

Supplemental Information for:

Host-microbiome associations of native and invasive small mammals across a tropical urban-rural ecotone

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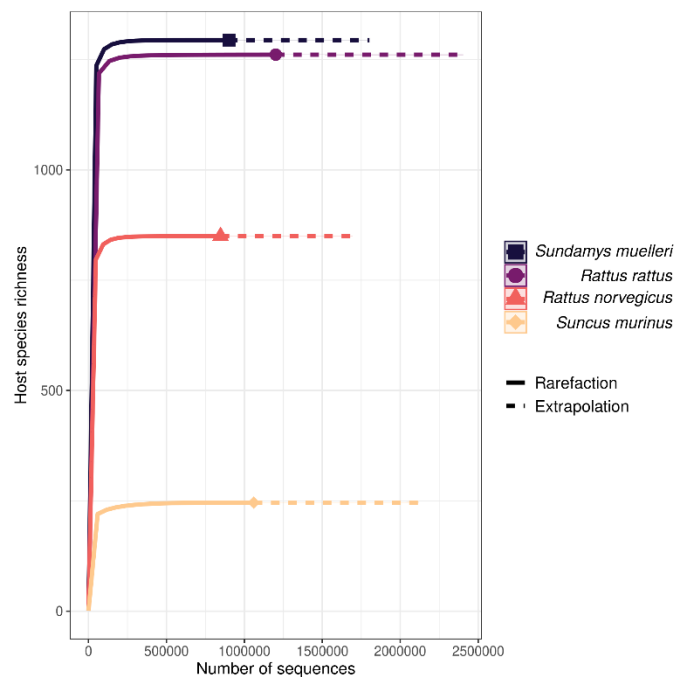


Figure S1: Alpha diversity rarefaction curves based on the cumulative number of amplicon sequence variants (ASV) recorded in fecal samples from the four focal host species. Estimates are based on Hill numbers, solid lines refer to the rarefied samples, dashed lines to extrapolated predictions of the same number of samples.

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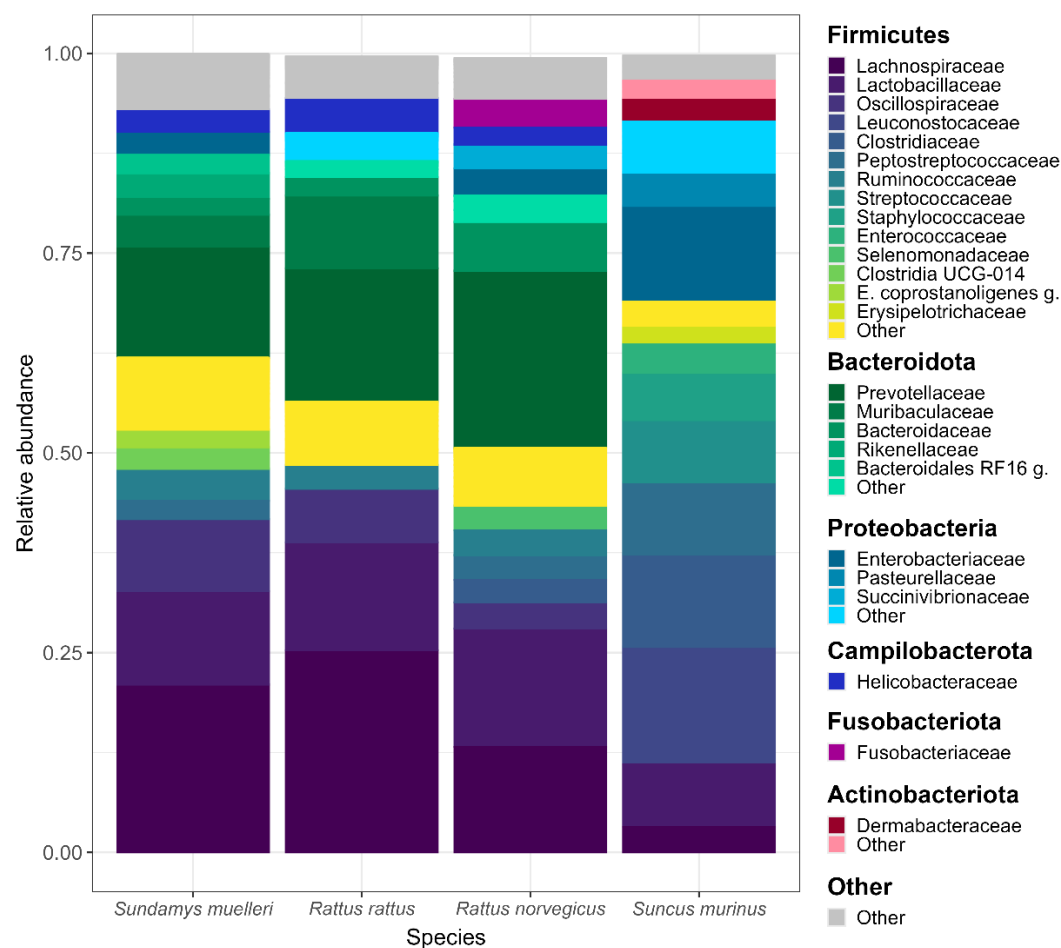


Figure S2: Faecal bacterial composition aggregated at family taxonomic level for four small mammal species from Borneo. Compositions are based on the relative proportion of amplicon sequence variants assigned to different taxonomic groups pooled for all individuals of each host species (Sample sizes: *Sund. muelleri*: n = 58, *R. rattus*: n = 74, *R. norvegicus*: n = 49, *Sunc. murinus*: n = 55). For each host species, families representing less than 2% relative abundance were clustered together as 'Other'.

(*E. coprostanoligenes* g.: Eubacterium coprostanoligenes group; Bacteroidales RF16 g.: Bacteroidales RF16 group.).

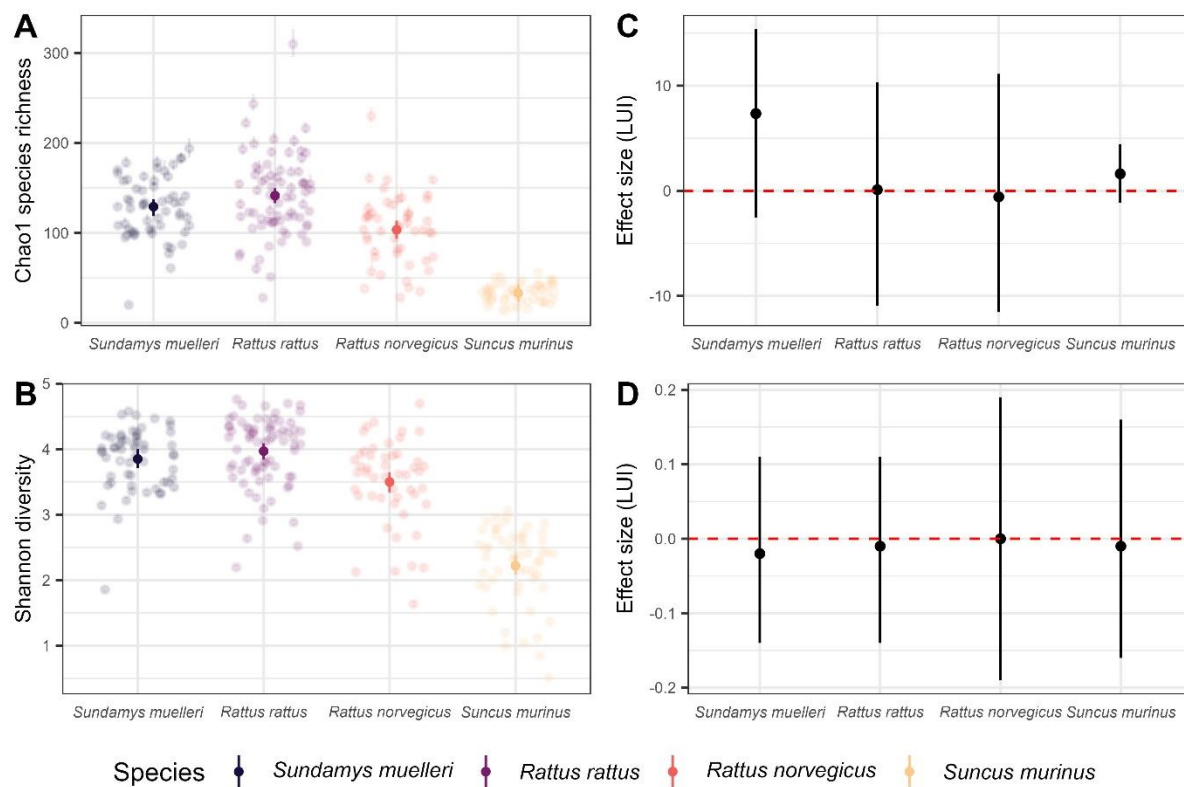


Figure S3: (A and B) Plots of the estimates of relative alpha diversity measures (Chao1 species richness and Shannon diversity) for each host individual, for the four studied small mammal species. Dark point and error bars represent the expected average estimate based on a generalised linear model and an iterative model fit to rarefied data as described in the methods. (C and D) Plots of the coefficient estimates of LUI as linear predictor for the variation in Chao1 and Shannon metrics depicted in A and B. Points represent the mode and lines represent the 95% CI, based on separate generalised linear models and iterative model fits for each species.

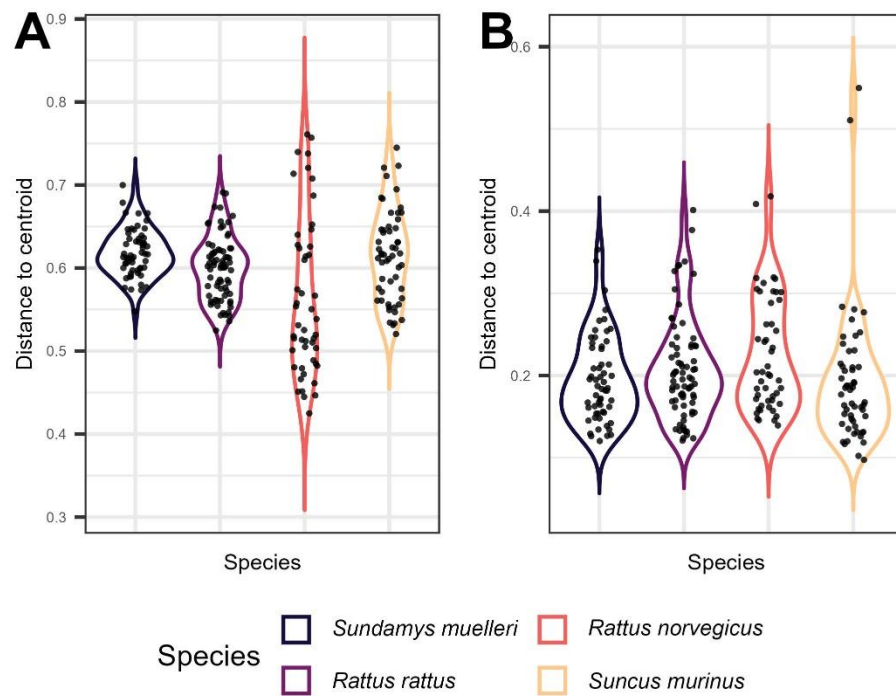


Figure S4: plots of beta dispersion, i.e. distance to species centroid defined using non-metric multidimensional scaling (NMDS), for the four studied small mammal species. (Sample sizes: $n = 58$ for *Sund. muelleri*, $n = 74$ for *R. rattus*, $n = 49$ for *R. norvegicus*, $n = 55$ for *Sunc. murinus*).

Table S1: Average estimate of alpha diversity (Chao1 species richness and Shannon diversity) and effect of land use intensity (LUI) on bacterial assemblages in host individuals for the four studied small mammal species. Values are reported as mode [95% CI], based on generalised linear models and an iterative model fit to rarefied data as described in the methods.

Species	Alpha diversity average estimates		LUI effect size	
	Chao1 species richness	Shannon diversity	Chao1 species richness	Shannon diversity
<i>Sundamys muelleri</i>	129.16 [118.69 – 137.49]	3.85 [3.71 – 4.00]	7.35 [-2.52 – 15.39]	-0.02 [-0.14 – 0.11]
<i>Rattus rattus</i>	141.58 [133.23 – 149.67]	3.97 [3.84 – 4.09]	0.11 [-10.95 – 10.33]	-0.01 [-0.14 – 0.11]
<i>Rattus norvegicus</i>	103.47 [93.30 – 113.70]	3.50 [3.34 – 3.65]	-0.57 [-11.53 – 11.13]	-0.00 [-0.19 – 0.19]
<i>Suncus murinus</i>	33.16 [23.56 – 42.95]	2.22 [2.08 – 2.38]	1.62 [-1.14 – 4.45]	-0.01 [-0.16 – 0.16]

Table S2: Proportion of variance in Bray Curtis and weighted UniFrac beta diversity of microbial assemblages from distinct host species explained by land use intensity (LUI) and spatial proximity of capture locations in Generalised dissimilarity models. Means and 95% confidence intervals of the variance explained are reported for those iteratively fitted models that returned estimates (models did not converge when fitted for both Bray Curtis and weighted UniFrac microbial assemblage dissimilarity in *Rattus rattus*).

Predictor variable	Rat species		
Bray Curtis			
	<i>Sundamys muelleri</i>	<i>Rattus rattus</i>	<i>Rattus norvegicus</i>
LUI	0.01 [0 – 0.03]	NA	7.14 [6.88 – 7.41]
Spatial proximity	0.38 [0.29 – 0.44]	NA	0
LUI $\cap$ Spatial proximity	0	NA	0
Unexplained	99.60 [99.50 – 99.70]	NA	92.80 [92.60 – 93.20]
Weighted UniFrac			
	<i>Sundamys muelleri</i>	<i>Rattus rattus</i>	<i>Rattus norvegicus</i>
LUI	0.01 [0.01 – 0.02]	NA	3.50 [3.31 – 3.76]
Spatial proximity	0.16 [0.12 – 0.22]	NA	0

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LUI $\cap$ Spatial proximity	0	NA	0
Unexplained	99.80 [99.80 – 99.90]	NA	96.50 [96.20 – 96.70]

Table S3: Analysis of similarity (ANOSIM) comparing microbiome assemblage of host species based on Bray Curtis and weighted UniFrac dissimilarity measures. The smallest R values and relative host species pairs are reported in bold; the most related species pairs and relative values are underlined.

Comparisons	Bray-Curtis		Weighted UniFrac	
	R value	p-value	R value	p-value
<i>Sundamys muelleri</i> – <i>Rattus rattus</i>	0.42	0.001	0.10	0.001
<i>Sund. muelleri</i> – <i>R. norvegicus</i>	0.69	0.001	0.33	0.001
<u><i>R. rattus</i> – <i>R. norvegicus</i></u>	<u>0.72</u>	<u>0.001</u>	<u>0.29</u>	<u>0.001</u>
<i>Sund. muelleri</i> – <i>Suncus murinus</i>	0.86	0.001	0.81	0.001
<i>R. rattus</i> – <i>Sunc. murinus</i>	0.91	0.001	0.78	0.001
<i>R. norvegicus</i> – <i>Sunc. murinus</i>	0.70	0.001	0.65	0.001

Table S4: results of Tukey's HSD (Honest Significant Difference) test used to compare beta dispersion among host species. The difference in terms of observed means for each pair of species, with 95% CI, and the adjusted p-values are reported on the left for Bray-Curtis and on the right for weighted UniFrac.

	Bray-Curtis		Weighted UniFrac	
	Difference in observed means [CI]	Adjusted p- value	Difference in observed means [CI]	Adjusted p-value
<i>Sundamys muelleri</i> – <i>Rattus rattus</i>	0.02 [0.01, 0.04]	0.25	0.01 [- 0.02, 0.04]	0.75
<i>Sund. muelleri</i> – <i>R. norvegicus</i>	0.05 [0.03, 0.08]	< 0.001 ***	0.03 [0, 0.06]	0.10
<i>R. rattus</i> – <i>R. norvegicus</i>	0.04 [0.01, 0.06]	< 0.01 **	0.02 [- 0.01, 0.05]	0.47
<i>Sund. muelleri</i> – <i>Suncus murinus</i>	< 0.01 [- 0.02, 0.03]	1	< 0.01 [- 0.03, 0.04]	1
<i>R. rattus</i> – <i>Sunc. murinus</i>	0.01 [- 0.01, 0.04]	0.46	0.02 [- 0.02, 0.05]	0.58
<i>R. norvegicus</i> – <i>Sunc. murinus</i>	0.05 [0.02, 0.08]	< 0.001 ***	0.03 [0, 0.07]	0.06

Table S5: Results from generalized linear models indicating the effect of land use intensity on Bray-Curtis dispersion (on the left) and weighted UniFrac dispersion (on the right), for each studied small mammal species.

	Bray-Curtis dispersion				Weighted UniFrac dispersion			
	Estimate	SE	T value	p-value	Estimate	SE	T value	p-value
	<i>Sundamys muelleri</i>							
Intercept	0.62	0.01	55.94	< 0.001 ***	0.19	0.02	9.37	< 0.001 ***
LUI	0.01	0.03	0.26	0.80	0.01	0.05	0.29	0.77
	<i>Rattus rattus</i>							
Intercept	0.60	0.02	31.70	< 0.001 ***	0.20	0.03	6.49	< 0.001 ***
LUI	0	0.04	-0.11	0.92	0.02	0.06	0.27	0.79
	<i>Rattus norvegicus</i>							
Intercept	0.74	0.05	13.88	< 0.001 ***	0.29	0.04	6.86	< 0.001 ***
LUI	-0.24	0.07	-3.36	< 0.01 **	-0.10	0.06	-1.64	0.11
	<i>Suncus murinus</i>							
Intercept	0.58	0.03	17.15	< 0.001 ***	0.18	0.05	3.48	< 0.01 **
LUI	0.06	0.06	1.03	0.31	0.03	0.09	0.27	0.79

Table S6: differentially abundant ASVs in relation to LUI identified in the four studied species using ANCOM-BC2 method ($adj\ p < 0.05$). ASVs that passed the sensitivity test for pseudo-count addition are reported in bold.

(LFC: log fold change; SE: standard error; SS: sensitivity test).

	Species	Host species	LFC	SE	W test	Adj p	SS
1	<i>Prevotellaceae spp.</i>	<i>Sundamys muelleri</i>	-14.39	1.62	-8.88	0.025	FALSE
2	<i>Desulfovibrionaceae spp.</i>	<i>Sundamys muelleri</i>	-12.40	1.61	-7.68	0.025	FALSE
3	<i>Clostridium perfringens</i>	<i>Sundamys muelleri</i>	18.35	1.64	11.20	0.050	FALSE
4	<i>Prevotellaceae UCG-001 spp.</i>	<i>Rattus norvegicus</i>	-8.00	0.74	-10.75	0.005	FALSE
5	<i>Muribaculaceae spp.</i>	<i>Rattus norvegicus</i>	-7.81	0.72	-10.92	0.005	FALSE
6	<i>Bacteroides sartorii</i>	<i>Rattus norvegicus</i>	-7.73	0.81	-9.54	< 0.001	TRUE
7	<i>Holdemanella spp.</i>	<i>Rattus norvegicus</i>	-7.57	0.71	-10.70	0.001	FALSE
8	<i>Alloprevotella spp.</i>	<i>Rattus norvegicus</i>	-6.66	0.86	-7.73	0.017	FALSE
9	<i>Terrisporobacter spp.</i>	<i>Rattus norvegicus</i>	-6.65	0.71	-9.34	0.005	TRUE
10	<i>Bacteroides spp.</i>	<i>Rattus norvegicus</i>	-6.49	0.70	-9.31	0.022	FALSE
11	<i>Paeniclostridium spp.</i>	<i>Rattus norvegicus</i>	-6.37	0.81	-7.86	0.017	FALSE
12	<i>Lachnospiraceae spp.</i>	<i>Rattus norvegicus</i>	-5.27	0.75	-7.05	0.013	FALSE
13	<i>Erysipelotrichaceae spp.</i>	<i>Rattus norvegicus</i>	-5.26	0.75	-7.04	0.036	FALSE
14	<i>Bacteroides rodentium</i>	<i>Rattus norvegicus</i>	-5.21	0.72	-7.27	0.035	FALSE
15	<i>Lachnospiraceae NK4A136 group spp.</i>	<i>Rattus norvegicus</i>	-4.91	0.71	-6.88	0.022	FALSE
16	<i>Eubacterium siraeum group spp.</i>	<i>Rattus norvegicus</i>	-4.88	0.75	-6.52	0.023	FALSE
17	<i>Muribaculaceae spp.</i>	<i>Rattus norvegicus</i>	-4.71	0.70	-6.72	0.039	FALSE
18	<i>Treponema succinifaciens</i>	<i>Rattus norvegicus</i>	-4.39	0.94	-4.69	0.010	FALSE
19	<i>Eubacterium coprostanoligenes group spp.</i>	<i>Rattus norvegicus</i>	-4.15	0.75	-5.57	0.013	TRUE
20	<i>Prevotellaceae UCG-001 spp.</i>	<i>Rattus norvegicus</i>	-4.14	0.74	-5.61	0.011	FALSE

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21	<i>Blautia</i> spp.	<i>Rattus norvegicus</i>	-4.10	0.73	-5.62	0.022	FALSE
22	<i>Muribaculaceae</i> spp.	<i>Rattus norvegicus</i>	-4.09	0.69	-5.89	0.011	FALSE
23	<i>Bacteroides</i> spp.	<i>Rattus norvegicus</i>	-4.07	0.97	-4.21	0.035	TRUE
24	<i>Rikenellaceae</i> RC9 group spp.	<i>Rattus norvegicus</i>	-4.00	0.85	-4.72	0.013	FALSE
25	<i>Prevotellaceae</i> UCG-001 spp.	<i>Rattus norvegicus</i>	-3.98	0.83	-4.80	0.008	FALSE
26	<i>Gastranaerophilales</i> spp.	<i>Rattus norvegicus</i>	-3.88	0.72	-5.42	0.035	FALSE
27	<i>Lactococcus garvieae</i>	<i>Rattus norvegicus</i>	-3.79	0.76	-4.98	0.041	FALSE
28	<i>Lachnospiraceae</i> spp.	<i>Rattus norvegicus</i>	-3.56	0.70	-5.11	0.040	FALSE
29	<i>Prevotellaceae</i> NK3B31 group spp.	<i>Rattus norvegicus</i>	-3.36	0.74	-4.55	0.047	FALSE
30	<i>Muribaculaceae</i> spp.	<i>Rattus norvegicus</i>	-3.28	0.72	-4.54	0.010	FALSE
31	CAG-352 spp.	<i>Rattus norvegicus</i>	-3.17	0.73	-4.35	0.019	FALSE
32	UCG-005 spp.	<i>Rattus norvegicus</i>	-3.08	0.74	-4.14	0.022	FALSE
33	<i>Lachnospiraceae</i> spp.	<i>Rattus norvegicus</i>	-2.94	0.76	-3.88	0.043	FALSE
34	<i>Prevotella</i> spp.	<i>Rattus norvegicus</i>	-2.88	0.72	-3.98	0.041	FALSE
35	<i>Treponema berlinense</i>	<i>Rattus norvegicus</i>	-2.79	0.81	-3.43	0.041	FALSE
36	<i>Bacteroides vulgatus</i>	<i>Rattus norvegicus</i>	-2.76	0.87	-3.17	0.035	FALSE
37	<i>Coprococcus</i> spp.	<i>Rattus norvegicus</i>	-2.74	0.71	-3.85	0.043	FALSE
38	<i>Lachnospira</i> spp.	<i>Rattus norvegicus</i>	-2.73	0.86	-3.18	0.046	FALSE
39	<i>Muribaculaceae</i> spp.	<i>Rattus norvegicus</i>	-2.68	0.71	-3.78	0.021	FALSE
40	NK4A214 group spp.	<i>Rattus norvegicus</i>	-2.57	0.71	-3.63	0.043	FALSE
41	NK4A214 group spp.	<i>Rattus norvegicus</i>	-2.56	0.69	-3.72	0.046	FALSE
42	<i>Bacteroides</i> spp.	<i>Rattus norvegicus</i>	-2.56	0.70	-3.66	0.043	FALSE
43	<i>Lachnospiraceae</i> spp.	<i>Rattus norvegicus</i>	-2.49	0.71	-3.52	0.039	FALSE
44	<i>Oscillibacter</i> spp.	<i>Rattus norvegicus</i>	-2.44	0.70	-3.48	0.035	FALSE
45	<i>Ruminococcus torques</i> group spp.	<i>Rattus norvegicus</i>	-2.19	0.69	-3.19	0.048	FALSE
46	<i>Parabacteroides</i> spp.	<i>Rattus norvegicus</i>	-2.08	0.70	-2.99	0.049	FALSE

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47	<i>Romboutsia</i> spp.	<i>Rattus norvegicus</i>	2.09	0.71	2.96	0.046	TRUE
48	<i>Phascolarctobacterium</i> spp.	<i>Rattus norvegicus</i>	2.22	0.70	3.17	0.046	FALSE
49	<i>Anaerostipes</i> spp.	<i>Rattus norvegicus</i>	2.42	0.71	3.42	0.048	TRUE
50	<i>Romboutsia</i> spp.	<i>Rattus norvegicus</i>	2.46	0.88	2.82	0.043	FALSE
51	<i>Bacteroides</i> spp.	<i>Rattus norvegicus</i>	2.81	0.85	3.29	0.048	FALSE
52	<i>Bacteroidales</i> RF16 group spp.	<i>Rattus norvegicus</i>	2.82	0.73	3.88	0.023	FALSE
53	<i>Veillonella</i> spp.	<i>Rattus norvegicus</i>	2.91	0.74	3.92	0.013	FALSE
54	<i>Bacteroides</i> spp.	<i>Rattus norvegicus</i>	2.97	0.71	4.20	0.029	TRUE
55	<i>Sutterella</i> spp.	<i>Rattus norvegicus</i>	3.00	0.81	3.69	0.013	TRUE
56	<i>Subdoligranulum</i> spp.	<i>Rattus norvegicus</i>	3.32	0.77	4.30	0.026	TRUE
57	<i>Collinsella</i> spp.	<i>Rattus norvegicus</i>	3.71	0.84	4.41	0.022	TRUE
58	<i>Eubacterium nodatum</i> group spp.	<i>Rattus norvegicus</i>	3.72	0.69	5.37	0.023	FALSE
59	<i>Lactobacillus</i> spp.	<i>Rattus norvegicus</i>	3.74	1.39	2.69	0.047	TRUE
60	<i>Lactobacillus</i> spp.	<i>Rattus norvegicus</i>	3.79	0.75	5.04	0.017	FALSE
61	<i>Fusobacterium</i> sp.	<i>Rattus norvegicus</i>	3.90	0.70	5.56	0.022	FALSE
62	<i>Succinivibrio</i> spp.	<i>Rattus norvegicus</i>	3.96	0.79	5.01	0.005	FALSE
63	<i>Prevotellaceae</i> NK3B31 group spp.	<i>Rattus norvegicus</i>	4.10	0.74	5.51	0.035	FALSE
64	<i>Staphylococcus</i> spp.	<i>Rattus norvegicus</i>	4.17	0.81	5.17	0.005	FALSE
65	<i>Oscillospiraceae</i> spp.	<i>Rattus norvegicus</i>	4.22	0.70	6.00	0.019	FALSE
66	<i>Erysipelotrichaceae</i> spp.	<i>Rattus norvegicus</i>	4.48	0.87	5.18	0.017	FALSE
67	<i>Mogibacterium</i> spp.	<i>Rattus norvegicus</i>	4.81	0.71	6.75	0.022	FALSE
68	<i>Clostridium sensu stricto</i> 1 spp.	<i>Rattus norvegicus</i>	5.05	0.99	5.13	0.002	TRUE
69	<i>CAG-352</i> spp.	<i>Rattus norvegicus</i>	5.12	0.70	7.33	0.035	FALSE
70	<i>Bacteroides</i> spp.	<i>Rattus norvegicus</i>	5.47	0.71	7.71	0.017	FALSE
71	<i>Ruminococcus</i> spp.	<i>Rattus norvegicus</i>	5.57	0.71	7.83	0.011	FALSE
72	<i>Eubacterium hallii</i> group spp.	<i>Rattus norvegicus</i>	6.12	0.70	8.74	0.024	TRUE

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73	<i>Eubacterium coprostanoligenes group spp.</i>	<i>Rattus norvegicus</i>	6.33	0.71	8.94	0.013	FALSE
74	<i>Helicobacter cholecystus</i>	<i>Rattus norvegicus</i>	6.73	0.69	9.71	0.006	FALSE
75	<i>Gastranaerophilales spp.</i>	<i>Rattus norvegicus</i>	12.58	0.70	17.90	0.010	FALSE
76	<i>Alloprevotella spp.</i>	<i>Rattus norvegicus</i>	16.84	0.71	23.70	0.006	FALSE
77	<i>Weissella spp.</i>	<i>Suncus murinus</i>	-13.97	2.14	-6.54	0.003	FALSE
78	<i>Turicibacter spp.</i>	<i>Suncus murinus</i>	-8.30	2.03	-4.09	0.011	FALSE
79	<i>Candidatus Amphibiichlamydia</i>	<i>Suncus murinus</i>	-8.28	2.23	-3.72	0.033	FALSE
80	<i>Enterobacteriaceae spp.</i>	<i>Suncus murinus</i>	-7.21	2.15	-3.36	0.039	FALSE
81	<i>Clostridium baratii</i>	<i>Suncus murinus</i>	9.11	2.13	4.28	0.040	FALSE
82	<i>Clostridium sensu stricto 1 spp.</i>	<i>Suncus murinus</i>	15.26	2.20	6.93	0.033	FALSE
83	<i>Cellulosilyticum spp.</i>	<i>Suncus murinus</i>	17.64	2.13	8.29	0.003	FALSE
84	<i>Romboutsia spp.</i>	<i>Suncus murinus</i>	18.68	2.13	8.78	0.003	FALSE