Comparison of gut microbiome in the Chinese mud snail (*Cipangopaludina chinensis*) and the invasive golden apple snail (*Pomacea canaliculata*)

Zihao Zhou [1,2,3] , Hongying Wu [3] , Dinghong Li [3] , Wenlong Zeng [3] , Jinlong Huang [Corresp., 1, 2, 3, 4] , Zhengjun Wu [Corresp. 1, 2, 3]

1 Ministry of Education, Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Gui Lin, GuangXi, China

2 Guangxi Normal University, Guangxi Key Laboratory of Rare and Endangered Animal Ecology, GuiLin, GuangXi, China

3 Guangxi Normal University, College of Life Sciences, GuiLin, GuangXi, China

4 Guangxi Normal University, Guangxi Key Laboratory of Landscape Resources Conservation and Sustainable Utilization in Lijiang River BasinInstitute for Sustainable Development and Innovation, GuiLin, GuangXi, China

Corresponding Authors: Jinlong Huang, Zhengjun Wu
Email address: jl_huang@163.com, wu_zhengjun@aliyun.com

Background. Gut microbiota play a critical role in nutrition absorption and environmental adaptation and can affect the biological characteristics of host animals. The invasive golden apple snail (*Pomacea canaliculata*) and native Chinese mud snail (*Cipangopaludina chinensis*) are two sympatric freshwater snails with similar ecological niche in southern China. However, gut microbiota comparison of interspecies remains unclear. Comparing the difference of gut microbiota between the invasive snail *P. canaliculata* and native snail *C. chinensis* could provide new insight into the invasion mechanism of *P.canaliculata* at the microbial level.

Methods. Gut samples from 20 golden apple snails and 20 Chinese mud snails from wild freshwater habitats were collected and isolated. The 16S rRNA gene V3-V4 region of the gut microbiota was analyzed using high throughput Illumina sequencing.

Results. The gut microbiota dominantly composed of Proteobacteria, Bacteroidetes, Firmicutes and Epsilonbacteraeota at phylum level in golden apple snail. Only Proteobacteria was the dominant phylum in Chinese mud snail. Alpha diversity analysis (Shannon and Simpson indices) showed there were no significant differences in gut microbial diversity, but relative abundances of the two groups differed significantly ($P < 0.05$). Beta diversity analysis (Bray Curtis and weighted UniFrac distance) showed marked differences in the gut microbiota structure ($P < 0.05$). Unique or high abundance microbial taxa were more abundant in the invasive snail compared to the native form. Functional prediction analysis indicated that the relative abundances of functions differed significantly regarding cofactor prosthetic group electron carrier and vitamin biosynthesis, amino acid biosynthesis, and nucleoside and nucleotide biosynthesis ($P < 0.05$). These results suggest an enhanced potential to adapt to new habitats in the invasive snail.

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5 Zi-Hao Zhou[1, 2, 4], Hong-Yin Wu[4], Ding-Hong Li[4], Wen-Long Zeng[4], Jin-Long Huang[1, 2, 3, 4 *],

6 Zheng-Jun Wu[1, 2, 4, *]

7 [1] Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection

8 (Guangxi Normal University), Ministry of Education, Guilin, Guangxi, China

9 [2] Guangxi Key Laboratory of Rare and Endangered Animal Ecology, Guangxi Normal

10 University, Guilin, Guangxi, China

11 [3] Guangxi Key Laboratory of Landscape Resources Conservation and Sustainable Utilization in

12 Lijiang River Basin Institute for Sustainable Development and Innovation, Guangxi Normal

13 University, Guilin, Guangxi, China

14 [4] College of Life Sciences, Guangxi Normal University, Guilin, Guangxi, China

16 *Corresponding author:

17 Jin-Long Huang (jl_huang@163.com), Zheng-Jun Wu (wu_zhengjun@aliyun.com)

18 Address: College of Life Sciences, Guangxi Normal University, Guilin, 541006, China.

20 ## Abstract

21 **Background.** Gut microbiota play a critical role in nutrition absorption and environmental

22 adaptation and can affect the biological characteristics of host animals. The invasive golden

23 apple snail (*Pomacea canaliculata*) and native Chinese mud snail (*Cipangopaludina chinensis*)

24 are two sympatric freshwater snails with similar ecological niche in southern China. However,

25 gut microbiota comparison of interspecies remains unclear. Comparing the difference of gut

26 microbiota between the invasive snail *P. canaliculata* and native snail *C. chinensis* could provide

27 new insight into the invasion mechanism of *P.canaliculata* at the microbial level.

28 **Methods.** Gut samples from 20 golden apple snails and 20 Chinese mud snails from wild

29 freshwater habitats were collected and isolated. The 16S rRNA gene V3-V4 region of the gut

30 microbiota was analyzed using high throughput Illumina sequencing.

31 **Results.** The gut microbiota dominantly composed of Proteobacteria, Bacteroidetes, Firmicutes

32 and Epsilonbacteraeota at phylum level in golden apple snail. Only Proteobacteria was the

33 dominant phylum in Chinese mud snail. Alpha diversity analysis (Shannon and Simpson indices)

34 showed there were no significant differences in gut microbial diversity, but relative abundances
35 of the two groups differed significantly ($P < 0.05$). Beta diversity analysis (Bray Curtis and
36 weighted UniFrac distance) showed marked differences in the gut microbiota structure ($P < 0.05$).
37 Unique or high abundance microbial taxa were more abundant in the invasive snail compared to
38 the native form. Functional prediction analysis indicated that the relative abundances of
39 functions differed significantly regarding cofactor prosthetic group electron carrier and vitamin
40 biosynthesis, amino acid biosynthesis, and nucleoside and nucleotide biosynthesis ($P < 0.05$).
41 These results suggest an enhanced potential to adapt to new habitats in the invasive snail.

42 **Keywords:** *Pomacea canaliculata*, *Cipangopaludina chinensis*, Invasion species, Gut
43 microbiota, 16S rRNA gene, High-throughput sequencing, Snail

Introduction

45 In recent years, gut microbiota research has made considerable progress (Gallo et al., 2020b),
46 highlighting the crucial involvement of gut microbes in many mammalian biological processes,
47 such as nutrition absorption, behavior, and intestinal development (Chen et al., 2021b; Li et al.,
48 2019; Lize et al., 2013). Continuous interactions between the gut microbiota and host have
49 evolved into a dynamic microbial ecological complex (Brestoff and Artis, 2013). Unlike in
50 terrestrial vertebrates, gut microbiota in aquatic animals are more variable (Ringo et al., 2016)
51 and sensitive to changing conditions (Zhang et al., 2020).

52 The association between hosts and gut microbiota can contribute to our understanding of
53 longevity, metabolism, development, and physiology of invertebrates (Lee and Hase, 2014). In
54 shrimp, gut microbiota have been suggested to affect visible nutrient acquisition and disease
55 incidence, which is a major contributor to population restriction (Xiong, 2018). Similarly, gut
56 microbiota in other aquatic invertebrates, such as *Daphnia*, play an important role in
57 improvement of high temperature resistance and antiviral ability (Akbar et al., 2021).
58 Cellulolytic bacteria in the giant African snail (*Achatina fulica*) gut play an important role in
59 cellulose decomposition (Pinheiro et al., 2015). The gut microbiota is important for organisms to
60 adapt to new environment as well. Previous studies have shown that gut microbiota in invasive
61 species have greater potential to invade the new habitats (Gallo et al., 2020a; Qu et al., 2020).
62 For instance, the snail *Potamopyrgus antipodarum* have greater core microbial taxa as an
63 invasive species (Bankers et al., 2021). Therefore, comparing the diversity and structure of the
64 gut microbiota between sympatric invasive and native freshwater species may help clarify the
65 underlying mechanisms related to invasion.

66 Various studies have shown that the gut microbiota can effectively resist internal pathogen
67 invasion and can promote successful biological invasion into new environments (Becker et al.,
68 2021; Habineza et al., 2019; Zhang et al., 2018). The golden apple snail (*Pomacea canaliculata*)

69 is listed among the 100 worst invasive alien species worldwide by the International Union for
70 Conservative of Nature and the Invasive Species Specialist Group (Chen et al., 2011a). This snail
71 causes serious damage to aquatic crops, as well as to wetland floral diversity and ecosystem
72 functioning (Carlsson et al., 2004; Lowe et al., 2000). The Chinese mud snail (*Cipangopaludina*
73 *chinensis*) is a native species and popular aquatic food source in China. Chinese mud snail feeds
74 mainly on plant debris, whereas the golden apple snail has a voracious appetite for vegetation,
75 including rice (*Oryza sativa*) crops (She et al., 2013). Both snail species occupy similar
76 freshwater habitats, but with different feeding habits in the wild. Whether the differences in
77 feeding habits are closely related to gut microbiota remains unclear. Gut microbiota are closely
78 related to host growth performance (Fan et al., 2019). It has been shown that the gut microbiota
79 of golden apple snails can be impacted by developmental stage, gender, and gut part (Li et al.,
80 2019; Lyra et al., 2018). However, few studies have explored the differences and abundance of
81 gut microbiota in the two sympatric invasive and native freshwater snails. Additionally, we
82 speculated that the gut microbiota may be important for the invasion and adaptation of invasive
83 snails such as the apple snail *P. canaliculata* to new environments. Therefore, to better
84 understand the gut microbiota community in the two freshwater snails, and investigate the
85 invasive mechanism of golden apple snail at microbial level, we compared the differences in gut
86 microbiota between the invasive golden apple snail and native Chinese mud snail using 16S
87 rRNA sequencing on the Illumina MiSeq platform. This study is of importance for the
88 understanding of invasion mechanism of *P. canaliculata*.

Materials and Methods

Sample collection and treatment

91 A total of 20 Chinese mud snails and 20 golden apple snails were collected from the Xiangsi
92 River (25.0459 °N, 110.1128 °E) in Huixian village, Guilin city, Guangxi, China in June 2021.
93 This research was approved by the ethics committee of Guangxi Normal University (IACUC-
94 202111-003). All snails were collected from shallow water, and sampled by fishing net. The
95 average shell heights of *P. canaliculata* and *C. chinensis* were 3.8 ± 0.3 cm and 5.3 ± 0.3 cm,
96 respectively. The *P. canaliculata* (PC group) and *C. chinensis* (CC group) snails were
97 maintained in 1 L of ultra-pure water in a 25 °C room for 5–7 days. All snails were fed with
98 Chinese cabbage (*Brassica rapa*). Prior to dissection, the snails were starved for 24 h to
99 minimize the amount of partially digested food in the gut.

100 The shells of snails were cleaned with 70% ethanol two times and ultra-pure water three
101 times. Shell destruction, dissection, and gut extraction were performed on a clean bench. Due to
102 the small size of snail guts, five snail's guts were pooled as a single sample, to make sure the
103 sample adequacy and experiment accuracy was qualified. All samples were repeated four times.

104   A total of eight samples were named with PC-1, PC-2, PC-3, PC-4 and CC-1, CC-2, CC-3, CC-4,
105   respectively. Samples were stored in 5-ml aseptic centrifuge tubes at −80 °C before DNA
106   extraction.

107   **16S rRNA gene sequencing**

108   The gut microbial DNA was extracted using a Fast DNA SPIN Extraction Kit (MP Biomedicals,
109   USA) following the manufacturer's instructions. The isolated DNA was stored at −80 °C until
110   polymerase chain reaction (PCR). The DNA concentration and molecular size were measured
111   using UV spectrophotometry (Eppendorf, BioPhotometer, Germany) and 0.8% agarose gel
112   electrophoresis, respectively. The hypervariable V3-V4 region of the 16S rRNA gene was
113   amplified by PCR using universal bacterial primers (338F: 5'-ACTCCTACGGGAGGGAGCA-
114   3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). Each primer included barcode sequences to
115   promote the sequencing of products. The DNA (20 ng) of each sample was amplified in 25-μl
116   reactions consisting of 0.25 μl of Q5 high-fidelity DNA polymerase (NEB, Ipswich, UK), 5 μl of
117   5-fold reaction buffer, 5 μl of 5-fold high GC buffer, 0.5 μl of dNTP Mix (10 mM), 2 μl of
118   template DNA, 1 μl of each primer (10 mM), and 10.25 μl of ddH$_2$O. The PCR conditions were
119   as follows: an initial denaturation at 98 °C for 5 min, 25 cycles of 98 °C denaturation for 10 s, 50
120   °C annealing for 30 s, 72 °C extension for 30 s, and a final extension at 72 °C for 5 min. The
121   PCR products were detected by 2% agarose gel electrophoresis and purified with an AxyPrep
122   DNA Gel Extraction Kit (Axygen, New York, USA). Quantification of PCR products was
123   performed using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA). A TruSeq Nano
124   DNA LT Library Prep Kit (Illumina, USA) was used to establish the DNA library. The library
125   was sequenced using a MiSeq Reagent Kit v3 (6 000-cycles-PE) (Illumina, USA) on the MiSeq
126   platform by Personal Biotechnology Co., Ltd. (Shanghai, China).

127   **Data and analysis**

128   The DADA2 method was used for quality control (Callahan et al., 2016). QIIME2 (version:
129   2019.4) software was used to remove primer sequences, sequences shorter than 150 bp, and
130   chimera sequences. The obtained high-quality sequences were clustered into operational
131   taxonomic units (OTUs) at 97% identity using VSEARCH software (v2.13.4) (Torbjørn et al.,
132   2016). The OTUs were annotated using the 16S rRNA database tool (SILVA v132) in QIIME2
133   (version: 2019.4). The lowest sequence number of OTU abundance was standardized for further
134   study. Raw sequencing reads were submitted to the National Center for Biotechnology
135   Information (NCBI) BioProject database (PRJNA756881).

136   **Alpha and beta diversity estimation**

137   The alpha and beta diversity indices represent the diversity of species within and between
138   biotopes, respectively (Whittaker, 1972). QIIME2 (version: 2019.4) was used to calculate the
139   alpha indexes including community richness (Chao1 and Observed species), diversity (Shannon

140 and Simpson), evolutionary population diversity (Faith's PD), evenness (Pielou's evenness). The

141 calculation methods can be obtained at http://scikit-

142 bio.org/docs/latest/generated/skbio.diversity.alpha.html#module-skbio.diversity.alpha. We used

143 R software to sketch box plots of estimators with an intuitive form. Significance between groups

144 was tested using the Wilcoxon rank-sum and Dunn tests.

145   To estimate beta diversity and similarity, differences in relative abundance of OTUs between

146 two groups were detected using the nonmetric multidimensional scaling (NMDS). Analysis of

147 similarities (ANOSIM) based on Bray-Curtis distance and weighted UniFrac distance with 999

148 permutations was used to determine similarities between two groups. Differences in bacterial

149 abundances (from phylum to family) between the two species were tested using linear

150 discriminant analysis effect size (LEfSe).

151 **Functional prediction and statistical analysis of gut microbiota**

152 Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2

153 (PICRUSt2) software (https://github.com/picrust/picrust2/wiki) was used to predict community

154 functions of the gut microbiota (Douglas et al., 2020). Greengenes ID corresponding to each

155 OTU was searched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and was

156 classified to the relevant KEGG pathway. Numbers of functional genes in each pathway were

157 calculated to compare functional enrichment in gut microbiota between the two groups.

158 Statistical analyses were conducted using independent-samples t-test in SPSS (v19.0) at a

159 significance level of $P = 0.05$.

160

Results

162 **Gut microbial diversity and composition in *P. canaliculata* and *C. chinensis***

163 A total of 737 690 high-quality sequences were obtained from all eight snail DNA samples (PC

164 1-4, and CC 1-4) after quality control and filtration, including 391 551 reads from the PC group

165 and 346 139 reads from the CC group (ranging from 46 498–187 205 reads per sample). We re-

166 sampled all samples based on the smallest number of reads (46 498) to correct for differences in

167 read number. The rarefaction curve of species based on the Shannon index reached asymptote,

168 indicating that sequencing depth basically covered all species in the samples (Figure S1).

169   Filtered sequences were clustered into OTUs at a 97% identity, and we obtained 5 911 valid

170 OTUs after removing those with relative abundances < 0.001%, including 4 927 of *P.*

171 *canaliculata* and 1 080 of *C. chinensis*. Comparing these OTUs with the SILVA database, 6 007

172 OTUs were annotated to 29 phyla, 52 classes, 123 orders, 247 families, 575 genera, and 963

173 species. For the PC group, the 4 927 OTUs were annotated to 27 phyla, 49 classes, 111 orders,

174 239 families, 553 genera, and 894 species. For the CC group, the 1 080 OTUs were annotated to

175   12 phyla, 18 classes, 44 orders, 67 families, 115 genera, and 150 species (Figure S2). In addition,
176   the Venn diagram indicated that 96 OTUs existed in both groups. Compared to the CC group
177   (984), however, the number of specific OTUs was much higher in the PC group (4 831) (Figure
178   S3).
179   Based on alpha diversity analysis, the Shannon and Simpson indices indicated that gut
180   microbiota diversity did not differ significantly between the two groups. However, the Chao1
181   and Observed species indices indicated that gut microbiota abundance was significantly higher in
182   the PC group than in the CC group ($P < 0.05$) (Figure 1).
183   Differences in beta diversity were evaluated using NMDS analysis based on Bray-Curtis and
184   weighted UniFrac distances. Results showed that the PC and CC groups could be isolated from
185   each other, and intergroup distance was higher than intragroup distance based on both calculation
186   methods (Figure 2A, B). Moreover, ANOSIM revealed significant differences between the PC
187   and CC groups (Bray-Curtis R = 0.396, $P = 0.027$, NMDS stress = 0.0000982; weighted UniFrac
188   R = 1, $P = 0.03$, NMDS stress = 0.0000776), as shown in Figure 2C, D. Similar results were
189   observed from principal coordinate analysis (PCoA) based on Bray-Curtis and weighted UniFrac
190   distances (Figure S4).

191   **Taxonomic composition of gut microbiome in *P. canaliculata* and *C. chinensis***
192   From all eight samples, the top 10 gut microbiota phyla were shown in Figure 3 and Table S1.
193   Comparing bacterial composition between the PC and CC groups, microbes with a relative
194   abundance >2% were defined as dominant phyla. Results showed that Proteobacteria was the
195   dominant phylum in both groups, accounting for 53.78% ± 15.59% in the PC group and 98.94%
196   ± 0.20% in the CC group. In the PC group, three other phyla were found with a relative
197   abundance > 2%, including Bacteroidetes (23.19% ± 11.38%), Firmicutes (16.00% ± 6.91%),
198   and Epsilonbacteraeota (4.28% ± 2.53%). For the CC group, Proteobacteria was the only phylum
199   with a relative abundance > 2%. Of the top 10 phyla, only seven existed in the CC group, with
200   Spirochaetes, Tenericutes, and Fibrobacteres not detected.
201   At the genus level, the top 10 genera were shown in Figure 4 and Table S2. In the PC group,
202   the dominant genus was *Chryseobacterium* (14.62% ± 13.24%), followed by *Lactococcus* (9.78%
203   ± 8.18%), *Uliginosibacterium* (9.73% ± 6.16%), *Aquitalea* (6.13% ± 3.13%), *Novispirillum* (4.11%
204   ± 2.39%), *Sulfurospirillum* (3.50% ± 2.24%), *Dechloromonas* (2.46% ± 2.03%), and *Bacteroides*
205   (2.17% ± 1.95%) (Figure 4 and Table S2). In the CC group, the dominant genera were
206   *Enterobacter* (6.73% ± 2.31%) and *Hafnia-Obesumbacterium* (2.49% ± 2.18%). Only four of the
207   top 10 genera were found in the CC group, with *Chryseobacterium*, *Uliginosibacterium*,
208   *Aquitalea*, *Novispirillum*, *Sulfurospirillum* and *Dechloromonas* not detected. In addition, based
209   on LEfSe analysis (LDA > 2), 10 phyla and 87 genera were identified showing significant
210   differences (Figure S5).

Functional prediction of gut microbiota

Gut microbiota functions were predicted using PICRUSt2. From the KEGG database, a total of 7186 genes were classified into seven level-1 pathways and 60 level-2 pathways. Of these 60 pathways, 17 were involved in generation of precursor metabolite energy, 15 were involved in degradation/utilization/assimilation, 12 were involved in biosynthesis, 10 were involved in metabolic clusters, two were involved in macromolecule modification, two were involved in glycan pathways, and two were involved in detoxification (Figure 5). From the level-2 results, cofactor, prosthetic group, electron carrier, and vitamin biosynthesis (16.28% ± 0.38%), amino acid biosynthesis (13.42% ± 0.11%), fatty acid and lipid biosynthesis (9.43% ± 0.60%), and nucleoside and nucleotide biosynthesis (11.53% ± 0.16%) were the most abundant functions (Figure 5). Among the top abundant functions, cofactor prosthetic group electron carrier and vitamin biosynthesis (t = -2.861, P = 0.029), amino acid biosynthesis (t = 17.057, P < 0.01), nucleoside and nucleotide biosynthesis (t = 10.457, P < 0.01), fatty acid and lipid biosynthesis (t = 2.887, P = 0.028), carboxylate degradation (t = -13.277, P < 0.01), secondary metabolite degradation (t = -10.064,P < 0.01), carbohydrate degradation (t = -12.963, P < 0.01), nucleoside and nucleotide degradation (t = 2.533, P = 0.044), aromatic compound degradation (t = -3.500, P = 0.013), glycolysis (t = 3.540, P = 0.012), amino acid degradation (t = -3.725, P = 0.010), C1 compound utilization and assimilation (t = 4.288, P = 0.005), and aromatic compound biosynthesis (t = 22.984, P < 0.01) were significantly different between the PC and CC group (Figure S6).

Discussion

The composition and diversity of gut microbiota can be affected by a variety of factors, such as the environment, food sources and species (Bibbo et al., 2016; Jin et al., 2017). Previous study on the gut microbiota of freshwater *Radix auricularia* and *Planorbella trivolvis* snails found that Proteobacteria is dominant in both, but microbial diversity differs significantly, which may be due to disparate sampling sites (Hu et al., 2018). In contrast, a six-month study found no significant differences in alpha diversity between invasive red-eared slider turtles (*Trachemys scripta elegans*) and native Chinese three-keeled pond turtles (*Chinemys reevesii*) (Qu et al., 2020). In addition, environment plays an important role to the gut microbiota of the same invasive species as well. It was reported that there were significant differences in the gut microbial community structure of the invasive fish *Oreochromis mossambicus* from the different habitats (Gaikwad et al., 2017). A recent study also showed that the core microbial taxa of invasive freshwater snail *P. antipodarum* in invaded habitat were larger than that in origin habitat (Bankers et al., 2021). These studies suggest that the growth environment is a vital factor for gut microbial diversity. In the current study, we found no significant differences in gut

246 microbiota diversity between the two freshwater snails, which were likely due to their similar
247 habitats. However, the Chao1 and Observed species indices showed marked differences in gut
248 microbiota abundance between the two groups, with significantly higher abundance in the PC
249 group than in the CC group. Previous research has found that dietary changes are not
250 significantly correlated with alpha diversity but are positively correlated with beta diversity (Li
251 et al., 2016). The beta diversity in this study was significantly different between golden apple
252 snails and Chinese mud snails. Golden apple snails have a more extensive diet than Chinese mud
253 snails (Morrison and Hay, 2011), which may be a dominant factor related to the significant
254 differences in beta diversity between the two species. Furthermore, the gut microbiota structure
255 was more complex in the PC group than in the CC group. It was suggested that more complex
256 microbial structure may be able to support the golden apple snail to adapt to the new habitats
257 more rapidly and flexibly.

258   In this study, the top three phyla in the two groups were Proteobacteria, Bacteroidetes, and
259 Firmicutes, followed by Epsilonbacteraeota, Spirochaetes, and Acidobacteria. Comparing our
260 results with other studies, we found that dominant microbiota were quite similar at the phylum
261 level (Chen et al., 2021a; Li et al., 2019). We compared results from two relevant studies and
262 found five phyla (Proteobacteria, Bacteroidetes, Tenericutes, Actinobacteria, and Firmicutes) in
263 common (Chen et al., 2021a; Li et al., 2019). However, Epsilonbacteraeota and Acidobacteria
264 were only found in our study (Chen et al., 2021a; Li et al., 2019). Epsilonbacteraeota is widely
265 known for clinical relevance and chemolithotrophy and is usually found in sulfide-rich sediments
266 (Waite et al., 2017). Acidobacteria is one of the most abundant soil bacteria with a unique
267 ecological function and is found in a wide variety of environments, including under extreme and
268 polluted conditions (Liu et al., 2017; Navarrete et al., 2015). Epsilonbacteraeota and
269 Acidobacteria were only found in our study, which may be due to environmental differences or
270 differences in long-term food sources of the golden apple snails. Further relevant environmental
271 experiments are needed to screen for factors that may contribute to this phenomenon. Moreover,
272 based on other studies on freshwater snails (e.g., *Radix auricularia*, *Planorbella trivolvis*, and
273 *Bulinus africanus*), the compositions of dominant gut microbiota are very similar at the phylum
274 level (Hu et al., 2018; Hu et al., 2021; Van Horn et al., 2012). Although intestinal microbiota
275 diversity is found in disparate snails, there appear to be core microbes shared among the above-
276 mentioned snails.

277   Proteobacteria was the dominant microbiota in both groups, and primary microbiota in other
278 Gastropoda like *Potamopyrgus antipodarum* and *Achatina fulica* (Cardoso et al., 2012b; Takacs-
279 Vesbach et al., 2016). Moreover, Proteobacteria is related to environmental adaptation due to its
280 ability to secrete lipase, protease, and amylase (Pemberton et al., 1997). Our results also showed
281 that the Proteobacteria genus *Enterobacter* was dominant at the genus level. *Enterobacter* is

282 considered a cellulolytic genus (Chen et al., 2021a; Pawar et al., 2015), with certain members,
283 such as the *Enterobacter* sp. strain Bisph2, able to degrade glyphosate (an organic phosphine
284 herbicide) (Benslama and Boulahrouf, 2016), and others, such as *Enterobacter* spH1, able to
285 degrade glucose and glycerin into value-added products (Gueell et al., 2015). Chinese mud snails
286 live in paddy fields, lakes, and rivers (Nakanishi et al., 2014) and primarily feed on diatoms and
287 plant debris (Cui et al., 2012), with no reports of carnivorous behavior has been reported yet.
288 Proteobacteria can help in diatom and plant debris digestion. Therefore, we speculate that the
289 relatively simple "lifestyle" of Chinese mud snails does not require a complex gut microbiota
290 composition.

291 In contrast, the diets of golden apple snails are more complex and include plants and aquatic
292 invertebrates, such as *Biomphalaria peregrina*, *Biomphalaria straminea*, and *Physa acuta*
293 (Cazzaniga, 1990; Kwong et al., 2009). More complicated diets require more digestion-related
294 bacteria. In addition to digestion-related Proteobacteria, both Bacteroidetes and Firmicutes were
295 related to nutrient absorption (Sommer et al., 2016; Thomas et al., 2011). Bacteroidetes can
296 degrade high molecular weight organic matter, while Firmicutes was related to the degradation
297 of lipids and dietary fiber (Chen et al., 2011b; Evans et al., 2011). Both these were highly
298 abundant in golden apple snails, indicating that they had more effective material decomposition
299 and energy absorption than the Chinese mud snail. The functions of these two phyla have also
300 been explored in invertebrates (Wang et al., 2020b). In the current study, average Bacteroidetes
301 and Firmicutes abundances were significantly higher in the PC group (23.20% and 16.00%,
302 respectively) than in the CC group (0.34% and 0.49%, respectively). The *Chryseobacterium*
303 genus was only found in the PC group, and the relative abundance of *Lactococcus* was
304 significantly higher in the PC group than in the CC group. *Chryseobacterium* can decompose
305 lignocellulose (Carlos et al., 2018; Puentes-Tellez and Salles, 2018; Weiss et al., 2021). In
306 addition, study on the American cockroach (*Periplaneta americana*) reported that
307 *Chryseobacterium* is only found in a high-fiber diet, indicating that *Chryseobacterium* can
308 decompose fiber (Dugas et al., 2001). As gram-positive bacteria, *Lactococcus* species are
309 recognized as safe microorganisms for food production and can produce L-lactic acid through
310 acidification to provide energy (Casalta and Montel, 2008). Unique or high abundance microbial
311 taxa were related to the material decomposition and capacity utilization, which indicated that the
312 golden apple snail can digest more substances to be more successful at invasion. Therefore, the
313 higher relative abundance and complex composition of gut microbiota in *P. canaliculata* can be
314 one of the reasons for its high survival and adaptability, and thus its successful invasion.

315 Among the top 10 dominant phyla, three existed in the PC group only: i.e., Spirochaetes,
316 Fibrobacteres, and Tenericutes. In recent years, less attention has been paid to the function of
317 spirochetes, with most studies limited to pathogens involved in Lyme disease, recurrent fever,

318 and syphilis (Gattorno et al., 2019; Hook, 2017; Radolf et al., 2012). In the current study, we
319 speculate that the Spirochaetes bacteria are parasites rather than functional bacteria in the golden
320 apple snails. Fibrobacteres is a well-known primary degrader of cellulose in the intestinal tract of
321 herbivores and can hydrolyze polymer using a distinctive set of glycoside hydrolases and binding
322 domains (Rahman et al., 2016). Tenericutes is suggested to be involved in carbohydrate storage,
323 carbon fixation, and environmental response (Wang et al., 2020a). In this study, both
324 Fibrobacteres and Tenericutes were unique dominant microbiota in golden apple snail, which
325 may be related to their high invasiveness.

326   Functional KEGG predictions indicated that many functions were significantly different
327 between the PC and CC groups, including cofactor, prosthetic group, electron carrier, vitamin
328 biosynthesis, amino acid biosynthesis, nucleoside and nucleotide biosynthesis, fatty acid and
329 lipid biosynthesis, and carboxylate degradation, consistent with previous studies on indigenous
330 species *Helix pomatia* and invasive species *Achatina fulica* (Cardoso et al., 2012a; Nicolai et al.,
331 2015).. Of these functions, the relative abundance in the PC group was significantly higher than
332 that in the CC group, including amino acid biosynthesis, fatty acid and lipid biosynthesis,
333 aromatic compound biosynthesis, and C1 compound utilization and assimilation. Gut microbiota
334 are crucial to host amino acid homeostasis and health (Mardinoglu et al., 2015). Several genera
335 are known to play crucial roles in amino acid biosynthesis, including *Fusobacterium*,
336 *Bacteroides*, and *Veillonella* (Lin et al., 2017). Additionally, *Lactococcus* and *Bacteroides* are
337 associated with fatty acid and lipid biosynthesis (Liu and Meng, 2008; Tanca et al., 2018). In this
338 study, the relative abundances of *Bacteroides* and *Lactococcus* were significantly higher in the
339 PC group and are suggested to be the main factors for the biosynthesis of basic elements. In
340 addition to fatty acid and lipid biosynthesis, *Lactococcus*, a dominant genus in our study, also
341 produces exopolysaccharide and aromatic compounds (Casalta and Montel, 2008). Our results
342 indicated that the two species showed considerable differences in gut microbiota functions. The
343 higher gene functions in PC group were focused on nutrient utilization, indicating that the gut
344 microbiota in golden apple snail has higher nutrient conversion capacity than the Chinese mud
345 snail, which helps the golden apple snail to improve the survivability in unstable environment.

Conclusions

347 Our study provided a general understanding of the gut microbial community characteristics
348 between sympatric invasive golden apple snail and native Chinese mud snail. We found that
349 microbial abundance and community structure was significantly different between golden apple
350 snail and Chinese mud snail. The abundance and community structure of gut microbiota in
351 golden apple snail was higher and more complex than in Chinese mud snail, suggesting that the
352 golden apple snail may have the ability to digest a wider variety of food. In addition, we found

353 some specific bacteria phyla and genera of golden apple snail gut microbiota, which can promote
354 the ability to absorb and transform nutrients helping golden apple snail to be more invasive.
355 These results revealed that the structure and function in microbiota composition are the reasons
356 why golden apple snail readily adapts to the new habitats.

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Competing interests

372 The authors declare that they have no competing interests.

Authors Contributions

374 Zhou ZH, Huang JL and Wu ZJ designed the study;
375 Zhou ZH, Wu HY, Li DH and Zeng WL performed the experiments and data analysis;
376 Zhou ZH drafted the manuscript;
377 Zhou ZH, Wu HY, Li DH, Zeng WL, Huang JL and Wu ZJ discussed the study design and data
378 analysis;
379 Huang JL and Wu ZJ revised the manuscript.

Availability of data and materials

381 The data are available at the National Center for Biotechnology Information(NCBI)
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References

Akbar S, Huang J, Zhou Q, Gu L, Sun Y, Zhang L, Lyu K, Yang Z. 2021. Elevated temperature and toxic Microcystis reduce *Daphnia* fitness and modulate gut microbiota. *Environmental Pollution* **271** DOI: 10.1016/j.envpol.2020.116409.

Bankers L, Dahan D, Neiman M, Adrian Tucci C, Frost C, Hurst G.D.D, King K.C. 2021. Invasive freshwater snails form novel microbial relationships. *Evolutionary Applications* **14**:770-780 DOI: 10.1111/eva.13158.

Becker A.A.M.J, Hill K.C, Butaye P. 2021. Unraveling the Gut Microbiome of the Invasive Small Indian Mongoose (*Urva auropunctata*) in the Caribbean. *Microorganisms* **9** DOI: 10.3390/microorganisms9030465.

Benslama O, Boulahrouf A. 2016. High-quality draft genome sequence of *Enterobacter* sp. Bisph2, a glyphosate-degrading bacterium isolated from a sandy soil of Biskra, Algeria. *Genomics data* **8**:61-66 DOI: 10.1016/j.gdata.2016.03.005.

Bibbo S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, Cammarota G. 2016. The role of diet on gut microbiota composition. *European Review for Medical and Pharmacological Sciences* **20**:4742-4749.

Brestoff J.R, Artis D. 2013. Commensal bacteria at the interface of host metabolism and the immune system. *Nature Immunology* **14**:676-684 DOI: 10.1038/ni.2640.

Callahan B.J, McMurdie P.J, Rosen M.J, Han A.W, Johnson A.J.A, Holmes S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods: Techniques for life scientists and chemists* **13** DOI: 10.1038/NMETH.3869.

Cardoso A.M, Cavalcante J.J.V, Cantao M.E, Thompson C.E, Flatschart R.B, Glogauer A, Scapin S.M.N, Sade Y.B, Beltrao P.J.M.S.I, Gerber A.L, Martins O.B, Garcia E.S, de Souza W, Vasconcelos A.T.R. 2012a. Metagenomic Analysis of the Microbiota from the Crop of an Invasive Snail Reveals a Rich Reservoir of Novel Genes. *Plos One* **7** DOI: 10.1371/journal.pone.0048505.

Cardoso A.M, Cavalcante J.J.V, Vieira R.P, Lima J.L, Grieco M.A.B, Clementino M.M, Vasconcelos A.T.R, Garcia E.S, de Souza W, Albano R.M, Martins O.B. 2012b. Gut Bacterial Communities in the Giant Land Snail *Achatina fulica* and Their Modification by Sugarcane-Based Diet. *Plos One* **7** DOI: 10.1371/journal.pone.0033440.

Carlos C, Fan H, Currie C.R. 2018. Substrate Shift Reveals Roles for Members of Bacterial Consortia in Degradation of Plant Cell Wall Polymers. *Frontiers in Microbiology* **9** DOI: 10.3389/fmicb.2018.00364.

Carlsson N.O.L, Nmark C.B, And L.A, Hansson. 2004. Invading herbivory: the golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* **85**:1575-1580 DOI: 10.1890/03-3146.

Casalta E, Montel M.-C. 2008. Safety assessment of dairy microorganisms: the *Lactococcus* genus. *International journal of food microbiology* **126**:271-3 DOI: 10.1016/j.ijfoodmicro.2007.08.013.

Cazzaniga N.J. 1990. Predation of *Pomacea canaliculata* (Ampullariidae) on adult

424   *Biomphalaria peregrina* (Planorbidae). *Annals of tropical medicine and parasitology*
425   **84**:97-100 DOI: 10.1080/00034983.1990.11812439.

426 **Chen L, Li S, Xiao Q, Lin Y, Li X, Qu Y, Wu G, Li H. 2021a.** Composition and diversity of
427   gut microbiota in *Pomacea canaliculata* in sexes and between developmental stages.
428   *BMC Microbiology* **21** DOI: 10.1186/s12866-021-02259-2.

429 **Chen L, Xu H, Li H, Wu J, Ding H, Liu Y. 2011a.** Isolation and Characterization of Sixteen
430   Polymorphic Microsatellite Loci in the Golden Apple Snail *Pomacea canaliculata*.
431   *International Journal of Molecular Sciences* **12**:5993-5998 DOI: 10.3390/ijms12095993.

432 **Chen X, Lu D, Li Z, Yue W, Wang J, Jiang X, Han H, Wang C. 2021b.** Plant and Animal-
433   Type Feedstuff Shape the Gut Microbiota and Metabolic Processes of the Chinese Mitten
434   Crab *Eriocheir sinensis*. *Frontiers in Veterinary Science* **8**. DOI:
435   10.3389/fvets.2021.589624.

436 **Chen Y, Penner G.B, Li M, Oba M, Guan L.L. 2011b.** Changes in Bacterial Diversity
437   Associated with Epithelial Tissue in the Beef Cow Rumen during the Transition to a
438   High-Grain Diet. *Applied and Environmental Microbiology* **77**:5770-5781 DOI:
439   10.1128/aem.00375-11.

440 **Cui J, Shan B, Tang W. 2012.** Effect of periphyton community structure on heavy metal
441   accumulation in mystery snail (*Cipangopaludina chinensis*): A case study of the Bai
442   River, *China. Journal of Environmental Sciences* **24**:1723-1730 DOI: 10.1016/s1001-
443   0742(11)60976-5.

444 **Douglas G.M, Maffei V.J, Zaneveld J.R, Yurgel S.N, Brown J.R, Taylor C.M, Huttenhower**
445   **C, Langille M.G.I. 2020.** PICRUSt2 for prediction of metagenome functions. *Nature*
446   *Biotechnology* **38**:685-688 DOI: 10.1038/s41587-020-0548-6.

447 **Dugas J.E, Zurek L, Paster B.J, Keddie B.A, Leadbetter E.R. 2001.** Isolation and
448   characterization of a *Chryseobacterium* strain from the gut of the American cockroach,
449   *Periplaneta americana*. Archives of microbiology **175**:259-262 DOI:
450   10.1007/s002030000243.

451 **Evans N.J, Brown J.M, Murray R.D, Getty B, Birtles R.J, Hart C.A, Carter S.D. 2011.**
452   Characterization of Novel Bovine Gastrointestinal Tract *Treponema* Isolates and
453   Comparison with Bovine Digital Dermatitis Treponemes. *Applied and Environmental*
454   *Microbiology* **77**:138-147 DOI: 10.1128/aem.00993-10.

455 **Fan J, Chen L, Mai G, Zhang H, Yang J, Deng D, Ma Y. 2019.** Dynamics of the gut
456   microbiota in developmental stages of *Litopenaeus vannamei* reveal its association with
457   body weight. *Scientific Reports* **9** DOI: 10.1038/s41598-018-37042-3.

458 **Gaikwad S.S, Shouche Y.S, Gade W.N. 2017.** Deep Sequencing Reveals Highly Variable Gut
459   Microbial Composition of Invasive Fish *Mossambicus Tilapia* (Oreochromis
460   mossambicus) Collected from Two Different Habitats. *Indian Journal of Microbiology*
461   **57**:235-240 DOI: 10.1007/s12088-017-0641-9.

462 **Gallo B.D, Farrell J.M, Leydet B. 2020a.** Use of next generation sequencing to compare simple
463   habitat and species level differences in the gut microbiota of an invasive and native
464   freshwater fish species. *PeerJ* **8** DOI: 10.7717/peerj.10237.

465 **Gallo B.D, Farrell J.M, Leydet B.F. 2020b.** Fish Gut Microbiome: A Primer to an Emerging
466   Discipline in the Fisheries Sciences. *Fisheries* **45**:271-282 DOI: 10.1002/fsh.10379.
467 **Gattorno M, Hofer M, Federici S, Vanoni F, Bovis F, Aksentijevich I, Anton J, Ignacio**
468   **Arostegui J, Barron K, Ben-Cherit E, Brogan P.A, Cantarini L, Ceccherini I, De**
469   **Benedetti F, Dedeoglu F, Demirkaya E, Frenkel J, Goldbach-Mansky R, Gul A,**
470   **Hentgen V, Hoffman H, Kallinich T, Kone-Paut I, Kuemmerle-Deschner J,**
471   **Lachmann H.J, Laxer R.M, Livneh A, Obici L, Ozen S, Rowczenio D, Russo R,**
472   **Shinar Y, Simon A, Toplak N, Touitou I, Uziel Y, van Gijn M, Foell D, Garassino C.,**
473   **Kastner D, Martini A, Sormani M.P, Ruperto N, Eurofever Registry P. 2019.**
474   Classification criteria for autoinflammatory recurrent fevers. *Annals of the Rheumatic*
475   *Diseases* **78**:1025-1032 DOI: 10.1136/annrheumdis-2019-215048.
476 **Gueell E.J, Maru B.T, Chimentao R.J, Gispert-Guirado F, Constanti M, Medina F. 2015.**
477   Combined heterogeneous catalysis and dark fermentation systems for the conversion of
478   cellulose into biohydrogen. *Biochemical Engineering Journal* **101**:209-219 DOI:
479   10.1016/j.bej.2015.06.004.
480 **Habineza P, Muhammad A, Ji T, Xiao R, Yin X, Hou Y, Shi Z. 2019.** The Promoting Effect
481   of Gut Microbiota on Growth and Development of Red Palm Weevil, *Rhynchophorus*
482   *ferrugineus (Olivier)* (Coleoptera: Dryophthoridae) by Modulating Its Nutritional
483   Metabolism. *Frontiers in Microbiology* **10** DOI: 10.3389/fmicb.2019.01212.
484 **Hook E.W, III. 2017.** Syphilis. *Lancet* **389**:1550-1557 DOI: 10.1016/s0140-6736(16)32411-4.
485 **Hu Z, Chen X, Chang J, Yu J, Tong Q, Li S, Niu H. 2018.** Compositional and predicted
486   functional analysis of the gut microbiota of *Radix auricularia* (Linnaeus) via high-
487   throughput Illumina sequencing. *PeerJ* **6** DOI: 10.7717/peerj.5537.
488 **Hu Z, Tong Q, Chang J, Yu J, Li S, Niu H, Ma D. 2021.** Gut bacterial communities in the
489   freshwater snail *Planorbella trivolvis* and their modification by a non-herbivorous diet.
490   *PeerJ* **9** DOI: 10.7717/peerj.10716.
491 **Jin Y, Wu S, Zeng Z, Fu Z. 2017.** Effects of environmental pollutants on gut microbiota.
492   *Environmental Pollution* **222**:1-9 DOI: 10.1016/j.envpol.2016.11.045.
493 **Kwong K.-L, Chan R.K.Y, Qiu J.-W. 2009.** The potential of the invasive snail Pomacea
494   canaliculata as a predator of various life-stages of five species of freshwater snails.
495   *Malacologia* **51**:343-356 DOI: 10.4002/040.051.0208.
496 **Lee W.-J, Hase K. 2014.** Gut microbiota-generated metabolites in animal health and disease.
497   *Nature Chemical Biology* **10**:416-424 DOI: 10.1038/nchembio.1535.
498 **Li H, Li T, Beasley D.E, Hedenec P, Xiao Z, Zhang S, Li J, Lin Q, Li X. 2016.** Diet Diversity
499   Is Associated with Beta but not Alpha Diversity of Pika Gut Microbiota. *Frontiers in*
500   *Microbiology* **7** DOI: 10.3389/fmicb.2016.01169.
501 **Li L.H, Lv S, Lu Y, Bi D.-Q, Guo Y.-H, Wu J.-T, Yue Z.-Y, Mao G.-Y, Guo Z.-X, Zhang Y,**
502   **Tang Y.-F. 2019.** Spatial structure of the microbiome in the gut of *Pomacea canaliculata*.
503   *BMC Microbiology* **19** DOI:10.1186/s12866-019-1661-x.
504 **Lin R, Liu W, Piao M, Zhu H. 2017.** A review of the relationship between the gut microbiota
505   and amino acid metabolism. *Amino Acids* **49**:2083-2090 DOI: 10.1007/s00726-017-2493-

506   3.

507 **Liu C, Dong Y, Hou L, Deng N, Jiao R. 2017.** Acidobacteria Community Responses to
508   Nitrogen Dose and Form in Chinese Fir Plantations in Southern China. *Current*
509   *Microbiology* **74**:396-403 DOI: 10.1007/s00284-016-1192-8.

510 **Liu H, Meng X. 2008.** Influence of acid adaptation on membrane-fatty acid profile and
511   expression of membrane proteins in Lactococcus lactis. *Wei sheng wu xue bao = Acta*
512   *microbiologica Sinica* **48**:1459-65.

513 **Lize A, McKay R, Lewis Z. 2013.** Gut microbiota and kin recognition. *Trends in Ecology &*
514   *Evolution* **28**:325-326 DOI: 10.1016/j.tree.2012.10.013.

515 **Lowe S, Browne M, Boudjelas S, De Poorter M. 2000.** 100 of the world's worst invasive alien
516   species a selection from the Global Invasive Species Database. *The Invasive Species*
517   *Specialist Group of the Species Survival Commission of the World Conservation*
518   *Union*:**12** pp.

519 **Lyra M.L, Bletz M.C, Haddad C.F.B, Vences M. 2018.** The Intestinal Microbiota of Tadpoles
520   Differs from Those of Syntopic Aquatic Invertebrates. *Microbial Ecology* **76**:121-124
521   DOI: 10.1007/s00248-017-1109-5.

522 **Mardinoglu A, Shoaie S, Bergentall M, Ghaffari P, Zhang C, Larsson E, Backhed F,**
523   **Nielsen J. 2015.** The gut microbiota modulates host amino acid and glutathione
524   metabolism in mice. *Molecular Systems Biology* **11** DOI: 10.15252/msb.20156487.

525 **Morrison W.E, Hay M.E. 2011.** Feeding and growth of native, invasive and non-invasive alien
526   apple snails (*Ampullariidae*) in the United States: Invasives eat more and grow more.
527   *Biological Invasions* **13**:945-955 DOI: 10.1007/s10530-010-9881-x.

528 **Nakanishi K, Takakura K.-I, Kanai R, Tawa K, Murakami D, Sawada H. 2014.** Impacts of
529   environmental factors in rice paddy fields on abundance of the mud snail
530   (*Cipangopaludina chinensis laeta*). *Journal of Molluscan Studies* **80**:460-463 DOI:
531   10.1093/mollus/eyu054.

532 **Navarrete A.A, Venturini A.M, Meyer K.M, Klein A.M, Tiedje J.M, Bohannan B.J.M,**
533   **Nuesslein K, Tsai S.M, Rodrigues J.L.M. 2015.** Differential Response of *Acidobacteria*
534   *Subgroups* to Forest-to-Pasture Conversion and Their Biogeographic Patterns in the
535   Western Brazilian Amazon. *Frontiers in Microbiology* **6** DOI:
536   10.3389/fmicb.2015.01443.

537 **Nicolai A, Rouland-Lefevre C, Ansart A, Filser J, Lenz R, Pando A, Charrier M. 2015.**
538   Inter-population differences and seasonal dynamic of the bacterial gut community in the
539   endangered land snail *Helix pomatia* (Gastropoda: Helicidae). *Malacologia* **59**:177-190
540   DOI: 10.4002/040.059.0101.

541 **Pawar K.D, Dar M.A, Rajput B.P, Kulkarni G.J. 2015.** Enrichment and Identification of
542   Cellulolytic Bacteria from the Gastrointestinal Tract of Giant African Snail, *Achatina*
543   *fulica*. *Applied Biochemistry and Biotechnology* **175**:1971-1980 DOI: 10.1007/s12010-
544   014-1379-z.

545 **Pemberton J.M, Kidd S.P, Schmidt R. 1997.** Secreted enzymes of Aeromonas. *FEMS*
546   *microbiology letters* **152**:1-10.

547 **Pinheiro G.L, Correa R.F, Cunha R.S, Cardoso A.M, Chaia C, Clementino M.M, Garcia**
548   **E.S, de Souza W, Frases S. 2015.** Isolation of aerobic cultivable cellulolytic bacteria
549   from different regions of the gastrointestinal tract of giant land snail *Achatina fulica*.
550   *Frontiers in Microbiology* **6** DOI: Artn 86010.3389/Fmicb.2015.00860.
551 **Puentes Tellez P.E, Salles J.F. 2018.** Construction of Effective Minimal Active Microbial
552   Consortia for Lignocellulose Degradation. *Microbial Ecology* **76**:419-429 DOI:
553   10.1007/s00248-017-1141-5.
554 **Qu Y.F, Wu Y.Q, Zhao Y.T, Lin L.H, Du Y, Li P, Li H, Ji X. 2020.** The invasive red-eared
555   slider turtle is more successful than the native Chinese three-keeled pond turtle: evidence
556   from the gut microbiota. *PeerJ* **8** DOI: 10.7717/peerj.10271.
557 **Radolf J.D, Caimano M.J, Stevenson B, Hu L.T. 2012.** Of ticks, mice and men: understanding
558   the dual-host lifestyle of Lyme disease spirochaetes. *Nature Reviews Microbiology*
559   **10**:87-99 DOI: 10.1038/nrmicro2714.
560 **Rahman N.A, Parks D.H, Vanwonterghem I, Morrison M, Tyson G.W, Hugenholtz P. 2016.**
561   A Phylogenomic Analysis of the Bacterial Phylum Fibrobacteres. *Frontiers in*
562   *Microbiology* **6** DOI: 10.3389/fmicb.2015.01469.
563 **Ringo E, Zhou Z, Vecino J.L.G, Wadsworth S, Romero J, Krogdahl A, Olsen R.E,**
564   **Dimitroglou A, Foey A, Davies S, Owen M, Lauzon H.L, Martinsen L.L, de**
565   **Schryver P, Bossier P, Sperstad S, Merrifield D.L. 2016.** Effect of dietary components
566   on the gut microbiota ofaquatic animals. A never-ending story? *Aquaculture Nutrition*
567   **22**:219-282 DOI: 10.1111/anu.12346.
568 **She Y.Z, Wu J.P, Zhang Y, Peng Y, Mo L, Luo X.J, Mai B.X. 2013.** Bioaccumulation of
569   polybrominated diphenyl ethers and several alternative halogenated flame retardants in a
570   small herbivorous food chain. *Environmental Pollution* **174**:164-170 DOI:
571   10.1016/j.envpol.2012.11.024.
572 **Sommer F, Stahlman M, Ilkayeva O, Arnemo J.M, Kindberg J, Josefsson J, Newgard C.B,**
573   **Frobert O, Backhed F. 2016.** The Gut Microbiota Modulates Energy Metabolism in the
574   Hibernating Brown Bear *Ursus arctos*. *Cell Reports* **14**:1655-1661 DOI:
575   10.1016/j.celrep.2016.01.026.
576 **Takacs-Vesbach C, King K, Van Horn D, Larkin K, Neiman M. 2016.** Distinct Bacterial
577   Microbiomes in Sexual and Asexual *Potamopyrgus antipodarum*, a New Zealand
578   Freshwater Snail. *Plos One* **11** DOI: 10.1371/journal.pone.0161050.
579 **Tanca A, Abbondio M, Palomba A, Fraumene C, Marongiu F, Serra M, Pagnozzi D,**
580   **Laconi E, Uzzau S. 2018.** Caloric restriction promotes functional changes involving
581   short-chain fatty acid biosynthesis in the rat gut microbiota. *Scientific Reports* **8** DOI:
582   10.1038/s41598-018-33100-y.
583 **Thomas F, Hehemann J.H, Rebuffet E, Czjzek M, Michel G. 2011.** Environmental and gut
584   Bacteroidetes: the food connection. *Frontiers in Microbiology* **2** DOI:
585   10.3389/fmicb.2011.00093.
586 **Torbjørn R, Tomáš F, Ben N, Christopher Q, Frédéric M. 2016.** VSEARCH: a versatile open
587   source tool for metagenomics. *PeerJ* **4** DOI: 10.7717/peerj.2584.

588 **Turnbaugh P.J, Ley R.E, Mahowald M.A, Magrini V, Mardis E.R, Gordon J.I. 2006.** An
589   obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*
590   **444**:1027-31 DOI: 10.1038/nature05414.
591 **Van Horn D.J, Garcia J.R, Loker E.S, Mitchell K.R, Mkoji G.M, Adema C.M, Takacs-**
592   **Vesbach C.D. 2012.** Complex intestinal bacterial communities in three species of
593   planorbid snails. *Journal of Molluscan Studies* **78**:74-80 DOI: 10.1093/mollus/eyr038.
594 **Waite D.W, Vanwonterghem I, Rinke C, Parks D.H, Zhang Y, Takai K, Sievert S.M,**
595   **Simon J, Campbell B.J, Hanson T.E, Woyke T, Klotz M.G, Hugenholtz P. 2017.**
596   Comparative Genomic Analysis of the Class Epsilonproteobacteria and Proposed
597   Reclassification to *Epsilonbacteraeota* (phyl. nov.). *Frontiers in Microbiology* **8** DOI:
598   10.3389/fmicb.2017.00682.
599 **Wang Y, Huang J.M, Zhou Y.L, Almeida A, Finn R.D, Danchin A, He L.S. 2020a.**
600   Phylogenomics of expanding uncultured environmental Tenericutes provides insights into
601   their pathogenicity and evolutionary relationship with Bacilli. *BMC Genomics* **21** DOI:
602   10.1186/s12864-020-06807-4.
603 **Wang Y, Liu M, Wang B, Jiang K, Wang M, Wang L. 2020b.** Response of the *Litopenaeus*
604   *vananmei* intestinal bacteria and antioxidant system to rearing density and exposure to
605   Vibrio paraheamolyticus E1. *Journal of Invertebrate Pathology* **170** DOI:
606   10.1016/j.jip.2020.107326.
607 **Weiss B, Oliveira Souza A.C, Lima Constancio M.T, Alvarenga D.O, Pylro V.S, Alves**
608   **L.M.C, Varani A.M. 2021.** Unraveling a Lignocellulose-Decomposing Bacterial
609   Consortium from Soil Associated with Dry Sugarcane Straw by Genomic-Centered
610   Metagenomics. *Microorganisms* **9** DOI: 10.3390/microorganisms9050995.
611 **Whittaker R.H. 1972.** Evolution and measurement of species diversity. *Taxon* **21**.
612 **Xiong J. 2018.** Progress in the gut microbiota in exploring shrimp disease pathogenesis and
613   incidence. *Applied Microbiology and Biotechnology* **102**:7343-7350 DOI:
614   10.1007/s00253-018-9199-7.
615 **Zhang F, Sun X.X, Zhang X.C, Zhang S, Lu J, Xia Y.M, Huang Y.H, Wang X.J. 2018.** The
616   interactions between gut microbiota and entomopathogenic fungi: a potential approach
617   for biological control of *Blattella germanica (L.)*. *Pest Management Science* **74**:438-447
618   DOI: 10.1002/ps.4726.
619 **Zhang Y, Li Z, Kholodkevich S, Sharov A, Chen C, Feng Y, Ren N, Sun K. 2020.** Effects of
620   cadmium on intestinal histology and microbiota in freshwater crayfish (*Procambarus*
621   *clarkii*). *Chemosphere* **242** DOI: 10.1016/j.chemosphere.2019.125105.
622

624 **Figure 1** Alpha diversity index summary. Chao 1 and Observed species indexes represent

625 community richness; Shannon and Simpson represent diversity; Faith's PD represent the

626 diversity of evolution; Pielou's evenness represent evenness; Good's coverage represent

627 coverage. * *P* < 0.05 represent significant level.

628

629 **Figure 2** NMDS and ANOSIM analysis based on (A, C) Bray-Curtis and (B, D) weighted

630 UniFrac distances of gut microbiota on OTU level. The NMDS ordination revealed a significant

631 difference based on Bray-Curtis (*P* = 0.034) and on weighted UniFrac distances (*P* = 0.031)

632 between PC and CC groups. The ANOSIM revealed a significant difference based on Bray-

633 Curtis (*P* = 0.027) and on weighted UniFrac distances (*P* = 0.03) between PC and CC groups.

634 Part of the dots have overlapped (A and B).

635

636 **Figure 3** Relative abundance of top 10 OTU's for *P. canaliculata* (PC) and *C. chinensis* (CC) at

637 the phylum level.

638

639 **Figure 4** Relative abundance of top 10 OTU's for *P. canaliculata* (PC) and *C. chinensis* (CC) at

640 the genus level.

641

642

643 **Figure 5** Gut microbiota predictive metabolic functions from KEGG database in all samples.

644 The color-bar indicates gene function (level 1). Detailed descriptions are shown on the left side

645 (level 2). The lines at the top of the figure have shown the KEGG level as well.

646

647

Figure 1

Alpha diversity index summary.

Chao 1 and Observed species indexes represent community richness; Shannon and Simpson represent diversity; Faith's PD represent the diversity of evolution; Pielou's evenness represent evenness; Good's coverage represent coverage. * $P<0.05$ represent significant level.
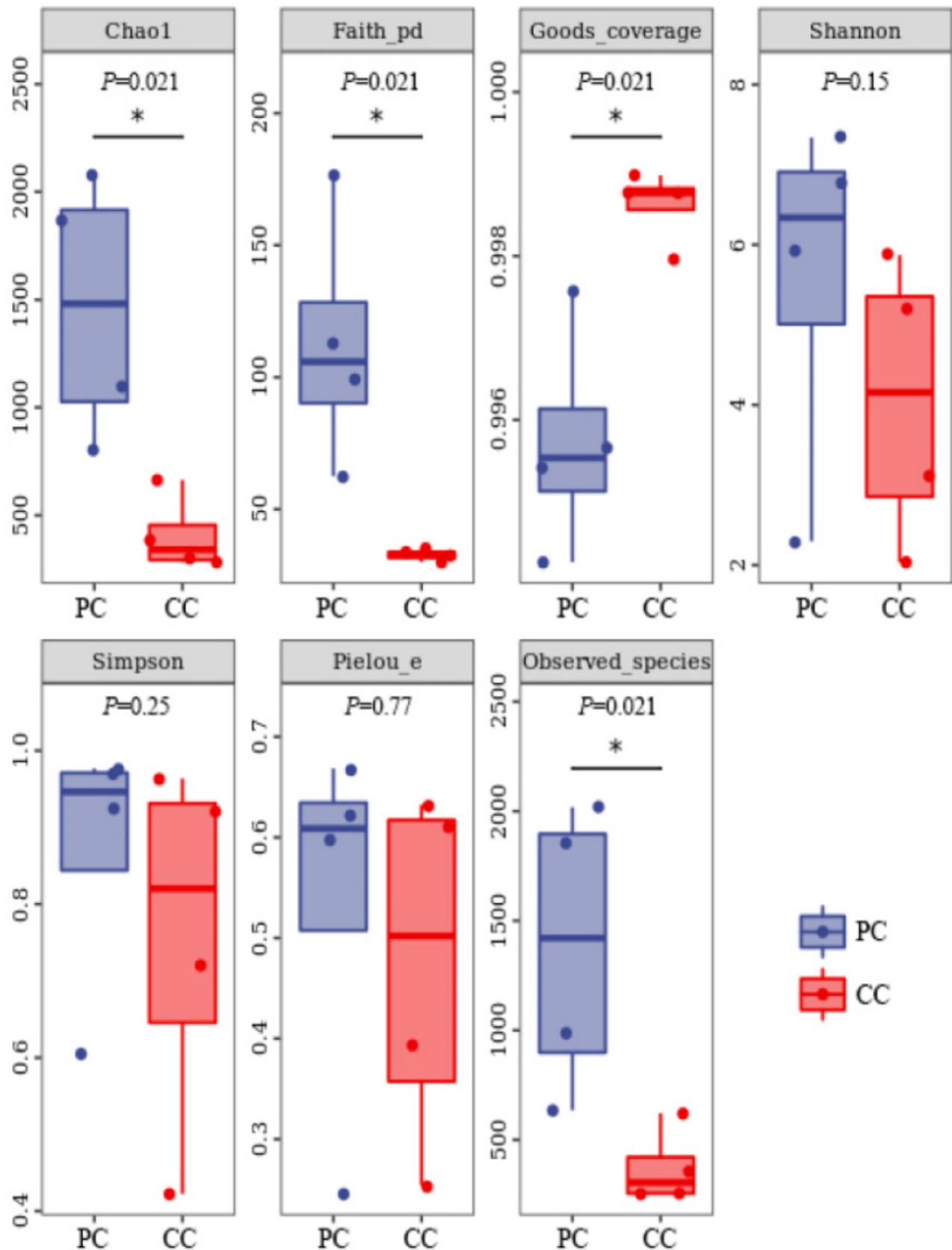
Figure 2

NMDS and ANOSIM analysis based on (A, C) Bray-Curtis and (B, D) weighted UniFrac distances of gut microbiota on OTU level.

The NMDS ordination revealed a significant difference based on Bray-Curtis ($P=0.034$) and on weighted UniFrac distances ($P=0.031$) between PC and CC groups. The ANOSIM revealed a significant difference based on Bray-Curtis ($P=0.027$) and on weighted UniFrac distances ($P=0.03$) between PC and CC groups. Part of the dots have overlapped (A and B).
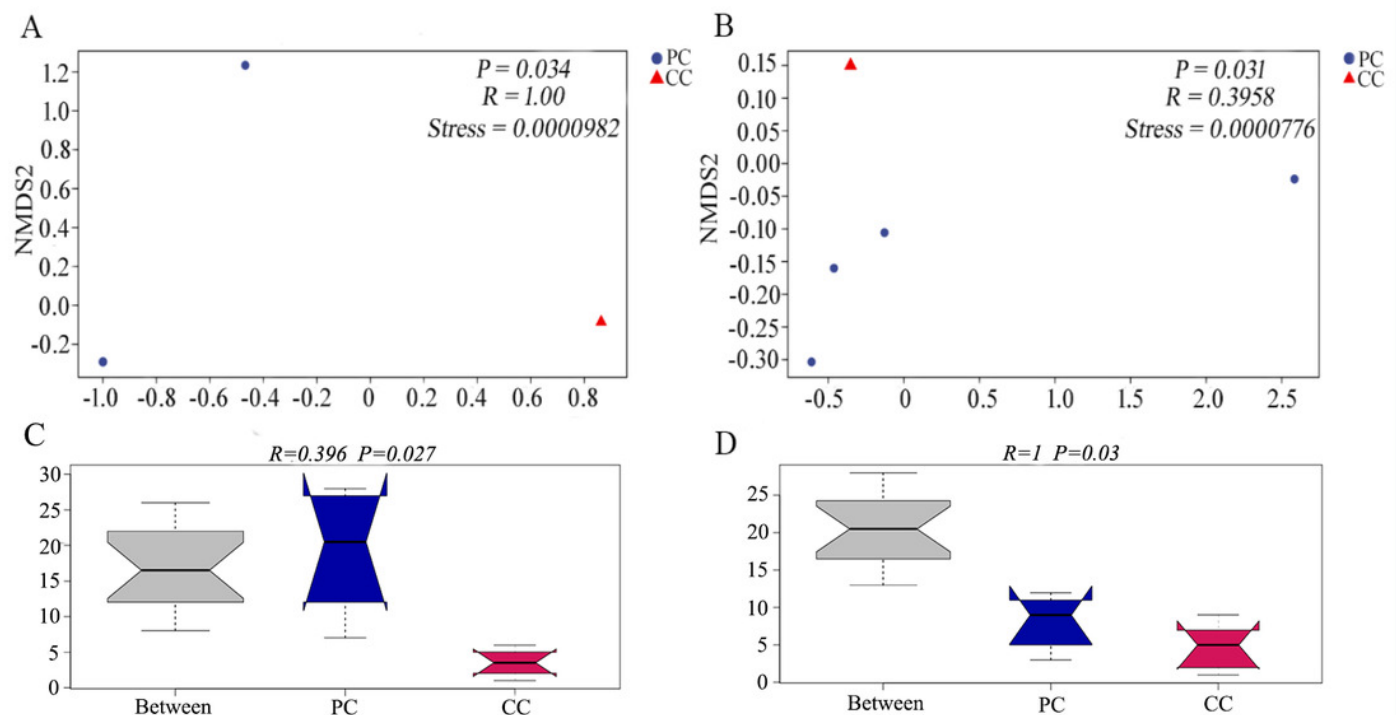
Figure 3

Relative abundance of top 10 OTU's for *P. canaliculata* (PC) and *C. chinensis* (CC) at the phylum level.
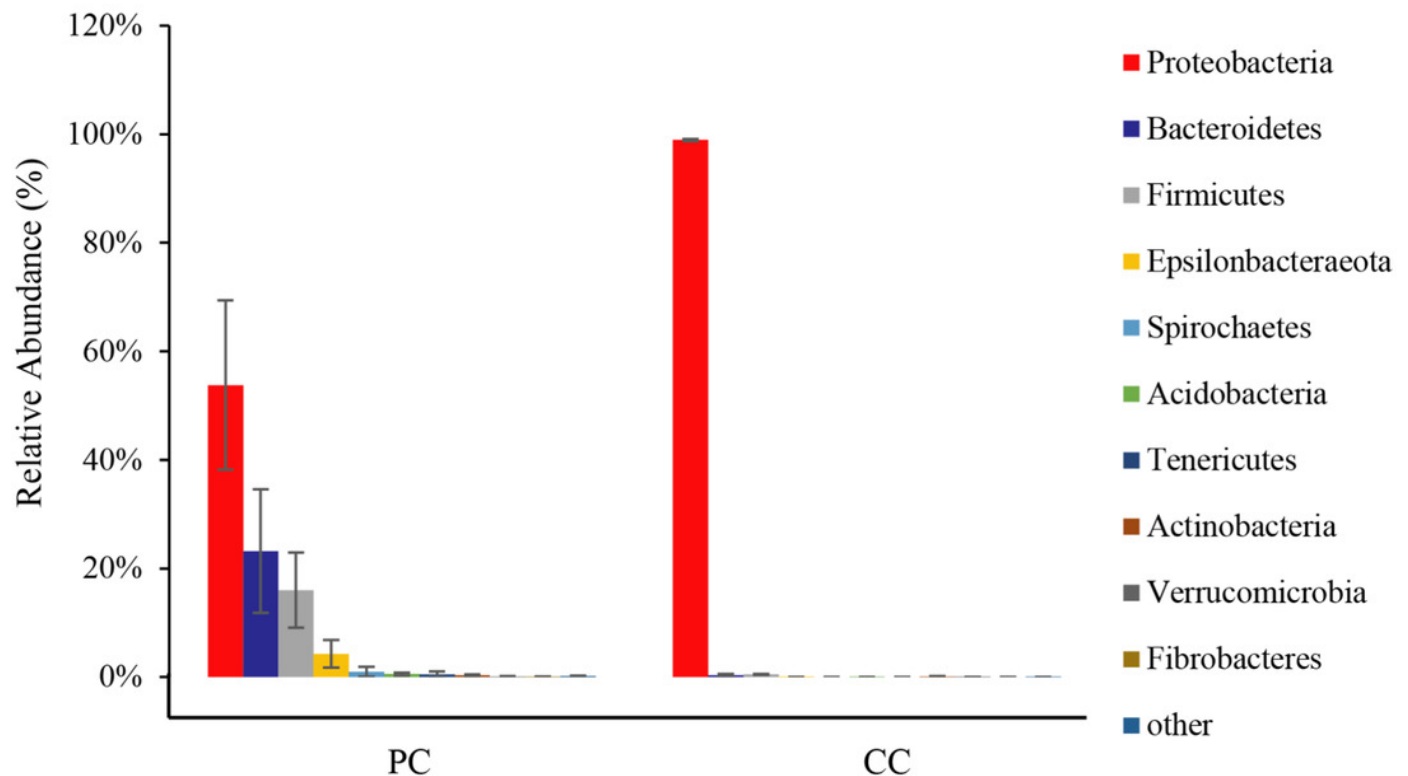
Figure 4

Relative abundance of top 10 OTU's for *P. canaliculata* (PC) and *C. chinensis* (CC) at the genus level.
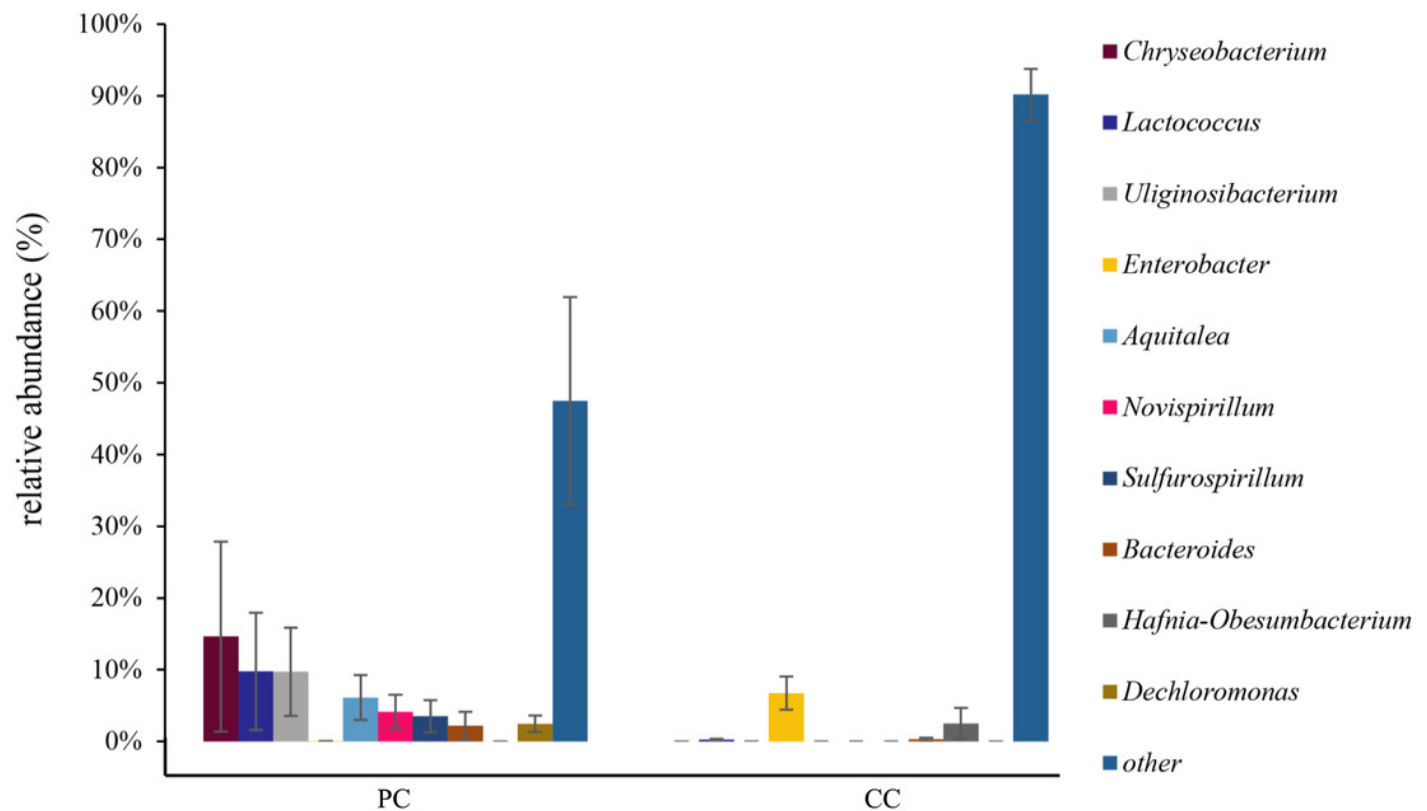
Figure 5

Gut microbiota predictive metabolic functions from KEGG database in all samples.

The color-bar indicates gene function (level 1). Detailed descriptions are shown on the left side (level 2). The lines at the top of the figure have shown the KEGG level as well.

Figure: Bar chart of Relative Abundance by Level 2 and Level 1 categories.

Level 2 | **Level 1**

Biosynthesis:
- Amine and Polyamine Biosynthesis
- Amino Acid Biosynthesis
- Aminoacyl-tRNA Charging
- Aromatic Compound Biosynthesis
- Carbohydrate Biosynthesis
- Cell Structure Biosynthesis
- Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis
- Fatty Acid and Lipid Biosynthesis
- Metabolic Regulator Biosynthesis
- Nucleoside and Nucleotide Biosynthesis
- Other Biosynthesis
- Secondary Metabolite Biosynthesis

Degradation/Utilization/Assimilation:
- Alcohol Degradation
- Aldehyde Degradation
- Amine and Polyamine Degradation
- Amino Acid Degradation
- Aromatic Compound Degradation
- C1 Compound Utilization and Assimilation
- Carbohydrate Degradation
- Carboxylate Degradation
- Chlorinated Compound Degradation
- Degradation/ilization/Assimilation - Other
- Fatty Acid and Lipid Degradation
- Inorganic Nutrient Metabolism
- Nucleoside and Nucleotide Degradation
- Polymeric Compound Degradation
- Secondary Metabolite Degradation

Deloxification:
- Antibiotic Resistance
- methanol oxidation to carbon dioxide

Generation of Precursor Metabolite and Energy:
- 1,5-anhydrofructose degradation
- Electron Transfer
- Entner-Duodoroff Pathways
- ethylmalonyl-CoA pathway
- Fermentation
- formaldehyde oxidation |
- Glycolysis
- glyoxylate cycle
- isopropanol biosynthesis
- methyl ketone biosynthesis
- methylaspartate cycle
- Pentose Phosphate Pathways
- Photosynthesis
- Respiration
- superpathway of glycolysis and Entner-Doudoroff
- superpathway of glycolysis, pyruvate dehydrogenase, TCA, and glyoxylate bypass
- TCA cycle

Glycan Pathways:
- Glycan Biosynthesis
- Glycan Degradation

Macromolecule Modification:
- Nucleic Acid Processing
- Protein Modification

Metabolic Clusters:
- L-glutamate and L-glutamine biosynthesis
- O-antigen building blocks biosynthesis (E. coli)
- phospholipases
- pyrimidine deoxyribonucleotide phosphorylation
- pyrimidine deoxyribonucleotides biosynthesis from CTP
- pyrimidine deoxyribonucleotides de novo biosynthesis I
- pyrimidine deoxyribonucleotides de novo biosynthesis III
- pyrimidine deoxyribonucleotides de novo biosynthesis IV
- superpathway of L -aspartate and L-asparagine biosynthesis
- tRNA charging

X-axis: Relative Abundance (0, 6000, 12000, 18000, 24000, 30000, 36000)