Biological Invasions

Revealing the mechanism of native and non-native snail coexistence through gut microbiome composition and structure

--Manuscript Draft--

Manuscript Number:	BINV-D-24-00082
Full Title:	Revealing the mechanism of native and non-native snail coexistence through gut microbiome composition and structure
Article Type:	Invasion note
Keywords:	coexistence; gut microbiome; non-native snail; Rumina decollata
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Funding Information:	key project of the open competition of Forestry Administration of Jiangsu Province (LYKJ[2022]01) — Dr. Honghua Ruan National College Students Innovation and Entrepreneurship Training Program (202310298062Z) — Ms. Yawen Bai
Abstract:	Elucidating the coexistence mechanisms of species is essential for the conservation of biodiversity. However, it is unclear which roles certain species play in a given ecosystem. This study revealed a noteworthy phenomenon, where a non-native snail (Rumina decollata - a highly invasive species reported by various countries) coexisted in large numbers with two local snail species (Bradybaena ravida and Euphaedusa aculus) without exhibiting apparent competition or predation. To understand the mechanism behind the coexistence of non-native and native species in large numbers, the compositions and structures of the gut microbiomes of R. decollata, B. ravida, and E. aculus (collected in Nanjing, China) were analyzed. The composition of the E. aculus gut microbiome was similar to that of R. decollata, while it was quite distinct from that of B. ravida. This implied that the gut microbiomes of snails were highly adaptable to variable environmental conditions, while being minimally influenced by host genetics. Another finding of this study was the observation of adaptive changes in

	the gut microbiome of R. decollata subsequent to its introduction. These changes may have been attributed to changes in the feeding behavior of R. decollata, which led to modifications in its ecological niche. The coexistence of R. decollata with native snails may have been due to a sufficient environmental carrying capacity, which prevented the emergence of strong competition. However, the specific dietary changes of R. decollata and its propagation still need to be continuously monitored to protect ecological security.
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Revealing the mechanism of native and non-native snail coexistence through gut microbiome composition and structure

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Abstract

Elucidating the coexistence mechanisms of species is essential for the conservation of biodiversity. However, it is unclear which roles certain species play in a given ecosystem. This study revealed a noteworthy phenomenon, where a non-native snail (*Rumina decollata* - a highly invasive species reported by various countries) coexisted in large numbers with two local snail species (*Bradybaena ravida* and *Euphaedusa aculus*) without exhibiting apparent competition or predation. To understand the mechanism behind the coexistence of non-native and native species in large numbers, the compositions and structures of the gut microbiomes of *R. decollata*, *B. ravida*, and *E. aculus* (collected in Nanjing, China) were analyzed. The composition of the *E. aculus* gut microbiome was similar to that of *R. decollata*, while it was quite distinct from that of *B. ravida*. This implied that the gut microbiomes of snails were highly adaptable to variable environmental conditions, while being minimally influenced by host genetics. Another finding of this study was the observation of adaptive changes in the gut microbiome of *R. decollata* subsequent to its introduction. These changes may have been attributed to changes in the feeding behavior of *R. decollata*, which led to modifications in its ecological niche. The coexistence of *R. decollata* with native snails may have been due to a sufficient environmental carrying capacity, which prevented the emergence of strong competition. However, the specific dietary changes of *R. decollata* and its propagation still need to be continuously monitored to protect ecological security.

Keywords: coexistence; gut microbiome; non-native snail; *Rumina decollata*

Introduction

The introduction of, or invasion by non-native species into local environments often has serious impacts on their biodiversity. The decollate snail, *Rumina decollata* (Stylommatophora: Achatinidae), which is native to the Mediterranean region **(Cowie, 2001)** is a gastropod species known for its high level of invasiveness. The expansion of its range is likely attributed to both unintentional and deliberate human dispersal, resulting in its widespread propagation to numerous countries and islands across Europe, Asia, Africa, and the Americas **(Pizá et al., 2023)**. Further, it has been reported in China and found in large quantities over the last century **(Chang and Chung, 1993; Francesco and Lagiglia, 2007)**. Although China has not yet designated it as an invasive species, the potential risk of its invasion cannot be ignored. *R. decollata* exhibits omnivorous and detritivorous feeding habits **(Cardillo et al., 2018)** and has been observed to prey on other land snails, worms, and insects **(McDonnell et al., 2016; Cardillo et al., 2018)**.

The introduction of non-native species often disrupts the ecological dynamics of original ecosystems. If they are highly competitive for resources or exert significant predation pressure on native species, this can lead to a significant decline, or even the extinction of entire populations of native species, resulting in the loss of biodiversity **(Davis, 2003)**. However, in this study (conducted in late autumn) (Fig. 1) *R. decollata* was found to congregate in large numbers with two native snail species (*Bradybaena ravida* and *Euphaedusa aculus*).

As is the case with other organisms, invasive species coexist and engage with their microbiome, which plays a critical role in their biology and ecology **(Bahrndorff et al., 2016)**. Although the microbiomes of various host species have been extensively investigated, their assessment in the context of invasion has been relatively limited **(Goddard-Dwyer et al., 2021)**. It has been proposed that an increased variability or diversity of functions exhibited by the gut microbiomes of invasive species might potentially enhance their capacity to adapt to a wider array of nutritional niches toward facilitating their establishment **(Bahrndorff et al., 2016; Goddard-Dwyer et al., 2021; Chiarello et al., 2022)**. An analysis of the gut microbiomes of non-native *R. decollata* snails may provide insights into why they can congregate and thrive in large numbers along with native snails such as *B. ravida* and *E. aculus*.

Materials and methods

The experimental models for this study were collected in late October 2022 in Nanjing (118°48'44"E, 32°4'55"N) from a sample of 10 snails of three different species (Fig. 1). Following morphological and molecular identification, the collected samples were transferred to sterile petri dishes to obtain fecal samples, which were the promptly frozen at -80°C pending analyses.

A total of 24 fecal samples (six from *B. ravida*, eight from *E. aculus*, and 10 from *R. decollata*) were collected for the extraction of DNA from the microbiome, which were sent to Shanghai Personal Biotechnology Co., Ltd for next-generation sequencing on the V3-V4 regions of the 16S rRNA genes. Among them, five samples of *B. ravida*, five of *E. aculus*, and nine of *R. decollata* were successfully sequenced. Library construction and sequencing were performed using the NovaSeq6000 platform. High-quality sequences were clustered into Amplicon Sequence Variants (ASV) using a threshold of ~100% sequence identity. These ASVs were then compared with the Greengenes database for taxonomic annotation **(DeSantis et al., 2006)**. Bioinformatics analyses were performed using the genescloud tools (https://www.genescloud.cn).

Results

A total of 1,831,631 raw reads were obtained from 19 fecal samples. Following the elimination of chimeras and filtering out of low-quality sequences, 1,605,345 high-quality sequences remained for further analysis. In terms of sequence volume, these sequences represented 87.65% of the initial dataset.

At the phylum level, the dominant bacterial phyla of *B. ravida* were Proteobacteria (54.03%), Tenericutes (34.93%), and Cyanobacteria (3.71%); for *E. aculus*, Proteobacteria (66.07%), Actinobacteria (13.92%), and Bacteroidetes (8.85%); and for *R. decollata*, Proteobacteria (68.63%), Bacteroidetes (9.30%), and Cyanobacteria (6.80%) (Fig. 2a). At the genus level, the dominant bacterial genera of *B. ravida* were

Mycoplasma (34.93%), *Agrobacterium* (3.61%), and *Pseudomonas* (1.38%); for *E. aculus*, *Agrobacterium* (4.49%), *Bacillus* (4.20%), and *Flavobacterium* (4.04%); and for *R. decollata*, *Agrobacterium* (4.08%), *Sphingobacterium* (3.73%), and *Massilia* (3.53%) (Fig. 2a).

For alpha diversity analysis (Fig. 2b), the Chao1, Shannon, Simpson indices and observed species indicated that the diversity of the *E. aculus* gut microbiome had the highest values. Although the abundance of the *B. ravida* gut microbiome was found to be greater than that of *R. decollata*, the diversity of the *R. decollata* gut microbiome was higher. Samples from *E. aculus* and *R. decollata* tended to cluster together based on the Bray-Curtis PCoA (Fig. 2c). However, there was a considerable distance observed between the *B. ravida* and *E. aculus*+*R. decollata* gut microbiome samples, which suggested significant differences in their community structures. Conversely, the gut microbiome community structures of *E. aculus* and *R. decollata* appeared to be relatively similar. The LefSe analysis results (Fig. 2d) revealed that the microbiome of *R. decollata* was characterized by the presence of significant taxa, including *Sphingomonas*, *Microbacterium*, *Pediococcus*, and *Methylobacterium*.

Discussion

Among the prominent microbiome in *R. decollata* (Fig. 2d), *Methylobacterium* was reported to be present in *Bombyx mori* **(Hao et al., 2019)**, which are known to be herbivorous. Additionally, *Sphingomonas* has the capacity to metabolize lignin **(Masai et al., 2007)**. Thus, the presence of these bacteria indicated a potential feeding behavior of *R. decollata* toward herbivorous in China.

It is noteworthy that *B. ravida* and *E. aculus* are a more closely related native species **(Zhao et al., 2023)** in contrast to *R. decollata*, which is a non-native species with a more distant genetic relationship **(Zhao et al., 2023)**. The composition of the *E. aculus* gut microbiome closely resembled that of *R. decollata*, while being significantly distinct that of *B. ravida* (Figs. 2a, c). Further, the compositions of *R. decollata* and *Achatina fulica* gut microbiomes (counterpart members of the family Achatinidae) revealed significant differences. The core *A. fulica* microbiome consisted of *Lactococcus* (26.3%), *Citrobacter* (15.1%), and *Mycoplasma* (12.4%) **(Xie et al., 2023)**. This finding indicated that the gut microbiota of non-native snails exhibited significant adaptability to environmental conditions and were minimally affected by host genetics.

When considering potential factors that contributed to the similarity in gut microbiomes between *R. decollata* and *E. aculus*, it was speculated that *R. decollata* (known for its broad dietary preferences) initially consumed other snails **(Cowie, 2001)**, but shifted its diet when food was scarce **(Cardillo et al., 2018)**. *B. ravida* is strictly herbivorous **(Fang et al., 2021; Zhang et a., 2022)**, while *E. aculus* has a broader diet and feeds on plants, microflora, and humus **(Helwerda and Schilthuizen, 2014)**. Consequently, the ecological niche of *R. decollata* might undergo modifications following its introduction/invasion. This may translate to adaptive changes in the composition of its gut microbiome, showing a tendency to be more akin to *E. aculus*, which also has a broad diet, while differing significantly from *B. ravida*. Studies have shown that although non-native species have the potential to carry gut microbes that are co-introduced from their native range, they may also acquire new gut microbes locally **(Parker et al., 2006)**. Thus, the ability of the organism to adapt to the environment is enhanced **(Chiarello et al., 2022)**.

It is generally believed that there are competitive or predatory relationships between native and non-native species in the same community **(Evans, 2021; Soares et al., 2021)**. The gut microbiome of *R. decollata* revealed a potential transition in its diet from being primarily carnivorous, to herbivorous or saprophagous; thus, the interspecific relationship between them should not be predation. The gut microbiomes of *R. decollata* and *E. aculus* were similar while they coexisted in large numbers, which was likely due to a considerable environmental carrying capacity. Although *R.*

decollata is not currently designated as invasive in China, and has not shown obvious competition or predation relationships with native species, the further monitoring of its specific dietary changes and future population trends are essential for the protection of China's ecological security.

Declarations

Author Contributions

LHY conceived the study. RHH and BYW acquired the funds. CYK, WJC, LTT, and LZY conducted the sampling. XW, BYW, and XTJ carried out the bioinformatics analysis. XW drafted the manuscript. RHH and LHY reviewed and revised the manuscript. All authors approved the final manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

All data used or analyzed during this study are available from the corresponding author upon reasonable request. Representative nucleic acid sequences reported in this paper have been submitted to NCBI (https://www.ncbi.nlm.nih.gov/) GenBank database under the accession number PRJNA1047744.

Funding

This work was supported by the key project of the open competition of Forestry Administration of Jiangsu Province (LYKJ[2022]01) and the National College Students Innovation and Entrepreneurship Training Program (202310298062Z).

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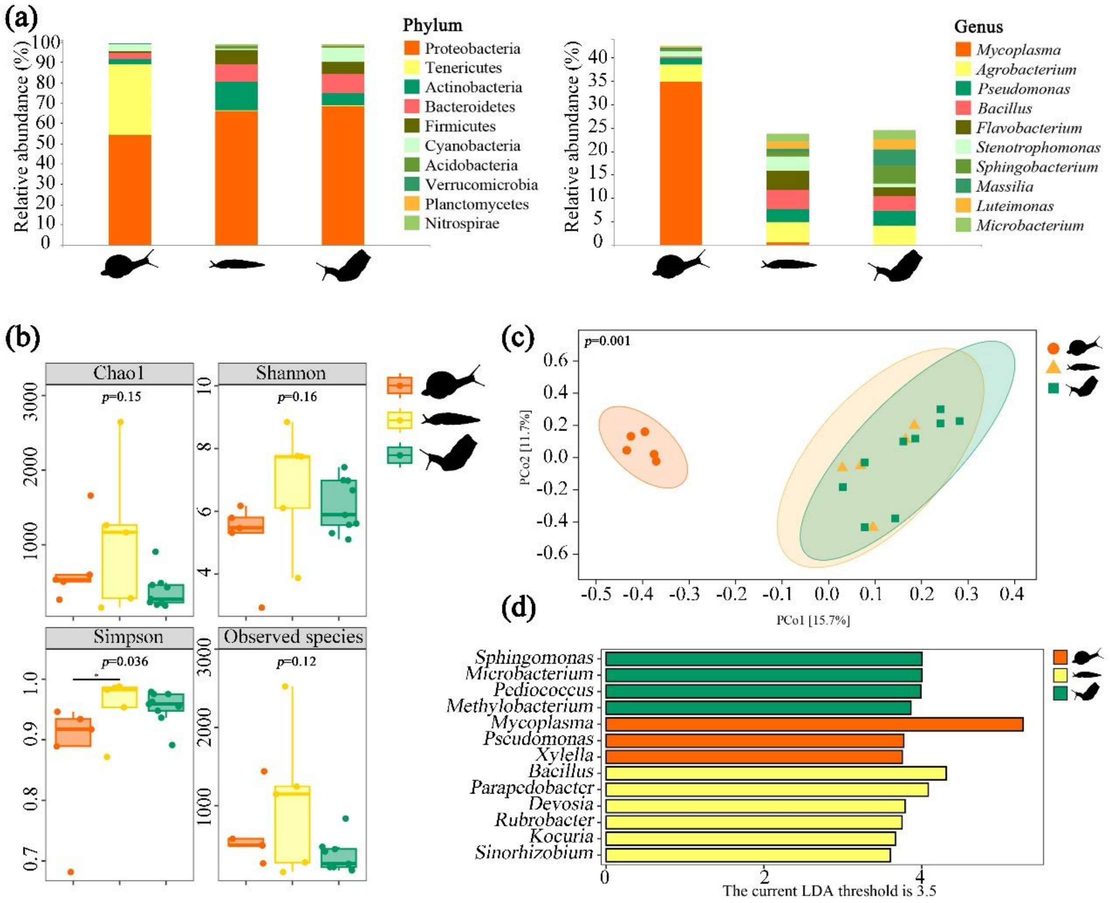
Figures

Fig. 1 Ecological image showing *Bradybaena ravida*, *Euphaedusa aculus*, and *Rumina decollata* congregated during the late autumn, with photographs of the three organisms.

Fig. 2 Relative microbiome abundances at the phylum and genus levels (Top 10) (a); Alpha diversity of *Bradybaena ravida*, *Euphaedusa aculus*, and *Rumina decollata* (b); Beta diversity of *B. ravida*, *E. aculus*, and *R. decollata* (c); LEfSe analysis of *B. ravida*, *E. aculus*, and *R. decollata* at genus level (d).

Fig. 1 Photograph showing *Bradybaena ravida*, *Euphaedusa aculus*, and *Rumina decollata* congregated during late autumn.



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February 13, 2024

Biological Invasions
Dear Editor,

I wish to submit an original paper titled “**Revealing the coexistence of native and non-native snails using composition and structure of gut microbiome**” for publication in the *Biological Invasions*. The paper was coauthored by Wei Xu, Yawen Bai, Tangjun Xu, Yukun Chen, Jiachen Wang, Tengteng Li, Zeyang Liu, Hongyi Liu, and Honghua Ruan.

Elucidating the coexistence mechanisms of species is essential for the conservation of biodiversity. However, it is unclear which roles certain species play in a given ecosystem. This study revealed a noteworthy phenomenon, where a non-native snail (*Rumina decollata* - a highly invasive species reported by various countries) coexisted in large numbers with two local snail species (*Bradybaena ravida* and *Euphaedusa aculus*) without exhibiting apparent competition or predation. To understand the mechanism behind the coexistence of non-native and native species in large numbers, the compositions and structures of the gut microbiomes of *R. decollata*, *B. ravida*, and *E. aculus* (collected in Nanjing, China) were analyzed. The composition of the *E. aculus* gut microbiome was similar to that of *R. decollata*, while it was quite distinct from that of *B. ravida*. This implied that the gut microbiomes of snails were highly adaptable to variable environmental conditions, while being minimally influenced by host genetics. Another finding of this study was the observation of adaptive changes in the gut microbiome of *R. decollata* subsequent to its introduction. These changes may have been attributed to changes in the feeding behavior of *R. decollata*, which led to modifications in its ecological niche. The coexistence of *R. decollata* with native snails may have been due to a sufficient environmental carrying capacity, which prevented the emergence of strong competition. However, the specific dietary changes of *R. decollata* and its propagation still need to be continuously monitored to protect ecological security.

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. We have read and understood your journal’s policies, and we believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare.

Thank you for your consideration. I look forward to hearing from you.

Sincerely,

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