ARTICLE

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Research on feeding habits and stomach fungi in *Eothenomys miletus* from Hengduan mountain regions

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Author contributions

Wan-Long Zhu conceived research, participated in design and coordination, and drafted a manuscript. Bo-Wen Yan and Wan-Long Zhu conducted research on plant detection of gastric contents and fungi detection in the stomach. All authors have contributed to this article and approved the submitted version.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

E. Miletus, *Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan; %RA, Relative Abundance; %FOO, Frequency Of Occurrence.

Citation

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Abstract

Eothenomys miletus (*E. miletus*) is one of the rodent species found in Yunnan, and it has caused significant harm to local agricultural production. In this study, we aimed to investigate the differences in feeding habits and stomach fungi of *E. miletus* across different areas in the Hengduan mountain regions. By exploring the main factors affecting the feeding habits and fungi of *E. miletus*, this study provides a theoretical basis for the prevention and control of this species. We collected *E. miletus* specimens from five regions, namely Deqin, Xianggelila, Lijiang, Jianchuan, and Ailaoshan. We measured their feeding habits and the types of fungi in their stomachs using high-throughput sequencing technology. The results showed that *E. miletus* primarily fed on Poaceae, Oxalidaceae, Asteraceae, and Fabaceae. Food diversity of *E. miletus* in Ailaoshan and Jianchuan was significantly lower than in the other three regions. As for stomach fungi, Ascomycota and Basidiomycota were the absolute dominant sectors. Changes in the diversity of fungi in different regions were consistent with changes in food diversity. The diversity of fungi in Ailaoshan and Jianchuan regions was lower than in the other three regions. These findings suggest that the feeding habits of *E. miletus* in different regions were affected by plant species, which, in turn, affects the diversity of fungi in their stomachs.

Keywords: *Eothenomys miletus*; Hengduan mountain; feeding habits; fungi

Labeled objective

Understanding how animals interact with their environment and conducting population ecology research require analysis of animal feeding behavior [1]. The study of animal feeding behavior encompasses several aspects, such as feeding habits, feeding time, food composition, and food utilization [2]. Factors such as habitat type, availability and quality of food resources, predation risk, and interference from human activity can all influence an animal's feeding habits [3]. Feeding habits of sympatric rodents can also be influenced by other related species. For instance, studies on two *Eospalax* species in the same area revealed that *Eospalax cansus* can avoid competition with its relatives by adopting a more diversified diet, while *Eospalax smithii* can control more available food resources in the area due to their larger population size [4]. Furthermore, the same species from different environments may adapt to their surroundings by altering their feeding strategies. Studies on *Cryptomys hottentotus* in various environments have shown that the feeding habits of *Cryptomys hottentatus* in arid areas with poor food resources are more complex than those in areas with adequate humidity and abundant food resources. They can compensate for the lack of food by broadening the width of their food niche [5].

With the increasing development of gut microbiology research in recent years, there has been a rise in studies on various rodent gut microorganisms. However, most of these studies focus on prokaryotes in animal gut microbiota, with relatively few majoring in eukaryotes, such as fungi, in the animal digestive tract. Sun et al. found that fungi play a crucial role in the intestinal tract of animals [6]. Fungi, as decomposers in the ecosystem, have a powerful ability to break down plant fibers and lignin and convert them into substances that can be used directly by other flora in the stomach. This is as important for phytophagous animals as the study of their gut microbes [7, 8]. In our previous studies, we have investigated the physiology, morphology, and microorganisms of the alimentary canal of *Eothenomys miletus* (*E. miletus*) in different regions [9, 10]. However, there have been no reports on the feeding habits and fungal diversity in the stomach of *E. miletus*. Understanding the feeding habits and fungal diversity in the stomach is of great significance in comprehending the energy metabolism of *E. miletus* in its natural environment.

Since the Quaternary glaciation, the activities of glaciers have resulted in the uplift of the Qinghai-Tibet Plateau and the formation of the Hengduan Mountains, leading to the evolution of climate characteristics from humid and warm to dry and cold. Furthermore, the north-south direction of the mountains and altitude greatly influence the region. As altitude increases, temperature decreases and precipitation increases, resulting in distinct dry and wet seasons, small annual temperature differences, large daily temperature fluctuations, and a three-dimensional climate, which in turn contributes to the diversity of vegetation and animal habitats [11]. *E. miletus*, belonging to the genus *Eothenomys* in Arvicolinae, is an inherent species of the Hengduan Mountains and an endemic species of China. Voles of this species are predominantly nocturnal [12, 13]. Previous reports on the feeding habits of rodents have utilized research methods such as direct observation, dissection, stool microscopic tissue analysis, and gastric content analysis [1]. However, with the advancement of high-throughput sequencing technology and the use of DNA barcodes, it is now possible to combine DNA barcodes with high-throughput sequencing, thereby allowing for species identification at the taxonomic level, making it particularly suitable for studying feeding habits of species that are challenging to observe or inhabit special habitats [14]. In this study, we employed 16S rRNA to investigate the feeding habits of *E. miletus* at the molecular level for the first time, and combined it with 18S rRNA to explore the relationship between its feeding habits and gastric fungi. This research can provide a theoretical foundation for understanding the survival and adaptation strategies of *E. miletus*.

Methods

Sample collection

E. miletus were captured in Deqin (DQ), Xianggelila (XGLL), Lijiang (LJ), Jianchuan (JC), and Ailaoshan (ALS) during the winter of 2020. All animals were healthy adult individuals in the non-reproductive period, and all animal procedures were conducted in accordance with the regulations of the Animals Care and Use Committee of the School of Life Sciences at Yunnan Normal University. This study was approved by the Committee (13-0901-011). Table 1 provides detailed information on the geographic location, climatic characteristics, and sample size of each sample point.

Measurement of morphological indicators

The morphological indicators were measured with reference to the methods of Yang et al. and Xia et al. Including body mass (accurate to 0.01 cm), body length (accurate to 0.01 g) [15, 16].

DNA extraction

We collected the contents of the stomach and extracted the fecal DNA using rapid purification kit (QIAamp Fast DNA Stool Mini Kit). Amplification was performed using 16S and 18S primers, respectively. The primers for 16S were rbcL and trnH-psbA, rbcL-F: CTTACCAGYCTTGATCGTTACAAAGG, rbcL-R: GTAAAATCAAGTCCACCRCG; trnH: ACTGCCTTGATCCACTTGGC, psbA: CGAAGCTCCATCTACAAATGG; The primers for 18S were SSU0817F, SSU1196R, SSU0817F: TTAGCATGGAATRAATAGGA, SSU1196R: TCTGACCTGGAGTTCC [17, 18].

Bioinformatics analysis

The Illumina MiSeq/NovaSeq platform was utilized for performing paired-end sequencing of community DNA fragments, and the QIIME platform was employed for processing and analyzing the raw data. Initially, the double-ended sequences were merged using Flash software, and each sample was assigned a unique barcode label. During the merging process, low-quality sequences (sequences with a length less than 300 or a base mass fraction less than 30) were removed. Next, the QIIME feature table rake function was applied to normalize the sequencing depth to 95% of the minimum sample sequence size.

Table 1 Geographical locations and main conditions for five populations of *E. miletus*

Regions	Sample number	Longitude and latitude	Altitude/m	Annual average temperature/°C	Winter temperature/°C	Precipitation/ mm	Vegetation types
DQ	9	99°03'75" E, 28°35'14" N	3459	4.7	3.0	633.7	Alpine meadows
XGLL	10	99°83'16" E, 27°90'73" N	3321	5.5	4.5	984.2	Subalpine meadows
LJ	10	100°22'90" E, 26°87'53" N	2478	12.6	10.5	975.0	Subalpine meadows and shrub lands
JC	11	99°75'03" E, 26°43'95" N	2590	13.9	11.0	987.3	Leafy bushes
ALS	10	100°42'49" E, 24°90'30" N	2217	19.7	17.5	597.0	Savannah shrubs

E. Miletus, *Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

Evaluation of the feeding habits

The dietary habits of *E. miletus* were assessed using the Relative Abundance (%RA) and Frequency Of Occurrence (%FOO) methods. Species with a %RA less than 0.01% and those with a lower %FOO were considered incidental food during the analysis, as their inclusion may introduce deviations [19]. Moreover, analyzing only the %FOO size may overestimate the importance of rare food types. To evaluate the food composition of *E. miletus* at the family and genus levels, the %RA and %FOO methods were combined.

Prediction and analysis of fungal function

Using the PICRUSt2 software, we were able to predict the functional metabolic pathways of gastric content fungi in *E. miletus* from the five regions studied. The results were annotated using the KEGG database.

Data analyses

Calculation of Relative Abundance and Frequency Of Occurrence. Relative Abundance (RA) = $(N_i/\Sigma N_i) \times 100\%$, Frequency Of Occurrence (FOO) = $(N_i/N) \times 100\%$.

Feeding habits of *E. miletus* in different regions. The correlation between regions and food is represented by a chord diagram.

α, β diversity. α diversity: estimate the diversity through three diversity indicators: Chao 1, Shannon, and Simpson. β diversity: using the ASV/OTU table flattened by QIIME2, call the “qiime diversity core metrics physical” command to calculate four different distance matrices, and perform a principal coordinate analysis of these distance matrices. The community structure is described using unweighted and weighted UniFrac distance matrices. The unweighted UniFrac distance depends on phylogenetic relationships and OTU species abundance, while species deletion/presence and phylogenetic relationships are considered by the weighted UniFrac. The figures were drawn in https://www.genescloud.cn.

α correlation between diversity index and body weight and length. The data were analyzed using SPSS 26.0 software, and the correlation was analyzed using a bivariate method and * was considered statistically significant, ** was considered extremely significant correlation.

Results

Food composition of *E. miletus*

At the family level, the %RA values for Poaceae, Oxalidaceae, Asteraceae, and Fabaceae are 28.77%, 19.97%, 18.76%, and 14.79%, respectively. The %FOO for these families is 100%, indicating that they are important components of *E. miletus*’ diet. In fact, the sum of these four families accounts for as much as 82.29% of the diet, suggesting that they are major contributors to *E. miletus*’ food composition (Figure 1).

At the genus level, *E. miletus* in all five regions was found to consume plants from the genera *Oxalis*, *Cenchrus*, and an unknown genus in the *Asteraceae* family, with %RA values of 19.97%, 19.44%, and 18.30%, respectively, and a %FOO of 100% for all three genera. Additionally, the %RA values for *Lotus*, an unknown genus in the *Poaceae* family, and *Oxyria* were found to account for a certain proportion, but their %FOO value was only 60%, indicating that *E. miletus* feeds on these in only three of the five regions (Figure 2).

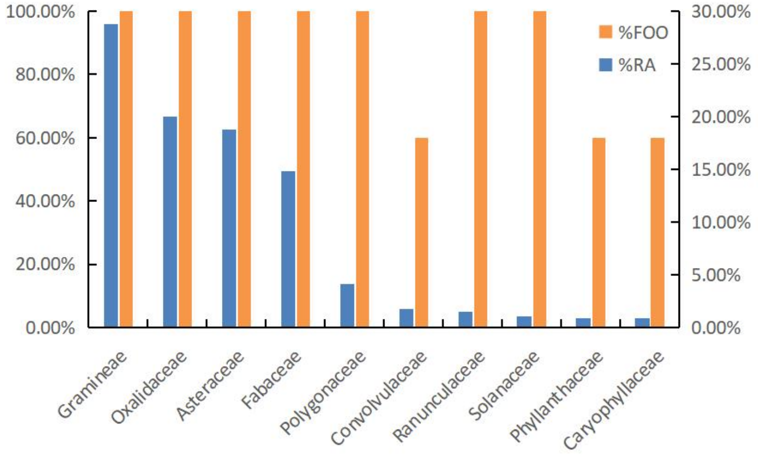

Figure 1 The family level food composition of the *E. miletus* (%RA and %FOO methods). *E. Miletus*, *Eothenomys miletus*; %RA, Relative Abundance; %FOO, Frequency Of Occurrence.

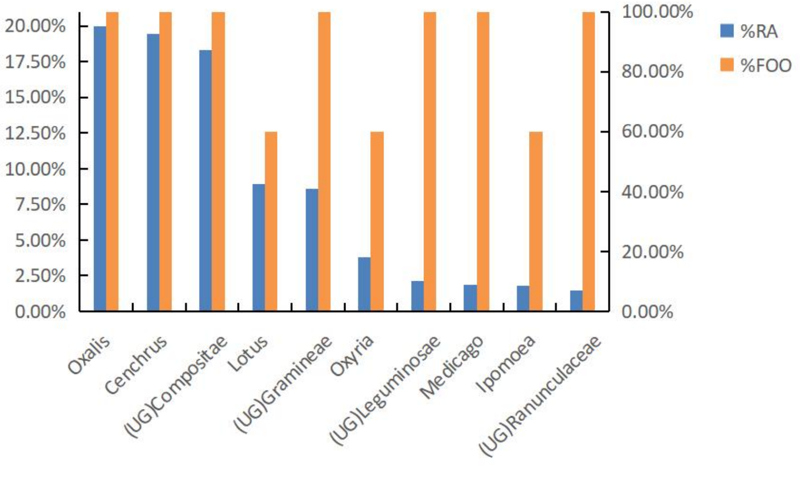

Figure 2 The genus level food composition of the *E. miletus* (%RA and %FOO methods). *E. Miletus*, *Eothenomys miletus*; %RA, Relative Abundance; %FOO, Frequency Of Occurrence.

Comparison of food composition types in *E. miletus* in different regions

Figure 3 presents the correspondence analysis of the feeding habits of *E. miletus* at the family level in five regions, and Figure 4 depicts the correspondence analysis of the feeding habits of *E. miletus* at the genus level in the same five regions. The results indicate that *E. miletus* primarily relies on four families and five genera as their main food sources, but owing to variances in vegetation across different regions, there are discernible differences in their food preferences and composition.

Analysis of food diversity in *E. miletus* in different regions

α analysis of feeding in five regions. Significant differences were observed in the α diversity (Chao 1, Shannon, and Simpson) ($P < 0.01$, Figure 5) between *E. miletus* samples from different regions. The results showed that *E. miletus* from LJ, XGLL, and DQ had higher food diversity compared to ALS and JC, with *E. miletus* from JC displaying the lowest food abundance. It can be speculated that the high altitude and low temperature of XGLL, combined with the lack of vegetation during winter sampling, may have forced *E. miletus* to seek more complex food sources to meet its nutritional requirements. In contrast, JC, with its relatively lower altitude and abundant rainfall throughout the year, had a more diverse and abundant food supply, making it easier for *E. miletus* to meet its dietary needs. This observation is consistent with the sampling site conditions.

Correlation analysis between diversity indices and body weight and length of *E. miletus* indicated a negative correlation between body size and the Chao 1, Shannon, and Simpson indices, with the difference being highly significant ($P < 0.01$) (Table 2). The results suggest that larger *E. miletus* specimens had lower food diversity. Larger specimens have better food gathering abilities, allowing them to occupy the main food sources in their respective regions.

β analysis of feeding in five regions. The principal coordinate analysis results show that (unweighted, Figure 6A, and weighted, Figure 6B) only samples from JC and ALS are clustered together. This result may indicate that the plant species diversity of ALS and JC is relatively low, and the food of *E. miletus* in these two regions is basically clustered together, while the plant differences between different regions in the other three regions are significant, which is also consistent with the α The results of diversity analysis correspond to each other.

Gastric fungal composition of *E. miletus*

Fungal DNA was extracted using high throughput sequencing and PCR products were amplified. After the removal of low-quality sequences, chimeras, monomers, and chloroplasts, the remaining sequences were standardized to 594131. The composition of the gastric fungal flora in *E. miletus* was analyzed based on the classification results of OUT. At the phylum level, the dominant fungi in the stomach of *E. miletus* in the five regions are shown in Figure 7, mainly Ascomycota (62.13%) and Basidiomycota (36.28%). At the genus level, the dominant genera of fungi in the stomach of *E. miletus* in the five regions are mainly *Candida* (8.54%) and *Cladosporium* (6.51%), except for *unclassified Ascomycota* (28.81%) and *unclassified Basidiomycota* (26.54%), as shown in Figure 8.

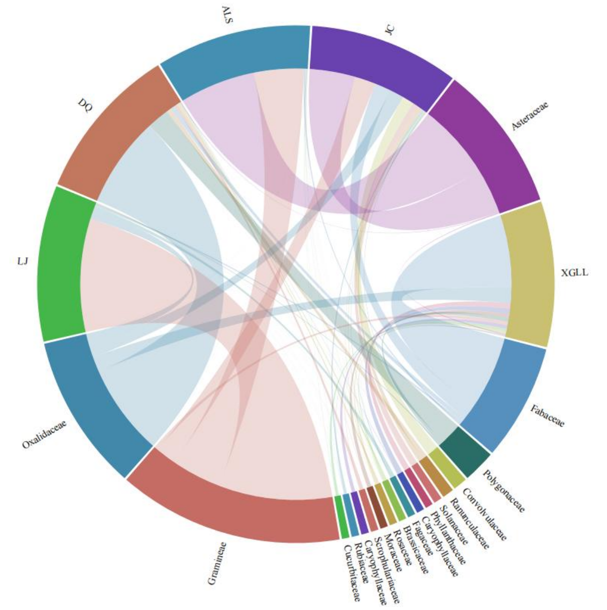

Figure 3 Chord diagram of feeding habits of *E. miletus* in different regions (Family Level). *E. Miletus*, *Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

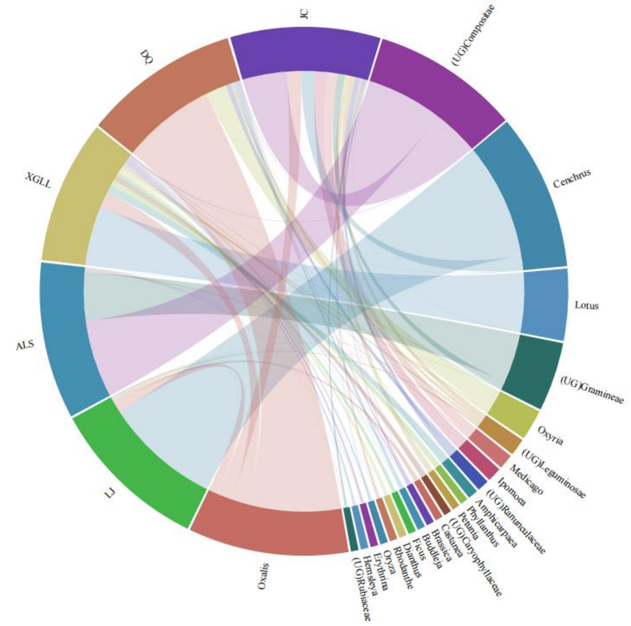

Figure 4 Chord diagram of feeding habits of *E. miletus* in different regions (Genus Level). *E. Miletus*, *Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

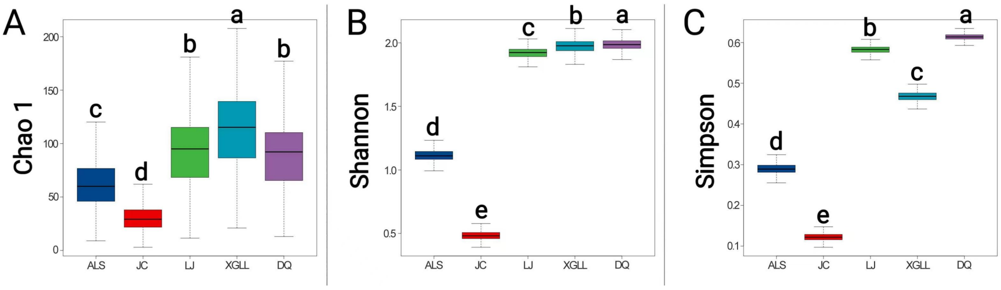

Figure 5 α diversity of food of the *E. miletus* in different regions. A, Chao1 Diversity; B, Shannon Diversity; C, Simpson Diversity. *E. Miletus*, *Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

Table 2 Food composition of *E. miletus* α diversity between diversity index and body weight and length

Alpha diversity indices	Body weight	Body length
Chao 1	−0.631**	−0.429**
Shannon	−0.655**	−0.528**
Simpson	−0.625**	−0.576**

*, was considered statistically significant, **, was considered extremely significant correlation. *E. Miletus*, *Eothenomys miletus*

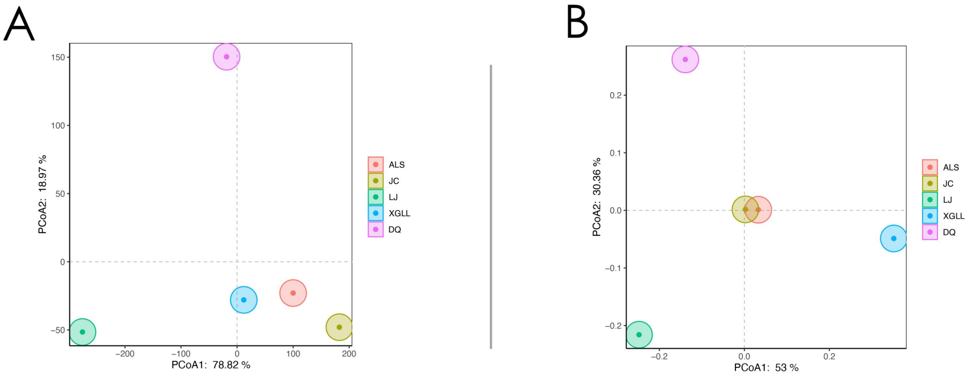

Figure 6 β diversity of food for *E. miletus* in different regions. A, unweighted; B, weighted. *E. Miletus*, *Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

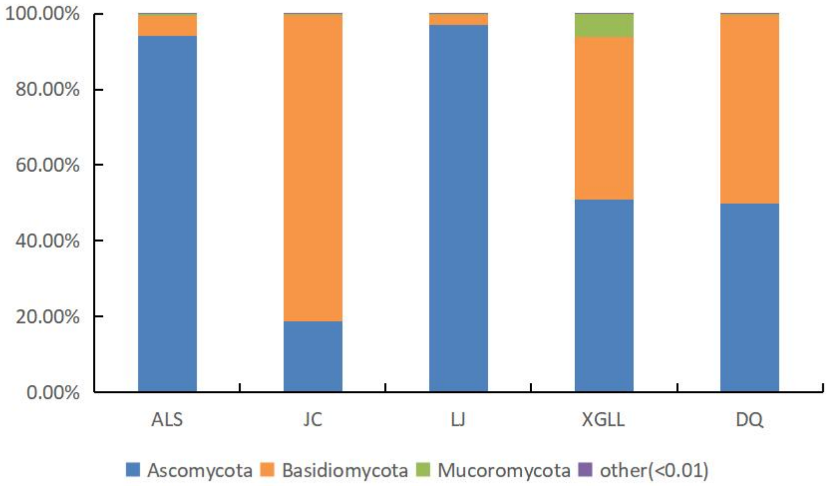

Figure 7 Level composition of fungus phyla in the stomach of *E. miletus* in different regions. *E. Miletus*, *Eothenomys miletus;* DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

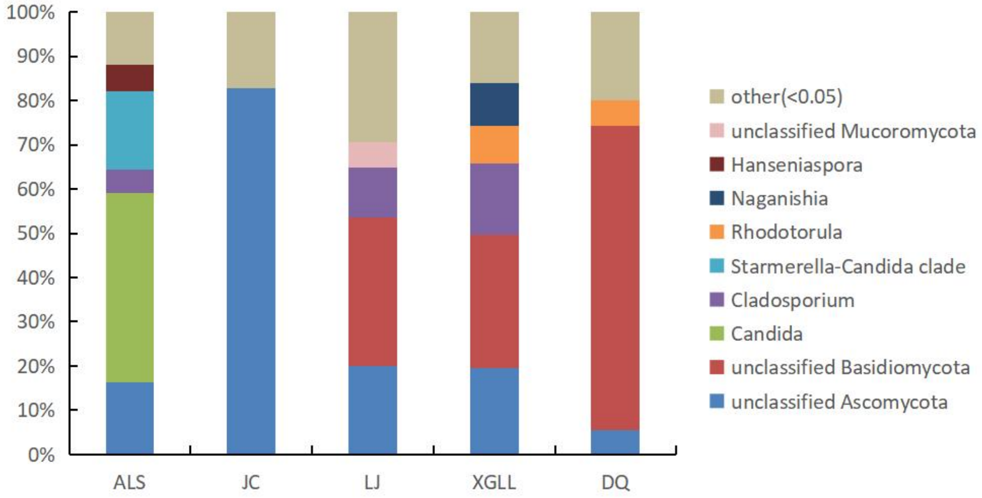

Figure 8 Level composition of fungus genus in the stomach of *E. miletus* in different regions. *E. Miletus*, *Eothenomys miletus;* DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

Analysis of fungi diversity in gastric content of *E. miletus*

Fungi α diversity index. There were significant differences in α diversity (Chao 1, Shannon, and Simpson) among the five regions ($P < 0.01$) (Figure 9). The diversity of fungi in the stomach of *E. miletus* was highest in LJ and XGLL, and lowest in the JC region, which corresponds to the feeding habits of *E. miletus* in each region. The diversity of fungi can affect the digestion of plant fibers by *E. miletus*. The greater the number of plant species consumed by *E. miletus*, the higher the diversity index of the fungi in its gut.

Fungi β diversity index. The principal coordinate analysis results indicate that, in the unweighted graph (Figure 10A) of *E. miletus* fungal flora, the DQ and LJ clusters are closely clustered together, while in the weighted graph (Figure 10B), the clusters of ALS and JC are more closely clustered. This pattern is similar to that observed in the α diversity of regional feeding habits, where regions with higher food diversity show closer clustering distances of fungi in the stomach of *E. miletus*.

Prediction and analysis of fungi function

Among the top 20 pathways, the primary function of fungi in the stomach of *E. miletus* is metabolism, constituting 91.13% of the total pathways, with biosynthesis accounting for 48.90% and catabolism accounting for 42.23%. Of these, only the lipoic acid metabolism pathway (ko00785), fatty acid biosynthesis pathway (ko00061), sulfur relay system (ko04122), and D-alanine metabolism pathway (ko00473) exhibit the highest abundance in LJ, while the remaining pathways exhibit the highest abundance in DQ (Table 3).

Conclusions

Feeding habits of *E. miletus*

Most methods for studying the feeding habits of rodents are based on microscopic tissue analysis, which, although allowing for quantitative analysis of feeding habits without the need to track individual animals, requires researchers to possess professional knowledge and the ability to accurately analyze plant debris in the animal's stomach. Moreover, some fragments may not be accurately separated due to excessive digestion time, which can significantly impact results and require considerable effort from researchers [20]. In contrast, this study used DNA barcode technology, a method for accurately and rapidly identifying the species animals feed on through short, standardized DNA sequence analysis [21]. This technology has previously been used by Zhang et al. to study the feeding habits of two zokors in Liupanshui, where they found that both zokors fed primarily on roots of herbaceous plants at the family level [4]. Similarly, DNA barcode technology was used to study the feeding habits of Marmota caudata, where chloroplast gene fragments were used as molecular markers [22]. In this study, DNA barcode technology was also used to analyze the feeding habits of *E. miletus* in five regions. Results indicated that *E. miletus* fed mainly on Poaceae, Oxalidaceae, Asteraceae, and Fabaceae, suggesting that herbs were their primary food source, consistent with other rodents [23]. Due to the low lignification of herbaceous plants and their small size, which are easy for rodents to ingest, the number of *E. miletus* captured near herbaceous plants in the five regions was higher than that near other plant species.

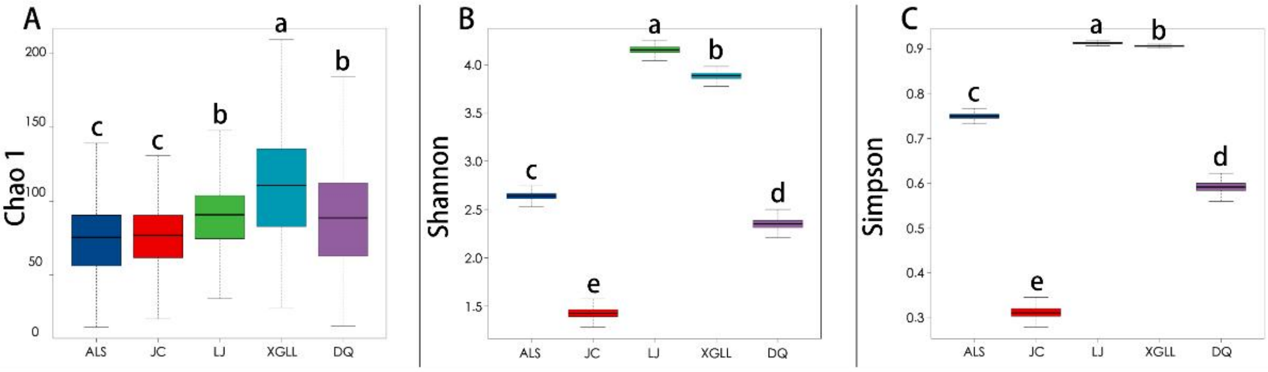

Figure 9 Alpha diversity of stomach fungi in *E. miletus* from different regions. A, Chao 1 Diversity; B, Shannon Diversity; C, Simpson Diversity. *E. Miletus, Eothenomys miletus*; DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

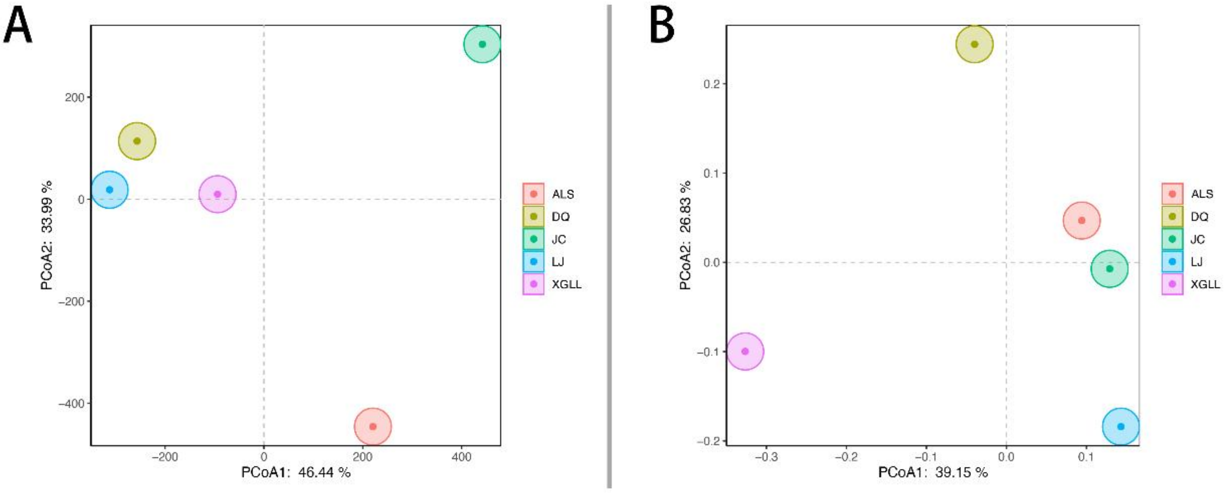

Figure 10 Beta diversity of gastric fungi in *E. miletus* in different regions. A, unweighted; B, weighted. *E. Miletus*, *Eothenomys miletus;* DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

Table 3 Functions of PICRUSt2 of stomach contents fungi in *E. miletus* predicting the top 20 MetaCyc database abundance

Rank	Pathway ID	ALS	JC	LJ	XGLL	DQ
1	ko01051	17.00	27.50	22.33	16.50	51.00
2	ko00290	12.64	18.14	20.10	8.95	29.14
3	ko00660	12.00	17.92	19.00	7.58	28.50
4	ko00770	9.87	11.53	16.12	6.27	21.67
5	ko00785	13.00	10.00	19.17	5.00	16.50
6	ko00061	14.08	8.12	17.92	3.42	16.77
7	ko00030	10.27	9.48	13.87	4.95	18.64
8	ko00072	10.75	8.63	11.71	0.00	25.88
9	ko04122	10.09	10.27	16.33	3.82	15.59
10	ko00473	9.33	7.50	18.33	6.67	14.17
11	ko00471	9.00	8.00	15.16	5.00	18.50
12	ko02030	11.08	10.23	8.44	6.69	18.73
13	ko00250	9.44	9.76	11.87	2.96	19.91
14	ko00620	9.95	8.65	14.13	3.18	17.61
15	ko00970	7.66	10.38	13.02	4.36	17.91
16	ko00730	9.45	8.59	15.48	3.27	16.32
17	ko00550	9.74	7.82	14.27	4.42	16.82
18	ko00340	8.00	9.42	12.84	4.37	17.34
19	ko00300	8.20	9.18	11.14	5.80	16.50
20	ko00670	7.71	8.21	12.18	4.21	18.29

E. Miletus, *Eothenomys miletus;* DQ, Deqin; XGLL, Xianggelila; LJ, Lijiang; JC, Jianchuan; ALS, Ailaoshan.

Feeding habits of *E. miletus* in different regions

Based on the α and β diversity in the five regions, the food diversity of *E. miletus* in LJ, XGLL, and DQ was significantly higher than that in ALS and JC. Clustering analysis of the feeding habits of ALS and JC showed that these two regions were grouped together, which was consistent with the local sampling site observations. The higher winter temperatures and more lush plant growth in JC and ALS meant that *E. miletus* in these regions had less foraging pressure and did not need to find a variety of plants to meet their needs, resulting in lower food diversity compared to the other three regions.

Combining *E. miletus*'s body shape with α diversity, we found that larger-sized individuals had lower food diversity. Previous research in the laboratory on *E. miletus* body shape in different regions revealed that individuals in ALS and JC were larger than those in LJ, XGLL, and DQ [24]. The food composition of *E. miletus* in these regions was also less diverse than that in LJ, XGLL, and DQ. Larger rodents occupy a higher niche, which allows them to occupy more food resources [25]. As a result, they may not need to eat and search for a wide range of food types to meet their needs, resulting in lower food diversity. Conversely, smaller rodents occupy a lower niche and need to eat a more diverse range of foods to meet their needs, resulting in higher food diversity.

Fungi in the stomach of *E. miletus* from different regions

E. miletus is a burrowing rodent species that primarily resides in shallow tunnels on the surface of the soil, which have relatively complex structures. Soil harbors the highest concentration of fungi in the natural environment, and the most suitable soil depth for fungal survival is approximately 10 cm in the surface layer [26]. Therefore, the type of fungi in the stomach of *E. miletus* is mainly influenced by the fungi types present in the soil of its habitat. Studies on digestive tract fungi in *Lasiopodomys brandtii* and zokor have shown that the dominant phyla are Ascomycetes and Basidiomycetes, consistent with the results of the present study [27, 28]. The use of 18S rRNA technology to analyze fungi in the stomach of *E. miletus* revealed that *Ascomycetes* and *Basidiomycetes* were also the predominant fungi.

Fungi possess a stronger ability to digest lignin than bacteria, and the diversity of fungi in phytophagous animals is influenced by their food diversity [29]. Combining food and fungi α diversity analysis, we found that two types of diversity exhibit a clear consistency, namely, the greater the food diversity, the greater the number of fungal species present in the stomach. This finding confirms that the diversity of fungi in the stomach is indeed influenced by food diversity. During functional prediction, it was observed that the primary functions of gastric fungi are biosynthesis (48.90%) and catabolism (42.23%). This indicates that metabolic pathways constitute the vast majority of fungal functions, suggesting that fungal physiological activities are robust, and that this biological activity promotes the digestive and metabolic activities of *E. miletus*. However, presently, there are few reports on the study of digestive tract fungi in rodents such as *E. miletus*, and most of the research focuses on intestinal microorganisms, leaving a gap in the research of digestive tract fungi in rodents. Therefore, the study of digestive tract fungi in *E. miletus* in this article lays the groundwork for future, more detailed research.

In conclusion, the feeding habits of *E. miletus* in various regions of the Hengduan Mountains are affected by the local environment. Plant growth is impacted by poor winter climate conditions. As a result, the poorer the climate conditions, the greater the food diversity of *E. miletus*, which in turn influences the diversity of fungi in its stomach. This finding further supports that the plant species present in the region where they reside are the primary factors affecting the feeding habits and fungi in the stomach of *E. miletus*.

References

1. Yong ZY, Guo C, Zhang MW, Wang Y, Li B. Significance and methodology of rodent's food habit research: A review. *Chin J Ecol* 2011;30(11):2637–2645. (Chinese) Available at: http://doi.org/10.13292/j.1000-4890.2011.0400
2. Zhu J, Yan ZT. Foods and food bases of the muskrat, Ondatra Zibethica linnaeus. *Curr Zool* 1965;4:352–363. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=3uoqIhG8C44YLTlOAiTRKth5mPLKqXjbyzE23kHsboNdN_s9Z9MF5gJAzByM2zbGmLRurf7nfSlSMYgNVDCJaKLPnntzy9l&uniplatform=NZKPT&src=copy
3. Wang Z, Zhang XH. Advances in the selection of foraging grounds for wild animals. *Chin J Ecol* 2014;33(11):3150–3156. (Chinese) Available at: http://doi.org/10.13292/j.1000-4890.20141022.007

4. Zhang XX, Zou Y, Zou X, Xu ZG, Nan XN, Han CX. DNA metabarcoding uncovers the diet of subterranean rodents in China. *PLoS One* 2022;17(4):e0258078. Available at: http://doi.org/10.1371/journal.pone.0258078
5. Robb GN, Harrison A, Woodborne S, Bennett NC. Diet composition of two common mole-rat populations in arid and mesic environments in South Africa as determined by stable isotope analysis. *J Zool* 2016;300(4):257–264. Available at: http://doi.org/10.1111/jzo.12378
6. Sun SS, Sun L, Wang K, et al. The gut commensal fungus, Candida parapsilosis, promotes high fat-diet induced obesity in mice. *Commun Biol* 2021;4(1):1220. Available at: http://doi.org/10.1038/s42003-021-02753-3
7. Joblin KN, Campbell GP, Richardson AJ, Stewart CS. Fermentation of barley straw by anaerobic rumen bacteria and fungi in axenic culture and in co-culture with methanogens. *Lett Appl Microbiol* 1989;9(5):195–197. Available at: http://doi.org/10.1111/j.1472-765X.1989.tb00323.x
8. Cheng YF, Shi QC, Sun RL, et al. The biotechnological potential of anaerobic fungi on fiber degradation and methane production. *World J Microbiol Biotechnol* 2018;34(10):155. Available at: http://doi.org/10.1007/s11274-018-2539-z
9. Zhang HJ, Hou DM, Mei L, Chen LX, Wang ZK, Zhu WL. Differences of body composition and morphology of the gastrointestinal tract in Eothenomys miletus at different areas from the Hengduan Mountains in summer. *Acta Theriol Sin* 2018;38(5):499–503. (Chinese) Available at: http://doi.org/10.16829/j.slxb.150168
10. Yan BW, Jia T, Wang ZK, Zhu WL. Comparative research of intestinal microbiota diversity and body mass regulation in Eothenomys miletus from different areas of Hengduan mountain regions. *Front Microbiol* 2022;13:1026841. Available at: http://doi.org/10.3389/fmicb.2022.1026841
11. Zhu WL, Cai JH, Lian X, Wang ZK. Adaptive character of metabolism in Eothenomys miletus in Hengduan Mountains region during cold acclimation. *J Therm Biol* 2010;35(8):417–421. Available at: http://doi.org/10.1016/j.jtherbio.2010.09.002
12. Liu YT, Jia T, Ren Y, Wang ZK, Zhu WL. Roles of Ghrelin and Leptin in Body Mass Regulation under Food Restriction Based on the AMPK Pathway in the Red-Backed Vole, Eothenomys miletus, from Kunming and Dali Regions. *Animals* (*Basel*) 2022;12(23):3333. Available at: http://doi.org/10.3390/ani12233333
13. Gong XN, Jia T, Zhang H, Wang ZK, Zhu WL. Physiological and behavioral responses of Eothenomys miletus in different elevations of Hengduan Mountain to high-sugar diet. *Chin J Zool* 2021;56(4):569–581. (Chinese) Available at: http://doi.org/10.13859/j.cjz.202104009
14. Pompanon F, Deagle BE, Symondson WO, Brown DS, Jarman SN, Taberlet P. Who is eating what: diet assessment using next generation sequencing. *Mol Ecol* 2011;21(8):1931–1950. Available at: http://doi.org/10.1111/j.1365-294X.2011.05403.x
15. Yang QS, Xia L, Ma Y, Feng ZJ, Quan GQ. Standard of measurement for skulls of mammals I: basic measurement. *Chin J Zool* 2005;3:50–56. (Chinese) Available at: http://doi.org/10.13859/j.cjz.2005.03.011
16. Xia L, Yang QS, Ma Y, Feng ZJ, Zhou LZ. Animal skull measurement standard III: Rodentia, Rabbitforma. *Chin J Zool* 2006;5:68–71. (Chinese) Available at: http://doi.org/10.13859/j.cjz.2006.05.013
17. Liu G, Ning Y, Xia XF, Gong MH. The application of high-throughput sequencing technologies to wildlife diet analysis. *Acta Eco Sin* 2018;38(9). (Chinese) Available at: http://doi.org/10.5846/stxb201706151092
18. Anderson IC, Cairney JWG. Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environ Microbiol* 2004;6(8):769–779. Available at: http://doi.org/10.1111/j.1462-2920.2004.00675.x
19. Šturm MB, Smith S, Ganbaatar O, et al. Isotope analysis combined with DNA barcoding provide new insights into the dietary niche of khulan in the Mongolian Gobi. *PLoS One* 2021;16(3):e0248294. Available at: http://doi.org/10.1371/journal.pone.0248294
20. Wang QY, Zhang YM, Wei WH, Bian JH. Study on feeding habits of plateau Myospalax. *Acta Theriol Sin* 2000;20(3):193–199. (Chinese) Available at: http://doi.org/10.16829/j.slxb.2000.03.005
21. Hebert PDN, Ratnasingham S, de Waard JR. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc Biol Sci* 2003;270(Suppl 1):S96–S99. Available at: http://doi.org/10.1098/rsbl.2003.0025
22. Zhang XM, Zhang AB. DNA barcoding and its application in the identification of diets of insect herbivores. *Acta Entomol Sin* 2017;60(1):104–119. (Chinese) Available at: http://doi.org/10.16380/j.kcxb.2017.01.012
23. Su JH, Ji WH, Xu CL, Nan ZB. The ecological characteristics of food habits for four herbivores in Gannan Meadow. *Chin J Zool* 2017;52(3):381–389. (Chinese) Available at: http://doi.org/10.13859/j.cjz.201703003
24. Liao YQ, Jia T, Zhu WL. Bone size and its effect on body mass in Eothenomys miletus from the Hengduan Mountain region. *J Vertebr Biol* 2023;72(22066). Available at: http://doi.org/10.25225/jvb.22066
25. Wang LQ, Zhang FS, Yang YP, Dong WH. Niche characteristics of rodentsin different habitats in Kubuqi Sandy Land. *Chin J Grassl* 2020;42(5):151–156. (Chinese) Available at: http://doi.org/10.16742/j.zgcdxb.20190264
26. Luo ZX, Chen W, Gao W. Fauna of China. Beijing: Science Press; 2000:89–95. (Chinese) ISBN: 7-03-007384-3/Q·864
27. Zhao ZY. *Diversity analysis and isolation and identification of intestinal fungi from Microtus brandti*. Jilin Agricultural University; 2022. (Chinese) Available at: http://doi.org/10.27163/d.cnki.gjlnu.2022.000027
28. Zou Y, Liang NN, Zhang XX, Han CX, Nan XN. Functional differentiation related to decomposing complex carbohydrates of intestinal microbes between two wild zokor species based on 16SrRNA sequences. *BMC Vet Res* 2021;17(1):216. Available at: http://doi.org/10.1186/s12917-021-02911-z
29. Sigoillot JC, Berrin JG, Bey M, et al. Fungal Strategies for Lignin Degradation. *Adv Bot Res* 2012;61:263–308. Available at: http://doi.org/10.1016/B978-0-12-416023-1.00008-2