Marine Environmental Research 212 (2025) 107605

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Characterisation of the gut microbiome and surveillance of antibiotic resistance genes in green sea turtles (*Chelonia mydas*)

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ARTICLE INFO

Keywords:
Green sea turtles
Gut microbiome
Antibiotic resistance
Antibiotic resistance genes
Environmental pollution
Gulf of Thailand

ABSTRACT

Green sea turtles (*Chelonia mydas*) are globally endangered marine herbivores that maintain the health of seagrass and coastal ecosystems. Their populations are declining due to human activities, including environmental pollution, which can disrupt gut microbial communities and compromise nutrition, immunity, and overall health. In this study, cloacal swabs from 139 green sea turtles categorised as captive juveniles, captive adults and wild stranded animals in the Gulf of Thailand, were analysed via shotgun metagenomic sequencing to elucidate bacterial taxonomic diversity and ARG profiles. In captive juveniles, Pseudomonadota was the most abundant phylum, followed by Ascomycota and Basidiomycota. In captive adults, Pseudomonadota exhibited an even greater predominance, with only minor contributions from unclassified bacteria and other taxa. In wild stranded green sea turtles, Pseudomonadota was dominant in their gut microbiome, but this was accompanied by notable levels of Actinomycetota, Bacteroidota, and Bacillota. Stranded turtles exhibited highest microbial diversity and variability, while captive adult turtles showed the lowest. Resistome profiling also revealed significant differences in the relative abundance of antibiotic resistance genes across all three groups. MacB (macrolide resistance) was the most abundant gene overall, with the highest abundance observed in juveniles (4.8 %). Stranded turtles exhibited elevated levels of TetA(58) (tetracycline resistance, 2.6 %) and msbA (nitroimidazole resistance, 2.2 %), while adults showed the greatest enrichment of Ecol_fabG_TRC (triclosan resistance, 3.8 %) and TxR (tetracycline resistance, 3.6 %). These data demonstrate that marked variability existed in the gut microbiome and resistome of green sea turtles across different life stages in captive or wild environments. This offers critical insights for the development of targeted conservation strategies and health management practices for both wild and captive green sea turtles. Strategies to mitigate the spread of antibiotic resistance should be developed.

1. Introduction

Green sea turtles (*Chelonia mydas*) are herbivorous reptiles that primarily consume algae and seagrasses in marine environments (Velasquez Vacca, 2023). The gut microbes of green sea turtles play a crucial role in digestion of their feed, nutrient absorption, and overall health by aiding in the breakdown of complex plant polysaccharides, synthesising essential vitamins, and modulating immune responses (Howell and Shaver, 2021; Iurk et al., 2024). However, increasing anthropogenic disturbances, including habitat destruction, climate change, pollution, and exposure to environmental contaminants, may disrupt microbial homeostasis due to altered microbial diversity. This disruption can have profound effects on host physiology and disease susceptibility and may ultimately compromise conservation efforts (Arienzo, 2023). Among these stressors, antibiotic residues in marine ecosystems have raised significant concerns because of the potential to select and promote the proliferation of antibiotic-resistant bacteria and the spread of antibiotic resistance genes (ARGs) across marine species

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https://doi.org/10.1016/j.marenvres.2025.107605
Received 24 July 2025; Received in revised form 7 October 2025; Accepted 7 October 2025
Available online 8 October 2025

(Drane et al., 2021; Vezeau and Kahn, 2024). The presence of ARGs not only raises concerns for the health of animals, but it also underscores the potential for wild animals to amplify the environmental spread and ecological cycling of antibiotic resistance (Chen et al., 2022; Vezeau and Kahn, 2024).

Green sea turtles are occasionally recovered as stranded on the seashore or in surrounding shallow waters, showing signs of illness or abnormal behaviour (Hart et al., 2006). Stranded or captured sea turtles with health complications are kept in rehabilitation centres in which animals are frequently treated with broad-spectrum antibiotics both prophylactically and therapeutically against microbiological infections without prior antibiotic susceptibility testing (Escobedo-Bonilla et al., 2022). This is due to the acute nature of infections and significant debilitation upon entrance to rehabilitation facilities (Carini et al., 2017). The use of antibiotics increases bacterial selection pressure, promoting the development of antibiotic resistance and it may also compromise the health of treated animals by disrupting the intestinal microbiome. In green sea turtles, compromised intestinal microbiome can increase the risk of intestinal disease and malnutrition as the microbial community plays an opportunistic role in digestion and nutrient assimilation from the seagrass diet (Ahasan, 2017; Bloodgood et al., 2020). In turn, this increases the green sea turtle's susceptibility to bacterial infection, resulting in a treatment cascade (Baquero et al., 2008; Carini et al., 2017). In previous studies, the gut microbiome of green sea turtles has been characterised primarily using 16S rRNA gene sequencing across different geographical locations (Ahasan, 2017; Ahasan et al., 2018; Campos et al., 2018; Díaz-Abad et al., 2022; McDermid et al., 2020). However, the selection of primers that bind to the 16S rRNA gene during amplification is always a challenge and may significantly impact the characterisation of the microbial community (Rausch et al., 2019; Tremblay et al., 2015). Moreover, the accuracy of amplicon-based approaches are limited to taxonomic resolution at the genus level (Ranjan et al., 2016). In contrast, shotgun metagenomic sequencing randomly reads the whole metagenome to produce genomic assemblies and species-level designations, providing additional information about microbial diversity (Simon et al., 2019). To date, only two studies have used metagenomic techniques to investigate the gut microbiome and ARGs in green sea turtles. One assessed both the gut microbiome and resistance genes (Chen et al., 2022), while the other focused specifically on ARGs (Niu et al., 2024b). Both studies were limited by small sample sizes. The primary objective of the current study was to utilise shotgun metagenomic sequencing to comprehensively characterise the gut microbiome and resistome of both wild and captive green sea turtles from the Gulf of Thailand. By incorporating a considerably larger sample size, this study reveals how developmental stage, residence in captivity, and potential exposure to anthropogenic contaminants including antibiotics in the wild, may affect microbial diversity and antibiotic resistance dynamics. The broader aim is to provide insights into the microbial ecology, and the impact of human-induced environmental change for this endangered turtle species in the Gulf of Thailand.

2. Methodology

2.1. Ethics statement

The Sample collection from both wild and captive green sea turtles was conducted with approval from the James Cook University Animal Ethics Committee (Permit no. A2931), Mahidol University Animal Ethics Committee (Permit no. MUVS-2023-10-66), and the Department of Fisheries, Bangkok, Thailand (Permit no. KS0510.54). Turtles were restrained for sampling without the use of anaesthesia and handled carefully to minimise stress and ensure their well-being under the supervision of experienced Thai aquatic veterinarians.

2.2. Study site

The samples were collected from turtles at the Sea Turtle Sanctuary (Lat.:12°36′54.9″N; Long.:101°41′23.3″E). The sanctuary is part of the Royal Sea Turtle Conservation Project under the initiative of Her Majesty Queen Sirikit. It is located on Ko Man Nai Island in the Rayong Gulf of Thailand. This facility plays a critical role in conserving endangered sea turtles by providing a safe environment for nesting, hatching, and rehabilitation.

2.3. Animal categorisation

A total of 139 live green sea turtles were included in this study. Based on life stage and the wild or captive status, turtles were classified into three groups: captive juveniles (n = 119), captive adults (n = 7), and wild-stranded individuals (n = 13). Each group was further subdivided into three subgroups (juvenile: J1, J2 and J3; adult: A1, A2 and A3; stranded: S1, S2 and S3) for sample pooling purposes. The captive juveniles were reared from hatchlings obtained from Kram Island, and adults rescued from entanglement in fishing gear in the coastal regions of Chonburi (13.1701° N, 100.5611° E), Rayong (12.6926° N, 101.1777° E), Chanthaburi (12.6039° N, 102.0915° E), and Trat (12.4102° N, 102.4630° E). The wild-stranded turtles were often found floating or entangled in fishing gear and brought to the sanctuary from the same coastal areas. For captive turtles, morphometric measurements, including body weight and curved carapace length (CCL), were recorded following protocols established by the Queensland Department of Environment and Heritage Protection (DEHP, 2013). Due to logistical constraints under field conditions, body weight and demographic data were not recorded for some wild green sea turtles prior to sampling. Detailed grouping and morphometric data that were obtainable are provided in Supplementary Table S1 and as previously reported (Ghafoor et al., 2025)

2.4. Sample collection

The samples from green sea turtles were collected in February 2024 and May 2024 (Supplementary Table S1). Deep cloacal swabs from individual green sea turtles in each categorised group were collected and then pooled into 50 mL phosphate-buffered saline (PBS) in falcon tubes by a slight agitation. To minimise the risk of exterior contamination, the cloacal area was flushed with 70 % ethanol before sample collection. A sterile polyester swab was then carefully inserted into the cloaca (approximately 5–10 cm depending on size of animals) and gently rolled to ensure adequate sampling of the cloacal contents (Ahasan et al., 2017a,b). The samples were kept in an icebox to maintain a temperature of approximately 4 °C and then transported to the laboratory at Kasetsart University (Bangkok, Thailand) within 13 h.

2.5. DNA extraction, library preparation and sequencing

DNA was extracted from 9 pooled samples using the QIAamp PowerFecal Pro DNA Kit (Cat. No. 51804, QIAGEN, Germany) following the manufacturer's instructions. The extracted DNA samples were stored at −80 °C until further analysis by Azenta Life Sciences, China. Genomic DNA (200 μg) from each sample was subjected to fragmentation using a Covaris system, generating fragments with an average size of 300bp. The fragmented DNA was subjected to end repair, 5′ phosphorylation, and 3′ adenylation using the end prep enzyme mix. Subsequently, adapters were ligated to the DNA fragments: the P7 adapter (read1: AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC) and the P5 adapter (read2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT). Size selection of the adapter-ligated fragments was performed using DNA Cleanup beads. The resulting libraries were amplified using P5 and P7 primers for 8 cycles, after which the amplified products were purified and validated on an Agilent 2100 Bioanalyzer. Shotgun sequencing was conducted on

the Illumina NovaSeq S4 platform using a paired-end 150 bp (PE150) configuration, which generated 10 gigabases (GB) of raw data per sample (Huang et al., 2024).

2.6. Data pre-processing and quality control

The raw sequence data were generated in the form of image files and base calls, which were processed into FASTQ format using bcl2fastq (version 2.17.1.14) (Huang et al., 2024). The quality of sequencing reads was assessed using FastQC (version 0.11.8), and low-quality reads ($Q < 20$) or reads with adapter contamination were trimmed using cutadapt (version 1.9.1) (Martin, 2011). Additionally, host-derived sequences were removed by mapping the clean reads to the host genome with BWA (version 0.7.12) and discarding any aligned reads(Li and Durbin, 2009, 2010).

2.6.1. De novo assembly and gene prediction

The filtered data underwent de novo assembly using MEGAHIT (version 1.1.3) (Li et al., 2016), which employs memory-efficient algorithms based on de Bruijn graph construction for complex metagenomic data. Multiple K-mer sizes (59, 79, 99, 119, 141) were tested to optimise the assembly. Pre-assemblies generated with varying K-mer values were assessed, and the optimal assembly for each sample was selected and merged to generate a complete final assembly. Protein-coding genes were predicted using Prodigal (v3.02) (Hyatt et al., 2010). Predicted sequences from all samples were pooled and clustered with MMseqs2 at 95 % nucleotide identity and 95 % alignment coverage to construct a non-redundant unigene catalog. Clean reads were aligned to this reference using SOAPaligner (v2.21) (Li et al., 2008), and unigene abundance was quantified based on mapped read counts normalised by gene length.

2.6.2. Taxonomic annotation of microbial communities

To determine the taxonomic composition of the microbial communities, unigene sequences were aligned against the NCBI non-redundant (NR) protein database using DIAMOND (v0.8.15.77) (Buchfink et al., 2015). An E-value threshold of $\leq$1e−5 was applied to ensure that only significant matches were considered (Li et al., 2019; Liu et al., 2024; Yang et al., 2022). Taxonomic assignments were made based on the best-scoring protein hits, with lineage information obtained from the corresponding NR annotations. The species-level classification of each unigene was combined with gene abundance data to calculate the relative abundance of microbial taxa across hierarchical taxonomic ranks, including phylum, order, family, genus, and species. For each sample, the abundance of each taxon was determined by summing the abundances of all unigenes assigned to that taxon for obtaining a detailed and quantitative profile of the microbial community.

2.6.3. Identification and quantification of antibiotic resistance genes (ARGs)

Antibiotic Resistance genes were identified by aligning predicted protein sequences against the Comprehensive Antibiotic Resistance Database (CARD) using the DIAMOND BLASTP algorithm (v0.8.15.77) (Buchfink et al., 2015), with a stringent e-value cutoff of $\leq$1e-5 to ensure high-confidence matches. CARD provides a curated and structured ontology of resistance determinants, which enables functional annotation of ARGs based on resistance mechanism and antibiotic class. Only the top-scoring alignment for each unigene was retained to minimise false-positive assignments. The relative abundance of each ARG was calculated by integrating gene-level read counts with CARD-based annotations for the comprehensive profiling of the resistome across three groups.

2.6.4. Statistical analysis for differences in microbial and ARG diversity

Alpha diversity was assessed using Shannon, Simpson, and Chao1 indices to evaluate microbial richness and evenness, or ARG profiles. Rarefaction curves and Rank-Abundance plots were generated to ensure sufficient sequencing depth and representation of the microbial diversity. Beta diversity was assessed using Principal Coordinates Analysis (PCoA) based on genus-level abundance for the microbiome, and the Bray-Curtis dissimilarity metrics for ARGs. Differences were statistically evaluated using Permutational Multivariate Analysis of Variance (PERMANOVA) and Analysis of Similarities (ANOSIM). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Sequencing and metagenome variations in green sea turtles

Sequencing metrics varied across life stages and conditions, with differences in read length, GC content, and N50 value, a metric for the quality and contiguity of assemblies. Wild stranded turtles exhibited the highest variability, while captive adults showed relatively longer reads and higher GC content (Supplementary Table S2). Metagenomic analysis revealed the highest unigene diversity in wild stranded turtles, followed by captive juveniles, while captive adults exhibited the lowest complexity.

The shared unigene analysis identified 6931 unigenes between captive juveniles and wild stranded turtles, 5664 between captive juveniles and captive adults, and only 172 between captive adults and wild stranded turtles. A core set of 549 unigenes was conserved across all three groups (Fig. 1).

3.2. Gut bacterial community composition

Taxonomic profiling of the gut microbiome across captive juvenile, captive adult, and wild stranded green sea turtles revealed distinct composition differences at multiple taxonomic levels. Bacterial dominance was observed across all groups, comprising 90.9 %, 99.8 %, and 99.5 % of total taxa in captive juveniles, captive adults, and wild stranded turtles respectively. Captive Juveniles uniquely exhibited 8.8 % eukaryotic sequences, while archaea (0.09 %) and viruses (0.3 %) were only detected in stranded individuals.

At the phylum level (Fig. 2a), *Pseudomonadota* dominated all groups, with the highest relative abundance observed in captive adults (98.4 %), followed by captive juveniles (86.8 %) and wild stranded animals (55.0 %). Captive juveniles exhibited lower proportions of *Ascomycota* (4.3 %), *Basidiomycota* (4.2 %), and *Bacillota* (3.0 %), whereas stranded turtles showed greater microbial diversity, with increased relative abundances of *Actinomycetota* (13.8 %), *Bacteroidota* (13.7 %), *Bacillota* (6.7 %), and *Campylobacterota* (4.7 %). At the family level (Fig. 2b), *Enterobacteriaceae* (54.1 %) and *Moraxellaceae* (8.6 %) were predominant in captive juveniles, while *Pseudomonadaceae* (67.7 %) were predominant in captive adults. Wild stranded green sea turtles exhibited a distinct family composition, with notable enrichment of *Shewanellaceae* (19.9 %) and *Neisseriaceae* (10.4 %). At the genus level (Fig. 2c), *Citrobacter* was the most abundant in captive juveniles (47.3 %), *Pseudomonas* was dominant in captive adults (67.5 %), and wild stranded green sea turtles had higher proportions of *Shewanella* (19.9 %) and *Corynebacterium* (11.5 %). Species-level profiling (Fig. 2d) identified *unclassified Citrobacter* (40.7 %) as the most abundant species in captive juveniles, *unclassified Pseudomonas* (51.2 %) in captive adults, and *unclassified Shewanella* (7.6 %) and *Shewanella baltica* (6.8 %) in wild stranded green sea turtles.

3.3. Alpha diversity in bacterial communities

Wild stranded green sea turtles tended to exhibit higher species count than captive juvenile and captive adult groups (Fig. 3a), although no significant differences in alpha diversity were observed among the groups (Chao1 test, $p = 0.11$) (Fig. 3b). Wild stranded animals represented by samples S1, S2, S3 and particularly S2, showed the highest richness with an Abundance-based Coverage Estimator (ACE) and

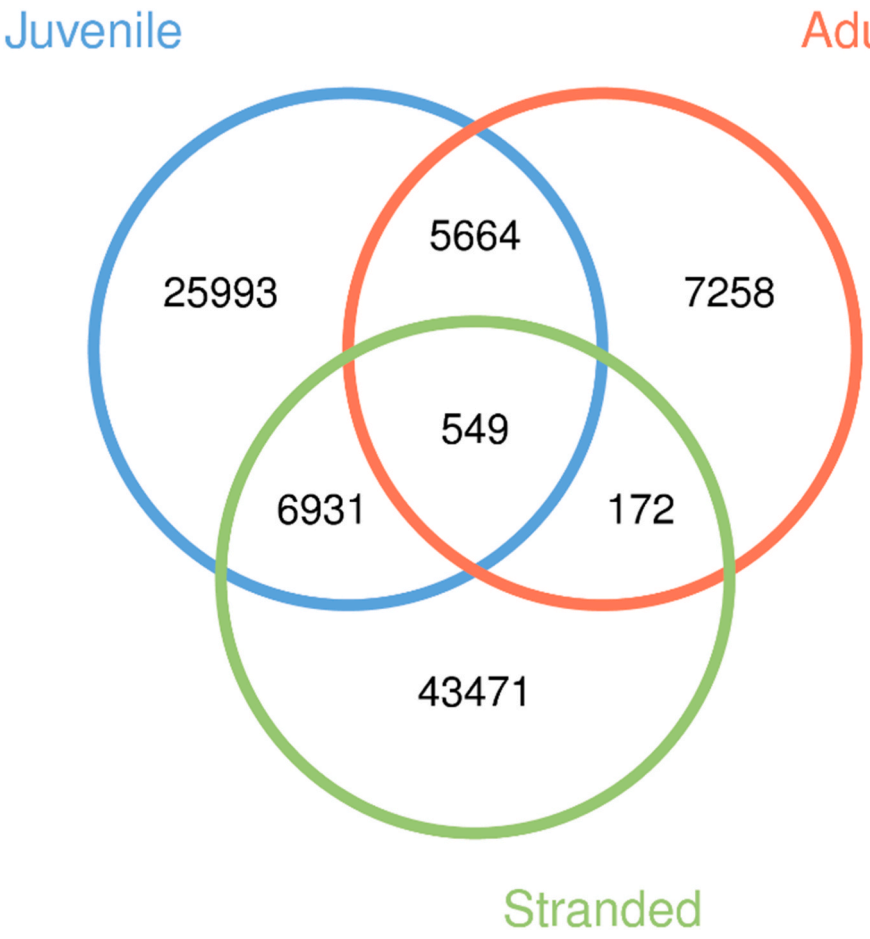

Fig. 1. Venn diagram illustrating the distribution of unique and shared microbial taxa among captive juvenile, captive adult, and wild stranded sea turtles. Each circle represents a different group, with numbers indicating the count of taxa unique to each group or shared between two or all three groups.

Chao1 values of 1307 and 1302, respectively. Samples from captive juvenile and adult green sea turtles and especially A1 (captive adult), had much lower richness, with ACE and Chao1 values of 64 and 61, respectively.

The Shannon and Simpson indices reflected similar patterns, with S2 in the wild stranded group exhibiting the highest diversity (Shannon = 7.487, Simpson = 0.976), while A1 in the captive adult group had the lowest (Shannon = 0.739, Simpson = 0.195). Good's coverage remained near 1 across all samples (Table 1). The rank abundance curve demonstrated that sample S2 and S3 in the wild stranded group had the highest microbial diversity, with a smoother and longer curve, while samples from captive juvenile and adult groups showed a steeper decline, indicative of lower diversity (Fig. 3c)

3.4. Beta diversity in bacterial communities

PCoA revealed distinct microbial community structures between the different groups of green sea turtles. Wild stranded turtles exhibited greater dispersion along the PCoA axes, which indicates higher microbial diversity and greater dissimilarity within the group (Fig. 4a). In contrast, captive juvenile and adult turtles demonstrated tighter clustering with more homogeneous microbial compositions. ANOSIM showed a moderate to strong dissimilarity between groups, with an R-value of 0.597 and a statistically significant p-value of 0.011 (Fig. 4b). In addition, PERMANOVA indicated significant differences in community composition across the three groups ($R^2 = 0.553$, $p = 0.01$).

3.5. Antibiotic resistance profiles

In captive juvenile turtles, the most prevalent resistance genes were those associated with macrolides (MacB, 4.8 %), followed by triclosan (Ecol_fabG_TRC, 2.6 %) and tetracycline (TxR, 2.5 %). Similarly, in captive adults, the ARG associated with macrolides resistance (MacB, 4.7 %), remained dominant with triclosan (Ecol_fabG_TRC, 3.8 %) and tetracycline (TxR, 3.6 %) as the next most abundant. In stranded animals, macrolide resistance (MacB, 3.1 %) was the most prevalent, followed by tetracycline resistance (TetA(58), 2.5 %) and nitroimidazole resistance (msbA, 2.2 %) (Fig. 5).

In addition, in all three experimental groups considered in this study, there was a high proportion of many different ARGs with very low relative abundance ranging from 0 to 0.6 % in captive juveniles, 0–0.7 % in captive adults, and 0–0.6 % in wild stranded green sea turtles (Fig. 5). Cumulatively, these very low-abundance ARGs accounted for majority of the resistance profiles comprising 60.4 %, 54.9 %, and 60.0 % of ARGs in captive juveniles, captive adults, and wild stranded green sea turtles respectively (Fig. 5). The top three antimicrobial classes represented by these very low abundance ARGs varied across the three groups. In captive juvenile turtles, genes encoding for aminocoumarin resistance (novA) and epoxide antibiotics such as fosfomycin (Abau_AbaF) were the most abundant followed by polyketides such as erythromycin and rapamycin (Ecol_EFTu_ENC), and fluoroquinolone (patB), resistance genes. In captive adults, β-lactam (Ngon_pilQ_BLA) resistance was dominant, followed by polymyxin (basS), mupirocin (Saur_ileS_MUP), and bacitracin (bcrA) resistance genes. In wild stranded turtles, tetracycline (tetB(P)) resistance was predominant, followed by fusidic acid (Saur_fusE_FA) and polyketide (Ecol_EFTu_ENC) resistance genes. A

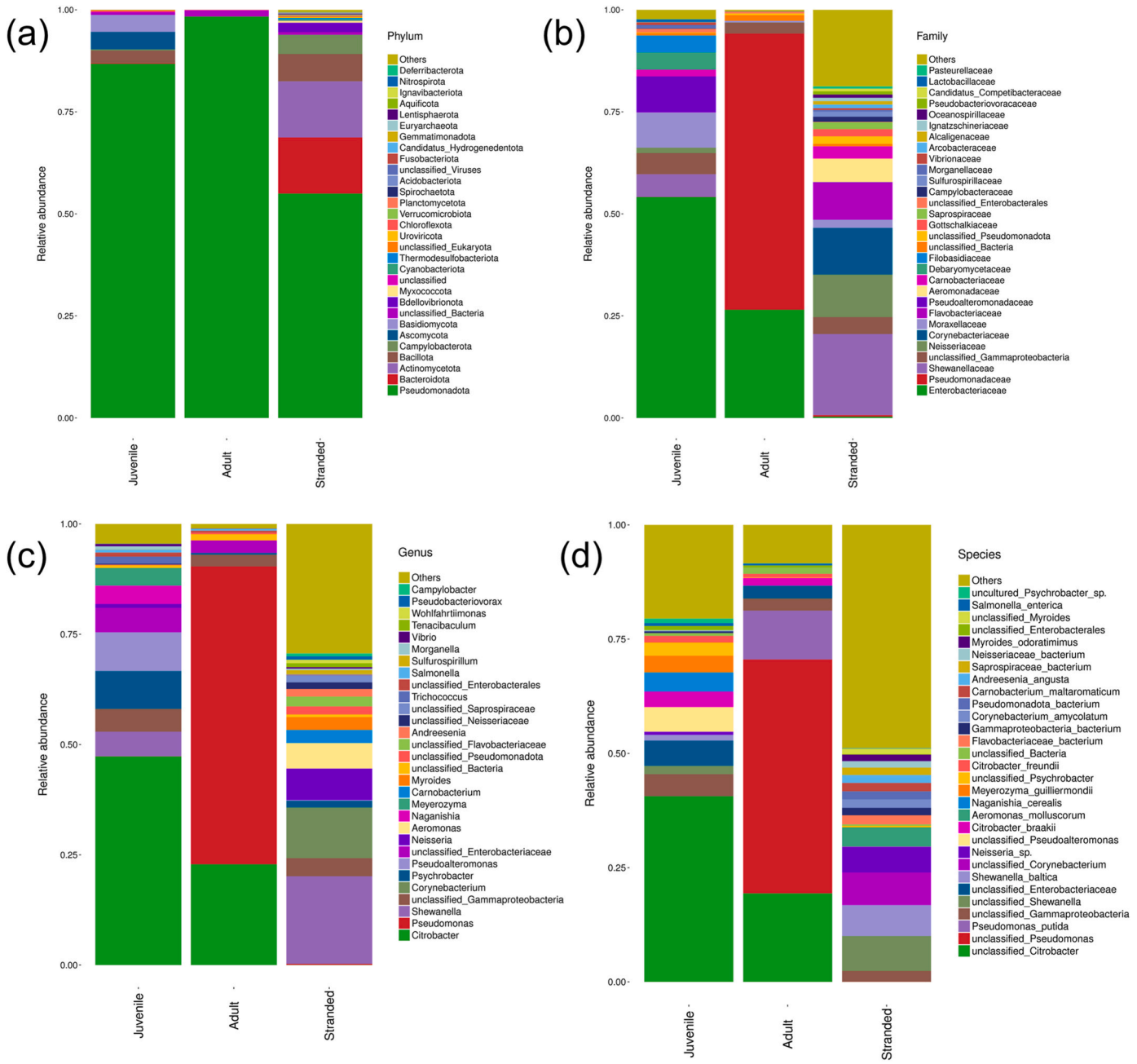

Fig. 2. Taxonomic profiling of the gut microbiome (a) at phylum level, (b) at family level, (c) at genus level, (d) at species level.

comprehensive resistome profile, which includes all annotated ARGs with relative abundances, is provided in Supplementary Table S3.

3.6. Alpha and beta diversity of ARGs in gut microbiome in green sea turtles

Alpha diversity analysis of CARD-annotated genes (Chao1 and Shannon indices) showed no significant differences in ARGs richness or evenness across the three groups ($p > 0.05$) (Supplementary Fig. S1). Beta diversity analysis demonstrated significant differences in ARGs composition across groups. PCoA revealed distinct clustering, with juveniles forming a compact group, while adults displayed greater dispersion. Stranded green sea turtles occupied a non-overlapping ordination space, which indicates substantial divergence in resistome composition. ANOSIM further confirmed significant compositional dissimilarities among groups with ($R = 0.49$, $p = 0.006$) (Fig. 6).

4. Discussion

Green sea turtles are listed as Endangered on the International Union for Conservation of Nature's Red List of Threatened Species (IUCN). Therefore, it is imperative to identify the main factors affecting the health of these animals. The microorganisms inhabiting the gastrointestinal tract in these animals are strongly associated with health (Campos et al., 2018). Studies have profiled the bacterial communities in the gut of sea turtles using 16S rRNA gene sequencing (Ahasan, 2017; Ahasan et al., 2018; Biagi et al., 2019; Campos et al., 2018; McDermid et al., 2020), while metagenomic sequencing has been applied in only one, with limited sample sizes (Chen et al., 2022). The present study provides a more comprehensive analysis of the bacteriome and ARG profile based on nine pools of cloacal swabs from a total of 139 green sea turtles in the Gulf of Thailand. Studied animals were stratified as captive juveniles, captive adults and wild stranding that were mostly juveniles.

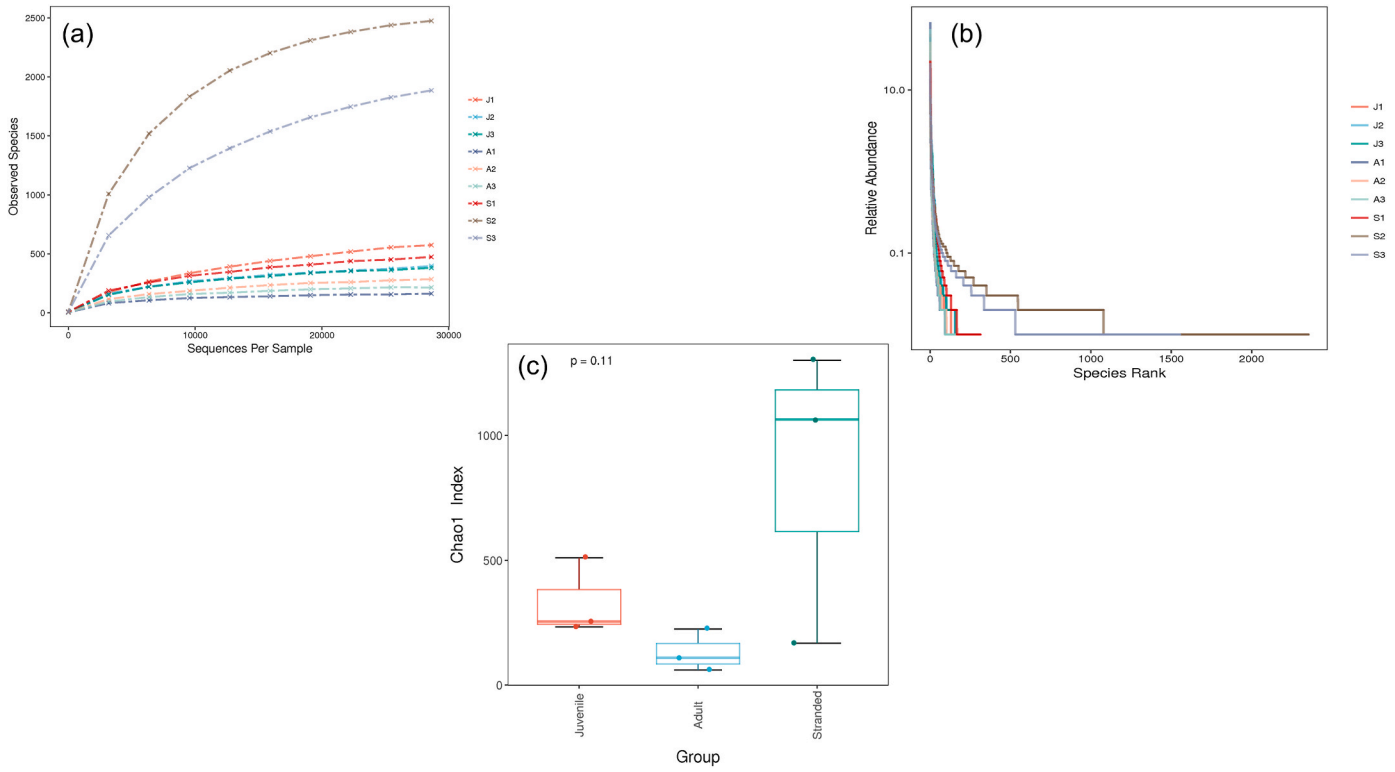

Fig. 3. Alpha diversity of the gut microbiome in wild stranded, captive juvenile, and captive adult green sea turtles. Rarefaction curves (a) show higher species richness in stranded animals. Chao1 estimates (b) indicate no significant differences in alpha diversity among groups (Chao1 test, P = 0.11), although stranded sample S2 exhibited the highest richness. Rank abundance curves (c) reveal greater microbial evenness and richness in wild stranded turtles compared to captive juvenile and captive adult groups.

Table 1
Statistical analysis of Alpha diversity.

Sample	Ace	Chao1	Shannon	Simpson	Goods coverage
J1	505.282	510.146	2.641	0.666	1
J2	279.428	254.204	2.633	0.775	1
J3	231.495	233.182	2.273	0.65	1
A1	64.282	60.60	0.739	0.195	1
A2	243.04	225.00	1.899	0.628	1
A3	111.382	108.833	1.34	0.429	1
S1	168.018	167.353	2.011	0.604	1
S2	1307.43	1301.497	7.487	0.976	0.997
S3	1056.109	1063.674	4.995	0.857	0.999

In all three groups, Pseudomonadota was the most predominant phyla, but sub-phyla bacterial diversity and community composition was significantly different across the groups. This pattern is consistent with the observation by Chen et al. (2022), who also reported dominance by Pseudomonadota. Members of this phyla are commonly associated with environmental stress and dietary fluctuations, as documented in previous studies (Bloodgood et al., 2020; McMaken et al., 2023). The elevated presence of this phyla in the gastrointestinal tract is often associated with dysbiosis, and may reflect underlying pathological conditions in both humans and animals (Campos et al., 2018; Samuelson et al., 2020; Shin et al., 2015). More specifically, the dysregulated proliferation of some specific species in Pseudomonadota such as *Klebsiella pneumoniae*, *Helicobacter pylori*, and *Escherichia coli* are context-dependent and may be linked to immune, metabolic, and gastrointestinal disorders shaped by host and environmental conditions.

In captive juvenile turtles examined here, other notable phyla present were Ascomycota, Basidiomycota, and Bacillota albeit at relatively low abundances. Ascomycota and Basidiomycota are fungal taxa but research on fungi in gut microbiome of juvenile green turtles remains limited; existing studies suggest some members of these taxa may have opportunistic ecological or pathological roles (Cafarchia et al., 2020; Chai et al., 2023). The detection of these fungal phyla in the gut of captive green sea turtles suggests potential environmental acquisition with mechanistically unknown roles within the microbiome (Niu et al., 2024). Additionally, the relatively low abundance of *Bacillota* in green sea turtles in captivity may challenge previous hypotheses regarding their major role in cellulose digestion. While *Bacillota* are known for their capacity to degrade plant polysaccharides (Ahasan et al., 2017a,b), our findings align with those of a previous study demonstrating that both green and loggerhead turtles exhibit the lowest relative abundance of *Bacillota* and, that *Bacillota* may not be as critical to cellulose digestion in herbivorous reptiles as previously assumed (Scheelings et al., 2020). Moreover, other studies have shown that dominance by *Bacillota* is not a universal feature of herbivorous vertebrates, including other reptiles (Dill-McFarland et al., 2016; Fogel, 2015; Raulo, 2015; Scheelings et al., 2020). Furthermore, it has also been reported that juvenile turtles often harbor a simpler microbial community due to their developing gut environment and dietary transitions (Arthur et al., 2008; Niu et al., 2024a; Price et al., 2017; Scheelings, 2019). Our findings emphasise the need for further research to elucidate the metabolic contributions of alternative microbial taxa in juvenile green turtle digestion and their role in shaping gut microbiome maturation during ontogenesis.

The gut microbiome of captive adult turtles exhibited a secondary dominant proportion of unclassified bacterial taxa, alongside a notably low abundance of Urovirocota and Actinomycetota. Urovirocota, is a recently identified viral phylum consisting of bacteriophages with double-stranded DNA genomes that infect bacterial hosts and have been detected in the microbiomes of various vertebrates (Benler et al., 2021; Lu et al., 2024). These bacteriophages can influence bacterial community dynamics by selectively lysing specific bacteria, thereby shaping microbial composition. Actinomycetota, a diverse bacterial phylum

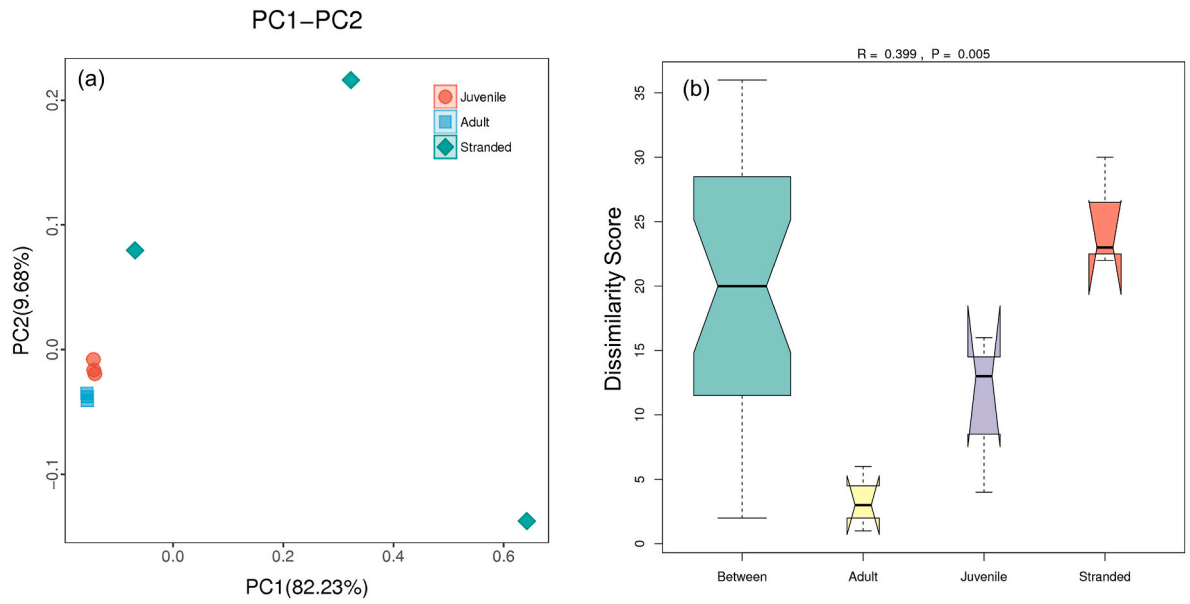

Fig. 4. Beta diversity of gut microbiome in green sea turtles. (a) PCoA reveals distinct clustering among groups, with wild stranded turtles showing greater dispersion. (b) ANOSIM and PERMANOVA indicate significant differences in community composition.

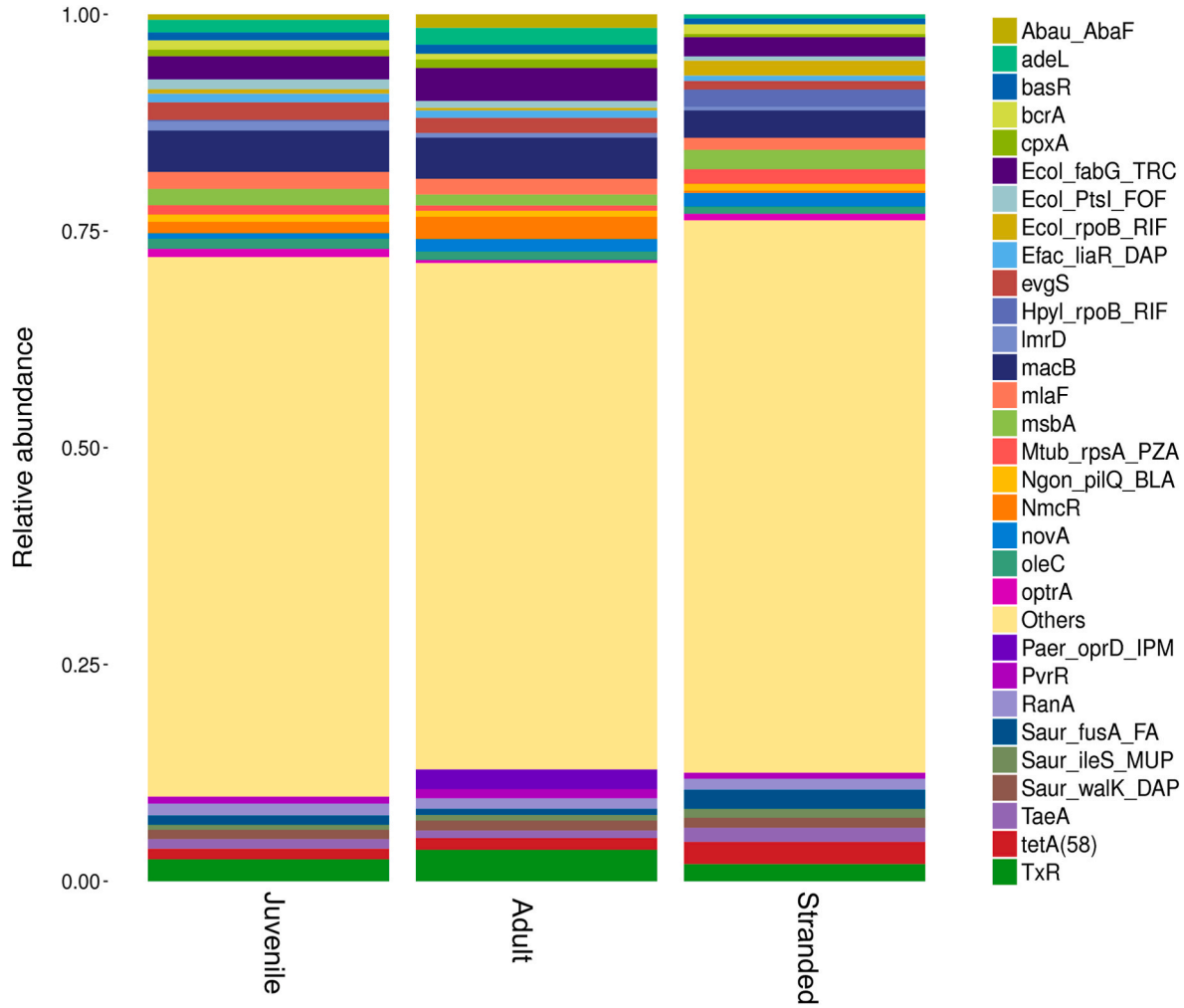

Fig. 5. Relative abundance of antibiotic resistance genes identified in the gut microbiome of captive juveniles, captive adults, and wild stranded green sea turtles.

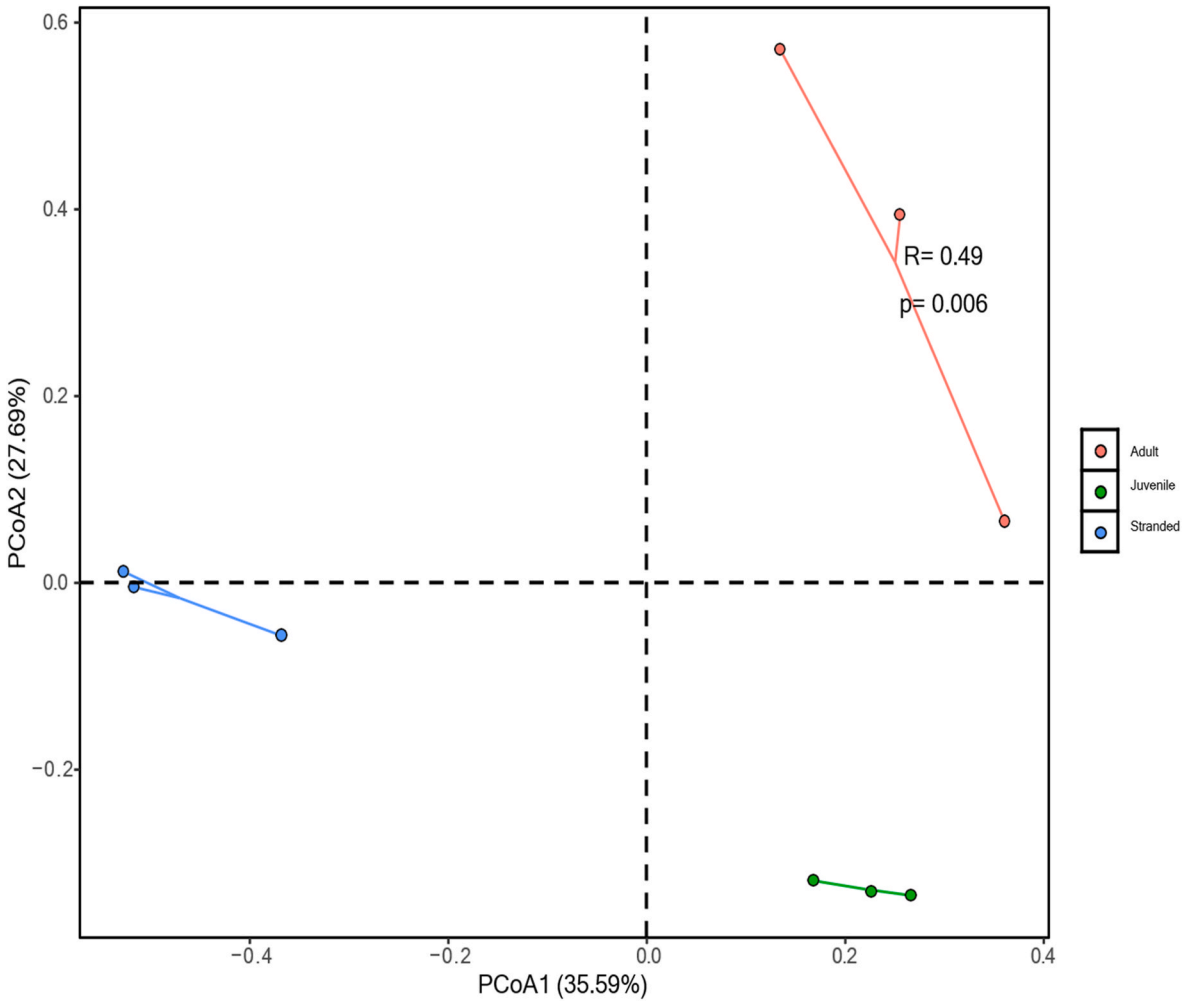

Fig. 6. Illustrating the principal coordinates analysis of antibiotics resistant genes-based beta diversity in the gut microbiome of green sea turtles. Distinct clustering is observed among captive adults (red), captive juveniles (green), and wild stranded (blue) animals. R and p-values denote ANOSIM results.

known for its role in secondary metabolite production, was also present at low relative abundance. Members of this phyla are known for producing antibacterial and antifungal compounds which help protect turtle eggs from fungal pathogens such as *Fusarium falciforme* (Sarmiento-Ramírez et al., 2014). Stranded green sea turtles exhibited a distinct gut microbial profile, characterised by a high relative abundance of Actinomycetota as a second dominant phylum, followed by Bacteroidota, Bacillota, and Campylobacterota. This composition aligns with prior 16S rRNA gene sequencing studies, which reported Bacteroidota and Bacillota as prominent constituents of the gut microbiome in stranded sea turtles (Ahasan et al., 2017a,b). These observations suggest an overall conservation in microbial shifts associated with stranding events.

At genus level, distinct microbial profiles were observed across the three groups of green sea turtles studied. In captive juveniles, *Citrobacter* emerged as the predominant genus. This finding aligns with previous work (Guo et al., 2022), that reported species of the *Citrobacter* genus being dominant on the carapaces of healthy juvenile green turtles. *Citrobacter* spp. has also been implicated in disease processes, including carapacial ulcers and systemic infections (Goldberg et al., 2016; Inurria et al., 2024), and may act as both a dominant coloniser and an opportunistic pathogen depending on host and environmental conditions.

Stranded green turtles exhibited elevated relative abundances of *Shewanella* and *Corynebacterium*. While some *Shewanella* spp. are recognised as part of the normal gut microbiome in sea turtles (Al-Bahry et al., 2011; Foti et al., 2009), *Shewanella* and *Corynebacterium* are increasingly associated with opportunistic infections in marine reptiles with pathogenic potential influenced by host immunity and environmental factors. (Magiorakos et al., 2012; Paździor, 2016; Tsai et al., 2008). Their presence in stranded green sea turtles in the current study likely reflects an opportunistic colonisation since these organisms can act both as colonisers and pathogens. Consistent with this postulation, *Shewanella* spp. were identified in cloacal samples from loggerhead turtles in the Western Mediterranean (Blasi et al., 2020), in prehospitalisation samples from turtles at a rehabilitation centre in Australia (Ahasan et al., 2018), and in tracheal lavages from Kemp's ridley turtles in USA (McNally et al., 2021). Similarly, *Corynebacterium* spp., though less frequently reported, have been implicated in chronic infections in green sea turtles (Ahasan, 2017; Turtle, 2010). In one case, *Corynebacterium* spp. was isolated from bilateral shoulder abscesses in an adult green turtle, alongside *Nocardia* spp. and alpha-hemolytic *Streptococcus* spp. (Turtle, 2010), suggesting a potential role in deep-seated, polymicrobial infections. These observations underscore the opportunistic nature of both *Shewanella* and *Corynebacterium*, and their emergence in stranded turtles further emphasises the likelihood of vulnerability in immunocompromised hosts to otherwise commensal or environmentally derived bacteria.

This study also presents a comparative analysis of antibiotic resistance genes in captive juveniles, captive adults and wild stranding green sea turtles that were mostly juveniles. The Alpha diversity analysis of CARD-annotated genes showed no significant differences in ARGs richness or evenness across the three groups, but beta diversity analysis

demonstrated significant differences in ARGs composition across the three groups. These differences in the broader classes of antibiotic resistance may be attributed to exposure to varying environmental pollutants in the Gulf of Thailand and thus contributing to observed resistance patterns. For example, the macrolide-specific efflux pump gene MacB emerged as the most dominant ARG across all groups, particularly in captive juveniles and captive adults, which suggests pervasive macrolide resistance likely driven by chronic environmental exposure to antibiotic residues from agricultural runoff or aquaculture (Milaković et al., 2019; Okeke et al., 2022). In a previous study conducted by Senta et al. (2021), it was demonstrated that macrolide residues can persist in surface and alluvial aquifer sediments for more than ten years after their discharge into the aquatic environment. These antibiotics commonly enter aquatic environments via untreated wastewater and agricultural runoff (Burnett et al., 2019; Wang et al., 2021; Wattayakorn, 2006). Studies have demonstrated that such discharges contribute to the accumulation of antibiotic residues in canal and river systems, which ultimately flow into the Gulf of Thailand (Mrozik et al., 2019; Tewari et al., 2013; Wang et al., 2021). In this study, MacB was the most abundant resistance gene across all three groups, consistent with patterns reported in a previous metagenomic analysis of gut bacteria from captive green and hawksbill turtles in China, where MacB was also the most prevalent resistance gene (Chen et al., 2022). This highlights the ecological persistence of macrolide antibiotics across diverse host species and environments. Adult green sea turtles in captivity also exhibited elevated tetracycline resistance (TxR), whereas wild stranded turtles showed a marked increase in TetA(58) and multiple ARGs associated with resistance to last-resort antibiotics including pleuromutilins, oxazolidinones, and daptomycin. Given that the health status of the stranded turtles was not assessed, the relationship between health, antibiotic resistance, and environmental factors remains unclear. Future studies investigating both healthy and stranded wild populations, alongside environmental samples, are needed to clarify these associations. In the current study, the widespread detection of resistance genes against other biocides such as triclosan (Ecol_fabG_TRC) may highlight the significant impact of anthropogenic pollution on marine ecosystems. Triclosan has been identified as a predominant contaminant in fish samples in Gulf of Thailand and this chemical disrupts reproductive functions in aquatic organisms (Chokki Veettil et al., 2024; Juksu et al., 2019). Additionally, triclosan exposure can shifts the bacterial community composition in marine periphyton at environmentally relevant concentrations (Martin et al., 2020), and it is known to promote the transfer and persistence of ARGs in aquatic environments (Lu et al., 2022). In the Gulf of Thailand, comparable anthropogenic activities such as industrial discharges, agricultural runoff, and tourism have been identified as source of pollutants into marine ecosystems. In addition, the Map Ta Phut industrial hub with its industrial estates, ports, and factories, is a major source of pollutants, including heavy metals, nutrients, and volatile organic compounds, and all these anthropogenic activities have the potential to significantly impact the Gulf`s marine ecosystems (Burnett et al., 2019; Wattayakorn, 2006). These pollutants may create environmental conditions that promote the proliferation and dissemination of antibiotic resistance within marine environments.

Despite the comprehensive analyses presented in this study, some limitations exist, and these should be addressed in future studies. First, sample sizes across groups were unbalanced, primarily due to conservation policies requiring the prompt release of rehabilitated turtles, which restricted sampling to opportunistic collections. This limitation may have introduced bias in statistical evaluations. Second, sampling was confined to a short time frame, preventing assessment of temporal variation in both microbiome composition and ARG profiles. Third, direct measurements of antibiotics and other biocides in the study regions were not feasible due to logistical constraints. Indirect evidence points to microbial shifts and ARG emergence, but direct quantification of these compounds would provide stronger support for causality. Future studies should therefore incorporate longitudinal sampling across seasons and include direct quantification of antibiotics and biocides to more accurately capture temporal dynamics and identify causal drivers.

Furthermore, ARGs that are presumably associated with bacteria in the gut but the close association between individual ARGs and bacteria species are not known. This could be elucidated using highly sophisticated sequencing approaches such as Hi-C sequencing that maps chromatin interactions across entire genomes. Additionally, the functional relevance of the identified ARGs was not assessed, and sequencing of RNA transcripts which was beyond the scope of the current study, would help to confirm the activity of detected ARGs within the microbiome. Host-and environment-specific factors influencing the microbiome were not fully explored, therefore metatranscriptomics or metaproteomics could offer a more comprehensive understanding of microbial function. Finally, the potential role of mobile genetic elements (MGEs) in ARG transfer was not examined; whole-genome sequencing combined with mobilome analysis could clarify the dynamics of ARG dissemination within microbial communities.

5. Conclusion

This study offers a comprehensive metagenomic analysis of the gut microbiome and resistome across different life stages and health statuses of green sea turtles in the Gulf of Thailand. The findings highlight significant shifts in microbial composition and antibiotic resistance profiles and, the likely influence of developmental stage and differences in the environments inhabited by the turtles. The functional significance of the microbial changes has not been characterised and should be focused for future studies. The resistome analysis revealed widespread resistance to macrolides and tetracyclines in juveniles and adults, while stranded turtles showed higher levels of resistance to last-resort antibiotics, including pleuromutilin and oxazolidinones. These results indicate that green sea turtles may serve as a reservoir for clinically relevant antibiotic resistance genes. These observations are potentially linked to environmental pollution, and this raises a concern of microbial evolution in marine species. The study highlights the critical need for integrating resistome surveillance into conservation strategies to mitigate emerging infectious threats and safeguard biodiversity in a changing environment.

CRediT authorship contribution statement

Dawood Ghafoor: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Orachun Hayakijkosol:** Writing – review & editing, Supervision, Data curation. **Noppadol Prasetsincharoen:** Resources, Methodology. **Carla C.M. Chen:** Writing – review & editing, Supervision. **Muhammad Noman:** Writing – review & editing, Formal analysis. **Poommate Chomchat:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Robert Kinobe:** Writing – review & editing, Supervision, Resources, Funding acquisition, Data curation, Conceptualization.

Funding

This work was supported by the MRF Competitive Funding Research Activity Grant, James Cook University, Australia and Boonthum Animal Hospital, Pathum Thani, Thailand [grant number 01/2024].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We gratefully acknowledge the support of the Department of Marine and Coastal Resources and the Department of Fisheries of Thailand for their assistance in facilitating this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107605.

Data availability

Data will be made available on request.

References

Ahasan, M.S., 2017. Gut bacterial communities in healthy and compromised green turtles (*Chelonia mydas*) and an alternative treatment for gastrointestinal disorders James Cook University]. https://doi.org/10.4225/28/5af27978657f0.

Ahasan, M.S., Picard, J., Elliott, L., Kinobe, R., Owens, L., Ariel, E., 2017a. Evidence of antibiotic resistance in enterobacteriales isolated from green sea turtles, *chelonia Mydas* on the great barrier reef. Mar. Pollut. Bull. 120 (1–2), 18–27. https://doi.org/10.1016/j.marpolbul.2017.04.046.

Ahasan, M.S., Waltzek, T.B., Huerlimann, R., Ariel, E., 2017b. Fecal bacterial communities of wild-captured and stranded green turtles (*chelonia mydas*) on the great barrier reef. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 93 (12), fix139. https://doi/10.1093/femsec/fix139.

Ahasan, M.S., Waltzek, T.B., Huerlimann, R., Ariel, E., 2018. Comparative analysis of gut bacterial communities of green turtles (*chelonia mydas*) pre-hospitalization and post-rehabilitation by high-throughput sequencing of bacterial 16S rRNA gene. Microbiol. Res. 207, 91–99.

Al-Bahry, S.N., Mahmoud, I.Y., Al-Zadjali, M., Elshafie, A., Al-Harthy, A., Al-Alawi, W., 2011. Antibiotic resistant bacteria as bio-indicator of polluted effluent in the green turtles, *chelonia mydas* in Oman. Mar. Environ. Res. 71 (2), 139–144. https://doi.org/10.1016/j.marenvres.2010.12.005.

Arienzo, M., 2023. Progress on the impact of persistent pollutants on marine turtles: a review. J. Mar. Sci. Eng. 11 (2), 266. https://doi/10.3390/jmse11020266.

Arthur, K.E., Boyle, M.C., Limpus, C.J., 2008. Ontogenetic changes in diet and habitat use in green sea turtle (*chelonia mydas*) life history. Mar. Ecol. Prog. Ser. 362, 303–311. https://doi.org/10.3389/fevo.2023.1139441.

Baquero, F., Martínez, J.-L., Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19 (3), 260–265. https://doi.org/10.1016/j.copbio.2008.05.006.

Benler, S., Yutin, N., Antipov, D., Rayko, M., Shmakov, S., Gussow, A.B., Pevzner, P., Koonin, E.V., 2021. Thousands of previously unknown phages discovered in whole-community human gut metagenomes. Microbiome 9, 1–17. https://doi.org/10.1186/s40168-021-01017-w.

Biagi, E., D'Amico, F., Soverini, M., Angelini, V., Barone, M., Turroni, S., Rampelli, S., Pari, S., Brigidi, P., Candela, M., 2019. Faecal bacterial communities from mediterranean loggerhead sea turtles (*Caretta caretta*). Environ. microbiol. reports 11 (3), 361–371. https://doi.org/10.1111/1758-2229.12683.

Blasi, M.F., Migliore, L., Mattei, D., Rotini, A., Thaller, M.C., Alduina, R., 2020. Antibiotic resistance of gram-negative bacteria from wild captured loggerhead sea turtles. Antibiotics 9 (4), 162. https://doi.org/10.3390/antibiotics9040162.

Bloodgood, J.C., Hernandez, S.M., Isaiah, A., Suchodolski, J.S., Hoopes, L.A., Thompson, P.M., Waltzek, T.B., Norton, T.M., 2020. The effect of diet on the gastrointestinal microbiome of juvenile rehabilitating green turtles (*Chelonia mydas*). PLoS One 15 (1), e0227060. https://doi.org/10.1371/journal.pone.0227060.

Buchfink, B., Xie, C., Huson, D.H., 2015. Fast and sensitive protein alignment using DIAMOND. Nat. Methods 12 (1), 59–60. https://doi.org/10.1038/nmeth.3176.

Burnett, W.C., Sola, P., Chanyotha, S., Bidorn, B., Kritsananuwat, R., Chinfak, N., 2019. Tracing underground sources of pollution to coastal waters off map Ta phut, Rayong, Thailand. Mar. Pollut. Bull. 148, 75–84. https://doi.org/10.1016/j.marpolbul.2019.07.071.

Cafarchia, C., Paradies, R., Figueredo, L.A., Iatta, R., Desantis, S., Di Bello, A.V.F., Zizzo, N., van Diepeningen, A.D., 2020. Fusarium spp. in loggerhead sea turtles (*Caretta caretta*): from colonization to infection. Veterinary Pathology 57 (1), 139–146. https://doi.org/10.1177/0300985819880347.

Campos, P., Guivernau, M., Prenafeta-Boldú, F.X., Cardona, L., 2018. Fast acquisition of a polysaccharide fermenting gut microbiome by juvenile green turtles *Chelonia mydas* after settlement in coastal habitats. Microbiome 6, 1–11. https://doi.org/10.1186/s40168-018-0454-z.

Carini, A., Ariel, E., Picard, J., Elliott, L., 2017. Antibiotic resistant bacterial isolates from captive green turtles and in vitro sensitivity to bacteriophages. Int. J. Microbiol 2017 (1), 5798161. https://doi.org/10.1155/2017/5798161.

Chai, C.X., Samat, A., Mohd-Taib, F.S., Bharudin, I., Tim, J., 2023. Fungal infection of sea turtle eggs in the sea turtle hatcheries in peninsular Malaysia. Fungal Ecology 63, 101243. https://doi.org/10.1016/j.funeco.2023.101243.

Chen, Y., Xia, Z., Li, H., 2022. Metagenomic comparison of gut communities between hawksbills (*Eretmochelys imbricata*) and green sea turtles (*Chelonia mydas*). Arch. Microbiol. 204 (8), 450. https://doi.org/10.1007/s00203-022-03073-8.

Chokki Veettil, P., Nikarthil Sidhick, J., Kavungal Abdulkhader, S., Ms, S.P., Kumari Chidambaran, C., 2024. Triclosan, an antimicrobial drug, induced reproductive impairment in the freshwater fish, Anabas testudineus (Bloch, 1792). Toxicol. Ind. Health 40 (5), 254–271. https://doi.org/10.1177/07482337241242510.

Díaz-Abad, L., Bacco-Mannina, N., Miguel Madeira, F., Serrao, E.A., Regalla, A., Patrício, A.R., Frade, P.R., 2022. Red, gold and green: microbial contribution of rhodophyta and other algae to green turtle (*Chelonia mydas*) gut microbiome. Microorganisms 10 (10), 1988. https://doi.org/10.3390/microorganisms10101988.

Dill-McFarland, K.A., Weimer, P.J., Pauli, J.N., Peery, M.Z., Suen, G., 2016. Diet specialization selects for an unusual and simplified gut microbiota in two-and three-toed sloths. Environ. Microbiol. 18 (5), 1391–1402. https://doi.org/10.1111/1462-2920.13022.

Drane, K., Huerlimann, R., Power, M., Whelan, A., Ariel, E., Sheehan, M., Kinobe, R., 2021. Testudines as sentinels for monitoring the dissemination of antibiotic resistance in marine environments: an integrative review. Antibiotics 10 (7), 775. https://doi.org/10.3390/antibiotics10070775.

Escobedo-Bonilla, C.M., Quiros-Rojas, N.M., Rudín-Salazar, E., 2022. Rehabilitation of marine turtles and welfare improvement by application of environmental enrichment strategies. Animals 12 (3), 282. https://doi.org/10.3390/ani12030282.

Fogel, A.T., 2015. The gut microbiome of wild lemurs: a comparison of sympatric lemur catta and Propithecus verreauxi. Folia Primatol. 86 (1–2), 85–95. https://doi.org/10.1159/000369971.

Foti, M., Giacopello, C., Bottari, T., Fisichella, V., Rinaldo, D., Mammina, C., 2009. Antibiotic resistance of gram negatives isolates from loggerhead sea turtles (*Caretta caretta*) in the central Mediterranean Sea. Mar. Pollut. Bull. 58 (9), 1363–1366. https://doi.org/10.1016/j.marpolbul.2009.04.020.

Ghafoor, D., Kinobe, R., Chen, C.C., Prasetsincharoen, N., Chomchat, P., Sangkachai, N., Hayakijkosol, O., 2025. Characterisation of the antimicrobial resistance profile of culturable Gram-negative bacterial isolates from green sea turtles (*Chelonia mydas*) in the gulf of Thailand. Mar. Environ. Res., 107466 https://doi.org/10.3390/ani15111594.

Goldberg, D.W., Cegoni, C.T., Rogério, D.W., Wanderlinde, J., Lima, E.P.E., Silveira, R.L., Jerdy, H., de Carvalho, E.C.Q., 2016. Fatal Citrobacter Coelomitis in a Juvenile Green Turtle (*Chelonia Mydas*): a Case Report.

Guo, Y., Chen, H., Liu, P., Wang, F., Li, L., Ye, M., Zhao, W., Chen, J., 2022. Microbial composition of carapace, feces, and water column in captive juvenile green sea turtles with carapacial ulcers. Front. Vet. Sci. 9, 1039519. https://doi.org/10.3389/fvets.2022.1039519.

Hart, K.M., Mooreside, P., Crowder, L.B., 2006. Interpreting the spatio-temporal patterns of sea turtle strandings: going with the flow. Biol. Conserv. 129 (2), 283–290. https://doi.org/10.1016/j.biocon.2005.10.047.

Howell, L.N., Shaver, D.J., 2021. Foraging habits of green sea turtles (*Chelonia mydas*) in the northwestern Gulf of Mexico. Front. Mar. Sci. 8, 658368. https://doi.org/10.3389/fmars.2021.658368.

Huang, K., Zhang, X., Xiong, N., Sun, L., Zhao, X., Zhou, K., Wu, J., 2024. First metagenomic sequencing for the analysis of microbial community populations of adults and pupae of *Melophagus ovinus* in Xinjiang, China. Front. Vet. Sci. 11, 1462772. https://doi.org/10.3389/fvets.2024.1462772.

Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinf. 11 (1), 119. https://doi.org/10.1186/1471-2105-11-119.

Inurria, A., Suárez-Pérez, A., Calabuig, P., Orós, J., 2024. Citrobacter freundii-associated lesions in stranded loggerhead sea turtles (*Caretta caretta*). Veterinary Pathology 61 (1), 140–144. https://doi.org/10.1177/03009858231183983.

Iurk, V.B., Ingles, M., Correa, G.S., Silva, C.R., Staichak, G., Pileggi, S.A.V., Christo, S.W., Domit, C., Pileggi, M., 2024. The potential influence of microplastics on the microbiome and disease susceptibility in sea turtles. Sci. Total Environ., 174298 https://doi.org/10.1016/j.scitotenv.2024.174298.

Juksu, K., Zhao, J.-L., Liu, Y.-S., Yao, L., Sarin, C., Sreesai, S., Klomjek, P., Jiang, Y.-X., Ying, G.-G., 2019. Occurrence, fate and risk assessment of biocides in wastewater treatment plants and aquatic environments in Thailand. Sci. Total Environ. 690, 1110–1119. https://doi.org/10.1016/j.scitotenv.2019.07.097.

Li, D., Luo, R., Liu, C.-M., Leung, C.-M., Ting, H.-F., Sadakane, K., Yamashita, H., Lam, T.-W., 2016. MEGAHIT v1. 0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102, 3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.

Li, H., Durbin, R., 2009. Fast and accurate short read alignment with burrows–wheeler transform. Bioinformatics 25 (14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.

Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with burrows–wheeler transform. Bioinformatics 26 (5), 589–595. https://doi.org/10.1093/bioinformatics/btp698.

Li, R., Li, Y., Kristiansen, K., Wang, J., 2008. SOAP: short oligonucleotide alignment program. Bioinformatics 24 (5), 713–714. https://doi.org/10.1093/bioinformatics/btn025.

Li, W., Ni, J., Cai, S., Liu, Y., Shen, C., Yang, H., Chen, Y., Tao, J., Yu, Y., Liu, Q., 2019. Variations in microbial community structure and functional gene expression in bio-treatment processes with odorous pollutants. Sci. Rep. 9 (1), 17870. https://doi.org/10.1038/s41598-019-54281-0.

Liu, Z., Liu, J., Geng, J., Wu, E., Zhu, J., Cong, B., Wu, R., Sun, H., 2024. Metatranscriptomic characterization of six types of forensic samples and its potential application to body fluid/tissue identification: a pilot study. Forensic Sci. Int.: Genetics 68, 102978. https://doi.org/10.1016/j.fsigen.2023.102978.

Lu, J., Yu, Z., Ding, P., Guo, J., 2022. Triclosan promotes conjugative transfer of antibiotic resistance genes to opportunistic pathogens in environmental microbiome. Environ. Sci. Technol. 56 (21), 15108–15119. https://doi.org/10.1016/j.biortech.2024.131522.

Lu, X., Gong, G., Zhang, Q., Yang, S., Wu, H., Zhao, M., Wang, X., Shen, Q., Ji, L., Liu, Y., 2024. Metagenomic analysis reveals high diversity of gut viromes in yaks (*Bos grunniens*) from the Qinghai-Tibet Plateau. Commun. Biol. 7 (1), 1097. https://doi.org/10.1038/s42003-024-06798-y.

Magiorakos, A.-P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M., Giske, C., Harbarth, S., Hindler, J., Kahlmeter, G., Olsson-Liljequist, B., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infection 18 (3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Martin, E.K., Kemal, S., Henrik, N.R., Alexander, E., Natalia, C., Henrik, J.C., Thomas, B., Hans, B., Erik, K., 2020. Triclosan changes community composition and selects for specific bacterial taxa in marine periphyton biofilms in low nanomolar concentrations. Ecotoxicology 29, 1083–1094. https://doi.org/10.1007/s10646-020-02246-9.

Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17 (1), 10–12. https://doi.org/10.14806/ej.17.1.200.

McDermid, K.J., Kittle III, R.P., Veillet, A., Plouviez, S., Muehlstein, L., Balazs, G.H., 2020. Identification of gastrointestinal microbiota in Hawaiian green turtles (*Chelonia mydas*). Evol. Bioinf. Online 16, 1176934320914603. https://doi.org/10.1177/1176934320914603.

McMaken, C.M., Burkholder, D.A., Milligan, R.J., Lopez, J.V., 2023. Potential impacts of environmental bacteria on the microbiota of loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtle eggs and their hatching success. MicrobiologyOpen 12 (3), e1363. https://doi.org/10.1002/mbo3.1363.

McNally, K.L., Bowen, J.L., Brisson, J.O., Kennedy, A., Innis, C.J., 2021. Evaluation of the respiratory microbiome and the use of tracheal lavage as a diagnostic tool in Kemp's ridley sea turtles (*Lepidochelys kempii*). Animals 11 (10), 2927. https://doi.org/10.3390/ani11102927.

Milaković, M., Vestergaard, G., González-Plaza, J.J., Petrić, I., Šimatović, A., Senta, I., Kublik, S., Schloter, M., Smalla, K., Udiković-Kolić, N., 2019. Pollution from azithromycin-manufacturing promotes macrolide-resistance gene propagation and induces spatial and seasonal bacterial community shifts in receiving river sediments. Environ. Int. 123, 501–511. https://doi.org/10.1016/j.envint.2018.12.050.

Mrozik, W., Vinitnantharat, S., Thongsamer, T., Pansuk, N., Pattanachan, P., Thayanukul, P., Acharya, K., Baluja, M.Q., Hazlerigg, C., Robson, A.F., 2019. The food-water quality nexus in periurban aquacultures downstream of Bangkok, Thailand. Sci. Total Environ. 695, 133923. https://doi.org/10.1016/j.scitotenv.2019.133923.

Niu, X., Lin, L., Zhang, T., An, X., Li, Y., Yu, Y., Hong, M., Shi, H., Ding, L., 2024. Comparison of the intestinal flora of wild and artificial breeding green turtles (*Chelonia mydas*). Front. Microbiol. 15, 1412015. https://doi.org/10.3389/fmicb.2024.1412015.

Niu, X., Lin, L., Zhang, T., An, X., Li, Y., Yu, Y., Hong, M., Shi, H., Ding, L., 2024b. Research on antibiotic resistance genes in wild and artificially bred green turtles (Chelonia mydas). Sci. Total Environ. 954, 176716. https://doi.org/10.1016/j.scitotenv.2024.176716.

Okeke, E.S., Chukwudozie, K.I., Nyaruaba, R., Ita, R.E., Oladipo, A., Ejeromedoghene, O., Atakpa, E.O., Agu, C.V., Okoye, C.O., 2022. Antibiotic resistance in aquaculture and aquatic organisms: a review of current nanotechnology applications for sustainable management. Environ. Sci. Pollut. Control Ser. 29 (46), 69241–69274. https://doi.org/10.1007/s11356-022-22319-y.

Paździor, E., 2016. *Shewanella putrefaciens*–a new opportunistic pathogen of freshwater fish. J. Veterinary Res 60 (4), 429–434. https://doi.org/10.1515/jvetres-2016-0064.

Price, J.T., Paladino, F.V., Lamont, M.M., Witherington, B.E., Bates, S.T., Soule, T., 2017. Characterization of the juvenile green turtle (*Chelonia mydas*) microbiome throughout an ontogenetic shift from pelagic to neritic habitats. PLoS One 12 (5), e0177642. https://doi.org/10.1371/journal.pone.0177642.

Ranjan, R., Rani, A., Metwally, A., McGee, H.S., Perkins, D.L., 2016. Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. Biochem. Biophys. Res. Commun. 469 (4), 967–977. https://doi.org/10.1016/j.bbrc.2015.12.083.

Raulo, A., 2015. Social Behavior and Gut Microbiota in Wild red-bellied Lemurs (*Eulemur Rubriventer*): in Search of the Role of Immunity in the Evolution of Sociality.

Rausch, P., Rühlemann, M., Hermes, B.M., Doms, S., Dagan, T., Dierking, K., Domin, H., Fraune, S., Von Frieling, J., Hentschel, U., 2019. Comparative analysis of amplicon and metagenomic sequencing methods reveals key features in the evolution of animal metaorganisms. Microbiome 7, 1–19. https://doi.org/10.1186/s40168-019-0743-1.

Samuelson, M.M., Pulis, E.E., Ray, C., Arias, C.R., Samuelson, D.R., Mattson, E.E., Solangi, M., 2020. Analysis of the fecal microbiome in Kemp's ridley sea turtles *Lepidochelys kempii* undergoing rehabilitation. Endanger. Species Res. 43, 121–131. https://doi.org/10.3354/esr01043.

Sarmiento-Ramírez, J.M., van der Voort, M., Raaijmakers, J.M., Dieguez-Uribeondo, J., 2014. Unravelling the microbiome of eggs of the endangered sea turtle *Eretmochelys imbricata* identifies bacteria with activity against the emerging pathogen *Fusarium falciforme*. PLoS One 9 (4), e95206. https://doi.org/10.1371/journal.pone.0095206.

Scheelings, T.F., 2019. The Microbiota of Sea Turtles. Monash University.

Scheelings, T.F., Moore, R.J., Van, T.T.H., Klaassen, M., Reina, R.D., 2020. Microbial symbiosis and coevolution of an entire clade of ancient vertebrates: the gut microbiota of sea turtles and its relationship to their phylogenetic history. Animal Microbiome 2, 1–12. https://doi.org/10.1186/s42523-020-00034-8.

Shin, N.-R., Whon, T.W., Bae, J.-W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33 (9), 496–503. https://doi/10.1016/j.tibtech.2015.06.011.

Simon, H.Y., Siddle, K.J., Park, D.J., Sabeti, P.C., 2019. Benchmarking metagenomics tools for taxonomic classification. Cell 178 (4), 779–794. https://doi/10.1016/j.cell.2019.07.010.

Tewari, S., Jindal, R., Kho, Y.L., Eo, S., Choi, K., 2013. Major pharmaceutical residues in wastewater treatment plants and receiving waters in Bangkok, Thailand, and associated ecological risks. Chemosphere 91 (5), 697–704. https://doi.org/10.1016/j.chemosphere.2012.12.042.

Tremblay, J., Singh, K., Fern, A., Kirton, E.S., He, S., Woyke, T., Lee, J., Chen, F., Dangl, J.L., Tringe, S.G., 2015. Primer and platform effects on 16S rRNA tag sequencing. Front. Microbiol. 6, 771. https://doi.org/10.3389/fmicb.2015.00771.

Tsai, M.-S., You, H.-L., Tang, Y.-F., Liu, J.-W., 2008. *Shewanella* soft tissue infection: case report and literature review. Int. J. Infect. Dis. 12 (6), e119–e124. https://doi.org/10.1016/j.ijid.2008.03.020.

Turtle, G.S., 2010. Bilateral chronic shoulder infections in an adult. J. Herpetol. Med. Surg. 20 (4). https://doi.org/10.5818/1529-9651-20.4.105.

Velasquez Vacca, A., 2023. Tracking historical changes in the trophic ecology of the green turtle" *Chelonia mydas*" in the Hawaiian islands. https://hdl.handle.net/2445/196921.

Vezeau, N., Kahn, L., 2024. Current understanding and knowledge gaps regarding wildlife as reservoirs of antimicrobial resistance. Am. J. Vet. Res. 85 (6). https://doi.org/10.2460/ajvr.24.02.0040.

Wang, A., Guo, X., Morimoto, A., Maetani, K., Tanoue, R., Tong-U-Dom, S., Buranapratheprat, A., 2021. Transport and dilution of fluvial antibiotic in the upper gulf of Thailand. Environ. Pollut. 288, 117779. https://doi.org/10.1016/j.envpol.2021.117779.

Wattayakorn, G., 2006. Environmental issues in the gulf of Thailand. In: The Environment in Asia Pacific Harbours. Springer, pp. 249–259.

Yang, Q., Cahn, J.K., Piel, J., Song, Y.-F., Zhang, W., Lin, H.-W., 2022. Marine sponge endosymbionts: structural and functional specificity of the microbiome within euryspongia arenaria cells. Microbiol. Spectr. 10 (3). https://doi.org/10.1128/spectrum.02296-21.