

Supplementary Material 2

Dietary niche breadth influences the effects of urbanization on the gut microbiota of sympatric rodents

Jason L. Anders^{a,b*}, Alexis M. Mychajliw^c, Mohamed Abdallah Mohamed Moustafa^{d,e}, Wessam Mohamed Ahmed Mohamed^d, Takashi Hayakawa^{f,g}, Ryo Nakao^d, Itsuro Koizumi^f

^aGraduate School of Environmental Science, Hokkaido University, N10W5 Sapporo, Hokkaido 060-0810, Japan

^bCenter for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, PO Box 1066. Blindern, 0316 Oslo, Norway

^cMiddlebury College, Departments of Biology and Environmental Studies, 276 Bicentennial Way, Middlebury, VT 05753, United States

^dLaboratory of Parasitology, Graduate School of Infectious Diseases, Faculty of Veterinary Medicine, Hokkaido University, N18W9, Sapporo, Hokkaido 060-0818, Japan

^eDepartment Animal Medicine, South Valley University, 83523, Egypt,

^f Faculty of Environmental Earth Science, Hokkaido University, N10W5 Sapporo, Hokkaido 060-0810, Japan

^gJapan Monkey Centre, Inuyama, Aichi 484-0081, Japan

*Corresponding author: Jason L. Anders, E-mail: j.l.anders@ibv.uio.no

Bacterial Phylum level description

Firmicutes and Bacteroidota were the two most abundant phyla in all three regions of the lower GIT of both host species comprising 80% of all microbes in *A. speciosus* and 90% in *M. rufocaninus* in both the natural and urban populations. Campilobacterota was the third most abundant in these same gut regions in *A. speciosus* except in the rectum of urban animals where Proteobacteria was the third most dominant phylum. Spirochaetota was the third most abundant phylum in the cecum, colon, and rectum of *M. rufocaninus* with little variation in relative abundance between natural and urban populations. Interestingly, although Firmicutes, Bacteroidota, and Proteobacteria were the three most abundant phyla in the small intestine of *A. speciosus*, Firmicutes was more abundant in the natural habitat with 71% as opposed to 58% in the urban parks, while Bacteroidota showed the opposite trend with 13% and 25% in the natural and urban habitats respectively. A similar but weaker trend was found in *M. rufocaninus*; Bacteroidota made up 41% and 54% in the natural and urban sites respectively, while Firmicutes comprised 34% and 26% of all microbes in the natural and urban sites respectively.

Differential abundance analyses using ANCOM-II

A secondary differential abundance analyses was conducted comparing the relative abundance of microbial genera in each gut region between natural and urban populations using ANCOM-II as described by Kaul et al. (2017) and the R-script written by Lin (2019). We found that few microbial genera exhibited differential abundance between habitat type in either host species (Tables S12, S13). Notably, those genera with a large difference in average relative abundances such as Tyzerella (42.87%, 29.19% in natural and urban respectively) and Lactobacillus (2.44%, 8.15% averaged in natural and urban respectively) in the small intestine of *A. speciosus* were not identified as differentially abundant (Table S10). This in combination with the significantly different microbial structure between urban and natural habitats based on our beta-diversity analyses (Tables S8, S9) indicate that ANCOM-II may be too conservative an analysis for the present study.

References

Kaul A, Mandal S, Davidov O, Peddada SD. (2017) Analysis of Microbiome Data in the Presence of Excess Zeros. Front Microbiol. 8, 2114. doi: 10.3389/fmicb.2017.02114

Frederick Huang Lin (2019) Huanglin/Ancom: Third release of Ancom (v2.1). Zenodo. <https://doi.org/10.5281/zenodo.3577802>

Supplemental Figures

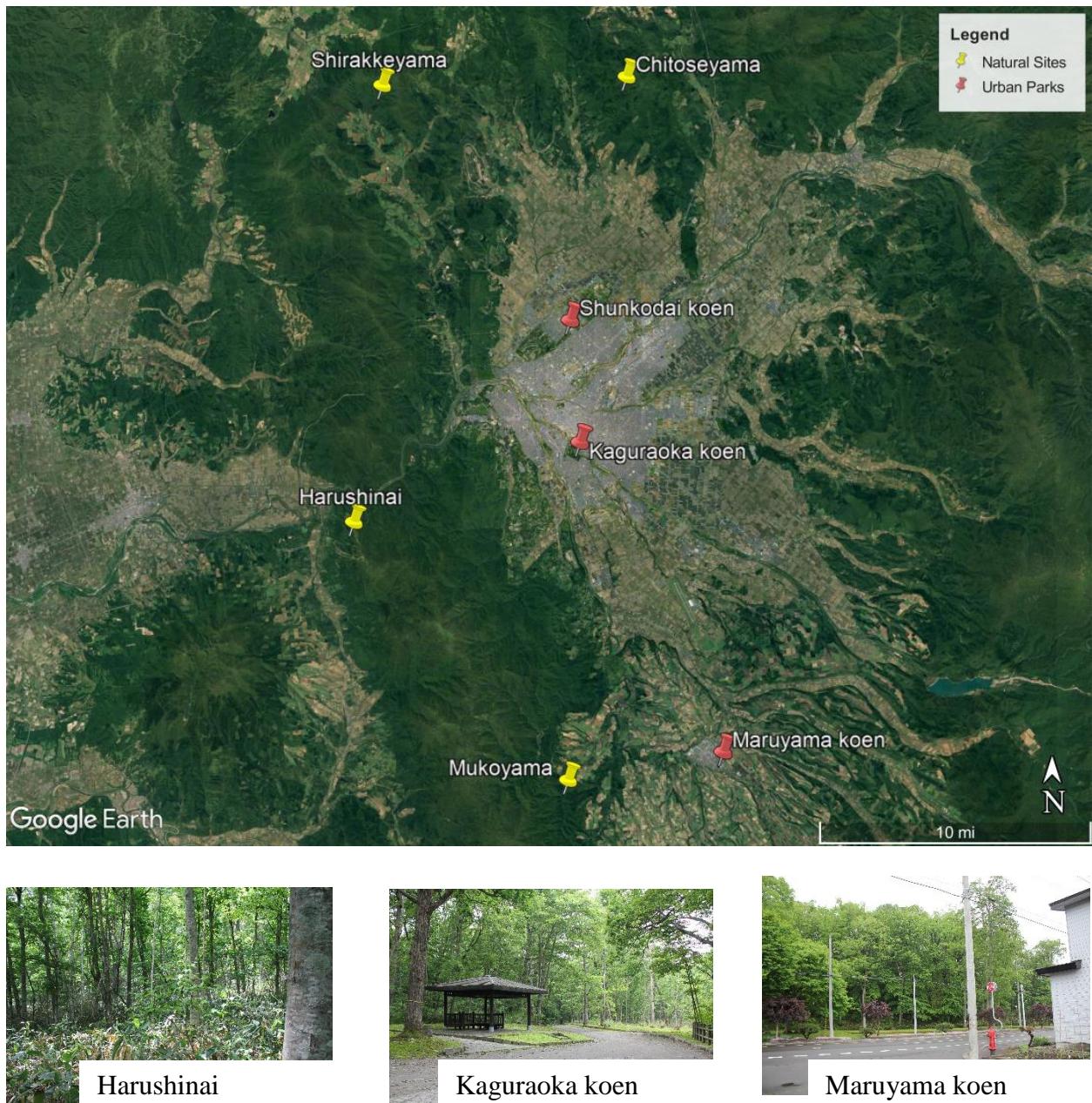


Figure S1: A satellite image showing the location of field sites and pictures of three sites (two urban and one natural). The map was created using Google Earth Pro (version 7.3.3, <https://www.google.com/earth/releases/>). Map data: Google, SIO, NOAA, U.S. Navy, NGA, GEBCO, © 2020 ZENRIN Image Landsat/ Copernicus

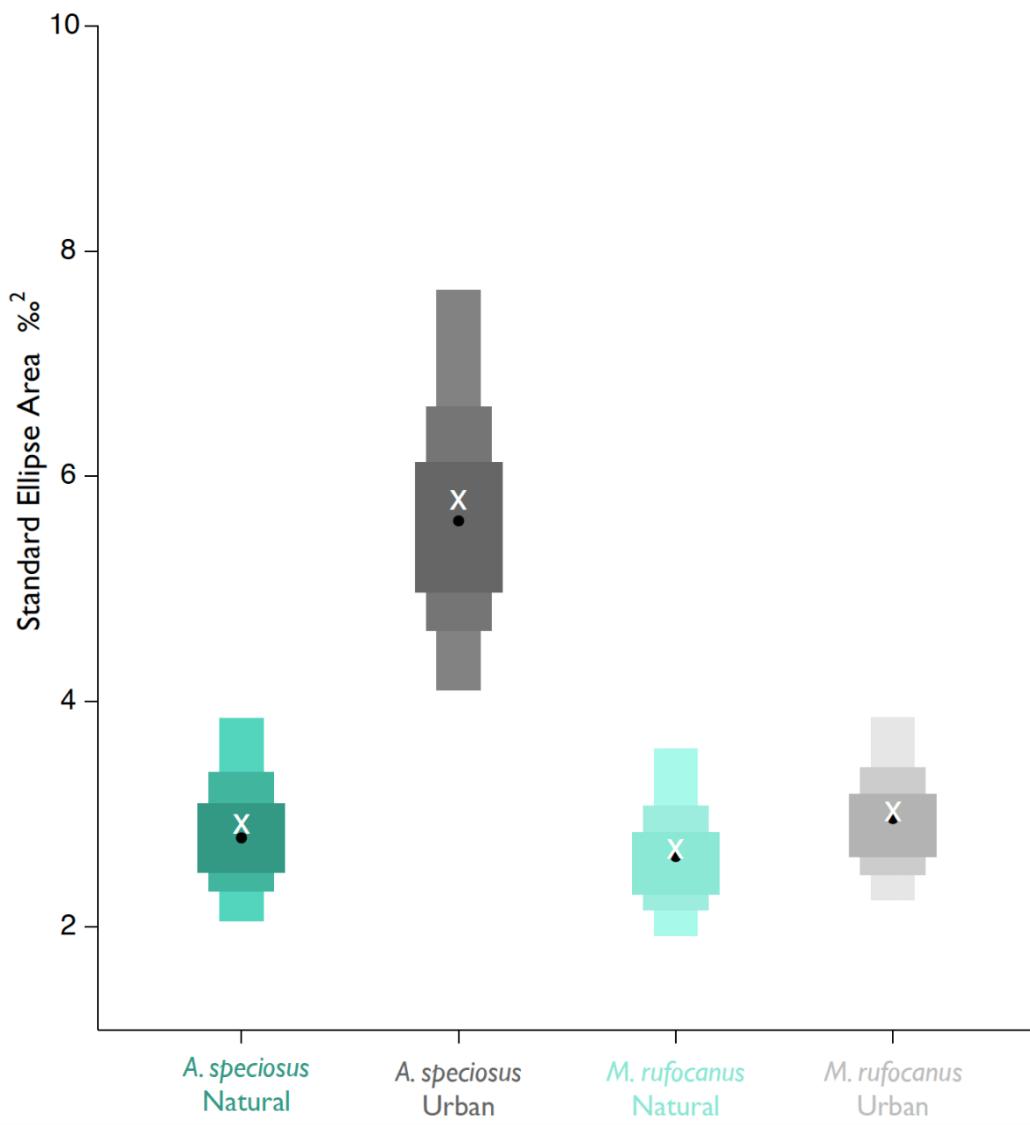


Figure S2: Bayesian standard ellipse area (SEA_b) for each group after 20,000 posterior draws where the boxes indicate 50%, 75%, and 95% confidence intervals and the white X is the maximum likelihood estimate of the small sample size corrected standard ellipse area (SEA_c).

Supplemental Tables

Habitat type	Site	Latitude	Longitude	<i>A. speciosus</i>	<i>M. rufocanus</i>
Natural	Chitoseyama	43° 56' 30.64"	142° 24' 10.09"	6	16
	Harushinai	43° 42' 19.82"	142° 12' 08.28"	5	20
	Mukoyama	43° 34' 07.41"	142° 21' 34.57"	11	3
	Shirakkeyama	43° 56' 14.15"	142° 13' 21.56"	20	4
Urban	Kaguraoka koen	43° 44' 52.24"	142° 22' 05.94"	19	13
	Shunkodai koen	43° 48' 45.38"	142° 21' 40.40"	16	29
	Maruyaama koen	43° 35' 00.68"	142° 28' 23.54"	6	8
Total				83	93

Table S1: Site information and number of animals captured at each.

Species	Small Intestine		Cecum		Colon		Rectum	
	Natural	Urban	Natural	Urban	Natural	Urban	Natural	Urban
<i>A. speciosus</i>	42	39	27	16	41	39	25	16
<i>M. rufocanus</i>	43	49	14	17	38	45	16	16
Total	85	88	41	33	79	84	41	32

Table S2: Number of samples per gut region for both *A. speciosus* and *M. rufocanus*.

		<i>A. speciosus</i>		<i>M. rufocanus</i>	
		Natural	Urban	Natural	Urban
$\delta^{13}\text{C}$	Mean \pm StD	-27.27 \pm 0.60	-27.35 \pm 1.28	-25.50 \pm 0.75	-24.86 \pm 0.70
	Min	-28.4	-30.59	-27.36	-27.02
	Max	-26.02	-22.90	-24.08	-23.20
$\delta^{15}\text{N}$	Mean \pm StD	2.53 \pm 1.55	3.76 \pm 1.46	2.51 \pm 1.17	2.22 \pm 1.36
	Min	0.59	1.40	0.59	-0.04
	Max	6.22	6.43	6.56	6.60

Table S3: Summary statistics (mean, standard deviation, minimum, maximum) of stable isotopes from natural and urban populations of the two rodent species.

	Natural		Urban	
	<i>As</i>	<i>Mr</i>	<i>As</i>	<i>Mr</i>
TA	8.97	11.8	22.84	15.73
SEA	2.83	2.61	5.63	2.95
SEAc	2.9	2.68	5.77	3.01

Table S4: Estimates of isotopic niche width as measured in per mil (‰) for total area (TA), standard ellipse area (SEA), and small sample size corrected SEA (SEAc) of natural and urban populations of *A. speciosus* (As) and *M. rufocanus* (Mr).

Gut region	Variable	<i>A. speciosus</i>			<i>M. rufocanus</i>		
		b	SE	p	b	SE	p
Small Intestine	Habitat type (Urban)	0.203	0.148	0.229	0.159	0.12	0.244
	Sex (Male)	0.181	0.139	0.198	-0.032	0.117	0.787
	Age	-0.066	0.153	0.665	-0.329	0.125	0.01
	$\delta^{13}\text{C}$	-0.015	0.072	0.839	-0.22	0.077	0.006
	$\delta^{15}\text{N}$	-0.053	0.048	0.272	0.03	0.044	0.495
	Habitat type (Urban)	0.028	0.137	0.843	-0.081	0.184	0.676
Cecum	Sex (Male)	0.194	0.066	0.006	0.016	0.093	0.867
	Age	-0.075	0.092	0.417	-0.179	0.095	0.075
	$\delta^{13}\text{C}$	0.03	0.032	0.363	-0.124	0.054	0.032
	$\delta^{15}\text{N}$	-0.029	0.024	0.229	0.043	0.034	0.224
Colon	Habitat type (Urban)	-0.001	0.055	0.988	0.06	0.056	0.335
	Sex (Male)	0.123	0.041	0.003	-0.054	0.039	0.163
	Age	0.079	0.046	0.088	-0.092	0.042	0.029
	$\delta^{13}\text{C}$	-0.022	0.026	0.399	-0.038	0.027	0.16
	$\delta^{15}\text{N}$	0.008	0.015	0.587	0.037	0.015	0.016
	Habitat type (Urban)	-0.028	0.091	0.771	-0.075	0.096	0.472
Rectum	Sex (Male)	0.239	0.065	0.001	-0.102	0.064	0.131
	Age	0.103	0.086	0.244	-0.038	0.064	0.56
	$\delta^{13}\text{C}$	0.024	0.03	0.426	-0.116	0.034	0.003
	$\delta^{15}\text{N}$	0.019	0.023	0.428	0.046	0.021	0.044

Table S5: Linear mixed effects model comparing ASV abundance diversity of the gut microbiome within each gut region between natural and urban populations. Bolded values indicate significance.

Gut region	Variable	<i>A. speciosus</i>			<i>M. rufocanus</i>		
		b	SE	p	b	SE	p
Small Intestine	Habitat type (Urban)	0.057	0.109	0.623	-0.017	0.065	0.807
	Sex (Male)	0.09	0.075	0.236	-0.01	0.056	0.863
	Age	-0.115	0.084	0.175	-0.108	0.061	0.083
	$\delta^{13}\text{C}$	-0.016	0.041	0.698	-0.046	0.038	0.222
	$\delta^{15}\text{N}$	-0.034	0.026	0.189	-0.008	0.021	0.707
	Habitat type (Urban)	-0.047	0.082	0.591	-0.063	0.084	0.484
	Sex (Male)	0.068	0.03	0.029	0.011	0.048	0.828
	Age	-0.072	0.041	0.091	-0.096	0.049	0.067
Cecum	$\delta^{13}\text{C}$	0.017	0.015	0.253	-0.069	0.028	0.023
	$\delta^{15}\text{N}$	-0.005	0.011	0.674	0.029	0.018	0.119
	Habitat type (Urban)	-0.024	0.031	0.472	0.008	0.028	0.785
	Sex (Male)	0.041	0.021	0.058	-0.026	0.022	0.252
Colon	Age	-0.005	0.024	0.844	-0.069	0.024	0.005
	$\delta^{13}\text{C}$	0.001	0.014	0.969	-0.033	0.015	0.035
	$\delta^{15}\text{N}$	0.009	0.008	0.242	0.021	0.009	0.019
	Habitat type (Urban)	-0.039	0.055	0.513	-0.09	0.042	0.085
Rectum	Sex (Male)	0.082	0.032	0.016	-0.018	0.032	0.577
	Age	0.014	0.043	0.745	-0.051	0.032	0.133
	$\delta^{13}\text{C}$	0.011	0.015	0.472	-0.047	0.017	0.015
	$\delta^{15}\text{N}$	0.006	0.012	0.627	0.035	0.011	0.004

Table S6: Linear mixed effects model comparing Faith's phylogenetic diversity of the gut microbiome within each gut region between natural and urban populations. Bolded values indicate significance.

Gut region	Dissimilarity index	<i>A. speciosus</i>				<i>M. rufocanus</i>			
		SS	μ^2	F	p	SS	μ^2	F	p
Small Intestine	Unweighted unifrac	0	0	0.0012	0.975	0.0147	0.0147	4.0267	0.039
	Weighted unifrac	0.0266	0.0266	1.6628	0.213				
Cecum	Unweighted unifrac	0.0066	0.0066	3.1382	0.082	0.0004	0.0004	0.3016	0.599
	Weighted unifrac	0	0	0.0013	0.974				
Colon	Unweighted unifrac	0	0	0.009	0.935	0.0002	0.0002	0.0944	0.756
	Weighted unifrac	0	0	0.0022	0.971				
Rectum	Unweighted unifrac	0.0057	0.0057	2.5459	0.118	0	0	0.0243	0.88
	Weighted unifrac	0.0001	0.0001	0.0159	0.901				

Table S7: PERMDISP results comparing the level of within habitat type dispersion of the gut microbial community. Bolded values indicate significance.

Dissimilarity index	Variable	Small Intestine				Cecum			
		SS	R ²	F	p	SS	R ²	F	p
Unweighted Unifrac	Habitat type	0.2468	0.0238	1.8441	0.026	0.1872	0.0483	2.1116	0.003
	Sex	0.1861	0.018	1.3906	0.097	0.0825	0.0212	0.9299	0.568
	Age	0.1735	0.0167	1.2967	0.14	0.0965	0.0249	1.0879	0.283
	δ ¹³ C	0.1161	0.0112	0.8678	0.606	0.1061	0.0273	1.1968	0.183
	δ ¹⁵ N	0.125	0.0121	0.9338	0.464	0.0806	0.0208	0.9093	0.639
Weighted Unifrac	Habitat type	0.2383	0.0299	2.303	0.04	0.0616	0.0243	1.0755	0.36
	Sex	0.0755	0.0095	0.73	0.624	0.0598	0.0236	1.0438	0.375
	Age	0.0932	0.0117	0.901	0.484	0.1548	0.0611	2.7049	0.016
	δ ¹³ C	0.0868	0.0109	0.8385	0.495	0.0475	0.0188	0.8305	0.565
	δ ¹⁵ N	0.0399	0.005	0.3859	0.909	0.0378	0.0149	0.6602	0.75
Dissimilarity	Variable	Colon				Rectum			
		SS	R ²	F	p	SS	R ²	F	p
Unweighted Unifrac	Habitat type	0.2481	0.0347	2.8492	0.001	0.186	0.0472	1.9672	0.002
	Sex	0.1087	0.0152	1.2479	0.111	0.1132	0.0287	1.1967	0.172
	Age	0.1683	0.0235	1.9331	0.001	0.1198	0.0304	1.2671	0.129
	δ ¹³ C	0.154	0.0215	1.7692	0.005	0.1012	0.0257	1.0699	0.327
	δ ¹⁵ N	0.0858	0.012	0.9858	0.482	0.0771	0.0196	0.8154	0.805
Weighted Unifrac	Habitat type	0.2153	0.038	3.1725	0.004	0.1175	0.0423	1.7684	0.078
	Sex	0.1349	0.0238	1.9883	0.036	0.1205	0.0434	1.8133	0.071
	Age	0.2767	0.0488	4.0775	0.001	0.109	0.0393	1.6408	0.098
	δ ¹³ C	0.1	0.0176	1.4741	0.141	0.0515	0.0186	0.7753	0.62
	δ ¹⁵ N	0.0716	0.0126	1.0559	0.358	0.0651	0.0235	0.9796	0.442

Table S8: PERMANOVA results comparing the gut microbiome of the different gut regions between natural and urban populations of *A. speciosus*. Bolded values indicate significance.

Dissimilarity index	Variable	Small Intestine				Cecum			
		SS	R ²	F	p	SS	R ²	F	p
Unweighted Unifrac	Habitat type	0.2815	0.0255	2.3093	0.002	0.1287	0.0469	1.454	0.046
	Sex	0.0867	0.0079	0.7113	0.905	0.0984	0.0359	1.1119	0.233
	Age	0.2114	0.0192	1.7346	0.024	0.1315	0.048	1.4855	0.035
	δ ¹³ C	0.3657	0.0332	3.0002	0.001	0.2389	0.0872	2.7	0.001
	δ ¹⁵ N	0.1406	0.0128	1.1532	0.203	0.1415	0.0516	1.5994	0.013
Weighted Unifrac	Habitat type	0.7677	0.0505	4.5194	0.009	0.0857	0.068	2.0661	0.039
	Sex	0.113	0.0074	0.6652	0.6	0.0666	0.0528	1.6051	0.113
	Age	0.2801	0.0184	1.649	0.144	0.0715	0.0568	1.725	0.074
	δ ¹³ C	0.4957	0.0326	2.9186	0.03	0.0735	0.0583	1.7714	0.086
	δ ¹⁵ N	0.1435	0.0094	0.845	0.462	0.0382	0.0303	0.9203	0.507
Dissimilarity	Variable	Colon				Rectum			
		SS	R ²	F	p	SS	R ²	F	p
Unweighted Unifrac	Habitat type	0.1915	0.0266	2.2349	0.001	0.1269	0.0487	1.5046	0.037
	Sex	0.1145	0.0159	1.3367	0.058	0.1134	0.0435	1.345	0.076
	Age	0.1435	0.0199	1.6747	0.011	0.118	0.0453	1.3996	0.052
	δ ¹³ C	0.23	0.0319	2.6839	0.001	0.2145	0.0824	2.5438	0.001
	δ ¹⁵ N	0.141	0.0196	1.6449	0.007	0.125	0.048	1.4815	0.039
Weighted Unifrac	Habitat type	0.1296	0.0344	2.7989	0.014	0.0892	0.0678	2.0539	0.064
	Sex	0.0424	0.0113	0.9164	0.453	0.099	0.0752	2.2793	0.04
	Age	0.0744	0.0198	1.6077	0.109	0.0598	0.0454	1.3761	0.203
	δ ¹³ C	0.035	0.0093	0.755	0.618	0.0697	0.053	1.6049	0.137
	δ ¹⁵ N	0.0363	0.0096	0.7837	0.608	0.0212	0.0161	0.4876	0.883

Table S9: PERMANOVA results comparing the gut microbiome of the different gut regions between natural and urban populations of *M. rufocanus*. Bolded values indicate significance

		<i>A. speciosus</i>			
Gut region	Microbial genus	Natural		Urban	
		Avg. relative abundance (%)	LDA	Avg. relative abundance (%)	LDA
Small Intestine	Tyzzerella	42.87	4.83	29.19	
	Mycoplasma	10.4	4.62	1.97	
	Lactobacillus	2.44		8.15	4.53
	Mucispirillum	0.04	3.55	0.02	
	Oscillospirales UCG_010	< 0.01		0.01	3.54
	Prevotellaceae UCG_003	0.10	2.73	< 0.01	
	Bifidobacterium	0		0.01	2.63
	Pseudomonas	0.07	2.59	< 0.01	
	Ochrobactrum	0		< 0.01	2.38
	Lachnospiraceae GCA_900066575	< 0.01		0.02	2.11
	Lachnospiraceae UCG_006	< 0.01		0.03	2.11
	Providencia	< 0.01	2.07	0	
	Ruminococcus	0.01		0.03	2.07
	Desulfovibrio	0.01		0.03	2.05
Cecum	Lachnospiraceae NC2004 group	0		0.02	2
	Prevotellaceae UCG_003	2.77	4.18	0.09	
	Treponema	2.07	4.02	0.47	
	Escherichia-Shigella	1.23		2.9	3.97
	Mycoplasma	1.88	3.92	0.29	
	Prevotellaceae Ga6A1 group	1.75	3.81	0.64	
	Lachnospiraceae UCG_001	0.03		0.19	2.91
	Cerasicoccus	0.03		0.17	2.64
	Butyricicoccus	0.04		0.07	2.27

Table S10: More abundant bacterial genera in the a) small intestine, b) cecum, c) colon, d) and rectum of *A. speciosus* in the natural and urban habitats based on LEfSe analysis.

		<i>A. speciosus</i>			
Gut region	Microbial genus	Natural		Urban	
		Avg. Relative Abundance (%)	LDA	Avg. Relative Abundance (%)	LDA
Colon	Mycoplasma	2.4	3.99	0.511	
	Prevotellaceae UCG_003	1.57	3.87	0.1	
	Prevotellaceae Ga6A1 group	1.47	3.63	0.82	
	Mucispirillum	1.56	3.62	0.89	
	Treponema	1.08	3.58	0.49	
	Bifidobacterium	0		< 0.01	3.43
	Anaerostipes	0		0.29	3.13
	Faecalibaculum	< 0.01		0.07	2.6
	Parasutterella	0.04		0.1	2.49
	Butyricoccus	0.029		0.09	2.49
Rectum	Frisingicoccus	0		< 0.01	2.05
	Oscillospiraceae NK4A214 group	0.02		0.05	2.05
	Prevotellaceae UCG_003	2.94	4.16	0.01	
	Prevotellaceae Ga6A1 group	2.26	3.9	0.78	
	Treponema	1.55	3.81	0.22	
	Mucispirillum	1.22	3.55	0.51	
	Ruminococcus	0.75		0.68	3.48
Rectum	Anaerostipes	0		0.71	3.35
	Lachnospiraceae UCG_001	0.03		0.22	3.07
	Oscillospiraceae UCG_005	0		< 0.01	2.61
	Lachnoclostridium	0.03		0.06	2.26
	Anaerovoracaceae, Family XIII, UCG_001	0		< 0.01	2.07

Table S10 (cont.): More abundant bacterial genera in the a) small intestine, b) cecum, c) colon, d) and rectum of *A. speciosus* in the natural and urban habitats based on LEfSe analysis.

<i>M. rufocanus</i>					
Gut region	Microbial genus	Natural		Urban	
		Avg. relative abundance (%)	LDA	Avg. relative abundance (%)	LDA
Small Intestine	Ureaplasma	9.84	4.44	4.18	
	Helicobacter	3.5	4.2	0.18	
	Campylobacteria	0.2	4.2	< 0.01	
	Mycoplasma	4.89	4.07	4.47	
	Exiguobacterium	0.01	3.8	< 0.01	
	Coxiella	0.04	3.57	0.01	
	Mucispirillum	0.02	3.22	< 0.01	
	Escherichia-Shigella	0.42	3.19	0.24	
	Monoglobus	< 0.01		0.03	3.17
	Ehrlichia	0.08	3.14	0	
	Staphylococcus	0.01	3.12	< 0.01	
	Candidatus Lariskella	0.01	2.76	< 0.01	
	Methylobacterium-Methylorubrum	0			2.21
	Lachnoclostridium	< 0.01		0.01	2.12
Cecum	Oscillibacter	0.02	2.09	0.01	
	Oscillospiraceae UCG_005	0.01	2.02	< 0.01	
	Alistipes	2.23	3.73	1.3	
	Odoribacter	0.88	3.49	0.32	
	Colidetribacter	0.99		1.28	3.24
	Erysipelatoclostridiaceae UCG_004	0.01	3.21	< 0.01	
	Eubacterium xylanophilum group	0.31	3.13	0.08	
	Anaerovoracaceae, Family XIII, AD3011	0.02	3.02	< 0.01	
	Desulfovibrio	0.21		0.35	2.95
	Lachnospiraceae, FCS020 group	< 0.01		0.03	2.89
Colon	Tuzzerella	0.15	2.81	0.04	
	Oscillospiraceae UCG_005	0.08	2.8	0.05	
	Lachnospiraceae, NK4B4 group	< 0.01		0.06	2.73
	Blautia	< 0.01		0.03	2.73

Table S11: More abundant bacterial genera in the a) small intestine, b) cecum, c) colon, d) and rectum of *M. rufocanus* in the natural and urban habitats based on LEfSe analysis.

<i>M. rufocanus</i>						
Gut region	Microbial genus	Natural		Urban		
		Avg. relative abundance (%)	LDA	Avg. relative abundance (%)	LDA	
Colon	Lachnospiraceae, NK4A136 group	5.26		7.85	4.08	
	Helicobacter	1.42		2.48	3.78	
	Alistipes	2.94	3.68	2.01		
	Odoribacter	0.98	3.32	0.47		
	Gastranaerophilales	0.87	3.15	0.6		
	Eubacterium xylanophilum group	0.31	3.04	0.1		
	Allobaculum	< 0.01		0.16	2.91	
	Mucispirillum	0.31	2.85	0.21		
	Erysipelatoclostridiaceae UCG_004	0.01	2.67	< 0.01		
	Intestinimonas	0.19		0.25	2.46	
Rectum	Peptococcus	0.11	2.35	0.07		
	Tuzzerella	0.06	2.3	0.02		
	Lachnospiraceae, NK4B4 group	0.04		0.07	2.22	
	Anaerovorax	< 0.01	2.07	0		
	Oscillospiraceae UCG_005	0.05	2.04	0.03		
	Ruminococcus	1.57	3.69	0.53		
	Eubacterium xylanophilum group	1.05	3.68	0.14		
	Peptococcus	0.18	2.68	0.09		
	Oscillospiraceae UCG_005	0.12	2.67	0.03		
	Anaerovoracaceae, Family XIII, AD3011	0.03	2.54	< 0.01		
	Lachnospiraceae, NK4B4 group	0.05		0.09	2.38	
	Tuzzerella	0.05	2.32	0.02		
	Bilophila	0.02	2.2	< 0.01		

Table S11 (cont.): More abundant bacterial genera in the a) small intestine, b) cecum, c) colon, d) and rectum of *M. rufocanus* in the natural and urban habitats based on LEfSe analysis.

<i>A. speciosus</i>						
Gut Region	Genus	W	cut-off value			
			0.9	0.8	0.7	0.6
Small Intestine	Spirochaetaceae	74	FALSE	FALSE	TRUE	TRUE
Cecum	Prevotellaceae Ga6A1 group	74	FALSE	TRUE	TRUE	TRUE
Cecum	Treponema	87	TRUE	TRUE	TRUE	TRUE
Colon	Treponema	93	TRUE	TRUE	TRUE	TRUE
Rectum	PrevotellaceaeGa6A1group	76	FALSE	FALSE	TRUE	TRUE
Rectum	Treponema	92	TRUE	TRUE	TRUE	TRUE

Table S12: Differentially abundant microbial genera between natural and urban populations of *A. speciosus* using ANCOM-II. Cutoff values indicate less conservative (0.6) to more conservative (0.9) analysis in terms of controlling for false discovery rate. TRUE indicates differential abundance and FALSE indicates no differential abundance.

<i>M. rufocanus</i>						
Gut Region	Genus	W	cut-off value			
			0.9	0.8	0.7	0.6
Small Intestine	Eggerthellaceae	71	FALSE	TRUE	TRUE	TRUE
Small Intestine	Christensenellaceae	71	FALSE	TRUE	TRUE	TRUE
Small Intestine	Coxiella	80	TRUE	TRUE	TRUE	TRUE
Cecum	Lachnospiraceae FCS020 group	77	FALSE	FALSE	TRUE	TRUE
Cecum	Lachnospiraceae NK4B4 group	77	FALSE	FALSE	TRUE	TRUE
Colon	Rs-E47 termite group	96	TRUE	TRUE	TRUE	TRUE
Colon	Tuzzerella	77	FALSE	FALSE	TRUE	TRUE
Rectum	xylanophilumgroup	71	FALSE	FALSE	TRUE	TRUE

Table S13: Differentially abundant microbial genera between natural and urban populations of *M. rufocanus* using ANCOM-II. Cutoff values indicate less conservative (0.6) to more conservative (0.9) analysis in terms of controlling for false discovery rate. TRUE indicates differential abundance and FALSE indicates no differential abundance.