

Comparison of Symbiotic Bacterial Community of Soft Corals *Sarcophyton* and *Sinularia* of the Hainan Province, (South China Sea, China)

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Abstract

Changes in the microbial community associated with environmental impacts can lead to opportunistic infections, coral disease and death. Diversity analysis and community comparison were performed on 23 collected soft Coral specimens from South China Sea surrounding Hainan Province (China) based on Illumina MiSeq sequencing. The results showed that Proteobacteria was the main symbiotic bacteria in soft corals. In the same geographical location, the diversity and abundance of symbiotic bacteria in the genus *Sinularia* are higher than genus *Sarcophyton*. Unlike *Sinularia*, the genus *Sarcophyton* is more inclined to Tenericutes. Furthermore, the same coral species has different bacterial community structure in different environments. The temperature difference between sampling points at 2 °C is the main factor affecting the results. A large number of Endozoicomonas found in stone corals have not become the dominant bacteria associated with soft corals. Coral-related pathogenic bacteria were not found in this investigation. This study provided a baseline for future studies of soft coral microbiomes, and assessment of functions of host metabolites and soft coral holobionts. Our result documented that same coral species in each locality represent identical pattern of bacterial diversity and community

Introduction

Alcyonacea is an order of Coelenterata, Anthozoa, and Octocorallia. The sea area near Hainan Island is rich in soft coral resources, and their symbiotic microbes have become an important target for the study of marine natural products (Hassan et al., 2019). Symbiotic microorganisms play an important role in driving the nutritional transformation and community succession of coral reef ecosystems and provide new ideas for scientists to develop new marine drugs (Sang et al., 2019).

As an important part of the symbiotic microbial system, symbiotic bacteria play a key role in the material cycle (Lesser et al., 2004), energy flow (Mao-Jones et al.,

2010), and healthy growth of coral ecosystems (Mahmoud & Kalendar, 2016). During coral bleaching, some nitrogen-fixing bacteria in mucus can replace the algae to feed coral organisms. For example, *Oculinary patagonica* in the Mediterranean Sea can synthesize organic matter and supply coral tissue to help corals survive during the crisis of lost zooxanthella (Teplitski & Ritchie, 2009). Moreover, probiotics can also promote the ecological balance of flora in or around the host (Merrifield et al., 2010). The coral symbiotic bacteria community is a complex dynamic combination, and this complex community structure is susceptible to many factors. Regional differences, eutrophication, diseases (Rosenberg et al., 2009) and other driving forces can lead to changes in coral symbiotic microbial community

structure. For example, McKew et al. (2012) found that the same species of corals in the Caribbean and Indonesian seas have different commensal bacterial community, and the diversity and abundance of symbiotic bacteria are significantly different. Thurber et al. (2009) pointed out that temperature, dissolved organic carbon content, and acidity can affect the symbiotic bacterial community structure of corals. Furthermore, the composition of the coral symbiotic bacteria is affected by environmental factors. Thus, the structure of the coral symbiotic bacteria community can better elucidate the growth condition of the corals. In a study of coral symbiotic bacteria under healthy and bleached conditions, Yu et al. (2019) found that some pathogens (*Vibrio*, *Pseudospirillum*, *Alteromonas*, and *Coxiella*) are present in albino individuals at high rates. Guest et al. (2016) pointed out that the breaking of the balance of microbial structure would increase the susceptibility of corals to disease, as well as the stability of the community or the biomarker that indicates the risk of bleaching.

Environmental factors can alter relationships among coral hosts and its bacterial community. Neulinger et al. (2008) suggested there was no indicative dissimilarity between the bacterial communities of deep-sea stony coral *Lophelia* from different localities. In contrast, bacterial diversity of *Porites* and *Acropora* from two geographical localities in Caribbean Sea (Mexico) and Indo-Pacific (Indonesia) were significantly different (McKew et al. 2012). Additionally, samples of soft coral *Scleronephthya gracillimum* from different geographical sites represented distinguished differentiation in bacterial diversity (Seonock et al. 2017). It is evident that environmental factors can alter the diversity structure of bacteria at different geographical areas.

Based on high throughput sequencing of extender, which has been widely used in the study of microbial diversity of coral symbiosis, more microbial groups have been found. Liang et al. (2017) obtained a high-throughput sequencing library of 16S rRNA gene to analyze the bacterial community structure of various massive and dendritic corals collected from the Xinyi Reef in the Nansha Islands of the South China Sea. They

obtained the symbiotic bacteria database of the Nansha reef-building corals in China. The commensal bacterial species have their own preferences in terms of their choice of corals to inhabit (Liang et al., 2017). In this study, the bacterial community structures of 23 soft coral samples collected from Ganzhe island (18°45'N, 110°30'E), Dazhou Island (18°40'N, 110°28'E), and Ximao Island (18°15'N, 109°22'E) were analyzed by 16S rRNA gene high throughput sequencing. Although bacterial community of stony coral have been studied well, there is a lack of information about soft coral. This study performed to explore the diversity of bacterial community with different localities from South China Sea (Hainan Province coral reefs, China) in two common host soft corals genera (*Sarcophyton* and *Sinularia*). The main aim is to investigate diversity of symbiotic bacterial associated with localities and coral species.

Materials and Methods

Coral Collection

23 soft coral specimens of *Sinularia* and *Sarcophyton* were collected from the coral reef areas in Ganzhe Island (18°45'31.21"N; 110°29'56.98"E), Dazhou Island (18°40'43.87"N; 110°28'45.68"E), and Ximao Island (18°14'9.03"N; 109°22'27.06"E) (Figure 1). The sampling distance was more than 10 meters, and three samples were collected per individual. The collected specimens were washed with aseptic seawater and placed in aseptic plastic bags. All specimens were stored briefly at low temperature (0 °C–4 °C) until laboratory DNA extraction. The coral species were identified in laboratory and compared with bone needles and COI + *igr1*+ *msh1* barcode (Zhou et al., 2019). Species information is shown in Table 1.

DNA Extraction

The crown tissue of *Sarcophyton* (2.5×2.5 cm) and finger tissue of *Sinularia* (2-3 cm) were cut with a pair of scissors. The total DNA (about 50 mg, including tissue and mucus) was extracted from marine animal genomic DNA extraction kit (Tsingke, Guangzhou, China). The

Table 1. Coral species list in this study

Location	Total No	Genus	Species	No	Abb.
Ximao Island (XI)	6	<i>Sarcophyton</i>	<i>S. trocheliophorum</i>	3	XI_Satr01; XI_Satr02; XI_Satr03
			<i>S. grandilobata</i>	2	XI_Sigr01; XI_Sigr02
		<i>Sinularia</i>	<i>S. querciformis</i>	1	XI_Siqu03
			<i>S. crassum</i>	1	GI_Sacr04
Ganzhe Island (GI)	8	<i>Sarcophyton</i>	<i>S. glaucum</i>	2	GI_Sagl05; GI_Sagl09
			<i>S. cherbonnieri</i>	2	GI_Sach06; GI_Sach10
		<i>Sinularia</i>	<i>S. wanannensis</i>	3	GI_Siwa10; GI_Siwa11; GI_Siwa12
			<i>S. ehrenbergi</i>	2	DI_Saeh07; DI_Saeh11
		<i>Sarcophyton</i>	<i>S. crassum</i>	1	DI_Sacr08
Dazhou Island (DI)	9		<i>S. maxima</i>	2	DI_Sima04; DI_Sima06
			<i>S. humilis</i>	1	DI_Sihu05
		<i>Sinularia</i>	<i>S. querciformis</i>	3	DI_Siqu07; DI_Siqu08; DI_Siqu09



Figure 1. Sample collection location. Ganzhe Island (GI), Dazhou Island (DI), and Ximao Island (XI). Map data ©2020 Google.

instructions on the kit were strictly followed. The prepared DNA is stored at -80°C after quality detection.

PCR Amplification and Illumina MiSeq Sequencing

Using DNA as template, the V3-V4 variable region of bacterial 16S rRNA gene was amplified by PCR with forward primer 338F(5'-ACTCCTACGGGAGGCAGCAG-3') and reverse primer 806R(5'-GGACTACHVGGGTWTCTAAT-3') (Mori et al., 2014; Xu et al., 2016). PCR was carried out in a total volume of 50 μl containing 48 μl Taq polymerase (2 \times Easy Taq[®] PCR SuperMix, Code#AS111 +dye, TransGen Biotech CO., Ltd. CHN), 1 μl solution of DNA and 0.5 μl of each primer. PCR amplification was carried out following Liang et al (2017). The quality of PCR products were considered using 2% agarose gel and it was purified by Gel extraction kit (Axygen Scientific, Inc; CA, USA) following its instruction manual before sequencing. Finally, the duplicate samples were combined and the PE2 \times 300

library was constructed according to the standard operating procedures of the Illumina MiSeq platform. Sequencing was performed on the Illumina MiSeq PE300 platform (Majorbio, Shanghai, China).

Data Analysis

According to the method of Liang et al. (2017), raw dataset were standardized using the software Trimmomatic v.0.39 (Bolger et al., 2014) to eliminate the reads with low quality and homopolymer inserts (<20). The remaining high-quality sequences were subjected to OTU cluster analysis and taxonomic analysis utilizing the RDP (Ribosomal Database Project) by USEARCH (UPARSE) (Woo et al., 2017). Based on the results of OTU cluster analysis, the diversity of single specimen (Alpha diversity) was obtained by Alpha diversity index analysis of sample clustering results by software Mothur version v.1.30.1 with 1000 iterations (Schloss et al., 2011). The coverage, abundance, and diversity of microbial

community were reflected by Good's species coverage (Coverage), community richness (Ace), and community diversity (Shannon) index. Taxonomy was identified and compared via SILVA database (Quast et al., 2013) following Qiime platform. Additionally, the Principal Coordinate Analysis (PCoA) was performed using the beta diversity index the OTUs level (Liang et al., 2017).

Results

Twenty-three specimen raw reads were obtained by high throughput sequencing, with a total of 1 175 650 valid sequences. The effective sequences of each specimen were more than 27 000, and the length of the sequences was 421–460 bp. The coverage index for all specimen databases was greater than 99%, and the sequencing results accurately reflected the true flora of the specimens. The specimen alpha-diversity index was listed in Table 2.

The average OTU number of genus *Sinularia* in both Ximao Island and Ganzhe Island was significantly higher than that of genus *Sarcophyton*, and the average OTU number of genus *Sinularia* in Dazhou Island was slightly lower than that of genus *Sarcophyton*. Nine specimens had OTU numbers greater than 500, of which six specimens were from genus *Sinularia* and three from genus *Sarcophyton*. On the contrary, less than 300 OTU samples were from the genus *Sarcophyton*. The values of Shannon index of all specimens were between 1.24 and 5.10 which the GI_Sagl09 specimen and the XI_Sigr02 specimen were respectively the minimum and maximum values. The difference of the large numerical value indicated a great difference in the diversity of the

symbiotic bacteria in different specimens. The difference of community richness ACE index between specimens was about the same as that of the Shannon index, except for a few cases such as the XI_Satr02 and XI_Satr03 specimens.

A total of 46 bacterial phyla were detected in 23 examined coral specimens which 24 bacterial phyla were shared among all coral hosts (Figure 2). The dominant phyla of all specimens were Proteobacteria (39.99%), Tendericutes (22.35%), Firmicutes (14.13%), Bacteroidetes (9.58%) and Acinetobacteria (8.29%) (Figure 3). After cluster analysis of each specimen, the abundance of symbiotic Proteobacteria was found to be the highest in the genus *Sinularia* from Dazhou Island. Its abundance was more than 60% (Figure 4). The specimen with the highest abundance was Si06 (92.8%). Proteobacteria was detected in other specimens, and the minimum abundance was still more than 10%. Unlike the genus *Sinularia*, the symbiotic bacteria of the *Sarcophyton* genus were more abundant. In addition to the large number of Proteobacteria, Bacteroidetes were maintained at a higher abundance (more than 30%) in the three specimens of the *Sarcophyton* genus from Ximao Island. The remaining nine specimens of genus *Sarcophyton* were rich in Tendericutes. In addition, Cyanobacteria were also found in all specimens, and their abundance was the highest in the DI_Saeh07 specimens (13.5%).

At the class level (Figure 5), Mollicutes, Gammaproteobacteria, and Alphaproteobacteria were the most abundant in all specimens. Among them, Mollicutes was more abundant in the genus *Sarcophyton* than genus *Sinularia*, whereas

Table 2. Numbers of sequences, operational taxonomic units (OTUs) (97%) and diversity estimates of bacteria associated with different corals

Location	Abb.	No. of. Seq	OTUs	ACE	Chao	Coverage	Shannon
Ximao Island	XI_Satr01	36530	478	591.26	591.06	0.996304	3.55
	XI_Satr02	33589	344	435.80	459.12	0.997023	3.49
	XI_Satr03	47633	356	471.77	469.29	0.997733	3.08
	XI_Sigr01	43586	866	892.66	913.63	0.998555	4.94
	XI_Sigr02	35024	942	972.04	987.02	0.997887	5.10
	XI_Siqu03	83226	659	681.71	696.14	0.999519	5.03
Ganzhe Island	GI_Sacr04	72077	279	290.18	289.91	0.999778	3.00
	GI_Sagl05	77683	324	333.60	347.75	0.999743	2.70
	GI_Sach06	88922	324	332.75	347.75	0.999775	2.02
	GI_Sagl09	41178	220	230.54	239.00	0.999514	1.24
	GI_Sach10	74655	352	356.14	358.00	0.999826	2.29
	GI_Siwa10	77457	518	528.61	528.00	0.999793	5.01
	GI_Siwa11	58289	392	397.96	401.75	0.999777	4.35
	GI_Siwa12	31863	309	324.56	337.88	0.999310	4.73
Dazhou Island	DI_Saeh07	58289	520	553.43	567.53	0.999262	3.61
	DI_Sacr08	54505	648	658.70	660.83	0.999596	4.92
	DI_Saeh11	27271	495	504.33	512.65	0.999083	4.06
	DI_Sima04	33513	548	674.00	641.44	0.995554	2.93
	DI_Sihu05	36534	424	583.70	593.03	0.995867	2.74
	DI_Sima06	37338	398	499.86	501.71	0.996759	2.50
	DI_Siqu07	32416	587	639.41	646.07	0.997100	3.06
	DI_Siqu08	43343	450	482.38	498.89	0.998639	2.85
	DI_Siqu09	50729	606	629.26	651.23	0.999034	3.89

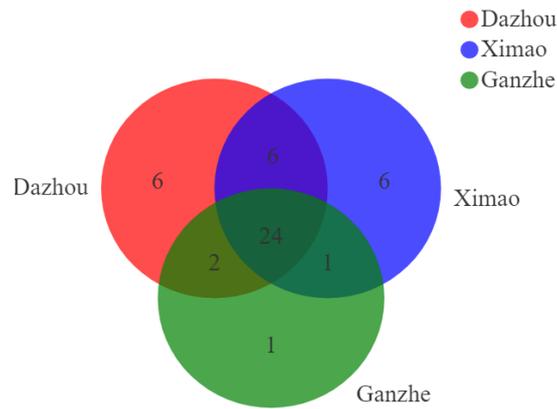


Figure 2. Venn diagram analysis. Different colors represent different groups (different islands), overlapping parts represent unique phyla in multiple groups (different islands), no overlapping parts represent phyla specific to the grouping (different islands), and numbers represent the corresponding number of phyla.

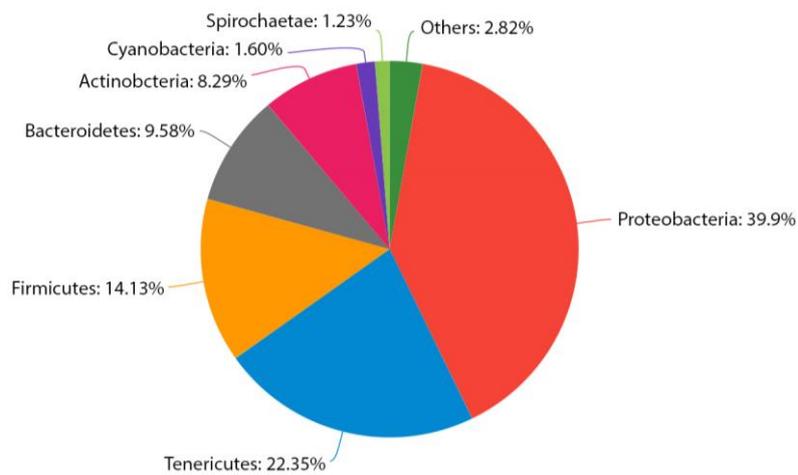


Figure 3. Microbial community pieplot on phylum level: All samples. “others” represent the bacterial phyla with abundances of less than 0.01%.

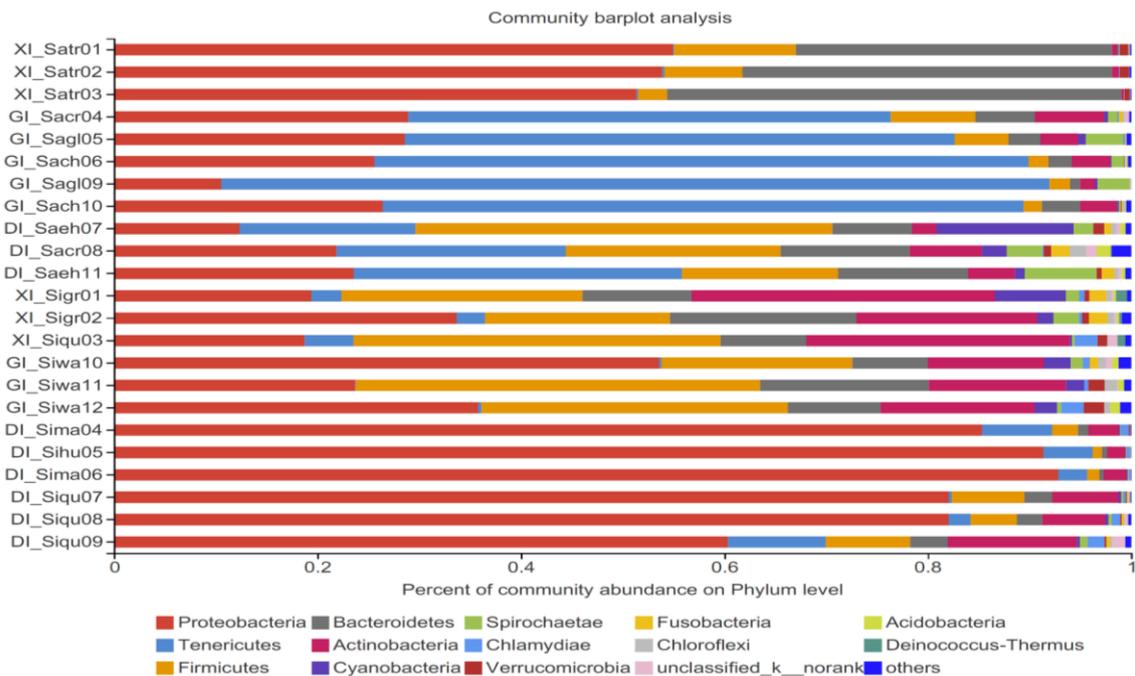


Figure 4. Bacterial composition profiles. Taxonomic classification of bacterial reads retrieved from different coral species on phylum level. “others” represent the bacterial phyla with abundances of less than 0.01%.

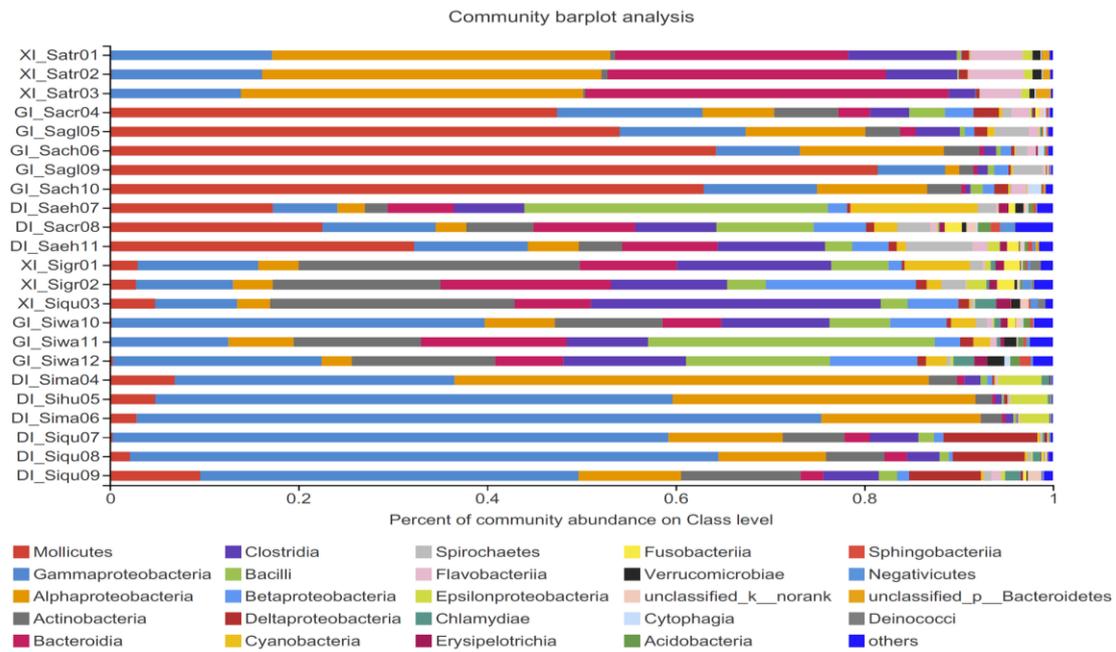


Figure 5. Bacterial composition profiles. Taxonomic classification of bacterial reads retrieved from different coral species on Class level. “others” represent the bacterial phyla with abundances of less than 0.01%.

Gammaproteobacteria were more abundant in genus *Sinularia* than genus *Sarcophyton*. Mollicutes comprised 81.4% of the total bacterial population in the GI_Sagl09 specimen, but was only 9.6% in the most highly abundant specimen DI_Siqu09 in the genus *Sinularia*. Gammaproteobacteria has a maximum abundance values of 72.6% and 17.1% in genus *Sinularia* and genus *Sarcophyton* respectively.

Totally identified bacterial were divided in 408 families (Figure 6) whereas 336 and 345 belonged to *Sarcophyton* and *Sinularia*, respectively. Spiroplasmataceae, Rhodobacteraceae, and Halomonadaceae are dominant flora with abundance percentages of 20.8%, 9.5%, and 4.0%, respectively. Rhodobacteraceae is a potential pathogen of coral. In addition, the abundance of symbiotic bacteria showed high similarity in the same coral genus at the same sampling point. For example, Rhodobacteraceae was most abundant in the genus *Sarcophyton* collected from Ximao Island, whereas Spiroplasmataceae were most abundant in genus *Sarcophyton* collected from Ganzhe Island. Unlike genus *Sarcophyton*, symbiotic bacteria in all specimens that were collected from Ximao Island and Ganzhe Island showed high diversity and do not contain major bacterial families.

PCoA analysis obviously showed seven separated groups (Figure 7). Although result cannot display a relationship between bacterial diversity and localities, it can be generally documented that in each locality bacterial characteristics of same coral species were clustered in separated group.

Discussion

At present, stony coral has been investigated by many experts for its unique reef-building capacity (Lee

et al., 2012; Li et al., 2013; Li et al., 2014). Although soft corals have high potential medicinal value (Li et al., 2019), there is no equivalent research report. This study collected two genera from three coral reefs of Ganzhe Island, Dazhou Island, and Ximao Island of Wanning City, Hainan Province, and a total of 23 soft coral specimens from nine species. A database of 23 commensal bacteria was obtained.

Significant differences in symbiotic bacteria in different species of stony corals have been confirmed (Hong et al., 2009). This study also found that this variability also exists in soft corals, and the three sea areas in this survey all showed this. There were significant differences in the abundance and diversity of symbiotic bacteria between different genera, but the differences in abundance and diversity of symbiotic bacteria in different species of a genus were not obvious (Table 2). The reason was unclear. Using the same method, Liang et al. (2017) found 55 bacteria phyla in 25 stone coral specimens, whereas in 23 soft coral specimens in this study, we detected 46 phyla. More bacterial symbiosis leads to greater uncertainty. Gardner et al. (2013) pointed out that for each additional species, the probability of instability increases by 2^{n-1} . Pollock et al. (2019) found that the reduction of coral bacterial community is more beneficial to coral immune function. Although this finding does not prove that the gradual replacement of the coverage of stony corals by soft corals in the waters around Hainan Island was due to the existence of fewer symbiotic bacteria, the symbiotic relationship between bacteria and genus *Sarcophyton* or genus *Sinularia* was more stable than that of bacteria and stony corals.

Consistent with previous studies, the dominant symbiotic bacteria of soft corals in the South China Sea is Proteobacteria (39.99%), but its abundance in soft

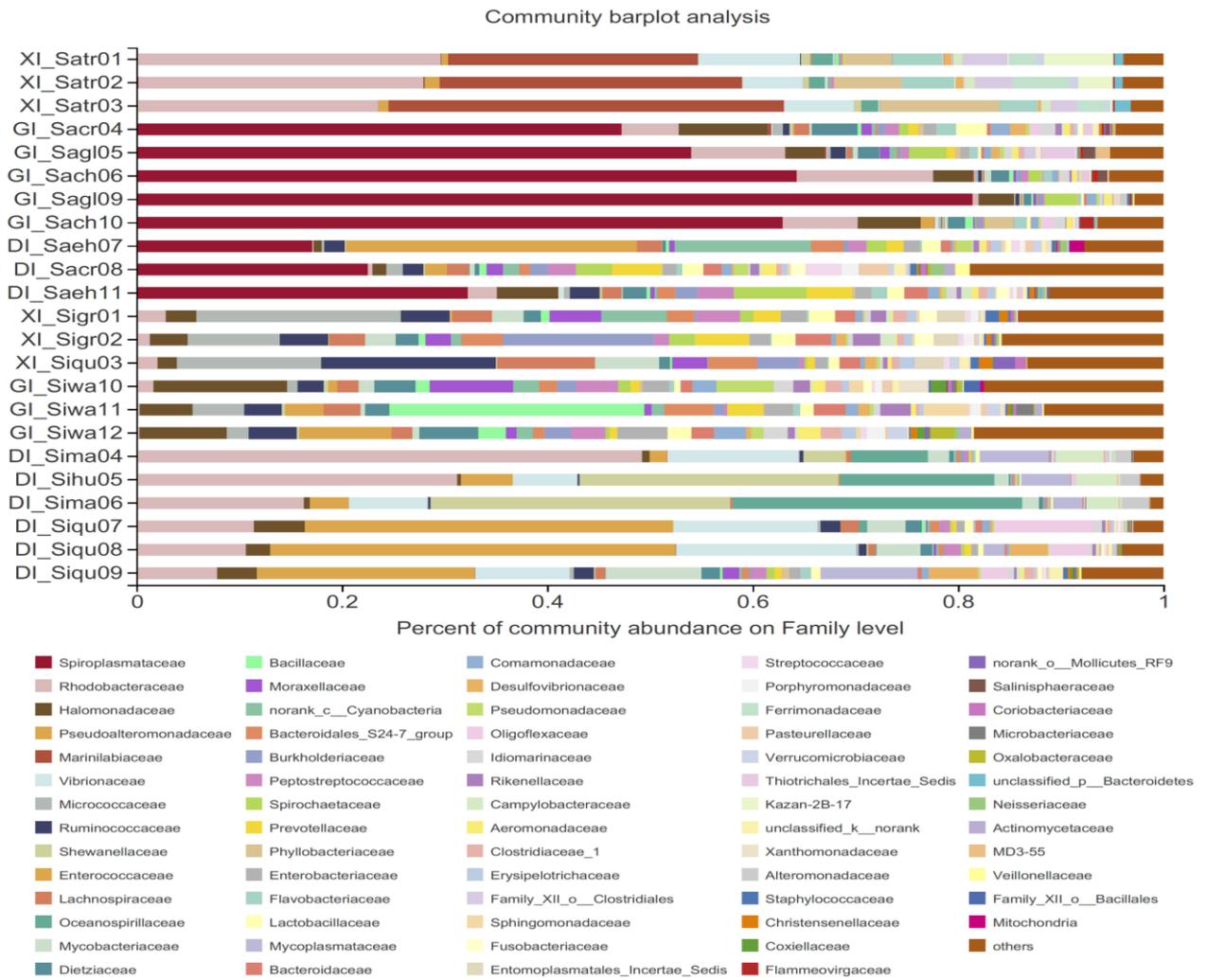


Figure 6. Bacterial composition profiles. Taxonomic classification of bacterial reads retrieved from different coral species on Family level. “others” represent the bacterial phyla with abundances of less than 0.01%.

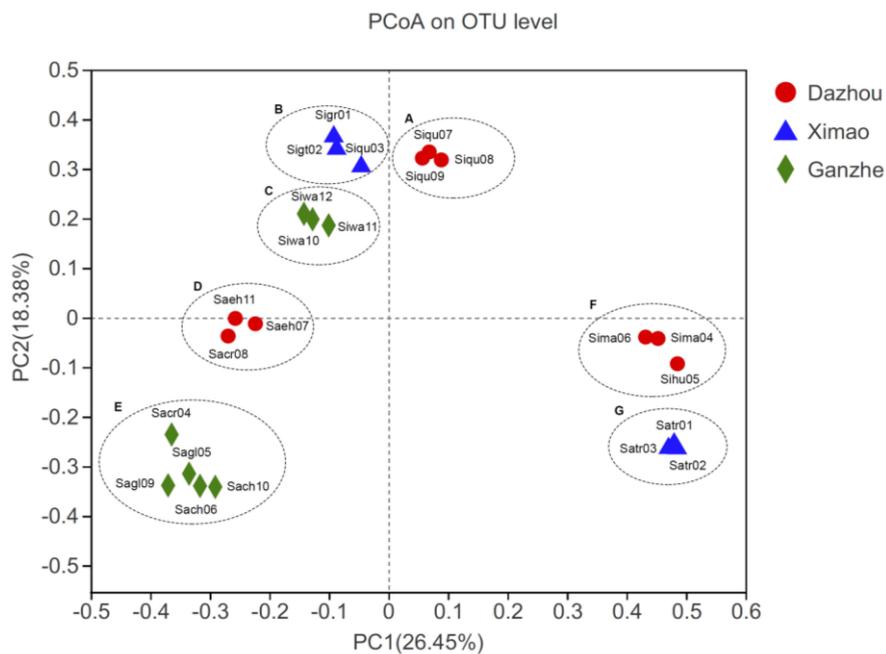


Figure 7. Principal co-ordinates analysis (PCoA) plot based on the OTU level from 25 coral species. The scatter plot is of principal coordinate 1 (PC1) vs. principal coordinate 2 (PC2). PC1 and PC2 represent the principal factors affecting bacterial composition associated with corals.

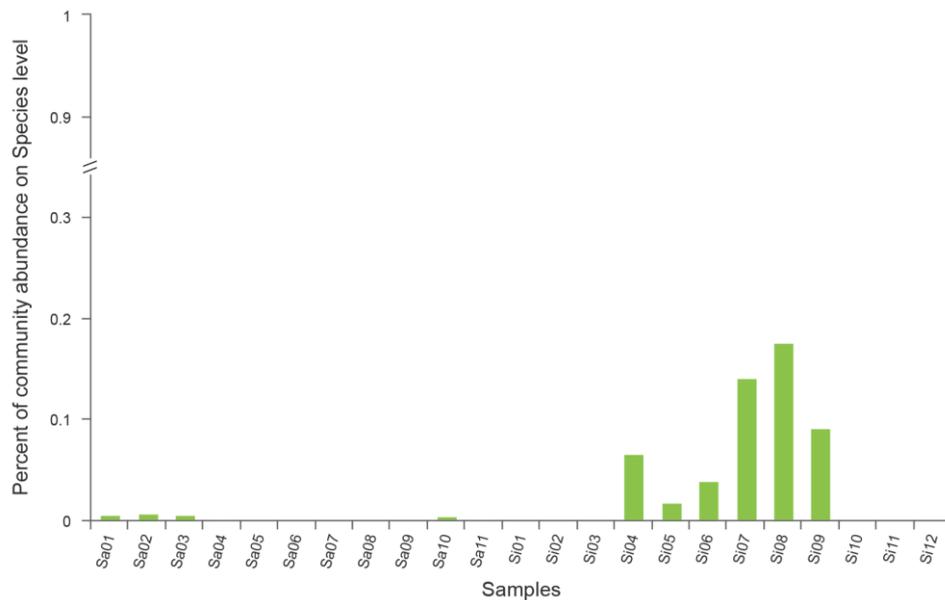


Figure S1. *Vibrio fortis* composition profiles.

corals is slightly lower than stony corals (52.56%). Compared with previous studies on symbiotic bacteria of stony corals, the presence of a large number of *Tenericutes* in the soft corals of the genus *Sarcophyton* genus has not been reported in studies on coral symbiotic bacteria near Hainan Island (Figure 4). Weiler et al. (2018) argue that *Tenericutes* has a relationship with cold-water corals that play an important role in the nitrogen cycle. However, in the genus *Sinularia* of the same waters, the abundance of such bacteria was lower, and even some species did not contain *Tenericutes*. At the same time, we found a large number of *Vibrio fortis* in the *Sinularia* genus in Dazhou Island (Figure S1). Many *Vibrio* species are considered to be potential coral pathogens, such as *Vibrio shiloi* (Kushmaro, Fine, & Rosenberg, 1966), *Vibrio coralliilyticus* (Ben-Haim, Zicherman-Keren, & Rosenberg, 2003), *Vibrio carchariae* (Ritchie & Smith, 1995), and *Vibrio alginolyticus* (Cervino et al., 2004). However, no report has indicated that *V. fortis* is also a coral pathogen. García-Amado et al. (2011) also confirmed that *V. fortis* is a common bacteria in seawater living at specific depths and a natural component of microbial community in marine redox environments. *Sarcophyton* and *Sinularia* have their own symbiotic bacteria, which are different from those of stony coral, thereby providing a theoretical basis for the further study of the genetic evolution of soft corals.

Chan et al. (2019) found that different coral species have different bacterial community, and environmental conditions are the main driving forces for early coral microbial community. This study confirmed that the same genus or the same species of soft corals that had different geographical locations, contained various bacterial community. Unlike the previous results on the genus *Sarcophyton* in the Red Sea (Lee et al., 2012), *Endozoicomonas* is not a dominant bacterial species and has low abundance among the *Sarcophyton* corals in the waters around Hainan. Symbiotic zooxanthellae can

affect the abundance of *Endozoicomonas* (Pantos et al., 2015), and the composition of different zooxanthellae may be one of the causes of this variation. The impact of different geographical environments on coral symbiotic bacteria is multifaceted. First, Woo et al. (2017) believed that differences in latitude are potential causes of coral symbiotic bacteria diversity. When latitude changes exceed 10°, it should be considered as an important factor affecting the results. In this study, the three sampling points are located near the 18° north latitude, which we believe is no longer a factor affecting the bacteria. Secondly, the composition of coral-associated bacteria is influenced by temperature and season (Hong et al., 2009). Based on the sampling time concentrated in September, a temperature difference of 2 °C exists between the temperatures at the Sanya sampling point and that at Wanning sampling point, which may lead to the richness in symbiotic bacteria in cold water corals, such as *Tenericutes*, as seen in the specimens from Dazhou Island and Ganzhe Island.

In a specific area, the unique breeding of corals often affects their symbiotic bacterial community. For example, *Stylophora pistillata* allows microorganisms to be transferred vertically from the parent to the offspring (Sharp, Distel, & Paul, 2012). Human activities, such as diving and fisheries, are also factors that influence the microbial composition of coral reef ecosystems (Kelly et al., 2014). However, we are not sure whether these incentives are direct factors influencing the geographical differences in soft coral symbiotic bacteria. Thus, direct experimental evidence is needed. Future studies on soft coral-associated microbes should consider host reproductive strategies, metabolites, and environmental factors.

In conclusion, although each locality represents special bacterial diversity, same coral species in each locality represent similar pattern of bacterial community.

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