FEMS Microbiology Ecology, 93, 2017, fiw204

doi: 10.1093/femsec/fiw204
Advance Access Publication Date: 3 October 2016
Research Article

RESEARCH ARTICLE

Analysis of bacterial composition in marine sponges reveals the influence of host phylogeny and environment

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One sentence summary: Linking sponge phylogeny to associated bacteria.
Editor: Julie Olson

ABSTRACT

Bacterial communities associated with sponges are influenced by environmental factors; however, some degree of genetic influence of the host on the microbiome is also expected. In this work, 16S rRNA gene amplicon sequencing revealed diverse bacterial phylotypes based on the phylogenies of three tropical sponges (*Aplysina fulva, Aiolochroia crassa* and *Chondrosia collectrix*). Despite their sympatric occurrence, the studied sponges presented different bacterial compositions that differed from those observed in seawater. However, lower dissimilarities in bacterial communities were observed within sponges from the same phylogenetic group. The relationships between operational taxonomic units (OTUs) recovered from the sponges and database sequences revealed associations among sequences from unrelated sponge species and sequences retrieved from diverse environmental samples. In addition, one Proteobacteria OTU retrieved from *A. fulva* was identical to sequences previously reported from *A. fulva* specimens collected along the Brazilian coast. Based on these results, we conclude that bacterial communities associated with marine sponges are shaped by host identity, while environmental conditions seem to be less important in shaping symbiont communities. This is the first study to assess bacterial communities associated with marine sponges in the remote St. Peter and St. Paul Archipelago using amplicon sequencing of the 16S rRNA gene.

Keywords: Aplysinidae; Chondrosidae, Associated symbionts; Verongimorpha; Saint Peter and Saint Paul Archipelago; Brazil

INTRODUCTION

Sponges (phylum Porifera) are the oldest multicellular animals still extant on Earth. These animals evolved over 600 million years ago and currently encompass over 8600 species that inhabit different marine and freshwater ecosystems (Webster and Thomas 2016). The evolutionary success of sponges is closely linked to their capacity to maintain a rich community of

Received: 14 April 2016; **Accepted:** 2 October 2016

microorganisms that can comprise up to 40% of their biomass (Hentschel *et al.* 2012). These microorganisms play key roles in nutrient cycling (Fan *et al.* 2012; Ribes *et al.* 2012) and are considered important modulating agents of sponge fitness by promoting host health and the ability to adapt to environmental stresses (Erwin and Thacker 2008; Hoffmann *et al.* 2009; Turque *et al.* 2010; Simister *et al.* 2012b; Fan *et al.* 2013; Webster *et al.* 2013b). In addition, sponge holobionts represent a valuable source of novel genetic and metabolic material, generating interest in the chemical, genetic and ecological exploitation of these animals. However, the microbial systems found in sponges remain poorly understood (Hardoim and Costa 2014).

To fully understand sponge biology, a detailed investigation of sponge-associated microbial communities is essential. Specifically, the ecological and evolutionary factors that influence the structure and dynamics of these communities must be described. Bacteria–sponge interactions display a high degree of host specificity and are often stable across spatiotemporal scales or under different environmental conditions (Hentschel *et al.* 2002; Taylor *et al.* 2004; Thiel *et al.* 2007; Montalvo and Hill 2011; Erwin *et al.* 2012; Pita *et al.* 2013a, b; Simister *et al.* 2013; Cárdenas *et al.* 2014; Hardoim and Costa 2014), although variations have been observed in some host species (Wichels *et al.* 2006; Turque *et al.* 2010; Cao *et al.* 2012; White *et al.* 2012; Luter *et al.* 2015; Morrow *et al.* 2015; Weigel and Erwin 2015; Morrow, Fiore and Lesser 2016). Host–microbe association specificity has been investigated using a wide variety of techniques (Taylor *et al.* 2004; Simister *et al.* 2012a, 2013; Hardoim and Costa 2014; Reveillaud *et al.* 2014), which have revealed that the microbial communities that are associated with sponges consist of a specific mix of generalist and specialist symbionts (Erwin *et al.* 2012; Thomas *et al.* 2016). However, the ecological and evolutionary significance of these associations remain unclear.

Many studies have surveyed microbial diversity across various host species, including tropical sponges, to characterise the spatiotemporal distributions of microbes and their associations with host health, phenotype, physiology and ecology (Friedrich *et al.* 2001; Webster *et al.* 2001, 2010; Montalvo and Hill 2011; Erwin *et al.* 2012; Schmitt *et al.* 2012; Burgsdorf *et al.* 2014; Cuvelier *et al.* 2014; Gloeckner *et al.* 2014; Hardoim and Costa 2014; Luter, Gibb and Webster 2014; Olson, Thacker and Gochfeld 2014; Reveillaud *et al.* 2014; Choudhury *et al.* 2015; Gao *et al.* 2015; Blanquer *et al.* 2016; Thomas *et al.* 2016). The following general conclusions have been obtained: (i) microbes are host specific (Schmitt *et al.* 2012); (ii) microbes associated with sponges differ from those found in planktonic communities in surrounding water (Hardoim *et al.* 2012); (iii) despite phylogenetic differences, microbes share functional characteristics that allow them to exist symbiotically (Fan *et al.* 2012); and (iv) some sponge species harbour a high microbial abundance (HMA), whereas other species maintain a low microbial abundance (LMA) (Gloeckner *et al.* 2014).

Microbial communities associated with sponge species have been extensively studied. Recent surveys have evaluated correlations between host phylogeny and bacterial community profiles from closely related sponges collected at different locations (Erpenbeck *et al.* 2002; Montalvo and Hill 2011; Erwin *et al.* 2012; Schmitt *et al.* 2012; Schöttner *et al.* 2013; Pita *et al.* 2013b; Easson and Thacker 2014; Reveillaud *et al.* 2014; Thomas *et al.* 2016). Overall, these studies have revealed a small core microbial community characterised by generalist symbionts as well as highly specific host–bacteria associations extending to rare taxa in the sponge microbiome (Reveillaud *et al.* 2014). Furthermore, these studies revealed that sponge microbiomes are highly diverse and shaped by both host and environmental factors (Erwin *et al.* 2012; Schmitt *et al.* 2012; Pita *et al.* 2013b). However, few studies have compared sponge microbial communities in closely related species that coexist in close spatial proximity (Erwin *et al.* 2012; Hardoim *et al.* 2012; Webster *et al.* 2013a; Cuvelier *et al.* 2014; Naim *et al.* 2014). The major driving forces regulating microbial diversity and the evolutionary history of these communities remain unclear; therefore, understanding the microbial patterns that are present in closely related sponge species is of special interest (Hardoim *et al.* 2012; Webster and Thomas 2016).

In the present survey, the phylogenies of three marine sponges (*Aiolochroia crassa*, *Aplysina fulva* and *Chondrosia collectrix*) that live in close spatial proximity were examined to determine the bacterial species that are specific and shared among phylogenetically related host sponges. To accomplish this, the compositions of the bacterial communities associated with 12 sponge specimens were characterised. Sponge samples were collected from the St. Peter and St. Paul Archipelago (SPSPA) in Brazil. To ensure the accurate identification of target sponges, host phylogenetic relatedness was assessed using sequences from the standard CO1 barcoding fragment. This is the first study to assess bacterial communities associated with marine sponges in the SPSPA using amplicon sequencing of the 16S rRNA gene.

MATERIALS AND METHODS

Study site

The SPSPA is one of the smallest and most isolated archipelagos in the world. It is located ∼1000 km from the city of Natal, Rio Grande do Norte State, North-eastern Brazil (0°55′02′N, 29°20′44′W) and is a federal Environmental Protected Area maintained by the Brazilian Navy. The archipelago is composed of 10 small islands and islets distributed across a 1.5-ha area (Fig. 1). The rocky shores of the archipelago are mainly devoid of unconsolidated substrates, with the exception of one cove that forms gradually from a 3 to 20 m depth and includes a mosaic of rocky and gravel bottom. The unique marine fauna harbours high endemism of fishes and sponges and have attracted growing attention from researchers (Edwards and Lubbock 1983; Moraes 2011). Despite the high local fishing pressure, the SPSPA suffers relatively little anthropogenic disturbance, at least with respect to industrial and domestic pollutants, due to its isolation from the mainland and sparse human occupation.

Sponge and seawater sampling

Twelve sponge specimens (i.e. four replicates from three sponge species) located at a similar depth (14–15 m) and within a few meters of each other were sampled during a single dive within a protected area at the cove of SPSPA, all inhabited the same rocky substrate. The temperature of the water was 25°C, and the specimens were living in an area that was exposed to light. A small fragment from each specimen was collected and placed separately into a 50-mL Falcon tube containing seawater; the tubes were then stored on ice for transport to the laboratory. The sampled sponges were previously identified as belonging to the families Aplysinidae: *Aiolochroia crassa* (Hyatt 1875) and *Aplysina fulva* (Pallas 1766) and Chondrosidae: *Chondrosia collectrix* (Schmidt 1870), all from the subclass Verongimorpha (Moraes 2011; Erpenbeck *et al.* 2012). Phylogenetic inference methods utilizing sequences of the standard CO1 barcoding fragment were used to aid in the molecular identification of the sponge species (Fig. S1,

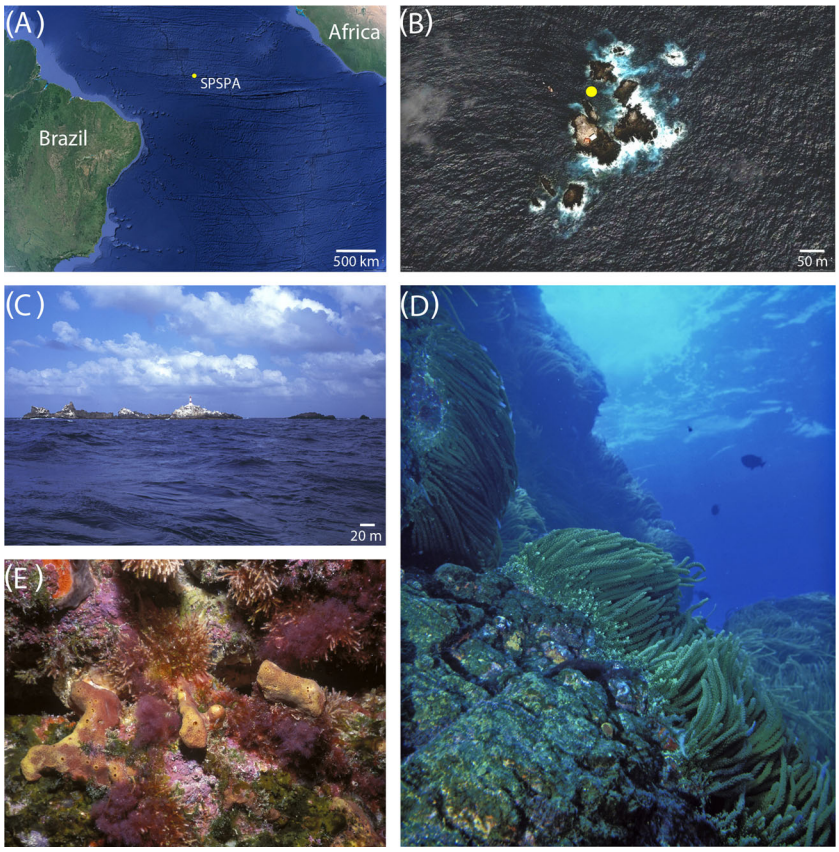

Figure 1. The SPSPA in the Equatorial Atlantic (0°55′02′N, 29°20′44′W) and *in situ* aspects of the sampling site. **(A)** Map showing the archipelago position; **(B)** satellite image of the SPSPA showing the sampling site at the cove (yellow dot); **(C)** west view of the SPSPA; **(D)** rocky shore at the cove at a 15-m depth; **(E)** close-up of benthos showing *Ap. fulva* and *C. collectrix* (massive yellow in the centre and encrusting black in the left upper corner, respectively). (A and B) Satellite images from (SIO NOAA, US Navy, NGA GEBCO)/Google Earth Pro software; (C–E) *in situ* photographs by F. Moraes.

Supporting Information). Five litres of seawater were collected from the sponge-sampling site using a Van Dorn bottle; one litre was immediately filtered through a 0.22-μm polycarbonate membrane (Millipore, MA, USA). This process was repeated four times, resulting in four replicates. After processing, all the samples were stored at –20°C until being submitted to DNA extraction. Seawater samples surrounding the sponges were collected for comparison to sponge symbionts.

Bacterial community analysis using 16S rRNA gene sequencing

In the laboratory, the seawater was drained from the Falcon tubes containing the sponges, and each sponge was individually rinsed three times in separate beakers with sterile artificial seawater (33 g l^{-1} Red Sea Salt®, Red Sea, Houston, TX, USA) to eliminate loosely attached microorganisms. The samples were dissected into fine pieces that included both ectosomal and choanosomal sections using sterilised scalpels. Total DNA was isolated from 0.25 g samples of dissected sponge tissue and from the membranes used to filter the seawater samples using a Power Soil DNA Isolation Kit (Mo Bio, Carlsbad, CA, USA) according to the manufacturer's instructions.

The V6 hypervariable region of the 16S rRNA gene sequence was PCR amplified using the primer set 967F (CAA CGC GAA GAA CCT TAC C; Sogin *et al.* 2006) and 1193R (CGT CRT CCC CRC CTT CC; Wang and Qian 2009) to generate amplicons of ~230 bp. The PCR was performed according to Kavamura *et al.* (2013). After amplification, each reaction was purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA). Emulsion PCR was performed using an Ion OneTouch 2 with an Ion Template PGM OT2 400 Kit (Life Technologies, Carlsbad, USA) according to the manufacturer's instructions. The amplicon libraries were sequenced using an Ion 316 Chip Kit v2 on an Ion Torrent PGM system. After sequencing, all reads were filtered using PGM software to remove low-quality and polyclonal sequences, yielding a total of 218 223 sequences. The sequence file (.fastq) was exported and analysed as described below.

Processing of sequences and taxonomic assignment

Raw sequences were quality filtered (average quality score = 25, window size = 5 bases, and maximum number of homopolymers = 6) and trimmed to a minimum length of 200 bp using Galaxy software (https://main.g2.bx.psu.edu). After processing, 120 179 sequences were analysed using the Quantitative Insights Into Microbial Ecology toolkit (QIIME), version 1.8 (Caporaso *et al.* 2010b) to identify operational taxonomic units (OTUs) with 97% similarity using the UCLUST method (Edgar 2010). A representative sequence from each OTU was aligned against the Greengenes core set using the NAST algorithm (Caporaso *et al.* 2010a). Chimeric sequences were removed using the UCHIME method (Edgar *et al.* 2011). Taxonomic assignment was performed using the UCLUST taxonomy assigner method with the Greengenes reference sequence database (McDonald *et al.* 2012). Non-target sequences (i.e. chloroplast, mitochondria and singleton

DNA) were removed from the dataset. After processing, 86 288 sequences were assigned to 2112 different OTUs (Table S1, Supporting Information), and a sample versus OTU table was created and used as input data for downstream analysis.

Statistical analysis of sequencing data

The numbers of sequence reads per phylum and class were normalised to the smallest sampling effort, which included 3040 sequences in each dataset and was expressed as a percentage of the total for each sample. Alpha diversity metrics were computed (observed species, chao1, Shannon and inverse Simpson) among specimens using R software (R-project 2014) with the Phyloseq package (McMurdie and Holmes 2013). To evaluate the effects of host phylogeny on bacterial community composition in the Aplysinidae sponges, we determined whether the microbiota of the sympatric *A. crassa* and *Ap. fulva* specimens shared a greater number of phylotypes than with *C. collectrix* (the nearest sister taxon to Aplysinidae in this study). We further determined whether these communities were distinct from the surrounding seawater. The rarefied datasets were analysed using weighted UniFrac (Lozupone *et al.* 2011) and Bray–Curtis distances (Bray and Curtis 1957) and clustered by principal coordinates analysis (PCoA). To evaluate the significance of the sample groupings based on these distance metrics, PERMANOVA was employed using the PAST software package, with significance values based on 10 000 permutations and adjusted using Bonferroni *post hoc* analysis for multiple pairwise comparisons. An average Bray–Curtis dissimilarity analysis was performed using a script customised in QIIME toolkit. The data were also statistically analysed using two-sample *t* tests for all comparisons. The extents to which the OTUs were shared or unique among the sample groups were visualised using Venn diagrams constructed using (i) a dataset composed of all the OTUs obtained from the sponges and seawater (2112 OTUs) and (ii) a dataset encompassing only the OTUs that were found in all four replicates of each sponge species (130 OTUs). For the second dataset, OTUs that were detected simultaneously in the sponges and the seawater samples were excluded as well as those that were sporadically detected in a single sample sponge species. This higher stringency dataset provided insight into which OTUs occurred stably in the sponges at the time of sample collection. Species-specific associations were based on the OTUs that were detected in all four replicates of a sponge species but not in any other analysed sponge species or in the surrounding seawater. These OTUs were compared to their best matches from the NCBI databases using BLAST (Altschul *et al.* 1990).

Nucleotide sequence accession numbers

The bacterial 16S rRNA gene sequences obtained in this study are publicly available in the MG-RAST server under accession numbers 4696821.3 to 4696836.3. The sponge CO1 sequences were deposited in GenBank under accession numbers KX034567 to KX034578.

RESULTS

Bacterial richness and diversity estimations

The Good's coverage indices obtained for the three sponge species were 96.8 ± 0.6%, 96.2 ± 0.2% and 96.0 ± 0.6% for *Aplysina fulva*, *Chondrosia collectrix* and *Aiolochroia crassa*, respectively, and the indices were 94.8 ± 1.0% for the seawater samples. Under our sequencing depth, the observed bacterial richness differed significantly between the sponges and the seawater (OTU numbers ranging from 181 to 322 and from 281 to 444, respectively; $P < 0.05$). Chao1 richness estimates for the sponges varied from 313 to 527 OTUs and from 399 to 613 OTUs for the seawater ($t = -3.98$, $P = 0.001$). The Shannon–Wiener indices for the sponge samples were lower on average (4.3–5.7), but they were not significantly different than the indices for the seawater (4.8 and 6.3) ($t = -0.56$, $P = 0.57$). Based on the observed species and the Chao1 alpha diversity metrics, the water samples presented the greatest species richness, followed by the *A. crassa*, *Ap. fulva* and *C. collectrix* samples (Fig. S2, Supporting Information).

Bacterial community composition based on 16S rRNA gene sequence analysis

The taxonomic profiles of the bacterial communities are summarised at the phylum and class levels in Fig. 2a. The OTUs were taxonomically assigned to 20 bacterial phyla in *Ap. fulva*, 19 in *A. crassa*, 13 in *C. collectrix* and 15 in seawater. The mean values (considering all replicates) revealed that Actinobacteria, Acidobacteria, Chloroflexi, Proteobacteria and Gemmatimonadetes were the most abundant phyla in the sponge community (>1% relative abundance of the reads) (Fig. 2b). The candidate phyla Poribacteria and PAUC34f were particularly enriched in *Ap. fulva*, and the phylum Bacteroidetes was enriched in *C. collectrix*. More than 95% of the bacterial reads from the seawater samples were affiliated with two dominant phyla: Proteobacteria (87%) and Bacteroidetes (8%). When the bacterial communities at the lower taxonomic levels were examined, 63 classes and 115 orders were recovered from all datasets. Class Acidimicrobiia was preferentially enriched in *A. crassa*. The phyla Chloroflexi and Acidobacteria varied in abundance among the three species. The classes within these phyla also varied among the sponges: Anaerolineae (Chloroflexi) was more abundant in *Ap. fulva* and *C. collectrix* than in *A. crassa*. Gammaproteobacteria (Proteobacteria) was also highly represented in the seawater samples. In contrast, purple sulfur bacteria (order: Chromatiales) within the phylum Proteobacteria were more abundant in the sponges than in the seawater samples.

Relationship between host phylogeny and bacterial communities

The results indicated that the three sponge species harboured bacterial communities that were distinct from those in the water column and also differed from each other (PERMANOVA, $F = 6.0035$, $P = 0.0001$). However, less bacterial dissimilarity was found within the sponges of the Aplysinidae family (PERMANOVA, $F = 3.3881$, $P = 0.0291$) when compared to its nearest relative Chondrosidae (Fig. 3). The average Bray–Curtis dissimilarity corroborated the previous findings acquired by PCoA analysis and added more evidence regarding the stability of the bacterial communities within the studied sponge species. The level of variation among replicates for the bacterial community inhabiting *C. collectrix* (~30% average Bray–Curtis dissimilarity) was more stable, while the bacterial communities in *Ap. fulva* and *A. crassa* showed higher interindividual variation (~60% and 45% average Bray–Curtis dissimilarity, respectively). Moreover, the bacterial assemblages in the Aplysinidae host showed a lower level of dissimilarity (~80% average Bray–Curtis dissimilarity) compared to those in *C. collectrix* (~95% average Bray–Curtis dissimilarity) (Fig. 3c).

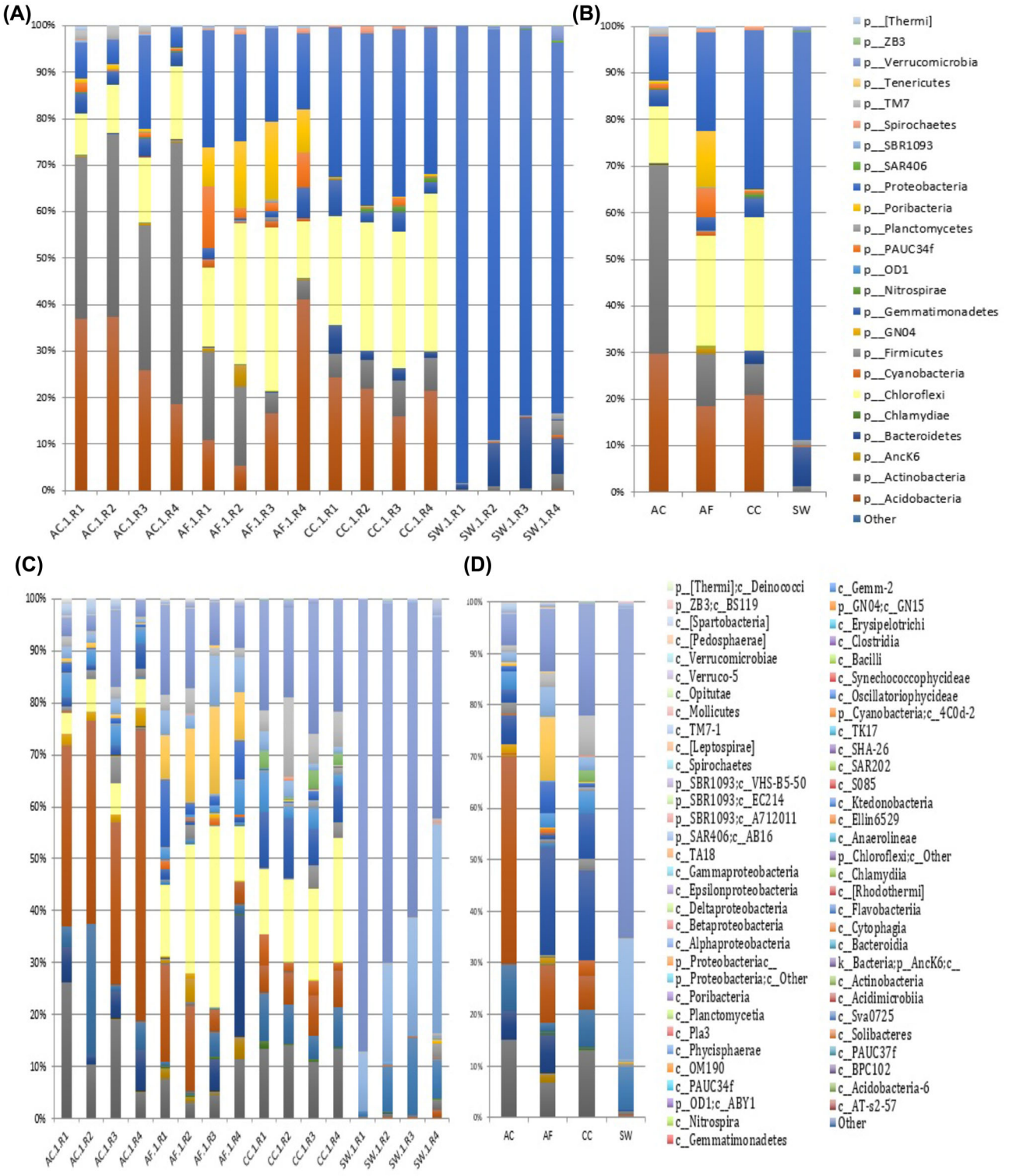

Figure 2. Taxonomic assignments at the phylum (**A** and **B**) and class (**C** and **D**) levels showing the relative abundance (%) of the 16S rRNA gene sequences from *A. crassa* (AC), *Ap. fulva* (AF), *C. collectrix* (CC) sponges and seawater (SW) samples. Bars represent the contributions of bacterial groups for each replicate ($n = 4$) (A and C) and for the average of each treatment (B and D).

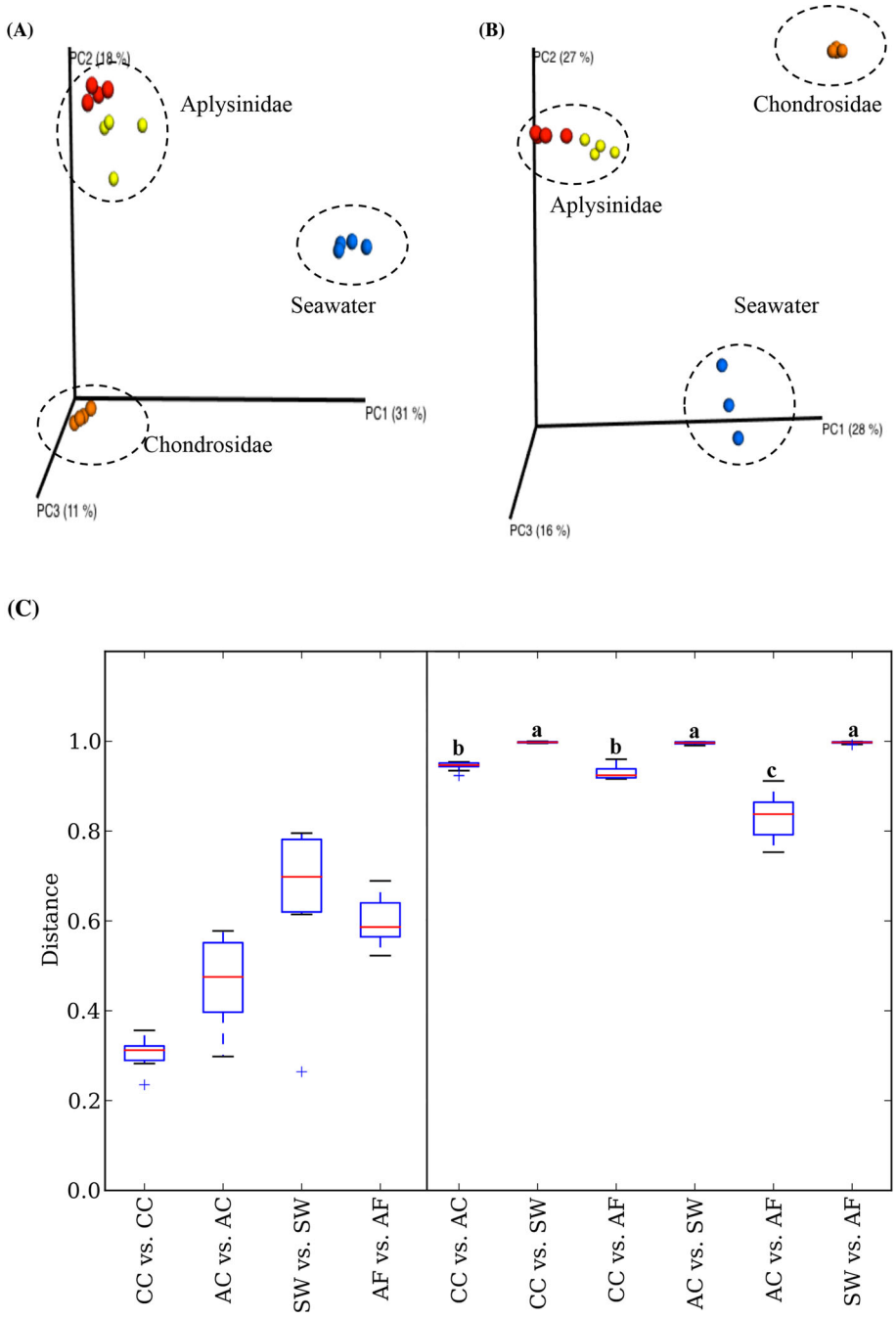

Figure 3. PCoA of sponges and seawater bacterial communities at 97% similarity, based on UCLUST clustering using (**A**) Bray–Curtis and (**B**) Unifrac distances. In both cases, there are three main groups: one composed of seawater samples (light blue dots) and two corresponding to the Aplysinidae family, composed of *A. crassa* and *Ap. fulva* (red and yellow dots, respectively), and the Chondrosidae family, composed of *C. collectrix* (orange dots). The box plot represents the average Bray–Curtis dissimilarity between the groups of samples (**C**).

More OTUs are shared among sponges from the same phylogenetic group

Among the 2112 OTUs present in this dataset, 1259 OTUs (59.6%) were strictly associated with sponges and 745 OTUs (35.3%) were associated with seawater. A total of 108 OTUs (5.1%) were shared between the sponges and seawater (Fig. 4a). Noticeably, *A. crassa* and *Ap. fulva* (Aplysinidae) shared more OTUs than did Aplysinidae and *C. collectrix*; there was also less sharing of OTUs between the sponges and the seawater.

A second Venn diagram was constructed to represent the OTUs that occurred in all four replicates of each sponge species. This new dataset contained 130 OTUs from the sponges and displayed high specificity for each sponge, with 54, 25 and 20 OTUs found in *C. collectrix*, *A. crassa* and *Ap. fulva*, respectively (Fig. 4b). Surprisingly, only five OTUs (8.7% of a total of 63 971 reads from the sponges) were shared among all the sponges. This core community contained generalist members of the phyla Gemmatimonadetes, Proteobacteria (Gammaproteobacteria), Acidobacteria, Actinobacteria (Acidimicrobiia) and Candidatus PAUC34f.

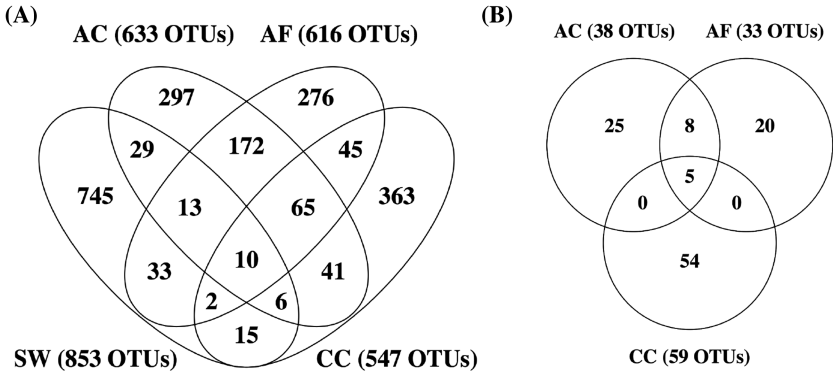

Figure 4. (**A**) Venn diagram showing the number of shared and exclusive OTUs from a total of 2112 OTUs for *A. crassa* (AC), *Ap. fulva* (AF), *C. collectrix* (CC) sponges and seawater (SW) samples. A total of 65 OTUs are shared among all three sponges, and only 10 OTUs can be found in all samples. AC and AF belong to the same family and share more OTUs (172) than the other two comparisons ((AC × CC = 41) and (AF × CC = 45)). (**B**) Venn diagram showing the number of stable and exclusive OTUs from a total of 130 OTUs. Only five OTUs are shared among all three species, and eight OTUs are shared exclusively between *A. crassa* and *Ap. fulva*, while none of these OTUs are shared with *C. collectrix*, their nearest relative.

Comparisons of these 130 OTUs in relation to other sequences deposited in GenBank revealed three patterns of affiliation, including 69 OTUs from different sponge species, 17 OTUs from sponge coral and 26 sequences related to diverse environmental samples (i.e. seawater and sediment) (Supplementary file, Table S2). Interestingly, one OTU related to Gammaproteobacteria that was retrieved from *Ap. fulva* was identical to sequences previously reported for specimens from the same species collected along the Brazilian coast (Table S2), while another OTU related to *Synechococcus* has been widely detected in tropical sponges from the Caribbean coast, including in *Aplysina* hosts.

DISCUSSION

Taxonomic richness and bacterial community diversity

The bacterial richness estimation found in our sponge samples varied from 181 to 322 OTUs, which is concordant with the ranges observed for other sponge species (Jackson *et al.* 2012; Schmitt *et al.* 2012; Cuvelier *et al.* 2014; Moitinho-Silva *et al.* 2014). A maximum of 364 different OTUs from a single sponge was detected in a study evaluating 32 sponge species collected at eight different locations worldwide (Schmitt *et al.* 2012). In the Mexican area of the Caribbean Sea, the sponge *Aiolochroia crassa* (279 OTUs) had a bacterial richness comparable to our samples of *A. crassa* (254–313 OTUs) (O'Connor-Sánchez *et al.* 2014). In contrast, lower bacterial richness (94–105 OTUs) was found in a three-year sampling study of the temperate sponge *Sarcotragus spinosulus*, with similar sequence coverage as that found in our study (Hardoim and Costa 2014).

In this study, more OTUs were identified in seawater than in sponges. This is concordant with previous studies, in which higher bacterial richness and dominance were observed in seawater (Cuvelier *et al.* 2014; Hardoim and Costa 2014; Moitinho-Silva *et al.* 2014; Ribes *et al.* 2015). Conversely, however, some studies have demonstrated higher bacterial richness and diversity in sponges (Webster *et al.* 2010; Lee *et al.* 2011; Hardoim *et al.* 2012; Thomas *et al.* 2016). In this sense, sponge tissues serve as a specific ecological niche that contains a higher abundance of bacterial groups that remain stable for long periods. Recently, a global survey of marine sponges revealed that these animals greatly contribute to the total microbial diversity of the world's oceans, with microbial richness estimates ranging from 50 to 3820 distinct OTUs per host (Thomas *et al.* 2016).

Overall, the three sponge species studied here contained similar bacterial diversity indices, concordant with reports from other host sponges (Cuvelier *et al.* 2014). Recently, Easson and Thacker (2014) found a strong correlation between bacterial diversity indices and Aplysinidae host phylogeny, suggesting that a significant phylogenetic signal exists across hosts.

OTU-level community composition of sponge-associated bacteria

In general, the phyla identified in this study were similar to those observed in previous studies of bacterial communities associated with sponges (Hardoim *et al.* 2009; Montalvo and Hill 2011; Schmitt *et al.* 2011, 2012; Simister *et al.* 2012a; Webster and Taylor 2012; Cleary *et al.* 2013, 2015; Bayer, Kamke and Hentschel 2014; Easson and Thacker 2014; Naim *et al.* 2014; O'Connor-Sánchez *et al.* 2014; de Voogd *et al.* 2015). Although the phyla Cyanobacteria and Bacteroidetes were ranked as minor groups using our sequencing approach, a high abundance of OTUs related to these phyla was previously detected in *Aplysina fulva* using a clone library of 16S rRNA gene sequences (Hardoim *et al.* 2009). Although the candidate phylum Poribacteria was detected in all sponge species examined in this study, it was not previously detected in microbiomes associated with *Ap. fulva* and *A. crassa* using the SILVA and RDP databases (Easson and Thacker 2014; O'Connor-Sánchez *et al.* 2014). However, the lack of Poribacteria in the above-referenced studies may be attributed to the use of different primers and bioinformatics pipelines. The candidate phylum Poribacteria was originally discovered in marine sponges (Fieseler *et al.* 2004) and has since been detected among many different sponge species, including members of the Aplysinidae family (Lafi *et al.* 2009). Poribacteria also has been widely found at low abundance in non-sponge habitats (Taylor *et al.* 2013). However, a recent study suggested that Poribacteria are not active when not associated with a host sponge (Moitinho-Silva *et al.* 2014). Additionally, single-cell genomic approaches have revealed a wide range of metabolic strategies and adaptations for symbiosis in Poribacteria associated with *Ap. aerophoba* (Siegl *et al.* 2011; Kamke *et al.* 2013, 2014). These same studies have proposed that Poribacteria are well adapted to the sponge environment.

In this study, the dominant OTUs were classified as Acidimicrobiia (Actinobacteria), Acidobacteria group 6, Anaerolineae (Chloroflexi) and Chromatiales (Gammaproteobacteria). Class

Acidimicrobiia was preferentially enriched in *A. crassa* and contributed the most dissimilarity between the sponges and the seawater. Hardoim and Costa (2014) also identified Acidimicrobiia as a stable and abundant group in *Sarcotragus spinosulus*. The marine Acidimicrobiia clade comprises photoheterotrophic bacteria with planktonic lifestyles that are well adapted to oligotrophic marine waters (Mizuno, Rodriguez-Valera and Ghai 2015). Acidobacteria and Chloroflexi are the most common bacterial phyla in HMA sponges belonging to many different phylogenetic lineages (Schmitt *et al.* 2011; Giles *et al.* 2013; Bayer, Kamke and Hentschel 2014; O'Connor-Sánchez *et al.* 2014). Purple sulfur bacteria within the order Chromatiales encompass microaerophilic or anaerobic species capable of performing anoxygenic photosynthesis (Imhoff 2005).

The dominance of these groups in sponges may indicate that they play specific roles in host biology. Although these groups have been detected in HMA sponges from diverse habitats, including tropical and temperate environments (Schmitt *et al.* 2011; Cleary *et al.* 2013; Cárdenas *et al.* 2014; Kennedy *et al.* 2014), very few isolated representatives have been recovered (Brück *et al.* 2010).

Association of bacterial community with host phylogeny and surrounding seawater

Molecular identification of the sponge species studied here permitted robust interpretation of the results concerning host taxonomy and the associated bacterial communities. Several studies have demonstrated that different sponges from the same habitat harbour distinct bacterial communities (Hardoim *et al.* 2012; Cleary *et al.* 2013; Naim *et al.* 2014). Consistent with these findings, the bacterial community compositions in the three sponge species evaluated here differed from one another and from that in the seawater surrounding the specimens. However, such dissimilarity often decreases when members of the same taxonomic and phylogenetic groups are considered (Easson and Thacker 2014) and when considering eukaryotic hosts that support complex bacterial consortia (animals and plants) (Ochman *et al.* 2010; Bouffaud *et al.* 2012, 2014; Moeller *et al.* 2013).

Our results demonstrated that two sponge species belonging to Aplysinidae presented less dissimilarity in their bacterial communities when compared to those in *Chondrosia collectrix*, the nearest relative (Fig. 3). Schöttner *et al.* (2013) also observed a positive relationship between bacterial community dissimilarity and phylogenetic distance in Geodiidae sponges (Porifera: Heteroscleromorpha) using ARISA profiles. These authors suggested that closely related hosts possess greater similarity in their bacterial communities than more distantly related hosts. Likewise, Montalvo and Hill (2011) detected similar bacterial communities for two phylogenetically close species of *Xestospongia* (*X. muta* and *X. testudinaria*), which inhabit the Atlantic and Pacific Oceans, respectively. The referenced study concluded that bacterial speciation occurred within sponge hosts that reflected vertical transmission through evolutionary time. Erwin *et al.* (2012) also detected a phylogenetic signal in two sympatric and phylogenetically related species of the genus *Ircinia*.

Despite Reveillaud *et al.* (2014) having shown that similar symbiont communities exist within the same host species across broad geographic and bathymetric scales, closely related species of *Hexadella* (Verongida; Ianthellidae), specifically *Hexadella pruvoti* and *H.* cf. *dedritifera*, did not maintain a high degree of similarity in their bacterial communities when compared to *H. dedritifera*, the phylogenetically closest species. This finding was consistent with a global study that surveyed sponge symbionts across 32 different host species, including *Aplysina* hosts. In the referenced study, no clear relationship was observed between host phylogeny and microbial community similarity; however, a tropical biogeographical clade was detected, indicating that subpopulations of bacterial lineages that are defined by temperature or water salinity likely reside in sponges (Schmitt *et al.* 2012). Recently, a growing body of evidence has demonstrated that biogeographical factors greatly affect many aspects of sponge biology (Schmitt *et al.* 2012; Burgsdorf *et al.* 2014; Luter *et al.* 2015; Weigel and Erwin 2015; Morrow, Fiore and Lesser 2016), although it is also important to note that shifts in microbial communities might vary from species to species, as has been reported in other studies (Hentschel *et al.* 2002; Taylor *et al.* 2005; Montalvo and Hill 2011; Pita *et al.* 2013a, b). This suggests that evolutionary processes rather than biogeographic factors have resulted in the spatial stability of sponge-associated bacterial communities. In the present study, the examined sponge specimens coexisted in close spatial proximity in a region quite isolated from the mainland; therefore, we can assume that the sponges grew under similar environmental conditions. As such, there were likely no great differences in environmental conditions that could explain the dissimilarities in the bacterial assemblages. Moreover, continued research on this topic has provided support for the hypothesis that a host sponge's evolutionary history plays a significant role in shaping the diversity of its symbiont community, despite strong selective forces for divergent microbiome composition (Easson and Thacker 2014; Thomas *et al.* 2016).

Shared and specific bacterial OTUs

In the current work, the three sponge species studied harboured 99 specific and stable OTUs (54, 25 and 20 in *C. collectrix*, *A. crassa* and *Ap. fulva*, respectively). Comparisons of these OTUs in relation to other sequences deposited in GenBank revealed some overlap with microbial sequences obtained from other sponges, corals, sediments and seawater. Erwin, Olson and Thacker (2011) identified the same patterns in an extensive study of the phylogenetic diversity and host specificity of bacteria inhabiting tropical sponges. Interestingly, one OTU related to Gammaproteobacteria that was retrieved from *Ap. fulva* in this study was previously found in the same sponge species in a sample collected along the Brazilian coast (Hardoim *et al.* 2009). In addition, an OTU retrieved from *Ap. fulva* that was closely related to *Synechococcus spongiarum* was compared using BLAST analysis to a sequence in GenBank, and the results revealed that this OTU has been widely detected in tropical sponges from the Caribbean coast, including in *Aplysina* hosts (Steindler *et al.* 2005; Erwin and Thacker 2008; Pita *et al.* 2013a; Olson, Thacker and Gochfeld 2014).

The above results suggest that a stable association exists among the bacterial communities that are found within *Aplysina* spp., which are often related to the health status of the host (Erwin and Thacker 2008; Olson, Thacker and Gochfeld 2014). In addition *S. spongiarum* has been detected in at least 40 sponge species and represents the largest sponge-specific cluster identified to date (Simister *et al.* 2012a; Gao *et al.* 2014). Vertical transmission of *S. spongiarum* from parents to offspring has been reported for other sponges (Oren, Steindler and Ilan 2005; Usher *et al.* 2005). The genome sequence of this cyanobacterium suggests that its symbiotic interaction with sponge hosts has caused the loss of genes involved in several nonessential functions, culminating in a reduction of the bacterium's genome

(Burgsdorf *et al.* 2015). This finding supports the hypothesis that sponges and their associated bacteria are co-evolving.

CONCLUSION

The present study is the first to investigate the dissimilarities that are found in bacterial communities inhabiting closely related sponge species that live in close spatial proximity in the SPSPA in Brazil. We further demonstrated that the profiles of sponge-associated bacterial communities differ not only between sponge species but also relative to the surrounding seawater. However, a reduced dissimilarity was observed in bacterial communities within *Aplysinidae* species when compared to *Chondrosidae*, the nearest relative to these species. Furthermore, by comparing bacterial communities in sympatric sponges from closely related hosts, we speculate that factors specific to each sponge species might act as selective forces that shape the phylogenetic profiles of bacterial assemblages. It is necessary to highlight that the minor dissimilarity found among closely related sponge species does not necessarily imply that symbionts co-evolve with their hosts. More research is needed to fully explore the degree to which closely related sponge hosts share symbiont communities.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

ACKNOWLEDGEMENTS

The authors are grateful the Fernando Rodrigues da Silva for assistance with sponge sampling. They would like to thank Dr Fernando Dini Andreote and Dr Tatiana Alves Rigamonte for critical reading in the previous version of this manuscript. Finally, the authors also thank the PROARQUIPELAGO Research Program and Brazilian Navy for technical support during the expedition to St. Peter and St. Paul Archipelago.

FUNDING

This work was supported by the National Council for Scientific and Technological Development [CNPq 405413/2012-0]. DTS received a FAPESP graduate scholarship [2013/25505-8]. FCM received a CAPES Postdoctoral scholarship [88887.091725/2014-01]. DBG and VNK were supported by a post- doctoral fellowships funded by FAPESP [2014/26131-7; 2013/08144-1]. RGT received a FAPESP Young scientist fellowship [2013/03158-4].

Conflict of interest. None declared.

REFERENCES

Altschul SF, Gish W, Miller W *et al.* Basic local alignment search tool. *J Mol Biol* 1990;**215**:403–10.

Bayer K, Kamke J, Hentschel U. Quantification of bacterial and archaeal symbionts in high and low microbial abundance sponges using real-time PCR. *FEMS Microbiol Ecol* 2014;**89**:679–90.

Blanquer A, Uriz MJ, Cebrian E *et al.* Snapshot of a bacterial microbiome shift during the early symptoms of a massive sponge die-off in the western Mediterranean. *Front Microbiol* 2016;**7**:752.

Bouffaud M, Kyselková M, Gouesnard B *et al.* Is diversification history of maize influencing selection of soil bacteria by roots? *Mol Ecol* 2012;**21**:195–206.

Bouffaud ML, Poirier MA, Muller D *et al.* Root microbiome relates to plant host evolution in maize and other Poaceae. *Environ Microbiol* 2014;**16**:2804–14.

Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 1957;**27**:325–49.

Brück WM, Brück TB, Self WT *et al.* Comparison of the anaerobic microbiota of deep-water Geodia spp. and sandy sediments in the Straits of Florida. *ISME J* 2010;**4**:686–99.

Burgsdorf I, Erwin PM, López-Legentil S *et al.* Biogeography rather than association with cyanobacteria structures symbiotic microbial communities in the marine sponge Petrosia ficiformis. *Front Microbiol* 2014;**5**:1–11.

Burgsdorf I, Slaby BM, Handley KM *et al.* Lifestyle evolution in cyanobacterial symbionts of sponges. *MBio* 2015;**6**:e00391–15.

Cao H, Cao X, Guan X *et al.* High temporal variability in bacterial community, silicatein and hsp70 expression during the annual life cycle of Hymeniacidon sinapium (Demospongiae) in China's Yellow Sea. *Aquaculture* 2012;**358-359**:262–73.

Caporaso JG, Bittinger K, Bushman FD *et al.* PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 2010a;**26**:266–7.

Caporaso JG, Kuczynski J, Stombaugh J *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010b;**7**:335–6.

Cárdenas CA, Bell JJ, Davy SK *et al.* Influence of environmental variation on symbiotic bacterial communities of two temperate sponges. *FEMS Microbiol Ecol* 2014;**88**:516–27.

Choudhury JD, Pramanik A, Webster NS *et al.* The pathogen of the Great Barrier Reef sponge Rhopaloeides odorabileis a new strain of Pseudoalteromonas agarivorans containing abundant and diverse virulence-related genes. *Mar Biotechnol* 2015;**17**:463–78.

Cleary DF, Becking LE, de Voogd NJ *et al.* Habitat- and host-related variation in sponge bacterial symbiont communities in Indonesian waters. *FEMS Microbiol Ecol* 2013;**85**:465–82.

Cleary DF, de Voogd NJ, Polónia AR *et al.* Composition and predictive functional analysis of bacterial communities in seawater, sediment and sponges in the Spermonde Archipelago, Indonesia. *Microb Ecol* 2015;**70**:889–903.

Cuvelier ML, Blake E, Mulheron R *et al.* Two distinct microbial communities revealed in the sponge Cinachyrella. *Front Microbiol* 2014;**5**:581.

de Voogd NJ, Cleary DFR, Polónia ARM *et al.* Bacterial community composition and predicted functional ecology of sponges, sediment and seawater from the thousand islands reef complex, west Java, Indonesia. *FEMS Microbiol Ecol* 2015;**91**:fiv019.

Easson CG, Thacker RW. Phylogenetic signal in the community structure of host-specific microbiomes of tropical marine sponges. *Front Microbiol* 2014;**5**:532.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010;**26**:2460–1.

Edgar RC, Haas BJ, Clemente JC *et al.* UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011;**27**:194–200.

Edwards A, Lubbock R. Marine zoogeography of St. Paul´s Rocks. *J Biogeogr* 1983;**10**:65–72.

Erpenbeck D, Breeuwer J, van der Velde H *et al.* Unravelling host and symbiont phylogenies of halichondrid sponges (Demospongiae, Porifera) using a mitochondrial marker. *Mar Biol* 2002;**141**:377–86.

Erpenbeck D, Sutcliffe P, Cook S *et al.* Horny sponges and their affairs: on the phylogenetic relationships of keratose sponges. *Mol Phylogenet Evol* 2012;**63**:809–816.

Erwin PM, Olson JB, Thacker RW. Phylogenetic diversity, host-specificity and community profiling of sponge-associated bacteria in the northern Gulf of Mexico. *PLoS One* 2011;**6**:e26806.

Erwin PM, Pita L, López-Legentil S *et al*. Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Appl Environ Microb* 2012;**78**:7358–68.

Erwin P, Thacker R. Phototrophic nutrition and symbiont diversity of two Caribbean sponge-cyanobacteria symbioses. *Mar Ecol Prog Ser* 2008;**362**:139–47.

Fan L, Liu M, Simister R *et al*. Marine microbial symbiosis heats up: the phylogenetic and functional response of a sponge holobiont to thermal stress. *ISME J* 2013;**7**:991–1002.

Fan L, Reynolds D, Liu M *et al*. Functional equivalence and evolutionary convergence in complex communities of microbial sponge symbionts. *P Natl Acad Sci USA* 2012;**109**:E1878–87.

Fieseler L, Horn M, Wagner M *et al*. Discovery of the novel candidate phylum 'Poribacteria' in marine sponges. *Appl Environ Microb* 2004;**70**:3724–32.

Friedrich AB, Fischer I, Proksch P *et al*. Temporal variations of the microbial community associated with the Mediterranean sponge Aplysina aerophoba. *FEMS Microbiol Ecol* 2001;**38**:105–13.

Gao Z, Wang Y, Tian R *et al*. Pyrosequencing revealed shifts of prokaryotic communities between healthy and disease-like tissues of the Red Sea sponge *Crella cyathophora*. *PeerJ* 2015;**3**:e890.

Gao Z, Wang Y, Tian R *et al*. Symbiotic adaptation drives genome streamlining of the cyanobacterial sponge symbiont 'Candidatus Synechococcus spongiarum'. *MBio* 2014;**5**:e00079–14.

Giles EC, Kamke J, Moitinho-Silva L *et al*. Bacterial community profiles in low microbial abundance sponges. *FEMS Microbiol Ecol* 2013;**83**:232–41.

Gloeckner V, Wehrl M, Moitinho-Silva L *et al*. The HMA-LMA dichotomy revisited: an electron microscope survey of 56 sponge species. *Biol Bull* 2014;**227**:78–88.

Hardoim CC, Costa R. Temporal dynamics of prokaryotic communities in the marine sponge Sarcotragus spinosulus. *Mol Ecol* 2014;**23**:3097–112.

Hardoim CC, Costa R, Araujo FV *et al*. Diversity of bacteria in the marine sponge Aplysina fulva in Brazilian coastal waters. *Appl Environ Microb* 2009;**75**:3331–43.

Hardoim CC, Esteves AI, Pires FR *et al*. Phylogenetically and spatially close marine sponges harbour divergent bacterial communities. *PLoS One* 2012;**7**:e53029.

Hentschel U, Hopke J, Horn M *et al*. Molecular evidence for a uniform microbial community in sponges from different oceans. *Appl Environ Microb* 2002;**68**:4431–40.

Hentschel U, Piel J, Degnan SM *et al*. Genomic insights into the marine sponge microbiome. *Nat Rev Microbiol* 2012;**10**:641–54.

Hoffmann F, Radax R, Woebken D *et al*. Complex nitrogen cycling in the sponge Geodia barretti. *Environ Microbiol* 2009;**11**:2228–43.

Imhoff JF. Order I. Chromatiales *ord. nov*. In: Brenner DJ, Krieg NR, Staley NR *et al*. (eds). *Bergeýs Manual of Systematic Bacteriology: The Proteobacteria), Part B (The Gammaproteobacteria*, 2nd edn, vol. **2**. New York: Springer, 2005, 1–3.

Jackson SA, Kennedy J, Morrissey JP *et al*. Pyrosequencing reveals diverse and distinct sponge-specific microbial communities in sponges from a single geographical location in Irish waters. *Microb Ecol* 2012;**64**:105–16.

Kamke J, Rinke C, Schwientek P *et al*. The candidate phylum Poribacteria by single-cell genomics: new insights into phylogeny, cell-compartmentation, eukaryote-like repeat proteins, and other genomic features. *PLoS One* 2014;**9**:e87353.

Kamke J, Sczyrba A, Ivanova N *et al*. Single-cell genomics reveals complex carbohydrate degradation patterns in poribacterial symbionts of marine sponges. *ISME J* 2013;**7**:2287–300.

Kavamura VN, Taketani RG, Lançoni MD *et al*. Water regime influences bulk soil and Rhizosphere of Cereus jamacaru bacterial communities in the Brazilian Caatinga biome. *PLoS One* 2013;**8**:e73606.

Kennedy J, Flemer B, Jackson SA *et al*. Evidence of a putative deep sea specific microbiome in marine sponges. *PLoS One* 2014;**9**:e91092.

Lafi FF, Fuerst JA, Fieseler L *et al*. Widespread distribution of Poribacteria in Demospongiae. *Appl Environ Microb* 2009;**75**:5695–9.

Lee OO, Wang Y, Lafi FF *et al*. Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. *ISME J* 2011;**5**:650–64.

Lozupone C, Lladser ME, Knights D *et al*. UniFrac: an effective distance metric for microbial community comparison. *ISME J* 2011;**5**:169–72.

Luter HM, Gibb K, Webster NS. Eutrophication has no short-term effect on the Cymbastela stipitata holobiont. *Front Microbiol* 2014;**5**:216.

Luter HM, Widder S, Botté ES *et al*. Biogeographic variation in the microbiome of the ecologically important sponge, Carteriospongia foliascens. *PeerJ* 2015;**3**:e1435.

McDonald D, Price MN, Goodrich J *et al*. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 2012;**6**:610–8.

McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One* 2013;**8**:e61217.

Mizuno CM, Rodriguez-Valera F, Ghai R. Genomes of planktonic Acidimicrobiales: widening horizons for Marine Actinobacteria by Metagenomics. *MBio* 2015;**6**:e02083–14.

Moeller AH, Peeters M, Ndjango JB *et al*. Sympatric chimpanzees and gorillas harbor convergent gut microbial communities. *Genome Res* 2013;**23**:1715–20.

Moitinho-Silva L, Bayer K, Cannistraci CV *et al*. Specificity and transcriptional activity of microbiota associated with low and high microbial abundance sponges from the Red Sea. *Mol Ecol* 2014;**23**:1348–63.

Montalvo NF, Hill RT. Sponge-associated bacteria are strictly maintained in two closely related but geographically distant sponge hosts. *Appl Environ Microb* 2011;**77**:7207–16.

Moraes F. *Esponjas das Ilhas Oceânicas Brasileiras*. Rio de Janeiro: Museu Nacional, 2011.

Morrow KM, Bourne DG, Humphrey C *et al*. Natural volcanic CO_2 seeps reveal future trajectories for host–microbial associations in corals and sponges. *ISME J* 2015;**9**:894–908.

Morrow KM, Fiore CL, Lesser MP. Environmental drivers of microbial community shifts in the giant barrel sponge, Xestospongia muta, over a shallow to mesophotic depth gradient. *Environ Microbiol* 2016;**18**:2025–38.

Naim MA, Morillo JA, Sørensen SJ *et al*. Host-specific microbial communities in three sympatric North Sea sponges. *FEMS Microbiol Ecol* 2014;**90**:390–403.

O'Connor-Sánchez A, Rivera-Domínguez AJ, De los Santos-Briones C *et al*. Acidobacteria appear to dominate the microbiome of two sympatric Caribbean sponges and one Zoanthid. *Biol Res* 2014;**47**:1–6.

Ochman H, Worobey M, Kuo C.-H *et al*. Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLoS Biol* 2010;**8**:e1000546.

Olson JB, Thacker RW, Gochfeld DJ. Molecular community profiling reveals impacts of time, space, and disease status on the bacterial community associated with the Caribbean sponge Aplysina cauliformis. *FEMS Microbiol Ecol* 2014;**87**:268–79.

Oren M, Steindler L, Ilan M. Transmission, plasticity and the molecular identification of cyanobacterial symbionts in the Red Sea sponge Diacarnus erythraenus. *Mar Biol* 2005;**148**:35–41.

Pita L, Erwin PM, Turon X *et al*. Till death do us part: stable sponge-bacteria associations under thermal and food shortage stresses. *PLoS One* 2013a;**8**:e80307.

Pita L, Turon X, López-Legentil S *et al*. Host rules: spatial stability of bacterial communities associated with marine sponges (Ircinia spp.) in the Western Mediterranean Sea. *FEMS Microbiol Ecol* 2013b;**86**:268–76.

Reveillaud J, Maignien L, Eren MA *et al*. Host-specificity among abundant and rare taxa in the sponge microbiome. *ISME J* 2014;**8**:1198–209.

Ribes M, Dziallas C, Coma R *et al*. Microbial diversity and putative diazotrophy in high- and low-microbial-abundance Mediterranean sponges. *Appl Environ Microb* 2015;**81**:5683–93.

Ribes M, Jiménez E, Yahel G *et al*. Functional convergence of microbes associated with temperate marine sponges. *Environ Microbiol* 2012;**14**:1224–39.

Schmitt S, Deines P, Behnam F *et al*. Chloroflexi bacteria are more diverse, abundant, and similar in high than in low microbial abundance sponges. *FEMS Microbiol Ecol* 2011;**78**:497–510.

Schmitt S, Tsai P, Bell J *et al*. Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *ISME J* 2012;**6**:564–76.

Schöttner S, Hoffmann F, Cardenas P *et al*. Relationships between host phylogeny, host type and bacterial community diversity in cold-water coral reef sponges. *PLoS One* 2013;**8**:e55505.

Siegl A, Kamke J, Hochmuth T *et al*. Single-cell genomics reveals the lifestyle of Poribacteria, a candidate phylum symbiotically associated with marine sponges. *ISME J* 2011;**5**:61–70.

Simister RL, Deines P, Botté ES *et al*. Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. *Environ Microbiol* 2012a;**14**:517–24.

Simister R, Taylor MW, Rogers KM *et al*. Temporal molecular and isotopic analysis of active bacterial communities in two New Zealand sponges. *FEMS Microbiol Ecol* 2013;**85**:195–205.

Simister R, Taylor MW, Tsai P *et al*. Thermal stress responses in the bacterial biosphere of the Great Barrier Reef sponge, Rhopaloeides odorabile. *Environ Microbiol* 2012b;**14**:3232–46.

Sogin ML, Morrison HG, Huber JA *et al*. Microbial diversity in the deep sea and the underexplored 'rare biosphere'. *P Natl Acad Sci USA* 2006;**103**:12115–20.

Steindler L, Huchon D, Avni A *et al*. 16S rRNA phylogeny of sponge-associated cyanobacteria. *Appl Environ Microb* 2005;**71**:4127–31.

Taylor MW, Schupp PJ, Dahllof I *et al*. Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. *Environ Microbiol* 2004;**6**:121–30.

Taylor MW, Schupp PJ, de Nys R *et al*. Biogeography of bacteria associated with the marine sponge Cymbastela concentrica. *Environ Microbiol* 2005;**7**:419–33.

Taylor MW, Tsai P, Simister RL *et al*. 'Sponge-specific' bacteria are widespread (but rare) in diverse marine environments. *ISME J* 2013;**7**:438–43.

Thiel V, Leininger S, Schmaljohann R *et al*. Sponge-specific bacterial associations of the Mediterranean sponge Chondrilla nucula (Demospongiae, Tetractinomorpha). *Microb Ecol* 2007;**54**:101–11.

Thomas T, Moitinho-Silva L, Lurgi M *et al*. Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun* 2016;**7**:11870.

Turque AS, Batista D, Silveira C *et al*. Environmental shaping of sponge associated archeal communities. *PLoS One* 2010;**5**:e15774.

Usher KM, Sutton DC, Toze S *et al*. Inter-generational transmission of microbial symbionts in the marine sponge Chondrilla australiensis (Demospongiae). *Mar Freshwater Res* 2005;**56**:125–31.

Wang Y, Qian PY. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One* 2009;**4**:e7401.

Webster N, Luter HM, Soo RM *et al*. Same, same but different: symbiotic bacterial associations in GBR sponges. *Front Microbiol* 2013a;**3**:1–11.

Webster N, Pantile R, Botté E *et al*. A complex life cycle in a warming planet: gene expression in thermally stressed sponges. *Mol Ecol* 2013b;**22**:1854–68.

Webster NS, Taylor MW. Marine sponges and their microbial symbionts: love and other relationships. *Environ Microbiol* 2012;**14**:335–46.

Webster NS, Taylor MW, Behnam F *et al*. Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environ Microbiol* 2010;**12**:2070–82.

Webster NS, Thomas T. The sponge Hologenome. *MBio* 2016;**7**:e00135–16.

Webster NS, Wilson KJ, Blackall LL *et al*. Phylogenetic diversity of bacteria associated with the marine sponge Rhopaloeides odorabile. *Appl Environ Microb* 2001;**67**:434–44.

Weigel BL, Erwin PM. Intraspecific variation in microbial symbiont communities of the sun sponge, Hymeniacidon heliophila, from intertidal and subtidal habitats. *Appl Environ Microb* 2015;**82**:650–8.

Wichels A, Würtz S, Döpke H *et al*. Bacterial diversity in the breadcrumb sponge Halichondria panicea (Pallas). *FEMS Microbiol Ecol* 2006;**56**:102–18.

White JR, Patel J, Ottesen A *et al*. Pyrosequencing of bacterial symbionts within Axinella corrugata sponges: diversity and seasonal variability. *PLoS One* 2012;**7**:e1936.