Depth effect on the prokaryotic community assemblage associated with sponges from different rocky reefs

Bárbara González-Acosta [Equal first author, 1], **Aarón Barraza** [Equal first author, 2], **César Guadarrama-Analco** [1], **Claudia J Hernández-Guerrero** [1], **Sergio Francisco Martínez-Díaz** [1], **César S Cardona-Felix** [3], **Ruth Noemí Aguila-Ramírez** [Corresp. 1]

[1] Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas, La Paz, Baja California Sur, México

[2] CONACYT-Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California Sur, México

[3] CONACYT-Instituto Politécnico Nacional, La Paz, Baja California Sur, México

Corresponding Author: Ruth Noemí Aguila-Ramírez
Email address: raguilar@ipn.mx

Background. Sponge microbiomes are essential for the function and survival of their host, they produce biologically active metabolites, therefore, they are ideal candidates for ecological, pharmacological, and clinical research. Implementing next-generation sequencing (NGS) has revealed that many factors, including the environment and host properties, determine the composition and structure of symbiotic communities across time and space. The controls of this variation are not well described. This study assessed the microbial communities associated with two marine sponges of the genera *Aplysina* (Nardo, 1834) and *Ircinia* (Nardo, 1833) in rocky reefs from Punta Arena de la Ventana (Gulf of California) and Pichilingue (La Paz Bay) in the coast of Baja California Sur, Mexico to determine the relative importance of environment and host in structuring the microbiome of sponges. **Methods.** Specimens of *Aplysina* sp were collected by scuba diving at two different depths, 10 m and 2 m, while *Ircinia* sp samples were collected at 2 m. The DNA of sponge-associated prokaryotes was extracted from 1 cm^3 of tissue, purified and sent for 16S amplicon sequencing. Primer trimmed pair-ended microbial 16S rDNA gene sequences were merged using Ribosomal Database Project (RDP) Paired-end Reads Assembler. Chao1, Shannon and Simpson (alpha) biodiversity indices were estimated, as well permutational analysis of variance (PERMANOVA), and Bray-Curtis distances. **Results.** The most abundant phyla differed between hosts. Those phyla were: Proteobacteria, Acidobacteria, Cyanobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, and Planctomycetes. In *Ircinia* sp the dominant phylum was Acidobacteria. We found that depth was the main factor influencing the microbial community. Analysis of similarities (ANOSIM) showed a significant difference between the microbial communities from different depths. Cluster analysis suggested that depth was more important than host in structuring the sponge microbiome.

Depth effect on the prokaryotic community assemblage associated with sponges from different rocky reefs

Bárbara González-Acosta[1Ç], Aarón Barraza[2Ç], César Guadarrama-Analco[1], Claudia Judith Hernández-Guerrero[1], Sergio F. Martínez-Díaz[1], César Salvador Cardona-Félix[3], Ruth Noemí Aguila-Ramírez[1]*

[1] Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas. La Paz, Baja California Sur 23060, México.
[2] CONACYT-Centro de Investigaciones Biológicas del Noroeste. La Paz, Baja California Sur, México.
[3] CONACYT-Instituto Politécnico Nacional-Centro Interdisciplinario de Ciencias Marinas. La Paz, Baja California Sur, México.

[Ç.] These authors contributed equally to this work.

*Corresponding Author:
Ruth Noemí Aguila-Ramírez[1]
Av. Instituto Politécnico Nacional S/N. Playa Palo de Santa Rita, La Paz, Baja California Sur, 23069, México
Email address: raguilar@ipn.mx

Abstract

Background. Sponge microbiomes are essential for the function and survival of their host, they produce biologically active metabolites, therefore, they are ideal candidates for ecological, pharmacological, and clinical research. Implementing next-generation sequencing (NGS**)** has revealed that many factors, including the environment and host properties, determine the composition and structure of symbiotic communities across time and space. The controls of this variation are not well described. This study assessed the microbial communities associated with two marine sponges of the genera *Aplysina* (Nardo, 1834) and *Ircinia* (Nardo, 1833) in rocky reefs from Punta Arena de la Ventana (Gulf of California) and Pichilingue (La Paz Bay) in the coast of Baja California Sur, Mexico to determine the relative importance of environment and host in structuring the microbiome of sponges.

Methods. Specimens of *Aplysina* sp were collected by scuba diving at two different depths, 10 m and 2 m, while *Ircinia* sp samples were collected at 2 m. The DNA of sponge-associated prokaryotes was extracted from 1 cm^3 of tissue, purified and sent for 16S amplicon sequencing. Primer trimmed pair-ended microbial 16S rDNA gene sequences were merged using Ribosomal Database Project (RDP) Paired-end Reads Assembler. Chao1, Shannon and Simpson (alpha) biodiversity indices were estimated**,** as well permutational analysis of variance (PERMANOVA), and Bray-Curtis distance**s**.

Results. The most abundant phyla differed between hosts. Those phyla were: Proteobacteria, Acidobacteria, Cyanobacteria, Chloroflexi, Actinobacteria, Bacteroidetes, and Planctomycetes. In *Ircinia* sp the dominant phylum was Acidobacteria. We found that depth was the main factor influencing the microbial community. Analysis of similarities (ANOSIM) showed a significant difference between the microbial communities from different depths. Cluster analysis suggested that depth was more important than host in structuring the sponge microbiome.

Introduction

Marine sponges (MS) inhabit shallow to mesophotic ecosystems and harbor on diverse symbionts (Taylor et al., 2007; Simister et al., 2012) that reach up to 50% of their total weight (Hentschel et al., 2003; Usher et al., 2004). The frequent and abundant presence of bacteria especially within the sponge mesohyl led authors to address these bacteria as symbionts (Vacelet, 1975; De Vos et al., 1995; Burja et al., 1999; Imhoff & Stöhr, 2003). Sponge microbiomes are

essential for their host's function (metabolic), health and survival (Lurgi et al., 2019). Furthermore, there is evidence of the production of biologically active metabolites by sponges-associated bacteria, being an important function in this association (Imhoff & Stöhr, 2003). Therefore, they are ideal candidates for ecological, pharmacological and clinical research. Sponge tissues host many symbionts, including heterotrophic bacteria, facultative anaerobes, dinoflagellates, cyanobacteria, archaea, fungi, and even viruses (Webster & Hill, 2001; Schippers et al., 2012). Support between 15 to several tenths phyla but the source of this variation in diversity is not well described (Taylor et al., 2007; Webster & Thomas, 2016; Villegas-Plazas et al., 2019).

Implementing next-generation sequencing (NGS) approaches to characterize marine sponge microbial communities has notoriously increased precision and quantity of the taxonomic complexity associated to these marine organisms (Schmitt et al., 2011; Webster & Taylor, 2012; Reveillaud et al., 2014). Moreover, the complex microbial communities of MS have been unveiled through NGS coupled with microbial diversity analyses to highlight that sponge microbiomes are largely host-specific and often stable across temporal scales under specific environmental conditions (Morrow et al., 2015; Weigel & Erwin, 2015; Morrow, Fiore & Lesser, 2016; Cleary et al., 2019). Recent research suggests that depth are the main drivers of the structure of ocean microbiome (Sunagawa et al., 2015). However, for symbioses, one would expect a strong microbial community differentiation to emerge across host species (Lurgi et al., 2019). Sponges can inhabit from shallow to mesophotic ecosystems, in deep water they are apparently less influenced by abiotic factors (Kahng, Copus & Wagner, 2014; Olson & Kellogg, 2010). Otherwise, in shallow ecosystems these abiotic factors could influence the sponges, thus, also on their associated microbial communities. Some studies have determined sponge associated microbial community changes at different water depths from shallow (0-30 m) to mesophotic areas (30-150 m) (Olson & Kellogg, 2010; Lesser, Slattery & Leichter, 2009; Kahng, Copus & Wagner, 2014). Though the specificity of the sponge microbiota appears more related with host phylogeny, differences in depth can be showing variance between microbial communities in shallow and deep reefs (Steinert et al., 2016)

However, to our knowledge no studies are available that evaluate whether among the same shallow water sponges (0-30 m) the community varies according to its range of distribution.

Although changes in abiotic factors are not as evident, as it could occur in mesophotic zones (30-150 m), the depth gradient could influence the composition of the microbial community associated with these sponges (Olson & Gao, 2013; Steiner et al., 2016).

Aplysina species are often associated with shallow rocky reefs. This species belongs to the Verongiida order and are distributed along the East Pacific from Mexico to Panama (Caballero-George et al., 2010; Cruz-Barraza et al., 2012). *Ircinia*, they are conspicuous and abundant in areas exposed to light in rocky-coral biotopes (Parra-Velandia & Zea, 2003) and more abundant in localities near sources of continental discharge with greater turbidity and load of organic material in suspension (Zea, 1994). In previous studies with sponges of these genera (*Aplysina* and *Ircinia*) from the Gulf of California, differences were observed in the biological activity of sponges and their associated bacteria, between sponges of the same genus and between genera (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012), it was considered that these differences were probably related to the site and the depth at which they were collected. For this reason, this study assessed the microbial communities associated with two marine sponges of the genera *Aplysina* (Nardo, 1834) and *Ircinia* (Nardo, 1833) in rocky reefs from Punta Arena de la Ventana (Gulf of California) and Pichilingue (La Paz Bay) in the coast of Baja California Sur, Mexico to determine the relative importance of environment and host in structuring the microbiome of sponges.

Materials & Methods

Specimens of *Aplysina* and *Ircinia* sponges previously collected to evaluate their biological activity (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012) were used for this study.

Aplysina sp specimens (n = 8) were collected in triplicate by scuba diving in Punta Arena, Baja California Sur, Mexico (24 ° 03 '40 "N and 109 ° 49 '52" W) at different water depths (2 m - 10 m). For this study, the depth of 2 m was considered shallow and 10 m as deep (Apl-S: 2m; Apl-D 10 m).

Three specimens in triplicate of *Ircinia* sp were collected in the Pichilingue locality inside La Paz Bay in Baja California Sur (24 ° 16 '08" N and 110 ° 19 '39" W) at 2 m depth (Fig. 1) (Permit SEMARNAT-08-049b Positive Ficta). The sponge samples were placed in sterile plastic bags and transferred to ice. In the laboratory, the epibiont organisms were removed and washed three times with sterile natural sea water, the outermost layer or pinacoderm was separated with a scalpel and pieces were cut from different areas of the sponges according to the suggestion by Friedrich et al. (2001), placed in tubes and frozen at -20 ºC. The identification of the sponges was carried out by Dr. Cristina Vega Juárez from the Bentos Laboratory of the Institute of Marine Sciences and Linmology of the Universidad Autónoma de México (UNAM), using dichotomous keys and published bibliography for the East Pacific on sponge taxonomy (Goméz et al, 2002; Cruz-Barraza & Carballo-Cenizo, 2008; Carballo-Cenizo & Cruz-Barraza, 2010).

Total DNA extraction

Pieces of approximately 1 cm^3 of sponge mesohyl were taken from each of the samples and their replicates to form a composite sample; they were finely fragmented with a scalpel; 500 µL of TE buffer (10 mM Tris-HC1 containing ethylenediaminetetraacetic acid (EDTA) 1 mM Na2, Thermo Fisher Scientific, Waltham, MA, USA) were added and sonicated for 10 min to detach the bacteria (Bransonic 3510). Then, they were centrifuged at 8000x g for 10 min, and the supernatant was placed in another 2 ml tube for DNA extraction. After that, the method of Phenol: Chloroform: isomilic was used (Sambrook & Russell, 2002; Caamal-Chan et al., 2019). The DNA was resuspended in 50 µl of TE buffer (pH 8.0). Total DNA was treated with RNase A (10 mg mL^{-1}, Promega, Madison, WI, USA) at 37 °C for 30 min. The integrity of the DNA was analyzed by agarose gel electrophoresis. Purity (λ 260 nm / 280 nm ratio) and quantity were evaluated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). DNA samples were stored at -20 °C.

16S V4 rDNA sequencing

Purified DNA was sent to the Next Generation Sequencing Core at Argonne National Laboratory, Argonne, IL, USA for amplicon sequencing. Briefly, the microbial 16S rDNA gene V4 regions were amplified using primer set 515F (5′-GTGC CAGCMGCCGCGGTAA-3′) and 806R (5′-GGAC TACHVGGG TWTCTAAT-3′) following the method described by Kozich et

al. (2013). Amplicons of 16S rDNA gene V4 regions were generated using Illumina MiSeq 500-cycle kit with Illumina MiSeq sequencing platform (San Diego, CA, USA).

Sequence processing and microbial diversity analysis

Primer trimmed pair-end bacterial 16S rDNA gene sequences were merged using Ribosomal Database Project (RDP) Pair-end Reads Assembler. The assembled sequences with an expected maximum error adjusted Q score less than 25 over the entire sequence were eliminated. VSEARCH (v2.4.3, 64 bit) was used to remove chimeras de novo, followed by removing chimeras by reference using RDP 16S rDNA gene (Rognes et al., 2016). High quality and chimera-free sequences were then clustered at 97% sequence similarity by CD-HIT (4.6.1) and RPD Classifier with a confidence cutoff at 50% (Cole et al., 2014). These sequences resulted in the identification of unique operational taxonomic units (OTUs) and their abundance in each sample (Wang et al., 2007; Fu et al., 2012; Bonder et al., 2012; Chen et al., 2013). The resulting operational taxonomic unit (OTU) table was then processed to be analyzed with R programming language, using various packages and custom scripts (www.r-project.org). Chao1 and Shannon and Simpson (alpha) biodiversity indices were estimated with the package 'iNEXT' (Hsieh, Ma & Chao, 2016). For data normalization, the frequency of best hits to each individual taxon for each metagenome was divided by the total number of hits per sample. PERMANOVA statistical analysis was performed with the 'adonis' function with the package 'vegan'. Bray-Curtis distance estimations were calculated using the 'vegdist' function, as well as principal coordinate analysis using the 'pcoa' function with the package 'vegan' (Oksanen et al., 2014).

Results

Sequencing run metrics

From all the samples sequenced, 379 392 reads were generated; after processing, 85 818 low-quality reads and chimeras were removed to keep high-quality pair-end-assembled reads, of which 146 787 reads could be assigned to prokaryotic taxa. The sequencing effort was assessed by Good's coverage analysis with a mean value for all sample reads of 70.64% ± 0.055% and a completeness analysis (full coverage reached below 5000 reads) (*Fig. S1*). A total of 1 102 OTUs were obtained by similarity clustering at 99% nucleotide identity and 786 OTUs after singleton removal within each sample. The raw sequence reads are deposited in NCBI BioProject

Database Accession number: PRJNA760541.
(https://dataview.ncbi.nlm.nih.gov/object/PRJNA760541?reviewer=ht1lj2o5cn5tkk782fcjqloktp)

Microbial communities associated with sponges

The resulting OTUs for this study showed that seven phyla were the most abundant among all mesohyl samples from *Aplysina* sp (10 m depth), *Aplysina* sp and *Ircinia* sp (2 m depth) with a minimal presence of Archaea (0.56% - 1.65% of the total classified reads). Proteobacteria represented the most abundant phylum (85.84%) for *Aplysina* sp (10 m) and was among the most abundant for *Aplysina* sp and *Ircinia* sp (2 m) samples (31.42% and 31.35%, respectively) (Fig. 2a).

Acidobacteria represented the most abundant (47.11%) phylum for *Ircinia* sp samples and also the most abundant (9.23%) for *Aplysina* sp (2 m) sample (Fig. 2a). Cyanobacteria was among the most abundant (4.69% - 21.73%) phylum for all samples, which was the second most abundant (21.73%) for *Aplysina* sp (2 m) sample (Fig. 2a). Chloroflexi was among the most abundant (15.96%) phylum for *Aplysina* sp at shallow water depth (Fig. 2a). Actinobacteria showed a higher abundance of 2- and 3-fold than *Aplysina* sp (10 m) for *Aplysina* sp and *Ircinia* sp samples at shallow depth (Fig. 2a). Bacteroidetes was among the most abundant phylum for *Aplysina* sp (4.56%) and *Ircinia* sp samples (Fig. 2a). Planctomycetes was among the most abundant phylum for shallow *Aplysina* sp (4.44%) samples (Fig. 2a). Interestingly, 30.1% of the total OTUs were shared among all samples analyzed (Fig. 2b). *Aplysina* sp (2 m) samples showed the highest amount of specific OTUs (24.5%), followed by *Ircinia* sp 13.2% and *Aplysina* sp (10 m) 11.7% samples (Fig. 2b). Both *Aplysina* sp samples (2 and 10 m) showed the highest ratio of exclusively shared OTUs (10.4%) (Fig. 2b).

Microbial community diversity and depth effect on sponge species

To determine bacterial taxonomic diversity, richness, and evenness of the microbial communities associated to sponges -both 2 and 10 m samples- alpha diversity indices were estimated by the OTUs rarefaction sampling curves (Fig. 3a) and through rarefaction (interpolation) and extrapolation (R/E). Sampling curve analysis for Chao1 (order q = 0) (Fig. 3b), Shannon (order q = 1) (Fig. 3c), and Simpson (order q = 2) (Fig. 3d) indices showed differences due to curve

clustering of the samples analyzed (Fig. 3). Furthermore, the principal coordinates analysis (PCoA) and constrained correspondence analysis (CCA) were performed to determine the clustering of the *Aplysina* sp (10 m), *Aplysina* sp (2 m), and *Ircinia* sp samples of microbial communities. The clustering either for PCoA and CCA (Fig. 4a-b, respectively) showed two well-defined and discrete groups based on sample depth regardless of the species (*Aplysina* sp or *Ircinia* sp).

To estimate depth and species effect on the bacterial community structures for deep and shallow *Aplysina* sp and *Ircinia* sp samples, a permutational analysis of variance (PERMANOVA) was applied. The PERMANOVA analysis showed that depth was the main factor influencing the microbial community structures in the sponge samples ($R_2 = 0.507$, $P = 0.008$), and sponge species did not have a significant effect ($R_2 = 0.184$, $P = 0.122$). Moreover, the PERMANOVA analysis for depth interaction species showed that depth ($R_2 = 0.507$, $P = 0.004$) was the main factor influencing microbial community structures regardless of sponge species ($R_2 = 0.110$, $P = 0.110$). Furthermore, an analysis of similarities (ANOSIM) was performed to determine differences among the microbial communities. The ANOSIM analysis showed a significant difference between depths in the microbial communities (Fig. 5). Finally, an unsupervised bi-clustering analysis was performed based on sample correlation to estimate the degree of relationship among samples and support the beta diversity analyzed (PCoA and CCA). Interestingly, the outcome of this approach also highlights sponge sample clustering based on depth instead of sponge species (*Fig. S2, Fig. S3*).

Discussion

This study characterized for the first time the associated prokaryotic communities with *Aplysina* and *Ircinia* mesohyl sponges from the Gulf of California. These sponges were initially collected to evaluate their potential production of bioactive compounds and to isolate the bacteria associated with them. Differences were observed in the biological activity of these sponges and cultivable bacteria isolated between the sponges of the same genera and between genera (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012), it was considered that these differences were probably related to the site and the depth at which they were collected, for this reason it was considered that the characterization of the prokaryotic communities from both

sponges by sequencing the 16S gen, would allow us to know the diversity of bacteria and archaea associated with the sponges and thus be able to explain the differences found in the antimicrobial activity tests. *Ircinia* is only found in shallow areas without a depth slope, unlike *Aplysina*, which is distributed in a rocky reef that ranges from 2 to 10 m deep. However, when analyzing the results of the sequencing, a similarity was found between the diversity of bacterial community of the sponges collected in the shallowest areas, therefore, the analysis was carried out focusing on the effect of depth on the bacterial diversity of these two genera of sponges.

A variation was found in relative abundance of the bacterial phyla associated with *Aplysina* sp at different depths. Archaea were present in a low abundance percentage. This low abundance could have been since specific primers for archaea were not used. For example, other authors (Chaib De Mares et al., 2017) have mentioned that when bacterial-specific primers were used, only 6% of the readings were classified as Archaea. On the other hand, when Archaea-specific primers were used, this proportion was 89%. The phylum Thaumarchaeota was the most abundant within archaea and was found in all samples. However, the 2-meter *Aplysina* samples were more abundant. This phylum – Thaumarchaeota – is known to comprise Nitrifying archaea, which was highly abundant in 14 investigated sponge species (Dat et al., 2018).

The most abundant phylum at both depths were Proteobacteria, which was evidenced that it was the predominant (85%) phylum for the deeper water samples (Fig. 2a). Proteobacteria have also been previously reported as a prominent group of sponge-associated microbial communities and highly abundant in the marine environment whether as planktonic or as symbiotic organisms (Li et al., 2006; Jasmin et al., 2015; Dat et al., 2018). This phylum has a direct role contributing to biogeochemical cycles through extracellular enzyme production besides performing some symbiotic functions in sponges, such as nitrogen fixation and secondary metabolite production for the chemical defense of the host (Stabili et al., 2014). Furthermore, the Cyanobacteria and Chloroflexi phyla showed a high proportional abundance in the *Aplysina* samples from the shallow zone. Those phyla have also been characterized with a remarkable transcriptional activity of genes directly involved in photosynthesis and carbon fixation and also responsible for converting ammonia into nitrate in marine sponges (Han, Li & Zhang, 2013; Bibi & Azhar,

2021). The high proportion of these bacteria in the shallowest sponges could be explained because the light intensity required for photosynthesis is greater in this area (Erwin et al., 2012; Souza et al., 2017; Glasl et al., 2019; Fiore et al., 2020).

As reported by Hardoim et al. (2021), the prokaryotic communities associated with *A. caissara* and *A. fulva* were very similar among these two species and dominated by Chloroflexi, Proteobacteria, Crenarchaeota, and Acidobacteria. In contrast, in this study, Chloroflexi were only represented with a high relative abundance in 2-m *Aplysina* samples and Crenarchaeota did not represent an important component in any of the samples. However, these same authors (Hardoim et al., 2021) mentioned that community composition was largely different for *Aplysina* species. For instance, the most abundant phyla encountered in *A. fulva, A. cauliformis, A. archeri, A. cavenicola,* and *A. aerophoba* sampled in seven distinct sites were assigned to Proteobacteria, Chloroflexi, unclassified bacteria, Acidobacteria, and Actinobacteria (Thomas et al., 2016), or *A. fulva* collected in Brazil with the community dominated by Cyanobacteria, Proteobacteria, and Chloroflexi (Hardoim et al., 2009), which coincides with the phyla with the highest relative abundance in the 2-m *Aplysina* samples collected at Punta Arena BCS.

Global bacterial community structure for shallow samples has a similar proportion of Proteobacteria phylum (Fig. 2a). For *Ircinia* sp samples Acidobacteria and Proteobacteria were the most abundant phyla, which is consistent with the results of different studies (Mohamed, 2007, Schmitt et al., 2007; Schmitt et al., 2008; Mohamed et al., 2008; Lee et al., 2011; Yang et al., 2011; Pita Galán, 2014; Hardoim et al., 2012; Pita, López-Legentil & Erwin, 2013; Engelberts et al., 2020) where they report that the core bacterial community associated with this genus is made up of seven phyla Proteobacteria, Acidobacteria, Cyanobacteria, Bacteroidetes, Actinobacteria, Firmicutes and Nitrospira. Despite being a very abundant and diverse group, the Acidobacteria phylum is not as well studied as Proteobacteria, so very little information is available on the species belonging to this phylum in marine environments. The advance in the functional genome analysis, such as that reported by Engelberts et al. (2020). These authors analyzed specific genes involved in metabolic pathways and biogeochemical cycles and found that some species of Acidobacteria participate in denitrification, nitrification, ammonification, metabolism of the taurine, exopolysaccharide production and synthesis of B complex vitamins.

This phylum has also been found in a high percentage in sponge species, such as *Xestospongia testudinaria* and *Luffariella variabilis*, but these bacteria had not been reported as predominant in sponges of the genus *Ircinia* (Webster et al., 2013) in most studies, Proteobacteria and Cyanobacteria are mentioned as the main components of the community associated with different species of *Ircinia* (Hardoim & Costa, 2014). It should be noted that this study would be the first report where the abundance of Acidobacteria is greater than that of proteobacteria for the genus *Ircinia*. For the shallow *Aplysina* samples, the most abundant were Proteobacteria, Cyanobacteria, Chloroflexi and Acidobacteria; deep *Aplysina* samples were dominated only by the Proteobacteria phylum. These prokaryotic taxa with a high relative abundance in this study are also abundant in other marine sponges (Moitinho-Silva et al., 2017; Dat et al., 2018).

In coral ecosystems the sponge-associated microbial community was observed to be more influenced by host identity (Steinert et al., 2016). This study found that sponges of different species (*Aplysina* and *Ircinia*) collected at different depths (2 m and 10 m) share a high proportion of OTUs (Fig. 2b). Albeit the *Aplysina* samples at 10 m and those at 2 m showed characteristic and discrete OTUs distributions (Fig. SF3). Environmental variability is an important factor in the sponge microbial community. A stable isotopic analysis in giant barrel sponge *Xestospongia muta* showed changes in the relationship $_{15}N$ / $_{13}C$ in sponges as depth increased (transition from dependency on photoautotrophy to heterotrophy), leading to a more stable microbial community along the depth gradient (Morrow et al., 2016).

A high proportion (~30%) of shared OTUs for *Aplysina* and *Ircinia* might be due to the ecophysiological similitudes among those species, since they are inhabiting similar reef areas, high diversity of organisms and undergoing the same selective pressures (Souza et al., 2017; Pearman et al., 2019; Turon et al., 2019). However, a higher proportion of exclusively shared OTUs for *Aplysina* samples was expected for both depths (10.4%) since those samples belong to the same species, and because other studies have found that host-specific prokaryotic communities are stable despite geographical and temporal differences (Erwin et al., 2015; Hardoim & Costa, 2014; Dat et al., 2018), to a greater extent in this case which is the same location and at the same time. Also is worth noting that approximately the same proportion of exclusively shared OTUs are shared between *Aplysina* at shallow and deep depths, respectively.

Several phyla are stable in *Aplysina* samples collected at different depths, but their relative abundance percentage differs markedly. In the deepest samples a clear predominance of Proteobacteria is observed, while in those at 2 m, the highest abundance percentage is divided mainly into three phyla, which include Proteobacteria, Cyanobacteria and Chloroflexi. Although this study does not have data on environmental parameters, other studies have found that the temperature difference between shallow areas of the reef and deep sites averaged 4^{o} C (from 3 m to 91 m deep), which was unlikely to affect sponge-microbial communities. Studies examining the effect of elevated temperatures found no change (at sub-lethal temperatures) in sponge bacterial communities during short term experiments (Webster et al., 2008; Simister et al., 2012; Steinert et al., 2016). Therefore, and due to the presence of bacteria of the phylum Cyanobacteria and Chloroflexi in greater abundance at 2 m, might imply that light intensity plays an important role in community changes, as other authors have suggested (Lesser et al., 2010).

Alpha diversity indices for richness and evenness estimated with rarefaction (interpolation) and extrapolation (R/E) sampling curves showed the clustering for *Aplysina* deep samples and overlapping both *Aplysina* and *Ircinia* shallow samples (Fig. 3). The beta diversity PCoA and CCA analyses showed a clustering directly related with depth instead of a relationship among sponge species supported with PERMANOVA and ANOSIM analyses (Fig. 3-4). The clustering analyses (PCoA and CCA) showed two well-defined groups, one corresponding to *Aplysina* bacteria collected in deeper areas and the other one that includes *Aplysina* and *Ircinia* bacteria from the shallow area. Interestingly, these findings are in accordance with the variation in the bacterial community structure assemblage, which is influenced directly by environmental factors, such as depth, temperature, and light intensity and not by the host sponge species (Maldonado & Young, 1998; Thoms et al., 2003; Olson & Gao, 2013; Morrow, Fiore & Lesser, 2016; Thomas et al., 2016; Pearman et al., 2019; Souza et al., 2017). These results differ from those reported in other studies, such as the reported by Gantt et al. (2019) found that bacterial communities exhibited a high degree of host specificity with greater intraspecific than interspecific similarity between locations and detected a significant effect of location on microbial diversity and composition within each host sponge species.

Despite the sample size differences among *Aplysina* for 2 m and 10 m depth, we were able to determine significant differences due to the characteristic prokaryotic structure assemblage for every depth analyzed (Fig. 2a, Fig. 5, and SF3).The sponge microbiome characterization has the full potential to be applied as a tool to determine environmental perturbations, such as those derived from pollution, xenobiotics spills, red tides, and others derived from the global climate change that has been affecting in the last years.

Conclusions

The environment plays a fundamental role to provide the appropriate conditions to sustain and preserve life that inhabits it. Marine ecosystems are not the exception, and it is even more relevant that the environmental conditions play an essential role to influence directly into the biological diversity, especially for those organisms that are attached to the marine ground, such as corals, sponges, algae.

Therefore, the performed microbial diversity analysis showed that depth was more important than host in structuring the *Aplysina* and *Ircinia* microbiome

Acknowledgments

The authors thank José Borges Souza for his help in taking samples, Cristina Vega Juárez by identifying the sponges, COFAA (Comisión de Operación y Fomento de Actividades Académicas del Instituto Politécnico Nacional) IPN and EDI (Estímulo al Desempeño Académico) fellows and Diana Fischer for English edition.

References

Aguila-Ramírez RN. 2012. Caracterización y bioactividad de bacterias asociadas a la esponja *Aplysina gerardogreeni* del Golfo de California. Doctoral Thesis. Centro Interdisciplinario de Ciencias Marinas. Instituto Politécnico Nacional, México.

Bibi F, Azhar EI. 2021. Analysis of bacterial communities in sponges and coral inhabiting Red Sea, using barcoded 454 pyrosequencing. Saudi Journal of Biological Sciences 28: 847-854. https://doi.org/10.1016/j.sjbs.2020.11.021

Bonder MJ, Abeln S, Zaura E, Brandt BW. 2012. Comparing clustering and pre-processing in taxonomy analysis. Bioinformatics 28: 2891–2897. https://doi.org/10.1093/bioinformatics/bts552

Burja AM, Webster NS, Murphy PT, Hill RT. 1999. Microbial symbionts of Great Barrier Reef sponges. Memories of the Queensland Museum 44:63-75

Caamal-Chan MG, Loera-Muro A, Castellanos T, Aguilar-Martínez CJ, Marfil-Santana MD, Barraza A. 2019. Analysis of the bacterial communities and endosymbionts of natural populations of *Bemisia tabaci* in several crop fields from Mexico semi-arid zone. Annals of Microbiology. https://doi.org/10.1007/s13213-019-01483-6.

Caballero-George CJ, Bolaños EO, Carballo JL, Cruz-Barraza JA, Arnold E. 2010. Protocol to isolate sponge-associated fungi from tropical waters and an examination of their cardioprotective potential. Current Trends in Biotechnology and Pharmacy 4:881–889.

Carballo-Cenizo JL, Cruz-Barraza JA. 2010. "A revision Of the genus *Mycale* (Poecilosclerida: Mycalidae) from the Mexican Pacific Ocean". Contributions to Zoology 79:165-191. https://doi:10.1163/18759866-07904003

Chaib De Mares M, Sipkema D, Huang S, Bunk B, Overmann J, van Elsas JD. 2017. Host specificity for bacterial, archaeal and fungal communities determined for high- and low-microbial abundance sponge species in two genera. Frontiers in Microbiology. 8:1-13. https://doi.org/10.3389/fmicb.2017.02560

Chen W, Zhang CK, Cheng Y, Zhang S, Zhao H. 2013. A comparison of methods for clustering 16S rRNA sequences into OTUs. PLoS ONE 8: e70837. https://doi.org/10.1371/journal.pone.0070837

Cleary DFR, Swierts T, Coelho FJRC, Polonia ARM, Huang YM, Ferreira MRS, Putchakarn S, Carvalheiro L, van der Ent E, Ueng JP, Gomes NCM, de Voogd NJ. 2019. The sponge microbiome within the greater coral reef microbial metacommunity. Nature Communications 10: 1644. https://doi.org/10.1038/s41467-019-09537-8.

Cole JR, Wang Q, Fish J, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR, Tiedje JM. 2014. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res 42: D633-42. https://doi.org/10.1093/nar/gkt1244.

Cruz-Barraza JA, Carballo JL, Rocha-Olivares A, Ehrlich H, Hog M. 2012. Integrative taxonomy and molecular phylogeny of genus *Aplysina* (Demospongiae: Verongida) from Mexican Pacific. PLOS ONE 7:e42049. https://doi.org/ 10.1371/journal.pone.0042049.

Dat TTH, Steinert G, Thi KCN, Smidt H, Sipkema D. 2018. Archaeal and bacterial diversity and community composition from 18 phylogenetically divergent sponge species in Vietnam. PeerJ 6:e4970. https://doi.org/10.7717/peerj.4970.

De Vos L, Riitzler K, Boury-Esnault N, Donadey C, Vacelet J. 1995. Atlas of sponge morphology. Smithsonian Institution Press, Washington

Engelberts JP, Robbins SJ, de Goeij JM, Aranda M, Bell SC, Webster NS. 2020. Characterization of a sponge microbiome using an integrative genome-centric approach. The ISME Journal 14(5); 1100-1110. https://doi.org/10.1038/s41396-020-0591-9

Erwin PM, Pita L, López-Legentil S, Turon X. 2012. Stability of sponge-bacteria over large seasonal shifts in temperature and irradiance. Applied and Environmental Microbiology 78: 7358-7368. https://doi.org/10.1128/AEM.02035-12.

Erwin PM, Coma R, López-Sendino P, Serrano E, Ribes M. 2015. Stable symbionts across the HMA-LMA dichotomy: low seasonal and inter-annual variation in sponge-associated bacteria from taxonomically diverse hosts. FEMS Microbiology 91:1-11. https://doi.org/10.1093/femsec/fiv115

Fiore CL, Jarett JK, Steinert G, Lesser MP. 2020. Trait-based comparison of coral and sponge microbiomes. Scientific Reports 10: 2340. https://doi.org/10.1038/s41598-020-59320-9.

Friedrich AB, Fischer I, Proksch P, Hacker J, Hentschel U. 2001. Temporal variation of the microbial community associated with the mediterranean sponge *Aplysina aerophoba*. FEMS Microbiology Ecology 38:105-113

Fu L, Niu B, Zhu Z, Wu S, Li W. 2012. CD-HIT: Accelerated for clustering the next-generation sequencing data. Bioinformatics 28: 3150–3152. https://doi.org/10.1093/bioinformatics/bts565.

Gantt SE, McMurray SE, Stubler AD, Finelli CM, Pawlik JR, Erwin PM. 2019. Testing the relationship between microbiome composition and flux of carbon and nutrients in caribbean coral reef sponges. Microbiome 7: 124. https://doi.org/10.1186/s40168-019-0739-x.

Gándara-Zamudio A. 2011. Actividad antibacteriana y citotóxica de diferentes morfologías de la esponja *Aplysina gerardogreeni* de Punta Arena de la Ventana, BCS, México. Tesis de Licenciatura. Universidad Autónoma de Baja California Sur, México.

Glasl B, Bourne DG, Frade PR, Thomas T, Schaffelke B, Webster NS. 2019. Microbial indicators of environmental perturbations in coral reef ecosystems. Microbiome 7: 94. https://doi.org/10.1186/s40168-019-0705-7.

Gómez P, Carballo JL, Vázquez LE, Cruz JA. 2002. New records for the sponge fauna (Porifera, Demospongiae) of the Pacific Coast of Mexico (East Pacific Ocean). Proceedings of the Biological Society of Washington 115(1): 223-237.

Han M, Li Z, Zhang F. 2013. The ammonia oxidizing and denitrifying prokaryotes associated with sponges from different sea areas. Microbial Ecology 66: 427–436. https://doi.org/10.1007/s00248-013-0197-0.

Hardoim CCP, Costa R, Araujo FV, Hajdu E, Peixoto R, Lins U, Rosado AS, Van Elsas, JD. 2009. Diversity of bacteria in the marine sponge *Aplysina fulva* in Brazilian coastal waters. Applied and Environmental Microbiology 75(10): 3331-3343. https://doi.org/10.1128/AEM.02101-08

Hardoim CC, Esteves AI, Pires FR, Gonçalves JM, Cox CJ, Xavier JR, Costa R. 2012. Phylogenetically and spatially close marine sponges harbour divergent bacterial communities. PLoS One, 7(12); e53029. https://doi.org/10.1371/journal.pone.0053029

Hardoim CC, Costa R. 2014. Microbial communities and bioactive compounds in marine sponges of the family Irciniidae—a review. Marine Drugs 12(10): 5089-5122. https://doi.org/10.3390/md12105089

Hardoim CCP, Lôbo-Hajdu G, Custódio MR, Hardoim PR. 2021. Prokaryotic, Fungal, and Unicellular Eukaryotic Core Communities across Three Sympatric Marine Sponges from the Southwestern Atlantic Coast are Dominated largely by Deterministic Assemblage Processes. Frontiers in Microbiology 12:1162. https://doi.org/10.3389/fmicb.2021.674004

Hentschel U, Fieseler L, Wehrl M, Gernert C, Steinert M, Hacker J, Horn M. 2003. Microbial diversity of marine sponges. Progress in Molecular and Subcellular Biology 37: 59-88. https://doi.org/10.1007/978-3-642-55519-0_3.

Hsieh TC, Ma KH, Chao A. 2016. iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). Methods in Ecology and Evolution 7: 1451-1456. https://doi.org/10.1111/2041-210X.12613.

Imhoff JF, Stöhr R. 2003. Sponge-Associated Bacteria: General Overview and Special Aspects of Bacteria Associated with *Halichondria panicea*. Progress in Molecular and Subcellular Biology, 35-57. https://doi:10.1007/978-3-642-55519-0_2

Jasmin C, Anas A, Nair S, Hong Y. 2015. Bacterial diversity associated with *Cinachyra cavernosa* and *Haliclona pigmentifera*, cohabiting sponges in the coral reef ecosystem of gulf of Mannar, Southeast Coast of India. PLOS ONE 10:e0123222. https://doi.org/10.1371/journal.pone.0123222.

Kahng SE, Copus JM, Wagner D. 2014. Recent advances in the ecology of mesophotic coral ecosystems (MCEs). Current Opinion in Environmental Sustainability 7: 72-81. https://doi.org/10.1016/j.cosust.2013.11.019.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79: 5112-5120. https://doi.org/10.1128/AEM.01043-13.

Lee OO, Wang Y, Yang J, Lafi FF, Al-Suwailem A. Pei-Yuan, 2011. Pyrosequencing reveals highly diverse and species-specific microbial communities in sponges from the Red Sea. ISME J. 5: 650–664. https://doi.org/10.1038/ismej.2010.165

Lesser MP, Slattery M, Leichter JJ. 2009. Ecology of mesophotic coral reefs. Journal of Experimental Marine Biology and Ecology 375: 1- 8. https://doi.org/10.1016/j.jembe.2009.05.009.

Lesser MP, Slattery M, Stat M, Ojimi M, Gates RD, Grottoli A. 2010 Photoacclimatization by the coral *Montastraea cavernosa* in the mesophotic zone: light, food, and genetics. Ecology 91:990–1003 https://doi.org/10.1890/09-0313.1

Lurgi M, Thomas T, Wemheuer B, Webster N, Montoya JM. 2019. Modularity and predicted functions of the global sponge-microbiome network. Nature Communications 10: 992 https://doi.org/10.1038/s41467-019-08925-4

Li ZY, He LM, Wu J, Jiang Q. 2006. Bacterial community diversity associated with four marine sponges from the South China Sea based on 16S rDNA-DGGE fingerprinting. Journal of Experimental Marine Biology and Ecology 329: 75–85. https://doi.org/10.1016/j.jembe.2005.08.014

Maldonado M, Young CM. 1998. Limits on the bathymetric distribution of keratose sponges: a field test in deep water. Marine Ecology Progress Series 74: 123-139. www.jstor.org/stable/24831760.

Mohamed NM. 2007. Ecophysiology of microbial communities associated with marine sponges *Ircinia strobilina* and *Mycale laxissima*. University of Maryland, College Park.

Morrow KM, Bourne DG, Humphrey C, Botté ES, Laffy P, Zaneveld J, Uthicke S, Fabricius KE, Webster NS. 2015. Natural volcanic CO2 seeps reveal future trajectories for host-microbial associations in corals and sponges. The ISME Journal 9: 894-908. https://doi.org/10.1038/ismej.2014.188.

Morrow KM, Fiore CL, Lesser MP. 2016. Environmental drivers of microbial community shifts in the giant barrel sponge, *Xestospongia muta*, over a shallow to mesophotic depth gradient. Environmental Microbiology 18: 2025-2038. https://doi.org/10.1111/1462-2920.13226.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2014. Vegan: community ecology package. R package version 2.2-0. Available at http://CRAN.Rproject.org/package=vegan.

Olson JB, Kellogg CA. 2010. Microbiology of corals, sponges, and algae in mesophotic coral environments. FEMS Microbiology Ecology 73: 17-30. https://doi.org/10.1111/j.1574-6941.2010.00862.x

Olson JB, Gao X. 2013. Characterizing the bacterial associates of three Caribbean sponges along a gradient from shallow to mesophotic depths. FEMS Microbiology Ecology 85: 74-84. https://doi.org/10.1111/1574-6941.12099.

Ortíz-Aguirre I. 2012. Actividad biológica de esponjas y su relación con la complejidad de la comunidad bentónica en la Bahía de La Paz, B.C.S., México. Master Thesis. Centro Interdisciplinario de Ciencias Marinas. Instituto Politécnico Nacional, México.

Parra-Velandia FJ, Zea S. 2003. Comparación de la abundancia y distribución de algunas características de las esponjas del género *Ircinia* (Porifera: Demospongiae) en dos localidades contrastantes del área de Santa Marta, Caribe colombiano. Boletín de Investigaciones Marinas y Costeras 32: 75-91.

Pearman JK, Aylagas E, Voolstra CR, Anlauf H, Villalobos R, Carvalho S. 2019. Disentangling the complex microbial community of coral reefs using standardized autonomous reef monitoring structures (ARMS). Molecular Ecology 28: 3496-3507. https://doi.org/10.1111/mec.15167.

Pita L, López-Legentil S, Erwin PM. 2013. Biogeography and host fidelity of bacterial communities in *Ircinia* spp. from the Bahamas. Microbial ecology, 66(2): 437-447. https://doi.org/10.1007/s00248-013-0215-2

Pita Galán, L. 2014. Biogeography of sponge-associated bacterial communities and resilence to anthropogenic perturbations= Biogeografía de las comunidades bacterianas asociadas a esponjas y su resiliencia frente a perturbaciones antropogénicas. PhD Thesis. Universidad de Barcelona, España.

Reveillaud J, Maignien L, Eren AM, Huber JA, Apprill A, Sogin ML, Vanreusel A. 2014. Host-specificity among abundant and rare taxa in the sponge microbiome. The ISME Journal 8, 1198–1209 doi: 10.1038/ismej.2013.227

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. 2016. VSEARCH: a versatile tool for metagenomics. PeerJ 4: e2584. https://doi.org/10.7717/peerj.2584.

Sambrook J, Russell DW. 2002. Molecular Cloning A Laboratory Manual. 3rd Edition, Science Press, Beijing, 461-471.

Schippers KJ, Sipkema D, Osinga R, Smidt H, Pomponi SA, Martens DE, Wijffels RH. 2012. Cultivation of sponges, sponge cells and symbionts: achievements and future prospects. Advances in Marine Biology 62: 273-337. https://doi.org/10.1016/B978-0-12-394283-8.00006-0.

Schmitt S, Weisz JB, Lindquist N, Hentschel U. 2007.Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. Applied and Environmental Microbiology 73: 2067-2078. https://doi.org/10.1128/AEM.01944-06

Schmitt S, Angermeier H, Schiller R, Lindquist N, Hentschel U. 2008. Molecular microbial diversity survey of sponge reproductive stages and mechanistic insights into vertical transmission of microbial symbionts. Applied and Environmental Microbiology 74: 7694-7708. https://doi.org/10.1128/AEM.00878-08.

Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N, Perez T, Rodrigo A, Schupp PJ, Vacelet J, Webster N, Hentschel U, Taylor MW. 2011. Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. The ISME Journal 6: 564–576. https://doi.org/10.1038/ismej.2011.116.

Simister RL, Deines P, Botté ES, Webster NS, Taylor MW. 2012. Sponge-specific clusters revisited: a comprehensive phylogeny of sponge-associated microorganisms. Environmental Microbiology 14: 517–524. doi: 10.1111/j.1462-2920.2011.02664.x

Souza DT, Genuário DB, Silva FSP, Pansa CC, Kavamura VN, Moraes FC, Taketani RG, Melo IS. 2017. Analysis of bacterial composition in marine sponges reveals the influence of host phylogeny and environment. FEMS Microbiology Ecology 93: fiw204. https://doi.org/10.1093/femsec/fiw204.

Stabili L, Giangrande A, Pizzolante G, Caruso G, Alifano P. 2014. Characterization of Vibrio diversity in the mucus of the polychaete *Myxicola infundibulum* (Annelida, Polichaeta). Microbial Ecology 67: 186–194. https://doi.org/10.1007/s00248-013-0312-2.

Steinert G, Taylor MW, Deines P, Simister RL, de Voogd NJ, Hoggard M, Schupp PJ. 2016. In four shallow and mesophotic tropical reef sponges from Guam the microbial community largely depends on host identity. PeerJ 4:e1936. https://doi.org/10.7717/peerj.1936.

Sunagawa S, Coelho LP, Chaffron S, Kultima JR, Labadie K, Salazar G, Velayoudon D. 2015. Structure and function of the global ocean microbiome. Science, 348(6237): 1261359. https://doi.org/10.1126/science.1261359

Taylor MW, Radax R, Steger D, Wagner M. 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. Microbiology and Molecular Biology Reviews 71:295–347. https://doi.org//10.1128/MMBR.00040-06.

Thomas T, Moitinho-Silva L, Lurgi M, Björk JR, Easson C, Astudillo-García C, Olson JB, Erwin PM, López-Legentil S, Luter H, Chaves-Fonnegra A, Costa R, Schupp PJ, Steindler L, Erpenbeck D, Gilbert J, Knight R, Ackermann G, Lopez, JV, Taylor MW, Thacker RW, Montoya JM, Hentschel U, Webster NS. 2016. Diversity structure and convergent evolution of the global sponge microbiome. Nature Communications 7: 11870. https://doi.org/10.1038/ncomms11870.

Thoms C, Horn M, Wagner M, Hentschel U, Proksch P. 2003. Monitoring microbial diversity and natural product profiles of the sponge *Aplysina cavernicola* following transplantation. Marine Biology 142: 685-692. https://doi.org/10.1007/s00227-002-1000-9.

Turon M, Cáliz J, Triado-Margarit X, Casamayor EO, Uriz MJ. 2019. Sponges and their microbiomes show similar community metrics across impacted and well-preserved reefs. Frontiers in Microbiology 10: 1961. https://doi.org/10.3389/fmicb.2019.01961.

Usher KM, Fromont J, Sutton DC, Toze S. 2004. The biogeography and phylogeny of unicellular cyanobacterial symbionts in selected sponges from Australia and the Mediterranean. Microbial Ecology 48, 167– 177. https://doi.org/ 10.1007/s00248-003-1062-3.

Vacelet J. 1975. Etude en mircrosopie electronique de l'association entre bacteries et spongiaires du genre Verongia (Dictyoceratida). Journal of Microscopic Biology Cellular 23:271-288

Verdugo Díaz G. 2004. Respuesta ecofisiológica del fitoplancton ante la variabilidad ambiental en una Bahía subtropical de Baja California Sur, México. PhD Thesis, Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas, México.

Villegas-Plazas M, Wos-Oxley ML, Sanchez JA. Pieper DH, Thomas OP, Junca H. 2019. Variations in Microbial Diversity and Metabolite Profiles of the Tropical Marine Sponge *Xestospongia muta* with Season and Depth. Microbial Ecology 78, 243–256. https://doi.org/10.1007/s00248-018-1285-y.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73: 5261-5267. https://doi.org/10.1128/AEM.00062-07.

Webster NS, Hill RT. 2001. The culturable microbial community of the Great Barrier Reef sponge *Rhopaloeides odorabile* is dominated by an α-Proteobacterium. Marine Biology 138: 843–851. https://doi.org/10.1007/s002270000503.

671

672 Webster NS, Xavier JR, Freckelton M, Motti CA, Cobb R. 2008. Shifts in microbial and
673 chemical patterns within the marine sponge *Aplysina aerophoba* during a disease outbreak.
674 Environmental microbiology, 10(12); 3366-3376.

675

676 Webster NS, Taylor MW, Behnam F, Lücker S, Rattei T, Whalan S, Horn M, Wagner M. 2010.
677 Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge
678 symbionts. Environmental Microbiology 12: 2070-2082. https://doi.org/ 10.1111/j.1462-
679 2920.2009.02065.x

680

681 Webster NS, Taylor MW. 2012. Marine sponges and their microbial symbionts: love and other
682 relationships. Environmental Microbiology 14: 335–346. https://doi.org/10.1111/j.1462-
683 2920.2011.02460.x

684

685 Webster NS, Luter HM, Soo RM, Botté ES, Simister RL, Abdo D, Whalan S. 2013. Same, same
686 but different: symbiotic bacterial associations in GBR sponges. Frontiers in Microbiology 3:444.
687 https://doi.org/10.3389/fmicb.2012.00444.

688

689 Webster NS, Thomas T. 2016. The sponge hologenome. MBio 7: e00135-16.
690 https://doi.org/10.1128/mBio.00135-16

691

692 Weigel BL, Erwin PM. 2015. Intraspecific variation in microbial symbiont communities of the
693 sun sponge, *Hymeniacidon heliophila*, from intertidal and subtidal habitats. Applied and
694 Environmental Microbiology 82: 650-658. https://doi.org/10.1128/AEM.02980-15.

695

696 Yang J, Sun J, Lee OO, Wong YH, Qian PY. 2011. Phylogenetic diversity and community
697 structure of sponge-associated bacteria from mangroves of the Caribbean Sea. Aquatic Microbial
698 Ecology, 62(3): 231-240. https://doi.org/10.3354/ame01473

699

700 Zea S. 1994. Patterns of coral and sponge abundance in stressed coral reefs at Santa Marta,
701 Colombian Caribbean: 257-264. In Soest, RWM. van, TMG. van Kempen y JC Braekman
702 (Eds.): Sponges in Time and Space. Balkema, Rotterdam.

Figure 1

Geographic localization of sponge sample collecting. Pichilingue and Punta Arena locations in Baja California Sur.

Each station is indicated in red. White bar represents 100 km. Map data © Google, Data SIO, NOAA, U.S. Navy, NGA, GEBCO, Image Landsat/Copernicus. Data LDEO-Columbia, NFS, NOAA

Figure 2

Microbial assemblage of *Aplysina spp.* and *Ircinia spp.* at different depths.

a) Microbial diversity structure and b) Venn diagram for OTUs shared between *Aplysina spp*. and *Ircinia spp.* samples at different depths.

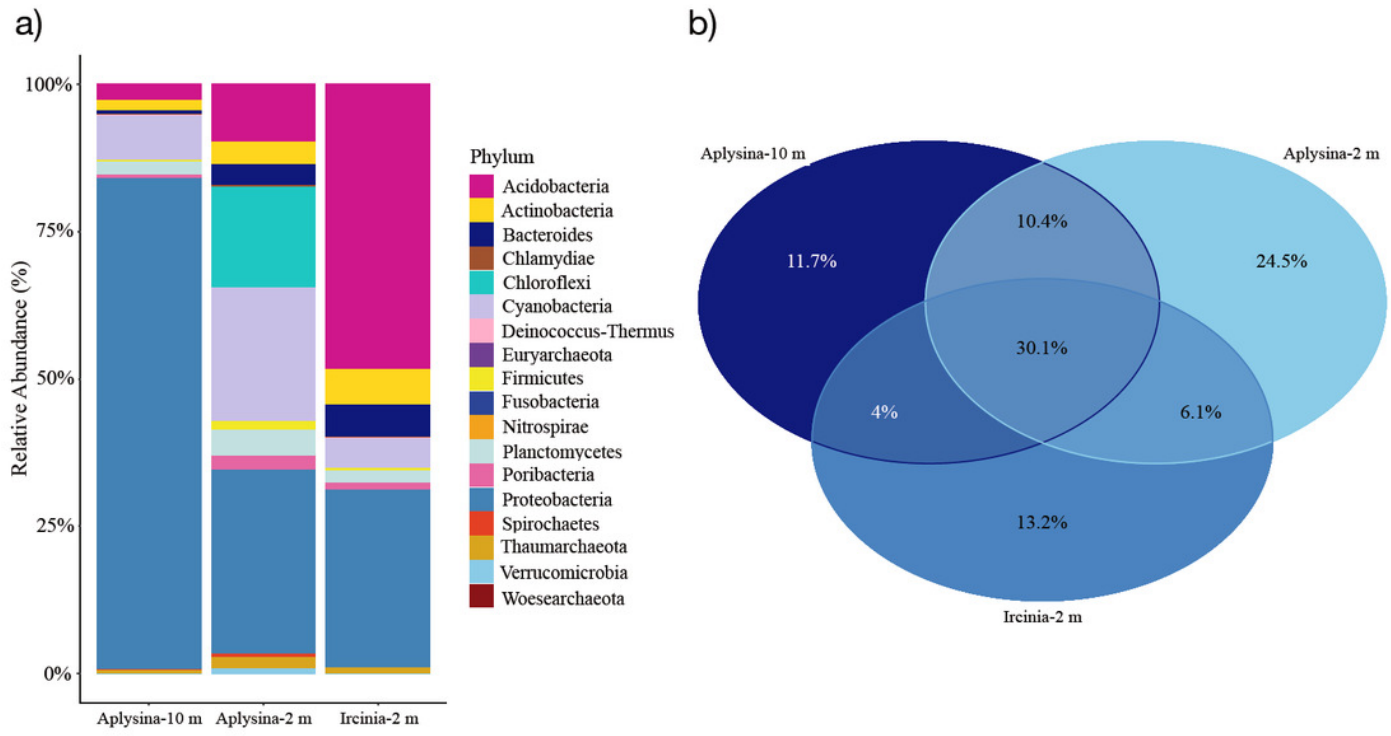

Figure 3

Rarefaction sampling curves and alpha diversity estimations for *Aplysina sp* and *Ircinia sp* samples at different depths

a) Rarefaction sampling curves, b) Chao1 index estimations (q = 0), c) Shannon index estimations (q = 1), d) Simpson index estimations through rarefaction (interpolation) and extrapolation (R/E) sampling curves

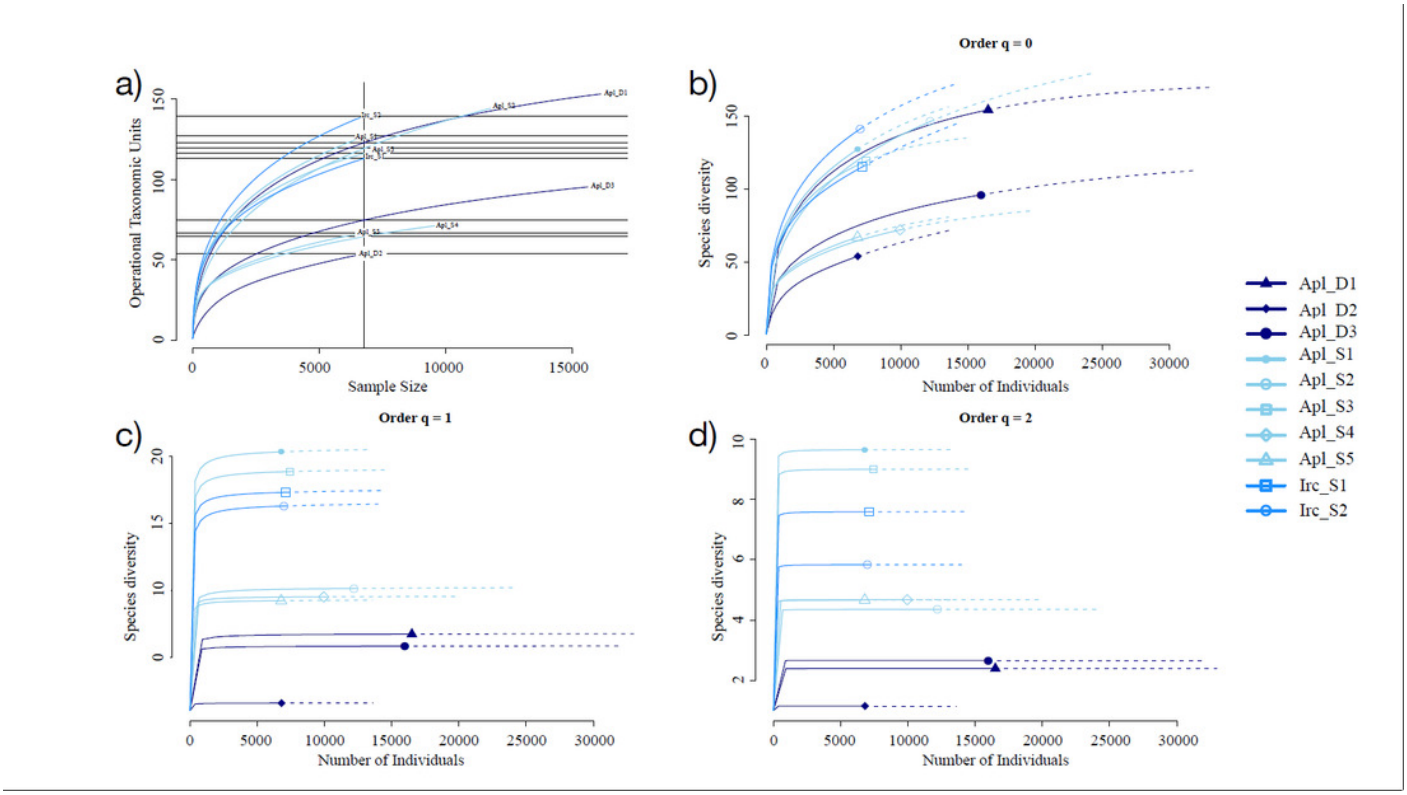

Figure 4

Sample clustering analysis of *Aplysina sp* and *Ircinia sp* samples at different depths

a) Principal coordinates analysis and b) canonical correspondence analysis for *Aplysina sp* and *Ircinia sp* samples at different depths

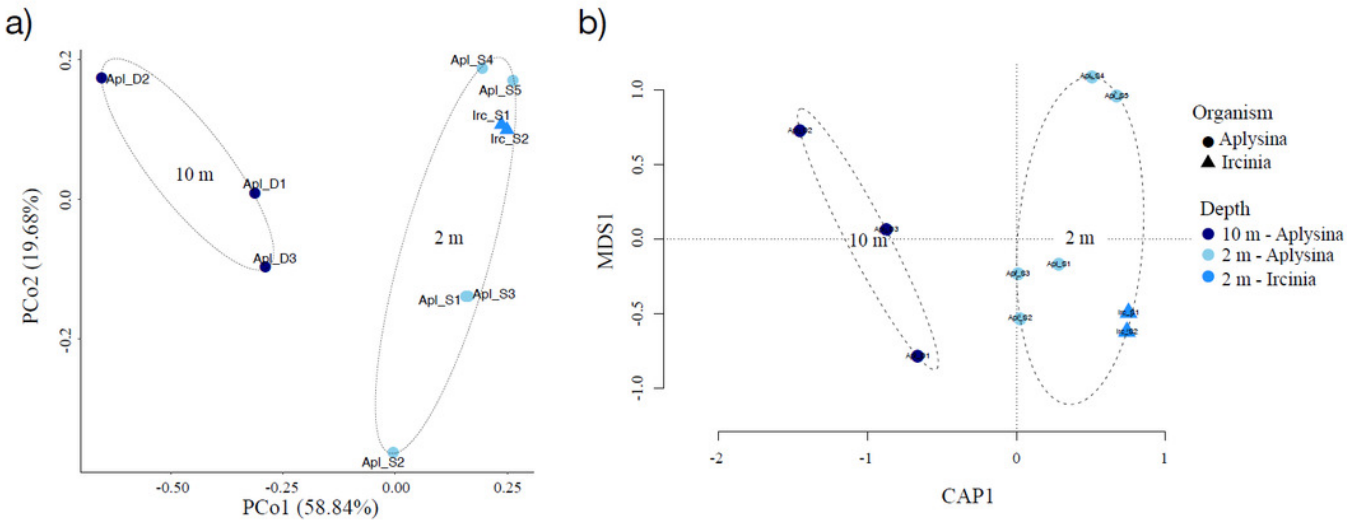

Figure 5

Analysis of similitude (ANOSIM) between sponge samples. ANOSIM analysis showed differences due to depth between *Aplysina sp* and *Ircinia sp* samples