Original Article

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The Gut Microbiome of Wild Lemurs: A Comparison of Sympatric *Lemur catta* and *Propithecus verreauxi*

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Key Words

Diet · Feeding physiology · *Lemur catta* · *Propithecus verreauxi* · Microbiome · Bacteria, gastrointestinal · Gut flora · Fecal microbes · Dietary adaptation

Abstract

Mammalian gut microbes are invaluable to the host's metabolism, but few researchers have examined gut microbial dynamics under natural conditions in wild mammals. This study aims to help fill this knowledge gap with a survey of the natural variation of the gut microbiome in 2 wild lemur species, *Lemur catta* and *Propithecus verreauxi*. The wild *L. catta* were also compared to a captive population to discern the effect of habitat within a species. Gut microbial DNA was extracted from fecal samples collected in Madagascar and the Vienna Zoo and sequenced. The wild and captive *L. catta* had distinct microbial communities, likely due to differences in diet and development between their populations. The wild *L. catta* and *P. verreauxi* also had distinct gut microbiomes, due to a change in microbial abundance, not composition. Within each lemur species, there was abundant variation between individuals and from the dry to the wet season. The intraspecific and temporal microbial variation requires more investigation, with changes in diet a likely contributor.

Introduction

The gut microbiome is an incredibly variable community of mostly bacteria and archaea [Rajilić-Stojanović, 2007]. Different mammalian species harbor unique microbial assemblages, due to their diets and phylogeny [Ley et al., 2008; Muegge et al., 2011]. Importantly, herbivores' gut microbes supplement the enzymes necessary for digesting plant polysaccharides, which they lack endogenously [Stevens and Hume, 1998].

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In humans, while changing diet affects the gut flora, geography also has an influence, with distant populations having distinct gut communities [Yatsunenko et al., 2012; David et al., 2014]. Within a population there can be large interindividual gut microbial variation, attributed to diet or environment [Yatsunenko et al., 2012], as well as intraindividual temporal variation [Costello et al., 2009].

Most gut microbiome research is on humans due to health implications, yet several primate gut microbiomes have also been investigated, revealing species-specific gut microbial communities [Eckburg et al., 2005; Frey et al., 2006; Uenishi et al., 2007; Ley et al., 2008; Ochman et al., 2010; Xu et al., 2010; Yildirim et al., 2010; Degnan et al., 2012; Moeller et al., 2012; McCord et al., 2013]. Comparisons of captive and wild populations of primate species have also found significant interpopulation gut microbial differences [Amato et al., 2013].

The gut microbiome can be altered by diet and environment, yet this variation in mammals has been thoroughly studied only in captive animals. To better comprehend the natural variation of the gut microbiome, long-term studies of wild populations are necessary. I compared wild and captive populations of *Lemur catta* to understand if captive gut microbiomes are representative of those in their wild counterparts. I then catalogued the variability in 2 wild lemur species, *L. catta* and *Propithecus verreauxi*. These sympatric species overlap in environmental exposure to microbes, yet their distinct diets may cause their gut microbiomes to be divergent. The goal of this study was to determine the natural inter- and intraspecific variation in the gut microbial communities in these lemur species over time. I hypothesized that the interspecific gut microbial changes would be greater than the intraspecific changes in *L. catta* and *P. verreauxi* and that the gut microbial composition would change seasonally in both lemur species. I additionally hypothesized that there would be prominent differences between the wild and captive *L. catta* populations' gut microbiomes.

Methods

The ring-tailed lemur *(L. catta)* and Verreaux's sifaka *(P. verreauxi)* are strepsirrhines endemic to Madagascar. *P. verreauxi* is predominantly a folivore [Richard et al., 2002; Rajilić-Stojanović, 2007], while *L. catta* is an opportunistic omnivore [Sauther et al., 1999; Ley et al., 2008; Muegge et al., 2011]. The Bezà Mahafaly Special Reserve is a deciduous tropical dry forest in southwest Madagascar with a habitat gradient from gallery forest in the east to xeric habitat in the west [Sussman and Rakotozafy, 1994; Stevens and Hume, 1998; Sussman et al., 2012]. The Bezà Mahafaly Special Reserve is highly seasonal, with a single wet season (October to April) and a single dry season (May to September) annually [Sauther and Cuozzo, 2009] and contains sympatric populations of *L. catta* and *P. verreauxi* [Sussman, 1991].

Noninvasive fecal sampling is a good analog to direct sampling of the gut microbiome [Eckburg et al., 2005; Costello et al., 2009]. Feces were collected from 6 wild *L. catta* and 6 *P. verreauxi* individuals at Bezà Mahafaly Special Reserve at 3 time points (during the dry season, at the onset of the wet season and late into the wet season) from October 2011 through February 2012 for a total of 18 samples per species (online suppl. table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000369971). To evaluate intraspecific microhabitat effects, samples were collected from a western (*L. catta* Blue and *P. verreauxi* Fano) and an eastern group (*L. catta* Red and *P. verreauxi* Vavy) of each species, then preserved in 90% ethanol [Frantzen et al., 1998]. Five fecal samples were collected from captive *L. catta* at the Vienna Zoo (Austria) in November 2012 and preserved in ethanol, though identification of which lemur provided each fecal sample was not possible.

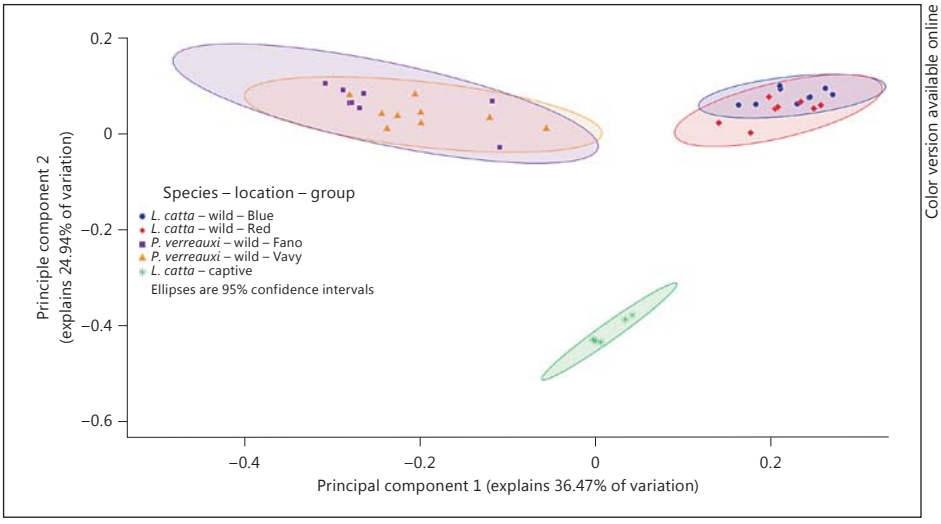

Fig. 1. Gut microbial β-diversity by species and location.

DNA extraction, PCR and genetic sequencing followed the protocols of the Earth Microbiome Project (http://www.earthmicrobiome.org/emp-standard-protocols/). PCR primers (515 forward/806 reverse) targeted the V4 region of the microbial 16S rDNA. The amplicons were sequenced with the Illumina HiSeq 2500 platform.

The sequence data were analyzed using the Quantitative Insights into Microbial Ecology pipeline [Eckburg et al., 2005; Frey et al., 2006; Uenishi et al., 2007; Ley et al., 2008; Caporaso et al., 2010; Xu et al., 2010; Yildirim et al., 2010], version 1.7.0. Quality filtering excluded sequences with a Phred quality score below 3. Chimeric sequences were detected in the quality-filtered sequences using USEARCH 6.1 with a 97% operational taxonomic unit (OTU) match to the October 2012 Greengenes database [DeSantis et al., 2006] and then removed. OTUs were picked using a combined reference- and de novo-based approach.

The β-diversity is a measure of the overlap among different microbial communities based on shared taxa and was calculated at a depth of 15,824 sequences. Samples were clustered together into unweighted pair group method with arithmetic mean (UPGMA) trees using the β-diversity distance matrices. The rarefied consensus UPGMA tree was used to create a sample clustering dendrogram. Principal component analysis used unweighted UniFrac distances (a β-diversity metric) [Lozupone and Knight, 2005] to show the overlap between two groups' samples. The microbial abundances of each population and season were compared using a Mann-Whitney U test [Mann and Whitney, 1947], since the data are nonparametric, with a Bonferroni correction for multiple comparisons [Remis et al., 2001].

Permits were obtained for fecal collection and export, and the research was approved by the Institutional Animal Care and Use Committee at the University of Southern California.

Results

Captive and Wild L. catta

The captive and wild *L. catta* samples yielded 34,243,832 sequences grouped into 1,933 unique OTUs. Of these OTUs, 32.3% were unique to the wild *L. catta*, with 5.8%

exclusive to the captive *L. catta*, and 61.9% discovered in both populations (online suppl. fig. 1). Samples from wild and captive *L. catta* could be unambiguously differentiated in the UPGMA dendrogram (online suppl. fig. 2). Using principal component analysis, the wild and captive *L. catta* clearly separate from one another, with no overlap between their 95% confidence intervals (fig. 1).

At the phylum level, there are clear microbial abundance differences between the wild and captive *L. catta* (fig. 2a), with significantly more *Firmicutes*, *Actinobacteria* and *Euryarchaeota* in the wild *L. catta* and significantly more *Bacteroidetes* and *Spirochaetes* in the captive *L. catta* (fig. 2b). There were much greater intersample differences among the wild population (fig. 3), though the captive samples varied greatly in *Proteobacteria* and *Spirochaetes* abundance.

Wild L. catta and P. verreauxi

The Madagascar samples yielded 43,729,047 sequences grouped into 1,973 unique OTUs. 12.8% of these OTUs were unique to *L. catta*, with 7.7% exclusive to *P. verreauxi*, and 79.5% discovered in both species (online suppl. fig. 1). Samples from *L. catta* and *P. verreauxi* could be unambiguously differentiated in the UPGMA dendrogram (online suppl. fig. 2). Within each species, there was abundant overlap between groups. Using principal component analysis, the *P. verreauxi* and wild *L. catta* samples clearly separate from one another, with no overlap between their 95% confidence intervals (fig. 1). Again, within each species, there was large overlap between the groups.

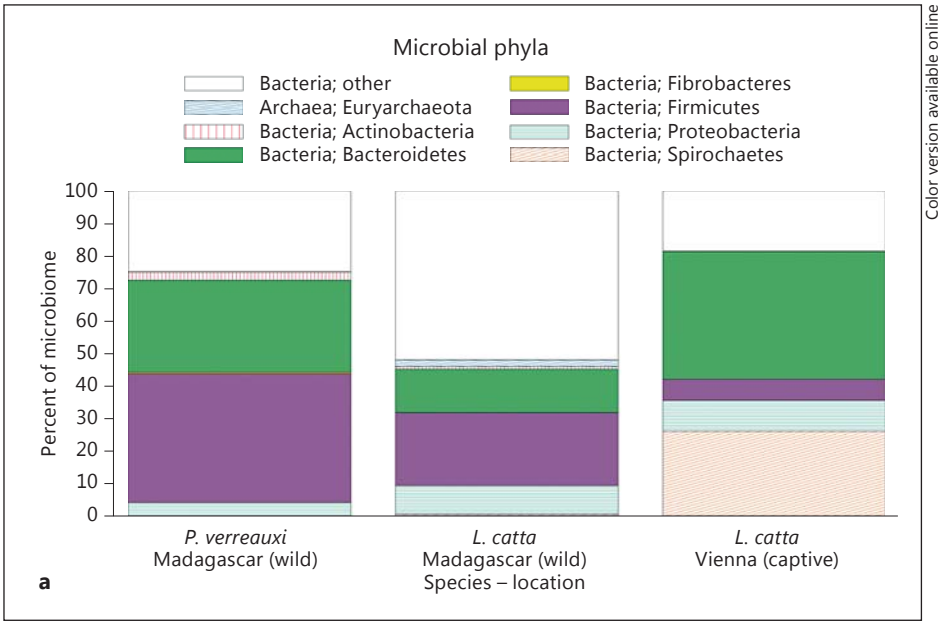

Fig. 2. a Average gut microbial abundance by species and location.

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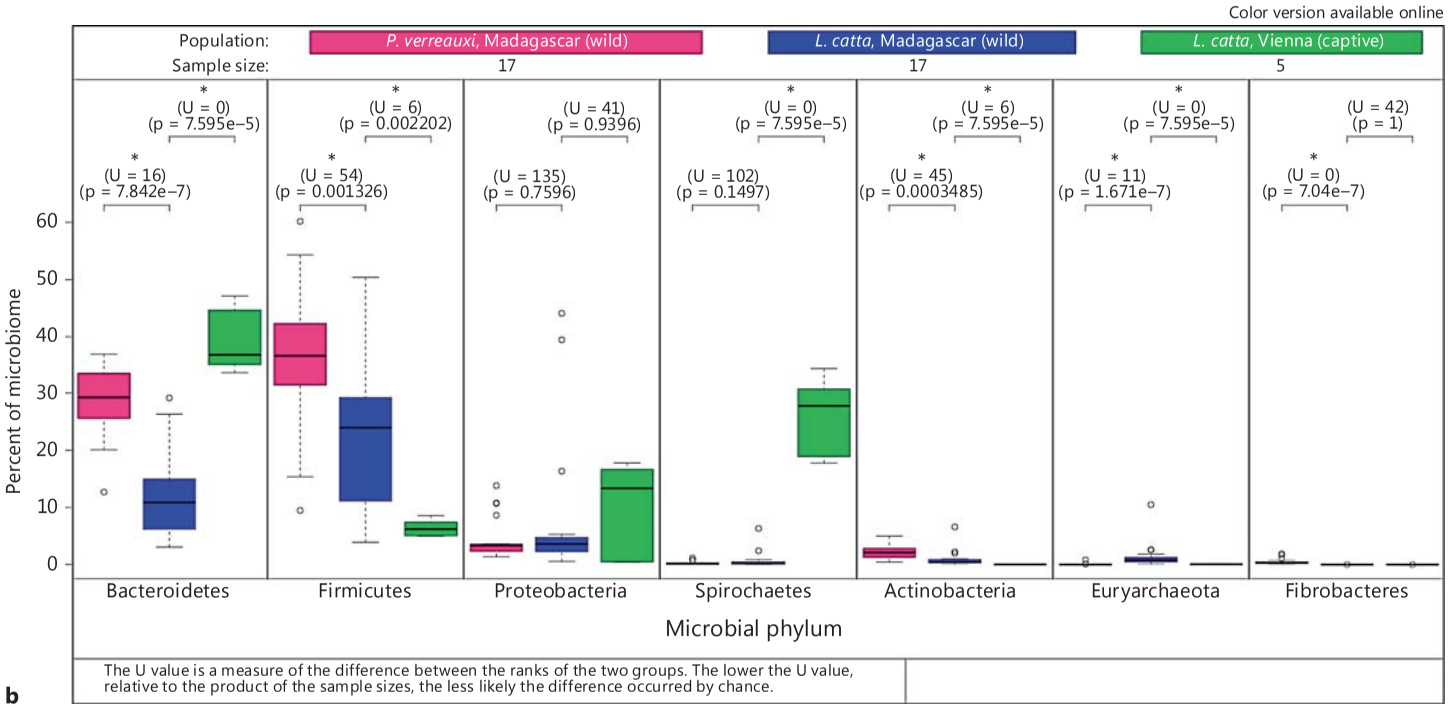

Fig. 2. b Average gut microbial abundance by species and location. The Mann-Whitney U test was used to compare the wild *L. catta* and *P. verreauxi* and to compare the wild and captive *L.* catta. * $p < 0.05$: statistically significantly different.

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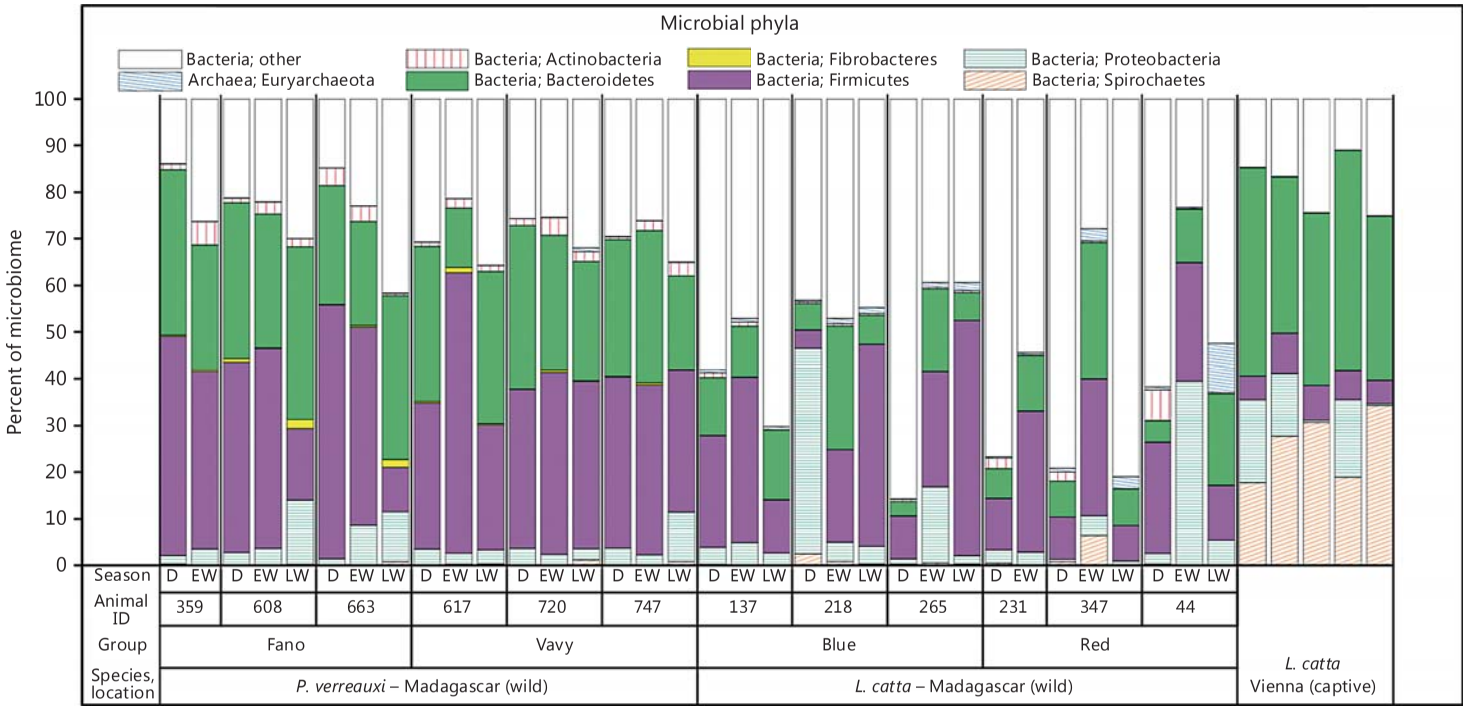

Fig. 3. Gut microbial abundance by sample. D = Dry season; EW = early wet season; LW = late wet season.

At the phylum level, there are clear differences between the average microbial abundances of the wild *L. catta* and *P. verreauxi* populations (fig. 2a), with significantly more *Bacteroidetes*, *Firmicutes* and *Actinobacteria* in *P. verreauxi* and significantly more *Proteobacteria* and *Euryarchaeota* in *L. catta* (fig. 2b). There were noticeable intersample differences within each population, with more variability in the *L. catta* than in *P. verreauxi* (fig. 3). In particular, the abundance of *Firmicutes* varied widely. The intersample microbial differences appeared to be due to seasonal changes more than consistent interindividual differences, with intraspecific samples clustering by time rather than by individual (online suppl. fig. 2).

Seasonally, there were diverse changes in different microbial phyla between the wild lemur populations (fig. 4a, b). The abundances of the 2 dominant phyla, *Bacteroidetes* and *Firmicutes*, both increased from the dry to wet season in *L. catta*, while these phyla decreased in abundance in *P. verreauxi* over the same period. Both lemur species showed an increased prevalence of *Euryarchaeota* in their gut microbiomes in the wet season. *P. verreauxi* also had increased populations of *Actinobacteria* and *Spirochaetes* in its gut in the wet season. *Actinobacteria* showed the reverse trend in *L. catta*, with a decrease in abundance from the dry to the wet season.

Discussion

Wild and Captive L. catta

The large separation between the gut microbiomes of wild and captive *L. catta* was reflected in their microbial abundances. This finding supports my hypothesis of large interpopulation gut microbial differences. This suggests that the environment has a strong influence on the composition of these gut microbial communities. Similar population-specific differences have been observed between wild and captive populations [Uenishi et al., 2007; Nakamura et al., 2011]. Diet has also been shown to cause broad shifts in the gut flora [David et al., 2014]. While phylogeny significantly influences the gut microbiome [Ochman et al., 2010], diet [David et al., 2014] and development [Dominguez-Bello et al., 2011] also play strong roles in determining the community composition and structure within the gut. So, it is not surprising that geographically and environmentally separate populations of *L. catta* would contain highly distinct microbiomes.

The captive *L. catta* at the Vienna Zoo show a distinctive microbial profile compared to the captive population at the St. Louis Zoo (the only other sequenced *L. catta* gut microbiome) [Ley et al., 2008]. The disparity between these 2 captive populations is similar in scale to the difference between each of these populations to the wild *L. catta* analyzed here.

Wild L. catta *and* P. verreauxi

The clear gut microbial differences between the wild *L. catta* and *P. verreauxi* are attributed to microbial abundance (fig. 2a), not composition (fig. 1). This finding is consonant with my hypothesis and research on other wild primates, where the gut microbial communities are differentiated by host species above all other factors [Moeller et al., 2013]. Similar to other mammals [Ley et al., 2008], *Bacteroidetes* and *Firmicutes* dominated the wild lemur guts. The wild *L. catta* samples had twice as many unclassified bacteria, which may contribute to the lower overall levels of *Bacteroidetes* and *Fir-*

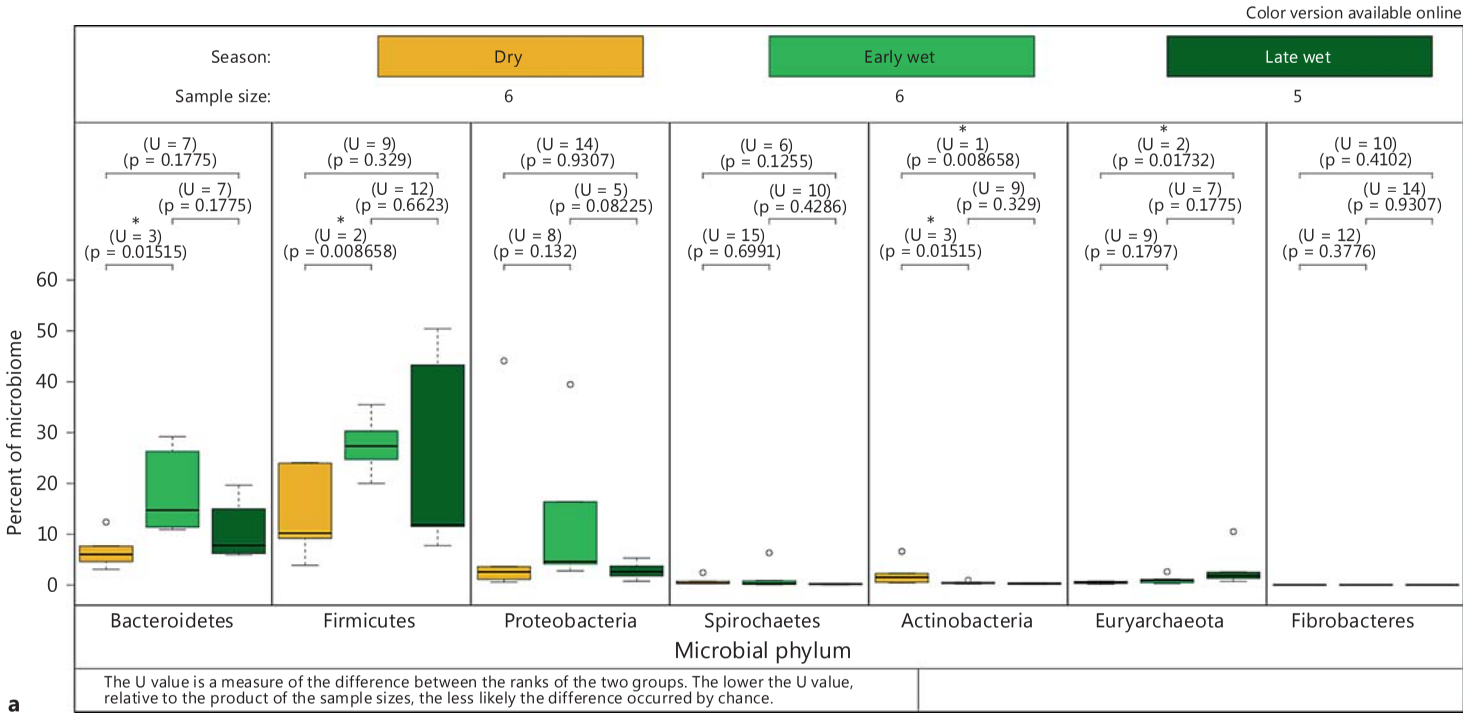

Fig. 4. a Average gut microbial abundance by season in wild *L. catta*. The Mann-Whitney U test was used to compare the wild *L. catta* gut microbial abundances in the dry season, the early wet season and the late wet season. * $p < 0.05$: statistically significantly different.

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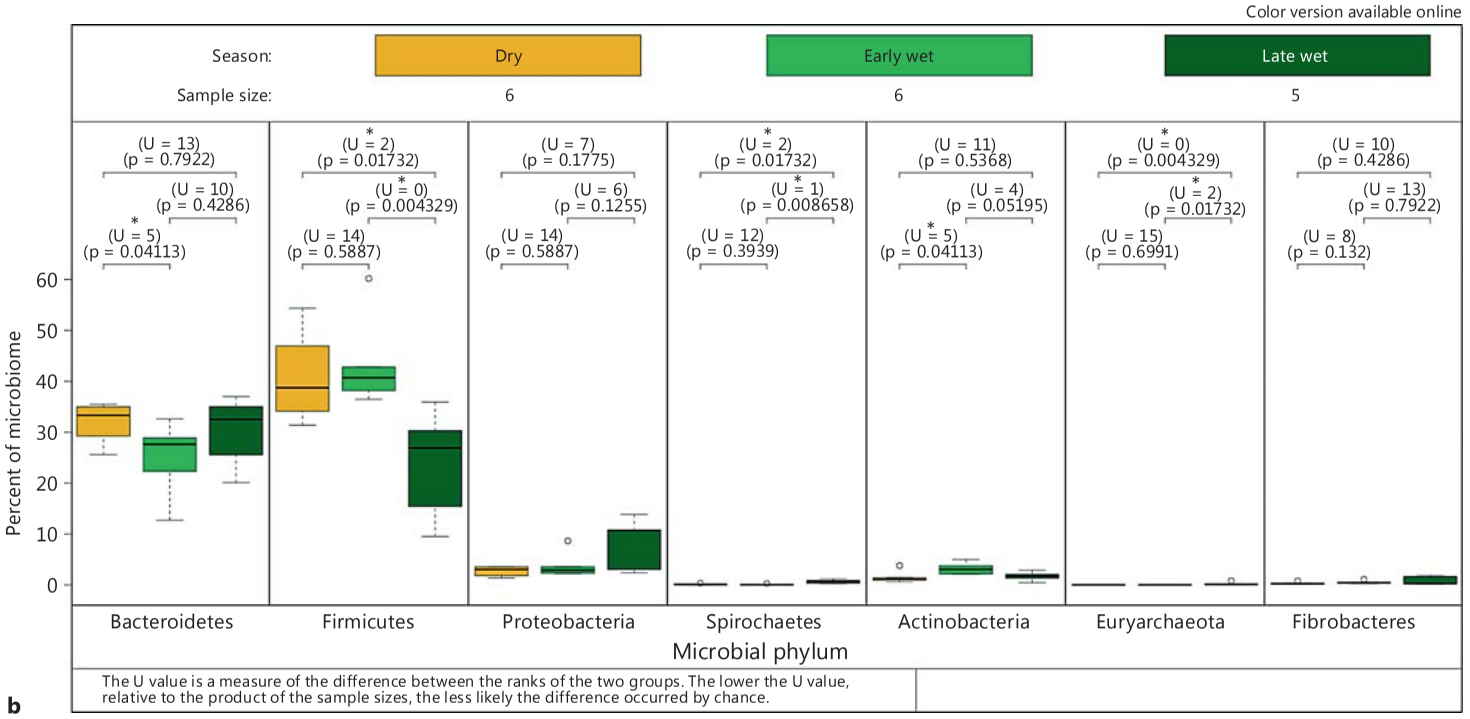

Fig. 4. b Average gut microbial abundance by season in wild *P. verreauxi*. The Mann-Whitney U test was used to compare the wild *P. verreauxi* gut microbial abundances in the dry season, the early wet season and the late wet season. * $p < 0.05$: statistically significantly different.

micutes in this population. Similar to the high endemism of plant and animal species in Madagascar [Myers et al., 2000], I suspect that there are endemic microbes on this island nation, consistent with microbial biogeographic theory [Martiny et al., 2006]. Due to the paucity of microbiome research in Madagascar, there are likely many uncharacterized endemic microbes, including several within the lemur gut.

The gut microbial communities are dynamic and highly variable between individuals and temporally (fig. 3, 4a, b). Interindividual microbial diversity is a natural feature of complex populations [Yatsunenko et al., 2012], so environmental factors, including diet, may only be able to explain part of the gut microbial variation. Despite this interindividual microbiome variability, each lemur species had a unique set of significant changes in the abundance of microbial phyla in their guts between the seasons. I posit that the gut microbial variability seen in wild *L. catta* and *P. verreauxi* is partly due to their seasonally shifting diets [Sauther, 1998], since short-term diet changes can cause reversible changes in the gastrointestinal flora [David et al., 2014]. Future studies are required to elucidate the detailed relationship between temporal changes, the gut microbial community and dietary shifts in wild primates.

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