The Effects of Host Taxon, Hybridization, and Environment on the Gut Microbiome of *Callithrix* Marmosets

Callithrix Gut Microbiome

Joanna Malukiewicz[1,,2], Reed A. Cartwright[3,16], Jorge A. Dergam[4], Claudia S. Igayara[5], Sharon Kessler[6,7], Silvia B. Moreira[8] , Leanne T. Nash[9], Patricia A. Nicola[2], Luiz C.M. Pereira[2], Alcides Pissinati[8], Carlos R. Ruiz-Miranda[10], Andrew T. Ozga[11,12], Christian Roos[13,14], Daniel L. Silva[5], Anne C. Stone[9,11,15], Adriana D. Grativol[10]

Primate Genetics Laboratory, German Primate Research Center, Leibniz Institute for Primate Research, Goettingen, Germany[1]
Centro de Conservacao e Manejo de Fauna da Caatinga, Universidade Federal do Vale do Sao Francisco, Petrolina PE, Brazil[2]
The Biodesign Institute, Arizona State University, Tempe, AZ, USA[3]
Department of Animal Biology, Federal University of Vicosa, Vicosa, AZ, USA[4]
Guarulhos Zoo, Guarulhos, SP, Brazil[5]
Department of Psychology, University of Stirling, Sterling, Scotland[6]
Department of Anthropology, Durham University, Durham, UK24[7]
Primatology Center of Rio de Janeiro, Guapimirim, RJ, Brazil[8]
School of Human Evolution and Social Change, Arizona State University, Tempe, AZ, USA[9]
Laboratorio das Ciencias Ambientais, Centro de Biociencias e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes RJ, Brazil[10]
Center for Evolution and Medicine, Arizona State University, Tempe, Arizona, USA[11]
Halmos School of Natural Sciences and Oceanography, Nova Southeastern University, Fort Lauderdale, Florida, USA[12]
Gene Bank of Primates and Primate Genetics Laboratory, German Primate Research Center, Leibniz Institute for Primate Research, Goettingen, Germany[13]
Gene Bank of Primates, German Primate Research Center, Leibniz Institute for Primate Research, Goettingen, Germany[14]
Institute of Human Origins, Arizona State University, Tempe, Arizona, USA[15]
School of Life Sciences, Arizona State University, Tempe, AZ USA[16]

Corresponding Author: Joanna Malukiewicz
jmalukie@gmail.com

Abstract

Microbiome studies show that host taxon, diet, and environment influence gut bacteria. However, these factors are rarely studied in animal hybrids and exudivores (which nutritionally exploit indigestible oligosaccharides). To investigate the effects of host taxon, hybridization, and environment on gut microbiota, we conducted 16S V4 ribosomal sequencing of the gut microbiome of marmosets (*Callithrix*), non-human primate (NHP) specialist exudivores that also hybridize. We sampled 59 wild, translocated, and captive pure and hybrid *Callithrix*, including endangered *C. aurita*. Gut microbiome diversity differed significantly between hybrids and non-hybrids, but host environment had the strongest overall effect on the gut microbiome. Captive marmosets showed relatively reduced gut microbiome diversity. Wild *Callithrix* had the highest relative abundance of *Bifidobacterium*, which process host-indigestible carbohydrates, while captive marmosets had the highest relative abundance of Enterobacteriaceae, a family containing several pathogenic bacteria. The wild marmoset gut microbiome was enriched predictively for carbohydrate metabolism functions, while that of captive marmosets was enriched for nucleotide and amino acid metabolism function. Our findings show that carbohydrate metabolism is integral to the composition and function of the wild exudivore gut microbiome. Further, captivity perturbs the exudivore gut microbiome, raising implications for captive host health and endangered exudivore conservation.

Introduction

The role of the gut microbiome in host physiology and health has been studied widely in humans [1-4] and increasingly in non-human primates (NHPs) and other animals [5-9]. While these studies show that host taxon, diet, and environment are key influences on animal gut bacteria, hybrid and exudivore hosts are underrepresented in such work. Hybridization may perturb host fitness by disrupting interactions between the host genome and gut microbiome from breakage of parental co-adapted gene complexes in hosts [10-11]. Exudivores subsist mainly on relatively indigestible oligosaccharides of tree gums or, if they gouge, hardened saps, that require fermentation for digestion. Their gut microbiomes may be exclusively dedicated to carbohydrate metabolism. Thus, hybridization and exudivory represent intriguing venues to round out our understanding of animal gut microbiomes.

Hybridization is well documented among NHPs and other animals [12-19]. A few studies of captive, natural, and experimental animals show that hybrid gut microbiomes differ from those of parental species [e.g., 10, 11, 20]. Human activities increasingly facilitate anthropogenic hybridization, which may lead to the rise of animal host populations with compromised viability due to perturbed gut microbiomes. Few data are available on anthropogenic hybrid gut microbiomes, and such data will increase comparative datasets that facilitate our understanding of how hybridization may impact animal health.

In terms of animal dietary strategies, gut microbiomes have been mostly studied in folivores, frugivores, and omnivores. For NHPs exploiting such diets, gut microbiome composition is more sensitive to environmental effects than that of other animals [6, 9,

21]. Further, captive diets have lower fiber and plant content than what NHPs eat in nature [6,9], which may perturb the gut microbiome of captive populations and negatively impact host health. Indeed, regardless of natural diet, captive NHP gut microbiome composition tends to resemble that of humans consuming a Western diet [6]. This diet may cause dysbiosis and be directly associated with chronic diseases that drive morbidity and mortality in industrialized countries [22]. For threatened species, captivity serves a vital conservation role, and a particular concern is whether a "westernized" gut microbiome profile would compromise host digestive or immune function for captive hosts reintroduced into the wild [9].

A number of NHPs are exudivores [23-26], and some exudivore primate species are endangered or vulnerable (e.g., *Allocebus trichotis, Microcebus berthae, M.ravelobensis, Nycticebus bengalensis, C. aurita and C. flaviceps*) [27] and some are known to hybridize [e.g., *Callithrix*: 19; *Microbecus*: 28]. Studies of the exudivore gut microbiome from the wild are rare [e.g., *Nycticebus pygmaeus*: 29] and even rarer in captivity [e.g., an individual captive *Callithrix jacchus*: 30]. Further investigation of the gut microbiome of wild exudivore NHPs will provide baseline data, which could improve conservation and management of natural host populations. Additionally, similar data from captive exudivores could facilitate understanding of exudivore welfare in captivity and during reintroduction initiatives.

Our study focused on marmosets (*Callithrix*), a genus of six species considered to be specialist exudivores due to several morphological adaptations [23, 25, 31]. Two species are critically endangered, *C. flaviceps* and *C. aurita*, though conservation plans underway for both species include reintroductions of captive individuals into the wild

[32]. *Callithrix* species are naturally allopatric, but hybridize at species contact points [19]. Nonetheless, anthropogenic hybridization occurs widely among *Callithrix* species due to illegal pet trade and exotic species introduction outside natural species ranges [19].

Here we applied 16S ribosomal (rRNA, V4 region) gene amplicon sequencing of *Callithrix* anal microbiotas, and investigated microbiome composition and predictive functional profiles of the *Callithrix* gut microbiome. Anal swabs were sampled from a total of 59 individuals across four species (*C. aurita*, *C. geoffroyi*, *C. penicillata*, and *C. jacchus*) and three anthropogenic hybrid types (*C. aurita* x *Callithrix* spp., *C. jacchus* x *C.* penicillata, *C. penicillata* x *C. geoffroyi*). These individuals were sampled in the wild as well as from captive facilities where animals were born in captivity or translocated from the wild. This multifaceted dataset gives us a unique opportunity to study how host taxon, hybridization, and environment shape the gut microbiota in specialist exudivore primates. We hypothesized that: (1) *Callithrix* hybrids have less diverse gut microbiomes than their parental species; (2) *Callithrix* hosts found in similar environments possess more similar gut microbiome diversity and predictive function; (3) captive *Callithrix* have less diverse gut microbiomes than wild *Callithrix*; and (4) as specialist exudivores, *Callithrix* hosts show gut microbial composition and predictive function profiles that reflect a relatively strong bias toward carbohydrate metabolism.

Materials and Methods

Sample Collection

We collected anal swabs between 2015 and 2016 from 59 adult individuals (Table 1 and Supplementary Table 1). Figure 1 and Supplementary Table 1 show sampling

locations for: (1) wild marmosets in Viçosa, Minas Gerais (MG), Berilo, MG, and Guiricema, MG; (2) captive-born marmosets housed at the Guarulhos Zoo, Guarulhos, São Paulo (SP), CEMAFAUNA Wild Animal Triage Center, Petrolina, Pernambuco (PE), and Rio de Janeiro Primatology Center (CPRJ), Guapimirim, Rio de Janeiro (RJ); (3) a wild group from Natividade, RJ that was caught and housed at CPRJ; (4) and a wild group from Ilha d'Agua Island, Rio de Janeiro, RJ that was caught and kept at SERCAS (Setor de Etologia, Reintrodução e Conservação de Animais Silvestres) at Universidade Estadual do Norte Fluminense (UENF), Campos dos Goytacazes, RJ.

Marmoset sampling was authorized by the Brazilian Environmental Ministry (SISBIO protocol #47964-2), the Arizona State University IACUC (protocol# 15-144R) and a CPRJ internal review. Wild animals were captured with auto-close Tomahawk style traps baited with banana. All sampled animals were immobilized by injection of ketamine (10 mg/kg of body weight) into the inner thigh intramuscular region. Animals were photographed, weighed, measured, and kept under veterinary observation. 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. Under field conditions, the storage buffer was kept at room temperature and then at 4C in the laboratory. After sample collection, animals were returned to cages, given a banana, and allowed to recover. All recovered wild marmosets were released at the original capture site.

Specimens were identified as pure *C. aurita*, *C. geoffroyi*, *C. jacchus* and *C. geoffroyi* or hybrid (*C. aurita* x *Callithrix* spp., *C. jacchus* x *C. penicillata*, and *C. penicillata* x *C. geoffroyi*) based on phenotypic characters following descriptions [34-37],

and whole genome genotyping (unpublished data). Specimens were further classified by their environment as wild (captured as free-range individual), translocated (born in the wild but later put into captivity), or captive (born and raised in captivity based on information from captive facilities).

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 [38]. 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 [39]. 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.

Bioinformatics and Statistical Analysis

Sequence data were demultiplexed using default parameters in Qiime2-2018.6 (https://qiime2.org) [40]. The DADA2 Qiime2 plug-in [41] 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 sub-operational taxonomic units (sOTUs) [42]. MAAFT and FastTree Qiime2 plug-ins [43,44] aligned and produced a phylogenetic tree of feature sequences, which was then mid-pointed rooted. Qiime2's q2-diverity plug-in was used to carry out alpha diversity analyses (Shannon's diversity index, observed OTUs, and Pielou's Evenness) and calculate beta diversity weighted UniFrac distance [45]. Based on rarefaction, we set the minimum sampling depth to 45000 reads for alpha and beta diversity measures. Box-plots were generated for each alpha diversity measure according to host taxon, hybrid status, and environment. Kruskal-Wallis group and pairwise tests determined statistical significance for each category. UniFrac principle coordinate analysis (PCoA) plots were also generated according to host taxon, hybrid status, and environment. Statistical significance of UniFrac PCoA plots within each category was determined by PERMANOVA group and pairwise tests [46]. All pairwise comparisons were corrected with the Benjamini–Hochberg procedure [47]. The corrected P-value was considered significant at $p<0.05$.

Taxonomic composition of samples was determined with the QIIME2 q2-feature-classifier plug-in, which was trained on the Greengenes OTU database [version 13_8, 48] at the 99% level. Reads were limited to 500 nucleotides from the portion of the 16S V4

region bound by the 515F/806R primer pair. Afterward, QIIME2 was used to generate bar plots of bacterial taxonomic abundance based on host taxon, hybrid status, and environment.

The QIIME2 gneiss plug-in [49] was used to carry out differential abundance analysis of bacterial taxa within and among hosts based on host taxon classification, hybrid status, and environment. Briefly, partitions were made for microbes that commonly co-occur together via Ward hierarchical clustering, which then underwent isometric log ratio transformation to control for false positives by testing “balances” (i.e., changes in log ratios between microbial abundances) [42]. Linear regression tested for balances between microorganisms using host taxon, hybrid status, and environment as co-variants by the ordinary least-squares (OLS) method.

PICRUST 1.1.3 [50] 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 [50]. Initially, the Greengenes reference data was 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 [51]. This program reconstructs microbial pathway abundances from metagenomic data using the KEGG Orthology catalogue [52, 53, 54]. The HUMAnN KEGG pathway modules output was used in LefSe [55] on the Huttenhower lab Galaxy server (http://huttenhower.sph.harvard.edu/galaxy/) to identify how abundance of modules differ between marmosets categorized by taxon, hybrid status, and

environment with linear discriminant analysis (LDA) coupled with effect size. These differences were then plotted in GraPhlAn 0.9 [56].

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, Hybridization, 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 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 diversity index values of hybrid marmosets were significantly higher than those of non-hybrid marmosets (Figure 3A; Kruskal-Wallis pairwise test, H=10.22, p =0.001). Pairwise differences between hybrids and non-hybrids were significant for both observed OTU number (Figure 3B; Kruskal-Wallis pairwise test, H=7.24, p=0.007) and evenness (Figure 3C; Kruskal-Wallis pairwise test, H=8.10, p =0.004) (Figures 3B and 3C). Shannon's index differed significantly among wild, translocated, and captive marmosets (Figure 4A; 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 4B; 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 4C; Kruskal-Wallis one-way 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, Hybridization, and Environment

PCoA plots of weighted Unifrac distances suggested that hybridization and environment more strongly affect host differences in gut microbial composition than taxon affiliation (Figures 5A-C). The first PCoA axis explained 33.02% of gut microbial variation and the second axis explained 19.49%. In separating the samples by taxon (Figures 5A), 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). In Figure 5B, hybrid status showed most non-hybrids clustering in the lower right PCoA quadrant whereas hybrids were more prominent in the lower and upper left quadrants. These differences for hybrid status were significant (PERMANOVA, pseudo-F = 4.43, p-value=0.003). Finally, 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 5C). 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).

Differential OTU abundance based on Host Taxon, Hybridization, and Environment

The most abundant bacterial classes among hosts (Figure 6, Supplementary Figure S1) were Gammaproteobacteria, Epsilonproteobacteria, Bacteroidia, Actinobacteria, Clostridia, and Alphaproteobacteria; although, many other bacterial classes were recognized. Hosts showed variation in both bacterial class and abundance among individuals (Supplementary Figure S1A), particularly in *C. aurita*, *C. geoffroyi*, and *C. penicillata*- taxa that were sampled across different locations and environments. In considering host hybrid status, Gammaproteobacteria was particularly abundant in pure hosts and Epsilonproteobacteria and Bacteroidia are more prominent in hybrid hosts (Supplementary Figure S1B). Finally, a strong pattern of bacterial taxonomic abundance

(limited to class for brevity) emerges upon classifying hosts by environment (Figure 6). 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.

Using a multivariate response linear regression on the "balance" of the microbial community of the *Callithrix* gut model, we evaluated the effect of different types of taxa, hybrid status, and environmental categories on the microbiome. Together, these categories explained 25.15% of the variation of the *Callithrix* gut microbiota, with environment having the strongest effect by being a covariate that explained 6% of the variation (Supplementary Table 12). A good fit of this "balance" model to our data is indicated by predicted points lying within the same region as the original microbial community and the residuals have roughly the same variance as the predicted points (Supplementary Figure S2).

Predictive Metagenomic and Metabolic Characterization of Marmoset Gut Microbiome

Metagenome prediction by PICRUSt using 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 collectively present in all samples (Supplementary Table 13). 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). A "secondary" set of 37 modules were present at <90% coverage in no less than one but no more than three sampled marmoset hosts (Supplementary Table 14). Examples of secondary modules present across sampled individuals at Level 3 KEGG BRITE hierarchies include: aromatic amino acid metabolism (2 modules), central carbohydrate metabolism (4 modules), cofactor and vitamin biosynthesis (3 modules), cysteine and methionine metabolism (2 modules), histidine metabolism (2 modules), and mineral and organic ion transport system (2 modules).

Several KEGG modules were differently abundant for host taxonomy, hybrid status, and environment (Figures 7-8, Supplementary Figures S3-S6). A total of 57 modules differed for taxa (Supplementary Figure S3 and S4), with *C. jacchus x C. penicillata* hybrids having the largest number of differently abundant modules and *C. jacchus* possessing the second highest number. Hybrids and non-hybrids showed a total of 63 differentially abundance modules (Supplementary Figures S5 and S6), with the former being overwhelmingly different in abundance of modules for metabolism of nucleotides, carbohydrates, and amino acids. Differences among the three groups of host

environment were not particularly concentrated around certain categories but rather spread out across clades of modules.

Discussion

Factors that Potentially Influence *Callithrix* Gut Microbiome Diversity

Our data showed that host hybrid status and environment more strongly shaped *Callithrix* gut microbiome diversity than host taxon classification. While host taxa differed significantly in microbial diversity measures, few pairwise taxa comparisons were significant. The gut microbiome of hybrid *Callithrix* was significantly more diverse that of non-hybrids. Captive marmosets had significantly less diverse gut microbiomes than their wild and translocated counterparts. As observed in other NHPs, captive marmosets showed the most reduced gut microbiome diversity in terms of species richness and evenness. However, the strong "humanization" effect of captivity reported for the gut microbiome of other NHPs was not entirely evident in our study.

Regarding hybridization effects on gut bacteria, changes in the hybrid gut microbiome relative to parental species may cause negative epistasis between microbial and chromosomal genes [10]. For example, *Nasonia* wasps and *Mus* mouse hybrids possessed altered microbiomes relative to parental species and showed aberrant expression of immune genes [10,11]. Further, *Nasonia* hybridization was lethal and *Mus* hybridization resulted in higher histopathology of caecal tissue in hybrids relative to parental species [10,11]. Aberrant immune gene expression in animal hybrids may make them more susceptible to infectious disease than parental species, and alterations in the gut microbiome due to hybridization may negatively affect hybrid fitness.

Anthropogenic *Callithrix* hybrids are fertile, tend to form hybrids swarms and are frequently present in large Brazilian urban areas and rural landscapes [19, 57; Malukiewicz, pers. obs.], and they possessed more diverse gut microbiomes than non-hybrids. In some cases, hybridization may also lead to favorable physiological functions that can drive adaptation, genetic novelty, or speciation [58]. Our data suggest show that hybridization does not perturb microbial diversity in *Callithrix,* and increased diversity of the gut microbiome may actually be favorable for hybrid host physiology, fitness, and health. Thus, the connection between the gut microbiome and host functions should be further studied in *Callithrix* hybrids to determine whether hybridization promotes host health and fitness in specialized exudivores.

Considering host environment, the lack of a strong "humanization" effect of captivity in *Callithrix* is likely related to differences in their dietary strategy as specialist exudivores to that of other NHPs. *Callithrix* possesses morphological adaptations for gouging gum from trees [59-61] and can spend more than 50% of their feeding time on gums in the wild (e.g., *C. aurita* [62]; *C. jacchus* [63]). However, captive *Callithrix* husbandry often omits gums from the marmoset diet or uses it as an enrichment ingredient [26]. Regular provisioning of exudates also did not apply to captive and translocated marmosets we sampled across four captive facilities, whose generalized *Callithrix* diets are shown in Table 3. One concern for a lack of gum in the diet of captive specialist exudivores is the development of health issues as well as a negative impact on breeding and survivability [26, 64]. Thus, our results suggest that provisioning of gum in the diet of captive specialized exudivores may improve host welfare and conservation

reintroduction programs by maintaining gut microbiomes closer to that of wild populations.

Bacterial Composition and Abundance of the *Callithrix* Gut Microbiome Relative to Other NHP and Mammals

We focus here on differences in composition and abundance of bacterial taxa in wild, translocated, and captive *Callithrix* (Table 4), as host environment showed a stronger effect on these aspects of the gut microbiome than host taxon classification and hybrid status. In a single captive *C. jacchus* fecal sample, Albert et al. [30] found Proteobacteria (37.1%), Firmicutes (33.0%), Bacteroidetes (28.1%), and Actinobacteria (<0.04%) as the major gut microbiome phyla. Proteobacteria was also the most abundant taxon among our captive and translocated hosts, although with a higher average abundance per individual than that of [30]. For another NHP specialist exudivore, the pygmy loris (*Nycticebus pygmaeus*), two wild individuals possessed Bacteroidetes (41.19%), Proteobacteria (30.43%), and Actinobacteria (10.98%) as major gut microbiome bacterial phyla [29]. Overall, these results suggest that Actinobacteria may be a gut microbe typically present in wild specialist exudivores, while Proteobacteria and Bacteroidetes are common in specialist exudivores regardless of environment.

The marmoset gut microbiome seems to differ in bacterial taxa abundance from that of non-gummivores. In an early study of mostly captive NHPs and other mammals, bacterial gut microbiome composition consisted of Firmicutes (65.7%), Bacteroidetes (16.3%), Proteobacteria (8.8%), Actinobacteria (4.7%) and some less abundant taxa [65]. In another captive NHP sample, Provotella and Bacteroides were the most abundant gut

microbes, in line with the modern human gut microbiome [6]. In a recent large sampling of mammals, including several NHPs, the gut microbiome of captive hosts was largely characterized by Bacteroidetes, class Bacilli (Firmicutes), Gammaproteobacteria (Proteobacteria), and class Verruco-5 (Verrucomicriobia) [9]. On the other hand, Actinobacteria, Prevotella (Bacterioidetes), and class Clostridia (Firmicutes) were most abundant for wild hosts [9].

The relatively higher abundances of gut Actinobacteria in our sampled wild marmosets are notable. Within this phylum, *Bifidobacterium* was the most common genus found across all host environments, but particularly among wild hosts. This bacterial genus is common in animal gastrointestinal tracts and metabolizes host-indigestible carbohydrates by aiding their importation into cells [30, 66]. 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* [30]. The relatively higher presence of *Bifidobacterium* in wild marmosets within our dataset is likely associated with a higher intake of tree gums by wild marmosets than in captivity- underlining the importance of *Bifidobacterium* in marmoset carbohydrate metabolism and gummivory adaptations.

As several strains of Proteobacteria are pathogenic, the high occurrence of these bacteria across our dataset is of note, particularly differences in Proteobacteria taxa present between captive, translocated, and wild marmosets. Wild marmosets had an average abundance of *Helicobacter* of 21% and <1% of Enterobacteriaceae. For translocated marmosets, average abundance was 24% *Helicobacter* and 16% Enterobacteriaceae while captive marmosets had 12% *Helicobacter* and 41%

Enterobacteriaceae. Previous studies have identified potentially pathogenic *Helicobacter* strains in captive marmosets [67-69]. In humans, *Helicobacter* is thought to cause hepatic or gastrointestinal disease [70]. However, marmosets infected with *Helicobacter* do not necessarily show clinical symptoms [68], or may show clinical symptoms of gastric disease but not be infected with *Helicobacter* [67, 69]. Enterobacteriaceae is frequently associated with intestinal diseases and contains a number of pathogenic bacterial strains of *Salmonella*, *Escherichia*, and *Shigella* [71, 72]. Although Enterobacteriaceae species were not determined for our sample, potentially pathogenic Enterobacteriaceae were previously found in captive and urban *Callithrix* [73]. Thus, future *Callithrix* microbiome research should identify which Proteobacteria species are present in the *Callithrix* gut and how close *Callithrix*-human contact affects the transmission host dynamics of these bacteria.

Predictive Functional Aspects of the *Callithrix* Gut Microbiome

The principle core predictive functional KEGG modules expressed in all sampled marmosets were related to central carbohydrate metabolism, purine metabolism, pyrimidine metabolism, cofactor and vitamin biosynthesis, among other modules. Given the differences in the principal bacterial taxa present in the gut of captive, translocated, and wild marmosets, similar functions may be carried out by various bacteria despite differences in host environment. Core predictive modules for carbohydrate metabolism were involved with glycolysis and the pentose phosphate pathway, pathways essential for the production of ATP and NADPH. For predictive cofactor and vitamin biosynthesis modules, biosynthesized products included pantothenate, riboflavin, biotin as well as NAD.

The gut microbiome of wild marmosets was particularly enriched for predictive modules involved in carbohydrate metabolism including that of glycolysis and pentose phosphate biosynthesis. While such modules are part of the core predictive functional modules discussed above, they are over-represented in wild marmosets likely due to the relatively high abundance of *Bifidobacterium* in their gut microbiome. Carbohydrate metabolism was also the most abundant functional category present in the two wild pygmy lories discussed above [29]. On the other hand, predictive functional KEGG modules enriched in the gut microbiome of captive and translocated marmosets were those involved in nucleotide and amino acid metabolism and environmental information processing. Enrichment of these particular predictive modules in the gut microbiome of captive and translocated marmosets may be related to their diets. Since gums were not a normal part of the marmoset diet in any of the captive facilities we sampled, the marmoset gut microbiome seems to have partially redirected functional and metabolic activities to those that differ from the wild.

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 NHP and non-NHP animal exudivores. Marmosets are maintained in captivity for biomedical research [74], in zoological collections, and as part of a recent conservation program in Brazil for *C. aurita* [32]. Several challenges arise in maintaining captive *Callithrix* such as marmoset wasting syndrome [26], a rise in obesity in captive populations [75], and lack of a standardized

marmoset diet. Additionally, it is concerning that our results indicated a relatively large abundance of Enterobacteriaceae in the gut microbiome of captive, but not wild, marmosets. In the case of biomedical studies, data from unhealthy marmosets may bias research findings, and captive-bred marmosets reintroduced into the wild may not be fit to survive with gut microbiome profiles different from their wild counterparts.

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 [26]. 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. As studies of the exudivore gut microbiome are generally rare, such issues are also applicable to other captive NHPs that have evolved to exploit this nutritional strategy. More studies are needed to understand better the health and reproductive consequences of omitting a major component of the natural diet of specialized exudivores in captivity.

To corroborate the findings of this study, future research should prospectively investigate the specialized exudivore gut microbiome with a larger host sampling across different taxa and environments, as well as through metagenomics and transcriptomics. A systematic study of the effects of different diets on the marmoset gut microbiome, including those with and without gum consumption, would facilitate understanding of how nutrition affects marmoset health and particularly their gastrointestinal health. For

Callithrix, such data will add to our understanding of the evolution of marmosets as exudivores, as well as help guide conservation efforts for *C. aurita* and *C. flaviceps*. Overall, such information will expand baseline gut microbiome data available for wild and translocated/captive specialized exudivores to allow for the development of new tools to improve the management, welfare, and conservation of these animals.

Acknowledgements

This work was supported by a Brazilian CNPq Jovens Talentos Postdoctoral Fellowship, an American Society of Primatologists Conversation Small Grant, and an International Primatological Society Research Grant. We thank Vanner Boere, Ita de Oliveira e Silva, Veronica Souza, Rodrigo S. Carvalho, the Guarulhos Zoo staff, the CEMAFAUNA staff, the CPRJ staff, SERCAS staff, and AMLD staff for assistance with wild and captive populations. We are very grateful to Cecil M. Lewis and Tanvi Honap and the LMAMR staff at University of Oklahoma for donation of PCR primers. We thank Camila Molina for comments on prior versions of this work.

Conflict of Interests

The authors declare no conflict of interests.

References

1. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR et al. Enterotypes of the human gut microbiome. *Nature* 2011; **473(7346)**:174-80.

2. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature* 2012;**486(7402)**:207-14.

3. Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, et al. Human genetics shape the gut microbiome. *Cell* 2014;**159(4)**:789-99.

4. Dominianni C, Sinha R, Goedert JJ, Pei Z, Yang L, Hayes RB et al. Sex, body mass index, and dietary fiber intake influence the human gut microbiome. *PLoS One* 2015; **10(4)**:e0124599.

5. McKenna P, Hoffmann C, Minkah N, Aye PP, Lackner A, Liu Z et al. The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. *PLoS Pathog* 2008;**4(2)**:e20.

6. Clayton JB, Vangay P, Huang H, Ward T, Hillmann BM, Al-Ghalith GA et al. Captivity humanizes the primate microbiome. *Proc Natl Acad Sci USA* 2016;**113(37)**:10376-81.

7. García-Amado MA, Shin H, Sanz V, Lentino M, Martínez LM, Contreras M et al. Comparison of gizzard and intestinal microbiota of wild neotropical birds. *PLoS One* 2018;**13(3)**:e0194857.

8. Ingala MR, Simmons NB, Wultsch C, Krampis K, Speer KA, Perkins SL. Comparing Microbiome sampling methods in a wild mammal: fecal and intestinal samples record different signals of host ecology, evolution. *Front Microbiol* 2018;**9**:803.

9. McKenzie VJ, Song SJ, Delsuc F, Prest TL, Oliverio AM, Korpita TM et al. The effects of captivity on the mammalian gut microbiome. *Integr Comp Biol* 2017;**257(4)**:690-704.

10. Brucker RM, Bordenstein SR. The Hologenomic Basis of Speciation: Gut Bacteria Cause Hybrid Lethality in the Genus *Nasonia*. *Science* 2013;**341**:667.

11. Wang J, Kalyan S, Steck N, Turner LM, Harr B, Künzel S et al. Analysis of intestinal microbiota in hybrid house mice reveals evolutionary divergence in a vertebrate hologenome. *Nat Commun* 2015;**6**:6440.

12. Arnold ML. Natural hybridization and evolution. Oxford University Press: New York, USA, 1997.

13, Detwiler KM, Burrell AS, Jolly, CJ. Conservation implications in hybridization in African cercopithecine monkeys. *Int J Primatol* 2005;**26**:661–684.

14. Mallet J. Hybridization as an invasion of the genome. *TREE* 2005;**20**:229–237.

15. Zinner D, Groeneveld LF, Keller C, Roos C. Mitochondrial phylogeography of baboons (*Papio* spp.): Indication for introgressive hybridization? *BMC Evol Biol* 2009;**9**:8.

16. Kelaita MA, Cortés-Ortiz L. Morphological variation of genetically confirmed *Alouatta pigra* × *A. palliata* hybrids from a natural hybrid zone in Tabasco, Mexico. Am J Phys Anthropol 2013;**150(2)**:223-34.

17. Carneiro J, Rodrigues LDF, Schneider H, Sampaio I. Molecular data highlight hybridization in squirrel monkeys (Saimiri, Cebidae). *Genet Mol Biol* 2016;**39**:539–546.

18. Wall JD, Schlebusch SA, Alberts SC, Cox, LA, Snyder-Mackler,N, Nevonen KA, et al. Genome wide ancestry and divergence patterns from low-coverage sequencing data reveal a complex history of admixture in wild baboons. Mol Ecol 2016;**25**:3469–3483.

19. Malukiewicz J. A review of experimental, atural, and anthropogenic hybridization in *Callithrix* marmosets. Int J Primatol 2019;**40**:72

20. Li W, Liu J, Tan H, Yang C, Ren L, Liu Q, et al. Genetic Effects on the Gut Microbiota Assemblages of Hybrid Fish From Parents With Different Feeding Habits. *Front Microbiol* 2018;**9**:2972.

21. Nagpal R, Shively CA, Appt SA, Register TC, Michalson KT et al. Gut microbiome composition in non-human primates consuming a Western or Mediterranean diet. *Front Nutr* 2018;**5**:28.

22. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA et al. Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 2005;81(2):341–354.

23. Nash LT. Dietary, behavioral, and morphological aspects of gummivory in primates. *Yearb Phys Anthropol* 1986;**29**:113-137.

24. Nash LT, Burrows AM. Introduction: advances and remaining sticky issues in the understanding of exudativory in primates. In: Burrow AM, Nash LT (eds). The Evolution of Exudativory in Primates. (Springer-Verlag, New York, 2010) pp. 1-23.

25. Smith A. Exudativory in primates: interspecific patterns. In: Burrow AM, Nash LT (eds). The Evolution of Exudativory in Primates. (Springer-Verlag, New York, 2010) pp. 45-87.

26. Cabana F, Maguire R, Hsu CD, Plowman A. Identification of possible nutritional and stress risk factors in the development of marmoset wasting syndrome. *Zoo Biol* 2018;**37(2)**:98-106.

27, IUCN. (2018). The IUCN Read List of Threatened Species. Version 2016–3. www.iucnredlist.org (accessed 23 January 23, 2018).

28. Hapke A, Gligor M, Rakotondranary SJ, Rosenkranz D, Zupke O. Hybridization of mouse lemurs: different patterns under different ecological conditions. *BMC Evol Biol* 2011;**11**:297.

29. Xu B, Xu W, Yang F, Li J, Yang Y, Tang X et al. Metagenomic analysis of the pygmy loris fecal microbiome reveals unique functional capacity related to metabolism of aromatic compounds. *PLoS One* 2013;**8(2**):e56565.

30. Albert K, Rani A, Sela DA. The comparative genomics of *Bifidobacterium callitrichos* reflects dietary carbohydrate utilization within the common marmoset gut. Microb Genom 2018; **4(6)**: e000183

31. Rowe N, Myers M (eds). All the Worlds Primates. (Pogonias Press, Charlestown, 2016).

32. Carvalho RS, Bergallo HG, Cronemberger C, Guimarães-Luiz T, Igayara-Souza CA, Jerusalinsky L, et al. *Callithrix aurita*: A tiny primate on the edge of extinction in the Brazilian Atlantic Forest. *Neotropical Primates* 2018; **24**:1–8.

33. Hershkovitz P. Living new world monkeys (Platyrrhini): with an introduction to primates. (University of Chicago Press, Chicago, 1977).

34. Coimbra-Filho AF, Pissinatti A, Rylands AB. (1993). Experimental multiple hybridism and natural hybrids among *Callithrix* species from eastern Brazil. In: Rylandss

AB (eds.) *Marmosets and tamarins: Systematics, behaviour, and ecology*. Oxford University Press: New York, 1993, pp 95–120.

35. Fuzessy LF, Silva IO, Malukiewicz J, Silva FFR, Pônzio MC, Boere V, et al. Morphological variation in wild marmosets (*Callithrix penicillata* and *C. geoffroyi*) and their hybrids. *Evol Biol* 2014;**41**:480–493.

36. Malukiewicz J, Boere V, Fuzessy LF, Grativol AD, French JA, Silva, IO et al. Hybridization effects and genetic diversity of the common and black-tufted marmoset (*Callithrix jacchus* and *C. penicillata*) mitochondrial control region. *Am J Phys Anthropol* 2014;**155**:522–536.

37, Carvalho RS. Conservação do saguis-da-serra-escuro (*Callithrix aurita* (Primates)) – Analise molecular e colormetrica de populações do gênero Callithrix e seus híbridos. PhD dissertation 2015, Universidade do Estado do Rio de Janeiro.

38. Zoentendal EG, Heilig HGH, Klaasssens ES, Booijink CCGM, Kleerebezem M, Smidt H, et al. Isolation of DNA from bacterial samples of the human gastrointestinal tract. *Nat. Protoc.* 2006;**1**:870-873.

39. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J* 2012;**6(8)**:1621-4.

40. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 2010;7**(5)**:335-336

41. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2:High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;**13(7)**:581-3.

42. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J et al. Best practices for analysing microbiomes. Nat Rev Microbiol 2018;**16(7)**:410-422.

43. Katoh K, Standley DM. Mafft multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 2013;**30(4)**:772–780.

44. Price MN, Dehal PS, Arkin AP. Fasttree 2–approximately maximum-likelihood trees for large alignments. *PloS One* 2010;5(3):e9490.

45. Lozupone CA, Hamady M, Kelley ST, Knight R. Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Appl Environ Microbiol* 2007;**73(5)**:1576-85.

46. Anderson MJ. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef: Statistics Reference Online 2017; https://doi.org/10.1002/9781118445112.stat07841

47. Benjamini Y, Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B* 1995;**57(1)**: 289–300.

48. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbol 2006; **2(7)**:5069-72.

49. Morton JT, Sanders J, Quinn RA, McDonald D, Gonzalez A, Vázquez-Baeza Y et al. Balance trees reveal microbial niche differentiation. *MSystems* 2017;**2(1)**:e00162–16.

50. Langille MGI, Zaneveld J, Caporaso, JG, McDonald D, Knights D, Reyes J et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnol* 2013;**8**:1-10.

51. Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL et al. Metabolic reconstruction for metagenomic data and its application to the human microbiome. *PLoS Comput Biol* 2012;**8(6)**:e1002358.

52. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res* 2002;**28(1):**27-30.

53. Kanehisa M, Tanabe M, Sato Y, and Morishima, K.; KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res* 2017;45:D353-D361.

54. Kanehisa M, Sato Y, Furumichi M, Morishima K, Tanabe M.; New approach for understanding genome variations in KEGG. *Nucleic Acids Res.* 2019;**47**:D590-D595.

55. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011;**12(6)**:R60.

56. Asnicar F, Weingart G, Tickle TL, Huttenhower C, Segata N. Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *PeerJ* 2015; doi: 10.7717/peerj.1029.

57. Ruiz-Miranda CR, Affonso AG, Martins A, Beck B. Distribuição do sagui (*Callithrix jacchus)* nas áreas de ocorrência do mico-leão-dourado (*Leontopithecus rosalia*) no estado do Rio de Janeiro. *Neotropical Primates*. 2000; **8**: 98–101.

58. Seehausen O, Butlin RK, Keller I, Wagner CE, Boughman JW, Hohenlohe PA, et al. Genomics and the origin of species. *Nat Rev Genet* 2014; 15:176-92.

59, Rylands AB, Faria, DS. Habitats, feeding ecology, and home range size in the genus *Callithrix*. In: Rylands AB (ed.), Marmosets and tamarins: Systematics, behaviour, and ecology. Oxford University Press:New York, USA, 1993, pp 95–120.

60. Power ML, Oftedal, OT. Differences Among Captive Callitrichids in the Digestive Responses to Dietary Gum. *Am J Primatol* 1996;**40**:131-144.

61. Taylor AB, Eng CM, Anapol FC, Vinyard CJ. The functional correlates of jaw-muscle fiber architecture in tree-gouging and nongouging callitrichid monkeys. *Am J Phys Anthropol* 2018;**139(3)**:353-67.

62. Martins MM, Setz EZ. (2000). Diet of Buffy Tufted-Eared Marmosets (*Callithrix aurita*) in a Forest Fragment in Southeastern Brazil. *Int J Primatol* 2000;**21**:467-476.

63. Pinheiro HLN, Pontes ARM. Home range, diet, and activity patterns of common marmosets (*Callithrix jacchus*) in very small and isolated fragments of the Atlantic Forest of northeastern Brazil. *Int J Ecolog* 2015; Article ID 685816 http://dx.doi.org/10.1155/2015/685816

64. Cabana F, Nekaris KAI. Diets high in fruits and low in gum exudates promote the occurrence and development of dental disease in pygmy slow loris (*Nycticebus pygmaeus*). *Zoo Biol* 2015; **34**:547–553.

65. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS et al. Evolution of mammals and their gut microbes. 2008;**320(5883)**:1647-51.

66. O'Callaghan A, van Sinderen D. Bifidobacteria and their role as members of the human gut microbiota. *Front Microbiol* 2016;**7**:925.

67. Mello MF, Monteiro AB, Fonseca EC, Pissinatti A, Ferreira AM. Identification of *Helicobacter* sp. in gastric mucosa from captive marmosets (*Callithrix* sp.; callitrichidae, primates). *Am J Primatol* 2005;**66(2)**:111-8.

68. Won YS, Vandamme P, Yoon JH, Park YH, Hyun BH, Kim HC et al. *Helicobacter callitrichis* sp. nov., a novel Helicobacter species isolated from the feces of the common marmoset (*Callithrix jacchus*). *FEMS Microbiol Lett* 2007; **271(2)**:239-44.

69. Shen Z, Feng Y, Sheh A, Everitt J, Bertram F, Paster BJ. Isolation and characterization of a novel Helicobacter species, *Helicobacter jaachi* sp. nov., from common marmosets (*Callithrix jaachus*). *J Med Microbiol* 2015;**64**:1063–1073.

70. Makola D, Peura DA, Crowe SE. *Helicobacter pylori* infection and related gastrointestinal diseases. *J Clin Gastroenterol* 2007;**6**:548-558.

71. Paterson DL. Resistance in gram-negative bacteria: enterobacteriaceae. *Am J Med* 2006;**119(6 Suppl 1):**S20-8

72. Kang E, Crouse A, Chevallier L, Pontier SM, Alzahrani A, Silué N *et al.* Enterobacteria and host resistance to infection. *Mamm Genome* 2018;**29(7-8)**:558-576.

73. Verona CES. Parasitos em sagüi-de-tufo-branco (*Callithrix jacchus*) no Rio de Janeiro. PhD Dissertation 2015 . Fundação Oswaldo Cruz.

74. Marini R, Wachtman L, Tardif S, Mansfield K, Fox J (eds). The common marmoset in captive and biomedical research. (Academic Press, Cambridge, 2018).

75. Power ML, Ross CN, Schulkin J, Tardif SD. The development of obesity begins at an early age in captive common marmosets (*Callithrix jacchus*). *Am J Primatol.* 2012;**74(3)**:261-9.

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**

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

Table 3. List of captive 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

Table 4. 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

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.

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 and color-coding 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 hybrid status showing Shannon diversity index (A), number of observed OTUs (B), and Pielou E evenness (C). Classifications and color-coding along the x-axis are H/Red=hybrids and P/Dark Blue=pure species.

Figure 4. 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 and color-coding along the x-axis are C/Red=captive, T/Dark Blue=translocated, and W/Orange=wild.

Figure 5. PCoA of weighted Unifrac distances by host taxon (A), hybrid status (B) and environment (C). Abbreviations and color codes in plot legends are the same as in Figures 1-3.

Figure 6. 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.

Figure 7. 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.

Figure 8. 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.

Supplementary Figure S1. (A). Relative abundances of gut bacterial classes by taxon (A). Taxon abbreviations are the same as in Figure 1. (B) Relative abundances of gut bacterial classes by hybrid status. Hybrid status abbreviations are the same as in Figure 2. In both A and B, colors within each bar represent bacterial classes with top colors representing greater bacterial relative abundance than those taxa at the bottom of each bar.

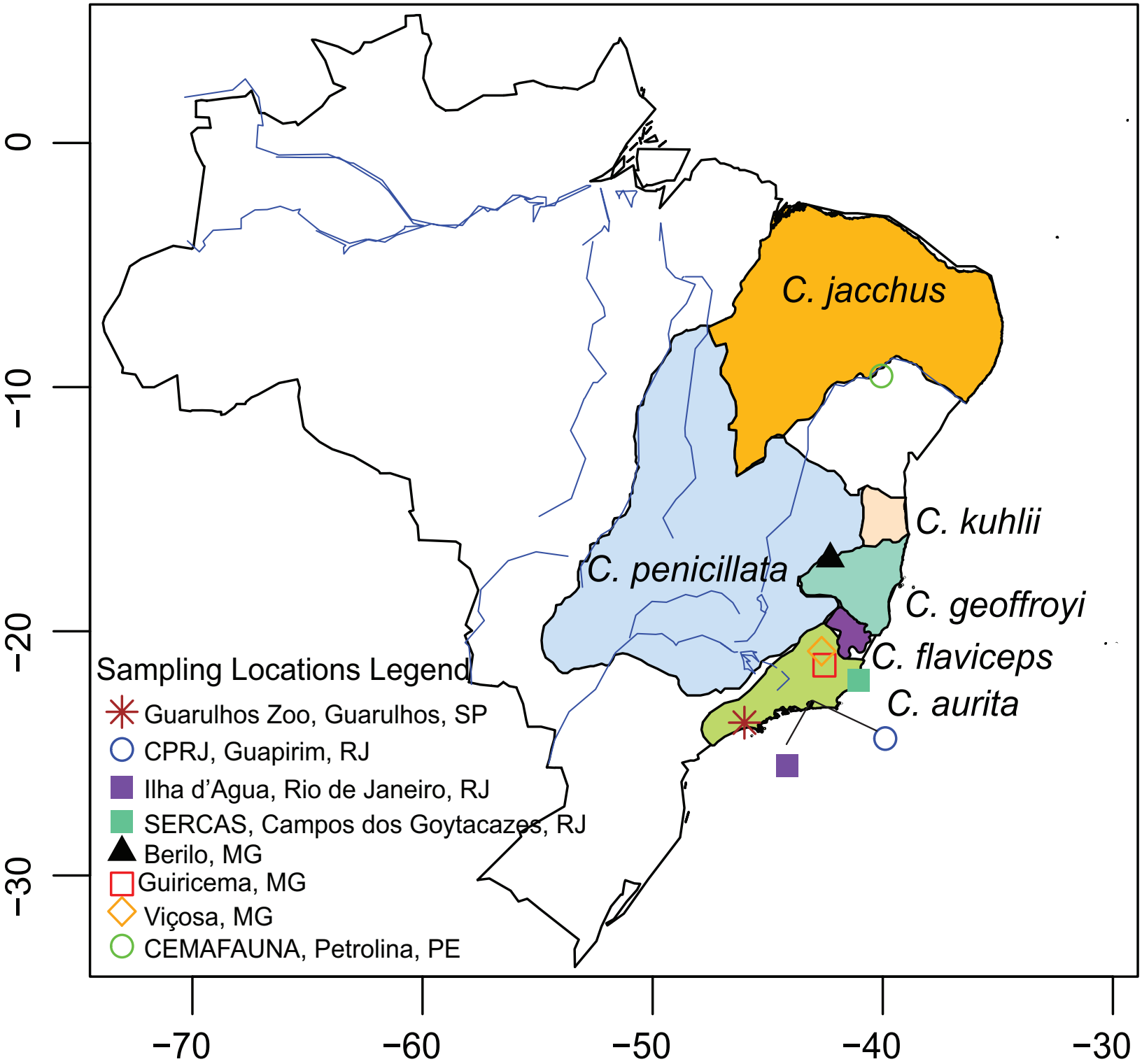
Supplementary Figure S2. Prediction plots of the top two balances for the OLE regression model, with the prediction residuals from the model projected onto these two balances.

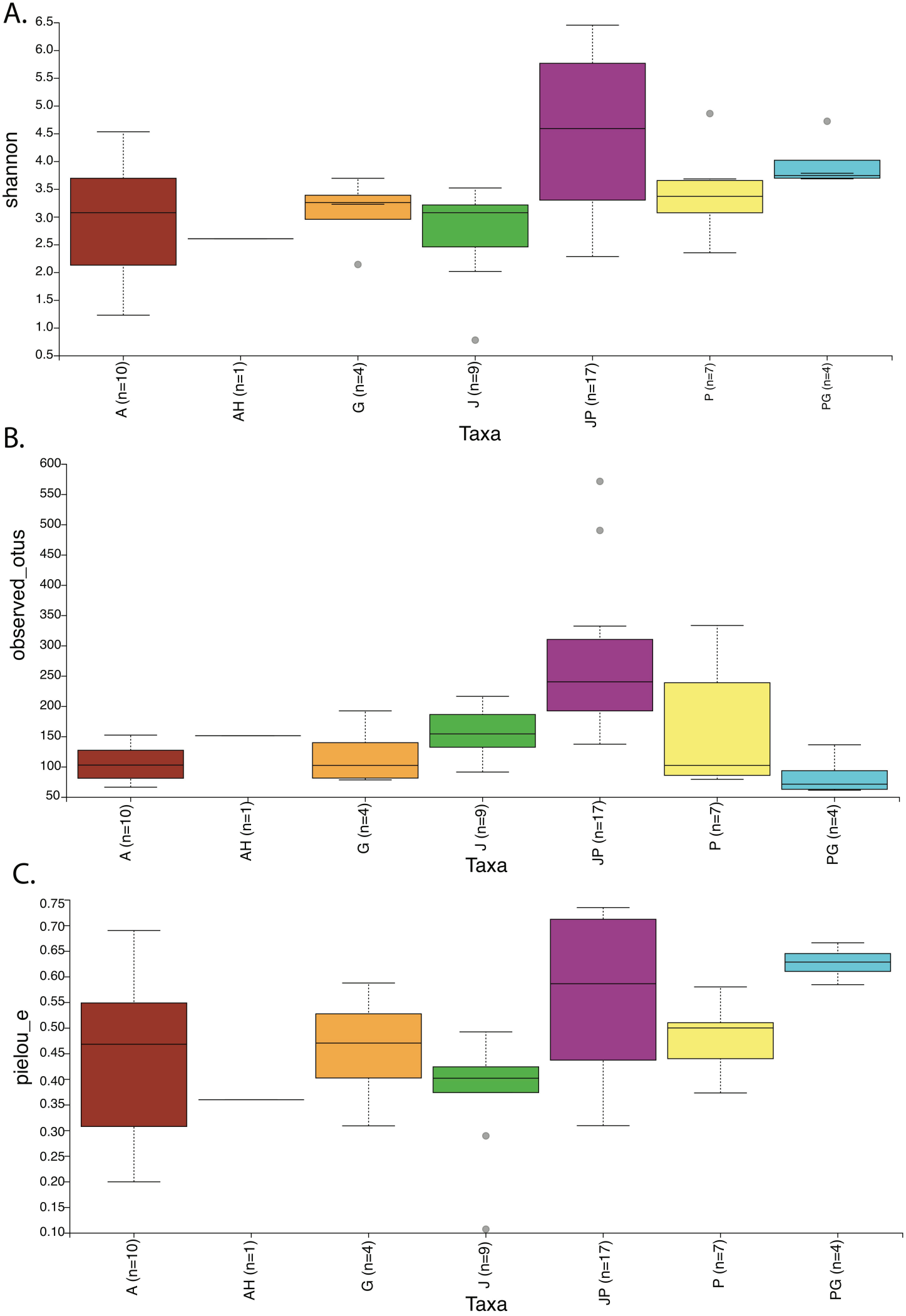
Supplementary Figure S3. HUMAnN metabolic reconstruction of marmoset fecal microbiome based on host taxonomy. The cladogram shows 3 KEGG BRITE hierarchical levels with an outer, generalized level, and ending with the inner-level annotated by letters that are detailed further in the accompanying legend. Circles not differentially abundant in any species are clear.

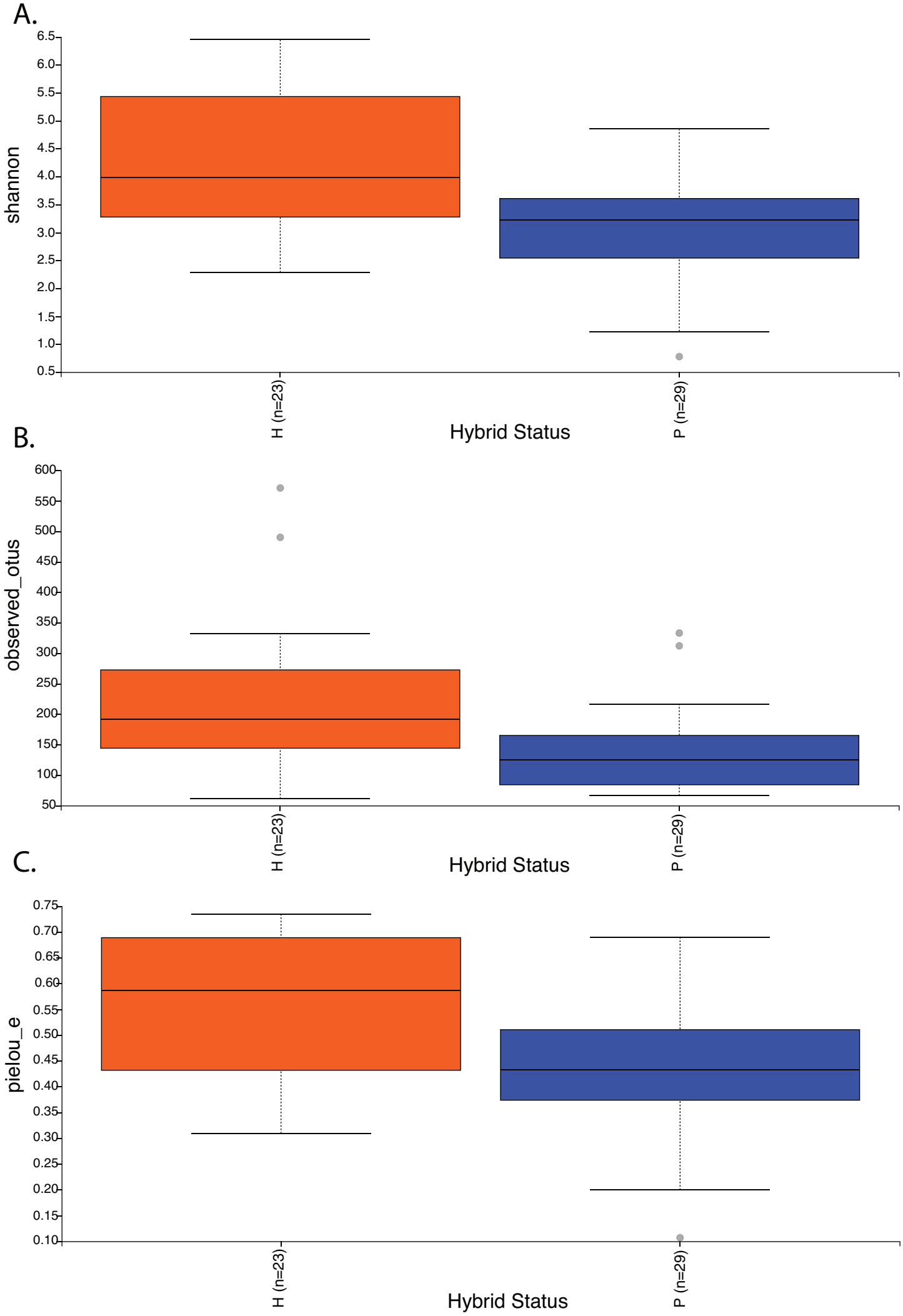
Supplementary Figure S4. Differentially abundant KEGG metabolic modules inferred by LEfSe are colored by their associated taxon classification, which follow the same abbreviations as Figure 1. The plot shows LDA scores resulting from LeFSe analysis for differentially abundant KEGG modules across host taxon classifications.

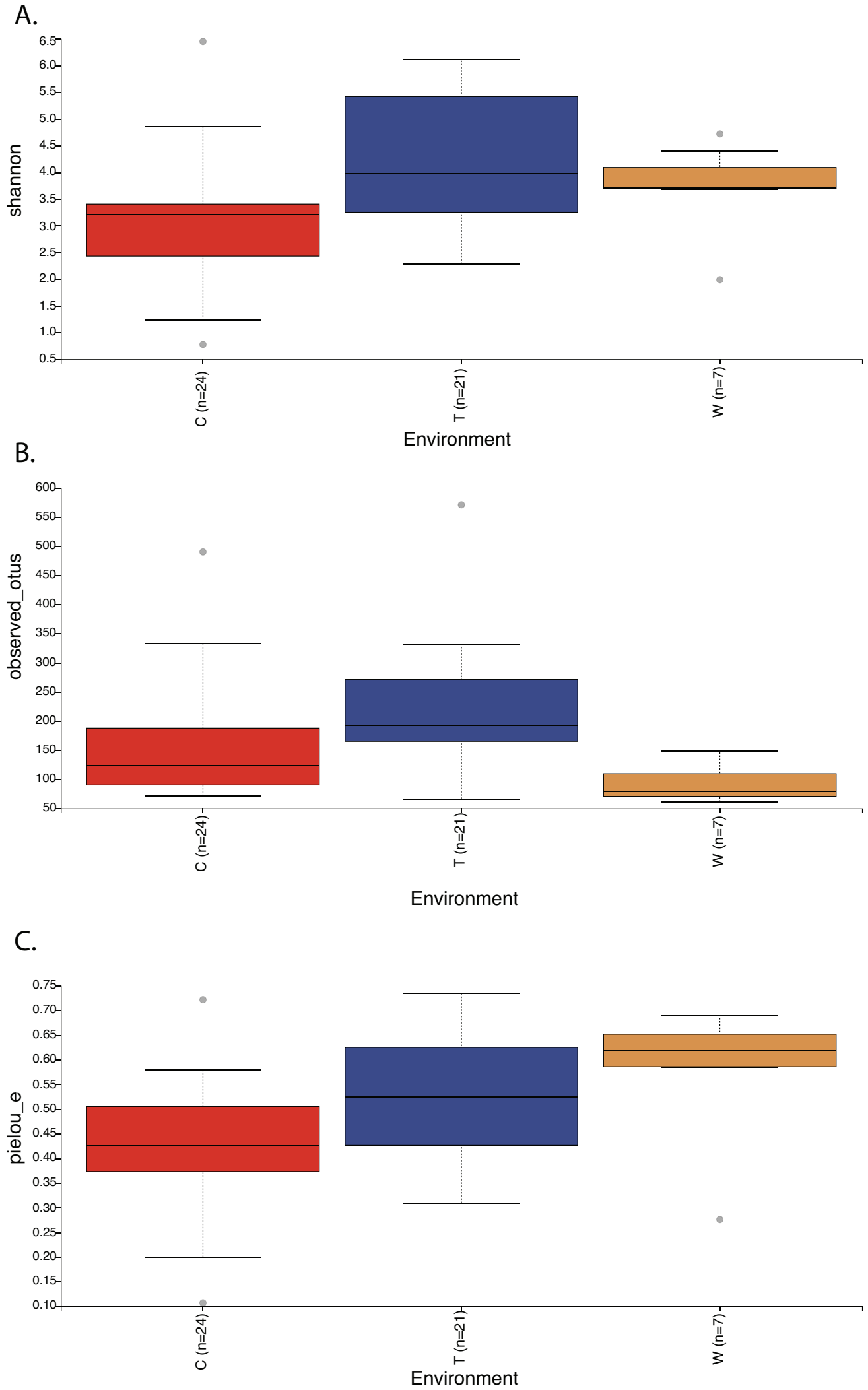
Supplementary Figure S5. HUMAnN metabolic reconstruction of marmoset fecal microbiome based on host hybrid status. The cladogram shows 3 KEGG BRITE hierarchical levels with an outer, generalized level, and ending with the inner-level annotated by letters that are detailed further in the accompanying legend. Circles not differentially abundant in any species are clear.

Supplementary Figure S6. Differentially abundant KEGG metabolic modules inferred by LEfSe are colored by their associated hybrid classification, which follow the same abbreviations as Figure 3. The plot shows LDA scores resulting from LeFSe analysis for differentially abundant KEGG modules across host hybrid status.

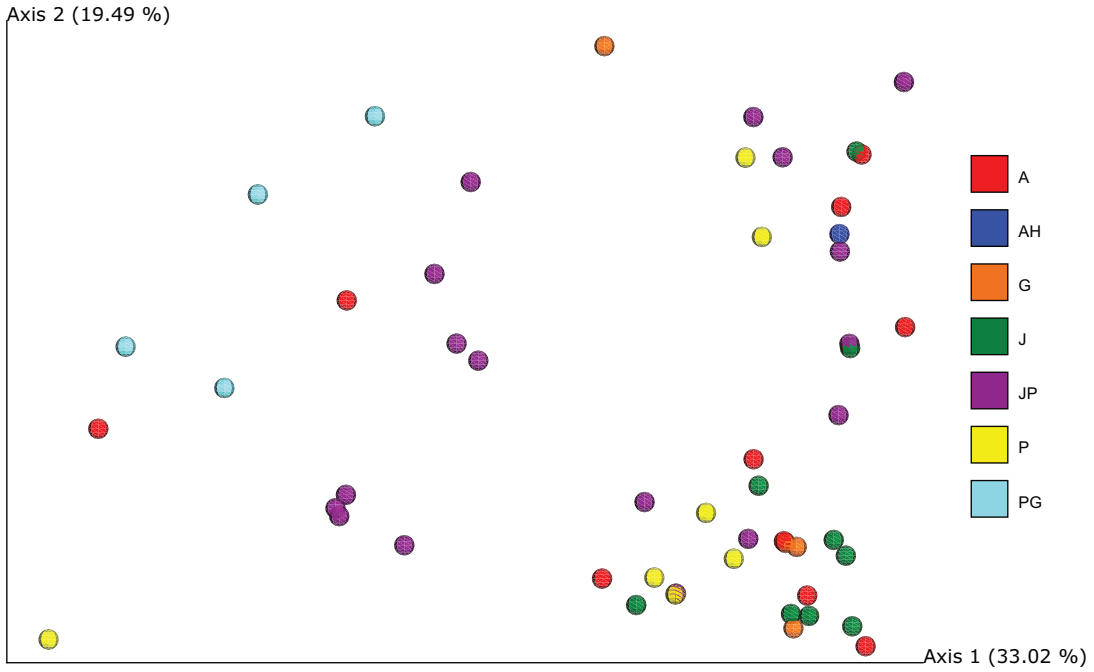

C. jacchus
C. kuhlii
C. penicillata
C. geoffroyi
C. flaviceps
C. aurita
Sampling Locations Legend
Guarulhos Zoo, Guarulhos, SP
CPRJ, Guapirim, RJ
Ilha d'Agua, Rio de Janeiro, RJ
SERCAS, Campos dos Goytacazes, RJ
Berilo, MG
Guiricema, MG
Viçosa, MG
CEMAFAUNA, Petrolina, PE
0
−10
−20
−30
−70
−60
−50
−40
−30


A.
shannon
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
A (n=10)
AH (n=1)
G (n=4)
J (n=9)
JP (n=17)
P (n=7)
PG (n=4)
Taxa
B.
observed_otus
600
550
500
450
400
350
300
250
200
150
100
50
A (n=10)
AH (n=1)
G (n=4)
J (n=9)
JP (n=17)
P (n=7)
PG (n=4)
Taxa
C.
pielou_e
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
A (n=10)
AH (n=1)
G (n=4)
J (n=9)
JP (n=17)
P (n=7)
PG (n=4)
Taxa

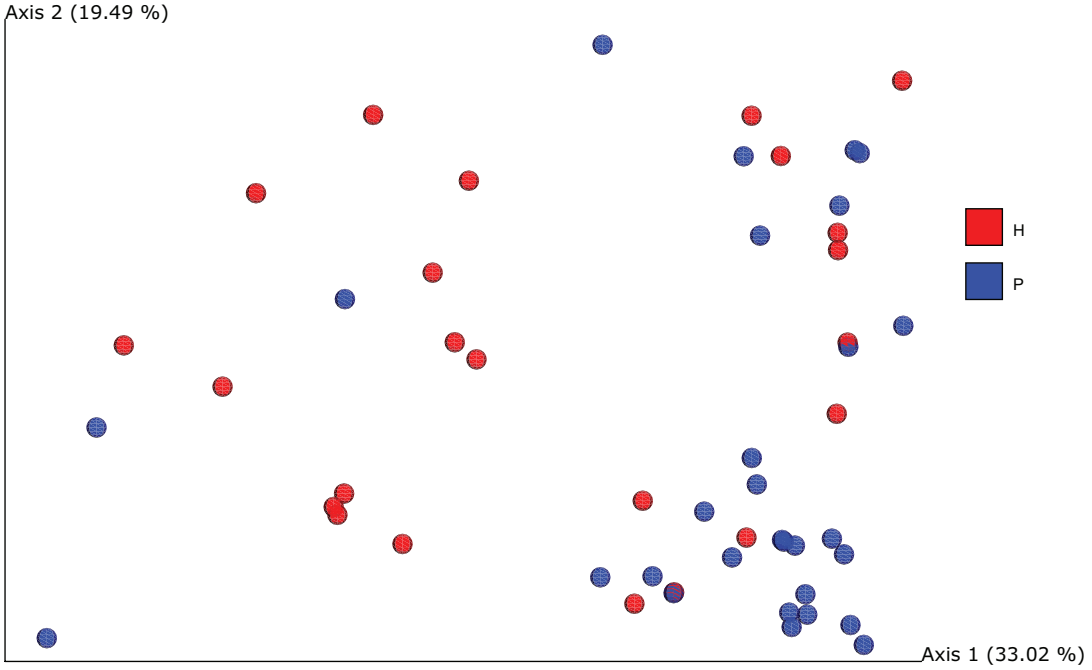

A.
shannon
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
H (n=23)
P (n=29)
Hybrid Status
B.
observed_otus
600
550
500
450
400
350
300
250
200
150
100
50
H (n=23)
P (n=29)
Hybrid Status
C.
pielou_e
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
H (n=23)
P (n=29)
Hybrid Status


A.
shannon
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
C (n=24)
T (n=21)
W (n=7)
Environment
B.
observed_otus
600
550
500
450
400
350
300
250
200
150
100
50
C (n=24)
T (n=21)
W (n=7)
Environment
C.
pielou_e
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
C (n=24)
T (n=21)
W (n=7)
Environment

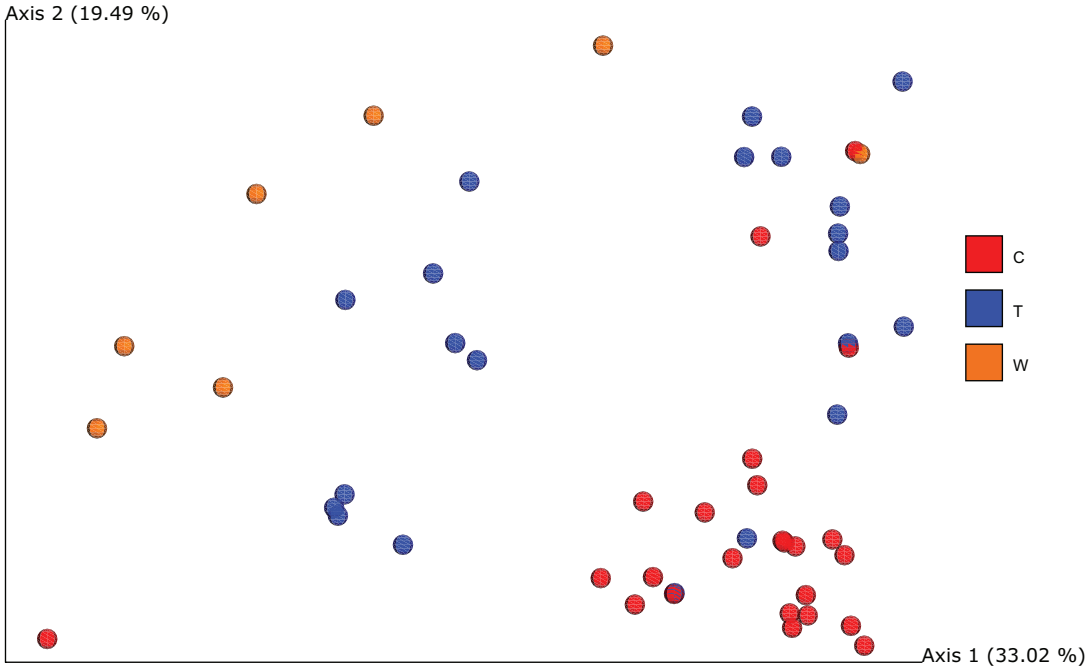
A.

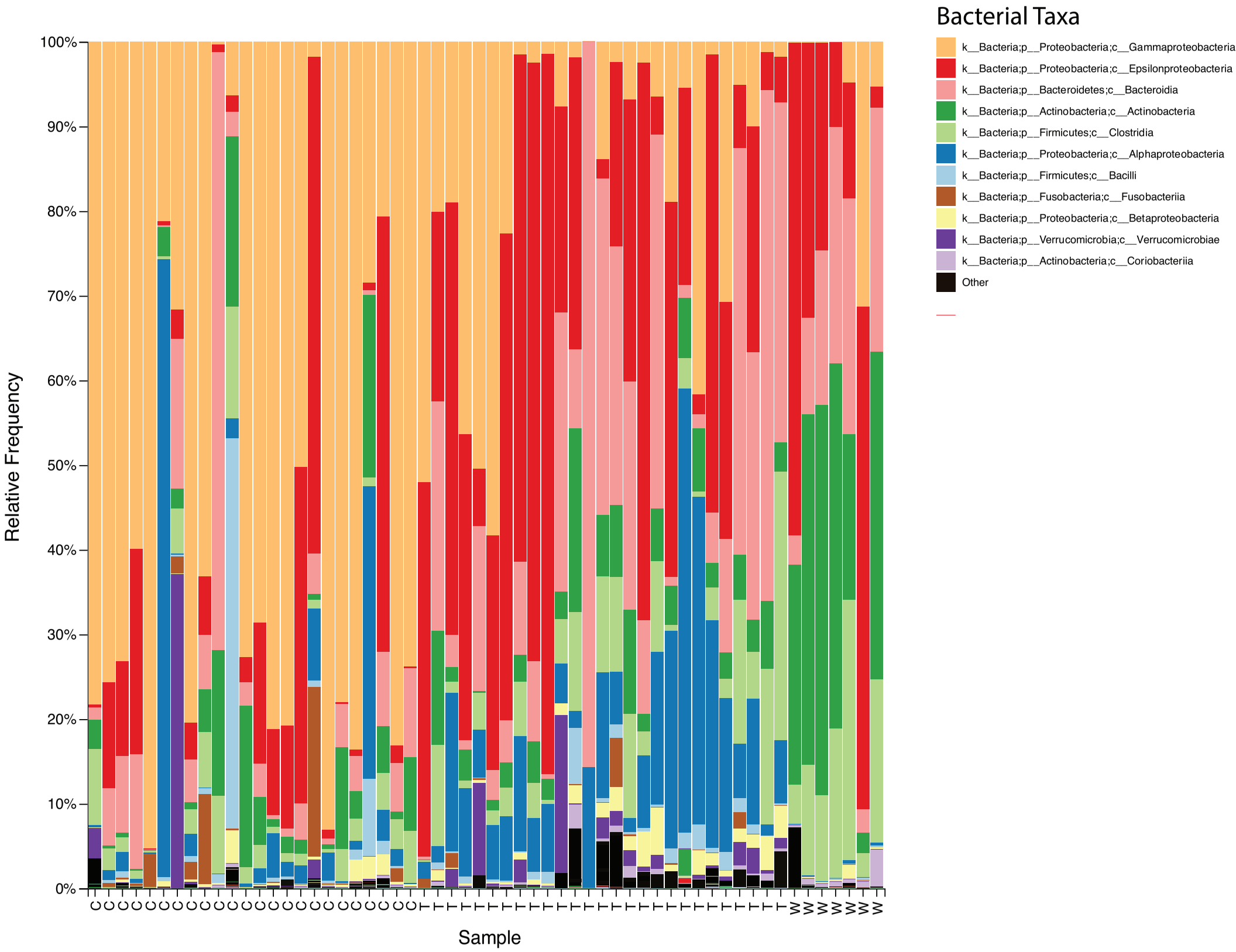

Axis 2 (19.49 %)
Axis 1 (33.02 %)
A
AH
G
J
JP
P
PG

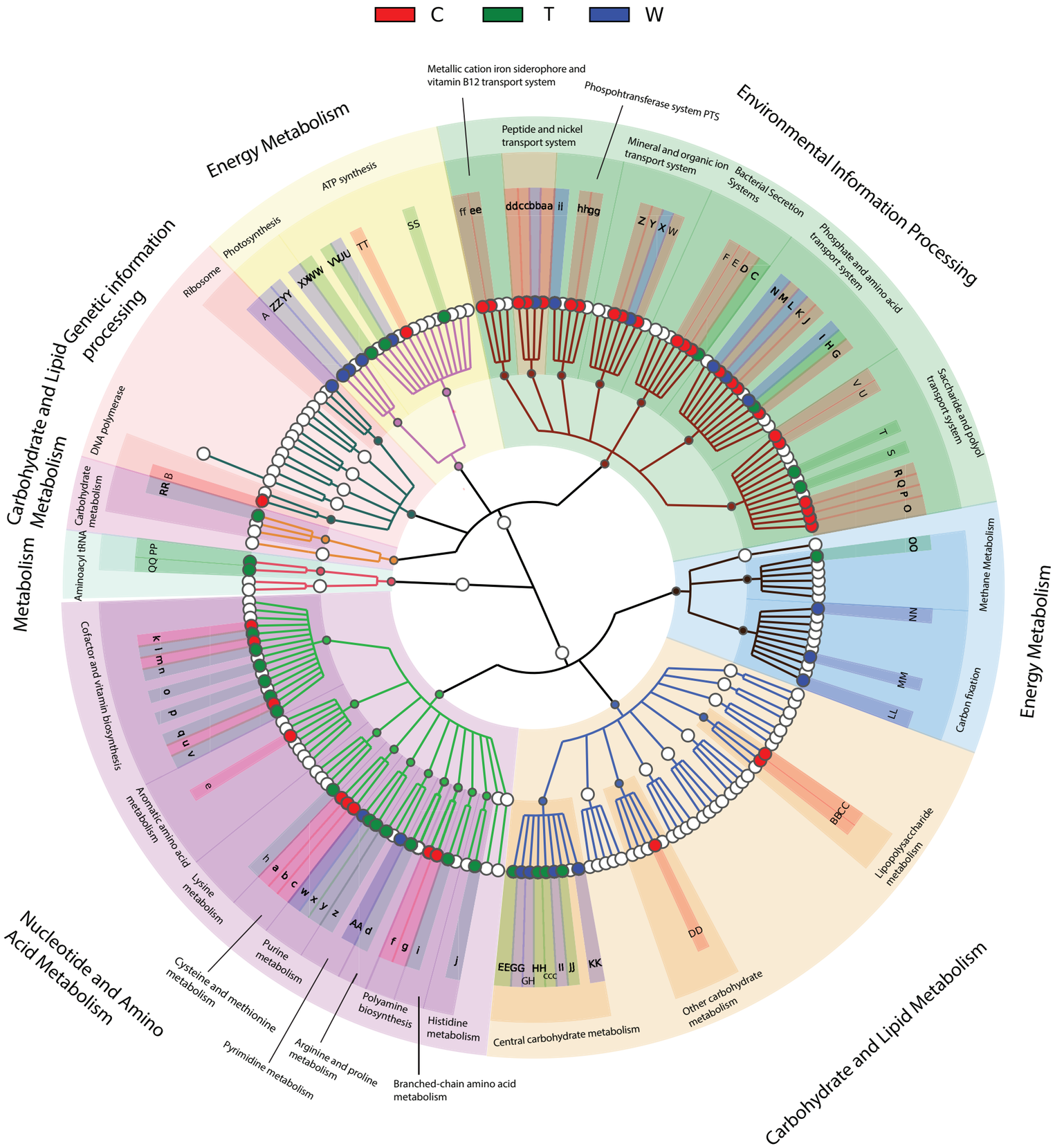
B.


Axis 2 (19.49 %)
Axis 1 (33.02 %)
H
P

C.


Axis 2 (19.49 %)
Axis 1 (33.02 %)
C
T
W


Bacterial Taxa
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria
k__Bacteria;p__Bacteroidetes;c__Bacteroidia
k__Bacteria;p__Actinobacteria;c__Actinobacteria
k__Bacteria;p__Firmicutes;c__Clostridia
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria
k__Bacteria;p__Firmicutes;c__Bacilli
k__Bacteria;p__Fusobacteria;c__Fusobacteriia
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria
k__Bacteria;p__Verrucomicrobia;c__Verrucomicrobiae
k__Bacteria;p__Actinobacteria;c__Coriobacteriia
Other
Relative Frequency
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Sample



A:M00178 Ribosome bacteria
AA:M00051 Uridine_monophosphate biosynthesis glutamine + PRPP => UMP
BB:M00064 ADP-L-glycero-D-manno-heptose_biosynthesis
C:M00335 Sec secretion system
CC:M00060 Lipopolysaccharide biosynthesis KDO2-lipid A
D:M00331 Type II general secretion system
DD:M00061 Uronic acid metabolism
E:M00326 RTX toxin transport system
EE:M00001 Glycolysis Embden-Meyerhof pathway glucose => pyruvate
F: M00336 Twin-arginine translocation Tat system
G:M00229 Arginine transport system
GG:M00007 Pentose phosphate pathway oxidative phase fructose 6P => ribulose 5P
G :M00006 Pentose phosphate pathway oxidative phase glucose 6P => ribulose 5P
H:M00222 Phosphate transport system
HH:M00009 Citrate_cycle_TCA_cycle_Krebs_cycle
I:M00237 Branched-chain amino acid transport system
II:M00002 Glycolysis core module involving three-carbon compound
J:M00277 PTS system N-acetylgalactosamine-specific II componentI
JJ:M00003 Gluconeogenesis oxaloacetate =>fructose-6P
K:M00230 Glutamate/aspartate transport system
KK:M00004 Pentose phosphate pathway Pentose phosphate cycle
L:M00323 Urea transport system
LL:M00166 Reductive pentose phosphate cycle RuBP + CO2 => glyceraldehyde-3P M00166
M:M00226 Histidine transport system
MM:M00165 Reductive pentose phosphate cycle Calvin cycle
N:M00322 Neutral amino acid transport system
NN:M00167 Reductive pentose phosphate cycle glyceraldehyde-3P => RuBP
O:M00219 AI-2 transport system
OO:M00174 Methane oxidation methylotroph methane => CO2
P:M00197 Putative fructooligosaccharide transport system
PP:M00360 Aminoacyl-tRNA_biosynthesis_prokaryotes
Q:M00194 Maltose/maltodextrin transport system
QQ:M00359 Aminoacyl-tRNA_biosynthesis_eukaryotes
R:M00202 Oligogalacturonide transport system
RR:M00311 2-oxoglutarate:ferredoxin oxidoreductas
S:M00207 Putative multiple sugar transport system
SS:M00149 Succinate_dehydrogenase_prokaryotes
T:M00200 Putative sorbitol/mannitol transport system
TT:M00150 Fumarate_reductase_prokaryotes
U:M00198 Putative sn-glycerol-phosphate transport system
UU:M00157 F-type ATPase bacteria
V:M00220 Rhamnose_transport_system
VV:M00156 Cytochrome_c_oxidase_cbb3-type
W:M00302 2-AminoethylPhosphonate transport system
WW:M00144 NADH quinone_oxidoreductase_prokaryotes
X:M00321 Bicarbonate transport system
XX:M00161 Photosystem_II
Y:M00185 Sulfate transport system
YY:M00164 ATP synthase
Z:M00300 Putrescine transport system
ZZ:M00163 Photosystem I
a:M00034 Methionine salvage pathway
aa:M00324 Dipeptide transport system
b:M00017 Methionine biosynthesis apartate => homoserine => methionine
bb:M00239 Peptides/nickel transport system
ccc:M00011Citrate cycle second carbon oxidation 2-oxoglutarate => oxaloacetate
c:M00035 Methionine degradation
cc:M00349 Microcin C transport system
d: M0028 Ornithine biosynthesis glutamate_=> ornithine
dd:M00348 Glutathione transport system
e:M00025 Tyrosine biosynthesis chorismate => tyrosine
ee:M00245 Cobalt transport system
f: M00133 Polyamine biosynthesis arginine => agmatine =>_putrescine => spermidine
ff:M00317 Manganese/iron_transport_system
g:M00136 GABA biosynthesis prokaryotes putrescine => GABA
gg:M00276 PTS system mannose-specific II component
h:M00016 Lysine biosynthesis succinyl-DAP pathway aspartate => lysine
hh:M00278 PTS system sorbose-specific II component
i:M00019 Valine/isoleucine biosynthesis pyruvate => valine/2-oxobutanoate => isoleucine
ii:M00277 PTS system N-acetylgalactosamine-specific II component
j:M00026 Histidine biosynthesis PRPP => histidine M00026
k:M00124 Pyridoxal biosynthesis erythrose-4P => pyridoxal-5P
l:M00125 Riboflavin biosynthesis GTP => riboflavin/FMN/FAD
m:M00127 PTS system N-acetylgalactosamine-specific II componentI
n:M00119 Pantothenate biosynthesis valine/L-aspartate => pantothenate
o:M00123 Biotin biosynthesis pimeloyl-ACP/CoA => biotin
p:M00115 NAD biosynthesis aspartate => NAD
q:M00116 Menaquinone biosynthesis chorismate => menaquinone
u:M00117 Ubiquinone biosynthesis prokaryotes chorismate => ubiquinone
v:M00126 Tetrahydrofolate biosynthesis GTP => THF
w:M00048 Inosine monophosphate biosynthesis PRPP + glutamine => IMP
x:M00050 Guanine ribonucleotide biosynthesis IMP => GDPGTP
y:M00049 Adenine ribonucleotide biosynthesis IMP => ADPATP
z:M00053 Pyrimidine deoxyribonuleotide biosynthesis CDP/CTP => dCDP/dCTPdTDP/dTTP

C
T
W
M00239_Peptides_nickeltransportsystem
M00051_Uridinemonophosphateb [..]thesis_glutamine__PRPP___UMP
M00022_Shikimatepathway_phos [..]ate_erythrose_4P__chorismate
M00006_Pentosephosphatepathw [..]ephase_glucose6P__ribulose5P
M00164_ATPsynthase
M00157_F_typeATPase_bacteria
M00004_Pentosephosphatepathway_Pentosephosphatecycle_
M00002_Glycolysis_coremoduleinvolvingthree_carboncompounds
M00048_Inosinemonophosphatebiosynthesis_PRPP_glutamine__IMP
M00007_Pentosephosphatepathw [..]vephase_fructose6P__ribose5P
M00237_Branched_chainaminoacidtransportsystem
M00178_Ribosome_bacteria
M00322_Neutralaminoacidtransportsystem
M00323_Ureatransportsystem
M00321_Bicarbonatetransportsystem
M00163_PhotosystemI
M00161_PhotosystemII
M00167_Reductivepentosephosp [..]ycle_glyceraldehyde_3P__RuBP
M00277_PTSsystem_N_acetylgalactosamine_specificIIcomponent
M00166_Reductivepentosephosp [..]_RuBP_CO2__glyceraldehyde_3P
M00165_Reductivepentosephosphatecycle_Calvincycle_
M00125_Riboflavinbiosynthesis_GTP__riboflavin_FMN_FAD
M00123_Biotinbiosynthesis_pimeloyl_CoA__biotin
M00003_Gluconeogenesis_oxaloacetate__fructose_6P
M00001_Glycolysis_Embden_Meyerhofpathway__glucose__pyruvate
M00119_Pantothenatebiosynthe [..]ne_L_aspartate__pantothenate
M00115_NADbiosynthesis_aspartate__NAD
M00028_Ornithinebiosynthesis_glutamate__ornithine
M00011_Citratecycle_secondcarbonoxidation
M00009_Citratecycle_TCAcycle_Krebscycle_
M00126_Tetrahydrofolatebiosynthesis_GTP__THF
M00144_ComplexI_NADHdehydrogenase__NADHdehydrogenaseI
M00149_ComplexII_succinatede [..]tase__succinatedehydrogenase
M00207_Multiplesugartransportsystem
M00026_Histidinebiosynthesis_PRPP__histidine
M00222_Phosphatetransportsystem
M00050_Guaninenucleotidebiosynthesis_IMP__GDP_dGDP_GTP_dGTP
M00311_2_oxoglutarate_ferredoxinoxidoreductase
M00049_Adeninenucleotidebiosynthesis_IMP__ADP_dADP_ATP_dATP
M00360_Aminoacyl_tRNAbiosynthesis_prokaryotes
M00359_Aminoacyl_tRNAbiosynthesis_eukaryotes
M00053_Pyrimidinedeoxyribonu [..]CDP_CTP__dCDP_dCTP_dTDP_dTTP
M00016_Lysinebiosynthesis_aspartate__lysine
M00335_Sec_secretion_system
M00156_ComplexIV_Cytochromec [..]cytochromecoxidase_cbb3_type
M00200_Sorbitol_mannitoltransportsystem
M00019_Leucinebiosynthesis_p [..]e__2_oxoisovalerate__leucine
M00174_Methaneoxidation_methylotroph_methane__CO2
M00116_Menaquinonebiosynthesis_chorismate__menaquinone
M00276_PTSsystem_mannose_specificIIcomponent
M00331_TypeIIgeneralsecretionsystem
M00197_Putativesugartransportsystem
M00198_sn_Glycerol3_phosphatetransportsystem
M00045_Histidinedegradation_ [..]ormiminoglutamate__glutamate
M00061_Uronicacidmetabolism
M00194_Maltose_maltodextrintransportsystem
M00117_Ubiquinonebiosynthesi [..]yotes_chorismate__ubiquinone
M00245_Cobalttransportsystem
M00025_Tyrosinebiosynthesis_chorismate__tyrosine
M00185_Sulfatetransportsystem
M00064_ADP_L_glycero_D_manno_heptosebiosynthesis
M00124_Pyridoxalbiosynthesis_erythrose_4P__pyridoxal_5P
M00133_Polyaminebiosynthesis [..]tine__putrescine__spermidine
M00060_Lipopolysaccharidebiosynthesis_KDO2_lipidA
M00035_Methioninedegradation
M00150_ComplexII_succinatede [..]reductase__fumaratereductase
M00225_Lysine_arginine_ornithinetransportsystem
M00317_Manganese_irontransportsystem
M00219_AI_2transportsystem
M00202_Oligogalacturonidetransportsystem
M00127_Thiaminebiosynthesis_AIR__thiamine_P_thiamine_2P
M00220_Rhamnosetransportsystem
M00278_PTSsystem_sorbose_specificIIcomponent
M00349_MicrocinCtransportsystem
M00336_Twin_argininetranslocation_Tat_system
M00034_Methioninesalvagepathway
M00300_Putrescinetransportsystem
M00230_Glutamate_aspartatetransportsystem
M00348_Glutathionetransportsystem
M00302_2_Aminoethylphosphonatetransportsystem
M00229_Argininetransportsystem
M00226_Histidinetransportsystem
M00324_Dipeptidetransportsystem
M00136_GABAbiosynthesis_prokaryotes_putrescine__GABA
M00017_Methioninebiosynthesi [..]tate__homoserine__methionine
M00326_RTXtoxintransportsystem
M00260_DNApolymeraseIIIcomplex_bacteria
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
LDA SCORE (log 10)