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RESEARCH ARTICLE

A comparison of prokaryote communities inhabiting sponges, bacterial mats, sediment and seawater in Southeast Asian coral reefs

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One sentence summary: Prokaryote communities were sampled from 17 sponge species, bacterial mats, sediment and seawater in Southeast Asian coral reef habitats in order to assign HMA or LMA status and compare compositional variation among species.

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ABSTRACT

In the present study, we used Illumina sequencing to explore the prokaryote communities of 17 demosponge species and how they compare with bacterial mat, sediment and seawater samples (all sampled from coral reef habitat in Taiwan and Thailand). The studied sponge species formed three clusters. Operational taxonomic unit (OTU) richness and evenness were by far highest in the sediment and bacterial mat biotopes. There were pronounced differences in OTU richness and evenness among clusters and also considerable variation among certain host species within clusters. Additionally, the relative abundance of some prokaryotic taxa also differed among clusters with Poribacteria, e.g., being recorded in all sponge species, but with very low relative abundances in species of two of the three clusters. This sponge-associated phylum was, however, recorded at relatively high mean abundance in bacterial mat samples, which also housed relatively high abundances of actinobacterial and Chloroflexi members. Our results support high microbial abundance (HMA) status of the species *Aaptos lobata*, *Hyrtios erectus*, *Pseudoceratina purpurea* and *Xestospongia testudinaria* and low microbial abundance (LMA) status of the species *Acanthella cavernosa*, *Echinodictyum asperum*, *Jaspis splendens*, *Ptilocaulis spiculifer*, *Stylissa carteri* and *Suberites diversicolor*. Other species (*Agelas cavernosa*, *Agelas nemoechinata*, *Acanthostylotella cornuta*, *Paratetilla* sp., *Hymeniacidon* sp. and *Haliclona cymaeformis*) deviated somewhat from the typical HMA/LMA dichotomy and formed a strongly supported cluster.

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INTRODUCTION

Benthic sessile communities have undergone massive compositional shifts in recent decades under the influence of a range of perturbations including climate change, pollution, overfishing, coral mining and coastal development (Gardner *et al.* 2003; Hughes *et al.* 2003; Cleary *et al.* 2014, 2016; Polónia *et al.* 2015a; de Bakker *et al.* 2017; Cleary 2017). Various studies have reported an increase in sponge density (Bell *et al.* 2013; Loh *et al.* 2015; McMurray, Finelli and Pawlik 2015). Importantly, sponge microbial communities play critical roles in sponge growth and metabolism, production of secondary metabolites, modification of water column chemistry and adaptation to changing environmental conditions (Wulff 2001; Hentschel, Usher and Taylor 2006; Taylor *et al.* 2007; Bell 2008; Fan *et al.* 2012; Hentschel *et al.* 2012; Maldonado *et al.* 2016).

Sponges have long been classified according to their symbiont abundance and diversity as bacterial sponges versus non-symbiont sponges or high microbial abundance (HMA) versus low microbial abundance (LMA) sponges (Vacelet and Donadey 1977; Reiswig 1981; Hentschel *et al.* 2002, 2003). HMA sponges generally contain abundant and diverse microbial communities, while LMA sponges generally contain low abundance and low diversity microbial communities. In addition to microbial abundance and diversity, these groups also differ in terms of mesohyl density (denser in HMA), aquiferous canals (wider in LMA), choanocyte chambers (larger in LMA), pumping rates (higher in LMA) and the presence of some polyketide synthase genes (only found in HMA) (Vacelet and Donadey 1977; Hochmuth *et al.* 2010). LMA and HMA sponges also house compositionally distinct microbial communities (Bayer, Kamke and Hentschel 2014; Cleary *et al.* 2015, 2018; de Voogd *et al.* 2015, 2018; Polónia *et al.* 2015b; Moitinho-Silva *et al.* 2017). Bayer, Kamke and Hentschel (2014) and Moitinho-Silva *et al.* (2017) noted that certain phyla were much more prevalent in HMA as opposed to LMA sponges (e.g. Chloroflexi, Poribacteria and Actinobacteria) and suggested that they were 'HMA indicators'. In addition to previously reported HMA (Chloroflexi, Poribacteria and Actinobacteria) indicators, they also identified additional indicators including Acidobacteria, PAUC34f, Gemmatimonadetes, SAR202, Anaerolineae and Acidimicrobiia. LMA sponges, in turn, were characterised by greater abundances of Bacteroidetes, Planctomycetes, Firmicutes, Alphaproteobacteria, Betaproteobacteria and Flavobacteriia. Although HMA or LMA status was initially based on transmission electron microscopy, this has not always proved effective at classifying sponge species to LMA or HMA status. For example, Gloeckner *et al.* (2014) remarked on the variable density of bacteria in HMA sponges with species belonging to the order Verongida housing densely packed bacterial communities in their mesohyl tissue, while species of the presumed HMA genera *Ircinia* and *Agelas* only housed moderately dense microbial consortia. In addition to electron microscopy, 16S rRNA gene sequencing has been shown to be a very useful technique in determining HMA or LMA status of the host sponge (Gloeckner *et al.* 2014).

In the present study, we used 16S rRNA gene sequencing to explore and compare the prokaryote communities of 17 sponge species, sediment, seawater and bacterial mat samples collected from sites in Taiwan and Thailand and to make preliminary assignments of HMA or LMA status for sponge species based on compositional data. The 17 sponge species were: *Aaptos lobata*, *Acanthella cavernosa*, *Acanthostylotella cornuta*, *Agelas cavernosa*, *Agelas nemoechinata*, *Echinodictyum asperum*, *Haliclona cymaeformis*, *Hymeniacidon sp.*, *Hyrtios erectus*, *Jaspis splendens*, *Neopetrosia sp.*, *Paratetilla sp.*, *Pseudoceratina purpurea*, *Ptilocaulis spiculifer*, *Stylissa carteri*, *Suberites diversicolor* and *Xestospongia testudinaria*. Our main objectives were to (1) compare prokaryote communities of sponges with communities found in bacterial mats, seawater and sediment based on 16s rRNA gene sequencing data, (2) identify the most abundant operational taxonomic unit (OTU)s and major higher prokaryote taxa found in sponge species, (3) determine the overlap of abundant OTUs among sponge species and between sponge species and environmental samples, (4) assess to what extent sponge species form strongly supported clusters based on compositional data and (5) extend the current knowledge of the HMA-LMA dichotomy among phylogenetically distant and closely related sponge species.

MATERIALS AND METHODS

Location

All sponge, bacterial mat, sediment and seawater samples were collected from various locations in Taiwan and Thailand. Supplementary Table 1 (Supporting Information) provides details on the sampling locations and dates, gps coordinates, diversity indices, and relative abundances of selected prokaryote taxa in each sample. All locations were coral reef habitat. A detailed description of the Taiwanese sampling sites can be found in Coelho *et al.* (2018) and Huang *et al.* (2016b).

Sampling

In the present study, we sampled 17 shallow water sponge species, bacterial mats, sediment and seawater (Table 1) in coral reef habitat in the Penghu Islands, Taiwan, from 25th to 29th of July, 2014; and in sites close to Phuket, Pattaya and Koh Tao, Thailand from 8th to 21st of August 2014 (Fig. 1 and Supplementary Table 1, Supporting Information). Additional samples were collected from Penghu from 24th of July to the 6th of August, 2016. For the environmental samples, sediment and seawater were collected at all sites, while bacterial mats were only collected from a single site in Thailand (Table 1). The sponge species were identified by NJ de Voogd, using classical morphological techniques, as *Acanthella cavernosa* (Dendy, 1922) (order: Bubarida); *Echinodictyum asperum* (Ridley and Dendy, 1886), and *Ptilocaulis spiculifer* (Lamarck, 1814) (order: Axinellida); *Jaspis splendens* (de Laubenfels, 1954), and *Paratetilla* sp. (order: Tetractinellida); *Stylissa carteri* (Dendy, 1889) (order: Scopalinida); *Agelas cavernosa* (Thiele, 1903); *Agelas nemoechinata* (Hoshino, 1985), and *Acanthostylotella cornuta* (Topsent, 1897) (order: Agelasida), *Aaptos lobata* (Calcinai, Bastari, Bertolino and Pansini, 2017); *Hymeniacidon* sp. and *Suberites diversicolor* (Becking and Lim, 2009) (order: Suberitida); *Haliclona cymaeformis* (Esper, 1806); *Xestospongia testudinaria* (Lamarck, 1815), and *Neopetrosia* sp. (order: Haplosclerida); *Hyrtios erectus* (Keller, 1889) (order: Dictyoceratida); and *Pseudoceratina purpurea* (Carter, 1880) (order: Verongida). Three species, namely, *S. carteri*, *H. erectus* and *X. testudinaria* were collected in multiple locations. A total of 2–10 replicates were sampled per biotope (Table 1). Subsequent statistical analyses were only performed using biotopes with at least three replicates. Sponges were photographed *in situ*, collected

Table 1. List of biotopes (bacterial mats, sediment, seawater and sponge species) sampled and number of samples collected from the main sampling locations in Koh Tao, Thailand, Pattaya, Thailand, Phuket, Thailand and the Penghu archipelago, Taiwan.

Type	Biotope	Koh Tao, Thailand	Pattaya, Thailand	Phuket, Thailand	Taiwan
Sponge	*Aaptos lobata*				2
Sponge	*Agelas cavernosa*				3
Sponge	*Acanthella cavernosa*			3	
Sponge	*Acanthostylotella cornuta*				2
Sponge	*Agelas nemoechinata*				2
Sponge	*Echinodictyum asperum*				3
Sponge	*Haliclona cymaeformis*				4
Sponge	*Hymeniacidon sp.*				2
Sponge	*Hyrtios erectus*	3	2	3	
Sponge	*Jaspis splendens*				3
Sponge	*Neopetrosia sp.*	1	3		
Sponge	*Paratetilla sp.*				2
Sponge	*Pseudoceratina purpurea*	2			
Sponge	*Ptilocaulis spiculifer*				4
Sponge	*Stylissa carteri*			3	4
Sponge	*Suberites diversicolor*				3
Sponge	*Xestospongia testudinaria*	3	1	2	3
Environmental	Bacterial mat	3			
Environmental	Sediment	1	3	1	4
Environmental	Seawater	2	2	3	3

using scuba diving, brought back to the laboratory and preserved in 95% ethanol for further identification and molecular work. All specimens have been deposited at Naturalis Biodiversity Center, Leiden, the Netherlands. Sponges were sampled with an apple corer or cut with a dive knife in order to sample the surface and interior. In addition to sponges, we sampled sediment, seawater and bacterial mats. Sediment was sampled with a syringe, extracting the first 5 cm. Bacterial mats were scraped off the surface sediment, partially or in their entirety depending on the size. The seawater prokaryote community was sampled by first collecting 1 l of seawater at ~ 1 m depth and subsequently filtering this through a Millipore® White Isopore Membrane Filter (0.22 μm pore size).

DNA extraction and sequencing

For prokaryotes, the 16S rRNA gene V3V4 variable region PCR primers 341F 5′-CCTACGGGNGGCWGCAG-3′ and 785R 5′-GACTACHVGGGTATCTAATCC-3′ (Klindworth *et al.* 2013) with expected amplicon size of 444 bp and barcode on the forward primer were used in a 28 cycle PCR assay (5 cycle used on PCR products) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. A blank control, in which no tissue was added to the Lysing Matrix E tubes, was also included in the samples. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads. Pooled and purified PCR product was used to prepare the DNA library following the Illumina TruSeq DNA library preparation protocol. Next-generation, paired-end sequencing was performed at mrDNA Molecular Research LP (http://www.mrdnalab.com/; last checked 18 November 2016) on an Illumina MiSeq device (Illumina Inc, San Diego, CA, USA) following the manufacturer's guidelines. Sequences from each end were joined following Q25 quality trimming of the ends followed by reorienting any 3'-5' reads back into 5'-3', and removal of short reads (<150 bp). The resultant files were analysed using the QIIME (Quantitative Insights Into Microbial Ecology; Caporaso *et al.* 2010) software package (http://www.qiime.org/; last checked 20 January 2017).

16S sequencing analysis

In QIIME, fasta and qual files were used as input for the split_libraries.py script. Default arguments were used except for the minimum sequence length, which was set at 250 bps after removal of forward primers and barcodes. In addition to user-defined cut-offs, the split_libraries.py script performs several quality filtering steps (http://qiime.org/scripts/split_libraries.html). OTUs (97% similarity cut-off) were selected using UPARSE with usearch10 (Edgar 2013). The UPARSE sequence analysis tool (Edgar 2013) provides clustering, chimera checking and quality filtering on de-multiplexed sequences. Chimera checking was performed using the UCHIME algorithm (Edgar *et al.* 2011). Reads were filtered with the -fastq_filter command and the following arguments -fastq_trunclen 250 -fastq_maxee 0.5 -fastq_truncqual 15. Sequences were then dereplicated and sorted using the -derep_fulllength and -sortbysize commands. OTU clustering was performed using the -cluster_otus command. In QIIME, representative sequences were selected using the pick_rep_set.py script using the 'most_abundant' method. Potential contaminants were removed from the OTU table if they occurred at least two times in the blank control. This conservative measure was chosen because of observations of bleeding between samples from Illumina sequencing and the appearance of abundant reads in blank controls with very low counts (Mitra *et al.* 2015; Sinha 2017). OTUs not classified as Bacteria or Archaea or classified as chloroplasts or mitochondria were also removed. Taxonomy was assigned to reference sequences of OTUs using default arguments in the assign_taxonomy.py script in QIIME using the SILVA_128_QIIME_release database and the uclust classifier method (Quast *et al.* 2013).

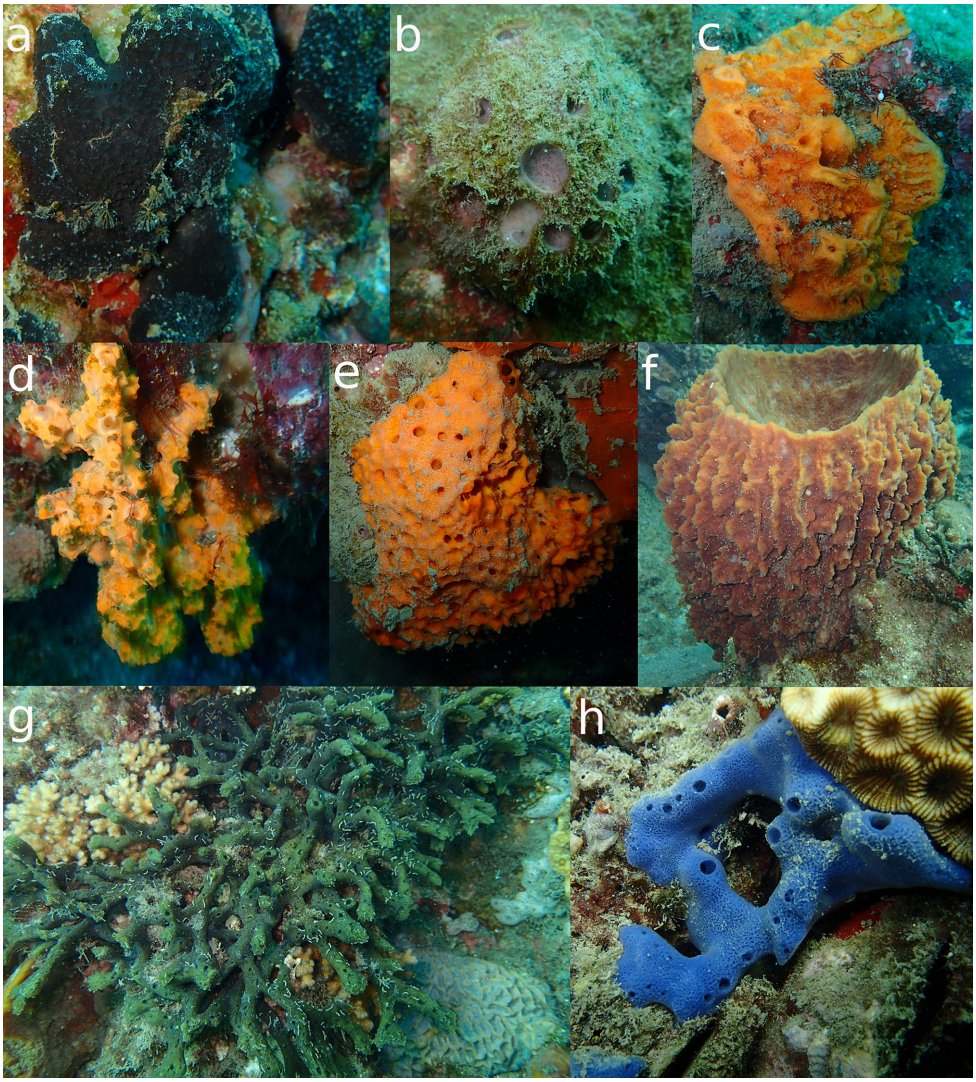

Figure 1. Underwater photographs of selected species sampled in the present study: **(A)** *H. erectus*, **(B)** *Paratetilla* sp., **(C)** *J. splendens*, **(D)** *S. carteri*, **(E)** *A. cavernosa*, **(F)** *X. testudinaria*, **(G)** *H. cymaeformis* and **(H)** *Neopetrosia* sp.c

We used the make_otu_table.py script in QIIME to generate a square matrix of OTUs x SAMPLES and rarefied this to 10 000 sequences per sample with the single_rarefaction.py script in QIIME. This rarefied table was subsequently used as input for further analyses using the R package (R Core Team 2013). Sequence Identifiers of closely related taxa of the most abundant OTUs were downloaded using the NCBI Basic Local Alignment Search Tool (BLAST) command line 'blastn' tool with the -db argument set to nt (Zhang *et al.* 2000). BLAST identifies locally similar regions between sequences, compares sequences to extant databases and assesses the significance of matches; evolutionary relationships can subsequently be inferred. Each run produces a list of hits based on significant similarity between pairs of sequences, i.e., the target sequence and taxa present in the database (or no hits if no significantly similar sequences are found). A discussion of how significance is determined can be found at http://www.ncbi.nlm.nih.gov/BLAST/tutorial/Altschul-1.html. The sequences generated in this study can be downloaded from the NCBI SRA: SRP109605, SRP133416, SRP133417 and SRP133418.

Statistical analysis

A table containing the OTU counts per sample was imported into R using the read.csv() function. This table was used to compare community composition, estimate richness and assess the relative abundance of the most abundant higher taxa (based on total number of sequences per taxon).

For compositional analyses, the OTU table was transformed using the decostand() function in vegan (Oksanen et al. 2019) with the method argument set to 'Hellinger'. With this transformation, the OTU table is adjusted such that subsequent analyses preserve the chosen distance among objects (samples in this case). The OTU table was transformed because of the inherent problems with the Euclidean-based distance metric, which is frequently used in cluster analyses (Legendre and Gallagher 2001). The Hellinger (Rao 1995) distance was chosen because it gave very good results in comparison to various distance metrics. In particular, it gave low weights to rare species, was monotonically related to the geographic distance along a model gradient, and reached an asymptote for sites with no species in common. It also produced little 'horseshoe effect' or inward

folding of sites at opposite ends of the gradient, in ordinations (Legendre and Gallagher 2001). A distance matrix was subsequently created with the vegdist() function in vegan using the Hellinger-transformed OTU table as input and the method argument set to 'euclidean'.

Variation in OTU composition was assessed with Principal Coordinates Analysis (PCO) using the cmdscale() function in R with the Hellinger-transformed distance matrix as input. Ordinations were produced for all samples (including sponge species, sediment, seawater and bacterial mats) and only including sponge species. Variation in OTU composition among sponge species was tested for significance using the adonis() function in vegan. In the adonis analysis, the Hellinger-transformed OTU table was the response variable with 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. We used the simper() function in vegan to identify significantly discriminating OTUs between pairs of biotopes based on the $\log_e (x + 1)$ transformed OTU table and 999 permutations. Discriminating OTUs contribute most to differences in composition between biotopes.

In addition to PCO, we used unconstrained, hierarchical clustering to identify strongly supported clusters with the pvclust package (Suzuki and Shimodaira 2015). The pvclust package provides validation procedures to test the uncertainty of a classification (Borcard, Gillet and Legendre 2018), namely, calculating the bootstrap probability (BP) and the approximately unbiased P values (AU) based on multiscale bootstrap resampling. High AU values indicate that a given cluster is strongly (e.g. AU $\geq$ 90) or significantly (AU $\geq$ 95) supported by the data. In pvclust, a hierarchical clustering dendrogram was produced using the pvclust() function with the method.dist argument set to 'euclidean' and the method.hclust set to 'ward.D2' (Ward 1963). Input for the function consisted of the Hellinger-transformed OTU table. Ward's method (Ward 1963) minimises within-group sum of squares. With the above procedure, we were interested in assessing to what extent sponge species formed strongly supported clusters and if there was evidence for clustering at a higher level, e.g., HMA versus LMA species.

We tested for significant differences in the relative abundance of selected prokaryote higher taxa, OTU richness, evenness and the relative abundance of seawater (OTUs found in seawater, but not sediment) and environmental (OTUs found in seawater and/or sediment) OTUs among sponge species with an analysis of deviance using the glm() function in R. In order to study the distribution of seawater and environmental OTUs among sponge species, we created a subset of the total dataset only including OTUs with >100 sequences due to the high abundance of rare OTUs, which were only found in a single sample. A number of these variables included an excess of zero counts in the samples, therefore, we set the family argument to 'tweedie' (Tweedie 1984) with var.power = 1.5 and link.power = 0 (a compound Poisson–Gamma distribution). Using the glm model, we tested for significant variation among biotopes using the anova() function in R with the F test. We used the emmeans() function in the emmeans library (Lenth 2017) to perform multiple comparisons of mean abundance among biotopes using the false discovery rate (fdr) method in the adjust argument. A heatmap was constructed to visualise the distribution of the most abundant OTUs (≥3000 sequences) using the heatmap2() function in the R package gplots (http://www.cran.r-project.org/). 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; Accessed 26 September 2011).

RESULTS

Sequencing yielded 850 000 (after rarefaction to 10 000 sequences per sample) sequences binned into 19 329 OTUs after quality control and excluding OTUs assigned to chloroplasts and mitochondria. The number of phyla varied from 26 in *A. nemoechinata* to 66 in sediment. Among sponges, most phyla (42) were recorded in *Neopetrosia* sp. The number of classes varied from 49 in *A. lobata* to 154 in sediment. Among sponges, most classes were found in *S. carteri* at 89.

Proteobacteria were the most abundant phylum overall with 435 231 sequences and 8726 OTUs followed by Chloroflexi (84 247 sequences; 670 OTUs), Cyanobacteria (6 183 761 418 sequences; 272 OTUs), Actinobacteria (61 087 sequences; 401 OTUs) and Bacteroidetes (39 993 sequences; 2174 OTUs). Planctomycetes and Acidobacteria, with 2164 and 894 OTUs respectively, were among the most diverse phyla, but were less abundant in terms of sequences at 16 509 and 34 919 respectively. Supplementary Table 2 (Supporting Information) provides information on the number of sequences and OTUs recorded for all phyla and the major proteobacterial classes.

There was a highly significant difference in composition among sponge species (adonis: $F_{16,46} = 12.72$; $P < 0.001$; $R^2 = 0.816$; Fig. 2a and b). Host species identity thus explained more than 80% of the variation in composition. The first axis separated samples of the sponge species *A. lobata*, *H. erectus*, *P. purpurea* and *X. testudinaria* (cluster 1) from samples of the sponge species *Acanthella cavernosa*, *E. asperum*, *J. splendens*, *Neopetrosia* sp., *P. spiculifer*, *S. carteri* and *S. diversicolor* (cluster 2) with samples of *Agelas cavernosa*, *A. nemoechinata*, *A. cornuta*, *H. cymaeformis*, *Hymeniacidon* sp. and *Paratetilla* sp. (cluster 3) intermediate. The second axis separated samples of *H. cymaeformis*, *Hymeniacidon* sp. and *Paratetilla* sp. from remaining sponge species. In Fig. 2c and d, which included bacterial mat, sediment and seawater samples, it can be seen that samples from the cluster 3 species *H. cymaeformis* and *Hymeniacidon* sp. clustered closer to sediment samples while samples from cluster 2 species clustered closer to seawater samples. The three bacterial mat samples varied widely along the first axis of variation and were intermediate between sediment and sponge samples along axis-2.

The clusters mentioned above are also evident in a hierarchical clustering analysis. The major division in the analysis separated cluster 1 samples from cluster 2 and 3 samples. There was also strong support for cluster 2 samples (AU = 90) and cluster 3 samples (AU = 94). Within cluster 3, there was also strong support for a *H. cymaeformis*, *Hymeniacidon* sp. and *Paratetilla* sp. cluster (AU = 91) and a separate *Agelas cavernosa*, *A. nemoechinata*, *A. cornuta* cluster (AU = 93). The strongest support, however, was at the host species level. In cluster 1, AU values were 97 for *X. testudinaria* and 100 for *A. lobata* and *P. purpurea* samples. *Hyrtios erectus* formed two strongly supported clusters (AU ≥ 97). There was also significant structuring among geographical populations of *X. testudinaria* and *H. erectus* (Fig 3). For example, for *H. erectus*, samples from the geographically proximate (in terms of sea distance) locations of Pattaya and Koh Tao formed a strongly supported cluster (AU = 99), which was distinct from samples from the more distant Phuket (AU = 97). In cluster 2 and 3, the species *S. diversicolor*, *J. splendens*, *P. spiculifer*, *E. asperum*, *Agelas cavernosa*, *A. nemoechinata*, *A. cornuta*, *H. cymaeformis*, *Hymeniacidon* sp. and *Paratetilla* sp. all formed strongly supported (AU >

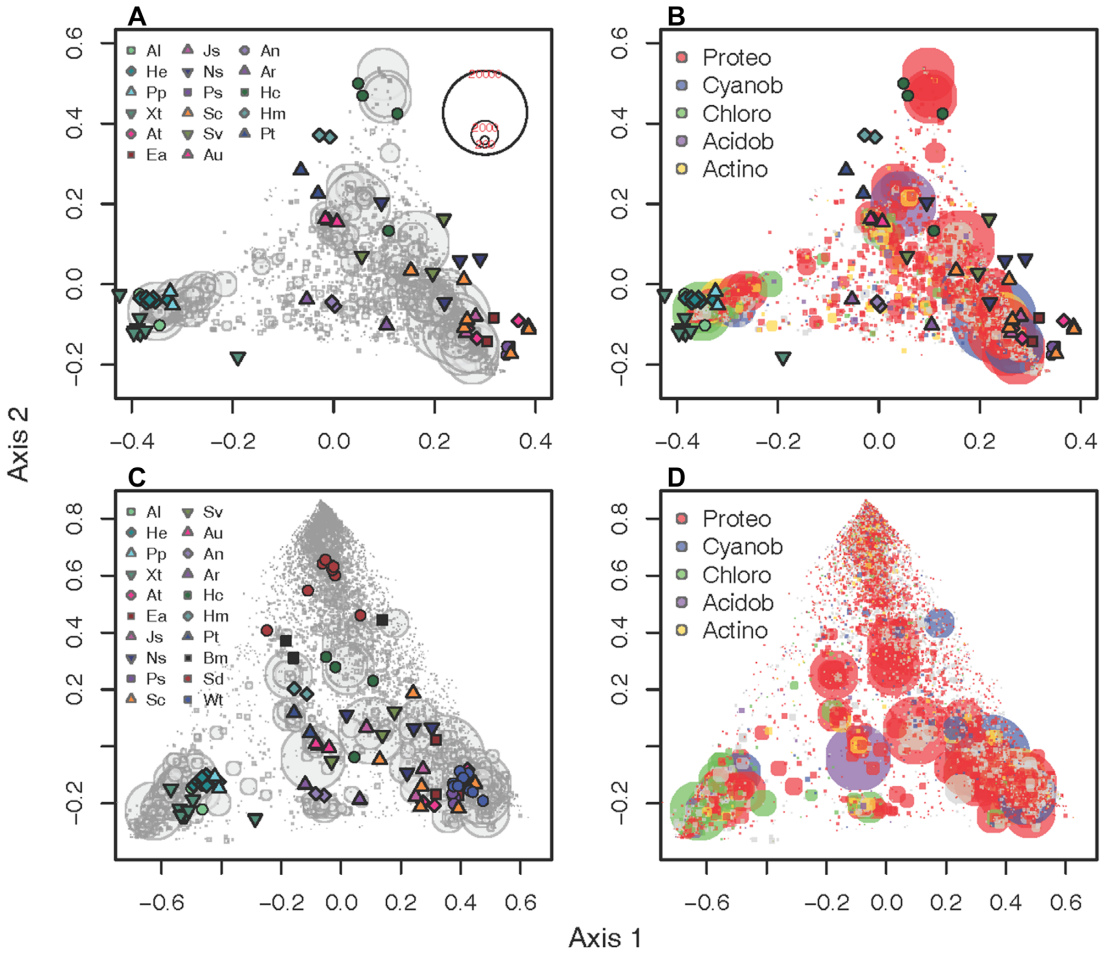

Figure 2. Ordination showing the first two axes of the principal coordinates analysis (PCO) of prokaryote OTU composition for sponge species. The eigenvalues for the axes are 7.96 (variance explained = 23.3%) and 3.34 (variance explained = 9.8%), respectively. In figure 2a, light grey symbols represent operational taxonomic unit (OTU) scores with the symbol size representing their abundance (number of sequence reads). In figure 2b the color of the symbol indicates their taxonomic affiliation: Proteo: Proteobacteria, Cyanob: Cyanobacteria, Chloro: Chloroflexi, Acido: Acidobacteria and Actino: Actinobacteria. Figures 2c and 2d show the ordination for the first two axes of the PCO for sponge species, bacterial mats, sediment and seawater. Codes refer to *A. lobata* (Al), *H. erectus* (He), *P. purpurea* (Pp), *X. testudinaria* (Xt), *A. cavernosa* (Au), *A. nemoechinata* (An), *A. cornuta* (Ar), *A. cavernosa* (At), *E. asperum* (Ea), *J. splendens* (Js), *Neopetrosia* sp. (Ns), *P. spiculifer* (Ps), *S. carteri* (Sc), *H. cymaeformis* (Hc), *Hymeniacidon* sp. (Hm), *Paratetilla* sp. (Pt), *S. diversicolor* (Sv), bacterial mat (Bm), sediment (Sd) and seawater (Wt).

99) sub-clusters. This was, however, not the case for the cluster 2 species *Acanthella cavernosa*, *S. carteri* or *Neopetrosia* sp. (Fig 3) suggesting pronounced variation in the prokaryote composition of these species.

The clusters seen in Fig. 3 are also evident in a heatmap of the most abundant OTUs (≥3000 sequences; Fig. 4). Note that this heatmap is based on untransformed OTU counts and not Hellinger-transformed data. The two main clusters in Fig. 4 include the same samples as the main clusters in Fig. 3. The main difference is between clusters 2 and 3. In Fig. 4, *H. cymaeformis*, *Hymeniacidon* sp. and *Paratetilla* sp. form a distinct cluster whereas the agelasids *Agelas cavernosa*, *A. nemoechinata* and *A. cornuta*, cluster together with cluster 2 species. Figs 4 and 5 highlight OTUs that are particularly abundant in a given sponge species or sets of species. These include sets of significantly ($P < 0.001$) discriminating OTUs identified using SIMPER analysis (Fig 5). Supplementary Table 3 (Supporting Information) provides the results of the SIMPER analysis including pairwise comparisons between pairs of biotopes.

In the SIMPER analysis, we only included biotopes with at least three samples. OTUs 44, 116, 122, 123, 132, 158, 168, 171, 187, 262 significantly discriminated *H. erectus* and *X. testudinaria* from *Acanthella cavernosa*, *E. asperum*, *J. splendens*, *P. spiculifer*, *S. carteri*, *Neopetrosia* sp., *S. diversicolor and H. cymaeformis* (Fig. 5 and Supplementary Table 3, Supporting Information). None of these, however, discriminated *H. erectus* and *X. testudinaria* from bacterial mat samples. These OTUs were assigned to the Actinobacteria, SBR1093, Proteobacteria (Alpha-, Gamma- and Deltaproteobacteria) and Chloroflexi (TK10 and SAR202) and had high sequence similarities (>99%) to organisms obtained from the sponge species *Plakortis halichondrioides*, *Aplysina cauliformis*, *Rhopaloeides odorabile*, the coral *Porites lutea* and an endolithic community. Supplementary Table 4 (Supporting Information) provides a list of the most abundant OTUs and results from BLAST searches using representative sequences from these OTUs. Certain, but not all, of these OTUs were also relatively abundant in *A. nemoechinata* and *A. cornuta*, which were not tested due to the low number of replicates. A number of OTUs (25, 113, 138, 173, 185 and 1001) were relatively abundant in the species *Acanthella cavernosa*, *E. asperum*, *J. splendens*, *P. spiculifer*, *S. carteri*, *Neopetrosia* sp., but these were all shared with seawater and also present, albeit less abundant in other sponge species.

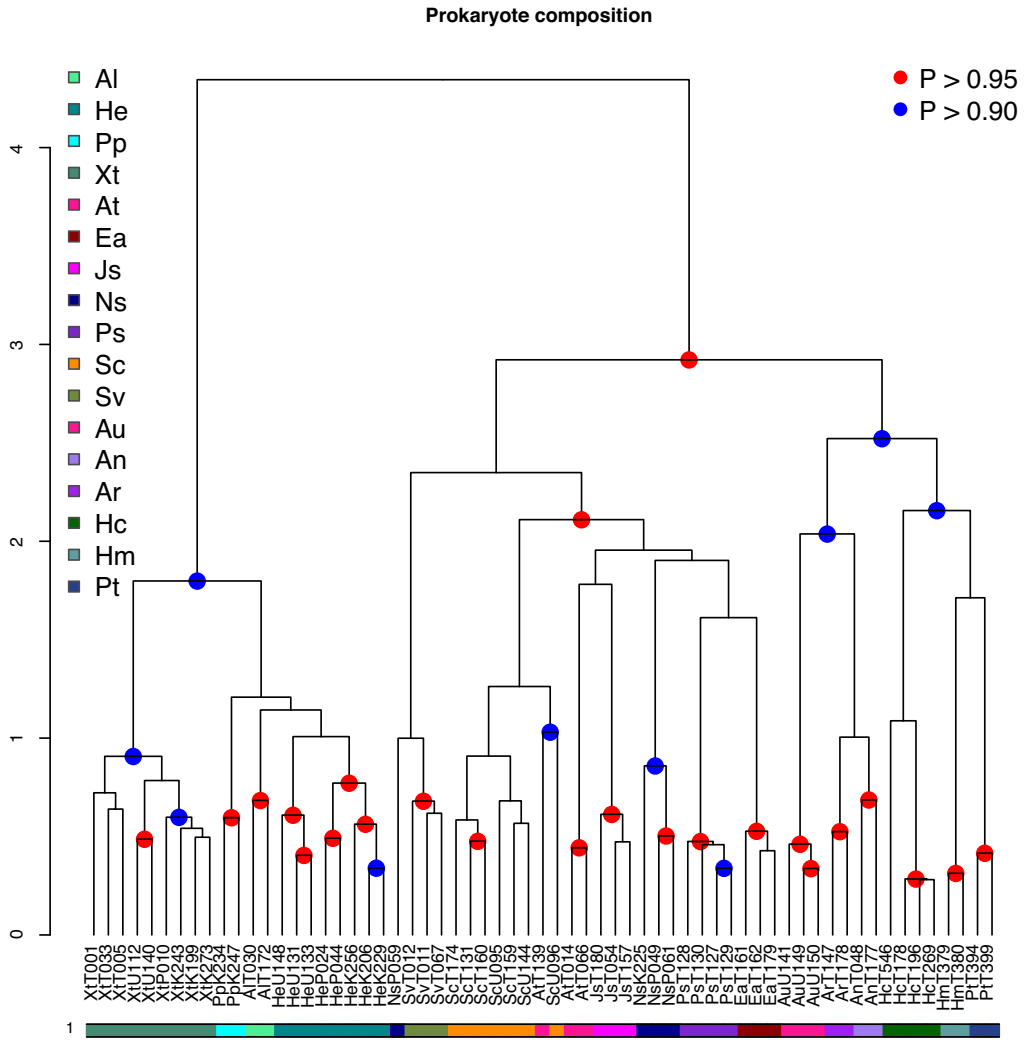

Figure 3. Unclassified, cluster dendrogram. Clusters with AU values > 90 are highlighted by blue symbols at the nodes while AU values > 95 are highlighted by red symbols at the nodes. The first two letters of the sample codes of the leaves refer to the sponge species: *A. lobata* (Al), *H. erectus* (He), *P. purpurea* (Pp), *X. testudinaria* (Xt), *A. cavernosa* (Au), *A. nemoechinata* (An), *A. cornuta* (Ar), *A. cavernosa* (At), *E. asperum* (Ea), *J. splendens* (Js), *Neopetrosia* sp. (Ns), *P. spiculifer* (Ps), *S. carteri* (Sc), *H. cymaeformis* (Hc), *Hymeniacidon* sp. (Hm), *Paratetilla* sp. (Pt) and *S. diversicolor* (Sv). The bar at the bottom of the dendrogram is coloured according to the colours in the upper left legend. For more information about the samples, see Supplementary Table 1 (Supporting Information).

The sponge species *Acanthella cavernosa, E. asperum*, *J. splendens*, *P. spiculifer*, *S. carteri, Neopetrosia* sp., *S. diversicolor and H. cymaeformis* all contained small subsets of highly abundant, significantly discriminating OTUs. OTU-62, assigned to the Sva0996 marine group (Actinobacteria), significantly discriminated *A. cavernosa* from all tested biotopes. It had 94% sequence similarity to an organism obtained from the sponge *Poecillastra compressa*. OTU-41, assigned to the PAUC26f genus (Acidobacteria), significantly discriminated *Ag. cavernosa* from all other tested biotopes. Note that OTU-41 was also relatively abundant in the other agelasids *A. nemoechinata* and *A. cornuta*, but both of these sponge species only included two samples and were not tested.

OTU-109, assigned to the Nitrosomonadaceae (Betaproteobacteria), significantly discriminated *E. asperum* from all tested biotopes. It had 95% sequence similarity to an organism obtained from the sponge *Tsitsikamma favus*. OTUs 91, 130 and 167, assigned to the Tectomicrobia, JTB255 marine benthic group (Gammaproteobacteria) and Gammaproteobacteria, respectively, significantly discriminated *J. splendens* from all tested biotopes. These OTUs had sequence similarities ranging from 93 to 97% with organisms obtained from sediment and a hypersaline basin. OTUs 45 and 64, assigned to the HTA4 (Gammaproteobacteria) and Nitrosomonadaceae, respectively, significantly discriminated *Neopetrosia* sp. from all tested biotopes. These OTUs had sequence similarities ranging from 98–99% to organisms obtained from the sponge species *Haliclona* sp. (Supplementary Table 4, Supporting Information).

OTUs 153 and 154, assigned to the Gammaproteobacteria and Nitrosomonadaceae, respectively, significantly discriminated *P. spiculifer* from all tested biotopes. These OTUs had sequence similarities ranging from 95–96% to organisms obtained from the sponge species *Raspailia topsenti* and *Haliclona* sp. OTUs 61, 96, 102 and 133, assigned to the HOC36 (Gammaproteobacteria), ARKDMS-49 (Proteobacteria), E01–9C-26 marine group (Gammaproteobacteria) and genus *Cenarchaeum* (Thaumarchaeota), significantly discriminated *S. carteri* from all tested biotopes. These OTUs had sequence similarities ranging from 98 to >99% with organisms obtained from the sponge species *Axinella* sp., *S. carteri* (from the Red

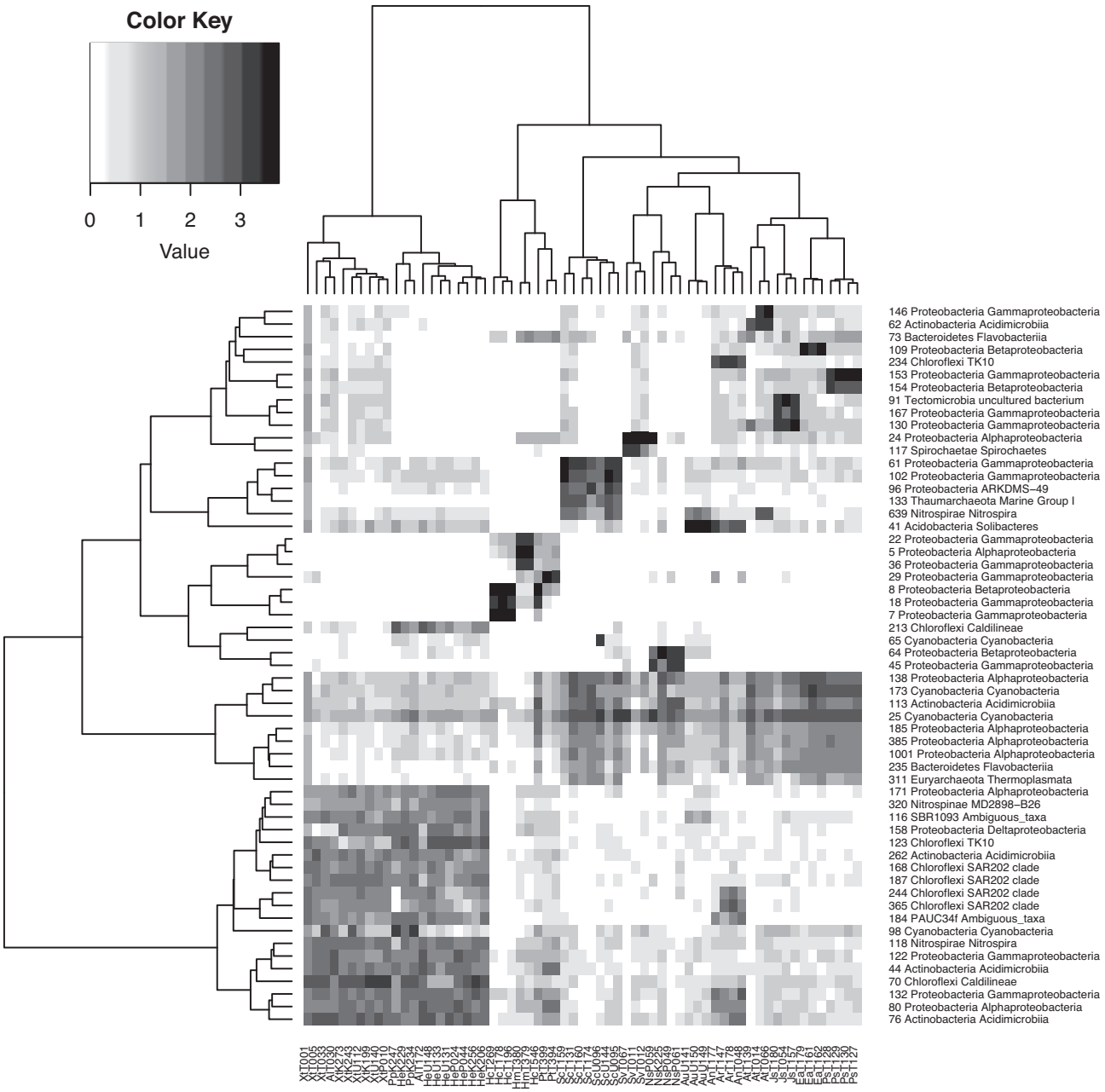

Figure 4. Heatmap of the most abundant OTUs found in *A. lobata* (Al), *H. erectus* (He), *P. purpurea* (Pp), *X. testudinaria* (Xt), *A. cavernosa* (Au), *A. nemoechinata* (An), *A. cornuta* (Ar), *A. cavernosa* (At), *E. asperum* (Ea), *J. splendens* (Js), *Neopetrosia* sp. (Ns), *P. spiculifer* (Ps), *S. carteri* (Sc), *H. cymaeformis* (Hc), *Hymeniacidon* sp. (Hm), *Paratetilla* sp. (Pt) and *S. diversicolor* (Sv). The grey scale 'color key' represents abundance on a log10 scale. The dendograms for rows and columns were generated using the hclust function in R, which applies a hierarchical cluster analysis using the complete linkage method and based on a euclidean dissimilarity matrix of the most abundant OTUs and Ward's clustering function.

Sea), *Phakellia fusca* and *Xestospongia exigua*. OTUs 7, 8 and 18, assigned to the Gammaproteobacteria, Nitrosomonadaceae (Betaproteobacteria) and Oceanospirillales (Gammaproteobacteria), respectively, significantly discriminated *H. cymaeformis* from all tested biotopes. These OTUs had sequence similarities ranging from 98 to >99% with organisms obtained from the Caribbean sponge species *Callyspongia vaginalis* and Indo-West Pacific *Gelliodes carnosa*. OTUs 24 and 117, assigned to the Rhodospirillaceae (Alphaproteobacteria) and *Spirochaeta* 2 genus (Spirochaetae), respectively, significantly discriminated *S. diversicolor* from all tested biotopes. OTU-24 had 98% sequence similarity to an organism obtained from marine sediment and OTU-117 had 94% sequence similarity to an organism obtained from the sponge species *Tsitsikamma favus* (Supplementary Table 4, Supporting Information).

In order to estimate the amount of environmental OTUs found in sponges, we created a dataset only including all OTUs >100 sequences ($OTUs_{100}$). This subset included 677 $OTUs_{100}$ and 749 650 sequences (88.2% of all sequences). Of the 697 $OTUs_{100}$, 588 (86.9%) representing 677 065 sequences (90.3%) were recorded in sediment samples, 481 (71.0%) representing 638 580 sequences (85.2%) in seawater samples and 625 (93.1%)

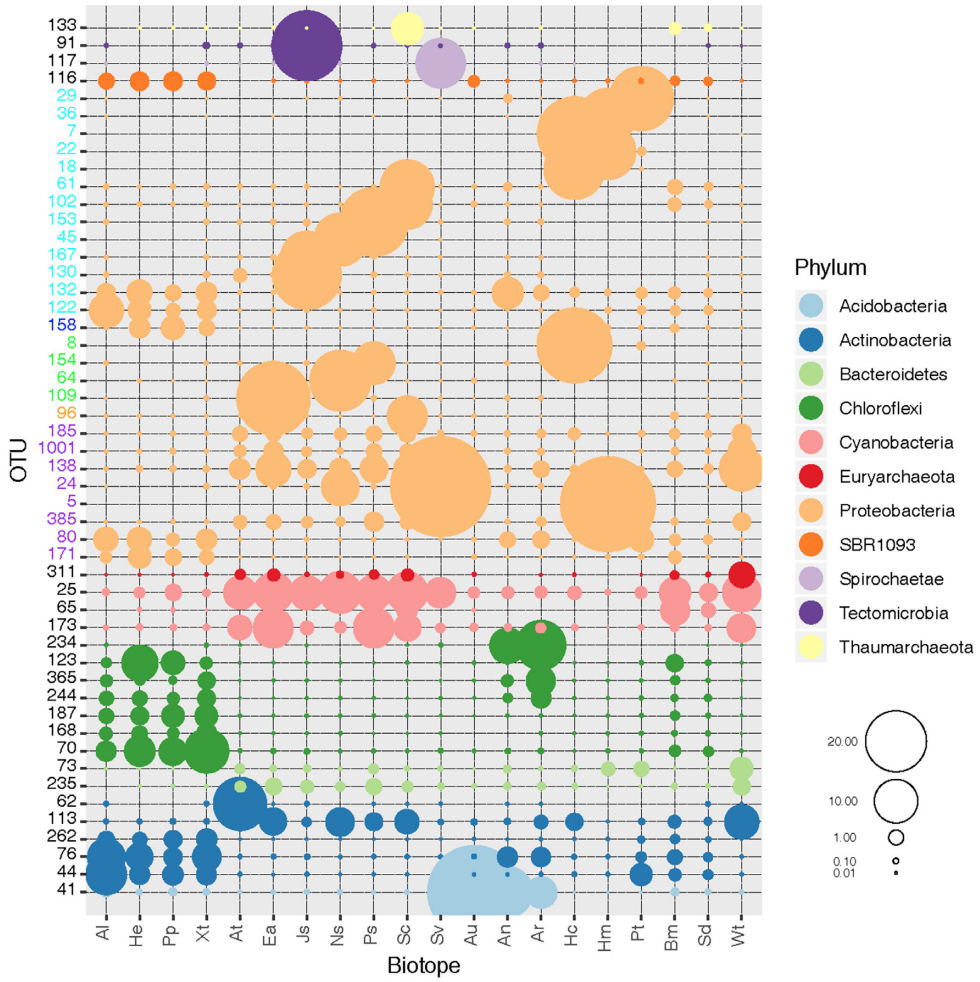

Figure 5. Relative abundance of significantly discriminating OTUs between pairs of biotopes identified using Simper ($P < 0.001$) and colour-coded according to prokaryote phylum for *A. lobata* (Al), *H. erectus* (He), *P. purpurea* (Pp), *X. testudinaria* (Xt), *A. cavernosa* (Au), *A. nemoechinata* (An), *A. cornuta* (Ar), *Acanthella cavernosa* (At), *E. asperum* (Ea), *J. splendens* (Js), *Neopetrosia* sp. (Ns), *P. spiculifer* (Ps), *S. carteri* (Sc), *H. cymaeformis* (Hc), *Hymeniacidon* sp. (Hm), *Paratetilla* sp. (Pt), *S. diversicolor* (Sv), bacterial mat (Bm) sediment (Sd) and seawater (Wt). The circle size of the OTU is proportional to the mean percentage of sequences per biotope as indicated by the symbol legend in the bottom right corner of the figure. The y-axis numbers shows the OTU number and have been colour coded for the proteobacterial OTUs in order to identify class assignment; purple: Alphaproteobacteria, orange: ARKDMS-49, green: Betaproteobacteria, blue: Deltaproteobacteria and cyan: Gammaproteobacteria.

representing 697 865 sequences (92.3%) in environmental samples (seawater and/or sediment). The relative abundance of seawater OTUs was significantly higher in the cluster 2 sponge species *Acanthella cavernosa*, *E. asperum*, *J. splendens*, *P. spiculifer* and *S. carteri* than all other tested biotopes (Fig. 6U). The relative abundance of seawater OTUs was also significantly higher in *H. erectus* than *X. testudinaria* (Supplementary Table 3, Supporting Information). The relative abundances of environmental (sediment and/or seawater) OTUs was significantly lower in the species *X. testudinaria* and *H. cymaeformis* than all other species (Fig. 6V). Supplementary Table 5 (Supporting Information) provides results of the emmeans analysis including pairwise comparisons between pairs of biotopes.

OTU richness was by far highest in the sediment and bacterial mat biotopes followed by the sponge species *E. asperum*, *J. splendens* and *P. spiculifer* and lowest in the species *A. lobata*, *Agelas cavernosa*, *A. nemoechinata* and *S. diversicolor* (Fig. 6 and Supplementary Table 5, Supporting Information). The sponge species *E. asperum* had significantly higher richness than all the tested cluster 1 and 3 species. Evenness was also highest in the sediment and bacterial mat biotopes followed by the species *A. lobata*, *H. erectus* and *X. testudinaria* and lowest in the species *H. cymaeformis*, *Hymeniacidon* sp., *J. splendens* and *S. diversicolor*. Evenness was significantly higher in the cluster 1 species *H. erectus* and *X. testudinaria* than all tested cluster 2 and 3 species.

There were also significant differences in the relative abundances of all of the most abundant phyla among biotopes (Fig. 6 and Supplementary Table 5, Supporting Information). The cluster 1 species *H. erectus* and *X. testudinaria* had significantly higher relative abundances of Chloroflexi, Acidobacteria (with the exception of *Agelas cavernosa*), Gemmatimonadetes, PAUC34f, SBR1093 and Poribacteria than all other biotopes. Note, however, that the untested agelasids *A. nemoechinata* and *A. cornuta* also had relatively high abundances of Chloroflexi, Acidobacteria, Gemmatimonadetes, PAUC34f and SBR1093, but not Poribacteria.

Overall, cluster 2 sponges were characterised by higher relative abundances of Proteobacteria than cluster 1 sponges (with the exception of *Acanthella cavernosa*). Cluster 2 species

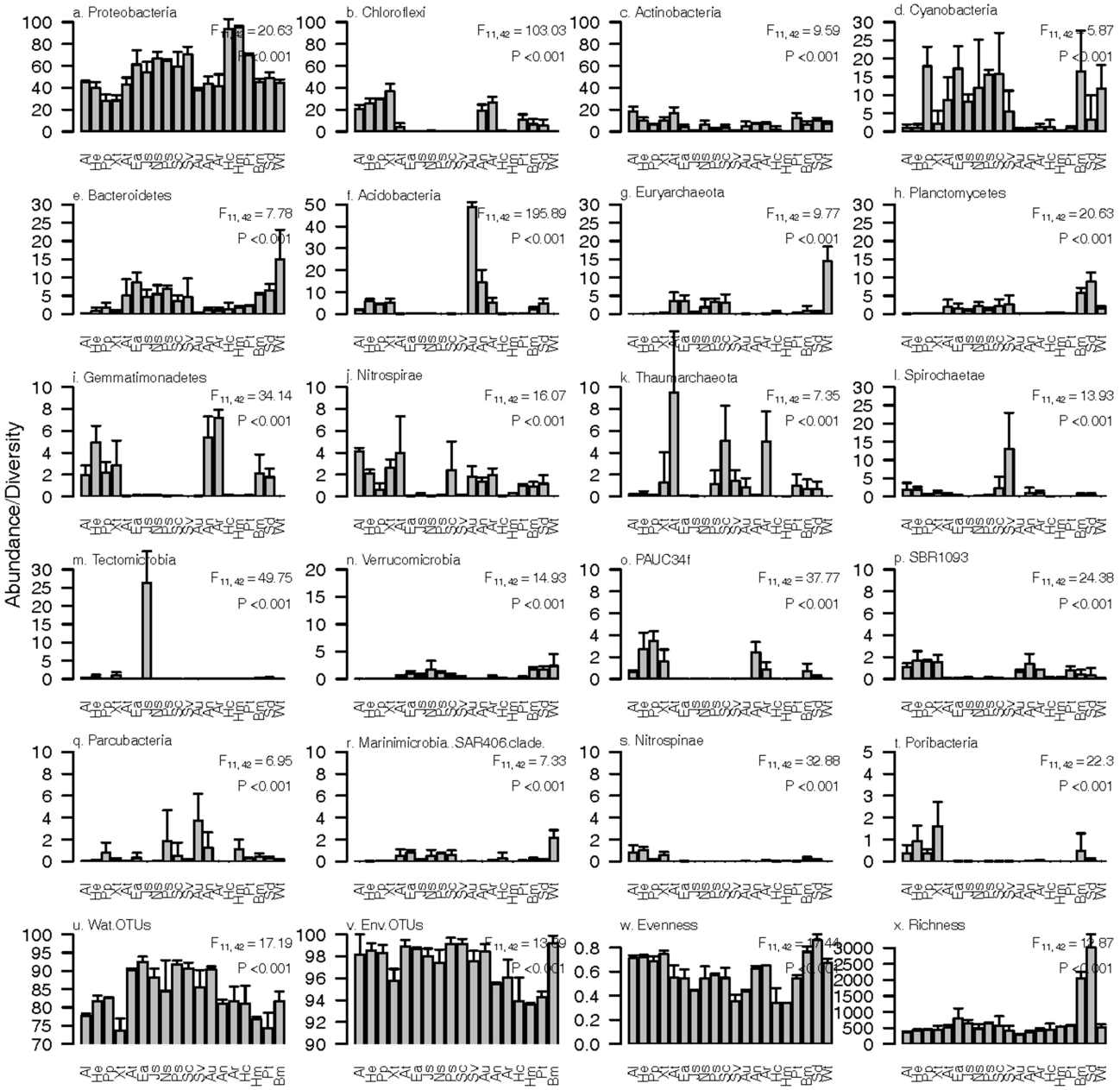

Figure 6. Mean (error bars represent a single standard deviation) relative abundance of (A) Proteobacteria, (B) Chloroflexi, (C) Actinobacteria, (D) Cyanobacteria, (E) Bacteroidetes, (F) Acidobacteria, (G) Euryarchaeota, (H) Planctomycetes, (I) Gemmatimonadetes, (J) Nitrospirae, (K) Thaumarchaeota, (L) Spirochaetae, (M) Tectomicrobia, (N) Verrucomicrobia, (O) PAUC34f, (P) SBR1093, (Q) Parcubacteria, (R) Marinimicrobia, (S) Nitrospinae, (T) Poribacteria, (U) seawater OTUs, (V) environmental OTUs (found in sediment and/or water), (W) evenness and (X) richness in the following biotopes: *A. lobata* (Al), *H. erectus* (He), *P. purpurea* (Pp), *X. testudinaria* (Xt), *Ag. cavernosa* (Au), *A. nemoechinata* (An), *A. cornuta* (Ar), *A. cavernosa* (At), *E. asperum* (Ea), *J. splendens* (Js), *Neopetrosia* sp. (Ns), *P. spiculifer* (Ps), *S. carteri* (Sc), *H. cymaeformis* (Hc), *Hymeniacidon* sp. (Hm), *Paratetilla* sp. (Pt), *S. diversicolor* (Sv), bacterial mat (Bm) sediment (Sd) and seawater (Wt). Results of the GLM analyses are presented in the top right of the subfigures.

also had significantly higher abundances of Bacteroidetes, Euryarchaeota (with the exception of *J. splendens*), Planctomycetes and Marinimicrobia (with the exception of *J. splendens*). Cluster 3 species generally differed from cluster 2 species with significantly lower abundances of Cyanobacteria, Euryarchaeota and Planctomycetes. Certain taxa were also particularly abundant in a given biotope. For example, the relative abundance of Acidobacteria was significantly higher in *Agelas cavernosa* than all other biotopes and the relative abundance of Tectomicrobia was significantly higher in *J. splendens* than all other biotopes

DISCUSSION

Samples from sponge species separated into three clusters with one cluster containing samples of the species *A. lobata*, *H. erectus*, *P. purpurea* and *X. testudinaria* (cluster 1). The second cluster consisted of species which were compositionally more similar to seawater samples (cluster 2: *A. cavernosa*, *E. asperum*, *J. splendens*, *Neopetrosia* sp., *P. spiculifer*, *S. carteri* and *S. diversicolor*) and the third cluster consisted of species that were intermediate in composition to cluster 1 and 2 species (e.g. *Agelas caver-*

nosa, *A. nemoechinata*, *A. cornuta*) and included species that were compositionally more similar to sediment samples (*Hymeniacidon* sp. and *H. cymaeformis*). Cluster 3, furthermore, included two strongly supported subclusters, one consisting of the agelasids *Agelas cavernosa*, *Agelas nemoechinata*, *Acathostylotella cornuta* and the other consisting of the remaining species *Hymeniacidon* sp., *Paratetilla* sp. and *H. cymaeformis*.

Cluster support was strongest at the host species level. All sponge species, with the exceptions of *Acanthella cavernosa*, *Neopetrosia* sp. and *S. carteri* formed significantly supported clusters. This supports a number of previous studies highlighting the importance of host identity in structuring prokaryote communities (Cleary *et al.* 2015, 2018; Steinert *et al.* 2016; Moitinho-Silva *et al.* 2017; Souza *et al.* 2017). Moitinho-Silva *et al.* (2017), e.g., found that variation in the composition of the sponge microbiome was largely explained by host identity, followed by HMA-LMA status and geographical variation within species (Moitinho-Silva *et al.* 2017).

Notwithstanding the greater influence of seawater on the prokaryote communities of cluster 2 species, they were dominated by relatively few OTUs, some of which were similar to organisms previously found in other sponge species. These OTUs were also relatively rare in seawater. The number of these abundant, discriminating OTUs varied from just 1 in *E. asperum* to 2 in *P. spiculifer*, 3 in *J. splendens* and *Neopetrosia* sp. and 4 in *S. carteri* (Fig. 6). Like cluster 2 species, certain cluster 3 species were also dominated by small subsets of dominant OTUs. *Haliclona cymaeformis* and *Hymeniacidon* sp., e.g. housed 3 dominant OTUs each while *Paratetilla* sp. and *S. diversicolor* housed one and two dominant OTUs, respectively. Other cluster 3 species (namely the agelasids) were more similar to cluster 1 species with respect to OTU dominance. Previous studies have also found that LMA sponges are characterised by the dominance of a limited set of OTUs in each sponge species, which are similar to sequences of organisms previously obtained from other LMA sponges (Giles *et al.* 2013; Poppell *et al.* 2014; Cleary *et al.* 2015, 2018, 2013). LMA sponges are also known to house prokaryote communities that are similar to those of seawater samples. For example, Moitinho-Silva *et al.* (2014) found that sequence data of *S. carteri* contained more sea water (~24%) sequences than *X. testudinaria* (~6%) in line with our results, although our higher percentage of seawater sequences in cluster 2 species (Fig. 3) was due to the removal of all OTUs <100 sequences. Taken together, our results suggest that cluster 2 species are LMA sponges and *S. carteri* and *S. diversicolor* have, in fact, previously been identified as LMA sponges (Gloeckner *et al.* 2014; Lurgi *et al.* 2019).

Cluster 1 species were compositionally distinct from cluster 2 and 3 species and differed significantly in the relative abundance of selected higher taxa. Cluster 1 species had significantly higher relative abundances of Chloroflexi (with the exceptions of *Agelas cavernosa* and *Paratetilla* sp.), Actinobacteria (with the exception of *Agelas cavernosa*), Acidobacteria (with the exception of *Agelas cavernosa*), Gemmatimonadetes, SBR1093, PAUC34f and Poribacteria than tested cluster 2 and 3 species.

Chloroflexi, Acidobacteria, Poribacteria and Actinobacteria are considered to be HMA indicators (Schmitt *et al.* 2011; Moitinho-Silva *et al.* 2017). Schmitt *et al.* (2011), e.g. showed that Chloroflexi were more abundant and diverse in HMA than LMA sponges and that HMA sponges contained similar Chloroflexi communities. In particular, Schmitt *et al.* (2011) found that the specific Chloroflexi lineages they found in HMA sponges were absent in other biotopes including seawater, algae, ascidians and LMA sponges. Although present in species from all clusters, there was pronounced variation among species in each cluster including the abundance of selected OTUs. There were also differences in the abundance of actinobacterial OTUs between pairs of species from both clusters.

Another pronounced difference between cluster 1, 2 and 3 species was the significantly lower relative abundance of Poribacteria in species from the latter two clusters. Poribacteria have mainly been associated with marine sponges (Hentschel *et al.* 2012; Thomas *et al.* 2016), but are also present in other hosts (Cleary *et al.* 2019). Arellano *et al.* (2013) did not detect members of Poribacteria in the sponge species *Myxilla methanophila* collected at two different cold-seep locations (550 m depth) in the Gulf of Mexico. The authors proposed two justifications for the lack of members of this candidate phylum: lack of specificity of the primer pair used for this phylum or the fact that Poribacteria have only been detected in shallow water sponge species. In the present study, Poribacteria were recorded in all sponge species, but their relative abundance was very low in cluster 2 and 3 species. They were, however, relatively abundant in all cluster 1 species, which would seem to rule out the lack of primer specificity as a justification. Samples of all species in the present study were also collected at similar depths. Previous studies have reported on the absence (Hochmuth *et al.* 2010) or low abundance (Bayer, Kamke and Hentschel 2014; Moitinho-Silva *et al.* 2017) of Poribacteria in LMA as opposed to HMA species. Although based on a limited set of species, Bayer, Kamke and Hentschel (2014) reported 5 orders of magnitude more Poribacteria in their sampled HMA sponges (*Aplysina aerophoba*, *Chrondrosia reniformis*, *Xestospongia muta*, *X. testudinaria* and *Ircinia felix*) than in their sampled LMA sponges (*Dysidea avara*, *S. carteri* and *Callyspongia vaginalis*). In the present study, the relative abundance of Poribacteria varied from 0.36 ± 0.19 (*P. purpurea*) to 1.61 ± 1.11 (*X. testudinaria*) for cluster 1 species and 0.00 ± 0.00 (*Paratetilla* sp.) to 0.06 ± 0.01 for cluster 2 and 3 species. Interestingly, Poribacteria were also recorded at relatively high mean abundances in bacterial mat samples although there was considerable variation among samples (0.49 ± 0.79).

Cluster 3 species were somewhat intermediate in composition to cluster 1 and 2 species. This included samples of the agelasids *Agelas cavernosa*, *A. cornuta* and *A. nemoechinata*. According to Gloeckner *et al.* (2014), in a study using transmission electron microscopy and DAPI-counting to determine the presence of microorganisms in the mesohyl matrix of several sponge species, the order Agelasida exclusively consisted of HMA species. However, as mentioned in the introduction, members of the genus *Agelas* only housed moderately dense microbial consortia in contrast to the high density consortia of other HMA sponge species (Gloeckner *et al.* 2014). In the present study, the agelasids in cluster 3 housed prokaryote communities distinct from species in clusters 1 and 2 and somewhat intermediate between both clusters as judged by the relative position of the sample points along axis 1 of Fig. 3. Although only three species belonging to different genera of agelasids were sampled in the present study, we provide some evidence that they deviate from other typical HMA species such as *X. testudinaria*.

The three agelasid species examined in the present study are also very different in colour and morphology. *Agelas nemoechinata* is a very common species and can be locally abundant in the southern Penghu islands. It is a large massive brown coloured sponge with several slightly raised oscules that can grow up to 1 m in height. The other *Agelas* species, *A. cavernosa*, is a very

small, common, cryptic, bright orange sponge that grows irregularly bearing digitiform outgrowths with a single, large oscule at the tips. The other agelasid sponge species examined was *A. cornuta*. This species was previously placed in a different order, Poecilosclerida, but DNA barcoding revealed that this species is a true agelasid sponge species (Morrow and Cardenas 2015). *Acantostylotellta cornuta* is a rare, but locally abundant, light green to brown sponge species, and grows thinly encrusting under coral overhangs.

In addition to the agelasids, the species *Hymeniacidon* sp., *Paratetilla* sp. and *H. cymaeformis* also were intermediate in composition along axis 1 between cluster 1 and 2 species. These species are distinct from each other. The *Paratetilla* species is probably a new sponge species and is a very common sponge in the Penghu Islands, but has not been observed outside Taiwan. It is globular and the exterior is white but the interior can be brown to purple in colour. It often grows on a sandy bottom and is covered by a thin layer of sediment. *Haliclona cymaeformis* is a bright green branching species and lives in association with a red macroalgae *Ceratodictyon spongiosum*. The sponge and algal species only occur in symbiosis and both species have never been observed without the other. The association is widespread and the species occurs from the Madagascar Sea to New Caledonia (van Soest *et al.* 2019) and mainly in very shallow water. The *Hymeniacidon* sponge species was not identified to species level, and is also probably a new species. This species, yellow in colour, was only found at one location growing thickly encrusting and exposed, out of the water, in the intertidal area. *Paratetilla* species and *Hymeniacidon* sp. were both found in shallow, turbid, silty environments.

Taken together, our data support the LMA status of the cluster 2 species *Acanthella cavernosa*, *E. asperum*, *J. splendens* and *P. spiculifer*, in addition to the previously identified LMA species *S. carteri* and *S. diversicolor*. Our results also support the HMA status of the cluster 1 species *A. lobata* and *P. purpurea*, in addition to the previously identified HMA species *H. erectus* and *X. testudinaria* (Gloeckner *et al.* 2014; Lurgi *et al.* 2019). The cluster 3 species, which consisted of the agelasids *Agelas cavernosa, A. nemoechinata* and *A. cornuta* in addition to *Hymeniacidon* sp., *Paratetilla* sp. and *H. cymaeformis* were compositionally distinct.

Moitinho-Silva *et al.* (2017) previously showed that there was more variation in the microbial composition of LMA as opposed to HMA species in line with the results of our study. In the present study, all sponge species formed strongly supported clusters with the exceptions of *Acanthella cavernosa*, *Neopetrosia* sp. and *S. carteri*. We also found evidence of geographical structuring in the HMA species *X. testudinaria* and *H. erectus*. Swierts, Cleary and de Voogd (2018) also identified significant geographical structuring of prokaryote communities associated with *X. testudinaria* across the Indopacific. Our results suggest that this may also apply to *H. erectus*.

The composition of the bacterial mats in the present study was highly variable although Cyanobacteria were the most abundant element. The bacterial mats also housed relatively high abundances of actinobacterial and Chloroflexi OTUs found in cluster 1 sponge species and a relatively high abundance of Poribacteria compared to cluster 2 and 3 species as mentioned previously. The dominant cyanobacterial OTUs in the mats were assigned to the genera *Synechococcus* and *Trichodesmium*. In Schiermonnikoog, the Netherlands, Cardoso *et al.* (2017) identified Cyanobacteria and Proteobacteria as the major taxa in coastal microbial mats followed by Bacteroidetes and Chloroflexi. They also identified an OTU assigned to the genus *Trichodesmium* as among the most active component of their dataset. *Trichodesmium* spp., however, are planktonic gas-vaculated cyanobacteria and they suggested the organism in their dataset may be a close relative of *Trichodesmium* that has adapted to a benthic life style.

CONCLUSION

The present study used 16S rRNA gene sequence data to test to what extent sponges could be given HMA or LMA status in line with previous studies (Hentschel *et al.* 2002, 2003; Cleary *et al.* 2015, 2018; de Voogd *et al.* 2015, 2018; Polónia *et al.* 2015b; Moitinho-Silva *et al.* 2017). Our results indicate LMA status for the species *Acanthella cavernosa*, *E. asperum*, *J. splendens*, *P. spiculifer*, *S. carteri* and *S. diversicolor*, and HMA status for the species *A. lobata*, *H. erectus*, *P. purpurea* and *X. testudinaria*. A number of species, however, did not appear to fit neatly within the HMA/LMA dichotomy and contained prokaryote communities intermediate between HMA and LMA species, some of which were compositionally closer to sediment samples. Our study also revealed high richness in bacterial mat communities and certain similarities between bacterial mat and sponge samples. Future studies would, thus, benefit from a more in-depth study of coral reef bacterial mats.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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Conflict of interest. None declared.

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