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Gut microbiome profile of *Namalycastis* sp. at Setiu wetland in Terengganu, Malaysia

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Abstract. *Iehata S, Hamzah SR, Azmi SS, Ibrahim YS. 2025. Gut microbiome profile of* Namalycastis *sp. at Setiu wetland in Terengganu, Malaysia. Biodiversitas 26: 356-365.* Marine worm regulates the surrounding sediment microbial composition structure and diversity. While there are increasing reports on marine worm-related microbiome assemblage and their functional role in the ecosystem, more knowledge still needs to be reported on the gut microbiome associated with the Nypa worm (*Namalycastis* sp.). The present study conducted 16S rRNA gene amplicon sequencing to investigate the microbiome community of the Nypa worm gut as well as the environmental sample (rotten Nypa and surrounding water) and analyzed potential microbial functions using functional annotation of prokaryotic taxa (FAPROTAX). Principal coordinates analysis (PCoA) and upset plot results revealed the obvious relationship between microbiome compositions associated with *Namalycastis* sp. gut and their habitat (Nypa). Similarly, some genera, such as genus *Demequina*, were found to be in higher abundance in *Namalycastis* sp. gut and Nypa. This higher abundance could indicate a symbiotic relationship or a specific adaptation to the Nypa environment. Functional predictions based on FAPROTAX indicated that Nypa worms possess higher potentials for aromatic hydrocarbon degradation, aromatic compound degradation, aliphatic non-methane hydrocarbon degradation, and hydrocarbon degradation than environmental samples. The findings in this study suggested that the Nypa worm gut is a reservoir of several beneficial bacteria, such as *Rhodococcus* and *Saccharimonadales*, which create a unique microbial environment that facilitates the microbiological degradation of organic compounds. In addition, the genus *Rhodococcus* and candidatus *Xiphinematobacter* would become potential biomarkers to monitor the shifting environmental condition of mangroves.

Keywords: 16S rRNA gene amplicon sequencing, biomarker, microbiome, polychaete, *Rhodococcus*, Setiu wetland

INTRODUCTION

Marine worms (polychaetas) have a vital role in ecosystems, including bioturbation (Tian et al. 2019; Gilbert et al. 2021). They also significantly influence the biogeochemical cycle (Sizmur et al. 2013; Kristensen et al. 2014). Furthermore, marine worms are a crucial resource for recreational fish bait (Cole et al. 2018) and aquaculture diet (Wibowo et al. 2020) due to their storage of high-quality polyunsaturated fatty acids (PUFAs) such as arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Wibowo et al. 2020; Pairohakul et al. 2021). In addition, Wang et al. (2019) suggested that polychaetes could potentially be a partial alternative to fishmeal and fish oil, and their cultivation using aquaculture waste could enhance sustainable fish aquaculture. Meanwhile, the Nypa worm is known as one of the major microbenthic components in the estuary and is used as fish bait (Junardi et al. 2014). Lim et al. (2021) stated that *Namalycastis* sp. was preferable prey to the mud crab and the possibility of reducing the impact of aquaculture waste on the environment using fish culture waste as *Namalycastis* sp. diet.

At present, several studies have suggested that polychaete-derived bacteria play a crucial role in various bioremediation processes, including biosurfactant production (Rizzo et al. 2013; Markande et al. 2014; Rizzo et al. 2014), and degradation of petroleum hydrocarbon (Sampaio et al. 2019), polystyrene (PS) (Zhao et al. 2024) and Polycyclic Aromatic Hydrocarbons (PAHs); (Wang et al. 2020a). Similarly, Shankar et al. (2015) indicated that the polychaete-derived bacteria possessed antibacterial and biofilm-inhibiting bioactive compounds. There are also some reports on microbial isolation, such as Actinomycetes and lactic acid bacteria as probiotic candidates from Nypa worm (Yanti et al. 2020a; Yanti et al. 2020b; Priscilla et al. 2022).

In this decade, the implementation of metagenomic analysis is widely used for microbial community investigation to provide microbial composition profiles and contributions to the biogeochemical cycle (Faust et al. 2015; Kerrigan et al. 2019; Grossart et al. 2020). So far, there are some reports on the impact of polychaete gut microbiome on the environment and vice versa. Marine polychaete *Capitella teleta* changed the microbial structure and function in sediment and suggested the possibility of the impact of gut microbial fermentation on the benthic ecosystem (Jang et al. 2021). In addition, Fuirst et al. (2021) revealed that a tube made by the decorator worm *Diopatra cuprea* creates a unique microbial community to enhance marine benthic

biogeochemical processes. On the other hand, Neave et al. (2012) mentioned that heavy metal-resistant bacterial abundance associated with marine polychaete *Ophlina* sp. is influenced by heavy metal concentration in sediment. Furthermore, Hochstein et al. (2019) reported that *C. teleta* grown in PAH-contaminated sediment increased aromatic compound degradation and detected a high abundance of PAH-degrading bacteria. Thus, polychaete constructs symbiotic relations with some microbes to regulate or facilitate biogeochemical cycles in marine benthic environments.

However, there is still a lack of study on the microbiome associated with Nypa worm and their functional role, unlike other worms such as *C. teleta*. Setiu Wetland, which constitutes the largest coastal wetland complex on the east coast of Peninsular Malaysia, has conducted several kinds of research on environmental pollution, such as heavy metal (Talukder et al. 2022; Sallan et al. 2023) and microplastic (Ibrahim et al. 2021). Thus, understanding the polychaete microbial community is essential to assessing marine benthic environmental conditions. This study conducted the 16S rRNA gene amplicon sequence analysis to assess the microbiome of the Nypa worm gut inhabiting rotten Nypa and its environmental sample (Nypa and water sample) from the Setiu Wetland at Terengganu, Malaysia. In addition, the predicted metabolic function of the microbiome related to the Nypa worm gut and its environmental sample was annotated using the functional annotation of prokaryotic taxa (FAPROTAX) database (Louca et al. 2016). Moreover, understanding the microbiome composition of *Namalycastis* sp. might provide information on beneficial bacteria, such as potential probiotics to improve the polychaete aquaculture production and prevent potentially pathogenic bacterial risk.

MATERIALS AND METHODS

Samples collection and genomic DNA extraction

Nypa worm (*Namalycastis* sp.) from the decaying fronds of *Nypa fruticans*, decaying *N. fruticans*, and water samples were collected from four different sites at Setiu Wetlands, Terengganu, Malaysia (Table 1, Figure 1). *Namalycastis* sp. and *N. fruticans* were kept in the sterile specimens' bag, and water samples were collected into sterilized blue cap bottles. Thereafter, these samples were transported to the laboratory on ice within 2 h of sample collection. All samples of *Namalycastis* sp. surface were sterilized by immersion in 75% ethanol, washed twice with sterile 0.01 M phosphate buffer saline (PBS) (Sigma-Aldrich Chemie GmbH, Munich, Germany) to remove loosely attached debris, and then the gut contents were aseptically squeezed into a sterile centrifuge tube. The 100 mL water sample was filtered onto 0.22 um filter paper and kept in a sterile centrifuge tube. Decaying *N. fruticans* were aseptically cut into small slips and transferred to a sterile centrifuge tube. Thereafter, bacterial genomic DNA from each sample was extracted using a Mobio Powersoil DNA isolation kit (Qiagen, USA) according to the manufacturer's instructions.

Table 1. Coordinate points of the sampling location for Nypa worms (*Namalycastis* sp.), Nypa, and water samples in the Setiu wetland, Terengganu, Malaysia

Station	Latitude	Longitude
1	5º43’21.08” N	102º40’13.87” E
2	5º41’30.77” N	102º41’54.98” E
3	5º39’49.27” N	102º43’57.69” E
4	5º37’10.34” N	102º47’31.51” E

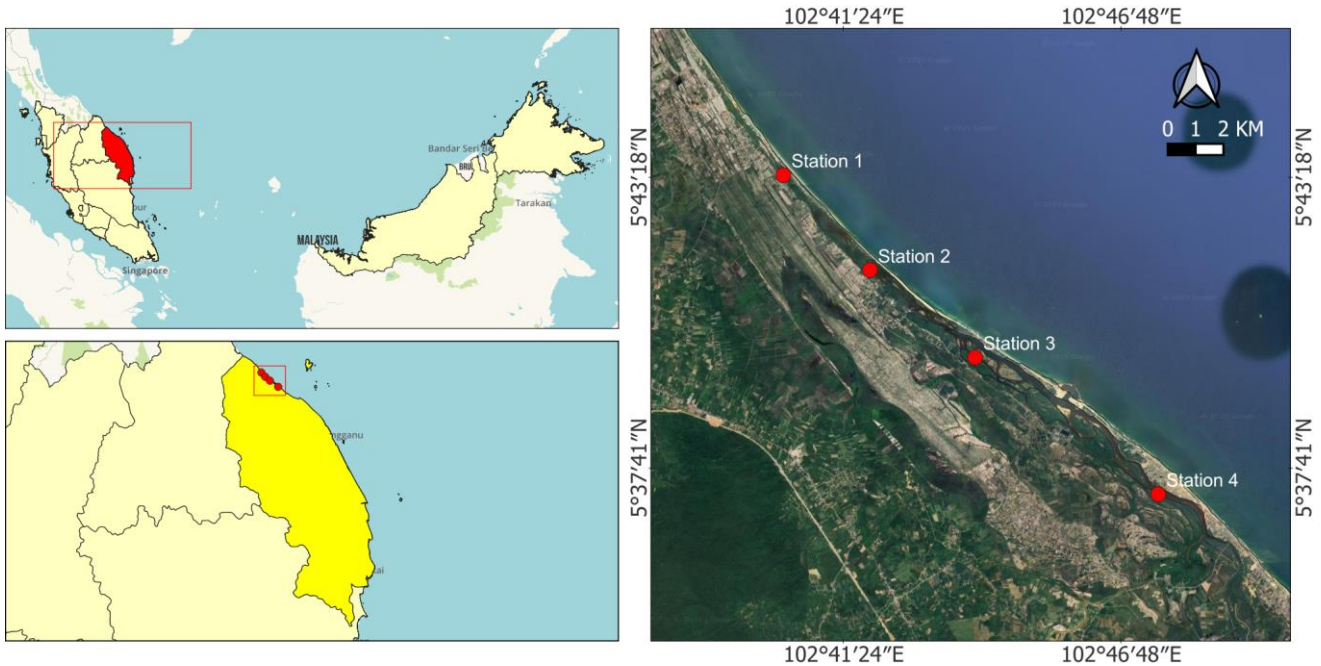


Figure 1. Sampling sites of Nypa worm (*Namalycastis* sp.), Nypa, and water samples in Setiu wetland, Terengganu, Malaysia

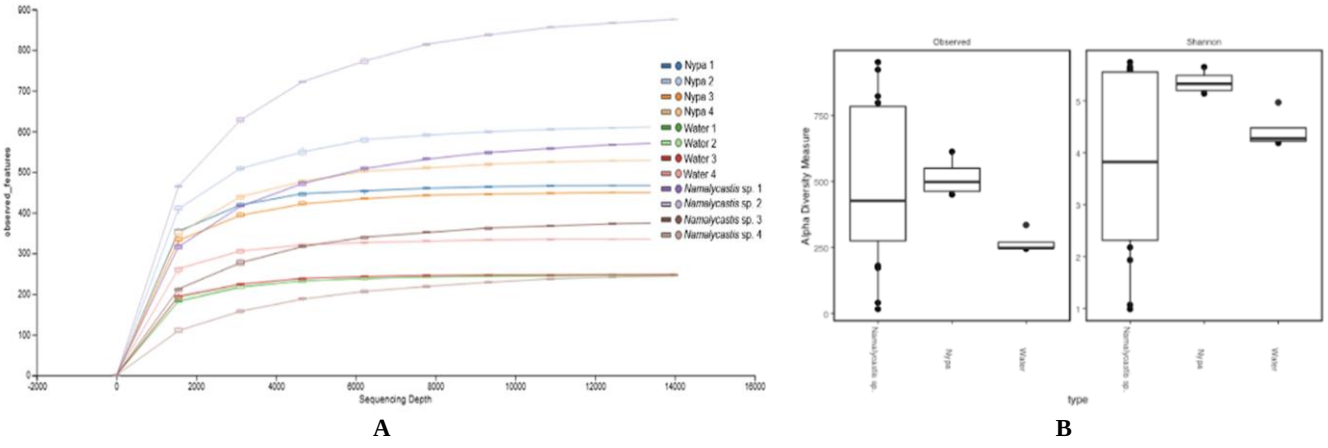
Microbial 16S rRNA gene amplicon sequencing and bioinformatics analysis

Extracted DNA samples were amplified in the V4 variable region of the 16S rRNA gene using a primer (515F: 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACNVGGGTWTCTAAT-3') (Walters et al. 2016) with 8bp barcode to each sample. All PCR reactions were conducted in 20 µL mixtures with 4 µL of 5×FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 5 µM of forward and reverse primers (0.8 µL), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. The PCR cycle condition was performed as follows: initial denaturation for 2 min at 95°C followed by 25 cycles of 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and a final extension at 72°C for 5 min. PCR amplicons were visualized with 2% agarose gel electrophoresis and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The purified PCR amplicons were then quantified using QuantiFluor™-ST (Promega, USA). The quantified amplicons were generated sample libraries, and then sample libraries were pooled with the equimolar amount and paired-end sequenced (2x250 bp) using an Illumina MiSeq (Illumina, USA). Pairs-end reads fastq files from the original DNA fragments were imported into QIIME2 2021.2 (Bolyen et al. 2019), trimmed with cutadapt by q2-cutadapt (Martin 2011), and then trimmed reads were denoising with DADA2 (Callahan et al. 2016). Thereafter, all generated amplicon sequence variants (ASVs) were aligned and taxonomically assigned to Eukaryote and not to bacterial or archaeal lineage, which were excluded for further analysis. After rarefied at 13986, principal coordinates analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) based on Bray Curtis dissimilarity were analyzed using Qiime2 core-metrics-phylogenetic command and then visualized using the R version 4.1.1. Upset plots to identify shared or unique microbes were calculated using R version 4.1.1. Taxonomic assignment of the ASVs was used as a q2-feature-classifier against SILVA 138 (Bokulich et al. 2018). The ASV table and taxonomic classification table were imported to the MicrobiomeAnalyst (Chong et al. 2020) to generate linear discriminant analysis (LDA) effect size (LEfSe) ($P<0.05$; LDA score>2.0) at genus level for bacterial taxa to identify the differential abundance of core microbiome and discover biomarker between Nypa worm and environmental sample (Nypa and Water sample). The annotation of potential function associated with the microbiome of each sample was analyzed using FAPROTAX (Louca et al. 2016). The 16S rRNA gene amplicon dataset of raw sequence data has been deposited in the Sequence Read Archive in the BioProject PRJNA826274.

RESULTS AND DISCUSSION

Microbial diversity differences in the Nypa worm (*Namalycastis* sp.) gut and environmental sample

The 16S rRNA gene amplicon sequencing using the V4 region of extracted DNA from Nypa worm (*Namalycastis* sp.) gut, Nypa, and water samples showed a total of 468,651 quality filtered sequences and 10,800 observed ASV across all samples. The rarefaction curve of each sample reaching the plateau indicated that the sequencing depth in this study was enough to represent the microbial diversity regardless of the sample (Figure 2A). Observed ASV and Shannon's index was ranging from 269±44 to 515±74 and 3.751±1.748 to 5.360±0.230, respectively (Figure 2B). PCoA based on Bray Curtis dissimilarity revealed the significant differences in the microbiome between *Namalycastis* sp. gut and environmental samples (Nypa and environmental sample) (Figure 3).



A **B**

Figure 2. The rarefaction curves (A) and α-diversity analysis (B) of nypa worm (*Namalycastis* sp.) gut, Nypa, and Water samples related microbial community from Setiu wetland, Terengganu, Malaysia

Pairwise PERMANOVA result revealed that microbial community between *Namalycastis* sp. and Nypa was closer than between *Namalycastis* sp. and water sample (*Namalycastis* sp. and Nypa: pseudo-F-value: 1.872078; p: 0.019; q: 0.024, *Namalycastis* sp. and water sample: pseudo-F-value: 4.101853; p: 0.001; q: 0.003), although there are significantly difference of each sample. Similarly, the upset plot result indicated that the number of shared ASVs between *Namalycastis* sp. and Nypa (823 ASVs) is higher than that between *Namalycastis* sp. and the water sample (37 ASVs). However, each sample has a lot of unique ASVs (Figure 4).

Microbial taxonomic composition related to the Nypa worm (*Namalycastis* sp.) gut and environmental sample

At the phylum level, Proteobacteria was identified as one of the predominant phylum in *Namaycastis* sp. gut, Nypa, and water samples, representing 28.60% to 61.42% (Figure 5A). The dominant group (more than 10% of relative abundance) in *Namaycastis* sp. gut was Actinobacteriota, Firmicutes, and Proteobacteria (11.65, 19.16, and 28.60%, respectively). On the other hand, Bacteroidota and Proteobacteria were observed as a predominant phylum in Nypa and water samples, accounting for 18.48 and 53.73% for Nypa and 11.55 and 61.42% for a water sample, respectively (Figure 5A). *Namalycastis* sp. gut is composed of 15 genera with more than 1% of relative abundance. On the other hand, Nypa and water samples were different in composition from *Namalycastis* sp. gut and composed of 16 and 21 genera with more than 1% relative abundance (Figure 5.B).

The dominant genus in the *Namalycastis* sp. gut was unclassified Bacilli (14.03%), *Sulfurovum* (8.76%), unclassified Bacteria (8.43%), unclassified Rhodobacteraceae (6.82%), *Vibrio* (4.23%), *Rhodococcus* (3.53%), *Photobacterium* (3.31%) (Figure 5B). Nypa sample was predominated by unclassified Rhodobacteraceae (13.79%) and Blrii41 (6.16%), unclassified Flavobacteraceae (4.49%), *Vibrio* (4.00%), and A4b (3.68%). On the other hand, unclassified Rhodobacteraceae (18.57%), unclassified Gammaproteobacteria (13.34%), unclassified Comamonadaceae (5.88%), Chloroplast (3.70%), Marine Group II (3.56%), unclassified Ectothiorhodospirales (3.20%), and Cryomorphaceae; g__uncultured (3.17%) were composed as dominant genus in water sample. Interestingly, some genera, such as *Romboutsia* and *Escherichia-Shigella*, were only detected from *Namalycastis* sp. gut microbiome, and a few genera, such as *Demequina* were detected from only *Namalycastis* sp. gut and Nypa microbiome sample (Table 2).

LEfSe analysis ($p<0.05$; LDA score>2.0) was conducted to identify genus-level core microbiome as a biomarker among *Namalycastis* sp. gut, Nypa, and water samples (Figure 6). *Namalycastis* sp. gut was found in two genera as biomarkers composed of the genus *Rhodococcus* and Candidatus *Xiphinematobacter* (Figure 6). Four different genera, such as *Woeseia* and *Demequina*, were biomarkers for the microbiome associated with the Nypa sample, whereas seven different genera, including Candidatus *Aquiliuna*, *Marinobacterium*, and *Synechococcus* CC9902, were biomarkers for water sample related microbiome.

Predicted functional profiles of microbiome associated with the Nypa worm (*Namalycastis* sp.) gut and environmental sample

The generated ASVs were annotated against the FAPROTAX database to understand predicted microbial functional groups. Four predicted functional genes (chemoheterotrophy, aerobic chemoheterotrophy, fermentation, and nitrate reduction) were composed of the most predicted functions of all samples (Figure 7A). Four functional predictions involving hydrocarbon degradation (aromatic hydrocarbon degradation, aromatic compound degradation, aliphatic non-methane hydrocarbon degradation, and hydrocarbon degradation) in *Namalycastis* sp. gut were remarkably higher (>3.5-fold abundance) than those in environmental samples, indicating the unique capabilities of this species (Figure 7.A).

In the minor functional group, xylanolytic, cellulolytic, and chitinolytic activities exhibited substantially higher relative abundance in the *Namalycastis* sp. gut and Nypa sample than in the water sample (0.608 to 0.614% in *Namalycastis* sp. gut and 0.463 to 0.588% in Nypa sample, whereas 0.014% in water sample). In addition, a sulfur cycle-related functional group such as sulfate and sulfite respiration, thiosulfate respirate, respiration of sulfur compounds, and dark oxidation of sulfur compounds were only detected from *Namalycastis* sp. gut and Nypa samples. In contrast, nitrate and nitrogen respiration were higher in *Namalycastis* sp. gut and water sample (Figure 7.B).

Table 2. High abundant ASVs in the *Namalycastis* sp. gut at Genus level with >0.5% relative abundance

	***Namalycastis* sp.**	**Nypa**	**Water**
unclassified Bacteria	8.43	0.47	0.00
Actinomarinales;f__uncultured; g__uncultured	1.99	0.80	0.01
Rhodococcus	3.53	0.00	0.00
Demequina	1.62	2.02	0.00
Sulfurovum	8.76	0.04	2.40
unclassified Bacilli	14.03	0.01	0.00
Pir4 lineage	1.07	0.59	0.00
unclassified Alphaproteobacteria	0.64	2.93	0.07
unclassified Rhizobiales	1.56	1.52	0.00
unclassified Rhizobiaceae	0.90	1.47	0.09
unclassified Rhodobacteraceae	6.82	13.79	18.57
unclassified Gammaproteobacteria	1.61	1.12	13.34
Photobacterium	3.31	0.58	0.04
Vibrio	4.23	4.00	1.13
unclassified Spirochaetaceae	2.70	0.04	0.00
Candidatus *Xiphinematobacter*	1.12	0.11	0.00
unclassified Opitutaceae	1.43	0.00	0.00
unclassified Ilumatobacteraceae	0.79	0.44	0.00
Lysinimicrobium	0.70	0.58	0.00
unclassified Peptostreptococcaceae	0.61	0.00	0.00
Romboutsia	0.96	0.00	0.00
unclassified Saccharimonadales	0.73	0.63	0.00
Saccharimonadales	0.63	0.08	0.00
unclassified Pirellulaceae	0.54	0.13	0.00
Escherichia-Shigella	0.55	0.00	0.00

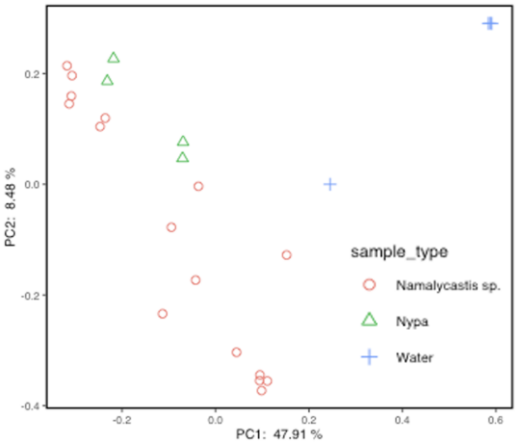


Figure 3. Principal coordinates analysis (PCoA) of the microbial community from different habitats based on Bray Curtis dissimilarity from Setiu wetland, Terengganu, Malaysia

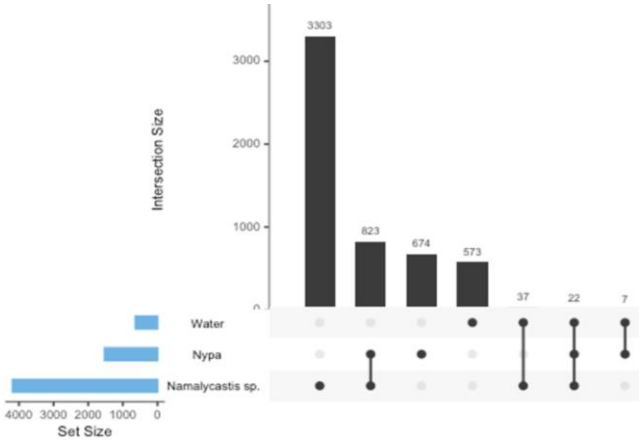


Figure 4. UpSet plot on the shared and unique ASVs in polychaete (*Namalycastis* sp.) gut and environmental (Nypa and Water) samples

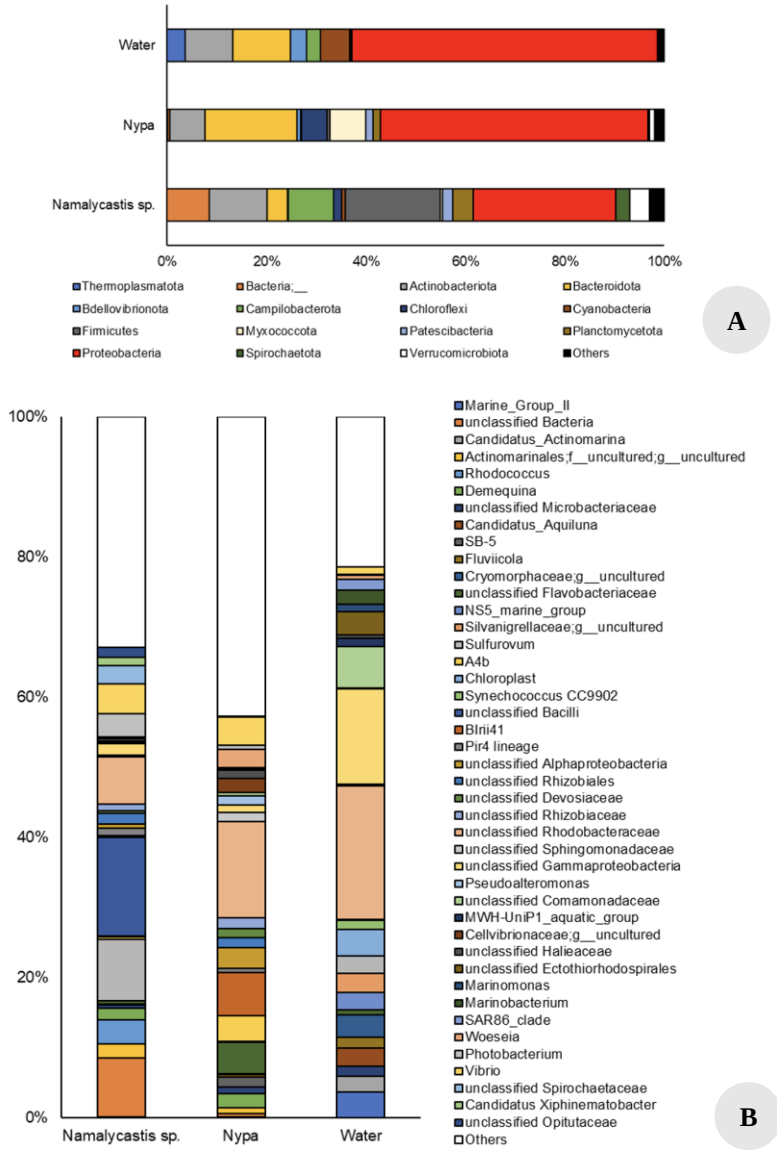


Figure 5. Taxonomic diversity and relative abundance of 16S rRNA gene amplicon sequences associated with microbial community composition of polychaete (*Namalycastis* sp.) gut, Nypa, and Water sample from Setiu wetland, Terengganu, Malaysia. The microbial community showed relative abundance at (A) Phylum level and (B) Genus level with rare taxa cut off at <1% relative abundance on each sample and rare taxon with less than 1% are classified as others

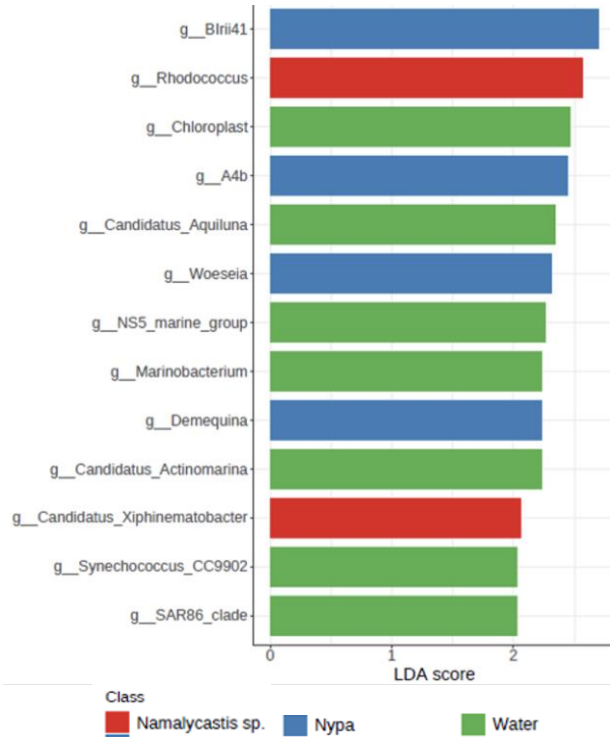


Figure 6. Core genera of the microbial community associated with Polychaete (*Namalycastis* sp.) gut and Environmental sample (Nypa and Water sample) from Setiu wetland, Terengganu, Malaysia discovered the LEfSe analysis (P<0.05, LDA score>2)

Discussion

Namalycastis sp. digestive tract enriches beneficial bacteria that can be applied as probiotics in aquaculture (Yanti et al. 2020b; Priscilla et al. 2022). This study provided the microbial biodiversity and community composition of the Nypa worm (*Namalycastis* sp.) gut and their surrounding environment (water and Nypa) using 16S rRNA gene amplicon sequencing in the Setiu Wetland, Terengganu. Our upset plot and PCoA results (Figures 3 and 4) revealed highly shared diversity between the Nypa worm gut and Nypa-associated microbiome compared to the Nypa worm gut and water-related microbiome.

Genus *Demequina* was detected only from *Namalycastis* sp. gut and Nypa microbiome sample (Table 2), which this genus is known as cellulolytic bacteria (Bienhold et al. 2013; Duan et al. 2019; Li et al. 2019). Moreover, several functional groups related to carbon degradation, such as cellulolytic, chitinolytic, and xylanolytic activity, were highly detected from *Namalycastis* sp. gut and Nypa samples. Meanwhile, sulfur cycle-related functional groups such as sulfate and respirate, thiosulfate respirates, and respiration of sulfur compounds were detected only from *Namalycastis* sp. gut and Nypa samples (Figure 7.B). These results might indicate the possibility that the microbiome composition of the Nypa worm gut was more closely related to the Nypa microbiome rather than water samples. This is supported by Vijayan et al. (2019), whose microbial diversity was closer to the tube than seawater and shared core operational taxonomic unit (OTU) with the tube compared to the seawater sample. In addition, the polychaete gut microbiome has some impacts on the sulfur cycle and degrades complex carbon (Jang et al. 2021).

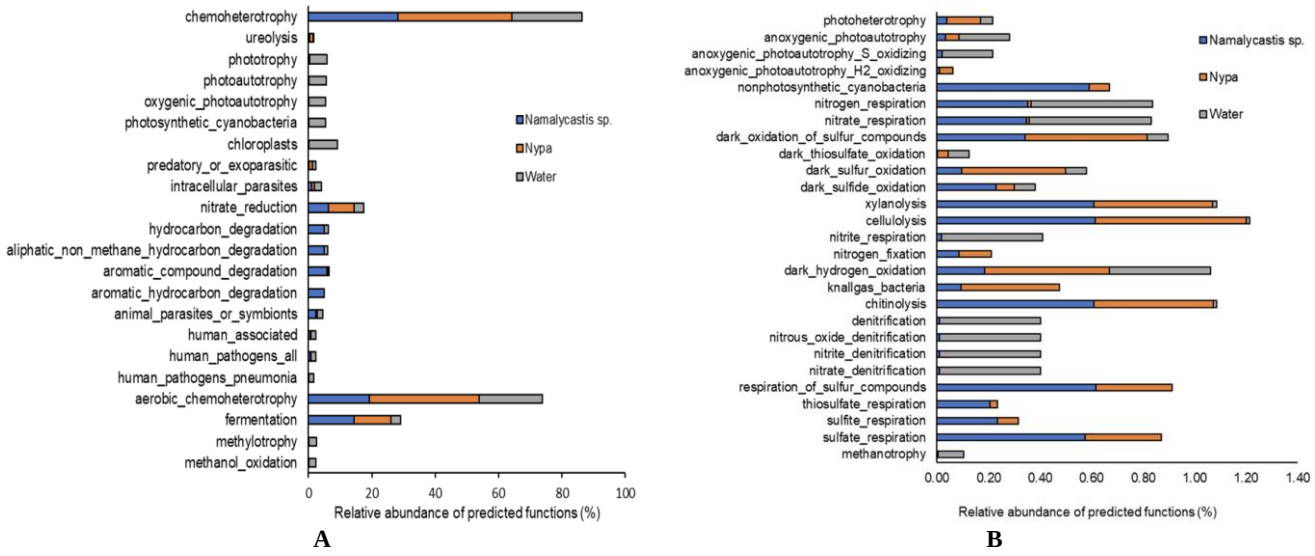


Figure 7. Mean relative abundances of major functional annotation groups in the microbial community of *Namalycastis* sp. gut, Nypa, and water sample (A) major functional groups (>1% relative abundance) and (B) minor functional groups (0.1-1% relative abundance)

In this study, three phyla composed of Actinobacteriota, Firmicutes, and Proteobacteria were the dominant group in *Namaycastis* sp. gut, which is consistent with previous cultured analysis on *Namaycastis* sp. gut (Priscilla et al. 2022). The genus *Rhodococcus*, unclassified Opitutaceae, unclassified Peptostreptococcaceae, *Escherichia-shigella* and *Romboutsia* were detected only *Namalycastis* sp. gut, and four genera (Candidatus *Xiphinematobacter*, *Sulfurovum*, *Photobacterium*, and Saccharimonadales) were higher abundance in *Namalycastis* sp. gut, where their abundance were >3.5 times compare to environmental sample. In addition, the abundance of the genus *Rhodococcus* and candidatus *Xiphinematobacter* were enriched in *Namalycastis* sp. gut as revealed by LEfSe analysis. It has been reported that the genera *Rhodococcus*, Saccharimonadales, and *Escherichia-Shigella* were members of the plastisphere (Rüthi et al. 2020; Xie et al. 2021; Du et al. 2022). Li et al. (2022) demonstrated that *Escherichia-Shigella* increased their abundance in the earthworm *Eisenia fetida* gut when exposed to polystyrene microplastics. Similarly, Zhang et al. (2022) reported that the genus *Rhodococcus* was uniquely found in biodegradable plastic. Chen et al. (2022) revealed that Saccharimonadales was one of the dominant bacteria of Low-Density Polyethylene (LDPE), Polyethylene Terephthalate (PET) and Polystyrene (PS), and demonstrated biodegradation of organic contamination. Auta et al. (2018) reported that the genus *Rhodococcus* possessed biodegradation activity of plastic, including LDPE and Polypropylene (PP). Moreover, the superworm (*Zophobas morio*) gut microbiome, including *Rhodococcus*, involves the degradation of polystyrene (Sun et al. 2022). At Setiu Wetland, there are few reports on the sustainable detection of microplastic from polychaete (*Namalycastis* sp.), which is ingested through their food web (Hamzah et al. 2021; Ibrahim et al. 2021; Anuar et al. 2022). These results might suggest that ingesting microplastic increases the plastic-attached bacterial abundance in *Namalycastis* sp. gut in this study. This is supported by previous reports that plastic leaches facilitate bacterial growth and affect bacterial diversity by providing dissolved organic matter (Romera-Castillo et al. 2018; Sheridan et al. 2022).

Genus *Rhodococcus* is known for the degradation activity of a variety of hydrocarbons such as PAHs (Song et al. 2011; Lang et al. 2016; Igun et al. 2019), lignin and their derivates (Jiang et al. 2022), phenol (Gu et al. 2018), and steroids substrates (Ye et al. 2019). Wang et al. (2020a) indicated the possibility of different types of organic pollutant degradation by marine sediment worm *Nereis succinea* gut microbiome. Similarly, Saccharimonadales was considered one of the hydrocarbon degradation bacteria (Chen et al. 2019; Chen et al. 2021). These results are congruent with our FAPROTAX result, which is a high abundance of hydrocarbon degradation such as aromatic hydrocarbon degradation, aromatic compound degradation, aliphatic non-methane hydrocarbon degradation, and hydrocarbon degradation. Thus, the Nypa worm might construct a host-microbial relationship with the hydrocarbon-degrading bacteria *Rhodococcus*. However, further investigation is needed to isolate these hydrocarbon degradation bacteria from the Nypa worm. Genus *Romboutsia* (Family Peptostreptococcaceae) is detected from Chinese mitten crab gut (Wang et al. 2020b), soft coral (Clever et al. 2022), and mangrove sediment (Fernández-Cadena et al. 2020). Chao et al. (2020) suggested the utilization of *Romboutsia* as one of the biomarkers against tetracycline (TC) toxicity to the earthworm due to a strong relationship with antioxidant enzymes: catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). Furthermore, the genus *Romboutsia* has the ability to produce a wide range of metabolic compounds, such as short-chain fatty acids, due to the fermentation of amino acids or carbohydrates (Zeibich et al. 2018; Gerritsen et al. 2019).

In this study, *Romboutsia* was detected only in the Nypa worm gut microbiome, and fermentation function by FAPROTAX analysis is higher than in environmental samples. These indicated that this bacterium might contribute to Nypa worm gut nutrition by fermentation. Candidatus *Xiphinematobacter* is known as the endosymbiont of nematode *Xiphinema* (Mobasseri et al. 2019) and is considered a bioindicator of *Alnus nepalensis* (alder) growth stage (Krishna et al. 2020). Sulfur oxidizing bacteria *Sulfurovum* is associated with marine invertebrate symbionts such as coastal marine worms (Ruehland and Dubilier 2010), marine nematodes (Bellec et al. 2020), and deep-sea water gastropods (Miyazaki et al. 2020). In addition, Huang et al. (2021) suggested that *Sulfurovum* positively correlates with chemocline parameters (DOC, nutrients, and sulfide). Similarly, Fernández-Cadena et al. (2020) reported that the abundance of *Sulfurovum* was enriched when heavy metal increased. In this study, the genus *Sulfurovum* was detected from the Nypa worm gut and water sample, indicating the possibility that this genus might be a bioindicator for monitoring environmental factors. Our result suggested a high abundance of possible pathogenic bacteria such as *Vibrio*, *Photobacterium*, and *Escherichia-Shigella* (4.23%, 3.31%, and 0.55%, correspondingly) detected from *Namalycastis* sp. gut. This is consistent with different polychaete results in which *Photobacterium* and *Vibrio* were higher in polychaete (*Sabella spallanzanii*) gut than in seawater (Licciano et al. 2007).

In conclusion, this study observed Nypa worm gut and their environment-associated microbiome using the 16S rRNA gene amplicon sequence approach and functional predictions analysis using FAPROTAX database. Nypa worm gut was a diverse microbiome composed of potentially beneficial and pathogenic bacteria. The results in this study suggested that several beneficial bacteria, such as *Rhodococcus* and *Romboutsia*, might have an essential role in the Nypa worm gut by degrading several carbon complexes and producing a variety of metabolic compounds to support host nutrition. In addition, some bacteria, such as *Rhodococcus* and Candidatus *Xiphinematobacter*, could be used as biomarkers to monitor environmental factors, potentially revolutionizing the field of environmental science and microbiology.

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