Host and Microbiome Genetic Diversity in Callithrix jacchus and other Brazil Marmosets

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9/18/2018

Callithrix

- 6 allopatric species (***C.aurita***, *C. geoffroyi*, *C. jacchus*, *C. penicillata*, ***C. flaviceps***, ***C. kuhlii***)
- The *Callithrix* genus is less than 2.5 million years old (Perelman et al., 2011; Malukiewicz et al., 2017), and marmoset species show incomplete, but variable levels of reproductive isolation (Coimbra-Filho et al., 1993)
- Marmosets hybridize naturally and anthropogenically

A. C. *aurita*, B. C. *penicillata*, C. C. *jacchus*

The Natural Distribution of *Callithrix*

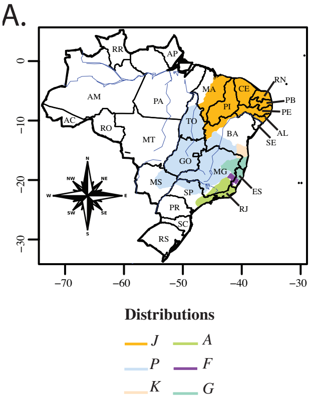


Figure 1: Malukeiwicz, in press

Natural (B) and Anthropogenic (C) Hybridization in *Callithrix*

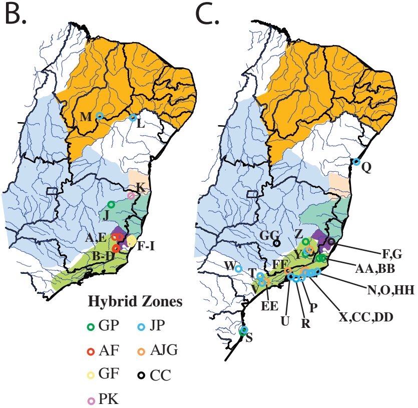


Figure 2: Malukiewicz, in press

Sampling for Low Coverage Whole Genome Data

Species	No	Origin
C. aurita	6	MG SP RJ
C. jacchus	7	Guarulhos Zoo/SP CEMAFAUNA/PE
C. geoffroyi	5	MG ES CPRJ/RJ
C. penicillata	8	DF MG PE

Population Structure of *Callithrix*

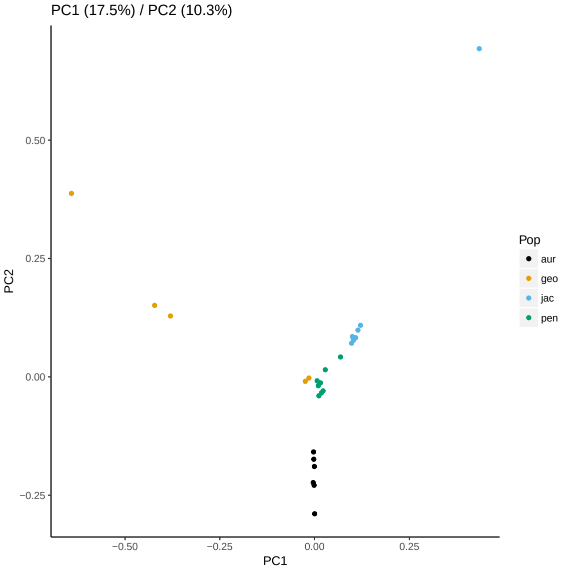


Figure 3: PCA of Low Coverage Whole Genome Sequence

Whole Genome Nucleotide Diveristy *C. jacchus*

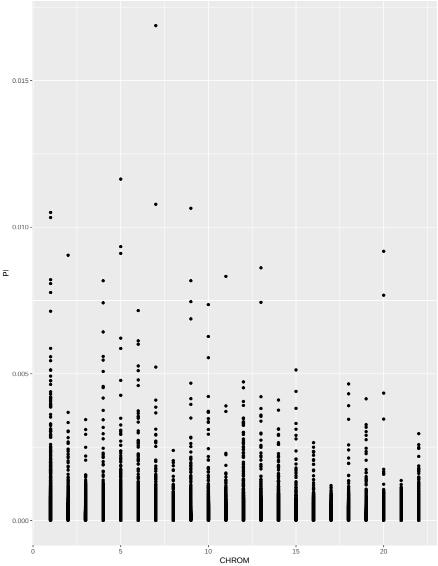


Figure 4: Whole Genome Nucleotide Diversity

Whole Genome Nucleotide Diveristy *C. penicillata*

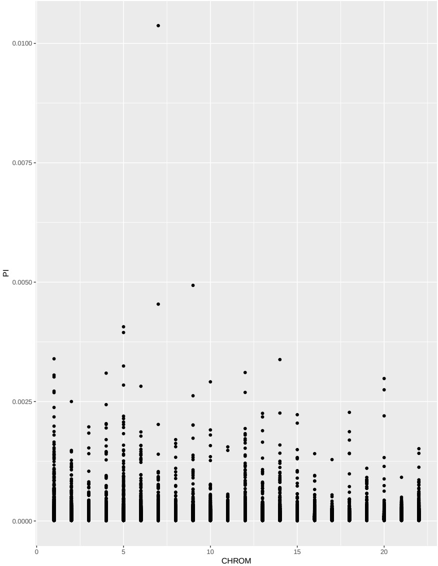


Figure 5: Whole Genome Nucleotide Diversity

Whole Genome Nucleotide Diveristy *C. aurita*

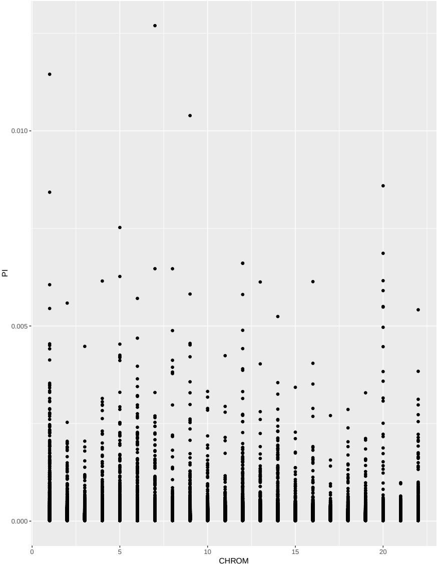


Figure 6: Whole Genome Nucleotide Diversity

Whole Genome Nucleotide Diveristy *C. geoffroyi*

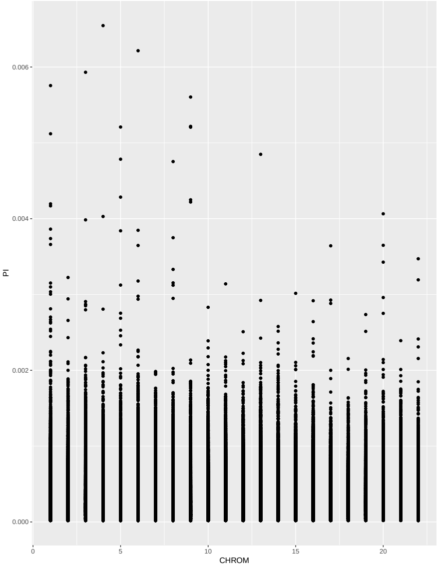


Figure 7: Whole Genome Nucleotide Diversity

Heterozygosity of Brazilian *C. jacchus*

INDV	O.HOM.	E.HOM.	N_SITES	F
BJT87	577478	414968.5	727465	0.52004
BJT86	566762	414914.0	727372	0.48598
BJT100	594487	414888.6	727319	0.57484
BJT157	349177	414854.3	727289	-0.21021
BJT165	594597	414955.5	727445	0.57487
BJT166	596660	414974.3	727470	0.58140
BJT167	585228	415012.9	727548	0.54463

Heterozygosity of Brazilian *C. penicillata*

INDV	O.HOM.	E.HOM.	N_SITES	F
BJT41	135590	105850.4	184885	0.37629
cpe025	163542	105963.1	185062	0.72794
BJT160	167398	105971.7	185092	0.77637
BJT8	146757	105873.5	184915	0.51724
cpe030	131277	105844.4	184867	0.32184
BJT40	154217	105877.3	184927	0.61151
BJT10	173637	106048.2	185207	0.85384
BJT159	151847	105916.8	184993	0.58083

Heterozygosity of Brazilian *C. aurita*

INDV	O.HOM.	E.HOM.	N_SITES	F
BJT107	373513	267194.3	467604	0.53051
BJT6	347774	267099.5	467422	0.40272
BJT65	361034	267142.0	467506	0.46861
BJT83	401317	267187.1	467571	0.66936
BJT114	387977	267147.0	467510	0.60306
BJT109	362619	267166.9	467556	0.47633

Heterozygosity of Brazilian *C. geoffroyi*

INDV	O.HOM.	E.HOM.	N_SITES	F
BJT102	1908466	1111558	1982879	0.91460
BJT170	892743	1111558	1982879	-0.25113
BJT171	902671	1111558	1982879	-0.23974
BJT169	923617	1111558	1982879	-0.21570
BJT143	1812546	1111558	1982879	0.80451

The Gut Microbiome

- The role of the gut microbiome in host physiology, nutrition, and health has been studied widely in humans (e.g., The Human Microbiome Project Consortium)
- Similar work in non-human primates (NHPs) and other animals is increasing (e.g., McKenna et al., 2008; Clayton et al., 2016; McKenzie et al., 2018).
- These various studies show that host taxaonomy, diet, and lifestyle/environment are key factors that influence gut microbiome abundance and composition.

Obligate Exudivory and Microbiomes

- A number of NHPs, including *Callithrix* marmosets are obligate exudivores (Cabana et al., 2018)
- They subsist on indigestible oligosaccarides of tree gums or hardened saps as a large part of their diet
- Studies of their gut microbiome the wild and captivity are rare
- Thus, gut microbioata assemblages in exudivore NHPs are not as well understood as that for other NHP dietary strategies.
- What are the effects of host taxonomy, hybridizaion, and habitat on the *Callithrix* gut microbiome?

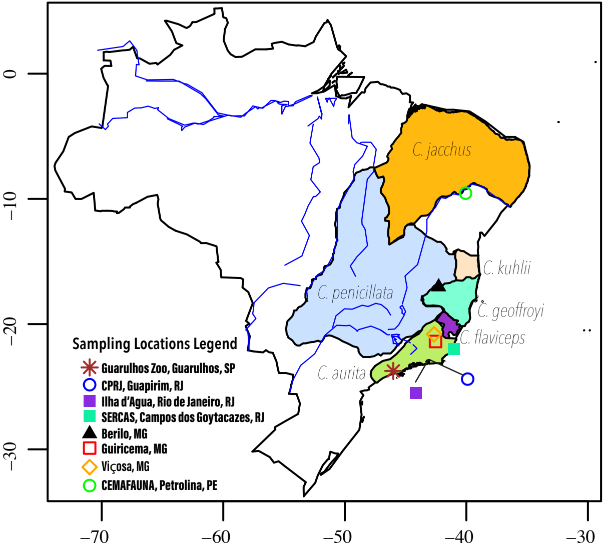
Methodology

- Anal swabs were collected between 2015 and 2016 from 59 individuals comprising four species and three hybrid types
- Bacterial DNA extraction from *Callithrix* anal swabs followed a modified version the phenol-chloroform-based protocol of Zoetendal et al. (2005).
- V4 region of the bacterial 16S rRNA gene was amplified in triplicate using the barcoded primer set 515f/806r
- PCR amplicons were sequenced on an Illumina MiSeq for 2x250 cycles
- Qiime2 (Caporaso et al., 2010) was used to analyze microbiomal alpha diversity, beta diveristy, and differential abundance analysis
- PICRUST 1.1.3 (Langille et al., 2013), HUManN 0.99 (Abubucker et al., 2012), LEfSe (Segata et al., 2010), GraPhlAn 0.9 (Asnicar et al., 2015), and STAMP v2.1.3(Parks et al. 2014) were used to analyzed predictive functional profiles of the 16S rRNA data

Sampling Totals

Classification	Count
C. aurita	10
C. geoffroyi	4
C. jacchus	9
C. penicillata	7
C. jacchus x C. penicillata	23
C. penicillata x C. geoffroyi	5
C. aurita hybrid	1

Sampling Locations



Results

- 1,1123,817 sequence reads were obtained with an average of 188,539 (+/- 129,170 SD) reads per sampled marmoset
- After quality filtering, a total of 9,063,712 reads remained within an 15,3622 (103648 +/- SD) average reads per sample
- Merging of paired-end sequences gave a total of 843,615 merged reads, with 142,891 (96076 +/- SD) reads per sample

Beta Diversity - Weighed UniFrac

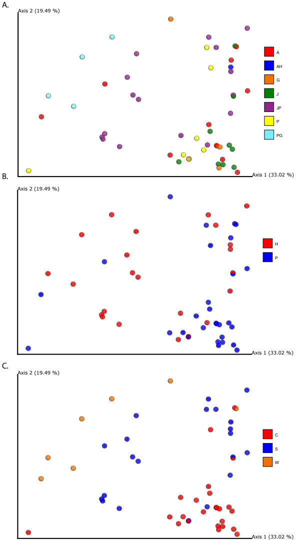


Figure 9: Weighed Unifrac PCoA by A. host taxonomy, B. hybrid status, C. and habitat

Effects of Host Taxonomy, Hybridization, and Habitat on Differential Abundances of Gut Bacterial Taxa

X	mse	Rsquared	R2diff
Intercept	25.8818	0.2362	0.0153
species[T.AH]	25.7934	0.2388	0.0127
species[T.G]	25.8623	0.2367	0.0147
species[T.J]	26.1926	0.2270	0.0245
species[T.JP]	25.9248	0.2349	0.0166
species[T.P]	26.0994	0.2297	0.0217
species[T.PG]	25.7753	0.2393	0.0121
hybrid_status[T.P]	25.7929	0.2388	0.0127
captive[T.S]	26.5108	0.2176	0.0339
captive[T.W]	26.3902	0.2212	0.0303

Alpha Diversity for Host Habitat

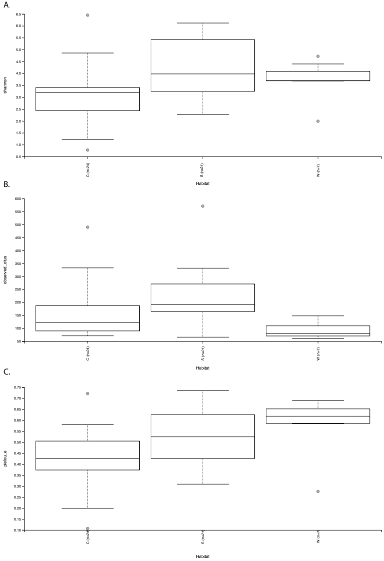
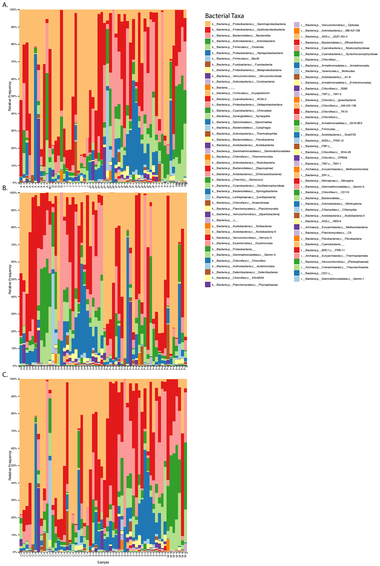


Figure 10: Boxplots of A. Shannon diversity index B. OTUs, and C. Pielou E

Relative abundances of gut bacterial classes by host taxon, hybrid status, and habitat



Microbiome Family-Level Taxanomic Composition by Host Habitat

- Captive hosts are dominated by Enterobacteriaceae (class Gammaproteobacteria)
- Semi-captive hosts are dominated by Helicobacteraceae and Campylobacteraceae (class Epsilonproteobacteria), Bacteroidaceae (class Bacterioidia),and Sphingomonadaceae (class Alphaproteobacteria)
- Wild hosts are dominated by Helicobacteraceae and Campylobacteraceae, Bacterioidia (family undetermined), Bifidobacteriaceae (class Actinobacteria), and Veillonellaceae (class Clostridia)

Examples of Core KEGG Modules Among Marmoset Hosts

- 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

Metabolic reconstruction of marmoset gut microbiome by host habitat

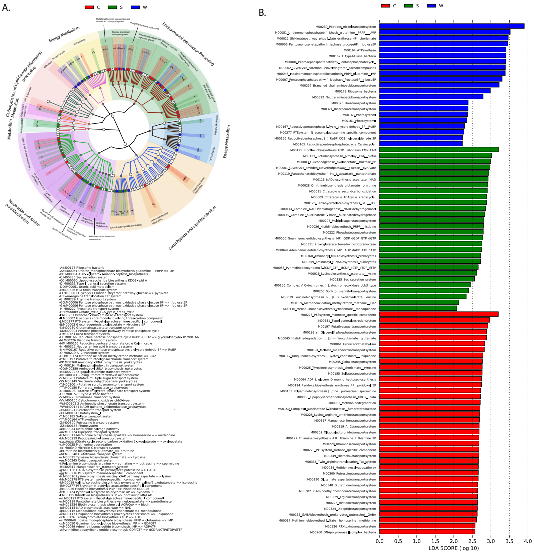


Figure 11: A: The cladogram in Category 3 KEGG BRITE hierarchical level

Captive Marmoset Diet

- 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

Wild Marmoset Diet

- Native *C. aurita* devoted feeding rime to gums (50.5%), fruits (11%), and animal prey (38.5%)(Martins and Setz, 2000)
- Exotic *C. penicillata* predominantly fed on animal prey (70%), followed by exudate (16%), fruit (8%), and supplemental (5%) (Zago et al., 2013.
- Native *C. jacchus* ate 83.4% plant species, animal protein mostly from insects (16.6%), and the diet was based almost exclusively on gums (Pinheiro and Pontes, 2015).
- Native *C. penicillata* in Goiânia, GO used a total to of 109 individual exudates sources belonging to 15 tree species in 10 families. *Tapirira guianensis* (Anacardiaceae) and *Croton urucurana* (Euphorbiaceae) were the species most used by marmosets (75%) (Lamoglia, 2015)