

First detection of porcine associated bacterium Inconstantimicrobium in a reptilian host: gastrointestinal microbiome characterisation of healthy and unhealthy green turtles (*Chelonia mydas*) using full-length 16S rRNA sequencing

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Short Report

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First detection of porcine associated bacterium *Inconstantimicrobium* in a reptilian host: gastrointestinal microbiome characterisation of healthy and unhealthy green turtles (*Chelonia mydas*) using full-length 16S rRNA sequencing.

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18 Abstract

19 Background

20 Green sea turtles (*Chelonia mydas*) rely on hindgut bacterial fermentation to digest a high-cellulose diet. Gastrointestinal disease is a common
21 cause of morbidity in stranded individuals; despite this the associated microbial communities remain poorly characterised. Most prior turtle
22 microbiome studies have used short-read amplicon sequencing, which limits taxonomic resolution at genus and species level. Here we applied
23 PacBio HiFi full-length 16S rRNA sequencing to characterise the gastrointestinal microbiome of one healthy and two debilitated green turtles
24 from Hervey Bay, Queensland, Australia.

25 Results

26 Cloacal samples were collected from three green turtles: a healthy adult (HB-G25); an unhealthy juvenile (HB-G26) with suspected parasitism,
27 dysbiosis, emaciation and renal failure; and an unhealthy sub-adult (Jethro (143717)) with suspected dysbiosis and severe
28 intestinal stasis. Full-length 16S rRNA amplicons were sequenced by PacBio HiFi to a target depth of 50,000 reads per sample and processed
29 with the pb-16S-nf pipeline in QIIME 2, with taxonomy assigned against the SILVA, GTDB and RefSeq/RDP databases. Healthy adult (HB-G25)
30 showed a mixed *Bacteroidota/Firmicutes_A/Proteobacteria* profile; unhealthy juvenile (HB-G26) was dominated by *Proteobacteria* (92.4%); and
31 unhealthy sub-adult (Jethro (143717)) was dominated by *Firmicutes_A* (77.0%), driven by a bloom of fermentative *Clostridia* including
32 *Clostridium*, an uncharacterised *Lachnospirales* genus (*RUG472*), *Cellulosilyticum* and *Inconstantimicrobium*. Beta diversity showed near-
33 complete separation between individuals (Bray-Curtis 0.986–0.999). *Inconstantimicrobium porci*, previously reported only from pig intestine
34 and agricultural soil, was detected here. A total of 25 ASVs assigned to *Inconstantimicrobium porci* (phylum, *Firmicutes A*; family *Clostridiaceae*)
35 were detected in unhealthy sub-adult (Jethro (143717)), comprising 3.2% relative abundance (1,151 reads). This genus was absent in unhealthy
36 juvenile HB-G26 and present at only trace levels in the healthy adult HB-G25 (135 reads). Unhealthy sub-adult (Jethro (143717)) had the

highest ASV richness (375) and Shannon index ($H' = 4.53$, tied with healthy adult (HB-G25)); unhealthy juvenile (HB-G26) had the lowest richness (183) and the lowest Shannon index of $H' = 3.90$. Healthy adult (HB-G25) had moderate ASV richness (279) and a high Shannon index of $H' = 4.53$.

Conclusion

The clostridial overgrowth observed in unhealthy sub-adult (Jethro (143717)) is mechanistically consistent with fermentative gas production and dysbiosis. *Inconstantimicrobium porci*, is described as a commensal anaerobe of the pig intestine with inferred fermentative capacity based on its phylogenetic placement within *Clostridiaceae*. It is hypothesised that exposure of these bacteria may be ascribed to increased terrestrial runoff caused by recent flooding events. Unhealthy juvenile (HB-G26)'s low ASV richness and Shannon diversity are consistent with dysbiosis reported in other debilitated juvenile green turtles, whereas HB-G25's moderate richness and more evenly distributed community reflect profiles described in healthy adult green turtles. These findings show the value of species-specific microbiome reference data for chelonian clinical assessment and dysbiosis recognition.

Keywords: green turtle, gastrointestinal microbiome, ileus, dysbiosis, *Inconstantimicrobium*, *Firmicutes*, *Proteobacteria*, *Bacteriodes*, *Clostridium*

55 **Background**

56 Green sea turtles (*Chelonia mydas*) are important marine megaherbivores whose nutrition depends heavily on microbial fermentation within
57 the gastrointestinal tract. Their seagrass and algal dominated diets contain complex structural carbohydrates that cannot be efficiently digested
58 by the host alone, making a functional gut microbiome essential for nutrient acquisition, gastrointestinal health, and ultimately survival.
59 Despite this critical role, the intestinal microbiota of green turtles remains relatively underexplored, notably in the context of health, disease
60 and clinical presentation [1-3].

61 This knowledge gap is significant in Australia, where disease and pathogen threats to sea turtle populations are still considered poorly
62 understood within conservation and wildlife health frameworks [4-7]. Increasing ecological stressors, including habitat degradation, flooding,
63 and other stressors, are likely playing a role in compromising health in wild sea turtles and may individuals susceptible to disease through
64 immunosuppression [8]. In sea turtles, this immunosuppression may allow opportunistic pathogens to proliferate and disturb normal microbial
65 communities, increasing the risk of dysbiosis and associated pathology [9-11]. As responses to stress entail complex interactions among the
66 immune, nervous, and endocrine systems, a more integrated understanding of host-microbe relationships is needed to more accurately
67 interpret disease process in sea turtles [8, 12, 13].

68 In practice, assessing disease prevalence and health status in sea turtles is difficult. Wild populations are challenging to sample, many
69 individuals inhabit remote marine environments, and health assessments frequently vary considerably between studies, limiting the
70 establishment of consistent baseline health parameters [2, 14-18]. However, recent advances in sequencing technology and the wider
71 availability of less-invasive sampling techniques such as cloacal swabs, have created new opportunities to investigate gastrointestinal
72 microbiome profiling as a practical and informative tool for wildlife health assessment.

73

74 To date, only limited research has examined how gut microbial communities differ between healthy and diseased sea turtles. Ahasan et
75 al., reported that intestinal flora in sick turtles from the northern Great Barrier Reef, Queensland, Australia was dominated by *Proteobacteria*,
76 whereas healthy turtles showed a higher relative abundance of *Firmicutes*, followed by *Bacteroides* [1]. These results indicate that microbial
77 composition may shift in association with disease status, yet what constitutes a healthy gastrointestinal microbiome in green turtles remains
78 poorly defined, particularly for stocks within South-East Queensland (SEQ), Australia. Moreover, prior sea turtle microbiome studies have relied
79 on short-read amplicon sequencing of one or two 16S rRNA hypervariable regions, which constrains taxonomic assignment [1]. This is especially
80 relevant in clinical settings in South-East Queensland, where severe gastrointestinal ileus, intestinal stasis, and obstipation are major
81 contributors to morbidity in stranded sea turtles, including those admitted to the Australia Zoo Wildlife Hospital (Bowtell et al., manuscript in
82 prep).

83 Against this background, the present study surveys the gut microbiome of three green sea turtles representing contrasting health states
84 in SEQ. These include a healthy wild-caught turtle used as a reference for presumed normal microbial composition (HB-G25); an unhealthy
85 juvenile (HB-G26), presenting in the field with suspected dysbiosis, renal failure secondary to dehydration, and severe emaciation; and an
86 unhealthy sub-adult (Jethro (143717)), with suspected dysbiosis and clinical signs consistent with severe intestinal stasis. By comparing
87 microbial profiles across these individuals, this study intends to contribute preliminary insight into how gastrointestinal microbial communities
88 may reflect or relate to health status, disease processes, and clinical pathology in green turtles from the region. Generating foundational
89 microbiome data for both healthy and compromised green turtles an important step toward improving evidence-based clinical assessment and
90 treatment. More broadly, understanding the gut microbiome may help clarify links between environmental stress, gastrointestinal dysfunction,
91 and disease susceptibility in sea turtles, supporting both wildlife rehabilitation and conservation management in South-East Queensland.

92

93 **Methods**

94 This pilot study adopted a descriptive, cross-sectional design across two sampling events. Three green sea turtles (*Chelonia mydas*) were
95 sampled across two collection events, within one month of each other, in Hervey Bay, Queensland, Australia (Figure 1). HB-G25 (adult) and HB-
96 G26 (juvenile), were sampled in September 2025 with HB-G26 subsequently being transported to AZWH. Unhealthy sub-adult (Jethro
97 (143717)) was rescued from Hervey Bay and admitted to AZWH, where a cloacal swab was collected upon admittance in October 2025.

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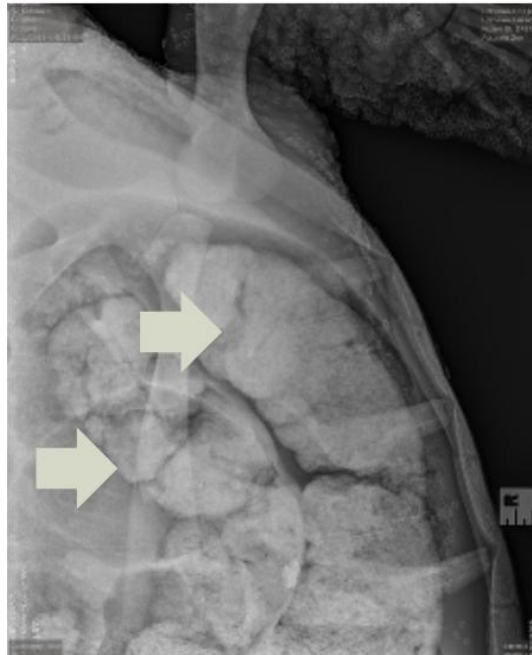
100



101 **Figure 1.** Satellite map showing the locations of the three turtles in this study, Hervey Bay, Queensland, Australia. Markers denote a healthy
 102 adult HB-G25 (green; 25.23299°S, 152.66899°E), an unhealthy sub-adult (Jethro (143717)) (red; 25.26855°S, 152.763541°E), and an unhealthy
 103 juvenile HB-G26 (yellow; 25.23627°S, 152.6712°E). Basemap imagery: Esri World Imagery.

104 ***Morphometrics***

105 HB-G25 was a healthy adult green turtle with no evidence of illness during in-field examination. HB-G26 was an unhealthy juvenile that was
106 subsequently rescued and found to be suffering from floating syndrome, suspected parasite infection (spirorchidiasis), dysbiosis and renal
107 failure due to dehydration on veterinary examination. Unhealthy sub-adult (Jethro (143717)) a sub-adult, presented with severe
108 gastrointestinal ileus, intestinal fecaliths, and gastrointestinal gas on diagnostic imaging (Figure 2). This was consistent with clinically significant
109 dysbiosis; however, the patient was in average body condition at the time of admission (Table 1).



117 **Figure 2.** X-ray image on admission of unhealthy sub-adult Jethro (143717). Arrows showing the top right carapace direct view of fecalith
118 formation.

119

120 **Table 1.** Morphometrics and clinical characteristics of three green sea turtles (*Chelonia mydas*) included in this study. Weight for healthy adult
 121 (HB-G25) could not be determined due to the size of the turtle in-field but as been estimated as 130kg+ based on the curved carapace length
 122 using guidelines from Limpus [19]. Body condition score is measured out of 5, according to standard guidelines from Norton & Wyneken [20].
 123 Emaciated = 1, poor = 2, average = 3, good = 4, obese = 5. It should be noted that both unhealthy juvenile (HB-G26) and unhealthy sub-adult
 124 (Jethro (143717)) both did not survive.

Individual	Health status	Cause of illness	Age class	Curved carapace length (cm)	Weight (kg)	Body condition score (/5) *
HB-G25	Healthy	N/A	Adult	118.3	130+	4
HB-G26	Unhealthy	Suspected spirorchidiasis & Dysbiosis	Juvenile	45.2	7.8	1
Jethro (143717)	Unhealthy	Dysbiosis	Sub-adult	81.0	50.0	3

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129 ***Microbiome sampling***

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131 Sample collection was taken directly from the cloaca following Bloodgood et al. [2], Scheelings, [21] & Ahasan et al., [1]. After health
132 assessment, turtles were positioned on a treatment table with the caudal end hanging off the edge, allowing easy access to the
133 cloaca. A sterile aluminium Copan CLASSIQSwabs™ swab was inserted into the cloaca to enter the distal colon [22]. For healthy adult
134 (HB-G25) & unhealthy juvenile (HB-G26), the swab end was stored in an Eppendorf tube with 0.5 mL RNAlater solution. After three
135 days kept in refrigeration (as per manufacturer recommendations), these samples were placed in –80 °C freezers until DNA
136 extraction. For unhealthy sub-adult (Jethro (143717)) the sample was placed into an Eppendorf tube and immediately stored in a -80
137 °C freezer. These samples were stored for 3 months until DNA extraction occurred. Extracted samples were stored for two months
138 until sequencing. No anaesthesia was administered for the purposes of sample collection. Two individuals (HB-G26 and Jethro
139 (143717)) were subsequently euthanised at Australia Zoo Wildlife Hospital under veterinary direction, as a welfare measure due to
140 deterioration in health in care; this was unrelated to the present study and occurred after cloacal samples had been collected.
141 Euthanasia was performed via intravenous administration of alfaxalone (Alfaxan; Jurox Pty Ltd, Australia) followed by pentobarbitone
142 sodium (Lethabarb; Jurox Pty Ltd, Australia) as per veterinary dosage and direction.

143 ***DNA extraction and library preparation***

144 Total genomic DNA was extracted from each sample using Blood and Tissue Kit (Qiagen, Hilden, Germany) according to manufacturer's
145 instructions. Full-length 16S rRNA amplicons, approximately 1,490 bp, were generated using the universal primers 27F
146 (AGRGTTYGATYMTGGCTCAG) and 1492R (AAGTCGTAACAAGGTARCY). Libraries were prepared and sequenced on a PacBio Sequel II system

147 (Pacific Biosciences, Menlo Park, CA, USA) by the Australian Genome Research Facility (AGRF, Brisbane, Australia), generating HiFi reads with a
148 per-read accuracy of $\geq$ Q20.

149 ***Bioinformatic analysis***

150 Raw reads were processed by the Australian Genomic Research Facility (AGRF) using the pb-16S-nf pipeline (v0.6; Nextflow), which
151 implements a QIIME 2 framework. Primers were trimmed using Cutadapt, and reads failing quality thresholds ($Q < 20$, length $< 1,000$ bp or $>$
152 $1,600$ bp, maxEE > 10) were excluded. Denoising and amplicon sequence variant (ASV) inference were performed with DADA2 using pseudo-
153 pooling. Taxonomy was assigned using a consensus approach across three classifiers: VSEARCH (97% identity; GTDB r207), and Naïve Bayes
154 classifiers trained on SILVA v138.1, GTDB r207, and RefSeq/RDP databases. Beta diversity distance matrices (Bray-Curtis dissimilarity,
155 unweighted and weighted UniFrac) were generated using QIIME 2's diversity plugin (core-metrics-phylogenetic) at the same rarefaction depth;
156 observed ASV richness and the Shannon index were calculated directly in RStudio (version 4.5.2) from the rarefied ASV table.

157 ***Statistical analysis***

158 Alpha diversity, the diversity of taxa within a single sample, was assessed for each turtle using observed amplicon sequence variant (ASV)
159 richness and the Shannon diversity index, calculated following rarefaction to an equal sequencing depth across samples. Principal coordinates
160 analysis (PCoA) of intestinal bacterial beta diversity across three green sea turtles was performed. Beta diversity is defined as the variation in
161 community composition between samples, in contrast to alpha diversity, which describes the diversity of taxa within a single sample [23].
162 Ordination plots were calculated for Bray-Curtis dissimilarity, unweighted UniFrac distance, and weighted UniFrac distance as per Lozupone &
163 Knight [24] and Lozupone et al. [25]. Given the small sample size ($n = 3$), all results are presented descriptively using RStudio, (R version 4.5.2).

164

165 **Results**

166 All three samples were sequenced to a target depth of 50,000 reads per sample. Demultiplexing efficiency was near-perfect across all samples.
167 Read quality was high, with median per-read Q-scores above Q30 and median read lengths of 1,478–1,501 bp, consistent with high-quality full-
168 length 16S rRNA amplicon recovery (Table 2). The best consensus taxonomy per ASV was retained. Amplicon sequence variants represent a
169 unique DNA sequence detected after denoising. Each ASV represents one unique microbial taxon or close relatives to this taxon. Samples were
170 rarefied to 35,531 reads, corresponding to the minimum retained library size across samples, prior to diversity analyses (Table 2). Phylum-level
171 gastrointestinal bacterial community composition was demonstrated for each turtle (Figure 3 & Table 3). Beta diversity was calculated using
172 Bray-Curtis dissimilarity and unweighted and weighted UniFrac distances (Figure 4). Alpha diversity was assessed using observed ASVs and the
173 Shannon index (Figure 5 & 6). Two negative controls were processed and sequenced alongside this sequencing batch, yielding 149 and 182
174 reads respectively, low relative to the approximately 50,000 raw reads obtained per biological sample (Table 2). Sequences recovered from
175 each control were highly diverse, with no single sequence dominating either control. A small minority of reads in each control (6/149 and
176 6/182) were nearly identical to abundant ASVs detected in the biological samples.

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182 **Table 2.** Sequencing output and quality filtering statistics for three green sea turtles (*Chelonia mydas*). Input reads were quality filtered and
183 chimera-checked using DADA2. Retained reads represent high-quality, non-chimeric amplicon sequence variants (ASVs) used in downstream
184 diversity analyses. All samples were rarefied to 35,531 reads prior to alpha and beta diversity calculations. *Unhealthy juvenile (HB-G26)
185 showed a higher chimera removal rate relative to other samples, likely reflecting template skew toward the few dominant, closely related taxa
186 in this low-diversity sample, which favours both chimera formation and detection by DADA2's de novo chimera-detection algorithm. A
187 rarefaction depth of 35,531 reads was selected for alpha and beta diversity analyses, retaining all three samples at full coverage. †No-template
188 controls (NTCs) were not processed through the DADA2 pipeline; only raw demultiplexed read counts are reported.

Sample	Input reads	Post-filter	Filter pass %	Non-chimeric	Retained %	ASVs
Heathy adult (HB-G25)	49,971	46,839	93.7%	35,957	71.9%	279
Unhealthy, juvenile (HB-G26)	49,965	46,577	93.2%	31,281	62.6%*	183
Unhealthy sub-adult (Jethro (143717)	49,965	46,702	93.5%	35,673	71.4%	375
Neg5 (NTC)	149	-	-	-	-	-
Neg6 (NTC)	182	-	-	-	-	-

189

190 Healthy adult HB-G25 gut microbiome were predominantly from the phyla *Bacteroidota* (35.1%), *Proteobacteria* (21.5%), *Firmicutes_A*
191 (19.5%), and *Campylobacterota* (17.3%) (Figure 3, Table 3). Unhealthy juvenile HB-G26 largely comprised *Proteobacteria* (92.4%), with minor
192 contributions from *Campylobacterota* (2.4%), *Bacteroidota* (2.0%), and *Firmicutes* (2.0%). The predominant genera were *Serratia* (28.1%),
193 *Pseudomonas* (26.8%), *Providencia* (20.1%) (Figure 5). Unhealthy sub-adult (Jethro (143717)) harboured a distinct microbial community
194 dominated by *Firmicutes_A* (77.0%) and *Bacteroidota* (14.4%), with minimal *Proteobacteria* (1.9%) (Figure 3, Table 3). At the genus level for
195 Unhealthy sub-adult (Jethro (143717)), this community was formed primarily of obligate fermentative anaerobes, including *Clostridium*
196 (19.2%), *RUG472* (12.7%), *Bact-08* (8.4%), *Cellulosilyticum* (5.5%), and *Heteroclostridium* (3.4%) (Figure 5).

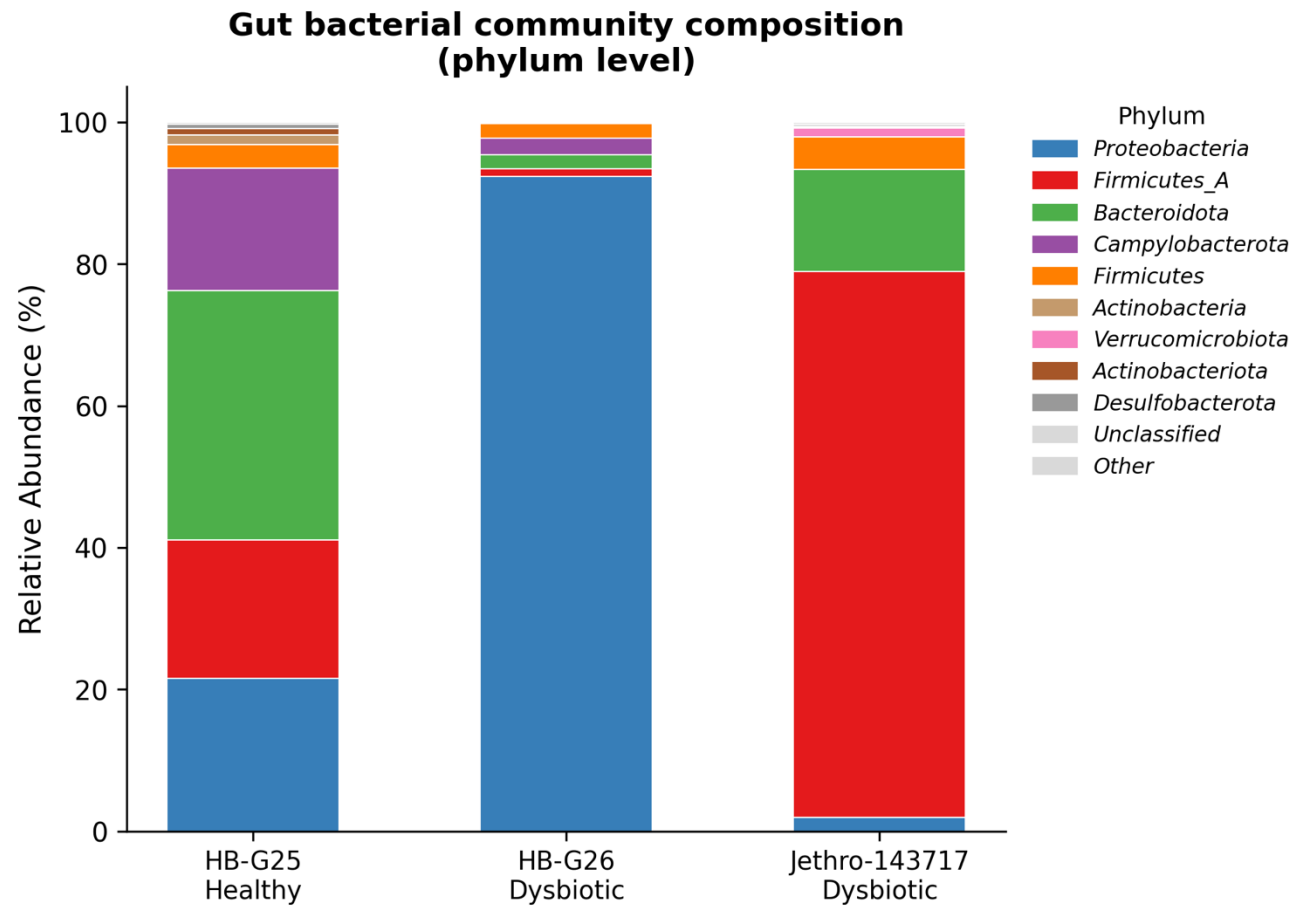


Figure 3. Phylum-level gastrointestinal bacterial community composition of three green sea turtles (*Chelonia mydas*), Healthy adult (HB-G25), Unhealthy juvenile (HB-G26) & Unhealthy sub adult (Jethro (143717)). Relative abundance (%) of the ten most abundant bacterial phyla

212 detected across all samples. Phyla contributing less than 0.1% across all samples are grouped as "Other". Table 3 applies a stricter $\geq 0.5\%$
213 threshold per sample, which is why it lists fewer phyla than shown here.

214 **Table 3.** Phylum-level relative abundance (%) of dominant bacterial taxa detected in the gastrointestinal microbiome of three green sea turtles
215 (*Chelonia mydas*) Healthy adult (HB-G25), Unhealthy juvenile (HB-G26), Unhealthy Sub adult (Jethro (143717)). Only phyla contributing $\geq 0.5\%$
216 relative abundance in at least one sample are shown.

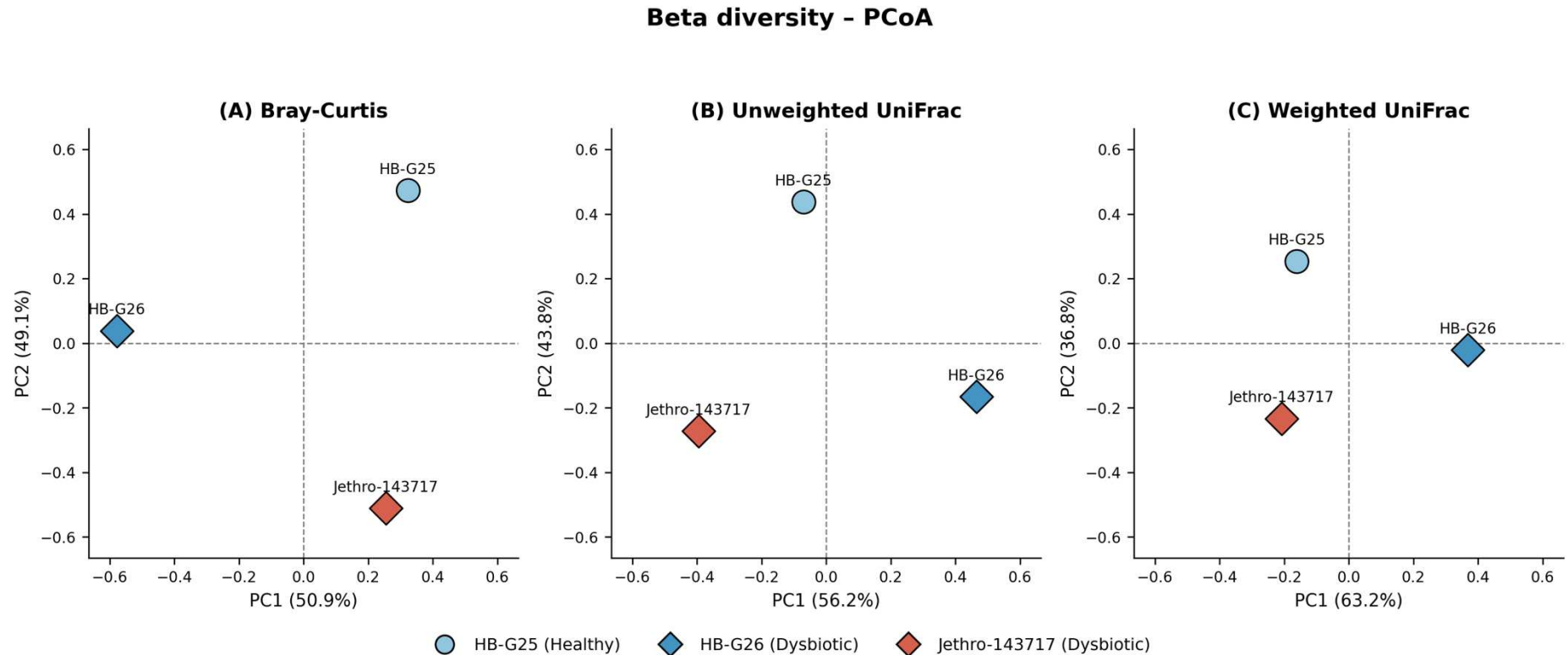
Phylum	Healthy Adult HB-G25	Unhealthy Juvenile HB-G26	Unhealthy Sub adult Jethro (143717)
<i>Firmicutes_A</i>	19.5%	1.0%	77.0%
<i>Firmicutes</i>	3.3%	2.0%	4.5%
<i>Proteobacteria</i>	21.5%	92.4%	1.9%
<i>Bacteroidota</i>	35.1%	2.0%	14.4%
<i>Campylobacterota</i>	17.3%	2.4%	<0.1%
<i>Verrucomicrobiota</i>	<0.1%	<0.1%	1.3%

Phylum	Healthy Adult HB-G25	Unhealthy Juvenile HB-G26	Unhealthy Sub adult Jethro (143717)
<i>Actinobacteriota</i>	2.3%	<0.1%	0.1%
<i>Desulfobacterota</i>	0.6%	<0.1%	<0.1%

217

218 Beta diversity analysis showed differences among all three individuals. The Bray-Curtis dissimilarities were 1.00 (healthy adult (HB-G25)
219 vs unhealthy juvenile (HB-G26)) indicating that the two samples are completely different and share no species in common, 1.00 (unhealthy
220 juvenile (HB-G26) vs unhealthy sub-adult (Jethro (143717))), and 0.99 (healthy adult (HB-G25) vs unhealthy sub-adult (Jethro (143717)))
221 signifying that there is some species in common between unhealthy sub-adult (Jethro (143717)) and the other two turtles (Figure 4).
222 Unweighted UniFrac (which ignores abundance and is highly sensitive to the presence of rare or low-abundance microbes) distances (0.81,
223 0.87, and 0.78; healthy adult (HB-G25) vs unhealthy juvenile (HB-G26); unhealthy juvenile (HB-G26) vs unhealthy sub-adult (Jethro (143717))),
224 and healthy adult (HB-G25) vs unhealthy sub-adult (Jethro (143717))), respectively), indicated high dissimilarity, with about 80% of the
225 evolutionary branches present across the samples unique to each individual. Weighted UniFrac (which accounts for both the
226 presence/absence of bacteria and their relative abundance) distances of (0.60, 0.61, and 0.49) (0.60, 0.61, and 0.49) (healthy adult (HB-G25) vs
227 unhealthy juvenile (HB-G26), unhealthy juvenile (HB-G26) vs unhealthy sub-adult (Jethro (143717))), and healthy adult (HB-G25) vs unhealthy

228 sub-adult (Jethro (143717))), respectively) indicated moderate similarity, and although the samples have very different lists of rare or minor
229 bacteria, their dominant, high-abundance bacteria are highly similar or share a close evolutionary history.
230



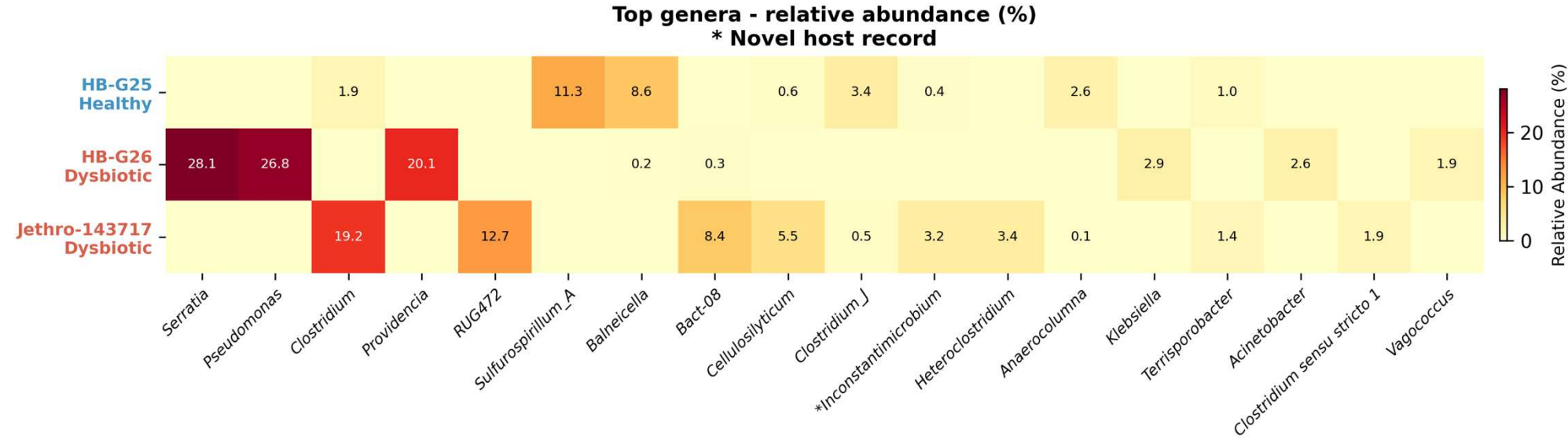
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232 **Figure 4.** Principal coordinates analysis (PCoA) of gastrointestinal bacterial beta diversity across three green sea turtles (*Chelonia mydas*).

233 Ordination plots are shown for (A) Bray-Curtis dissimilarity, (B) unweighted UniFrac distance, and (C) weighted UniFrac distance, all calculated

234 following rarefaction to 35,531 reads. The percentage of variance explained by each principal coordinate axis is indicated. Circle denotes
235 healthy adult (HB-G25), blue diamond denotes unhealthy juvenile (HB-G26); the red diamond denotes unhealthy sub-adult (Jethro (143717)).

236 The relative abundance heat map gives indication to which genera are most prevalent in each turtle (Figure 5). It indicates the
237 occurrence of *Sulfurospirillum_A* in healthy adult HB-G25, *Serratia*, *Pseudomonas* & *Providencia* in unhealthy juvenile HB-G26 and *Clostridium*
238 in unhealthy sub-adult (Jethro (143717)). A total of 25 ASVs assigned to *Inconstantimicrobium porci* (phylum *Firmicutes_A*, family
239 *Clostridiaceae*) were detected in the unhealthy sub-adult (Jethro (143717)), comprising 3.2% relative abundance (1,151 reads) (Figure 5). This
240 genus was absent in unhealthy juvenile HB-G26 and present at only trace levels in the healthy adult HB-G25 (0.4%; 135 reads, 25 ASVs).

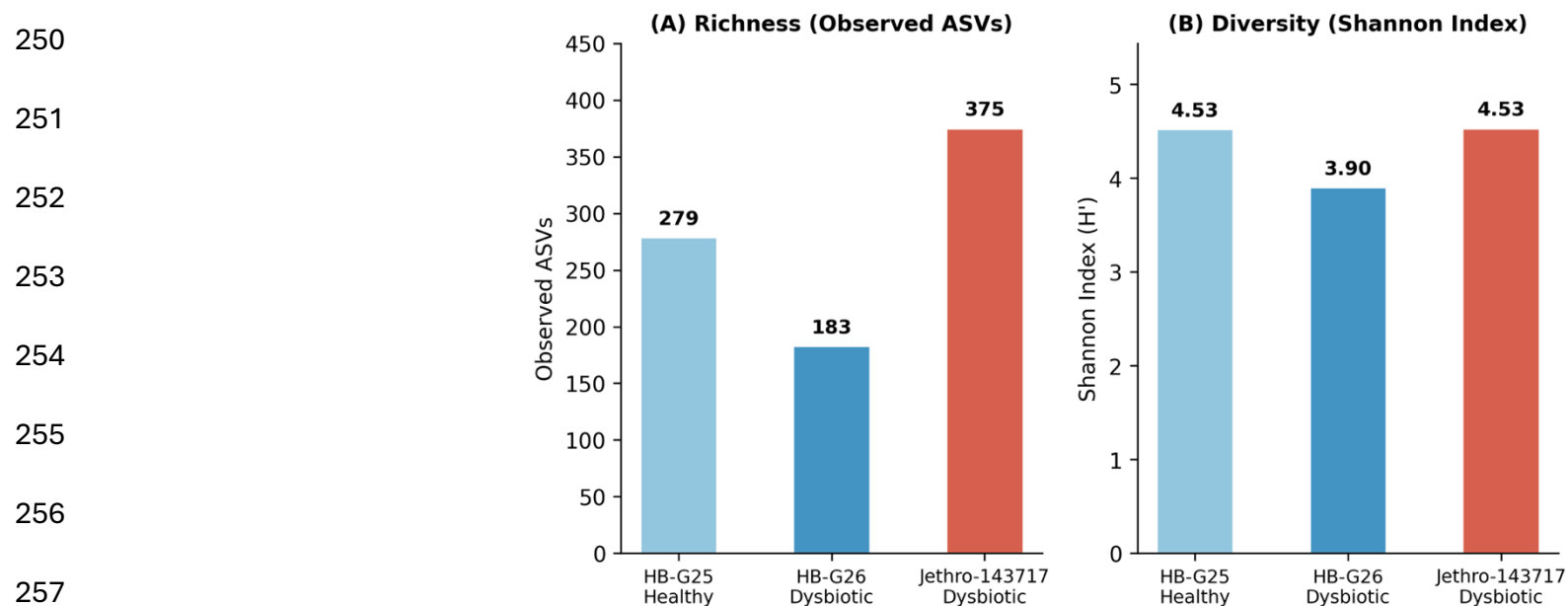


241 **Figure 5.** Relative abundance (%) of the 18 most abundant bacterial genera detected across the healthy, un-healthy and dysbiotic (*Chelonia*
242 *mydas*) gastrointestinal microbiomes. Cell values represent relative abundance (%) per sample; empty cells indicate absence or abundance

243 below 0.05%. Genera are ordered by total abundance across all samples. Asterisk (*) denotes a novel host record for *Inconstantimicrobium*
244 *porci*.

245 Based on alpha diversity metrics for gastrointestinal bacterial communities for the three individuals, unhealthy sub adult (Jethro
246 (143717)) had the highest number of observed ASVs (375) and the highest Shannon index ($H' = 4.53$) of the three turtles. Healthy adult HB-G25
247 had a moderate ASV richness (279 ASVs) and an equally high Shannon index ($H' = 4.53$). Unhealthy juvenile HB-G26 had the lowest ASV
248 richness (183) and the lowest Shannon value ($H' = 3.90$) (Figure 6).

249 **Alpha diversity metrics**



258 **Figure 6.** Alpha diversity metrics for gastrointestinal bacterial communities of three green sea turtles (*Chelonia mydas*). (A) Observed amplicon
259 sequence variants (ASVs) and (B) Shannon diversity index (H') per individual, calculated following rarefaction to 35,531 reads. Samples are
260 colour-coded by health status and age: light blue healthy adult (HB-G25), dark blue unhealthy juvenile (HB-G26), red unhealthy sub adult
261 (Jethro (143717)).

262

263 Discussion

264 The gastrointestinal microbiomes of the three green turtles examined in this study differed markedly in taxonomic composition and overall
265 community structure, with near-complete separation between individuals in community composition (beta diversity); alpha diversity (within-
266 sample richness and evenness) was less clearly differentiated, with the healthy adult and the unhealthy sub-adult showing similar Shannon
267 diversity despite their contrasting clinical presentations. This pronounced inter-individual variation is consistent with previous reports showing
268 substantially high microbiome heterogeneity in sea turtles [26] and supports the importance of characterising intestinal microbial communities
269 across a range of health states rather than relying on single individual or pooled comparisons [1]. Despite the limited sample size, the
270 microbiome profiles observed here broadly aligned with clinical presentation. The healthy adult, HB-G25, exhibited a community structure
271 similar with profiles previously reported for wild, clinically normal green turtles, characterised by dominance of *Bacteroidota* and *Firmicutes*
272 [1]. The unhealthy juvenile, HB-G26, showed a *Proteobacteria*-dominant microbiome, reduced ASV richness, and the lowest Shannon diversity
273 of the three individuals. This feature is consistent with dysbiosis, defined as a disruption of normal gastrointestinal microbial balance involving
274 reduced diversity and/or overrepresentation of potentially opportunistic taxa [27], and closely mirrors patterns reported in stranded or
275 clinically compromised green turtles in previous studies [1, 2, 22, 28, 29]. Together, these results support the emerging view that
276 *Proteobacteria* dominance may represent a useful microbiome-based indicator of compromised health in *Chelonia mydas*.

277

278 The microbiome of the unhealthy sub-adult, Jethro (143717) presented a contrasting form of dysbiosis. Although this individual
279 displayed severe intestinal stasis and did not exhibit a microbiome profile comparable to the healthy turtle, dysbiosis in this case was not
280 associated with reduced diversity. Unhealthy sub-adult (Jethro (143717)) showed the highest observed ASV richness and a Shannon diversity
281 equivalent to that of the healthy adult, but the microbial community was restructured around *Firmicutes_A*, particularly fermentative
282 Clostridia-class anaerobes. This suggests that the gastrointestinal dysfunction may not always appear as a simple collapse in diversity, but
283 rather as a substantial compositional shift toward taxa favoured under altered physiological conditions, such as prolonged intestinal transit
284 time and anaerobic fermentation. Comparable patterns have been observed in other host systems, where pathological states are associated
285 with compositional shifts, rather than diversity reduction [30]. HB-G25 being a clinically healthy individual, showed a composition consistent
286 with the *Bacteroidota/Firmicutes*-dominant profile reported in wild-captured healthy green turtles [1]. The distinction between healthy adult
287 HB-G25 and unhealthy sub-adult (Jethro (143717)) is therefore notable: while the unhealthy juvenile HB-G26's profile shows a depleted and
288 taxonomically restricted microbiome, the sub-adults profile appears to represent a fermentative reorganisation associated with severe stasis
289 [41-43]. These contrasting dysbiotic state the importance of interpreting microbiome disruption in relation to specific clinical syndromes rather
290 than assuming a single microbial signature of ill health.

291

292 A particularly important finding was the detection of *Inconstantimicrobium porci* in a sea turtle, representing, to our knowledge, the
293 first record of this genus in a reptilian host using full-length 16S rRNA sequencing. Originally described as a fermentative anaerobe from the
294 porcine gastrointestinal tract by Wylensek et al. [31], *I. porci* was present at elevated relative abundance in unhealthy sub-adult (Jethro
295 (143717)), the turtle with severe gastrointestinal stasis, and was detected only at trace levels in the healthy adult (HB-G25). This distribution
296 raises the possibility that *Inconstantimicrobium* may persist as a low-abundance commensal bacterium in clinically healthy green turtles, but

297 with the capacity to proliferate opportunistically under conditions that favour obligate anaerobic fermentation, such as periods of reduced gut
298 motility. Although its clinical significance is unable to be determined from the present study alone, its association with a turtle exhibiting
299 severe intestinal dysfunction makes it a taxon of interest. Whether this organism represents an environmentally acquired bacterium, a
300 transient coloniser, or a previously unrecognised member of the green turtle gut microbiota remains unresolved. Nonetheless, the detection
301 of *I. porci* stresses the value of species-level taxonomic resolution in wildlife microbiome studies, particularly where newly described, poorly
302 characterised, or potentially clinically relevant taxa may otherwise remain undetected.

303

304 One potential source of irregular environmental exposure is through flooding. Flooding is a natural process with thoroughly
305 documented ecological benefits, including the delivery of nutrients and minerals to downstream environments. However, when catchments are
306 heavily modified or polluted, flood magnitude increases and sediment discharge becomes more frequent and intense, overwhelming these
307 benefits and delivering nutrients and contaminants in excess [32]. Between 2021 and 2022, three consecutive La Niña events impacted
308 Queensland, producing widespread flooding across the Fraser Coast region and generating the largest surge recorded from the Mary River in
309 over a century [33]. The resulting sediment plumes were extensive, and the persistent turbidity caused the loss of some of the most
310 ecologically significant seagrass meadows on Australia's east coast [34-36]. Beyond the physical impacts of sediment deposition, flood-driven
311 resuspension of sediments is also associated with the mobilisation and transport of pathogenic organisms into coastal waters [37, 38]. Flood-
312 driven deterioration of coastal water quality is well documented to produce spikes in pathogens including *E. coli*, *Cryptosporidium* spp., and
313 *Enterococcus* spp., among others [39]. These organisms are associated with gastrointestinal illness and ear infections in humans, and
314 considerable research has examined exposure risks for swimmers at coastal beaches [39]. However, the implications of these pathogen spikes
315 for disease susceptibility in sea turtles remain comparatively poorly understood.

316

317 To our knowledge, this study is the first to apply PacBio HiFi full-length 16S rRNA sequencing to characterise the gastrointestinal
318 microbiome of *Chelonia mydas*. Compared with conventional short-read sequencing approaches, targeting only one or two hypervariable
319 regions, full-length amplicon sequencing provides substantially improved taxonomic resolution and enables assignment at or near species
320 level. This was central to the identification of *Inconstantimicrobium porci*, a recently described genus that may not have been resolved using
321 standard Illumina-based methods. In the context of wildlife health, such resolution has direct practical value, as distinguishing closely related
322 microbial taxa may be critical for identifying organisms associated with normal gastrointestinal function, opportunistic overgrowth, or disease
323 states.

324

325 Taken together, these findings highlight the potential utility of the gut microbiome as a complementary tool for health assessment in
326 green sea turtles. The three turtles in this study each showed distinct microbial profiles that broadly reflected their clinical condition: a
327 *Bacteroidota/Firmicutes*-dominant community in the healthy adult, a low-diversity *Proteobacteria*-dominant profile in the unhealthy juvenile,
328 and a *Firmicutes_A*-rich fermentative community in the sub-adult with intestinal stasis. Although these observations are necessarily
329 preliminary, they suggest that microbiome profiling may help distinguish different forms of gastrointestinal dysfunction, identify candidate
330 microbial indicators of compromised health, and improve interpretation of disease processes in stranded or rehabilitating turtles. More
331 broadly, integrating microbiome data into clinical and conservation frameworks might provide a valuable means for developing evidence-
332 based diagnostics and treatment strategies for sea turtles in South-East Queensland.

333 ***Limitations***

334 The small sample size ($n = 3$) restricts the robustness of scientific analysis and limits the generalisability of the findings; consequently, results
335 should be regarded as descriptive and hypothesis-generating rather than definitive. Furthermore, health status and life stage were fully

336 confounded in this sample: the healthy individual (HB-G25) was an adult, whereas both unhealthy individuals (HB-G26, Jethro (143717)) were a
337 juvenile and sub-adult respectively, so observed microbiome differences cannot be unambiguously attributed to health status alone. This is a
338 particularly important caveat in green turtles, which undergo a well-documented ontogenetic diet shift from an omnivorous diet as oceanic-
339 stage juveniles to a predominantly herbivorous diet as they recruit to neritic foraging grounds as sub-adults and adults [40], a shift that could
340 independently shape gut microbiome composition irrespective of health status. As this is full-length and has better taxonomic resolution, it is
341 difficult to compare to papers that have only looked at V3-V4 regions. Although full-length 16S rRNA sequencing offers enhanced taxonomic
342 resolution compared to short-read methods, it does not provide insight into functional capacity. As a result, the metabolic roles of key taxa
343 such as *Inconstantimicrobium porci* and the uncharacterised *RUG472* lineage are inferred from genomic data of related organisms rather than
344 directly demonstrated. Reliance on cloacal swab sampling rather than segmental gastrointestinal sampling throughout the gastrointestinal
345 tract further limits the ability to assess regional microbiome variation, which is known to be substantial in sea turtles. Lastly, the clinical
346 significance of *Inconstantimicrobium porci* detection cannot be determined solely from this study, as its presence may indicate opportunistic
347 colonisation, a commensal relationship, or an active contribution to pathology. Finally, this pilot study did not incorporate a positive (mock
348 community) sequencing control. Two negative (no-template) controls were processed and sequenced by AGRF alongside this sequencing batch.
349 Sequences recovered from each control were highly diverse, with no single sequence dominating either control, a pattern reflective with low-
350 level trace or reagent-derived DNA rather than carryover of one contaminant organism. A small minority of reads in each control (6/149 and
351 6/182) were nearly identical to abundant ASVs detected in the biological samples, indicating a low level of cross-contamination or index-
352 hopping typical of multiplexed amplicon sequencing runs; most reads did not match any ASV detected in the biological samples. Sequence data
353 from both controls are included in the repository submission alongside the biological sample data. Cloacal/gut content is also a comparatively
354 high-biomass sample type, for which such low-level contamination is unlikely to meaningfully affect the recovered community signal.
355 Nonetheless, without a mock-community positive control, we cannot formally verify expected-community recovery accuracy for this specific

356 run, and we recommend that future studies in this system include a positive control processed and sequenced alongside biological samples.
357 Samples collected as part of this broader study will incorporate positive controls.
358

359 ***Future direction***

360 Future studies should use culture and isolate *Inconstantimicrobium porci* from reptile hosts to allow direct characterisation of its fermentative
361 capacity and antibiotic susceptibility profile, which has clinical relevance if this organism proves to be a consistent feature of dysbiotic green
362 turtle intestinal communities. Due to the challenges in sampling in remote environments and difficulty in the laborious process of capturing
363 sea turtles, culturing would allow a biobank of isolates for future studies and analysis. Broader surveillance across species, populations, and
364 geographic regions will be essential to further characterise microbiome differences between healthy, compromised, and dysbiotic individuals
365 across the full range of sea turtles found in south-east Queensland. This should be paired with environmental sampling, including seawater,
366 sediment and food from foraging sites to distinguish host-associated from environmentally acquired taxa.

367 ***Conclusions***

368 This study provides preliminary evidence that the gut microbiome of *Chelonia mydas* varies markedly with health status and clinical
369 presentation, illustrating its potential relevance to both sea turtle medicine and conservation. Using PacBio HiFi full-length 16S rRNA
370 sequencing, we identified distinct microbial community structures in a healthy adult, an unhealthy juvenile, and an unhealthy sub-adult with
371 severe intestinal stasis, demonstrating that dysbiosis may manifest not only as reduced diversity, but also as substantial compositional
372 restructuring. The healthy turtle (HB-G25) exhibited a microbiome broadly consistent with profiles reported for clinically normal green turtles,
373 whereas the compromised individuals showed divergent microbial signatures associated with poor health, including *Proteobacteria* dominance

374 in the juvenile and a fermentative, *Firmicutes_A*-rich community in the sub-adult. Notably, the detection of *Inconstantimicrobium porci* in a sea
375 turtle represents the first record of this genus in a reptilian host and underlines the value of full-length sequencing for revealing previously
376 unrecognised taxa of possible clinical importance. Although limited by sample size, these findings demonstrate the promise of microbiome
377 profiling as a tool for improving understanding of gastrointestinal dysfunction, refining clinical interpretation, and supporting the development
378 of microbiome-informed health assessment in green turtles from South-East Queensland.

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394 **Declarations**

395 ***Ethics approval and consent to participate***

396 Animal ethics and welfare review was completed internally by the University of the Sunshine Coast Animal Ethics Committee (ANS24113). For
397 the wild-caught individuals (HB-G25 and HB-G26), a scientific purposes permit was granted by the Department of Environment under section
398 211 of the Nature Conservation (Animals) Regulation 2020 specifically for this research: WA0060686. For unhealthy sub-adult (Jethro
399 (143717)), this research was conducted under the AZWH rehabilitation & research permit: WA0055632

400 ***Consent for publication***

401 Not applicable.

402 ***Availability of data and materials***

403 The 16S rRNA amplicon sequencing data generated and analysed during this study, including reads from the no-template negative control, are
404 available in the NCBI Sequence Read Archive (SRA) under BioProject accession PRJNA1501626. SRA accessions are as follows: SRR39821233
405 (<https://www.ncbi.nlm.nih.gov/sra/?term=SRR39821233>), SRR39821234 (<https://www.ncbi.nlm.nih.gov/sra/?term=SRR39821234>),
406 SRR39821235 (<https://www.ncbi.nlm.nih.gov/sra/?term=SRR39821235>), SRR39821236
407 (<https://www.ncbi.nlm.nih.gov/sra/?term=SRR39821236>), SRR39821237 (<https://www.ncbi.nlm.nih.gov/sra/?term=SRR39821237>). The R
408 scripts used for statistical analysis are deposited in a public repository figshare (<https://doi.org/10.6084/m9.figshare.33433663>) No
409 metabolomics data were generated or analysed in this study.

410

411 ***Competing interests***

412 The authors declare no known conflict of interests.

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416 ***Authors' contributions***

417 J. Bowtell: Conceptualisation, sample collection, laboratory & analysis, downstream diversity analysis, data interpretation, writing- original
418 draft.

419 N. Blais: Sample collection, laboratory assistance, review & editing

420 A. Hammond: Sample collection, review & editing

421 Z. Egerton: Sample collection, review & editing

422 E. Kay: Supervision, review & editing

423 C. Madden: Review & editing

424 M. Jelocnik: Supervision, assistance with laboratory supplies & extraction. Review & Editing

425 H. Duong: Assistance with laboratory supplies & extraction. Review & Editing

426 K.A. Townsend: Conceptualisation, sample collection, data interpretation, writing, review & editing, supervision

427 *All authors read and approved the final manuscript.*

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