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Comparative Study on the Differences in Gut Microbiota Function and Body Mass Regulation in *Tupaia belangeri* between Two Regions: The Role of Ecological Factors

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ABSTRACT

Background: The gut microbiota plays an important regulatory role in the growth and development of animals and the gut microbiota structure of wild animals in different regions exhibits different adaptation patterns. Investigating the gut microbiota structure and body weight of *Tupaia belangeri* from different regions can provide deeper insights into their adaptation patterns.

Methods: This study investigated the impacts of environmental variables, specifically temperature and humidity, on the composition of gut microbiota and key physiological parameters in *T. belangeri* across distinct geographic regions. In the current research, body mass and the intestinal microbiota were determined in *T. belangeri* between two regions (Chuxiong, CX; Mile, ML).

Result: The results were as follows: 1) Body mass in ML was significantly higher than that of in CX; 2) There were differences in the dominant phyla and genera of gut bacterial communities between the two regions; 3) Comparative analysis of biodiversity indices revealed non-significant divergence between the two study sites, although differences in endemic genera; 4) Pathogenic bacteria were found to varying degrees in the intestinal tracts in both regions. Overall, we believe that *T. belangeri* can adapt to different environmental temperatures or humidity in the two regions by adjusting its body mass and altering the structural diversity of its gut microbiota.

Key words: Adaptation, Body mass regulation, Community composition, Intestinal microbiota, *Tupaia belangeri*.

INTRODUCTION

Intestinal microbiota plays an important regulatory role in the growth and development of animals, participating in the digestion and absorption of nutrients, regulate the intestinal environment and affecting the hosts' health (Duarte *et al.*, 2025). Some studies have shown that environmental factors such as temperature, humidity or altitude were the main factors leading to differences in the gut microbiota structure of the same mammals in different regions (Zhan *et al.*, 2019). For instance, studies have shown that environmental differences and food properties lead to significant differences in gut biodiversity and cellulose digestion capabilities between the two regions of giant pandas (Zhan *et al.*, 2019). Therefore, clarifying the impact of environmental factors on gut microbiota and the interrelationship between environmental factors and gut microbiota can help understand the adaptation of species to different environments and further understand the reasons for the evolution of host adaptation to the environment.

Mammals can enhance their adaptability to natural environmental variations by regulating their physiological characteristics, thereby increasing their survival ability in the field mainly manifested in body mass, food intake (Jia *et al.*, 2024; Geng and Zhu, 2024). When faced with different environments, small mammals typically adjust their body weight to alter their overall survival (Welman *et al.*, 2022). Significant differences in body weight were observed in *Eothenomys miletus* from five regions of the Hengduan Mountains, suggesting that this may be related to factors such as humidity and temperature in their habitats (Yan *et al.*, 2022). Therefore, the same species can exhibit different body weight regulation in different regions, showing phenotypic plasticity. Understanding the body weight changes of animals in different environments is beneficial for clarifying the adaptation mode of the species.

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Tupaia belangeri belongs to the family *Tupaiidae* and is a diurnal small mammal unique to the Eastern Ocean, widely distributed in South Asia, Southeast Asia and southwestern China, it serves as an animal model for human metabolic diseases in biomedical research (Wang *et al.*, 2022; Yang *et al.*, 2024). In previous studies, our laboratory explored the effects of different environmental factors on the energy metabolism of *T. belangeri* (Hou *et al.*, 2022; Yang *et al.*, 2024), However, there is limited research on the gut microbiota composition and its correlation with body weight regulation of the *T. belangeri* in different regions. Therefore, this study selected Mile City (ML) and Chuxiong City (CX), as sampling points, as these significant differences in humidity and temperature, as sampling points (Table 1). Will *T. belangeri* adapt to different environments by adjusting body weight and gut microbiota? Based on the previous research background, we propose the following scientific hypotheses: 1). The body weight regulation patterns of *T. belangeri* vary across different regions, with ML having a higher body weight than CX; 2) The gut microbiota of *T. belangeri* in different regions demonstrate variations in composition and diversity, with higher diversity in ML than in CX; 3) The body weight and the differences in gut microbiota of the *T. belangeri* were related to the varying humidity and temperature in the two regions. This investigation provides mechanistic insights into how geographic isolation shapes gut microbial communities in *T. belangeri*, thereby advancing our comprehension of host-microbe co-evolution and environmental resilience.

MATERIALS AND METHODS

Sampling of experimental animals

In this experiment, 20 *T. belangeri* were collected in December 2023 in CX and ML, Yunnan Province, obtain animal body weight on site and collect feces and excrement. All experimental animals are adult individuals in the non-reproductive period. The specific information captured is shown in Table 1. The experiment was conducted at the School of Life Sciences, Yunnan Normal University.

Determination of body mass

The *T. belangeri* was weighed using an electronic balance (AB204-S model, Switzerland) with an accuracy of 0.01 g.

DNA extraction

Quickly collect 0.1 grams of rectal feces and excrement, use Ezup column soil DNA extraction kit (Sangon Biotech, China) to extract sample DNA according to the manufacturer's instructions, quantify the extracted DNA using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, IL, USA) (Mu *et al.*, 2024).

High throughput sequencing

Determine that a nucleic acid concentration higher than 10 ng/uL and a purity (A260/A180) greater than 1.8 in the purified PCR product are valid samples. Mix the effective DNA samples equimolar and use the Illumina Miseq platform (Illumina, Sandiego, CA, USA) from Beijing Novogene Co., Ltd. for sequencing (Zhang *et al.*, 2023).

Bioinformatics analysis

Obtain a 2×250 bp double terminal sequence through Illumina Miseq platform sequencing and process and analyze the raw data using the QIIME platform. And the sequences of all samples were standardized using the "Daisy Chopper" script code (Li *et al.*, 2017).

Data analysis

Microbial community composition

Bacterial community described by creating a bar chart using Origin 2024.

α, β diversity

Chao 1 and Shannon diversity were tested using Mann Whitney test in SPSS 23 to determine if the differences between the two groups were significant. β Diversity: Using QIIME, the community structure was described based on Bray curtis and jaccard distance matrices. Visualize diversity using origin.

Venn diagram

Analysis of venn graph implemented online in Venn 2.1.

Correlation analysis

PERMANOVA analysis is also used to compare the effects of different environmental factors on CX and ML bacterial communities.

Enrichment analysis

Use the "Pheatmap" and "ComplexHeatMap" in R4.3.3 to draw a heatmap.

Permanova analysis

Using the Vegan software package and employing Permanova to evaluate the relationship between dominant genera and climate factors.

Network analysis

A network analysis was conducted using R version 3.6.2 and Gephi software version 0.9.2 to identify significant interactions ($P<0.05$, $|r|>0.4$) between variables (Zhang *et al.*, 2023).

Table 1: Climate information of sampling sites.

	Latitude	Longitude	Sex ratio (♂:♀)	Altitude	Temperature	Relative humidity
CX	25°1′20″N	101°30′7″E	6:4	1845m	16°C	36%
ML	24°31′15″N	103°24′24″E	1:1	1781m	23°C	77%

Determine the impact of randomness and deterministic processes on community assembly

The process of community assembly is quantified through improved random ratio (MST) based on zero model (Ormerod *et al.*, 2016). According to the neutral community model (NCM), use the package "Hmisc" to calculate the community assembly process (Song *et al.*, 2022).

Use SPSS 26.0 software package for data analysis

All data conform to normal distribution and homogeneity of variance. Because there is no significant difference in the measurement indicators between males and females of the *T. belangeri*, the data will be combined and used. An independent sample t-test was used to analyze body weight and $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Differences in weight regulation of *T. belangeri* in two regions

It was found that there was a significant difference in body weight of the *T. belangeri* between the two regions ($t=6.142$, $P<0.01$); The body mass of *T. belangeri* in CX is significantly smaller than that in ML (Fig 1).

The maintenance of animal body weight relies on the balance between energy consumption and acquisition, with various environmental factors influencing these processes, such as temperature and humidity (Li *et al.*, 2003). For instance, species like *E. miletus* and *Apodemus chevrieri* lose body weight to ensure survival in cold temperatures (Zhu and Gao, 2017; Jia and Zhu, 2021). In winter, the ambient temperature in CX was lower than that in ML, which resulted in higher temperatures and abundant food resources in ML. Therefore, *T. belangeri* did not need to reduce its body weight to reduce absolute energy consumption. Moreover, the average relative humidity in ML was higher than that in CX, Research had shown that under high temperature or high humidity environments, animals' evaporative heat dissipation and energy consumption were reduced and they can maintain a higher body weight (Li *et al.*, 2013). Through gut microbiota analysis, it was found that Firmicutes had significant differences between the two regions. studies have shown that the ratio of Firmicutes and Bacteroidetes is correlated with host body weight (Du *et al.*, 2013), explaining the mutual influence and regulation between gut microbiota and body mass of *T. belangeri*. Therefore, the difference in mass between the two regions was a response to the differences in environmental temperature and humidity conditions.

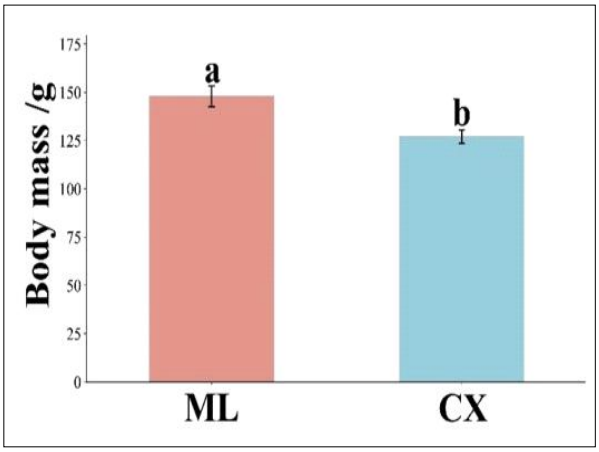


Fig 1: Comparison of body mass of *T. belangeri* in ML and CX.

Microbial community composition

Phylum-level analysis revealed that the dominant phylum of gut microbiota in the *T. belangeri* in CX were Proteobacteria, Bacteroidetes and Firmicutes, the average relative abundance as follows 79.69%, 8.56% and 4.98%. The dominant phylum of gut microbiota in the *T. belangeri* in ML were Proteobacteria, Firmicutes and Spirochaetes; the average relative abundance was 68.36%, 14.84% and 9.20%, respectively. There was a significant difference in the relative abundance of Firmicutes between CX and ML (Mann Whitney U test, $P<0.05$, Fig 2).

At the door-level taxonomic, the dominant genus of gut microbiota in the *T. belangeri* in CX were as follows: *Escherichia* (64.31%), *Bacteroides* (5.37%) and *Flexispira* (3.12%). The dominant genus of gut microbiota in the *T. belangeri* in ML were *Escherichia* (44.14%), *Campylobacter* (12.04%) and *Bacillus* (5.48%). However, there was no significant difference in the relative abundance of dominant genera between the two regions (Mann Whitney U test, $P>0.05$, Fig 3).

The gut microbiota comprises a diverse microbial population that undergoes dynamic changes and engages in mutually beneficial symbiosis with the host over extended periods, depending on its state (Zhang *et al.*, 2017). The results indicated that the dominant phyla in *T. belangeri* from CX at the phylum level were Proteobacteria, Bacteroidetes and Firmicutes, which were Proteobacteria, Firmicutes and Spirochaetes in ML and the relative abundance of Firmicutes showed significant differences. The difference in relative abundance of Firmicutes between the two regions may be related to environmental temperature, studies had shown that bacteria with carbohydrate and energy metabolism pathway related functions in the gut of Macaca thibetana in winter and spring significantly increase in spring, enabling them to quickly recover from severe energy loss experienced in winter (Sun *et al.*, 2016). Furthermore, the relative abundance of Proteobacteria was the highest in the two regions of *T. belangeri*, which may be related to their feeding on corn, rice and other crops. Studies have shown that dietary resources rich in plant-based substances are usually associated with a higher proportion of Firmicutes and Proteobacteria (Kuo *et al.*, 2024). *Escherichia* is a dominant genus in both regions, it belongs to the phylum Proteobacteria and was a common and important bacterium in the gut that helps stabilize the gut microbiota, increase intestinal peristalsis and enhance digestive capacity. But it is also an opportunistic pathogen, but when

the host's immune system is weakened or it invades tissues and organs outside the intestine, it can cause infectious diseases such as sepsis (Shin *et al.*, 2015). This warns local authorities to strengthen management and monitoring of the *T. belangeri* to reduce the risk of disease spread.

Analysis of microbial community α and β diversity

The Mann-Whitney U test revealed no significant differences in gut microbiota diversity metrics between *T. belangeri* populations from the two study regions (Chao1 index: P= 0.12; Shannon index: P= 0.07, Fig 4). The distribution of β diversity in the gut microbiota of the *T. belangeri* in CX and ML was scattered on the PCoA map, with no obvious clustering trend (P>0.05, Fig 5).

Distribution of common and unique microorganisms in different regions

There was a total of 135 genera (55.79%) of *T. belangeri* in CX and ML. There were 36 genera (14.88%) unique to *T. belangeri* in CX and 71 genera (29.34%) unique to *T. belangeri* in ML. ML had a higher number of gut microbiota belonging to the endemic genus (Fig 6).

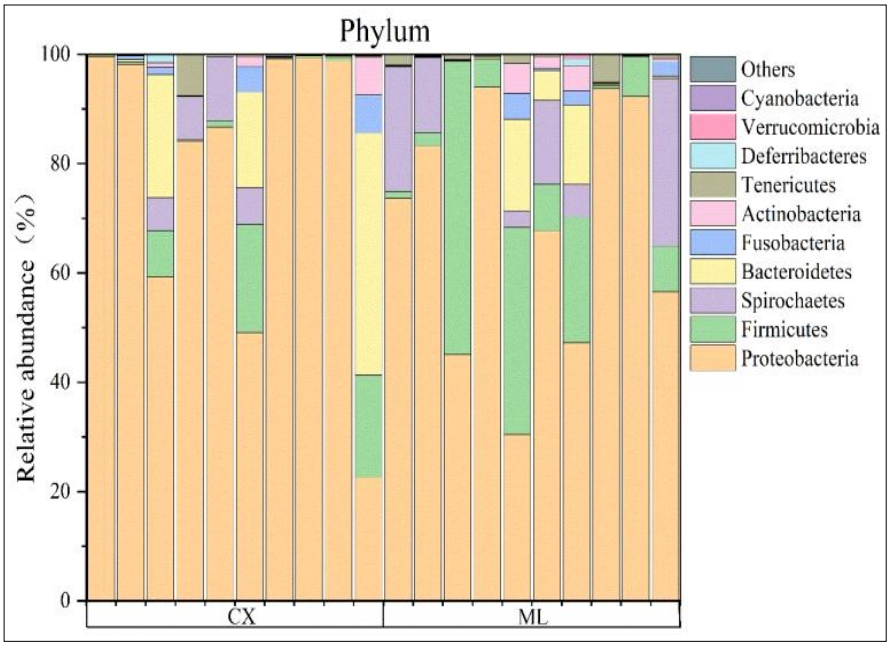


Fig 2: Analysis of gut microbiota composition door of *T. belangeri* in CX and ML.

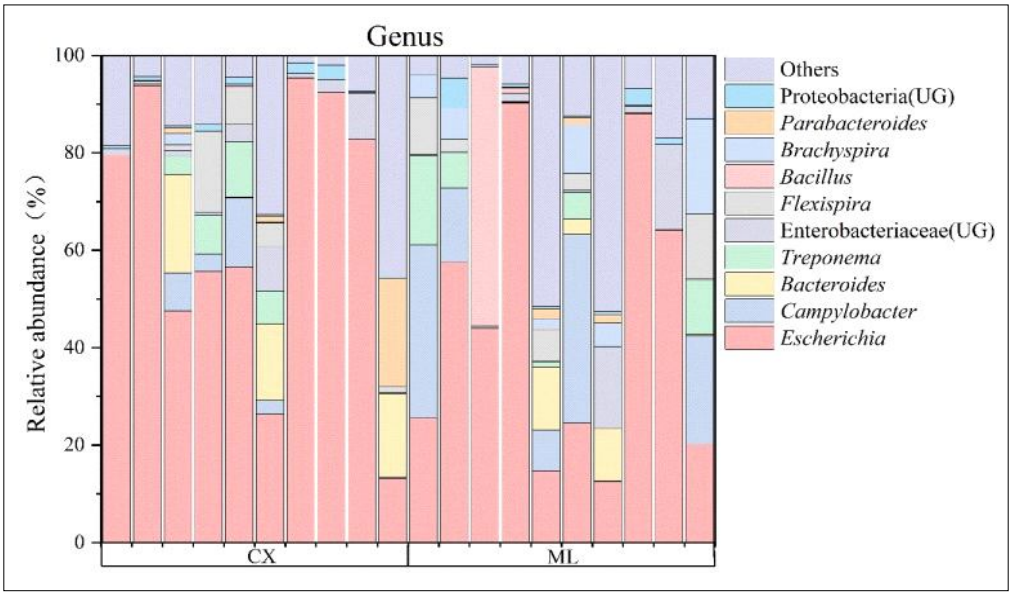


Fig 3: Analysis of the composition of gut microbiota gene in *T. belangeri* in CX and ML.

Analysis of microbial enrichment differences in different regions

Escherichia, *Enterobacteriaceae* (UG), *Yersinia*, *Slackia*, *Morganella* and *Providencia* were significantly enriched in CX ($P<0.05$). Adlercreutzia, Clostridiales (UG), Proteobacteria (UG), *Brachyspira*, *Campylobacter* and *[Ruminococcus]* were significantly enriched in ML ($P<0.05$, Fig 7).

The diversity index describes the statistical measure of microbial community diversity, including the richness and evenness of microbial species (Duarte *et al.*, 2025). A series of research results all indicated that the structure and composition of gut microbiota was closely related to the environment. Although there was no significant difference in β diversity and β diversity between the two regions, but there are CX and ML had differences in their unique gut microbiota. ML had a higher environmental temperature in winter than CX, which also means that ML had more abundant food resources in winter, *T. belangeri* can consume a more diverse range of food, thus requiring a more structurally complex and diverse gut microbiota with more species. Moreover, it was found that the impact of gut microbiota between the two regions was almost equal, indicating that the difference in gut microbiota structure between the two may be related to the different humidity and temperature levels between the two regions, which was one of the adaptation and regulation methods of the *T. belangeri* to different environments.

Influencing factors of gut microbiota

Body weight had a significant impact on its gut microbiota ($P<0.05$), compared with altitude and gender, temperature and humidity have a greater impact on the gut microbiota of *T. belangeri* in CX and ML ($P>0.05$, Fig 8).

Assembly process of gut microbiota

Quantifying the assembly process of gut microbiota in *T. belangeri* in CX and cities using corrected random rate (MST), the MST values of the gut microbiota of the *T. belangeri* in CX and ML were mostly below the threshold line of 0.5, indicating that deterministic processes play a more important role in both communities. And there was no significant difference in MST values between the CX and the ML (Mann Whitney U test, $P>0.05$, Fig 9).

Co-occurrence network of gut microbiota

This network analysis included the top 200 OTUs with relative abundance and based on Gephi 0.10, constructed a network with 200 nodes and 3030 edges, among them, there are 2998 positive edges (98.94%) and 32 negative edges (1.06%), with different colors representing different

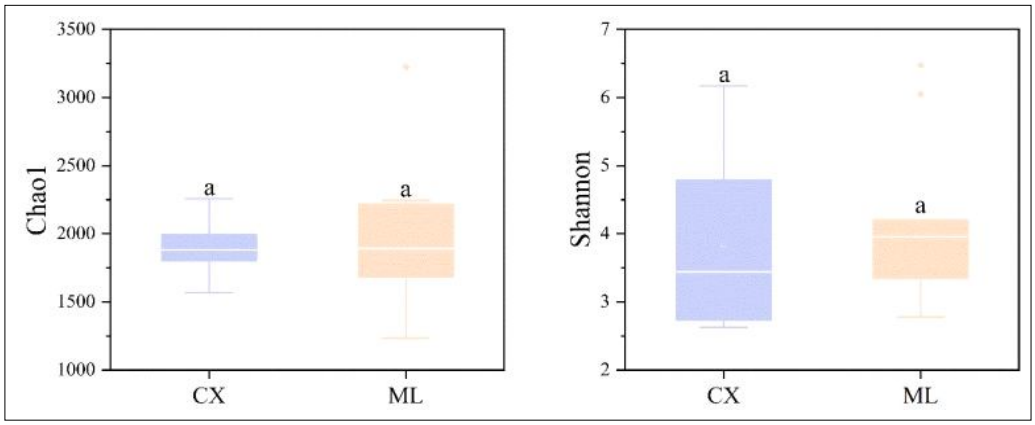


Fig 4: α diversity of gut microbiota in *T. belangeri* in CX and ML.

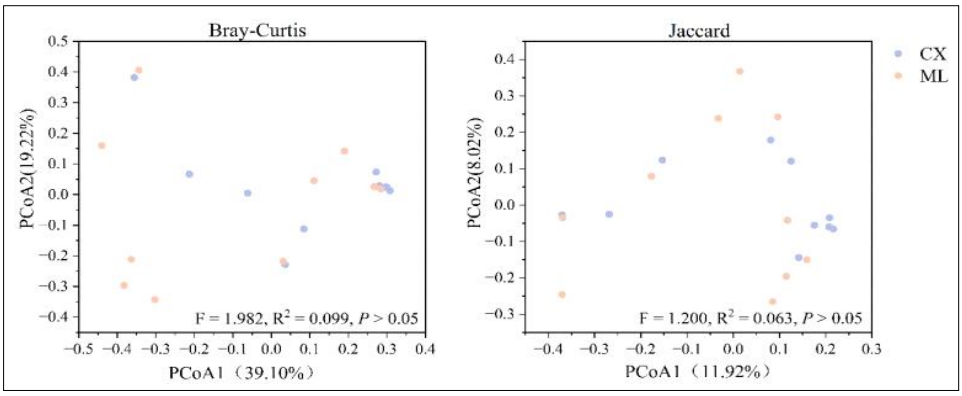


Fig 5: Gut microbiota β diversity of *T. belangeri* in CX and ML.

modules. This may mean that the microorganisms in the OTUs co-occurrence network are dominated by cooperative relationships (Fig 10). Further construct dominant OTUs co-occurrence networks for the top 200 relative abundance of *T. belangeri* in CX and ML and calculate their network topology characteristics (Table 2). The average degree and graph density of the co-occurrence network in CX were higher than those in ML and the average path length is shorter than that in ML, indicating that the co-occurrence network in CX had stronger connectivity, closer relationships and higher modularity than that in ML. The correlation between dominant OTUs in the gut of the *T. belangeri* in CX was stronger than that in ML.

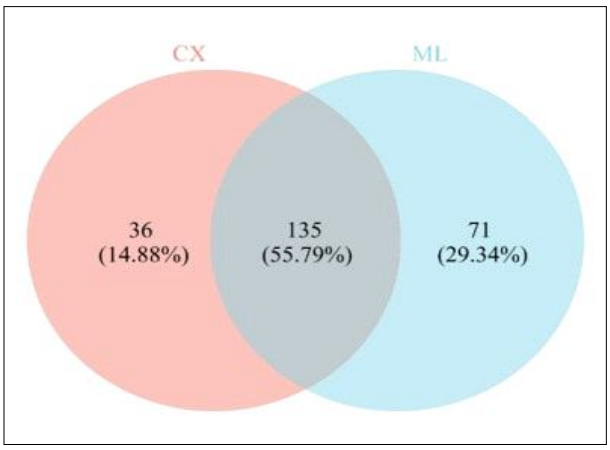


Fig 6: Venn diagram of common and unique gut microbiota in *T. belangeri* from different regions.

Opportunistic pathogenic bacteria in the gut

The bubble chart showed the opportunistic pathogenic bacteria in the gut of the *T. belangeri* in CX and ML. The size of the bubbles represents the relative abundance of the opportunistic pathogenic bacteria and the color represents the grouping. A total of 25 opportunistic pathogenic bacterial genera were detected, including 14 species detected in the gut of the *T. belangeri* in CX and 16 species detected in ML (Fig 10). It was found that

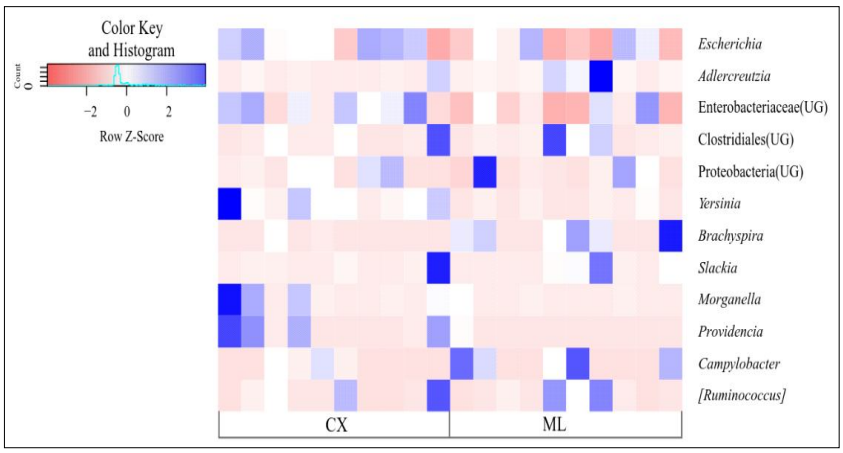


Fig 7: Significant differences in gut microbiota between *T. belangeri* in CX and ML.

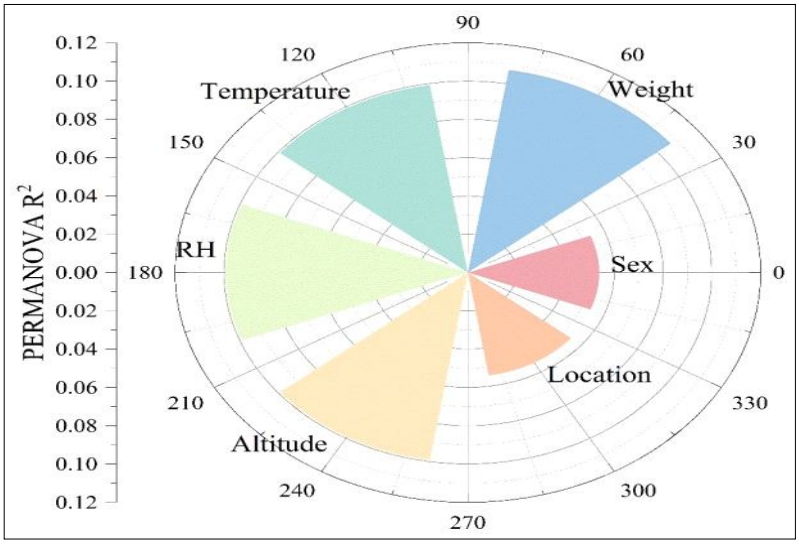


Fig 8: Influencing factors of gut microbiota in *T. belangeri* in CX and ML.

Escherichia was widely present in the gut of *T. belangeri* in both regions. *Bacillus* and *Campylobacter* had higher relative abundance in ML, while *Bacteroides* had higher relative abundance in CX (Fig 11).

Abnormalities in the gut microbiota was associated with various diseases. For instance, inflammatory bowel disease is related to the decrease in relative abundance of Clostridia and an overall decline in bacterial diversity (Koch and Schmid-Hempel, 2011; Dominianni *et al.*, 2015). This indicates that the stability of the microbial community in the body and the importance of maintaining the stability of gut microbiota after changes in gut bacterial community

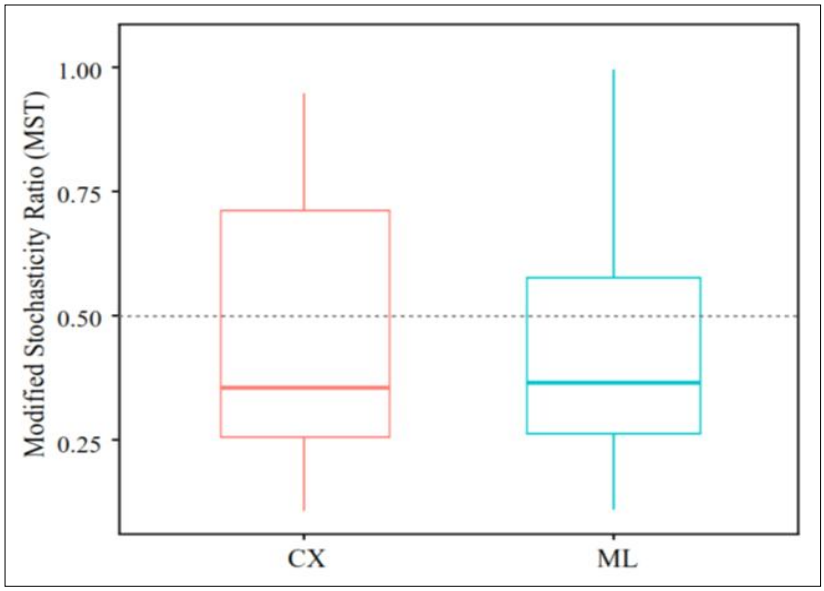


Fig 9: Corrected random rates of gut microbiota in *T. belangeri* in CX and ML.

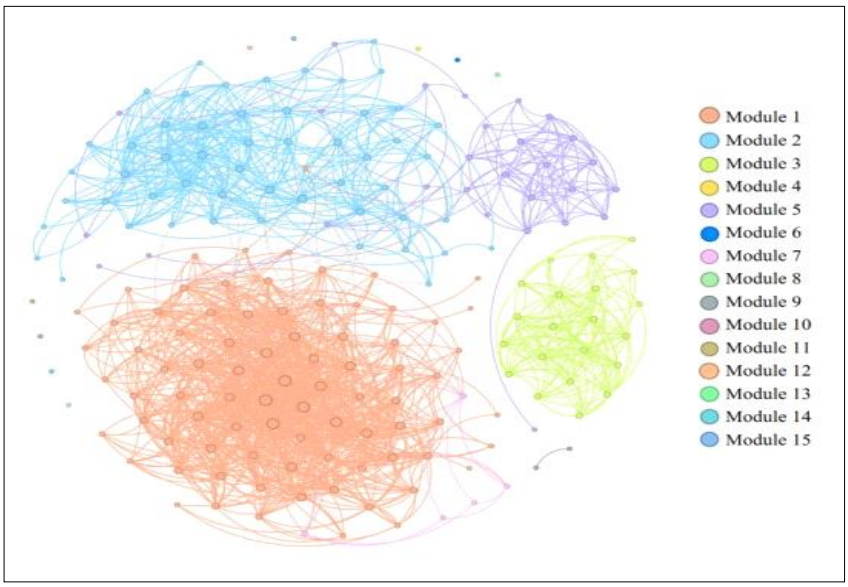


Fig 10: Co-occurrence network of dominant OTUs in the gut of *T. belangeri* in all regions.

Table 2: Network topology characteristics of dominant OTUs in the gut of *T. belangeri* in CX and ML.

Group	Nodes	Edges	Average degree	Average path length	Graph diameter	Graph density	Clustering coefficient	Modularity
Total	200	3030	15.15	3.379	9	0.076	0.646	0.578
CX	200	3996	19.98	2.793	9	0.165	0.672	1.376
ML	200	3865	19.325	3.136	7	0.144	0.721	0.501

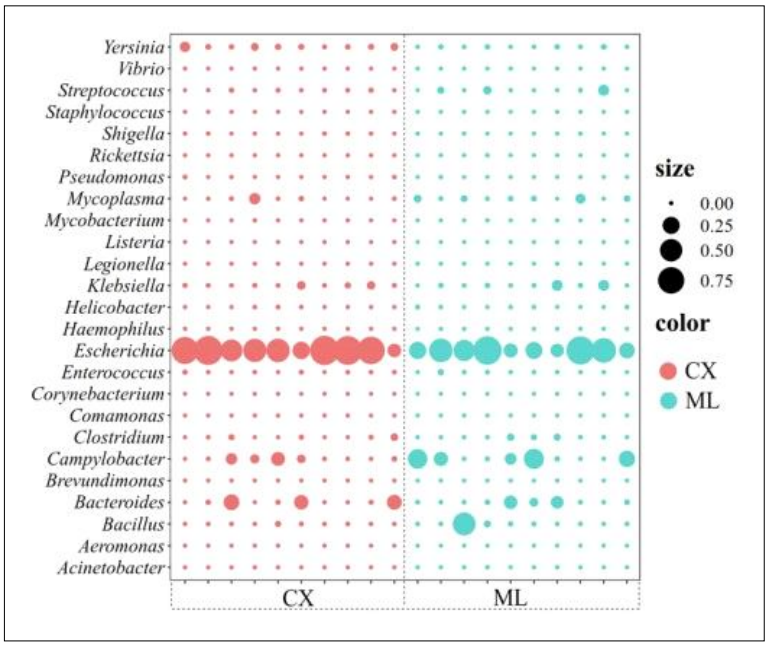


Fig 11: Opportunistic pathogenic bacteria in the gut of *T. belangeri* in CX and ML.

structure or catastrophic depletion of certain microbial communities in the gut are crucial. In our study, a total of 25 opportunistic pathogenic bacteria were detected, among which *Escherichia* was widely present in two regions. This is a common pathogenic bacterium of the Proteobacteria phylum. When it captures pathogenic or toxic factors from the outside, it can cause tissue and organ inflammation or diarrhea, sepsis, *etc*. in animals (Shin *et al.*, 2015). Bacteroides was relatively more abundant in the gut of the *T. belangeri* in CX. This bacterium can enhance the host's innate immune response (Bry *et al.*, 1996; Ley *et al.*, 2005; Corthier *et al.*, 1985). But when it is distributed outside the intestine, it can lead to host diseases such as diarrhea, osteomyelitis, endogenous abdominal abscess, tonsillitis, *etc*. (Hesham *et al.*, 2007; Swidsinski *et al.*, 2007). The different speculations of pathogenic bacteria between two regions may be related to their environment, such as temperature, water quality, food resources, *etc*. This also warns the local government to strengthen monitoring and management of the *T. belangeri* to avoid the occurrence of zoonotic diseases.

CONCLUSION

In conclusion, *T. belangeri* in ML and CX adapt to different environmental temperatures and humidity by adjusting their body mass and changing the structural diversity of their gut microbiota. Moreover, there were different pathogenic bacteria in the intestines in both places, which warns the local government to pay attention to the monitoring and management of wild *T. belangeri*. The ability of wild animals to quickly and effectively regulate their physiological characteristics in different environments will directly affect their survival. The present study helps to understand the adaptive evolution of the *T. belangeri* in different environments.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures were within the rules of Animals Care and Use Committee of School of Life Sciences, Yunnan Normal University. This study was approved by the committee (13-0901-011).

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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