EXAMINING MICROBIAL TRANSFER BETWEEN HUMAN AND NON-HUMAN PRIMATES USING A ONE HEALTH APPROACH

By

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ABSTRACT

The health of humans, wildlife, plants, and their environments are interconnected, and this concept is fundamental promoting public health and conservation goals globally. The One Health approach defines the relationship of humans, plants, animals and our shared environments, positioning health at the core. This approach aids conservation management as facilities can look at health holistically, focusing not only on the health of the captive populations but also of the staff, volunteers, and the public. This is particularly important for the conservation of endangered species, such as lemurs. In Madagascar, lemur populations represent 20% of the worlds primate species and are endangered due to habitat fragmentation, deforestation, and anthropogenic factors. Captivity gives endangered populations a chance at rehabilitation and humans the opportunity to observe and learn from their behaviors, physiology, and development. Although

captivity holds many benefits, we must think about how factors introduced by conservatory settings influence the health of captive animals and the humans who interact with them. Past studies have documented the transfer of microbes is documented to occur between humans and their pets. In this study, I examined the extent to which microbiome exchange occurred between human and non-human primates observed at the Duke Lemur Center (DLC) in Durham, North Carolina, USA. I hypothesized that (i) lemurs exposed to human microbiomes frequently (i.e., daily) and housed in captive only settings will exhibit altered gut microbial compositions and higher microbial diversity of human associated taxa than free-ranging lemurs that interact rarely with humans and maintain spatial proximity or no human contact. I also hypothesized that (ii) humans working in Duke Lemur Center departments with high levels of contact between NHPs will harbor an altered skin microbiome, composed of bacterial taxa associated with lemur species. To test this hypothesis, I collected fecal samples from four lemur populations, which included blue-eyed black lemurs (*Eulemur flavifrons*), Coquerel's sifakas *(Propithecus coquereli*), crowned lemurs (*Eulemur coronatus),* and ring-tailed lemurs (*Lemur catta*). This research was approved by the East Carolina University's Institutional Animal Care and Use Committee (IACUC) protocol number: AUP#P110. Human forehead and hand skin swab samples from 13 interns over five timepoints were provided voluntarily from the DLC summer interns who worked in various departments across the facility. These protocols were approved by East Carolina University's Intuitional Review Board (#UMCIRB 22-000802). The gradient of lemur-human interaction intensity across DLC department was done to ensure a variety of frequency of interaction with the lemur populations. There were seven husbandry interns, four research interns, one education intern and one paleo-primatology intern. I extracted genomic DNA from human skin and lemur fecal samples and conducted 16S rRNA gene PacBio HiFi

long-read sequencing. Results showed that human-associated taxa were observed to a greater extent in the gut microbiomes of enclosed lemurs where there was more frequent contact with humans. Variation in diversity was observed in the free-ranging, terrestrial population, *Lemur catta*, who I observed in the field as approaching gated areas and trails that people often occupied. In human forehead samples, taxa associated with the phylum Bacteroides were observed. Both lemur fecal samples and human skin samples harbored Bacteroides, demonstrating the presence of non-endemic microbes in lemur guts. In humans, this revealed that animal caretakers can exhibit altered microbial diversity due to contact intensity with lemurs since taxa from the Bacteroides phylum were observed in high relative abundance on the human skin microbiome of the animal husbandry interns only, which is contrary to past studies. Understanding the mechanisms of microbiome transmissions among humans and non-human primates is especially important for the conservation of captive non-human primates and the health of the humans managing captive populations. Transmission of microbes among humans and non-human primates can provide key information on how to best serve these captive populations and manage facilities to ensure the best outcomes for rehabilitation and conservation of species.

EXAMINING MICROBIAL TRANSFER BETWEEN HUMAN AND NON-HUMAN PRIMATES USING A ONE HEALTH APPROACH

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INTRODUCTION

The health of humans, wildlife, plants, and their environments are interconnected. This fundamental concept is essential for effectively promoting public health and conservation goals globally (Trinh et al., 2018). As such, the One Health approach explicitly addresses the interconnection of health between humans, plants, animals and their shared environments for protecting public health and supporting conservation goals on a global scale (Panel (OHHLEP) et al., 2022). The One Health approach can be useful in managing facilities to ensure the health and safety of animals, caretakers, volunteers, and the public, especially for critically endangered species. Captivity of endangered species in conservatory settings like animal sanctuaries and zoos give captive populations a chance at rehabilitation and survival, and humans the opportunity to observe and learn about their behavior, biology, and evolution (Moss and Esson, 2010). Specifically, the One Health approach guides the care and management of non-human primate (NHP) health in captivity; requiring humans to view health holistically, thus shaping the way we approach conservation strategies for NHPs in captivity (Cosby et al., 2024). This is particularly important for lemurs, which are considered the most threatened group of mammals on the planet. The five lemur families which inhabit Madagascar represent 20% of the world's primate species, and the role that conservatories and zoos play in the lemur conservation is critically important (Schwitzer et al., 2014).

Animal interactions at facilities such as zoos, conservation centers, and sanctuaries can directly influence both NHP and human behavior. Studies show that animal interactions can drive pro-conservation behavior in humans, specifically those that were able to experience and develop a connection with the animal during a visit (Skibins and Powell, 2013). But variation in species interactions of captive populations exists. For example, lemur populations, can perceive

human interactions differently. Lemurs in captivity are not only influenced by human interactions but habitat design and enrichment also play major roles (Collins et al., 2017). Free-ranging populations are not only given the chance to roam in a more natural setting but are also viewed more positively by public visitors. Naturalistic zoo-exhibit design provides direct benefits to captive animals by promoting naturalistic behaviors via more stimulating habitats (Chih Mun et al., 2013). The option to retreat from humans can provide the lemurs with a sense of security thus having a more positive effect on lemur behavior (Chih Mun et al., 2013). Management of facilities and their mission for providing environmental enrichment to lemur populations is key to successful research, care, and conservation. A study highlighting the Fota Wildlife Park in Ireland, analyzed the effects of visitors on ring-tailed lemurs housed in a 'walkthrough,' free-ranging environment. This study found that the lemurs were more likely to exhibit stressful behaviors when visitors were present; however, in the absence of visitors, lemurs exhibited normal behavior (Collins et al., 2017). In addition, researchers cited that the lemurs were affected by environmental variables in the same way, favoring endemic conditions over the latter, thus reiterating the need for facility founders and managers to understand the full scope of lemur behaviors in the wild and in captivity, and the effects of conditions on lemur health (Collins et al., 2017). Behavioral patterns of lemurs in captivity can be observed across species. For example, crowned lemurs (*Eulemur coronatus*) at the Newquay Zoo in the United Kingdom, were observed performing preferential behavior to visitors engaging in feeding activity over zookeepers who had scheduled interaction with the lemurs (Jones et al., 2016). Over the study, it was concluded that crowned lemurs exhibited altered behaviors to recognizable humans over those unfamiliar and gave insight to habitual behavioral changes for lemurs in captivity (Jones et al., 2016). The effects of habitat types and human interactions on the

behaviors of captive lemurs are a crucial factor to the environmental aspect of the One Health strategy.

In addition to species interactions, the environment, which includes abiotic geochemical resources and living components, influences plants and animals. Specifically, the soil is a source of commensal, beneficial, and pathogenic microorganisms that are recognized as key drivers of health across populations (Banerjee and van der Heijden, 2022). It is documented that microbiome exchange occurs between the environment and hosts and between hosts (Trinh et al., 2018). For example, among free-ranging baboons (*Papio nubis* and *P. cynocephalus*), soil properties explain variation in gut microbial communities more than diet or social interactions (Grieneisen et al., 2019). Movement and direct contact with soil can facilitate exchange between animal and soil microbiomes.

Another connection of the environment to the host is through diet. Diet directly influences the gut microbial microbiome and diet changes in captivity can lead to the loss of endemic microbes (Greene et al., 2021). Westernization of human diets has been paralleled to captivity in NHPs as gut composition changes. Bacteria in the genera *Bacteroides* and *Prevotella* dominate the human gut microbiota, and these bacterial taxa have been observed in higher abundances in captive compared to wild NHPs (Clayton et al., 2016). Discussions surrounding gut-dysbiosis and captivity are shifting to include environmental variables and the concept of cross-species exchange to account for the vast diversity of microbes that can be present in captive habitats. Captive populations that consume less fiber, mimic the human microbiome, while wild and free-ranging captive populations that consume more plant material and soil have higher fiber consumption (Clayton et al., 2016). Fiber consumption has been highlighted as a baseline for understanding gut microbial diversity across species. *Eulemur coronatus* and *Eulemur flavifrons*

have similar dietary composition as they are both frugivorous and consume fiber in lower abundances. Anatomically, these lemurs have shorter gastrointestinal tracts and exhibit a greater abundance of simple-fiber metabolizers including a variety of *Prevotella species* (Greene et al., 2019). In contrast, Coquerel's sifakas *(Propithecus coquereli)* are frugo-folivorous in the wild, and in captivity, this species is supplemented by seasonal foliage and orchard vegetables. This diet contributes to greater signatures of fiber degradation, the loss of endemic microbes, and an increase in low-abundance taxa suggesting that captive populations have an overall advantage at gaining a diverse group of microbes (Greene et al., 2021). A recent study from the Duke Lemur Center, found that Coquerel's sifaka in forest enclosures adapted natural foraging strategies of young and mature leaves, fruits, nuts, and flowers from 39 plant species (Greene et al., 2023). This reiterates the need for knowledgeable facilities management and the importance of natural behaviors being retained in captive settings.

Understanding the role of microorganisms in maintaining both human and animal health and as participants in a myriad of ecological processes, will help tailor the use of One Health strategies in conservation. Since microbial community assembly depends on dispersal, drift, and selection, discussions on microbiome composition rely on understanding the relative importance of these different processes (Nemergut et al., 2013). We know that host-associated microbiomes are essential for improved host nutrition and metabolism, immune function, mood, and physical health (Lynch and Pedersen, 2016). But when host microbiome composition is disrupted, loss of beneficial function and potential for gains in unwanted functions can result (Belizário and Faintuch, 2018). Cross-species exchanges of gut microbes could provide insights into how interactions with other animals influence human health, behavior, and physiology (Moran et al., 2019). The intensity of cross-species interactions with interaction frequency can be seen as a

benchmark of microbial exchange between humans and NHPs. There are minimal studies of this transmission, but the primary evidence for cross-species exchange of microbes is seen from pets in the home. Humans and their pet dogs and cats share similar gut microbes which demonstrates that the exchange from other species is possible (Song et al., 2013). The significance of gut microbiome exchange between humans and NHPs would be much greater due to close evolutionary relations and similar physiologies that humans and NHPs share. Understanding the mechanisms of microbiome transmissions among humans and NHPs using a One Health framework is especially important for the conservation of endangered captive NHPs and the health of the humans managing captive populations (Amato et al., 2019).

While we know that gut dysbiosis can be dependent on factors such as inter-species interactions, environment, and diet, more work examining cross-species interactions and microbial exchanges is needed to understand how to better serve animals in captivity and their human caretakers. In this thesis, I ask the research question how does the intensity of human and lemur interactions influence the human microbiome? The project goal was to understand the extent that microbiome exchange occurs between humans and NHPs at the Duke Lemur Center (DLC) in Durham, North Carolina. The DLC facilitates the conservation of NHPs and houses ~250 lemurs across 18 species. The DLC exhibits the widest diversity of lemurs in the world, outside of their endemic home of Madagascar (Yoder, 2017). The DLC aims to further our understanding of the biology and behavior of lemurs to support vibrant lemur communities and has done so successfully since their establishment in 1966 (Yoder, 2017). The specific objectives of this study were to (1) characterize lemur gut and human skin microbiomes and (2) examine microbiome transfer between human and NHP hosts. I hypothesized that (i) lemurs exposed to human microbiomes frequently (i.e., daily) and housed in captive only settings will exhibit

altered gut microbial compositions and higher microbial diversity of human associated taxa than free-ranging lemurs that interact rarely with humans and maintain spatial proximity or no human contact. I also hypothesized that (ii) humans working in Duke Lemur Center departments with high levels of contact between NHPs will harbor an altered skin microbiome, composed of bacterial taxa associated with lemur species.

METHODS

Experimental Design

I collected fecal samples from the Duke Lemur Center in Durham, NC, at the end of May 2022 throughout July 2022. I collected 5 fecal samples per individual for the following species: blue-eyed black lemurs (*Eulemur flavifrons*), crowned lemurs (*Eulemur coronatus*), Coquerel's sifakas (*Propithecus coquereli*) and ring-tailed lemurs (*Lemur catta*). This research was approved by the East Carolina University's Institutional Animal Care and Use Committee (IACUC) protocol number: AUP#P110. Each of these species lives in various environments from enclosed habitats to free-ranging terrestrial and arboreal habitats. Feces were collected immediately after defecation and transferred into labeled sample tubes with 95% ethanol as solution (Amato et al., 2013). The human subjects research involved the collection of biological samples and human survey data. These protocols were approved by East Carolina University's Intuitional Review Board (#UMCIRB 22-000802). Human skin samples were collected from 13 summer interns working at the Duke Lemur Center. Participants were asked to voluntarily complete questionnaires as an intake benchmark. To represent a gradient of interaction intensity, interns were chosen across departments. There were seven husbandry interns, four research interns, one education intern, and one paleo-primatology intern. Five timepoints throughout the summer were selected as sampling dates; June 1st, June 7th, June 14th, June 28^{th,} and July 26th. These dates represented different phases of the internship with June 1st being one week prior to the start, June 7th was the start date of the internship and the dates following were 2, 4-, and 8-week increments. For the human samples, I asked participants to dip the swab heads into a buffer solution, vigorously swab the forehead or palms and rotate the swab heads for 1 min., and store the swabs in a collection tube with buffer solution. I stored all sample types at -20 °C until the end of the

internship program and then transferred samples to -80 °C until DNA extraction was completed.

DNA Extraction

I extracted DNA from a subset of the human swab samples, specifically forehead, collected on dates June 1st, June 14th and July 26th to represent the beginning, middle, and end of the internships program. Human skin and lemur fecal samples were extracted using the Qiagen PowerSoil Kit following the manufactures protocol. Human swabs were extracted with modifications according to published methods (Manus et al., 2022). The Katherine Amato lab extracted DNA from lemur fecal samples using the Qiagen DNeasy PowerSoil Pro Kit.

Amplicon Sequencing of Human and Lemur Microbiomes

I used the DNA extracted from the human skin swabs and the lemur feces obtained from the template in PCR reactions. The DNA was amplified in a 48-well plate with unique barcoded primers in each well. Primers were pre-loaded into each well thus making the location of each sample correspond to its unique barcode. To obtain PCR products that were amplified twice (50µL PCR libraries), PCR reactions were completed under the conditions of The Earth Microbiome Project library preparation using modified 515F/926R primer adapters '/5Phos/GCATCCACTCACGTGTGATATGTGYCAGCMGCCGCGGTAA' (forward) and '/5Phos/GCATCCGCTGTATACACGCTCCCRAMCTGTCTCACGACG' (reverse) to target the full length 16S rRNA gene (Parada et al., 2016; Quince et al., 2011). These amplicons were sequenced with the HiFi mode using PacBio's latest sequencing platform, the PacBio Revio, which produces amplicons that are 10-30kb long with a 99.999% accuracy. The use of PacBio full-length sequencing enables a higher resolution taxonomic evaluation of human skin and

lemur fecal samples. This method is known to produce a more accurate view of the microbial community and higher percentage of classifications to the species level compared to sequencing variable regions of the 16S rRNA gene (Castinel et al., 2022).

Processing Amplicon Sequences

I used the DADA2: Fast and accurate sample inference from amplicon data with single-nucleotide resolution pipeline to process the amplicon sequences (Callahan et al., 2016). Amplicon sequence variants (ASVs) were identified using the dada2 pipeline and QIIME 2 (Hall and Beiko, 2018). The dada2 pipeline was employed to align demultiplexed sequences, denoise ASVs, filter and trim and assign taxonomy. The forward and reverse sequences were aligned using 'CutAdapt' and the dada2 workflow was followed to obtain a feature table, taxonomy table, and ASV table. These outputs were then read into the R statistical environment (Rv4.4.1, RStudio version 2024.04.2+764) and used in a QIIME 2 workflow with packages such as phyloseq (McMurdie and Holmes, 2013) , ggplot2 (Wickham, 2016) , tidyverse (Wickham et al., 2019) and vegan (Oksanen et al., 2012).

Statistical Analyses

For statistical analyses, I used R to compute bacterial species diversity metrics (e.g., Shannon H' diversity) and compute Bray-Curtis dissimilarity and weighted and unweighted UniFrac distances (Lozupone et al., 2011) to account for phylogenetic relationships between bacteria. I compared lemur fecal bacterial Shannon diversity across environment type (free-ranging (arboreal/terrestrial) and enclosed) and across lemur species bacterial species diversity (across lemur species) using the analysis of variance (ANOVA) with species and habitat as fixed effects.

I also compared the human skin microbiome across intern departments using an ANOVA with intern department as the fixed effect.

To test for beta diversity, unweighted and weighted UniFrac distances were computed. Unweighted distances accounted for ratio of the presence/absence of a bacterial species to the phylogenetic branch length and weighted distances accounted for species relative abundance metrics to branch length, corrected with abundance difference. I visualized the composition of the lemur and human skin bacterial communities using a principal coordinates analysis (PCoA) based on a Bray-Curtis dissimilarity to examine lemur bacterial communities across species and habitat and examine human skin bacterial communities across intern departments. I used the permutational multivariate analysis of variance (PERMANOVA) to measure the variation in lemur fecal bacterial community composition according to habitat type, human contact intensity, and species difference. I also ran a PERMANOVA to examine the variation in human skin bacterial community composition according to DLC intern department.

RESULTS

I characterized lemur gut and human skin microbiomes to test the hypothesis that lemurs exposed to human microbiomes frequently (i.e., daily) and housed in captive only settings will be more distinct compared to free-ranging lemurs. The lemur microbial diversity measured using the Shannon's diversity index and the Simpson's Evenness index, tended to be higher for lemurs that lived in enclosed environments compared to free-ranging arboreal and free-ranging terrestrial populations (ANOVA, Shannon: $F_{2,51}$=7.069, P=0.002; Simpson: $F_{2,51}$=4.282, P=0.019) (Fig. 2, Table S1). Host species identity influenced lemur fecal microbial diversity to a lesser degree (ANOVA, Shannon population: $F_{1,51}$=3.036, P=0.087; Simpson population: $F_{1,51}$=3.145, P=0.082) (Fig. 2, Table S1). According to lemur species, microbial alpha diversity was highest in *E. coronatus* and *E. flavifrons* compared to *L. catta* and *P. coquereli* (Fig. 3). As expected, lemur species identity influenced gut microbial community composition (PERMANOVA, species: R^2=0.076, P=0.001; Fig. 4; Table S2). The lemur populations that were free-ranging and arboreal were the most distinct from the closed and free-ranging terrestrial (PERMANOVA, population: R^2=0.229, P=0.001; Fig. 4). An unexpected observation was the similarity among the gut microbiomes of free-ranging *L. catta* and enclosed *E. coronatus* species (Fig. 4). In the field, I observed that the ring-tailed lemurs more readily interacted with humans. For example, *L. catta* individuals were observed approaching the gated areas and feeding areas when humans were present, while *E. coronatus* individuals were housed in a walk-out caged setting and a dual cage setting, which is public facing.

I also characterized the human forehead microbiomes according to the department where the interns worked over 8 weeks. For the skin microbiome, I observed a variable impact of department influence on Shannon's diversity and Simpson's evenness (ANOVA, Shannon:

$F_{2,51}$=1.737, P=0.202; Simpson: $F_{2,51}$=2.528, P=0.105) (Fig. 5, S1). The associated DLC department did however influence the human forehead microbiome. The microbial community composition of the interns in closer proximity to lemurs (Research, Husbandry) are more similar in composition compared to interns that worked in the Administration department (education, paleo-primatology) (Fig. 6; PERMANOVA, department: R^2=0.105, P=0.027). An unexpected result was the overall bacterial community composition pattern which showed points with similar compositions regardless of collection date and population (Fig. 6; PERMANOVA, department: R^2=0.049, P=0.958).

The five main bacterial species represented in the lemur gut microbiome were from the phyla Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria, and Spirochaetota (Fig. S1, S3). In captive lemurs, taxa from the phyla Bacteroidetes and Firmicutes, are seen more frequently, as they are a nonendemic taxa for the lemur gut and are present in the human gut microbiome. The taxonomic distribution of the human skin microbiome included taxa from the phyla Actinobacteriota, Firmicutes, and Bacteroidetes (Fig.2, S4). Specifically, the abundance of species from the phylum Bacteroidetes in lemurs was higher than in humans (Fig. S1, S3). A similar pattern was observed when viewing the abundance of Actinobacteria from humans (Fig S2, S4).

Comparatively, both lemur gut and human skin microbiomes exhibited shared bacterial species, specifically those from the Firmicutes and Bacteroidetes phyla. (Fig.S3, S4). Lemur fecal samples showed a high abundance of taxa from Bacteroidetes and the human skin samples from the Husbandry and Research departments showed the presence of Bacteroidetes, demonstrating that the frequency of interaction between human caregivers and primates can alter their microbiome and the transmission of microbes can occur

DISCUSSION

The goal of this project was to examine the extent that microbiome exchange occurs between humans and lemurs at the Duke Lemur Center. I tested the hypothesis that lemurs exposed to human microbiomes frequently (i.e., daily) and housed in captive only settings will exhibit altered gut microbial compositions and higher microbial diversity of human associated taxa than free-ranging lemurs that interact rarely with humans and maintain spatial proximity or no human contact. Based on the results, the lemur populations that were exposed to humans more frequently (enclosed habitat, free-ranging terrestrial) did exhibit altered gut microbiome composition and had a higher diversity of microbes compared to populations with less frequent interaction (free-ranging arboreal) (Fig. 2). It is documented that captive NHPs exposed to more human-like conditions and diets are expected to exhibit a higher amount of shared bacterial species with humans (Manara et al., 2019). I predicted based on previous studies at the Duke Lemur Center, there would be approximately 5 bacterial species shared across the sample populations from different habitats. The five main bacterial species (ASVs) represented in the lemur gut microbiome were from the Phylum Bacteroidetes, Cyanobacteria, Firmicutes, Proteobacteria and Spirochaetota (Fig.S3). Presence of Bacteroides is indicative of lemurs in captivity as in previous studies, it is documented that lemurs in captivity lose their native microbiome and become dominated with bacteria from the genera *Prevotella* and *Bacteroides*, which are dominant genera from the human gut microbiome (Clayton et al., 2016).

The Coquerel's sifakas represent the free-ranging arboreal population, and here had the higher alpha diversity of the free-ranging lemurs. As they are a frugo-folivorous and foraging population, their diet variability allows them to be introduced to a wider range of microbes than those consuming primarily captive diets (Greene et al., 2021). The ring-tailed lemurs were

observed with more similar composition to enclosed populations. Through field observations, ring-tailed lemurs tended to be closer to gated and trail areas, thus encountering humans more often than the arboreal population. The diets of the blue-eyed black (*Eulemur flavifrons*), crowned (*Eulemur coronatus*), and ring-tailed lemurs (*Lemur catta*) are more similar in composition as they are primarily herbivores. Ring-tailed lemurs tend to consume diets higher in fruits and/or carbohydrates as preferential diets have been seen in captive populations of this species (Hansell et al., 2020). This is in alignment with my results of ring-tailed lemur microbial diversity being closer to that of the enclosed populations at the DLC.

I also tested the hypothesis that humans working in Duke Lemur Center departments with high levels of contact between NHPs will harbor an altered skin microbiome, composed of bacterial taxa associated with lemur species. Based on the results, interns in departments with higher contact intensity tended to exhibit a higher microbial diversity than those with lesser contact. Specifically, husbandry interns exhibited the widest range of microbial diversity which aligns with the job classification as they directly cared for the lemurs. Administrative jobs included both the education and paleo-primatology department which spent more time away from lemur populations. Based on the results, these populations exhibited the lowest microbial diversity. Previous studies that focused on characterizing the human skin microbiome have found three indicator species that are of high relative abundance; these species are *Staphylococcus epidermidis*, *Corynebacterium spp.*, and *Propionibacterium acnes* (Ross et al., 2018). Therefore, I expected the human skin samples of interns in frequent contact with NHPs to encompass these human-associated bacterial taxa. The human skin microbiome was dominated by bacterial taxa from the phyla Actinobacteria, Firmicutes, and Bacteroidetes. It is striking that these gut-specific bacterial taxa were identified abundantly in adult skin microbiomes. Variations in human skin

microbiota are explained by intrinsic and extrinsic factors. This variation begins in the womb, as infants acquire microbes from their mother. Birthing method, such as vaginal delivery versus Cesarean delivery can impact the diversity of microbes in infants (Skowron et al., 2021). It is documented that infant skin harbors a microbiome consisting of taxa primarily from the mother with higher signatures of bacteria from the phyla Firmicutes and Bacteroidetes; however as we age our skin microbiome shifts to include higher proportions of bacterial from the phyla Actinobacteria and Proteobacteria. (Skowron et al., 2021). Host physiology is an intrinsic factor that can impact the diversity of the skin microbiome as studies highlight oily skin areas such as the forehead, to harbor more lipophilic bacteria compared to drier regions such as the forearm, which can possess a more diverse microbiome (Dimitriu et al., 2019). The use of antibiotics, over-the-counter medications, skin care and lifestyle are all extrinsic factors that can impact the skin microbiome (Ferček et al., 2021). Environmental conditions directly affect the skin microbiome. Research suggests that exposed body sites harbor a higher diversity of microbes compared to those that are covered. It is also documented that networks of bacteria collected from exposed sites show higher connectivity (Dimitriu et al., 2019). Host physiology, diet, and environmental effects influence the human skin microbiome, and underscores the use of the One Health approach for understanding human health.

Understanding microbial diversity, variation, and transmission across human and animal populations is an undertaking that many scientists have dedicated their lives to learning and contributing to. In this project, I was able to gain a deeper interpretation of human skin and lemur fecal microbiomes. One Health allows us to view variation across factors that are all interconnected. To gain a comprehensive understanding of the impacts of microbiome exchange

on host health, we must view health holistically and take measures to protect not only ourselves but the environments we interact with.

LIMITATIONS OF EXPERIMENT

As the sampling of human biological data was completed by interns, voluntarily, under (#UMCIRB 22-000802), even sample collection across departments could not be ensured. Participants were given protocols to follow when sampling either their forehead or palm, however, I observed variations in 'swab dirtiness', suggesting that some skin samples were more robust than others. Provided human sample data was used in groups, by department, to provide a more even sampling as explained in the experimental design.

FUTURE DIRECTIONS

This project characterized the lemur gut microbiome and the human skin microbiome to understand the extent that microbiome exchange occurs between humans and NHPs, at the Duke Lemur Center. The specific objectives were to (1) characterize lemur gut and human skin microbiomes and (2) examine microbiome transfer between human and NHP hosts. Additional analysis needs to be done to understand the extent of microbiome transfer between human and NHP hosts as the presence of non-endemic taxa (i.e., Bacteroidetes) were observed in the lemur gut and were also present on the human skin microbiome (Figure S3).

As we gain a deeper understanding of how captive NHP populations are influenced not only by habitat but also diet, analyses on the functionality of newly introduced microbes will provide a deeper look at the implications of microbiome transfer between hosts. Plant data from not only the foods provided to lemurs at the DLC but also environmental components could provide additional insight into the lemur gut microbial composition. Previous studies have shown that lemur–plant interactions were highly trait structured, and the loss of both lemur and plant species threatened the establishment of mutualistic association (DeSisto and Herrera, 2022). The thesis

project provides a benchmark for understanding microbiome transfer between human and NHP hosts in captivity, which aids the conservation and management strategies of captive lemurs.

FIGURES

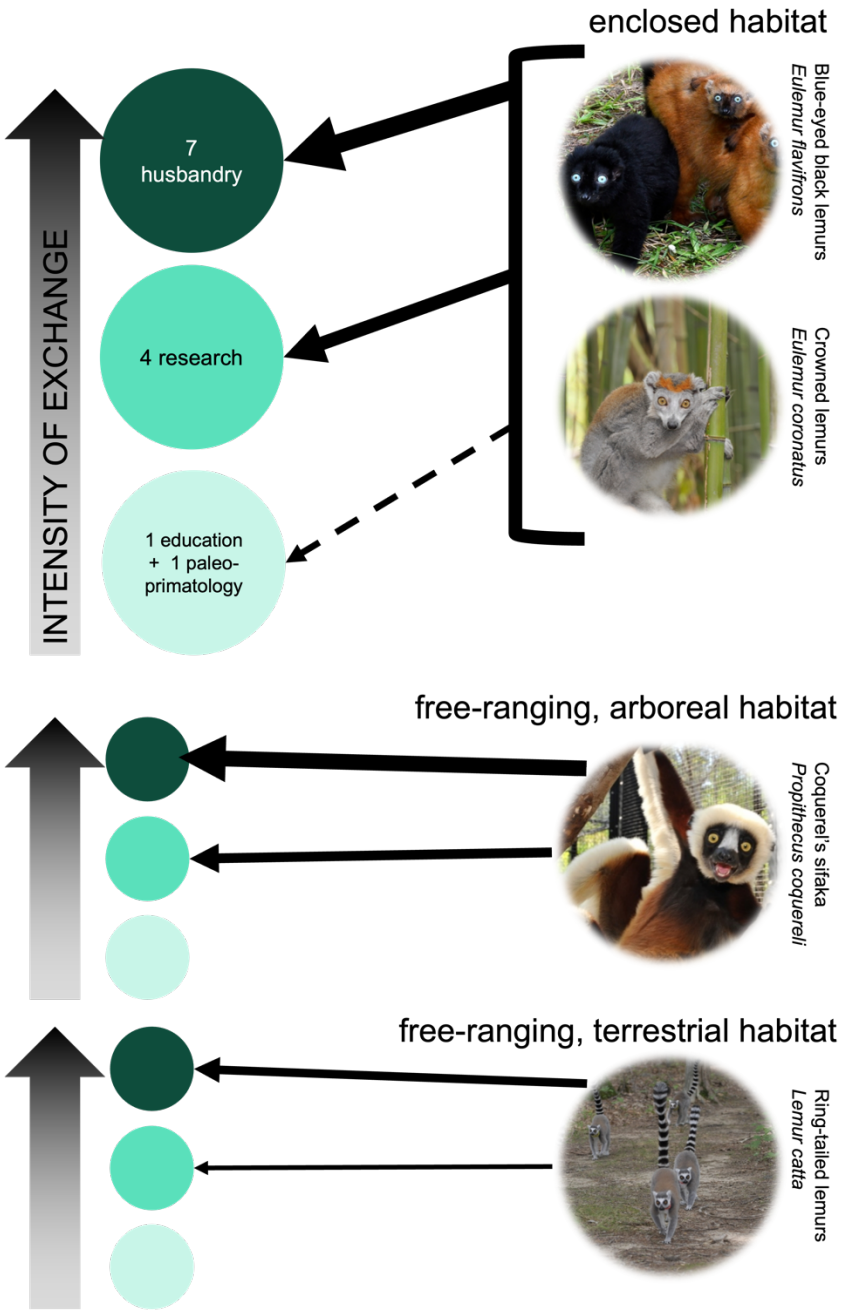

Figure 1. Experimental design diagram that represents 13 interns who participated in human microbiome sampling. Seven of the interns were in the husbandry department, encompassing the highest amount of contact, represented by the bold solid arrow, with the lemur population across both free-ranging and enclosed habitats. Four interns were in the research department, exhibiting the second highest amount of contact with lemur populations, represented by the thin bolded arrow. The last two interns were in the education and paleoanthropology department (administrative), located in the office space(s) at the DLC, represented by the dashed arrow, the lowest amount of contact.

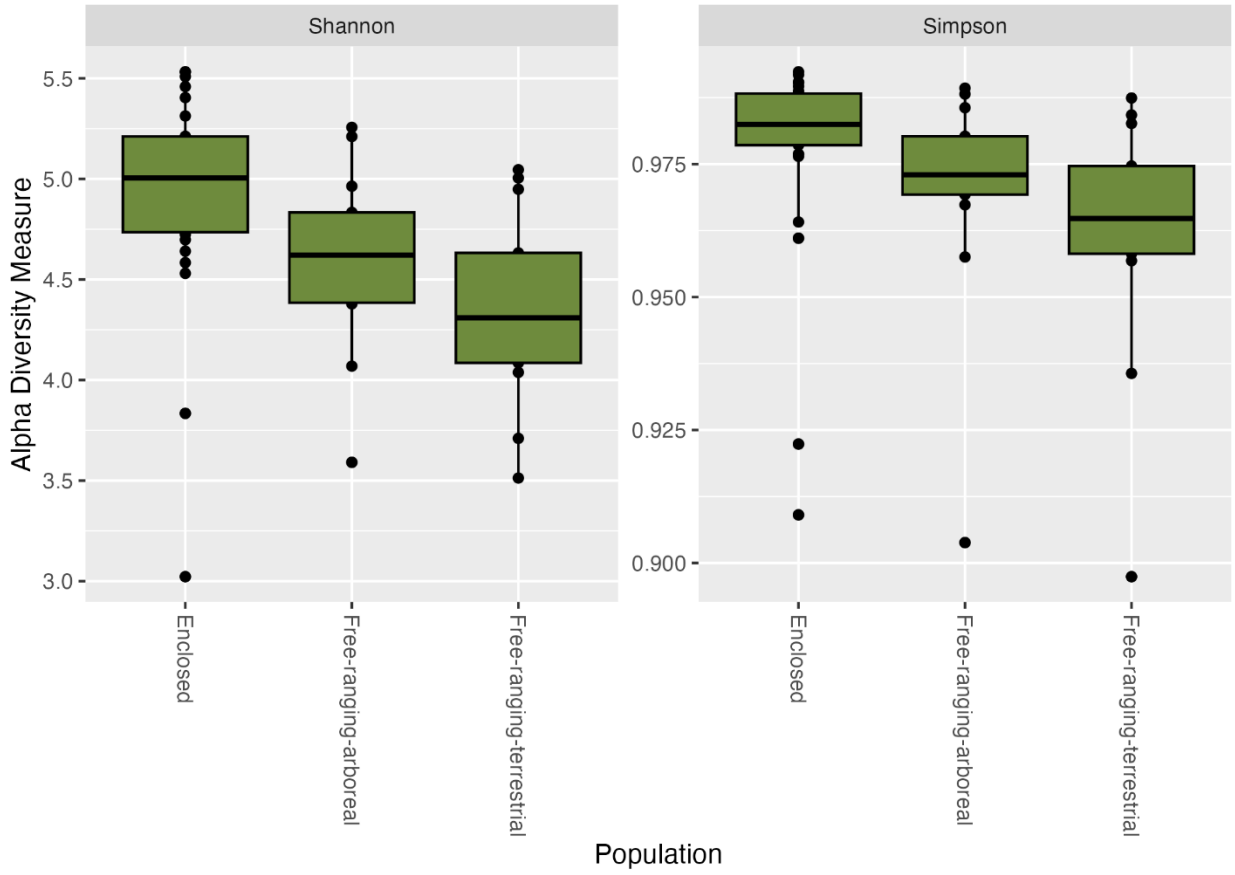

Figure 2. Boxplots summarizing the lemur gut microbial diversity based on the Shannon Diversity Index (H') and the Simpson's Evenness Index (1-D) according to the environment where the lemur populations (Enclosed only, Free-ranging arboreal, Free-ranging terrestrial) spends time. The boxplot is a visual representation of 5 key summary statistics: the median, the 25% and 75% percentiles, and the whiskers, which represent the feasible range of the data as determined by 1.5 × the interquartile range. Symbols represent individual data points.

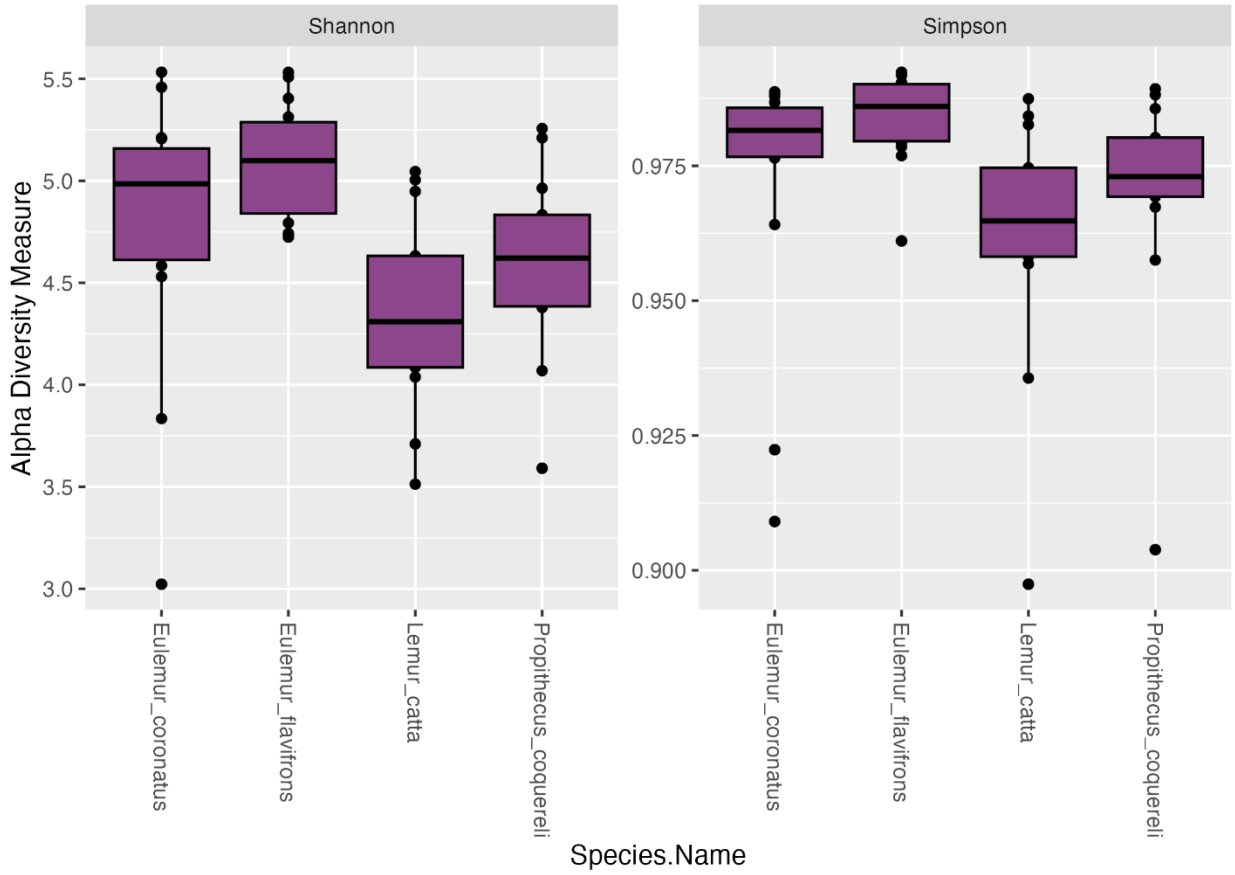

Figure 3. Boxplots for lemur gut microbial diversity based on the Shannon Diversity Index (H') (left) and Simpson's Evenness Index (1-D) (right) according to species. The boxplot is a visual representation of 5 key summary statistics: the median, the 25% and 75% percentiles, and the whiskers, which represent the feasible range of the data as determined by 1.5 × the interquartile range. Symbols represent individual data points.

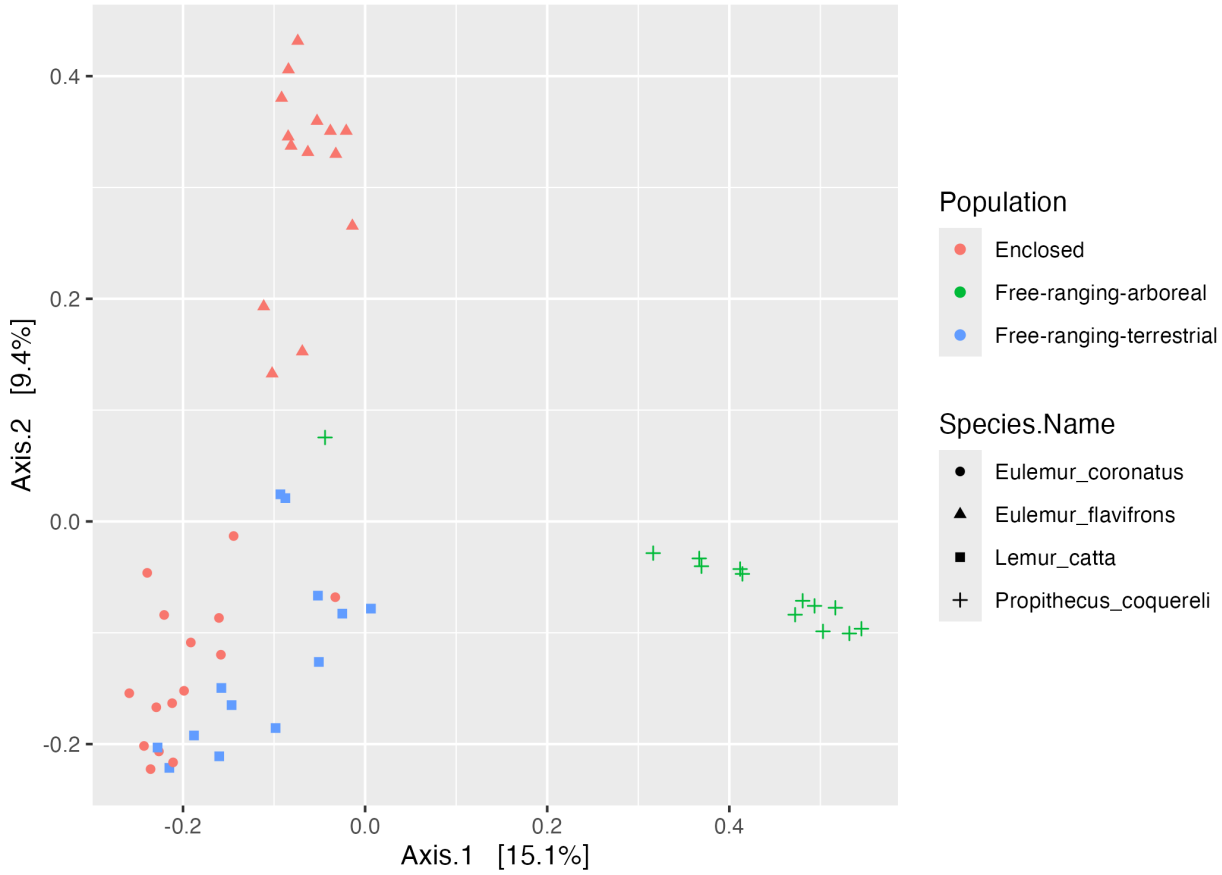

Figure 4. Ordination based on a principal coordinates analysis (PCoA) depicting microbial community composition according to lemur population. Symbols are colored according to population (red = Enclosed, green = Free-ranging arboreal, blue = Free-ranging terrestrial) and shape according to lemur species (circle = *Eulemur coronatus*, triangle = *Eulemur flavifrons*, square = *Lemur catta*, plus = *Propithecus coquereli*).

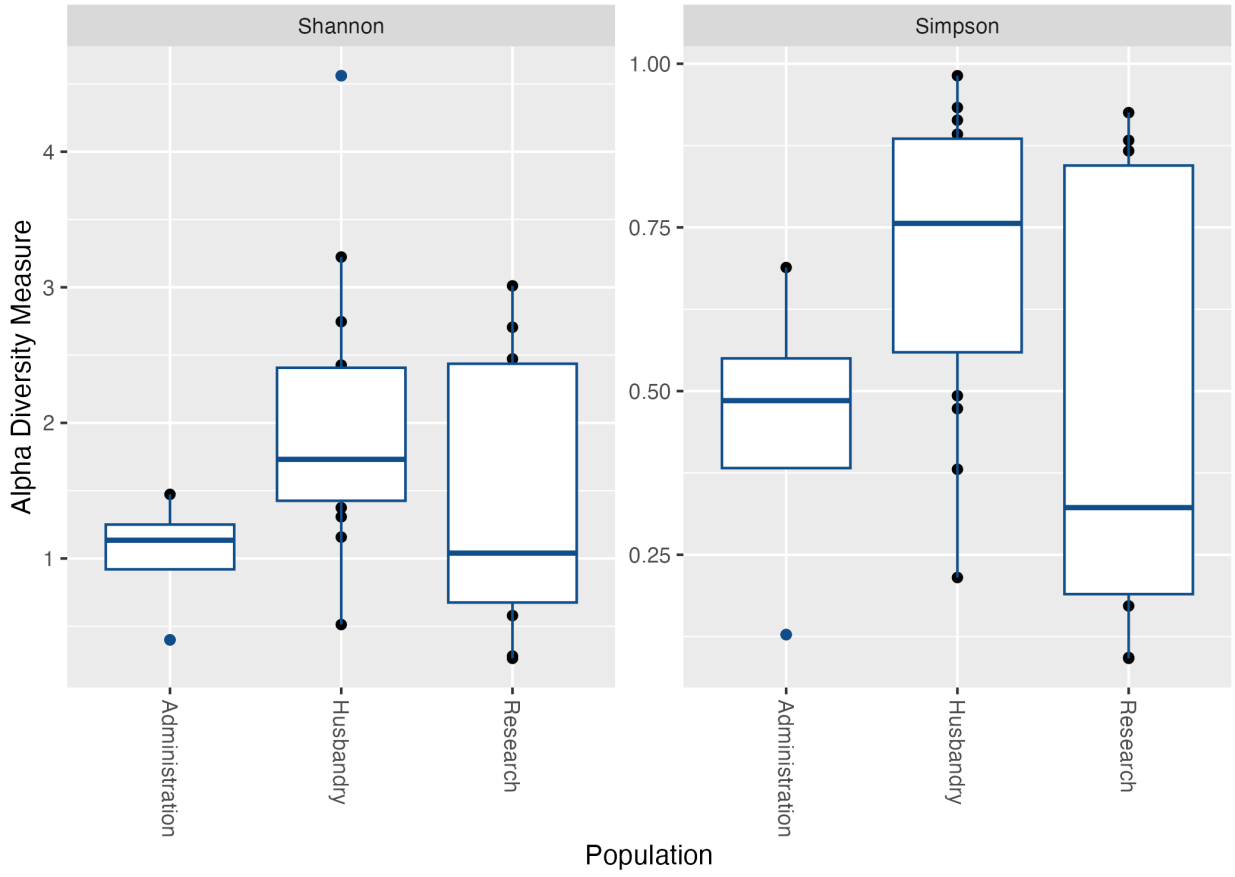

Figure 5. Boxplots summarizing the human skin microbial diversity based on the Shannon Diversity Index (H') and Simpsons Evenness Index (1-D) according to the DLC department where the intern worked (Administration, Husbandry, Research). The boxplot is a visual representation of 5 key summary statistics: the median, the 25% and 75% percentiles, and the whiskers, which represent the feasible range of the data as determined by 1.5 × the interquartile range. Symbols represent individual data points.

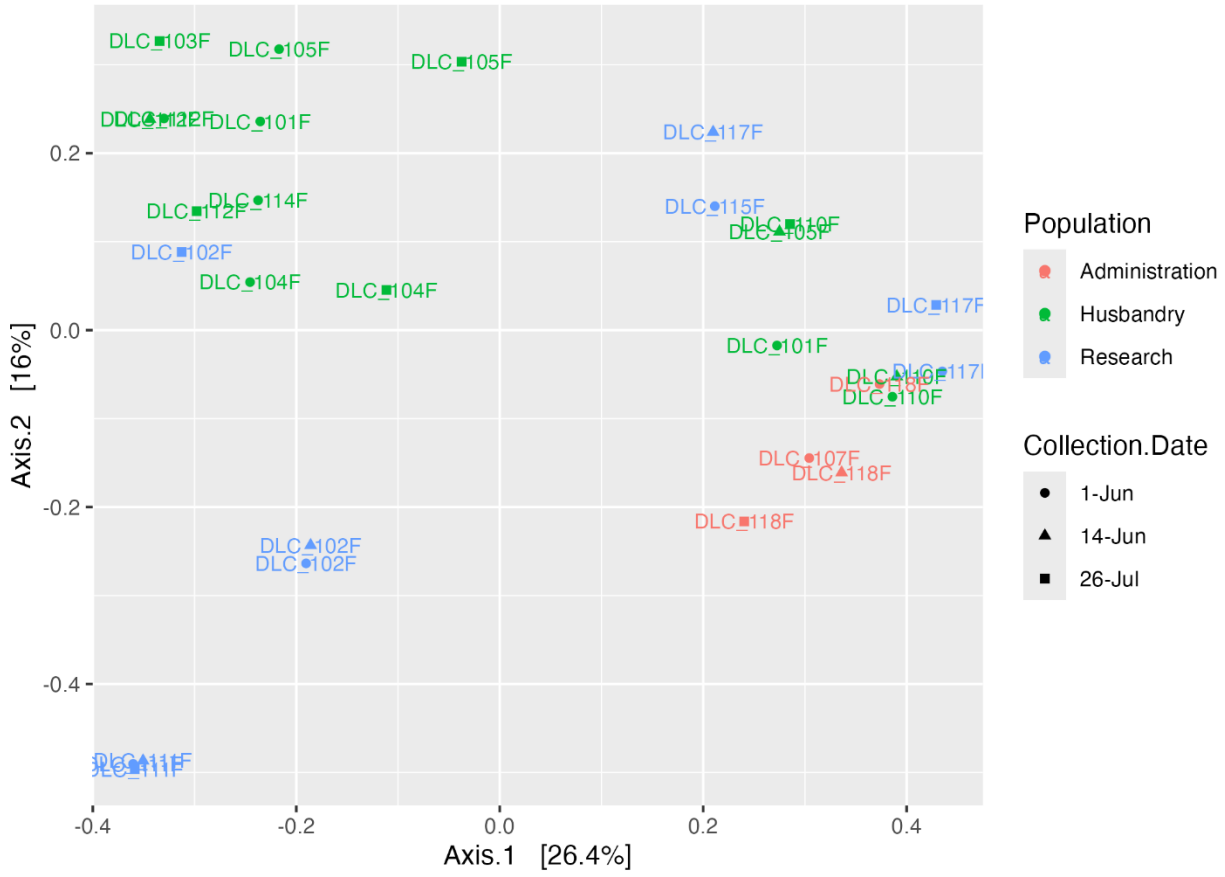

Figure 6. Ordination based on a principal coordinates analysis (PCoA) depicting human skin microbial community composition according to intern department. Symbols are colored according to intern population (red = Administration, green = Husbandry, blue = Research) and shape according to sample date (circles = June 1st, triangle = June 14th square = July 26th).

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APPENDIX A: SUPPLEMENTAL MATERIAL

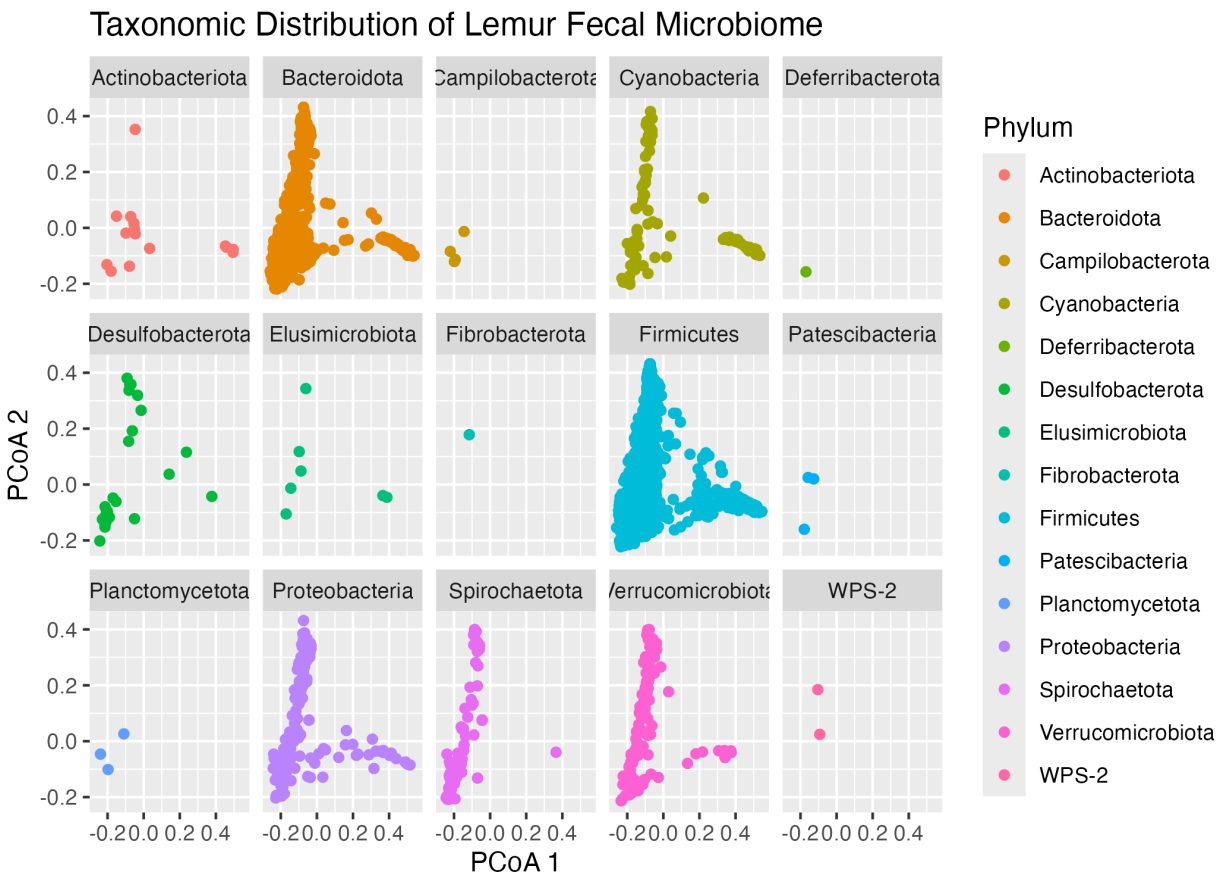

Figure S1. Ordination based on a principal coordinates analysis depicting the taxonomic distribution of the lemur gut microbiome. Each point represents a unique amplicon sequence variant (ASV) identified in the data set and grouped and colored according to phylum-level classification.

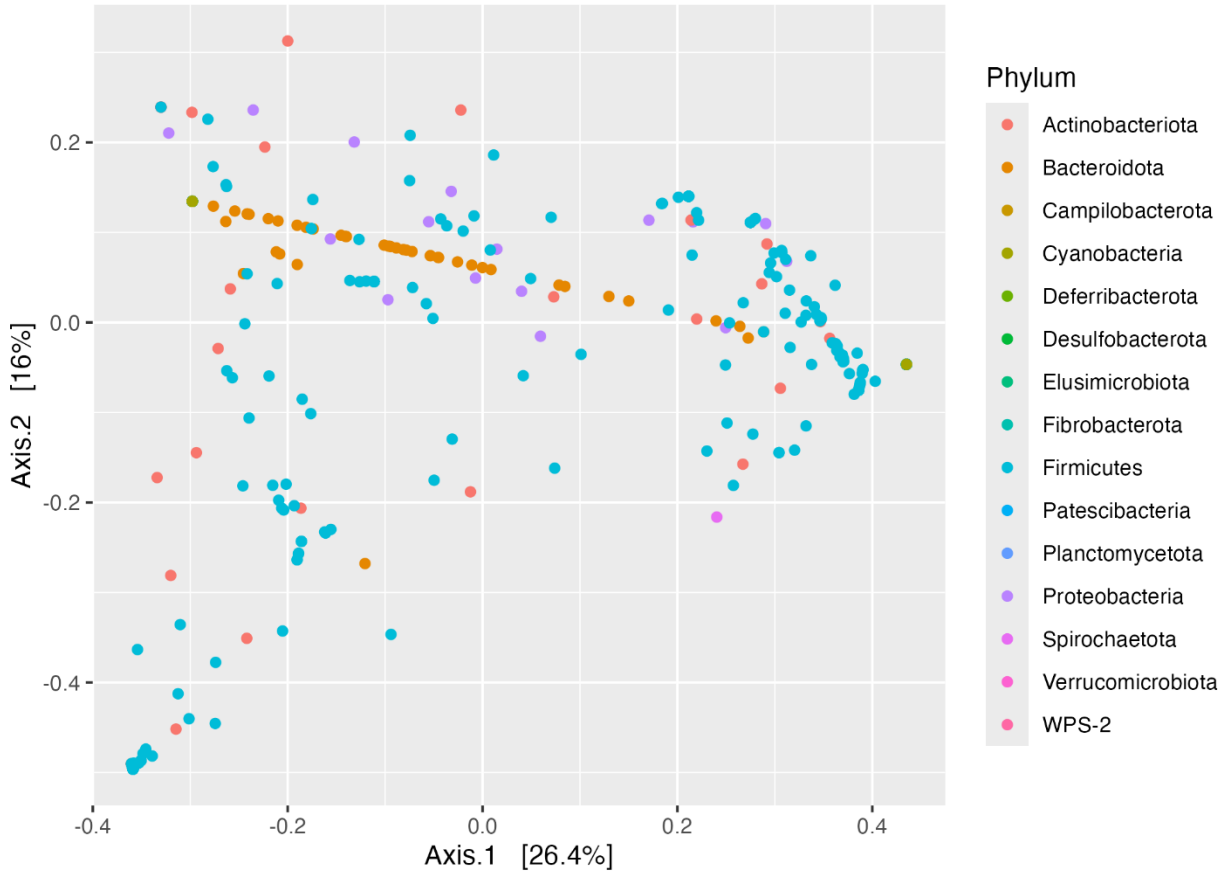

Figure S2. Ordination based on a principal coordinates analysis (PCoA) depicting the taxonomic distribution of the human skin microbiome. Each point represents a unique amplicon sequence variant (ASV) identified in the data set and grouped and colored according to phylum-level classification.

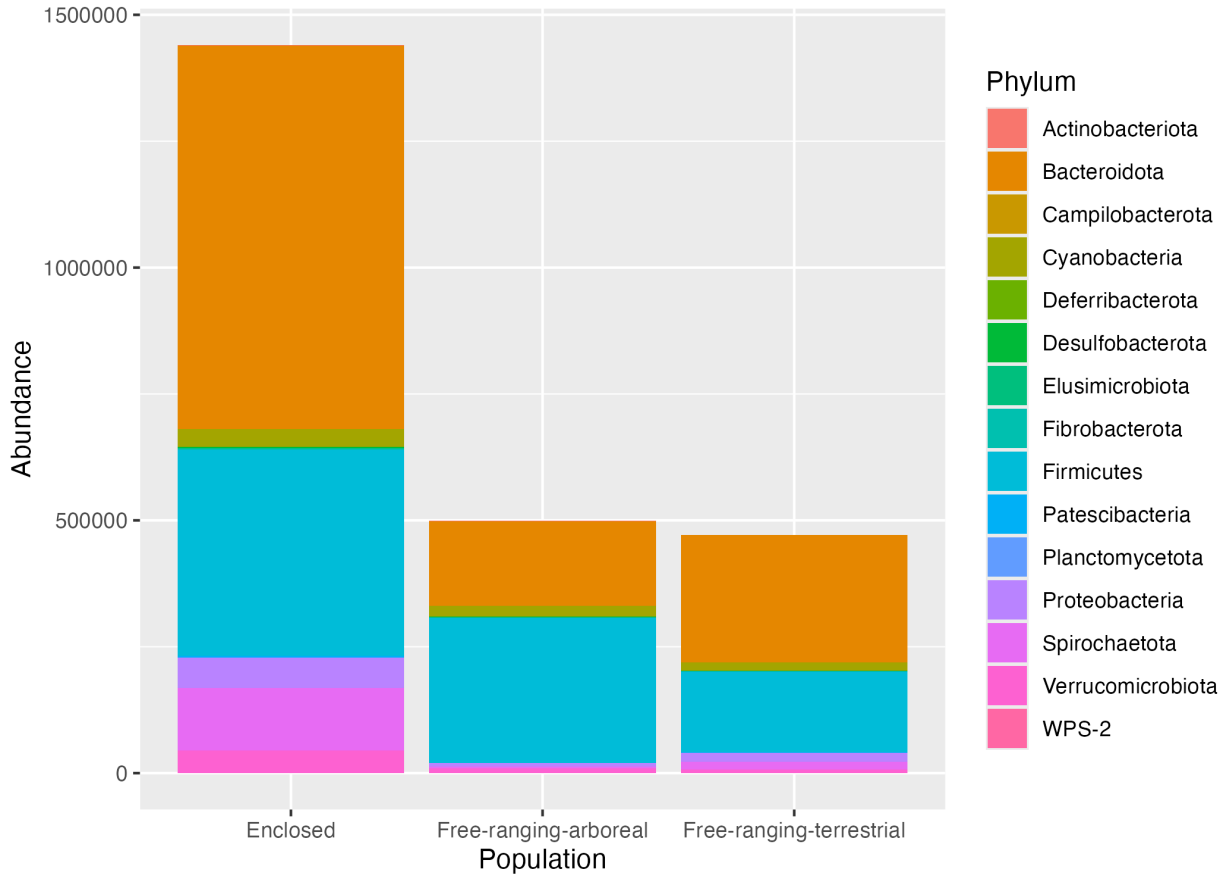

Figure S3. Stacked abundance bar chart based on taxonomy abundance of the lemur gut microbiome. Each bar represents a population (ie. Enclosed, Free-ranging-arboreal, free-ranging-terrestrial). Bacterial taxa are grouped and colored according to phylum-level classification.

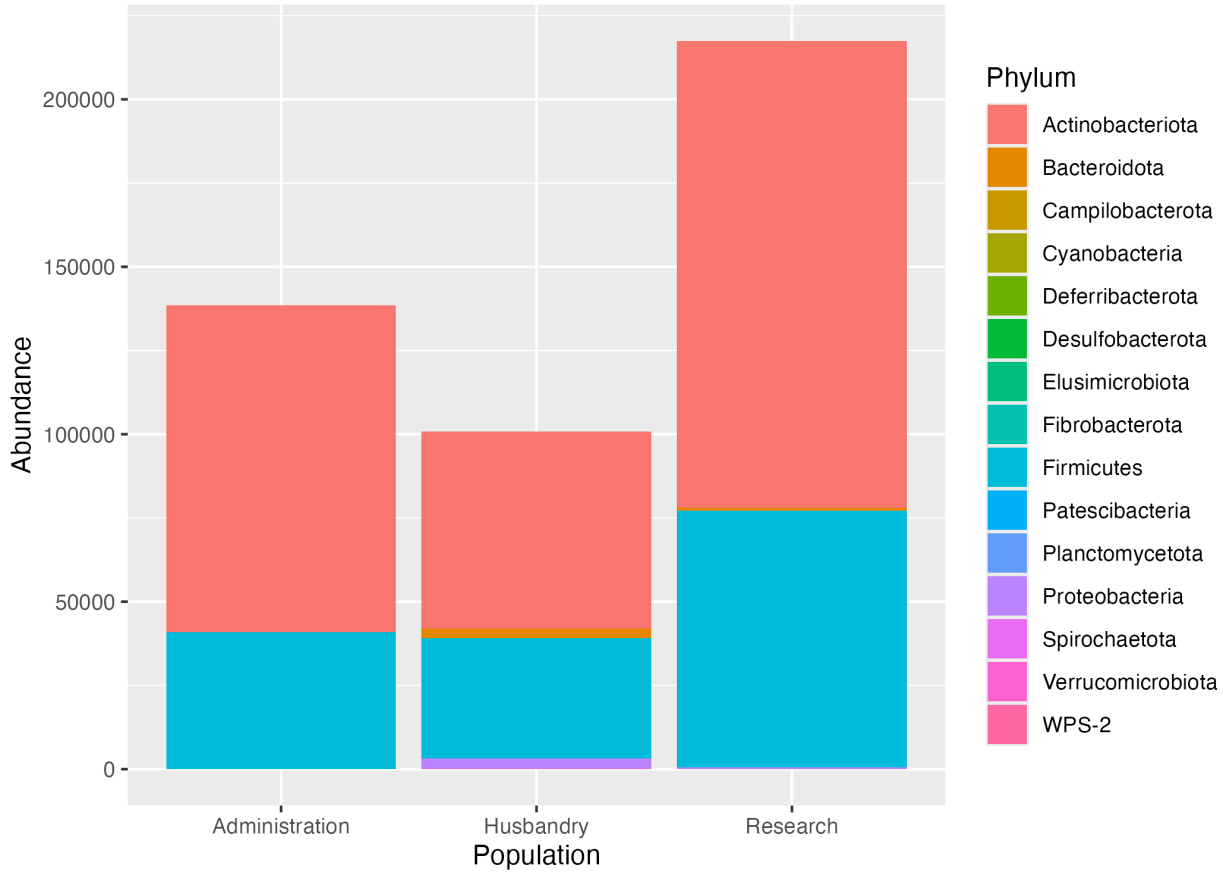

Figure S4. Stacked abundance bar chart based on taxonomy abundance of the human skin microbiome. Each bar represents a population (i.e., Administration, Husbandry, Research). Bacterial taxa are grouped and colored according to phylum-level classification.

Table S1. Summary of analysis of variance table comparing bacterial Shannon diversity (A) and Inverse Simpson's diversity (B) among lemur gut microbiomes according to population (enclosed only, free-ranging arboreal, free-ranging terrestrial) and lemur species and comparing Shannon diversity (C) and Inverse Simpson's diversity (D) among human skin microbiomes according to population (DLC intern department) and collection date.

(A) lemur Shannon diversity ~ Population * Species.Name

Factor	df	Sum Sq	Mean Sq	Fval	Pr(>F)
Population	2	3.313	1.657	7.069	0.002
Species.Name	1	0.711	0.711	3.036	0.087
Residuals	51	11.953	0.234		

(B) lemur Simpson's evenness ~ Population * Species.Name

Factor	df	Sum Sq	Mean Sq	F-value	Pr(>F)
Population	2	0.024	0.012	4.282	0.019
Species.Name	1	0.009	0.009	3.145	0.082
Residuals	51	0.141	0.003		

(C) human Shannon diversity ~ Population * Collection.Date

Factor	df	Sum Sq	Mean Sq	F-value	Pr(>F)
Population	2	3.756	1.878	1.737	0.202
Collection.Date	2	0.931	0.465	0.430	0.656
Population:Collection Date	4	1.286	0.322	0.297	0.876
Residuals	20	21.625	1.081		

(D) human Simpson's evenness ~ Population * Collection.Date

Factor	df	Sum Sq	Mean Sq	F-value	Pr(>F)
Population	2	0.033	0.166	2.528	0.105
Collection.Date	2	0.014	0.007	0.106	0.9
Population:Collection.Date	4	0.040	0.010	0.151	0.96
Residuals	20	1.310	0.065		

Table S2. Summary of multivariate analyses for lemur gut and human skin microbiomes. PERMANOVA comparing lemur gut microbiome (A) patterns among lemur species and populations (enclosed only, free-ranging arboreal, free-ranging terrestrial) and human skin microbiome (B) patterns among DLC intern department and collection date.

(A) lemur microbiome ~ Population * Species.Name

Fixed Effect	df	SumOfSqs	R^2	F-value	Pr(>F)
Population	2	4.784	0.229	8.419	0.001
Species.Name	1	1.576	0.076	5.546	0.001
Residual	51	14.490	0.695		
Total	54	20.850	1.000		

(B) human microbiome ~ Population * Collection.Date

Fixed Effect	df	SumOfSqs	R^2	F-value	Pr(>F)
Population (intern department)	2	0.979	0.105	1.484	0.027
Collection Date	2	0.457	0.049	0.693	0.958
Residual	20	7.915	0.846		
Total	28	9.350	1.000		

APPENDIX B: IACUC Approval Letter

Animal Care and Use Committee
003 Ed Warren Life Sciences Building | East Carolina University | Greenville NC 27834 - 4354
252-744-2436 office | 252-744-2355 fax

July 7, 2022

James Loudon, Ph.D.
Department of Anthropology, ECU

Subject: Protocol P110, original approval date 05/23/2022

Dear Dr. Loudon:

The amendment#1 to your Animal Use Protocol entitled, "The impacts of humans on lemur gut microbiomes at the Duke Lemur Center" (AUP#P110) was reviewed by this institution's Animal Care and Use Committee on 07/07/2022. The following action was taken by the Committee:

"Approved as submitted"

Please contact Aaron Hinkle prior to any hazard use

A copy of the protocols is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP/Amendment and are familiar with its contents.

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/GD

enclosure

APPENDIX C: IRB Review Letter

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board
4N-64 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · **rede.ecu.edu/umcirb/**

Notification of Initial Approval: Expedited

From: Biomedical IRB
To: James Loudon
CC:
Date: 5/26/2022
Re: UMCIRB 22-000802
Human and lemur gut microbiomes

I am pleased to inform you that your Expedited Application was approved. Approval of the study and any consent form(s) occurred on 5/26/2022. The research study is eligible for review under expedited category # 3,7. The Chairperson (or designee) deemed this study no more than minimal risk.

As the Principal Investigator you are explicitly responsible for the conduct of all aspects of this study and must adhere to all reporting requirements for the study. Your responsibilities include but are not limited to:

1. Ensuring changes to the approved research (including the UMCIRB approved consent document) are initiated only after UMCIRB review and approval except when necessary to eliminate an apparent immediate hazard to the participant. All changes (e.g. a change in procedure, number of participants, personnel, study locations, new recruitment materials, study instruments, etc.) must be prospectively reviewed and approved by the UMCIRB before they are implemented;

2. Where informed consent has not been waived by the UMCIRB, ensuring that only valid versions of the UMCIRB approved, date-stamped informed consent document(s) are used for obtaining informed consent (consent documents with the IRB approval date stamp are found under the Documents tab in the ePIRATE study workspace);

3. Promptly reporting to the UMCIRB all unanticipated problems involving risks to participants and others;

4. Submission of a final report application to the UMICRB prior to the expected end date provided in the IRB application in order to document human research activity has ended and to provide a timepoint in which to base document retention; and

5. Submission of an amendment to extend the expected end date if the study is not expected to be completed by that date. The amendment should be submitted 30 days prior to the UMCIRB approved expected end date or as soon as the Investigator is aware that the study will not be completed by that date.

The approval includes the following items:

Name	Description
Loudon Informed Consent Submission No More Than Minimal Risk 5.12.22.pdf	Consent Forms
Loudon IRB Participant Information Page 5.23.22.pdf	Surveys and Questionnaires
Loudon IRB Questionnaire 5.23.22.pdf	Surveys and Questionnaires
Loudon IRB Skin Microbiome Collection 5.5.22.pdf	Other Medical Procedures/Considerations
Recruitment and Contact Scripts Examining Bi Directional May 23 2022.pdf	Recruitment Documents/Scripts
Research Protocol Examining Bi Directional May 24 2022.pdf	Other Medical Procedures/Considerations
Research Protocol Examining Bi Directional May 5 2022.pdf	Study Protocol or Grant Application

For research studies where a waiver or alteration of HIPAA Authorization has been approved, the IRB states that each of the waiver criteria in 45 CFR 164.512(i)(1)(i)(A) and (2)(i) through (v) have been met. Additionally, the elements of PHI to be collected as described in items 1 and 2 of the Application for Waiver of Authorization have been determined to be the minimal necessary for the specified research.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.