Comparison and diversity of gut microbiota between invasive golden apple snail (*Pomacea canaliculata*) and native Chinese mud snail (*Cipangopaludina chinensis*)

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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 in southern China. Comparing the gut microbiota of these two species could help clarify the invasive mechanism of golden apple snails at the microbial level.

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

Results. In total, 27 phyla were identified in the *P. canaliculata* (PC) group, including dominant Proteobacteria (average relative abundance of 53.78% ± 15.59%), Bacteroidetes (23.19% ± 11.38%), and Firmicutes (16.00% ± 6.91%). In total, 12 phyla were identified in the *C. chinensis* (CC) group, although only Proteobacteria (98.94% ± 0.20%) had a relative abundance >2%. 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 ($P < 0.05$). 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 ($P < 0.05$).

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Abstract

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

Introduction

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

The association between hosts and gut microbiota can contribute to our understanding of the longevity, metabolism, development, and physiology of invertebrates (Lee and Hase 2014). In shrimp, gut microbiota are suggested to affect visible nutrient acquisition and disease incidence, which is a major contributor to population restriction (Xiong 2018). Similarly, gut microbiota in other aquatic invertebrates, such as *Daphnia*, play an important role in improvement of high-temperature resistance and antiviral ability (Akbar et al. 2021). Cellulolytic bacteria in the giant African snail (*Achatina fulica*) gut play an important role in cellulose decomposition (Pinheiro et al. 2015). Therefore, comparing the diversity and structure of the gut microbiota between sympatric invasive and native freshwater species may help clarify the underlying mechanisms related to invasion.

Various studies have shown that the gut microbiota can effectively resist internal pathogen invasion and can promote successful biological invasion into new environments (Zhang et al. 2018, Habineza et al. 2019, Becker et al. 2021). The golden apple snail (*Pomacea canaliculata*) is listed among the 100 worst invasive alien species worldwide by the International Union for Conservative of Nature and the Invasive Species Specialist Group. This snail causes serious damage to aquatic crops, as well as to wetland floral diversity and ecosystem functioning

(Carlsson et al. 2004, Lowe et al. 2000). The Chinese mud snail (*Cipangopaludina chinensis*) is a native species and popular aquatic food in China. Both snail species occupy similar freshwater habitats, but with different feeding habits. Chinese mud snails feed mainly on plant debris, whereas the golden apple snail has a voracious appetite for vegetation, including rice (*Oryza sativa*) crops (She et al. 2013). Gut microbiota are closely related to host growth performance (Fan et al. 2019). In addition, research has shown that the gut microbiota of golden apple snails can be impacted by developmental stage, sex, and gut part (Lyra et al. 2018, Li et al. 2019). However, few studies have explored the differences and abundance of gut microbiota in sympatric invasive and native species. In this study, we compared the gut microbiota between the invasive golden apple snail and native Chinese mud snail using 16S rRNA sequencing on the Illumina MiSeq platform. Comparing differences in gut microbes between these two species may help elucidate the potential causes of apple snail invasion.

Materials and Methods

Sample collection and treatment

A total of 20 Chinese mud snails and 20 golden apple snails were collected from the Xiangsi River (25.0459 °N, 110.1128 °E) in Huixian village, Guilin city, Guangxi, China in June 2021. The average shell heights of *P. canaliculata* and *C. chinensis* were 3.8 ± 0.3 mm and 5.3 ± 0.3 mm, respectively. The *P. canaliculata* (PC group) and *C. chinensis* (CC group) snails were maintained in 1 L of ultra-pure water in a 25 °C room for 5–7 days. All snails were fed with Chinese cabbage (*Brassica rapa*). Prior to dissection, the snails were starved for 24 h to minimize the amount of partially digested food in the gut.

The shells of snails were cleaned with 70% ethanol two times and ultra-pure water three times. Shell destruction, dissection, and gut extraction were performed on a clean bench. The guts of five snails were mixed as a single sample, with four replicates. Samples were stored in 5-ml aseptic centrifuge tubes at −80 °C before DNA extraction.

16S rRNA gene sequencing

The gut microbiota DNA was extracted using a Fast DNA SPIN Extraction Kit (MP Biomedicals, USA) following the manufacturer's instructions. The isolated DNA was stored at −80 °C until polymerase chain reaction (PCR). The DNA concentration and molecular size were measured using UV spectrophotometry (Eppendorf, BioPhotometer, Germany) and 0.8% agarose gel electrophoresis, respectively. The hypervariable V3-V4 region of the 16S rRNA gene was amplified by PCR using universal bacterial primers (338F: 5'-ACTCCTACGGGAGGGAGCA-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). Each primer included barcode sequences to promote the sequencing of products. The DNA (20 ng) of each sample was amplified in 25-μl reactions consisting of 0.25 μl of Q5 high-fidelity DNA polymerase (NEB, Ipswich, UK), 5 μl of

5-fold reaction buffer, 5 μl of 5-fold high GC buffer, 0.5 μl of dNTP Mix (10 mM), 2 μl of template DNA, 1 μl of each primer (10 mM), and 10.25 μl of ddH_2O. The PCR conditions were as follows: initial denaturation at 98 °C for 5 min followed by 25 cycles of 98 °C for 10 s, 50 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 5 min to ensure the complete extension of PCR products. The PCR products were detected by 2% agarose gel electrophoresis and purified with an AxyPrep DNA Gel Extraction Kit (Axygen, New York, USA). Quantification of PCR products was performed using a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA). A TruSeq Nano DNA LT Library Prep Kit (Illumina, USA) was used to establish the DNA library. The library was sequenced using a MiSeq Reagent Kit v3 (6 000-cycles-PE) (Illumina, USA) on the MiSeq platform by Personal Biotechnology Co., Ltd. (Shanghai, China).

Data and analysis

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

Alpha and beta diversity estimation

The alpha and beta diversity indices represent the diversity of species within and between biotopes, respectively (Whittaker 1972). Alpha diversity indices, including community richness (Chao1 and Observed species), diversity (Shannon and Simpson), diversity of evolution population (Faith's PD), evenness (Pielou's evenness), and coverage (Good's coverage), were calculated using QIIME2. The calculation methods can be obtained at http://scikit-bio.org/docs/latest/generated/skbio.diversity.alpha.html#module-skbio.diversity.alpha. We used R software to sketch box plots of estimators with an intuitive form. Significance between groups was tested using the Wilcoxon rank-sum and Dunn tests.

To estimate beta diversity or similarity, differences in relative abundance of OTUs between two groups were detected using the nonmetric multidimensional scaling (NMDS). Analysis of similarities (ANOSIM) based on Bray-Curtis distance and weighted UniFrac distance with 999 permutations was used to determine similarities between two groups. Differences in bacterial abundances (from phylum to family) between the two species were tested using linear discriminant analysis effect size (LEfSe).

Functional prediction and statistical analysis of gut microbiota

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2) software (https://github.com/picrust/picrust2/wiki) was used to predict community

functions of the gut microbiota (Douglas et al. 2020). Greengenes ID corresponding to each OTU was searched in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and was classified to the relevant KEGG pathway. Numbers of functional genes in each pathway were calculated to compare functional enrichment in gut microbiota between the two groups. Statistical analyses were conducted using independent-samples *t*-test in SPSS (v19.0) at a significance level of $\alpha = 0.05$.

Results

Diversity and composition of *P. canaliculata* and *C. chinensis* gut microbiota

A total of 737 690 high-quality sequences were obtained from all eight snail DNA samples after quality control and filtration, including 391 551 reads from the PC group and 346 139 reads from the CC group (ranging from 46 498–187 205 reads per sample). We re-sampled all samples based on the smallest number of reads (46 498) to correct for differences in read number. Rarefaction curves based on the Shannon index for all samples ranged from 99.43%–99.90%, indicating that sequencing depth basically covered all species in the samples (Figure S1).

Filtered sequences were clustered into OTUs at a 97% identity, and we obtained 5 911 valid OTUs after removing those with relative abundances <0.001%, including 4 927 of *P. canaliculata* and 1 080 of *C. chinensis*. Comparing these OTUs with the SILVA database, 6 007 OTUs were annotated to 29 phyla, 52 classes, 123 orders, 247 families, 575 genera, and 963 species. For the PC group, the 4 927 OTUs were annotated to 27 phyla, 49 classes, 111 orders, 239 families, 553 genera, and 894 species. For the CC group, the 1 080 OTUs were annotated to 12 phyla, 18 classes, 44 orders, 67 families, 115 genera, and 150 species (Figure S2). In addition, the Venn diagram indicated that 96 OTUs existed in both groups. Compared to the CC group (984), however, the number of specific OTUs was much higher in the PC group (4 831) (Figure S3).

Based on alpha diversity analysis, the Shannon and Simpson indices indicated that gut microbiota diversity did not differ significantly between the two groups ($P > 0.05$). However, the Chao1 and Observed species indices indicated that gut microbiota abundance was significantly higher in the PC group than in the CC group ($P < 0.05$) (Figure 1).

Taxonomic composition of *P. canaliculata* and *C. chinensis* gut microbiota

From all eight samples, the top 10 gut microbiota phyla were Proteobacteria (76.36% ± 11.18%), Bacteroidetes (11.78% ± 6.81%), Firmicutes (8.25% ± 4.34%), Epsilonbacteraeota (2.14% ± 1.42%), Spirochaetes (0.50% ± 0.47%), Acidobacteria (0.28% ± 0.16%), Tenericutes (0.26% ± 0.25%), Actinobacteria (0.23% ± 0.09%), Verrucomicrobia (0.05% ± 0.03%), and Fibrobacteres (0.03% ± 0.03%) (Figure 2).

Comparing bacterial composition between the two groups, microbes with a relative abundance >2% were defined as dominant phyla. Results showed that Proteobacteria was the dominant phylum in both groups, accounting for 53.78% ± 15.59% in the PC group and 98.94% ± 0.20% in the CC group. In the PC group, three other phyla were found with a relative abundance >2%, including Bacteroidetes (23.19% ± 11.38%), Firmicutes (16.00% ± 6.91%), and Epsilonbacteraeota (4.28% ± 2.53%). For the CC group, Proteobacteria was the only phylum with a relative abundance >2%. Of the top 10 phyla, only seven existed in the CC group, with Spirochaetes, Tenericutes, and Fibrobacteres not detected (Figure 2).

At the genus level, the top 10 genera were *Chryseobacterium* (7.31% ± 6.72%), *Lactococcus* (5.02% ± 4.19%), *Uliginosibacterium* (4.86% ± 3.39%), *Enterobacter* (3.39% ± 1.66%), *Aquitalea* (3.06% ± 1.86%), *Novispirillum* (2.06% ± 1.35%), *Sulfurospirillum* (1.75% ± 1.23%), *Bacteroides* (1.25% ± 0.97%), *Hafnia-Obesumbacterium* (1.25% ± 1.12%), and *Dechloromonas* (1.23% ± 0.71%) (Figure 3). In the PC group, the dominant genus was *Chryseobacterium* (14.62% ± 13.24%), followed by *Lactococcus* (9.78% ± 8.18%), *Uliginosibacterium* (9.73% ± 6.16%), *Aquitalea* (6.13% ± 3.13%), *Novispirillum* (4.11% ± 2.39%), *Sulfurospirillum* (3.50% ± 2.24%), *Dechloromonas* (2.46% ± 2.03%), and *Bacteroides* (2.17% ± 1.95%) (Figure 3). In the CC group, the dominant genera were *Enterobacter* (6.73% ± 2.31%) and *Hafnia-Obesumbacterium* (2.49% ± 2.18%). Only four of the top 10 genera were found in the CC group, with *Chryseobacterium*, *Uliginosibacterium*, *Aquitalea*, *Novispirillum*, *Sulfurospirillum* and *Dechloromonas* not detected (Figure 3). In addition, based on LEfSe analysis (LDA > 2), 10 phyla and 87 genera were identified as showing significant differences (Figure S4).

Differences in gut microbiota between two snail species

Differences in beta diversity were evaluated using NMDS analysis based on Bray-Curtis and weighted UniFrac distances. Results showed that the two groups could be isolated from each other, and intergroup distance was higher than intragroup distance based on both calculation methods (Figure 4A, B). Moreover, ANOSIM revealed significant differences between the PC and CC groups (Bray-Curtis R = 0.396, P = 0.027, NMDS stress = 0.0000982; weighted UniFrac R = 1, P = 0.03, NMDS stress = 0.0000776), as shown in Figure 4C, D. Similar results were observed from principal coordinate analysis (PCoA) based on Bray-Curtis and weighted UniFrac distances (Figure S5).

Function 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 ($F = 50.279$, $t = -2.861$, $P = 0.029$), amino acid biosynthesis ($F = 0.300$, $t = 17.057$, $P < 0.01$), nucleoside and nucleotide biosynthesis ($F = 4.503$, $t = 10.457$, $P < 0.01$), fatty acid and lipid biosynthesis ($F = 4.901$, $t = 2.887$, $P = 0.028$), carboxylate degradation ($F = 0.256$, $t = -13.277$, $P < 0.01$), secondary metabolite degradation ($F = 21.674$, $t = -10.064$，$P < 0.01$), carbohydrate degradation ($F = 0.889$, $t = -12.963$, $P < 0.01$), nucleoside and nucleotide degradation ($F = 0.018$, $t = 2.533$, $P = 0.044$), aromatic compound degradation ($F = 10.152$, $t = -3.500$, $P = 0.013$), glycolysis ($F = 6.002$, $t = 3.540$, $P = 0.012$), amino acid degradation ($F = 0.320$, $t = -3.725$, $P = 0.010$), C1 compound utilization and assimilation ($F = 1.292$, $t = 4.288$, $P = 0.005$), and aromatic compound biosynthesis ($F = 0.992$, $t = 22.984$, $P < 0.01$) 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. 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). Moreover, gut microbiota diversity and abundance are reported to differ significantly in invasive *Oreochromis mossambicus* fish from different habitats (Gaikwad et al. 2017). 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). 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 microbiota diversity between the two freshwater snails, which is likely due to their similar habitats. However, the Chao1 and Observed species indices showed marked differences in gut microbiota abundance between the two groups, with significantly higher abundance in the PC group than in the CC group. Previous research has found that dietary changes are not significantly correlated with alpha diversity but are positively correlated with beta diversity (Li et al. 2016). In our study, the beta diversity results showed that the intestinal microbial community structure of samples within the same species was highly similar, but there were significant differences in the community structure of samples among different species ($P < 0.05$). Golden apple snails have a more extensive diet than Chinese mud snails (Morrison and Hay 2011), which may be a dominant factor related to the

significant differences in beta diversity between the two species. Furthermore, the gut microbiota structure was more complex in the PC group than in the CC group.

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

Proteobacteria was the dominant microbiota in both groups, and primary microbiota in other Gastropoda like *Potamopyrgus antipodarum* and *Achatina fulica* (Cardoso et al. 2012b, Takacs-Vesbach et al. 2016). Moreover, Proteobacteria is related to environmental adaptation due to its ability to secrete lipase, protease, and amylase (Pemberton et al. 1997). Our results also showed that the Proteobacteria genus *Enterobacter* was dominant at the genus level. *Enterobacter* is considered a cellulolytic genus (Pawar et al. 2015, Chen et al. 2021a), with certain members, such as the *Enterobacter* sp. strain Bisph2, able to degrade glyphosate (an organic phosphine herbicide) (Benslama and Boulahrouf 2016), and others, such as *Enterobacter* spH1, able to degrade glucose and glycerin into value-added products (Gueell et al. 2015). Chinese mud snails live in paddy fields, lakes, and rivers (Nakanishi et al. 2014) and primarily feed on diatoms and plant debris (Cui et al. 2012), with no reports of carnivorous behavior has been reported yet. Proteobacteria can help in diatom and plant debris digestion. Therefore, we speculate that the relatively simple “lifestyle” of Chinese mud snails does not require a complex gut microbiota composition.

In contrast, the diets of golden apple snails are more complex and include plants and aquatic

invertebrates, such as *Biomphalaria peregrina*, *Biomphalaria straminea*, and *Physa acuta* (Cazzaniga 1990, Kwong et al. 2009). More complicated diets require more digestion-related bacteria. In addition to digestion-related Proteobacteria, both Bacteroidetes and Firmicutes are related to nutrient absorption (Thomas et al. 2011, Sommer et al. 2016). Bacteroidetes functions in the degradation of high molecular weight organic matter, while Firmicutes is related to the degradation of lipids and dietary fiber, indicating that the relative proportions of Firmicutes and Bacteroidetes are closely related to the feeding habits and metabolism of the host (Chen et al. 2011, Evans et al. 2011). Previous research has shown that the relative abundance of Bacteroidetes is lower in fat rats than normal rats, while Firmicutes is higher in fat rats than normal rats (Turnbaugh et al. 2006). In addition, the ratio of these two phyla can have a significant influence on energy absorption in rats (Turnbaugh et al. 2006). The functions of these two phyla have also been explored in invertebrates (Wang et al. 2020b). In the current study, average Bacteroidetes and Firmicutes abundances were significantly higher in the PC group (23.20% and 16.00%, respectively) than in the CC group (0.34% and 0.49%, respectively). The *Chryseobacterium* genus was only found in the PC group, and the relative abundance of *Lactococcus* was significantly higher in the PC group than in the CC group. *Chryseobacterium* can decompose lignocellulose (Carlos et al. 2018, Puentes-Tellez and Salles 2018, Weiss et al. 2021). In addition, study on the American cockroach (*Periplaneta americana*) reported that *Chryseobacterium* is only found in a high-fiber diet, indicating that *Chryseobacterium* can decompose fiber (Dugas et al. 2001). As gram-positive bacteria, *Lactococcus* species are recognized as safe microorganisms for food production and can produce L-lactic acid through acidification to provide energy (Casalta and Montel 2008). Therefore, the higher relative abundance and complex composition of gut microbiota in *P. canaliculata* may be one of the reasons for its high survival and adaptability, and thus its successful invasion.

Among the top 10 dominant phyla, three existed in the PC group only: i.e., Spirochaetes, Fibrobacteres, and Tenericutes. In recent years, less attention has been paid to the function of spirochetes, with most studies limited to pathogens involved in Lyme disease, recurrent fever, and syphilis (Radolf et al. 2012, Hook 2017, Gattorno et al. 2019). In the current study, we speculate that the Spirochaetes bacteria are parasites rather than functional bacteria in the golden apple snails. Fibrobacteres is a well-known primary degrader of cellulose in the intestinal tract of herbivores and can hydrolyze polymer using a distinctive set of glycoside hydrolases and binding domains (Rahman et al. 2016). Tenericutes is suggested to be involved in carbohydrate storage, carbon fixation, and environmental response (Wang et al. 2020a). In this study, both Fibrobacteres and Tenericutes were unique dominant microbiota in golden apple snails, which may be related to their high invasiveness.

Functional KEGG predictions indicated that many functions were significantly different

between the PC and CC groups, including cofactor, prosthetic group, electron carrier, vitamin biosynthesis, amino acid biosynthesis, nucleoside and nucleotide biosynthesis, fatty acid and lipid biosynthesis, and carboxylate degradation, consistent with previous studies on indigenous species *Helix pomatia* and invasive species *Achatina fulica* (Cardoso et al. 2012a, Nicolai et al. 2015).. Of these functions, the relative abundance in the PC group was significantly higher than that in the CC group, including amino acid biosynthesis, fatty acid and lipid biosynthesis, aromatic compound biosynthesis, and C1 compound utilization and assimilation. Gut microbiota are crucial to host amino acid homeostasis and health (Mardinoglu et al. 2015). Several genera are known to play crucial roles in amino acid biosynthesis, including *Fusobacterium*, *Bacteroides*, and *Veillonella* (Lin et al. 2017). Additionally, *Lactococcus* and *Bacteroides* are associated with fatty acid and lipid biosynthesis (Liu and Meng 2008, Tanca et al. 2018). In this study, the relative abundances of *Bacteroides* and *Lactococcus* were significantly higher in the PC group and are suggested to be the main factors for the biosynthesis of basic elements. In addition to fatty acid and lipid biosynthesis, *Lactococcus*, a dominant genus in our study, also produces exopolysaccharide and aromatic compounds (Casalta and Montel 2008). Our results indicated that the two species showed considerable differences in gut microbiota functions.

Conclusions

Our study showed significant differences in the relative abundance and community structure of the gut microbiota between the golden apple snail and Chinese mud snail. Our results provide new insights and theoretical evidence for the invasion mechanism of *P. canaliculata*. Thus, greater attention should be paid to the connection between the environment and gut microbiota of *P. canaliculata* in future studies.

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Competing interests

The authors declare that they have no competing interests.

Authors Contributions

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

Availability of data and materials

The data are available at the National Center for Biotechnology Information（NCBI）BioProject: PRJNA756881.

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Figure 1 Alpha diversity indexes summary.

Figure 2 Relative abundance of top 10 OTU's for *P. canaliculata* and *C. chinensis* at the phylum level.

Figure 3 Relative abundance of top 10 OTU's for *P. canaliculata* and *C. chinensis* at the genus level.

Figure 4 NMDS and ANOSIM analysis based on (A, C) Bray-Curtis and (B, D) weighted UniFrac distances of gut microbiota on OTU level.

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

Figure S1 Rarefaction Curve based on Shannon index.

Figure S2 The content of operational taxonomic units (OTUs) and different bacterial taxonomic units of each sample.

Figure S3 Venn diagram of shared and unique OTUs among different groups.

Figure S4 The linear discriminant analysis (LDA) of two groups with the LDA >2.

Figure S5 PCoA analysis based on (a) unweighted and (b) weighted Unifrac distances of gut microbiome on OTU level.

Figure S6 The prediction of two groups in KEGG database in different annotation abundance.

Figure 1

Alpha diversity indexes summary.

Note：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.

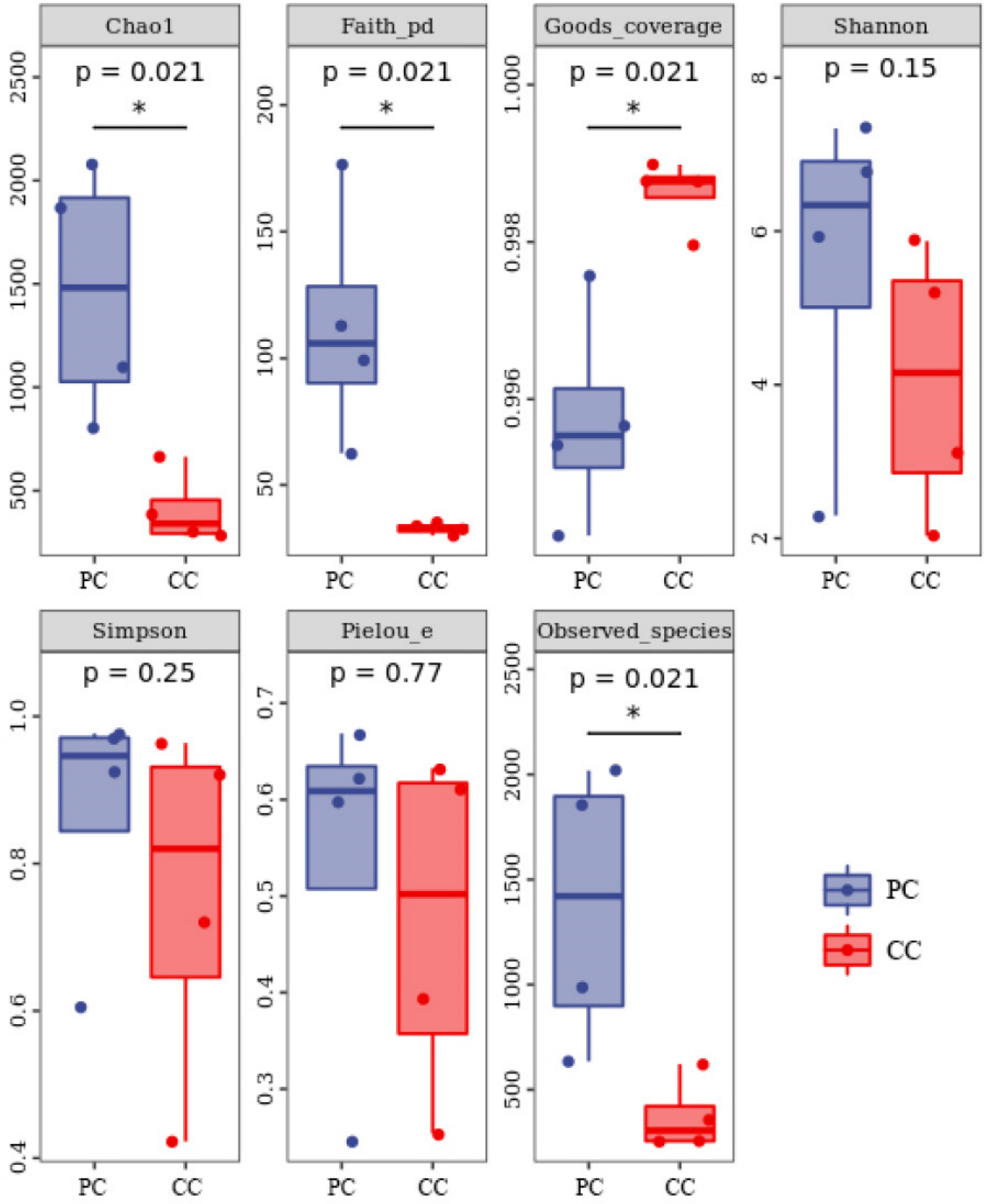
Chao1
p = 0.021
*
2500
2000
1500
1000
500
PC
CC
Faith_pd
p = 0.021
*
200
150
100
50
PC
CC
Goods_coverage
p = 0.021
*
1.000
0.998
0.996
PC
CC
Shannon
p = 0.15
8
6
4
2
PC
CC
Simpson
p = 0.25
1.0
0.8
0.6
0.4
PC
CC
Pielou_e
p = 0.77
0.7
0.6
0.5
0.4
0.3
PC
CC
Observed_species
p = 0.021
*
2500
2000
1500
1000
500
PC
CC
PC
CC

Figure 2

Relative abundance of top 10 OTU's for *P. canaliculata* and *C. chinensis* at the phylum level.

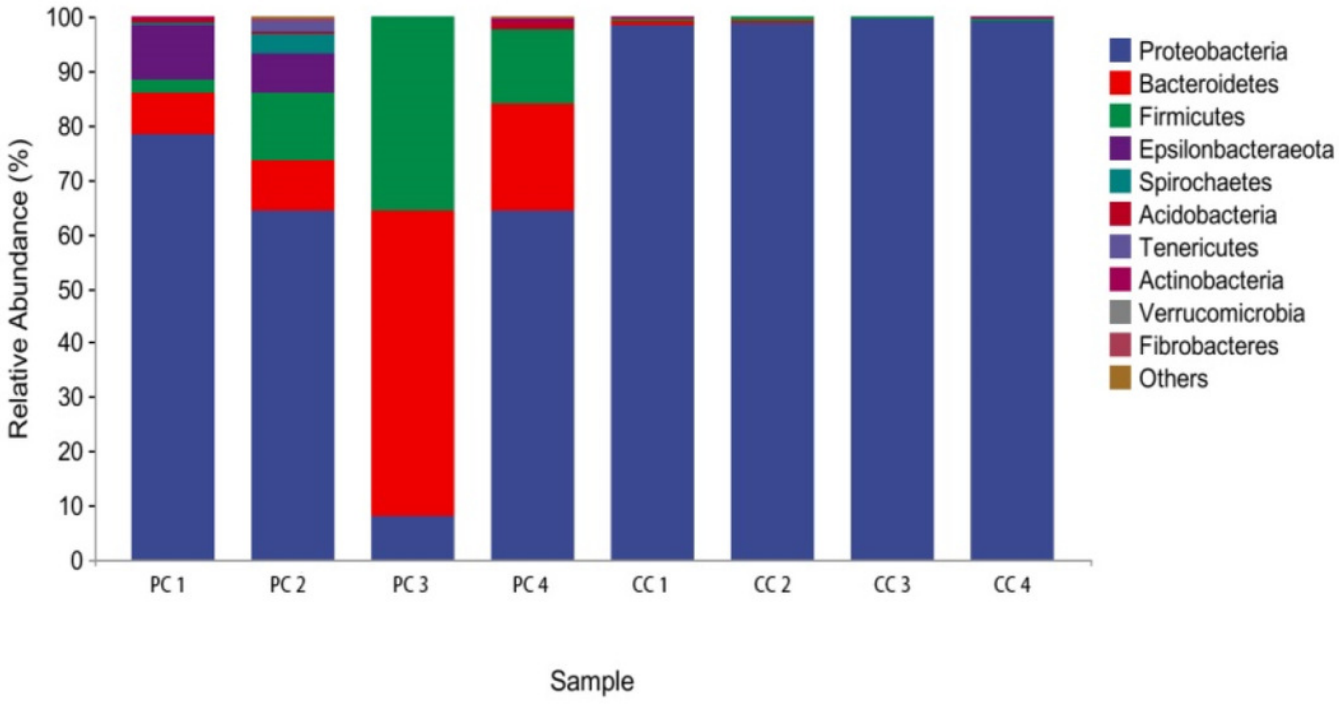

Figure 3

Relative abundance of top 10 OTU's for *P. canaliculata* and *C. chinensis* at the genus level.

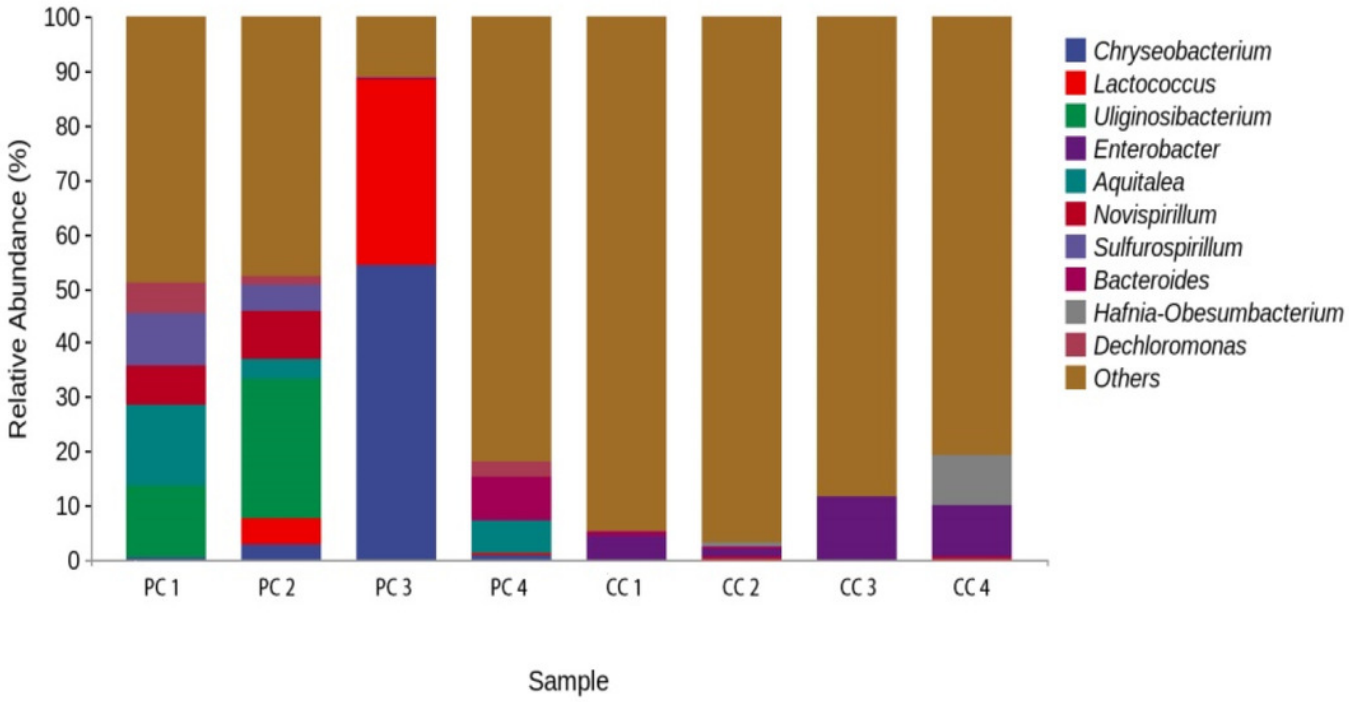

Figure 4

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

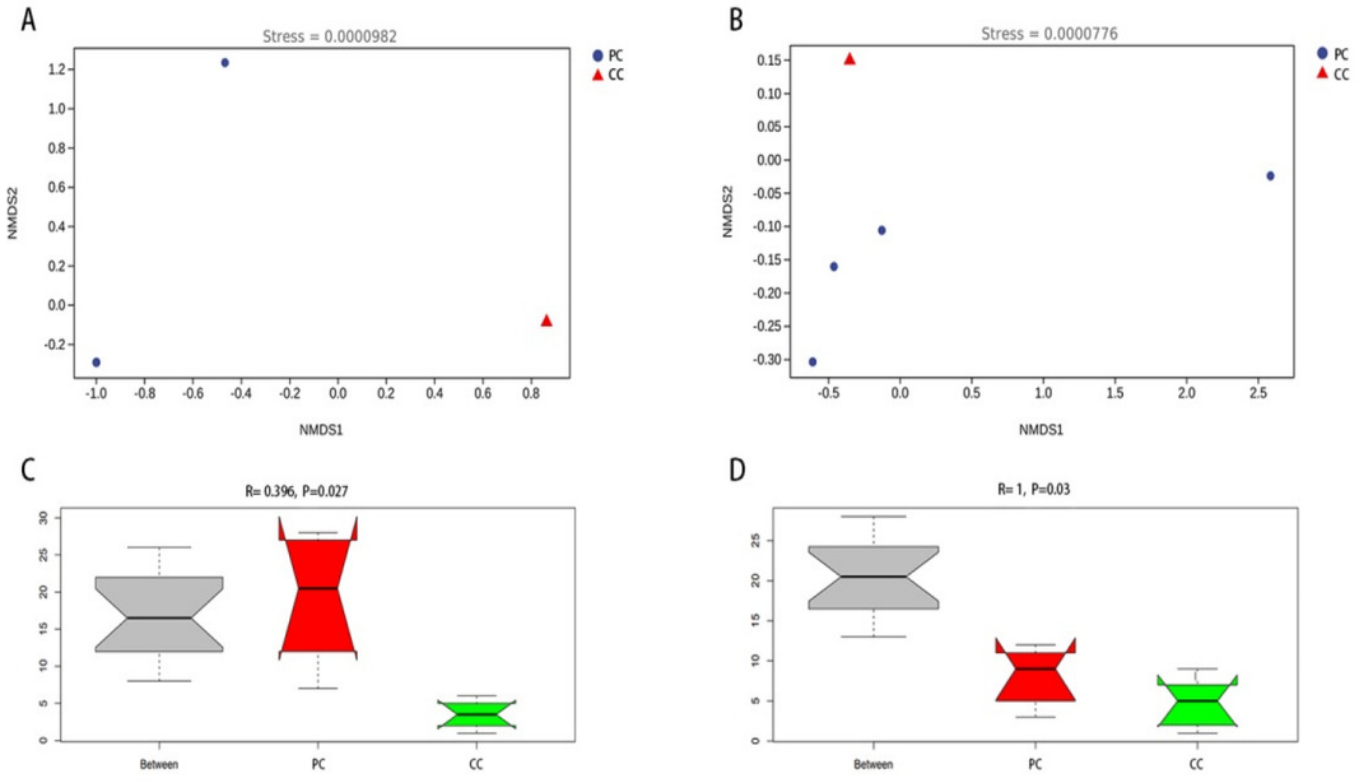

Figure 5

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


Level 2
Level 1
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
Biosynthesis
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/Utilization/Assimilation - Other
Fatty Acid and Lipid Degradation
Inorganic Nutrient Metabolism
Nucleoside and Nucleotide Degradation
Polymeric Compound Degradation
Secondary Metabolite Degradation
Degradation/Utilization/Assimilation
Antibiotic Resistance
methanol oxidation to carbon dioxide
Detoxification
1,5-anhydrofructose degradation
Electron Transfer
Entner-Duodoroff Pathways
ethylmalonyl-CoA pathway
Fermentation
formaldehyde oxidation I
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
Generation of Precursor Metabolite and Energy
Glycan Biosynthesis
Glycan Degradation
Glycan Pathways
Nucleic Acid Processing
Protein Modification
Macromolecule Modification
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
Metabolic Clusters
0
6000
12000
18000
24000
30000
36000
Relative Abundance