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

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Abstract

Background. Sponge microbiomes are essential for the function and survival of their host and produce biologically active metabolites, therefore, they are ideal candidates for ecological, pharmacological and clinical research. Next-generation sequencing (NGS) has revealed that many factors, including the environment and host, determine the composition and structure of these symbiotic communities but 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 10 m and 2 m; *Ircinia* sp samples were collected at 2 m. DNA of sponge-associated prokaryotes was extracted from 1 cm³ 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. Depth was the main factor influencing the microbial community, as analysis of similarities (ANOSIM) showed a significant difference between the microbial communities from different depths.

Conclusion. Microbial diversity analysis showed that depth was more important than host in structuring the *Aplysina* sp and *Ircinia* sp microbiome. This observation contrast with previous reports that the sponge microbiome is highly host specific.

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). Frequent and abundant presence of bacteria,

32 especially within the sponge mesohyll, led authors to address these bacteria as symbionts

33 (Vacelet, 1975; De Vos et al., 1995; Burja et al., 1999; Imhoff & Stöhr, 2003). Sponge

34 microbiomes are essential for their host's function (metabolic), health and survival (Lurgi et al.,

35 2019). Furthermore, production of biologically active metabolites by sponges-associated

36 bacteria, is an important function in this association (Imhoff & Stöhr, 2003). Therefore, they are

37 ideal candidates for ecological, pharmacological and clinical research. Sponge tissues host many

38 symbionts, including heterotrophic bacteria, facultative anaerobes, dinoflagellates,

39 cyanobacteria, archaea, fungi, and viruses (Webster & Hill, 2001; Schippers et al., 2012). These

40 microbial communities included dozens of phyla. Variation in diversity of these microbiomes is

41 not well described (Taylor et al., 2007; Webster & Thomas, 2016; Villegas-Plazas et al., 2019).

42

43 Next-generation sequencing (NGS) approaches to characterize marine sponge microbial

44 communities has dramatically increased precision and quantity of surveys of the taxonomic

45 complexity of these microbiomes (Schmitt et al., 2011; Webster & Taylor, 2012; Reveillaud et

46 al., 2014) and revealed that sponge microbiomes are largely host-specific and stable across

47 temporal scales under specific environmental conditions (Morrow et al., 2015; Weigel & Erwin,

48 2015; Morrow, Fiore & Lesser, 2016; Cleary et al., 2019). Recent research suggests that depth

49 drivers of the structure of ocean microbiome (Sunagawa et al., 2015). However, for symbioses,

50 one would expect a strong microbial community differentiation to emerge across host species

51 (Lurgi et al., 2019). Sponges can inhabit from shallow to mesophotic ecosystems, in deep water

52 they are apparently less influenced by abiotic factors (Kahng, Copus & Wagner, 2014; Olson &

53 Kellogg, 2010). In shallow water these abiotic factors could influence the sponges, and their

54 associated microbial communities. Some studies have determined sponge associated microbial

55 community changes at different water depths from shallow (0-30 m) to mesophotic areas (30-150

56 m) (Olson & Kellogg, 2010; Lesser, Slattery & Leichter, 2009; Kahng, Copus & Wagner, 2014).

57 Though the specificity of the sponge microbiota appears more related with host phylogeny,

58 microbial communities in shallow and deep reefs vary (Steinert et al., 2016)

59 However, to our knowledge no studies are available that evaluate whether among the same

60 shallow water sponges (0-30 m) the community varies according to its range of distribution.

61 Although changes in abiotic factors are not as evident, as it could occur in mesophotic zones (30-

62   150 m), the depth gradient could influence the composition of the microbial community

63   associated with these sponges (Olson & Gao, 2013; Steiner et al., 2016).

64

65   *Aplysina* species are often associated with shallow rocky reefs. This species belongs to the

66   Verongiida order and are distributed along the East Pacific from Mexico to Panama (Caballero-

67   George et al., 2010; Cruz-Barraza et al., 2012). *Ircinia*, they are conspicuous and abundant in

68   areas exposed to light in rocky-coral biotopes (Parra-Velandia & Zea, 2003) and more abundant

69   in localities near sources of continental discharge with greater turbidity and load of organic

70   material in suspension (Zea, 1994). In previous studies with sponges of these genera (*Aplysina* sp

71   and *Ircinia* sp) from the Gulf of California, differences were observed in the biological activity

72   of sponges and their associated bacteria, between sponges of the same genus and between genera

73   (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012). These differences appear

74   related to site and depth. For this reason, this study assessed the microbial communities

75   associated with two marine sponges of the genera *Aplysina* (Nardo, 1834) and *Ircinia* (Nardo,

76   1833) in rocky reefs from Punta Arena de la Ventana (Gulf of California) and Pichilingue (La

77   Paz Bay) in the coast of Baja California Sur, Mexico to determine the relative importance of

78   environment and host in structuring the microbiome of sponges.

79

80

81   **Materials & Methods**

82   Specimens of *Aplysina* sp and *Ircinia* sp sponges previously collected to evaluate their biological

83   activity (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012) were used for

84   this study.

85

86   *Aplysina* sp specimens ($n = 8$) were collected in triplicate by scuba diving in Punta Arena, Baja

87   California Sur, Mexico (24 ° 03 '40 "N and 109 ° 49 '52" W) at different water depths (2 m - 10

88   m). For this study, the depth of 2 m was considered shallow and 10 m as deep (Apl-S: 2m; Apl-D

89   10 m).

90

91   Three specimens in triplicate of *Ircinia* sp were collected in the Pichilingue locality inside La

92   Paz Bay in Baja California Sur (24 ° 16 '08" N and 110 ° 19 '39" W) at 2 m depth (Fig. 1)

93   (Permit SEMARNAT-08-049b Positive Ficta). Sponge samples were placed in sterile plastic

94   bags and transferred to ice. In the laboratory, the epibiont organisms were removed and washed

95   three times with sterile natural sea water, the outermost layer or pinacoderm was separated with a

96   scalpel and pieces were cut from different areas of the sponges according to the suggestion by

97   Friedrich et al. (2001), placed in tubes and frozen at -20 ºC. Dr. Cristina Vega Juárez from the

98   Bentos Laboratory of the Institute of Marine Sciences and Linmology of the Universidad

99   Autónoma de México (UNAM), identified the sponges using dichotomous keys and published

100 bibliography for the East Pacific on sponge taxonomy (Gómez et al, 2002; Cruz-Barraza &

101 Carballo-Cenizo, 2008; Carballo-Cenizo & Cruz-Barraza, 2010).

102

103 Total DNA extraction

104 Pieces of approximately 1 cm^3 of sponge mesohyl were taken from each of sample and their

105 replicates to form a composite sample; composites were then finely fragmented with a scalpel;

106 500 µL of TE buffer (10 mM Tris-HC1, 1mM disodium ethylenediaminetetraacetic acid

107 (EDTA), Thermo Fisher Scientific, Waltham, MA, USA) were added and sonicated for 10 min to

108 detach the bacteria (Bransonic 3510). Then, the mixture was centrifuged at 8,000x g for 10 min,

109 and the supernatant was placed in another 2 ml tube for DNA extraction with phenol:

110 chloroform: isoamyl following Sambrook & Russell (2002) and Caamal-Chan et al. (2019).

111 Recovered DNA was resuspended in 50 µl of TE buffer (pH 8.0) and treated with RNase A (10

112 mg mL^{-1}, Promega, Madison, WI, USA) at 37 °C for 30 min. The integrity of the DNA was

113 analyzed by agarose gel electrophoresis. Purity (λ 260 nm / 280 nm ratio) and quantity were

114 evaluated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

115 USA). DNA samples were stored at -20 °C.

116

117 16S V4 rDNA sequencing

118 Purified DNA was sent to the Next Generation Sequencing Core at Argonne National

119 Laboratory, Argonne, IL, USA for amplicon sequencing. Briefly, the microbial 16S rRNA gene

120 V4 regions were amplified using primer set 515F (5′-GTGC CAGCMGCCGCGGTAA-3′) and

121 806R (5′-GGAC TACHVGGG TWTCTAAT-3′) following the method described by Kozich et

122 al. (2013). Amplicons of 16S rRNA gene V4 regions were generated using Illumina MiSeq 500-

123 cycle kit with Illumina MiSeq sequencing platform (San Diego, CA, USA).

124

125   Sequence processing and microbial diversity analysis

126   Primer trimmed pair-end bacterial 16S rDNA gene sequences were merged using Ribosomal

127   Database Project (RDP) Pair-end Reads Assembler. The assembled sequences with an expected

128   maximum error adjusted Q score less than 25 over the entire sequence were eliminated.

129   VSEARCH (v2.4.3, 64 bit) was used to remove chimeras de novo, followed by removing

130   chimeras by reference using RDP 16S rDNA gene (Rognes et al., 2016). High quality and

131   chimera-free sequences were then clustered at 97% sequence similarity by CD-HIT (4.6.1) and

132   RPD Classifier with a confidence cutoff at 50% (Cole et al., 2014). These sequences resulted in

133   the identification of unique operational taxonomic units (OTUs) and their abundance in each

134   sample (Wang et al., 2007; Fu et al., 2012; Bonder et al., 2012; Chen et al., 2013). The resulting

135   operational taxonomic unit (OTU) table was then processed to be analyzed with R programming

136   language, using various packages and custom scripts (www.r-project.org). Chao1 and Shannon

137   and Simpson (alpha) biodiversity indices were estimated with the package 'iNEXT' (Hsieh, Ma

138   & Chao, 2016). For data normalization, the frequency of best hits to each individual taxon for

139   each metagenome was divided by the total number of hits per sample. PERMANOVA and

140   ANOSIM statistical analysis were performed with the 'adonis' and 'anosim' functions,

141   respectively, with the package 'vegan'. Bray-Curtis distance estimations were calculated using

142   the 'vegdist' function, as well as principal coordinate analysis using the 'pcoa' function with the

143   package 'vegan' (Oksanen et al., 2014).

144

145   **Results**

146   Sequencing run metrics

147   From all the samples sequenced, 379,392 reads were generated; after processing, 85,818 low-

148   quality reads and chimeras were removed to keep high-quality pair-end-assembled reads, of

149   which 146,787 reads could be assigned to prokaryotic taxa. The sequencing effort was assessed

150   by Good's coverage analysis with a mean value for all sample reads of $71\% \pm 0.1\%$ and a

151   completeness analysis (full coverage reached below 5000 reads) (*Fig. S1*). A total of 1,102

152   OTUs were obtained by similarity clustering at 99% nucleotide identity and 786 OTUs after

153   singleton removal within each sample. Raw sequence reads are deposited in NCBI BioProject

154 Database Accession number: PRJNA760541.

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

156

157 Microbial communities associated with sponges

158 The resulting OTUs for this study showed that seven phyla were the most abundant among all

159 mesohyl samples from *Aplysina* sp (10 m depth), *Aplysina* sp and *Ircinia* sp (2 m depth) with a

160 minimal presence of Archaea (0.56% - 1.65% of the total classified reads). Proteobacteria

161 represented the most abundant phylum (86%) for *Aplysina* sp (10 m) and was among the most

162 abundant for *Aplysina* sp and *Ircinia* sp (2 m) samples (31% for both) (Fig. 2a).

163

164 Acidobacteria represented the most abundant (47%) phylum for *Ircinia* sp samples and also the

165 most abundant (9%) for *Aplysina* sp (2 m) sample (Fig. 2a). Cyanobacteria was among the most

166 abundant (5% - 22%) phylum for all samples, which was the second most abundant (22%) for

167 *Aplysina* sp (2 m) sample (Fig. 2a). Chloroflexi was among the most abundant (16%) phylum for

168 *Aplysina* sp at shallow water depth (Fig. 2a). Actinobacteria showed a higher abundance of 2-

169 and 3-fold than *Aplysina* sp (10 m) for *Aplysina* sp and *Ircinia* sp samples at shallow depth (Fig.

170 2a). Bacteroidetes was among the most abundant phylum for *Aplysina* sp (5%) and *Ircinia* sp

171 samples (Fig. 2a). Planctomycetes was among the most abundant phylum for shallow *Aplysina*

172 sp (5%) samples (Fig. 2a). Interestingly, 30% of the total OTUs were shared among all samples

173 analyzed (Fig. 2b). *Aplysina* sp (2 m) samples showed the highest amount of specific OTUs

174 (25%), followed by *Ircinia* sp 13% and *Aplysina* sp (10 m) 12% samples (Fig. 2b). Both

175 *Aplysina* sp samples (2 and 10 m) showed the highest ratio of exclusively shared OTUs (10%)

176 (Fig. 2b).

177

178 Microbial community diversity and depth effect on sponge species

179 Sampling curve analysis for Chao1 (order q = 0) (Fig. 3b), Shannon (order q = 1) (Fig. 3c), and

180 Simpson (order q = 2) (Fig. 3d) indices showed differences due to curve clustering of the

181 samples analyzed (Fig. 3). Furthermore, the principal coordinates analysis (PCoA) and

182 constrained correspondence analysis (CCA) (Fig. 4a-b, respectively) showed two well-defined

183 and discrete groups based on sample depth regardless of the species (*Aplysina* sp or *Ircinia* sp).

184

185   PERMANOVA analysis showed that depth was the main factor influencing the microbial

186   community structures in the sponge samples ($R_2 = 0.507$, $P = 0.008$). Sponge species did not

187   have a significant effect ($R_2 = 0.184$, $P = 0.122$). Moreover, the PERMANOVA analysis for

188   depth interaction species showed that depth ($R_2 = 0.507$, $P = 0.004$) was the main factor

189   influencing microbial community structures regardless of sponge species ($R_2 = 0.110$, $P =$

190   0.110). ANOSIM analysis also showed a significant difference between depths in the microbial

191   communities (Fig. 5) and unsupervised bi-clustering analysis based on sample correlation to

192   estimate the degree of relationship among samples supported this beta diversity analysis (PCoA

193   and CCA) (*Fig. S2, Fig. S3*).

194

Discussion

196   This study characterized for the first time prokaryotic communities associated with *Aplysina* sp

197   and *Ircinia* sp mesohyl sponges from the Gulf of California. These sponges were initially

198   collected to evaluate their potential production of bioactive compounds and to isolate the bacteria

199   associated with them. Differences were observed in the biological activity of these sponges and

200   cultivable bacteria isolated between the sponges of the same genera and between genera

201   (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012) appeared related to the

202   site and depth at which they were collected, for this reason the prokaryotic communities from

203   both sponges was characterized by high-throughput sequencing the 16S rRNA gene fragments.

204

205   *Ircinia* sp is only found in shallow areas without a depth slope; *Aplysina* sp, is distributed in a

206   rocky reef that ranges from 2 to 10 m deep. Preliminary analysis the results of the sequencing,

207   showed similarity between bacterial communities of sponges collected in the shallowest areas,

208   therefore, the analysis was carried out focusing on the effect of depth on the bacterial diversity of

209   these two genera of sponges.

210

211   Variation was found in relative abundance of the bacterial phyla associated with *Aplysina* sp at

212   different depths. Archaea were present in a low abundance percentage. This low abundance

213   could have been since specific primers for archaea were not used. Chaib De Mares et al. (2017)

214   reported that when bacterial-specific primers were used, only 6% of the readings were classified

215   as Archaea. On the other hand, when Archaea-specific primers were used, this proportion was

216 89%. The phylum Thaumarchaeota was the most abundant within archaea and was found in all
217 samples and was most abundant in *Aplysina* sp samples collected at 2 meters. This phylum –
218 Thaumarchaeota – comprises nitrifying archaea and is highly abundant in sponge microbiomes
219 (Dat et al., 2018).

220

221 The most abundant phylum at both depths were Proteobacteria (Fig. 2a). Proteobacteria are a
222 prominent group of sponge-associated microbial communities and highly abundant in the marine
223 in plankton organisms (Li et al., 2006; Jasmin et al., 2015; Dat et al., 2018). This phylum
224 contributes to biogeochemical cycles through extracellular enzyme production and performs
225 some symbiotic functions in sponges, such as nitrogen fixation and secondary metabolite
226 production for the chemical defense of the host (Stabili et al., 2014). Furthermore, Cyanobacteria
227 and Chloroflexi showed a high proportional abundance in the *Aplysina* sp samples from the
228 shallow zone. These phyla mediate photosynthesis and carbon fixation and responsible for
229 converting ammonia into nitrate in marine sponges (Han, Li & Zhang, 2013; Bibi & Azhar,
230 2021). The high proportion of these bacteria in the shallowest sponges could be explained
231 because light intensity (Erwin et al., 2012; Souza et al., 2017; Glasl et al., 2019; Fiore et al.,
232 2020).

233

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

246

247   Global bacterial community structure for shallow samples has a similar proportion of

248   Proteobacteria phylum (Fig. 2a). For *Ircinia* sp samples Acidobacteria and Proteobacteria were

249   the most abundant phyla, which is consistent with the results of several studies (Mohamed, 2007,

250   Schmitt et al., 2007; Schmitt et al., 2008; Lee et al., 2011; Yang et al., 2011; Hardoim et al.,

251   2012; Pita, López-Legentil & Erwin, 2013; Pita, 2014; Engelberts et al., 2020) where they report

252   that the core bacterial community associated with this genus is made up of seven phyla

253   Proteobacteria, Acidobacteria, Cyanobacteria, Bacteroidetes, Actinobacteria, Firmicutes and

254   Nitrospira. Despite being a very abundant and diverse group, the Acidobacteria phylum is not as

255   well studied as Proteobacteria, so very little information is available on the species belonging to

256   this phylum in marine environments. Engelberts et al. (2020) analyzed specific genes involved in

257   metabolic pathways and biogeochemical cycles and found that some species of Acidobacteria

258   participate in denitrification, nitrification, ammonification, metabolism of the taurine,

259   exopolysaccharide production and synthesis of B complex vitamins. This phylum has also been

260   found in a high percentage in sponge species, such as *Xestospongia testudinaria* and *Luffariella*

261   *variabilis*, but these bacteria had not been reported as predominant in sponges of the genus

262   *Ircinia* (Webster et al., 2013). In most studies of the community associated with different species

263   of *Ircinia*, Proteobacteria and Cyanobacteria dominate (Hardoim & Costa, 2014). It should be

264   noted that this study would be the first report where the abundance of Acidobacteria is greater

265   than that of Proteobacteria for the genus *Ircinia*. For the shallow *Aplysina* sp samples, the most

266   abundant were Proteobacteria, Cyanobacteria, Chloroflexi and Acidobacteria; deep *Aplysina* sp

267   samples were dominated only by the Proteobacteria phylum. These prokaryotic taxa with a high

268   relative abundance in this study are also abundant in other marine sponges (Moitinho-Silva et al.,

269   2017; Dat et al., 2018).

270

271   In coral ecosystems host identity controls the sponge-associated microbial community (Steinert

272   et al., 2016). This study found that sponges of different species (*Aplysina* sp and *Ircinia* sp)

273   collected at different depths (2 m and 10 m) share a high proportion of OTUs (Fig. 2b). Albeit

274   the *Aplysina* sp samples at 10 m and those at 2 m showed characteristic and discrete OTUs

275   distributions (Fig. SF3). Environmental variability is an important factor in the sponge microbial

276   community. Stable isotopic analysis in giant barrel sponge *Xestospongia muta* showed changes

277   in the relationship $_{15}N$ / $_{13}C$ in sponges as depth increased (transition from dependency on

278   photoautotrophy to heterotrophy), leading to a more stable microbial community along the depth
279   gradient (Morrow et al., 2016).
280
281   A high proportion (~30%) of shared OTUs for *Aplysina* sp and *Ircinia* sp might be due to the
282   ecophysiological similitudes among those species, since they are inhabiting similar reef areas
283   and face similar selective pressures (Souza et al., 2017; Pearman et al., 2019; Turon et al., 2019).
284   However, a higher proportion of exclusively shared OTUs for *Aplysina* sp samples was expected
285   for both depths (10%) since those samples belong to the same species, and because other studies
286   have found that host-specific prokaryotic communities are stable despite geographical and
287   temporal differences (Erwin et al., 2015; Hardoim & Costa, 2014; Dat et al., 2018).
288   Approximately the same proportion of exclusively shared OTUs are shared between *Aplysina* sp
289   at shallow and deep depths, respectively. Several phyla are stable in *Aplysina* sp samples
290   collected at different depths, but their relative abundance percentage differs markedly. In the
291   deepest samples a clear Proteobacteria predominated, while in the shallowest samples three
292   phyla, Proteobacteria, Cyanobacteria and Chloroflexi, predominated. Although this study does
293   not have data on environmental parameters, other studies have found that the temperature
294   difference between shallow areas of the reef and deep sites averaged 4º C (from 3 m to 91 m
295   deep), which was unlikely to affect sponge-microbial communities. Studies examining the effect
296   of elevated temperatures found no change (at sub-lethal temperatures) in sponge bacterial
297   communities during short term experiments (Webster et al., 2008; Simister et al., 2012; Steinert
298   et al., 2016). Therefore, and due to the presence of bacteria of the phylum Cyanobacteria and
299   Chloroflexi in greater abundance at 2 m, might imply that light intensity plays an important role
300   in community changes, as other authors have suggested (Lesser et al., 2010).
301
302   Alpha diversity indices for richness and evenness estimated with rarefaction (interpolation) and
303   extrapolation (R/E) sampling curves showed the clustering for *Aplysina* sp deep samples and
304   overlapping both *Aplysina* sp and *Ircinia* sp shallow samples (Fig. 3). The beta diversity PCoA
305   and CCA analyses showed a clustering directly related with depth instead of a relationship
306   among sponge species supported with PERMANOVA and ANOSIM analyses (Fig. 3-4). The
307   clustering analyses (PCoA and CCA) showed two well-defined groups, one corresponding to
308   *Aplysina* sp bacteria collected in deeper areas and the other one corresponding to *Aplysina* sp and

309 *Ircinia* sp bacteria from the shallow area. These findings are in accordance with the variation in

310 the bacterial community structure assemblage, which is influenced directly by environmental

311 factors, such as depth, temperature, and light intensity and not by the host sponge species

312 (Maldonado & Young, 1998; Thoms et al., 2003; Olson & Gao, 2013; Morrow, Fiore & Lesser,

313 2016; Thomas et al., 2016; Pearman et al., 2019; Souza et al., 2017). These results differ from

314 those reported in other studies. For example, Gantt et al. (2019) reported that bacterial

315 communities exhibited a high degree of host specificity.

316

Conclusions

318 Microbial diversity analysis showed that depth was more important than host in structuring the

319 *Aplysina* sp and *Ircinia* sp microbiome.

320

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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.
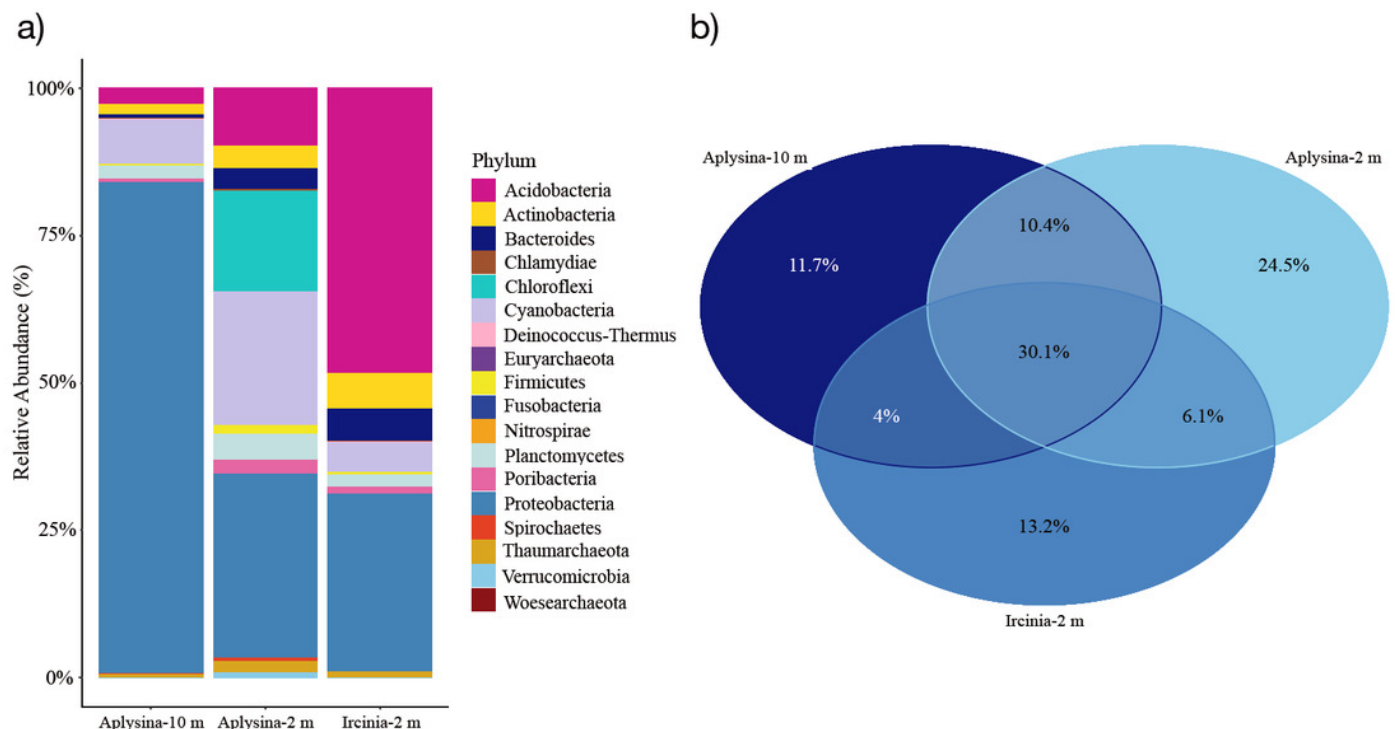
Figure 3

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
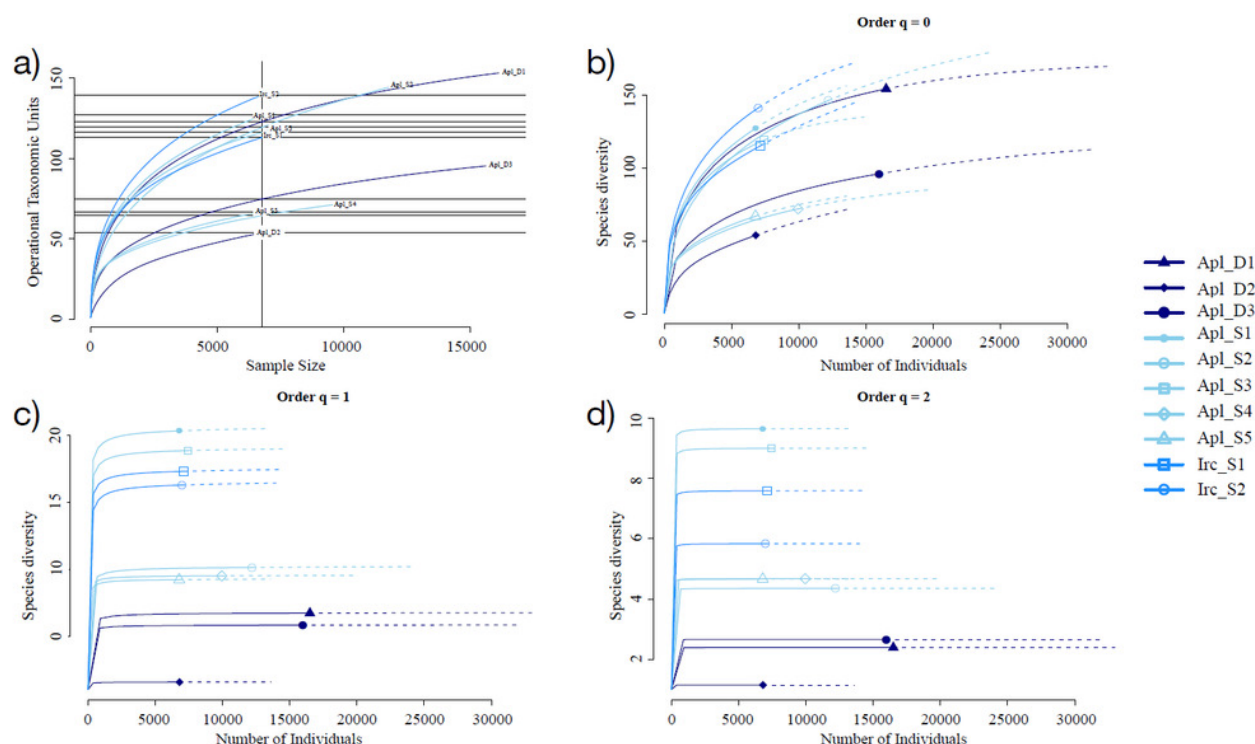
Figure 4

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

/* minimal */
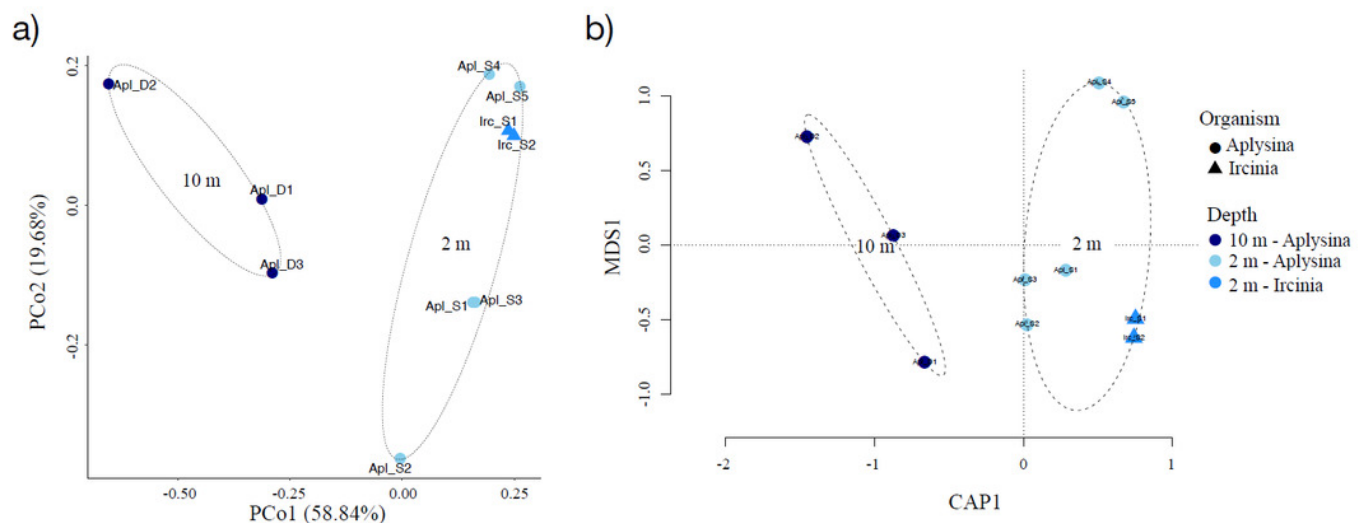
Figure 5

Analysis of similitude (ANOSIM) between sponge samples. ANOSIM analysis showed differences between *Aplysina* sp at 2m depth and *Aplysina* sp and *Ircinia* sp at 10 m depth samples.