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Metagenomic Insights into the Gut Microbial Communities and Functional Dynamics of *Bohadschia marmorata* in Barobo, Surigao del Sur, Mindanao

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Abstract

Studies on the identification, functional roles, and environmental factors shaping the gut microbiota composition of the sea cucumber *Bohadschia marmorata* remain scarce. This study aims to (i) characterize the gut bacterial community structure of *B. marmorata* from Barobo, Surigao del Sur using 16S rRNA gene-based metagenomic analysis, (ii) describe microbial diversity and community composition, and (iii) identify bacterial taxa with potential relevance to probiotic development, opportunistic pathogenicity, and food safety. In November 2024 and February 2025, adult *Bohadschia marmorata* were collected from seagrass beds in Barobo, Surigao del Sur, with six individuals selected for gut microbiome analysis. The V3–V4 region of the bacterial 16S rRNA gene was sequenced using the Oxford Nanopore MinION platform and analyzed with QIIME2, SILVA, and PICRUSt2. The gut microbiota was dominated by Pseudomonadota (~60%), followed by Bacillota (~25%) and Bacteroidota (~10%). Functional profiling revealed enrichment in energy metabolism, carbohydrate degradation, amino acid metabolism, and oxidative phosphorylation. Several taxa, including *Bacillus velezensis*, *Ruminococcus*, and *Pseudomonas*, showed potential probiotic and biotechnological relevance. These findings provide baseline information for sustainable aquaculture, biomonitoring, and the ecological understanding of *B. marmorata*.

Introduction

Sea cucumber fisheries constitute an important livelihood in the Philippines, supporting numerous small-scale and marginal fishers (Arriesgado et al., 2022; Jontila, 2023). Historical records indicate that the Philippines has maintained continuous sea cucumber production since the 1950s and currently ranks among the leading exporters of processed sea cucumber products in Southeast Asia (Alejandro, 2019; Altamirano, 2022). Approximately 100 sea cucumber species occur in Philippine waters, of which at least 31 are commercially important (Jontila et al., 2017), underscoring both their economic and ecological significance.

Among these, *Bohadschia marmorata* is a widely distributed sea cucumber species of medium commercial value, reported from multiple regions including Zamboanga Peninsula, Surigao del Sur, Palawan, Eastern Visayas, and Basilan (Cabansag, 2014;

Jontila et al., 2017; Arriesgado et al., 2022; Jaafar et al., 2018). This species inhabits intertidal to shallow subtidal seagrass beds with sandy to sandy–muddy substrates and exhibits burrowing behavior that actively modifies sediment structure and chemistry. Through bioturbation, feeding, and excretion, *B. marmorata* influences the cycling and bioavailability of nutrients such as nitrogen, phosphorus, and carbon, thereby contributing to benthic ecosystem functioning (Hou et al., 2017).

Sea cucumbers are deposit- and suspension-feeders that ingest sediment-associated organic matter and harbor diverse gut microbial communities. These microbiota are involved in digestion, nutrient metabolism, and host health, and may include taxa that produce bioactive secondary metabolites with antibacterial, antifungal, immunomodulatory, or cytotoxic properties (Purcell et al., 2016; Chen, 2021, 2023). Studies on other holothurian species, such as *Apostichopus japonicus, Holothuria leucospilota*, and *H. atra*, have revealed complex and variable gut microbiomes, including bacterial taxa with potential probiotic applications in aquaculture (Zhang et al., 2012, 2013; Kim et al., 2017; Wang et al., 2023). However, sea cucumber-associated microbiota may also include opportunistic or transient pathogenic bacteria with implications for food safety and environmental health (Kim et al., 2017).

As aquaculture continues to expand globally, disease outbreaks associated with intensified farming practices pose significant challenges to sustainability and productivity (Bondad-Reantaso et al., 2023). Microorganisms that confer health benefits to the host have emerged as an eco-friendly strategy to enhance growth, immunity, and disease resistance in cultured species (Munir et al., 2022). In this context, the gut microbiota of sea cucumbers represents a promising but underexplored source of candidate probiotic microorganisms for aquaculture applications.

Despite the ecological and economic importance of *B. marmorata*, information on its gut microbiome remains scarce, particularly from tropical environments. Most existing studies rely on culture-dependent approaches that capture only a fraction of microbial diversity, whereas metagenomic analysis provides a comprehensive, culture-independent framework for characterizing microbial community structure and potential function.

This study aims to (i) characterize the gut bacterial community structure of *B. marmorata* from Barobo, Surigao del Sur, using 16S rRNA gene-based metagenomic analysis, (ii) describe microbial diversity and community composition, and (iii) identify bacterial taxa with potential relevance to probiotic development, opportunistic pathogenicity, and food safety. As *B. marmorata* is currently listed as Data Deficient by the IUCN and remains unevaluated by CITES (Palomares & Pauly, 2025), this work provides baseline microbiome data that contributes to ecological understanding, sustainable aquaculture development, and future conservation and management strategies.

Methodology

B. marmorata Sample Collection and Experimental Design

The experimental organism *B. marmorata* (Figure 1) is the top commercially important sea cucumber in Barobo, Surigao del Sur (Figure 2), as indicated by catch landing data reported by Arriesgado et al. (2025). Sampling was conducted during two distinct periods: November 2024 and February 2025, and serves as replicates.

In each sampling month, a total of 30 adult individuals of *B. marmorata* (wet weight: 80–100 g) were collected from seagrass beds in Barobo, Surigao del Sur, through gleaning and handpicking. From these collections, three individuals per month (n= 3) were randomly selected for gut microbiome analysis, resulting in a total of six individuals subjected to metagenomic sequencing across all sampling periods. Each individual sea cucumber was initially treated as an independent biological unit prior to pooling, while

Figure 1. Top commercially important sea cucumber in Barobo, Surigao del Sur (Arriesgado et al., 2022) *Bohadschia marmorata* together with its corresponding gut structure.

pooled samples represented the analytical unit for sequencing.

The selected individuals were dissected individually using sterile scalpels under aseptic conditions. Gut contents were carefully extracted by gently squeezing the digestive tract and were transferred into sterile containers. The three gut samples in each month were pooled. Samples were maintained at low temperature during transport to minimize microbial proliferation and were subsequently stored at −80°C upon arrival at the metagenomics laboratory until further DNA extraction and sequencing.

DNA Extraction, Quality Assessment, and 16S rRNA Gene Amplification

Genomic DNA was extracted from individual gut tissue samples of *B. marmorata* using the Qiagen DNA easy Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's protocol with minor modifications to optimize DNA yield and purity. DNA extraction and initial quality assessment were conducted at the Philippine Genome Center–Mindanao.

The integrity of extracted DNA was evaluated by agarose gel electrophoresis. DNA samples were mixed with Vivantis 6× loading dye and resolved on a 1% agarose gel stained with gel red alongside a Vivantis 1 kb DNA ladder. Electrophoresis was performed using a Cleaver Scientific system at 100 V for 30 min, and gels were visualized under UV illumination using a Vilber NanoPac 300 Gel Documentation System. For microbial community profiling, the bacterial 16S rRNA gene was amplified targeting the V3–V4 hypervariable regions which provide high taxonomic resolution for bacterial identification. Amplification was performed using universal bacterial primers 341F (5'-ACTCCTACGGGA GGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') supplied with the Oxford Nanopore Technologies (ONT) 16S Barcoding Kit. PCR amplification was performed at the Philippine Genome Center–Mindanao, with each sample amplified in triplicate to ensure reproducibility. The PCR conditions followed the Oxford Nanopore Technologies (ONT) recommended protocol, consisting of an initial denaturation step, followed by cyclic denaturation, annealing, and extension, and a final extension step. The amplification parameters were as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 40 s, annealing at 55°C for 2 min, and extension at 72°C for 1 min; and a final extension at 72°C for 7 min (Tabardillo et al., 2025). The resulting amplicons were verified by agarose gel electrophoresis prior to library preparation to confirm successful amplification and the expected amplicon size (~450 bp).

Nanopore Sequencing and Library Preparation

Amplicon libraries were prepared using the ONT 16S Barcoding Kit (SQK-16S024) according to the manufacturer's instructions. Individual samples were uniquely barcoded and pooled equimolarly prior to sequencing. Sequencing was performed on an ONT MinION Mk1B platform using FLO-MIN106 (R9.4.1) flow cells. Sequencing runs were conducted for approximately 72 h and monitored in real time using MinKNOW software v23.07.15.

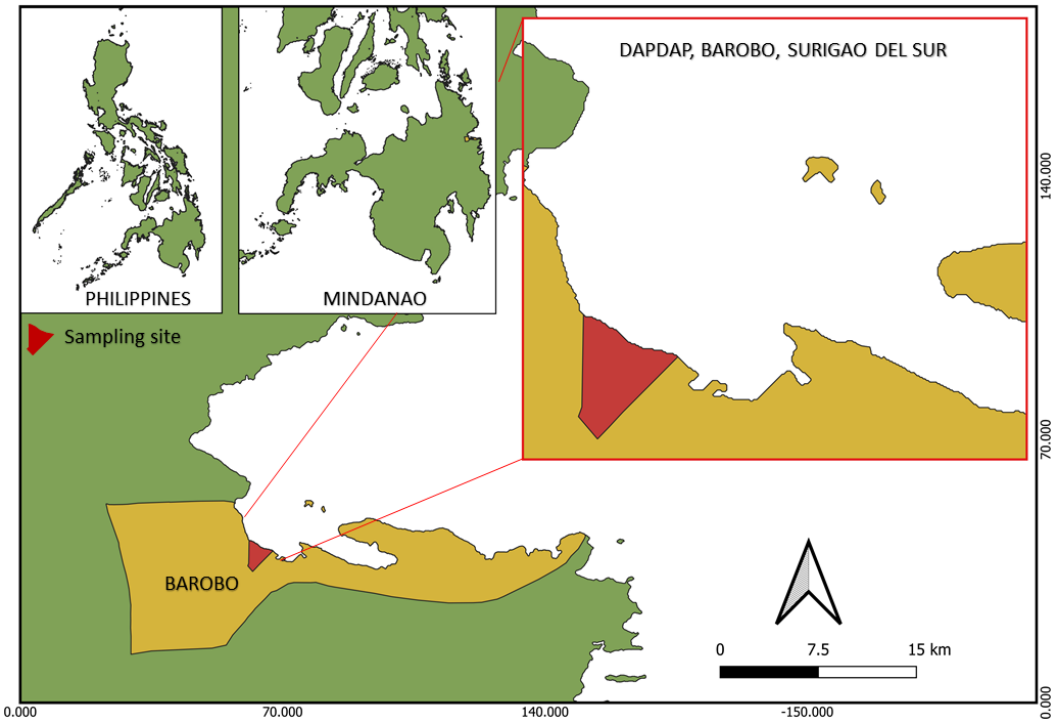

Figure 2. Sampling site for the collection of commercially important sea cucumber species, with concurrent assessment of water quality parameters and habitat characteristics.

Base Calling, Quality Control, and Bioinformatics Analysis

Raw electrical signal data were base called and demultiplexed using Guppy v7.1.4 (embedded in MinKNOW) with the fast basecalling model. Demultiplexed reads were exported in compressed FASTQ format, with an expected read length of approximately 450 bp.

Quality filtering and trimming were performed based on read quality profiles, removing reads with a minimum average Phred score <Q10 and retaining sequences within the expected amplicon length range. These thresholds were selected to balance read quality and sequencing depth while minimizing the loss of informative reads.

High-quality reads were subjected to denoising and chimera removal using the DADA2 algorithm implemented in QIIME2 (v2023.9). Truncation and filtering parameters were selected based on inspection of per-base quality score distributions to retain high-confidence sequence regions while excluding low-quality tails. Following denoising and chimera filtering, optimum reads were retained, resulting in a set of high-resolution amplicon sequence variants (ASVs).

Singleton ASVs and very low-abundance features (<0.01% of total reads across all samples) were removed to reduce the influence of sequencing artifacts and spurious taxa on downstream diversity analyses.

Taxonomic assignment of ASVs was performed using a naïve Bayes classifier trained on the SILVA 138 reference database, trimmed to the V3–V4 region of the bacterial 16S rRNA gene. This database was selected due to its comprehensive coverage and widespread use for bacterial community profiling. Relative abundance tables were generated at multiple taxonomic levels to characterize gut microbial community composition.

A comprehensive analytical report, including taxonomic profiles, relative abundance summaries, and visualization outputs, was generated to facilitate interpretation of the microbial community structure.

Collection of Physico-chemical Parameters and Habitat Description

The physico-chemical parameters of seawater were measured during the November 2024 and February 2025 sampling periods using a calibrated multi-parameter water quality probe. Parameters recorded included temperature, salinity, pH, and dissolved oxygen. All measurements were conducted in situ at the sea cucumber collection sites located in Turtle Island, Barobo, Surigao del Sur.

The sampling area represents the natural benthic habitat of *B. marmorata*, which predominantly occupies intertidal to shallow subtidal zones and is commonly associated with heterogeneous substrates, including seagrass meadows dominated by *Thalassia hemprichii*, sandy bottoms, and coralline substrates. These environments provide both trophic resources and structural refuge for the species. Water-quality measurements were taken approximately 10–20 cm above the sediment–water interface to accurately characterize the microenvironment directly experienced by *B. marmorata.*

Functional Prediction of Microbial Communities

Functional profiles of the gut microbiota were predicted from 16S rRNA gene sequences using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States), core software. The pipeline infers metagenomic functional content by placing amplicon sequence variants into a reference phylogeny and predicting gene family abundances based on closely related reference genomes.

Predicted gene families were annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and collapsed into KEGG Orthology (KO) terms and higher-level KEGG pathways. Relative abundances of predicted pathways were normalized and visualized using heatmaps to compare functional patterns across taxa.

Functional predictions derived from PICRUSt2 represent inferred metabolic potential rather than direct measurements of gene abundance or expression. As such, these predictions are subject to limitations associated with reference genome availability, taxonomic assignment accuracy, and the use of marker-gene data. Consequently, the inferred functions should be interpreted as putative metabolic capabilities, not experimentally validated microbial activities.

Diversity Analysis

Alpha diversity was calculated using amplicon sequence variant (ASV) relative abundance data and included richness (S), Shannon diversity (H’), Simpson index (λ), inverse Simpson index, Berger–Parker dominance, Pielou’s evenness, effective number of species, and Faith’s phylogenetic diversity. These metrics were used to characterize microbial richness, evenness, and dominance within individual samples.

Beta diversity was assessed using both abundance-based (Bray–Curtis) and presence–absence–based (Jaccard) dissimilarity indices, as well as phylogeny-based UniFrac distances. Community-level patterns were visualized using principal coordinates analysis (PCoA).

Due to the limited number of biological replicates, formal statistical testing of group differences (e.g., PERMANOVA) was not performed. Accordingly, diversity metrics were interpreted descriptively to characterize overall gut microbial community structure, and no inferential claims regarding temporal or environmental differences were made.

Results and Discussion

Overview of Gut Microbial Community Composition

16S rRNA gene sequencing revealed that the gut microbiota of *B. marmorata* was dominated by Pseudomonadota (≈60%), followed by Bacillota (≈25%) and Bacteroidota (≈10%), while minor phyla collectively contributed less than 5% (Figure 3). This phylum-level distribution aligns with the microbial consortia of sediment-feeding holothurians such as *Apostichopus japonicus* and *Holothuria scabra* (Zhao et al., 2024; Zhang et al., 2022).

The prevalence of Pseudomonadota likely reflects their metabolic versatility and environmental ubiquity. This group is known for driving biogeochemical processes, including the sulfur cycle, carbon and nitrogen metabolism, and organic matter degradation (Zhou et al., 2020; Ge et al., 2024). In this study, Pseudomonadota was the most abundant phylum in the gut microbial community of *B. marmorata*. Similar dominance of Pseudomonadota has been reported in other tropical sea cucumber species, including *Holothuria atra, Stichopus chloronotus,* and *S. monotuberculatus* (Wang et al., 2023). Members of this phylum are known to be metabolically versatile in environmental and host-associated communities, and although functional roles cannot be inferred directly from 16S data, their prevalence suggests they may contribute indirectly to nutrient processing and gut ecology.

Bacillota and Bacteroidota, common in the digestive systems of detritivores, are associated with fermentation and polysaccharide breakdown. These processes are vital for nutrient assimilation from detrital substrates (Félix et al., 2025). Such microbial diversity provides metabolic buffering capacity that allows *B. marmorata* to thrive in low-nutrient sediments. In sea cucumbers, these bacterial groups are often associated with the posterior intestine, where they may contribute to digestion and energy production. Consistent with this pattern, Bacteroidota have also been detected in *Apostichopus japonicus* (Lu et al., 2023) and *Holothuria glaberrima* (Pagán- Jiménez et al., 2019c). Metagenomic sequencing has also revealed their occurrence in the foregut (FG) and midgut (MG) regions, following Proteobacteria, Actinobacteria, and Firmicutes (Quintanilla-Mena et al., 2022).

Community Structure and Functional Implications

At finer taxonomic resolution, the community was dominated by Alphaproteobacteria, Gammaproteobacteria, and Bacilli, with

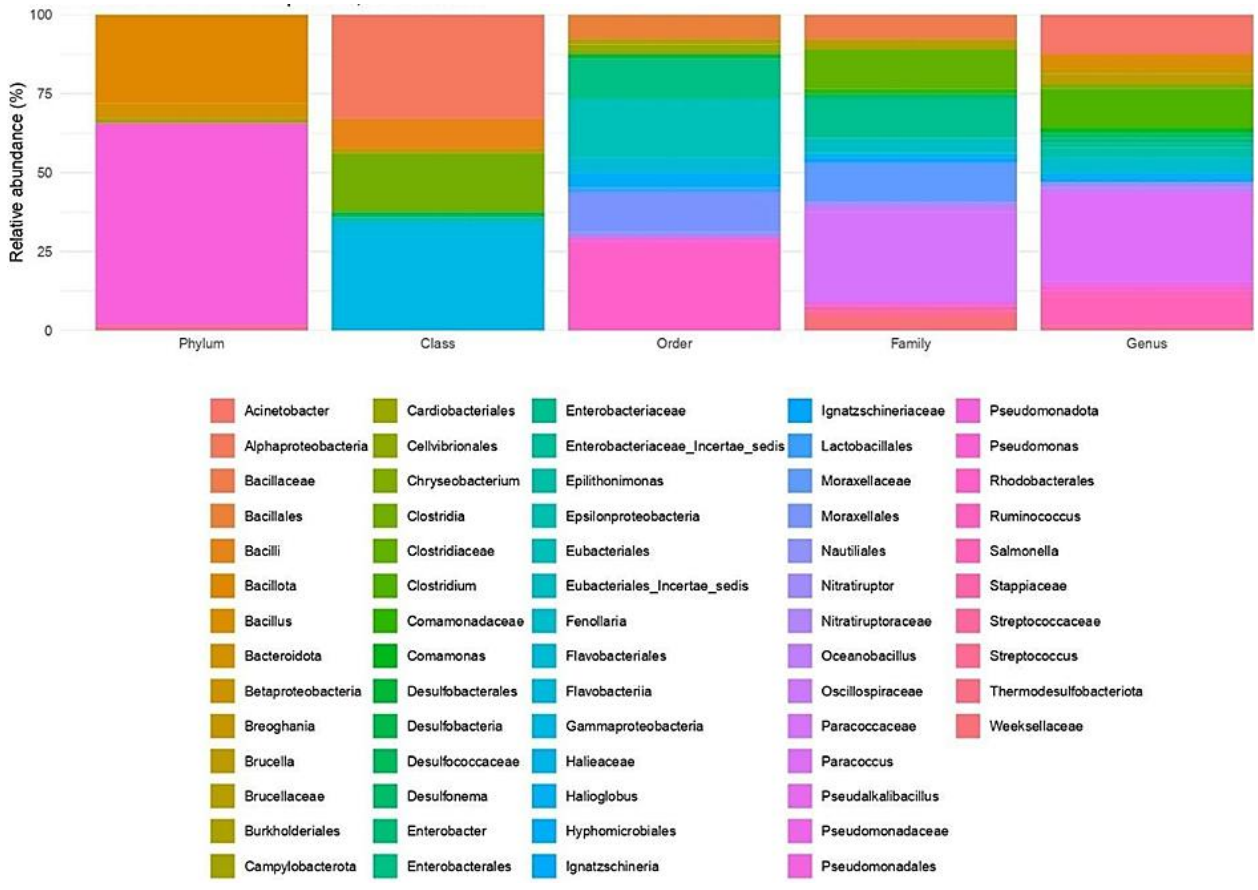

Figure 3. Relative abundance of gut bacterial taxa of *Bohadschia marmorata* at the phylum, class, order, family, and genus levels based on 16S rRNA gene sequencing. Each colored segment represents a distinct bacterial taxon, with bar heights indicating their proportional contribution to the overall gut microbial community.

Rhodobacterales, Moraxellales, and Bacillales being particularly abundant. Families such as Rhodobacteriaceae, Moraxellaceae, and Bacillaceae accounted for a large fraction of total reads. Prominent genera included *Paracoccus, Acinetobacter, Bacillus*, and *Streptococcus.*

Members of this group are known for their contributions to the host regeneration, as observed in *Sclerodactyla briareus* (Weigel, 2020). They also participate in organic matter decomposition, nitrogen cycling, sulfur and carbon cycling (Dyksma et al., 2016; Jia et al., 2024c), and phosphorus solubilization, contributing to both environmental nutrient dynamics and *B. marmorata* gut health (Mandic-Mulec et al., 2015). Moreover, these groups also exhibit high stress tolerance and exhibit adaptability to diverse marine conditions, persist in extreme environments, and contribute to nutrient recycling and gut stability (Márquez et al., 2011; Mandic-Mulec et al., 2015b).

This composition suggests a synergistic network of bacterial guilds engaged in nutrient remineralization, biofilm formation, and redox-dependent energy cycling, all of which are important for holothurian detritivory (Wang et al., 2023). Members of Rhodobacterales are phototrophic sulfur oxidizers that contribute to the detoxification of sulfide-rich sediments, while Bacillales and Moraxellales are involved in extracellular enzyme production and the degradation of complex organic polymers (Yamazaki et al., 2020).

The gut environment of *B. marmorata* harbors a metabolically resilient microbial community capable of responding dynamically to changing oxygen and substrate conditions. This adaptive flexibility parallels strategies observed in other benthic deposit feeders and supports the concept of microbiome-mediated metabolic plasticity in echinoderm digestion (Figure 3).

Core and Transient Taxa: Ecological and Environmental Interpretation

The "core microbiome" of *B. marmorata*, comprising taxa consistently detected across individuals, was dominated by *Paracoccus, Bacillus*, and *Pseudomonas*. These genera are metabolically versatile and participate in carbon and nitrogen cycling, as well as antioxidant protection of the host (Zhao et al., 2024).

Given the resolution limits of 16S rRNA amplicon sequencing, assignments to potentially pathogenic taxa should be interpreted cautiously and reflect sequence similarity rather than confirmed species-level identification. Their occurrence, consistent with observations in *Holothuria edulis* and *Holothuria scabra*, likely represents environmental exposure rather than stable colonization (Arfatahery, 2023). Because *B. marmorata* is a sediment-feeding species, these transient bacteria may originate from ingested material. Stress-induced dysbiosis studies in *Apostichopus japonicus* have shown that shifts in low-abundance taxa correlate with increased oxidative stress and immune modulation, indicating their value as bioindicators of environmental stress (Cui et al., 2024) (Figure 4).

Functional Potential and Ecosystem Roles

Functional annotation (KEGG-based) showed enrichment in energy metabolism, carbohydrate degradation, amino acid turnover, and oxidative phosphorylation. These results are similar to those

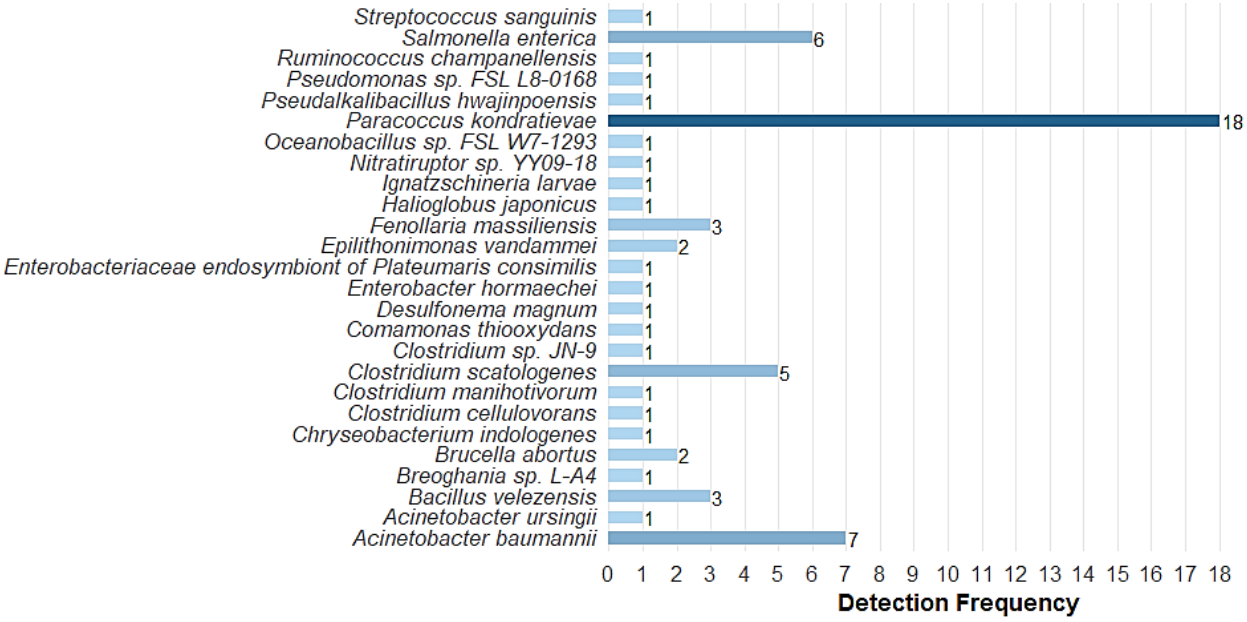

Figure 4. Detection frequency of core bacterial species identified in the gut of *Bohadschia marmorata* based on 16S rRNA gene sequencing. Bars represent the number of samples in which each species was detected, highlighting dominant and recurrent taxa within the gut microbiome.

reported in *Holothuria* and *Stichopus* species, reflecting the conserved metabolic framework of holothurian gut consortia (Zhao et al., 2024). The enrichment of two-component regulatory systems and stress-response pathways indicates a high capacity for adaptive regulation under fluctuating sedimentary redox and nutrient conditions. These systems enhance microbial resilience in benthic environments characterized by varying oxygen and organic loads (Zhang et al., 2022).

This metabolic flexibility supports the ecological role of the *B. marmorata* gut microbiome in nutrient remineralization and sediment bioturbation. Through microbial loop interactions, the microbiome enhances ecosystem productivity and sediment health, reinforcing the species' function as a benthic ecosystem engineer (Félix et al., 2025) (Figure 5).

Probiotic and Biotechnological Potential

The key finding of this study is the detection of *Bacillus velezensis* in the gut of *B. marmorata*. This type of gram-positive and spore-forming bacterium can produce diverse enzymes useful for fermented foods and enzyme production (protease, cellulase, and phytase). It also synthesizes antibacterial compounds (Su et al., 2023) and produces antibiotics that inhibit the growth of pathogens (Jin et al., 2017). It is a non-toxic and harmless biocontrol agent. Moreover, *B. velezensis* has garnered considerable attention as a promising probiotic, capable of modulating intestinal microbiota and enhancing intestinal barrier function (Zhang et al.,2019).

Another genus of interest found in this study is *Ruminococcus*, which is considered a potential next-generation probiotic (NGP) due to its ability to degrade complex carbohydrates and contribute to gut health. On the other hand, *Pseudomonas* (proteobacteria), a gram-negative aerobic bacterium or facultative symbiotic anaerobe, is also considered a potential probiotic. It is highly effective against the 'infectious hematopoietic necrosis virus' in the fish gut (Y. Li et al., 2020).

B. velezensis, *Ruminococcus,* and *Pseudomonas* are recognized for their probiotic and bioactive potential in aquaculture and biotechnology (Sanjeewa & Herath, 2023). Although these taxa suggest promising probiotic potential, functional validation requires strain isolation, genomic characterization, and controlled host trials. Future studies should integrate metabolomics, metatranscriptomics, and in vivo assays to confirm probiotic efficacy and host-microbe interaction mechanisms.

Diversity and Environmental Relevance

Diversity indices (Shannon H'= 2.70; Simpson λ= 0.12; Pielou's evenness= 0.83) revealed a balanced microbial consortium with high evenness and functional redundancy (Table 1). Similar diversity has been observed in *Apostichopus japonicus* under stable conditions, while environmental stress leads to reduced

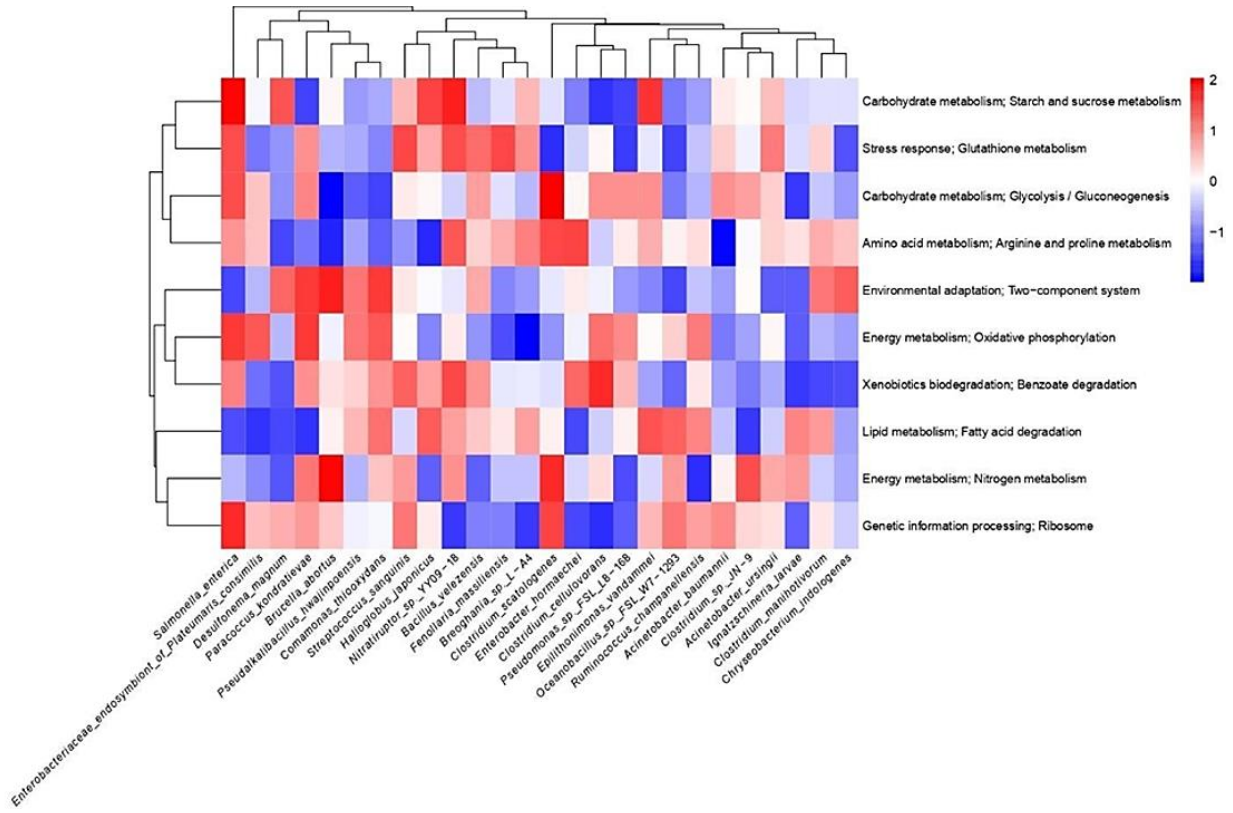

Figure 5. KEGG-based functional prediction heatmap of bacterial species identified in the gut of *Bohadschia marmorata*, showing the relative enrichment of metabolic and cellular pathways across taxa.

Table 1. Summary of the diversity indices across samples from sea cucumber *B. marmorata*

Diversity indices	*B. marmorata*
Berger-Parker Index	0.28
Pielou's Evenness	0.83
Effective Species	14.78
Inverse Simpson	8.57
Simpson Index (λ)	0.12
Shannon Index (H')	2.70
Richness (S)	26
Total Count (N)	64

richness and dominance of opportunistic taxa (Cui et al., 2024). These results suggest that microbiome diversity and evenness can act as early indicators of environmental disturbance. Incorporating microbiome-based assessments into conservation programs for *B. marmorata* may enhance the detection of habitat degradation and pollution in tropical benthic ecosystems.

Conclusion

The findings suggest that the *B. marmorata* gut microbiome functions both as a metabolic extension of the host and as a responsive indicator of environmental quality. This dual role emphasizes the ecological importance of holothurian-microbiome interactions and highlights the potential of microbiome analysis in marine biomonitoring. Future research should integrate multi-omics approaches, including metagenomics and metabolomics, to unravel the co-evolutionary and ecological mechanisms that underpin host-microbiome symbioses in benthic invertebrates (Hamel et al., 2024).

Ethical Statement

All sea cucumber (*Bohadschia marmorata*) samples were collected legally under a Gratuitous Permit issued by the Bureau of Fisheries and Aquatic Resources (BFAR), the Philippine government agency responsible for the protection and management of aquatic organisms. All procedures followed institutional and national guidelines for the responsible use of marine organisms in research.

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Author Contribution

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Original draft Preparation: Jomar F. Besoña, Marjel M. Broce

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Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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