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ABOUT COVER

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AIMS AND SCOPE

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ORIGINAL ARTICLE

Basic Study

Comparative analysis of the gut microbiota of wild adult rats from nine district areas in Hainan, China

Li-Na Niu, Guan-Nan Zhang, Duan-Duan Xuan, Chong Lin, Zi Lu, Pei-Pei Cao, Shao-Wen Chen, Yong Zhang, Xiu-Ji Cui, Shou-Kui Hu

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Li-Na Niu, Guan-Nan Zhang, Pei-Pei Cao, Yong Zhang, Xiu-Ji Cui, Key Laboratory of Tropical Translational Medicine of Ministry of Education, Hainan Medical University, Haikou 571199, Hainan Province, China

Li-Na Niu, Guan-Nan Zhang, Pei-Pei Cao, Yong Zhang, Xiu-Ji Cui, The University of Hong Kong Joint Laboratory of Tropical Infectious Diseases, Hainan Medical University, Haikou 571199, Hainan Province, China

Li-Na Niu, Guan-Nan Zhang, Pei-Pei Cao, Yong Zhang, Xiu-Ji Cui, Department of Pathogen Biology, Hainan Medical University, Haikou 571199, Hainan Province, China

Duan-Duan Xuan, Department of Laboratory, Xinxiang First People's Hospital, Xinxiang 453000, Henan Province, China

Chong Lin, Zi Lu, Shao-Wen Chen, Department of Laboratory, The Second Affiliated Hospital of Hainan Medical University, Haikou 570311, Hainan Province, China

Shou-Kui Hu, Department of Clinical Laboratory, Peking University Shougang Hospital, Beijing 100144, China

Corresponding author: Shou-Kui Hu, MD, Professor, Department of Clinical Laboratory, Peking University Shougang Hospital, No. 9 Jinyuanzhuang Road, Shijingshan District, Beijing 100144, China. shoukuihu@163.com

Abstract

BACKGROUND

Wild rats have the potential to hold zoonotic infectious agents that can spread to humans and cause disease.

AIM

To better understand the composition of gut bacterial communities in rats is essential for preventing and treating such diseases. As a tropical island located in the south of China, Hainan province has abundant rat species. Here, we examined the gut bacterial composition in wild adult rats from Hainan province.

METHODS

Fresh fecal samples were collected from 162 wild adult rats, including three

species (*Rattus norvegicus*, *Leopoldamys edwardsi*, and *Rattus losea*), from nine regions of Hainan province between 2017-2018.

RESULTS

We analyzed the composition of gut microbiota using the 16S rRNA gene amplicon sequencing. We identified 4903 bacterial operational taxonomic units (30 phyla, 175 families, and 498 genera), which vary between samples of different rat species in various habitats at various times of the year. In general, Firmicutes were the most abundant phyla, followed by Bacteroidetes (15.55%), Proteobacteria (6.13%), and Actinobacteria (4.02%). The genus *Lactobacillus* (20.08%), unidentified_*Clostridiales* (5.16%), *Romboutsia* (4.33%), unidentified_*Ruminococcaceae* (3.83%), *Bacteroides* (3.66%), *Helicobacter* (2.40%) and *Streptococcus* (2.37%) were dominant.

CONCLUSION

The composition and abundance of the gut microbial communities varied between rat species and locations. This work provides fundamental information to identify microbial communities useful for disease control in Hainan province.

Key Words: Hainan; Rat; Fecal microbiome; Microbial community; 16S rRNA

Core Tip: Several researches have investigated the microbial communities in arthropods but few researches have investigated microbial diversity in wild rats' gut in Hainan province. Here, by 16S rRNA gene amplicon sequencing we compared the gut bacterial communities in fecal samples from 162 wild rats of three species and nine geographic locations in Hainan.

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INTRODUCTION

The laboratory rat has been used as a useful experimental model for biomedical sciences since the last century. Rats were domesticated as early as the Edo period[1]. Gut microbiota is composed of myriad microbes (archaea, bacteria, fungi, and viruses) that interact with each other and with their host, playing important roles in the host's metabolism, physiology, homeostasis, health, and disease development[2]. There is an increase in studies of the laboratory rat gut microbiota[3-9], but very few on wild rats[10].

Wild rats pose a serious threat to public health because they are reservoirs for a number of zoonotic pathogens[11-13] and frequently and closely interact with people, domestic animals, and other animals.

Only a small number of researchers have looked into the microbial diversity in the guts of wild rats in the province of Hainan, compared to the number of researchers who have looked into the microbial communities in arthropods. Here, by 16S rRNA gene amplicon sequencing we compared the gut bacterial communities in fecal samples from 162 wild rats of three species and nine geographic locations in Hainan.

MATERIALS AND METHODS

Rat collection and morphology identification

Between December 2017 and November 2018, wild adult rats were collected using mousetraps in rat cages from nine geographic locations in Hainan province (L1-L9: L1 = Yinggeling; L2 = Huangjingjiaoling; L3 = Danzhou; L4 = Dongfang; L5 = Haikou; L6 = Jianfengling; L7 = Baisha; L8 = Sanya; L9 = Lingao) (Figure 1 and Supplementary Table 1). The trap sites we selected included mountains, residential areas, and farmland. When the team got back to the lab, they based their determination of gender on the morphology of the external reproductive organs. Based on DNA barcode analysis, a polymerase chain reaction (PCR) assay was used to confirm the identification of the species of rats. The characteristics of all rats are recorded, including acquisition time, location, sex, weight, and adult or

immaturity (Supplementary Table 1).

Molecular identification of rats

The SDS method was used for DNA extraction. The PCR assay was performed to identify rat species using the primer 5′-TACCATGAGGACAAATATCATTCTG-3′ and 5′-CCTCCTAGTTTGTTAGGGATTGATCG-3′[14]. Amplification was performed in 25 μL total volume, including DNA template (2 μL), each primer (0.5 μL), 10 × Gene Amp PCR Buffer (2.5 μL), dNTP (1.25 mmol/L) (2 μL), rTaq DNA polymerase (5 U/μL) (0.5 μL) and double distilled water (17 μL). The PCR procedure was as follows: (1) Initial denaturation 95 ℃ (5 min); (2) 35 cycles each of denaturation 94 ℃ (30 s); (3) primer annealing 51 ℃ (30 s); and (4) extension 72 ℃ (30 s). It took 5 min to complete the extension step's 35th cycle. The mouse species was determined through a blast to the NCBI nucleotide database, and the amplification products were identified using Sanger sequencing.

Tissue and fecal sampling

CO_2 was used for euthanizing rats. Intestinal tissue was dissected for each rat species, rinsing three times in sterile normal saline. In the meantime, samples of feces were taken from the intestinal tissues. Then, samples were immediately collected in a brain heart infusion medium with glycerol and subsequently stored at -80 ℃ until analysis.

16S rRNA microbial profiling preparation

Fecal samples weighing about 50 mg were used for 16S rRNA microbial profiling analyses. Following the manufacturer's instructions, microbial DNA was extracted from the fecal samples using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany). The PCR products were examined using electrophoresis. Thermo GeneJET Gel Extraction Kit was used to recover the PCR products after running them on 2% agarose gels in TAE buffer. Until analyses, all of the extracted DNA was kept at -20 ℃.

The hypervariable regions V3-V4 of the bacterial 16S rRNA were amplified with primers 5′-CCTAYGGGRBGCASCAG-3′ and 5′-GGACTACNNGGGTATCTAAT-3′. PCR reactions were performed in a 30 μL volume consisting of Phusion® High-Fidelity PCR Master Mix with GC buffer (New England Biolabs) (15 μL), forward and reverse primers (0.2 μM), and template DNA (10 ng). The PCR reaction was done with the following conditions: (1) Initial denaturation at 98 ℃ for 1 min; (2) 30 cycles of denaturation at 98 ℃ for 10 s; (3) annealing at 50 ℃ for 30 s; (4) elongation at 72 ℃ for 30 s; and (5) final extension 72 ℃ for 5 min. Sequencing libraries were made with an Ion Plus Fragment Library Kit (48 rxns) (Thermo Scientific) according to the manufacturer's protocols. The Qubit 2.0 Fluorometer (Thermo Scientific) was used to evaluate the library's quality. Sequencing was done on the Ion S5TM XL platform last.

16S rRNA microbial profiling analysis

Based on the unique barcode, single-end reads were assigned to samples and truncated by cutting off the barcode and primer sequence. The Cutadapt quality control process involved treating the raw reads with quality filtering under specific filtering conditions in order to produce high-quality clean reads [15]. Then, we compared the reads with the Silva database, and reference database[16] using UCHIME Algorithm[17] to detect and remove chimera sequences[18] to obtain clean reads.

Uparse software performed analyses of microbial profiling[19]. Sequences with a 97% or higher similarity to one another were found to the same Operational taxonomic unit (OTU). We screened the representative sequence of each OTU for further annotation. Taxonomic data was annotated at various taxonomic levels using the Silva Database, which uses the Mothur algorithm[16]. We used the MUSCLE software to conduct multiple sequence alignment to study the difference between the dominant species in different groups[20]. We normalized OTUs abundance using the standard sequence number of the sample with the least sequences and summarized the OTU abundance in a table. Subsequently, we analyzed the alpha diversity (α-diversity) and beta diversity (β-diversity) based on this normalized data.

Bacterial diversity analysis

In the complexity of species diversity, we applied α-diversity through 6 indices (Observed-species, Chao1, ACE, Shannon, Simpson, Good's coverage). Chao1 and ACE were selected as the Chao1 and ACE estimators to determine richness. The Shannon index and Simpson index were used to calculating Shannon diversity and Simpson diversity. Using Good's coverage, Coverage was chosen to describe the depth of the Sequencing.

We applied β-diversity for evaluating the similarity of different samples rather than describing within-sample diversity like α-diversity. We calculated β-diversity on both Weighted and Unweighted unifrac. The dimension of the original variables was reduced using principal component analysis. Principal coordinate analysis (PCoA) was performed to visualize complex, multidimensional data and was displayed to visualize microbial community structure relationships. Firstly, we obtained a distance matrix among samples in weighted or unweighted unifrac. Secondly, we transformed the distance matrix to a new set of orthogonal axes. Finally, the first principal coordinate demonstrated the maximum variation factor, and the second principal coordinate demonstrated the second maximum

one, and so on. We performed Adonis analysis to know to what extent location explained variation in microbial composition. For significant difference analysis, either the USEARCH software (https://www.drive5.com/usearch) or R software (version 4.0.3) was used.

RESULTS

Rat species from different sampling areas

Among the 162 wild rats collected by traps from nine district areas which covered almost half of Hainan province (Figure 1), three rat species (*Rattus norvegicus*, *Leopoldamys edwardsi*, and *Rattus losea*) were predominant (Table 1 and Supplementary Table 1). These rat species showed distinct geographic distributions. *Rattus norvegicus* (brown rat) was mainly found in farmland (Danzhou, Dongfang, Baisha, and Sanya) and residential areas (Haikou); *Leopoldamys edwardsi* (Edward's long-tailed rat) was exclusively found in mountain areas in Yinggeling, Huangjingjiaoling, and Jianfengling; *Rattus losea* (Lesser rice-field rat) was only found from mountains in Lingao area (Table 1).

Gut bacterial species composition among rat species

A total of 162 fecal samples (one from each rat) were collected from the field-collected wild adult rats and subjected to bacterial profiling analyses, and estimating the bacterial community composition of these samples involved 16S rRNA gene amplicon sequencing. A total of 13326753 raw reads (mean ± SE = 82263.91 ± 8277.45 per sample) were obtained from the 162 samples that were sequenced (Supplementary Table 2). Among them, 12537453 clean reads (mean ± SE = 77391.69 ± 7604.97 per sample) remained for subsequent analyses after quality control processing. These sequences were divided into 4903 OTUs that belonged to 30 phyla, 175 families, and 498 genera (Supplementary Table 3). Among those OTUs that were defined to species level (Supplementary Table 3), some notoriously famous bacteria were found, such as *Bacillus anthracis* (OUT_426), *Bacteroides pyogenes* (OUT_3846), *Clostridium perfringens* (OUT_8), *Streptococcus suis* (OUT_410), and *Vibrio cholerae* (OUT_3092).

As shown in Figure 2A, sequences originating from Firmicutes (average 65.76%, Supplementary Table 4) were dominant, followed by Bacteroidetes (15.55%), Proteobacteria (6.13%), Actinobacteria (4.02%), Unclassified bacteria (3.68%), Tenericutes (1.93%), Fusobacteria (1.00%), Spirochaetes (0.88%), Chlamydiae (0.27%), Melainabacteria (0.17%), and so on (detailed in Supplementary Table 4, those < 0.15% were pooled together as "Others" in Figure 2A). Although Bacteroidetes were the second most abundant on average, this was not the case for samples from Danzhou, Haikou, Baisha, and Lingao (Supplementary Table 4).

Following a similar trend, Clostridia (averaged 32.72%) and Bacilli (averaged 26.48%) were the top two classes of bacteria in most samples (Figure 2B), but Clostridia only ranked fourth in samples from the Jianfengling area, and Bacteroidia was the 2nd most abundant in samples from Yinggeling, Huangjingjiaoling and Sanya (Supplementary Table 5).

According to the average value, the most abundant family was Lactobacillaceae (20.08%), followed by Ruminococcaceae (12.03%), Lachnospiraceae (8.11%), Muribaculaceae (6.33%), Peptostreptococcaceae (5.61%), unidentified_Clostridiales (5.58%), Erysipelotrichaceae (5.50%), Bacteroidaceae (3.66%), Enterobacteriaceae (3.25%), and Streptococcaceae (2.71%) (detailed in Supplementary Table 6, those < 2.5% were pooled together as "Others" in Figure 2C). Compared to the profiles for the bacterial phylum and class, the profiles for the actual values were more complex. Lactobacillaceae was the most abundant in samples from five areas (Danzhou, Dongfang, Jianfengling, Baisha, and Lingao), Ruminococcaceae was most abundant in samples from Yinggeling and Huangjingjiaoling, and Peptostreptococcaceae and Bacteroidaceae were the richest in samples from Haikou and Sanya, respectively (Supplementary Table 6).

As illustrated in Figure 2D and detailed in Supplementary Table 7, the genus with the highest OTU abundance in the feces of rats was those (with relative abundance less than 0.5%) designated as 'others' (average 26.80%), followed by *Lactobacillus* (20.08%), unidentified_*Clostridiales* (5.16%), *Romboutsia* (4.33%), and so on. In samples from eight different areas, "Others" or *Lactobacillus* ranked first and second, but not in samples from the Haikou area, which had the highest concentrations of unidentified *Clostridiales* and *Romboutsia*.

As shown in Figure 3, the heatmap color-coded the different OUT compositions in samples of the nine groups (Figure 3A); all of the samples contained unique bacterial OTUs, according to the upset plot (Figure 3B), but those from the Jianfengling region had the highest numbers of both (618 for unique and 153 for common with the Lingao area).

Gut bacterial diversity of rat

Alpha diversity indices were performed to detect whether bacterial diversity and richness significantly varied among samples of different rat species and geographic locations. The bacterial species richness, represented by Chao1 and ace denoting the number of species rather than the abundance of each species was highest for samples from the Yinggeling area (Table 2). Statistics were conducted for the Chao1 values because the values of both indices (Chao1 and ace) changed in a remarkably similar way across

Table 1 Location, species, number, season, and habitat of trapped rats

Site	Rat species	Number of midgut samples	Sampling season	Trap sites
YGL	LE	32	2018.11	Mountains
HJJL	LE	25	2018.11	Mountains
DZ	RN	12	2017.12	Farmland
DF	RN	12	2018.01	Farmland
HK	RN	13	2017.12	Residential areas
JFL	LE	13	2018.06	Mountains
BS	RN	12	2018.01	Farmland
SY	RN	23	2018.05	Farmland
LG	RL	20	2018.11	Mountains

YGL: Yinggeling; HJJL: Huangjingjiaoling; DZ: Danzhou; DF: Dongfang; HK: Haikou; JFL: Jianfengling; BS: Baisha; SY: Sanya; LG: Lingao; LE: Leopoldamys edwardsi; RN: Rattus norvegicus; RL: Rattus losea.

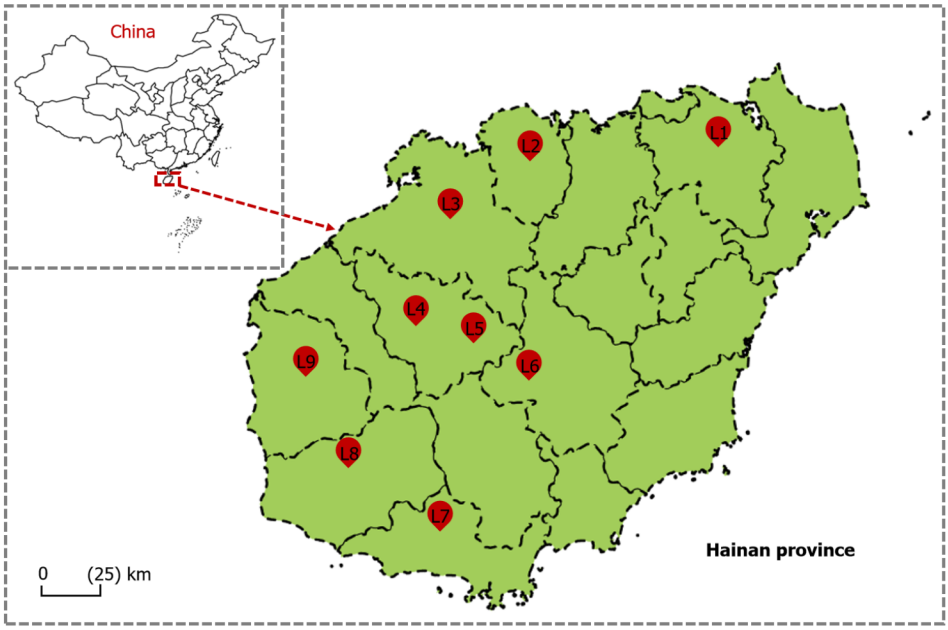

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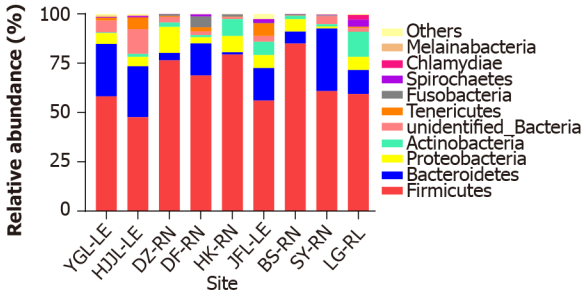
Figure 1 Sketch map of Hainan province. The map shows the locations of the nine collection study sites. L1 = Yinggeling, mountain; L2 = Huangjingjiaoling, mountain; L3 = Danzhou, residential areas; L4 = Dongfang, residential areas; L5 = Haikou, residential areas; L6 = Jianfengling, mountain; L7 = Baisha, residential areas; L8 = Sanya, residential areas; L9 = Lingao, farmland.

the nine groups. Except for Lingao and Jianfengling, samples from Yinggeling and Huangjingjiaoling, the two highest among all, had significantly more bacteria than the other five samples (Figure 4A). Taking Yinggeling as an example, it was most significantly higher ($P < 0.0001$) than those from Danzhou, Dongfang, Haikou, Sanya and Baisha, and significant ($P < 0.05$) compared to Lingao and Jianfengling (Figure 4A). In addition, samples from the Jianfengling area had higher bacterial richness compared to those from Danzhou and Sanya, and those from the Lingao area had higher richness than those from Danzhou and Sanya. There was no significant difference between other groups if not mentioned above.

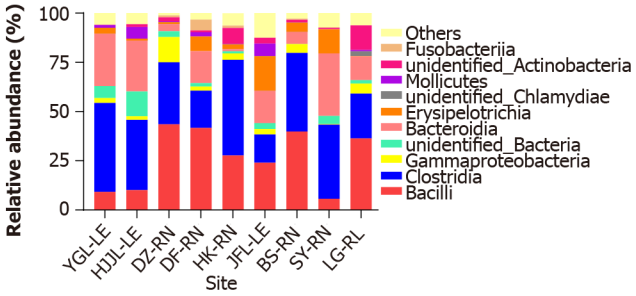
Species diversity, represented by Shannon diversity and Simpson diversity, indicates species richness and evenness in the community. As the two indices changed accordingly (Table 2), values of the Shannon diversity were used to do the statistics. In comparison to samples from Danzhou ($P < 0.0001$), Dongfang ($P < 0.01$), Haikou ($P < 0.001$), and Sanya ($P < 0.05$), samples from the Yinggeling area showed significant differences. Although similar in its bacterial composition pattern to those from the Yinggeling area, the species diversity of samples from the Huangjingjiaoling area was less significant with samples from Danzhou ($P < 0.001$) and Haikou ($P < 0.05$). In contrast to the insignificant results

A



Taxonomy	YGL	HJJL	DZ	DF	HK	JFL	BS	SY	LG
Firmicutes	58.24%	47.63%	76.45%	68.80%	79.36%	56.07%	84.96%	60.89%	59.41%
Bacteroidetes	26.57%	25.85%	3.72%	16.23%	1.15%	16.48%	6.12%	31.77%	12.10%
Proteobacteria	5.41%	4.69%	13.20%	3.09%	8.31%	6.61%	6.23%	0.99%	6.68%
Actinobacteria	0.32%	1.55%	2.22%	1.13%	8.53%	6.70%	1.77%	1.18%	12.78%
unidentified_Bacteria	6.10%	12.52%	2.86%	1.76%	1.04%	2.97%	0.08%	4.02%	1.80%
Tenericutes	1.39%	5.96%	0.46%	2.20%	0.19%	6.35%	0.07%	0.05%	0.71%
Fusobacteria	0.07%	0.02%	0.95%	5.50%	1.22%	0.19%	0.60%	0.39%	0.04%
Spirochaetes	0.25%	0.67%	0.13%	1.07%	0.01%	1.88%	0.06%	0.34%	3.51%
Chlamydiae	0.00%	0.00%	0.00%	0.01%	0.01%	0.00%	0.00%	0.02%	2.43%
Melainabacteria	0.74%	0.38%	0.01%	0.11%	0.07%	0.09%	0.02%	0.05%	0.05%

B



Taxonomy	YGL	HJJL	DZ	DF	HK	JFL	BS	SY	LG
Lactobacillaceae	8.27%	10.02%	34.03%	26.94%	14.94%	21.44%	29.09%	3.08%	32.91%
Ruminococcaceae	21.12 %	21.43%	2.30%	9.52%	3.23%	7.09%	21.31%	15.04%	7.21%
Lachnospiraceae	17.80%	10.17%	0.84%	5.66%	3.06%	3.87%	9.50%	18.17%	3.94%
Muribaculaceae	12.39 %	18.82%	0.39%	3.04%	0.34%	12.41%	2.22%	2.67%	4.70%
Peptostreptococcaceae	0.96%	0.04%	10.46%	2.08%	21.22%	0.31%	5.08%	1.09%	9.23%
unidentified_Clostridiales	2.55%	1.51%	17.77%	1.07%	20.65%	1.00%	2.95%	1.19%	1.53%
Erysipelotrichaceae	3.06%	1.00%	0.88%	7.44%	2.42%	17.58%	4.77%	12.27%	0.11%
Bacteroidaceae	1.10%	0.38%	0.40%	8.28%	0.17%	0.39%	0.69%	21.30%	0.25%
Enterobacteriaceae	1.24%	1.33%	12.68%	1.74%	2.98%	0.71%	4.43%	0.21%	3.89%
Streptococcaceae	0.37%	0.03%	3.49%	7.38%	6.86%	0.93%	3.36%	0.88%	1.04%

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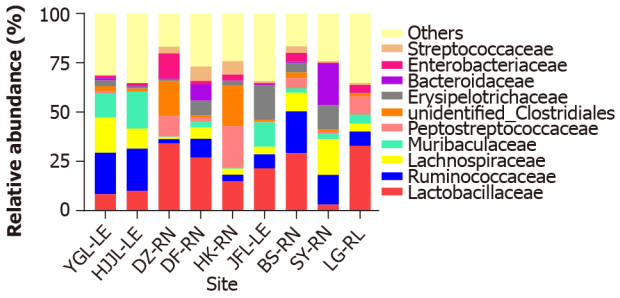
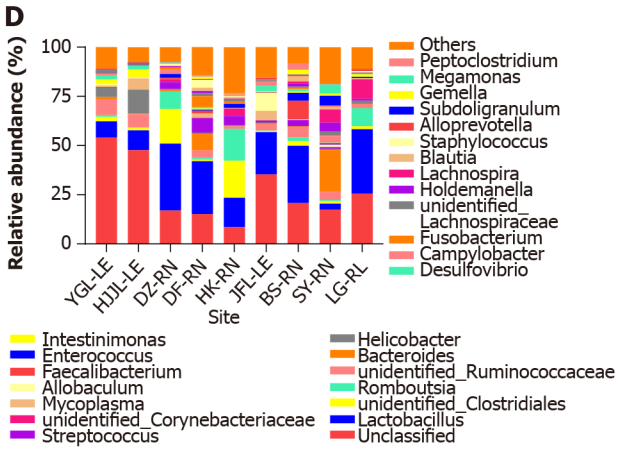


Taxonomy	YGL	HJJL	DZ	DF	HK	JFL	BS	SY	LG
Bacilli	9.24%	10.09%	43.51%	41.70%	27.73%	24.06%	39.85%	5.75%	36.39%
Clostridia	45.17%	35.68%	31.53%	18.95%	48.56%	14.30%	39.92%	37.65%	22.69%
Gammaproteobacteria	2.43%	1.89%	12.87%	2.03%	3.28%	2.76%	4.52%	0.35%	5.09%
unidentified_Bacteria	6.10%	12.52%	2.86%	1.76%	1.04%	2.97%	0.08%	4.02%	1.80%
Bacteroidia	26.57%	25.85%	3.72%	16.23%	1.15%	16.48%	6.12%	31.77%	12.10%
Erysipelotrichia	3.06%	1.00%	0.88%	7.44%	2.42%	17.58%	4.77%	12.27%	0.11%
unidentified_Chlamydiae	0.00%	0.00%	0.00%	0.01%	0.01%	0.00%	0.00%	0.02%	2.43%
Mollicutes	1.39%	5.96%	0.46%	2.20%	0.19%	6.35%	0.07%	0.05%	0.71%
unidentified_Actinobacteria	0.12%	1.42%	2.07%	0.85%	8.12%	2.92%	1.28%	0.62%	12.47%
Fusobacteriia	0.07%	0.02%	0.95%	5.50%	1.22%	0.19%	0.60%	0.39%	0.04%



Taxonomy	YGL	HJJL	DZ	DF	HK	JFL	BS	SY	LG
Others	54.03%	47.66%	16.93%	15.13%	8.51%	35.32%	20.79%	17.39%	25.42%
Lactobacillus	8.27%	10.02%	34.03%	26.94%	14.92%	21.44%	29.08%	3.08%	32.91%
unidentified_Clostridiales	2.23%	1.43%	17.63%	0.68%	18.88%	0.59%	2.45%	1.08%	1.46%
Romboutsia	0.96%	0.03%	8.87%	0.97%	16.15%	0.29%	1.91%	0.67%	9.13%
unidentified_Ruminococcaceae	8.06%	6.70%	0.32%	3.85%	1.26%	3.19%	4.81%	4.21%	2.06%
Bacteroides	1.10%	0.38%	0.40%	8.28%	0.17%	0.39%	0.69%	21.30%	0.25%
Helicobacter	4.79%	12.13%	0.61%	0.72%	0.58%	0.58%	0.02%	0.59%	1.59%
Streptococcus	0.26%	0.02%	3.45%	7.29%	4.46%	0.91%	3.19%	0.86%	0.90%
unidentified_Corynebacteriaceae	0.01%	0.00%	1.47%	0.30%	3.83%	0.00%	0.07%	0.01%	9.93%
Mycoplasma	0.86%	5.78%	0.06%	1.98%	0.04%	4.88%	0.02%	0.02%	0.55%

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Figure 2 Mean relative abundances of bacterial phylum, class, family, and genus associated with three rat species at different sites. A: Phylum; B: Class; C: Family; D: Genus. Families with an abundance of less than 2.50% and Phyla with an abundance of less than 0.15% were combined to form the category "Others." YGL: Yinggeling; HJJL: Huangjingjiaoling; DZ: Danzhou; DF: Dongfang; HK: Haikou; JFL: Jianfengling; BS: Baisha; SY: Sanya; LG: Lingao; LE: Leopoldamys edwardsi; RN: Rattus norvegicus; RL: Rattus losea.

among sample groups not mentioned above, the species diversity of samples from the Danzhou area was significantly different ($P < 0.05$) from those from the Lingao area (Figure 4B).

Variation of gut bacterial communities among study locations

Distance metrics were used to estimate differences between rat species and geographical locations in bacterial community profiles. The results showed a significant difference in microbiota among the three rat species and nine locations based on unweighted unifrac and weighted unifrac distances. When analyzing the fecal microbiota from various study sites, we used PCoA plots to visualize its composition. Using Unweighted Unifrac distance, two PCoA coordinates percent variation explained PCo1 (21.33%) and PCo2 (6.98%) of the total variation, respectively. We observed significant differences in fecal microbial β-diversity among communities from different locations, including *Leopoldamys edwardsi* from Yinggeling, Huangjingjiaoling, and Jianfengling; *Rattus norvegicus* from Danzhou, Baisha, and Sanya. *Rattus norvegicus* from the Dongfang area does not cluster tightly. There were no observable differences between Rattus norvegicus from Danzhou and Haikou (Figure 5A). Two PCoA coordinates based on Weighted Unifrac distance percent variation revealed PCo1 (44.41%) and PCo2 (10.69%), respectively (Figure 5B). These differences were mainly due to the geographical location, which included a plethora of variables (rat species, habitat, season, diet, *etc.*).

Heatmaps based on Bray-Curtis distances and Weighted unifrac distances, respectively, showed the diversity and similarity of microbial communities (Figure 5C and D). As shown in Figure 5C, the most similar (0.508, the lowest in Supplementary Table 8) intestinal bacterial community structure was between samples from Jianfengling and Huangjingjiaoling (highlighted with blue triangles in

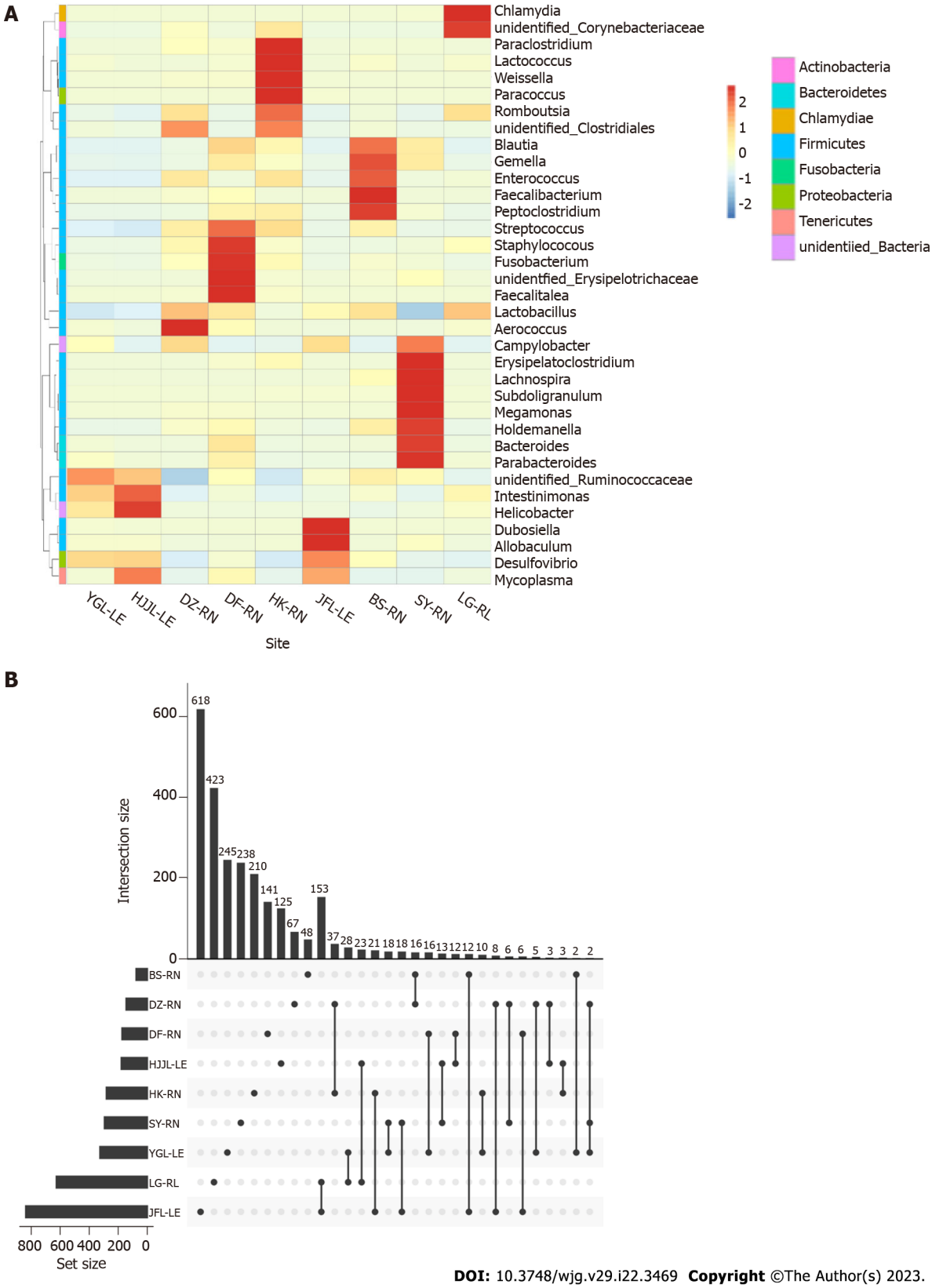

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Figure 3 Heatmap and upset plot featuring the rat gut bacterial community. A: The heatmap in log scale depicting the gut bacterial community of rat feces obtained with open reference operational taxonomic unit (OTU) picking methods. Blue colors represent high abundance, and red colors represent low abundance; white indicates absence; B: Visualizing the relationships between OTUs in all rat species across all sites using an upset plot. The Sangerbox tools, a free online platform for data analysis, were used to create the plot (http://www.sangerbox.com/tool). YGL: Yinggeling; HJJL: Huangjingjiaoling; DZ: Danzhou; DF: Dongfang; HK: Haikou; JFL: Jianfengling; BS: Baisha; SY: Sanya; LG: Lingao; LE: Leopoldamys edwardsi; RN: Rattus norvegicus; RL: Rattus losea.

Table 2 Richness and diversity (mean ± SD) of the rat intestinal content bacterial community

Group	Observed_species	Chao1	Ace	Shannon	Simpson
YGL-LE	662.16 ± 185.6	853.76 ± 201.05	862.86 ± 191.94	6.30 ± 1.19	0.95 ± 0.05
HJJL-LE	654.16 ± 207.22	827.96 ± 227.51	835.31 ± 217.91	5.83 ± 1.88	0.88 ± 0.15
DZ-RN	213.42 ± 128.99	340.60 ± 168.92	348.04 ± 161.47	3.70 ± 1.14	0.82 ± 0.10
DF-RN	369.50 ± 177.23	490.55 ± 186.68	511.53 ± 179.36	4.59 ± 1.39	0.87 ± 0.12
HK-RN	346.85 ± 188.71	492.23 ± 226.98	511.22 ± 234.13	4.19 ± 1.59	0.80 ± 0.21
JFL-LE	533.77 ± 209.11	696.02 ± 238.19	700.85 ± 227.48	5.25 ± 1.17	0.89 ± 0.07
BS-RN	424.17 ± 126.51	550.92 ± 164.77	574.32 ± 159.53	5.04 ± 1.07	0.89 ± 0.09
SY-RN	342.91 ± 129.91	458.86 ± 162.56	466.06 ± 153.41	5.12 ± 1.01	0.92 ± 0.06
LG-RL	532.85 ± 221.85	715.35 ± 259.97	747.67 ± 259.51	5.30 ± 1.31	0.90 ± 0.09

YGL: Yinggeling; HJJL: Huangjingjiaoling; DZ: Danzhou; DF: Dongfang; HK: Haikou; JFL: Jianfengling; BS: Baisha; SY: Sanya; LG: Lingao; LE: Leopoldamys edwardsi; RN: Rattus norvegicus; RL: Rattus losea.

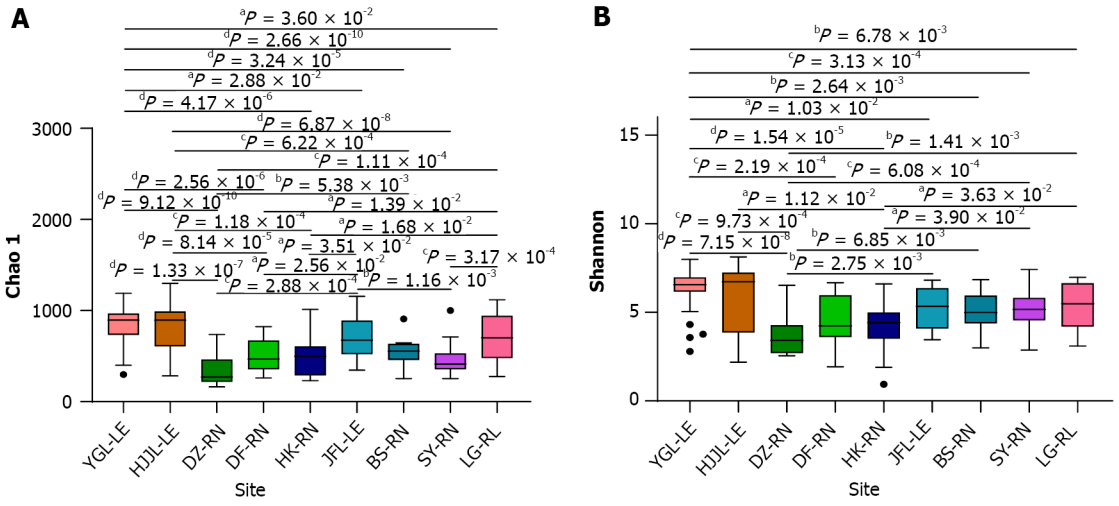

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Figure 4 Boxplot representation of observed species. Boxplots show the distribution of bacteria between rat samples categorized under different locations and rat species. A: Boxplot representation of chao1; B: Boxplot representation of Shannon diversity. Values from all statistical tests are available in additional Table 2. YGL: Yinggeling; HJJL: Huangjingjiaoling; DZ: Danzhou; DF: Dongfang; HK: Haikou; JFL: Jianfengling; BS: Baisha; SY: Sanya; LG: Lingao; LE: Leopoldamys edwardsi; RN: Rattus norvegicus; RL: Rattus losea. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $^{d}P < 0.0001$.

Figure 5C), followed by that between Baisha and Dongfang (0.57), and those of Danzhou area (0.61-0.63, Supplementary Table 8) with Baisha, Dongfang, Haikou, and Lingao (Figure 5C). Conversely, samples from the Sanya area were most dissimilar to those from Huangjingjiaoling (0.91), Lingao (0.89), and Haikou (0.88). As shown in Figure 5D, the lowest difference (0.16, Supplementary Table 9) was observed between samples from Yinggeling and Huangjingjiaoling (highlighted by the blue triangles), suggesting the bacteria in these two groups of samples were evolutionary more similar. Conversely, the bacteria in the samples from the Sanya area were evolutionary most distant (highlighted by the stars in Figure 5D) to those from Danzhou and Haikou (0.51 and 0.57, respectively; Supplementary Table 9).

DISCUSSION

Through the same procedure from sample collection and processing to sequencing, the gut bacterial communities differed at all levels (phylum, class, family, and genus) among fecal samples of wild rats from nine geographical locations, three different habitats, and rat species, and two seasons (winter *vs* summer). According to scant research, diet uncontrollable for wild rats is a significant determinant of gut bacterial composition[21-23], which differs for rats living in diverse habitats in every location at all

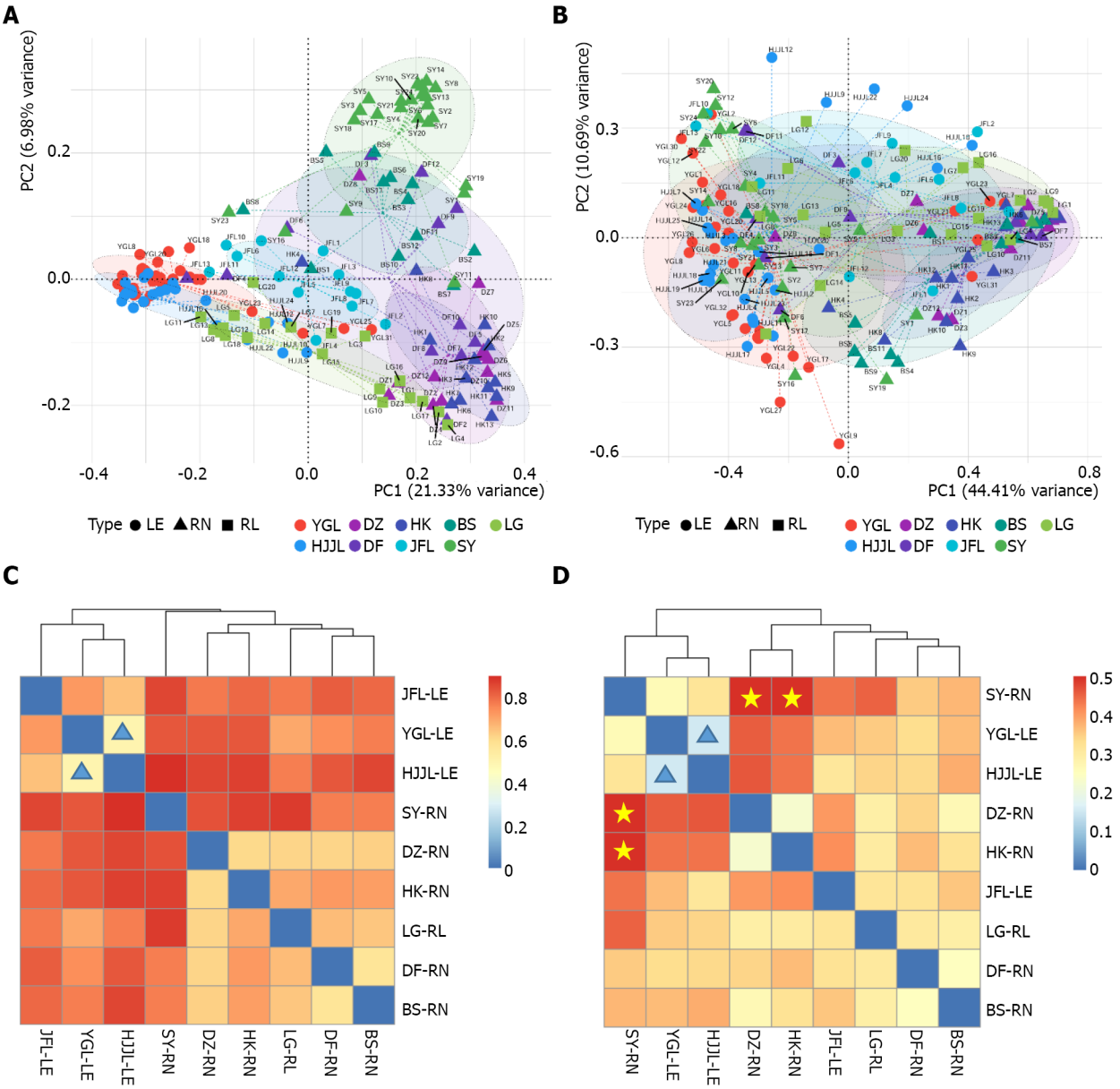

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Figure 5 Principal coordinate analysis plot based on unweighted Unifrac distance and weighted Unifrac distance matrix depicting differences in the composition of the gut microbiota from different rat species and locations. A: unweighted Unifrac distance; B: Weighted Unifrac distance; C and D: Colors represent community profiles of location. Matrix heatmap of the Bray-Curtis distances (C) and the weighted unifrac beta diversity (D) between the microbial communities of rats from nine different areas. YGL: Yinggeling; HJJL: Huangjingjiaoling; DZ: Danzhou; DF: Dongfang; HK: Haikou; JFL: Jianfengling; BS: Baisha; SY: Sanya; LG: Lingao; LE: Leopoldamys edwardsi; RN: Rattus norvegicus; RL: Rattus losea.

seasons. There were quite a few striking findings from our study. As the only representative samples of residential area (Haikou) rats, their gut bacterial composition was different at the family and genus level, richest with *Peptostreptococcaceae* and unidentified_*Clostridiales* at the family level, and with unidentified_*Clostridiales* and *Romboutsia* at the genus level. Although collected from the same rat species (*Rattus norvegicus*) during the same winter season, the bacterial profiles of the four groups of samples from different areas (Baisha, Dongfang, Danzhou, and Haikou) were distinctly different at the class, family, and genus level, revealing the complexity of factors (*e.g.*, habitat, farmland *vs* residential area) behind the bacterial community. The general bacterial profile of samples taken from the same species of rat (*Leopoldamys edwardsi*) in the summer in one mountain area (Yinggeling) varied from samples taken in the winter from the other two mountain areas (Huangjingjiaoling and Jianfengling), indicating that season (and thus a variety of factors) played a significant role in determining their gut bacterial composition.

Several studies report that Firmicutes, Bacteroidetes, and Proteobacteria are the most abundant phyla, and *Lactobacillus* is the predominant genus in fecal samples from both wild (*Rattus norvegicus*) and laboratory (Sprague-Dawley) rats[7,9], a pattern similar to our results. Fusobacteria was interestingly

the fourth most prevalent phylum in[10], which is most consistent with findings from one of our nine groups (Dongfang) (Figure 2B). Although of the same species (*Rattus norvegicus*), sample size (12), and similar climate, rats from the Dongfang area of this study were trapped in January from farmland on an isolated island contrasting to all year round (one mouse per month) from a residential area in an inland metropolitan city[10].

Although we identified some potential bacterial pathogens by their species names and reputation in the samples (Supplementary Table 3), a profile distinct from the results of other studies[11,12], they still need to be tested for virulence and pathogenesis. Because wild rats carry some bacteria (*Bacillus anthracis*, *Vibrio cholerae,* and *Yersinia pestis*)[11,12] and this study] can cause a serious outbreak or even a pandemic, it would be prudent to start a regular survey program at some strategic points to monitor the distribution of sentinel rodents and the target pathogens the animals may transmit.

The ideal comparison should only have one factor, but this seems like an impossible task for our current research. Admittedly our study had some limitations, to name a few: (1) As some variables, like sex, age, and so on, were difficult, if not impossible, to control as a baseline investigation; (2) despite the interesting findings (having the highest *Corynebacteriaceae* in all samples) of the gut bacterial composition in *Rattus losea*, it was from only one area (Lingao); (3) the summer samples were collected from two different rat species (*Rattus norvegicus vs Leopoldamys edwardsi*,) in two different habitats (farmland *vs* mountain) from only one area each, making the results less conclusive and comparable; and (4) samples from residential rats were only from Haikou area, making some of the results less comparable.

CONCLUSION

The study revealed the structure of gut bacterial communities by sampling wild rats' feces. In general, the gut microbiota was different in both composition and abundance in samples from the nine areas of Hainan of three wild rat species, three habitats (mountain, farmland, and residential area), and two seasons (summer and winter). This study provides fundamental information for identifying microbial communities that will be useful for disease control in Hainan Province.

ARTICLE HIGHLIGHTS

Research background

Wild rats are potential reservoirs for zoonotic infectious agents which can be transmitted to and cause diseases in humans.

Research motivation

As a tropical island locating in the south of China, Hainan province has abundant rat species.

Research objectives

To better understand the composition of gut bacterial communities in rats is essential for preventing and treating such diseases. Here, we examined the gut bacterial composition in wild adult rats from Hainan province.

Research methods

Fresh faecal samples were collected from 162 wild adult rats, including three species (*Rattus norvegicus*, *Leopoldamys edwardsi*, and *Rattus losea*), from nine regions of Hainan province between 2017-2018.

Research results

We analyzed the composition of gut microbiota using the 16S rRNA gene amplicon sequencing. We identified 4,903 bacterial operational taxonomic units (30 phyla, 175 families, and 498 genera), which is different among samples of different rat species in different habitat during different season. In general, Firmicutes were the most abundant phyla, followed by Bacteroidetes (15.55%), Proteobacteria (6.13%) and Actinobacteria (4.02%). The genus *Lactobacillus* (20.08%), unidentified_*Clostridiales* (5.16%), *Romboutsia* (4.33%), unidentified_*Ruminococcaceae* (3.83%), *Bacteroides* (3.66%), *Helicobacter* (2.40%) and *Streptococcus* (2.37%) were dominant.

Research conclusions

Among locations for rat species, the gut microbial communities were different in composition and abundance. This work provides fundamental information to identify microbial communities useful for disease control in Hainan province.

Research perspectives

The study revealed the structure of gut bacterial communities by sampling wild rats' feces. In general, the gut microbiota was different in both composition and abundance in samples from the nine areas of Hainan of three wild rat species, three habitats (mountain, farmland and residential area), and of two seasons (summer and winter). This work provides fundamental information to identify microbial communities which will be useful for disease control in Hainan province.

FOOTNOTES

Author contributions: Niu LN, Zhang GN, Xuan DD, Lin C and Lu Z contributed equally; Niu LN contributed to the conceptualization; Zhang GN, Xuan DD, and Cao PP performed the data curation; Lu Z, Chen SW, and Zhang Y contributed to the formal analysis; Cao PP, Cui XJ, and Lu Z performed the investigation; Niu LN, and Lin C performed the methodology; Hu SK, and Lin C contributed to the supervision; Niu LN, Zhang GN, Xuan DD, and Hu SK wrote the original draft; All authors contributed to the article and approved the submitted version.

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Institutional animal care and use committee statement: The study was approved by the Animal Ethics Committee of Hainan Medical University, China. We confirmed that all methods were performed under the relevant guidelines and regulations.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The study's datasets have been added to the Sequence Read Archive repository (SRA). This item has accession number PRJNA767903.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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ORCID number: Shou-Kui Hu 0000-0001-6339-3954.

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REFERENCES

1 **Hulme-Beaman A**, Orton D, Cucchi T. The origins of the domesticate brown rat (Rattus norvegicus) and its pathways to domestication. *Anim Front* 2021; **11**: 78-86 [PMID: 34158992 DOI: 10.1093/af/vfab020]
2 **Chen Y**, Zhou J, Wang L. Role and Mechanism of Gut Microbiota in Human Disease. *Front Cell Infect Microbiol* 2021; **11**: 625913 [PMID: 33816335 DOI: 10.3389/fcimb.2021.625913]
3 **Brooks SP**, McAllister M, Sandoz M, Kalmokoff ML. Culture-independent phylogenetic analysis of the faecal flora of the rat. *Can J Microbiol* 2003; **49**: 589-601 [PMID: 14663493 DOI: 10.1139/w03-075]
4 **Manichanh C**, Reeder J, Gibert P, Varela E, Llopis M, Antolin M, Guigo R, Knight R, Guarner F. Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. *Genome Res* 2010; **20**: 1411-1419 [PMID: 20736229 DOI: 10.1101/gr.107987.110]
5 **Ferrario C**, Statello R, Carnevali L, Mancabelli L, Milani C, Mangifesta M, Duranti S, Lugli GA, Jimenez B, Lodge S, Viappiani A, Alessandri G, Dall'Asta M, Del Rio D, Sgoifo A, van Sinderen D, Ventura M, Turroni F. How to Feed the Mammalian Gut Microbiota: Bacterial and Metabolic Modulation by Dietary Fibers. *Front Microbiol* 2017; **8**: 1749 [PMID: 28955319 DOI: 10.3389/fmicb.2017.01749]
6 **Flemer B**, Gaci N, Borrel G, Sanderson IR, Chaudhary PP, Tottey W, O'Toole PW, Brugère JF. Fecal microbiota variation across the lifespan of the healthy laboratory rat. *Gut Microbes* 2017; **8**: 428-439 [PMID: 28586297 DOI: 10.1080/19490976.2017.1334033]

7 **Li D**, Chen H, Mao B, Yang Q, Zhao J, Gu Z, Zhang H, Chen YQ, Chen W. Microbial Biogeography and Core Microbiota of the Rat Digestive Tract. *Sci Rep* 2017; **8**: 45840 [PMID: 28374781 DOI: 10.1038/srep45840]

8 **Nagpal R**, Wang S, Solberg Woods LC, Seshie O, Chung ST, Shively CA, Register TC, Craft S, McClain DA, Yadav H. Comparative Microbiome Signatures and Short-Chain Fatty Acids in Mouse, Rat, Non-human Primate, and Human Feces. *Front Microbiol* 2018; **9**: 2897 [PMID: 30555441 DOI: 10.3389/fmicb.2018.02897]

9 **Pan H**, Guo R, Zhu J, Wang Q, Ju Y, Xie Y, Zheng Y, Wang Z, Li T, Liu Z, Lu L, Li F, Tong B, Xiao L, Xu X, Li R, Yuan Z, Yang H, Wang J, Kristiansen K, Jia H, Liu L. A gene catalogue of the Sprague-Dawley rat gut metagenome. *Gigascience* 2018; **7** [PMID: 29762673 DOI: 10.1093/gigascience/giy055]

10 **He WQ**, Xiong YQ, Ge J, Chen YX, Chen XJ, Zhong XS, Ou ZJ, Gao YH, Cheng MJ, Mo Y, Wen YQ, Qiu M, Huo ST, Chen SW, Zheng XY, He H, Li YZ, You FF, Zhang MY, Chen Q. Composition of gut and oropharynx bacterial communities in Rattus norvegicus and Suncus murinus in China. *BMC Vet Res* 2020; **16**: 413 [PMID: 33129337 DOI: 10.1186/s12917-020-02619-6]

11 **Himsworth CG**, Parsons KL, Jardine C, Patrick DM. Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. *Vector Borne Zoonotic Dis* 2013; **13**: 349-359 [PMID: 23590323 DOI: 10.1089/vbz.2012.1195]

12 **Easterbrook JD**, Kaplan JB, Vanasco NB, Reeves WK, Purcell RH, Kosoy MY, Glass GE, Watson J, Klein SL. A survey of zoonotic pathogens carried by Norway rats in Baltimore, Maryland, USA. *Epidemiol Infect* 2007; **135**: 1192-1199 [PMID: 17224086 DOI: 10.1017/S0950268806007746]

13 **Zhao W**, Zhou H, Yang L, Ma T, Zhou J, Liu H, Lu G, Huang H. Prevalence, genetic diversity and implications for public health of Enterocytozoon bieneusi in various rodents from Hainan Province, China. *Parasit Vectors* 2020; **13**: 438 [PMID: 32878633 DOI: 10.1186/s13071-020-04314-9]

14 **Verma SK**, Singh L, Novel universal primers establish identity of an enormous number of animal species for forensic application.
Molecular Ecology Notes 2010; 3: 28-31 [DOI: 10.1046/j.1471-8286.2003.00340.x]

15 **Martin M**. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J* 2011; **17** [DOI: 10.14806/ej.17.1.200]

16 **Quast C**, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 2013; **41**: D590-D596 [PMID: 23193283 DOI: 10.1093/nar/gks1219]

17 **Edgar RC**, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011; **27**: 2194-2200 [PMID: 21700674 DOI: 10.1093/bioinformatics/btr381]

18 **Haas BJ**, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ; Human Microbiome Consortium, Petrosino JF, Knight R, Birren BW. Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. *Genome Res* 2011; **21**: 494-504 [PMID: 21212162 DOI: 10.1101/gr.112730.110]

19 **Edgar RC**. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013; **10**: 996-998 [PMID: 23955772 DOI: 10.1038/nmeth.2604]

20 **Edgar RC**. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004; **32**: 1792-1797 [PMID: 15034147 DOI: 10.1093/nar/gkh340]

21 **Queipo-Ortuño MI**, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM, Cardona F, Casanueva F, Tinahones FJ. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One* 2013; **8**: e65465 [PMID: 23724144 DOI: 10.1371/journal.pone.0065465]

22 **Liu X**, Blouin JM, Santacruz A, Lan A, Andriamihaja M, Wilkanowicz S, Benetti PH, Tomé D, Sanz Y, Blachier F, Davila AM. High-protein diet modifies colonic microbiota and luminal environment but not colonocyte metabolism in the rat model: the increased luminal bulk connection. *Am J Physiol Gastrointest Liver Physiol* 2014; **307**: G459-G470 [PMID: 24970777 DOI: 10.1152/ajpgi.00400.2013]

23 **Qi X**, Xu W, Guo M, Chen S, Liu Y, He X, Huang K. Rice- or pork-based diets with similar calorie and content result in different rat gut microbiota. *Int J Food Sci Nutr* 2017; **68**: 829-839 [PMID: 28320221 DOI: 10.1080/09637486.2017.1301889]

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