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REVIEW ARTICLE

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The importance of scale in comparative microbiome research: New insights from the gut and glands of captive and wild lemurs

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Abstract

Research on animal microbiomes is increasingly aimed at determining the evolutionary and ecological factors that govern host–microbiome dynamics, which are invariably intertwined and potentially synergistic. We present three empirical studies related to this topic, each of which relies on the diversity of Malagasy lemurs (representing a total of 19 species) and the comparative approach applied across scales of analysis. In Study 1, we compare gut microbial membership across 14 species in the wild to test the relative importance of host phylogeny and feeding strategy in mediating microbiome structure. Whereas host phylogeny strongly predicted community composition, the same feeding strategies shared by distant relatives did not produce convergent microbial consortia, but rather shaped microbiomes in host lineage-specific ways, particularly in folivores. In Study 2, we compare 14 species of wild and captive folivores, frugivores, and omnivores, to highlight the importance of captive populations for advancing gut microbiome research. We show that the perturbational effect of captivity is mediated by host feeding strategy and can be mitigated, in part, by modified animal management. In Study 3, we examine various scent-gland microbiomes across three species in the wild or captivity and show them to vary by host species, sex, body site, and a proxy of social status. These rare data provide support for the bacterial fermentation hypothesis in olfactory signal production and implicate steroid hormones as mediators of microbial community structure. We conclude by discussing the role of scale in comparative microbial studies, the links between feeding strategy and host–microbiome coadaptation, the underappreciated benefits of captive populations for advancing conservation research, and the need to consider the entirety of an animal's microbiota. Ultimately, these studies will help move the field from exploratory to hypothesis-driven research.

RESEARCH HIGHLIGHTS

- Comparing gut microbiomes in diverse lemurs shows that community structure reflects host phylogeny at broad scales, but when controlling for evolutionary time, feeding strategy underlies the strength of host-microbe coadaptation at narrow scales.

- Host feeding strategy mediates captivity-induced microbial "dysbiosis" but management techniques that promote naturalized foraging can mitigate negative, species-specific consequences.
- Glandular microbiomes in captive and wild lemurs vary by host species, sex, social rank, and body site and likely contribute to the distinct chemical signals associated with each of these traits.

KEYWORDS
ecology, evolution, feeding strategy, olfactory signals, strepsirrhine

1 | INTRODUCTION

Animal microbiomes, the complex communities of microorganisms that inhabit virtually every body site (Ding & Schloss, 2014), are among the most prominent research foci of our time (Bordenstein & Theis, 2015; Costello, Stagaman, Dethlefsen, Bohannan, & Relman, 2012; Foster, Schluter, Coyte, & Rakoff-Nahoum, 2017). Implicated in myriad aspects of vertebrate life (McFall-Ngai et al., 2013), microbiomes perform countless functions for hosts, from conferring genetic and enzymatic potential that host genomes lack (Flint, Scott, Duncan, Louis, & Forano, 2012; LeBlanc et al., 2013) to specifically interacting with the brain and immune systems to mediate emotional (Cryan & Dinan, 2012; Mayer, Tillisch, & Gupta, 2015) and physical wellbeing (Stecher & Hardt, 2011; Thaiss, Zmora, Levy, & Elinav, 2016). Indeed, animals are now understood to have evolved in a "bacterial world" (McFall-Ngai et al., 2013), with the long, intertwined history between hosts and their microbes shaping their respective evolutionary trajectories.

To date, much of the literature has been focused on the gut microbiome (hereafter "GMB") of humans and model systems and its relation to the health concerns of modern society (Clemente, Ursell, Parfrey, & Knight, 2012; Kau, Ahern, Griffin, Goodman, & Gordon, 2011; Turnbaugh et al., 2009). The study of microbiota from other body sites (e.g. skin, the oral cavity, and vagina), albeit less mainstream, further accentuates the diverse ways in which microbes can impact host health (Cho & Blaser, 2012). Recent years have seen microbiome science rapidly expand across disciplines: wildlife biologists, including primatologists, now study microbiomes to gain insights into the behavior (Archie & Theis, 2011), evolution (Fraune & Bosch, 2010; Suzuki, 2017), and conservation (Stumpf et al., 2016) of their study subjects. With this expansion, the field is also increasingly moving from exploratory to hypothesis-driven research; however, many of the current and emerging questions, particularly about the evolutionary and ecological mechanisms that govern animal microbiomes, could be profitably examined using comparative approaches and a broader representation of host systems.

The lemurs of Madagascar constitute a nontraditional primate group that offers the evolutionary and ecological resolution necessary to test emerging hypotheses. Lemurs represent one of nature's great experiments and have long fascinated scientists, both for the mysterious circumstances that led to their evolution (Horvath et al., 2008) and for the impressive diversity characterizing extinct and extant species (Jungers et al., 2002; Mittermeier et al., 2008). Millions of years ago, early lemurs are thought to have rafted from continental Africa to Madagascar (Ali & Huber, 2010), which was geographically isolated and devoid of mammalian life. From these founding events (see Gunnell et al., 2018), lemurs radiated across the island's microhabitats, filling numerous ecological niches (Martin, 1972). Today, Lemuroidea comprises over 100 extant species from 15 genera and five families and is among the most diverse groups of primates worldwide (Figure 1). From the early naturalists to modern researchers, the unusual history and varied ecology of lemurs has made them key subjects for studies of macro-biodiversity (Jolly & Sussman, 2006). It is now increasingly clear that lemurs are an equally compelling group for studies of micro-biodiversity.

Research on lemur-associated microbial consortia and their links to host evolution and ecology is burgeoning, but thus far with a singular emphasis on the GMB. Researchers have documented within-species variation in GMB structure relative to seasonal food availability (Springer et al., 2017), habitat quality (Bennett et al., 2016), and social dynamics (Perofsky, Lewis, Abondano, Di Fiore, & Meyers, 2017; Raulo et al., 2018). Increasingly, researchers have adopted small-scale comparative approaches, investigating GMB composition across host populations, species, and habitat types (Greene et al., in review; Perofsky, Lewis, & Meyers, 2019; Umanets et al., 2018). Opportunities for longitudinal sampling and experimental manipulation using captive populations have facilitated studies on the timing of microbiome acquisition across host ontogenetic development (McKenney, Rodrigo, & Yoder, 2015), patterns of recovery from enteric infection (McKenney, Greene, Drea, & Yoder, 2017), and response to changing dietary quality (Greene, McKenney, O'Connell, & Drea, 2018). While these studies have contributed important insights, the lemurs' unique potential for wide-scale, comparative analyses across species and microbiota remains largely untapped.

Here, we present three, broadly tuned, comparative studies of lemur microbiomes. In our first study, we explore the gut consortia of four families of wild lemurs representing eight genera and 14 species to show that effects of host phylogenetic placement and feeding strategy on GMB structure vary depending on the scale of analysis

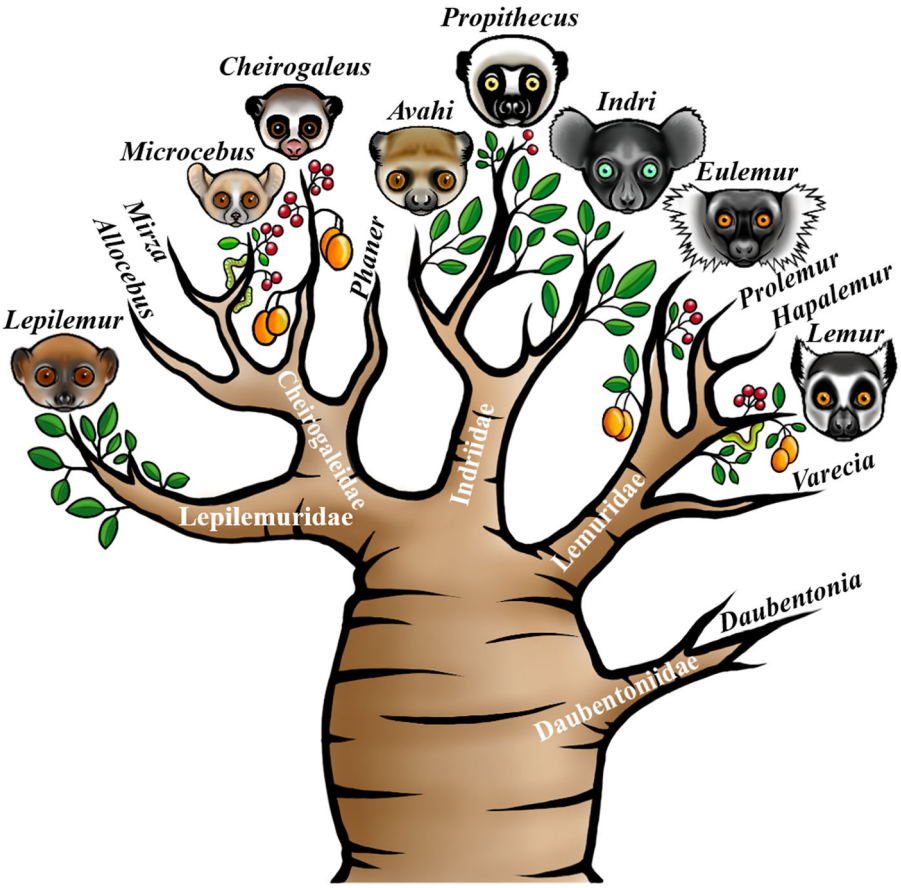


FIGURE 1 Illustration of the lemur family "tree of life" at genus-level resolution. Faces represent each genus included across the three studies and dietary items represent their feeding strategies. Illustrated by Sally Bornbusch

used to reflect host phylogeny. In our second study, we compare the gut consortia of 14 species comprising wild and captive populations to show that perturbational effects of captivity can be modulated by host feeding strategy and, thus, can be attenuated by management techniques. In our third study, we maintain a wild and captive species comparison, but shift attention to the lemurs' understudied glandular microbiomes. We relate microbial variation across several scent glands to host sociodemographic factors to suggest a microbial contribution to host chemical communication and social behavior. Conducting these studies across analytical scales demonstrates the power of comparative research, while highlighting the benefits of lemurs as nontraditional model systems for examining host–microbiome symbioses.

2 | GENERAL METHODS

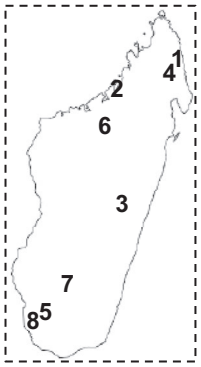
2.1 | Study subjects and sampling

The study subjects included 261 juvenile or adult lemurs representing 19 host species, eight genera, and four families (Tables 1 and 2). Our wild subjects were 170 lemurs from 14 species living freely at eight sites across Madagascar. Our 91 captive subjects included sifakas (*Propithecus* spp.), brown lemurs (*Eulemur* spp.), and ring-tailed lemurs (*Lemur catta*), all housed at the Duke Lemur Center (DLC) in Durham, North Carolina. At the time of sampling, the captive animals were socially housed in standard indoor/outdoor pens (146 m^2/animal). A subset of the sifakas ($n = 23/29$) and brown lemurs ($n = 14/51$) gained additional access to large forested enclosures (0.6–5.8 ha) in which they could semi-free range and forage ad libitum. For sifakas, the standard diet included a fiber-rich chow, nuts or beans, sweet potato or corn, vegetables, kale or collard greens, and local foliage harvested from the surrounding North Carolinian woods. For brown lemurs and ring-tailed lemurs, the standard diet included a protein and fat-rich chow, fresh fruits, and vegetables. Water was freely available.

We collected a total of 289 fecal samples during animal follows or routine captures, conducted from 2013 to 2017, as previously described (Greene et al., 2018, in review; Junge et al., 2017; Table 1). We also collected 56 glandular secretion samples from a subset of 35 adult lemurs (Table 2), obtained during routine captures of sifakas (*Propithecus diadema*) in September 2014 and of woolly lemurs (*Avahi Laniger*) in March 2015 (Junge et al., 2017), and during handling of awake, gently restrained ring-tailed lemurs at the DLC in November 2016 (Scordato & Drea, 2007). Members from each species of this latter subset contributed genital (i.e., labial or scrotal) secretions; the male sifakas additionally contributed sternal secretions (Lewis & van Schaik, 2007) and the male ring-tailed lemurs additionally contributed brachial secretions from both the left and right shoulders (Scordato & Drea, 2007). To collect genital and sternal secretions, we used sterile, rayon swabs that we gently swiped across the glandular field. To obtain the brachial secretions, we

TABLE 1 Classification, location, and sampling of wild and captive lemurs in Studies 1 and 2

Host family	Host species	Sample origin	n	
			Lemurs	Samples
Wild subjects				
Cheirogaleidae	*Cheirogaleus* sp.	[1] Tsihomanaomby PA	3	5
	Microcebus danfossi	[2] Anjajavy PA	1	1
Indriidae	*Avahi laniger*	[3] Ambatovy CZ	20	27
	Indri indri	[3] Ambatovy CZ	16	29
	Propithecus candidus	[4] Marojejy NP	10	10
	Propithecus coquereli	[2] Anjajavy PA	15	15
	Propithecus diadema	[3] Ambatovy CZ	23	27
	Propithecus verreauxi	[5] Beza-Mahafaly SR	3	3
Lemuridae	*Eulemur albifrons*	[4] Marojejy NP	14	14
	Eulemur fulvus	[2] Anjajavy PA	6	6
	Eulemur mongoz	[6] Ankarafantsika NP	2	2
	Eulemur rubriventer	[4] Marojejy NP	11	11
	Lemur catta	[7] Isalo NP	11	11
	Lemur catta	[8] Tsimanampetsotsa NP	29	29
Lepilemuridae	*Lepilemur Mustelinus*	[3] Ambatovy CZ	6	
Captive subjects				
Indriidae	*Propithecus coquereli*	Duke Lemur Center	29	29
Lemuridae	*Eulemur collaris*	Duke Lemur Center	6	6
	Eulemur coronatus	Duke Lemur Center	14	14
	Eulemur flavifrons	Duke Lemur Center	14	16
	Eulemur macaco	Duke Lemur Center	2	2
	Eulemur mongoz	Duke Lemur Center	11	11
	Eulemur rubriventer	Duke Lemur Center	2	2
	Eulemur rufifrons	Duke Lemur Center	2	2
	Lemur catta	Duke Lemur Center	11	11



Note. CZ: conservation zone; NP: national park; PA: protected area; SR: special reserve.

first extruded the secretions by gently squeezing the glandular pocket.

We placed fresh feces immediately in preservation buffer (OMNIgene.GUT; DNA Genotek, Ottawa, Canada; or 96% ethanol) at room temperature or stored them in sterile tubes at −20°C or −80°C. All swabs of glandular secretions were immediately placed in sterile tubes at −20°C or −80°C. Storage conditions for samples collected in Madagascar were maintained during transit to the United States, where upon arrival at Duke University, they were stored with samples collected at the DLC, at −80°C, until analysis.

Our procedures were approved by the Institutional Animal Care and Use Committee of Duke University (Protocols: A168-14-07, A208-14-02, A111-16-05, A127-16-06, A007–17-01, A152-17-06, A263-17-12) and by Madagascar's Ministère de l'Environnement, de l'Ecologie et des Forêts (Permits: 197/13, 085/14, 068/15, 038/16,

TABLE 2 Classification, location, and sampling of wild and captive lemurs in Study 3

Host species	Sample origin	n		Sample sizes		
		Females	Males	Genital	Sternal	Paired brachial
Avahi laniger	Madagascar: Ambatovy CZ	7	0	7	0	0
Propithecus diadema	Madagascar: Ambatovy CZ	8	10	11	8	0
Lemur catta	Duke Lemur Center	0	10	10	0	20

Note. CZ: conservation zone.

162/16, 083/17, 136/17, 035/18, 136-EA05MG15). This study complies with the American Society of Primatologists Principles for Ethical Treatment of Non-Human Primates.

2.2 | Sequencing and bioinformatics

We extracted genomic DNA from all samples using commercial kits (DNeasy PowerSoil Kit; Qiagen, Hilden, Germany), and shipped standard aliquots to Argonne National Laboratories (Lemont, IL), where the v4 region of the 16S rRNA gene was amplified via the 515f-806r primer set, and sequenced on Illumina's MiSeq platform (Caporaso et al., 2012; McKenney, Greene, et al., 2017). Raw sequences were analyzed via our published bioinformatics pipeline using the Quantitative Insights Into Microbial Ecology (QIIME) package (v1.9.1; Caporaso et al., 2010; McKenney, Greene, et al., 2017). We retained samples that were sequenced to a depth of minimally 10,000 reads, with each sample covered by 47,657 reads, on average (Standard Deviation: 16,452). From the samples retained, we picked Operational taxonomic units (OTUs) using the de novo UClust method and based on 97% sequence similarity. OTU taxonomy was assigned using the Greengenes database (v13_8) via the summarize.taxa.py function in QIIME. OTU profiles and taxonomic assignments were used in downstream statistical analyses (see Section 2 within Studies 1–3 for statistical details).

3 | STUDY 1: PHYLOGENETIC AND ECOLOGICAL PATTERNS IN LEMUR GUT MICROBIOMES REFLECT SCALE OF ANALYSIS

3.1 | Introduction

A major goal of GMB researchers is to improve our understanding of the evolutionary and ecological mechanisms by which consortia are established and mediated (Clayton, Gomez, et al., 2018; Groussin et al., 2017). One topic of discussion involves the respective roles of host phylogenetic relationships and feeding ecology (Delsuc et al., 2014; Groussin et al., 2017; Ley et al., 2008; Nishida & Ochman, 2018). On the one hand, the role of phylogenetic placement dominates when analyses are scaled broadly across mammalian taxa (Amato et al., 2018; Groussin et al., 2017; Ley et al., 2008; Nishida & Ochman, 2018; Perofsky et al., 2019) or within lineages that are characterized by limited species diversity (Ochman et al., 2010). On the other hand, shared feeding strategies can lead to GMB convergence across distant relatives (Delsuc et al., 2014). Moreover, lineages that have faced environmental or dietary challenges during the speciation process have seemingly experienced faster host–microbiome coadaptation than have comparable lineages radiating under more stable conditions (Greene et al., in review; Nishida & Ochman, 2018). Thus, in some cases, the hosts' feeding strategies, diets, or environments appear to shape the GMB within the constraints of their phylogenetic placement, whereas in certain cases, the influence of these ecological factors can override the signal of host phylogeny.

Among primates, lemurs offer a unique opportunity to tease apart the relative contributions to GMB structure of host phylogeny and feeding strategy. Notably, of the ~110 extant species, 89 (or 81%) stem from just six highly speciose genera within four families, including the mouse lemurs (*Microcebus* spp.) and dwarf lemurs (*Cheirogaleus* spp.) from Cheirogaleidae, the woolly lemurs (*Avahi* spp.) and sifakas (*Propithecus* spp.) from Indriidae, the brown lemurs (*Eulemur* spp.) from Lemuridae, and the sportive lemurs (*Lepilemur* spp.) from Lepilemuridae. The species within these genera diverged over relatively similar evolutionary timescales (Dos Reis et al., 2018; Herrera & Dávalos, 2016). Today, they routinely live in sympatry across Madagascar's distinct habitats and display a diversity of feeding strategies (Figure 1). We recently compared GMB structure across two of these host genera, the folivorous sifakas (*Propithecus* spp.) and frugivorous brown lemurs (*Eulemur* spp.; Greene et al., in review). Scaling analyses across 12 host species, we showed that sifakas and brown lemurs harbored distinct GMBs; however, within host genera, microbial community structure better related to habitat type than to host phylogeny, and the effect was stronger for folivores than for frugivores. We attributed these patterns to the extreme microendemism in plant composition across Madagascar (Wilmé, Goodman, & Ganzhorn, 2006) and to the specialized microbial metabolism required to facilitate folivory (Clayton, Gomez, et al., 2018; Flint, Bayer, Rincon, Lamed, & White, 2008).

Here, we expand this line of research across a greater diversity of host genera (Table 1). Specifically, we use a GMB database newly amassed from fecal samples comparably collected and analyzed from wild populations of 14 lemur species, representing each of the six speciose host genera as well as two monotypic genera, *Lemur* and *Indri*. Our study subjects stem from four host families, including Indriidae and Lepilemuridae that convergently evolved folivory as a feeding strategy, and Cheirogaleidae and Lemuridae that both contain lineages of omnivores and frugivores (Richard & Dewar, 1991). Consistent with previous research (Amato et al., 2018; Greene et al., in review), we expect the subjects' GMBs to show clear links to host phylogeny, and for the GMBs of each lemur family to have a distinct membership, regardless of shared feeding strategies. We then narrow our focus to the host-GMB relationships occurring within one clade each of the folivores and non-folivores (i.e., Indriidae and Lemuridae, respectively), and include a family-specific outgroup for the sifakas and brown lemurs, namely woolly lemurs and ring-tailed lemurs, respectively. Within the clade of folivores, sifakas and woolly lemurs diverged at the same time as brown lemurs and ring-tailed lemurs diverged within the clade of non-folivores (Dos Reis et al., 2018; Herrera & Dávalos, 2016). If folivory were a stronger driver of host-GMB coadaptation than frugivory or omnivory, then, when controlling for evolutionary time, we would expect the microbial consortia of sifakas and woolly lemurs to be more disparate than the consortia of brown lemurs and ring-tailed lemurs.

3.2 | Statistical analyses

We centered our statistical analyses around two types of data, GMB similarity and taxonomic composition. With regard to the former, we used all OTUs to calculate beta diversity (i.e., unweighted UniFrac [UUF] distances) that captures the similarity in microbial composition between pairs of samples by reflecting OTU presence (Lozupone, Lladser, Knights, Stombaugh, & Knight, 2011). We asked if there was a signal of host phylogenetic relatedness in the GMBs across all lemurs. We used Mantel tests to compare the average UUF distances between each pair of host species to their published divergence times (Herrera & Dávalos, 2016). We implemented these analyses using the ade4 package (version 1.7–4; Dray & Dufour, 2007), the RStudio program (version 1.1.463, RStudio, Inc., Boston, MA), and R software (version 3.3.3, R Development Core Team, 2017; www.r-project.org). We used nonparametric Wilcoxon's tests implemented in Rstudio to compare UUF distances to host phylogenetic level, including between host families, genera, species, and individuals. The former three categories comprised values averaged across species and the latter category comprised each pairwise comparison entered individually. We then repeated the above analyses within the subset of samples from the brown lemurs and ring-tailed lemurs (i.e., the Lemuridae family) and from the sifakas and woolly lemurs (i.e., the Indriidae family).

From our assignments of microbial taxonomy, we retained the relative abundance of "major" microbial phyla and genera (OTUs; i.e., microbial taxa that accounted for >1% of sequences, on average across individuals in one host group). We tested if the major taxa were enriched in particular hosts using linear discriminant analysis effect size (LEfSe; Segata et al., 2011), with an additional, conservative correction factor for multiple testing (Benjamini & Hochberg, 1995). Using the full data set, we tested if host family was associated with microbial enrichment, whereas within the subsets of data from the indriids and lemurids, we tested for microbial enrichment within families at the level of host genus.

3.3 | Results

3.3.1 | GMB patterns relative to host phylogenetic relationships

Across all lemur subjects, there was a strong signal of host phylogenetic relatedness in the GMB (Figure 2). Mantel tests comparing host phylogenetic divergence to UUF distances revealed an overall significant relationship, such that more closely related lemurs shared more similar GMBs ($r = 0.810$, $p = 0.001$; Figure 2a). When collapsing species comparisons at each phylogenetic level, this effect was driven by differences at shallow phylogenetic resolutions (Figure 2b). There was no difference in GMB similarity (i.e., UUF distances) when comparing species from different families to confamiliar species from different host genera ($W = 416$, $z = -0.494$, $p = 0.621$); however, confamiliar species from different genera harbored less similar GMBs compared with congenerics from different species ($W = 168$, $z = -5.193$, $p < 0.001$). GMBs were also less similar between species from the same host genera than within species ($W = 123$, $z = -2.470$, $p = 0.014$).

With regard to microbial taxonomic composition, at the microbial phyla level, all lemur GMBs showed dominance by the Bacteroidetes and Firmicutes phyla, which together accounted for 63.4% of sequences, on average, across host families (Figure 2c). Eight other phyla each accounted for minimally 1% of sequences in one host lineage, with Actinobacteria and Proteobacteria being the most abundant. Unassigned taxa (i.e., those that could not be mapped to a kingdom of life) were also present in relatively large proportions within the consortia of indriids and, to a lesser extent, lepilemurids and lemurids.

Compared with the analyses of microbiota at phylum-level resolution, those focused on microbial genera showed greater differences in the presence/absence and abundance of taxa between host lineages (Figure 2d). With regard to the presence or absence of taxa, only five microbial genera were found in the GMBs of 100% of the lemurs. These included *Prevotella*, unassigned genera within the Coriobacteriaceae, Lachnospiraceae, and Ruminococcaceae families, and an unassigned genus within an unassigned family of Clostridiales. With regard to the relative abundance of taxa, we identified 36 major microbial taxa, 31 of which were significantly enriched in particular host families (Table 3). The GMBs of cheirogaleids and lemurids were associated with significant enrichment for 15 and 10 microbes, respectively, which stemmed from diverse microbial phyla. In contrast, the GMBs of indriids were only significantly enriched for three taxa; those of lepilemurids were only enriched for two taxa—the unassigned Clostridiales taxon and *Adlercreutzia*—which together comprised, on average, 70% of all lepilemurid sequences.

3.3.2 | GMB patterns relative to host feeding strategy

Our data set provided little evidence of GMB convergence between lemur families that shared similar feeding strategies. Nevertheless, the importance of feeding strategy emerged when we narrowed analyses within single clades of folivores (sifakas and woolly lemurs from Indriidae) and non-folivores (brown lemurs and ring-tailed lemurs from Lemuridae; Figure 3). Within this subset of samples, there was a strong signal of host phylogenetic placement on GMB structure: Mantel tests showed a significant relationship between host phylogenetic divergence and UUF distances, averaged across species comparisons ($r = 0.907$, $p = 0.001$; Figure 3a). Moreover, when collapsing species comparisons at host phylogenetic levels (Figure 3b), the GMB differences between species of sifakas were significantly smaller than were the differences between sifakas and woolly lemurs, and the differences between species of brown lemurs were significantly smaller than were the differences between brown lemurs and ring-tailed lemurs ($W = 24$, $z = -2.593$, $p = 0.009$, for both comparisons). Of particular relevance was the finding that GMB dissimilarity was significantly greater between sifakas and woolly lemurs than between brown lemurs and ring-tailed lemurs ($W = 16$, $z = -2.189$, $p = 0.029$). Consistent with our previous research (Greene et al., in review), GMB dissimilarity was greater between sifaka species than between brown lemur species ($W = 1$, $z = -2.853$, $p = 0.004$).

With regard to microbial taxonomic composition, sifakas and woolly lemurs harbored only 21 major taxa in their GMBs (Figure 3c),

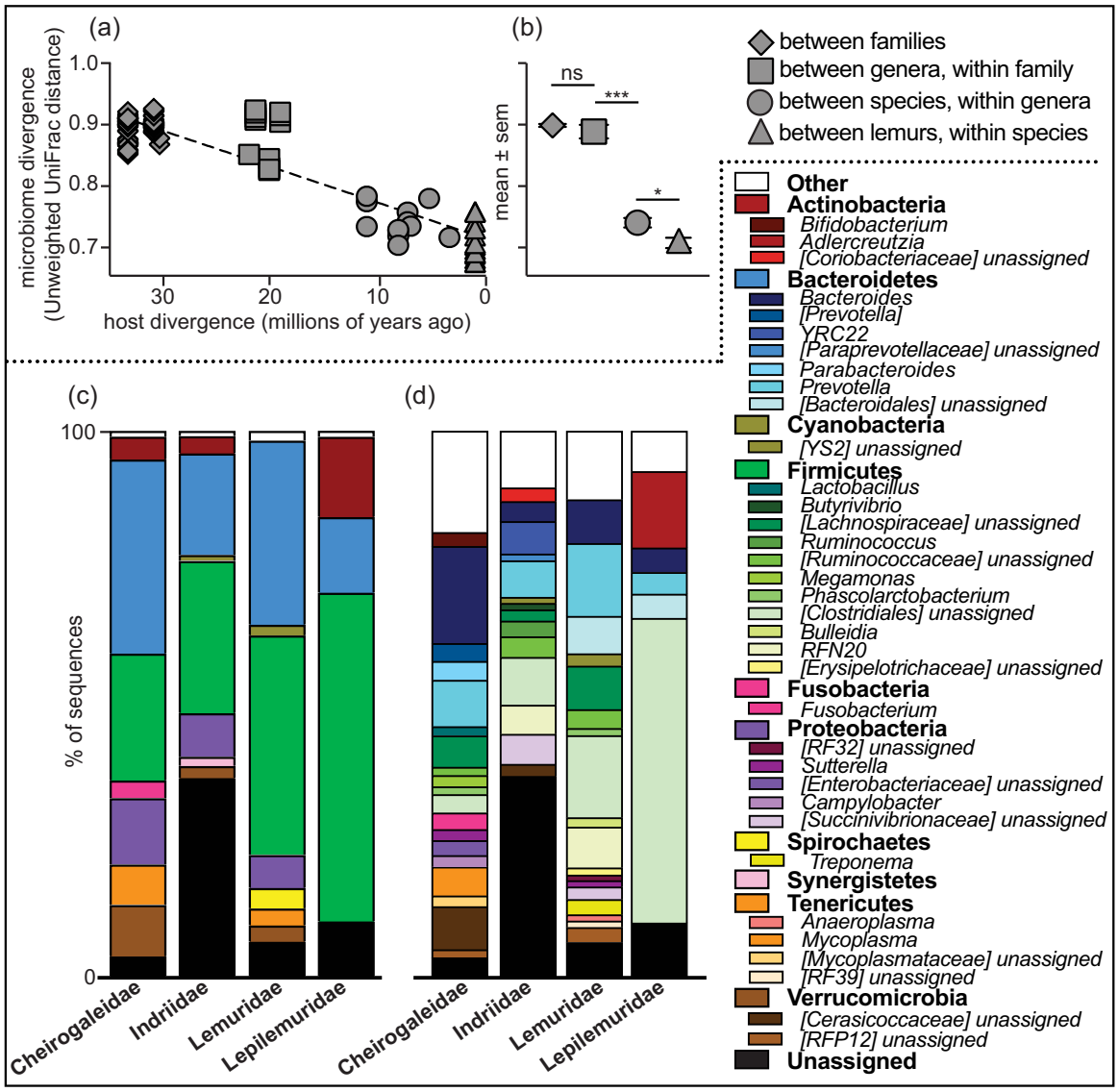


FIGURE 2 Gut microbiome structure relative to host phylogenetic relationships across the 14 lemur species in Study 1. Depicted are unweighted UniFrac distances graphed relative to host (a) published host divergence times (Herrera & Dávalos, 2016) and (b) host phylogenetic levels, for all pairwise comparisons. At the level of the host family, major microbial taxa (i.e., those that accounted for >1% of sequences in minimally one host lineage) are shown at the (c) microbial phylum and (d) genus levels, with color families referring to the former and distinct shades referring to the latter. $^{*}p < 0.05$, $^{***}p < 0.001$

19 (or 90.4%) of which were enriched in either host genera (Table 4). Brown lemurs and ring-tailed lemurs harbored a total of 31 major taxa (Figure 3d), 26 (or 83.9%) of which were enriched in either host genera (Table 4).

3.4 | Discussion

Across lemurs, stemming from diverse families, genera, and species, we found the structure of the GMB to reflect host phylogeny, with more closely related hosts sharing a more similar GMB composition. This effect was largely driven by differences at or below the host genus level, such that two species harbored equally dissimilar GMBs whether they belonged to different genera within the same family or to different taxonomic families. When scaling our analyses at the broadest level possible for lemurs (i.e., at the level of host family), we found little evidence of GMB convergence between the two families of folivores, the Indriidae and Lepilemuridae, or between the two families of non-folivores, the Cheirogaleidae and Lemuridae. When narrowing our analytical scope within lemur lineages that diverged over comparable timescales, however, we found that folivorous hosts consistently harbored more disparate GMBs than did non-folivorous hosts. Indeed, the equivalent GMB dissimilarity within and between confamiliar species was largely driven by the greater disparity between folivores at the level of host genus. Together, these data provide support for the hypothesis that feeding strategy underlies the strength of host-GMB coadaptation, with the specialized microbial metabolism required to facilitate folivory (Flint et al., 2008; Wong, De Souza, Kendall, Emam, & Jenkins, 2006) exerting a stronger

TABLE 3 Microbial taxa significantly enriched in the gut microbiomes of lemur families

	Microbial taxon			
Host family	**Phylum**	**Order**	**[Family] genus**	**Log (LDA)**
Cheirogaleidae	Actinobacteria	Bifidobacteriaceae	*Bifidobacterium*	4.40***
	Bacteroidetes	Bacteroidales	[Paraprevotellaceae] *Prevotella*	4.26**
			Bacteroides	4.91***
			Parabacteroides	4.24***
	Firmicutes	Lactobacillales	*Lactobacillus*	4.30***
		Clostridiales	*Megamonas*	4.14***
			Phascolarctobacterium	3.88***
	Fusobacteriua	Fusobacteriales	*Fusobacterium*	4.51***
	Proteobacteria	RF32	Unassigned	3.77***
		Burkholderiales	*Sutterella*	4.07***
		Campylobacterales	*Campylobacter*	4.11***
		Enterbacteriales	[Enterbacteriaceae] unassigned	4.27***
	Tenericutes	Mycoplasmataceae	*Mycoplasma*	4.53***
			Unassigned	4.09***
	Verrucomicrobia	Cerasicoccales	[Cerasicoccaceae] unassigned	4.64***
Indriidae	Actinobacteria	Coriobacteriales	[Coriobacteriaceae] unassigned	4.14***
	Bacteroidetes	Bacteroidales	*YRC22*	4.48***
	Firmicutes	Clostridiales	[Ruminococcaceae] unassigned	4.22***
	Unassigned	Unassigned	Unassigned	5.25***
Lemuridae	Bacteroidetes	Bacteroidales	*Prevotella*	4.70***
			Unassigned	4.56***
	Cyanobacteria	YS2	Unassigned	4.08***
	Firmicutes	Clostridiales	[Lachnospiraceae] unassigned	4.60***
		Erysipelotrichales	*Bulleidia*	3.99***
			RFN20	4.58***
			Unassigned	3.87***
	Spirochaetes	Spirochaetales	*Treponema*	4.21***
	Tenericutes	Anaeroplasmatales	*Anaeroplasma*	3.80***
		RF39	Unassigned	3.81***
	Verrucomicrobia	WCHB1_41	[RFP12] unassigned	4.18***
Lepilemuridae	Actinobacteria	Coriobacteriales	*Adlercreutzia*	4.82***
	Firmicutes	Clostridiales	Unassigned	5.42***

**Taxa enriched at $p < 0.01$.
***Taxa enriched at $p < 0.001$.

force relative to the more relaxed microbial requirements for either frugivory or omnivory.

Most of the dominant microbes identified were either present or were significantly enriched in only one host family. Indeed, few microbes were common across all lemurs. Nevertheless, many of the microbial taxa enriched in particular lemur families were those that are commonly related to feeding strategy and diet. For example, the GMBs of cheirogaleids and lemurids were enriched for the following taxa: *Bacteroides*, *Prevotella*, and other taxa that are linked to diets rich in simple fibers, proteins, and fats (Chen et al., 2017; Wu et al., 2011); *Proteobacteria* members that are likely to be linked to oxygen tolerance (Shin, Whon, & Bae, 2015) stemming from shorter gastrointestinal systems; and *Verrucomicrobia* members that are associated with fruit consumption (Amato et al., 2016; Gomez et al., 2016). In contrast, the GMBs of lepilemurids primarily comprised just five microbial taxa and were dominated by a single taxon within the Clostridiales order. That the folivorous lepilemurids have a simple GMB, a short gastrointestinal tract, and have been observed to use cecotrophic behavior (Hladik & Charles-Dominique, 1974), suggests that they may have evolved a unique microbial strategy—they appear to be reliant on one cellulolytic taxon to process their leafy diets. Unlike the GMBs of lepilemurids, those of indriids had a greater taxonomic diversity but a significant enrichment for unassigned microbes. Relative to the microbiomes of humans and other anthropoid primates (Amato et al., 2018), the predominance of unassigned microbial taxa in lemur, and particularly indriid, microbiomes (Perofsky et al., 2017; Springer et al., 2017) merits mention. Perhaps the long isolation of Madagascar from other landmasses, and the leafy diets and complex gastrointestinal morphology specific to indriids, gave rise to a wealth of microbes that await characterization.

These results underscore the importance of phylogenetic scale when designing evolutionary and ecological studies on host-associated GMBs. A broad focus at the resolution of microbial phyla, and host order or family may offer key insights into the overarching patterns of host-GMB symbioses that reflect deep time (Amato et al., 2018; Groussin et al., 2017), but such phylogenetic breadth may obscure the dynamics occurring within lineages relative to more recent changes in host ecology (Greene et al., in review; McKenney, Maslanka, Rodrigo, & Yoder, 2018). Such differences across studies might indicate a case of ecological fallacy (Freedman, 1999), which occurs when patterns characterizing the aggregate whole are opposite to those operating within the contributing parts. Accordingly, when host-GMB interaction is studied too broadly, the role of host phylogeny might mask that of feeding strategy or other ecological factors (Amato et al., 2018; Groussin

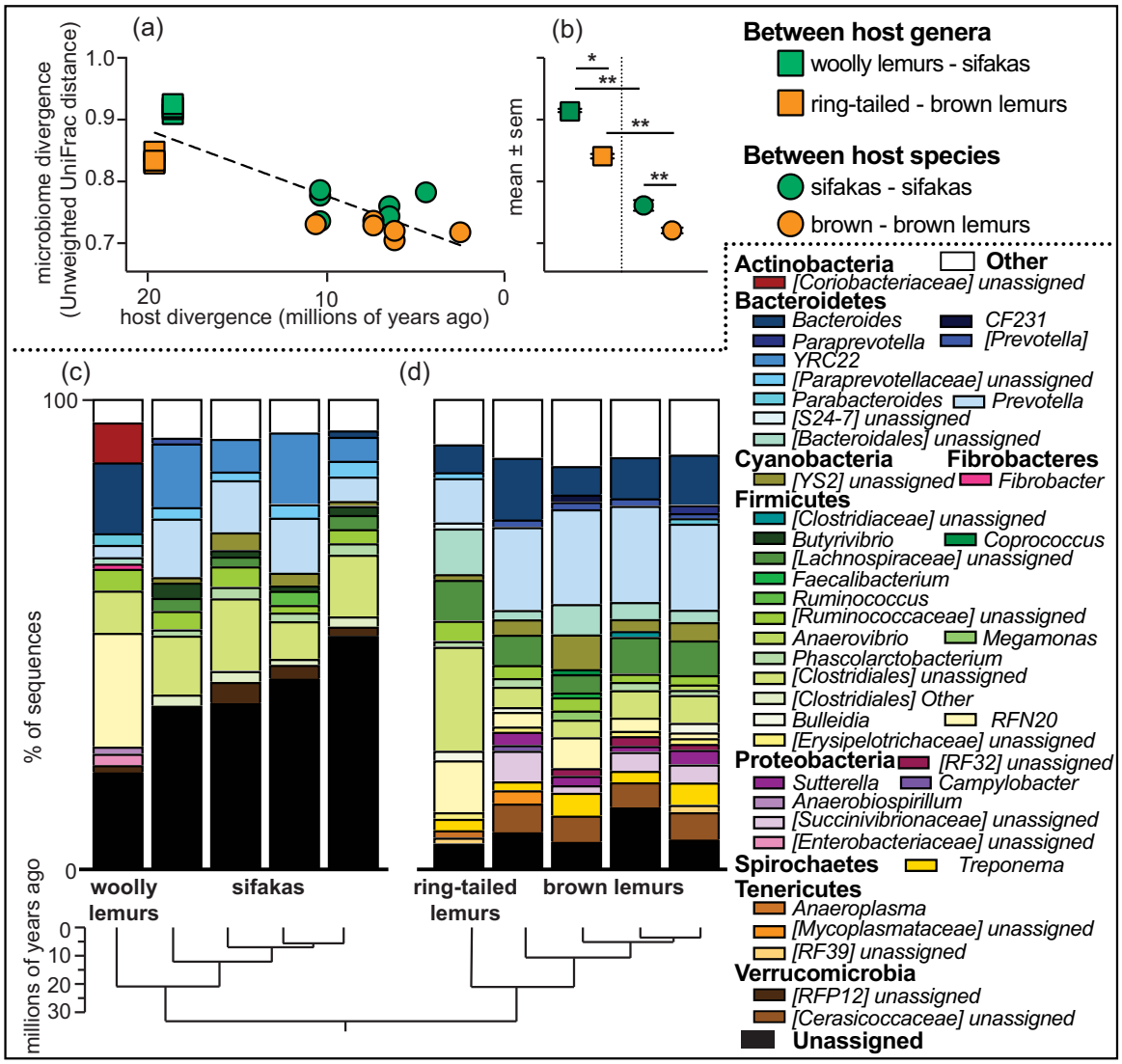


FIGURE 3 Gut microbiome structure relative to host phylogenetic relationships across single clades of folivores (Indriidae: woolly lemurs and sifakas) and non-folivores (Lemuridae: ring-tailed lemurs and brown lemurs) in Study 1. Depicted are unweighted UniFrac distances graphed (a) relative to published host divergence times (Herrera & Dávalos, 2016) and (b) within and between the lineage of folivores (green) and non-folivores (orange). At the level of host species, major microbial taxa (i.e., those that accounted for >1% of sequences in minimally one host species) are shown at the level of microbial genus for (c) folivores and (d) non-folivores, with color families referring to the microbial phyla and distinct shades referring to specific Operational Taxonomic Units. $^{*}p < 0.05$, $^{**}p < 0.01$

et al., 2017). When controlling for evolutionary time and looking within lineages, however, the importance of environments and feeding strategies emerge (Greene et al., in review; Nishida & Ochman, 2018).

4 | STUDY 2: FEEDING STRATEGIES MEDIATE GUT MICROBIOME DIFFERENCES BETWEEN WILD AND CAPTIVE LEMURS

4.1 | Introduction

One of the most consistent findings from studies comparing hosts across environments is that captive animals harbor dysbiotic GMBs that are depleted, imbalanced, or "humanized" (i.e., show an enrichment for human-associated taxa) compared with those of their wild counterparts (Clayton et al., 2016; Kohl et al., 2017; Kohl, Skopec, & Dearing, 2014; McKenzie et al., 2017). In addition, captive animals can show increased susceptibility to pathogens. One proposed mechanism to explain captivity-induced dysbiosis centers around changes in dietary composition. Notably, the routine use of chows, domesticated produce, and carbohydrate-based treat items generally result in fiber-limited, but sugar-rich (i.e., "westernized") diets (Clayton, Al-Ghalith, et al., 2018). For herbivores (animals that consume a broadly plant-based diet) and folivores (animals that consume a specifically foliage-based diet), increasing plant-fiber consumption can mitigate the microbial consequences associated with captivity, as evidenced by improved GMB diversity, stability, membership, and nutrient production (Clayton, Al-Ghalith, et al., 2018; Greene et al., 2018; Kohl et al., 2014). A second mechanism proposed to explain captivity-induced dysbiosis centers

TABLE 4 Microbial taxa enriched in the gut microbiomes of host genera

	Microbial taxon			
Host taxon	**Phylum**	**Order**	**[Family] genus**	**Log (LDA)**
Analysis I: Indriidae				
Woolly lemurs (outgroup)	Actinobacteria	Coriobacteriales	[Coriobacteriaceae] unassigned	4.65***
	Bacteroidetes	Bacteroidales	*Bacteroides*	4.88***
			Parabacteroides	4.12***
			Unassigned	3.98***
	Fibrobacteres	Fibrobacterales	*Fibrobacter*	3.92***
	Firmicutes	Clostridiales	[Ruminococcaceae] unassigned	3.93*
		Erysipelotrichales	*RFN20*	5.12***
	Proteobacteria	Aeromondales	*Anaerobiospirillum*	4.32***
		Enterobacteriales	[Enterobacteriaceae] unassigned	4.20*
Sifakas	Bacteroidetes	Bacteroidales	*Prevotella*	4.68***
			YRC22	4.79***
			[Paraprevotellaceae] unassigned	4.18***
	Cyanobacteria	YS2	Unassigned	4.13***
	Firmicutes	Clostridiales	*Butyrivibrio*	4.15***
			[Lachnospiraceae] unassigned	3.95***
			Ruminococcus	3.73*
			Phascolarctobacterium	4.07***
			Unassigned	4.33***
			Other	4.15***
Analysis II: Lemuridae				
Ring-tailed lemurs (outgroup)	Bacteroidetes	Bacteroidales	*CF231*	3.72*
			[Paraprevotellaceae] unassigned	3.87***
			[S24_7] unassigned	3.84***
			Unassigned	4.58***
	Firmicutes	Clostridiales	[Ruminococcaceae] unassigned	4.10***
			Unassigned	4.96***
		Erysipelotrichales	*Bulleidia*	3.72**
			RFN20	4.67***
			[Erysipelotrichaceae] unassigned	3.62*
	Tenericutes	Anaeroplasmatales	*Anaeroplasma*	8.82***
Brown lemurs	Bacteroidetes	Bacteroidales	*Paraprevotella*	3.85***
			[Paraprevotellaceae] *Prevotella*	4.04***
			Bacteroides	4.45***
			Parabacteroides	4.07***
			Prevotella	4.68***
	Cyanobacteria	YS2	Unassigned	4.20***
	Firmicutes	Clostridiales	[Clostridiaceae] unassigned	3.62***
			Coprococcus	4.05***
			Faecalibacterium	3.96***
			Anaerovibrio	3.90***
			Megamonas	3.95***
	Proteobacteria	RF32	Unassigned	3.56*
		Burkholderiales	*Sutterella*	4.23***
		Campylobacterales	*Campylobacter*	3.94***
		Aeromondales	[Succinivibrionaceae] unassigned	4.36***
	Verrucomicrobia	WCHB1_41	[RFP12] unassigned	4.50***

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

around changes in environmental conditions. For instance, host populations that are maintained under sterilized conditions, which are routinely prescribed antibiotics, or which are geographically separated from their wild counterparts are likely to have lost their native microbes (Trevelline, Fontaine, Hartup, & Kohl, 2019). Granting captive animals exposure to more naturalized housing conditions or environmental substrates can shift host-associated microbiota (Trevelline et al., 2019), with potential implications for animal health (Van Bonn et al., 2015).

Although researchers have often emphasized the negative consequences of captivity to host GMBs, captive populations nevertheless provide a critical resource for microbiome research. For example, experimental manipulations in captive woodrats (*Neotoma* spp.) have shown that dietary tannins shape GMB diversity and function (Kohl & Dearing, 2016; Kohl, Stengel, & Dearing, 2015). Studies in captive monkeys have established linkages between GMBs and disease states (McKenna et al., 2008), breastfeeding (O'Sullivan et al., 2013), and westernized or fibrous diets (Albert, Rani, & Sela, 2018; Amato et al., 2015), and have informed methods development and provided validation for future field studies (Hale, Tan, Knight, & Amato, 2015). Capitalizing on the potential for longitudinal and experimental research

at the DLC, members of our team have related GMB structure to (a) specific sites along the gastrointestinal tract (Greene & McKenney, 2018), (b) enteric infection with *Cryptosporidium* and subsequent antibiotic administration (McKenney, Greene et al., 2017), (c) host feeding strategy across infant ontogenetic development (McKenney et al., 2015) and in adulthood (McKenney, O'Connell, Rodrigo, & Yoder, 2017), (d) bamboo specialization (McKenney et al., 2018), and (e) shifts in dietary quality (Greene et al., 2018).

Here, by using lemurs that are characterized by diverse feeding strategies—folivores (sifakas), frugivores (brown lemurs), and omnivores (ring-tailed lemurs; Table 1)—we address the two potential mechanisms of captivity-induced microbial dysbiosis, westernized diets versus loss of native microbial consortia. With regard to the former, we ask if shifts in the abundance of *Bacteroides* and *Prevotella*, microbes that are respectively associated with proteins and fats versus non-cellulolytic plant fibers (Wu et al., 2011) vary across the hosts' environments relative to feeding ecology. We then ask if unassigned taxa (i.e., those that do not map to sequences generated from humans, model organisms, or captive wildlife) similarly vary across environments and hosts. We use these unassigned taxa as a proxy for "native" microbes. Although they could include microbes that are, as yet, unidentified, they more likely reflect those endemic to Madagascar, given their prolific abundance in the GMBs of wild lemurs (Figures 2 and 3c,d). For *Bacteroides*, *Prevotella*, and unassigned taxa, we further ask if allowing captive lemurs the opportunity to forage freely on local vegetation and acquire environmental microbes may potentially shift gut microbes towards a more natural abundance.

4.2 | Statistical methods

We used nonparametric Wilcoxon's tests, implemented in Rstudio, to test for differences in the relative abundance of taxa across environmental conditions separately for each host genus (Table 1). For sifakas and brown lemurs, we further applied Wilcoxon's tests within captive subjects to ask if forest access was associated with differences in taxonomic relative abundance within host genera.

4.3 | Results

4.3.1 | Lemur GMBs under wild versus captive conditions

The relative abundances of both *Bacteroides* and *Prevotella* in lemur GMBs differed between captive animals and their wild counterparts (Figure 4a,b); however, the nature of the differences varied by host feeding strategy. Compared to their wild counterparts, captive folivores (i.e., the sifakas) had a significantly greater proportion of *Bacteroides* ($W = 1{,}572$, $z = -7.282$, $p < 0.001$), but a significantly smaller proportion of *Prevotella* ($W = 171$, $z = -5.890$, $p < 0.001$); captive frugivores (i.e., the brown lemurs) had a significantly smaller proportion of *Bacteroides* ($W = 217$, $z = -5.834$, $p < 0.001$), but a

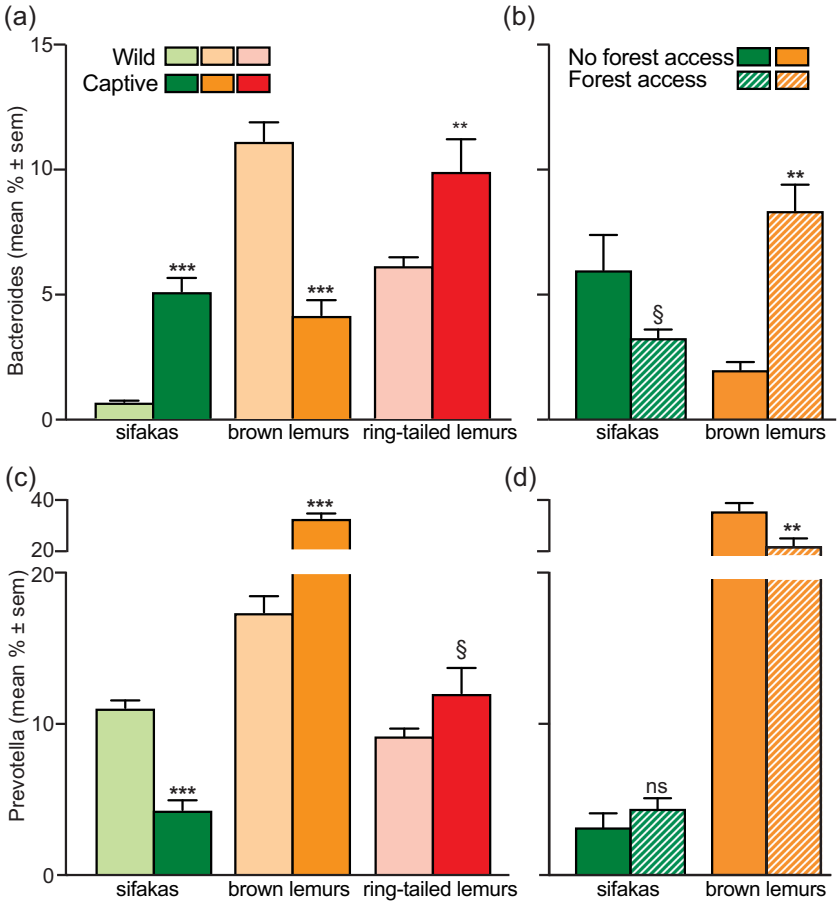


FIGURE 4 The proportion of *Bacteroides* and *Prevotella* in the gut microbiomes of folivores (sifakas; green), frugivores (brown lemurs; orange), and omnivores (ring-tailed lemurs; red) in Study 2. (a,c) The subjects were either living freely in Madagascar (pale colors) or in captivity at the Duke Lemur Center (dark colors), and (b,d) those in captivity either lacked (solid bars) or gained (striped bars) forest access. $^{\S}p < 0.1$, $^{**}p < 0.01$, $^{***}p < 0.001$

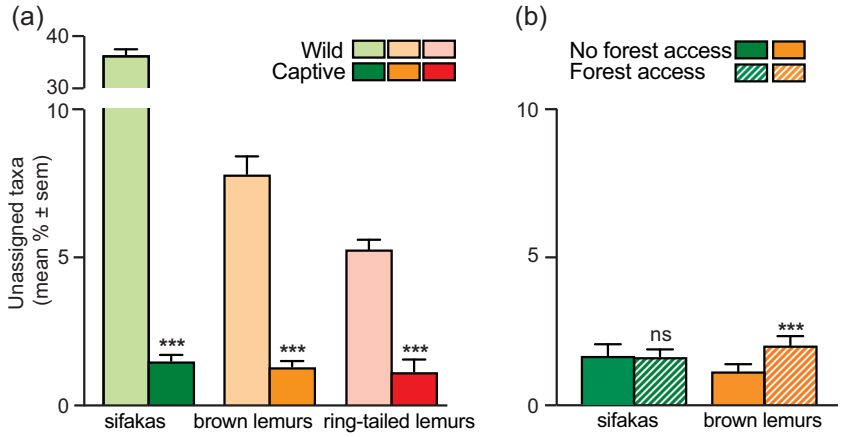


FIGURE 5 The proportion of unassigned microbes in the gut microbiomes of folivores (sifakas; green), frugivores (brown lemurs; orange), and omnivores (ring-tailed lemurs; red) in Study 2. (a) The subjects were either living freely in Madagascar (pale colors) or in captivity at the Duke Lemur Center (dark colors), and (b) those in captivity either lacked (solid bars) or gained (striped bars) forest access. *** $p < 0.001$

significantly greater proportion of *Prevotella* ($W = 1{,}492$, $z = 5.479$, $p < 0.001$); and, captive omnivores (i.e., the ring-tailed lemurs) had a significantly greater proportions of *Bacteroides* ($W = 348$, $z = 3.103$, $p = 0.003$) and modestly greater proportions of *Prevotella* ($W = 299$, $z = 1.80$, $p = 0.072$).

The relative abundance of unassigned taxa was also reduced in captive lemurs compared with their wild counterparts (Figure 5a). On average, the unassigned taxa in the GMBs of wild sifakas, brown lemurs, and ring-tailed lemurs, respectively, represented 36.9%, 8.1%, and 5.3% of sequences, whereas the unassigned taxa in the GMBs of captive members of the same hosts represented just 1.6%, 1.3%, and 1.2% of sequences, respectively. These differences were statistically significant (sifakas: $W = 0$, $z = -7.663$; brown lemurs: $W = 34$, $z = -7.460$; ring-tailed lemurs: $W = 18$, $z = -5.407$; all $p < 0.001$).

4.3.2 | The effect of forest access on the GMBs of captive lemurs

The relative abundances of both *Bacteroides* and *Prevotella* in lemur GMBs also differed between captive animals that were granted versus denied forest access (Figure 4c,d). Compared to their peers that were denied forest access, captive sifakas that were granted forest access had modestly smaller proportions of *Bacteroides* ($W = 104$, $z = -0.657$, $p = 0.062$), whereas brown lemurs granted forest access had significantly greater proportions of *Bacteroides* ($W = 130$, $z = 2.943$, $p = 0.003$), but smaller proportions of *Prevotella* ($W = 402$, $z = -2.635$, $p = 0.008$). The proportion of unassigned taxa was also significantly greater for captive brown lemurs granted forest access compared with their peers lacking forest access ($W = 112$, $z = 3.352$, $p < 0.001$); however, for sifakas, forest access did not alter the abundance of unassigned taxa ($W = 72$, $z = 0.131$, $p = 0.90$; Figure 5b).

4.4 | Discussion

By comparatively examining gut microbial taxa across diverse lemurs and environmental conditions, we show that host feeding strategy can mediate the perturbational effect of captivity. Our results indicate that both dietary and environmental mechanisms contribute to captivity-induced dysbiosis. Consistent with previous research (Clayton, Al-Ghalith, et al., 2018; Clayton et al., 2016), the captive lemurs had different proportions of *Bacteroides* and *Prevotella*, and diminished proportions of unassigned taxa, compared with their wild peers. The direction and magnitude of these wild/captive differences were host specific, however, with the specialists showing greater "effects" of captivity compared to the generalists. As consummate omnivores (Sauther, Sussman, & Gould, 1999), ring-tailed lemurs perhaps harbor GMBs that are better able to buffer the consequences of captivity, a finding that is consistent with reports of wild ring-tailed lemurs having GMBs that appear to be somewhat resistant to anthropogenic habitat disturbance (Bennett et al., 2016). We further showed that management techniques can mitigate the microbial consequences of nonnative diets and artificial environmental conditions. Working to restore or "rewild" the GMBs of captive animals has clear implications for improving host health and for promoting the ecological validity of future microbiome research. Moreover, studying the ecological processes by which host GMBs can be restored would be relevant for conservation strategies targeting anthropogenically disturbed wildlife and could be yet another benefit of research on captive populations.

Supporting the dietary mechanism of microbial dysbiosis, we found group-specific differences between captive and wild populations in their abundance of *Bacteroides* and *Prevotella*, microbes that, respectively, reflect dietary proteins and fats, and simple fibers (Wu et al., 2011). For sifakas—folivores that can flexibly rely on available fruit (Sato et al., 2016)—the total restriction of fruit and fruit-fiber consumption in captivity likely underlies the reduced abundance of *Prevotella* in captive animals. In contrast, the increased abundance of *Bacteroides* in captive sifakas could derive from the added dietary fat and protein obtained via their consumption of beans and nuts. For brown lemurs—frugivores that can flexibly fallback on immature fruits and foliage (Sato et al., 2016)—the year-round provisioning of orchard fruits to captive animals likely underlies their increased abundance of *Prevotella*. Wild and orchard fruits generally differ in composition, with the former characterized by greater fiber and the latter containing mostly flesh, with little rind, seeds, or pulp. The reduced abundance of *Bacteroides* in captive brown lemurs might indicate a deficit in fat and protein intake.

Supporting the environmental mechanism of microbial dysbiosis, we found that the proportion of unassigned taxa, which routinely comprises 6–37% of GMBs in wild lemurs, was diminished to 1–2% in captive lemurs. Moreover, the "naturally occurring" difference in unassigned taxa between wild folivores and wild non-folivores was absent in their peers living under captive conditions. The reduced abundance of unassigned taxa in captive lemurs could derive from the routine sterilization of their food and enclosures (intended to remove potential pathogens) or from their potentially repeated exposure to antibiotics. The microbiomes of current-day captive animals, like their hosts (Grogan, Sauther, Cuozzo, & Drea, 2017), might additionally reflect a remnant "founder effect" in the original pool of wild-caught subjects, with the loss of microbial diversity over time being compounded by relatively infrequent microbial influxes from new wild animals or captive animals transferred from other facilities. If native gut microbes were outcompeted by local "environmental" taxa and/or failed to be replenished, captive populations could evidence the depletion of native taxa. Such depletion could, in turn, underlie certain health problems common to captive animals (McKenney, Greene, et al., 2017; Trevelline et al., 2019). It would be interesting to know if a similar depletion in native microbes occurs in captive populations housed in close geographical proximity to their endemic range. More generally, these results support the idea that microbiome science could be beneficially incorporated into current conservation aims (Stumpf et al., 2016; Trevelline et al., 2019).

The relationship between host feeding strategy, concurrent diet, and environmental condition in differentially perturbing the GMBs of captive lemurs is further evidenced by our results from subjects that gained access to forested enclosures. Notably, for both folivores and frugivores, the opportunity to forage freely (Abhau, 2007) was associated with microbial abundances that more closely mimicked natural occurrences. For semi-free rangers, an increased consumption of young leaves or wild fruits, and a reduced consumption of orchard produce, could explain the differential proportions of both *Prevotella* and *Bacteroides*. For semi-free ranging brown lemurs, the increase in unassigned taxa could potentially stem from the colonization of microbes endemic to North Carolina's forests or from the proliferation of microbes endemic to Madagascar that survived the transition into captivity and persisted as reservoirs. Ultimately, these results support both dietary and environmental mechanisms of captivity-induced dysbiosis that can be mitigated via animal management strategies.

5 | STUDY 3. BEYOND THE GUT: SCENT-GLAND MICROBIOMES VARY BY BODY SITE AND HOST TRAITS

5.1 | Introduction

As evidenced in the previous studies, mammalian (including primate) microbiome research has been focused on the gut, reflecting the preeminence of dietary processing to host health (Clayton, Gomez, et al., 2018; Ley et al., 2008; Wu et al., 2011). Nevertheless, microbes are ubiquitous and enormously versatile, and are able to colonize most body sites (e.g. mouth, skin, genitals, scent glands; Dewhirst et al., 2010; Schommer & Gallo, 2013; Stumpf et al., 2013; Theis et al., 2013). Although the structure and function of these "other" microbiomes are often less well studied, owing in part to the necessarily more obtrusive sampling methods, they are no less significant to host health (Ichinohe et al., 2011; Schommer & Gallo, 2013). For example, in haplorhine primates, the structure of genital microbiomes varies according to species, age, sexuality, and reproductive/hormonal state, with potentially significant consequences for disease risk and reproductive health (Miller, Livermore, Alberts, Tung, & Archie, 2017; Stumpf et al., 2013; Uchihashi et al., 2015; Yildirim et al., 2014). Remarkably, various microbiomes can also affect host life-history traits (Fraune & Bosch, 2010) and social behavior (Archie & Theis, 2011; Ezenwa, Gerardo, Inouye, Medina, & Xavier, 2012), either directly, such as via the gut/brain axis (Cryan & Dinan, 2012; Mayer et al., 2015), or more indirectly, such as by affecting chemical cues that animals use to communicate (Archie & Theis, 2011; Ezenwa & Williams, 2014).

Whereas sociality has been linked to GMBs in lemurs, in that their consortia reflect social-group membership (Bennett et al., 2016; Springer et al., 2017), grooming partners (Perofsky et al., 2017; Raulo et al., 2018), and scent-marking frequencies (Perofsky et al., 2017), there have been, as yet, no studies of lemur glandular microbiomes. Indeed, of the various microbial communities inhabiting animal bodies, the glandular microbiota is among the least well studied, particularly in primates, and its linkages to individual traits and olfactory signals, and ultimately social behavior, remain poorly understood. In only two studies of social carnivorans (hyenas and meerkats) have researchers combined genetic and chemical analyses to show that the molecular composition of the host's glandular bacteria predictably covaries with the chemical composition of its glandular odorants (Leclaire, Jacob, Greene, Dubay, & Drea, 2017; Theis et al., 2013). Similar support for the fermentation hypothesis of olfactory signaling (Albone & Perry, 1976), which posits that microbes contribute to host odorants via anaerobic fermentation, could derive from similar examination of lemur glandular microbiomes. Olfactory signaling and glandular scent marking have a prominent role in strepsirrhines: Lemurs scent mark from numerous glandular scent sources across body sites (Schilling, 1979) that produce specific chemical signatures and convey information about hosts, including immutable traits like sex and genetic makeup, and transient conditions like health and reproductive state (Drea, 2015; Harris, Boulet, Grogan, & Drea, 2018). Whether or not glandular microbiomes are correlated to, or metabolically contribute to, the production of glandular odorants in lemurs remains to be explored.

Just as we probed lemur GMBs across species and at various scales of analysis in our first two studies, so too will we now comparatively examine lemur glandular microbiomes across multiple levels of analysis. Here, we focus on host features, from species, to sociodemographic groups (e.g., sex and social status), to individuals (possessing different glands). We begin by examining wild and sympatric sifakas and woolly lemurs to determine if genital microbiomes vary by species and sex. Whereas little is known about scent marking in woolly lemurs (Schilling, 1979), sifakas of both sexes routinely scent mark using anogenital glands

(Lewis, 2005; Pochron, Morelli, Scirbona, & Wright, 2005) and deposit sex-specific chemical signatures (Greene & Drea, 2014). Next, we examine the sternal exudates that can naturally stain the chest of male sifakas: Typically, dominant or breeding males have stained chests, whereas subordinate males do not (Lewis & van Schaik, 2007). Because these two "classes" of exudates are chemically distinct or bear a "chemical signature" of social status (Drea et al., 2013), we ask if they contain a similarly distinct "microbial signature," as would be predicted by the fermentation hypothesis. Finally, within species, we ask if different types of male glandular secretions are also microbially distinct. For this question, we compare the sternal versus genital secretions of wild sifakas, and the chemically distinct brachial versus genital secretions of captive ring-tailed lemurs (Scordato, Dubay, & Drea, 2007). The latter species comparison allows us to ask if glandular patterns evident in wild animals are also evident in captive animals.

5.2 | Statistical methods

We centered our statistical analyses of secretion samples (Table 2) around two types of data, microbial community similarity and taxonomic composition. We used all OTUs to calculate UUF distances. We performed four analyses of similarity on UUF distances in QIIME to compare microbial community similarity in (a) the genital secretions of wild, female sifakas and woolly lemurs; (b) the genital secretions of wild, male and female sifakas; (c) the genital and sternal secretions of wild, male sifakas (from both stained and unstained males); and (d) the genital and brachial secretions of captive, male ring-tailed lemurs. For the male sifakas, we further used Kruskal–Wallis and Dunn's multiple comparison tests on UUF distances, implemented in GraphPad Prism (GraphPad Software; San Diego, CA), to determine which of the three "types" of glandular secretions were most distinct. We used principle coordinate analysis of UUF distances to visualize variation in microbiome composition. We also retained the relative abundances of "major" OTUs that represented >1% of sequences, on average across individuals, in the microbiomes of minimally one glandular source. On these major taxa, we used separate LEfSe analyses within the four subsets of samples with the correction factor for multiple testing to determine the microbes that were significantly enriched relative to host traits.

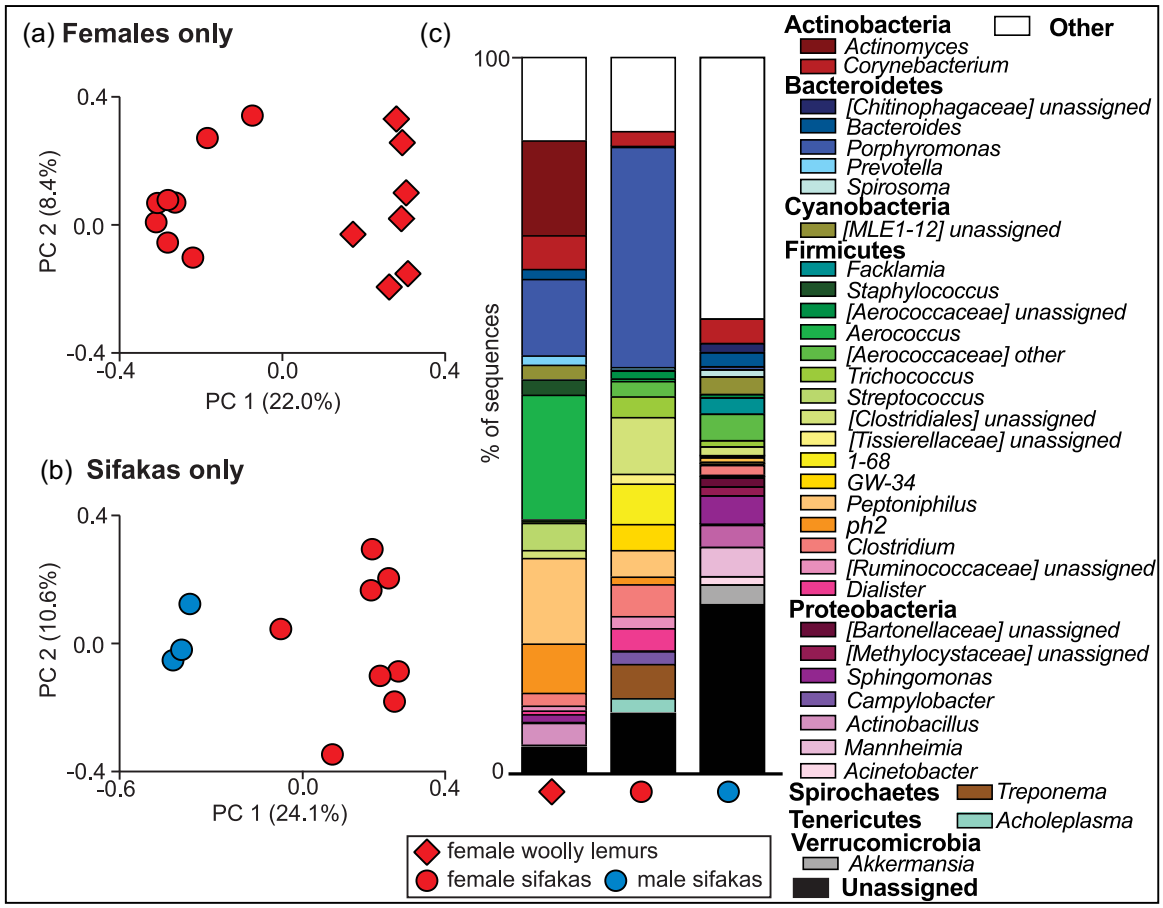


FIGURE 6 Glandular microbiomes in the genital secretions of woolly lemurs (diamonds) and sifakas (circles), including females (red) and males (blue) in Study 3. Depicted are principle coordinates (PCs) of unweighted UniFrac measures of the genital microbiomes graphed relative to host (a) species for the labial secretions of females, and (b) sex for the homologous genital secretions of sifakas. The major microbial taxa (i.e., those that accounted for >1% of sequences in minimally one glandular source across subjects) are shown (c) at the level of microbial genus relative to scent source, with color families depicting microbial phyla and distinct shades reflecting specific microbial Operational Taxonomic Units

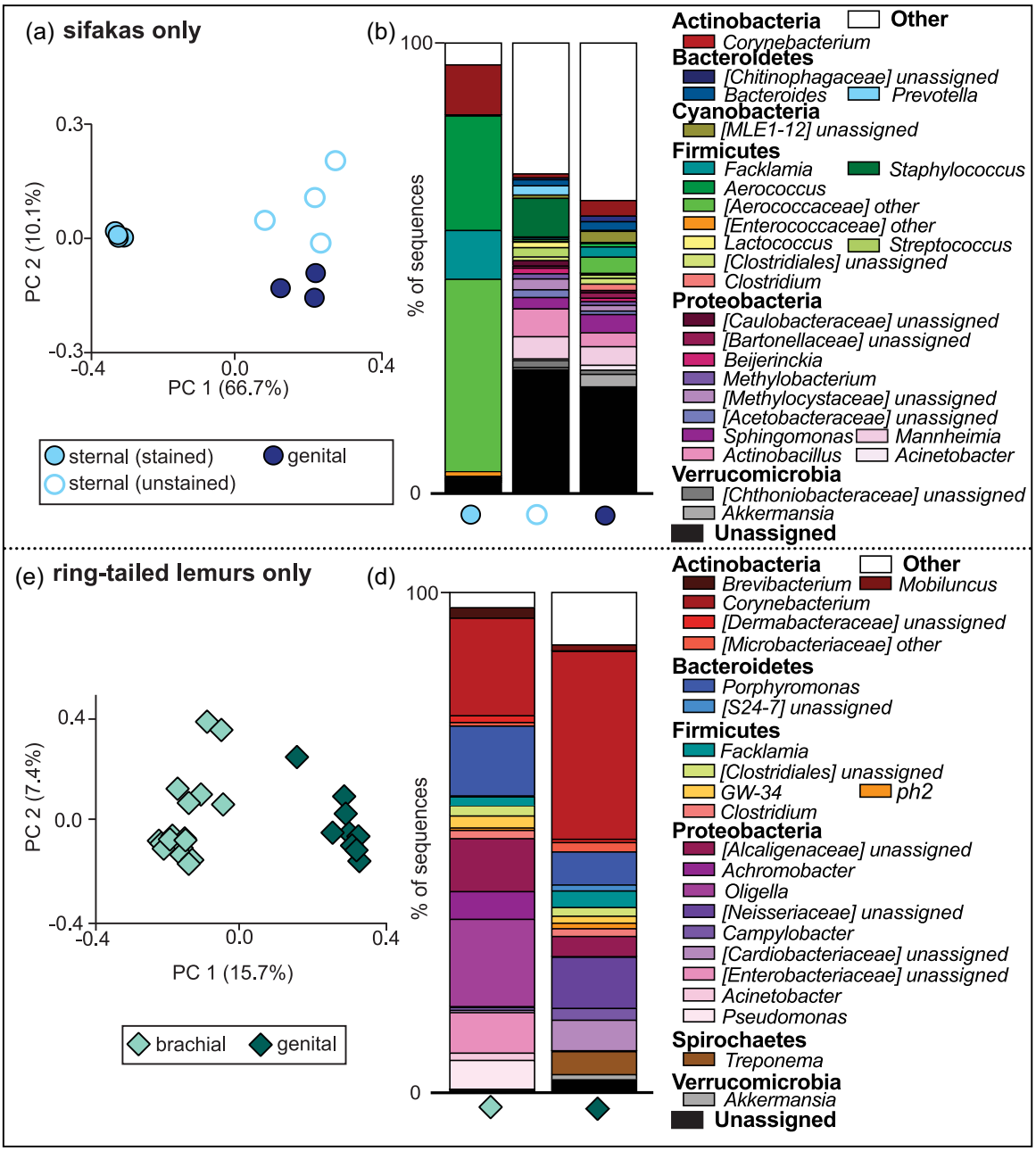


FIGURE 7 Glandular microbiomes in the secretions of (a, b) male sifakas (circles) and (c, d) ring-tailed lemurs (diamonds) in Study 3. Depicted are principle coordinates (PCs) of unweighted UniFrac distances calculated for (a) the scrotal (dark blue) and sternal secretions (light blue) of male sifakas with stained (closed) and unstained (open) chests as well as from (c) the brachial (turquoise) and scrotal (teal) secretions of male ring-tailed lemurs. The major microbial taxa (i.e., those that accounted for >1% of sequences in minimally one glandular source across subjects) are shown at the level of microbial genus relative to scent source for (b) sifakas and (d) ring-tailed lemurs, with color families depicting microbial phyla and distinct shades reflecting specific microbial Operational Taxonomic Units

5.3 | Results

5.3.1 | General patterns of glandular microbial membership

Across subjects, glandular microbiomes comprised primarily the Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes phyla, with minor contributions from Cyanobacteria, Verrucomicrobia, and Spirochaetes. Additionally, unassigned taxa (as defined in Study 1) were prevalent in the glandular consortia of wild, but not captive, lemurs (Figures 6 and 7).

5.3.2 | Species and sex differences in glandular microbiota

The genital microbiomes of wild lemurs varied by host species and sex (Figure 6). For wild, female woolly lemurs and sifakas, the overall

TABLE 5 Microbial taxa enriched in the genital microbiomes of female lemurs

	Microbial taxon			
Host species	**Phylum**	**Order**	**[Family] genus**	**Log (LDA)**
Woolly lemurs	Actinobacteria	Actinomycetales	*Actinomyces*	4.91**
	Bacteroidetes	Bacteroidales	*Bacteroides*	4.07**
	Cyanobacteria	MLE1_12	Unassigned	4.22*
	Firmicutes	Bacillales	*Staphylococcus*	4.15*
		Lactobacillales	*Aerococcus*	4.95**
			Streptococcus	4.30**
		Clostridiales	*Peptoniphilus*	4.67**
			ph2	4.56*
	Proteobacteria	Sphingomonadales	*Sphingomonas*	4.08*
		Pasteurellales	*Actinobacillus*	4.33**
Sifakas	Bacteroidetes	Bacteroidales	*Porphyromonas*	5.09*
	Firmicutes	Lactobacillales	*Facklamia*	4.01*
			Trichococcus	4.30*
			Other	4.15*
		Clostridiales	*Clostridium*	4.33*
			GW_34	4.46**
			1_68	4.55**
			Dialister	4.36**
			Unassigned	4.66**
	Spirochaetes	Spirochaetales	*Treponema*	4.50*

*$p < 0.05$.
**$p < 0.01$.

composition in genital microbiomes was significantly different between host species ($R = 0.994$, $p = 0.005$; Figure 6a). For wild sifakas, the secretions from homologous genital glands (i.e., labial and scrotal microbiomes) were compositionally distinct ($R = 0.935$, $p = 0.005$; Figure 6b). Across the genital secretions of wild hosts, 34 taxa each accounted for >1% of sequences, on average, in minimally one glandular source (Figure 6c). Twenty of these major taxa were significantly enriched in the genital consortia of either woolly lemurs or sifakas (Table 5), and 15 were significantly enriched in the genital secretions of either female or male sifakas (Table 6).

5.3.3 | Putative social status differences in glandular microbiota

The microbial assemblages of the sternal glands in wild, male sifakas varied dramatically between males based on a proxy of their social status (Figure 7a,b). Notably, the stained males (that were presumably dominant or breeding) produced sternal secretions that had a microbial composition significantly different from that characterizing unstained (or presumably subordinate) males ($R = 1.0$, $p = 0.031$; Figure 7a). The sternal secretions of stained males were characterized by only five major bacterial genera, including *Corynebacterium*, *Aerococcus*, *Facklamia*, and unidentified taxa in the Aerococcaceae and Enterococcaceae families (Figure 7b). *Proteobacteria* were conspicuously absent from the consortia of stained males. In contrast, the sternal microbiomes of unstained males were more diverse and comprised Proteobacteria (particularly *Actinobacillus*) and Firmicutes (particularly *Staphylococcus*) members. The proportion of unassigned taxa was significantly greater, by nearly 12-fold, in the sternal microbiomes of unstained males ($\log(LDA) = 5.29$, $p = 0.04$) than of stained males (Figure 7b).

TABLE 6 Microbial taxa enriched in the genital microbiomes of sifakas

	Microbial taxon			
Host sex	**Phylum**	**Order**	**[Family] genus**	**Log (LDA)**
Females	Bacteroidetes	Bacteroidales	*Porphyromonas*	5.25*
	Firmicutes	Clostridiales	*GW_34*	4.32*
			Peptoniphilus	4.35*
			1_68	4.53*
			Dialister	4.32*
Males	Actinobacteria	Actinomycetales	*Actinomyces*	3.84*
	Bacteroidetes	Bacteroidales	*Bacteroides*	4.28*
	Cyanobacteria	MLE1_12	Unassigned	4.41*
	Firmicutes	Bacillales	*Staphylococcus*	4.37*
		Lactobacillales	*Streptococcus*	4.06*
	Proteobacteria	Sphingomonadales	*Sphingomonas*	4.60*
		Pasteurellales	*Actinobacillus*	4.47*
			Mannheimia	4.67*

*$p < 0.05$.

TABLE 7 Microbial taxa enriched across glandular sources within male lemurs

	Microbial taxon			
Glandular source	**Phylum**	**Order**	**[Family] genus**	**Log (LDA)**
Analysis I: sifakas				
Sternal (stained)	Firmicutes	Lactobacillales	*Aerococcus*	5.10*
			Facklamia	4.79*
			Other	5.36*
Sternal (unstained)	Firmicutes	Bacillales	*Staphylococcus*	4.93*
		Lactobacillales	*Streptococcus*	4.20*
	Proteobacteria	Caulobacterales	[Caulobacteraceae] unassigned	4.36*
		Rhizobiales	*Beijerinckia*	4.10*
			Methylobacterium	4.12*
			[Methylocystaceae] unassigned	4.27*
		Rhodospirillales	[Acetobacteraceae] unassigned	4.22*
		Pasteurellales	*Actinobacillus*	4.65*
			Mannheimia	4.55*
	Verrucomicrobia	Chthoniobacterales	[Chthoniobacteraceae] unassigned	4.22*
Genital	Cyanobacteria	MLE1_12	Unassigned	4.34*
	Proteobacteria	Rhizobiales	[Bartonellaceae] unassigned	4.16*
		Sphingomonadales	*Sphingomonas*	4.52*
	Verrucomicrobia	Verrucomicrobiales	*Akkermansia*	4.38*
Analysis II: ring-tailed lemurs				
Brachial	Actinobacteria	Actinomycetales	*Brevibacterium*	4.29**
	Proteobacteria	Burkholderiales	*Oligella*	5.00*
Genital	Actinobacteria	Actinomycetales	*Corynebacterium*	5.08*
	Proteobacteria	Neisseriales	[Neisseriaceae] unassigned	4.82**
		Campylobacterales	*Campylobacter*	4.36**
		Cardiobacteriales	[Cardiobacteriaceae] unassigned	4.60**
	Spirochaetes	Spirochaetales	*Treponema*	4.58**

*$p < 0.05$.
**$p < 0.01$.

5.3.4 | Individual gland differences in microbiota

The sternal and genital secretions of male sifakas were compositionally distinct ($R = 0.78$, $p = 0.001$; Figure 7a,b) and enriched for different, major microbial taxa (Table 7). These differences were driven largely by the sternal microbiomes of stained males: Pairwise comparisons between the glandular sources of males indicated that the sternal microbiomes of unstained males were more similar to genital microbiomes than they were to the sternal microbiomes of stained males ($W = 4$, $z = 4.94$, $p < 0.001$).

The differences evident in wild, male sifakas between their glandular microbiomes were mirrored in captive, male ring-tailed lemurs, although the latter findings involved different types of glands. Ring-tailed lemurs harbored distinct microbiomes between their brachial and genital secretions (Figure 7c,d). Microbiome composition varied significantly between brachial and genital secretions ($R = 0.759$, $p = 0.001$; Figure 7c), seven major taxa were significantly enriched in either brachial or genital secretions (Table 7).

5.4 | Discussion

Symbiotic relationships in animal-associated microbiomes other than the GMB may be similarly important drivers of social behavior (Ezenwa et al., 2012). We report on differences in glandular microbiomes according to the species, sex, social or breeding status, and type of gland. All of these differences may underlie host social interaction, specifically through microbial contributions to the chemical composition of host scent signals. Variation in the production and circulation of reproductive hormones, which have long been implicated in the mediation of host-secreted olfactory compounds (Drea, 2015), may influence the composition of glandular microbiota, thereby affecting their volatile contribution to host signals. Here, we uncovered several patterns of covariation between hosts and their glandular microbiomes that would be consistent with this putative mechanism.

First, the genital microbiomes of lemurs comprised fermentative anaerobes, such as those from the Clostridiales, Bacteroidales, Lactobacillales orders, which are also abundant in the glandular microbiota of carnivorans (meerkats: Leclaire et al., 2017; badgers: Sin, Buesching, Burke, & Macdonald, 2012; hyenas: Theis et al., 2013). The relative abundance of these taxa, as well as overall microbiome composition, varied by host species and by sex. These general patterns reprise findings in various strepsirrhines, including sifakas and ring-tailed lemurs, in that chemical signatures also vary by species, sex, and gland of origin (delBarco-Trillo, Sacha, Dubay, & Drea, 2012; Greene & Drea, 2014; Scordato et al., 2007). The differences we observed between types of glandular secretions are consistent with the microbial differences observed between epithelial microhabitats (Council

et al., 2016) and further suggest functional differences between the various glands and their products (Drea & Scordato, 2008).

Second, our visual characterization of the sternal gland "dimorphism" in male diademed sifakas was supported by dramatic microbial differences between the sternal secretions of stained and unstained males. Notably, stained males harbored a simple glandular microbiome, dominated by lactic-acid bacteria in the Aerococcaceae family, as well as *Corynebacterium*, whereas unstained males harbored a richer glandular microbiome, comprising taxa like *Staphylococcus* and many Proteobacteria members. Relative to the sternal consortia of stained males, those of unstained males were more comparable with the male's genital (or scrotal) consortia, both showing similarities with previously described skin microbiota (Cosseau et al., 2016). Evidence of sternal gland dimorphism in diademed sifakas, similar to that described for the related Verreaux's sifaka (Lewis & van Schaik, 2007) suggests that variation in these glands may be attributed to a comparable underlying mechanism. If so, status-associated testosterone concentrations (Lewis, 2009) may affect the chemical quality of male sifaka olfactory signals (Drea et al., 2013), potentially through androgenic effects on sternal microbiota. Such a mechanism could have broad applicability for explaining effects of social status on chemical signatures across species (e.g. Drea et al., 2013; Hayes, Richardson, & Wyllie, 2003; Kruczek, 1997; Setchell et al., 2010). Moreover, it would be consistent with the role of circulating gonadal and adrenal hormones in governing microbiome structure, more broadly (Brotman, Ravel, Bavoil, Gravitt, & Ghanem, 2014; Sudo, 2014). These ideas could be tested by manipulating reproductive hormones under controlled conditions to examine the effects on microbial communities.

Beyond addressing the particular relevance of microbiomes to host communication and social behavior, the present study illustrates the potential richness of information to be gained through comparative study of diverse and underrepresented microbiomes. Indeed, these analyses merely scratched the surface of glandular microbial complexity. As with the study of nontraditional models and the countless unassigned microbial taxa they host, the study of underrepresented microbiomes also reveals the limits of our understanding of this burgeoning field and highlight the need for continued bioprospection. Our comparisons across wild and captive populations draw attention to the benefits of dual field and laboratory approaches. That gland-specific consortia were evident both in wild and captive subjects shows that certain patterns of microbial diversity may be immutable: Whereas dietary or environmental constraints imposed by captivity may alter GMBs (Clayton, Al-Ghalith, et al., 2018; Study 2), comparable effects of captivity may not be universal across microbiomes. Thus, instead of viewing captive animals as generally unrepresentative of the natural situation, they could be more profitably seen as providing an untapped resource for testing hypotheses about the nature of host–microbiome relations.

5.5 | Conclusions

Capitalizing on the phylogenetic and ecological diversity of lemurs, we have showcased the comparative approach for exploring host–microbiome symbioses across multiple scales of analysis. Our first study contributes to the debate over the relative influence of host phylogenetic placement versus feeding ecology on GMB structure (Groussin et al., 2017; Nishida & Ochman, 2018). We specifically showed that both constraints shape GMB membership but the phylogenetic scale of analyses can influence the results and their interpretation. Our findings are consistent with recent reports across mammals (Groussin et al., 2017; Ley et al., 2008; Nishida & Ochman, 2018; Perofsky et al., 2019) and primates (Amato et al., 2018), including lemurs (Greene et al., in review), that, at gross scales and when feeding strategies are convergent, phylogeny is a strong predictor of GMB structure. Nevertheless, when examining dynamics within lineages of folivores or herbivores compared with frugivores or omnivores, feeding strategy more strongly underlies host–microbiome coadaptation. This contradiction perhaps stems from the prediction that convergent feeding strategies should result in structurally convergent GMBs, rather than expecting different lineages to solve similar challenges in different ways. Of all the feeding strategies available to animals, folivory and herbivory fundamentally rely on microbial enzymes to ferment complex fibers, like cellulose, into nutrients (Flint et al., 2008). That metabolic synergy between host and microbes is required to sustain folivory evokes the concept of a holobiont, where the unit of selection is the collective genome of hosts and their microbiota (Bordenstein & Theis, 2015). Although the hologenome theory of evolution is a subject of debate (Douglas & Werren, 2016; Moran & Sloan, 2015; Theis et al., 2016), it might be most applicable when hosts are wholly reliant on microbial action for sustenance and survival.

In our second study, we scaled our analyses across environments to probe the dietary and environmental mechanisms that potentially underlie captivity-induced microbial dysbiosis. Although captivity perturbed the GMBs of all lemurs, the strength and direction of the effect was dependent on host feeding strategy and the opportunity for free-choice foraging. We suggest that naturalizing the lifestyles of captive animals could help rewild their GMBs. From a conservation perspective the notion of rewilding is exciting, as captive populations provide a genetic "safety net" for endangered wildlife and their natural microbiota (Trevelline et al., 2019). Microbial tools can thus contribute to the optimization of animal diets, nutrition, and health (Greene et al., 2018; Stumpf et al., 2016). Ultimately, a better understanding of how captivity perturbs GMBs relative to host traits could lead to improved management techniques with clear conservation implications. Given certain logistical constraints of working with wildlife, further research on captive animals could provide insight into the mechanisms that mediate host–microbiome dynamics.

In our third study, we comparatively explored glandular microbiomes across species, sexes, proxies of social status, and scent sources. Although situated at different body sites, many of the microbial metabolic processes within the gut and glands are similar, but their function for hosts may be site-specific. Indeed, microbes allow hosts to profitably use their capacity for anaerobic metabolism, that is fermentation, in numerous ways. In the gut, fiber is fermented to produce energy (Wong et al., 2006), whereas

in scent glands, the by-products of fermentation become olfactory signals (Theis et al., 2013). That microbes contribute to mammalian olfactory signals via fermentation is a well-established hypothesis (Albone & Perry, 1976), supported by more recent analyses in which chemical and microbial composition have been correlated (Leclaire et al., 2017; Theis et al., 2013). Here, we add to this literature by showing that broad patterns in chemical differences between lemur scent signals, across scales, are mirrored by broad patterns in microbial differences. Our finding that a proxy of male social status, which has been correlated to testosterone concentrations (Lewis, 2009), is evidenced in sternal chemistry (Drea et al., 2013) and glandular microbiomes, suggests an additional role for circulating steroid hormones in underlying microbiome structure. Because microbial products in scent glands can also serve health benefits (Martín-Vivaldi et al., 2010), the interplay occurring in scent glands between microbial metabolism that promotes host health versus producing signals of permanent and transient host health (Harris et al., 2018) is an area that deserves greater research attention.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

L. K. G. and C. M. D. conceived of and designed the studies with input from all authors. L. K. G., S. L. B., R. L. H., S. R. G., and C. M. D. collected samples. E. A. M. developed sequencing and bioinformatics protocols with A. D. Y. L. K. G. and S. L. B. performed sample and data analysis. L. K. G. and C. M. D. drafted the manuscript, and S. L. B. contributed to the final preparation, including illustrating Figure 1.

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REFERENCES

Abhau, A. (2007). *The survival of a Malagasy lemur species Propithecus verreauxi coquereli in captivity: The vital role of a self-selected plant diet* (unpublished doctoral dissertation). Technischen Universität Carolo-Wilhelmina zu Braunschweig, Braunschweig.

Albert, K., Rani, A., & Sela, D. A. (2018). The comparative genomics of Bifidobacterium callitrichos reflects dietary carbohydrate utilization within the common marmoset gut. *Microbial Genomics*, *4*, e000183. https://doi.org/10.1099/mgen.0.000183

Albone, E. S., & Perry, G. C. (1976). Anal sac secretion of the red fox, *Vulpes vulpes*; volatile fatty acids and diamines: Implications for a fermentation hypothesis of chemical recognition. *Journal of Chemical Ecology*, *2*, 101–111. https://doi.org/10.1007/BF00988029

Ali, J. R., & Huber, M. (2010). Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature*, *463*, 653–656. https://doi.org/10.1038/nature0876

Amato, K. R., Martinez-Mota, R., Righini, N., Raguet-Schofield, M., Corcione, F. P., Marini, E., & Leigh, S. R. (2016). Phylogenetic and ecological factors impact the gut microbiota of two Neotropical primate species. *Oecologia*, *180*, 717–733. https://doi.org/10.007/s00442-015-3507-z

Amato, K. R., Sanders, J. G., Song, S. J., Nute, M., Metcalf, J. L., Thompson, L. R., & Leigh, S. R. (2018). Evolutionary trends in host physiology outweigh dietary niche in structuring primate gut microbiomes. *The ISME Journal*, *1*, 576–587. https://doi.org/10.1038/s41396-018-0175-0

Amato, K. R., Yeoman, C. J., Cerda, G., Schmitt, C., Cramer, J. D., Miller, M. E. B., & Leight, S. R. (2015). Variable responses of human and non-human primate gut microbiomes to a Western diet. *Microbiome*, *3*, 53. https://doi.org/10.1186/s40168-015-0120-7.

Archie, E. A., & Theis, K. R. (2011). Animal behaviour meets microbial ecology. *Animal Behaviour*, *82*, 425–436. https://doi.org/10.1016/j.anbehav.2011.05.029

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*, 289–300. https://doi.org/10.2307/2346101

Bennett, G., Malone, M., Sauther, M. L., Cuozzo, F. P., White, B., Nelson, K. E., & Amato, K. R. (2016). Host age, social group, and habitat type influence the gut microbiota of wild ring-tailed lemurs (*Lemur catta*). *American Journal of Primatology*, *78*, 883–892. https://doi.org/10.1002/ajp.22555

Van Bonn, W., LaPointe, A., Gibbons, S. M., Frazier, A., Hampton-Marcell, J., & Gilbert, J. (2015). Aquarium microbiome response to ninety-percent system water change: Clues to microbiome management. *Zoo Biology*, *34*, 360–367. https://doi.org/10.1002/zoo.21220

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLOS Biology*, *13*, e1002226. https://doi.org/10.1371/journal.pbio.1002226

Brotman, R. M., Ravel, J., Bavoil, P. M., Gravitt, P. E., & Ghanem, K. G. (2014). Microbiome, sex hormones, and immune responses in the reproductive tract: Challenges for vaccine development against sexually transmitted infections. *Vaccine*, *32*, 1543–1552. https://doi.org/10.1016/j.vaccine.2013.10.010

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., & Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*, 335–336. https://doi.org/10.1038/nmeth.f.303

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal*, *6*, 1621–1624. https://doi.org/10.1038.ismej.2012.8

Chen, T., Long, W., Zhang, C., Liu, S., Zhao, L., & Hamaker, B. R. (2017). Fiber-utilizing capacity varies in Prevotella-versus Bacteroides-dominated gut

microbiota. *Scientific Reports*, *7*, 2594. https://doi.org/10.1038/s41598-017-02995-4

Cho, I., & Blaser, M. J. (2012). The human microbiome: At the interface of health and disease. *Nature Reviews Genetics*, *13*, 260–270. https://doi.org/10.1038/nrg3182

Clayton, J. B., Al-Ghalith, G. A., Long, H. T., Van Tuan, B., Cabana, F., Huang, H., & Johnson, T. J. (2018). Associations between nutrition, gut microbiome, and health in a novel nonhuman primate model. *Scientific Reports*, *8*, 11159. https://doi.org/10.1038/s41598-018-29277-x

Clayton, J. B., Gomez, A., Amato, K., Knights, D., Travis, D. A., Blekhman, R., & Johnson, T. J. (2018). The gut microbiome of nonhuman primates: Lessons in ecology and evolution. *American Journal of Primatology*, *80*, e22867. https://doi.org/10.1002/ajp.22867

Clayton, J. B., Vangay, P., Huang, H., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., & Knights, D. (2016). Captivity humanizes the primate microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 10376–10381. https://doi.org/10.1073/pnas.1521835113

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The impact of the gut microbiota on human health: An integrative view. *Cell*, *148*, 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Cosseau, C., Romano-Bertrand, S., Duplan, H., Lucas, O., Ingrassia, I., Pigasse, C., & Jumas-Bilak, E. (2016). *Proteobacteria* from the human skin microbiota: Species-level diversity and hypotheses. *One Health*, *2*, 33–41. https://doi.org/10.1016/j.onehlt.2016.020.002

Costello, E. K., Stagaman, K., Dethlefsen, L., Bohannan, B. J., & Relman, D. A. (2012). The application of ecological theory toward an understanding of the human microbiome. *Science*, *336*, 1255–1262. https://doi.org/10.1126/science/1224203

Council, S. E., Savage, A. M., Urban, J. M., Ehlers, M. E., Skene, J. P., Platt, M. L., & Horvath, J. E. (2016). Diversity and evolution of the primate skin microbiome. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20152586. DOI: 10.1098/rspb.2015.2586

Cryan, J. F., & Dinan, T. G. (2012). Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience*, *13*, 701–712. https://doi.org/10.1038/nrn3346

delBarco-Trillo, J., Sacha, C. R., Dubay, G. R., & Drea, C. M. (2012). Eulemur, me lemur: The evolution of scent-signal complexity in a primate clade. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*, 1909–1922. https://doi.org/10.1098/rstb.2011.0225

Delsuc, F., Metcalf, J. L., Wegener Parfrey, L., Song, S. J., González, A., & Knight, R. (2014). Convergence of gut microbiomes in myrmecophagous mammals. *Molecular Ecology*, *23*, 1301–1317. https://doi.org/10.1111/mec.12501

Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W.-H., & Wade, W. G. (2010). The human oral microbiome. *Journal of Bacteriology*, *192*, 5002–5017. https://doi.org/10.1128/JB.00542-10

Ding, T., & Schloss, P. D. (2014). Dynamics and associations of microbial community types across the human body. *Nature*, *509*, 357–360. https://doi.org/10.1038/nature13178

Douglas, A. E., & Werren, J. H. (2016). Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio*, *7*, e02099–02015. https://doi.org/10.1128/mBio.02099–15 s

Drea, C. M. (2015). D'scent of man: A comparative survey of primate chemosignaling in relation to sex. *Hormones and Behavior*, *68*, 117–133. https://doi.org/10.1016/j.ybeh.2014.08.001

Dray, S., & Dufour, A. (2007). The ade4 Package: Implementing the Duality Diagram for Ecologists. *Journal of Statistical Software*, *22*, 1–20. https://doi.org/10.18647/jss.v022.i04

Drea, C. M., Boulet, M., Delbarco-Trillo, J., Greene, L. K., Sacha, C. R., Goodwin, T. E., & Dubay, G. R. (2013). The "secret" in secretions: Methodological considerations in deciphering primate olfactory communication. *American Journal of Primatology*, *75*, 621–642. https://doi.org/10.1002/ajp.22143

Drea, C. M., & Scordato, E. S. (2008). Olfactory communication in the ringtailed lemur (*Lemur catta*): Form and function of multimodal signals. In J. L. Hurst, R. L. Beyon, S. C. Roberts, & T. D. Wyatt (Eds.), *Chemical Signals in Vertebrates* 11, pp. 91–102). New York: Springer. https://doi.org/10.1007/978-0-387-73945-8_8.

Ezenwa, V. O., Gerardo, N. M., Inouye, D. W., Medina, M., & Xavier, J. B. (2012). Animal behavior and the microbiome. *Science*, *338*, 198–199. https://doi.org/10.1126/science.1227412

Ezenwa, V. O., & Williams, A. E. (2014). Microbes and animal olfactory communication: Where do we go from here? *BIOESSAYS*, *36*, 847–854. https://doi.org/10.1002/bies.201400016

Flint, H. J., Bayer, E. A., Rincon, M. T., Lamed, R., & White, B. A. (2008). Polysaccharide utilization by gut bacteria: Potential for new insights from genomic analysis. *Nature Reviews Microbiology*, *6*, 121–131. https://doi.org/10.1038/nrmicro1817

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes*, *3*, 289–306. https://doi.org/10.4161/gmic19897

Foster, K. R., Schluter, J., Coyte, K. Z., & Rakoff-Nahoum, S. (2017). The evolution of the host microbiome as an ecosystem on a leash. *Nature*, *548*, 43–51. https://doi.org/10.1038/nature2329

Fraune, S., & Bosch, T. C. (2010). Why bacteria matter in animal development and evolution. *BIOESSAYS*, *32*, 571–580. https://doi.org/10.1002/bies.200900192

Freedman, D. A. (1999). Ecological inference and the ecological fallacy. In N. J. Smelser, & P. B. Baltes (Eds.), *International encyclopedia of the social & behavioral sciences* Vol *6*, pp. 4027–3030). Amsterdam: Elsevier.

Gomez, A., Rothman, J. M., Petrzelkova, K., Yeoman, C. J., Vlckova, K., Umaña, J. D., & Leigh, S. R. (2016). Temporal variation selects for diet-microbe co-metabolic traits in the gut of *Gorilla* spp. *The ISME Journal*, *10*, 514–526. https://doi.org/10.1038/ismej.2015.146

Greene, L. K., Clayton, J. B., Rothman, R. S., Semel, B. P., Semel, M. A., Gillespie, T. R., & Drea, C. M. Local habitat, not phylogenetic relatedness, predicts gut microbiome structure within frugivorous and folivorous lemur lineages. *Biology Letters*. in review

Greene, L. K., & Drea, C. M. (2014). Love is in the air: Sociality and pair bondedness influence sifaka reproductive signalling. *Animal Behaviour*, *88*, 147–156. https://doi.org/10.1016/j.anbehav.2013.11.019

Greene, L. K., & McKenney, E. A. (2018). The inside tract: The appendicular, cecal, and colonic microbiome of captive aye-ayes. *American Journal of Physical Anthropology*, *166*, 960–967. https://doi.org/10.1002/ajpa.23481

Greene, L. K., McKenney, E. A., O'Connell, T. M., & Drea, C. M. (2018). The critical role of dietary foliage in maintaining the gut microbiome and metabolome of folivorous sifakas. *Scientific Reports*, *8*, 14482. https://doi.org/10.1038/s41598-018-32759-7

Grogan, K. E., Sauther, M. L., Cuozzo, F. P., & Drea, C. M. (2017). Genetic wealth, population health: Major histocompatibility complex variation in captive and wild ring-tailed lemurs. *Ecology and Evolution*, *7*, 7638–7649. https://doi.org/10.1002/ece3.3317

Groussin, M., Mazel, F., Sanders, J. G., Smillie, C. S., Lavergne, S., Thuiller, W., & Alm, E. J. (2017). Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. *Nature Communications*, *8*, 14319. https://doi.org/10.1038/ncomms14319

Gunnell, G. F., Boyer, D. M., Friscia, A. R., Heritage, S., Manthi, F. K., Miller, E. R., & Seiffert, E. R. (2018). Fossil lemurs from Egypt and Kenya suggest an African origin for Madagascar's aye-aye. *Nature Communications*, *9*, 3193. https://doi.org/10.1038/s41467-018-05648-w

Hale, V. L., Tan, C. L., Knight, R., & Amato, K. R. (2015). Effect of preservation method on spider monkey (*Ateles geoffroyi*) fecal

microbiota over 8 weeks. *Journal of Microbiological Methods*, *113*, 16–26. https://doi.org/10.1016/j.mimet.2015-03-021

Harris, R. L., Boulet, M., Grogan, K. E., & Drea, C. M. (2018). Costs of injury for scent signalling in a strepsirrhine primate. *Scientific Reports*, *8*, 9882. https://doi.org/10.1038/s41598-018-27322-3

Hayes, R., Richardson, B., & Wyllie, S. (2003). To fix or not to fix: The role of 2-phenoxyethanol in rabbit, *Oryctolagus cuniculus*, chin gland secretion. *Journal of Chemical Ecology*, *29*, 1051–1064. https://doi.org/10.1023/A:1023836319677

Herrera, J. P., & Dávalos, L. M. (2016). Phylogeny and divergence times of lemurs inferred with recent and ancient fossils in the tree. *Systematic Biology*, *65*, 772–791. https://doi.org/10.0193/sysbio.syw035

Hladik, C. M., & Charles-Dominique, P. (1974). The behavior and ecologyof the sportive lemur (*Lepilemur mustelinus*) in relation to its dietarypeculiarities. In D. Chivers, & J. Herbert (Eds.), *Prosimian Biology* (pp. 23–27). London: Duckworth.

Horvath, J. E., Weisrock, D. W., Embry, S. L., Fiorentino, I., Balhoff, J. P., Kappeler, P., & Yoder, A. D. (2008). Development and application of a phylogenomic toolkit: Resolving the evolutionary history of Madagascar's lemurs. *Genome Research*, *18*, 489–499. https://doi.org/10.1101/gr.726520

Ichinohe, T., Pang, I. K., Kumamoto, Y., Peaper, D. R., Ho, J. H., Murray, T. S., & Iwasaki, A. (2011). Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proceedings of the National Academy of Sciences of the United States of America*, *108*, 5354–5359. https://doi.org/10.1073/pnas.1019378108

Jolly, A., & Sussman, R. W. (2006). Notes on the history of ecological studies of Malagasy lemurs. In L. Gould, & M. L. Sauther (Eds.), *Lemurs: Ecology and adaptation* (pp. 19–39). New York: Springer.

Junge, R. E., Williams, C. V., Rakotondrainibe, H., Mahefarisoa, K. L., Rajaonarivelo, T., Faulkner, C., & Mass, V. (2017). Baseline health andnutrition evaluation of two sympatric nocturnal lemur species (*Avahi laniger and Lepilemur mustelinus*) residing near an active mine site atAmbatovy, Madagascar. *Journal of Zoo and Wildlife Medicine*, *48*, 794–803. https://doi.org/10.1638/2016-0261

Jungers, W. L., Godfrey, L. R., Simons, E. L., Wunderlich, R. E., Richmond, B. G., & Chatrath, P. S. (2002). Ecomorphology and behavior of giant extinct lemurs from Madagascar. In J. M. Plavcan, R. F. Kay, C. P. van Schaik, & W. L. Jungers (Eds.), *Reconstructing behavior in the primate fossil record* (pp. 71–411). New York: Kluwer Academic/Plenum Press. https://doi.org/10.1007/978-1-4615-1348-8_10.

Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L., & Gordon, J. I. (2011). Human nutrition, the gut microbiome and the immune system. *Nature*, *474*, 327–336. https://doi.org/10.1038/nature10213

Kohl, K. D., Brun, A., Magallanes, M., Brinkerhoff, J., Laspiur, A., Acosta, J. C., & Bordenstein, S. R. (2017). Gut microbial ecology of lizards: Insights into diversity in the wild, effects of captivity, variation across gut regions and transmission. *Molecular Ecology*, *26*, 1175–1189. https://doi.org/10.111/mec.13921

Kohl, K. D., & Dearing, M. D. (2016). The woodrat gut microbiota as an experimental system for understanding microbial metabolism of dietary toxins. *Frontiers in Microbiology*, *7*, 1165. https://doi.org/10.3389/fmicb.2016.01165

Kohl, K. D., Skopec, M. M., & Dearing, M. D. (2014). Captivity results in disparate loss of gut microbial diversity in closely related hosts. *Conservation Physiology*, *2*, cou009. https://doi.org/10.1093/conphys/cou009

Kohl, K. D., Stengel, A., & Dearing, M. D. (2015). Inoculation of tannin-degrading bacteria into novel hosts increases performance on tannin-rich diets. *Environmental Microbiology*, *18*, 1720–1729. https://doi.org/10.1111/1462-2920.12841

Kruczek, M. (1997). Male rank and female choice in the bank vole, *Clethrionomys glareolus*. *Behavioural Processes*, *40*, 171–176. https://doi.org/10.1016/S0376-6357(97)00785-7

LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., & Ventura, M. (2013). Bacteria as vitamin suppliers to their host: A gut microbiota perspective. *Current Opinion in Biotechnology*, *24*, 160–168. https://doi.org/10.1016/j.copbio.2012.08.005

Leclaire, S., Jacob, S., Greene, L. K., Dubay, G. R., & Drea, C. M. (2017). Social odours covary with bacterial community in the anal secretions of wild meerkats. *Scientific Reports*, *7*, 3240. https://doi.org/10.1038/s41598-017-03356-x

Lewis, R. J. (2005). Sex differences in scent-marking in sifaka: Mating conflict or male services? . *American Journal of Physical Anthropology*, *128*, 389–398. https://doi.org/10.1002/ajpa.20206

Lewis, R. J. (2009). Chest staining variation as a signal of testosterone levels in male Verreaux's Sifaka. *Physiology & Behavior*, *96*, 586–592. https://doi.org/10.1016/j.physbeh.2008.12.020

Lewis, R. J., & van Schaik, C. P. (2007). Bimorphism in male Verreaux's sifaka in the Kirindy Forest of Madagascar. *International Journal of Primatology*, *28*, 159–182. https://doi.org/10.1007/s10764-006-9107-3

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. *Science*, *320*, 1647–1651. https://doi.org/10.1126/science.1155725

Lozupone, C., Lladser, M. E., Knights, D., Stombaugh, J., & Knight, R. (2011). UniFrac: An effective distance metric for microbial community comparison. *The ISME Journal*, *5*, 169–172. https://doi.org/10.1038/ismej.2010.133

Martin, R. D. (1972). Review lecture: Adaptive Radiation and Behaviour of the Malagasy Lemurs. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, *264*, 295–352. https://doi.org/10.1098/rstb.1972.0013

Martín-Vivaldi, M., Peña, A., Peralta-Sánchez, J. M., Sánchez, L., Ananou, S., Ruiz-Rodríguez, M., & Soler, J. J. (2010). Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proceedings of the Royal Society B: Biological Sciences*, *277*, 123–130. https://doi.org/10.1098/rspb.2009.1377

Mayer, E. A., Tillisch, K., & Gupta, A. (2015). Gut/brain axis and the microbiota. *The Journal of Clinical Investigation*, *125*, 926–938. https://doi.org/10.1172/JCI76304

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., & Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 3229–3236. https://doi.org/10.1073/pnas.1218525110

McKenna, P., Hoffmann, C., Minkah, N., Aye, P. P., Lackner, A., Liu, Z., & Bushman, F. D. (2008). The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. *PLOS Pathogens*, *4*, e20. https://doi.org/10.1371/journal.ppat.0040020

McKenney, E., O'Connell, T. M., Rodrigo, A., & Yoder, A. D. (2017). Feeding strategy shapes gut metagenomic enrichment and functional specialization in captive lemurs. *Gut Microbes*, *9*, 202–217. https://doi.org/10.1080/19490976.2017.140876

McKenney, E. A., Greene, L. K., Drea, C. M., & Yoder, A. D. (2017). Down for the count: Cryptosporidium infection depletes the gut microbiome in Coquerel's sifakas. *Microbial Ecology in Health and Disease*, *28*, 1335165. https://doi.org/10.1080/16512235.2017.1335165

McKenney, E. A., Maslanka, M., Rodrigo, A., & Yoder, A. D. (2018). Bamboo specialists from two mammalian orders (Primates, Carnivora) share a high number of low-abundance gut microbes. *Microbial Ecology*, *76*, 272–284. https://doi.org/10.1007/s00248-017-1114-8

McKenney, E. A., Rodrigo, A., & Yoder, A. D. (2015). Patterns of gut bacterial colonization in three primate species. *PLOS One*, *10*, e0124618. https://doi.org/10.1371/journal.pone.0124618

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., & 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

Miller, E. A., Livermore, J. A., Alberts, S. C., Tung, J., & Archie, E. A. (2017). Ovarian cycling and reproductive state shape the vaginal microbiota in wild baboons. *Microbiome*, *5*, 8. https://doi.org/10.1186/s40168-017-0228-z

Mittermeier, R. A., Ganzhorn, J. U., Konstant, W. R., Glander, K., Tattersall, I., Groves, C. P., & Rasoloarison, R. M. (2008). Lemur diversity in Madagascar. *International Journal of Primatology*, *29*, 1607–1656. https://doi.org/10.1007/s10764-008-9317-y

Moran, N. A., & Sloan, D. B. (2015). The hologenome concept: Helpful or hollow? *PLOS Biology*, *13*, e1002311. https://doi.org/10.1371/journal.pbio.1002311

Nishida, A. H., & Ochman, H. (2018). Rates of gut microbiome divergence in mammals. *Molecular Ecology*, *27*, 1884–1897. https://doi.org/10.1111/mec.14473

Ochman, H., Worobey, M., Kuo, C.-H., Ndjango, J.-B. N., Peeters, M., Hahn, B. H., & Hugenholtz, P. (2010). Evolutionary relationships of wild hominids recapitulated by gut microbial communities. *PLOS Biology*, *8*, e1000546. https://doi.org/10.1371/journal.pbio.1000546

O'Sullivan, A., He, X., McNiven, E. M., Haggarty, N. W., Lönnerdal, B., & Slupsky, C. M. (2013). Early diet impacts infant rhesus gut microbiome, immunity, and metabolism. *Journal of Proteome Research*, *12*, 2833–2845. https://doi.org/10.1021/pr4001702

Perofsky, A. C., Lewis, R. J., Abondano, L. A., Di Fiore, A., & Meyers, L. A. (2017). Hierarchical social networks shape gut microbial composition in wild Verreaux's sifaka. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20172274. https://doi.org/10.1098/rspb.2017.2274

Perofsky, A. C., Lewis, R. J., & Meyers, L. A. (2019). Terrestriality and bacterial transfer: A comparative study of gut microbiomes in sympatric Malagasy mammals. *The ISME Journal*, *13*, 50–63. https://doi.org/10.1038/s41396-018-0251-5

Pochron, S. T., Morelli, T. L., Scirbona, J., & Wright, P. C. (2005). Sex differences in scent marking in *Propithecus edwardsi* of Ranomafana National Park, Madagascar. *American Journal of Primatology*, *66*, 97–110. https://doi.org/10.1002/ajp.20130

R Core Team. (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Raulo, A., Ruokolainen, L., Lane, A., Amato, K., Knight, R., Leigh, S., & Tecot, S. R. (2018). Social behaviour and gut microbiota in red-bellied lemurs (*Eulemur rubriventer*): In search of the role of immunity in the evolution of sociality. *Journal of Animal Ecology*, *87*, 388–399. https://doi.org/10.1111/1365-2656.12781

Dos Reis, M., Gunnell, G. F., Barba-Montoya, J., Wilkins, A., Yang, Z., & Yoder, A. D. (2018). Using phylogenomic data to explore the effects of relaxed clocks and calibraiton strategies on divergence time estimation: Primates as a test case. *Systematic Biology*, *67*, 594–615. https://doi.org/10.1093/sys-bio/syy001

Richard, A. F., & Dewar, R. E. (1991). Lemur ecology. *Annual Review of Ecology and Systematics*, *22*, 145–175. https://doi.org/10.1146/annurev.es.22.110191.001045

Sato, H., Santini, L., Patel, E. R., Campera, M., Yamashita, N., Colquhoun, I. C., & Donati, G. (2016). Dietary flexibility and feeding strategies of *Eulemur*: A comparison with *Propithecus*. *International Journal of Primatology*, *37*, 109–129. https://doi.org/10.1007/s10764-015-9877-6

Sauther, M. L., Sussman, R. W., & Gould, L. (1999). The socioecology of the ringtailed lemur: Thirty-five years of research. *Evolutionary Anthropology*, *8*, 120–132. https://doi.org/10.1002/(SICI)1520-6505(1999)8:4<120::AID-EVAN3>3.0.CO;2.-O

Schilling, A. (1979). Olfactory communication in prosimians. In G. A. Doyle, & R. D. Martin (Eds.), *The study of prosimian behavior* (pp. 461–542). New York: Academic. https://doi.org/10.1016/B978-0-12-222150-7.X5001-6.

Schommer, N. N., & Gallo, R. L. (2013). Structure and function of the human skin microbiome. *Trends in Microbiology*, *21*, 660–668. https://doi.org/10.1016/j.tim.2013.10.001

Scordato, E. S., & Drea, C. M. (2007). Scents and sensibility: Information content of olfactory signals in the ringtailed lemur, *Lemur catta*. *Animal Behaviour*, *73*, 301–314. https://doi.org/10.1016/j.anbehav.2006.08.006

Scordato, E. S., Dubay, G., & Drea, C. M. (2007). Chemical composition of scent marks in the ringtailed lemur (*Lemur catta*): Glandular differences, seasonal variation, and individual signatures. *Chemical Senses*, *32*, 493–504. https://doi.org/10.1093/chemse/bjm018

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, *12*, R60. https://doi.org/10.1186/gb-2011-12-6-r60

Setchell, J. M., Vaglio, S., Moggi-Cecchi, J., Boscaro, F., Calamai, L., & Knapp, L. A. (2010). Chemical composition of scent-gland secretions in an Old World monkey (*Mandrillus sphinx*): Influence of sex, male status, and individual identity. *Chemical Senses*, *35*, 205–220. https://doi.org/10.1093/chemse/bjp105

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

Sin, Y. W., Buesching, C. D., Burke, T., & Macdonald, D. W. (2012). Molecular characterization of the microbial communities in the subcaudal gland secretion of the European badger (*Meles meles*). *FEMS Microbiology Ecology*, *81*, 648–659. https://doi.org/10.1111/j.1574-6941.2012.01396.x

Springer, A., Fichtel, C., Al-Ghalith, G. A., Koch, F., Amato, K. R., Clayton, J. B., & Kappeler, P. M. (2017). Patterns of seasonality and group membership characterize the gut microbiota in a longitudinal study of wild Verreaux's sifakas (*Propithecus verreauxi*). *Ecology and Evolution*, *7*, 5732–5745. https://doi.org/10.1002/ece3.3148

Stecher, B., & Hardt, W. D. (2011). Mechanisms controlling pathogen colonization of the gut. *Current Opinion in Microbiology*, *14*, 82–91. https://doi.org/10.1016/j.mib.2010.10.003

Stumpf, R., Gomez, A., Amato, K., Yeoman, C., Polk, J., Wilson, B., & Leigh, S. R. (2016). Microbiomes, metagenomics, and primate conservation: New strategies, tools, and applications. *Biological Conservation*, *199*, 56–66. https://doi.org/10.1016/j.biocon.2016.03.035

Stumpf, R. M., Wilson, B. A., Rivera, A., Yildirim, S., Yeoman, C. J., Polk, J. D., & Leigh, S. R. (2013). The primate vaginal microbiome: Comparative context and implications for human health and disease. *American Journal of Physical Anthropology*, *152*, 119–134. https://doi.org/10.1002/ajpa.22395

Sudo, N. (2014). Microbiome, HPA axis and production of endocrine hormones in the gut. *Advances in Experimental Medicine and Biology*, *817*, 117–194. https://doi.org/10.1007/978-1-4939-0897-4_8

Suzuki, T. A. (2017). Links between natural variation in the microbiome and host fitness in wild mammals. *Integrative and Comparative Biology*, *57*, 756–769. https://doi.org/10.1093/icb/icx104

Thaiss, C. A., Zmora, N., Levy, M., & Elinav, E. (2016). The microbiome and innate immunity. *Nature*, *535*, 65–74. https://doi.org/10.1038/nature18847

Theis, K. R., Dheilly, N. M., Klassen, J. L., Brucker, R. M., Baines, J. F., Bosch, T. C., & Lloyd, E. A. (2016). Getting the hologenome concept right: An eco-evolutionary framework for hosts and their microbiomes. *mSystems*, *1*, e00028–00016. https://doi.org/10.1128/mSystems.00028-16

Theis, K. R., Venkataraman, A., Dycus, J. A., Koonter, K. D., Schmitt-Matzen, E. N., Wagner, A. P., & Schmidt, T. M. (2013). Symbiotic bacteria appear to mediate hyena social odors. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 19832–19837. https://doi.org/10.1073/pnas.1306477110

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*, 20182448. https://doi.org/10.1098/rspb.2018.2448

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: A metagenomic analysis of humanized gnotobiotic mice. *Science Translational Medicine*, *1*, 6ra14. https://doi.org/10.1126/scitranslmed.3000322

Uchihashi, M., Bergin, I., Bassis, C., Hashway, S., Chai, D., & Bell, J. (2015). Influence of age, reproductive cycling status, and menstruation on the vaginal microbiome in baboons (*Papio anubis*). *American Journal of Primatology*, *77*, 563–578. https://doi.org/10.1002/ajp.22378

Umanets, A., de Winter, I., IJdema, F., Ramiro-Garcia, J., van Hooft, P., Heitkönig, I. M., & Smidt, H. (2018). Occupancy strongly influences faecal microbial composition of wild lemurs. *FEMS Microbiology Ecology*, *94*, fiy017. https://doi.org/10.1093/femsec/fiy017

Wilmé, L., Goodman, S. M., & Ganzhorn, J. U. (2006). Biogeographic evolution of Madagascar's microendemic biota. *Science*, *312*, 1063–1065. https://doi.org/10.1126/science.1122806

Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: Fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology*, *40*, 235–243. https://doi.org/10.1097/00004836-200603000-00015

Wu, G. D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S. A., & Lewis, J. D. (2011). Linking long-term dietary patterns with gut microbial enterotypes. *Science*, *334*, 105–108. https://doi.org/10.1126/science.1208344

Yildirim, S., Yeoman, C. J., Janga, S. C., Thomas, S. M., Ho, M., Leigh, S. R., & Stumpf, R. M. (2014). Primate vaginal microbiomes exhibit species specificity without universal Lactobacillus dominance. *The ISME Journal*, *8*, 2431–2444. https://doi.org/10.1038/ismej.2014.90

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