Received: 15 May 2023 | Revised: 12 April 2024 | Accepted: 24 April 2024

DOI: 10.1002/edn3.559

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

WILEY

ORIGINAL ARTICLE

# Assessing the gut microbiome and the influence of host genetics on a critically endangered primate, the northern muriqui (*Brachyteles hypoxanthus*)

Naiara Guimaraes Sales[1] | Mariane da Cruz Kaizer[2,3] | Samuel S. Browett[4] | Sofia I. Gabriel[5,6] | Allan D. McDevitt[1,7]

[1]School of Science, Engineering and Environment, University of Salford, Salford, UK

[2]National Institute of the Atlantic Forest (INMA), Santa Teresa, Brazil

[3]Caparaó Muriqui Project, Rede Eco-Diversa Para Conservação da Biodiversidade, Tombos, Brazil

[4]Molecular Ecology Research Group, Eco-Innovation Research Centre, School of Science and Computing, Waterford Institute of Technology, Waterford, Ireland

[5]CESAM – Centro de Estudos Do Ambiente e Do Mar, Departamento de Biologia Animal, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal

[6]Departamento de Biologia da Universidade de Aveiro, Aveiro, Portugal

[7]Department of Natural Resources and the Environment, School of Science and Computing, Atlantic Technological University, Galway, Ireland

**Correspondence**
Naiara Guimaraes Sales, School of Science, Engineering and Environment, University of Salford, Salford, UK.
Email: n.guimaraessales@salford.ac.uk; naiarasl@gmail.com

## Abstract

The Northern muriqui (*Brachyteles hypoxanthus*) is one of the world's most critically endangered primates, with only ~1000 mature individuals remaining in the wild. Habitat loss and hunting have led to its sharp decline, making conservation efforts crucial. Analyses of gut microbiomes in wild populations can provide valuable information on host health and vulnerability, and ultimately, contribute to baseline knowledge toward improving conservation programs and reintroduction efforts. In this study, we analyzed the microbiome (16S rRNA metabarcoding) of fecal samples belonging to 53 uniquely genotyped individuals from three social groups from the Caparaó National Park, aiming to provide the first assessment of the microbiome diversity and composition for this species. Our results showed the muriqui gut microbiome was predominantly composed of the phyla Bacteroidetes and Firmicutes, with the dominant classes represented by Bacteroidia and Clostridia. High similarity in bacterial diversity and composition was found for individuals from distinct groups, suggesting a negligible geographical effect at the fine spatial scale analyzed. No significant effect of host genotype heterozygosity levels on microbiota diversity was recovered, but a significant influence of genetic distance on microbiota community structure and composition was demonstrated. Our findings stress the importance of considering associations between host genetics and the microbiome and suggest that the analyzed populations host a similar microbiome composition. This detailed microbiome assessment can aid conservation actions, including future anthropogenic impact assessments and animal reintroductions.

**KEYWORDS**
16S rRNA, amplicon sequencing, Atlantic Forest, bacteria, endemic, microbiota, threatened species

## 1 | INTRODUCTION

Recent assessments on global biodiversity have seen a steep rise in the number of endangered species, forecasting overwhelming and unprecedented extinction rates in the near future. According to the latest IUCN report, over 42,000 species are threatened with extinction (IPBES, 2019; IUCN, 2022). Habitat fragmentation and hunting are among the main threats to species survival, and in one of the world's biodiversity hotspots, the Atlantic Forest, the impact of these threats has pushed several species close to the brink of extinction (Ribeiro et al., 2009). Among these highly threatened species is the Northern muriqui (*Brachyteles hypoxanthus*), the largest extant species of the platyrrhine primates. This primate, endemic to the Brazilian Atlantic Forest, is currently one of the world's most critically endangered primates, with an estimated population of less than 1000 mature individuals left in only a dozen forest fragments (de Melo et al., 2021; Strier et al., 2017).

Contemporary conservation strategies, aiming at protecting threatened species, are crucial and have targeted multiple approaches including the creation of forest corridors, translocation of individuals to increase genetic diversity between populations, and ex-situ breeding programs (Chazdon et al., 2020; Franquesa-Soller et al., 2022a; Kierulff et al., 2012). Nevertheless, these efforts might be disrupted not only by the limited availability of habitat but also due to low success rates in both translocation actions and captivity/post-release scenarios related to animal reproduction and health (Beck, 2019; Campera et al., 2020).

Recently, the role of microorganisms in influencing animal health has led to a discussion of the importance of microbiome studies in threatened species conservation (West et al., 2019). Host-microbiome interactions are known to exert powerful influences on the health, physiology, behavior, and fitness of its host, and due to the complexity of these relationships, the host and its associated microbiota might often be considered metaorganisms (Bosch & McFall-Ngai, 2011; Lynch & Hsiao, 2022). The bacterial microbiome plays a key functional role within the host organism with many types of bacteria being present in the gut microbiota. These include commensal bacteria known to act on the host's immunological system (Forsythe & Bienenstock, 2010), beneficial bacteria which provide an essential function for health (e.g., acting on vitamin and short-chain fatty acids synthesis – McKenney et al., 2018), or the opportunistic ones that might be linked to the onset of diseases (West et al., 2019). Thus, fluctuations in microbiota composition might lead to host phenotype changes (Henry et al., 2021), with healthier individuals showing highly dynamic microbial communities whereas the onset or development of diseases may be due to dysbiosis of the microbiota (Gilbert et al., 2016; McKenzie et al., 2018).

Given the host-microbiome interaction, investigating the microbiome linked to the host's biological information and habitat might shed light on the responses to environmental changes, and subsequently direct required species management and conservation actions (Couch & Epps, 2022). In this context, microbiome research has great potential to improve conservation efforts and its application in a more ecological context might increase the understanding of the effect of several factors such as climate and land-use changes, infectious disease, and captivity and translocations on species' response and survival (Trevelline et al., 2019). These efforts combined might represent a turning point for conservation outcomes, much needed in the face of the current biodiversity crisis. Despite the current understanding of the microbiome's significance on species conservation, studies addressing its role remain mostly restricted to research on humans (The Integrative HMP Research Network Consortium, 2019), whereas the understanding of the microbiome in threatened species and its effect on host health, fitness and survival are still in their infancy (Bahrndorff et al., 2016; West et al., 2019). Therefore, there is an urge to gather novel noninvasive information, providing baseline knowledge to help inform management decisions and conservation action plans. Due to intense deforestation and fragmentation, connectivity between habitats can be severely impacted, leading to the isolation of entire populations or individuals. This is especially relevant to the Critically Endangered muriqui, with females being unable to disperse in search of new conspecific groups (i.e., due to the absence of suitable habitats or barriers to migration). Therefore, translocations are likely to be vital for the long-term persistence of this species (Oliveira, Murray, et al., 2020; Oliveira, Tabacow, et al., 2020). Among the few forest remnants known to harbor muriqui populations, the Caparaó National Park harbors newly identified groups of muriqui that were recently discovered, and it is now considered one of the key areas for this species' persistence (Kaizer et al., 2022).

In this study, we characterized the muriqui gut microbiome diversity and provided a comparison among different groups inhabiting both sides of the Caparaó National Park to evaluate the similarities and discrepancies in their microbiota composition. Additionally, to investigate the potential influence of host genetic diversity on the microbiome, the effect of individual homozygosity on the microbiota's alpha diversity and the influence of genetic distance on the bacterial community composition was analyzed. Considering the lack of knowledge about the microbiota of this and other endangered species in general, this study has the potential to advance management practices to increase the success of conservation actions and/or future animal translocations.

## 2 | MATERIALS AND METHODS

### 2.1 | The Northern Muriqui, *Brachyteles hypoxanthus*

Considered one of the most critically endangered primates on Earth, this highly social species is known to live in large cooperative groups of up to 100 individuals (Chaves et al., 2011). Their herbivorous diet consists primarily of fruits, leaves, and flowers and therefore, this species plays a crucial role in forest ecology by dispersing seeds through their dietary choices. Unusually for primates, females are known to disperse away from their natal group whereas

males exhibit philopatric behavior (Strier et al., 2006). Additionally, northern muriquis are arboreal and spend most of their lives in the treetops of the Atlantic Forest in Brazil, a habitat that is rapidly dwindling due to deforestation. Therefore, their conservation is a critical concern for preserving biodiversity in this region. Due to the extensive habitat loss and hunting pressure, the Northern muriqui experienced a sharp population decline of over 80% in the past 70 years (de Melo et al., 2021; Strier et al., 2017). With the northern muriquis facing increasing threats and their populations in critical condition, management and conservation efforts are more urgent than ever.

## 2.2 | Field sampling

Samples were collected at Caparaó National Park (CNP) (Southeastern Brazil, 20°37′–20°19′ S; 41°43′–41°55′ W) between 2018 and 2020. This protected area is characterized by the presence of a chain of mountains dividing the park into two areas, harboring unhabituated groups of the Northern muriqui (*B. hypoxanthus*) known to occur on both sides of the park. In this study, samples from three groups were analyzed, including one muriqui group inhabiting the western side of the park (Vale do Aleixo, VA, $n = 37$ samples), and two groups occurring in two different valleys on the eastern side of the park including Vale do Santa Marta (VSM, $n = 10$ samples) and Vale do Facão de Pedra (VFP, $n = 6$ samples) (Figure 1, Table S1). The vegetation for the western side of the park (i.e., VA site) is characterized by a seasonal semi-deciduous montane forest, whereas the eastern side (i.e., VSM and VFP sites) comprises a montane ombrophilous dense forest (Kaizer et al., 2022).

To characterize the gut microbiome of individuals from this critically endangered species, fecal samples were collected opportunistically at three different sites (Table S1), under an approved license (SISBIO 54795-1). All samples were collected noninvasively and fresh beneath animals that had recently defecated and were obtained without disturbing the animals. Upon collection, samples were immediately stored in RNAlater (Ambion) using equal volumes and kept at −20°C until shipment on dry ice to the UK before DNA extraction.

## 2.3 | Individual and population genetic analyses

All individuals included in the microbiome analyses were genotyped using a set of 14 nuclear microsatellite loci (see Table S2). DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen) following the human DNA analysis protocol. For full details of the genotyping protocol, see the Data S1 section.

Individual identity was estimated in CERVUS v 3.0.7 (Kalinowski et al., 2007). To ensure samples represented different individuals,

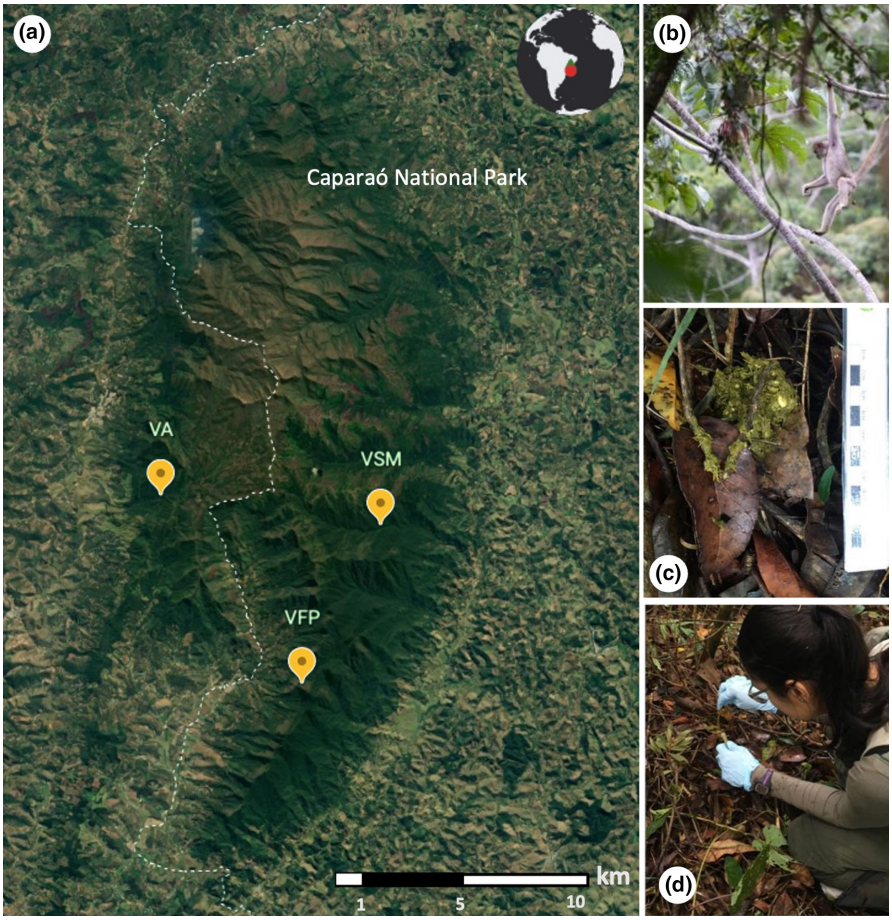


FIGURE 1 (a) Map of Caparaó National Park (CNP), Brazil, including locations of the study sites: Vale do Aleixo (VA), Vale do Santa Marta (VSM) and Vale do Facão de Pedra (VFP). (b) Study species (*Brachyteles hypoxanthus*), (c) Fresh fecal sample, and (d) Mariane da Cruz Kaizer undertaking noninvasive fecal sampling.

duplicates were identified by searching for matching genotypes using the probability of identity assuming a full-sibling relationship (PIDsib, <0.05) and removing genotypes if they matched at 10 or more loci, without mismatches.

Individual and social group-level observed heterozygosity ($H_o$) and homozygosity by loci (HL) were calculated using Cernicalin (Aparicio et al., 2006), and pairwise Nei's genetic distances were calculated in GenAlEx 6.5 (Peakall & Smouse, 2012), for all groups and all individuals.

## 2.4 | 16S rRNA amplicon sequencing

The characterization of bacterial communities was conducted based on the amplicon sequencing of ~250 bp of the 16S rRNA region using the 515F and 806R primers (Caporaso et al., 2011). Briefly, PCRs were conducted in duplicates in a final volume of 15 μL per reaction, using the Solis BioDyne 5× HOT FIREPol® Blend Master Mix (3 μL), 2 μM primers (4 μL), 2 μL of sample DNA. Thermocycler conditions were as described in Antwis et al. (2021): 95°C for 10 min; 25 cycles of 95°C for 30 s, 55°C for 20 s and 72°C for 30 s; and a final extension of 72°C for 8 min. Negative (PCR blanks, $N = 2$) and positive (ZymoBIOMICS Microbial Community DNA Standard, $N = 1$) controls were also included, totalling 56 samples. PCR replicates were combined and purified using HighPrepTM PCR clean-up beads (MagBio) using a 1:1.5× ratio, according to the manufacturer's instructions. PCR products were checked on an Agilent 2200 TapeStation, DNA concentration was measured by fluorometric analysis (Qubit, Thermo Fisher Scientific) and samples were equimolarly pooled (normalized to 30 ng per sample). The final library was sequenced using the Illumina platform (2 × 300 bp, MiSeq v3 kit) along with samples from an unrelated project.

## 2.5 | Bioinformatics and statistical analyses

Bioinformatics analyses were conducted in RStudio (R version 4.1.3), following the DADA2 workflow (Callahan et al., 2016; please see the Data S1 for detailed information on the filtering steps). The 16S rRNA gene amplicon sequencing yielded a total of 2,502,995 raw sequence reads, with a contig length of ~253 bp after merging paired-end reads. Sequence variants (SVs) with lengths >260 bp (0.004% of total sequences) along with chimaeras (0.031% of total reads) were removed. Taxonomic assignment was based on the SILVA v132 database (Quast et al., 2013; Yilmaz et al., 2014). DADA2 identified 10 unique SVs in the sequenced mock community sample including 8 bacterial isolates and two yeasts. ASVs that were not taxonomically assigned to the kingdom "Bacteria" (e.g., Archaea) or that were classified to the class "Chloroplast" or the family "Mitochondria" were removed from the dataset. No taxa-specific filter was applied, and therefore both transient and resident bacteria were included in the downstream analyses. After all filtering steps, the final SV table, taxonomy table and sample metadata were exported to the phyloseq package (McMurdie & Holmes, 2014), including a total of 1,954,164 reads and a mean of 34,394 reads per sample (ranging from 5950 to 61,008 reads/sample).

Statistical analyses and data visualization were performed in RStudio v461 using the phyloseq, vegan and ggplot2 packages (McMurdie & Holmes, 2014; Oksanen et al., 2018; R Core Team, 2021). Rarefaction curves were used to analyze the expected number of amplicon sequence variants (ASVs) detected per sample according to the sequencing depth. To account for differences in sequencing depth (Figure S1) and to allow comparisons with other studies, alpha – and beta-diversity analyses were computed through the traditional rarefaction method as in Baniel et al. (2021). The rarefied dataset was obtained at a subsampling depth of 12,300 reads and thus, one sample (<12,300 reads) was excluded from the downstream analyses. Accumulation curves were obtained for rarefied data, including all samples analyzed, to visualize the expected number of taxa detected according to the sampling size (Figure S2).

Considering the ongoing discussion on the use of rarefaction to adjust and account for discrepancies across samples (McMurdie & Holmes, 2014; Willis, 2019), an additional dataset was obtained using the clean count data (i.e., prior to rarefaction) and transformed using proportions (i.e., dividing the reads obtained for each ASV by the total number of reads in the sample, also known as Total Sum Normalization or TTS, McKnight et al., 2018). All analyses obtained from proportions transformed data are included and discussed in the Data S1.

To determine the diversity and composition of bacterial communities, bar plots of relative read abundance were obtained for Phyla and Class, and alpha and beta diversity indices were calculated at the ASV level. Alpha diversity was estimated using four indices (observed richness, Chao1, Shannon's and Simpson diversity), and the association between studied sites and the alpha diversity matrices was investigated using the Kruskal–Wallis test, following Shapiro–Wilk normality test results (all $p = <0.05$). To investigate the association between alpha diversity and individual-level observed homozygosity ($H_o$), a Pearson's correlation test was used.

Beta diversity was calculated using the Bray–Curtis and UniFrac distance metrics (weighted and unweighted, Lozupone & Knight, 2005). The Bray–Curtis is a dissimilarity measure based on abundance data, whereas the Unweighted UniFrac distances are based on the presence/absence of different taxa considering the phylogenetic relationships, and the Weighted UniFrac additionally incorporates the taxa abundance information. For the latter analyses, phylogenetic trees were obtained using the *ape* package. The *betadisper* function was used to test the multivariate homogeneity of group dispersion, and the statistical difference in beta dispersion between groups was investigated through the *permutest*. Due to the varied sample sizes and unequal beta dispersion between groups, the ANOSIM (Analysis of Similarities) test was used to assess statistical differences in microbial beta diversity across different locations, using 999 permutations. Patterns of separation between samples were visualized through Principal Coordinates Analysis (PCoA)

based on the Bray-Curtis, Unweighted and Weighted UniFrac dissimilarity matrices.

A consensus approach based on two differential abundance methods was used following Nearing et al. (2022) to ensure robust biological interpretations. Initially, a conservative approach was implemented by applying a prevalence-based filtration and removing taxa which featured in less than 10% of all samples. Differential abundance testing was conducted for muriqui social groups on the nonrarefied dataset using the R package DeSeq2 (Love et al., 2014). A Wald test with Benjamini–Hochberg adjustment and only ASVs that remained statistically significant ($p < 0.05$) were retained. Then, ANCOM-BC2 (Lin & Peddada, 2024) was applied for multiple pairwise comparisons of social groups. ANCOM analysis controls for false discovery rates (FDR) by applying a sensitivity analysis to investigate the effect of pseudo-count addition to zeros. The default Holm-Bonferroni method (Holm, 1979) was used, and only taxa passing the sensitivity score (SS) filter were considered.

Analysis of the effect of geographical distance on microbiome compositions was conducted considering the dissimilarity between groups (i.e., herein represented by using distinct social groups sampled in different locations inhabiting both east and west sides of the park) for both alpha and beta diversity indices. The association between the microbiome composition (UniFrac/Bray–Curtis distances) and the influence of host genetics on microbial community structure was investigated through the Mantel test based on Spearman's rank correlations (999 permutations), testing the relationship between individual-level genetic distances and Bray–Curtis/UniFrac distances.

## 3 | RESULTS

In total, muriqui fecal samples from 53 uniquely identified individuals (inferred from microsatellite genotyping) were successfully sequenced and the curated 16S rRNA dataset included 651,900 reads after all quality control filtering, classification, and rarefaction. Across the microbiota of all muriqui sampled in this study, 1339 ASVs were retrieved, representing at least nine Phyla, 18 Classes, and 56 Orders.

Based on the alpha-diversity metrics obtained, the bacterial microbiota profile retained for all analyzed groups revealed Bacteroidetes as the most abundant phylum accounting for 58.33% of all sequences, Firmicutes were about half as abundant with 25.77% of the reads, followed by Proteobacteria (9.55%), Tenericutes (2.71%) and Cyanobacteria (1.95%) (Figure 2, Tables S3–S5). A similar pattern of phyla compositional abundance was obtained across most of the individuals analyzed, with only four individuals showing a read abundance >20% for Proteobacteria, from which two individuals showed a higher abundance of this phylum over Bacteroidetes/Firmicutes (MC104, MC112 – Figure 2a). When comparing the social groups analyzed, only samples collected from Vale do Aleixo had a visible presence of Actinobacteria.

Overall, the microbiota profile at the Class level was also very similar across most individuals sampled. The Bacteroidetes retrieved from the muriqui gut represented a diverse array of Families, the great majority from the Bacteroidia which was also the most abundant Class with 99.99% of the reads recovered for this Phylum. For the Firmicutes, most of the highly abundant families belonged to the Clostridia (76.23%) and Erysipelotrichia (12.39%) Classes. Although accounting for only 4.85% of the total reads recovered for Firmicutes, Bacilli showed a greater predominance in one of the analyzed individuals (MC10). Gammaproteobacteria which was the most abundant for the Proteobacteria (92.31% of the reads), was also predominant in two of the analyzed samples (MC104, MC112), and for all other individuals, Bacteroidia was the most abundant (i.e., 51/53–96% of the samples analyzed) (Figure 2b, Table S4).

Analyses of microbial community alpha diversity, based on the ASV level, showed similar ASV richness for all groups analyzed (Figure 3, Table S5). The VFP site had the highest diversity for the observed and Shannon indices (Observed = 277.33 ± 53.65, Shannon = 4.41 ± 0.51), followed by VSM for the Observed and Chao1 indices, respectively (252.40 ± 37.43, 259.40 ± 40.23) and VA for the Simpson, alongside VFP (0.95 ± 0.04, 0.95 ± 0.05, respectively). Yet, no significant statistical differences were found among the analyzed groups for the four indices (Observed $p = 0.549$, Chao1 $p = 0.517$, Shannon $p = 0.135$, Simpson $p = 0.121$, Table S6).

To determine whether the social group influenced the community composition of the microbiome, three beta diversity measures (Bray–Curtis, Unweighted and Weighted UniFrac) were obtained and compared across the analyzed groups (Table S7). Although significant differences were retrieved from the PERMANOVA test ($p < 0.05$, Table S7), the variance partitioning test (PERMUTEST) showed a greater compositional variance within the groups, with significantly different dispersions retrieved between VFP-VA and VFP-VSM (overall, Bray–Curtis $p = 0.006$, Unweighted UniFrac $p = 0.034$, and Weighted UniFrac $p = 0.018$). A further comparison obtained through the ANOSIM test revealed no significant difference across the analyzed groups (Bray–Curtis $p = 0.311$, Unweighted UniFrac $p = 0.284$, Weighted UniFrac $p = 0.534$). Therefore, the composition of muriqui gut microbial communities showed similarities across the three analyzed sites, as revealed by beta diversity showing the overlap between individuals from different groups and with no clustering by sampling location (Figure 4).

Despite not showing a significantly different microbiota community, the distribution of social groups influenced the differential abundance of some microbial taxa (Figures S3 and S4). DeSeq analyses identified 15 ASVs as differentially abundant ($p < 0.05$) between VA and VFP, five between VA and VSM and zero when comparing VFP and VSM sites. In contrast to that, ANCOM-BC2 (SS filter applied) detected nine taxa between VA and VFP, one between VA and VSM and one between VFP and VSM (Figure S4). To avoid FDRs and allow for robust biological inferences, only taxa detected by both DeSeq and ANCOM-BC2 analyses were considered. This consensus approach revealed VFP as the social group with the greatest

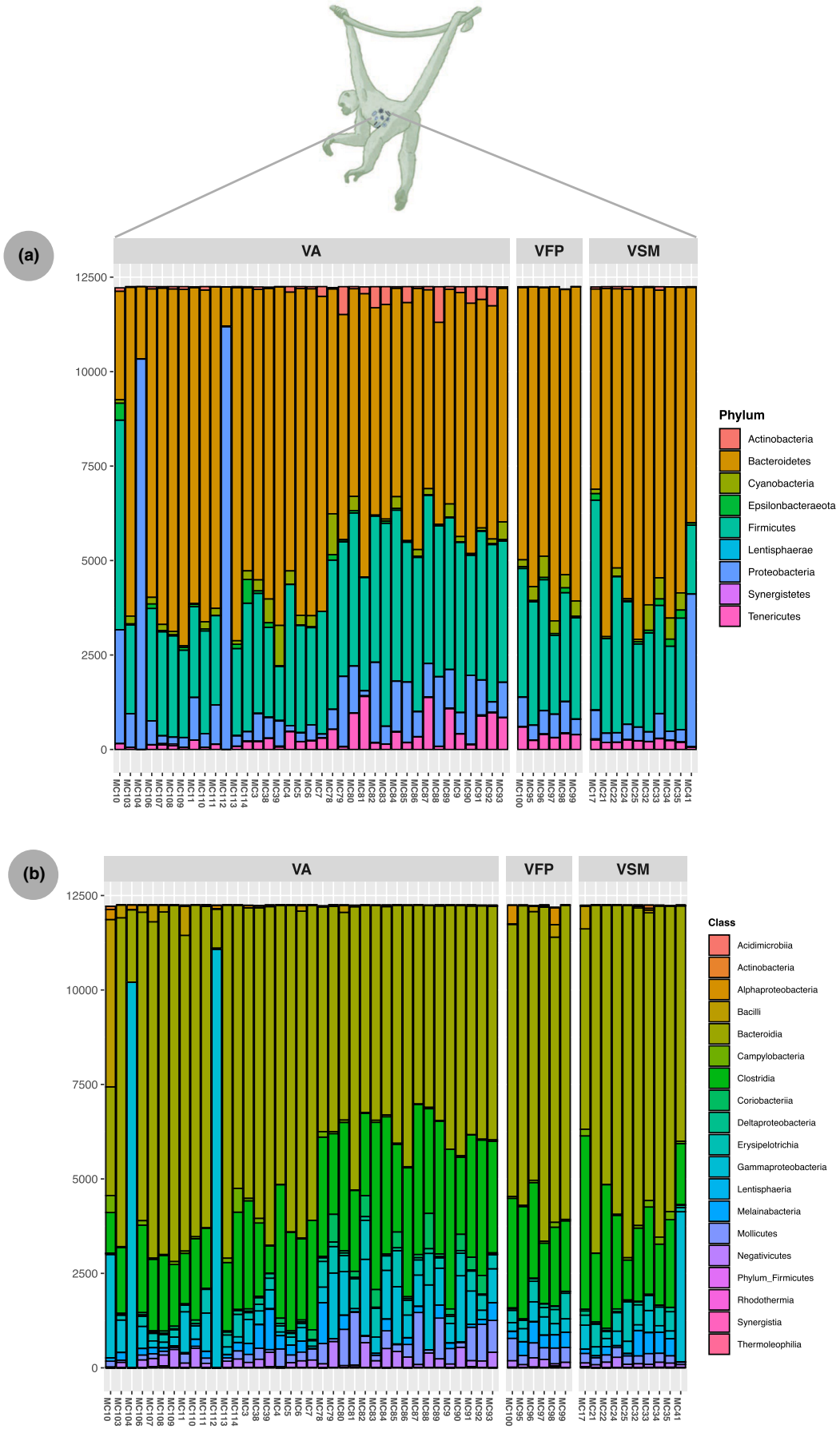

**FIGURE 2** Northern muriqui gut microbiome profiles obtained from three groups (VA – Vale do Aleixo, VFP – Vale do Facão de Pedra, VSM – Vale de Santa Marta). (a) Muriqui bacterial composition showing the mean proportion of reads assigned to Phyla for each individual and including the analyzed groups. (b) Bacterial composition showing the mean proportion of reads retrieved for each Class for all individuals and analyzed groups.

amount of ASVs showing a differential abundance, including seven taxa differentially abundant when compared to VA, with two showing a higher abundance in VA (*Mycoplasma*, Lachnospiraceae_ND3007_group) and the remaining five being more abundant in VFP (*Selenomonas*, *Olsenella*, Family_XIII_UCG-001, Eggerthellaceae and Atopobiaceae). Only Family_XIII_UCG-001 was differentially abundant when comparing VFP and VSM, with a greater abundance shown in VSM.

Finally, the interplay between host genetics and the gut microbiome was investigated using both alpha and beta microbial diversities. The homozygosity coefficients under investigation in this study ($H_o$ – observed homozygosity per individual, HL – homozygosity by loci) did not indicate a relationship with bacterial ASV richness for any of the indices analyzed (Observed, Chao1, Shannon, Simpson, all $p => 0.05$, Table S8). However, a significant correlation was retrieved when comparing genetic distances and beta diversity distances (Mantel test, Bray–Curtis $p=0.003$ $r=0.22$; Unweighted UniFrac $p=0.04$ $r=0.13$; Weighted UniFrac $p=0.002$ $r=0.25$, Table S9). Stronger positive correlations were found when comparing the muriqui genetic distances to beta diversity dissimilarity metrics that take into account the abundance (i.e., Weighted UniFrac and Bray–Curtis), with the highest $r$ value obtained for the Weighted UniFrac distance, which considers the taxa abundance and phylogenetic relationships ($r=0.25$), followed by Bray–Curtis distance ($r=0.22$) and Unweighted UniFrac ($r=0.13$).

Therefore, these results demonstrate that although heterozygosity does not seem to affect the microbiota alpha diversity, there is an influence of genetic relatedness on microbial community structure, considering both microbiome composition measured through beta diversity estimates and genetic distance between individuals.

## 4 | DISCUSSION

Investigations into the gut microbiome of wild populations are considered an emerging priority for assessing the health of these populations in the face of ever-increasing anthropogenic disturbances (West et al., 2019). Identifying potential endogenous and exogenous factors that might play a role in shaping the microbiome is paramount, especially when considering the effect of microbiota on an animal's health status and the possible implications for the conservation and management of endangered species (Bahrndorff et al., 2016; Jiménez & Sommer, 2016). In this context, we analyzed the gut microbiome of 53 muriqui individuals from the Caparaó National Park, Brazil. We aimed to provide an initial assessment of this critically endangered species' microbiome and in particular, to investigate the putative effect of group distribution (i.e., geographical location) and host genetics on their microbial structure and composition.

Overall, we observed that Bacteroidetes was the predominant phyla followed by Firmicutes, with the dominant classes represented by Bacteroidia and Clostridia, with both phyla and classes being commonly found in primate guts (Clayton et al., 2018; Nagpal et al., 2018; Yao et al., 2021; Zhao et al., 2023). Among the individuals analyzed, 7.40% ($n=4$, MC10, MC104, MC112 and MC41) showed differences in their bacterial community composition, with the abundance of phylum Proteobacteria (class Gammaproteobacteria) accounting for >20% of the total reads. The overgrowth of Proteobacteria, which include numerous pathogenic genera of bacteria, has been suggested as a signature of dysbiosis and disease in humans, including metabolic disorders, inflammation, and cancer (Rizzatti et al., 2017; Shin et al., 2015). This compositional shift might reflect host or environmental changes that could lead to dysbiotic gut microbiomes or natural variations in the group such as different feeding strategies (Mallott et al., 2018), the age of the individuals (Reveles et al., 2019), reproductive state (Sun et al., 2020), or social status (Rudolph et al., 2022) for example. A comparison of the three social groups showed the presence of Actinobacteria only in individuals inhabiting Vale do Aleixo. Actinobacteria, even though usually found in low abundance, has a major role in maintaining gut homeostasis (Binda et al., 2018; Sarkar, 2022).

Two out of the four individuals (MC104 and MC112) had the lowest alpha diversity values recovered among the analyzed groups (MC104/MC112 – Observed=40/88, Chao1=40/92.5, Shannon=2.17/2.63, Simpson=0.78/0.88). Low alpha diversity has been previously detected together with dysbiosis of the gut microbiome for captive primates and linked to dietary changes and increased stress levels (Frankel et al., 2019; McKenzie et al., 2017). However, dysbiosis remains poorly reported for wild primates (and indeed, most wild species) and further investigations are required to understand patterns in wildlife populations more generally (Ghosh et al., 2021). Dietary changes could also be linked to compositional shifts found for the other two individuals showing higher bacterial diversity (MC10 and MC41). Similar compositional shifts between Bacteroidetes and Proteobacteria have been previously reported and linked to distinct food intakes for the Arunachal macaque, with a high prevalence of Bacteroidetes in animals who foraged upon cultivated crops in contrast to an enriched Proteobacteria for groups feeding on human leftover food remains (Ghosh et al., 2021).

Muriquis have a predominantly folivorous and frugivorous diet. Studies assessing the muriqui's diet showed a diet composition including resources such as leaves, fruits, flowers, and additional items (e.g., twigs, tree barks, stem) (de Carvalho-Jr et al., 2004). Knowledge in feeding behavior remains limited for *Brachyteles*, especially for the Northern muriqui. However, variation in the percentage of feeding records was previously recorded for groups inhabiting distinct areas (i.e., higher intake of flowers in Fazenda Esmeralda than in Estação

(a)

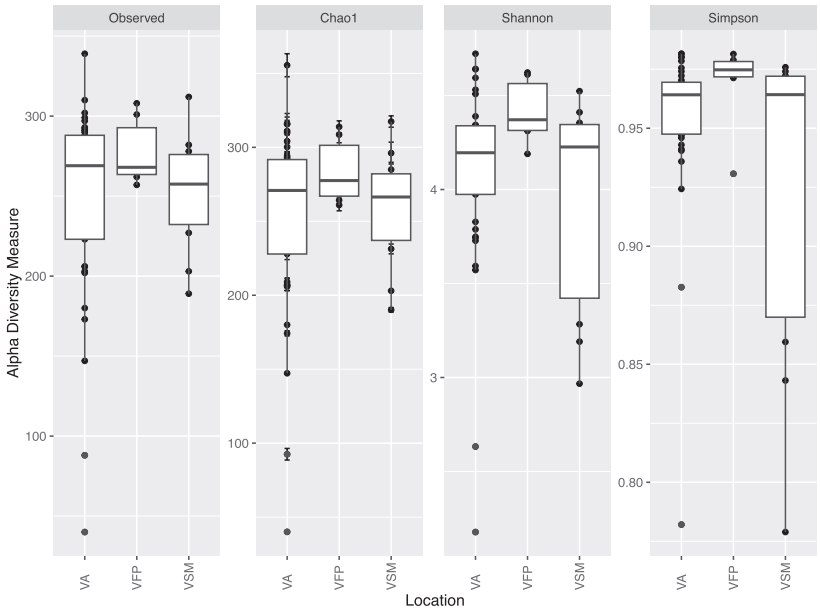


**FIGURE 3** Alpha diversity measures estimated by Observed (ASV richness), Chao1, Shannon and Simpson indices, including (a) Boxplot comparing the alpha-diversity estimates obtained using four indices (Observed, Chao1, Shannon, and Simpson) for each analyzed group and (b) the estimated mean and standard deviation values (in brackets).

(b)

| | Observed | Chao1 | Shannon | Simpson |
|---|---|---|---|---|
| **VA** | 247.16 (56.58) | 255.01 (58.96) | 4.08 (0.52) | 0.95 (0.05) |
| **VFP** | 277.33 (53.65) | 255.37 (55.64) | 4.41 (0.51) | 0.95 (0.04) |
| **VSM** | 252.40 (37.17) | 259.40 (40.23) | 3.93 (0.49) | 0.93 (0.06) |

Biológica de Caratinga which had a comparatively higher proportion of fruit consumption; de Carvalho-Jr et al., 2004); Rímoli & Ades, 1997), and shifts toward a more folivorous diet when fruits are scarce were reported and linked possibly to changes in rainfall and food resource availability (Strier, 1991). Yet, the diet of muriquis inhabiting the CNP and possible diet shifts remain largely unknown to date.

Although no significant difference was found for the alpha diversity measures recovered among the analyzed groups, the overall values were lower than reported for other primates of the Atelidae family (McKenzie et al., 2017; West et al., 2019). In this study, the muriqui showed Shannon bacterial diversity ranging from 3.93 to 4.41, whereas for both *Alouatta* spp. and *Ateles* spp. with values above six recovered for wild individuals and values above four were retrieved for individuals in captivity (McKenzie et al., 2017). Still, a lowered bacterial diversity has been observed in primates living in captivity or fragmented areas (Amato et al., 2013; Barelli et al., 2020; Trosvik et al., 2018; Zhao et al., 2023) and the small group sizes inhabiting this forest remnant might have contributed to this reduction in gut microbial diversity. It is important to note, however, that the alpha diversity values herein retrieved were in line with results obtained by Harrison et al. (2021), in which values <4 (Shannon diversity) were retrieved for muriqui, with this species showing a more diverse microbial community when compared to other mammal species (e.g., capuchin monkey, eastern black rhino). Therefore, a more in-depth comparative analysis of wild primate bacterial diversity, alongside an investigation of the underlying factors responsible for shaping microbiome diversity, is needed.

Microbial community composition across analyzed groups demonstrated no significant differences. However, a differential abundance was recovered for some of these groups, especially when comparing VA and VFP (Table S10). The highest amount of over-represented taxa was seen in the VFP group, including ASVs from Olsenella, Selenomonas, Family_XIII_UCG-001, Eggerthellaceae and Atopobiaceae. In contrast to that, the VA group had two over-represented ASV with *Mycoplasma* and Lachnospiraceae_ND3007_ group being detected in a significantly greater amount when contrasted to VFP (ANCOM-BC2 lfc = 2.03 and 1.63, respectively). In the VSM group, only Family_XIII_UCG-001 showed a significantly greater abundance in comparison with the VA groups, and no differentially abundant taxa was retrieved between VSM and VFP.

Taxa showing a lower abundance in VA included the families Atopobiaceae, Eggerthellaceae, and the genera Selenomonas, Olsenella and Family_XIII_UCG-001. Across these families, several taxa have been previously linked to gut health such as playing an important role in plant secondary compound metabolism (e.g., Eggerthellaceae, Greene et al., 2020), acting in the fermentation of lactate and soluble sugars (e.g., intestinal *Selenomonas*, Hespell et al., 2006, Sawanon et al., 2011,), and playing a role in fiber

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences
WILEY



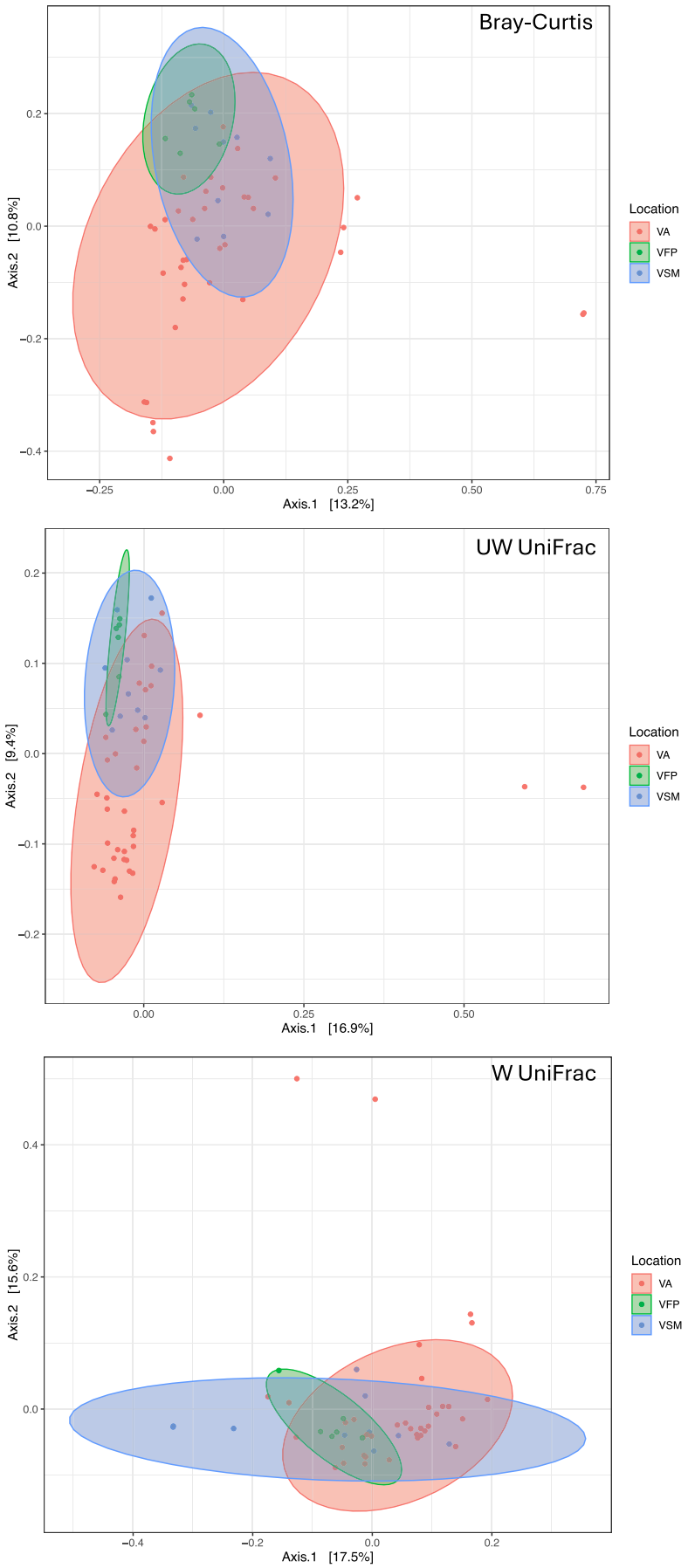


**FIGURE 4** Visualization of beta diversity through Principal Coordinates Analysis (PCoA), including Bray–Curtis, Unweighted UniFrac, and Weighted UniFrac distances.

digestion, host lipid metabolism and energy storage in high-fat diets (e.g., *Olsenella*, Houtkamp et al., 2023, Kaakoush, 2015).

The highly abundant *Mycoplasma* found for the VA group represents a commensal group of bacteria widely detected in association with the mucous membrane of mammals, however, the occurrence of potential opportunistic infections associated with these bacteria has been previously reported including platyrrhine primates (e.g., detection of *Mycoplasma* sp. in an *Alouatta caraya* individual showing normochromic and macrocytic anemia, Bonato et al., 2015). These differential abundances could provide some insights regarding animals' gut health and/or the effect of environmental conditions, including food availability and foraging behavior. On that account, a more detailed functional analysis of bacterial communities including metagenomic analysis and metabolomic profiling is key.

The gut microbiome itself is shaped not only by extrinsic conditions (i.e., diet, lifestyle, habitat heterogeneity) but also by intrinsic factors (i.e., sex, age, genotype) (Amato et al., 2013; Bahrndorff et al., 2016; Dubois et al., 2017; Wasimuddin et al., 2017). In this context, the host microbiome has a two-way route, both influencing the host's immune system and being influenced by the host's genetic diversity (Montero et al., 2021). The association between genetic differences among hosts and in shaping their susceptibility to pathogens is well known. Yet, the contribution of host genes to the microbiome remains mostly unclear (Tabrett & Horton, 2020).

Considering the role of geography in shaping the gut microbiome has not been seen herein at this microgeographic scale, an investigation of the correlation between host genetics and microbiome diversity and composition was performed. In this study, 14 nuclear microsatellite loci were used to investigate the association between the genetic makeup and gut bacterial communities of the host. No effect of muriqui genetic diversity on microbial diversity was obtained, as demonstrated by the absence of a correlation between individual heterozygosity ($H_o$ – observed heterozygosity, HL – homozygosity by loci) and bacterial ASV richness (Observed, Chao1, Shannon, Simpson, all $p \geq 0.05$). However, the influence of host genetics on microbiome was demonstrated through a significant positive correlation retrieved between beta diversity indices and genetic distances (Mantel test, $p < 0.05$), with the highest r value retrieved for the Weighted UniFrac distance, highlighting the importance of considering taxa abundance and phylogenetic relationships. These results demonstrate that microbial community structure may be influenced by genetic relatedness, as previously reported by Grieneisen et al. (2021) where a high gut microbiome heritability was demonstrated after analyzing 16,000 baboon gut microbiome profiles obtained for 585 individuals during a 14-year-long study.

The results obtained herein offer an interesting insight into the Northern muriqui's management and conservation. The demonstrated influence of host genetics on the microbiome and the absence of geographic filtering (no difference retrieved between analyzed social groups) might indicate the possible movement of individuals between these groups. Female muriquis are known to emigrate from their natal groups, a rare life history strategy among primates (Strier et al., 2015). This dispersal pattern could help explain the similarities found among the analyzed groups due to genetic and microbiota exchange, thus, indicating the possible dispersal of females between groups despite the geographical barriers (i.e., a mountain chain splitting the area into two sides – western and eastern; Figure 1).

Anthropogenic disturbances are known to heavily impact wildlife populations in a plethora of forms, including changes in host microbiomes (Zhu et al., 2021). Disruptions in the gut microbiome composition have been demonstrated, including the domestication and "humanization" of the wildlife gut microbiome (Dillard et al., 2022; Prabhu et al., 2020), and the shift in the microbiota community composition due to an increase in disease-associated taxa and a decrease of beneficial bacteria (Wasimuddin et al., 2022). On top of that, population bottlenecks lead to the loss of genetic variation, increased inbreeding and therefore, resulting in a less diverse microbiome. The constrained microbiome diversity must be the focus of discussion, alongside population size and genetic diversity, when dealing with threatened populations, as it may play a significant role in host fitness and adaptability (Ørsted et al., 2022). Species reintroductions/translocations represent one of the key effective strategies deployed toward the conservation of threatened species with reduced population sizes (Zhu et al., 2021). Despite the efforts made in implementing ex-situ programs mimicking natural conditions, captive animals are often kept under various conditions that might greatly differ from their natural environments. In this context, investigating their wildlife microbiome is paramount to provide subsidies for comparisons with animals kept in captivity before the start of release programs, especially considering the role of microbiota in nutrition uptaking and general host health.

## 5 | CONCLUSIONS

Here, we have provided a characterization of the gut microbiome of one the world's most endangered primates, the Northern muriqui. No effect of geographic distance on the microbiota composition was detected, as high similarities for both alpha and beta diversities were recovered among the three groups inhabiting the Caparaó National Park. Host heterozygosity recovered from neutral nuclear markers (i.e., microsatellites) also did not seem to exert any influence on microbiota diversity. However, an association between microbiome composition and genetic distance was demonstrated. Therefore, the microbiome did not seem to be affected by the geographic distance but may be associated with genetic relatedness, indicating a possible exchange of individuals between groups due to female dispersal.

Considering the baseline microbiome data provided herein, further investigations should be carried out to more fully understand the change in microbiota putatively associated with the human encroachment that might disturb the host microbiome and consequently, lead to a decline in the population's health status. In this context, the microbiome could then be used as an early warning of threats to muriqui, since negative changes in the microbiome composition might render these animals susceptible to diseases, facilitating

co-infections and the spread of zoonotic diseases, ultimately jeopardizing the species' survival (Brändel et al., 2018).

## AUTHOR CONTRIBUTIONS

NGS and ADM conceptualized and designed the study. MCK collected the fecal samples and performed laboratory work concerning microsatellite genotyping. NGS performed the lab analyses concerning the microbiome. NGS processed the sequence data and performed the statistical analysis. SSB advised on the laboratory work and statistical analyses. NGS, ADM, and SIG wrote the original draft. All authors contributed to writing, reviewing, and editing the text.

## ACKNOWLEDGMENTS

We acknowledge the support provided by the Caparaó National Park, and Claudio Novaes for the invaluable help in data collection.

## FUNDING INFORMATION

Not applicable.

## CONFLICT OF INTEREST STATEMENT

The authors declare that there are no competing interests.

## DATA AVAILABILITY STATEMENT

Raw files and associated metadata can be downloaded from FigShare at https://doi.org/10.6084/m9.figshare.22086218.v2. RScripts used to analyze the data for this manuscript are available at https://github.com/ngsales/Muriqui_microbiome/.

## CONSENT FOR PUBLICATION

Not applicable.

## ORCID

*Naiara Guimaraes Sales* https://orcid.org/0000-0002-2922-3561
*Allan D. McDevitt* https://orcid.org/0000-0002-2677-7833

## REFERENCES

Amato, K. R., Yeman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., Gaskins, H. R., Stumpf, R. M., Yildirim, S., Torralba, M., Gillis, M., Wilson, B. A., Nelson, K. E., White, B. A., & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (*Alouatta pigra*) gastrointestinal microbiomes. *The ISME Journal*, *7*, 1344–1353. https://doi.org/10.1038/ISMEJ.2013.16

Antwis, R. E., Beresford, N. A., Jackson, J. A., Fawkes, R., Barnett, C. L., Potter, E., Walker, L., Gaschak, S., & Wood, M. D. (2021). Impacts of radiation exposure on the bacterial and fungal microbiome of small mammals in the Chernobyl exclusion zone. *The Journal of Animal Ecology*, *90*, 2172–2187. https://doi.org/10.1111/1365-2656.13507

Aparicio, J. M., Ortego, J., & Cordero, P. J. (2006). What should we weigh to estimate heterozygosity, alleles or loci? *Molecular Ecology*, *15*, 4659–4665. https://doi.org/10.1111/j.1365-294X.2006.03111.x

Bahrndorff, S., Alemu, T., Alemneh, T., & Lund Nielsen, J. (2016). The microbiome of animals: Implications for conservation biology. *International Journal of Genomics*, *2016*, 5304028. https://doi.org/10.1155/2016/5304028

Baniel, A., Amato, K. R., Beehner, J. C., Bergman, T. J., Mercer, A., Perlman, R. F., Petrullo, L., Reitsema, L., Sams, S., Lu, A., & Snyder-Mackler, N. (2021). Seasonal shifts in the gut microbiome indicate plastic responses to diet in wild geladas. *Microbiome*, *9*(1), 26. https://doi.org/10.1186/s40168-020-00977-9

Barelli, C., Albanese, D., Stumpf, R. M., Asangba, A., Donati, C., Rovero, F., & Hauffe, H. C. (2020). The gut microbiota communities of wild arboreal and ground-feeding tropical primates are affected differently by habitat disturbance. *mSystems*, *5*, e00061-20. https://doi.org/10.1128/mSystems.00061-20

Beck, B. B. (2019). *Unwitting travelers: A history of primate reintroduction*. Salt Water Media.

Binda, C., Lopetuso, L. R., Rizzatti, G., Gibiino, G., Cennamo, V., & Gasbarrini, A. (2018). Actinobacteria: A relevant minority for the maintenance of gut homeostasis. *Digestive and Liver Disease*, *50*(5), 421–428. https://doi.org/10.1016/j.dld.2018.02.012

Bonato, L., Figueiredo, M. A. P., Gonçalves, L. R., Machado, R. Z., & André, M. R. (2015). Occurrence and molecular characterization of *Bartonella* spp. and hemoplasmas in neotropical primates from Brazilian Amazon. *Comparative Immunology, Microbiology and Infectious Diseases*, *42*, 15–20. https://doi.org/10.1016/j.cimid.2015.09.0010147-9571/

Bosch, T. C., & McFall-Ngai, M. J. (2011). Metaorganisms as the new frontier. *Zoology*, *114*(4), 185–190.

Brändel, S. D., Tschapka, M., Page, R., Rasche, A., Corman, V. M., Drosten, C., & Sommer, S. (2018). Astrovirus infections induce age-dependent dysbiosis in gut microbiomes of bats. *The ISME Journal*, *12*, 2883–2893. https://doi.org/10.1038/s41396-018-0239-1

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Campera, M., Brown, E., Imron, M. A., & Nekaris, K. A. I. (2020). Unmonitored releases of small animals? The importance of considering natural dispersal, health, and human habituation when releasing a territorial mammal threatened by wildlife trade. *Biological Conservation*, *242*, 108404. https://doi.org/10.1016/j.biocon.2019.108404

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, *108* Suppl 1(Suppl 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

Chaves, P. B., Alvarenga, C. S., Possamai, C. D. B., Dias, L. G., Boubli, J. P., Strier, K. B., Mendes, S. L., & Fagundes, V. (2011). Genetic diversity and population history of a critically endangered primate, the northern muriqui (*Brachyteles hypoxanthus*). *PLoS One*, *6*(6), e20722.

Chazdon, R. L., Cullen, L., Padua, S. M., & Padua, C. V. (2020). People, primates and predators in the Pontal: From endangered species conservation to forest and landscape restoration in Brazil's Atlantic Forest. *Royal Society Open Science*, *7*(12), 200939. https://doi.org/10.1098/rsos.200939

Clayton, J. B., Gomez, A., Amato, K., Knights, D., Travis, D. A., Blekhman, R., Knight, R., Leigh, S., Stumpf, R., Wolf, T., Glander, K. E., Cabana, F., & Johnson, T. J. (2018). The gut microbiome of nonhuman primates: Lessons in ecology and evolution. *American Journal of Primatology*, *80*(6), e22867.

Couch, C. E., & Epps, C. W. (2022). Host, microbiome, and complex space: Applying population and landscape genetic approaches to gut microbiome research in wild populations. *Journal of Heredity*, *113*, 221–234. https://doi.org/10.1093/jhered/esab078

de Melo, F. R., Boubli, J. P., Mittermeier, R. A., Jerusalinsky, L., Tabacow, F. P., Ferraz, D. S., & Talebi, M. (2021). *Brachyteles hypoxanthus* (amended version of 2019 assessment). The IUCN Red Listof

Threatened Species 2021: e.T2994A191693399. https://doi.org/10.2305/IUCN.UK.2021-1.RLTS.T2994A191693399.en

de Carvalho Jr, O., Ferrari, S. F., & Strier, K. B. (2004). Diet of a muriqui group (*Brachyteles arachnoides*) in continuous primary forest. *Primates*, *45*, 201–204.

Dillard, B. A., Chung, A. K., Gunderson, A. R., Campbell-Staton, S. C., & Moeller, A. H. (2022). Humanization of wildlife gut microbiota in urban environments. *BioRxiv*. https://doi.org/10.1101/2022.01.05.475040

Dubois, G., Girard, C., Lapointe, F. J., & Shapiro, B. J. (2017). The Inuit gut microbiome is dynamic over time and shaped by traditional foods. *Microbiome*, *5*, 151. https://doi.org/10.1186/s40168-017-0370-7

Forsythe, P., & Bienenstock, J. (2010). Immunomodulation by commensal and probiotic bacteria. *Immunological Investigations*, *39*(4–5), 429–448. https://doi.org/10.3109/08820131003667978

Frankel, J. S., Mallott, E. K., Hopper, L. M., Ross, S. R., & Amato, K. R. (2019). The effect of captivity on the primate gut microbiome varies with host dietary niche. *American Journal of Primatology*, *81*, 1–9. https://doi.org/10.1002/ajp.23061

Franquesa-Soler, M., Aristizabal, J. F., Andresen, E., del Burgo, I. V., Shedden-Gonzalez, A., & Rodriguez-Luna, E. (2022). Long-term assessment of the translocation of an endangered primate into an agroforestry system. *Endangered Species Research*, *48*, 235–246. https://doi.org/10.3354/esr01195

Ghosh, A., Thakur, M., Sharma, L. K., & Chandra, K. (2021). Linking gut microbiome with the feeding behavior of the Arunachal macaque (*Macaca munzala*). *Scientific Reports*, *11*, 21926. https://doi.org/10.1038/s41598-021-01316-0

Gilbert, J. A., Quinn, R. A., Debelius, J., Xu, Z. Z., Morton, J., Garg, N., Jansson, J. K., Dorrestein, P. C., & Knight, R. (2016). Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature*, *535*, 94–103. https://doi.org/10.1038/nature18850

Greene, L. K., Bornbusch, S. L., McKenney, E. A., Harris, R. L., Gorvetzian, S. R., Yoder, A. D., & Drea, C. M. (2019). The importance of scale in comparative microbiome research: New insights from the gut and glands of captive and wild lemurs. *American Journal of Primatology*, *81*, e22974.

Grieneisen, L., Dasauri, M., Gould, T. J., Bjork, J. R., Grenier, J., Jansen, D., Gottel, N., Gordon, J. B., Learn, N. H., Gesquiere, L. R., Wango, T. L., Mututua, R. S., Warutere, J. K., Siodi, L'i., Gilbert, J. A., Barreiro, L. B., Alberts, S. C., Tung, J., Archie, E. A., & Blekhman, R. (2021). Gut microbiome heritability is nearly universal but environmentally contingent. *Science*, *373*, 181–186. https://doi.org/10.1126/science.aba5483

Harrison, X. A., Antwis, R. E., Mcdevitt, A. D., Dunn, J. C., Griffiths, S. M., Benvenuto, C., Birtles, R., Boubli, J. P., Bown, K., Bridson, C., Brooks, D. R., Browett, S. S., Carden, R. F., Chantrey, J., Clever, F., Coscia, I., Edwards, K. L., Ferry, N., Goodhead, I., … Antwis, R. E. (2021). Fungal microbiomes are determined by host phylogeny and exhibit widespread associations with the bacterial microbiome. *Proceedings of the Royal Society B: Biological Sciences*, *288*, 20210552. https://doi.org/10.1098/rspb.2021.0552

Henry, L. P., Bruijning, M., Forsberg, S. K. G., & Ayroles, J. F. (2021). The microbiome extends host evolutionary potential. *Nature Communications*, *12*(1), 5141. https://doi.org/10.1038/s41467-021-25315-x

Hespell, R. B., Paster, B. J., & Dewhirst, F. E. (2006). The genus *Selenomonas*. In M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, & E. Stackebrandt (Eds.), *The prokaryotes* (pp. 982–990). Springer. https://doi.org/10.1007/0-387-30744-3_33

Holm, S. (1979). A simple sequentially rejective multiple-test procedure. *Scandinavian Journal of Statistics*, *6*, 65–70.

Houtkamp, I. M., van Zijll Langhout, M., Bessem, M., Pirovano, W., & Kort, R. (2023). Multiomics characterisation of the zoo-housed gorilla gut microbiome reveals bacterial community compositions shifts, fungal cellulose-degrading, and archaeal methanogenic activity. *Gut Microbiome*, *4*, e12. https://doi.org/10.1017/gmb.2023.11

IPBES. (2019). Global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services. E. S. Brondizio, J. Settele, S. Díaz, and H. T. Ngo (editors). IPBES secretariat, Bonn, Germany. 1148 pages. https://doi.org/10.5281/zenodo.3831673

IUCN. (2022). *The IUCN red list of threatened species. Version 2022-2*. Retrieved January 20, 2023, from https://www.iucnredlist.org

Jiménez, R. R., & Sommer, S. (2016). The amphibian microbiome: Natural range of variation, pathogenic dysbiosis, and role in conservation. *Biodiversity and Conservation*, *26*(4), 763–786. https://doi.org/10.1007/s10531-016-1272-x

Kaakoush, N. (2015). Insights into the role of Erysipelotrichaceae in the human host. *Frontiers in Cellular and Infection Microbiology*, *5*, 84. https://doi.org/10.3389/fcimb.2015.00084

Kaizer, M., Alvim, T., Novaes, C., McDevitt, A., & Young, R. (2022). Snapshot of the Atlantic Forest canopy: Surveying arboreal mammals in a biodiversity hotspot. *Oryx*, *56*(6), 825–836. https://doi.org/10.1017/S0030605321001563

Kalinowski, S. T., Taper, M. L., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *16*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kierulff, M. C. M., Ruiz-Miranda, C. R., Oliveira, P. P., Beck, B. B., Martins, A., Dietz, J. M., Rambaldi, D. M., & Baker, A. J. (2012). The Golden lion tamarin *Leontopithecus rosalia*: A conservation success story. *International Zoo Yearbook*, *46*, 36–45. https://doi.org/10.1111/j.1748-1090.2012.00170.x

Lin, H., & Peddada, S. D. (2024). Multigroup analysis of compositions of microbiomes with covariate adjustments and repeated measures. *Nature Methods*, *21*, 83–91. https://doi.org/10.1038/s41592-023-02092-7

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNAseq data with DESeq2. *Genome Biology*, *15*, 1–21. https://doi.org/10.1186/s13059-014-0550-8

Lozupone, C., & Knight, R. (2005). UniFrac: A new phylogenetic method for comparing microbial communities. *Applied and Environmental Microbiology*, *71*(12), 8228–8235. https://doi.org/10.1128/AEM.71.12.8228-8235.2005

Lynch, J. B., & Hsiao, E. Y. (2022). Microbiomes as sources of emergent host phenotypes. *Science*, *365*(6460), 1405–1409. https://doi.org/10.1126/science.aay0240

Mallott, E. K., Amato, K. R., Garber, P. A., & Malhi, R. S. (2018). Influence of fruit and invertebrate consumption on the gut microbiota of wild white-faced capuchins (*Cebus capucinus*). *American Journal of Physical Anthropology*, *165*(3), 576–588. https://doi.org/10.1002/ajpa.23395

McKenney, E. A., Koelle, K., Dunn, R. R., & Yoder, A. D. (2018). The ecosystem services of animal microbiomes. *Molecular Ecology*, *27*(8), 2164–2172. https://doi.org/10.1111/MEC.14532

McKenzie, V. J., Kueneman, J. G., & Harris, R. N. (2018). Probiotics as a tool for disease mitigation in wildlife: Insights from food production and medicine. *Annals of the New York Academy of Sciences*, *1429*, 18–30. https://doi.org/10.1111/nyas.13617

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., Alexiev, A., Amato, K. R., Metcalf, J. L., Kowalewski, M., Avenant, N. L., Link, A., di Fiore, A., Seguin-Orlando, A., Feh, C., Orlando, L., Mendelson, J. R., Sanders, J., & Knight, R. (2017). The effects of captivity on the mammalian gut microbiome. *Integrative and Comparative Biology*, *57*, 690–704. https://doi.org/10.1093/icb/icx090

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2018). Methods for normalizing microbiome data: An ecological perspective. *Methods in Ecology and Evolution*, *10*, 389–400. https://doi.org/10.1111/2041-210X.13115

McMurdie, P. J., & Holmes, S. (2014). Waste not, want not: Why rarefying microbiome data is inadmissible. *PLoS Computational Biology*, *10*, e1003531. https://doi.org/10.1371/journal.pcbi.1003531

Montero, B. K., Uddin, W., Schewensow, N., Gillingham, M. A. F., Ratovonamana, Y. R., Rakotondranary, S. J., Corman, V., Drosten, C., Ganzhorn, J. U., & Sommer, S. (2021). Evidence of MHC class 1 and II influencing viral and helminth infection via the microbiome in a non-human primate. *PLoS Pathogens*, *17*, e1009675. https://doi.org/10.1371/journal.ppat.1009675

Nagpal, R., Shively, C. A., Appt, S. A., Register, T. C., Michalson, K. T., Vitolins, M. Z., & Yadav, H. (2018). Gut microbiome composition in non-human primates consuming a Western or Mediterranean diet. *Frontiers in Nutrition*, *5*, 28. https://doi.org/10.3389/fnut.2018.00028

Nearing, J. T., Douglas, G. M., Hayes, M. G., MacDonald, J., Desai, D. K., Allward, N., Jones, C. M. A., Wright, R. J., Dhanani, A. S., Comeau, A. M., & Langille, M. G. I. (2022). Microbiome differential abundance methods produce different results across 38 datasets. *Nature Communications*, *13*, 342. https://doi.org/10.1038/s41467-022-28034-z

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. M., Wagner, H., & Oksanen, M. J. (2018). Package 'vegan'. Community ecology package, version, 2(3).

Oliveira, B. C. M., Murray, M., Tseng, F., & Widmer, G. (2020). The fecal microbiota of wild and captive raptors. *Animal Microbiome*, *2*, 15. https://doi.org/10.1186/s42523-020-00035-7

Oliveira, P., Tabacow, F. P., Gasparotto, V. P. O., teixeira, E. P. T., Testa, M. F., Teixeira, D. S., Ferreira, A. I. G., Barros, T. P., Moreira, L. S., Pereira, P., de Melo, F. R., & de Melo, F. C. S. A. (2020). Relato de caso envolvendo a translocação de uma fêma de Brachyteles hypoxanthus (Primates, Atelidae) para um recinto semi-natural como estratégia de conservação da espécie. *Neotropical Primates*, *26*(2), 56–62.

Ørsted, M., Yashiro, E., Hoffmann, A. A., & Kristensen, T. N. (2022). Population bottlenecks constrain host microbiome diversity and genetic variation impeding fitness. *PLoS Genetics*, *18*(5), e1010206. https://doi.org/10.1371/journal.pgen.1010206

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539.

Prabhu, V. R., Wasimuddin, V. R., Kamalakkannan, R., Arjun, M. S., & Nagarajan, M. (2020). Consequences of domestication on gut microbiome: A comparative study between wild gaur and domestic Mithun. *Frontiers in Microbiology*, *11*, 133. https://doi.org/10.3389/FMICB.2020.00133

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA genedatabase project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), 590–596. https://doi.org/10.1093/nar/gks1219

R Core Team. (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Rímoli, J., & Ades, C. (1997). Estratégias de forrageamento de um grupo de muriquis (*Brachyteles arachnoides*, Primates, Cebidae) da Estação Biológica de Caratinga-MG. In S. F. Ferrari & H. Schneider (Eds.), *A Primatologia no Brasil—5* (pp. 39–57). Sociedade Brasileira de Primatologia.

Reveles, K. R., Patel, S., Forney, L., & Ross, C. N. (2019). Age-related changes in the marmoset gut microbiome. *American Journal of Primatology*, *81*(2), e22960. https://doi.org/10.1002/ajp.22960

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J., & Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, *142*, 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Rizzatti, G., Lopetuso, L. R., Gibiino, G., Binda, C., & Gasbarrini, A. (2017). Proteobacteria: A common factor in human diseases. *BioMed Research International*, *2017*, 1–7. https://doi.org/10.1155/2017/9351507

RStudio Team. (2020). *RStudio: Integrated development for R*. RStudio, PBC. http://www.rstudio.com/

Rudolph, K., Schneider, D., Fichtel, C., Daniel, R., Heistermann, M., & Kappeler, P. M. (2022). Drivers of gut microbiome variation within and between groups of a wild Malagasy primate. *Microbiome*, *10*, 28. https://doi.org/10.1186/s40168-021-01223-6

Sarkar, P. (2022). Isolation of gut Actinobacteria from fecal and tissue samples. In D. Dharumadurai (Ed.), *Methods in actinobacteriology* (pp. 45–50). Springer Protocols Handbooks. Humana. https://doi.org/10.1007/978-1-0716-1728-1_10

Sawanon, S., Koike, S., & Kobayashi, Y. (2011). Evidence for the possible involvement of Selenomonas ruminantium in rumen fiber digestion. *FEMS Microbiology Letters*, *325*, 170–179. https://doi.org/10.1111/j.1574-6968.2011.02427.x

Shin, N. R., Whon, T. W., & Bae, J. W. (2015). Proteobacteria: Microbial signature of dysbiosis in gut microbiota. *Trends in Biotechnology*, *33*(9), 496–503. https://doi.org/10.1016/j.tibtech.2015.06.011

Strier, K. B. (1991). Diet in one group of woolly spider monkeys, or muriquis (*Brachyteles arachnoides*). *American Journal of Primatology*, *23*(2), 113–126.

Strier, K. B., Possamai, C. B., & Mendes, S. L. (2015). Dispersal patterns of female northern muriquis: Implications for social dynamics, life history, and conservation. In T. Furuichi, J. Yamagiwa, & F. Aureli (Eds.), *Dispersing primate females: Life history and social strategies in male-philopatric species* (pp. 3–22). Springer Japan.

Strier, K. B., Possamai, C. B., Tabacow, F. P., Pissinatti, A., Lanna, A. M., Rodrigues de Melo, F., Moreira, L., Talebi, M., Breves, P., Mendes, S. L., & Jerusalinsky, L. (2017). Demographic monitoring of wild muriqui populations: Criteria for defining priority areas and monitoring intensity. *PLoS One*, *12*(12), e0188922. https://doi.org/10.1371/journal.pone.0188922

Strier, K. B., Boubli, J. P., Possamai, C. B., & Mendes, S. L. (2006). Population demography of northern muriquis (*Brachyteles hypoxanthus*) at the Estação Biológica de Caratinga/Reserva Particular do Patrimônio Natural-Feliciano Miguel Abdala, Minas Gerais, Brazil. *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *130*(2), 227–237.

Sun, B., Xu, X., Xia, Y., Cheng, Y., Mao, S., Xiang, X., Xia, D., Wang, X., & Li, J. (2020). Variation of gut microbiome in free-ranging female tibetan macaques (*Macaca thibetana*) across different reproductive states. *Animals*, *11*(1), 39. https://doi.org/10.3390/ani11010039

Tabrett, A., & Horton, M. W. (2020). The influence of host genetics on the microbiome [version 1; peer review: 2 approved]. *F1000Research*, *9*(F1000 faculty rev), 84. https://doi.org/10.12688/f1000research.20835.1

The Integrative HMP (iHMP) Research Network Consortium. (2019). The integrative human microbiome project. *Nature*, *569*, 641–648. https://doi.org/10.1038/s41586-019-1238-8

Trevelline, B. K., Fontaine, S. S., Hartup, B. K., & Kohl, K. D. (2019). Conservation biology needs a microbial renaissance: A call for the consideration of host-associated microbiota in wildlife management practices. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1895), 20182448.

Trosvik, P., Rueness, E. K., De Muinck, E. J., Moges, A., & Mekonnen, A. (2018). Ecological plasticity in the gastrointestinal microbiomes of Ethiopian Chlorocebus monkeys. *Scientific Reports*, *8*, 20. https://doi.org/10.1038/s41598-017-18435-2

Wasimuddin, M. H., Ratovonamana, Y. R., Rakotondranary, S. J., Ganzhorn, J. U., & Sommer, S. (2022). Anthropogenic disturbance impacts gut microbiome homeostasis in a Malagasy primate.

*Frontiers in Microbiology*, *21*(13), 911275. https://doi.org/10.3389/fmicb.2022.911275

Wasimuddin, S. M., Melzheimer, J., Thalwitzer, S., Heinrich, S., Wachter, B., & Sommer, S. (2017). Gut microbiomes of free-ranging and captive Namibian cheetahs: Diversity, putative functions and occurrence of potential pathogens. *Molecular Ecology*, *26*, 5515–5527. https://doi.org/10.1111/mec.14278

West, A. G., Waite, D. W., Deines, P., Bourne, D. G., Digby, A., McKenzie, V. J., & Taylor, M. W. (2019). The microbiome in threatened species conservation. *Biological Conservation*, *229*, 85–98. https://doi.org/10.1016/j.biocon.2018.11.016

Willis, A. D. (2019). Rarefaction, alpha diversity, and statistics. *Frontiers in Microbiology*, *10*, 2407. https://doi.org/10.3389/fmicb.2019.02407

Yao, L., Li, X., Zhou, Z., Shi, D., Li, Z., Li, S., Yao, H., Yang, J., Yu, H., & Xiao, Y. (2021). Age-based variations in the gut microbiome of the Shennongjia (Hubei) Golden snub-nosed monkey (*Rhinopithecus roxellana hubeiensis*). *BioMed Research International*, *2021*, 6667715. https://doi.org/10.1155/2021/6667715

Zhao, G., Qi, M., Wang, Q., Hu, C., Li, X., Chen, Y., Yang, J., Yu, H., Chen, H., & Guo, A. (2023). Gut microbiome variations in *Rhinopithecus roxellanae* caused by changes in the environment. *BMC Genomics*, *24*(1), 62. https://doi.org/10.1186/s12864-023-09142-6

Zhu, L., Wang, J., & Bahrndorff, S. (2021). Editorial: The wildlife gut microbiome and its implication for conservation biology. *Frontiers in Microbiology*, *12*, 697499. https://doi.org/10.3389/fmicb.2021.697499

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Guimaraes Sales, N., da Cruz Kaizer, M., Browett, S. S., Gabriel, S. I., & McDevitt, A. D. (2024). Assessing the gut microbiome and the influence of host genetics on a critically endangered primate, the northern muriqui (*Brachyteles hypoxanthus*). *Environmental DNA*, *6*, e559. https://doi.org/10.1002/edn3.559