Supplementary Information for

Fur seal microbiota are shaped by the social and physical environment, show mother-offspring similarities and are associated with host genetic quality

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Datasets S1 to S18

Supplementary Information Text

Material and Methods

Bacterial DNA extraction protocol

Kit: Mo Bio BiOstic Bacteremia DNA Isolation Kit
Step 1: Vortex sample tube (swab in Amies medium) and spin down to collect liquid at bottom of tube. Remove swab and transfer liquid into new 2 mL collection tube (ca 800 µL). Centrifuge at 13,000 x g for 2 min to pellet bacteria and discard supernatant (biohazard waste).
Step 2: Add 450 µL solution CB1 (pre-warmed to 55°C) and resuspend pellet by pipetting up and down. Transfer lysate into 2 mL MicroBead Tube. Vortex 10 sec and place in 70°C water bath for 15 min.
Step 3: Transfer tube to homogeniser and shake for 10 min at 50Hz.
Step 4: Centrifuge tube to pellet debris at 10,000 x g for 1 min. Transfer supernatant to new 2 mL collection tube.
Step 5: Add 100 µL solution CB2 and vortex. Incubate for 5 min at RT and centrifuge at 10,000 x g for 1 min. Transfer supernatant to new collection tube.
Step 6: Add 1 mL of solution CB3. Vortex and pulse centrifuge to collect all liquid from lid.
Step 7: Load 530 µL lysate onto Spin filter column and centrifuge at 10,000 x g for 1 min. Discard flow-through and place filter column back into collection tube. Repeat until all lysate is loaded.
Step 8: Transfer filter column to new collection tube and wash with 500 µL solution CB4. Spin at 10,000 x g for 1 min. Discard flow-through and place filter column back into collection tube.
Step 9: Repeat wash step as above (step 8).
Step10: Centrifuge at 13,000 x g for 2 min to dry filter column.
Step11: Transfer filter column to new collection tube. Elute with 50 µL solution CB5 (do not heat) directly onto centre of the column membrane. Incubate at RT for 5 min. Centrifuge at 10,000 x g for 1 min.
Step 12: Discard filter column and store genomic DNA at -20 to -80°C.

16S V3-V4 amplicon sequencing

For the amplicon PCR (amplification of the 16S target region) each reaction contained 1X KAPA HiFi HotStart Ready Mix (KAPA Biostystems, Wilmington, Massachusetts), 0.1 µM of each primer, and 5.0 µL of bacterial DNA extract in a final volume of 25 µL. The cycling conditions were: initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and a final extension step at 71°C for 5 min. A positive control of 10^6 *E. coli* cells, and a negative control containing PCR-grade water were run along-side the samples. PCR results were inspected by running 5 µL of product on a 2% agarose gel. For samples, which showed very strong bands accompanied by a smear, PCR was repeated with half the initial volume of DNA extract. For samples, with very faint bands, PCR was repeated and the products of both amplifications pooled together. Amplicon PCR products were cleaned using AMPure XP beads following the Illumina 16S Metagenomics Sequencing Library Preparation guideline document. Elution volume of 10 mM Tris-HCl pH 8.0 varied between 35 and 20 µL depending of the strength of the bands observed on the gel. After the cleanup, 1 µL of PCR product was run on a Bioanalyzer DNA 1000 chip (Agilent Technologies, Inc., Waldbronn, Germany) for each sample to verify the size of the product at ~550 bp. Dual-indeces and Illumina sequencing adapters were attached to the amplicons during index PCR

using the Nextera XT Index Kit (lllumina, Inc., San Diego, CA, USA). Each 50 µL reaction contained 1X KAPA HiFi HotStart Ready Mix, 5 µL of amplicon PCR product, and 5 µL of index Primer 1 (N7XX) and Primer 2 (N5XX); the thermocycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 8 cycles of denaturation at 95° C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec, and a final extension step at 72°C for 5 min. Index PCR cleanup was performed as described above with an elution volume of 27.5 µL. For each sample 1 µL of a 1:50 dilution was run on a Bioanalyzer DNA 1000 chip to verify the expected size of ~630 bp. Quantification of all libraries was performed using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Molecular Probes, Eugene, OR, USA) on a TECAN Infinite Reader M200. Very highly concentrated samples (as calculated from the Bioanalyzer readings) were diluted 1:10 prior to the assay. The DNA concentration in nM was calculated as described in the Illumina 16S Metagenomics Sequencing Library Preparation guideline document based on the average library size reading from the Bioanalyzer, and the DNA concentration in ng/µL determined with the PicoGreen assay. All 96 libraries were then diluted to a final DNA concentration of 4 nM and pooled for the MiSeq run.

OTU generation pipeline

Forward and reverse reads were first merged into a single sequence using the -fastq_mergepairs command with the following parameters: staggered pairs were discarded, 3' ends of each read were truncated at the first Q score ≤5, aligned regions were allowed a maximum difference of 25% and 30 mismatches, and minimum length and maximum length were restricted to 380 and 520, respectively (the V3-V4 target region is expected to be ~460 bp long but can vary substantially in length in different bacterial taxa). Due to the suboptimal quality of the raw reads a high percentage of mismatches was allowed during the merging to increase the number of total merged sequences. Sequences that contained a high error rate as a result of this low threshold were filtered during the very stringent quality filtering step and are not expected to cause problems in downstream analyses. Then, forward and reverse primers were removed from the merged sequences using Cutadapt version 1.9.1 (Martin, 2011) in two consecutive steps. First, the forward primer, V3-V4-341F, was removed using the 5' anchor flag (-g ^). Simultaneously, we searched potential reverse-complemented sequences for the reverse primer, V3-V4-805R, in forward orientation with the 5' anchor flag. Sequences that were trimmed with the reverse primer flag were reverse complemented with the fastx_reverse_complement command from the FastX toolkit version 0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/). Second, the reverse-complemented reverse primer was removed using a 3' anchor flag (-a $; or forward primer with 5' anchor flag). Both trimming steps allowed for a maximum error rate of 0.2, and required a minimum overlap of 10 bp. Sequences that were not trimmed in either step were discarded from further analyses. After primer trimming, low quality sequences were filtered using the USEARCH -fastq_filter command with an expected error threshold of 1.0 (Edgar & Flyvbjerg, 2015). High quality sequences were then dereplicated to retain only unique sequences using the -fastx_uniques command. We clustered all sequences that were ≥97% identical to each other into 97% operational taxomonic units (OTUs) using the cluster_otus command with -minsize 2 to remove singleton sequences. This command includes the removal of chimeric reads thus no separate chimera removal was performed. OTUs were classified using the sintax command (Edgar, 2016) with the RDP training set with species names (provided on the USEARCH manual pages) and a confidence cutoff of 0.8. This database was given preference over larger more commonly used 16S datasets (e.g. SILVA or Greengenes) based on the USEARCH manual recommendations to choose a small database with

authoritative classification over a large databases containing computational predictions for taxa (which would result in low confidence annotations when using another prediction algorithm like sintax for the classification of OTUs). We then searched for OTUs with low confidence classifications at domain and phylum level (bootstrap values <1.0) and aligned the corresponding sequences against the NCBI nt database using BLASTn from the standalone blast+ tools version 2.6.0 (Camacho et al., 2009). Alignment hits that did not contain the terms "16S" or "bacterium" were manually inspected and subjected to reciprocal BLASTn searches against the web-based NCBI nt database. Sequences that could not be assigned to bacterial 16S were discarded. Additionally, we searched the sintax classifications for chloroplast and mitochondrial assignments and discarded the associated OTUs. Overall, 63 OTUs were removed from the dataset. Finally, an OTU table was generated for the final set of OTUs by mapping the merged and trimmed raw reads (not quality filtered) of each sample against the OTUs with a 97% identity threshold using the usearch_global command. OTUs that were represented by less then 0.005% of all reads (Bokulich et al., 2013) were trimmed from the table (-min_otu_freq 0.00005).

Analysis of OTUs

We calculated the Jost index as a measure for alpha-diversity. The Jost index is based on a family of metrics called Hill numbers of parameter q, where q determines how abundance is weighted (Chao, Chiu, & Jost, 2010). These indices are transformed into the effective number of species (which is defined as the number of equally-common species required to give a particular value of an index). If q = 0, it is equivalent to species richness, which is a commonly used diversity index in microbiome studies, but is invalid for our analysis due to the removal of singletons in the OTU clustering step. Thus we report the Jost index of order 1 (q = 1), which is equivalent to the Shannon index and measures the number of common OTUs in the sample (Chao et al., 2010).

Functional analysis of OTUs

In order to provide insights into the functional capacity of the Antarctic fur seal skin microbiome, we utilised the PICRUSt software package (Langille et al., 2013) on the Langille Lab PICRUSt Galaxy Instance (Afgan et al., 2016) to predict metagenome functional content from our 16S rRNA amplicons. PICRUSt analysis requires OTUs generated from closed-reference picking. Thus, we created a new OTU table from the merged and primer-trimmed sequences using the pick_closed_reference_otus.py script in QIIME based on OTUs with at least 97% identity to the Greengenes database v. 3.18. The table was trimmed with the filter_otus_from_otu_table.py to remove OTUs with less than 0.005% of all reads mapped. We then rarefied the OTU table to the smallest read count (3,117) and uploaded it to the Galaxy server. Next we normalised the table using normalize_by_copy_number.py to account for variantion in 16S copy number abundance. Functional predictions were performed by running predict_metagenomes.py with KEGG Orthologs. The accuracy of the predictions was assessed by calculating the nearest sequenced taxon index (NSTI), which is defined as the sum of phylogenetic distances for each organism in the OTU table to its nearest relative with a sequenced reference genome (Langille et al., 2013). The overall NSTI score of 0.075 ± 0.022 SD calculated for our samples was deemed sufficient for reliable functional predictions (Langille et al., 2013). The predicted metagenomes were collapsed to pathway hierarchy level 3 using categorize_by_function.py. The resulting .biom file was then transformed into a STAMP-profile using biom_to_stamp.py (Comeau, Douglas, & Langille, 2017) and analysed within STAMP v. 2.1.3 (Parks, Tyson, Hugenholtz, & Beiko, 2014). We performed a Welch's t-test (two-sided) to test for significant differences in the mean

proportions of KEGG orthologue counts between FWB and SSB and subjected the resulting *p*-values to FDR correction according to (Benjamini & Hochberg, 1995). Differences in functional predictions among fur seal individuals were visualised by principal component analysis (PCA). We converted the .biom file from the categorize_by_function.py output into a .txt file and PCA was performed with the prcomp function in R. Prior to PCA, we removed KEGG categories that were not represented by at least one hit. The data were also Log(x+0.0001)-transformed as described above and centred and scaled to zero mean and unit variance. Separate PCAs were performed for hierarchical levels 1 to 3.

Results

PICRUSt functional predictions

In order to run the PICRUSt functional analysis we generated a new OTU table using closed-reference picking against the Greengenes database v. 3.18. This new OTU table contained 761 OTUs (after trimming) with 2,460,219 mapped sequences (mean depth 25,627). The results of the PICRUSt analysis suggest that the observed difference in bacterial composition between the two breeding colonies is also reflected at the functional level. PCA performed with functional predictions at hierarchical level 3 grouped the beaches into two partially overlapping clusters along PC1 and PC2 (Figure S9). KEGG orthologues within “Metabolism”, “Cellular Processes”, and “Organismal Systems” top-level functional pathways were significantly more abundant at FWB ($p<0.01$), whereas “Genetic Information Processing” was significantly enriched at SSB (Figure S10). At 3rd level hierarchical pathways both breeding colonies differed significantly in 157 categories (Figure S11).

Supplementary Figures

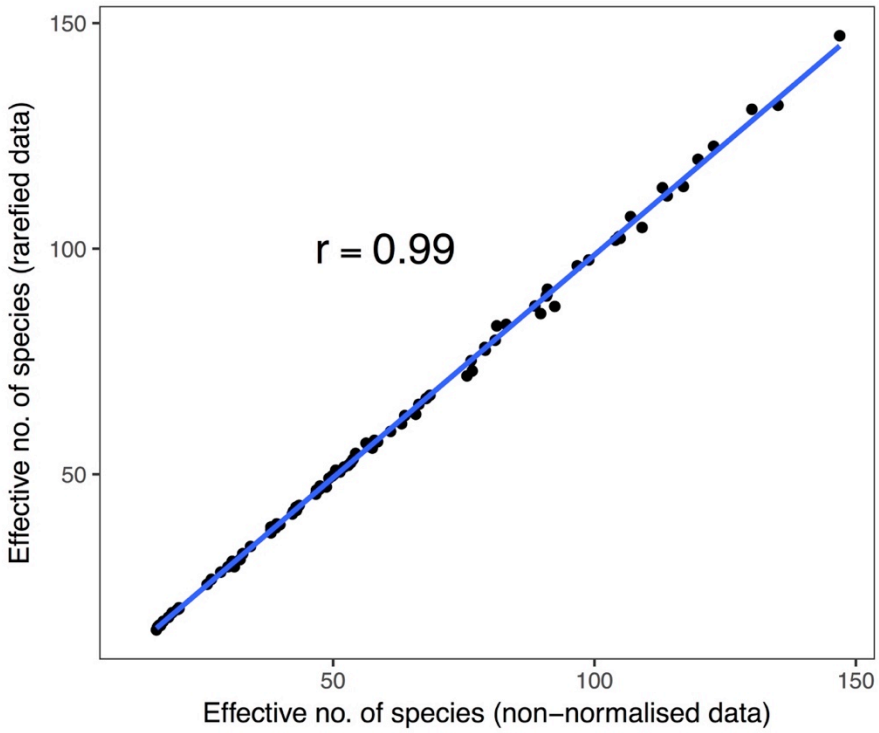

Figure S1. Correlation of alpha diversity estimates calculated from the non-normalised and rarefied OTU tables.

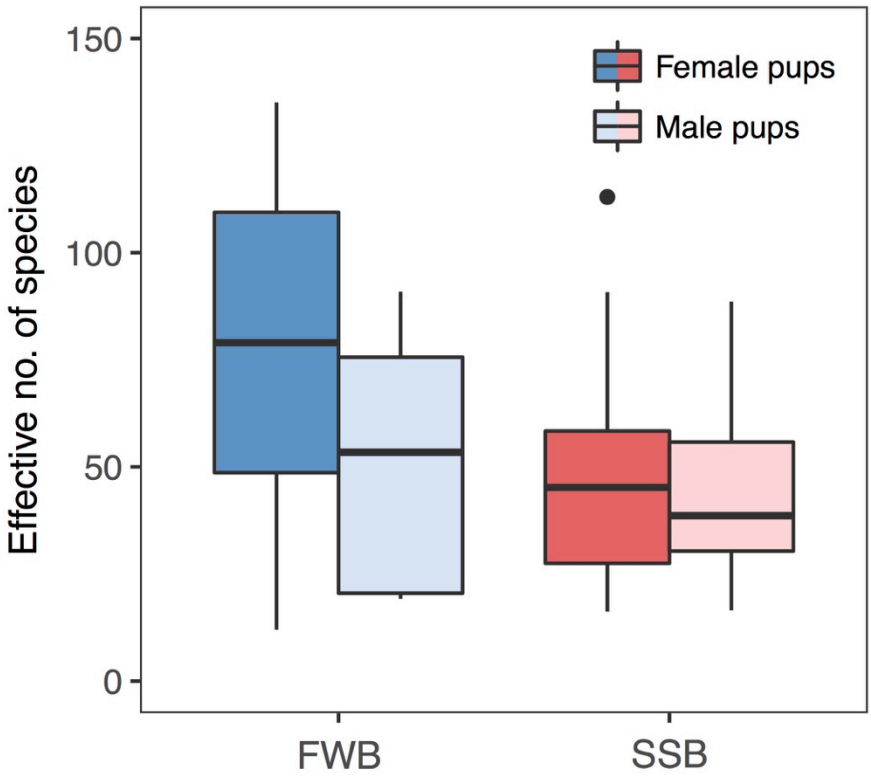

Figure S2. Comparison of alpha diversity estimates (given as effective number of bacterial species) between male and female pups. Data are shown separately for the two breeding colonies (FWB: freshwater beach, SSB: special study beach).

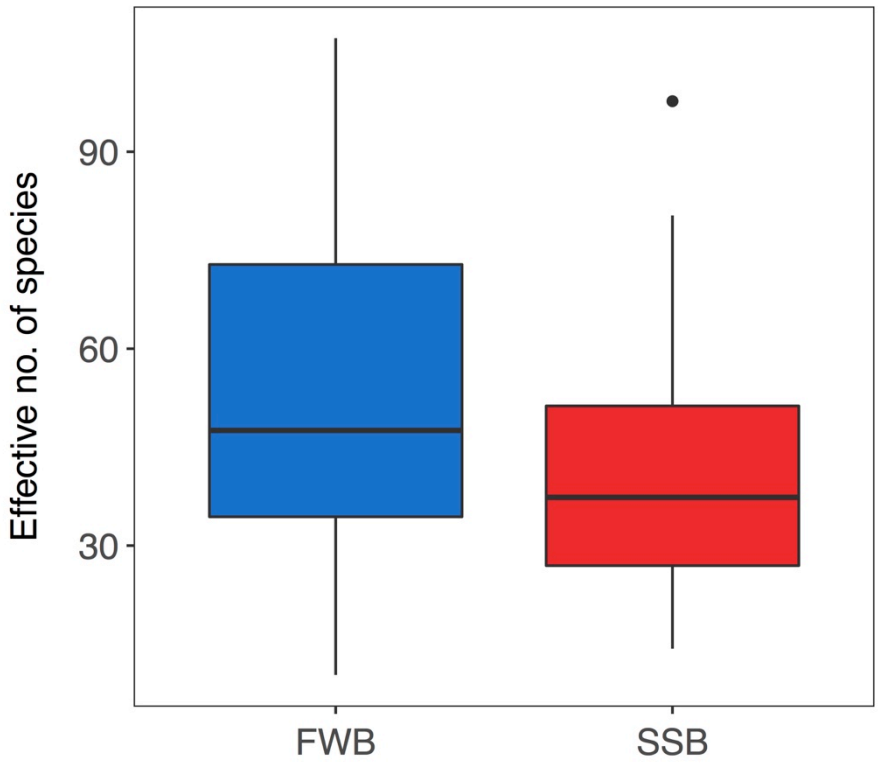

Figure S3. Comparison of alpha diversity estimates (given as effective number of bacterial species) between the two breeding colonies (FWB: freshwater beach, SSB: special study beach) based on OTUs belonging to the four dominant phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria.

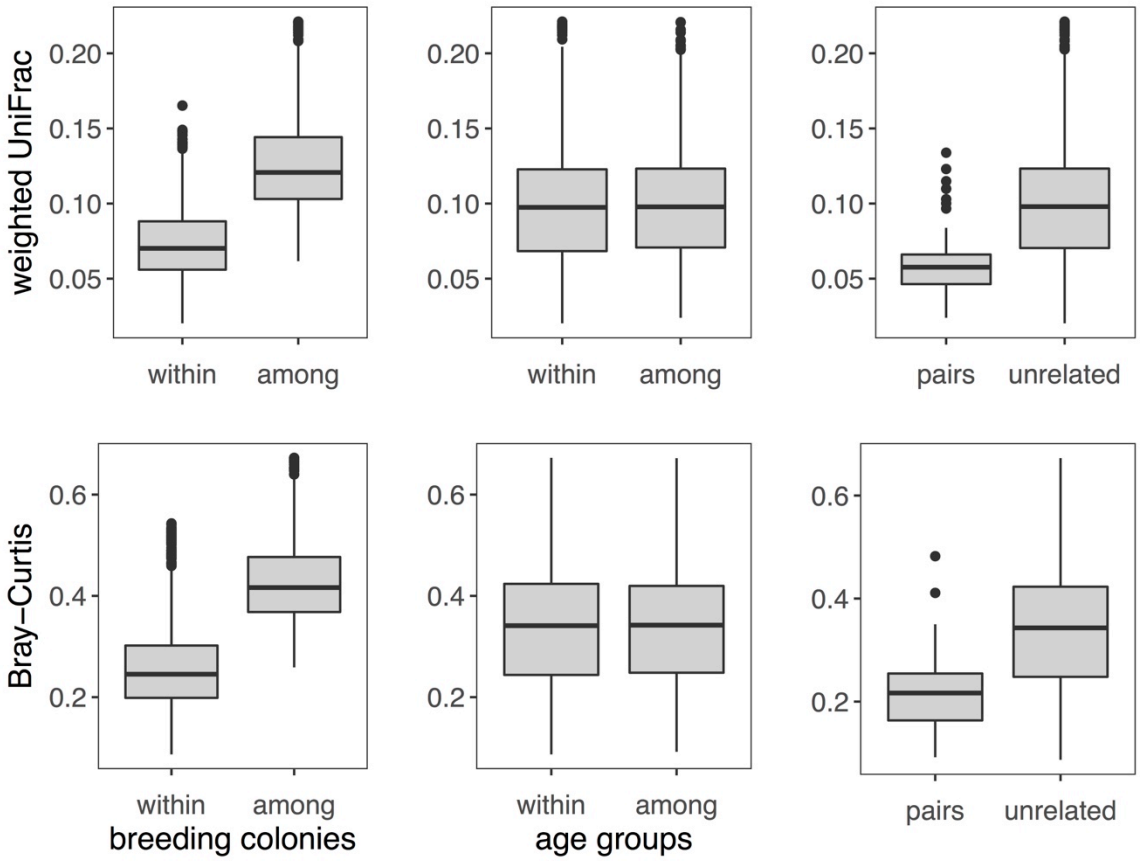

Figure S4. Weighted UniFrac and Bray-Curtis distances within and among breeding colonies, age groups as well as between mother-pup pairs and unrelated individuals (note: here unrelated refers to all pairwise comparisons between an individual and all other 94 individuals for which no mother-pup relationship was recorded).

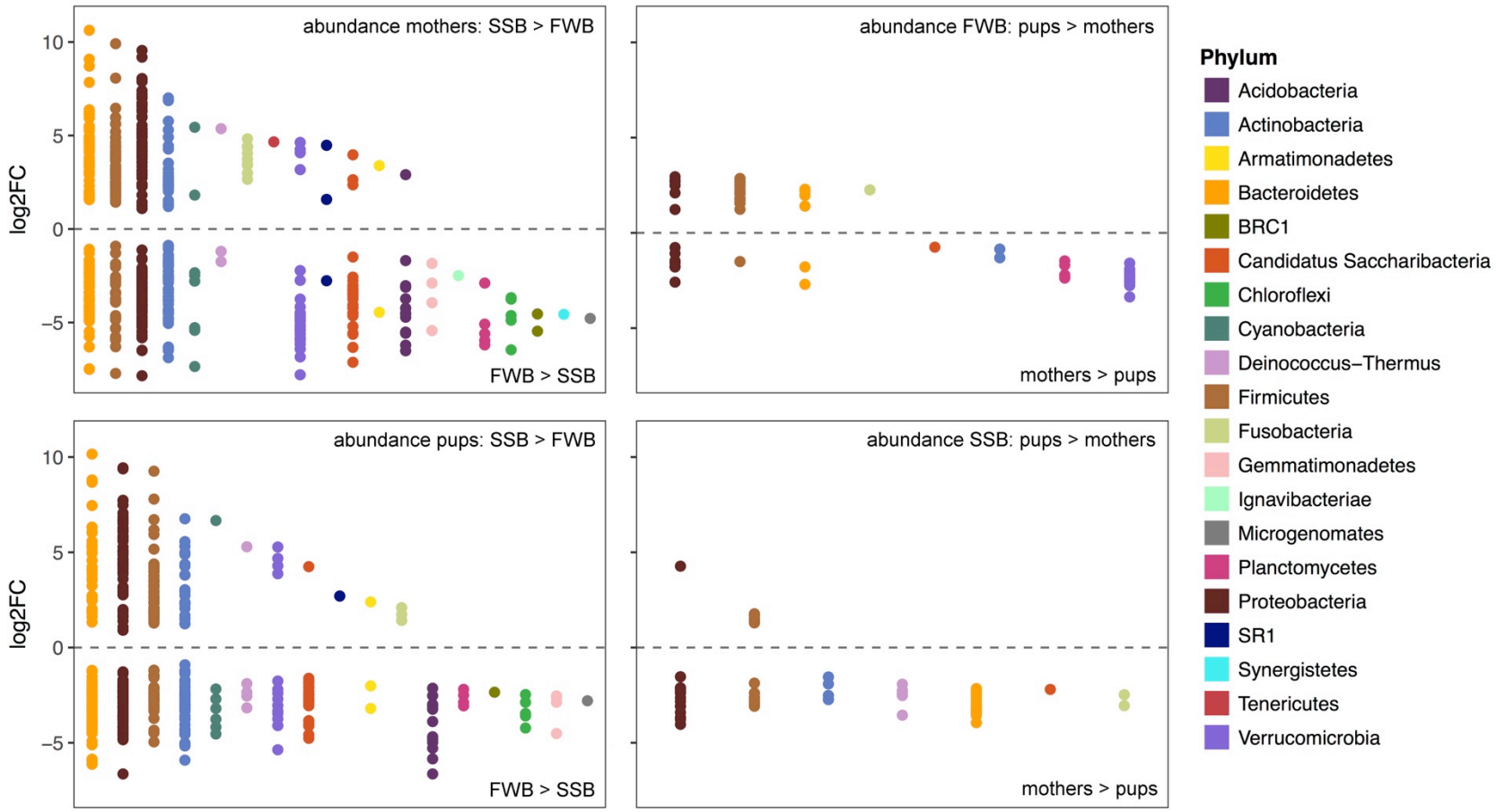

Figure S5. Differential abundance analysis. The left top and bottom panels show OTUs that are significantly differentially abundant between mothers of the two breeding colonies and pups of the two breeding colonies, respectively. The right top and bottom panels show OTUs that are significantly differentially abundant between the two age groups at FWB and SSB, respectively. OTU phylum membership is represented by the different colours. OTUs with a log2-fold-change larger than zero are significantly more abundant at SSB/in pups, and OTUs with a log2-fold-change smaller than zero are significantly more abundant at FWB/in mothers. Only OTUs with reliable phylum classification are shown.

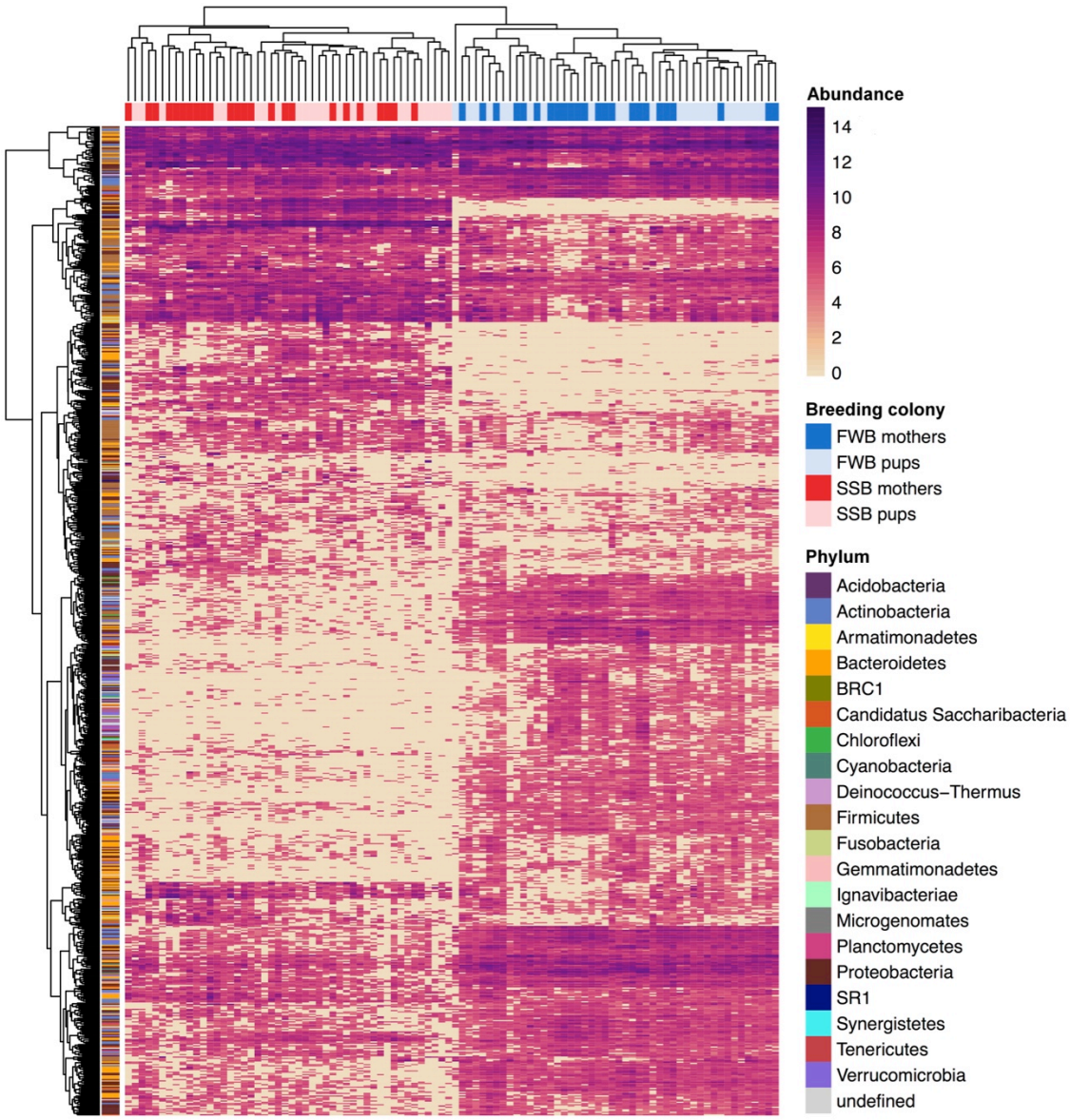

Figure S6. Heatmap showing broad patterns of OTU abundance. Each column corresponds to one individual and each row corresponds to an OTU. Abundance is represented by the Log(x + 0.0001)-transformed CSS normalised OTU counts and was grouped using complete linkage hierarchical clustering of Euclidean distances. The horizontal bar above the plot indicates which breeding colony and age group an individual belongs to. The vertical bar on the left-hand side of the plot represents the phylum membership of each OTU.

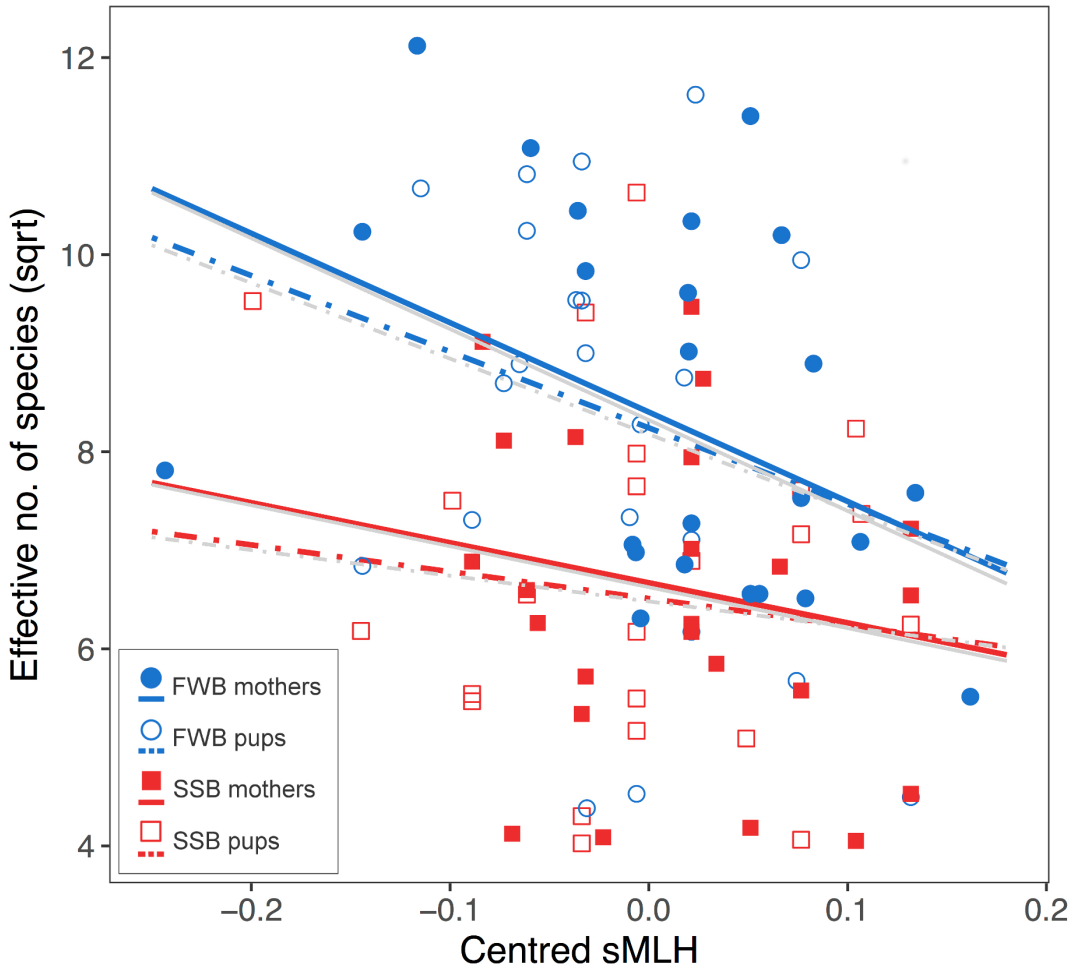

Figure S7. Relationship between bacterial alpha diversity (square root transformed effective number of species) and individual heterozygosity (sMLH, centered around the mean). Plotted are the raw data together with the regression lines from a linear mixed model, in which the effect of heterozygosity on alpha diversity was tested while controlling for both colony and age, including interactions (not significant) between sMLH and breeding colony, and sMLH and age class. Additional grey lines represent tightly clustered regression lines from 100 linear mixed models, where for each model the alpha diversity response variable was calculated from a different OTU table randomly rarefied to 10,000 reads (multiple rarefactions).

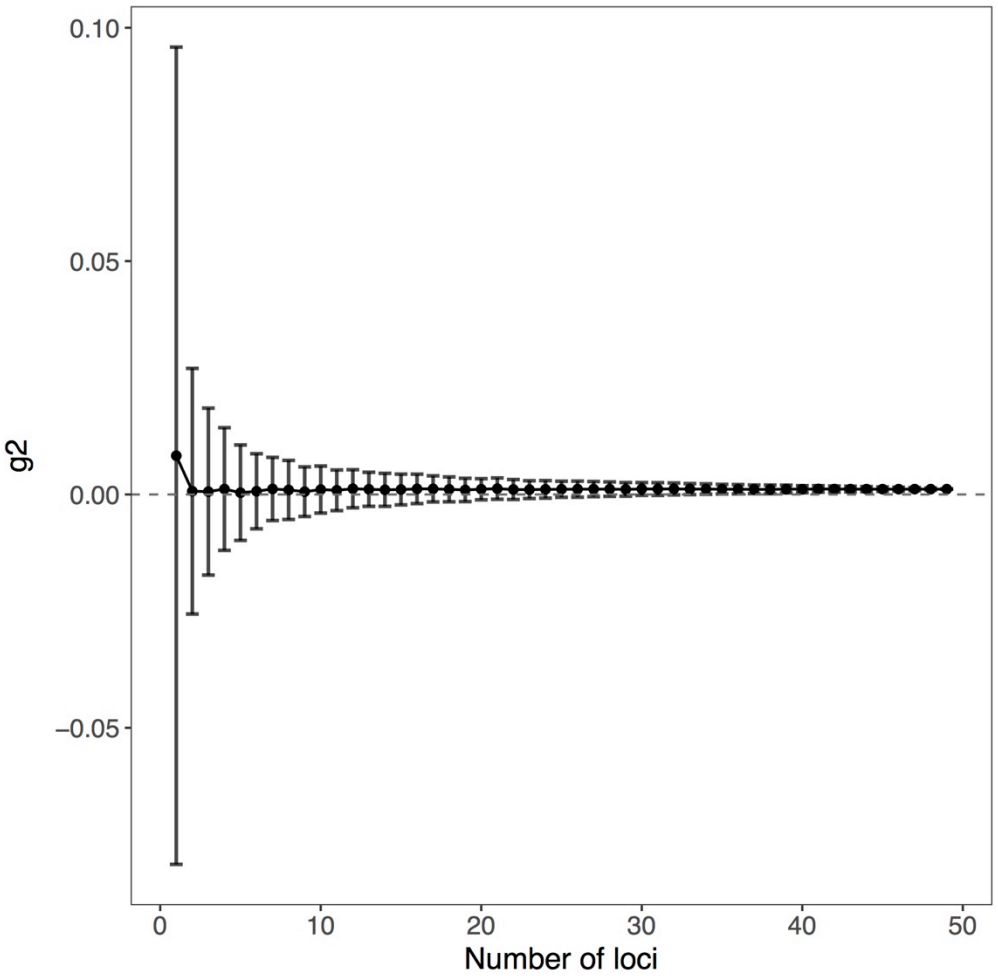

Figure S8. Two-locus heterozygosity disequilibrium, g_2, computation from a gradually increasing random subset of microsatellite loci.

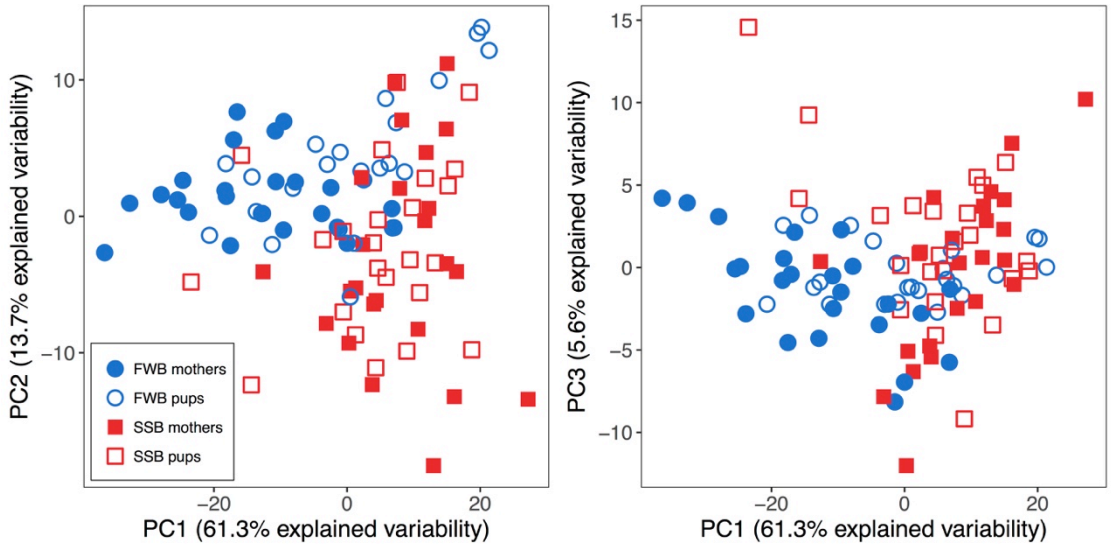

Figure S9. Principal component analysis of PICRUSt functional predictions at hierarchical level 3.

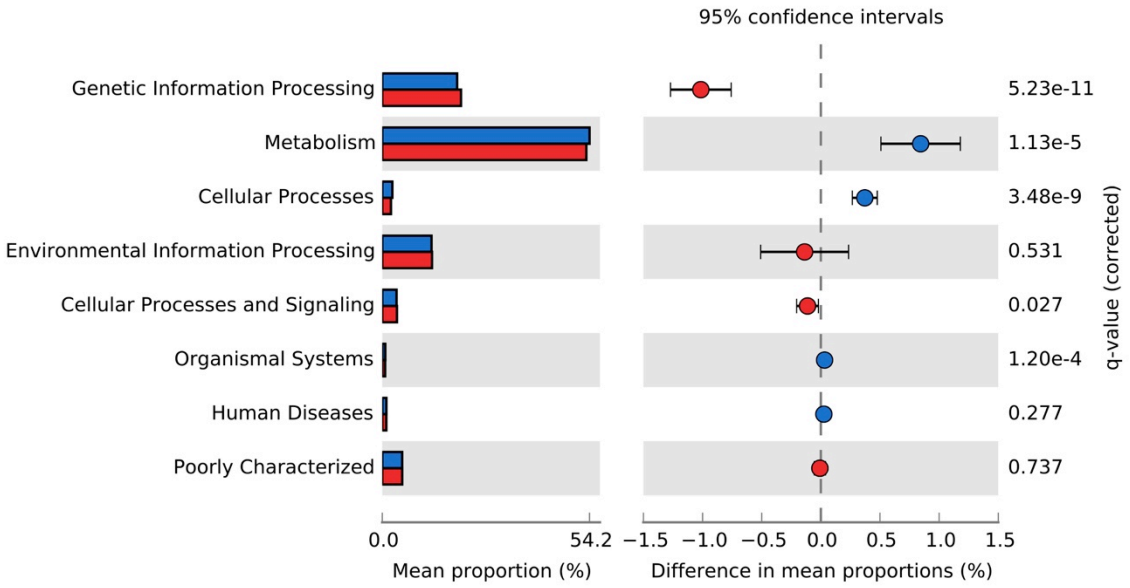

Figure S10. Statistical analysis of PICRUSt predictions of KEGG top-level (level 1) functional pathways. The bar plot indicates the mean proportion of KEGG orthologues of each major pathway for FWB (blue) and SSB (red) individuals. The right panel shows the differences in mean proportions between the two groups and associated 95% confidence intervals, as well as the FDR corrected *p*-values.

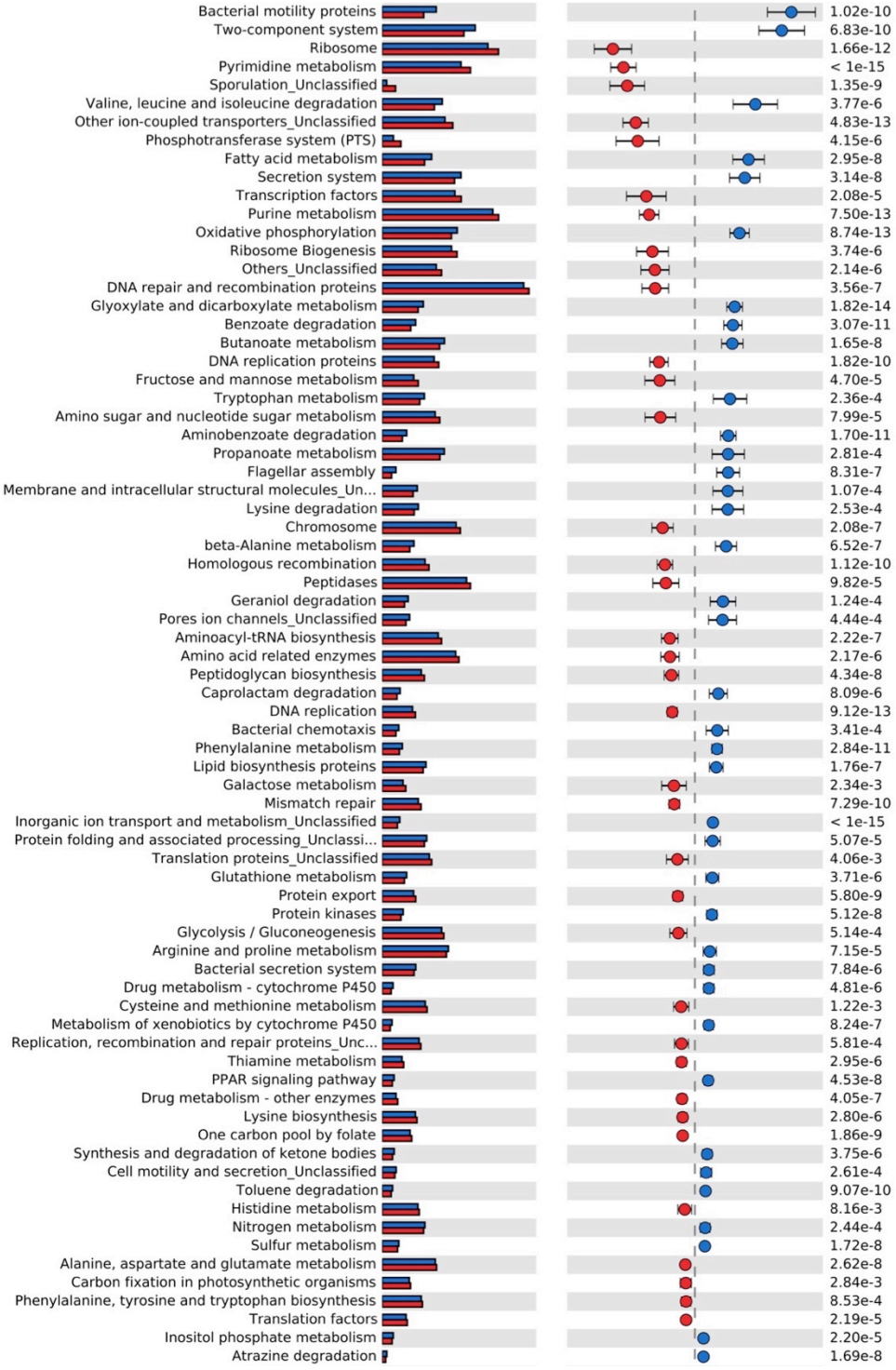

Figure S11. Statistical analysis of PICRUSt predictions of KEGG functional pathways at the 3rd hierarchical level. The bar plot indicates the mean proportion of KEGG orthologues of each pathway for FWB (blue) and SSB (red) individuals. The right panel shows the differences in mean proportions between the two groups and associated 95% confidence intervals, as well as the FDR corrected p-values. Only pathways with significantly different mean proportions are shown ($p<0.01$).

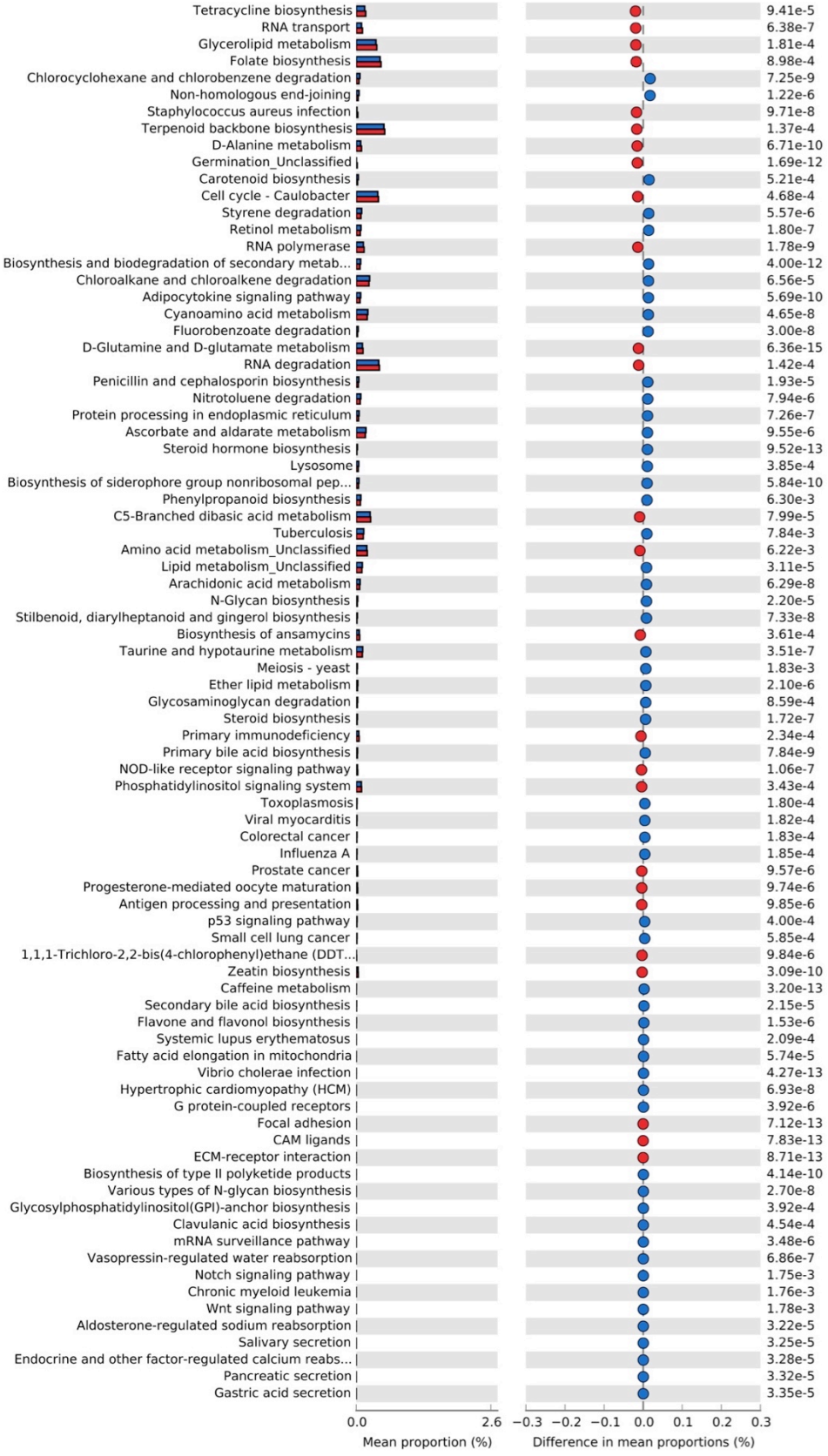

Figure S11. continued.

Supplementary Tables

Table S1. Sample information for 48 Antarctic fur seal mother-pup pairs.

Mother analysisID	Pup analysisID	Mother tagID	Mother sampleID	Pup tagID	Pup sampleID	Beach
M10	P10	W9166 mum	W9166 mum	W9166 pup	W9166 pup	FWB
M12	P12	W9165 mum	W9165 mum	W9165 pup	W9165 pup	FWB
M16	P16	W9164 mum	W9164 mum	W9164 pup	W9164 pup	FWB
M19	P19	W9173 mum	W9173 mum	W9173 pup	W9173 pup	FWB
M2	P2	W9132 mum	W9132 mum	W9132 pup	W9132 pup	FWB
M20	P20	W9172 mum	W9172 mum	W9172 pup	W9172 pup	FWB
M21	P21	W9135 mum	W9135 mum	W9135 pup	W9135 pup	FWB
M22	P22	W9171 mum	W9171 mum	W9171 pup	W9171 pup	FWB
M23	P23	W9168 mum	W9168 mum	W9168 pup	W9168 pup	FWB
M24	P24	W9134 mum	W9134 mum	W9134 pup	W9134 pup	FWB
M26	P26	W9175 mum	W9175 mum	W9175 pup	W9175 pup	FWB
M27	P27	W9133 mum	W9133 mum	W9133 pup	W9133 pup	FWB
M28	P28	W9167 mum	W9167 mum	W9167 pup	W9167 pup	FWB
M29	P29	W9176 mum	W9176 mum	W9176 pup	W9176 pup	FWB
M3	P3	W9138 mum	W9138 mum	W9138 pup	W9138 pup	FWB
M31	P31	W9170 mum	W9170 mum	W9170 pup	W9170 pup	FWB
M32	P32	W9174 mum	W9174 mum	W9174 pup	W9174 pup	FWB
M34	P34	W9178 mum	W9178 mum	W9178 pup	W9178 pup	FWB
M35	P35	W9169 mum	W9169 mum	W9169 pup	W9169 pup	FWB
M4	P4	W9163 mum	W9163 mum	W9163 pup	W9163 pup	FWB
M6	P6	W9162 mum	W9162 mum	W9162 pup	W9162 pup	FWB
M7	P7	W9137 mum	W9137 mum	W9137 pup	W9137 pup	FWB
M8	P8	W9136 mum	W9136 mum	W9136 pup	W9136 pup	FWB
M9	P9	W9161 mum	W9161 mum	W9161 pup	W9161 pup	FWB
M11	P11	W8287 mum	AGF14024	W8287 pup	AGP14028	SSB
M1	P1	W8282 mum	AGF14019	W8282 pup	AGP14234	SSB
M13	P13	W8285 mum	AGF14022	W8285 pup	AGP14153	SSB
M14	P14	W8265 mum	AGF14001	W8265 pup	AGP14170	SSB
M15	P15	W8283 mum	AGF14020	W8283 pup	AGP14050	SSB
M17	P17	W8284 mum	AGF14021	W8284 pup	AGP14146	SSB
M18	P18	W8289 mum	AGF14025	W8289 pup	AGP14235	SSB
M25	P25	W8264 mum	AGF14002	W8264 pup	AGP14148	SSB
M30	P30	W8276 mum	AGF14013	W8276 pup	AGP14313	SSB
M33	P33	W8274 mum	AGF14011	W8274 pup	AGP14295	SSB
M37	P37	W8272 mum	AGF14009	W8272 pup	AGP14179	SSB

M38	P38	W8279 mum	AGF14016	W8279 pup	AGP14308	SSB
M39	P39	W8281 mum	AGF14018	W8281 pup	AGP14323	SSB
M40	P40	W8268 mum	AGF14005	W8268 pup	AGP14207	SSB
M41	P41	W8266 mum	AGF14003	W8266 pup	AGP14201	SSB
M42	P42	W8280 mum	AGF14017	W8280 pup	AGP14096	SSB
M43	P43	W8277 mum	AGF14014	W8277 pup	AGP14306	SSB
M44	P44	W8278 mum	AGF14015	W8278 pup	AGP14317	SSB
M45	P45	W8271 mum	AGF14008	W8271 pup	AGP14237	SSB
M46	P46	W8269 mum	AGF14006	W8269 pup	AGP14229	SSB
M47	P47	W8273 mum	AGF14010	W8273 pup	AGP14301	SSB
M48	P48	W8270 mum	AGF14007	W8270 pup	AGP14227	SSB
M49	P49	W8275 mum	AGF14012	W8275 pup	AGP14180	SSB
M5	P5	W8286 mum	AGF14023	W8286 pup	AGP14067	SSB

FWB – freshwater beach, SSB – special study beach.

Table S2. Microsatellite loci used for genotyping 48 Antarctic fur seal mother-pup pairs.

Locus	Mix	T_a in °C	N_a	H_o	HWE *p*-values		Reference
					pups	mothers	
Pv9	1	53	10	0.82	1.000	1.000	(Allen *et al.*, 1995)
Hg6.3	1	53	12	0.88	0.609	0.609	(Allen et al., 1995)
Hg8.10	1	53	5	0.45	1.000	1.000	(Allen et al., 1995)
Hg1.3	1	53	12	0.80	0.488	0.488	(Gemmell *et al.*, 1997)
M11a	1	53	17	0.92	1.000	1.000	(Hoelzel, 1999)
PvcA	1	53	7	0.80	0.609	0.609	(Coltman *et al.*, 1996)
Zcwb07	1	53	10	0.87	1.000	1.000	(Hoffman *et al.*, 2007)
Agaz2	1	53	10	0.83	0.609	0.609	(Hoffman, 2009)
Ag3	2	60	2	0.37	1.000	1.000	(Hoffman *et al.*, 2008)
Agaz6	2	60	4	0.67	0.609	0.609	(Hoffman, 2009)
OrrFCB7	2	60	10	0.87	0.773	0.773	(Buchanan *et al.*, 1998)
Ag2	2	60	7	0.79	0.488	0.488	(Hoffman et al., 2008)
OrrFCB2	2	60	11	0.85	0.488	0.488	(Buchanan et al., 1998)
Lw10	2	60	13	0.91	1.000	1.000	(Davis *et al.*, 2002)
Zcwc01	2	60	11	0.85	0.488	0.488	(Hoffman et al., 2007)
Agaz5	2	60	3	0.59	0.892	0.892	(Hoffman, 2009)
ZcwCgDhB.14	2	60	6	0.82	0.954	0.954	(Hernandez-Velazquez et al., 2005)
SSL301	3	60	13	0.91	1.000	1.000	(Huebinger *et al.*, 2007)
Ag7	3	60	7	0.79	0.488	0.488	(Hoffman et al., 2008)
Agt10	3	60	3	0.34	1.000	1.000	(Hoffman et al., 2008)
ZcwCgDh4.7	3	60	11	0.84	0.721	0.721	(Hernandez-Velazquez et al., 2005)
Zcwe05	3	60	9	0.76	0.609	0.609	unpublished
Ag1	3	60	10	0.86	0.488	0.488	(Hoffman et al., 2008)
OrrFCB8	3	60	8	0.77	0.609	0.609	(Buchanan et al., 1998)
Agt47	3	60	3	0.48	1.000	1.000	(Hoffman & Nichols, 2011)
Zcwf07	4	60	8	0.79	0.488	0.488	(Hoffman et al., 2007)
ZcwD02	4	60	13	0.91	0.609	0.609	(Wolf *et al.*, 2005)
ZcwCgDh1.8	4	60	8	0.70	0.775	0.775	(Hernandez-Velazquez et al., 2005)
Aa4	4	60	7	0.68	0.609	0.609	(Gemmell et al., 1997)
ZcCgDh5.8	4	60	13	0.86	0.488	0.488	(Hernandez-Velazquez et al., 2005)
Agaz3	4	60	6	0.54	1.000	1.000	(Hoffman, 2009)
962-1	5	60	4	0.53	0.488	0.488	unpublished
554-6	5	60	2	0.17	1.000	1.000	unpublished
Zcwa12	5	60	18	0.86	0.609	0.609	(Hoffman et al., 2007)
PvcE	5	60	14	0.88	0.721	0.721	(Coltman et al., 1996)
Zcwb09	5	60	12	0.86	0.773	0.773	(Wolf et al., 2005)
agaz10	5	60	10	0.73	0.488	0.488	(Hoffman, 2009)
Mang44	5	60	6	0.72	0.609	0.609	(Sanvito et al., 2013)

Mang36	5	60	4	0.07	1.000	1.000	(Sanvito et al., 2013)
Zcwe03	6	60	9	0.78	0.949	0.721	(Wolf et al., 2005)
Zcwe04	6	60	11	0.83	0.299	0.699	(Hoffman et al., 2007)
101-26	7	60	6	0.72	0.100	0.699	unpublished
928-4b	7	60	9	0.82	0.554	1.000	unpublished
507-11	7	60	3	0.45	0.967	0.895	unpublished
Zcwa05	8	60	15	0.90	0.949	1.000	(Hoffman et al., 2007)
Zcwe12	8	60	9	0.79	1.000	0.723	(Hoffman et al., 2007)
ZcwCgDh3.6	8	60	3	0.21	1.000	1.000	(Hernandez-Velazquez et al., 2005)
Hg6.1	8	60	12	0.88	1.000	1.000	(Allen et al., 1995)
Zcwc11	8	60	12	0.89	0.362	0.752	(Wolf et al., 2005)
Lc28	8	60	7	0.83	0.949	1.000	(Davis et al., 2002)

P-values (after FDR correction) for Hardy-Weinberg equilibrium (HWE) tests are shown separately for mothers and pups. Mix indicates the PCR mastermix the loci were amplified in. Ta – annealing temperature, Na – number of alleles, Ho – observed heterozygosity.

Table S3. Bacterial phyla detected in the *A. gazelle* skin microbiome.

Phylum	Read count	Abundance (%)	No. of OTUs
Proteobacteria	1,231,373	38.80	210
Bacteroidetes	695,050	21.90	165
Firmicutes	676,701	21.32	134
Actinobacteria	360,848	11.37	104
Deinococcus-Thermus	32,948	1.04	6
Cyanobacteria	32,410	1.02	11
Verrucomicrobia	31,885	1.00	35
Candidatus Saccharibacteria	29,301	0.92	36
Fusobacteria	26,987	0.85	9
Acidobacteria	24,372	0.77	18
undefined	17,517	0.55	33
Planctomycetes	3,892	0.12	5
Gemmatimonadetes	2,502	0.08	4
Chloroflexi	2,386	0.08	5
SR1	1,641	0.05	3
Tenericutes	1,259	0.04	2
Armatimonadetes	1,101	0.03	3
BRC1	760	0.02	2
Microgenomates	263	0.01	1
Synergistetes	183	0.01	1
Ignavibacteriae	171	0.01	1

Table S4. The Antarctic fur seal skin core microbiome. The first 29 OTUs were recovered in all sampled individuals. Together, all 123 OTUs represent the core microbiome present in 90% of the sampled individuals.

OTU	Phylum	Family	Genus	Abundance
Otu1	Proteobacteria	Moraxellaceae	*Psychrobacter*	17.28
Otu3	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	5.5
Otu22	Proteobacteria	Moraxellaceae	*Psychrobacter*	3.79
Otu4	Firmicutes	Carnobacteriaceae	*Jeotgalibaca*	2.89
Otu2253	Proteobacteria	Moraxellaceae	*Psychrobacter*	2.83
Otu6	Actinobacteria	Intrasporangiaceae	unassigned	2.47
Otu13	Proteobacteria	Moraxellaceae	*Psychrobacter*	2.12
Otu29	Firmicutes	Streptococcaceae	*Streptococcus*	1.6
Otu16	Actinobacteria	Propionibacteriaceae	unassigned	1.29
Otu15	Firmicutes	Clostridiales Incertae Sedis XI	*Tissierella*	0.99
Otu31	Actinobacteria	Micrococcaceae	*Arthrobacter*	0.88
Otu11	Actinobacteria	Micrococcaceae	*Arthrobacter*	0.84
Otu14	Firmicutes	Clostridiaceae 1	*Clostridium* sensu stricto	0.83
Otu5	Firmicutes	Peptostreptococcaceae	*Clostridium XI*	0.8
Otu26	Deinococcus-Thermus	Deinococcaceae	*Deinococcus*	0.67
Otu18	Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	0.6
Otu19	Firmicutes	Clostridiaceae 1	*Clostridium* sensu stricto	0.59
Otu78	Firmicutes	Carnobacteriaceae	*Atopostipes*	0.59
Otu17	Bacteroidetes	Flavobacteriaceae	unassigned	0.59
Otu36	Actinobacteria	Microbacteriaceae	*Agrococcus*	0.59
Otu401	Proteobacteria	Moraxellaceae	*Psychrobacter*	0.57
Otu1771	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	0.5
Otu25	Firmicutes	Carnobacteriaceae	*Carnobacterium*	0.46
Otu32	Firmicutes	Planococcaceae	*Sporosarcina*	0.44
Otu43	Actinobacteria	Acidimicrobiaceae	*Ilumatobacter*	0.37
Otu82	Bacteroidetes	Flavobacteriaceae	unassigned	0.34
Otu72	Proteobacteria	Rhodobacteraceae	unassigned	0.21
Otu145	Actinobacteria	Microbacteriaceae	*Leifsonia*	0.2
Otu488	Actinobacteria	Nocardioidaceae	*Nocardioides*	0.11
Otu9	Bacteroidetes	Flavobacteriaceae	*Gelidibacter*	1.79
Otu7	Bacteroidetes	Flavobacteriaceae	unassigned	1.74
Otu12	Firmicutes	Planococcaceae	unassigned	1.31
Otu24	Bacteroidetes	Flavobacteriaceae	unassigned	1.04
Otu83	Proteobacteria	Comamonadaceae	*Polaromonas*	0.88
Otu90	Proteobacteria	Pasteurellaceae	*Otariodibacter*	0.86

OTU	Phylum	Family	Genus	Abundance
Otu60	Proteobacteria	Comamonadaceae	unassigned	0.73
Otu8	Firmicutes	Peptostreptococcaceae	*Clostridium XI*	0.68
Otu995	Proteobacteria	Pasteurellaceae	*Otariodibacter*	0.54
Otu93	Proteobacteria	Rhodobacteraceae	unassigned	0.49
Otu51	Proteobacteria	Neisseriaceae	*Neisseria*	0.47
Otu38	Firmicutes	Clostridiaceae 1	unassigned	0.46
Otu35	Proteobacteria	Moraxellaceae	*Acinetobacter*	0.44
Otu54	Bacteroidetes	Bacteroidaceae	*Bacteroides*	0.4
Otu129	Bacteroidetes	Chitinophagaceae	unassigned	0.4
Otu80	Firmicutes	Carnobacteriaceae	*Atopobacter*	0.34
Otu21	Cyanobacteria	Family IV	*GpIV*	0.32
Otu28	Fusobacteria	Fusobacteriaceae	*Fusobacterium*	0.29
Otu141	Proteobacteria	Xanthomonadaceae	*Thermomonas*	0.27
Otu57	Proteobacteria	Enterobacteriaceae	*Escherichia/Shigella*	0.26
Otu84	Actinobacteria	Intrasporangiaceae	unassigned	0.26
Otu50	Actinobacteria	unassigned	unassigned	0.26
Otu59	Firmicutes	Clostridiales Incertae Sedis XI	*Anaerococcus*	0.26
Otu47	Firmicutes	Clostridiales Incertae Sedis XI	*Tissierella*	0.25
Otu96	Actinobacteria	Iamiaceae	*Aquihabitans*	0.25
Otu45	Firmicutes	Ruminococcaceae	unassigned	0.24
Otu52	Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	0.24
Otu53	Firmicutes	Lachnospiraceae	*Blautia*	0.24
Otu102	Proteobacteria	Burkholderiaceae	unassigned	0.24
Otu68	Actinobacteria	Nocardioidaceae	*Nocardioides*	0.23
Otu101	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	0.23
Otu46	Actinobacteria	unassigned	unassigned	0.21
Otu76	Deinococcus-Thermus	Deinococcaceae	*Deinococcus*	0.2
Otu86	Actinobacteria	Dietziaceae	*Dietzia*	0.2
Otu39	Firmicutes	Lachnospiraceae	unassigned	0.2
Otu88	Actinobacteria	Actinomycetaceae	*Arcanobacterium*	0.19
Otu157	Firmicutes	Planococcaceae	unassigned	0.19
Otu74	Firmicutes	Erysipelotrichaceae	*Erysipelothrix*	0.19
Otu2175	Proteobacteria	Comamonadaceae	*Polaromonas*	0.19
Otu73	Bacteroidetes	Chitinophagaceae	unassigned	0.18
Otu339	Proteobacteria	Comamonadaceae	unassigned	0.17
Otu49	Firmicutes	Lachnospiraceae	unassigned	0.17
Otu228	Bacteroidetes	unassigned	unassigned	0.17

OTU	Phylum	Family	Genus	Abundance
Otu85	Bacteroidetes	Flavobacteriaceae	*Flavobacterium*	0.16
Otu55	Firmicutes	Eubacteriaceae	*Eubacterium*	0.16
Otu213	Verrucomicrobia	Verrucomicrobiaceae	*Luteolibacter*	0.16
Otu146	Bacteroidetes	Bacteroidaceae	*Bacteroides*	0.16
Otu69	Actinobacteria	Intrasporangiaceae	*Ornithinicoccus*	0.14
Otu189	Firmicutes	Clostridiaceae 1	*Clostridium* sensu stricto	0.14
Otu122	Actinobacteria	Micrococcaceae	unassigned	0.14
Otu214	Proteobacteria	Moraxellaceae	*Psychrobacter*	0.13
Otu103	Proteobacteria	Xanthomonadaceae	*Dokdonella*	0.13
Otu58	Proteobacteria	Methylobacteriaceae	*Methylobacterium*	0.12
Otu1883	Proteobacteria	Comamonadaceae	*Rhodoferax*	0.12
Otu209	Actinobacteria	Dermacoccaceae	unassigned	0.12
Otu130	Firmicutes	Ruminococcaceae	unassigned	0.12
Otu105	Firmicutes	Clostridiales Incertae Sedis XI	*Helcococcus*	0.11
Otu89	Actinobacteria	unassigned	unassigned	0.11
Otu65	Candidatus Saccharibacteria	unassigned	Saccharibacteria genera incertae sedis	0.11
Otu115	Firmicutes	Streptococcaceae	*Streptococcus*	0.11
Otu167	Bacteroidetes	Cytophagaceae	*Hymenobacter*	0.11
Otu120	Candidatus Saccharibacteria	unassigned	Saccharibacteria genera incertae sedis	0.11
Otu143	Actinobacteria	Iamiaceae	*Aquihabitans*	0.1
Otu135	Firmicutes	Erysipelotrichaceae	*Erysipelothrix*	0.09
Otu75	Fusobacteria	Fusobacteriaceae	unassigned	0.09
Otu201	Firmicutes	unassigned	unassigned	0.09
Otu95	Firmicutes	Clostridiaceae 1	*Clostridium* sensu stricto	0.08
Otu177	Candidatus Saccharibacteria	unassigned	Saccharibacteria genera incertae sedis	0.08
Otu174	Bacteroidetes	Chitinophagaceae	*Ferruginibacter*	0.08
Otu108	Actinobacteria	Nocardiaceae	*Rhodococcus*	0.08
Otu156	Proteobacteria	Xanthomonadaceae	unassigned	0.08
Otu110	Proteobacteria	Rhodobacteraceae	unassigned	0.07
Otu133	Proteobacteria	unassigned	unassigned	0.07
Otu236	Actinobacteria	Acidimicrobiaceae	*Ilumatobacter*	0.07
Otu136	Actinobacteria	Coriobacteriaceae	*Collinsella*	0.07
Otu629	Proteobacteria	Comamonadaceae	unassigned	0.06
Otu234	Actinobacteria	Nakamurellaceae	*Nakamurella*	0.06
Otu207	Actinobacteria	Corynebacterineae incertae sedis	*Tomitella*	0.06
Otu148	Firmicutes	Lachnospiraceae	*Clostridium XlVb*	0.06

OTU	Phylum	Family	Genus	Abundance
Otu121	Firmicutes	Ruminococcaceae	*Butyricicoccus*	0.06
Otu222	Proteobacteria	Xanthomonadaceae	*Lysobacter*	0.06
Otu1017	Proteobacteria	Xanthomonadaceae	*Rhodanobacter*	0.05
Otu691	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	0.05
Otu211	Actinobacteria	Nocardiaceae	unassigned	0.05
Otu270	Bacteroidetes	Cyclobacteriaceae	*Algoriphagus*	0.05
Otu1469	Bacteroidetes	Flavobacteriaceae	*Chryseobacterium*	0.04
Otu1703	Firmicutes	Lachnospiraceae	*Blautia*	0.04
Otu2423	Bacteroidetes	Chitinophagaceae	unassigned	0.04
Otu365	Actinobacteria	Nocardioidaceae	*Aeromicrobium*	0.04
Otu332	Actinobacteria	unassigned	unassigned	0.03
Otu458	Actinobacteria	Microbacteriaceae	unassigned	0.03
Otu308	Actinobacteria	Demequinaceae	*Demequina*	0.03
Otu429	Actinobacteria	unassigned	unassigned	0.03
Otu612	Actinobacteria	Microbacteriaceae	unassigned	0.02

Table S5. Results of a linear mixed model testing for differences in alpha-diversity (response variable – calculated either from all 788 OTUs or from a subset of 613 OTUs belonging to the four dominant phyla) between breeding colonies (FWB, SSB) and age group (mother, pup) (fixed effects). Pair ID (53 groups; unrelated pairs were coded as separate groups) was included as a random effect. Regression coefficients and variance components and their standard errors (S.E.) are reported. Significance was determined by performing likelihood ratio tests.

Trait	**Parameters**	**Estimates**	**S.E.**	***t*-value**	***p*-value**
All OTUs					
alpha diversity	Intercept	8.28	0.35		
$N_{obs} = 96$	Breeding colony SSB	-1.64	0.43	-3.81	2.8×10^{-4}
$N_{Colony/Age} = 24$	Age pup	-0.17	0.34	-0.49	0.625
$R^2_{(m)} = 0.16$	σ^2_{PairID}	0.90			
$R^2_{(c)} = 0.37$	$\sigma^2_{Resid.}$	2.76			
OTUs from dominant phyla only					
alpha diversity	Intercept	7.09	0.31		
$N_{obs} = 96$	Breeding colony SSB	-0.91	0.38	-2.41	0.017
$N_{Colony/Age} = 24$	Age pup	0.07	0.29	0.26	0.796
$R^2_{(m)} = 0.07$	σ^2_{PairID}	0.79			
$R^2_{(c)} = 0.34$	$\sigma^2_{Resid.}$	1.96			

$R^2_{(m)}$ – marginal R_2 - is the variance explained by the fixed factors, and $R^2_{(c)}$ – conditional R_2 - is the variance explained by both, the fixed and the random factors.

Table S6. Results of a linear model testing for differences in alpha-diversity (response variable) between female and male pups with breeding colony (FWB, SSB) included as fixed effect.

Trait	**Parameters**	**Estimates**	**S.E.**	***t*-value**	***p*-value**
alpha diversity	Intercept	8.32	0.47	17.73	
$N_{obs} = 48$	Breeding colony SSB	-1.27	0.58	-2.17	0.035
$N_{Colony/Sex} = 12$	Sex male	-1.02	0.60	-1.70	0.096

Table S7. Characterisation of significantly differentially abundant OTUs (overrepresented at SSB) assigned to the phylum Fusobacteria.

OTU	Family	Genus	Species	Blastn hit[a], sequence identity	Disease associations	Reference
Otu28	Fusobacteriaceae	Fusobacterium	undefined	*F. mortiferum* strain CCUG 14475, 100%	Associated with colorectal cancer in humans	George et al., 1981
Otu67	Leptotrichiaceae	Streptobacillus	*S.moniliformis*	*Oceanivirga salmonicida*, 97%	*S. moniliformis* can cause rat bite fever, *O. salmonicida* extracted from multifocal tissue necrosis in Atlantic salmon	Eisenberg et al., 2016; Elliott, 2007
Otu56	Fusobacteriaceae	Fusobacterium	*F. perfoetens*	-	Associated with colorectal cancer in humans	Kostic et al., 2012
Otu99	Fusobacteriaceae	Fusobacterium	undefined	*Leptotrichia sp.* feline oral taxon, 99%; *F. russii* strain 15_439_34918, 99%	Associated with a wide-variety of infections in humans	George et al., 1981
Otu75	Fusobacteriaceae	undefined	undefined	-	-	
Otu210	Fusobacteriaceae	Fusobacterium	*F. equinum*	*F. gonidiaformans* ATCC 25563, 100%	Associated with a wide-variety of infections in humans	George et al., 1981
Otu372	Fusobacteriaceae	Fusobacterium	undefined	*F. nucleatum* strain WAL 9696, 100%	Associated with a wide-variety of infections in humans; associated with colorectal cancer in humans	Citron, 2002; George et al., 1981
Otu369	Leptotrichiaceae	Leptotrichia	*L. goodfellowii*	*Leptotrichia sp.* canine oral taxon, 99%; *L. goodfellowii* strain LB57 97%	Can cause infections in immunocompromised patients	Matias et al., 2016
Otu1543	Leptotrichiaceae	Streptobacillus	*S. moniliformis*	*Leptotrichia sp.* ES2714_GLU, 99%	Can cause rat bite fever	Elliott, 2007

[a] Best result from a NCBI Blastn search against the nt database which is not uncultured bacterium or similar.

Table S8. Results of a linear mixed model investigating the relationship between alpha diversity (response variable) and heterozygosity (fixed effect) while controlling for breeding colony (FWB, SSB) and age group (mother, pup) (fixed effects). Pair ID (53 groups; unrelated pairs were coded as separate groups) was included as a random effect. Regression coefficients and variance components and their standard errors (S.E.) are reported. Significance was determined by performing likelihood ratio tests.

Trait	**Parameters**	**Estimates**	**S.E.**	***t*-value**	***p*-value**
alpha diversity	Intercept	8.37	0.34		
$N_{obs} = 95$	cHeterozygosity	-6.06	2.43	-2.50	0.017
	Breeding colony SSB	-1.68	0.40	-4.22	7.7×10^{-5}
	Age pup	-0.29	0.37	-0.78	0.425
$R^2_{(m)} = 0.22$	σ^2_{PairID}	0.39			
$R^2_{(c)} = 0.30$	$\sigma^2_{Resid.}$	3.04			

$R^2_{(m)}$ – marginal R_2 - is the variance explained by the fixed factors, and $R^2_{(c)}$ – conditional R_2 - is the variance explained by both, the fixed and the random factors.

Table S9. Results of a linear mixed model investigating the relationship between alpha diversity (response variable) and heterozygosity (fixed effect) while controlling for breeding colony (FWB, SSB) and age group (mother, pup) (fixed effects) including interactions between sMLH and breeding colony, and sMLH and age. Pair ID (53 groups; unrelated pairs were coded as separate groups) was included as a random effect. Regression coefficients and variance components and their standard errors (S.E.) are reported. Significance was determined by performing likelihood ratio tests.

Trait	**Parameters**	**Estimates**	**S.E.**	***t*-value**	***p*-value**
alpha diversity	Intercept	8.37	0.34		
$N_{obs} = 95$	cHeterozygosity	-9.85	3.85	-2.56	
	Breeding colony SSB	-1.68	0.40	-4.28	
	Age pup	-0.29	0.37	-0.78	
	cHeterozygosity * Colony SSB	7.75	4.91	1.58	0.106
	cHeterozygosity * Age pup	-0.32	4.99	-0.06	0.943
$R^2_{(m)} = 0.24$	σ^2_{PairID}	0.31			
$R^2_{(c)} = 0.31$	$\sigma^2_{Resid.}$	3.09			

$R^2_{(m)}$ – marginal R_2 - is the variance explained by the fixed factors, and $R^2_{(c)}$ – conditional R_2 - is the variance explained by both, the fixed and the random factors.

Additional Supporting Information

Additional Dataset S1: Collection of scripts for the OTU processing pipeline.

Additional Dataset S2: Summary statistics for the OTU processing pipeline.

Additional Dataset S3: R Markdown file containing all the R code used for the analyses of the Antarctic fur seal microbiome.

Additional Dataset S4: R markdown input file containing sequencing statistics.

Additional Dataset S5/S5.1: R markdown input file containing genotypes at 50 microsatellite loci for 95 fur seal individuals (pup P22 was removed due to large number of missing genotypes).

Additional Dataset S6: R markdown input file containing pairwise relatedness estimates.

Additional Dataset S7: R markdown input file containing the OTU RDP classifications obtained with the USEARCH sintax command.

Additional Dataset S8: R markdown input file containing the OTU RDP classifications prepared for phyloseq input.

Additional Dataset S9: R markdown input file containing the trimmed OTU table for all samples.

Additional Dataset S10: R markdown input file containing the trimmed and rarefied OTU table for 94 samples.

Additional Dataset S11: R markdown input file containing metadata for all samples.

Additional Dataset S12: R markdown input file containing alpha diversity estimates (Effective number of species–Jost1) calculated from the non-normalised and rarefied OTU tables in USEARCH for all sampled individuals.

Additional Dataset S13: R markdown input file containing alpha diversity estimates (Effective number of species–Jost1) calculated for OTUs belonging to the main phyla.

Additional Dataset S14: R markdown input file containing a phylogenetic tree needed for UniFrac distance calculations (created with FastTree in QIIME).

Additional Dataset S15: R markdown input file containing pupping locations at SSB.

Additional Dataset S16: R markdown input file containing alpha diversity estimates (Effective number of species–Jost1) calculated for 100 rarefied OTU tables in USEARCH for all sampled individuals.

Additional Dataset S17: R markdown input file containing NSTI values calculated from PICRUSt.

Additional Dataset S18: R markdown input file containing read counts for KEGG functional predictions calculated with PICRUSt.

References

Afgan, E., Baker, D., van den Beek, M., Blankenberg, D., Bouvier, D., Cech, M., . . . Goecks, J. (2016). The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2016 update. *Nucleic Acids Research, 44*(W1), W3-W10. doi:10.1093/nar/gkw343

Allen, P. J., Amos, W., Pomeroy, P. P., & Twiss, S. D. (1995). Microsatellite variation in grey seals (*Halichoerus grypus*) shows evidence of genetic differentiation between two British breeding colonies. *Molecular Ecology, 4*(6), 653-662.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B, 57*, 289-300.

Bokulich, N. A., Subramanian, S., Faith, J. J., Gevers, D., Gordon, J. I., Knight, R., . . . Caporaso, J. G. (2013). Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods, 10*(1), 57-59. doi:10.1038/nmeth.2276

Buchanan, F. C., Maiers, L. D., Thue, T. D., De March, B. G., & Stewart, R. E. (1998). Microsatellites from the Atlantic walrus *Odobenus rosmarus rosmarus*. *Molecular Ecology, 7*(8), 1083-1085.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., & Madden, T. L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics, 10*, 421. doi:10.1186/1471-2105-10-421

Chao, A., Chiu, C. H., & Jost, L. (2010). Phylogenetic diversity measures based on Hill numbers. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 365*(1558), 3599-3609. doi:10.1098/rstb.2010.0272

Citron, D. M. (2002). Update on the Taxonomy and Clinical Aspects of the Genus *Fusobacterium*. *Clinical Infectious Diseaeses, 35*((Suppl 1)), S22-27.

Coltman, D. W., Bowen, W. D., & Wright, J. M. (1996). PCR primers for harbour seal (*Phoca vitulina concolour*) microsatellites amplify polymorphic loci in other pinniped species. *Molecular Ecology, 5*(1), 161-163.

Comeau, A. M., Douglas, G. M., & Langille, M. G. (2017). Microbiome Helper: a custom and streamlined workflow for microbiome research. *mSystems, 2*(1), e00127-00116. doi:10.1128/mSystems.00127-16

Davis, C. S., Gelatt, T. S., Siniff, D., & Strobeck, C. (2002). Dinucleotide microsatellite markers from the Antarctic seals and their use in other pinnipeds. *Molecular Ecology Notes, 2*, 203-208.

Edgar, R. C. (2016). SINTAX, a simple non-Bayesian taxonomy classifier for 16S and ITS sequences. *BioRxive*. doi:http://dx.doi.org/10.1101/074161

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics, 31*(21), 3476-3482. doi:10.1093/bioinformatics/btv401

Eisenberg, T., Kämpfer, P., Ewers, C., Semmler, T., Glaeser, S. P., Collins, E., . . . Palmer, R. (2016). *Oceanivirga salmonicida* gen. nov., sp. nov., a member of the Leptotrichiaceae isolated from Atlantic salmon (*Salmo salar*). *International Journal of Systematic and Evolutionary Microbiology, 66*(6), 2429-2437. doi:doi:10.1099/ijsem.0.001050

Elliott, S. P. (2007). Rat Bite Fever and *Streptobacillus moniliformis*. *Clinical Microbiology Reviews, 20*(1), 13-22.

Gemmell, N. J., Allen, P. J., Goodman, S. J., & Reed, J. Z. (1997). Interspecific microsatellite markers for the study of pinniped populations. *Molecular Ecology, 6*(7), 661-666.
George, W. L., Kirby, B. D., Sutter, V. L., Citron, D. M., & Finegold, S. M. (1981). Gram-negative anaerobic Bacilli: their role in infection and patterns of susceptibility to antimicrobial agents. II. Little-known *Fusobacterium* species and miscellaneous genera. *Reviews of Infectious Diseases, 3*(3), 599-626.
Hernandez-Velazquez, F. D., Galindo-Sanchez, C. E., Taylor, M. I., J., D. l. R.-V., Cote, I. M., Schramm, Y., . . . Rico, C. (2005). New polymorphic microsatellite markers for California sea lions (*Zalophus californianus*). *Molecular Ecology Notes, 5*(1), 140-142. doi:10.1111/j.1471-8286.2004.00858.x
Hoelzel, A. R. (1999). Alpha-male paternity in elephant seals. *Behavioral Ecology and Sociobiology, 46*, 298-306.
Hoffman, J. I. (2009). A panel of new microsatellite loci for genetic studies of Antarctic fur seals and other otariids. *Conservation Genetics, 10*, 989-992.
Hoffman, J. I., Dasmahapatra, K. K., & Nichols, H. J. (2008). PERMANENT GENETIC RESOURCES: Ten novel polymorphic dinucleotide microsatellite loci cloned from the Antarctic fur seal *Arctocephalus gazella*. *Molecular Ecology Resources, 8*(2), 459-461. doi:10.1111/j.1471-8286.2007.01993.x
Hoffman, J. I., & Nichols, H. J. (2011). A novel approach for mining polymorphic microsatellite markers *in silico*. *PLoS One, 6*(8), e23283. doi:10.1371/journal.pone.0023283
Hoffman, J. I., Steinfartz, S., & Wolf, J. B. W. (2007). Ten novel dinucleotide microsatellite loci cloned from the Galápagos sea lion (*Zalophus californianus wollebaeki*) are polymorphic in other pinniped species. *Molecular Ecology Notes, 7*, 103-105.
Huebinger, R. M., Louis, E. E., Gelatt, T. S., Rea, L. D., & Bickham, J. W. (2007). Characterization of eight microsatellite loci in Steller sea lions (*Eumetopias jubatus*). *Molecular Ecology Notes, 7*(6), 1097-1099.
Kostic, A. D., Gevers, D., Pedamallu, C. S., Michaud, M., Duke, F., Earl, A. M., . . . Meyerson, M. (2012). Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Research, 22*(2), 292-298. doi:10.1101/gr.126573.111
Langille, M. G., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., . . . Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology, 31*(9), 814-821. doi:10.1038/nbt.2676
Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal, 17*(1), 10-12. doi:10.14806/ej.17.1.200
Matias, W. R., Bourque, D. L., Niwano, T., Onderdonk, A. B., & Katz, J. T. (2016). Subacute Bacterial Endocarditis with *Leptotrichia goodfellowii* in a Patient with a Valvular Allograft: A Case Report and Review of the Literature. *Case Reports in Infectious Diseases, 2016*, 5. doi:10.1155/2016/3051212
Parks, D. H., Tyson, G. W., Hugenholtz, P., & Beiko, R. G. (2014). STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics, 30*(21), 3123-3124. doi:10.1093/bioinformatics/btu494
Sanvito, S., Dueñes Meza, A., Schramm, Y., Cruz Hernández, P., Esquer Garrigos, Y., & Galimberti, F. (2013). Isolation and cross-species amplification of novel

microsatellite loci in a charismatic marine mammal species, the northern elephant seal. *Conservation Genetics Resources, 5*(1), 93-96

Wolf, J. B. W., Tautz, D., Caccone, A., & Steinfartz, S. (2005). Development of new microsatellite loci and evaluation of loci from other pinniped species for the Galápagos sea lion (*Zalophus californianus wollebaeki*). *Conservation Genetics, 7*(3), 461-465.