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Bacterial diversity of *Spheciospongia* spp. from Rancabuaya Beach, West Java, Indonesia, using the NGS approach

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Abstract. *Srikandace Y, Wahhaab A, Rakhmat AF, Kamarisima, Putri SP, Aditiawati P. 2025. Bacterial diversity of* Spheciospongia *spp. from Rancabuaya Beach, West Java, Indonesia, using the NGS approach. Asian J Trop Biotechnol 22: 7-15*. For the first time, the bacterial community associated with *Spheciospongia* spp. from Rancabuaya Beach in Garut was identified using Next-Generation Sequencing (NGS). The study focused on the bacterial diversity present on the sponge's body surfaces (SEK1, SEK2), within their inner tissues (SEN1, SEN2), and in the surrounding seawater (SW). The results revealed that SW had the highest number of species, with 198 detected, followed by SEN2 and SEN1, with 193 and 165 species, respectively. In contrast, SEK1 and SEK2 contained 32 and 26 bacterial species, respectively. The analysis also showed that SW possessed the highest diversity and evenness indices at the genus level, with values of 2.95 and 0.79, respectively, indicating the absence of a dominant genus. Conversely, SEK1 and SEK2 exhibited low evenness indices (0.33 and 0.24) and low variety indices (0.80 and 0.54), indicating that the community lacks a balance of species. SEN1 and SEN2 demonstrated moderate genus diversity, but their evenness and dominance varied. The dominant phyla across all samples were *Proteobacteria* and Bacteroidota. Notably, the NGS method identified several actinomycete genera for the first time, including *Micrococcus*, *Leucobacter*, *Egibacter*, *Lawsonella*, *Nakamurella*, and *Corynebacterium*. A recent study confirmed that NGS provides a rapid, sensitive, and high-quality method for determining microbial communities.

Keywords: Actinobacteria, diversity, dominance, evenness, indices, next-generation sequencing, *Spheciospongia*

INTRODUCTION

Mangrove sponges play a crucial role in the ecosystem by providing habitats, shelter, and food sources for marine microorganisms. Sponge-associated microbial communities are diverse and exhibit mutualistic relationships with one another, acting as symbionts or pathogens (Bibi et al. 2020). This mutualistic relationship involves the sponge supplying organic particles as nutrition for microorganisms, while microorganisms produce metabolites that inhibit pathogen growth on the sponge (Li et al. 2023; Mehbub et al. 2024). The sponge can also produce bioactive compounds as nutrition for microorganisms and to prevent pathogens (Varijakzhan et al. 2021; Hong et al. 2022). Microorganisms associated with sponges can account for up to 40% of the body weight of particular sponge species, with their population densities being 100 to 10,000 times higher than in surrounding seawater (Sugden et al. 2022). These microbial communities, often located within the sponge's mesohyl, support the sponge's metabolism and produce beneficial molecules, including antimicrobials and other biologically active substances (Dat et al. 2021; Sugden et al. 2022).

The bacterial communities associated with various sponge species have been the subject of numerous studies, employing both culture-dependent and culture-independent methods. Approximately 1% of the bacteria can be effectively cultured in the laboratory, while other bacteria have been discovered through culture-independent approaches based on the 16S rRNA gene (Ito et al. 2019; Ahmad et al. 2024). Actinomycete bacteria are important microorganisms and are still being explored to uncover their potential compounds. Due to the challenges of isolating and culturing actinomycetes in the laboratory, Next-Generation Sequencing (NGS) facilitates the identification of additional actinomycete species and the development of suitable media to isolate them from sponges. NGS has enhanced our understanding of sponge symbiont diversity, revealing that unculturable bacteria can make up 99% of any environment (Bibi et al. 2020; Abbas and Mahmoud 2022).

The NGS approach enables the investigation of microbial communities from various geographical locations, providing deeper insights into the diversity of bacterial populations (Waterworth et al. 2021). Moreover, NGS is a powerful tool for studying bacteria, providing an in-depth, unbiased, and accurate analysis of bacterial communities, revealing complex interactions, diverse species, and functional roles

(Tan et al. 2015). It provides a far more detailed and comprehensive picture of the microbial world than traditional methods, driving discoveries in microbiology and ecology (Satam et al. 2023). NGS enables the exploration of new Actinomycetes or Streptomyces species by allowing researchers to perform comprehensive metagenomic surveys, identify novel genes, and discover previously unknown bacterial strains that may produce bioactive compounds (Alam et al. 2021). Several structural profiles of bacterial communities in sponges have been identified through NGS using the Illumina platform. The bacterial diversity in the freshwater sponges *Eunapius carteri* and *Corvospongilla lapidosa* had 14 phyla, with over 2,900 OTUs for *C. lapidosa* and 980 OTUs for *E. carteri* (Gaikwad et al. 2016). The sponge *Callyspongia* sp., from the Thousand Islands, Indonesia, exhibited a low abundance of Actinomycetes (0.09%) and was dominated by Proteobacteria, which comprised 82% of the community (Retnowati et al. 2021). The bacterial community associated with the sponge *Haliclona oculata* (Linnaeus, 1759) includes 17 phyla, while *Amphius huxleyi* contains 13 phyla. Proteobacteria are the dominant phylum in both sponge species, though other phyla are present in varying abundances (Dat et al. 2021). Therefore, the Illumina platform is chosen for its high throughput, accuracy, and cost-effectiveness, making it ideal for studying the diverse bacterial communities found in sponges and other environments.

The bacteria associated with marine sponges are well-documented, but the symbiotic bacteria found in coastal sponges remain largely unexplored. Rancabuaya Beach in Garut, West Java, has limited human activity, making it an ideal site for study. *Spheciospongia* spp. can be easily found attached to coral rocks. This beach, situated adjacent to the Indian Ocean, boasts coral reefs, powerful waves, a gently sloping seabed, and a pristine white sand shoreline with crystal-clear and blue water. Thus, the current study aimed to investigate the bacterial community of the widespread coastal water and the surface of *Spheciospongia* spp. through the NGS method. This culture-independent and high-throughput method offers an efficient and powerful approach to uncovering the hidden biodiversity of these bacteria.

MATERIALS AND METHODS

Study area

Recent research was conducted at Rancabuaya Beach in the Garut District of West Java, Indonesia (Figure 1).

Procedures

Sponge and water sampling

The samples were taken from the supralittoral to mesolittoral zones using no specialized equipment and were transferred into sterile plastic containers. Beach water was also collected and placed in a 1 L sterile bottle. All samples were stored in a cooler bag, transported to the laboratory, and processed immediately. Collecting sponges did not require permission from the authorities, as it pertained to species that were neither endangered nor protected.

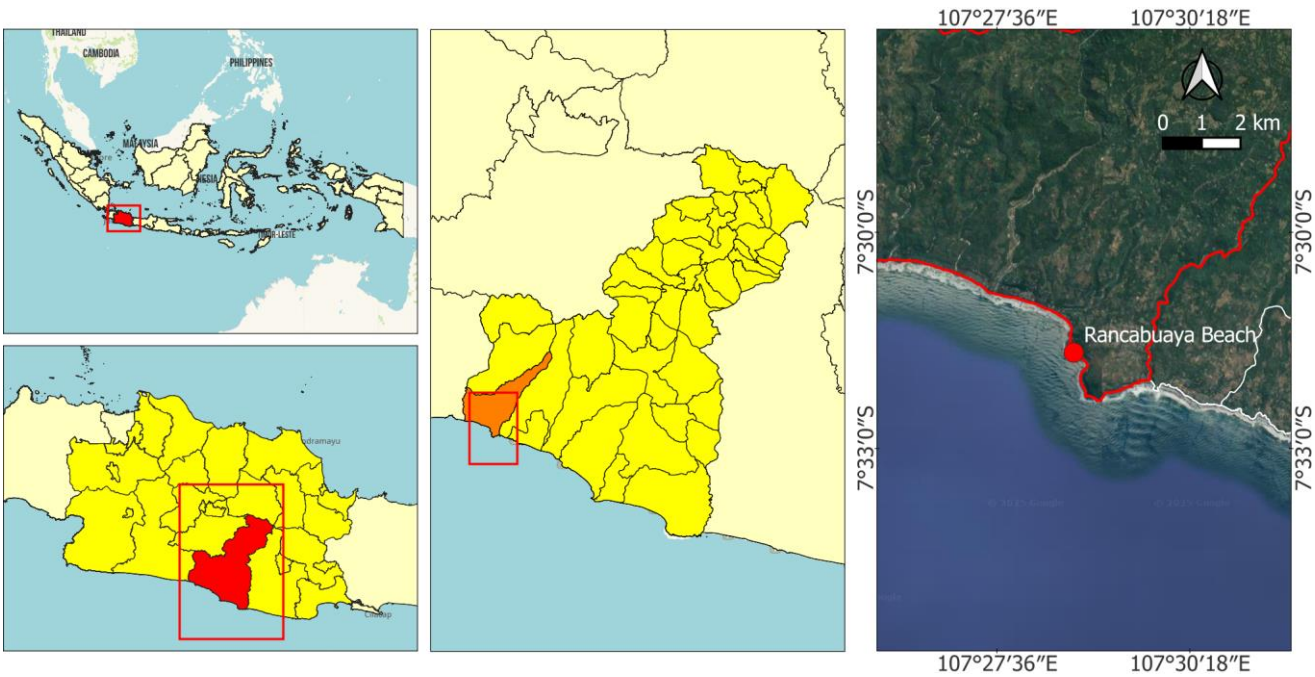

Figure 1. The location of the sampling site is in Rancabuaya Beach, Caringin, West Java, Indonesia

DNA extraction from sponges

Total genomic DNA was extracted from two sponges and seawater samples using a combination of bead-beating and the phenol: chloroform: isoamyl alcohol method (Djurhuus et al. 2017). The surfaces and inside of the sponge bodies were cut, crushed, and dissolved in artificial sterile seawater. The samples were then centrifuged at 8000 rpm for 5 minutes to separate the crushed sponge material from the sterile seawater containing bacteria, resulting in four sponge samples: SEK1, SEK2, SEN1, and SEN2. All samples were concentrated by centrifugation at 8000 rpm for 5 minutes. The pellets from each sample were dissolved in 1 mL of sterile Phosphate-Buffered Saline (PBS). Bacterial DNA was extracted from the pellets using the phenol-chloroform: isoamyl method. The recovered DNA was resuspended in 20 µL of TE buffer (pH 8.0). The integrity of the DNA was assessed by agarose gel electrophoresis. Purity (measured as the λ 260 nm/280 nm ratio) and quantity were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The DNA samples were stored at -20°C for PCR amplification. The purification of PCR products was performed using the Promega Wizard PCR Clean-Up Protocol procedure. DNA was visualized using electrophoresis with a 2% agarose gel in 1X TBE buffer and a 1 kbp DNA ladder marker (Promega).

16S rRNA amplification

The 16S rRNA genes were amplified by polymerase chain reaction (PCR) using primers 27F (GAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) in the first-stage PCR. The PCR mixture consisted of 12.5 µL GoTaq Green Master Mix (Promega), 1 µL 1492r primer (10 pM), 1 µL 27f primer (10 pM), 1 µL sample DNA, and 25 µL Nuclease-Free Water (NFW) in a final volume of 50 µL. PCR products were purified using the QIAquick PCR kit (Qiagen, Germany) according to the manufacturer's instructions. The cycling conditions are as follows: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute, with a final extension of 5 minutes at 72°C. The gene-specific sequences of 16S V3 and V4 regions were submitted for amplicon sequencing using the Illumina MiSeq platform. The construction of a 16S rRNA library and subsequent Illumina NGS sequencing analysis were conducted by Macrogen Korea, utilizing the purified PCR products as a template. Furthermore, the second-stage PCR reaction utilized the Nextera XT DNA Library Preparation Kit following the two-step cycling standard protocol recommended by the manufacturer.

Data analysis

Sequencing data analysis: OTU clustering and taxon annotation

Based on the unique barcode of each sample, paired-end reads were assigned. The samples were then truncated by removing the barcode and primer sequences, and the reads were merged using FLASH v1.2.7. OTUs were performed with QIIME2 2017.4. Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin, then denoised using DADA2. The sequences were clustered into Operational Taxonomic Units (OTUs) with a similarity of ≥97%. The most frequent sequence within each OTU was selected and screened to obtain the representative sequence. All Amplified Sequence Variants (ASVs) were aligned using MAFFT. Taxonomy assigned to ASVs using feature classifier q2 (Bokulich et al. 2018).

Diversity, evenness, and dominance index calculation

The diversity index (H'), evenness index (E), and dominance index were calculated using the equation formula (Roswell et al. 2021) and classified according to Table 1.

$$H' = \sum_{i=1}^{s} \frac{ni}{N} \ln \frac{ni}{N}$$

$$E = \frac{H'}{lnS}$$

$$D = \Sigma \frac{ni}{N}$$

RESULTS AND DISCUSSION

Sponges collection

The sponges collected belong to *Spheciospongia*, as shown in Figure 2. The condition of the coastal waters was characterized by clear blue water, an odorless quality, a salinity ranging from 2.70 to 3.10 ‰, and a pH of 8.22. *Spheciosponga sp.1* exhibited a light brown underside and a green upper surface, with body surface and internal tissues designated SEK1 and SEN1 samples. *Spheciospongia* sp.2 displayed a bright yellow upper and lower body surface, with its body surface and internal tissues categorized as SEK2 and SEN2 samples. Seawater samples were coded SW.

Table 1. Diversity index value criteria

Index	Value	Criteria
Diversity	H'<1	Low
	1<H'<3	Moderate
	H'>1	High
Evenness	E<1	Low
	1<E<3	Moderate
	E>1	High
Dominance	D<1	Low
	1<D<3	Moderate
	D>1	High
H': Diversity Index	E: Evenness Index	D: Dominance Index
S: No. of species	H': Diversity Index	ni: Individual number
ni: Individual number	ln: Natural logarithm	N: Total individual number
N: Total individual number	S: No. of species	

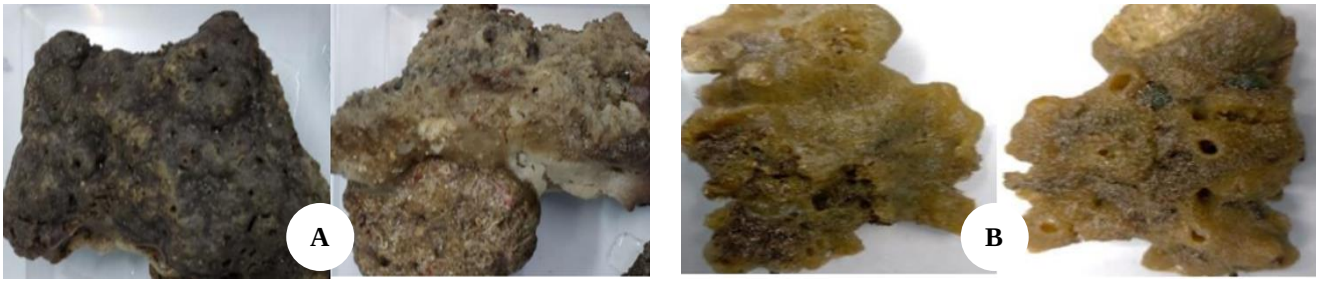

Figure 2. The sponge collection from Rancabuaya beach: A. *Spheciospongia* sp.1; B. *Spheciospongia* sp.2

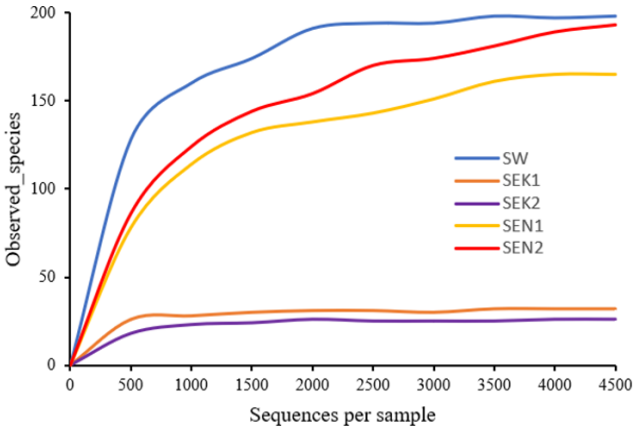

Figure 3. Rarefaction curves showing observed species richness in samples

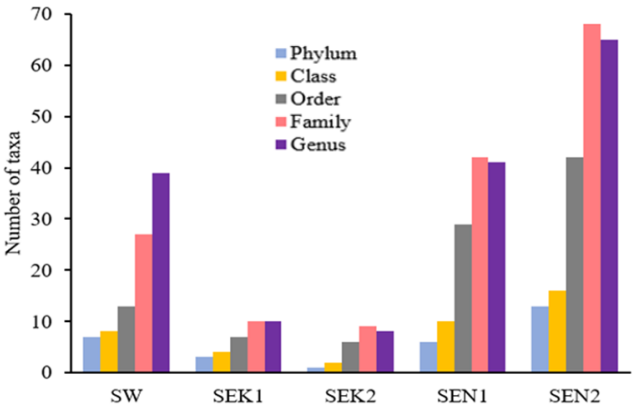

Figure 4. Number of phyla to genera of bacteria observed in samples

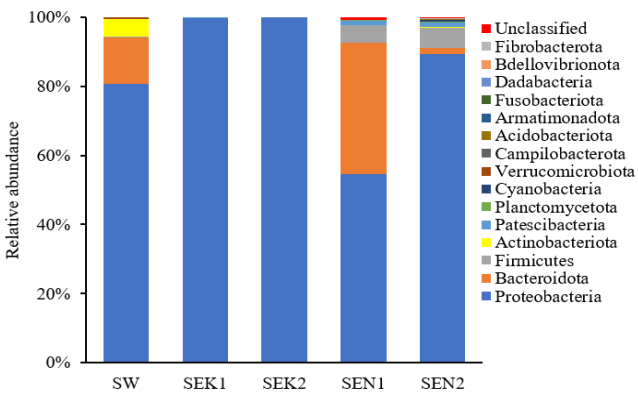

Figure 5. The relative abundances of 15 dominant phyla

Bacterial diversity associated with *Sphesiospongia* spp

The rarefaction curve displayed a horizontal asymptote, indicating that the number of bacterial species (OTUs) detected in each sample had high sequence coverage (Figure 3). In SW, the highest number of bacterial species was detected, totaling 198 species, followed by SEN2 with 193 species and SEN1 with 165 species. Meanwhile, samples SEK1 and SEK2 contained 32 and 26 bacterial species, respectively. The highest abundance of bacteria was found within the sponge, with SEN1 and SEN2 accounting for 83.76 and 88.13% of the total bacterial population, respectively. Bacterial abundance was low on the SEK1 and SEK2, but the highest abundance was observed in SW. Clear saturated plains with adequate coverage and complete sorting (100%) from all samples. According to Willis (2019), A rarefaction curve that approaches a horizontal asymptote, indicating sufficient sampling, can be used to evaluate the observed species richness.

Figure 4 depicts the diversity of microbial communities in different sponge samples. The SW sample contained 7 phyla, 8 classes, 13 orders, 27 families, and 39 genera. The SEK1 and SEK2 samples had 3 and 1 phyla, 4 and 2 classes, 7 and 6 orders, 10 and 9 families, and 10 and 8 genera, respectively. In the SEN1 and SEN2 samples, 6 and 13 phyla, 10 and 16 classes, 29 and 42 orders, 42 and 68 families, and 41 and 65 genera were identified. These findings suggest that sponges create a suitable environment for bacterial growth, resulting in a rich diversity of microbial communities, particularly in the SEN1 and SEN2 samples. These results suggest that sponges serve as a rich organic source in their body tissues, creating a suitable habitat for bacteria. This is evidenced by the higher number of genera identified in the SEN1 and SEN2 samples.

The relative abundance of the top 15 bacterial taxa at the phylum level is shown in Figure 5. Other identified phyla were categorized as Others, while Unclassified was used for unknowns. The predominant phylum found in all samples was Proteobacteria, with the following percentages: SW (80.78%), SEK1 (99.77%), SEK2 (100%), SEN1 (54.49%), and SEN2 (89.27%). In the SW sample, the three dominant phyla were Proteobacteria (80.78%), Bacteroidota (13.47%), and Actinobacteria (5.03%). All SEK samples were also primarily dominated by Proteobacteria. In contrast, the SEN samples were characterized by three dominant phyla: Proteobacteria, Bacteroidota, and Firmicutes. Specifically, SEN1 contained Proteobacteria (54.49%), Bacteroidota (38.19%), and Firmicutes (5.10%), while

SEN2 had Proteobacteria (89.27%), Bacteroidota (1.90%), and Firmicutes (5.90%). No 100% phyla abundance was detected in SEN1 (97.78%) and SEN2 (97.07%), possibly due to insufficient amplification or detection of some fragments of DNA, which could have led to the loss of some sequence information. NGS technology may introduce errors, deletions, insertions, and substitutions. These mistakes may show discrepancies between the actual and observed sequences (Das et al. 2023). Therefore, to detect sequence variants, NGS shows a confidence interval of approximately 98-100% for the identified taxonomy, which may influence the abundance of the detected microorganisms (Rojahn et al. 2022). Proteobacteria is the largest phylum in the bacterial domain, while Firmicutes, Bacteroidetes, and Actinobacteria are the dominant phyla associated with eukaryotes (Taylor et al. 2007).

The dominant class of bacteria across all samples in Figure 6 belonged to the Proteobacteria phylum, specifically Gammaproteobacteria, with the following percentages: SW (71.90%), SEK1 (99.26%), SEK2 (99.85%), SEN1 (54.06%), and SEN2 (84.53%). Alphaproteobacteria were detected only in the SW sample (5.03%), ranking as the fourth most dominant class after Bacteroidia (13.47%) and Actinobacteria (8.88%). In SEK1 and SEK2, small percentages of Actinobacteria were detected (0.51% and 0.15%), making them the second dominant class in those samples. For SEN1, the second and third most common classes were Bacteroidia (38.19%) and Actinobacteria (0.40%). In SEN2, the second and third most prevalent classes were Actinobacteria (4.70%) and Bacteroidia (1.90%).

Gammaproteobacteria emerged as the dominant class compared to Alphaproteobacteria due to their paraphyletic nature, while Alphaproteobacteria are monophyletic (Orata et al. 2018). This makes Gammaproteobacteria the largest and most diverse group within the Proteobacteria phylum (Orata et al. 2018; Nguyen et al. 2023). Furthermore, Bacteroidetes bacteria are often detected in sponges, but their functions in host development remain largely unknown (Li et al. 2021). In marine sponges, Actinobacteria represent a diverse group of microorganisms capable of producing bioactive substances that help protect the sponge from predators (Retnowati et al. 2021).

Figure 7 shows that in the SW sample, three dominant orders were identified: Oceanospirillales (26.92%), Alteromonadales (20.57%)-with the Pseudoalteromonadaceae family making up 11.22%—and Flavobacteriales (13.38%). In the SEK1, SEK2, and SEN2 samples, Alteromonadales was the most prevalent order, with percentages of 84.64, 91.12, and 74.79%, respectively. In contrast, two dominant orders were found in SEN1: Flavobacteriales (37.93%) and Alteromonadales (32.26%). Additionally, several bacterial orders, including Rhodobacterales, Burkholderiales, Nitrosococcales, Rhizobiales, Micrococcales, and Caldalkalibacillales, were not present in SW but were found in SEK and SEN.

Seawater is primarily composed of bacteria from the orders Alteromonadales, Flavobacteriales, Rhodobacterales, Sphingobacteriales, Verrucomicrobiales, and Vibrionales (Rygaard et al. 2017; Rufino and Procópio 2021). These dominant bacterial orders require a variety of mineral sources for their growth. Seawater contains essential minerals such as magnesium (Mg), calcium (Ca), potassium (K), chromium (Cr), selenium (Se), zinc (Zn), and vanadium (V). Additionally, these bacteria play a crucial role in the decomposition of organic matter, contributing to the nutrient availability in seawater. The concentration of these inorganic materials tends to increase with sea depth (Nani et al. 2016).

The Pectobacteriaceae (13.52%), a family of Enterobacterales, is the most prevalent in SW, as shown in Figure 8. The Pseudoalteromonadaceae family is the most abundant in SEK1 and SEK2, accounting for 81.29% and 86.24%, respectively. Flavobacteriaceae is the most prevalent family in SEN1 (38.15%), whereas Pseudoalteromonadaceae again accounts for the highest percentage in SEN2 (70.12%). Only in SEN and SEK were the families Rhodobacteraceae, Alcaligenaceae, and Shewanellaceae present. Pseudoalteromonadaceae and Flavobacteriaceae are commonly found in marine water and sponges (Delgadillo-Ordoñez et al. 2022). Pectobacteriaceae are typically found in brackish water and river systems (Hugouvieux-Cotte-Pattat et al. 2024).

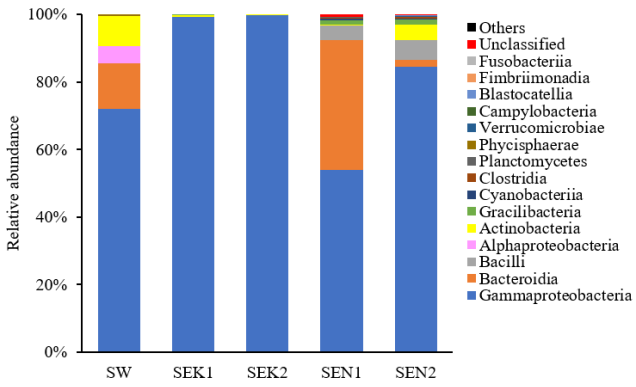

Figure 6. The relative abundances of 15 dominant classes

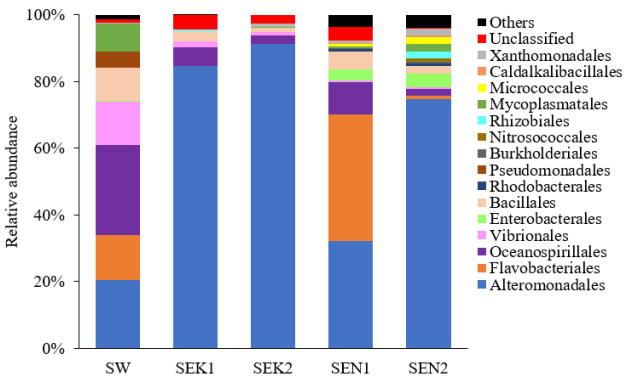

Figure 7. The relative abundances of 15 dominant orders

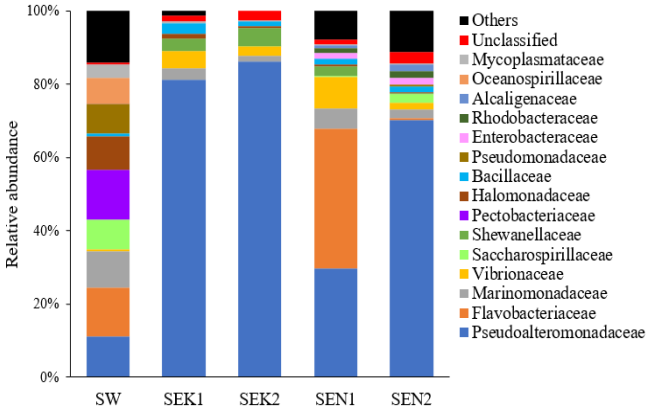

Figure 8. The relative abundances of 15 dominant families

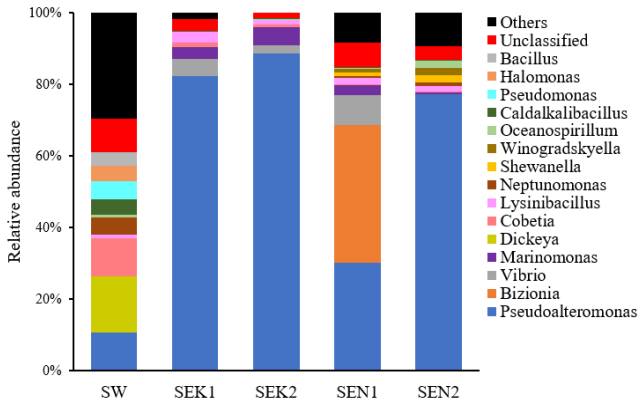

Figure 9. The relative abundances of 15 dominant genera

The abundance of Pectobacteriaceae along river courses is due to the suitability of temperature, nitrate availability, and dissolved organic carbon concentration in the water. Pectobacteriaceae are prevalent in freshwater environments, often causing disease outbreaks in aquatic plants (Ben Moussa et al. 2022). Pseudoalteromonadaceae exhibit versatile metabolic capabilities, making them highly adaptable to various ecological habitats, and play a crucial role in the marine environment. Ecologically, *Pseudoalteromonas* forms a biofilm in ecosystems and engages in predator-like interactions within microbial communities as a defense mechanism (Bowman 2007). Flavobacteriaceae is a group of bacteria found in soil, freshwater, and marine environments. This group is well known for its motility, which is associated with fish diseases. Several species within this family also have the potential for bioremediation of chemical compounds and organic materials, such as hydrocarbons. However, some species are pathogenic to fish (Skovhus et al. 2007).

SW was primarily dominated by *Dickeya*, which makes up 15.69% of the bacterial community in Figure 9. In contrast, SEK1, SEK2, and SEN2 were dominated by *Pseudoalteromonas*, comprising 82.26, 88.55, and 77.27%. *Bizionia* sp., found only in SEN1, accounted for approximately 38.39%. *Dickeya* was exclusively found in SW and was not present in sponges. In addition to beach water, *Dickeya* can be found in river water, as well as in sponges and lake water (Watanabe et al. 2015; Laport et al. 2019; Hugouvieux-Cotte-Pattat et al. 2024). *Pseudoalteromonas* spp. exclusively found in marine environments and are associated with eukaryotic hosts, including shellfish, pufferfish, tunicates, and sponges. The widespread presence of *Pseudoalteromonas* spp. across different habitats demonstrated adaptive strategies and survival capabilities (Holmström and Kjelleberg 1999; Sonnenberg and Haugen 2021). Additionally, *Bizionia argentinensis* sp. nov. and other *Bizionia* spp. have been isolated from Terra Nova Bay in the Ross Sea, and marine bacteria *Cliona* sp. were isolated from the marine sponge *Grantia celata* and Arctic Fjord seawater (Bercovich et al. 2008; Li et al. 2015; Savoca et al. 2019).

Structure the community of bacteria at the genus level

The diversity, evenness, and dominance indices provide insights into community structure. The bacterial diversity index across all samples ranged from low to moderate (Table 2). In SW, there was a high genus population evenness (0.79), with no single genus dominating, indicating a stable community. In contrast, low evenness values were observed in SEK1 (0.33), SEK2 (0.24), and SEN2 (0.31), accompanied by a high dominance index (0.60-0.79). Meanwhile, SEN1 exhibited moderate evenness (0.51) and a low dominance value (0.25). According to Strong (2016), the Shannon-Wiener diversity index (H') is categorized as low diversity ($H'<1$), moderate diversity ($1\leq H'\leq 3$), and high diversity ($H'>3$). The evenness index (E') is classified as low ($E<0.4$), moderate ($0.4\leq E\leq 0.6$), and high ($E>0.6$). The dominance index is categorized as low ($0\leq D\leq 0.3$), moderate ($0.3\leq D\leq 0.6$), and high ($0.6\leq D\leq 1$).

Actinomycetes diversity in sponges and coastal water

The most abundant actinobacteria families in the sponge and coastal waters varied. Actinomycete genera were more prevalent and detected in sponge bodies than in seawater, likely due to differences in nutrient availability. It indicated that the same sponge symbiont bacteria or actinomycetes vary even within the same habitat. Actinomycetes were more prevalent in the Sponge's Inner Tissue (SEN) and seawater (SW). A few actinomycetes were observed on the sponge body's surface (SEK) (Figure 10).

Table 2. Diversity index of the bacteria community associated with sponges at the genus level

Index	SW	SEK1	SEK2	SEN1	SEN2
Diversity	2.95	0.80	0.54	1.92	1.28
Category	Moderate	Low	Low	Moderate	Moderate
Evenness	0.79	0.33	0.24	0.51	0.31
Category	High	Low	Low	Moderate	Low
Dominance	0.07	0.68	0.79	0.25	0.60
Category	Low	Moderate	High	Low	Moderate

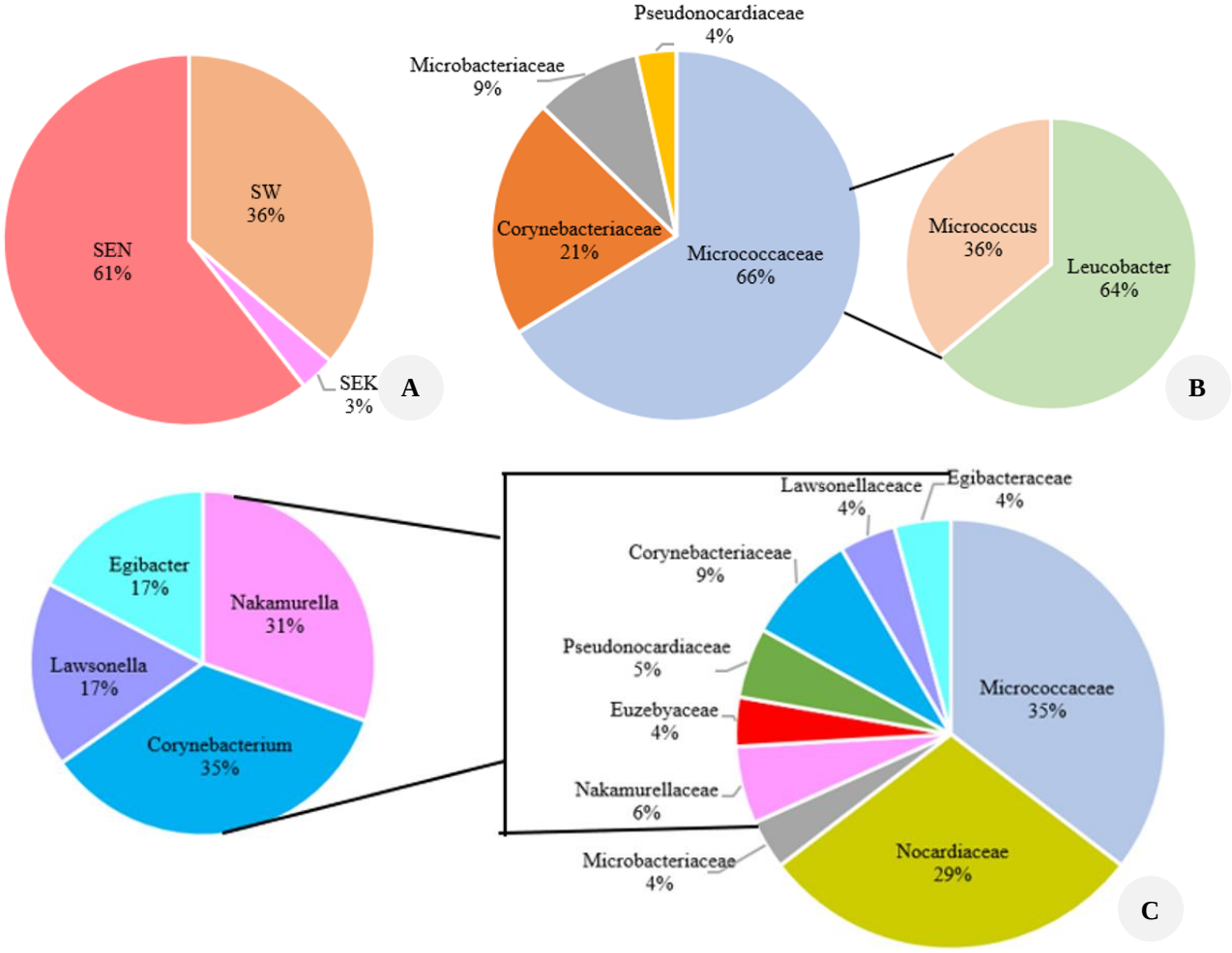

Figure 10. The abundance of the actinobacteria community: A. SW, SEK, SEN; B. two genera belonging to Micrococcaceae in SW; and C. Four genera to certain families in SEN

The SW sample consisted of 4 families, with 2 genera—*Leucobacter* and *Micrococcus*—belonging to the Micrococcaceae family. In SEK, only Micrococcaceae was identified (see Suppl). Nine families were discovered in the SEN sample, with the Micrococaceae and Nocardiaceae families being the most abundant. A recent study reveals that *Micrococcus*, *Leucobacteria*, *Egibacter*, *Lawsonella*, *Nakamurella*, and *Corynebacteria* were discovered using the NGS method. Not all genera could be identified among all the Actinobacteria families identified in this study.

Micrococcaceae are widely recognized as originating from various terrestrial environments, marine sponges, cyanobacterial layers, and marine sediments (Palomo et al. 2013). Additionally, Micrococcaceae have been identified in several sponge species, including *Callyspongia aerizusa*, *Haliclona atra*, *H. fascigera*, *Biemna* sp., *Paratetilla* sp., *Pseudoceratina* sp., *Scopalina hapalia*, *Plakortis kenyensis*, and *Tetrapocillon minor*, all found at a depth of 10 meters in Tanzania (Helber et al. 2018). The abundance of Corynebacteriaceae exceeds 50% in sediments compared to seawater (Sulardiono et al. 2018). A similar study showed approximately 6% Corynebacteriaceae and 1% Nocardiaceae in the sponge *Callyspongia* sp. collected from a depth of 16 meters off Panggang Island in the Thousand Islands, Indonesia (Retnowati et al. 2021). In addition, Nocardiaceae have been reported in various environments and are commonly isolated from freshwater, saltwater, dust, soil, and decaying organic matter (Brown-Elliott et al. 2006). A recent study demonstrates that NGS technology can effectively identify rare actinobacteria groups associated with *Spheciospongia* spp. that use traditional culturing methods. Additionally, utilizing NGS technology to describe bacterial diversity has significantly improved the understanding of the community structure within sponge-associated bacteria, especially actinobacteria.

In conclusion, the highest bacterial abundance was found in the internal tissue of the sponge (SEN), compared to the sponge surface (SEK) and the surrounding seawater (SW). The two main phyla detected were Proteobacteria and Bacteroidota, with the predominant classes being Gammaproteobacteria and Bacteroidia. The dominant families associated with sponges and seawater included Micrococcaceae, Corynebacteriaceae, and Pseudonocardiaceae. The NGS method was the first to

identify genera such as *Corynebacterium*, *Leucobacter*, *Egibacter*, *Lawsonella*, and *Nakamurella*. Many prokaryotes associated with the sponge remain unculturable and previously unknown. However, NGS techniques now enable the determination of bacterial diversity and the development of media for isolating specific bacteria from sponges. This approach also facilitates the exploration of these bacteria, particularly actinomycetes, as potential producers of bioactive compounds.

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