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Metagenomic characterization of the gut microbiota of Actinopyga echinites reveals ecological functions and untapped biotechnological potential

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Abstract

Host-associated microbiomes are increasingly recognized as key drivers of ecosystem functioning, yet the microbial diversity of tropical sea cucumbers remains poorly understood. This study provides the first metagenomic characterization of the gut microbiota of the vulnerable (IUCN Red List) sea cucumber, *Actinopyga echinites*, from the Philippines, and explores its ecological and biotechnological significance. Gut samples collected from Barobo, Surigao del Sur, were analyzed using Oxford Nanopore 16S rRNA sequencing and bioinformatic profiling. The gut microbiome was dominated by *Bacillota* and *Pseudomonadota*, with *Clostridia*, *Bacilli*, and Gammaproteobacteria representing the major taxonomic groups. Diversity analyses revealed a moderately diverse but highly even microbial community (Shannon index = 2.45; Pielou's evenness = 0.95), indicating a stable and functionally balanced gut ecosystem. Core microbial taxa included *Morganella morganii* and *Bacillus spp*., while several environmentally derived taxa reflected continuous recruitment from sediment-associated habitats. Functional prediction analyses suggested strong capacities for carbohydrate degradation, protein turnover, nutrient remineralization, and anaerobic fermentation, highlighting the microbiome's contribution to host nutrition and benthic nutrient cycling. Notably, several bacterial taxa were associated with the production of hydrolytic enzymes, antimicrobial peptides, and biosurfactants with potential applications in aquaculture, biotechnology, pharmaceuticals, and environmental bioremediation. The coexistence of fermentative, probiotic, and metabolically versatile microorganisms suggests that the gut of *A. echinites* functions as a microbial bioreactor linking host physiology to ecosystem processes. These findings establish a critical baseline for understanding host–microbe interactions in tropical holothurians and identify *A. echinites* as a promising reservoir of microbial resources for sustainable conservation initiatives and marine biotechnology.

Introduction

Sea cucumbers (Holothuroidea) comprise roughly 1,700 species and are important benthic deposit-feeders and bioturbators in tropical and temperate marine ecosystems (Purcell et al. 2016; Harini et al. 2024; Félix et al. 2025). Through continuous ingestion, bioturbation, and reworking of surface sediments, these echinoderms regulate nutrient remineralization, accelerate organic matter decomposition, and modify sediment biogeochemistry, thereby contributing substantially to ecosystem productivity and stability (MacTavish et al. 2012; Purcell et al. 2016). In addition to their ecological importance, sea cucumbers sustain valuable commercial fisheries and aquaculture industries and have emerged as promising sources of bioactive compounds with pharmaceutical, nutraceutical, cosmetic, and biotechnological applications (Anderson et al. 2010; Rahman and Yusoff 2017). Nevertheless, increasing international demand for dried sea cucumber products (bêche-de-mer or trepang) has intensified harvesting pressure throughout the Indo-Pacific, leading to population declines in numerous commercially exploited species (Purcell et al. 2011).

The Philippines represents one of the principal centers of sea cucumber diversity in the Indo-Pacific region, supporting extensive artisanal fisheries with at least 47 species identified as comercially valuable

(Choo, 2008; Labe, 2009; Arriesgado et al. 2022a). Among these, *Actinopyga echinites* (Jaeger, 1833) is an ecologically important deposit-feeding holothurian distributed across shallow coral reef flats, seagrass meadows, and sandy coastal habitats (Purcell et al. 2023). Besides supporting coastal livelihoods in Mindanao (Arriesgado et al. 2022a; Arriesgado et al. 2022b; Sornito et al. 2022), the species performs essential ecosystem functions by enhancing sediment turnover, stimulating nutrient recycling, and facilitating organic matter processing (Purcell et al. 2016; Pasquini et al. 2021; Sadoul et al. 2022). However, continued exploitation has contributed to population declines, resulting in its classification as Vulnerable on the IUCN Red List (Conand, 2014). Although previous investigations have substantially improved understanding of its fisheries, abundance, and aquaculture potential (Arriesgado et al. 2022a; Sornito et al. 2022; Besoña et al. 2026; Alejandro 2019), little is known about the microbial communities associated with its digestive system.

Growing evidence demonstrates that host-associated microbiomes constitute an integral component of marine invertebrate biology. Rather than acting solely as transient microbial assemblages, gut microbiota participates in digestion, nutrient assimilation, immune regulation, and physiological adaptation while simultaneously influencing ecosystem-level biogeochemical processes (Amaro et al. 2010; Pan et al. 2024; Abyaba et al. 2024). In sea cucumbers, intestinal microorganisms degrade complex organic substrates and facilitate the recycling of carbon and nutrients within benthic sediments. Studies of *H. atra*, *H. leucospilota*, *B. marmorata* and other tropical holothurians have consistently reported gut microbiomes dominated by metabolically versatile bacterial groups involved in organic matter transformation and sediment nutrient cycling (Gao et al. 2021; Besoña et al. 2026). Likewise, variation in gut microbial diversity has been associated with differences in host growth and physiological condition in cultured *Holothuria scabra* (Tabardillo et al. 2025), showing the ecological importance of host–microbe interactions in holothurian biology.

The rapid development of high-throughput sequencing technologies has transformed the study of host-associated microbial communities by enabling comprehensive taxonomic characterization and functional prediction without reliance on culture-dependent approaches. Amplicon-based metagenomic analyses have identified microbial lineages associated with nutrient metabolism, antimicrobial compound production, xenobiotic degradation, and other processes with considerable ecological and industrial significance (Jia et al. 2024; Choo et al. 2023; Weigel, 2020). Despite these advances, no study has characterized the gut microbiome of *A. echinites* from Philippine waters, leaving a substantial gap in understanding how microbial communities contribute to the ecology, conservation, and potential biotechnological value of this vulnerable tropical holothurian.

To address this knowledge gap, this study employed Oxford Nanopore-based 16S rRNA metagenomic sequencing to characterize the gut bacterial community of *A. echinites* collected from Barobo, Surigao del Sur, Philippines. Specifically, the study aimed to (i) determine the taxonomic composition and diversity of gut-associated bacterial communities, (ii) predict their ecological functions and biotechnological potential, and (iii) evaluate their relevance to nutrient cycling and host ecology. By providing the first metagenomic characterization of the gut microbiota of *A. echinites* in the Philippines,

this study establishes an important baseline for understanding host–microbe interactions in tropical sea cucumbers while identifying microbial resources with potential applications in sustainable aquaculture, marine biotechnology, and biodiversity conservation.

Materials and Methods

Sample collection and experimental design

Adult *Actinopyga echinites* were obtained from seagrass habitats in Barobo, Surigao del Sur, Philippines (Fig. 1), where the species represents one of the principal components of the local sea cucumber fishery based on recent catch landing assessments (Arriesgado et al. 2025). Collection of *Actinopyga echinites* specimens was authorized under BFAR Gratuitous Permit No. GP-0330-2025 issued by the Bureau of Fisheries and Aquatic Resources (BFAR), Philippines. Field collections were conducted during two independent sampling periods, November 2024 and February 2025, with each sampling event treated as a biological replicate for subsequent microbiome analyses.

During each collection period, 30 adult individuals (wet weight: 80–100 g) were manually collected via gleaning and handpicking from shallow seagrass beds. Following collection, three individuals from each sampling event (n = 3) were randomly selected for gut microbiome characterization, giving a total of 9 individuals included in the metagenomic analysis. Each selected specimen was treated as an independent biological replicate throughout sample processing.

Specimens designated for microbiome analysis were processed immediately under sterile laboratory conditions. Each individual was dissected separately using sterilized scalpels, after which gut contents were aseptically recovered by gently expressing the digestive tract into sterile collection tubes. Gut material from the three selected individuals in each sampling period was pooled to generate a representative sample for sequencing. Throughout transport, samples were maintained at chilled temperatures to minimize microbial alteration and were transferred to a − 80°C freezer upon arrival at the laboratory, where they remained until DNA extraction and downstream sequencing.

DNA extraction, DNA quality assessment, and 16S rRNA gene amplification

Total genomic DNA was isolated from gut samples of *A. echinites* using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). DNA extraction followed the manufacturer's protocol with minor modifications designed to maximize DNA yield and purity for downstream sequencing applications. All molecular procedures, including DNA extraction and preliminary quality evaluation, were carried out at the Philippine Genome Center–Mindanao.

DNA integrity was subsequently assessed by agarose gel electrophoresis. Each DNA extract was combined with Vivantis 6× loading dye before separation on a 1% agarose gel alongside a Vivantis 1 kb

DNA ladder. Electrophoresis was performed using a Cleaver Scientific electrophoresis system operated at 100 V for 30 min. Gel images were visualized under ultraviolet illumination using a Vilber NanoPac 300 Gel Documentation System to verify DNA quality and confirm that extracted nucleic acids were suitable for downstream amplification.

The bacterial 16S rRNA gene was amplified to profile gut microbial communities, targeting the V3–V4 hypervariable regions because of their high taxonomic resolution for bacterial identification. PCR amplification employed the universal bacterial primer set supplied with the Oxford Nanopore Technologies (ONT) 16S Barcoding Kit. Amplification reactions were conducted at the Philippine Genome Center–Mindanao, with each biological sample processed in triplicate to improve reproducibility. Thermal cycling conditions followed the protocol recommended by ONT. Successful amplification was confirmed by agarose gel electrophoresis before library preparation, and PCR products exhibited the expected fragment size of approximately 450 bp.

Nanopore sequencing and library preparation

Sequencing libraries were generated using the Oxford Nanopore Technologies 16S Barcoding Kit (SQK-16S024) following the manufacturer's recommendations. Amplicons from individual samples were assigned unique barcode sequences and pooled at equimolar concentrations before sequencing. Library sequencing was performed using an ONT MinION Mk1B instrument equipped with FLO-MIN106 (R9.4.1) flow cells. Sequencing runs were maintained for approximately 72 h, with instrument performance and sequencing output continuously monitored via MinKNOW software (v23.07.15).

Basecalling, quality control, and bioinformatics analysis

Raw nanopore signal files were converted to nucleotide sequences and demultiplexed with Guppy v7.1.4 integrated into the MinKNOW environment, using the fast-basecalling model. Demultiplexed reads were exported as compressed FASTQ files, producing amplicons with an expected read length of approximately 450 bp. To improve downstream analytical accuracy, sequencing reads were subjected to quality filtering and length trimming. Reads with an average Phred quality score below Q10 were discarded, while sequences falling within the expected amplicon length were retained. These filtering criteria were selected to preserve sequencing depth while minimizing the inclusion of low-quality reads. High-quality reads were processed using the DADA2 algorithm implemented in QIIME2 (v2023.9), with denoising and chimera removal performed simultaneously. Filtering and truncation parameters were optimized after inspecting base-quality profiles to retain high-confidence sequence information while excluding low-quality terminal regions. This workflow generated a final dataset composed of high-resolution amplicon sequence variants (ASVs). To minimize analytical noise introduced by sequencing artefacts, singleton ASVs together with features representing less than 0.01% of the total sequencing reads were excluded before downstream community analyses. Taxonomic identities of retained ASVs were assigned using a naïve Bayes classifier trained against the SILVA 138 reference database trimmed

to the bacterial V3–V4 region. Relative abundance tables were subsequently generated across multiple taxonomic ranks to characterize the composition of the gut microbial community. The resulting analytical pipeline produced taxonomic summaries, abundance matrices, and graphical outputs that formed the basis for subsequent ecological interpretation.

Functional prediction of microbial communities

Potential metabolic functions of the gut microbiota were inferred from 16S rRNA gene sequences using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States). This analytical framework predicts metagenomic functional profiles by placing amplicon sequence variants within a reference phylogeny and estimating gene family abundances from closely related genomes.

Predicted gene families were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, then summarized into KEGG Orthology (KO) groups and higher-order metabolic pathways. Relative pathway abundances were normalized and displayed as heatmaps to facilitate comparisons among dominant bacterial taxa.

Because PICRUSt2 infers functional potential from phylogenetic relationships rather than directly sequencing microbial genomes, predicted pathways should be regarded as estimates of metabolic capability rather than direct measurements of gene abundance or expression. Consequently, these functional profiles were interpreted as putative metabolic potential that requires confirmation through genome-resolved metagenomic or transcriptomic analyses.

Diversity analysis

Microbial alpha diversity was quantified from ASV abundance data using species richness (S), Shannon diversity (H'), Simpson dominance (λ), inverse Simpson diversity, Berger–Parker dominance, Pielou's evenness, effective number of species, and Faith's phylogenetic diversity. Collectively, these complementary metrics were used to evaluate microbial richness, dominance, evenness, and phylogenetic diversity within individual gut communities.

Variation in community composition among samples was assessed using Bray–Curtis, Jaccard, and UniFrac distance metrics representing abundance-based, presence–absence, and phylogeny-based dissimilarity, respectively. Patterns of beta diversity were visualized through principal coordinates analysis (PCoA).

Accordingly, diversity analyses were interpreted descriptively to characterize overall community composition without making inferential comparisons among sampling periods or environmental conditions.

The raw 16S rRNA gene sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1437922. The data are

currently under embargo and will be publicly accessible from 1 January 2027.

Results

Taxonomic composition of gut microbiota

The gut microbiota of *A. echinites* comprised three bacterial phyla, with *Bacillota* representing the dominant phylum, followed by *Pseudomonadota*, while *Acidobacteriota* accounted for only a minor proportion of the community (Fig. 2). At the class level, the bacterial assemblage was primarily composed of *Clostridia, Bacilli*, and *Gammaproteobacteria*, whereas *Alphaproteobacteria* and *Terriglobia* occurred at relatively lower abundances.

Greater taxonomic resolution at the order level revealed that the gut microbiota was mainly represented by *Eubacteriales*, *Hyphomicrobiales*, *Bacillales*, *Lactobacillales*, *Enterobacterales*, and *Sphingomonadales*, with several additional orders contributing smaller proportions of the bacterial community (Fig. 2). At the family level, *Clostridiaceae*, *Erythrobacteraceae*, *Morganellaceae*, *Lactobacillaceae*, *Hyphomonadaceae*, *Brucellaceae*, *Peptostreptococcaceae*, *Rhizobiaceae*, *Bacillaceae*, *Candidatus Korobacteraceae*, and *Natronincolaceae* were identified, although their relative abundances varied considerably.

Genus-level classification showed a heterogeneous bacterial community comprising *Morganella, Limosilactobacillus, Priestia, Erythrobacter, Hyphomonas, Serpentinicella, Brucella*, and *Candidatus Liberibacter*, together with several less abundant taxa (Fig. 2). No single genus dominated the microbial assemblage, indicating that multiple bacterial genera contributed to the overall gut microbial composition.

Detection frequency of dominant bacterial taxa

The frequency of occurrence of bacterial taxa across biological replicates revealed differences in the consistency of individual members of the gut microbiota (Fig. 3). Among the identified taxa, *Morganella morganii* and *Bacillus anthracis* were detected in all three biological replicates (detection frequency = 3), representing the most consistently observed bacterial species in the gut microbiome of *A. echinites*.

Clostridium botulinum was detected in two of the three biological replicates (detection frequency = 2), indicating an intermediate occurrence level. In contrast, the remaining bacterial taxa were each detected in a single biological replicate (detection frequency = 1). These taxa included *Brucella abortus*, *Candidatus Korobacter versatilis*, *Candidatus Liberibacter solanacearum, Clostridium sp.* JN-1, *Erythrobacter* uncutlured, *Fenollaria massiliensis, Hyphomonas* sp. KY3, *Limosilactobacillus panis, Priestia aryabhattai*, and *Serpentinicella alkaliphila* (Fig. 3).

Predicted functional profiles of the gut microbiota

PICRUSt2-based functional prediction identified multiple metabolic pathways associated with the dominant bacterial taxa detected in the gut microbiota of *A. echinites* (Fig. 4). Hierarchical clustering of

standardized functional abundances (z-scores) revealed considerable variation in the predicted metabolic profiles among bacterial taxa, indicating differences in their potential functional contributions.

Predicted functions encompassed a broad range of metabolic processes, including glutathione metabolism (stress response), oxidative phosphorylation (energy metabolism), ribosome-associated genetic information processing, starch and sucrose metabolism, glycolysis/gluconeogenesis, arginine and proline metabolism, fatty acid degradation, nitrogen metabolism, benzoate degradation, and pathways associated with environmental adaptation (Fig. 4).

Variation in pathway abundance was evident among taxa. Higher standardized abundances (positive z-scores; red) were observed for selected bacterial taxa in pathways related to carbohydrate metabolism, amino acid metabolism, lipid metabolism, nitrogen metabolism, and xenobiotic biodegradation, whereas lower standardized abundances (negative z-scores; blue) characterized other taxa for the same functional categories. Similarly, pathways involved in energy metabolism, genetic information processing, and stress response exhibited heterogeneous distributions across the bacterial community. Hierarchical clustering further grouped bacterial taxa according to similarities in their predicted functional profiles and simultaneously clustered metabolic pathways with comparable abundance patterns.

Alpha diversity of the gut microbiota

Alpha diversity analysis revealed a total of 18 detected bacterial taxa, corresponding to a species richness (S) of 13 and an effective number of species of 11.56 (Table 1). The gut microbiota exhibited a Shannon diversity index (H') of 2.45, an inverse Simpson index of 10.13, and a Simpson dominance index (λ) of 0.10. Community evenness was high, as indicated by a Pielou's evenness (J') value of 0.95, whereas the Berger–Parker dominance index was 0.17 (Table 1).

Table 1
Alpha diversity of the gut microbiota of *Actinopyga echinites*. Diversity was quantified using species richness (S), Shannon diversity (H'), Simpson dominance (λ), inverse Simpson diversity, Berger–Parker dominance, Pielou's evenness (J'), effective number of species, and total detected taxa (N), providing complementary measures of microbial richness, evenness, and dominance within the gut bacterial community.

Diversity indices	***Actinopyga echinites***
Berger-Parker Index	0.17
Pielou's Evenness (J')	0.95
Effective Species	11.56
Inverse Simpson	10.13
Simpson Index (λ)	0.10
Shannon Index (H')	2.45
Richness (S)	13
Total Count (N)	18

Discussion

Gut microbial community composition

The gut microbiota of *A. echinites* is dominated by *Bacillota* and *Pseudomonadota*, with minor representation of *Acidobacteriota*. Within *Bacillota*, *Clostridia* and *Bacilli* are predominant, including fermentative and probiotic taxa such as *Limosilactobacillus* and *Priestia* (Ouwehand et al. 2002; Gupta et al. 2020). Members of *Clostridiaceae* and *Lactobacillaceae* support anaerobic degradation of carbohydrates and may aid host digestion and immune modulation (Flint et al. 2012).

Pseudomonadota, represented by Gammaproteobacteria and Alphaproteobacteria, includes facultative anaerobes and environmental generalists such as *Morganella* and *Erythrobacter* (Newton et al. 2013). Additional families, such as *Candidatus Korobacteraceae* and *Brucellaceae*, highlight metabolic versatility and potential host interactions. The microbiota exhibits fermentative capacity, environmental adaptability, and roles in nutrient cycling and gut homeostasis, consistent with microbial assemblages in other holothurians (Zhang et al. 2012).

Community structure and functional implications

The gut microbiota of *A. echinites* showed a limited but stable core community, with *Morganella morganii* and *Bacillus anthracis* consistently detected across replicates, indicating a close association with the

host (Liu et al. 2016). Notably, *M. morganii* is widely recognized as an opportunistic pathogen in humans and a frequent cause of nosocomial infection (Shi et al. 2022; Falagas et al. 2006). Its persistence can be attributed to the nutrient-rich, low-oxygen environment of the intestine and the sediment-feeding behavior of *A. echinites*, suggesting a possible commensal or symbiotic role, synthesizing poly-beta-hydroxybutyrate (PHB), which may modulate the intestinal microbiota and contribute to their growth (Yamazaki et al. 2016). The role of *B. anthracis* in marine environments remains unclear; it may exist as a dormant spore, a contaminant, or an adapted symbiont. The consistent detection of *M. morganii* across biological replicates suggests that this bacterium may maintain a stable association with the gut environment of *A. echinites*. Although the present study provides no direct evidence of vertical transmission or genome reduction, this repeated occurrence raises the possibility of an early host-associated evolutionary relationship. If *M. morganii* is maintained across successive host generations, long term adaptation to the host environment could potentially lead to genome reduction through processes consistent with muller's Ratchet as described for obligate endosymbiotic bacteria (Naito & Pawlowska, 2016). *Clostridium botulinum* was less consistently detected but may contribute to anaerobic fermentation and protein degradation (Erbguth, 2004).

Other taxa, including *Serpentinicella alkaliphila*, *Priestia aryabhattai*, *Limosilactobacillus panis*, *Hyphomonas sp*., *Fenollaria massiliensis*, uncultured *Erythrobacter*, *Clostridium sp*., *Candidatus Liberibacter solanacearum*, *Candidatus Korobacter versatilis*, and *Brucella abortus*, appeared sporadically, likely acquired through sediment ingestion. The detection of soil- and plant-associated bacteria further supports environmental acquisition via benthic feeding. The occurrence of potentially pathogenic taxa (*B. anthracis*, *C. botulinum*, *B. abortus*) warrants further investigation to clarify whether they are passive colonizers or metabolically active members of the microbiome. *A. echinites* hosts a stable core microbiota alongside variable, environmentally derived taxa, with potential roles in digestion, immune regulation, and adaptation to benthic habitats.

Ecosystem roles and biotechnological potential

Anaerobic bacteria, such as *C. botulinum* and *Limosilactobacillus panis*, produce hydrolytic enzymes, including proteases, cellulases, and lipases, that are well suited to low-oxygen marine sediment environments (Verardi et al. 2023; Dalmaso et al. 2015). According to Wu et al. (2024), these enzymes exhibit significant potential for waste bioconversion, biofuel production, and industrial fermentation due to their stability under extreme environmental conditions. Previous findings by Matsushita and Okabe (2001) also identified *L. panis* as an anaerobic bacterium capable of hydrolyzing polysaccharides, lipids, and proteins by secreting glycosyltransferases, esterases, and lipases. The use of enzymes from extremophilic microorganisms is particularly valuable in industrial biotechnology, as these enzymes retain activity under harsh conditions, including high temperatures, fluctuating pH, elevated salinity, and pressure (Niehaus et al. 1999). In addition, some gut-associated bacteria secrete enzymes, including proteases, lipases, and amylases, that enhance nutrient assimilation and gut health in aquaculture species (Gao et al. 2014). Collectively, these properties underscore the potential of anaerobic bacteria as key contributors to nutrient recycling and sustainable biotechnological processes. Furthermore, several

gut-associated bacteria, including *Priestia aryabhattai and Fenollaria massiliensis*, are known to synthesize secondary metabolites such as antimicrobial peptides (AMPs), biosurfactants, and signaling molecules (Sun et al. 2021; Sultan et al. 2025). These metabolites hold considerable promise for pharmaceutical applications, natural product discovery, and antifouling solutions in aquaculture systems. AMPs are widely distributed in nature and possess broad-spectrum antibacterial, antiviral, and anticancer properties, making them viable alternatives to conventional antibiotics due to their lower risk of inducing drug resistance (Li et al. 2023). Similarly, biosurfactants, amphiphilic compounds produced by microorganisms, can reduce interfacial and surface tension and have shown significant potential in biomedical and pharmaceutical fields, including drug delivery, antimicrobial applications, and biofilm inhibition (Ceresa et al. 2023).

The occurrence of these bioactive and enzyme-producing bacteria within the gut microbiota of *A. echinites* shows their biotechnological importance for developing eco-friendly industrial, aquaculture, and medical applications. These findings suggest the concept that the sea cucumber gut ecosystem serves as a reservoir of functionally diverse microorganisms with considerable potential for future exploration in biotechnology and sustainable marine resource utilization.

Diversity and environmental relevance

The gut microbiota of *A. echinites* exhibited moderate bacterial diversity coupled with remarkably high community evenness, suggesting a balanced and functionally stable microbial ecosystem. Alpha diversity metrics revealed a Shannon diversity index (H') of 2.45, species richness (S) of 13, and an effective number of species of 11.56, indicating that the gut environment supports a moderately diverse assemblage of microorganisms despite the relatively low taxonomic richness observed (Table 1). The effective number of species, which translates Shannon diversity into the equivalent number of equally abundant taxa, suggests that most detected taxa contribute meaningfully to community structure rather than being represented by rare or transient organisms.

The high Pielou's evenness value (J = 0.95) demonstrates that bacterial taxa were distributed relatively uniformly throughout the gut microbiome. Such high evenness is generally considered an indicator of ecological stability because no single taxon overwhelmingly dominates community composition (Pielou, 1966). This observation is further supported by the low Berger–Parker dominance index (0.17) and Simpson dominance value (λ = 0.10), which indicate limited dominance by any individual microbial taxon. The corresponding inverse Simpson index (10.13) also suggests a relatively diverse community with multiple coexisting taxa contributing to ecosystem functions.

The observed diversity pattern is consistent with the ecological feeding strategy of *A. echinites*. As a deposit-feeding holothurian, this species continuously ingests sediment particles rich in organic matter and microorganisms, thereby facilitating the recruitment of a broad spectrum of environmental bacteria into the digestive tract (Purcell et al. 2016; Pan et al. 2024). Previous studies have demonstrated that sea cucumber guts function as microbial bioreactors where ingested microbes interact with resident bacterial communities to facilitate nutrient recycling and organic matter degradation (Amaro et al. 2010;

Pan et al. 2024). The relatively high evenness observed in the present study suggests that the gut environment provides ecological niches that support diverse microbial functions, including carbohydrate fermentation, protein degradation, and nutrient remineralization.

Compared with other tropical sea cucumbers, the diversity observed in *A. echinites* falls within the range reported for wild deposit-feeding holothurians. Gao et al. (2021) reported that the gut microbiota of *Holothuria atra* and *H. leucospilota* consisted of diverse bacterial assemblages dominated by *Proteobacteria* and *Firmicutes*, reflecting adaptation to sediment-based feeding. Similarly, Wang et al. (2023) demonstrated that tropical sea cucumbers harbor complex gut microbial communities associated with nutrient metabolism and environmental adaptation. The moderate richness but high evenness detected in *A. echinites* may indicate a specialized, yet stable microbial consortium adapted to the physicochemical conditions of seagrass-associated sediments in Barobo, Surigao del Sur.

From an ecological perspective, balanced microbial communities are often associated with greater functional redundancy, whereby multiple taxa perform similar ecological roles. Functional redundancy enhances resilience against environmental disturbances and contributes to the stability of ecosystem processes (Allison & Martiny, 2008). In the gut of *A. echinites*, the coexistence of fermentative taxa such as *Limosilactobacillus* and *Clostridium*, together with environmentally derived taxa including *Erythrobacter* and *Hyphomonas*, suggests complementary roles in organic matter decomposition and nutrient transformation. Such microbial activities may enhance the host's capacity to process sedimentary organic matter while simultaneously influencing nutrient fluxes within benthic ecosystems.

The relatively low dominance values observed in this study also indicate that the gut microbiome is not strongly influenced by opportunistic bacterial blooms, which are often associated with dysbiosis or environmental stress (Shreiner et al. 2015). Instead, the microbial assemblage appears to be maintained through dynamic interactions between host selection mechanisms and continuous environmental recruitment. This finding supports the hypothesis that sea cucumber microbiomes are shaped by both host-associated and sediment-derived microbial communities, resulting in a stable yet adaptable gut ecosystem (Weigel, 2020; Pan et al. 2024).

The diversity metrics indicate that *A. echinites* hosts a moderately diverse, highly even, and ecologically balanced gut microbiota. Such a community structure likely contributes to digestive efficiency, nutrient cycling, and host health while providing resilience against environmental fluctuations. These findings further provide the ecological importance of sea cucumber-associated microbiomes and highlight their potential to maintain benthic ecosystem functioning and support future biotechnological applications.

Conclusion

This study provides the first metagenomic insight into the gut microbiota of the vulnerable sea cucumber *A. echinites* in the Philippines, revealing a diverse and highly balanced microbial community with important ecological and biotechnological functions. The dominance of fermentative and metabolically versatile bacteria highlights the role of microbiomes in nutrient cycling, organic matter

degradation, and host digestive processes. Moreover, the detection of taxa associated with hydrolytic enzymes, antimicrobial compounds, and biosurfactants underscores the untapped potential of *A. echinites* as a reservoir of valuable microbial resources. These findings advance our understanding of host–microbe interactions in tropical sea cucumbers and provide a foundation for future conservation strategies, sustainable aquaculture development, and marine biotechnology applications.

Declarations

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Competing Interests

The authors declare that they have no competing financial or non-financial interests that could have influenced the work reported in this paper.

Author Contributions

J.F.B., E.M.A., F.F.F., and F.L.P. designed the study. J.F.B., M.M.B., M.B.S., E.J.A.T., M.F.B., and D.M.A. performed the experiments and collected the data. J.F.B. analyzed the data. J.F.B. wrote the original draft of the manuscript. E.M.A., F.F.F., R.Y.C., and F.L.P. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics Approval

Specimen collection was conducted under BFAR Gratuitous Permit No. GP-0330-2025, issued by the Bureau of Fisheries and Aquatic Resources (BFAR), Philippines. All collection activities complied with the conditions of the permit and applicable national regulations governing the collection of aquatic wildlife for scientific research.

Data Availability

The raw 16S rRNA gene sequencing data generated during this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject accession number PRJNA1437922. The dataset is currently under embargo and will become publicly available on 1 January 2027. All other data generated or analyzed during this study are included in this published article.

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Figures



Figure 1

Adult specimen of *A. echinites* collected from seagrass habitats in Barobo, Surigao del Sur, Philippines. The inset illustrates the dissected digestive tract from which gut material was obtained for metagenomic characterization.

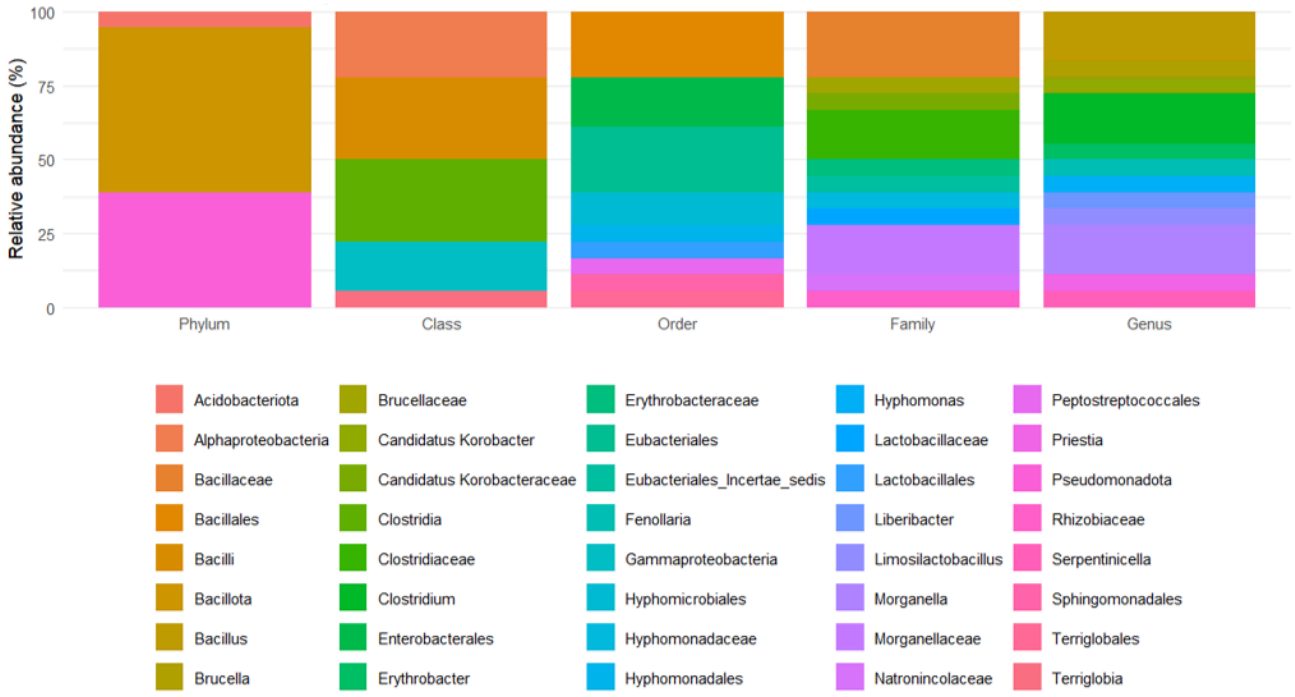


Figure 2

Taxonomic composition of the gut microbiota of *A. echinites*across five hierarchical taxonomic levels. Stacked bar plots illustrate the relative abundance (%) of bacterial taxa at the phylum, class, order, family, and genus levels derived from Oxford Nanopore 16S rRNA sequencing.

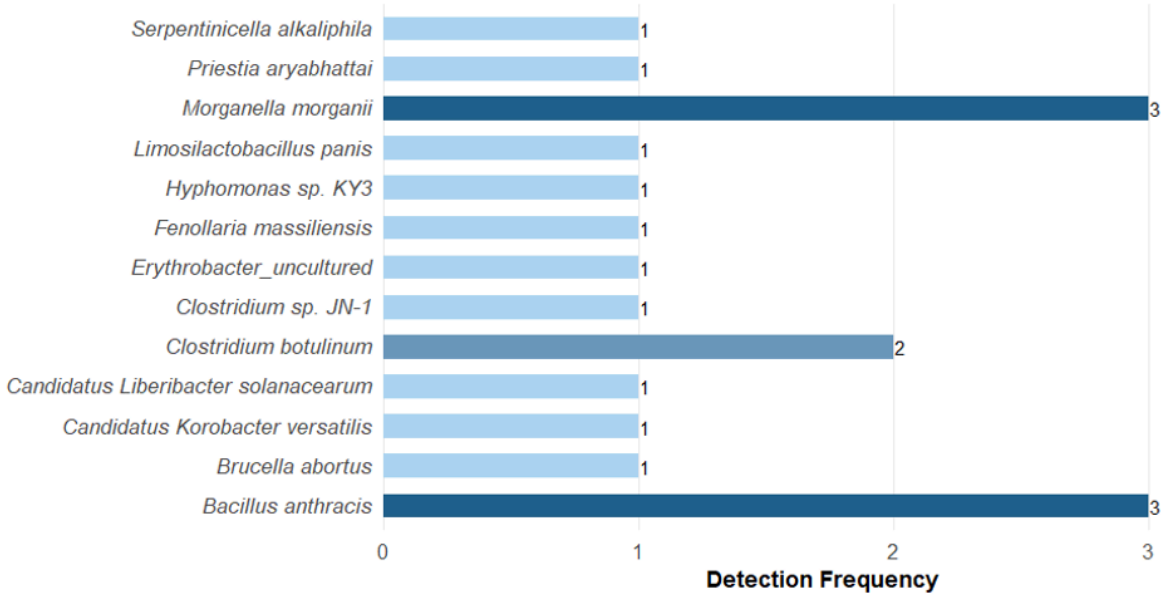


Figure 3

Detection frequency of bacterial taxa identified in the gut microbiota of *A. echinites* across biological replicates. Numbers at the end of each bar indicate the number of replicates in which each taxon was detected.

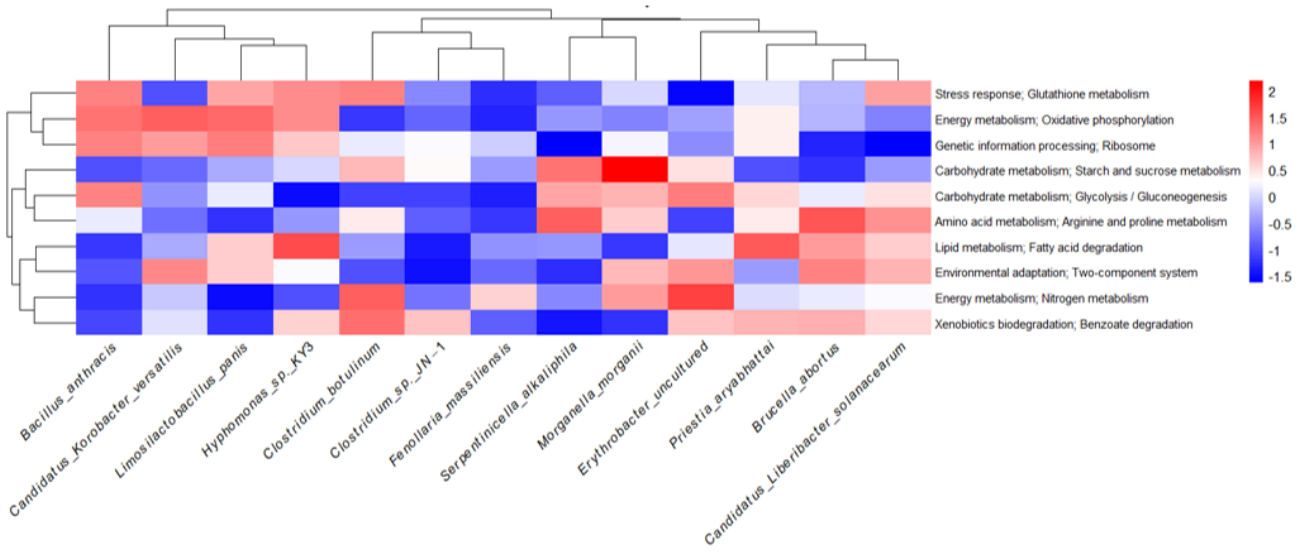


Figure 4

Heatmap showing the predicted functional profiles of dominant bacterial taxa identified in the gut microbiota of Actinopyga echinites based on PICRUSt2 analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations. Rows represent predicted metabolic pathways, and columns represent bacterial taxa. The colour gradient indicates standardized relative functional abundance (z-score), with red denoting higher predicted abundance and blue indicating lower predicted abundance.