Space, time, and captivity: quantifying factors influencing the microbiome of an alpine ungulate

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Abstract

The community of microorganisms in the microbiome is affected by host species, diet, and environment and is linked to normal functioning metabolism, immune system, and development. Although the microbiome fluctuates in response to host demands and environmental changes, there are core groups of microorganisms that remain relatively constant throughout the hosts lifetime. Ruminants are mammals that rely on highly specialized digestive and metabolic modifications, including microbiome adaptations, to persist in extreme environments. We assayed the gut microbiome of four mountain goat (*Oreamnos americanus*) populations in western North America. We quantified microbiome diversity and composition between groups in the wild and captivity, across space, and over time. There were no differences in community evenness or diversity across groups, although we observed a decreasing diversity trend across the summer. Pairwise dissimilarity estimates grouped the captive population distinctly from the wild populations, and moderately grouped the southern wild distinctly from the two northern wild populations. We identified 33 genera modified by captivity, with major differences in key groups associated with cellulose degradation that likely reflect differences in diet. Our findings are consistent with other ruminant studies and provide baseline microbiome data in this enigmatic species, offering valuable insights into the health of wild alpine ungulates.

Keywords: *Oreamnos americanus*, microbiome, ruminant, microorganisms, rumen, goat

Summary: This study characterizes the microbiome of mountain goat (Oreamnos americanus) populations across space, time, and the effects of captivity to offer more insights into the health of alpine wildlife.

Introduction

The microbiome is a dynamic community of microorganisms that colonize organisms from birth onwards. The gut microbiome at a given time is affected by the age, species, diet, nutrition, and environment of the host and is directly linked to host health, including metabolism, immunity, and development (Nishida & Ochman, 2017). Although much of the gut microbiome is a transient response to changes in the host, core groups of microorganisms are found in stable relative abundances throughout the life of the host and the proportions of these groups act like a

signature of the host's diet, health, and environment (Gogul et al., 2018). The stable relative abundances of the core groups are directly related to the function and the demand of the core groups, such as the relative ratio of *Firmicutes* to *Bacteroidetes*, the dominant core groups in mammal gut microbiomes, can be used to discern between carnivorous and herbivorous mammals since each group is responsible for different metabolic demands (Kreisinger et al., 2018; Muegge et al., 2011).

Recent work has proposed the notion of a holobiome, that is an amalgamation of the host genome with the microbiome, that collectively influence the phenotype of a host. Unlike previous models the phenotype is not based solely on an individuals' genome, but a result of complex interactions between the hosts' genome, microbiome, and environment (Carthey et al., 2018; Sharpton, 2018). This interaction is particularly relevant when it comes to rapidly changing environments, where for example the host microbiome of coral was critical in adapting to warming temperatures (Ziegler et al. 2017). Similarly, measuring signatures in the gut microbiome over time in a single population or between populations, such as between captive and wild populations of mammals, can help effectively monitor health (Bahrndorff et al., 2016; Bik et al., 2016). Many captive populations of mammal species suffer from poor health and some of these poor health conditions have been correlated to gut microbiome dysbiosis and decreased diversity in groups responsible for normal health (Li et al., 2018; McKenzie et al., 2017). Actions that target the correction of the microbiome, meaning promoting signatures of core groups that are closer to what is seen in relevant wild and healthy populations, have been suggested as a feasible approach to help alleviate and manage health issues (Li et al., 2018).

Ruminants are herbivorous hooved mammals (ungulates) with specialized anatomical and physiological adaptations to accommodate the cellulolytic fermentation of low-nutrition, high-fiber plant materials (de Tarso et al., 2016). Many extant ruminant populations have been domesticated such as cow (*Bos taurus*), goat (*Capra hircus*), sheep (*Ovis aries*), bison (*Bison bison bison*), reindeer (*Rangifer tarandus*), and yak (*Bos grunniens*). Agricultural practices surrounding domesticated ruminants are one of the leading global causes of environment change and destruction and it has facilitated increased transmission rates of ruminant-specific diseases and parasites into and between populations of domesticated, captive, and wild ruminants (Li et al., 2017; Rojas-Downing et al., 2017). Many of the remaining wild ruminant species are facing

population declines and genetic diversity loss that are directly and indirectly driven by human activity, including agricultural-related activities that have promoted predation, disease and parasitism expansions, environmental change, and have led to significant habitat loss (Smith et al., 2016; Martin, 2001). Monitoring the microbiome of ruminant populations serves as an important tool that can be used to inform management and conservation bodies of diet selection, adaptation to environmental change, and disease and parasitism trends.

The North American mountain goat (*Oreamnos americanus*) is a large, long-lived alpine and sub-alpine ruminant and a symbolic icon of mountain wilderness. This big game species is found in fragmented, and occasionally isolated, sub-populations across the Western Cordillera mountain ranges from Alaska, USA through two Canadian provinces to Wyoming, USA (Festa-Bianchet, 2008). Mountain goats uniquely use their habitats, showing mixed seasonal and sexual habitat selection preferences, which possibly lends to the low genetic variability across their genomes (Shafer et al., 2012; Ortego et al., 2011; Poole & Heard, 2003). Their long lifespans, along with seasonally and sexually dependent habitat selection, makes mountain goats a unique study system for understanding the how the evolution of the microbiome to support a highly adapted and unique alpine specialist.

We assayed the gut microbiome of four western North American mountain goat populations, of which one was a captive population, to measure the alpha and beta diversity and taxonomic composition (Figure 1). Statistical comparisons were used to detect the influence of captivity, space and time on the diversity and composition of the gut microbiome. This is the first study to establish a microbiome profile for both captive and for wild mountain goat populations. There is evidence that species of origin (evolution) and diet choice are drivers behind the average composition of the microbiome, meaning that there should be similar profiles across all populations (Sharpton, 2018; Hale et al., 2018). Howeever, captivity generally decreases diversity measures in mammals and there is evidence of seasonality influencing mammalian microbiomes (McKenzie et al., 2017; Sun et al., 2016; Carey et al., 2013). Based on current knowledge, we predicted that the effect of captivity would drive the strongest differences in the diversity and the composition in mountain goat gut microbiomes, followed by seasonality and biogeography.

Methods

Study species

Mountain goats are part of the Bovidae family and inhabit the mountains of western North America. Mountain goats are an important trophy and subsistence game species and populations are managed for harvest and non-consumptive uses. Although they are economically and ecologically important, mountain goats remain one of the most difficult animals to study large North America (Festa-Bianchet & Côté, 2008; Lowrey et al., 2017). Mountain goats can be described as generalist foragers relative to some other ruminant species, but it has been shown that do clearly show preferential selection of forage under some circumstances (Côté and Festa-Bianchet, 2003). During their daily movements, mountain goats spread an abundant amount of compact, dry pellets (feces) that are well preserved in alpine environment they occupy.

Study sites

The Calgary Zoo, Alberta, Canada (CZ; 51°N, 114°W) is one of Canada's oldest accredited zoos. The zoo houses a small group of captive mountain goats that are fed year-round an *ad libidum* diet of herbivore pellets and mixed hay. This group of mountain goats are accessible for research and veterinary purposes. Caw Ridge, Alberta (CR; 54N, 119W) is an alpine and sub-alpine habitat in western Canada with a regional population of wild mountain goats that have been extensively monitored since 1989 (Ortego et al., 2011; Mainguy, Côté, & Coltman, 2009; Festa-Bianchet & Côté, 2008). As of 2017, CR was home to about 30 marked mountain goat individuals. This landscape only provides high quantities of quality forage during the summer months (June-August), while the rest of the year it is covered in snow or ice (Festa-Bianchet & Cote, 2008). Altitudinal migration does not occur in this population. Ship Mountain and Yes Bay (YB; 56N, -132W) are both located in the Cleveland Peninsula, a small region in southeast Alaska, United States. Wild individuals on the Cleveland Peninsula are genetically and morphologically distinct from mainland populations (Breen et al., submitted). Notably, mountain goats from this region are impacted by a maritime climate and spend time at low elevations (Fox & Smith, 1988; Smith & Raedeke, 1982). Available diet information for the populations are found in Supplementary Table S1.

Sample collection and DNA extraction

Fresh mountain goat fecal samples were collected from SM, YB, CR, and CZ during the summer months (June-August 2016). Samples were collected at CR after observing groups of mountain goats defecate, where those in Alaska were collected from areas where mountain goats were observed. All samples (N=54), except those from CR, were stored immediately in 70% ethanol (EtOH) and frozen at -20°C in plastic 50 ml conical tubes for preservation. CR samples were stored individually in plastic sample bags at -20°C. All surfaces were sterilized with 90% EtOH and 10% bleach solution to prevent environmental contamination. A small portion of each single fecal sample (~1/3) was digested over night at 56°C in 20 ul proteinase K and 180 ul Buffer ATL from the Qiagen DNeasy Blood & Tissue Kit. The gDNA was extracted from the digest by the Qiagen AllPrep PowerFecal DNA/RNA Kit following manufacturers protocol (Qiagen 80244).

Quality assessment and library preparation

Species identification was confirmed for SM samples as the population is sympatric with Sitka black-tailed deer (*Odocoileus hemionus sitkensis*). The mitochondrial D-Loop was amplified using L15527 and H00438 primers and sequenced on an ABI3 3730 (Wu et al. 2003). The consensus sequences generated in BioEdit (v 7.0.4.1) were screened using NCBI BLAST to identify the species. For all known mountain goat fecal samples DNA concentrations were measured with a Qubit 3.0 Fluorometer per manufacturers protocol (Invitrogen). Samples were concentrated if the extracted concentration of gDNA was below 3 ng/ul. The validated Illumina 16S rRNA Metagenomic Sequencing Library Preparation (#15044223 rev.B) protocol was then followed for library preparation (Rimoldi, Terova, Ascione, Giannico, & Brambilla, 2018). The 16S ribosomal ribonucleic acid (16S rRNA) hypervariable region, specifically the V3 and V4 regions, were targeted with four variants of 341F and 805R primers using the primers designed by Jakobsson et al., 2014. A unique combination of Nextera XT indexes, index 1 (i7) and index 2 (i5) adapters, were assigned to each sample for multiplexing and pooling of 96 samples.

Four replicates of each sample of fecal gDNA were amplified in 25 ul PCR using the 341F and 805R primers (Jakobsson et al., 2014). The replicated amplicons for each sample were combined into a single reaction of 100 ul and purified using a QIAquick PCR Purification Kit

(Qiagen, 28104). Sample indexes were annealed to the amplicons using an 8-cycle PCR reaction to produce fragments approximately 630 bp in length. Aliquots of 100 ng DNA for each sample were pooled together and purified with the QIAquick PCR Purification Kit for a final volume of 50 ul. The final purified library was validated by TapeStation (Agilent, G2991AA) and sequenced in 300 bp pair-end reads on an Illumina MiSeq sequencer at the Genomic Facility of Guelph University (Guelph, Ontario).

Analysis

Bioinformatics and taxonomic evaluation

The quality of the raw sequences was assessed with FastQC (v 0.11.4) and the low-quality cut-off for forward and reverse reads was determined. Forward and reverse reads were then imported into QIIME2 (v 2018.6). The quality control function DADA2 was used to truncate forward and reverse reads and perform denoising, and the detection and removal of chimeras. The results of DADA2 with only forward, reverse, and merged reads were analyzed independently. Sequencing data were grouped by status (captive or wild), population (CZ, CR, SM, or YB), and collection time (samples from CR collected in June, July, or August) for analytical purposes.

Samples within the groupings were normalized to the sample with the fewest contigs and diversity estimates were calculated in QIIME2. Alpha diversity estimates of community richness included Shannon Index and observed OTUs and estimates of community evenness included Pielou's evenness. Pairwise sample dissimilarity estimates (beta diversity), which are estimates of community dissimilarity, included Bray-Curtis, unweighted UniFrac, and weighted UniFrac dissimilarity distance matrices. The taxonomy, to the species level, of all sample reads were assigned using Silva 132 reference taxonomy database. The relative proportion of *Firmicutes* to *Bacteroidetes* was calculated for each of the grouped data.

Statistical analysis and visualization

Differences in community richness and evenness by the grouping (population, status, or collection month) were tested with the Kruskal-Wallis rank sum test and p-values were adjusted using Benjamini & Hochberg correction (q-values; Storey, 2002). All taxonomic analysis and

visualization were computed with the unclassified reads removed. A Wilcoxon rank sum test with continuity correction was used to assess the differences in relative abundance of taxonomic classifications based on origin with all unclassified sequences removed. A Kruskal-Wallis rank sum test was used to assess the differences in relative abundance of taxonomic classifications based on collection month. Differences were treated as significant if the p-value was < 0.01 and a post-hoc (Dunn) test was conducted to determine where the differences occurred between the three collection times. Statistical PERMANOVA tests were conducted using the ADONIS function from the R package Vegan (v 2.5.2) on Bray Curtis dissimilarity matrices to test for the presence of shifts in the microbiome communities between groups. A principle coordinates analysis (PCoA) was conducted using Bray Curtis dissimilarity matrices. Data were visualized with the ggplot2 (v 2.3.0.0) package in RStudio (v 3.4.1).

Results

Samples and quality filtering

A total of 54 fecal samples were selected for this study, however seven fecal samples from SM were excluded because of > 95% identification as black-tailed Sitka deer and five were excluded for quality reasons. A total of ~ 5.37 million paired-end reads were generated from the remaining 42 fecal samples (SRA accession number XXXXXX). FastQC analysis indicated that both forward and reverse reads lost quality > 250 bp in length (Phred score < 25), so all reads were trimmed to a length of 250 bp and following DADA2 quality filtering, resulted in 896,534 high quality overlapping reads (contigs) kept for taxonomic and diversity analyses. The sequence breakdown for each group can be seen in Supplementary Table S2. Analyses were also conducted on each read set individually, but we only report the results for the merged reads as the patterns were qualitatively similar (just considerably more data). Contigs that were classified to Archaeal lineages (6,013 contigs) were removed from the analysis. The remaining 890,521 contigs were classified into 3,886 unique 16S rRNA ribosomal sequence variants (RSVs) with at least 1 representative sequence, and 3,854 unique RSVs with at least 10 representative sequences.

Microbiome communities between captive and wild mountain goats

The three alpha diversity metrics did not show any differences between the captive and wild populations (q-value > 0.89 for all comparisons; Table 1; Figure 2). There were more unique classifications in wild than in captive mountain goats (Supplementary Table S3). Based on relative abundances the top two phyla for captive and wild mountain goats were *Firmicutes* and *Bacteroidetes*, with the remaining of the top five only differing by *Spirochaetes* for captive and by *Proteobacteria* for wild mountain goats (Table 2; Table 3). The important distinguishing taxonomic differences (p-value < 0.01) between captive and wild mountain goats arose from 3 phyla, 4 classes, 4 orders, 14 families, 33 genera, and 26 species of bacteria (Supplementary Table S4; Figure 3). A 4-way PERMANOVA of the Bray Curtis dissimilarity matrix indicated that the captive and wild mountain goats had significant shifts (p<0.001) in their microbiome communities (Table 4; Figure 2).

Microbiome communities across four distinct mountain goat populations

The three alpha diversity metrics did not show any differences between the four populations (q-value > 0.57 for all comparisons; Table 1; Figure 2). At the phylum level there were one more unique classification in Alberta mountain goats than in Alaskan mountain goats (Supplementary Table S2). At the genus level there were more unique taxonomic classifications in CZ than in all other populations, with SM having the fewest (Supplementary Table S5). Based on relative abundances the top two phyla for all four mountain goat populations were *Firmicutes* and *Bacteroidetes* (Table 2), with the remaining top five phyla being the most different in SM, which differed by two unique phyla (*Tenericutes* and *Lentisphaerae*; Table 2). The top five genera, with varying relative abundance, were the same for all populations except CZ, which differed with one unique genus (*Bacteroides*; Table 3). A 4-way PERMANOVA of the Bray Curtis dissimilarity matrix indicated that the four populations of mountain goats had significant shifts (p<0.001) in their microbiome communities (Table 4) and a principle components analysis (PCoA) grouped the CZ samples along PC1 differently than the other three populations that were geographically structured (Figure 4).

Microbiome communities in a single mountain goat population across time

The three alpha diversity metrics showed moderate differences between the collection months at CR (q-value = 0.04 for all comparisons; Table 1; Figure 2), with a trend toward decreasing

diversity as the summer progressed. The significant differences (q-value < 0.01) in the taxonomies observed between June-July, June-August, and July-August at CR were contributed by 3 classes, 4 families, 3 genera, and 3 species (Supplementary Table S5). There was no difference in the phyla or orders observed between any months. Based on relative abundances the top two phyla were *Firmicutes* and *Bacteroidetes*, with the remaining of the top five phyla only differing by the occurrence of *Planctomycetes* in August (Table 2). The top five genera, with varying relative abundances, were different by one unique genus in June and by two unique genera in July and August (Table 3). A 4-way PERMANOVA of the Bray Curtis dissimilarity matrix indicated that the collection months had shifts in their microbiome communities (p=0.018; Table 4) and a PCoA was unable to differentially separate samples based on collection month (Supplementary Figure S1).

Discussion

The structure of the gut microbiome is influenced by a multitude of biotic and abiotic host-specific factors, including genetics, diet, environment, and health status (Bahrndorff et al., 2016). The gut microbiome interacts with the host via genes, proteins, or metabolites and is important for maintaining normal health and physiology (Feng et al., 2018) and there is evidence that the microbiome is more tightly linked with fitness (survival and successful reproduction) than to dietary niches (Amato et al., 2018). Thus, there is a clear body of evidence that links the gut microbiome to the health of individuals and these links may be useful tools in guiding population management in captive and wild populations (Bahrndorff et al., 2016). This is the first study to characterize the microbiome of wild mountain goats, while also quantifying how status (captive or wild), spatial distribution, and time influence such communities. These data should inform both basic and applied research of ungulate microbiomes, and potentially inform the management of this enigmatic species by identifying the composition of microbial populations in wild, healthy individuals that could be used as a target. The non-invasive nature of sampling, particularly with respect to disturbance of mountain goats and their difficult terrain, is an attractive element of health monitoring with respect to this species.

Comparing diversity metrics

Most microbiome comparisons to date indicate significant decreases in the alpha diversity (diversity and evenness) in captive ruminant populations; however, there are studies that show no changes or even increases in diversity in captive populations in other mammals (McKenzie et al., 2017; Kohl et al., 2014). In Bovidae, McKenzie et al. (2017) showed no statistical difference between captive and wild groups, which was also observed in mountain goats. This finding suggests that, for the mountain goats at the Calgary Zoo, captivity has played little effect on overall microbiome diversity. There are, however, clear shifts observed between the captive and wild mountain goat microbiome communities (Figure 3).

The Shannon index, a standard measure of community diversity, for mountain goats was comparable to other unique high-altitude ruminant populations, such as muskoxen (*Ovibos moschatus*), Bactrian camel (*Camelus bactrianus*), and Norwegian reindeer (*Rangifer tarandus tarandus*), but less comparable to roe deer (*Capreolus capreolu*) and dzo (*Bos grunniens* and *Bos taurus* hybrid; He et al., 2018; Salgado-Flores et al., 2016). Although there is evidence of convergent evolution of the microbiome in high-altitude ruminants, the microbiome community diversity we measured through the Shannon index is not in agreeance with this evidence (Zhang et al., 2016). There are strong seasonal dynamics in activity, diet, and body condition which could lead to disparity between the microbiome community diversity we measured in mountain goats and other studies from high-altitude ruminant species. In particular, mountain goats appear to lose and regain a significant amount of body mass (up to 30-40%) between winter and summer (Côté and Festa-Bianchet, 2003). Such dynamics may provide important context for understanding seasonal changes in the microbiome. For example, during spring and early summer animals (particularly parturient females) may be oriented to obtaining, absorbing, and utilizing nutrients whereas later in late-summer and fall may be storing nutrients for periods of winter scarcity.

The dominance of *Firmicutes* and *Bacteroidetes* phyla is consistent with other ruminant studies (O' Donnell et al., 2017). There were minimal changes in the relative percent *Firmicutes* (from 75% to 72%) and increases in the relative classified *Bacteroidetes* (from 16% to 17%) from wild to captive mountain goats, which is consistent with the general mammalian trends observed in McKenzie et al. (2017). The *Firmicutes* to *Bacteroidetes* ratio (F/B) has been used to give a rough estimate of the function of the microbiome; for example, a higher F/B ratio in

treatment groups compared to control groups in mice and humans has generally been linked with diseases such as obesity, and an elevated body mass index (BMI; Koliada et al., 2017). Koliada et al., (2017) suggested that the association between increase F/B ratios and elevated BMIs arises from *Firmicutes*, linked with nutrition absorption and circulation, being more efficiently able to participate in energy utilization than *Bacteroidetes*, that are linked with nutrition storage. Therefore, increases in the relative abundance of *Firmicutes* can significantly contribute to the hosts' elevated BMI phenotype. The diets of ruminants are typically low in nutrients, especially during winter months, and the elevated F/B ratio in mountain goats, relative to some other ruminants, suggests that the metabolism efforts of mountain goat microbiomes are driven to obtain and absorb, rather than store, nutrition. Alternatively, a finding of elevated F/B ratio could also be indicative of other stressors, such as recovery from the cost of winter survival or costs of lactation.

Actinobacteria, another phylum associated with body condition (e.g. BMI) and a phylum that may contribute to the immune system of the host, is a typical subdominant phylum seen in ruminants (Koliada et al., 2017; Ventura et al., 2007). However, *Actinobacteria* was not seen as a subdominant phylum in captive (0.68%) or wild (0.91%) populations of mountain goats which is consistent with observations in wild Sika Deer (*Cervus nippon hortulorum*) and wild forest musk deer microbiomes (Guan et al., 2017). The top genera in wild mountain goat microbiomes included *Ruminococcaceae UCG-01*0, *Ruminococcaceae UCG-005* and *Christensenellaceae R-7 group*, which were also top groups seen in wild populations of sika deer and forest musk deer microbiomes (Guan et al., 2017; Li et al., 2017). We speculate that these genera are essential for normal gut function in wild ruminant populations although specific functions are unknown.

Detectable shifts in microbiome community compositions

There were significant shifts in the microbiome community composition between the four different population groups. However, an R^2 value of 0.09 suggests that there are multiple factors, beyond population origin driving the shifts seen in the microbiome communities. Interestingly, although the microbiome communities between captive and wild groups of mountain goats were significantly different, there was less variation explained by captive and wild group differences (R^2=0.03) than between different population groups. The spatial

differences thus are more prominent in shaping microbial composition than that of captivity, and our model fit is consistent with other comparisons of captive and wild groups of ruminants (McKenzie et al., 2018). Here we speculate that the northern latitude or geographic isolation of the Alaskan population has contributed to reduced microbiome diversity in terms of species number (Shafer et al., 2012; Table S3).

At the phylum level, the most significant ($p < 0.001$) shifts in microbiome community composition between captive and wild groups were seen in *Planctomycetes* (increased in wild), and *Fibrobacteres* and *Spirochaetes* (both increased in captive). Until recently, *Planctomycetes* were thought to be strictly environmental, but they have been linked to herbivorous microbiomes and are elevated in forage-based diets (Auffret et al., 2017; Baldo et al., 2017; Cayrou et al., 2013). The presence of *Fibrobacteres* is well established in herbivorous and omnivorous mammal microbiomes as *Fibrobacteres spp.* participates in cellulose degradation and nitrogen fixing (Rahman et al., 2015; Ransom-Jones et al., 2012). *Spirochaetes* are endosymbionts and also involved in cellulose degradation and nitrogen fixing, which are both fundamental cycles in the degradation of vegetation and cellulose-rich materials (Van de Water et al., 2016). Collectively, these shifts at the phyla level are consistent with diet alterations between captive and wild animals with similar patterns seen in black-tailed Sitka deer (Guan et al., 2017).

Interestingly, sample time at CR had the largest effect (R^2=0.15; p=0.018). Notable, the most different genera ($p < 0.001$) between June and July was the *Eubacterium hallii group* (increased in July); similarly, *Clostridiales family XIII AD3011 group* and *Eubacterium hallii groups* increased in July. Between July and August, *Erysipelatoclostridium* increased. The *Eubacterium hallii group* is a commonly observed genus in mammal microbiomes and plays a role in glycolysis (Engels et al., 2016); we speculate that the increase of *Eubacterium hallii group* in July relative to June or August might be associated with shifts in forage quality during the peak of summer at CR. While the core microbiome appears relatively constant in the mountain goat, at the local-level there are clear shifts in individual bacteria that reflect changes in the microhabitat over time.

Conclusion

We are beginning to understand how the gut microbiome influences the host and is, in turn, influenced by the host. There is a need to understand how to best apply this knowledge to aid the management of mammal populations (Li et al., 2018; O' Donnell et al., 2017). The direct connection of the gut microbiome to both individual and population health makes gut microbiome assays an important tool for monitoring the health and disease trends of domesticated, captive, and wild populations of mammals (Bahrndorff et al., 2016; Jiménez & Sommer, 2017). This study suggests that there are clear differences in microbiome community composition, but not diversity, that can be best explained by a combination of factors, including status, seasonality and population of origin. The diversity and taxonomic findings are consistent with other recent ruminant studies, including the microbiomes measured in sika deer, muskoxen, and forest musk deer. The baseline microbiome data generated here thus has the potential to provide valuable insight into the health of wild mountain goat populations and can be applied to guide population management decisions on mountain goat health in the future.

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Figure 1. Geographical distribution of study sites in Alaska and Canada. With blue representing Calgary Zoo, Alberta; green representing Caw Ridge, Alberta; yellow representing Ship Mountain, Alaska; and purple representing Yes Bay, Alaska. The size of the points reflects the relative number of samples that originate from the study site.

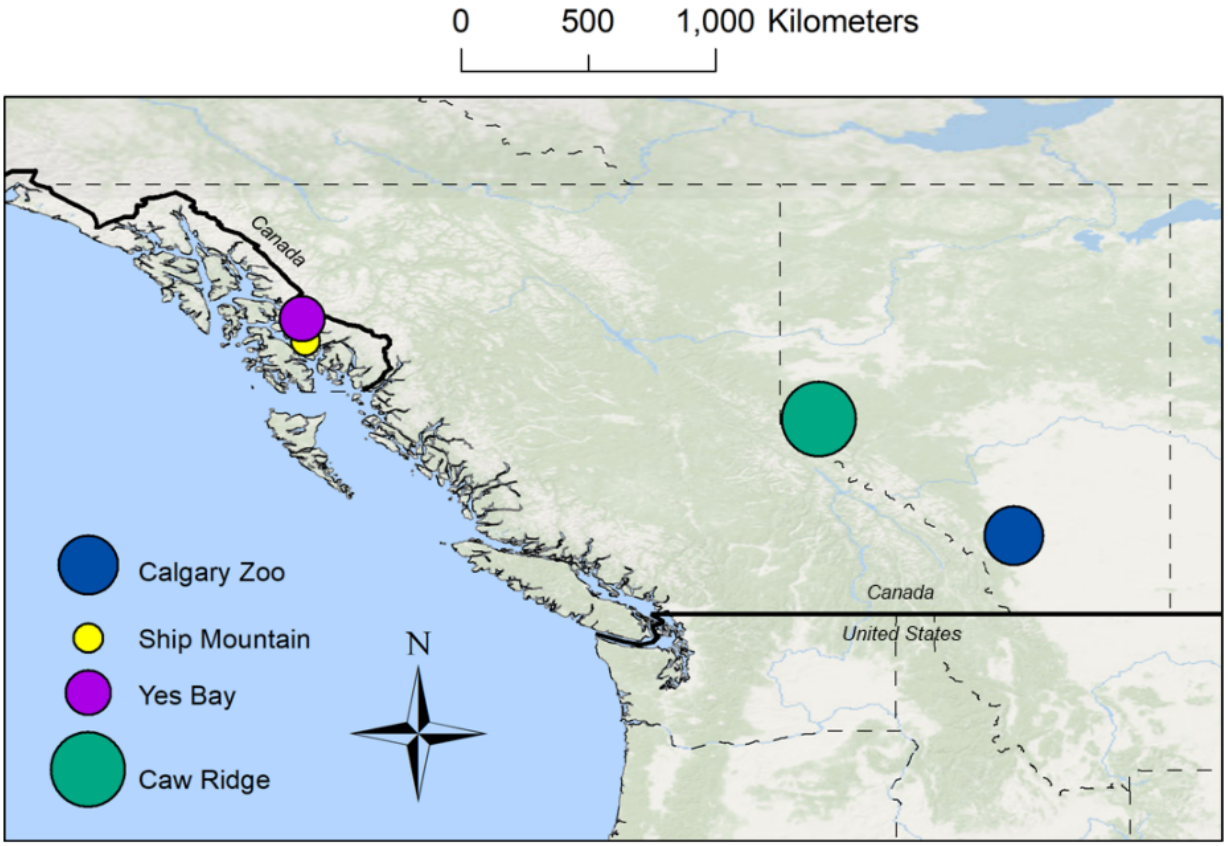

Figure 2. Calculated alpha diversity measures on rarified samples for captive and wild, spatial, and temporal comparisons: (A) Shannon Index reflecting community diversity, (B) Pielou's community evenness, and (C) observed number of operational taxonomic units (OTUs).

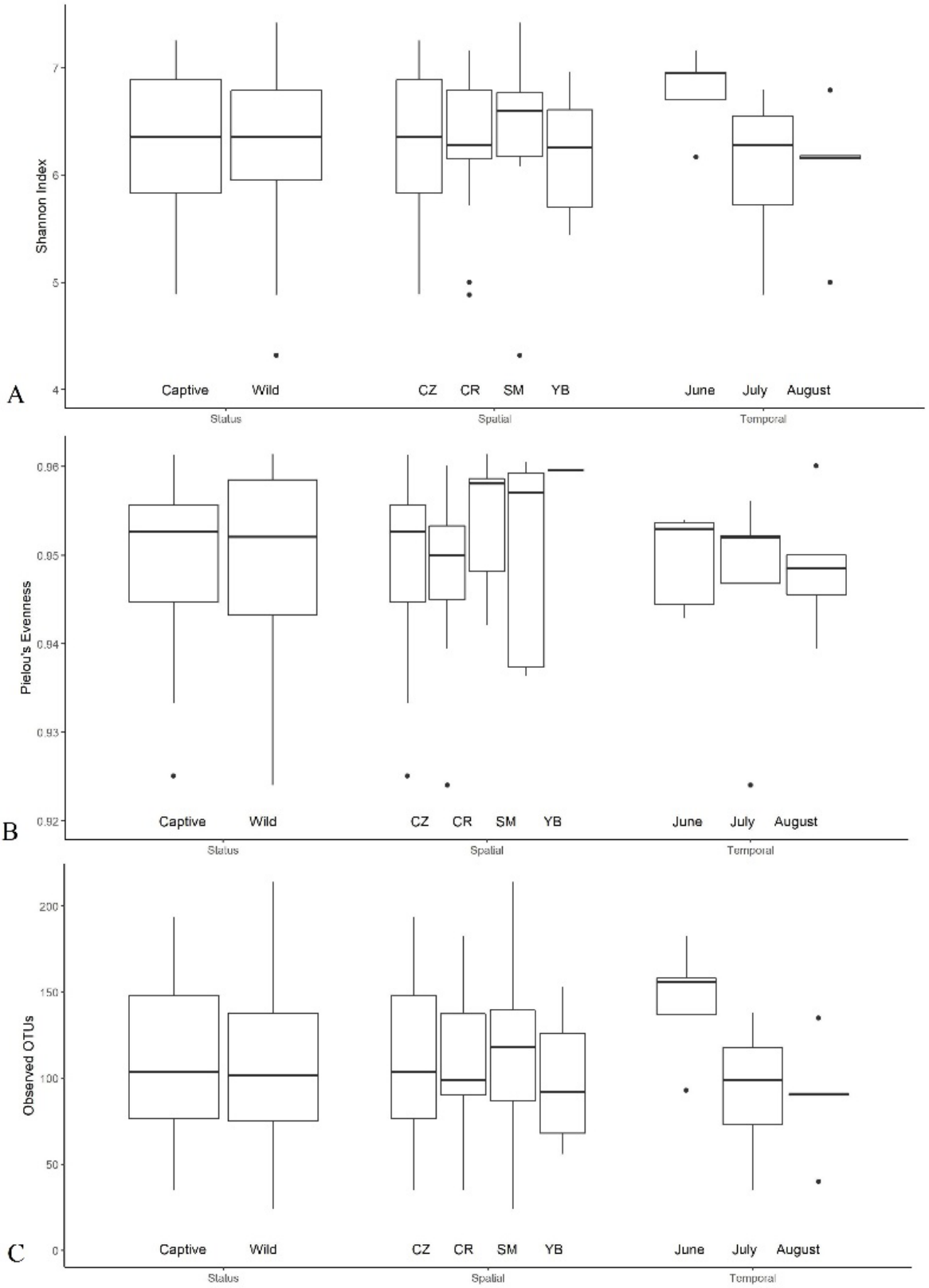
A
Shannon Index
7
6
5
4
Captive
Wild
CZ
CR
SM
YB
June
July
August
Status
Spatial
Temporal
B
Pielou's Evenness
0.96
0.95
0.94
0.93
0.92
Captive
Wild
CZ
CR
SM
YB
June
July
August
Status
Spatial
Temporal
C
Observed OTUs
200
150
100
50
0
Captive
Wild
CZ
CR
SM
YB
June
July
August
Status
Spatial
Temporal

Figure 3. The difference in relative percent abundance of significant genera (p-value < 0.01) that differ between captive and wild populations of mountain goats and their taxonomic breakdown at the phylum and class levels.

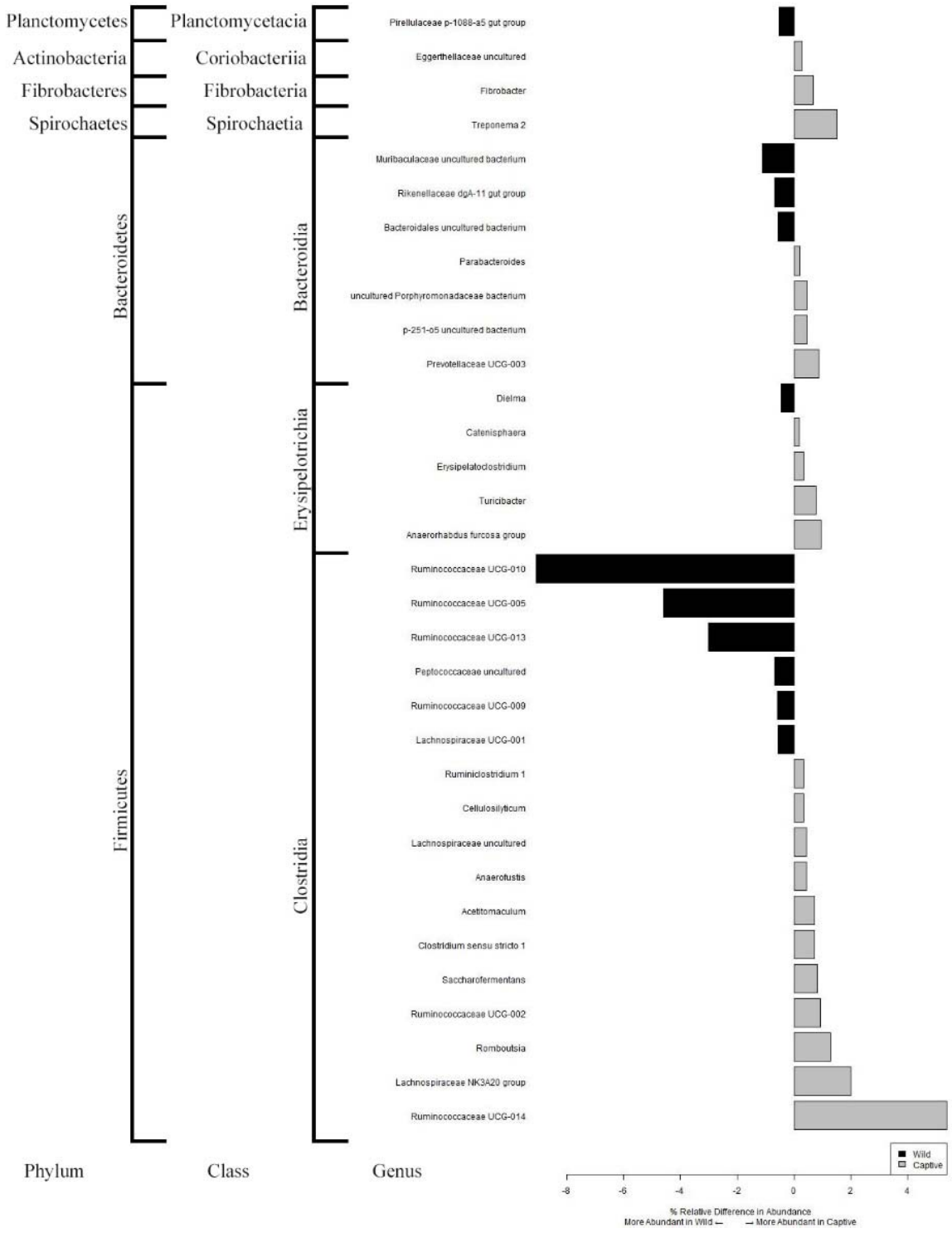
Planctomycetes
Actinobacteria
Fibrobacteres
Spirochaetes
Bacteroidetes
Firmicutes
Planctomycetacia
Coriobacteriia
Fibrobacteria
Spirochaetia
Bacteroidia
Erysipelotrichia
Clostridia
Pirellulaceae p-1088-a5 gut group
Eggerthellaceae uncultured
Fibrobacter
Treponema 2
Muribaculaceae uncultured bacterium
Rikenellaceae dgA-11 gut group
Bacteroidales uncultured bacterium
Parabacteroides
uncultured Porphyromonadaceae bacterium
p-251-o5 uncultured bacterium
Prevotellaceae UCG-003
Dielma
Catenisphaera
Erysipelatoclostridium
Turicibacter
Anaerorhabdus furcosa group
Ruminococcaceae UCG-010
Ruminococcaceae UCG-005
Ruminococcaceae UCG-013
Peptococcaceae uncultured
Ruminococcaceae UCG-009
Lachnospiraceae UCG-001
Ruminiclostridium 1
Cellulosilyticum
Lachnospiraceae uncultured
Anaerofustis
Acetitomaculum
Clostridium sensu stricto 1
Saccharofermentans
Ruminococcaceae UCG-002
Romboutsia
Lachnospiraceae NK3A20 group
Ruminococcaceae UCG-014
Phylum
Class
Genus
Wild
Captive
-8
-6
-4
-2
0
2
4
% Relative Difference in Abundance
More Abundant in Wild ←
→ More Abundant in Captive

Figure 4. Principal components analysis (PCoA) of Bray Curtis dissimilarity index values. The first component explains 3.34% of variation and separates the captive population (Calgary Zoo), from the wild populations. The second component explains 3.15% of the variation and moderately separates Caw Ridge, a southern wild population, from both of the northern wild populations.

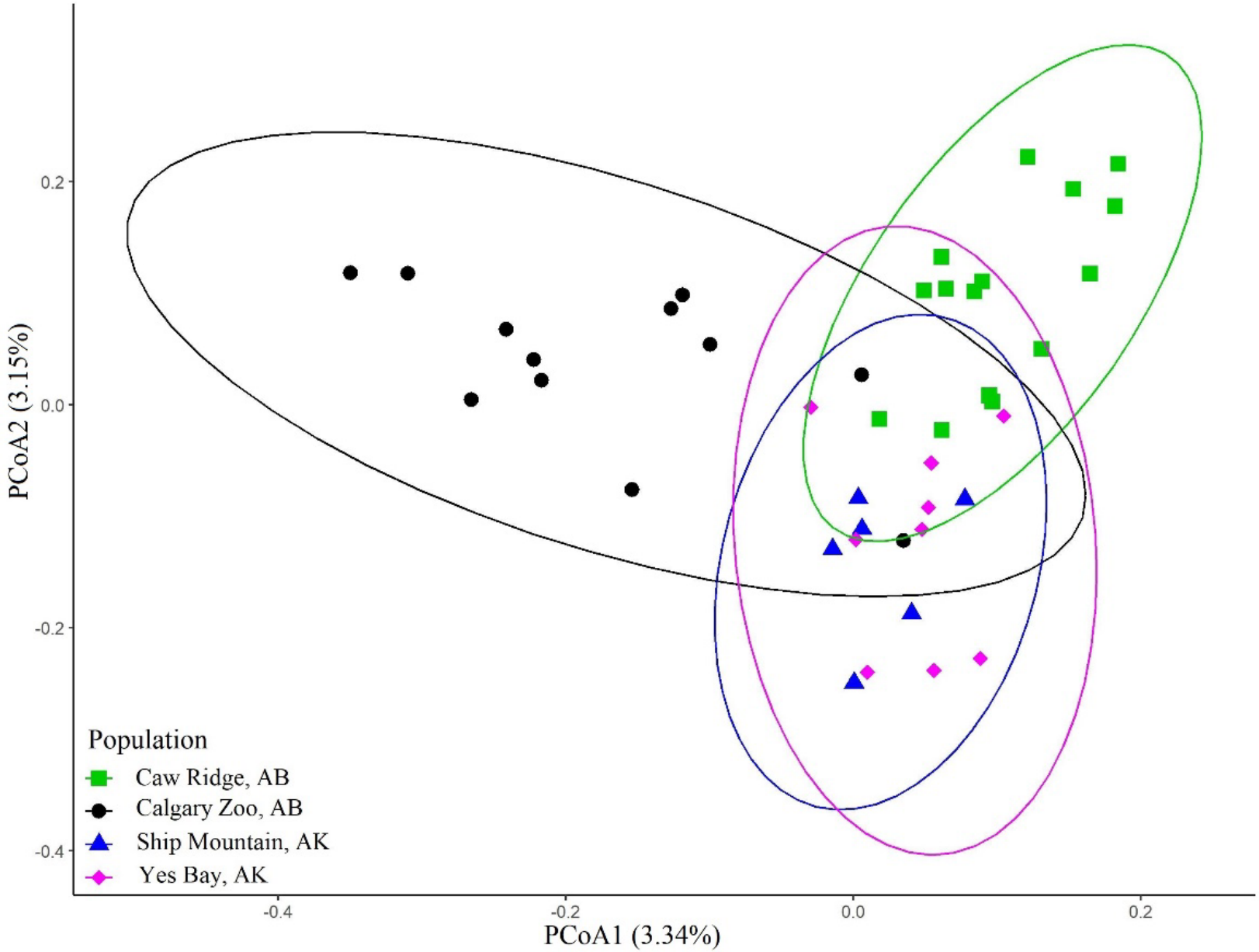
0.2
0.0
-0.2
-0.4
PCoA2 (3.15%)
-0.4
-0.2
0.0
0.2
PCoA1 (3.34%)
Population
Caw Ridge, AB
Calgary Zoo, AB
Ship Mountain, AK
Yes Bay, AK

Table 1. Alpha diversity analysis for the four mountain goat populations (Calgary Zoo, Alberta = CZ; Caw Ridge, Alberta = CR; Ship Mountain, Alaska = SM; and Yes Bay, Alaska = YB), for captive and wild mountain goats, and for the different collection months at CR. Alpha diversity metrics include observed OTUs, Shannon Index (community diversity), and Pielou's evenness (community evenness). All corrected p-values (q-values) were > 0.89 for all comparisons.

Group	*N*	Observed OTUs	Shannon Index	Pielou's Evenness
Status				
Captive	12	1,348	6.28±0.82	0.95± 0.01
Wild	30	3,239	6.27±0.72	0.95± 0.01
Population				
CZ	12	1,348	6.27± 0.82	0.95± 0.01
CR	15	1,637	6.29±1.06	0.95± 0.01
SM	6	700	6.30±0.68	0.95± 0.01
YB	9	902	6.22±0.59	0.95± 0.01
Month				
June	5	713	6.79±0.35	0.95± 0.01
July	5	467	6.06±0.76	0.95± 0.01
August	5	449	6.06±0.65	0.95± 0.01

Table 2. The top five microbiome phyla based on relative abundance for the four mountain goat populations (Calgary Zoo, Alberta = CZ; Caw Ridge, Alberta = CR; Ship Mountain, Alaska = SM; and Yes Bay, Alaska = YB), for captive and wild mountain goats, and for the different collection months at CR. The relative *Firmicutes* to *Bacteroidetes* ratios are also reported. Bolded groups represent a unique classification among groups.

Group						
Status			*F/B Ratio*			
Captive	*Firmicutes* 72%	*Bacteroidetes* 17%	4.35	*Patescibacteria* 4.6%	***Spirochaetes*** 1.6%	*Verrucomicrobia* 1.1%
Wild	*Firmicutes* 75%	*Bacteroidetes* 16%	4.73	*Patescibacteria* 2.5%	*Verrucomicrobia* 1.6%	***Proteobacteria*** 1.3%
Population						
CZ	*Firmicutes* 72%	*Bacteroidetes* 17%	4.35	*Patescibacteria* 4.6%	***Spirochaetes*** 1.6%	*Verrucomicrobia* 1.1%
CR	*Firmicutes* 77%	*Bacteroidetes* 14%	5.63	*Patescibacteria* 3.8%	*Verrucomicrobia* 2.1%	***Actinobacteria*** 1.4%
SM	*Firmicutes* 71%	*Bacteroidetes* 20%	3.54	*Proteobacteria* 2.8%	***Tenericutes*** 1.7%	***Lentisphaerae*** 0.93%
YB	*Firmicutes* 76%	*Bacteroidetes* 17%	4.49	*Proteobacteria* 2.1%	*Patescibacteria* 1.6%	*Verrucomicrobia* 1.5%
Month						
June	*Firmicutes* 74%	*Bacteroidetes* 18%	4.18	*Patescibacteria* 3.5%	*Verrucomicrobia* 1.3%	*Actinobacteria* 0.93%
July	*Firmicutes* 86%	*Bacteroidetes* 5.8%	14.77	*Actinobacteria* 3.2%	*Patescibacteria* 3.1%	*Verrucomicrobia* 0.97%
August	*Firmicutes* 74%	*Bacteroidetes* 15%	4.81	*Patescibacteria* 5.4%	*Verrucomicrobia* 3.2%	***Planctomycetes*** 0.67%

Table 3. The top five microbiome genera, with relative percent classification, for all four mountain goat populations, for captive and wild mountain goats, and for the different collection months at Caw Ridge, Alberta. Bolded groups represent a unique classification among groups.

Group					
Status					
Captive	*Ruminococcaceae UCG-014* 12%	*Eubacterium coprostanoligenes group* 8%	*Ruminococcaceae UCG-005* 6%	***Bacteroides*** 6%	*Christensenellaceae R-7 group* 5.6%
Wild	***Ruminococcaceae UCG-010*** 11%	*Ruminococcaceae UCG-005* 11%	*Eubacterium coprostanoligenes group* 6.5%	*Ruminococcaceae UCG-014* 6.2%	*Christensenellaceae R-7 group* 6.1%
Population					
CZ	*Ruminococcaceae UCG-014* 11.7%	*Eubacterium coprostanoligenes group* 8.1%	*Ruminococcaceae UCG-005* 6.1%	***Bacteroides*** 6.1%	*Christensenellaceae R-7 group* 5.6%
CR	*Ruminococcaceae UCG-005* 11.5%	*Christensenellaceae R-7 group* 9.7%	*Ruminococcaceae UCG-014* 8.8%	*Eubacterium coprostanoligenes group* 6.2%	*Ruminococcaceae UCG-013* 5.1%
SM	*Ruminococcaceae UCG-010* 21.3%	*Ruminococcaceae UCG-005* 9.5%	*Alistipes* 8.8%	*Eubacterium coprostanoligenes group* 5.8%	*Ruminococcaceae UCG-013* 4.4%
YB	*Ruminococcaceae UCG-010* 21.0%	*Ruminococcaceae UCG-005* 10.4%	*Eubacterium coprostanoligenes group* 8.0%	*Ruminococcaceae UCG-013* 7.2%	*Alistipes* 4.8%
Month					
June	*Christensenellaceae R-7 group* 10%	*Ruminococcaceae UCG-014* 10%	*Ruminococcaceae UCG-005* 10%	***Ruminococcaceae UCG-013*** 6%	*Bacteroides* 6%
July	*Christensenellaceae R-7 group* 13%	*Ruminococcaceae UCG-005* 12%	*Ruminococcaceae UCG-014* 9%	***Ruminococcaceae NK4A214 group*** 6%	***Eubacterium brachy group*** 5%
August	*Ruminococcaceae UCG-014* 11%	*Ruminococcaceae UCG-005* 11%	***Eubacterium coprostanoligenes group*** 10%	***Candidatus Saccharimonas*** 7%	*Bacteroides* 6%

Table 4. Results of 4-way, 2-way, and 3-way PERMANOVA comparisons of what?? of Bray Curtis dissimilarity matrix for all four mountain goat populations, for captive and wild mountain goats, and for the different collection months at Caw Ridge, Alberta, respectively.

Group	#perm	DF (factor:total)	SS	MS	F.model	p-value	R^2
Status	999	1:41	0.6702	0.67016	1.3953	0.001	0.034
Population	999	3:41	1.8011	0.60036	1.2626	0.001	0.091
Month	999	2:14	0.9960	0.49801	1.0631	0.018	0.151