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Insights into the composition of gut microbiota in response to environmental temperature: The case of the Mongolia racerunner (*Eremias argus*)

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ABSTRACT

Gut microbiota are essential to maintain host health and fitness largely through its influence with behavior, development and reproduction of host, particular among the amniotic ectothermic reptiles. Global warming effects on the composition of the host's gut microbiota in some taxa are generally known. Previous studies on reptiles in both natural habitats and lab-reared individuals have demonstrated that temperature changes can alter the composition and function of the host's gut microbiota. However, these effects on wild-caught reptiles are not well-understood. This study investigated changes in the composition of gut microbiota of wild-caught lizards (*Eremias argus*), which were experimentally exposed to two different temperatures. The results showed that the increase in temperature altered the gut microbiota; both the groups showed an altered and destabilized composition of gut microbiota in response to their adaptive states. The warming did not significantly alter the relative abundances of the main gut microbial communities in both the lizard genders, but increased those of predicted pathogenic bacterial genera, including *Acinetobacter*, *Anaerotruncus,* and *Dehalobacterium*. These results provided insights into the ecological adaptations of *Eremias argus*, but the body health and fitness of wild populations, concerning temperature changes, warrant further investigations.

1. Introduction

Global warming has a far-reaching impact on the health and fitness of animals (Ockendon et al., 2014), including heat-mediated changes in the reproduction, metabolism, immunity, and behavior of animals (Petford and Alexander, 2021; Scheun et al., 2021), especially reptiles (Zhang et al., 2020). For instance, variation in temperature can alter the plasticity of animals' gender and body shape (Li et al., 2020; Şahina and Kuyucu, 2021) by decreasing oxidative stress and immunosuppression, which might cause changes in telomere length and accelerate species extinction (Cahill et al., 2012; Han et al., 2020; Velando et al., 2021). It has been extensively reported that environmental temperature shapes the composition of gut microbiota in animals, including both invertebrates and vertebrates (Bestion et al., 2017; Ferguson et al., 2018; Moeller et al., 2020; Moghadam et al., 2018). Yet, very few studies have focused on the effects of global warming on the gut microbiota of reptiles.

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Gut microbiota consists of a dynamic and balanced microbial community in the host organism and plays an essential role in the nutrition, metabolism, and immunity of the host against potential pathogens (Keenan et al., 2013; Nicholson et al., 2012; Siddiqui et al., 2022). Gut microbiota varies with diet, way of living, and environment (De Filippo et al., 2010; Kau et al., 2011; Kohl et al., 2017; Tang et al., 2020; Wu et al., 2011). Previous studies have shown that warming has altered the composition of gut microbiota in wild-caught lizards throughout their whole life history, resulting in a decreased diversity and increased pathogenic bacteria (Bestion et al., 2017). In some cases, these changes have affected the thermal physiology of the host (Moeller et al., 2020). Although studies on the microbiota of reptiles have focused on the endogenous microbial communities (Colston and Jackson, 2016; Eliades et al., 2021), the composition of gut microbiota has been least explored. The effects of temperature on the gut microbiota of wild lizards are still unclear.

Mongolia racerunner (*Eremias argus*) is a typical oviparous animal, belonging to the family Lacertidae and genus *Eremias*, and has widely distributed from China to Mongolia, Russia, and South Korea (Kim et al., 2012a, 2012b; Park et al., 2014). It inhabits forests, shrublands, grasslands, wetlands, rocky areas, and deserts and prefers warm, dry, sunny, and sandy environmental conditions, which increase its body temperature in order to sustain fundamental life viability (Orlova et al., 2019). Mongolia racerunner eats grasshoppers, beetles, ants, leafhoppers, moths, flies, bees, and spiders as its diet (Huang et al., 2016). Globally, Mongolia racerunner has been categorized as Least Concern (Orlova et al., 2019) and is a national protected animal of significant ecological, scientific, and social value in China (http://www.forestry.gov.cn/main/3951/20170315/957039.html, Accessed 20 February 2022). With climate changes coupled with overgrazing and patterns of land use, their habitats are under enormous pressure with severe degradation and fragmentation. Therefore, in this context, the survival and adaptation of these terrestrial reptiles need further attention.

According to the fourth conference report of the Intergovernmental Panel on Climate Change (IPCC), global warming will continuously increase in the future with an expected rise in global temperature by 1.1–6.4 °C by the end of this century (IPCC et al., 2007). Based on this observation, it was predicted that global warming might alter the composition of the lizard's gut microbiota in response to the increase in environmental temperature. Therefore, first, this study aimed to characterize the composition and abundance of the gut microbiota of wild-caught Mongolia racerunner. Second, we aimed to explore changes in the composition of gut microbiota under different temperature trials. Our study might provide decisive evidence on the effects of global warming on the gut microbiota of lizards in different temperate zones.

2. Materials and methods

2.1. Sources of lizard

Mongolia racerunner species, which were used in this study (n = 70, including 40 females and 30 males), were collected using an artificial trap (Pelozuelo and Frerot, 2006) (Fig. 1) in May 2019 in Maliankou district Yinchuan, Ningxia Hui autonomous region

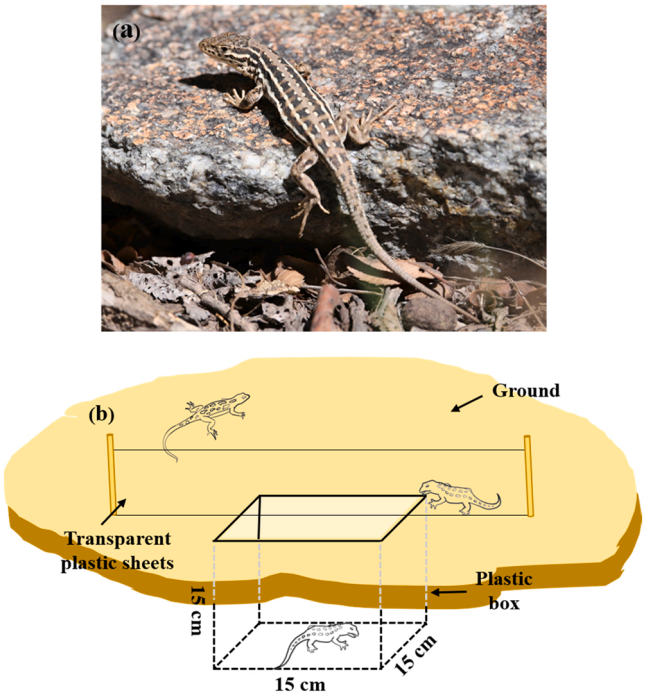

Fig. 1. (a) Mongolia racerunner (*Eremias argus*); (b) Schematic representation of an artificial trap used in this study. None of the lizards were killed during collection and experiments.

(38°34′23.66″N, 105°56′31.93″E; 1,200–1,400 m a.s.l.), China; this region has an average annual temperature of − 0.9 ℃ and average yearly rainfall of 420 mm. The habitats in these areas are deserts and semi-desert grasslands (Zhang et al., 2013). If the lizards were observed to have mating and breeding (oviposition) behaviors, they were excluded from the experiments. This was done for eliminating the deviation of experimental results. Finally, a total of 42 (24 females and 18 males) lizards were included in this study.

The detailed capturing procedure is as follows. A hole was dug near the burrows, where lizards were expected to live and plastic boxes (size: 15 × 15 × 15 cm) with an upward-opening were placed. A transparent plastic sheet was placed vertically on the upper center of the traps and both the ends were fixed on the column. All the traps were connected in series using a plastic sheet. When the lizard crawled along with the plastic sheet, it fell into the trap and was caught successfully. The gender of each lizard was identified and a particular number was allocated. This approach minimized the chances of injuries to lizards during their capturing. A total of 30 traps were set with a distance interval of 0.5 m between two traps.

2.2. Housing condition and experimental design

All the captured lizards were subsequently transferred to the Key Laboratory of Conservation Biology, National Forestry and Grassland Administration, Harbin, China. Before starting the experiment, the animals were randomly divided into warming group (W; n = 20, average snout-vent length = 53.98 ± 7.45 mm, average body mass = 3.90 ± 0.46 g; 12 females and 8 males) and control group (C; n = 22, average snout-vent length = 53.48 ± 4.66 mm, average body mass = 3.85 ± 0.39 g; 12 females and 10 males). The lizards were kept in plastic boxes (55 ×40 ×32 cm) having a sandy substrate of about 3-cm depth. The lizards in both the groups were kept at 12/12 h light/dark cycles (light period 7:00 am – 7:00 pm) and a constant temperature of 30 °C for at least two weeks. Meanwhile, food was provided *ad libitum*, including mainly yellow mealworm (*Tenebrio molitor*) larvae and a small amount of nutrients, enriched with specialized reptile calcium, multivitamins powder, and water. Throughout the experimental period, the temperature of the C group was kept constant at 30 °C, while that of the W group was set to 36.27 °C in order to perform a 3-month warming training experiment (Figs. S1 and S2). Fresh fecal samples were collected from the whole group in the last seven days of the experimental period. Sterile filter papers were placed into the boxes and 2–3 grains of fresh fecal samples were collected into sterilized tubes immediately after defecation using sterilized tweezers. The fecal samples were stored at − 80 °C before genomic DNA extraction.

2.3. DNA extraction and sequencing

Total genomic DNA extraction from the fecal samples was performed using an Omega Stool DNA kit (Omega bio-tek, GA, USA). The DNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of extracted DNA was then confirmed on 1% agarose gel. The V3–V4 hypervariable region of *16S rRNA* gene was amplified using universal primers (338F: 5'-ACTCCTACGGGAGGCAGCA-3'; 806R: 5'-GGACTACHVGGGTWTCTAAT-3'). PCR reaction mixture contained 2 μl of DNA template, 5 μl of Q5 reaction buffer (5X), 5 μl of GC buffer (5X), 2 μl (2.5 mM) of dNTPs, 1 μl (10 uM) of each forward and reverse primer, 0.25 μl of Q5 DNA Polymerase and 8.75 μl of dd·H_2O. The PCR reaction conditions were as follows: initial denaturation at 98 °C for 2 min; 25 cycles of denaturation at 98 °C for 15 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; and final elongation at 72 °C for 5 min. The purified PCR products were sent to Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) for sequencing on an Illumina MiSeq platform with MiSeq Reagent Kit v3 (600 cycles), generating paired-end 2 × 250 bp sequence reads. The minimum sequence read length was set to be ≥ 150 bp and the sequences with ambiguous base N were excluded. Operational Taxonomic Units (OTUs) were clustered with 97% identity threshold using UPARSE (v9.2.64). The chimeric sequences were identified and removed using USEARCH (v5.2.236, http://www.drive5.com/usearch/). The taxonomy of each *16S rRNA* gene sequence was analyzed using Ribosomal Database Project (RDP) Classifier against the Silva 16S rRNA database (SSU119, http://www.arb-silva.de) with a confidence threshold of 70%.

2.4. Statistical analyses

Alpha microbial diversity indices, including Observed species and Shannon indices, were performed after being tested for Student's *t* tests/Kruskal–Wallis test using QIIME2 (v2019.4, https://view.qiime2.org/). ANOVA was used to test the effects of temperature state, gender, and interactions. The differences in the structure of gut microbiota between the groups were compared using principal coordinates analysis (PCoA) based on the weighted and unweighted UniFrac distances. Linear discriminant analysis (LDA) effect size (LEfSe) analysis was conducted to identify the significant differences in the relative abundances of bacterial taxa between the two different samples with the consideration of standard tests for statistical significance (Segata et al., 2011). Similarities among the different samples were identified by cluster analysis, which classified the samples, belonging to varying conditions, into multiple groups based on the OTU abundances. All the statistical analyses were performed using IBM SPSS software v22.0. The differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. Effects of warming on the gut Alpha microbial diversity

A total of 5,361,657 effective sequence reads, ranging from 50 to 435 bp were obtained. The majority of the sequence reads lengths were more than 150 bp (Fig. S3). A total of 799–1,760 OTUs per sample were obtained with a 97% sequence identity threshold. The

rarefaction curves of the OTUs gently reached saturation, indicating that the number of samples taken in this study was reasonable and could better reflect the diversity of microbiota, and the sequencing depth covered almost all the species in the sample required for covering the bacterial diversity (Fig. S4). The species accumulation curves tended to be gentle, suggesting that the sample size in this study was sufficient. A further increase in sample size would not result in identifying new species (Fig. S5). The results also showed that the Rank abundance curve did not show a sharp decline, rather it was uniform and regular, indicating that the species composition in the samples was relatively constant with the rich composition of microorganisms in each sample (Fig. S6).

In this study, the Good's coverage index of all the treatment groups was more than 99%, and the diversity coverage in the sample reached a high level. The gut microbiota of the two groups was relatively uniform with little difference in the Pielou index between the two groups. Warming reduced the Chao1 index from 1,492.226 to 1,238.259 ($t = 4.145$, $P = 0.000$), Observed species index from 1,302.855 to 1,055.660 ($t = 4.032$, $P = 0.000$), and Shannon index from 6.424 to 6.033 ($t = 2.209$, $P = 0.04$), indicating that the warming reduced the abundance and diversity of gut microbiota of the lizards (Table 1).

Two-way ANOVA was used to analyze the differences in the richness and diversity of the lizard's gut microbiota in each group. The increase in temperature significantly affected the richness ($df = 1$, $F = 15.963$, $P = 0.000$) and diversity ($df = 1$, $F = 4.598$, $P = 0.038$) of gut microbiota, among which, gender had no significant role ($P = 0.190$; $P = 0.532$), and no significant interaction was observed between temperature and gender ($P = 0.684$; $P = 0.233$) (Table S1).

There were significant differences in the richness ($z = 3.350$, $P = 0.001$) and diversity ($z = 2.795$, $P = 0.005$) of gut microbiota between the W and C groups. The gender-specific differences in the richness (male: $z = 2.540$, $P = 0.011$; female: $z = 2.221$, $P = 0.026$) and diversity (male: $z = 3.288$, $P = 0.000$) of gut microbiota between the groups were significant, except for the difference in the diversity of gut microbiota among females, which was insignificant ($z = 1.212$, $P = 0.225$) (Fig. 2).

3.2. warming altered the composition and structure of lizard's gut microbiota

A total of 18,439 OTUs were detected in the 42 fecal samples. A total of 3,452 OTUs were shared between the W and C groups, while 1,263 OTUs were shared between the male and female groups (Fig. 3a and b). There were 32 bacterial phyla (female 27 and male 29) in the W group and 30 bacterial phyla (female 27 and male 23) in the C group. The most abundant phyla in the two groups were Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Verrucomicrobia, which accounted for more than 99% of the gut microbiota in any group and constituted the predominant bacterial phyla of the gut microbiota of *E. argus*. Comparatively, warming reduced the abundances of Bacteroidetes and Actinobacteria among the female lizards' gut microbiota and increased those of Firmicutes, Proteobacteria, and Verrucomicrobia. Among the phyla of male lizards' gut microbiota, the relative abundance of Bacteroidetes decreased, while those of Firmicutes, Proteobacteria, Actinobacteria, and Verrucomicrobia increased. However, the difference between these groups was insignificant (Fig. 4a).

A total of 492 genera (female 383 and male 419) were annotated in the C group, while 424 genera (female 371 and male 307) were annotated in the W group. *Bacteroides*, *Parabacteroides*, *Desulfovibrio*, *Bilophila*, and *Odoribacter* were the most abundant genera in each group. After heat treatments, the relative abundances of gut microbiota altered at the genus level. The relative abundances of *Bacteroides* and *Parabacteroides* decreased, while that of *Desulfovibrio* increased, but the difference was not significant ($P > 0.05$). As compared to the C group, the relative abundances of *Parabacteroides*, *Parabacteroides*, and *Desulfovibrio* in the gut microbiota of female lizards decreased, while those of *Odoribacter* and *Bilophila* increased. Meanwhile, the relative abundances of *Odoribacter*, *Parabacteroides*, and *Bilophila* decreased, while that of *Desulfovibrio* increased in the W group (Fig. 4b).

The heatmap of the top 50 genera, which was developed based on Bray–Curtis dissimilarity, revealed the differences in relative abundances and aggregations of dominant genera in each group (Fig. 5). The heatmap showed that the composition and relative abundance of gut microbiota were different among all groups. However, the increase in the abundance of genera *Acinetobacter*, *Anaerotruncus*, and *Dehalobacterium* might negatively affect the health of lizards. Based on the weighted and unweighted UniFrac distances, PCoA showed that the W group had a higher similarity in the floral structure as compared to the C group. The composition of female lizards' gut microbiota between the two groups showed a particular difference (Fig. 6).

LEfSe analysis was performed to analyze the species with significant differences between the groups. The LEfSe distribution bar

Table 1
Number of the diversity indicators of gut microbiomes isolated from the lizards fed different traits.

Group	Chao1	Observed species	Faith_pd	Simpson	Shannon	Pielou_e	Good's coverage
C	1492.226 ± 234.767^{A}	1302.855 ± 240.297^{A}	92.174 ± 11.467^{A}	0.943 ± 0.071^{a}	6.424 ± 0.726^{a}	0.622 ± 0.064^{a}	0.995 ± 0.001^{A}
W	1238.259 ± 147.977^{B}	1055.660 ± 135.864^{B}	83.073 ± 5.571^{B}	0.940 ± 0.034^{a}	6.033 ± 0.484^{b}	0.601 ± 0.047^{a}	0.996 ± 0.001^{B}
C-M	1448.237 ± 235.922^{a}	1243.110 ± 219.251^{ab}	93.880 ± 11.976^{a}	0.966 ± 0.011^{a}	6.620 ± 0.313^{a}	0.645 ± 0.023^{a}	0.995 ± 0.001^{b}
C-F	1528.884 ± 237.604^{a}	1351.150 ± 255.544^{a}	90.752 ± 11.350^{ab}	0.924 ± 0.093^{a}	6.260 ± 0.928^{ab}	0.602 ± 0.080^{a}	0.995 ± 0.001^{b}
W-M	1226.989 ± 163.634^{b}	1021.113 ± 135.153^{b}	83.012 ± 5.468^{b}	0.944 ± 0.018^{a}	5.965 ± 0.382^{b}	0.597 ± 0.033^{a}	0.996 ± 0.001^{ab}
W-F	1245.773 ± 143.825^{b}	1078.417 ± 137.757^{b}	83.113 ± 5.880^{b}	0.937 ± 0.042^{a}	6.079 ± 0.553^{ab}	0.604 ± 0.056^{a}	0.996 ± 0.001^{a}

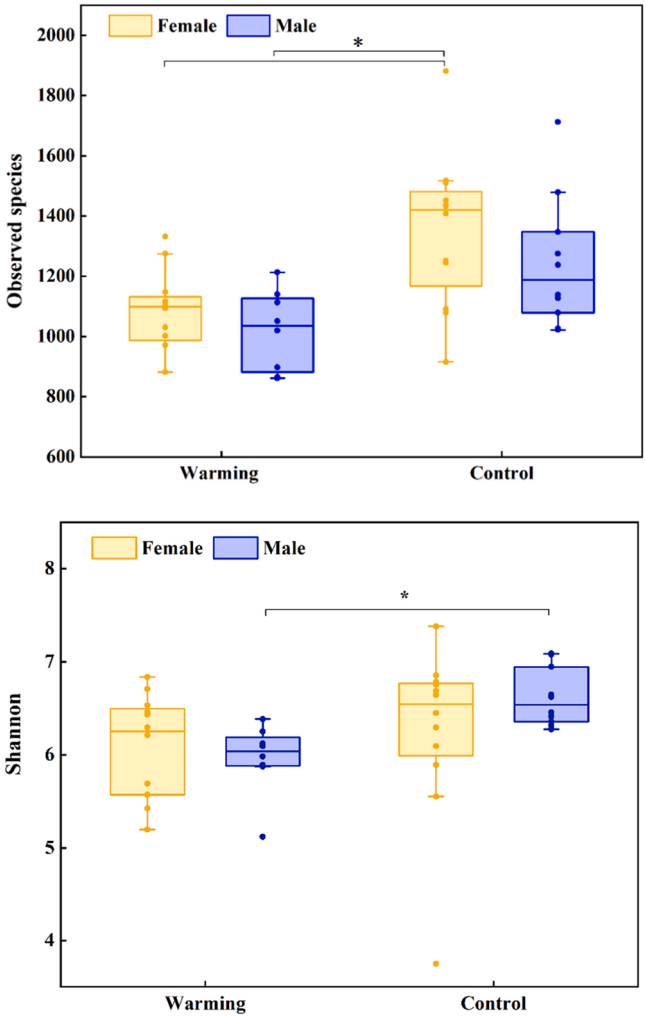

Fig. 2. Alpha diversity indices (Observed species and Shannon index) among the group (All values represent mean ± SD, Significance (Student's t tests) $P < 0.05$ indicated by *, $P < 0.01$ indicated by **).

chart revealed that the longer the bar, the more significant the difference in the taxa (Fig. 7a). There were 13 genera, which showed differences in gut microbiota in the C group, including the genera *Roseburia*, *Adlercreutzia*, *Janthinobacterium*, and *Butyricicoccus*, which had the most obvious differences (LDA score >2). Accordingly, only six genera and *Eggerthella* had the most significant difference in the W group. A hierarchical taxonomic relationship of the main taxonomic units from the genus (outer circle) to phylum (inner circle) of gut microbiota was constructed using a taxonomic cladogram (Fig. 7b). There were significant differences in some members of gut microbial communities in the C group, including Bacteroidetes, Patulibacteraceae, S24_7, Sinobacteraceae, Aerococcaceae, Pilonospiraceae, Alteromonadaceae, Moraxellaceae, *Rhodococcus*, *Adlercreutzia*, *AF12*, *Facklamia*, *Roseburia*, *rc4_4*, *Butyricicoccus*, *Paracoccus*, *Janthinobacterium*, *Cellvibrio*, *Citrobacter*, *Serratia*, and *Psychrobacter*. However, in the group, CFB_26, BA008, Christensenellaceae, Purpuromonas, Lycidaceae, Planococcaceae, Ruminoco, *Eggerthella*, *Ammoniphilus Rummeliibacillus*, *Macrococcus*, *Allobaculum*, and *Flexispira* showed significant differences.

4. Discussion

This study showed that the phyla Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Verrucomicrobia accounted for more than 99% of the gut microbiota in each sample of the C group, indicating that these bacterial phyla were the predominant phyla in *E. argus*. Moreover, the abundance of annotated species in gut microbiota decreased after heat treatments, but the phyla with the highest abundances were still Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Verrucomicrobia. This suggested that warming could affect the composition of gut microbiota of the *E. argus*, but its predominant microbiota was retained.

As reported in many studies, Bacteroidetes and Firmicutes are the dominant bacterial phyla in the gut microbiota of most terrestrial mammals (Jiang et al., 2021; Sun et al., 2016; Waite et al., 2012; Zhu et al., 2020). The current study results were also in agreement

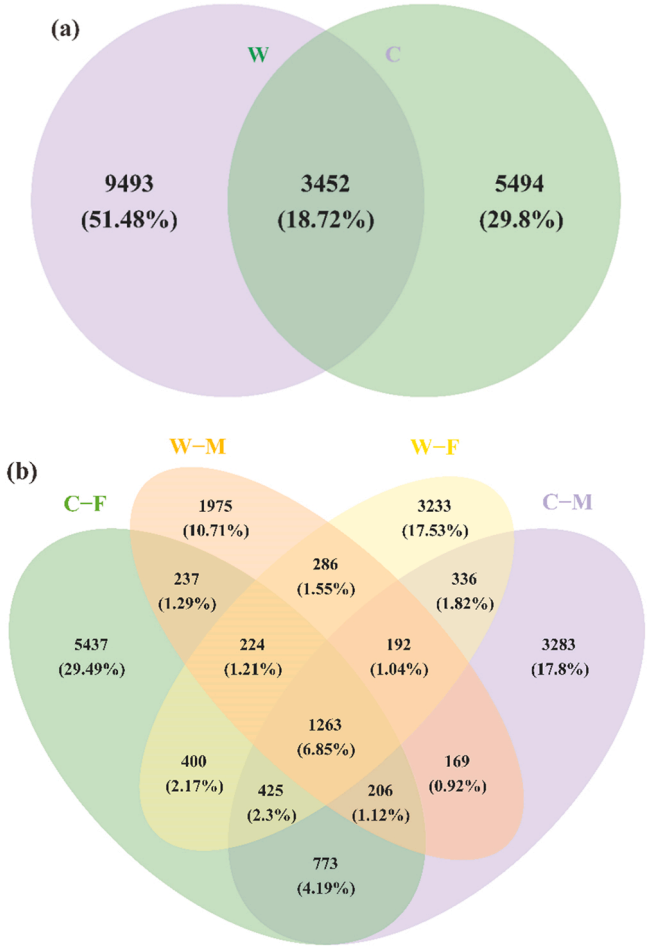

Fig. 3. (a and b) Core and specific OTUs of lizard fecal sample. Each ellipse represents a group. The number of OTUs (core OTUs) shared among all the groups is shown in the center and the number of specific OTUs is shown in the non-overlapping proportions of each ellipse.

with the previous findings on the gut microbiota of reptiles with slight differences, which might be due to differences in species, environment, and sampling methods. Despite the variances in the origin, size, and health conditions of loggerhead sea turtles (*Caretta caretta*), Firmicutes, Bacteroidetes, and Proteobacteria were the predominant phyla among their gut microbiota (Arizza et al., 2019). However, a study by Biagi et al. (2019) showed that Firmicutes and Fusobacteria were the main bacterial phyla; this difference might be due to the difference in environmental conditions. Among the gut microbiota of herbivorous reptiles, such as Seychelles giant tortoises (*Aldabrachelys gigantea*) raised in natural and controlled environments, the major phyla included Bacteroidetes, Firmicutes, and Spirochaetes (Sandri et al., 2020). Similar findings were observed in lizards. For instance, a study on the composition of gut microbiota of wild crocodile lizard (*Shinisaurus crocodinurus*) showed that the phyla Bacteroidea, Firmicutes, and Proteobacteria accounted for the highest abundance, which was the core microbiota (Jiang et al., 2017), but among captive populations, their gut microbiota mainly composed of phyla Firmicutes and Proteobacteria (Tang et al., 2020).

Some studies showed that ambient temperature could alter the composition of gut microbiota of fish and frogs in *in-vitro* (Kohl and Yahn, 2016; Li et al., 2018). When the ambient temperature increased by 6 ℃, the dominant bacteria families in the fish gut microbiota changed from Micavibrio, Comamonadaceae, and Saprospiraceae to Pseudomonadacea, Alcaligenaceae. Microbacteriaceae (Soriano et al., 2018). The relative abundance of Planctomycetes and Mycobacterium in the gut microbiota of *Rana pipiens* tadpoles increased after heat treatments (Kohl and Yahn, 2016). As compared to housing at 10 °C and 15 °C, the OTU abundance of gut microbiota of *Plethodon cinereus* decreased by 24.9% and its diversity decreased by 8.3% at 20 °C (Fontaine et al., 2018). In addition, an increase in the temperature by 2–3 °C in a semi-natural environment resulted in a 34% decrease in the gut microbial diversity of *Zootoca vivipara*, which showed that the increase in temperature might negatively impact the survival of the host (Bestion et al., 2017). In this study, although the main members of the lizard's gut microbiota were almost the same at the phylum, family, and genus levels, there were differences in their relative abundances at each level after 5.5 °C change in each group. Moreover, the relative abundance and alpha diversity index of gut microbiota in the W group were 246.54 and 0.390, respectively, which were lower than those in the C group. In agreement with the previous studies, a general conclusion was drawn that a temperature rise was inversely proportional to the diversity of the gut microbiota of reptiles. The current study results again confirmed that ambient temperature was an essential factor,

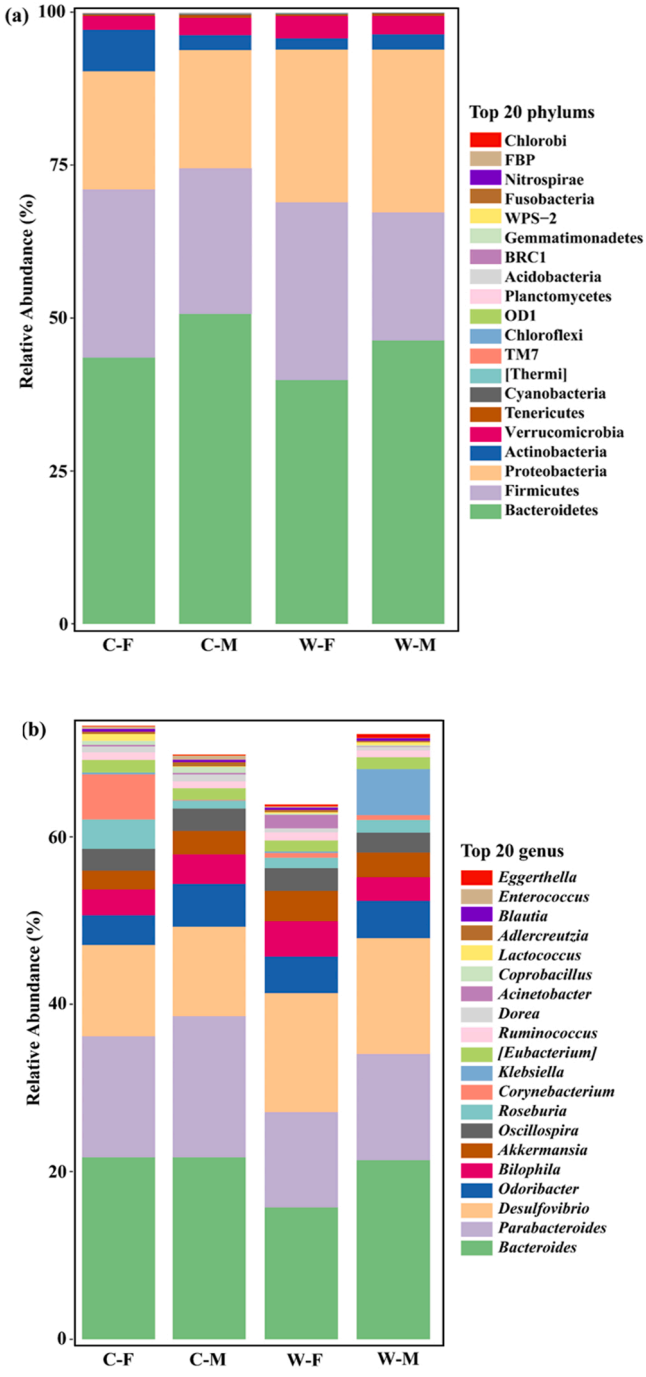

Fig. 4. Analysis of gut microbiota of lizard. Relative abundances of gut microbiota at the phylum (a) and genus (b) level in each group.

affecting the physiological and biochemical functions of animals by significantly altering the composition of their microbiota.

Most of the gut microbial species are beneficial to the host and promote the development of gut-associated lymphoid tissues, which is an important antigen. However, some gut microbial species might cause harm to the host. For example, some gut microbial species act as opportunistic pathogens, and their excessive aggregation might increase the risk of host infection (Kostic et al., 2014). Previous studies on the gut microbiota of ectotherm animals have also shown that the relative abundance of bacterial genera decreased with the increase in temperature, but the pathogenic bacterial groups increased, respectively (Fontaine et al., 2018; Kohl and Yahn, 2016). In this study, *Acinetobacter*, *Anaerorhabdus*, and *Dehalobacterium* were the dominant bacterial genera in the gut microbiota of female lizards in the W group, which are commonly found in the abscess of the host visceral organs or aging process and are mostly pathogenic

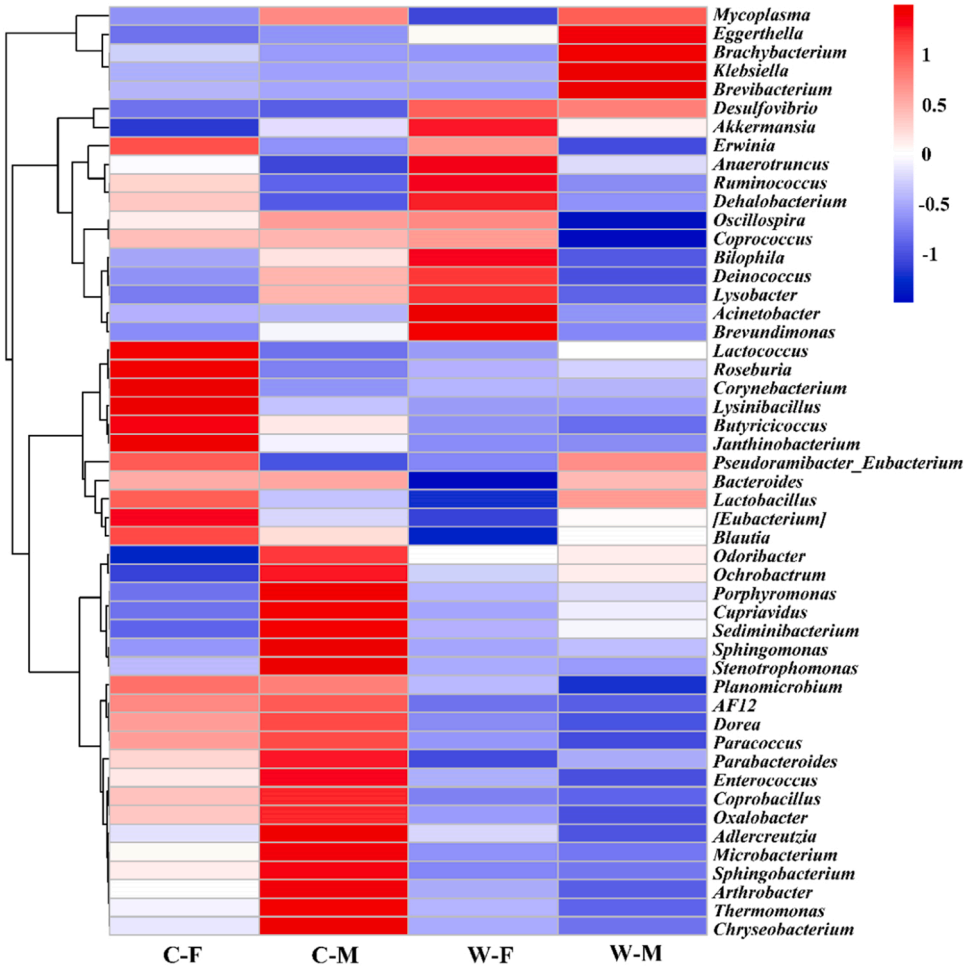

Fig. 5. Heatmap, revealing the trends of dynamics of gut microbial community with the top 50 bacterial genera in each group.

(Soriano et al., 2018). Warming reduced the richness and diversity of gut microbiota. The abundance of pathogenic bacteria increased in the lizards, suggesting that an increase in the temperature might negatively affect the gut microbiota of lizards and harm their health.

This study also focused on the composition of gut microbiota in different genders of lizards and found differences in the gut microbiota between males and females. The abundance and diversity of gut microbiota among the females were higher and the effects of heat treatments on the gut microbiota in females were more prominent. At present, studies on the factors, causing the differences in the gut microbiota between different genders of reptiles, are lacking. Some researchers have proposed that, like a genetic trait, gender can affect the structural composition of the host's gut microbiota by interacting with sex hormones and gender-specific immune responses (Markle et al., 2013; Valeri and Endres, 2021). Therefore, it was speculated that the differences in hormone levels and gender-specific resistance performance also existed in reptile groups, which jointly affected the structure and composition of gut microbiota in different genders.

5. Conclusions and recommendations

The results obtained in the current study provided insights into the ecological adaptations of lizards. The current study was focused on the effects of heat treatments on the composition and diversity of gut microbiota of Mongolia racerunner (*Eremias argus*), aiming to investigate the variation in the gut microbiota of ectotherms in the context of global warming. The results suggested that the gut microbiota reshaped with the increase in temperature, leading to an altered and destabilized composition of gut microbiota in response to their adaptive states. Meanwhile, some predicted pathogenic communities were found after the warming. This study also showed that the differences in gender-specific hormone levels and immune performance also existed in reptiles, which jointly affected the structure and composition of gut microbiota. Therefore, future studies should focus on the correlations among gender, gut microbiota, hormone levels, and immunity. In addition, studies should also focus on ecological conservation and adaptation mechanisms of these environmentally sensitive ectotherms.

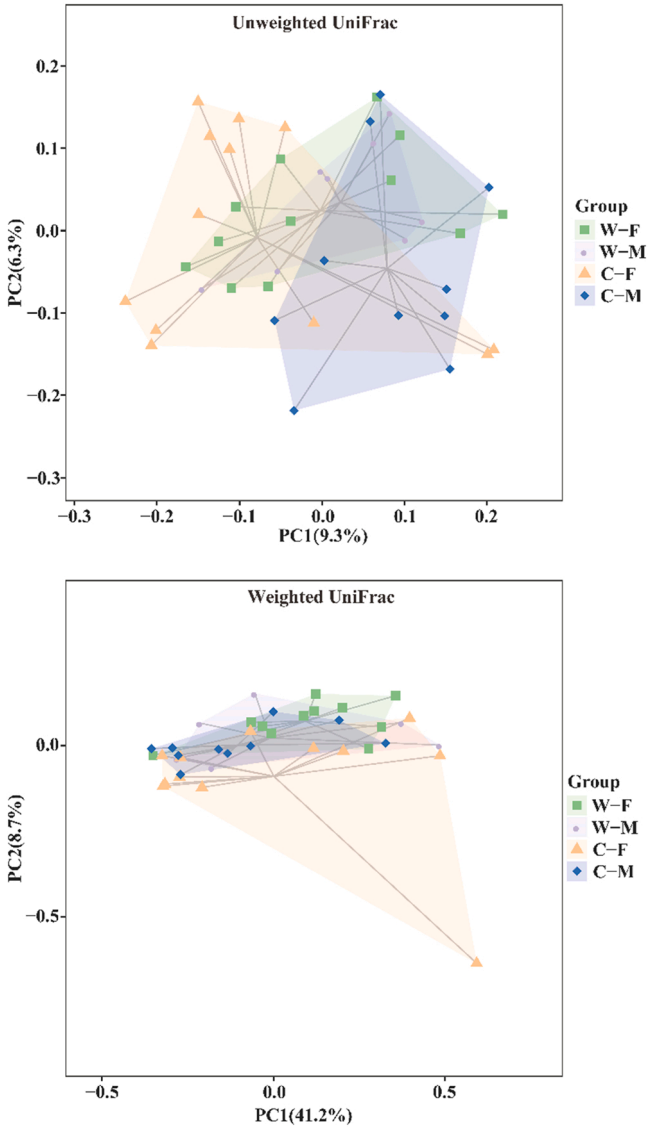

Fig. 6. Unweighted and weighted UniFrac distance PCoA of the lizard's gut microbiota of C group (C-Female and C-Male) and W group (W-Female and W-Male). Each point represents the gut microbiota of a lizard identified in a single fecal sample. Different colors indicate collections from the different groups.

Ethics statement

The animal study was reviewed and approved by the Board of Ethical Committee for Experimental Animals, Northeast Forestry University (No. 2022028).

CRediT authorship contribution statement

Zhirong Zhang: Conceptualization, Methodology, Validation, Writing - original draft, Review and editing. **Qian Zhu**: Conceptualization – experimental design, Methodology, Formal analysis, Data curation. **Junda Chen**: Conceptualization – experimental design, Drawing, Visualization. **Romaan Hayat Khattak**: Writing – review, and editing. **Zongzhi Li**: Drawing, Visualization. **Liwei Teng**: Conceptualization – experimental design, Methodology, Supervision, Writing - review, and editing. **Zhensheng Liu**: Conceptualization, Methodology, Supervision, Funding acquisition, Project administration, Writing - review, and editing. All authors contributed to the writing and reviewing of the manuscript and agreed to the published version of the manuscript.

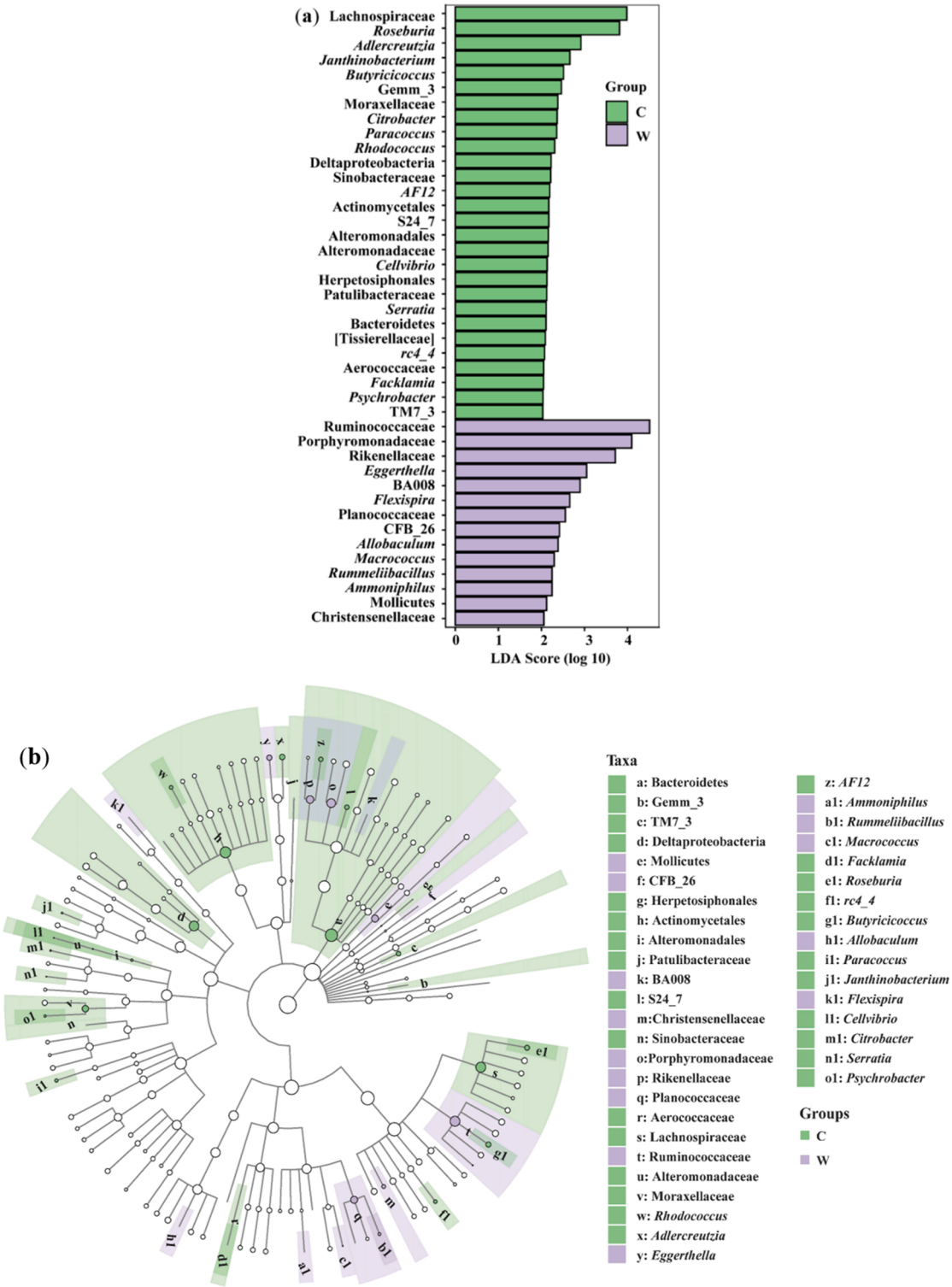

Fig. 7. Unique composition of lizards' gut microbiota. (a) Bar chart showing the log-transformed LDA scores of bacterial taxa identified by LEfSe analysis, where only the taxa, meeting an LDA threshold value of > 2, are shown. (b) Taxonomic cladogram, indicating the phylogenetic distribution of microbial lineages associated with each group. Differences are represented in unique colors in each group. Circles represent the phylogenetic levels from phylum to genus (OTUs) inside out, where each circle's diameter is proportional to the taxon's abundance.

Declaration of competing interest

The authors declare no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

All *16S* rRNA gene sequences produced for this study are available in the NCBI Bioproject database (PRJNA825498).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.gecco.2022.e02125.

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