

Gut region influences the diversity and interactions of bacterial communities in pikas (*Ochotona curzoniae* and *Ochotona daurica*)

Huan Li¹, Tongtong Li², Aileen Berasategui³, Junpeng Rui², Xiao Zhang⁴,
Chaonan Li², Zhishu Xiao⁵, *, Xiangzhen Li², *

1. Fujian Provincial Key Laboratory of Soil Environmental Health and Regulation, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002, China
2. Key Laboratory of Environmental and Applied Microbiology, CAS; Environmental Microbiology Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Sichuan, 610041, PR China
3. Biochemistry Department, Max Planck Institute for Chemical Ecology, 07745, Jena, Germany.
4. State Key Laboratory of Integrated Management of Pest Insects and Rodents in Agriculture, Institute of Zoology, Chinese Academy of Sciences, Beichen West Road, Chaoyang, District, Beijing 100101, China
5. College of Environment and Planning, Henan University, Jinming Avenue, Kaifeng, 475004, China.

*Corresponding author, Z. Xiao, E-mail: xiaozs@ioz.ac.cn; X. Li, lixz@cib.ac.cn

Running title: Bacterial biogeography of pika gastrointestinal tract

Table S1. Sample information across 224 samples described in this study

Group name	Anatomical region	Common name	Altitude(m)	Body weight ^a	NO. ^b	Habitat	Sampling site	District
1000_Mouth	Mouth	Daurian pikas	1000	111±12	12	Typical steppe	Xiwuqi	Inner Mongolia
1000_Stomach	Stomach	Daurian pikas	1000	111±12	12	Typical steppe	Xiwuqi	Inner Mongolia
1000_Small-intestine	Small-intestine	Daurian pikas	1000	111±12	12	Typical steppe	Xiwuqi	Inner Mongolia
1000_Caecum	Caecum	Daurian pikas	1000	111±12	12	Typical steppe	Xiwuqi	Inner Mongolia
1000_Colon	Colon	Daurian pikas	1000	111±12	12	Typical steppe	Xiwuqi	Inner Mongolia
1000_Plant	NA	Plant(<i>Stipa sp.</i> , <i>Kochiaprostrata sp.</i> , <i>Convolvunlus ammannii</i> , <i>Setaria viridis</i>) ^c	1000	NA	4	Typical steppe	Xiwuqi	Inner Mongolia
1000_Soil	NA	Soil	1000	NA	5	Typical steppe	Xiwuqi	Inner Mongolia
1198_Mouth	Mouth	Daurian pikas	1198	151±8	14	Typical steppe	Baiyinxile	Inner Mongolia
1198_Stomach	Stomach	Daurian pikas	1198	151±8	14	Typical steppe	Baiyinxile	Inner Mongolia
1198_Small-intestine	Small-intestine	Daurian pikas	1198	151±8	14	Typical steppe	Baiyinxile	Inner Mongolia
1198_Caecum	Caecum	Daurian pikas	1198	151±8	14	Typical steppe	Baiyinxile	Inner Mongolia
1198_Colon	Colon	Daurian pikas	1198	151±8	14	Typical steppe	Baiyinxile	Inner Mongolia
1198_Plant	NA	Plant(<i>Stipa sp.</i> , <i>Kochiaprostrata sp.</i> , <i>Cleistogenes squarrosa</i> , <i>Chenopodium acuminatum</i>) ^c	1198	NA	4	Typical steppe	Baiyinxile	Inner Mongolia
1198_Soil	NA	Soil	1198	NA	5	Typical steppe	Baiyinxile	Inner Mongolia
4331_Mouth	Mouth	Plateau pikas	4331	135±14	13	Alpine meadow	Guoluo	Qinghai-Tibet Plateau
4331_Stomach	Stomach	Plateau pikas	4331	135±14	13	Alpine meadow	Guoluo	Qinghai-Tibet Plateau
4331_Small-intestine	Small-intestine	Plateau pikas	4331	135±14	13	Alpine meadow	Guoluo	Qinghai-Tibet Plateau
4331_Caecum	Caecum	Plateau pikas	4331	135±14	13	Alpine meadow	Guoluo	Qinghai-Tibet Plateau
4331_Colon	Colon	Plateau pikas	4331	135±14	13	Alpine meadow	Guoluo	Qinghai-Tibet Plateau
4331_Plant	NA	Plant(<i>Kobresia humilis</i> , <i>Elymus nutans</i> , <i>Potentilla anserine</i> , <i>Carex moorcraftii</i> , <i>Thalictrum petaloideum</i>) ^c	4331	135±14	5	Alpine meadow	Guoluo	Qinghai-Tibet Plateau
4331_Soil	NA	Soil	4331	135±14	6	Alpine meadow	Guoluo	Qinghai-Tibet Plateau

^a The data for body weight were expressed as Mean±SEM

^b The number of samples in each group

^c The dominant plant species in each sampling site

Table S2 The pH values (Mean±SE) of luminal contents across the gut regions in pikas.

Gut region	Plateau Pikas	Daurian Pikas	P value
Mouth	NA	NA	NA
Stomach	2.22±0.09	2.77±0.12	0.003
Small intestine	6.32±0.12	6.43±0.04	0.18
Caecum	6.56±0.15	6.59±0.06	0.64
Colon	6.64±0.18	6.62±0.10	0.79

One -way ANOVA was used to compare the difference in the same gut region between Plateau pikas and Daurian pikas

Significance is indicated by $P < 0.05$

Table S3. Two-way ANOVA was used to examine the effect of host species and gut regions in shaping the relative abundance of four dominant phyla and genera in the pika digestive tract.

Phyla Factor	Bacteroidetes		Firmicutes		Spirochaetes		Proteobacteria	
	F	P-value	F	P-value	F	P-value	F	P-value
Species	17.347	< 0.001	9.466	< 0.001	0.534	0.587	2.398	0.094
Gut region	21.439	< 0.001	21.161	< 0.001	7.358	< 0.001	121.397	< 0.001
species * Gut region	1.069	0.387	2.893	0.005	1.674	0.107	1.432	0.186
Genera Factor	<i>Prevotella</i>		<i>Oscillospira</i>		<i>Streptococcus</i>		<i>Ruminococcus</i>	
	F	P-value	F	P-value	F	P-value	F	P-value
Species	90.646	< 0.001	0.058	0.809	2.674	0.104	12.327	0.001
Gut region	29.765	< 0.001	10.469	< 0.001	20.405	< 0.001	5.724	< 0.001
species * region	8.23	< 0.001	1.393	0.238	2.25	0.065	0.954	0.434

Significance is indicated by P < 0.05

Table S4. Two-way ANOVA was used to examine the effect of host species and gut regions in shaping the alpha diversity measures of microbial communities in the pika digestive tract.

Phyla Factor	Shannon diversity		Evenness		Observed Species		Phylogenetic diversity	
	F	P-value	F	P-value	F	P-value	F	P-value
Species	6.093	0.014	8.562	0.004	1.368	0.244	0.707	0.402
Gut region	24.549	<0.001	24.145	<0.001	22.12	<0.001	19	<0.001
species * Gut region	0.234	0.919	0.258	0.904	0.249	0.91	0.429	0.787

Significance is indicated by $P < 0.05$

Table S5. PERMANOVA analysis showing the effects of various factors in shaping the bacterial communities of pika digestive tract.

PERMANOVA	Daurian pikas				Plateau pikas	
Altitude	1000		1198		4331	
	R ²	P-value	R ²	P-value	R ²	P-value
Weighted UniFrac distance						
Gut regions	0.517	<0.001	0.5	<0.001	0.482	<0.001
Individual	0.1	0.131	0.114	0.063	0.145	0.023
Weight	0.015	0.158	0.019	0.065	0.003	0.635
Sex	0.014	0.152	0.018	0.065	0.0001	0.994

Significance is indicated by P < 0.05

Table S6. PERMANOVA analysis summarizing the divergence of bacterial community in the different gut regions of pikas based on weighted UniFrac distance

Host species		Daurian pikas				Plateau pikas	
Altitude		1000m		1198m		4331m	
Gut regions	Sample location	R ²	P-value	R ²	P-value	R ²	P-value
Mouth-Stomach	Adjacent Sites	0.72	<0.001	0.562	<0.001	0.54	<0.001
Stomach-Small-intestine	Adjacent Sites	0.062	0.228	0.077	0.085	0.12	0.035
Small-intestine-Caecum	Adjacent Sites	0.215	0.002	0.462	<0.001	0.29	0.002
Caecum-Colon	Adjacent Sites	0.032	0.563	0.023	0.629	0.007	1
Mouth-Small-intestine	Non-Adjacent Sites	0.648	<0.001	0.655	<0.001	0.31	0.002
Mouth-Caecum	Non-Adjacent Sites	0.562	<0.001	0.478	<0.001	0.52	<0.001
Mouth-Colon	Non-Adjacent Sites	0.562	<0.001	0.548	<0.001	0.54	<0.001
Stomach-Caecum	Non-Adjacent Sites	0.383	<0.001	0.31	<0.001	0.57	<0.001
Stomach-Colon	Non-Adjacent Sites	0.447	<0.001	0.363	<0.001	0.56	<0.001
Small-intestine-colon	Non-Adjacent Sites	0.289	<0.001	0.47	<0.001	0.3	0.002

P values have been corrected using the false discovery rate control.

Significance is indicated by $P < 0.05$

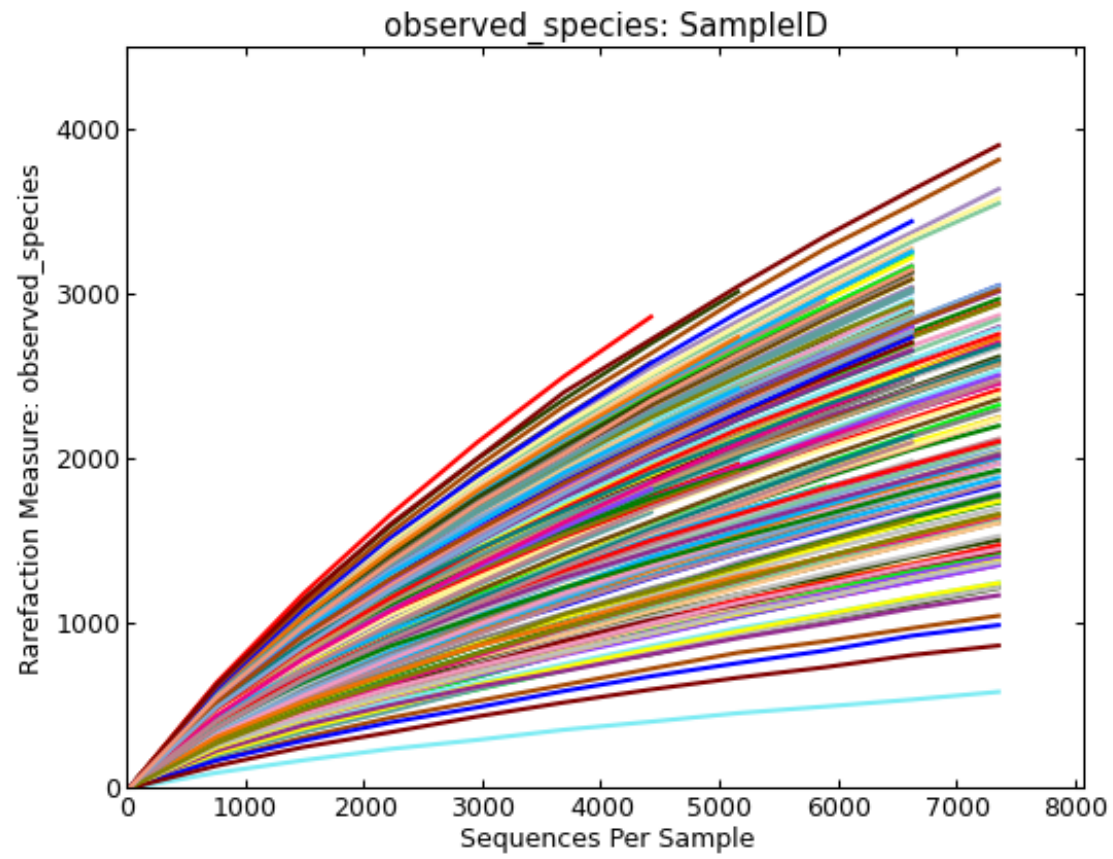


Figure S1. The OTU-level rarefaction curves of observed species across all samples.

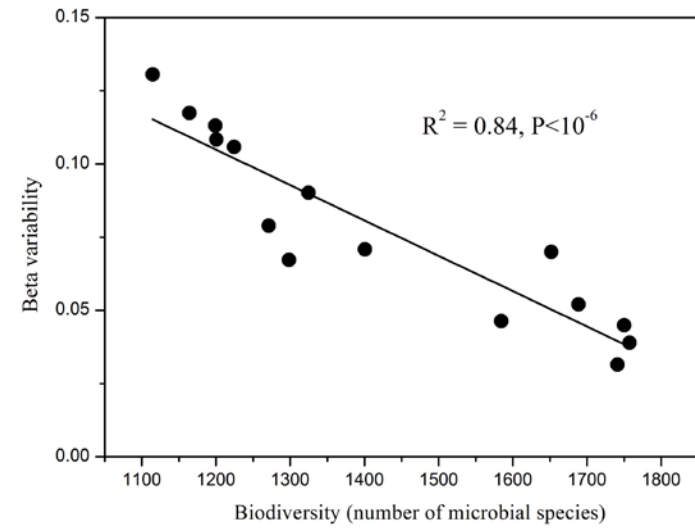
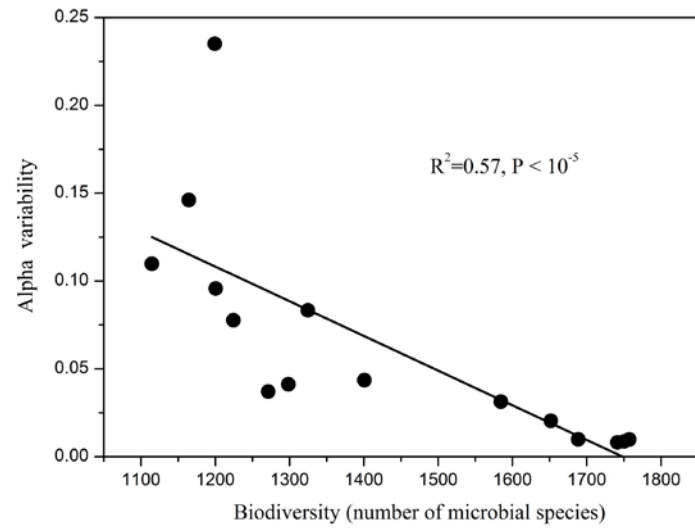


Figure S2. Linear regression relationship between microbial biodiversity (observed species) and alpha variability or beta variability.