The Gut Microbiome of Exudivorous Wild and Captive Marmosets

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Abstract

Among mammals, captive dietary specialists like primate folivores are prone to gastrointestinal distress and suffer the greatest gut microbiome diversity losses relative to wild individuals. Less is known about the gut microbiome of mammalian exudivores, which exploit tree gums and sap, and increased knowledge could improve management of wild exudivores and facilitate captive exudivore welfare. A number of primates, including *Callithrix* marmosets, represent key mammalian exudivores. Captive marmosets commonly develop gastrointestinal distress symptoms that in human diseases are linked to microbiome dysbiosis. Thus, we studied wild and captive *Callithrix* gut microbiome composition and predictive function through 16S V4 ribosomal subunit sequencing of 59 wild, translocated, and captive pure and hybrid *Callithrix.* We found that host environment had a stronger effect on the gut microbiome than host taxon. Captive marmosets showed relatively reduced gut microbiome diversity. Wild *Callithrix* gut microbiomes were enriched for carbohydrate function and *Bifidobacterium,* which process host-indigestible carbohydrates. Captive marmosets had the highest relative abundance of Enterobacteriaceae, a family containing several pathogenic bacteria. Captive marmosets also showed gut microbiome composition aspects seen in human gastrointestinal diseases. Thus, captivity may perturb the exudivore gut microbiome, which raises implications for captive exudivore welfare and calls for modified husbandry practices.

Keywords

exudivore, non-human primate, microbiome, Neotropical, microbiome, *Bifidobacterium*

Introduction

The mammalian gut microbiome plays an important role in host physiology [1-2], and microbiome dysbiosis may negatively impact host health [2-7]. Closely related mammals generally share similar gut microbiome composition (GMC), which is usually enriched for bacteria associated with the main macronutrients of a host's feeding strategy [8-10]. Yet, the environment significantly alters individual host microbiomes, as evidenced by differences in microbiome composition between wild and captive conspecifics [9, 11-14]. Gut microbiome studies of captive and wild mammals show that non-human primates (NHPs) experience relatively large losses of native gut microbiome diversity in captivity compared to the wild [6,11]. Additionally, folivorous NHPs are especially prone to GI problems in captivity [15], and among humans and non-human primates (NHPs) dysbiosis in GMC has been tied to gastro-intestinal (GI) diseases [7,15,16].

A number of mammals are exudivorous [16], particularly primates [18], but their gut microibome remains less studied relative to other mammals. However, the Brazilian *Callithrix* marmosets, obligate NHP exudivores, are excellent models to improve our understanding of exudivore gut microbiome. *Callithrix* species are endemic to Brazil and in the wild subsist on hard to digest oligosaccharides of tree gums or, if they gouge, hardened saps, that require fermentation for digestion [19, 20]. Marmosets are regularly maintained in captivity as biomedical research models [21], captive breeding of *C. aurita* (one of the 25 most endangered primates in the world [22]), and due to illegal pet trafficking [23, 24]. In captivity, marmosets commonly develop symptoms of GI distress referred to by some authors as marmoset wasting syndrome) like chronic diarrhea, chronic enteritis, and chronic colitis without clear pathogenesis

[25-28]. As mammalian dietary specialists, it is likely that captivity causes gut microbiome dysbiosis in *Callithrix,* which in turn may be implicated in the pathogenesis of GI distress.

Given these problems, further investigation of the gut microbiome of mammalian exudivores is necessary and will provide baseline data to improve their welfare in captivity. A necessary first step towards understanding diseased GMC profiles is defining baseline GMC variation of non-diseased individuals [2,29]. Thus, comparing GMC of wild and captive conspecifics is an important step for such approaches. Up to now, *Callithrix* gut microbiome studies have focused on captive *C. jacchus* to identify specific bacterial strains [30-33] and on how life history, social, or laboratory conditions affect the GMC [34-36]. Here, we determine gut microbiome profiles of wild and translocated/captive *Callithrix* sampled throughout Brazil. We applied 16S ribosomal subunit (rRNA, V4 region) gene amplicon sequencing of *Callithrix* anal microbiota, and investigated GMC and gut microbiome predictive functional profiles. Anal swabs were sampled from 59 individuals of four species and three hybrid types (Table 1) that were sampled in the wild and Brazilian captive facilities (Figure 1). We hypothesize that: (1) *Callithrix* taxa share similar bacterial taxa as part of host GMC; (2) captive *Callithrix* have less diverse GMC than wild *Callithrix*; and (3) *Callithrix* GMC and predictive functional profiles are strongly biased toward carbohydrate metabolism.

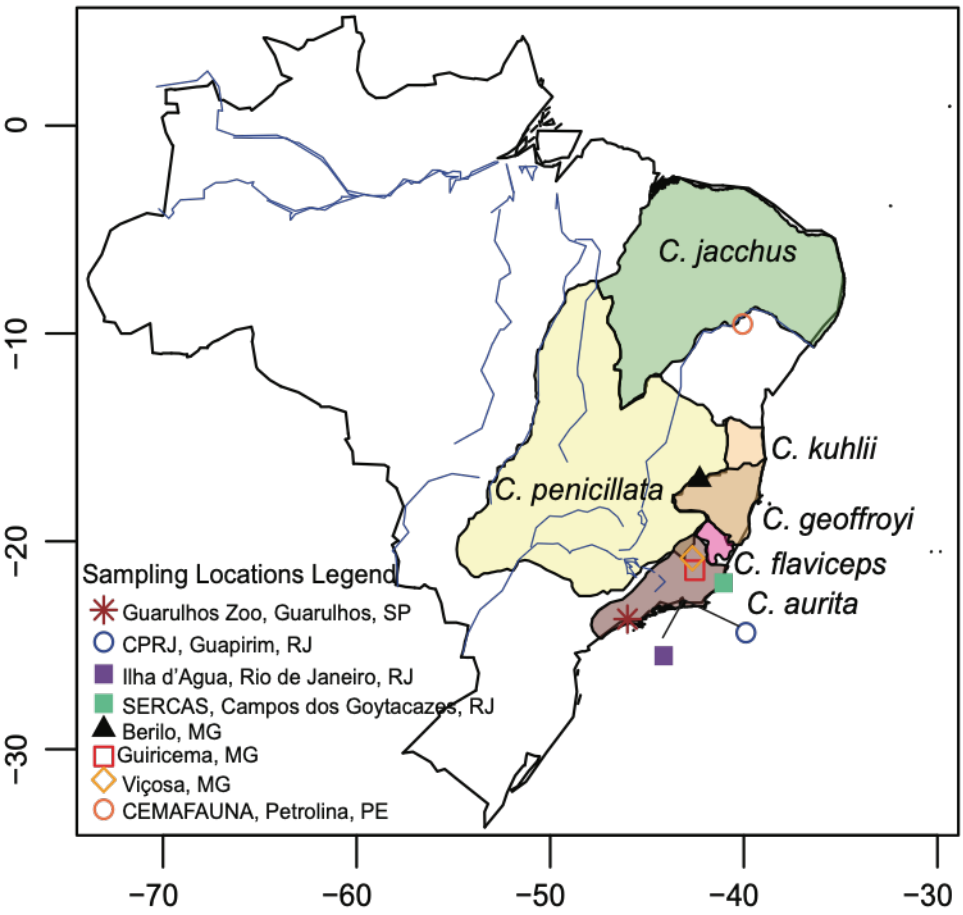

Figure 1. Natural *Callithrix* ranges and sampling locations in Brazil. Sampling locations are represented by different colored shapes and species names are written next to their respective ranges. Legend abbreviations are as follows: MG: Minas Gerais, Rio de Janeiro: RJ, Pernambuco: PE, São Paulo: SP; CPRJ: Centro de Primatologia do Rio de Janeiro; CEMAFAUNA: Centro de Conservação e Manejo da Fauna da Caatinga.

Table 1. Host count distribution by taxon, hybrid status, and environment categories

Classification	Species	Hybrid	Captive	Translocated	Wild
C. aurita	10	0	5	3	2
C. geoffroyi	4	0	3	0	1
C. jacchus	9	0	9	0	0
C. penicillata	7	0	6	1	0
C. jacchus x *C. penicillata*	0	23	1	22	5
C. penicillata x *C. geoffroyi*	0	5	0	0	0
C. aurita x *Callithrix* sp.	0	1	0	1	0
Total	**30**	**29**	**24**	**27**	**8**

Materials and Methods

Sample Collection

We collected anal swabs between 2015 and 2016 from 59 adult individuals (Figure 1, Table 1 and Supplementary Table 1). Marmoset sampling was authorized by the Brazilian Environmental Ministry (SISBIO protocol #47964-2), the Arizona State University IACUC (protocol# 15-144R). Wild animals were captured with Tomahawk style traps baited with bananas. All sampled animals were immobilized by injection of ketamine (10 mg/kg of body

weight) into the inner thigh intramuscular region. All were photographed, weighed, measured, examined clinically by veterinarians, and deemed healthy upon examination. Copan FLOQSwab tips were inserted and rotated in the anal region and then each swab was submerged in a storage buffer (50 mM Tris pH 8.0, 50 mM EDTA, 50 mM Sucrose, 100 mM NaCl, 1% SDS) for several seconds and then discarded. After processing, animals were returned to cages, and allowed to recover. Wild marmosets were released at original capture sites. Specimens taxon identification followed phenotype descriptions in [37-40], and confirmed by whole genome genotyping for some individuals (unpublished data). Specimens were also classified by their environment as wild (captured as free-range individuals), translocated (born wild but later put into captivity), or captive (born and raised in captivity).

Sample Processing and Sequencing

Bacterial DNA extraction from *Callithrix* anal swabs was carried out by adding 0.3g zirconia beads to tubes containing storage buffer and swabbed material following a modified phenol-chloroform protocol [41]. Modifications included beating the samples on a vortex fitted with a horizontal vortex adaptor (#13000-V1-24, Mo Bio, Carlsbad, CA, USA) for 10 minutes at step "2Aiii," precipitating samples in 100% ethanol in step "2Axvi," and rehydrating DNA pellets in 25 uL low TE buffer at step "2Axxii." Extracted DNA was quantified on a Qubit3 (Life Technologies, Carlsbad, CA, USA) with a dsDNA HS Assay Kit (Life Technologies). Then the V4 region of the bacterial 16S rRNA gene was amplified in triplicate using the barcoded primer set 515f/806r [42]. Genetic samples have been registered in the Brazilian CGen SISGEN database (Supplementary Table 2). Amplicon triplicates were combined for each individual and then pooled in equimolar amounts into a multiplexed Illumina sequencing library. The library was purified with a Zymo DNA Concentrator and Cleaner-5 (#D4013, Zymo Research, Irving,

CA, USA) and size selected for 375-380 base pairs with Agencourt Ampure XP (#A63880, Beckman Coulter, Indianapolis, IN, USA) magnetic beads. Following international regulations, DNA extraction and library preparation was carried out at the Federal University of Viçosa, Brazil and then sequenced at Arizona State University, USA on an Illumina MiSeq for 2x250 cycles. The dataset supporting the conclusions of this article is available in the NCBI SRA repository under Bioproject SUB6302859.

Bioinformatics and Statistical Analysis

Code for bioinformatics analysis described below for Qiime2-2018.6 and Qiime2-2020.2 [43] is available at https://github.com/Callithrix-omics/callithrix_microbiome. Data were demultiplexed using default parameters in Qiime2-2018.6. The DADA2 Qiime2-2018.6 plug-in [44] was used to quality-filter and trim sequences and join paired-end reads. Upon trimming, the first 10 and last 30 nucleotides were removed from reverse reads due to low base quality. These steps resulted in feature tables of DNA sequences and their per-sample counts, termed operational taxonomic units (OTUs). MAAFT and FastTree Qiime2-2018.6 plug-ins [45,46] aligned and produced a phylogenetic tree of feature sequences, which was then mid-pointed rooted.

Qiime2-2018.6's q2-diversity plug-in was used to estimate alpha diversity measures (Shannon's diversity index, observed OTUs, and Pielou's Evenness) and calculate beta diversity weighted UniFrac distance [47]. Based on rarefaction, we set the minimum sampling depth to 45000 reads for alpha and beta diversity analyses. Box-plots were generated for each alpha diversity measure according to host taxon and environment. Kruskal-Wallis group and pairwise tests determined statistical significance for each category. Statistical differences between host

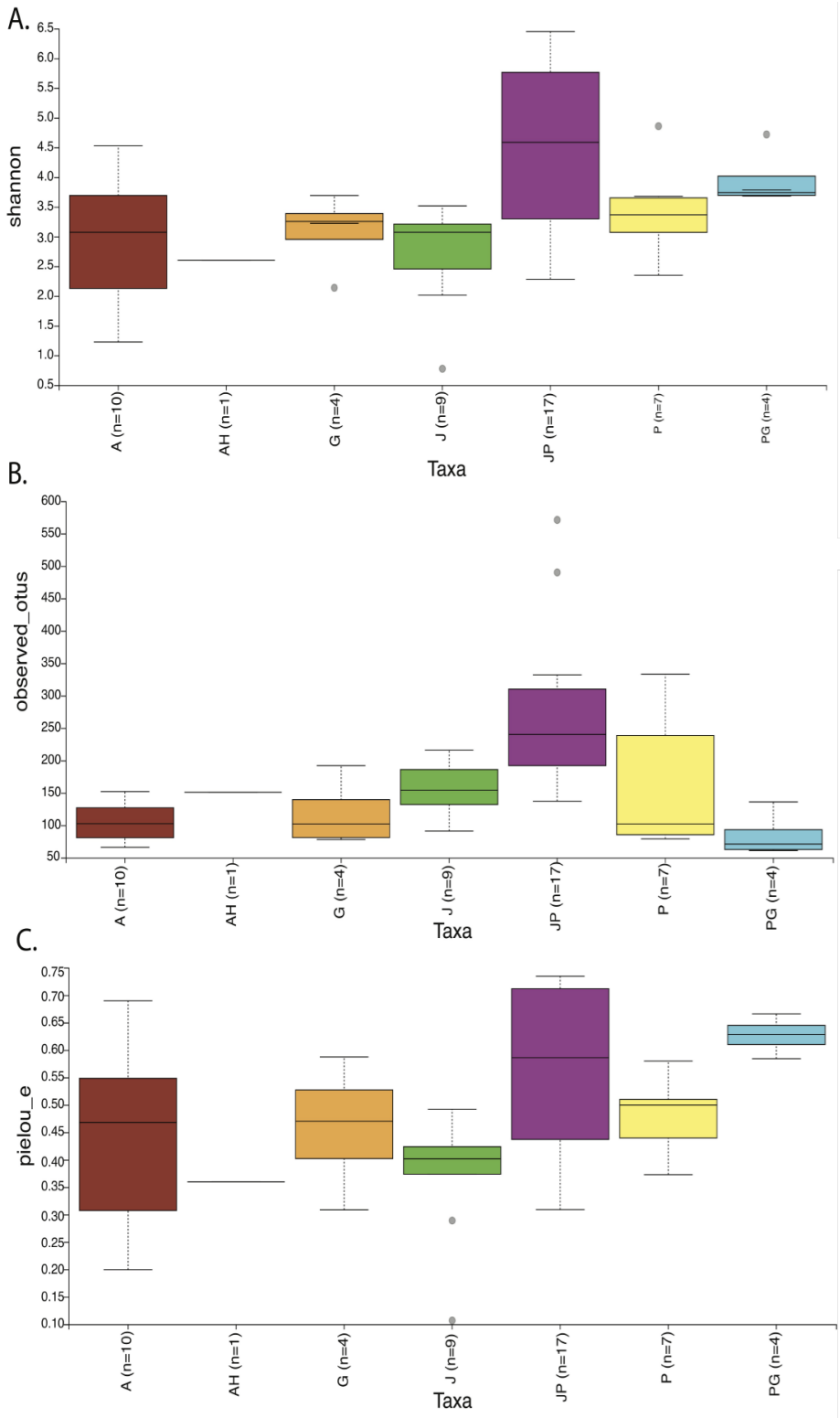

Figure 2. Boxplots of gut microbiome species alpha diversity by host taxon showing Shannon diversity index (A), number of observed OTUs (B), and Pielou E evenness (C). Classifications along the x-axis are A/Red=*C. aurita*, AH=*C. aurita* hybrid, G/Orange= *C. geoffroyi*, J/Green= *C. jacchus*, P/Yellow= *C. penicillata*, JP/Purple= *C. jacchus* x *C. penicillata* hybrids, and PG/ Blue= *C. penicillata* x *C. geoffroyi* hybrids.

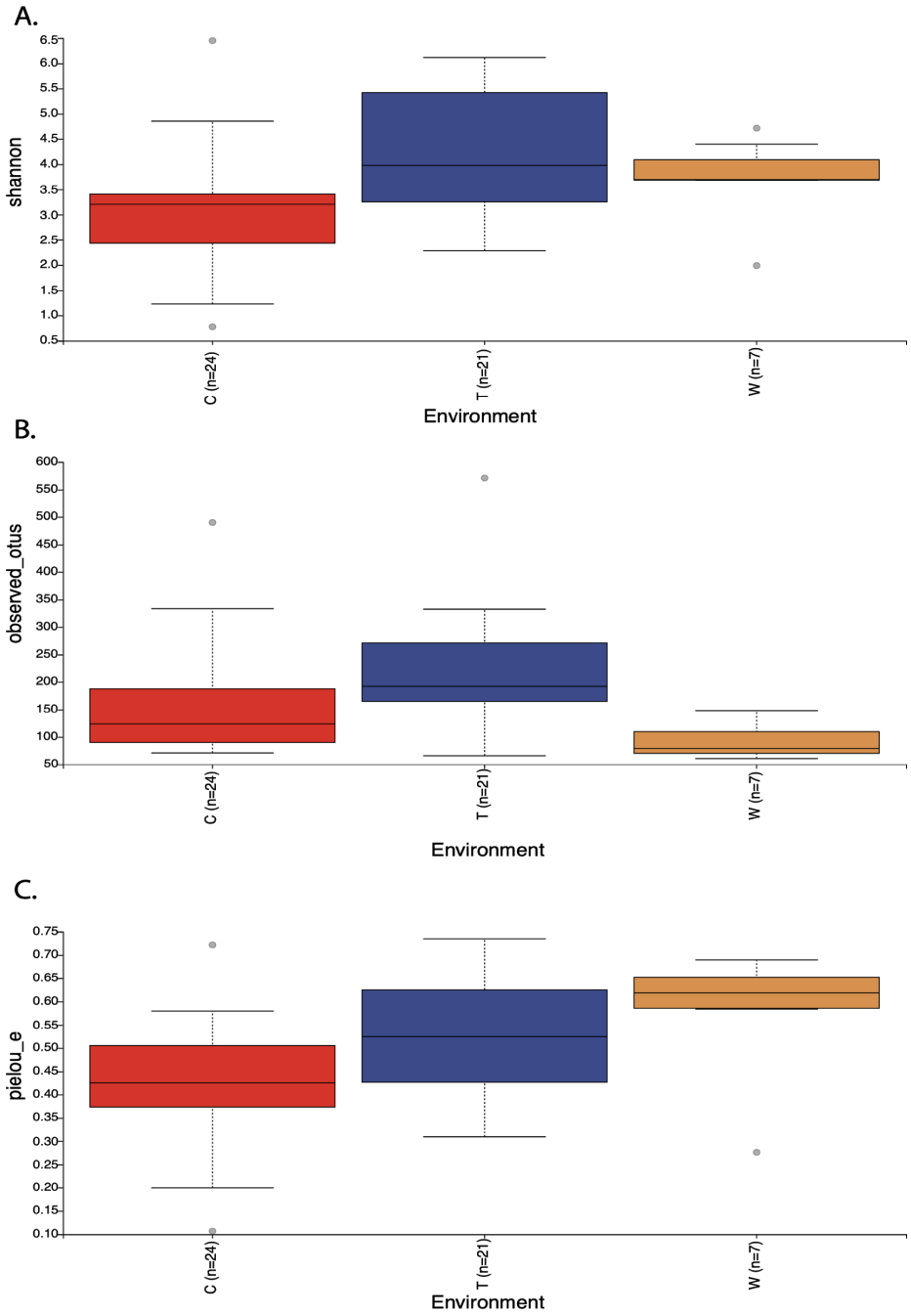

Figure 3. Boxplots of gut microbiome species alpha diversity by host environment showing Shannon diversity index (A), number of observed OTUs (B), and Pielou E evenness (C). Classifications along the x-axis are C/Red=captive, T/Dark Blue=translocated, and W/Orange=wild.

sex, taxon, and environmental classifications were tested using adonis PERMANOVA test for beta group significance based on weighted UniFrac distances with the Qiime2-2020.2 adonis plug-in [48,49]. Weighted UniFrac principle coordinate analysis (PCoA) plots were generated according to host taxon and environment. Statistical significance of UniFrac PCoA plots within each category was determined by PERMANOVA group and pairwise tests [50]. All pairwise comparisons were corrected with the Benjamini–Hochberg procedure [51] (Benjamini and Hochberg, 1995). The corrected P-value was considered significant at $p<0.05$.

Taxonomic composition of samples was determined with the Qiime2-2018.6 q2-feature-classifier plug-in, which was trained on the Greengenes OTU database (version 13_8) [52] at the 99% level. Reads were limited to 500 nucleotides from the portion of the 16S V4 region bounded by the 515F/806R primer pair. Afterward, Qiime2-2018.6 was used to generate bar plots of bacterial taxonomic abundance based on host taxon and environment. The core gut OTUs that were present in at least 50% of all sampled hosts was determined with the Qiime2-2018.6 feature-table core-features visualizer and OTU taxonomic classifications were based on the q2-feature-classifier classifications. We used linear discriminant analysis coupled with effect size (LEfSe) on the Huttenhower lab Galaxy server (http://huttenhower.sph.harvard.edu/galaxy/), with LDA score>2.5 and P-value<0.05, to determine which OTUs have differential abundance between wild, captive, and translocated marmosets.

PICRUST 1.1.3 [53] was used for predictive functional profiling of bacterial communities via a phylogeny-based approach to correlate sampled bacterial species to the Greengenes 13_5 99% OTU reference dataset. Initially, the Greengenes reference data were used in QIIME2 to make a closed-reference OTU table from our sequence reads and converted to biom format. The OTU table was normalized by 16s rRNA copy number in PICRUST and the

extrapolated metagenome results then were input into HUMAnN 0.99 [54]. This program reconstructs microbial pathway abundances from metagenomic data using the KEGG Orthology catalogue [55-57]. The HUMAnN KEGG pathway module (functional units of genes) output was used in LefSe [58] on the Huttenhower lab Galaxy server (http://huttenhower.sph.harvard.edu/galaxy/) to identify how abundance of modules differ between marmosets categorized by taxon and environment with linear discriminant analysis (LDA) coupled with effect size. These differences were then plotted in GraPhlAn 0.9 [59].

Results

A total of 11,123,817 sequence reads was obtained with an average of 188,539 (129,170 +/- SD) reads per sample. After quality filtering, 9,063,712 reads remained with an average 153,622 (103,648 +/- SD) reads per sample. Afterward, merging of paired-end sequences produced 843,615 reads, with an average of 142,891 (96,076 +/- SD) reads per sample. This information is detailed in Supplementary Table 3.

Gut Microbiome Alpha Diversity based on Host Taxon and Environment

Rarefaction excluded a total of seven individuals from alpha diversity analysis (Supplementary Table 1). Although taxa differed significantly in Shannon's diversity index (Figure 2A; Kruskal-Wallis group test, H=16.88, p=0.01), Kruskal-Wallis pairwise tests were not significant (Supplementary Table 4). The number of observed OTUs among taxa ranged from 61 to 571 (Supplementary Table 1), and median values varied significantly among taxa (Figure 2B; Kruskal-Wallis group test, H=28.16, p=0.00). For pairwise comparisons, the median observed

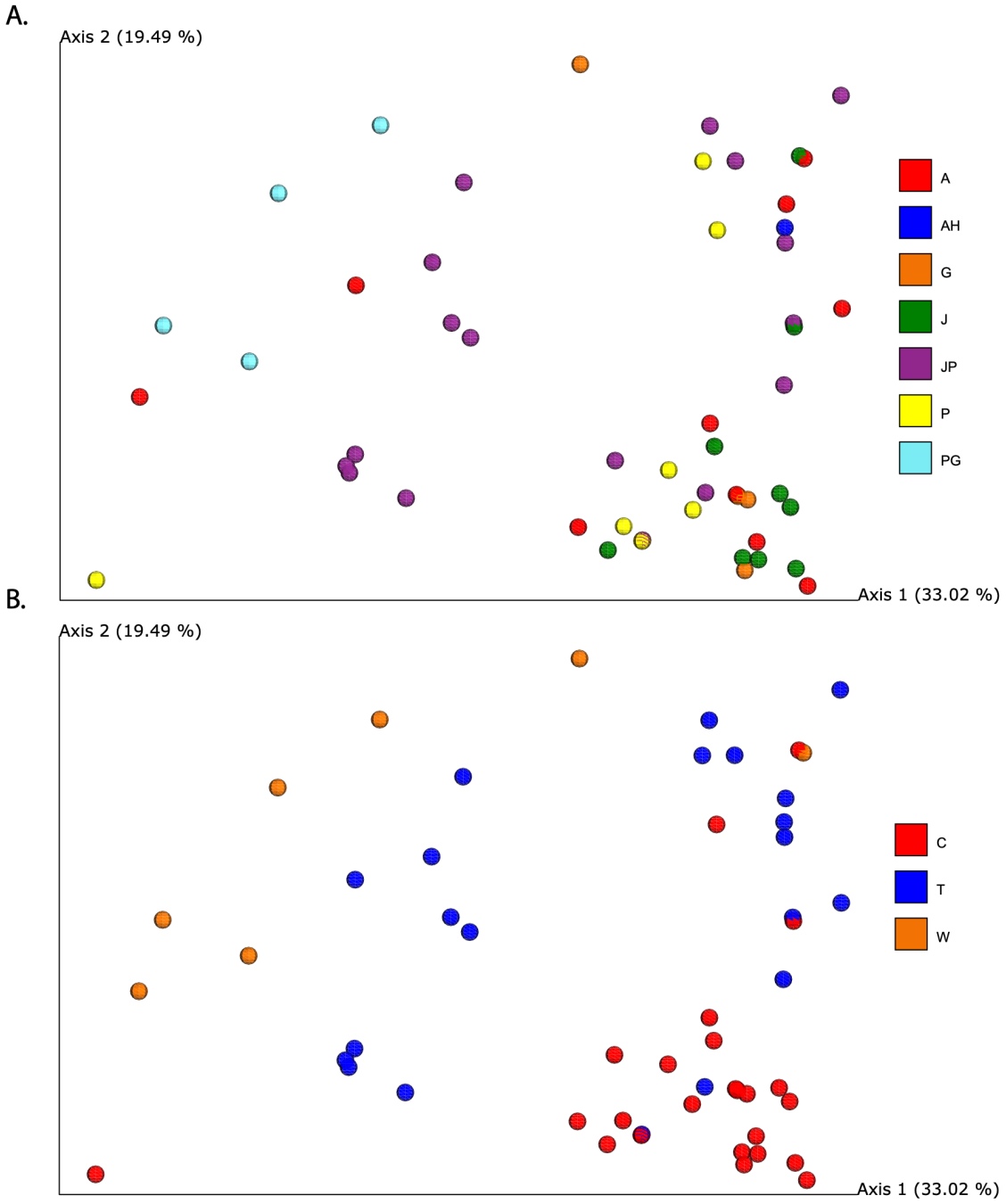

Figure 4. PCoA of weighted Unifrac distances by host taxon (A) and environment (B). Abbreviations and color codes in plot legends are the same as in Figures 2-3.

OTUs in *C. jacchus* x *C. penicillata* hybrids were significantly different from that of *C. aurita* and *C. penicillata* x *C. geoffroyi* hybrids (Supplementary Table 5). Although taxa differed significantly in species evenness (Figure 2C; Kruskal-Wallis group test, H=16.41, p=0.01), subsequent pairwise tests were not significant (Supplementary Table 6). Shannon's index differed significantly among wild, translocated, and captive marmosets (Figure 3A; Kruskal-Wallis group test, H=9.85, p=0.007), and pairwise differences were significant between captive vs. translocated and captive vs. wild groups (Supplementary Table 7). Differences in median observed OTUs were significant among host environment categories (Figure 3B; Kruskal-Wallis group test, H=15.95, p=0.00), specifically between translocated vs. captive and translocated vs. wild (Supplementary Table 8). Species evenness differences were significant among host environments (Figure 3C; Kruskal-Wallis group test, H=10.03, p=0.007), with captive vs. translocated and captive vs. wild comparisons showing significant pairwise differences (Supplementary Table 9).

Gut Microbiome Beta Diversity based on Host Taxon and Environment

No differences in gut microbiome composition were observed between marmoset male and female hosts (adonis, F=1.03, df=2, R^2=0.04, p-value= 0.4). Gut microbiome composition between host taxa was significant when hybrids were included (adonis, F=2.66, df=2, R2=0.26, p-value=0.001), but no differences were detected between *Callithrix* species (adonis, F=0.76, df=3, R2=0.10, p-value=0.75). Lastly, 27% of variation in marmoset gut microbiome composition could be significantly attributed to differences between host environments (adonis,

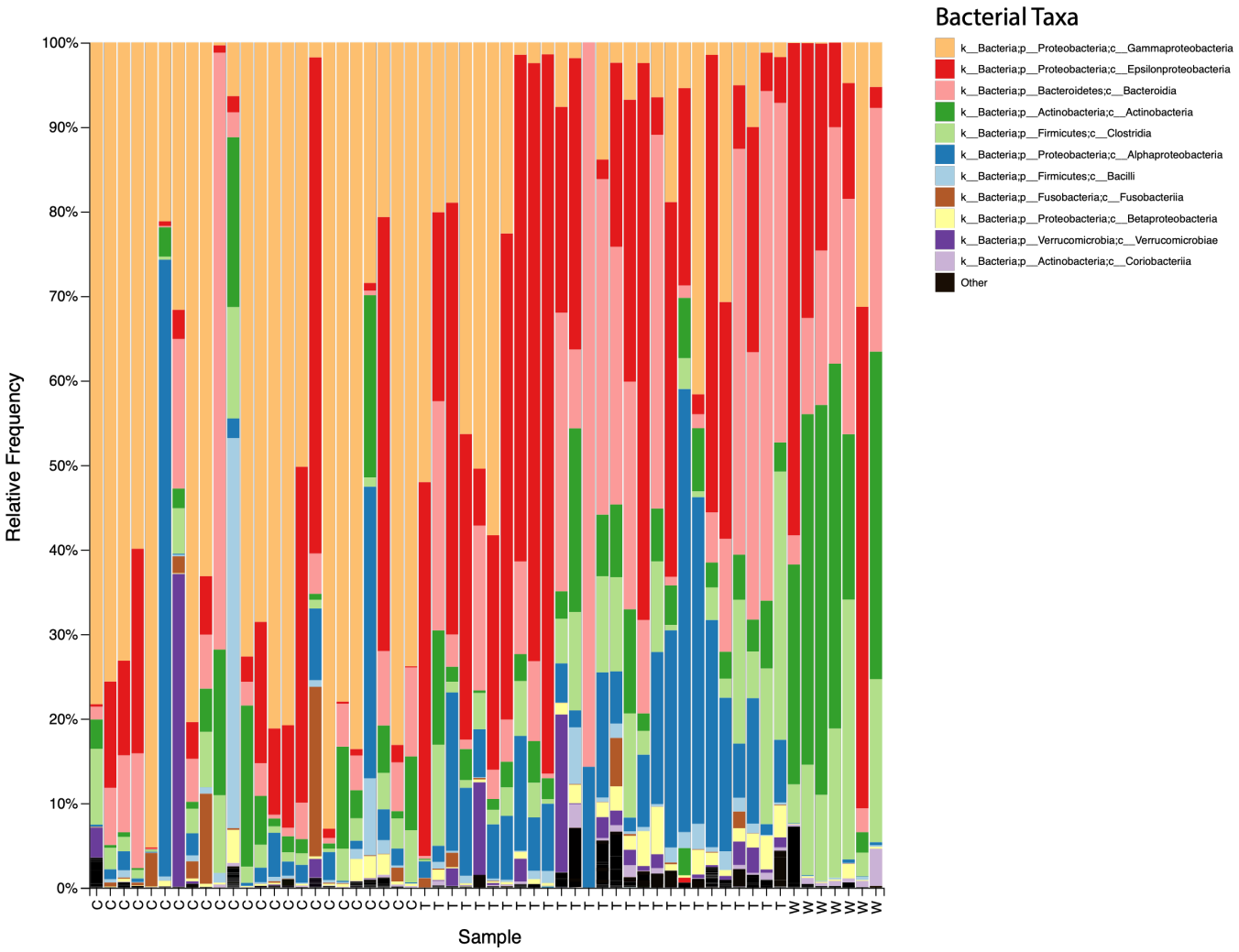

Figure 5. Relative abundances of gut bacterial classes by environment. Each bar represents a marmoset host, and hosts are classified as C (captive), T (translocated), and W (wild). Colors within each bar represent the top 11 most abundant bacterial classes. Black represents all other bacterial classes. Top colors represent greater bacterial relative abundance than those taxa at the bottom of each bar.

F=8.88, df=6, R^2=0.26, p-value=0.001). PCoA plots of weighted Unifrac distances also suggested that environment more strongly effects host differences in gut microbial composition than taxon affiliation (Figures 4A-B). The first PCoA axis explained 33.02% of gut microbial variation and the second axis explained 19.49%. In separating the samples by taxon (Figure 4A), some taxa showed loose clustering, but most of them showed interdispersion in the upper and lower right quadrants. Distances among taxa were significant (PERMANOVA, pseudo-F= 2.67, p-value=0.002), but there were few significant pairwise comparisons among them (Supplementary Table 10). Separation of hosts according to environment resulted in the most prominent clustering patterns between wild, translocated, and captive samples, particularly along the first axis (Figure 4B). Weighted Unifrac distances between samples by environment were significant at both the group level (PERMANOVA, pseudo-F=8.88, p-value=0.001), as well as for all pairwise comparisons (Supplementary Table 11). When only captive and translocated pure species hosts were analyzed together most samples clustered together along both the first and second components (Supplementary Figure 1A-B), regardless of species category. Group level (PERMANOVA, pseudo-F=1.20, p-value=0.257) nor pairwise weighted Unifrac distances were significant between host species (Supplementary Table 12). There were also no significant differences between captive and translocated hosts (PERMANOVA, pseudo-F=1.89, p-value=0.128).

Differential OTU Abundance based on Host Taxon and Environment

The core bacterial classes among hosts (Figure 5, Supplementary Figure 2; Supplementary Table 13) included Gammaproteobacteria, Epsilonproteobacteria, Bacteroidia,

Table 2. Core predictive functional bacterial KEGG modules present among all sampled marmoset hosts at a coverage of >90%

Module ID	KEGG Module Definition	Level 3 KEGG Brite Category
M00001	Glycolysis (Embden-Meyerhof pathway), glucose => pyruvate	Central carbohydrate metabolism
M00002	Glycolysis, core module involving three-carbon compounds	Central carbohydrate metabolism
M00003	Gluconeogenesis, oxaloacetate => fructose-6P	Central carbohydrate metabolism
M00004	Pentose phosphate pathway (Pentose phosphate cycle)	Central carbohydrate metabolism
M00006	Pentose phosphate pathway, oxidative phase, glucose 6P => ribulose 5P	Central carbohydrate metabolism
M00007	Pentose phosphate pathway, non-oxidative phase, fructose 6P => ribose 5P	Central carbohydrate metabolism
M00049	Adenine nucleotide biosynthesis, IMP => ADP/dADP,ATP/ dATP	Purine Metabolism<
M00050	Guanine ribonucleotide biosynthesis IMP => GDPGTP	Purine Metabolism<
M00051	Uridine_monophosphate biosynthesis glutamine + PRPP => UMP	Pyrimidine Metabolism
M00115	NAD biosynthesis aspartate => NAD	Cofactor and vitamin biosynthesis
M00119	Pantothenate biosynthesis valine/L-aspartate => pantothenate	Cofactor and vitamin biosynthesis
M00123	Biotin biosynthesis, pimeloyl-CoA => biotin	Cofactor and vitamin biosynthesis
M00125	Riboflavin biosynthesis, GTP => riboflavin/FMN/FAD	Cofactor and vitamin biosynthesis
M00126	Tetrahydrofolate biosynthesis, GTP => THF	Cofactor and vitamin biosynthesis
M00157	F-type ATPase, bacteria	ATP Synthesis

M00164	ATP synthase	Photosynthesis
M00222	Phosphate transport system	Phosphate and Amino Acid Transport System
M00229	Arginine transport system	Phosphate and Amino Acid Transport System
M00299	Spermidine/putrescine transport system	Mineral and Organic Ion Transport System
M00360	Aminoacyl-tRNA_biosynthesis prokaryotes	Aminoacyl tRNA

Actinobacteria, Clostridia, and Alphaproteobacteria among others. Hosts showed variation in both bacterial class and abundance among individuals (Supplementary Figure 2), particularly in *C. aurita*, *C. geoffroyi*, and *C. penicillata*- taxa that were sampled across different locations and environments. Then, a strong pattern of bacterial taxonomic abundance (limited to class for brevity) emerges upon classifying hosts by environment (Figure 5). At the family level these patterns are dominated by: (1) Enterobacteriaceae (class Gammaproteobacteria) in captive hosts; (2) families Helicobacteraceae and Campylobacteraceae (class Epsilonproteobacteria), Bacteroidaceae (class Bacterioidia), and Sphingomonadaceae (class Alphaproteobacteria) in translocated hosts; and (3) Helicobacteraceae and Campylobacteraceae, Bacterioidia (family undetermined), Bifidobacteriaceae (class Actinobacteria), and Veillonellaceae (class Clostridia) in wild hosts.

LEfSe differential gut microbiome bacteria abundance testing between hosts found several microbe genera to differ between wild, captive, and translocated hosts (Supplementary Figure 3). The wild marmoset gut microbiome was enriched for 8 bacterial genera, including *Megasphaera*, *Campylobacter*, *Bifidobacterium* with the former having the highest LDA score. For captivity, 12 bacteria taxa were enriched for this host environmental category, with the Enterobacteriaceae family

having the highest LDA score followed by *Providencia.* For translocated hosts, 41 bacterial taxa were enriched for this host environment, with *Helicobacter* having the highest LDA score.

Predictive Metagenomic and Metabolic Characterization of Marmoset Gut Microbiome

Metagenome prediction by PICRUSt gave an average weighed NSTI value across hosts of 0.035 and host scores ranged from 0.008 to 0.147 (Supplementary Table 14). The 16S rRNA gene amplicon data analyzed in QIIME2 resulted in predicted proteins classified as KEGG orthologos (KOs) with a total of 6909 KOs across all hosts. Using these KOs with HUMAnN for metabolic reconstruction detected 113 KEGG modules (functional units of genes) collectively present in all samples (Supplementary Table 15). Among these modules, 20 were present across all samples at a coverage of >90% and were identified as core modules (Table 2). The core modules were found to be involved with the following Level 3 KEGG BRITE hierarchies: central carbohydrate metabolism (6 modules), purine metabolism (2 modules), pyrimidine metabolism (1 module), cofactor and vitamin biosynthesis (5 modules), ATP synthesis (1 module), photosynthesis (1 module), phosphate and amino acid transport system (2 modules), mineral and organic ion transport system (1 module), and aminoacyl tRNA (1 module).

Several KEGG modules were differently abundant for host taxonomy and environment (Figures 6-7, Supplementary Figures 4-5). A total of 57 modules differed for taxa (Supplementary Figure 4 and 5), with *C. jacchus x C. penicillata* hybrids having the largest number of differently abundant modules and *C. jacchus* possessing the second highest number. Differences among the three groups of host environment were not particularly concentrated around certain categories but rather spread out across clades of modules.

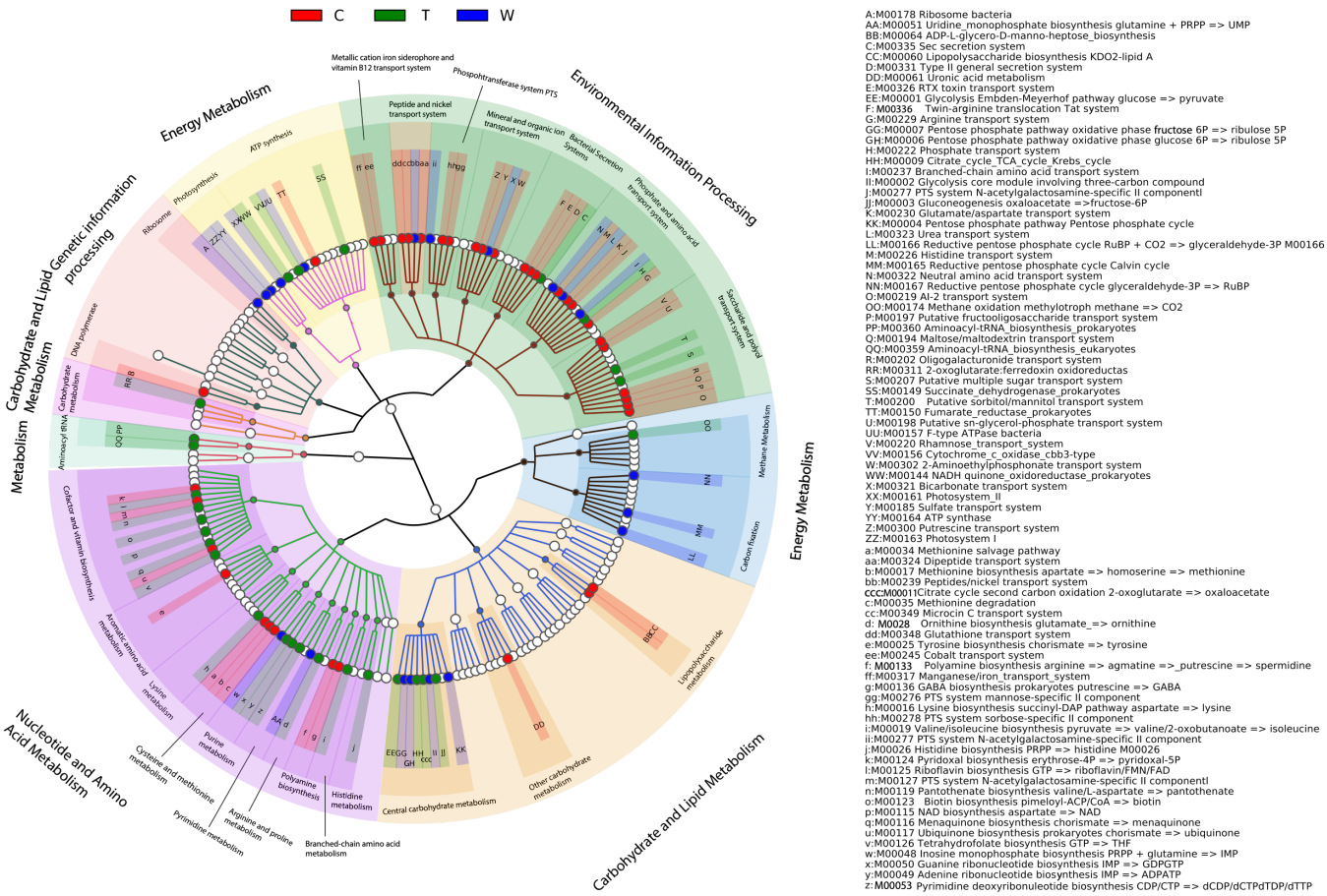

Figure 6. HUMAnN metabolic reconstruction of marmoset fecal microbiome based on host environment. The cladogram shows 3 KEGG BRITE hierarchical levels with an outer, generalized level, and ending with the inner-level annotated by letters that are detailed further in the accompanying legend. Circles not differentially abundant in any species are clear. Environment classifications and colors follow the same abbreviations as Figures 3.

Figure 7. Differentially abundant KEGG metabolic modules inferred by LEfSe are colored by their associated environment classification, which follow the same abbreviations as Figures 3. The plot shows LDA scores resulting from LeFSe analysis for differentially abundant KEGG modules across host environment.

Discussion

Our results revealed that in marmosets as other animals, both host environment and taxonomy shape the gut microbiome [60-63]. However, host environment in this study showed stronger influence on marmoset GMC than host taxonomy, perhaps due to how closely related *Callithrix* species are as a recent primate radiation [64,65]. As host environment had the strongest effect on marmoset GMC within our study, the below discussion focuses largely on this factor.

The Wild *Callithrix* Gut Microbiome is Associated with Carbohydrate Metabolism

As exudivores, wild *Callithrix* regularly exploit tree gums or hardened saps [19,20,66,67]. Morphological adaptations of marmosets for exudivory include a well-developed caecum for fermentation of plant exudates [68] and a lower-anterior dentition "tooth scraper" to perforate tree bark and lianas [69]. In line with such morphological adaptations to exudate exploitation, our data show that the wild *Callithrix* gut microbiome is geared towards carbohydrate metabolism.

While wild and captive/translocated marmosets possessed similar classes of bacterial phyla (Table 3), bacterial abundance differed starkly between host environment. Unlike non-captive marmosets, wild marmosets had a GMC enriched for the Bifidobacteriaceae family (Actionobacterium), which includes genera like *Bifibacterium* that are common in animal gastrointestinal tracts and metabolize host-indigestible carbohydrates [70,71]. Wild marmosets gut microbiomes were also enriched for other bacteria involved in carbohydrate metabolism like *Megasphaera* and *Pectinatus* (both Firmicutes), which are actually known as important beer spoilage bacteria [72].

Table 3. Percentages of most abundant bacterial taxa present in marmoset hosts according to host environment

Phylum	Captive (%)	Translocated (%)	Wild (%)
Actinobacteria	6	6	32
Bacteroidetes	8	20	17
Firmicutes	6	7	14
Proteobacteria	76	63	35

The principle core predictive functional KEGG modules expressed in all sampled marmosets were related to central carbohydrate metabolism as well as purine metabolism, pyrimidine metabolism, cofactor and vitamin biosynthesis. Core predictive modules in both wild and non-wild hosts for carbohydrate metabolism were involved with glycolysis and the pentose phosphate pathway, pathways essential for the production of ATP and NADPH. However, the gut microbiome of wild marmosets was particularly enriched for predictive modules involved in carbohydrate metabolism including that of glycolysis and pentose phosphate biosynthesis, perhaps due to enrichment of gut microbes like Actinobacteria in wild hosts.

Captive Marmoset GMC Mirrors Aspects of Human GI Distress

While methodological differences between studies need to be taken into consideration, certain trends arise in GMC of captive marmosets in our study and captive *C. jacchus* from previous studies. Actinobacteria was not the most abundant gut bacterial taxa for most captive *Callithrix* and there is increased abundance of gut Proteobacteria among captive marmosets here and other studies [36,70]. One study of captive *C. jacchus* uniquely compared gut microbiome composition between a specific pathogen free colony, housed under conditions that minimized outside contamination, with a

conventionally housed group [34]. Many barrier marmosets had relatively high abundance of *Bifibacterium* (approximately 1% to 80%), while *Fusobacterium* B (phylum Fusobacteria) was dominant in conventionally housed hosts [34].

The GMC of captive and translocated marmosets shows similarity to certain aspects of the GMC of human GI diseases associated with gut microbiome dysbiosis [7,18,73,74]. Crohn's Disease (CD) generally affects the terminal ileum and colon [75,76] and GMC of CD sufferers is less diverse relative to healthy humans [18,77,78]. The gut microbiome of CD patients is enriched for bacterial taxa that include Enterobacteriaceae and Veillonellaceae, and decreased in Bifidobacteriaceae [18,79]. We also observed similar patterns in non-wild marmosets, relative to wild counterparts. Enterobacteriaceae is frequently associated with intestinal diseases and contains a number of pathogenic bacterial strains of *Salmonella*, *Escherichia*, and *Shigella* [80,81] and potentially pathogenic Enterobacteriaceae were previously found in captive and urban *Callithrix* [82]. Translocated marmosets GMC showed higher abundance of Bacteroidetes and Clostridia, a pattern also seen in ulcerative colitis [79], a GI disease which usually involves rectal and colon inflammation [75,76].

Importance of *Bifidobacterium* and Natural Diets for the Exudivore Gut

Data from this and previously published studies suggest that *Bifidobacterium* is an important gut microbe for animal hosts that nutritionally exploit plant exudates. Further, high abundance of *Bifidobacterium* in gut microbiomes of exudivore specialists may be a biomarker for host gut microbiome eubiosis. In the specialist exudivore the pygmy loris (*Nycticebus pygmaeus*), two wild individuals possessed Actinobacteria (10.98%) as major gut microbiome bacterial phyla [83], and gut microbiome function was enriched for carbohydrate metabolism. *Bifidobacterium* was also the most abundant and enriched gut bacterial taxa in wild individuals of *Nycticebus javanicus,* another primate

exudivore [84]. The genomes of three isolates of *Bifidobacterium callitrichos* from a captive *C. jacchus* fecal sample contained predicted genes associated with galactose and arabinose metabolism, which are major constituents of tree gums eaten by *C. jacchus* [70]. In 3 US captive facilities, *C. jacchus* collectively shared four species of *Bifidobacterium* which possessed genes encoding ATP-binding cassette proteins, which are important for nutrient transport that may be specific to marmoset gut [32].

One concern for a lack of tree gums in the diet of captive specialist exudivores is the development of health issues as well as a negative impact on breeding and survivability [85,86]. Current captive *Callithrix* husbandry, often omits gums from the marmoset diet or uses it as an enrichment ingredient (Table 4) [86]. In humans suffering from GI diseases, increasing plant-based foods and dietary fiber, resulted in increasing microbiome diversity, remission of GI symptoms, and decreasing risk of GI [7,87]. Such diets may increase gut abundance of bacteria such as *Bifibacterium* that produce short chain fatty acids like butyrate, which may guard against proliferation of pathogenic bacteria in the gut and decrease chronic inflammation [7,87]. Lack of access to a natural-wild diet for marmosets and other exudivory specialists may also promotes loss of native gut microbe *Bifidobacterium* and enrichment of potentially pathogenic bacterial strains of Enterobacteriaceae.

Table 4. Diet collectively fed to marmoset hosts in sampled captive facilities

Fruits	Papaya, Orange, Banana, Apple, Pear, Avacado, Kiwi, Melon, Mango
Carbs	Sweet Potato, Potato, Beets
Veggies	Cucumber, Eggplant, Pumpkin, Chuchu, Cauliflower, Carrots
Proteins	Cooked Chicken, Cooked Egg

Animal studies increasingly recognize the need to include host-microbiome associations in management of captive populations [2,17,88]. It has also been demonstrated, as in mice and wood-rats,

that feeding more natural diets to individuals in captivity helped maintain host GMC profiles closer to wild, free-ranging hosts [88,89]. Plausibly, GI distress in captive *Callithrix* may be related to unnatural diets that lower abundance of *Bifidobacterium* and increase presence of potentially pathogenic bacteria Enterobacteriacea thereby driving dysbiosis in the gut microbiome. Thus, based on such observations, captive facilities should consider provisioning of gum in the diet of specialized exudivores to improve host welfare by maintaining gut microbiomes closer to that of wild populations.

Conclusion

The results of this study have given us an initial genus-level look into the gut microbiome of specialized exudivorous NHPs. Our major findings indicate that, just as in other primates, the gut microbiome of *Callithrix* is sensitive to environmental factors, and these findings likely apply more broadly to other animal exudivores. Our findings also suggest that carbohydrate metabolism is a core function of the *Callithrix* gut microbiome. Yet, although marmosets are specialized exudivores, there is a wide gap between the nutritional intake of marmosets in the wild and captivity, where the marmoset physiology for gummivory is generally not considered. From an evolutionary and health point of view, this disconnect between wild and captive *Callithrix* diets leaves open several questions concerning maladaptation of the marmoset digestive system, perturbation of gut microbiota, malabsorption of nutrients, and long term health effects. More studies are needed to understand better the health and reproductive consequences of omitting as well as increasing gum intake by specialized exudivores in captivity. Also, systematic studies are needed to better understand whether *Bifidobacterium* plays a central role in maintaining euobisis in the gut microbiomes of *Callithrix* and other exudivory specialists. Overall, such information will expand baseline gut microbiome data available for wild and non-wild

exudivores to allow for the development of new tools to improve the management, welfare, and conservation of animal exudivores.

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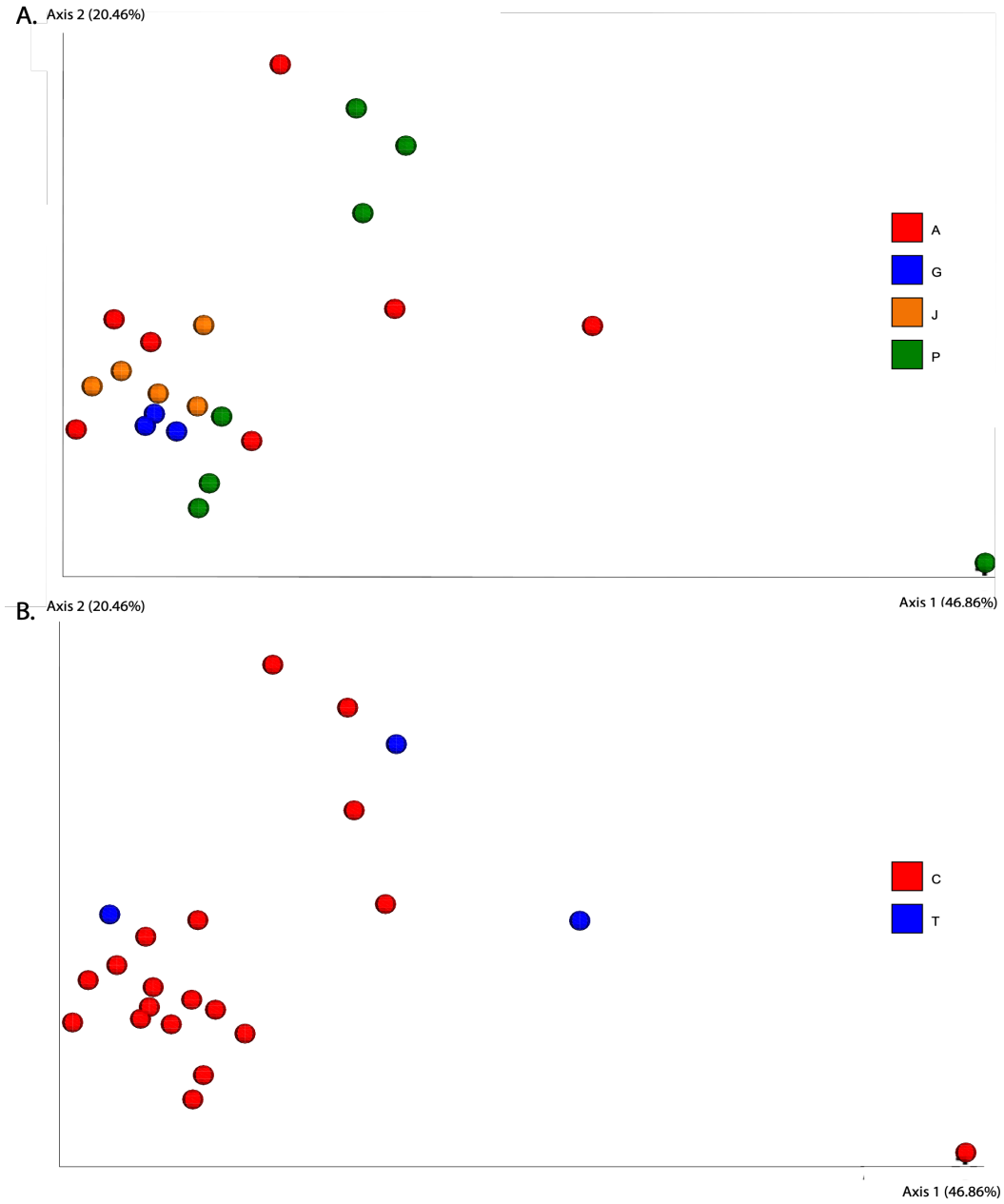
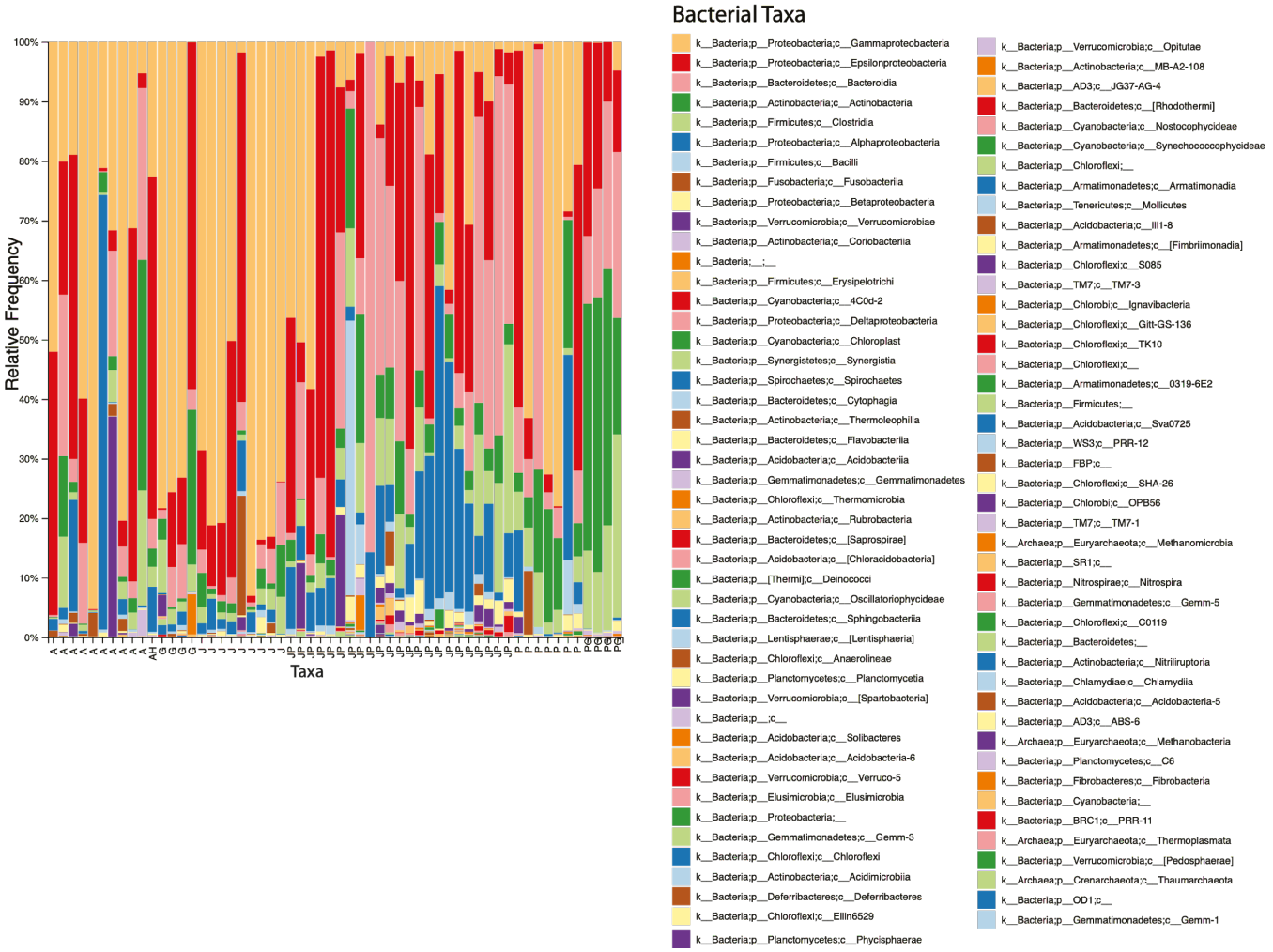
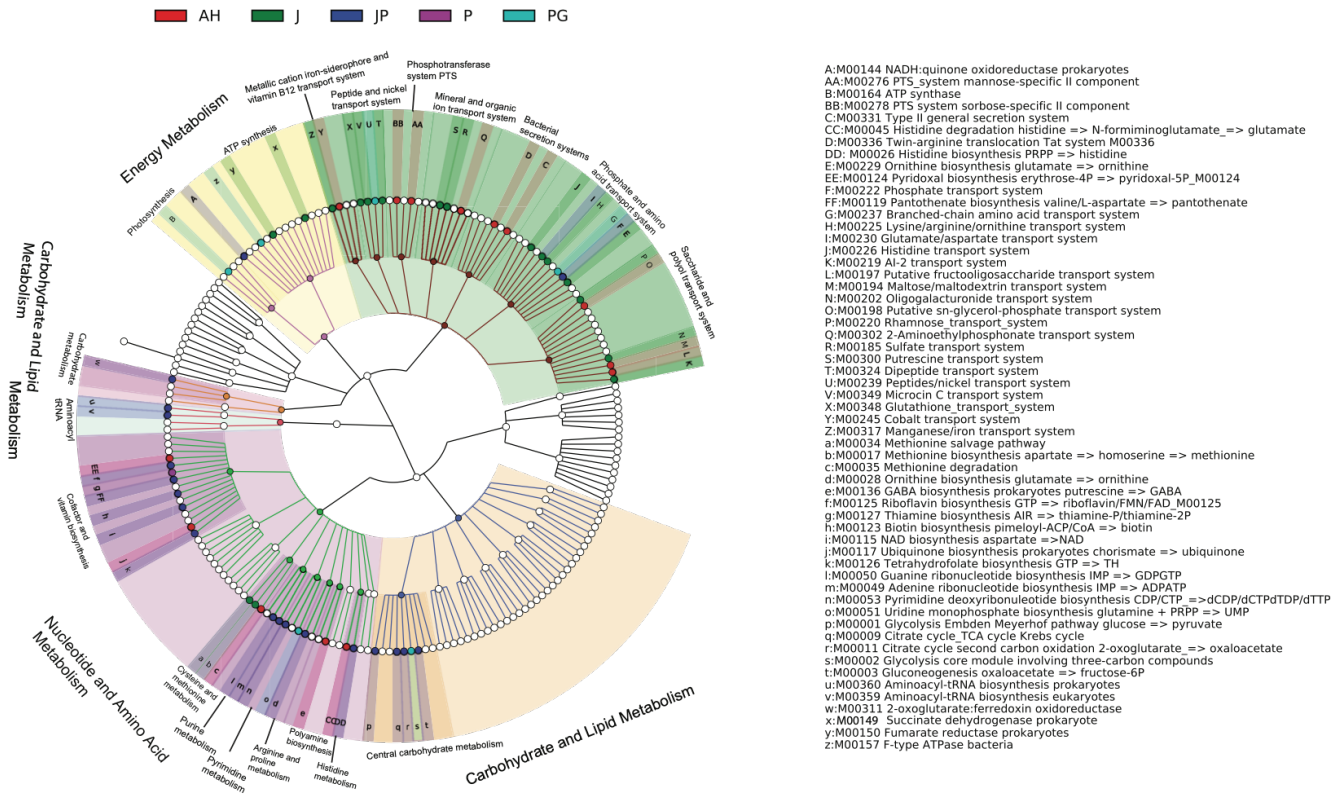


Supplementary Figure 1. PCoA of weighted Unifrac distances by host taxon (A) and environment (B) for non-hybrid individuals only. Classifications along the x-axis are A/Red=*C. aurita*, G/blue= *C. geoffroyi*, J/Orange= *C. jacchus,* P/blue= *C. penicillata.* In panel A, classifications along the x-axis are C/Red=captive, T/Dark Blue=translocated, and W/Orange=wild. In panel B, classifications along the x-axis are C/Red=captive, T/Dark Blue=translocated.

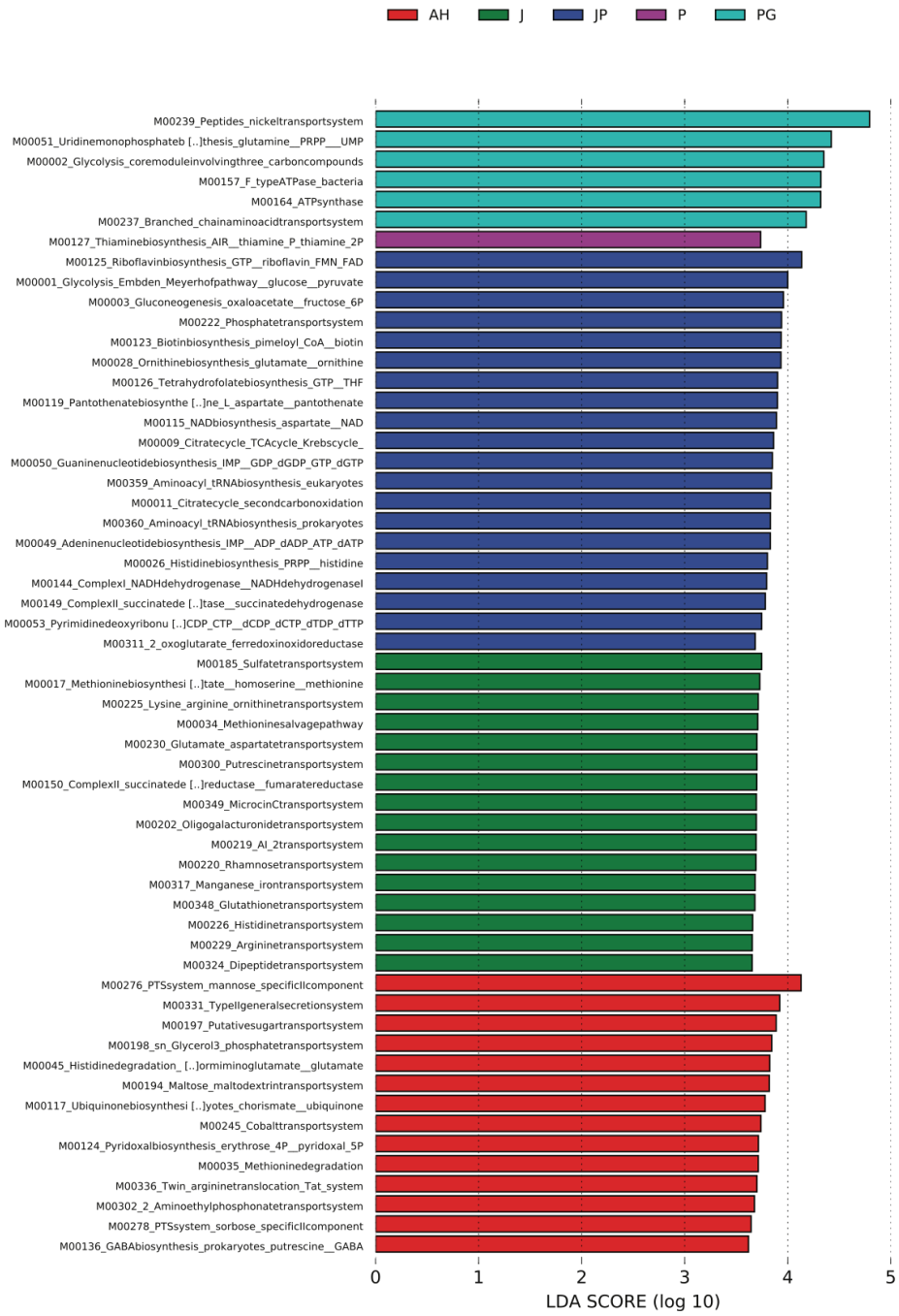


Supplementary Figure 2. Relative abundances of gut bacterial classes by taxon. Each bar represents a marmoset host, and hosts are classified as A=*C. aurita*, AH=*C. aurita* hybrid, G=*C. geoffroyi*, J= *C. jacchus*, P=*C. penicillata*, JP=*C. jacchus* x *C. penicillata* hybrids, and PG=*C. penicillata* x *C. geoffroyi* hybrids. Top colors represent greater bacterial relative abundance than those taxa at the bottom of each bar.

Supplementary Figure 3. Differentially abundant bacterial abundances inferred by LEfSe are colored by their associated environment classification, C/Red=Captive, T/Green=Translocated, W/Blue=Wild.



Supplementary Figure 4. HUMAnN metabolic reconstruction of marmoset fecal microbiome based on host taxon. The cladogram shows 3 KEGG BRITE hierarchical levels with an outer, generalized level, and ending with the inner-level annotated by letters that are detailed further in the accompanying legend. Circles not differentially abundant in any species are clear. Taxon classifications and colors are as AH/ Red=*C. aurita* hybrid, J/Green=*C. jacchus*, P/Purple=*C. penicillata*, JP/Blue=*C. jacchus* x *C. penicillata* hybrids, PG/sea foam green=*C. penicillata* x *C. geoffroyi.*



Supplementary Figure 5. Differentially abundant KEGG metabolic modules inferred by LEfSe are colored by their associated host taxon classification, which follow the same abbreviations as Supplementary Figure 4. The plot shows LDA scores resulting from LeFSe analysis for differentially abundant KEGG modules across host taxa.