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ORIGINAL ARTICLE



Compositional analysis of archaeal communities in high and low microbial abundance sponges in the Misool coral reef system, Indonesia

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ABSTRACT

The high/low microbial abundance (HMA/LMA) dichotomy in sponges has been the subject of several studies over recent years, but few studies have analysed this dichotomy in terms of the sponge archaeal community and function. Using a 16S rRNA gene barcoded pyrosequencing approach and predictive functional analysis (PICRUSt) we compared the archaeal composition, richness and predicted function of one HMA sponge (*Xestospongia testudinaria*), one LMA sponge (*Stylissa carteri*) and one sponge species of unknown microbial abundance (*Aaptos lobata*). Although most of the archaeal sequences were assigned to the Crenarchaeota phylum, *S. carteri* had the highest percentage of sequences assigned to the Euryarchaeota phylum. Variation among sponge species explained >85% of the variation in archaeal operational taxonomic unit (OTU) composition with each sponge species forming a distinct cluster. There were significant differences in predicted PICRUSt profiles among sponge species, suggesting that archaeal communities present in the studied sponge species may perform different functions. *X. testudinaria* and *A. lobata* were similar both in terms of OTU and KEGG orthologues composition, which may indicate that *A. lobata* is a HMA sponge. Additionally, some of the most enriched functions seem to be related to traits associated with high and low microbial abundance sponges.

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Introduction

Marine sponges are abundant, conspicuous sessile filter-feeders which harbour exceptionally high microbial (Archaea, Bacteria, and Eukaryota) densities (Diaz and Rützler 2001) within their mesohyl (Mointinho-Silva et al. 2014). By actively filtering large volumes of seawater, sponges contribute to benthic–pelagic coupling and to water column composition alteration (e.g. secondary metabolite emanation, nutrient transformations; e.g. Bell 2008; Hoffmann et al. 2009; McMurray et al. 2014).

It has been assumed that part of the sponge functional repertoire has its origin not in the sponge itself but in its symbionts (e.g. Freeman and Thacker 2011; Ribes et al. 2012, 2015). Although not completely understood, due to the lack of studies of cultured endosymbionts, sponge-associated microorganisms are likely involved in nutrient transport and utilization (denitrification; nitrification, ammonium oxidation;

carbon fixation); degradation of benzoic compounds; biosynthesis of secondary metabolites, antibiotics, cofactors and vitamins; redox sensing and response (Thomas et al. 2010; Fan et al. 2012; Hentschel et al. 2012). This functional repertoire benefits sponges by enhancing nutrient transfer; growth rates; aiding in metabolic waste processing; and providing protection against ultraviolet light and disease (Simpson 1984; Hentschel et al. 2002; Holmes and Blanch 2007; Erwin and Thacker 2008; Cebrian et al. 2011; Webster and Taylor 2012; Freeman et al. 2013). Additionally, these microbial-mediated metabolic functions play important ecological roles in coral reefs (e.g. Yahel et al. 2003; Hoffmann et al. 2009; de Goeij et al. 2013). Most microbial community studies of coral reefs have, however, focused on bacterial communities and less attention has been paid to archaeal communities. The same holds for sponge studies where the archaeal communities are still less studied, and thus less understood, than bacterial communities.

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Archaea members can dominate the microbial community in some sponges; for example, in *Drummacidon mexicana* and *Inflatella pellicula*, Archaea account for more than 60% of all prokaryote cells (Preston et al. 1996; Jackson et al. 2013). Environmental factors may influence the sponge archaeal community (Turque et al., 2010), although there is some indication that the sponge archaeal community is related to sponge phylogeny (Schmitt et al. 2008; Steger et al. 2008). Zhang et al. (2014), studied sympatric sponges from the Mediterranean (collected in different seasons) and the Caribbean and showed specificity and persistence of archaeal symbionts within sponge species. One of the archaeal members most frequently associated with sponges belongs to the phylum Thaumarchaeota (Brochier-Armanet et al. 2008). This phylum includes members that are able to convert ammonium to nitrite and fix CO₂ through ammonia oxidation. These characteristics suggest that Archaea may play critical roles in sponge hosts. These roles are likely linked with the sponge nitrogen metabolism and consequently with the nitrogen cycle (Zhang et al. 2014). However, the exact roles played by Archaea are still largely unknown.

Sponge species differ substantially in terms of the abundance of their microbial symbiont communities (e.g. Hentschel et al. 2003; Kamke et al. 2010; Bayer et al. 2014a). While some species have a number of microorganisms in the order of 10⁹ cells per gram wet weight of sponge ('high microbial abundance' – HMA) others have a number of microorganisms similar to that found in the surrounding seawater ('low microbial abundance' – LMA), i.e. 10⁵ to 10⁶ cells per gram wet weight of sponge (Hentschel et al. 2012). This dichotomy is not only related to microbial abundance but also to sponge morphology and physiology, microbial diversity, microbial composition and microbial specificity (Erwin et al. 2015 and references therein). HMA sponges host highly diverse microbial communities (29 bacterial phyla; 2 Archaeal phyla; Bayer et al. 2014a); while LMA sponges, in contrast, host less diverse microbial communities that, to a certain extent, are similar to that of the surrounding seawater (Schmitt et al. 2007).

Some studies suggested that HMA and LMA sponges are dominated by distinct microbial members. Most of the HMA and LMA sponge indicators are, however, bacterial members (Bayer et al. 2014b; Moitinho-Silva et al. 2017). For Archaea, Moitinho-Silva et al. (2017), for example, reported that a Nitrosopumilus Operational Taxonomic Unit (OTU) was more abundant in LMA than in HMA sponges while Bayer et al. (2014b) reported that the archaeal *amoA* gene

was slightly less abundant in HMA sponges. There are conflicting results among studies as to which factors better explain the microbial community composition of sponges; and it remains uncertain which is the most important factor influencing sponge microbial communities (host microbial abundance groups or host phylogeny; Blanquer et al. 2013; Schöttner et al. 2013).

The influence of microbial abundances in sponge function remains largely unknown as well as the ecological consequences of a functional HMA/LMA dichotomy. Bayer et al. (2014b) suggested that the HMA/LMA dichotomy extends to the functional gene level, however, few specific differences exist when individual genes were inspected. Most of the studies of sponge microbial HMA/LMA dichotomy have concentrated on bacterial communities, little is known about the existence of an archaeal dichotomy between HMA and LMA sponges.

In the present study, we assessed the composition of Archaea in three sponge species: the demosponges *Stylissa carteri* (Scopalinida: Scopalinidae; LMA; Giles et al. 2013), *Xestospongia testudinaria* (Haplosclerida: Petrosiidae; HMA; Gloeckner et al. 2014) and *Aaptos lobata* (Suberitida: Suberitidae; unknown microbial abundance), collected from three reef sites in the coral reef system of South East Misool, Papua, eastern Indonesia. Our main goals were to compare archaeal richness, composition and putative function between HMA and LMA sponges using a 16S rRNA gene bar-coded pyrosequencing approach and a predictive metagenomic approach.

Materials and methods

Data collection

Triplicate samples of *Stylissa carteri*, *Aaptos lobata* and *Xestospongia testudinaria* were collected by snorkelling and scuba from 13 to 18 September 2013 in South East Misool, Raja Ampat region, West Papua province in Indonesia (Figure 1).

Stylissa carteri (Dendy, 1889) is a medium-sized bright orange sponge with a variable growth form (from thickly flabellate to bushy and digitate). It has a soft consistency and is easily torn with multiple small oscules distributed across its sponge body. *Xestospongia testudinaria* (Lamarck, 1815) is one of the largest known sponges and has a barrel to volcano-shaped morphology with a single large osculum. Its consistency is crumbly to stony and often very difficult to tear. *Aaptos lobata* (Calcinai et al. 2017) occurs in shallow coral reef ecosystems (de Voogd

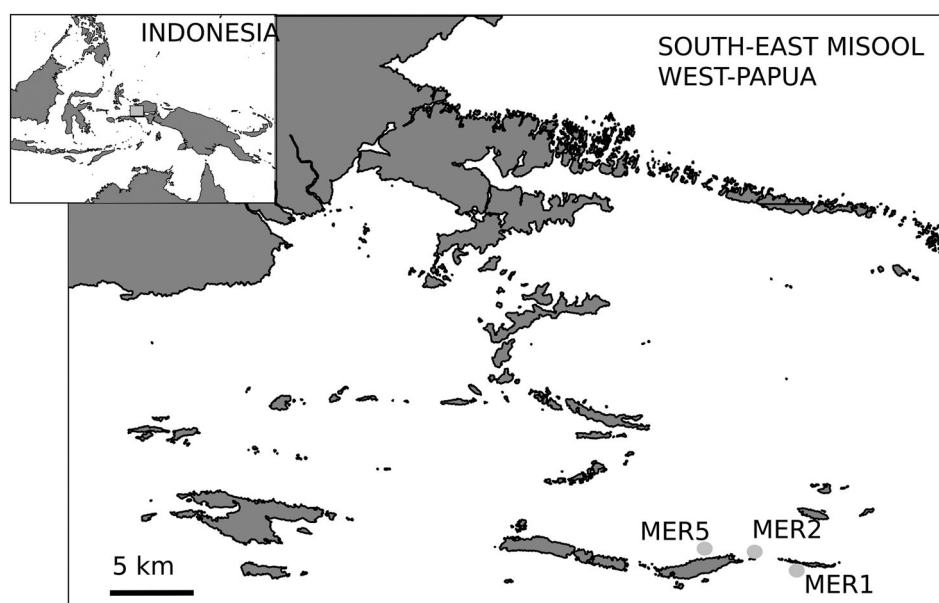


Figure 1. Map of the study area (Misool coral reef system) showing the study sites (Mer1; Mer2; Mer5).

and Cleary 2008) and has a thick irregular lobate growth form with numerous small oscules. The exterior is dark brown coloured and the interior is canary yellow. The sponge turns dark brown when taken out of the water. The consistency is very compact and firm, but easily cut. All three sponge species are common and have a wide distribution range in the Indo-Pacific region.

Sampling took place by the islands of Kalig and Boo (Mer1 (2°13'6.18"S, 130°33'52.74"E), Mer2 (2°13'7.32"S, 130°33'36.66"E), Mer5 (2°13'12.00"S, 130°31'37.00"E)) in the southern region. The locations had high coral cover along a slope or wall. One specimen of each sponge species was collected at the Mer1, Mer2 and Mer5 locations. Sponges were pre-identified in the field, photographed *in situ* and preserved in 95% ethanol for morphological identification by LB and NdV and deposited in the sponge collection of Naturalis Biodiversity Center, Leiden, the Netherlands. Samples of *S. carteri*, *A. lobata* and *X. testudinaria* were collected including segments of surface and interior in order to sample, as much as possible, the whole archaeal community (Cleary et al. 2013; Polónia et al. 2014). Stored in 95% EtOH (Cleary et al. 2013), all samples were kept at temperatures lower than 4° C. Once in the laboratory, samples were stored at –80°C until DNA extraction.

DNA extraction and pyrosequencing

We isolated PCR-ready genomic DNA from *S. carteri*, *A. lobata* and *X. testudinaria* using the FastDNA® SPIN

Kit (MPbiomedicals) following manufacturer's instructions and previously published methods (Polónia et al. 2014, 2015). Succinctly, 500 mg of sponge tissue were transferred to Lysing Matrix E tubes containing a mixture of ceramic and silica particles and lysed in the FastPrep® Instrument (Q Biogene) for 80 s at speed 6.0. Prior to 454 pyrosequencing, the amplicons of the archaeal 16S rRNA gene were obtained using the Archaea specific primers ARC344f-mod and Arch958R-mod (Pires et al. 2012). Using the amplicons of the archaeal 16S rRNA gene as template, the V3–V4 regions were amplified using barcoded fusion primers (524F-10-ext, Arch958R-mod; Pires et al. 2012). Following previous studies (Polónia et al. 2016, 2017), the barcoded pyrosequencing libraries were analysed using the QIIME (Quantitative Insights Into Microbial Ecology; Caporaso et al. 2010) software package (<http://www.qiime.org/>; last checked 20.3.2015). Briefly, 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 (set at 218 bp after removal of forward primers and barcodes); backward primers were removed using the 'truncate only' argument and a sliding window test of quality scores was enabled with a value of 50 as suggested in the QIIME description for the scrip.

Greengenes (gg_13_5; <http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>) considers Thaumarchaeota (Brochier-Armanet et al. 2008, 2012) a class of the Crenarchaeota phylum; for this reason, throughout this study, we will follow the Greengenes taxonomy and refer Thaumarchaeota as a class. The DNA sequences

generated in this study can be downloaded from the NCBI SRA: SRP069346.

Predictive metagenome analysis

To predict the metagenome of each sample, we used PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States; Langille et al. 2013). PICRUSt is a bioinformatics tool that uses marker genes, in this case 16S rRNA, to predict metagenome gene functional content. In order to test data for statistical significance, biological consistency and effect size relevance among sponge species we used the linear discriminant analysis (LDA) effect size (LEfSe) method (Segata et al. 2011). A detailed description of these methods has been published previously (Cleary et al. 2013; Langille et al. 2013; Polónia et al. 2014, 2015). PICRUSt results, instead of measuring actual gene presence/expression and function are predictive and thus provide information on putative enrichment and function. Care, thus, must still be taken in the interpretation of these results. LEfSe results are presented hierarchically using histograms (Segata et al. 2011). In addition to metagenomic data, we also obtained the weighted Nearest Sequenced Taxon Index (NSTI) scores for each sample. NSTI index is a way to evaluate the prediction accuracy of PICRUSt by averaging the branch length separating an OTU from a reference OTU (Langille et al. 2013).

Statistical analysis

Sequences not classified as Archaea were removed prior to statistical analysis. We used a self-written function in R (Gomes et al. 2010) to estimate rarefied OTU richness for each sample. Care, however, should be taken in the interpretation of richness estimates based on sequence data given the prevalence of sequencing errors (Edgar 2013). We assessed the distribution of OTUs in sponge species using a Venn diagram with the `venn()` function of the `venneuler` package in R. The OTU abundance matrix was $\log_e(x + 1)$ transformed (in order to normalize the distribution of the data) and a distance matrix was constructed using the Bray–Curtis index with the `vegdist()` function in the VEGAN package (Oksanen et al. 2009) in R. Sample diversity was assessed with rarefied species richness (S) (Gotelli and Colwell 2001) and Shannon's (H') diversity index (Shannon and Weaver 1949) using the `diversity()` and `specnumber()` functions of the Vegan package (Oksanen et al. 2009). The Chao1 richness estimator was calculated using the `estimateR()` function of the same package. Variation in OTU

composition among sponge species (*S. carteri*, *A. lobata* and *X. testudinaria*) was assessed with Principal Coordinates Analysis (PCO) using the `cmdscale()` function in R with the Bray–Curtis distance matrix as input. Variation among sponge species was tested for significance using the `adonis()` function in VEGAN. In the adonis analysis, the Bray–Curtis distance matrix of species composition was the response variable with the sponge species 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. Detailed descriptions of the functions used here can be found in R (e.g. `?cmdscale`) and online in reference manuals (<http://cran.r-project.org/web/packages/vegan/index.html>; 29.5.2015). The heatmap was generated using the function `heatmap2()` in the R package `gplots` (<http://www.cran.r-project.org/>). The OTUs were log transformed and clustered according to their occurrence by UPGMA hierarchical clustering.

Results

The sequencing effort yielded 34,882 archaeal sequences, which were assigned to 38 archaeal OTUs after quality control, OTU picking and removal of chimeras and sequences not assigned to the archaeal domain. Of the total 38 OTUs, 12 had less than five sequences and of these four were singletons (one sequence per OTU).

Higher taxon abundance

Most of the sponge (*Xestospongia testudinaria* – 97.79%; *Aaptos lobata* – 97.48%; *Stylissa carteri* – 82.90%) archaeal sequences were assigned to the Crenarchaeota phylum, Thaumarchaeota class, Cenarchaeales order (except *A. lobata* with 97.46% assigned to Cenarchaeales and 0.017% assigned to Nitrososphaerales order) and Cenarchaeaceae family (Figure 2; Figures S1 and S2). 82.50% of the *S. carteri* archaeal sequences were assigned to the Cenarchaeum genus while 97.78% and 96.48% of the *X. testudinaria* and *A. lobata* archaeal sequences respectively were assigned to the Nitrosopumilus genus.

OTU richness was highest in *S. carteri* and lowest in *X. testudinaria* (Figure S3). An asymptote was not achieved for any of the sponge species indicating that true richness was not assessed in the studied samples. However, due to the presence of amplification and sequencing artifacts, richness estimates based on

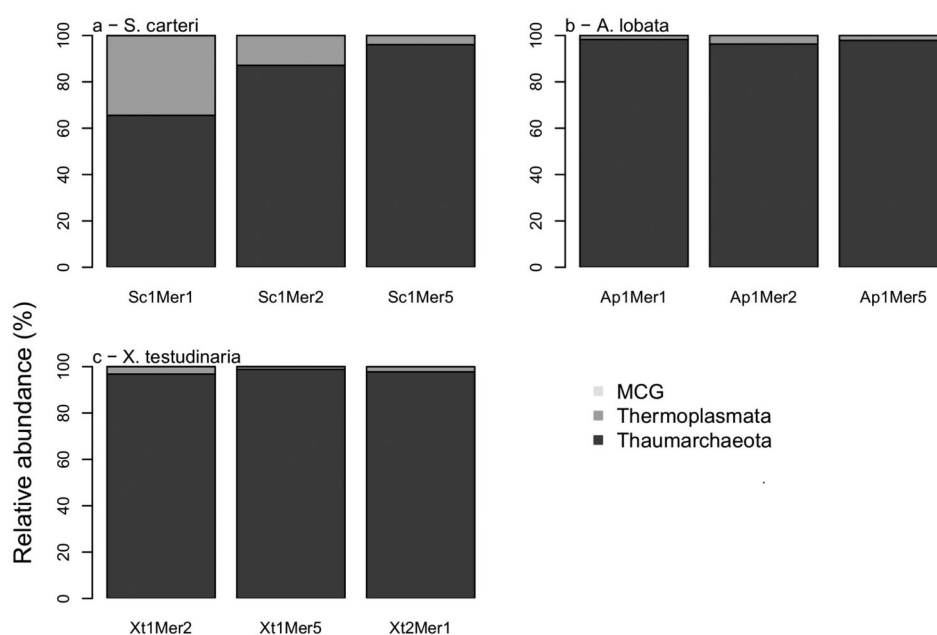


Figure 2. Mean relative abundance of the most abundant archaeal classes from *S. carteri* (Sc), *A. lobata* (Ap) and *X. testudinaria* (Xt).

454 sequencing should be treated conservatively (Edgar 2013).

Stylissa carteri from the site Mer1 had a lower percentage of sequences assigned to the Crenarchaeota phylum (65.55%) when compared to the samples collected at the sites Mer2 and Mer5 (87.10% and 96.05%).

Most of the OTUs were shared among all three sponge species (15 OTUs; Figure 3). *Stylissa carteri* had eight exclusive OTUs; *A. lobata* six exclusive OTUs and *X. testudinaria* only one exclusive OTU. Four OTUs were exclusively shared between *A. lobata* and

X. testudinaria and only one OTU was exclusively shared between *A. lobata* and *S. carteri*.

The first (OTU-4), third (OTU-167) and fourth (OTU-526) most abundant OTUs were assigned to the genus *Nitrosopumilus* and were closely related to archaeal phylotypes associated with the marine sponges *Holoxea* sp. (99.07%) and *Xestospongia muta* (99.75%; 99.01%) (Figure 5; Table SI). The second most abundant OTU (OTU-3) was assigned to the *Cenarchaeum symbiosum* species and was closely related (100%) to archaeal phylotypes associated with the marine sponge *Phakellia fusca*. The fifth most abundant OTU (OTU-2) was assigned to the Marine group II family and was closely related (100%) to archaeal phylotypes associated with the coral *Diploria labyrinthiformis* (Figure 4; Table SI).

The Shannon's diversity index (H), species richness (S), Pielou's evenness (J), Chao1 index (S.chao1), archaeal reads and nearest sequenced taxon index (NSTI) values for the sponge species sampled are shown in Table I. The highest mean values of species richness and Chao 1 estimate were detected in *S. carteri* samples while *X. testudinaria* samples had the highest mean values of H, J and NSTI. *Aaptos lobata* had the highest mean number of archaeal reads.

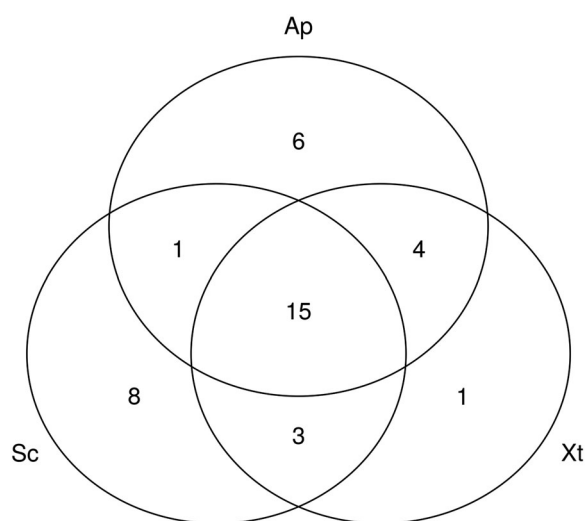


Figure 3. Venn diagram showing the amount of archaeal OTUs shared among the studied sponge species: *S. carteri* (Sc), *A. lobata* (Ap) and *X. testudinaria* (Xt).

Importance of sponge species in structuring composition

There was a highly significant difference in archaeal composition among sponge species ($F_{2,6} = 17.11$, $P < 0.01$, $R^2 = 0.851$). Variation among sponge species

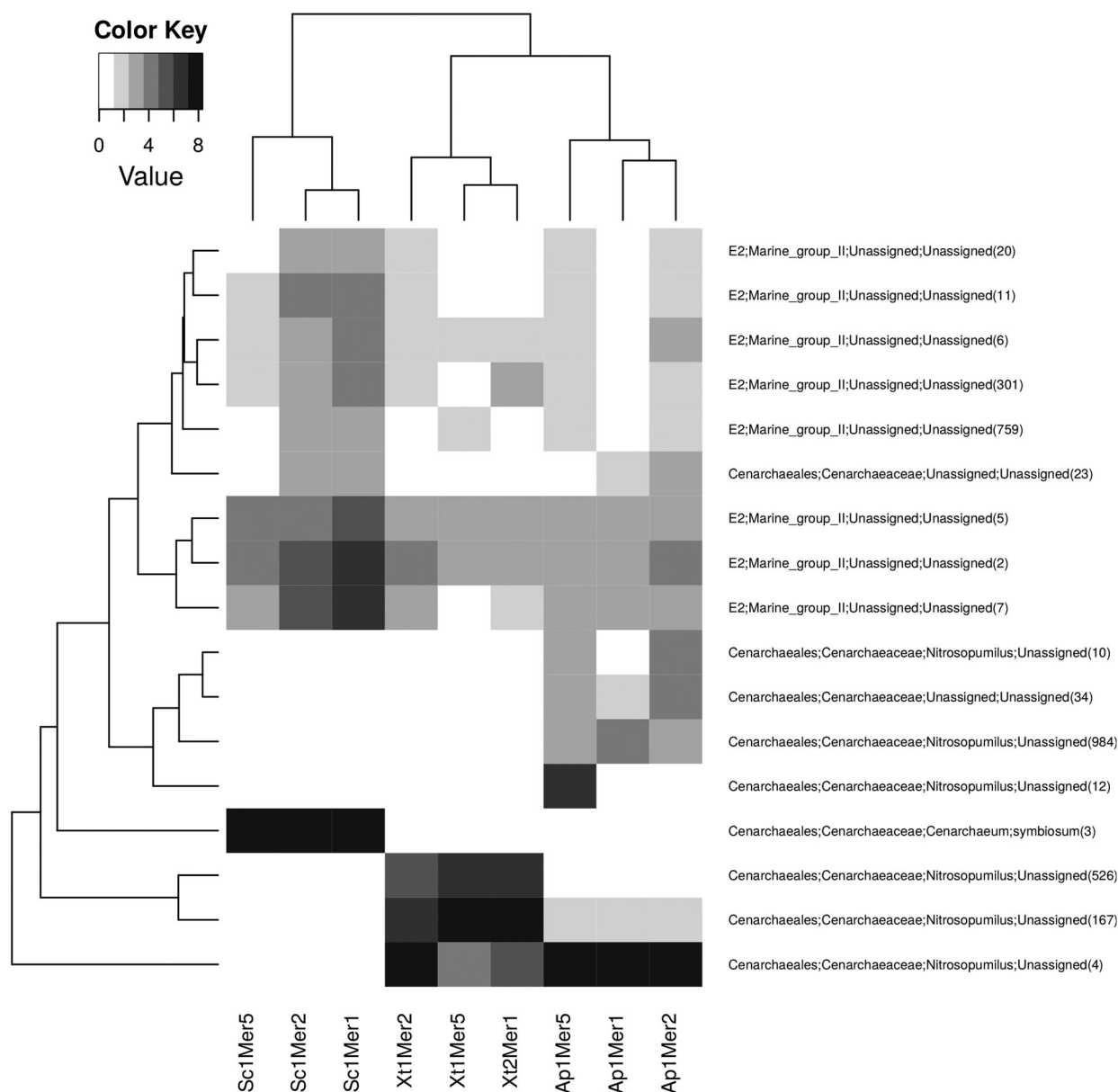


Figure 4. Heatmap showing the abundance of dominant OTUs (sequence reads ≥ 50 sequences). Xt: *X. testudinaria*; Ap: *A. lobata*; Sc: *S. carteri*.

thus explained >85% of the variation in archaeal composition. A PCO ordination (Figure 5) of the first two axes showed three distinct clusters representing samples from: (1) *S. carteri*; (2) *A. lobata* and (3) *X. testudinaria*. Axis 1 of the PCO ordination separated *S. carteri* samples from *A. lobata* and *X. testudinaria* samples. Axis 2 separated *X. testudinaria* samples from *A. lobata* samples with samples from *S. carteri* intermediate.

Predictive metagenome analysis

The PICRUSt and LEfSe analyses revealed significant differences in predicted enrichment among sponge

samples for a number of functional top level categories (Figure S4), subcategories and individual pathways (Figures 6 and 7). Note that, as referred to above, since PICRUSt results are predictive, care must be taken in the interpretation of these results.

In terms of KEGG categories (Figure 7), *S. carteri* samples were significantly enriched for the metabolism of cofactors and vitamins, especially the porphyrin and chlorophyll metabolism and pantothenate and CoA biosynthesis pathways, oxidative phosphorylation and sulphur metabolism (energy metabolism pathways), aminoacyl-tRNA biosynthesis and basal transcription factors (genetic information processing pathways) and bacterial chemotaxis (cell motility pathway).

Table I. Individual, mean and standard deviation values of the Shannon's diversity index (H), Species richness (S), Pielou's evenness (J), Chao1 index, Archaeal Reads and Nearest Sequenced Taxon Index (NSTI) for the studied samples.

Sample	Shannon's diversity index (H)			Species richness (S)			Pielou's evenness (J)			Chao1 index			Archaeal Reads			Weighted NSTI		
	H	Mean	Standard Deviation	S	Mean	Standard Deviation	J	Mean	Standard Deviation	S.chao1	Mean	Standard Deviation	Reads	Mean	Standard Deviation	NSTI	Mean	Standard Deviation
Sc1Mer1	1.357	0.777	0.440	26	21.0	4.08	0.417	0.2	0.13	33.500	24.4	6.97	3840	3645.0	418.16	0.08	0.068	0.01
Sc1Mer2	0.680			21			0.223			23.000			4031			0.065		
Sc1Mer5	0.293			16			0.106			16.600			3064			0.058		
Ap1Mer1	0.244	0.450	0.173	18	20.3	2.62	0.084	0.1	0.06	18.125	22.8	5.82	3657	4025.3	326.67	0.095	0.092	0.01
Ap1Mer2	0.440			24			0.138			31.000			4451			0.095		
Ap1Mer5	0.668			19			0.227			19.250			3968			0.085		
Xt1Mer2	1.007	0.892	0.088	15	16.3	1.25	0.372	0.3	0.04	18.000	20.3	2.18	3540	3957.0	297.73	0.096	0.096	0.00
Xt1Mer5	0.795			16			0.287			19.750			4115			0.095		
Xt2Mer1	0.874			18			0.302			23.250			4216			0.095		

Xestospongia testudinaria samples were significantly enriched for several carbohydrate metabolism pathways, namely glycolysis/gluconeogenesis, citrate cycle (TCA cycle), pentose and glucuronate interconversions and pyruvate, propanoate, pentose phosphate and starch and sucrose metabolisms; additionally *X. testudinaria* samples were also enriched for the methane metabolism (energy metabolism), tryptophan metabolism, lysine degradation (amino acid metabolism), limonene and pinene degradation (metabolism of terpenoids and polyketides), chlorocyclohexane and chlorobenzene degradation and caprolactam degradation (xenobiotics biodegradation and metabolism) and the porphyrin and chlorophyll metabolism pathways (metabolism of cofactors and vitamins). *Aaptos lobata* samples, in turn, were significantly enriched for the phenylalanine metabolism pathway.

There was a significant difference among sponge species concerning KEGG ortholog (KO) composition ($F_{2,6} = 30.08$, $P < 0.01$, $R^2 = 0.909$). Variation among sponge species thus explained almost 91% of the variation in KO composition. A PCO ordination (Figure 8) of the first two axes showed two distinct clusters representing samples from: (1) *S. carteri*; (2) *A. lobata* and *X. testudinaria*. Axis 1 of the PCO ordination separated *S. carteri* samples from *A. lobata* and *X. testudinaria* samples. Axis 2 separated the *S. carteri* sample Sc1Mer5 from all the other *S. carteri*, *A. lobata* and *X. testudinaria* samples.

Discussion

The development of next-generation sequencing (NGS) technologies has allowed the characterization of sponge microbial communities at unprecedented levels. The sponge microbial community is now known to be diverse, stable and distinct from the surrounding environment (Hentschel et al. 2002, 2012). Despite little evidence of a clear dichotomy between HMA and LMA sponges, the microbial community of the HMA sponge has been characterized as highly abundant and diverse in contrast to LMA sponges, which host communities that are generally considered to be similar to that of the surrounding environment. Little is, however, known about the existence of an archaeal dichotomy between HMA and LMA sponges.

In line with several studies, the archaeal community of sponges was dominated by Crenarchaeota (Holmes and Blanch 2007; Lee et al. 2011; Polónia et al. 2014, 2015). Nevertheless, our results showed distinct archaeal communities among the studied sponge species but higher similarity between *X. testudinaria* and *A. lobata*. While *X. testudinaria* and *A. lobata*

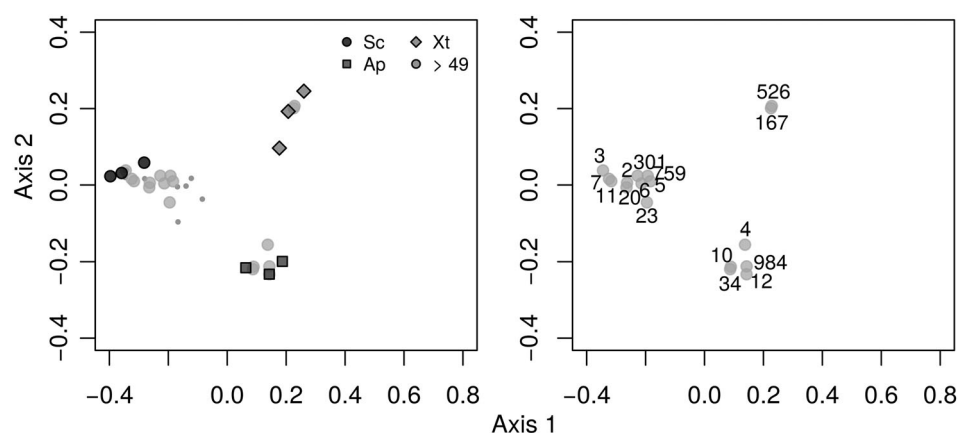


Figure 5. Ordination showing the first and second axes of the PCO analysis for archaeal OTU composition. Symbols represent samples from *S. carteri* (Sc), *A. lobata* (Ap) and *X. testudinaria* (Xt). Numbers refer to OTU numbers in Table S1. The small light grey circles represent OTUs with <49 sequences while the larger light grey circles represent OTUs ≥ 50 sequences.

shared the most abundant OTUs overall and coincidentally the most abundant OTU of each other, the most abundant OTU of *Stylissa carteri* (OTU-3) was not encountered in any other sponge species. In addition to this, OTU-3 represented almost 82% of the *S. carteri* archaeal community (with 8858 sequences) suggesting that *Cenarchaeum symbiosum* is likely to make a most pronounced contribution to *S. carteri* functional aspects. Moitinho-Silva et al. (2014) also linked the dominant members of the microbial community of *S. carteri* to the highly expressed functions attributed to its microbiome. *Cenarchaeum symbiosum* has been reported either as a strict autotroph, or as a mixotroph using both carbon dioxide and organic material as carbon sources (Hallam et al. 2006). It is likely that these are the main functions performed by this organism in *S. carteri*.

Xestospongia testudinaria and *A. lobata* had similar OTU composition. The most abundant OTU overall (OTU-4) was exclusively found in *X. testudinaria* (2484 sequences) and *A. lobata* (10,863). This OTU was assigned to the genus *Nitrosopumilus*. *Nitrosopumilus maritimus* has been reported as a chemolithoautotrophic archaeon, which aerobically oxidizes ammonia to nitrite (nitrification; Könneke et al. 2005). It is likely that these are the main functions performed by *Nitrosopumilus* members in both sponge species.

The most abundant shared OTUs were almost all (2, 5, 6, 7, 11, 20, 301, 759) assigned to the family Marine Group II (Euryarchaeota, Thermoplasmata, E2). The ecological role of the Marine Group II is still largely unknown due to the absence of cultured representatives of this group. It is believed that the organisms assigned to this group live heterotrophically (Zhang et al. 2015). Through the analysis of a nearly complete genome of a Marine Group II representative, Iverson

et al. (2012) suggested these organisms are photo-heterotrophic, motile and polymer (lipids and proteins) degraders.

NSTI scores were generally low but higher for *X. testudinaria* and *A. lobata*. According to Langille et al. (2013), PICRUSt still produces accurate results for samples with a mean NSTI score of 0.17. Here, all the mean NSTI values were lower than 0.17, thus providing relatively accurate and interesting insights into putative archaeal community functioning.

In general *X. testudinaria* and *A. lobata* had a similar KO composition that was, in turn, distinct from that of *S. carteri*. Archaeal communities of *X. testudinaria* and *A. lobata* were similarly enriched in most pathways (Figure S4 and Figure 7). Based on the assumption that the prediction of the HMA-LMA status can be based on the microbiome profiles of sponges (Moitinho-Silva et al. 2017), these results, together with the above referred compositional similarity, suggest that *A. lobata* is a HMA sponge, although this remains to be proven.

According to LEfSe analysis, *X. testudinaria* showed a significant enrichment in seven of the 15 carbohydrate metabolism pathways namely glycolysis/gluconeogenesis, citrate cycle (TCA cycle), pentose and glucuronate interconversions and pyruvate, propanoate, pentose phosphate and starch and sucrose metabolisms (Figure 6). These results are in accordance with Freeman and Thacker (2011) and support the generally accepted idea that HMA sponges rely more heavily on their symbionts to acquire energy, and especially carbon, than LMA sponges, which rely more heavily on their high pumping rates (Weisz et al. 2007). Freeman and Thacker (2011) reported that *Aplysina cauliformis* and *Neopetrosia subtriangularis* (two HMA sponges) obtained about 77% of their carbon needs

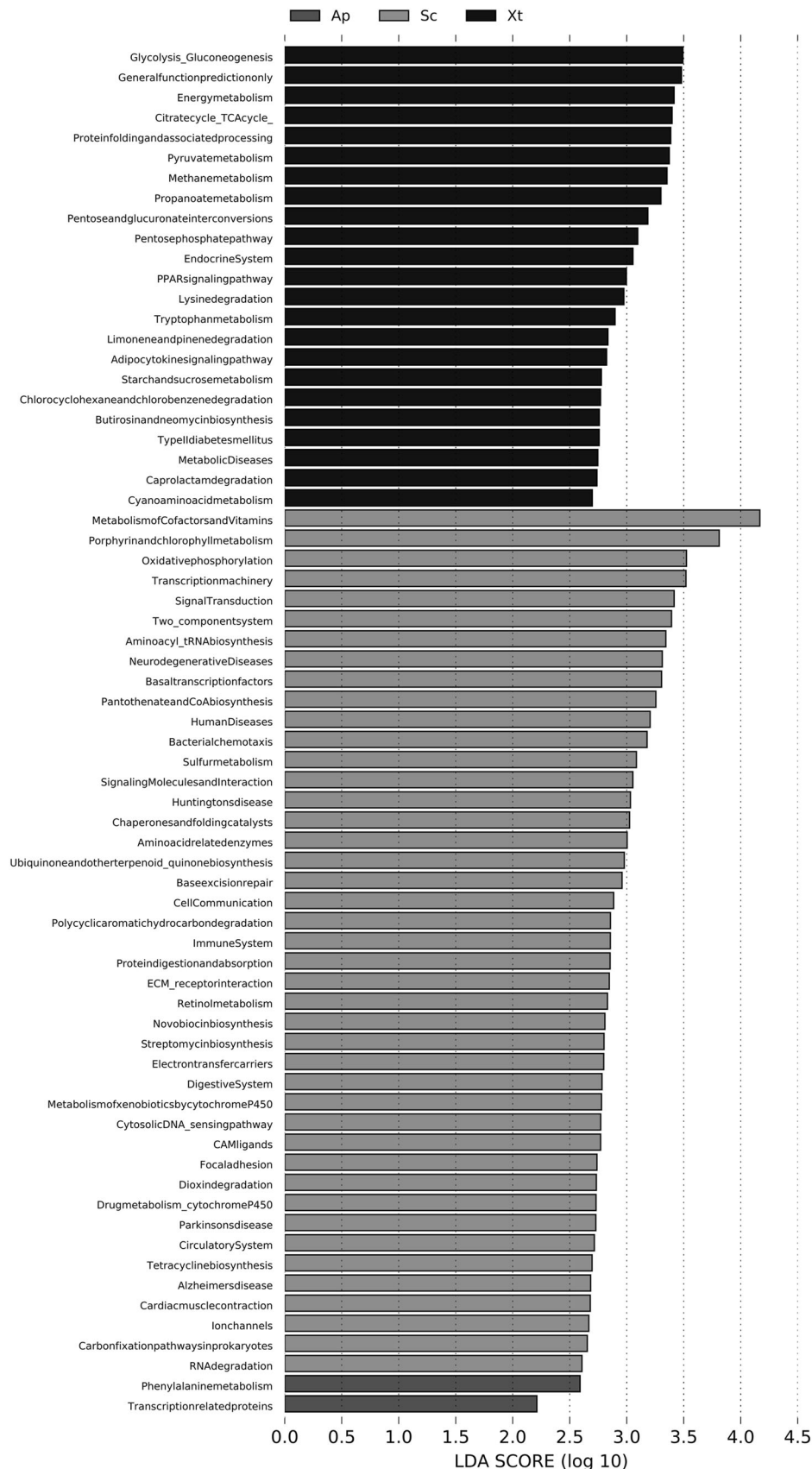


Figure 6. Histogram of the LDA scores ranking the statistically and biologically different archaeal categories, subcategories and pathways according to the effect size. KEGG categories, subcategories and individual pathways coloured dark grey indicate significant enrichment in *A. lobata* while those coloured light grey indicate significant enrichment in *S. carteri* and those coloured black indicate significant enrichment in *X. testudinaria*.

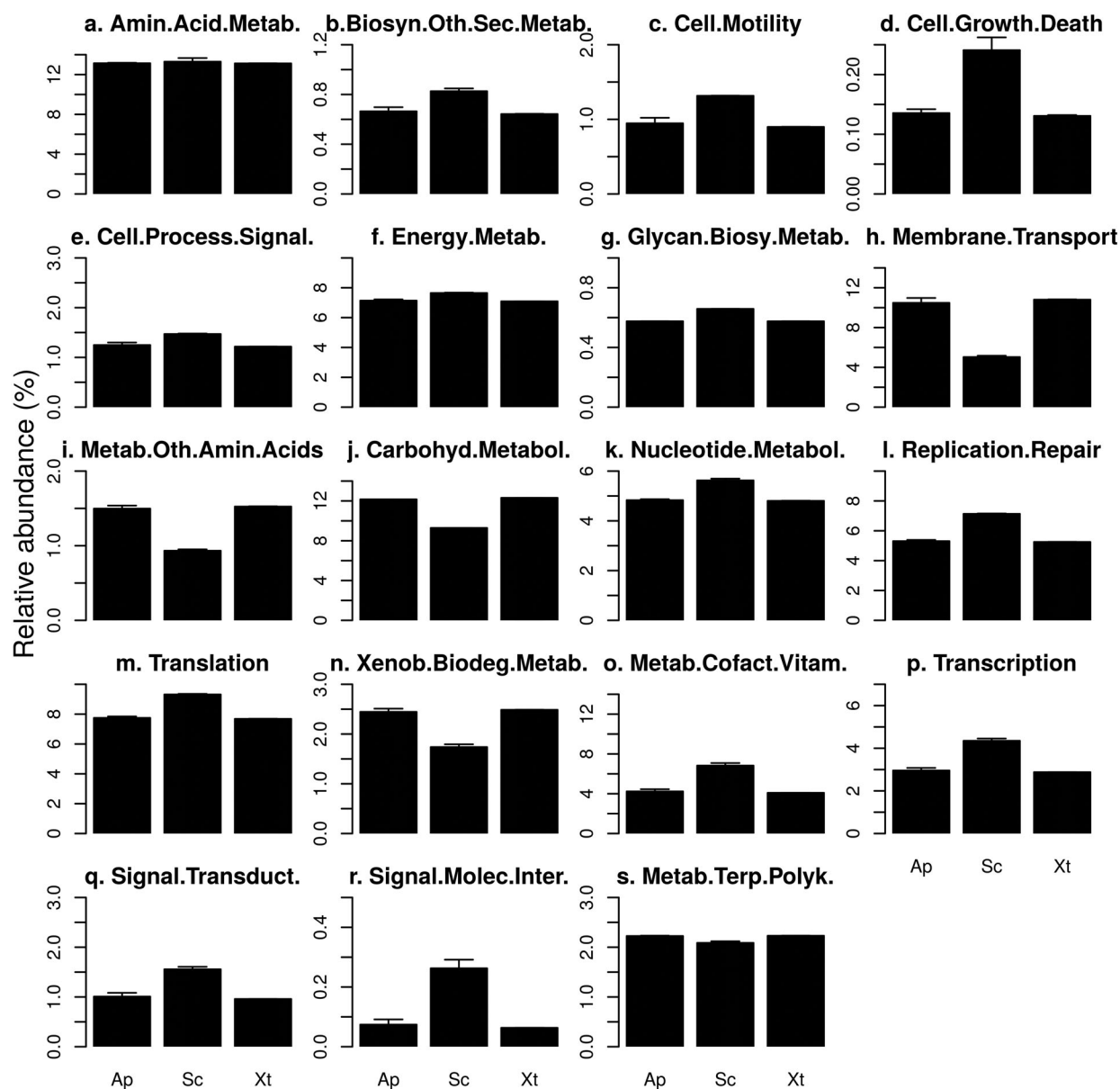


Figure 7. Mean relative abundance of predicted gene counts for selected functional individual pathways for samples from *S. carteri* (Sc), *A. lobata* (Ap) and *X. testudinaria* (Xt). Error bars represent a single standard deviation. The individual pathways shown include the following KEGG pathways: (a) Amino acid metabolism; (b) Biosynthesis of other Secondary Metabolites; (c) Cell Motility; (d) Cell Growth and Death; (e) Cellular Processes and Signalling; (f) Energy Metabolism; (g) Glycan Biosynthesis and Metabolism; (h) Membrane Transport; (i) Metabolism of other Amino Acids; (j) Carbohydrate Metabolism; (k) Nucleotide Metabolism; (l) Replication and Repair; (m) Translation; (n) Xenobiotics Biodegradation and Metabolism; (o) Metabolism of Cofactors and Vitamins; (p) Transcription; (q) Signal Transduction; (r) Signalling Molecules and Interaction; (s) Metabolism of Terpenoids and Polyketides.

from their microbial cells while only 27% is obtained by the LMA sponge *Niphates erecta*.

Additionally, *X. testudinaria* was also significantly enriched in pathways such as limonene and pinene degradation (metabolism of terpenoids and polyketides) and chlorocyclohexane, chlorobenzene and caprolactam degradation (xenobiotics biodegradation and metabolism). By degrading these compounds Archaea obtain energy and, at the same time, remove toxic compounds from the sponge host tissue. This may suggest

sponges, and in particular *X. testudinaria*, as xenobiotic degraders and thus as bioremediators. The degradation of toxic compounds was previously associated with other sponge species, namely, *Cymbastela coralliophila*, *Rhopaloeides odorabile* and *Cymbastela concentrica* (Fan et al. 2012).

S. carteri samples were significantly enriched in genetic information processing pathways namely: transcription (basal transcription factors) and translation (aminoacyl-tRNA biosynthesis) pathways; and in

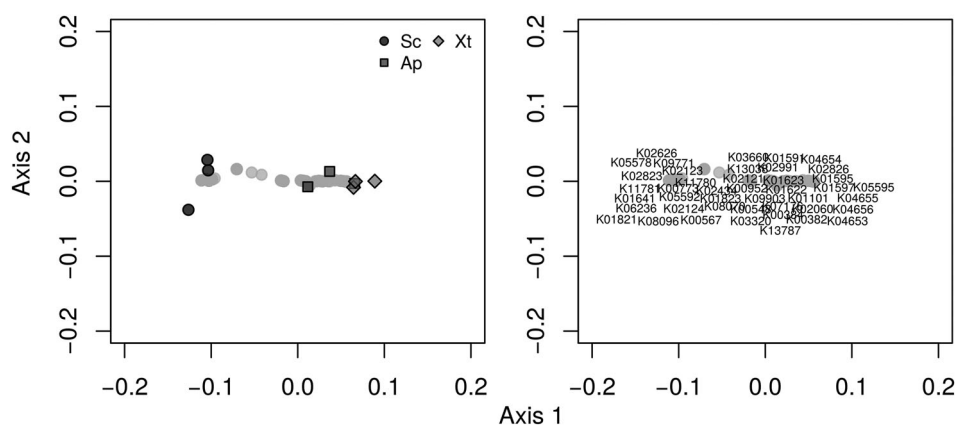


Figure 8. Ordination showing the first two axes of the PCO analysis for KO composition. Symbols represent samples from *S. carteri* (Sc), *A. lobata* (Ap), *X. testudinaria* (Xt). Numbers refer to KO numbers.

environmental information processing pathways (signalling molecules and interaction and signal transduction: two-component system).

Genetic information processing is particularly important when organisms are exposed to endogenous and/or exogenous factors that can damage DNA (Eggleston 2007). Previous studies have shown that sponge microbes were enriched in proteins related to stress responses (Thomas et al. 2010; Fan et al. 2012; Liu et al. 2012; Moitinho-Silva et al. 2014) and Fan et al. (2012) suggested that sponge symbionts acquired resistance mechanisms to deal with the stressful conditions found within the sponge environment. It is possible that due to their morphology and to the high volumes of water filtered by LMA sponges, these sponges represent a less protected and stable environment for Archaea than HMA sponges. Supporting this idea, *S. carteri* samples were enriched in signal transduction systems, which play important roles in the adaptation of organisms to new environmental conditions (sensing the environment and responding by altering gene expression) and thus predicted to be enriched in organisms living in unstable environments. The two-component system (part of the signal transduction system and also significantly enriched in *S. carteri*) has been reported as a system in which organisms respond, through gene regulation, to changes in nutrient (carbon, phosphorus, nitrogen, etc.) and oxygen availability in the environment (Chang and Stewart 1998; Oyserman et al. 2016a, 2016b).

The present study suggested the existence of a HMA/LMA dichotomy in archaeal compositional and functional levels. The archaeal community of *A. lobata* was very similar to that of *X. testudinaria* both in terms of OTU and KO composition which suggests that *A. lobata* is a HMA sponge. This, however, needs to be

confirmed in further studies. This study also suggests that some of the most enriched functions identified in the sponge archaeal communities may be related to the different morphologies and life strategies associated with LMA and HMA sponge species.

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