responsible for the transport of sugars, lipid, proteins, nitrogen, and others substrates across microbial membranes. The high expression of these transporters can be more important in water or sediment biotopes where the competition for nutrients is greater and its availability lower. These transporters can confer an important competitive advantage maximizing the archaeon nutrient uptake ability. Fan et al. [28] recently showed ABC transporters to be more abundant in sponge symbionts when compared to planktonic microbes;

however, their study included both archaeal and bacterial microbiomes. In the present study, ABC transporters were most abundant in the sediment biotope as was the top level functional category "environmental information processing." ABC transporters were also more abundant in *S. massa* than seawater or *X. testudinaria*. The higher expression of ABC transporters in *S. massa* suggests that this sponge is a relatively nutrient poor environment when compared to *X. testudinaria*.

Xenobiotic biodegradation and metabolism was one of the functional subcategories significantly enriched in seawater biotopes. Some of the functional individual pathways with a high relative abundance in this biotope were aminobenzoate, caprolactam, chloroalkane and chloroalkene, and nitrotoluene degradation. However, some functional individual pathways of this functional subcategory were found in high relative abundance in sponge biotopes and particularly in *S.massa*, namely, atrazine and toluene degradation. These organic compounds are used in a vast number of industries (metal, paint, textile, wood, and chemical) and also in agriculture (herbicides) and are considered important environmental contaminants. These enter the aquatic environment largely through agricultural and industrial runoff [55]. These findings suggest that communities of Archaea may be relevant xenobiotic degraders and act as bioremediators in polluted environments. The high relative abundance of enzymes involved in xenobiotic degradation in *S. massa* when compared to *X. testudinaria* may be the result of both species occupying distinct habitats; the shallow distribution of *S. massa* may make it more subject to anthropogenic pollutants than *X. testudinaria*. With the degradation of these xenobiotic compounds, archaeal symbionts obtain carbon, nitrogen, and energy while promoting the removal of toxic compounds from the sponge host tissue. Proteins thought to be involved in the degradation of aromatic compounds were previously found in the sponge species *Cymbastela coralliophila*, *Rhopaloeides odorabile*, and *Cymbastela concentrica* [28]. Despite not being a xenobiotic, limonene may also be considered an environmental contaminant. Limonene degradation was enriched in nonhost biotopes. Limonene is a monoterpene produced both biogenically and anthropogenically; it is used industrially in metal, electronic, printing, and paint industries as a substitute for other solvents (chlorinated or hydrocarbons). In aquatic environments, this compound presents high acute toxicity to some aquatic organisms (fish and daphnia) and may bioaccumulate [29].

Sponge symbionts have been shown to produce antibiotic compounds with a protective function against potential sponge pathogens and competitors [30, 86]. Here, sponge archaeal communities were enriched in the function of tetracycline biosynthesis; an antibiotic with antibacterial activity toward pathogenic microorganisms. Additionally, *S. massa* symbionts showed high expression of human diseases and more specifically infectious diseases subcategories (bacterial, viral, and parasitic) and significant enrichment in amoebiasis and tuberculosis individual pathways. Several studies have linked sponge-isolated compounds with the treatment of human diseases [79]. It has been assumed that these compounds are synthesized not by the sponge itself but by their symbionts [30, 86]. Several studies have reported marine sponge secondary metabolites with antibacterial activity against *Mycobacterium tuberculosis* [16, 66] and antiamoebic properties against amoebic parasites [46, 56]. These results suggest that *S. massa* is a potential source of secondary metabolites with activities against vectors of human diseases.

Stylissa massa symbionts were also significantly enriched with pathways associated with amino acid metabolism (biosynthesis of valine, isoleucine, leucine, phenylalanine, tyrosine, and tryptophan) and metabolism of cofactors and vitamins. Besides being building blocks for proteins, amino acids are also considered precursors for the production of secondary metabolites [10, 23]. For example, leucine seems to induce bacitracin synthetase while tryptophan induces the dimethylallyltryptophan production in ergot alkaloid biosynthesis (Haavik and Froyshov and Krupinski *et al.* reviewed in [23]). This can be a justification for the higher expression of genes encoding the biosynthesis of amino acids in sponge biotopes where secondary metabolite production is higher. Pathways associated with the biosynthesis of other secondary metabolites were significantly enriched in *X. testudinaria*. *S. massa* seems also to rely more on their archaeal symbionts for the acquisition of very important compounds as vitamins and cofactors than on their filter-feeding activity. For example, vitamin B12, which needs to be acquired by sponges, is also an important cofactor in the Wood–Ljungdahl pathway, which is a mechanism used by sulfate reducers and methanogens to convert carbon compounds to organic carbon [75, 78].

Our study is the first to assess archaeal composition in different sponge hosts, seawater, and sediment in a coral reef environment. As such, we provide novel insights into the distribution of Archaea. OTU composition differed significantly among biotopes, and there were marked differences in the number of OTUs found in each biotope. The sediment biotope in particular harbored the greatest number of OTUs and a phylogenetically diverse archaeal community. Our phylogenetic tree, furthermore, provides evidence that sponges host phylogenetically distinct archaeal assemblages. The abundant OTUs from *X. testudinaria* formed a distinct and well supported cluster in our phylogenetic tree. Several significant differences were observed in functional pathways between archaeal communities of both sponge species and between nonhost archaeal communities. The major differences in functional pathways were, however, between sponge and nonhost biotopes. The results of our PICRUSt and LEfSe analysis suggested that phylogenetically distinct archaeal communities tend to perform specific roles in biotopes

occupying the same physical environment. Our results also suggested different nutritional strategies in nonhost and sponge Archaea and a clear interdependence between sponge hosts and archaeal symbionts in terms of nutrient acquisition. With the exception of the methane and nitrogen metabolic pathways, all energy metabolic pathways were enriched in sponges when compared to nonhost biotopes. This indicates the importance of nonhost and sponge biotopes in structuring archaeal community composition. It also suggests that Archaea from nonhost and sponge biotopes may play complementary roles in important ecosystem functions such as nutrient cycling. Further studies are needed to assess the importance of sponge and other archaeal communities in nutrient dynamics and other ecosystem functions in coral reef environments.

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References

1. Angel R, Claus P, Conrad R (2012) Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. ISME J 6:847–862. doi:10.1038/ismej.2011.141
2. Baker BJ, Lesniewski RA, Dick GJ (2012) Genome-enabled transcriptomics reveals archaeal populations that drive nitrification in a deep-sea hydrothermal plume. ISME J 6:2269–2279. doi:10.1038/ismej.2012.64
3. Barns SM, Delwiche CF, Palmer JD, Pace NR (1996) Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. P Natl Acad Sci USA 93:9188–9193. doi:10.1073/pnas.93.17.9188
4. Bartlett T (2013) Small Scale Experimental Systems for Coral Research: Considerations, Planning, and Recommendations. NOAA Technical Memorandum NOS NCCOS 165 and CRCP 18, Charleston, p 68
5. Bell J (2008) The functional roles of marine sponges. Estuar Coast Shelf S 79:341–353. doi:10.1016/j.ecss.2008.05.002
6. Bowen JL, Morrison HG, Hobbie JE, Sogin ML (2012) Salt marsh sediment diversity: a test of the variability of the rare biosphere among environmental replicates. ISME J 6:2014–2023. doi:10.1038/ismej.2012.47
7. Brochier-Armanet C, Gribaldo S, Zivanovic Y, Confalonieri F, Forterre P (2005) *Nanoarchaea*: representatives of a novel archaeal phylum or a fast-evolving euryarchaeal lineage related to *Thermococcales*? Genome Biol 6:R42. doi:10.1186/gb-2005-6-5-r42
8. Brochier-Armanet C, Boussau B, Gribaldo S, Forterre P (2008) Mesophilic *Crenarchaeota*: proposal for a third archaeal phylum, the *Thaumarchaeota*. Nat Rev Microbiol 6:245–252. doi:10.1038/nrmicro1852
9. Brochier-Armanet C, Gribaldo S, Forterre P (2011) Spotlight on the *Thaumarchaeota*. ISME J 6:227–230. doi:10.1038/ismej.2011.145
10. Bromke MA (2013) Amino acid biosynthesis pathways in diatoms. Metabolites 3:294–311
11. Cao H, Li M, Hong Y, Gu J (2011) Diversity and abundance of ammonia-oxidizing Archaea and bacteria in polluted mangrove sediment. Syst Appl Microbiol 34:513–523. doi:10.1016/j.syapm.2010.11.023
12. Capone D, Dunham S, Horrigan S, Duguay L (1992) Microbial nitrogen transformations in unconsolidated coral reef sediments. Mar Ecol-Prog Ser 80:75–88. doi:10.3354/meps080075
13. Cleary DFR, Becking LE, Voogd NJ, Pires AC, Polonia AR, Egas C, Gomes NCM (2013) Habitat and host-related variation in sponge bacterial symbionts communities in Indonesian waters. FEMS Microbiol Ecol. doi:10.1111/1574-6941.12135
14. Cleary DFR, De Vantier L, Vail L, Manto P, de Voogd NJ, Rachello-Dolmen PG, Tuti Y, Budiyanto A, Wolstenholme J, Hoeksema BW et al (2008) Relating variation in species composition to environmental variables: a multi-taxon study in an Indonesian coral reef complex. Aquat Sci 70:419–431. doi:10.1007/s00027-008-8077-2
15. Cleary DFR, Suharsono, Hoeksema B (2006) Coral diversity across a disturbance gradient in the Pulau Seribu reef complex off Jakarta, Indonesia. Biodivers Conserv 15:3653–3674. doi:10.1007/s10531-004-4692-y
16. Copp BR, Pearce AN (2007) Natural product growth inhibitors of *Mycobacterium tuberculosis*. Nat Prod Rep 24:278–297. doi:10.1039/b513520f
17. Costa R, Keller-Costa T, Gomes NC, da Rocha UN, van Overbeek L, van Elsas JD (2013) Evidence for selective bacterial community structuring in the freshwater sponge *Ephydatia fluviatilis*. Microb Ecol 65:232–244. doi:10.1007/s00248-012-0102-2
18. Dang H, Zhang X, Sun J, Li T, Zhang Z, Yang G (2008) Diversity and spatial distribution of sediment ammonia-oxidizing *Crenarchaeota* in response to estuarine and environmental gradients in the Changjiang Estuary and East China Sea. Microbiology 154:2084–2095. doi:10.1099/mic.0.2007/013581-0
19. de Voogd N, Becking L, Cleary D (2009) Sponge community composition in the Derawan Islands, ne Kalimantan, Indonesia. Mar Ecol Prog Ser 396:169–180. doi:10.3354/meps08349
20. DeLong E (1992) Archaea in coastal marine environments. P Natl Acad Sci USA 89:5685–5689. doi:10.1073/pnas.89.12.5685
21. DeLong E, Pace N (2001) Environmental diversity of bacteria and Archaea. Syst Biol 50:470–478. doi:10.1080/106351501750435040
22. DeLong E, Wu K, Prézelin B, Jovine R et al (1994) High abundance of Archaea in Antarctic marine picoplankton. Nature 371:695–697. doi:10.1038/371695a0
23. Demain AL (1998) Induction of microbial secondary metabolism. Int Microbiol 259–264
24. Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996. doi:10.1038/NMETH.2604
25. Elkins JG, Podar M, Graham DE, Makarova KS, Wolf Y, Randau L, Hedlund BP, Brochier-Armanet C, Kunin V, Anderson I et al (2008) A Korarchaeal genome reveals insights into the evolution of the Archaea. P Natl Acad Sci USA 105:8102–8107. doi:10.1073/pnas.0801980105
26. Erwin PM, Thacker RW (2008) Cryptic diversity of the symbiotic cyanobacterium *Synechococcus spongiarum* among sponge hosts. Mol Ecol 17:2937–2947. doi:10.1111/j.1365-294X.2008.03808.x
27. Erwin P, Olson J, Thacker R (2011) Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern gulf of Mexico. PloS one 6:e26806. doi:10.1371/journal.pone.0026806
28. Fan L, Reynolds D, Liu M, Stark M, Kjelleberg S, Webster NS, Thomas T (2012) Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. P Natl Acad Sci USA 109:E1878–E1887. doi:10.1073/pnas.1203287109

29. Filipsson AF, Bard J, Karlsson S (1998) Concise International Chemical Assessment Document 5: Limonene, vol 5. World Health Organization, Geneva, pp 1–36
30. Flemer B, Kennedy J, Margassery LM, Morrissey JP, O'Gara F, Dobson ADW (2012) Diversity and antimicrobial activities of microbes from two Irish marine sponges, *Suberites carnosus* and *Leucosolenia sp.* J Appl Microbiol 112:289–301. doi:10.1111/j.1365-2672.2011.05211.x
31. Francis C, Roberts K, Beman J, Santoro A, Oakley B (2005) Ubiquity and diversity of ammonia oxidizing Archaea in water columns and sediments of the ocean. P Natl Acad Sci USA 102: 14683–14688. doi:10.1073/pnas.0506625102
32. Freeman CJ, Thacker RW (2011) Complex interactions between marine sponges and their symbiotic microbial communities. Limnol Oceanogr 56:1577–1586. doi:10.4319/lo.2011.56.5.1577
33. Grossart HP, Frindte K, Dziallas C, Eckert W, Tang KW (2011) Microbial methane production in oxygenated water column of an oligotrophic lake. P Natl Acad Sci USA 108:19657–19661. doi:10.1073/pnas.1110716108
34. Hentschel U, Hopke J, Horn M, Friedrich A, Wagner M, Hacker J, Moore B (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microb 68: 4431–4440. doi:10.1128/AEM.68.9.4431-4440.2002
35. Hentschel U, Usher K, Taylor M (2006) Marine sponges as microbial fermenters. FEMS Microbiol Ecol 55:167–177. doi:10.1111/j.1574-6941.2005.00046.x
36. Hentschel U, Piel J, Degnan SM, Taylor MW (2012) Genomic insights into the marine sponge microbiome. Nat Rev Microbiol 10:641–654. doi:10.1038/nrmicro2839
37. Hoffmann F, Radax R, Woebken D, Holtappels M, Lavik G, Rapp HT, Schlaeppy ML, Schleper C, Kuypers MMM (2009) Complex nitrogen cycling in the sponge *Geodia barretti*. Environ Microbiol 11:2228–2243. doi:10.1111/j.1462-2920.2009.01944.x
38. Hohmann-Marriott MF & Blankenship RE (2011) Evolution of Photosynthesis In Merchant, SS and Briggs, WR and Ort, D, editor, Annu Rev Plant Biol 62: 515–548. doi: 10.1146/annurev-arplant-042110-103811
39. Holmes B, Blanch H (2007) Genus-specific associations of marine sponges with Group I *Crenarchaeotes*. Mar Biol 150:759–772. doi: 10.1007/s00227-006-0361-x
40. Huber H, Hohn MJ, Rachel R, Fuchs T, Wimmer VC, Stetter KO (2002) A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. Nature 417:63–67. doi:10.1038/417063a
41. Jackson SA, Kennedy J, Morrissey JP, OGara F, Dobson AD (2012) Pyrosequencing reveals diverse and distinct sponge-specific microbial communities in sponges from a single geographical location in Irish waters. Microb Ecol 64:105–116. doi:10.1007/s00248-011-0002-x
42. Kanehisa M, Goto S (2000) KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30. doi:10.1093/nar/28.1.27
43. Kendall M, Wardlaw G, Tang C, Bonin A, Liu Y, Valentine D (2007) Diversity of Archaea in marine sediments from Skan Bay, Alaska, including cultivated methanogens, and description of *Methanogenium boonei sp.* nov. Appl Environ Microb 73:407–414. doi:10.1128/AEM.01154-06
44. Kiene RP (1991) In Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, Halomethanes. American Society for Microbiology, Washington DC, pp 111–146
45. Kozubal MA, Romine M, deM Jennings R, Jay ZJ, Tringe SG, Rusch DB, Beam JP, McCue LA, Inskeep WP (2012) *Geoarchaeota*: a new candidate phylum in the Archaea from high-temperature acidic iron mats in Yellowstone National Park. ISME J 7:622201. doi:10.1038/ismej.2012.132
46. Lakshmi V, Saxena A, Mishra SK, Mishra M, Srivastava S, Ghoshal S (2009) Antiamebic activity of marine sponge *Haliclona exigua* (Krikpatrick). Bangladesh J Pharmacol 4:55–59. doi:10.3329/bjp.v4i1.1083
47. Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Thurber RLV, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 31:814. doi:10.1038/nbt.2676
48. Lee O, Wang Y, Yang J, Lafi F, Al-Suwailem A, Qian P (2011) Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. ISME J 5:650–664. doi:10.1038/ismej.2010.165
49. Lee Y, Lee J, Lee H (2001) Microbial symbiosis in marine sponges. J Microbiol 39:254–264
50. Legendre P, Gallagher E (2001) Ecologically meaningful transformations for ordination of species data. Oecologia 129:271–280. doi:10.1007/s004420100716
51. Li D, Xu Y, Shao C, Yang R, Zheng C, Chen Y, Fu X, Qian P, She Z, Voogd N et al (2012) Antibacterial bisabolane-type sesquiterpenoids from the sponge-derived fungus *Aspergillus sp.* Mar Drugs 10:234–241. doi:10.3390/md10010234
52. Marty D, Nival P, Yoon W (1997) *Methanoarchaea* associated with sinking particles and zooplankton collected in the Northeastern tropical Atlantic. Oceanol Acta 20:863–869
53. Massana R, DeLong E, PA C (2000) A few cosmopolitan phylotypes dominate planktonic archaeal assemblages in widely different oceanic provinces. Appl Environ Microb 66:1777–1787. doi:10.1128/AEM.66.5.1777-1787.2000
54. Metcalf WW, Griffin BM, Cicchillo RM, Gao J, Janga SC, Cooke HA, Circello BT, Evans BS, Martens-Habbena W, Stahl DA, van der Donk WA (2012) Synthesis of methylphosphonic acid by marine microbes: a source for methane in the aerobic ocean. Science 337: 1104–1107. doi:10.1126/science.1219875
55. Murdock JN, Shields JFD, Lizotte JRE (2013) Periphyton responses to nutrient and atrazine mixtures introduced through agricultural runoff. Ecotoxicology 22:215–230. doi:10.1007/s10646-012-1018-9
56. Nakisah MA, Muryany MYI, Fatimah H, Fadilah RN, Zalilawati MR, Khamsah S, Habsah M (2012) Anti-amoebic properties of a Malaysian marine sponge *Aaptos sp* on *Acanthamoeba castellanii*. World J Microb Biot 28:1237–1244. doi:10.1007/s11274-011-0927-8
57. Nunoura T, Takaki Y, Kakuta J, Nishi S, Sugahara J, Kazama H, Chee GJ, Hattori M, Kanai A, Atomi H et al (2011) Insights into the evolution of Archaea and eukaryotic protein modifier systems revealed by the genome of a novel archaeal group. Nucleic Acids Res 39:3204–3223. doi:10.1093/nar/gkq1228
58. Offre P, Spang A, Schleper C (2013) Archaea in biogeochemical cycles. Annu Rev Microbiol 67:437–457. doi: 10.1146/annurev-micro-092412-155614
59. Oksanen J, Kindt R, Legendre P, O'Hara B, Simpson G, Solymos P, Stevens M, Wagner H (2009) vegan: Community ecology package. R package version 1.15–2. URL: http://CRAN.R-project.org/package=vegan
60. Pires A, Cleary D, Almeida A, Cunha Â, Dealtry S, Mendonça-Hagler L, Smalla K, Gomes N (2012) Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented archaeal diversity in mangrove sediment and rhizosphere samples. Appl Environ Microb 78:5520–5528. doi:10.1128/AEM.00386-12
61. Pitcher A, Villanueva L, Hopmans E, Schouten S, Reichart G, Damsté J (2011) Niche segregation of ammonia-oxidizing Archaea and anammox Bacteria in the Arabian Sea oxygen minimum zone. ISME J 5:1896–1904. doi:10.1038/ismej.2011.60
62. Preston C, Wu K, Molinski T, DeLong E (1996) A psychrophilic Crenarchaeon inhabits a marine sponge: *Cenarchaeum symbiosum* gen. nov., sp. nov. P Natl Acad Sci USA 93:6241–6246. doi:10.1073/pnas.93.13.6241
63. Previsic A, Walton C, Kucinic M, Mitrikeski PT, Kerovec M (2009) Pleistocene divergence of Dinaric Drusus endemics (*Trichoptera*,

Limnephilidae) in multiple microrefugia within the Balkan Peninsula. Mol Ecol 18:634–647. doi:10.1111/j.1365-294X.2008.04046.x
64. Prosser JI, Nicol GW (2008) Relative contributions of Archaea and bacteria to aerobic ammonia oxidation in the environment. Environ Microbiol 10:2931–2941. doi:10.1111/j.1462-2920.2008.01775.x
65. Qian P, Wang Y, Lee O, Lau S, Yang J, Lafi F, Al-Suwailem A, Wong T (2010) Vertical stratification of microbial communities in the Red Sea revealed by 16s rDNA pyrosequencing. ISME J 5:507–518. doi:10.1038/ismej.2010.112
66. Quideau S, Lebon M, Lamidey A (2002) Enantiospecific synthesis of the antituberculosis marine sponge metabolite (+)-puupehenone. The arenol oxidative activation route. Org Lett 4:3975–3978. doi:10.1021/ol026855t
67. Rachello-Dolmen P, Cleary D (2007) Relating coral species traits to environmental conditions in the Jakarta Bay/Pulau Seribu reef system, Indonesia. Estuar Coast Shelf S 73:816–826. doi:10.1016/j.ecss.2007.03.017
68. Radax R, Rattei T, Lanzen A, Bayer C, Rapp HT, Urich T, Schleper C (2012) Metatranscriptomics of the marine sponge *Geodia barretti*: tackling phylogeny and function of its microbial community. Environ Microbiol 14:1308–1324. doi:10.1111/j.1462-2920.2012.02714.x
69. Reeburgh WS (2007) Oceanic methane biogeochemistry. Chem Rev 107:486–513. doi:10.1021/cr050362v
70. Rohde S, Gochfeld D, Ankisetty S, Avula B, Schupp P, Slattery M (2012) Spatial variability in secondary metabolites of the indo-pacific sponge *Stylissa massa*. J Chem Ecol 38:463–475. doi: 10.1007/s10886-012-0124-8
71. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362. doi:10.1038/nrmicro1635
72. Rusch A, Hannides A, Gaidos E (2009) Diverse communities of active bacteria and Archaea along oxygen gradients in coral reef sediments. Coral Reefs 28:15–26. doi:10.1007/s00338-008-0427-y
73. Schmitt S, Angermeier H, Schiller R, Lindquist N, Hentschel U (2008) Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Appl Environ Microb 74:7694–7708. doi:10.1128/AEM.00878-08
74. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, Huttenhower C (2011) Metagenomic biomarker discovery and explanation. Genome Biol 12:R60. doi:10.1186/gb-2011-12-6-r60
75. Seravalli J, Kumar M, Ragsdale S (2002) Rapid kinetic studies of acetyl-CoA synthesis: evidence supporting the catalytic intermediacy of a paramagnetic NiFeC species in the autotrophic Wood-Ljungdahl pathway. Biochemistry 41:1807–1819. doi:10.1021/bi011687i
76. Sharp KH, Eam B, Faulkner DJ, Haygood MG (2007) Vertical transmission of diverse microbes in the tropical sponge *Corticium sp.* Appl Environ Microb 73:622–629. doi:10.1128/AEM.01493-06
77. Siboni N, Ben-Dov E, Sivan A, Kushmaro A (2008) Global distribution and diversity of coral associated Archaea and their possible role in the coral holobiont nitrogen cycle. Environ Microbiol 10:2979–2990. doi:10.1111/j.1462-2920.2008.01718.x
78. Siegl A, Kamke J, Hochmuth T, Piel J, Richter M, Liang C, Dandekar T, Hentschel U (2011) Single-cell genomics reveals the lifestyle of *Poribacteria*, a candidate phylum symbiotically associated with marine sponges. ISME J 5:61–70. doi:10.1038/ismej.2010.95
79. Sipkema D, Franssen M, Osinga R, Tramper J, Wijffels R (2005) Marine sponges as pharmacy. Mar Biotechnol 7:142–162. doi:10.1007/s10126-004-0405-5
80. Sogin ML, Morrison HG, Huber JA, Mark Welch D, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". P Natl Acad Sci USA 103:12115–12120. doi:10.1073/pnas.0605127103
81. Spang A, Hatzenpichler R, Brochier-Armanet C, Rattei T, Tischler P, Spieck E, Streit W, Stahl DA, Wagner M, Schleper C (2010) Distinct gene set in two different lineages of ammonia-oxidizing Archaea supports the phylum *Thaumarchaeota*. Trends Microbiol 18:331–340. doi:10.1016/j.tim.2010.06.003
82. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) Mega5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. doi:10.1093/molbev/msr121
83. Tavaré S (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. Lect Math Life Sci 17:57–86
84. Taylor MW, Hill RT, Hentschel U (2011) Meeting Report: 1st International Symposium on Sponge Microbiology. Mar Biotechnol 13:1057–1061. doi:10.1007/s10126-011-9397-0
85. Taylor MW, Tsai P, Simister RL, Deines P, Botte E, Ericson G, Schmitt S, Webster NS (2013) Sponge-specific bacteria are widespread (but rare) in diverse marine environments. ISME J 7:438–443. doi:10.1038/ismej.2012.111
86. Taylor M, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiol Mol Biol R 71:295–347. doi:10.1128/MMBR.00040-06
87. Team RDC (2013) R: a language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. version 2.15. URL: http://www.R-project.org/
88. Turque A, Batista D, Silveira C, Cardoso A, Vieira R, Moraes F, Clementino M, Albano R, Paranhos R, Martins O et al (2010) Environmental shaping of sponge associated archaeal communities. PLoS One 5:e15774. doi:10.1371/journal.pone.0015774
89. Urakawa H, Martens-Habbena W, Stahl DA (2010) High abundance of ammonia-oxidizing Archaea in coastal waters, determined using a modified DNA extraction method. Appl Environ Microb 76:2129–2135. doi:10.1128/AEM.02692-09
90. Valentine DL (2007) Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nat Rev Microbiol 5:316–323. doi:10.1038/nrmicro1619
91. Webster NS, Taylor MW (2012) Marine sponges and their microbial symbionts: love and other relationships. Environ Microbiol 14:335–346. doi:10.1111/j.1462-2920.2011.02460.x
92. Webster N, Negri A, Munro M, Battershill C (2004) Diverse microbial communities inhabit Antarctic sponges. Environ Microbiol 6:288–300. doi:10.1111/j.1462-2920.2004.00570.x
93. Webster N, Taylor M, Behnam F, Lucker S, Rattei T, Whalan S, Horn M, Wagner M (2010) Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. Environ Microbiol 12:2070–2082. doi:10.1111/j.1462-2920.2009.02065.x
94. Wemheuer B, Wemheuer F, Daniel R (2012) RNA-based assessment of diversity and composition of active archaeal communities in the German Bight. Archaea-An Int Microbiol J. doi:10.1155/2012/695826
95. Wilkinson C (1983) Net primary productivity in coral-reef sponges. Science 219:410–412. doi:10.1126/science.219.4583.410
96. Wuchter C, Abbas B, Coolen M, Herfort L, Van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl G, Middelburg J et al (2006) Archaeal nitrification in the ocean. P Natl Acad Sci USA 103:12317–12322. doi:10.1073/pnas.0600756103
97. Wulff J (2001) Assessing and monitoring coral reef sponges: why and how? B Mar Sci 69:831–846
98. Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203–214. doi:10.1089/10665270050081478
99. Zhou X, Xu T, Yang X, Huang R, Yang B, Tang L, Liu Y (2010) Chemical and biological aspects of marine sponges of the genus *Xestospongia*. Chem Biodivers 7:2201–2227. doi:10.1002/cbdv.201000024