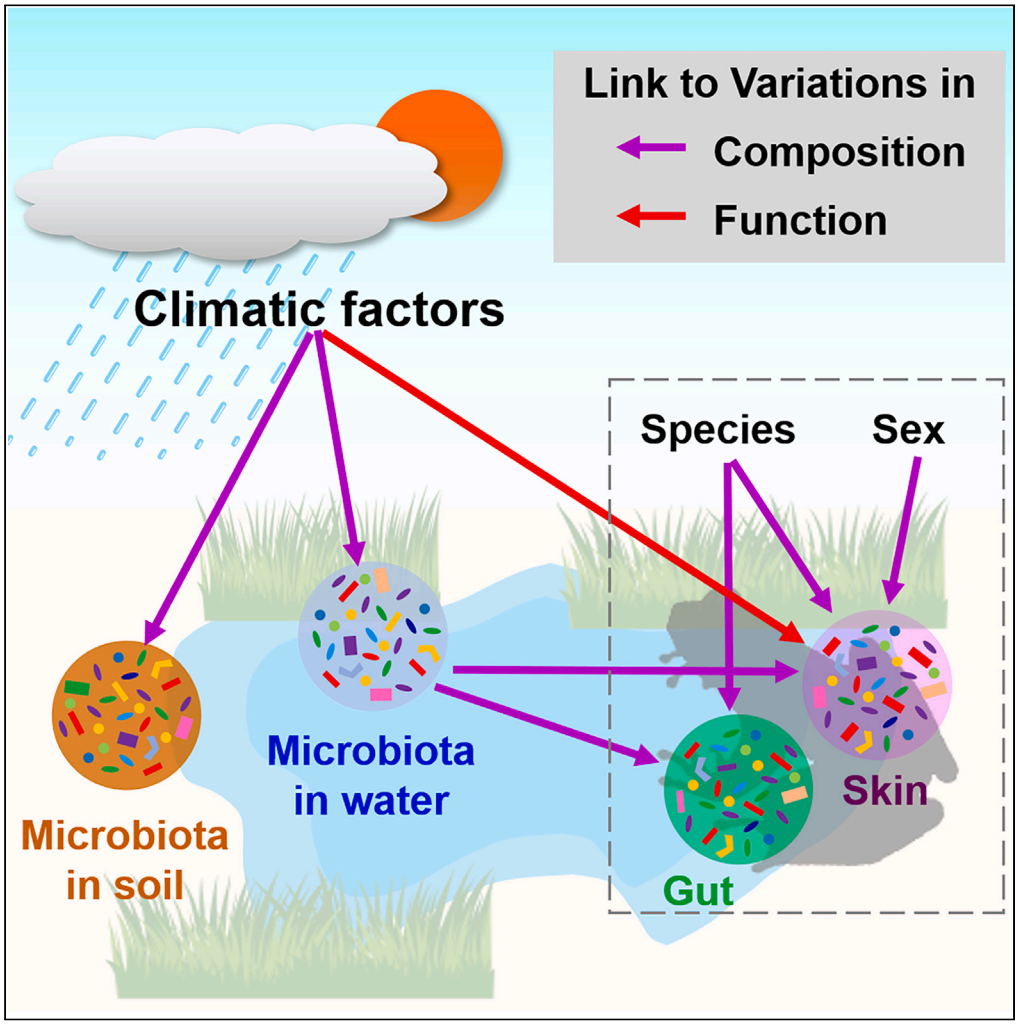
iScience

CellPress
OPEN ACCESS

Article

Microbial diversity in mountain-dwelling amphibians: The combined effects of host and climatic factors



Wei Zhu, Liming Chang, Meihua Zhang, Qiheng Chen, Lulu Sui, Cheng Shen, Jianping Jiang

changlm@cib.ac.cn (L.C.)
jiangjp@cib.ac.cn (J.J.)

Highlights

Climatic factors drive variations in water and soil microbiota

Climatic factors are associated with variations in amphibian microbiota

Water microbiota constitute a significant source of amphibian microbiota

Water microbiota serve as a bridge between climatic factors and host microbiota

Zhu et al., iScience 27, 109907
June 21, 2024
https://doi.org/10.1016/j.isci.2024.109907

iScience

CellPress
OPEN ACCESS

Article

Microbial diversity in mountain-dwelling amphibians: The combined effects of host and climatic factors

Wei Zhu,[1] Liming Chang,[1,3,*] Meihua Zhang,[1] Qiheng Chen,[1,2] Lulu Sui,[1,2] Cheng Shen,[1] and Jianping Jiang[1,2,*]

SUMMARY

Comprehending the determinants of host-associated microbiota is pivotal in microbial ecology. Yet, the links between climatic factors and variations in host-associated microbiota necessitate further clarification. Mountain-dwelling amphibians, with limited dispersal abilities, serve as valuable models for addressing these questions. Our study, using 126 amphibian-associated microbial samples (64 gut and 62 skin) and 101 environmental microbial samples (51 soil and 50 water) from the eastern Tibetan Plateau, revealed host factors as primary drivers of the variations in host-associated microbiota. However, climatic factors contributed to additional variations in gut microbial beta-diversity and skin microbial function. Water microbiota were identified as a significant contributor to the amphibian-associated microbiomes, with their climate-driven variations mediating an indirect association between the variations in climatic factors and host-associated microbiota. These findings extend our understanding of the assembly of host-associated microbiota in amphibians, emphasizing the significance of microbiota in evaluating the impact of climate change on animals.

INTRODUCTION

The host-associated microbiota of vertebrates play pivotal roles in maintaining host health.[1–4] Their plasticity in community structure and function contributes significantly to host ability to adapt to changing or extreme environments,[5–8] offering unique insight into the adaptive potential of animals to environmental changes. Thus, the compositional and functional diversity of host-associated microbiota hold importance for the conservation and management of wild animals.[9,10]

Identifying the determinants of the diversity and function of host-associated microbiota is a crucial focus in microbial ecology.[11] Relevant research encompasses a wide range of organisms, including mammals,[12,13] fish,[14,15] and amphibians.[16,17] Host factors, such as phylogeny, genetics, sex, diet, and age, are well established factors that shape the community structure of host-associated microbiota.[18–22] Additionally, environmental factors of microhabitats, including pH, salinity, and particularly the temperature, also contribute to the variations of host-associated microbiota.[8,23–27] Recently, attention has been paid to the potential association between geographical climate (e.g., annual temperature and precipitation) and microbial variations in wild vertebrates.[22,28–30] This potential association allows for exploration of the diversity patterns of vertebrate commensal microbiota at a broader spatial scale,[30] and it holds implications for understanding the animals' environmental adaptation in the context of climate change.[31]

Existing evidences suggest a close association between the variations in climatic factors and the composition of environmental microbiota (e.g., soil and water microbiota).[32–34] Environmental microbiota serve as a significant source for the skin and gut microbiota of animals.[35–37] Consequently, we may speculate that climate-dependent variations in environmental microbiota may influence the composition of host-associated microbiota, indicating a potential indirect association between climatic factors and animal-associated microbiota. Hence, considering environmental microbiota in the association network is essential to provide a comprehensive perspective on the assembly of host-associated microbiota.[17]

Amphibians are vulnerable to climate change and their wild populations are undergoing significant decline.[38–40] Understanding the connections between their microbial diversity and climatic factors may be helpful in assessing their survival status. Moreover, amphibians exhibit limited dispersal capacity,[41,42] implying their physiological adaptation to local climates over time. Indeed, amphibian populations from distinct geographic regions often exhibit differences in their physiology, metabolism,[43,44] and microbiota.[45–47] However, scant attention has been given to exploring the potential influence of climatic factors on the variations in commensal microbiota.[48] Mountain-dwelling amphibians experience highly variable climate conditions, making them suitable models for studying the associations

[1]CAS Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, China
[2]University of Chinese Academy of Sciences, Beijing 100049, China
[3]Lead contact
*Correspondence: changlm@cib.ac.cn (L.C.), jiangjp@cib.ac.cn (J.J.)
https://doi.org/10.1016/j.isci.2024.109907

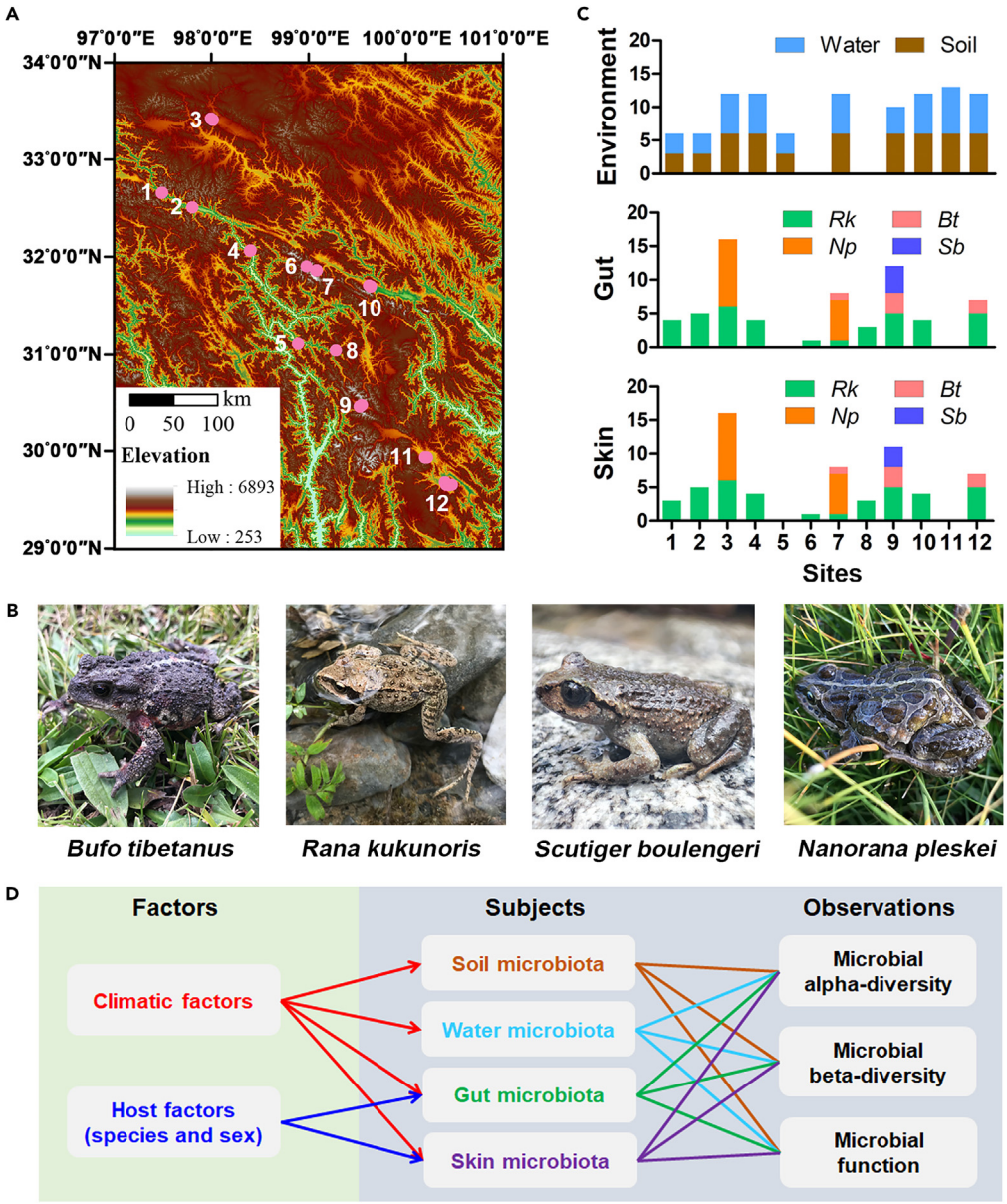


Figure 1. Sample information

(A) Study area and sampling sites.

(B) Photos of the four amphibian species.

(C) Bar plots showing the sample size at each collection site. *Rk*, *R. kukunoris*; *Bt*, *B. tibetanus*; *Np*, *N. pleskei*; *Sb*, *S. boulengeri*.

(D) A flowchart illustrating the factors and observations in our data analyses.

between host-associated microbiota and climatic factors. Additionally, these animals encounter unique environmental challenges, such as strong UV radiation in high-latitudes. Considering that some molecules produced by commensal microbiota can shield or repair UV damage,[49] it is intriguing to investigate whether the skin microbiota of high-latitude amphibians possess special metabolic functions to protect their exposed skin.

The Qinghai–Tibet Plateau, the world's highest and largest plateau in the world,[50] encompasses parts of four global biodiversity hotspots.[51] Its vast size and wide elevational range (1,000 to over 8,000 m) create extensive ecological gradients in temperature and precipitation,[52] providing a natural laboratory for studying organism-environment interactions. In this study, we conducted a regional survey in the eastern Tibetan Plateau, establishing twelve geographical sites and sampling the gut and skin microbiota of four amphibian species (*Bufo tibetanus*, *Rana kukunoris*, *Scutiger boulengeri*, and *Nanorana pleskei*) (Figure 1, Table 1). We analyzed the similarity and associations between environmental (water and soil microbiota of the animals' habitats) and host-associated microbiota (i.e., gut and skin), and explored

Table 1. Basic information of the four amphibian species

Species	Altitude (m)		Breeding season	Living habit
	Record	This study (Mean ± SD)		
Rana kukunoris	2000–4400	3859 ± 309[b]	March-May	The edge of still water and other moist environments.
Scutiger boulengeri	2700–5100	4110[ab]	May-August	The edge of streams and pools.
Bufo tibetanus	2300–4300	4007 ± 174[ab]	April-June	Among the rocks or weeds in the alpine grassland, farmland and forest edge.
Nanorana pleskei	3300–4500	4196 ± 18[a]	May-June	The edge of streams and pools, or among the weeds

The altitudes at which we caught these animals were analyzed with one-way ANOVA and LSD post-hoc test. Different letters denote significant difference between groups.

the significance of host and climatic factors in explaining variations in microbial diversity, composition, and function. Our hypotheses were: (1) while host factors (e.g., body site and species) primarily drive the variations in host-associated microbiota, climatic factors may account for additional variations; and (2) environmental microbiota might act as mediators in the associations between climatic factors and host-associated microbiota.

RESULTS

Comparative analyses on different types of microbiota

The bacterial phyla with relative abundances exceeding 10% were Firmicutes (34.8%), Proteobacteria (32.5%), and Bacteroidetes (25.2%) in amphibian gut microbiota; Proteobacteria (93.0%) in amphibian skin microbiota; Proteobacteria (63.0%) in water microbiota; and Proteobacteria (27.1%), Planctomycetes (14.1%), Bacteroidetes (11.7%), Acidobacteria (11.1%), and Actinobacteria (10%) in soil microbiota (Figure 2). Soil microbiota exhibited the highest alpha-diversity, followed by water, gut, and skin microbiota (Figure 2B). Microbial composition significantly differed between sample types ($F_{3,226} = 29.4$, $R^2 = 0.28$, $p < 0.001$, PERMANOVA). Gut and skin microbiota were more similar to water microbiota in composition than to soil microbiota (Figure 2C). Source tracking analyses indicated that water microbiota, but not soil microbiota, significantly contributed to amphibian skin (92.0%) and gut (18.5%) microbiota (Figures 2D–2F). The prediction rate of water microbiota to skin microbiota was significantly higher in *N. pleskei* than in *R. kukunoris* (Figure 2F).

In comparison to skin microbiota, gut microbiota exhibited a higher relative abundance of Firmicutes, Spirochaetae, and Tenericutes, while having a lower relative abundance of Proteobacteria (Figure 2A). Gut microbiota also displayed higher and lower relative abundance of genes involved in primary metabolism (e.g., carbohydrate and lipid metabolism) and secondary metabolism (e.g., metabolism of terpene, terpenoid and steroids and antibiotics biosynthesis), respectively (Figure S2A). Alpha- and beta-diversity indices of gut microbiota did not correlate with those of skin microbiota from the same individuals (Figures S2B and S2C).

Association of climatic factors with environmental microbiota

The alpha-diversity of both soil and water microbiota showed no significant association with climatic factors (Figures 3A and S3A).

The beta-diversity (unweighted UniFrac distances) of soil microbiota was significantly associated with both temperature- ($F_{1,47} = 2.38$, $R^2 = 0.04$, $p < 0.001$) and precipitation-related climatic factors ($F_{1,47} = 5.56$, $R^2 = 0.10$, $p < 0.001$) (Figures 3A and S3B). The distribution of soil samples in the PCoA scatterplot exhibited a climate-dependent pattern, particularly associated with the precipitation-related factor (Figure 3B). Bacterial genera whose abundances significantly correlated ($p < 0.001$ for both Spearman and Pearson correlations) with precipitation-related climatic factor included *Pirellula*, *Pir4 lineage*, and *Planctomyces* in the soil (Figure 3C). The beta-diversity of water microbiota varied with the climatic temperature-related factor ($F_{1,46} = 2.68$, $R^2 = 0.05$, $p < 0.001$) and mean diurnal range ($F_{1,46} = 1.74$, $R^2 = 0.03$, $p < 0.05$) (Figures 3A, 3B, and S3B). Bacteria whose abundance varied with temperature-related factor included *Candidatus* and *Endomicrobium* in the water ($p < 0.001$ for both Spearman and Pearson correlations; Figure 3C).

The variations in microbial functions of both soil and water samples could not be predicted by the climatic indices in this study (Figures 3A and S3C).

Effects of host factors on amphibian microbiota

The alpha-diversity of amphibian gut microbiota remained unaffected by host and climatic factors. However, the skin microbial alpha-diversity was significantly influenced by host species and sex (Figures 4A, S4A, and S4B). Among the four species, *N. pleskei* exhibited the highest skin microbial alpha-diversity, differing significantly from *R. kukunoris*. Regarding sex, females displayed markedly higher skin microbial alpha-diversity than juveniles (frogs having not reached sexual maturity) and males ($F_{2,53} = 3.57$, $p < 0.05$, LSD post-hoc test following mixed linear model), with juveniles exhibiting the lowest diversity (Figure 4B).

For beta-diversity, host species emerged as the primary factor driving the variations of both gut and skin microbiota (Figures 4A, S4C, and S4D). Specifically, we observed significant differences in gut microbial composition between *N. pleskei* and the others, as well as in skin microbial composition between *N. pleskei* and *R. kukunoris* ($F_{1,52} = 5.04$, adjusted $p < 0.05$, PERMANOVA; Figures 4C and 4D). The gut

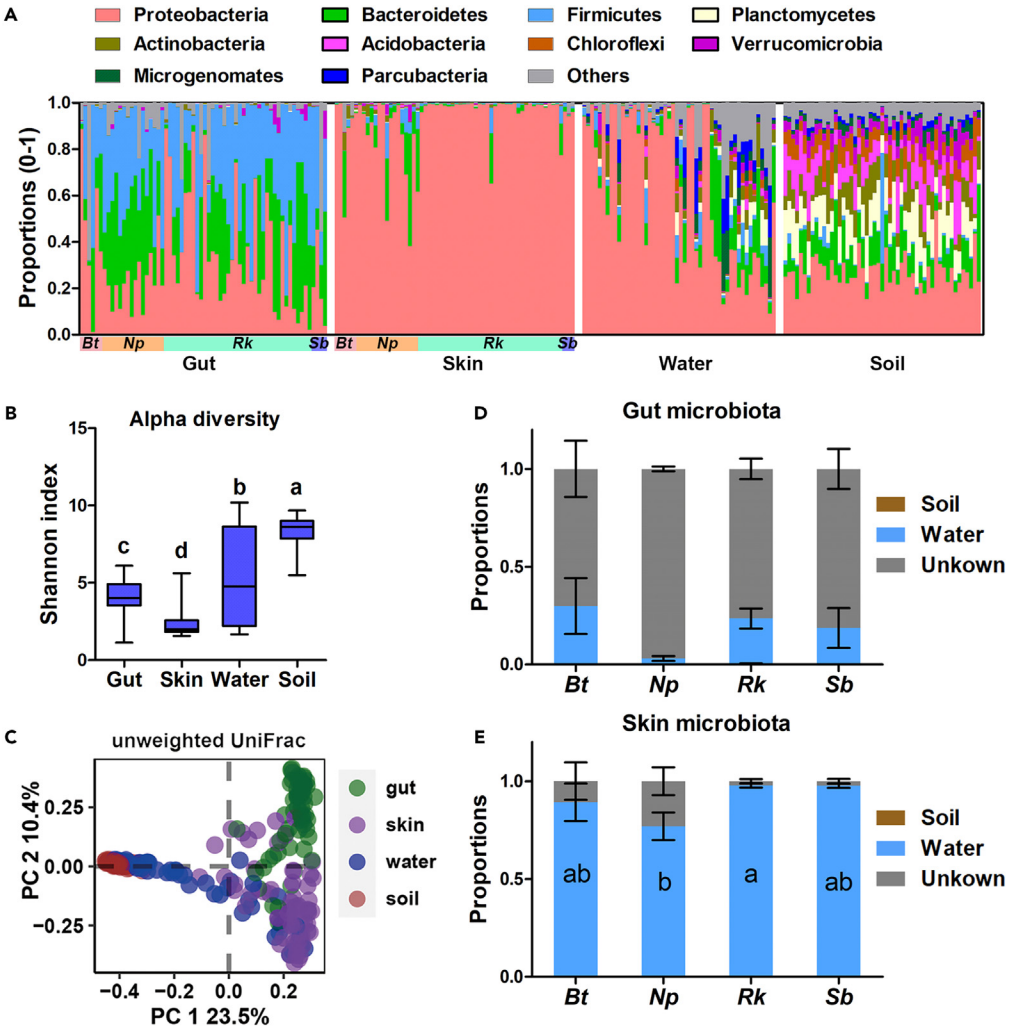


Figure 2. Comparative analyses on microbial diversity between different type of samples

(A) Microbial composition at phylum level.

(B) Microbial alpha-diversity based on Shannon index. Each value represents the mean ± SE. Different letter denote significant differences between groups ($p < 0.05$, LSD post hoc test following linear mixed model with collection site as a random factor).

(C) PCoA scatterplots presenting the similarity in microbial beta-diversity (unweighted UniFrac distance) between samples.

(D–E) Results of source-track analyses showing the contribution of environmental microbiota to amphibian microbiota. Each value represents the mean ± SE. Different letter denote significant differences in the proportions of water-derived microbiota between groups ($p < 0.05$, LSD post hoc test following linear mixed model with collection site as a random factor).

microbiota of *N. pleskei* exhibited a higher relative abundance of *Acidaminococcaceae*, *Coprobacillus*, Desulfovibrionaceae, *Mycoplasmatales*, and *Eubacterium* compared to the others three species; additionally, the skin microbiota of *N. pleskei* displayed a higher relative abundance of *Clostridia*, *Aeromonas*, and Pseudomonadaceae in their skin microbiota (adjusted $p < 0.05$, screened by LEfSe and further confirmed by Kruskal-Wallis test; Figures S4E and S4F). Climatic factors (temperature-related factor and mean diurnal range) and host sex contributed to additional variations in gut and skin microbial beta-diversity, respectively (Figures 4A, S4C, and S4D), indicating combined effects of host and climatic factors on amphibian microbiota. We observed significant pairwise differences in skin microbial beta-diversity among female, male, and juvenile individuals (Figures 4E and 4F). *Bacteroides*, *Parabacteroides*, *Lelliottia*, *Alistipes*, and Fimbriimonadaceae showed significant contributions to the inter-sex differences in the skin microbiota, being most abundant in females, followed by males, and nearly absent in juveniles (Figure S4G).

The variations in microbial functions of both gut and skin samples were independent of host and climatic factors (Figures 4A, S5A, and S5B). However, we observed interspecies differences in the microbial functional redundancy of skin samples ($F_{3,29} = 4.29$, $p = 0.013$, mixed linear model; Figures S5C and S5D), with *R. kukunoris* displaying a significantly lower level than *N. pleskei* ($p < 0.05$, LSD post-hoc test; Figure S5E).

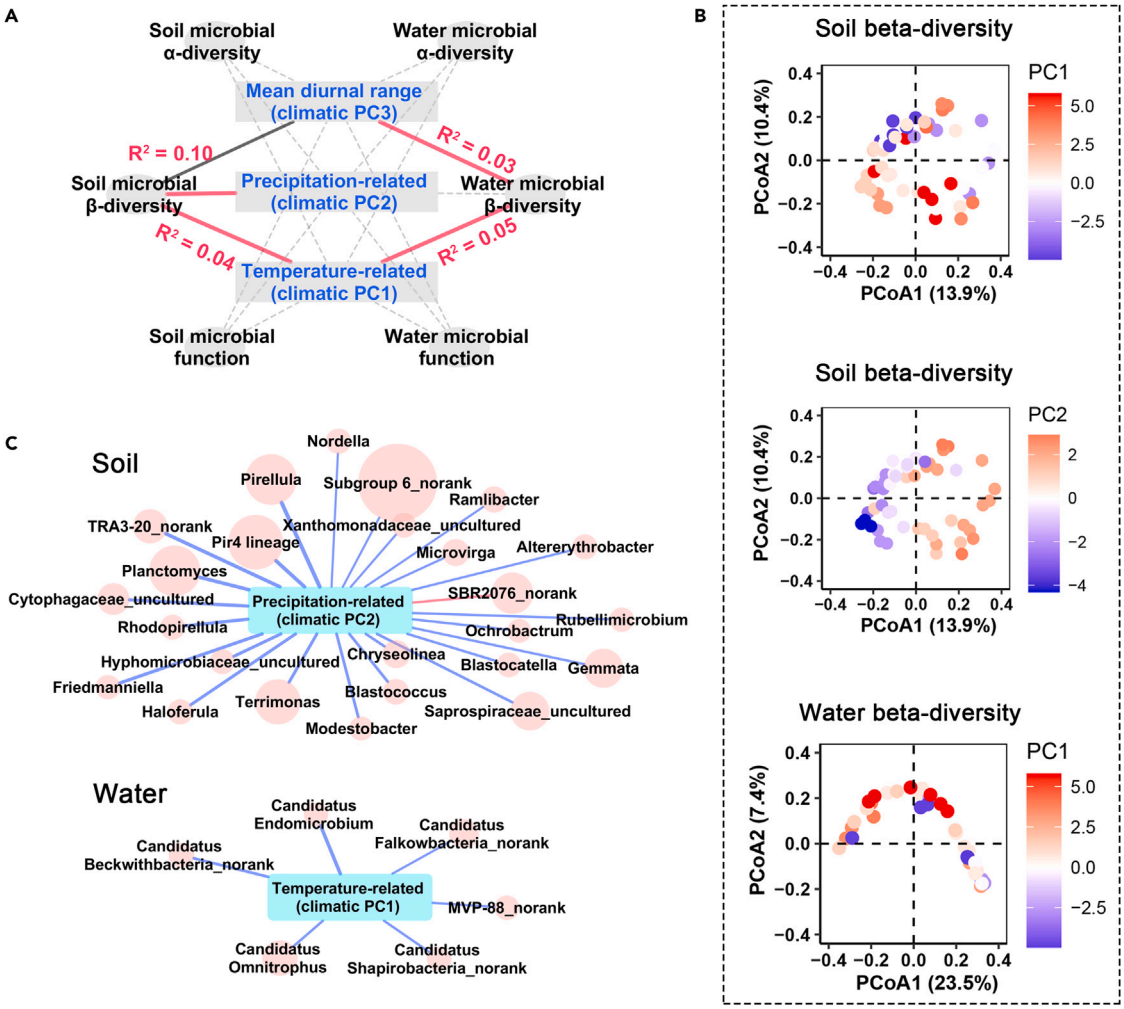


Figure 3. Associations of climatic factors with soil and water microbiota

(A) A network summarizing the associations between climatic indices and microbial diversity or function. The red, black, and gray-dashed colors of edges represent significant ($p < 0.05$), marginally significant ($0.05 < p < 0.1$), and insignificant associations ($p > 0.1$), respectively. Associations of climatic indices with microbial alpha-diversity, beta-diversity, and functions were analyzed with mixed linear models (climatic grids as random effects), PERMANOVA (unweighted UniFrac distance), and PERMANOVA (Bray-Curtis distances), respectively (detailed in Figure S3).

(B) PCoA scatterplots illustrate the variation in microbial beta-diversity (unweighted UniFrac distance) with climatic indices.

(C) Networks depict the bacterial genera whose abundance significantly varied with the climatic indices. A correlation was considered significant if $p < 0.001$ for both Spearman and Pearson correlations. The size of the nodes denotes the relative abundance of each bacterial genus; the width of edges denotes the absolute R value (Spearman correlation) of each correlation; blue and red colors of the edges indicate positive and negative correlations respectively.

Associations between climatic factors and amphibian microbiota

As previously mentioned, climatic factors explained additional variations in amphibian microbiota beyond those accounted for by host factors. To minimize the influence of host-related interference, we specifically examined the associations between climatic factors and microbial variations in a single species, *R. kukunoris*, which had samples collected from ten distinct sites.

The alpha-diversity of both skin and gut microbiota did not vary with climatic factors (Figures 5A and S6), whereas the beta-diversity of gut microbiota showed significant associations with diurnal mean range ($F_{1,32} = 1.69$, $p < 0.05$, PERMANOVA; Figures 5A, 5B, and S7A), consistent with the findings at multi-species level. For instance, the relative abundance of *Paucimonas* in the gut microbiota varied with mean diurnal range (Figure S7B). Moreover, the skin microbial function of *R. kukunoris* exhibited a significant association with precipitation-related climatic factor ($F_{1,31} = 3.57$, $p < 0.05$, PERMANOVA; Figures 5C and S7C). Functional items that positively correlated with precipitation-related factor included 'inositol phosphate metabolism', 'streptomycin biosynthesis', 'neomycin, kanamycin and gentamicin biosynthesis', 'N-glycan biosynthesis', and 'indole alkaloid biosynthesis', while those negatively correlated with precipitation-related factor included 'nitrogen metabolism', 'taurine and hypotaurine metabolism', 'biosynthesis of vancomycin group antibiotics', 'biofilm formation', 'biosynthesis of siderophore group nonribosomal peptides', 'nonribosomal peptide structure', and 'cyanoamino acid metabolism' (Figure 4D).

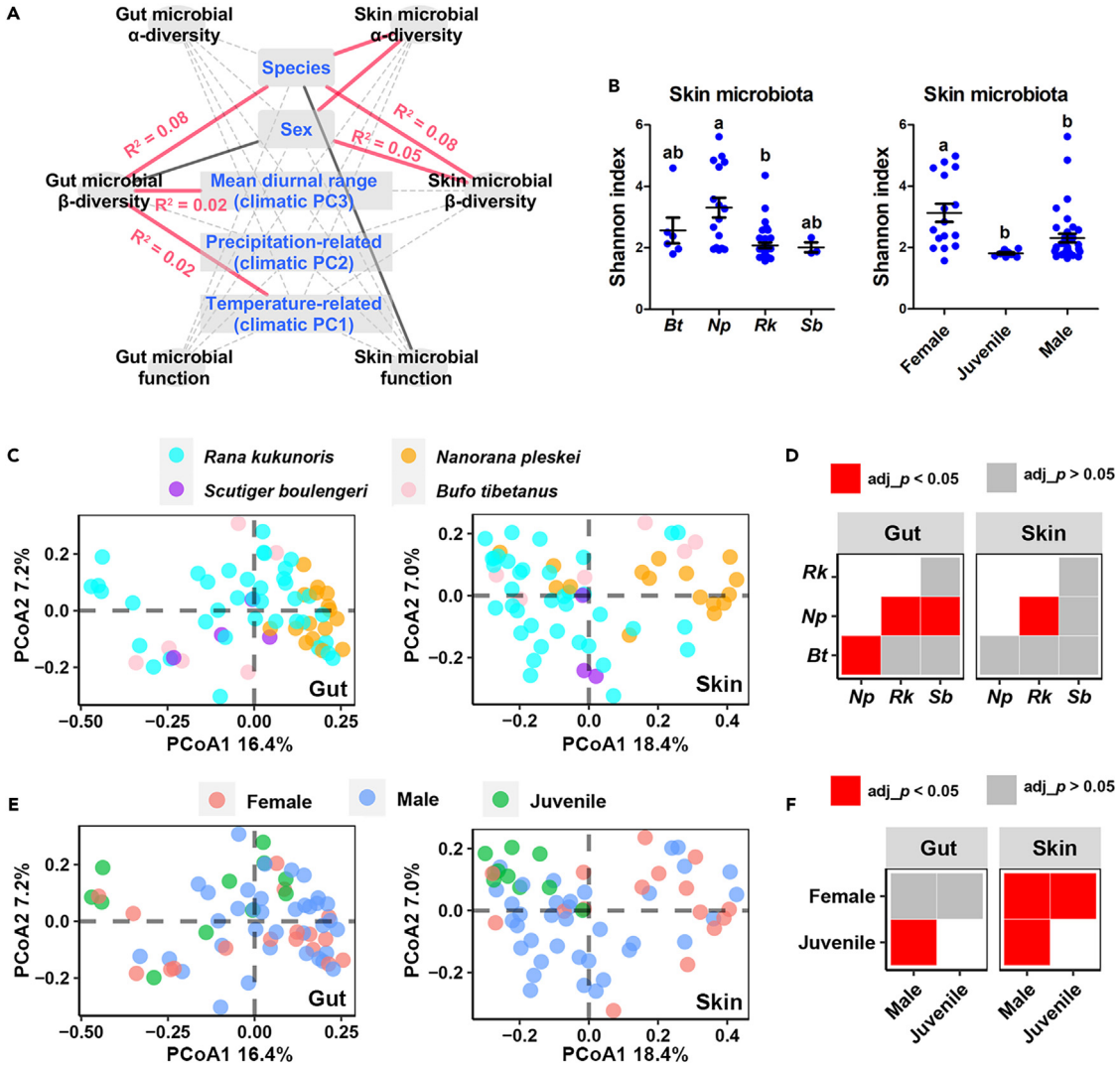


Figure 4. Variations in amphibian gut and skin microbiota with host factors

(A) A network summarizing the associations between climatic indices and microbial diversity or function. Red, black, and gray-dashed colors of edges denote significant ($p < 0.05$), marginally significant ($0.05 < p < 0.1$), and insignificant associations ($p > 0.1$), respectively. Associations of host and climatic factors with microbial alpha-diversity, beta-diversity, and functions were analyzed with mixed linear models (climatic grids as random effects), PERMANOVA (unweighted UniFrac distance), and PERMANOVA (Bray-Curtis distances), respectively.

(B) Variations in amphibian microbiota with host factors (i.e., species and sex). Different letters denote significant differences between groups ($p < 0.05$, LSD post-hoc tests following mixed linear model).

(C) PCoA scatterplot showing the variation in microbial beta-diversity with host species.

(D) Pairwise comparison in microbial beta-diversity between species (pairwise PERMANOVA and BH correction).

(E) PCoA scatterplot showing the variation in microbial beta-diversity with host sex.

(F) Pairwise comparison in microbial beta-diversity between sexes (pairwise PERMANOVA and BH correction).

Given the significant role of water microbiota as a source for the host-associated microbiota, we constructed a SEM to explore whether climatic-dependent variations in water microbiota might mediate potential associations between climatic factors and *R. kukunoris* microbiota (Figure 6A). Results indicated that temperature-related climatic factor and mean diurnal range were significant predictors for the variations in beta-diversity of water microbiota, which subsequently influenced the variations in beta-diversity of *R. kukunoris* gut and skin microbiota (Figure 6B).

DISCUSSION

Recognizing the crucial role of commensal microbiota in animal health and environmental adaptation, understanding the factors shaping their community structure is vital for animal conservation in the context of climate change. Currently, there is a scarcity of studies exploring the

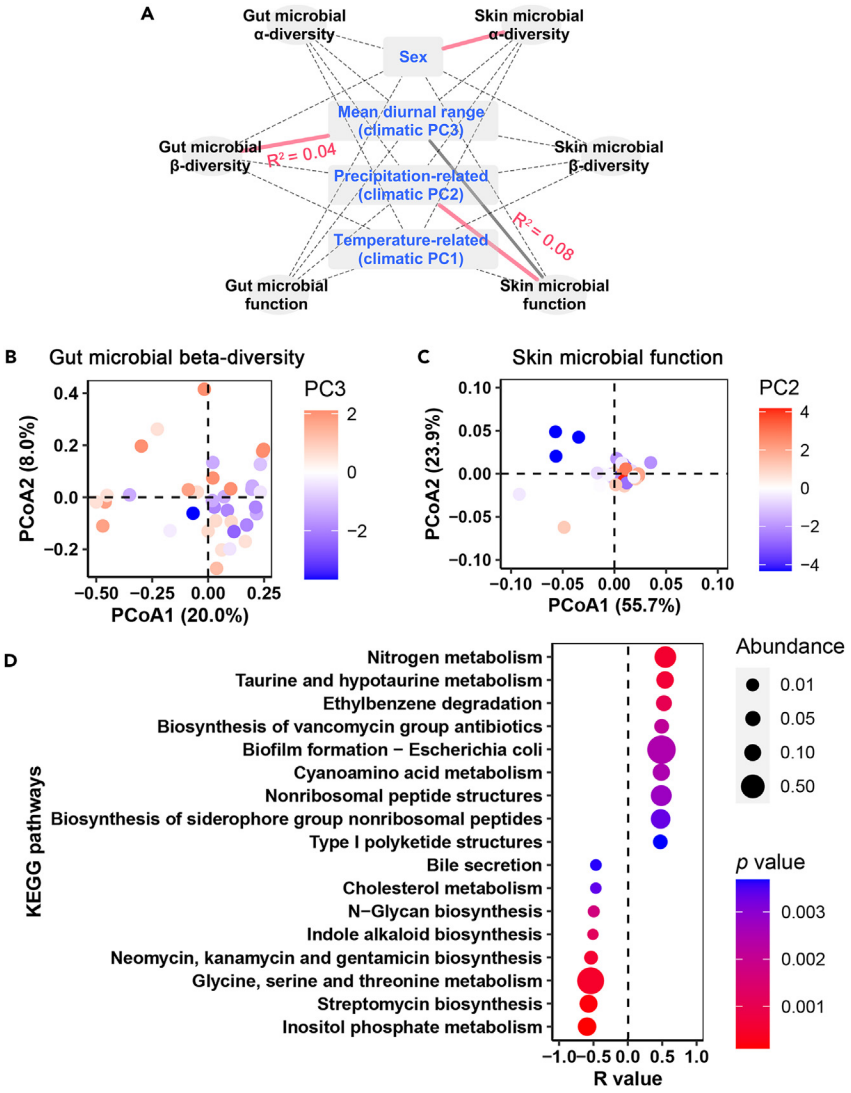


Figure 5. Variations in *R. kukunoris* gut and skin microbiota with climatic factors

(A) A network summarizing the associations between climatic indices and microbial diversity or function. The red, black, and gray-dashed colors of edges denote significant ($p < 0.05$), marginally significant ($0.05 < p < 0.1$), and insignificant associations ($p > 0.1$), respectively. The associations of host and climatic factors with microbial alpha-diversity, beta-diversity, and functions were analyzed with mixed linear models (climatic grids as random effects), PERMANOVA (unweighted UniFrac distance), and PERMANOVA (Bray-Curtis distances), respectively.

(B) PCoA scatterplot depicting the variation in gut microbial beta-diversity with mean diurnal range (climatic PC3).

(C) PCoA scatterplot illustrating the variation in skin microbial function with precipitation-related climatic factor (PC2).

(D) Skin microbial functions significantly correlated ($p < 0.005$ in both Pearson and Spearman correlations) with precipitation-related climatic factor.

potential links between climatic factors and host-associated microbiota, and our understanding of the ecological processes involved is limited. This study investigates the factors influencing the assembly of host-associated microbiota in mountain-dwelling amphibians, with a specific focus on the role of climatic factors in driving microbial variations. The findings support our two hypotheses: host and climatic factors jointly shape the community structure of commensal microbiota, and climate-driven variations in environmental microbiota mediate associations between climatic factors and host-associated microbiota. This research underscores the need for a comprehensive approach considering both host-specific and environmental factors in conservation strategies, particularly in the face of climate change.

Host factors emerged as the primary determinant shaping the diversity of gut and skin microbiota. There were prominent differences in the composition and function between skin and gut microbiota of mountain-dwelling amphibians. This suggested that the body site (e.g., gut and skin), from which the microbiota were sampled, was a major factor influencing the community structure of host-associated microbiota.[14] Analogous to low-elevation amphibian species,[45,53] Firmicutes, Proteobacteria, and Bacteroidetes stood out as the dominant bacterial phyla in the gut, whereas Proteobacteria constituted a remarkable high proportion in the skin microbiota. The gut microbiota of these species

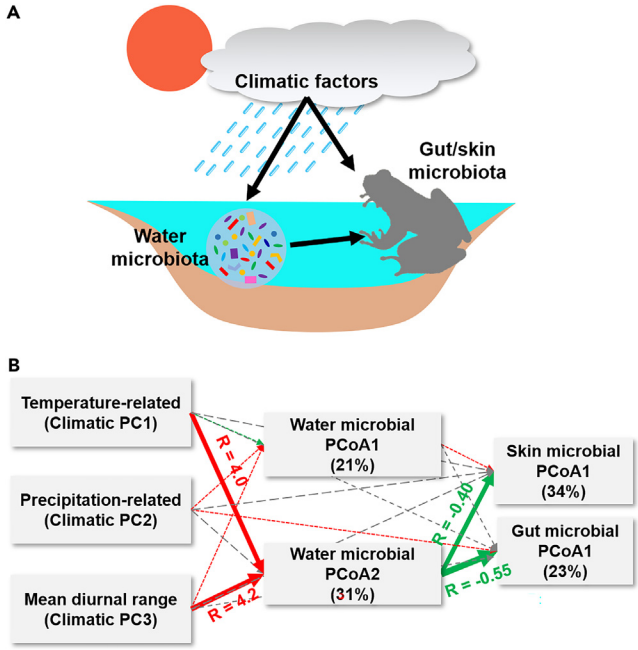


Figure 6. Water microbiota mediated associations between *R. kukunoris* microbiota and climatic factors

(A) A schematic diagram illustrating the potential paths in our SEM. Climatic factors may be directly or indirectly linked to the host-associated microbiota, with water microbiota potentially acting as mediators.

(B) Simplified SEM presenting the roles of water microbiota in mediating the associations between climatic factors and *R. kukunoris* microbiota (AIC = 45.876, BIC = 73.262). The percentages below the items indicate the explanation rate, presenting the proportion of the total variance explained by the simplified model. Dashed gray paths were removed from the simplified model, while the red and green paths were retained, which denote positive and negative correlations, respectively. The solid paths met the specified significance level ($p < 0.05$) in the SEM, and their width reflects the absolute R value.

harbored abundant genes associated with primary metabolism, especially carbohydrate metabolism, while their skin microbiota featured terpene, terpenoid, and steroids biosynthesis. Terpene, terpenoid, and steroid derivatives serve diverse functions, including stress alleviation, maintenance of cell membrane integrity, photoprotection, attraction or repulsion of organisms, host growth promotion, and defense.[54] Consequently, the observed differences in composition and function between gut and skin microbiota align with their respective roles in host health; the gut microbiota play an important role in host digestion and nutrient absorption, whereas the skin microbiota function as a barrier in pathogen defense and UV protection.[49,55,56] Given the intense UV stress in the mountain environments and the exposed skin of amphibians, the skin microbiota might play a crucial role in the adaption of mountain-dwelling amphibians to high altitudes.

When we focused on a specific type of microbiota, such as gut or skin microbiota, host species emerged as the primary factor accounting for variations in their beta-diversity. This finding is logical, as host species in this study likely reflect both the host's phylogeny and diet preferences, two well-known drivers of host-associated microbial community composition.[57,58] Interestingly, despite the significant interspecies variations in the beta-diversity of gut and skin microbiota, their functions did not significantly differ across species. This suggests that the microbiota of mountain-dwelling amphibians perform common and essential functions. Host species was also a significant factor explaining variations in the alpha-diversity and function redundancy of skin microbiota, but not gut microbiota. Skin microbiota of *R. kukunoris* and *S. boulengeri* exhibited relatively lower alpha-diversity and functional redundancy than that of *N. pleskei*, indicating that the functional stability of their skin microbiota might be more susceptible to disruption. Additionally, the host's sex played a significant role in explaining variations in both alpha- and beta-diversity of skin microbiota. Juveniles exhibited the lowest alpha-diversity in their skin microbiota and lacked some common bacteria found in adults. We speculated that juveniles might need exposure to more diverse environments[59] or direct skin contact with adults, such as mating after reaching sexual maturity,[60] to acquire these missing microbes. Regardless, the skin microbiota of amphibian juveniles appeared underdeveloped and potentially vulnerable to external disturbances. The species- and sex-depended variations in amphibian microbiota highlighted the importance of considering both taxonomy and ontogeny when evaluating the impacts of environmental changes on high-altitude amphibians.[61]

In the case of a particular species, individuals from different geographic region always exhibit differences in their commensal microbiota.[62,63] However, whether these variations are partly driven by the climatic factors has been rarely explored. In this study, we analyzed the microbiota of field amphibians, and found that climatic factors accounted for additional variations in their commensal microbiota beyond those explained by the host factors, suggesting that host and climatic factors jointly shaped the microbiota of mountain-dwelling amphibians. This aligns with our earlier investigations conducted on laboratory-acclimated lizards collected from various geographic sites.[64,65] Kueneman et al.[30] reported that amphibian skin alpha-diversity was consistently correlated with temperature-associated climatic factors. This diverged

from our observations, possibly owing to the fact that our species were from a mountainous area and that the two studies were conducted at different geographic levels. Another study conducted by Woodhams et al.,[28] encompassing multiple animal clades such as mammal, amphibian, fish, and insect, suggested that digestive-associated microbiomes are primarily explained by variations in climatic factors, whereas skin-associated microbiomes are explained by host factors such as phylogeny/immune complexity and trophic level/diet, plus climate. This finding aligns with our observations related to microbial function, although it contrasts with our results regarding alpha- and beta-diversity. These complexities highlight that the influences of climatic factors on host-associated microbiota are multifaceted and may depend on animal clades and geographic scales.

It was interesting to find that precipitation-related climatic PC was associated with functional variations in *R. kukunoris* skin microbiota. This climatic factor was correlated with the abundances of genes involved in various antibiotics biosynthesis processes, including streptomycin, vancomycin, neomycin, kanamycin, and gentamicin, and non-ribosomal peptides. These microbial small-molecule products exhibit antibiotic activities.[66] Given that precipitation is potentially linked to the spread and prevalence of amphibian pathogens,[67,68] the climate-dependent variations in antibiotics biosynthesis within skin microbiota might play a role in environmental adaptation of amphibians. To test this hypothesis, further studies could focus on examining trends in environmental pathogens and skin microbial functions along an annual precipitation gradient.

Our results indicated that climatic factors drove the variations in the beta-diversity of environmental microbiota. These results align with previous research, suggesting that soil and water microbial taxa can respond to changes in temperature and water availability,[69–71] thereby mediating the impacts of climate changes on the biogeochemical cycling and plant growth.[72,73] Notably, water microbes emerged as a significant source of the host-associated microbiota in mountain-dwelling amphibians, while soil microbiota did not, despite both being in contact with amphibian skin. This suggested that host-associated microbiota were likely under selective pressure, potentially exerted by the host factors. Our results indicated that the climate-dependent variations in water microbiota might mediate an indirect association between climatic factors and amphibian microbiota. This understanding provides a mechanistic perspective on the alignment between host-associated microbiota and the local climate, deepening our comprehension of the impact of climate change on mountain ecosystems by offering a systematic view on the relationships between different ecological elements.

Conclusion

Host-associated microbiota of mountain-dwelling amphibians vary with both host and climatic factors, making it essential to consider these factors when discussing animal resistance and susceptibility to climate change. Environmental microbiota, being a major source of host-associated microbiomes, play a crucial role in mediating the associations between climatic factors and host-associated microbiota. This indirect association offers a mechanistic understanding of the alignment between host-associated microbiota and the local climate. Identifying the relationships among different ecological elements in habitats is meaningful for presenting a systematic perspective on the assembly of host-associated microbiota in amphibians, and it may also provide valuable insights into the environmental adaptation of animals.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL
  - Animals
- METHOD DETAILS
  - Sample collection and environmental information
  - 16S rRNA gene amplicon sequencing
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.109907.

ACKNOWLEDGMENTS

This work was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (STEP, Grant No. 2019QZKK05010503); National Natural Science Foundation of China (31900327), and China Biodiversity Observation Networks (Sino BON or Sino BON – Amphibian & Reptile).

AUTHOR CONTRIBUTIONS

W.Z.: Conceptualization, Investigation, Writing - original draft, Writing - review and editing, Funding acquisition, Project administration, Resources. L.C.: Investigation, Writing - original draft, Writing - review and editing, Resources. M.Z.: Investigation, Resources. Q.C.: Investigation, Resources. L.S.: Investigation, Resources. C.S.: Investigation, Resources. J.J.: Conceptualization, Investigation, Writing - review and editing, Funding acquisition, Project administration.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: July 12, 2023
Revised: November 30, 2023
Accepted: May 2, 2024
Published: May 3, 2024

REFERENCES

1. Rooks, M.G., and Garrett, W.S. (2016). Gut microbiota, metabolites and host immunity. Nat. Rev. Immunol. *16*, 341–352. https://doi.org/10.1038/nri.2016.42.
2. Hooper, L.V., Littman, D.R., and Macpherson, A.J. (2012). Interactions Between the Microbiota and the Immune System. Science *336*, 1268–1273. https://doi.org/10.1126/science.1223490.
3. Sonnenburg, J.L., and Bäckhed, F. (2016). Diet-microbiota interactions as moderators of human metabolism. Nature *535*, 56–64. https://doi.org/10.1038/nature18846.
4. Tremaroli, V., and Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. Nature *489*, 242–249. https://doi.org/10.1038/nature11552.
5. Zhang, Z., Xu, D., Wang, L., Hao, J., Wang, J., Zhou, X., Wang, W., Qiu, Q., Huang, X., Zhou, J., et al. (2016). Convergent evolution of rumen microbiomes in high-altitude mammals. Curr. Biol. *26*, 1873–1879. https://doi.org/10.1016/j.cub.2016.05.012.
6. Mohamed, A.R., Ochsenkühn, M.A., Kazlak, A.M., Moustafa, A., and Amin, S.A. (2023). The coral microbiome: towards an understanding of the molecular mechanisms of coral–microbiota interactions. FEMS Microbiol. Rev. *47*, fuad005. https://doi.org/10.1093/femsre/fuad005.
7. Littleford-Colquhoun, B.L., Weyrich, L.S., Kent, N., and Frere, C.H. (2019). City life alters the gut microbiome and stable isotope profiling of the eastern water dragon (*Intellagama lesueurii*). Mol. Ecol. *28*, 4592–4607. https://doi.org/10.1111/mec.15240.
8. Baldassarre, L., Ying, H., Reitzel, A.M., Franzenburg, S., and Fraune, S. (2022). Microbiota mediated plasticity promotes thermal adaptation in the sea anemone *Nematostella vectensis*. Nat. Commun. *13*, 3804. https://doi.org/10.1038/s41467-022-31350-z.
9. Wei, F., Wu, Q., Hu, Y., Huang, G., Nie, Y., and Yan, L. (2019). Conservation metagenomics: a new branch of conservation biology. Sci. China Life Sci. *62*, 168–178. https://doi.org/10.1007/s11427-018-9423-3.
10. Huang, G., Qi, D., Yang, Z., Hou, R., Shi, W., Zhao, F., Li, Z., Yan, L., and Wei, F. (2024). Gut microbiome as a key monitoring indicator for reintroductions of captive animals. Conserv. Biol. *38*, e14173. https://doi.org/10.1111/cobi.14173.
11. Hernández-Gómez, O., and Hua, J. (2023). From the organismal to biosphere levels: environmental impacts on the amphibian microbiota. FEMS Microbiol. Rev. *47*, fuad002. https://doi.org/10.1093/femsre/fuad002.
12. Faith, J.J., Mcnulty, N.P., Rey, F.E., and Gordon, J.I. (2011). Predicting a Human Gut Microbiota's Response to Diet in Gnotobiotic Mice. Science *333*, 101–104.
13. David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature *505*, 559–563. https://doi.org/10.1038/nature12820.
14. Minich, J.J., Härer, A., Vechinski, J., Frable, B.W., Skelton, Z.R., Kunselman, E., Shane, M.A., Perry, D.S., Gonzalez, A., McDonald, D., et al. (2022). Host biology, ecology and the environment influence microbial biomass and diversity in 101 marine fish species. Nat. Commun. *13*, 6978. https://doi.org/10.1038/s41467-022-34557-2.
15. Minich, J.J., Petrus, S., Michael, J.D., Michael, T.P., Knight, R., and Allen, E.E. (2020). Temporal, Environmental, and Biological Drivers of the Mucosal Microbiome in a Wild Marine Fish, Scomber japonicus. mSphere *5*, e00401–e00420. https://doi.org/10.1128/mSphere.00401-20.
16. Tong, Q., Cui, L.Y., Hu, Z.F., Du, X.P., Abid, H.M., and Wang, H.B. (2020). Environmental and host factors shaping the gut microbiota diversity of brown frog *Rana dybowskii*. Sci. Total Environ. *741*, 140142. https://doi.org/10.1016/j.scitotenv.2020.140142.
17. Bernardo-Cravo, A.P., Schmeller, D.S., Chatzinotas, A., Vredenburg, V.T., and Loyau, A. (2020). Environmental factors and host microbiomes shape host–pathogen dynamics. Trends Parasitol. *36*, 616–633. https://doi.org/10.1016/j.pt.2020.04.010.
18. Davenport, E.R. (2020). Genetic Variation Shapes Murine Gut Microbiota via Immunity. Trends Immunol. *41*, 1–3. https://doi.org/10.1016/j.it.2019.11.009.
19. Khan, A.A., Yurkovetskiy, L., O'Grady, K., Pickard, J.M., de Pooter, R., Antonopoulos, D.A., Golovkina, T., and Chervonsky, A. (2019). Polymorphic Immune Mechanisms Regulate Commensal Repertoire. Cell Rep. *29*, 541–550.e4. https://doi.org/10.1016/j.celrep.2019.09.010.
20. Adams, N.E., Becker, M.A., and Edmands, S. (2021). Effect of Geography and Captivity on Scat Bacterial Communities in the Imperiled Channel Island Fox. Front. Microbiol. *12*, 748323. https://doi.org/10.3389/fmicb.2021.748323.
21. Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'Connor, M.P., Rosen, G.L., Knight, R., Kilham, S.S., and Russell, J.A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol. Ecol. *21*, 3363–3378. https://doi.org/10.1111/j.1365-294X.2012.05552.x.
22. Greenspan, S.E., Peloso, P., Fuentes-González, J.A., Bletz, M., Lyra, M.L., Machado, I.F., Martins, R.A., Medina, D., Moura-Campos, D., Neely, W.J., et al. (2022). Low microbiome diversity in threatened amphibians from two biodiversity hotspots. Anim. Microbiome *4*, 69. https://doi.org/10.1186/s42523-022-00220-w.
23. Zhu, L., Zhu, W., Zhao, T., Chen, H., Zhao, C., Xu, L., Chang, Q., and Jiang, J. (2021). Environmental temperatures affect the gastrointestinal microbes of the Chinese giant salamander. Front. Microbiol. *12*, 543767. https://doi.org/10.3389/fmicb.2021.543767.
24. Greenspan, S.E., Migliorini, G.H., Lyra, M.L., Pontes, M.R., Carvalho, T., Ribeiro, L.P., Moura-Campos, D., Haddad, C.F.B., Toledo, L.F., Romero, G.Q., and Becker, C.G. (2020). Warming drives ecological community changes linked to host-associated microbiome dysbiosis. Nat. Clim. Chang. *10*, 1057–1061. https://doi.org/10.1038/s41558-020-0899-5.
25. Li, J., Bates, K.A., Hoang, K.L., Hector, T.E., Knowles, S.C.L., and King, K.C. (2023). Experimental temperatures shape host microbiome diversity and composition. Glob. Chang. Biol. *29*, 41–56. https://doi.org/10.1111/gcb.16429.
26. Longo, A.V., and Zamudio, K.R. (2017). Temperature variation, bacterial diversity and fungal infection dynamics in the amphibian skin. Mol. Ecol. *26*, 4787–4797. https://doi.org/10.1111/mec.14220.
27. Kohl, K.D., and Yahn, J. (2016). Effects of environmental temperature on the gut microbial communities of tadpoles. Environ. Microbiol. *18*, 1561–1565. https://doi.org/10.1111/1462-2920.13255.
28. Woodhams, D.C., Bletz, M.C., Becker, C.G., Bender, H.A., Buitrago-Rosas, D., Diebboll,

H., Huynh, R., Kearns, P.J., Kueneman, J., Kurosawa, E., et al. (2020). Host-associated microbiomes are predicted by immune system complexity and climate. Genome Biol. *21*, 23. https://doi.org/10.1186/s13059-019-1908-8.

29. Schmeller, D.S., Cheng, T., Shelton, J., Lin, C.F., Chan-Alvarado, A., Bernardo-Cravo, A., Zoccarato, L., Ding, T.S., Lin, Y.P., Swei, A., et al. (2022). Environment is associated with chytrid infection and skin microbiome richness on an amphibian rich island (Taiwan). Sci. Rep. *12*, 16456. https://doi.org/10.1038/s41598-022-20547-3.
30. Kueneman, J.G., Bletz, M.C., McKenzie, V.J., Becker, C.G., Joseph, M.B., Abarca, J.G., Archer, H., Arellano, A.L., Bataille, A., Becker, M., et al. (2019). Community richness of amphibian skin bacteria correlates with bioclimate at the global scale. Nat. Ecol. Evol. *3*, 381–389. https://doi.org/10.1038/s41559-019-0798-1.
31. Williams, C.E., Williams, C.L., and Logan, M.L. (2023). Climate change is not just global warming: Multidimensional impacts on animal gut microbiota. Microb. Biotechnol. *16*, 1736–1744. https://doi.org/10.1111/1751-7915.14276.
32. Jansson, J.K., and Hofmockel, K.S. (2020). Soil microbiomes and climate change. Nat. Rev. Microbiol. *18*, 35–46. https://doi.org/10.1038/s41579-019-0265-7.
33. Freeman, C., Gresswell, R., Guasch, H., Hudson, J., Lock, M.A., Reynolds, B., Sabater, F., and Sabater, S. (1994). The role of drought in the impact of climatic change on the microbiota of peatland streams. Freshw. Biol. *32*, 223–230. https://doi.org/10.1111/j.1365-2427.1994.tb00878.x.
34. Minich, J.J., Morris, M.M., Brown, M., Doane, M., Edwards, M.S., Michael, T.P., and Dinsdale, E.A. (2018). Elevated temperature drives kelp microbiome dysbiosis, while elevated carbon dioxide induces water microbiome disruption. PLoS One *13*, e0192772. https://doi.org/10.1371/journal.pone.0192772.
35. Zhu, W., Lv, Y., Zhang, Q.D., Chang, L.M., Chen, Q.H., Wang, B., and Jiang, J.P. (2023). Cascading effects of Pb on the environmental and symbiotic microbiota and tadpoles' physiology based on field data and laboratory validation. Sci. Total Environ. *862*, 160817. https://doi.org/10.1016/j.scitotenv.2022.160817.
36. Xu, L., Xiang, M., Zhu, W., Zhang, M., Chen, H., Huang, J., Chen, Y., Chang, Q., Jiang, J., and Zhu, L. (2020). The behavior of amphibians shapes their symbiotic microbiomes. mSystems *5*, e00626-20. https://doi.org/10.1128/mSystems.00626-20.
37. Hyde, E.R., Navas-Molina, J.A., Song, S.J., Kueneman, J.G., Ackermann, G., Cardona, C., Humphrey, G., Boyer, D., Weaver, T., Mendelson, J.R., et al. (2016). The Oral and Skin Microbiomes of Captive Komodo Dragons Are Significantly Shared with Their Habitat. mSystems *1*, e00046. https://doi.org/10.1128/mSystems.00046-16.
38. Carey, C., and Alexander, M.A. (2003). Climate change and amphibian declines: is there a link? Divers. Distrib. *9*, 111–121. https://doi.org/10.1046/j.1472-4642.2003.00011.x.
39. Blaustein, A.R., Walls, S.C., Bancroft, B.A., Lawler, J.J., Searle, C.L., and Gervasi, S.S. (2010). Direct and Indirect Effects of Climate Change on Amphibian Populations. Diversity *2*, 281–313. https://doi.org/10.3390/d2020281.
40. Luedtke, J.A., Chanson, J., Neam, K., Hobin, L., Maciel, A.O., Catenazzi, A., Borzée, A., Hamidy, A., Aowphol, A., Jean, A., et al. (2023). Ongoing declines for the world's amphibians in the face of emerging threats. Nature *622*, 308–314. https://doi.org/10.1038/s41586-023-06578-4.
41. Semlitsch, R.D. (2008). Differentiating Migration and Dispersal Processes for Pond-Breeding Amphibians. J. Wildl. Manage. *72*, 260–267. https://doi.org/10.2193/2007-082.
42. Pittman, S.E., Osbourn, M.S., and Semlitsch, R.D. (2014). Movement ecology of amphibians: A missing component for understanding population declines. Biol. Conserv. *169*, 44–53. https://doi.org/10.1016/j.biocon.2013.10.020.
43. Tan, S., Li, P., Yao, Z., Liu, G., Yue, B., Fu, J., and Chen, J. (2021). Metabolic cold adaptation in the Asiatic toad: intraspecific comparison along an altitudinal gradient. J. Comp. Physiol. B *191*, 765–776. https://doi.org/10.1007/s00360-021-01381-x.
44. Yang, W., Qi, Y., and Fu, J. (2016). Genetic signals of high-altitude adaptation in amphibians: a comparative transcriptome analysis. BMC Genet. *17*, 134. https://doi.org/10.1186/s12863-016-0440-z.
45. Xu, L.L., Chen, H., Zhang, M., Zhu, W., Chang, Q., Lu, G., Chen, Y., Jiang, J., and Zhu, L. (2020). Changes in the community structure of the symbiotic microbes of wild amphibians from the eastern edge of the Tibetan Plateau. MicrobiologyOpen *9*, e1004. https://doi.org/10.1002/mbo3.1004.
46. Walker, D.M., Hill, A.J., Albecker, M.A., McCoy, M.W., Grisnik, M., Romer, A., Grajal-Puche, A., Camp, C., Kelehear, C., Wooten, J., et al. (2020). Variation in the Slimy Salamander (Plethodon spp.) Skin and Gut-Microbial Assemblages Is Explained by Geographic Distance and Host Affinity. Microb. Ecol. *79*, 985–997. https://doi.org/10.1007/s00248-019-01456-x.
47. Song, X., Zhang, J., Song, J., and Zhai, Y. (2021). Decisive Effects of Life Stage on the Gut Microbiota Discrepancy Between Two Wild Populations of Hibernating Asiatic Toads (Bufo gargarizans). Front. Microbiol. *12*, 665849. https://doi.org/10.3389/fmicb.2021.665849.
48. Hernandez-Gomez, O., and Hua, J. (2023). From the organismal to biosphere levels: environmental impacts on the amphibian microbiota. FEMS Microbiol. Rev. *47*. https://doi.org/10.1093/femsre/fuad002.
49. Souak, D., Barreau, M., Courtois, A., André, V., Duclairoir Poc, C., Feuilloley, M.G.J., and Gault, M. (2021). Challenging Cosmetic Innovation: The Skin Microbiota and Probiotics Protect the Skin from UV-Induced Damage. Microorganisms *9*, 936. https://doi.org/10.3390/microorganisms9050936.
50. Herzschuh, U., Birks, H.J.B., Jian, N., Yan, Z., Hongyan, L., Xingqi, L., and Grosse, G. (2009). Holocene land-cover changes on the Tibetan Plateau. Holocene *20*, 91–104. https://doi.org/10.1177/0959683609348882.
51. Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A., and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature *403*, 853–858. https://doi.org/10.1038/35002501.
52. Favre, A., Päckert, M., Pauls, S.U., Jähnig, S.C., Uhl, D., Michalak, I., and Muellner-Riehl, A.N. (2015). The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. Biol. Rev. *90*, 236–253. https://doi.org/10.1111/brv.12107.
53. Tong, Q., Hu, Z.F., Du, X.P., Bie, J., and Wang, H.B. (2020). Effects of Seasonal Hibernation on the Similarities Between the Skin Microbiota and Gut Microbiota of an Amphibian (*Rana dybowskii*). Microb. Ecol. *79*, 898–909. https://doi.org/10.1007/s00248-019-01466-9.
54. Avalos, M., Garbeva, P., Vader, L., van Wezel, G.P., Dickschat, J.S., and Ulanova, D. (2022). Biosynthesis, evolution and ecology of microbial terpenoids. Nat. Prod. Rep. *39*, 249–272. https://doi.org/10.1039/d1np00047k.
55. Bletz, M.C., Kelly, M., Sabino-Pinto, J., Bales, E., Van Praet, S., Bert, W., Boyen, F., Vences, M., Steinfartz, S., Pasmans, F., and Martel, A. (2018). Disruption of skin microbiota contributes to salamander disease. Proc. Biol. Sci. *285*, 20180758. https://doi.org/10.1098/rspb.2018.0758.
56. Woodhams, D.C., Bletz, M., Kueneman, J., and McKenzie, V. (2016). Managing Amphibian Disease with Skin Microbiota. Trends Microbiol. *24*, 161–164. https://doi.org/10.1016/j.tim.2015.12.010.
57. Youngblut, N.D., Reischer, G.H., Walters, W., Schuster, N., Walzer, C., Stalder, G., Ley, R.E., and Farnleitner, A.H. (2019). Host diet and evolutionary history explain different aspects of gut microbiome diversity among vertebrate clades. Nat. Commun. *10*, 2200. https://doi.org/10.1038/s41467-019-10191-3.
58. Amato, K.R., G Sanders, J., Song, S.J., Nute, M., Metcalf, J.L., Thompson, L.R., Morton, J.T., Amir, A., J McKenzie, V., Humphrey, G., et al. (2019). Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. ISME J. *13*, 576–587. https://doi.org/10.1038/s41396-018-0175-0.
59. Walke, J.B., Becker, M.H., Loftus, S.C., House, L.L., Cormier, G., Jensen, R.V., and Belden, L.K. (2014). Amphibian skin may select for rare environmental microbes. ISME J. *8*, 2207–2217.
60. Smith, C.C., and Mueller, U.G. (2015). Sexual transmission of beneficial microbes. Trends Ecol. Evol. *30*, 438–440.
61. Komoroske, L.M., Connon, R.E., Lindberg, J., Cheng, B.S., Castillo, G., Hasenbein, M., and Fangue, N.A. (2014). Ontogeny influences sensitivity to climate change stressors in an endangered fish. Conserv. Physiol. *2*, cou008. https://doi.org/10.1093/conphys/cou008.
62. Zouache, K., Raharimalala, F.N., Raquin, V., Tran-Van, V., Raveloson, L.H.R., Ravelonandro, P., and Mavingui, P. (2011). Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. FEMS Microbiol. Ecol. *75*, 377–389. https://doi.org/10.1111/j.1574-6941.2010.01012.x.
63. Huang, S., and Zhang, H. (2013). The Impact of Environmental Heterogeneity and Life Stage on the Hindgut Microbiota of *Holotrichia parallela* Larvae (Coleoptera: Scarabaeidae). PLoS One *8*, e57169. https://doi.org/10.1371/journal.pone.0057169.
64. Zhu, W., Shi, X., Qi, Y., Wang, X., Chang, L., Zhao, C., Zhu, L., and Jiang, J. (2022). Commensal microbiota and host metabolic divergence are associated with the adaptation of Diploderma vela to spatially heterogeneous environments. Integrative Zoology *n/a*. Integr. Zool. *17*, 346–365. https://doi.org/10.1111/1749-4877.12590.

65. Zhu, W., Qi, Y., Wang, X., Shi, X., Chang, L., Liu, J., Zhu, L., and Jiang, J. (2022). Multi-omics approaches revealed the associations of host metabolism and gut microbiome with phylogeny and environmental adaptation in mountain dragons. Front. Microbiol. *13*, 913700. https://doi.org/10.3389/fmicb.2022.913700.
66. Walsh, C.T. (2004). Polyketide and Nonribosomal Peptide Antibiotics: Modularity and Versatility. Science *303*, 1805–1810. https://doi.org/10.1126/science.1094318.
67. Kolby, J.E., Ramirez, S.D., Berger, L., Griffin, D.W., Jocque, M., and Skerratt, L.F. (2015). Presence of amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) in rainwater suggests aerial dispersal is possible. Aerobiologia *31*, 411–419. https://doi.org/10.1007/s10453-015-9374-6.
68. Ron, S.R. (2005). Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the New World 1. Biotropica *37*, 209–221.
69. Bell, C.W., Acosta-Martinez, V., McIntyre, N.E., Cox, S., Tissue, D.T., and Zak, J.C. (2009). Linking Microbial Community Structure and Function to Seasonal Differences in Soil Moisture and Temperature in a Chihuahuan Desert Grassland. Microb. Ecol. *58*, 827–842. https://doi.org/10.1007/s00248-009-9529-5.
70. Bell, C., McIntyre, N., Cox, S., Tissue, D., and Zak, J. (2008). Soil Microbial Responses to Temporal Variations of Moisture and Temperature in a Chihuahuan Desert Grassland. Microb. Ecol. *56*, 153–167. https://doi.org/10.1007/s00248-007-9333-z.
71. Brandão, J., Weiskerger, C., Valério, E., Pitkänen, T., Meriläinen, P., Avolio, L., Heaney, C.D., and Sadowsky, M.J. (2022). Climate Change Impacts on Microbiota in Beach Sand and Water: Looking Ahead. Int. J. Environ. Res. Publ. Health *19*, 1444. https://doi.org/10.3390/ijerph19031444.
72. Dubey, A., Malla, M.A., Khan, F., Chowdhary, K., Yadav, S., Kumar, A., Sharma, S., Khare, P.K., and Khan, M.L. (2019). Soil microbiome: a key player for conservation of soil health under changing climate. Biodivers. Conserv. *28*, 2405–2429. https://doi.org/10.1007/s10531-019-01760-5.
73. Sheik, C.S., Beasley, W.H., Elshahed, M.S., Zhou, X., Luo, Y., and Krumholz, L.R. (2011). Effect of warming and drought on grassland microbial communities. ISME J. *5*, 1692–1700. https://doi.org/10.1038/ismej.2011.32.
74. Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics *30*, 2114–2120.
75. Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics *27*, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.
76. Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods *10*, 996–998. https://doi.org/10.1038/nmeth.2604.
77. Edgar, R.C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics *26*, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.
78. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. *41*, D590–D596. https://doi.org/10.1093/nar/gks1219.
79. Wemheuer, F., Taylor, J.A., Daniel, R., Johnston, E., Meinicke, P., Thomas, T., and Wemheuer, B. (2020). Tax4Fun2: prediction of habitat-specific functional profiles and functional redundancy based on 16S rRNA gene sequences. Environ. Microbiome *15*, 11. https://doi.org/10.1186/s40793-020-00358-7.
80. R Core Team (2019). R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing).
81. Wickham, H. (2009). Ggplot2: Elegant Graphics for Data Analysis (Springer Publishing Company, Incorporated).
82. Knights, D., Kuczynski, J., Charlson, E.S., Zaneveld, J., Mozer, M.C., Collman, R.G., Bushman, F.D., Knight, R., and Kelley, S.T. (2011). Bayesian community-wide culture-independent microbial source tracking. Nat. Methods *8*, 761–763. https://doi.org/10.1038/nmeth.1650.
83. Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., et al. (2020). Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. *18*, e3000411. https://doi.org/10.1371/journal.pbio.3000411.
84. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011). Guide for the Care and Use of Laboratory Animals, 8th edition (National Academies Press (US)). https://doi.org/10.17226/12910.
85. Martin, M. (2011). CUTADAPT removes adapter sequences from high-throughput sequencing reads. EMBnet J. *17*, 10–12. https://doi.org/10.14806/ej.17.1.200.
86. Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. Nat. Methods *7*, 335–336. https://doi.org/10.1038/nmeth.f.303.
87. Dixon, P. (2003). VEGAN, a package of R functions for community ecology. J. Veg. Sci. *14*, 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x.
88. Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., and Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. Genome Biol. *12*, R60. https://doi.org/10.1186/gb-2011-12-6-r60.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Four amphibian species including *Bufo tibetanus*, *Rana kukunoris*, *Scutiger boulengeri*, and *Nanorana pleskei*	Collected in Aug. 2020 at the eastern edge of the Tibetan Plateau	N/A
Water and soil microbiota of four amphibian species' habitats	Collected in Aug. 2020 at the eastern edge of the Tibetan Plateau	N/A
Host-associated microbiota of four amphibian species (i.e., gut and skin)	Collected in Aug. 2020 at the eastern edge of the Tibetan Plateau	N/A
Critical commercial assays		
QIAamp DNA Stool Mini kit	Qiagen	N/A
Qubit dsDNA HS Assay Kit	Life Technologies, Carlsbad, CA, United States	N/A
MagicPure Size Selection DNA Beads	TransGen Biotech, Beijing, China	N/A
Deposited data		
Sequencing data and relevant files	This paper	Genome Sequence Archive: [CRA011106]
Software and algorithms		
Lima v1.7.0	N/A	https://github.com/pacificbiosciences/barcoding
Cutadapt 1.9.1	Marcel Martin	https://cutadapt.readthedocs.io/en/stable/
Trimmomatic 0.33	Bolger et al.[74]	https://github.com/timflutre/trimmomatic
UCHIME 8.0	Edgar et al.[75]	http://drive5.com/uchime
USEARCH v10.0	Edgar[76,77]	http://www.drive5.com/usearch/
QIIME 1.9	Bolyen et al.	https://qiime.wordpress.com/
SILVA132	Quast et al.[78]	https://www.arb-silva.de/
Tax4Fun2	Wemheuer et al.[79]	https://doi.org/10.1101/490037
IBM SPSS v21.0	IBM, Armonk, NY, USA	https://www.ibm.com/cn-zh/spss
R[80]	R Core Team[80]	https://www.r-project.org
Cytoscape 3.5.0	Shannon et al.	http://cytoscape.org
Graphpad prism 5	GraphPad Software	https://www.graphpad.com
ggplot2	Wickham[81]	https://github.com/tidyverse/ggplot2
Source-Tracker 0.9.5	Knights et al.[82]	https://doi.org/10.1038/nmeth.16500.9.5
Mantel (vegan package in R)	N/A	https://github.com/vegandevs/vegan
Other		
Climatic factor data	This paper	WorldClim v 2.1 database

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Liming Chang, changlm@cib.ac.cn.

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Sequencing data and relevant files have been uploaded to Genome Sequence Archive (https://ngdc.cncb.ac.cn/gsub/): [CRA011106]. The other raw data have been uploaded to figshare: [https://doi.org/10.6084/m9.figshare.25650198.v1]. These data are publicly available as of the date of publication.
- This paper does not report original code.
- Any additional information required to reanalyse the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL

Animals

The samples were collected in Aug. 2020 at the eastern edge of the Tibetan Plateau (Figure 1A). The study area spans over 480 km, representing the largest distance between collection sites, and encompasses an elevational range of over 1,000 m. A total of 101 environmental samples (51 soil and 50 water) and 126 frog-associated microbial samples (64 gut and 62 skin samples from four species) were collected from 12 sampling sites (Figure 1B). For a given species, individuals with much smaller body size than the adults were classified as juveniles. To prevent artificial and cross-contamination, disposable plastic gloves were used during animal capture. All animal protocols in this study were reviewed and approved by the Animal Ethical and Welfare Committee of Chengdu Institute of Biology, Chinese Academy of Sciences (permit number: 2020-AR-JJP-01), in compliance with the ARRIVE guidelines 2.0[83] and Guide for the Care and Use of Laboratory Animals (8th edition) published by National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals.[84]

METHOD DETAILS

Sample collection and environmental information

The skin microbiota were sampled immediately upon captured, with each animal rinsed three times with sterile water to remove potential transient bacteria. Using three sterile swabs, we sampled the back, side, and abdomen of each animal, respectively.[45] These swabs were then transferred to 2 mL aseptic centrifuge tubes containing 1.5 mL 95% ethanol. Subsequently, the animals were euthanized with MS-222. The sex of the adults was determined after dissection. Gut contents were collected with sterile tweezers and transferred to 2 or 50 mL (depending on the animal's size) aseptic centrifuge tubes containing 95% ethanol. New gloves were used for each microbial sample of each individual. All the animals were collected near water bodies, and corresponding soil and water samples were also collected. Each geographical site may include multiple soil or water samples (Figure 1C). Each environmental sample was obtained by equally mixing soil or surface water collected from 3 to 5 points, selected using the equidistant sampling method along the banks of water bodies, with points located more than 1 m apart. For each point, the water and soil samples were collected approximately 1 m inward and outward from the water's edge, respectively. Additional soil and water sampling was skipped if the next animal was within 200 m of the last capture. Subsequent protocols for collection of environmental microbial samples followed the methods described previously.[45] Detailed sample information has been uploaded to figshare (https://doi.org/10.6084/m9.figshare.25650198.v1).

We recorded the longitude and latitude coordinates for each sample and obtained 19 corresponding climatic factors by querying the WorldClim v 2.1 database (at a resolution of 30 s). These factors included annual mean temperature (bio1), mean diurnal range (bio2), isothermality (bio3), temperature seasonality (bio4), maximum temperature of warmest month (bio5), minimum temperature of coldest month (bio6), temperature annual range (bio7), mean temperature of wettest quarter (bio8), mean temperature of driest quarter (bio9), mean temperature of warmest quarter (bio10), mean temperature of coldest quarter (bio11), annual precipitation (bio12), precipitation of wettest month (bio13), precipitation of driest month (bio14), precipitation seasonality (bio15), precipitation of wettest quarter (bio16), precipitation of driest quarter (bio17), precipitation of warmest quarter (bio18), and precipitation of coldest quarter (bio19). These data have been uploaded to figshare (https://doi.org/10.6084/m9.figshare.25650198.v1). To reduce the dimensions of the climate data, we conducted principal component analysis (PCA) to extract the first three principal components (PCs). These components accounted for 57.7%, 21.6%, and 16.1% of the total variance (accumulated explanation rate >95%; Figure S1). PC1 was primarily influenced by temperature-related factors (e.g., bio11, bio9, bio4, and bio9); PC2 by precipitation-related factors (e.g., bio13, bio16, and bio18); and PC3 by bio2. Thus, these three climatic PCs were named as temperature-related factor, precipitation-related factor, and mean diurnal range, respectively.

16S rRNA gene amplicon sequencing

The DNA extraction was performed using a QIAamp DNA Stool Mini kit (Qiagen) following the manufacturer's instructions. A DNA extraction (blank) control was included in the process. Nucleic acid integrity was verified through electrophoresis, and the concentration and purity of each DNA sample was determined using a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, United States). The entire 16S rRNA gene was amplified using primers 27F (AGRGTTTGATYNTGGCTCAG) and 1492R (TASGGHTACCTTGTTASGACTT) primers. PCR thermocycling conditions included an initial denaturation at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 60 s, with a final extension step at 72°C for 7 min. A DNA extraction (blank) control was also included for each PCR plate. PCR products were visualized on 1% agarose gels and quantified using a Nanodrop (Thermo Fisher Scientific, USA). A total of 227 samples meet the following criteria: (1) high DNA integrity; (2) DNA concentrations >10 ng/μL; (3) DNA total quality >100 ng. Blank controls showed no visible bands and had a DNA

amount of less than 1 ng/μL. The products were purified with MagicPure Size Selection DNA Beads (TransGen Biotech, Beijing, China). High-throughput sequencing was performed on a PacBio Sequel II platform by Mingke Biotechnology Co., Ltd. (Hangzhou, China). Circular consensus sequences (CCSs) were identified with lima v1.7.0, and primers were removed using Cutadapt 1.9.1 (identity ≥80% and coverage ≥80%).[85] Quality control was performed using Trimmomatic (version 0.33; sliding window, 50 bp),[74] and chimera was removed using UCHIME 8.0.[75] Sequences with >97% identity were considered operational taxonomic unit (OTU) using USEARCH v10.0.[76,77] The QIIME 1.9 pipeline was used to process the data and generate OTU tables.[86] Each OTU was classified by annotation against the SILVA132 database.[78] All the sequenced samples were remained, and sequencing depth ranged from 4,715 to 44,240 CCSs. The absolute abundance of OTUs was normalized to a standard sequence number, corresponding to the sample with the least sequences (4,715 in this study). Microbial alpha-diversity, beta-diversity, and functional diversity indices were calculated to assess the variations of microbiota (Figure 1D). The alpha-diversity (e.g., Shannon index) and dissimilarity matrices (e.g., unweighted UniFrac distances) were produced using the QIIME pipeline. Bacterial functions and functional redundancy of gut microbiota were predicted using Tax4Fun2.[79] This generated a table illustrating the relative abundance of each functional item in every sample. Subsequently, we converted this abundance table into a Bray-Curtis distance matrix using the vegan package in R.[87] This transformation aimed to facilitate the analysis of potential associations between factors and the functional diversity of microbiota, employing the same approach as applied to microbial beta-diversity (detailed below). The related data tables have been uploaded to figshare (https://doi.org/10.6084/m9.figshare.25650198.v1).

QUANTIFICATION AND STATISTICAL ANALYSIS

We conducted all the statistical analyses using IBM SPSS v21.0 (IBM, Armonk, NY, USA) and R.[80] Normality of the data was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests, and homoscedasticity across groups was evaluated using Levene's tests. Benjamini-Hochberg (BH) correction was applied to adjust p values of multiple comparison. Correlation networks were constructed using Cytoscape 3.5.0. Graphs were generated using Graphpad prism 5 and ggplot2.[81]

Initially, we characterized the microbial composition and functions of each microbiota type (soil, water, gut, and skin) and analyzed their potential associations. Alpha-diversity was analyzed with mixed linear models (fixed factors: sample types; random factors, the ID of each sample's collection site), followed by LSD post-hoc tests. The differences in beta-diversity between samples were assessed using permutational multivariate analysis of variance (PERMANOVA; factors, sample types; permutation, 9,999; sum of square, type II; based on unweighted UniFrac distances). Potential microbe transmission between environmental and host-associated microbiota was analyzed using SourceTracker 0.9.5.[82] Associations in the variations of alpha-diversity and beta-diversity between skin and gut samples from the same individuals were analyzed using Spearman correlation and Mantel (vegan package in R), respectively.[87] Principal coordinate analyses (PCoA, based on dissimilarity matrices) were employed to visualize beta-diversity dissimilarity. Differential microbial functions between microbiota types were identified using Kruskal-Wallis.

Subsequently, we examined the contributions of host and climatic factors to the variations in diversity and function of each type of microbiota. Alpha-diversity was analyzed using mixed linear models (fixed factors: host effects; covariates, climatic PC1−3; random factors, ID of climatic grids), followed by LSD post-hoc test. Influences of host and climatic factors on microbial beta-diversity (unweighted UniFrac distances) and functions (Bray-Curtis distances) were analyzed using PERMANOVA (factors: host factors and climatic PC1−3; sum of square: type II; permutation: 9,999). PCoA was used to visualize beta-diversity or microbial function dissimilarity. Further differential analyses were conducted for significant factors identified through PERMANOVA. Differential microbes between species or sexes were screened using Kruskal-Wallis tests (adjusted $p < 0.05$) and LEfSe ($p < 0.05$ and adjusted LDA >3).[88] We combined Pearson and Spearman correlations to screen the differential microbes or functions correlating with the climatic factors (PC1−3).

Finally, structural equation model (SEM) was constructed to explore whether the environmental microbiota potentially mediated the associations between climatic factors and amphibian microbial community structure. The first two principal coordinate axes (PCoA1 and PCoA2) were used to represent the overall variations in water microbiota beta-diversity (water PCoA1, 23.5%; water PCoA2, 7.4%). PCoA1 was used to represent the overall variations in gut (16.4%) and skin (18.4%) microbiota beta-diversity. In the SEM, each animal individual was considered as an observation, and its skin and gut microbiota corresponded one-to-one. The average values were calculated for the water or soil microbiota from the same climatic grids, to better present the overall environmental microbial diversity within each climatic grid. We hypothesized that climatic factors might be directly associated with the host-associated microbiota, or indirectly associated with them through influencing the environmental microbiota. The full models were simplified by removing non-significant paths until the Akaike information criterion (AIC) and Bayesian information criterion (BIC) values no longer decreased.