Yu et al. *Zool. Res.* 2025, 46(1): 139−151
https://doi.org/10.24272/j.issn.2095-8137.2024.284
https://cstr.cn/32248.14.zr.2024.284

ZR | Zoological Research

Article Open Access

Reciprocal translocation experiments reveal gut microbiome plasticity and host specificity in a Qinghai-Xizang Plateau lizard

Wei Yu[1,2,#], Jing Yang[2,#], Li-Wei Teng[1,3,#], Xiao-Long Zhao[2,4], Ze-Yu Zhu[1,2], Shuang Cui[1], Wei-Guo Du[2], Zhen-Sheng Liu[1,3,*], Zhi-Gao Zeng[2,*]

[1] *College of Wildlife and Protected Area*, *Northeast Forestry University*, *Harbin*, *Heilongjiang* 150040, *China*
[2] *Key Laboratory of Animal Ecology and Conservation Biology*, *Institute of Zoology*, *Chinese Academy of Sciences*, *Beijing* 100101, *China*
[3] *Key Laboratory of Conservation Biology*, *National Forestry and Grassland Administration*, *Harbin*, *Heilongjiang* 150040, *China*
[4] *School of Ecological and Environmental Sciences*, *Institute of Eco-Chongming*, *Shanghai Institute of Wildlife Epidemics*, *East China Normal University*, *Shanghai* 200241, *China*

ABSTRACT

Animal adaptation to environmental challenges is a complex process involving intricate interactions between the host genotype and gut microbiome composition. The gut microbiome, highly responsive to external environmental factors, plays a crucial role in host adaptability and may facilitate local adaptation within species. Concurrently, the genetic background of host populations influences gut microbiome composition, highlighting the bidirectional relationship between host and microbiome. Despite this, our understanding of gut microbiome plasticity and its role in host adaptability remains limited, particularly in reptiles. To clarify this issue, we conducted a reciprocal translocation experiment with gravid females of the Qinghai toad-headed lizards (*Phrynocephalus vlangalii*) between high-altitude (2 600 m a.s.l.) and superhigh-altitude (3 600 m a.s.l.) environments on Dangjin Mountain of the Qinghai-Xizang Plateau, China. One year later, we assessed the phenotypes and gut microbiomes of their offspring. Results revealed significant plasticity in gut microbiome diversity and structure in response to contrasting elevations. High-altitude conditions increased diversity, and maternal effects appeared to enable high-altitude lizards to maintain elevated diversity when exposed to superhigh-altitude environments. Additionally, superhigh-altitude lizards displayed distinct gut microbiome structures with notable host specificity, potentially linked to their lower growth rates. Overall, these findings underscore the importance of the gut microbiome in facilitating reptilian adaptation to rapid environmental changes across altitudinal gradients. Furthermore, this study provides critical insights into microbial mechanisms underpinning local adaptation and adaptative plasticity, offering a foundation for future research on host-microbiome interactions in evolutionary and ecological contexts.

Keywords: Gut microbiome; Plasticity; Host specificity; *Phrynocephalus vlangalii*; Reciprocal translocated experiment

INTRODUCTION

The gut microbiome is critical for host fitness and health, underpinning numerous biological processes and mediating interactions between organisms and their environments (Gensollen et al., 2016; Melaku et al., 2021; Zhang et al., 2020). Its composition and abundance are shaped by a complex interplay of dietary inputs, genetic factors, and environmental dynamics, reflecting the multifaceted nature of host-microbiome relationships (Chen et al., 2022; He et al., 2018; Moeller et al., 2020; Smith et al., 2015; Vasconcelos et al., 2023; Williams et al., 2022). Changes in environmental conditions and host genetics create selective pressures that influence microbial community dynamics (Carrier & Reitzel, 2018; Fietz et al., 2018; Nielsen et al., 2023). However, despite extensive research, the plasticity and specificity of the gut microbiome remain poorly understood, particularly in the context of environmental adaptation.

Organisms adapt to environmental variation through various

Received: 29 October 2024; Accepted: 20 January 2025; Online: 21 January 2025

Foundation items: This work was supported by the National Natural Science Foundation of China (31861143023, 31872252) and Strategic Priority Research Program of the Chinese Academy of Sciences (XDA20050201)

[#]Authors contributed equally to this work

*Corresponding authors, E-mail: zhenshengliu@163.com; zengzhg@ioz.ac.cn

mechanisms such as local adaptation and phenotypic plasticity, which often coexist in nature (Becheler et al., 2022; Catullo et al., 2019; Čupić et al., 2023; Ma et al., 2018; Petipas et al., 2021; Swanson et al., 2023). Local adaptation involves the evolution of traits suited to specific environmental conditions, providing a heritable advantage in distinct habitats (Johnson et al., 2022; Kawecki & Ebert, 2004). Conversely, phenotypic plasticity enables a single genotype to express different phenotypes in response to varying environmental stimuli (Catullo et al., 2019; Pigliucci, 2005), which may persist throughout an individual's life (Baldo et al., 2023). Recent studies have increasingly focused on the molecular pathways underlying phenotypic plasticity and its role in ecological and evolutionary processes (Krist et al., 2021; Regan & Sheldon, 2023; Schuster et al., 2021; Sun et al., 2021). Reciprocal translocation experiments, derived from common garden experiments, involve the transfer of individuals between native and non-native habitats to investigate the influence of genetic and environmental factors on population-level variation (Hao et al., 2021; Ho et al., 2020; Johnson et al., 2022; Lane et al., 2019). Multiple studies have employed this approach to examine variation in growth, behavior, and survival among animal populations (Iraeta et al., 2006, 2008; Niewiarowski & Roosenburg, 1993; Ortega et al., 2017; Sears, 2005). However, most studies have focused on organismal responses to environmental changes, overlooking the potential contributions of the gut microbiome to adaptive processes.

Host adaptation is influenced by both the host genome and gut microbiome, both of which contribute to survival and fitness in changing environments (Martínez et al., 2018; Rennison et al., 2019; Uren Webster et al., 2018). Unlike the relatively stable genome, the gut microbiome is highly dynamic, responding rapidly to external factors through plastic changes that facilitate adaptation (Baniel et al., 2021; Carrier & Reitzel, 2018; Khakisahneh et al., 2020; Nielsen et al., 2023; Yang et al., 2024). This microbial flexibility plays a key role in phenotypic plasticity (Alberdi et al., 2016; Baniel et al., 2021; Carrier & Reitzel, 2018; Khakisahneh et al., 2020), enabling hosts to adjust dietary and metabolic processes to align with environmental demands (Baniel et al., 2021; Hicks et al., 2018; Mallott et al., 2022). Ectotherms, in particular, demonstrate highly dynamic gut microbiomes, which typically enhance their ability to thrive in novel environments (Bletz et al., 2016; Fontaine et al., 2022; Piazzon et al., 2020; Zhu et al., 2024a). Beyond this plasticity, the composition of the microbiome is closely linked to the phylogeny of the host (Ley et al., 2008; Rennison et al., 2019), potentially influencing evolutionary trajectories and driving reproductive isolation, thereby constraining local adaptation within host populations (Fietz et al., 2018; Greyson-Gaito et al., 2020; Rennison et al., 2019). Host specificity in gut microbiomes further underscores their adaptive importance (Chen et al., 2023; Mazel et al., 2024), with ectotherms frequently showing preferential associations with specific bacterial species (Powell et al., 2016; Sauers & Sadd, 2019). These microbial partnerships can provide adaptive benefits, enhancing host survival and ecological success (Chung et al., 2012; Sauers & Sadd, 2019). However, while the adaptive role of microbiome plasticity is well recognized, its effects on host phenotypic plasticity and local adaptation remain underexplored, leaving critical gaps in our understanding of adaptive evolution.

The Qinghai toad-headed lizard (*Phrynocephalus vlangalii*) is an endemic viviparous reptile inhabiting the northeastern Qinghai-Xizang Plateau in China, across a broad altitudinal range of 2 000 to 4 500 m a.s.l. (Zhao et al., 1999). Populations living at these contrasting elevations experience abrupt environmental gradients, making them ideal models for studying adaptive evolution (Serén et al., 2023). While previous studies have explored behavioral adaptations (Zhao et al., 2022; Zhu et al., 2020), physiological responses (Zhu et al., 2020, 2021), life history traits (Lu et al., 2018a, 2018b; Yu et al., 2023), and genomic features (Sun et al., 2018; Wu et al., 2022) in this species, few studies have explored the plasticity and specificity of their gut microbiomes, critical factors in adaptation to extreme environments given the direct impact of the gut microbiome on physiology, immune responses, and behavior (Du et al., 2022; Ho et al., 2020; Zeng et al., 2020). Environmental stressors encountered by *P. vlangalii* populations likely drive adaptation through a combination of genetic factors and the plasticity and host specificity of their gut microbiomes, which can directly impact survival and fitness (Alberdi et al., 2016; Baniel et al., 2021; Carrier & Reitzel, 2018). For example, in the eastern water dragon (*Intellagama lesueurii*), the gut microbiome exhibits plasticity in response to dietary changes associated with urbanization, specifically increased plant and fat intake (Littleford-Colquhoun et al., 2019). Similarly, the eastern river shrimp (*Macrobrachium nipponense*) shows gut microbiome plasticity when transitioning between lake and river habitats (Chen et al., 2017). The genetic background of hosts also shapes the gut microbiomes, contributing to host specificity. For example, populations of Mexican tetras (*Astyanax mexicanus*) from distinct habitats maintain different microbiome profiles even under uniform laboratory conditions, reflecting differences in host genetics (Riddle et al., 2024).

Populations of *P. vlangalii* inhabiting different altitudes display marked differences in gut microbiome composition (Zhang et al., 2018), suggesting the influence of both environmental plasticity and genetic backgrounds on microbial community structure. Reciprocal translocation experiments offer a robust approach for exploring the contributions of phenotypic plasticity and genetic determination to adaptive traits (Johnson et al., 2022; Kawecki & Ebert, 2004). To investigate the role of gut microbiome plasticity and host specificity in adaptation, a reciprocal translocation experiment was conducted on *P. vlangalii* populations at two contrasting altitudes. We hypothesize that, following translocation, the gut microbiota of individuals introduced to a novel environment will adapt to resemble that of the local population. However, genetic differences between populations may also assert a strong influence, leading to distinct microbial profiles compared to those of native individuals. In addition, the interaction between microbiome plasticity and host specificity may affect the host phenotype, potentially driving changes in migrant traits to align with those of the local population. By integrating environmental and genetic perspectives, this approach aims to elucidate the mechanisms underlying the interactions among environmental factors, genetic variations, and gut microbiome plasticity.

MATERIALS AND METHODS

Reciprocal translocation experiment

Between early June and early July 2020, 60 late-pregnant *P. vlangalii* females were captured from two distinct altitudinal

sites on Dangjin Mountain, Aksai County, Gansu Province, China. These locations were categorized as superhigh altitude (3 600 m a.s.l.) and high altitude (2 600 m a.s.l.), based on the global elevation classification system of Price et al. (2013). The superhigh-altitude site (N39°18'53", E94°15'40") was situated near the summit of the south slope of Dangjin Mountain in an alpine steppe ecosystem. In contrast, the high-altitude site (N39°24'42", E94°14'12") was situated at the northern base of the mountain within a temperate steppe zone (Zhu et al., 2024b). Despite being separated by only 30 km, the two locations exhibited marked climatic differences. Superhigh-altitude conditions were characterized by lower average daily temperatures and higher average daily humidity compared to high-altitude areas. While overall light intensity was similar between the sites, the timing of peak light intensity varied. At the high-altitude site, maximum light intensity occurred between 1000h and 1200h, while in the superhigh-altitude site, maximum light intensity peaked between 1300h and 1600h (Yu et al., 2023).

A reciprocal translocation experiment was conducted with a 2×2 factorial design that combined two populations of *P. vlangalii* (superhigh- and high-altitude females) with two environmental conditions (superhigh- and high-altitude habitats) (Figure 1A). The experimental groups were categorized as follows: high-high lizards (high-altitude lizards in their native environment), superhigh-superhigh lizards (superhigh-altitude lizards in their native environment), high-superhigh lizards (high-altitude lizards translocated to superhigh-altitude habitats), and superhigh-high lizards (superhigh-altitude lizards translocated to high-altitude habitats). At each study site, outdoor semi-natural circular enclosures (radius r=0.85 m) were constructed using transparent plastic sheets. These enclosures replicated the natural vegetation of their respective habitats and were covered with nets to prevent predation by birds.

Following capture, each lizard was measured for snout-vent length (SVL) using digital vernier calipers (PD-151, Prokit's Industries Co., Ltd., Taiwan, China) and for body mass (BM) with an electronic balance (ES-08B, Shanghai Hochoice Apparatus Manufacturer Co., Ltd., Shanghai, China). Individual identification was achieved through toe-clipping, and lizards were assigned to enclosures based on the experimental design, with two individuals per enclosure. Of the 60 pregnant females captured from the high-altitude population, half were placed in high-altitude enclosures, while the remainder were translocated to superhigh-altitude enclosures. Similarly, 60 pregnant females captured from the superhigh-altitude population were divided between superhigh-altitude and high-altitude enclosures. Consistent rearing was maintained in each enclosure, with food (*Tenebrio molitor* larvae and adults) provided *ad libitum* every two days.

Between July and August 2020, the captured females gave birth to their neonates, which were toe-clipped for individual identification. The neonates were measured for SVL and BM at birth and before overwintering. Cannibalism was not observed in this species, and stomach content analyses revealed no evidence of conspecific consumption (Zhao et al., 1999). Previous studies have indicated that mothers and offspring of this species share burrows (Qi et al., 2012). Through regular monitoring of enclosures, interactions between mothers and offspring were documented. Phenotypic data were collected from the offspring to evaluate developmental outcomes. As *P. vlangalii* is a viviparous species, it was not possible to establish germ-free conditions. To address this limitation, gut microbiota data from the offspring were analyzed to minimize the potential confounding effects of historical colonization.

In May and September 2021, the SVL and BM of juveniles were measured again. Resting metabolic rates were recorded in August 2021. Survival status was documented during these measurements, with survival coded as 1 and death as 0. Growth rates for SVL and BM were calculated for each individual using the formula $\ln(\text{measurement}_2/\text{measurement}_1)/(\text{date}_2\text{-date}_1)$ (Sun et al., 2018). Body condition was quantified using the scaled mass index (SMI) (Peig & Green, 2009), which accounts for the allometric relationship between SVL and BM. This index normalizes each individual's mass to the mean body size of the population. Data for males and females were analyzed separately, with sex identified based on ventral tail-tip coloration (orange for females, black for males) (Glavaš et al., 2020; Peig & Green, 2009; Zhao et al., 1999). The SMI was calculated using the following formula:

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}} \quad (1)$$

where M_i and L_i are the BM and SVL of lizard i, respectively; L_0 is an arithmetic mean value for the study population; b_{SMA} is the scaling exponent estimated by the SMA regression of M on L; and $\widehat{M}_i$ is the predicted BM for lizard i when the SVL is L_0.

From 19 to 25 August 2021, 32 lizard juveniles (eight per group) were randomly selected for field measurements of carbon dioxide production rates using closed-flow respirometry (FOXBOX, Sable Systems International, Henderson, GA, USA). These measures were conducted to estimate resting metabolic rates, which provide a critical measure of maintenance energy costs in ectotherms (Jiang et al., 2024; Rutschmann et al., 2024). Before testing, lizards were fasted for 48 h to standardize metabolic conditions and acclimated to temperatures of 18, 27, and 36°C (in random order) for 1 h. The temperature of 36°C closely approximates the preferred temperature of both studied populations (Zhu et al., 2024b). Closed-flow respirometry was performed in a 286 mL thermostatic chamber to measure carbon dioxide production and construct a thermal function curve for the metabolic rates of juveniles at set temperatures. To avoid possible biases from repeated measurements at the same temperature, the body temperature of each lizard was measured once a day. To minimize the impact of circadian rhythms, resting metabolic rate was only measured between 0900h and 1800h each day. The resting metabolic rate was calculated using the following formula:

$$\text{RMR} = \text{VCO}_2 \times \text{volume}/\text{body mass} \quad (2)$$

where VCO_2 is the CO_2 production rate, measured as percentage per hour (%/h), in a closed-circuit system. After the experiment, the female lizards were returned to their original field enclosures. All collection and handling procedures were approved by the Animal Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences (IOZ-IACUC-2023-153).

Fecal collection and gut microbiota analysis

From 16 to 21 August 2021, fecal samples were collected from lizards to analyze their gut microbiome. Sampling was

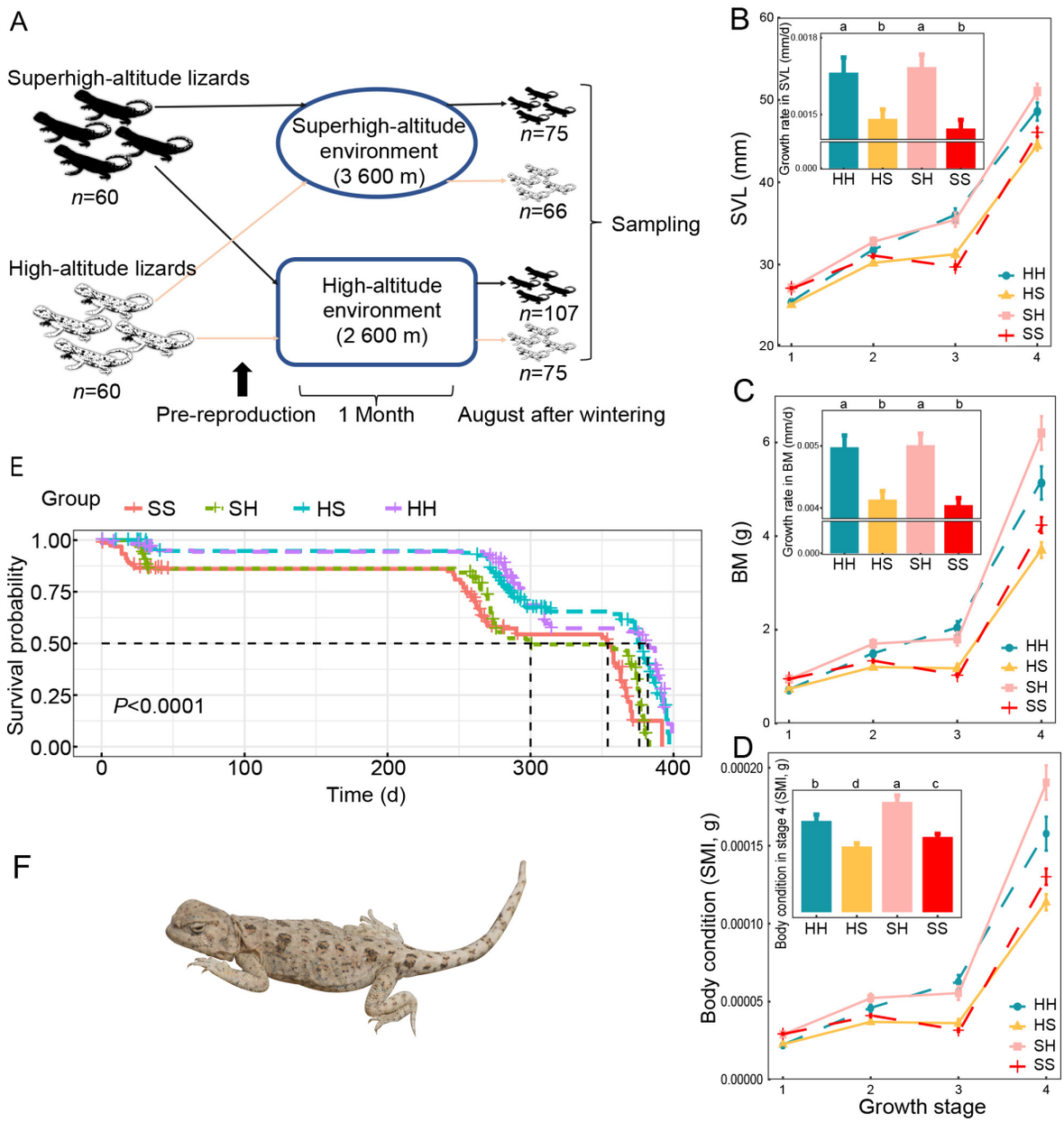

Figure 1 Experimental design and phenotypic changes

A: Reciprocal translocation experimental design. B–E: Phenotypic changes in each group in reciprocal translocation experiment: B: Snout-vent length (SVL); C: Body mass (BM); D: Body condition (SMI, scaled mass index); E: Kaplan-Meier survival curve, with time on the X-axis representing number of days after lizard births (July–September 2020). F: Photo of *Phrynocephalus vlangalii* (by Wei Yu). [a] & [b] represent differences between different treatment groups. Data are shown as mean±standard error (SE). HH and HS represent high-altitude lizards inhabiting native high-altitude environments and translocated to superhigh-altitude environments, respectively. SS and SH represent superhigh-altitude lizards inhabiting native superhigh-altitude environments and translocated to high-altitude environments, respectively. Numbers 1–4 on the X-axis represent four stages of lizard growth, respectively: after birth in 2020 (1), before wintering in 2020 (2), after emerging from hibernation in 2021 (3), and before wintering in 2021 (4).

conducted on sunny days between 1100h to 1600h. During this period, each lizard was individually transferred to a small, sterilized plastic container placed near its enclosure. Fecal matter produced during the observation period was promptly collected, ensuring minimal contamination. A total of 32 fecal samples were collected, with eight samples from each experimental group. The samples were immediately transferred to sterile tubes containing RNAlater for preservation. After collection, each lizard was returned to its enclosure. The collected samples were transported to the Institute of Zoology, Chinese Academy of Sciences, Beijing, and stored at –80°C for testing and analysis of gut microbiota.

DNA extraction, amplification, and sequencing were performed by Personal Biotechnology (China). Complete DNA samples were initially extracted using the E.Z.N.A™ Mag-Bind Soil DNA Kit (M5635, Omega, USA) following the manufacturer's protocols. DNA quality and concentration were assessed using a Quantifluor-ST fluorometer (Promega, E6090, USA) and Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, P7589, USA) at 260 nmol/L and 280 nmol/L, respectively. Additionally, DNA integrity was confirmed through 1.2% agarose gel electrophoresis.

The bacterial 16S rRNA gene V3–V4 hypervariable region was amplified using forward (338F: 5'-ACTCCTACGGGAG GCAGCA-3') and reverse primers (806R: 5'-GGACTACHVG GGTWTCTAAT-3'). The polymerase chain reaction (PCR) (25 mL) consisted of 1 mL of template DNA, 1 mL of amplicon PCR forward primer (10 mmol/L), 1 mL of amplicon PCR reverse primer (10 mmol/L), 2 mL of dNTP (2.5 mmol/L), 0.25 mL of Fast Pfu DNA Polymerase, 5 mL of 2×buffer, and 14.75 mL of ddH_2O. Thermal cycling was performed as follows: initial denaturation at 98°C for 5 min, followed by 25 cycles of denaturation at 98°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 45 s, with a final extension step at 72°C for 5 min. Subsequently, PE250 paired-end sequencing was performed using the Illumina MiSeq platform (Illumina San Diego, USA) after the DNA libraries were mixed.

Raw sequencing reads were processed using QIIME 2 (v.2020.11.1) (Bolyen et al., 2019). Adapters were trimmed using Cutadapt (v.3.1) to remove sequences with at least 90% base overlap (Martin, 2011). The Divisive Amplicon Denoising Algorithm DADA2 (v.1.18.0) pipeline was utilized for sequence quality control, including filtering low-quality reads, denoising reads, merging forward and reverse reads, removing chimeric reads, and identifying amplicon sequence variants (ASVs) (Callahan et al., 2016). MAFFT (v.7.475) (Katoh et al., 2002) and FastTree (v.2.1.10) (Price et al., 2009) were used to align sequences and construct the phylogenetic tree. Taxonomic classification of ASVs was performed using the Greengenes reference database (v.13-8-99) (Desantis et al., 2006).

Statistical analysis

All statistical analyses were performed using R (v4.2.2). Data normality was assessed with the Shapiro-Wilk test, and homogeneity of variance was evaluated using the Bartlett test. Where necessary, data were normalized by exponential or logarithmic transformation. To explore the effects of population and rearing environment on juvenile growth rate, a linear mixed model was employed, with population and rearing environment as fixed factors and enclosures as random factors. Generalized linear mixed models, with a gamma distribution family, were used to analyze the effects of population and rearing environment on body condition (measured by SMI), with population, rearing environment, and growth stage as fixed factors and enclosures as random factors. Model selection was conducted using Akaike Information Criterion (AIC) (Supplementary Table S1), and *post hoc* tests were performed using the emmeans package. Repeated measures analysis of variance, conducted using the ez package in R, was used to investigate the effects of population and rearing environment on offspring resting metabolic rate, with population and rearing environment as fixed factors and rearing temperature as repeated measurement factors. Survival analysis was conducted using the Cox proportional risk model to detect the effects of population and rearing environment on survival probabilities, with model selection based on AIC. Kaplan-Meier survival curves were generated to visualize differences in survival rates between groups.

Microbial alpha diversity (Chao1 richness and Shannon diversity) was calculated using the vegan package, with the effects of population and environment assessed through linear models. Paired comparisons were performed using the nonparametric Wilcoxon rank sum test (Wilcoxon, 1945). For beta diversity, the Bray-Curtis distance was calculated, and permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the influence of population and environment on microbial community composition. Principal coordinate analysis (PCoA) was conducted to visualize beta diversity patterns, with PC1 and PC2 compared using a *t*-test. Differences in intergroup microbial structure were compared using analysis of similarities (ANOSIM). Relative abundances at the phylum and gene levels were visualized using a bar chart. Differences in microbial composition among groups were explored using nonparametric tests implemented using the PMCMRplus package.

Gut microbiota plasticity was evaluated by determining whether microbial compositions of translocated lizards shifted toward those of native lizards. Host specificity was defined as the stability of microbial communities within a host, independent of environmental variation. Linear discriminant analysis effect size (LEfSe) was used to identify biomarkers distinguishing the gut microbiota of local and non-native lizards at both superhigh and high altitudes, providing insights into microbial abundance patterns specific to each host environment.

Spearman correlation analysis, conducted using the psych package, was used to determine the relationships between gut microbial composition (at the phylum, class, and genus levels) and growth rate (SVL and BM) or body condition. This approach was selected for its suitability in analyzing non-normally distributed data and its ability to capture monotonic relationships. Results were visualized using heatmaps.

To explore the functional implications of the observed plasticity and host specificity, differences in the abundance of microbial communities in the intestines and their corresponding functions were analyzed. *T*-test was used to compare differences in the relative abundance of microbes in the different Kyoto Encyclopedia of Genes and Genomes (KEGG) functional categories, and results were visualized with extended error bar plots, highlighting key differences in microbial function across groups.

RESULTS

Specific growth rate

The SVL growth rate in juvenile lizards was affected by the rearing environment ($F_{1,\ 55.89}$=17.96, P<0.001), but not by the population source ($F_{1,\ 90.95}$=0.30, P=0.59). Similarly, the BM growth rate was affected by the rearing environment ($F_{1,\ 57.60}$=29.95, P<0.001), but not by the population source ($F_{1,\ 92.56}$=0.39, P=0.54). Juveniles reared in the high-altitude environment exhibited accelerated growth in both SVL and BM, whereas those in the superhigh-altitude environment experienced reduced growth rates (Figure 1B, C).

Body condition and resting metabolic rates

Resting metabolic rates were not significantly affected by population source ($F_{1,\ 28}$=1.01, P=0.32), rearing environment ($F_{1,\ 28}$=2.97, P=0.10), or their interaction ($F_{1,\ 28}$=2.85, P=0.10; Supplementary Figure S1). However, resting metabolic rates increased significantly with rising temperatures in both the superhigh- and high-altitude environments ($F_{2,\ 56}$=163.33, P<0.001; Supplementary Figure S1).

The body condition (SMI) of lizards was significantly influenced by population source (χ^2=1 339.6, P<0.001), rearing environment (χ^2=20 543.7, P<0.001), growth stage

(χ^2=127 174.5, P<0.001), and the interaction between population and environment (χ^2=112.8, P<0.001; Figure 1D). At the end of the experiment, significant differences in body condition were observed among treatment groups (P<0.001), with SMI size rankings as follows: superhigh-high lizards>high-high lizards>superhigh-superhigh lizards>high-superhigh lizards (Figure 1D).

Survival rate

The survival rate of lizards was significantly impacted by population source (χ^2=53.63, P<0.001), but not by rearing environment (χ^2=0.24, P=0.62; Figure 1E). High-altitude lizards exhibited higher survival rates than superhigh-altitude lizards, regardless of rearing environment (P<0.001; Supplementary Figure S2A, D, E). Translocation did not significantly affect survival rates, regardless of population source (P>0.05; Supplementary Figure S2B, C).

Microbial alpha diversity

Gut microbiome alpha diversity, measured through Chao1 richness and Shannon diversity, revealed significant patterns. High-high lizards exhibited significantly higher gut Chao1 richness than superhigh-superhigh lizards (W=58, P<0.01), but no significant difference was observed in gut Shannon diversity between the two populations (W=46, P=0.16; Figure 2A, B). Linear model analyses indicated that rearing environment significantly affected gut Chao1 richness ($F_{1,\ 28}$=12.03, P<0.01), but not by population source ($F_{1,\ 28}$=1.72, P=0.20) or their interaction ($F_{1,\ 28}$=0.04, P=0.85). Similarly, gut Shannon diversity was affected by rearing environment ($F_{1,\ 28}$=8.97, P<0.01) but not by population source ($F_{1,\ 28}$=1.66, P=0.21) or their interaction ($F_{1,\ 28}$=0.02, P=0.88). High-altitude environments increased both the gut Shannon diversity (W=52, P<0.05) and Chao1 richness (W=64, P<0.001) in superhigh-altitude lizards (Figure 2A, B). Conversely, superhigh-altitude environments did not significantly affect the gut Shannon diversity (W=44, P=0.23) or Chao1 richness (W=50, P=0.06) of high-altitude lizards, although slight decreases were observed (Figure 2A, B).

Microbial beta diversity

Significant differences in gut microbiome structure were observed between superhigh- and high-altitude lizards ($F_{1,\ 14}$=2.19, P<0.001; Figure 2C; Supplementary Figure S3). After reciprocal translocations, the gut microbiome structure in both populations underwent substantial changes. These changes were significantly impacted by population source ($F_{1,\ 28}$=1.47, P<0.01) and rearing environment ($F_{1,\ 28}$=2.24, P<0.001), with no significant interaction between the two factors ($F_{1,\ 28}$=0.03, P=0.49; Figure 2C). Even at superhigh altitudes, the gut microbiome structures of the two populations

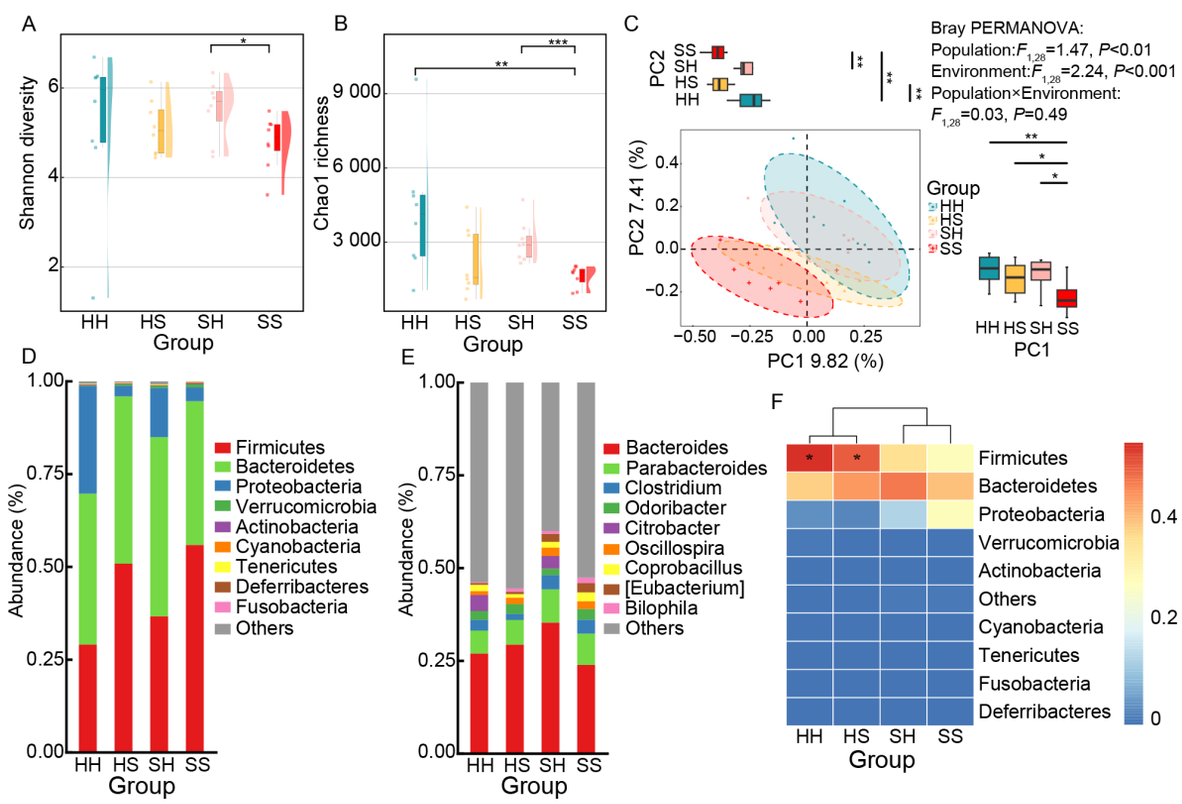

Figure 2 Gut microbiota diversity and composition in lizards

A, B: Changes in alpha diversity of gut microbiota in each group during reciprocal translocation experiments. A: Shannon diversity; B: Chao1 richness. C: Principal coordinates analysis (PCoA) and PERMANOVA analysis based on Bray-Curtis distance of each group in reciprocal translocation experiment, with two sets of grouped boxplots representing PC1 and PC2, illustrating distinctions among groups. D, E: Relative abundance of gut microbial composition. D: Phylum; E: Genus. F: Heatmap of inter-group differences in gut microbiota abundance at phylum level for *Phrynocephalus vlangalii*. Top hierarchical clustering tree indicates clustering relationships between groups, with asterisks denoting strong correlations between gut microbiota and samples. Asterisks indicate significant differences between two groups, *: P<0.05; **: P<0.01; ***: P<0.001. Data are mean±standard error (SE). HH and HS represent high-altitude lizards inhabiting native high-altitude environments and translocated to superhigh-altitude environments, respectively. SS and SH represent superhigh-altitude lizards inhabiting native superhigh-altitude environments and translocated to high-altitude environments, respectively.

remained distinct (Figure 2C; Supplementary Figure S3).

Gut microbiota composition

Analysis of fecal samples from the lizards revealed the presence of 24 phyla and 194 genera in their gut microbiota. The dominant phyla across both superhigh- and high-altitude lizards were *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, with their relative abundances corresponding to the respective rearing environments (Figure 2D). *Firmicutes* was particularly predominant in lizards originating from superhigh-altitude environments, while *Proteobacteria* showed lower relative abundance (Figure 2D; Supplementary Figure S4). Notably, the genus *Citrobacter* was significantly more abundant in high-altitude environments, irrespective of where the population originated, and less prevalent in superhigh-altitude environments (Figure 2E; Supplementary Figure S5).

Plasticity of gut microbiota

LEfSe analysis identified key biomarkers distinguishing superhigh- and high-altitude local populations, consistent with observed abundance differences between groups at the phylum and genus levels (Figure 3; Supplementary Figures S4, S5). The genera *Bacteroides* and *Citrobacter* were identified as key biomarkers for local high-altitude populations, while the phylum *Firmicutes* was the primary biomarker for the superhigh-altitude populations (Figure 3A). Translocation experiments demonstrated gut microbiota plasticity. In high-altitude lizards translocated to superhigh altitudes, *Citrobacter* abundance decreased significantly, while *Firmicutes* abundance increased significantly (Figure 3B). Conversely, in superhigh-altitude lizards translocated to high altitudes, *Firmicutes* abundance showed a marked decrease, while *Bacteroides* showed a marked increase (Figure 3C). LEfSe analysis further revealed that the class *Erysipelotrichia* was a host-specific bacterial group uniquely associated with superhigh-altitude lizards (Figure 3A, D).

Functional prediction of gut microbiota

KEGG enrichment analysis revealed significant shifts in metabolic pathways associated with gut microbial plasticity following translocation. When high-altitude lizards were translocated to superhigh-altitude environments, the beta-alanine metabolism pathway was significantly up-regulated, while the steroid biosynthesis pathway was significantly down-regulated (Figure 4B). When superhigh-altitude lizards were translocated to high-altitude environments, four metabolic pathways were significantly up-regulated, including folate biosynthesis, riboflavin metabolism, beta-alanine metabolism, and oxidative phosphorylation, and five metabolic pathways were significantly down-regulated, including base excision repair, D-arginine and D-ornithine metabolism, steroid biosynthesis, proteasome, meiosis-yeast (Figure 4C). No differential metabolic pathways were observed between the superhigh- and high-altitude populations within the high-altitude environment. However, in the superhigh-altitude environment, seven metabolic pathways were enriched in high-altitude lizards, and five metabolic pathways were enriched in superhigh-altitude lizards (Figure 4D). Notably, high-altitude lizards exhibited enrichment in the riboflavin metabolism pathway, while superhigh-altitude lizards showed enrichment in the fructose and mannose metabolism pathway.

DISCUSSION

Phenotypic plasticity is a key mechanism by which species adapt to novel and fluctuating environments (Alberdi et al., 2016; Fontaine & Kohl, 2023; Khakisahneh et al., 2020; Marsh et al., 2022; Uren Webster et al., 2018). Extensive research has highlighted the importance of plasticity in life history traits, behavior, physiology, and molecular mechanisms in environmental adaptation (Du, 2006; Iraeta et al., 2006, 2008; Lane et al., 2019; Sears, 2005; Sun et al., 2018, 2022; Wong et al., 2021). However, the plasticity of the gut microbiome and its influence on host adaptability remain underexplored, particularly in the context of rapidly changing environments. This study explored the plasticity and host specificity of the gut microbiome in *P. vlangalii* and their potential implications for host fitness. The findings aligned with our predictions that the gut microbiome of *P. vlangalii* exhibited remarkable plasticity and underwent substantial alterations following translocation. Both environmental plasticity and population genetic factors significantly influenced the gut microbiome structure. Furthermore, specific microbial markers and functional pathways associated with microbiota plasticity demonstrated possible effects on host fitness, suggesting a functional role of gut microbial adaptation in host resilience to environmental shifts.

To mitigate the potential confounding effects of historical colonization, which could obscure the plasticity of the gut microbiome in response to new environments, our analysis focused exclusively on the offspring of translocated lizards. Historical colonization refers to the stable microbial community present before translocation, which may persist and influence subsequent microbial changes (Uren Webster et al., 2020). Although this approach limits direct observations of initial microbial shifts in adults, it provides a more accurate assessment of how gut microbiota respond to environmental changes without pre-existing microbial interference. Overall, these results highlight the dual influence of environmental plasticity and host specificity in shaping the gut microbiome of *P. vlangalii*, with changes in the gut microbiome likely playing pivotal roles in enhancing host fitness.

Our findings revealed that gut microbiome diversity was higher in the high-altitude lizards than in the superhigh-altitude lizards (Figure 2B). The dominant phyla in both populations were *Bacteroidetes* and *Firmicutes* (Figure 2D), consistent with previous research (Zhang et al., 2018). However, the gut microbiome of high-altitude lizards was distinguished by the significant presence of *Citrobacter* within the phylum *Proteobacteria* (Figure 2D, E). LEfSe analysis identified *Citrobacter* and *Bacteroides* as key biomarkers in high-altitude lizards, whereas *Firmicutes* was the key biomarker in superhigh-altitude lizards (Figure 3A). Mechanistically, *Citrobacter* has been shown to enhance host energy storage by modulating the gut microbial community (Melaku et al., 2021; Zhang et al., 2020), while *Bacteroides* has been shown to induce gut IgA production for immune regulation (Yang et al., 2020). The enrichment of these microbes in high-altitude lizards highlights their potential importance in optimizing energy metabolism and strengthening immunity under these environmental conditions. Additionally, functional enrichment analysis indicated that the nine metabolic pathways enriched in the gut microbiome of lizards at high altitudes were mainly related to energy production, lipid, and protein synthesis (Figure 4A). A more diverse gut microbiome may enhance energy regulation and fat storage, as suggested in previous studies (Sommer et al., 2016; Wang et al., 2020). This functional capacity aligns with the observed higher growth rates, improved body condition (SMI), and greater

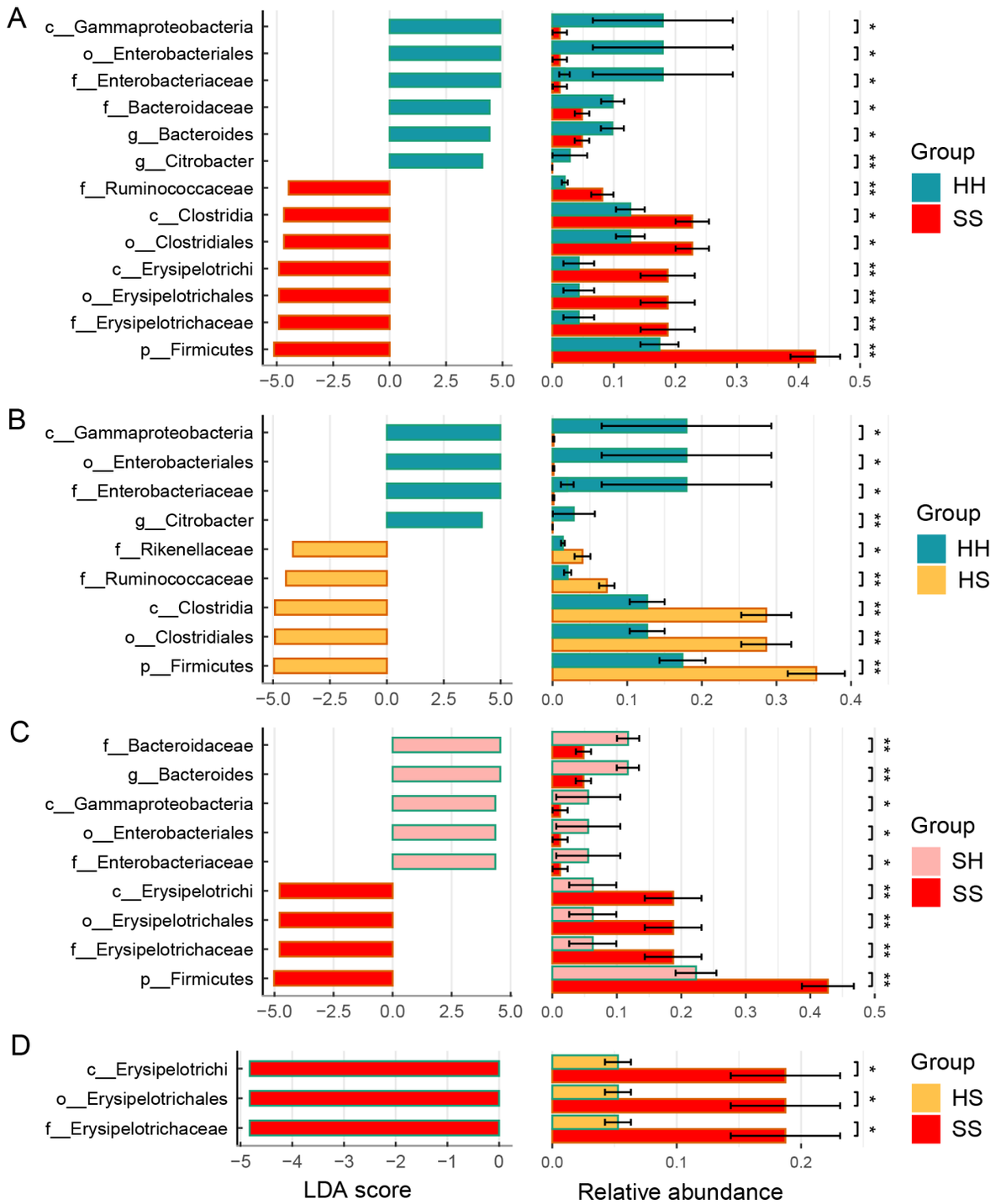

Figure 3 Differences in bacterial taxa of *Phrynocephalus vlangalii* determined using linear discriminant analysis of effect size (LEfSe)

A: Comparisons between source populations. B, C: Comparisons of plasticity. D: Host-specificity comparisons. Left: LDA score plots, Right: Corresponding Kruskal-Wallis single-factor tests of abundance among groups. Asterisks indicate significant differences between two groups, *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$. HH and HS represent high-altitude lizards inhabiting native high-altitude environments and translocated to superhigh-altitude environments, respectively. SS and SH represent superhigh-altitude lizards inhabiting native superhigh-altitude environments and translocated to high-altitude environments, respectively.

survival rates in high-altitude lizards compared to superhigh-altitude lizards (Figure 1; Supplementary Figure S2A). The elevated presence of *Citrobacter* and the increased Shannon diversity in high-altitude lizards may contribute to more effective energy management and better overall fitness. Interestingly, high-altitude lizards tend to experience lower parasitic infection rates (Megía-Palma et al., 2020), which may influence the composition of their gut microbial communities. However, further investigation is needed to elucidate the interplay between parasitic infections and gut microbiome composition in these populations.

Do environmental changes drive the gut microbiota of translocated lizards to resemble those of native populations? Our reciprocal translocation experiments demonstrated that the alpha diversity of the gut microbiota in *P. vlangalii* was influenced only by rearing environment, highlighting the

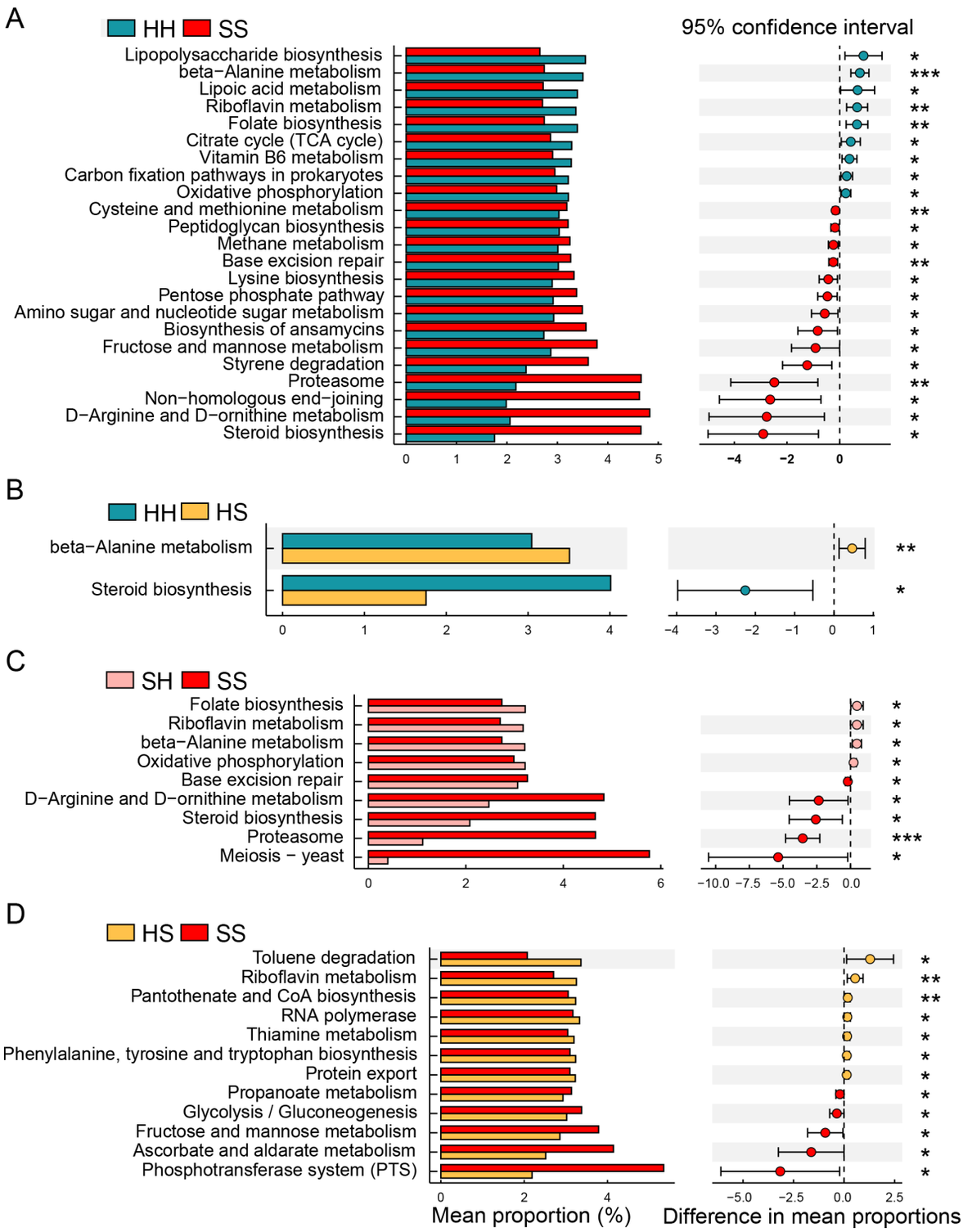

Figure 4 Relative abundance of KEGG orthologs in *Phrynocephalus vlangalii* detected by PICRUSt2 analysis

A: Comparisons between source populations. B, C: Comparisons of plasticity. D: Host-specificity comparisons (no significant difference in KEGG ortholog relative abundance between HH and SH). Asterisks indicate significant differences between two groups, *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$. HH and HS represent high-altitude lizards inhabiting native high-altitude environments and translocated to superhigh-altitude environments, respectively. SS and SH represent superhigh-altitude lizards inhabiting native superhigh-altitude environments and translocated to high-altitude environments, respectively.

remarkable plasticity of gut microbes to altitudinal shifts (Figure 2A, B). This finding underscores the crucial role of environmental factors, particularly altitude, in shaping gut microbiota composition. For instance, lizards residing at high altitudes (2 900 m) exhibited greater microbial diversity than their counterparts at superhigh altitudes (4 250 m), consistent with previous research linking altitudinal gradients with microbial diversity (Zhang et al., 2018).

Our results further revealed a significant increase in alpha diversity (Chao1 richness and Shannon index) in superhigh-

altitude lizards translocated to high-altitude environments. This increase may be attributed to the improved environmental conditions, such as higher temperature and oxygen availability at lower altitudes. Conversely, the alpha diversity of high-altitude lizards remained stable even after translocation to superhigh altitudes, maintaining higher levels. This stability may be related to maternal microbial transmission, which has been shown to influence gut microbiota composition and persist into adulthood (Ren et al., 2017).

Environmental factors played a dominant role in determining the composition of gut microbiota, as evidenced by a hierarchical clustering heatmap of species composition (Figure 2F). Both superhigh- and high-altitude lizards exhibited significant changes in beta diversity following translocation, reflecting strong microbial plasticity (Figure 2C; Supplementary Figure S3). However, high-altitude lizards translocated to superhigh altitudes displayed no changes along the PC2 axis, indicating that certain gut microbial components did not change with the environment and were host specific (Figure 2C). Analysis of population sources and rearing environments revealed that the structure of the gut microbial communities was shaped by both environmental plasticity and host specificity (Figure 2C; Supplementary Figure S3). High-altitude environments were associated with enhanced microbial diversity, while high-altitude lizards appeared to sustain elevated diversity levels even under superhigh-altitude conditions, likely due to maternal effects. Overall, these findings indicate that gut microbial composition in *P. vlangalii* is shaped by both plastic responses to environmental conditions and intrinsic host-specific factors. While the gut microbiota of translocated lizards adjusts to resemble that of native populations, host-specific components persist.

Our findings reveal that after high-altitude lizards were translocated to superhigh altitudes, their growth rates and body condition declined, while survival remained unaffected (Figure 1; Supplementary Figure S2B). This decline coincided with a significant reduction in the abundance of *Citrobacter* and a marked increase in the abundance of *Firmicutes* (Figures 2D, E, 3B; Supplementary Figure S5). *Citrobacter* is known to enhance host energy storage by influencing the gut microbial community and increasing the availability of glucose and lipids, crucial for growth and metabolic function (Zhang et al., 2020). Conversely, *Firmicutes*, involved in the production of short-chain fatty acids (SCFAs) such as butyrate, which modulate immune function, exhibited a negative correlation with growth in both BM and SVL (Ridaura et al., 2013; Turnbaugh et al., 2006). This negative correlation may reflect a trade-off where heightened immune activity diverts resources from growth and metabolic demands. These findings were supported by metabolic analysis. Notably, after high-altitude lizards were translocated to superhigh altitudes, beta-alanine metabolism significantly increased, while steroid biosynthesis significantly decreased (Figure 4B). This metabolic shift likely reduces fat storage, potentially promoting growth (Nie et al., 2023). However, the reduced body condition observed at superhigh altitudes compared to high altitudes (Figure 1D: HH>HS) indicates that these changes may not fully mitigate the challenges of superhigh-altitude environments. In contrast, when superhigh-altitude lizards were translocated to high altitudes, their growth rates and body condition improved, although their survival rates remained unchanged (Figure 1B–D; Supplementary Figure S2C). This improvement was accompanied by a significant decrease in the abundance of *Firmicutes* and a marked increase in the abundance of *Bacteroides* (Figures 2D, E, 3C; Supplementary Figure S4). While *Firmicutes* contributes SCFAs with immune modulatory functions, *Bacteroidetes* promotes IgA production, enhancing immune regulation (Ridaura et al., 2013; Turnbaugh et al., 2006). The high-altitude environment fostered greater microbial diversity, as evidenced by increased Chao1 indices when superhigh-altitude lizards were translocated to high altitudes (Figure 2B). Enhanced microbial diversity is associated with improved energy metabolism and fat storage (Sommer et al., 2016; Wang et al., 2020). Briefly, we identified considerable plasticity in the gut microbiome of *P. vlangalii*. High-altitude environments induced greater gut microbiome diversity, while superhigh-altitude environments induced the opposite trend. Our results also indicated that *Citrobacter* may be beneficial for host growth, while *Erysipelotrichia* and *Firmicutes* were negatively correlated with host growth (Supplementary Figure S6), potentially reflecting adaptations to resource-limited environments (Zhu et al., 2024a). Evaluating the ecological significance of these phenotypic changes is challenging due to the complexity of underlying biological mechanisms. Future studies should prioritize experimental manipulation of the gut microbiome to validate its effects on growth and other physiological traits. Integrating host metabolomics and transcriptomics will provide deeper insights into how the gut microbiome influences host survival, physiological functions, and adaptive significance of phenotypic plasticity.

Our findings also demonstrated that the gut microbiota of both superhigh- and high-altitude lizards exhibited strong host specificity. Notably, growth rates did not significantly differ between populations, regardless of the rearing environment. However, superhigh-altitude lizards exhibited lower survival rates but better body condition compared to their high-altitude counterparts (Figure 1B–D; Supplementary Figure S2D, E). Beta diversity analysis revealed that population origin significantly influenced the gut microbiome structure, with clear differences along the PC2 axis in the superhigh-altitude environment between the two populations (Figure 2C). This stable association between the gut microbiome and population origin underscores the host specificity of the gut microbial community in these lizards. While these findings suggest a potential influence of both altitude and population origin on microbial community structure, sampling additional populations across varying altitudes could further clarify whether these effects are altitude-specific or site-dependent.

LEfSe analysis identified *Erysipelotrichaceae* as a key biomarker in the gut microbiome of superhigh-altitude lizards (Figure 3D). This bacterial family has been implicated in lipid metabolism disorders and inflammation (Kaakoush, 2015; Wu et al., 2021), potentially contributing to the lower survival rates observed in superhigh-altitude lizards (Supplementary Figure S2D, E). Furthermore, metabolic pathway analysis revealed distinct patterns between the two populations. High-altitude lizards exhibited enrichment in seven metabolic pathways, primarily related to protein production (Figure 3D). Conversely, superhigh-altitude lizards showed enrichment in five metabolic pathways, including the propanoate metabolism pathway, which is related to obesity (Jiao et al., 2018). These findings may partially explain the better body condition observed in superhigh-altitude lizards. Furthermore, a significant negative correlation was detected between *Erysipelothrix* abundance

and host growth (Supplementary Figure S6B). This association suggests that the host-specific gut microbiota of superhigh-altitude lizards may limit their growth rate, reflecting a trade-off between maintaining body condition and achieving growth in resource-limited environments.

In summary, our findings revealed significant differences in the gut microbiome between superhigh- and high-altitude populations of *P. vlangalii*, primarily driven by environmental factors related to altitude. Translocation experiments demonstrated pronounced plasticity in the gut microbiome, emphasizing its adaptability to environmental changes and its potential influence on host fitness. Notably, the closer the gut microbiome of translocated lizards resembled that of the local altitude population, the better their growth, body condition, and survival performance. In superhigh-altitude lizards, the host-specific gut microbiota composition, characterized by the presence of *Erysipelotrichaceae* and enrichment in lipid metabolism pathways associated with obesity, likely contributed to their lower survival rates despite better body condition compared to high-altitude lizards. These findings underscore the critical role of gut microbiota in mediating host physiological responses and highlight the vulnerability of superhigh-altitude populations to environmental pressures that shape gut microbiomes. Future studies should include mid-term monitoring of gut microbiota in translocated populations to assess whether microbial communities gradually converge with those of the local population or whether there is a persistent historical legacy effect (Baldo et al., 2023). Such insights will provide a deeper understanding of the long-term dynamics of gut microbiota in response to environmental changes. While this study did not deeply explore the mechanisms by which gut microbes influence adaptive phenotypes, existing studies have shown that gut microbes can affect host phenotypes by regulating metabolism and physiological functions (Sommer et al., 2016; Wang et al., 2020). Future studies should focus on the potential of microbial transplantation to facilitate phenotypic transitions between high- and low-altitude environments and assess its role in enhancing adaptability. Such studies will help clarify the functional contributions of gut microbiota to host adaptation in diverse and challenging ecosystems.

DATA AVAILABILITY

The metadata and raw sequence reads have been deposited in the NCBI SRA database (PRJNA1141194), China National Center for Bioinformation (PRJCA032768), and Science Data Bank databases (DOI: 10.57760/sciencedb.j00139.00134).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Z.G.Z., Z.S.L., L.W.T., W.G.D., and W.Y. conceived and designed the studies. W.Y., J.Y., Z.Y.Z., X.L.Z., and S.C. collected and analyzed the data; W.Y., J.Y., L.W.T., S.C., and Z.G.Z. wrote the manuscript, and all authors contributed to revisions. All authors read and approved the final version of the manuscript.

ACKNOWLEDGMENTS

We thank Bao-Jun Sun for advice on experimental design and manuscript preparation and Shuo Liu for help with data analysis. We thank Zhong-Wen Jiang for suggestions on manuscript revisions.

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