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An analysis of the bacterial community in and around scleractinian corals of Phu Quoc Island, Vietnam

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Abstract :

Corals harbour a myriad of microorganisms, many of which play a beneficial role for their host. To date, many of these microbes have not been identified, and information is also lacking on their origin, in particular their potential presence in the surrounding seawater or sediment. In this study, we used 16S rRNA gene sequencing to investigate the bacterial communities associated with three genera of scleractinian coral (Acropora, Lobophyllia and Porites) of the coast of Phu Quoc Island in Vietnam. We surveyed the bacterial communities on the mucous layer of these corals, as well as in the water column and the surface sediment in their vicinity, which we considered as five biotopes: Acropora, Lobophyllia, Porites, water column, and sediment. Overall, we identified 29 phyla, 50 classes, 114 orders, 254 families, and 402 genera across all samples. Proteobacteria were dominant in most of the biotopes, while Desulfobacterota and Bacteroidota were mainly found in the sediment. Bacteriome analysis based on amplicon sequence variants (ASVs) suggested that five genera (Algicola, Algicola bacteriolytica, Alteromonas, Catenococcus, and Vibrio) were the core bacteria in the three coral biotopes, but there were no shared ASVs across all five biotopes investigated. Additionally, linear discriminant analysis revealed that 23 biomarkers differed significantly across the five biotopes, with coral biotopes having the highest diversity of bacterial taxa (15 biomarkers), followed by seawater (4 biomarkers) and sediment biotopes (4 biomarkers). These findings highlight that the composition of the coral bacteriome is significantly different from that of nearby seawater and sediment samples, and that the composition may be specific to the coral host.

Keywords : Coral, Sediment, Seawater, Bacterial diversity, Core microbiome, Phu Quoc Island

1. Introduction

Coral reefs, one of the most biologically diverse marine ecosystems, are complex ecological communities made up of a variety of coral species and their surrounding environment (Zhang et al., 2021). Studies have shown that coral reefs play a vital role, providing habitats for a wide variety of organisms (Elliff and Silva, 2017), as well as protecting the coastline from storm waves and erosion (Mhuantong et al., 2019). However, coral is vulnerable to environmental changes caused by human activity: for example, increasing temperatures due to global warming can make corals more susceptible to disease and lead to a reduction in coral biodiversity (Mhuantong et al., 2019; Rosenberg et al., 2007). Disruption or imbalance in the composition of the microbial community can also cause disease in corals (MacKnight et al., 2021; Ritchie, 2006). Given the increasing environmental stress on coral, it is urgent to study the functional role and contribution of the components of the coral reef ecosystem to better understand how they interact with each other and how they are changing.

Corals have a complex symbiotic relationship with diverse living organisms, including viruses, archaea, bacteria, symbiotic dinoflagellates (zooxanthellae), and fungi, which together form the coral holobiont (Bourne et al., 2009; Rohwer et al., 2002; Rosenberg et al., 2007). Of these, the bacterial community is a particularly critical component. Bacteria are involved in most of the physiological functions of corals, including food digestion, nutrient absorption, immune system development, and pathogen defence (Rohwer et al., 2002; Shnit-Orland et al., 2010). Microbes can be found in a variety of microniches in the coral holobiont,

including the coral's surface mucous layer, tissue, and skeleton (Li et al., 2014; Rosenberg et al., 2007). To distinguish these microniches from non-host biotopes such as sediment and seawater, they are generally referred to as "host biotopes".

The development of molecular tools, next-generation sequencing (NGS), and metagenomic technology is increasing our understanding of the interactions between microbes and their hosts. These tools have allowed the study of coral-associated bacterial diversity and composition in many parts of the world, including Southeast Asia (Mhuantong et al., 2019; Pootakham et al., 2017), the Pacific Ocean (Yang et al., 2020), Western Australia (Bernasconi et al., 2019), the Red Sea (Osman et al., 2020), the South China Sea (Qi et al., 2022; Zhang et al., 2015), and the Indian Ocean (Wambua et al., 2021). A few of these studies have demonstrated that coral bacterial communities differ from those in sediment (Dong et al., 2022) and seawater surrounding the coral (Zhang et al., 2015), and that specific bacterial taxa may be found in some corals.

The coral ecosystem in Vietnam is highly diverse, with at least 366 coral species belonging to 70 genera identified to date (Latypov, 2005, 2011). Studies on the coral microbiome in this region have mainly concentrated on microbial diversity and composition (Bettarel et al., 2018; Mien et al., 2019; Pham et al., 2015) or the bacterial community's antimicrobial activities (Mien et al., 2020), while less attention has been paid to the microbes in nearby sediment and seawater, although the myriad of microbial communities in coral, seawater, and sediment differ. For the purpose of this study, we assume that coral species contain microbial communities that differ from those in seawater and sediment. Through examining specific bacterial amplicon sequence variants (ASVs) in the different compartments, the study aimed to identify the core bacteriomes, the dominant bacterial ASVs, and bacterial taxa that were shared across samples. As Phu Quoc Island has a diverse range of coral ecosystems (Tin et

al., 2014), the results allow valuable insights into the bacterial communities in corals and their surroundings, which could be a useful springboard for further studies.

2. Materials and Methods

2.1. Sampling site and methods

The samples (sediment, seawater, and scleractinian coral) were taken off the coast of Vietnam's Phu Quoc Island (9°55'20.6"N, 104°01'16.4"E), located in the lower Gulf of Thailand, in May 2020. During one sampling occasion (via scuba diving), a total of 19 samples were collected, 9 from individual corals (3 from each of the 3 coral types), 5 from the sediment, and 5 from the water column at a depth of 5 to 10 m. We considered these in our analyses as five separate biotopes: *Acropora muricata, Lobophyllia, Porites,* water column, and sediment.

For the coral samples, we selected only healthy coral branches, which were obtained from three scleractinian coral species: *Acropora muricata* (AF, n = 3) species, *Lobophyllia* (LB, n = 3), and *Porites* (PO, n=3) genera. Mucus collection was carried out as described in previous studies (Bettarel et al., 2018; Naumann et al., 2010), by taking coral nubbins out of the water, rinsing them with autoclaved and 0.2µm filtrated seawater, and exposing them to the air for 3 minutes. To avoid contamination or dilution by seawater, the mucus production of the coral sample for the first 30 seconds was eliminated. After this, the mucus samples were collected with sterile syringes, transferred to sterile cryotubes, immediately fixed with 30% glycerol solution at a ratio of 1:1, and stored at -20°C until analysis.

The seawater and sediment samples were collected at a distance of less than 2 m from the corals, and then transferred into sterilized 15-mL Falcon tubes. They were then stored at -20°C until analysis in the lab.

2.2. Bacterial DNA extraction and 16S rRNA gene sequencing

The total DNA was isolated separately from the coral mucus, the sediment (1 g), and the seawater (from material captured in the membrane filter) samples using the Easy-DNA™ gDNA Purification Kit (Invitrogen, Thermo Fisher Scientific, USA) following the manufacturer's instructions. The purification and quantity of extracted bacterial DNA were assessed by a NanoDrop 2000c spectrophotometer (Thermo Scientific, USA), and DNA quality was checked by running on 1% agarose gel electrophoresis. The DNA was then diluted to 10 ng μl^{-1} with TE buffer and stored at -20°C until analysis.

To amplify the bacterial 16S *rRNA* gene from coral mucus, seawater, and sediment samples, a region of approximately 470 bp covering the V4–V5 hypervariable regions of the ribosomal DNA was targeted using the universal bacterial primer sets 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3) (Parada et al., 2016). The polymerase chain reaction (PCR) amplifications were carried out in an Eppendorf 6331 Nexus Gradient MasterCycler Thermal Cycler (Hampton, New Hampshire, USA) as follows: 94°C for 1 min at the denaturation step, followed by 30 cycles of 94°C for 30 sec, then 55°C for 30 sec, 72°C for 1 min, and the final extension of 72°C for 5 min. All amplicons were checked for size and quality by agarose gel electrophoresis before using the Illumina MiSeq platform to perform sequencing of the *16S rRNA* gene. To assess whether bacterial DNA used for analysis was contaminated during the DNA extraction and polymerase chain reaction (PCR) stages, we used distilled water as the negative control in the PCR, as it contained all reaction components except for the template DNA.

2.3. Raw data processing and statistical analyses

The result of amplicon sequencing generated 846,011 raw reads from the 19 samples; the DADA2 pipeline was used for data processing. This included filtering and trimming low–quality sequences, denoising, removing chimeras, constructing the ASV table, and assigning

taxonomy, as detailed in the protocol described by Callahan et al. (2016). Barcodes, primers, low–quality sequences (QC < 20), and sequences shorter than 200 bp or longer than 500 bp were removed. After screening and denoising, a total of 334,503 sequences remained, with a median of 18,526 and a mean of 17,605 sequences per sample (min = 5,810; max = 32,557). These were then clustered into ASVs based on a 97% similarity to the V4–V5 region of the 16S rRNA gene in the SILVA version 138.1 database (https://www.arb-silva.de/) (Quast et al., 2013). To further clean datasets, singletons, unassigned taxa, and taxa classified as chloroplast and mitochondria were also removed, resulting in 334,417 sequences (corresponding to 3,497 distant ASVs) that were retained for the alpha diversity analysis. The Good's coverage index was calculated as (1 – [ASV singletons/ASVs total])* 100. For observed ASVs, this index was greater than 95.64%, indicating that the sample size was sufficient to capture the majority of bacterial diversity (Table 1).

To compare the diversity in the samples, alpha diversity indices such as observed ASVs, Chao1 richness, and the Shannon index were calculated with a cut-off value of 5,799 reads per sample, which was the smallest number of reads per sample obtained (Fig S1). Alpha diversity indices were then estimated for overall differences using the non–parametric Kruskal–Wallis test with the function kruskal.test within R software (Ogle et al., 2022), while Dunn's test was conducted following the Kruskal-Wallis test for multiple pairwise comparisons between the biotopes. A log (x+1) transformation was used to normalize the distribution of the data before performing the beta diversity analysis. Differences in bacterial community composition between the five biotopes were visualized with principal coordinate analysis (PCoA) using the plot_ordination function with the Bray–Curtis dissimilarity method and clustered using the ggclust function in R. Variation between biotopes was tested for significance with the adonis2 function in R (with the number of permutations set at 9999). All

146 statistical analysis was performed by R software (R Core Team, 2020) and the R package
147 vegan 2.6-2 (Oksanen et al., 2022).

148 The comparative examination of relative abundance primarily focused on the dominant phyla
149 (mean relative abundance > 1%) and genera (mean relative abundance > 2%). As the majority
150 of datasets did not follow an assumption of normality distribution, a comparison of relative
151 abundance between biotopes was undertaken using the Kruskal–Wallis test. The *p*-value was
152 adjusted for multiple comparisons using the Benjamini and Hochberg procedure and then
153 represented as a *q*-value. A *q*-value of <0.05 was considered statistically significant. To
154 determine if there were statistically significant differences in the bacterial communities of
155 coral, sediment, and seawater, STAMP software (v2.1.3) with Welch's t-test (corrected $p <$
156 0.05) was used (Parks et al., 2014).

157 Linear discriminant analysis effect size (LEfSe) was used to detect the most differentially
158 abundant taxa across all the biotopes, and the abundance box plot was visualized using the
159 diff_analysis and ggdiffbox functions in R (MicrobiotaProcess package) (Xu and Yu, 2022).
160 The linear discriminant analysis (LDA) score threshold of the bacterial taxa was set to 4.5 for
161 all the samples. The *p*-value in the LDA was corrected for multiple hypothesis testing using
162 the Benjamini and Hochberg false discovery rate (FDR) correction (White et al., 2009). The
163 resulting *p*-value, FDR, and LDA scores are shown in Table S2.

164 The core ASVs in coral microbiomes were determined using several percentage cut-offs
165 ranging from 30% to 100% (Hernandez-Agreda et al., 2017). In this study, the presence of an
166 ASV in at least 70% of samples was chosen as a conservative representation of the core
167 bacteriome. To identify both the unique and shared ASVs in the biotopes in our study, an
168 UpSet diagram was generated using the microbiomeutilities and ComplexHeatmap packages
169 in R.

3. Results

3.1. Diversity of bacterial communities

In our analysis of the diversity of the coral microbiome as well as in the seawater and sediment were investigated using 16S rRNA gene sequences. Alpha diversity for each sample was estimated through diversity indexes such as observed ASVs, Chao1 richness, and Shannon, which indicated a wide range of values. In general, the number of observed ASVs was found to be highest in *Lobophyllia* samples (LB1, LB2), followed by sediment samples (SE5, SE3), and then seawater samples (WA2, WA5) (Table 1). The Chao1 indices ranged from 201.0 for *Acropora muricata* (AF2) to 717.5 for *Lobophyllia* (LB1), while the Shannon index had the smallest value (3.77) for *Porites* (PO1) and the largest (5.70) for sediment (SE1) samples. A similar trend was seen in mean bacterial richness (Table 1), which was highest in *Lobophyllia* samples (585.7 ± 84.0 for observed ASVs and 615.5 ± 95.1 for Chao1) and lowest in seawater samples (200.2 ± 4.0 for observed ASVs and 207.4 ± 4.6 for Chao1). The Shannon index showed the lowest mean in *Porites* samples (3.8 ± 0.1) and the highest in sediment samples (5.6 ± 0.1).

Beta diversity was visualized using PCoA, which enabled a comparison of bacterial communities between samples. The results of the analysis indicated a distribution of bacteria divided into three groups based on their community structure. Coral-associated bacterial communities were on the positive side of PC1 and PC2, sediment samples were on the negative side of PC1 and PC2, while seawater samples were on the negative side of PC1 and the positive side of PC2. The coral samples also tended to be grouped closely together compared to the seawater and sediment biotopes (Fig. 1a). This was further supported by

hierarchical clustering with coral samples belonging to the same group, distinguishing them from seawater and sediment samples (Fig. 1b).

PERMANOVA (ADONIS, F = 22.39, R^2 = 0.86, p = 1E-04) indicated significant differences in bacterial composition between the samples taken from the five biotopes.

3.2. Bacterial community composition

The bacterial community composition associated with the different samples was analyzed using high-throughput sequencing technology. Based on the SILVA 16S rRNA gene database, a total of 3,497 bacterial ASVs were identified from 19 samples, with a similarity cut-off value of 97%. Of the 3,497 ASVs, the sediment biotope had the highest number of ASVs (1,364), followed by the *Lobophyllia* (1,315), *Porites* (465), and *Acropora muricata* (427) biotopes, whereas the lowest number of ASVs was observed in the seawater biotope (377). Only five ASVs appeared in all biotopes (Fig. S2). The 3,497 bacterial ASVs were then taxonomically classified into 29 phyla, 50 classes, 114 orders, 254 families, and 402 genera.

At the phylum level, while 29 phyla were detected, *Proteobacteria* and *Bacteroidota* accounted for more than 60% of the sequences (Fig. 2a). On the whole, *Proteobacteria* was the predominant phylum in most of the samples, with a relative abundance ranging from 29.2% in a sediment sample (SE2) to 76.8% in a *Lobophyllia* sample (LB1). *Desulfobacterota* (21.7–27.8%) was the most abundant phylum in the sediment samples, whereas it was a minor taxon in coral samples, accounting for less than 7.1% of relative abundance (Fig. 2a). There was a noticeable difference in the relative abundance of *Bacteroidota* between biotopes, beginning at 6.3% in an *Acropora muricata* sample (AF1),

gradually increasing from 13.2% in *Lobophyllia* (LB1) to 25.1% in seawater (WA5) samples, and peaking at 30.7% in a sediment sample (SE5). Other phyla, including *Firmicutes, Spirochaetota, Bdellovibrionota,* and *Calditrichota*, were also found in most of the biotopes, with a relative abundance lower than 6% (Fig. 2a).

The differences in relative abundance of bacterial phyla were examined using the Kruskal–Wallis test (corrected with $q < 0.05$, Benjamini–Hochberg method). In the findings, 12 out of 29 phyla exhibited statistically significant differences between the five biotopes (Table 2).

The mean relative abundance of *Proteobacteria* in *Lobophyllia* samples (0.76 ± 0.01) was higher than that in sediment samples (0.34 ± 0.05), whereas the abundance of *Desulfobacterota* in *Lobophyllia* samples was only 0.03, which was significantly lower compared to that in sediment samples (0.24 ± 0.02). Interestingly, *Actinobacteriota* was a highly abundant phylum in seawater samples (0.1 ± 0.01), but insignificant in other biotopes (below 1% in relative abundance) (Table 2).

At the genus level, the dominant genera in coral biotopes differed from those in seawater and sediment (Fig. 2b). While *Algicola, Vibrio,* and *Thalassotalea* were mainly found in coral samples, the *Rhodobacteraceae* strain *HIMB11* and *Candidatus_Actinomarina* were the dominant genera in seawater samples, and the *Sva0081* marine benthic group was the most abundant genus in sediment samples (Fig. 2b).

A statistical analysis of the top 30 genera (relative abundance greater than 2%) revealed significant differences in the mean relative abundance of bacterial genera between the five biotopes based on the Kruskal–Wallis test (corrected with $q < 0.05$, Benjamini–Hochberg method) (Table S1). In general, *Acropora muricata* and seawater samples exhibited a higher

241 number of dominant genera than the other samples. In particular, the top five genera,
242 including *Vibrio* (0.17 ± 0.03), *Thalassotalea* (0.1), *Malaciobacter* (0.08 ± 0.01),
243 *Thalassolituus* (0.07 ± 0.01) and *Halarcobacter* (0.06 ± 0.01) were remarkably higher in the
244 *Acropora muricata* samples compared with the other four biotopes. Likewise, *HIMB11*,
245 *Candidatus_Actinomarina,* the *NS4* marine group, the *NS5* marine group, and *Clade_Ia* were
246 significantly different between biotopes; the abundance of these was highest in the seawater
247 biotope with an average relative abundance of more than 5%. In the *Porites* biotope, *Algicola*
248 (0.3 ± 0.02) and *Marinifilum* (0.06 ± 0.01) were the most abundant bacterial genera, with a
249 higher dominance than in other biotopes (Kruskal–Wallis test, $q < 0.05$).

250 To compare the composition and relative abundance of dominant bacterial genera (mean
251 relative abundance > 2%) in more detail, we divided the five biotopes into three groups: coral
252 (including all nine coral samples), seawater (WA), and sediment (SE). Differences in
253 bacterial relative abundance between groups were observed at the genus level based on the
254 Welch t-test with STAMP software (corrected p-value < 0.05). The results of the statistical
255 analysis revealed that there was a highly significant difference in the relative abundance of
256 bacterial genera between groups and that each had its own distinct dominant genera (Fig. 3).
257 The bacteria in the coral group consisted mainly of *Algicola, Vibrio, Thalassotalea,* and
258 *Halarcobacter* (Fig. 3a). In contrast, the seawater was dominated by *HIMB11*,
259 *Candidatus_Actinomarina,* the *NS4* marine group, the *NS5* marine group, *Clade_Ia*, and
260 *Muricata* (Fig. 3b), while the sediment was dominated by the *Sva0081* benthic group,
261 *Woeseia,* and *Candidatus_Thiobios* genera (Fig. 3c).

262 *3.3. Biomarker analysis based on bacterial community abundance*

263 We designed a linear discriminant analysis to detect differentially abundant bacteria taxa in
264 the five biotopes by comparing bacterial contents across all biotopes using biomarkers in the

genomic data. In this comparison of the bacterial components in all five samples, a total of 23 bacterial taxa were identified (LDA score ≥ 4.5), with significant taxonomic variation between the samples (Fig. 4, Table S2). The analysis showed that the *Porites* samples had the greatest diversity of bacterial taxa of all the biotopes, while the number of biomarkers in *Lobophyllia* was the smallest, with only two biomarkers (Fig. 4).

This analysis found 23 bacterial biomarkers across all the taxonomic units (from species to phylum) with significant differences in relative abundance and the LDA score (Fig. 4). Of these, 10 biomarkers were identified in the *Porites* biotope: *Arcobacteraceae* (LDA = 4.51, FDR = 0.01), *Campylobacterota* (LDA = 4.54, FDR = 0.01), *Campylobacteria* (LDA = 4.54, FDR = 0.01), *Campylobacterales* (LDA = 4.54, FDR = 0.01), *Bacteroidales* (LDA = 4.57, FDR = 0.01), *Gammaproteobacteria* (LDA = 4.68, FDR = 0.01), *Algicola* (LDA = 4.81, FDR = 0.01), *Enterobacterales* (LDA = 4.98, FDR = 0.01), *Pseudoalteromonadaceae* (LDA = 4.82, FDR = 0.01) and *Algicola bacteriolytica* (LDA = 4.75, FDR = 0.01). In *Acropora muricata* samples, biomarkers included the phylum *Proteobacteria* (LDA = 5.10, FDR = 0.01), class *Alphaproteobacteria* (LDA = 5.01, FDR = 0.01), and order *Rickettsiales* (LDA = 4.87, FDR = 0.03). In seawater and sediment, each biotope had four biomarkers, whereas *Lobophyllia* had only two: *Terasakiellaceae* (LDA = 4.52, FDR = 0.02) and *Rhodospirillales* (LDA = 4.62, FDR = 0.02). Furthermore, the species *Algicola bacteriolytica* was found to be the lowest taxonomic unit in this study (Fig. 4, Table S2).

3.4. The core microbiome of Biotopes

Taxa present in at least 70% of the samples (with greater than 0.1% abundance) were defined as the core microbiota of the bacterial community. Using UpSet diagrams, we identified a total of 694 core ASVs across all samples, accounting for 19.8% of the total (3,497 ASVs). However, significant proportions of unique ASVs were detected in each biotope: 72 in

289 *Acropora muricata*, 79 in sediment, 119 in *Porites*, 124 in seawater, and 134 in *Lobophyllia*
290 (Fig. 5).
291
292 The *Lobophyllia* samples had the highest number of core ASVs (201), followed by *Porites*
293 (153) and seawater (153) samples, while *Acropora muricata* and sediment samples had the
294 lowest ASVs, with less than 100 for each (Fig. 5). We found no shared ASVs between
295 seawater and sediment samples. In contrast, the three types of coral showed similarity in core
296 bacteriome composition, with a total of 33 shared core ASVs (7 for *Acropora muricata* and
297 *Lobophyllia*, 9 for *Acropora muricata* and *Porites*, 12 for *Porites* and *Lobophyllia*, and 5 for
298 all three biotopes). Surprisingly, none of the ASVs were shared across all five biotopes, and
299 only one ASV was shared by four biotopes. However, five core ASVs were found to be
300 shared by all three coral biotopes: ASV2, ASV5, ASV46, ASV49, and ASV83. Four of these
301 ASVs were classified at the genus level (*Algicola, Vibrio, Alteromonas,* and *Catenococus*),
302 and only one was classified to species level (*Algicola bacteriolytic*) (Table S2).
303
304 **4. Discussion**
305 A number of studies have demonstrated that highly diverse microbial communities inhabit
306 coral reefs (Ceh et al., 2011; Hussien et al., 2019; Li et al., 2013). However, most of these
307 have investigated the diversity and function of the microbial communities associated with
308 coral and other hosts, while little attention has been given to the free-living bacteria present in
309 marine habitats such as the seawater and sediment surrounding coral reefs. Our study sought
310 to provide information on this through a comparative analysis of bacterial communities
311 present in five biotopes in the Gulf of Thailand.
312 *4.1. Differences in bacterial diversity and community structure*

We found that the coral-associated bacterial communities were highly diverse and significantly different from those in the seawater and sediment samples. Based on richness and Shannon evenness, benthic communities had the highest bacterial diversity of all the biotopes, followed by two corals (*Porites* and *Acropora muricata*), and seawater was the least diverse, except for the *Lobophyllia* biotope (Table 1). This is in line with the results reported by Kemp et al. (2015). Previous studies have suggested that sediment could be regarded as a microbial seed bank and that the bacterial community inhabiting the sediment shares a similar taxonomic composition with other biotopes such as algae, corals, and sea cucumbers (Cleary et al., 2019). Other studies have shown that sediment samples are rich in nutrients, as they accumulate large amounts of organic and inorganic compounds (Chaudhari et al., 2020; Dong et al., 2022), which could explain why our sediment samples had a more diverse bacterial population than the other samples.

A PCoA plot and clustering dendrogram also demonstrated the variation in the bacterial community structure of coral, sediment, and seawater biotopes (Fig. 1). In line with previous studies (Kemp et al., 2015; Schöttner et al., 2012, 2009), our findings revealed a clear separation in bacterial composition and community structure between biotopes, suggesting a divergence between non-host biotopes and host biotopes. However, it was found that host biotopes (the corals) tended to cluster more closely together on the PCoA plot than the other biotopes, suggesting that coral biotopes could contribute to stabilizing the microbial community structure. Notably, although forming distinct clusters, samples from *Lobophyllia* were closer to seawater and sediment than other coral biotopes (Fig. 1). Moreover, the core bacteriome analysis showed that populations of bacteria inhabiting *Lobophyllia* samples shared significantly more ASVs with sediment (13) and seawater (22) than with the other coral biotopes, and their distributional positions were closer to seawater and sediment than to

the other coral samples (Fig. 5). This suggests that the bacterial population in the *Lobophyllia* biotope could be affected by the surrounding sediment and seawater.

4.2. Common and specific bacteriome composition

Proteobacteria are thought to play an important role in various biogeochemical cycles, including nitrogen and carbon cycles, sulfur metabolism, and nutrient turnover (Quach et al., 2021; Zhou et al., 2020). In line with several previous studies (Carlos et al., 2013; Cleary et al., 2019), this study found that most of the samples were dominated by the *Proteobacteria* phylum. However, this finding contrasts with that of Kemp et al. (2015), which found a higher proportion of *Cyanobacteria* in marine biotope bacterial communities (Kemp et al., 2015). This difference may be due to the different hosts and geographic sites. Besides, the decline in relative abundance of *Proteobacteria* was found to be accompanied by a gradual increase in relative abundance of *Bacteroidota,* which reflected the shift in the components of bacterial communities across biotopes.

One of the main goals of our study was to identify bacteria that existed in specific biotopes and core taxa. Some of the biotopes we sampled had a high prevalence of specific bacterial taxa. For example, in the coral biotopes, there was a relatively high abundance of bacterial taxa belonging to the genera *Algicola, Vibrio, Alteromonas,* and *Catenococus,* and the species *Algicola bacteriolytic*. These formed part of the core bacteriome we identified. The STAMP statistical analysis also indicated that *HIMB11*, *Candidatus_Actinomarina*, the *NS4* marine group, *NS5* marine group*, Clade_Ia*, and *Formosa* genera were mainly found in seawater, while the *Sva0081* benthic group, *Woeseia,* and *Candidatus_Thiobios* accounted for a higher proportion of the bacterial community in the sediment. This indicates that each biotope harbors different bacterial taxa that play particular functional roles or are involved in the biotope's particular biogeochemical processes.

Although few investigations have highlighted the functional role of the genus *Algicola* in coral, its presence in diseased coral samples has been demonstrated in a number of findings (Becker et al., 2022; Meyer et al., 2019; Séré et al., 2016). Likewise, *Vibrio* (Becker et al., 2022; Meyer et al., 2019; Séré et al., 2016), *Alteromonas* (Séré et al., 2016), and *Catenococus* (Fifer et al., 2022) genera have been detected in disease-associated coral samples. According to Cervino et al. (2004) and Frydenborg et al. (2014), *Vibrio* could exist in the coral holobiont as an opportunistic bacterium when environmental conditions change. *Alteromonas* and *Thalassotalea* genera, on the other hand, benefit coral organisms by participating in metabolic pathways that allow the coral holobiont to absorb sources of carbohydrates (Kim et al., 2020), sulfur, and carbon more efficiently (Liu et al., 2018; Raina et al., 2009). In our study, all coral samples were in good health. Thus, the presence of bacteria (such as *Algicola* and *Vibrio*) may indicate that they are opportunistic pathogens that have no effect on host health.

Sediment samples consisted mostly of bacterial taxa from the marine benthic group, which are concerned with biogeochemical cycles such as carbon, sulfur, nitrogen, and sediment remineralization. In this study, the prevalence of the genera *Sva0081* sediment group, *Woeseia, Candidatus Thiobios* (Fig. 3), and *Thiogranum* (Table S1) in the sediment samples agrees with previous studies of sediments from other regions, including Valdibora Bay (Adriatic Sea), Mesoamerican Reef, Boihai Sea, and Bismarck Sea (Demko et al., 2021; Guo et al., 2022; Meier et al., 2019; Paliaga et al., 2019). Furthermore, the LDA result suggested that biomarkers in sediment biotope were the members of the phylum *Desulfobacterota,* which are recognized as sulfate–reducing (Flieder et al., 2021) and hydrocarbon–degrading microorganisms (Zhang et al., 2021). It can be said that the microbes associated with the sediment biotope were quite diverse and enriched by marine benthic groups, indicating that such bacteria may play an essential part in the metabolism pathways of marine sediments.

Seawater samples had the lowest bacterial diversity and composition among the five biotopes, with just three phyla (mean relative abundance>1%, Table 2). We discovered a higher proportion of the phylum *Actinobacteriota* in seawater column samples. This result is in contrast to the study of Kuang et al. (2015), who found that the phylum *Actinobacteriota* predominated in coral samples. Members of the actinobacterial group have previously been identified as potential sources of bioactive and antimicrobial compounds (Mahmoud and Kalendar, 2016). Some genera (Fig. 3b) in our findings were previously described in the study of Kopprio et al. (2021), including *NS4* marine group*, NS5* marine group*, HIMB11,* and *Formosa*. Accordingly, these genera lived in low oxygen environments and were potential indicators of eutrophication status in Vietnam's Cam Ranh and Van Phong Bays. As a result, the ubiquitous presence of actinobacterial members (such as *Actinobacteriota* and *Candidatus_Actinomarina*) and the genera *NS4* marine group*, NS5* marine group*, HIMB11,* and *Formosa* in the seawater samples surrounding coral reefs may be regarded as potential bacterial markers of environmental quality and host health.

5. Conclusion

This study is one of the few to date to examine not only the coral bacteriome but the bacteriome of its surroundings and whether and how these are interrelated. We were able to identify the bacterial communities associated with three genera of scleractinian coral (*Acropora, Lobophyllia,* and *Porites*), as well as those in the nearby sediment and seawater. While there were significant differences in the relative abundance of dominant bacteria in these different biotopes, *Proteobacteria* were dominant in most of them; however, there were no shared bacteria across all five biotopes. Coral biotopes had the highest diversity of taxa (15 biomarkers), followed by seawater (4 biomarkers) and sediment biotopes (4 biomarkers). It should be noted that sampling was only conducted in one session during one season, so

411 temporal variation in bacterial communities was not assessed. Sampling was also only carried
412 out in one location, and only healthy coral was selected. Further studies would be valuable to
413 broaden knowledge on the microbiome of these essential marine ecosystems, which are
414 currently facing numerous threats.
415
416 **Credit Author Statement**
417 **Duong Huy Nguyen**: Methodology, Software, Formal analysis, Data Curation, Writing -
418 Original Draft, Writing - Review & Editing. **Yvan Bettarel**: Project administration,
419 Resources, Writing - Review & Editing. **Ha Hoang Chu**: Supervision, Resources, Writing -
420 Review & Editing. **Van Ngoc Bui**: Conceptualization, Supervision, Project administration,
421 Funding acquisition, Resources, Writing - Review & Editing.
422
423 **Declaration of competing interest**
424 The authors declare that they have no known competing financial interests or personal
425 relationships that could have appeared to influence the work reported in this article.
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431
432 **Data Availability**
433 Database will be made available if required.
434

435 **References**

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Tables

Table 1. Alpha diversity estimates of bacterial communities associated with each sample analyzed in this study

*Samples were rarefied to 5,799 sequences before calculation of diversity metrics. Different letters indicate significant differences among biotopes based on Kruskal test and Dunn's test pairwise comparisons with p value < 0.05.

Table 2. Statistical analysis of the top 12 phyla (with relative abundance > 1%) detected across five biotopes (AF = *Acropora muricata,* LB = *Lobophyllia*, PO = *Porites*, SE = sediment, WA = seawater).

Figure Captions

Fig. 1. Cluster analyses of bacterial communities from five biotopes based on the Bray–Curtis dissimilarity. (a) Principal coordinate analysis (PCoA); (b) Hierarchical clustering dendrogram (Ward algorithm). PCoA1 explained 29.2% and PCoA2 27.6% of the total variation in bacterial community structure.

Fig. 2. Taxonomic classification and top 20 bacterial taxa in relative abundance across biotopes (phyla, Fig. 2a and genera Fig. 2b).

Fig. 3. STAMP analysis (Welch's t-test, $p < 0.05$) of significant differences of the dominant genera in three different groups (coral, water, and sediment). Comparison of genera between coral and sediment (a), coral and seawater (b), and sediment and seawater groups (c). Bars indicate the standard deviation, and corrected *p*-values are indicated to the right. For each comparison, the mean proportion of genera (left) and difference in mean proportions (right) were represented.

Fig. 4. Linear discriminant analysis effect size (LEfSe) results on biotope bacteriomes. On the left, the abundance distribution of biomarkers from five biotopes is shown as a boxplot ($p < 0.05$, Kruskal–Wallis test). On the right, each dot represents the mean of $\log_{10}$ (LDA score) of a biomarker with the highest abundance.

Fig. 5. UpSet plot showing intersections between the bacteria community in the five different biotopes. Numbers to the left represent the number of core ASVs in each group, while numbers above the graph show the unique and shared ASVs.

Figure 1

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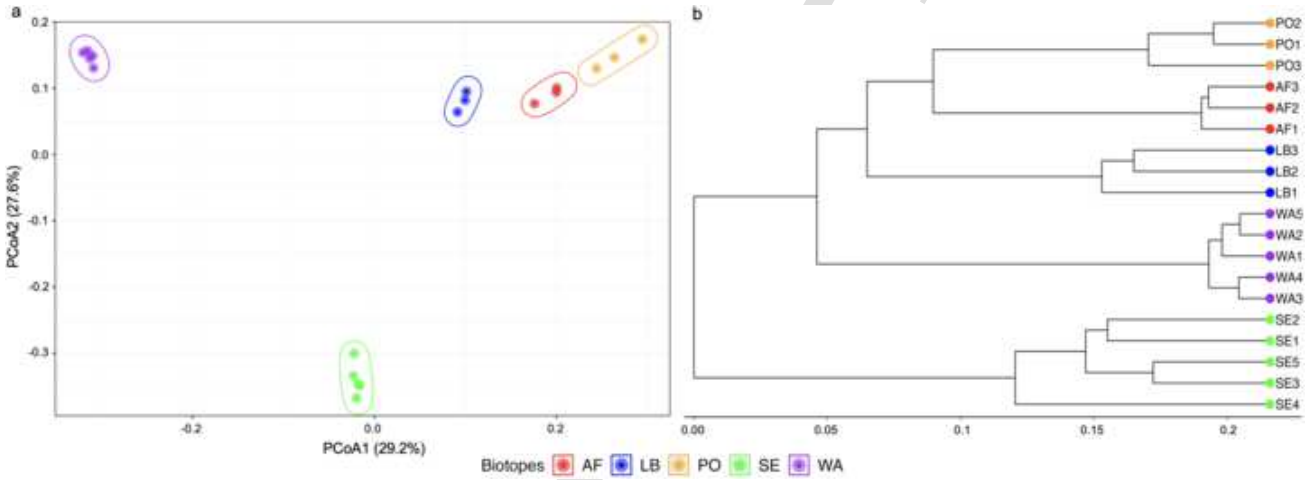

Figure 2

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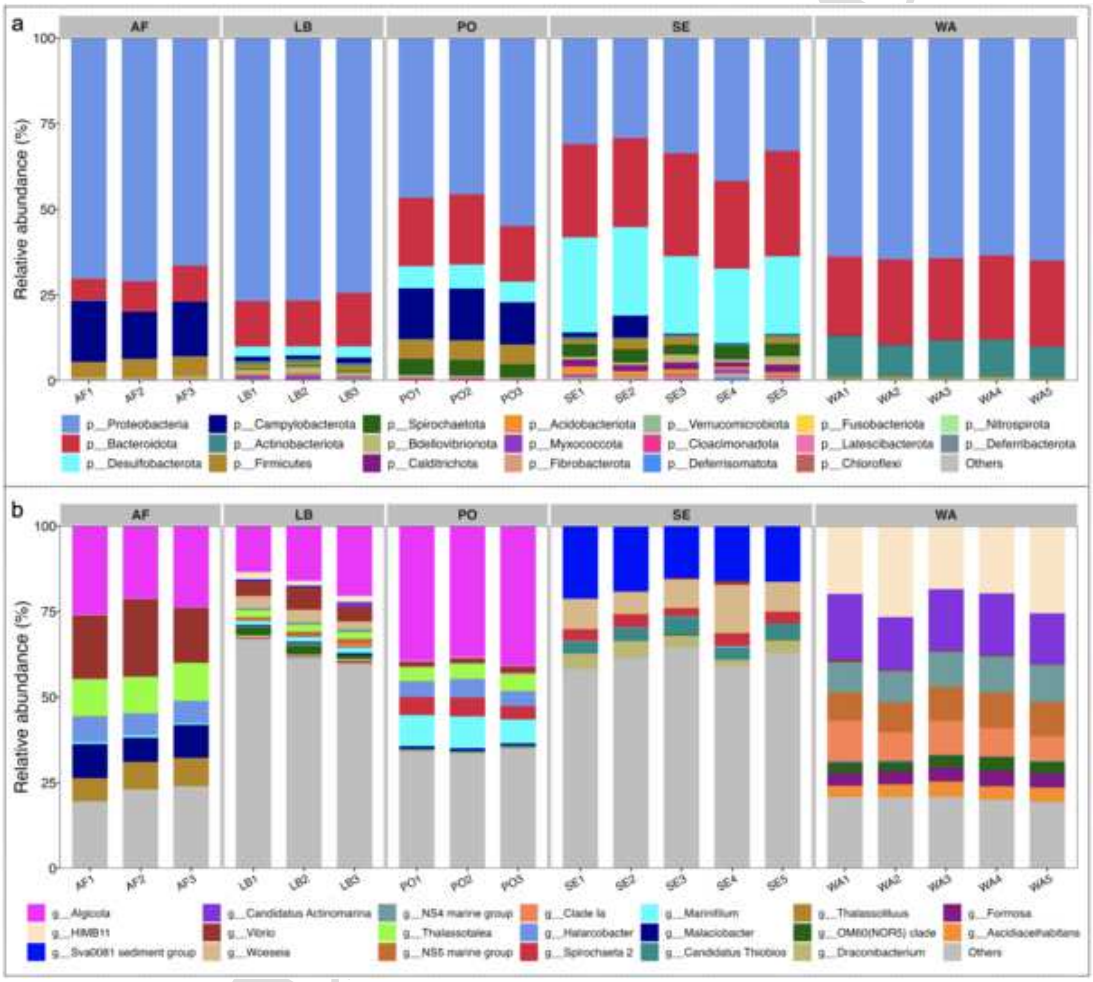

Figure 3

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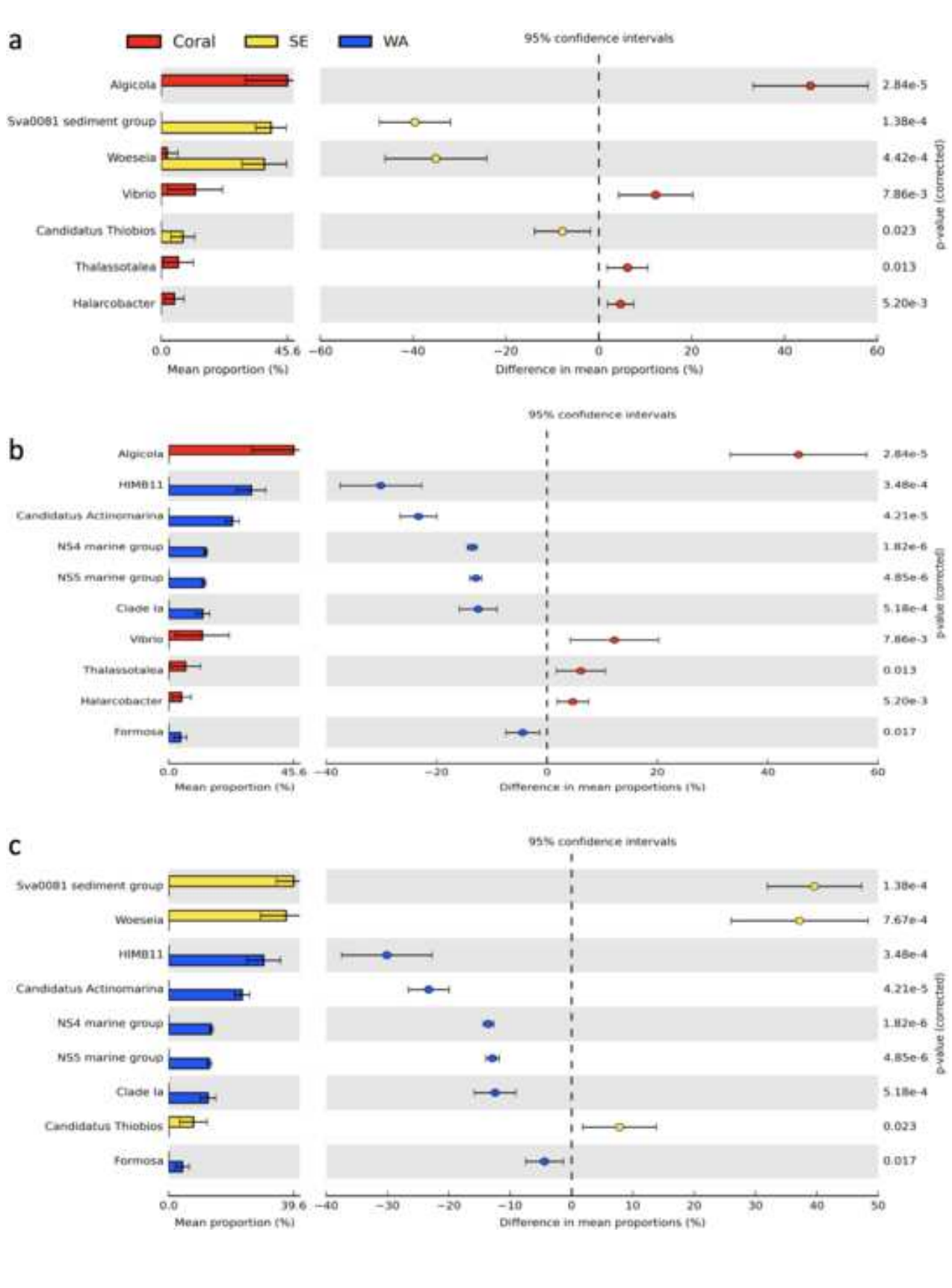

Figure 4

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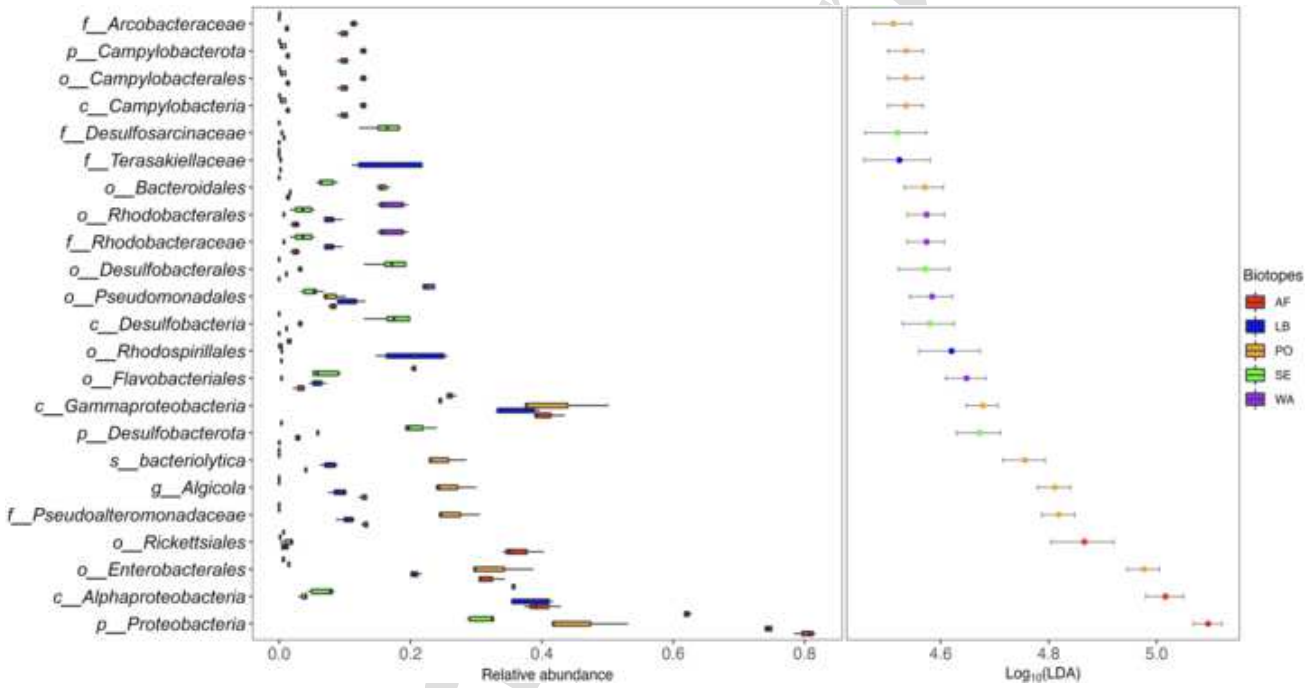

Figure 5

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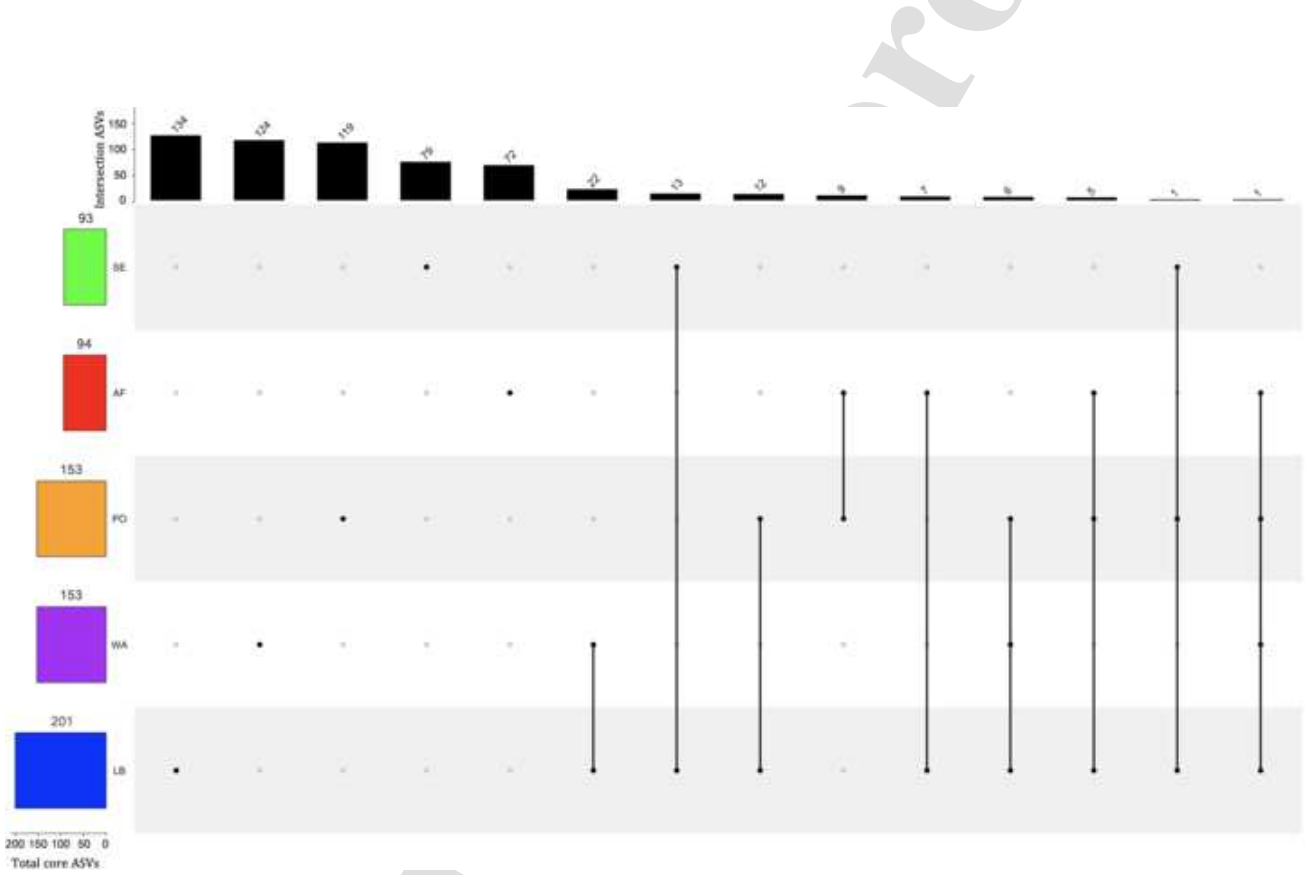

Table:

Title: **An analysis of the bacterial community in and around scleractinian corals of Phu Quoc Island, Vietnam**

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Table 1. Alpha diversity estimates of bacterial communities associated with each sample analyzed in this study

Sample	Biotope	Sequence Reads*	ASVs Observed	Chao1 Richness	Shannon Index	Good's Coverage
AF1	*Acropora muricata*	21,081	227	241.78	3.90	98.69
AF2		18,834	194	201.00	3.97	99.49
AF3		21,736	217	222.04	4.00	98.64
Mean (standard deviation) for *Acropora muricata* samples			212.7(16.9)^{ab}	221.6(20.4)^{ab}	4.0(0.1)^{a}	
LB1	*Lobophyllia*	22,922	676	717.46	5.44	100.00
LB2		19,100	571	599.71	5.24	98.62
LB3		21,198	510	529.32	4.77	98.84
Mean (standard deviation) for *Lobophyllia* samples			585.7(84.0)^{a}	615.5(95.1)^{a}	5.2(0.3)^{ab}	
PO1	*Porites*	22,983	228	239.54	3.77	98.70
PO2		19,033	222	240.12	3.78	100.00
PO3		32,555	302	326.57	3.95	98.70
Mean (standard deviation) for *Porites* samples			251.0(44.6)^{a}	268.7(50.1)^{a}	3.8(0.1)^{ab}	
SE1	Sediment	9,558	417	423.12	5.70	95.64
SE2		10,929	409	426.88	5.49	98.80
SE3		10,746	435	449.00	5.64	97.10
SE4		5,799	315	324.71	5.39	99.10
SE5		10,474	437	444.91	5.66	99.77
Mean (standard deviation) for sediment samples			402.6(50.4)^{c}	413.7(51.0)^{b}	5.6(0.1)^{b}	
WA1	Seawater	15,885	200	213.91	4.48	99.01
WA2		17,240	195	206.14	4.37	98.48
WA3		18,491	206	210.23	4.50	99.52
WA4		18,525	201	204.27	4.51	100.00
WA5		17,328	199	202.60	4.43	100.00
Mean (standard deviation) for seawater samples			200.2(4.0)^{b}	207.4(4.6)^{c}	4.5(0.1)^{c}	

Table 2 Statistical analysis of the top 12 phyla (with relative abundance > 1%) detected across five biotopes (AF = *Acropora muricata*, LB = *Lobophyllia*, PO = *Porites*, SE = sediment, WA = seawater)

Taxa	Relative abundance (Mean ± S.D)					*p*-value	*q*-value
	AF	LB	PO	SE	WA		
Proteobacteria	0.69±0.02	0.76±0.01	0.49±0.05	0.34±0.05	0.64±0.01	1.80E-03	4.65E-03
Bacteroidota	0.08±0.02	0.14±0.01	0.19±0.02	0.28±0.02	0.24±0.01	1.80E-03	4.65E-03
Desulfobacterota	0	0.03±0	0.06±0	0.24±0.02	0	1.80E-03	4.65E-03
Campylobacterota	0.15±0.02	0.01±0	0.14±0.01	0.04±0.03	0	2.80E-03	4.65E-03
Actinobacteriota	0	0	0	0	0.1±0.01	2.20E-03	4.65E-03
Firmicutes	0.05±0.01	0.01±0	0.05±0	0.02±0.01	0	7.10E-03	7.10E-03
Spirochaetota	0	0	0.04±0	0.04±0	0	3.10E-03	4.65E-03
Bdellovibrionota	0	0.01±0	0	0.02±0	0	4.40E-03	5.28E-03
Calditrichota	0	0	0	0.02±0	0	4.30E-03	5.28E-03
Acidobacteriota	0	0	0	0.01±0	0	1.80E-03	4.65E-03
Myxococcot	0	0.01±0	0	0.01	0	5.40E-03	5.89E-03
Fibrobacterota	0	0	0	0.01	0	2.60E-03	4.65E-03

Credit Author Statement

Duong Huy Nguyen: Methodology, Software, Formal analysis, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Yvan Bettarel**: Project administration, Resources, Writing - Review & Editing. **Ha Hoang Chu**: Supervision, Resources, Writing - Review & Editing. **Van Ngoc Bui**: Conceptualization, Supervision, Project administration, Funding acquisition, Resources, Writing - Review & Editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: