

Supplementary Material

Appendix S1. Read numbers and total base pairs for each barcode in the January 2017 sequencing run.

Sample	Total reads	Total Mbp	Mean length
OB2	19907	14.62	734
WP11	10164	9.63	947
WP5	8237	6.78	823
LB7	7548	7.04	933
OB13	3644	3.63	995
WP9	2954	2.4	814
OB5	2850	2.06	721
WP8	2801	2.32	827
LB6	2531	1.6	632
OB7	2473	1.87	756
LB5	1641	1.16	705
LB3	1554	0.99	636
None	16673	13.01	781
Total	82977	67.1	809

Appendix S2. Read numbers and total base pairs for each barcode in the March 2017 sequencing run.

Sample	Total reads	Total Mbp	Mean length
LB1	17820	9.21	517
LB8	16923	13.13	776
WP2	10511	7.00	666
LB4	8684	4.92	567
OB11	5689	3.40	598
WP10	1563	0.99	633
OB12	1479	0.89	604
WP12	1309	0.78	596
LB2	1127	0.76	676
WP3	637	0.73	1141
OB14	541	0.37	683
OB10	435	0.24	555
None	29432	21.33	725
Total	96150	63.75	663

Appendix S3. Characteristics of alignments for reads assigned to the Primate family. Many reads are both long and have high identity, suggesting that they are not false positive assignments, but contamination.

Rat	Read length	Mean read accuracy	% ID	Alignment length	Genus
WP10	428	0.91	94.0	314	<i>Homo</i>
WP10	782	0.92	93.8	657	<i>Homo</i>
OB5	365	0.90	93.2	249	<i>Homo</i>
LB3	515	0.95	92.6	462	<i>Homo</i>
WP10	510	0.90	92.0	460	<i>Homo</i>
WP10	704	0.90	91.1	587	<i>Homo</i>
WP10	467	0.89	90.3	402	<i>Homo</i>
WP10	494	0.89	89.7	388	<i>Homo</i>
WP10	339	0.88	89.6	269	<i>Homo</i>
WP10	446	0.88	89.6	326	<i>Homo</i>
WP10	327	0.91	89.1	210	<i>Homo</i>
OB5	415	0.89	88.8	277	<i>Homo</i>
OB5	561	0.86	88.7	257	<i>Homo</i>
WP11	434	0.86	88.7	301	<i>Homo</i>
WP10	486	0.88	88.2	365	<i>Homo</i>
WP10	613	0.90	88.2	526	<i>Homo</i>
WP10	563	0.87	88.2	457	<i>Homo</i>
WP10	1373	0.91	87.7	1337	<i>Homo</i>
WP11	526	0.91	87.5	473	<i>Homo</i>
OB14	478	0.89	86.9	373	<i>Homo</i>
OB5	715	0.89	86.8	673	<i>Homo</i>
WP10	475	0.87	86.7	362	<i>Homo</i>
WP10	398	0.86	86.5	259	<i>Homo</i>
WP10	377	0.88	85.3	251	<i>Homo</i>
WP9	558	0.87	85.2	508	<i>Homo</i>
WP10	429	0.86	84.8	276	<i>Homo</i>
WP10	322	0.81	84.5	174	<i>Homo</i>
WP8	723	0.84	83.1	438	<i>Homo</i>
LB8	965	0.86	80.4	245	<i>Rhinopithecus</i>
LB5	3042	0.94	79.4	1018	<i>Cebus</i>
WP2	464	0.93	77.3	216	<i>Homo</i>
LB8	671	0.90	73.9	406	<i>Aotus</i>

Appendix S4. SIMPER analysis of family contributions to group similarities.

Family	Average Abundance	Average Similarity	Similarity/SD	Percentage contribution	Group
Hymenolepididae	3.37	6.87	0.34	51.2	LB
Solanaceae	1.57	1.48	0.34	11.1	LB
Fabaceae	1.74	1.41	0.44	10.5	LB
Arecaceae	2.86	7.11	1	33.4	OB
Poaceae	2.87	4.82	0.55	22.7	OB
Fabaceae	1.17	1.98	0.51	9.3	OB
Phasianidae	1.79	1.67	0.34	7.9	OB
Poaceae	5.08	17.61	0.62	72.1	WP

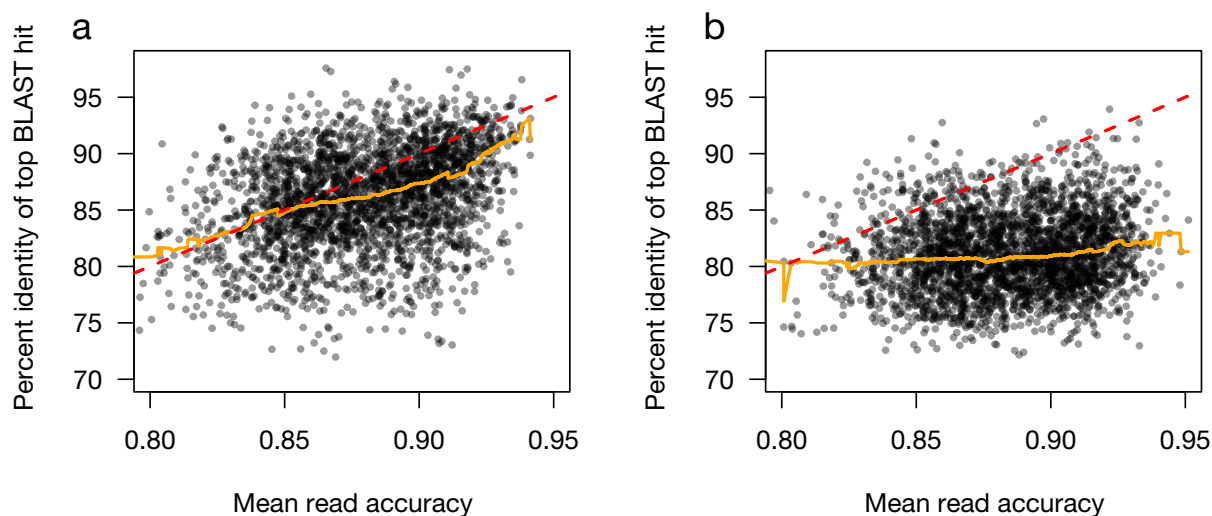
Appendix S5. SIMPER analysis of family contributions to group dissimilarities.

Species	Av Abund Group1	Avg Abund Group2	Avg. Diss	Diss/SD	% contrib	Group1	Group2
Poaceae	1.95	5.08	15.15	1.04	16.74	LB	WP
Poaceae	2.87	5.08	11.29	1.26	13.78	OB	WP
Hymenolepididae	3.37	0.48	10.8	0.73	11.93	LB	WP
Hymenolepididae	3.37	0.29	9.37	0.79	10.32	LB	OB
Poaceae	1.95	2.87	8.37	1.1	9.22	LB	OB
Arecaceae	0.05	2.86	6.99	1.41	7.7	LB	OB
Arecaceae	2.86	1.31	5.92	1.29	7.23	OB	WP
Fabaceae	1.74	1.05	6.14	0.67	6.78	LB	WP
Podocarpaceae	0	2.38	5.34	0.83	5.9	LB	WP
Podocarpaceae	0.71	2.38	4.82	0.99	5.88	OB	WP
Fabaceae	1.74	1.17	4.87	0.81	5.37	LB	OB
Fabaceae	1.17	1.05	4.31	0.84	5.26	OB	WP

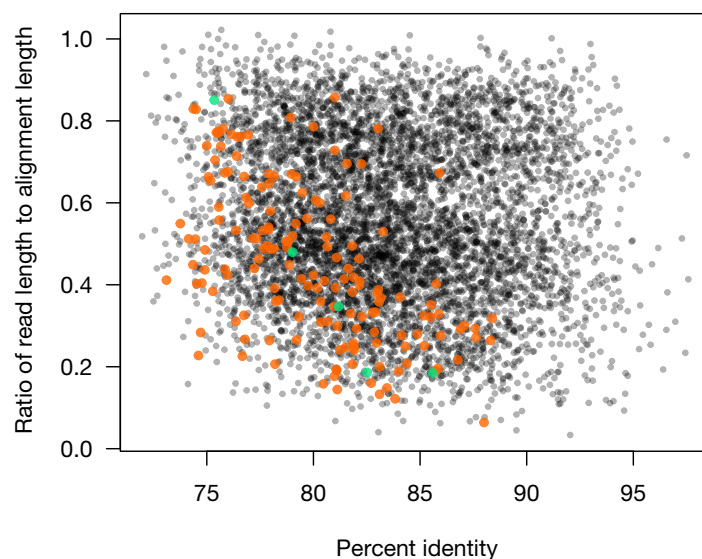
Appendix S6. Characteristics of alignments for reads that are likely false positive assignments. Most are short or have low identity, suggesting that they are false positive assignments. The exceptions are the reads matching Buthidae, which we hypothesise are due to the rats' predation of the sister taxa harvestmen. Octopodidae, Salmonidae, and Poeciliidae (guppies and similar aquaria fish) are possible but improbable prey items.

Rat	Read length	Mean read accuracy	% ID	Alignment length	Genus	MEGAN family
WP5	1285	0.90	82.9	298	Centruroides	Buthidae
OB11	1874	0.90	88.0	664	Centruroides	Buthidae
WP5	1711	0.93	79.3	762	Centruroides	Buthidae
WP11	859	0.93	86.1	151	Octopus	Octopodidae
WP2	516	0.86	81.4	172	Oncorhynchus	Salmonidae
OB12	424	0.90	85.7	140	Xiphophorus	Poeciliidae
OB12	643	0.84	89.3	177	Xiphophorus	Poeciliidae

Appendix S7. Correlation of read accuracy with alignment characteristics. Only rat reads exhibit a clear positive relationship between accuracy and percent identity. (a) indicates the relationship for reads assigned to *Rattus* and (b) for *Mus*. The orange line indicates a running median; the red dotted line is the $y = x$ line, which is expected if accuracy corresponds exactly to percent identity.



Appendix S8. Alignment characteristics of true positive and false positive taxon assignments at the family level. False positive taxon assignments, *Cricetideaa* (orange) and *Spalacidae* (green), have lower percent identity and shorter alignment lengths than true positive taxon assignments, e.g. *Muridae* (black). Only a single false positive taxon assignment had a read length to alignment length ratio greater than 0.5 and a percent identity greater than 85%. This suggests that further filters or methodologies (e.g. decision tree analysis using different read and alignment characteristics) could, if necessary, decrease false positive rates even further.



Appendix S9. Table of read BLAST hits and assigned MEGAN taxa with reads reclassified at the family or order level by filtering on read length to alignment length ratio and percent identity; in attached .txt file.

Appendix S10. Table of read BLAST hits and assigned MEGAN taxa with no filters applied; in attached .txt file.

