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

Bárbara González-Acosta[1C], Aarón Barraza[2C], 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.

[C] 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 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.

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

32   essential for their host's function (metabolic), health and survival (Lurgi et al., 2019).

33   Furthermore, there is evidence of the production of biologically active metabolites by sponges-

34   associated bacteria, being an important function in this association (Imhoff & Stöhr, 2003).

35   Therefore, they are ideal candidates for ecological, pharmacological and clinical research.

36   Sponge tissues host many symbionts, including heterotrophic bacteria, facultative anaerobes,

37   dinoflagellates, cyanobacteria, archaea, fungi, and even viruses (Webster & Hill, 2001; Schippers

38   et al., 2012). Support between 15 to several tenths phyla but the source of this variation in

39   diversity is not well described (Taylor et al., 2007; Webster & Thomas, 2016; Villegas-Plazas et

40   al., 2019).

41

42   Implementing next-generation sequencing (NGS) approaches to characterize marine sponge

43   microbial communities has notoriously increased precision and quantity of the taxonomic

44   complexity associated to these marine organisms (Schmitt et al., 2011; Webster & Taylor, 2012;

45   Reveillaud et al., 2014). Moreover, the complex microbial communities of MS have been

46   unveiled through NGS coupled with microbial diversity analyses to highlight that sponge

47   microbiomes are largely host-specific and often stable across temporal scales under specific

48   environmental conditions (Morrow et al., 2015; Weigel & Erwin, 2015; Morrow, Fiore & Lesser,

49   2016; Cleary et al., 2019). Recent research suggests that depth are the main drivers of the

50   structure of ocean microbiome (Sunagawa et al., 2015). However, for symbioses, one would

51   expect a strong microbial community differentiation to emerge across host species (Lurgi et al.,

52   2019). Sponges can inhabit from shallow to mesophotic ecosystems, in deep water they are

53   apparently less influenced by abiotic factors (Kahng, Copus & Wagner, 2014; Olson & Kellogg,

54   2010). Otherwise, in shallow ecosystems these abiotic factors could influence the sponges, thus,

55   also on their associated microbial communities. Some studies have determined sponge associated

56   microbial community changes at different water depths from shallow (0-30 m) to mesophotic

57   areas (30-150 m) (Olson & Kellogg, 2010; Lesser, Slattery & Leichter, 2009; Kahng, Copus &

58   Wagner, 2014). Though the specificity of the sponge microbiota appears more related with host

59   phylogeny, differences in depth can be showing variance between microbial communities in

60   shallow and deep reefs (Steinert et al., 2016)

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

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

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

64   150 m), the depth gradient could influence the composition of the microbial community

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

66

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

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

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

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

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

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

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

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

75   (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012). It was considered that

76   these differences were probably related to the site and the depth at which they were collected.

77   For this reason, this study assessed the microbial communities associated with two marine

78   sponges of the genera *Aplysina* (Nardo, 1834) and *Ircinia* (Nardo, 1833) in rocky reefs from

79   Punta Arena de la Ventana (Gulf of California) and Pichilingue (La Paz Bay) in the coast of Baja

80   California Sur, Mexico to determine the relative importance of environment and host in

81   structuring the microbiome of sponges.

82

83

84   **Materials & Methods**

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

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

87   this study.

88

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

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

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

92   10 m).

93

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

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

96   (Permit SEMARNAT-08-049b Positive Ficta). The sponge samples were placed in sterile plastic

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

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

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

100 Friedrich et al. (2001), placed in tubes and frozen at -20 ºC. The identification of the sponges

101 was carried out by Dr. Cristina Vega Juárez from the Bentos Laboratory of the Institute of

102 Marine Sciences and Linmology of the Universidad Autónoma de México (UNAM), using

103 dichotomous keys and published bibliography for the East Pacific on sponge taxonomy (Gómez

104 et al, 2002; Cruz-Barraza & Carballo-Cenizo, 2008; Carballo-Cenizo & Cruz-Barraza, 2010).

105

106 Total DNA extraction

107 Pieces of approximately 1 cm^3 of sponge mesohyl were taken from each of the samples and their

108 replicates to form a composite sample; they were finely fragmented with a scalpel; 500 µL of TE

109 buffer (10 mM Tris-HC1 containing ethylenediaminetetraacetic acid (EDTA) 1 mM Na2,

110 Thermo Fisher Scientific, Waltham, MA, USA) were added and sonicated for 10 min to detach

111 the bacteria (Bransonic 3510). Then, they were centrifuged at 8000x g for 10 min, and the

112 supernatant was placed in another 2 ml tube for DNA extraction. After that, the method of

113 Phenol: Chloroform: isomilic was used (Sambrook & Russell, 2002; Caamal-Chan et al., 2019).

114 The DNA was resuspended in 50 µl of TE buffer (pH 8.0). Total DNA was treated with RNase A

115 (10 mg mL^{-1}, Promega, Madison, WI, USA) at 37 °C for 30 min. The integrity of the DNA was

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

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

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

119

120 16S V4 rDNA sequencing

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

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

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

124 806R (5′-GGAC TACHVGGG TWTCTAAT-3′) following the method described by Kozich et

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

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

127

128 Sequence processing and microbial diversity analysis

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

147

148 **Results**

149 Sequencing run metrics

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

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

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

153 by Good's coverage analysis with a mean value for all sample reads of 70.64% ± 0.055% and a

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

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

156   singleton removal within each sample. The raw sequence reads are deposited in NCBI BioProject

157   Database Accession number: PRJNA760541.

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

159

160   Microbial communities associated with sponges

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

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

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

164   represented the most abundant phylum (85.84%) for *Aplysina* sp (10 m) and was among the most

165   abundant for *Aplysina* sp and *Ircinia* sp (2 m) samples (31.42% and 31.35%, respectively) (Fig.

166   2a).

167

168   Acidobacteria represented the most abundant (47.11%) phylum for *Ircinia* sp samples and also

169   the most abundant (9.23%) for *Aplysina* sp (2 m) sample (Fig. 2a). Cyanobacteria was among the

170   most abundant (4.69% - 21.73%) phylum for all samples, which was the second most abundant

171   (21.73%) for *Aplysina* sp (2 m) sample (Fig. 2a). Chloroflexi was among the most abundant

172   (15.96%) phylum for *Aplysina* sp at shallow water depth (Fig. 2a). Actinobacteria showed a

173   higher abundance of 2- and 3-fold than *Aplysina* sp (10 m) for *Aplysina* sp and *Ircinia* sp

174   samples at shallow depth (Fig. 2a). Bacteroidetes was among the most abundant phylum for

175   *Aplysina* sp (4.56%) and *Ircinia* sp samples (Fig. 2a). Planctomycetes was among the most

176   abundant phylum for shallow *Aplysina* sp (4.44%) samples (Fig. 2a). Interestingly, 30.1% of the

177   total OTUs were shared among all samples analyzed (Fig. 2b). *Aplysina* sp (2 m) samples

178   showed the highest amount of specific OTUs (24.5%), followed by *Ircinia* sp 13.2% and

179   *Aplysina* sp (10 m) 11.7% samples (Fig. 2b). Both *Aplysina* sp samples (2 and 10 m) showed the

180   highest ratio of exclusively shared OTUs (10.4%) (Fig. 2b).

181

182   Microbial community diversity and depth effect on sponge species

183   To determine bacterial taxonomic diversity, richness, and evenness of the microbial communities

184   associated to sponges -both 2 and 10 m samples- alpha diversity indices were estimated by the

185   OTUs rarefaction sampling curves (Fig. 3a) and through rarefaction (interpolation) and

186   extrapolation (R/E). Sampling curve analysis for Chao1 (order q = 0) (Fig. 3b), Shannon (order q

187   = 1) (Fig. 3c), and Simpson (order q = 2) (Fig. 3d) indices showed differences due to curve

188   clustering of the samples analyzed (Fig. 3). Furthermore, the principal coordinates analysis

189   (PCoA) and constrained correspondence analysis (CCA) were performed to determine the

190   clustering of the *Aplysina* sp (10 m), *Aplysina* sp (2 m), and *Ircinia* sp samples of microbial

191   communities. The clustering either for PCoA and CCA (Fig. 4a-b, respectively) showed two

192   well-defined and discrete groups based on sample depth regardless of the species (*Aplysina* sp or

193   *Ircinia* sp).

194

195   To estimate depth and species effect on the bacterial community structures for deep and shallow

196   *Aplysina* sp and *Ircinia* sp samples, a permutational analysis of variance (PERMANOVA) was

197   applied. The PERMANOVA analysis showed that depth was the main factor influencing the

198   microbial community structures in the sponge samples ($R_2 = 0.507$, $P = 0.008$), and sponge

199   species did not have a significant effect ($R_2 = 0.184$, $P = 0.122$). Moreover, the PERMANOVA

200   analysis for depth interaction species showed that depth ($R_2 = 0.507$, $P = 0.004$) was the main

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

202   0.110). Furthermore, an analysis of similarities (ANOSIM) was performed to determine

203   differences among the microbial communities. The ANOSIM analysis showed a significant

204   difference between depths in the microbial communities (Fig. 5). Finally, an unsupervised bi-

205   clustering analysis was performed based on sample correlation to estimate the degree of

206   relationship among samples and support the beta diversity analyzed (PCoA and CCA) (*Fig. S2,*

207   *Fig. S3*).

208

209   **Discussion**

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

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

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

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

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

215   (Gándara-Zamudio, 2011; Aguila-Ramírez, 2012; Ortíz-Aguirre, 2012).

216

217   It was considered that these differences were probably related to the site and the depth at which

218   they were collected, for this reason it was considered that the characterization of the prokaryotic

219   communities from both sponges by sequencing the 16S rRNA, would allow us to know the

220   diversity of bacteria and archaea associated with the sponges and thus be able to explain the

221   differences found in the antimicrobial activity tests.

222

223   *Ircinia* sp is only found in shallow areas without a depth slope, unlike *Aplysina* sp, which is

224   distributed in a rocky reef that ranges from 2 to 10 m deep. However, when analyzing the results

225   of the sequencing, a similarity was found between the diversity of bacterial community of the

226   sponges collected in the shallowest areas, therefore, the analysis was carried out focusing on the

227   effect of depth on the bacterial diversity of these two genera of sponges.

228

229   A variation was found in relative abundance of the bacterial phyla associated with *Aplysina* sp at

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

231   could have been since specific primers for archaea were not used. For example, other authors

232   (Chaib De Mares et al., 2017) have mentioned that when bacterial-specific primers were used,

233   only 6% of the readings were classified as Archaea. On the other hand, when Archaea-specific

234   primers were used, this proportion was 89%. The phylum Thaumarchaeota was the most

235   abundant within archaea and was found in all samples. However, the 2-meter *Aplysina* sp

236   samples were more abundant. This phylum – Thaumarchaeota – is known to comprise

237   nitrifying archaea, which was highly abundant in 14 investigated sponge species (Dat et al.,

238   2018).

239

240   The most abundant phylum at both depths were Proteobacteria, which was evidenced that it was

241   the predominant (85%) phylum for the deeper water samples (Fig. 2a). Proteobacteria have also

242   been previously reported as a prominent group of sponge-associated microbial communities and

243   highly abundant in the marine environment whether as planktonic or as symbiotic organisms (Li

244   et al., 2006; Jasmin et al., 2015; Dat et al., 2018). This phylum has a direct role contributing to

245   biogeochemical cycles through extracellular enzyme production besides performing some

246   symbiotic functions in sponges, such as nitrogen fixation and secondary metabolite production

247   for the chemical defense of the host (Stabili et al., 2014). Furthermore, the Cyanobacteria and

248   Chloroflexi phyla showed a high proportional abundance in the *Aplysina* samples from the

249   shallow zone. Those phyla have also been characterized with a remarkable transcriptional

250   activity of genes directly involved in photosynthesis and carbon fixation and also responsible for

251   converting ammonia into nitrate in marine sponges (Han, Li & Zhang, 2013; Bibi & Azhar,

252   2021). The high proportion of these bacteria in the shallowest sponges could be explained

253   because the light intensity required for photosynthesis is greater in this area (Erwin et al., 2012;

254   Souza et al., 2017; Glasl et al., 2019; Fiore et al., 2020).

255

256   As reported by Hardoim et al. (2021), the prokaryotic communities associated with *A. caissara*

257   and *A. fulva* were very similar among these two species and dominated by Chloroflexi,

258   Proteobacteria, Crenarchaeota, and Acidobacteria. In contrast, in this study, Chloroflexi was only

259   represented with a high relative abundance in 2-m *Aplysina* sp samples and Crenarchaeota did

260   not represent an important component in any of the samples. However, these same authors

261   (Hardoim et al., 2021) mentioned that community composition was largely different for *Aplysina*

262   species. For instance, the most abundant phyla encountered in *A. fulva, A. cauliformis, A.*

263   *archeri, A. cavenicola,* and *A. aerophoba* sampled in seven distinct sites were assigned to

264   Proteobacteria, Chloroflexi, unclassified bacteria, Acidobacteria, and Actinobacteria (Thomas et

265   al., 2016), or *A. fulva* collected in Brazil with the community dominated by Cyanobacteria,

266   Proteobacteria, and Chloroflexi (Hardoim et al., 2009), which coincides with the phyla with the

267   highest relative abundance in the 2-m *Aplysina* sp samples collected at Punta Arena BCS.

268

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

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

271   the most abundant phyla, which is consistent with the results of different studies (Mohamed,

272   2007, Schmitt et al., 2007; Schmitt et al., 2008; Lee et al., 2011; Yang et al., 2011; Hardoim et

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

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

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

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

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

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

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

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

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

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

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

284   *Ircinia* (Webster et al., 2013) in most studies, Proteobacteria and Cyanobacteria are mentioned as

285   the main components of the community associated with different species of *Ircinia* (Hardoim &

286   Costa, 2014). It should be noted that this study would be the first report where the abundance of

287   Acidobacteria is greater than that of Proteobacteria for the genus *Ircinia*. For the shallow

288   *Aplysina* sp samples, the most abundant were Proteobacteria, Cyanobacteria, Chloroflexi and

289   Acidobacteria; deep *Aplysina* sp samples were dominated only by the Proteobacteria phylum.

290   These prokaryotic taxa with a high relative abundance in this study are also abundant in other

291   marine sponges (Moitinho-Silva et al., 2017; Dat et al., 2018).

292

293   In coral ecosystems the sponge-associated microbial community was observed to be more

294   influenced by host identity (Steinert et al., 2016). This study found that sponges of different

295   species (*Aplysina* sp and *Ircinia* sp) collected at different depths (2 m and 10 m) share a high

296   proportion of OTUs (Fig. 2b). Albeit the *Aplysina* sp samples at 10 m and those at 2 m showed

297   characteristic and discrete OTUs distributions (Fig. SF3). Environmental variability is an

298   important factor in the sponge microbial community. A stable isotopic analysis in giant barrel

299   sponge *Xestospongia muta* showed changes in the relationship $_{15}N / _{13}C$ in sponges as depth

300   increased (transition from dependency on photoautotrophy to heterotrophy), leading to a more

301   stable microbial community along the depth gradient (Morrow et al., 2016).

302

303   A high proportion (~30%) of shared OTUs for *Aplysina* sp and *Ircinia* sp might be due to the

304   ecophysiological similitudes among those species, since they are inhabiting similar reef areas,

305   high diversity of organisms and undergoing the same selective pressures (Souza et al., 2017;

306   Pearman et al., 2019; Turon et al., 2019). However, a higher proportion of exclusively shared

307   OTUs for *Aplysina* sp samples was expected for both depths (10.4%) since those samples belong

308   to the same species, and because other studies have found that host-specific prokaryotic

309   communities are stable despite geographical and temporal differences (Erwin et al., 2015;

310 Hardoim & Costa, 2014; Dat et al., 2018), to a greater extent in this case which is the same

311 location and at the same time. Also is worth noting that approximately the same proportion of

312 exclusively shared OTUs are shared between *Aplysina* sp at shallow and deep depths,

313 respectively. Several phyla are stable in *Aplysina* sp samples collected at different depths, but

314 their relative abundance percentage differs markedly. In the deepest samples a clear

315 predominance of Proteobacteria is observed, while in those at 2 m, the highest abundance

316 percentage is divided mainly into three phyla, which include Proteobacteria, Cyanobacteria and

317 Chloroflexi. Although this study does not have data on environmental parameters, other studies

318 have found that the temperature difference between shallow areas of the reef and deep sites

319 averaged 4° C (from 3 m to 91 m deep), which was unlikely to affect sponge-microbial

320 communities. Studies examining the effect of elevated temperatures found no change (at sub-

321 lethal temperatures) in sponge bacterial communities during short term experiments (Webster et

322 al., 2008; Simister et al., 2012; Steinert et al., 2016). Therefore, and due to the presence of

323 bacteria of the phylum Cyanobacteria and Chloroflexi in greater abundance at 2 m, might imply

324 that light intensity plays an important role in community changes, as other authors have

325 suggested (Lesser et al., 2010).

326

327 Alpha diversity indices for richness and evenness estimated with rarefaction (interpolation) and

328 extrapolation (R/E) sampling curves showed the clustering for *Aplysina* sp deep samples and

329 overlapping both *Aplysina* sp and *Ircinia* sp shallow samples (Fig. 3). The beta diversity PCoA

330 and CCA analyses showed a clustering directly related with depth instead of a relationship

331 among sponge species supported with PERMANOVA and ANOSIM analyses (Fig. 3-4). The

332 clustering analyses (PCoA and CCA) showed two well-defined groups, one corresponding to

333 *Aplysina* sp bacteria collected in deeper areas and the other one that includes *Aplysina* sp and

334 *Ircinia* sp bacteria from the shallow area. Interestingly, these findings are in accordance with the

335 variation in the bacterial community structure assemblage, which is influenced directly by

336 environmental factors, such as depth, temperature, and light intensity and not by the host sponge

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

338 Lesser, 2016; Thomas et al., 2016; Pearman et al., 2019; Souza et al., 2017). These results differ

339 from those reported in other studies, such as the reported by Gantt et al. (2019) found that

340 bacterial communities exhibited a high degree of host specificity with greater intraspecific than

341   interspecific similarity between locations and detected a significant effect of location on

342   microbial diversity and composition within each host sponge species.

343

344   Despite the sample size differences among *Aplysina* sp for 2 m and 10 m depth, we were able to

345   determine significant differences due to the characteristic prokaryotic structure assemblage for

346   every depth analyzed (Fig. 2a, Fig. 5, and SF3).

347

348   Conclusions

349   The environment plays a fundamental role to provide the appropriate conditions to sustain and

350   preserve life that inhabits it. Marine ecosystems are not the exception, and it is even more

351   relevant that the environmental conditions play an essential role to influence directly into the

352   biological diversity, especially for those organisms that are attached to the marine ground, such

353   as corals, sponges, algae.

354   Therefore, the performed microbial diversity analysis showed that depth was more important

355   than host in structuring the *Aplysina* sp and *Ircinia* sp microbiome

356

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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.

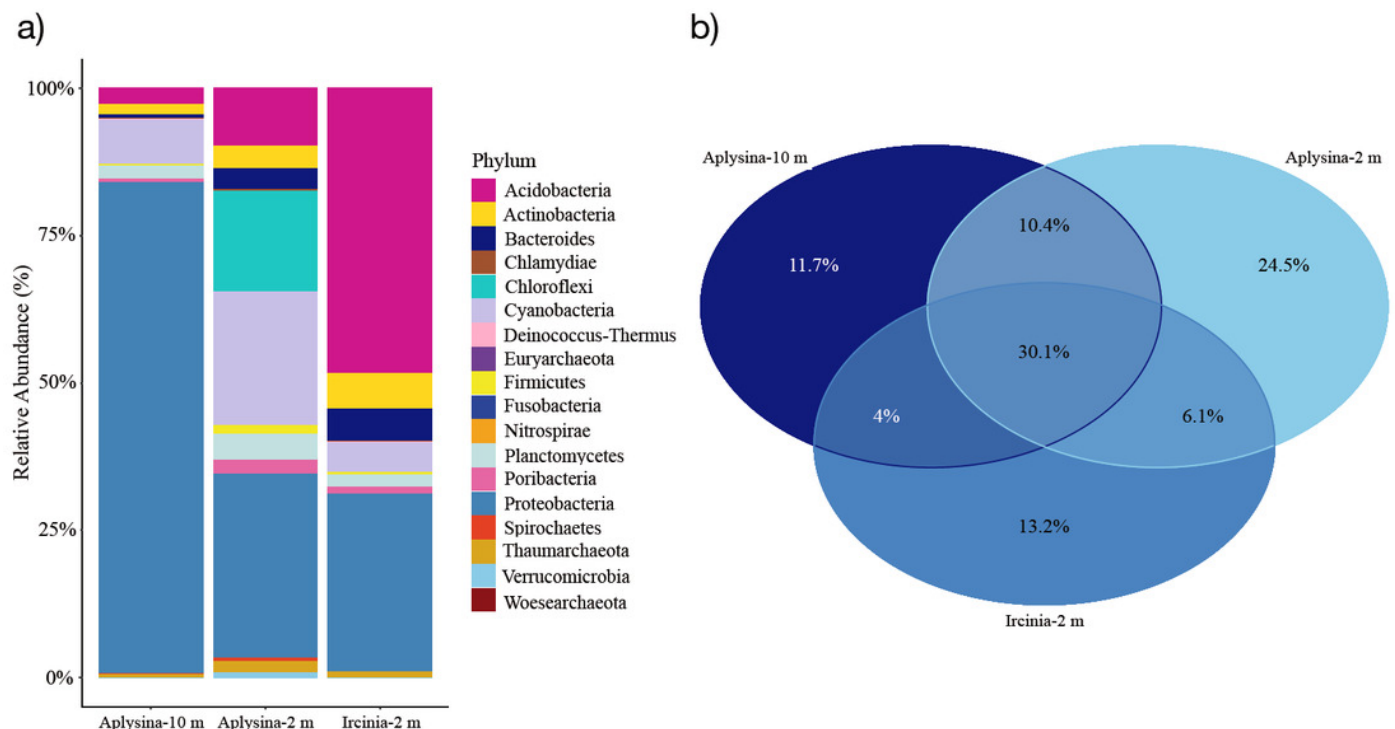
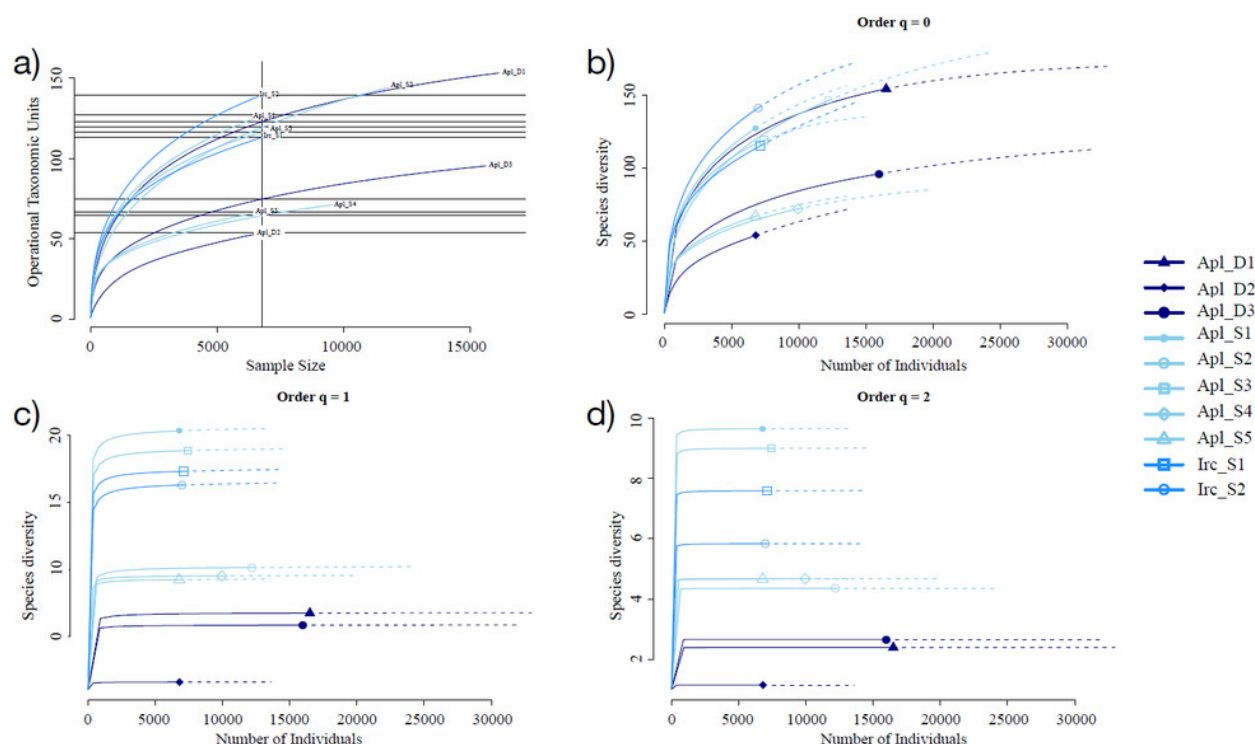



Figure 3

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

asonably# 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

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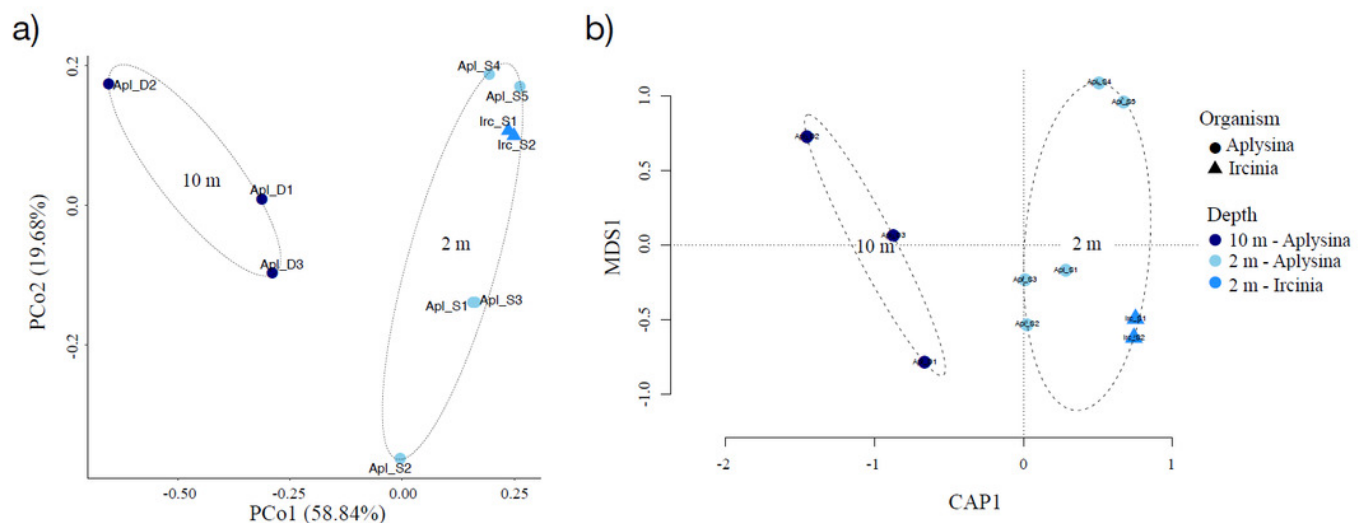
Figure 5

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.